Thesis for the Degree of Doctor of Philosophy

Fermentative hydrogen and methane productions using membrane bioreactors

Julius Gbenga Akinbomi
Abstract

The role of energy as a stimulant for economic growth and environmental sustainability of any nation has made the focus on green fuels, including fermentative hydrogen (bioH₂) and methane (bioCH₄), to be a priority for the World’s policy makers. Nigeria, as the most populous African country, with worsening energy crisis, can benefit from the introduction of the bioH₂ and bioCH₄ technologies into the country’s energy mix, since such technologies have the potential of generating energy from organic wastes such as fruit waste.

Fruit waste was studied in detail in this work because of its great economic and environmental potential, as large quantities of the wastes (10–65% of raw fruit) are generated from fruit consumption and processing. Meanwhile, bioH₂ and bioCH₄ productions involving anaerobic microorganisms in direct contact with organic wastes have been observed to result in substrate and product inhibitions, which reduce the gas yields and limit the application of the technologies on an industrial scale. For example, in this study, the first experimental work to determine the effects of hydraulic retention times and fruit mixing on bioH₂ production from single and mixed fruits revealed the highest cumulative bioH₂ yield to be equivalent to 30% of the theoretical yield. However, combining the fermentation process with the application of membrane encapsulated cells and membrane separation techniques, respectively, could reduce substrate and product inhibitions of the microorganisms. This study, therefore, focused on the application of membrane techniques to enhance the yields of bioH₂ and bioCH₄ productions from the organic wastes.

The second experimental work which focused on reduction of substrate inhibition, involved the investigation of the effects of the PVDF membrane encapsulation techniques on the bioH₂ and bioCH₄ productions from nutrient media with limonene, myrcene, octanol and hexanal as fruit flavours. The results showed that membrane encapsulated cells produced bioCH₄ faster and lasted longer, compared to free cells in limonene. Also, about 60% membrane protective effect against myrcene, octanol and hexanal inhibitions was obtained. Regarding bioH₂ production, membrane encapsulated cells, compared to free cells, produced higher average daily yields of 94, 30 and 77% with hexanal, myrcene and octanol as flavours, respectively. The final part of the study, which was aimed at reducing product inhibition, involved the study of the effects of membrane permeation of volatile fatty acids (VFAs) on the bioreactor hydrodynamics in relation to bioH₂ production. The investigation revealed that low transmembrane pressure of 10⁴Pa was required to achieve a 3L h⁻¹ m⁻² critical flux with reversible fouling mainly due to cake layer formation, and bioH₂ production was also observed to restart after VFAs removal.

The results from this study suggest that membrane-based techniques could improve bioH₂ and bioCH₄ productions from fermentation media with substrate and product inhibitions.

Keywords: Encapsulation, Inhibition, hydrodynamics, hydrogen, methane, fruit flavour, Membrane bioreactor
List of Publications

The thesis is founded on the results presented in the following articles:


**Paper IV:** Akinbomi, J., Wikandari, R., Taherzadeh, M.J. Enhanced fermentative hydrogen and methane productions from inhibitory-fruit flavour medium with membrane-encapsulated cells (submitted)


Statement of Contributions

Julius Akinbomi’s contributions to each of the above publications are:

**Paper I:** Responsible for part of the experimental work, data analyses and manuscript writing

**Paper II:** Responsible for the experimental work, data analyses and manuscript writing

**Paper III:** Responsible for part of the experimental work, data analyses and manuscript writing

**Paper IV:** Conceived the idea together with the co-authors as well as responsible for the experimental work, data analyses and manuscript writing

**Paper V:** Responsible for the literature survey, data collection and manuscript writing

**Paper VI:** Responsible for part of the literature survey, data collection and manuscript writing
Reflection on My PhD Journey

Having gone through a challenging, but exciting and rewarding PhD experience, I thought it was good to reflect on my journey and write briefly on it.

➢ My motivation for coming to Sweden for PhD study in Resource Recovery

The journey of the PhD programme began in Nigeria with the aim of working at the frontier of biogas technology in Nigeria. During the period, Nigeria was really in need of affordable technology that could guarantee stable power supply to her population. Besides, the country was also seeking strategies to efficiently manage the huge amount of waste that was inevitably being turned out on a daily basis through the activities of her teeming population. Having read about how electricity could be generated through biogas from organic wastes, and how by adopting the green fuel technology, the increasing energy demands of the growing world population could be met without exhausting the natural resources and polluting the environment, I was motivated to choose biogas as a course of study.

➢ My dream as a teacher in Lagos State University (LASU), Lagos, Nigeria

As a teacher in one of the Universities in Nigeria, Lagos State University (LASU), I aspired to be among the professional teachers that would help in improving the quality of teaching and learning in Nigeria by influencing innovative and entrepreneurial students who would be able to put Nigeria and the World at large on a sustainable footing. Knowing fully well that teachers are employed not just to teach but to teach thoroughly at a high professional standard and that good teaching is often informed by good research, I made plans to start my PhD study immediately after my Master’s degree programme in Chemical Engineering. Few months before the start of my PhD programme in Faculty of Engineering, LASU, Nigeria, Professor Mohammad Taherzadeh and Dr Kayode Adekunle came to LASU to enlighten us on the resource recovery programme going on at University of Borås, Sweden, and share with us how Borås as a city uses biogas for vehicle fuel and combined heat and power (CHP). Not long after the seminar presentation, four PhD students including me were informed of the opportunity of going to Sweden for PhD study in Resource Recovery through student exchange programme between Nigeria and Sweden. When I heard about it, - ‘it was like a dream come true’
➢ The first year of my PhD study

The journey from Nigeria to Sweden was such an eventful one, as I was not alone but with three other PhD students. We got to Sweden during winter period; it was my first time of seeing snow falling as I had never experienced it in Nigeria. I was not used to the very cold weather at the beginning so it was really tough for me to adapt to the cold weather. But afterwards, I got acclimated to the varying weather conditions.

My PhD programme in Sweden, which focused on biohydrogen and biogas production from organic wastes and agricultural residues, was supervised by a full-fledged Professor in Resource Recovery, Mohammad Taherzadeh. My first year was quite busy as the course load was intense, and I also had to conduct some experiments in the laboratory in order to get acquainted with the equipment as well as the safety measures involved while using the equipment. However, it was worthwhile to devote much time to the various lectures, presentations and laboratory work.

➢ The second year of my PhD study

During the second year of the PhD programme, I went to Nigeria with the aim of setting-up a mini biogas laboratory unit in my home country University for easy cross-border green fuel technology. However, due to the reorganisation that was going on during the period, the project was not feasible. Nonetheless, it is anticipated that in the nearest future, the project will be feasible as it could be a launch pad for the development and dissemination of green fuel technology in Nigeria. Meanwhile, my stay in Nigeria was not an idle period as I was busy writing some articles together with some PhD students on the experiments carried out the previous year.

➢ The third year of my PhD study

During the third year of the PhD studies, I was in France at Blaise Pascal University, Clermont Ferrand, for a six-month student exchange programme. The research in France was carried out in collaboration with a PhD student, Zaineb Trad, under the supervision of Professor Christophe Vial and Professor Christian Larroche. The aim of the research was to develop an innovative anaerobic membrane bioreactor (AnMBR) with combined benefits of external and immersed AnMBRs for simultaneous production of biohydrogen and volatile fatty acids (VFAs). The bioreactor did not only allow VFAs to be removed for further applications with minimal
modification to the hydrodynamics, but also prevented the inhibition of biohydrogen production by total VFAs.

Beyond the boundary of academic activities, my short duration in France gave me the opportunity to learn about the French culture, including its geography, history, religion, food, among others. One thing I find common to most countries in Europe is the importance they attach to their indigenous languages. Unlike in Sweden where people are not reluctant to speak English to you, French people are not enthusiastic about speaking English with foreigners. Although I know how difficult it is for students to have the mastery of foreign languages during the beginning of their carriers in foreign countries, the language barrier actually motivates foreign students to be determined to learn the languages of their host countries so that it will help them in interpersonal interactions.

➢ The final period of my PhD study

The remaining period of the PhD programme was spent in Sweden for the completion of other relevant research work.

Looking back, the PhD experience has been, though challenging, a fulfilling one for me! It is true that PhD journey is not a bed of roses and never a straight forward one. There is hardly any research without something going wrong at one stage or another. However, according to an adage that says ‘Life is 10% of what happened to us and 90% of how we react to it’, it is the attitude of the researcher that determines the eventual success or failure of the research work. Consequently, the PhD experience has taught me many lessons, including:

• being able to bring creative ideas into realisation
• ability to learn new skills and expertise required for the research and cope with difficulties encountered during the research, and
• being able to take criticism and turn research failure into success

- One thing is certain; the PhD study has been a pleasant and exciting one for me!
Acknowledgements

First of all, I want to express my profound gratitude to my main supervisor, Professor Mohammad Taherzadeh for his guidance, thorough supervision, constructive criticisms, expert advice, encouragement and support. He took time out of his extremely busy schedule to read my manuscripts and dissertation as well as bringing to my attention the details that needed to be addressed. In fact, the success of this research is due, in no small measure, to the support I received from him. Words cannot really express how grateful I am to you Sir. I am also grateful to Dr. Tomas Brandberg for his assistance during my PhD studies.

I am thankful to Lagos State University, Nigeria and University of Borås, Sweden for giving me the opportunity and support to have my PhD studies in Sweden. I also want to show my appreciation to Professor S.A. Sanni and Dr. Kayode Adekunle who initiated the collaboration between the two Universities. My special thanks go to my examiner, Professor Kim Bolton and Dr Päivi Yltervo for reading and making useful suggestions for the final draft of this thesis. Regarding my colleagues in Swedish Centre for Resource Recovery and other departments at the University of Borås as well as in France (Blaise Pascal University), I have had the privilege on different occasions of meeting many intelligent Post-doc, PhD and Master’s students since the inception of my PhD programme in Resource Recovery in 2011. Although they are too numerous to name here, I appreciate their contributions to the success of my study. To all the members of staff at University of Borås, I am grateful for the conducive research environment that was made available to me. I am also indebted to my two supervisors when I was in France, Professor C. Larroche and Professor C. Vial, as well as the PhD student, Zaineb.

My special thanks go to my darling wife, Dayo, for her patience and support, and also for taking good care of our princess, Feyi, during my absence. I am also grateful to my in-laws. And to my precious mother and late father of blessed memory; you are my role models. You taught me from my childhood to be good, responsible, strong, determined and prepared to pursue and achieve my lifetime goals. According to one of your sayings, ‘if there is a will there will be a way’- I will forever be grateful to you. To all my big sisters and brothers, I appreciate you all for your endless love, prayers, supports and encouragements. I am indeed grateful.

---To my God-the source of my strength- THANK YOU LORD!
**Nomenclature**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>AnMBRs</td>
<td>Anaerobic membrane bioreactors</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>bioH₂</td>
<td>Fermentative hydrogen</td>
</tr>
<tr>
<td>bioCH₄</td>
<td>Fermentative methane</td>
</tr>
<tr>
<td>C</td>
<td>Concentration</td>
</tr>
<tr>
<td>C/N</td>
<td>Carbon to Nitrogen ratio</td>
</tr>
<tr>
<td>C:N:P:S</td>
<td>Proportion of carbon, nitrogen, phosphorus and sulphur</td>
</tr>
<tr>
<td>CHP</td>
<td>Combined heat and power</td>
</tr>
<tr>
<td>CH₃COOH</td>
<td>Acetate</td>
</tr>
<tr>
<td>CH₃CH₂COOH</td>
<td>Propionate</td>
</tr>
<tr>
<td>CH₃CH₂CH₂COOH</td>
<td>Butyrate</td>
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<tr>
<td>CH₃CHOHCOOH</td>
<td>Lactate</td>
</tr>
<tr>
<td>CH₃CH₂OH</td>
<td>Ethanol</td>
</tr>
<tr>
<td>C₆H₁₂O₆</td>
<td>Glucose</td>
</tr>
<tr>
<td>CH₃COCOOH</td>
<td>Pyruvate</td>
</tr>
<tr>
<td>CoA</td>
<td>Coenzyme A</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>COD</td>
<td>Chemical oxygen demand</td>
</tr>
<tr>
<td>D</td>
<td>Diffusion coefficient</td>
</tr>
<tr>
<td>E(t)</td>
<td>Exit age distribution</td>
</tr>
<tr>
<td>ΔE</td>
<td>Change in internal energy</td>
</tr>
<tr>
<td>ECMBRs</td>
<td>External cross-flow membrane bioreactors</td>
</tr>
<tr>
<td>ESMBRs</td>
<td>Externally submerged membrane bioreactors</td>
</tr>
<tr>
<td>Fe</td>
<td>Iron</td>
</tr>
<tr>
<td>fd (ox)</td>
<td>Oxidised ferredoxin</td>
</tr>
<tr>
<td>fd (red)</td>
<td>Reduced ferredoxin</td>
</tr>
<tr>
<td>ΔG</td>
<td>Free energy change</td>
</tr>
<tr>
<td>HRT</td>
<td>Hydraulic retention time</td>
</tr>
</tbody>
</table>
$\Delta H$ Enthalpy change

$H_2$ Hydrogen

$H^+$ Proton

$H_2S$ Hydrogen sulphide

ISMBRs Internally submerged membrane bioreactors

$J$ Filtrate flux

$K$ Potassium

$m$ Maximum acceptable absolute value of mix relative deviation

$N$ Nitrogen

$\text{NAD}^+$ Oxidised Nicotiamide adenine dinucleotide

$\text{NADH}$ Reduced Nicotiamide adenine dinucleotide

$\text{Ni}$ Nickel

$N_2$ Nitrogen

$\text{NH}_3$ Ammonia

$O_2$ Oxygen

$\text{OLR}$ Organic loading rate

$\Delta P$ Applied pressure (Transmembrane pressure)

$\text{PFRO}$ Pyruvate-ferredoxin oxidoreductase

$\text{PFL}$ Pyruvate-formate lyase

PVDF Poly (vinylidene fluoride)

$\text{PTFE}$ Poly (tetrafluoroethylene)

PE Polyethylene

$\text{PP}$ Polypropelene

$P$ Phosphorus

$P_f$ Feed pressure

$P_p$ Permeate pressure

$P_r$ Retentate pressure

$\text{P2G}$ Power-to-Gas

$q$ Heat

$\text{LCFA}$ Long chain fatty acids

$R$ Gas constant
Re  Cake layer or external fouling
Ri  Irreversible adsorption and pore plugging
Rm  Intrinsic membrane resistance
RT  Total resistance
RTD  Residence Time Distribution
ΔS  Entropy change
Se  Selenium
t  Time
T  Temperature
TMP  Transmembrane pressure
ΔV  Change in volume
x  Thickness
w  Total work done
W  Tungsten
μ  Viscosity
VFA(s)  Volatile fatty acid(s)
# Table of Contents

Abstract iii  
List of Publications iv  
Reflection on my PhD Journey v  
Acknowledgements viii  
Nomenclature ix  
Table of Contents xii  

## Chapter 1. Introduction

1.1. Background 1  
1.2. Objectives and Scope 2  
1.3. Thesis Structure 4  
1.4. Contribution of the Thesis 5  
1.5. Research ethics and social aspects 5  

## Chapter 2. Fermentative process for hydrogen and methane productions  

2.1. Basics of fermentation process 7  
2.2. Dark fermentation: a pathway to effective biomethane production 9  
2.2.1. Hydrogen production methods 9  
2.2.2. Microbiology of hydrogen, volatile fatty acids and methane productions 12  
2.2.2.1. Hydrogen 13  
2.2.2.2. Volatile fatty acids 14  
2.2.2.3. Methane 14  
2.2.3. Thermodynamics of fermentative hydrogen and methane productions 15  
2.3. Factors influencing fermentative hydrogen and methane productions 18  
2.3.1. Nature of feedstock 19  
2.3.2. Medium pH and alkalinity 19  
2.3.3. Inoculum pretreatment 20  
2.3.4. Complexity of the seed cultures 21  
2.3.5. Temperature 21
2.3.6. Retention times and organic loading rates 22
2.3.7. Inhibitors 22
2.3.8. Mixing 23
2.3.9. Hydrogen partial pressure 24
2.3.10. Nutrient supplementation 24
2.4. End-use technologies for fermentative hydrogen and methane 25
  2.4.1. Attractive qualities of hydrogen and methane as energy carriers 26
2.5. Implications of fermentative hydrogen and methane for technological applications 26
  2.5.1. Process limitations 27
  2.5.2. Infrastructure barriers 28

Chapter 3. Feedstocks for fermentative hydrogen and methane productions 31
3.1. Feedstocks suitability for hydrogen and methane productions 31
  3.1.1. Non-lignocellulosic feedstocks 31
  3.1.2. Lignocellulosic feedstocks 32
3.2. Types of feedstocks for hydrogen and methane productions 33
  3.2.1. Agriculture crop wastes 33
  3.2.2. Livestock manure 34
  3.2.3. Municipal solid waste 34
  3.2.4. Industrial wastes and municipal wastewa ter 34
3.3. Inhibitory effects of fruit flavours and volatile fatty acids 36
  3.3.1. Inhibitory effects of fruit flavours 36
    3.3.1.1. Proposed mechanism of flavour toxicity to bacteria 36
    3.3.1.2. Adaptation of bacteria to toxic environment 37
  3.3.2. Inhibition of volatile fatty acids 38
3.4. Limiting the inhibitory effects of fruit flavours and volatile fatty acids 38
  3.4.1. Control measure to limit fruit flavour inhibition during fermentation 38
  3.4.2. Control measure to limit volatile fatty acids inhibition during fermentation 39
Chapter 4. Membrane processes for improvement of fermentative hydrogen and methane productions

4.1. Membrane classification

4.1.1. Application of PVDF membrane in fermentative hydrogen and methane productions

4.2. Influence of membrane permeability on membrane performance

4.3. Encapsulation technology for cell retention and inhibition control

4.4. Application of hollow fibre membrane configuration for VFA permeation

4.5. Limitations of membrane technology: Membrane fouling and cost

4.6. Implications of membrane applications in this study

Chapter 5. Bioreactor hydrodynamics for fermentative hydrogen and methane productions

5.1. Ideal and real reactors

5.2. Mixing in bioreactors

5.2.1. Mixing and mean circulation times

5.3. Residence time distribution measurement

5.4. Membrane filtration

6. Conclusions and Future Work

6.1. Conclusions

6.2. Future Work

References
CHAPTER 1

Introduction

1.1. Background

Humanity is endowed with diverse resources in form of materials, energy, services and knowledge, which could be utilised for maximal benefits. Meanwhile, the usage of some of these resources such as fossil fuels has resulted in negative consequences including resource depletion, global climate change and environmental pollution, which could ultimately threaten human existence. In contrast, resources such as wastes, which are inevitably generated in large amounts from daily human activities such as food consumption, farming activities and industrial processing, could be a source of huge wealth for a nation, without any negative consequences, if the wastes are properly and efficiently utilised. For instance, Nigeria is the most populous country in Africa with over 165 million people and an annual growth rate of about 2.8% (Paper V) but the country faces worsening energy crisis with 60% of the population having no access to the national power supply while those that have access to the power supply experience frequent power outages. Besides, Nigeria also has the challenge of inefficient waste management system for the huge amount of wastes inevitably generated daily by the teeming population of the country. Therefore, Nigeria could benefit immensely from a technology that could effectively turn wastes into affordable energy for the people (Paper V). Although there are various waste management techniques, such as recycling, composting, landfill and incineration, anaerobic digestion offers numerous benefits which include minimal environmental impact and waste valorisation for production of energy carriers (hydrogen, methane and ethanol, among others), organic fertilizers and other valuable products (1, 2).

During anaerobic digestion, the initial step of producing hydrogen from organic wastes before using the metabolites (mainly volatile fatty acids) as building blocks of valuable compounds (biomethane, biolipids and microbial fuel cells), enables efficient valorisation of the organic wastes. The traditional single stage of anaerobic digestion to generate only methane for energy usage does not allow for efficient and optimal utilization of the feedstock for energy production. It has been observed that only 30% of the methane production during anaerobic digestion.
digestion is produced from carbon dioxide reduction using hydrogen while more than 70% of the methane production comes from acetic acid conversion by heterotrophic methanogenic archaea (3-5). Consequently, high concentration of hydrogen and carbon dioxide is left unconverted in the digester and only a small portion of the hydrogen produced ends up being consumed by the hydrogen consuming microorganisms. In this regard, anaerobic digestion process could be better utilised if more energy in the form of hydrogen, in addition to methane, could be obtained from the process (6). However, for efficient anaerobic digestion and high productivity, especially during continuous process, it is often necessary to retain bacterial cells for a long time to obtain high cell density and protect the microorganisms from substrate and product inhibitions (Papers I and IV). In Papers I and IV, hydrophilic poly (vinylidene fluoride) (PVDF) membranes with pore size of 0.1μm were used to hold and restrict the movement of the fermentative bacteria and archaea for efficient performance. It is also often required to constantly remove some portion of the fermentation broth to prevent product inhibition of the process (Paper III). In the study carried out in Paper III, hydrophilic PVDF hollow fibre membrane module operated in the cross-flow ‘outside-in’ mode and placed in a recirculation loop while coupled to a 5-L mechanically stirred tank reactor, was used to extract volatile fatty acids (VFAs) from the fermentation broth. The integration of bacterial cell retention and product recovery during continuous fermentation processes could be effectively achieved by using membrane techniques, which have the benefits of increasing cell concentration and reducing substrate and product inhibitions.

1.2. Objectives and Scope

Industrial production of combined hydrogen and methane via dark fermentation process is still extremely limited due to low hydrogen and methane yields obtained from various laboratory research works. The low yield has been attributed to the unfavourable energetics of the hydrogen and methane productions as well as the tendency of the fermentation process to naturally produce cell biomass (7). Consequently, most fermentative organisms only produce a relatively small amount of hydrogen along with other fermentation products including acetate, butyrate, butanol and acetone, resulting in suboptimal methane production. Acetate production allows the formation of adenosine triphosphate (ATP), while formation of other reduced products allows the oxidation of nicotinamide adenine dinucleotide (NADH), which is necessary to maintain redox balance in the fermentation process. Other factors including environmental and process...
parameters, inefficient substrate conversion, substrate and product inhibition also contribute to the low hydrogen yield from the fermentation process (8).

Considering the yield-related challenges associated with fermentative hydrogen production and subsequent methane production during fermentation process, this research, therefore, intended to improve the yields of fermentative hydrogen and methane through membrane control of substrate (Papers I and IV) and product (Paper III) inhibitions (Figure 1.1) as well as using varying operational parameters (Paper II). Moreover, in order to determine the feasibility and sustainability of future commercial production of hydrogen and biogas productions in Nigeria as well as other parts of the world, in terms of feedstock availability and bioreactors suitability, reviews were conducted on biogas development in Nigeria (Paper V) and also on various types of membrane bioreactors that could be used for ethanol and biogas production (Paper VI).

![Figure 1.1. Schematic diagram for the scope of the research](image)

The aim of this research was achieved through the investigation of the following activities: (i) Demonstration of the protective effects of hydrophilic PVDF membranes on fermentative bacteria against inhibitory effects of fruit flavour media during fermentation process (Papers I and IV),
(ii) Evaluation of the potential of hydrogen yield enhancement from fruit fermentation through varying hydraulic retention times and fruit mixing (Paper II),

(iii) Investigation of the feasibility of employing membrane VFA permeation to improve fermentative hydrogen production without any major modification of the hydrodynamics in the anaerobic membrane bioreactor system (Paper III),

(iv) Assessment of feedstock availability for commercial production of biogas production in Nigeria (Paper V),

(v) Study of membrane bioreactors suitable for biogas production (Paper VI).

Generally, hydrogen and methane productions can be enhanced using a suitable microbial species, process modification, efficient bioreactor design and genetic techniques. The scope of this research was, however, limited to the application of process modification and bioreactor design for the improvement of fermentative hydrogen and methane productions.

1.3. Thesis Structure

The thesis is organised into two parts: the first part provides information on the basic principles that the research work was based on, while the second part comprises of the six articles from the research. The experimental work mainly focused on two subject areas with regard to fermentative hydrogen and methane productions. The first area was on the application of membrane encapsulation techniques to protect bacteria from substrate inhibition and thereby enhance the hydrogen and methane production potential of the bacteria. The second area of the research focused on reducing the effect of product inhibition on bacteria through process parameters and membrane permeation of volatile fatty acids with consequent improvement on the fermentative hydrogen production.

The chapters included in the first part of the thesis are as follows:

**Chapter 1** introduces the main reasons for conducting research on the investigated subject as well as the intended objectives of the research.

**Chapter 2** lays the foundation for the research problem with literature review on fermentation process and thermodynamics for hydrogen and methane productions including determining factors, end-use technologies as well as the implications of the technology applications.
Chapter 3 provides information on potential feedstock for fermentative hydrogen and methane productions along with the inhibitory effects of fruit flavours and VFAs.

Chapter 4 describes membrane processes and the underlying principles of membrane encapsulation and VFAs permeation.

Chapter 5 relates fluid hydrodynamics in the bioreactors to the bioreactor performance and the effectiveness of the fermentation process.

Chapter 6 summarises the main research conclusions and provides directions for future work.

1.4. Contribution of the Thesis

Generation of hydrogen and methane through anaerobic fermentation process has been established as an environmentally-friendly and non-energy intensive technique that could play a significant role in the future green and zero-emission world. Fermentative methane technology on an industrial scale has been around for decades in most advanced countries, while fermentative hydrogen production is not yet commercialised due to the challenge of low hydrogen yield from the process. Research efforts have therefore been intensified to find ways of improving, not only hydrogen yields, but also methane yields from fermentation of diverse organic compounds. The results from this research are significant in the area of reducing the effects of substrate and product inhibitions on the fermentative hydrogen and methane productions, thereby, enhancing the yields of the two energy carriers. Moreover, the study provides insight into the antimicrobial effects of fruit flavours on both fermentative hydrogen and methane and can be used as a guide for improving the gas yields during commercial applications.

1.5. Research ethics and social aspects

In view of the growing global threats of energy insecurity and climate change due to greenhouse gas emissions, coupled with the inefficient waste management system, especially, in most third world countries, the research was focused on how to efficiently recover resources in terms of energy or useful products from waste materials while simultaneously reducing environmental pollution. It is anticipated that efficient production of fermentative hydrogen and
methane could be used as a tool in tackling global challenges including energy insecurity, climate crises and inefficient waste management system. Energy carriers, such as fermentative hydrogen and methane, provide utility in terms of energy, and its effective demand by consumers will depend on factors including cost effectiveness, appropriateness of the technology, availability, reliability, efficiency and technical potentials. Therefore, the effects of these variables, especially, membrane techniques and process enhancement, were, therefore, investigated during this study (Papers I - IV) for the improvement of fermentative hydrogen and methane productions.

Nevertheless, there are some considerable ethical problems related to commercial application of biofuels such as fermentative hydrogen and methane. It is believed that increasing demand for biofuel production may simultaneously cause the rise in demand for arable lands used for growing food crops, thereby, leading to food shortage. Growing biofuel crops in arable lands may compete with food production for arable land, water and plant nutrients, which may create more problems including increase in global market price for food making it beyond the reach of poor people. In addition, there is a risk of environmental pollution due to application of fertilizer and pesticides in the production of biofuel crops. In other words, ethical dilemmas often arise in the process of tackling some of the global crises including management of natural resources, energy, climate changes and food crises. Consequently, it is necessary to consider the effects of the development of any green technology on various actors (people, animals and the natural world) in such a way that the green technology being developed will have the least possible damage, if any, to the various actors.

Regarding ethical issues relating to my research, the food-versus-biofuel problem is not a barrier, as organic waste materials that are produced from daily human activities, are the potential feedstocks for the production of the biofuels. Furthermore, during the research work, ethical norms, including honesty, objectivity, integrity, carefulness, openness, respect for intellectual property, confidentiality, reliable publication and competence, which govern research conduct in my fields were strictly adhered to in order to make the results of the investigations acceptable to the general public (9, 10).
CHAPTER 2

Fermentative process for hydrogen and methane productions

The fermentation process for the production of hydrogen and methane is an anaerobic digestion process in which complex organic feedstock is broken down microbially into simpler substances in the absence of oxygen.

2.1. Basics of fermentation process

Anaerobic fermentation process is carried out by different species of anaerobic microorganisms in several successive steps, with each step depending on the preceding one. The fermentation process consists of four steps, which include hydrolysis, acidogenesis, acetogenesis and methanogenesis \(^{(11)}\) (Figure 2.1). In hydrolysis, the complex components in the feedstock including carbohydrates, proteins and fats, are initially broken down by extracellular enzymes into their respective monomeric units- glucose (sugars), amino acids and fatty acids. The extracellular enzymes including amylases, proteases and lipases, are secreted by various strains of hydrolytic bacteria to break down the complex compounds into soluble compounds that could easily be transported across the bacterial cell membrane. The enzymes production as well as feedstock particle size, duration of enzyme-particle contact, pH, among others, determine the rate of the hydrolysis process \(^{(12, 13)}\). The products of the hydrolysis are converted by acidifying bacteria into volatile fatty acids (VFAs), alcohols, carbon dioxide (CO\(_2\)), hydrogen (H\(_2\)) and ammonia (NH\(_3\)) during acidogenesis \(^{(14)}\). The acidifying bacteria, which are a mixture of facultative and obligatory bacteria, are important in creating an anaerobic condition during the fermentation process, as the facultative anaerobes have the potential of using up the oxygen that might have been mistakenly introduced into the process along with the feed.

During acetogenesis, low molecular weight VFAs are converted by acetogenic bacteria into acetic acid (CH\(_3\)COOH), CO\(_2\) and H\(_2\), which can be easily utilised by methanogenic archaea \(^{(15)}\). Acetogenesis requires efficient and continuous removal of hydrogen formed from the fermentation process, as the process can only be favoured thermodynamically at low partial pressure of hydrogen \(^{(11)}\). This was one of the reasons why recovery of fermentative hydrogen
during dark fermentation process was given a priority during this research study (Papers II, III and IV) as it could make the fermentation process cost effective in terms of the energy recovery. At the final stage, methanogenic archaeal group uses three biochemical pathways, namely, acetotrophic, hydrogenotrophic and methylotrophic pathways, to produce methane from the products of previous stages including CH$_3$COOH, CO$_2$, H$_2$, formate, methanol and methylamine. It has however be shown that more than 70% of the methane production in anaerobic digestion comes from acetate conversion (3, 16, 17).

The four phases during fermentation process, namely, hydrolysis, acidification, acetogenesis and methanogenesis can all take place in a bioreactor resulting in one-stage fermentation. Alternatively, the four phases may be divided into two parts called two-stage
fermentation process. The first part including hydrolysis, acidogenesis and acetogenesis, takes place in the first bioreactor, while the second part called methanogenesis takes place in the second reactor (18). The two-stage fermentation is appropriate for combined hydrogen and methane productions as it allows for the optimisation of process parameters including pH and temperature, which differ for both hydrogen and methanogenic archaea (19-21). The recovery of hydrogen as energy carrier during anaerobic fermentation studies is usually done in a two-stage fermentation process in which VFAs, produced as the by-products of the dark fermentation process in the first reactor, are further degraded to produced other valuable products including hydrogen, methane, biodiesel and bioplastics.

Fermentation process for hydrogen and methane productions can be operated in batch, fed-batch or continuous mode (22, 23). Batch reactor is operated as a closed culture system, in which the bioreactor is filled with nutrients and other additives at the beginning of the process; thereafter, the reactor is sealed for the digestion process to complete. Then the fermentation products are recovered at the end of the process. In this study, fermentative methane productions from feedstocks containing flavour compounds were investigated using batch fermentation processes (Papers I and IV). In fed-batch fermentation, critical elements of the nutrient solutions are added to the bioreactor, while the fermentation broth remains in the bioreactor until the end of the fermentation process (24, 25). Regarding continuous fermentation process, the bioreactor is operated as an open system in which substrates are added continuously to the bioreactor with simultaneous withdrawal of the digested sludge. Fermentative hydrogen production in this study was investigated using continuous fermentation process, as the process was more effective in preventing the growth of hydrogen consuming microorganisms such as methanogenic archaea. However, the mode of operation was not completely a continuous operation as the substrate feeding and effluent withdrawals were done only once each day during the experiment.

2.2. Dark fermentation: a pathway to effective biomethane production

2.2.1. Hydrogen production methods

Various industrial methods for hydrogen production exist including steam reforming of methane, thermo-chemical water splitting and biomass gasification, pyrolysis and ‘Power-to-Gas’ (P2G) techniques (Figure 2.2). The P2G is a new technique of hydrogen production that is
now attracting researchers’ attention because of its potential to store excess electricity in form of gas fuel by the conversion of electrical power to gas fuels. In the P2G techniques, different pathways exist including ‘Power-to-Hydrogen’, ‘Power-to-Methane’ and ‘Power-to-Syngas’. In the Power-to-Hydrogen, the hydrogen produced from the electrolysis of water is used directly as transport fuel or for other purposes (26). Regarding Power-to-Methane’, the hydrogen produced from the electrolysis is combined with CO₂ to form methane using a methanation reaction (Sabatier or biological methanation) (27-31). In the Power-to-Syngas’, the hydrogen formed from the electrolysis of water is combined with CO₂ in a conversion reactor to produce a mixture of gases, including hydrogen, carbon monoxide and water, which is called syngas (32).

Figure 2.2. Hydrogen production techniques
Meanwhile, most of the above techniques employed in the industrial hydrogen production, are energy intensive and non-environmentally friendly techniques. Consequently, low-energy techniques that are also non-polluting are currently being focused on as alternative hydrogen production techniques. The low-energy techniques include dark fermentation (33, 34), biophotolysis, photo-fermentation (35, 36) microbial electrolysis and enzymatic techniques. Biophotolysis is a water splitting process using green algae or cyanobacteria via direct and indirect routes with light as the energy source. Molecular oxygen and hydrogen are produced by utilising inorganic CO$_2$ in the presence of sunlight and water. In photo-fermentation, hydrogen is produced through the activities of photosynthetic bacteria, which have the ability to utilise diverse substrates, ranging from inorganic to organic acids with light as the energy source. Microbial electrolysis involves the application of external electric potential to enhance hydrogen production from microbial cells using various organic substrates. Among the biological techniques, dark fermentation seems like a promising alternative as future commercial hydrogen production process because, unlike other biological methods, it does not require light energy and has lower energy demands as well as having higher hydrogen production rate (37). Moreover, it is simple and robust and has the potential for small footprint (7, 38, 39).

Dark fermentation is different from other biological processes in the sense that it uses organic substrates as both energy and carbon sources. It is a process that involves the microbial conversion of organic substrates to biohydrogen in an oxygen-free environment. It is called dark fermentation because the fermentation takes place in the absence of light, unlike photo-fermentation, that is a light-dependent process. In normal anaerobic digestion process, dark fermentation usually occurs together with methanogenesis, as hydrogen and acetate produced during dark fermentation process are used as substrates for methanogenic archaea (40). However, when the two processes occur together in a single-stage system, energy recovery is usually inefficient as hydrogen produced is consumed by hydrogen consuming microorganisms leaving only methane as the only gaseous energy carrier that could be recovered. Moreover, the two processes differ in terms of the required nutrients, optimal environmental conditions, growth kinetics, among others; hence, any disturbance in the process optimal conditions can affect the efficiency of the microorganisms community. If the dark fermentation is, however, separated from the methane forming process, for example, in a two-stage system, overall energy extraction
from the substrate conversion could be increased with the additional energy obtained through methane generation from the by-products of the hydrogen fermentation.

2.2.2. Microbiology of hydrogen, volatile fatty acids and methane productions

Anaerobic fermentation of organic compounds for energy production and cell growth usually involves electron generation, which must be disposed off to electron acceptors. In dark fermentation reactions, which take place in an environment that lacks terminal electron acceptors such as oxygen, sulphate, nitrate and ferric iron, redox balance is maintained by the production of molecular hydrogen (H₂) with protons (H⁺) from water serving as electron acceptor. Electrons released during the conversion of organic compounds into series of degraded and oxidised intermediate compounds, are utilised to convert coenzymes such as nicotiamide adenine dinucleotide (NAD⁺) to their reduced form. The reduced coenzyme returns back to its oxidised form by reducing intermediate compounds including pyruvate (Figure 2.3). Reduction equivalents including formate, reduced ferredoxin and nicotiamide adenine dinucleotide (NADH) function as electron donors to hydrogen (41).

**Figure 2.3.** Metabolic process during dark fermentative hydrogen production
2.2.2.1. Hydrogen

During anaerobic fermentation process, the bacteria break down organic compounds into pyruvate, which is further degraded with the aid of either of two enzymes, namely, pyruvate-formate lyase (PFL) and pyruvate-ferredoxin oxidoreductase (PFRO) (Equations 2.1 & 2.2). The most commonly used enzyme during fermentative hydrogen production or fermentation involving obligate or thermophilic bacteria, is PFRO.

\[
\textit{Pyruvate} + \textit{CoA} \xrightarrow{\text{PFL}} \textit{Acetyl} - \textit{CoA} + \textit{formate} \quad 2.1
\]

\[
\textit{Pyruvate} + \textit{CoA} \xrightarrow{\text{PFRO}} \textit{Acetyl} - \textit{CoA} + \textit{CO}_2 + 2 \textit{fd (red)} \quad 2.2
\]

The pyruvate generated from fermented sugars is cleaved by pyruvate ferredoxin oxidoreductase in the presence of coenzyme A (CoA) to generate acetyl CoA, reduced ferredoxin and CO\(_2\) (Equation 2.3), while the reduced ferredoxin generated, catalyses hydrogen formation (Equations 2.4 & 2.5).

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 2\text{NAD}^+ \rightarrow 2\text{CH}_3\text{COCOOH (pyruvate)} + 2\text{NADH} + 2\text{H}^+ \quad 2.3
\]

\[
\text{Pyruvate} + \text{CoA} + 2\text{fd (ox)} \rightarrow \text{Acetyl} - \text{CoA} + 2\text{fd (red)} + \text{CO}_2 \quad 2.4
\]

\[
2\text{H}^+ + \text{fd (red)} \rightarrow \text{H}_2 + \text{fd (ox)} \quad 2.5
\]

Hydrogen production is related to the activity of an iron-sulphur protein called ferredoxin, an electron carrier of low redox potential. The metabolic process for hydrogen production is dependent on the reduction of the metabolite ferredoxin, which in turn depends on the recycling of ferredoxin through oxidation. The transfer of electrons from NADH to reduced ferredoxin ensures the continuation of the recycling process of ferredoxin (42). The fermentative bacteria need to regenerate the cytoplasmic electron carrier NAD to maintain the glycolysis. Three main classes of hydrogen forming enzymes including [FeFe]-hydrogenase, [NiFe]-hydrogenase and nitrogenase, catalyse the recycling processes of ferredoxin (42). In clostridium bacteria, the hydrogen production is mostly due to [FeFe]-hydrogenase with activity that is hundred times higher than [NiFe]-hydrogenase and a thousand times higher than nitrogenase (43, 44).
2.2.2.2. Volatile fatty acids

During dark fermentation, the main aqueous products are acetate, propionate and butyrate, while formate, lactate, valerate and caproate are also produced as minor acidogenic products (45). The acetyl-CoA produced during the cleavage of PFRO, is the essential intermediate in the production of both volatile fatty acids and solvents. When volatile fatty acids are generated, there are no reductions that could prevent the reduced ferredoxin from transferring electrons to a hydrogenase that permits the use of protons as a final acceptor; thus, the ferredoxin is re-oxidised and molecular hydrogen is released from the cell. However, under certain unfavourable conditions such as high hydrogen partial pressure, the formation of hydrogen is limited, and carbon flow to acid production pathway is switched to the solvent production pathway, which involves reduction. As a result, ferredoxin is unable to transfer electrons to a hydrogenase for hydrogen production, thereby the cell is forced to channel electrons through NADH:ferredoxin oxidoreductase (NADH consumption) to form some reduced compounds such as lactate, ethanol and butanol, resulting in a lowered hydrogen yield (42, 46). This is why it is better to have two-stage fermentation process, which allows continuous removal of hydrogen from the process, while the metabolic products from the first stage are used for methane production, thereby leading to efficient energy recovery.

2.2.2.3. Methane

High proportion of methane production in an anaerobic digester occurs from the use of acetate and hydrogen by methane-forming bacteria. Aceticlastic cleavage of acetate and reduction of CO₂ are the two major pathways to methane production. The pathways involving propionic and butyric acids fermentation only have minor contribution to methane production. There are three principal groups of methane-forming bacteria including hydrogenotrophic, acetotrophic and methylotrophic methanogens. Hydrogenotrophic methanogens use hydrogen to convert CO₂ into methane, acetotrophic methanogens split acetate into methane and CO₂, while methylotrophic methanogens grow on substrates that contain methyl group including methanol and methylamines. The acetotrophic methanogens reproduce more slowly than the hydrogenotrophic methanogens and are adversely affected by the accumulation of hydrogen. The maintenance of low partial hydrogen pressure in an anaerobic digester is, therefore, favourable for the activity of acetotrophic methanogens (47).
2.2.3. Thermodynamics of fermentative hydrogen and methane productions

The thermodynamics of fermentation process determines the bioH₂ and bioCH₄ yields from the process. As every chemical reaction involves loss or gain of electrons, the bacteria involved in the fermentation process conserve their energy through the coupling of ATP for the breakdown of organic compounds in their environment. The amount of energy released during the process depends on the distance between the electron donor and electron acceptor, as the coupling of two reactions cannot occur if they are separated from each other. The energy released is necessary for various bacterial activities including mass transport of molecules across bacterial cell membrane. In essence, bacterial metabolism involves energy transformation, and the energy transfer mechanism is based on thermodynamics principles (first and second laws of thermodynamics) (40, 48). The total energy involved in bacterial metabolism (oxidation and reduction reactions) must be conserved to maintain the integrity of the bacteria as stated in the first laws of thermodynamics, which states that total amount of energy in nature, is constant. In other words, heat (q) added to a system of given energy content must appear as a change in the internal energy (ΔE) of the system or in the total work carried out by the system on the surrounding (w) (Equations 2.6 & 2.7).

$$q = ΔE + w \quad \text{(2.6)}$$
$$ΔE = q - w \quad \text{(2.7)}$$

When heat addition to a system also results in volume change (ΔV) at a constant pressure (P), the change in internal energy can be referred to as enthalpy (ΔH) (Equations 2.8 & 2.9).

$$ΔH = ΔE + PΔV \quad \text{(2.8)}$$
or \hspace{1cm} $$ΔH = q - w \quad \text{(2.9)}$$

The second law of thermodynamics expresses that all reactions that occur proceed in a direction that the degree of randomness, commonly referred to as entropy (S) of the universe, increases to the maximum possible towards an equilibrium position (Equations 2.10 & 2.11).
\[ \Delta S = \frac{q}{T} \]  \hspace{1cm} 2.10

or \[ q = T \Delta S \]  \hspace{1cm} 2.11

where q, T and \( \Delta S \) represent heat, temperature and entropy change.

The combination of first and second law of thermodynamics (Equation 2.12) indicates that the tendency to attain position of maximum entropy is the driving force of all processes including the biological processes, and heat is either given up or absorbed by the system and its environment to enable them to reach a state of maximum entropy.

\[ \Delta H = T \Delta S - w \]  \hspace{1cm} 2.12

The change in heat (enthalpy) and entropy are related by the free energy (Equations 2.13 & 2.14), which is the energy released to perform useful work.

\[ \Delta G = \Delta H - T \Delta S \]  \hspace{1cm} 2.13

\[ \Delta G = -w \] (since \( \Delta H = T \Delta S - w \))  \hspace{1cm} 2.14

The values of free energy change (\( \Delta G \)) determine the spontaneity of a reaction. Free energy values that are greater than zero (\( \Delta G > 0 \)), equal to zero (\( \Delta G = 0 \)) or less than zero (\( \Delta G < 0 \)) represent that the reactions are not spontaneous, at equilibrium or spontaneous, respectively. As given in the equation, increase in temperature of a reaction process could make the reaction spontaneous depending on the enthalpy change of the system. As a consequence, most of the experiments investigated in this study were carried out at thermophilic temperatures (55°C) (Papers I, II & IV). Regarding the transfer of substances through cell membranes and other surfaces, the exchange free energy (\( \Delta G \)) for the transport of a mole of substance of concentration, \( C_1 \), from one place to another where it is present at \( C_2 \) is given as (Equation 2.15)

\[ \Delta G = RT \log \frac{C_2}{C_1} \]  \hspace{1cm} 2.15
The reaction is favourable when $\Delta G$ is negative, that is, when $C_2$ is less than $C_1$. In the absence of intervening factors, an equilibrium will be reached where $C_2 = C_1$, resulting in $\Delta G$ being equal to zero.

In a dark fermentation process, 12 mol H$_2$ per mol glucose could theoretically be obtained from the complete conversion of glucose to H$_2$ and carbon dioxide (Equation 2.16). But, the reaction is not thermodynamically favourable due to the production of a large quantity of metabolic products (VFAs, alcohols and lactate) associated with hydrogen production. The thermodynamic constraints make the maximum attainable hydrogen yields to be 4 and 2 mol/mol glucose if the associated metabolism products are acetate and butyrate, respectively (Equations 2.17 & 2.18). However, the fermentation with only acetate as the main organic acid (Equation 2.18) has higher theoretical yield than with other organic acids as products (Figure 2.4) under equilibrium conditions (7). Acetate and hydrogen are not the only fermentation products formed during the process, other secondary fermentation products such as ethanol, butyrate and lactate are also formed, thereby reducing the molar yield of the hydrogen production (49).

\[
\begin{align*}
C_6H_{12}O_6 + 6H_2O & \rightarrow 12H_2 + 6CO_2 \quad (\Delta G^\circ = +3.2 \text{ kJ}) \\ 
C_6H_{12}O_6 + 2H_2O & \rightarrow 4H_2 + 2CO_2 + 2CH_3COOH \text{ (acetate)} \quad (\Delta G^\circ = -206 \text{ kJ}) \\ 
C_6H_{12}O_6 & \rightarrow 2H_2 + 2CO_2 + CH_3CH_2CH_2COOH \text{ (butyrate)} \quad (\Delta G^\circ = -254 \text{ kJ})
\end{align*}
\]

The actual yield during the real experiment is often less than the maximum theoretical yield. For example, in this study (Paper II), experiment was conducted to explore means of increasing hydrogen production from fruit wastes including orange, apple, banana, grape and melon. The highest yield obtained was from the fruit mixture with equal weight proportion at an operating temperature of 55°C and HRT of 5 days, and the yield was just 30% of the theoretical yield (Paper II). The low yield might be attributed to the tendency of anaerobic fermentation processes to form other secondary fermentation products including ethanol, propionate and lactate, which consume hydrogen by uptake hydrogenases (7).
2.3. Factors influencing fermentative hydrogen and methane productions

The production of hydrogen and methane during fermentation process is facilitated by the concerted action of various anaerobic microorganisms. The microorganism efficiency regarding the gas production depends on several factors: nature of feedstock, inoculum pretreatment, medium pH and alkalinity, temperature, solid and hydraulic retention times, organic loading rates, hydrogen partial pressure, mixing, inhibitors, carbon to nitrogen ratio (C/N) and inoculum to substrate ratio (ISR) (50, 51). The factors are known to influence the microbial metabolism processes and thereby determine the processing time, production rate, yield and relative composition of hydrogen and methane generated from the fermentation process.
2.3.1 Nature of feedstock

The characteristics of feedstock including composition, C/N ratio and particle size affect the feedstock biodegradability, yield and rate of hydrogen and methane productions during anaerobic process. The microorganisms use the feedstock as a source of energy, electron acceptors and building blocks for new cell growth. The amount of the major components of feedstock including proteins, lipids, carbohydrates (monosaccharides, disaccharides and polysaccharides) and lignin, influences the ease or difficulty of the biodegradation of the feedstock (52). Readily degradable feedstocks, such as low molecular sugars, food waste, among others, degrade faster than fats, proteins and lignocellulosic materials. Lignocellulosic materials usually require pretreatment prior to their digestion due to the presence of lignin that tightly binds cellulose and hemicelluloses together. Various pretreatment methods that could enhance the biodegradation of biomass include physical, chemical or biological methods (53). Feedstock composition also affects the C/N of the feedstock with the optimum C/N ratio for anaerobic digestion reported to be in the range of 20 - 30: 1 (54). Very high C/N ratio leads to low biogas production due to low protein formation that affects the energy and structural metabolism of the microorganisms in terms of the substrate degradation efficiency. On the other hand, low C/N ratio increases ammonia concentration which could possibly result in ammonia/ammonium inhibition of the fermentation process (52). Furthermore, particle size of feedstock also plays a significant role in the biodegradation of the feedstock as small particle size provides high surface area for microorganism activities (55). A particle size of 2 mm was reported to be optimum for biodegradation of some feedstock (56). In Paper III, ground straw was sieved to a particle size of 2mm before the particles were used as substrate in the investigation of the effect of VFA permeation on biohydrogen production (Paper III).

