OPTIMIZATION OF 2,3-BUTANE-DIOL PRODUCTION IN A BIOREACTOR BY *BACILLUS AMYLOLIQUEFACIENS*

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ABSTRACT

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Due to the depleting fossil fuel reserves and the ever changing oil prices, the production of 2,3-butanediol has shifted towards more biological methods. Current studies are experimenting with various strains of bacteria and carbon sources to find the optimal production method. Finding the right balance in the production could make it possible to produce 2,3-butanediol in a larger scale. For this purpose, the production of 2,3-butanediol by the bacteria *Bacillus amyloliquefaciens* was optimized.

Three experiments were performed in a 15 litre fermenter during a three-week period at the Ostfalia University of Applied Sciences in Wolfenbüttel, Germany. Each of the tests had the same starting medium and measured parameters with the addition of one or two feedings of sucrose. By adjusting the different parameters, such as the oxygen percentage in the medium, air flow and pH, an aerobic growth phase and an aerobic production phase could be achieved.

The results of the optimization of the production of 2,3-butanediol show that sucrose can be used to replace glucose as the main carbon source. However, there was no significant increase in the production of 2,3-butanediol with the addition of the feeding. While adjusting the availability of oxygen a clear growth and production phase can be distinguished. The results were positive although there is need for further experiments with more advanced analytical methods and upgraded measuring devices.

Key words: 2,3-butanediol, optimization, *Bacillus amyloliquefaciens*,

CONTENTS

1 INTRODUCTION

This experimental research was done in order to study the production of 2,3-butanediol (23BD) in a bioreactor. This included optimization of the different parameters from oxygen percentage in the medium to pH regulation. All the experimental parts were conducted in a laboratory at Ostfalia University of Applied Sciences which is located in Wolfenbüttel, Germany. The literature research aims to give background on the product, the microorganism used and the production pathway. Prior experiments on the topic are discussed after the literature research.

1.1 2,3-butanediol

Biotechnological production of 2,3-butanediol (23BD) has been on the increase ever since the rising oil prices and the decreasing fossil fuel reserves. The multiple applications available for 23BD make it an interesting development prospective. Even though there are already several studies on the microorganisms producing 23BD and there has been numerous laboratory scale experiments, more research is required to make the process run more smoothly and become profitable. (Celinska, 2009)

23BD is a chemical commonly produced from oil although there are few strains of bacteria capable of synthesizing the product. The need for 1,3-butadiene (13BD) and methyl ethyl ketone during World War II increased the interest for 23BD production. Especially since 13BD is an essential monomer for synthetic rubber. After the war the production continued mainly petro chemically, although already before the war time small scale pilot fermentations were performed biologically with *Klebsiella oxytoca* and *Bacillus polymyxa*. In the further studies the chosen bacteria remained mostly as the same strains which were studied initially. (Um & Kim, 2013)

There is a wide range of chemical products produced from 23BD such as methyl ethyl ketone (fuel additive), gamma-butyrolactone and 1,3-butadiene (Köpke at al., 2011). Several other potential uses for 23BD are anti-freezing agents, solvents, plastic and flavouring agent in food products. Through esterification 23BD forms precursors for polyurethane which is then further used in many different products from drugs to lotions (Syu, 2001). Köpke et al (2011) suggests that the downstream products of 23BD could potentially have 32 million tons per annum in the global market with value of $43 billion in sales.

To understand the potential of 23BD it is important to view its different characteristics. 23BD has a high boiling point of 180-184°C, a low freezing point of -60°C and the chemical formula of $CH_3CH(OH)CH(OH)CH_3$. Usually found in liquid from but crystalline is also possible. It is colourless and odourless. Due to the high boiling point, removing 23BD from the fermentation medium requires large amounts of water making the purification process one of the difficult aspects of the production process (Voloch, n.d.). There are three isomeric forms of 23BD, one of which possess the low freezing point and could potential be used in anti-freezing agents. The three isomeric forms can be seen in the figure 1 below (Syu, 2001). Picture 1 below represents the retention times captured from a 23BD by HPLC. This is a representation as to how the different isomers can be recognised yet it is impossible to define which of the three isomers are present. The difficulty is to determine which one of the peaks represent which of the isomers.

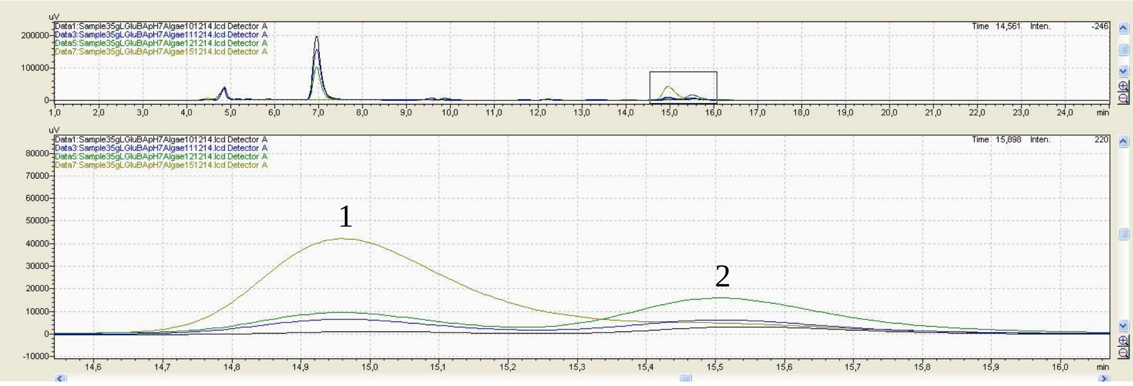
$$\begin{array}{c} CH_3 \\ | \\ H - C - OH \\ | \\ HO - C - H \\ | \\ CH_3 \end{array} \qquad \begin{array}{c} CH_3 \\ | \\ HO - C - H \\ | \\ H - C - OH \\ | \\ CH_3 \end{array}$$

2*S*,3*S*-2,3-butanediol 2*R*,3*R*-2,3-butanediol

$$\begin{array}{c} CH_3 \\ | \\ H - C - OH \\ | \\ H - C - OH \\ | \\ CH_3 \end{array}$$

meso-2,3-butanediol

FIGURE 1. The three different stereoisomers of 23BD (Voloch et al, n.d.)



PICTURE 1. Points 1 and 2 show the two retention times for 23BD representing two unidentified isomers, measured with the HPLC.

1.1.1 Microorganisms and pathway

23BD production by fermentation has been concentrated to certain bacterial strains *Klebsiella pneumoniae, Klebsiella oxytoca,* and *Serratia marcescens*. These bacteria have been proven to produce 23BD in an efficient way through the mixed acid fermentation pathway (Yang et al., 2012). In the case of *Klebsiella pneumoniae*, it has been studied to be able to utilize glucose, galactose, mannose, xylose, arabinose and certain disaccharides as a substrate. These substrates can be found in the cellulose and hemicellulose components of wood and agriculture residue, making the bacteria highly attractive for industrial production of 23BD. (Yu & Saddler, 1985) However, all of the bacterial strains mentioned above have been identified as opportunistic pathogens by World Health Organisation (WHO), making the industrial applications demanding. (Li et al., 2013)

Due to the pathogenicity of the many bacteria used to produce 23BD, the interest to find an organism which is generally regarded as safe (GRAS) and could produce 23BD has been on the rise. Bacterial strains such as *Bacillus licheniformis*, *Paenibacillus polymyxa* and *Bacillus amyloliquefaciens* have been isolated and proven to produce 23BD in a manner comparable to the pathogenic bacteria. However, the GRAS bacteria have their own challenges, one of them being a lower fermentation temperature of 30-40°C. The temperature lowers the cost of heating the fermenter but increases the risk of contamination. Thermophilic fermentation used with the pathogenic bacteria mentioned above is usually operated at 50-60°C reducing the risk of contamination by other bacteria. (Li et al., 2013)

The organisms used for 23BD production have been shown to follow the mixed acid fermentation pathway. A simplified version of the metabolic pathway can be seen in figure 2. The start of the pathway is glycolysis where glucose is converted to pyruvate. (Müller, 2008) After which two enzymes α-acetolactate synthase catalyses the condensation of two pyruvate molecules with a single decarboxylation. Decarboxylation is an essential process for all nutrients which are used by biological cells and organisms for source of energy in the catabolic metabolism (Frey & Hegeman, 2007). The single decarboxylation allows the formation of α-acetolactate and α-acetolactate decarboxylase that decarboxylates the latter one to acetoin and further to 23BD. (Giovannini et al., n.d.)

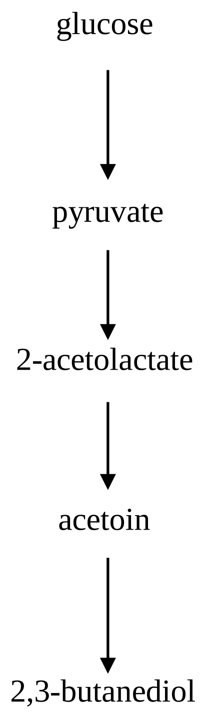
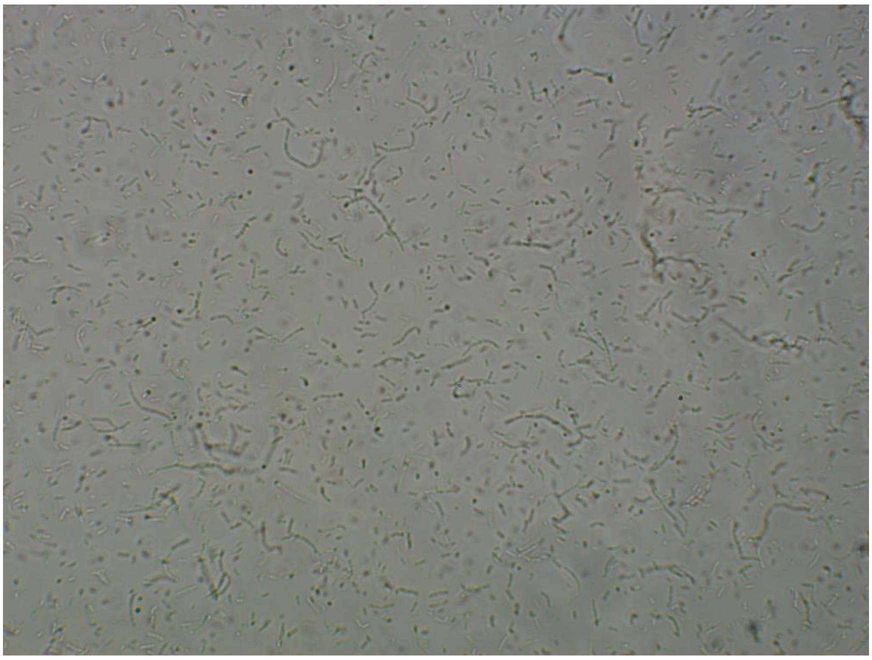


FIGURE 2. Simplified metabolic pathway of 23BD by anaerobic bacteria. (Müller, 2008)

1.2 Choosing the bacteria *Bacillus amyloliquefaciens*

The bacteria were initially chosen to be worked with due to it not being pathogenic which was an important requirement for the production. The non-pathogenic bacteria were not only chosen due to safety reasons but also because of specific laboratory requirements. The genus *Bacillus* is characterized by Gram-positive which explained as the bacteria having much larger cell wall or peptidoglycan which stains blue-purple. The bacteria are aerobic or facultative anaerobic, rod shaped, see the picture 2 below, and form spores. The genus contains more than 60 species which have been identified based on physiological and nutritional tests. (Wulff et al. 2002)



PICTURE 2. Microscopic view of *Bacillus amyloliquefaciens*.

When choosing the particular bacteria, literature research was done on previous experiments to see benefits of using this particular strain. *Bacillus amyloliquefaciens* was observed to produce 23BD without significant amounts of by-products. Only small amounts of acetate and lactate had been recorded. This strain of *Bacillus* does not produce glycerol or ethanol which could hinder the carbon utilization. In overall *Bacillus amyloliquefaciens* seemed to provide a good platform to work on and optimize the fermentation parameters. (Alam et al. 1989)

2 PRELIMINARY RESEARCH AND EXPERIMENTS

Prior to the experiment in the bioreactor, three stages of experimental research had been performed. They give explanations for the reasoning behind the choices done when planning and setting up the bioreactor experiments.

