UMEÅ UNIVERSITY

Minimizing Liquid Waste in Peptide Synthesis

A NEW APPLICATION FOR THE ROTATING BED REACTOR

By

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Abstract

Peptide drugs are used to treat a broad spectrum of diseases such as cancer and HIV [1] and have many more promising applications, such as new vaccines against SARS-CoV-2 [2]. The most popular manufacturing method for peptides is *solid-phase peptide synthesis* (SPPS) [3]. The main drawback of SPPS is that it is a costly and wasteful process [4].

SpinChem is a company that provides technology solutions for chemical processes. Recently, SpinChem has started investigating if their *Rotating Bed Reactor* (RBR) is suitable for peptide synthesis. The goal of this project is to investigate how the RBR can make processes like SPPS more resource-efficient. The idea is that the RBR-system can maximize the *solid-phase to liquid ratio* (STL). The STL is the ratio of the volume of solid-phase material and the volume of liquid. By maximizing the STL, it is possible to manufacture peptides using less solvents and chemicals. The main quest of the project is formulated into a single question:

How does a high STL affect the efficiency of the RBR-system?

To answer the question, Minitab's statistical software and *design of experiments* (DOE) will be used to plan and perform experiments in both lab- and industrial scales. DOE factorial experiments are used to gain as much information as possible about the new RBR-system. The results are analyzed and summarized to make a solid foundation for the continued work on the new RBR application.

Peptide synthesis efficiency in the RBR-system is measured using ionic adsorption. The ionic adsorption rate is measured in both lab-scale and industrial-scale experiments. In the lab-scale experiments, the decrease of ions was on average 86,5% after just 15 s with an average STL of 0,936. The industrial-scale experiments showed a similar result where the average decrease in ions was 92,9% after 20 s with an average STL of 0,947. It was concluded that the RBR-system can reduce the consumption of washing-solvent in SPPS by up to 82%.

Acknowledgments

First and foremost, I would like to express my gratitude towards Emil Byström and Erik Löfgren at SpinChem AB. Your guidance and questions have helped steer the project in the right direction. And your trust and encouragement have let me test my knowledge and grow into the role of an engineer.

I would also like to thank André Nylund and Jonas Bygdén at Umeå Plåtslageri AB for letting me use your tools and workshop. Your support made it possible to manufacture prototypes of an unusually high standard and has contributed to the projects' success.

Lastly, I would like to thank Artem Iakunkov for providing valuable advice on the report and writing. Your feedback has not only increased the quality of the report but has also given me further insight into the world of academic writing.

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1 Introduction

Peptides are a type of molecule that has recently experienced a revival of interest in the pharmaceutical industry [5]. Peptide drugs are already used to treat several well-known diseases such as cancer, HIV, and multiple sclerosis (MS) [1]. It might even be suitable for new vaccines against SARS-CoV-2 [2].

One of the challenges with peptide drugs is the costly and wasteful manufacturing process. A peptide molecule consists of a chain of amino acids that can vary in length from two to fifty. The most common manufacturing technique is *solid-phase peptide synthesis* (SPPS) [3]. In SPPS, the peptides are attached to a granular resin submerged in liquid. The peptides are built by extending the chain of amino acids one step at a time. The resin must be washed thoroughly between each building step, generating large amounts of contaminated fluids and hazardous solvents [4]. Besides trying to find new, less toxic materials, it might be good to make the existing methods more resource-efficient.

SpinChem is a company based on the technology of their patented *rotating bed reactor* (RBR). The technology is still relatively new but already has many applications. Today, most of the applications of the RBR focus on treating large amounts of liquid using as little solid-phase material as possible. However, SpinChem has identified the possibility that the RBR could also be used for processes like SPPS.

1.1 The goal of this project

This project is the first to study the possibilities of applying the RBR in chemical processes where it is desirable to maximize the *solid-phase to liquid ratio* (STL). The STL is the ratio between the volume of solid-phase material and the volume of liquid. In addition to the STL, the other fundamental property is the efficiency of the RBR-system. If the reactional efficiency is too low, the system is useless. The main quest of the project can be formulated in a single question:

How does a high STL affect the efficiency of the RBR-system?

At first, there will be a theoretical analysis of the problem, but the project's primary focus is to build prototypes and perform experiments to evaluate the RBR-system for STL maximization. The prototypes will be user-friendly to make the experiments easier and demonstrate proof of concept.

The efficiency and STL of the RBR-system will be tested at different scales. There will be a motivation to what materials and methods are used to test the system. The results will be analyzed and compared to understand how to maintain efficiency while maximizing the STL.

1.2 Project timeline

To make it easier for the reader to follow the progress, **Figure 1.1** shows a simplified timeline of the thesis project. Both the project and report can naturally be divided into two parts, the lab- and industrial-scale. In the conclusions, there will be a comparison of the two.

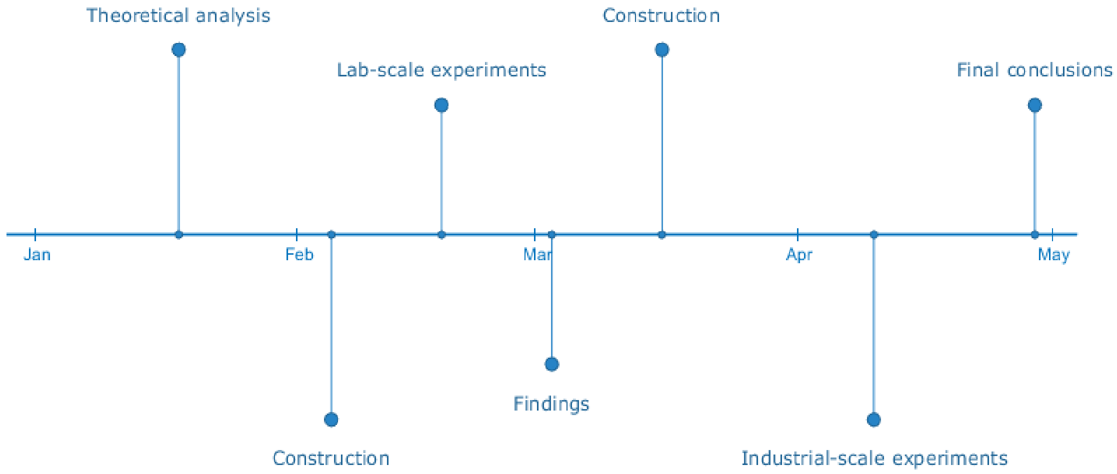

Figure 1.1: A simple timeline to help the reader follow along in the progress of the project. Most sections in the report will be divided into either of the two parts; the lab-scale experiments or the industrial-scale experiments.

2 Theory

The theory related to this project contains three parts. The first part goes through the theory needed to understand the RBR and its new application. The second part is related to the specific theories necessary to understand the experiments and measurement method. In the last part, the experimental methods used while planning and analyzing will be discussed.

2.1 The Rotating Bed Reactor

There is a wide range of applications for the RBR, but its principle is the same. The reactor consists of a hollow cylinder filled with a packed bed of the solid phase, a permeable granular material. On the inside and outside of the reactor, a fine membrane keeps the solid-phase material inside the RBR but allows the liquid to flow through it. When the RBR is rotated, the centrifugal force will create a liquid flow through the solid-phase bed. In the top and bottom, inlets allow the liquid to circulate through the reactor. All of this can be seen in **Figure 2.1**.

