Enzymatic glucose production from fine sieve material recovered from domestic waste water

"Reusing used toilet paper"

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Abstract

The pursuit of a circular economy has led to the introduction of finescreens in Waste Water Treatment Plants (WWTP). The high cellulose content of the fine sieve material (FSM) collected in the screenings of domestic waste water (Ruiken, Breuer, Klaversma, Santiago, & van Loosdrecht, 2013), shows the promise of being enzymatically converted into glucose. However, this process is currently economically unfeasible, were the enzyme costs are considered to be a major barrier (Liu, Zhang, & Bao, 2016). In an attempt to design a method in which the enzyme usage is limited to reach economic feasibility, immobilization by cellulose binding domains and dialysis membrane were evaluated. By performing a market evaluation an estimation on the potential market size and possible production was made, which led to a cost price of € 200.00 per metric ton of glucose. To determine the cost price for glucose derived from FSM, a cost analysis on a production facility with a potential annual production of 10,000 ton of glucose was made. With a cost price of € 365.68 per ton of glucose, economic feasibility was not reached. However, potential further research on the composition of FSM shows the promise of reducing the cost price to € 225.84, which might be sufficient to reach economic feasibility.

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Introduction

To achieve the introduction of a circular economy it is required to reach a new view concerning the use of raw materials. An environmental sustainable operation can only be achieved if the linear economy of mining raw materials, producing product and discharging waste is changed into a closed cycle, in which the discharge of an operation can be used as the starting material for a new process.

This new view on recycling and reuse of raw materials is the driving force on the introduction of finescreens in Waste Water Treatment Plants (WWTP). The additional filtration of waste water leads to the collection of fine sieve material (FSM), consisting of a high cellulose content mainly originating from used toilet paper (Ruiken et al., 2013). The removal of the FSM results in an increase in performance of the WWTP, it increases the capacity of the plant while the required aeration energy is reduced (Roest, 2018). Furthermore, the extracted cellulose fibers can be used for several different applications. For instance, using the extracted cellulose fibers as a filtering aid in the dewatering of sludge to increase the concentration of organic waste streams from which biogas can be derived, to be decomposed and used as fertilizer, and to be reused as a raw material. (Wouters, Euverink, Neef, van Opijnen, & Poiesz, 2017).

Another possible use of the cellulose fibers recovered from fine sieve material is the production of bioplastics and as a raw material for bioplastics. The first step into developing such an application is the conversion of the cellulose fibers into its sugars, to be used as a source in fermentation or as the starting point in the development of chemical building blocks. With a bio-based economy in mind, the enzymatic conversion of cellulose retrieved from FSM to produce glucose which can be used as a raw material for several applications, would fit perfectly in an attempt to transform the economy from linear to circular. However, the enzymatic conversion of cellulose to glucose is not yet economically feasible.

This study will investigate whether an economically feasible method for the enzymatic conversion of cellulose retrieved from fine sieve material can be achieved.

Problem determination

Problem statement

The use of enzymes to convert the cellulose fibers present in FSM into glucose promises an increase in the value of the materials. However, the enzymatic conversion of cellulose to glucose is not yet commercially feasible due to low process efficiency, the complexity and the costs of cellulases, the enzyme used (Vynios et al., 2009).

Problem owner analysis

The water boards are considered to be the problem owners. In the Netherlands, these regional government bodies are responsible for managing water levels, the waterways, the water barriers, the quality of water and the sewage treatment. None of these operations are profitable, which makes the addition of a profitable operation desirable. If the enzymatic production of glucose from fine sieve material is determined to be economically feasible, the water boards might have the potential to produce a profit.

System description

The first part of the research will be limited to researching the possible enzymatic glucose production from fine sieve materials. This results in the following graphic depiction of the system studied:

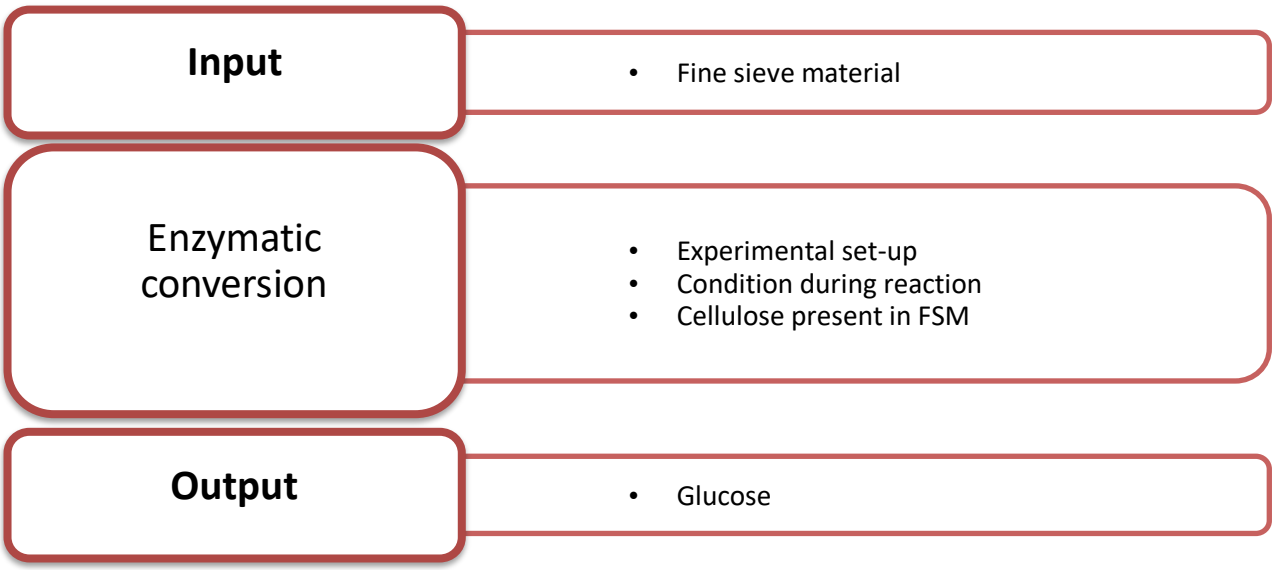

Figure 1: System description

The fine sieves are placed out of the scope of this research, only the produced FSM is taken as the input of the system. The enzymatic conversion is a function of several elements. The experimental set-up will have a profound effect on the amount of enzyme needed if different immobilization methods are used. The reaction conditions, the acidity, temperature, substrate and enzyme loading, and duration, will influence the amount of converted FSM into glucose, which will be limited to the amount of cellulose present in the FSM.

Stake-holder analysis

The Cellulose Assisted Dewatering of Sludge (CADoS) project is a collaboration of several Dutch industries, research institutes and water authorities based on the harvesting of cellulose fibres from raw municipal waste water to promote the dewatering of sludge. This project initiated the full-scale trial of finescreen technology in WWTP Ulrum to investigate the impact of finescreen technology on the biological treatment of waste water, which has the potential to increase the capacity and reduce the aeration energy significantly. In addition to the operational benefits, the CADoS project is researching the possibility of using the screenings for further processing, in the production of biogas and possibly as the raw material for bioplastics (Wouters et al., 2017). The production of bioplastics requires the hydrolysis of the cellulose chains into its reducing sugar, glucose. If the enzymatic conversion of cellulose present in FSM can be converted into glucose, this application might be in reach.

The ENTEG research group of Prof. Dr. Gert-Jan Euverink is involved in the CADoS project by researching the potential biogas production through anaerobic microbial fermentation. The dewatered sludge, a concentrated organic waste stream, has the potential to yield biogas in an efficient manner. Furthermore, as the first supervisor of this integration project, Prof. Dr. Gert-Jan Euverink is interested in additional applications of the FSM retrieved from waste water which would increase the contribution of the ENTEG research group to the CADoS project and the development of a biobased economy.

The introduction of a commercially feasible enzymatic conversion of fine sieve materials to glucose will benefit the WWTP themselves. The return on investment in the introduction of fine sieve technology increases if the enzymatic production of glucose from fine sieve material is economically feasible. The investment needed to introduce the sieve technology will carry less uncertainty and thus more appealing for the WWTP. This will eventually lead to quicker adaptation of fine sieve technology and its benefits, reducing the WWTP environmental impact while increasing the capacity.

The major use of glucose is in the food industry. However, glucose retrieved from fine sieve material does not sound appealing towards consumers to be eaten. Glucose retrieved from fine sieve material could be used in non-food applications as the raw material for the production of bioplastics or in the biofuel industry. Using fine sieve material as a source for glucose instead of raw materials suitable for consumption removes the discussion if turning a food source into a non-food product is just. These industries will thus benefit a glucose stream which does not compete with the food industry and can be considered as a stake-holder.

Design steps

During this research the regulative design cycle of the OBS method is used. This cycle consists of six iterative steps, the problem mess, problem choice, diagnose, design, implementation and evaluation. During the empirical research on the evaluation of the required enzyme several iterations of this cycle will be needed to ensure an optimal final design.

If the overall research is considered the cycle is limited to the design step, since the implementation will not be executed.

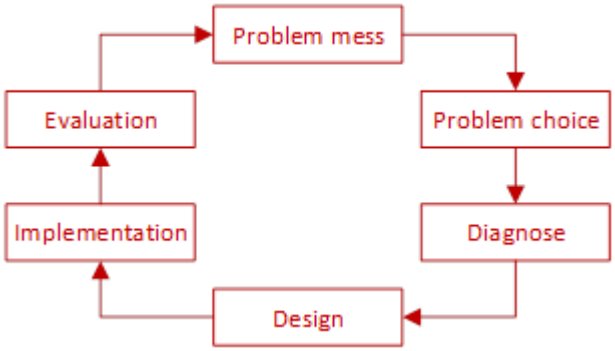

Figure 2: Regulative cycle

Research goal

Finding a method to enzymatically produce glucose from cellulose present in fine sieve materials and determine the economic feasibility by performing a market evaluation and estimating the costs of production by designing a full-scale chemical plant design.

Research question

The goal mentioned above result in the following main research question:

What method can be used to produce glucose from fine sieve material on an economically feasible scale?

Conceptual research design

To understand the difficulties which might be encountered in the process of answering the research question, the chemistry behind the enzymatic production needs to be studied further.