2.1 Start of the project

Prior to the set-up for the bioreactor initial research was done on finding the proper bacterial strain for the 23BD production and completing a laboratory scale experiment. The following information is based on experiments and a written project report 2,3-Butanediol production by *Bacillus amyloliquefaciens* by Manninen (2015) from autumn 2014. The initial laboratory tests were based on earlier study done by Alam et al. (1989).

The experiments were performed to see if the strain of bacteria could produce similar results as in the reference experiment. The approach was conducted in 100 ml shaking flasks, at 37°C, with 80 rpm, in the presence of oxygen. The experiments included testing different glucose concentrations and pH levels to find the optimal fermentation conditions. The fermentation products were analysed using a high-performance liquid chromatograph and the turbidity was measured with a spectrophotometer.

Testing different glucose concentrations from 10 to 90 g/L showed that the optimal concentration for the highest biomass development was 70 g/L. The highest yield of 23BD was produced from 50g/L of glucose. For further testing of different pH levels, the concentration of 70 g/L of glucose was chosen to ensure long production time. In the second part of the experiments different initial pH level were tested. The pH level was adjusted only at the beginning and ranged from 3 to 9. Based on the test results pH of 7 was chosen as the optimal. Although the pH was later decided to be 6 due to the medium conditions having a starting pH close to 6. During the end of the experiment a short test with algae biomass as an added substrate was tested.

The algae were dried and added to half amount of the chosen glucose concentration. However, results didn't show a significant change in the production and further testing time would have been required. The experiments overall showed promising results for further development for the 23BD production using the bacteria *Bacillus amyliliquefaciens*.

2.2 Further development

After the initial test phase, a group of German students from Ostfalia University of Applied Sciences continued the work during spring 2015. The aim of the work was to find alternative substrates and have a series of laboratory scale tests to see if there would be any possible replacements. The following information is based on the work done at a laboratory in Germany and a written project report Media optimization for 2,3-Butanediol production by *Bacillus amyloliquefaciens* by Goltz et al. (2015).

The tests were based on the initial work with same working methods as in the initial epxeriments. For the test, initial amount of glucose was chosen to be 60 g/L. During the tests various carbon sources were tested at different temperatures. This included adding red and/or green algae, waste water from local paper mill or corn steep liquor to the medium with half of the initial glucose to see if it would enhance the production. Results showed that glucose is essential to the growth of the bacteria alongside the production of 23BD and therefore could not be replaced by the substrates tested. However, red and green algae showed slight enhancing effect to the 23BD production and should be studied further. Overall the results gained showed that more repetitions and better analytical study should be performed. Furthermore, the exact metabolic process of 23BD production in the chosen bacteria should be clarified.

2.3 Preparations for the upscaling

The second study showed that there is a possibility to enhance the production of 23BD production by adding red and/or green algae to the medium. However, a possible replacement to glucose was not found. It was suggested that sucrose or as commonly known house hold sugar could serve as a cheaper alternative. This was further studied early summer 2015 at a laboratory in Ostfalia University of Applied Sciences.

Series of tests were performed to see if sucrose could replace glucose as the main carbon source. Alongside this experiment, further test with adding algae to the medium were performed. Due to a contamination in the cultivation batch the results from the algae experiment could not be clarified. However, small improvement to the 23BD production could be noticed and more tests should be performed. On the other hand, sucrose proved to be a viable replacement to glucose with the production of 23BD with sucrose being at times even higher than with glucose. It was decided that the next step should be to see if sucrose would still produce good amount of 23BD when moving the experiment to a larger scale.

3 AIM OF THE EXPERIMENTAL RESEARCH

The aims of the research were to confirm the change of carbon source from glucose to sucrose in a larger scale set up, optimize different parameters (oxygen percentage, pH, air flow) to further develop the process and test the possibility of having aerobic growth phase and anaerobic production phase.

4 MATERIALS AND METHODS

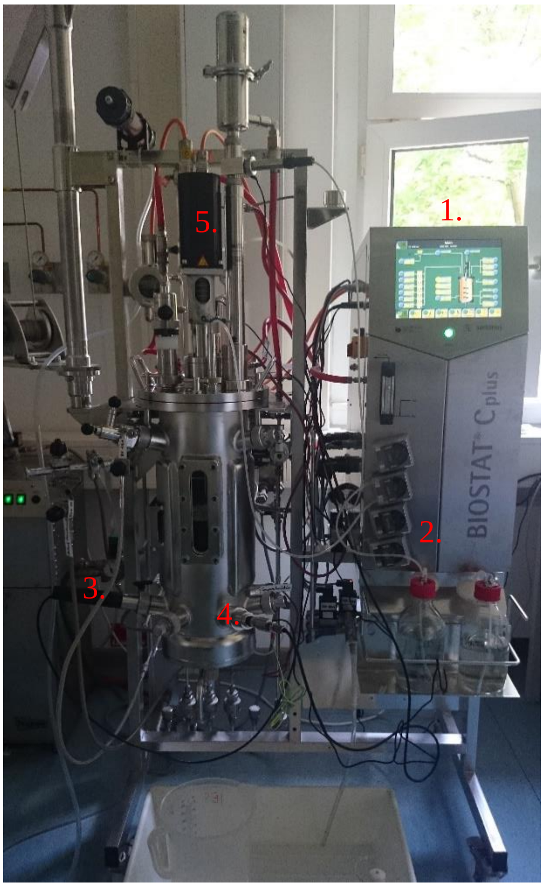
4.1 Nutrients and Chemicals

A medium consisting of household sugar/sucrose (60 g/L), peptone (10 g/L), yeast extract (5 g/L) and NaCl (2 g/L) was used for nutrients in the bioreactor tests. The pH regulation was achieved with NaOH (5.0 M). Additionally, few drops of silicone anti-foaming agent was added before leaving the bioreactor to run overnight.

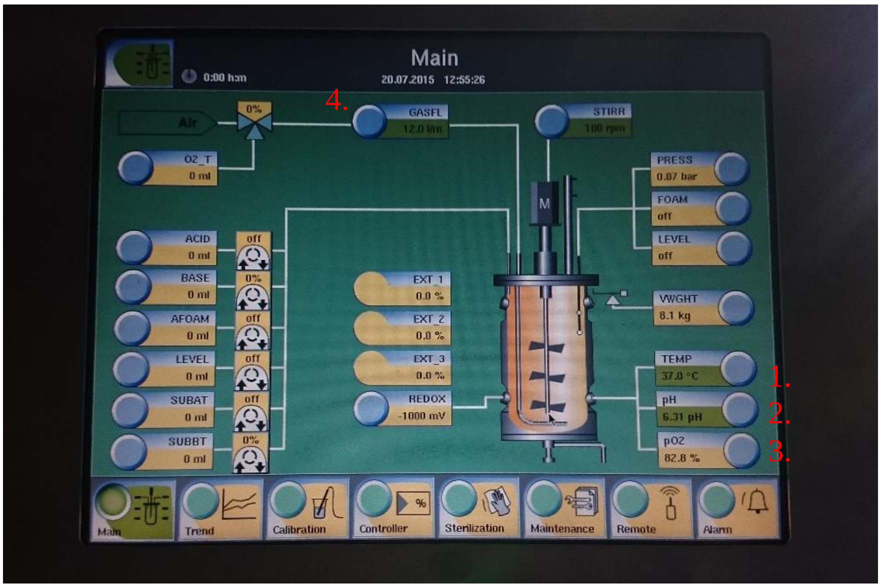
4.2 Set-up and Operation

Pure strain of *Bacillus amyloliquefaciens* was cultivated under sterile conditions into 250 ml shaking flasks with a medium consisting of glucose (60 g/L), peptone (10 g/L), yeast extract (5 g/L) and NaCl (2 g/L) in 200 ml. The flasks were incubated at 100 rpm and 37°C for 24 to 48 hours. The cultures were checked under the microscope prior the tests. A 15 litre fermenter or bioreactor, picture 3 and 4, (BIOSTAT ® Cplus, Sartorius, Germany) was used during the tests. 8 litres of the nutrient medium was prepared and sterilized in the bioreactor after which the medium was inoculated with the culture. The tests were run at 100 rpm with even temperature of 37°C.

During the tests the oxygen percentage was adjust first to ensure aerobic conditions in the fermenter. After the aerobic phase the oxygen percentage was released and the air flow was controlled to create anaerobic phase as seen in figure 3. During the first two runs of the bioreactor the O_2 percentage in the medium was regulated to 50 % during the first 24 hours with no adjustment to air flow. After the initial stage the air flow was adjusted to 4 L/m with no control of oxygen percentage and in the second run the air flow was adjusted to 2 L/m. In the last run the air flow was controlled at 12 L/m for the first 24 hours and lowered to 2 L/m, without O_2 % regulation. The bacteria were fed in the morning with 200 ml solution of household sugar which had been sterilized in an autoclave. During the last setup, pH was regulated automatically to 6.



PICTURE 3. Bioreactor set-up used during all the test phases. (1. operating screen, 2. pH regulation, 3. pH sensor, 4. oxygen sensor, 5. stirring)



PICTURE 4. Bioreactor main operating screen. (1. temperature, 2. pH, 3. oxygen percentage, 4. airflow, 5. stirring)

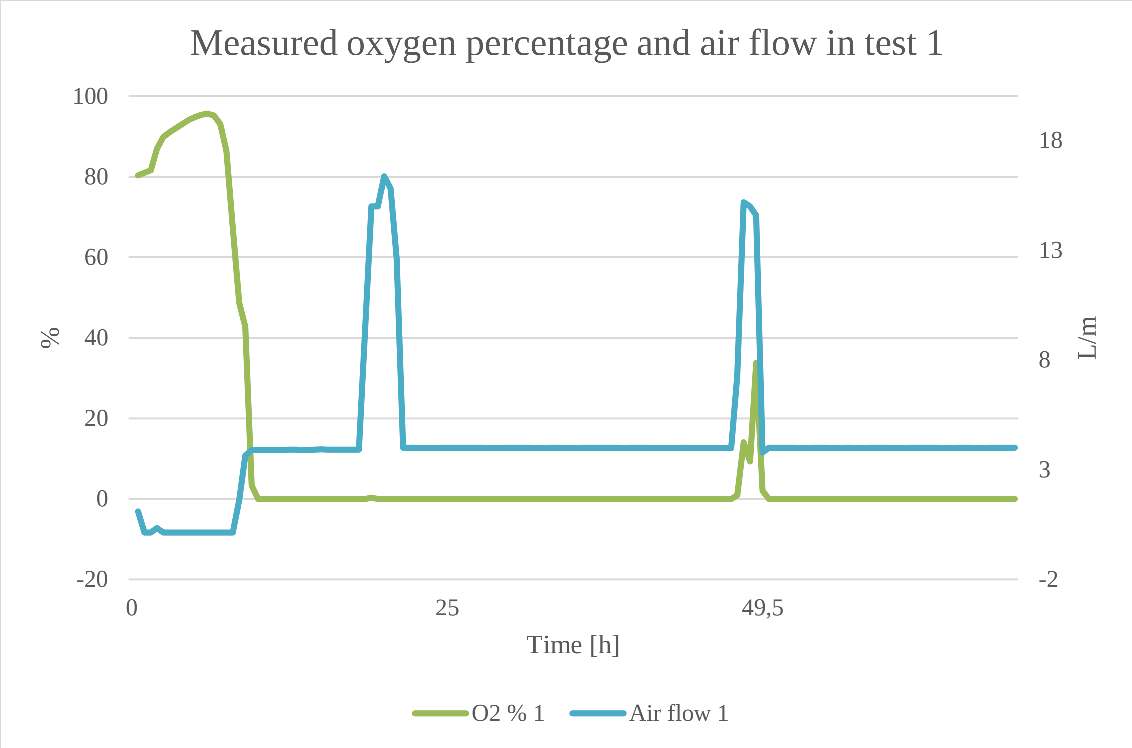


FIGURE 3. Graph showing the measured values of oxygen percentage and air flow in the bioreactor.

