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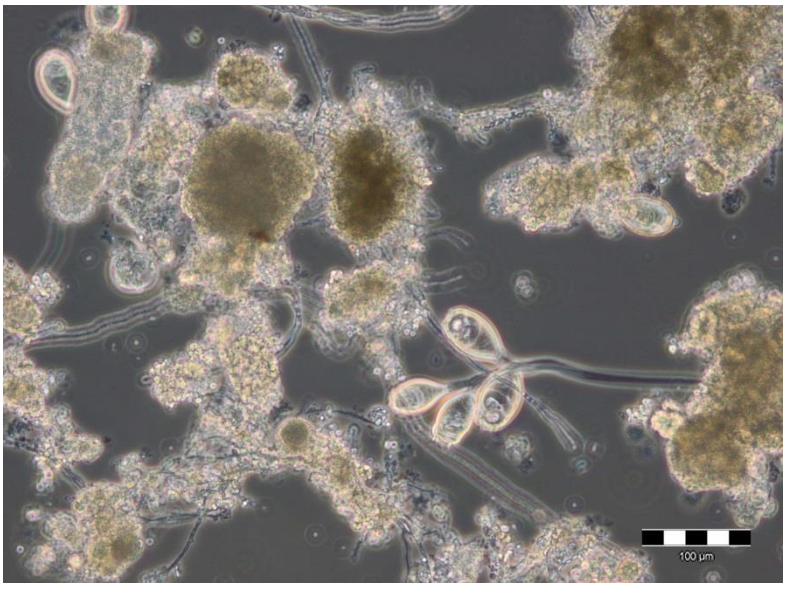

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Department of Civil and Environmental Engineering
Division of Water Environment Technology
Chalmers University of Technology
SE-412 96 Gothenburg
Sweden
Telephone + 46 (0)31-772 1000

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Abstract

The activated sludge process is one of the most important parts of conventional wastewater treatment plants. Wastewater is treated biologically in activated sludge basins. Activated sludge can remove soluble contaminants, primarily organics compounds and nitrogen, from the wastewater stream. The activated sludge is a mixture of microorganisms, inorganic and organic compounds aggregated to flocs. Nitrogen and organics can be removed from wastewater by oxidation or physical adsorption by activated sludge. Physical adsorption of organics and nitrogen by activated sludge takes place as soon as the wastewater has contact with the sludge mixture. The purpose of this thesis is to investigate the adsorption ability of different activated sludge.

Three sequencing batch reactors (SBR) were operated in order to produce different kinds of activated sludge mixtures. Reactor one and two were fed with carbon source compound and reactor three was fed with nitrogen source compound. It was found that the flocs properties were changing significantly in these three reactors during three months of operation. The sludge in reactor three had finer and smaller flocs in comparison with the sludge in reactor one and two. Due to the different feed compounds, the dominant community of bacteria inside reactor one and two were heterotrophic bacteria and the major bacteria community in reactor three were nitrifying bacteria.

The adsorption tests were conducted for the sludge mixtures of reactor one, two and three with glucose, ammonium, and milk powder as model compounds. Sodium azide was used to inhibit bacterial oxidation during the adsorption tests. The results showed that the sludge in reactor one was more capable of removing glucose and ammonium from synthetic wastewater. On the other hand, sludge in reactor three could remove higher concentration of milk powder in the first minutes of mixing in comparison with sludge in reactor one and two. Furthermore, the adsorption rate per unit of time for different adsorption tests show that higher concentration of organic and nitrogen were removed in the first minutes of contact in comparison with the next hours of mixing.

Key words: activated sludge, wastewater, wastewater treatment plant, biology, adsorption, organics, nitrogen, SBR,

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1 Introduction

1.1 Background

In the past, wastewater was transported away from people, and was released to surface water without treatment. However, municipalities began to construct organized wastewater treatment systems in order to reduce health problems and undesirable aesthetics such as odours and to minimize environmental issues as consequence of contaminated waters.

The conventional wastewater treatment process is divided into preliminary, primary and secondary treatment steps. In the preliminary step, large objects such as trash and papers can be removed by bar screens in order to protect downstream operation. Then, sand particles are usually removed in grit chambers. The primary treatment consists of primary sedimentation tanks that can remove floating and settleable solids, which reduce the amount of chemical oxygen demand (COD). The secondary treatment includes the activated sludge basins and secondary settlers. In the activated sludge basins COD and nitrogen converted biologically. Flocs which are spontaneously formed in activated sludge basins can be removed in secondary settlers. Primary and secondary sludge wasted from the system are treated in sludge treatment units. Usually, sludge is treated in an anaerobic digester where complex organic compounds are broken down during fermentation. Methane gas is a main product produced in anaerobic digester during sludge treatment (Metcalf and Eddy 2003). Figure 1.1 illustrates the treatment process.

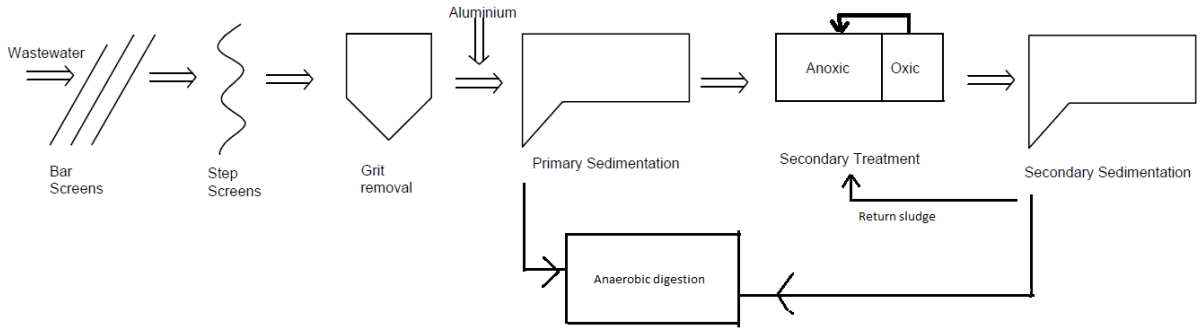


Figure 1.1 The Schematic drawing of a typical wastewater plant.

Activated sludge is the most common way to treat wastewater biologically. It is used to remove soluble contaminants, primarily organics compounds and nitrogen, from the wastewater stream. The activated sludge is a mixture of microorganisms, inorganic and organic compounds aggregated to flocs. Microorganisms oxidize organic matter for growth and survival and thereby convert dissolved and particulate material to biomass that can be separated from the wastewater in the secondary sedimentation step. The efficiency of the activated sludge process depends on the degradation rate of pollutants in the aeration basin and the settleability of the flocs in secondary sedimentation tank.

The increasing amount of nitrogen compounds in wastewater can lead to eutrophication of surface water if discharged untreated. Nitrogen removal can be achieved by nitrification and denitrification in the treatment plant. Nitrification is the biological oxidation of the ammonium to nitrite and then nitrate. Afterwards the nitrate is reduced to nitrogen gas in the denitrification process (equation 1).

However, these processes become a limiting step in conventional treatment system because of the very low growth rate of nitrifies as obligate autotrophic bacteria (Matatoshi et al. 1997). It can cause severe problems to the nitrifiers when a shock loads of high organic materials or toxic compound such as heavy metals is added to the nitrification basin without pre-treatment. High concentration of organic compounds leads to lower nitrification efficiency due to loss of ammonium by assimilation of heterotrophs or because the fast-growing heterotrophs outcompete the slow-growing nitrifiers in the system.

$(NH_4 \rightarrow NO_2 \rightarrow NO_3 \rightarrow NO_2 \rightarrow NO \rightarrow N_2O \rightarrow N_2)$ Equation 1

Population growth increases the amount of wastewater produced by a society. Increase in amount of wastewater leads to increase in the needed capacity of the treatment plants. In this situation one solution is to increase the activated sludge basin capacity. Aeration of the activated sludge basins is responsible for a large portion of the electricity consumption in wastewater treatment plants, so more energy must be invested in order to treat more wastewater. The energy efficiency of wastewater treatment plants has become an important factor in both economic and environmental aspects recently. Providing energy for treating wastewater is a significant part of the total cost that should be considered when designing an individual treatment plant (Muga and Mihelcic 2008).

A lot of organic substances and nutrients like nitrogen and phosphorus reach the treatment plant every day. In the activated sludge process, microbial breakdown of organics produce large amounts of carbon dioxide, which is released to the air without any recovery or conversion. A better way to utilize the organic substances reaching a wastewater treatment plant would be using them for energy generation in the anaerobic digestion process. Direct anaerobic digestion of dilute organic substances is challenging, especially in temperate climates. Instead, the conventional activated sludge process could potentially be modified to enhance organics removal by adsorption rather than oxidation to carbon dioxide. Furthermore, ammonium could potentially also be removed from wastewater by adsorption. This would help to make wastewater treatment plants more energy efficient and reduce the amount of greenhouse gases (GHG) emitted.

1.2 Aim and goal

To design an efficient treatment system it is necessary to know the physical, chemical and biological processes which take place in the activated sludge system. The main goal of this thesis is to investigate biosorption (physical adsorption) of organic matter and ammonium on activated sludge flocs. It is known that there will be a rapid uptake of organic matters by the activated sludge as soon as wastewater comes in contact with sludge flocs (Boehnke et al. 1997). However the purpose of this thesis is to increase the knowledge of the mechanisms and kinetic relationships governing the biosorption process. In details, the specific objectives of this thesis are to:

- Investigate the adsorption capacity of the different kinds of activated sludge.
- Determine the adsorption isotherms.
- Examine the adsorption ability of inactive and active sludge.

- Study relationship between adsorption ability and extracellular polymeric substances (EPS).

2 Literature review

There are some studies which investigate adsorption of heavy metals, dyes and some other hazardous chemicals. Usually they investigate the adsorption of these chemicals to dead biomass instead of live activated sludge (Maurya et al. 2006). Removal of organic matter (i.e. biochemical oxygen demand BOD) and nitrogen are the most important processes in municipal wastewater treatment plants. However, there are few scientific studies which have investigated the adsorption of organic matter and ammonium onto activated sludge.

2.1 Adsorption

Adsorption is a separation technique in which molecules or particles are bound to a surface. The surface can be in different phases and features. In this thesis the adsorption of organic compounds and ammonium onto activated sludge is of interest. The performance of the adsorption method depends on the adsorbent-adsorbate equilibrium concentration and the rate at which mass transfers happen at constant temperature (Mannarswamyet al. 2009). The relationship is usually described by an adsorption isotherm. One of the earliest isotherms which can correlate data for adsorption was introduced by Freundlich in 1909. This model is usually used to describe the adsorption in wastewater systems. The Freundlich equation is as follows:

$$\frac{x}{m} = K C_e^{\,1/n}$$

where x/m is the concentration of the adsorbed material per unit weight of adsorbent, C_e is the equilibrium concentration of adsorbate in solution (mg/L), K (mg/g)(known as Freundlich parameter) and n are constants.

In 1916, Irving Langmuir published a new model isotherm:

$$\frac{C_e}{x/m} = \frac{1}{a * b} + \left(\frac{1}{a}\right) * C_e$$

where C_e and x/m are the same as above and a and b are constants. Langmuir assumptions for developing this isotherm are: (1) there are limited numbers of adsorption sites on the adsorbent of equal energy (2) adsorption is reversible. When using these two methods we should be aware that both isotherms are only valid over limited range of adsorbate concentration (Reynolds 1996)

2.2 Organic carbon adsorption

There was an observation from Boehnke et al. (1997) that illustrates when raw wastewater is mixed with activated sludge, there is a dramatic decrease in the concentration of organic matter in the first few minutes. He reported that in the first minute of contact, 40% of the organic load can be removed and after 10 minutes the removal efficiency can increase up to 70%. As this amount of time is not sufficient for oxidizing the organic matter, the phenomenon could be adsorption of organic matter to

the activated sludge (Boehnke et al. 1997). Another process that is designed base on physical adsorption is called adsorption-biooxidation (A-B) which was developed in Germany (Boehnke et al 1998). It was done in two steps. First the wastewater is fed to a high rate activated sludge process with a short hydraulic retention time (HRT) (about 30 minutes) and short solid retention time (SRT). This stage is preferably operated at oxygen content close to zero. Therefore, physical adsorption is the dominating process for removal of BOD in this stage. Then the effluent from this stage is fed to a second activated sludge process with longer SRT, where the wastewater is polished and nutrient removal can be achieved. The A-B process is a suitable alternative to standard activated sludge technology. It can reduce COD and BOD, while being extremely resistant to toxic shocks and pH fluctuations. Also, the capability of breaking down complex chemical pollutants make it suited for the treatment of industrial wastewaters. There are more than fifty treatment plants that use this process in order to treat the municipal and industrial wastewater (Boehnke et al 1998).

Tan and Chua investigated (1997) the adsorption of chemical oxygen demand (COD) to activated sludge. They established a process which can help measure the COD adsorption capacity (CAC) of the activated sludge. They determined the CAC by mixing the sewage with activated sludge and measured the rapid reduction of the COD per unit mass of activated sludge. They observed that the CAC increased with flow along the aeration unit. The fact behind this observation is that the sludge in the inlet of the aeration unit was probably overloaded with substrate so it had a lower adsorption capacity. The CAC could be used to estimate the air supply for aeration unit in order to improve and facilitate the activated sludge process and prevent the bulking problems (Tan and Chua 1997).

There is another study which investigated the activated sludge as flocculants for an advanced primary treatment process (Zhao et al. 2000). They conducted this research due to the idea that the activated sludge costs less in comparison with the chemical flocculants. They achieved to 70-80% COD removal and 80-95% removal of suspended solids in the primary treatment step by using activated sludge as flocculants.

2.3 Ammonium adsorption

There are some studies conducted about adsorption of ammonium to activated sludge and biofilms. Ammonium is well-known as a complex that can adsorb to various organic and inorganic compounds in e.g. soil (Wang and Øien 1986) and sediment systems (Nielsen et al. 1996). Nielsen et al. (1996) investigated the adsorption of ammonium to activated sludge in two treatment plants. They observed that ammonium could adsorb to activated sludge flocs in both treatment plants. They determined that NH_4^+ was adsorbed to activated sludge in concentrations up to 0.5 mg N per g suspended solids.

There is also a study which compares the adsorption of ammonium to activated sludge, aerobic granules and anammox granules (Bassin et al. 2011). Bassin et al. (2011) found that the adsorption of NH_4^+ to activated sludge and anammox granular is around 0.2 mgNH_4-N/gVSS while aerobic granular sludge had higher adsorption capacity between 0.9 and 1.7 mgNH_4-N/gVSS. They reported that there is no significant difference in ammonium adsorption in lab scale reactors which was operated at different temperature (20 and 30 °C). Bassin et al. (2011) observed that there was a considerable decrease in adsorption of ammonium when the concentration of salt was increased in the solution. Ammonium has a positive surface charge. Therefore, the competition between salt (i.e. Na^+) and

ammonium for binding to the negatively charged groups in the EPS or microbial cell walls can lead to a significant decrease in adsorption of ammonium.

Schwitalla et al. (2008) studied ammonium adsorption in full scale sequencing batch reactors (SBR).They showed that adsorption and desorption of the ammonium played a significant role in ammonium mass balance. They reported an adsorption from 0.07 to 0.2mg NH4-N/gVSS to activated sludge flocs.

Wik (1999) studied the ammonium adsorption in a trickling filter. He estimated an ammonium adsorption of 2.7 mg NH_4-N/m2 when the ammonium concentration was 15mg NH_4-N/L in influent. Temmink et al (2001) studied the ammonium adsorption in biofilm reactors. They observed that when the influent ammonium concentration was 52±20mg NH_4-N/L or 38±20mg NH_4-N/L there was an ammonium adsorption of 9 or 21% respectively.

2.4 Extracellular polymeric substance (EPS)

The sludge floc has complex and heterogeneous composition. One of the major components of the activated sludge is extracellular polymeric substances (EPS) (Frølund et al., 1996). Two main components of the EPS are protein and carbohydrates (Sheng et al, 2010). Moreover, humic substances is another the key component of the EPS in activated sludge (Frølund et al., 1996). The characteristics of the sludge completely depend on the environment which is surrounding the sludge flocs. The environmental condition has an impact on the EPS, because it originates from metabolism or lysis of microorganism and wastewater itself (Urbain et al., 1993). In other words, inducing certain conditions can cause specific changes. Environmental parameters like pH, wastewater composition and available cations can affect the chemical and physical characteristic of EPS (Nielsen et al 1997).The EPS provide a highly hydrated gel matrix around a microbial cell (Wingendar et al., 1999). The EPS matrix plays a significant role in the physiochemical properties of the microbial aggregates such as adsorption ability (Sheng et al., 2010). The EPS matrix is always negatively charged and could bind with the positively charged components like ammonium through electrostatic interactions (Esparza-Soto and Westerhoff, 2003). The activated sludge flocs have a dynamic double-layered EPS structure of the loosely bound EPS (LB-EPS) diffused from the tightly bound EPS (TB-EPS) that surrounds the cells (Jorand et al., 1995). Humic substances have a lower binding strength and capability in comparison with protein. Loosely bound EPS has higher fraction of protein than tightly bound EPS, hence LB-EPS may have a greater binding capability than the TB-EPS. (Pan et al. 2010).

Ye et al. (2011) investigated the influences of the different carbon source on EPS and flocculation, settling and dewatering properties of activated sludge. They reported that there is no significant correlation between the LB-EPS and TB-EPS or poly saccharides level and flocculation, settleability and dewaterability of the activated sludge.

The literature overview above shows that the adsorption of organic matter and ammonium are important phenomena in activated sludge processes. As for adsorption of organic matter, there are some processes such as contact stabilization and adsorption bio oxidation that relies on the ability of

the activated sludge to rapidly uptake organic substances. However, because of the lack of scientific knowledge in the field of adsorption capacity of activated sludge, the thesis project proposed here is warranted. Our study can help optimize the process of adsorption of organic matter and ammonium to activated sludge, which will allow us to design more efficient wastewater treatment plants, both in terms of energy consumption and pollutant removal.

3 Methodology

The experimental parts of the project were done in the Environmental Chemistry laboratory at the department of Civil and Environmental Engineering, Chalmers University of Technology. The laboratory tests are divided to five parts:

- Initial adsorption tests
- Set up sequencing batch reactors (SBR)
- Improving the method
- Final adsorption tests
- Monitoring tests (i.e. EPS, TSS)
- Analytical methods

The activated sludge was collected from the Rya wastewater treatment plant in the city of Gothenburg. During the adsorption experiments, glucose as dissolved organics, milk powder as colloidal organics or ammonium chloride as nitrogen source was added to the activated sludge.

3.1 Initial adsorption tests

Preliminary experiments were conducted to examine adsorption of glucose, milk powder, and ammonium onto fresh and active activated sludge harvested directly from the treatment plant. These results could then serve as a comparison for adsorption using sludge from the SBRs.

For the initial adsorption tests the activated sludge was collected from the Rya wastewater treatment plant and used the same day. The activated sludge mixtures were centrifuged and the supernatant was removed. The settled activated sludge was resuspended in tap water (In the first experiment a mixture of a buffer and a trace complex was used instead of tap water (see Table 3.1)). Then the suspension was divided into 6 beakers, each of a volume of 800mL. The sludge and water inside each beaker was mixed by a stirrer and left over night in order to let the sludge acclimatize to the new conditions. Air was pumped only to three of these six beakers during the night.

Table 3.1. Composition of buffer and trace element solution.

Buffer	K_2HPO_4 6 g/L	NaH_2PO_4 1.88 g/L	$MgSO_4$ 0.5 g/L	$CaCl_2*2H_2O$ 0.015 g/L	$FeSO_4*7H_2O$ 0.01 g/L			
Trace	H_3BO_3 0.05 g/L	$ZnCl_2$ 0.05 g/L	$CuCl_2*2H_2O$ 0.038 g/L	$MnSO_4*4H_2O$ 0.05 g/L	$(NH_4)6Mo_7O_{24}*4H_2O$ 0.05 g/L	$AlCl_3$ 0.05 g/L	$CoCl_2*6H_2O$ 0.05 g/L	$NiCl_2$ 0.05 g/L

The first step of this adsorption test was to let the sludge settle in the beakers. After that, 400 mL of supernatant was removed from each beaker and 400 mL of the glucose, milk powder or ammonium solution with a concentration of 200mg C-N per litre was added to the activated sludge. The solutions made by dissolving the compounds in tap water (Table 3.1). Furthermore, 220mg NaCl was added to tap water in order to achieve the same conductivity as the original activated sludge (625µs/cm). Samples were taken from each beaker after 1 min, 10 min, 25 min, 50 min and 160 min time intervals (see figure 3.1). These samples were centrifuged at 4000g for 5 minutes. Supernatant were collected and stored in 15mL tubes. By measuring the total organic carbon (TOC) and total nitrogen (TN) in the original wastewater and component solutions, it is possible to calculate the theoretical initial TOC and TN. The average of these two numbers is represented as a theoretical initial concentration for

the mixture. Samples were analysed by TOC and TN analyser device (TOC-V, Shimadzu). The difference between the initial TOC (TN) and the one which was measured from time intervals represented the adsorbed part. However, it is hard to find out whether the reduction of TOC (TN) in the beakers after each sampling was due to adsorption of carbon (nitrogen) to activated sludge. This uncertainty originated from the fact that there should be some oxidation of the carbon/nitrogen by some groups of heterotrophs and nitrifying bacteria in the activated sludge. However, this primary experiment could shows whether there was adsorption of C or N in the first seconds when the new wastewater came in contact with the activated sludge.

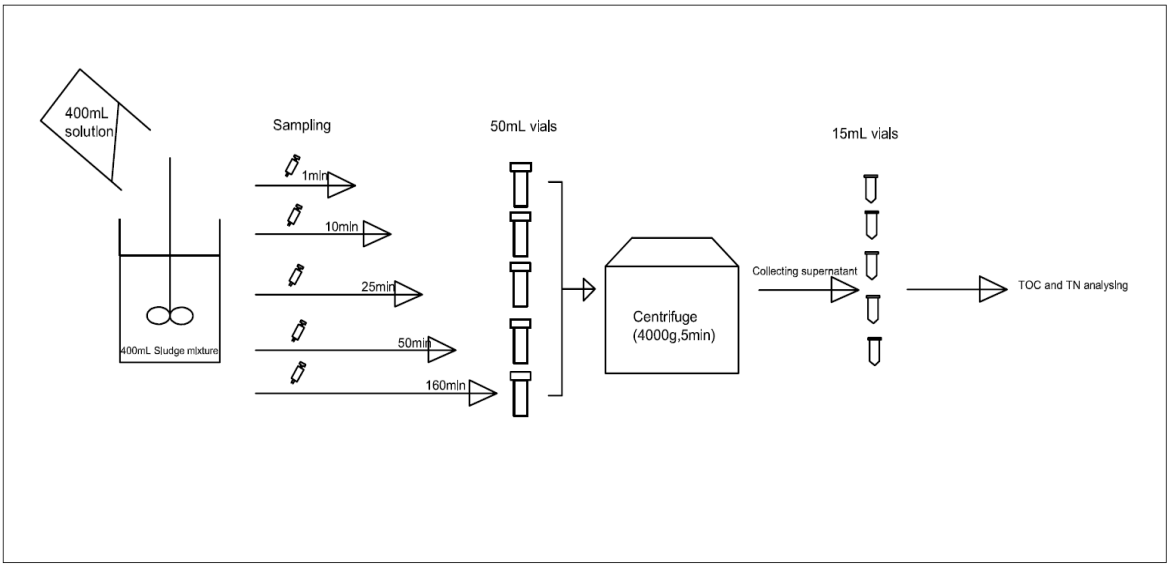


Figure 3.1. A schematic drawing of initial adsorption test.

3.2 SBR set up

One of the important goals of this thesis is to investigate the adsorption capacity of different activated sludge mixtures in contact with different compounds. To produce different kinds of activated sludge, it was decided to run three sequencing batch reactors with the volume of 8 litres each. They were fed for about two months with different chemical components. Two of these reactors were fed with carbon source and one with nitrogen/ammonium source. The carbon source solution which was used to feed reactor number one is composed of glucose, sodium chloride, di-potassium phosphate, sodium bicarbonate and ammonium chloride in tap water. Reactor number two was fed with the same component solution which was added to reactor number one, but instead of sodium chloride, calcium chloride was added (Table 3.2). Reactor number three was fed with ammonium chloride, sodium bicarbonate and di-potassium phosphate (Table 3.2). Table 3.2 illustrates the dosage of chemicals which was added to each reactor every two days for an experimental period of two months. During these two months the reactors were aerated with air pump and were mixed at a speed of 145 min^{-1}.

Table 3.2. Composition of synthetic wastewater solutions fed to the SBRs. The compounds were mixed in tap water.

Chemical(mg/L)/Reactor	Reactor one	Reactor two	Reactor three
sodium chloride	5332	-	-
calcium chloride	-	1176	-
sodium bicarbonate	3100	3100	10750
di-potassium phosphate	128	128	128
Ammonium chloride	456	456	3420
Glucose	8000	8000	-

The reactors were fed every two days. First the aeration pump and mixing unit were turned off. Then the reactor was left for 10 minutes. The sludge settled during this time. Then, 4L of supernatant was removed from the activated sludge reactors and 4L of the solutions which are mentioned above were added to the reactors. Before and after feeding, two samples were collected from each reactor for TOC and TN analysis.

The main reason that the reactors were fed with different components is to produce different kinds of activated sludge with different characteristics mainly in terms of extracellular polymeric substances (EPS) composition but also with different floc structure.

3.3 Improving the method

3.3.1 Adsorption

In order to improve the adsorption test we decided to do the adsorption test in 50mL tubes and triplicate samples for each time interval. A shaking table with a rotation speed of 150 rpm was used for mixing the activated sludge and solution during the adsorption tests. The centrifuge with 4000g was used for 5 minutes for each sample to separate the activated sludge.

A specific amount of activated sludge was collected from each reactor. Then 40 mL of this activated sludge was poured in 50mL tubes and left to settle. Then 20mL of the supernatant from each tube was removed. The 20mL mixture of the solution which was of interest to study for adsorption (glucose, milk powder, or ammonium) was added to the activated sludge and the sample tubes were left on the shaking table for a certain time intervals. For each activated sludge mixture, 5 time intervals were investigated and for each time intervals 3 replicate samples were used. After each time interval the sample was centrifuged. The supernatant was collected for analysis. The main idea that 50mL tubes were used instead of beakers was that less sludge would be used and it would be possible to run three replicate samples at the same time.The collected supernatant was analysed by TOC analyser.