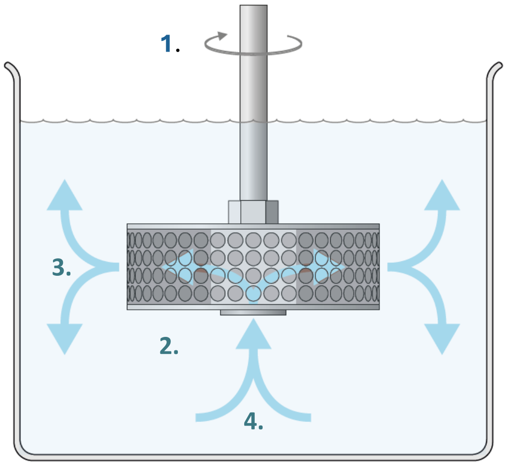

Figure 2.1: The working principles of the RBR. A hollow cylinder is filled with a packed bed solid-phase material and is **1)** rotated in a liquid. **2)** The liquid and packed bed's co-rotation causes a centrifugal force that pushes the liquid through the packed bed. **3)** The liquid flows through the outer membrane of the RBR, and **4)** is sucked into the reactor again at the top and bottom of the RBR. Courtesy of SpinChem [6].

The benefits of using an RBR compared to traditional methods such as stirred tank reactors are that it has a faster process, higher yield, and reduced consumption of reagents [6]. Another benefit is that the solid-phase is stationary in RBR, and hence there is minimal grinding of the material.

2.2 Defining the system

In its usual applications, the RBR system can be divided into the reactor and the vessel. For the application of maximizing the STL, a center volume centerpiece is added to the system. The center volume displaces some of the volume inside the reactor that otherwise would have been filled with liquid. **Figure 2.2** shows the different geometrical parameters in the RBR-system.

It was decided to use SpinChems existing RBR-models since it is infeasible to investigate all ten geometrical parameters simultaneously. Choosing the existing RBR-models limits the number of controllable parameters to four, the so-called clearance parameters. The two height clearances dH_{Top} and dH_{Bottom} are the distances between the top and bottom of the RBR and the lid and bottom of the vessel. dC is the distance between the outside of the center volume and the inside of the RBR. The last clearance parameter, dR, is the distance between the outside of the RBR and the inside of the vessel.

The final simplification was to set the two height clearances to be the same, i.e., $dH_{Top} = dH_{Bottom} = dH$. This simplification reduces the number of parameters to study to only dR, dH, and dC. Changing any of these three clearance parameters will affect the flow and amount of liquid in the system.

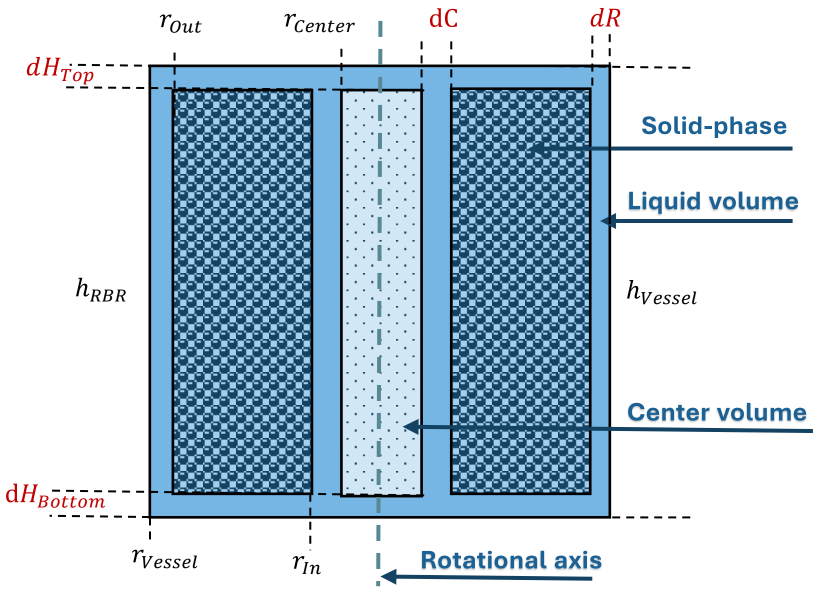

Figure 2.2: Cross-section of the vessel, RBR, and center volume. There are ten geometrical parameters, and the parameters in red show the remaining parameters when using SpinChems existing RBR models for the experiments.

2.3 The solid-phase to liquid ratio

The system can be divided into several sub-volumes, which must be calculated when deriving a theoretical expression for the STL. The two most straightforward volumes are the cylindrical volumes of the vessel and the center volume. The vessels volume is calculated as

$$V_{Vessel} = \pi r_{Vessel}^2 (h_{RBR} + dH_{Top} + dH_{Bottom}), \quad (2.1)$$

and the volume of the center volume is calculated as

$$V_{Center} = \pi r_{Center}^2 h_{RBR}. \quad (2.2)$$

The volume of the solid-phase compartment inside the RBR can be calculated using the formula for a hollow cylinder

$$V_{RBR} = \pi (r_{Out}^2 - r_{In}^2) h_{RBR}. \quad (2.3)$$

By combining equations (2.1), (2.2), and (2.3), it is possible to construct another sub-volume. V_{Out} is the volume inside the vessel but outside of the RBR and center volume. It is calculated as

$$V_{Out} = V_{Vessel} - V_{RBR} - V_{Center}. \quad (2.4)$$

The volume V_{Out} is filled with liquid, but there is also room for some liquid between the resin beads in the solid-phase compartment. So, to take this into account, it is necessary to introduce the concept of packing factor, PF, which is a measurement of how much of the available volume that the solid-phase will occupy. Since PF is used as a unitless scaling factor, its value is $0 \leq PF \leq 1$. Using PF makes it possible to create an expression for the volume of the solid-phase material that fits inside the solid-phase compartment of the reactor, it is

$$V_{RBR}^{Solid} = PF \cdot V_{RBR}. \tag{2.5}$$

The volume inside the solid-phase compartment that is not occupied by resin will be filled with liquid, and it can be calculated as

$$V_{RBR}^{Liquid} = (1 - PF) \cdot V_{RBR}. \tag{2.6}$$

Finally, by combining the three sub-volumes derived in equations (2.4), (2.5), and (2.6), it is possible to calculate one of the main properties of the system, the solid-phase to liquid ratio:

$$STL = \frac{V_{RBR}^{Solid}}{V_{RBR}^{Liquid} + V_{Out}}, \tag{2.7}$$

which is the property that this project aims to maximize. Later in the experiment, the STL will be determined empirically by measuring the volumes of the liquid and solid-phase. Equation (2.7) can be used to calculate the STL for a range of parameter values. **Figure 2.3** shows how the STL changes with the two parameters dR and dH for a system based on a S14 RBR model and $PF = 0{,}70$.