4.3 Analytical Methods and sample preparation

Biomass concentration was studied spectrophotometrically (Thermo Scientific, Miltiskan™ GO, USA) by measuring turbidity at 600 nm. The fermentation products were analysed using a high-performance liquid chromatograph, HPLC (SHIMADZU, Japan). Pure acetic acid, 23BD, glucose, fructose and household sugar were used to create the standards for HPLC. In preparations for the standards three different concentrations g/L were made of each product including two sets of solutions were made; acetic acid, 23BD, glucose and fructose in one and acetic acid, 23BD and household sugar in one. The height of the peak was produced by the HPLC, which is based on retention time, was plotted against the concentration in g/L for each standard. Calibration curve was created by using LabSolutions program (SHIMADZU, Japan). Samples for the HPLC were centrifuged at 3000 x g for 10 min after which the supernatant was filtered through a 0,22-µm filter before injecting the sample into a vial. Two parallel samples were analysed at each point of sampling. Due to analysing sugars the temperature in the column was set between 70 and 80°C. The column used in the HPLC was Organic Acid in HPLC Column made of Polystyrol-divinilbenzol Copolymer of the company CS-Chromatographie Service GmbH.

5 RESULTS AND DISCUSSION

The following results and discussion presents the data collected during each of the three experiments. The full tables of the collected data can be found in the appendices 1, 2 and 3.

5.1 23BD production and nutrient depletion

The HPLC was set-up to measure two retention times for 23BD. The results presented below are the sum of both measured values. In the picture 1 on page 6, the two retention times are shown. The two peaks are believed to represent two different isomers of 23BD (Bo et al. 2011). In this research, the different isomers in the HPLC were not determined. Increased production rate of 23BD was observed at the beginning of each test as shown in figure 4. After 40 hour point the production was detected to have slowed down. With the first and second test, the results reached a production amounts of 36,9 g/L and 37,1 g/L of 23BD when in the last test the production reached only 28,7 g/L. There was no significant increase in the production after each feeding. In the second test a slight decrease in the 23BD amount was detected after the feeding.

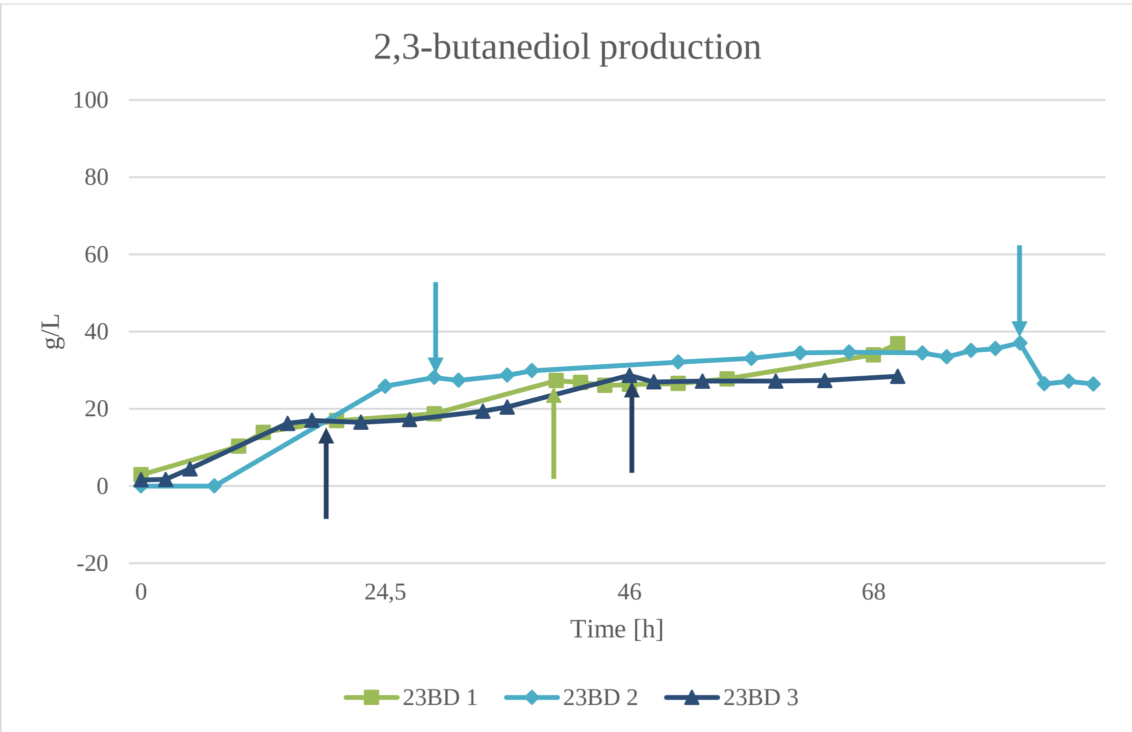


FIGURE 4. 23BD production during the three tests. The markers represent the sample collection while the arrows represent points of feeding.

In comparison to the 23BD production sucrose was depleted, figure 5, as the production increased. It was observed that in first test within 24 hours of each feeding including the start of the bioreactor, sucrose was utilised almost completely. In the second and third test the utilization of sucrose is slower and not complete. It is important to note that some of the values presented from the HPLC are negative values which indicates that the actual concentrations of the samples were below the lower end of the standard curve and exact concentration could not been generated.

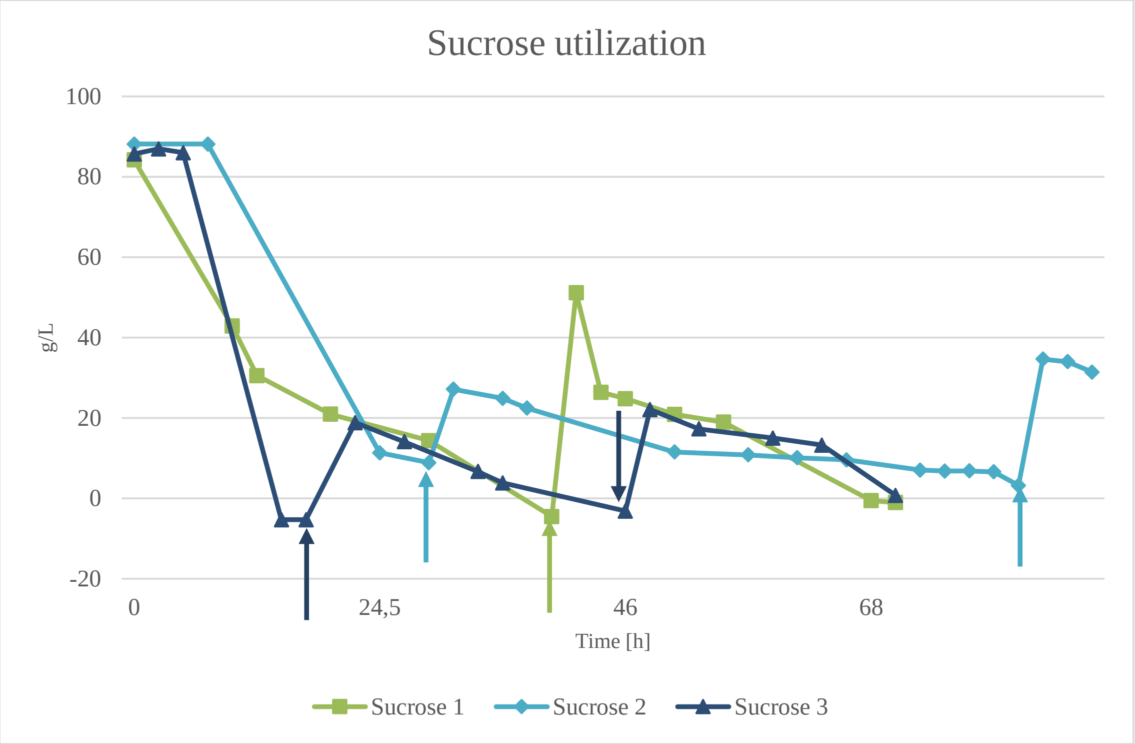


FIGURE 5. Sucrose utilization during the three tests. Arrows represent points of feeding.

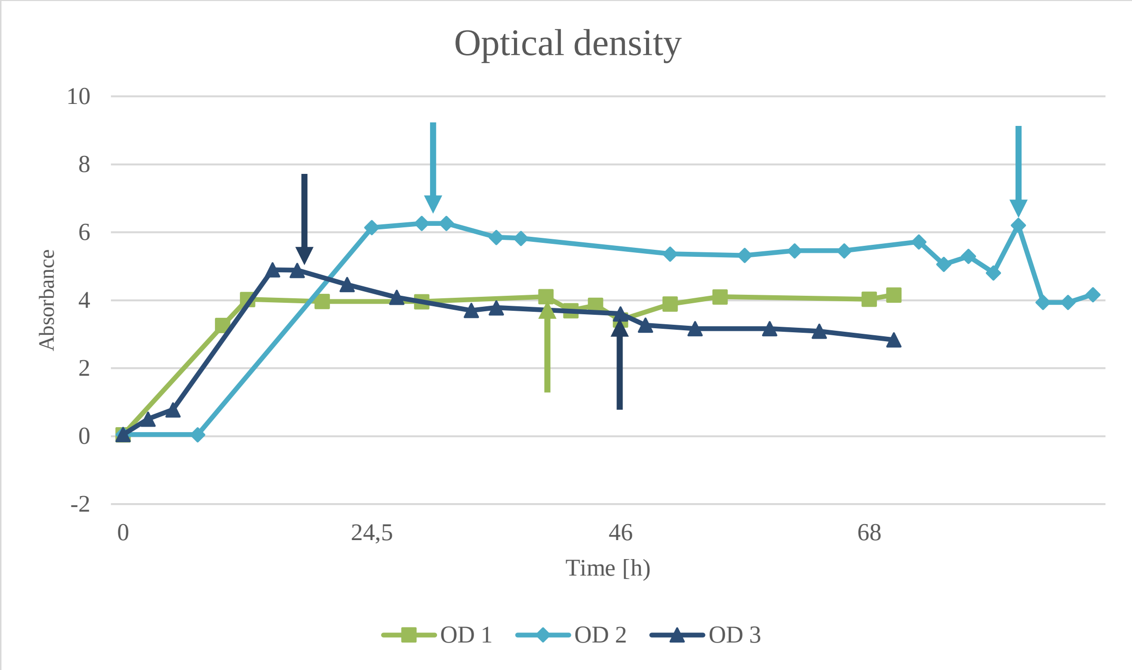


FIGURE 6. The optical density measured in all the three tests. The markers represent the sample collection while the arrows represent the points of feeding.

The initial growth phase is visible in all the figures 5, 6 and also in the following figure 7 presented below. From the 23BD production, sucrose utilization and optical density one can see that after the initial lag phase there is rapid growth of the bacteria which has also activated the production of 23BD. Figure 6 shows that after 24 hours the bacteria has reached its stationary phase depleting its carbohydrate source. It is clear from this information that *Basillus amyloliquefacien* is able to utilize sucrose in similar manner as glucose producing similar results. Early studies had shown that from 100 g of glucose, approximately 33g of 23BD could be produced in a typical fermentation procedure (Alam et al. 1989). When comparing results, table 1, from previous research sucrose can be accepted as a replacement for glucose. This gives an idea on the comparison since exactly the same tests haven’t been performed at this stage but should be treated critically. There are slight differences in the fermentation processes that effect the result and should be taken into consideration such as size difference of the bioreactor and difference on the air flow rate.

TABLE 1. Comparison on results gained from previous research with glucose and from the current research with sucrose. (Alam et al. 1989)

	Initial carbon source concentration [g/L]	Time [h]	Concentration of 23BD [g/L]
Previous research	57	55	21,90
Current research	60	54	34,69 (test 2)

During each of the experiments sucrose was observed to decrease while glucose and fructose levels were increasing. Figure 7 below shows the development during test 1 of sucrose breaking down to glucose and fructose. The bacteria *Bacillus amyloliquefaciens* possess the ability to metabolize sucrose as a carbon source through the extracellular enzyme levansucrase. The enzyme is produced during the active growth phase allowing the bacteria to break down sucrose to be able to utilize the glucose. (Mäntsälä. 1982) It could be assumed that the initial lag phase before growth phase is due to the bacteria activating and producing the enzyme to break the sucrose.

