Leachate treatment and anaerobic digestion using aquatic plants and algae

Emma Ström

Master’s programme
Science for Sustainable Development

Master’s Thesis, 30 ECTS credits
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Supervisor: Andreas Berg

2010
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Abstract
Phytoremediation as a way to control and lessen nutrient concentrations in landfill leachate is a cheap and environmentally sustainable method. Accumulated nutrients in the plants can then be removed by harvesting and anaerobically digesting the biomass. This study presents two aquatic plants (L. minor (L.) and P. stratiotes (L.)) and one microalgae species (C. vulgaris (L.)), their capacities for growth and nutrient removal in leachate from Häradsudden landfill, Sweden, are investigated. The biogas potential of the two plants is determined via anaerobic digestion in a batch run, followed by a lab-scale reactor run for L. minor only. Results show that growth in leachate directly from the landfill is not possible for the selected species, but at a leachate dilution of 50% or more. Nutrients are removed in leachates with plants to a higher extent than in leachates without, yet the actual amounts do not differ notably between plant species. L. minor proves a better choice than P. stratiotes despite this as growth is superior for L. minor under the experimental conditions of this study. Considering biogas production, L. minor gives more methane than P. stratiotes according to the results from the batch run. The former is however not suitable for large-scale anaerobic digestion unless as an additional feedstock due to practical cultivation issues.

KEYWORDS: Anaerobic digestion, Landfill leachate, Lemna minor, Phytoremediation, Pistia stratiotes

List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>DW</td>
<td>Dry weight</td>
</tr>
<tr>
<td>GC</td>
<td>Gas chromatograph/y</td>
</tr>
<tr>
<td>FID</td>
<td>Flame ionization detector</td>
</tr>
<tr>
<td>HRT</td>
<td>Hydraulic retention time</td>
</tr>
<tr>
<td>OLR</td>
<td>Organic loading rate</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SRT</td>
<td>Sludge retention time</td>
</tr>
<tr>
<td>TS</td>
<td>Total solids</td>
</tr>
<tr>
<td>VFA</td>
<td>Volatile fatty acid</td>
</tr>
<tr>
<td>VS</td>
<td>Volatile solids</td>
</tr>
</tbody>
</table>

1. Introduction

Throwing it out the window, sweeping it under the rug, sending it off to a far-away land… When it comes to waste, “what you cannot see does not exist” seems to have been the predominating idea throughout history. Consequences were for later. Now, what with the global cry for cheap and lasting energy, waste has gone from something to get rid off to a potent resource and behaviors are starting to change.

In Sweden, sorting waste is a habit more often than not nowadays and landfilling diminishes. Still, cleaning up after those who came before and making sure that our present actions do not impede those of the future is a task not easily carried out. Landfills will not vanish just because no one is there to see them and aging waste leach pollutants as time goes by, spreading environmental and health-wise risks to the surroundings.

A serious environmental hazard, landfill leachate brings toxic levels of metals, nutrients and resilient compounds into the soil or nearby water bodies (Abbas et al, 2009; Di Iaconi et al,
While landfilling is strictly regulated in Sweden today, both regarding what is deposited and how, any leachate still has to be purified to acceptable quality before entering the ecosystem (Naturvårdsverket, 2010).

One step toward sustainability is to incorporate methods that make use of or mimic nature’s pathways. When it comes to purification of polluted waters like leachate, phytoremediation is one such method. It involves plants accumulating or transforming pollutants from the landfill and passing on cleaner water (Blaylock and Huang, 2000). Not only is phytoremediation a cheap low-maintenance method, it can enhance the aesthetic value of an otherwise not so appealing landfill area (Nagendran et al, 2006; Susarla et al, 2002).

Harvesting of the plants might be needed in order to keep the pollutants out of the system as they have accumulated in the biomass (Susarla et al, 2002). To remain within a sustainable framework, the plants should then be made use of rather than thrown away or burnt. A possible method gaining attention in Sweden is anaerobic digestion, a means to get biogas for fuel or electricity. It is a viable option economically as well as environmentally.

The project described in this report is a joint venture by companies Econova Biotech AB and Scandinavian Biogas Fuels AB in the county of Östergötland, Sweden. Econova Biotech AB manages a landfill, Häradsudden, where a leachate purification system using mainly phytoremediation and airing is to be instigated during 2010. Scandinavian Biogas Fuels AB with experiences in the biogas field assists through investigating anaerobic digestion as a sustainable end-use of phytoremediation plants. A co-operation between said companies could lead to improved environmental conditions on and around Häradsudden landfill as well as in the county as a whole; a solution that closes production cycles and minds the future consequences.

1.1 Aim
As the long-term goal is to create a stable eco-cycle at Häradsudden with water treatment and biogas production as the main features, suitable plants for phytoremediation in Häradsudden’s leachate pond and for later biogas production were the focal points of this project. The aim was to determine the suitability of the selected plants to grow in Häradsudden leachate and if they had biogas potential enough to be used in energy production after being harvested.

1.2 Research questions
A stepwise range of research questions was set up in order to answer the aim in a complete and comprehensible way. The research questions were as follows:
- What plants or algae known for their nutrient uptake ability can be grown in the Swedish climate at a satisfactory rate without themselves posing a threat to the environment through invasive spreading?
- Under what climatic conditions (temperature, lighting) are the selected plants or algae able to grow in leachate from Häradsudden landfill?
- To what extent are the selected plants able to take up nutrients, mainly nitrogen and phosphorus, under the experimental conditions?
- What amount of methane can be produced using the selected plants or algae as feed substrate for anaerobic digestion in a) a batch run and b) a lab-scale reactor?
2. Background

2.1 Leachate in landfills

Landfill leachate is commonly characterized as water that has percolated through the landfill, where the water can come from precipitation, groundwater seepage or from the wastes in the landfill (Ali et al, 2004; Renou et al, 2008a). The water passing through the waste masses is polluted with nutrients and toxic substrates like heavy metals, loosened from their origins through biodegradation or other chemical processes (Jones et al, 2006). Landfill leachate is thus considered an environmental hazard as the pollutants spread into the surroundings, affecting the local biota and in cases of transport via groundwater or other aquatic systems even farther away (Abbas et al, 2009; Jones et al, 2006).

Depending on the amount of time wastes have been allowed to degrade undisturbed, different processes take place within the waste masses and thus releasing different kinds of pollutants. As biodegradation takes place, organic matter is stepwise broken down into smaller compounds, eventually releasing methane, the other major environmental problem for landfills (Ahn et al, 2002). A few characteristics can be used to determine the state of the landfill leachate. These include but are not limited to total solids (TS), pH, ammonium, total nitrogen content and heavy metals. As the landfill ages, degraded organic solids are flushed away, consequently raising ammonia levels when nitrogen compounds are released. This makes it possible to determine a site-specific course of treatment if the landfill history is known. (Abbas et al, 2009; Jones et al, 2006; Renou et al, 2008a)

As leachates differ so much even within landfills, a universal solution to the problem will probably not be found. However, methods are continuously reviewed and developed to meet this environmental issue (Abbas et al, 2009; Renou et al, 2008a; Nagendran et al, 2006). Treatments within the recycling, filtering, biological and chemical areas are most common (Abbas et al, 2009; Renou et al, 2008a), but phytoremediation –the use of plants in the purifying process- has gained attention as a viable option for landfills (Jones et al, 2006; Kim and Owens, 2010; Nagendran et al, 2006).

2.2 Phytoremediation

Phytoremediation is a collective term including all forms of purifying or remedying methods where growing plants are used (Sadowsky, 1999; Susarla et al, 2002). Through a multitude of available biochemical processes, pollutants and nutrient levels are dealt with by living plants that are self-sustaining and simultaneously prevent soil erosion and present aesthetic values to the area, among other advantages (Susarla et al, 2002). The processes are listed in Table 1 below and may vary in efficiency depending on the site and landfill composition (Nagendran et al, 2006; Susarla et al, 2002).

Table 1. Phytoremediation methods summarized from the works of Nagendran with colleagues (2006) and Susarla with colleagues (2002).

<table>
<thead>
<tr>
<th>Method</th>
<th>Process</th>
</tr>
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<tbody>
<tr>
<td>Phytocapping</td>
<td>Plants hold soil together and prevents rainwater from turning into leachate, limiting pollutant movement</td>
</tr>
<tr>
<td>Phytoaccumulation/-extraction/</td>
<td>Plants take up pollutants and nutrients and store them. Requires harvest of plants at</td>
</tr>
<tr>
<td>-sorption</td>
<td></td>
</tr>
</tbody>
</table>


**Phytovolatization**
Plants process hazardous substrates into volatile forms, allowing them to leave the soil or water through evaporation.

**Phytotransformation/-degradation**
Plants process hazardous substrates into less dangerous compounds. Degraded substrates may be released or stored.

**Phytostabilization**
Plants alter soil environment via the roots to cause stabilization of hazardous substrates in the ground. No harvest required.

**Phyto-/Hydraulic pumping**
Plants take up amounts of water enough to prevent leachate movement and its effects.

**Rhizo(sphere) degradation**
Microbial and fungal activity in the root systems of the plants degrade hazardous substrates into less dangerous compounds.

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Not only does leachate quality influence the choice of method, but plant resilience and growth characteristics do as well. Daylight and temperature may restrict use of one or more phytoremediation methods in temperate zones (Song et al, 2006; Vermaat and Sand-Jensen, 1987; Vindbaek Madsen and Brix, 1997). On the other hand, invasive spreading by the fast-growing plants often used is limited when temperatures are low outside of the cultivation areas (Hallstan, 2005).

Phytoaccumulation, as mentioned in Table 1, requires harvest at regular intervals to remove the contaminants from the area. The biomass must then be processed in order to prevent the high levels of pollutants and nutrients from entering the biological circulatory system in some other way. If the contaminants are desirable, extraction from the biomass can be done (Susarla et al, 2002). Using the plant matter as feedstock for fish or cattle is possible if pollution levels are within acceptable ranges (Leng et al, 1995). Turning the plants into ethanol (Mishima et al, 2008) or biogas (Verma et al, 2007) are attractive options as the energy market expands, since the nutrients and heavy metals would be used up in the process.

### 2.3 Nitrification and denitrification

While nitrogen is quite abundant in the atmosphere in the form of N\(_2\), plants can only take up the highly important nutrient as ammonium (NH\(_4^+\)) or nitrate (NO\(_3^-\)) ions. Mineralization is the process where organically bound nitrogen is turned into ammonium by bacteria and fungi. From there, nitrification and denitrification are the two most important pathways for nitrogen (The Water Planet Company, 2010; Pidwirny, 2010), unless plants or other organisms are present to absorb nitrogen in its various forms.

