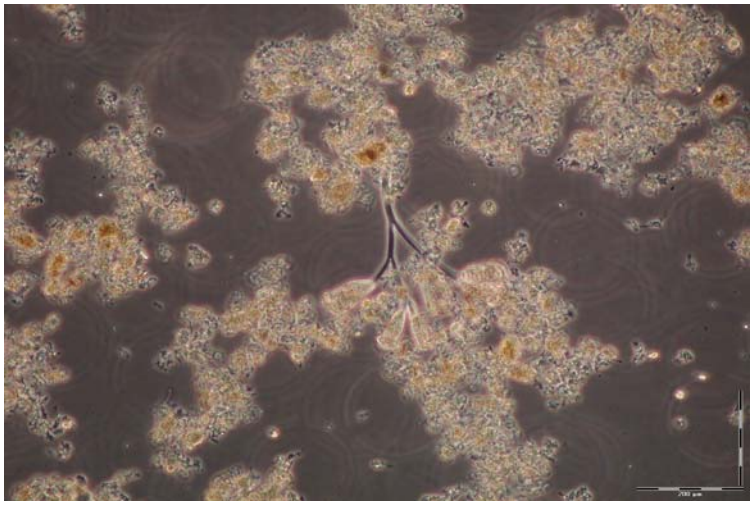
CHALMERS

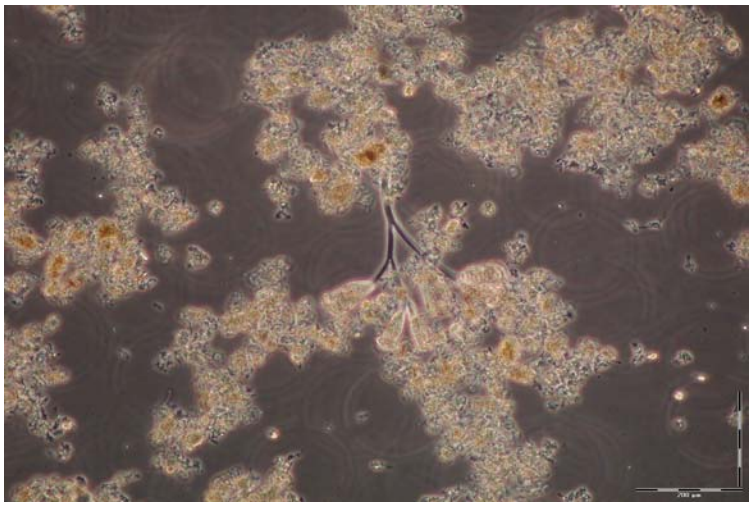


Study of temperature effects on activated sludge floc stability

Master's Thesis in the International Master's Programme
Applied Environmental Measurement Techniques

RASHED IBRAHIM MANASSRA

Civil and Environmental Engineering
Water Environment Technology
CHALMERS UNIVERSITY OF TECHNOLOGY
Göteborg, Sweden 2006
Master's thesis 2006:6



Study of temperature effects on activated sludge floc stability

Master thesis in Applied Environmental Measurement Techniques

By

Rashed Ibrahim Manassra

under super vision of

Dr. Britt-Marie Wilén

Chalmers University of Technology
Department of civil and environmental Engineering
Water Environment Technology

Göteborg, Sweden 2006
Master's Thesis 2006:6

Acknowledgements

I would like to warmly thank my project supervisor Dr. Britt-Marie Wilén, at the department of Civil and Environmental Engineering/ Water Environment Technology at Chalmers University of Technology, for the time she has devoted to me, for her numerous sensible advise, for her endless source of help and support and for making sure my project went well. I also would like to thank the examiner of the thesis Professor Greg Morrison, Director of Master degree Programme of Applied Environmental Measurement Techniques, head of Water Environment Technology department, for his assistance and taking time to read the thesis.
I also would like to extend my thanks to all my teachers at Chalmers University for their assistance and guidance during my study. I am also thankful for the members at the Rya Wastewater Treatment Plant in Göteborg and Jesper and Mona at Chalmers laboratory for their help and kind treatment during my work.

Dedication

I would like to dedicate this work to my parents, brothers, Gassan and Ismail, and all my sisters for their endless encouragement and support they have given. The thought of affection and loving memories made me able to undertake the hectic and challenging task of master thesis.

To all my relatives, cousins and Friends
I dedicate this work

Abstract

Temperature is an important parameter in biological wastewater treatment because the effects it has on the microbial growth and activity. The influence of either decreasing the temperature (5°C, 10°C and 15°C) or increasing it to a moderate high temperature(30°C) on the degree of deflocculation of the activated sludge flocs was studied. The activated sludge flocs were exposed to controlled shear conditions in two parallel lab scale batch reactors in short term experiments (0-3 h) under aerobic and anaerobic conditions. Floc disintegration was measured as turbidity in the supernatant after centrifugation. The composition of the turbidity was analyzed in terms of total and dissolved biopolymers (carbohydrate, protein and humic substances). The results show that, when activated sludge acclimatized to a temperature of about 20ºC was exposed to different low temperatures (5ºC, 10ºC and 15ºC) under aerobic conditions, the flocs deflocculated significantly more compared to at 20ºC. However, when the same experiments were carried out under anaerobic conditions, it was found that the activated sludge flocs deflocculated more at 20ºC than at 5ºC, 10ºC and 15ºC. The concentration of total biopolymers measured in terms of protein, carbohydrate and humic substances followed the turbidity development and was higher at 5ºC, 10ºC and 15ºC than at 20ºC under aerobic conditions, while it was higher at 20ºC than at 5ºC, 10ºC and 15ºC under anaerobic conditions. Higher concentrations were found for all total organic fractions under anaerobic conditions. The dissolved biopolymers were released more at 20°C than at 5°C, 10°C and 15°C under aerobic conditions. The concentrations of dissolved polymers were also higher at 30°C than at 20°C. The dissolved biopolymers followed the turbidity curve for anaerobic conditions and they were always higher at 20°C than at 5°C, 10°C and 15°C. The concentrations of Na in the supernatant were rather similar under aerobic and anaerobic conditions. The concentration of K were slightly higher under anaerobic conditions than under aerobic conditions. Under aerobic conditions there was a slight increase in concentration and there was a trend towards higher concentrations at higher temperatures. The increased in concentration was particularly high at 30ºC. Similar observations were made for anaerobic conditions.Very low concentrations of Fe were found in the supernatant at aerobic conditions, whereas slightly higher concentrations were observed for anaerobic conditions. It was observed that, the concentration of Ca, Mg and in some cases Fe ions decreased with the time of the experiments in both test reactors and reference reactors under both aerobic and anaerobic conditions.

1.0. Chapter One: Literature review

1.1. Introduction

Removal of organic matter and nutrients such as phosphorus and nitrogen from sewage is the main concern of the wastewater treatment. If too much untreated sewage or other organic matter is added to any water body such as lakes, streams or sea, the levels of dissolved oxygen will drop to levels too low to support sensitive species of aquatic biota and other aquatic life. Generally, wastewater is treated physically, by separating coarse and floating solids, chemically, by adding precipitants for phosphorus removal, and biologically by means of the activated sludge process. In this process the microorganisms metabolize and transform organic and inorganic substances into environmentally acceptable forms, and they proliferate and grow as flocs. The activated sludge must be settled and separated from the treated water before it is discharged into any receiving water body. The effectiveness of settling of activated sludge from the treated mixed liquor is mainly dependent on the ability of the activated sludge to form flocs. Two processes are important for the successful operation of a biological wastewater treatment plant, the transformation of dissolved and particulate wastewater components into biomass and solid liquid separation by sedimentation.

The efficiency of solid liquid separation in the activated sludge process is determined by the capacity of sludge biomass to remain flocculated and to settle and compact fast. When the activated sludge flocculates poorly, the level of suspended solids in the effluent increases, and it is often associated with weak floc structures. Deflocculation (erosion of small particles from the flocs) and poor bioaggregation can be the result of transient operating conditions and environmental stresses such as shift in temperature, toxic compounds, metals, dissolved oxygen concentration, pH, ionic strength, shift in substrate loading and nutrient characteristics or concentration.

The temperature is very important in biological wastewater treatment systems because the effects it has on the microbial growth. While most microorganisms are able to exist over a broad temperature range, there is usually an optimum temperature at which each species grows best. In treatment plants, a slow adaptation occurs due to seasonal changes in temperature. However, sudden changes in temperature affect the microbial activity which might affect processes like flocculation due to changed surface properties of the microbial cells.

Wastewater treatment plants based on the activated sludge process are subject to transient operating conditions. The hydraulic loading varies significantly due to diurnal variations and to inflow and infiltration of storm water due to rain events. At different flows, the composition of the wastewater varies due to dilution with stormwater or varying load of domestic wastewater. When the flow changes, the composition and characteristics of the wastewater change as well. This has impact on the biological processes in the plant, such as changed concentration of carbon source for the denitrification but it has also impact on the physical properties of the particles in the activated sludge due to for instance changed ionic strength and temperature. It has been observed that the effluent turbidity often increases when the temperature in the wastewater decreases as a result of rain events. The temperature also has a seasonal variation where the wastewater temperature in Sweden varies from approximately 8°C in the winter to 21°C in the summer (Wilén *et al.,* 2005). It was observed that the suspended solids concentration in the effluent is higher during the winter. Also during

rain events, the temperature in the water decreased and an immediate increase in turbidity could also often be observed. It was speculated that this could be due to a decreased microbial activity, which in previous research has been found to affect the floc stability (Wilén et al. 2000).

1.2. Aim of the thesis (objectives)

The purpose of this thesis was to study the effect of temperature on the stability of activated sludge flocs when a controlled shear rate was applied under aerobic and anaerobic conditions. The composition of the released floc matter was also to be investigated and quantified in terms of total and dissolved biopolymers (protein, humic substances and carbohydrates), and cations such as, Mg^{2+}, Ca^{2+}, K^{+}, Na^{+} and Fe^{3+} into the solution under aerobic and/or anaerobic conditions.

2.0. Chapter two: Literature review

2.1. Wastewater composition

Wastewater is produced from industries, municipalities, and storm water; it varies significantly in both composition and quantity with time. The composition of domestic wastewater varies due to variations in the discharged amounts of substances and dilution with storm water. Wastewater components can be divided into three main groups; physical, chemical and biological. Physical pollutants are categorized as, solids, colour, odour, and conductivity, each of these categories might have chemical or biological sources and it can be removed by chemical or biological methods. Chemical pollutants can be organic or inorganic; the organic pollutants are more biodegradable and they can be categorized according to the rate of biodegradation into three categories; (1) readily biodegradable compounds which have low molecular weight and can be immediately metabolised by bacteria like, methanol, ethanol, volatile fatty acids, monosaccharides and lower amino acids; (2) slowly biodegradable compounds which can be hydrolysed by extracellular enzymes before they can be entered into the cell; and (3) Unbiodegradable compounds, which remain in the effluent after passing through the biological treatment process, and they can be dissolved or adsorbed to the flocs. Chemical pollutants can be found in settable, floatable, suspended or dissolved solids. Biological pollutants are cellular in nature and have a combination of organic and inorganic constituents.

The discharge of organic pollutants causes oxygen depletion in the receiving waterbody, and discharge of nutrients such as phosphorous and nitrogen causes excess growth of algae that produce toxins and causes oxygen depletion in the receiving water (eutrophication).

2.2. Wastewater treatment

In the wastewater treatment plant the contaminants are reduced to a certain allowable concentration. The basic principle of the wastewater treatment plant is to convert dissolved compounds into solids, which can be removed from the treated water. The treatment process is generally passing through series of stages as follows (Figure A).

Primary treatment is a physical process to separate coarse and floating solids, gravels and grasses during the passage of wastewater through a bar screen, generally followed by primary settling in tanks allowing larger particles to settle. The settled particles receive further treatment as sludge, the remaining wastewater flows into the next stage.

Secondary treatment is based on biological and/or chemical processes which are used to remove organic matter and nutrients such as nitrogen and phosphorous. Organic matter can be removed by metabolic activities of microorganisms. Nitrogen is mainly removed biologically whereas phosphorus can be removed biologically and by chemical precipitation. Bioreactors are mostly compartmentalized to provide three environmental zones suitable for the growth of certain species of microorganisms. The three zones are: the anaerobic zone, which has no dissolved oxygen, the anoxic zone, which has no dissolved oxygen but has nitrate and/or nitrite; and the aerobic zone, which has dissolved oxygen. In biological treatment the bacteria grow either suspended in aggregates, which are known as activated sludge flocs, or in biofilms. The most common method is the activated sludge process, which is based on suspended growth of bacteria.

Tertiary treatment is a physicochemical process to further reduce the inorganic nutrients such as nitrogen and phosphorous, suspended particles, and disease causing organisms from the effluent. It can include filtration, precipitation and chlorination.

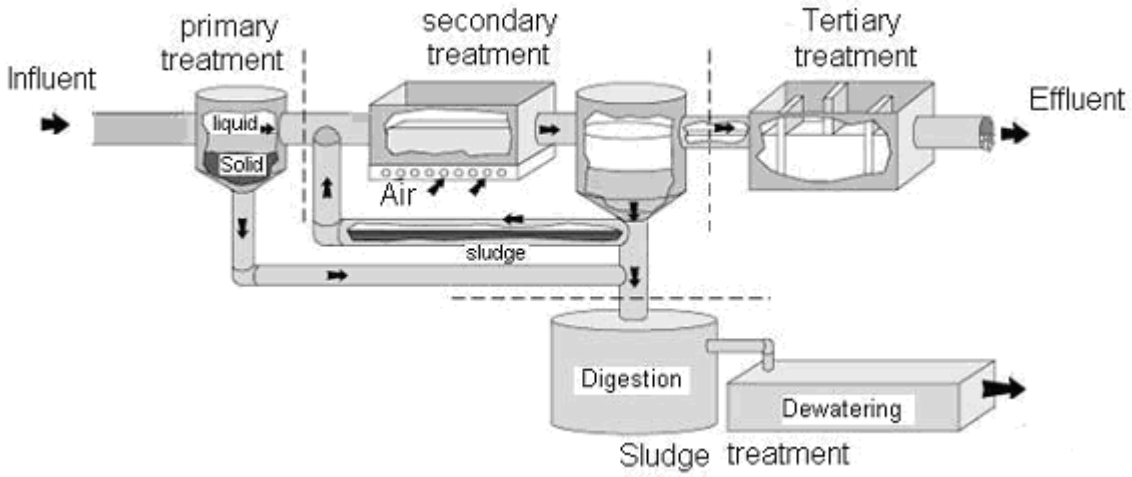


Figure A: Schematic view of a conventional wastewater treatment plant

2.3. The activated sludge process

The activated sludge process is a biological method for wastewater treatment that is performed by a variable and mixed community of microorganisms. The carbonaceous organic matter in the wastewater provides an energy source for the production of new cells for a mixed population of microorganisms. The microbes convert carbon into cell tissue and oxidize the organic matter into carbon dioxide and water. In addition, microorganisms may exist in activated sludge that obtains energy by oxidizing ammonia nitrogen to nitrate nitrogen in the process known as nitrification. This process takes place if the solids retention time (SRT) of the sludge is long enough for these slow growing microorganisms to exist. The presence of anoxic zones are necessary for denitrifying bacteria to exist, i.e. the ones that convert nitrate or nitrite to nitrogen gas which is the last step in the biological nitrogen removal.

Flocs are formed spontaneously when wastewater is aerated, and when the aeration is stopped the flocs starts to settle, and the supernatant can be discharged and the flocs can be reused to treat a new portion of wastewater (figure B). The ability of bacteria to form flocs that has a density higher than water enables separation of the sludge from the treated water, something which is used in the secondary settlers which is often the last treatment step in activated sludge plants.