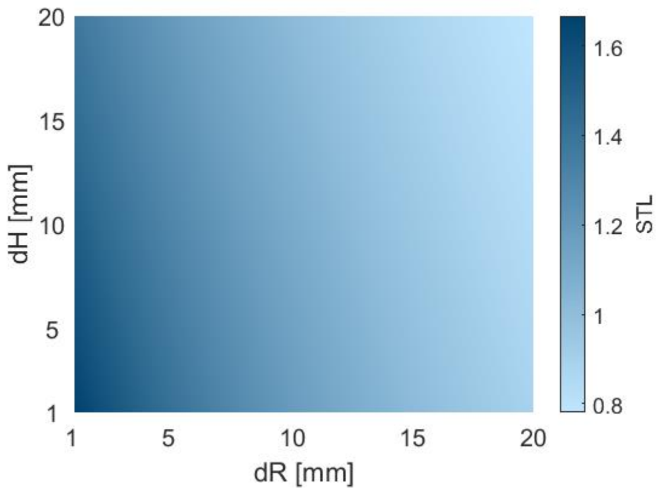

Figure 2.3: Example of how the solid-phase to liquid ratio changes with the clearance parameters dR and dH when using SpinChems S14 RBR. To be able to visualize this in 2D, the center clearance dC has been set to 10 mm and $PF = 0{,}70$.

2.4 Measuring the efficiency of the reactors

Since SPPS is complex, expensive, and uses toxic chemicals, deionization of tap water is used to determine the efficiency of the reactor. The deionization is performed using a resin meant for ionic adsorption. The rate of deionization is measured using electrolytic conductivity.

2.4.1 Measuring the reaction rate in the resin

There is no easy way to measure the reaction rate in the resin directly. However, since it is a closed system, it can be measured implicitly by investigating the liquid. The electrolytic conductivity, κ [µS/cm] (micro-Siemens per centimeter), of the water is proportional to the concentrations of conductive ions. The electrolytic conductivity is proportional to the concentration of ions in the liquid $[A]$ (parts-per-million). According to [7], the concentration can be approximated as

$$[A] \approx 0{,}64 \cdot \kappa. \tag{2.8}$$

The resins adsorption rate depends on $[A]$, and the conductivity decreases faster in the vessels with small liquid volumes. To compensate for the difference in volume, it is more beneficial to study the reaction rate constant, K_{Liquid} $[s^{-1}]$ (per second), a constant that quantifies the rate and direction of the reaction in the liquid.

From previous experiments at SpinChem, it has been observed that ionic adsorption usually follows the pattern of a first-order reaction. For a first-order reaction, K_{Liquid} is determined from the slope of the semi-logarithmic decrease of the concentration [8]. In **Figure 2.4**, two graphs are demonstrating the expected behavior.

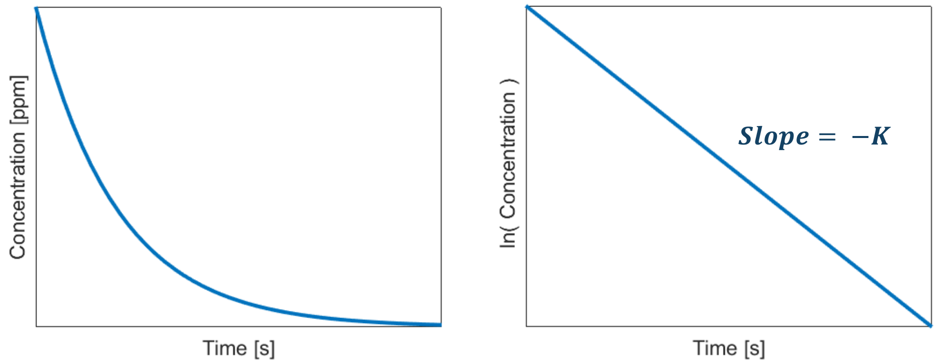

Figure 2.4: Left: A theoretical example of the exponential decrease of reactants in a first-order reaction. **Right:** The linear decrease in the semi-logarithmic concentration confirms that it is a first-order reaction. The slope of the curve gives the reaction rate constant.

In the main experiments, the conductivity is measured at $t_0 = 0\ s$ and t_{Final}. And by doing so, it is possible to calculate the linear decrease as

$$Slope = -K_{Liquid} = \frac{\Delta Y}{\Delta X} = \frac{\ln([A]_{Final}) - \ln([A]_0)}{t_{Final} - t_0} = \ln\left(\frac{[A]_{Final}}{[A]_0}\right) / \ t_{Final}. \tag{2.9}$$

In SPPS, it is the reaction in the solid-phase that is important. So, the final step is to translate K_{Liquid} to the reaction rate constant for the resin, K_{Solid}. It is reasonable to assume that all ions leaving the liquid volume must go into the solid-phase. The translation from K_{Liquid} to K_{Solid} is

$$K_{Solid} = -\frac{V_{Liquid}}{V_{Solid}} \cdot K_{Liquid}, \tag{2.10}$$

where V_{Solid} $[m^3]$ and V_{Liquid} $[m^3]$ are the volumes of the two phases. K_{Solid} is the primary indicator of the RBR-systems efficiency and will be used to compare the reaction efficiency of the different-sized vessels. In the results, K_{Solid} is normalized by its maximum value to make it easier to see the effect of the clearance parameters.

2.4.2 Initial and long-term performance of the resin

Previous resin-based experiments at SpinChem have shown that the resin has a higher adsorption rate when it is new [9]. The performance of the resin gradually decreases during the first use cycles. After the initial decrease, there is a period where the performance of the resin is approximately constant. So, some measurements will be required to make sure that the main experiments can be performed in the constant-performance region of the resin.

2.5 Design of experiments

Now that all the necessary equations are derived, the next step is to plan the experiments. The goal of the experiments is to collect information about how the three clearance parameters affect the STL and efficiency of the reactor. One way to get as much information as possible is to use *Design of Experiments* (DOE), a set of well-established statistical methods for planning, performing, and analyzing experiments.

The two main experiments have both been performed as 2^k-full-factorial experiments, where k is the number of controllable parameters (factors). Each factor is tested at two different levels, the so-called low and high factor levels. By testing all combinations of the factor levels, it is possible to gain information both about the effects of the factors themselves and how they might interact with each other. See **Figure 2.5** for a representation of a factorial experiment with three parameters and two factor levels.

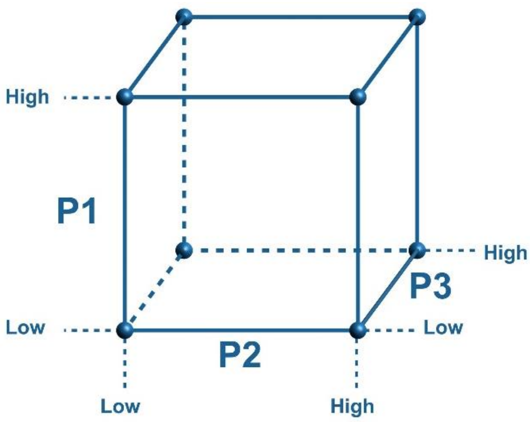

Figure 2.5: A 2^3-factorial experiment for the three factors P1, P2, and P3. Each corner of the cube represents a unique combination of the factor levels.