Nitrification is the conversion of ammonium via nitrite to nitrate, a two-step process generally viewed as one since the overall reaction rate is fast enough to leave only minimal levels of nitrite present at any time (Madigan and Martinko, 2000). Autotrophic bacteria of genus *Nitrosomonas* and *Nitrobacter* perform these two steps, respectively (Pidwirny, 2010). Optimal pH for nitrification lies between 7.5 and 8.5 and temperatures are most favorable between 30 and 35°C although a range between 10 and 40°C ensures functionality (The Water Planet Company, 2010). The chemistry of the process is as follows (Madigan and Martinko, 2000; The Water Planet Company, 2010):
Denitrification is the further conversion of nitrate into nitrogen gas, carried out by heterotrophic bacteria (Pidwirny, 2010). This process occurs when oxygen levels are low and nitrate serves as the foremost oxygen source for the organisms. Optimal pH for the latter ranges from 7.0 to 8.5 and preferred temperatures are 5-30°C. A carbon source has to be available for denitrifiers to thrive. Below is the chemical outline for the denitrification process, where methanol is used as representing any carbon source (Madigan and Martinko, 2000; The Water Planet Company, 2010):

\[
6 \text{NO}_3^- + 5 \text{CH}_3\text{OH} \rightarrow 3 \text{N}_2 + 5 \text{CO}_2 + 7 \text{H}_2\text{O} + 6 \text{OH}^-
\]

Nitrification can also occur through the use of carbonate and oxygen, therefore causing a lowering of alkalinity in the surrounding environment. Denitrification on the other hand increases alkalinity and also counters pH-decreases, thus mitigating the effects of nitrification. (The Water Planet Company, 2010)

2.4 Biogas production through anaerobic digestion

Biogas production through anaerobic digestion of organic material not only makes use of the nutrients, but also degrades the organic matter significantly (Ecofys Bio Energy group, 2008; Hronich et al, 2008; Verma et al, 2007).

Anaerobic digestion can be used in several different kinds of digesters, but the processes within are the same for all kinds. Micro-organisms living in the digestate make use of nutrients in their immediate environment to degrade fats, carbohydrates, lipids and proteins (and to some extent fibers) with methane and carbon dioxide as end products. It is the absence of oxygen that allows these chemical pathways to occur; had it been an aerobic process there would have been different end products. (Fachagentur Nachwachsende Rohstoffe e.V. (FNR hereafter), 2009; Reith et al, 2003; Wilkie, 2004)

The biogas process can be run at different temperatures, in which case the organism cultures vary depending on what their optimal growth temperature is. Psychrophilic (10-20°C), mesophilic (20-40°C) or termophilic (50-60°C) organisms are used, although the two latter systems are most common. (FNR, 2009; Reith et al, 2003; Weiland, 2010)

The micro-organisms are responsible for the chemical degradation depicted in Figure 1:

![Diagram of the biochemical process of anaerobic digestion as derived from the work of Gujer and Zehnder (1983)](attachment:Fig_1.png)

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**Fig. 1.** The biochemical process of anaerobic digestion as derived from the work of Gujer and Zehnder (1983)
The respective degradation steps are named hydrolysis, acidogenesis, acetogenesis and methanogenesis. Different microorganisms handle each separate step. Hydrolysis is generally viewed as the rate determining step, but accumulations of any substance in the chain indicates that something is not working within the digester. (FNR, 2009; Gujer and Zehnder, 1983; Reith et al, 2003)

Sludge retention time (SRT) and hydraulic retention time (HRT) are aspects determining to what extent the above processes should be allowed to run. The former is the time microorganisms spend in the digester, thereby controlling the biogas formation and acidification of the digestate. The latter is principally the amount of time that the feedstock remains inside the digester. There is a relationship between SRT and HRT dependent upon temperatures and feed stock used, where they can be of the same time span or the SRT exceeding the HRT. (Gerardi, 2003; Reith et al, 2003)

Temperature, SRT and HRT and organic load rate (OLR) are the main parameters externally controlled when producing biogas. The OLR determines how much dry weight of the feed stock should be added to the digester at each loading moment (the regularity of these vary depending upon digester type). (FNR, 2009; Reith et al, 2003) Other parameters that allow check-ups and control of the processes include pH, total and volatile solids (TS and VS), volatile fatty acid (VFA) accumulation, hydrogen and ammonia content, conductivity and gas production (with methane content analyzed separately) of the digester (FNR, 2009; Gerardi, 2003).

The biogas is purified to remove carbon dioxide, hydrosulphides and water vapor that lowers efficiency and can impede technical equipment, leaving the desired methane. The gas can then be turned into electricity, heat or vehicle fuel. The residues in the digester can in most cases be used as an odor-free, environmentally friendly fertilizer for cultivation, if proper care has been taken to remove pathogens from the substrate prior to or post-digestion. (FNR, 2009; Reith et al, 2003; Wilkie, 2004)

2.5 Häradsudden landfill
Häradsudden landfill is situated outside of Norrköping, Sweden, and was installed in 1977 according to Malin Asplund at Econova Biotech AB (personal contact, 2010-05-07). Municipal, vegetative, industrial and construction wastes are handled at Häradsudden, as well as wastes from water treatment from industries and the municipality. Asbestos is also accepted at the landfill. (Econova Biotech AB, 2010)

Leachate treatment is one of the major projects at the landfill, where a system of phytoremediation and airing is investigated (this report being part of the pre-evaluation) as a possible method to purify the water. At the present, leachate from Häradsudden is treated at the municipal waste water treatment plant in Norrköping. (Econova Biotech AB, 2010)

2.6 The plants
Two aquatic plants and one microalgae species were used in this experiment, selected for their nutrient uptake efficiency and growth capacity among other factors (see section 3).
2.6.1 *Pistia stratiotes* (L.)

![Fig 2. Pistia stratiotes. Photo taken by author during the cultivation experiment.](image)

The free-floating aquatic plant *Pistia stratiotes* (L.) is also known as water lettuce, forming rosettes up to 15 cm across that may resemble ordinary lettuce (Coelho et al, 2005, Fonkou et al, 2002). The plant is known for its efficient nutrient uptake ability (Lu et al, 2010), something coupled to its fast-growing capacity that in many places has turned water lettuce into a strongly invasive plant (Coelho et al, 2005; Šajna et al, 2007). It forms dense mats on the water surface and grows at a rate of 60-110 t DW ha\(^{-1}\) yr\(^{-1}\) (Mishima et al, 2008). As it is native to South America, Swedish temperatures are below *P. stratiotes’* optimal range most of the year, which has prevented it from spreading although use in aquariums and garden ponds is common (Hallstan, 2005).

2.6.2 *Lemna minor* (L.)

![Fig 3. Lemna minor. Photo taken by author during the cultivation experiment.](image)

*Lemna minor* (L.) is also a free-floating plant although much smaller than the above; it is among the smallest flowering plants known. Fronds are less than a cm across and *L. minor* sprouts only one root per plant (Dalu and Ndamba, 2003). It grows rapidly; under favorable conditions it can double its biomass in two days or less and produce 10-30 t DW ha\(^{-1}\) year\(^{-1}\).
(Leng et al, 1995). This leads to the forming of dense mats on the surface of the water body that efficiently inhibits subsurface-organisms from oxygen access through atmospheric interactions (Driever et al, 2005). Coupled to this is the high nutrient removal capacity of \textit{L. minor}, making it a suitable plant for water treatments (Dalu and Ndamba, 2003).

\textit{L. minor} is one of four species in the family \textit{Lemnaceae}, in which all plant types are known by the common name duckweed (Dalu and Ndamba, 2003). \textit{L. minor} is sometimes labeled “common duckweed” (Anderberg and Anderberg, 2010).

2.6.3 \textit{Chlorella vulgaris} (L.)

\textit{C. vulgaris} (L.) is a strand of green microalgae commonly used in laboratories. Today, it is a common ingredient in health food for humans as well as is extensively studied for waste water treatment abilities. (Şen et al, 2005) \textit{C. vulgaris} is one of the fastest growing microalgae species (Kim and Lee, 2009), which makes it a promising organism to use in sustainability issues where nutrient uptake is essential, for example. It has been proposed that phytoremediation of waste waters might improve with the additional use of \textit{C. vulgaris} (Bich et al, 1998; Valderrama et al, 2002), which was also why the microalgae was included in this study.

3. Materials and Methods

3.1 Plant cultivation

A literature study was done in order to determine a number of possible plants and algae for the project. A total of twenty species were selected as suitable, out of which three were settled upon for the experiment: \textit{Lemna minor} (aquatic plant), \textit{Pistia stratiotes} (aquatic plant) and \textit{Chlorella vulgaris} (freshwater microalgae). These plants were selected on the basis of high growth, low invasiveness, easy harvesting, good nutrient uptake ability and resilience to low temperature and high levels of nutrients in the water, as according to the literature. \textit{L. minor} and \textit{P. stratiotes} were bought from private aquariums in Sweden and shipped by mail. \textit{C. vulgaris} had been grown at the lab before and was already in place. Upon arrival, all plants
were kept in a nutrient solution (100% Z8 including Gaffron’s micronutrient mix (Kotai, 1972)).

Two different test set-ups were used for plant cultivation. A total of around 120L leachate was used throughout both test set-ups together, drawn from Häradsudden landfill and delivered prior to test start. Initial concentrations of nutrients and other values of the raw leachate from Häradsudden are shown in Table 2 below.

Table 2. Initial values of the raw leachate drawn from Häradsudden landfill. Standard deviations not available due to one-point measurements.

<table>
<thead>
<tr>
<th>pH</th>
<th>NH$_4^+$ (mg/L)</th>
<th>NO$_3^-$ (mg/L)</th>
<th>PO$_4^{3-}$ (mg/L)</th>
<th>Alkalinity (mg CaCO$_3$/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.04</td>
<td>315</td>
<td>23.5</td>
<td>0.75</td>
<td>3040</td>
</tr>
</tbody>
</table>

Lighting intensity was tested and found to be suitable at 700 lux for the first run, but was raised to 900 lux for the second cultivation since plants seemed to have adjusted to the lighting levels and were thought to grow better with higher intensity. 150W Halolux halogen lamps were used. The light regime was kept at an approximate 16:8 schedule, apart from the weekends where a 24-hour day resp. night had to be used due to practical reasons. The temperature was kept at 18°C. Plants were grown in containers with an 18.2x18.2cm area (330 cm$^2$) and 10 cm water depth.