The success of the activated-sludge process depends on establishing a mixed community of microorganisms that will aggregate and adhere in a process known as bioflocculation. Different types of activated sludge solids separations problems occur, such as bulking sludge due to the excessive proliferation of filamentous bacteria, foaming due to growth of certain microorganisms and poor flocculation properties of the microorganisms. These problems often indicate an imbalance in the biological component of the process. In the ideal and healthy biological treatment system, filamentous organisms grow in low to moderate number within a floc (a large aggregate of floc-forming bacteria) and give it strength.

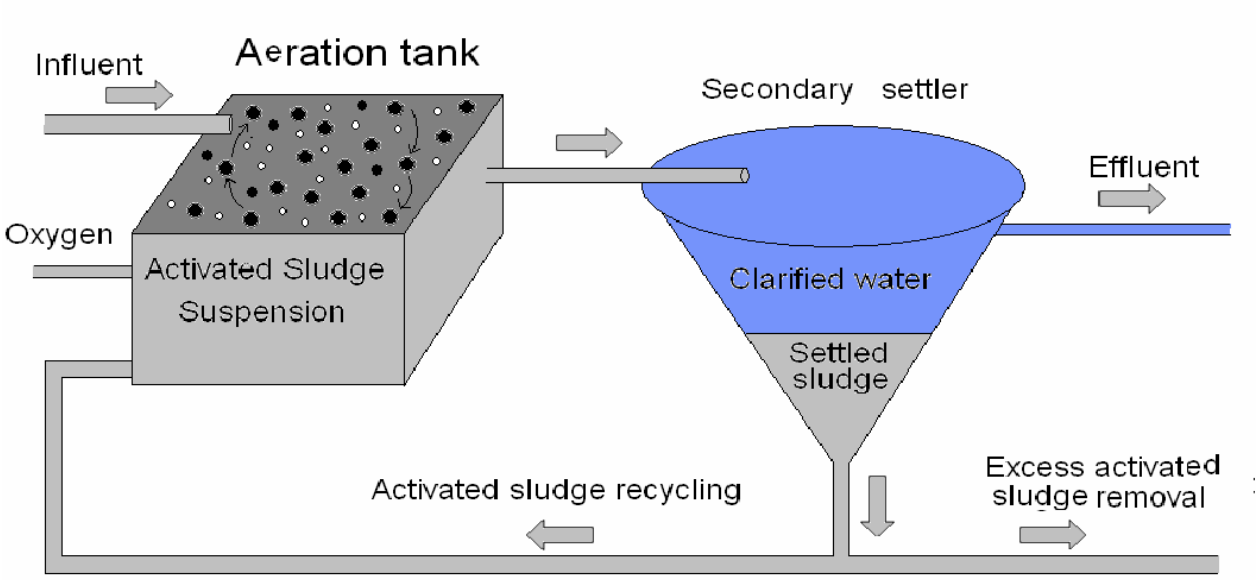


Figure B: Schematic view of the activated sludge process

2.4. Composition of the activated sludge flocs

Activated sludge flocs are complexes of different living microorganisms (mainly bacteria), dead cells, undigested large organic fragments trapped in the flocs, and an inorganic fraction (Eikelboon and Buijsen, 1981) (figure C and D). The flocs contain approximately 30-40% inorganic matter and 60- 70% organic matter, of which the extracellular polymeric substances (EPS) compose about 50-60%. The organic fraction of the sludge contains approximately 50% protein, 20-30% humic substances, and 10-30% carbohydrate and the other organic fraction can be uronic acid and nucleic acid (Nielsen and Keiding, 1998). Protein and polysaccharide account for 75-89% of the EPS composition (Tsuneda et al., 2003).

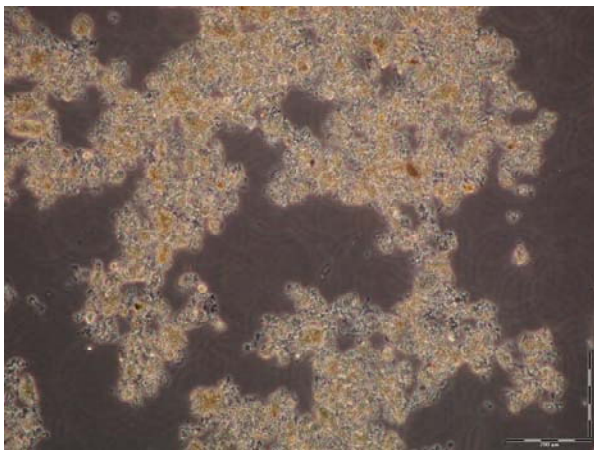


Figure C: Activated sludge flocs images (taken by light microscope, 10x).

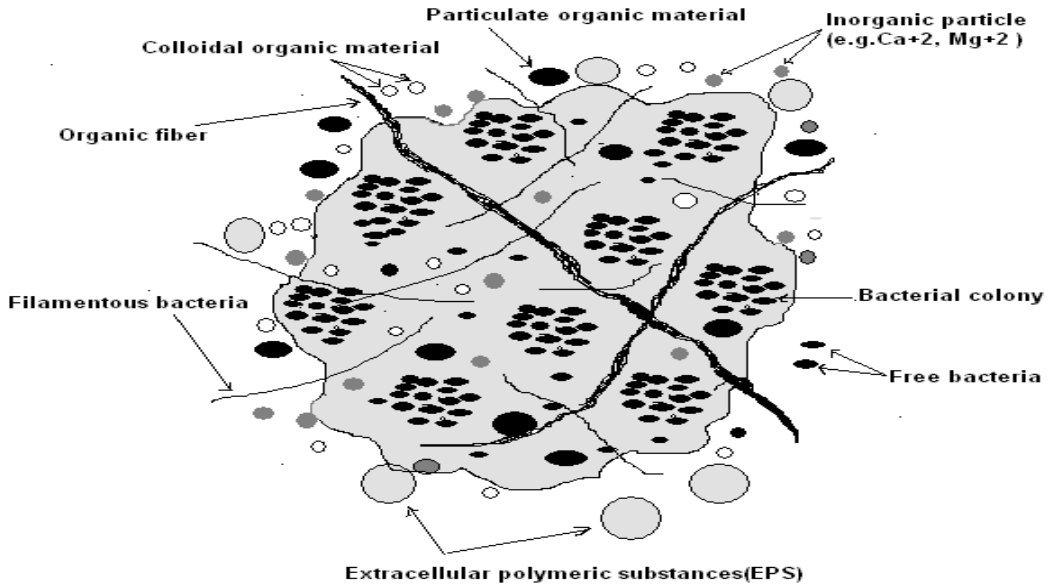


Figure D: Schematic drawing of the of the activated sludge floc**.**

2.5. The organisms in biological treatment plants

A very diversified group of organisms are involved in the biological processes in treatment plants (Eikelboon and Buijsen, 1981). Not all organisms are identified, so it is only possible to list which species are present. Bacteria constitute the majority of the microorganisms present in activated sludge. Bacteria that require organic compounds for their supply of carbon and energy (heterotrophic bacteria) predominate; whereas bacteria that use inorganic compounds for cell growth (autotrophic bacteria) occur in proportion to concentrations of carbon and nitrogen. Both aerobic and anaerobic bacteria may exist in the activated sludge, but the preponderance of species is facultative, able to live in either the presence of or lack of dissolved oxygen. The main task of the bacteria is to degrade the dissolved organic matter and they contribute to the degradation of suspended organic matter through the production of extracellular enzymes (exoenzymes).

Fungi are also constituent of activated sludge. These multicultural organisms metabolise organic compounds and can successfully compete with bacteria under certain environmental conditions in a mixed culture. Rotifers, and protozoans are also residents of activated sludge. The latter microorganisms are represented largely by ciliated species, but flagellated protozoans and amoebae may also be present. Protozoans graze on bacteria, fungi, algae and suspended organic matter and fulfil an important role for the secondary settling of the wastewater, they also serve as indicators of the activated sludge condition, and ciliated species are instrumental in removing Escherichia coli from sewage. Additionally, viruses of human origin may be found in raw sewage influent, but a large percentage appears to be removed by the activated sludge process.

2.6. Extracellular polymeric substances (ESP)

Extracellular polymeric substances (EPS) or exopolymers are construction materials of microbial aggregates in activated sludge flocs or biofilms. EPS are different classes of macromolecules such as polysaccharides, proteins, nucleic acids, phospholipids and other polymeric compounds which fill and form the spaces between the microbial cells in the activated sludge flocs (Garnier et al., 2005), and they contain various functional groups including carboxyl, amino and phosphate groups (Tsuneda et al., 2003). The EPS keep the organisms together in a three-dimensional gel like hydrated matrix by week physicochemical interactions such as electrostatic, hydrophobic, Van der Waals and hydrogen interactions (Garnier et al., 2005). The exact function of the EPS is not completely explained and clarified because of its extremely heterogeneous nature (Tsuneda et al., 2003).

The physicochemical properties of EPS enable them to play an important role in sludge flocculation and settling properties (Garnier et al., 2005), and they play a significant role in formation and function of microbial aggregates, including adhesion phenomena, matrix structure formation and microbial physicochemical process (Tsuneda et al., 2003). It has been suggested that polysaccharides play a major role in flocculation due to their negatively charged groups that are bridged by divalent cations. In many studies, proteins are found to be the main component within the sludge with a 4-5-protein-to-polysaccharide ratio and it has been found that they play a role in floc formation. The role of protein for the floc formation is explained by hydrophobic interactions and polyvalent cation bridge, both enhancing the stability of biopolymers network. EPS play an important role in the structure and water retention of activated sludge and biofilms (Neyens et al., 2003).

2.7. Factors affecting deflocculation and suspended solids in effluents

2.7.1 Temperature

It has been found that sludge deflocculation increases and the flocculation physicochemical properties deteriorate under temperature shift from 30ºC – 45ºC (Morgan-Sagastume and Allen, 2005). They found that, up-shifts in temperature from 35ºC to 45ºC had three major effects; an increase in effluent soluble chemical oxygen demand (SCOD) and effluent suspended solids (ESS) concentrations and deterioration of the sludge settling characteristics such as poorer sludge compressibility and settleability, high sludge volume index (SVI) and settleability (low zone settling velocity) by promoting filament proliferation. They also found that, the temperature shifts and periodic temperature oscillation (31.5ºC - 40ºC) causes a more negatively charged sludge, a shift in filamentous organism population and a reduction in protozoan/metazoan concentration and diversity (Morgan-Sagastume and Allen, 2003). It has also been found that decreased microbial activity caused by a temperature reduction leads to increased deflocculation of activated sludge (Wilén *et al.*, 2000).

2.7.2. Ion effect

Previous studies show that the ion strength has a major effect on flocculation and deflocculation of activated sludge flocs. It has been found that the floc structure stability and sludge settleability increase by addition of divalent cations ($Ca^{2+}$and Mg^{2+}) to the sludge and it was explained that those cations play an important role in improving bioflocculation by cationic bridges. More compact flocs and stronger floc structure were obtained by magnesium enrichment (Morgan-Sagastume and Allen, 2005), and the addition of potassium (0.1 g/l)

created a noticeable improvement of sludge settleability and lead to the inhibition of microfloc formation (Muller, *et al.,* 2002). Settling and dewatering properties have been found to be improved through the addition of calcium and magnesium, and a considerable improvement in the floc density was observed through the addition of magnesium, whereas high concentrations of sodium ions and low concentration of divalent cations in the influent of wastewater treatment plant were observed in the sludge of poor settling and dewatering properties (Murthy *et al.*, 1999).

Iron has also been found to affect floc stability. Reduction of Fe (III) to Fe (II) can take place under anaerobic conditions (Wilén *et al.,* 2000). It has been found that Fe (III), which is produced as a result of oxidation of Fe (II), has a minor role in reflocculation and improvement of flocculation properties (Wilen *et al.*, 2004). However, removing Fe (III) from the sludge matrix by adding sulphide results in strong deflocculation. This phenomenon was explained due to either lack of aerobic microbial activity or due to change in the local physicochemical conditions mediated by anaerobic microbial activity (Wilen *et al.*, 2000). Furthermore, iron should be linked to the flocculation in a similar way as calcium and magnesium and therefore Fe(III) should form stronger bridges to the EPS than Fe(II).

2.7.3. Shear forces

Hydrodynamic shear exists in all biological wastewater treatment systems as a result of aeration and mechanical mixing. It plays a very important role in the formation of aerobic granules (Liu and Tay, 2002), where weak hydrodynamic shear did not lead to the formation of aerobic granules while higher shear lead to formation of more compact, rounder smaller and stronger aerobic granules (Garnier et al., 2005). A similar effect on aggregate characteristics is probably also seen for activated sludge flocs. The hydrodynamic shear is also important in microbial attachment and self-immobilization processes; mild shear provides a favourable condition for attachment of the microbial cells (Liu and Tay, 2002) as well as mild shear (400-600 rpm) results in larger floc size and lower sludge volume index (SVI)(Liu *et al.,* 2005). The increase in shear rate, leads to floc breakage as either erosion or fragmentation (Jarvis *et al.,* 2005). The sludge production rate decreases with the increasing shear rate while the exopolysaccharide production of the microbial cells increase with shear rate (Liu and Tay, 2002).

2.7.4. Anaerobic conditions

Previous studies have shown that the activated sludge deflocculates when it is exposed to anaerobic conditions (Wilen *et al.,* 2000). It was explained as growth of anaerobic or facultative anaerobic bacteria between the flocs or dying of aerobic organisms in the flocs. The inhibition of the eucariotic population or an inhibition of extracellular polymer production results in an increase in deflocculation and turbidity under anaerobic conditions. Besides, the hydrolysis of organics in the EPS matrix, which takes place under anaerobic conditions, may lead to degradation of the floc matrix. Biological reduction of Fe (III) by microorganisms under anaerobic conditions is another important cause for deflocculation (Nielsen *et al.,* 1996).

3.0. Chapter three: Experimental work (Materials and Methods)

3.1. Activated sludge samples

The activated sludge was taken from the Rya wastewater treatment plant (WWTP) in Göteborg, Sweden. The Rya WWTP, serving about 750 000 pe, is an activated sludge treatment plant designed for biological nitrogen removal utilizing pre-denitrification and post-nitrification in a trickling filter (Balmér et al., 1998). Phosphorus is removed by chemical precipitation by ferrous sulphate ($FeSO_4$). The plant is operated at a low solids retention time (SRT), 2-4 days. The flow to the plant varies considerably from 175,000 to 1,425,000 m^3/d with an average daily flow of about 350,000 m^3/d).

The sludge samples were brought immediately to the laboratory, located at the WWTP, after sampling and the experiments were carried out directly thereafter.

3.2. Experimental set up

The activated sludge was thickened twice to get higher turbidity in the supernatant during the shear test and to obtain a clear difference during the deflocculation of the activated sludge flocs. Three-litre reactors filled up with 2 litre of sludge were used in the experimental study, 132 mm in diameter with 4 baffles (14 mm wide) to maintain a homogenous shearing of the activated sludge (Figure E). A pitch bladed paddle (∅ 45 mm, projected area 15.27 cm^2) was placed 1.5 cm from the bottom of the reactor, and an electronic mixer was controlling its speed. The stirring speed was fixed at 700 rpm to create a G-value of 1700 s^{-1} during the whole experimental period. The reactors were sealed with covers to avoid oxygen coming in from the surface. Air was bubbled into the reactors to obtain aerobic conditions, while compressed nitrogen was bubbled to obtain anaerobic conditions. Each experiment, under either aerobic or an aerobic conditions, was carried out in duplicate. Aliquots were taken with a syringe from the reactors and centrifuged in a centrifuge tube at 2100 rpm for 2 minutes to leave only single bacteria and small floc fragments in the supernatant. The turbidity was measured as the absorbance of the supernatant at 650 nm with a spectrophotometer.

