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Industrial process for the conversion of bread waste into a substrate for the red micro alga Galdieria sulphuraria in the production of Nanoglycogen

S.J.M van Eekert
S2896311
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1st supervisor: Prof. dr. M.J.E.C van der Maarel
2nd supervisor: dr. ing. H. Kloosterman

Abstract

An industrial process for the conversion of bread waste to a substrate for the red micro alga *G. sulphuraria* in order to produce Nanoglycogen was designed. It was aimed to investigate if bread waste can compete with glycerol as a substrate for the *G. sulphuraria.* The influence of the variables reaction time, enzyme concentration and temperature on the hydrolysis process were investigated for economically optimal process conditions. The optimum conditions found are T = 20 ºC, t = 24 hr and enzyme concentration = 0.8 g/kg. This resulted in a glucose solution of 47.8 g/L with an estimated price of € 4.83 per kg glucose, which is 43 times higher than the price of glycerol. It this IP it is concluded that bread waste cannot compete with glycerol as a substrate for the *G. sulphuraria* in the production of Nanoglycogen

Table of contents

Preface

Due to unstainable economic growth, which relies on the exploitation of limited fossil resources, there is searched for new biomass resources. It appears that algae are a good alternative to terrestrial crops due to the high biomass productivity without using the fertile agricultural land. Therefore, competition with other crops is eliminated (Posten & Chen., 2016; Dismukes et al., 2008). Alga can be used, directly as a food source (Radmer, 1996) or as a source for biodiesel (Wijffels and Barbosa, 2010). Some alga produces high-value products like pigments and polysaccharides (Borowitzka, 2013). One of these high value end products is Nanoglycogen produced by the *Galdieria sulphuraria* (Martínez García, 2017). It is aimed by the research group Aquatic Biotechnology and Bioproduct engineering (University of Groningen) to produces Nanoglycogen on industrial scale. In order to commercialize Nanoglycogen research is done on different growing substrates for the *G. sulpuraria*. The *G. sulphuraria* can grow on glycerol (Martínez García, 2017). Glycerol is a residual product of the biodiesel industry and purchase costs are around €0,11 kg. Bread waste arise as new promising substrate after research of Sloth et al. (2017). Before the bread waste can be used as a substrate it has to be pretreated. At the moment, there are no insights into the costs of using bread waste as substrate for the *G. sulphuraria.*

It is asked if bread waste can compete with glycerol on an industrial scale as a substrate for the *G. sulphuraria* to produce Nanoglycogen. To answer this question lab research is done to investigate the influence of the variables enzyme, temperature and reaction time on the hydrolysis process of bread waste. Next, an industrial design was proposed, including procurement and production, based on this design a model was built to optimize the glucose production with the lowest possible production costs.

Chapter 1 will discuss the research design and will address among others the problem the research problem, the system description, and research questions. Chapter 2 explores the influence of the different variables on the hydrolysis process of bread waste into glucose. In chapter 3 an industrial design is proposed. Based on this design and the results of chapter 2 the glucose production , and the production costs are estimated. The estimation is done by optimizing a model from the design process. Chapter 4 gives a total cost analysis. A price of € 4.83 per kg produced glucose is found, which is 43 times more expensive than glycerol. From this result, there is concluded that bread waste cannot compete with glycerol as a substrate for the *G. sulphuraria.*

Chapter 1: Research Design

In this section, an introduction is given with reason and back ground on the research topic. Next the research problem is given analyzed by the use of a system description, causal diagram and a stakeholder analysis. From this the research goal is defined and the main research question is formulated.

1.1 Introduction

Due to unstainable economic growth, which relies on the exploitation of limited fossil resources, there is searched for new biomass resources. It appears that algae are a good alternative to terrestrial crops due to the high biomass productivity without using the fertile agricultural land. Therefore, competition with other crops is eliminated (Posten & Chen., 2016; Dismukes et al., 2008). In the past years, algae were used directly as a food source for the production of polysaccharides (Radmer 1996) or as a source for biodiesel. Although lately biodiesel production from algae was in the center of attention (Wijffels and Barbosa, 2010; Miao & Wu 2006). Algae can produce a wide range of high-value products like pigments, polysaccharides, and polyunsaturated fatty acids. (Borowitzka, 2013).

Despite, these promising features some difficulties are encountered during cultivation of the algae. Most microalgae species are full phototrophs, and their growth is inextricably linked to the availability of light (Martínez García, 2017). Mass cultivation of these microalgae can be done in open ponds or closed photobioreactors with artificially supplied light. (Lee et al., 2001). These cultivation methods come with high costs. Usually, this type of microalga cultivation is associated with low biomass yields and the processes are not economically feasible (Borowitzka, 1992). Heterotrophic cultivation of alga is much more attractive. During Heterotrophic cultivation cells are grown in the dark using an organic compound as carbon and energy source. This cultivation method results in much higher biomass yields and is, therefore, more cost-effective (Perez-Garcia et al., 2011).

Only a few algae can grow heterotrophic. Among this alga, the red microalga *Galdieria sulphuraria* is found. The *G. sulphuraria* grows acidophilic at pH 2 and can grow on a wide range of carbon sources including monosaccharides, sugar alcohols, organic acids and amino acids. (Gross & Scharrenberger, 1995).

The research group Aquatic Biotechnology and Bioproduct engineering at the University of Groningen is researching the high-value end products that can be extracted for the *Galdieria sulphuraria.* One promising carbohydrate found is highly branched glycogen, named Nanoglycogen (Martínez García, 2017).

Nanoglycogen

Many organisms use polysaccharides as a way of storing cellular energy**.** The most commonly used storage polysaccharides in nature are starch and glycogen. Land plants and green algae mainly produce starch. Glycogen can be found in bacteria, yeast and animal muscle and liver cells. Starch and glycogen are both built from O-linked glucose units but differ in structure. Starch is composed of the polymers amylopectin and amylose. Amylopectin is a branched polymer and amylose a linear polymer.

When glycogen is compared to amylopectin (figure 1.1), they look very similar. Both have α-(1-4) joined glucose with branched attached through α-(1-6) bonds (Martínez García, 2017). Compared to amylopectin the glycogen has a greater proportion of branching linkages 7-18% depending on the source it comes from (Martínez García Stuart, & van der Maarel, 2016). The short side chains result in a water-

soluble conformation that is different from that of starch (Manners, 1991a). This conformation gives the molecule an industrial advantage. The molecule is soluble in cold water and easy accessible for enzymes. This could lower production costs associated with the pre-treatment of starch. At the moment the use of glycogen is limited because micro-organism that accumulated glycogen under condition of limiting growth (Preiss, 1984). Now *the Galdieria sulphuraria* is found as new glycogen producer (Martínez García Stuart, & van der Maarel, 2016). The glycogen from the *Galdieria sulpuraria* is highly branched and has very short side chains with a DP 4-10 (Martínez García et al., 2016) and therefore is called Nanoglycogen (figure 1.2). The production of Nanoglycogen on industrial scale becomes more interesting when more research is done on the applications of Nanoglycogen.

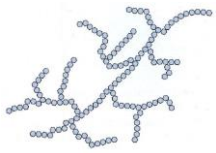

Figure 1.1: structure of amylopectin

Figuur 1.2: Nanoglycogen

Application of Nanoglycogen

Nanoglycogen can be used as slowly digestible carbohydrate in sports drinks or as an osmotic agent in peritoneal dialysis. The advantages of Nanoglycogen used in sports drinks compared to other carbohydrates is their slower degradation by digestive enzymes which leads to a more gradual glucose concentration appearance in the bloodstream and lower insulin response (Takii, Ishihara, Kometani, Okada, & Fushiki, 1999). The branched glucose polymers have a negligible contribution to the osmotic value due to their high molecular weight, even at high concentrations. Therefore, it can be used in hypotonic sports drinks (Takii et al., 2005). There is already a commercialized product Cluster Dextrin widely sold as quickly absorbed but slowly metabolize carbohydrate ingredient that provides a constant energy supply during exercise (Martínez García, 2017). The price of cluster dextrin is estimated on €28 /kg (appendix A)

In the case of peritoneal dialysis, the NanoGlycogen can be used as an osmotic agent instead of glucose. The advantage over glucose is that the NanoGlycogen is not readily absorbed in the bloodstream creating a long-lasting osmotic gradient (Martínez García, 2017). Baxter Healthcare produces a commercially available peritonea dialysis solution, Extraneal®, which also caused an long-lasting osmotic gradient. The solution contains Icodextrin, which is a glucose polymer with less than 10% of α 1-6 linkages (Moberly et al., 2002). The estimated kg prices of Icodextrin is €371 (appendix A)

Commercialization of Nanoglycogen

As the properties of glycogen are very promising, it is aimed by the research group to commercialize the product. Commercialization of glycogen can only be done if the value of the end product exceeds the production costs. To lower the production costs research is done on different growing substrates for the *G. sulphuraria* end the corresponding Nanoglycogen accumulation.

A promising substrate is glycerol (Martínez García, 2017). The *G. sulphuraria* can grow on pure and crude glycerol if an extra nitrogen source is added (Martínez García, 2017). In both cases the alga produces glycogen. Glycerol is a major byproduct in the biodiesel production process, which is yielded at

10 wt% of the biodiesel production (X. Fan, 2010). The global biodiesel market is estimated to have a size of 37 billion cubic meters in 2016, which comes to 3.7 billion gallons of crude glycerol a year (L. Wang et al, 2006). The produced glycerol is expensive to purify for use in pharmaceutical, cosmetics and foods industry, there is sought to alternative methods for it's disposal. The market is flooded with excessive crude glycerol. As a result, is sold for €0,05 – 0,11 / kg (Johnson and Taconi, 2007).

Bread waste as a substrate for the *Galdieria sulphuraria*

Due to an inspiring article of sloth et al., 2017, where the G. sulphuraria is grown on food waste from restaurants and bakeries to produce pyocyanin, using food waste a substrate in the commercialization of Nanoglycogen is considered as a new possibility

Food waste discharged from restaurants, households and the food industry is accounted for 2 billion kilos per year in the Netherlands (nos.nl). The environmental consequences of this are unforeseeable. Food waste now accounts for more than one quarter of the total freshwater consumption and ~300 million barrels of oil per year (K.D Hall and J. Guo, 2009). However, this waste is a rich source of nutrients, e.g., soluble sugar, starch, lipid, proteins for microbial growth (Moon, 2011). Therefore, it is interesting to search for new recycling possibilities.

In this report, there is looked explicitly at recycling bread waste by the use of the G. sulphuraria. The Galdieria is not able to use the bread waste material immediately as a substrate. Therefore the waste needs to be hydrolyzed. Hydrolysis is performed with the commercially available enzyme Stargen^{tm} 002 produced by Dupont.

