A comparison of the effects glucose and fructose have on the growth rate of activated sludge in an activated sludge process.

Name: Kristoffer Alexander Krogerus
School: Mattlidens Gymnasium
Country: Finland

Abstract

I decided to make my investigation in the area of wastewater treatment, as wastewater and pollution has become a growing problem globally. I wanted to study biological wastewater treatment, and this led me to the activated sludge process. I was sceptical at first about the effectiveness of the process, which led me to my research question: **Will the growth rate of activated sludge in an activated sludge process differ depending on whether glucose or fructose is given to it as BOD, and which monosaccharide will increase the growth rate more?**

I decided to compare the effects fructose and glucose have on the activated sludge process and my final objective was to calculate the growth rates of activated sludge fed by glucose and fed by fructose. I had to design a method using the change of substrate concentration in the tank as a basis, i.e. the amount of fructose and glucose consumed by the sludge, because through research I found an expression for the growth rate dependent on the susbstrate concentration, Monod's equation. I scanned through various methods of measuring the substrate concentration in water, and using the density turned out to be the simplest and cheapest. I then used the change of substrate concentration in Monod's equation to receive a calculated value for the growth rate, and to derive at my conclusion: The growth rate of activated sludge in an activated sludge process differs whether fructose or glucose is given to it as BOD, and glucose will increase the growth rate more. Thus I was able to show to myself that the process really works, and that the effectiveness of the process depends on the substances that are to be oxidised (i.e. broken down).

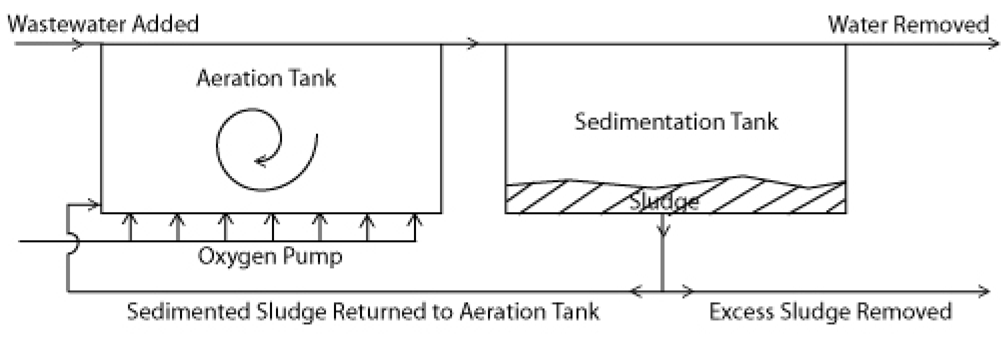
Table of Contents

Introduction

As all living organisms depend on water as a key requirement of survival, it makes the preservation of the water quality important. Factors negatively affecting the quality of the water found in nature include the wastewater produced by public sewage, industries, and during agricultural and urban runoffs (due to precipitation).[1] Among the defects this pollution brings is that it helps breed bacteria and other perhaps harmful micro-organisms, as a consequence of the substances in the pollution, which give nutrients and energy to micro-organisms, and it can bring poisonous substances into the water, which can threaten the surrounding ecosystem and its members. This has led to the fact that many new techniques of treating water has arisen, including the biological wastewater treatment method called the Activated Sludge Process. Other techniques exist in different areas (chemical), but the benefits of the Activated Sludge Process include that it is the only process that effectively removes substances which put a biological oxygen demand, i.e. micro-organisms will strive to break down these substances, which requires oxygen. Therefore I decided to make my investigation in the Activated Sludge Process, and my research question is: Will the growth rate of activated sludge in an activated sludge process differ depending on whether glucose or fructose is given to it as BOD, and which monosaccharide will increase the growth rate more?

An outline of the Activated Sludge Process

Figure 1 - Illustration representing the continous activated sludge system.



[1] Eckenfelder Jr., Wesley, 1980:1

Biological wastewater treatment includes aerobic and anaerobic methods, where the aerobic is known as the Activated Sludge Process. The Activated Sludge Process is a continuous process of biological wastewater treatment, where micro-organisms, found in the wastewater, use up solved, colloid and solid organic compounds found in the water as an energy source for cell respiration and division (i.e. asexual reproduction) in an aerated tank (to make the system aerobic) at a specific temperature. The 'activated' micro-organisms are known as activated sludge. The process also includes the sedimentation of the sludge, so that the treated water (water without any organic material nor micro-organisms) can be removed and let out into nature, without risk of polluting public waters. The sludge is then returned to the aeration tank, and reused and concentrated. As the process is continuous new wastewater is added constantly, while treated water and excess sludge is removed. I have illustrated the system in Figure 1.

The organics, which are biodegradable, are known as BOD, the biological oxygen demand, which comes from the fact that the process strives to remove substances which put an oxygen demand on the system.[2] Around one third of the organics, BOD, are broken down by enzymes and are oxidized in cell respiration, giving the following simplified reaction (Reaction 1):

(Reaction 1):

Organics (BOD) + O_2 + N + P $\rightarrow$ cells + CO_2 + H_2O + non-degradable soluble remains + ΔH (Energy)

The activation tank can be compared to a small ecosystem where an equal state of fungi, bacteria, protozoa and metazoans exist. The age of the sludge (i.e. the time it circulates in the system before it is removed as excess sludge) also affects the microbiological composition of the sludge, as older sludge consists of more advanced organisms than younger sludge, which is due to the fact that higher organisms need more time to develop, and more advanced organisms can feed on the less advanced organisms. Like all ecosystems, the sludge is also affected by abiotic factors, as the temperature, pH, and oxygen and nutrient levels, which affect the micro-organism's enzyme functions (as all enzymes work the best at specific conditions).[3]

---

[2] http://www.college.ucla.edu/webproject/micro7/studentprojects7/Rader/asludge2.htm (18.08.2005)
[3] ibid (09.10.2005)

Simple carbohydrate metabolism in micro-organisms

The biological oxidation of organic compounds into ATP molecules (used as energy) and other simpler organic compounds by bacteria for use in biosynthetic[4] and assimilatory[5] reactions is known as heterotrophic metabolism, and starts with the process of glycolysis.[6]

The process of glycolysis[7], carried out by prokaryotes and eukaryotes, can take several pathways in converting monosaccharides into energy. These pathways include the one common in cyanobacteria and eukaryotes, the Embden-Meyerhof pathway, and the bacteria specific pentose phosphate pathway and Entner-Doudoroff pathway.[8] The oxidation of glucose in the Embden-Meyerhof pathway takes place in the following way:[9,10]

Glucose reacts with ATP to form Glucose-6-phosphate and ADP under the presence of the enzymes Hexokinase and Glucokinase. Glucose-6-phospate is then converted into Fructose-6-phospate with the enzyme Phosphohexose Isomerase. Fructose-6-phosphate again reacts with ATP in the presence of the enzyme Phosphofructokinase-1, to form ADP and Fructose-1,6-bisphosphate. Fructose-1,6-bisphosphate is then hydrolysed with the help of Aldolase to form Glyceraldehyde-3-phosphate (G3P) and Dihydroxyacetone Phosphate (DHAP), which both are molecules containing of 3 carbon atoms (the original glucose molecule containing 6). DHAP can then be catalysed by Triose Phosphate Isomerase into G3P (and vice versa), as DHAP and G3P exist in equilibrium. The following steps are repeated once per G3P molecule produced in the previous step: G3P reacts with NAD^+, H^- and P to give NADH, H^+ and 1,3-Bisphosphoglycerate, catalysed by the enzyme Glyceraldehyde-3-phosphate Dehydrogenase. 1,3-Bisphosphoglycerate then reacts with ADP to form 3-Phosphoglycerate and ATP, catalysed by Phosphoglycerate Kinase. 3-Phosphoglycerate is then transformed into 2-Phosphoglycerate under the presence of

4 A reaction producing a chemical compound by a living organism
5 A reaction which adds elements to the organisms biomass
6 http://gsbs.utmb.edu/microbook/ch004.htm (20.08.2005)
7 The conversion of monosaccharides into pyruvate
8 http://gsbs.utmb.edu/microbook/ch004.htm (20.08.2005)
9 http://web.indstate.edu/thcme/mwking/glycolysis.html (04.09.2005)
10 Green: Carbohydrate backbone. Blue: Enzyme. Dark Red: Energy-related or waste substance.