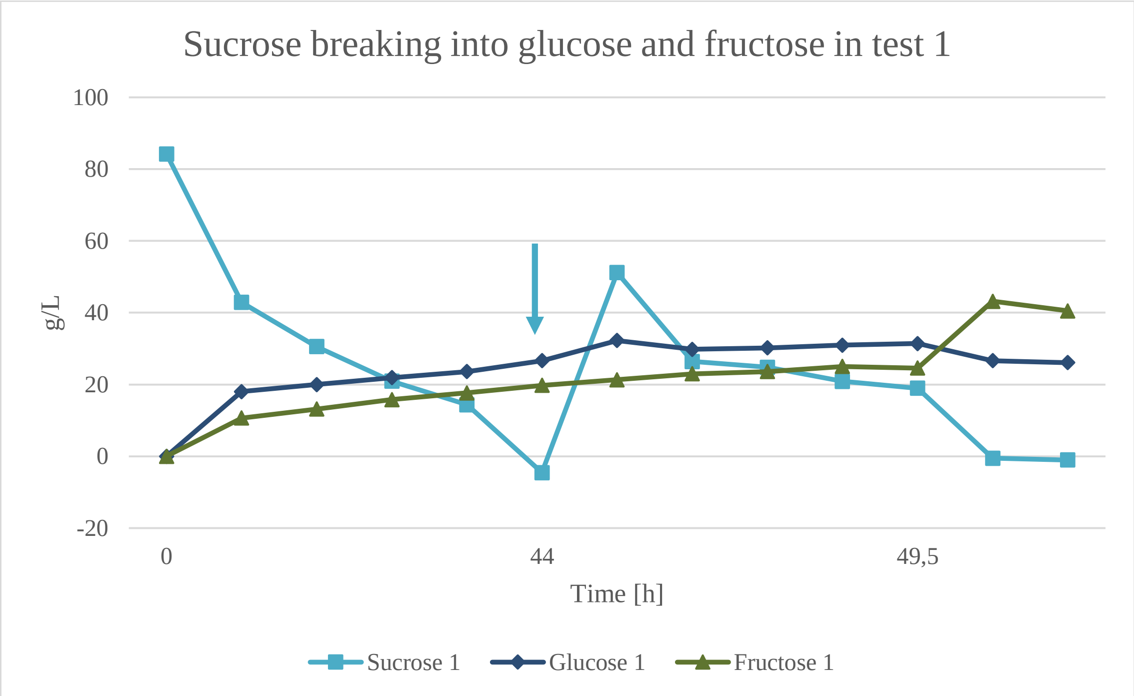


FIGURE 7. Development of sucrose breaking into glucose and fructose during test 1. The markers represent the sample collection while the arrow represents the point of feeding.

5.2 Adjusting oxygen percentage, air flow and pH

After adjusting the oxygen percentage to 50 % and the sensor sowing closer to 100 %, the percentage in each test dropped to zero after approximately 10 hours into the run as seen in the figure 8 below. During the growth phase the air flow was not adjusted. Due to the laboratory circumstances ensuring that there would not be problems during the night time the air flow was adjusted to a maximum of 4 L/m for the growth phase during the first test. In the second and third test the maximum was kept at 12 L/m during the growth phase. After the growth phase the air flow was adjusted to 2 or 4 L/m to have anaerobic conditions.

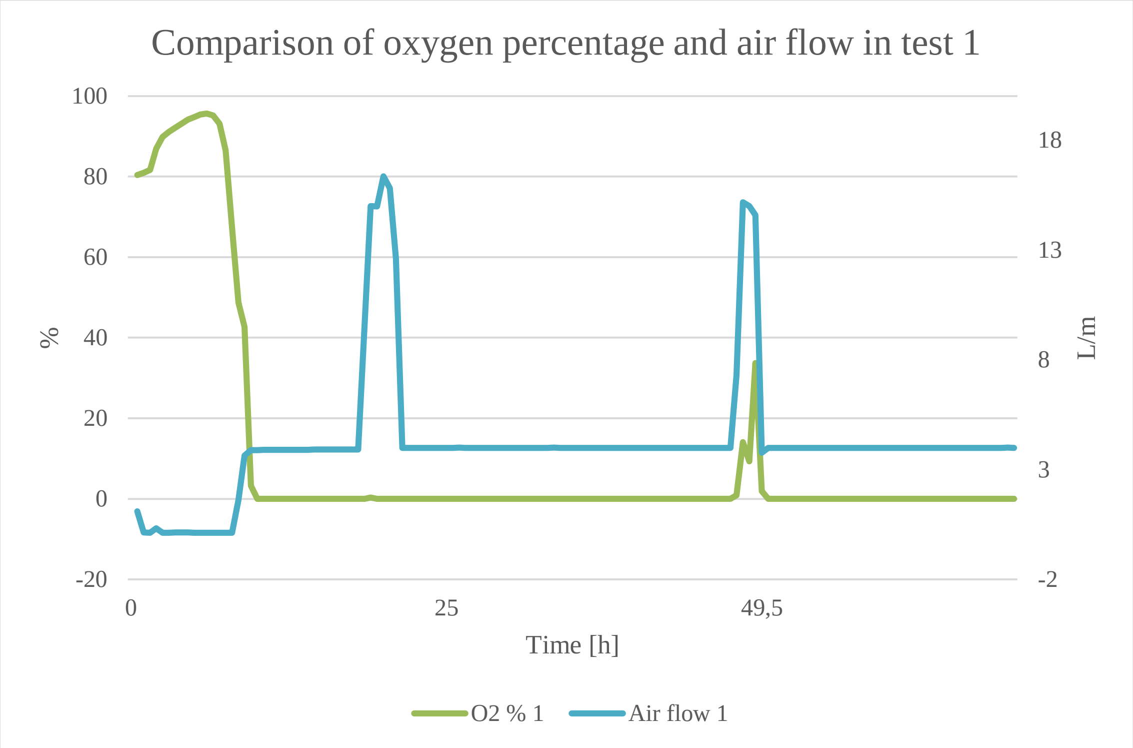


FIGURE 8. Comparison of the measured oxygen percentage in the medium to the air flow.

The drop in the oxygen percentage is related to the growth phase. During the experiment there was a first aerobic growth phase and second anaerobic production phase. The reason for why the oxygen percentage drops all the way to zero in unknown. Based on the air flow there should be oxygen in the medium which can also been seen when the air flow was adjusted to 20 L/m the oxygen percentage rose momentarily. There is possibility that the added anti-foaming agent could be responsible for changing the conditions inside the bioreactor. Some anti-foaming agents are known to affect the surface properties resulting in changes to the permeability of the medium. Silicon-based anti-foaming agents have been studied to negatively affect the mass transfer coefficient, gas hold up and gas velocity in the medium. (Routledge. 2012) This could explain as to why the sensor showed zero percent as the effect happened during the night time and the anti-foaming agent had been added to it prior this time. The sensor was tested out of the bioreactor and it was tested to be working. Immediately after being put back into the medium the oxygen percentage dropped gradually to zero.

Adjustments to the pH were also made as seen the figure 9 below. During the first run of the bioreactor, the pH was not adjusted. After approximately 10 hours the pH started drop for the starting value of approximately 6,3. In the second test the was adjusted to 6 to see if the bacteria were active and still producing 23BD and acetic acid. The level of acetic acid stayed low throughout each test without any significant changes and is therefore not presented here in the results. In the last run the pH was adjusted to 6 due to the possibility that the low pH is inhibiting the bacterial growth and 23BD production. After the first feeding the pH was allowed to drop naturally to see the if the bacteria are active. At the end of the test the pH was also allowed to drop.

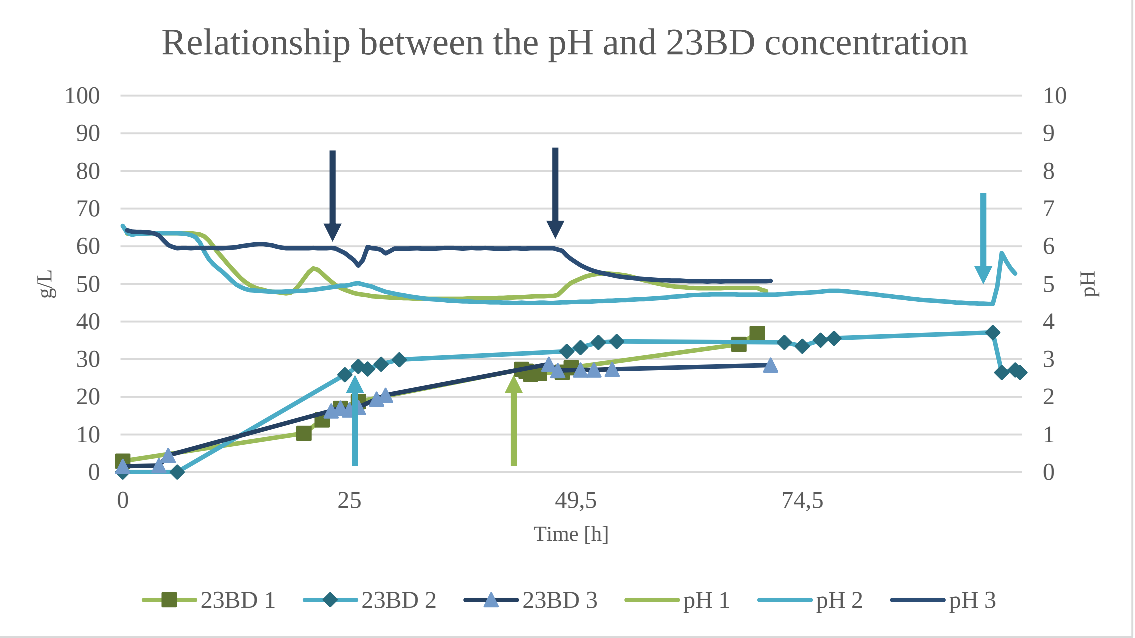


FIGURE 9. pH level measured during the three tests and the relationship to 23BD concentration. The three lines on top represent pH while the three below represent 23BD. The markers represent the sample collection while the arrows represent the points of feeding.

The drop in the pH is due to the production of acetic acid. As the measurements for the pH was done by the bioreactor system and from that a more precise moment when acetic acid and 23BD is produced can be determined. There were small fluctuations in the pH in each test which can be to some part explained to be due to the feeding. The feeding dilutes the media in the bioreactor and can lead to small rise in the pH. Comparing the results and observing the 23BD production it seems that the regulation of pH might not be necessary to the process. This can be also seen in a study done by Alam et al. (1989) where it was shown that there was no significant change in the production of 23BD when having controlled or uncontrolled pH level.

5.3 Combined analysis

Each test included data collected from the HPLC, bioreactor and spectrophotometer. Figure 10 shows the combined data from test one. The full graph shows the relationships between the different parameters in the tests. The sudden drop in oxygen percentage with the correlation to the optical density shows the start of the growth phase. The availability of oxygen determines the pathway in use; cell assimilation, respiration or fermentation (Alam et al., 1989) The drop in pH indicates production of acetic acid and 23BD. Based on the data it is possible to have a fed-batch for producing 23BD though it looks like there is either unidentified inhibiting factor or the bacteria have reached their maximum growth and start to die due to unfavourable conditions.

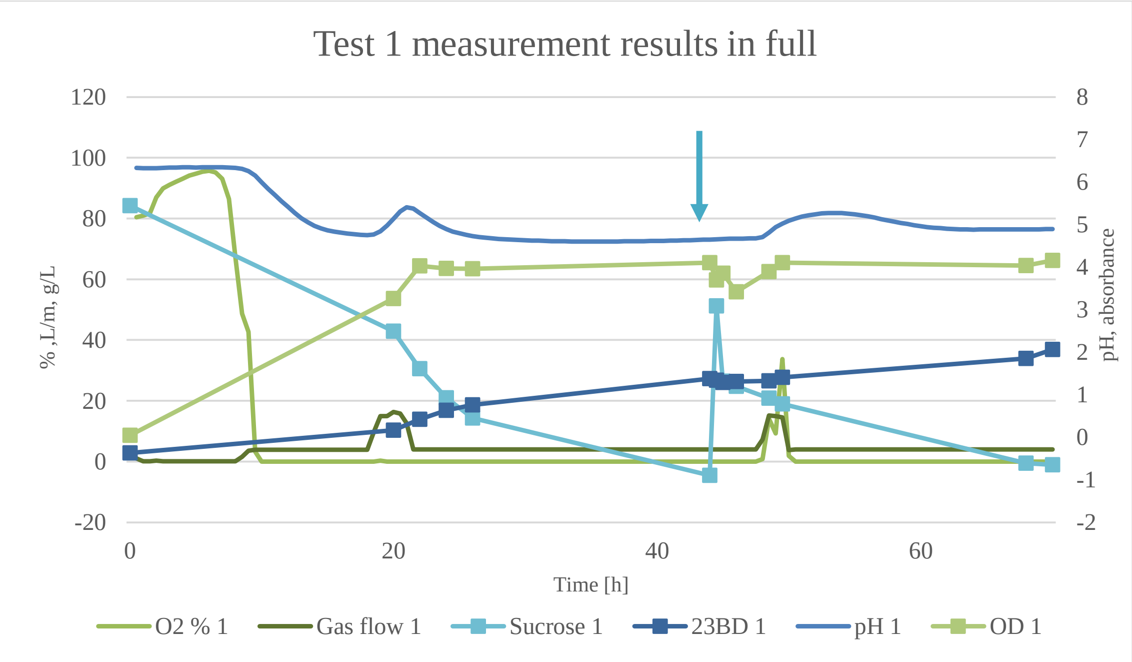


FIGURE 10. The full results of the measurement during test 1. The markers represent the sample collection while the arrow represents the point of feeding.