Bread waste consists mainly out of water (46 %) carbohydrates (43 %) and proteins (9.5 %) (ah.nl). The starch needs to be broken down into glucose molecules. This process is very similar to the fermentation process for bioethanol production.

Borgesius

A large bread waste producer in the Netherlands is Borgesius, an industrial bakery who sells bread to restaurants and supermarkets. The unsold bread is taken back by the bakery; this is called return-bread. The return-bread is recycled and turned into cattle feed (figure 1.3). The previous years the regulations among cattle feed have become stricter (European Commission of Food and Feed Safety). The bread does not contain animal remains, wherefore the coming years no significant consequences about the cattle feed regulations will occur. Although borchesius is interested in new methods to recycle bread waste into valuable end products, because this increases the breadwaste value.

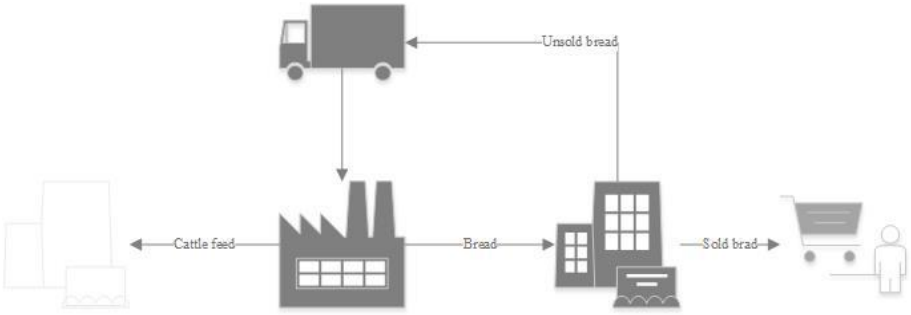

Figure 1.3 The bread cycle

1.2 Problem definition

It is aimed by the research group to commercialize Nanoglycogen extracted from the *Galdieria sulphuraria* and to produce this on an industrial scale. The *Galdieria sulphuraria* can grow on a wide range of substrates (Gross & Scharrenberger, 1995). It is known that the *Galdieria sulphuraria* can grow on glycerol and under these conditions accumulates Nanoglycogen. In the search for feasible substrates, bread waste appears. The bread waste cannot be used immediately as a substrate and has to be hydrolyzed into glucose molecules.

Before the waste can be used on industrial scale to produce Nanoglycogen more insights are needed in the hydrolysis process. Also, the design of the industrial hydrolysis process of bread waste is unknown. Therefore, no insights into the production costs are available, which are needed to indicate if bread waste can compete with glycerol as a substrate for the *Galdieria sulphuraria* in the production of Nano glycogen.

The problem described above can be considered as a technical problem. The technical problem consists of designing an industrial process for the hydrolysis of bread waste. No social problem can be identified since it consists only of a problem in which the research group is involved, without conflicting other parties.

1.3 Problem owner

The research group Aquatic Biotechnology and Bioproduct engineering is identified as problem owner. The research group has high influence in solving the problem in the search for substrates that can be used on an industrial scale for the growth of the *Galdieria sulphuraria*, by researching different substrates. However, the group has moderate interest in the solution because the researchers will not immediately have an advantage from the invention, except brand awareness.

1.4 Problem Context

To get a more in-depth sight in the problem context an overview of the Nanoglycogen production based on bread waste is given in (figure 1.4). First, the bread is hydrolyzed, and a glucose solution is produced. This glucose solution is used to grow the *Galdieria sulphuraria*. Next, the glycogen is extracted from the *Galdieria sulphuraria.*

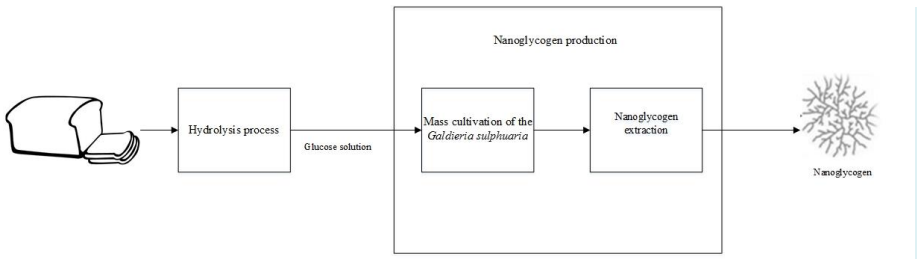

Figure 1.4: Overview of the Nano glycogen production with bread waste as a substrate

Met opmerkingen [IB1]: Misschien nog in 1 zin nog duidelijk wat nu het probleem is. Als dat niet kan dan gewoon zo laten

Met opmerkingen [IB2]: Best wel duidelijk allemaal! Loopt echt heel veel beter in elkaar over dan vorige keer. Alleen omdat je de figuren nog niet hebt genoemd een beetje onoverzichtelijk. Ik weet nog niet helemaal welk je beter kan laten zien, want hier doe je het groteren en later zoom je in. Ja is denk ik wel beter zo. Maar eerst was het me nog niet heel duidelijk dat je het juist alleen over hydrolysis ging hebben en niet over de rest. Maar later was dat wel helemaal duidelijk

More insights into the bread waste hydrolysis process need to be created, to indicate if bread waste can compete with glycerol as a substrate for the *Galdieria sulphuraria*. The hydrolysis process is a subsystem of the Nanoglycogen production. The problem is considered an instrumental problem. The hydrolysis process that needs to be designed can be considered as the instrument leading to a glucose solution as an output. Efficacy of a system is the ability to achieve a goal, in this case, the contribution to the higher lever system the Nanoglycogen production. The hydrolysis system only contributes to the goal Nanoglycogen production when it produces a useful substrate for the *Galdieria sulphuraria*. Otherwise the system is useless. It is known that the *G. sulphuraria* can grow on bread waste (Sloth et al., 2017). However, it is assumed the *G. sulphuraria* produces Nanoglycogen if bread waste is used as a substrate, which is not investigated yet.

1.5 System description

The larger system for the production of Nanoglycogen from bread waste material is shown in figure 1.4. First, the bread waste is hydrolyzed, and a glucose solution will remain. Next, the *G. sulphuraria* will be grown on the glucose solution, and the glycogen will be extracted. The *G. Sulphuraria* is considered as a black box. It is not known precisely how the G. sulphuraria produce the glycogen. The understanding of this phenomena is beyond the scope of this project.

It is assumed that the growing and the extraction procedure for the substrates, bread waste, and glycerol, will be comparable and will have equal costs. The difference in economic feasibility will be accused by the prices of the growing substrate and the Nano-glycogen yield for the different substrates.
To narrow the scope of this integration project, there is chosen to focus on the optimization of the hydrolysis of the bread waste to glucose on an industrial scale. With the underlying idea the more glucose is produced, the more the alga can grow, and the more glycogen is produced. A detailed system description of the hydrolysis process is given in figure 1.5

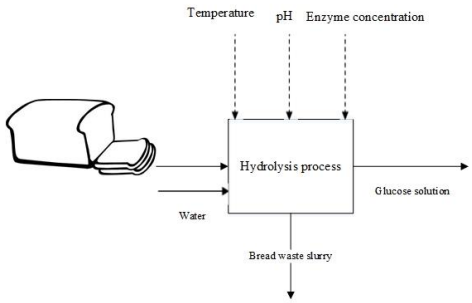

Figure 1.5: system description of the nano glycogen production with bread waste as a growing sub-strate

Bread and water are the inputs of the process. The output, glucose solution, is influenced by the variables pH, temperature and enzyme concentration. The other output stream is bread waste slurry. This slurry is considered waste.
is

1.6 Causal diagram

A causal diagram is implemented to get a more in-depth insight into the problem. In a causal loop diagram (figure 1.6) variables are denoted by arrows denoting the causal influences among the variables. A polarity (+/-) is assigned and may negative or positive. If the cause increases the effect also increases, then this is a positive link. A negative link follows the inverse principle (J.D. Sterman, 2000). It is important to underline that link polarities describe the structure of the system, but not necessarily the actual behavior of the variables. In the diagram, essential variables are identified for Nanoglycogen production from bread waste.

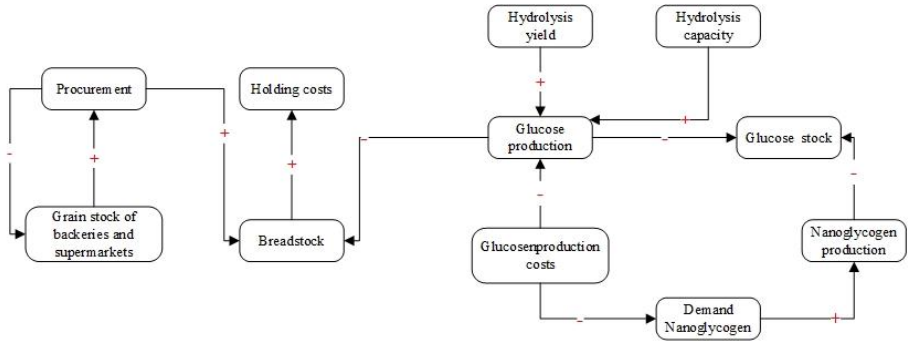

Figure 1.6: Causal loop diagram for glucose production from bread waste

From this, the glucose production costs and the glucose production are identified as the most important variable for the Nano glycogen production. In addition, the relevance of the procurement becomes clear. Without procurement, there is no bread stock and no glucose can be produced.

1.7 Stakeholder analysis

For the problem analysis, the stakeholders for the system at hand are chosen. A stakeholder of the problem is a person, group of persons or an institution affected by treating the problem (Wieringa R.J., 2014). Each stakeholder has different interests and influence on the problem. The following stakeholders for the defined problem were indicated: Research group Aquatic Biotechnology and Bioproduct technology, University of Groningen, Borgesius bakery and sports drink producers. The stakeholders are represented in figure 1.6.

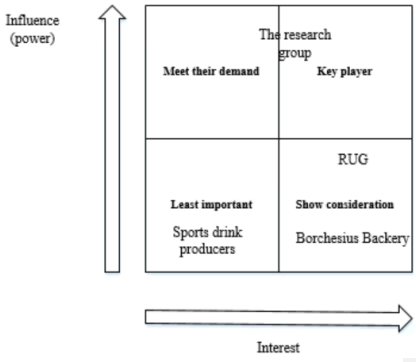

Figure 1.6 : Stakeholders categorized according to influence and interest

Research group Aquatic Biotechnology and Bioproduct engineering: The research group aims to evaluate the production of Nanoglycogen made of hydrolyzed bread waste material. They are willing to develop new applicable technologies. The researchers have high influence in solving the problem because it is their research. The research group also has little interest in solving the problem. Even so, when the problem is solved, they will not immediately affect by it.