The 2^k-factorial experiment results in a linear regression model for how the response variable depends on the factors. However, in the RBR-system, it is reasonable to expect that the response is not linear with respect to the factors. But usually a linear model is considered enough for the initial screening of a system [10].

When performing factorial experiments, it is recommended to randomize the run-order of the samples to stop any systematic errors from skewing the data. Another helpful practice is to use blocking, i.e., the addition of a categorical variable that explains any unintended effects from a change in the process. Blocking will be used to ensure that there are no significant effects of changing the resin between the replicates.

To make the DOE process as simple as possible, Minitab's statistical software is used to design and analyze the experiments. In Minitab, it is easy to generate factorial experiments, collect data, and analyze and visualize the results.

3 Experiments

The experiments are divided into two parts, the lab-scale experiments and the industrial-scale experiments. In each part, the main objective is to perform a factorial experiment to determine how the clearance parameters affect the efficiency and STL. However, the lab-scale experiment also includes some extra measurements to investigate the properties of the resin.

3.1 Lab-scale experiments

The main lab-scale experiment is a 2^3-full factorial experiment testing the effects and interactions of the three clearance parameters. It was decided to use one of SpinChems smaller reactors, the S3 RBR, which has a solid-phase compartment of 69 ml. The S3 has a height of 32 mm and an outer diameter of 70 mm. The inner diameter of the S3 RBR is 32 mm. The resin used for the experiment was Lewatit® NM 60, an ion exchange resin from Lanxess used to purify water.

3.1.1 Choosing the factor levels

The different levels of the clearance parameters were chosen based on collective intuition and curiosity to be as small as possible but still allowing a decent flow through the resin. The factor levels for the lab-scale experiment are shown in **Table 3.1**.

Table 3.1: The low and high factor levels chosen for the three parameters studied in the 2^3-full factorial experiment. Together these factor levels can be combined into eight unique combinations. The height of the S3 RBR is 32 mm, and the outer radius is 35 mm. The inner radius is of the RBR is 16 mm.

Parameter	Low [mm]	High [mm]
dR	2	4
dH	1	2
dC	2	4

3.1.2 Design and construction

The goal of the design was to create a system that was accurate, waterproof, and easy to fill and empty. A CAD model was created to 3D-print the vessels, lid, and fixture mount, and how this looked is shown in **Figure 3.1**. One detail that is difficult to see in the CAD model is the four vertical baffles added to the inside of the vessel walls. The purpose of the baffles is to increase the vertical flow of the liquid and improve the reactor's efficiency.

The total number of parts was seventeen, five for the lid and fixture mount and three for each of the four different-sized vessels. All seventeen parts were 3D-printed in PETG-plastic, which is easy to work with, waterproof, and durable. A ball bearing was added between the lid and driveshaft to reduce friction and increase the longevity of the prototype.

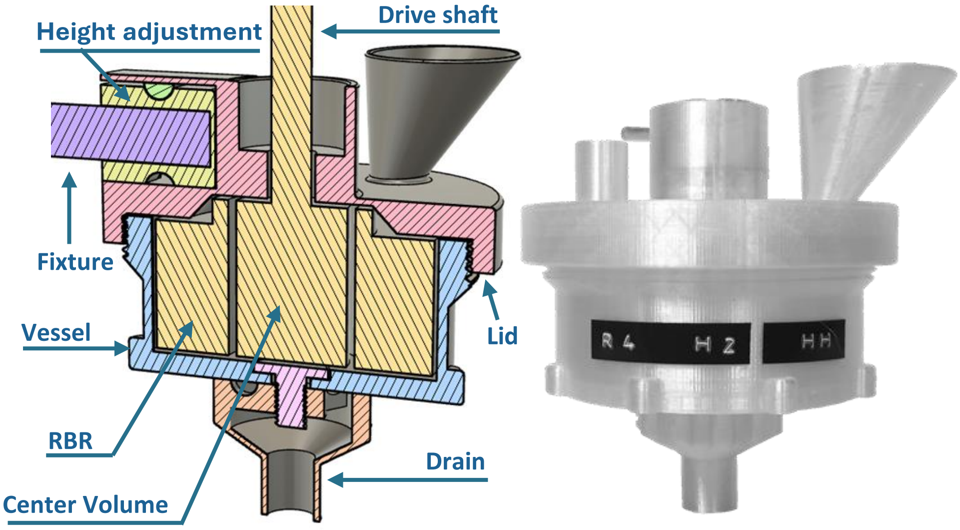

Figure 3.1: Left: Cross-section of the CAD model used to create the prototype vessel in the lab-scale experiment together with the S3 RB. **Right:** Image of the 3D-printed lid and one of the four vessels.

3.1.3 Determining the runtime and reaction order

A test series was conducted to find a suitable runtime for the factorial experiment. The decrease in conductivity of the water was measured for seven different runtimes from zero to two minutes. The same measurement series will also be used to determine if the reaction is a first-order reaction.

3.1.4 Performance of the resin

As mentioned in the theory section, it is reasonable to expect that the resin's ability to adsorb ions decreases for each use cycle. To study this behavior, the RBR was loaded with resin and run for 35 cycles of 15 s in the largest vessel. The vessel was filled with new water in each cycle, and the conductivity of the water was measured before and after each cycle.

3.1.5 Description of runs experimental runs

Before the actual experiment, some preparations had to be made. Two days before the experiment, a large container was filled with tap water to allow its temperature to stabilize to be constant during the whole experiment. Another preparation was to pre-swell the resin in deionized water.

Besides the preparations, the experiment can be broken down into six steps as follows:

1. Fill the RBR with resin and mount the RBR on the driveshaft of the electrical motor.
2. Run RBR in water equivalent to 10 filled vessels to eliminate initial effects in the resin performance.
3. Mount the correct vessel, fill it with water and run the RBR at 400 RPM for 15 s.
4. Collect the water and measure its weight and electrolytic conductivity.
5. Repeat steps 3-4 for the seven remaining parameter combinations.
6. Repeat steps 1-5 for the two remaining experimental blocks/replicates.

3.2 Industrial-scale experiments

Based on the success of the lab-scale experiment, it was decided that the following prototypes would be constructed on a much larger scale. SpinChem has an RBR model called S14, which has room for 14 liters of solid-phase material. The S14 is 310 mm high and has an outer diameter of 330 mm. The inner diameter of the S14 is 190 mm. Except for the size, the main difference between S14 and S3 is that S14 is proportionally twice as high.

3.2.1 Choosing the factor levels

A relevant finding from the lab-scale experiment was that the radius of the center volume, dC, had a relatively small effect on the reaction rate in the resin. In the industrial-scale experiment, dC is held constant at 10 mm to simplify the experiment and construction of the prototype.

The electrolytic conductivity decreased by at least 80% during the 15 s runtime in the lab-scale experiment. The reaction rate was considered high enough that it would not be necessary to investigate larger clearances. For the industrial-scale experiment, the factor levels were chosen proportionally smaller than in the lab-scale experiment. The factor levels chosen for the industrial-scale experiment are shown in **Table 3.2**.

Table 3.2: The factor levels for the lab-scale experiment. The center clearance, dC, was held constant at 10 mm. The height of the S14 RBR is 310 mm, and the outer radius is 165 mm. The inner radius is 95 mm.