3.2 The first cultivation set-up
Four different combinations of plants were tested in 100% leachate: 1) P. stratiotes 2) L. minor 3) P. stratiotes + C. vulgaris and 4) L. minor + C. vulgaris. Triplicates of each combination were made, and then doubled to keep an identical half of the test set-up on a 5% CO$_2$-addition during lit hours. Approximately 10 individuals of L. minor, 1-3 individuals of P. stratiotes and 6 mL (the amount determined from earlier growth experiments with this algae) nutrient solution containing C. vulgaris were added to each container. Watering was meant to take place on a need-basis, using more leachate, but no such occasion had time to occur. The test had been planned to run for 30 days, but was terminated after one week due to no remaining live plants.

3.3 The second cultivation set-up
Three different concentrations of leachate were used; 10, 30 and 50%, which were prepared by diluting 100% leachate with tap water. Triplicates for each leachate concentration were kept for P. stratiotes and L. minor respectively. Plants were never combined, but C. vulgaris was added to one container per triplicate in order to determine possible effects of combining algae with plants. This gave a total of 18 containers, six per leachate concentration. See Figure 5 for a set-up overview.

<table>
<thead>
<tr>
<th>10% leachate</th>
<th>30% leachate</th>
<th>50% leachate</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. stratiotes</td>
<td>L. minor</td>
<td>P. stratiotes</td>
</tr>
<tr>
<td>P. stratiotes</td>
<td>L. minor</td>
<td>P. stratiotes</td>
</tr>
<tr>
<td>P. stratiotes +C. vulgaris</td>
<td>L. minor +C. vulgaris</td>
<td>P. stratiotes +C. vulgaris</td>
</tr>
</tbody>
</table>

Fig. 5. Set-up for the second cultivation run with leachates of different concentrations
Around 10 *L. minor* individuals, 1 *P. stratiotes* individual and 6 mL nutrient solution containing *C. vulgaris* were added to each container. Amounts were based on plant availability (due to the death of all plants in cultivation run one and the winter season in Sweden, there was only so many plants available) and for algae, experiences from earlier growth in nutrient solution. Refilling containers to keep water depth at 10 cm was done with corresponding concentrations of leachate the first two times (on days 4 and 8), which resulted in increased leachate concentrations (as the main reason for water depth decrease being evaporated water rather than plant uptake, leaving nutrients at the same levels but in less volume) that seriously stressed the plants. These concentration increases were adjusted by exchanging the refill volume of leachate (660 mL) in each container with distilled water on day 10. Refilling was thenceforth done with distilled water only to keep nutrient levels safely below the plant capacity limit. No additional CO₂ was used. The cultivation was run for 29 days.

### 3.4 Controls
Throughout the entire cultivation period, controls of 1) *P. stratiotes*, 2) *L. minor*, 3) *P. stratiotes + C. vulgaris* and 4) *L. minor + C. vulgaris* were grown in a nutrient solution (100% Z8 with Gaffron’s micronutrient mix included) to ensure that plants and algae were able to grow under the lighting and temperature conditions set.

Also, a control of leachate activity at different concentrations was initiated during the second cultivation run, in order to compare nutrient concentration changes when no plants or algae were present with the changes in the test set-up. Triplicates of 10, 30 and 50% leachate respectively, without any plants or algae, were kept for 30 days under the same conditions as for the cultivation. The same measurements that were carried out for the second cultivation run were performed on the leachate controls (see section 3.5).

### 3.5 Plant cultivation measurements

#### 3.5.1 Growth
Surface cover was measured at the start of each week. *P. stratiotes* cover was approximated using a ruler to determine cm² increases, while *L. minor* individuals were counted and the number compared to the previous week. *C. vulgaris* was only checked by visual estimation to determine if the algae were still alive and growing or not, no further measurements were taken on that account. Photos were taken to visually compare plant and leachate status between weeks. Harvesting was never necessary. Controls were not measured in detail but checked regularly by visual estimation to confirm continuous growth.

#### 3.5.2 Accumulated biomass (TS and VS)
Upon arrival, around 0.2 g wet weight (ww) of *P. stratiotes*, 0.3-0.4 g ww of *L. minor* and 2 g ww of *C. vulgaris* were weighed before being put into 105°C for 24 hours and then weighed again to determine TS. The remains were put into 550°C for two hours and the residues were weighed a final time to get the VS of the samples. TS and VS contents were compared to the final measurement where plants (not algae) from all containers were placed in 105°C for 25 hours after the second cultivation run. VS was assumed to remain constant from the initial measurement and were thus not checked again.
3.5.3 NH$_4^+$, NO$_3^-$, PO$_4^{3-}$ and alkalinity of leachate

Ammonium, nitrate, phosphate and alkalinity were measured using analysis cuvettes of the LCK series from Hach-Lange. Samples were added to cuvettes specifically made for each analysis respectively, allowed to react with the analysis-specific reagent inside (see www.hach-lange.com for detailed information) and were then run through a Hach-Lange DR2800 spectrophotometer to determine substrate concentrations. All leachates were stirred prior to sampling. The mentioned compounds and alkalinity were first measured on the initial 100% leachate and from this, corresponding initial values were calculated for the lower concentrations in the second cultivation run. Ammonium and nitrate were further checked on days 10 and 18 for the second cultivation run and leachate controls. Upon finishing the cultivation at day 30, all compounds and alkalinity were tested again. During the test period, new ammonium cuvettes with lower measuring ranges had to be ordered due to much lower ammonium levels in the leachate than expected.

3.5.4 Total nitrogen in plants and sediment

A few individual plants from _P. stratiotes_ and _L. minor_ were dried for 48 hours in 55°C before the first cultivation run, in order to have a base value of total nitrogen content in the plant types (see nitrogen determination details below). At the end of cultivation run two, all plants (but not algae) from all containers were dried in 105°C for 25 hours (this only included plants from the second run since the plants in the first test had died _en masse_), and then ground by hand to homogenize the samples. 2mm of sediment had appeared during the cultivation run, which contained settled leachate solids mixed with algae and plant parts. A square of 4x4 cm sediment was gathered from each container, dried in 55°C for 48 hours and sent for analysis at a the Evolutionary Biology Centre in Uppsala, Sweden, along with all the plant samples. Total nitrogen and carbon was analyzed there using a Costech element analyzer of the ECS 4010 series, in which samples were oxidized, reduced, dried and then run through a chromatograph column for separation. Results were interpreted using the computer program EAS Clarity by Costech.

3.5.5 Water color and turbidity

The second cultivation run and leachate controls were checked for color change and decreased turbidity. By visual estimation, water characteristics were noted at the beginning of each week, with regards to color, turbidity and sedimentation. Digital photos were taken at each estimation date to make comparison possible.

3.5.6 pH of leachate

Measurements were taken on the leachate upon arrival and on day 1 of cultivation run. Three out of five containers were sampled and values were assumed to represent leachate in all five containers. Thereafter pH was taken on each container twice a week; Mondays and Thursdays. pH was taken using an inoLab pH 730 device of the WTW series, which was calibrated each Monday. The electrode was a Hamilton Polilyte Bridge Lab electrode capable of measuring pH 2-14. A buffer solution of pH 7.96 +/- 0.05 at 25°C was used as reference. All leachates were stirred prior to sampling.

One pH adjustment was carried out for the second cultivation run on day 24, for test containers only and not controls, since pH had been rising steadily and in some containers drew close to 10 which would be taxing on the plants. A pH of 9.41 (a mid-level value at the previous pH measurement) was used to calculate the amount of hydrochloric acid (HCl) needed. With the aim to lower pH from 9.41 to around 8.0, trials with 20 and 40 mL of leachate were performed to which small amounts (0-0.15 mL) of HCl was added until a
satisfactory amount had been determined. As 0.15 mL 0.1M HCl was needed to lower pH 1.63 units in 40 mL of leachate, 12.4 mL HCl was required for each leachate container of 3300 mL.

3.6 Biogas potential of plants

3.6.1 The batch process
To investigate the biogas potential of *P. stratiotes* and *L. minor*, a batch run was performed before going to the reactor stage. Due to the limited growth of plants in leachates, control plants grown on nutrient solution Z8 100% (including Gaffron’s micronutrient mix) (Kotai, 1972) were used. *P. stratiotes* and *L. minor* were rinsed of as much algae as possible and cut into pieces approximately 2-4mm² in size. A small portion of each plant type were weighed and dried in 105°C for 24 hours, then weighed again and put into 550°C for another two hours in order to determine TS and VS of the samples.

Working volumes for the batch were 0.1 L in 0.32 L anaerobic glass bottles with a rubber stopper and metal screw-on cap. Organic loads for *P. stratiotes* and *L. minor* were 1.6 g VS/L and 2.1 g VS/L respectively, and triplicates made for each plant. Digestion sludge from the Nykvarn waste water treatment plant was used as inoculum to provide the bacterial cultures for the biogas process. Bottles were flushed with argon to remove all oxygen before substrates, inoculum, nutrient solutions and water was added. Boiled distilled water was used to give equal working volumes in all bottles. The gaseous phase was then changed from argon to a mix of nitrogen and carbon dioxide (80% resp 20%). Whatman paper (at 5.0 g VS/L) was used as a control substrate in a triplicate of its own. Further controls included one triplicate with inoculum only and one with boiled water and 50 mL methane (incubated methane samples) where the latter also contained an overpressure. In total there were fifteen batch bottles, which were then put in a dark room heated to 37°C to incubate.

The mean result from the inoculum-only controls was subtracted from the results of the bottles with plants, in order to determine the biogas potential of the respective plants. The incubated methane sample results should not change during the batch run, since changes of 15% or more would indicate analysis errors that renders the measurement unusable. Measurements for keeping track of biogas production were done on days 1, 4, 6, 12, 20 and 32. All samples were taken in a 37°C environment with a working lamp lit for the duration of sampling only.

3.6.2 Pressure measurements
Pressures, to measure gas formation in the bottles, were checked using a Testo 312-3 pressure meter. A needle was attached to the end of the gas tube in order to penetrate the rubber stopper without releasing any gas into the surroundings. The Testo 312-3 then measures the difference between the atmospheric pressure and that inside the bottle in question, and is capable of pressures from 0 to 6000 hPa. For the methane-only control bottles, pressure was only measured on day 1 and then assumed to remain constant for the remainder of the batch run.

3.6.3 Methane content
The methane content of the produced biogas was measured by GC-FID; a gas chromatograph with a flame ionization detector. The machine was a 5880A series GC-FID from Hewlett Packard. Injections were made manually. The column used was a Poraplot T column and the carrier gas used was N₂ at a speed of 130 mL/min. Injection temperature was 150°C, oven
temperature 80°C and the detector temperature 150°C for the first two measurements and 250°C thereafter.