The schematics of the enzymatic conversion of cellulose is depicted in *Figure 3*. Cellulose, a polymer composed of D-glucose units linked by 1,4-1,4-glycosidic bonds, is crystalline in nature due to the tight configuration of the cellulose chains. The enzymatic conversion of the cellulose chains starts with the cleavage of the non-covalent interaction between the different cellulose chains by endocellulase, followed by the hydrolysis of the individual cellulose fibers into smaller sugars by exocellulase. The smaller carbohydrates, cellobiose, cellotetrose or higher order glucose chains, are converted into glucose with the help of β-glucosidase (Golan, 2011).

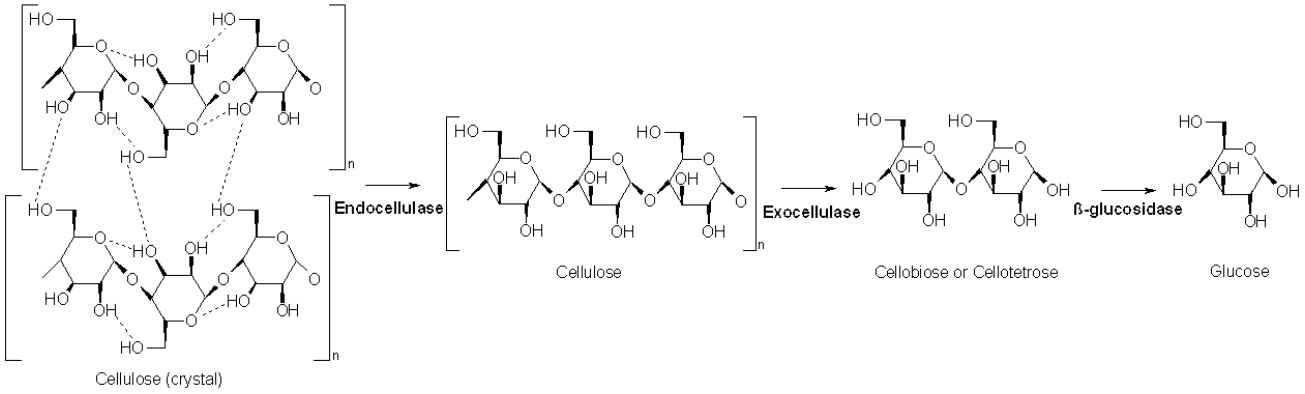

Figure 3: Enzymatic conversion of cellulose into glucose (Golan, 2011)

Most cellulose degrading enzymes consist of two functional groups, a binding site named the Cellulose Binding Domain (CBD), which ensures the correct positioning of the enzyme on the cellulose fibers, and an active site which performs the actual hydrolyzation of the cellulose (Linder & Teeri, 1996). The binding sites are vital to the activity of the enzyme. Removal of the binding site has shown to have a profound effect on the activity of the enzyme, resulting in a decrease in activity up to 50% compared to the initial activity (Tomme et al., 1988). The research of Linder and Teeri has proven that the binding of the CBD's to the cellulose is a reversible process, by diluting the cellulose enzyme suspension the bonded enzyme can be retrieved from the mixture. However, if continuous binding can be achieved while the cellulose is converted continuously, reuse of the enzyme might be possible.

Furthermore, the formed saccharides have shown to severely inhibit the enzyme activity, leading to a decrease in the sugar yields. Cellobiose and glucose have been proven to be inhibitors, where converting cellobiose to glucose reduces the inhibition significantly (Holtzapple, Cognata, Shu, & Hendrickson, 1990).

Conceptual casual model

The conceptual casual model is used to determine the underlying problems of the goal. To determine the economic feasibility of the enzymatic glucose productions both the costs and the potential revenues will need to be investigated.

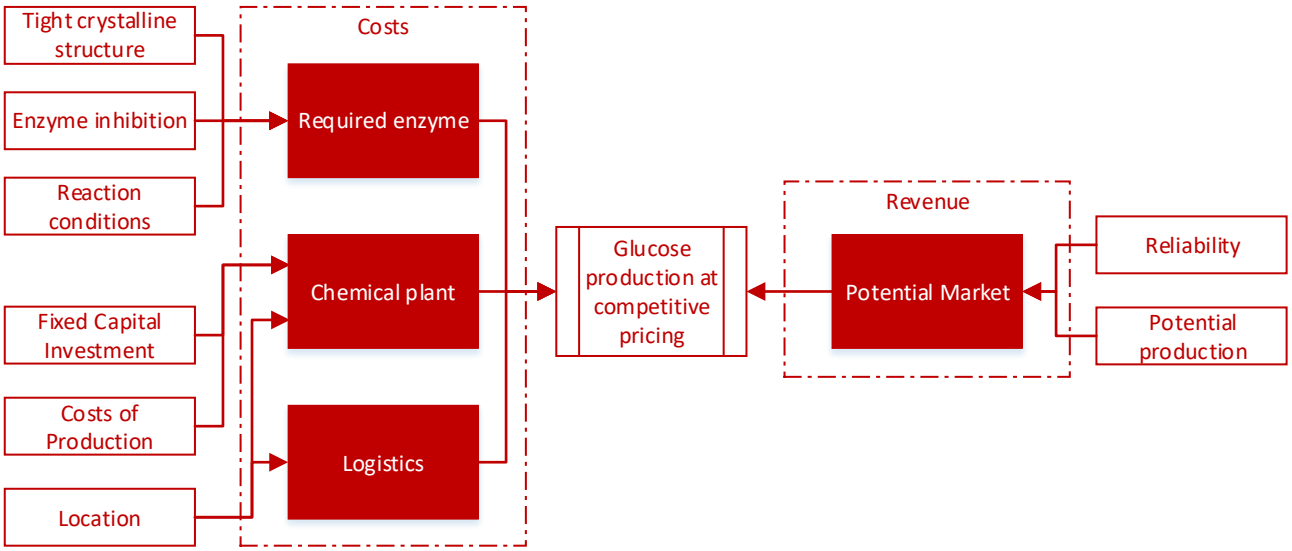

Figure 4: Conceptual casual model

To achieve a greater understanding of variables in the conceptual model the different elements are discussed briefly.

Required enzyme

The costs of cellulase is one of the major barriers for the commercialization of sugar production from cellulose containing biomass (Liu et al., 2016). Reducing the amount of required enzyme will influence the economic feasibility significantly.

The activity of the enzyme is influenced by several underlaying variables: the conditions of the reaction, the inhibition of the enzyme and the tight crystalline structure of celluloses. To reduce the required amount of enzyme the activity of the enzyme is needed to be optimal, which requires optimal reaction conditions and limited inhibition. Furthermore, the introduction of a method which enables the reuse of the enzyme would reduce the needed amount significantly.

Logistics

The location of the production site might have a major influence on the expected costs. Two scenarios need to be considered, an on-site production plant and the use of a central production plant.

Chemical plant

The costs of the chemical plant are divided into the fixed capital investment, which included the purchase and installment cost of the equipment needed to produce glucose from fine sieve materials, and the costs of production, including the running costs such as energy use, the required labor costs, depreciation and transportation costs. The choice concerning the location of the plant will be of major influence on the transportation costs and the required scale of production.

The elements which will influence the costs of production in the conceptual model can be divided into two different segments. An empirical research based on the workings of the enzymatic glucose conversion and the development of a business case.

Potential market

To produce a revenue a potential targetable market needs to exist. Potential clients which might consider glucose produced from fine sieve material as a raw material are needed to ensure a revenue. However, to be competitive compared to other glucose sources, the reliability should be high and the potential capacity for the production of glucose from fine sieve material needs to be sufficient.

The elements which will influence the costs of production and the potential revenue in the conceptual model can be divided into two different segments. An empirical research based on the workings of the enzymatic glucose conversion, mainly orientated towards reducing the amount of required enzyme, and the development of a business case, in which the logistics, chemical plant and the potential market will be discussed.

Adapted research question

The discussion of the elements presented in the conceptual casual model leads to the following adaptation of the initial research question:

> *Can a method be developed in which the enzyme usage is limited such that the enzymatic production of glucose becomes economically feasible?*

With the following sub questions:

> *Can the CBDs be exploited as an effective immobilization technique?*
>
> *Is there a market for glucose produced from FSM?*
>
> *Will the implementation of an immobilization technique on a large-scale lead to economic feasibility?*

Materials and methods

Cellulose conversion measurements

To measure the conversion of the cellulose two measurement methods are used to determine both the glucose concentration and the concentration of the reducing sugars. By measuring both the reducing sugars and the glucose concentration, the concentration of oligosaccharides can be determined since these are detected by the reducing sugar measurement, while remaining undetected in the glucose measurement.

Reducing sugar concentration

To determine the concentration of the reducing sugars the Nelson Somogyi method is used. This method utilizes the reducing property of the aldehyde or keto groups present in a reducing sugar. When a reducing sugar is heated with the presence alkaline copper tartrate, the copper is reduced from cupric to cuprous state, resulting in the formation of cuprous oxide. By treating the cuprous oxide with arsenomolybdic acid, molybdenum blue is produced.

All used solutions were prepared according to the method of McClearly and McGeough (McCleary & McGeough, 2015). 175 µL of a sample was diluted with 175 µL distilled water. 250 µL of solution D was added after which the mixture was placed in a water bath at 100°C for 15 minutes. The solution was removed from the boiling water and 1.5 mL of solution E was added, followed by vigorous mixing. The mixture was left to react at room temperature for 10 minutes, after which it was again mixed vigorously. The absorbance of the solution was measured at 520 nm using a spectrophotometer. In order to determine the reducing sugar concentration, the absorbance was compared to the absorbance of a calibration curve produced with six samples containing a varying glucose concentration, ranging from 0 to 0.44 mg/mL.

Glucose concentration determination

The glucose concentration is measured with the Megazyme D-Glucose kit (GOPOD Format). The assay employs high purity glucose oxidase and peroxidase which converts the glucose present in a sample to quinoneimine dye with a 1:1 ratio through a 2 stepped reaction.