The experiments were performed in two parallel reactors; the first one was used as a test reactor, which was exposed to different low temperatures (5°C, 10°C and 15°C) and a moderate high temperature (30°C). The other reactor was operated as a reference reactor at ambient temperature (20°C). The target temperature of the activated sludge in the test reactor was obtained by placing the reactor in a temperature controlled water bath. A U-shaped steel pipe was inserted into the reactor and water from the water bath was pumped through to speed up the transmission of the heat or cold from the water bath to the activated sludge inside the reactor.

The reactors were run for 3 hours including two phases:
***Start up phase*:** during this phase, the sludge was stirred at low speed, 100 rpm, with bubbling of air under aerobic conditions and nitrogen gas under anaerobic conditions, for one hour to obtain the target temperature.

Testing phase: after obtaining the target temperature the stirring speed was increased to 700 rpm and 10 samples were taken during regular intervals for 2 hours; 10 minutes interval between the first seven samples and 20 minutes between each of the three last samples.

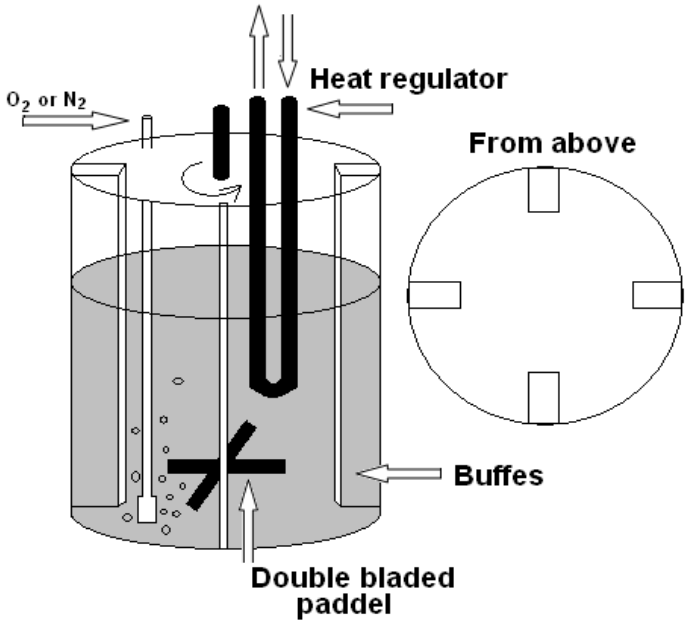


Figure E: Schematic view of the test activated sludge reactor (3 litre reactor filled up with 2 litres of sludge). The reference reactor is identical to the test reactor, but without heat regulator.

Turbidity measurements and filtration

The turbidity was measured on the spectrophotometer as the absorbance at 650 nm after 2 minutes of centrifugation at 2100 rpm. After removing the residual solids by centrifugation, half of the supernatant was filtered through 0.45-µm filters to analyse the dissolved biopolymers (protein, humic substances and carbohydrate), whereas the rest was left without filtration to analyse the total biopolymers. Both filtrated and unfiltrated samples were frozen immediately to avoid the biological degradation of biopolymers.

Dissolved oxygen, conductivity and pH measurements

Dissolved oxygen concentration was measured to make sure that the oxygen was present during the aerobic period and absent during the anaerobic period at the end of each experiment. Conductivity and pH were measured at the end of each experiment.

3.3. Analytical methods for biopolymers measurements (composition of turbidity and sludge)

The frozen supernatant was defrosted and analysed for biopolymers (carbohydrates, proteins and humic substances) as dissolved and total fractions. The total sludge were defrosted, homogenized, by using the ultrasonic devise (2 minutes for each sample with range between 60-100W), and measured for total carbohydrates, protein and humic substances (total sludge was diluted 20 times for measuring protein and humus and 10 times for measuring carbohydrates). Carbohydrates were determined by using the anthrone method, with glucose standard as described by (Frølund *et al.*, 1996). Protein and humic substances were determined by a modified Lowry method (Frølund *et al.*, 1996). All samples were measured in duplicate.

3.4. Preparation of samples for Ions measurement by using ICP-MS

Sludge samples
The cations in the total sludge and filtrated supernatant were measured as follows: The sludge samples were homogenized and diluted 25 times. Concentrated HNO_3 (0.8 ml) and milliQ water (7.2 ml) were added to 2 ml homogenised sludge in a glass test tub. The sludge was then digested at 120°C for 2 hours by using HACH method. The samples were filtered through 0.45 µm filters. The samples were then diluted 100 times (9.9 ml milliQ water was added to 100µl from the filtered samples) and 100µL of 0.1mg/l internal standard (Rh) and 100µL of concentrated nitric acid (69%) were added. The ions measured by using ICP-MS. Total iron in the total sludge was analysed by ICP_MS and by Ferrozine/Hepes method.

Filtrated supernatant samples
The filtrated supernatant samples were prepared without digestion as follows: 100µl supernatant sample, 100µl internal standard (0.1mg/L Rh) and 100µl concentrated nitric acid were mixed in a test tube, then 9.7ml milliQ water was added.

3.5. Test and control experiments

Shear Test
Deflocculation at different shear intensities (300rpm, 500 rpm, 700 rpm and 900 rpm) and two different temperatures (20°C and 10°C) were investigated under aerobic conditions for 1 hour before the experiments were curried out, to select the suitable stirring level which gives a stable development in turbidity.

Control tests
Three control experiments were performed in order to help in the interpretation of some results. The control experiments were:

1) Sodium azide addition (NaN_3)
Sodium azide, an inhibitor which stops the microbial activity, was added as described below to assess the relative effect on the physical sludge properties, mainly viscosity, of the drop in temperature from 20 to 5°C. The reference reactor and the test reactors were run at room temperature 20°C and 5°C, respectively. A mixture of 2 g NaN_3 dissolved in 25 ml milliQ water was added to each reactor. Both reactors were aerated for 1 hour at low stirring speed of 100 rpm, and then the shear speed increased to 700 rpm for 2 hours and samples were taken during regular intervals and centrifuged as described earlier.

2) Deflocculation under aerobic and anaerobic conditions at the same temperature (5°C)
Two reactors were run at 5°C and 700 rpm; the first was run under aerobic conditions and the other under anaerobic conditions, for 2 hours after the ending of the start up phase. This experiment was carried out in order to check out the effect of aerobic and anaerobic conditions on the degree of deflocculation when the biological activity was negligible.

3) pH control test
Four different shear experiments were carried out at different pH (7, 7.5, 8 and 8.5) to assess its impact on deflocculation of the activated sludge. It was observed that the activated sludge deflocculated more under anaerobic conditions than under aerobic conditions, at the same time as it was observed that the pH increased more at the end of the anaerobic experiment

than under aerobic conditions. Activated sludge flocs reactors were run at room temperature (20°C) and stirring speed of 700 rpm, for 2 hours after the start up phase. The pH was adjusted during the test phase (2 hours) by using 0.5M HCl and 0.5M NaOH.

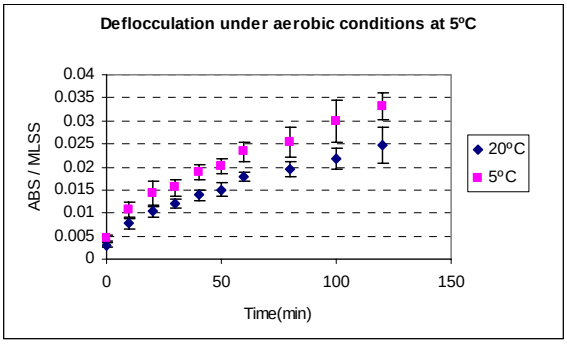
4.0. Chapter four: Results and Discussion

The effects of either decreasing the temperature (5ºC, 10ºC and 15ºC) or increasing it to a moderate high temperature (30ºC), on the degree of deflocculation of the activated sludge were investigated in short-term experiments (2 hours) under both aerobic and anaerobic conditions. The results from the different tests are summarized in the following paragraphs.

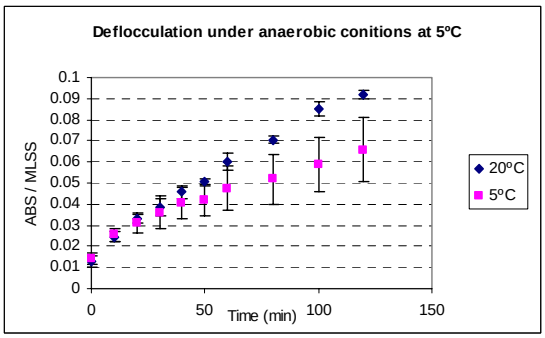
4.1. Deflocculation under aerobic and anaerobic conditions at different temperature

When activated sludge acclimatized to a temperature of about 20ºC was exposed to different low temperatures (5ºC, 10ºC and 15ºC) under aerobic conditions, the flocs deflocculated significantly more compared to at 20ºC (Figure 1a, c, e). When the same experiments were carried out under anaerobic conditions, it was found that the activated sludge flocs deflocculated more at 20ºC than at 5ºC, 10ºC and 15ºC (Figure1b, d, f). The difference in deflocculation development between all tested low temperatures (5ºC, 10ºC and 15ºC) and the reference reactor operated at 20ºC was much higher under anaerobic conditions than under aerobic conditions. This indicates that anaerobic conditions have a relatively larger effect on the floc stability than exposure to low temperatures. Since the experiments were carried out on different days, the deflocculation curves cannot be directly compared since the sludge floc structure could have been different. However, the degree of deflocculation was always higher for the anaerobic conditions.

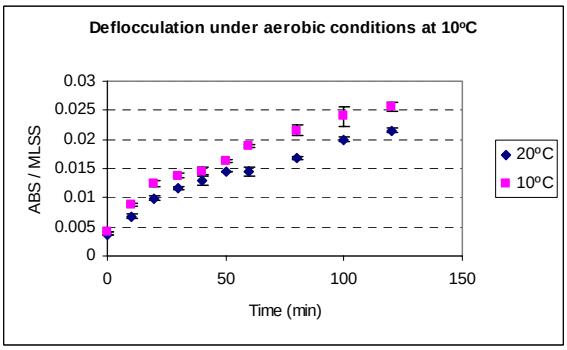
For anaerobic conditions it was found that the difference in deflocculation development started to occur after about one hour of shear whereas for the aerobic conditions a difference was observed right from the beginning of the shearing, and this difference became higher after two hours under anaerobic conditions. This result indicates that there is some lag phase before the anaerobic microbial activity is started. The results show that even a minor decrease in temperature leads to an increased deflocculation. The standard deviation was very small for most duplicated experiments carried out under both aerobic and anaerobic conditions. A slight increase in standard deviation was observed when the activated sludge flocs were exposed to 5°C under both aerobic and anaerobic conditions.


Deflocculation under aerobic conditions at 5ºC
ABS / MLSS
Time(min)
20ºC
5ºC

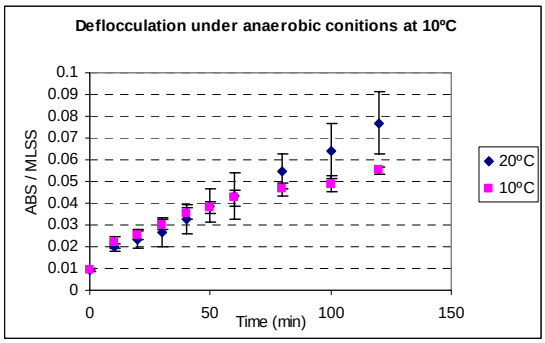
a)


Deflocculation under anaerobic conitions at 5ºC
ABS / MLSS
Time (min)
20ºC
5ºC

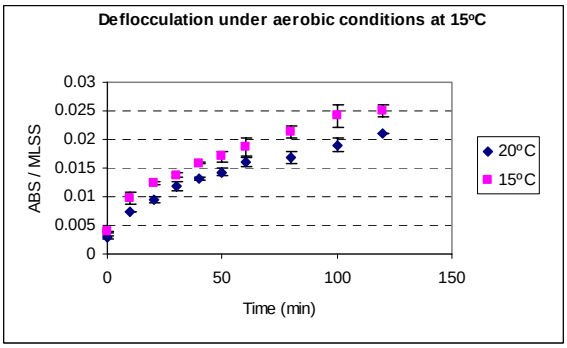
b)


Deflocculation under aerobic conditions at 10ºC
ABS / MLSS
Time (min)
20ºC
10ºC

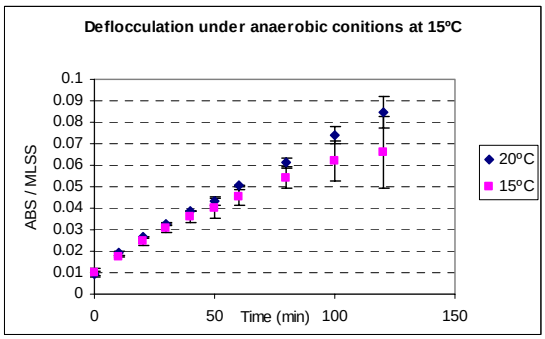
c)


Deflocculation under anaerobic conitions at 10ºC
ABS / MLSS
Time (min)
20ºC
10ºC

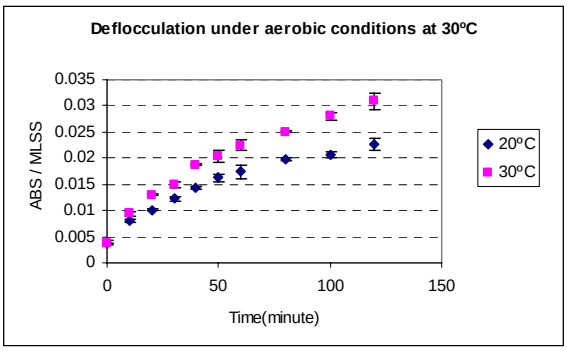
d)


Deflocculation under aerobic conditions at 15ºC
ABS / MLSS
Time (min)
20ºC
15ºC

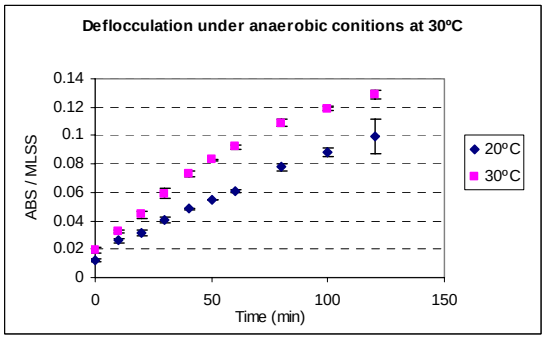
e)


Deflocculation under anaerobic conitions at 15ºC
ABS / MLSS
Time (min)
20ºC
15ºC

f)


Deflocculation under aerobic conditions at 30ºC
ABS / MLSS
Time(minute)
20ºC
30ºC

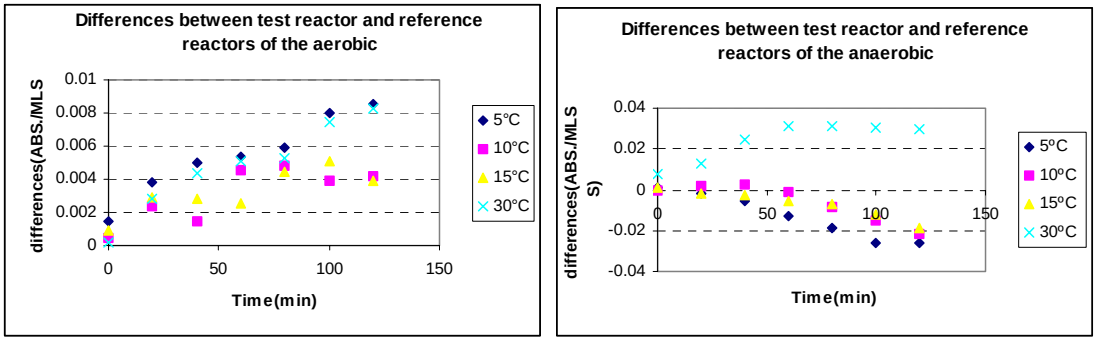
g)


Deflocculation under anaerobic conitions at 30ºC
ABS / MLSS
Time (min)
20ºC
30ºC

h)

Figure 1 Deflocculation under aerobic and anaerobic conditions under different temperatures (a) aerobic conditions and 5 °C; (b) anaerobic conditions and 5°C; (c) aerobic conditions and 10°C; (d) anaerobic conditions and 10°C; (e) aerobic conditions and 15°C; (f) anaerobic conditions and 15°C; (g) aerobic conditions and 30°C; and (h) anaerobic conditions and 30°C.