The amount of 23BD produced depend on the availability of the substrate and whether the bacteria are active. Due to the laboratory regulations collecting samples during night time was not possible. This meant that some important data was not collected. Especially in the beginning the starting point for growth and production of 23BD would be valuable information. With continuous data it would be possible to observe and predict the maximum growth and the need for feeding. The current data does not show if the peak had been already missed leading the bacteria to go to the stationary phase and some would already start to die. Once then fed the bacteria would not be active to produce 23BD at the maximum capacity.

The other clear problem faced during the tests was the drop in oxygen percentage. It is unclear as to what caused the sensor to not detect any oxygen in the medium which in turn made it harder to know if the bacteria were getting enough or too much oxygen during both aerobic growth phase and the anaerobic production phase.

Regarding of the pH regulation the results did not change. It was stated in a study by Alam et al. (1989) that with uncontrolled pH there is a clear fast drop in the pH which slows down the growth of the bacteria. As seen in the figure 10 as the pH reaches a certain level the growth of the bacteria slows down. It could be useful to only adjust pH during the growth phase. In figure 9 it can be seen that constant pH regulation could hinder the 23BD production but this should be tested to see if there is a connection.

5.4 Future work

The current state of the optimization of 23BD requires further studies to find the optimal conditions for the production. Running the bioreactor multiple times for different lengths and collecting data is vital. Especially having continuous data collection available is important to adjust the feeding times and the aerobic growth phase and the anaerobic production phase. Longer run in the bioreactor could provide more information on the maximum capabilities of the single inoculum. The next step would be to find out if the fed-batch could be turned into a continuous test and to see what option could be the best. Options such as replacing 80% of the medium to try and reactivate the bacteria allowing it more room to grow or having smaller quantities with more frequent feedings. It could also be that with this strain of bacteria that continuous is not possible and that there is a limit with the fed-batch as to how long the same inoculum could be used.

Apart from continuing the experiments, updating the equipment is an important step. Finding the reason for the drop in oxygen percentage or finding a new way to measure the oxygen percentage in the medium. Alongside the oxygen sensor it would be useful to invest on sensor to automatically measure the sucrose concentration in the medium. This would speed the process and there would not be need to predict or guess the proper time for the feeding. Further test for the need of pH regulations should be conducted.

Important to consider the options for downstream processing and trying to find the best possible addition to the process to make it complete. To further research the downstream processes it would be beneficial to identify other by-products in the process. Also calculating the cost of the production at the moment and compare the price of the product with the price in the market and make an assessment. Finding and replacing some of the medium components with cheaper options. Such as the peptone which is relatively expensive compared to the other components.

6 CONCLUSIONS

The experiments completed in the bioreactor presents sucrose as a cheaper and viable option to fully replace glucose. The bacteria break down the sucrose into glucose and fructose and are then able to utilize the glucose as the carbon source. Using the different phases for the production; aerobic and anaerobic, have shown to be successful although further experiments are required.

The analysis of the results shows good levels of 23BD produced in each test. Even though the feeding did not seem to rapidly increase the production of 23BD, the added sucrose solution kept the process running.

Future work on the project should be done on the matter of collecting continuous data to find more accurate feeding times to ensure maximum utilization of the bacteria. Updating the equipment for the oxygen sensor and for measuring sucrose concentration. Also studying possible replacements for the medium components and making cost calculations of the process with comparison to current market price.

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APPENDICES

Appendix 1. Table showing all the data collected during experiment 1.

Age (h)	pH 1	O2 % 1	Pressure 1	Stirring 1	Gas flow 1	Temperatu	Weight1	OD 1	Sucrose 1	23BD 1	Glucose 1	Fructose 1	Acetic acid 1
0								0,046	84,198	2,891	0,000	0,000	0,000
0,5	6,3345125	80,386	0,039	99,700	1,094	36,960	7,713						
1	6,32600335	80,956	-0,001	99,600	0,132	36,997	7,650						
1,5	6,32450167	81,641	-0,001	99,700	0,128	37,013	7,664						
2	6,32649168	86,910	0,001	99,800	0,331	36,998	7,678						
2,5	6,33547834	89,869	0,002	99,700	0,125	36,990	7,628						
3	6,34150165	91,082	0,002	99,700	0,126	36,990	7,620						
3,5	6,34349168	92,141	0,002	99,700	0,132	36,997	7,635						
4	6,34699666	93,093	0,002	99,600	0,134	36,998	7,642						
4,5	6,34550832	94,101	0,002	99,700	0,131	36,997	7,643						
5	6,34100667	94,804	0,002	99,700	0,128	36,998	7,635						
5,5	6,34448169	95,399	0,003	99,600	0,127	36,997	7,635						
6	6,34850498	95,670	0,003	99,700	0,129	36,998	7,635						
6,5	6,34600333	95,193	0,003	99,700	0,129	36,997	7,642						
7	6,34450167	93,089	0,003	99,600	0,129	36,990	7,650						
7,5	6,34051166	86,464	0,004	99,600	0,128	36,968	7,643						
8	6,33052166	67,342	0,003	99,600	0,127	36,982	7,635						
8,5	6,30904998	48,731	0,006	99,600	1,603	37,012	7,635						
9	6,26061162	42,711	0,010	99,700	3,629	37,013	7,635						
9,5	6,15224988	3,280	0,012	99,800	3,888	37,005	7,635						
10	5,99577165	0,000	0,013	99,800	3,888	37,005	7,628						
10,5	5,83825335	0,000	0,014	99,800	3,895	37,005	7,620						
11	5,68924334	0,000	0,014	99,800	3,899	37,005	7,620						
11,5	5,54822668	0,000	0,014	99,800	3,894	37,005	7,613						
12	5,412725	0,000	0,014	99,800	3,895	37,005	7,612						
12,5	5,27822333	0,000	0,014	99,800	3,903	37,005	7,627						
13	5,15419003	0,000	0,014	99,800	3,902	37,005	7,642						
13,5	5,05065503	0,000	0,014	99,800	3,897	37,005	7,643						
14	4,96762169	0,000	0,014	99,800	3,897	37,005	7,605						
14,5	4,9065817	0,000	0,014	99,800	3,910	37,005	7,582						
15	4,86605336	0,000	0,014	99,800	3,918	36,998	7,590						
15,5	4,83554834	0,000	0,014	99,800	3,909	36,997	7,597						
16	4,80953834	0,000	0,014	99,800	3,907	37,012	7,583						
16,5	4,79152168	0,000	0,014	99,800	3,912	37,013	7,575						
17	4,77702666	0,000	0,014	99,800	3,908	36,998	7,575						
17,5	4,761525	0,000	0,014	99,800	3,904	36,990	7,567						
18	4,74951501	0,000	0,014	99,800	3,909	36,997	7,553						
18,5	4,76443507	0,000	0,035	99,800	9,545	36,998	7,544						
19	4,84330178	0,322	0,056	99,800	14,984	37,005	7,508						
19,5	4,97625504	0,000	0,056	99,700	14,979	37,005	7,478						
20	5,13720837	0,000	0,066	99,600	16,347	36,997	7,477	3,261	42,856	10,279	18,041	10,649	0,402
20,5	5,30822166	0,000	0,076	99,700	15,802	36,998	7,323						
21	5,40595314	0,000	0,044	99,700	12,593	36,990	7,265						
21,5	5,37514984	0,000	0,012	99,600	3,987	36,997	7,239						
22	5,27468497	0,000	0,012	99,700	3,991	37,050	7,242	4,028	30,541	13,871	19,993	13,150	0,409
22,5	5,16318667	0,000	0,012	99,700	3,990	37,043	7,133						
23	5,05816335	0,000	0,012	99,600	3,983	36,990	7,095						
23,5	4,96714002	0,000	0,012	99,600	3,981	36,975	7,080						
24	4,89260836	0,000	0,013	99,600	3,985	36,960	7,070	3,968	20,941	16,898	21,904	15,792	0,421
24,5	4,83657836	0,000	0,013	99,600	3,988	36,960	6,825						
25	4,79356501	0,000	0,014	99,700	3,990	36,975	6,795						
25,5	4,75705667	0,000	0,014	99,800	3,991	36,990	6,777						
26	4,72903668	0,000	0,015	99,700	3,993	36,990	6,727	3,962	14,354	18,696	23,598	17,669	0,421
26,5	4,70952834	0,000	0,015	99,700	3,989	36,997	6,567						
27	4,69153166	0,000	0,014	99,800	3,987	36,998	6,532						
27,5	4,67352834	0,000	0,014	99,700	3,990	36,997	6,533						
28	4,66051501	0,000	0,014	99,600	3,990	37,005	6,532						
28,5	4,65051833	0,000	0,014	99,600	3,984	36,990	6,540						
29	4,64400335	0,000	0,015	99,600	3,985	36,990	6,533						
29,5	4,63701999	0,000	0,015	99,600	3,989	36,998	6,510						
30	4,63050168	0,000	0,015	99,600	3,988	36,990	6,510						
30,5	4,62601332	0,000	0,015	99,600	3,992	36,997	6,540						
31	4,62000667	0,000	0,015	99,700	3,990	36,998	6,540						
31,5	4,616505	0,000	0,015	99,800	3,990	36,983	6,532						
32	4,61201	0,000	0,015	99,800	3,986	36,982	6,540						
32,5	4,60800334	0,000	0,015	99,700	3,982	37,005	6,533						
33	4,60600333	0,000	0,015	99,600	3,988	37,005	6,525						
33,5	4,60151166	0,000	0,015	99,700	3,993	36,997	6,525						
34	4,60099002	0,000	0,015	99,700	3,989	37,012	6,510						
34,5	4,60001331	0,000	0,015	99,600	3,984	37,005	6,495						
35	4,59799335	0,000	0,015	99,700	3,985	36,990	6,495						