Phosphoglycerate Mutase. 2-Phosphoglycerate is transformed into Phosphoenolpyruvate by the enzyme Enolase. Phosphoenolpyruvate then reacts with ADP to form Pyruvate and ATP, catalysed by Pyruvate Kinase, giving the following combined reaction (Reaction 2):

(Reaction 2):

Glucose + 2 ADP + 2 NAD^+ + 2 P -----> 2 Pyruvate + 2 ATP + 2 NADH + 2 H^+

The oxidation of fructose takes place in a similar way, where fructose reacts in the following way to produce G3P (see oxidation of glucose), from where the pathway follows the same steps as glucose's pathway:[11,12]

Fructose reacts with ATP under the presence of Fructokinase to form Fructose-1-Phosphate and ADP. Fructose-1-Phosphate is then split into Glyceraldehyde and DHAP with the help of Fructose-1-P Aldolase. Glyceraldehyde then reacts with ATP to form G3P and ADP, catalysed by Triose Kinase, while DHAP is transformed into G3P with the help of Triose Phosphate Isomerase. G3P then enters the glucose pathway.

Through glycolysis micro-organisms are able to convert 1 mole glucose, as well as 1 mole fructose, into 2 moles of ATP, 2 moles of NADH and 2 moles Pyruvate. The 2 moles of NADH can be further synthesized in Oxidative Phosphorylation to produce either 4 or 6 moles ATP, while the 2 moles of Pyruvate can be oxidized through the TCA cycle to form a further 30 moles of ATP. Therefore the total ATP yield of the oxidation of 1 mole glucose or fructose is 36 or 38 moles of ATP.[13]

Before reproduction the micro-organism's cell increases slightly in size, leading to an increase in respiration and use of organics. This means that growth rate of micro-organisms is affected by the amount of substrates it can absorb and convert into energy.

---

[11] Green: Carbohydrate backbone. Blue: Enzyme. Dark Red: Energy-related or waste substance.
[12] http://web.indstate.edu/thcme/mwking/non-glucose-sugar-metabolism.html (04.09.2005)
[13] http://web.indstate.edu/thcme/mwking/glycolysis.html (04.09.2005)

Planning

My experiment consisted of two parts, whereas the first was to simulate the activated sludge process used in wastewater plants to maintain the growth of activated sludge, needed and researched during the experiment. The second part consisted of the methods needed to answer the research question, which included the measuring of sludge's monosaccharides consumption, and investigating the change of biomass.

Research Question:

Will the growth rate of activated sludge in an activated sludge process differ depending on whether glucose or fructose is given to it as BOD, and which monosaccharide will increase the growth rate more?

Hypothesis:

Both prokaryotic (e.g. bacteria) and eukaryotic (e.g. protozoa) organisms carry out the oxidation of organic compounds to pyruvate through glycolysis, which means that glucose and fructose should both affect the growth rate of the sludge, as they both are carbohydrates, and are both oxidized in the process of glycolysis. As reproduction and living require energy, the oxidation of fructose and glucose should both affect the growth rate, while fructose has less 'steps' in its oxidative pathway than glucose, and has a less complex pathway, which would mean the conversion of fructose to pyruvate is faster than the conversion of glucose to pyruvate. My hypothesis is hence that the growth rate of activated sludge will differ depending on whether fructose or glucose is given to it, and that fructose will increase the growth rate more than glucose.

Variables:

Independent: The monosaccharide used as BOD. In the experiment fructose and glucose are used.

Dependent: The measured growth rate of the micro-organisms found in the activated sludge.

Controlled:

- The daily addition of BOD into both tanks is kept equal for both tanks, making the measurement of the growth rate more accurately. This is achieved by giving an equal amount of fructose and glucose (in mass[14]) to the tanks.
- The volume of both tanks is equal, 1.5 liters, along with the volume of water inside the tanks, 1.2 liters. The source of water of both tanks is the same, so that the environment affects the sludge in the same way, giving a more accurate answer to the research question.
- The aeration of both tanks is also kept the same, by dividing the cable from the pump with a Y-piece, into two cables, each placed into the two tanks, giving equal aeration.
- The amount of living organisms in both samples are attempted to being kept at the same level and of the same species. The organisms in both tanks are originally from the same ecosystem/tank (used when establishing the activated sludge from the mud collected from the lake bed) and was split equally by mass to the two new tanks.
- The amount of nutrients (hen faeces) given to both samples in mass is also the same, to make sure that the growth rate will be affected equally by the nutrients, as a difference in nutrients given can affect the growth rate differently.

Material Used:

Tools:

- *2 Cylinders*, with a volume of 1.5 liters, used as aeration tanks in the simulation of the activated sludge process. One tank was used per monosaccharide.
- *Aquarium Pump*, used to oxidise the water, by pumping air through it. The same pump is used for both cylinders with a Y-cable.
- *Litre Measure*, for use in removal and addition of water to the system.

[14] Both compounds have the same molecular mass, samples of the same mass contain equal amounts of glucose and fructose

- *Light Microscope*, made by Motic, magnification 40-400x, to look for life in the sludge.
- *8 plastic bottles*, with a volume of 100 ml, for storing of samples.
- *50 ml burette*, to use in density measurement.
- *Analytical Scales*, to use in density measurement and sample weighing.
- *Beakers,* volumes of 250 ml, for use as containers.

Substances:

- *Mud and Water* collected from the lake bed of Myllylampi, a small oligotrophic lake in the area of Lohja in southern Finland, 200 meters from Lohjanjärvi, the greatest lake in the area. This is used to start of sludge growth.
- *Sucrose*, used as simulated BOD during experiment.
- *Glucose*, used as simulated BOD during experiment.
- *Fructose*, used as simulated BOD during experiment.
- *Hen faeces,* given as nutrients.

Method:

- The process was initiated by collecting the mud from the lake bed. This was then placed into a 1.5 liter tank, and water was added until the volume of the contents was 1.2 liters. The aquarium pump cable was then placed into the tank, with the cable-end at the bottom of the tank. As an initial BOD addition, 1 teaspoon (tsp) of sucrose was added to the process, together with one pellet of hen faeces for the necessary minerals and nutrients.
- Each day during a 4 week period, the aeration pumps were removed, thus stopping the air flow, and the water circulation. This led to the sedimentation of the sludge, making it possible to exchange approx. 1 liter of water (to add new natural nutrients). After the water was changed sucrose was added (between 0.5-2 tsps per day) and along with one pellet of hen faeces.
- The activated sludge was split into two equal parts, and added to the two cylinders, equal in volume. Water is added to both tanks until the aqueous volume is 1.2 liters. The aeration pump is connected to both tanks, having the pump as a common source, using a Y-piece to divide the air cable.