After shaking the batch bottles to release any gas trapped in the liquid, 1 mL gas samples were taken with a syringe and put in a 31.7 mL sample vial through its rubber septum. 0.3 mL gas samples were then manually injected and run on the GC-FID, triplicate runs for each sample vial. Standards used contained 0.07%, 0.63% and 1.71% methane and were run five times each, using the best four values to determine the standard curve.

3.7 The reactor process

*L. minor* was selected for a lab-scale reactor test after the batch run. Plants were grown on 100% Z8 nutrient solution in a constantly lit (at 500 lux, which was defined by experiments by others in the same room) 20°C climate room and harvested regularly, upon which they were kept at 4°C until used in reactor feed portions. Portions were made for four-seven days at a time, to ensure available feed material each day. Prepared portions were kept frozen until the day before use, when they were put in a fridge to defrost. Feed portions included 9.9 g ww biosludge and 11.45 g ww organic household waste apart from varying amounts (12-26 g ww) of *L. minor* (varying due to different TS and VS values of the plants from different containers, ages or nutrient levels), as well as 0.5 mL Fe-II to enhance performance. Plants were initially cut with a scissors to pieces of 2-4 mm², but were later prepared by using an immersion blender which rendered the plant material into a mush with some whole single leaves and roots. Feeding was done daily at approximately the same time each day.

OLR for the first four days was 0.5 g VS/L and was then increased to 1.0 g VS/L for the remainder of the reactor run. HRT for the later OLR was 20 days, and the reactor was run for one such period of time plus the initial four days at lower OLR (i.e. a total of 24 days). Since inoculum from a previous lab-scale reactor with similar feedstock was used, no upstart period was necessary.

A continuously stirred tank reactor (CSTR) with 1L working volume was used, where the small volume was due to limited plant material. The stirrer was a Eurostar power-b top stirrer from IKA labortechnik, and stirring took place for 15 minutes three times during each 24-hour-period as well as 10 minutes prior to and after feeding. The stirring speed was 300 rpm which was enough to mix the reactor sludge without causing a vortex in the sludge A vortex would have lowered the sludge level below the stirrer water lock and caused oxygen to enter the digester, i.e. disrupting the anaerobic processes. Sludge withdrawal and feedstock additions were done using a syringe attached to an otherwise closed rubber tube leading directly into the digester. A custom made gas meter using a water displacement technique was attached to the reactor to measure amount of gas produced, and from there the gas was led into a ventilation system. The entire set-up was kept at 37°C in darkness, apart from working lights during feeding.

3.7.1 Gas measurements

Total gas produced was measured using the custom made gas meter, which was checked before feeding and reset after to avoid accidental beats due to handling of the reactor. The gas meter showed a number of beats (caused by the gas passing through the gas meter) that corresponded to a certain amount of biogas, which was then calculated.

Methane, carbon dioxide, oxygen and hydrogen sulphide were to be measured weekly during the reactor run. A gas tight balloon was attached to the gas meter to collect the gas produced,
and gas was gathered for five days before measurement took place. Gas content was measured using Biogas Check from Geotech in the climate room of 37°C, where the contents of the balloon were pumped through the machine and compounds registered and shown on a display.

However, too small amounts of gas were produced weekly for the measuring to be accurate with the Geotech device. Instead, a syringe was inserted directly into the reactor and gas withdrawn at the end of the test period. A triplicate of 31.7 mL bottles with 1 mL reactor gas was then run on the GC-FID, three runs per bottle. This gave the methane content of the reactor. A triplicate from the balloon was also run, and standards according to the description in section 3.6.3.

3.7.2 pH
pH was measured on the withdrawn digester sludge twice a week, using the same instruments and routines as described in section 3.5.6. Samples were adjusted to 25°C as well before measuring.

3.7.3 VFAs
VFAs measured were ethanol, acetic, propionic, isobutyric, butyric, isovaleric, n-valeric, isocapronic, n-capronic and hepatonic acids, which was done twice a week. 1.5 mL of withdrawn digester sludge was centrifuged at 12000 rpm for 10 minutes, after which 0.4 mL of the supernatant was mixed with 0.04 mL internal standard. The prepared sample was then run through a 6890 series GC-FID from Hewlett Packard. Samples were injected using a 6890 series Agilent autoinjector. The column used was a BP21 capillary column by SGE Australia (30m x 0.32mm (0.25µm)), which is a special column for analyzing VFAs dissolved in water. The carrier medium was nitrogen gas at a flow of 2 mL/min. Temperatures were 150°C for the injection chamber, 50-200°C (on a pre-set program) in the oven and 250°C for the detector. Results were integrated and analyzed using the computer program Chromeleon Client v6.20 by Dionex. Acids of amounts above 0.5 mM were considered, all others merely noted.

3.7.4 Ammonium
Ammonium was measured once a week using analysis cuvettes (the LCK series) from Hach-Lange, instruments and routines as described in section 3.5.3. Withdrawn digester sludge was diluted 1:20 before adding it to the cuvette.

3.7.5 TS and VS
Each Monday, TS and VS of both reactor sludge and harvested L. minor were determined; the former in order to keep an eye on TS and VS reduction within the digester, the latter in order to correctly adjust food recipes for the week. Samples of both substrates were weighed before being put into 105°C for 24 hours and then weighed again to determine TS. The remains were put into 550°C for two hours and the residues were weighed a final time to get the VS of the samples.

3.8 Calculations

3.8.1 TS and VS

\[
TS(\%) = \frac{\text{(crucible weight + sample dry weight) - crucible weight}}{\text{sample wet weight}} \times 100
\]
3.8.2 Total nitrogen content of plants

\( N_{\text{tot}} \) (g/m²) for \( P. \text{stratiotes} \):  
\[
(\text{plant DW g/size m}^2) \times N_{\text{tot}} \%
\]

\( N_{\text{tot}} \) (g/m²) for \( L. \text{minor} \):  
Size assumed to be 0.12 cm² for all plant individuals.  
1) \( 0.12 \times \text{no. of plants} \)  
2) \( (\text{plant DW g/size m}^2) \times N_{\text{tot}} \% \)

3.8.3 \( \text{CH}_4 \) produced by \( L. \text{minor} \) in the lab-scale digester

The lab-scale digester produced an average of 1340 mL biogas day\(^{-1} \) at 37°C. Specific biogas production day\(^{-1} \) (i.e. at 0°C) was achieved by a 6% compensation factor as determined by Scandinavian Biogas Fuels AB:  
\[
1340 \text{ mL/day} \times 0.94 = 1260 \text{ mL/day}
\]

Biosludge and organic household waste amounts below were as according to collected data by Scandinavian Biogas Fuels AB (2010).  
Biosludge: 200 mL specific biogas production g\(^{-1} \) VS  
Organic household waste: 600 mL specific biogas production g\(^{-1} \) VS  
\( L. \text{minor} \): x mL specific biogas production g\(^{-1} \) VS  
Substrates added were biosludge, organic household waste and \( L. \text{minor} \), at 1 g OLR each.  
\[
1 \text{ g} \times 200 \text{ mL/g VS} + 1 \text{ g} \times 600 \text{ mL/g VS} + 1 \text{ g} \times x \text{ mL/g VS} = 1260 \text{ mL}
\]
\[
x \text{ mL} = 1260 \text{ mL} - 800 \text{ mL} = 460 \text{ mL}
\]

According to measurements, 41% of the biogas produced in the lab-scale digester was \( \text{CH}_4 \).  
\[
460 \text{ mL/day} \times 0.41 = 190 \text{ mL/day}
\]

4. Results

4.1 Plant cultivation

4.1.1 Growth: cultivation run one

Cultivation run one, where plants were grown in 100% leachate, proved quite unsuccessful. In four days, all plants in all containers were dead after having showed no growth and yellowing or withering spots from early on. \( C. \text{vulgaris} \) was visibly affected although some flocks might have still been alive after four days as opposed to the complete death of the plants. Additional carbon dioxide did nothing to aid the plants. Plants in containers with \( C. \text{vulgaris} \) were equally withering and dead as those without after four days. It was clear that \( L. \text{minor} \) and \( P. \text{stratiotes} \) could not grow in 100% leachate from Häradsudden landfill at its present state (i.e. prior to the phytoremediation system being instigated).

4.1.2 Growth: cultivation run two

Plants were able to grow in all leachates in the second cultivation run, where concentrations of 10, 30 and 50% leachate were used. Of the three concentrations, 50% leachate proved
hardest on the plants, while 10% leachate seemed most favorable. The graphs in Figures 6 and 7 show the mean growths of plants in respective leachate without any special attention to \textit{C. vulgaris}-containers, since the results of those did not deviate from the results of containers without algae.

As seen in Figure 6, \textit{L. minor} had a doubling time of around seven days during the second week. Doubling times receded after that, although plants in 10 and 30\% leachates still grew. For 50\% leachate, growth was declining somewhat after two weeks. Reproduction of plants in said leachate seemed dysfunctional or inhibited. Containers with added \textit{C. vulgaris} showed no extraordinary capabilities growth-wise, the only difference being in 50\% leachate where \textit{L. minor} individuals kept a dark green color throughout the test period as opposed to the sometimes yellowing and much lighter plants in 50\% leachate containers without the algae.

\textit{P. stratiotes} proved more restricted by the climatic conditions and leachate concentrations than \textit{L. minor}, and growth (in Figure 7 seen as cm$^2$ covered) was slow if at all noticed. Though some increase of surface cover was seen in 10\% leachate containers, plants in 50\% leachate were stressed (withering or corrosion damages) throughout the entire test period and even showed a decrease in surface covered compared to initial amounts.

It is worth noting however, that leachate of 50\% concentration was not the definite maximum for these plants to grow in. The two additions of leachate when refilling containers (see section 3.3) raised the concentration to 67\%. These additions and adjustments thereof are not visible in the trendlines in Figures 6 and 7 depicting growth, but plants showed signs of stress (mainly yellowing and corrosion), indicating that the limit of Häradsudden leachate lies around 67\%.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Fig_6}
\caption{Mean growths (as number of plants) of \textit{L.minor} in leachates of 10, 30 and 50\% concentrations. Standard errors are shown as error bars. Note that the horizontal axis starts on day 1 and not 0, due to a correction of numbers of plants in all containers on day 1. $\blacklozenge$ = 10\% leachate, $\blacksquare$ = 30\% concentrations, $\blacktriangle$ = 50\% concentrations}
\end{figure}
Controls of both *L. minor* and *P. stratiotes*, with and without *C. vulgaris* grew continuously and notably during the test period, but were not measured other than by visual estimation. Microalgae increase in control containers was strong and invasive.