2.3.2 Medium pH and alkalinity

The pH of the fermentation broth plays a significant role in the effectiveness of the fermentation process as it directly affects the activities of the bacteria involved in the fermentation process. Generally, methanogens are more sensitive to acidic conditions than other anaerobic bacteria involved in the fermentation process. Hydrogen and methane productions during fermentation process require different pH values of 5.5 - 6.5 (57) and 6.5 - 8.2 (1, 58, 59), respectively. Anaerobic fermentation has a natural way of controlling the pH of the medium
through the buffering system of the dissolution of carbon dioxide and ammonia (alkalinity) to control the high and low pH fermentation media respectively (60, 61). However, the process buffering system is often overwhelmed by the nature and loading rate of the feedstock, thereby requiring external measures for the pH regulation. The initial pH values of the substrate media used for hydrogen (Papers II, III and IV) and methane productions (Papers I and IV) during this study were on average 5.5 and 6.8, respectively.

In Paper IV, the initial pH range for all the reactors for the continuous hydrogen fermentation was from 5.2 to 5.9. However, gradual reduction in the pH values of the fermenting media below 5.0 was observed at the beginning of the experiment, which could be attributed to the production of organic acids associated with the hydrogen formation during the fermentation process (62). The pH profile indicated that the pH values for all the reactors did not vary significantly but were nearly constant throughout the experiment, with an average value of 4.40 ± 0.04. This could possibly imply that the daily effluent withdrawal from the reactor system could have prevented the accumulation of organic acids that could have led to drastic reduction in the pH value of the fermentation media. Moreover, it could also be due to the adaptation potential of fermentative microorganisms to the inhibitory fermentative media.

2.3.3. Inoculum pretreatment

Several methods of inoculum pretreatment, including heat shock treatment, acid/base treatment, as well as using chemical inhibitors such as 2-bromoethanesulfonic acid (BESA), acetylene and chloroform, have been used to improve hydrogen and methane productions during fermentation process. Effective hydrogen production often requires initial pretreatment of the seed inoculum in order to suppress the activities of the hydrogen consuming bacteria, since they are usually in syntrophic association with hydrogen producing bacteria. However, it has been observed that inoculum pretreatment alone could not sustain the inhibition of the hydrogen consuming processes for a long period of time. Operation of a fermentation process with pretreated inoculum in the medium, at initial pH of 5.5, has been proved to be effective in the inhibition of the hydrogen consuming processes such as methanogenesis and homoacetogenesis (63). In this study, hydrogen producing bacteria was heat-pretreated at 100°C for 15 min. The initial pH of the mixture of the heat-pretreated inoculum and the substrate was then adjusted to around 5.5 before they were used for the continuous hydrogen production from the medium.
containing inhibitory flavour compounds. This was to ensure that the growth of the hydrogen consuming microorganisms such as methanogenic archaea, were effectively inhibited during the process (Papers II, III and IV)

2.3.4. Complexity of the seed cultures

Hydrogen production can also be influenced by the nature of the seed cultures, which can either be pure cultures or mixed cultures. Pure culture fermentation involves a single species of seed culture throughout the fermentation process, while mixed culture fermentation is carried out by multiple strains of seed culture. In pure culture, there is limited interference by the hydrogen consuming bacteria, such as sulphate-reducing, homoacetogenic and methane producing bacteria. However, special care needs to be taken when pure culture is used as it can easily be contaminated by hydrogen consuming bacteria. One important benefit of mixed cultures is their ability to utilise a variety of substrates due to the presence of diverse microorganisms. Consequently, mixed cultures were used in all the anaerobic fermentation processes (batch and continuous) involved in this study (Papers I, II, III and IV)

2.3.5 Temperature

Anaerobic fermentation processes for hydrogen and methane productions can be run at different temperatures including psychrophilic temperature (less than 20°C), mesophilic temperature (30 - 42°C, usually 35°C), thermophilic (50 - 60°C, usually 55°C) and hyperthermophilic (>80°C) (60, 64-66). It is, however, important that temperature should be kept constant when operating fermentation process in any of the temperature range, as temperature fluctuations can reduce the gas production. Compared with mesophilic process, thermophilic process is faster and more efficient with higher gas production as it increases compounds solubility and enhances reaction rates. However, thermophilic process is more energy intensive and sensitive to any disturbance in terms of environmental and operational parameters (67). Temperature influences the physicochemical characteristics of the fluid medium as well as the growth rate of the bacteria in the anaerobic bioreactor (68). In this study, most of the experiments were operated at thermophilic temperature 55°C (Papers I, II and IV), except the experiment conducted to investigate the effects of membrane permeation of VFAs on bioreactor hydrodynamics and hydrogen production, which was operated at mesophilic temperature (Paper
The reactor used for the study in Paper III was, however, equipped with a two-stage impeller to ensure uniformity in temperature and other process parameters.

2.3.6. Retention times and organic loading rates

Retention times, including solids and hydraulic retention times (SRT and HRT), are the average times that the solid and liquid part of feedstocks, respectively, spend in the digester before they are removed. They are often dependent on the nature of the feedstock, the temperature of the process, digester volume and organic loading rate. Feedstocks that are easily degraded will require shorter times than those that are not easily degraded. The SRT and HRT of feedstock in a completely mixed bioreactor without recycling are generally the same. The range of HRT of anaerobic digester for solid waste treatment can vary from 3 to 55 days (Paper II) according to the nature of the feedstock, process temperature and bioreactor configuration (52), while SRT, especially for high rate digesters, can range from 10 to 20 days (69). The technique of low HRT and pH is often employed during continuous hydrogen production for effective elimination of hydrogen consuming bacteria such as methanogens (70, 71). In Paper III, effects of mixing and varying HRTs of 3, 5 and 8.6 days of fruit wastes (orange, apple, banana, grape and melon) on bioH$_2$ in a continuous process at 55°C for 47 days were investigated. Although it was observed that there was no statistically significant effect of the interaction of HRT and fruit mixing on bioH$_2$, there was an improvement in cumulative bioH$_2$ yields from all the feedstocks when HRT was 5 days while fruit mixture with equal fruit proportion produced the highest cumulative bioH$_2$ yield of 513mL/g VS (30% of the theoretical yield).

The organic loading rate (OLR), which is the quantity of feedstock volatile solids fed into a fixed digester volume within a period of time, is related to HRT value. For a constant volatile solid (VS) of a feedstock, low HRT is coupled with high OLR, while for a varied VS, OLR value can vary at the same HRT rate. The OLR of a continuous fermentation process is an important parameter that should be managed effectively, as very low or high OLR could result in lower gas production or VFA accumulation, respectively (11).

2.3.7. Inhibitors

Several substances including antibiotics, disinfectants and detergents, food preservatives, organic substances, ammonia, sulphide, oxygen, heavy metals, among others, can act as
inhibitors during fermentation process since they could cause inhibition of bacterial growth and performance (11, 72). The sources of the inhibitors are either associated with fresh feedstocks such as fruit flavours in fruit wastes (Papers I, II and IV) or as by-products of the bacterial metabolism activities such as VFAs (Paper III). The effects of these inhibitors are influenced by factors including the adaptation ability of the bacteria to the inhibition (acclimation), the absence or presence of other inhibitors (antagonism or synergism), and variation in process parameters (11).

2.3.8. Mixing

During fermentation processes, three phase reactions including liquid-solid, gas-solid and gas-liquid, exist inside the bioreactors. Mixing of bioreactors facilitates the transfer of energy, nutrients and metabolites within the bioreactors thereby ensuring efficient reactions among the three phases. In the absence of mixing, slurry inside the digester tends to accumulate to form scum layer on the surface, thereby preventing the upward movement of the gas. Mixing enhances the fermentation process by providing adequate contact among the fresh feedstock, bacteria and nutrients as well as reducing scum build-up (73, 74). Mixing also facilitates physical and chemical uniformity of digested sludge by preventing the formation of dead zones due to particle deposition. However, optimisation of the mixing conditions is necessary, as gentle mixing is good to avoid bacterial shearing and inhibition while vigorous agitation is needed to achieve biomass or solid suspension in a bioreactor (75).

In Paper III, a two-stage impeller consisting of a bottom impeller with four-blade disk turbine, and the top impeller with a three-bladed pitched turbine, was employed to promote uniform flow discharge in all directions within the membrane bioreactor during the experiment to investigate the effects of VFA permeation on the bioreactor hydrodynamics and hydrogen production. The stirred tank reactor, which was coupled to the hollow fibre-membrane module, was observed to be instantaneously mixed, in comparison to the fluid in the recirculation loop. The results not only showed that the properties of the fluid in the stirred tank were unaffected by the presence of the recirculation loop, but also showed that the stirring in the tank had no apparent effect on the mixing properties of the loop. Besides, the loop exhibited nearly the same behaviour with and without permeate extraction, provided the recirculation flow rate was more than ten times as high as the withdrawal flow rate (Paper III).
2.3.9. Hydrogen partial pressure

Hydrogen production pathways are very sensitive to hydrogen partial pressure as accumulation of hydrogen in the reactor headspace may increase the partial pressure of hydrogen in the reactor system. During dark fermentation process, the disposal of electrons via pyruvate-ferredoxin oxidoreductase or NADH-ferredoxin oxidoreductase and hydrogenase might be affected by the corresponding NADH and acetyl-CoA levels as well as environmental conditions such as high hydrogen partial pressure. When hydrogen concentration increases, the law of mass action limits the formation of hydrogen and the cell is forced to channel electrons through NADH: ferredoxin oxidoreductase, shifting the metabolic to produce more reduced metabolites and solvents including ethanol, lactate, propionic acid, and butanol, resulting in reduction in the hydrogen production (76-78). As solvent production involves reductions, ferredoxin is unable to transfer electrons to a hydrogenase for H₂ evolution.

In the presence of high concentration of hydrogen, the formation of reduced metabolites and solvents including propionate, lactate and ethanol are more thermodynamically favourable than acetate and butyrate formations (Equations 2.17 – 2.21) (7, 41, 43, 49, 79-81). Although ethanol formation is less thermodynamically favourable, its formation under some conditions yields no hydrogen (Equation 2.21). For this reason, it is necessary to avoid high partial pressure of hydrogen that can force the cell to switch from acidogenic to solventogenic fermentation. In this study, hydrogen partial pressure was controlled by the continuous removal of the generated hydrogen from the bioreactor system, and also by daily mixing of the bioreactors (Papers II, III and IV)

\[
\begin{align*}
C_6H_{12}O_6 + 2H_2 & \rightarrow 2CH_3CH_2COOH + 2H_2O \quad \text{(propionate)} \quad (-279.4kJ) \quad 2.19 \\
C_6H_{12}O_6 & \rightarrow 2CH_3CHOHCOOH + H^+ \quad \text{(Lactate)} \quad (-225.4kJ) \quad 2.20 \\
C_6H_{12}O_6 & \rightarrow 2CH_3CH_2OH + 2CO_2 \quad \text{(Ethanol)} \quad (-164.8kJ) \quad 2.21
\end{align*}
\]

2.3.10. Nutrient supplementation

In addition to the feedstock supplied during fermentation process, microorganisms also require some nutrients including nitrogen, phosphorus (P), potassium (K), iron (Fe), copper, cobalt, manganese, calcium, molybdenum, vanadium, magnesium, sodium, nickel (Ni), selenium
(Se), tungsten (W) and zinc in certain proportions, for effective bacterial metabolism and growth. Nitrogen and phosphorus are macronutrients that are required in large quantities by anaerobic microorganisms. For example, nitrogen is an essential component of amino acids and is required for optimal growth of the microorganisms. For high-strength and low-loading organic wastes, the ratio of chemical oxygen demand (COD), nitrogen and phosphorus that are mostly used are 1000:7:1 and 350:7:1, respectively (11). Micronutrients such as cobalt, iron and nickel, among others, are necessary as they are incorporated in enzyme systems for proper substrate degradation and conversion into hydrogen and methane. Cobalt, for example, is required as an activator of enzymes during hydrogen and methane productions. Iron is an important component of hydrogenases, the enzymes involved in the production of hydrogen.

Metal ions such as magnesium and sodium are also required for transport across cell membrane and as cofactors of other enzymes. Yeast extract is often used to supply some of the nutrients to the microorganism as it contains amino acids, minerals and vitamins including, the B vitamins, biotin and folic acid. However, for these nutrients to be biologically available to anaerobic microorganisms, they must be present in the nutrient media in soluble form and not as precipitates. Besides, the available nutrient must not be in excess as excess of these nutrients can inhibit the efficiency of the anaerobic microorganisms (11, 82). For instance, for effective fermentation of municipal solid wastes, the average optimal values (based on dry basis) of nutrient supplementation for C/N, C/P and C/K have been given as 20 - 30, 150 - 300 and 40 - 100, respectively while the concentrations of Fe, Ni, Se, and W, have been reported to be 100 - 5000, 5 - 20, 0 - 0.05 and 0.05 - 1 mg/kg, respectively (83).

2.4. End-use technologies for fermentative hydrogen and methane

The huge dependency of world’s energy needs on fossil fuels has resulted in vast depletion of fossil fuel reserves, coupled with the increased carbon dioxide emission from the combustion of the fossil fuels. The negative impact of fossil fuel usage could be reduced if renewable energy carriers including biohydrogen and biomethane are used as replacement or complement to fossil fuels (84, 85).
2.4.1. Attractive qualities of hydrogen and methane as energy carriers

Biohydrogen and biomethane are biofuels that are formed from recently living organisms called biomass, or their metabolites, as opposed to materials enclosed in geological formation for a long time before their transformation to fossil fuels. Other biofuels include biodiesel and bioethanol, among others. Biogas is a colourless gas produced through anaerobic decomposition of organic material by microorganisms. Depending on the nature of the organic materials and operating conditions, the gas composition includes methane (bioCH$_4$), carbon dioxide (CO$_2$), nitrogen (N$_2$), Oxygen (O$_2$), hydrogen sulphide (H$_2$S), and ammonia (NH$_3$) with composition of 40 - 75%, 25 - 40%, 0.5 - 2.5%, 0.1 - 1%, 0.1 - 0.5% and 0.1 - 0.5%, respectively (1, 86). Upgrading of biogas through cleaning and removal of trace components including water (H$_2$O), H$_2$S, NH$_3$ and CO$_2$, results in bioCH$_4$ with properties close to natural gas, and hence can be used for exactly the same end uses as that of natural gas.

Biomethane has various end-uses including heating, cooking, electricity and automotive fuels as well as serving as feedstock for the production of various products in chemical process industries (87). The gas could be used for combined heat and power (CHP) on site or be fed-in into the existing natural gas grid as a substitute or supplement to natural gas. Biomethane can also be transported by trucks as compressed gas or in liquid form. Regarding hydrogen, it has high energy content per unit mass (88) as well as having a non-greenhouse gas, water vapour, as its only combustion product (84, 89-96). Hydrogen has also been shown to be a versatile fuel as it can be used directly as gas in hydrogen fuel cells (84) and in adapted internal combustion engines, or stored as liquid or a metal hydride (97) for future purposes. Hydrogen gas can also be transmitted alone through natural gas pipelines (98) or be mixed with methane to form a more efficient fuel called hythane. Furthermore, hydrogen can be used in the production of syngas for electricity generation or diesel production. Besides its application as energy source, hydrogen can also be used as reactant in hydrogenation process, ammonia, methanol and syngas production, among others (99).

2.5. Implications of fermentative hydrogen and methane for technological applications

Despite the numerous benefits of application of dark fermentation for hydrogen and subsequent methane generation, the actualisation of the technology for commercial use is still limited by process and infrastructural challenges. These barriers must be overcome before the
industrial production of fermentative hydrogen and methane can be economical. For example, in the study carried out in Paper V regarding the feasibility of the development and dissemination of biogas production in Nigeria, it was found that despite the huge potential for electricity generation from organic biomass feedstock (Table 2.1), more than 60% of the population does not have access to the national power supply because they are not connected to the grid system. People that are even connected to the grid system often experience frequent power outages (Paper V). The non-existence of green fuels in the energy mix of the country has been attributed to lack of favourable policy formulation and implementation. The role of the government in stimulating the market penetration of green fuel technology cannot be overrated. Government intervention, regulatory mechanisms for green fuel technology, increased awareness level and capacity building are necessary for the development of green fuel technology in Nigeria (Paper V).

**2.5.1. Process limitations**

Although biomethane from biogas has been used in Europe and US on a large scale for various end-user technologies, including, vehicle fuel and CHP, the gas supply is very low, compared to the increasing demand for heat, electricity and transport fuels, however, in most developing countries, the technology is nearly non-existent.

**Table 2.1. Theoretical electricity generation from available biomass feedstock in Nigeria (Paper V)**

<table>
<thead>
<tr>
<th>Biomass feedstock</th>
<th>Total potential biomass feedstock (tonnes * 10⁶)</th>
<th>Quantity of available biomass feedstock (tonnes * 10⁶)</th>
<th>BMP of biogas produced based on 0.7m³/kg VS at 35°C (m³ * 10⁶)</th>
<th>Potential electricity production based on 3.73 kWh / m³CH₄ kWh x 10⁹</th>
<th>Electricity production (Terawatt hour, TWh)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agriculture crop wastes</td>
<td>171.86</td>
<td>51.56</td>
<td>20.77</td>
<td>77.47</td>
<td>77.47</td>
</tr>
<tr>
<td>Livestock manure</td>
<td>32.40</td>
<td>9.79</td>
<td>3.69</td>
<td>13.76</td>
<td>13.76</td>
</tr>
<tr>
<td>Livestock abattoir waste</td>
<td>0.83</td>
<td>0.83</td>
<td>0.34</td>
<td>1.27</td>
<td>1.27</td>
</tr>
<tr>
<td>Organic MSW</td>
<td>33.12</td>
<td>33.12</td>
<td>13.27</td>
<td>49.50</td>
<td>49.50</td>
</tr>
<tr>
<td>Human waste</td>
<td>86.12</td>
<td>86.12</td>
<td>34.29</td>
<td>127.90</td>
<td>127.90</td>
</tr>
<tr>
<td>Total</td>
<td>324.33</td>
<td>181.42</td>
<td>72.36</td>
<td>270</td>
<td>270</td>
</tr>
</tbody>
</table>
Regarding fermentative hydrogen, it is still at the research phase due to the process challenges including low production rates and yields, inefficient substrate conversion, as well as substrate and product inhibitions. During dark fermentation, more reduced compounds including ethanol, propionic acid and lactic acids are often produced as fermentation products in place of hydrogen. Moreover, hydrogen production during fermentation process is associated with the production of volatile fatty acids, which at very high concentrations could permeate through the bacterial cells causing cell lyses (100).

2.5.2. Infrastructure barriers

The widespread application of fermentative hydrogen and methane as major energy carriers in transportation, combined heat and power (CHP), and domestic activities, among others, will depend on the availability of infrastructure required for its production, storage and transportation. The production, distribution and storage of fermentative hydrogen and methane require good infrastructure. Concerning fermentative methane, the infrastructure is available, as it can be conditioned for transportation through the existing natural gas grid. Alternatively, stand-alone systems can be used where fermentative methane is used at the site of production for the generation of heat and electricity. With regard to the infrastructures for hydrogen transport, distribution and storage are nearly non-existent; the existing infrastructures could also be adapted for their utilisation in the short-term, prior to the development of the actual infrastructure for hydrogen distribution (99). Hydrogen and methane could be mixed together to form an improved gas mixture referred to as bio-hythane, which can then be transported in the existing natural gas distribution system. However, hydrogen is a light gas with poor specific volume energy density, compared to other fuels. Its low volumetric energy content limits its storage efficiency. The current hydrogen storage technique including liquefaction and compression at 350 to 700 bar in steel tanks are not efficient as it results in energy loss of 10% H\textsubscript{2} during compression and 30 to 40% in liquefaction. Alternative storage methods for hydrogen are being developed whereby H\textsubscript{2} is bound in storage materials through absorption, adsorption or chemical reactions. The feasibility of application of fermentative hydrogen in stand-alone systems is, therefore, limited for distant locations due to challenges involved in storing hydrogen (99).

In view of the increasing greenhouse gas emissions and the challenges of meeting the increasing energy demand without resource depletion, efforts should be intensified in the
establishment of a good and more coherent framework including stable political government, effective policy, investment programmes, financial support schemes and utilisation strategies, among others, to accelerate fermentative hydrogen and methane development and ensure its sustainability. The government needs to create a platform for fermentative hydrogen and methane technology to have competitive benefits including lower costs, flexibility, excellent performances, and sustainability, among others, over fossil fuels energy. The development of the technology could make the energy supply available, adequate, affordable, convenient and reliable especially for people in developing countries (101).
CHAPTER 3

**Feedstocks for fermentative hydrogen and methane productions**

The interesting fact about fermentative hydrogen and methane productions is that all organic feedstocks could be used as potential feedstock for their production \( (102) \); however, the gas production and quality depend on the composition of the feedstocks.

### 3.1. Feedstocks suitability for hydrogen and methane productions

Feedstock composition is an important factor that influences the performance of microorganisms and subsequent gas production and quality, during fermentation process for hydrogen and methane productions. The proportion of carbon, nitrogen, phosphorus and sulphur (C:N:P:S) in the feedstock must be such that it provides for the needs of the microorganisms in terms of energy sources and development of new cells, as the mass ratio of C:N:P:S in microorganism biomass is approximately 100:10:1:1 \( (60) \). The optimal nutrient ratio for carbon, nitrogen, sulphur and phosphorus (C: N: P: S) has been reported as 500: 15:5:3 and 600:15:5:3 for acidogenesis and methanogenesis, respectively \( (60, 103) \). The C:N ratio must not be too high or too low, or else, the fermentation process can suffer from nitrogen deficiency or ammonia inhibition, respectively \( (104, 105) \). Thus, based on biodegradation, feedstock can be classified into two major groups, namely, non- lignocellulosic and lignocellulosic feedstock.

#### 3.1.1. Non-lignocellulosic feedstocks

Non-lignocellulosic feedstocks are biomass material that are not lignified and therefore can be easily degraded by microorganisms. The main compositions of non-lignocellulosic feedstocks include carbohydrates, proteins and lipids. The hydrolysis rate of carbohydrate is faster, when compared to lipid and protein \( (106) \). Carbohydrate-rich feedstocks contain diverse sugars including monosaccharides (glucose, fructose, etc), disaccharides (sucrose and lactose) and polysaccharides (cellulose, hemicelluloses, starch and glycogen). Monosaccharides and disaccharides are easily and rapidly broken down into VFAs, \( \text{H}_2 \), \( \text{CO}_2 \) and alcohols by anaerobic microorganisms and could lead to VFA accumulation if it is not controlled \( (18, 107-109) \). Most
polysaccharides, except starch, are slowly degraded during fermentation process due to their structure. Starch consists of straight and branched chains of D-glucose units that form a structure that could be easily degraded by microorganisms. D-glucose isomer occurs naturally in higher living organisms unlike L-glucose isomer, which is synthesised in the laboratory. Although both starch and cellulose are polymeric forms of D-glucose units, they differ in the orientation of the 1, 4-glycosidic linkages of the glucose units. In starch, the orientation of all the glucose repeating units are the same; in other words, the glucose units are connected by alpha linkages (alpha 1, 4 linkages). On the contrary, each repeating glucose unit in cellulose is rotated 180 degrees around the axis of the polymer backbone chain, forming glycosidic linkages called beta linkages (beta 1, 4 linkages). In addition, cellulose is mostly linear chains of glucose molecules unlike starch with chains of glucose units that can either be linear, branched or a mix. Consequently, the differences in the orientations and nature of branching of the glycosidic linkages in starch and cellulose influence their chemical and physical properties, especially their biodegradation (110).

Regarding protein rich feedstocks, they consist of long chains of amino acids, which are broken down during fermentation process to release ammonia depending on the temperature or pH of the medium. High concentration of ammonia could become inhibitory to the fermentation process, as the dissolution of the ammonia in the fermentation medium raises the pH of the medium, thereby affecting the activities of the microorganism (16, 106, 111-113). The hydrolysis rate of protein is slower than carbohydrate and lipid degradation (114). Lipid-rich feedstocks consist of different forms of fatty acids and glycerol including milk, cheese, meat, palm oil and vegetable oils (115). During fermentation process, lipids are hydrolysed into long chain fatty acids (LCFA) which form foam at high concentration due to their surface-active properties (116, 117). Moreover, fats can easily bind to microorganism cells, thereby making the attached cells prone to wash-out (116). Hence, high concentration of lipids, though very energy-rich, can be inhibitory to microorganisms during fermentation process (118-121).

3.1.2. Lignocellulosic feedstocks

Lignocellulosic biomass consists of three major components, namely, cellulose, hemicelluloses and lignin as well as small fraction of ash and soluble substances called extractives. Cellulose is a polymer of glucose with a crystalline structure of long chains of glucose forming microfibrils that cannot be easily degraded. In contrast, hemicellulloses could be
easily degraded into the component sugars consisting of several five- and six-carbon sugars. In plants, lignin acts as glue that holds cellulose and hemicelluloses together. Lignocellulosic feedstocks are often subjected to various physical, thermal, chemical and biological pretreatments to improve their digestibility (53). During hydrolysis of lignocelluloses, only cellulose and hemicellulose components are converted into fermentable sugars, while lignin remains unconverted since it is a polymer of recalcitrant aromatic lignin units including \( p \)-hydroxyphenyl, guaiacyl, and syringyl units (122, 123).

### 3.2. Types of feedstocks for hydrogen and methane productions

Nutritional requirements as well as energy required for several anaerobic bacteria responsible for hydrogen and methane production could be obtained from organic feedstocks including agricultural crop wastes, livestock manure, sewage sludge, municipal solid wastes, organic industrial wastes and wastewater, among others. The feedstocks contain high organic contents that make them suitable and fermentable (124). In this study, both real and synthetic substrates supplemented by other nutrients were used for the fermentation processes. In Paper I, synthetic substrate, which includes acetic acid, butyric acid, propionic acid, methanol and glucose, mixed at a mass ratio of 3:1:1:1:1, as well as the tested fruit flavour (limonene), were used. Similarly, in Paper IV, synthetic substrate which contained 20g/L each of glucose, yeast extract and nutrient broth, as well as synthetic fruit flavours including hexanal, myrcene and octanol were used. In contrast, real substrates including fruit wastes (orange, apple, banana, grape and melon) and wheat straw were used in Papers II and III, respectively.

#### 3.2.1. Agricultural crop wastes

Agricultural crop wastes including straws, stalks, and bark generated during farming operation and processing of crops such as corn, rice, potatoes, and fruits, among others, have the potential to generate hydrogen and methane (125). Some agricultural crop wastes such as fruit and potatoes are easily degradable (18), while others such as straws are lignocellulosic with high contents of cellulose, hemicelluloses and lignin; therefore, they are difficult to digest by microorganism without some forms of pretreatments to break down their complex structure (20, 53, 126). Lignocellulosic crop wastes with high C/N ratio, such as, apple pomace, corn cobs, fruit wastes and rice hulls, with average C/N ratios of 48, 98, 40 and 121 (127), respectively;
could be mixed with livestock manure, including poultry and pig manure, for effective gas production during fermentation process (128).

3.2.2. Livestock manure

Animal wastes contain diverse nutrients suitable for the growth of anaerobic microorganisms; however, the nutrient compositions vary for different animal wastes. Poultry and pig manures, for example, contain higher concentration of protein than cattle manure, while cattle manure contain less organic content than manures from pigs and poultry (111, 129, 130). Most livestock manures have low C/N ratios because of the high nitrogen content. For example, broiler litter, cattle, laying hens, sheep, swine and turkey litter, have average C/N ratios of 14, 19, 6, 16, 14 and 16, respectively (127). If livestock manure is used as sole feedstock during fermentation process, there could, therefore, be problems with ammonia inhibition or low gas production. As a result, co-digestion of livestock manures with other feedstock is usually employed for efficient fermentation process (131).

3.2.3. Municipal solid waste

Municipal solid waste (MSW) consists of solid wastes from residential, commercial, institutional and industrial sources with the exception of construction waste. The composition of the waste depends on factors such as the source of the waste, the standard of living and habits of residents, as well as sorting method and the climatic conditions. The generated MSW usually has both organic and inorganic components, and therefore, requires sorting and removal of the inorganic components including plastics, glass, and metals, as well as construction wastes before the waste is used as feedstock for anaerobic digestion. The C/N ratios of MSW vary depending on the sources of the wastes, for instance, the average range of C/N ratios of food wastes, night soil and sewage sludge are reportedly given as 14 - 16, 6 - 10 and 5 - 16, respectively (127).

3.2.4. Industrial wastes and municipal wastewater

Industrial wastes and municipal wastewater including organic wastewater from industries, schools, hospitals, government parastatal, sewage sludge from septic tanks or sewers and slaughterhouse wastes are potential feedstocks for hydrogen and methane productions during fermentation process. Slaughterhouse wastes contain high concentration of protein and fat, which
include suspended organic solids such as grease, hair, feathers, manure and undigested feed. Although the gas production potential of slaughter wastes is higher than animal manure, the high protein and fat contents often lead to inhibition due to ammonia and LCFA during fermentation process (132-134). Therefore, slaughterhouse wastes are often co-digested with feedstock with lower protein and fat contents to improve the gas production during the fermentation process. Application of sewage sludge as feedstock for fermentation process is an effective waste management system as it helps to get rid of pathogenic organisms that could be harmful to people if the sludge is discarded in the environment. However, the gas production potential of sewage sludge is lower than that of livestock manure as it contains lower organic content. Moreover, the sludge could contain substances such as heavy metals and organic pollutants that could be inhibitory to anaerobic microorganisms since the sludge comes from wastewater that contains faeces, urine and laundry waste. The average values of typical composition of untreated wastewater, including total solids, suspended solids, biochemical oxygen demand (BOD₅ at 20°C), chemical oxygen demand (COD), total organic carbon (TOC), nitrogen, free ammonia, phosphorus and alkalinity (as CaCO₃), among others, have been reported to be 720, 220, 220, 500, 160, 40, 25, 8 and 100 mg/L, respectively (135).

Generally, feedstocks with high or low C/N ratios could cause thermodynamics imbalance during fermentation process. When high C/N ratio feedstocks, such as agricultural crop wastes, are solely used as substrates during fermentation process, the low nitrogen content is rapidly consumed thereby affecting the natural potential of the process to control the acidity of the process. In the same vein, when low C/N ratio feedstocks, including livestock manure, are used exclusively as substrates during fermentation process, excess nitrogen is released causing increase in the process alkalinity and subsequent thermodynamics shift towards the production of reduced metabolites including propionate, lactate and ethanol (Equations 2.19 -2.21) Therefore, to optimise the fermentation process, mixture of feedstocks with high and low C/N ratios are often used as substrates.
3.3. Inhibitory effect of fruit flavours and volatile fatty acids

3.3.1. Inhibitory effects of fruit flavours

The availability of fruit waste in large quantities with 10-65% of the raw fruits generated as wastes from the fruit consumption and processing (136); as well as the high content of the fruit organic matter content; makes it a potential feedstock for anaerobic fermentation process. Naturally, plants fend off parasitic attack using several defence mechanisms including the production of a range of volatile organic compounds which often show antimicrobial activity against a wide range of bacteria, yeasts and moulds. The antimicrobial characteristic of plants has been known for a long period of time with Chinese using plants in medicinal therapies as far back as 5000 years ago and Egyptians using plants for food preservation and in mummification in 1550 BC (137, 138). In flowering plants, fruits produce various volatile organic compounds including esters, alcohols, aldehydes, ketones, lactones and terpenoids, among others, which make up their characteristic aroma and flavour characteristics (139). Although the fruit flavours in plants are present in limited quantity with a range of 0.001 - 0.01% of the fresh fruit weight (140, 141), their toxicity against microorganism depends on their threshold concentration and interaction with other compounds. Previous research activities on the effects of fruit flavour have confirmed the toxicity of fruit flavours against microorganisms (142-147).

The various factors that contribute to the flavour quality of fruits include genetics, harvest maturity, environmental conditions, post-harvest handling and storage (148, 149). The antimicrobial effect of fruit flavour compounds can be beneficial regarding the improvement of food shelf life; the effect can also be detrimental especially during anaerobic digestion of feedstock containing the flavour compounds since they reduce the bacterial effectiveness (150).

3.3.1.1 Proposed mechanism of flavour toxicity to bacteria

Flavour compounds initiate their antimicrobial activity against bacteria through their interaction with bacterial cell membrane. The toxicity of a flavour compound during fermentation process depends on its cell membrane permeability. The hydrophobic nature of most flavour compounds allows them to interact with the cell membrane and accumulate within the phospholipid bilayer of the bacteria. The integrity of the cell is lost if the concentration of the accumulated flavour compound exceeds a tolerable limit (151-159).
The proposed mechanism through which a flavour compound develops its toxicity against bacteria includes: degradation of bacterial cell wall, disruption of cell membrane, leakage of cell contents, and depletion of proton motive force as well as cytoplasm coagulation. The effects eventually lead to loss of cell viability and death (159) (Figure 3.1).

![Figure 3.1. Proposed Antimicrobial mechanisms of flavour compounds](image)

3.3.1.2. Adaptation of bacterial to toxic environment

Bacteria can adapt to an inhibitory medium for a while as long as the inhibitor concentration is kept constant. The flavour inhibition could be acute or chronic; it is acute if the bacterial is exposed to a relatively high concentration within a short time, but it is chronic if the toxicity results from gradual and long exposure of the bacteria to the toxic compound. The duration of the chronic toxicity during fermentation process depends on the contact time and the ratio of the toxic compound to the bacterial population (153, 160).

As bacterial resistance cannot be sustained by itself for a long period of time (153); one of the objectives of using membrane encapsulated bacteria during the fermentation process in this research (Papers I and IV) was to reduce the exposure period of bacteria to the flavour compounds. During the research, the bacteria was protected against the flavour compounds by using a hydrophilic polyvinylidene fluoride (PVDF) membrane, which allowed water soluble nutrients to diffuse while repelling the flavour compounds.
3.3.2. Inhibition of volatile fatty acids

During fermentation process, different microbial activities take place simultaneously, with the fermentative bacteria depending on one another activities. Slight process imbalances due to variation in operational parameters including temperature, retention time, OLR, among others (161), could result in accumulation of VFAs, the most important intermediates during fermentation process (162). However, above certain threshold, VFAs could be toxic to the activities of the fermentative bacteria, as it has been shown that glucose fermentation could be inhibited at total VFA concentration above 4g L⁻¹ (163, 164). Propionic and butyric acids inhibition are more toxic than acetic acid inhibition with propionic acid concentrations over 3 g L⁻¹ having the potential to cause digester failure (165). VFA toxicity occurs as a result of the increase in the undissociated form of the VFAs, which could diffuse into the bacterial membrane with consequent dissociation, leading to reduction in the cytoplasmic pH inside the bacteria. In a single stage digester, there is likelihood of VFA accumulation especially during the digestion of easily degradable feedstock. The reason for this is that methanogens in low concentration are not able to metabolise acetate until their number increases exponentially.

3.4. Limiting the inhibitory effects of fruit flavours and volatile fatty acids

Hydrogen and methane productions from organic feedstock during fermentation process are often not optimized due to substrate and product inhibitions, which affect the efficiency of the fermentative bacteria.

3.4.1. Control measure to limit the fruit flavour inhibition during fermentation

The hydrophobic nature of most flavour compounds enables them to create partition in the phospholipids of the bacterial cell membrane, thereby rendering them permeable and leading to the leakage of cell contents. Hence, it is expected that enclosing bacteria in hydrophilic polymers may help to reduce the penetration of the flavour compounds since flavour components have lower hydrophilicity and larger molecule sizes than water. Therefore, in this work (Papers I and IV), synthetic encapsulating sachets made of hydrophilic PVDF membranes were used to enclose the fermentative bacteria. Each flat sheet membrane with pore size, thickness and diameter of 0.1 µm, 125 µm and 90 mm, respectively; was cut and folded into rectangular dimensions with width and length of 3 and 6 cm, respectively. The membranes were heat-sealed
with heating and cooling times of 5.0 and 5.5s, respectively. One side of the membrane sachet was left open for cell insertion after which the opening was sealed to form a membrane capsule. The substrates used during the experiments were synthetic media, while the flavour compounds including limonene, hexanal, myrcene and octanol, were used as the inhibitory substances (Papers I and IV). The experimental set-ups for the study are shown in Figures 3.2 and 3.3. The protective effects of the membrane in the media containing the flavour compounds were investigated by comparing the gas production potentials (bioH₂ and bioCH₄₄) of both encapsulated and free cells. In the limonene containing medium, membrane-encapsulated cells, compared with the free cells, produced methane faster and were able to survive the effects of the inhibitory flavour medium at a loading rate of 15g COD L⁻¹ d⁻¹ for a longer period. The free cells failed completely at an OLR of 7.5 COD L⁻¹ d⁻¹. In fermentation broth containing myrcene, octanol and hexanal, methane cumulative yields of 182 ± 15, 111 ± 81 and 150 ± 24 mL/ COD were obtained from encapsulated cells while no methane production was observed from the free cells. Regarding hydrogen production, average daily yields of 179 ± 26, 198 ± 16 and 189 ± 17 mL/ COD were produced from encapsulated cells in medium containing myrcene, octanol and hexanal, respectively, while average yields of 133 ± 77, 88 ± 71 and 68 ± 76 mL/ COD were produced from the free cells. It was observed that though free cells of bioH₂ producing bacteria were able to produce reasonable amounts of bioH₂ regardless of the flavour inhibitors, the amount of bioH₂ produced was less, compared to that of encapsulated cells.

3.4.2. Control measure to limit the volatile fatty acids inhibition during fermentation

In recent times, the VFAs produced during anaerobic fermentation process have been utilised as a suitable carbon substrate for denitrification and the production of biodegradable plastics, electricity via microbial fuel cell, biogas, hydrogen as well as lipids for biodiesel (I66). Hence, VFAs are valuable substrates, and their production during fermentation process can be regulated through filtration for effective fermentation process. Various strategies have been employed to limit VFA inhibition, including varying operational parameters such as temperature, pH, organic loading rate, and mixing, with filtration being increasingly used because of its benefits (I67). In this study, VFA inhibition on anaerobic microorganisms during fermentation was limited by extracting the VFA using PVDF hollow fibre membrane (Paper III). The VFA permeation was carried out using an externally submerged hollow-fibre membrane module
coupled with a mechanically-stirred tank reactor (Figure 3.4). The effects of the membrane permeation of the VFA on the bioreactor hydrodynamics and hydrogen productions were investigated. Furthermore, mixing and transmembrane pressure (TMP) across the membrane bioreactor system were also studied as a function of the operating conditions, while focusing on the cleaning procedure to assess the feasibility of the filtration process. The results showed an improved biohydrogen production with a defined VFA permeate extraction, as the biohydrogen production was observed to restart after VFA extraction. While low recirculation flow rate and TMP values maximised the permeate flux, the fouling mechanism due to cake layer was observed to be reversible. The cleaning procedure based on gas scouring and backwashing with the substrate was defined, while low TMP of $10^4$ Pa was required to achieve a $3 \text{ L h}^{-1} \text{m}^{-2}$ critical flux. Additionally, it was observed that the hydrodynamic properties in the bioreactor were not significantly modified.
Figure 3.2. Experimental setup for investigating the effect of membrane-encapsulated cells on fermentative methane production from inhibitory-fruit flavour medium (Paper 1 and IV)
(a) Empty membrane; (b) Encapsulated cells (width position); (c) Encapsulated cells (length position); (d) Batch process (bioreactors with encapsulated cells); (e) Batch process (bioreactors with free cells) 
(f) Semi-continuous process setup:
1- Membrane bioreactor with encapsulated cells, 2- Warm water jacket, 3 – Water heater,
4- Volumetric gas analyser, 5- Purge container, 6 – Feed container, 7 - Peristaltic pump
8 -Data acquisition system
Figure 3.3. Experimental setup for investigating the effect of membrane-encapsulated cells on fermentative hydrogen production from inhibitory-fruit flavour medium (Paper IV)

Figure 3.4. Set-up for concurrent extraction of volatile fatty acids and biohydrogen production (Paper III)
CHAPTER 4

Membrane processes for improvement of fermentative hydrogen and methane productions

Membrane is a thin barrier that controls the permeation of a chemical species using differences in concentration, pressure and/or electrical potential gradient between the two compartments they separate (168). The benefits of membrane application include the prevention of washout of slow-growing bacteria (169) and the protection of bacteria from toxic effects of substrate and product inhibitions.

4.1. Membrane classification

The distinctiveness of membrane depends on its properties including pore size and shape, symmetry, and the membrane materials. Regarding membrane materials, membranes can consist of organic or inorganic materials (170). Organic membranes are produced using various polymers such as cellulose acetate, polysulfone, polyamide, polyethylene (PE), polypropylene (PP), polytetrafluoroethylene (Teflon PTFE), polyethersulfone and polyvinylidene fluoride (PVDF); however, inorganic membranes refer to membranes made of materials such as ceramic, carbon, silica, zeolite, oxides (alumina and zirconia) and metals such as palladium, silver, among others (171). Most membranes are usually made of hydrophobic polymers owing to their qualities including chemical resistance, low swelling, low fouling tendency and good separation performance. Common hydrophobic membranes that are often employed are PVDF, PTFE, PE, PP and zeolites (172). However, hydrophobic membranes are usually hydrophilised to allow water permeation during fermentation process.

Membranes are also classified according to their filtration techniques including microfiltration, ultrafiltration, nanofiltration and reverse osmosis, depending on their pore size distribution (Papers I, III and IV). Membranes used for microfiltration generally have pore size of approximately 0.03 to 10 microns, with an operating pressure between 100 and 400 kPa while the range of membrane pore size for ultrafiltration membranes is approximately 0.002 to 0.1 microns, with an operating pressure between 200 and 700 kPa. Nanofiltration membranes have
pore size between 0.0001 to 0.01 microns with operating pressure between 600 kPa and 1000 kPa. Reverse osmosis filtration technique is able to retain almost all molecules, except water, due to size of the pore which ranges from 0.0001 to 0.001 microns (173, 174).

There are several types of membrane module configurations, which are either planar or cylindrical in geometry. The planar or flat sheet configurations include plate-and-frame and spiral-wound modules while cylindrical configurations include hollow fibre and tubular modules. Hollow fibre and tubular modules have shell and tube configurations, but hollow fibre modules have greater number of tubes with smaller diameters than the tubes in tubular modules. The tubular is often applied to enhance turbulent flow. The fibres in hollow fibre modules may consist of several hundreds to over 10,000 fibres with the fibres bundled together longitudinally, potted in a resin on both ends and encapsulated in a pressure vessel. The mode of operation of the hollow fibre module could be inside-out or outside-in. In inside-out the feed flow enters through the centre of the fibre (lumen) and is filtered radially through the fibre wall, while outside-in operation, the feed flow enters from outside the fibre into the inside of the fibre. The fibre modules have the benefit of high filtration surface area per unit volume; however, flux maintenance and membrane fouling are key issues during filtration process (175). The spiral-wound module consists of flat sheet membrane wound around a central perforated tube. It is often used for cross-flow filtration. The flat sheet modules consist of flat and thin-film composite sheets with a thin layer being supported on a thicker layer that has wider pore. They are cheap and usually disposable.

As reviewed in Paper VI, anaerobic membrane bioreactors (AnMBRs) could be configured in three different ways: internally submerged membrane (ISMBR), externally submerged membrane (ESMBR) (Paper VI) and external cross-flow membrane (ECMBR) bioreactors (172, 176). In ISMBR, the membrane is placed directly inside the fermentor, however, in ESMBR, the membrane is placed in a separate container different from the fermentor. The benefit of ESMBR is that it is easier to clean than ISMBR, but it often requires pump for sludge recirculation during the fermentation process (177). Unlike the submerged membrane bioreactors, the ECMBR is operated in a cross-flow mode in which the fermented broth is pumped parallel to the membrane surface. Although ECMBR has the benefit of reducing the frequency of fouling formation, sustaining the continuous operation is energy intensive (177).
4.1.1. Application of PVDF membrane in fermentative hydrogen and methane productions

PVDF is a commonly used membrane in microfiltration and ultra-filtration membrane processes because of its low price and excellent qualities including high hydrophobicity, mechanical strength, thermal stability, as well as, chemical and ultra violet resistance (178). The membrane is chemically stable to a wide range of chemical compounds such as inorganic acids, oxidants, halogens, aromatic, aliphatic and chlorinated solvents. The PVDF membrane is a semi-crystalline polymeric membrane consisting of both crystalline and amorphous phases with crystalline part responsible for its excellent thermal stability, while the amorphous part is responsible for the flexibility of the membrane (172). The macromolecular straight chain of PVDF is enveloped by fluorine and hydrogen atoms (-CH₂-CF₂-), with fluorine atoms, being a high electronegative element, providing high dissociation energy of the C-F bonds, which makes the membrane to have higher thermal stability compared to other polymeric membranes (179). PVDF has a glass transition temperature ($T_g$) of around $-39^\circ C$, a melting point temperature of around $160^\circ C$ and a thermal decomposition temperature of above $316^\circ C$.

PVDF also exhibits piezoelectric properties which make it to be valuable as sensor and actuator materials. However, the intrinsic hydrophobic nature of PVDF makes it prone to organic fouling and low wettability with high resistance to water flow. As a result, several membrane modification techniques including blending, surface coating, irradiation grafting and plasma modification, are used to incorporate hydrophilicity into hydrophobic PVDF membranes to enhance their performances (180). Considering the excellent quality of PVDF membranes, this study, therefore, used hydrophilic PVDF membranes in all the experiments investigated. Two different membrane configurations, including flat sheet and hollow fibre modules were employed in the membrane encapsulation and VFA extraction, respectively.

4.2 Influence of membrane permeability on membrane performance

The effectiveness of membrane for VFA permeation and protection against inhibitory effects of substrate and product inhibition during fermentation process, which was the focus of this research, depends on the permeability of the membrane. The membrane permeation of molecules is influenced by the nature of the membrane materials (pore size, hydrophobicity/hydrophilicity, free volume and filler particles) and solubility of the permeants.
(molecular size, concentration, among others) (181-183). The ability of a membrane to regulate the permeation of various molecules through it is an important feature that is employed in separation processes. The permeation process can either follow solution-diffusion model where the permeants dissolve and diffuse through the membrane, or pore flow model where the permeants pass through the membrane pores. In this study, water permeability of the PVDF membrane was determined using distilled water. An average value of 0.048 mL/min of pure water permeability was obtained for the PVDF membrane.