The results show that the differences in the absorbance/mixed liquor suspended solid (absorbance at 750nm/MLSS) between the test reactor and reference reactor under aerobic conditions were rather similar for activated sludge exposed to 5°C and to 30°C (Figure 2a, 1g), so it could be concluded that low temperature (5°C) and a moderate high temperature (30°C) have similar effects on deflocculation under aerobic conditions. However, the differences were significantly higher at 30°C than at 5°C under anaerobic conditions (Figure 2b). This indicates that the sludge flocs deflocculate slightly more at 5°C than at a moderate high temperature (30°C) and ambient temperature (20°C) under aerobic conditions, while the sludge flocs deflocculated substantially more at high temperature (30°C) than at 5°C and other low temperature (10°C and 15°C) under anaerobic conditions. The differences between the reference and test reactors showed that the sludge flocs deflocculated more at ambient temperature (20°C) than at all low temperatures under anaerobic conditions. The difference in absorbance values between the test and reference reactor increased with decreasing temperature under aerobic conditions. On the contrary, for the experiments carried out at anaerobic conditions, the difference in turbidity was negative which means that the flocs deflocculated less at decreased temperatures (Figure 2b). At increased temperature (30°C) the difference was positive showing an increased deflocculation at higher temperatures.



a) b)

Figure 2: Differences in absorbance at 650 nm/mixed liquor suspended solid (MLSS) between the test reactor and reference reactor under (a) aerobic condition; and (b) anaerobic conditions.

4.2. Composition of the eroded fractions

4.2.1. Total eroded biopolymers

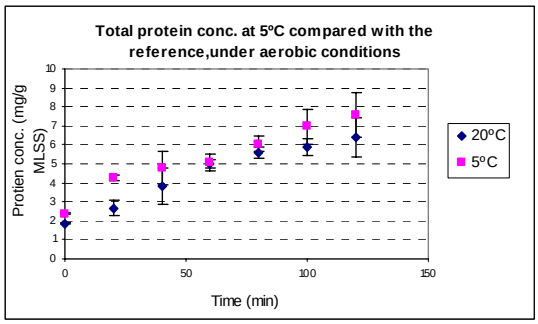
The analysis of the supernatant during the deflocculation of the activated sludge flocs at the different low temperatures (5ºC, 10ºC and 15ºC) showed that the concentration of total biopolymers (i.e. the total composition of the supernatant which includes the dissolved fraction) has the same trend as the turbidity under both aerobic conditions and anaerobic conditions. The concentration of the total biopolymers, protein, carbohydrates and humic substances, were higher at 5ºC, 10ºC and 15ºC than at 20ºC under aerobic conditions, while their concentration were higher at 20ºC than at 5ºC, 10ºC and 15ºC under anaerobic

conditions. Higher concentration values were found for all total fractions under anaerobic conditions.

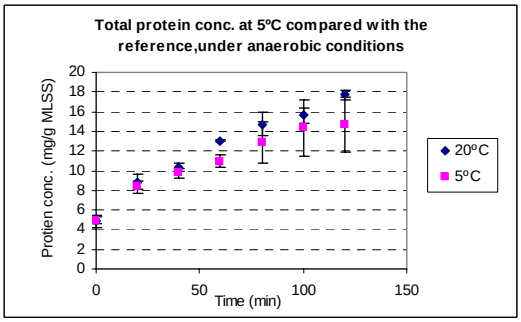
It was observed in the sludge that was exposed to different low temperatures (5ºC, 10ºC and 15ºC) under aerobic conditions, that the difference in concentration of humic substances (Figure 4a, c, e) and carbohydrates between the test reactor and the reference reactor (Figure 5a, c, e) was relatively small. For the anaerobic conditions, there was a larger difference in the total concentration between test reactor and reference reactor for all biopolymers. However, there was a larger difference in concentration of protein and humic substances at the different low temperatures (5ºC, 10ºC and 15ºC).

It was found that the concentration of particulate biopolymers present in the supernatant during the deflocculation at a high temperature (30ºC) corresponded well to the turbidity under both aerobic and anaerobic conditions. The concentrations for all particulate biopolymers were higher at 30ºC under anaerobic conditions than under aerobic conditions. Furthermore, the concentrations of biopolymers were higher under both aerobic and anaerobic conditions at high temperature (30ºC) than at the three different low temperatures. At high temperature the biopolymer concentration showed the same trend under both aerobic and anaerobic conditions, i.e. the sludge deflocculated more at 30ºC than at 20ºC (figures 3g,h, 4g,h, 5g,h). A slight difference in the development of the concentration of humic substances was observed under aerobic conditions at 30ºC (figure 4g), whereas a significantly higher difference was found for the protein and carbohydrates (Figures 3g and 5g). The relative concentration of the total fractions was higher under anaerobic conditions at 30°C than at 5°C, 10°C and 15°C.

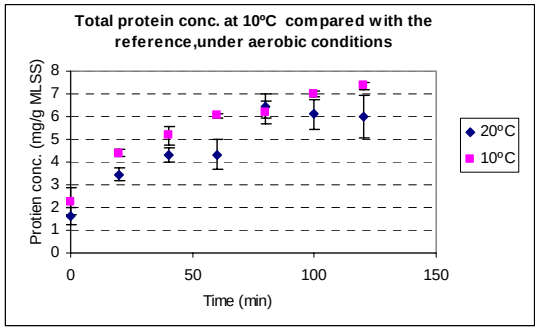
The standard deviation of the concentrations of total biopolymers of the two duplicated experiments was very small under both aerobic and anaerobic conditions at 10, 15 and 20°C and at 30°C, whereas it was a bit larger at 5°C under both aerobic and anaerobic conditions. The highest differences in concentrations were found for 30°C for both aerobic and anaerobic conditions.



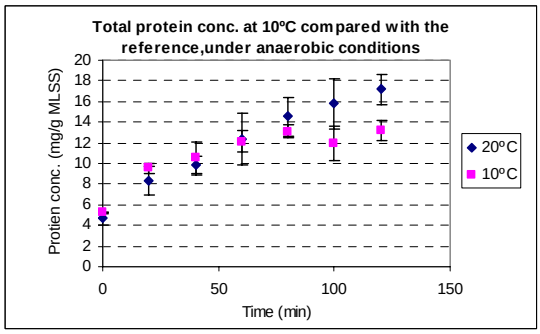
a)



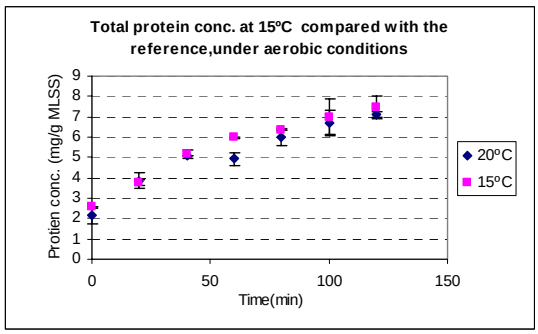
b)



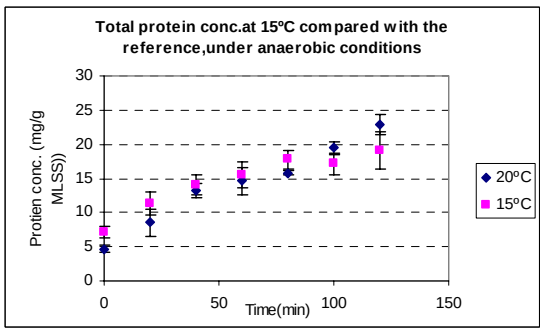
c)



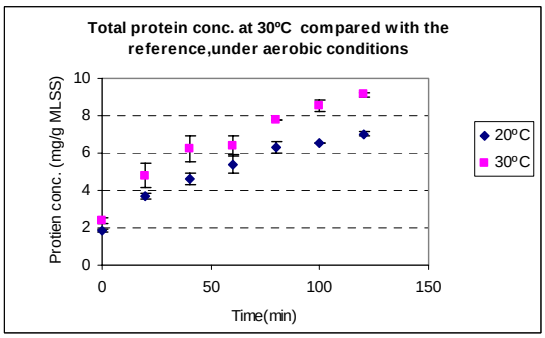
d)



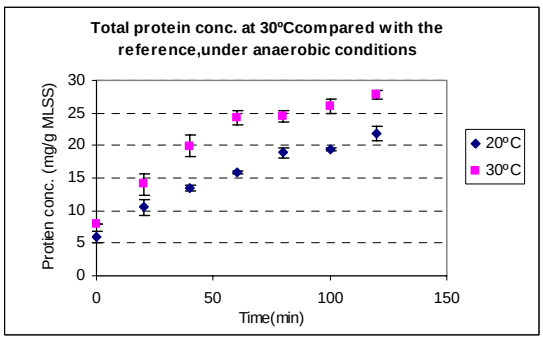
e)



f)



g)



h)

Figure 3 Concentration of total protein in the organic fraction of the supernatant (a) aerobic conditions and 5 °C; (b) anaerobic conditions and 5°C; (c) aerobic conditions and 10°C; (d) anaerobic conditions and 10°C; (e) aerobic conditions and 15°C; (f) anaerobic conditions and 15°C; (g) aerobic conditions and 30°C; and (h) anaerobic conditions and 30°C.

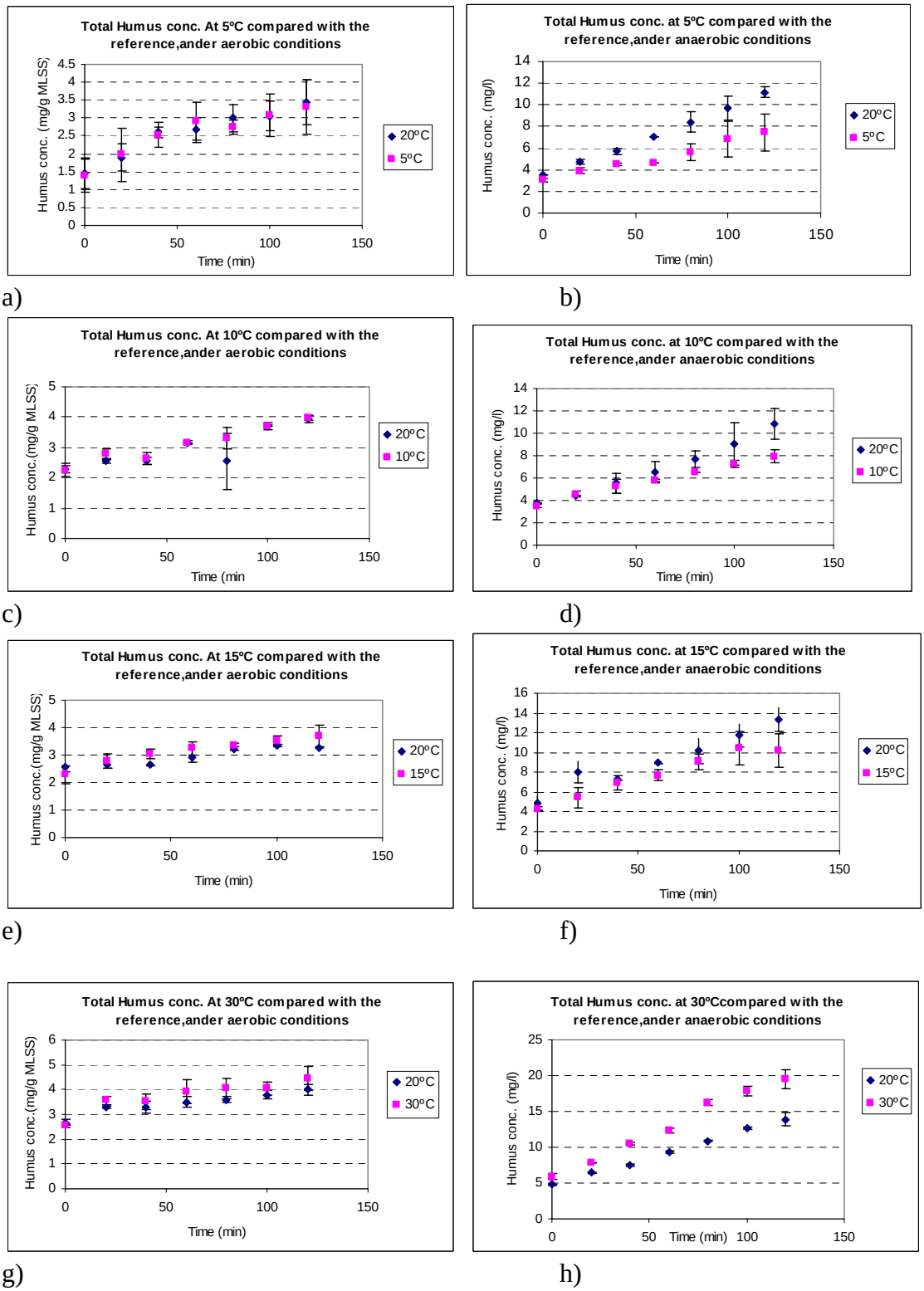
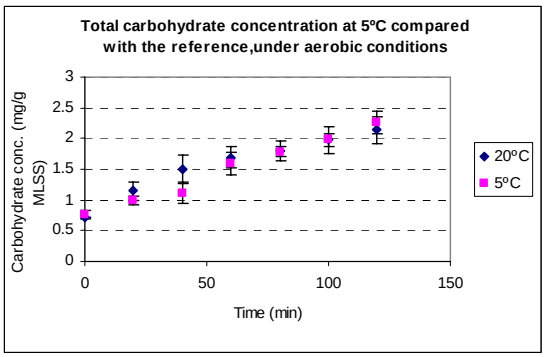
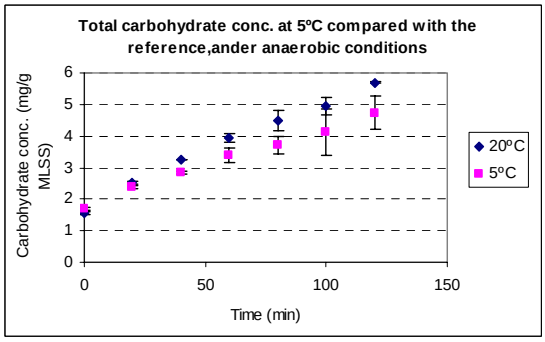


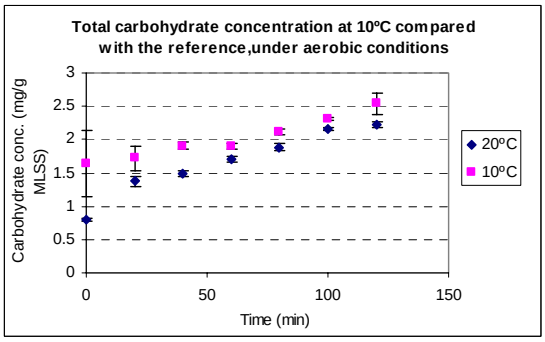
Figure 4 Concentration of total humic substances in the organic fraction of the supernatant (a) aerobic conditions and 5 °C; (b) anaerobic conditions and 5°C; (c) aerobic conditions and 10°C; (d) anaerobic conditions and 10°C; (e) aerobic conditions and 15°C; (f) anaerobic conditions and 15°C; (g) aerobic conditions and 30°C; and (h) anaerobic conditions and 30°C.