University of Groningen: The University of Groningen is funding the project. Therefore, they have interest in new insights coming from the project. The university aims to conduct high minded and changing research. Secondly, they can make a profit on the inventions by selling them as patents.

Sports drink producers: The production of Nanoglycogen gives new possibilities for sports drink producers. The Nanoglycogen is different from the current available carbohydrates and sugars on the market. In addition, they could sell their product under a green label. Sports drink producers know about the new possibilities of nano glycogen. However, they have low interest because it is unknown if the nano glycogen can be produced at industrial scale. Also, they have low influence in the research because they are not involved.

Borgesius, industrial bakery: Borgesius is the supplier of the bread waste material. Nowadays the return-bread is sold to the cattle feed industry. Borgesius is of opinion al the bread they produce need to be eaten. Borgesius is interested in the project because new ways to recycle bread waste gives more value to the waste.

1.8 Goal and scope

The main goal of the project is to indicate if bread waste can compete with glycerol as substrate for the *G. Sulphuria* in the production of Nanoglycogen. This goal can be divided in a knowledge goal and design goal. First, the effect of the variables pH, temperature and enzyme concentration on hydrolysis of bread waste needs to be investigated which is seen as the knowledge goal. In addition, an industrial process for the hydrolysis of bread waste needs to be designed including the logistic process and a cost analysis, which is the design goal. In the industrial design it is aimed to minimize the production costs. The scope contains both the recycling sector and the sports drink or medical sector.

1.9 Research and project design

In this integration project, the rigor cycle and the design cycle from Hevner (Hevner, 2007) will be used. First, the rigor cycle is applied during lab scale experiment. During the experiments, the effect of the variables pH, temperature, and enzyme concentration on the hydrolysis of bread waste are aimed to be determined. Information on bread waste is gained from interviews.

Next, the information gained from the rigor cycle will be used to design an industrial process, which minimizes the costs of hydrolyzing. In this process, mathematical modeling in excel is used to describe the process. Based on this design a cost analysis is made to indicate the production costs. This information will be used to make a comparison between the different substrates. As validation of the model, the *G. sulphuraria* is grown on the output of bread hydrolysis process.

From the set goals in the previous section the following research questions arise:

Main research question

Can on industrial scale hydrolyzed bread waste, be used as a substrate for the G. Sulphuraria, compete in an economic way with glycerol in the production of Nanoglycogen?

Knowledge questions

-What are the effects of the variables time, enzyme concentration and temperature on hydrolysis of bread waste using Stargen 002™?
- What is the economically optimum industrial design of the hydrolysis process of bread waste?
- What are the production costs of the hydrolysis of bread waste?

Met opmerkingen [IB3]: Deze komt uit het niets opeens, misschien kan je dit al even eerder noemen als je de definitie hiervan noemt?

Chapter 2: Hydrolysis of bread waste by the use of Stargen 002^{tm}

2.1 Introduction

In the search for new ways to recycle biomass the algae appear. One of these interesting algae is the *Galdieria sulphuraria*. Different starting materials for the *G. sulphuraria* are already investigated. The *G. sulphuraria* can grow on glucose (Martínez García, 2017) on sugar beet mass (Schmidt et al.,2005), effluents from waste water treatment plants (Selvaratnam et at., 2014) and on food waste from restaurants and bakeries (Sloth et al., 2017).

In this research, there is singularly focused on bread waste. Bread waste consists mainly of starch (50 %) and proteins (9%) (ah.nl). The starch content is hydrolyzed into glucose molecules, with the use of the enzyme Stargen 002^{tm} (figure 2.1). Stargen^{TM}002 is a particular starch hydrolyzing enzyme blend produced by DuPont. Stargen^{TM}002 is capable of hydrolyzing raw starch granules through a synergistic action by an acid α-amylase and a glucoamylase from *Trichoderma reese*i (J.M.M. Adams et al., 2012; Dupont Stargen^{TM} information sheet). At the moment no research papers have been identified on Stargen^{TM} 002. The yield of conversion with Stargen depends on the variables time, glucose concentration and temperature (Dupont). It is aimed to understand the influence of the variables: temperature and enzyme concentration on the starch conversion. The final goal of this research is to provide information for the hydrolysis of bread waste on an industrial scale.

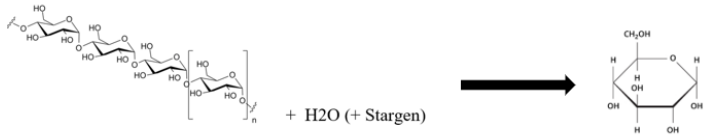

Figure 2.1 The hydrolysis of starch

2.2 Methods

Characterisation and preparation of the bread waste

Dry weight of the bread waste was determined by freeze-drying the material for 72 hours. The dry weight measurement was carried out three times. The known composition (appendix B) of the wet weight material is used to calculate the composition of the dry weight material.

To prepare the bread samples, 150 g (wet weight) of bread (whole grain bread, COOP) was suspended in 1 L demineralized water and was acidified with HCl to PH 4. After that, the suspension was autoclaved for 20 minutes at 121 ^{o}C and divided into portions of 50 grams in 500 ml flasks.

Hydrolysis and measurements of the glucose concentration

Hydrolysis of carbohydrates in the bakery was carried out by the use of different concentrations Stargen^{TM} 002 (gram Enzyme/ kg wet weight bread) and 20, 30 and 40 temperatures. Samples were taken regularly during hydrolysis and analyzed for the presence of glucose by the use of the GOPOD reagent. The GOPOD reagent was prepared according to the instructions of Megazyme. 300 µl of GOPOD and 10 µl of sample were incubated for 20 minutes at 50 ^{o}C degrees. The glucose concentration was measured as absorbance at 510 nm on a spectrophotometer (Spectramax Plus 384). Each analysis was measured in fourfold. The yield is the percentage of starch converted into glucose (eq. 1)

$$Yield = \frac{Glucose\ (g/l)}{Starch(g/l)} * 100\% \quad (1)$$

Influence of the parameters enzyme concentration, temperature and reaction time on the glucose yield.

To estimate the influence of the enzyme concentration, the temperature and the reaction time on the glucose production all the data of the measuring points were filled in Excel 2016. The glucose concentration was seen as the depended variable. Temperature, reaction and enzyme concentration were seen as independent variables. Regression analysis in excel was applied using the data. A 95% confidence level was used, and the line was forced to 0. The influence of the variables can be described by equation 2. A linear relation is assumed between the glucose concentration and the variables temperature, reaction time and enzyme concentration. Because the solution has an average starting concentration of 5,2 g glucose/liter, it is decided to run multivariable regression from 900 to 84200 s, and the starting concentration is considered constant.

$$glucose\ concentration\left(\frac{g}{l}\right) = 5{,}2 + a * temperature\ (K) + b * reaction\ time\ (s) + c *\ enzyme\ (\frac{g}{kg}) \quad (2)$$

Indication of industrial properties of the bread waste mixture

The density of the reaction mixture was determined by weighing 100 ml of the reaction mixture. A size cylinder was used to measure the volume of the bread waste mixture. The weight fraction of the output after distillation was determined by centrifuging reaction mixture samples. The supernatant was removed, and the slurry part was weighed.

2.3 Results and discussion

Dry weight and composition

Bread waste consisted of regular whole grain bread. The dry matter content was 54,7% (w/w) of which was 79,5 % was dry organic matter. 92,1% of this dry organic matter was known as starch 2,5 % sugars and 3.9 % as lipids. When there is assumed all the starch present can be converted into glucose and a weight increase of 11,5 % [2], due the addition of water during hydrolysis is taken into account, a maximum concentration of 85 g/L can be reached.

Bakery waste hydrolysates

The results of the hydrolysis over time at respectively 20, 30 and 40 ^{o}C are shown in figure 2.2, 2.3.The standard deviations can be found in Appendix C. First, an excess of the enzyme was used to determine the highest glucose yield possible for each temperature. The enzyme concentration was 30 times higher than the advised enzyme concentration by Dupont. Next, the enzyme concentrations were lowered to find an optimum. After 24 hours of hydrolysis, the process was considered as being completed. At 20 ^{O}C the lowest yields were achieved (table 2.1). The lower concentrations (0,8g/kg; 1,2 g/kg and 1.6 g.kg) kept increasing over time, while the mixtures with a higher concentration of enzyme show a faster increase in glucose in the beginning. At 30 ^{o}C and 40 ^{o}C higher yields were achieved, all concentrations kept increasing over the full 24 hours. From the mixtures at 30 ^{o}C and 40 ^{o}C can be concluded that a higher enzyme concentration leads to a higher yield. This cannot be concluded from the experiments at

20 °C, which is probably too low for the proper working of the enzyme. Also, it was found the higher the temperature, leads to higher yields.
The yields after 24 hours are shown in figure 2.4.

In a recent study of Sloth et al. (2017,) at 50 °C and application of stirring higher glucose concentrations of 75 g/L were found. Other studies which used Stargen™ found a conversion of cassava starch of 33 % (H. Hargono, 2018) and conversion of maize between 40 – 80 % (J. Adams, 2012) at respectively 30 and 32 °C. These results are comparable with the results shown figure 2.4

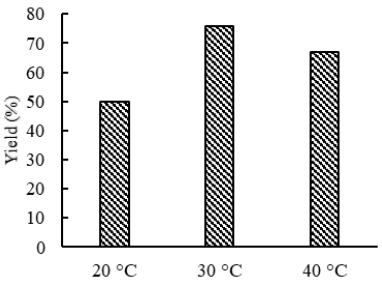

Figure 2.4: Glucose yield after the hydrolysis of bread waste after 24 hours

The maximum glucose concentration found is 65 g/L (48 g/kg, 30°C)

At the moment the experiment was considered finished, the bread mixture had retained its brown color. Therefore it is concluded that not all starch is hydrolyzed and some starch cannot be hydrolyzed.

During the experiment different essential variables were recognized for the process which accuses deviations in the measurements. First, the way of grinding the material has a high influence on the sampling. Second, the samples are not homogenous because no continuous stirring was applied during the experiments as it was seen in (Sloth et al., 2017) were higher yields were achieved.