4.3. Encapsulation technology for cell retention and inhibition control

Encapsulation of bacterial cell is a technique used for restricting bacterial mobility within a polymeric semi-permeable membrane. In encapsulation, the microorganisms are restricted by the membrane walls, however, the cells move around within the membrane (184, 185). In this technique, the membrane is semi-permeable as it allows substrates and nutrient to pass through it depending on the membrane pore size, while retaining the microorganism. The benefits of encapsulation technique is that anaerobic microorganism can be retained in the digester longer without being washed out during continuous operation, thereby giving the cells sufficient time to grow and degrade the substrate supplied (186). Additionally, the microorganisms could be easily recovered at the end of the fermentation process, thereby reducing the purification cost (187). Encapsulation also encourages the use of small reactor system since the concentration of the microorganism is higher than in free cells. It also acts as a protective barrier for the microorganism against the effect of substrate and product inhibitions (188). In this study (Papers I and IV), encapsulation technique was used to confine the movement of the bacteria within the walls of flat sheet PVDF membrane (Figure 4.1). The technique, however, has some limitations which include occasional cell leakage and inefficient diffusion of substrate to the microorganism in the membrane as well as the diffusion of product away from the microorganism and out to the external medium (187, 189).
Besides encapsulation, other methods of restricting bacterial movement either completely or to a small limited region include adsorption, covalent binding, entrapment and crosslinking. Adsorption is a reversible immobilisation technique as it is based on weak binding forces between the support and the microorganisms. Due to the weak binding forces, which may include van der Waals forces, ionic or hydrogen bonds, high rate leakage and unstable binding are common occurrences (189). Adsorption is prone to changes in environmental conditions, for example, pH, temperature and ionic strength, among others. Unlike encapsulation, entrapment is an irreversible immobilisation technique in which microorganisms are entrapped in a support matrix or inside a membrane. Cross-linking is also an irreversible method of immobilisation that is support-free but it involves the joining of microorganism to one another to form a large structure (190). The method is cost effective, and it increases the volumetric activity of the microorganisms. Covalent binding involves the formation of a covalent bond between the microorganisms and the support material. In this technique, microorganism leakage is minimized as a result of the strong linkage between the support and the organisms (186, 187).
4.4. Application of hollow fibre membrane configuration for VFA permeation

Accumulation of volatile fatty acids (VFA) during dark fermentation is a major challenge as it has been attributed, among other factors, to inhibition of microbial cell growth and substrate consumption, leading to low hydrogen yield (191). Nonetheless, different kinds of separation techniques have been applied to separate VFAs from the fermentation broth, such as ion exchange (192), electrodialysis (193, 194), adsorption (195), liquid-liquid extraction (196), and membrane separation (197), but most of the separation techniques are expensive and inefficient. However, with improvement in membrane structure and qualities, membrane costs have become inexpensive and affordable (198). Moreover, membranes have the advantage of coupling VFA separation with biomass retention during anaerobic fermentation process, thereby maximizing bioenergy production.

In hollow fibre membrane bioreactor, optimising module packing density is crucial as it reduces the number of fibre modules as well as the size of the membrane tank, hence, reducing the capital and operation costs of the reactor system. When submerged hollow fibre membrane is used during anaerobic digestion process, the filtration is often in outside-inside mode. In outside-inside filtration mode, an uneven distribution of the transmembrane pressure (TMP) may develop along the module fibres, as increased fibre length and ratio of outside diameter to inside diameter along with reduced fibre inner diameter, could increase the non-uniformity of the flux distribution. The calculated TMP values based on the suction pressure at the outlet could be different from the actual TMP along the fibres, thereby affecting the accuracy of the membrane permeability (199).

4.5. Limitations of membrane technology: Membrane fouling and cost

The resistance to fluid flow through the membrane during filtration process is often due to membrane fouling, which is a term that is used to describe the loss of membrane throughput. Generally, fouling occurs when particulate, colloidal or soluble materials are deposited inside membrane pores or surface. Membrane fouling is a major barrier to membrane application in fermentation processes as it is associated with flux or permeate flow reduction, low permeate quality and increased operational costs due to increased energy consumption (200). Normally, membrane bioreactors (MBRs) are designed for the maintenance of constant flux but when membrane fouling sets in, it causes increase in TMP and decrease in flux to the point that
membrane cleaning or replacement is required. Membrane fouling is influenced by factors such as sludge characteristics, operational parameters and membrane qualities (201). Although, membrane fouling cannot be entirely avoided during membrane filtration process, the frequency of its occurrence could be reduced through physical cleaning such as relaxation and backwashing or chemical cleaning. Chemical cleaning of membrane is more effective in removing membrane fouling than physical cleaning, but frequent use of chemical cleaning can damage the membrane and shorten the membrane life-time (202-204). Previously, membrane cost was part of the barrier to the application of membrane technology, but extensive research on membrane improvement has resulted in cheap and affordable membrane in recent times (170, 198). However, operating costs associated with membrane fouling abatement is still the main barrier to the application of membrane technology (201).

4.6. Implications of membrane applications in this study

The applications of membranes in this study was done in two parts; the first part, which focused on reduction of substrate inhibition, involved the investigation of the effects of the PVDF membrane encapsulation techniques on the bioH₂ and bioCH₄ production from fruit waste media. The results of methane production from the thermophilic batch and continuous fermentation of nutrient media with limonene, myrcene, octanol and hexanal as fruit flavours showed that membrane encapsulated, compared to free cells, produced methane faster with limonene as flavour. The encapsulated cells were able to survive the effects of the inhibitory activity of limonene flavour medium at a loading rate of 15g COD L⁻¹d⁻¹ for a longer period even after the free cells had completely failed at an OLR of 7.5g COD L⁻¹d⁻¹ (Paper I). Likewise, with myrcene, octanol and hexanal as fruit flavours, bioCH₄ cumulative yields of 182 ± 15, 111± 81 and 150 ± 24 mL/g COD, respectively, were obtained from encapsulated cells while no CH₄ production was observed from free cells during batch fermentation process. About 60% membrane protective effect against myrcene, octanol and hexanol inhibitions was obtained (Paper IV).

Regarding bioH₂ production, average daily yields of 68 ± 76, 133 ± 77, 88 ± 71 mL/g COD were produced from the free cells with hexanal, myrcene and octanol, respectively, while average yields of 189 ± 16, 179 ± 26 and 198 ± 17 mL/g COD were produced from encapsulated cells containing hexanal, myrcene and octanol respectively (Paper IV). This indicated that
membrane encapsulated cells, compared to free cells, produced higher daily yields of 94, 30 and 77% for hexanal, myrcene and octanol as flavours, respectively. It was observed that though free cells of bioH₂ producing bacteria were able to produce reasonable amount of bioH₂ regardless of the flavour inhibitors, the amount of bioH₂ produced was less compared to that of encapsulated cells (Paper IV).

The final part of this study, which was aimed at reducing product inhibition, involved the study of the effects of membrane permeation of volatile fatty acids (VFAs) on the bioreactor hydrodynamics in relation to bioH₂ production. The investigation revealed that low transmembrane pressure of $10^4$ Pa was required to achieve a $3 \text{ L.h}^{-1}\text{m}^{-2}$ critical flux with reversible fouling mainly due to cake layer formation, while bioH₂ production was observed to restart after VFAs removal (Paper III). The results from this study suggest that membrane-based techniques could actually improve bioH₂ and bioCH₄ productions from fermentation media with substrate and product inhibitions.
CHAPTER 5

Bioreactor hydrodynamics for fermentative hydrogen and methane productions

5.1. Ideal and real reactors

Ideal reactors are model systems analogous to effective reactors for which transport and mixing processes can be exactly described mathematically. On the contrary, the transport and mixing processes in real reactors can only be roughly known due to different flow effects that could occur, causing dead zones (stagnant region) and channelling (short-circuits or bypassing) (Figure 5.1). Dead zone is a region with low or no mixing in a mixed reactor, while channelling occurs when a portion of the reactants is transported to the output in a shortest time possible without taking part in the reaction process (205). The occurrence of dead zone and channelling in a reactor affects the reactor performance and reduces the product yield.

Figure 5.1. Fluid flow in non-ideal reactor
5.2. Mixing in bioreactors

Mixing is a physical process, which is designed to reduce non-uniformities in fluids through the prevention of gradients in the fluid properties including temperature and concentration, among others. Mixing also facilitates physical and chemical uniformity of digested sludge by preventing the formation of dead zones due to particle deposition. It prevents gas bubbles being trapped in the fermentation broth. However, mixing should not be strong as it can cause shearing and wash-out of the microorganisms, thereby, leading to loss of microorganism activity (75, 206, 207). For effective performance during fermentation process, bioreactors are often equipped with impellers to facilitate uniform mixing and good contact between the substrate and microorganisms, as well as to prevent sedimentation of the substrate (73, 74). Apart from mechanical mixers, mixing can also be carried out through gas injection or recirculation of digested slurry with the aid of pressure pumps. However, for effective mixing to occur, the fluid distributed by impeller or other techniques must pass over the entire bioreactor in a good time. Moreover, the velocity of the fluid leaving the mixer must be able to take the fluid to the extreme part of the reactor. Effectiveness of mixing could be evaluated using two parameters, including mixing time and mean circulation time (208, 209).

5.2.1 Mixing and mean circulation times

Mixing time ($t_m$) is the time required to attain homogenisation to the molecular scale. However, since molecular scale measurement could not be determined experimentally, terminal mixing time is often determined. Terminal mixing time is the time required to achieve a given degree of homogeneity on the scale of observation, starting from the completely non-integrated state. Experimentally, measurement of mixing time is done through tracer technique, whereby the tracer (usually a pulse of electrolyte solution) is injected into the reactor. The mixing time is taken as the time at which the tracer concentration ($C_t$) at the measurement location has almost attained the expected final mean tracer concentration ($C_f$). Mathematically, the mixing time is defined as the time from tracer addition to the time when the tracer concentration is given as (Equation 5.1):

$$\frac{C_t - C_i}{C_f} = m$$ 5.1
where \( m \) is the maximum acceptable absolute value of the relative deviation of the mix. At the beginning of the experiment, \( m = 1.0 \), while at the end of the experiment when complete homogeneity has been attained, \( m = 0 \). The lowest value of \( m \) at the end of the experiment which can be accurately measured is 0.05 (5%). Mixing time can be identified from the response curve as the time after which the concentration of the tracer (\( C_i \)) differs from the final concentration (\( C_f \)) by less than 5% of the total concentration difference (\( C_f - C_i \)). The mean circulation time is the main time interval between two consecutive peaks of maximum concentration of tracer on the response curve (Figure 5.2) during the measurement of residence time distribution.

**Figure 5.2.** A typical response curve of Mixing and mean recirculation times

### 5.3. Residence time distribution measurement

In real reactors, fluid molecules spend different times before their exit from the reactor, with some molecules finding their way to the exit immediately as they enter the reactor, while other molecules stay for a long time before their exit from the reactors. Residence time
distribution (RTD) of a reactor is a probability distribution function that describes the duration that a fluid molecule spends inside the reactor (210, 211). It is usually expressed as an exit age distribution, $E(t)$ (Equation 5.1).

$$\int_0^\infty E(t) \, dt = 1$$

The average residence time for all the fluid molecules is given as (eq 5.2):

$$\bar{t} = \int_0^\infty t \cdot E(t) \, dt$$

In the absence of dead zones and channelling, the average residence time calculated from RTD will be equal to the hydraulic retention time (HRT) calculated from the total reactor volume and the volumetric flow rate of the fluid. Measurement of residence time distribution of fluid molecules in a reactor provides insight regarding the mixing performance of the reactor and consequently, facilitates the effective reactor design or retrofit that allows for a high and sustainable organic loading rate, a short hydraulic retention time and maximal hydrogen and methane yields (212).

**5.4. Membrane filtration**

Membrane filtration can be carried out using one or two techniques which include dead-end and cross-flow filtration depending on the direction of the flow of the feed suspension that is being filtered. In dead-end filtration, the direction of the feed suspension to be filtered is perpendicular to the membrane surface, while, in cross-flow filtration, the direction of feed suspension is tangential or parallel to the membrane surface. Dead-end filtration is effective for separating out particles with low concentration from feed suspension, and it is often carried out in batch mode where the mass of the deposited material or retentate increases until the material can no longer be deposited again. Hydraulic resistance of the retentate increases with filtration time, which results in the decrease of the filtration rate across the membrane. Cross-flow filtration is usually operated using multi-sheet, hollow fibre or tubular membrane configurations (213, 214). Darcy’s law of filtration expresses membrane performance during filtration in terms of the filtrate flux ($J$), which is the volume of the filtrate that passes through the unit membrane.
area in unit time (Equation 5.3), where \( \Delta P \), \( \mu \) and \( R_T \) represent the applied pressure (transmembrane pressure), filtrate viscosity and total resistance to the fluid flow (215). The total resistance, based on the resistance-in-series model, is a combined resistance of three resistances, including intrinsic membrane resistance \( (R_m) \), reversible resistance due to cake layer or external fouling \( (R_c) \), and internal fouling resistance due to irreversible adsorption and pore plugging \( (R_i) \) (Equation 5.4). Darcy’s law highlights that the permeate flux through a porous membrane is directly proportional to the transmembrane pressure \( (TMP) \) and the membrane area, but is inversely proportional to the membrane resistance due to fouling and to feed viscosity (216). The expression for the TMP is given by Equation 5.5, where \( P_f \), \( P_r \) and \( P_p \) are feed, retentate and permeate pressures, respectively.

\[
J = \frac{\Delta P}{\mu R_T} = \frac{TMP}{\mu R_T} \tag{5.3}
\]

\[
R_T = R_m + R_c + R_i \tag{5.4}
\]

\[
TMP = \frac{P_f + P_r}{2} - P_p \tag{5.5}
\]

The filtrate flux through the membrane materials decreases with the occurrence of membrane fouling. Hence, flux or filtration efficiency, is directly affected by membrane fouling with a consequent decrease in system productivity and increase in operating cost. The rate of membrane fouling could be reduced by carrying out filtration process below the critical flux and by simultaneously maintaining high shear rate through velocity gradient or gas sparging close to the membrane. Membrane fouling can also be reduced by using appropriate membrane configuration and modules, as in the case of hollow fibre membrane modules (217). In this study; during the investigation of the effects of membrane permeation of VFAs on bioreactor hydrodynamics and bioH\(_2\) production (Paper III), the experimental set-up was designed to enable both gas scouring (bubbling of CO\(_2\) through membrane surface) and back-washing with permeate to control membrane fouling.
In the first study of the cleaning procedures in Paper III, CO$_2$ was injected for 5 minutes after a filtration step of 15 minutes using the digestate; then, the same cycle was repeated. In the second study (Paper III), the same procedure was applied, but CO$_2$ cleaning procedure was replaced by permeate backwashing. Backwashing was obtained using the reversible flow of the withdrawal peristaltic pump, which, however, presented the drawback of using a fraction of the filtrate for backwashing. The fouling was studied by following the permeate flow rate over time as a function of the rotation speed of the withdrawal pump. The effectiveness of cleaning was assessed through the evolution of the permeate flow rate over several filtration-cleaning cycles. The results showed that backwashing was slightly more efficient for recovering the initial density flux (4.45 L h$^{-1}$m$^{-2}$), since the permeate flux after backwashing remained above 4.40 L h$^{-1}$m$^{-2}$, while it was about 4.30 L h$^{-1}$m$^{-2}$ after CO$_2$ injections. This implied that fouling was probably reversible, but the cleaning remained incomplete using only CO$_2$ injections. Moreover, the results also revealed that low transmembrane pressure of $10^4$Pa was required to achieve a 3 L h$^{-1}$m$^{-2}$ critical flux with reversible fouling mainly due to cake layer formation, while bioH$_2$ production was observed to restart after VFAs removal (Paper III).
CHAPTER 6

Conclusions and Future Work

6.1. Conclusions

Fermentative hydrogen and methane are energy carriers that are neutral with regard to CO$_2$ emissions and they could, therefore, play a significant role in the development of a sustainable global climate. Although utilization of the fermentative hydrogen and methane as separate energy carriers offers numerous benefits including low fossil-fuel dependency, effective waste management system, reduction of greenhouse gas emission and energy accessibility, the usage of the mixture of hydrogen and methane has the potential of providing more benefits than individual utilization. The mixture of hydrogen and methane as fuel offers synergistic benefits of the two energy carriers, in the sense that, hydrogen complements the drawbacks of methane that affect its combustion efficiency, including narrow flammability range, high ignition temperature, and slow burning speed. Presently, the market of fermentative hydrogen as a fuel is non-existent, while that of fermentative methane is still at the beginning stages even with the commercial applications in some advanced countries. The major barriers associated with the widespread applications of fermentative hydrogen and methane as fuels include low gas yields and availability of required infrastructure for gas production (Paper V), distribution and storage. This research has focused on how to contribute to the facilitation of widespread application of fermentative hydrogen and methane through yield improvement.

The low yields of hydrogen and methane productions during fermentation process are partly due to substrate and product inhibitions. This study, therefore, investigated the potential of employing membrane technology and varying operational parameters in yield enhancement of fermentative hydrogen and methane. The major results from this study are summarized as follows:

- Enclosing fermentative bacteria in hydrophilic PVDF membrane prevented bacterial wash-out as well as protected the bacteria against harsh environment such as inhibitory flavour medium. Compared with the free cells, membrane-encapsulated cells produced methane faster and were able to survive the effects of the inhibitory flavour medium at a
loading rate of 15 g COD L⁻¹d⁻¹ for a longer period even after the free cells had completely failed at an OLR of 7.5 g COD L⁻¹d⁻¹.

- With myrcene, octanol and hexanal as fruit flavours, CH₄ cumulative yields of 182 ± 15, 111± 81 and 150 ± 24 mL/g COD, respectively, were obtained from encapsulated cells, while no CH₄ production was observed from free cells during batch fermentation. Regarding bioH₂ production, average daily yield of 68 ± 76, 133 ± 77, 88 ± 71 mL/g COD were produced from free cells with hexanal, myrcene and octanol, respectively, while average yield of 189 ± 16, 179 ± 26 and 198 ± 17 mL/g COD were produced from encapsulated cells containing hexanal, myrcene and octanol, respectively. It was observed that though free cells of bioH₂ producing bacteria were able to produce reasonable amounts of bioH₂ regardless of the flavour inhibitors, the amount of bioH₂ produced was less, compared to that of encapsulated cells.

- Meanwhile, a study conducted to evaluate effects of hydraulic retention time (HRT) and fruit mixing on bioH₂ production from single and mixed fruits indicated that there was no statistically significant effect of the interaction of HRT and fruit mixing on bioH₂ yield. However, it was observed that there was an improvement in cumulative bioH₂ yield from all the feedstocks when HRT was 5 days, while fruit mixture with equal fruit proportion produced the highest cumulative bioH₂ yield of 513mL/g VS (30% of the theoretical yield).

- The investigation of the effect of membrane permeation of VFAs on the hydrodynamics in the bioreactor in relation to bioH₂ production from glucose and straw as separate feedstock in a submerged anaerobic bioreactor revealed that low transmembrane pressure of 10⁴Pa was required to achieve a 3 Lh⁻¹m⁻² critical flux with reversible fouling mainly due to cake layer formation, while bioH₂ production was observed to restart after VFAs removal.

The results from this research suggest that membrane-based techniques and varying operational parameters could improve the yields of hydrogen and methane productions from fermentation media with substrate and product inhibitions. It is, therefore, expected that application of the knowledge from this research along with the provision of government support
scheme in various countries will facilitate the widespread commercial production and utilisation of fermentative hydrogen and methane in terms of the economic viability of the technology.

6.2. Future Work

Based on the results from this research work, research activities stated below will be the direction of future work for further development of fermentative hydrogen and methane technology.

- During the research, hydrogen and methane fermentation were carried out separately. However, to obtain higher yield and determine the overall substrate conversion efficiency for energy utilisation, the partially digested sludge consisting of VFAs still need to be utilised to extract additional energy from it. The future work will therefore involve combining hydrogen and methane productions in a two-stage system where VFAs extracted or the digested sludge from stage one will be used as substrates for the second stage. It will therefore be possible to evaluate accurately the economic feasibility of the process.

- Part of the research work in this thesis involved investigation of the hydrodynamic and yield effects of VFA permeation using external immersed membrane module, it will be interesting to study the effects using internally submerged membrane module

- In the experiment conducted to investigate the effect of fruit flavour inhibition on the bacterial activities as linked to hydrogen production, it was observed that hydrogen production from encapsulated cells remained relatively constant for three days, after feed input and effluent withdrawal had stopped. The hydrogen production, however, declined after the three days. Therefore, it may be interesting to investigate if intermittent loading of feedstock with inhibitors can improve the microorganism adaptation to the inhibitory compounds, possibly giving the microorganisms enough time to neutralise the effect of the inhibitors without affecting the hydrogen yield.

- Extensive studies with modelling should be done on the membrane permeability of fruit flavour on bacteria cell membrane, with the aim of determining the direct relationship
between the concentration of the inhibitory compounds and the survival duration as well as the surviving tactics of the microorganisms. Furthermore, since the production of hydrogen is associated with other reduced metabolites, other than acetic acid, which has the highest theoretical hydrogen yields; modelling approach will also be applied to establish the direct relationship among the secondary metabolites and feedstock compositions as well as process parameters.

- Further studies will also be conducted on how to improve the efficiency of the microbial activities. From previous research studies, it has been found that sole pretreatment of seed sludge could not sustain the flourish and proliferation of the hydrogen producing microorganisms for a long period of time, as the hydrogen production decreased after running the experiment for a while. It will, therefore, be interesting to find the optimal factor parameters that could be combined for effective long-term maintenance of hydrogen producing bacteria during fermentation process.

- During the experiment on the effects of fruit flavour inhibition on hydrogen and methane production, synthetic fruit flavour compounds were used. It will be interesting in the future to use the flavour compounds extracted directly from the fruit wastes for the investigation.

- Although this study showed that membrane encapsulated cells could improve the yields of fermentative hydrogen and methane productions, the membrane could, however, restrict the growth of the anaerobic microorganism during the doubling period. Therefore, it will be interesting to study the effects of the membrane wall restriction on the growth of the microorganisms in relation to the yields of the fermentative hydrogen and methane production.
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Paper I
Biogas production by encased bacteria in synthetic membranes: protective effects in toxic media and high loading rates

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Biogas production by encased bacteria in synthetic membranes: protective effects in toxic media and high loading rates

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A bioreactor including encased digesting bacteria for biogas production was developed, and its performance in toxic media and under high organic loading rates (OLRs) was examined and compared with traditional digestion reactors. The bacteria (3 g) were encased and sealed in $3 \times 6 \text{ cm}^2$ PVDF (polyvinylidene fluoride) membranes with a pore size of 0.1 $\mu$m, and then several sachets were placed in the reactors. They were then examined in toxic medium containing up to 3% limonene as a model inhibitor in batch reactors, and OLRs of up to 20 g COD/L.day in semi-continuous digestions. The free and encased cells with an identical total bacterial concentration of 9 g in a medium containing 2% limonene produced at most 6.56 and 23.06 mL biogas per day, respectively. In addition, the digestion with free cells completely failed at an OLR of 7.5 g COD/L.day, while the encased cells were still fully active with a loading of 15 g COD/L.day.

Keywords: biogas; synthetic membrane; encapsulation; cell containment; inhibitor

Introduction

Biogas or biomethane is a renewable energy source with several applications, for example, car fuel, heating, cooking, or electricity production. Biogas consists mainly of methane and carbon dioxide, but may also contain minor impurities of other components [1]. The anaerobic digestion process and production of methane consists of hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Typically, methanogenesis is more sensitive to the environment than the earlier stages [2]. Moreover, the doubling time of hydrolysis and acidogenesis in bacteria is about 1.0–1.5 days, while acetogens and methanogens need about 1–4 and 5–15 days doubling time, respectively [3]. Consequently, the methane-forming bacteria need a longer retention time and are very sensitive to the process conditions; hence, they can easily be washed out. Therefore, if the dilution rate of methane-forming bacteria in the digester is too high, or if the withdrawal of digester sludge is premature, the population size of methane-forming bacteria is greatly reduced.

Other process challenges, such as low methane yield and an unstable process, can often occur during the anaerobic digestion process. An important factor is toxicity and the presence of inhibitor compounds in the substrates or wastes. The impact of the toxic substance is that it mostly inhibits the performance of bacteria in the digester by disturbing bacterial growth, resulting in anaerobic reactor upset or failure [4]. There are many inhibitors that play an important role in the digestion process, including: ammonia, light, heavy metals, and organic compounds such as long-chain fatty acids and phenol [4–6]. In the anaerobic digestion of fruit waste, D-limonene is an example of an important inhibitor of bacterial activities [7]. Limonene is a hydrocarbon classified as a cyclic terpene, which is a major component of peel oil in citrus fruits. Not only does limonene cause odours in citrus fruits, it is also well known as an antimicrobial agent [8–10]. In the field of ethanol production, limonene was reported as being an inhibitor of ethanol productivity [11,12]. Furthermore, it also inhibits the digestion of bacteria and inhibits biogas production [13]. It is common to pre-treat the substrate or use cell protection methods in order to reduce or prevent these inhibitory effects and avoid washing out of the cells [7,14,15].

Retaining the bacteria inside the digester by immobilization could be a solution to these problems. Cell immobilization is an attractive method for maintaining a high cell concentration in the reactor. In addition, immobilizing microbial cells at a high density not only improves the productivity of a bioreactor, but the microbial cells immobilized in a polymeric membrane can also be protected from harsh environmental conditions such as pH, temperature, organic solvents, and toxic components. Immobilized microbial cells can also be handled more easily and recovered from the solution without difficulty [16]. Continuous processes can be operated at a high cell density without loss.
of microbial cells even at high dilution rates, which results in a higher bioreactor volumetric productivity [3]. Among various cell immobilization methods, encapsulation, or cell containment, is highly attractive. Upon encapsulation, the cells are retained in a capsule made by a membrane permeable to nutrients and metabolites [17]. The necessary properties of the membrane include having a good substrate and easier product transfer, protection of the cells, and no leakage from the capsules. Encapsulation has been applied to various bioprocesses such as whole-cell biocatalysts, artificial cells, and biosorbents [16,18]. Another type of cell encapsulation has cells contained behind a barrier, and can be achieved by using microporous membrane filters, by entrapment of cells in a microcapsule, or by cell immobilization onto an interaction surface of two immiscible liquids [16,19–22]. This method could also be an interesting way to retain the cells by using a synthetic polymeric membrane for continuous processes, and to protect the cells in order to enhance biogas productivity. However, we have found no report in the literature on using encapsulation or cell containment technology with digesting bacteria in continuous process and cell protection testing for biogas production.

The aims of this work were to investigate the performance of methane-producing bacteria encased in synthetic polymeric membranes in a long-term digester with different organic loading rates (OLRs) for rapid biogas production, and to investigate its protective effect against limonene. The total volume of biogas and methane, and the total amounts and composition of the volatile fatty acids (VFAs) were also determined regularly.

Materials and methods

Anaerobic culture preparation

Anaerobic cultivations were prepared following a method described elsewhere [23,24]. The inoculum was obtained from a 3000 m³ municipal solid waste digester operating at thermophilic (55 °C) conditions (Borås Energy & Environment AB, Sweden). It was then degassed by pre-incubating at 55 °C for 3 days. The active anaerobic culture was homogenized and passed through a sieve with a pore size of 1.0 mm in order to separate any remaining large particles. The sludge was then centrifuged at 14,000 × g for 10 min to separate the supernatant and bacteria, which were thereafter used as an inoculum for cell containment in different experiments.

Synthetic medium

The nutrients for methanogenic bacteria were mimicked from the normal products of the first stage in digestion, i.e. the hydrolysis. The composition of this synthetic medium was the carbon source, including acetic acid, propionic acid, butyric acid, methanol, and glucose mixed at a mass ratio of 3:1:1:1:1; a basal medium [25] was used for addition of nutrients necessary for the anaerobic cultures.

The composition of the medium is shown in Table 1. The pH of the mixture of synthetic substrates was adjusted to 7.0 ± 0.5.

Membrane sachet preparations and cell containment procedure

A cell containment technique was employed following the method previously described [26]. Flat plain PVDF (polyvinylidene fluoride, Durapore®) membranes supplied by Thermo Fisher Scientific Inc. (Sweden) were used as a synthetic membrane supporting material. The membranes were cut into rectangular shapes of 6 × 6 cm and folded to create membrane pockets of 3 × 6 cm². These membrane sheets were then heat-sealed (HPL 450 AS, Hawo, Germany) on two sides with heating and cooling times of 4.5 and 4.5 s, leaving one side open for the insertion of the inoculums. Bacterial inoculums (3 g) were then injected carefully into the synthetic membrane pockets, and sealed accordingly. The sachets containing the inoculum were used immediately for biogas production.

Protective effect of sachets in batch anaerobic digestion process

The batch digestion processes were performed in order to examine the protective effect of the sachets on the bacteria against limonene as the inhibitor. Limonene (Fluka, Sweden) was mixed with a synthetic medium in concentrations of 0 (control), 1, 2, and 3% (v/v). In each digesting reactor, three sachets were placed in a synthetic medium. The reactors used were serum glass bottles with 150 mL working volume, closed with butyl rubber seals and plastic caps.

Table 1. Composition of the basal medium used to supply nutrients in the synthetic medium.

<table>
<thead>
<tr>
<th>Components</th>
<th>Amount (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄Cl</td>
<td>1200</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>400</td>
</tr>
<tr>
<td>KCl</td>
<td>400</td>
</tr>
<tr>
<td>Na₂S·9H₂O</td>
<td>300</td>
</tr>
<tr>
<td>CaCl₂·2H₂O</td>
<td>50</td>
</tr>
<tr>
<td>CaCl₂·2H₂O</td>
<td>50</td>
</tr>
<tr>
<td>(NH₄)₂HPO₄</td>
<td>80</td>
</tr>
<tr>
<td>FeCl₃·4H₂O</td>
<td>40</td>
</tr>
<tr>
<td>CoCl₂·6H₂O</td>
<td>10</td>
</tr>
<tr>
<td>KI</td>
<td>10</td>
</tr>
<tr>
<td>MnCl₂·4H₂O</td>
<td>0.5</td>
</tr>
<tr>
<td>CuCl₂·2H₂O</td>
<td>0.5</td>
</tr>
<tr>
<td>ZnCl₂</td>
<td>0.5</td>
</tr>
<tr>
<td>AlCl₃·6H₂O</td>
<td>0.5</td>
</tr>
<tr>
<td>Na₂MoO₄·2H₂O</td>
<td>0.5</td>
</tr>
<tr>
<td>H₂BO₃</td>
<td>0.5</td>
</tr>
<tr>
<td>NiCl₂·6H₂O</td>
<td>0.5</td>
</tr>
<tr>
<td>Na₂WO₄·2H₂O</td>
<td>0.5</td>
</tr>
<tr>
<td>Na₂SeO₃</td>
<td>0.5</td>
</tr>
<tr>
<td>Cysteine</td>
<td>10</td>
</tr>
</tbody>
</table>
Anaerobic digestions with free cells were performed in parallel with identical conditions as reference. The headspace of each bottle was flushed with 80% nitrogen and 20% carbon dioxide gas mix to attain the anaerobic conditions and help to keep the pH neutral at the beginning of the process [23]. Digestions were carried out under thermophilic conditions at 55 ± 1°C in an incubator for 10 days. All digesters were shaken two times per day in order to have better contact between the inoculums and substrate.

Table 2. Experimental set-up of organic loading rates condition during the semi-continuous process of testing free and encased methane-producing bacteria during anaerobic digestion.

<table>
<thead>
<tr>
<th>Organic loading rate (OLR) (gCOD/L.day)</th>
<th>Working volume of reactor (mL)</th>
<th>Synthetic medium strength (g COD/L)</th>
<th>Volume of medium (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>1100</td>
<td>9.33</td>
<td>294</td>
</tr>
<tr>
<td>5.0</td>
<td>1100</td>
<td>9.33</td>
<td>588</td>
</tr>
<tr>
<td>7.5</td>
<td>1100</td>
<td>9.33</td>
<td>884</td>
</tr>
<tr>
<td>10.0</td>
<td>1100</td>
<td>9.33</td>
<td>1100</td>
</tr>
<tr>
<td>15.0</td>
<td>1100</td>
<td>9.33</td>
<td>177</td>
</tr>
<tr>
<td>20.0</td>
<td>1100</td>
<td>9.33</td>
<td>354</td>
</tr>
</tbody>
</table>

Effect of high loading rates in semi-continuous anaerobic digestion process

A long retention time of, for example, 30 days is usually a challenge in anaerobic digestion processes, resulting in large digestion reactors and challenging their economical feasibility. In this work, the efficiency of encased bacteria at different feeding rates of synthetic wastewater [27] was investigated, and compared with free cells in a long-term, semi-continuous process.

In order to investigate the performance of cells encased in synthetic sachets from a long-term perspective, a semi-continuous process mode was employed (Figure 1). In fact, the reactor was fed continuously by a recirculating pump. However, considering the system boundary around both the digester and the recirculating pump, the system can be defined as semi-continuous. The reactors were made in-house from Plexiglas®, had a total inner volume of 1.5 L and were fitted with rubber seals and an outlet for the biogas. In this experiment, 37 sachets containing a total of 111 g bacteria were added into 1.1 L medium in each reactor. In parallel, the same amount of the bacteria was used as inoculum in the reference reactor using free cells. Thermophilic conditions were maintained at 55 ± 1°C throughout the process by passing warm water from a water bath through the reactor jacket (Figure 1). The OLR was then gradually increased by increasing the volume of medium fed (Table 2). Each loading rate was maintained for 7 days, giving a total digestion process of 42 days. During the digestion, the medium was circulated continuously with a flow rate of 150 mL/min. The key parameters to monitor anaerobic digestion processes are normally biogas production and volatile fatty acid accumulation, which gives an indication of how well the digesting bacteria can handle the degradation of a substrate [24,28].

Analytical methods

The volume of biogas was automatically recorded using a data acquisition system (AMPTS, Bioprocess Control Sweden AB, Sweden). The VFAs were analysed using a HPLC system (Waters, Milford, MA) equipped with an ion-exchange column (Aminex HPX-87H, Bio-Rad, Hercules, CA) working at 60°C using 5 mM sulphuric acid as an eluent with a flow rate of 0.6 mL/min, and a UV detector (Waters 2414) for detection of the VFAs. The methane was quantified using a gas chromatograph (Auto system, Perkin-Elmer, USA) on a packed column (Perkin-Elmer, 6' × 1.8” OD, 80/100 Mesh, USA) and a thermal conductivity detector (Perkin-Elmer, USA) with an injection temperature of 150°C. Nitrogen at a flow rate of 20 mL/min at 60°C was used as carrier gas. A 0.25 mL syringe (VICI, precision sampling Inc., USA) was employed for the gas sampling. The obtained peak area was compared with a standard methane gas analysed at the same condition (STP: 273.15 K, 101.325 kPa). All experiments were operated in duplicate and the results are shown as means ± standard deviation.

Results and discussion

Protective effect of the sachet membranes

The presence of toxic compounds is a challenge for digestion processes. A variety of inorganic and organic materials, which are commonly present in industrial biogas processes, can be toxic in anaerobic digesters. D-limonene, a major component of peel oil in citrus fruits, is an antibacterial
agent, which is effective in killing bacteria such as *Mycobacterium tuberculosis* and *Streptococcus mutans* and a variety of fungi at very low concentrations [8,29–31]. In this work, the efficiency of encased methane-producing bacteria in membrane sachets was investigated, using 0–3% limonene as a model inhibitor. Methane production was determined regularly, with results shown in Figure 2.

Daily methane production by the encased bacteria increased dramatically up until the fifth day of digestion for most treatments, regardless of the limonene concentration. Then, it decreased until the last day of digestion, limited by the amount of substrate given. With both encased cells and free cells, a limonene concentration of 3% tended to impact on the lowest methane production compared with 2%, 1% and 0%. However, methane production from free cells with different concentrations of limonene was substantial. The maximum methane production from free cells was seen on the fifth day of digestion, with methane volumes at 0, 1, 2, and 3% limonene being 23.65, 20.03, 23.06, and 19.00 mL, respectively. It should be noted that the additional barrier of the membrane cases did not affect the diffusion of substrate into the encased cell and products out of it. These results reveal that methane-producing bacteria encased in a synthetic polymeric membrane (PVDF) are better protected against the inhibitor as opposed to free cells.

**Biogas production at different organic loading rates**

Anaerobic digestion in a semi-continuous digester was performed with encased cells and free cells as a reference. It was performed under thermophilic conditions for 42 days while at the same time increasing the OLR until no gas production occurred.

At the beginning of the process, the suspended sludge containing methane-producing bacteria sedimented at the bottom of the reactor even though the substrate was always circulated through the reactor. Biogas was produced and the bubbles rose to the top of the reactor. As part of the semi-continuous process, a certain volume of the medium (Table 1) in the reactor was withdrawn and replaced with fresh medium, causing some free cells to be washed out, which was not the case for the encased cells in the sachets. The sachets containing bacteria swelled immediately after the first day of digestion, resulting in an increase of the sachet volume inside the reactor (Figure 3). Moreover, gas bubbles were clearly observed in the medium throughout the digestion process, which indicates that gas was produced inside the sachets and subsequently released. Most of the sachets stayed intact and had no cell leakage throughout the process.

The results of digestion are presented in Figure 4(a). With low OLR (2.5 g COD/L.day), biogas was produced continuously by both the free and the encased cells, although there was a substantial difference in their biogas production rates. The average daily biogas productions from the encased and free cells were 916 and 137 mL, respectively. After 7 days, the organic load was doubled to 5.0 g COD/L.day. This resulted in increasing biogas production by both the free and encased methane-producing bacteria. The same trend of more biogas from encased cells than free cells was observed, which was 1837 vs. 729 mL per day, respectively. On the other hand, when the loading rate was increased to 7.5 g COD/L.day, the biogas production by the encased cells still increased to an average of 2889 mL per day. In contrast, for the reactor with free cells, the biogas production declined as the loading rate was increased to 7.5 g COD/L.day, and digestion completely failed by the 18th day.

With encased cells in the semi-continuous processes, the loading rate was further increased to 10, 15, and 20 g COD/L.day. Biogas was still produced continuously,
despite these very high OLRs. The average biogas produced at loading rates of 10 and 15 g COD/L.day were 3552 and 5441 mL per day, respectively. On the other hand, at an OLR of 20 g COD/L.day biogas production decreased dramatically. The average biogas volume produced at this OLR was 3606 mL. The maximum substrate tolerance level refers to the highest organic load tolerated by the microbial community without any decrease in their activity. In a similar study for a traditional biogas process, Liu et al. [32] reported a maximum and stable biogas production rate of 4.25 m$^3$ (m$^3$ d)$^{-1}$ at an OLR of 6.0 kg VS/m$^3$.day by thermophilic anaerobic digestion of municipal biomass waste. However, a higher OLR of 8.0 kg VS/m$^3$.day resulted in a maximum methane production rate of 2.94 m$^3$/m$^3$.day [33]. In our study, the highest load introduced to the encased reactors was 20 g COD/L.day, after which daily biogas production declined.

These results reveal that the encased methane-forming bacteria were able to tolerate higher OLR than the free cells, and were able to produce more biogas in a semi-continuous process. This means that anaerobic digestion systems with encased methane-forming bacteria have a better system capacity to prevent washing out of cells, and to produce biogas faster than the free cells.

**Degradation of volatile fatty acids by free cells and encased cells**

VFAs serve as substrate for methane-forming bacteria, resulting in the production of methane. Their production and consumption can give an accurate idea of the balance, in the sense that the different bacteria can have sufficient production and consumption rates. Moreover, high concentrations of VFAs can inhibit the anaerobic process [4]; therefore, it is important to monitor these compounds. In this work, the degradation and composition of total VFAs was regularly analysed in the culture of both free and encased cells during the semi-continuous processes. The results are summarized in Figure 4(b).

At low OLR of 2.5 g COD/L.day, the total VFAs in the reactors of both the free and the encased cells decreased continuously from 5 g/L to 2.34 and 0.92 g/L, respectively, in accordance with the increasing biogas production. As the loading rate increased to 5 g COD/L.day, the total VFA concentrations in both the encased and free cell cultures were stable between the 8th and 14th day of digestion. However, the values started to increase for the free cells, from 2.47 to 5.70 g/L, when the OLR was increased to 7.5 g COD/L.day. This is an indication that the anaerobic process had failed, since no biogas was produced from the VFAs and washing out of cells could be observed. On the other hand, the encased cells were able to keep the levels of total VFAs low as the loading rate was increased from 5 to both 7.5 and 10 g COD/L.day. The variation in the measured level ranged only from 1.45–0.25 g/L. As the OLR was increased to 15 g COD/L.day, the deterioration of total VFAs slightly decreased, meaning that higher levels of VFAs were detected. Nevertheless, the concentrations increased continuously during the last period.
Figure 4. The digestion performance of free and encased methane-forming bacteria in a semi-continuous anaerobic process. (a) daily biogas productions and (b) accumulated total volatile fatty acids (VFAs).

(20 g COD/L.day), meaning that the highest tolerable concentration of the cells had been reached. From the 35th day until the end of the experiment, the total VFAs concentration in the bioreactor increased from 10 to 40 g/L. With increasing OLRs, the anaerobic reactor suffered from an increase of VFA concentration, resulting in inhibition of the methanogenic bacteria [33]. Forbes et al. [34] reported that an OLR of 9 g COD/L.day at a hydraulic retention time (HRT) of 1 day made the thermophilic bioreactor unstable, shown by a high concentration of VFAs in the effluent. Wijekoon et al. [35] showed the total VFA concentration to increase from 2.5 to 4.7 and 7.0 g/L with increased OLR of 5.1 to 8.1 and 12 kg COD/L.day, and the process was not stable at an OLR of 12 kg COD/L.day due to high accumulated VFAs.

The composition of VFAs, including acetate, propionate, and butyrate, was also investigated. For the encased bacterial system, the results (Figure 5) show that acetate, propionate and butyrate concentrations decreased dramatically, even though the organic loading was increased continuously from 2.5 to 10.0 g COD/L.day. However, when an OLR of 15 or 20 g COD/L.day was introduced to the reactor, acetate, propionate, and butyrate accumulated, starting with propionate (Figure 5(b)). In contrast, Figure 5(a) shows the trend of acetate, propionate, and butyrate being consumed by free cells in the semi-continuous anaerobic reactor. Acetate, propionate, and butyrate concentrations decreased slightly during the 7 days of 2.5 g COD/L.day. It then increased when 7.5 g COD/L.day was added to the reactor.

Finally, it is worth noting the lower VFA values in the reactor with the encased cells compared with the free cells (Figure 5). This means higher degradation of VFAs by encased bacteria than that of the suspended cells. This
Conclusion
Encasing methane-producing bacteria in semi-permeable membrane (PVDF) is a successful technique for enhancing biogas production. PVDF had the ability to retain the cells in the reactor. A protective effect of the PVDF membranes against limonene, a potent inhibitor of microorganisms, was also obtained. It was shown that the encased bacteria could produce methane faster than free cells from substrates containing limonene concentrations of 0, 1, 2, and 3%. Encased bacteria were able to produce more biogas than free cells during the process, lasting for 42 days with the maximum OLR of 20 g COD/L day, while the free cells had already failed at OLR 7.5 g COD/L day. However, further research is required to investigate the potential of different kinds of membrane for cell encasement, in order to improve the industrial development of biogas production, and anaerobic digestion processes longer than 42 days should be studied in order to investigate the life span of the membrane. Furthermore, the protective effect of sachets towards other inhibitors of the anaerobic digestion process would be interesting to study.

Acknowledgements
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[2] Griffin ME, McMahon KD, Mackie RI, Raskin L. Methanogenic population dynamics during start-up of...


Evaluation of Fermentative Hydrogen Production from Single and Mixed Fruit Wastes

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Abstract: The economic viability of employing dark fermentative hydrogen from whole fruit wastes as a green alternative to fossil fuels is limited by low hydrogen yield due to the inhibitory effect of some metabolites in the fermentation medium. In exploring means of increasing hydrogen production from fruit wastes, including orange, apple, banana, grape and melon, the present study assessed the hydrogen production potential of singly-fermented fruits as compared to the fermentation of mixed fruits. The fruit feedstock was subjected to varying hydraulic retention times (HRTs) in a continuous fermentation process at 55 °C for 47 days. The weight distributions of the first, second and third fruit mixtures were 70%, 50% and 20% orange share, respectively, while the residual weight was shared equally by the other fruits. The results indicated that there was an improvement in cumulative hydrogen yield from all of the feedstock when the HRT was five days. Based on the results obtained, apple as a single fruit and a fruit mixture with 20% orange share have the most improved cumulative hydrogen yields of 504 (29.5% of theoretical yield) and 513 mL/g volatile solid (VS) (30% of theoretical yield), respectively, when compared to other fruits.

Keywords: whole fruit wastes; singly-digested fruits; mixing proportion; biohydrogen; retention time; significant effect
1. Introduction

The quest for renewable, efficient and environmentally-friendly alternative energy sources to fossil fuels has stimulated intense research studies on fermentative hydrogen (H$_2$) production from biomass. Among other renewable energy sources (solar, hydro-power, wind and geothermal), fermentative H$_2$ production has received remarkable interest due to its striking properties, including having very high energy content per unit mass and being a clean energy carrier, as it forms only water vapor during combustion [1–3]. Fermentative H$_2$ production can facilitate the quick transition of the hydrocarbon-based economy to a hydrogen-based economy, especially in the transport sector. Fuel cell electric vehicles powered by fermentative H$_2$ are zero emission vehicles that could be used as green energy technology to tackle the challenge of depleted fossil fuel reserves and pollution associated with conventional transport fuels. Furthermore, the considerable attention on fermentative H$_2$ is also due to the reliability of the continuous supply of feedstock, which is inevitably generated from daily human and animal activities. Among the feedstock available for H$_2$ production, fruit wastes have relative great economic and environmental potential due to the large quantities of wastes generated from fruit consumption and industrial processing (10%–65% of raw fruit) [4]. The application of fruit wastes as feedstock for H$_2$ production is an eco-friendly process, since littered fruit wastes could constitute a health nuisance to people and the environment. Meanwhile, due to low H$_2$ yield from the fermentation process, most of the hydrogen currently used in various industrial applications is obtained from non-green sources, including steam reforming of natural gas, water electrolysis and coal gasification [5–7]. Low H$_2$ yield during the fermentation process is attributed to, among other factors, the natural tendency of the fermentation process to be optimized to produce cell biomass instead of H$_2$. In a dark fermentation process, 12 mol H$_2$/mol glucose could be theoretically obtained from complete conversion of glucose to H$_2$ and carbon dioxide (Equation (1)), but the reaction is thermodynamically impossible due to the production of a large quantity of by-products (volatile fatty acids (VFAs), alcohols and lactate) associated with H$_2$ production. The thermodynamic constraints make the maximum attainable H$_2$ yields to be 4 and 2 mol/mol glucose if the associated by-products are acetate and butyrate, respectively (Equations (2) and (3)) [8,9]:

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 6\text{H}_2\text{O} \rightarrow 12\text{H}_2 + 6\text{CO}_2 \quad (\Delta G^\circ = +3.2 \text{ kJ}) \tag{1}
\]

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 2\text{H}_2\text{O} \rightarrow 4\text{H}_2 + 2\text{CO}_2 + 2\text{CH}_3\text{COOH} (\text{acetate}) \quad (\Delta G^\circ = -206 \text{ kJ}) \tag{2}
\]

\[
\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2\text{H}_2 + 2\text{CO}_2 + \text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} (\text{butyrate}) \quad (\Delta G^\circ = -254 \text{ kJ}) \tag{3}
\]

The low H$_2$ from the fermentation process could also be attributed to feedstock inhibition including the antimicrobial inhibition of flavor compounds in fruit. During the ripening process, fruits usually produce flavor compounds (esters, alcohols, aldehydes, ketones, lactones and terpenoids), which are used as natural defense mechanisms in plants against microbial invasion (Table 1) [10–24]. Although the amount of fruit flavors in fruits is not high (usually 0.001%–0.01% of the fruit’s fresh weight), the antimicrobial effect of the flavor compounds cannot be ignored. The positive effect of the flavor compounds in fruit is responsible for fruit freshness and the long shelf life of fruits, while a negative effect has been observed to be responsible for the inhibition of bacterial activities during the fermentation process. For instance, D-limonene, which is a citrus flavor belonging to a class of terpenoids, was found
to have an antimicrobial effect at a very low concentration of 0.01% w/v [11,25–27] and to cause the failure of the anaerobic digestion process, even at a very low concentration of 400 µL/L [26]. As a result, some strategic approaches have been developed to mitigate the antimicrobial effect of limonene and, consequently, to improve bioenergy production during anaerobic digestion. In a study by Youngsukkasem et al. [28], an encapsulated membrane was effectively used to reduce the inhibitory effect of D-limonene on the fermentative bacteria. Membrane-encased bacteria were observed to tolerate up to 3% limonene in the synthetic medium with consequent higher methane production than free cells. Wikandiri et al. [29] also developed a membrane bioreactor that could tolerate between 5 and 10 g/L of limonene in the feedstock for biomethane generation. Similarly, various techniques, including feedstock and inoculum pretreatment, optimal inoculum-to-substrate ratio, gas sparging, bioreactor design and a two-stage fermentation system, among others, have been employed for improving the recovery efficiency of fermentative H₂ from food wastes, especially fruit wastes [30–33].