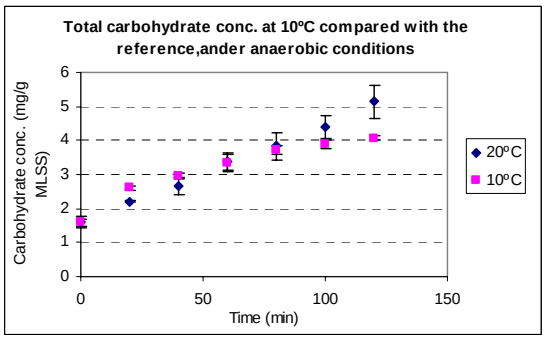
a)



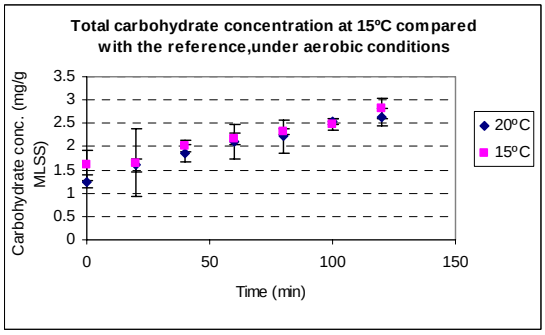
b)



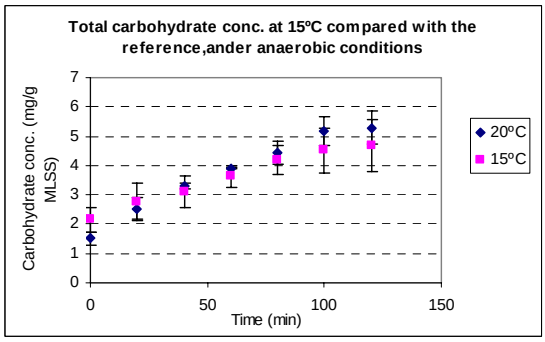
c)



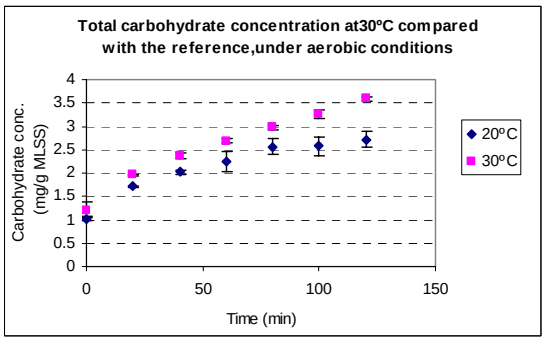
d)



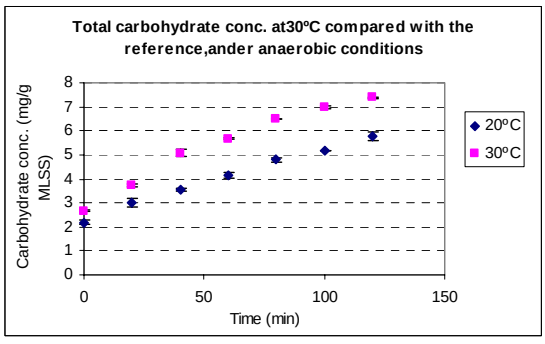
e)



f)



g)



h)

Figure 5 concentration of total carbohydrates in the organic fraction of the supernatant (a) aerobic conditions and 5 °C; (b) anaerobic conditions and 5°C; (c) aerobic conditions and 10°C; (d) anaerobic conditions and 10°C; (e) aerobic conditions and 15°C; (f) anaerobic conditions and 15°C; (g) aerobic conditions and 30°C; and (h) anaerobic conditions and 30°C.

The calculations of the ratio between the different fractions protein:humic substances: carbohydrate in the eroded total biopolymers in the supernatant are summarized in Table 1. It can be seen that there was relatively more total protein in the supernatant under both aerobic and anaerobic conditions at all the examined low and high temperatures. The was relatively more proteins and humic substances in the supernatant at low temperatures (5ºC and 10ºC) than at high temperature (30ºC), whereas the opposite were observed under anaerobic conditions. The ratios between the protein, carbohydrates and humic substances were roughly the same under aerobic and anaerobic conditions at 5ºC. The particles deflocculated under anaerobic conditions at 10ºC, 15ºC and 30ºC contain relatively more protein and humic substances than particles deflocculated under aerobic conditions at the same temperatures. More protein and humic substances were released under aerobic conditions at 5ºC than at 30ºC, while the opposite was observed under anaerobic conditions.

When looking at the protein:humic substances:carbohydrate ratio for the total sludge, it contained relatively more protein and less humic substances. The ratio of the total humic substances released into the supernatant under aerobic conditions was close to ratios of the humic substances in the total sludge at all temperatures. The ratios of the total protein released into the supernatant under both aerobic and anaerobic conditions were lower than its ratios in the total sludge.

Table 1 Ratio between the total eroded biopolymers in the supernatant after 2 hours under both aerobic and anaerobic conditions at different temperatures.

	Ratio under aerobic conditions			Ratio under anaerobic conditions		
Temperature	Total Protein	Total Humus	Total Carbohydrate	Total Protein	Total Humus	Total Carbohydrate
5ºC	3.3481	1.4634	1	3.1019	1.5725	1
10ºC	2.9013	1.5676	1	3.2324	1.9446	1
15ºC	2.6438	1.3192	1	4.0612	2.1605	1
20°C	2.7457	1.5209	1	3.6409	2.2484	1
30ºC	2.5451	1.2426	1	3.7634	2.6455	1

Table 2 Ratio of the total biopolymers in the total sludge

	Ratio under aerobic conditions			Ratio under anaerobic conditions		
Temperature	Total Protein	Total Humus	Total Carbohydrate	Total Protein	Total Humus	Total Carbohydrate
5ºC	4.1105	1.4684	1	4.5633	1.4509	1
10ºC	4.1921	1.4563	1	4.3204	1.3155	1
15ºC	5.1029	1.1459	1	3.9777	1.3615	1
30ºC	4.8671	1.6487	1	5.8675	1.2572	1

The concentration of the compounds protein, humic substances and carbohydrate were well correlated to the turbidity that is seen as high values of the regression coefficient r^2 (Figure 6). When looking at the correlations for the different compounds it can be seen that the correlation for the lowest temperature (5°C) deviated from the other temperatures, especially for experiments carried out at aerobic conditions; the turbidities were relatively higher for the different concentrations compared to at higher temperatures. For the anaerobic conditions, similar but smaller differences were observed for protein and humic substances. These results indicate that the different compounds are released differently at different temperatures.

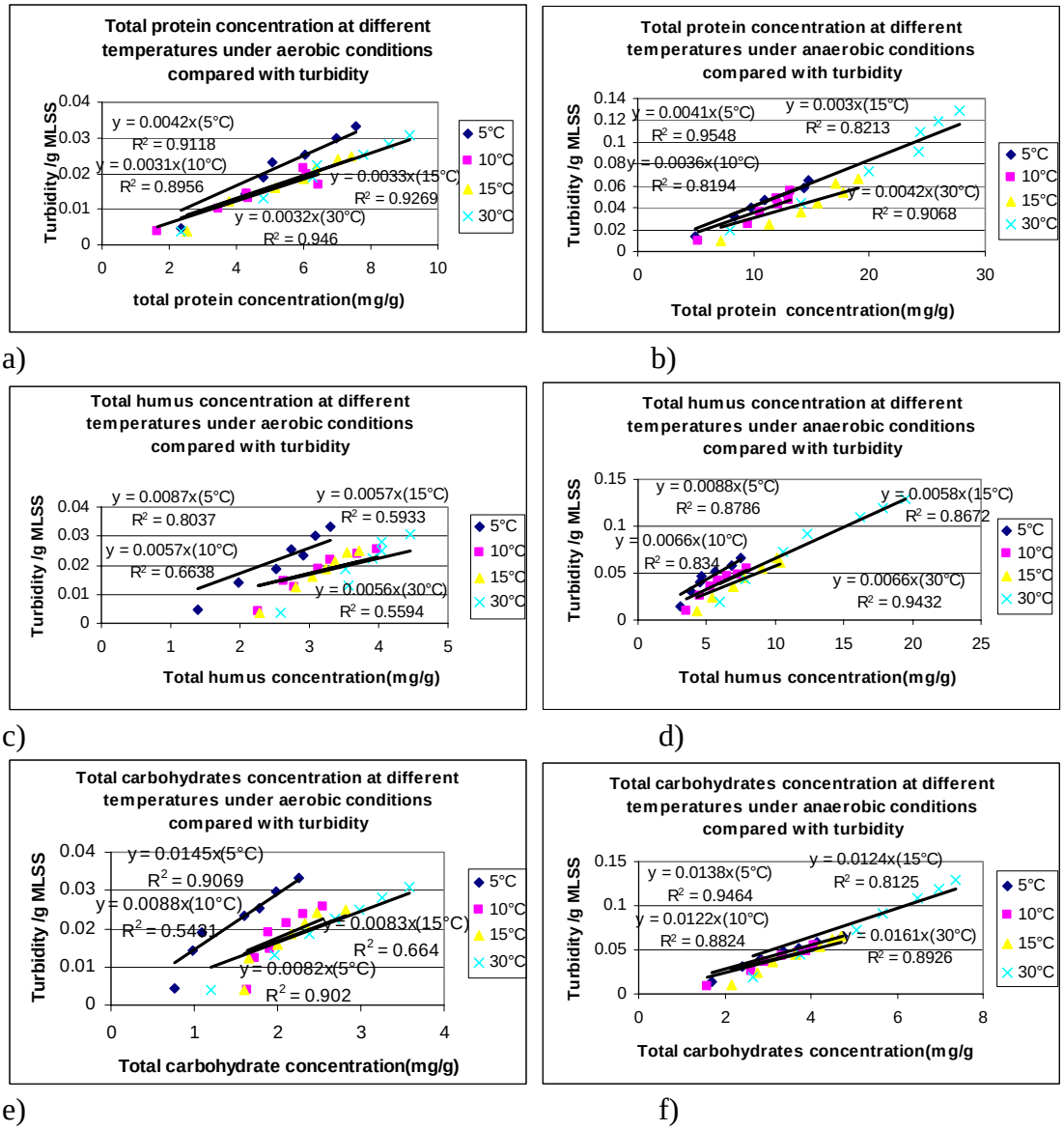


Figure 6 Correlation between total biopolymer fraction and turbidity under aerobic and anaerobic conditions (a) total protein under aerobic conditions; (b) total protein under anaerobic conditions; (c) total humic substances under aerobic conditions; (d) total humic substances under anaerobic conditions; (e) total carbohydrate under aerobic conditions; (f) total carbohydrate under anaerobic conditions.

4.2.2. Dissolved eroded biopolymers

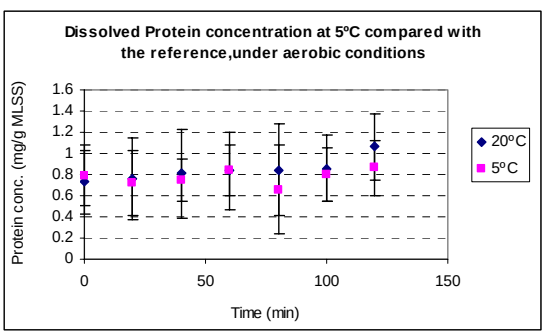
It was observed that the dissolved biopolymers (protein, humic substances and carbohydrates) were released more at 20°C than at 5°C, 10°C and 15°C under aerobic conditions (figure 7a,c, e, 8a,c, e, 9a,c, e). This could be interpreted as the dissolved fractions were more firmly bound to the flocs than the particulate fractions at lower temperatures. The concentrations of dissolved polymers were also higher at 30°C than at 20°C. The reason to why the concentrations of biopolymers were higher in the reference reactor operated at 20°C could be that the polymers dissolve better at higher temperatures due to a lower viscosity. The difference in concentration development was lesser for dissolved protein than for dissolved humic substances and carbohydrates at 5ºC, 10°C and 15°C under aerobic conditions (figure

7a, 8a and 9a). The dissolved biopolymers followed the turbidity curve for anaerobic conditions and they were always higher at 20°C than at 5°C, 10°C and 15°C (figures 10b,d,f). No such correlation was observed for aerobic conditions (10a,c,e). The concentration also increased relatively more under anaerobic conditions than under aerobic conditions.

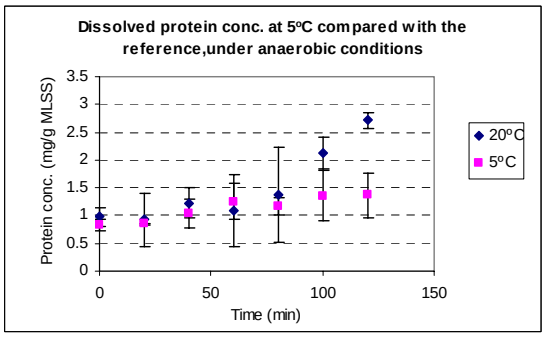
The concentration of dissolved biopolymers did not change much under aerobic conditions at low temperatures; they only increased slightly with shearing time, and it was observed that the concentrations were much higher under anaerobic conditions than under aerobic conditions from starting point until terminating the experiments. This indicates that the anaerobic processes occurring gives rise to a weaker floc structure and hence a higher release of polymers to the bulk phase.

The results show that the concentration of dissolved biopolymers has the same trend as the turbidity under both aerobic and anaerobic conditions at high temperature (30ºC). The concentrations were substantially higher under anaerobic conditions than under aerobic conditions. Furthermore, the concentration of the dissolved fractions were higher at 30°C than at low temperature (5°C, 10°C and 15°C) and ambient temperature (20ºC). The difference in the development of dissolved protein, humic substances and carbohydrate concentration between test and reference reactors was higher under anaerobic conditions than under aerobic conditions. The concentration of dissolved protein and humic substances remained approximately constant during the whole experimental period under aerobic conditions at high temperature (30ºC) in a similar way as at low temperature, while there was a noticeable increase in the concentration of dissolved carbohydrate (figure 9g).

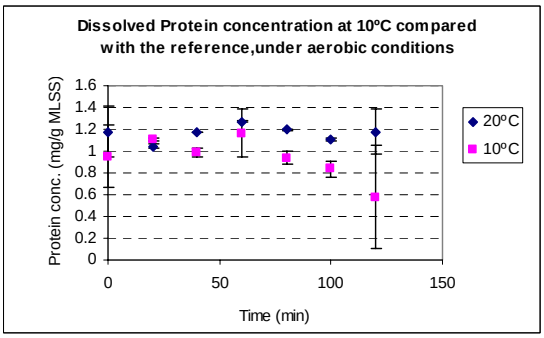
The standard deviation of the concentrations of dissolved biopolymers of the two duplicated experiments was very small under both aerobic and anaerobic conditions. It was, however, obviously larger at 5ºC under both aerobic and anaerobic conditions.