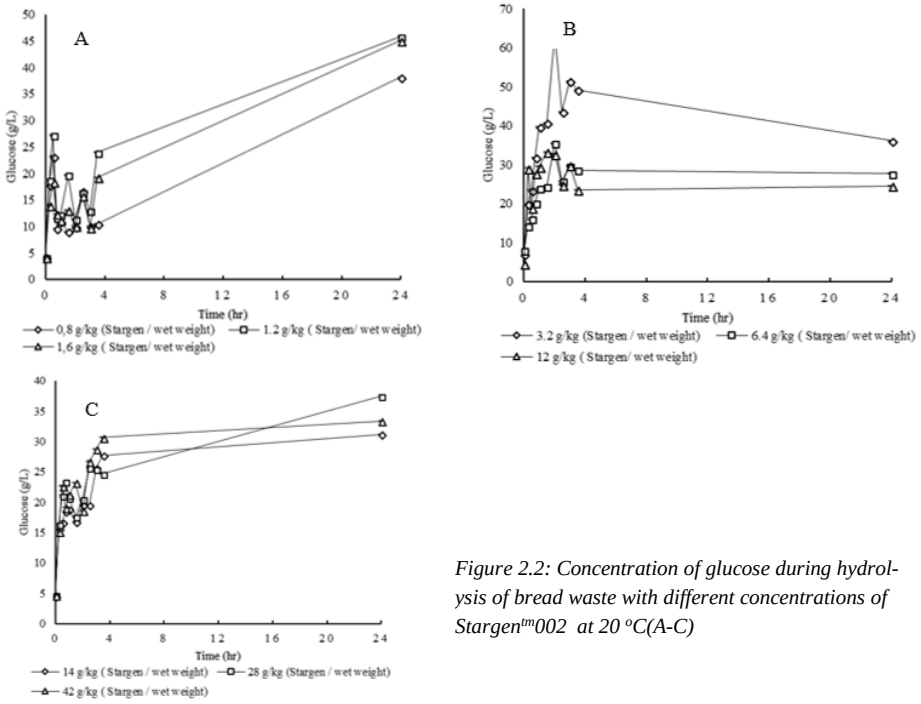

Figure 2.2: Concentration of glucose during hydrolysis of bread waste with different concentrations of Stargen^{tm}002 at 20 °C(A-C)

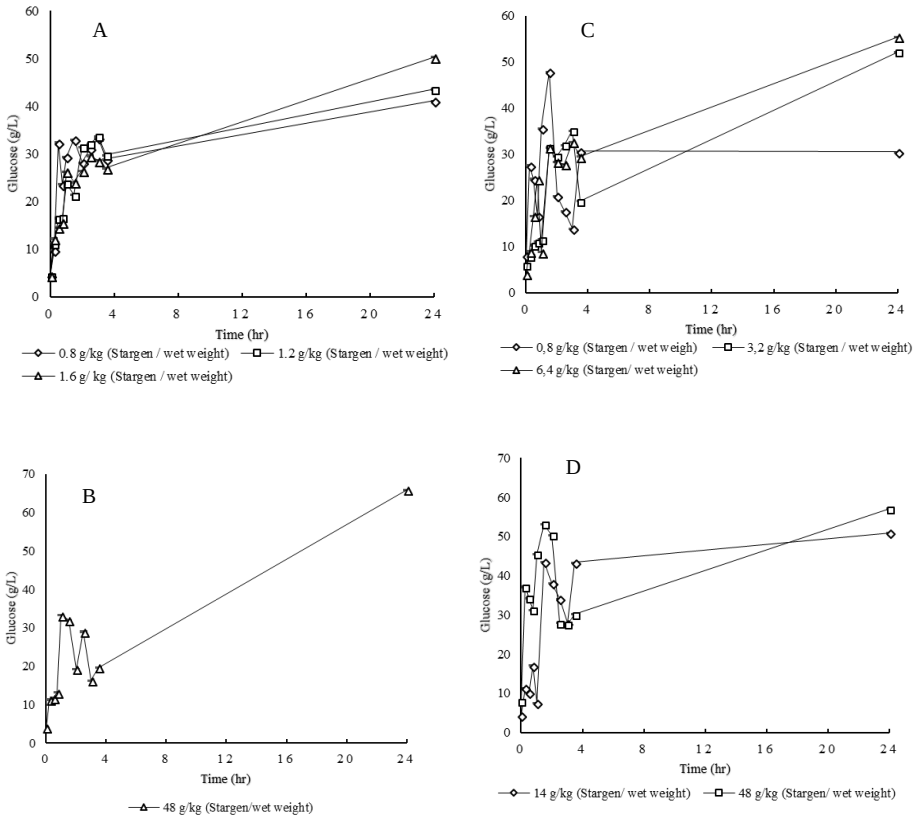

Figure 2.3: Concentration of glucose during hydrolysis of bread waste with different concentrations of Stargen™ 002 at 30 ºC (A-B) and 40 ºC (C-D)

Multivariable regression

The results of the multivariable regression are shown in table 2..1 Based on the Dupont information sheet an linear relationship is assumed. The linear regression gives an R square value of 0.870 which means that 87% of the variation is caused by the variables time, enzyme and temperature. The model can be considered significant based on the p-value of the F test which is way below 0.05.

R square	0.870	
Significance F	$2.1*10^{-102}$	
	P-value	**Coefficient**
Temperature (a)	$9.09*10^{-18}$	0,000263
Reaction time (b)	0.000535	0,144884
Enzyme (c)	$1*10^{-56}$	0,067519

Table 2.1 Result multivariable regression on the variables temperature, reaction time and enzyme concentration

Al the P values of the independent variables are below 0.15 which allows working with the coefficients to predict the glucose concentration on industrial scale eq 2. Reaction time and Enzyme concentration have the largest coefficient what means they have the most influence on the glucose concentration.

Industrial parameters

For the application and energy calculation, information on the volume and density of the reaction mixture is needed. A density of 1062 kg/m^3 was found, and the increase in volume after adding the bread is 11.3 %.

2.4 Conclusion

The conversion of starch from bread waste depends mainly on the variables of temperature, reaction time. In general, a higher the temperature in the range of 20 – 40 ^{o}C, a longer reaction time, and a higher enzyme concentration leads to higher conversion. Conversion yields of 76% (30 ^{o}C) and 67% (40^{o}C) are found after 24 hours.

Chapter 3: Industrial design

The overall scope of this project is to evaluate the efficiency of an industrial process to produce glucose from bread waste on an industrial scale with the final aim to use it as a growing substrate for the Galdieria sulphuraria. Efficiency is evaluated by total the production costs per kg glucose. In this section, the procurement and the variable production costs of glucose production from bread waste on an industrial scale are calculated.

The calculations are done by designing an industrial scale production process based on the lab experiments in chapter 1. A model is built, which relates the glucose production to the production costs to minimize the variable costs during the production process. Based on the optimum bread waste quantity an optimum order policy approached.

First, the logistics are discussed. The logistics involved several stages, among the most important the supplier selection, definition of the order quantity, the purchase frequency of raw material and the inventory control (M.A. Rendon-Sagardi, M.A. Sanchez-Raminez C, 2014). Next, the industrial process design is proposed and optimized.

3.1 Procurement

The process uses bread waste to produce a glucose solution. For a continuous process, an uninterrupted supply of bread waste needs to be guaranteed. The procurement involves several stages among the most importation supplier selection the order quantity, the purchase frequency of the raw material and the inventory control. (M.A. Rendon-Sagardi, M.A. Sanchez-Raminez C, 2014). In this section, there is searched for an optimum order policy to minimize the procurement costs. First, the supplier is selected, and the maximum possible order quantity is defined. Next, an inventory model is proposed according to economic order quantity model. It was found that the transportation costs for bread are too high compared to its value. The minimum cost found per kg bread waste was € 0,84.

3.1.1 Supplier selection

As a supplier, the industrial bakery Borgesius is chosen. The bakery for the region of Groningen is located in Sappemeer. Borgesius daily produce the bread and distributes it over the supermarkets. With each delivery, the unsold bread is taken back to the factory. Only regular bread can be returned to the bakery, products as apple turnovers, sausage, and pizza rolls are not allowed and have to be disposed by the supermarkets. When the returned bread arrives at the bakery, it can be distributed to glucose plant, which will also be located at Sappemeer industrial to reduce transportation costs.

For bread waste, it is considered that the supplier provides 2%-3% of the total production as bread waste (Personal communication Janet Borgesius, Commercial director Borgesius, May 15, 2018), which gives an estimated maximum supply of 720 pieces of bread (554kg/day) and 3360 (184 kg/day) rolls a day (appendix C). The production of the bread can be assumed constant. The price of bread waste can fluctuate due to the fluctuations in the world grain price and the bread waste demand. At the moment al the bread waste is bought by the cattle feed industry (Personal communication Janet Borgesius, Commercial director Borgesius, May 15, 2018).

3.1.2 Definition of the order quantity

The maximum order quantity relies on Borgesius' maximum bread waste production (738 kg/day). This is enough to realize a chemical reactor of 5470 L(appendix D), but relying on the full production comes with several disadvantages. The increased demand for bread waste will cause a price increase. Secondly,

there is no precise information on fluctuations in the production. Therefore, there is chosen to use 25%-75 % of the maximum supply.
The bread waste material has an age of 1 day when it is delivered to the factory and it is assumed that bread cannot stored longer than four days before it decays and is affected by fungi. A mathematical model is built, to determine the optimal order quantity.

3.1.3 Inventory control: The economic order quantity model (EOQ)

To determine the optimum inventory policy the economic order quantity model with lead times is used (Taha H., 2011) The EOQ - model is a deterministic model, which assumes that the inventory level is decreasing over time. Usually, this model is not used for perishable goods. However, the demand is constant. Therefore it can be calculated how long the bread waste is in inventory when FIFO (First in, First out) is applied.
It is assumed the bread waste need to be ordered a week before the delivery, so not all bread waste is automatically delivered to the cattle feed industry. From Janet Borgesius, the commercial director of Borgesius, (personal communication, May 15, 2018) is known that price of unpacking and sorting the bread waste is 0,10 euro per whole bread. This is seen as the purchase price for the bread because the bread waste, in general, has no value. Holding costs (Carrying costs) are estimated at 25% of the purchase price (Taha H., 2011). Transporting costs are estimated at 400 euro per truck (Janet Borgesius, personal communication, May 15, 2018), This includes fuel and driver costs. The truck visits al clients and brings the bread waste to the hydrolysis plant in Sappemeer. It is assumed that a maximum load of one truck is 589 kg bread (Appendix D). When the bread waste exceeds the maximum truckload, then an extra truck needs to be used.