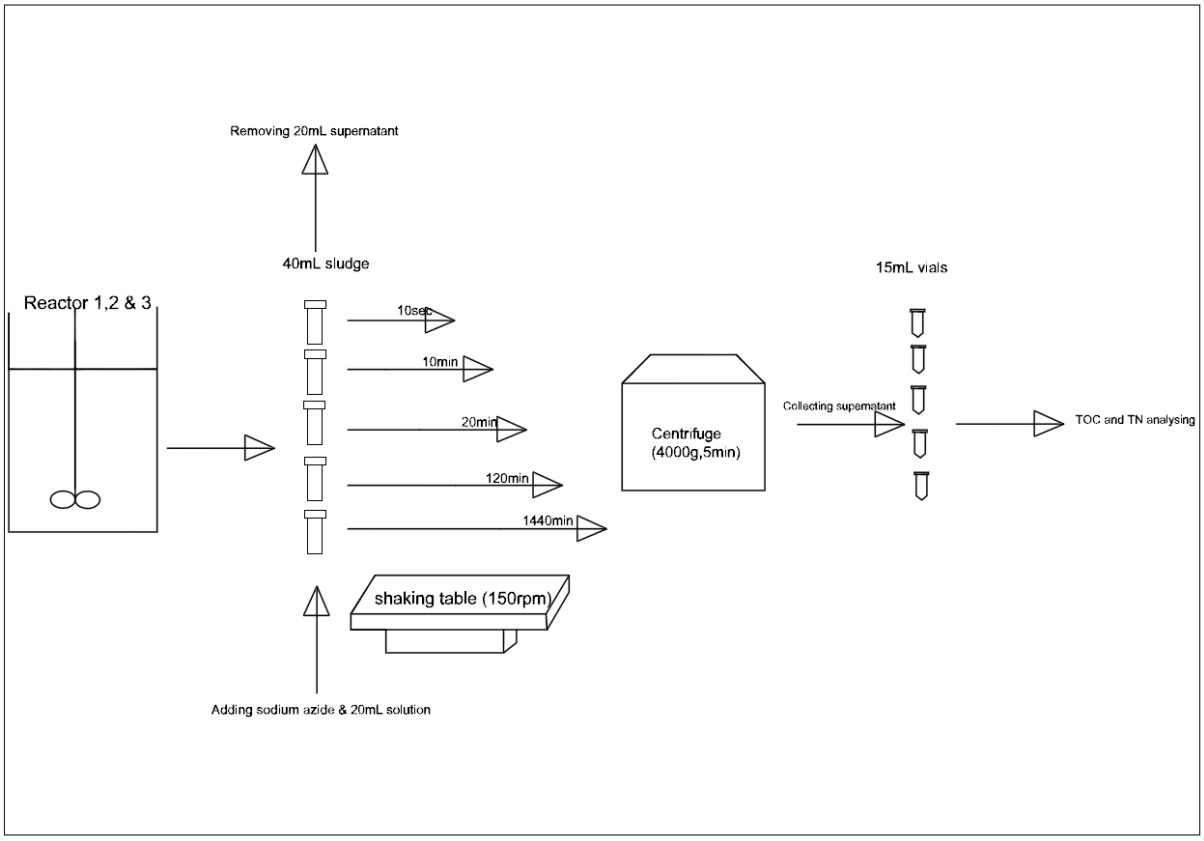
3.3.2 Improving the method to prevent unwanted oxidation

There should be an oxidation occurring as soon as the solution has contact with the activated sludge. The bacteria inside the system start to oxidize the glucose/milk powder/ammonium. To prevent the system from this phenomenon, it was decided to add the 1 g/L sodium azide to the activated sludge. Sodium azide can inhibit the bacteria which are inside the activated sludge, so they cannot oxidize the organics and ammonium (Barbot et al. 2010). Sodium azide was added after the 20mL supernatant was removed from 50mL activated sludge tubes. After adding the sodium azide samples were left for 2 minutes. During this time sodium azide could have a sufficient effect on the bacteria. The rest of the experiment is identical to previous experiments.

3.4 Final adsorption tests

The final adsorption tests were done exactly in the same way as the 50mL tube tests (see figure 3.2). In these tests sodium azide was added to inhibit the activity of the bacteria. The experiments were done frequently with different kinds of activated sludge which were produced in the reactors and also different concentrations of carbon sources in order to determine the adsorption isotherms.

TOC, TN, ammonium, nitrate and milk powder concentration inside the solutions were measured in order to estimate the adsorption capacity of activated sludge. These parameters were analysed by different analytical instruments which are presented in the analytical methods section of this chapter.



Figur 3.2. The Schematic drawing of final adsorption test.

3.5 Monitoring tests

3.5.1 The EPS test

Since the three reactors in this experiment were fed with different components, it was expected that EPS inside each reactor differs from the others. In order to monitor the changes in EPS characteristics the sonication/thermal extraction process (Li and Yang, 2007) was done frequently to extract the LB-EPS and TB-EPS. The activated sludge was centrifuged at 4000g for 5 minutes in a 50mL tube. The sludge pallet was resuspended in a NaCl solution (tap water and NaCl) which had similar conductivity of the original sludge suspension. After resuspension, this new sludge mixture was sheared by vortex for 1 minute and sonicated at 20 kHz and 330WL^{-1} for 2 minutes. Then the sample was shaken horizontally for 10 minutes at 150rpm by shaking plate. In order to separate the supernatant and the solid, the liquor was centrifuged for 10 minutes at 4000g. The collected supernatant was considered

as LB-EPS of this sludge sample. The residual sludge pellet left in the centrifuged tube was resuspended in the NaCl solution up to the original volume of 50mL, and sonicated for 3 minutes, then heated at 60° C for 30 minutes and finally centrifuged for 20 minutes at 4000g. The collected supernatant was considered as the TB-EPS of the sludge sample. The solids content of the sludge confined the quantity of the polymers which were extracted. Therefore, the amount of extracted EPS was normalized to the amount of sludge in each sample.

This EPS extraction process was done for each reactor frequently and the LB-EPS and TB-EPS were collected and analysed with TOC analyser and expressed as mg TOC (TN) g-1 VSS. The TOC and TN which was measured represented the carbohydrate and protein content changes of the EPS. The TSS and VSS of the sludge were also measured for each EPS extraction.

3.5.2 Investigating sludge activity

There was an experiment done in order to investigate the ability of oxidizing carbon and nitrogen by different kind of activated sludge from each reactor. Four beakers were filled up to 800 mL with the sludge from reactor one and three and left to settle for over 45 minutes.

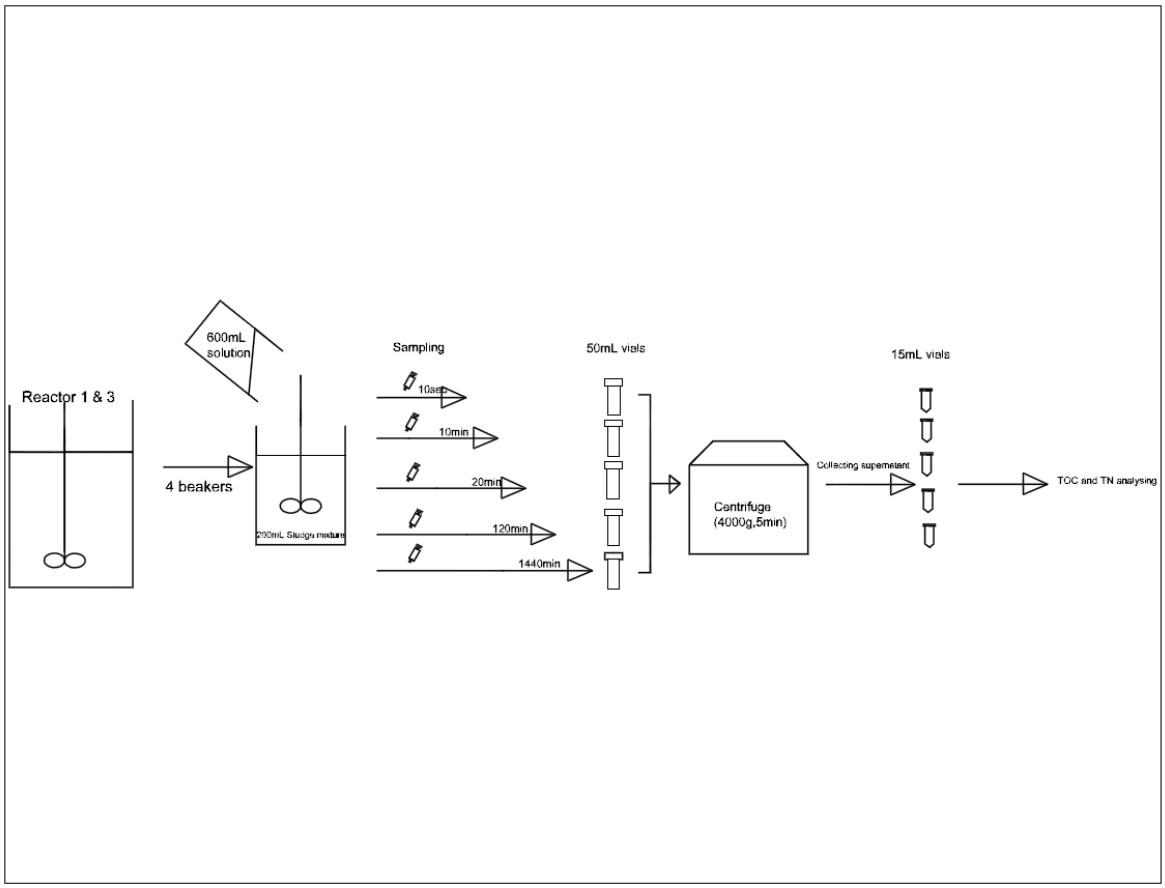
To prepare the carbon and nitrogen solution, glucose and ammonium was dissolved separately in the medium that was used for feeding the reactor number one and three (Table 3.3). The concentration of carbon and nitrogen in the mentioned solution was around 200mg C-N/L.

Table 3.3. The mixture inside beakers for oxidation test

	$C_6H_{12}O_6$ (mg/L)	K_2PO_4(mg/L)	NaCl(mg/L)	NH_4Cl(mg/L)	$NaHCO_3$(mg/L)
beaker 1 (reactor one)	500	30	1200	120	780
beaker 2 (reactor one)	-	30	1200	771	780
beaker 3 (reactor three)	500	30	-	-	2100
beaker 4 (reactor three)	-	30	-	771	2100

600mL supernatant of the sludge was removed. The prepared solution was added to the sludge and the mixing device started to mix them at 200 rpm in presence of air. Beaker 1 was filled with sludge from reactor one (fed with glucose component) and glucose solution. Beaker 2 was filled with same sludge and ammonium solution. Beaker 3 was a mixture of sludge from reactor three (which was fed with ammonium), and glucose solution. Beaker 4 was filled with the same sludge and ammonium solution. The process of sampling was done in five steps, approximately 10 sec, 10, 20, 120 and 1440 minutes after initial mixing. Sampling was done with a syringe.

After each step of sampling the collected samples were harvested by centrifugation (4000g, 5min) in 50mL tubes. The supernatant was stored in 15mL tubes (see figure 3.3). The samples which contained carbon were analysed by TOC analyser, the rest that contained nitrogen were examined with ion chromatography. The ion chromatograph was used to determine how much of ammonium was oxidized into nitrate or nitrite. The TSS and VSS were also measured for each beaker before adding the solution.



Figur 3.3. The Schematic drawing of the test for investigating the sludge activity.

The amount of decrease in TOC and TN shows the oxidation rate of the C and N by heterotroph and nitrifying bacteria inside the sludge. The purpose of this experiment was to show the differences between the sludge which was produced during 2 months operation of the SBRs. Furthermore, it can help investigate which kind of sludge (rich with heterotrophs or nitrifiers) is more capable to adsorb for example ammonium better than the others. Finally, it can help categorize the characteristics of sludge which is of interest to study in this project.

3.5.3 Monitoring the reactors

Monitoring of these three reactors was one of the important issues during this project. It was done with some simple tests. The TSS (VSS) was measured every two days. Also dissolved oxygen (DO), pH and conductivity of the sludge were measured with the DO sensor device (WTW multi 350i) three times a week. The changes of these three factors were insignificant during the period of two months after two weeks of running when the reactors got to a stable level. The Table 3.3 shows the average value of the DO, pH and conductivity for each reactor during this experiment.

Table 3.4 The average DO, Conductivity and pH for SBR.

	Reactor one	**Reactor two**	**Reactor three**
DO (mg/l)	7	7.5	9.5
Conductivity (µs/cm)	3200	1200	3300
pH	8.2	8.1	8.3

The TOC and TN inside the reactors were measured before and after feeding process. The monitoring results will be presented in the next chapter.

3.6 Analytical methods

3.6.1 TOC and TN analysis

TOC and TN concentrations were measured with a TOC-V analyser (Shimadzu). Preparation of samples before analysis was done by diluting the samples to a TOC concentration of less than 200mg/L.

First the samples were centrifuged (4000g) for 5 minutes. Then they were diluted with Milli-Q water into the appropriate concentration. EPS samples were filtered after the centrifugation. The filtration process was done before dilution. The size of filter which was used for EPS samples is 0.45µm.

3.6.2 Ion Chromatography

An ICS-900 ion chromatograph (Dionex) was used. Samples were prepared by taking 0.5 ml of supernatant collected from adsorption tests and diluting it up to 25mL with milli-Q water. Then 10mL of that was filtered through 0.45µm filters and was stored in special tubes. These tubes were put in the ion chromatograph in order to be analysed. The chromatography software calculated the concentrations of ammonium, nitrate and nitrite based on the results from standards with known concentrations.

3.6.3 Spectrophotometer

The spectrophotometer (Shimadzu) works with different wavelengths and measures the absorbance of light by the sample. The spectrophotometer was used to measure adsorption of milk powder (colloidal organic matter). Therefore, we needed to find the specific wavelength that can show the turbidity changes in our samples. First we found the optimum wavelength by drawing the curve of wave absorbance for different concentration of milk powder solution. The optimum wavelength is from 400 to 700 (400, 500, 600, and 700). Then it was possible to draw standard curve for the specific wavelength against different concentration. Afterward, by measuring the turbidity for each sample and comparing it with the standard curve, it was possible to calculate the expected milk powder concentration in each sample.

3.6.4 High-Performance Liquid Chromatograph (HPLC)

The HPLC was used in order investigate organic components present in the EPS samples. It has two different detectors: UV detector and refractive index detector. The EPS samples were filtered through the 0.45µm filters. Then, 0.1mL of the filtered sample were stored in small vials and put in the HPLC for analysing. The samples flowed through a specific column (Aminex HPX-87H) where the compounds were separated according to their retention time. The Aminex HPX-87H column is used for the analysis of carbohydrates found in solution with carboxylic acids, volatile fatty acids, short chain acids, alcohols, ketones and many neutral metabolic by-products. After that the liquids flowed through the detectors for analysis. By defining the standard curves for different compound it will be possible to calculate the concentration of different liquid compounds inside the samples.

4 Results and discussion

In this chapter, the results from the series of experiments are presented and discussed. The experiments were conducted in the Environmental Chemistry Laboratory at the department of Civil and Environmental Engineering, Chalmers University of Technology, between July 2012 and December 2012.

4.1 Initial experiments

In order to investigate whether activated sludge can rapidly adsorb organic substances and ammonium, four initial experiments were conducted. The results from this initial study could then be used as a comparison in the following experiments. These initial tests were done from July to August 2012.

4.1.1 Removal of glucose by activated sludge suspensions

In the first experiment sludge was resuspended in the mixture of buffer and trace solution (see Table 3.1). For adsorption test, glucose with concentration of 200mgC/L was dissolved in the solution above. Then the glucose solution was added to the sludge mixture. The test was done in 6 beakers, three of them were aerated and the others were not aerated. The diagram below shows the changes of TOC over a period of 3 hours.

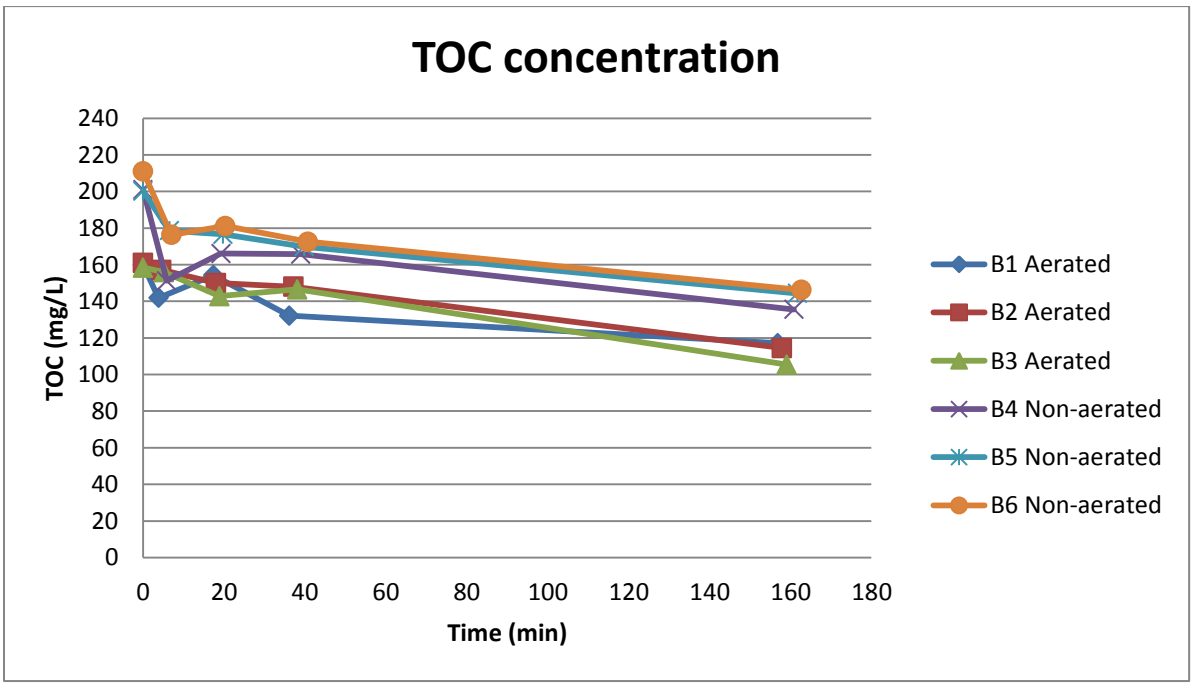


Figure 4.1 Removal of glucose in aerated and non-aerated beaker containing activated sludge.

The results show that the TOC in these beakers was decreased continuously over a period of 3 hours. The dramatic decrease in TOC concentration in the first minutes compared to the last part of the experiment indicates that adsorption took place as bacteria did not have the sufficient time to oxidize the organic compounds. The TSS was measured and equal to 4.2 g/L (TSS) and 2.2 g/L volatile suspended solid (VSS).

Table 4.1 shows the differences between the uptake rate (mgTOC/gVSS.min) of first minute and last 2 hours of mixing. The uptake rate in first minutes of contact is higher than the uptake rate in last 2 hours of mixing in all beakers.

Table 4.1. Uptake rate in first minute of contact and last 2 hours of mixing in 6 beakers.

	B1	B2	B3	B4	B5	B6
First minutes (mgTOC/gVSS.min)	1,340	0,238	0,138	1,933	0,754	1,069
Last 2 hours(mgTOC/gVSS.min)	0,044	0,096	0,117	0,085	0,072	0,074

During this test it was observed that the concentration of organic carbon in the resuspended sludge was higher than was expected and around 100mg/L in aerated beakers and 200mg/L in non-aerated beakers. One possible reason that could cause this problem was the buffer solution which the sludge was resuspended in caused deflocculation or release of organic substances by the sludge. Therefore a second experiment was conducted in order to investigate this phenomenon.

4.1.2 Release of TOC by activated sludge under varying conditions

In the second experiment, the initial TOC concentration was studied in three different activated sludge solutions: (1) original activated sludge which was taken from Rya WWTP, (2) sludge which was resuspended in buffer solution (see table 3.1) and (3) sludge that was resuspended in tap water. The experiment was done in both aerated and non-aerated conditions. The purpose of this experiment was to find the best conditions for conducting the adsorption tests. The adsorption tests should be conducted in a solution that has minimum impact on the sludge and allows reproducible experiment.

The Table 4.2 shows the results for the second experiment. The activated sludge was mixed under the mentioned condition in table 4.2 for 24 hours. The samples from each beaker were analysed in two ways, filtered and not filtered. Samples were filtered by 45um filter.

Table 4.2 Initial TOC concentration for different mixtures

Activated sludge mixture and condition	TOC conc. with filtration (mg/L)	TOC conc. without filtration (mg/L)
Tap water	8.9	10.04
Buffer and with aeration	29.7	49.9
Buffer and without aeration	34.0	51.8
Original WW and with aeration	16.2	16.7
Original WW and without aeration	14.9	14.9

According to the table above TOC concentration was increased when the sludge was resuspended in buffer solution in comparison with the original wastewater. However there was not a significant change in initial TOC concentration when the sludge was resuspended in tap water. Moreover, for sludge resuspended in tap water and original wastewater, TOC concentration did not vary significantly when the samples were filtered in comparison when they were not filtered. The purpose of filtration was to investigate if there were some colloidal particles inside the supernatant which could not be settled by centrifugation. As long as there is no difference in TOC concentration

between the filtered samples and non-filtered samples, we decided not to filter the samples in next experiments.

The conductivity of the mixtures was measured by conductivity sensor. The results are: 632µs/cm for original wastewater, 255µs/cm for tap water solution and 7600µs/cm for buffer solution. Higher conductivity could lead to higher turbidity and higher rate of disintegration of the flocs and in consequence release more TOC into activated sludge mixture (Rasmussen et al. 1994). Based on the results which were observed in this experiment we decided to use tap water solution with the same conductivity as original wastewater instead the buffer solution and aerated condition in next experiment. 215 mg NaCl was dissolved in tap water in order to increase the conductivity up to 632µs/cm

4.1.3 Removal of glucose, milk powder, and ammonium by activated sludge

The third experiment was done in order to compare the sludge that was either resuspended in tap water or the original sludge supernatant. Milk powder, ammonium and glucose were dissolved separately in tap water which had the same conductivity as the original wastewater. The concentration of carbon and nitrogen after dissolving in each solution was around 200mg/L. The adsorption test was done in 6 parallel beakers and 5 time intervals. The TSS and VSS in all beakers were measured and were around 2.3gTSS/L and 1.2gVSS/L. Figure 4.2 shows the removal of TOC by activated sludge for glucose and milk powder solution in a period of 150 minutes.

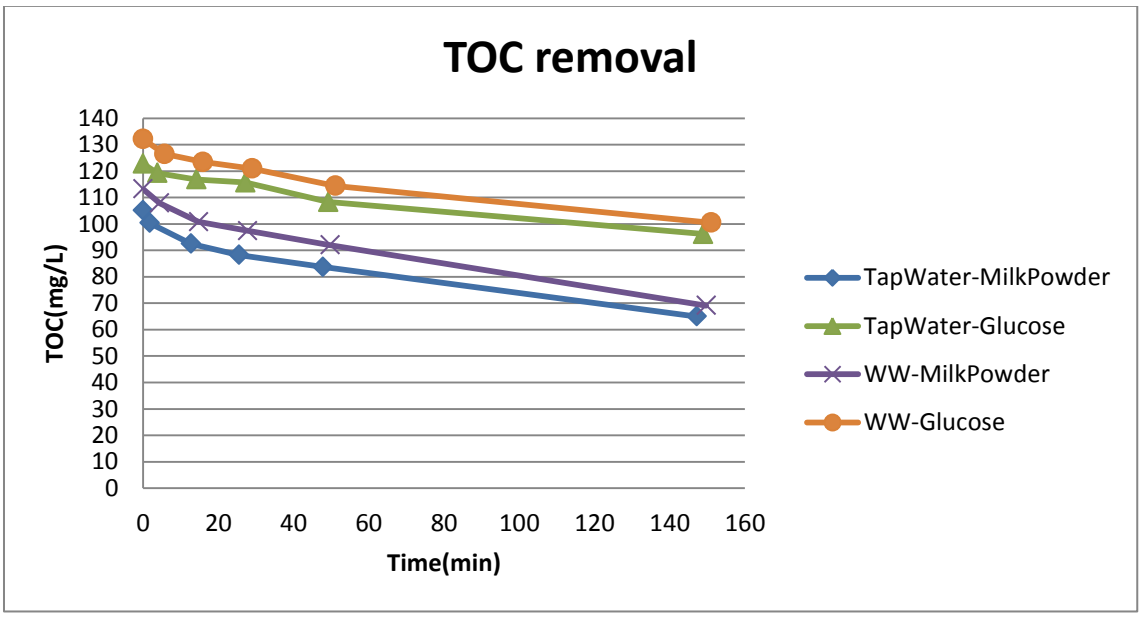


Figure 4.2 TOC changes for milk powder and glucose solution in contact with different sludge.

The graph illustrates that there was not considerable differences in TOC removal ability between tap water and original wastewater sludge mixture due to the same removal trend. It can be seen that TOC removal rate was substantially lower after a rapid decrease in the first minutes of contact. Figure 4.3 shows cumulative specific uptake of milk powder and glucose.

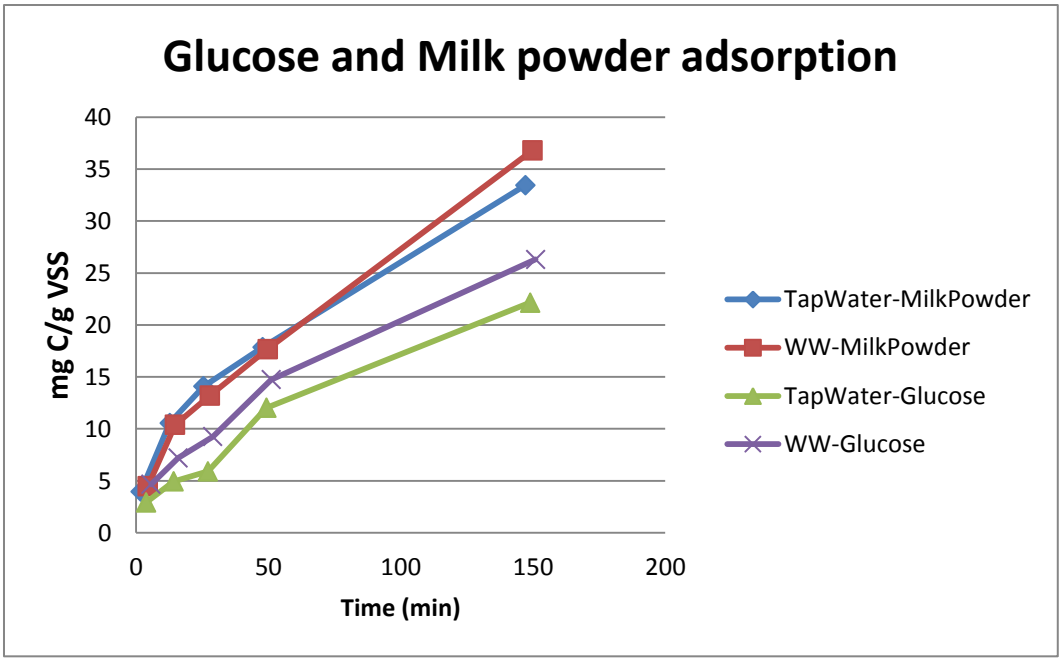


Figure 4.3 Milk powder and glucose removal rate for a period of time.

Table 4.3 shows the uptake rate of TOC per VSS per unit of time for first minutes of mixing and last 100 minutes of mixing. The results show that the uptake rate in first minutes of contact in all beakers was much higher than the uptake rate in last 100 minutes of mixing. The reason could be the oxidation of TOC and physical adsorption of TOC were happened simultaneously in the first minutes when the solution had contact with sludge.

Table 4.3. Uptake rate in first minute of contact and last 100 minutes of mixing in 4 beakers.

	TW-MP	WW-MP	TW-G	WW-G
First minutes (mgTOC/gVSS.min)	2,266	1,012	0,764	0,824
Last 2 hours(mgTOC/gVSS.min)	0,127	0,153	0,082	0,092

Total nitrogen was measured by TOC analyser in order to estimate the nitrogen removal ability of these two kinds of activated sludge mixture. The results are shown in the Figure 4.4. It was observed that there was not a significant variation in concentration of nitrogen in the mixtures. Therefore, the nitrogen removal ability of the sludge mixtures was low.