A 14 µL sample is placed in a microwell after which distilled water is added to reach a total volume of 50 µL. 250 µL GOPOD solution containing the glucose oxidase and peroxidase is added followed by a 20 min incubation at 37 °C. The absorbance of the sample is measured at 510 nm and the glucose concentration was determined by comparing the absorbance to a calibration curve of samples with glucose concentrations ranging from 0 to 1 mg/ml with an interval of approximately 0.14 mg/mL.

Buffer solution preparation

To stabilize acidity during the reaction, a sodium acetate buffer solution was prepared. 5.7 mL 17.5 M acetate solution was diluted with 994.3 mL distilled water to achieve an acetate solution with a molarity of 100 mM. Sodium hydroxide was added to achieve a pH of 5.09.

Enzyme activity determination

To estimate the activity of the Cellulase (Celluclast, SIGMA-ALDRICH C2730, ≥700 units/g) in Filter Paper Units (FPU) two experiments were performed with varying enzyme concentration. The 50 µL of the enzyme was diluted to a volume of 2000 µL with the previously prepared sodium acetate buffer (100mM, pH 5.09). 10 mg of crystalline cellulose was added. The suspension was placed in a hot water bath at 50°C and stirred vigorously to keep the cellulose in suspension. Samples were taken over a time period of 1350 minutes.

The experiment was repeated with 50 µL enzyme diluted to a volume of 1000 µL with buffer. 5 mg of crystalline cellulose was added to achieve a comparable substrate concentration and samples were taken over a period of 120 minutes. The glucose concentration was determined with the GOPOD method from which the FPU was determined according to the method described by Ghose (Ghose, 1987). Furthermore, the reducing sugar concentration of the mixture with the 25 µL/mL enzyme concentration were measured using the Nelson Somogyi method. Since a different substrate was used to evaluate the activity, namely crystalline cellulose instead of filter paper, the calculated FPU is considered to be an estimation.

Adsorption evaluation

In the attempt to evaluate whether the cellulose binding domains can be used as an effective immobilization technique, an experiment was performed to investigate the adsorption of the enzyme on FSM. In literature, adsorption levels of 90% have been reached with cellulose concentrations of above 60 mg/ml with an enzyme depicting an FPU of 2.72 units/ml activity (Mandels, Kostick, & Parizek, 1971). To discover if this level of adsorption can be reached on FSM, comparable reaction conditions are used.

Two suspensions containing 25 µL of cellulase (approximately FPU 6.8 units/mL) diluted with 30 µL of 100 mM acetate buffer (pH 5.09) with 9.9 mg of FSM were made, resulting in a FSM concentration of 180 mg/mL consisting of approximately 40-50% cellulose. The suspensions were mixed vigorously and left to incubate for 20 minutes at room temperature.

The suspensions were centrifuged for 2 minutes at 5000 RPM, after which the supernatant of one of the suspensions with a total volume of 43 µL was removed and placed in a reaction tube, from here on referred to as tube 1. 9.6 mg of FSM and 957 µL of buffer solution were added to tube 1. 990 µL of buffer solution was added to the solids. After mixing thoroughly the suspension was removed and placed in tube 2.

The remaining supernatant (36 µL) from the other suspension was discarded and replaced with buffer solution. After mixing, the suspension was left to incubate again for 20 min at room temperature. The suspension was centrifuged for 2 minutes at 5000 RPM and 45.7 µL of supernatant was discarded. 988 µL of buffer was added to the solids and placed in tube 3 after vigorous mixing.
All three tubes were placed in a water bath at 50°C while being mixed. Samples were taken over a time period of 24 hours. Glucose concentrations were determined according to the GOPOD method.
To determine whether the enzyme was bonded to the substrate or was simply present in the remaining liquid of the sediment, 10 mg of FSM was added to three 1 mL enzyme dilutions in acetate buffer with concentrations of 20.6 µL/mL ,4.4 µL/mL and 1.9 µL/mL. The suspensions were placed in a 50 °C water bath and samples were taken over a time period of 4 hours. Glucose concentrations were determined according to the GOPOD method.

Enzyme immobilization

Mass transfer rate membrane

4 mL of 20 mg/mL glucose solution was placed inside a 30 mm dialysis membrane (Spectra/Por Dialysis Membrane, MWCO: 6000-8000, nominal flat width 50mm). The membrane was placed in 215 mL distilled water at a temperature of 50°C which was continuously stirred. Samples were taken outside of the membrane over a time period of three hours of which the glucose concentrations were determined according to the GOPOD method.

Immobilization

100 µL cellulase (FPU 6.8 units/mL) was diluted with 3.9 mL sodium acetate buffer. After dilution the enzyme was placed in a 30 mm dialysis membrane (Spectra/Por Dialysis Membrane, MWCO: 6000-8000, nominal flat width 50mm) containing 80 mg FSM. The membrane was placed in 200 mL of buffer solution at a temperature of 45°C. Samples were taken over a time period of 4 hours of which glucose and reducing sugar concentration were measured according to the GOPOD and Nelson Somogyi method respectively. The experiment was repeated under the same condition with 703.5 mg additional FSM to investigate the influence of abundant substrate.

Membrane degradation

36 mg of finely cut dialysis membrane (Spectra/Por Dialysis Membrane, MWCO: 6000-8000, nominal flat width 50mm) was suspended in a 1 mL enzyme dilution (25 µL cellulase, 975 µL acetate buffer solution). The temperature of the suspension was kept at 45°C and samples were taken over a time period of 17.5 hours of which the glucose concentration was determined according to the GOPOD method.

Results and Discussion

Enzyme activity determination

The glucose and reducing sugar measurements of the 25 µL/mL enzymes solution are depicted in *Figure 5.*

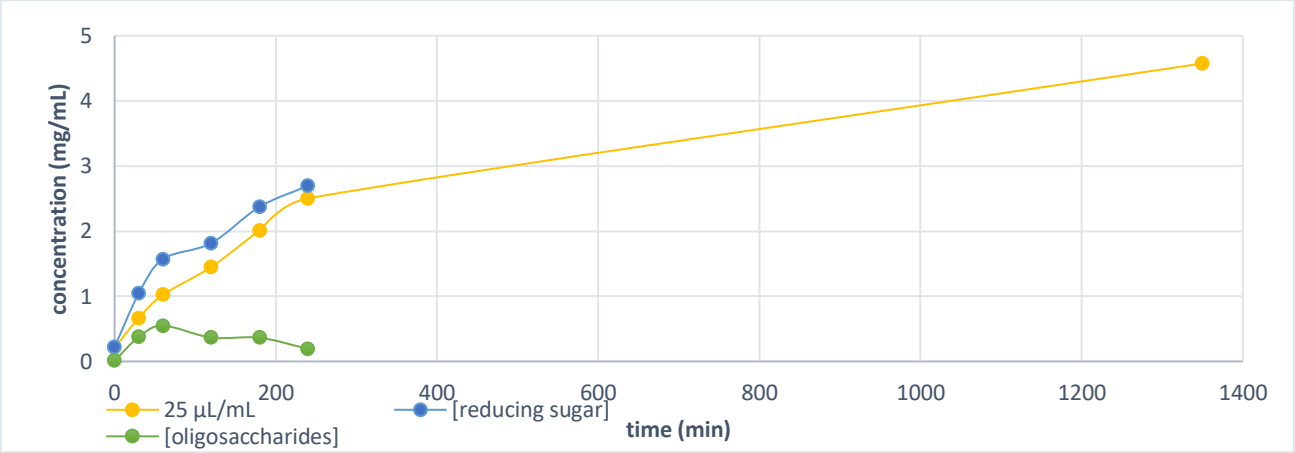

Figure 5: Glucose and reducing sugar concentration overtime with 25 µL/mL enzyme concentration

The initial high increase in sugar concentrations displays a high enzyme activity which reduces over time over time, due to substrate limitation and inhibition of the enzyme due to the formed saccharides (Holtzapple et al., 1990). The difference in reducing sugar concentration and glucose concentration diminishes over time, as shown by the decrease in the oligosaccharides concentration. This indicates a higher initial production of reducing sugars than the β-glucosidase can process, which are further reduced overtime until only glucose remains.

To estimate the enzyme activity the glucose concentrations of the first 120 minutes for both enzymes dilutions are plotted in *Figure 6*. A linear relationship is assumed during this period from which the liberated glucose was calculated and plotted over the enzyme concentration. An exponential relation between the enzyme concentration and the liberated glucose is assumed according to the FPU determination method of(Ghose, 1987). This is used to approximate the amount of enzyme needed to produce 2 mg of glucose within an hour, resulting in an enzyme concentration of 55 µL/mL, equal to an activity of 6.8 units/mL.

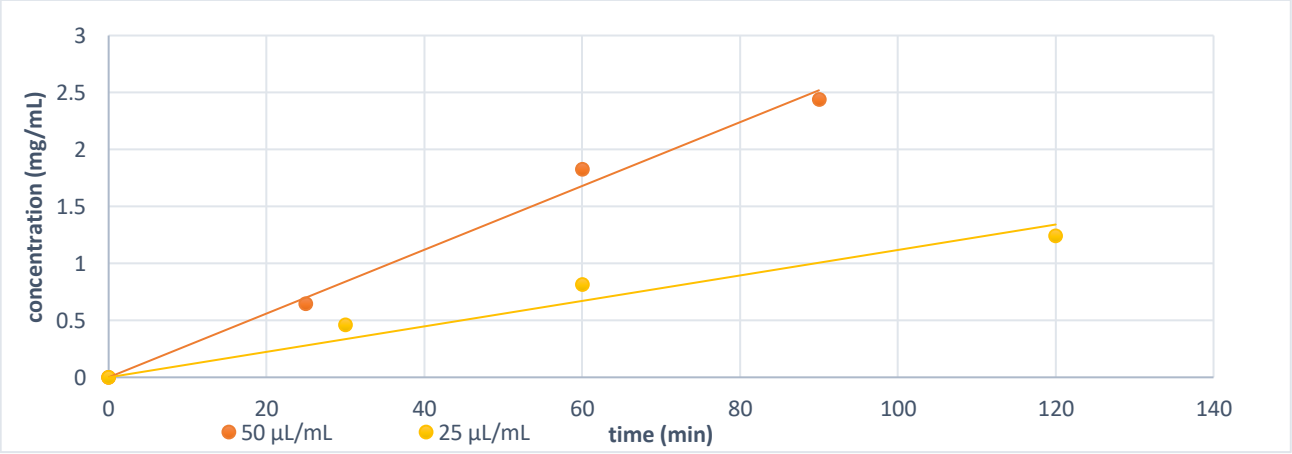

Figure 6: Glucose concentrations of the 50 µL/mL and 25 µL/mL enzyme dilutions over time

Adsorption determination

The glucose concentration over time of all three reactions is depicted in *Figure 7*.