The economic order quantity model relates optimum order quantity (y* = kg bread waste), the set-up costs (k), the holding costs (h) and the demand (D) and is given in equation 3a. The cycle time (t*) is given in equation 3b and depends on the optimum order quantity and the demand. By the use of the t* the effective lead time can be calculated (equation 3c and 3d). The effective lead time is the time between two consecutive orders. The input values for the model are given in table 3.1

Demand (D)	553 kg / day *
Holding costs (h)	€0,02 /day
Setup costs (K)	400

Table 3.1. Input values for the EOQ-model.
**optimum process quantity proposed in industrial process design*

$y^* = \sqrt{\frac{2KD}{h}}$ and Le (3a)

$t^* = \frac{y^*}{D}$ (3b)

$Le = L - nt^*$ if $int(\frac{L}{t^*}) \geq 1$ and $n = int(\frac{L}{t^*})$ (3c)

$Le = L$ if $int(\frac{L}{t^*}) \leq 1$ (3d)

3.1.4 Results and discussion

The EOQ- model gives an optimum order policy of ordering 4206 kg bread every 7.6 days. Unfortunately, this quantity is too large, and the bread cannot be stored for that long. The set-up costs are accusing this large order quantity. Due to the low value of bread it is only feasible to transport the bread in very large quantities. The volume of one truck is 558 kilo and the optimum order quantity is much higher than this volume. Every time, the bread waste quantity exceeds 558 kg an extra truck is needed and the set – up costs (K) increasing with 400 euro.

Therefore, another method is proposed. The procurement costs of the bread, including the set-up costs and the holding costs, are calculated with equation 4. The results are shown in the figure 3.1.The procurement costs decrease when the trucks are filled. The procurement costs increasing when order quantity exceeds the capacity of the truck is from figure 3.1 , there can be concluded that procurement costs are minimal only, when full trucks are used. A new order policy is derived from these results: Every day a batch of 553 kg is ordered, which is almost a full truck and the demand of bread waste per day . It is assumed that the bread is stored for one day. The new procurement costs is calculated according to equation 4. This policy gives a total cost of € 0.74 per kg bread waste.

$$procurement\ costs\ (€) = \frac{\frac{D}{558} * K + D * h}{D} \quad (4)$$

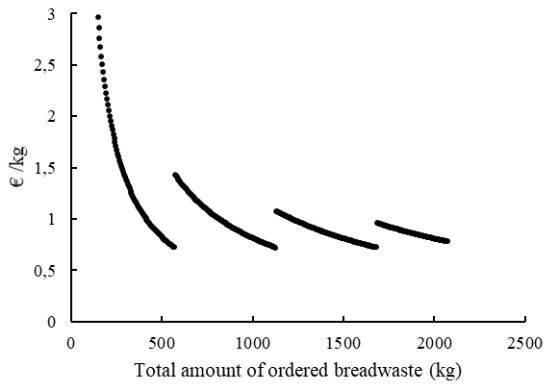

Figure 3.1: Procurement costs per kg bread waste

3.2 Process design

It is aimed to produce glucose from bread waste on industrial scale. In chapter 2 the hydrolysis of bread waste is performed on lab scale. Changes in technology, regulations, availability of materials and prices are making it difficult to simulate the results of a process on industrial scale, although information is available from the laboratory experiments. Only a few studies have focused on scale-up frameworks (F. Piccinno et al., 2016). The overall goal of this section is to develop a scale-up framework for the laboratory process and to find an economic optimum between the reaction circumstances and the bread waste input. Optimization is done by minimizing the variable costs of the production process per kg glucose produced (equation 5). The overall scope of the framework is not to analyze the process into detail, but to analyze the process at a larger scale from a technical perspective, therefore some simplifications are made for the sake of practicality.

$$variable\ cost\ per\ kg\ glucose = \frac{estimated\ glucose\ production}{energy\ costs + raw\ material\ costs} \quad (5)$$

First, a process design is proposed. Next, a model is built concluding all process steps. Whit this model the optimum bread feed, reaction circumstances and reactor dimensions are calculated to minimize the variable costs. It was found that the minimum variable production costs per kilo of glucose are €0.86.

3.2.1 Proposed process design

The experiments performed in chapter 1 can be considered as small batch reactors. Therefore a liquid phase batch process is chosen, which usually takes place in a batch reactor under stirring and if applicable heating or cooling. The batch process is a discontinuous process.
Based on this information the following industrial design is proposed (figure 3.2). First, the bread waste will be ground and transported to the first reactor. This transportation can be done by a conveyor belt or by hand. In this reactor vessel in tank stirring is applied and the suspension is homogenized. The suspension is pumped to the next reaction vessel, where a heated liquid batch reaction in an insulated batch reactor with an in-tank stirrer is applied. The liquid batch reaction is followed by pumping the slurry to the final step centrifugation. It is chosen to exclude the autoclaving from the process design because its very time and energy consuming. It is assumed fungi do not have infected the bread at the moment of processing and that the raw material is used immediately.

The material inputs are bread (reactant), water (solvent and reactant), Stargen 002 (enzyme) and HCl (pH controller) whereas the energy input is mainly required for stirring and heating of the reaction mixture. It is unclear how much HCL needs to be used per batch, because the pH of brown and white bread differs. However, it is known from the lab experiments these amounts are neglectable small. Therefore, the use of HCl is not included in the cost analysis. Furthermore, HCl is relatively cheap (Appendix E) and will not drastically affect the estimated production costs.

The main output of the reaction is a mixture of bread, glucose, water, and enzyme. The final output, after centrifugation, is a glucose solution and a bread waste stream. The glucose solution is used as a substrate. The possibilities for the bread waste slurry will be discussed in one of the following paragraphs. It is chosen to not recycle the heat.

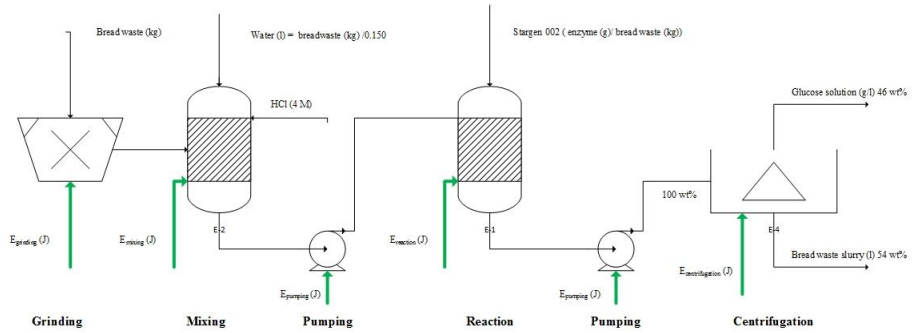

Figure 3.2: Material and energy input for the proposed process design

3.2.2 Process optimization - Method

Input values

The scale-up of each reactant is performed linearly, even the enzyme which is used in excess when concentrations above 1.2 g/kg are used, to ensure the same conversion results. The water is used as a solvent and a reactant at the same time. Also the solvent is scaled up linearly to achieve the same end concentrations achieved in the lab scale experiments.

Enzymes are crucial to the success of the reaction. The effect of the enzyme is affected by temperature and the acidic conditions. The purchase price of enzymes is relatively high, but they are used in small quantities. The enzyme will not be recycled,

The density of the suspension and the volume is calculated in chapter 2. A density of 1062 kg/m^3 was found, and the increase in volume after adding the bread is 11.3 %.

For the overall process, steady-state condition are assumed. Therefore the following mass balance has to be fulfilled after the optimization calculations. The influence of the enzyme and the acid on the mass balance are neglected because of the minimal quantities used compared to the reactant and the solvent.

Bread waste (kg) + water (kg) = Glucose solution (kg) + Bread waste slurry (kg)

Reactor size

The volume of the reactor is normally 10% larger than the volume of the reaction mixture. The volume of the reaction mixture depends on the amount of bread waste used. When the volume is known, the reactor dimensions can be calculated according to interpolating according to known reactions dimension (F. Piccioni et al.,) shown in table 3.2.

Physical entity	**Symbol**	**unit**	**1000 L**	**5000 L**
Reaction mixture Volume	V_{mix}	m^3	1	5
Height of reactor	L	m	1.119	1.913
Diameter of reactor	D	m	1	1.913
Surface area	A	m^2	5.899	17.249
Reactor volume	$V_{reactor}$	m^3	1.1	5.5

Table 3.2: Reactor dimensions (F. Picconno et al., 2016)

Energy Calculations

Reaction
The overall reaction performed is endotherm. The energy needed to produce 1 mole of glucose molecules is estimated at 80 kJ/mole (Appendix E). The reactor is continuously heated. Therefore it is assumed that this consumed energy during the reaction is included in the reactor design.

Grinding
The bread waste need to be ground before it can be mixed.. The energy consumption during grinding mainly depends on the particle size. As the particle size distribution is unknown a value needs to be estimated to approach the energy consumption. F. Picciono and R. Hischier (2016) calculated average values for various grinders based on the publication of Vauck and Muller (1994), this has resulted in a value between 8 and 16 kWh/ton of ground material. Since no information on the particle size is available, the upper value (16 kWh/ton) should be used as an approximation. The grinding energy (J) can be calculated according to equation 6.

$$E_{grinding}(J) = 16\ kWh * 3.6 * 10^3 * m_{bread} \quad (6)$$

Pumping
The reaction mixture has to be transported between process steps. Commonly, this transfer is done by pumping liquids through pipes. The change in hydraulic head is the energy consumption of the pump (Δh), which is influenced by the height difference between the starting and end point, pressure loss due to friction and the average speed of the fluid, pipe length and pipe diameter. The energy consumption of the pump can be calculated with pump efficiency, the mass to be transferred and the gravitational acceleration (g) equation 7.

$$E_{pump} = \frac{m*g*\Delta h}{\eta_{pump}} \quad (7)$$

To simplify the calculation the parameters of the pipe have been standardized (L= 15 m, d = 0,2 m) and steel is chosen as material of construction (k= 0.1 mm) (Vauck and Müller, 2000). The height difference was standardized to 4 m to overcome the height of the proposed reactrr certainly. A speed of 1 m/s at turbulent flow was assumed and efficiency of 0.75 (Vauck and Müller, 2000). This result in value of 55 J per kg of pumped material (equation 8) (F. Piccinno et al., 2016)

$$E_{pump} = 55\frac{J}{kg}*\text{m} \quad (8)$$

Mixing
In the design is chosen for separate reactors for mixing and the reaction. Different type of homogenizers like high pressure, ultrasonic and rotor-stator can be used. In this design there is chosen for a rotor-stator type, because this type is most commonly used. Homogenizing can be seen as stirring at very high shear rates, Therefore the standard equation for stirring energy (J) is applicable (equation 9). The parameters differ from regular stirring. The power number data is comparable to normal agitators (F. Picciono and R. Hischier, 2016) an average power number was calculated (Zhang et al., 2012 ; Myers et al., 2001). Compared to smaller agitators the impeller diameter of homogenizers are smaller. An average value for the diameters, shear rates was estimated by F. Picciono and R. Hischier (2016) based on producer data. Due to the increased shear rates, energy consumption is considerably higher than for regular mixing. Values for 1000 L and 5000 L reactors are provided. To estimate the matching values for the calculated reactor volume interpolating is used, assuming the values are linearly related. All values used during the mixing calculations are provided in table 3.3.