There was some contamination of *C. vulgaris* between leachate containers, but not to an extent that it turned into a problem. There was however another kind of algae, possibly originating from one of the private aquariums providing the test plants, that grew strongly in short time and appeared in some control and test containers. As this algae intertwined with plant roots and *C. vulgaris* it was not possible to remove it during the test period, which might have added to the protection of plant roots and definitely aided in nutrient removal. This new algae was light green and formed threads or networks in the water, and was assumed to be of the *Chlorophyta* genus although no closer speciation could be performed.

### 4.1.3 Biomass

*P. stratiotes* showed little change in biomass after cultivation in 10% leachate, while 30 and 50% leachate plants even lost TS content compared to initial values. The control, which was kept on nutrient solution Z8 the entire time, doubled the TS content while VS remained similar to the initial (see Table 3).

*L. minor* showed a notable increase in TS contents compared to the initial measurements, with triple amounts for 10% leachate, double for 30% leachate and quadruple for 50% leachate. TS of the control dropped and also showed a large decrease in VS.

Note that VS after the cultivation run was assumed to remain identical to initial values, since there was too little plant matter for both total nitrogen and VS analyses.
Table 3. Biomass change in plants as TS and VS of TS. Z8 nutrient solution was used. Sample percentages indicate which leachate concentration plants were cultivated in. Note that VS values for leachate samples were assumed to remain identical to the initial measurements. Standard deviations presented in parentheses.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial</th>
<th>28 days in Z8 + 30 days in leachate</th>
<th>59 days in Z8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TS (%)</td>
<td>VS (% of TS)</td>
<td>TS (%)</td>
</tr>
<tr>
<td>P. stratiotes 10%</td>
<td>9.6 (0.5)</td>
<td>87.9 (0.5)</td>
<td>9.6 (0.8)</td>
</tr>
<tr>
<td>P. stratiotes 30%</td>
<td>9.6 (0.5)</td>
<td>87.9 (0.5)</td>
<td>6.6 (0.2)</td>
</tr>
<tr>
<td>P. stratiotes 50%</td>
<td>9.6 (0.5)</td>
<td>87.9 (0.5)</td>
<td>6.6 (0.2)</td>
</tr>
<tr>
<td>P. stratiotes control</td>
<td>9.6 (0.5)</td>
<td>87.9 (0.5)</td>
<td>13.4 (0.0)</td>
</tr>
<tr>
<td>L. minor 10%</td>
<td>5.3 (1.2)</td>
<td>93.9 (0.8)</td>
<td>14.7 (1.2)</td>
</tr>
<tr>
<td>L. minor 30%</td>
<td>5.3 (1.2)</td>
<td>93.9 (0.8)</td>
<td>11.1 (1.1)</td>
</tr>
<tr>
<td>L. minor 50%</td>
<td>5.3 (1.2)</td>
<td>93.9 (0.8)</td>
<td>19.3 (0.3)</td>
</tr>
<tr>
<td>L. minor control</td>
<td>5.3 (1.2)</td>
<td>93.9 (0.8)</td>
<td>4.4 (0.0)</td>
</tr>
</tbody>
</table>

4.1.4 Total nitrogen in plants and sediments
Accumulated total nitrogen in plants was higher for P. stratiotes than for L. minor, as shown in Table 4. The former had a total nitrogen content of between 2 and 4 g/m² while the content of L. minor ranged between 1 and 2 g/m². Though of higher percentual content than for 10% leachate, actual nitrogen amounts were lowest for L. minor in 30% leachate, as seen in Table 4. Furthermore, P. stratiotes showed higher percentual nitrogen content for plants in 30% leachate although actual contents were in range as expected compared to the other leachate concentrations.

Table 4. Total nitrogen accumulated in plants during the second cultivation run (29 days), presented as % of dry weight and g/m². Means of total nitrogen in each plant community is shown, with standard deviations in parentheses. Ps = P. stratiotes, Lm = L. minor, 10, 30 and 50 indicate leachate concentrations.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Accumulated N&lt;sub&gt;tot&lt;/sub&gt; in plants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(%) of DW</td>
</tr>
<tr>
<td>Ps10</td>
<td>4.4 (0.4)</td>
</tr>
<tr>
<td>Ps30</td>
<td>5.8 (1.0)</td>
</tr>
<tr>
<td>Ps50</td>
<td>5.4 (0.4)</td>
</tr>
<tr>
<td>Lm10</td>
<td>2.6 (0.5)</td>
</tr>
<tr>
<td>Lm30</td>
<td>3.1 (0.4)</td>
</tr>
<tr>
<td>Lm50</td>
<td>4.5 (0.3)</td>
</tr>
</tbody>
</table>

No TS was measured for sediments, which was why no actual weights could be presented in Table 5. For all leachates, sediments held around 3% nitrogen of the total DW. The trend
followed plant concentrations well, with 10% leachate sediments having the highest percentage of nitrogen content and 50% leachate sediments the lowest.

Table 5. Total nitrogen in sediments at the end of the cultivation run (see section 3.5.4) presented as % of dry weight. Standard deviations are shown in parentheses. Ps = P. stratiotes, Lm = L. minor, 10, 30 and 50 indicate leachate concentrations.

<table>
<thead>
<tr>
<th>Sample</th>
<th>N_{tot} in sediments (% of DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ps10</td>
<td>3.0 (0.4)</td>
</tr>
<tr>
<td>Ps30</td>
<td>2.8 (0.0)</td>
</tr>
<tr>
<td>Ps50</td>
<td>2.5 (0.1)</td>
</tr>
<tr>
<td>Lm10</td>
<td>3.6 (0.7)</td>
</tr>
<tr>
<td>Lm30</td>
<td>3.0 (0.3)</td>
</tr>
<tr>
<td>Lm50</td>
<td>2.6 (0.1)</td>
</tr>
</tbody>
</table>

4.1.5 Phosphate in leachates
Diluting the leachate to the 10, 30 and 50% concentrations for cultivation run two caused the phosphate levels to fall below the measurement ranges of 0.5-5.0 mg/L and exact final values could not be achieved. Initial values for the new concentrations were recalculated from 100% leachate values and were low, although the control leachate values were a bit higher. Still, even for 50% control leachates, phosphates had decreased to below 0.5 mg/L after 29 days, a decrease of at least 26.5%.

Table 6. Phosphate levels for leachates with plants and control leachates. Ps = P. stratiotes, Lm = L. minor, Ctrl = Control, 10, 30 and 50 indicate leachate concentrations. Standard deviations are not available due to one-point measurements.

<table>
<thead>
<tr>
<th>Sample</th>
<th>PO_{4}^{3-}P (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ps10</td>
<td>0.1</td>
</tr>
<tr>
<td>Ps30</td>
<td>0.2</td>
</tr>
<tr>
<td>Ps50</td>
<td>0.4</td>
</tr>
<tr>
<td>Lm10</td>
<td>0.1</td>
</tr>
<tr>
<td>Lm30</td>
<td>0.2</td>
</tr>
<tr>
<td>Lm50</td>
<td>0.4</td>
</tr>
<tr>
<td>Ctrl10</td>
<td>0.1</td>
</tr>
<tr>
<td>Ctrl30</td>
<td>0.4</td>
</tr>
<tr>
<td>Ctrl50</td>
<td>0.7</td>
</tr>
</tbody>
</table>
4.1.6 Nitrate

Fig 8. Nitrate in leachates with P. stratiotes, of different concentrations over time. Standard errors are shown as error bars. ■ = initial leachate, ■ = leachate day 9, ■ = leachate day 17, ■ = leachate day 29, ■ = control leachate day 29 (Ctrl = control, d = day)

Fig 9. Nitrate in leachates with L. minor, of different concentrations over time. Standard errors are shown as error bars. ■ = initial leachate, ■ = leachate day 9, ■ = leachate day 17, ■ = leachate day 29, ■ = control leachate day 29 (Ctrl = control, d = day)
Nitrate (seen in Figures 8 and 9) in leachates with *P. stratiotes* and *L. minor* showed similar trends. A strong increase of nitrate concentrations could be seen after nine days, where 50% leachates gave the steepest increase and 10% leachates the lowest. For the remainder of the cultivation run, nitrate levels of 10% and 30% leachates for both plant types stayed approximately the same as the respective levels on day 9, while for 50% leachates there was a drop in nitrate concentrations from day 9 to 29.

Control leachates showed a major increase in nitrate concentrations at the end of the test period, at around three times as much as for leachates with plants.

### 4.1.7 Ammonium
Measurement ranges for the analysis cuvettes (47-130 mg/L and later 2-47 mg/L) limited the exact outcome of some ammonium samples, but as a way of showing the declining trend in ammonium levels results were satisfactory.

Table 7. Ammonium levels for leachates with plants and controls without plants. No measurements were done for controls on day 17. *Ps* = *P. stratiotes*, *Lm* = *L. minor*, *Ctrl* = Control. 10, 30 and 50 indicate leachate concentrations. No standard deviations are available due to initial one-point measurements and inexact results.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial leachate</th>
<th>Day 9</th>
<th>Day 17</th>
<th>Day 29</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ps</em>10</td>
<td>32</td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ps</em>30</td>
<td>95</td>
<td>B</td>
<td>e</td>
<td>e</td>
</tr>
<tr>
<td><em>Ps</em>50</td>
<td>158</td>
<td>e</td>
<td>l</td>
<td>B</td>
</tr>
<tr>
<td><em>Lm</em>10</td>
<td>32</td>
<td>o</td>
<td>w</td>
<td>l</td>
</tr>
<tr>
<td><em>Lm</em>30</td>
<td>95</td>
<td>o</td>
<td>w</td>
<td>o</td>
</tr>
<tr>
<td><em>Lm</em>50</td>
<td>158</td>
<td>4</td>
<td>2</td>
<td>w</td>
</tr>
<tr>
<td><em>Ctrl</em>10</td>
<td>32</td>
<td>7</td>
<td>n/a</td>
<td>2</td>
</tr>
<tr>
<td><em>Ctrl</em>30</td>
<td>95</td>
<td>n/a</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ctrl</em>50</td>
<td>158</td>
<td>74</td>
<td>n/a</td>
<td></td>
</tr>
</tbody>
</table>

As shown in Table 7, despite the variation of initial levels of ammonium in leachates with plants, after about half the cultivation time there was less than 2 mg/L left in all containers. This was a decrease of at least 93.5% for the 10% leachates, 98.0% for the 30% leachates and 98.5% for the 50% leachates, in both plant and control containers. No difference between plant types could be discerned, and the only notable value was the 50% control leachate which had not dropped as much as the rest on day 9.