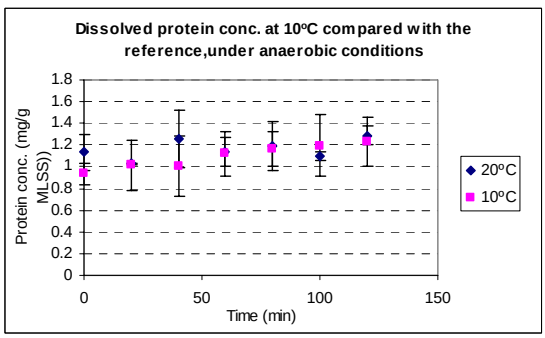
a)



b)



c)



d)

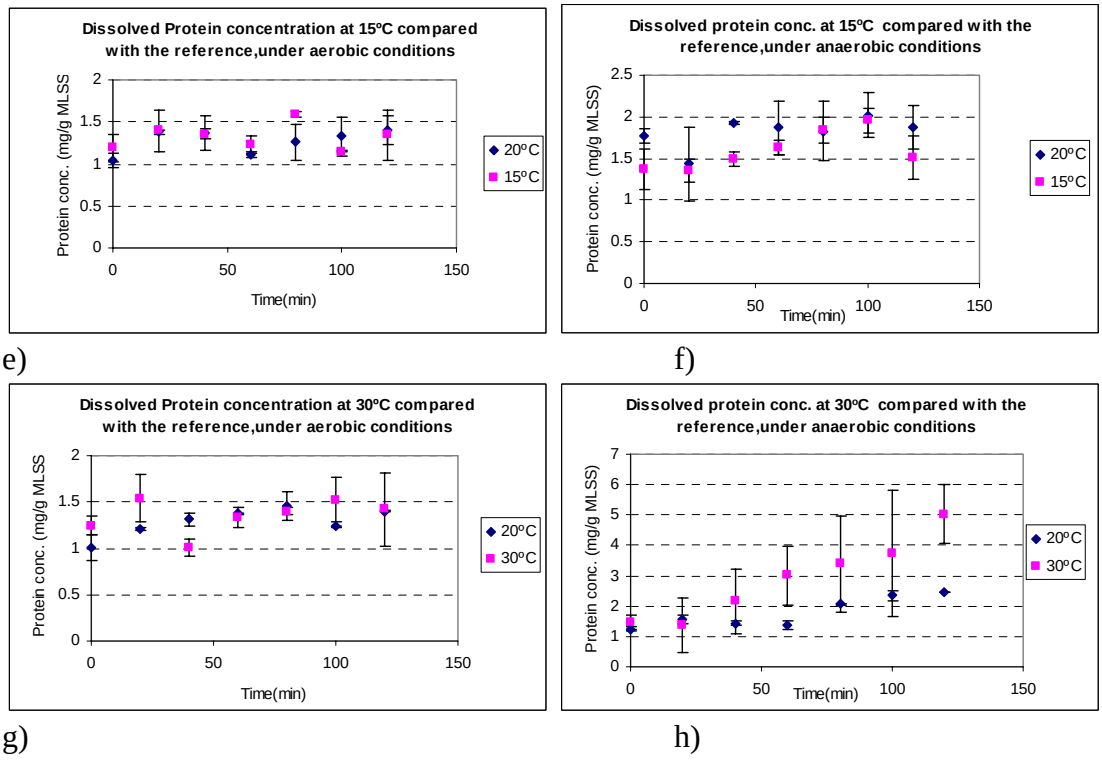
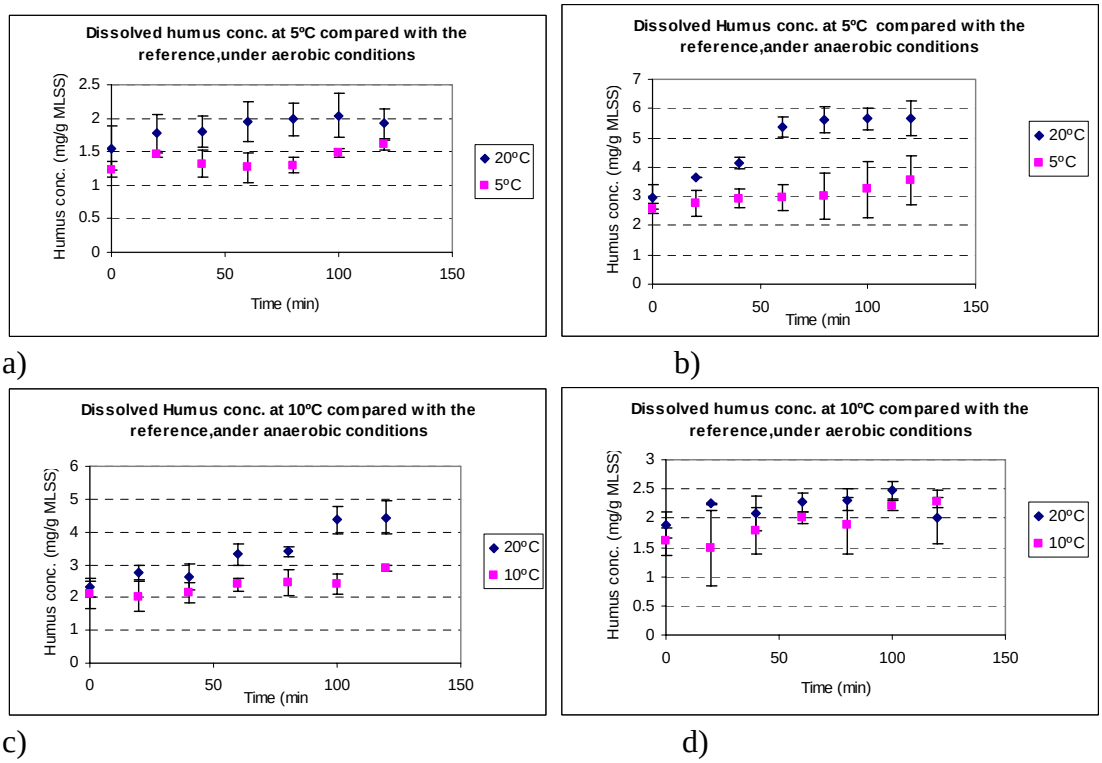
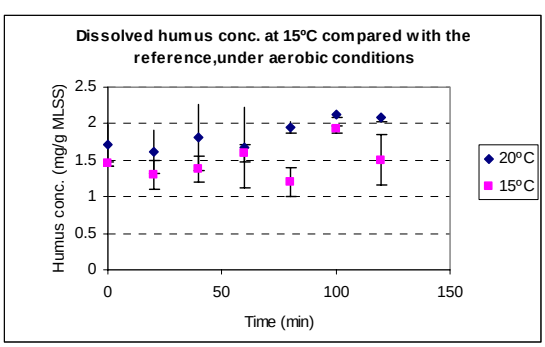
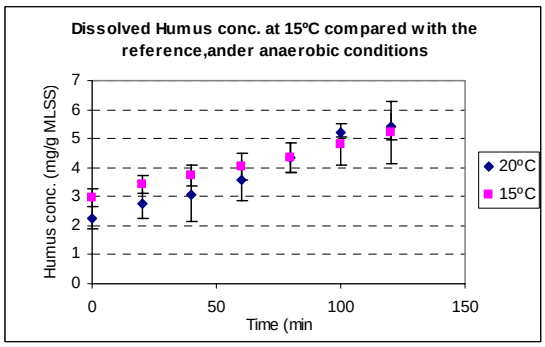


Figure 7 Concentration of dissolved protein in the organic fraction of the supernatant (a) aerobic conditions and 5 °C; (b) anaerobic conditions and 5°C; (c) aerobic conditions and 10°C; (d) anaerobic conditions and 10°C; (e) aerobic conditions and 15°C; (f) anaerobic conditions and 15°C; (g) aerobic conditions and 30°C; and (h) anaerobic conditions and 30°C.

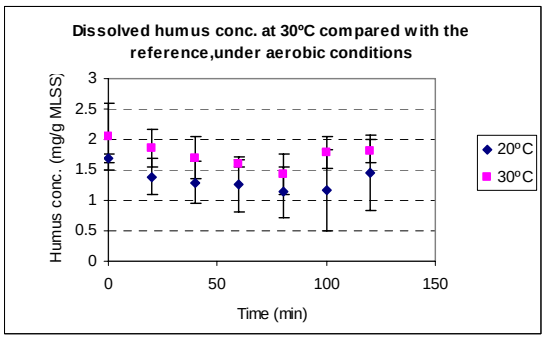




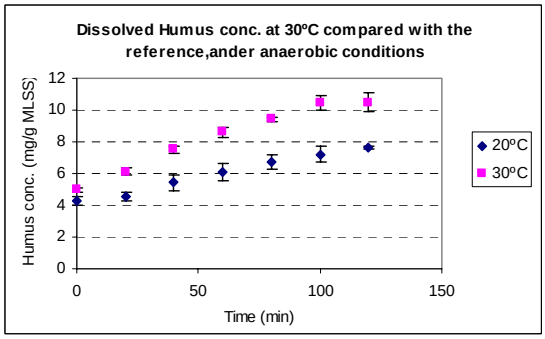
e)



f)

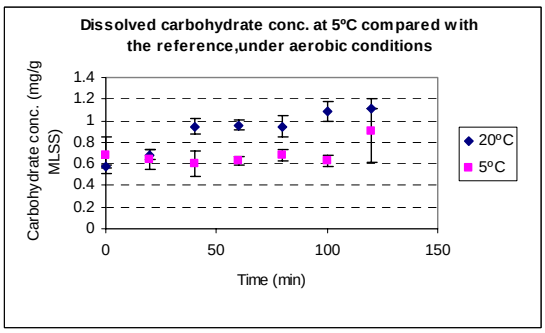


g)

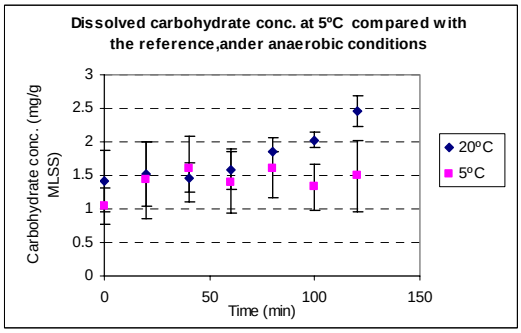


h)

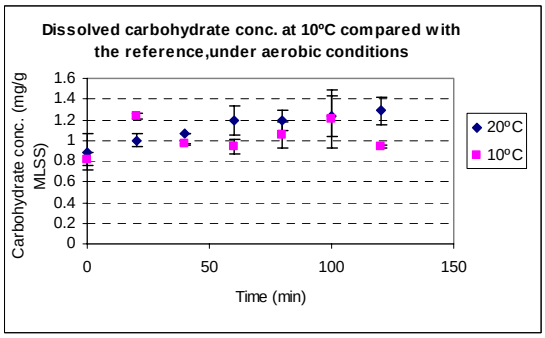
Figure 8: Concentration of dissolved humic substances in the organic fraction of the supernatant (a) aerobic conditions and 5 °C; (b) anaerobic conditions and 5°C; (c) aerobic conditions and 10°C; (d) anaerobic conditions and 10°C; (e) aerobic conditions and 15°C; (f) anaerobic conditions and 15°C; (g) aerobic conditions and 30°C; and (h) anaerobic conditions and 30°C.



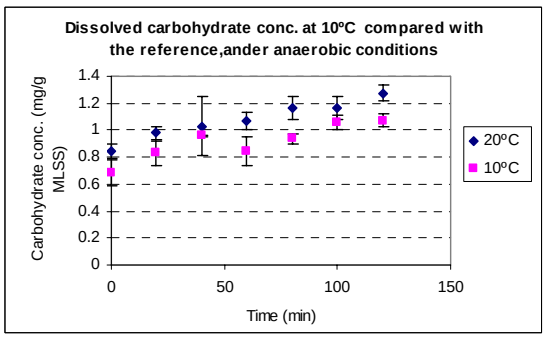
a)



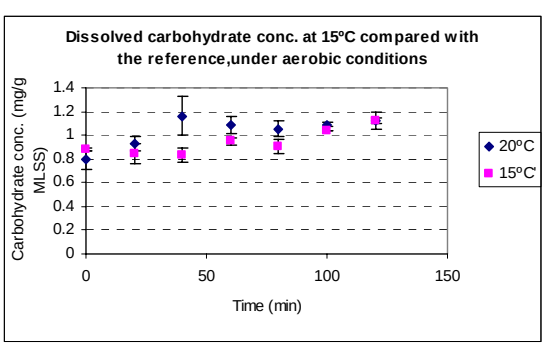
b)



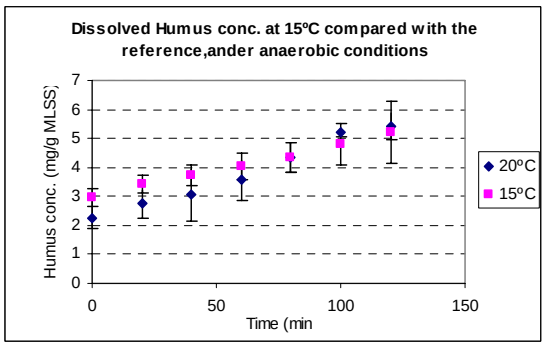
c)



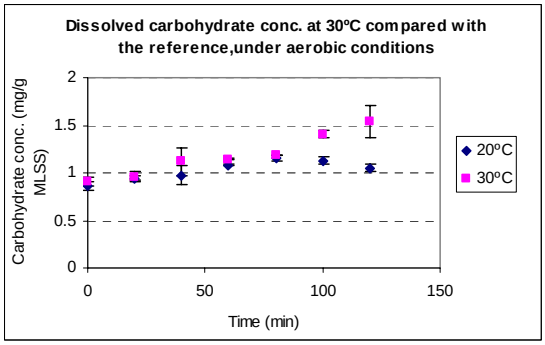
d)



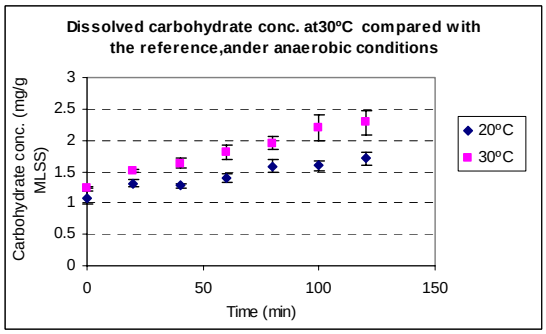
e)



f)



g)



h)

Figure 9 Concentration of dissolved carbohydrate in the organic fraction of the supernatant (a) aerobic conditions and 5 °C; (b) anaerobic conditions and 5°C; (c) aerobic conditions and 10°C; (d) anaerobic conditions and 10°C; (e) aerobic conditions and 15°C; (f) anaerobic conditions and 15°C; (g) aerobic conditions and 30°C; and (h) anaerobic conditions and 30°C.

The calculations of the ratio between the dissolved biopolymers protein:humic substances:carbohydrate shows that the dissolved humic substances were released more than the dissolved protein under both aerobic and anaerobic conditions at all low and high temperatures (Table 3), in spite of the higher ratio of protein in the total sludge (Table 2). The ratio of protein was a little bit lower than 1 at 5ºC, 10ºC and 30ºC under aerobic conditions and at 5ºC under anaerobic conditions. Slight differences in the ratio of the dissolved biopolymers were observed at 30ºC, 20ºC (ambient), and 15ºC under aerobic conditions, whereas significant differences were observed under anaerobic conditions especially at the high temperature. The ratio between the protein, carbohydrates and humic substances were more or less the same under aerobic conditions at 30ºC, while the ratio of dissolved humic substances were twice the concentration of protein, and 4.5 times higher than carbohydrate (Table 3). The ratios of dissolved protein eroded into the supernatant under both aerobic and anaerobic conditions were much less than the ratios of the total biopolymers in the sludge.

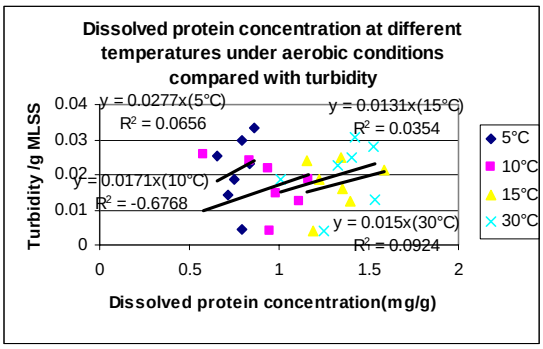
Table 3: Ratio between the dissolved biopolymers in the supernatant after 2 hours under both aerobic and anaerobic conditions at different temperatures.