Age (h)	pH 1	O2 % 1	Pressure 1	Stirring 1	Gas flow 1	Temperatu	Weight1	OD 1	Sucrose 1	23BD 1	Glucose 1	Fructose 1	Acetic acid 1
35,5	4,601495	0,000	0,015	99,700	3,988	36,997	6,488						
36	4,59901332	0,000	0,015	99,700	3,990	36,998	6,487						
36,5	4,59799002	0,000	0,015	99,800	3,987	36,990	6,495						
37	4,602495	0,000	0,015	99,700	3,987	36,983	6,488						
37,5	4,60499667	0,000	0,015	99,600	3,989	36,990	6,473						
38	4,607495	0,000	0,015	99,700	3,991	37,012	6,472						
38,5	4,609	0,000	0,015	99,700	3,990	37,005	6,465						
39	4,60999667	0,000	0,015	99,600	3,985	37,005	6,465						
39,5	4,61349167	0,000	0,015	99,600	3,987	37,005	6,473						
40	4,61550166	0,000	0,015	99,600	3,988	36,997	6,458						
40,5	4,61649501	0,000	0,015	99,600	3,990	37,005	6,450						
41	4,62148834	0,000	0,015	99,700	3,987	36,998	6,443						
41,5	4,62649499	0,000	0,015	99,800	3,984	36,990	6,442						
42	4,62750166	0,000	0,015	99,800	3,984	36,997	6,450						
42,5	4,63198335	0,000	0,015	99,800	3,987	37,012	6,443						
43	4,63849499	0,000	0,015	99,800	3,986	37,013	6,435						
43,5	4,64249167	0,000	0,015	99,800	3,988	37,005	6,443						
44	4,646495	0,000	0,015	99,700	3,989	37,005	6,472	4,107	-4,527	27,28	26,645	19,779	0,651
44,5	4,65099	0,000	0,015	99,600	3,982	36,959	6,414	3,699	51,177	26,82	32,267	21,33	0,634
45	4,65948167	0,000	0,015	99,700	3,985	37,020	6,146	3,852	26,405	26,056	29,779	22,994	0,628
45,5	4,66749166	0,000	0,014	99,700	3,986	37,012	5,913						
46	4,66850499	0,000	0,014	99,600	3,984	37,050	5,835	3,422	24,808	26,297	30,182	23,598	0,635
46,5	4,66899334	0,000	0,015	99,700	3,983	37,035	5,588						
47	4,67448834	0,000	0,016	99,800	3,984	36,990	5,536						
47,5	4,67799999	0,000	0,016	99,700	3,984	37,005	5,566						
48	4,70441174	0,874	0,046	99,700	7,291	36,990	5,537						
48,5	4,81023515	14,072	0,067	99,700	15,166	37,056	5,517	3,891	20,889	26,555	30,969	25,021	0,669
49	4,93734158	9,275	0,057	99,700	14,985	36,945	5,278						
49,5	5,02337164	33,749	0,032	99,700	14,577	37,065	5,190	4,104	18,947	27,749	31,384	24,595	0,701
50	5,08841163	1,928	0,009	99,700	3,772	36,986	5,001						
50,5	5,13941833	0,000	0,013	99,700	3,987	36,990	4,972						
51	5,18642499	0,000	0,014	99,600	3,987	36,997	4,980						
51,5	5,22145831	0,000	0,015	99,600	3,990	37,005	4,973						
52	5,24396666	0,000	0,015	99,600	3,991	36,990	4,980						
52,5	5,26297	0,000	0,016	99,700	3,990	36,997	4,973						
53	5,27349498	0,000	0,017	99,700	3,984	37,013	4,950						
53,5	5,27300666	0,000	0,016	99,600	3,985	37,005	4,957						
54	5,26900667	0,000	0,016	99,600	3,990	37,005	4,958						
54,5	5,25952498	0,000	0,016	99,600	3,990	37,005	4,943						
55	5,24253166	0,000	0,016	99,600	3,989	37,005	4,950						
55,5	5,22004332	0,000	0,016	99,700	3,986	37,005	4,965						
56	5,19404333	0,000	0,016	99,800	3,985	37,012	4,958						
56,5	5,16455499	0,000	0,016	99,700	3,990	37,005	4,950						
57	5,13006	0,000	0,017	99,600	3,989	36,997	4,950						
57,5	5,095555	0,000	0,017	99,700	3,983	36,998	4,935						
58	5,06554501	0,000	0,017	99,800	3,985	36,983	4,920						
58,5	5,03655166	0,000	0,017	99,800	3,990	36,982	4,927						
59	5,01252835	0,000	0,016	99,700	3,990	36,990	4,928						
59,5	4,98805331	0,000	0,016	99,700	3,989	37,005	4,920						
60	4,96053835	0,000	0,017	99,700	3,988	37,013	4,913						
60,5	4,94252168	0,000	0,016	99,700	3,986	36,998	4,905						
61	4,92752833	0,000	0,017	99,800	3,982	36,990	4,912						
61,5	4,91451501	0,000	0,016	99,700	3,988	36,990	4,913						
62	4,90451833	0,000	0,017	99,700	3,992	36,990	4,890						
62,5	4,894515	0,000	0,017	99,800	3,990	37,005	4,875						
63	4,88750834	0,000	0,017	99,800	3,987	37,005	4,875						
63,5	4,88400334	0,000	0,017	99,700	3,987	37,012	4,882						
64	4,883	0,000	0,017	99,600	3,990	37,020	4,883						
64,5	4,88599001	0,000	0,017	99,600	3,984	37,005	4,875						
65	4,88600998	0,000	0,017	99,600	3,984	36,998	4,875						
65,5	4,88399668	0,000	0,017	99,700	3,987	36,990	4,868						
66	4,88599667	0,000	0,017	99,700	3,988	36,990	4,867						
66,5	4,888495	0,000	0,017	99,700	3,987	36,997	4,875						
67	4,88800666	0,000	0,017	99,800	3,981	36,983	4,875						
67,5	4,88799334	0,000	0,017	99,800	3,982	36,975	4,875						
68	4,88900333	0,000	0,017	99,800	3,988	37,065	4,876	4,035	-0,517	33,971	26,635	43,2	0,876
68,5	4,88899667	0,000	0,017	99,800	3,989	36,978	4,715						
69	4,89049833	0,000	0,017	99,800	3,988	36,998	4,628						
69,5	4,892495	0,000	0,017	99,700	3,993	36,990	4,590						
70	4,89300333	0,000	0,017	99,600	3,989	37,083	4,587	4,159	-1,034	36,853	26,108	40,5	0,823
70,5	4,83568818	59,929	0,073	94,080	2,173	47,400	4,288						
71	4,80192355	105,969	0,795	99,600	0,144	117,655	3,886						

Appendix 2. Table showing all the data collected during experiment 2.

Age (h)	pH 2	O2 % 2	Pressure 2	Stirring 2	Temperatu	Weight 2	AIr flow 2	OD 2	Sucrose 2	23-BDO 2	Glucose 2	Fructose 2	Acetic acid 2
0	6,543	25,200	0,041	100,2	24,465	7,995	9,615	0,049	88,112	0	0,000	0,000	0,000
0,5	6,355	91,979	0,021	100,08663	33,521	7,948	1,8491248						
1	6,303	109,151	0,005	99,877538	37,004	7,752	0,1089441						
1,5	6,325	108,048	0,005	99,798573	37,002	7,679	0,1243691						
2	6,334	106,098	0,005	99,677538	36,993	7,650	0,1309011						
2,5	6,338	104,439	0,005	99,6	36,993	7,650	0,1374748						
3													
3,5	6,343	102,436	0,005	99,6	36,993	7,658	0,1421417						
4	6,343	101,657	0,005	99,6	37,005	7,644	0,1391417						
4,5	6,343	101,010	0,005	99,6	36,996	7,650	0,1361631						
5	6,345	99,779	0,005	99,6	37,000	7,651	0,1350214						
5,5	6,344	97,746	0,005	99,6	37,032	7,629	0,1369583						
6	6,343	103,463	0,005	99,6	37,031	7,442	3,3849442	0,045	88,112	0	0,000	0,000	0,000
6,5	6,337	99,725	0,005	99,601427	36,993	7,407	0,1433261						
7	6,325	65,831	0,006	99,721035	36,984	7,395	0,6070375						
7,5	6,297	49,217	0,018	99,677538	36,990	7,395	4,6953083						
8	6,242	46,581	0,038	99,601427	37,008	7,386	10,168812						
8,5	6,098	22,674	0,047	99,722462	36,993	7,371	12,019617						
9	5,848	0,292	0,048	99,8	36,993	7,347	12,021816						
9,5	5,656	0,000	0,050	99,8	37,005	7,344	12,026241						
10	5,522	0,000	0,050	99,8	36,996	7,332	12,016823						
10,5	5,419	0,000	0,051	99,8	36,990	7,320	12,026567						
11	5,319	0,000	0,051	99,8	36,990	7,311	12,024957						
11,5	5,203	0,000	0,051	99,8	37,008	7,287	12,016163						
12	5,085	0,000	0,051	99,8	37,011	7,284	12,015128						
12,5	4,988	0,000	0,051	99,8	37,005	7,271	12,025936						
13	4,916	0,000	0,051	99,801427	37,014	7,233	12,025695						
13,5	4,866	0,000	0,051	99,921035	36,993	7,224	12,024738						
14	4,835	0,000	0,051	99,876111	36,984	7,212	12,032511						
14,5	4,820	0,000	0,051	99,678965	36,990	7,200	12,035936						
15	4,810	0,000	0,051	99,722462	36,990	7,182	12,030553						
15,5	4,802	0,000	0,051	99,8	36,990	7,179	12,032575						
16	4,796	0,000	0,051	99,8	36,999	7,158	12,041404						
16,5	4,792	0,000	0,050	99,8	37,005	7,149	12,035858						
17	4,792	0,000	0,051	99,8	37,014	7,136	12,033738						
17,5	4,792	0,000	0,051	99,8	37,002	7,107	12,041447						
18	4,794	0,000	0,051	99,8	36,981	7,095	12,039468						
18,5	4,799	0,000	0,051	99,8	36,993	7,095	12,034377						
19	4,807	0,000	0,051	99,801427	36,996	7,068	12,05122						
19,5	4,815	0,000	0,051	99,921035	36,990	7,059	12,05012						
20	4,818	0,000	0,052	99,877538	36,990	7,045	12,040206						
20,5	4,831	0,000	0,052	99,8	36,999	7,026	12,042717						
21	4,842	0,000	0,052	99,8	37,005	7,020	12,048567						
21,5	4,856	0,000	0,052	99,8	37,005	7,002	12,041815						
22	4,873	0,000	0,052	99,8	37,005	6,990	12,035893						
22,5	4,890	0,000	0,052	99,8	36,996	6,972	12,027072						
23	4,908	0,000	0,052	99,8	37,008	6,951	12,042958						
23,5	4,926	0,000	0,051	99,798573	37,003	6,944	12,049476						
24	4,948	0,000	0,024	99,678965	37,077	6,930	10,306529						
24,5	4,952	0,008	0,034	99,722462	36,973	6,800	9,9666945	6,143	11,329	25,83	20,134	25,013	0,556
25	4,969	0,581	0,050	99,8	37,005	6,738	12,025807						
25,5	4,997	0,171	0,051	99,8	36,996	6,709	11,998845						
26	5,017	0,000	0,051	99,8	36,999	6,473	10,88463	6,263	8,848	28,096	20,020	25,554	0,573
26,5	4,986	0,000	0,025	99,798573	37,025	6,826	2,8277078						
26,75								6,263	27,139	27,367	25,363	33,349	0,549
27	4,927	0,000	0,009	99,678965	37,014	6,793	1,9901417						
27,5	4,874	0,000	0,009	99,722462	36,984	6,745	1,9871417						
28	4,829	0,000	0,009	99,798573	36,987	6,546	1,9842273	5,854	24,877	28,701	25,997	33,494	0,550
28,5	4,791	0,000	0,009	99,677538	36,996	6,458	1,9884466						
29	4,761	0,000	0,009	99,6	37,000	6,426	1,9865106						
29,5	4,733	0,000	0,009	99,6	37,081	6,437	1,9848369						
30	4,714	0,000	0,009	99,6	36,965	6,256	1,9859786	5,828	22,464	29,843	25,412	35,012	0,569
30,5	4,691	0,000	0,009	99,6	36,990	6,264	1,9841631						
31	4,672	0,000	0,009	99,6	36,999	6,252	1,9830428						
31,5	4,655	0,000	0,010	99,6	37,005	6,258	1,9866953						
32	4,635	0,000	0,010	99,6	37,014	6,270	1,9907727						
32,5	4,615	0,000	0,010	99,6	37,011	6,261	1,9865748						
33	4,604	0,000	0,010	99,601427	37,014	6,264	1,9903263						
33,5	4,595	0,000	0,010	99,721035	37,020	6,261	1,9931631						
34	4,585	0,000	0,011	99,677538	37,011	6,264	1,991893						
34,5	4,573	0,000	0,011	99,6	37,005	6,252	1,9828795						
35	4,564	0,000	0,011	99,6	36,996	6,240	1,9824894						