Comparing the total decrease in ammonium levels with the total increase of nitrate, assuming that all ammonium had turned to nitrate and that all nitrate came from nitrification, a ratio of ammonium remaining in leachates as nitrate could be determined. Ratios were much lower in leachates with plants than in controls without plants, as depicted in Table 8, but were quite similar between plant species. There was also a trend of lesser ammonium-to-nitrate the higher the leachate concentration.
Table 8. Ammonium remaining as nitrate in plant and control leachates, assuming that all ammonium has turned to nitrate and that all nitrate comes from nitrification of the initial ammonium. Ps = P. stratiotes, Lm = L. minor, Ctrl = Control. 10, 30 and 50 indicate leachate concentrations. Standard deviations were not possible to calculate due to one-point measures and inexact results as seen in Table 7.

<table>
<thead>
<tr>
<th>Sample</th>
<th>NH$_4^+$-N decrease (mg/L)</th>
<th>NO$_3^-$-N increase (mg/L)</th>
<th>NH$_4^+$-N remaining as NO$_3^-$-N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ps10</td>
<td>30</td>
<td>6</td>
<td>19</td>
</tr>
<tr>
<td>Ps30</td>
<td>93</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Ps50</td>
<td>156</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td>Lm10</td>
<td>30</td>
<td>5</td>
<td>17</td>
</tr>
<tr>
<td>Lm30</td>
<td>93</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Lm50</td>
<td>156</td>
<td>19</td>
<td>12</td>
</tr>
<tr>
<td>Ctrl10</td>
<td>50</td>
<td>25</td>
<td>83</td>
</tr>
<tr>
<td>Ctrl30</td>
<td>93</td>
<td>66</td>
<td>72</td>
</tr>
<tr>
<td>Ctrl50</td>
<td>156</td>
<td>87</td>
<td>56</td>
</tr>
</tbody>
</table>

4.1.8 Alkalinity

![Graph showing Alkalinity](image)

Fig 10. Mean alkalinites of different leachate concentrations, standard errors shown as error bars. ■ = initial leachate, ■ = control leachate day 29, ■ = P. stratiotes leachate day 29, ■ = L. minor leachate day 29 (Ps = P. stratiotes, Lm = L. minor, Ctrl = control, d = day)
Compared to initial alkalinity levels in all leachates, at the end of cultivation levels had been drastically lowered except for 10% leachate with plants which remained about the same (see Figure 10). There were no great differences between plant species or between controls and leachates with plants, apart from 10% leachate where the controls were much lower than all other leachates. Furthermore, the difference in alkalinity between 10, 30 and 50% leachates at initial measuring did not appear as pronouncedly for plant or control leachates on day 29.

4.1.9 Water color and turbidity

Initial leachates of all concentrations held a red tinge to the water and there was no sediment in the experiment containers. Leachates with plants followed a color-shift from red to clear via yellow, green and very dark in the four weeks of cultivation. This was most pronounced in 30% leachates followed by 10% leachates, while 50% leachates had the least pronounced color-shifts. There was no difference between plant types or between containers with or without algae. Control leachates without plants followed the same shift in color only much slower, and in four weeks the controls only reached the green stage and remained much browner and murkier than the leachates with plants had ever been.

Sediments appeared as a ~2 mm biofilm at the bottom of all containers with plants after around two weeks. Sediments followed the initial coloring of the leachates, but turned darker as time passed and more suspended matter settled. In the end of the cultivation period, sediments were what gave the impression of color to the leachate; waters were quite clear as mentioned above. Control leachates without plants never gained any sediment.

Fig 11a-d. Shifts in color and turbidity. The figure shows 30% leachate with L. minor at the time of a = 0 days b = 14 days c = 21 days and d = 28 days
4.1.10 pH
pH followed an identical trend for all leachates with plants throughout the cultivation period (see Figure 12). Apart from 10% leachates which were lower than the rest upon start-up, pH decreased to around pH 8.00 during the first week but then rose notably for all leachates to around pH 9.50. A pH adjustment was done on day 24 (see section 3.5.6), intended to lower pH by 1.5 but turned out barely notable at the following measurement on day 29. Instead, the graph shows a relative stability for all samples at pH 9.50 or slightly below.

Fig 12. pH of leachates with plants, standard errors shown as error bars. ◆ = L. minor, 10% leachate, ■ = L. minor, 30% leachate, ▲ = L. minor, 50% leachate, ◆ = P. stratiotes, 10% leachate, ▲ = P. stratiotes, 30% leachate, ● = P. stratiotes, 50% leachate

Fig 13. pH of leachate controls without plants, standard errors shown as error bars. ◆ = 10% leachate, ▲ = 30% leachate, ▲ = 50% leachate
Control leachates (in Figure 13) followed a similar pH trend but didn’t reach as high values as fast as the plant leachate samples. Also, no pH adjustment was performed on the control leachates but the 10% leachate dropped slightly and pH of the 30% and 50% leachates seemed about to flatten out at the end of the control run without any external input.

4.2 Batch results
Whatman paper as a control substrate gave gas production enough with a recognizable trend curve as compared to Scandinavian Biogas Fuels AB routines (2010) for the batch to be considered satisfactory. The inoculum was active and suited for anaerobic digestion, while incubated methane controls remained on the same level throughout the batch run, as desired.

![Graph showing methane production over time](image)

*(Fig 14. Mean CH₄ productions at 0°C as mL g⁻¹ VS. Standard errors shown as error bars. ♦ = L. minor, ■ = P. stratiotes, ▲ = Whatman paper)*

*P. stratiotes*, as seen in Figure 14, had initiated a notable gas production after only a few days, but leveled out soon thereafter. One of the triplicates remained lower than the other two throughout the batch run and was apparently fully digested sooner than the other two, causing a lower mean. That bottle also showed a dip at day 20 which was not mirrored in the total gas production and was ascribed to a measurement error. Mean values for *P. stratiotes* bottles at 0°C gave 225 ±18 mL methane g⁻¹ VS after 32 days.

*L. minor* had a satisfactory biogas production, and there even seemed to be some biomass left to digest at the end of the batch run as trend lines hadn’t completely leveled out. Triplicate samples showed good correlation between each other for methane production (see Figure 14). Mean values for *L. minor* bottles at 0°C gave 461 ±14 mL methane g⁻¹ VS after 32 days.

4.3 Reactor results

4.3.1 Gas production
As seen in Figure 15, gas production rose for the first three days after which the loading rate was changed and gas production stabilized. It remained between 320 and 420 mL g⁻¹ VS for
the rest of the experiment. The missing value on day 8 was due to a measurement error, leaving the produced gas amount undetermined.

![Graph showing biogas production over time](image)

**Fig 15. Specific biogas production of L. minor-fed lab-scale reactor as mL/g VS at 0°C.**

### 4.3.2 Methane content

The measurements using a gas tight balloon and the Biogas Check proved unsuccessful as too small amounts of gas were produced to give accurate readings. Instead gas sampled directly from the gas phase inside the reactor was run on the GC-FID at the end of the reactor run. This gave a methane concentration of 41 ±2.5% (SD in absolute percentages). Gas stored in the balloon was also tested and gave 25 ±2.5% (SD in absolute percentages), proving that the gas sampling had been unsatisfactory. As calculated in section 3.8.3, *L. minor* gave a production of 460 NmL biogas g⁻¹ VS which equaled 190 NmL methane g⁻¹ VS in this lab-scale reactor.

### 4.3.3 pH

pH values remained stable around 7.4-7.5 up until day 13, after which they dropped to below 7.1. The reactor adjusted this on its own by raising pH slightly in a few days without any external adjustments although extra monitoring was performed through extra pH measures. The highest point of recovery reached was 7.2 however, because on day 20 pH had dropped again to the same low values as earlier. No adjustments were made and the reactor run ended before any drastic increase or decrease could occur, leaving pH at just above 7.0.

### 4.3.4 VFA

The initial value of acetic acid was 1.8 mM, which was assumed to be a consequence of the routines and feedstock of the digester prior to this experiment starting. Thereafter, all acids including acetic acid were below 0.5 mM and considered non-existent to any harmful degree. On day 13, VFA concentrations rose, and there were indications of many acids although only acetic and propionic acids were considered. These were around 4 and 2 mM respectively. However, in only a few days time they had disappeared without any external adjustments. Near the end of the reactor run, acetic acid rose to 1.1 mM. There was a clear correlation
between low pH and increasing VFAs during the reactor run, although VFA appearances were delayed with a few days when pH fell below 7.1 near the end of the experiment.

4.3.5 Ammonium
There was a solid trend of ammonium diminishing in the reactor over time, with a proportional decrease of approximately 10% each week.

5. Discussion

5.1 Plant cultivation
It is worth noting that the outcome of this study might differ from cultivation and nutrient removal results in larger scale. Efficiencies regarding nutrient uptakes could be misleading since containers in the laboratory posed a small, enclosed environment in which changes were notable (Vermaat and Hanif, 1998). Growth as affected by the shifting weather and temperatures of an outdoor pond will most likely be different than in the controlled climate room of the laboratory. Therefore, results and the following discussion are to be seen as indications for large-scale cultivation, and not exact answers.

5.1.1 Growth
The results of the first cultivation run clearly showed that the used strains of *P. stratiotes*, *L. minor* or even *C. vulgaris* cannot grow in leachate from Häradsudden landfill unless some dilution takes place first. This is in line with the method capacity, as phytoremediation generally is used as a later purification step in a system for polluted soil or water (Nagendran et al, 2006; Susarla et al, 2002).

Vermaat and Hanif (1998) report a “surprisingly poor” (p. 2573) growth rate for *L. minor* and other duckweeds on domestic waste water, in line with the result of cultivation run one and two. As plants grew for Vermaat and Hanif (1998) in waste water with higher nutrient content than the Häradsudden leachate, this would indicate that plants in cultivation run one should have been able to survive. However, Vermaat and Hanif (1998) do not report any ammonium levels for the waste water, and as Jones with colleagues (2006) state, ammonium is often present in high amounts in landfill leachate, making it a toxic compound (Jones et al, 2006, p 827). Clarke and Baldwin (2002) deduced that concentrations of above 200 mg/L ammonia affect aquatic plants and as initial levels of ammonium in Häradsudden leachate were above 300 mg/L (assumed to be in a steady-state relation with ammonia, placing the latter at about the same concentration), this was likely one of the main compounds responsible for the swift death of all plants in cultivation run one. Other aspects that could have worked toward the too harsh conditions are salinity (Cross, 2002), elevated levels of heavy metals or other toxic compounds (Susarla et al, 2002), all of which were not analyzed in this experiment. *P. stratiotes* showed corroding effects on the leaves and roots that most likely had to do with salinity, and as Haller et al (1974) determine, this plant has a lower tolerance toward salinity than *L. minor*. Toward the end of the second cultivation period, pH had increased to above 9.5, which probably hampered growth of the plants even though they did not succumb to it.