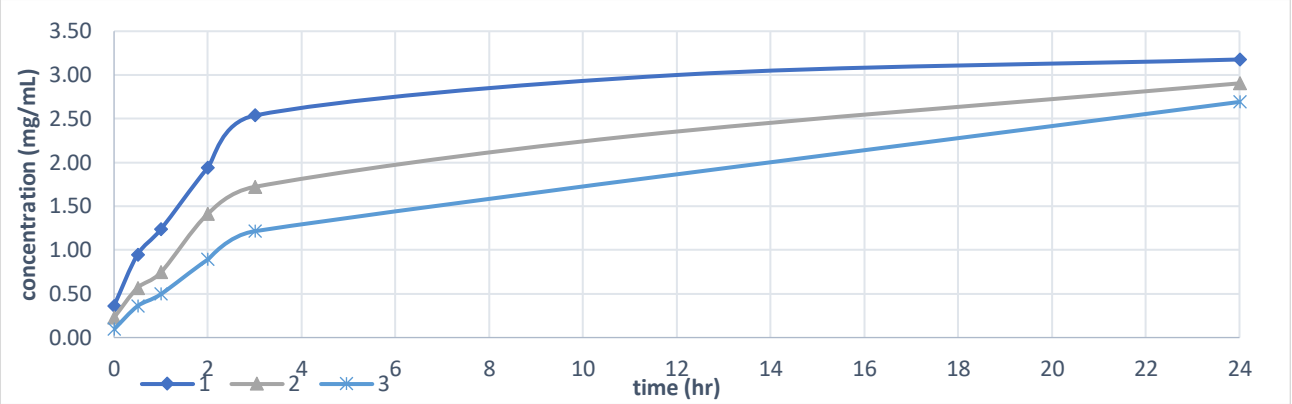

Figure 7:Glucose concentration over time for supernatant (1), solids (2) and double incubated solids (3)

The graph again depicted in *Figure 7* shows a high activity of the enzyme in all three samples during the first 3 hours, which reduces afterwards. A small dip can be noticed around the 1-hour mark, which can be contributed to an error which occurred with the heating plate. This resulted in a decrease in temperature to 33.6°C at a time of 45 minutes, reducing the enzyme activity. Even though the difference in glucose production is significant in the initial hours, after 24 hours the differences have decreased. This could be explained by the inhibition of the enzyme by the produced sugars, which are being produced at a higher rate by sample 1 compared to the other samples.

The results show that most of the enzyme activity remains in the supernatant, as the initial activity is much higher compared to the enzyme activity in the solids. The glucose concentration over time of the double incubated solids, displays an even further reduction in enzyme activity.

By weighing the suspension at every step of the incubation steps, the amount of remaining liquid in the solids was calculated. If no adsorption is assumed, the enzyme concentration in the remaining liquid is equal to the initial enzyme concentration. By assuming a density of 1.17 mg/ml of the enzyme solution, the theoretical amount of enzyme present is equal to 20.6 µL, 4.4 µL and 1.9 µL for tube 1, 2 and 3 respectively. During this calculation a density of 1.17 mg/ml of the enzyme was assumed. To evaluate whether the activity in the sediment and supernatant corresponds to the calculated amount of enzyme left in the supernatant and sediments, three reactions were followed with a 20.6 µL/mL, 4.4 µL/mL and 1.9 µL/mL enzyme concentration. The results of these experiments are depicted in *Figure 8*.

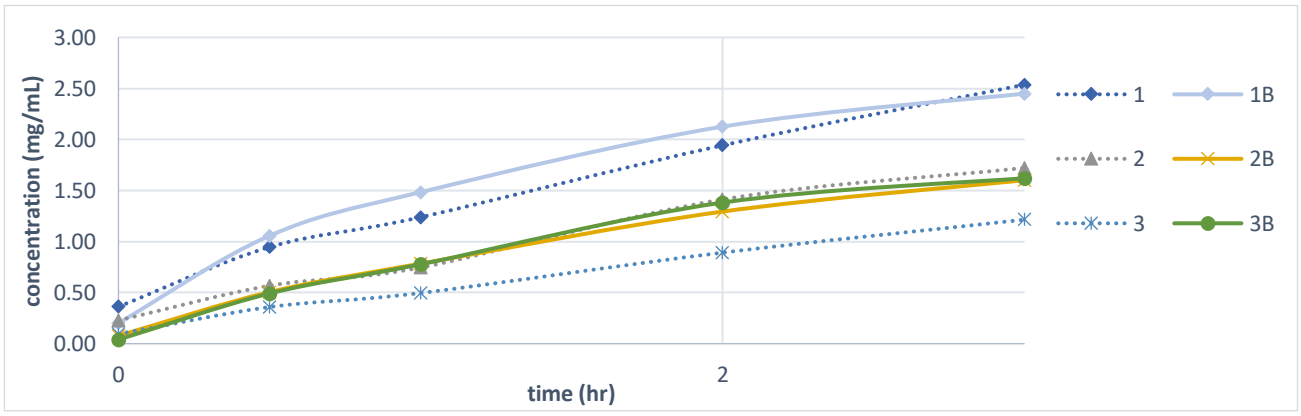

Figure 8: Glucose concentration over time for supernatant (1), solids (2) and double incubated solids (3) with the glucose concentration of their corresponding theoretical enzyme concentration over time (1B, 2B, and 3B)

When looking at the results in *Figure 8*, no significant difference in activity can be noticed between the results of the adsorption experiment and the experiment with their corresponding enzyme concentration if no adsorption has taken place for 1 and 2. The doubled incubated sediment depicts even less enzyme activity

compared to its theoretical enzyme concentration. From these results it can be concluded that no actual adsorption of the enzyme has taken place, since the activity present in the reactions corresponds to, or is lower than, the theoretical amount of enzyme present if no adsorption has taken place.

Enzyme immobilization

To estimate the occurred evaporation of buffer solution during the reaction, the difference between the initial volume and final volume of the permeate for the final immobilization experiment was measured. The evaporation was assumed to be linear over time. The calculated evaporation rate is assumed to be linearly dependent on the absolute temperature and surface contact area (Hisatake, Tanaka, & Aizawa, 1993). The surface contact area in each experiment is constant, where only the temperature differs between the mass transfer rate experiments and immobilization experiments. All further atmospheric conditions are assumed to be constant. This results in an evaporation rate of 0.1142 mL/min and 0.1125 mL/min for the mass transfer rate and immobilization experiments respectively.

Mass transfer rate

The results of the evaluation of the potential mass transfer rate of glucose through the dialysis membrane are depicted in *Figure 9*.

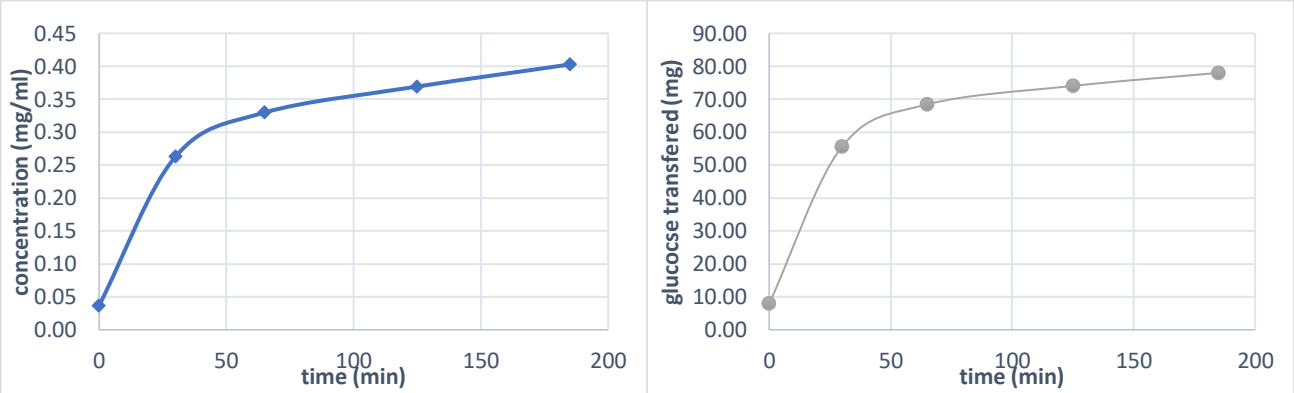

Figure 9: Glucose concentration in the permeate (left) and total glucose present in permeate (right)

Due to the high pore size of the membrane used (6000-8000 MWCO) and the relative high concentration gradient fast permeation of the membrane is achieved by the glucose. After three hours the almost all glucose present in the membrane was transferred through the dialysis membrane, which indicates no limitation in mass transfer with the used pore size. The high initial velocity of the mass transfer rate will most likely not be reached during the immobilization experiments, since the driving force of the mass transfer is concentration gradient over the membrane. During the immobilization experiments the gradient is developed over time with the production of glucose within the membrane, which would not result in the same behavior as depicted in *Figure 9* since a high concentration gradient is present from the start

Immobilization

The results of the immobilization experiment with an initial FSM concentration of 20 mg/mL within the membrane are depicted in *Figure 10.*

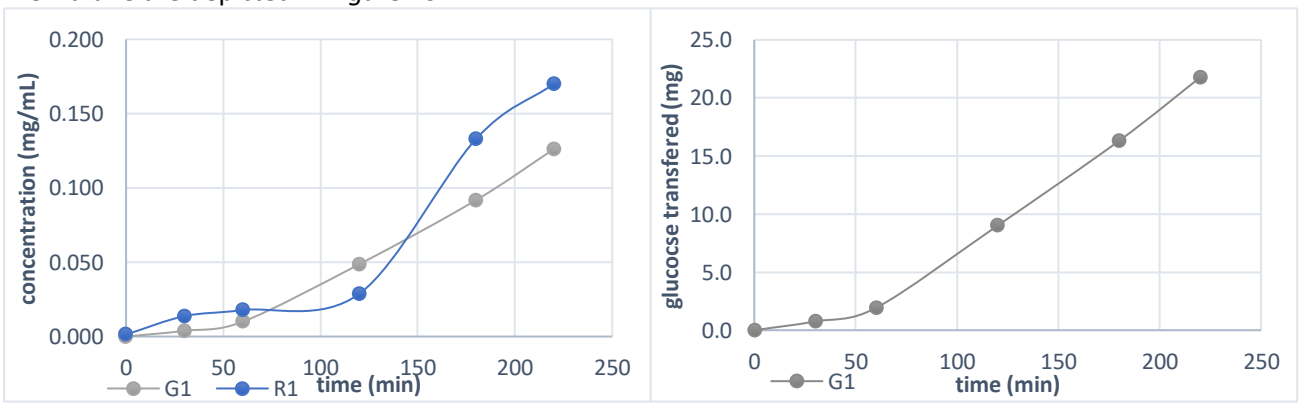

Figure 10: Glucose (G1) and reducing sugar (R1) concentration (left) and glucose permeated through membrane (right) over time

The reducing sugar concentration (R1) in *Figure 10* displays unexpected behavior, resulting in a lower concentration compared to the glucose measurements at 120 minutes. This would suggest a mistake in the measurement procedure of the reducing sugar measurement, since all other measurement points depict the expected behavior.