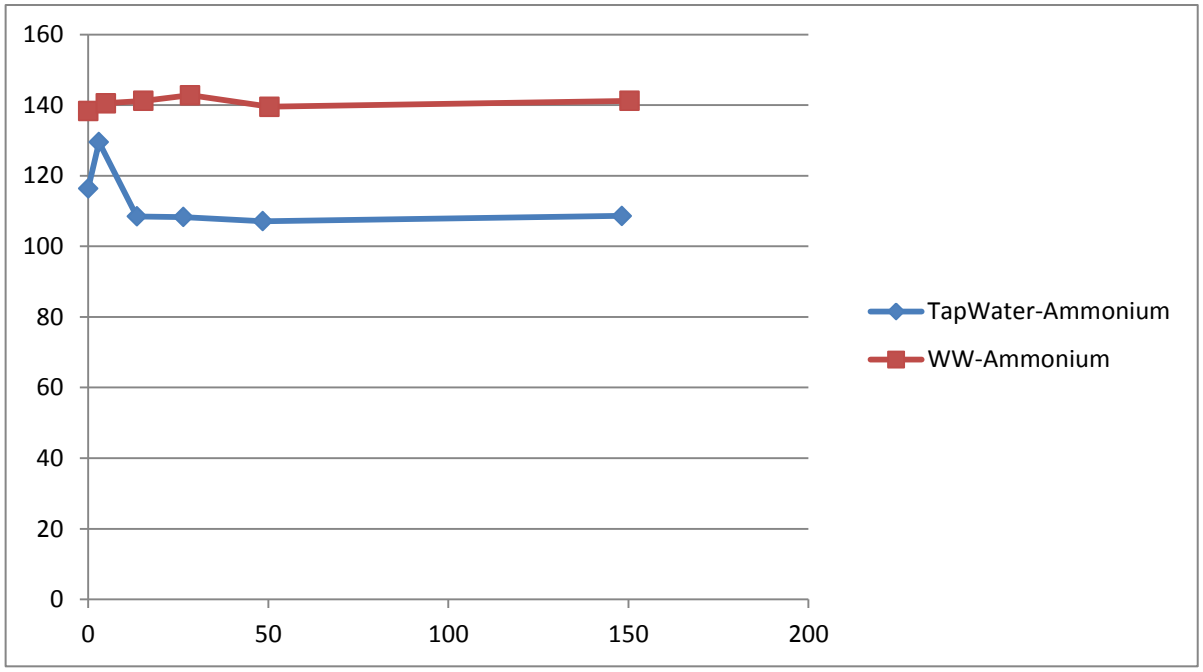


Figure 4.4 The TN changes over a period of time for different sludge mixtures.

4.1.4 Removal of glucose, milk powder, and ammonium by activated sludge in three replicate samples.

In order to avoid the error during the experiments, it was decided to do the further experiments with three replicates for a certain time interval. The fourth test was done in the same way as third experiment instead in 50mL tubes. Tap water was used as a solution. The figure below displays the changes in TOC after mixing the glucose and milk powder solution (200mg C/L) with the activated sludge mixture. Samples were taken in 6 time intervals for a period of 24 hours. The average TOC concentration of the replicates is shown in Figure 4.5 as a concentration of TOC in certain time.

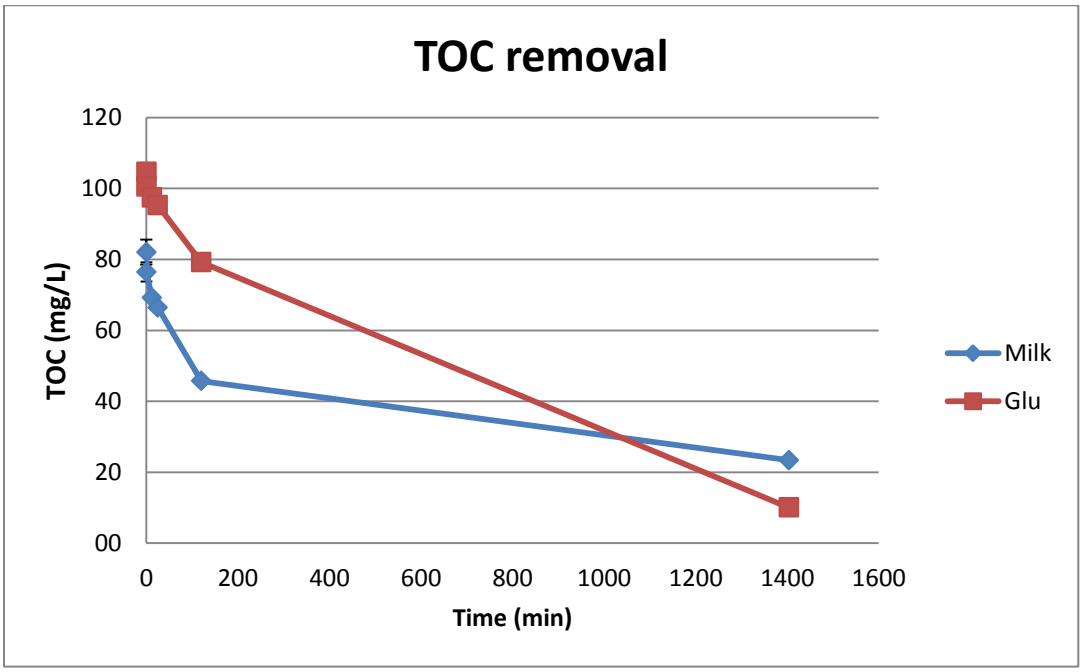


Figure 4.5. Variation of TOC in milk powder and glucose solution in contact by sludge. Error bars show standard deviations of three replicates.

These results show that the activated sludge could remove the milk powder and glucose very fast in the first minutes of contact and then the removal rate decreased slightly into a stable level after some hours. Figure 4.6 illustrates the removal rate of carbon and nitrogen for the milk powder, glucose and ammonium. It was observed that a fast uptake happened in the first minute of mixing the ammonium solution with sludge. Then after 10 minutes nitrogen could not be removed by activated sludge any more.

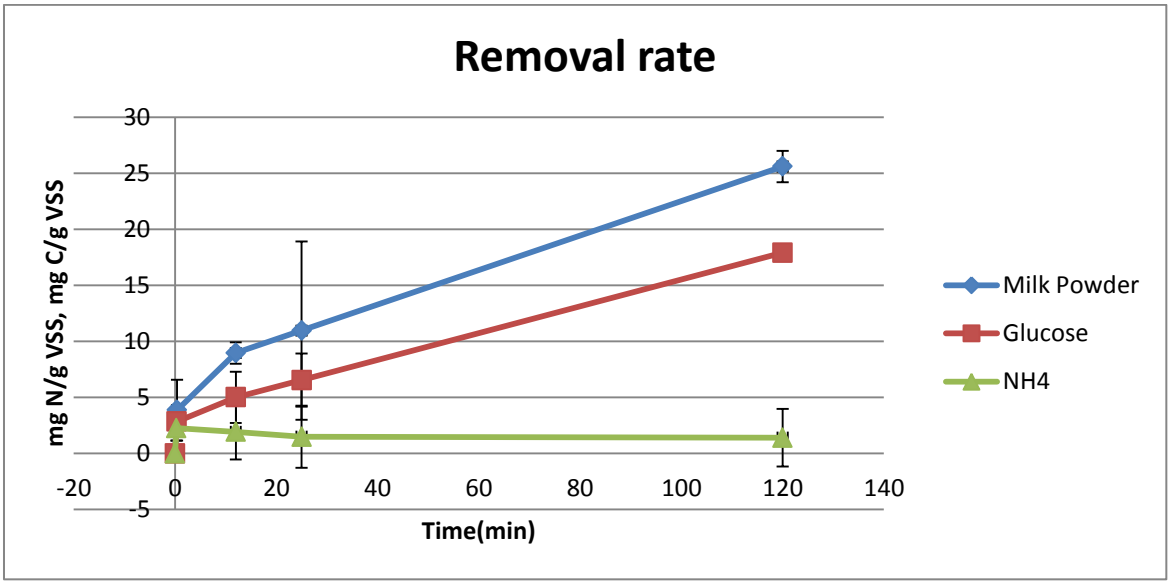


Figure 4.6. Removal rate of nitrogen and carbon. Error bars show standard deviations of three replicates.

It can be clearly seen that 3mgC/VSS.min and 4mg milk powder-C/VSS.min was removed after the solution had contact with sludge. 10 minutes after first contact 0.25mgC/gVSS.min was removed by activated sludge and this removal rate is lower than the first minute of contact. Then, there was 5mg milk powder-C/gVSS.min removed after 10 minutes. By comparing this uptake per minute with first minute uptake, it could be observed that the amount of carbon which was removed every minute in a period of 10 minutes is lower than the first uptake. The VSS of the sludge was 1.4 g/L. Since the first minute of contact is a short time for bacteria to oxidize the organic carbon it is assumed that the fast carbon removal should be a physical adsorption of carbon into activated sludge. However, the error bars for glucose and milk powder adsorption show that the results were inaccurate for the samples which were taken after 25 minutes of mixing.

4.2 SBR set up results

To investigate the effect of different types of sludge on adsorption capacity, activated sludge was cultivated in three SBRs under different conditions. The results from this experiment are divided into 3 parts. Each part represents the results for a specific reactor. The results include the TOC, TN, TSS, VSS and EPS monitoring over three months from 18th September to 18th of December 2012.

4.2.1 Reactor one

Reactor number one was fed with the solution which was shown in Table 3.1 in previous chapter. The TOC was measured every two days to monitor the carbon consumption ability of the bacteria. The results for TOC concentration plotted against time are shown in the Figure 4.7. The peaks in the graph represent the TOC concentration which was measured immediately after feeding the reactor.

The drops represent the TOC concentration 2 days after feeding, when the bacteria had time to consume the organic carbon.

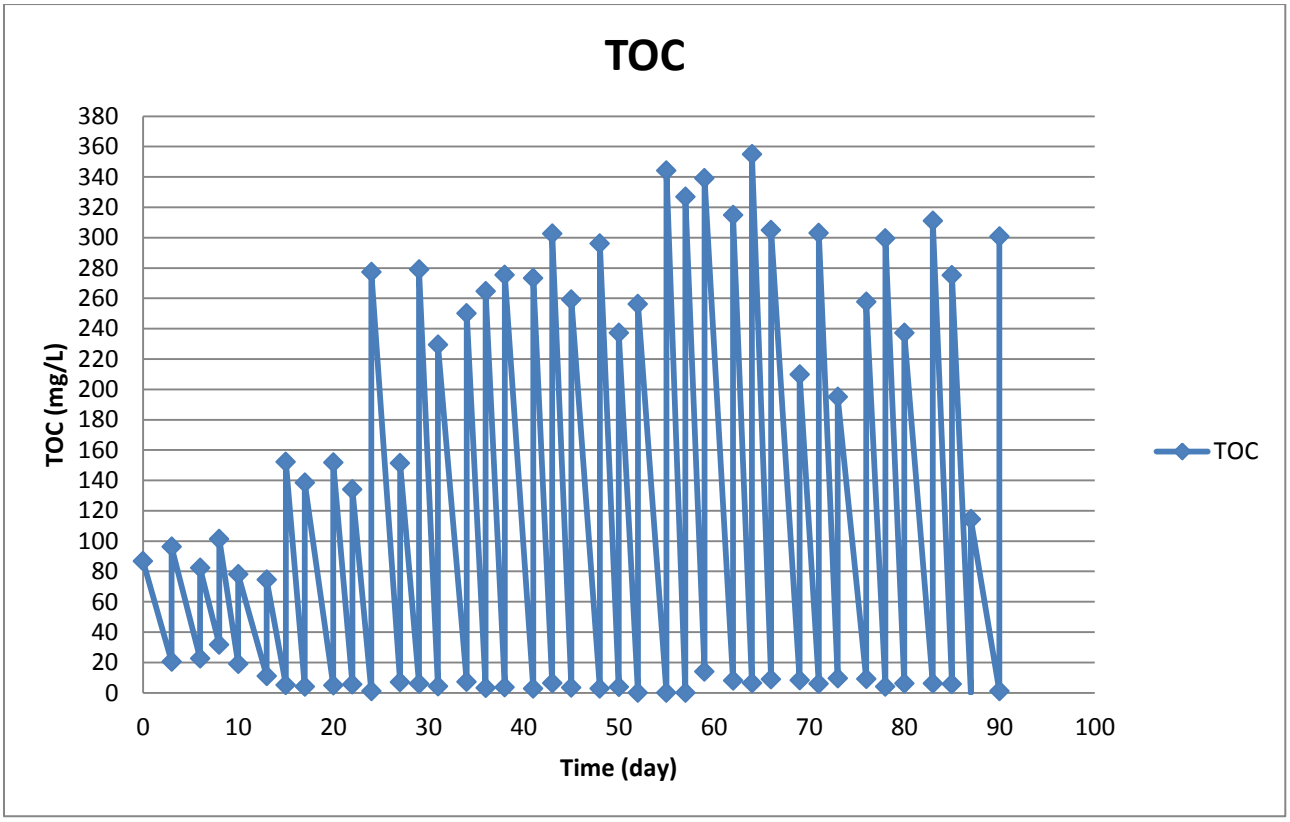


Figure 4.7. Variation of TOC in reactor one.

The initial concentration of glucose which was added to reactor one was 250mg/L. Then, during 15 days it was observed that glucose was consumed completely after each 2 days of feeding. However, there was not a significant change in sludge amount and characteristic. Therefore, it was decided to add 500mg/L glucose. The same results were reported one week after the concentration of the glucose was increased. At last, the glucose concentration was increased up to 1000mg/L. The glucose was consumed completely by bacteria but this time there were some changes in amount and characteristic of bacteria was observed. The changes that could be seen without special equipment, are; changing in colour, increasing in amount of TSS and producing larger flocs aggregates. The sludge colour turned black from light brown after 40 days. Furthermore, the TSS of reactor one started to increase from 2.5g/L to 3.5g/L. The variation of TSS over three months shows in Figure 4.8.

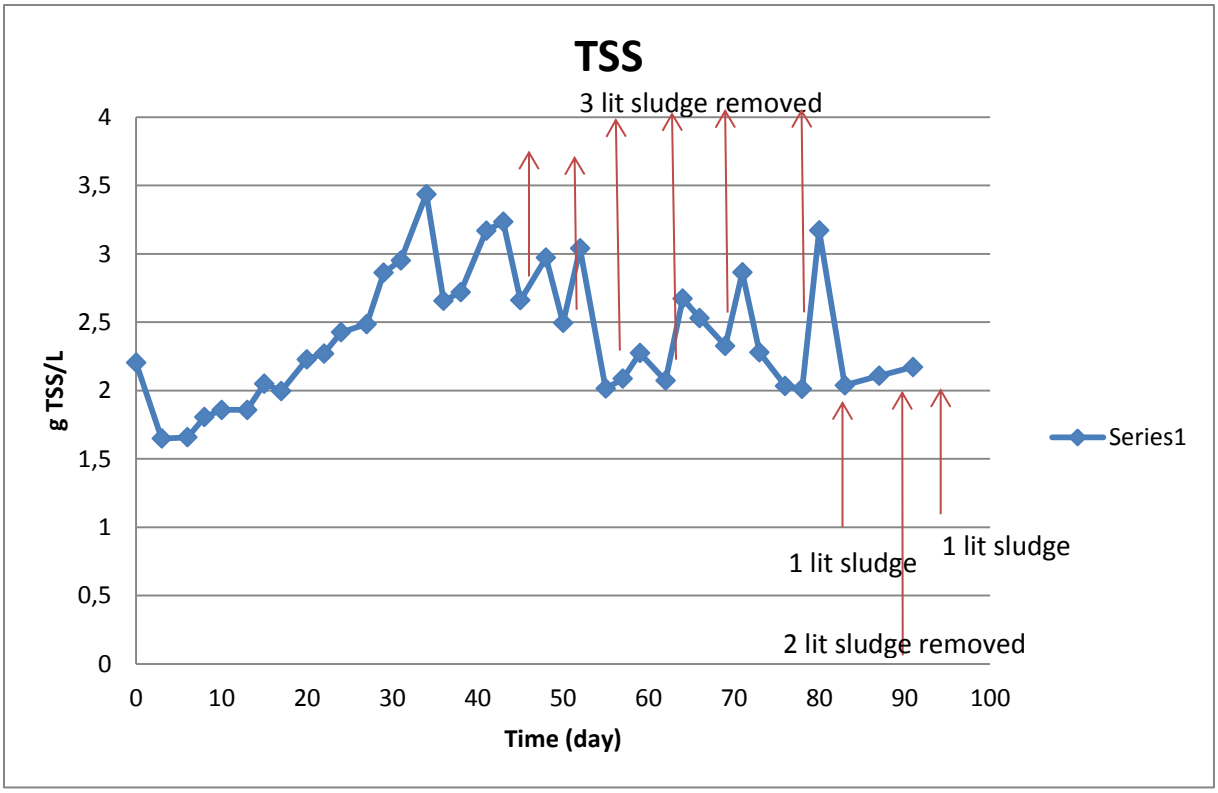


Figure 4.8. Variation of TSS in reactor one. Red arrows show occasions when sludge was removed from the reactor.

The growth rate of sludge increased after 42 days. Therefore, we tried to keep the TSS concentration around 2.2g/L (original concentration) by removing some amount of sludge mixture from the reactor. The VSS was measured the same time that TSS was measured. The VSS/TSS ratio shows the percentage of organic fraction of the sludge. For reactor one this ratio was 69% in the beginning of the study and reached to above 90% after 50 days. The dramatic increase of TSS shows that the bacteria which could survive by consuming glucose, dominated in the reactor and start to grow faster in comparison with the other communities. It was expected that the dominant group should be the heterotrophic bacteria. However, microbial community analysis using molecular techniques was not conducted. In consequence of TSS growth it was observed that the flocs became bigger in size. Furthermore, we decided to study the floc structure and appearance by using a microscope. The results from this study will be presented in chapter 4.6.

Solid retention time (SRT) was calculated based on the removal of sludge in reactor one and it is equal to 22.5 days. SRT is the average time the activated sludge solids are in the system. The ionic strength was calculated for sludge mixture in all reactors by considering the feed load composition. The ionic strength of sludge mixture in reactor one was equal to 0.136mol/L. For ionic strength of higher than 0.1 the sludge stability decreased (Zita and Hermansson 1994)

4.2.2 Reactor two

The process for feeding reactor two was the same as reactor one. The changes of TOC concentration in reactor two are shown in the Figure 4.9. The peaks and drops in this graph are representing the TOC concentration immediately and 2 days after feeding. The graph shows that the amount of glucose which was added to the reactor was consumed completely after 2 days by heterotrophic bacteria. Glucose concentration was increased in the feeding solution the same as in reactor one.

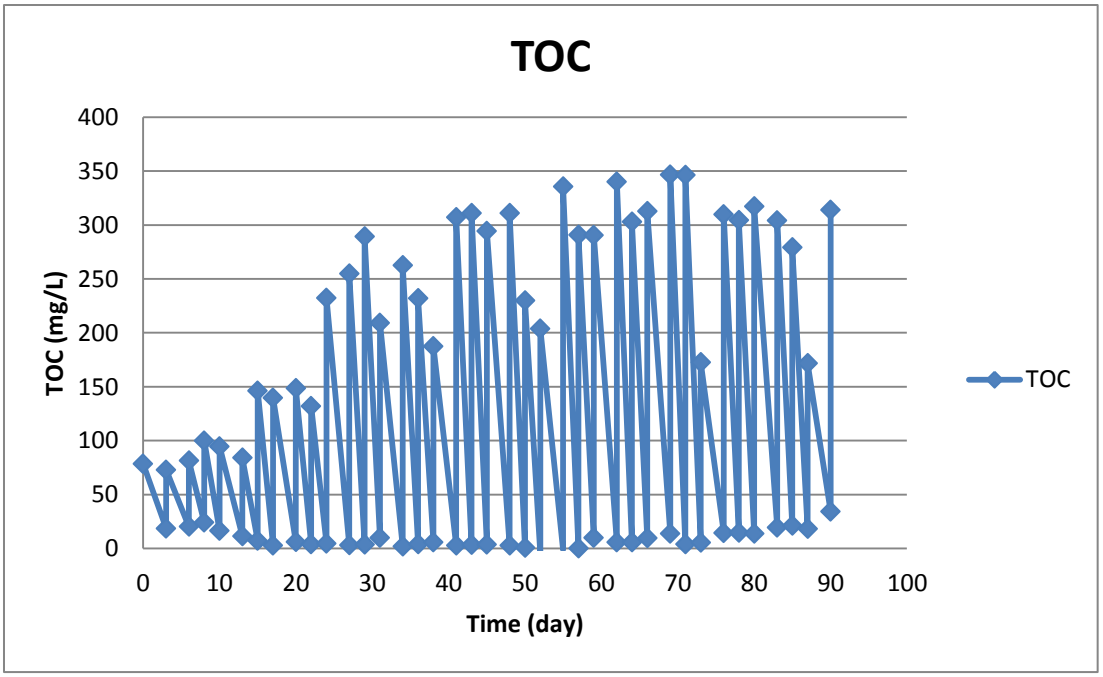


Figure 4.9. Variation of TOC in reactor two.

Same changes as reactor one were reported for reactor two. However, it took 67 days for the sludge colour in reactor two to change from light brown to dark brown. The TSS amount of reactor two was measured every two days. The Figure 4.10 shows the TSS changes against time.

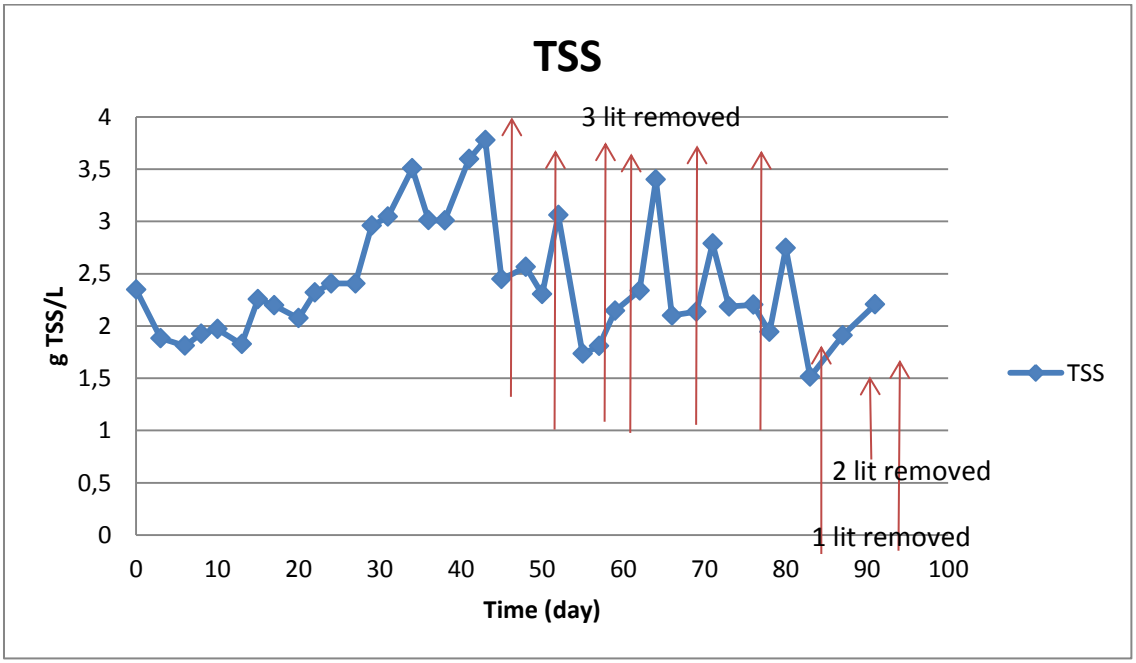


Figure 4.10. Variation of TSS in reactor two.

The sludge was removed from reactor two due to the increase in TSS concentration after a while. The trend of changing TSS in reactor two was almost similar to the trend in reactor one. The reason could be that because both reactors were fed with glucose. Furthermore, the heterotroph bacteria were the dominant microbial community inside reactor two. The VSS/TSS ratio for sludge mixture in reactor two was 70% in the beginning and reach to above 90% after 50 days. The SRT for reactor two

based on sludge removal rate was 22.8 days. The ionic strength of sludge mixture in reactor two was equal to 0.06mol/L.

4.2.3 Reactor three

Reactor three was fed with the solution which was presented in Table 3.1. The main difference between this reactor and reactor one and two was that this reactor was fed with ammonium instead of organic carbon (glucose). Furthermore, the nitrogen content was measured in reactor three with TOC analyser for total nitrogen and ion chromatograph for ammonium, nitrate, and nitrite ions. The ammonium inside the reactor three was converted to nitrate and nitrite after two days. It is also possible that some of the nitrate or nitrite was denitrified to nitrogen gas leaving the reactor; although no organic electron donor was added to support denitrification. Figure 4.11 shows the variations of TN reactor three. However, it should be noted that TN measurement alone could not illustrate the reactions carried out by the bacteria in reactor three.

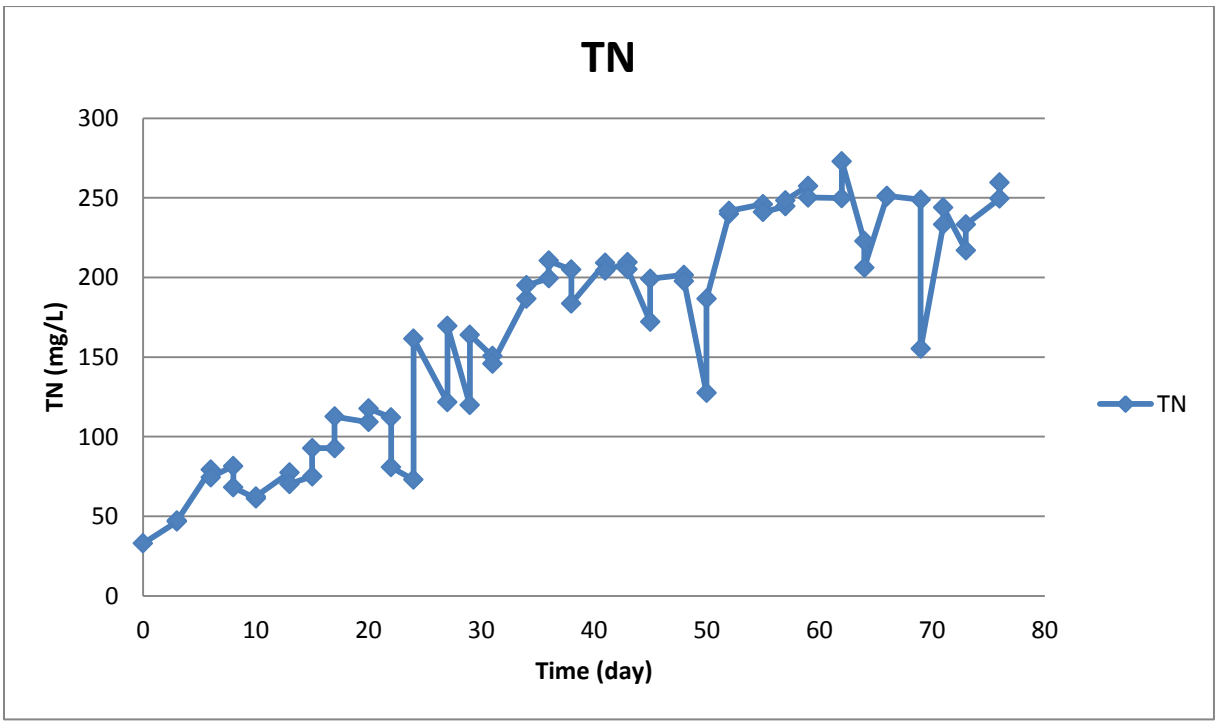


Figure 4.11. Variation of TN in reactor three.

In day 24 the concentration of ammonium chloride which was added to feed solution was increased to 215mg/L and after one week it was increased to 428mg/L. The samples were taken every 2 days one before feeding the reactor and one immediately after feeding the reactor. In order to study the rate of ammonium consumption by the bacteria inside reactor three, the samples were analysed by ion chromatograph. The Figure 4.12 and 4.13 show the ion chromatograph results.