Physical entity	Symbol	Unit	1000 L	5000 L
Impeller diameter	d	m	0.139	0.260
Power number of rotor	N_p	-	2.39	2.39
The rotational speed of the rotor	N	1/s	48.333	20
Efficiency of agitator	η_{stir}	%	90	90

Table 3.3: scale - dependent date for the calculation of the rotor-stator homogenizer energy draw (F. Picciono and R. Hischier, 2016)

$$E_{mix\ (J)} = \frac{Np * \rho_{mix} * N^3 * d^5 * t}{\eta_{mix}} \quad (9)$$

Mixed batch reactor

The energy consumption of the mixed batch reactors exists of two parts: the heating energy and the stirring energy. The total energy needed depends on many factors and can vary significantly depending on the reactor (type, size, surface, material, insulation) the heating element (type, size, surface, material, efficiency) and the reaction parameters (F. Picciono and R. Hischier, 2016). Many of these factors rely on the actual plant design, and an exact calculation is only possible if a detailed plant design is available. For the scale-up procedure, this complexity is handled by simplification focusing on main contributors and working with average sizes and designs. The heating energy required for the reactor (Q_{react}) is composed of the energy to raise the reaction mixture to the right temperature (Q_{heat}) and the heat loss (Q_{loss}) divided by the efficiency of the heating element (η_{heat}) (equation 10).

$$Q_{reaction} = \frac{Q_{heat} + Q_{loss}}{\eta_{heat}} \quad (10)$$

Heating energy (Q_{heat}) calculation

The bread waste is dissolved in excess of water. To ease the calculations, the influence of the bread waste on the specific heat capacity of the mixture is neglected, and the specific heat capacity of water is used during the calculations as seen in the scale-up framework of F. Piccinno et al. (2016). The specific heat capacity (C_p (J/kg*K)) is used to indicate the amount of energy required to obtain a temperature change of 1 K per unit mass (kg) of material. The reaction temperature is indicated by (T_r) and will be between the 293 and 313 K (Chapter 2). The water is stored in a tank and it is assumed it the temperature of the water equals the outside temperature. The average outside temperature in the Netherlands is 284.2 K (KNMI annual review 2017). The mass of the reaction mixture (m_{mix} (kg)) is calculated by the volume and the density of the mixture, this calculation can be found in chapter 2. The heating energy (J) can be calculated according to equation 11.

$$Q_{heat} = C_p * m_{mix} * (T_r - T_0) \quad (11)$$

Heat loss energy (Q_{loss}) calculation

Conduction of the reactor wall causes heat loss. The lost energy must be compensated with extra energy to keep the reaction mixture at the right temperature. The heat varies with the used heating system. As a simplification, the conduction through the reactor walls are neglected, and only the insulation layer is taken into consideration as proposed by F. Piccinno et al. (2016). The heat loss depends on the surface area of the reactor (A), the thickness of the insulation layer (s) the thermal conductivity of the insulation

material (k_a), the reaction time (t) and the temperature difference between inside (T_r) and outside (T_{out}) the reactor. The outside temperature is the temperature in the factory hall and is estimated at 289 K.
A cylindrical reactor size, is assumed, so that the surface area can be calculated with the height (L) and the diameter (D). The actual volume of the reactor exceeds typically 10 % of the reaction mixture volume (F. Picciono et al., 2016).

Different insulation materials with varying thicknesses can be used. A widely used insulator is glass fiber (F. Piccinno et al., 2016) which has a temperature range of 20-450 ° C and a thermal conductivity of between 0.032 and 0,052 (W/m.K) (Thirumaleshwar, 2009). The thermal conductivity is based on an insulation layer of 75 mm thickness (Perry and Green, 2008)

The heating elements efficiency was standardized at 75% for the 1000 L reactor (F. Piccinno et al., 2016). The efficiency increases with the size of the reactor, therefore a scaling factor is applied. The best approximation found is a scaling factor of 0.02 (Caduff et al., 2014). All values used are shown in table 3.4. During the calculations, it is assumed that all values are linearly related and interpolating is used to calculate the intermediate values.

The heat loss energy can be calculated according to equation 12

$$Q_{loss} = A * \frac{k_a}{s}(T_r - T_{out}) * t \quad (12)$$

equation 11 and 12 are substituted in equation 10 to calculate the heating energy required (eq. 13).

$$Q_{reaction} = \frac{Q_{heat} + Q_{loss}}{\eta_{heat}} = \frac{C_p * m_{mix} * (T_r - T_0) + A * \frac{k_a}{s}(T_r - T_{out}) * t}{\eta_{heat}} \quad (13)$$

Physical entity	**Symbol**	**unit**	**1000 L**	**5000 L**
Reaction mixture Volume	V_{mix}	m^3	1	5
Height of reactor	L	m	1.119	1.913
Diameter of reactor	D	m	1	1.913
Surface area	A	m^2	5.899	17.249
Reactor volume	$V_{reactor}$	m^3	1.1	5.5
Insulation material	-	-	Glass fiber	Glass fiber
Thermal conductivity of insulation	k_a	W/m.k	0.042	0.042
Insulation thickness	s	M	0.075	0.075
Heat transfer coefficient of insulation	k_a/s	$W/m^2.k$	0.56	0.56
Efficiency of heating element	η_{heat}	%	75	77
Impeller diameter	d	m	0.373	0.638
mixture temperature	K	K	283.9	283.9
Outside temperature	K	K	289	289
Reaction time	t	s	Variable	Variable

Table 1.4: scale-dependent data for the calculation of heating energy proposed by F. Piccinno et al., (2016)

Stirring energy

To ensure equal heat distribution during the reaction stirring is applied. The stirring energy (E_{stir}) is influenced by the power number (N_p), the density of the mixture (ρ_{mix}), the rotational velocity (N) the diameter of the impeller (d), the reaction time (t) and the stirring efficiency (η_{stir}). Their relation is shown in equation 9.

The power number is specific to a type of impeller and constant turbulent flow. In general, turbulent flow is used during mixing (F. Piccinno et al., 2016). It is assumed an axial flow impeller is used, corresponding power numbers found are in the range of 0.28 and 1.94 of which an average is calculated (Albright, 2009). The impeller diameter is calculated according to the rule of thumb that the impeller diameter is one-third of the reactor diameter. For rotational velocity, a standard value of 85 rpm is used and is based on equal tip speed.

Centrifugation

Centrifugation is used as liquid-solid separation method. In the industrial design, there is chosen for centrifugation instead of filtration because the particle size distribution is unknown. No estimations from the research are received about the energy intensity of the centrifugation, therefore, the upper value of 10 kWh/ton is used (F. Piccinno et al., 2016). The centrifugation energy can differ with a factor 10 (F. Piccinno et al., 2016. The centrifugation energy is calculated according equation 13.

$$E_{centrifugation} = 10\ kWh * 3{,}6 * 10^3 * m_{mix} \quad (13)$$

Optimization of the glucose production

It is aimed to minimize price per kilo of glucose produced due to the hydrolysis process. In chapter 2 a multivariable regression is applied to indicate the effect of the variables reaction time (t), temperature(K) and the enzyme dosage (enzyme (g)/wet weight (kg)) on the glucose production per liter (eq. 14). The process costs are calculated as the sum of the energy costs, the enzyme costs, energy costs and the cost of the bread waste (equation 15). The costs of these variables are shown in table 4.

$$estimated\ glucose\ production\ \left(\frac{g}{L}\right) = 5.2 + 0.0026 * t + 0.1449 * enzyme\ \left(\frac{g}{kg}\right) + 0.0752 * T_r \quad (14)$$

$$process\ costs\ (€) = \frac{E_{total}}{3.6*10^6} * 0.20 + m_{bread} * enzyme\ \left(\frac{g}{kg}\right) * 5.50 + \frac{m_{bread}}{0.150} * 10^{-3} * 0.625 \quad (15)$$

	Cost
Stargen 002	€ 5.50/kg
Bread waste	€ 0.1 / bread
Electric energy	€ 0.20 / kWh
Water	€ 0.625 / m^3

Table 2.6: costs of raw materials for the hydrolysis process

The variables are kg bread waste, temperature, enzyme dosage and reaction time. The constraints applied are shown in table 3.7.

Variables	
Temperature (K)	293≤ K ≤313
Enzyme dosage (enzyme (g)/wet weight (kg))	0.8≤ g/kg ≤48
Reaction time (s)	0≤ t ≤ 86400
Bread waste (kg)	0 ≤ kg ≤ 553

Table 3.7 constraints for the process optimization model

The variable cost per kilo of glucose is given in equation 16 and 17, which gives a nonlinear equation and is therefore solved with the function GRG nonlinear in excel to find the minimum.

$$variable\ cost\ per\ kilo\ glucose(€) = \frac{m_{mix} * 0.44\ wt\% * (estimate\ glucose\ prodcution)}{process\ costs} \quad (16)$$

$$variable\ cost\ per\ kilo\ glucose\ (€) = \frac{m_{mix} * 0.44\ wt\% * (5.2 + 0.0026 * t + 0.1449 * enzyme\left(\frac{g}{kg}\right) + 0.0752 * T_r)}{\frac{E_{total}}{3.6 * 10^6} * 0.20 + m_{bread} * enzyme\left(\frac{g}{kg}\right) * 5.50 + \frac{m_{bread}}{0.150} * 10^{-3} * 0.625} \quad (17)$$

3.2.3 Results and discussion

Process design

After optimization of the model an optimum value of the variables was found (table 3.8). Based on these values the mass and energy balances for the reactor could be completed and are summarized in (figure 3.3)

Variables	
Temperature (K)	293
Enzyme dosage (enzyme (g)/wet weight (kg))	0.8
Reaction time (s)	86400
Bread waste (kg)	553

Table 3.8 variable values after optimization after optimization

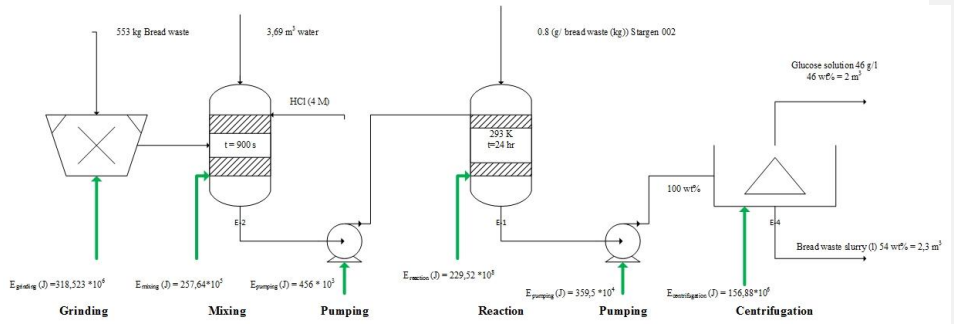

Figure 3.3: *Reactor design*

Proposed equipment design

Bases on the optimization of the production process the dimension of the two reactors can be indicated. During the calculation, cylindrical reactors were assumed. The reactor size is dependent on the bread waste input. The dimensions of the reactor (table 3.9) are calculated to make an indication of the fixed costs of the process.