The initial slow increase of glucose concentration in the permeate can be explained due to the absence of a concentration gradient over the membrane. The initial high enzyme activity within the membrane and the produced glucose concentration which follows is only measured after permeation. After approximately 60 minutes the concentration gradient seems to be sufficient to initiate a significant increase in the amount of sugars permeating the membrane, as a steady increase in glucose concentration is observed between the first and second hour.

During the third hour, FSM was found in the permeate which indicated a leak in the membrane, which renders the results inaccurate after 2 hours. The membrane is composed of cellulose acetate, which due to the additional acetyl groups, should require the presence of esterases and cellulase in order to be degraded to glucose (Puls, Wilson, & Hölter, 2011). This leads to two possible explanations for the formation of the leak, either esterases is present in the enzyme mixture, which might be possible since the exact composition is unknown, or there are unacetylated sections present in the cellulose acetate membrane.

The possibility of leakage in the cellulose membranes is confirmed by the suppliers of the membranes, who discourage the usage of cellulase with cellulose acetate membranes (Thermo Fisher Scientific, 2018).

In an attempt to delay the formation of leakages in the membrane the experiment was repeated with an initial FSM concentration inside the membrane of 175.9 mg/mL, of which the results are depicted in *Figure 11*.

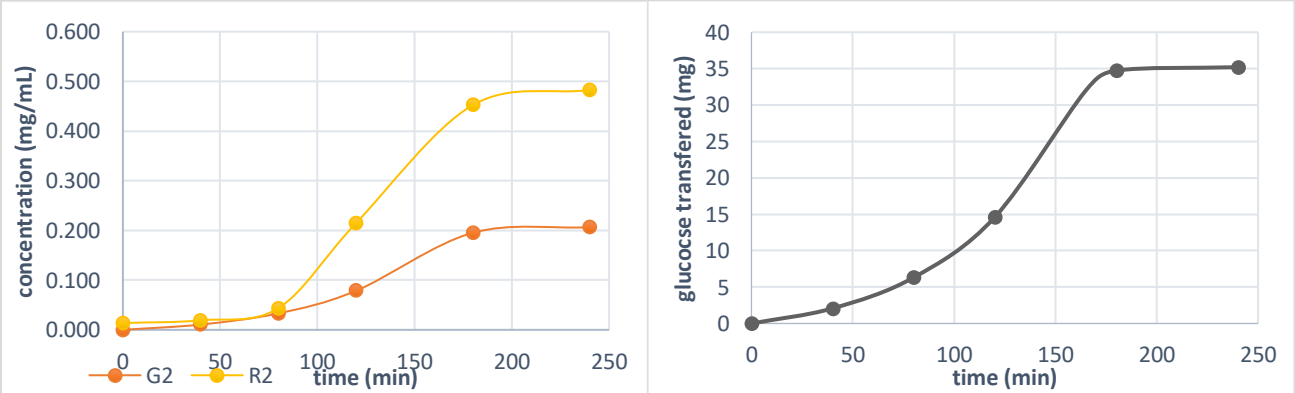

Figure 11: Glucose (G2) and reducing sugar (R2) concentration (left) and glucose permeated through membrane (right) over time

With the presence of a major substrate abundance, the probability of the enzyme degrading the membrane was expected to decrease. However, FSM was detected in the permeate during sampling at 3 hours, indicating the formation of leaks between 2 and 3 hours.

The difference in reducing sugar concentration and the glucose concentration is significantly higher compared to previously measured values over time (*Figure 5*). Furthermore, the glucose concentration is not approaching the reducing sugar concentration. The pore size is large enough to pass reducing sugars consisting of up to 45 linked glucose units. Due to the absence of β-glucosidase in the permeate, no further reduction takes place after the oligosaccharides have permeated the membrane.

After the integrity of the membrane was lost, the glucose and reducing sugar formation reduced while no reduction between the reducing sugar and glucose concentration can be noticed. After three hours a neglectable amount of liquid was left in the membrane when taken out of the solution. This explains the flattening of the concentration increase. If it is assumed that all enzyme and substrate has leaked from the membrane, the concentration of both the enzyme and substrate is reduced with a factor of 50.

Membrane degradation

To confirm if the membrane is degraded by cellulase, finely cut membrane was used as a substrate. The results are depicted in *Figure 12*.

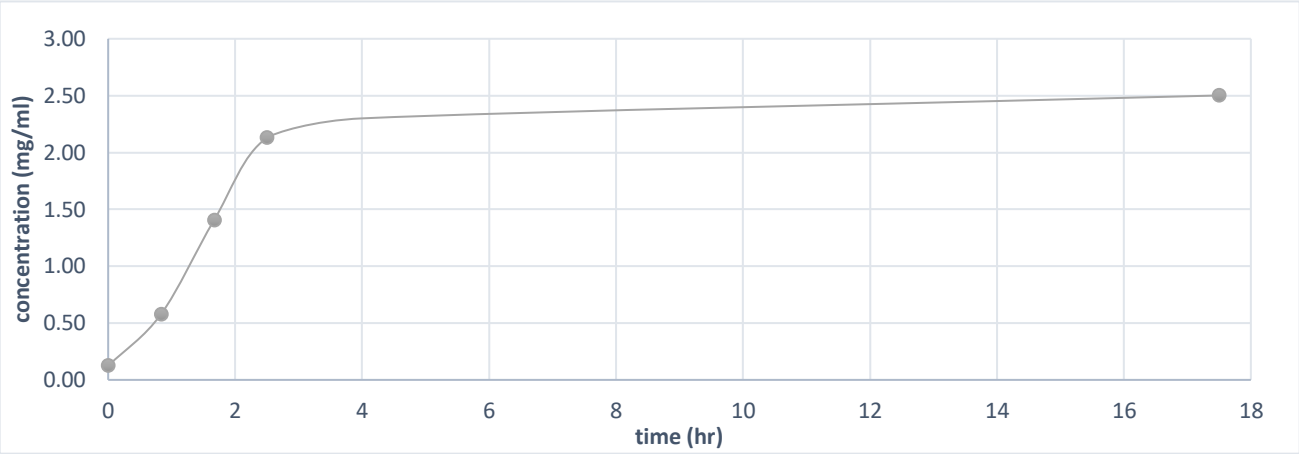

Figure 12: Glucose concentration over time with membrane as substrate

The results show an increase in glucose concentration over time. After 17 hours 2.5 mg of glucose was produced, a conversion of 6.25% of the initial substrate. This confirms the suspicion of the formation of leaks in the cellulose acetate membrane due to degradation by the enzyme.

Assumptions for upscaling

Due to the degradation of the membrane, none of the immobilization experiments resulted in a conversion rate over time which could be used as the parameter for the performance of a full-scale membrane reactor. Only the results over the first 2 hours could be deemed accurate. To extrapolate the results of the immobilization experiments, the trends depicted by the previous experiments depicted in *Figure 7* are used. The glucose concentration achieved at 2 hours for each reaction is approximately half of the finally reached glucose concentration. Although the experimental design of the adsorption experiments is completely different compared to the immobilization experiments, the achieved glucose concentration at 2 hours in the immobilization experiments is assumed to be the half of the final value in order to estimate the conversion rate.

By using the results of G1 depicted in *Figure 10*, the estimate final glucose production results in a value of 18 mg. This is equal to a 21.5% conversion of the FSM, or a 45% cellulose conversion if a cellulose content in the FSM of 47% is assumed. Furthermore, it is assumed that the final concentration and conversion is reached after 8 hours.

With the usage of a membrane to immobilize the enzyme, reusing the enzyme is assumed to be possible. The results of the adsorption test depicted no adsorption of the enzyme on the FSM. If the unconverted FSM is allowed to settle, a major part of the buffer solution containing the enzyme could be removed from the reactor. After the removal of the unconverted FSM, the enzyme solution could be reused with new FSM. During the full-scale approximation a recovery rate of 90% of the enzyme solution is assumed.

Economic feasibility determination

Market evaluation for glucose derived from fine sieve material

European sugar market development

The abolishment of the quota on sugar production in the European Union in 2017 will have a profound effect on the European sugar production and the sugar price. The sugar production in the first year after the abolishment is expected to increase with 22% from 16.8 million tons to 20.5 million tons. The additional supply will partly be exported and used to rebuild stocks. Furthermore, the additional production is expected to result in a reduction in the difference between the European and global sugar price to 40 euro/ton. The decreased difference will make the EU a less attractive export destination, mainly due to the 98 euro/ton duty imposed on imported sugar. Due to the increase in export and the expected decrease in import, the EU is expected to become a net exporter of sugar after the abolishment of the sugar quota.