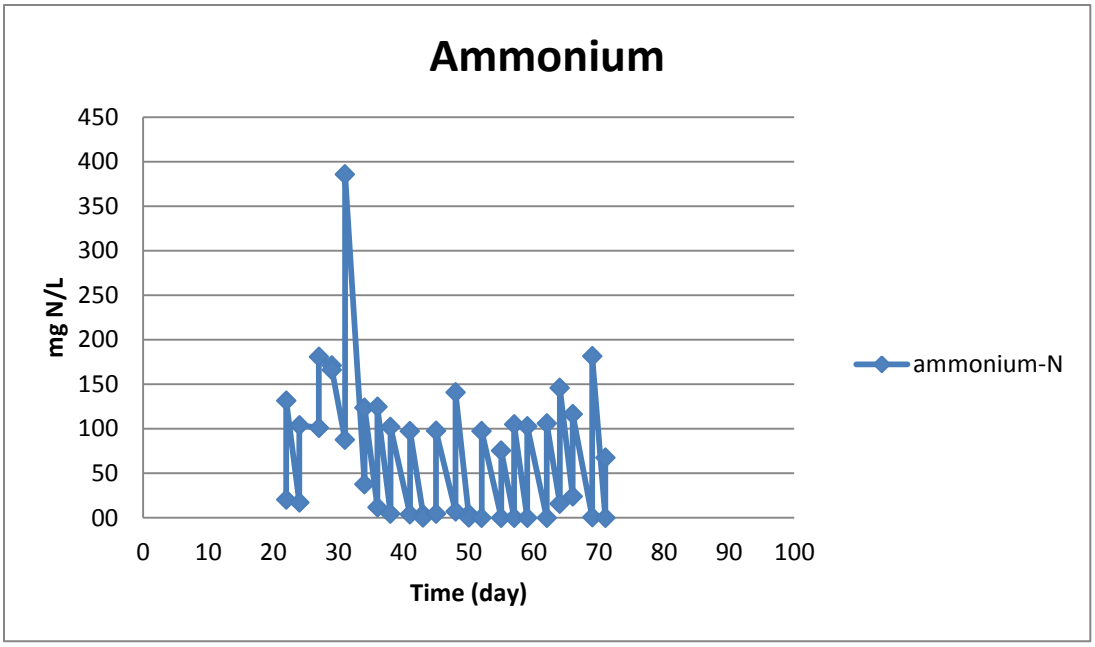


Figure 4.12. The changes of ammonium concentration in reactor three over a period of three months

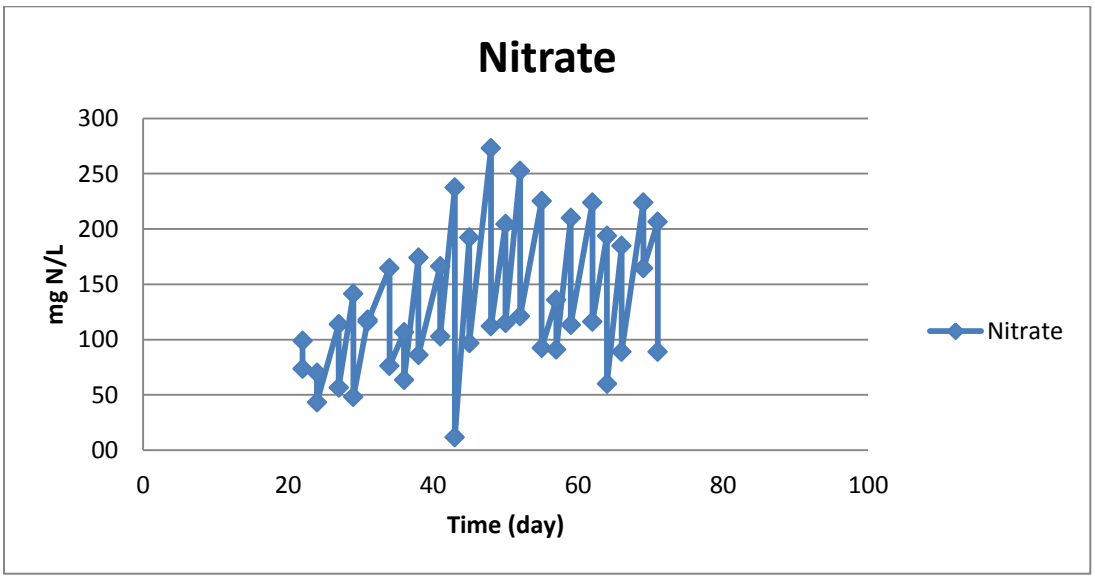


Figure 4.13. The changes of nitrate concentration in reactor three over a period of three months.

The peaks in the ammonium consumption graph shows the NH_4^+-N concentration in the samples which was taken after the reactor was fed, and the drops represent the nitrogen concentration after 2 days mixing. The results shows that after 30 days nitrifiers inside reactor three could be capable of converting all the ammonium to nitrate. However, from day 20 to 30 the ammonium was not consumed completely. One reason could be that the dominant community of the bacteria inside reactor three was not nitrifiers. In other words, the amounts of nitrifiers were not enough to convert all the ammonium to nitrate in two days. It should be noted that the high ammonium concentration measured on day 31 might be because of error that has happened during sampling. Figure 4.13 shows the NO_3^--N concentration during the operation of reactor three. The drops show the concentration after feeding and the peaks shows the nitrogen concentration after 2 days. The difference between one drop and one afterward peak shows the amount of product that was

converted during 2 days. By comparing the two diagrams in Figure 4.12 and 4.13, it can be observed that the ammonium was completely converted to nitrate during 2 days of mixing. For example in day 60, the ammonium concentration after feeding was 113mg N/L and after 2 days it reached to near zero. Then, the nitrate concentration in day 60 was 113 mg N/L and after 2 days it reached to 224mg N/L that means the nitrate concentration was increased 111mg N/L and this amount is equal to ammonium-nitrogen amount which was added to the system the same day. Moreover, the concentration of nitrite in reactor three was close to zero during the operation. The TSS changes during the operation of reactor three is shown in the Figure 4.14. The TSS in reactor three decreased from 2.2g/L in the beginning to 0.5g/L at the end of the project. One reason could be the low growth rate of nitrifies in comparison with the rate of decay of non-nitrifies in the original sludge inoculum. 60 days after operating this reactor VSS/TSS ratio for this reactor was around 75%. The physical shape of the sludge was changed during the project from large flocs to fine and small flocs. Also, the colour was changed from brown to light yellow during the operation. The ionic strength of sludge mixture in reactor three was equal to 0.19. Therefore, high degradation of the flocs was expected in this reactor.

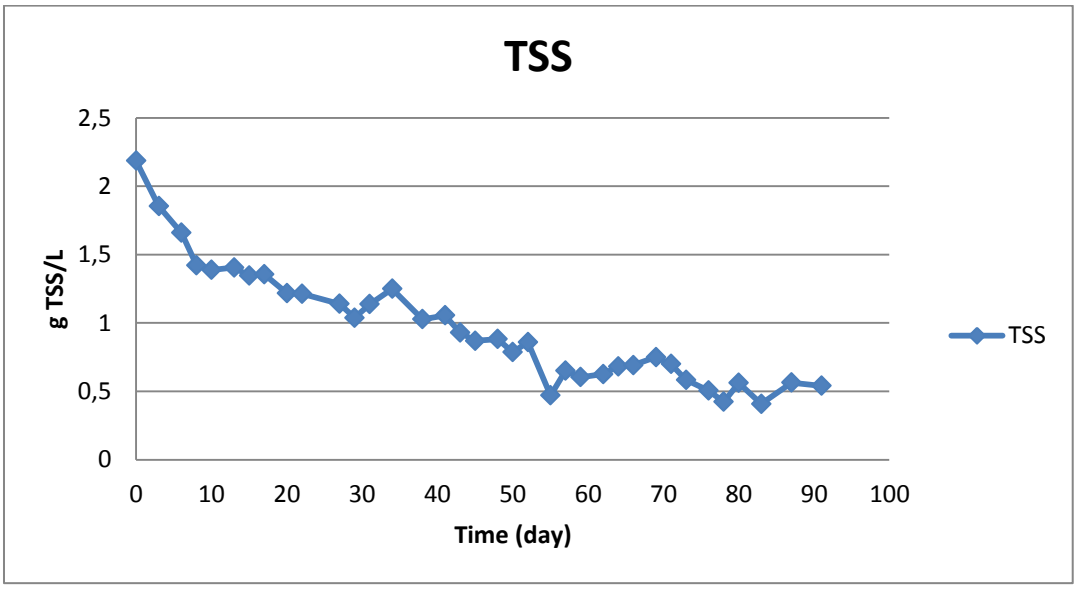


Figure 4.14. Variation of TSS in reactor three.

4.2.4 EPS results of SBR

EPS was another issue which was monitored during this study for all reactors.. The TN and TOC of the EPS samples were measured without filtration and are illustrated below for the three reactors. Carbohydrates and protein contain organic carbon. Therefore, the TOC value indicates the total EPS content. Proteins contain a lot of nitrogen so TN can be used as an indicator for protein content of EPS.

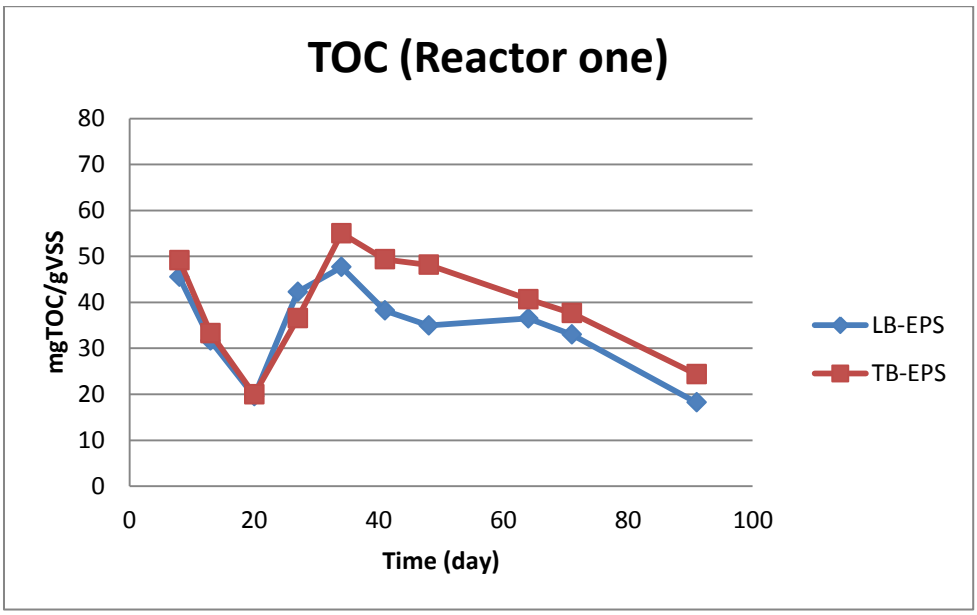


Figure 4.15. Variation of TOC in LB-EPS and TB-EPS samples of reactor one.

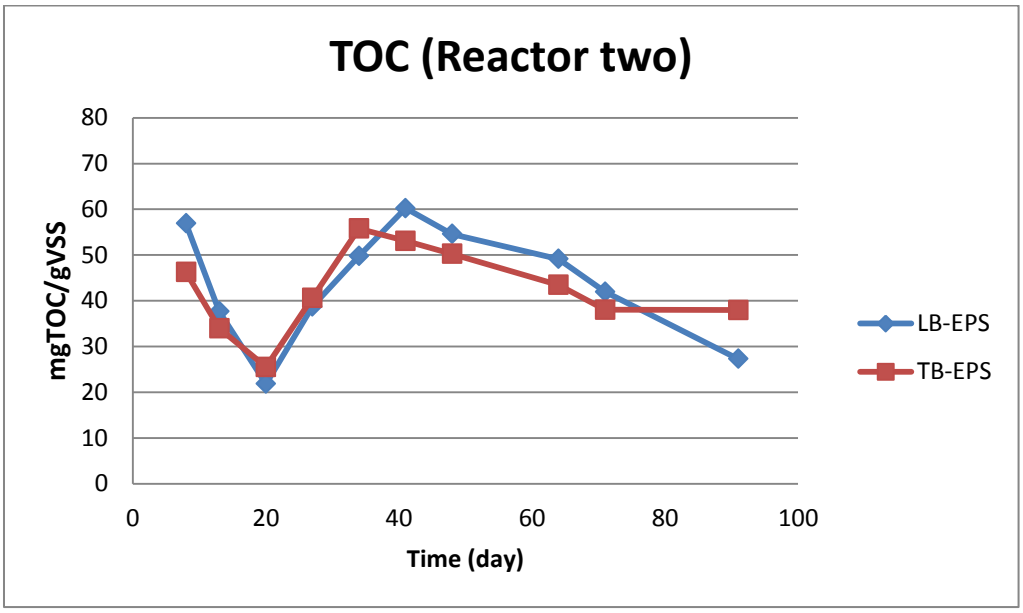


Figure 4.16. Variation of TOC in LB-EPS and TB-EPS samples of reactor two

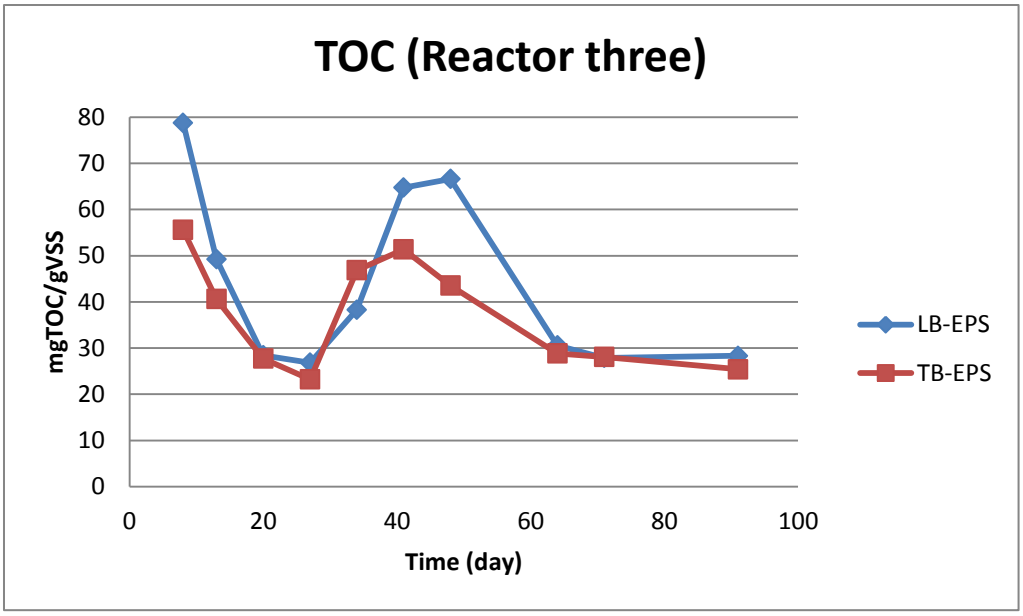


Figure 4.17. Variation of TOC in LB-EPS and TB-EPS samples of reactor three.

As shown in Figure 4.15 above, the TOC of LB-EPS and TB-EPS complex for reactor one was decreased from 50mg/gVSS to 20mg/gVSS from day 8 to day 20. The same trend has happened in reactor two and three between these days. Then after day 20 when the feed concentration was increased the TOC for LB-EPS and TB-EPS was increased temporarily. In reactor one it went up to about 50mg/gVSS and for reactor two this amount was around 60mg/gVSS. However, in reactor three there is a significant difference between LB-EPS and TB-EPS. The TOC concentration of TB-EPS reached to 50mg/gVSS and LB-EPS went to 65mg/gVSS in reactor three. Then the TOC concentration of LB-EPS and TB-EPS decreased dramatically in all reactors. These graphs show that increasing the feed load can have a significant effect on TOC concentration of EPS content but this effect is not permanent and will be back to the lower stable level as long as the bacteria acclimatize to the new condition. Figures 4.18, 4.19 and 4.20 illustrate the changes of TN in LB-EPS and TB-EPS samples of these three reactors.

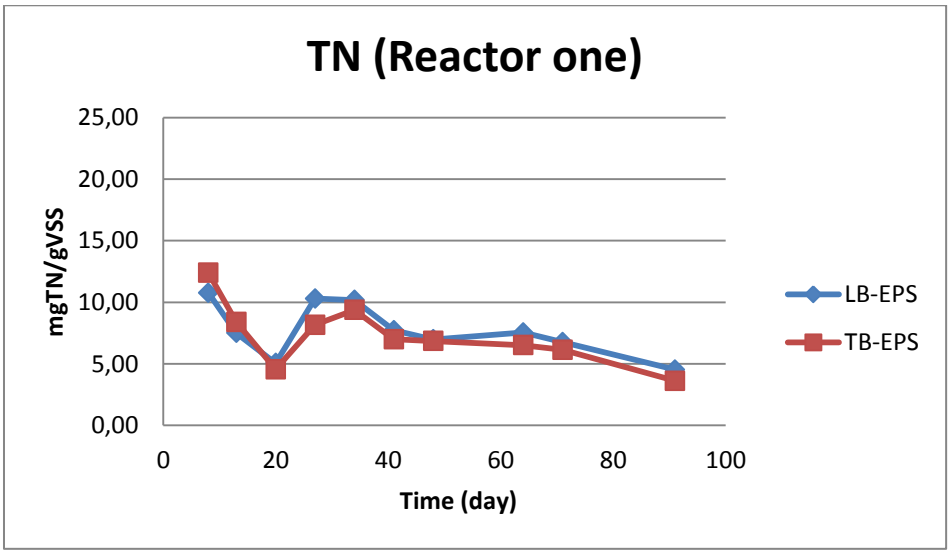


Figure 4.18. Variation of TN in LB-EPS and TB-EPS samples of reactor one.

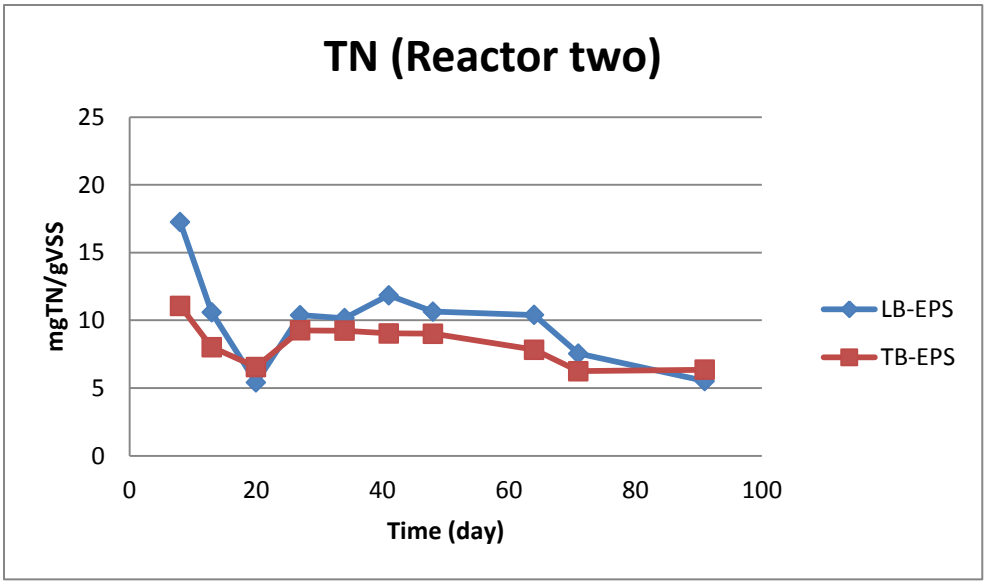


Figure 4.19. Variation of TN in LB-EPS and TB-EPS samples of reactor two.

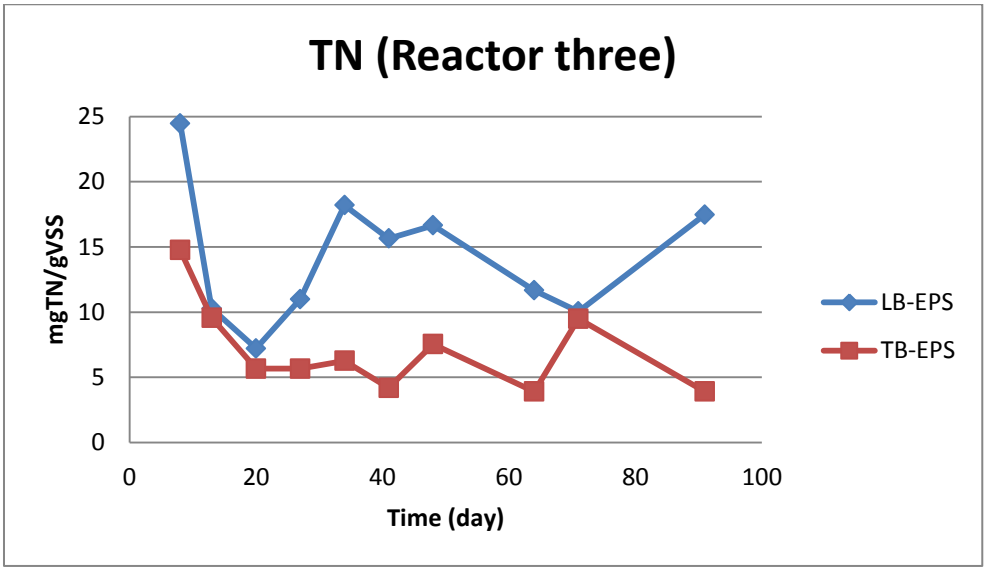


Figure 4.20. Variation of TN in LB-EPS and TB-EPS samples of reactor three

The changes in nitrogen concentration of EPS samples in reactor one had the same trend as TOC with a drop after 20 days and then a dramatic increase up to 10mg/gVSS when the feed load was increased. Then, the TN concentration decreased gradually to 4mg/gVSS at the end of the project. In reactor two the TN concentration of 17mg/gVSS for LB-EPS and 8mg/gVSS for TB-EPS dropped to 5mg/gVSS, afterward it followed a moderate level around 6.5mg/gVSS. The TN was decreased dramatically to 5 mg/gVSS after 20 days in reactor three. The concentration of nitrogen in LB-EPS samples went up to 18mg/gVSS after increasing the feed concentration and afterward it decreased to 9mg/gVSS with a slightly increase at the end of the project. However, the nitrogen concentration in TB-EPS flocculated moderately around 7 mg/gVSS. The results for reactor three show that the LB-EPS had more protein content than TB-EPS. LB-EPS and TB-EPS in reactor one and two had almost equal amounts of protein content. The results show that increased the feed concentration has a negligible effect on the nitrogen concentration of EPS samples in reactor two. However, the effect of feed load was noticeable in reactor one and three, and this effect was temporary.

4.2.5 EPS analysis with HPLC

The EPS samples from last date of sampling (17th December 2012) were analysed with HPLC. The results are shown in Figure 4.21, 4.22 and 4.23. Each peak in the HPLC outcome represents specific compound that was detected by detector.

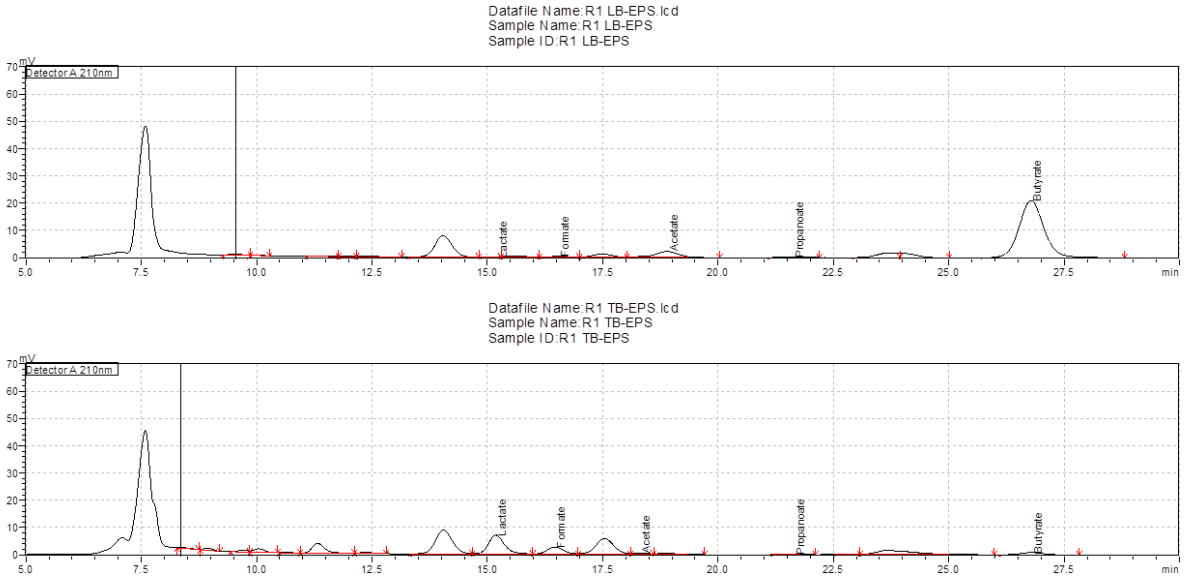


Figure 4.21. UV detector results of TB-EPS and LB-EPS samples: reactor one.

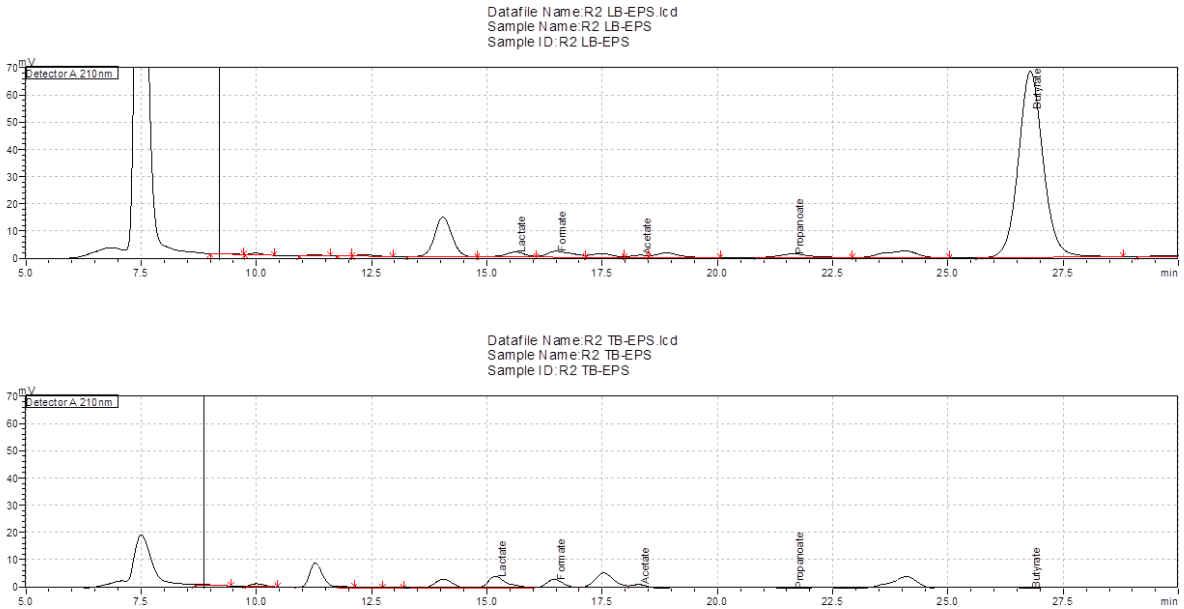


Figure 4.22. UV detector results of TB-EPS and LB-EPS samples: reactor two.