Table 1. Fruit flavors in some fruits.

<table>
<thead>
<tr>
<th>Fruit</th>
<th>Flavor compound</th>
<th>Flavor group</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange</td>
<td>Hexanal and nonanal</td>
<td>Aldehydes</td>
<td>[10]</td>
</tr>
<tr>
<td></td>
<td>Octanol, 3-methyl butanol</td>
<td>Alcohols</td>
<td>[10,23]</td>
</tr>
<tr>
<td></td>
<td>α-pinene, car-3-ene, myrcene and limonene</td>
<td>Terpenoid</td>
<td>[10,11]</td>
</tr>
<tr>
<td></td>
<td>Pentanone, heptanone, undecanone</td>
<td>Ketones</td>
<td>[12,13]</td>
</tr>
<tr>
<td></td>
<td>Butanal, hexanal and E-2-hexanal</td>
<td>Aldehydes</td>
<td>[12,13]</td>
</tr>
<tr>
<td>Banana</td>
<td>1-butanol, 2-pentanol, 3-methyl-1-butanol, 1-hexanol and eugenol</td>
<td>Alcohol</td>
<td>[12,13]</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate, butyl acetate, 2-methyl propyl acetate, hexyl acetate, hexyl butanoate and butyl butanoate</td>
<td>Esters</td>
<td>[12,13]</td>
</tr>
<tr>
<td></td>
<td>n-Hexanal, E-2-hexenal, nonanal, acetaldehyde</td>
<td>Aldehydes</td>
<td>[10,14]</td>
</tr>
<tr>
<td>Apple</td>
<td>Hexanol and butanol</td>
<td>Alcohols</td>
<td>[14]</td>
</tr>
<tr>
<td></td>
<td>Car-3-ene</td>
<td>Terpenoid</td>
<td>[10]</td>
</tr>
<tr>
<td></td>
<td>Ethyl butanoate, ethyl -2-methylbutanoate, hexyl acetate, etc.</td>
<td>Ester</td>
<td>[14,15,23]</td>
</tr>
<tr>
<td></td>
<td>Epicatechin</td>
<td>Polyphenol</td>
<td>[16,17]</td>
</tr>
<tr>
<td>Grape</td>
<td>Hexanal</td>
<td>Aldehyde</td>
<td>[10]</td>
</tr>
<tr>
<td></td>
<td>Octanol and hexanol</td>
<td>Alcohols</td>
<td>[14,18]</td>
</tr>
<tr>
<td></td>
<td>Hexyl acetate, ethyl acetate and ethyl hexanoate</td>
<td>Esters</td>
<td>[10,18,19]</td>
</tr>
<tr>
<td></td>
<td>Quercetin and epicatechin</td>
<td>Polyphenol</td>
<td>[16]</td>
</tr>
<tr>
<td>Melon</td>
<td>Nonanal, benzaldehyde and E-2-nonenal</td>
<td>Aldehydes</td>
<td>[20–22]</td>
</tr>
<tr>
<td></td>
<td>Ethyl 2-methyl propyl acetate and 2-methyl butyl acetate</td>
<td>Esters</td>
<td>[20–22]</td>
</tr>
</tbody>
</table>

Meanwhile, in most research work on fermentative H₂ from fruit wastes, fruit peels are often used as feedstock, since they are discarded during consumption and industrial processing. However, research should also be focused on the application of whole fruit wastes as feedstock, since large quantities of whole fruit wastes are generated during harvest, transportation and storage, due to microbial or pest attack. The objective of this study was therefore to investigate the effect of combined varying hydraulic retention time (HRT) and fruit mixing ratio on fermentative H₂ yield from whole fruit wastes in order to have an idea of how to manage whole fruit wastes feedstock for optimal fermentative hydrogen production. The potential of fruit biodegradability and fermentative hydrogen production could be enhanced or lessened by the characteristics of fruit mixtures (nutrient composition, carbon to nitrogen
ratio, toxicity) obtained when two or more fruits were combined. Besides nutrient composition, the inherent antimicrobial flavor compounds in fruits could also be a decisive factor in the overall effect. Unlike single fruit waste, mixed fruit wastes have the advantage of mutual interaction between various fruits in the fruit mixture, which might result in varying degrees of inhibitory and enhanced effects due to factors, such as additive (a combined effect equal to the sum of the individual effects), synergistic (a combined effect greater than the sum of the individual effects) and antagonistic effects (a combined effect less than the individual effects).

2. Results and Discussion

The performance of the fermentative H₂ production process from the whole fruit wastes of orange, banana, apple, grape and melon was evaluated by measuring the hydrogen yield and volatile fatty acid (VFA) productions. The hydraulic retention times (HRTs) were reduced as the organic loading rates (OLRs) were subsequently increased in the experiments. Although the mass of the different fruit wastes fed to the reactors were equal, their OLRs were not the same, because of different percentages of volatile solids in each fruit waste. The fermentation process was in three phases, Phase 1, 2 and 3, which corresponded to HRT of 8.6, 5 and 3 days, respectively. The continuous stirred tank reactors (CSTRs) were started up with an initial HRT of 8.6 days, which was the first phase of the fermentation process spanning a period of 15 days. During the second phase, which covered the period between the 16th and 30th days, the HRT was decreased from 8.6 down to five days. The HRT was furthered reduced to three days during the third phase, which spanned the period between the 31st and 47th days.

2.1. Hydrogen Production Yields from Singly-Digested Fruits

The hydrogen production from the singly-digested fruits was monitored throughout the three phases of the 47-day period. During the first phase (HRT of 8.6 days), hydrogen yields from the fermentation of individual fruits increased, except the yield from grape, which decreased sharply (Figure 1) due to the decrease in pH values. The highest (493 mL/gVS added; VS, volatile solid) and lowest (216 mL/gVS added) average hydrogen yields during the first phase were obtained from apple and melon, respectively. The hydrogen yields of all of the fruits increased during the second phase (HRT of five days), with apple and melon still producing the highest and lowest average hydrogen yields of 635 and 352 mL/gVS added, respectively. The average hydrogen yields during the third phase (HRT of three days), however, decreased with apple and grape, producing the highest and lowest yields of 440 and 182 mL/gVS, respectively.
2.2. Hydrogen Production Yields from Mixed Fruits

Hydrogen yields from the fermentation of the three fruit mixtures, including 70% (Mix 1), 50% (Mix 2) and 20% (Mix 3) orange share, also followed a similar pattern, but with better performance than the fermentation of individual fruits, except apple (Figure 2). During the first phase, hydrogen yields from Mix 1 and Mix 3 increased, but there was a slight decrease in hydrogen yield from Mix 2. The highest average hydrogen yield of 523 mL/gVS obtained during the first phase was from the fermentation of Mix 3. In the second phase, hydrogen yields from Mix 1 and Mix 2 increased, while there was a slight reduction in hydrogen yield from Mix 3. However, Mix 3 produced the highest average hydrogen yield of 553 mL/gVS at the end of the second phase. The hydrogen yields from the three fruit mixtures decreased during the last phase, with Mix 3 still producing the highest yield of 491 mL/gVS. Mix 3 seemed to perform averagely better than other mixed fruits in terms of hydrogen yield. This might be due to the ineffectiveness of the toxicity of the individual inhibitors as a consequent effect of the mutual interaction of the different flavor compounds in the fruit mixture. Besides, since limonene constitutes the major component of citrus essential oils, the reduction of orange percentage in Mix 3 could also impact the reduced antimicrobial effect of limonene in the mixture. Moreover, the use of an appropriate amount of nutrient combination in any mixture of fruit waste is necessary for optimal hydrogen yields from the fermentation process, as excess or insufficient nutrients may affect the stability and gas productivity of the process [34]. The macronutrients (Na, K, Ca and Mg), micronutrients (Fe, Co, Ni and Mo) and some vitamins are necessary for the cell growth and metabolic activities of fermentative microorganism [34–37].

![Hydrogen yields from the fermentation of single fruits.](image-url)
2.3. VFAs Production

The knowledge of the distribution of VFA compositions formed during the fermentation process is important, as it provides information about the metabolic pathways involved in the process. The distribution of VFA compositions produced during the fermentation of the fruit wastes showed that acetic and butyric acids were the dominant VFAs, while propionic, iso-butyric and iso-valeric were produced in very low amounts (Figure 3). At the end of the first phase of the initial OLR, the acetic acids produced from orange, banana, apple, grape, melon, Mix 1, Mix 2 and Mix 3 were 0.37, 0.62, 0.73, 0.75, 0.48, 0.56, 0.74 and 1.05 g/L, respectively. As the HRT decreased, the values of the acetic acid increased, except for grape and melon, which decreased during the second phase. At the end of the fermentation process, apple and Mix 3 produced significant amounts of acetic acids, which might be connected to their high cumulative hydrogen yields, as acetic acid production is often associated with hydrogen production [38,39].
2.4. Comparison of Hydrogen Yields and Acetic Acid Productions with Theoretical Values

In a biological \textit{in vivo} system, the theoretical maximum hydrogen yield that could be obtained from glucose at standard temperature and pressure is 4 mol H\textsubscript{2}/mol glucose when acetic acid is the only soluble metabolite \cite{40,41}. However, during dark fermentation, hydrogen production is often produced along with reduced metabolites, including alcohols, lactic, propionic and valeric acids, which are involved in the hydrogen consuming pathway, thereby leading to actual hydrogen yields being significantly lower than the theoretical values \cite{42–46}. On the other hand, high hydrogen yields are usually linked with the moderate accumulation of a mixture of acetic and butyric acids though accumulation of butyric acids, and its branched isomer could be an indication of process instability \cite{47}. In the present study, the hydrogen yields from the fermentation of all of the fruit substrates, except grape and melon, increased with the increase in acetic acid production as the HRT decreased from 8.6 down to five days (Table 2). This was expected as the concentration of volatile fatty acids usually increased with the increase in substrate loading, which correlated with the decrease in HRT from 8.6 down to five days \cite{39}. Meanwhile, the hydrogen yields decreased with the increase in acetic acid production, as the HRT was further decreased from five down to three days. In the comparison of actual yields to theoretical yields, the relative yield was calculated as given in Equation (4).

\[
\text{Relative Yield} = \left( \frac{\text{Actual yield}}{\text{Theoretical yield}} \right) \times 100
\] (4)
2.4.1. Relative Yield of Hydrogen Production

The theoretical yield of hydrogen was based on Equation (2) with the assumption that at standard temperature and pressure (STP), a maximum of four moles of hydrogen (H₂) could be produced from one mole of glucose when acetic acid was produced as the only reduced metabolite. The chemical oxygen demand (COD) equivalent of four moles of hydrogen is 1.4 L H₂/g COD of glucose at STP. In other words, 1.4 L of hydrogen gas could be generated through complete anaerobic degradation of 1 g COD of glucose at STP. The total COD of the individual fruit waste was calculated, and the equivalent theoretical yield was determined. The theoretical yield estimated and the actual yields obtained experimentally were then used to calculate the relative yield using Equation (4).

Table 2. Comparison of hydrogen yields and acetic acid production with theoretical values.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Average hydrogen yields</th>
<th>Average acetic acid production</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRT (d)</td>
<td>8.6</td>
<td>5.0</td>
</tr>
<tr>
<td>Fruit</td>
<td>AY (mL/gVS) RY (%)</td>
<td>AY (mL/gVS) RY (%)</td>
</tr>
<tr>
<td>Orange</td>
<td>279</td>
<td>16.1</td>
</tr>
<tr>
<td>Banana</td>
<td>389</td>
<td>22.7</td>
</tr>
<tr>
<td>Apple</td>
<td>493</td>
<td>28.9</td>
</tr>
<tr>
<td>Grape</td>
<td>347</td>
<td>20.5</td>
</tr>
<tr>
<td>Melon</td>
<td>216</td>
<td>12.6</td>
</tr>
<tr>
<td>Mix 1</td>
<td>268</td>
<td>15.5</td>
</tr>
<tr>
<td>Mix 2</td>
<td>270</td>
<td>15.7</td>
</tr>
<tr>
<td>Mix 3</td>
<td>523</td>
<td>30.5</td>
</tr>
</tbody>
</table>

AY, actual yield; RY, relative yield.

Comparing the actual yields to theoretical yields, maximum relative yields of hydrogen production obtained during the three phases were 37.3% and 32.3% from apple (single fruit fermentation) and Mix 3 (mixed fruit fermentation), respectively. For the whole fermentation period of 47 days, the maximum relative yields of hydrogen production were 29.5% and 30.0% from apple and Mix 3, respectively. On average, this indicates that only 30% of the chemical oxygen demand in the fruit mixture with Mix 3 could be converted into hydrogen (assuming the fruit wastes could be utilized as glucose). This low value might be due to the effects of the toxicity of the fruit flavors and soluble metabolites.

2.4.2. Relative Yield of Volatile Fatty Acids

The theoretical yield of acetic acid production was also based on Equation 2 with the assumption that at standard temperature and pressure (STP), a maximum of two moles of acetic acid (CH₃COOH) could be produced from one mole of glucose as the only reduced metabolite. The chemical oxygen demand (COD) equivalent of two moles of acetic acid is 0.666 g-O₂/L. In the case of acetic acid, the theoretical yield was based on 1 g COD of the fruit waste. The estimated values of the theoretical acetic acid production and the actual production of acetic acid were then used to calculate the relative yield.
Throughout the whole fermentation period of 47 days, the maximum acetic acid concentration was 1.62 g/L from Mix 3. Although the maximum acetic acid concentration was less than the inhibiting acetic concentration (greater than 2 g/L) [48], the decrease in hydrogen yield during the last fermentation period could have been due to the organic loading exceeding its threshold limit. At a high loading rate, acetic acid might accumulate and permeate the cell membrane of the hydrogen-producing bacteria with subsequent disruption in the activities of the bacteria.

The relative acetic acid yields corresponding to the maximum hydrogen yields were 136% and 172% for apple and 20% orange share, respectively. It appeared that the fermentative bacteria involved in the fermentation of apple and 20% orange share were able to maintain their cell physiological balance in the presence of extra acetic acids. The reason that some relative yields of acetic acid production were higher than 100% could be due to the fact that the theoretical yield was based on 1 g COD of glucose as feedstock, whereas in the real fruit feedstock, the nutrient composition included carbohydrate, protein and lipids that could have been converted into acetic acids.

2.5. Significant Effects of Varying Hydraulic Retention Times, Fruit Mixing and Their Interaction on Hydrogen Yield and Acetic Acid Production

The experiment was conducted to investigate the effects of hydraulic retention time (HRT) and fruit mixing, as well as their interactions on hydrogen yield and acetic acid production. The results of the two-way analysis of variance (ANOVA) are summarized in Tables 3 and 4. Based on the results obtained, the hypothesis tests on the effects of hydraulic retention time (HRT) and fruit mixing, as well as their interactions (HRT and fruit mixing interaction) on hydrogen yield indicated that the factors did not have significant effects since their $p$-values, 0.061, 0.259 and 0.763 (Table 3) for HRT, fruit mixing and HRT and fruit mixing interaction, respectively, were all greater than the chosen $\alpha$-level (0.05). The results were further corroborated with the Turkey pairwise comparisons for the difference in means (Table 4), which showed that the difference in means of hydrogen yield due to all of the factor levels were not significantly different, since their adjusted $p$-values were all greater than the chosen $\alpha$-level (0.05). Regarding the effects of the factors on acetic acid production, the hypothesis tests (Table 3) showed that HRT and fruit mixing really had significant effects on the production of acetic acids, since their $p$-values of 0.000 and 0.009 for HRT and fruit mixing, respectively, were lower than the chosen $\alpha$-level (0.05). On the contrary, the effect of the interaction of HRT and fruit mixing on acetic acid production was not statistically significant, since its $p$-value (0.830) was greater than the chosen $\alpha$-level (0.05).

The Tukey pairwise comparisons for the difference in means due to HRT (Table 4) showed that the adjusted $p$-values for the differences between the mean for HRT of three days and the means for HRT of five days (0.024) and 8.6 days (0.000) were all lower than the chosen $\alpha$-level (0.05), which indicated that these differences were significant. In the comparison of the difference in means for acetic acid production due to fruit mixing, the adjusted $p$-value (0.009) was lower than the chosen $\alpha$-level (0.05), indicating that the difference was statistically significant.

For the effect of the interaction of HRT and fruit mixing on acetic acid production, the adjusted $p$-values for the difference between the mean for mixed fruit operated at HRT of three days and the means for single fruits operated at HRT of five days (0.010) and 8.6 days (0.001) were lower than the
chosen α-level (0.05). Similarly, the adjusted \(p\)-values for single fruits (0.010) and mixed fruits (0.037), when operated separately at HRT of three and 8.6 days, were lower than the chosen α-level (0.05). Furthermore, the adjusted \(p\)-value (0.044) for the difference between the mean for mixed fruit operated at HRT of five days and the mean for single fruit operated at HRT of 8.6 days was lower than the chosen α-level (0.05), which indicated that the difference was statistically significant.

Meanwhile, as a consequence of the insignificant effect of fruit mixing on hydrogen yield, the fruit feedstock was further considered as individual substrates for analysis of variance (Table 5). Based on the results obtained, the hypothesis tests showed that both HRT and individual substrates had significant effects on hydrogen yields, since their \(p\)-values (0.001) were lower than the chosen α-level (0.05). Similarly, the effects of HRT and individual substrates on acetic acid production were statistically significant, since their \(p\)-values, 0.000 and 0.001 for HRT and individual substrate, respectively, were lower than the chosen α-level (0.05).

Table 3. Summary of two-way analysis of variance (ANOVA) for the effects of hydraulic retention time (HRT) and fruit mixing (Mix) on hydrogen yield and acetic acid production.

<table>
<thead>
<tr>
<th>Response</th>
<th>Factor Type</th>
<th>Factor Levels</th>
<th>Factor Values</th>
<th>df</th>
<th>Adj SS</th>
<th>Adj MS</th>
<th>(F)-Value</th>
<th>(p)-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen yield</td>
<td>HRT Fixed</td>
<td>3</td>
<td>3.0; 5.0; 8.6</td>
<td>2</td>
<td>3,8951</td>
<td>3.28</td>
<td>3.28</td>
<td>0.061</td>
</tr>
<tr>
<td></td>
<td>Mix Fixed</td>
<td>2</td>
<td>N; Y</td>
<td>1</td>
<td>16,187</td>
<td>1.36</td>
<td>1.36</td>
<td>0.259</td>
</tr>
<tr>
<td>HRT and mix interaction</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>3,266</td>
<td>0.27</td>
<td>0.27</td>
<td>0.763</td>
</tr>
<tr>
<td>Error</td>
<td></td>
<td></td>
<td></td>
<td>18</td>
<td>11,890</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid production</td>
<td>HRT Fixed</td>
<td>3</td>
<td>3.0; 5.0; 8.6</td>
<td>2</td>
<td>1.05371</td>
<td>0.526854</td>
<td>12.82</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Mix Fixed</td>
<td>2</td>
<td>N; Y</td>
<td>1</td>
<td>0.35219</td>
<td>0.352188</td>
<td>8.57</td>
<td>0.009</td>
</tr>
<tr>
<td>HRT and mix interaction</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>0.01551</td>
<td>0.007754</td>
<td>0.19</td>
<td>0.830</td>
</tr>
<tr>
<td>Error</td>
<td></td>
<td></td>
<td></td>
<td>18</td>
<td>0.73985</td>
<td>0.041103</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Tukey pairwise comparisons: response = \(H_2\) yield, acetic acid production; factor = HRT, mix and mix and HRT interaction.

<table>
<thead>
<tr>
<th>Response</th>
<th>Factor</th>
<th>Difference of factor levels</th>
<th>Difference of means</th>
<th>SE of difference</th>
<th>Simultaneous 95% CI</th>
<th>(t)-value</th>
<th>Adjusted (p)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(H_2) yield</td>
<td>HRT</td>
<td>5.0–3.0</td>
<td>131.8</td>
<td>56.3</td>
<td>(–12.0; 275.5)</td>
<td>2.34</td>
<td>0.076</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.6–3.0</td>
<td>15.3</td>
<td>56.3</td>
<td>(–128.4; 159.0)</td>
<td>0.27</td>
<td>0.960</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.6–5.0</td>
<td>–116.5</td>
<td>56.3</td>
<td>(–260.2; 27.3)</td>
<td>–2.07</td>
<td>0.125</td>
</tr>
<tr>
<td></td>
<td>Mix</td>
<td>YES–NO</td>
<td>53.6</td>
<td>46.0</td>
<td>(–42.9; 150.2)</td>
<td>1.17</td>
<td>0.259</td>
</tr>
<tr>
<td></td>
<td>Mix and HRT interaction</td>
<td>(NO 5.0)–(NO 3.0)</td>
<td>147.2</td>
<td>69.0</td>
<td>(–71.8; 366.2)</td>
<td>2.13</td>
<td>0.314</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(NO 8.6)–(NO 3.0)</td>
<td>56.6</td>
<td>69.0</td>
<td>(–162.4; 275.6)</td>
<td>0.82</td>
<td>0.960</td>
</tr>
</tbody>
</table>
Table 4. Cont.

<table>
<thead>
<tr>
<th>Response Factor</th>
<th>Difference of factor levels</th>
<th>Difference of means</th>
<th>SE of difference</th>
<th>Simultaneous 95% CI</th>
<th>t-value</th>
<th>Adjusted p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>YES 3.0–NO 3.0</td>
<td>91.5</td>
<td>79.6</td>
<td>(–161.4; 344.3)</td>
<td>1.15</td>
<td>0.855</td>
<td></td>
</tr>
<tr>
<td>YES 5.0–NO 3.0</td>
<td>207.8</td>
<td>79.6</td>
<td>(–45.0; 460.6)</td>
<td>2.61</td>
<td>0.145</td>
<td></td>
</tr>
<tr>
<td>YES 8.6–NO 3.0</td>
<td>65.5</td>
<td>79.6</td>
<td>(–187.4; 318.3)</td>
<td>0.82</td>
<td>0.960</td>
<td></td>
</tr>
<tr>
<td>NO 8.6–NO 5.0</td>
<td>–90.6</td>
<td>69.0</td>
<td>(–309.6; 128.4)</td>
<td>–1.31</td>
<td>0.774</td>
<td></td>
</tr>
<tr>
<td>YES 3.0–NO 5.0</td>
<td>–55.7</td>
<td>79.6</td>
<td>(–308.6; 197.1)</td>
<td>–0.70</td>
<td>0.980</td>
<td></td>
</tr>
<tr>
<td>YES 5.0–NO 5.0</td>
<td>60.6</td>
<td>79.6</td>
<td>(–192.2; 313.4)</td>
<td>0.76</td>
<td>0.971</td>
<td></td>
</tr>
<tr>
<td>YES 8.6–NO 5.0</td>
<td>–81.7</td>
<td>79.6</td>
<td>(–334.6; 171.1)</td>
<td>–1.03</td>
<td>0.903</td>
<td></td>
</tr>
<tr>
<td>YES 3.0–NO 8.6</td>
<td>34.9</td>
<td>79.6</td>
<td>(–218.0; 287.7)</td>
<td>0.44</td>
<td>0.998</td>
<td></td>
</tr>
<tr>
<td>YES 5.0–NO 8.6</td>
<td>151.2</td>
<td>79.6</td>
<td>(–101.6; 404.0)</td>
<td>1.90</td>
<td>0.434</td>
<td></td>
</tr>
<tr>
<td>YES 8.6–NO 5.0</td>
<td>8.9</td>
<td>79.6</td>
<td>(–244.0; 261.7)</td>
<td>0.11</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>YES 5.0–YES 3.0</td>
<td>116.3</td>
<td>89.0</td>
<td>(–166.3; 399.0)</td>
<td>1.31</td>
<td>0.778</td>
<td></td>
</tr>
<tr>
<td>YES 8.6–YES 3.0</td>
<td>–26.0</td>
<td>89.0</td>
<td>(–308.7; 256.7)</td>
<td>–0.29</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>YES 3.0–YES 5.0</td>
<td>–142.3</td>
<td>89.0</td>
<td>(–425.0; 140.3)</td>
<td>–1.60</td>
<td>0.610</td>
<td></td>
</tr>
</tbody>
</table>

Acetic acid

<table>
<thead>
<tr>
<th>HRT</th>
<th>Difference of means</th>
<th>SE of difference</th>
<th>Simultaneous 95% CI</th>
<th>t-value</th>
<th>Adjusted p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0–3.0</td>
<td>–0.305</td>
<td>0.105</td>
<td>(–0.572; –0.037)</td>
<td>–2.91</td>
<td>0.024</td>
</tr>
<tr>
<td>8.6–3.0</td>
<td>–0.528</td>
<td>0.105</td>
<td>(–0.795; –0.261)</td>
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<td>0.000</td>
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<tr>
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<td>(–0.491; 0.044)</td>
<td>–2.13</td>
<td>0.111</td>
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<tr>
<td>YES–NO</td>
<td>0.2502</td>
<td>0.0855</td>
<td>(0.0706; 0.4298)</td>
<td>2.93</td>
<td>0.124</td>
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</table>

Mix and HRT interaction

<table>
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<tr>
<th>Response Factor</th>
<th>Difference of factor levels</th>
<th>Difference of means</th>
<th>SE of difference</th>
<th>Simultaneous 95% CI</th>
<th>t-value</th>
<th>Adjusted p-value</th>
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<tbody>
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<td>YES 3.0–NO 3.0</td>
<td>–0.346</td>
<td>0.128</td>
<td>(–0.753; 0.061)</td>
<td>–2.70</td>
<td>0.124</td>
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<td>YES 5.0–NO 3.0</td>
<td>–0.506</td>
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<td>(–0.913; 0.099)</td>
<td>–3.95</td>
<td>0.010</td>
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<tr>
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<td>0.237</td>
<td>0.148</td>
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<td>(–0.789; 0.262)</td>
<td>–1.59</td>
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<td>YES 8.6–YES 3.0</td>
<td>–0.550</td>
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<td>–3.32</td>
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<td>(–0.812; 0.239)</td>
<td>–1.73</td>
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Table 5. Summary of two-way analysis of variance (ANOVA) for the effects of hydraulic retention time (HRT) and substrate.

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<th>Factor</th>
<th>Factor Type</th>
<th>Factor Levels</th>
<th>Factor Values</th>
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<th>Adj SS</th>
<th>Adj MS</th>
<th>F-Value</th>
<th>p-Value</th>
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<td>HRT</td>
<td>Fixed</td>
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<td>3.0; 5.0; 8.6</td>
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<td>83,069</td>
<td>41,534</td>
<td>11.52</td>
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<tr>
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<td>Fixed</td>
<td>8</td>
<td>Apple; Banana; Grape; Melon; Mix1; Mix 2; Mix 3; Orange</td>
<td>7</td>
<td>186,264</td>
<td>26,609</td>
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<td>Error</td>
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<td>23</td>
<td>319,805</td>
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<tr>
<td>Acetic acid production</td>
<td>HRT</td>
<td>Fixed</td>
<td>3</td>
<td>3.0; 5.0; 8.6</td>
<td>2</td>
<td>1.1074</td>
<td>0.55372</td>
<td>30.39</td>
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<tr>
<td>Mix</td>
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<td>Fixed</td>
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<td>Apple; Banana; Grape; Melon; Mix1; Mix 2; Mix 3; Orange</td>
<td>7</td>
<td>0.8524</td>
<td>0.12178</td>
<td>6.68</td>
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<tr>
<td>Error</td>
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<td>14</td>
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<td>0.01822</td>
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The Tukey pairwise comparisons for the difference in means of hydrogen yield due to HRT (Table 6) showed that the adjusted *p*-values for the differences between the mean for HRT of five days and the means for HRT of three days (0.001) and 8.6 days (0.007) were lower than the chosen α-level (0.05), which indicated that these differences were significant. For the effects of individual substrates on hydrogen yield, the Turkey pairwise comparisons showed that the adjusted *p*-values for the differences between the mean for apple and the means for grape (0.010), melon (0.010), Mix 1 (0.026) and orange (0.007) were all lower than the chosen α-level (0.05). In the same vein, the adjusted *p*-values for the difference between the mean for Mix 3 and the means for grape (0.010), melon (0.010), Mix 1 (0.026) and orange (0.007) were lower than the chosen α-level (0.05), which indicated that the mean differences were significant. Meanwhile, the Tukey comparison for the difference in means of acetic acid production due to HRT showed that the adjusted *p*-values for the differences between the mean for HRT of three days and the means for HRT of five days (0.001) and 8.6 days (0.0022) were lower than the chosen α-level (0.05). Furthermore, the adjusted *p*-value (0.021) for the difference between the mean for HRT of five days and 8.6 days was lower than the chosen α-level (0.05), which indicated that the difference was significant. In the case of the effects of individual substrates on acetic acid production, the adjusted *p*-values for the differences between the mean for Mix 3 and the means for grape (0.026), melon (0.000) and orange (0.007) were below the chosen α-level (0.05). Similarly, the adjusted *p*-values for the difference between the mean for melon and the means for apple (0.036) and Mix 2 (0.034) were below the chosen α-level (0.05), which showed that the mean differences were significant.
Table 6. Tukey pairwise comparisons: Response = H₂ yield, acetic acid production; factor = HRT, substrate.

<table>
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<tr>
<th>Response</th>
<th>Factor</th>
<th>Difference of Factor Levels</th>
<th>Difference of Means</th>
<th>SE of Difference</th>
<th>Simultaneous 95% CI</th>
<th>t-Value</th>
<th>Adjusted p-Value</th>
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<td>H₂ yield</td>
<td>HRT</td>
<td>5.0–3.0</td>
<td>135.6</td>
<td>30.0</td>
<td>(57.1; 214.2)</td>
<td>4.52</td>
<td>0.001</td>
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<tr>
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<td>8.6–3.0</td>
<td>25.6</td>
<td>30.0</td>
<td>(–52.9; 104.2)</td>
<td>0.85</td>
<td>0.677</td>
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<td>8.6–5.0</td>
<td>–110.0</td>
<td>30.0</td>
<td>(–188.5; –31.5)</td>
<td>–3.66</td>
<td>0.007</td>
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<tr>
<td>Substrate</td>
<td>Banana-Apple</td>
<td>–169.3</td>
<td>49.0</td>
<td>(–342.3; 3.6)</td>
<td>–3.45</td>
<td>0.057</td>
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<tr>
<td></td>
<td>Grape-apple</td>
<td>–218.3</td>
<td>49.0</td>
<td>(–391.3; –45.4)</td>
<td>–4.45</td>
<td>0.010</td>
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<tr>
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<td>Melon-Apple</td>
<td>–217.7</td>
<td>49.0</td>
<td>(–390.6; –44.7)</td>
<td>–4.44</td>
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<td>Mix 1-Apple</td>
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<td>(–364.0; –18.0)</td>
<td>–3.90</td>
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<td>Mix 2-Apple</td>
<td>–147.3</td>
<td>49.0</td>
<td>(–320.3; 25.6)</td>
<td>–3.01</td>
<td>0.123</td>
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<td>Mix 3-Apple</td>
<td>–0.3</td>
<td>49.0</td>
<td>(–173.3; 172.6)</td>
<td>–0.01</td>
<td>1.000</td>
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<tr>
<td></td>
<td>Orange-Apple</td>
<td>–227.3</td>
<td>49.0</td>
<td>(–400.3; –54.4)</td>
<td>–4.64</td>
<td>0.007</td>
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<tr>
<td></td>
<td>Grape-Banana</td>
<td>–49.0</td>
<td>49.0</td>
<td>(–222.0; 124.6)</td>
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<td>Melon-Banana</td>
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<td>49.0</td>
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<td>Mix 1-Banana</td>
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<td>(–194.6; 151.3)</td>
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<td>Mix 2-Banana</td>
<td>22.0</td>
<td>49.0</td>
<td>(–151.0; 195.0)</td>
<td>0.45</td>
<td>1.000</td>
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<tr>
<td></td>
<td>Mix 3-Banana</td>
<td>169.0</td>
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<td>(–4.0; 342.0)</td>
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<td>Melon-Grape</td>
<td>0.7</td>
<td>49.0</td>
<td>(–172.3; 173.6)</td>
<td>0.01</td>
<td>1.000</td>
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<td>(–102.0; 244.0)</td>
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<td>Mix 3-Grape</td>
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<td>49.0</td>
<td>(45.0; 391.0)</td>
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<td>0.010</td>
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<td>49.0</td>
<td>(–182.0; 164.0)</td>
<td>–0.18</td>
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<td>49.0</td>
<td>(–146.3; 199.6)</td>
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<td>Mix 2-Melon</td>
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<td>(–102.6; 243.3)</td>
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<td>(44.4; 390.3)</td>
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<td>–9.7</td>
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<td>(–0.386; 0.392)</td>
<td>0.03</td>
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<td>Mix 3-Apple</td>
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<td>(–0.606; 0.172)</td>
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<td>0.110</td>
<td>(–0.449; 0.329)</td>
<td>–0.54</td>
<td>0.999</td>
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</table>
Table 6. Cont.

<table>
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<tr>
<th>Response</th>
<th>Factor</th>
<th>Difference of Factor Levels</th>
<th>Difference of Means</th>
<th>SE of Difference</th>
<th>Simultaneous 95% CI</th>
<th>t-Value</th>
<th>Adjusted p-Value</th>
</tr>
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<tbody>
<tr>
<td>Melon-Banana</td>
<td>–0.333</td>
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<td>(–0.722; 0.056)</td>
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<td>0.110</td>
<td>(–0.362; 0.416)</td>
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<td>1.000</td>
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<td>0.110</td>
<td>(–0.309; 0.469)</td>
<td>0.73</td>
<td>0.995</td>
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<td>Mix 3-Banana</td>
<td>0.370</td>
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<td>(–0.019; 0.759)</td>
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<td>Orange-Banana</td>
<td>–0.140</td>
<td>0.110</td>
<td>(–0.529; 0.249)</td>
<td>–1.27</td>
<td>0.896</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melon-Grape</td>
<td>–0.273</td>
<td>0.110</td>
<td>(–0.662; 0.116)</td>
<td>–2.48</td>
<td>0.278</td>
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</tr>
<tr>
<td>Mix 1-Grape</td>
<td>0.087</td>
<td>0.110</td>
<td>(–0.302; 0.476)</td>
<td>0.79</td>
<td>0.991</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mix 2-Grape</td>
<td>0.140</td>
<td>0.110</td>
<td>(–0.249; 0.529)</td>
<td>1.27</td>
<td>0.896</td>
<td></td>
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</tr>
<tr>
<td>Mix 3-Grape</td>
<td>0.430</td>
<td>0.110</td>
<td>(0.041; 0.819)</td>
<td>3.90</td>
<td>0.026</td>
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</tr>
<tr>
<td>Orange–Grape</td>
<td>–0.080</td>
<td>0.110</td>
<td>(–0.469; 0.309)</td>
<td>–0.73</td>
<td>0.995</td>
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<tr>
<td>Mix 1-Melon</td>
<td>0.360</td>
<td>0.110</td>
<td>(–0.029; 0.749)</td>
<td>3.27</td>
<td>0.079</td>
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<tr>
<td>Mix 2-Melon</td>
<td>0.413</td>
<td>0.110</td>
<td>(0.024; 0.802)</td>
<td>3.75</td>
<td>0.034</td>
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<td>Mix 3-Melon</td>
<td>0.703</td>
<td>0.110</td>
<td>(0.314; 1.092)</td>
<td>6.38</td>
<td>0.000</td>
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<tr>
<td>Orange-Melon</td>
<td>0.193</td>
<td>0.110</td>
<td>(–0.196; 0.582)</td>
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<td>0.657</td>
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<tr>
<td>Mix 2-Mix 1</td>
<td>0.053</td>
<td>0.110</td>
<td>(–0.336; 0.422)</td>
<td>0.48</td>
<td>1.000</td>
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<td></td>
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<tr>
<td>Mix 3-Mix 1</td>
<td>0.343</td>
<td>0.110</td>
<td>(–0.046; 0.732)</td>
<td>3.12</td>
<td>0.102</td>
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<td>Orange-Mix 1</td>
<td>–0.167</td>
<td>0.110</td>
<td>(–0.556; 0.222)</td>
<td>–1.51</td>
<td>0.790</td>
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<td>Mix 3-Mix 2</td>
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<td>0.223</td>
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<td>Orange-Mix 2</td>
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<td>(–0.609; 0.169)</td>
<td>–2.00</td>
<td>0.516</td>
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<td>Orange-Mix 3</td>
<td>–0.510</td>
<td>0.110</td>
<td>(–0.899; –0.121)</td>
<td>–4.63</td>
<td>0.007</td>
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</tr>
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</table>

3. Experimental Section

3.1. Experimental Materials (Feedstock, Seed Sludge and Nutrient)

Whole fruit wastes, including apple (Malus sp.), banana (Musa sp.), grape (Vitis sp.), melon (Cucumis sp.) and orange (Citrus sp.), obtained from a local shop (Borås, Sweden), were used as feedstock for the anaerobic digestion process. The fruits as a whole (rind and inner part of the fruits) were ground separately in a kitchen blender (Waring Commercial, Torrington, CT, USA) before they were stored in a cold room (5 °C) to reduce their deterioration prior to the fermentation process. The hydrogen-producing microorganism (HPM) was prepared from sludge obtained from a thermophilic (55 °C) biogas plant (Borås Energi & Miljö AB, Borås, Sweden) that utilized the organic fraction of municipal solid waste as feedstock for biogas production. The HPM was enriched by adjusting the pH to 5.0 ± 0.1 coupled with heat treatment at 105 °C for 1 h. The nutrients used for the growth of HPM were a mixture of macro- and micronutrients, including (g/L): FeCl2·4H2O, 11.401; KH2PO4, 4.681; NH4Cl, 0.814; NaHCO3, 3.000; MgSO4·7H2O, 0.320; NiSO4·6H2O, 0.032; CaCl2, 0.050; Na2B4O7·10H2O, 0.007; (NH4)6Mo7O24·4H2O, 0.014; ZnCl2, 0.023; CoCl2·6H2O, 0.021; CuCl2·2H2O, 0.010.
3.2. Experimental Setup and Procedures

The fermentative production of hydrogen was carried out using eight continuous stirred tank bioreactors (CSTR) in order to evaluate the performance, in terms of hydrogen yields and VFA production, of the fermentative hydrogen production process from the whole fruit wastes. Each CSTR reactor (active volume of 3 L and headspace of 1 L) contained fermentation medium at 55 °C and a pH of 5.0 (Figure 4). Eight different fruit substrates were used, including single fruits (banana, apple, grape, melon, orange) and mixed fruits, which differed in the amount of orange in the mixtures. The weight distribution of the first, second and third fruit mixtures were 70%, 50% and 20% orange share, respectively, while the residual weight was shared equally by banana, apple, grape, melon and orange. A thermostatic water bath (GD 100, Grant instruments Ltd., Cambridgeshire, UK) was employed to provide the heating energy needed to maintain the required temperatures in the CSTRs. Prior to the start of the fermentation process, the fruit slurries were left to attain room temperature (22 °C) before they were fed into the bioreactors. A mixture of HPM, feedstock, distilled water, macro- and micro-nutrients in solution at a volumetric ratio of 8:2:6:4:1, respectively, was added to the CSTRs and left for three days without any daily feedstock feeding in order to adapt or acclimatize the inoculum to the fermentation environment. The fermentation process was started, after the third day, with the feeding of the fruit feedstock mixed with distilled water, buffer solution (1 M NaHCO₃), macro- and micro-nutrients at a ratio of 10:60:3:20:7, respectively, into the CSTR. The whole experiment was run for 47 days with an increase in the organic loading rate (OLR).

![Figure 4. Schematic diagram of the fermentative hydrogen production from single and mixed fruits.](image)

3.3. Analytical Procedures

The characteristics of the feedstock for the two-stage fermentation process, including total solid (TS), volatile solid (VS) and pH (Table 7), were determined according to standard methods [49]. The main nutrient compositions of the fruit wastes and their mixtures are presented in Table 8 according to the nutrient database of the U.S. Department of Agriculture [50]. The gas produced was measured
using the Automatic Methane Potential Testing System (AMPTS, Bioprocess Control AB, Lund, Sweden), which is based on the principle of water displacement and buoyancy, while the hydrogen composition of the gas produced was sampled with the aid of a 0.25-µL pressure-tight gas syringe (VICI, Baton Rouge, LA, USA) and analyzed using a gas chromatograph (GC, Perkin-Elmer, 710 Bridgeport Avenue, Shelton, CT, USA) equipped with a packed column (Perkin-Elmer, 6’ × 1.8” OD, 80/100, Mesh, 710 Bridgeport Avenue, Shelton, CT, USA) and a thermal conductivity detector (TCD, PerkinElmer, 710 Bridgeport Avenue, Shelton, CT, USA) set at 200 °C. The temperatures of the oven and injector were set at 75 °C and 150 °C, respectively, while nitrogen gas at a flow rate, temperature and pressure of 20 mL/min, 60 °C and 1 bar, respectively, was used as the carrier gas. The volatile fatty acids (VFA) in the effluent samples were measured using a high-performance liquid chromatograph (HPLC, Waters 2695, Waters Corporation, Milford, MA, USA) equipped with an RI detector (Waters 2414, Waters Corporation, Milford, MA, USA) and a biohydrogen-ion exchange column (Aminex HPX-87H, Bio-Rad, Hercules, CA, USA) operating at 60 °C and 0.6 mL/min and with 5 mM sulfuric acid as the effluent.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Apple</th>
<th>Banana</th>
<th>Grape</th>
<th>Melon</th>
<th>Orange</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS (%)</td>
<td>11.72 ± 0.02</td>
<td>16.36 ± 0.24</td>
<td>19.32 ± 0.24</td>
<td>8.93 ± 0.26</td>
<td>16.85 ± 0.30</td>
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<tr>
<td>VS (%)</td>
<td>11.53 ± 0.35</td>
<td>15.5 ± 0.05</td>
<td>18.68 ± 0.06</td>
<td>8.39 ± 0.16</td>
<td>16.26 ± 0.20</td>
</tr>
<tr>
<td>pH</td>
<td>3.90 ± 0.01</td>
<td>5.06 ± 0.02</td>
<td>3.67 ± 0.02</td>
<td>4.88 ± 0.01</td>
<td>4.04 ± 0.02</td>
</tr>
</tbody>
</table>

Table 7. Measured characteristics of fruit wastes. TS, total solid.

Table 8. Nutrient composition in 100 g of fruit.

<table>
<thead>
<tr>
<th>Fruit</th>
<th>Carbohydrate</th>
<th>Protein</th>
<th>Lipid</th>
<th>C:N Ratio *</th>
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<tbody>
<tr>
<td></td>
<td>g</td>
<td>%</td>
<td>g</td>
<td>%</td>
</tr>
<tr>
<td>Apple</td>
<td>14.06</td>
<td>97</td>
<td>0.27</td>
<td>2</td>
</tr>
<tr>
<td>Melon</td>
<td>9.09</td>
<td>93</td>
<td>0.54</td>
<td>6</td>
</tr>
<tr>
<td>Banana</td>
<td>22.84</td>
<td>94</td>
<td>1.09</td>
<td>5</td>
</tr>
<tr>
<td>Orange</td>
<td>15.50</td>
<td>90</td>
<td>1.30</td>
<td>8</td>
</tr>
<tr>
<td>Grape</td>
<td>18.10</td>
<td>95</td>
<td>0.72</td>
<td>4</td>
</tr>
<tr>
<td>Mix 1</td>
<td>15.71</td>
<td>92</td>
<td>1.10</td>
<td>6</td>
</tr>
<tr>
<td>Mix 2</td>
<td>15.77</td>
<td>95</td>
<td>0.59</td>
<td>3</td>
</tr>
<tr>
<td>Mix 3</td>
<td>15.92</td>
<td>94</td>
<td>0.78</td>
<td>5</td>
</tr>
</tbody>
</table>

* Estimated value.

4. Conclusions

The present study assessed the effect of hydraulic retention times and fruit mixing on biohydrogen production from fruit wastes in continuous stirred tank bioreactors. The results of the two-way analysis of variance indicated that there was no statistically-significant effect of the interaction of hydraulic retention time and fruit mixing on hydrogen yields and acetic acid production. However, the results established that significant improvement in hydrogen yields could be obtained when apple and Mix 3 were used as individual substrates. The results also indicated that operating the fermentative hydrogen production at a hydraulic retention time of 5 days could greatly increase hydrogen yield, as it reduced
the amount of acetic acid accumulated during the fermentation process. It could therefore be inferred that fermentation of apple or Mix 3 at hydraulic retention time of 5 days could be used to reduce the effect of bacterial inhibition due to flavor compounds in the fruits and thereby enhance hydrogen production from the fruit wastes.

Acknowledgments

The authors wish to express their gratitude to the Swedish Research Council (Sweden) and Lagos State University (Nigeria) for providing financial support during the research work. We are also grateful for the assistance given by Magnus Lundin on statistical analysis of the data and to Khamdan Cahyari for his support during the study.

Acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tr>
<td>AY</td>
<td>Actual yield</td>
</tr>
<tr>
<td>COD</td>
<td>Chemical oxygen demand</td>
</tr>
<tr>
<td>HPM</td>
<td>Hydrogen producing microorganism</td>
</tr>
<tr>
<td>MS</td>
<td>Mean square</td>
</tr>
<tr>
<td>Mix 1</td>
<td>70% orange mixed fruit</td>
</tr>
<tr>
<td>Mix 2</td>
<td>50% orange mixed fruit</td>
</tr>
<tr>
<td>Mix 3</td>
<td>20% orange mixed fruit</td>
</tr>
<tr>
<td>PY</td>
<td>Percent yield</td>
</tr>
<tr>
<td>SV</td>
<td>Source of variation</td>
</tr>
<tr>
<td>TVFA</td>
<td>Total volatile fatty acids</td>
</tr>
<tr>
<td>VS</td>
<td>Volatile solid</td>
</tr>
<tr>
<td>CIs</td>
<td>Confidence intervals</td>
</tr>
<tr>
<td>dF</td>
<td>degree of freedom</td>
</tr>
<tr>
<td>HRT</td>
<td>Hydraulic retention time</td>
</tr>
<tr>
<td>Mix</td>
<td>Mixing</td>
</tr>
<tr>
<td>OLR</td>
<td>Organic loading rate</td>
</tr>
<tr>
<td>SS</td>
<td>Sum of squares</td>
</tr>
<tr>
<td>TS</td>
<td>Total solid</td>
</tr>
<tr>
<td>VFA</td>
<td>Volatile fatty acids</td>
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Conflicts of Interest

The authors declare no conflicts of interest

References


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Development of a submerged anaerobic membrane bioreactor for concurrent extraction of volatile fatty acids and biohydrogen production

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c CNRS, UMR 6602, IP, F-63178 Aubière, France
d Swedish Centre for Resource Recovery, University of Borås, S-50190, Sweden

HIGHLIGHTS
- An externally-submerged anaerobic membrane reactor was used to produce BioH2.
- Mixing, transmembrane pressure (TMP) and fouling were investigated.
- TMP was low (10 kPa) and fouling was reversible, mainly due to cake layer formation.
- Gas scouring and backwashing with the substrate were used as a cleaning procedure.
- Biohydrogen production was shown to restart after removing VFAs in the permeate.