The initial increase in sugar production is expected to stabilize at a value of 18 million tons annually, an increase of 12% compared to current production. At the stabilized rate of production, the white sugar price is projected to average around 400 euro/ton, a reduction of 23% compared to the average price in the period of 2012 up until 2016. The raw sugar price is expected to average 300 euro/ton after the production is stabilized (Zaitegui Pérez et al., 2017), which, due to the high impurities present in the produced glucose, can be considered as the uppermost price for the produced glucose.

Potential production

By evaluating the purchases of toilet paper for a 5-student household over a time period of 157 days an estimation of the amount of toilet paper used per year for an individual was made, resulting in a value of 13 kg. By combining the estimated toilet paper use with the expected population of the Netherlands in 2030, the expected 49% of suspended solids retrieved by finescreen technology (Roest, 2018) and the adjustment in molecular weight of glucose in cellulose and as a separate molecule, a size estimation of the potential glucose production from FSM equals 0.101 Mt in 2030.

This estimation is considered to be an upper limit for the potential glucose production, since it assumes the complete conversion of all cellulose present in fine sieve material to glucose. Furthermore, it assumes that every WWTP uses finescreen technology and all collected FSM retrieved is used for this application.

Furthermore, from the estimated amount of toilet paper usage an estimation on the cellulose content of the FSM can be made. Waste water in the Netherlands contains 0.242 g/L of suspended solids (CBS StatLine, 2018a), whereas the total amount of cellulose from toilet paper derived from the estimations _equals 0.112 g/L, equal to 47%.

Potential market size

In order to evaluate the possible market for the enzymatic produced glucose from fine sieve material the potential applications for glucose produced from fine sieve material need to be evaluated. Due to the origin and the higher probability of containing impurities compared to other sources of sugar, glucose derived from fine sieve material is undesirable for food and pharmaceutical application.

One of the most promising targetable markets for the produced glucose is functioning as a feedstock from which other chemicals are derived. Nowadays most of the chemical building blocks such as ethylene, benzene and toluene are derived from fossil fuels, mainly from crude oil. Due to the depletion of the fossil fuel supply in the upcoming decades, a new feedstock for the production of chemical building blocks is needed. Biomass and the sugars within them show the promise of being the replacing source for crude oil.

Blaauw et al. have performed a study in which an estimation on the required biomass in the EU in 2030 is made to completely replace the derivation of chemical building blocks from fossil fuels. Three different growth scenarios were assumed in the changes in demand for the chemical building blocks in Europe, a low, moderate and strong growth scenario. The needed biomass to replace fossil fuels in 2030 on average was estimated at 155 Mt, 212 Mt, and 290 Mt for the low, moderate and strong growth scenario respectively (Blaauw, Bos, van Hal, Saygin, & Patel, 2013). In the estimations made by Blaauw et al., a process efficiency of 100% is assumed in the conversion of biomass towards chemical building blocks. Due to the unlikelihood of obtaining such an efficiency, the required biomass estimations are considered to be the minimal values.

In order to make a crude estimation of the needed sugar for the Dutch chemical industry for the derivation of chemical building blocks in 2030 additional data is required. The estimations made by Blaauw et al. are based on a sugar content of 75%. Furthermore, the chemical industry in the Netherlands has the ambition to increase the share of biomass derived chemical building blocks to 25%. Finally, the Dutch chemical industry makes up 7.6% of the European market.(Blaauw, van Haveren, Scott, & Bos, 2008). By incorporating these figures with the estimations of Blaauw et al, the estimated amount of sugar needed as the raw material for the derivation of chemical building blocks in 2030 is 2.2 Mt, 3 Mt and 4 Mt for the low, moderate and strong growth scenario for the chemical industry respectively.

Chemical plant design

To evaluate the economic feasibility of the enzymatic conversion of glucose retrieved from FSM, a costs analysis of a full-scale production facility must be made.

To derive a cost estimation of a chemical plant, clear boundaries of the design requirements are needed. When the limitations of the glucose derived from FSM are taken into account, the limited applicability and the presence of impurities, the costs of production should be limited to 200 euro/ton in order to be competitive towards other sugar sources. The upper limit of the potential production size is estimated at 0.101 Mt which assumes the presence of finescreen technology in every WWTP, with all fine sieve material purposed for the enzymatic glucose production. Due to these assumptions, the desired output is set at 10% of the potential market size in the design of the chemical plant.

If the desired output is produced, a 0.5%, 0.35% and 0.25% of the potential market size could be facilitated for the low, moderate and strong growth scenarios. Furthermore, if the desired output is combined with the assumed maximal production costs per ton of produced glucose, the variable costs should be limited to approximately 2 million euro's annually to break even.

Location

The location of the chemical plant has a significant impact on the design of the chemical plant and the costs in logistics. To limit the costs associated with logistics an on-site conversion of the retrieved FSM at the WWTP themselves would be ideal. However, there is not a WWTP in the Netherlands processes enough waste water in order to reach the scale of desired output. Only a few water boards are currently processing waste water volumes nearing the desired goal, which are depicted in *Table 1.*

Table 1: Statistics largest water boards in the Netherlands 2016 (CBS StatLine, 2018b)

	WWTP	**Capacity**	**Waste water influent**	
	amount	1000 v.e.	Volume (1000 m^3)	Percentage
Netherlands total	327	30122	1904878	100%
Waternet	12	2024	127103	6.7%
HHS Rijnland	22	2084	121488	6.4%
HHS Delfland	4	1938	133020	7.0%

The water boards depicted in *Table 1* are located in densely populated areas, where HHS Rijnland and HHS Delfland are located next to each other.

HHS Delfland does not only process the highest amount of waste water, it is achieved with the lowest amount of WWTP. To limit the costs associated with transportation of FSM, being located in the area of operation of HHS Delfland would result in the least amount of transport of FSM needed. Especially if a plant is built next to the WWTP with the highest capacity of HHS Delfland, WWTP Harnaschpolder. WWTP Harnaschpolder has a capacity of 1.3 million v.e., which makes up 65% of all waste water treated under the responsibility of the water board, equivalent to approximately 5% of the total volume of the waste water treated in the Netherlands. The needed transportation of FSM to reach the desired output would be reduced by approximately a quarter if the plant is located on or near the site of Harnaschpolder, neglecting transport of FSM within the WWTP and assuming a FSM conversion of 21%.

The remaining FSM which is needed to reach the desired output would need to be transported from the other WWTPs of HHS Delfland and neighboring water boards. Due to the densely populated area, it can be assumed that the needed FSM can be retrieved from WWTPs within a radius of 50 km.

Chemical plant design

The design of the chemical installation is depicted in *Figure 13.*

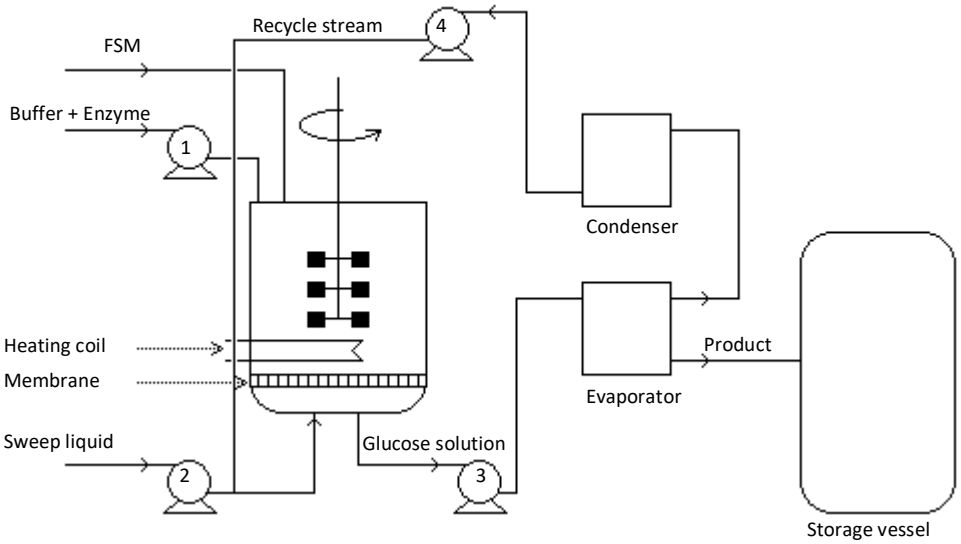

Figure 13: Design chemical membrane reactor

The desired production is set at 10.000 ton of glucose with a concentration of 90 wt%, a suitable concentration for industrial fermentation (Le Bot, Gouy, Kearsley, & Dziedzic, 1995). The cellulose content in FSM was deemed to be 47% and the FSM concentration of 20 kg/m^3 in the reactor is used, comparable to the immobilization experiments.

The used conversion rate was set at 21%, which is reached in 8 hours. Taking additional time needed for preparation and cleaning into consideration, only a single batch per day can be completed with a reaction time of 8 hours.

Similar membrane permeation rates are assumed as in the immobilization experiments. Furthermore, this is assumed to be reached with a single membrane suspended a certain height above the bottom of the reactor. The maximum glucose concentration allowed at the permeate side of the membrane is set to 0.05 kg/m^3, equal to the concentration of the membrane experiment at 2 hours. This will ensure a high concentration gradient over the membrane, resulting in a continuous driving force for the glucose to permeate. To simplify the calculations, the permeation through the membrane is assumed to be linear over time and no energy loss is assumed.

With the set goals and assumptions discussed above, the requirements for each element in *Figure 13* are depicted in *Table 2*.

Table 2: Required specifications elements chemical plant design from assumption

Element			Required size parameter
Reactor	[FSM]	(kg/m^3)	20
	FSM	(kg/day)	131145
	V_{RaM}	m^3	6557
	V_{RuM}	m^3	482
	Membrane	m^2	804
Flowrate	Buffer + enzyme	m^3/s	1.82
	Sweep liquid	L /s	0.24
	Glucose solution	L /s	40.66
	Product	L /s	0.24
	Recycle stream	L /s	40.43
Evaporator	T increase	°C	50
	Required A	m^2	2
Condenser	T decrease	°C	50
	Required A	m^2	2
Product storage	V	m^3	1250

The desired production of 10,000 ton of glucose a year requires approximately 13 ton of FSM a day with a conversion rate of 21%. Operated in a single batch and combined with the substrate concentration, the volume above the membrane (V_{RaM}) should be at least 6557 m^3.