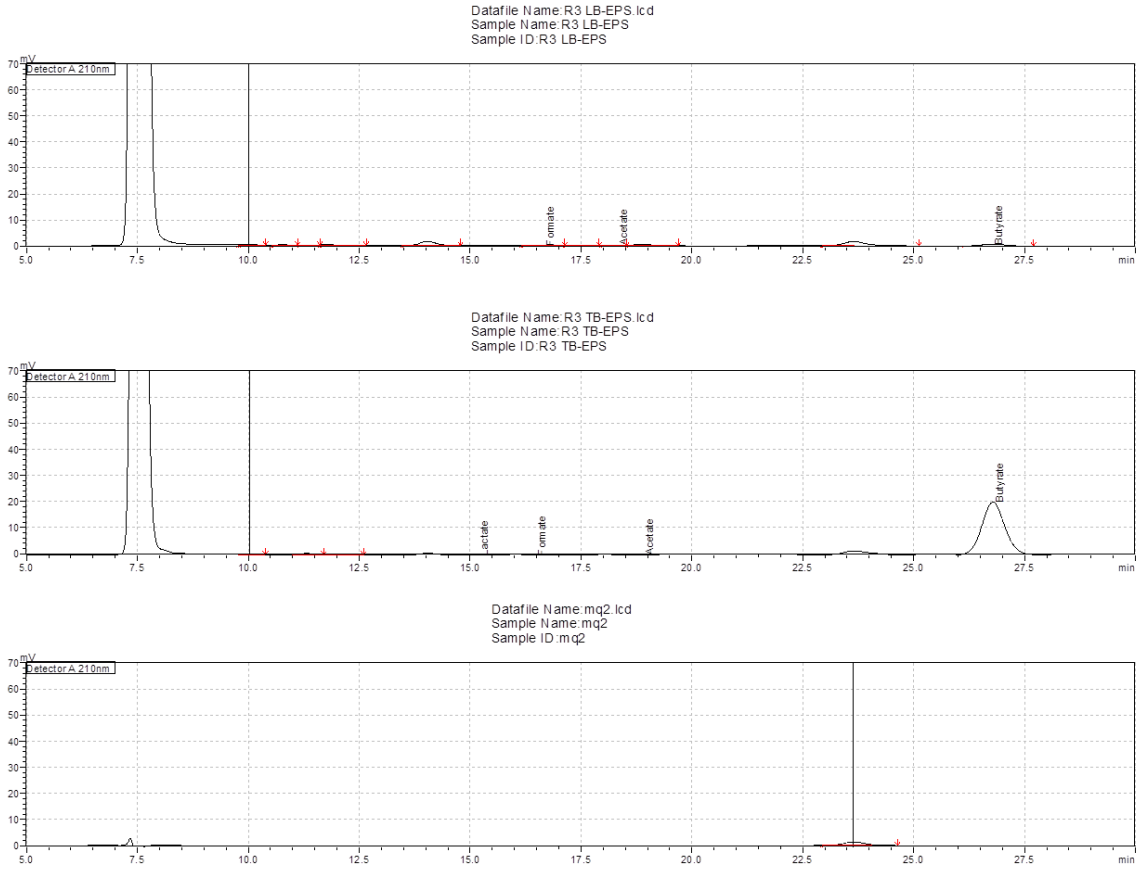


Figure 4.23. UV detector results of TB-EPS and LB-EPS samples: reactor three and milli-Q.

Figures above are shown the HPLC results for filtered EPS samples of reactor one, two and three. These results were analysed by the UV detector. Butyrate, or a compound with the same retention time,was the major component which was detected in LB-EPS samples of reactor one and two. The amount of butyrate in LB-EPS samples of reactor three was very low. However, this chemical was found as a major compound in TB-EPS of reactor three. Very low concentrations of carbohydrates such as acetate and glucose were detected in all samples. This observation could be explained that the LB-EPS in reactor one and two could adsorb compounds better than TB-EPS. Nevertheless, the component can attached easier to the TB-EPS in reactor three in comparison by LB-EPS.

The compounds which were detected by refractive index detector are shown in Figure 4.24, 4.25 and 4.26.

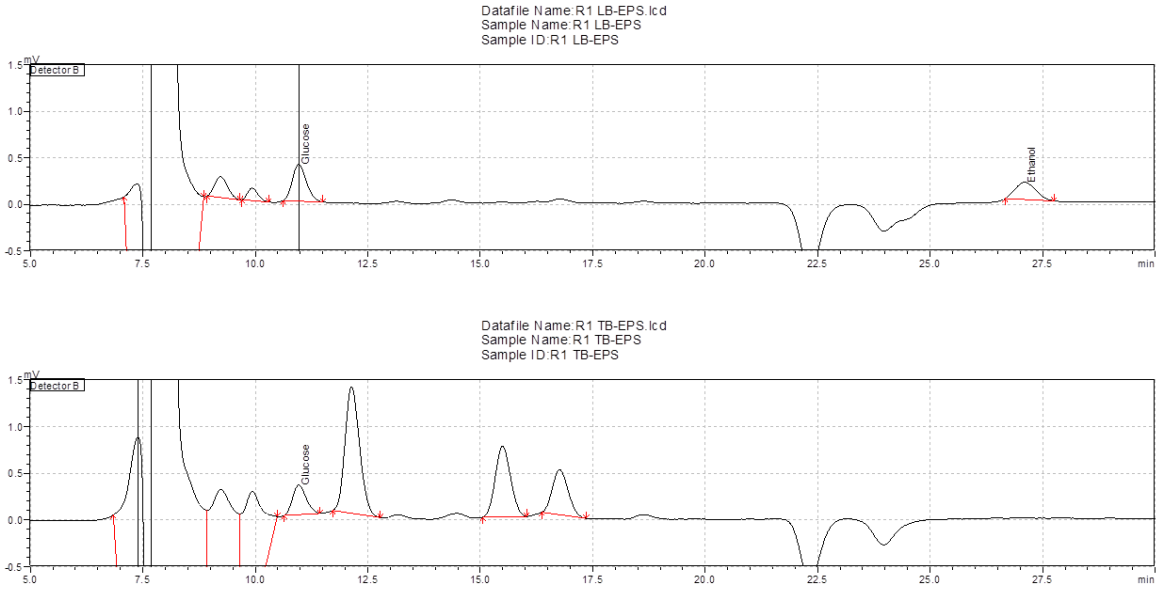


Figure 4.24. Refractive index detector results of TB-EPS and LB-EPS samples: reactor one.

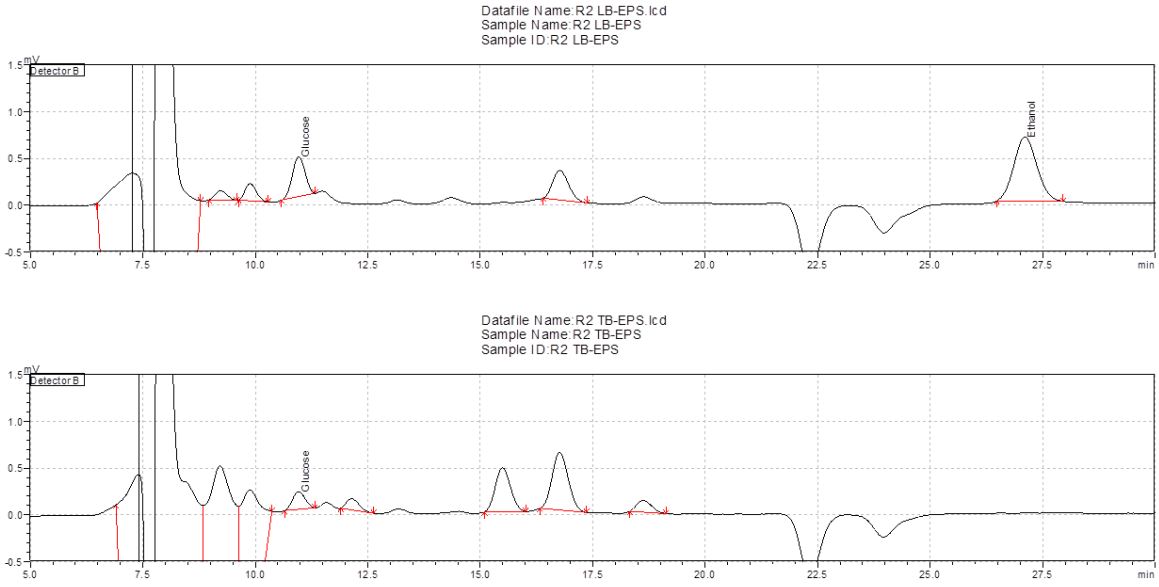


Figure 4.25. Refractive index detector results of TB-EPS and LB-EPS samples: reactor two.

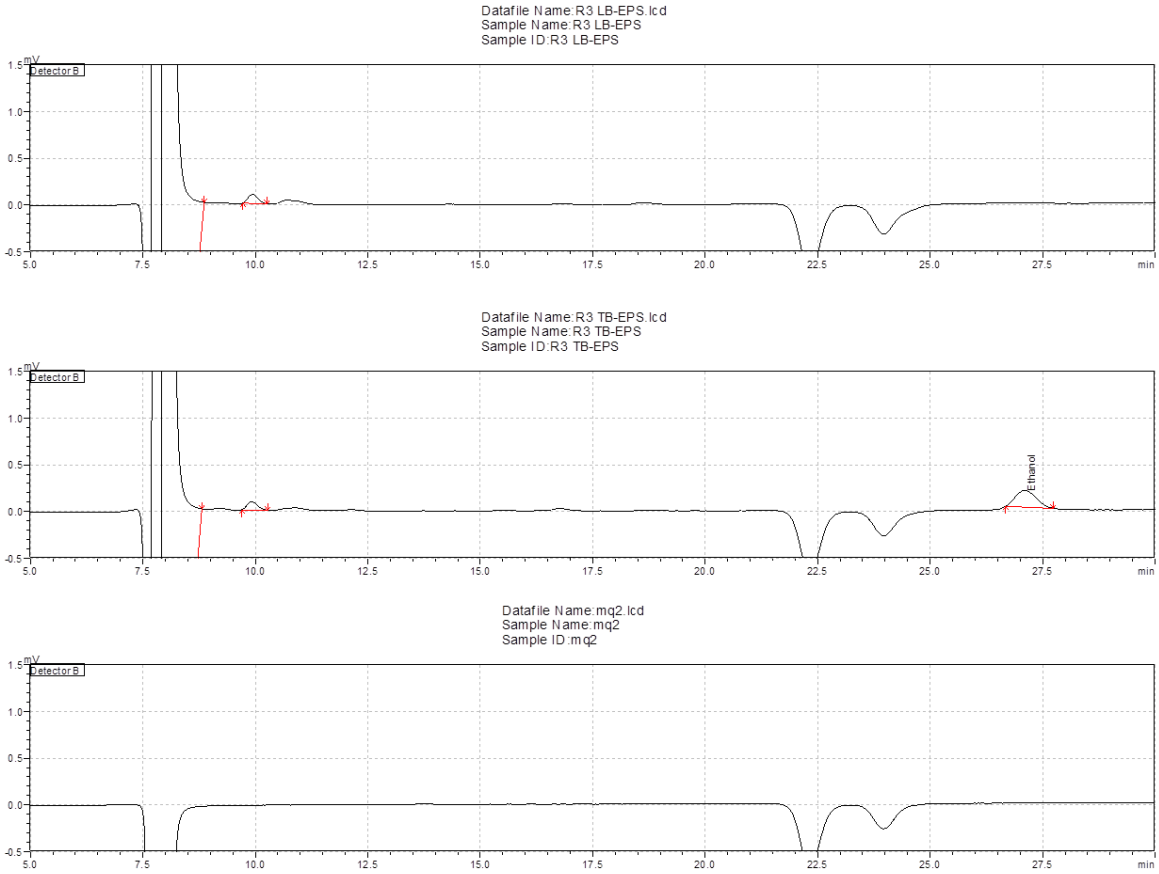


Figure 4.26. Refractive index detector results of TB-EPS and LB-EPS samples: reactor three and milli-Q.

The results show that some kinds of sugar components were detected in LB-EPS and TB-EPS of reactor one and two. However, these compounds were not found in reactor three samples. Ethanol, or a compound with the same retention time, was another compound which could be detected in LB-EPS samples from reactor one and two. Nevertheless, the minor amount of ethanol was found in TB-EPS of reactor three samples.

The HPLC diagrams show that there were major differences between the sludge which was fed with glucose (reactor one and two) and the sludge which was fed with ammonium (reactor three). These dissimilarities were presented by differences in physical characteristics and EPS contents.

4.3 Sludge ability of oxidation

This test was done 65 days after the reactors were operated. It was expected that the sludge characteristics had changed after this time period. The test was performed for reactor one and three which were supposed to have most differences in characteristics due to the different feed compounds. Changes in TOC were measured in order to determine the reduction of glucose in both reactors over a night. The nitrogen concentration was analysed as well, by measuring NH_4^+ and NO_3^- concentration in both reactors. The Figure 4.27 illustrates the TOC removal rate in both reactor one and reactor three over a period of 24 hours.

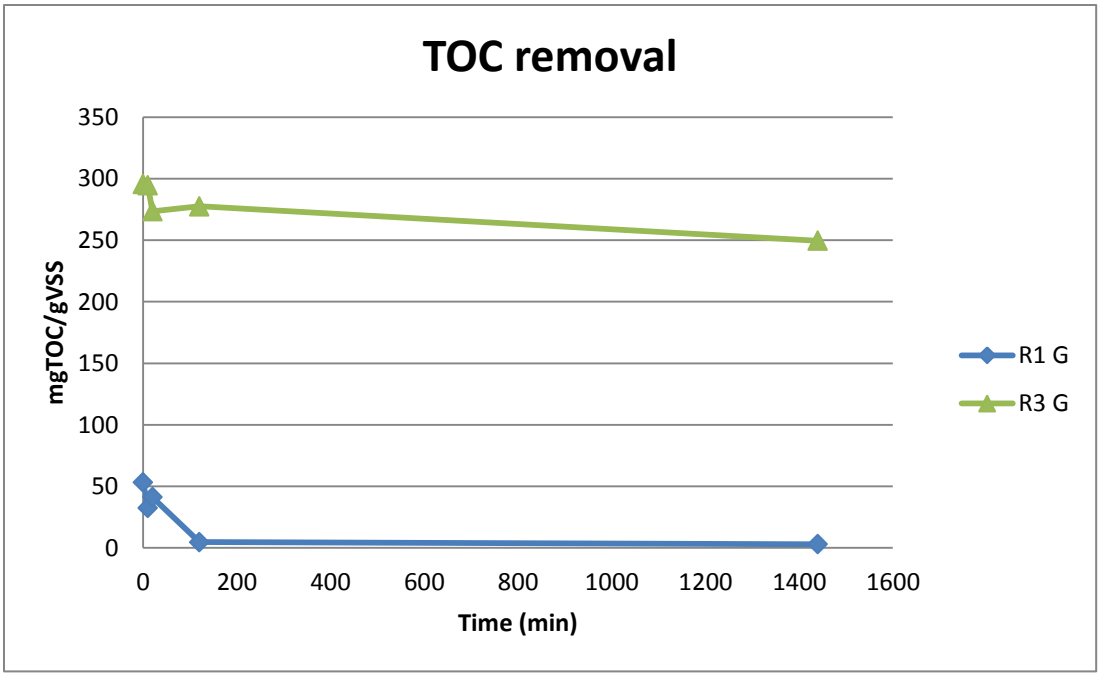


Figure 4.27. TOC removal rate in reactor one and three.

As shown in Figure 4.27 there was a dramatic drop in TOC concentration after two hours of contact in reactor one from 53mgTOC/gVSS to 4mgTOC/gVSS. However, during this time the TOC concentration changed from 295mgTOC/gVSS to 277mg TOC/gVSS in reactor three. In conclusion, the sludge which was fed with glucose (reactor one) was more capable to remove organic carbon than the other sludge which was fed with nitrogen source (reactor three). Both sludge mixtures reached a stable level after 2 hours of contact. In other words, the maximum removal of TOC was happened in first two hours. The VSS for reactor one was 2.2g/L and for reactor three was 0.3g/L. It was observed that the TOC was increased in reactor one after 10 minutes operation. This could be caused by error in method of sampling or error in procedure of TOC analysing.

The Figures 4.28 and 4.29 demonstrate the changes in NH_4^+-N and NO_3^--N concentration in this experiment. The ammonium concentration was changed from 80mgN/gVSS to 37mgN/gVSS after 24 hours in reactor one. However, that amount of ammonium which was consumed by bacteria was not converted to nitrate. The results for reactor one shows the nitrate production is negligible. Therefore, by considering the results for ammonium and glucose consumption by the bacteria in reactor one it can be concluded that the dominant bacteria community inside the reactor one was heterotrophs that could be able to consume carbon faster and better than ammonium for producing energy in order to grow.

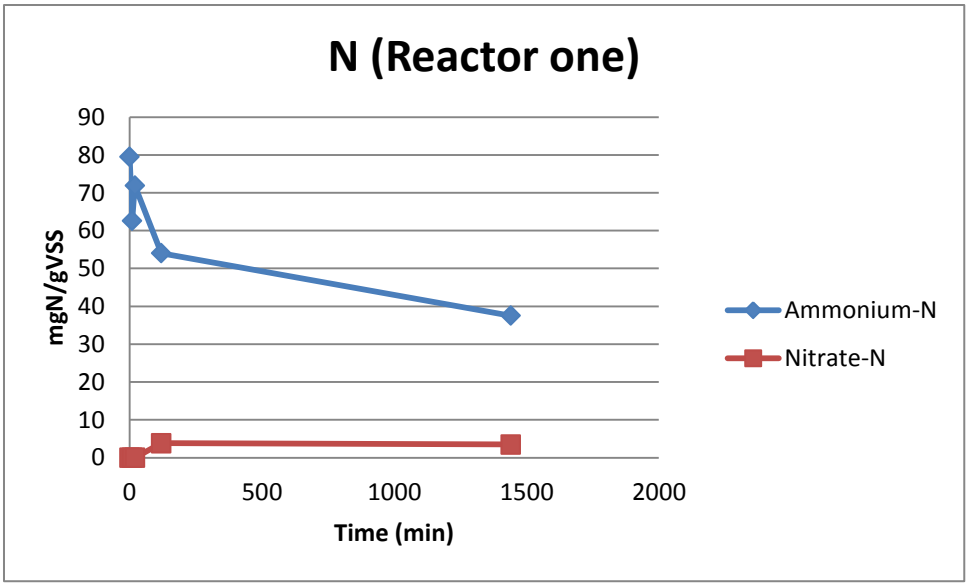


Figure 4.28. Changes in ammonium and nitrate concentration in reactor one.

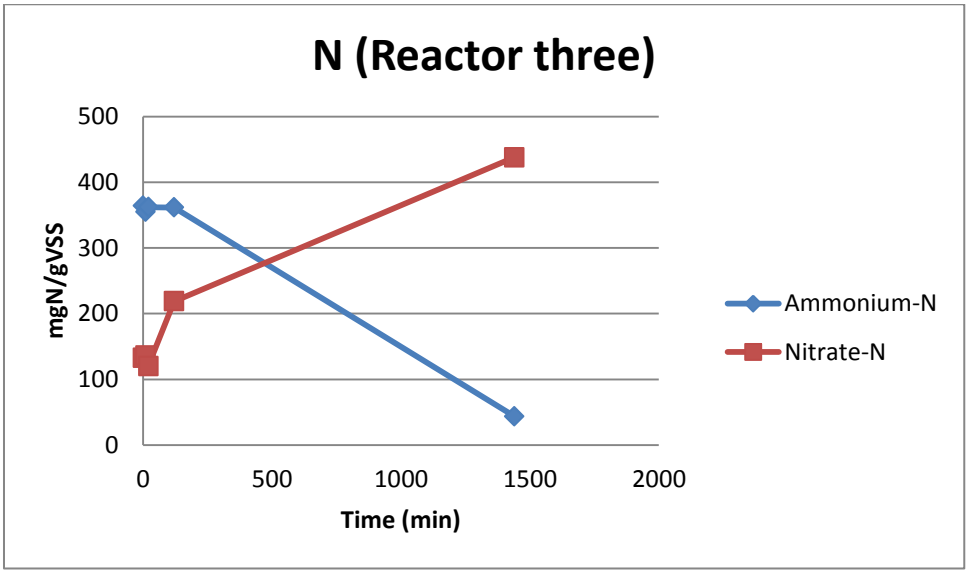


Figure 4.29. Changes in ammonium and nitrate concentration in reactor three.

The ammonium removal results for reactor three is shown in Figure 4.29.The concentration of NH_4^+-N was fell from 365mgN/gVSS in the beginning of mixing down to around 43mgN/gVSS over a period of 24 hours. In the other hand, the NO_3^--N concentration was increased from 132mgN/gVSS to 438mgN/gVSS. The results show that the ammonium was converted to nitrate entirely after 24 hours of mixing. Therefore, it might be concluded that the reactor three was enriched with nitrifying bacteria that were more capable to remove nitrogen than organic carbon.

4.4 Results of final adsorption tests

During the operation of the reactors, the adsorption tests were done frequently. The results for these experiments were divided in three parts according to the compound which was assessed for adsorbed to the sludge. The tests were done with and without sodium azide in order to investigate the adsorption capacity of active and inactive sludge.

4.4.1 Glucose adsorption

The results for adsorption of glucose onto activated sludge are shown in Figure 4.30. The first experiment was done on 16th of November 2012 with sodium azide and samples were taken in two steps for reactor one and three. The concentration of glucose in solution was 200mgC/L.

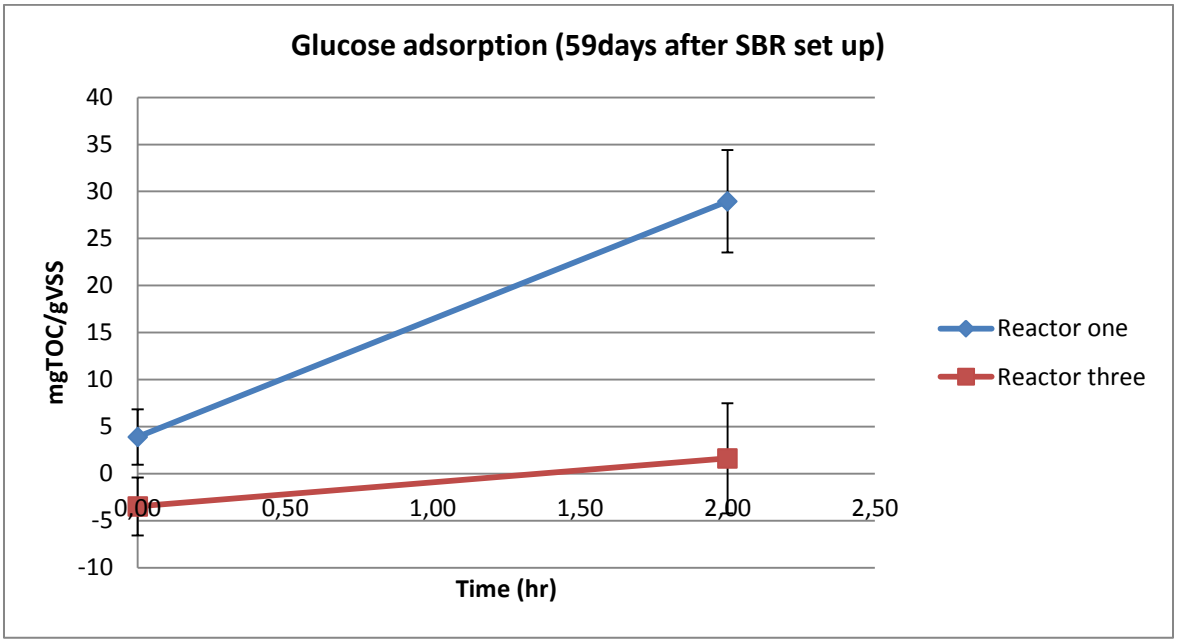


Figure 4.30. The adsorption of glucose in reactor one and three (with azide).

The first point at time zero in Figure 4.30 represents the amount of glucose that was adsorbed just after the solution had contact with the sludge. The amount of glucose that was adsorbed in reactor one increased from 3.8mgC/gVSS in the beginning to 28mgC/gVSS after 2 hours. This reduction of glucose was expected to be the physical adsorption due to the fact that the sodium azide could stop the bacteria from oxidizing the glucose. The process of adsorption was completely different in reactor three. It was observed that there were not any adsorption happened in reactor three for this experiment. The adsorption rate in reactor one was 3.8mgTOC/gVSS.min in first minute of contact and for last 2 hours of mixing it was equal to 0.2mgTOC/gVSS.min. The error bars for replicate samples were shown in figure 4.30. Samples which were taken after two hours had more variation in their outcomes.

Another experiment was done 2 weeks after the first experiment. The adsorption test was done for reactor one, two and three with sodium azide. The results of this experiment are displayed in Figure 4.31.

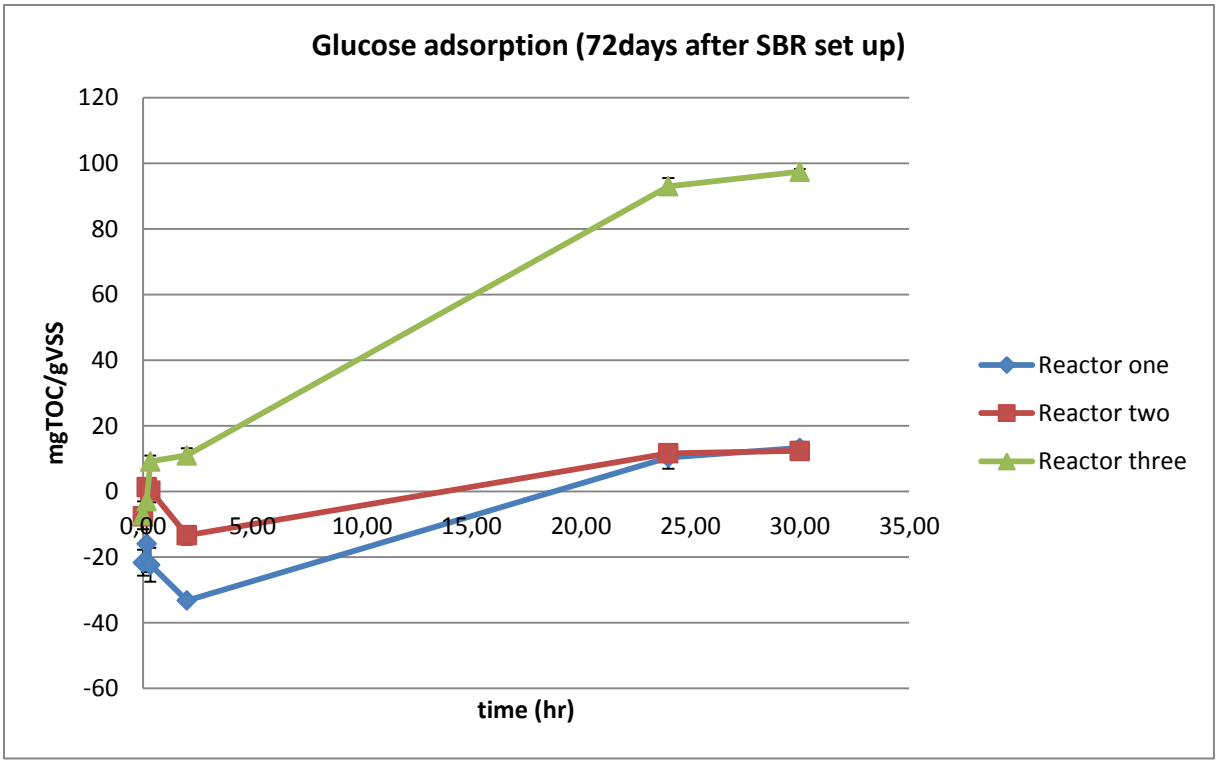


Figure 4.31. The adsorption of glucose in reactor one, two and three (with azide).

The results of this experiment were totally different from the previous test. The outcomes of reactor three shows that the glucose adsorbed onto activate sludge after 30 minutes of mixing. Then the adsorption rate was increased to 92mgTOC/gVSS after 24 hours of contact. On the other hand, the adsorption rate in reactor one and two shows that there was not any adsorption occurring during the first 20 hours of contact. However, 11mgTOC/gVSS was adsorbed in to sludge after 24 hours in reactor one and two. The negative points in Figure 4.31 illustrate that organic carbon was released in the mixture after mixing the solution with sludge. This phenomenon might be a deflocculation and disintegration of sludge flocs and need further investigations. The VSS for reactor one two and three were 0.6g/L, 0.6g/L and 0.2g/L.

In order to investigate the reason for carbon concentration increasing in the mixture after a while, the following experiment was done with lower concentration of glucose around 20mgC/L three weeks later. The lower concentrations of carbon did not need to be diluted; therefore, the results from TOC analysing would be more precise. The outcomes of this test are shown in Figure 4.32. The test was done for all reactors.