	Ratio under aerobic conditions			Ratio under anaerobic conditions		
Temperature	Dissolved Protein	Dissolved Humus	Dissolved Carbohydrate	Dissolved Protein	Dissolved Humus	Dissolved Carbohydrate
5ºC	0.9495	1.7683	1	0.9126	2.3849	1
10ºC	0.6106	2.4146	1	1.1477	2.6927	1
15ºC	1.1964	1.3376	1	1.0491	3.6289	1
20ºC	0.9557	1.7309	1	1.1138	1.7189	1
30ºC	0.9224	0.9429	1	2.1986	4.5834	1

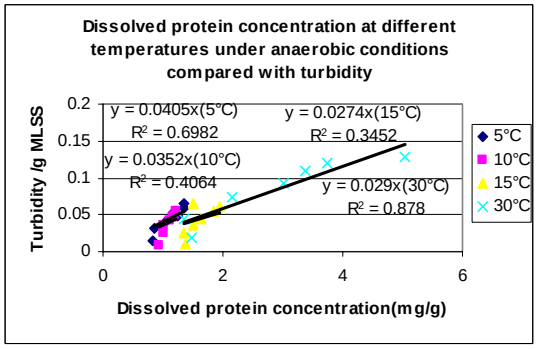
Table 4: Ratio of the total biopolymers in the total sludge.

	Ratio under aerobic conditions			Ratio under anaerobic conditions		
Temperature	Total Protein	Total Humus	Total Carbohydrate	Total Protein	Total Humus	Total Carbohydrate
5ºC	4.1105	1.4684	1	4.5633	1.4509	1
10ºC	4.1921	1.4563	1	4.3204	1.3155	1
15ºC	5.1029	1.1459	1	3.9777	1.3615	1
30ºC	4.8671	1.6487	1	5.8675	1.2572	1

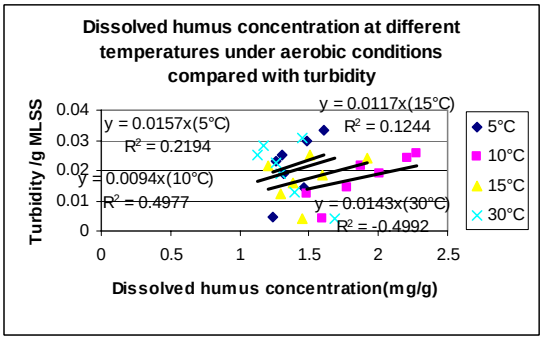
The concentrations of the dissolved biopolymers were less well correlated to the turbidity when the activated sludge deflocculated under aerobic conditions, which is seen as low values of the regression coefficient r^2 (Figure10). The correlations were generally much better during anaerobic (Figure 10a,d,f) compared to under aerobic conditions (Figure 10a,c, e) indicating a higher degree of release of dissolved fractions under anaerobic conditions. The results indicate that the different dissolved compounds are released differently at different temperatures under both aerobic and anaerobic conditions.



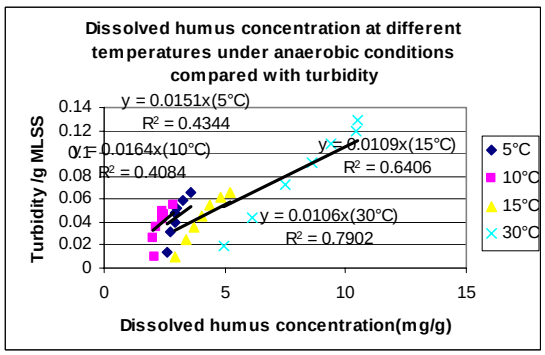
a)



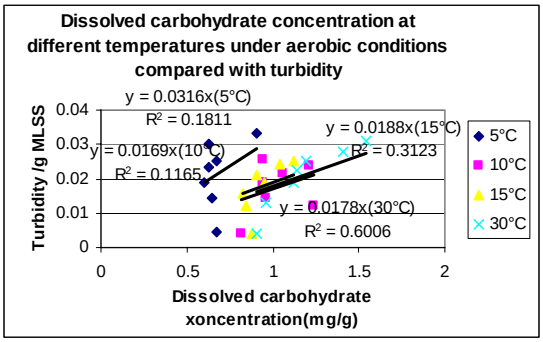
b)



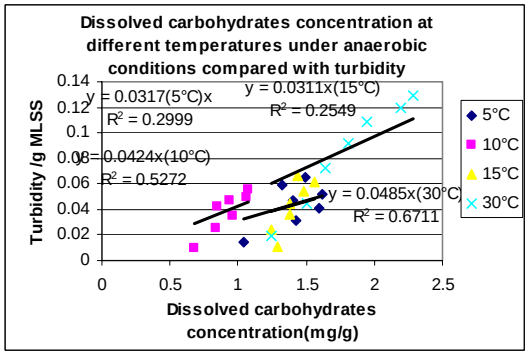
c)



d)



e)

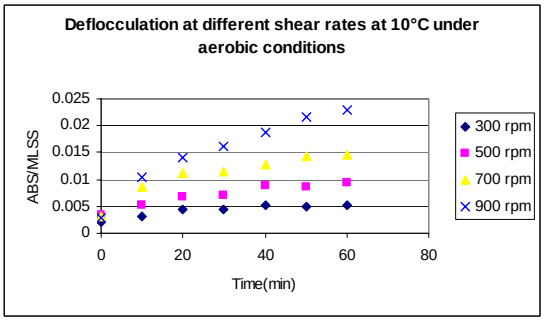


f)

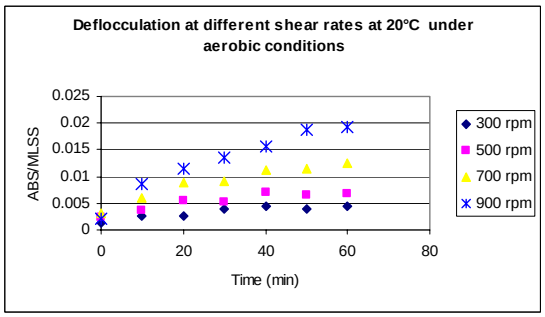
Figure 10 Correlation between dissolved biopolymer fraction and turbidity under aerobic and anaerobic conditions (a) dissolved protein under aerobic conditions; (b) dissolved protein under anaerobic conditions; (c) dissolved humic substances under aerobic conditions; (d) dissolved humic substances under anaerobic conditions ;(e) dissolved carbohydrate under aerobic conditions; (f) dissolved carbohydrate under anaerobic conditions.

4.3. Results of the control experiments

The results show that different shear rates have different effects on the deflocculation of the activated sludge. The deflocculation increased with the increasing in the shear rate (Figure 11). The results show that the sludge flocs are not completely disrupted and an equilibrium disintegration was not reached at 700 rpm at either 10 or 20°C.



a)



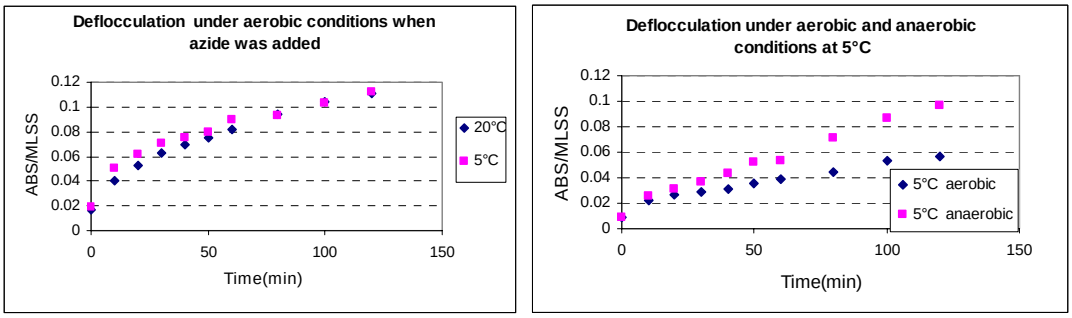
b)

Figure 11 Deflocculation under aerobic conditions at different shear rates (300, 500, 700 and 900 rpm (a) at the ambient temperature (20ºC); and (b) at 10ºC.

Azide inhibits the microbial activity and by adding it to activated sludge sheared at different temperatures, the physical effects of water viscosity on the sludge flocs can be assessed. Similar degrees of deflocculation were observed at shear tests carried out at 5ºC and 20ºC, when azide was added (Figure 12a). This indicates that the higher viscosity of the cold activated sludge has no significant effect on the physical properties of the sludge.

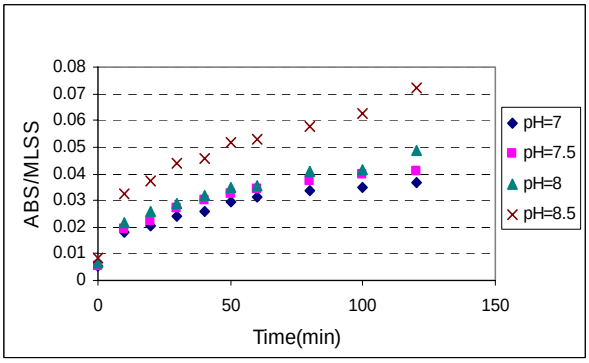
At 5ºC the microbial activity should be reduced to very low levels and therefore exposure to anaerobic or aerobic conditions should have no significant effect on the deflocculation. The results of the control test experiment showed, however, that the activated sludge flocs deflocculated more under anaerobic conditions than under aerobic conditions when they were exposed to 5ºC (figure 12b). It was found that the pH increased more under anaerobic conditions, to 8.2, while it was around 7.4 under aerobic conditions. It was observed that the

deflocculation rate of the activated sludge flocs increased with increasing pH in the control experiment (Figure 12c). At pH 7-7.5 the difference in turbidity development was very small. A slight increase was observed at pH 8 whereas at pH 8.5 it increased to high values. This could affect the interpretation of the reasons behind the increase in deflocculation and the biopolymers concentration under anaerobic conditions, since pH rose more under the anaerobic conditions than under the aerobic conditions; the changes in pH could have significant effects on floc stability and microbial activities. When looking at the data from the anaerobic conditions the pH increased beyond 7.5 up to 8.3. However, the pH was similar for the reference and test reactors in each experiment and therefore the results reflect effects of temperature. However, a comparison between the deflocculation under aerobic and anaerobic conditions is not possible due to the pH differences.



a)

b)



c)

Figure 12: Deflocculation under (a) aerobic conditions with azide addition (b) aerobic and anaerobic conditions at 5ºC (c) exposure to different pH and aerobic conditions.

4.4. Eroded (released) ions into the supernatant

Since iron salts were added to the sludge it contained large amounts of Fe, on an average 130 mg/gMLSS. The sludge contained the following concentrations of the other ions: 26 mg Na, 24 mg K, 13 mg Al, 12 mg Ca and 4.5 mg Mg per g MLSS. Before shearing had started the supernatant contained relatively high concentrations of Na compared to K which were of similar concentrations in the sludge. This indicates that Na binds weakly to the floc matrix or that it was present in the wastewater. The supernatant also contained relatively large concentrations of both Ca and Mg. On the other hand the supernatant contained very low concentrations of Fe whereas the concentrations of aluminium were slightly higher.

It was observed that the concentration of the different ions changed throughout the experiment in different ways. The concentration of Na was high in the water already at the start of the experiment. The concentration in the supernatant remained the same or increased slightly under aerobic conditions whereas it remained fairly unchanged under anaerobic conditions (figure 13). The concentrations were rather similar under aerobic and anaerobic conditions. The concentration of K was slightly higher under anaerobic conditions than under aerobic conditions (figure 16 a,c,e,g). Under aerobic conditions there was a slight increase in concentration and there was a trend towards higher concentrations at higher temperatures. The increased in concentration was particularly high at 30ºC. Similar observations were made for anaerobic conditions (figure 16 b,d,f,h).

The concentrations of Mg were similar under aerobic and anaerobic conditions (figure 14). There was a slight decrease in the concentration under both aerobic and anaerobic conditions. Under aerobic conditions there was a tendency towards higher concentrations in the supernatant at lower temperatures (figure 14 a,c,e,g). No such trend was found for anaerobic conditions (figure 14 b,d,f,h). The concentration of Mg in the supernatant was higher than the concentration of Ca and it was in a similar range under both aerobic and anaerobic conditions (figure 17). Just as for Mg, the concentration of Ca decreased during the shearing. For aerobic conditions there was no large difference in concentration between the different temperatures (figure 17 a,c,e,g). Similar observations were observed for anaerobic conditions (figure 17 b,d,f,h).

The concentration of Al was low but higher compared to the concentration of Fe (figure 15). The concentrations were also generally higher at higher temperatures, especially under anaerobic conditions. Very low concentrations of Fe were found in the supernatant at aerobic conditions (figure 18 a,c,e,g) whereas slightly higher concentrations were observed for anaerobic conditions (figure 18 b,d,f,h). The concentrations remained relatively constant throughout the experiments under both aerobic and anaerobic conditions.

It was observed that, the concentration of Ca, Mg and in some cases Fe ions decreased with the time of the experiments in both test reactors and reference reactors under both aerobic and anaerobic conditions (figure 14, 17 and 18). This could either have been due to binding with the dissolved biopolymers (carbohydrate, protein and humic substances), which showed the same trend or due to a chemical precipitation. Fe ions are strongly bound to the floc matrix and form hydroxide complexes at high pH. The concentration of Ca, Mg and Fe ions decreased more under anaerobic conditions than under aerobic conditions. This could happen as a result of the higher pH values observed under anaerobic conditions and this could have led to a larger precipitation compared to at aerobic conditions. Al and K increased during the time of experiments at all different temperature under both aerobic and anaerobic conditions (figure 15 and 16).

As a summary it can be concluded that the concentration of ions released to the supernatant during the deflocculation of activated sludge flocs in both test reactors and reference reactors were higher under anaerobic conditions than under aerobic conditions. The concentrations of those ions were similar in the total sludge for the different experiments.