Parameter	Low [mm]	High [mm]
dR	5	10
dH	5	10

3.2.2 Design and construction

The idea with the industrial-scale prototypes was not only to do the experiments but also to use them as proof of concept. Therefore, it was decided to make the vessels and center volume out of stainless steel. Moreover, to get a visual understanding of the flow created inside the reactor, the lid was made from 6 mm transparent plexiglass.

Since the S14 has four bolts sticking up from the top surface, the RBR and center volume was extended by adding 16 mm of plexiglass so that the top surface became smooth. The smaller parts such as the lid-axel connection, fixture mount, drain, and center volumes top and bottom were all 3D-printed using PETG-plastic. As for the lab-scale experiment, four baffles were added to the inside of the vessel walls. The CAD model and one prototype are shown in **Figure 3.2**.

Instead of manufacturing four different vessels, there are two vessels with different radii and two plastic rings. The rings can be added between the vessel and lid to test each vessel for two different heights.

In the industrial-scale prototypes, the driveshaft goes through the center volume and vessel bottom to be held in place by a slip bearing to reduce sidewise motions. The extra point of contact makes the RBR and vessel much more stable and allows for tighter clearances.

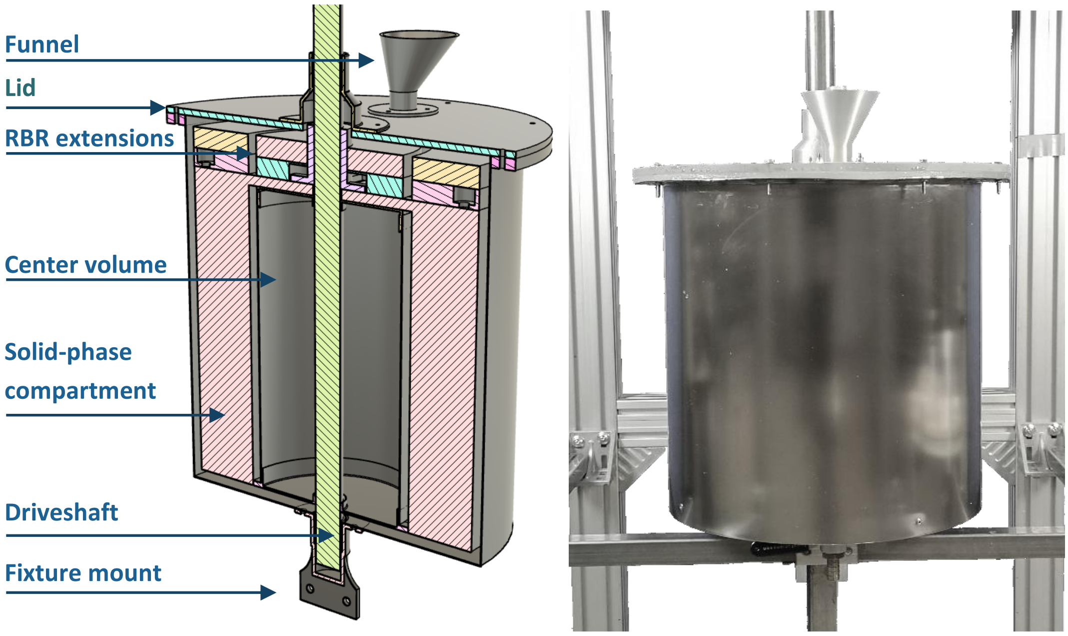

Figure 3.2: Left: A cross-section of the CAD model for the experimental setup in the industrial-scale experiment. **Right:** One of the prototypes constructed using 0,7 mm thick stainless steel with the lid made from 6 mm transparent plexiglass.

3.2.3 Description of the experimental procedure

The main difference from the lab-scale experiment is that the industrial-scale experiment only investigates the effects of dR and dH. The full factorial experiment requires 12 runs to cover the three replicates. Based on the measurements of the initial effects in the resin, it was assumed that the same resin could be used for all 12 runs.

As before, a large tank was filled with enough tap water to cover the whole experiment, and the resin was pre-swelled before filling the RBR.

The following list goes through the essential steps in the experiment:

1. Mount the RBR and center volume on the driveshaft, and then fill the RBR with resin.
2. Mount the largest vessel and wash the resin in 10 cycles of water by rotating the RBR at 220 RPM for 20 s.
3. Mount the correct vessel, fill it with water and rotate the RBR at 220 RPM for 20 s.
4. Collect the water and measure its weight and electrolytic conductivity.
5. Repeat steps 3-4 for the remaining 11 runs.

Lastly, the packing factor of the resin was determined by filling a test tube with a known volume of resin and adding water until the water level was in line with the top of the resin. By measuring the difference in weight, it is possible to determine the liquid volume between the resin beads.

4 Results

The result section follows the structure of the experimental section and has been divided into two parts corresponding to the lab-scale and industrial-scale results.

4.1 Lab-scale results

In the lab-scale results, there are four parts. First, the results from the experiments try to determine a suitable runtime and determine the reaction order. After this comes the results from the measurements of the resin's initial performance. The third part is the results from the full factorial experiment. Lastly, there is the analysis generated in Minitab.

4.1.1 Determining the runtime and reaction order

The decrease in electrolytic conductivity was measured for seven different runtimes, and the result is shown in **Figure 4.1**. For all the measurements, the smallest vessel was used with an average liquid weight of 32,7 g.

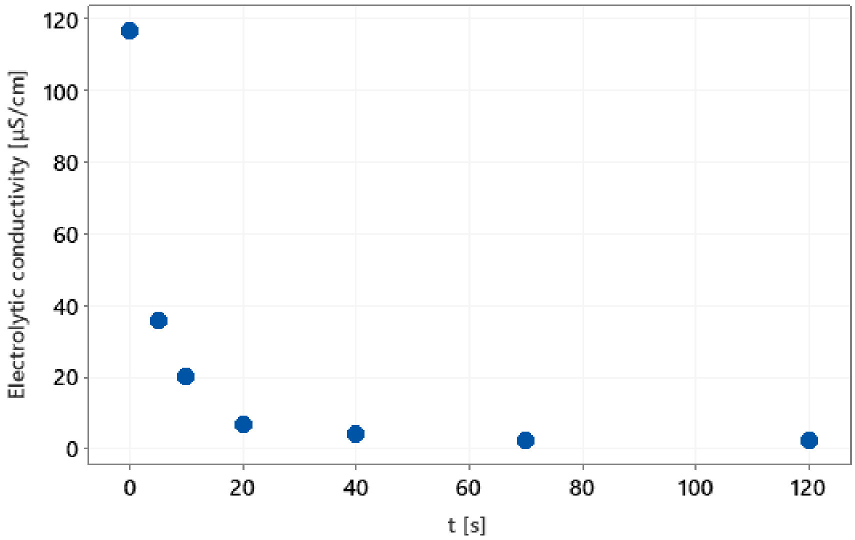

Figure 4.1: The decrease in electrolytic conductivity for different runtimes using the smallest vessel and Lewatit® NM 60 resin. It was decided that a runtime of 15 s was suitable for the lab-scale experiment.