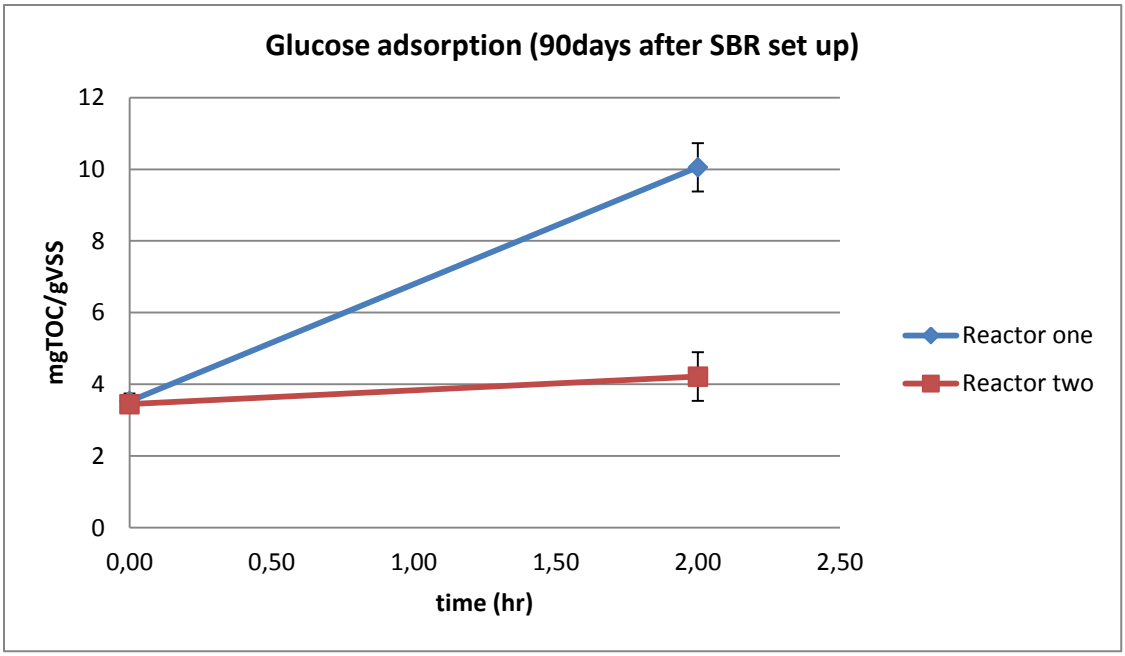


Figure 4.32. The adsorption of glucose in reactor one and two (with azide).

The Figure 4.32 shows that an adsorption occurred in reactor one and two during two hours of mixing. The adsorption rate changed from 3.4mgTOC/gVSS in first minute after mixing to 10mgTOC/gVSS after 2 hours. However, the rate of adsorption in reactor two was mostly constant around 4mgTOC/gVSS and did not have a significant change after two hours. The outcomes of reactor three were not realistic and there were only negative data for organic carbon concentration. The reason could be low concentration of carbon inside the supernatant of the samples. Error bars show that there was not a significant variation between the concentrations of replicate samples. The adsorption rate for first minute of contact in reactor one and two was equal to 3.4mgTOC/gVSS.min. For next two hours of mixing the adsorption rate per unit of time for reactor one and two was 0.055mgTOC/gVSS.min and 0.005mgTOC/gVSS.min.

These experiments show that the adsorption of glucose to different sludge was not following a specific trend. Moreover, there was not a significant correlation between the sludge EPS content and glucose adsorption capacity of activated sludge. However, it was observed that a noticeable amount of glucose was adsorbed to the different inactive sludge in these experiments.

4.4.2 Ammonium adsorption

The adsorption of ammonium into activated sludge was investigated in two experiments. The sodium azide was used in both tests. The first test was done on the 3rd of December 2012. Figure 4.33 and 4.34 shows the changes in ammonium-N concentration during 30 hours. The samples of these experiments were analysed with ion chromatograph.

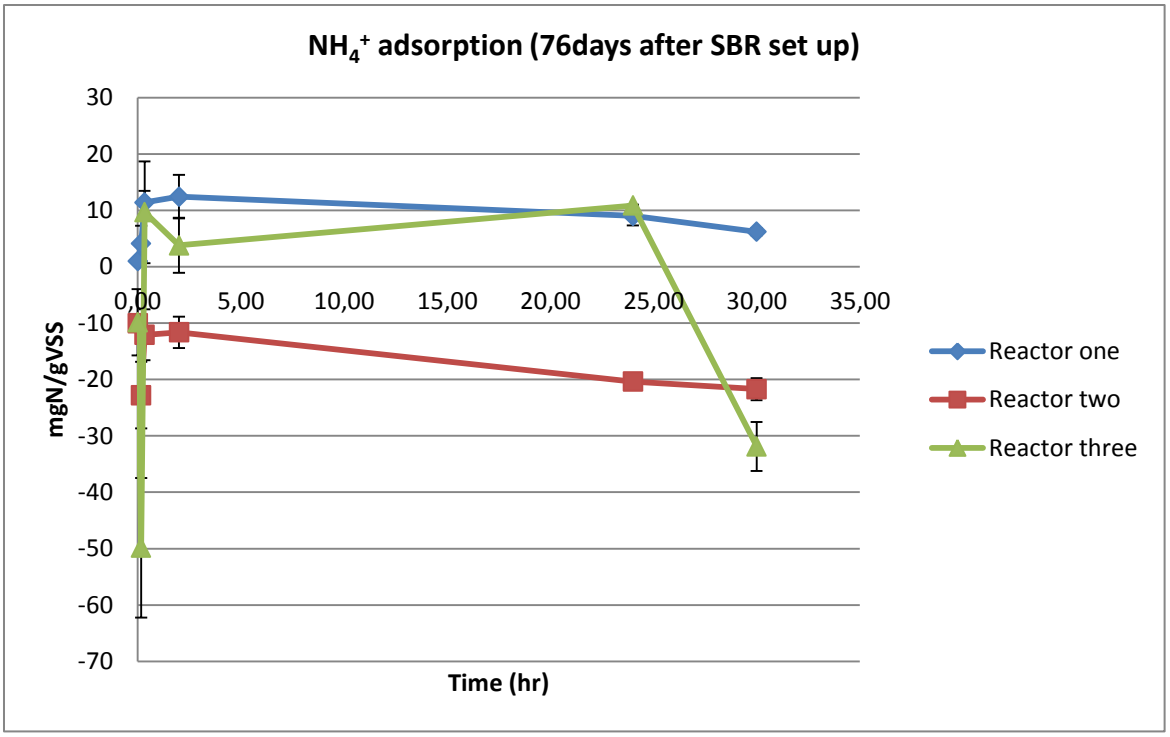


Figure 4.33. The adsorption of ammonium in reactor one, two and three (with azide).

The outcome for reactor one shows that the ammonium was adsorbed to the sludge in the first minute of mixing. The adsorbed concentration increased after 2 hours to 12.5mgN/gVSS. Afterward, the sludge began to release a small part of the adsorbed nitrogen into the mixture. The release of nitrogen was observed by ion chromatograph analysis. The disintegration of the sludge after 24 hours of mixing could lead to nitrogen releases. The results for reactor two show that the ammonium was not able to be adsorbed onto the sludge. The results display that the NH_4^+-N concentration in reactor two was increased first, then small part of it adsorbed again to sludge. However, it began to release the ammonium after a while. In reactor three the ammonium-N concentration increased after 10 minutes then it started to be adsorbed but after a while the sludge started to release some amount of ammonium again. These processes of releasing and adsorbing might have happened due to the analytical error or any fault during the sampling procedure or some unknown phenomenon. The rate of adsorption per unit of time in reactor one for first minute and 2hours of mixing were equal to 1mgN/gVSS.min and 0.095mgN/gVSS.min.

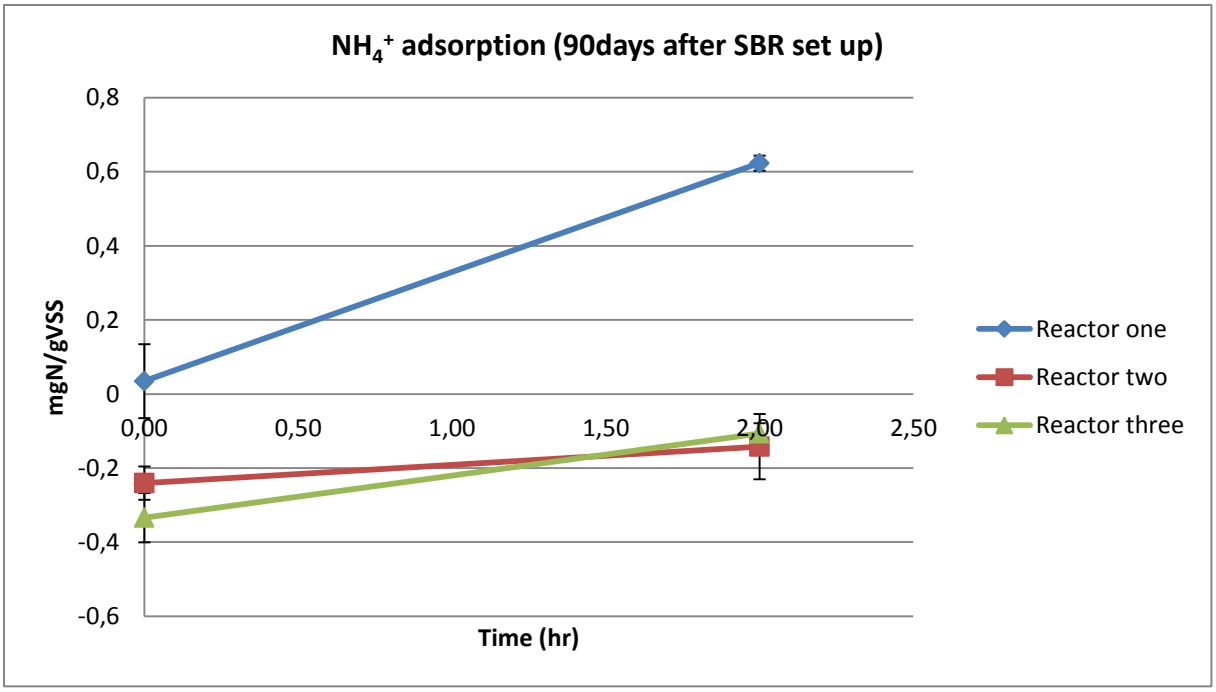


Figure 4.34. The adsorption of ammonium in reactor one, two and three (with azide).

Another ammonium adsorption experiment was done 2 weeks after the first test. The NH_4^+-N concentration in the solution was 20mgN/L and sodium azide was used as inhibitor. Two samples were taken during test one immediately and another one after 2 hours of mixing. The figure above presents the results of this experiment. 0.03mgN/gVSS was adsorbed immediately after mixing the solution with sludge from reactor one. Then, the adsorption rate increased up to 0.63mgN/gVSS after 2 hours. The outcomes for reactor two and three show that after mixing the solution with sludge the concentration of NH_4^+-N increased in the mixture immediately after mixing, and then it began to decrease in the next 2 hours. Table 4.4 shows the adsorption rate per unit of time for immediate adsorption and after 2 hours of mixing.

Table 4.4. Uptake rate in first minute of contact and 120 minutes of mixing in reactor one, two and three.

	Reactor one	**Reactor two**	**Reactor three**
First minute (mgN/gVSS.min)	0.03	---	---
Last 2 hours(mgN/gVSS.min)	0.005	0.0015	0.0007

The results of these two experiments show that the sludge inside reactor one, which was fed with glucose and had high conductivity, could be able to remove ammonium by physical adsorption. However the sludge inside reactor two which were fed with glucose and had lower conductivity could not remove ammonium. Furthermore, it was observed that the sludge which was fed with ammonium (reactor three) did not have capability of removing nitrogen via physical adsorption.

By considering the adsorption ability of sludge inside reactor one and the EPS content for the same sludge, it can be observed that there was not any significant relation between EPS content and adsorption. Moreover, it can be concluded that the maximum adsorption of nitrogen was happened

in reactor one after 2 hours of mixing. Nevertheless, the results are not precise enough to allow us to make a clear conclusion about the sludge from reactor two and three.

4.4.3 Milk powder adsorption

The adsorption of milk powder onto activated sludge was studied by performing a couple of experiments. The experiments were conducted with and without sodium azide. Some results were analysed by TOC analyser and the rest were analysed by a spectrophotometer.

The first experiment was done 7th of November 2012 (50 days after operating) with and without sodium azide. The concentration of milk powder-C which was added to the sludge was 200mgC/L. The experiment was performed with two replicates in 5 steps. 15ml tubes were used instead of 50mL tubes. The VSS for reactor one and three was 0.9g/L and 0.4g/L, respectively. The results of this test are shown in Figure 4.35.

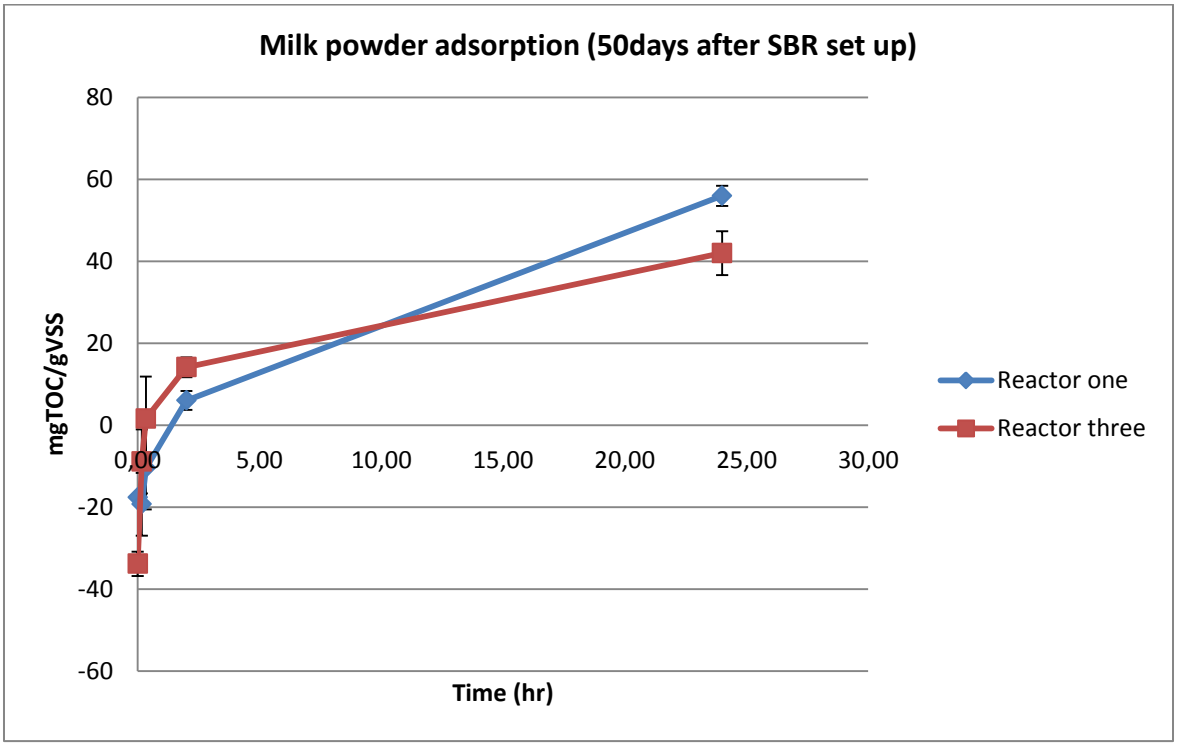


Figure 4.35. The adsorption of milk powder in reactor one and three (without azide).

As shown in Figure 4.35 carbon was removed by activated sludge in reactor one and three, after two hours. The removal rate of milk powder-C in reactor one is higher than the removal rate in reactor three after 2 to 24 hours of mixing (see Table 4.5).

Table 4.5. Uptake rate between 2 hours and 24 hours of mixing in reactor one and three.

	Reactor one	**Reactor three**
From first minute to 2 hours (mgN/gVSS.min)	0.19	0.4
From 2hours to24 hours(mgTOC/gVSS.min)	0.037	0.021

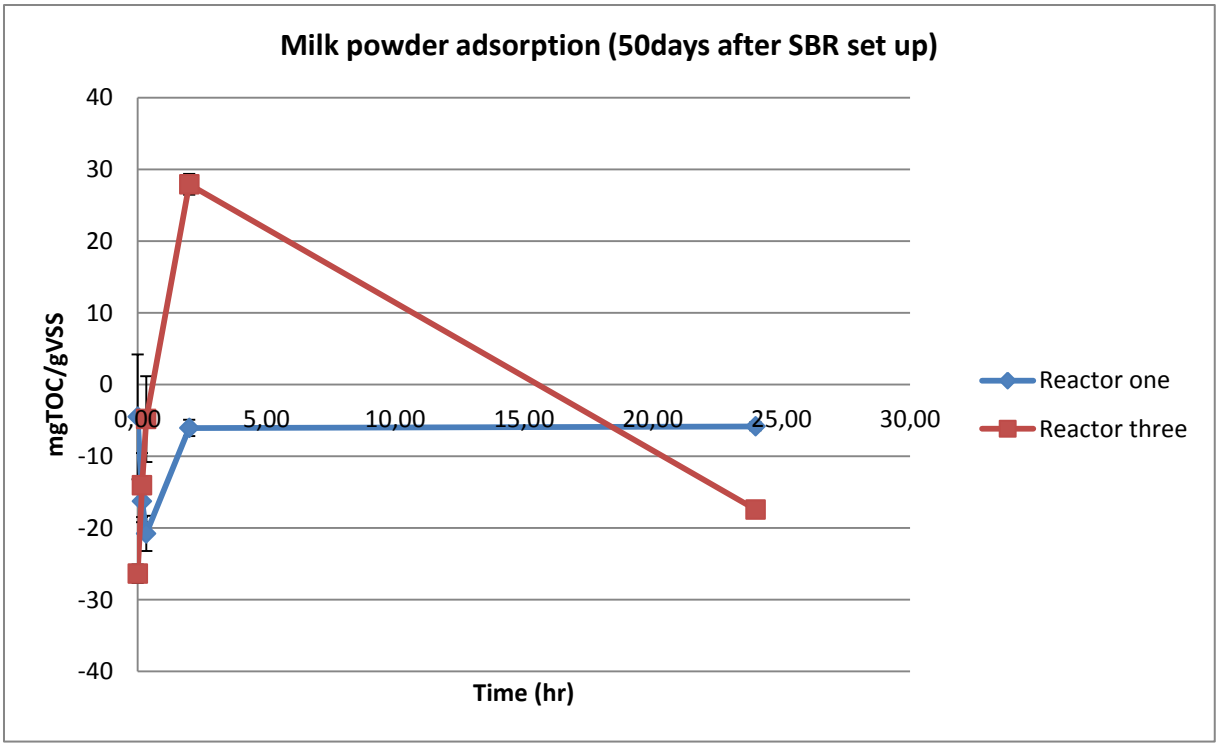


Figure 4.36. The adsorption of milk powder in reactor one and three (with azide).

The results for reactor one show that the carbon concentration inside the mixture increased after mixing the milk powder and sodium azide with the sludge. Then the sludge started to remove the carbon but after a while the carbon release began again. The reason could be deflocculating of flocs inside reactor one after mixing the solution with sludge. The outcome for reactor three is almost below zero except for 2hours sample which was around 30mgTOC/gVSS. The reason might be an error in test procedure, or the TOC analyser could not analyse milk powder samples.

By comparing these two graphs, it is observed that in both reactors when the sodium azide was not used, the carbon began to oxidize after two hours. Carbon was removed in first two hours in reactor three in both conditions. Therefore, it can be concluded that this reduction of carbon could be the physical adsorption.

Another test was done in 50mL tubes, a week later. As shown in Figure 4.37, the carbon was removed as soon as it had contact with the sludge from reactor two and three. The observation for reactor one shows that the carbon was not removed rapidly after mixing. After two hours of mixing the removal rate increased in reactor one and two up to around 70mgTOC/gVSS. However, carbon removal rate followed a stable trend without any changes in reactor three after 2hours. The rate of adsorption per unit of time in first minute of contact was higher than next 24 hours of mixing for reactor two and three (see Table 4.6).

Table 4.6. Uptake rate between first minute and 24 hours of mixing in reactor two and three.

	Reactor two	**Reactor three**
First minutes (mgTOC/gVSS.min)	7.8	13.3
Last 24hours(mgTOC/gVSS.min)	0.045	0.02

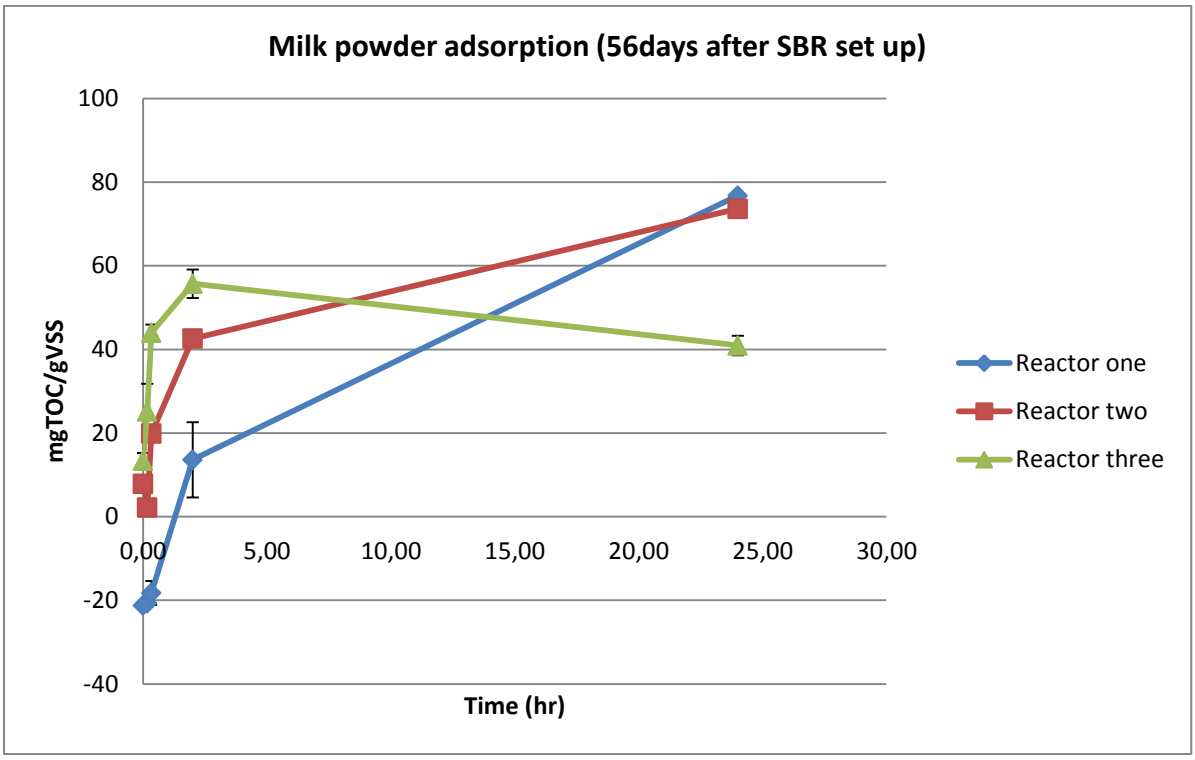


Figure 4.37. The adsorption of milk powder in reactor one, two and three (without azide).

The results were completely changed when sodium azide was added to sludge. As shown in Figure 4.38 after 10 minutes of contact between solution and sludge, the carbon started to be removed and it was continued until it reached a peak around 35mgTOC/gVSS for reactor three. Then, the carbon removal was not changed during next 24 hours. The results for reactor two show that 10mgTOC/gVSS was released during next 22 hours after 20mgTOC/gVSS was adsorbed in first 2 hours. In reactor one, the measured carbon concentration was higher than the initial one. It means that the carbon was released inside the mixture or there was an error occurring during the processes. It was expected that physical adsorption occured in reactor two and three in the first two hours due to the sodium azide.

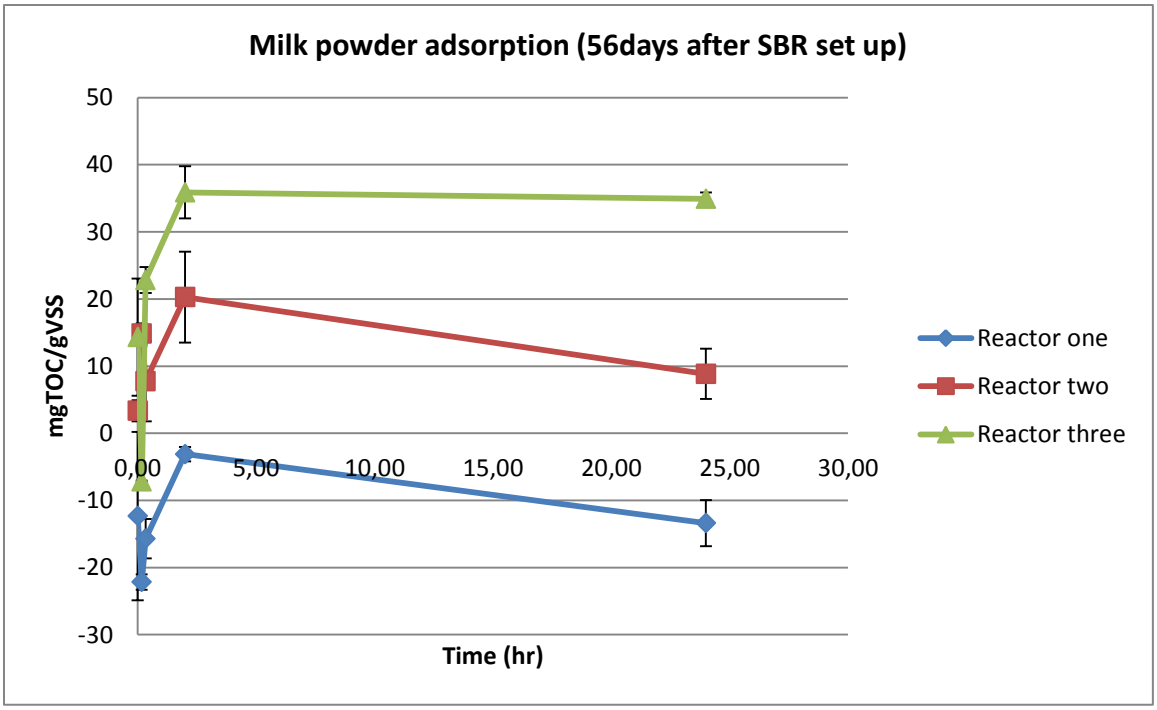
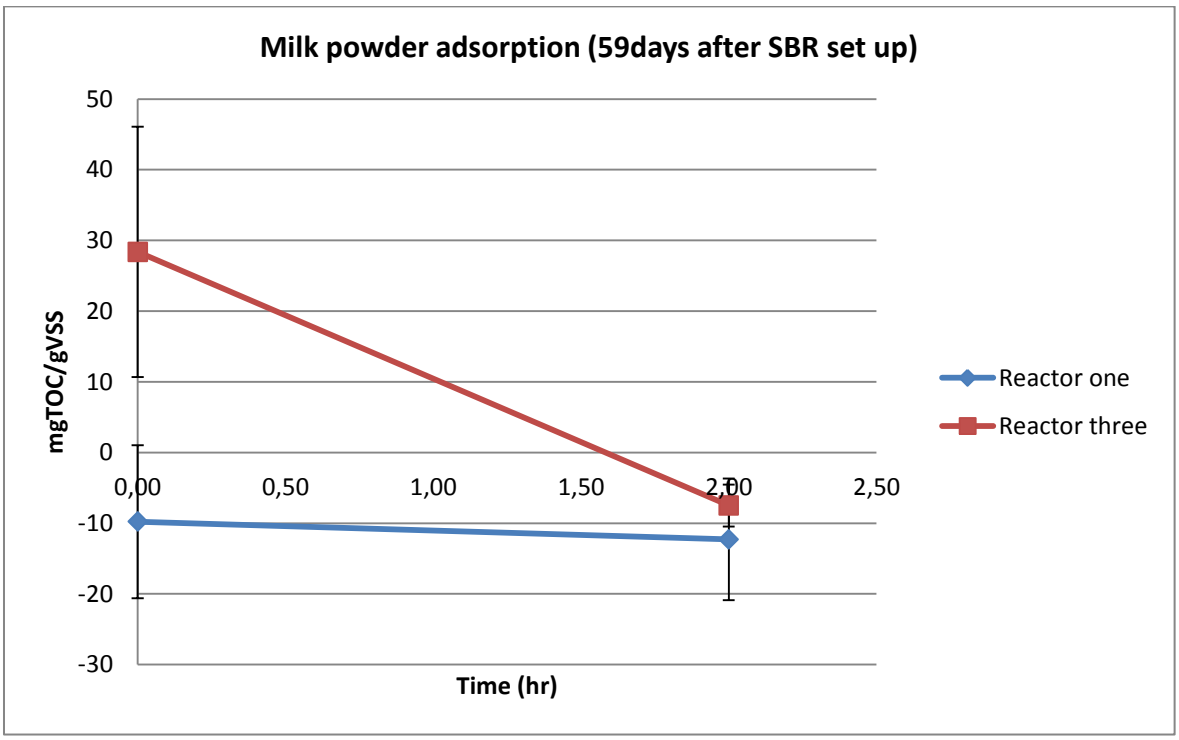


Figure 4.38. The adsorption of milk powder in reactor one, two and three (with azide).