In the second cultivation run *L. minor* and *C. vulgaris* grew, while *P. stratiotes* showed little if any increase in surface cover. The main reason for this slow growth was assumed to be the harsh conditions of growing on leachate in combination with temperature. Growth of *L. minor* and *P. stratiotes* in nutrient solution at 20°C (which was carried out in order to maximize biomass before advancing to the biogas step of the experiment) proved much better compared to controls on the same nutrient solution at 18°C. *P. stratiotes* according to Smirnova and
Mironova (2004) grows slowly in chemically challenging waters even though purifying effects can be satisfactory. It should manage to grow in 18°C as reported by Šajna with colleagues (2007), but there is a strong correlation between growth and temperature where the former increases if the surrounding environment gets warmer (Šajna et al, 2007). What with the Swedish climate, *P. stratiotes* might not be suitable for phytoremediation in outdoor ponds like the one at Häradsudden. *L. minor* on the other hand grows naturally in the county along with other species of the *Lemnaceae* family (Anderberg and Anderberg, 2010), and could probably adjust to the leachate conditions with time and better temperatures.

Algae, in this case *C. vulgaris*, are harder to harvest than floating plants and since containers with *C. vulgaris* showed no differing values compared to those without, there is no reason to include another microorganism in the leachate for purifying reasons. *L. minor* also has periphyton attached to the roots that enhances nutrient uptake (Vermaat and Hanif, 1998), which provides the extra effect sought for with the addition of microalgae in the experiment, although without the invasive spreading showed by *C. vulgaris*.

### 5.1.2 Biomass

As mentioned in section 4, there was not enough plant material to do a final VS measurement as well as total nitrogen, why VS values were assumed to remain identical to initial percentages. This was not optimal and final VS values would have made the estimation of plant uptake more correct, but the approximation will have to do for this discussion.

Looking at *P. Stratiotes*, TS decreased for the higher leachate concentrations. This could have to do with the bioavailability in 30 and 50% leachates possibly being lower than that of 10% leachate (Vermaat and Hanif, 1998). For control plants, increase in TS accompanied by a slight decrease of VS could be explained by the nutrient solution composition where a multitude of minerals were included. In the nutrient solution, there was much more microalgae growth than in leachates which could have enhanced TS increase for control plants.

*L. minor* showed the opposite trend compared to *P. stratiotes* with an increase in TS for leachate plants and decrease for control plants. Being a much smaller plant than *P. stratiotes*, it is possible that the extreme algae growth in control containers rather prevented *L. minor* controls from satisfactory nutrient uptake than enhanced it, causing them to lose biomass as can be seen by a lower TS as well as VS. Comparing the two species, *L. minor* seemed better off than *P. stratiotes* both when it came to surface cover and to biomass increase on Häradsudden leachate.

### 5.1.3 Total nitrogen in plants and sediments

*P. stratiotes* had twice the total nitrogen uptake than that of *L. minor*, as shown in Table 4. Some of the content could be accredited to the attached periphyton as discussed by Smirnova and Mironova (2004), since there was a notable amount of attached algae and other microorganisms on all *P. stratiotes*-individuals which only partly came off through rinsing with tap water. Lu with colleagues (2010) label *P. stratiotes* as having “great potential” (p 96) for removal of nutrients, and judging by the total nitrogen percentages of plant dry weight as seen in Table 4 compared to the growth capacity of 60-110 t ha⁻¹ yr⁻¹ as mentioned by Mishima and colleagues (2008), this plant definitely holds great promise for nitrogen removal. As stated in section 5.1.1 though, the Swedish might prove too taxing for *P. stratiotes*. This naturally lessens nitrogen uptake as well as that of other compounds (Lu et al, 2010).
*L. minor* has similar nitrogen accumulation in 10% and 30% leachates, although the amounts are slightly lower in the latter. This was due to different growth and TS in the varying leachate concentrations (see sections 4.1.2-3). Vermaat and Hanif (1998) state that slow-growing species and species growing slowly due to environmental harshness have the highest nutrient content after a test run of a few weeks. This is applicable both to *P. stratiotes* in all cases within this study, as well as for *L. minor* in 50% leachate. Due to “extraordinary” growth in 10% leachates (and thereby nutrient accumulation) and slow growth enhancing the nutrient uptake in 50% leachates as Vermaat and Hanif (1998) report, the lowest actual amounts would be found in 30% leachates as seen in Table 4.

Nitrogen content in sediments was quite even between leachate concentrations and plant types, as seen in Table 5. 10% leachate sediments contained most total nitrogen, which corresponds to the idea of nitrification and denitrification processes being most active in said leachate (see section 5.1.6). It is safe to assume that *C. vulgaris* had settled in the sediment as biofilm, thus being partly responsible for sediment nitrogen content through uptake of its own. Assuming that 10% leachate proved as favorable for the algae as for the plants, those containers would have held more *C. vulgaris* and thereby more total nitrogen in the sediment compared to leachates of 30 and 50%, as was also the case (see Table 5).

5.1.4 Phosphate in leachates
As the second cultivation run included diluted leachates, phosphate levels were lower than the available measuring range (<0.5 mg/L) from the start. This meant that no nutrient removal could be studied for phosphorus. It is safe to say that phosphorus was taken up by the plants and algae though, as nitrogen and phosphorus are commonly known to be the two nutrients necessary for all plant growth. Bioavailability should not be an issue as phosphorus present in landfill leachate should be accessible to plants (Renou et al, 2008a).

The measurement limit of 0.5 mg/L was, while not producing any exact values, enough for this study regarding nutrient concentration reductions in Häradsudden leachate. Econova Biotech AB has to treat the leachate to levels of 0.5 mg/L phosphorus maximum in order to be permitted to release it into the surroundings, as per municipal directives according to Malin Asplund at Econova AB (personal contact, 2010-05-07). If plant cultivation is to be used as a means of treating Häradsudden leachate, the water will have to be diluted to the extent that phosphorus levels automatically fall beneath the permitted concentration.

5.1.5 pH and ammonium
Vermaat and Hanif (1998) report a pH increase in waste water studies with *Lemnaceae* of up to pH 9.6, which matches the results in this study. There is no further discussion on this increase of pH by Vermaat and Hanif (1998), but Leng et al (1995) mention algal respiration as a carbon dioxide source in water (which raises pH) when photosynthesis is not enough to overcome carbon dioxide release. Algae were present in leachate containers with plants as noted in section 4 above. For control leachates without plants, judging by the murkiness of said waters and the similarity to the pH curve of leachates (see Figures 12 and 13), there were some microalgae present to facilitate a carbon dioxide release greater than photosynthesis even there.

The nitrification process instead lowers pH by turning ammonium into nitrate (The Water Planet Company, 2010), meaning that this process competed with algae respiration in affecting pH. Initially, algae biomass was low and there was a sufficient amount of
ammonium in each container to allow nitrification to lower pH, as seen in Figure 12. However, after nine days ammonium levels in leachates had diminished greatly and were low compared to initial amounts (as judged by 50% leachate values, see Table 7) and nitrification had to slow down markedly. This in combination with algae growth and its increasing carbon dioxide uptake caused pH to rise after ten days and remain high despite efforts to adjust it to a lower level. The only leachate differing from this pronounced trend was the 50% control leachate, in which ammonium levels decreased more slowly and adversely showed a less steep pH increase after day 10. Similar to these results, Vermaat and Hanif (1998) report a 99% decrease of ammonium after twelve days and a notable periphyton presence throughout the experiment, which in correlation with their pH of 9.6 supports the theory of the same processes occurring in this study.

For ammonium remaining in the leachate as nitrate (see Table 8), the trend of lower ratios the higher the leachate concentrations was ascribed to the inhibitory effect free ammonia has on nitrifiers (The Water Planet Company, 2010). What with the higher concentrations of ammonium in 30 and 50% leachates, it is safe to say that ammonia too was present in elevated amounts. This inhibited nitrifiers and caused a slower turn-over rate for nitrification, allowing ammonium to evaporate to the atmosphere rather than being nitrified.

Econova Biotech AB has a municipal directive to keep nitrogen in the form of ammonium below 50 mg/L in order to safely discharge leachate from the landfill. The results of this study showed that such levels can be accomplished with or without plants present; only the process is faster with plants than without. Furthermore, nitrate was transformed by denitrifiers or taken up by plants in leachates where plants were present, causing further purification of leachates in terms of total nitrogen.

5.1.6 Nitrate and alkalinity
Nitrification occurred for both plant and control leachates of all concentrations. For control leachates without plants however, nitrate levels rose steeply and then remained elevated, while leachates with plants showed a modest raise of nitrate content that slowly began to drop as time passed. This decrease in nitrate was ascribed to plant uptake as well as denitrification. As only leachates with plants provided enough anoxic sediment in which denitrifiers could thrive, the process needing an anaerobic environment to function (Madigan and Martinko 2010), nitrate amounts were lowered for the plant containers while nitrate remained unchanged in controls. Alkalinity further confirms this difference in nitrate processing between plant and control leachates, as alkalinity is consumed when nitrification occurs (The Water Planet Company, 2010). There was notable symmetry especially in control leachate nitrate increase compared to alkalinity decrease. Where nitrate concentrations went up, alkalinity went down.

For leachates with plants alkalinity decreased to similar levels as for controls, except for the 10% containers in which alkalinity remained close to initial values. For the latter, this was assumed to include changes over time not seen when just measuring initial and final values. What with nitrate concentrations in 10% leachate showing the greatest percentual increase compared to the other leachates (see Figure 10), denitrification would have occurred more strongly in 10% leachate in a similar way and causing alkalinity to rise back to its initial levels. This concentration change of calcium carbonate could not be seen in the graph as no continuous alkalinity measurements were done.
Alkalinity may also have been raised by other mechanisms (Abril and Frankignoulle, 2000). One such mechanism likely to have occurred in Häradsudden leachate was the anaerobic Fe(III)-reduction (Abril and Frankignoulle, 2000), as judging by the initial color of the leachate there was plenty of iron present (which was also mentioned by Malin Asplund at Econova Biotech AB (personal contact, 2010-05-07)).