Age (h)	pH 2	O2 % 2	Pressure 2	Stirring 2	Temperatu	Weight 2	AIr flow 2	OD 2	Sucrose 2	23-BDO 2	Glucose 2	Fructose 2	Acetic acid 2
35,5	4,554	0,000	0,011	99,6	36,999	6,231	1,9841203						
36	4,547	0,000	0,011	99,601427	37,014	6,252	1,9793475						
36,5	4,538	0,000	0,011	99,721035	37,011	6,252	1,9788797						
37	4,533	0,000	0,011	99,677538	36,996	6,231	1,9836953						
37,5	4,530	0,000	0,011	99,601427	36,990	6,234	1,9878155						
38	4,521	0,000	0,011	99,721035	37,008	6,231	1,9871417						
38,5	4,517	0,000	0,012	99,677538	37,020	6,234	1,9842059						
39	4,516	0,000	0,012	99,6	37,011	6,231	1,9866525						
39,5	4,514	0,000	0,012	99,6	36,996	6,215	1,9871845						
40	4,511	0,000	0,012	99,6	36,999	6,230	1,9877941						
40,5	4,509	0,000	0,012	99,6	36,987	6,231	1,9853261						
41	4,509	0,000	0,012	99,601427	36,993	6,225	1,9830642						
41,5	4,503	0,000	0,012	99,721035	36,996	6,225	1,9884894						
42	4,499	0,000	0,012	99,677538	36,999	6,225	1,9901203						
42,5	4,494	0,000	0,012	99,6	37,005	6,207	1,9854545						
43	4,494	0,000	0,012	99,6	37,005	6,213	1,9939146						
43,5	4,496	0,000	0,012	99,601427	37,005	6,234	1,9918153						
44	4,495	0,000	0,012	99,721035	37,005	6,230	1,9859786						
44,5	4,493	0,000	0,012	99,677538	37,014	6,198	1,9841631						
45	4,494	0,000	0,012	99,6	37,002	6,189	1,9830214						
45,5	4,496	0,000	0,012	99,6	36,999	6,195	1,9848369						
46	4,495	0,000	0,013	99,6	37,005	6,195	1,9859786						
46,5	4,495	0,000	0,013	99,601427	37,005	6,195	1,9842059						
47	4,494	0,000	0,013	99,721035	36,996	6,186	1,9866311						
47,5	4,499	0,000	0,012	99,677538	36,990	6,172	1,9852619						
48	4,504	0,000	0,013	99,6	37,008	6,171	1,9776176						
48,5	4,508	0,000	0,013	99,601427	37,002	6,065	1,984936	5,363	11,548	32,109	33,762	26,461	0,864
49	4,514	0,000	0,013	99,722462	36,999	6,014	1,9846737						
49,5	4,520	0,000	0,012	99,798573	37,014	5,965	1,9818369						
50	4,525	0,000	0,012	99,677538	37,001	5,789	1,9830214	5,321	10,809	33,054	31,831	29,518	0,855
50,5	4,529	0,000	0,013	99,6	36,972	5,724	1,9848369						
51	4,529	0,000	0,013	99,6	36,978	5,730	1,9859786						
51,5	4,534	0,000	0,013	99,6	36,999	5,741	1,9842059						
52	4,541	0,000	0,013	99,6	36,996	5,561	1,9866739	5,459	10,087	34,456	30,897	30,314	0,823
52,5	4,544	0,000	0,013	99,6	36,990	5,477	1,9890214						
53	4,549	0,000	0,013	99,6	36,990	5,460	1,9908155						
53,5	4,552	0,000	0,013	99,6	36,993	5,436	1,9901203						
54	4,556	0,000	0,012	99,601427	36,999	5,167	1,9853475	5,460	9,565	34,685	30,148	31,499	0,801
54,5	4,564	0,000	0,013	99,721035	37,005	5,127	1,9848155						
55	4,570	0,000	0,013	99,677538	37,014	5,142	1,9842487						
55,5	4,576	0,000	0,013	99,6	37,002	5,142	1,9903263						
56	4,585	0,000	0,013	99,6	36,990	5,130	1,9930561						
56,5	4,592	0,000	0,012	99,6	36,999	5,130	1,9828795						
57	4,596	0,000	0,012	99,6	37,005	5,121	1,9824894						
57,5	4,601	0,000	0,013	99,6	37,005	5,124	1,9842273						
58	4,608	0,000	0,013	99,6	36,996	5,139	1,9884466						
58,5	4,617	0,000	0,013	99,6	36,999	5,136	1,9865106						
59	4,626	0,000	0,013	99,6	37,005	5,148	1,9848369						
59,5	4,638	0,000	0,013	99,6	37,005	5,133	1,9860428						
60	4,651	0,000	0,013	99,601427	36,996	5,133	1,9896525						
60,5	4,661	0,000	0,013	99,722462	37,008	5,127	1,9900989						
61	4,671	0,000	0,012	99,798573	37,011	5,115	1,983532						
61,5	4,680	0,011	0,012	99,677538	37,014	5,115	1,9836953						
62	4,692	4,811	0,013	99,6	37,011	5,115	1,9877941						
62,5	4,701	29,727	0,013	99,6	37,005	5,133	1,9853689						
63	4,708	60,902	0,013	99,6	36,996	5,136	1,9866525						
63,5	4,711	84,916	0,013	99,6	36,999	5,130	1,9871631						
64	4,716	97,419	0,013	99,6	37,005	5,121	1,986						
64,5	4,720	102,599	0,013	99,6	37,005	5,124	1,9860428						
65	4,720	104,281	0,013	99,6	36,996	5,121	1,9896311						
65,5	4,717	104,568	0,013	99,6	36,999	5,124	1,9883261						
66	4,717	104,232	0,013	99,6	36,987	5,130	1,986						
66,5	4,719	103,692	0,013	99,6	36,984	5,130	1,9860214						
67	4,717	102,816	0,013	99,6	36,990	5,121	1,9878583						
67,5	4,714	101,447	0,013	99,6	37,008	5,124	1,9907941						
68	4,715	99,652	0,013	99,6	37,011	5,121	1,9883261						
68,5	4,714	97,123	0,013	99,6	36,987	5,106	1,986						
69	4,713	93,790	0,013	99,6	36,993	5,109	1,9860428						
69,5	4,711	89,430	0,013	99,601427	36,996	5,106	1,9896311						
70	4,710	83,788	0,013	99,721035	37,008	5,091	1,9883261						

Age (h)	pH 2	O2 % 2	Pressure 2	Stirring 2	Temperatu	Weight 2	Air flow 2	OD 2	Sucrose 2	23-BDO 2	Glucose 2	Fructose 2	Acetic acid 2
70,5	4,711	76,616	0,013	99,677538	37,011	5,085	1,9859358						
71	4,713	67,763	0,013	99,601427	37,005	5,085	1,9805962						
71,5	4,716	56,077	0,013	99,722462	36,996	5,104	1,9861205						
72	4,719	41,918	0,013	99,8	37,044	5,127	1,9864892						
72,5	4,727	26,170	0,013	99,798573	37,025	4,956	1,9830214	5,721	7,031	34,441	33,434	27,497	0,943
73	4,738	7,894	0,013	99,677538	36,999	4,857	1,9848155						
73,5	4,746	0,120	0,013	99,6	36,987	4,836	1,9841845						
74	4,750	0,000	0,012	99,6	36,978	4,831	1,9848155						
74,5	4,754	0,000	0,012	99,6	37,023	4,754	1,9841845	5,058	6,784	33,403	31,193	31,453	0,934
75	4,759	0,000	0,013	99,601427	36,978	4,617	1,9848797						
75,5	4,770	0,000	0,013	99,722462	36,999	4,659	1,9895883						
76	4,781	0,000	0,013	99,798573	36,975	4,605	1,9846951						
76,5	4,792	0,000	0,013	99,677538	37,014	4,347	1,9837167	5,296	6,82	35,08	30,157	32,562	0,896
77	4,806	0,000	0,013	99,6	36,999	4,305	1,9895669						
77,5	4,810	0,000	0,011	99,6	37,004	4,292	1,9829223						
78	4,813	0,000	0,011	99,6	37,024	3,993	1,9861205	4,810	6,589	35,577	29,269	34,419	0,886
78,5	4,812	0,000	0,013	99,6	37,002	3,960	1,9864678						
79	4,805	0,000	0,013	99,6	36,999	3,951	1,9812273						
79,5	4,797	0,000	0,013	99,6	37,014	3,963	1,985468						
80	4,783	0,000	0,013	99,6	37,002	3,957	1,9853689						
80,5	4,770	0,000	0,013	99,6	36,999	3,945	1,9866739						
81	4,758	0,000	0,013	99,6	37,005	3,954	1,9889786						
81,5	4,744	0,000	0,013	99,6	36,996	3,960	1,9871845						
82	4,732	0,000	0,013	99,6	36,999	3,951	1,9878797						
82,5	4,718	0,000	0,013	99,6	36,987	3,954	1,9926097						
83	4,700	0,000	0,013	99,6	36,984	3,951	1,989425						
83,5	4,687	0,000	0,013	99,6	36,999	3,945	1,9805534						
84	4,675	0,000	0,013	99,6	37,014	3,954	1,9825536						
84,5	4,658	0,000	0,013	99,6	37,011	3,960	1,9896953						
85	4,642	0,000	0,013	99,6	36,996	3,960	1,9938369						
85,5	4,631	0,000	0,013	99,6	36,999	3,933	1,9950214						
86	4,614	0,000	0,013	99,6	36,987	3,933	1,9967299						
86,5	4,601	0,000	0,013	99,6	36,975	3,945	1,9888153						
87	4,590	0,000	0,013	99,6	37,002	3,936	1,983						
87,5	4,578	0,000	0,013	99,6	37,011	3,939	1,9830856						
88	4,568	0,000	0,013	99,6	36,996	3,954	1,9902621						
88,5	4,555	0,000	0,013	99,6	36,999	3,960	1,9876737						
89	4,547	0,000	0,013	99,6	37,005	3,951	1,9848583						
89,5	4,540	0,000	0,013	99,6	37,005	3,954	1,9878155						
90	4,530	0,000	0,013	99,6	36,996	3,951	1,9872273						
90,5	4,524	0,000	0,013	99,6	36,999	3,954	1,9914466						
91	4,514	0,000	0,013	99,6	36,987	3,951	1,9894678						
91,5	4,502	0,000	0,013	99,6	36,984	3,954	1,9842059						
92	4,496	0,000	0,013	99,6	36,999	3,960	1,9866311						
92,5	4,489	0,000	0,013	99,6	37,014	3,942	1,9853261						
93	4,484	0,000	0,013	99,6	37,002	3,921	1,983						
93,5	4,480	0,000	0,013	99,6	36,981	3,924	1,9830214						
94	4,474	0,000	0,014	99,6	36,993	3,921	1,9848369						
94,5	4,474	0,000	0,013	99,6	37,005	3,916	1,9859786						
95	4,468	0,001	0,013	99,6	37,002	3,980	1,9842059						
95,5	4,466	0,341	0,014	99,6	36,992	4,790	1,9866097	6,212	3,217	37,051	34,19	24,905	1,284
96	4,927	0,000	0,014	99,6	37,018	5,651	1,9835748						
96,5	5,818	0,000	0,013	99,6	37,023	5,526	1,9872835	3,941	34,65	26,438	29,114	26,513	0,833
97	5,592	0,000	0,013	99,6	37,014	5,424	1,9865106						
97,5	5,413	0,000	0,011	99,6	37,010	5,414	1,9848369						
98	5,275	0,000	0,012	99,6	36,979	5,251	1,9860428	3,941	33,981	27,104	28,995	27,804	0,829
98,75								4,169	31,407	26,42	27,861	27,913	0,786

Appendix 3. Table showing all the data collected during experiment 3.