The first four data points in the runtime data are also used together with equation (2.8) to determine the reaction order. The result and corresponding linear regression model are shown in **Figure 4.2**.

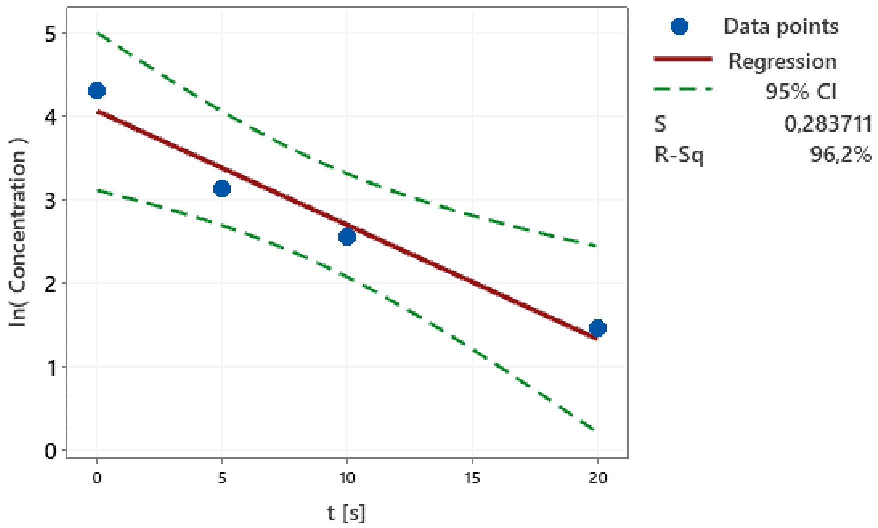

Figure 4.2: The approximate linearity of the semi-logarithmic concentration of ions confirms that the reaction is a first-order reaction. S is the average deviation from the linear regression line and R^2 is a measurement of how much of the variation in the response variable is explained by the linear model.

4.1.2 Initial performance of the resin

The initial and long-term performance of the resin's adsorption capacity is shown in **Figure 4.3**. In addition to the data points, there is an arbitrary regression model to make it easier to determine the constant performance region. The largest vessel was used for all measurements, resulting in an average sample weight of 68,7 g.

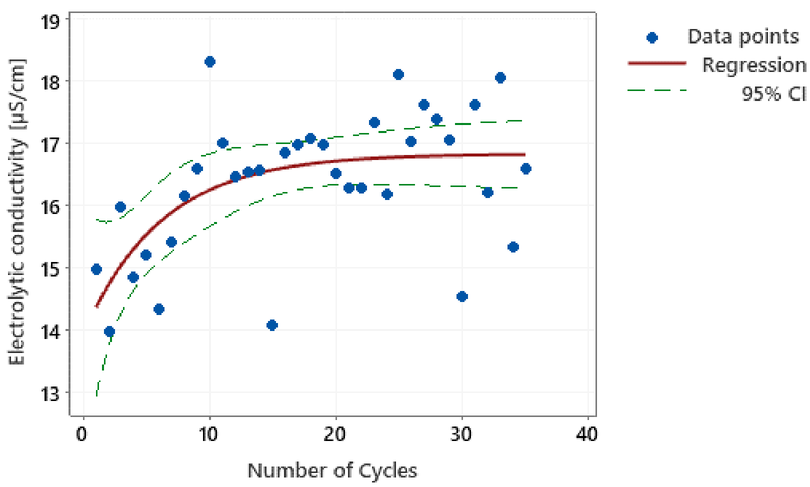

Figure 4.3: The decrease in the resin's performance together with an arbitrary regression model. Most of the loss of performance occurs in the first ten cycles, and afterward, there is a relatively stable region.

4.1.3 The full factorial experiment

The lab-scale experiment was run according to the description in the experimental section, and the measurements of electrolytic conductivity and liquid volume are shown in **Table 4.1:** Experimental plan and measurements from the lab-scale 2^3-full factorial experiment.

Table 4.1: Experimental plan and measurements from the lab-scale 2^3-full factorial experiment. The average decrease in conductivity was 86,5% after 15 s at 400 RPM.

Run Order	Blocks	dR [mm]	dH [mm]	dC [mm]	Conductivity [µS/cm]	Liquid volume [ml]
1	1	2	1	2	11,94	44
2	1	4	2	2	19,29	63
3	1	4	1	2	16,47	53
4	1	4	2	4	17,77	69
5	1	2	2	4	18,84	52
6	1	2	2	2	15,38	49
7	1	4	1	4	14,72	57
8	1	2	1	4	14,29	46
9	3	4	1	2	14,92	52
10	3	2	1	2	11,48	38
11	3	2	2	2	13,97	50
12	3	4	2	2	23,00	63
13	3	4	1	4	19,48	59
14	3	4	2	4	18,84	65
15	3	2	2	4	13,17	49
16	3	2	1	4	12,88	44
17	2	2	2	2	14,24	50
18	2	2	1	4	11,97	42
19	2	2	1	2	11,21	34
20	2	4	1	4	14,82	57
21	2	2	2	4	16,09	52
22	2	4	1	2	15,03	56
23	2	4	2	2	20,20	68
24	2	4	2	4	20,40	71

4.1.4 Minitab analysis of the full factorial experiment

The initial factorial model generated in Minitab showed that at a significance level of 95%, only the three main effects of the clearance parameters had a significant effect on K_{Solid}. This means that there was no effect from either the parameter interactions or the experimental blocks. Equation (4.1) shows the updated linear regression model generated in Minitab when excluding the insignificant terms.

$$K_{Solid} = 0{,}08304 + 0{,}01156 \cdot dR + 0{,}01137 \cdot dH + 0{,}00390 \cdot dC \quad (4.1)$$

In the regression model, 43,1% of the variation in K_{Solid} is explained by dR, 42,4% from dH, and that dC only accounts for the remaining 14,6% of the variation.

Another way to visualize the effects of the clearance parameters is to look at the cube plot in **Figure 4.4**. The values in the cube plot have been normalized by the maximum value to make it easier to compare the different reaction rate constants. The trend is that K_{Solid} is largest when all clearance parameters are at their maximum.

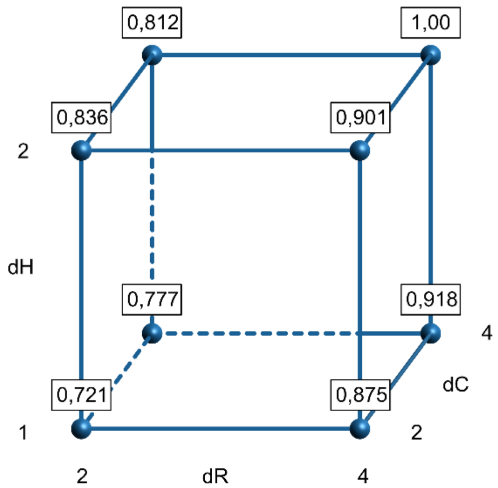

Figure 4.4: Cube plot showing the normalized mean values of K_{Solid} for the eight parameter combinations. When all clearances are as tight as possible, K_{Solid} is 27,9% lower than at the maximum.