- To determine the growth rate of the activated sludge, Monod's Equation is used (which uses the change in BOD concentration to calculate the growth rate), as well as a physically observing the change in volume of the sludge in both tanks.
- A sample (50 ml) is taken from both tanks, and added to a plastic bottle (samples are to be used later for density measurements). After this fructose is added to the first tank and glucose to the other tank (1g in each tank during Sample 1 (S1), 2g during Sample 2 (S2), and 3g during Sample 3 (S3)). The sugar is let to dissolve, and a sample from both tanks is taken and added to an empty plastic bottle (used for density measurement). The sludge is then left to consume and react with the sugar for 1 hour and 10 hours. After 1 hour and 10 hours new samples are taken from both tanks, and added to empty bottles (for density measurement), adding up to a total of 4 samples per tank per S1-S3.
- The previous step is repeated once per sugar sample (S1-S3).
- Due to a systematic error[15] I discovered in the burette a fourth sample (S4) had to be taken with new equipment and careful measuring to ensure accurate results.
- Density Measurement: A sample is added to a burette. The initial volume is recorded. Approximately 10 ml is poured from the burette into a beaker on an analytical scale, with the beaker's weight tared to zero weight. After the water has been dropped into the beaker, the exact change of volume is read off the burette and the mass of the sample is read off the scales, and recorded. The density is calculated by dividing the mass with the volume. The samples' densities are then compared with standard measurements, where I have measured the density of samples of water containing fructose and glucose in different[16] concentrations.
- Finally the physical change of sludge was noted, i.e. the increase/decrease in volume of the sludge between the days S1 and S4 was taken.
- Constantly any observations are observed in a log.

[15] Described on page 14.
[16] 0.1-1.0 g/L with 0.1 g/L intervals, and 1.0-2.5 g/L with 0.5 g/L intervals

Observation Log and Data Collection

Week 1 (10.7-16.7):

I started the Activated Sludge Process by gathering water from the lake bed of Myllylampi, by stirring around in the muddy bed, and collecting 0.5 L water in a bottle. I added the water to the aeration tank, and added tap water until a total volume of 1.2 L is reached. Color is brownish-grey. A strong smell of decay, as a result of anaerobic conditions in the lake. During sedimentation some bubbles rise from sludge to surface, as methane is released (as a result of anaerobic conditions).

Week 2 (17.7-23.7):

During week 2 the amount of daily sugar added to the sludge tank is increased, which has led to an increase of methane produced, as the oxygen pumps can not pump enough oxygen as is needed by the aerobic cell respiration reactions, therefore methane is produced and most of the sludge floats on the surface. The smell is stronger now.

Week 3 (24.7-30.7):

During this week the sugar addition is decreased with around a third, which led to the fact that in the end of the week all sludge had sunk again, and almost no smell was present, as a result of complete aerobic respiration.

Week 4 (31.7-6.8):

Appearance is similar to that of Week 3, but no smell.

Week 5 (7.8-13.8):

During a microscope observation of 35-day-old sludge, I looked at some samples of the sludge to search for any organisms, and life could be noted through moving *Difflugia* and *Paramecium*, while the pH of the activated sludge was measured to be 6.[17]

Week 6-8 (14.8-3.9):

Appearance similar to that of previous week. Sludge is prepared for analysis.

Week 9 (4.9-10.9):

For the trials (S1-S3), I took 8 samples in total, 4 samples from each sugar, the first being before addition, the second directly after the addition, the third 1 hour after

[17] Micro-organisms identified with the help of 'Planktonopas' *Suomen Kalastusyhdistys n:o 34,* by Järnefelt, Naulapää and Tikkanen and *Knuffa För Limnologi* by B. Andersson, M. Bengtsson, T. Elmqvist, O. Nordell, and L. Westin.

addition, and the fourth 10 hours after the addition of the respective sugars, fructose and glucose. The data from the density measurement of Sample 1 (S1) is found in Table 1.[18] Each sample's density was measured 2 or 3 times, depending on the preciseness of the result. The data from the density measurement of Sample 2 (S2) is found in Table 2 and Sample 3 (S3) in Table 3.

Week 10-13 (11.9-7.10):

After samples S1-3 were taken a systematic error was found in the burette, as air was collected in the bottom, and affecting the volume of water let out, and therefore the results, which can't be found reliable, and as seen in the Data Processing and Presentation section won't give any results. Therefore a fourth S4 (2.5g / 1.2 L) sample was made with a new burette, and carried out with great caution to ensure as accurate results as possible were gained. These are displayed in Table 4.

A new microscope investigation is carried out, and filamentous bacteria are spotted (Figure 6-7), and often imply that the system lacks oxygen, nutrients, or carbohydrates. The data from the density measurement of water with different concentrations of fructose and glucose are found in Table 5.

As the process was finished the volume of the sludge was compared visually in both tanks, and the volume of the glucose sludge was slightly greater (0.5 cm more) than the fructose sludge.

Table 1 - Data from Density Measurement of Sample 1, initially containing 1g sugar / 1.2 L (0.83 g/L)

Volumes in ml, +/-0.05ml	**Fructose**			
Masses in g, +/-0.001g	Standard Sample	Directly After S. A.	1 Hour After S.A.	10 Hours After S.A.
Initial Volume	9.8	11.05	12.75	13.45
Change in Volume	10.0	10.35	9.55	10.05
Change of Mass	9.657	10.162	9.544	9.983
Change in Volume	10.0	10.1	9.65	9.5
Change of Mass	10.035	10.080	9.630	9.540
Change in Volume	13.1	-	-	10.0
Change of Mass	13.099	-	-	9.968
	Glucose			
	Standard Sample	Directly After S. A.	1 Hour After S.A.	10 Hours After S.A.
Initial Volume	9.85	14.0	13.25	11.75
Change in Volume	10.05	9.85	10.1	9.8
Change of Mass	9.703	9.782	10.039	9.755
Change in Volume	9.9	9.45	9.65	9.95
Change of Mass	9.862	9.389	9.631	9.952
Change in Volume	-	9.75	-	9.45
Change of Mass	-	9.772	-	9.286

[18] S.A. refers to 'Sugar Addition', and will be used for the rest of the document

Table 2 - Data from Density Measurement of Sample 2, initially containing 2g sugar / 1.2 L (1.67 g/L)				
Volumes in ml, +/-0.05ml	**Fructose**			
Masses in g, +/-0.001g	Standard Sample	Directly After S. A.	1 Hour After S.A.	10 Hours After S.A.
Initial Volume	19.8	18.5	17.9	18.1
Change in Volume	9.9	10.1	10.0	10.0
Change of Mass	9.850	10.114	9.998	10.053
Change in Volume	10.15	10.0	10.1	10.0
Change of Mass	10.149	9.960	10.105	10.034
Change in Volume	9.9	10.1	10.15	11.1
Change of Mass	9.944	10.149	10.144	11.148
	Glucose			
	Standard Sample	Directly After S. A.	1 Hour After S.A.	10 Hours After S.A.
Initial Volume	18.5	17.7	17.2	18.9
Change in Volume	10.2	10.0	10.0	10.0
Change of Mass	10.131	9.985	9.863	9.896
Change in Volume	9.9	10.0	10.0	10.1
Change of Mass	9.930	9.996	9.957	10.166
Change in Volume	10.0	10.1	10.0	10.15
Change of Mass	10.032	10.180	10.036	10.178