Age (h)	pH 3	O2 % 3	Pressure 3	Stirring 3	Temperature 3	Weight 3	Air flow 3	Added base	OD 3	Sucrose 3	23BD 3	Glucose 3	Fructose 3	Acetic acid
0									0,051	85,638	1,575	0,000	0,000	0,000
0,5	6,423	65,303	0,039	98,353	37,088	7,910	10,870265	0,000						
1	6,388	120,185	0,074	99,600	36,990	7,740	11,85292	0,000						
1,5	6,381	120,431	0,072	99,600	37,005	7,916	11,902415	0,000						
2	6,376	119,630	0,069	99,600	36,990	8,118	11,929495	0,000						
2,5	6,372	118,409	0,069	99,600	36,975	8,058	11,938475	0,000						
3	6,361	114,960	0,069	99,600	36,975	8,067	11,97889	0,000						
3,5	6,336	106,907	0,036	99,600	37,012	8,036	11,62967	0,000						
4	6,282	82,224	0,036	99,600	37,020	8,076	10,509129	0,150	0,507	86,924	1,702	0,000	0,000	0,000
4,5	6,147	42,205	0,068	99,600	37,012	8,021	11,35357	0,300						
5	6,027	1,661	0,069	99,600	36,998	7,915	11,94299	0,474	0,783	85,98	4,447	0,000	0,000	0,421
5,5	5,976	0,000	0,069	99,600	37,037	7,775	10,659453	0,725						
6	5,947	0,000	0,070	99,600	36,990	7,583	11,952	0,875						
6,5	5,951	0,000	0,070	99,600	36,990	7,568	11,96097	1,000						
7	5,950	0,000	0,070	99,600	36,997	7,545	11,962525	1,075						
7,5	5,946	0,000	0,070	99,700	36,990	7,523	11,959485	1,125						
8	5,949	0,000	0,070	99,700	36,990	7,515	11,992405	1,175						
8,5	5,950	0,000	0,071	99,600	37,005	7,523	11,992595	1,225						
9	5,946	0,000	0,071	99,600	37,005	7,500	11,965495	1,275						
9,5	5,950	0,000	0,071	99,600	36,998	7,478	11,96102	1,350						
10	5,953	0,000	0,071	99,600	36,997	7,455	11,95799	1,425						
10,5	5,946	0,000	0,072	99,600	36,998	7,440	11,959505	1,475						
11	5,945	0,000	0,072	99,700	36,997	7,440	11,96099	1,525						
11,5	5,951	0,000	0,072	99,700	37,005	7,440	11,968485	1,550						
12	5,960	0,000	0,071	99,600	36,990	7,418	11,96403	1,550						
12,5	5,973	0,000	0,072	99,600	36,982	7,395	11,96098	1,550						
13	5,992	0,000	0,072	99,600	36,990	7,388	11,96401	1,550						
13,5	6,013	0,000	0,072	99,600	36,990	7,373	11,962495	1,550						
14	6,030	0,000	0,072	99,700	36,990	7,365	11,95802	1,550						
14,5	6,043	0,000	0,073	99,700	36,990	7,343	11,96695	1,550						
15	6,055	0,000	0,073	99,700	36,975	7,335	11,97602	1,550						
15,5	6,055	0,000	0,073	99,700	36,982	7,335	11,96103	1,550						
16	6,040	0,000	0,073	99,600	37,005	7,320	11,952	1,550						
16,5	6,020	0,000	0,073	99,700	36,998	7,313	11,956485	1,550						
17	5,991	0,000	0,073	99,800	36,990	7,283	11,953525	1,550						
17,5	5,960	0,000	0,073	99,700	37,005	7,260	11,944505	1,550						
18	5,947	0,000	0,074	99,600	37,013	7,260	11,95197	1,575						
18,5	5,946	0,000	0,074	99,600	37,005	7,230	11,95801	1,650						
19	5,948	0,000	0,074	99,600	36,998	7,215	11,953505	1,750						
19,5	5,945	0,000	0,074	99,600	36,997	7,215	11,9819	1,850						
20	5,944	0,000	0,074	99,600	37,005	7,200	11,97911	1,975						
20,5	5,946	0,000	0,074	99,600	37,005	7,193	11,94301	2,100						
21	5,952	0,000	0,074	99,700	37,005	7,170	11,944485	2,225						
21,5	5,949	0,000	0,074	99,700	37,005	7,155	11,949	2,350						
22	5,944	0,000	0,075	99,600	37,005	7,140	11,94601	2,475						
22,5	5,947	0,000	0,075	99,600	37,005	7,118	11,941505	2,625						
23	5,950	0,000	0,075	99,700	36,990	7,140	11,94	2,750	4,901	-5,32	16,23	28,619	25,828	1,253
23,5	5,940	0,000	0,042	99,700	37,079	7,168	8,8207925	2,900						
23,75									4,883	-5,333	17,004	28,39	25,915	1,282
24	5,819	0,000	0,010	99,600	37,043	7,037	1,987515	3,000						
24,25									4,465	18,84	16,524	34,208	29,897	1,219
24,5	5,629	0,000	0,010	99,600	36,939	7,173	1,987485	3,000						
25	5,483	0,000	0,010	99,600	37,020	7,257	1,987515	3,000	4,091	14,089	17,174	35,173	31,888	1,23
25,5	5,629	0,000	0,010	99,700	36,975	7,168	1,984495	3,349						
26	5,979	0,000	0,010	99,700	36,975	7,014	1,986	3,825						
26,5	5,943	0,000	0,010	99,600	37,005	6,983	1,984505	4,050						
27	5,938	0,000	0,010	99,600	36,990	6,975	1,984495	4,250	3,703	6,692	19,384	36,274	33,519	1,352
27,5	5,907	0,000	0,010	99,600	37,047	6,979	1,986	4,425						
28	5,809	0,000	0,010	99,600	36,975	6,860	1,986	4,500	3,783	3,854	20,452	36,586	34,41	1,388
28,5	5,866	0,000	0,010	99,600	37,055	6,761	1,986	4,650						
29	5,940	0,000	0,010	99,600	36,975	6,608	1,986	4,900						
29,5	5,933	0,000	0,010	99,600	36,990	6,578	1,984505	5,075						
30	5,934	0,000	0,010	99,600	36,997	6,585	1,984495	5,250						
30,5	5,934	0,000	0,010	99,600	36,998	6,608	1,986	5,425						
31														
31,5	5,943	0,000	0,011	99,600	36,982	6,600	1,987485	5,775						
32	5,938	0,000	0,011	99,600	36,998	6,600	1,987515	5,925						
32,5	5,935	0,000	0,011	99,600	36,997	6,607	1,984495	6,100						
33	5,935	0,000	0,011	99,600	37,012	6,608	1,986	6,275						
33,5	5,937	0,000	0,011	99,600	37,020	6,600	1,98301	6,450						
34	5,941	0,000	0,011	99,600	37,013	6,593	1,981495	6,625						
34,5	5,951	0,000	0,012	99,600	36,998	6,592	1,98599	6,800						
35	5,952	0,000	0,012	99,600	36,990	6,600	1,98302	6,975						

Age (h)	pH 3	O2 % 3	Pressure 3	Stirring 3	Temperature 3	Weight 3	Air flow 3	Added base	OD 3	Sucrose 3	23BD 3	Glucose 3	Fructose 3	Acetic acid 3
35,5	5,949	0,000	0,012	99,600	36,997	6,600	1,97999	7,125						
36	5,944	0,000	0,012	99,600	37,012	6,600	1,983	7,300						
36,5	5,937	0,000	0,012	99,600	37,005	6,607	1,983	7,475						
37	5,947	0,000	0,012	99,600	36,997	6,615	1,987485	7,650						
37,5	5,950	0,000	0,012	99,600	36,998	6,608	1,98901	7,825						
38	5,942	0,000	0,012	99,600	37,005	6,600	1,984505	7,975						
38,5	5,944	0,000	0,012	99,600	37,005	6,600	1,987485	8,125						
39	5,950	0,000	0,012	99,600	36,997	6,607	1,993495	8,300						
39,5	5,949	0,000	0,012	99,600	36,998	6,600	1,98902	8,475						
40	5,941	0,000	0,012	99,600	36,990	6,600	1,98599	8,625						
40,5	5,937	0,000	0,012	99,600	36,997	6,622	1,98302	8,775						
41	5,934	0,000	0,012	99,600	36,998	6,615	1,981485	8,925						
41,5	5,938	0,000	0,013	99,600	36,983	6,600	1,986	9,075						
42	5,948	0,000	0,013	99,600	36,990	6,600	1,986	9,225						
42,5	5,946	0,000	0,012	99,600	37,005	6,600	1,987495	9,375						
43	5,935	0,000	0,013	99,600	36,998	6,600	1,987505	9,525						
43,5	5,938	0,000	0,013	99,600	36,990	6,593	1,986	9,675						
44	5,944	0,000	0,013	99,600	36,997	6,592	1,984505	9,825						
44,5	5,948	0,000	0,012	99,600	37,005	6,600	1,98599	9,975						
45	5,944	0,000	0,012	99,600	36,998	6,607	1,990495	10,125						
45,5	5,944	0,000	0,013	99,600	37,005	6,600	1,98901	10,275						
46	5,947	0,000	0,013	99,600	37,005	6,592	1,98899	10,425	3,604	-3,167	28,673	32,188	37,917	1,667
46,5	5,944	0,000	0,013	99,600	37,058	6,589	1,990505	10,575						
46,75									3,269	22,077	26,951	34,443	46,814	1,471
47	5,875	0,000	0,013	99,600	36,991	7,002	1,989	10,650						
47,5	5,749	0,000	0,013	99,600	37,003	6,800	1,98302	10,650						
48	5,653	0,000	0,013	99,600	36,998	6,735	1,97999	10,650						
48,5	5,570	0,000	0,013	99,600	36,990	6,712	1,987485	10,650						
49	5,498	0,000	0,014	99,600	36,997	6,713	1,987515	10,650	3,165	17,286	27,167	34,34	49,344	1,455
49,5	5,437	0,000	0,013	99,600	37,107	6,696	1,987485	10,650						
50	5,386	0,000	0,013	99,600	37,013	6,545	1,992	10,650						
50,5	5,345	0,000	0,014	99,600	36,997	6,488	1,987515	10,650	3,168	15,017	27,142	34,439	49,907	1,455
51	5,310	0,000	0,014	99,600	36,975	6,459	1,983	10,650						
51,5	5,281	0,000	0,014	99,600	36,982	6,327	1,987485	10,650						
52	5,254	0,000	0,014	99,600	36,998	6,281	1,98901	10,650						
52,5	5,230	0,000	0,014	99,600	37,036	6,169	1,987495	10,650	3,093	13,241	27,34	34,834	51,006	1,494
53	5,208	0,000	0,015	99,600	36,990	6,053	1,987505	10,650						
53,5	5,188	0,000	0,014	99,600	36,990	6,030	1,987495	10,650						
54	5,174	0,000	0,014	99,600	36,997	6,030	1,987505	10,650						
54,5	5,164	0,000	0,014	99,600	37,012	6,030	1,987495	10,650						
55	5,150	0,000	0,014	99,600	37,020	6,037	1,98601	10,650						
55,5	5,137	0,000	0,014	99,600	37,005	6,038	1,98599	10,650						
56	5,126	0,000	0,014	99,600	36,997	6,030	1,989	10,650						
56,5	5,118	0,000	0,014	99,600	36,990	6,015	1,984515	10,650						
57	5,113	0,000	0,014	99,600	36,982	6,030	1,98299	10,650						
57,5	5,104	0,000	0,014	99,600	36,990	6,030	1,984505	10,650						
58	5,098	0,000	0,014	99,600	36,997	6,015	1,984495	10,650						
58,5	5,096	0,000	0,014	99,600	37,012	6,030	1,986	10,650						
59	5,089	0,000	0,014	99,600	37,020	6,023	1,987495	10,650						
59,5	5,085	0,000	0,014	99,600	37,013	6,008	1,989	10,650						
60	5,082	0,000	0,015	99,600	36,998	6,007	1,984515	10,650						
60,5	5,077	0,000	0,015	99,600	36,990	6,008	1,98	10,650						
61	5,073	0,000	0,014	99,600	36,997	6,000	1,981495	10,650						
61,5	5,072	0,000	0,014	99,600	36,990	6,000	1,984495	10,650						
62	5,071	0,000	0,014	99,600	36,990	5,993	1,986	10,650						
62,5	5,066	0,000	0,014	99,600	37,005	6,000	1,98899	10,650						
63	5,064	0,000	0,014	99,600	37,005	6,008	1,98901	10,650						
63,5	5,068	0,000	0,014	99,600	37,005	5,993	1,990485	10,650						
64	5,065	0,000	0,014	99,600	36,998	5,992	1,990515	10,650						
64,5	5,063	0,000	0,014	99,600	36,997	6,000	1,98301	10,650						
65	5,065	0,000	0,015	99,600	37,005	6,000	1,98598	10,650						
65,5	5,066	0,000	0,015	99,600	37,005	6,000	1,98901	10,650						
66	5,065	0,000	0,014	99,600	37,012	6,000	1,987495	10,650						
66,5	5,066	0,000	0,015	99,600	37,013	6,000	1,987505	10,650						
67	5,068	0,000	0,015	99,600	36,998	6,000	1,986	10,650						
67,5	5,068	0,000	0,015	99,600	36,997	5,993	1,984505	10,650						
68	5,069	0,000	0,015	99,600	36,998	5,985	1,983	10,650						
68,5	5,070	0,000	0,015	99,600	36,997	5,985	1,981505	10,650						
69	5,071	0,000	0,015	99,600	37,005	6,000	1,98299	10,650						
69,5	5,071	0,000	0,014	99,600	36,990	6,008	1,986	10,650						
70	5,075	0,000	0,014	99,600	36,982	5,985	1,98899	10,650	2,839	0,749	28,403	42,926	46,964	1,811