The same type of cube plot can also be used to visualize how the STL changes with the clearance parameters, and **Figure 4.5** shows the linear model for the STL at a PF of 70,1%. The STL values are not normalized since the magnitude of the STL at the different data points is of interest.

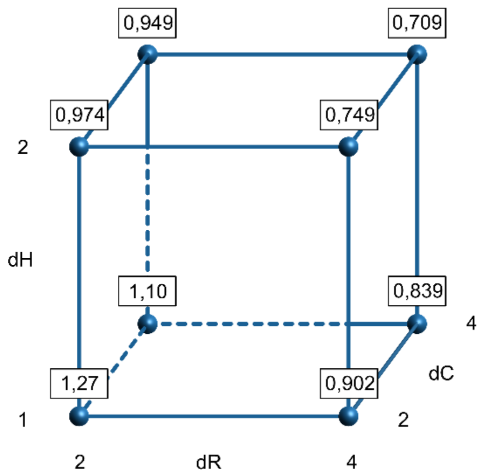

Figure 4.5: Cube plot showing the mean values of how the STL changes with the three clearance parameters in the lab-scale experiment. The STL is 79,1% higher for the smallest vessel compared to the largest vessel.

As a final summary of the lab-scale results is a combination of the two previous figures, **Figure 4.6** shows the effects of the clearance parameters on both the STL and K_{Solid}. The trend is that small clearances have a limiting effect on the reaction rate inside the reactor.

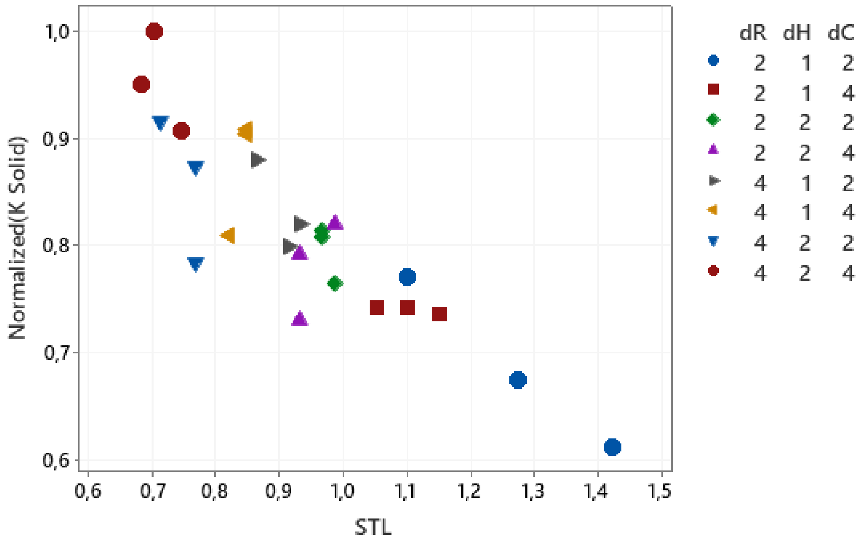

Figure 4.6: The relation between the reactional efficiency of the reactor and the STL for the different clearance parameters. K_{Solid} has been normalized by its largest value. The trend is that small clearances increase the STL but decrease K_{Solid}.

4.2 Industrial-scale results

The resin was washed in ten cycles using the largest vessel to eliminate the initial effects in its adsorption capacity. In the same process, a suitable RPM and runtime were determined to be 220 RPM at 20 s. Following the description in the experimental section resulted in the data shown in **Table 4.2**.

Table 4.2: Experimental plan and measurements in the industrial-scale experiment. The initial conductivity was measured separately to 106,2 µS/cm. The average decrease in conductivity was 92,9% after 20 s at 220 RPM.

Run Order	dR [mm]	dH [mm]	Conductivity [µS/cm]	Liquid weight [kg]
1	5	5	3,37	8,96
2	5	5	3,20	8,96
3	10	10	12,14	12,12
4	5	10	4,79	10,21
5	5	10	5,50	10,16
6	10	5	9,75	10,89
7	10	5	9,31	10,87
8	10	5	9,90	10,92
9	5	10	5,79	10,11
10	5	5	4,30	8,94
11	10	10	10,28	12,22
12	10	10	11,56	12,18

The factorial regression model generated in Minitab showed that at a significance level of 95%, only dR had a significant effect on K_{Solid}. There is a weak trend for dH, but it is not strong enough to statistically determine that dH affects K_{Solid}. In the cube plot shown in **Figure 4.7**, it is evident that dH does not have a strong effect on K_{Solid}.

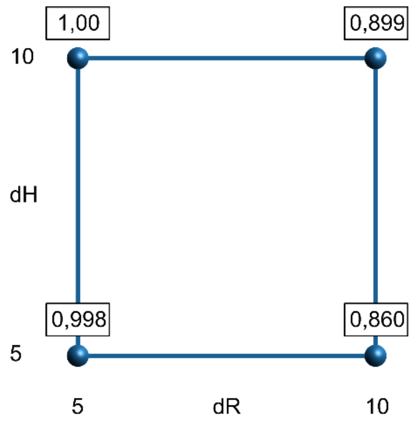

Figure 4.7: The normalized mean values for the three replicates of K_{Solid}. Only dR is statistically significant since the effect of dH is too small compared to the fluctuations in the data. K_{Solid} increase for smaller dR which indicates that a smaller radial clearance contributes to a more efficient flow through the solid-phase bed.

In addition to the factorial regression model for K_{Solid}, a new model was created to describes how the STL changes with dR and dH. As expected, both parameters significantly affect the STL, and the corresponding cube plot is shown in **Figure 4.8**.

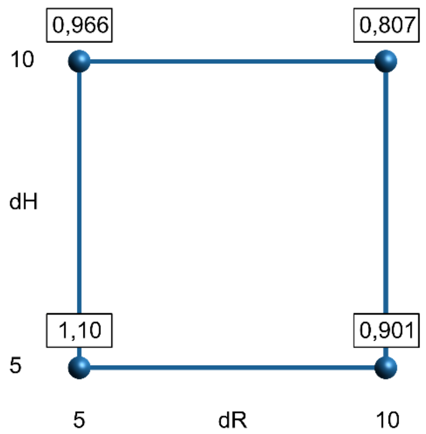

Figure 4.8: Visualization of how the STL depends on the two clearance parameters when using the S14 RBR from SpinChem. The STL is 36,3% higher for the smallest vessel compared to the largest vessel.

As for the lab-scale results, the final graph combines the two most essential parts of the project: the reactor's STL and efficiency. In **Figure 4.9**, the STL is plotted against the normalized values of K_{solid} and show the effect of the clearance parameters.