Table 3 - Data from Density Measurement of Sample 3, initially containing 3g sugar / 1.2 L (2.5 g/L)				
Volumes in ml, +/-0.05ml	**Fructose**			
Masses in g, +/-0.001g	Standard Sample	Directly After S. A.	1 Hour After S.A.	10 Hours After S.A.
Initial Volume	18.5	18.5	19.8	19.4
Change in Volume	10.0	9.95	10.0	10.1
Change of Mass	10.002	9.913	9.980	10.137
Change in Volume	10.0	10.1	10.15	10.05
Change of Mass	10.067	10.132	10.212	10.080
Change in Volume	10.0	10.1	10.0	10.45
Change of Mass	10.075	10.169	10.055	10.536
	Glucose			
	Standard Sample	Directly After S. A.	1 Hour After S.A.	10 Hours After S.A.
Initial Volume	15.9	18.4	19.8	19.8
Change in Volume	9.95	9.85	10.0	9.9
Change of Mass	9.948	9.820	10.030	9.845
Change in Volume	10.1	10.05	10.0	10.1
Change of Mass	10.121	10.087	10.004	10.096
Change in Volume	10.0	10.0	10.0	10.05
Change of Mass	10.070	10.057	10.040	10.050

Table 4 - Data from Density Measurement of Sample 4, initially containing 2.5g sugar / 1.2 L (2.08 g/L)				
Volumes in ml, +/-0.05ml	**Fructose**			
Masses in g, +/-0.001g	Standard Sample	Directly After S.A.	1 Hour After S.A.	10 Hours After S.A.
Initial Volume	19.0	20.0	20.0	19.0
Change in Volume	10.0	10.0	10.0	10.0
Change of Mass	10.107	10.126	10.118	10.103
Change in Volume	10.0	10.0	10.0	10.0
Change of Mass	10.104	10.119	10.112	10.102
Change in Volume	10.0	10.0	10.0	10.0
Change of Mass	10.109	10.130	10.127	10.110
	Glucose			
	Standard Sample	Directly After S.A.	1 Hour After S.A.	10 Hours After S.A.
Initial Volume	20.0	19.0	18.0	20.0
Change in Volume	10.0	10.0	10	10.0
Change of Mass	10.092	10.135	10.109	10.098
Change in Volume	10.0	10.0	10.05	10.0
Change of Mass	10.109	10.122	10.167	10.110
Change in Volume	10.0	10.0	10.05	10.0
Change of Mass	10.116	10.136	10.175	10.090

Table 5 - Data from Density Measurement of different fructose and glucose solutions (Volumes in ml, +/-0.05ml. Masses in g, +/-0.001g)

	Fructose													
Grams / Liter	**0**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1.0**	**1.5**	**2.0**	**2.5**
Initial Volume	3.4	4.9	8.0	8.0	9.0	8.0	9.0	8.0	10.0	9.5	9.0	8.0	8.0	7.0
Change in Volume	10.0	9.9	10.0	10.05	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Change of Mass	9.933	9.850	9.961	10.014	9.986	9.974	9.982	9.986	9.975	9.983	9.991	9.990	9.975	9.991
Change in Volume	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.1	10.0	10.0
Change of Mass	9.944	9.962	9.975	9.983	9.960	9.977	9.974	9.979	9.980	9.974	9.990	10.096	10.002	9.996
Change in Volume	9.5	10.0	10.0	9.95	9.95	10.0	10.05	10.0	10.0	10.0	10.5	10.0	10.05	10.0
Change of Mass	9.443	9.943	9.979	9.936	9.946	9.993	10.045	9.988	10.008	10.011	10.487	9.988	10.053	10.018
	Glucose													
Grams / Liter	**0**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1.0**	**1.5**	**2.0**	**2.5**
Initial Volume	3.4	8.5	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.5	8.0
Change in Volume	10.0	10.0	10.0	10.0	10.0	10.0	10.05	10.0	10.0	10.4	10.15	10.1	10.0	10.0
Change of Mass	9.933	9.957	9.981	9.976	9.985	9.995	10.040	9.963	9.983	10.381	10.139	10.091	9.992	10.007
Change in Volume	10.0	10.0	10.0	10.0	10.1	10.0	10.05	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Change of Mass	9.944	9.976	9.972	9.971	10.083	9.984	10.037	9.997	9.992	9.998	9.991	9.999	10.001	9.992
Change in Volume	9.5	10.0	10.0	10.0	9.9	10.05	10.0	10.05	10.0	10.0	10.2	10.0	10.0	10.0
Change of Mass	9.443	9.977	9.959	9.980	9.871	10.018	9.973	10.045	9.985	9.995	10.198	10.001	10.003	10.020

Figure 6 – Sample from glucose tank under microscope. 100x Magnification.

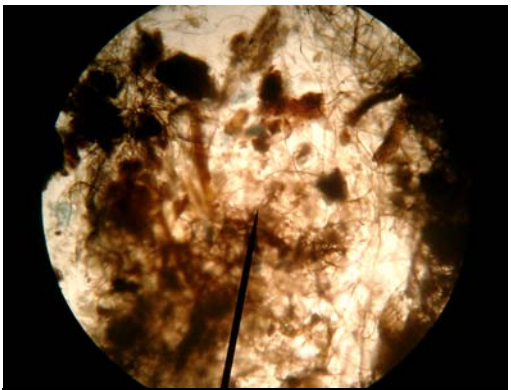


Figure 7 – Sample from fructose tank under microscope. 100x Magnification.

Data Processing and Presentation

The oxidation mechanism of the biological organic removal from the solution of mixed substrates is complex, and is generally described as a sequence of three processes, contact of cell and substrate, transport of substrate into cell and intermediate metabolism of the substrate.[19] This mechanism is built upon the micro-organism's oxidizing enzyme system. The transportation into the cell requires the substrate to be simple enough to pass through the plasma membrane, which means complex molecules need to be broken down by extracellular enzymes. When the substrates have entered the cell, the oxidation reactions start.

[19] Eckenfelder Jr., Wesley, 1980:270

The find the kinetics of organic removal in an activated sludge system one can use Monod's equation, which can be defined for the kinetics of single substrate removal (Equation 1):

Equation 1 – Monod's Equation
$\mu = \bar{\mu} \frac{S}{K_S + S}$
μ = Specific growth rate of organisms (1/hr)
$\bar{\mu}$ = Maximum growth rate of organisms (1/hr)
K_S = Monod's Constant. K_S is defined as the substrate concentration when the rate is one half of the maximum rate. (g/L)
S = Substrate concentration (g/L)
a = Biomass yield coefficient (g/g)
X_v = Biomass Concentration (g/L)
t = Time (hr)

This equation can be manipulated and presented in the following form when taking into account the change of substrate concentration:

$$\frac{S_0\text{-}S_e}{X_v t} = \frac{\bar{\mu}}{a} \frac{S_e}{K_s + S_e} \Rightarrow \bar{\mu} = a \frac{(S_0\text{-}S_e)}{X_v t} \frac{(K_s + S_e)}{S_e}$$

By entering values into the different variables of the equation I try to estimate the growth rate of the two systems. S_o and S_e will be the initial and final substrate concentrations respectively.

To find the change of substrate concentration (i.e. glucose and fructose concentration) I needed to compare the densities of the samples with the standard samples created, to try to obtain the approximate substrate concentration at the different times after solving, so that the change of concentration could be found. The densities were found by dividing the change in mass with the change in volume during titration, with the processed data of samples S1-S4 presented in Figures 8-17.[20]

[20] Test 1-3 being the samples taken during titration, and Average being the average of the samples.