GRAPHICAL ABSTRACT

ABSTRACT

The aim of this work was to study an externally-submerged membrane bioreactor for the cyclic extraction of volatile fatty acids (VFAs) during anaerobic fermentation, combining the advantages of submerged and external technologies for enhancing biohydrogen (BioH2) production from agrowaste. Mixing and transmembrane pressure (TMP) across a hollow fiber membrane placed in a recirculation loop coupled to a stirred tank were investigated, so that the loop did not significantly modify the hydrodynamic properties in the tank. The fouling mechanism, due to cake layer formation, was reversible. A cleaning procedure based on gas scouring and backwashing with the substrate was defined. Low TMP, 104 Pa, was required to achieve a 3 L h⁻¹ m⁻² critical flux. During fermentation, BioH2 production was shown to restart after removing VFAs with the permeate, so as to enhance simultaneously BioH2 production and the recovery of VFAs as platform molecules.

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1. Introduction

The benefits of biohydrogen (BioH2) production through dark fermentation process regarding reduction in greenhouse gas emission and valorization of organic waste materials cannot be downplayed. However, low yield and production rate of BioH2 have been the major barriers to the commercial-scale application of the
dark fermentation process. Among the contributing factors to low
BioH2 yield is the accumulation of volatile fatty acids (VFAs), which
results in a decrease in pH and consequent inhibition of the fer-
mentation process. VFAs, including acetic, propionic and butyric
acids, are usually produced through the main mechanism of the dark fermentation process, while other organic acids, such as
succinic, lactic and fumaric acids are produced when there is
imbalance during the digestion process. Therefore, regulation of
VFAs production by continuously removing them from the fermen-
tation medium is crucial for the process stability and efficiency.
Meanwhile, extracted VFAs from fermentation medium can be
used for the production of fuels and energy through biochemical
of thermochemical pathways. The VFAs can also find a direct usage as food additives in the food and the pharmaceutical industries (Venkata-Mohan and Pandey, 2013). Different techniques including
ion exchange (Gluszcz et al., 2004), adsorption (Joglekar et al., 2006), electrodeionization (Huang et al., 2007; Wang et al.,
2006), liquid-liquid extraction (Mostafa, 1999; Senol and Dramur,
2004), distillation (Mumtaz et al., 2008), esterification (Pereira et al., 2011), reactive extraction (Hong et al., 2001) and membrane
process (Wodaki and Nowaczyk, 1997) have been employed to
recover organic acids from fermentation broth. Among the various
techniques for VFAs recovery, membrane processes seem to be the
most efficient, eco-friendly and economic method. Membrane
filtration exhibits numerous benefits including small footprint,
and enhanced retention which enables sludge retention time to be
independently controlled from hydraulic retention time. It also
reduces the additional cost of disinfectant since it allows the
removal of microorganisms from VFAs to a certain degree for
subsequent treatment.

Generally, the efficiency and economics of membrane filtration
depend on membrane module design (tubular, plate and frame,
rotary disk or hollow fiber), pore size (microfiltration, ultrafiltra-
tion, nanofiltration or reverse osmosis), membrane material
(organic, inorganic, metallic, hydrophobicity or hydrophilicity),
filtration mode (dead end or cross mode), operating conditions
(flux, hydraulic retention time and sludge retention time) and
sludge characteristics (biomass concentration, pH, extracellular
polymeric substances and soluble microbial product). As a result,
many challenges are associated with the application of membrane
filtration process, among which the most important is membrane
fouling. Membrane fouling is caused by particles deposition, plug-
ging and narrowing of membrane pores and surfaces (Bae and Tak,
2005; Defrance et al., 2000). Flux and, hence, filtration efficiency is
directly affected by membrane fouling with a consequent decrease
in system productivity and increase in operating cost. Darcy’s law
highlights that the permeate flux through a porous membrane is
directly proportional to the transmembrane pressure (TMP) and
the membrane area, but is inversely proportional to the membrane
resistance due to fouling and to feed viscosity, as shown in Eq. (1)
(Field et al., 1995). In this equation, J is the permeate flux, μ the vis-
cosity of the liquid feed; TMP and R reflects the membrane pressure and the total resistance, are given by Eqs. (2) and (3).

\[
J = \frac{\text{TMP}}{\mu R}\label{eq1}
\]

\[
\text{TMP} = \frac{P_f + P_r - P_p}{2}\label{eq2}
\]

\[
R = R_m + R_{if} + R_g\label{eq3}
\]

In the above equations, \(P_f\), \(P_r\), \(P_p\), \(R_m\), \(R_{if}\) and \(R_g\) are the feed, retentate and permeate pressures, the clean membrane resistance,
and the external and internal fouling resistance, respectively. As
illustrated in Eq. (1), the rate of membrane fouling could be
reduced by carrying out filtration process below the critical flux,
and by maintaining simultaneously high shear rate through veloc-
ity gradient or gas sparging close to the membrane. Membrane
fouling can also be reduced by using appropriate membrane con-
figuration and modules, as in the case of hollow fiber membrane
modules which constitute a common membrane configuration
employed in many industrial membrane processes owing to its
excellent mass transfer qualities and high membrane surface area.

Among the two main membrane configurations including
submerged and side-stream membrane bioreactors (MBRs),
internally-submerged MBR is usually preferred to side-stream
MBRs owing to its advantages which include smaller footprint
and less energy requirement (Cote et al., 1997; Singhania et al.,
2012). However, in some commercial applications, side-stream
MBRs were selected when a higher frequency for membrane

---

List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>AnSMBR</td>
<td>anaerobic submerged membrane reactor</td>
</tr>
<tr>
<td>CSTR</td>
<td>continuous stirred tank reactor</td>
</tr>
<tr>
<td>RTD</td>
<td>residence time distribution</td>
</tr>
<tr>
<td>TMP</td>
<td>transmembrane pressure (Pa)</td>
</tr>
<tr>
<td>VFAs</td>
<td>volatile fatty acids</td>
</tr>
<tr>
<td>A</td>
<td>membrane surface area (m²)</td>
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<tr>
<td>C(t)</td>
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<tr>
<td>V</td>
<td>filtrate volume (m³)</td>
</tr>
<tr>
<td>θ</td>
<td>normalized time (-)</td>
</tr>
<tr>
<td>μ</td>
<td>water viscosity (Pa s)</td>
</tr>
<tr>
<td>ρ</td>
<td>liquid density (kg m⁻³)</td>
</tr>
<tr>
<td>σₑ</td>
<td>variance of the error of the flux model (1² h⁻² m⁻⁴)</td>
</tr>
</tbody>
</table>
| tₑ         | space time in the membrane module (s)
replacement is required at a given flow rate. An attractive alternative that combines the advantages of submerged and side-stream technologies is the externally-submerged membrane bioreactor: it can be operated at low pressure, while membrane cleaning and replacement are easier (Singhania et al., 2012). However, this has been disregarded in comparison to internally-submerged devices. Up to now, most of the previous research work on VFAs recovery from fermentation broths focused on the comparison of separation technologies used as a downstream treatment. The cyclic extraction of VFAs during acidogenic fermentation using an externally-submerged membrane coupled to the anaerobic digester has not been investigated in detail. Consequently, the aim of this work was to develop an original anaerobic externally-submerged membrane bioreactor (AnSMBR) for the concurrent cyclic extraction of VFAs and bioH₂ production. In addition, transmembrane pressure (TMP) was studied as a function of the operating conditions, while focusing on the cleaning procedure to assess the feasibility of the filtration process.

2. Methods

2.1. Experimental set-up

The anaerobic membrane reactor system employed for the membrane filtration experiments was composed of an externally-submerged hollow fiber microfiltration (MF) membrane module that was operated in the cross-flow “outside-in” mode and placed in a recirculation loop coupled to a 5-L mechanically stirred tank reactor (Fig. 1) from GPC (France). The cylindrical membrane module was made up of 142 free polyvinylidene fluoride (PVDF) fibers placed in 32.5 cm length housing with a surface area \( A = 0.155 \, \text{m}^2 \) and a 0.2 µm cut-off diameter. The volume of the recirculation loop was measured, about 0.5 L. The stirred tank (internal diameter of 17 cm and vessel height 35 cm) was equipped with a two-stage impeller: the bottom impeller was a four-blade disk (Rushton) turbine of 5.6 cm diameter and the top impeller was a three-bladed 45° pitched turbine of 8.8 cm diameter. The rotation speed of the impeller was varied from 20 to 150 rpm. The top impeller promotes equal flow discharge in the axial and the radial directions due to the 45° angle, while the bottom impeller promotes mainly a radial flow discharge. A peristaltic pump, denoted circulation pump, was used to impose the flow rate in the external membrane module. The permeate was recovered using another peristaltic pump, denoted withdrawal pump, that sucked the liquid phase, causing a negative gauge pressure inside the fibers. As a result, both the stirred tank and the loop could be operated under atmospheric pressure, which prevented fermentation inhibition by H₂ gas. The recirculation flow rate could be varied from 50 to 395.3 mL/min. This corresponded to space time values in the tank between 12 and 100 min, and to space time values in the membrane module between 1.2 and 10 min. For permeate recovery, the rotation speed of the withdrawal pump could be varied from 200 to 1000 rpm, which was equivalent to flow rates between 3 and 16 mL/min for water at atmospheric pressure. The permeate flow rate was estimated by recording the mass of permeate over time using an electronic balance.

2.2. Experimental procedures

The first task was to validate the design of the bioreactor including the potential application of an externally-submerged membrane module, the volume ratio between the tank and the module, and the range of recirculation and withdrawal flow rates. Mixing time and residence time distribution (RTD) analysis as well as TMP and permeate flow rate measurements were first carried out using water. The results from the experiments were useful for describing not only the hydrodynamic behavior of the membrane module for fouling prevention and the influence of the recirculation loop on the mixing properties of the stirred tank, but also for the optimization of the inlet and outlet positions of the recirculation loop in the tank. Experiments were later conducted to measure TMP and flow rates using the actual fermentation broth as it was done when water was used. All the experiments were done in triplicate.
2.2.1. Mixing and RTD analysis

A conductivity tracer technique was applied, in which distilled water and sodium chloride (NaCl) were used as the working fluid and the tracer, respectively. The conductivity tracer technique involved the manual injection of an approximated δ-Dirac pulse of 1.0 M NaCl solution in the reactor system and the subsequent detection of the tracer response signal using conductivity probes, such as the CDC 241-9 (cell constant, 0.913 cm⁻¹) and the CDC 749 (cell constant, 1.45 cm⁻¹), connected to a CDM210 conductivity-meter (Radiometer Analytical, France). Both mixing time and RTD (Residence Time Distribution) experiments were carried out in the stirred tank reactor, while only RTD was measured in the membrane module with and without permeate extraction. Data was recorded at 1 Hz frequency.

First, batch experiments were carried out to estimate mixing time as a function of the rotation speed of the impeller. These were compared to mixing time measurements in the presence of the recirculation loop. The mixing time \( \tau_{in} \) and mean circulation \( \tau_c \) times in the stirred tank were extracted from the experimental curves of tracer concentration vs. time as a function of recirculation flow rate and impeller rotation speed, as in Liu (2012). Then, RTD analysis was carried out in the membrane fiber bundle under continuous flow conditions, without and with permeate extraction, as a function of the impeller rotation speed in the tank, the circulation flow rate and the speed of the withdrawal pump.

2.2.2. Transmembrane pressure (TMP) and permeate flow rate measurements

Membrane filtration was driven in the cross-flow “outside-in” mode, i.e. with the filtration proceeding from outside to inside of the fibers, as the feed solution was being pumped from the membrane module. As a result, the flow rate of permeate, \( J \), was deduced by weighing the mass of permeate withdrawn over time. TMP was determined by measuring the pressure at the inlet and the outlet of the fiber module and on the permeate side of the membrane, using two absolute and one differential pressure sensors (Keller A.G, Germany). The differential pressure sensor and one absolute pressure sensor were placed close to the membrane inlet, while the other absolute pressure sensor was placed close to the outlet of the membrane unit. TMP was deduced from experimental data using Eqs. (4)–(8).

\[
\text{TMP} = \frac{(P_{in} + \rho g h_{in}) - (P_{out} + \rho g h_{out})}{2} - P_p
\]

(4)

where \( P_{in} \) is the inlet pressure (measured), \( P_{out} \) the outlet pressure (measured) and \( P_p \) the permeate pressure, while \( P_{diff} \) is the differential pressure (measured), defined as:

\[
P_{diff} = P_{in} - P_p
\]

(5)

As the fibers are vertical, gravitational potential energy must be accounted for and the difference of height, \( Ah = h_{out} - h_{in} \), between the outlet (top) and the inlet (bottom) of the fiber module emerges from Eq. (5). As a result, TMP can be expressed as:

\[
\text{TMP} = \rho g Ah + \frac{P_{out} - P_{in}}{2} + P_{diff}
\]

(6)

When there is no flow (fluid velocity \( v = 0 \)) through the membrane, Eq. (7) is obtained:

\[
-\rho g Ah = \frac{P_{out} - P_{in}}{2} + P_{diff} \quad j_{\nu=0}
\]

(7)

Finally, one deduces that:

\[
\text{TMP} = \frac{(P_{out} - P_{in})}{2} + P_{diff} \quad \text{for} \quad j_{\nu=0}
\]

(8)

which combines measurements when recirculation is stopped \( (\nu = 0) \) to data obtained during filtration.

2.2.3. Anaerobic digestion experiments

To obtain fermentation broth for filtration experiments, acidogenic fermentation was carried out using mixed cultures with a mesophilic bacterial consortium. Glucose (20 g/L) was initially used as the carbon source in order to enhance the reproducibility of BioH2 production using batch experiments. Other complementary nutrients included K2HPO4, 3H2O (6 g/L), KH2PO4 (6 g/L), (NH4)2SO4 (12 g/L) as the nitrogen source, CaCl2, 2H2O (0.15 g/L), FeSO4, 7H2O (0.4 g/L), MnSO4, 6H2O (0.15 g/L), NaCl (12 g/L), MgSO4 (1.2 g/L), ZnSO4 (8.2 g/L). pH was controlled at 6, through minute addition of KOH solution (8 M). Cultures were carried out at 35 °C, starting with a 100% CO2 atmosphere and redox potential about −350 mV. The mesophilic consortium promoted only BioH2 production because the methanogenic bacteria had been removed. The broth from the batch experiments was used, first, for testing fouling and cleaning procedures in the membrane module. Then, fed-batch fermentation experiments were carried out, with a cyclic extraction of the liquid phase using the membrane module during anaerobic digestion, so as to validate the design of the AnSMBR. Fermentation experiments involving wheat straw as the substrate (with the same complementary nutrients as with glucose) were also conducted to ensure that mixing conditions in the tank were compatible with the suspension of straw particles, while avoiding their circulation in the membrane module. Substrate addition (either 20 g/L glucose powder or 20 g/L grinded straw particles of about 2 mm size after sieving) was carried out using a funnel; rapid addition coupled to anaerobic and low redox conditions prevented contamination and did not disturb significantly the fermentation.

Glucose and the VFAs profile, both in the tank and in the permeate, were measured after sampling, using an HPLC device (Agilent Technologies 1100 series, USA). The HPLC was fitted with two columns (Rezex ROA 300 7.8 nm, Phenomenex, USA) mounted in a serial assembly inside an oven (50 °C) equipped with a refractometer as detector. The mobile phase was 2 mM sulfuric acid solution prepared in ultra-pure water with a flow rate of 0.7 mL/min (isocratic mode). For the analysis, 2 mL of sample was mixed with 250 mL of Ba(OH)2·8H2O (0.3 M) and 250 mL of ZnSO4·7H2O (5% w/v). The mixture was centrifuged at 10,000g for 5 min. The supernatant was filtered through a 0.45 mm cellulose acetate filter and injected for analysis. The gas phase (mainly BioH2 and CO2) was analyzed using an Agilent 3000 micro-GC gas analyser equipped with two capillary columns and two thermal conductivity detectors. Volumes of gas produced were measured using a volumetric flowmeter (Ritter, GC-1, Germany).

2.2.4. Analysis of fouling and membrane cleaning

The experimental set-up was designed to enable both gas scouring (bubbling of gas through membrane surface) and backwashing with permeate. In this work, CO2 was used as the gas phase, even though the final objective was to use the gas phase produced during the fermentation process, i.e. a mixture of CO2 and H2, for gas scouring. Backwashing was obtained using the reversible flow of the withdrawal peristaltic pump, which prevented the drawback to use a fraction of the filtrate for backwashing. Fouling was studied by following the permeate flow rate over time as a function of the rotation speed of the withdrawal pump. The effectiveness of cleaning was assessed through the evolution of the permeate flow rate \( J \) over several filtration-cleaning cycles.
3. Results and discussion

3.1. Validation of reactor design: hydrodynamics and mixing

3.1.1. Characterization of the stirred tank reactor

Mixing in the batch stirred tank bioreactor was studied, first, as a reference in order to determine the influence of the additional external membrane module on the behavior of the reactor. The evolution of the normalized salt concentration was measured near the impeller after tracer injection close to the free surface. The response curves always exhibited a similar pattern for impeller rotation speed between 20 and 150 rpm, as illustrated in Fig. 2 for 30 rpm. The mixing time \( t_m \) in this case was about 128 s, which was far smaller than the minimum residence time of the fluid in the tank when the recirculation pump was operated at its maximum flow rate, about 12.5 min. The curve showed that the tracer quickly reached the sensor with a circulation time \( t_c \) about 15 s. As expected, \( t_m \) and \( t_c \) decreased when higher impeller speed was applied (Fig. 2). Above 100 rpm, \( t_c \) became so small, so that it could not be measured any more, as it was about one-tenth of the mixing time, which was, roughly, inversely proportional to rotation speed, as the product between rotation speed \( N \) and \( t_m \) was about 70 (Table 1). Complementary experiments with straw particles showed that 100 rpm was the minimum speed to obtain a fully suspended solid phase. Similarly, additional experiments in which the recirculation loop was fed showed that the mixing behavior of the stirred tank remained unaffected by the recirculation. Consequently, the stirred tank appeared to be “instantaneously” mixed in comparison to the fluid in the recirculation loop. Experiments also validated the position of the aspiration point of the loop near the surface in order to prevent the presence of solids in the loop. In addition, Fig. 2 highlighted the typical mixing pattern of turbulent flow, which was in agreement with Reynolds number that reached 1200 for rotation speed at 30 rpm in the stirred tank. The Reynolds number of the stirred tank varied between \( 10^3 \) and \( 2 \cdot 10^4 \) for both impellers, considered separately, which covers the region from the transitional flow to fully-developed turbulent flow conditions. However, all the response curves presented a similar shape, which was in agreement with a gradual transition between the flow regimes usually observed in stirred tanks.

3.1.2. Characterization of the membrane module

As underlined above, the stirred tank appeared to be perfectly mixed in less than 155 s at the lowest rotation speed, 20 rpm, and in less than 30 s at the highest one, 150 rpm. This was far lower than the residence time in the tank in which the minimum value was 12.5 min for the maximum recirculation flow rate. As the volume of the membrane module was one-tenth of that of the tank, the minimum residence time in the module was 65 s, indicating that the mixing time in the tank might also be smaller than the residence time in the recirculation loop, for example at a flow rate at which the straw was fully suspended, i.e., 100 rpm (Table 1). This was the key information that confirmed that rotation speed in the stirred tank and recirculation flow rate could be chosen so that the membrane module did not affect the mixing properties and, therefore, the culture conditions in the AniMBR. As a result, mixing in the recirculation loop could be studied independently from the tank.

The normalized tracer response curves \( E(t) \) were measured by injecting the tracer at the inlet of the recirculation loop. Typical examples obtained without permeate extraction are shown in Fig. 3a. RTD curves were obtained when the tank was not mechanically stirred. However, similar results were also obtained when different rotation speeds were applied, which confirmed experimentally that the tank and the loop could be treated as independent systems. As expected, peak time became shorter with increasing flow rate in Fig. 3a. Meanwhile, when the curves were plotted vs. the normalized time \( \theta \) (i.e., time divided by the residence time in the loop), all the curves presented a similar shape. Without permeate extraction (Fig. 3b), all the typical curves exhibited a peak emerging at \( \theta = 0.5 \), followed by a long tail. A key point was that the experimental curves exhibited a shape rather close to the theoretical RTD observed for an empty pipe in the laminar flow regime (dashed curve in Fig. 3d), which could be expressed as a function of the Heaviside function \( H(\theta) \) as follows:

\[
E(t) = \frac{t_m^2}{2t_c} \int_0^\infty [1 - e^{-\theta}] \, d\theta = \frac{1}{2\theta_c} H(\theta - \frac{1}{2})
\]

This revealed that laminar flow conditions prevailed in the membrane module, indicating that the bundle mainly behaved as an empty tube, despite the presence of the fibers. As a matter of fact, the Reynolds number based on the diameter of the bundle was lower than 1000 at the highest flow rate. This showed that, even without the fibers, laminar flow conditions would prevail. As a result, it seemed that the surface area occupied by the fibers, which implies higher speed and higher viscosity in comparison to an empty pipe, did not change significantly the hydrodynamics. Similarly, as shown also in Fig. 3c, permeate extraction did not affect the trends, even when the recirculation flow rate was low. With water, the withdrawal flow rate was about 15 mL/min in Fig. 3c, which indicated that only 15% of the inflow was extracted when it was 100 mL/min while for higher recirculation flow rate, the permeate was less than 10% of the inlet flow. As a result, this explained why the RTD curves were unaffected by permeate extraction.

As a conclusion, the results not only showed that the properties of the fluid in the stirred tank were unaffected by the presence of the recirculation loop, but also showed that the stirring speed in the tank had no apparent effect on the mixing properties of the loop. In addition, the loop exhibited nearly the same behavior with and without permeate extraction, provided the recirculation flow rate was more than ten times as high as the withdrawal flow rate.

<table>
<thead>
<tr>
<th>Speed (rpm)</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
<th>100</th>
<th>150</th>
</tr>
</thead>
<tbody>
<tr>
<td>( t_m )</td>
<td>155</td>
<td>128</td>
<td>110</td>
<td>90</td>
<td>75</td>
<td>63</td>
<td>50</td>
<td>48</td>
<td>42</td>
<td>27</td>
</tr>
<tr>
<td>( t_c )</td>
<td>19</td>
<td>15</td>
<td>8</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig. 2. Illustration of normalized NaCl concentration-time curve at various impeller rotation speeds in the batch stirred tank.
As a consequent of these results, the AnSMBR could, therefore, be described as a perfectly mixed tank coupled to a recirculation loop which could be assimilated to a tubular laminar flow region and does not affect significantly the hydrodynamics of the liquid phase in the tank.

3.2. Validation of reactor design: filtration

The reactor design was validated using the results obtained from filtration and cleaning measurements of the microfiltration (MF) membrane using water and fermentation broth.

3.2.1. Characterization of membrane properties using water and broth

To quantify the hydraulic performance of the membrane module, different key parameters were investigated. First, a master TMP curve was established from measurements using water as a function of the flow rates imposed simultaneously by the withdrawal pump and the recirculation pump (Fig. 4a). This showed that an increase in TPM was observed when the permeate flow rate was increased, while TMP decreased when the recirculation flow rate in the loop was increased. The increase of TMP vs. $J$ was expected (Eq. (1)); TMP should be proportional to $J$, as illustrated qualitatively in Fig. 4a and quantitatively in Fig. 4b with a circulation flow rate of water at 50 mL/min. The slope for water in Fig. 4b enabled the membrane permeability to water ($L_p$) to be calculated using Eq. (10):

$$J_0 = L_p \cdot \text{TMP}$$  \hspace{1cm} (10)

Since the slope of the adjusted straight line was equal to $2.5 \cdot 10^{-3}$, ($L_p$) could be calculated and was equal to 250 L h$^{-1}$ m$^{-2}$ bar$^{-1}$, which was in agreement with the order of magnitude of ($L_p$) on ultrafiltration membranes. Consequently, $R_m$ was $1.45 \cdot 10^{12}$ m$^{-1}$. It must be mentioned that this value was obtained on a membrane that had already been used for filtration with the digestate and that had only been subjected to physical cleaning procedures. This highlighted the adequate properties of the PVDF membrane in terms of material and pore size, as these ($L_p$) and $R_m$ values were close to literature data for perfectly clean membranes, between 50 and 500 L h$^{-1}$ m$^{-2}$ bar$^{-1}$ (El-Rayess et al., 2012; Puspitasari et al., 2010; Yang et al., 2011).

Conversely, the decrease in TMP when the circulation flow rate of water, as the fluid phase, was increased seemed counter-intuitive. When the circulation flow rate was increased, pressure drop increased in the module; consequently, both the inlet pressure $P_{in}$ and the differential pressure $P_{diff}$ increased at constant permeate flow rate, while the outlet pressure remained nearly constant, close to atmospheric pressure. Meanwhile, an increase in TMP could be expected. As illustrated in Eq. (8); $P_{in}$ and $P_{diff}$ actually had opposite effects on TMP. $P_{in} - P_{out}$ was equal
to pressure drop which was proportional to \( \nu^2/2 \) in the bundle. While \( P_{\text{in}} \) was increased by the same pressure drop, it was also decreased by the kinetic term \( \nu^2/2 \) in the inlet tube where the fluid velocity was far higher than in the bundle. This effect seemed to be predominant and might explain why a lower TMP was required at constant \( J \) when the recirculation flow rate was increased. Consequently, the TMP value required to achieve the desired permeate flow rate could be reduced by increasing the circulation flow rate, indicating that increasing the circulation flow rate decreased the apparent membrane resistance \( R_m \) when water was used as the fluid phase in the AnSMBR.

Based on the result, experiments were carried out to determine the membrane properties using the fermentation broth. Two values of the recirculation flow rate were studied: 50 mL/min and 100 mL/min. As usually observed in tangential filtration due to fouling, the curves initially presented an increase in \( J \) as a function of TMP, followed by a plateau value above a TMP of 105 Pa which corresponded to the maximum permeation flow rate that could be achieved due to membrane fouling (Fig. 4b). As a result, any further increase in TMP did not increase \( J \), which was due to the accumulation of foulants on the surface and/or in the pores of the membrane. This figure also showed that the maximum \( J \) values were dependent on the recirculation flow rate, 2.7 L h\(^{-1}\) m\(^{-2}\) for 100 mL/min and about 4 L h\(^{-1}\) m\(^{-2}\) for 50 mL/min. In addition, for the same \( J \) value, TMP values of, at least, one order of magnitude higher were required with the fermentation broth than with distilled water. These results could be due to the higher viscosity of the broth (about twice as high as water) and from fouling that resulted in an increase of \( R_m \) in the linear region, roughly by a factor 5. It was also important to point out that the maximum \( J \) value decreased when the recirculation flow rate was decreased, which highlighted that higher shear rate in the module did not favor membrane cleaning; conversely, higher fluid velocity accelerated the accumulation of foulants.

As a conclusion, the hydraulic properties were determined both with distilled water and the digestate. The results demonstrated the feasibility of the filtration, provided irreversible fouling did not occur and the membrane module could be operated during anaerobic digestion. A key point was that TMP values required for permeate recovery remained low, about 10^4 Pa, which confirmed the opportunity to use an externally-submerged membrane reactor: contrary to side-stream membrane bioreactors, high TMP and shear were not needed for filtration.

3.2.2. Analysis of cleaning procedures

In order to evaluate the cleaning methods used for membrane after fouling, gas scouring was studied, first. CO\(_2\) was injected for 5 minutes after a filtration step of 15 minutes using the digestate; then, the same cycle was repeated. In a second experiment, the same procedure was applied, but using backwashing instead of CO\(_2\) injection. The results showed that backwashing was slightly more efficient for recovering the initial density flux (4.45 L h\(^{-1}\) m\(^{-2}\)), since the permeate flux after backwashing always remained above 4.4 L h\(^{-1}\) m\(^{-2}\), while it was about 4.3 L h\(^{-1}\) m\(^{-2}\) after three CO\(_2\) injections (Fig. 5a). This indicated that fouling was probably reversible, but that cleaning remained incomplete using only gas scouring. The lower effectiveness of gas scouring was demonstrated by an additional experiment in which a first filtration step of 145 min was applied, and then stopped for 70 min, with the aim to simulate a longer filtration cycle. Physical cleaning using CO\(_2\) injection was carried out just before starting a new filtration cycle. In Fig. 5b, the evolution of the permeate flux \( J \) vs. time using the digestate showed that the initial permeate flux in phase A, 4.5 L h\(^{-1}\) m\(^{-2}\), was almost recovered in phase C (4.2 L h\(^{-1}\) m\(^{-2}\)) after CO\(_2\) gas injection, but not totally, as expected. This, however, confirmed that fouling was mainly reversible, but the permeate flux declined rapidly in phase C in Fig. 5b, which highlighted that the kinetics of fouling became more rapid when the membrane was fouled the first time. A similar, but slower trend was observed with backwashing, which highlighted that cleaning should be applied frequently. In this case, using permeate for backwashing was not acceptable from a techno-economic point of view. A more attractive solution, therefore, involved using the substrate instead of the permeate in a “cyclic” process.

As a result, a methodology of membrane cleaning was defined which included flushing with CO\(_2\) for 3 min every 15 min during permeate extraction and backwashing with fresh culture medium when cyclic extraction was stopped. The consequence was that many filtration/physical cleaning cycles could be operated before there was a need for chemical methods. This procedure could also be applied in the presence of straw, provided a strainer was placed at the inlet and the outlet of the recirculation loop to avoid clogging the membrane, and that filtration was carried out with an inlet point close to the top of the reactor at a reduced rotation speed. These results also confirmed that PVDF membranes could be used in this process, which was in accordance with literature data (Nguyen et al., 2011) for low surface energy of PVDF; a
property that would promote the detachment of deposits and, thus, cleaning and unclogging of the membrane.

3.2.3. Analysis of fouling mechanisms

In the literature, many studies have tried to identify the mechanisms of membrane fouling in anaerobic membrane bioreactors (Charfi et al., 2012; Choo and Lee, 1998; He et al., 2005; Wang and Tarabara, 2008) in order to prevent particle fouling on the membrane surface or blocking in the membrane pores that caused a rapid decline in filtration flux, as well as a major difficulty for keeping a high performance. Different models were commonly used to describe the fouling mechanisms and their relationship with the biotic parameters (microorganisms can be attached, grow on the membrane surface and produce enough of extracellular polymeric substances to facilitate the development of a biofilm on the surface of the membrane) and the abiotic parameters (deposition of material within the pores of the membrane and/or on its surface). A first class of models was aimed at defining the relationship between the biological materials in the fluid and the effectiveness of separation techniques based on MF and ultrafiltration (UF). However, an alternative approach to identify the mechanisms of colloidal fouling based on Hermia’s analysis (Hermia, 1982) could be used to define a general equation for different fouling mechanisms causing the flux decline as illustrated in Eq. (11).

$$\frac{d^2t}{dV} = k\left(\frac{dt}{dV}\right)^n$$

(11)

where $k$ is constant and $n$ is the blocking index equal to 2, 3/2, 1 or 0 for complete blocking, intermediate blocking, standard blocking, and cake formation, respectively. The filtration time and the filtrate volume $V$ recovered at time $t$ per membrane unit area $A$ could then be related to the permeate flux (Eq. (12)).

$$J = \frac{1}{A} \frac{dV}{dt}$$

(12)

Combining Eqs. (12) and (11) gives:

$$\frac{dJ}{dt} = -kj_0f^{2-n}$$

(13)

This method is advantageous (Wang and Tarabara, 2008), as it provides the opportunity to use a single plot to identify all blocking mechanisms, and also determine how the data can be interpreted in terms of blocking laws. A significant disadvantage of the direct application of Eq. (13) is the high sensitivity of the value of $n$ with respect to the noise in the flux data, and therefore, its influence on the choice of the fouling mechanism. The mathematical form of the four conventional fouling mechanisms are summarized in Eqs. (14)–(17) and expressed as a function of $J_0$ (the initial flow rate) and $k$, a kinetic parameter.

$$J = J_0e^{-k_0f}$$ (Complete blocking, $n = 2$)

(14)

$$J = \frac{J_0}{k_0f + 1}$$ (Intermediate blocking, $n = 1$)

(15)

$$J = \frac{4J_0}{(k_0f + 1)^{3/2}}$$ (Standard blocking, $n = 3/2$)

(16)

$$J = \frac{J_0}{\left(2k_0f + 1\right)^{1/2}}$$ (Cake formation, $n = 0$)

(17)
These models have been related to experimental data (Fig. 5c and d) which corresponded to a clean membrane and to a membrane just after gas scouring in Fig. 5b, respectively. Experimental data was fitted by optimizing simultaneously $J_0$ and $k$ using a method of least squares based on the Levenberg–Marquardt algorithm that minimizes the difference between experimental $J$ and predicted $J_{pred}$ permeate flux values. The adjusted parameters of the predicted profiles in Fig. 5c and d were presented in Table 2 together with the variance of the error ($\sigma^2$) for each model. The analysis of the results obtained from the experiments on a clean membrane (Fig. 5c) showed that the cake formation model ($n=0$) with an optimized kinetic parameter $k_d = 2.1 \times 10^{-2} \text{ s m}^{-2}$ exhibited a better fit of the experimental data in comparison to the other models. Conversely, complete blocking presented the highest error in Table 2. The same models were used to predict fouling after CO$_2$ gas scouring (Fig. 5d). Although fouling was more rapid in this case, the plot displayed the same trends and fouling corresponded to the cake formation model. As a conclusion, these results clearly confirmed the reversibility of fouling, suggested in the previous section, and justified from a theoretical point of view that chemical cleaning was not often required and that a combination of gas scouring and backwashing constituted an adequate cleaning procedure.

### 3.3. Validation of reactor design: coupling filtration and fermentation

The validation of the AnSMBR design and operating conditions was carried out during anaerobic digestion experiments. Fig. 6 summarizes the evolution of the biogas volume and composition, pH, and VFAs concentrations in the liquid phase over time. This figure also showed that pH control was effective and that the biogas contained only CO$_2$ and BioH$_2$. Experiments were initially carried out as in a conventional batch anaerobic bioreactor, with periodic addition of glucose and withdrawal of digestate, mainly for chemical analysis. After a latent period, gas production started, mainly with CO$_2$. Hydrogen content in the biogas was significant after 400 h. For VFAs analysis, formate, acetate and butyrate were shown to be the main VFA compounds, while lactate could also be observed. On the contrary, propionate and succinate, isobutyrate, valerate and ethanol played a secondary role. Butyrate was always the most abundant VFA when BioH$_2$ production was efficient, which indicated that the reaction followed mainly the butyrate fermentation pathway to BioH$_2$. This was confirmed by the maximum BioH$_2$ yield of 1.1 mol H$_2$/mol glucose with a maximum production rate between 4 and 5 L H$_2$/day L for 20 g/L substrate with a maximum of 58% BioH$_2$ in the biogas during the experiments, the maximum being 42% in Fig. 6. However, a rapid accumulation of lactate emerged when the BioH$_2$ productivity decreased, which occurred after 1000 and 1500 h, respectively. A comparison with literature data (Table 3) shows that the AnSMBR of this study outperforms batch and fed-batch conventional bioreactors in terms of hydrogen production rate, which was expected due to the inhibition by VFAs that emerges in conventional reactors, as mentioned by Kargi and Pamukoglu (2009). The comparison of the gas phase composition also agrees with literature data, both in conventional and membrane bioreactors. Conversely, a comparison of the VFA profiles cannot be drawn, as this differs too strongly in the data of Table 3, which confirms that it depends primarily on the combined effects of the substrate and the microorganisms, rather than on the bioreactor configuration. Finally, Table 3 also shows that without optimization and in unfavourable fed-batch conditions, the BioH$_2$ production rate is close to the values reported in the literature on other external submerged membrane bioreactors, which confirms that the technology developed in this work is promising.

In this experiment, the reactor was operated as an AnSMBR after 1400 h while permeate withdrawal using the process defined in the previous sections was applied twice. The first filtration cycle
Table 3  
Comparison of hydrogen production by anaerobic cultures using different kinds of conventional bioreactors (batch, fed-batch, CSTR) and submerged membrane bioreactors.

<table>
<thead>
<tr>
<th>Reactor configuration</th>
<th>Membrane module characteristics</th>
<th>Micro-organism (substrate)</th>
<th>Feedstock content</th>
<th>T (°C)</th>
<th>HRT (h)</th>
<th>Permeate flux (L/m² h)</th>
<th>Effluent composition</th>
<th>H₂ production rate (L/d g substrate)</th>
<th>H₂ yield (mol H₂/mol hexose)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Submerged external hollow fiber membrane with 5-L fed-batch tank</td>
<td>Pore size: 0.2 μm Effective surface area: 0.155 m²</td>
<td>Mixed mesophilic microflora</td>
<td>Glucose or straw</td>
<td>20 g/L</td>
<td>35</td>
<td>3</td>
<td>(i) H₂, H₂O, H₂O₂, H₂S, CO₂</td>
<td>0.2–0.25 L/d g substrate</td>
<td>0.2</td>
<td>This study</td>
</tr>
<tr>
<td>Submerged flat sheet module with 5-L CSTR</td>
<td>Pore size: 0.45 μm Effective surface area: 0.1 m² Size: 240 x 340 x 10 mm Surface area: 0.025 m²</td>
<td>Seed sludge</td>
<td>Mixed microflora</td>
<td>52.7 g COD/L</td>
<td>55</td>
<td>10.5</td>
<td>0.8–1.0 L/m² d</td>
<td>(i) H₂, H₂O, H₂O₂, H₂S, CO₂</td>
<td>0.2 L/d g COD</td>
<td>2.2</td>
</tr>
<tr>
<td>Submerged hollow fiber module with 5-L CSTR</td>
<td>Pore size: 0.04 μm Effective surface area: 0.047 m²</td>
<td>Mixed microflora</td>
<td>Tofu processing waste</td>
<td>43.4 g COD/L</td>
<td>60</td>
<td>8</td>
<td>4.32 L/m² h</td>
<td>(i) H₂, H₂O, H₂O₂, H₂S, CO₂</td>
<td>0.3 L/d g COD</td>
<td>1.87</td>
</tr>
<tr>
<td>Submerged plate frame membrane with 5-L CSTR</td>
<td>Pore size: 0.45 μm Effective filtration area: 0.1 m²</td>
<td>Mixed mesophilic microflora</td>
<td>Glucose</td>
<td>16 g/L</td>
<td>35</td>
<td>9</td>
<td>4–5 L/m² h</td>
<td>(i) H₂, H₂O, H₂O₂, H₂S, CO₂</td>
<td>0.36 L/d g substrate</td>
<td>1.19</td>
</tr>
<tr>
<td>Reactor without membrane module</td>
<td>1.9 L – CSTR</td>
<td>Mixed microflora</td>
<td>Sucrose</td>
<td>11.1 g/L</td>
<td>35</td>
<td>4</td>
<td>(i) EtOH, VFA (C₂–C₆) (g)</td>
<td>1.3 L/d g substrate</td>
<td>3.31</td>
<td>Han et al. (2014)</td>
</tr>
<tr>
<td></td>
<td>2 L – fed batch</td>
<td>Anaerobic sludge</td>
<td>Wheat starch</td>
<td>20 g/L</td>
<td>35</td>
<td>–</td>
<td>(i) H₂, H₂O, H₂O₂, H₂S, CO₂</td>
<td>0.01 L/d g</td>
<td>3.1</td>
<td>Kargi and Pamukoglu (2009)</td>
</tr>
<tr>
<td></td>
<td>150 mL – batch</td>
<td>Cow dung compost</td>
<td>Wheat straw</td>
<td>5–53 g/L</td>
<td>36</td>
<td>–</td>
<td>(i) H₂, H₂O, H₂O₂, H₂S, CO₂</td>
<td>0.25 L/d g dried straw</td>
<td>68.1 mL H₂/g dried straw</td>
<td>Fan et al. (2006)</td>
</tr>
</tbody>
</table>

EtOH, ethanol; H₂C, acetic acid; HBu, butyric acid; HPr, propionic acid; HLa, lactic acid; HCa, caproic acid.
was operated at about 1400 h. In this period, the composition of the biogas still exhibited a high BioH2 content, but the volume of biogas produced was declining and the lactic acid concentration was increasing. The first consequence of membrane filtration was that the concentration of VFA decreased because the VFA profile of the permeate was similar to that of the broth, which was partially replaced by fresh substrate. This was followed by a peak of BioH2 content in the biogas and the rapid consumption of lactate anions. These trends were similar to those observed after a conventional addition of fresh substrate and withdrawal of digestate, for example, after the addition of glucose around 600 h. This result highlighted that the AnSMBR behaved as a conventional fed-batch anaerobic bioreactor in this case, although it ensured that biomass was maintained in the tank.

The second filtration cycle was operated around 1600 h. In this case, the amount of biogas produced had decreased and this contained almost exclusively CO2. The lactic acid concentration had increased sharply with the total amount of VFAs (Fig. 6). Filtration was used to remove and replace about one fourth of the reactor volume and the effect of dilution appeared clearly in the figure. Contrary to the previous cycle, bioH2 production did not restart immediately due to the inhibition by the high VFAs content. However, the increase of lactic acid and VFAs contents was delayed and by maintaining biomass in the bioreactor, a change of metabolic pathway was achieved, so that lactate could be consumed and used as a substrate for bioH2 production, with butyrate as the main by-product. As a result, bioH2 production restarted, which highlighted the versatility of the AnSMBR for BioH2 production. By comparing these results with those of the previous filtration cycle, one can conclude that bioH2 could be effectively produced by dark fermentation using the externally-submerged AnSMBR which seemed to be more versatile than a conventional fed-batch bioreactor. Besides, lactic acid concentration could be a good indicator of the time when a filtration cycle had to be started so as to optimize BioH2 productivity with the microbial consortium of this work.

4. Conclusions

In this work, the applicability of an original AnSMBR with an externally-submerged MF module for BioH2 production was assessed. Its geometry and operating conditions were chosen, so that the hydrodynamic properties in the fermenter were not significantly modified. Low recirculation flow rate and TMP values maximized permeate flux. The fouling mechanism, due to cake layer formation, was reversible. A cleaning procedure based on physical methods was defined. During fermentation, BioH2 production was shown to restart after removing VFAs with the permeate, so as to enhance simultaneously BioH2 production and the recovery of VFAs as platform molecules.

Acknowledgements

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References


Paper IV
Enhanced fermentative hydrogen and methane productions from inhibitory-fruit flavour medium with membrane-encapsulated cells

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Abstract: This study focused on the possibility of improving fermentative hydrogen and methane productions from inhibitory-fruit flavour medium using PVDF membrane encapsulated cells. Hexanal, myrcene and octanol that are naturally produced in fruits such as apple, grape, mango, orange, strawberry and plum, were investigated. Batch and semi-continuous fermentation processes at 55°C were carried out. Presence of 5g/L of myrcene, octanol and hexanal resulted in no methane formation by fermenting bacteria, while encapsulated cells in the membranes resulted in successful fermentation with 182, 111 and 150 mL/gCOD of methane respectively. These inhibitors were not so serious inhibitors on hydrogen producing bacteria. With free cells in the presence of 5g/L (final concentration) of hexanal, myrcene and octanol flavour media, average daily yields of 68, 133, 88 mL/gCOD of hydrogen were obtained. However, cell encapsulation further improved these hydrogen yields to 189, 179 and 198 mL/gCOD. The results from this study indicate that the yields of fermentative hydrogen and methane productions from inhibitory medium could be improved using encapsulated cells.

Keywords: Encapsulated bacteria; fruit flavours; membrane; hydrogen; methane; inhibition;

1. Introduction

The increasing energy demand and depleting fossil fuel reserves coupled with the global warming have stimulated a rapid growth in developing alternative energy sources that are sustainable, renewable and environmentally friendly. Energy carriers such as hydrogen and methane have been suggested as good substitutes for fossil fuels. Accordingly, various production pathways have been explored for hydrogen and methane productions including water electrolysis (power-to-gas), thermo-chemical processing, photo-chemical processing, photo-catalytic processing and photo-electrochemical processing [1], as well as biological methods including photo-fermentation [2, 3] and anaerobic
fermentation [4, 5]. Among the diverse production pathways, anaerobic fermentation via dark fermentation for hydrogen and methane production seems to be a promising option because of its low energy requirement, renewable and non-polluting qualities as well as being able to utilize organic residuals as carbon and energy sources. However, dark fermentation process is characterized by low hydrogen yield, which consequently affects methane production. This phenomenon has been attributed to factors; such as, substrate and product inhibitions, environmental and operating parameters as well as the tendency of the fermentation process to result in biomass production. The challenge with most of the hydrogen production pathways during dark fermentation is the problem of underutilization of substrate with only one-third of the substrate having potential to be converted to hydrogen while the remaining two-thirds forming organic acids and reduced compounds [6]. Moreover, some fermentative feedstocks often contain inhibitory compounds that tend to inhibit the feedstock degradability by anaerobic microorganisms.

Fruit waste has been widely utilized as feedstock during anaerobic fermentation due to its degradability and availability from the huge turnout of the wastes from human consumption and processing. However, the yields from the fruit fermentation processes are often low, which have been attributed, among other factors, to the flavour compounds inherently present in the fruits. Flavours are complex mixture of various organic compounds, including aldehydes, terpenoids, alcohols, ketones, lactones and esters; with antimicrobial activity against a wide range of bacteria, yeasts and molds [7-13]. The antimicrobial natures of fruit flavours are evident in their various applications including as food preservatives [14, 15] and alternative medicines [11, 16-21]. Previous research activities on the effect of fruit flavour also confirmed the toxicity of fruit flavour compounds [22-28]. The toxicity of flavour compounds against bacteria probably comes from the hydrophobic quality of flavour compounds that allows them to penetrate and bind with phospholipids of bacterial cell membrane as well as other cell organelles; thereby making them water permeable [29-34]. The cell integrity is lost if the concentration of the accumulated flavour compound exceeds a tolerable limit. Although the adaptive potential of bacterial against flavour compounds had been reported [35-37]; the hold-up time of the bacteria depends on the concentration of the flavour compounds and the exposure period of the bacteria to the flavour compounds, as the bacterial resistance cannot by itself be sustained for a long period of time [38]. Considering the hydrophobic nature of flavour compounds, a hydrophilic barrier can be created around the bacterial cells during fermentation process to prevent direct bacterial contact with the flavour compounds as well as to reduce bacteria exposure time to the flavour compounds. The technique of employing hydrophilic poly (vinylidene) fluoride barrier around anaerobic microorganism in a medium containing flavour compounds in order to reduce the antimicrobial effects of the flavour compounds during fermentation process; formed the basis of this study. Polyvinylidene fluoride
(PVDF) membrane is a semi-crystalline polymeric membrane consisting of both crystalline and amorphous phases with crystalline part responsible for its excellent thermal stability while the amorphous part is responsible for the flexibility of the membrane [39]. The membrane is chemically stable to a wide range of chemical compounds including inorganic acids, oxidants, halogens, aromatic, aliphatic and chlorinated solvents. However, the intrinsic hydrophobic nature of PVDF makes it to be prone to organic fouling and low wettability with high resistance to water flow. Consequently, several membrane modification techniques including blending, surface coating, irradiation grafting and plasma modification, are used to incorporate hydrophilicity into hydrophobic PVDF membranes to enhance their performances [40].