5.1.7 Water color and turbidity
As time passed, leachates shifted in color and became clearer. This had partly to do with sedimentation of suspended solids, as sediments appeared and grew to about 2 mm thick at the end of the cultivation run. What with the initial red color of all leachates, it was assumed that iron levels were high, something also commented on by Econova Biotech AB representative Malin Asplund (personal contact, 2010-05-07). As Upadhyay and colleagues (2007) show, iron is a metal most preferred by both *P. stratiotes* and *L. minor* when it comes to heavy metal uptake, and both species possess quite a capacity for removing it from polluted water (Upadhyay et al, 2007). This could be a source for the color change, along with algae growth that would have enhanced the green tinge.

5.2 Biogas production

5.2.1 Batch run
As seen in Figure 14, Whatman samples did not get fully digested in the 32 days of the batch run, which was ascribed to the low amount of inoculum used (0.1 L). Gas amounts produced in Whatman paper bottles were reasonable considering the low organic load both of Whatman paper and inoculum compared to earlier batch references done by Scandinavian Biogas Fuels AB. For incubated methane samples, values were 5-10% lower than expected, which incurs a similar understatement of sample values in addition to the aforementioned low organic load. This is not adhered to in stating of results here or in section 4.2, but noted as a point of interest.

Although *P. stratiotes* was relatively swiftly digested to begin with, showing a strong rise in amount of gas produced, it leveled off rather quickly and never reached any astounding amounts. According to Nipaney and Panholzer (1987), *P. stratiotes* can give up to 400 mL methane g⁻¹ VS and has been labeled an “excellent substrate” for methane production (Gunaseelan, 1997, p 111). In this study however, the aquatic plant proved less successful and gave only 225 mL methane g⁻¹ VS. It is possible that insufficient homogenization of the substrate was behind the difference in outcome, causing lessened availability of the biomass content in this study. Another explanation could be that *P. stratiotes* in Nipaney and Panholzer’s study (1987) were further matured than those of this experiment, which might have caused differences in biomass composition (i.e. more nutrients had accumulated in the mature plants than in this study, allowing the formation of more protein (Fonkou et al, 2002)). A combination of different nutrient content than in literature (Nipaney and Panholzer, 1987) and microorganisms not optimal for cellulose-containing substrates (Scandinavian Biogas Fuels AB, 2010) was most likely the cause in this study.

Compared to *P. stratiotes*, *L. minor* did not as good in terms of initial production rate potential. *P. stratiotes*, had reached 71% of its maximum amount mL of gas per gram added VS after 4 days’ digestion where *L. minor* had only reached 40% in the same amount of time. This still did not make the former a better candidate for biogas production, as *L. minor* produced 461± 14 mL methane g⁻¹ VS compared to the 225 ± 18 mL g⁻¹ VS of *P. stratiotes*. 
Jain and colleagues (1992) present a biogas potential of *L. minor* at little more than 100 mL g\(^{-1}\) added VS depending on what metal composition is used. Some metals cause lessened methane production while others enhance it (Jain et al, 1992), which could to some extent explain the higher potential found in this study. However, this study provided a *L. minor* methane potential of four to five times the ones reported by Jain and colleagues (1992), which presence of metals alone probably cannot explain. A factor to be considered as the main reason for these differing numbers was the VS amount of 66% that seemed dramatically low despite good correlation between samples (see Table 3). All VS values for *L. minor* during the reactor run that followed were above 76%, pointing to a misleading organic load for the batch. A measurement error or insufficient preparation of samples (i.e. drying) could have caused an understatement of VS values for *L. minor* that affected the loading and thus the methane production. Other explanations to this could be found in microorganism cultures (Bagi et al, 2007; Weiland, 2010) or the nutrient or chemical composition of the plants as they can vary greatly within the Lemna family (Landolt and Kandeler, 1987).

### 5.2.2 Reactor run

Results from the lab-scale reactor run showed *L. minor* producing 190 NmL methane g\(^{-1}\) VS. As with the batch results, Jain and colleagues (1992) present far lower values for the same species, ranging from 89 to 127 NmL g\(^{-1}\) VS depending on what heavy metals affect the plant. The discussion in section 5.2.1 will not be repeated here, but it is worth noting that reactor results were closer to literature values (Jain et al, 1992) which confirms the VS values of *L. minor* during the reactor run in this study being more accurate than those of the batch run.

Unfortunately the feeding process of the lab-scale reactor sometimes caused air to enter the digester, and the 41 ±2% methane content (SD as absolute percentages) of the biogas gotten directly from the digester gas phase was likely underestimated. Had productivity been affected to any larger degree though, VFAs would have increased as the anaerobic microorganisms became inhibited (Gerardi, 2003) As VFAs remained close to zero throughout the reactor run, the digester process was considered stable in that respect. Another factor that could have increased methane production was the microbial culture in the digester sludge, as cellulose digesters associated with plant degradation probably would have proven more efficient than random waste water microorganisms (Patience et al, 1983).

That pH decreased prior to any shift in VFA concentrations indicates another reason for the low pH values than process inhibition that causes VFAs (Griffin et al, 1998). It is possible that *L. minor* or the total feedstock composition(including biosludge and organic household waste had a lower pH to begin with. This, as the first HRT drew to an end and *L. minor* occupied a larger share of the digester sludge, could then have caused a lower pH in the digester. A low buffer capacity coupled to the increasing amount of new feedstock (of assumed low pH) might have influenced the digester further at that time (Griffin et al, 1998).

*L. minor* as a feedstock for anaerobic digestion was suitable with regards to methane potential and biogas produced, although would have been even better with a larger digester (i.e. without the feeding process risking air to enter the digester space) and possibly another inoculum more adept at degrading cellulose. However, *L. minor* works better as an addition to other substrates rather than on its own, as cultivation of the plant to get the amounts needed to run a reactor is time and space consuming (Clark et al, 1996). Also, methane amounts of 190 mL g\(^{-1}\) VS are low compared to many other substrates (Gunaseelan, 1997).
6. Conclusions

Of the species selected for phytoremediation, plants proved more practically advantageous than microalgae. Choosing a species native to the climate is preferred, as the locally present *L. minor* grew better and handled the harsh conditions of leachate better than the tropical *P. stratiotes*. None of the selected plants or algae could grow in 100% Häradsudden leachate, but showed better coping abilities in leachates of 10, 30 and 50% leachate. The lower the leachate concentration, the better the growth. Had a higher temperature been allowed during the experiment, growth would have increased further but then not mirroring the climatic conditions at Häradsudden landfill. For nutrient removal, there were no great differences between *L. minor* and *P. stratiotes*. Ammonium was swiftly nitrified in both leachates with plants and control leachates without, reaching below the allowed limit of 50 mg/L in little over a week. However, no further processing of nitrate was seen in control leachates without plants while denitrification did occur in leachates with plants, removing nitrate as well from the water. Total nitrogen amounts accumulated in plants was 2-4 g m\(^{-2}\) for *P. stratiotes* and 1-2 g m\(^{-2}\) for *L. minor*, but coupled to the better growth of the latter, *L. minor* was deemed most suitable for phytoremediation in leachate of this kind (if diluted to at least 50%). As leachates had to be diluted for plants to grow, phosphate concentrations were already below the allowed and measured limit of 0.5 mg/L upon cultivation start-up (which could have been growth limiting for the plants). Water color and turbidity shifted notably for leachates with plants during the four weeks of the experiment, while control leachates without plants barely showed any change in color and none in turbidity.

The batch experiment proved *P. stratiotes* worse off than *L. minor*; the former at around 225 NmL methane g\(^{-1}\) VS and the latter at around 461 NmL methane g\(^{-1}\) VS. This was less by *P. stratiotes* and more by *L. minor* than described in literature (Gunaseelan, 1997), which was ascribed to differing nutrient contents of the plants compared to studies by others (see section 5.2.1).

*L. minor* was selected for the anaerobic digester run, and produced 190 NmL methane g\(^{-1}\) VS. The amount would most likely have been larger with another feeding process, as air sometimes entered the digester when feeding. *L. minor* was considered a suitable feedstock addition for anaerobic digestion, but to run a large-scale reactor on this plant only would convey practical issues regarding cultivation, harvesting and drying.

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Appendices

Appendix 1: Recommendations for Econova Biotech AB

1. Species
Of the two species studied, *L. minor* proved better than *P. stratiotes* for nutrient removal considering growth capacity under the temperature and leachate conditions characteristic for Häradsudden landfill. However, as studied by Vermaat and Hanif (1998), two other species in the *Lemnaceae* family might prove even better for the cause; *Lemna gibba* L. and *Spirodela polyrhiza* L. Both species grow naturally in Östergötland and have the same characteristics as *L. minor* (Anderberg and Anderberg, 2010) only with higher nutrient uptake capacities and/or resiliencies to elevated levels of chemical compounds (Vermaat and Hanif, 1998).

2. Viability (economical and practical)
While harvesting of duckweed is easy (skimming it off the surface) and might not be required that often judging by growth rates in the leachate, nutrient removal per year using duckweed must be compared to that of using air pumps, as well as costs for the two methods considered. Though the phytoremediating method is next to gratis, the time of year when growth is possible (and viable) might be too short to make a difference, or growth too slow to remove nutrients of satisfactory amounts. Drawing spill heat pipes beneath the leachate pond to increase water temperature and prolong the duckweed season is a possible solution that further tips the scale towards phytoremediation, but costs must be weighed in here as well. The resilience of duckweed to cold is good though, and the species would not have to be replanted each year but rather pick up where it left off the year before (Anderberg and Anderberg, 2010).

3. End management
Harvesting of the duckweed needs to be done both to remove accumulated nutrients and to enable further growth of the culture (too high a density prevents growth, as presented by Leng and colleagues (1995)). Using duckweed for biogas production can definitely be done, but the amounts needed to sustain a biogas reactor are far too large to be possible with the cultivation area available. As an additional feed stock however, duckweed can make a contribution to biogas production in an existing reactor if allowed to dry a little and grinded or otherwise chopped up to increase bioavailability of the accumulated nutrients (see also Clark et al, 1996).

4. Further tests
As the new treatment system at Häradsudden was set in motion after the collection of leachate for this study, growth capacity in the cleaner leachate that now reaches the ponds should be investigated. Climatic conditions should also be studied to determine the ability of the duckweed to grow on site, for example by placing the preferred duckweed species in a container filled with leachate and placed next to the leachate pond. Also, growing duckweed in the same leachate but indoors, at room temperature by a window, could give an indication as to whether a temperature of around 20°C is better than the outdoor temperatures which often lie below the former (the mean temperature of the Norrköping area in July being 16-18°C according to SMHI (2010-05-29)). This would give test data for the possible consideration of spill heat pipes. Note that an indoor test should be performed in a closed area where ventilation is good, as evaporating ammonium causes a notable smell and could give headaches.