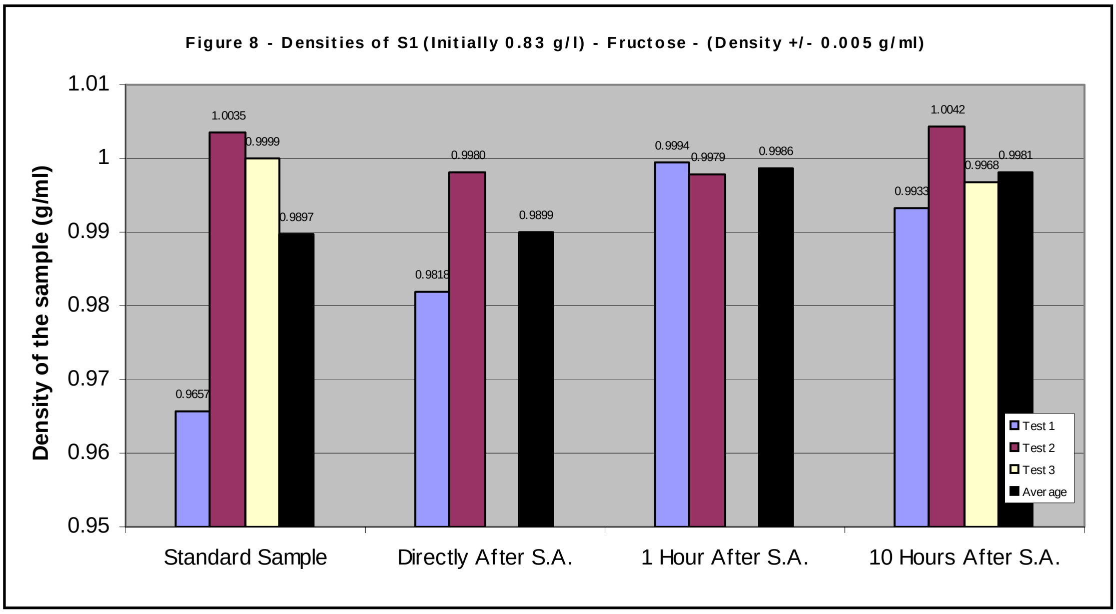


Figure 8 - Densities of S1 (Initially 0.83 g/l) - Fructose - (Density +/- 0.005 g/ml)
Density of the sample (g/ml)
1.01
1
0.99
0.98
0.97
0.96
0.95
0.9657
1.0035
0.9999
0.9897
0.9818
0.9980
0.9899
0.9994
0.9979
0.9986
0.9933
1.0042
0.9968
0.9981
Standard Sample
Directly After S.A.
1 Hour After S.A.
10 Hours After S.A.
Test 1
Test 2
Test 3
Aver age

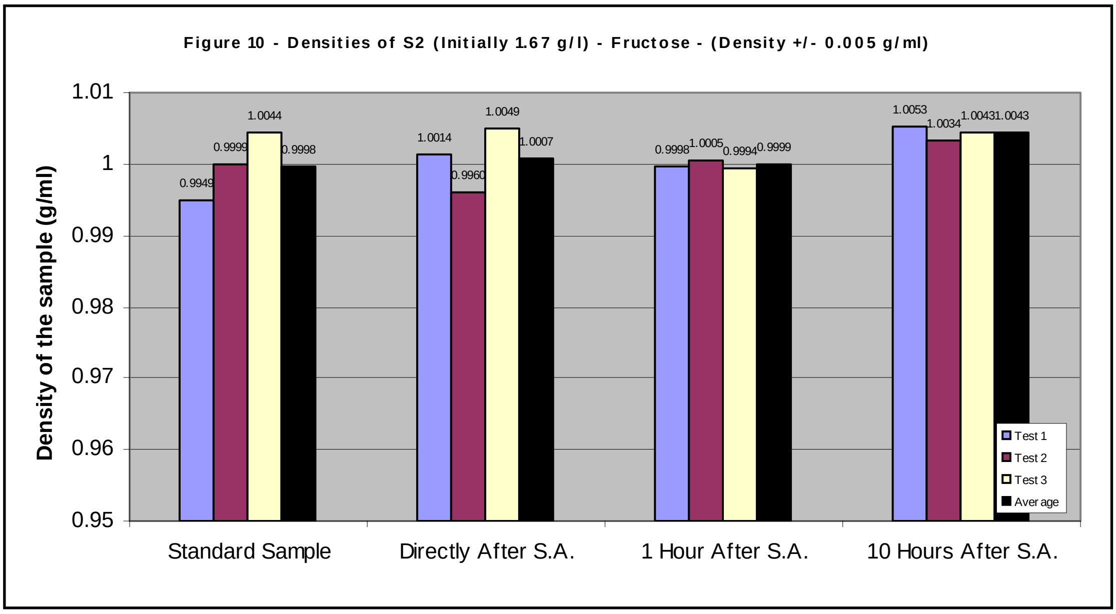


Figure 10 - Densities of S2 (Initially 1.67 g/l) - Fructose - (Density +/- 0.005 g/ml)
Density of the sample (g/ml)
1.01
1
0.99
0.98
0.97
0.96
0.95
0.9949
0.9999
1.0044
0.9998
1.0014
0.9960
1.0049
1.0007
0.9998
1.0005
0.9994
0.9999
1.0053
1.0034
1.0043
1.0043
Standard Sample
Directly After S.A.
1 Hour After S.A.
10 Hours After S.A.
Test 1
Test 2
Test 3
Aver age

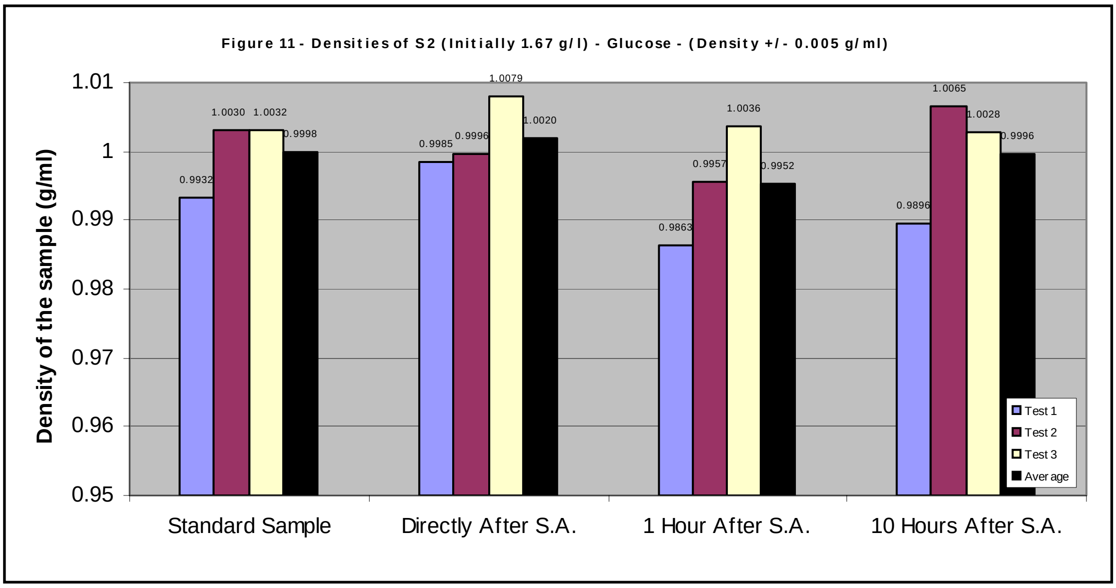


Figure 11 - Densities of S2 (Initially 1.67 g/l) - Glucose - (Density +/- 0.005 g/ml)
Density of the sample (g/ml)
1.01
1
0.99
0.98
0.97
0.96
0.95
0.9932
1.0030
1.0032
0.9998
0.9985
0.9996
1.0079
1.0020
0.9863
0.9957
1.0036
0.9952
0.9896
1.0065
1.0028
0.9996
Standard Sample
Directly After S.A.
1 Hour After S.A.
10 Hours After S.A.
Test 1
Test 2
Test 3
Aver age

Figure 12 - Densities of S3 (Initially 2.5 g/l) - Fructose - (Density +/- 0.005 g/ml)