In several studies involving fermentation processes, cell encapsulation has been proved to be an effective technique for cell stability, high biomass concentration, and enhanced fermentative hydrogen and methane production [41, 42]. Cell encapsulation is vital for cell survival and increased tolerance to toxic medium such as industrial wastewaters which contain toxic compounds including phenols, benzenes and halogenated aliphatics, among others [43]. Application of membrane in cell encapsulation has the potential of enhancing the total energy value of fermentation process which is among the main objectives of producing hydrogen and methane from the process [44, 45]. Meanwhile, there has been no previous report on the effects of using hydrophilic PVDF membranes for cell encapsulation on fermentative hydrogen from media containing hexanal, myrcene and octanol flavours though there were some reports on protective effects of membrane encapsulation on fermentative methane production from limonene contained media [22, 23]. The objective of this study was therefore to investigate the potential of using membrane-encapsulated cells to improve hydrogen and methane productions from media containing hexanal, myrcene and octanol during batch and semi-continuous fermentation processes. Enclosing fermentative microorganisms inside a hydrophilic membrane during fermentation process could reduce the bacterial exposure to the antimicrobial effects of fruit flavours, minimize the penetration of the fruit flavours, and thereby improve the yields of hydrogen and methane productions. Besides, information from further studies on the direct correlation between the concentration of flavour compounds and their corresponding antimicrobial effects could be applied in the health sector to combat the menace of malaria and dengue fever epidemic in tropical regions of the world.

2. Results and Discussion

Effective fermentative hydrogen and methane productions from fruit wastes during anaerobic digestion is often limited by the inherent fruit flavours, which act as fruit defense mechanism against microbial invasion. Hexanal, myrcene and octanol are fruit flavours that are naturally produced in
fruits such as apple, grape, mango, orange, strawberry and plum, which are essential parts of human diet. Consequently, large quantities of the slowly digestible fruit wastes are generated from their production, processing and consumption, thereby, constituting environmental pollution and health hazards to people. It would therefore be necessary to devise a technique for improving degradation of the fruit wastes and thereby increase hydrogen and methane production potential of the fruit wastes.

2.1. Effects of fruit flavours on methane production during batch fermentation process

The batch fermentation process for methane production of encapsulated and free cells from medium containing 0.5% w/v (5g/L) concentration of fruit flavours including hexanal, myrcene and octanol was carried out at 55°C for 11 days with manual mixing of the reactors twice a day. The results indicated no methane production from free cells directly in contact with all the fruit flavours at concentration of 0.5% w/v (Figure 1). On the contrary, cumulative methane yield of 182 ± 15, 111 ± 81 and 150 ± 24 mL/g COD were obtained from the encapsulated bacteria immersed in medium with myrcene, octanol and hexanal, respectively. The lowest methane yield was from octanol indicating that the inhibitory effect of octanol seemed to be stronger than that of hexanal and myrcene. Although this could be related to the solubility, size and chemical structure of the flavour compounds, which influence the flavour permeability; the mechanisms of inhibition during fermentation process are sometimes difficult to understand partly due to the various adaptive abilities of fermentative microorganisms.

Comparison of the accumulative methane production from encapsulated cells without flavour compounds (membrane control) and encapsulated cells with flavour compounds (membrane hexanal, membrane myrcene and membrane octanol); the results indicate that membrane protective effect against the flavour compounds could be given approximately as 60%. This implied that the membrane could protect more than half of the hydrogen production from being affected by the inhibitory effect of the flavour compounds. The results, therefore suggest that it is possible to improve biomethane production from medium with high concentration of fruit flavours using the technique of membrane-cell encapsulation technique.
2.2. Effects of fruit flavours on hydrogen production during semi-semi-continuous fermentation process

The inhibitory effects of three flavour compounds (hexanal, myrcene and octanol) on hydrogen production potentials of fermentative microorganisms were investigated during the semi-semi-continuous fermentation operated for 18 days at 55°C. The concentrations of the fruit flavour compounds were increased at interval of 5 days starting with 0.05g/L through 0.5 and finally 5g/L. After 15 days of fermentation process, feed supply and effluent withdrawal from the reactor were stopped in order to observe for three days how the system adjust to the previous loading of the inhibitory flavour medium. The average daily yields (Figure 2) and accumulated volumes of hydrogen (Figure 3) obtained from the fermentation process clearly showed the protective effects of employing encapsulated cells during anaerobic fermentation process. The average hydrogen productions from the encapsulated cells were higher than the productions from the free cells. Meanwhile, none of the flavour compounds used during the semi-semi-continuous fermentation process could be said to have the most inhibitory effects, as the degree of the inhibitory effects varied among the fruit flavour. For example, in batch fermentation process, octanol was found to have the most inhibitory effect while during the semi-semi-continuous process; both hexanal and myrcene were observed to have greater inhibitory effects than octanol. When the flavour compounds were exposed to the free cells, hexanal showed the
most inhibitory effect as indicated by the low average daily hydrogen yield of 68mL/gCOD, while among the encapsulated cells, myrcene showed the lowest average daily hydrogen yield of 179 mL/gCOD. The variation could be due to the complexity of antimicrobial mechanisms of flavour compounds coupled with the adaptive potential of the fermentative microorganisms.

At flavour concentration of 0.05 g/L, the inhibitory effects of the flavour compounds were not significant as the average hydrogen yields from the encapsulated and free cells were almost the same (Table 1). However, the inhibitory effects of the flavour compounds, especially among the free cells, were considerably significant when the concentration was increased to 0.5 g/L. The percentage reduction in average daily hydrogen yields from hexanal, myrcene and octanol were 77, 45 and 35% respectively (Table 2). The increase in the flavour concentration did not have much effect on encapsulated cells except when myrcene was used as the flavour which resulted in the reduction of the average daily hydrogen yield by 23%. When the flavour concentration was increased from 0.5 to 5 g/L, most of the cells including both encapsulated and free cells experienced improved activity regarding the increase in average daily hydrogen yields (Table 2). The reason might probably be due to the adaptive ability of the anaerobic microorganisms to the inhibitory medium as well as the potential of the microorganisms to degrade some of the flavour compounds. Meanwhile, when the supply of nutrient and withdrawal of effluent stopped, the average daily hydrogen production from the free cells dropped significantly except for free cells in hexanal medium which experienced yield increase. However, the average daily yields from the encapsulated cells did not experience much change after three days of ending the feed supply and effluent withdrawal.

Throughout the experiment, it might be worthwhile to state that the increase in the concentration of flavour compound in the fermentation medium did not significantly affect the average daily hydrogen yield from the encapsulated cells as the hydrogen production was nearly constant. It was also observed that, though free cells of hydrogen producing bacteria were able to produce reasonable amounts of hydrogen regardless of the flavour inhibitors, the amount of hydrogen produced was less compared with encapsulated cells. Based on the results, it could therefore be concluded that fermentative hydrogen and methane productions from inhibitory fruit flavour medium could be improved using the technique of membrane-cell encapsulation.
Figure 2. Semi-continuous fermentation process for daily hydrogen yield from substrate with fruit flavours in comparison to control experiment

Figure 3. Semi-continuous fermentation process for cumulative hydrogen volume from substrate with flavours in comparison to control experiment
Table 1. Average hydrogen yields at the three flavour concentrations

<table>
<thead>
<tr>
<th>Flavour compound</th>
<th>Average hydrogen yield (ml / gCOD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Free cells</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Hexanal</td>
<td>179.6  42.3  7.55  27.23</td>
</tr>
<tr>
<td>Myrcene</td>
<td>183.9  101.2  138.7  91.53</td>
</tr>
<tr>
<td>Octanol</td>
<td>126.2  81.5  100.9  14.4</td>
</tr>
<tr>
<td></td>
<td>Membrane</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Hexanal</td>
<td>176.5  193.2  196.4  192.7</td>
</tr>
<tr>
<td>Myrcene</td>
<td>197.9  152.5  187.9  175.6</td>
</tr>
<tr>
<td>Octanol</td>
<td>183.8  202.7  200.9  210.8</td>
</tr>
</tbody>
</table>

Table 2. Effects of change in flavour concentration on average hydrogen yield

<table>
<thead>
<tr>
<th>Flavour compound</th>
<th>Change in average hydrogen yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Increase of flavour concentration from 0.05 to 0.5g/L</td>
</tr>
<tr>
<td></td>
<td>Free cells</td>
</tr>
<tr>
<td>Hexanal</td>
<td>(-)  77</td>
</tr>
<tr>
<td>Myrcene</td>
<td>(-)  45</td>
</tr>
<tr>
<td>Octanol</td>
<td>(-)  35</td>
</tr>
<tr>
<td></td>
<td>Membrane</td>
</tr>
<tr>
<td>Hexanal</td>
<td>(+)  9</td>
</tr>
<tr>
<td>Myrcene</td>
<td>(-)  23</td>
</tr>
<tr>
<td>Octanol</td>
<td>(+)  9</td>
</tr>
</tbody>
</table>

2.3. Digestate pH values during the semi-semi-semi-continuous fermentation process

The pH plays an important role during fermentative hydrogen production as it affects the metabolic pathways in hydrogen production as well as limiting hydrogen consumption by hydrogenotrophic methanogens [46-49]. Hydrogen and methane productions during fermentation process require different pH values of 5.5 – 6.5 and 6.5 – 8.2, respectively. In this study, batch fermentation was used for methane production with the pH range of 6.8 - 7.2 while semi-continuous fermentation was used for hydrogen production with the range of initial pH range values of 5.2 to 5.9 [50]. During the semi-continuous fermentation, gradual reduction in the pH values of the fermenting media below 5.0 was observed at the beginning of the experiment, which could be attributed to the production of organic acids associated with the hydrogen formation during fermentation process [51]. The pH profile (Figure
4) indicated that the pH values for all the reactors did not vary significantly but were nearly constant throughout the experiment with an average value of 4.40 ± 0.04. This could possibly imply that there the daily effluent withdrawal from the reactor system could have prevented the accumulation of organic acids that could have led to drastic reduction in the pH value of the fermentation media. Moreover, it could also be probably due to the adaptation potential of fermentative microorganisms to the inhibitory fermentative media.

![Figure 4. Daily digestate pH values during the semi-continuous fermentation process](image)

2.4. Implication of membrane applications for cell encapsulation

Encapsulation techniques could have some limitations including the inefficient diffusion of nutrients to the microorganisms in the membrane as well as membrane fouling. It is often necessary to determine the water permeability of the membranes to be employed during fermentation process. The permeability results can also be useful in the determination of loss in membrane efficiency during after the fermentation process. In this study, an average value of 0.048 mL/min of pure water permeability was obtained for the PVDF membrane. This indicated that in a time period of one minute, the membrane could allow an approximate value of 0.048mL of distilled water to pass through it.. Membrane permeability is influenced by various factors including the membrane materials (pore size, hydrophobicity/hydrophilicity, free volume and filler particles) and solubility of the permeates [52-54].

The resistance to fluid flow through the membrane during filtration process is often due to membrane fouling, which is a term that is used to describe the loss of membrane throughput.
Generally, fouling occurs when particulate, colloidal or soluble materials are deposited inside membrane pores or surface. Membrane fouling is a major barrier to membrane application in fermentation processes as it is associated with flux or permeate flow reduction, low permeate quality and increased operational costs due to increased energy consumption. Membrane fouling is influenced by factors such as sludge characteristics, operational parameters and membrane qualities. Although, membrane fouling cannot be entirely avoided during membrane filtration process, the frequency of its occurrence could be reduced through physical cleaning such as relaxation and backwashing or chemical cleaning. Chemical cleaning of membrane is more effective in removing membrane fouling than physical cleaning, but frequent use of chemical cleaning can damage the membrane and shorten the membrane life-time. Previously, membrane cost was part of the barrier to the application of membrane technology, but extensive research on membrane improvement has resulted in cheap and affordable membrane in recent times. However, operating costs associated with membrane fouling abatement is still the main barrier to the application of membrane technology.

3. Experimental Section

3.1. Materials

3.1.1. Anaerobic sludge,

Anaerobic sludge used for the digestion was obtained from a 3000-m³ municipal solid waste thermophilic (55°C) digester at Borås Energy and Environment AB (Borås, Sweden). Prior to the start of the experiment, the sludge was incubated at 55°C for 3 days to allow the bacteria to be activated and digest the left-over carbon source. After the incubation, the sludge was thoroughly mixed and filtered with a screen of 1mm pore size to remove particles bigger than the pore size of the screen. For encapsulation purposes, the sludge was centrifuged at 14,000 x g for 10 min to separate the solid inoculum from the supernatant [22].

3.1.2. Membrane-encapsulation procedure

The synthetic encapsulating sachets for holding the bacteria were made of flat sheet hydrophilic polyvinylidene fluoride (PVDF) membranes (Durapore®, Thermo Fisher Scientific Inc., Sweden) with pore size, thickness and diameter of 0.1µm, 125 µm and 90 mm, respectively. The encapsulating sachets were prepared as described in previous report [22]. Each membrane was cut and folded into rectangular dimensions with width and length of 3 and 6cm respectively. The membranes were heat-sealed (HPL 450 AS, Hawo, Germany) with heating and cooling times of 5.5 s while leaving one side
left open for cell insertion after which the opening was sealed to form a membrane capsule. The sealing and cooling times for the membranes were 5.0 and 5.5 s, respectively. The fermentation process was carried out immediately the membrane encapsulation procedure was completed.

3.1.3. Nutrient medium and flavour compounds

The nutrient medium used during the fermentation process was a synthetic medium consisting of 20 g/L glucose (supplied by Merck), 20 g/L yeast extract (supplied by Merck) and 20 g/L nutrient broth (supplied by Sigma-Aldrich). The nutrient broth contained D (+)-glucose (1 g/L), peptone (15 g/L), sodium chloride (6 g/L) and yeast extract (3 g/L). The medium was sterilized by filtration through a 0.2 μm membrane before it was used for the fermentation process. The flavour compounds (supplied by Sigma-Aldrich), consisting of hexanal, myrcene and octanol, were used as inhibitors during the fermentation process.

3.2. Experimental set-up and procedures

The experiment was separated into two parts. The first part was batch fermentation for methane production while the second part was the semi-continuous fermentation process for hydrogen production. Both fermentation processes were carried out under thermophilic condition (55°C) and the same flavour compounds including hexanal, myrcene and octanol were used. The seed inoculum was incubated at 55°C for three days before it was employed for both batch and semi-continuous fermentation process digestion process [55].

3.2.1. Batch fermentation process for methane production

The reactors used for the batch fermentation of methane were 118 mL serum glass bottles with active volume of 53.5 mL and headspace of 65.5 mL. Each reactor was filled with 1.0 mL of filtered nutrient medium containing 20 g/L each of nutrient broth, yeast extract and D (+)-glucose. Three fruit flavour compounds, including hexanal, myrcene and octanol, were used with each having 0.5% w/v concentration prepared by dissolving 5 g of the inhibitor in 1 liter of distilled water. Fifty milliliters of the anaerobic sludge was measured and centrifuged from which 3 g pellet was used for the encapsulation. For each flavour investigated, the batch fermentation reactors were grouped into two categories, including, encapsulated and free cells. For encapsulated cells with inhibitor; the reactor bottle contained 3 g of the inoculum pellet encapsulated in the membrane, 47 ml of distilled water, 2.5 mL of the flavour compound (0.5% w/v) and 1 mL of nutrient medium. Regarding the free cells with inhibitor; the reactor bottle contained 50 mL of uncentrifuged inoculum and 2.5 mL of the flavour compound and 1 mL of nutrient medium. Besides the two groups of reactor bottles, other groups
included membrane and free cells controls; both of which differed from the first two groups by the replacement of the fruit flavour with 2.5 mL of distilled water. Blank reactors containing 50 mL of non-centrifuged inoculum and 3.5 mL of distilled water were also prepared. After filling the serum glass bottles with appropriate medium of pH between 6.8 and 7.2, they were closed with rubber seals and plastic caps. The bottle headspace was flushed with 80% nitrogen and 20% carbon dioxide to create anaerobic environment [56]. All the experiments were carried out in triplicates and incubated at 55°C in a water bath. During the course of the experiment, the reactor bottles were shaken manually two times everyday to enhance the fermentation activities.

3.2.2. Semi-continuous fermentation process for hydrogen production

The semi-continuous experiments were carried out using parallel 500 mL bioreactors and automatically gas volume recording system (AMPTS, Bioprocess Control Sweden AB, Sweden). Prior to the start of the semi-continuous experiment, the sludge for the fermentative hydrogen production was heat-pretreated at 100°C for 15 min and the initial pH adjusted to values between 5.2 and 5.9, as hydrogen production has been observed to be enhanced at the pH range [57]. An average of 32 g pellet of the inoculum sludge (equivalent to 5.6 g VSS/L) obtained from the centrifuged sludge was used separately for each reactor with free and encapsulated cells. Regarding the reactors with free cells, the inoculum pellet (5.6 g VSS/L) was added into each 500 mL glass reactor bottle (liquid volume of 450 mL) containing 300 mL of filtered nutrient medium and 97 mL of distilled water. The nutrient medium was composed of 20 g/L each of nutrient broth, yeast extract and D-glucose. The resulting mixture was thoroughly mixed so that the inoculum pellet could dissolve completely to form homogeneous mixture. The flavour compounds (myrcene, octanol and hexanal) were prepared in three different concentrations including 0.05, 0.5 and 5 g/L, after which, 21 mL of the prepared flavour solutions was added into each reactor. For encapsulated cell-reactors, the inoculum pellet (32g) was divided into eight equal portions (4 g each), which were inserted into eight membrane sachets. Each reactor bottle contained eight membrane sachets with each sachet enclosing 4 g of inoculum pellet. The whole experiment was started with the addition of lowest flavour concentration (0.05 g/L) while the gradual increase in concentration was done at an interval of 5 days. With the constant active volume of 450 mL and daily flow of 50 mL/d, the hydraulic retention time (HRT) throughout the experiment was 9 days. Throughout the experiment, the reactor bottles were shaken twice a day to ensure adequate contact among the nutrients, anaerobic cells and flavour compounds. The pH of the effluent withdrawn each day of the experiment was measured in order to gain insight into the state of the fermentation process during the experiment.
3.3. Analytical method

The volumes of biogas and hydrogen generated during the anaerobic fermentation processes were measured using a data acquisition system (AMPTS, Bioprocess Control Sweden AB, Sweden). The individual gas compositions were determined by using a 0.25 mL syringe (VICI, precious sampling Inc., USA) for the gas sampling while the gas quantification was done using a gas chromatograph (Perkin-Elmer, USA). The gas chromatograph was equipped with a packed column (Perkin-Elmer, 6’ x 1.8" OD, 80/100, Mesh, USA) and a thermal conductivity detector (Perkin-Elmer, USA) with an inject temperature of 150°C. Nitrogen at a flow rate of 20 mL/min at 60°C was used as carrier gas.

3.4. Membrane performance measurement

The ability of a membrane to regulate the permeation of various molecules through it is an important feature that is employed in separation processes. The driving forces producing movement of permeants, which could be concentration, pressure, temperature and electromotive force; are connected in such a way that overall driving force is the chemical potential. Membrane permeability determines the rates of movement of nutrients and inhibitors into the cells of the fermentative microorganisms as well as the discharge of the cell metabolism products. In this study, distilled water was used to determine the pure water permeability (PWP) parameter of the hydrophilic PVDF membranes used during the experimental work in this study. The time required for a definite quantity of distilled water to pass through the membranes was observed and recorded. The water flow rate through the membrane was calculated by dividing the volume of permeated water by the time required for the permeation. Since the experiment was carried out at room temperature (22°C), the temperature correction of 0.794 was used to adjust the values obtained from the permeability test.

4. Conclusions

The major barrier associated with the widespread applications of fermentative hydrogen and methane as fuels include, among others, the low yields of the gas production. The low yields have been partly attributed to substrate inhibition. This study, therefore, investigated the inhibitory effect of some flavour compounds in fruits, which is one of the important factors contributing to low hydrogen and methane yields during fermentation process of fruit wastes. The potential of employing membrane technology to improve the yields of hydrogen and methane from such process was explored. The results suggest that the membrane-based techniques could actually improve hydrogen and methane production from fermentation media with substrate inhibition. Compared with the free cells,
membrane-encapsulated cells produced methane faster and were able to survive the effects of the inhibitory flavour medium. Higher gas productions were also observed from encapsulated cells, when compared to free cells, in the inhibitory fruit flavour. However, it could be observed from the results obtained that the membrane could not completely protect the fermentative organism against the inhibitory effects of flavour compounds. Therefore, further membrane improvement is necessary to effectively protect the microorganism from the inhibitory fruit flavour medium.

Acknowledgments

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Author Contributions

Julius Akinbomi is the first author and responsible for designing, performing and writing of the article while Rachma Wikandari assisted in the designing of the experiment. Professor Mohammad Taherzadeh is the main supervisor of the first author responsible for guidance and supervision of the project from experimental designing to the revision of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References


Development and Dissemination Strategies for Accelerating Biogas Production in Nigeria

Julius Akinbomi, Tomas Brandberg, Sikiru A. Sanni, and Mohammad J. Taherzadeh

Following the worsening energy crisis of unreliable electricity and unaffordable petroleum products coupled with the increase number of poverty-stricken people in Nigeria, the populace is desperately in need of cheap alternative energy supplies that will replace or complement the existing energy sources. Previous efforts by the government in tackling the challenge by citizenship sensitization of the need for introduction of biofuel into the country’s energy mix have not yielded the expected results because of a lack of sustained government effort. In light of the shortcomings, this study assesses the current potential of available biomass feedstock for biogas production in Nigeria, and further proposes appropriate biogas plants, depending on feedstock type and quantity, for the six geopolitical zones in Nigeria. Besides, the study proposes government-driven biogas development systems that could be effectively used to harness, using biogas technology, the estimated 270 TWh of potential electrical energy from 181 million tonnes of available biomass, in the advancement of electricity generation and consequent improvement of welfare in Nigeria.

Keywords: Biogas; available feedstock; Nigerian’s prospect; Biogas-consultancy; Electricity

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INTRODUCTION

Energy accessibility is the catalyst for economic growth, development, and poverty alleviation, and it determines the level of social development in a country. Over the years, Nigeria has been facing numerous challenges including a severe electricity shortage, an inefficient waste management system, and environmental degradation. More than 60% of the population does not have access to the national power supply because they are not connected to the grid system; and even for those that are connected to the grid system, power outages are a common challenge (Kennedy-Darling et al. 2008; Okoye 2007). As a result of an unstable power supply, most people currently rely on generators for their supply of off-grid electricity. Inadequate and inaccessible energy services have compelled most industries and businesses that could not afford the high cost of running their business operations, to close down shop, a situation that has led to a surge in the number of impoverished or unemployed people. Also, owing to insufficient refining capacity to cope with the domestic demand, the Nigerian economy heavily relies on imported petroleum products. The heavy reliance of Nigerian economy on the fossil fuel market makes it vulnerable to any little instability in global oil market. For example,
following the recent halt in the importation of Nigerian crude oil by the United States of America due to the shale oil revolution, the Nigerian minister of petroleum had urged the country to adopt sustainable economic policies, as a matter of urgency, for fear of impending economic stress that the development might have in the future (TheScoopNG 2014).

Although the usage of fossil fuels products has contributed immensely to the global economic growth and development, the negative effects of its application in the area of health and environment are gradually overshadowing the economic benefits, coupled with the facts that fossil fuels are finite in supply and consequently the prices of their products are vulnerable to frequent increase. The frequent increase in the price of fossil fuel products has brought untold hardship to people in developing countries, not the least in Nigeria. Because of the increase in poverty, most people who could not afford the expensive fossil fuel products have resorted to the environmentally unfriendly practice of felling wood for cooking, causing dwindling forest reserves. Besides the challenge of electricity shortage, Nigeria also faces the problem of an inefficient management system of wastes, including agricultural, municipal solid waste (MSW), and sewage, among others. The wastes are generated daily in large quantities but are disposed in unhygienic and unsustainable ways such as burning, unsanitary land filling, or indiscriminate dumping of waste on the streets and drains. Landfilling, for example, has the potential of causing further water and air pollution through leachate and gases, which are the two main products generated from a landfill. An inefficient waste management system due to lack of technical expertise, regulatory setup, and adequate funds, has contributed to various environmental challenges currently being experienced in Nigeria. Consequently, environmental pollution, flooding, and disease epidemics from indiscriminate waste dumping on the streets and drains are common occurrences in the country (Amori et al. 2013; Leton and Omotosho 2004).

Nigeria has an estimated population of over 165 million people and an annual growth rate of about 2.8% (Factbook 2014; FAOSTAT 2014; Shaaban and Petinrin 2014). The country has a total area of 924,000 km², out of which 33.0% is arable land replanted after each harvest, while 3.1% is cultivated with permanent crops. The tropical climatic conditions in the country, which are characterized by high humidity in the south and high temperatures in the north with an average temperature of 27 °C, encourage large-scale agriculture. Because of the high population, huge amounts of waste are inevitably produced daily without an effective waste management system, and moreover more energy is required to satisfy the increasing energy demand. Meanwhile, the abundant waste generated daily can be utilized as energy resources for provision of adequate energy for the citizenry by the adoption of biogas technology. The technology offers numerous benefits, including provision of energy for cooking, heating, lighting, and as a vehicle fuel, job creation, income revenue generation, reduction of workload or drudgery for women, agricultural development, and air pollution reduction (Nyns 1986). Besides, the country also needs an effective waste management system to manage the huge amount of waste being generated daily in Nigeria. Application of biogas technology has the potential of maintaining a balance between production and consumption of waste and energy, since the technology is based on conversion of organic waste materials into energy in form of biogas. The warm climatic conditions are adequate
for anaerobic digestion process of organic wastes without the need for extra heating. Channeling wastes into biogas production could therefore be one of the most efficient ways of waste disposal, energy production, and environmental protection.

The news from Nigerian Finance Minister, on April 2014, that Nigeria is currently Africa’s largest economy and 26th largest in the World, comes with mixed feelings for many Nigerians. The positive aspect of the news is that the growth of the economy has placed Nigeria within reach of its vision 20:2020 to become one of the world’s top 20 economies by the year 2020. And this will definitely increase investment opportunities in the country. The negative aspect of the news is that the growth impact has not benefitted poorer members of society, as 60% of the population does not have access to energy and as such many people have become impoverished. Previous efforts by the government in finding solutions to the problems by citizenship sensitization of the need to introduce biofuel energy into the country’s energy mix have not yielded the expected results because of lack of sustained government effort. Little attention has been paid to the development of biogas technology in Nigeria, with only few units of biogas pilot plants developed by different research centres (Sambo 2005). The development and application of biogas technology have been hampered by a number of factors including storage difficulty of biomass residues, technical barriers, poor financial support from the government, and low levels of public awareness of the benefits of using biogas as an energy source. This study therefore aims at examining current biogas production potentials of Nigerian biomass resources, and proposing strategies for an accelerated biogas development in Nigeria.

Potential Assessment of Nigerian Biomass Feedstock for Biogas Production

Biogas is a colourless and odourless mixture of gases produced through anaerobic decomposition of organic materials by microorganisms, and depending on the nature of the organic materials and operating conditions, the gas composition includes methane, carbon dioxide, nitrogen, oxygen, hydrogen sulphide, and ammonia with compositions of 40-75%, 25-40, 0.5-2.5%, 0.1-1% 0.1-0.5%, and 0.1-0.5%, respectively (Salomon and Lora 2009; Weiland 2010). Biogas can be used to augment conventional energy sources for various purposes including cooking, heating, vehicle fuel, and electricity generation, while the sludge from the anaerobic process can be used as organic fertilizer. Potential biogas feedstocks that are available in Nigeria include agricultural crop and residues, livestock wastes, municipal solid wastes and sewage.

Biogas production from agricultural crop wastes

Agricultural crop wastes are potential sources of biogas energy, especially in Nigerian rural areas where nearly everyone practices farming. Nigeria produces a wide range of agricultural crops in large quantities for consumption and exportation, and consequently huge amount of residues are generated from the crops. Agricultural crop wastes may consist of rotten crops due to inadequate storage facilities. There are infected crops due to diseases and also residues produced from crop processing after harvest. Table 1 shows the average quantity of agricultural crop wastes from the production between year 2003 and 2012 in Nigeria.
Table 1. Biochemical Methane Potential (BMP) of Biogas from Average Crop Production between Year 2003 and 2012 in Nigeria

<table>
<thead>
<tr>
<th>Agricultural Crops</th>
<th>Average Production (Tonnes x 10^6)</th>
<th>Residue Type</th>
<th>Estimated Quantity of Potential Residues (Tonnes x 10^6)</th>
<th>Estimated Quantity of Unavailable Crop Residues (70% of Potential Crop Residues) (Tonnes x 10^6)</th>
<th>Estimated quantity of available crop residues (Tonnes x 10^6)</th>
<th>Actual vs. converted to biogas (51% of crop residue) (Tonnes x 10^6)</th>
<th>BMP of biogas produced based on 0.7 m^3/kg VS at STP (m^3 x 10^9)</th>
<th>BMP of biogas produced based on 0.7 m^3/kg VS at 35˚C (m^3 x 10^9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cassava</td>
<td>43.6</td>
<td>Cassava stalk</td>
<td>0.062</td>
<td>2.70</td>
<td>1.89</td>
<td>0.81</td>
<td>0.41</td>
<td>0.29</td>
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<tr>
<td></td>
<td></td>
<td>Cassava peelings</td>
<td>0.25</td>
<td>10.90</td>
<td>7.63</td>
<td>3.27</td>
<td>1.67</td>
<td>1.17</td>
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<tr>
<td>Cocoa beans</td>
<td>0.4</td>
<td>Cocoa husk</td>
<td>1</td>
<td>0.40</td>
<td>0.28</td>
<td>0.12</td>
<td>0.06</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cocoa pods</td>
<td>1</td>
<td>0.40</td>
<td>0.28</td>
<td>0.12</td>
<td>0.06</td>
<td>0.04</td>
</tr>
<tr>
<td>Coconut</td>
<td>0.2</td>
<td>Coconut shell</td>
<td>0.65</td>
<td>0.13</td>
<td>0.09</td>
<td>0.04</td>
<td>0.02</td>
<td>0.01</td>
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<td></td>
<td></td>
<td>Coconut husk</td>
<td>0.419</td>
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<td>0.02</td>
<td>0.01</td>
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<td>Maize</td>
<td>7.2</td>
<td>Maize stalk</td>
<td>2.30</td>
<td>16.56</td>
<td>11.59</td>
<td>4.97</td>
<td>2.53</td>
<td>1.77</td>
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<tr>
<td></td>
<td></td>
<td>Maize cob</td>
<td>0.27</td>
<td>1.94</td>
<td>1.36</td>
<td>0.58</td>
<td>0.30</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maize husks</td>
<td>0.30</td>
<td>2.16</td>
<td>1.51</td>
<td>0.65</td>
<td>0.33</td>
<td>0.23</td>
</tr>
<tr>
<td>Groundnut with shell</td>
<td>3.2</td>
<td>Groundnut husk/shell</td>
<td>0.42</td>
<td>1.34</td>
<td>0.94</td>
<td>0.40</td>
<td>0.21</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Groundnut straw</td>
<td>2.3</td>
<td>7.36</td>
<td>5.15</td>
<td>2.21</td>
<td>1.13</td>
<td>0.79</td>
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<tr>
<td>Millet</td>
<td>6.1</td>
<td>Millet straw</td>
<td>2</td>
<td>12.20</td>
<td>8.54</td>
<td>3.66</td>
<td>1.87</td>
<td>1.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Millet stalk</td>
<td>3</td>
<td>18.30</td>
<td>12.81</td>
<td>5.49</td>
<td>2.80</td>
<td>1.96</td>
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<tr>
<td>Oil palm fruit</td>
<td>8.4</td>
<td>Empty fruit bunch</td>
<td>0.24</td>
<td>2.02</td>
<td>1.41</td>
<td>0.61</td>
<td>0.31</td>
<td>0.22</td>
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<tr>
<td></td>
<td></td>
<td>Oil palm fibre</td>
<td>0.122</td>
<td>1.03</td>
<td>0.72</td>
<td>0.31</td>
<td>0.16</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oil palm shell</td>
<td>0.047</td>
<td>0.40</td>
<td>0.28</td>
<td>0.12</td>
<td>0.06</td>
<td>0.04</td>
</tr>
<tr>
<td>Rice, paddy</td>
<td>3.9</td>
<td>Rice husk</td>
<td>0.26</td>
<td>1.01</td>
<td>0.71</td>
<td>0.30</td>
<td>0.15</td>
<td>0.11</td>
</tr>
</tbody>
</table>

### Table 1. (cont’d). Biochemical Methane Potential (BMP) of Biogas from Average Crop Production between 2003 and 2012 in Nigeria

<table>
<thead>
<tr>
<th>Agricultural Crops</th>
<th>Average Production (Tonnes x 10^6)</th>
<th>Residue Type</th>
<th>Residue-to-Product Ratio (RPR)</th>
<th>Estimated Quantity of Potential Residues (Tones x 10^6)</th>
<th>Estimated Quantity of Unavailable Crop Residues (70% Of Potential Crop Residues) (Tones x 10^6)</th>
<th>Estimated Quantity of Available Crop Residues (Tones X 10^6)</th>
<th>Actual vs. Converted to Biogas (51% of Crop Residue) (Tones x 10^6)</th>
<th>BMP of Biogas Produced Based on 0.7 m^3/kgVS at 35°C (m^3 x 10^9)</th>
<th>BMP of Biogas Produced on 0.7 m^3/kgVS at STP (m^3 x 10^9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potatoes</td>
<td>0.9</td>
<td>Potatoes stem and leaves</td>
<td>0.4</td>
<td>0.36</td>
<td>0.25</td>
<td>0.11</td>
<td>0.06</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Sorghum</td>
<td>8.0</td>
<td>Sorghum straw</td>
<td>2</td>
<td>16.00</td>
<td>11.20</td>
<td>4.80</td>
<td>2.45</td>
<td>1.71</td>
<td>1.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sorghum stalk</td>
<td>2.62</td>
<td>20.96</td>
<td>14.67</td>
<td>6.29</td>
<td>3.21</td>
<td>2.24</td>
<td>2.53</td>
</tr>
<tr>
<td>Sugarcane</td>
<td>1.2</td>
<td>Sugar cane bagasse</td>
<td>0.3</td>
<td>0.36</td>
<td>0.25</td>
<td>0.11</td>
<td>0.06</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sugar cane straw</td>
<td>1.5</td>
<td>1.80</td>
<td>1.26</td>
<td>0.54</td>
<td>0.28</td>
<td>0.19</td>
<td>0.22</td>
</tr>
<tr>
<td>Soybeans</td>
<td>0.5</td>
<td>Soybeans straw and pods</td>
<td>3.5</td>
<td>1.75</td>
<td>1.23</td>
<td>0.53</td>
<td>0.27</td>
<td>0.19</td>
<td>0.21</td>
</tr>
<tr>
<td>Sweet potatoes</td>
<td>3.2</td>
<td>Sweet potatoes residues</td>
<td>0.36</td>
<td>1.15</td>
<td>0.81</td>
<td>0.35</td>
<td>0.18</td>
<td>0.12</td>
<td>0.14</td>
</tr>
<tr>
<td>Cocoyam</td>
<td>4.3</td>
<td>Cocoyam residues</td>
<td>0.36</td>
<td>1.55</td>
<td>1.09</td>
<td>0.47</td>
<td>0.24</td>
<td>0.17</td>
<td>0.19</td>
</tr>
<tr>
<td>Tobacco</td>
<td>0.02</td>
<td>Tobacco stem/stalks</td>
<td>2.0</td>
<td>0.04</td>
<td>0.03</td>
<td>0.01</td>
<td>0.01</td>
<td>0.0043</td>
<td>0.0048</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>1.7</td>
<td>Tomato stem</td>
<td>0.3</td>
<td>0.51</td>
<td>0.36</td>
<td>0.15</td>
<td>0.08</td>
<td>0.05</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tomato leaves</td>
<td>0.3</td>
<td>0.51</td>
<td>0.36</td>
<td>0.15</td>
<td>0.08</td>
<td>0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>Fresh vegetables</td>
<td>5.4</td>
<td>Vegetable residues</td>
<td>0.45</td>
<td>2.43</td>
<td>1.70</td>
<td>0.73</td>
<td>0.37</td>
<td>0.26</td>
<td>0.29</td>
</tr>
<tr>
<td>Mangoes, mangoostens</td>
<td>0.8</td>
<td>residues</td>
<td>1.8</td>
<td>1.44</td>
<td>1.01</td>
<td>0.43</td>
<td>0.22</td>
<td>0.15</td>
<td>0.17</td>
</tr>
<tr>
<td>Melon seed</td>
<td>0.5</td>
<td>residues</td>
<td>1.26</td>
<td>0.63</td>
<td>0.44</td>
<td>0.19</td>
<td>0.10</td>
<td>0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>Ginger</td>
<td>0.2</td>
<td>Ginger residue</td>
<td>1.15</td>
<td>0.23</td>
<td>0.16</td>
<td>0.07</td>
<td>0.04</td>
<td>0.02</td>
<td>0.03</td>
</tr>
</tbody>
</table>


---

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<table>
<thead>
<tr>
<th>Agricultural crops</th>
<th>Average Production (Tonnes x 10^6)</th>
<th>Residue type</th>
<th>Residue-to-Product Ratio (RPR)</th>
<th>Estimated Quantity of Potential Residues (Tonnes x 10^6)</th>
<th>Estimated Quantity of Unavailable Crop Residues (70% Of Potential Crop Residues)</th>
<th>Estimated Quantity of Available Crop Residues (Tonnes x 10^6)</th>
<th>Actual vs. Converted to Biogas (51% of Crop Residue)</th>
<th>BMP of Biogas Produced Based on 0.7m^3/kgVS at STP (m^3 x 10^9)</th>
<th>BMP of Biogas Produced Based on 0.7m^3/kgVS at 35°C (m^3 x 10^9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chili &amp; pepper (green)</td>
<td>0.7</td>
<td>residues</td>
<td>0.45</td>
<td>0.32</td>
<td>0.22</td>
<td>0.10</td>
<td>0.05</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>Cashews with shells</td>
<td>0.7</td>
<td>Cashew shell</td>
<td>2.10</td>
<td>1.47</td>
<td>1.03</td>
<td>0.44</td>
<td>0.22</td>
<td>0.16</td>
<td>0.18</td>
</tr>
<tr>
<td>Carrot and turnings</td>
<td>0.2</td>
<td>residues</td>
<td>0.45</td>
<td>0.09</td>
<td>0.06</td>
<td>0.03</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Coffee, green</td>
<td>0.004</td>
<td>Coffee husk</td>
<td>21</td>
<td>0.08</td>
<td>0.06</td>
<td>0.02</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Cowpea, dry</td>
<td>2.7</td>
<td>Cowpea husk</td>
<td>2.9</td>
<td>7.83</td>
<td>5.48</td>
<td>2.35</td>
<td>1.20</td>
<td>0.84</td>
<td>0.95</td>
</tr>
<tr>
<td>Fruit, Citrus</td>
<td>3.7</td>
<td>residue</td>
<td>1.8</td>
<td>6.66</td>
<td>4.66</td>
<td>2.00</td>
<td>1.02</td>
<td>0.71</td>
<td>0.80</td>
</tr>
<tr>
<td>Kolanuts</td>
<td>0.1</td>
<td>residues</td>
<td>1.8</td>
<td>0.18</td>
<td>0.13</td>
<td>0.05</td>
<td>0.03</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Okra</td>
<td>1.0</td>
<td>residues</td>
<td>0.45</td>
<td>0.45</td>
<td>0.32</td>
<td>0.14</td>
<td>0.07</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Onions, dry</td>
<td>1.2</td>
<td>residues</td>
<td>0.45</td>
<td>0.54</td>
<td>0.38</td>
<td>0.16</td>
<td>0.08</td>
<td>0.06</td>
<td>0.07</td>
</tr>
<tr>
<td>Pawpaw</td>
<td>0.8</td>
<td>residues</td>
<td>1.8</td>
<td>1.44</td>
<td>1.01</td>
<td>0.43</td>
<td>0.22</td>
<td>0.15</td>
<td>0.17</td>
</tr>
<tr>
<td>Pineapples</td>
<td>1.1</td>
<td>residues</td>
<td>1.8</td>
<td>1.98</td>
<td>1.39</td>
<td>0.59</td>
<td>0.30</td>
<td>0.21</td>
<td>0.24</td>
</tr>
<tr>
<td>Pulses</td>
<td>0.07</td>
<td>residues</td>
<td>1.9</td>
<td>0.13</td>
<td>0.09</td>
<td>0.04</td>
<td>0.02</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Plantain</td>
<td>2.7</td>
<td>Plantain residues</td>
<td>1.8</td>
<td>4.86</td>
<td>3.40</td>
<td>1.46</td>
<td>0.74</td>
<td>0.52</td>
<td>0.59</td>
</tr>
<tr>
<td>Seed cotton</td>
<td>0.5</td>
<td>Cotton stalks</td>
<td>3.52</td>
<td>1.76</td>
<td>1.23</td>
<td>0.53</td>
<td>0.27</td>
<td>0.19</td>
<td>0.21</td>
</tr>
<tr>
<td>Wheat</td>
<td>0.08</td>
<td>Straw</td>
<td>1.750</td>
<td>0.14</td>
<td>0.10</td>
<td>0.04</td>
<td>0.02</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Yams</td>
<td>33.7</td>
<td>Yam peelings</td>
<td>0.25</td>
<td>8.43</td>
<td>5.90</td>
<td>2.53</td>
<td>1.29</td>
<td>0.90</td>
<td>1.02</td>
</tr>
<tr>
<td>Total</td>
<td>171.86</td>
<td>120.30</td>
<td>51.56</td>
<td>26.29</td>
<td>18.41</td>
<td>20.77</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a* FAOSTAT, 2014;  
1 Kristoferson and Bokhalders 1991; Ryan and Openshaw 1991; Smeets et al. 2004; Webb 1979a; Webb 1979b  
2m-0 (Burke 2001; Deublein and Steinhauser 2008; Parkin and Owen 1986)
The method used for calculating the average quantity of crop wastes is based on a residue-to-product ratio (RPR) method in which the RPR for different crops are used to multiply annual production of each crop. The RPR ratio, which represents the amount of residues that could be obtained from a unit amount of crop harvested, were selected from different literature sources, since each source covered only some of the crops studied. Meanwhile, the available quantities of residues using the RPR ratios might not be the actual values in practice due to climatic variations coupled with the facts that different studies indicated varying RPR’s for the same crop; the quantity obtained could still be used as the best guide for policy makers to get a picture of the amount of residues that could be generated from each crop, since the RPR ratios made provisions for variations in crop, variety, climate and different farming activities. VS ratios were taken from literature sources different from those that the RPR ratios were taken from because information on VS ratios was not given in the literature containing RPRs ratios. Biochemical methane potential (BMP) was calculated based on the assumption that the waste could be taken as primary solids, and that a cubic metre of BMP could be obtained from one kg VS of the primary solids as given in Khanal (2008). The average quantity of crop residues obtained annually from the harvesting and processing of the agricultural crops was estimated to 172 million tonnes.

Meanwhile, about 70% of the residues generated during crop harvesting and processing are often used for other purposes such as soil mulch, fuel, building materials, and animal fodder (Dayo 2007; Jibrin et al. 2013). As regards animal fodder, the most commonly fed crop residues include cassava and yam peels, cowpea husk, and groundnut husks, brans, oilcakes, maize, millet, and sorghum stovers (DE-Leew 1997; Onwuka et al. 1997; Singh et al. 2011). Leguminous crop residues are often preferred to cereal residues as animal fodder because of their higher nutritive value, digestibility, crude protein content, and minerals (Owen 1994). The quantity of crop residues available for biogas production could therefore be reduced. In fact, it has been observed that during the rainy season, agricultural crop residues supply 58% of animal fodder (Jibrin et al. 2013). Taking the crop residues used for other purposes into consideration, the quantity of available crop residues for biogas production was estimated at approximately 52 million tonnes, from which 21 billion cubic metres of methane gas could be generated at 35 °C (Table 1).

**Biogas production from livestock waste: livestock manure and abattoir waste**

Livestock waste includes dead livestock due to diseases, livestock manure, slaughterhouse wastes such as hair, feather, bones, blood, undigested food, and meat from animal and poultry processing industries. Among the livestock reared in Nigeria, only cattle, goats, sheep, pigs, and chicken are produced in large quantities, as shown in Table 2a. The amount of animal dung that could be obtained from the average annual population of the livestock was estimated to be approximately 32 million tonnes, from which 3.7 billion cubic metres of methane gas could be produced. However, the available animal manure for biogas production may in reality be lower, since the considerable amounts of animal dung is often left on the grazing field to improve the soil quality.

Regarding abattoir waste, a huge amount is usually generated daily in Nigeria because of high consumption of meat by people. Often these wastes are not treated before being discharged into nearby streams and rivers, thereby constituting an environmental and health hazard to the people living in the neighbourhood. Compositions of abattoir wastes generally include animal blood, intestinal content, waste tissue, and bone. From
the common reared livestock in Nigeria, an estimated amount of 0.83 million tonnes (Table 2b) abattoir waste could be generated annually, which could be harnessed using biogas technology to produce about 0.34 billion cubic metres of methane gas.

**Biogas production from municipal solid waste (MSW)**

The quantity and composition of MSW generated in any particular region depends most importantly on factors such as people's lifestyles, standard of living, consumption patterns, local climate, as well as cultural and educational differences. The waste generation rate in low-income countries (developing countries) has been found to be within the range of 0.4 to 0.6 kg/person/day (Blight and Mbande 1996; Chandrappa and Das 2012; Cointrea 1982). This is similar to the waste generated rate of 0.44 to 0.66 kg/capita/day generated in some urban region in Nigeria (Ogwueleka 2009). The moisture and organic content of the waste generated in developing countries are reportedly reasonably high, which makes them to be suitable for anaerobic digestion (Babayemi and Dauda 2009).

In this study, the average waste generated rate of 0.62 kg/capita/day was used as a representative value for each person in Nigeria. To estimate the average quantity of MSW generated in Nigeria, the average waste generated rate of the six Nigerian Geo-Political zones including North-central, North-East, North-West, South-East, South-West, South-South was obtained from their six respective cities namely, Abuja (0.66 kg/capita/day), Bauchi (0.86), Kano (0.56), Aba (0.40), Lagos (0.63), and Port-Harcourt (0.6) (all kg/capita/day) (Adewunmi et al. 2005; Babayemi and Dauda 2009; Ogwueleka 2009; Usman and Mohammed 2012). An estimated value of 37 million tonnes organic MSW residues could be available for biogas production with BMP of 13 billion cubic metres (Table 3).

**Biogas production from human wastes**

Human waste, often called black water, consists of faeces and urine and forms part of sewage generated from a community. The other part of the sewage is called grey water, which represents wastewater from all sources including bathroom, kitchen, and laundry without human wastes (Katukiza et al. 2012; Uwidia and Ademoroti 2011). Unlike human wastes, grey water is often highly contaminated with different substances including domestic wastes such as soaps/detergents, shampoo, pharmaceuticals, and industrial wastes, which make them unsuitable as feedstock for biogas production without adequate pre-treatment, as they may cause failure of biogas digesters. Within Nigerian urban communities, pit latrines are common in low-income households (Chaggu et al. 2002; Howard et al. 2003; Kulabako et al. 2010; WHO and UNICEF 2010), while water closet toilets are common in middle and high-income households. In Nigerian rural communities, soil pit and open defecation are still the common forms of human waste disposal, since many rural dwellers do not have any form of toilets (Esrey et al. 1998). Pit latrines and water closet toilets are usually connected to septic tanks, which collect and transports human wastes into a soak away pit.