A product volume of 6.85 m^3 a day is needed to produce the desired amount of glucose with a concentration of 90 wt%, which is equal to a flowrate of 0.24 L/s over an 8 hour period. This amount is concentrated from an initial concentration of 5 wt%, which requires a flowrate of 40.66 L/s. The recycle stream is equal to the difference between the glucose solution stream and product stream, whereas the sweep liquid flow must be equal to the product stream during production. The required flowrate for the buffer and enzyme solution has been selected in order to fill the reactor within an hour, requiring a flowrate of 1.82 m^3/s.

By assuming a constant permeation rate during the 8-hour operating period, the set Glucose stream flow rate and the maximal glucose concentration present, the volume needed underneath the membrane (V_{RuM}) was calculated to be 482 m^3. In order to hold both V_{RuM} and V_{RaM}, the total volume of the reactor is set at 7500 m^3. To have a membrane dividing the V_{RuM} and V_{RaM} in the reactor, a membrane area of 804 m^2 is required at a height of 0.6 m.

The size of the required reactor, with a radius of 16 m and a height of 9.3 m, is incredible and could be impractical. This could be solved by using multiple reactors running in parallel. Using multiple reactors in parallel could increase the fixed capital investment. However, it will not have a profound effect on the costs of production and the calculated cost price, since only a small part of the fixed costs are realated to the fixed capital investment. To simplify the modelling in the determination of the economic feasibility, the choice was made to use a single reactor.

The required area of the condenser and evaporator were calculated by using the general equation for heat transfer across a surface, with an overall heat transfer coefficient of 1250 $W/m^2°C$ for the used steam, resulting in approximately 2 m^2.

Finally, the storage vessel for the product was sized to be able to hold a volume equal to half a year of production.

Economic evaluation

From the required size parameters, the fixed capital investment, variable costs of production and the fixed costs of production will be determined. All values retrieved in dollars were converted to euros with the average exchange rate of 2017, being equal to 1.13.

Fixed capital investment

The total costs of building a full-scale plant is generally known as the fixed capital investment. The fixed capital investment consists of four elements, the inside battery limits (ISBL) investments, the outside battery limits (OSBL) investments, the engineering costs and finally the contingency charges (Towler & Sinnott, 2012).

The ISBL investments are considered to be the costs of the plant itself, the purchase of equipment, their installation costs and miscellaneous overhead. The OSBL investments are all investments which are required outside of the plant, such as infrastructure and landscape changes needed to accommodate the new plant. Engineering costs include the costs of detailed design and other engineering services. Finally, contingency charges are extra costs added to allow variation from the costs estimate.

In the book of Towler and Sinnot, the ISBL investments are given as a function of the total purchased equipment, by factors proposed by Hand (Hand, 1958). This results in a crude estimation of the ISBL investments, in which the engineering costs have already been incorporated. The OSBL investment is considered to be 20% of the ISBL investments, and the contingency charges are assumed to be 10% of the summation of the ISBL and OSBL investments. By using these relations, the fixed capital investment can be approximated as a function of the purchased equipment.

In order to estimate the capital needed for the purchase of the required equipment, Towler and Sinnot describe a correlation between the cost of equipment and the size parameter, depicted in the formula below.

$$C_e = a + bS^n$$

Where C_e is equal to the costs in dollars, a and b are costs constants for the type of equipment, S represents the size parameter and n is the exponent for the type of equipment.

With the required size parameter depicted in *Table 2* and the formula above, the costs of the equipment was calculated, which are depicted in *Table 3.*

The costs estimate of the membrane was made with a price per squared meter, based on the membrane price and size used in the work of Bick et al. (Bick, Gillerman, Manor, & Oron, 2012).

Table 3: Equipment costs derived with the correlation between the cost of equipment and the size parameter, with the ISBL estimate derived from the installation factors (Hand, 1958)

Equipment		Equipment costs	ISBL estimate
Reactor	Tank	€ 495,823	€ 743,735
	Stirrer	€ 54,657	€ 218,626
	Membrane	€ 15,539	€ 62,156
Evaporator		€ 25,735	€ 90,074
Condenser		€ 2,389	€ 8,363
Product storage		€ 200,110	€ 500,274
Pump	1*	€ 270,559	€ 1,082,236
	2	€ 2,928	€ 11,712
	3	€ 6,545	€ 26,178
	4	€ 6,519	€ 26,077
	Total	**€ 1,080,804**	**€ 2,769,431**

*set of 14 parallel pumps used to limit costs

With the ISBL estimation of € 2,769,431 and the previously mentioned relations, the OSBL and the contingency charges can be calculated, resulting in € 553,886 and € 332,332 respectively. The total fixed capital investment

estimation, the summation of the ISBL (includes engineering costs), OSBL and contingency charges, is equal to € 3,655,649.

Costs of production

The costs of production consists of both the fixed costs, comprised of costs which are incurred regardless of the plants operation rate or output, and variable costs, costs proportional to the operation rate or output of the plant (Towler & Sinnott, 2012).

Fixed costs

Labor costs are a major part of the fixed costs. The minimal amount of positions required in a plant is three, one operator in the control room, one outside and one in the tank room. In order to operate continuously throughout the year with the allowance of free time for the operators requires 4.8 operators per position (Towler & Sinnott, 2012). The monthly wages are assumed to be € 3,000.00, with an additional direct salary overhead of 60%.

The depreciation is taken linearly over a time period of 10 years for all the equipment, except for the membrane, which is depreciated over a time period of 5 years (Bick et al., 2012).

The maintenance costs were estimated as 3% of the ISBL, whereas the Property taxes and insurances were assumed to be 1% of the ISBL.

Variable costs

The raw material usage comprises of the enzyme, water usage and the sodium acetate consumption. In the enzyme costs estimation a recovery rate of 90% is assumed, with a cost price equal to € 4.48 per kg (Kazi et al., 2010) and an enzyme substrate loading of 2%. The industrial price for drinking water for the cost price per m^3 of water, equal to € 0.92 (van der Zeijden, Muizer, Braaksma, & Pasaribu, 2009). For the sodium acetate a purchase cost of € 500.00 per ton is assumed.

The ideal energy for heating the required water, the evaporator, stirrer and pumps were calculated. To convert the ideal situation into a realistic estimation, a 50% efficiency was assumed. A cost per kWh of € 0.095 was used (Nillesen, 2014).

The transportation was calculated under the assumption of an average traveling distance of 50 km. The used costs per km per ton of material is € 0.18 (Schade et al., 2006).

The remaining FSM waste is treated as inert solids, which must be transported to landfills. The disposal rate was set at € 25.00 per ton excluding taxes, equal to € 43.32 per ton after taxes.

Table 4: Costs of production estimations, divided in fixed and variable costs

Fixed costs					
Labor costs including overhead		€	806,400		
Maintenance		€	83,083		
Property Taxes and insurance		€	27,694		
Deprecation		€	109,634		
			Total annual fixed costs	**€**	**1,026,812**
Variable costs					
Raw materials	Enzyme	€	470,227		
	Water	€	228,525		
	Sodium acetate	€	134,037		
Utilities	Energy	€	112,669		
Transport		€	314,133		
Waste disposal		€	1,415,778		
			Total annual variable costs	**€**	**2,675,369**
			Total annual costs of production	**€**	**3,702,181**

The total annual costs of production depicted in *Table 4* result in a cost price of € 365.68 per ton. At a cost price of € 365.68 per ton the process is deemed economically unfeasible, since the market evaluation determined the requirement of a cost price around € 200.00 per ton in order to be competitive towards other sugar sources. Furthermore, the accuracy and sensitivity of the estimations on the enzyme costs need to be considered. The assumed 90% recovery rate of the enzyme activity between the batches has not been researched. A reduction in the recovery rate of the enzyme will have a profound effect on the cost price, leading to an increase of € 5.10 per ton for every reduced percentage in the recovery rate. Furthermore, estimates in the enzyme prices per kg of protein ranges from $ 1.25 to $23.30 (Liu et al., 2016). With the relatively low used rate of € 4.48 (Kazi et al., 2010), the cost price estimation needs to be considered as a minimal value.

However, some opportunities for improvement are present in the current estimation. The costs for waste disposal is equal to 37% of the total annual costs of production. Research by the ENTEG group is performed on the composition of FSM besides cellulose, which is expected to consist of organic material. If this is the case, using the remaining solids as the feedstock to produce biogas might be possible. If this process is assumed to be costs efficient, the waste disposal costs could be removed completely, reducing the cost price to € 225.84, nearing the desired cost price of € 200.00. With such a cost price, economic feasibility of the enzymatic conversion of FSM might be reached.

Conclusion

During this research, an attempt was made to design a method in which the enzyme usage is limited such that the enzymatic production of glucose from FSM becomes economically feasible.

By evaluating the adsorption of cellulase on the FSM, it was concluded that the CBDs could not be used as an effective immobilization technique, since no adsorption of the cellulase on the cellulose occurred. In an attempt to create the possibility to reuse the enzyme, the enzyme was immobilized with the help of a dialysis membrane. However, the incompatibility of the enzyme with the cellulose acetate membrane led to the degradation of the dialysis membrane.

From the performed market evaluation, the potential glucose production from FSM was estimated to be equal to 0.101 Mton. Sugars used as the feedstock for the derivation of chemical building blocks was identified as a possible market for the produced glucose, with a market size ranging from 2.2 Mt, 3 Mt and 4 Mt for low, moderate and strong growth scenario's in 2030. Furthermore, the developments on the European sugar market resulted in a goal for the cost price, equal to € 200.00 per ton of glucose. At this rate the glucose derived from FSM is expected to be competitive compared to other, generally purer, sources of sugar.

To evaluate the economic feasibility of the enzymatic conversion of glucose retrieved from FSM, a costs analysis on a production facility with the potential annual production of 10,000 ton of glucose was made. This resulted in a fixed capital investment of € 3,655,649 with an annual cost of production equal of € 3,702,181, equal to a cost price of € 365.68 per ton of glucose. Due to the limitations in the made assumptions for upscaling, the determined cost price needs to be considered as a minimal value. With the determined cost price, economic feasibility in the enzymatic conversion of cellulose retrieved from FSM is not reached.