The next experiment was done with three replicates in two steps. The Figure 4.39 presents the results for this test.



Figur 4.39. The adsorption of milk powder in reactor one and three (with azide).

Milk powder-C was removed by sludge in reactor three immediately after mixing the solution and sludge. After two hours the changes in carbon concentration show that carbon was released into the mixture. The result for reactor one represents the release of carbon in sludge mixture instead of

adsorption. Furthermore, error bars for both diagrams show that the reliability of this experiment was very low.

The concentrations of milk powder from the next experiment were analysed with spectrophotometer. The reason was to investigate the adsorption ability of sludge from reactor one due to the fact that the previous results varied a lot. Next test was done with three replicates in two steps. The results are presented below.

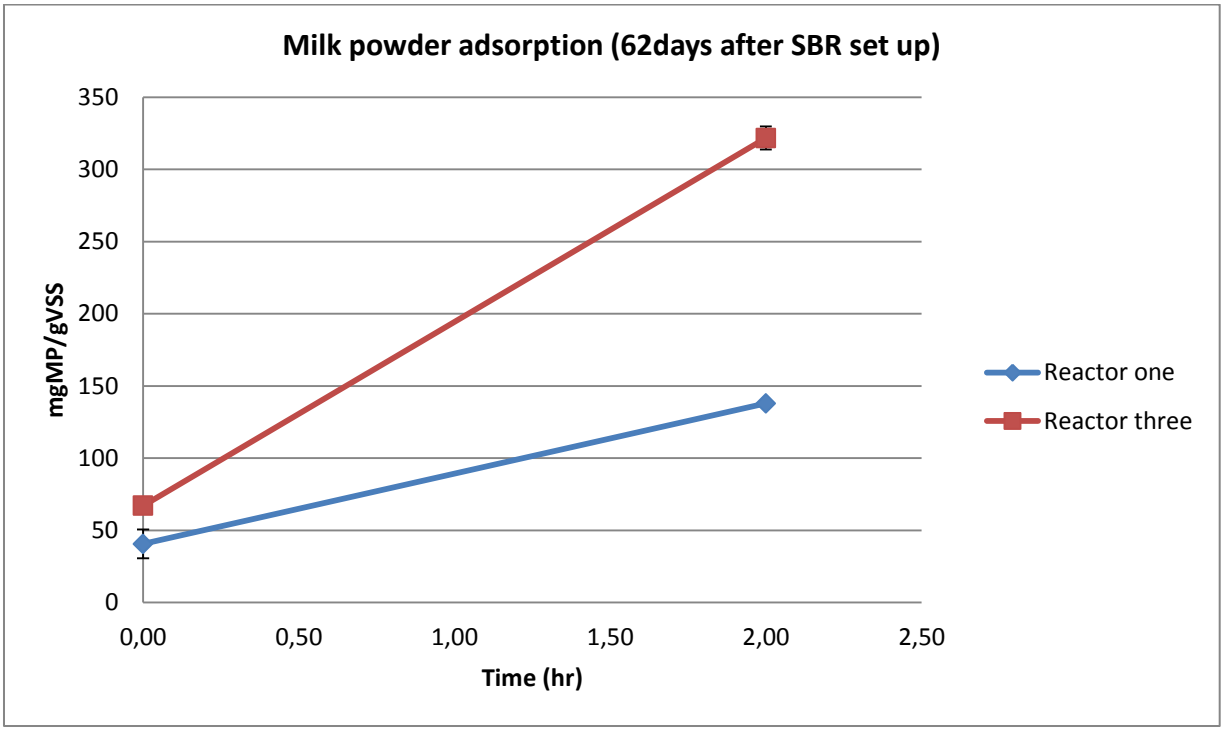


Figure 4.40. The adsorption of milk powder in reactor one and three (with azide)

The removal rate of milk powder in reactor one and three is shown in Figure 4.40. The results for reactor one was totally different from the outcomes which were observed earlier for this reactor. The results which were analysed by spectrophotometer show that milk powder could be removed when it had contact with sludge in reactor one. 40mgMP/gVSS was removed immediately after mixing, and after 2 hours this removal rate reached to 137mgMP/gVSS. It was observed that 67mgMP/gVSS was removed in first minute of contact in reactor three. Afterward the removal rate increased up to 321mgMP/gVSS. Therefore, it could be determined that the sludge in reactor three was more capable than sludge in reactor one for removing the milk powder after 2 hours of contact. The removal rate per unit of time for these two reactors is shown in table 4.7.The removal of milk powder could be a physical adsorption in this experiment due to the fact that sodium azide inhibits the bacteria. In other words, the microorganisms cannot oxidize the milk powder. The VSS of the sludge in reactor one and three were 1.17g/L and 0.28g/L.

Table 4.7. Uptake rate between first minute and 2 hours of mixing in reactor one and three.

	Reactor one	**Reactor three**
First minutes (mgMP/gVSS.min)	40	67
Last 2hours(mgMP/gVSS.min)	0.83	2.11

Spectrophotometer was used to analyse the samples from further milk powder adsorption experiments. The reason was that the outcomes from spectrophotometer were more realistic than the results which were analysed by TOC analyser. Moreover, milk powder is a colloidal compound that can settle in TOC analyser bottle or can attach to the walls of the bottle during analysing and can cause error in results. On the other hand, sodium azide might cause some disintegration of the flocs structure and might lead to release of TOC.

Another test was done in 5 sampling steps. The results were analysed by spectrophotometer. The VSS of the sludge in reactor one, two and three were 1.2g/L, 1.15g/L and 0.3g/L.

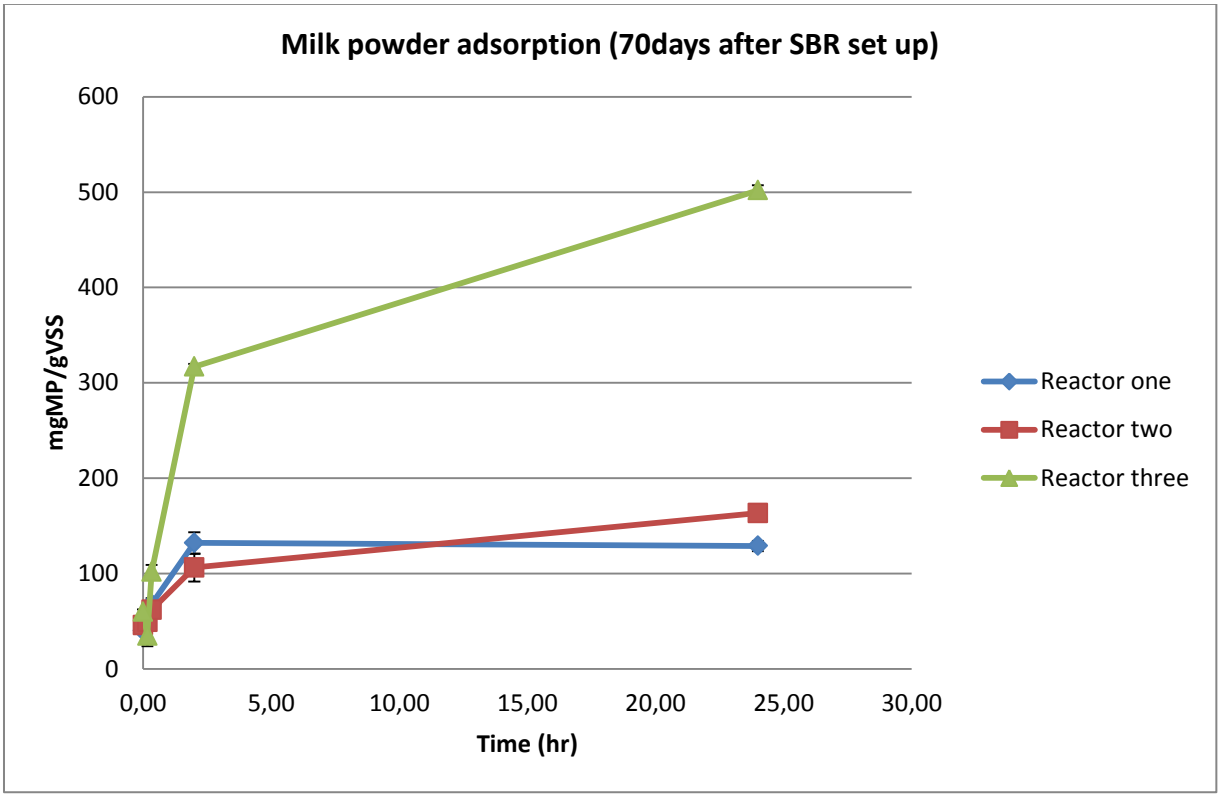


Figure 4.41. The adsorption of milk powder in reactor one, two and three (with azide)

As shown in Figure 4.41, about 50mgMP/gVSS was removed in all reactors immediately after mixing the solution with sludge. In reactor one the maximum milk powder adsorption (132mgMP/gVSS) was happened after 2 hours of mixing. Afterward, during the next 22 hours the milk powder adsorption graph followed constant condition which means that there was no adsorption taking place in the mixture. In reactor two, the milk powder was removed with a similar trend which was happened in reactor one. However, the removal rate of milk powder did not cease after 2 hours, and it was increased slightly. The rate of milk powder removal in the first two hours was higher than the next 22 hours in all reactors (see table 4.8). The removal rate increased significantly from 102mgMP/gTSS to 316mgMP/gVSS in 90minutes in reactor three. The results show that the sludge in reactor three began to remove more milk powder after 30 minutes in comparison with the other reactors. The removal of milk powder continued until it reached to 502mgMP/gVSS after 24 hours in reactor three.

Table 4.8 Uptake rate between first minute, 2hours and 24 hours of mixing in reactor one, two and three.

	Reactor one	Reactor two	Reactor three
First minutes (mgMP/gVSS.min)	40	45	59
First 2 hours(mgMP/gVSS.min)	0.76	0.5	2.13
Last 24hours(mgMP/gVSS.min)	Close to zero	0.04	0.14

This experiment reported that all kinds of sludge which were produced during these three months removed milk powder with a similar rate at the start of the adsorption. However, the removal rate was increased significantly with time in reactor three in comparison with the other reactors. One probable reason could be that the sludge in reactor three was finer than the sludge in reactor one and two. Therefore, it had more surface area in order to bind the milk powder particles. In other words, the sludge in reactor one and two had larger flocs that were saturated faster than flocs in reactor three.

There was not a correlation between EPS and milk powder adsorption capacity according to the milk powder adsorption tests and EPS content for different sludge.

4.5 Milk powder adsorption isotherm

Different milk powder concentrations were added to different kinds of sludge in order to investigate the milk powder adsorption isotherm. The first test was done for milk powder solution with three levels of concentration: 465mgMP/L, 232mgMP/L and 116mgMP/L. The isotherm diagrams of this experiment are shown in Figure 4.42 and 4.43. The linear for of Freundlich equation ($log\frac{x}{m} = \log K + \frac{1}{n} logC$) was used for drawing the isotherm graphs.

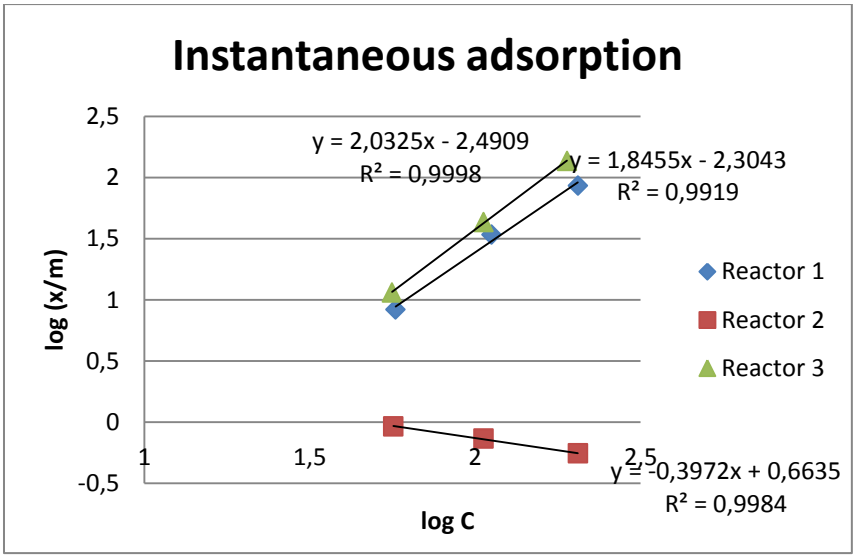


Figure 4.42. Freundlich adsorption isotherms of reactor one, two and three (Instantaneous adsorption).

The Figure 4.42 show the isotherm graphs for adsorption of milk powder by three different kinds of sludge. According to the diagram above the isotherm slope (1/n) for reactor one and three was

higher than 1, and this means that the adsorption capacity of the sludge would be increased faster when the load concentration was increased gradually. However, the intercept (logK) of these isotherms was a negetive value. The K value was 0.003 for reactor one and 0.005 for reactor three. The results for reactor two could not be fitted to the Freundlich isotherm model.

By comparing the numeric adsorption results of different load concentrations,it could be concluded that the higher concentration of milk powder in reactor three led to higher adsorption in first minutes of contacting. The same trend was expected for reactor one, but the adsorption capacity of sludge was lower than reactor three. However, in reactor two the higher concentraton of milk powder caused lower adsorption.

The Figure 4.43 presents the isotherms for milk powder adsorption after 2 hours of mixing for the above experiment. The Freundlich equation was used for drawing the isotherm diagram.

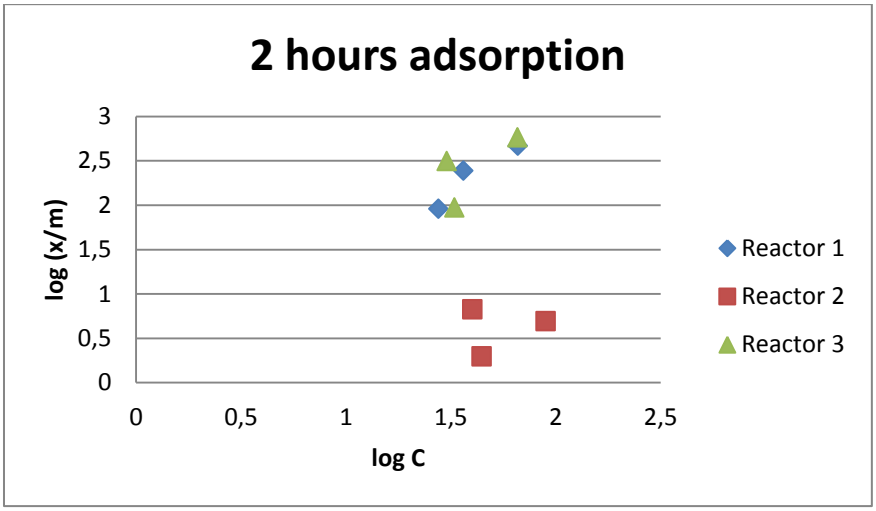


Figure 4.43. . Freundlich adsorption isotherms of reactor one, two and three (2 hours adsorption).

The results for 2 hours of mixing show that the isotherms did not follow a linear trend. Therefore the Freundlich equation could not be used to explain the adsorption isotherms for these samples.

The results of experiment above were plotted with Langmuir equation as well. As shown in Figure 4.44, the isotherms were plotted for instantaneous adsorption. The results for reactor one and three do not fit into the Langmuir equation model ($\frac{x}{c} = K.x_{max} - K.x$) due to the fact that the slope of the isotherm graph should be negative. However, the result for reactor two shows that maximum milk powder concentration which could be adsorbed was near 70mg/L. However it was not possible to determine the adsorption rate by considering the load concentration changes in one direction.

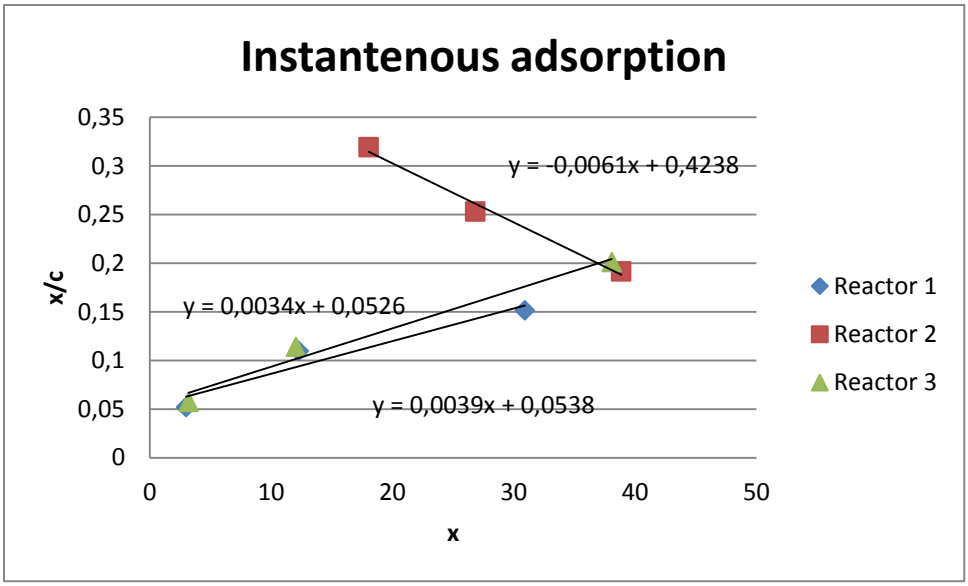


Figure 4.44. Langmuir adsorption isotherms of reactor one, two and three (Instantaneous adsorption).

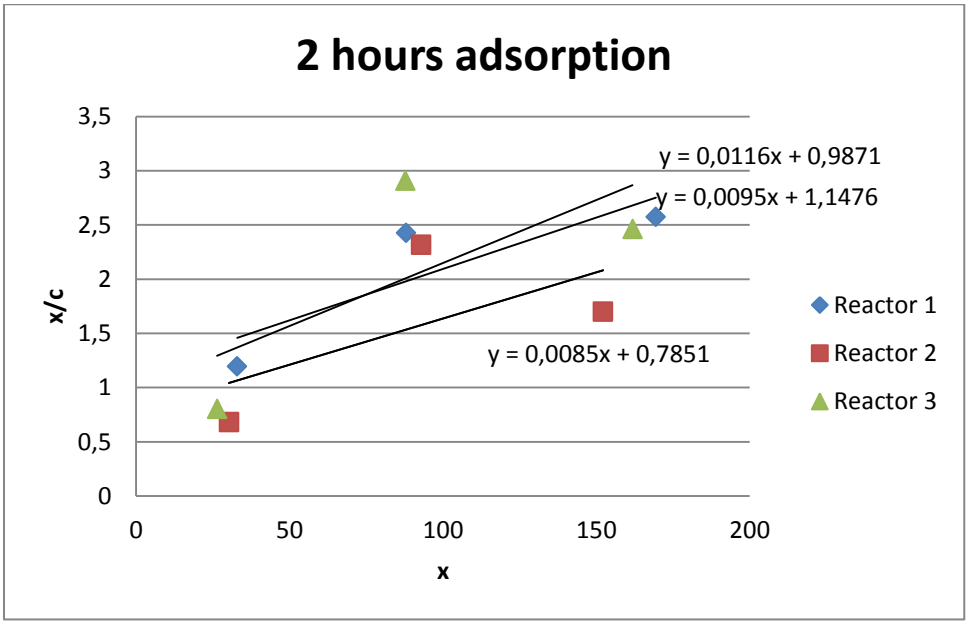


Figure 4.45. Langmuir adsorption isotherms of reactor one, two and three (2 hours adsorption).

The results for two hours contact could not be defined by the Langmuir equation as well (Figure 4.45). The linear correlation was not observed for milk powder adsorption after two hours of mixing.

Another experiment was done in order to investigate the milk powder adsorption isotherm. This test was done with 5 different milk powder concentrations for reactor one and three. Milk powder concentrations which were used are: 1860 mg MP/L, 930 mg MP/L, 465 mg MP/L, 232 mg MP/L and 116 mg MP/L. The Figure 4.46 shows the results of instantaneous adsorption isotherm. The results could be described by the Freundlich model.

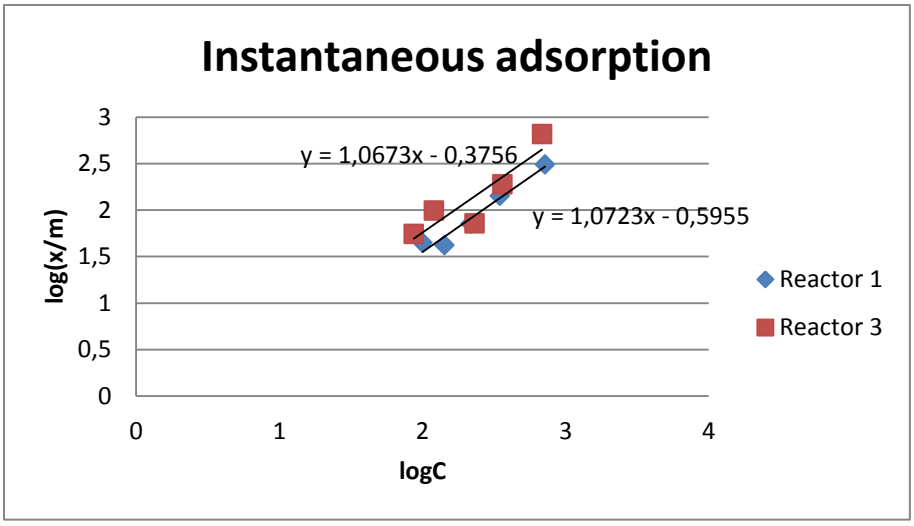


Figure 4.46. Freundlich adsorption isotherms of reactor one and three (Instantaneous adsorption).

As shown in Figure 4.46 both isotherm diagrams of instantaneous adsorption have slope (1/n) near 1 which means that the adsorption would increase when the load concentration increased. However, the rate of adsorption increased with the same pace as the load concentration was increased. The intercept (log K) for both isotherms were negative. The K value was 0.25 and 0.42 for reactor one and three. Higher values of K could lead to more adsorption capacity.
By comparing the numeric results of milk powder adsorption in both reactors it can be seen that the adsorption capacity in reactor three was higher in comparison with reactor one. The reason could be the differences in characteristic of the sludge which they had.

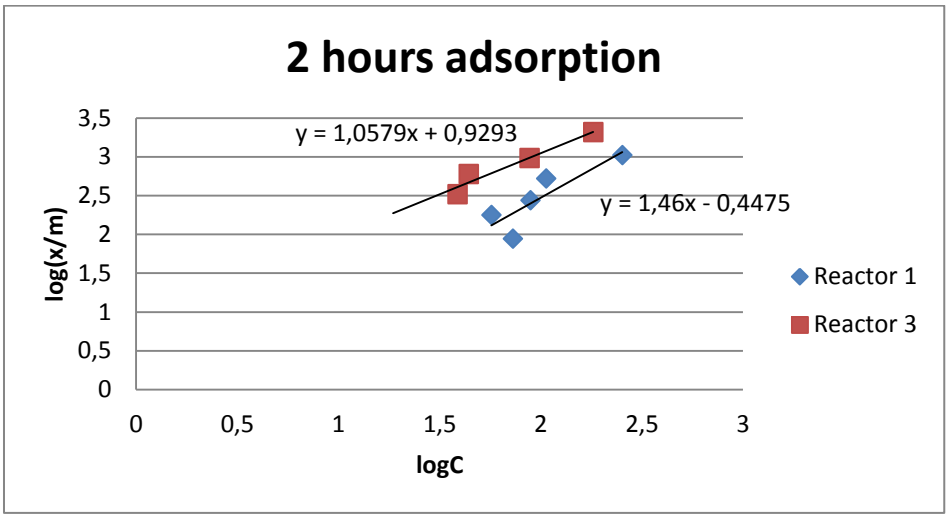
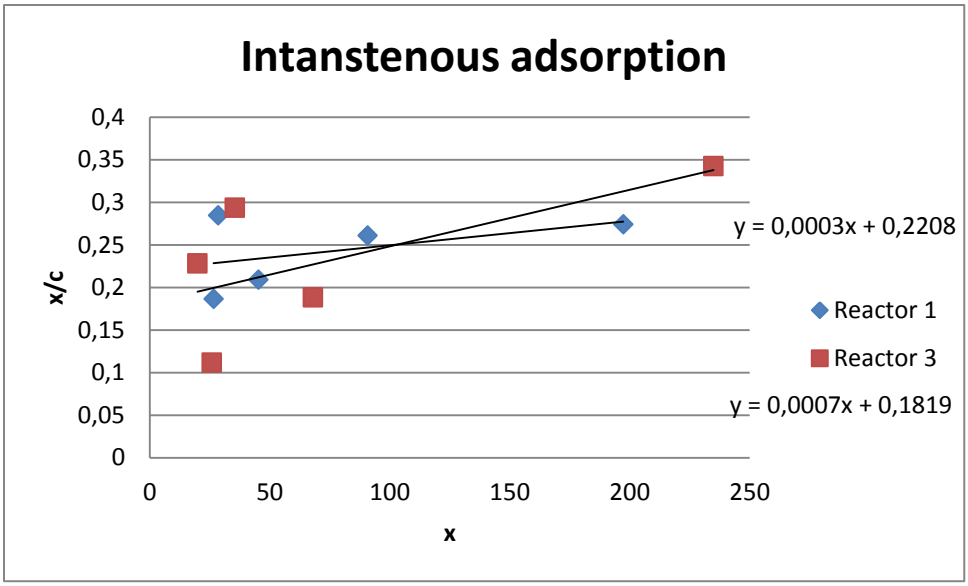


Figure 4.47. Freundlich adsorption isotherms of reactor one and three (2 hours adsorption).

As shown in figure 4.47 the results of two hours contact in reactor one is similar to instantaneous adsorption isotherm. However, in reactor three the intercept of the isotherm is positive and equal to 0.9293 (K=8.5). The Freundlich model for reactor three shows that the adsorption rate would be increased if the load concentration increases.
It can be concluded from these isotherms that the milk powder adsorption capacity of sludge which was fed with ammonium is higher than the sludge which was fed with glucose. The Figure 4.48 and 4.49 show the isotherms which was modified with Langmuir equation.



Figur 4.48. Langmuir adsorption isotherms of reactor one and three (Instantaneous adsorption).

By considering the Langmuir equation it can be observed that the results of this experiment could not be fitted to a Langmuir model due to the fact that the slope was not negative in diagrams. According to Figure 4.49 the results of 2 hours mixing had the same trend and could not be modified by Langmuir equation. Therefore, this experiment and the previous one show that the Langmuir model could not be used in order to model the milk powder adsorption isotherms for reactor one and three.