However, most septic and soakaway systems in Nigeria are not properly designed, located, operated, and maintained with consequent pollution of soil, surface water, and groundwater. Lack of good sanitation systems for disposing human wastes have been a major concern to many Nigerians and often facilitate the spread of diseases among people. Therefore, proper treatment of human waste before disposal is required, and this could be best achieved by anaerobic digestion.
### Table 2a. Methane Potential of Biogas from Average Livestock Population & Manure Production in Nigeria (2003 to 2012)

<table>
<thead>
<tr>
<th>Livestock</th>
<th>Stocks (Heads)</th>
<th>Annual Dry Dung from Each Animal (Tonnes)</th>
<th>Total Annual Animal Dung (Tonnes x 10^6)</th>
<th>Available Animal Dung (30% of Production) (kg x 10^6)</th>
<th>Actual vs. Converted to Biogas (% of Production)</th>
<th>Actual vs. Converted to Biogas (kg x 10^6)</th>
<th>BMP of Biogas Produced Based on 0.7 m^3/kgVS at STP (m^3 x 10^9)</th>
<th>BPM of Biogas Produced Based on 0.7 m^3/kgVS at 35°C (m^3 x 10^9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>16,571,801</td>
<td>1.0</td>
<td>16.60</td>
<td>4.98</td>
<td>48</td>
<td>2.39</td>
<td>1.67</td>
<td>1.89</td>
</tr>
<tr>
<td>Pigs</td>
<td>6,792,244</td>
<td>0.3</td>
<td>2.04</td>
<td>0.61</td>
<td>45</td>
<td>0.28</td>
<td>0.20</td>
<td>0.22</td>
</tr>
<tr>
<td>Goats</td>
<td>53,027,703</td>
<td>0.15</td>
<td>8.00</td>
<td>2.40</td>
<td>48</td>
<td>1.15</td>
<td>0.81</td>
<td>0.91</td>
</tr>
<tr>
<td>Sheep</td>
<td>34,030,382</td>
<td>0.15</td>
<td>5.11</td>
<td>1.53</td>
<td>48</td>
<td>0.73</td>
<td>0.51</td>
<td>0.58</td>
</tr>
<tr>
<td>Chicken</td>
<td>171,331,000</td>
<td>0.005</td>
<td>0.90</td>
<td>0.27</td>
<td>42</td>
<td>0.11</td>
<td>0.08</td>
<td>0.09</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>32.4</td>
<td>9.79</td>
<td></td>
<td></td>
<td></td>
<td>3.69</td>
</tr>
</tbody>
</table>

* (World-Bank 1977)

### Table 2b. Methane Potential of Biogas from Average Annual Abattoir Wastes in Nigeria Generated (2003 to 2012)

<table>
<thead>
<tr>
<th>Annual Slaughtered Livestock</th>
<th>Average Weight of Slaughtered Animal (kg)</th>
<th>Amount of Waste from each slaughtered Animal (35% of Animal Body Weight) (kg)</th>
<th>Annual Slaughtered Livestock Waste (kg x 10^9)</th>
<th>Actual vs. Converted to Biogas (% of the Manure Production)</th>
<th>Actual vs. Converted to Biogas (kg x 10^6)</th>
<th>BMP of Biogas Produced Based on 0.7 m^3/kgVS at STP (m^3 x 10^9)</th>
<th>BMP of Biogas Produced Based on 0.7 m^3/kgVS at 35°C (m^3 x 10^9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>2,541,377</td>
<td>353</td>
<td>123.6</td>
<td>0.31</td>
<td>51</td>
<td>0.158</td>
<td>0.111</td>
</tr>
<tr>
<td>Pigs</td>
<td>4,752,865</td>
<td>60</td>
<td>21.0</td>
<td>0.10</td>
<td>51</td>
<td>0.051</td>
<td>0.036</td>
</tr>
<tr>
<td>Goats</td>
<td>21,461,956</td>
<td>23</td>
<td>8.1</td>
<td>0.11</td>
<td>51</td>
<td>0.056</td>
<td>0.039</td>
</tr>
<tr>
<td>Sheep</td>
<td>13,628,125</td>
<td>33</td>
<td>11.6</td>
<td>0.16</td>
<td>51</td>
<td>0.082</td>
<td>0.057</td>
</tr>
<tr>
<td>Chicken</td>
<td>257,127,778</td>
<td>1.7</td>
<td>0.6</td>
<td>0.15</td>
<td>51</td>
<td>0.077</td>
<td>0.054</td>
</tr>
<tr>
<td>Total Waste</td>
<td></td>
<td></td>
<td>0.83</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* (Achoja, 2013; Adeshina et al., 2003; Akinfolarin and Okubanjo, 2010; | (World-Bank 1998)

### Table 3. Biochemical Methane Potential (BMP) of Biogas from Average MSW and Human Wastes Generated in Nigeria

<table>
<thead>
<tr>
<th>Waste</th>
<th>Waste Generated Rate (kg/capita/year)</th>
<th>Population (x10^5)</th>
<th>Annual Total Waste (kg x 10^9)</th>
<th>Actual vs. Converted to Biogas (% of the Manure Production)</th>
<th>Actual vs. Converted to Biogas (kg x 10^6)</th>
<th>BMP of Biogas Produced Based on 0.7 m^3/Kgvs at STP (m^3 x 10^9)</th>
<th>BMP of Biogas Produced Based on 0.7 m^3/kg at 35°C (m^3 x 10^9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic MSW</td>
<td>226.30</td>
<td>165</td>
<td>37.34</td>
<td>45</td>
<td>16.80</td>
<td>11.76</td>
<td>13.27</td>
</tr>
<tr>
<td>Total Human wastes</td>
<td>375.95</td>
<td>165</td>
<td>62.03</td>
<td>50.4</td>
<td>31.26</td>
<td>21.88</td>
<td>24.70</td>
</tr>
<tr>
<td>Urine</td>
<td>146.00</td>
<td>165</td>
<td>24.09</td>
<td>50.4</td>
<td>12.14</td>
<td>8.50</td>
<td>9.59</td>
</tr>
<tr>
<td>Feces</td>
<td>86.12</td>
<td>165</td>
<td>50.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

From the Nigerian population of about 165 million, it is estimated that 86 million tonnes human wastes (faeces and urine) could be obtained annually from which 128 billion cubic metres of methane gas could be produced (Table 3).

**Electricity Production Potential of Nigerian Biomass Feedstock**

Various studies have shown the existence of a strong relationship between human development and annual per capita energy consumption (Meisen and Akin 2008; NBS 2009). This indicates that the level of social development in a country is reflected in the level of electricity consumption. The potential for electricity energy generation from the biomass feedstock studied was estimated as 270 TWh for all the available biomass feedstock (Ostrem 2004), as given in Table 4.

**Table 4. Theoretical Electricity Generation from Available Biomass Feedstock in Nigeria**

<table>
<thead>
<tr>
<th>Biomass Feedstock</th>
<th>Total Potential Biomass Feedstock (tonnes x 10^6)</th>
<th>Quantity of Available Biomass Feedstock (tonnes x 10^6)</th>
<th>BMP of biogas produced based on 0.7 m^3/kgVS at 35°C (m^3 x 10^9)</th>
<th>Potential Electricity Production based on 3.73 kWh/m^3CH4 (kWh x 10^9)</th>
<th>Electricity production (Terawatt hour, TWh)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agricultural crop wastes</td>
<td>171.86</td>
<td>51.56</td>
<td>20.77</td>
<td>77.47</td>
<td>77.47</td>
</tr>
<tr>
<td>Livestock manure</td>
<td>32.40</td>
<td>9.79</td>
<td>3.69</td>
<td>13.76</td>
<td>13.76</td>
</tr>
<tr>
<td>Livestock abattoir waste</td>
<td>0.83</td>
<td>0.83</td>
<td>0.34</td>
<td>1.27</td>
<td>1.27</td>
</tr>
<tr>
<td>Organic MSW</td>
<td>33.12</td>
<td>33.12</td>
<td>13.27</td>
<td>49.50</td>
<td>49.50</td>
</tr>
<tr>
<td>Human waste</td>
<td>86.12</td>
<td>86.12</td>
<td>34.29</td>
<td>127.90</td>
<td>127.90</td>
</tr>
<tr>
<td>Total</td>
<td>324.33</td>
<td>181.42</td>
<td>72.36</td>
<td>270</td>
<td>270</td>
</tr>
</tbody>
</table>

\[(Ostrem 2004)\]

When considering Nigerian energy needs, average cooking energy demand per capita per day had been estimated at 0.26 m^3 of biogas (Adeoti et al. 2000), which is equivalent to 0.97 kWh of electricity per capita per day. In other words, annually, each person will need an average of 354 kWh of electricity, which could be satisfactorily obtained from the biomass feedstock studied. In fact, the estimated 270 TWh of electricity energy from all the available biomass feedstock could be used to satisfy the energy needs of about 763 million people, which are far greater than the Nigerian population. Large scale electricity generation from biogas powered generator will be a cheaper, easier, and more affordable source of cooking energy, as it will eliminate challenges including biogas storage, explosion risks, adaptability of other cooking stoves, among others, involved in using biogas cooking stoves.

**Biogas Energy Market in Nigeria: Current and Future**

The current sources of electricity in Nigeria are gas, hydropower, oil, coal with cooking, lighting, and running of electrical appliances, in line with the domestic activities that usually consume energy in most Nigerian households. A majority of people living in rural areas rely mostly on firewood, dried animal dung, crop residues, and charcoal for cooking because they could not afford the high cost of kerosene and LPG, while electricity is usually unreliable and inaccessible (IEA 2006). Even in the urban areas where electricity, LPG, and kerosene are available to many households, the usage of the energy sources for cooking depends on the household income, with people often giving
preference to low-cost energy source (Arthur et al. 2010; Davis 1998; Howells et al. 2005). Most people with little or no access to electricity rely majorly on fuel wood and charcoal (Abila 2012), while most low-income households in urban areas often prefer using charcoal to firewood because of its durability, availability, and less polluting nature (Sebokah 2009).

Despite the fact that biogas technology is a proven and established technology in many parts of the World such as Germany, United Kingdom, Switzerland, France, Austria, Netherlands, Sweden, Denmark, Norway, Republic of Korea, Finland, Republic of Ireland, Brazil, China, and India (Table 5); the rate of development of biogas technology in most African countries is still at a low ebb. The rapid development of biogas technology in most European countries could be linked to various strategies employed by the respective countries, and most especially by the Renewable Energy Directive (RES) proposed by the European Union, which sets a binding target for all Members States to reach a 20% share of renewable energies in the total energy consumption by 2020. Biogas technologies in Europe, United States, and Latin America are often on a large scale with biogas produced used for various applications such as electricity generation, district heating, injection into natural gas pipelines, and as transportation fuel in buses, cars and trains. However, in Asian and some African countries, biogas technologies are on a small or household scale with the produced biogas being used for domestic purposes such as cooking and lighting, among others (Peters and Thielmann 2008; Sorda et al. 2010).

In Nigeria, some biogas projects have been executed, including construction of biogas plants at Zaria prison in Kaduna, Ojokoro in Lagos, Mayflower School Ikene in Ogun State, and a biogas plant at Usman Danfodiyo University in Sokoto with capacity of the digesters ranges between 10 and 20 m³ (Abubakar 1990; Adeyanju 2008; Atuanya and Aigbirior 2002; Dangogo and Fernando 1986; Igoni et al. 2008; Ilori et al. 2007; Lawal et al. 1995; Odeyemi 1983; Ojolo et al. 2007; Sambo 2005). However, the biogas projects are yet to be commercialized, since most of them are either non-operational or still at the research stage. The failure of various pilot biogas programmes and a low level of biogas development and dissemination in Nigeria have been attributed to a number of factors including lack of policy formulation, ineffective implementation of existing biofuel policies, lack of government commitment, technical inadequacy (inaccessibility to spare parts, unskilled operators), ineffective waste management system, poor storage facility and transportation system, lack of continuity of previous biogas programme initiatives by the successive governments, inadequate structural facilities, and a low level of awareness of benefits accrued from biogas technology. The current energy situation in Nigeria shows that biogas energy is not yet part of Nigeria’s energy mix as the mix is currently dominated by fuel wood, petroleum products, and hydroelectricity.

Meanwhile, all hope is not lost, as this is a common experience with the introduction of new technologies, which often require fostering for a period of time before achieving their stable implementation in terms of ample social, environmental, and economic benefits. However, lessons should be drawn from the failed biogas projects and used in the future design and operation of biogas plants. Effort must be geared towards preventing failure of biogas plants, as this can do a great damage to market penetration of the technology since prospective users or customers of the technology can lose interest in making any investment in the technology. Furthermore, strategies that are being employed in developed countries to advance biogas technology could also be adapted in Nigeria too.
### Table 5. Comparison of Biogas Production Strategies in Different Countries

<table>
<thead>
<tr>
<th>S/N</th>
<th>Number of biogas plants at the end of 2013</th>
<th>Energy production (GWh/year)</th>
<th>Strategies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(A) Medium to Large scale Biogas Plant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Germany</td>
<td>9945</td>
<td>40970</td>
</tr>
<tr>
<td></td>
<td>Strategies that contribute to Germany being the largest biogas producer in Europe include: i) setting of target to use 30% renewable energy in electricity production and 14% in heat production by 2020. ii) Provision of electricity feed-in tariff (FIT) system that gives primary access to the grid and ensures producers a premium or tariff for the electricity produced. The FIT system was facilitated by the Gas Entry/Energy Management Act, which required natural grid operators to connect biomethane suppliers to the grid. iii) Exemption of production and use of biogas from Eco-tax. iv) Granting of investment subsidies and favourable loans to investor in biogas applications.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>United Kingdom</td>
<td>610</td>
<td>10494</td>
</tr>
<tr>
<td></td>
<td>Strategies include: i) Provision of FIT for electricity generation from August 2011. The FIT includes: €0.17 for up to 250kW, 0.13 €0.16 for &gt;251 kWh up to 500kWh and €0.11 for &gt;500kWh. ii) Awarding of double Renewable Obligation Certificates (ROCS) plants involved in anaerobic digestion (AD). iii) Provision of renewable heat incentive in form of tariff of €0.08 for biomethane injected into the natural gas grid and combusted downstream from April 2011. iv) Provision of Renewable Transport Fuel Obligation Certificates worth €0.13 in 2010.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Switzerland</td>
<td>600</td>
<td>1023</td>
</tr>
<tr>
<td></td>
<td>Strategies include: i) Provision of FIT system for electricity. ii) Fund provision from Swiss Gas Association, a voluntary support program, for biomethane injection in order to achieve the set target of injecting 300 GWh biomethane annually within 6 years. iii) Financial support for projects on reduction of greenhouse gas emissions.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>France</td>
<td>336</td>
<td>1273</td>
</tr>
<tr>
<td></td>
<td>Strategies include: i) Provision of FIT system for electricity produced from biogas with energy efficiency bonus and manure bonus included. The FIT incentives include 0.8580 to 0.14521 EUR/kWh for landfills, 0.1182 to 0.2110 EUR/kWh for AD plants, 45 to 95 EUR/MWh for biomethane from landfills, 69 to 125 EUR/MWh for upgrading the biogas to biomethane from AD plants.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1IEA Bioenergy (2014)
### Table 5. (cont’d). Comparison of Biogas Production Strategies in Different Countries

<table>
<thead>
<tr>
<th>S/N</th>
<th>Number of biogas plants at the end 2013</th>
<th>Energy production (GWh/year)</th>
<th>Strategies</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Austria 336</td>
<td>585</td>
<td>(i) Provision of FIT system which is supported by Green Electricity Law. The incentives include 0.1950 EUR/kWh up to 250 kWh, 0.1693 EUR/kWh from 250 - 500 kWh, 0.1334 EUR/kWh from 500 - 750 kWh, 0.1293 EUR/kWh for higher than 750 kW + 0.02 EUR/kWh if biogas is upgraded + 0.02 EUR/kWh if heat is used efficiently.</td>
</tr>
<tr>
<td>6</td>
<td>Netherland 252</td>
<td>n.a</td>
<td>There is a support scheme that favours large scale biogas applications.</td>
</tr>
<tr>
<td>7</td>
<td>Sweden 242</td>
<td>1589</td>
<td>Although Sweden has no feed-in tariff system, other support systems exist which include target setting of zero emission of greenhouse gases by 2050, provision of economic incentives including tax-free policy on emission of carbon dioxide, nitrous oxide and sulphur taxes during biogas production, green certificate system, free parking charge for biogas fueled vehicles and introduction of climate investment programme.</td>
</tr>
<tr>
<td>8</td>
<td>Denmark 167</td>
<td>1218</td>
<td>The strategies employed include a bottom-up approach, access to investment grants, implementation of energy taxes and introduction of various financial incentives for both upgraded biogas supplied to the natural gas grid and to purified biogas entering a town gas grid.</td>
</tr>
<tr>
<td>9</td>
<td>Norway 129</td>
<td>500</td>
<td>(i) Banning of landfilling biodegradables of since 2009 which led to increase in available biogas feedstock. (ii) Provision of delivery support system that gives 3.5 EUR per ton of manure delivered to biogas plants. (iii) Provision of biogas investment aid. (iv) Tax-exemption and investment aid for infrastructure related on biogas fueled vehicles.</td>
</tr>
<tr>
<td>10</td>
<td>Republic of Korea 78</td>
<td>1925</td>
<td>Although there are no tariffs or subsidies for biogas, there is Renewable Portfolio Standard (RPS) system implemented since 2012 which mandates 2% of the total power generation to be supplied using the appropriate kind of renewable energy. Moreover, 10% Value Added Tax (VAT) and 2% tariffs are charged when the mixture of CNG and biogas is sold.</td>
</tr>
<tr>
<td>11</td>
<td>Finland 73</td>
<td>569</td>
<td>Strategies include: (i) Establishment of Electric Market Authority to support new biogas plants, which produce more than 100 kVA, with a feed-in tariff which guarantees a minimum price of 83.50 EUR/MWh electricity and 50 EUR/MWh heat premium on top of basic subsidy if the generated heat is utilized, provided that the total efficiency is at least 50%. (ii) Financial support by the Ministry of Agriculture and Forestry for biogas plants built on farms aiming at producing their own energy and heat. (iii) Exemption of production and use of biogas from excise tax.</td>
</tr>
</tbody>
</table>

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Raven and Gregersen (2007)
Table 5. (cont’d). Comparison of Biogas Production Strategies in Different Countries

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(A) Medium to Large scale Biogas Plant</td>
</tr>
</tbody>
</table>
| 12  | Republic of Ireland                     | 30                            | Strategies include:
|     |                                        | n.a                           | (i) Implementation of landfill levy of 75 EUR/ton of waste landfilled to discourage landfilling activities. |
|     |                                        |                               | (ii) Requirement of population centres with an excess of 25 000 persons to provide collection of source segregated food waste in order to encourage digestion of organic fraction of municipal solid waste. |
|     |                                        |                               | (iii) There is FIT system which includes 0.15 EUR/kWh for AD CHP equal to or less than 500 kW; 0.13EUR/kWh for AD CHP greater than 500 kW; 0.11 EUR/kWh for AD (non CHP) equal to or less than 500 kW and 0.10 EUR/kWh for AD (non CHP) greater than 500 kW. |
| 13  | Brazil                                  | 22                            | Strategies include:
|     |                                        | 697                           | (i) Provision of credit to producers of biogas technologies. |
|     |                                        |                               | (ii) Provision of rural technical assistance to promote improvement of infrastructure associated with biogas technology. |
|     |                                        |                               | (B) Small-scale or household digesters |
| 14  | China                                   | 26.5 million\(^{w,x}\)       | Strategies |
| 15  | India                                   | 4 million                     | In most Asian countries, biogas programs developed rapidly because of significant financial and technical support provided by their governments and various aid agencies\(^{y,z}\). In Nepal, the support is through various policy instruments such as biogas support programme (BSP)\(^{aa}\) which is technologically standardized and free from political interference. Demand for biogas digesters was stimulated through subsidy and quality control mechanisms. When government financial support declined, Nepal generated financial support by developing biogas plants across the country as clean development mechanism (CDM) projects, and all these strategies have contributed to Nepal’s achievement of having the highest per capita biogas plant in the World\(^{v,ab,ac}\). Bangladeshi government has been actively involved in the development of biogas technology in the country. In China and India, drivers to rapid biogas development include strong government support, technical knowledge, availability of fermentation materials\(^{x}\), China’s principle of adaptability of materials of construction of biogas plants to locality contributed the rapid development of biogas technology in the country. |
| 16  | Nepal                                   | 268,464                       | |
| 17  | Vietnam                                 | 152,349                       | |
| 18  | Bangladesh                              | 26,311                        | |
| 19  | Cambodia                                | 19,173                        | |
| 20  | Indonesia                               | 7835                          | |
| 21  | Pakistan                                | 5357\(^{x}\)                 | |

\(^{w}\)Surendra et al. (2014); \(^{x}\) Chen et al. (2010); \(^{y}\) Bond and Templeton (2011); \(^{z}\) Kristoferson and Bokhalders (1991); \(^{ab}\) Gunnerston and Stuckey (1986); \(^{ac}\) BSP (2012)
Table 5. (cont’d). Comparison of Biogas Production Strategies in Different Countries

<table>
<thead>
<tr>
<th>Strategies</th>
<th>Number of small scale digesters as at 2007</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kenya</td>
<td>6749</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>5011</td>
</tr>
<tr>
<td>Tanzania</td>
<td>4980</td>
</tr>
<tr>
<td>Uganda</td>
<td>3083</td>
</tr>
<tr>
<td>Burkina Faso</td>
<td>2013</td>
</tr>
<tr>
<td>Senegal</td>
<td>334</td>
</tr>
<tr>
<td>Cameroon</td>
<td>159</td>
</tr>
<tr>
<td>Benin</td>
<td>42</td>
</tr>
</tbody>
</table>

Biogas technology in most African countries has recorded little success due to less availability of technical and operational support among other factors. Only few of the installed biogas plants are still operational. Few operational biogas plants often experience numerous downtimes.

*Surendra et al. (2014)*
Obviously, adequate preparation is needed for the pre-design, design, operation and post-design of the biogas plants in order to accelerate the development of biogas technology in Nigeria.

**Appropriateness of Digesters for Biogas Production in Nigeria**

Digester design is an important factor in the sustainability of biogas technology. Although a digester can be adapted to suit a purpose different from that for which it was made, for effective performance the type of digesters to be selected depends on several factors including the feedstock type and availability, purpose, operational factors, scale, bacterial growth system, temperature, and population, among others. Table 6 shows examples of digesters commonly used in different applications of biogas technology. Digesters in most developed countries are usually medium to large digesters, while digesters in most developing countries are mostly household or small-scale digesters.

There seems to be, therefore, a correlation between the scale of digester and biogas utilization; with gas utilization in most developing countries specifically for cooking and lighting, while gas utilization in most developed countries is for large scale electricity generation, heat, and vehicle fuels. The three common types of digesters used in most developing countries include Chinese fixed dome digester, Indian floating drum digester, and flexible balloon digester. Of these, the floating drum and fixed dome digesters installations are more robust and expensive than flexible balloon installations, which are cheap but subject to damage. Often, a trade-off needs to be made between choosing between expensive but robust, and cheap but non-durable designs.

In Nigeria, digester suitability could be based on feedstock type and availability, geopolitical zones, population, and climatic vulnerability (*i.e.* rainfall decline, coastal flooding, and erosion). The six geopolitical zones and the year 2011 zone-based population percentage of the states in Nigerian are shown in Fig. 1. According to feedstock type and availability, Table 7 indicates the potential agricultural feedstock that could be used for large-scale biogas in the six zones. In the North West, the major agricultural crops that could generate large quantity of residues for large-scale production of biogas include guinea corn, maize, millet, beans, rice, cotton and groundnut, cassava, and yam. According to the climatic vulnerability (Table 8), the zone is extremely vulnerable, so adequate storage facility is needed to ensure continuous supply of the feedstock, though there is significant irrigation system spread across the zone. For effective costs, time, and labour management, a very large biogas plant dedicated to electricity provision for the whole zone can be located at Gombe, which is a state at the centre of the North East zone. Moreover, since Northern Nigeria is notable for commercial livestock farming, residues from the major crops stated above and livestock manure could be co-digested in the proposed biogas plant. Meanwhile, the availability of livestock manure for biogas production will depend on government support for provision of ranches to prevent nomadic farming, which is the common livestock farming system in the northern Nigeria. Besides the proposed large biogas plant, each state in the region could also support installation of household and community biogas plants that could use municipal wastes, sewage, and household wastes as feedstock.

The major agricultural crops that could be produced in the other five zones and from which large quantities of residues could be generated are also given in Table 8. There is no significant difference in terms of the available feedstock for biogas production in the different zones in Northern Nigeria. There is, however, significant difference between the feedstock available in the Southern and Northern zones.
### Table 6. Classification of Digesters

<table>
<thead>
<tr>
<th>S/N</th>
<th>Basis of Classification</th>
<th>Digester</th>
<th>Description</th>
<th>Merits and demerits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mode of operation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Batch</td>
<td>Batch mode digester</td>
<td>It is filled completely and emptied completely after a fixed retention time.</td>
<td>(i) It is easy and does not require daily routine of feeding. (ii) The major demerit is the unsteady nature of the gas-output.</td>
</tr>
<tr>
<td></td>
<td>Semi-continuous</td>
<td>Semi-continuous mode digester</td>
<td>A portion of the culture media is withdrawn at intervals and fresh medium is added to the system.</td>
<td>(i) It is good for co-digestion of substrates with easy and difficult digestible components. (ii) It does not require much control. (iii) There is often a volume variation of the culture media which can affect its productivity.</td>
</tr>
<tr>
<td></td>
<td>Continuous</td>
<td>Continuous mode digester</td>
<td>It is fed and emptied often automatically at regular intervals.</td>
<td>(i) It is suitable for households where there is daily routine of activities. (ii) The gas output is constant. (iii) It often requires that substrate be fluid and homogeneous especially if it is automatic.</td>
</tr>
<tr>
<td>1</td>
<td>Total Solids</td>
<td>&lt;2%</td>
<td>Anaerobic lagoon digester</td>
<td>It has low capital cost. (ii) It is designed for emission and odour control. (iii) It is not designed to optimize biogas production. (iv) It has a poor bacteria to substrate contact leading to long retention time and low digestion time.</td>
</tr>
<tr>
<td></td>
<td>2-10%</td>
<td>Continuous stirred tank reactor</td>
<td>It is usually cylindrical with low height to diameter ratio with an incorporated mixer to ensure thorough mixing.</td>
<td>(i) It can accommodate wide range of solids. (ii) There is high potential of optimized biogas production. (iii) It has high capital and operational cost.</td>
</tr>
<tr>
<td></td>
<td>11-13%</td>
<td>Plug flow digester*</td>
<td>It is a linearly arranged reactors with inputs entering from one end, and effluents exiting on the other end with a retention time between 20 to 30 days.</td>
<td>(i) It does not require incorporated mixer as the inputs move in a slug (ii) Capital and operating costs are not extremely high (iii) The digester requires occasional cleaning due to down times (iv) The digester prefers non-fibrous feedstock such as animal excrements.</td>
</tr>
</tbody>
</table>

*Tomori (2012); *Ferrer et al. (2011)
Table 6. (Cont’d). Classification of Digesters\(^{ah}\)

<table>
<thead>
<tr>
<th>S/N</th>
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<th>Digester</th>
<th>Description</th>
<th>Merits and demerits</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Gas holder structure</td>
<td>Floating Gasholder</td>
<td>Floating drum digester(^a)</td>
<td>The digester has a movable gasholder that floats either directly on the slurry or in a water jacket of its own.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fixed gas holder</td>
<td>Fixed-dome digester(^b)</td>
<td>The digester has a fixed, non-movable gasholder that sits on top of the digester.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(i) It has the advantage of constant gas pressure as this depends on the weight of gasholder.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(ii) Its construction is easy.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(iii) Material costs of the steel gas holder are high.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(iv) It has a short life span since the steel parts are susceptible to corrosion.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(i) It has low construction costs.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(ii) It has a long life span if it is well-constructed since it is often constructed using non-rusting steel parts.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(iii) The gas is often ineffectively utilized as the gas pressure fluctuates significantly.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(iv) If the digester is not gas-tight, gas leakage may occur.</td>
</tr>
<tr>
<td>3</td>
<td>Stages of anaerobic digestion process</td>
<td>One-stage digestion process</td>
<td>One-stage digester</td>
<td>This is a digester with a single reactor where, all anaerobic processes take place.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Multiple stage processes</td>
<td>Multiple stage digester</td>
<td>This digester consists of two or more reactors where different anaerobic processes take place.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(i) It is less expensive to operate than multiple stage digester.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(ii) It is subjected to frequent disruptions due to occurrence of many reactions in the same reactor.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(i) It is more efficient than single stage system since it allows specialization of acid and methane producing bacteria.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(ii) Biogas production is optimized.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(iii) It is more expensive to run than single stage system.</td>
</tr>
<tr>
<td>4</td>
<td>Temperature</td>
<td>5-20 °C</td>
<td>Psychrophilic digester</td>
<td>It is a digester which is operated at a low temperature range of 5-20 °C.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30-35 °C</td>
<td>Mesophilic digester</td>
<td>It is a digester which is operated at a low temperature range of 30-35 °C.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50-60 °C</td>
<td>Thermophilic digester</td>
<td>It is a digester which is operated at a low temperature range of 50-60 °C.</td>
</tr>
</tbody>
</table>

\(^{ah}\)Tomori (2012); \(^{a}\)Singh and Sooch (2004); \(^{b}\)Santerre and Smith (1982)
### Table 6. (Cont’d). Classification of Digesters\(^{ah}\)

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<tbody>
<tr>
<td>5</td>
<td>Bacterial growth system</td>
<td>Suspended bacteria</td>
<td>In this digester, the bacteria are suspended and distributed throughout the digester.</td>
<td>There is high potential of wash out of bacteria if the system is used for continuous operation.</td>
</tr>
<tr>
<td></td>
<td>Attached bacteria</td>
<td>Fixed film growth digester</td>
<td>In this system, the bacteria are held by a media (e.g. membrane) for a long period.</td>
<td>(i) It allows long SRTs and short hydraulic retention times (HRTs). (ii) Biogas production is optimized.</td>
</tr>
<tr>
<td>6</td>
<td>Scale</td>
<td>Very small scale</td>
<td>Family scale or household biogas plants that cannot produce more than 100 kW</td>
<td>(i) It is simple, cheap, robust and easy to operate and maintain, and can be constructed with local materials. (ii) Millions of family scale digesters are operated in countries like China, India or Nepal.</td>
</tr>
<tr>
<td></td>
<td>Small or medium scale</td>
<td>Farm-scale biogas plants that can produce between 100 and 500 kW</td>
<td>It is designed to produce biogas at the community level for electricity, heat production and gas engine fuel. The feedstock is often from one or two neighbouring farms.</td>
<td>(i) The digester can be simple or complex with various sizes, designs and technologies. (ii) Many farm scale biogas plants are operated in European countries such as Germany, France, Austria, Denmark, and Sweden, among others.</td>
</tr>
<tr>
<td></td>
<td>Large scale</td>
<td>Centralized co-digestion biogas plants that can produce more than 500 kW</td>
<td>The digester is designed for co-digestion of many suitable feedstocks, and it is often centrally located to reduce costs, time and manpower required to transport feedstock and digestate to and from the digester, respectively.</td>
<td>(i) The digester is more profitable to generate higher agricultural, environmental and economic benefits for the society due to its economies of scale, higher capacity utilization and adequate professional management. (ii) This type of digesters is common in most developed countries including Denmark, USA, Germany, France, Sweden, among others. (iii) The demerit of large scale biogas plant is that if there is biological process inhibition there will be a total breakdown in the gas production for a period of time, since there is only one large digester. Furthermore, the installation cost is higher than small scale digester. (iv) High investment costs are often required because of the complex structure which include large reactor volumes, additional components including pumps, temperature regulators, pre-storage tank among others, included in their designs.</td>
</tr>
</tbody>
</table>

\(^{ah}\)Tomori (2012)
Fig. 1. Geopolitical zones and the zone-based population percentage of the states in Nigeria

<table>
<thead>
<tr>
<th>S/N</th>
<th>State</th>
<th>% Zone Population</th>
<th>S/N</th>
<th>State</th>
<th>% Zone Population</th>
<th>S/N</th>
<th>State</th>
<th>% Zone Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Abia</td>
<td>17.2</td>
<td>13</td>
<td>Ekiti</td>
<td>8.6</td>
<td>25</td>
<td>Nasarawa</td>
<td>8.9</td>
</tr>
<tr>
<td>2</td>
<td>Adamawa</td>
<td>16.5</td>
<td>14</td>
<td>Enugu</td>
<td>10.0</td>
<td>26</td>
<td>Niger</td>
<td>13.8</td>
</tr>
<tr>
<td>3</td>
<td>Akwa Ibom</td>
<td>18.8</td>
<td>15</td>
<td>Gombe</td>
<td>12.4</td>
<td>27</td>
<td>Ogun</td>
<td>13.6</td>
</tr>
<tr>
<td>4</td>
<td>Anambra</td>
<td>25.3</td>
<td>16</td>
<td>Imo</td>
<td>24.3</td>
<td>28</td>
<td>Ondo</td>
<td>12.4</td>
</tr>
<tr>
<td>5</td>
<td>Bauchi</td>
<td>24.7</td>
<td>17</td>
<td>Jigawa</td>
<td>12.0</td>
<td>29</td>
<td>Ogun</td>
<td>12.3</td>
</tr>
<tr>
<td>6</td>
<td>Bayelsa</td>
<td>8.0</td>
<td>18</td>
<td>Kaduna</td>
<td>16.9</td>
<td>30</td>
<td>Oyo</td>
<td>20.3</td>
</tr>
<tr>
<td>7</td>
<td>Benue</td>
<td>20.4</td>
<td>19</td>
<td>Kano</td>
<td>26.4</td>
<td>31</td>
<td>Plateau</td>
<td>15.1</td>
</tr>
<tr>
<td>8</td>
<td>Borno</td>
<td>22.1</td>
<td>20</td>
<td>Katsina</td>
<td>16.1</td>
<td>32</td>
<td>Rivers</td>
<td>25.0</td>
</tr>
<tr>
<td>9</td>
<td>Cross River</td>
<td>13.6</td>
<td>21</td>
<td>Kebbi</td>
<td>9.1</td>
<td>33</td>
<td>Sokoto</td>
<td>10.3</td>
</tr>
<tr>
<td>10</td>
<td>Delta</td>
<td>19.6</td>
<td>22</td>
<td>Kogi</td>
<td>15.9</td>
<td>34</td>
<td>Taraba</td>
<td>11.9</td>
</tr>
<tr>
<td>11</td>
<td>Ebonyi</td>
<td>13.2</td>
<td>23</td>
<td>Kwara</td>
<td>11.3</td>
<td>35</td>
<td>Yobe</td>
<td>12.4</td>
</tr>
<tr>
<td>12</td>
<td>Edo</td>
<td>15.0</td>
<td>24</td>
<td>Lagos</td>
<td>32.8</td>
<td>36</td>
<td>Zamfara</td>
<td>9.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>27</td>
<td>FCT, Abuja</td>
<td>9.1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 7. Geopolitical Zones and their Major Agricultural Crops Production in 2010\(^\text{ad}\)

<table>
<thead>
<tr>
<th>Geopolitical zones</th>
<th>Member States</th>
<th>Major Agricultural crops production in 2010 (production in thousand metric tons)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cassava</td>
</tr>
<tr>
<td>North East</td>
<td>Taraba</td>
<td>2,187.96</td>
</tr>
<tr>
<td></td>
<td>Borno</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Bauchi</td>
<td>18.38</td>
</tr>
<tr>
<td></td>
<td>Adamawa</td>
<td>14.19</td>
</tr>
<tr>
<td></td>
<td>Gombe</td>
<td>13.94</td>
</tr>
<tr>
<td></td>
<td>Yobe</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>2,234.47</td>
</tr>
<tr>
<td>North West</td>
<td>Kaduna</td>
<td>2,535.64</td>
</tr>
<tr>
<td></td>
<td>Kebbi</td>
<td>43.04</td>
</tr>
<tr>
<td></td>
<td>Zamfara</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Sokoto</td>
<td>3.71</td>
</tr>
<tr>
<td></td>
<td>Kano</td>
<td>4.16</td>
</tr>
<tr>
<td></td>
<td>Jigawa</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Katsina</td>
<td>54.77</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>2,641.32</td>
</tr>
<tr>
<td>North Central</td>
<td>Kogi</td>
<td>2,879.39</td>
</tr>
<tr>
<td></td>
<td>Niger</td>
<td>999.76</td>
</tr>
<tr>
<td></td>
<td>Benue</td>
<td>3,791.45</td>
</tr>
<tr>
<td></td>
<td>Kwara</td>
<td>1,012.16</td>
</tr>
<tr>
<td></td>
<td>Plateau</td>
<td>294.54</td>
</tr>
<tr>
<td></td>
<td>Nasarawa</td>
<td>1,157.30</td>
</tr>
<tr>
<td></td>
<td>FCT, Abuja</td>
<td>33.62</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>10,168.22</td>
</tr>
</tbody>
</table>

\(\text{ad}\) NBS (2012).
Table 7. (Cont’d). Geopolitical Zones and their Major Agricultural Crops Production in 2010\textsuperscript{ad}

<table>
<thead>
<tr>
<th>Geopolitical zones</th>
<th>Member States</th>
<th>Major Agricultural crops production in 2010 (production in thousand metric tons)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cassava</td>
</tr>
<tr>
<td>South West</td>
<td>Oyo</td>
<td>2,920.01</td>
</tr>
<tr>
<td></td>
<td>Ogun</td>
<td>1,519.79</td>
</tr>
<tr>
<td></td>
<td>Lagos</td>
<td>322.58</td>
</tr>
<tr>
<td></td>
<td>Ondo</td>
<td>2,205.09</td>
</tr>
<tr>
<td></td>
<td>Osun</td>
<td>834.34</td>
</tr>
<tr>
<td></td>
<td>Ekiti</td>
<td>1,377.65</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>9,179.46</td>
</tr>
<tr>
<td>South South</td>
<td>Akwa-Ibom</td>
<td>2,380.37</td>
</tr>
<tr>
<td></td>
<td>Bayelsa</td>
<td>33.04</td>
</tr>
<tr>
<td></td>
<td>Edo</td>
<td>504.43</td>
</tr>
<tr>
<td></td>
<td>Cross-River</td>
<td>3,302.47</td>
</tr>
<tr>
<td></td>
<td>Delta</td>
<td>1,810.60</td>
</tr>
<tr>
<td></td>
<td>Rivers</td>
<td>2,004.19</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>10,035.10</td>
</tr>
<tr>
<td>South East</td>
<td>Ebonyi</td>
<td>1,058.59</td>
</tr>
<tr>
<td></td>
<td>Enugu</td>
<td>2,831.63</td>
</tr>
<tr>
<td></td>
<td>Imo</td>
<td>2,181.93</td>
</tr>
<tr>
<td></td>
<td>Abia</td>
<td>415.74</td>
</tr>
<tr>
<td></td>
<td>Anambra</td>
<td>1,786.72</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>8,274.61</td>
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</table>
Table 8. Geopolitical Zones, Climatic Patterns, and Vulnerability of Major Agricultural Crops Production in 2010

<table>
<thead>
<tr>
<th>Geopolitical Zones</th>
<th>North East</th>
<th>North West</th>
<th>North Central</th>
<th>South West</th>
<th>South South</th>
<th>South East</th>
</tr>
</thead>
<tbody>
<tr>
<td>Projected Population in 2011</td>
<td>22,270,120</td>
<td>41,826,430</td>
<td>24,213,654</td>
<td>32,483,310</td>
<td>24,568,687</td>
<td>18,932,315</td>
</tr>
<tr>
<td>Member states and their zone-based population percentage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taraba</td>
<td>11.9</td>
<td>Kaduna</td>
<td>16.9</td>
<td>Kogi</td>
<td>15.9</td>
<td>Oyo</td>
</tr>
<tr>
<td>Borno</td>
<td>22.1</td>
<td>Kebbi</td>
<td>9.1</td>
<td>Niger</td>
<td>19.3</td>
<td>Ogun</td>
</tr>
<tr>
<td>Bauchi</td>
<td>24.7</td>
<td>Zamfara</td>
<td>9.2</td>
<td>Benue</td>
<td>20.4</td>
<td>Lagos</td>
</tr>
<tr>
<td>Adamawa</td>
<td>16.5</td>
<td>Sokoto</td>
<td>10.3</td>
<td>Kwaara</td>
<td>11.3</td>
<td>Ondo</td>
</tr>
<tr>
<td>Gombe</td>
<td>12.4</td>
<td>Kano</td>
<td>26.4</td>
<td>Plateau</td>
<td>15.1</td>
<td>Osun</td>
</tr>
<tr>
<td>Yobe</td>
<td>12.4</td>
<td>Jigawa</td>
<td>12.0</td>
<td>Nasarawa</td>
<td>8.9</td>
<td>Ekiti</td>
</tr>
<tr>
<td>Katsina</td>
<td>16.1</td>
<td>FCT, Abuja</td>
<td>9.1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Climatic condition</th>
<th>Temperature</th>
<th>Rainfall</th>
<th>Feedstock in high quantity for biogas production</th>
<th>Climate vulnerability Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean maximum temperature ranged from 31.1 to 42.6 °C while monthly minimum temperature ranged from 11.1 to 19.2 °C</td>
<td>Mean maximum temperature ranged from 31.1 to 42.6 °C while monthly minimum temperature ranged from 11.1 to 19.2 °C</td>
<td>Total rainfall varied from 300-1000mm</td>
<td>Guinea corn, maize, millet, beans, rice, cotton and groundnut, cassava, yam and commercial livestock wastes</td>
<td></td>
</tr>
<tr>
<td>Mean maximum temperature ranged from 31.1-42.6 °C while monthly minimum temperature ranged from 11.1-19.2 °C</td>
<td>Mean maximum temperature ranged between 31.1-42.6 °C temperature while monthly minimum temperature ranged from 20.0-24.1 °C</td>
<td>Total rainfall varied from 2000-3000mm</td>
<td>Guinea corn, millet, Maize, beans, groundnut, rice, cotton, soya beans, and commercial livestock wastes</td>
<td></td>
</tr>
<tr>
<td>Total rainfall varied from 2000-3000mm</td>
<td>Mean maximum temperature ranged between 31.1-42.6 °C temperature while monthly minimum temperature ranged from 20.0-24.1 °C</td>
<td>Total rainfall varied from 2000-3000mm</td>
<td>Yam, melon, cassava, maize, groundnuts, soya bean, rice, beans, guinea corn and commercial livestock wastes</td>
<td></td>
</tr>
<tr>
<td>Mean maximum temperature ranged between 31.1-42.6 °C temperature while monthly minimum temperature ranged from 20.0-24.1 °C</td>
<td>Mean maximum temperature ranged between 31.1-42.6 °C temperature while monthly minimum temperature ranged from 20.0-24.1 °C</td>
<td>Total rainfall varied from 2000-3000mm</td>
<td>Cassava, yam, melon, cocoyam, maize, rice and domestic livestock wastes</td>
<td></td>
</tr>
<tr>
<td>Total rainfall varied from 2000-3000mm</td>
<td>Mean maximum temperature ranged between 31.1-42.6 °C temperature while monthly minimum temperature ranged from 20.0-24.1 °C</td>
<td>Total rainfall varied from 2000-3000mm</td>
<td>Cassava, Yam, rice, cocoyam, maize, melon and domestic livestock wastes</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Climate vulnerability Index</th>
<th>1</th>
<th>3</th>
<th>4</th>
<th>6</th>
<th>5</th>
<th>2</th>
</tr>
</thead>
</table>

---

\(\text{a}^a\) NBS (2012); \(\text{b}^b\) Nigeria Climate Review Bulletin (2010); \(\text{c}^c\) Federal Ministry of Environment (Special Climate Unit) (2010)  
1, 2, 3, 4, 5, 6 – degree of relative climate vulnerability (1: extremely vulnerable; 6: least vulnerable)
While cereals and legumes are produced in large quantity in the Northern zones, root and tuber crops such as yam, cocoyam, and cassava are produced in large quantity in the Southern zones. The variation is due to the climatic variations in the different zones. The central states for which the proposed large scale biogas plants could be located in the North West, North Central, South West, South East, and South South are Katsina, Abuja, Oyo, Rivers, and Anambra. The areas chosen for the potential large scale biogas plants should be protected from flooding and other destructive events. Furthermore, livestock farming in all the southern zones are mainly domestic, unlike in the North where it is practiced on a large scale, so the livestock manure may be used for household or community biogas digesters. The digesters could also use sewage, household and municipal wastes as feedstock to biogas production for domestic purposes.

**Biogas Technology Development and Dissemination Strategies**

Development of a viable biogas market is a prerequisite to attracting biogas investors. For a biogas market to be viable, people or potential biogas users must be informed of the benefits accrued unto them by using biogas as energy source. The following strategies are necessary for development of viable biogas market.

*The need for government intervention*

The role of government in stimulating the market penetration of biogas technology cannot be overrated, and thus, for easy penetration of biogas energy into energy market, the government needs to play an active role in ensuring that the biogas energy is sufficient, efficient, affordable, steady, and dependable (Winkler et al. 2011). Government interventions through subsidy provisions and tax holidays are needed to reduce the initial cost of investing in biogas technology. Uninterrupted development of biogas technology and dissemination requires unwavering and long-term government support in many areas, including financial support, legislative support, and technical support. The high level of biogas technology in most developed countries as discussed in Table 5 has been attributed to favourable policy formulation and implementation (Palvas et al. 2010; Stehlik 2010). It is therefore obvious that government support and development of biogas technology are inseparable. Government has an important role to play in the creation of an enabling environment for private sector participation in biogas technology in such a way that the produced biogas will be affordable to meet energy needs of the citizenry. At the present oil price, the initial capital cost of biogas production investment is higher than that of fossil fuel products, which tends to make fossil fuel products more affordable than biogas. However, the biogas sector can be made more affordable than any other energy sources if appropriate government measures are put in place. Favourable policies including a ban on landfilling of organic waste, setting of a target for inclusion of a specific percentage of biogas energy in the Nigerian energy mix, financial remuneration in form of feed-in system incentives, tax exemption/holiday, tipping fees on treated wastes, green certificates, affordable connection fees to the biogas based grid system, low biogas price, subsidy, among others, should be promulgated and implemented so that demand for biogas as a product would be encouraged. Markets for the two main products from anaerobic digesters including biogas and organic fertilizer, should be developed so that the biogas technology would be financially profitable and economically attractive to would-be investors. The economic viability of biogas technology will depend on income generation ability of its two products including biogas for cooking, lighting and power and digestate for organic fertilizer, or for fishpond or
animal feed. Nigeria has about 36 states, a federal capital territory, and 760 local governments with 12, 19, and 5 states having between 6-17, 18-29, and 30-44 local governments respectively. In order to effectively accelerate the development and dissemination of biogas technology, government at all levels including all the three tiers of Nigerian government namely, federal, state, and local governments should bear the bulk of responsibility involved in the development and dissemination of biogas technology.

Increased awareness level and capacity building development

The level of awareness of the benefits of biogas technology needs to be raised, as many people are not acquainted with benefits associated with biogas technology. In the rural area, for example, some people still have the notion that food cooked using fuel wood tastes better than food cooked using other energy sources. There should also be a feedback mechanism whereby biogas credibility as perceived by users could be easily communicated. This speeds up dissemination of the technology. This could be achieved by setting up a monitoring system based on cell phone technology, since most people both in urban and rural areas now use cell phones. Furthermore, many people lack the technical know-how in operating and maintaining biogas plants. People should be trained to construct, operate, and maintain biogas plants for efficient and optimum production. It would also be wise to make use of locally available materials in Nigeria for biogas projects in order to reduce the difficulty involved in getting spare parts of plants and thereby ensure the sustainability of the biogas programme. Capacity building through technical training to enhance local capability in the operation and maintenance of biogas plants could be achieved through the establishment of biogas research institutes or consultancy centres where biogas operators and users can find answers to their various questions and most importantly obtain any urgent assistance they may need from seasoned biogas experts and consultants at the centres.

Regulatory mechanism for biogas market in Nigeria

In 2007, the Nigerian government, upon realizing the urgent need to incorporate biogas energy into her national energy mix, set up a national biofuels initiative under the Renewable Energy Division of Nigerian National Petroleum Corporation (NNPC), to coordinate the development of biofuel technology in the country. A ministry such as NNPC should therefore be organized and equipped to fill in the potential supply-demand gap by buying back excess biogas energy or supplying biogas energy in case of inadequacy as depicted in Fig. 2.
This is necessary since one of the main challenges to biogas dissemination in most countries where biogas technology is well developed is the cost of fuelling appliances using biogas as compared to fuelling it using other conventional energy sources such as petroleum products. Customer’s demand of biogas as a commodity or service that provides utility in terms of energy will depend on their willingness, attitude, and ability to pay for the commodity and the satisfaction derived from its usage. In other words, effective demand of biogas technology as a commodity will depend on factors that include its cost effectiveness, appropriateness, availability, reliability, efficiency, and technical potential. All of these factors need to be put into consideration for successful development and dissemination of biogas technology in Nigeria.