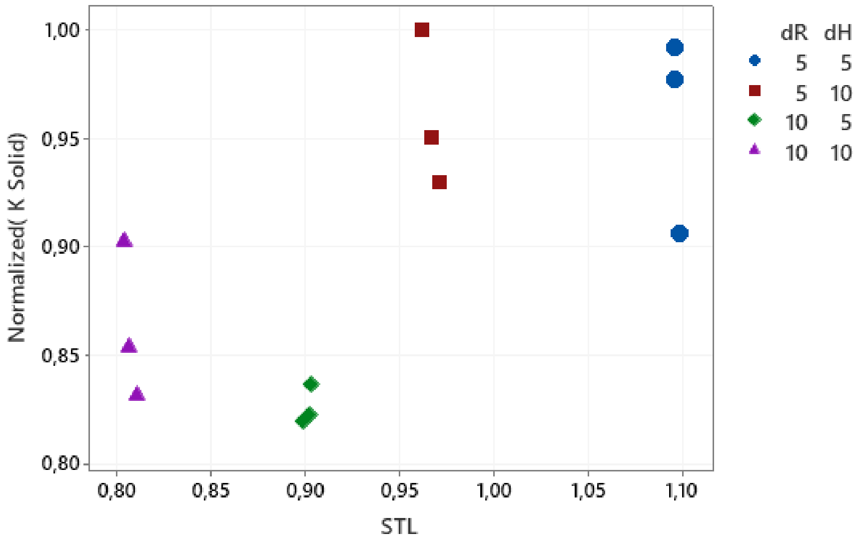

Figure 4.9: The relation between the reactional efficiency of the reactor and the STL for the different clearance parameters. K_{Solid} has been normalized by its largest value. The height clearance, dH, does not have a statistically significant effect on K_{Solid}. The trend is that small clearances increase the STL and K_{Solid}.

5 Discussion and conclusions

Let us start with the most exciting conclusion; the experiments show that the RBR technology is promising for chemical processes where it is desirable to maximize the STL. For the lab-scale experiment, the ionic concentration decreased on average 86,5% after only 15 s of treatment. Furthermore, it decreased on average 92,9% after 20 s of treatment in the industrial-scale experiment. With such high reaction rates, it is reasonable to expect that the RBR-system can be further optimized to achieve an even higher STL.

5.1 Sources of error

In **Figure 4.2**, the linearity of the logarithmic concentration of the ions is demonstrated. The data is not completely linear because there are several types of ions present in tap water. Different ions have different adsorption rates, slightly shifting the overall adsorption rate over time [11]. One way to make the reaction rate more consistent would be to exchange the tap water for a more uniform solution.

The next thing to discuss is the initial behavior of the resin that is shown in **Figure 4.3**. The experiments showed that the performance of the resin was stable enough after the first ten cycles. In hindsight, it would be better to wash the resin for a couple more cycles to get rid of even more of the initial effects and lower the fluctuations in the collected data.

In the factorial experiment, K_{Solid} was chosen as the primary measurement of the reactor's efficiency. However, the concept of "reaction rate in the solid-phase", K_{Solid}, might seem unconventional for people with insight into the field. The reason to use K_{Solid} is to simplify the theory and experiments. Since the project aims to study reactional efficiency and volumes, it is unnecessary to involve too many complicated theories and measurements.

It might also be relevant to discuss any difference in the theoretical and experimental values of the STL. The theoretical expression for the STL in equation (2.7) does not consider any material thicknesses and shapes that affect the STL. For example, the driveshaft and axle mounts are not included in the model, and the RBR itself has different material thicknesses associated with it.

In the measurements of the initial effects and the two factorial experiments, the fluctuations in K_{Solid} are quite large. It is reasonable to think that the most significant error source in the measurements is the runtime. A stopwatch was used to start and stop the electrical motor manually, and **Figure 4.1** show that the runtime has an enormous impact on the final conductivity. One way to avoid any time-related error would be to implement a digital system that can control the electric motor and RBR more precisely.

In the two cube plots showing the STL, **Figure 4.6** and **Figure 4.9**, there is some variation of the STL within the replicates. The relative fluctuations in the industrial-scale experiments are minor compared to the lab-scale. The variation is larger for the lab-scale because any air bubbles trapped in the RBR-system have a more significant effect on the smaller system.

5.2 Comparing the two scales

In the lab-scale experiment, all three clearance parameters had a significant effect on the reaction rate constant. In the industrial-scale experiment, only the radial clearance, dR, was significant. It is reasonable to believe that dH does affect the liquid flow and reaction rate. That dH is insignificant only means that its effect is minor compared to the variation in the data.

One considerable drawback with the two factorial experiments is that there are many differences between the lab-scale and industrial-scale experiments. Several parameters like temperature, RPM, geometrical proportions, materials, and others have been left out of the experiments. With so many unstudied parameters, the actual magnitude of K_{Solid} are not so valuable. The differences in magnitude are the reason that K_{Solid} is normalized in **Figure 4.4** and **Figure 4.7**. The normalization makes it easier to spot the trends in reactional efficiency.

In **Figure 4.6**, there is a negative trend for K_{Solid} with increasing STL. Reducing the clearances from 4 mm to 2 mm limits the liquid flow through the solid-phase bed and lowers the reaction rate constant. For the industrial-scale experiment, the trend is the opposite. In **Figure 4.9**, K_{Solid} increase when reducing the clearances from 10 mm to 5 mm. Given that the two trends are opposite, it is reasonable to conclude that it is not only the proportions of the clearances that are important. Other factors such as the physical properties of the liquid also play a significant role in flow rate.

5.3 The RBR compared to conventional methods for SPPS

By following the conventional method for automated SPPS in [12], it is possible to calculate the savings when applying the RBR-system. For each amino coupling cycle, at least 23 washing steps are required. Each washing step uses 32 l of solvents per mol of the produced peptide.

To produce 1 mol of peptides using a polystyrene resin with loading capacity of 0,7 mmol/g and a yield of 20%, we need 7,1 kg of resin. The volume of 7,1 kg polystyrene resin is 6,5 l [13]. The highest STL in the industrial-scale experiment was 1,10. To achieve the same STL using 6,5 l of resin requires 5,9 l of liquid.

So, if 1 mol of peptides is produced using the RBR-system for all 23 washing steps, 140 l of solvents are required. The conventional SPPS method uses 740 l of solvents to complete the 23 washing steps. This means that it is possible to reduce the consumption of washing-solvent by 82% using the RBR-system!

With the high efficiency and STL of the prototypes, it is worth investing more time and money in the RBR-system. Someone with good insight into the practice of SPPS would undoubtedly see the potential of the RBR. If not for the whole process, at least some parts can be improved by implementing the RBR technology. A common problem in SPPS is the grinding of the solid-phase material that occurs when stirring the tanks [14]. Avoiding grinding of solid-phase material is the reason that the RBR was invented in the first place!

Another way to use the RBR and combat hazardous waste is to treat and purify the solvents used in the conventional SPPS processes. Doing this makes it possible to reuse more of the liquids in the process, which would lower the cost and environmental impact.

5.4 Thoughts for the future

This thesis project shows that it takes quite some time to study only a few parameters, and the true number of system parameters is much higher than the ten parameters defined in **Figure 2.2**. For this reason, it might be a good idea to involve any potential customers early in the development process so that the system can be designed after customer preferences from the start.

As a finishing touch, I would like to leave a suggestion for SpinChem about what to do next. An easy but interesting experiment that would give valuable results would be to investigate how a smaller radial clearance affect the K_{Solid}. For the industrial-scale prototypes, a smaller clearance can easily be achieved by adding a layer of material to the inside of the smallest walls. This experiment would be helpful when trying to understand the non-linear effects of the clearance parameters on the flow rate in the RBR-system.

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