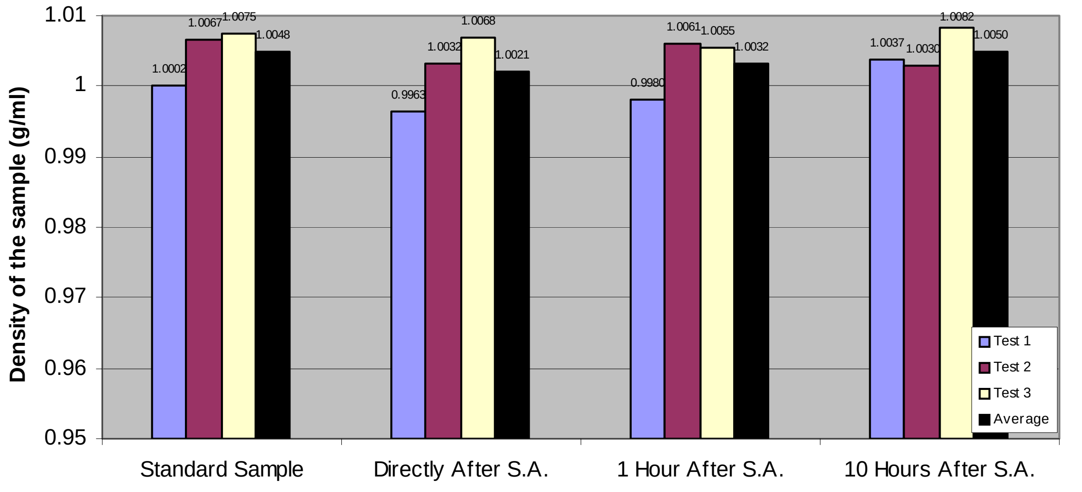


Figure 13 - Densities of S3 (Initially 2.5 g/l) - Glucose - (Density +/- 0.005 g/ml)

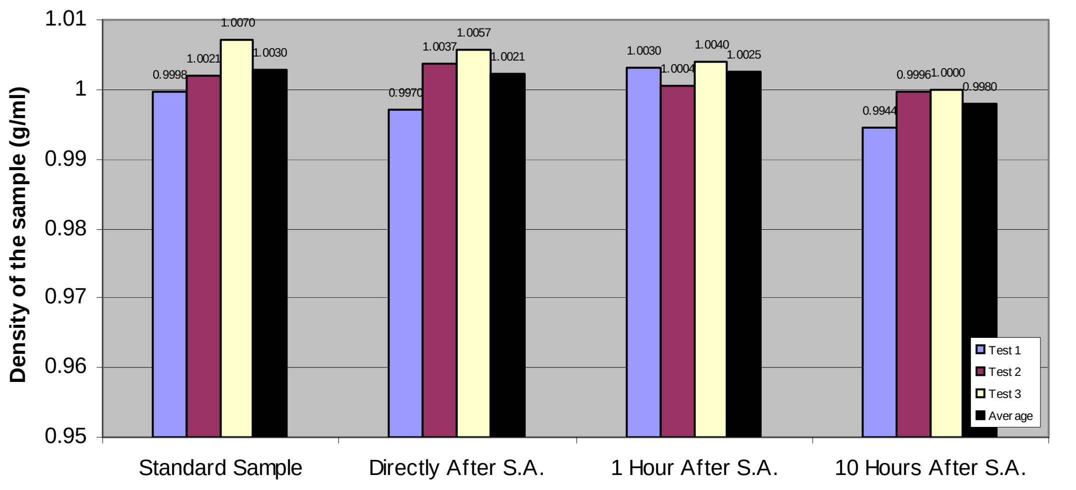
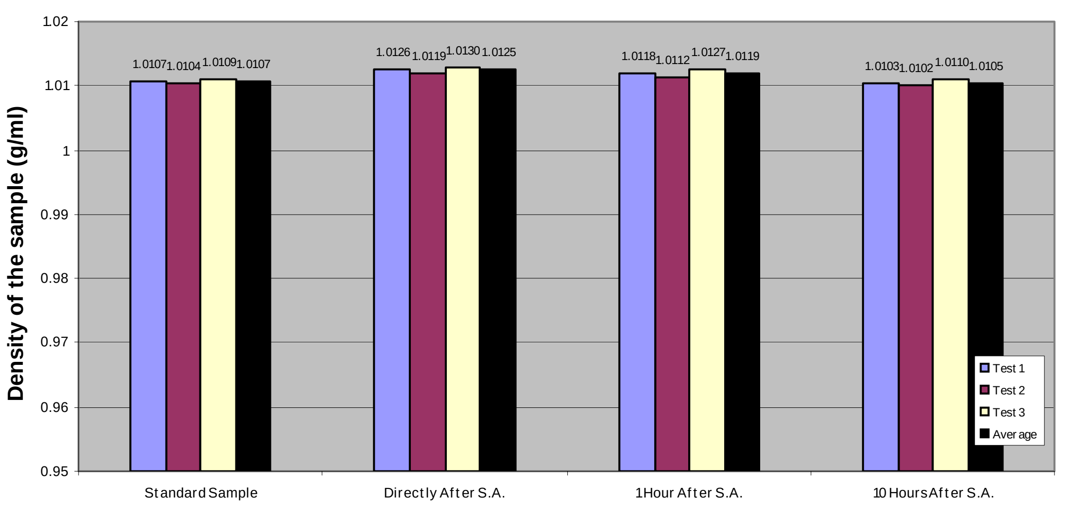


Figure 14 - Densities of S4 (Initially 2.08 g/l) - Fructose - (Density +/- 0.005 g/ml)



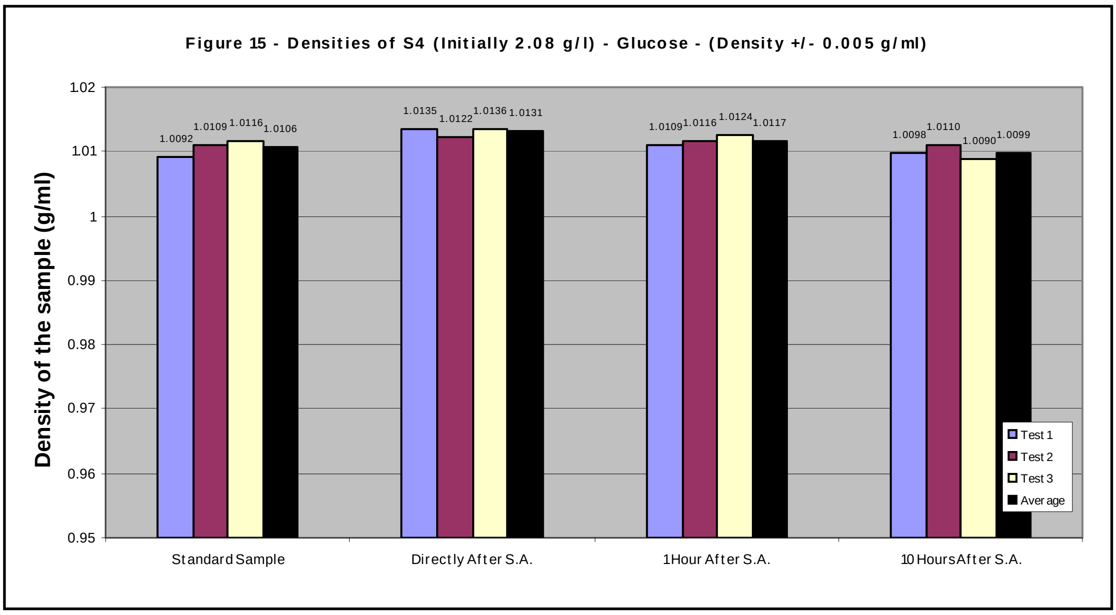


Figure 15 - Densities of S4 (Initially 2.08 g/l) - Glucose - (Density +/- 0.005 g/ml)