CONCLUSIONS

Assessment of different types of wastes in this study has shown that there is huge potential of biomass feedstock for commercial biogas production in Nigeria. The available Nigerian biomass feedstock includes agricultural crop wastes, livestock manure, abattoir waste, organic MSW, and human waste with potential quantity of 52, 10, 0.8, 33, and 86 million tonnes respectively. Total biochemical potential of biogas that could be generated from the biomass feedstock is 72 billion cubic metres, from which 270 TWh of electricity could be generated, which is enough to satisfy the annual electricity need of the Nigerian population. Furthermore, according to the geopolitical zoning of biomass and manure, northern zones have the potential for high production of cereal and legumes crop residues, while the southern zones have the potential for high production of root and tuber crops. It could be a wise idea to have a large centrally located biogas plants in each geopolitical zone that could be used to generate electrical energy to power each zone.

Presently, biogas energy has not been incorporated into the Nigerian energy mix since the current level of biogas technology in Nigeria is very low. Most of the few existing pilot scale digesters are currently non-operational, while the few biogas plants that are operational have frequent downtimes. The problem has been attributed to technical, economic, and social impediments including poor digester designs, management, maintenance, planning, monitoring, lack of awareness, and inadequate dissemination strategy. Most importantly among the barriers to the dissemination of biogas technology in Nigeria is the lack of support from the government in the area of policy promulgation (legislative framework) and implementation, provision of subsidies, soft loans, and tax incentives in addition to good structural facilities. Meanwhile, in order for Nigeria to meet and surpass the lowest threshold of energy accessibility of 100 kWh of electricity and 1200 kWh of modern fuels per person per year proposed by International Energy Agency (IEA) (AGECC 2010; GEA 2012; IEA 2011), concerted effort and shared responsibility from various stakeholders including policy makers (government), researchers, industries, educators, and end-users must be geared towards introduction of a successful and sustainable biogas technology to provide alignment between economic, social, environmental, and regulatory variables needed for the technology. The target of 80% electricity coverage by 2015 in the roadmap to power sector reforms may not be realizable if urgent measures on accelerating biogas development and dissemination are not put in place (Jonathan 2010). If barriers to the development of biogas technology could be surmounted, opportunities such as huge availability of biogas feedstock, favourable climate which promotes large scale
agriculture, huge population, among others, abounds for accelerated development of biogas technology in Nigeria. Strategies including creation of biogas research or consultancy centres and re-organization of a ministry such as NNPC to fill in the potential supply-demand gap by buying back excess biogas energy or supplying biogas energy in case of inadequacy; provisions of soft loans, promotion of specialized programmes focusing on technology and knowledge transfer from countries with well developed biogas technology, among others, will help in the acceleration and dissemination of biogas technology in Nigeria. The need for Nigeria to diversify her energy sources and toe the line of biogas technology by harmonizing biogas into the existing energy supply-chain cannot be over-emphasized in view of the country’s vision to be among the top 20 economies of the world by 2020. Given political will and government unwavering support coupled with the effective management of technical, social, political, legal, technical, human, cultural, and environmental factors, biogas technology could be evolved and disseminated to meet the daily energy needs of the Nigerian citizenry.

ACKNOWLEDGMENTS

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Membrane bioreactors’ potential for ethanol and biogas production: a review

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Membrane bioreactors’ potential for ethanol and biogas production: a review

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Companies developing and producing membranes for different separation purposes, as well as the market for these, have markedly increased in numbers over the last decade. Membrane and separation technology might well contribute to making fuel ethanol and biogas production from lignocellulosic materials more economically viable and productive. Combining biological processes with membrane separation techniques in a membrane bioreactor (MBR) increases cell concentrations extensively in the bioreactor. Such a combination furthermore reduces product inhibition during the biological process, increases product concentration and productivity, and simplifies the separation of product and/or cells. Various MBRs have been studied over the years, where the membrane is either submerged inside the liquid to be filtered, or placed in an external loop outside the bioreactor. All configurations have advantages and drawbacks, as reviewed in this paper. The current review presents an account of the membrane separation technologies, and the research performed on MBRs, focusing on ethanol and biogas production. The advantages and potentials of the technology are elucidated.

Keywords: membrane bioreactor; ethanol; biogas; filtration

Introduction

Industrial applications of membrane technology for biological applications have in the last decade flourished over the world. Several large companies, such as Kubota, Merck-Millipore, Pall, GE Healthcare, and Sartorius, are now providing membrane products aimed for highly different separation processes. Many membranes are also used in biological processes in the form of membrane bioreactors (MBRs). At present, most MBRs are employed in water or wastewater treatment, in order to achieve a quality of the effluent, sufficiently suitable for reuse or recycling.\[1,2\] MBRs for wastewater treatment are well developed, and are today used commercially in many countries for mostly dilute wastewater streams, while MBRs for ethanol production is still a new concept and need development for larger scale utilization.

Although there is widespread industrial application of membrane technology in wastewater treatment, the treatment process has been limited to aerobic biological process with only few industries employing anaerobic processes owing to the feed types, biological process and operational conditions of anaerobic MBR (AnMBR).\[3,4\] The complexity of the biological reactions and different types of microorganisms involved in anaerobic processes presents unique effects on the membrane fouling characteristics, when compared with the aerobic processes. Moreover, there is high probability of process failure due to the presence of inhibitory substances, such as heavy metals, chlorinated hydrocarbons, and cyanides, often present in feeding wastewaters or sludge. There is also additional cost of heating the AnMBRs to mesophilic or thermophilic temperatures especially during cold climates.\[5\] These challenges result in the limited industrial application of AnMBRs for wastewater treatment and biogas/ethanol production. However, the interest in application of anaerobic process in membrane reactors for wastewater treatment is increasing because the process unlike the aerobic process has the potential of energy recovery from the waste and reduction of greenhouse gas emissions.\[3\] Furthermore, AnMBR, unlike aerobic MBR (AeMBR) produces very low amount of residual sludge since it has low biomass yields and growth rates. With more exhaustive studies to address the challenges of anaerobic processes, full-scale industrial application of AnMBRs will be widespread. MBRs for water and wastewater treatment have been extensively covered in numerous other reviews,\[6–9\] and are therefore not in focus in this paper. Some MBRs producing biogas from wastewater is, however, mentioned, as some MBRs treating wastewater can be utilized for anaerobic biogas production.

One of the major complications in biotechnology and in biological processes, hampering a successful commercial process, is downstream processing, such as separation of products or biocatalysts from the spent medium or product purification.\[10\] Furthermore, product streams formed by
biological processes are often very dilute, and consist of a complex mixture of components. Downstream separation processes can therefore be both expensive and technically challenging.[10] As a consequence, bioprocesses in general, and downstream processing in particular, need to have their process steps enhanced and integrated, in order to improve yields, cut process time, and reduce operation and capital costs.[11]

In MBRs, a biological process is integrated with a permselective membrane. In the process of ethanol or biogas production, the membrane can either be used for separating cells from the medium, thereby increasing the biomass concentration in the bioreactor, to aid removal of the inhibitor, or to recover product in situ. Membrane separation techniques can also easily be coupled with continuous processes.[12] Continuous processes have several advantages compared with traditional batch operations, especially since they use smaller reactors or employ higher dilution rates, lowering capital as well as maintenance costs. The continuous process demands an overall low level of inhibitors in the culture medium and/or high cell concentrations,[13] which is accomplished with the MBR technology. Furthermore, slow-growing microorganisms in anaerobic biogas reactors, such as methanogenic bacteria, can benefit from being retained inside the bioreactor.

The aim of the present review was to introduce the MBR technology in ethanol and biogas processes, and to summarize the development of MBRs and the membrane technologies for these biofuels, along with their advantages and future potential.

Ethanol and biogas production

Ethanol and biogas (methane) are renewable and environmentally friendly fuels which can be used as an alternative to the traditional fossil fuels. Optimistically, the development of ethanol and biogas production can decrease the world’s dependence on fossil fuel, thereby reducing the excessive emissions of greenhouse gases, which accelerate global warming. As both ethanol and biogas can be produced from renewable feedstocks, such as agricultural, municipal and forest residues, their production does not contribute to the net emissions of carbon dioxide. The basic problem with ethanol and biogas production is the question of process economy and product yield. One interesting and promising approach is to use membrane technology and MBR processes, which, by assisting in achieving high cell densities, separating cells, products, or residual compounds in the process, greatly would improve the ethanol and biogas production economy.

In anaerobic digestion of waste materials, the amount of biogas (mainly methane and carbon dioxide) produced is mainly dependent on the interactions of different consortia of degrading microorganisms. The conversion of organic material to methane is immensely complex, and consists of four major stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The last stage, performed by methane-forming bacteria, is commonly the most sensitive step in the process, since methane-forming bacteria have a very slow growth rate and are sensitive to inhibitors, pH, and other process conditions. It is therefore important to prevent bacteria from being washed out from the reactor, and to reduce inhibitor levels. AnMBRs have proven to provide a successful technique for avoiding cell washout, longer retention times, and presence/accumulation of toxic compounds.[14]

Ethanol is currently produced mainly from sugar and starch-rich materials, which are also used as food and feed. The current global debate on food vs. fuel renders these types of raw materials little appeal for ethanol production. Being omnipresent, wastes and lignocellulosic residuals from municipalities, agriculture, and forest industries are deemed more suitable as raw materials.[15] Nevertheless, lignocelluloses are usually very recalcitrant, and need pre-treatment or complete hydrolysis into fermentable sugars prior to being utilized for production of, e.g. ethanol or biogas.

Various different sugar containing hydrolysates produced from lignocellulosic materials have been applied for ethanol production in MBRs. Lignocellulosic materials are abundant and thus of interest as feedstock for producing fermentable sugars for ethanol production. However, the material has to be hydrolysed by, e.g. acid or enzymatic hydrolysis, prior to fermentation. Dilute acid hydrolysis is performed at high temperature, with relatively low concentrations of, e.g. sulphuric acid. During the degradation process, several toxic compounds are formed, which could affect the fermentation process negatively. Numerous studies have therefore been carried out, striving to find ways to overcome the toxicity of hydrolysates, e.g. by using high cell densities,[16] MBR,[17,18] or detoxification.[19,20]

In the production of bulk fuels, such as ethanol and biogas, every step has to be optimized in order to obtain an economically viable large-scale production. It is therefore crucial to maximize productivity without cell cultures being destabilized due to, e.g. inhibition problems or energy-intensive product recovery. Continued development of MBRs will hopefully aid in resolving these issues.

Membrane concept

In order to separate components or cells in a liquid mixture by means of filtration, membranes are coupled with the bioreactors. The porous membrane is manufactured to contain ceramic, metallic, or polymeric material. The separation of the mixed compounds in the liquid is usually brought about by applying pressure or vacuum across the porous permselective membrane, but can also be prompted by a concentration gradient.[21,22] The membrane forms a barrier, allowing some components to pass the membrane more readily than others, and this selectivity is mostly determined by the pore size of the membranes,[1] but
Environmental Technology

other characteristics of the membranes, such as hydrophilicity, can also affect the selection, hindering hydrophilic (or hydrophobic) components to permeate the membrane.[23] Membranes are typically defined according to their separation mode, i.e. microfiltration, ultrafiltration, nanofiltration, and reverse osmosis. In MBRs, aimed at ethanol and biogas production, microfiltration and ultrafiltration membranes are employed.

Presently, there are two principally different MBR designs, where the membrane is either operated under direct pressure or vacuum. In the first configuration, with direct pressure, the membrane is placed in an external loop separated from the bioreactor (external cross-flow membrane) and a pump forces the bioreactor broth into the membrane module and to permeate through the membrane (Figure 1(a)).[22] The external cross-flow MBRs are operated in a cross-flow mode, where the liquid to be filtered flows with high velocity parallel to the membrane surface, which thereby also hinder cake formation on the membrane surface. This mode of operation reduces the fouling tendency of external cross-flow membranes,[24] and increases the flux through the membrane. Among the disadvantages of external cross-flow MBRs, are the significant energy amounts required for sustaining a continuous flow through the membrane,[1] and the complex reactor design can be mentioned.

In contrast, when the membrane is operated at lower than atmospheric pressure or vacuum, a pump can be used to pull permeate through the membrane. This configuration is usually named submerged or immersed, since the membrane is placed directly in the liquid (Figure 1). The advantages of submerged MBRs are that they usually require less energy to run compared with external cross-flow MBR.[1,22] The submerged MBR can, however, be problematic to operate at high particulate or cell concentrations, due to fouling. Usually a larger membrane surface area has to be applied in submerged than cross-flow MBRs.[1] A way to disrupt fouling and cake formation is to vigorously purge gas across the submerged membrane surface.[22]

There are two configurations in which the vacuum-driven submerged membranes can be designed. The membrane can either be immersed directly into the bioreactor (Figure 1(b)), or submerged in a separate container which is connected to the bioreactor (Figure 1(c)). The external chamber configuration with submerged membrane has the advantage of being easier to clean,[22] but require energy to pump the retentate back to the bioreactor.

When membranes do not allow components to pass through, the particles or cells in the concentrated liquid tend to attach and accumulate on the membrane surface, curbing the filtering process. Upon component accumulation, the flow through the membrane is reduced by different phenomena. In combination, these phenomena are referred to as fouling, where compounds deposit on the membrane surface or inside the membrane. Fouling is also the main obstacle for optimal membrane function.[1] MBRs operation is largely dependent on the membrane flux and usually overtime membrane fouling will result in a lowered permeate flux or an increased transmembrane pressure (TMP). If the membrane in the MBR is operated at a flux below a critical value, the TMP can be kept constant with no fouling of the membrane. If the flux is increased over a critical flux value, the TMP will increase rapidly together with membrane fouling. However, there is never any perfect non-fouling operation,
but by keeping the flux at a value lower than the critical flux (sustainable flux) can give a slower linear increase in TMP.[2,22] By monitoring the TMP overtime, the membrane module can also be cleaned prior to extensive fouling, which makes long cycles of continuous processes possible.

If the fluid to be filtered contains a large amount of solids and soluble compounds, as is the case during fermentation processes, the membrane flux tends to fall sharply with increasing time and cell growth,[12] Different techniques have been explored to overcome this problem, e.g. turbulent promoters,[25] rotating filters,[26,27] unsteady flows,[28] intermittent jets,[29] periodical inversion of flows,[30] ultrasound,[31] gas injection,[12,32] anti-fouling membranes,[2] and dynamic membranes.[33] Most of these methods increase the turbulence of the liquid near the membrane, which destabilizes the cake layer on the membrane surface, thereby improving the filtration flux. The most suitable method to maintain a high permeate flux is dependent on which membrane module is applied. For example, gas sparging has been shown to be effective to reduce concentration polarization and/or increase permeate flux in tubular, hollow-fibre, plate and frame (PF) and spiral wound (SW) membrane modules.[34]

Ultrafiltration membranes used for cell separation are reported to have a lower fouling tendency than microfiltration membranes, which may be attributed to differences in pore size. Since the pores of microfiltration membranes are in the same order of magnitude as the microorganisms, smaller cells can lodge themselves inside the larger pores, causing physical blockage of the pores.[35] However, if the pores are much smaller than the cells, as in ultrafiltration membranes, the shear force of the flow will force the cells to ‘roll off’ the surface.[35] Besides the cell concentration, other components in the media, such as proteins, carbohydrates, and particles, hold the potential to increase fouling rates.[35,36] In the present paper, the phenomena of fouling are not covered in further detail, as it is discussed immensely elsewhere.[2,8]

Membrane processes: development trends and perspectives

Membrane filtration is presently used within many different areas, such as biopharmaceutical processes, desalination, water treatment,[8] food and beverage manufacturing, industrial production of paints, adhesives, chemicals, etc.[35,37] The demands on the filtration processes and the filter membranes largely depend on the application. Regulations for filters and filtration processes are very strict in drug and other biopharmaceutical applications, whereas filters used in, e.g., paint, adhesive, and chemical industries are much less refined, and considerably cheaper. In the beverage industry, where it is essential to remove microorganisms to avoid contamination and consumer illness as well as microbial spoilage, microfiltration may be an alternative to pasteurization, given that an adequate membrane is used to filter the product.[37]

In bioprocesses, membrane separation is used for different upstream and downstream applications.[21,38] The interest in membranes for biotechnological applications is mainly driven by the demand for higher productivity and reduced production costs. Over the years, MBRs have proven to hold many advantages; they offer for instance high product yields, ascertained sterility, high biological activity, and superior separation efficiency. In addition, the process usually has a low energy consumption, enables a continuous operation, and is simple to operate and scale up. At present, membranes are applied in biotechnology for sterile filtration, liquid clarification, cell harvesting, virus removal, protein concentration, etc.[21]

Configuration of membranes in MBRs

The membranes for MBRs are produced in various configurations, such as PF, hollow-fibre, SW, and tubular geometries.[21,39] Hollow-fibre membranes have been applied at a laboratory scale, as they provide a large surface area per packing density.[21] In ethanol and biogas production processes, many different configurations have been tested at a small scale, several of these are described in this paper.

Hollow-fibre and tubular membrane reactors

Yeast has been successfully immobilized inside hollow-fibre membranes, which normally are used for ultrafiltration. Hollow-fibres membranes have hence been employed for the cultivation of yeast, bacteria, mammalian cells,[39] and enzymes.[40] Hollow-fibre membranes are generally used in bundles, joined and sealed with a cylindrical housing at each end. The housing enables separation of the extracapillary space from the fibre lumen. Cells have been cultivated in the extracapillary space of the module, while the medium was pumped through the lumen space, and nutrient molecules diffused through the fibre membrane. The porosity of the membrane needs to be selected in accordance with what the membrane must retain, i.e., the cells and the product molecules, or only the cells.[39]

Very high cell densities have been acquired in hollow-fibre reactors, since they offer very large surface area per volume.[41] The housing of the hollow-fibre membrane module allows the cells to escape shear forces and contamination, since the porosity of the membrane facilitates selective nutrients to permeate the extracapillary space.[39]

A previous study[40] describes how *Saccharomyces cerevisiae* was grown on the shell side of asymmetric-walled polysulfone membranes as well as on the surface of isotropic-walled polypropylene hollow-fibre membranes. The medium was continuously pumped through the hollow-fibre lumen in order to supply the cells with nutrients, and to remove products by diffusion. In the asymmetric
membranes, the yeast reached very high, tissue-like densities of more than $10^{10}$ cells/mL, and in some regions the cells accounted for almost 100% of the volume. However, the cell packing showed a radial distribution across the fibre wall, indicating that the cells located further than 100 μm from the lumen surface did not receive sufficient amount of glucose. In the asymmetric membranes, the yeast density reached approximately $3.5 \times 10^9$ cells/mL.[40] Notwithstanding that very high cell densities were reached, low nutrient and product transport rates curtailed the system performance, and over time, accumulation of CO$_2$ inside the reactor reduced the ethanol production as well. The highest ethanol productivity reported was 26 g/(L h).[40] However, much higher productivities have been achieved by, e.g. fermenting lactose in an MBR, using a cross-flow membrane. Cheryan and Mehaia [13] reached an ethanol productivity of 240 g/(L h) at a cell concentration of 90 g/L.

There are nevertheless some drawbacks with the use of hollow-fibres, such as fouling, clogging fibres, problems in accessing the cell mass, difficulty to sustain a well-defined fibre spacing, and rupturing fibres when cells grow and produce gas.[39] In external hollow-fibre modules, the high recirculation speed will furthermore result in high pumping costs, and may also damage the cells.[24]

Tubular membranes may be preferable to hollow-fibres, in order to avoid the risk of clogging fibres.[42,43] In a trial conducted by Escobar et al.[43] a set of ceramic tubular membranes was tested at pilot scale in a 7000 L MBR. Ceramic tubular membranes were applied as they can easily be backwashed, have high flux rates, and can be cleaned with aggressive chemicals if needed. The cell concentration inside the MBR was regulated and kept at below 120 g/L. By applying low fluxes (below 70 L/m$^2$h), the membranes could be operated successfully for approximately 4.5 days before cleaning. However, operating the membranes at higher fluxes required cleaning more often.[43]

**Plate and frame and spiral wound membrane reactors**

Even though PF membrane reactors have a lower surface area per volume compared with hollow-fibres, they hold most of the advantages of hollow-fibres. PF MBRs have been applied for many purposes in order to produce, e.g. antibodies or enzymes or to apply enzymes by themselves. These flat membranes can be used, e.g. in frames that are separated with a spacer creating a space between the membranes where cells or enzymes can be added.[44] In these MBRs, the space between the membranes is accessible and the cells can hence be replaced if necessary. Furthermore, the distance between the cells and the medium is easily controlled by changing the spacers thickness between the two flat membranes.[39] PF modules which do not have any spacer material between the membranes also exist.[45]

The PF membrane can also serve as a separation unit to separate, e.g. pectin, antibodies, cells, enzymes from a liquid in the same way as hollow-fibres [44] or to concentrate, e.g. fermentation broths.[46] For example, Pyle et al. [46] used both flat sheet and tubular membrane modules to concentrate a bakers’ yeast suspension up to 20% dry weight. In a study performed by Thuander,[47] a PF module from Alfa Laval was used to retain cells in the bioreactor during continuous fermentation of molasses. The PF module was both employed internally and externally to the bioreactor. During the cultivation, the membrane flux rapidly decreased down to 6.3–4.6 L/m$^2$h during the first hours, were it stayed during the main part of the cultivation. The yeast concentration increased steadily during cultivation and reached almost 15 g/L after around 60 h when using the external PF module.[47]

As in PF modules, SW modules also use flat sheet membranes. However, in SW modules the membrane sheets are tightly wound together around a central collector tube, usually with a mesh-like spacer between each sheet of membrane. The design of the SW modules makes them only suitable for use on feed streams that only contain fine suspended solids.[48] In ethanol production, SW modules have, e.g. been used to combine two separation processes, extraction and membrane permeation in a single module in order to lower the energy cost during ethanol separation. Offeman and Robertson [49] successfully developed a SW module where ethanol in the fermentation broth could be separated. In the module, one membrane contains solvent and extracts the ethanol from the broth. A second membrane can then by the help of a vacuum removed the ethanol from the solvent.[49] SW modules have also been applied during enzymatic hydrolysis of corn starch to produce clarified glucose syrup. The formed sugars can thereafter be utilized for ethanol production.[50]

**Membrane technology in ethanol and biogas processes**

**Ethanol production process**

Attaining a high ethanol productivity in the bioreactors is crucial for keeping the bulk chemical costs low. Keeping the cell density high is one way of achieving high productivity, but dilution rate and cell growth rate undermine this in conventional continuous cultivations. Today, centrifugation or filtration is the most preferred mode of separation of microbial cells at the industrial scale.[51] However, other cell retention methods, such as cell immobilization,[52–55] encapsulation,[53,56–59] or cross-flow membranes,[13,18] have also been utilized to maintain high biomass concentrations in the bioreactor. MBRs have been applied in several studies on cell recycling, with the purpose of gaining higher productivity.

MBRs have been examined for ethanol production, with the membranes coupled either internally or externally to the bioreactor,[18,60] and different types of membrane technologies and MBRs were tested with the aim of improving the fermentative production of ethanol. MBRs have also
been used for cell retention/recycling [13] to obtain high biomass concentrations in the bioreactors. Furthermore, extractive MBRs have been applied to remove inhibitors such as ethanol,[61,62] and pervaporation MBRs have been used to eliminate volatile inhibiting compounds from the cultivation broth.[63]

Mercier et al. [12] succeeded to reach a final yeast concentration of 150 g/L by performing a 100 h continuous fermentation, using cross-flow filtration to recycle cells back to the bioreactor. To avoid severe fouling and declining membrane flux at higher biomass concentrations, air was injected into the feed stream.[12] In contrast, Lafforgue et al. [42] were able to obtain as much as 345 g/L of yeast biomass in a bioreactor coupled to a microfiltration membrane for cell recycling. Within 100 h, the total biomass level had reached 300 g/L with a viability of 75%, and the system was stable for more than 50 h. While performing fermentation at very high yeast concentrations, both cell size and morphology were altered, and the viscosity of the broth was increased.[42]

Several studies on ethanol fermentation in MBRs have been conducted, using, e.g. glucose as the carbon source [17,60,61,64]; these are summarized in Table 1. Chang et al. [60] used an internal stainless steel filter, and could successfully produce ethanol from glucose at an average yield of 92.7% of the theoretical yield, with a productivity of 20 g/(L h) ethanol, much higher than achieved in traditional continuous systems.[70] The MBR was furthermore operated for 10 days without complications, and showed a stable ethanol production at an average cell concentration of approximately 55 g/L. The cell concentration was maintained at the same level by controlling dilution rate and bleed ratio. Several other substrates such as tapioca hydrolysate,[17] wood hydrolysate,[16,18] and lactose [13] have also been successfully used in MBRs for fermentation of ethanol.

Fermentation of the liquid part of enzymatically hydrolysed oak wood was successful in a continuous cultivation, using an MBR with a submerged membrane, and yielded an ethanol concentration of more than 70 g/L.[65] Prior to fermentation, the hydrolysate was concentrated up to 180 g/L glucose by vacuum evaporation, and sterilized at 60°C for 120 min. The low temperature applied was to avoid the formation of large amounts of toxic materials during the sterilization process. The maximum ethanol productivity during the continuous fermentation was 16.9 g/(L h).[65]

Removal of inhibitor from the fermentation processes

Fermentation can be inhibited by a high concentration of the product (e.g. ethanol), carbon sources (such as sugars), and other compounds that follow the process, such as furans or phenolic compounds present in the lignocellulosic hydrolysates. Product inhibition can be reduced by removing or degrading the toxic compounds from the broth in situ, using different methods. Continuous product recovery of volatile compounds can be carried out by creating a vacuum in the bioreactor, or in a separate chamber, where the volatile inhibitory compounds (e.g. ethanol) subsequently can be distilled from the broth.[71,72] Nonvolatile inhibitors can be removed by using conventional liquid extraction methods (the most commonly used method)[11,73,74] adsorption on ion-exchange resin,[75] activated carbon,[45,76,77] or polymeric adsorbents.[78]

Inhibitor levels are also possible to reduce by using MBRs. Since very high cell densities can be achieved in MBRs, the cells’ own capacity of performing in situ detoxification of some compounds can be utilized. For example, one study showed that a continuous yeast cultivation in an MBR was able to ferment a sugar solution containing up to 17.0 g/L furfural, without drastic changes of ethanol productivity. Furfural is known to severely affect the growth of yeast, even at low concentrations. However, by effectively keeping the yeast at a high density in the MBR enabled rapid degradation of the incoming furfural, leaving the permeated fluid from the MBR with merely low levels of furfural.[79]

The fermenting yeast can also be protected from toxic compounds in the medium by means of encapsulation. By enclosing the yeast inside spherical hydrophilic alginate membranes, hydrophobic inhibitors, such as limonene present in citrus waste, can be hindered to enter and negatively affect the cells. For example, Pourbafrani et al. [23] encapsulated yeast in this manner and were able to ferment a medium containing as much as 1.5% limonene.

A high concentration of ethanol tends to have inhibitory effects on the process, and the toxicity of ethanol is hence directly related to the sugar concentration in the medium.[80] As an extreme example, an ethanol-tolerant yeast strain of Saccharomyces diastaticus was able to yield a final ethanol concentration of 17.5% (v/v).[81] High ethanol concentrations result in lower batch yields, thus reducing the production capacity. On the other hand, recovering ethanol from a dilute fermentation broth requires a large amount of energy, resulting in increased processing costs.

Membrane-based extraction

Ethanol fermentation by means of membrane-based extraction has previously been reported.[61,82] The membrane separates the aqueous and solvent phases, thus eliminating formation of emulsions and a need to separate the aqueous and solvent phases in downstream processes. Both SW, PF and hollow-fibre membranes have been used to separate the culture medium from the extracting solvent. For instance, multi-membranes, composed of three separate flat sheet membranes, were employed for extractive ethanol fermentation by Cho and Shuler.[62] Applying tributyl phosphate as the extracting solvent, they succeeded to completely ferment 200 g/L glucose, using a 6:4 volume ratio of tributyl phosphate to medium. Franke and Sirkar [83] performed hollow-fibre membrane extractive
<table>
<thead>
<tr>
<th>Membrane configuration</th>
<th>Membrane material</th>
<th>Pore size/MWC</th>
<th>Microorganism</th>
<th>Working volume/reactor size</th>
<th>Biomass (g/L)</th>
<th>Productivity (g/(L h))</th>
<th>Medium</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Cell retention</td>
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<tr>
<td>Submerged</td>
<td>Porous stainless steel cylindrical tubes</td>
<td>2/10 μm</td>
<td><em>Saccharomyces cerevisiae</em> ATCC 24858</td>
<td>1.5 L</td>
<td>50–150</td>
<td>20</td>
<td>Glucose 100 g/L</td>
<td>[60]</td>
</tr>
<tr>
<td>Submerged</td>
<td>Porous stainless steel cylindrical tubes</td>
<td>2 μm</td>
<td><em>Saccharomyces cerevisiae</em></td>
<td>1.8 L</td>
<td>208</td>
<td>14.7</td>
<td>Glucose 100 g/L</td>
<td>[17]</td>
</tr>
<tr>
<td>Submerged</td>
<td>Ceramic cylindrical tubes</td>
<td>0.3 μm</td>
<td><em>Saccharomyces cerevisiae</em></td>
<td>1.5 L</td>
<td>1.5 × 10^9 cells/mL</td>
<td>28.4</td>
<td>Tapioca hydrolysates</td>
<td>Wood hydrolysate</td>
</tr>
<tr>
<td>Submerged</td>
<td>Ceramic cylindrical tubes</td>
<td>0.3 μm</td>
<td><em>Saccharomyces cerevisiae</em></td>
<td>1.5 L</td>
<td>58</td>
<td>13</td>
<td>Glucose 100 g/L</td>
<td>[66]</td>
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<tr>
<td>Submerged</td>
<td>Fluoro polymer</td>
<td>2 μm</td>
<td><em>Saccharomyces cerevisiae</em> (Bakers’ yeast)</td>
<td>140 L</td>
<td>15</td>
<td>–</td>
<td>Molasses</td>
<td>[47]</td>
</tr>
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<td>External cross-flow</td>
<td>Durapore filter (Millipore)</td>
<td>0.45 μm</td>
<td><em>Saccharomyces cerevisiae</em> ATCC 96581</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Wood hydrolysate</td>
<td>[18]</td>
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<tr>
<td>External cross-flow</td>
<td>Ceramic tubular</td>
<td>–</td>
<td><em>Saccharomyces cerevisiae</em></td>
<td>700 L</td>
<td>60–100</td>
<td>33</td>
<td>Dextrose 93–95 g/L</td>
<td>Glucose 150 g/L</td>
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<td>Ceramic/titanium oxide tubular</td>
<td>0.14 μm</td>
<td><em>Saccharomyces cerevisiae</em></td>
<td>2.4 L</td>
<td>300</td>
<td>–</td>
<td>Tapioca hydrolysates</td>
<td>Wood hydrolysate</td>
</tr>
<tr>
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<td>Ceramic tubular</td>
<td>50,000 MWC</td>
<td><em>Kluyveromyces fragilis</em> NRRL 2415</td>
<td>0.5–3 L</td>
<td>90</td>
<td>240</td>
<td>Lactose 50 g/L</td>
<td>[13]</td>
</tr>
<tr>
<td>External cross-flow</td>
<td>Ceramic tubular</td>
<td>150,000 MWC</td>
<td><em>Saccharomyces cerevisiae</em> CBS 8066</td>
<td>R1: 4.5 L R2: 1.5 L</td>
<td>R1: 59 R2: 156.8</td>
<td>4</td>
<td>Glucose 100 g/L</td>
<td>[64]</td>
</tr>
<tr>
<td>Hollow-fiber fermentor</td>
<td>–</td>
<td>50,000 MWC</td>
<td><em>Saccharomyces cerevisiae</em> NRRL-Y-132</td>
<td>625 mL</td>
<td>260</td>
<td>17</td>
<td>Glucose 100 g/L</td>
<td>[67]</td>
</tr>
<tr>
<td>Hollow-fiber fermentor</td>
<td>Polysulfone, polypropylene</td>
<td>10,000 MWC</td>
<td><em>Saccharomyces cerevisiae</em> ATCC 4126</td>
<td>–</td>
<td>10^{10} cells/mL</td>
<td>17</td>
<td>Glucose 10–89 g/L</td>
<td>[40]</td>
</tr>
<tr>
<td>Hollow-fiber fermentor</td>
<td>Polyamide</td>
<td>10,000 MWC</td>
<td><em>Saccharomyces cerevisiae</em> ATCC 24858</td>
<td>40 mL</td>
<td>6</td>
<td>10</td>
<td>Glucose 100 g/L</td>
<td>[41]</td>
</tr>
<tr>
<td>(B) Extractive fermentor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hollow-fiber fermentor</td>
<td>Celgard® X-20 (polypropylene)</td>
<td>–</td>
<td><em>Saccharomyces cerevisiae</em> NRRL-Y-132</td>
<td>75 mL</td>
<td>100–300</td>
<td>31.6</td>
<td>Glucose 300 g/L</td>
<td>[61]</td>
</tr>
<tr>
<td>(c) Pervaporation fermentation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Submerged</td>
<td>Silicalite</td>
<td>–</td>
<td>Dry bakers’ yeast (Oriental yeast)</td>
<td>500 mL</td>
<td>5.0 × 10^8 cells/mL</td>
<td>–</td>
<td>Glucose 100–120 g/L</td>
<td>[68]</td>
</tr>
<tr>
<td>Submerged</td>
<td>Silicalite-silicon rubber</td>
<td>–</td>
<td>Dry bakers’ yeast (Oriental yeast)</td>
<td>150 mL</td>
<td>10</td>
<td>–</td>
<td>Glucose 200 g/L</td>
<td>[69]</td>
</tr>
</tbody>
</table>

Note: MWC, molecular weight cutoff.
fermentation, with dibutyl phthalate as solvent. The advantage of extracting the inhibitors was however limited due to low glucose levels during the fermentation process. The full potential of extractive fermentation is only attained at high glucose concentrations. Kang et al. [61] used a feed medium holding a glucose concentration of 300 g/L in their extractive fermentation with hollow-fibre membranes, and the derived ethanol productivity increased significantly during the in situ solvent extraction, particularly when the solvent/substrate ratio was increased.

Improving the membrane separation technology of fermentation and distillation would greatly increase the potential for making ethanol production technically appealing and more economical. Ethanol separation need to be more cost-effective and less energy-intensive.[77] Online solvent extraction and pervaporation are techniques that use membranes to facilitate the separation of ethanol from the fermentation broth.

**Pervaporation of ethanol**

In the pervaporation process using hydrophobic membranes, components are transformed from liquid to vapour and transferred through a hydrophobic membrane. Selectivity is not based on the thermodynamic evaporation equilibrium, but on the differences in membrane sorption, and diffusion properties of the different components. Using hydrophobic pervaporation membranes for separating volatile organic compounds (e.g. ethanol) has become a fascinating alternative to conventional processes, such as distillation, extraction, adsorption, and stripping.[63] Pervaporation membranes can be connected either externally in a sub-stream, or be integrated into the bioreactor.

One of the virtues of coupling hydrophobic pervaporation membranes with the fermentation process is the elimination of volatile compounds exerting product inhibition, by selectively removing these compounds from its dilute aqueous medium. By keeping the inhibitory compounds below a critical level, the overall productivity is enhanced. Another advantage of the pervaporation technique is the ethanol concentration attained during the process. Since the permeate from the hydrophobic pervaporation membrane holds a high concentration of ethanol, the energy needed for further concentration and purification is low. Although ethanol and water can be effectively separated by distillation, this procedure constitutes an energy sink in the process.[77] As much as 60% of the energy obtained from combustion of ethanol may be required for the distillation separation process.[78]

Different membranes made of e.g. polyvinyl alcohol, polyurethane,[84] and silicalite,[68] have been utilized for pervaporation of ethanol during fermentation. Hydrophobic pervaporation membranes are usually made of silicons, and are hence impermeable to electrolytes. As a consequence, most organic acids and salts are enriched in the fermenter.[68]

By using a silicalite pervaporation membrane, Nomura et al. [68] succeeded to extract a permeate from fermentation broth, with a high concentration of ethanol. The permselectivity of the hydrophobic pervaporation membrane enabled a permeate containing 81.0% ethanol to be extracted from a fermentation broth, where the concentration of ethanol was 4.73%.[68] By-products, such as acidic compounds in the fermentation broth, can however adsorb onto the membrane silicalite surface, and negatively influence the pervaporation performance. This can be prevented by coating the surface with a thin layer of hydrophobic silicon rubber.[69]

**Application of membrane technology in biogas production**

All aerobic membrane technologies (AeMBRs) utilized so far for wastewater treatment can be used for biogas production in an anaerobic process with slight design modification of the AeMBR. AnMBR technology for wastewater treatment still needs to scale the hurdle of operational parameters including low temperatures and hydraulic retention times comparable to AeMBR technology.[85] However, AnMBR technology has received some degree of acceptance in the recent years as a substitute in place of AeMBR for wastewater and other waste streams. It is because of AnMBRs potential of energy recovery in addition to providing the same benefits as AeMBR with reduced energy, while AeMBRs require high energy input for aeration.[86] In AnMBR, emphasis is on biogas recovery along with waste treatment. The absence of oxygen as an electron acceptor in anaerobic process prompts the microbial systems to dispose electrons into methane (biogas).[14] Unlike AeMBRs which emits greenhouse gases such as carbon dioxide and nitrous oxide (if nitrifying/denitrifying), AnMBRs generate useful gases such as biohydrogen and biogas that could be used for energy production.[87] AnMBR technology is being applied for biohydrogen production since hydrogen has high energy content (142 kJ/g) and devoid of harmful emissions during utilization.[88] The advantage of the AnMBR technology in this area is that both biohydrogen and biogas could be produced using a single membrane system since in anaerobic digestion, the hydrogen production phase occurs briefly before the methanogenic phase occurs. The methanogenesis could be inhibited using various means including chemical inhibition, pH control, promotion of ferric-reducing conditions, control of hydrogen partial pressure, among others.[89,90]

Furthermore, membrane technology has been developed for biogas purification, which eliminates major problems commonly encountered in conventional methods of purification. For instance, high concentration of carbon dioxide in biogas makes its desulphurization difficult in conventional method that is based on a chemical reaction of hydrogen sulphide (H₂S) with quicklime or slaked lime in a solid or liquid form. This is because the carbon dioxide also reacts quickly with the quick and slaked lime making it insufficient for biogas desulphurization.[91] Moreover, conventional biogas
Table 2. Application of membrane technology for hydrogen and biogas production.

<table>
<thead>
<tr>
<th>Membrane configuration</th>
<th>Membrane characteristics</th>
<th>Medium</th>
<th>Temp (°C)</th>
<th>Permeate flux (L/m² h)</th>
<th>Hydrogen production rate (m³/m³ h)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Laboratory scale AnMBR for hydrogen production</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Submerged</td>
<td>Kubota Co, plate flame (FS) type of 0.45 μm pore size, 0.1 m² effective area and 240 × 340 × 10 mm module size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>35</td>
<td>5</td>
<td>2.43–2.56[^a]</td>
<td></td>
<td>[94]</td>
</tr>
<tr>
<td>External</td>
<td>US Filter C., tubular, ceramic, pore size:0.2–0.8 μm; membrane surface area:0.005 m² and membrane dimension of ID = 7 mm, OD = 10 mm; length = 250 mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>–</td>
<td>57–60</td>
<td>0.64</td>
<td></td>
<td>[95]</td>
</tr>
<tr>
<td>External</td>
<td>Deposition Sciences Inc., hollow fibre, MF, pore size: 0.2 μm</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>35</td>
<td>–</td>
<td>1.02–1.48</td>
<td></td>
<td>[96]</td>
</tr>
<tr>
<td>Submerged</td>
<td>MF, hollow fibre,</td>
<td></td>
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<tr>
<td></td>
<td>GE Water and Process Technologies, ZW-1 hollow-fibre UF membrane module, pore size, 0.04 μm</td>
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<tr>
<td>Submerged</td>
<td>Tofu processing waste</td>
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</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>60</td>
<td>4.32</td>
<td>12.81–19.86[^a]</td>
<td></td>
<td>[97]</td>
</tr>
<tr>
<td>(B) Laboratory and pilot scale AnMBR for methane production</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>External (P)</td>
<td>Stork, WFFX 0281, UF, MWCO: 100 kDa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Domestic wastewater</td>
<td>37</td>
<td>–</td>
<td>0–55</td>
<td></td>
<td>[99]</td>
</tr>
<tr>
<td>External (L)</td>
<td>Weir Envig. Tubular, UF, PES, MWCO: 20 kDa</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>Swine manure</td>
<td>–</td>
<td>5–10</td>
<td>2.26</td>
<td></td>
<td>[100]</td>
</tr>
<tr>
<td>External (P)</td>
<td>Stork, WFFX 0281, UF, MWCO: 100 kDa</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Domestic wastewater</td>
<td>37</td>
<td>3.5–13</td>
<td>&lt; 30</td>
<td></td>
<td>[101]</td>
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<tr>
<td>External (L)</td>
<td>Weir Envig. Tubular, UF, PES, MWCO: 20 kDa</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Swine manure</td>
<td>37</td>
<td>5–10</td>
<td>2–3</td>
<td></td>
<td>[102]</td>
</tr>
<tr>
<td>External (P)</td>
<td>Stork, WFFX 0281, UF, MWCO: 100 kDa</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Slaughterhouse wastewater</td>
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<td></td>
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<td></td>
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<tr>
<td></td>
<td>37</td>
<td>2.22–2.46</td>
<td>50–102</td>
<td></td>
<td></td>
<td>[103]</td>
</tr>
<tr>
<td>Submerged (L)</td>
<td>Zenon, capillary, UF, pore size: 0.1 μm</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Landfill leachate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
<td>[104]</td>
</tr>
<tr>
<td>Submerged (L)</td>
<td>Kubota, PE, FS, pore size: 0.4 μm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Municipal solid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>0.5–9</td>
<td>–</td>
<td></td>
<td></td>
<td>[105]</td>
</tr>
<tr>
<td>Submerged (L)</td>
<td>FS, PVDF, pore size:0.3 μm</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Kraft evaporator condensate plus methanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>5.6–12.5</td>
<td>–</td>
<td></td>
<td></td>
<td>[106]</td>
</tr>
</tbody>
</table>

Note: L = laboratory; P = Pilot.
[^a] Units are L/L/d.
[^b] Unit is mol/L/d.

upgrading techniques such as absorption or adsorption need high amount of energy and great space. Membrane system, on the other hand, can perform three separation steps of carbon dioxide (CO₂), and H₂S removal and dehydration in one step, thereby reducing the space needed. In a laboratory study by Harasimowicz et al., [91] CO₂ and methane gases were efficiently separated with hollow-fibre module membrane, A-2 type (UBE) supplied by UBE Europe GmbH. Two gas mixtures were used in the experiment with the first mixture consisting of 50% of both methane and CO₂, while the second mixture consisted of 68% of CH₄, 30% of CO₂, and 2% of H₂S. The result was 89.5% and 93.5% methane recovery in first and second mixtures, respectively. Polyimides are polymeric membranes that are commonly used for biogas purification because of its low manufacturing cost and chemical resistant to gases present.[92] Biogas has also been produced by encasing the methane-producing bacteria in synthetic and natural membranes.[93] This method enhanced the rate of biogas production by maintaining a high cell density in the reactor, and by protecting the cells from inhibition factors in the medium.[93] Table 2 shows the application of membrane technology for biogas and hydrogen production.

Market penetration of membrane technology

AeMBR technology had been commercialized since 1970s as external loop and later as immersed process for wastewater treatment, desalination, and water purification in countries such as UK/Ireland, France, Germany, Japan, China, South Korea, Iberia, Benelux, Italy, USA, Canada, and Mexico, making great contributions in the market. Companies including Ge-Zenon, Kubota, Mitsubishi Rayon, and Memcor have dominated the membrane market.[107–110] As of 2009, about 4400 AeMBRs had been installed in over 200 countries by Kubota, Mitsubishi Rayon, and Ge-Zenon.[110] The shared percentage of the number of installed membrane-based biogas plants installed between
Membrane materials are usually made of organic polymers, metallic and inorganic materials, e.g. ceramics. Although metallic and inorganic membranes have properties such as high tolerance to corrosion, abrasion, oxidation, and better fouling recovery when compared with organic polymers, polymeric membranes are more frequently used since they have lower costs. The choice of polymer, however, is often a compromise among the membrane properties which include hydrophilicity, ease of fabrication, cost, and robustness. Although hydrophobic polymers have good properties including chemical resistance, biocompatibility, low swelling, among others, hydrophilic membranes were found to be more prone to fouling than hydrophobic membranes since most reactions between foulants and membranes are hydrophilic in nature. Therefore, most hydrophobic membranes are hydrophilized to obtain some desirable qualities. Generally, polyolefins, polyethersulfone (PES), and polyvinylidene difluoride (PVDF) are polymers that are usually favoured.[85,86,104]

**Challenges and possible remedies for sustainability of the MBRs’ future**

The many benefits associated with the MBR technology makes it a reliable and valuable option, favourable over other waste management techniques. Some of the many advantages are footprint efficiency (reducing capital costs), high effluent quality, low-pressure system, high capacity, and the technology is easy to control. An important feature of the MBR technology is its ability to retain biomass when using high dilution rates, preventing biomass washout during the continuous process.[43]

In spite of the many advantages and the fast pace of development of MBR technology, commercial application is still confronted with some limitations, particularly for ethanol productions, membrane fouling constituting the major one. Membrane fouling is a barrier to any process involving membrane application since it causes decline in permeate flux and thereby increases energy input along with operating costs.[113] High particulate and cell concentrations, along with several antifoaming agents, such as polyoxyethylene, polyoxypropylene, oleyl ether, polyglycols, and silicon oils severely foul the membranes.[13,43]

Although anti-fouling measures such as biogas sparging,[114] agitation and aeration, and membrane vibration are used to reduce fouling rate in membrane operation, they cannot eliminate membrane fouling completely.[106] Unrelenting efforts are being made by various researchers to find ways of keeping membrane fouling to the minimum to avoid frequent cleaning thereby lengthening membrane lifespan.[115–119] MBRs techniques producing ethanol have rarely been scaled up and utilized on industrial scale, and are not as developed as MBRs in water and wastewater treatments. Biogas production in MBRs has in contrast been more successful by the combination of anaerobic digesters and MBRs utilizing different wastewaters, and has also been successfully installed on larger scale at several places. It is, therefore, important to continue transferring the knowledge from the successful wastewater MBR concepts and evaluate and solve the present problems in ethanol and biogas in order to improve the performance of the MBRs. Much work still remains before MBRs for ethanol and biogas production can be operated at high cell and/or particulate concentrations.

**Conclusion**

In MBRs, a biological process, such as, e.g. ethanol fermentation or anaerobic biogas production, is combined with a membrane separation technique. The membrane can be coupled with the bioreactor, either by submerging it inside the bioreactor culture, or by placing it in an external loop outside the bioreactor. The MBR allows the cell concentrations in the bioreactor to be significantly increased. Furthermore, product inhibition is diminished, product concentration is amplified, and the separation of product and/or cells after the process can be simplified. In addition, the productivity of ethanol fermentation can be increased profoundly when using an MBR instead of the traditional batch or continuous processes. Accordingly, MBR holds a recognizable potential for the development of faster and more economically viable processes to be applied in the production of ethanol and biogas.

**Acknowledgement**

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**References**