Further research on the composition of FSM might result in a cost-effective usage of the unconverted FSM. This would reduce the cost price to € 225.84, nearing the desired costs price of € 200.00. With a cost price of € 225.84, economic feasibility in the enzymatic conversion of FSM into glucose might be reached in the future.

References

Bick, A., Gillerman, L., Manor, Y., & Oron, G. (2012). Economic assessment of an integrated membrane system for secondary effluent polishing for unrestricted reuse. *Water, 4*(1), 219-236.

Blaauw, R., Bos, H., van Hal, J., Saygin, D., & Patel, M. (2013). *De biomassabehoefte van de chemische industrie in een biobasedeconomy* Wageningen UR-Food & Biobased Research.

Blaauw, R., van Haveren, J., Scott, E. L., & Bos, H. L. (2008). Biomass for the dutch chemical industry.

CBS StatLine. (2018a). Zuivering van stedelijk afvalwater; per provincie en stroomgebieddistrict. Retrieved from https://opendata.cbs.nl/statline/#/CBS/nl/dataset/7477/table?ts=1529230002461

CBS StatLine. (2018b). Zuivering van stedelijk afvalwater; per regionale waterkwaliteitsbeheerder. Retrieved from https://opendata.cbs.nl/#/CBS/nl/dataset/71476ned/table?ts=1527933331461

Ghose, T. (1987). Measurement of cellulase activities. *Pure and Applied Chemistry, 59*(2), 257-268.

Golan, A. E. (2011). Cellulase: Types and action, mechanism, and uses. In A. E. Golan (Ed.), (pp. 251-254). Hauppauge: Nova Science Publishers, Inc.

Hand, W. (1958). From flow sheet to cost estimate. *Petroleum Refiner, 37*(9), 331-334.

Hisatake, K., Tanaka, S., & Aizawa, Y. (1993). Evaporation rate of water in a vessel. *Journal of Applied Physics, 73*(11), 7395-7401.

Holtzapple, M., Cognata, M., Shu, Y., & Hendrickson, C. (1990). Inhibition of trichoderma reesei cellulase by sugars and solvents. *Biotechnology and Bioengineering, 36*(3), 275-287.

Kazi, F. K., Fortman, J., Anex, R., Kothandaraman, G., Hsu, D., Aden, A., & Dutta, A. (2010). Techno-economic analysis of biochemical scenarios for production of cellulosic ethanol.

Le Bot, Y., Gouy, P., Kearsley, M., & Dziedzic, S. (1995). Handbook of starch hydrolysis products and their derivatives., 155-177.

Linder, M., & Teeri, T. T. (1996). The cellulose-binding domain of the major cellobiohydrolase of trichoderma reesei exhibits true reversibility and a high exchange rate on crystalline cellulose. *Proceedings of the National Academy of Sciences of the United States of America, 93*(22), 12251-12255.

Liu, G., Zhang, J., & Bao, J. (2016). Cost evaluation of cellulase enzyme for industrial-scale cellulosic ethanol production based on rigorous aspen plus modeling. *Bioprocess and Biosystems Engineering, 39*(1), 133-140.

Mandels, M., Kostick, J., & Parizek, R. (1971). (1971). The use of adsorbed cellulase in the continuous conversion of cellulose to glucose. Paper presented at the *Journal of Polymer Science: Polymer Symposia, , 36*(1) 445-459.

McCleary, B. V., & McGeough, P. (2015). A comparison of polysaccharide substrates and reducing sugar methods for the measurement of endo-1, 4-β-xylanase. *Applied Biochemistry and Biotechnology, 177*(5), 1152-1163.

Nillesen, P. (2014). *Ministerie van economische zaken; prijsvergelijk elektriciteit.* (). Retrieved from http://www.rijksoverheid.nl/ministeries/ez/documenten-en-publicaties/rapporten/2013/10/03/prijsvergelijk-elektriciteit-nederland-duitsland.html

Puls, J., Wilson, S. A., & Hölter, D. (2011). Degradation of cellulose acetate-based materials: A review. *Journal of Polymers and the Environment, 19*(1), 152-165.

Roest, K. (2018). *Finescreen supported biological wastewater treat ment to enhance plant capacity.* (Report No. 11).Executive Agency for Small and Medium-sized Enterprises.

Ruiken, C. J., Breuer, G., Klaversma, E., Santiago, T., & van Loosdrecht, M. C. M. (2013). Sieving wastewater - cellulose recovery, economic and energy evaluation. *Water Research, 47*(1), 43-48.

Schade, W., Doll, C., Maibach, M., Peter, M., Crespo, F., Carvalho, D., . . . Afraz, N. (2006). COMPETE final report: Analysis of the contribution of transport policies to the competitiveness of the EU economy and comparison with the united states.

Thermo Fisher Scientific. (2018). Dialysis methods for protein research. Retrieved from https://www.thermofisher.com/nl/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/dialysis-methods-protein-research.html

Tomme, P., TILBEURGH, H., PETTERSSON, G., DAMME, J., VANDEKERCKHOVE, J., KNOWLES, J., . . . CLAEYSSENS, M. (1988). Studies of the cellulolytic system of trichoderma reesei QM 9414. *The FEBS Journal, 170*(3), 575-581.

Towler, G., & Sinnott, R. K. (2012). *Chemical engineering design: Principles, practice and economics of plant and process design* Elsevier.

van der Zeijden, P. T., Muizer, A. P., Braaksma, R. M., & Pasaribu, M. N. (2009). *Industriewater in nederland.* ().

Vynios, D. H., Papaioannou, D. A., Filos, G., Karigiannis, G., Tziala, T., & Lagios, G. (2009). Enzymatic production of glucose from waste paper. *Bioresources, 4*(2), 509-521.

Wouters, H., Euverink, G. J., Neef, R., van Opijnen, E., & Poiesz, W. (2017). *Cellulose assisted dewatering of sludge, research objectives and business case.* (). 10.13140/RG.2.2.10936.75528: Research Gate.

Zaitegui Pérez, D., Barel, S., Capkovicova, A., Hélaine, S., Lanos, B., Londero, P., . . . Schagen, M. (2017). *Eu agricultural outlook, for the agricultural markets and income 2017-2030.* ().European Commision, Agriculture and Rural Development.

Appendix A: Market evaluation data

Data market evaluation

Population of the Netherlands (CBS)	**2015**	**16940000**	
	2030	17836800	
Total domestic wastewater (2015)		1.96E+12	L
per person		115541	L
Toilet paper use per person		13.0	Kg
TSS		0.242	g/L
Percentage cellulose in TSS		47%	
Total cellulose in waste water	2015	0.221	Mt
	2030	0.232	Mt
Retrievable cellulose with finescreen	2015	0.088	Mt
	2030	0.093	Mt
Potential glucose production	2015	0.098	Mt
	2030	0.103	Mt
Potential market size 2030	low	2.2	Mt
	moderate	3	Mt
	high	4	Mt

Appendix B: Chemical Plant design constants and data

Stream specifications

		Product stream	Glucose stream	Recycle stream *	Sweep liquid**
Glucose	wt%	90%	5%		
m solution	m3	24997709	449958756		
Volume	m3	2499.77	427461		
Volume per day	m3	6.85	1171	1164	6.85
Flow	m3/hr	0.86	146	146	0.86
	L/s	0.24	40.66	40.43	0.24
***Equal to Glucose stream-Product stream **Equal to product stream**					

Installations factors per equipment (Hand, 1958)

Equipment type	Installation factor
Heat exchangers	3.5
Instruments	4
Pressure vessels	4
Pumps	4

Costs constants for equipment costs estimation (Towler & Sinnott, 2012)

	Size low	Size up	unit	a	b	n
Pump	0.2	500	L/s	3300	48	1.2
Evaporator	0.5	12	m2	29000	53.5	0.6
Tank	100	10000	m3	53000	2400	0.6
Propeller	5	75	kW	4300	1920	0.8
Double pipe heat exchanger	1	80	m2	500	1100	1

Equipment purchase costs and installation costs

Evaporator	Price	€ 29,081.09		
	Installation costs	€ 101,783.82		
Condensor	Price	€ 2,700.00		
	Installation costs	€ 9,450.00		
Tank (reactor)	Price	€ 560,280.30		
	Propellor	€ 61,761.86		
	Membrane	€ 17,559.05		
	Total	€ 639,601.21		
	installation costs	€ 1,078,383.17		
Tank product	Price	€ 226,123.99		
	installation costs	€ 565,309.97		
			Flowrate (L/s)	# pumps parallel
Pump 1	Price	€ 305,731.60	143	14
Pump 2	Price	€ 3,308.56	0.24	1
Pump 3	Price	€ 7,395.38	40.7	1
Pump 4	Price	€ 7,366.66	40.4	1
	Total	€ 323,802.20		
	installation costs	€ 971,406.61		

Appendix C: Economic Evaluation data

Economic evaluation data

Yearly goal glucose produced (KG)	10124072	kg
conversion	45%	
Cellulose content FSM	47%	
Glucose needed	22497938	kg
FSM needed (KG)	47867953	kg
Enzyme		
Enzyme price	4.486725664	euro/kg
needed	2802	kg
Cost per batch without reuse	€ 12,572.91	
reuse percentage	90%	
Enzyme costs yearly	€ 470,226.67	
E/S loading	2%	
Energy		
Temp during day	13.5	C
Energy needed for heating	85863.14	kWh
Evaporation	290555.31	kWh
Stirrer	204400	kWh
Pumps	12176	kWh
costs	€ 112,668.95	
Materials used		
Water	248397	m3/year
price	0.92	eur/m3
costs	€ 228,525.49	
Sodium acetate	302.92	ton
	500	eur/ton
	€ 134,036.95	
Transport		
price per tonkm	€ 0.18	euro/tonkm
ton FSM per year needed	35901	
km	50	
Waste disposal		
FSM waste	32682	ton
price per ton	€ 43.32	/ton
costs	€ 1,415,778	

Economic evaluation data continued

Labor	
positions	3
operators	14
Wages HSE/month	€ 3,000.00
overhead	60%
costs	€ 806,400.00
Maintenance	
ISBL	2769430.693
percentage	3%
costs	€ 83,082.92
Property Taxes and insurance	
percentage	1%
costs	€ 27,694.31