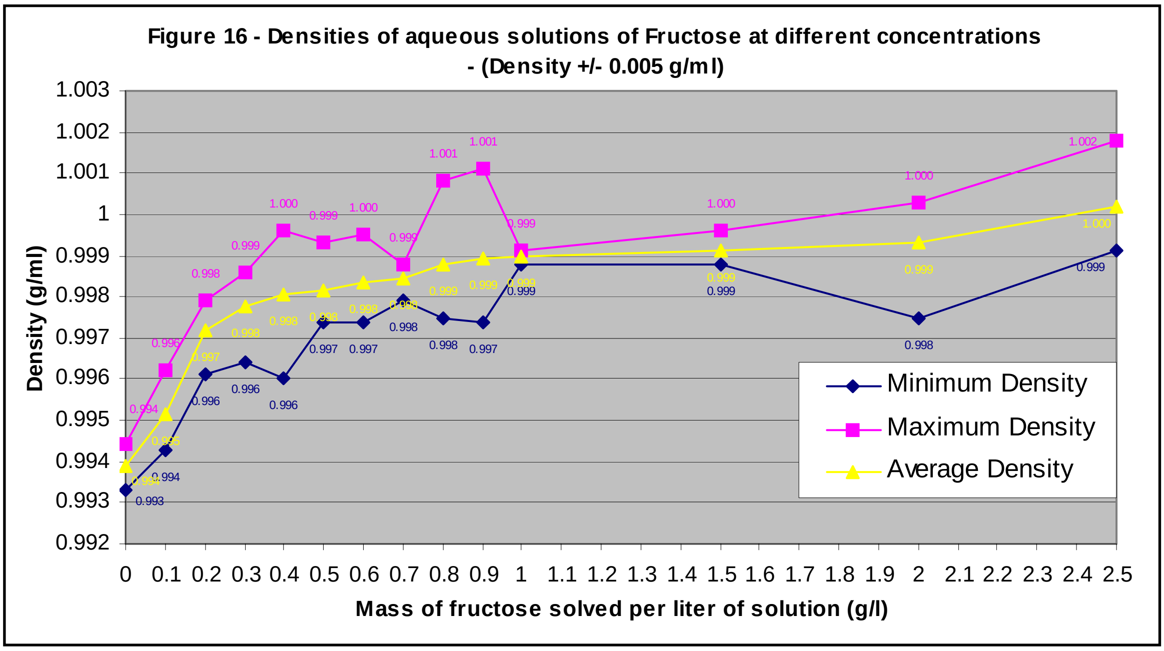


Figure 16 - Densities of aqueous solutions of Fructose at different concentrations - (Density +/- 0.005 g/ml)

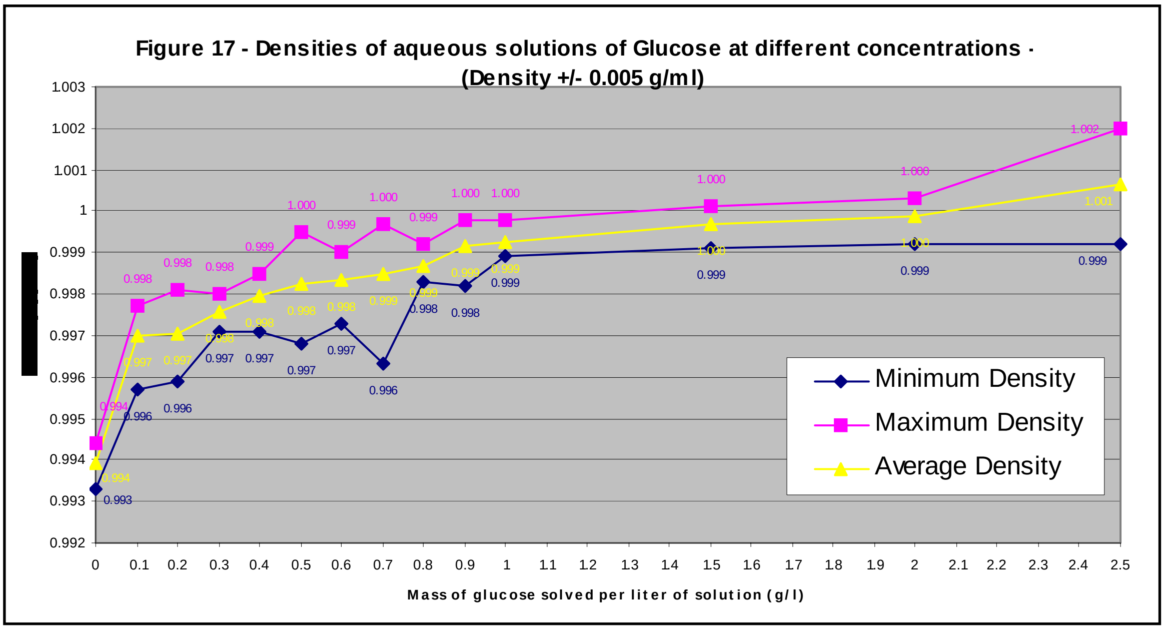


Figure 17 - Densities of aqueous solutions of Glucose at different concentrations - (Density +/- 0.005 g/ml)

As S1-3 show so imprecise results, and partly unrealistic results (i.e. the sugar contents is higher after 10 hours after solving than directly after solving (S3-Fructose)), I've decided to only use the data and results derived from S4 in my calculations, even though I won't be able to compare results and thus get the preciseness of any results achieved. When measuring the density of the samples from the process, one must take into consideration the fact that though sedimentation and careful removal of water from the tanks, the water was never sludge-free, but there was approximately an equal amount of sludge in every sample. Consequently every sample in S4 will have a density higher than that of only tap water with sugar, and I assumed that 1.3%[21] of the mass was sludge. I then compared Figures 14-15 with Figures 16-17, and came to the results displayed in Table 6.

Table 6 - The Approximate Sugar Contents of Sample S4 (Initially 2.08 g/l) (Density +/- 0.005 g/ml)		
	98.7% of Average Density of Fructose (g/ml)	Sugar Concentration (g/l)
S4 - Standard Sample	0.9975	0.2
S4 - Directly After S.A.	0.9993	2.0
S4 - 1 Hour After S.A.	0.9987	0.8
S4 - 10 Hours After S.A.	0.9974	0.2
	98.7% of Average Density of Glucose (g/ml)	Sugar Concentration (g/l)
S4 - Standard Sample	0.9974	0.3
S4 - Directly After S.A.	0.9999	2.0
S4 - 1 Hour After S.A.	0.9985	0.7
S4 - 10 Hours After S.A.	0.9968	0.1

These values of the sugar concentration can then be inserted into Monod's equation, together with the estimated[22] data found in Table 7, to calculate the Maximum Growth Rate of the system (Table 8).