The total energy used during the process is 124 kWh. Heating the reaction mixture takes 50 % of the total consumed energy (figure 3.4). Which is the minimum amount because the reaction mixture is heated to the minimum temperature of 20 ^{O}C.

	Mixing - Reactor	**Reaction – Reactor**
Height (m)	1.735	1.735
Diameter (m)	1.735	1.735
Reactor volume (m^3)	4.5	4.5
Impeller diameter (m)	0,232	0,578

Table 3.9 reactor dimensions

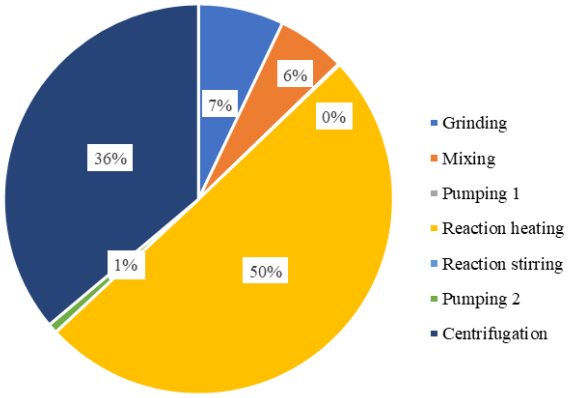

Table 3.4 Energy consumption per reaction step

Variable costs – Production process

The total energy used during the process is 124 kWh. Total production of 95.87 kilos of glucose per day was realized, with an estimated variable cost of € 0.86 per kilo of glucose. This price indication only includes the variable costs considered with the production process. It is important to emphasize that this only a price indication.

The transportation of the ground material is not included in the process design. Furthermore, the upper values for energy usage are used, causing high energy prices. During the process, equipment is underutilized which causes in practice higher production costs. A solution to this problem could be to increase the input with other food waste which can be hydrolyzed by the use of Stargen.

Output streams

Glucose solution

The process produces two output streams, a glucose solution, and bread waste slurry. The glucose solution is aimed to be used as a substrate for the *G. sulphuraria*. However, some containments are present in this solution. One of the most important is the enzyme Stargen 002 ™. The two main components of the enzyme glucoamylase and alpha amylase are toxic to algae with a concentration above 20 mg/l and 100 mg/l (SDS Stargen ™ 002, Genencor). These values are exceeded for al enzyme concentration used during the lab experiments. In other experiments where high concentrations of Stargen are used in the production of the substrate, the algae still grew (Sloth et al., 2017). Therefore this is not considered as a problem now.

The produced substrate does not contain a nitrogen source which can be used by the alga. Therefore the alga will not grow on this substrate (Sloth et al., 2017). This problem can be solved by adding the enzyme Fermgen™ produced by Dupont during the reaction process. Fermgen™ is anci proteolytic enzyme characterized by its ability to hydrolyze proteins under low pH conditions. The enzyme works in the same temperature and pH range as Stargen™. Another solution is adding $(NH_4)_2SO4_4$ to the growth medium (Sloth et al., 2017) to ensure growth of the *G. Sulphuraria.* This procedure is also applied when glycerol is used as a substrate (Martínez García, 2017).

The output glucose concentration according the model is 47 g/L. This is a relatively high sugar concentration for growing the *G. sulphuraria.* Normally growing media for the *Galdieria sulphuraria* contains 3-4 % of growing substrate (Personal communication, Alle van Wijk, Research group Aquatic Biotechnology and Bioproduct engineering, 7 June, 2018). However, 5% growing substrate is used by Martínez García to produce Nanoglycogen from glycerol. To reach lower concentrations the solution can be diluted before it is used as a substrate.

Bread waste slurry

The other output is bread waste slurry. It is assumed the slurry has no residual value and can not be sold. The daily production bread waste slurry is 2300 liter/day The slurry could be composted and provided as manure material to the local farmers. Another option is to sell the bread waste slurry yet to the cattle feed industry. However, more research has to be done on regulations and on the production process of cattle feed to have a clear idea whether this is a possible solution.

3.2.4 Design validation

To validate the output value, the *G. sulphuraria* is grown on the glucose solution. The glucose solution is produced under the proposed process conditions. It was found that the *G. sulphuraria* shows little growth on bread waste supplemented with a nitrogen source. OD of 7 is reached measured against water as blank. The real OD is probably lower. The full experiment and discussion of the results can be found in Appendix F.

Chapter 4: Total Cost analysis and economic feasibility

In this section, a complete cost analysis of the glucose production from bread waste is done, and the economic feasibility is discussed. An estimated price of €4.83 / kg glucose was found. The process was not considered economically feasible, and the product cannot compete with the currently available products on the market. However, when a new application for Nanoglycogen is found, a new market can be generated.

4.1 Cost analysis

The total cost of 1 kg produced glucose can be divided into fixed cost and variable costs. The calculations are based on a fulltime production process of 7 days, 24 hours per day. The yearly glucose production is based on the daily glucose production found in chapter 3 (95.8 kg/day). Oversight of the estimated costs can be found in Appendix G. The total cost found per kg/glucose is €4.83. The distribution of the various costs is shown in figure 4.1.

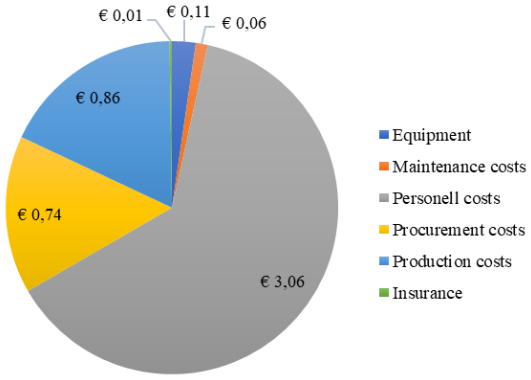

Figure 3.1 Distribution of different costs per kg/glucose

Fixed costs

Equipment, maintenance, insurance and personnel costs are considered as fixed costs. The cost of land or building rental vary widely between locations and are not considered in this project.

Difficulties were encountered during the search for the prices of equipment. It was not possible to gain accurate insight into equipment prices because they are based on personal offers. Therefore, it is chosen to estimate prices based on mass production companies from Asia who offer their equipment on the internet. It is assumed that the life of the devices is 10 years and that they are fully depreciated over this period. Maintenance is a fixed cost as the plant must be kept in good condition regardless of the level of production. Maintenance costs are estimated at 5 % of investment in equipment (Sinnott, R. K., & Towler, G. (2009)). All plants require insurance to cover third party liability as was potential plant damage, the insurance is estimated at 1% of the equipment investment (Sinnott, R. K., & Towler, G. (2009))

As the cost of equipment can be considered as the minimum price, insurance and maintenance costs are based on the equipment costs those will also be the minimum for this case. It needs to be taken into consideration that these costs are an approximation and the real values will be higher.

The number of workers depends on the batch sequence and extent of automation. A minimum of 3 workers is required according to (Sinnott, R. K., & Towler, G. (2009). Personnel costs are based on three full-time workers for 40 hours a week, with a net salary of € 13.- /hr. The personnel costs have a share of 47%. Automating the process could be advantageous. However, this will go up hand in hand with high investment costs. More research needs to be done to indicate of automatization will decrease the production costs. Another option to lower personnel cost is to move the entire process to low wage countries.

Corporate overhead charges as research and development, selling, marketing and administrative costs are not included because those costs are linked to a company and not directly to the production process.

Variable costs

As variable costs, the procurement and process costs are considered. These costs are described in chapter 3.

4.2 Economic feasibility

The total costs per kilo of glucose produced from bread waste are 4.83/kg. This is 43 times higher than the price of glycerol which is between €0,05 – 0,11 / kg (Johnson and Taconi, 2007). For the process to be economically feasible. The value of the end products need to compensate for the production costs Yields of 0.24 % glycogen on glycerol are found (Martínez García, 2017). It is assumed that the yield for glycogen on bread waste will be equal. Then the hydrolysis process cost will be € 2012.33 per kilo Nanoglycogen. It is unknown what the production costs for growing the alga and extraction of the Nanoglycogen are equal for both substrates. Based on the hydrolysis process and the estimated kilo prices (Appendix A) from cluster dextrin (€10/kg) and icodextrin (€371/kg), the Nanoglycogen produced from bread waste cannot compete with these products. However, research can be done on other applications for Nanoglycogen to create a new market.

General conclusion

In this research, there was searched if hydrolyzed bread waste can be produced on industrial scale as a substrate for the *G. sulphuraria* and can compete in economic efficiency with glycerol. Based on the designed process a price of €4.83/kg produced glucose is found, which is 43 times higher than glycerol, therefore is concluded that bread waste cannot compete with glycerol as a substrate for the *G. sulphuraria.* If the price of Nanoglycogen produced from bread waste is compared to competing products based on the hydrolysis process, the productions costs exceed both selling prices from Icodextrin and cluster dextrin. Therefore, it is advised to conduct more research on other applications for Nanoglycogen.