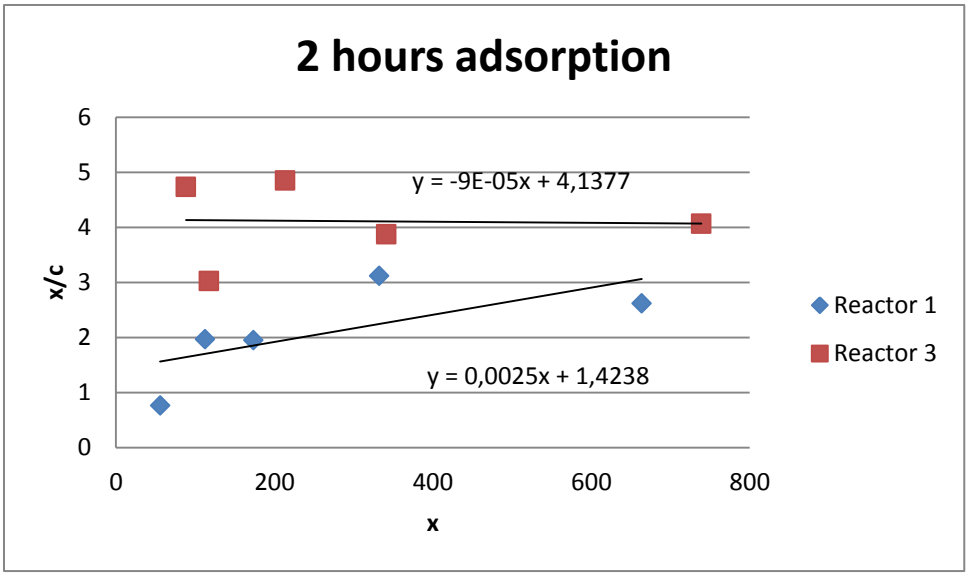


Figure 4.49. . Langmuir adsorption isotherms of reactor one and three (2 hours adsorption).

4.6 Microscopy

Some photos of the sludge flocs were taken by a microscope in order to evaluate their characteristics of the flocs and to be able compare the flocs which were produced in different reactors. The pictures were taken on the 26th of November 2012 (69 days after SBR set up) using a microscope at Rya wastewater treatment plant

4.6.1 Reactor one

Microscopic pictures were taken at different magnifications (Figure 4.50 and 4.51).

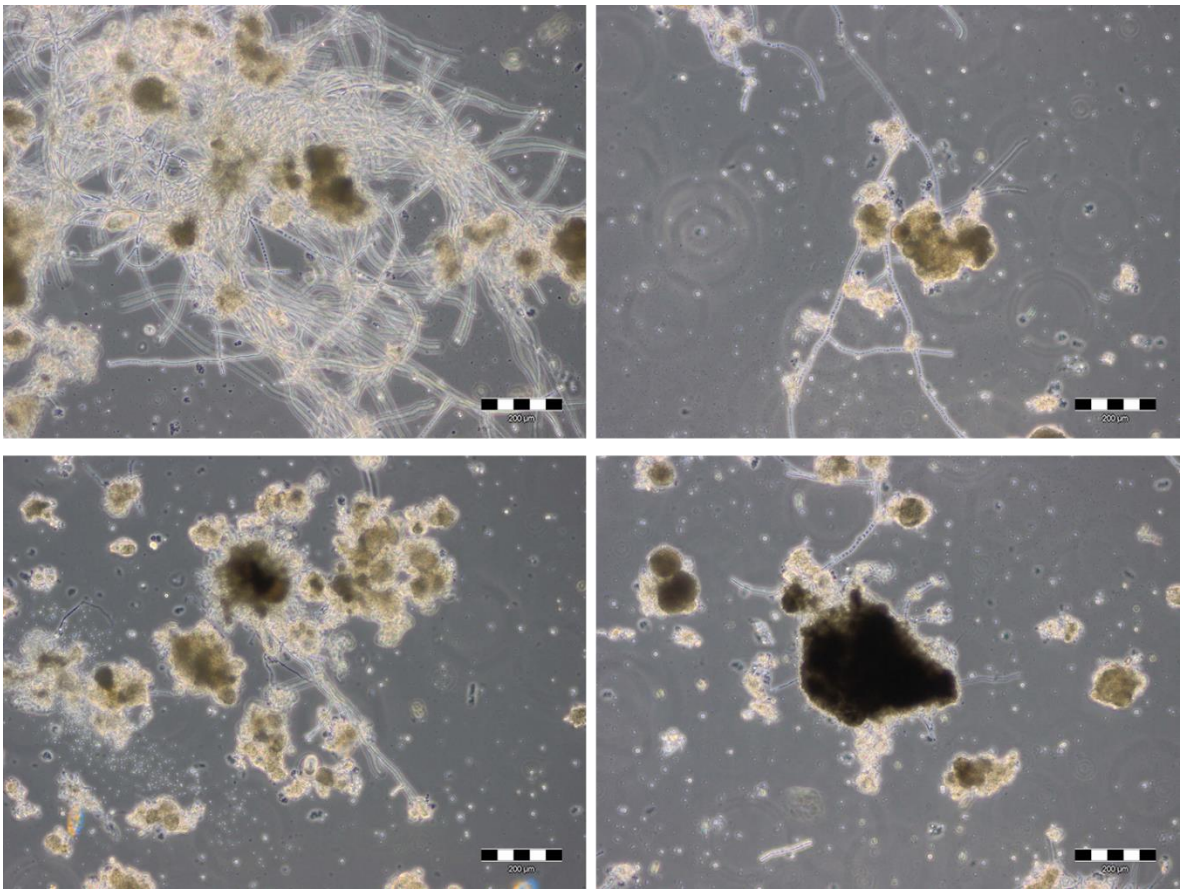


Figure 4.50. Microscopic pictures of the flocs in reactor one at 10Xmagnification. The scale bars represent 200 µm.

The flocs in reactor one were mostly greyish. As Figure 4.50 shows the flocs had dense structure and round shape with sharp edges. The sizes of the flocs varied between 50µm to 250µm and the size distribution of the flocs is wide with many small flocs present between the larger ones. Thick and long filaments were observed in the floc structure (Figure 4.50). However, these filaments were not present in all flocs.

During the microscopy it was observed that lots of protozoa were moving around and consumed small flocs and single bacteria. Furthermore, some free swimming ciliates and rotifers (metazoan) were observed under the microscope.

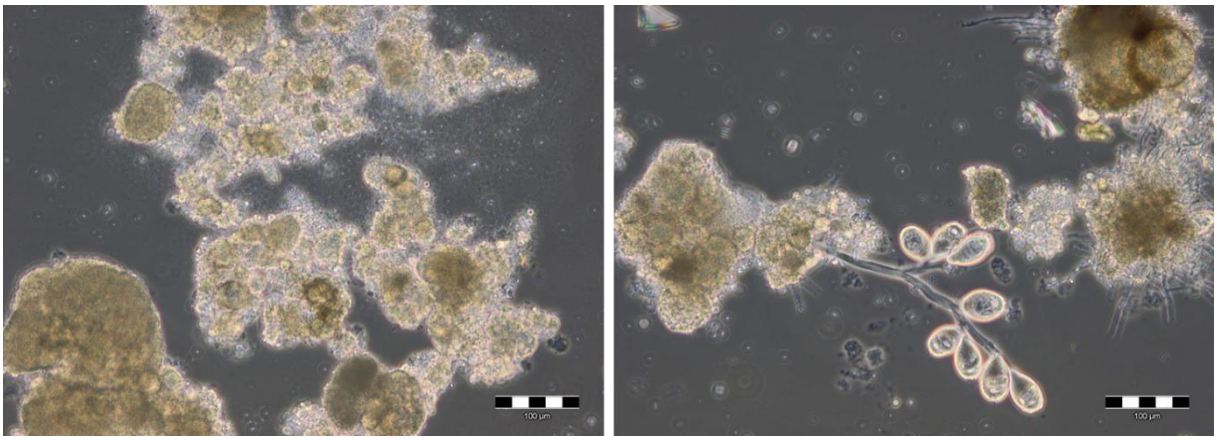


Figure 4.51. Microscopic pictures of the flocs in reactor one at 20X magnification. The scale bars represent 100 µm.

As shown in Figure 4.51 some flocs looked like dense granules. Furthermore, some filaments had stalked ciliates (Figure 4.51). These stalked ciliates could be seen with 20X microscopic magnitude.

4.6.2 Reactor two

The sludge in reactor two had brown colour with irregular shape and distribution. The Figure 4.52 shows that the flocs had sharp edges with sizes from 50 µm to 300µm.

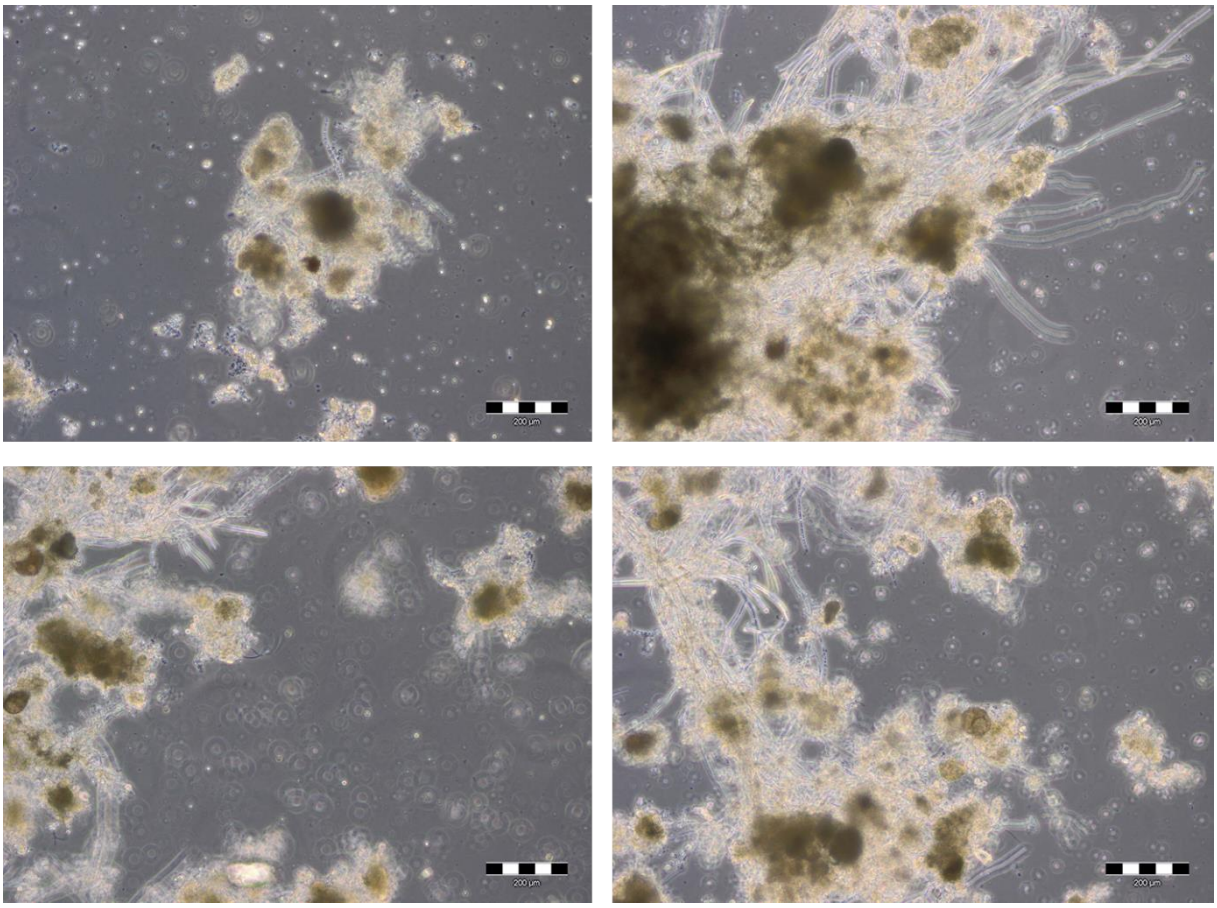


Figure 4.52. Microscopic pictures of the flocs in reactor two at 10X magnification. The scale bars represent 200 µm.

As shown in Figure 4.52 the flocs had dense structure. Figure 4.52 shows that the big flocs were surrounded by dense branches of thick and long filaments. Some swimming ciliates were observed inside the samples.

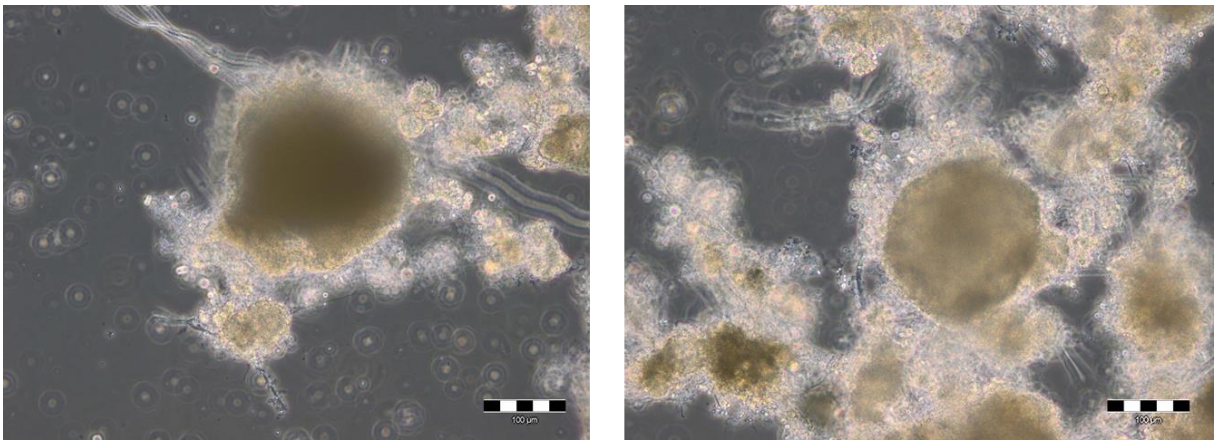


Figure 4.53. Microscopic pictures of the flocs in reactor two at 20X magnification. The scale bars represent 100 µm.

The Figure 4.53 shows that there were a few big rounded flocs in the sludge. These round shape flocs were surrounded by small flocs and filaments. They had darker colour under the microscope in comparison with the other parts of the sample.

4.6.3 Reactor three

The sludge in reactor three had red/brown colour. The sludge had round shape with sharp edges structure and medium size distribution. The flocs inside reactor three were smaller in size in comparison with the flocs in reactor one flocs. As shown in Figure 4.54 reactor three had a very well flocculated sludge with compact round colonies.

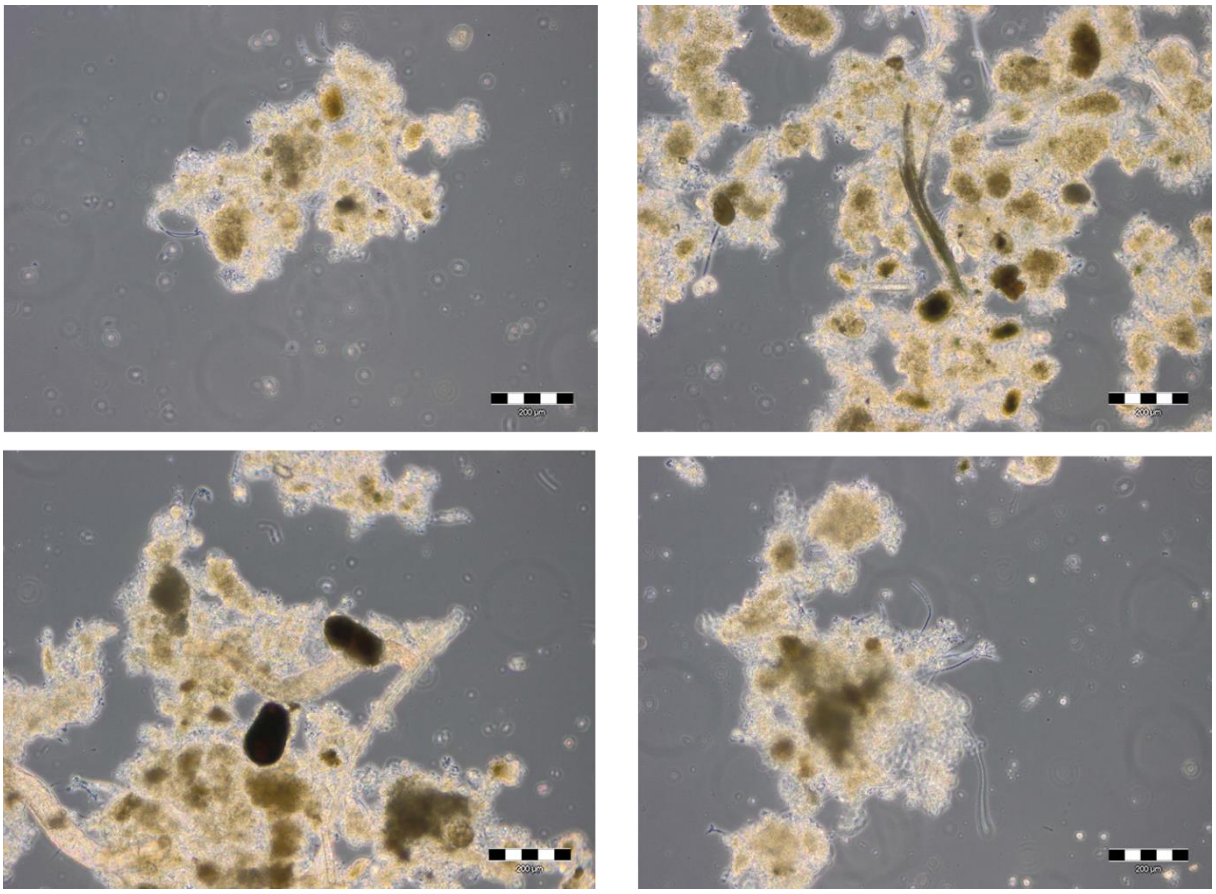


Figure 4.54. Microscopic pictures of the flocs in reactor three at 10X magnification. The scale bars represent 200 µm.

Figure 4.54 shows that protozoa lived inside the floc, and consumed it as a feed. Furthermore, there were not any free ciliates in reactor three. Lots of worms were observed inside the flocs. Figure 4.54 illustrates that the amount of filament in reactor three was lower than in reactor one and two. The filament in reactor three were mostly like branches which attached to the flocs in some places.

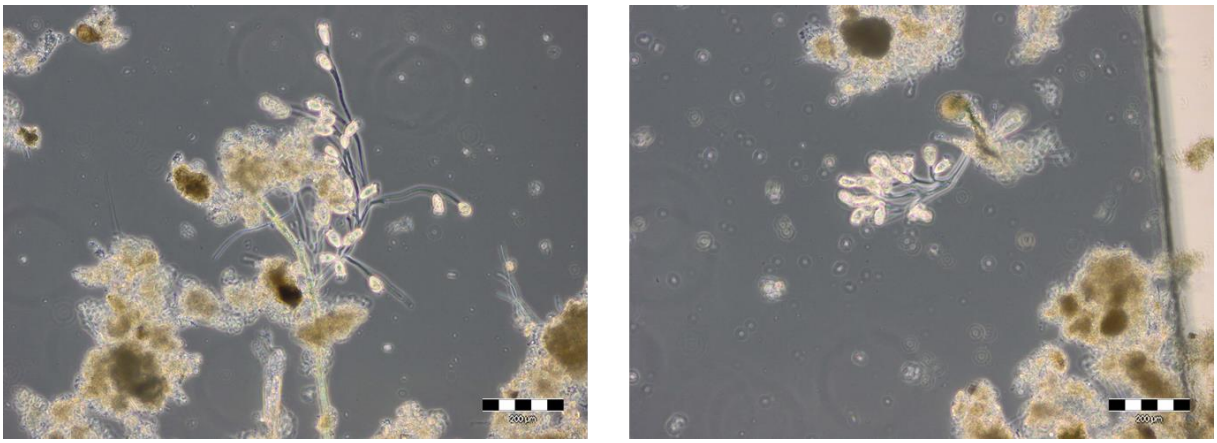


Figure 4.55. Microscopic pictures of the flocs in reactor three at 10X and 20X magnification.

Figure 4.55 shows that there were some stalked ciliates in the sludge. One green filament can be clearly seen in figure 4.55. This green filament could be a kind of algae which grew inside reactor three.

4.7 Comparing three reactors

Three different kinds of sludge were produced during this project. In this section we tried to compare the characteristics of the sludge in the three reactors by comparing the results of oxidation adsorption tests, EPS quantity and quality and microscopy analysis. At last, removal rate in different adsorption tests were compared for the different sludges.

Increasing the feed load had a significant effect on TOC concentration of EPS samples for all reactors. This means that the total amount of EPS increased when the feed load was increased. This increases in EPS-TOC was not permanent, and began to decrease when the bacteria acclimatized to the new concentration of feed. The TN concentration of EPS samples started to increase when the feed load was increased in reactor one and three. However, increasing the feed load did not have significant effect on TN concentration of EPS samples in reactor two which means that the protein content of EPS in reactor two did not change by increasing the feed load. All the changes in EPS-TN concentration were temporary. In conclusion, the EPS test showed that there was not a significant difference between reactor one, two and three. However, HPLC results showed that the characteristics of LB-EPS and TB-EPS were different in reactor one and two in comparison with reactor three.

The oxidation test outcomes show that the sludge which was fed with glucose was more capable to remove organic carbon compounds. In the other hand, the sludge which was fed with ammonium was more efficient for removing the nitrogen. The reason could be that heterotrophic bacteria grew inside reactor one when this reactor was fed with organic carbon. Moreover, nitrifying bacteria grew in reactor three when this reactor was fed with nitrogen source. Therefore, from results of oxidation test and SBR set up it could be concluded that the sludge in the reactors were completely different from each other in characteristics and abilities.

The microscopy analysis showed that the physical characteristics of the sludge in reactor one and two were mostly the same, since they were fed with organic carbon. However, the characteristics of the sludge in reactor three was completely different from the other reactors because this reactor was fed with nitrogen source. The size of the flocs in reactor one and two were similar and bigger in comparison with the flocs in reactor three. Moreover, the most distinguishable difference between the sludge characteristics was the filaments that were found in large numbers in reactor one and two in comparison with reactor three.

The adsorption rate per unit of time was calculated for all three reactors for different adsorption tests. The table 4.9 shows the results for adsorption rate of different compounds (glucose, milk powder and ammonium) for first minute after mixing the solution with sludge.

Table 4.9. Uptake rate in first minute of contact for different adsorption tests in reactor one, two and three.

	Reactor one	Reactor two	Reactor three
Glucose (59days) mgTOC/gVSS.min	3.8	N/A	---
Glucose (90days) mgTOC/gVSS.min	3.4	3.4	---
Ammonium (76days) mgN/gVSS.min	1	---	---
Ammonium (90days) mgN/gVSS.min	0.03	---	---
Milk powder (56days) mgTOC/gVSS.min	---	3.3	14.3
Milk powder (59days) mgTOC/gVSS.min	---	N/A	28.2
Milk powder (62days) mgMP/gVSS.min	40	N/A	67
Milk powder (70days) mgMP/gVSS.min	40	45	59

N/A: no adsorption test was done,
---: varied results were obtained

Table 4.9 shows that sludge inside reactor one (which was fed with carbon source) was more efficient for removing the glucose and ammonium. However, sludge inside reactor three (which was fed with nitrogen source) was more capable to remove milk powder. The concentration of milk powder which was removed by reactor three was higher than the removal rate in reactor one and two. The reason could be the more surface area that flocs in reactor three had due to their smaller size.

The milk powder adsorption isotherms showed that when the concentration of the milk powder in solution was increased the adsorption ability of the sludge in reactor one and three was increased. However, the adsorption ability of the sludge in reactor two was decreased when the milk powder concentration was increased in solution. The values for K and 1/n are shown in Table 4.10 and 4.11. A number in prentices indicates the number of reactor.

Table 4.10. K and 1/n values of Freundlich model for all reactors (first milk powder adsorption isotherm test).

	K	1/n
Instantaneous	0.003 (1) Not fitted (2) 0.005 (3)	1.85 (1) Not fitted (2) 2.03 (3)
After 2 hours	Not fitted (1) Not fitted (2) Not fitted (3)	Not fitted (1) Not fitted (2) Not fitted (3)

Table 4.11. K and 1/n values of Freundlich model for all reactors (second milk powder adsorption isotherm test).

	K	1/n
Instantaneous	0.25(1) 0.42(3)	0.94(1) 0.92(3)
After 2 hours	0.36(1) 8.5(3)	0.68(1) 0.95(3)

5 Conclusions

The primary studies of glucose and milk powder removal in 800mL beakers show that there were rapid removal happened during first minutes after mixing the solution with sludge mixture. Then, the removal rate continued with lower pace. The ammonium removal was not significant during mixing. One reason could be that there was no change in the amount of TN concentration when it was measured by TOC and TN analyser, because nitrifiers converted the ammonium to nitrate.

The results of the SBR set up show that the glucose and ammonium which were added to the reactors were completely consumed by bacteria. The TSS of reactor one and two started to increase after the glucose concentration was increased. Therefore, the excess sludge was removed from the reactors every time when the TSS was more than 2.5mg/L. The TSS in reactor three began to decrease after a while, probably due to the decay of heterotrophic biomass.

The glucose adsorption tests show that glucose adsorbed into the sludge from reactor one and two when the lower concentration of the glucose was added to the sludge. However, reactor three had incorrect results for this concentration. The sludge in reactor one and three had different responses in contact with higher concentrations of glucose. One experiment shows that the sludge in reactor one had ability to remove the glucose. However the results of the other experiment were completely different and there was not any adsorption observed. The same trend was observed for reactor three. In conclusion; it is hard to make a prediction about the adsorption of glucose to different kinds of activated sludge. The reason could be due to the high conductivity of these two reactors (reactor one and three). The high conductivity could lead to TOC releases and deflocculating. Another reason might be possible errors happened during sampling and analysing.

In all experiments, it was observed that ammonium adsorbed into sludge in reactor one. The sludge in reactor two was not capable to remove ammonium by physical adsorption. The results of reactor three were contradictory. In other words, one experiment showed that the adsorption of ammonium was happened in reactor three, but the other test did not show the same result. It can be concluded that the sludge in reactor one with high conductivity and enrichment of heterotrophs could remove ammonium by physical adsorption. It is hard to make a conclusion about the adsorption of ammonium in reactor two and three due to the fact that there was lack of data about the sources of error in experiments.

Some results which were measured by TOC analyser for milk powder adsorption were varied a lot and therefore, different measurement method was used. It was observed that the sludge in all reactors could remove milk powder rapidly in first minutes of contact. Then, the removal pace decreased. The sludge in reactor three could remove more milk powder in comparison with the sludge in reactor one and two. The reason could be the more surface area that the sludge in reactor three had because it was finer than sludge in reactor one and two.

The Freundlich and Langmuir adsorption model was adapted to the milk powder adsorption experiments. The milk powder adsorption for reactor two did not fit in to the Freundlich model. However, it could be explained by the Langmuir equation. The maximum milk powder concentration which could be removed by the sludge in reactor two was estimated near 70mg/L by using the Langmuir equation. The Freundlich model for reactor one and three showed that if the milk powder concentration was increased in solution, then the adsorption capacity of the sludge would be increased.

The method which was used for adsorption tests in this thesis need to be improved since the 50mL replicate samples gave quite different results in different experiment. This could be due to varying mixing conditions on the shaking table. The samples were moving on the shaking table, so pattern of mixing inside each tube was different from the others. This dissimilarity in mixing pattern can lead to possible errors and variation in results.

6 Recommendations

For further studies we recommend the followings:

1. An investigation of adsorption is better to be done in beakers instead of 50ml vials due to the mixing and settling problems which are possible to occur during the experiments. Furthermore, it is easier in beakers to monitor the air inside the sludge mixture by installing the pumps.
2. Use another inhibitor instead of sodium azide, since the sodium azide contains nitrogen itself and it can cause problems during ammonium adsorption tests.
3. More HPLC analysing can be performed for EPS samples in order to monitor the compounds inside the samples during the SBR monitoring.
4. Different organic carbon compounds can be used for feeding the reactors in order to cultivate more varied sludge mixtures.
5. Adsorption capacity of sludge with different TSS can be investigated in order to determine the effect of TSS on adsorption ability of sludge.
6. Consider the environmental effects such as temperature and pH in future study in the field of activated sludge adsorption capacity.

7 References

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