Table 7 – Estimated Data for variables and constants of Monod's equation.	
	Value
X_v (g/L)	3
a (g/g)	0.9
K_s (g/L)	4

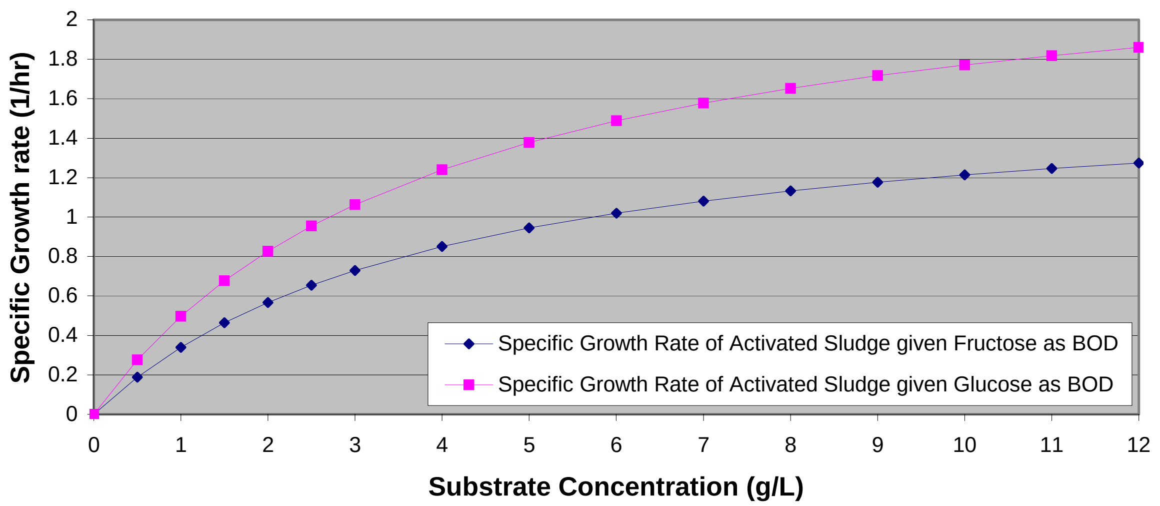
[21] This value makes the mass of sugar initially solved into the wastewater correspond to the mass of the wastewater obtained through the density measurements from the sample 'Directly after S.A.'

[22] The chosen values are both realistic and give precise results for the calculations.

Table 8 – Calculations of the growth rate of the activated sludge in the two tanks.[23]	
Fructose Tank:	Glucose Tank:
$\bar{\mu} = a \dfrac{(S_0 - S_e)}{X_v t} \dfrac{(K_s + S_e)}{S_e}$	$\bar{\mu} = a \dfrac{(S_0 - S_e)}{X_v t} \dfrac{(K_s + S_e)}{S_e}$
$\mu_1 = 0.9 \dfrac{(2.0 - 0.8)}{3 \cdot 1} \dfrac{(4 + 0.8)}{0.8} = 2.16\ hr^{-1}$	$\mu_1 = 0.9 \dfrac{(2.0 - 0.7)}{3 \cdot 1} \dfrac{(4 + 0.7)}{0.7} = 2.62\ hr^{-1}$
$\mu_2 = 0.9 \dfrac{(2.0 - 0.2)}{3 \cdot 10} \dfrac{(4 + 0.2)}{0.2} = 1.13\ hr^{-1}$	$\mu_2 = 0.9 \dfrac{(2.0 - 0.1)}{3 \cdot 10} \dfrac{(4 + 0.1)}{0.1} = 2.34\ hr^{-1}$
Average $\bar{\mu} = \dfrac{2.16 + 1.13}{2} = 1.70\ hr^{-1}$	Average $\bar{\mu} = \dfrac{2.62 + 2.34}{2} = 2.48\ hr^{-1}$

As the average $\bar{\mu}$'s differ, the growth rate of the sludge is different whether fructose or glucose is given to it as BOD, and as the $\bar{\mu}$ for glucose is higher, glucose increases the growth rate of the activated sludge more (Figure 18)[24]. This is then supported by that the amount of sludge in the glucose tank was greater than the amount of sludge in the fructose tank after the investigation were done, and initially they had been fairly equal.

Figure 18 - A Graph Mapping the Specific Growth Rates of Fructose and Glucose at Different Substrate Concentrations



[23] μ_1 corresponds to 1 hour after S.A., μ_2 corresponds to 10 hours after S.A.
[24] A graph mapping Equation 1 using the calculated growth rate values.

Conclusion and Evaluation

I came to the conclusion that the growth rate of activated sludge in an activated sludge process differs whether glucose or fructose is given to it as BOD, and that glucose will increase the growth rate more. The final values obtained for the growth rate in the fructose tank was 1.70 hr^{-1}, and for the glucose tank 2.48 hr^{-1}. Hence my hypothesis was proven wrong, which could be explained by the fact that even though the fructose pathway has less ‘steps’, the ‘steps’ might have higher energy requirements, thus making the glucose pathway more efficient. As both sugars are so similar in structure, they will also affect the growth rate in a similar way, other than if e.g. glucose and a non-sugar carbohydrate were studied.

Errors and Suggestions of Improvement

The greatest error in this investigation was the inaccurate method of determining the sugar concentration of the wastewater, making it difficult to determine an accurate growth rate of the activated sludge. This included the problem of that the samples from the aeration tanks contained more particles and sludge than tap water, thus the densities weren’t directly comparable, and assumptions had to be made. To improve this I could have used a better method of analysing the sugar concentration of a solution, which can include chemical sugar analysis or spectroscopy and when measuring the densities of water at different substrate concentrations, I could have used the same muddy water that started the process.

Another error is the fact that the aeration tanks did not receive equal amounts of oxygen, as the air cables clogged up, giving slightly different air amounts to both tanks. This could be improved by using two identical aquarium pumps, as well as changing cables daily, to ensure more equal aeration.

During sample-taking several problems arose, firstly when water was removed during sample-taking small amounts of sludge were removed, and thus I could not ensure constant sludge amounts, which I could have improved by filtering the water when taking the sample to remove as much sludge as possible, to ensure as constant sludge amount in the tanks as possible. Secondly as the samples were taken and frozen, the freezing wasn't instant, so oxidation of the sugars can have continued after the sample was taken, which could have been improved by carrying out the density measurement directly after the samples were taken.

During the density measurement the systematic error in the burette arose, followed by the fact that I hadn't taken enough samples. This could have been improved by repeating S1-3 with a new burette, and also by taking more samples at different substrate concentrations, giving more results, and hence a more accurate answer.

The tanks can have initially contained slightly different amounts of sludge, which affects the amount of sugar oxidized, and thus the growth rate. Even though the sludge was divided quite equally by volume, doesn't mean that it contained equal amounts of living organisms. This error is difficult to improve, but by repeating the method but giving the same sugar to both tanks I could use Monod's equation and my calculated growth rate to calculate the biomass of both tanks.

Finally I made assumptions of the values of the different constants and variables in Monod's equation, as well assuming them being equal in both tanks, which affects the growth rate calculated. This could have been improved by experimentally determining the different values.

Bibliography

Literature:

Andersson, B., Bengtsson, M., Elmqvist, T., Nordell, O., Westin, L., 1979, *Knuffa För Limnologi,* Gothenburg: Fältbiologerna

Eckenfelder Jr., W. Wesley, 1980, *Principles Of Water Quality Management,* Boston: CBI Publishing Company, Inc.

Järnefelt, Naulapää and Tikkanen, 1976, 'Planktonopas', *Suomen Kalastusyhdistys n:o 34,* Helsinki Univeristy

Electronic:

Jurtshuk Jr., Peter, *Medmicro Chapter 4,* [Online], [Accessed on 07.09.2005], available from: <URL: http://gsbs.utmb.edu/microbook/ch004.htm>

King, Michael W., *Glycolysis,* [Online], [Accessed on 07.09.2005], available from: <URL: http://web.indstate.edu/thcme/mwking/glycolysis.html>

King, Michael W., *Non-Glucose Carbons in Glycolysis,* [Online], [Accessed on 07.09.2005], available from: <URL: http://web.indstate.edu/thcme/mwking/non-glucose-sugar-metabolism.html>

UCLA College, *Activated Sludge,* [Online], [Accessed on 07.09.2005], available from: <URL: http://www.college.ucla.edu/webproject/micro7/studentprojects7/Rader/asludge2.htm >