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Appendix A- Price of competing products

Cluster dextrin

The price per kg is estimated at € 10 euro (Personal communication, Prof. M.J.E.C van der Maarel)

Ico dextrin

Information about extraneal dialyse fluid is gained from baxter.com The dialysis fluid contains 7.5 % icodextrin. The fluid is sold for $112.35 per 2 l. The average density of dextrins is 1.44 g/ml (C.Y. Takeiti et al., 2010). This gives an estimate kilo prices of €371 euro

Appendix B – wet weight bread composition

	per 100 g
Transvetzuren	0.01 g
Energie	1043 kJ
Energie	246 kcal
Vetten	1.5 g
Vetzuren, totaal verzadigd	0.4 g
Koolhydraten	47.3 g
Eiwitten	8.8 g
Zout	0.95 g
Voedingsvezel	4.2 g

Ah.nl

Appendix C – Standard deviations of the hydrolysis experiment

Enzyme concentration (Stargen / wet weight) / time(hr)	0	0,25	0,5	0,75	1	1,5	2	2,5	3	3,5	24
0,8 g/kg	0,001408	0,026641	0,177341	0,040166	0,026286	0,002992	0,00984	0,007808	0,007359	0,012604	0,016245
1.2 g/kg	0,005156	0,134024	0,153651	0,087946	0,072966	0,078845	0,010372	0,056125	0,018425	0,062496	0,1315
1,6 g/kg	0,004002	0,157022	0,095597	0,02736	0,04484	0,026815	0,027225	0,128897	0,019868	0,055736	0,027436
3.2 g/kg	0,00744	0,056884	0,059767	0,198604	0,027686	0,067213	0,030462	0,020027	0,033966	0,144928	0,006918
6.4 g/kg	0,009497	0,067734	0,073815	0,092693	0,088735	0,071057	0,083386	0,08093	0,021507	0,202977	0,001774
12 g/kg	0,008972	0,080727	0,073815	0,0699	0,077799	0,083412	0,090454	0,068263	0,126308	0,063141	0,014963
14 g/kg	0,009653	0,024201	0,012752	0,019697	0,014236	0,044092	0,038808	0,022516	0,086609	0,085374	0,019833
28 g/kg	0,349524	0,043966	0,094484	0,017973	0,012068	0,04702	0,032602	0,0426	0,025871	0,079171	0,038538
42 g/kg (	0,010295	0,038354	0,094725	0,021039	0,021307	0,013032	0,067108	0,16863	0,082092	0,024828	0,008842

Figure B.1 Standard deviations: concentration of glucose during hydrolysis of bread waste with different concentrations of Stargen™ at 20 °C

Enzyme concentration (Stargen / wet weight) / time(hr)	0	0,25	0,5	0,75	1	1,5	2	2,5	3	3,5	24
0.8 g/kg	0,028978	0,003574	0,031585	0,003243	0,046318	0,013029	0,022926	0,05977	0,016857	0,04877	0,095927
1.2 g/kg	0,049875	0,014729	0,104013	0,116992	0,009718	0,036227	0,051916	0,01726	0,138984	0,021144	0,075263
1.6 g/kg	0,140947	0,050479	0,015995	0,057893	0,050202	0,031533	0,042705	0,036577	0,066764	0,057528	0,043706
48 g/kg	0,002808	0,058417	0,007213	0,066915	0,06698	0,09192	0,025938	0	0,069048	0,025497	0,094425

Figure B.2 Standard deviations:concentration of glucose during hydrolysis of bread waste with different concentrations of Stargen™ at 30 °C

Enzyme concentration (Stargen / wet weight) / time(hr)	0	0,25	0,5	0,75	1	1,5	2	2,5	3	3,5	24
0,8 g/kg	0,344995	0,064247	0,074516	0,073515	0,257538	0,062375	0,043717	0,018334	0,012267	0,014763	0,171401
3,2 g/kg	0,016261	0,031276	0,075002	0,040118	0,042849	0,059452	0,054125	0,047609	0,01716	0,096115	0,043673
6,4 g/kg	0,002207	0,021972	0,306059	0,217847	0,049153	0,059452	0,092058	0,073941	0,01757	0,219028	0,110429
14 g/kg	0,002273	0,032504	0,02835	0,237977	0,027854	0,064921	0,152517	0,057628	0,076745	0,017268	0,059538
48 g/kg	0,344995	0,116408	0,137255	0,18205	0,135462	0,067256	0,062974	0,050577	0,038575	0,007379	0,070788

Appendix D – Calculations on the bread supply by Borgesius backery

Estimated maximum bread waste supply

Production capacity: 3000 bread per/ hour and 10 000 roll/hour

Production hours: a 12 hour 7 day a week production is assumed.

One whole bread has a weight of 0.77 kg (ah.nl) and a role has a weight of 0.022 kg (ah.nl)

From the breadwaste 3-4% is returned to the backery.

This gives an estimated production of 554 kg bread and 184 kg rolls per day.

Estimated reactor volume

For 0.150 kg of bread 1 liter water is used. This mixture has a volume of 1.113 liter. The maximum reactor volume for 738 kg breadwaste is 5470 L.

Maximum truck load

The load size of an truck is estimated on 12*2,55*2 m. This gives a total volume of 61.2 m^3.

The volume of one bread is 0.08 m^3, which means 765 breads (589 kilo) can be loaded in one truck.

Appendix E – Reaction energy calculations and HCl price

The bond dissociation energies are found in Comprehensive Handbook of chemical bond energies (Y.R. Luo). Breaking bonds costs energy. During the forming of bonds energy is released. The bond dissociation energies are approached based on known bond energies.

Bond	Based on	Dissociation energy
Reactant		
~CO-C~ (starch)	CH_3O-CH_4	346 kJ/mole
HO-H (water)	H_2O	493.4 kJ/mole
Product		
~CO-H (glucose)	C_2H_5CO-H	374.5 kJ/mole
~C-OH (glucose)	C_5H_9-OH	385 kJ/mole

Luo, Y . R ., Comprehensive Handbook of Chemical Bond Energies, CRC Press, Boca Raton, FL, 2007 .

HCl price

Diluted HCl can be bought for €2.31 /L toolsvoordeling.nl

Appendix F – Growth of the Galdieria sulphuraria on bread waste

Growth of the G. Sulphuraria on bread waste

In this section the growth of the *G. sulphuraria* on bread waste is discussed. The *G. Sulphuraria* belongs to the red micro Algae (Rhodophyta). Rhodophyta is a diverse group of organisms that contains both multicellular and unicellular species that can colonize a wide range of habitats as sulphur springs, fresh waters and even volcanic environment.

The most recent phylogenetic studies classify the red algae into two subphyla: Cyanidiophytina and Rhodophyntia. (Yoon et al.,2006; Yang et al). The Cyanidiophyntina contains one class (Cyanidiophyceae) and one order named Cyanidiales to which the *G. Sulphuraria* belongs.

The Cyanidiales show a broad metabolic flexibility. The *G. sulphuraria* can grow using photosynthesis or heterotrophically in complete darkness, using a wide range of carbon sources including sugar alcohols, mono saccharides, organic and amino acids (Gross and Schnarrenberger, 1995). The Galdieria can grow on sugar concentrations above 400 g/L, salt concentrations up top 2-3 M and pH values below 1 (Schmidt et al., 2005) and can therefore be cultivated under extreme conditions where the risk of contamination is relatively low Biomass productivities have in continuous flow reached 50 g/L/day (Graverholt and Eriksen, 2007) .This is very favourable as it is aimed to grow the Galdieria on industrial scale.

To validate the industrial process designed in chapter 3 it is investigated if the *G. sulphuraria* can grow on the produced substrate.

Methods

Preparation of the bread waste hydrolysates

The 1L of bread waste mixture is prepared as described in chapter 2. The hydrolysis conditions are the same as proposed in the industrial design in chapter 3 (T = 20 ^{o}C, Enzyme concentration = 0.8 g/kg Stargen, t= 24 hr). When the hydrolysis is considered finished, the bread waste mixture is centrifuged to separate the glucose solution. The sample was analysed on glucose by the use of GOPOD as described in chapter 2.

Strain and growth media.

Galdieria sulphuraria 108 was kindly provided from a stock culture of Tijmen Bovenmars (Master student, University of Groningen). Heterotrophic batch cultures were grown in 250 ml conical flasks containing 50 ml of liquid medium. The flasks were incubated in orbital shaking incubator operated at 150 rpm. The temperature was controlled at 40 ^{o}C. The cultures were grown in Allen medium with and without nitrogen source $(NH_4)SO_4$. As solvent the glucose solution was used. Each culture was measured in two-fold. The medium was brought to pH 2 by addition of 1 M HCl and autoclaved for 20 minutes at 121 ^{o}C.

Quantification of biomass

Biomass concentration was measured as absorbance (OD) at 800 nm on a Spectramax Plus 384. The samples were diluted in water when necessary. The samples were measured against water and growing medium.

Results and Discussion

The G. sulphuraria shows growth on bread waste (figure G.1). An OD around 7 is reached. This OD are overestimated because all samples are measured against water. The proteins in the original blank, the allen medium with glucose solution, started to denature over time and the blank became turbid. Due to the denaturation the blank could not be used anymore. The results are shown in figure G.1. The OD reached is less than the OD reached on glycerol , which was between 8 and 10 (Martínez García, 2017).

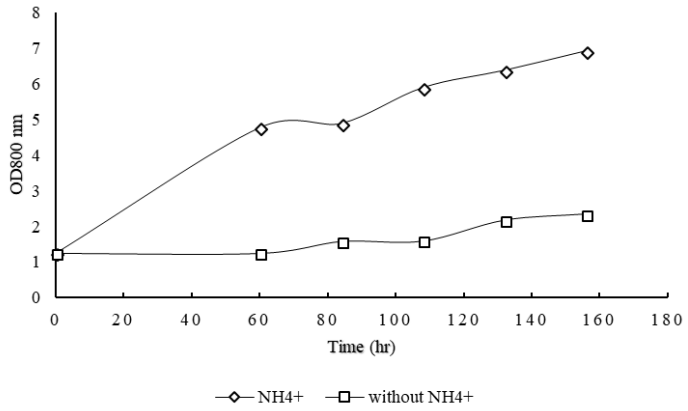

Figure G.1 Growth of the Galdieria sulphuraria on bread waste supplemented with and without NH4+

Appendix G – Cost analysis

Fixed costs		
Equipment		
- Reactor mixing	€ 7500[1]	€ 750 / year
- Reactor reaction	€ 7500[1]	€ 750/ year
- Grinder	€ 2000[2]	€ 200/ year
- Pump (2 x)	€ 1900[3]	€ 190/ year
- Centrifuge	€ 19600[1]	€ 19600/ year
Maintenance		€ 1150 / year
Insurance		€ 385 / year
Personnel costs		
- 3 full-time workers		€105456[4] / year

Variable costs		
Procurement costs	€ 0,74 / kg glucose	
Production costs	€ 0,86 / kg glucose	
Total cost per kg glucose		4,83 /kg glucose

[1] made-in-china.com, [2] aliexpress.com, [3] ebay.com [4] payrollplaats.nl