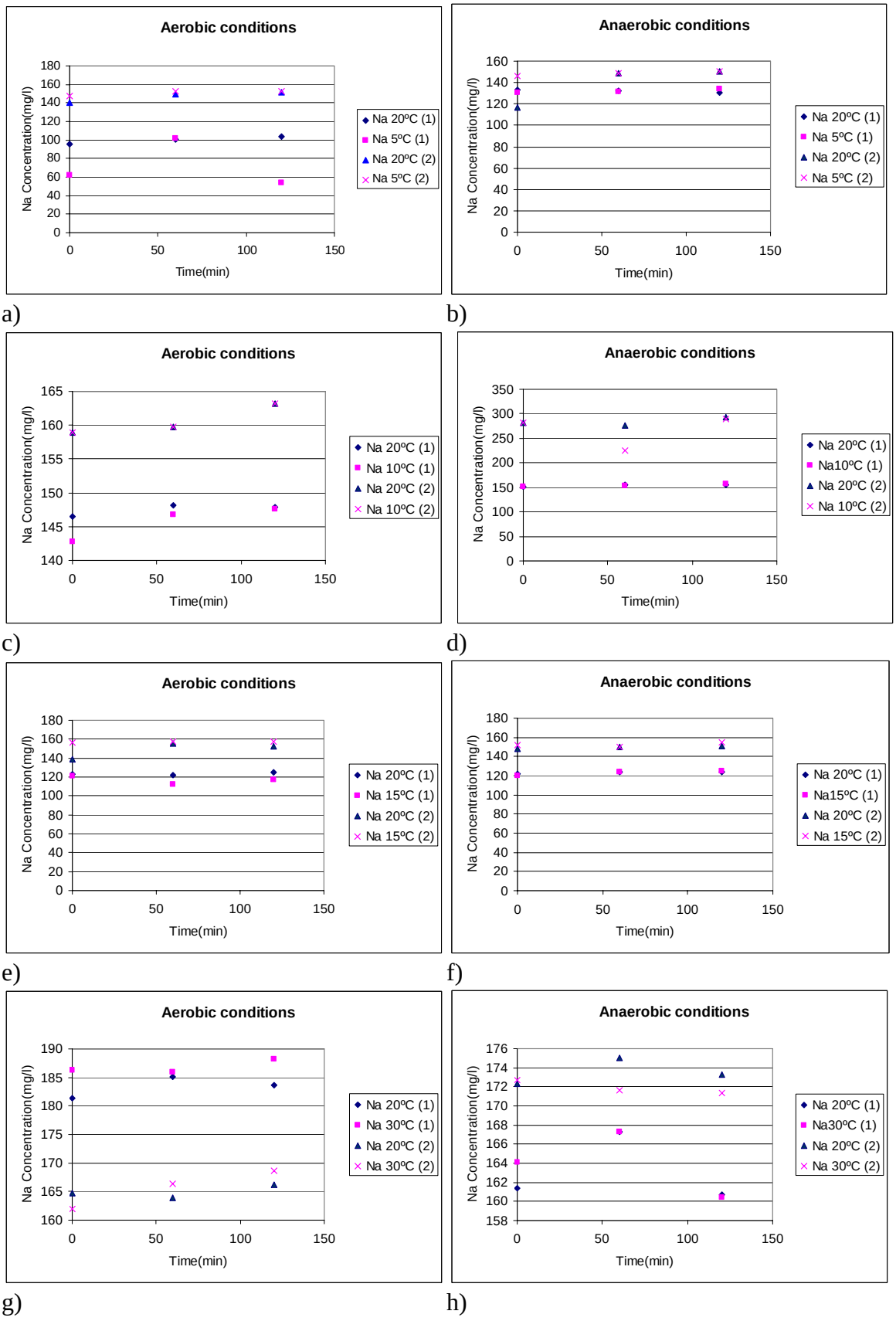
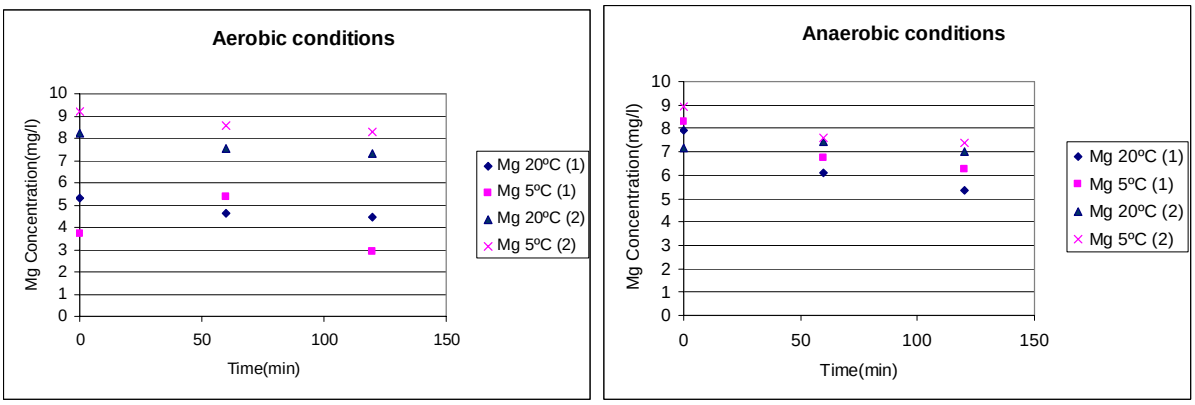
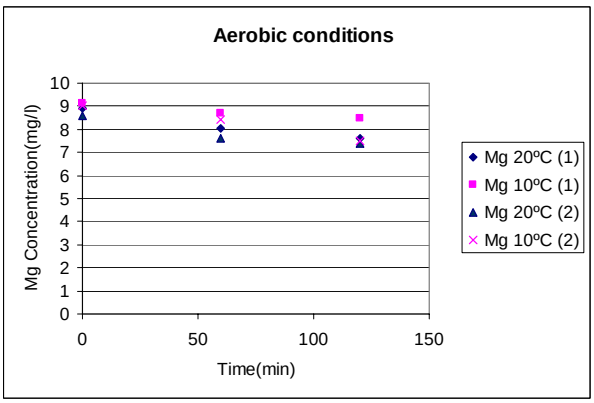


Figure 13 Concentration of Na ions, in mg/l, that eroded from activated sludge flocs into the supernatant during the deflocculation at 700 rpm shear strength (a) aerobic conditions and 5 °C; (b) anaerobic conditions and 5°C; (c) aerobic conditions and 10°C; (d) anaerobic conditions and 10°C; (e) aerobic conditions and 15°C; (f) anaerobic conditions and 15°C; (g) aerobic conditions and 30°C; and (h) anaerobic conditions and 30°C.

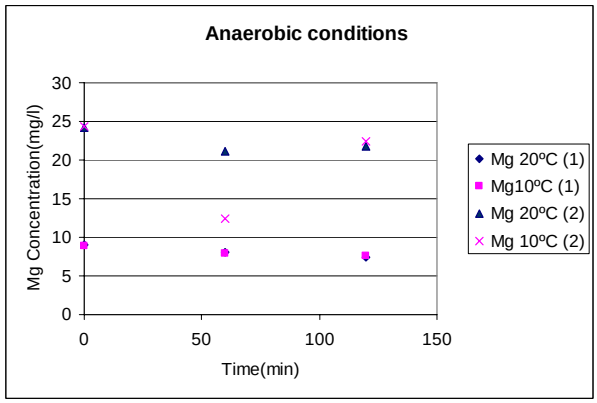


a)

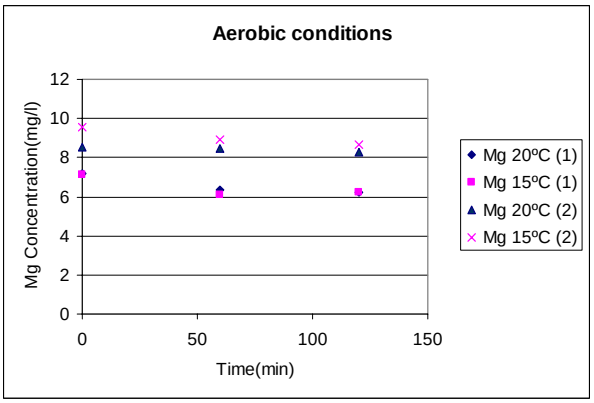
b)



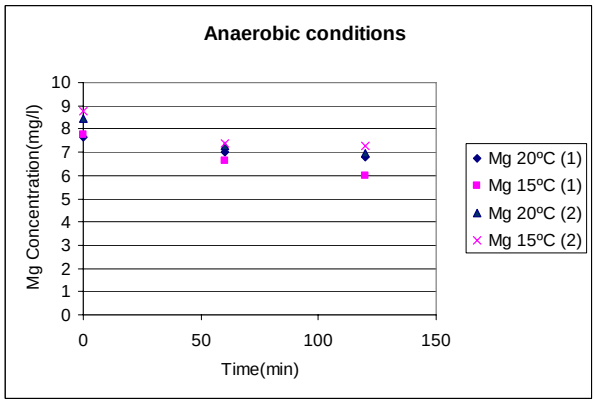
c)



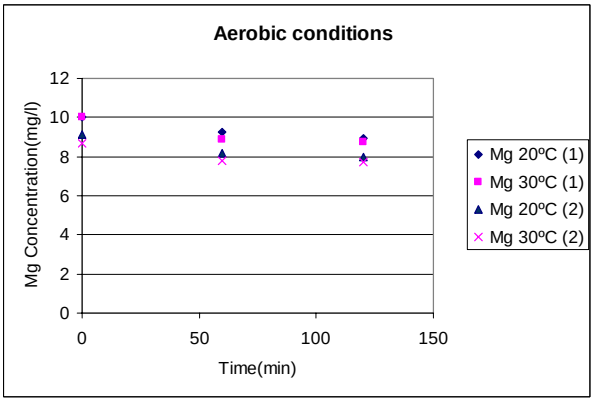
d)



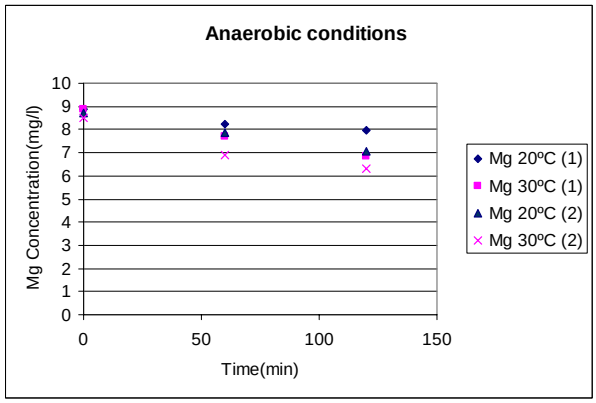
e)



f)



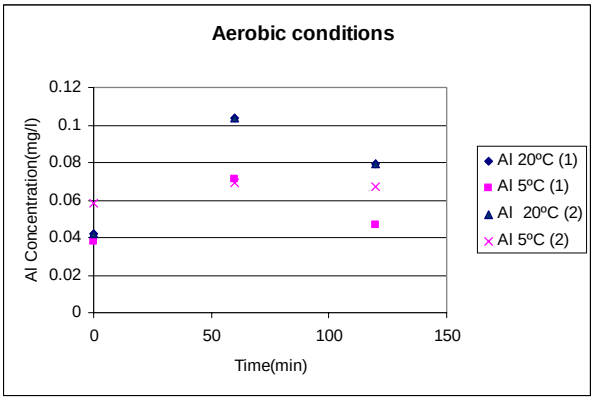
g)



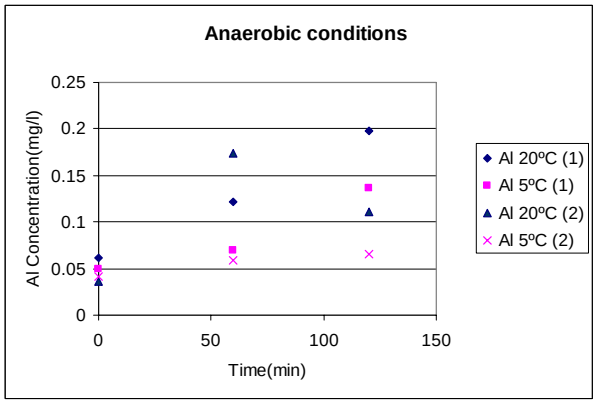
h)

Figure 14 Concentration of Mg ions, in mg/l, that eroded from activated sludge flocs into the supernatant during the deflocculation at 700 rpm shear strength (a) aerobic conditions and 5 °C; (b) anaerobic conditions

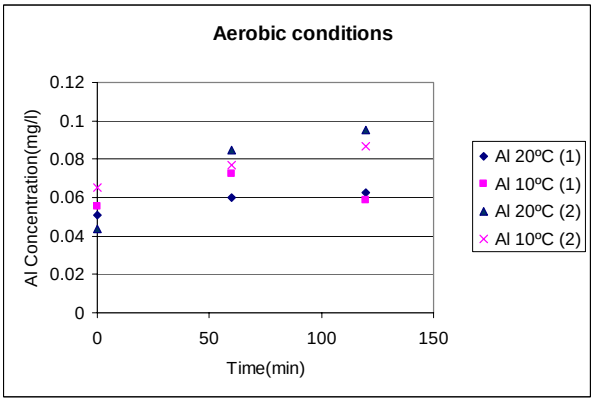
and 5°C; (c) aerobic conditions and 10°C; (d) anaerobic conditions and 10°C; (e) aerobic conditions and 15°C; (f) anaerobic conditions and 15°C; (g) aerobic conditions and 30°C; and (h) anaerobic conditions and 30°C.



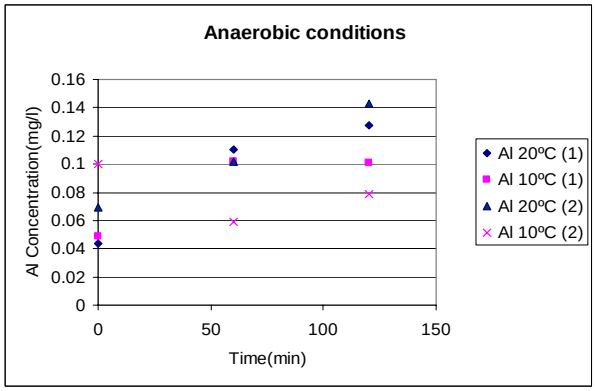
a)



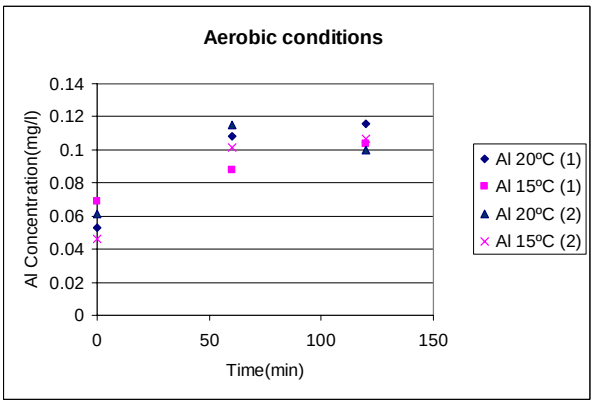
b)



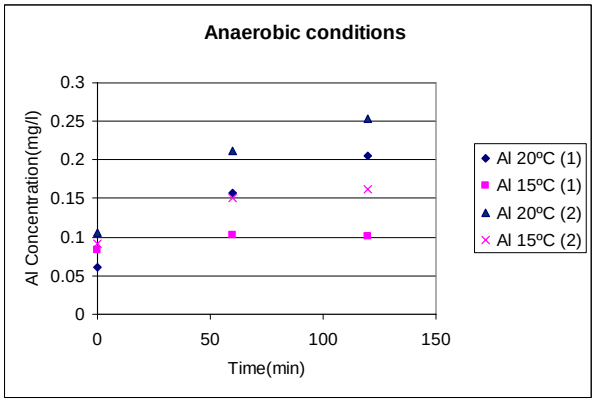
c)



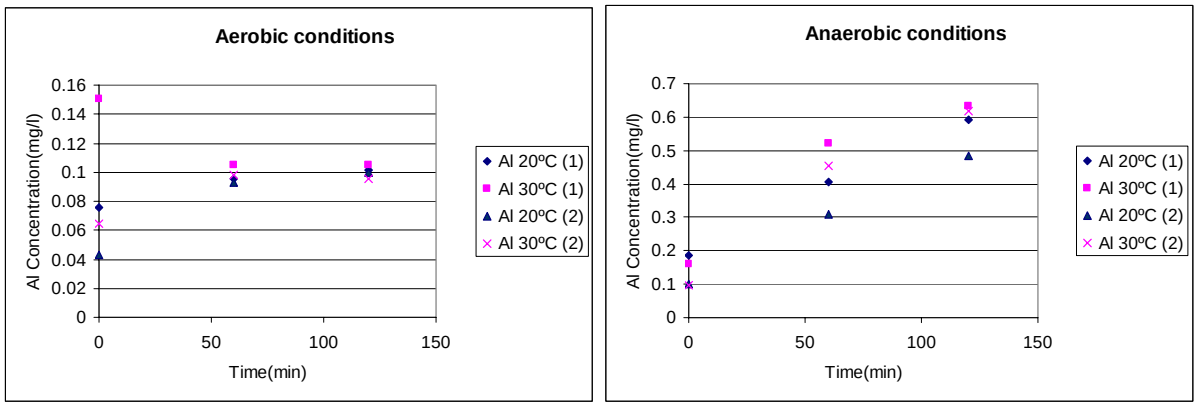
d)



e)

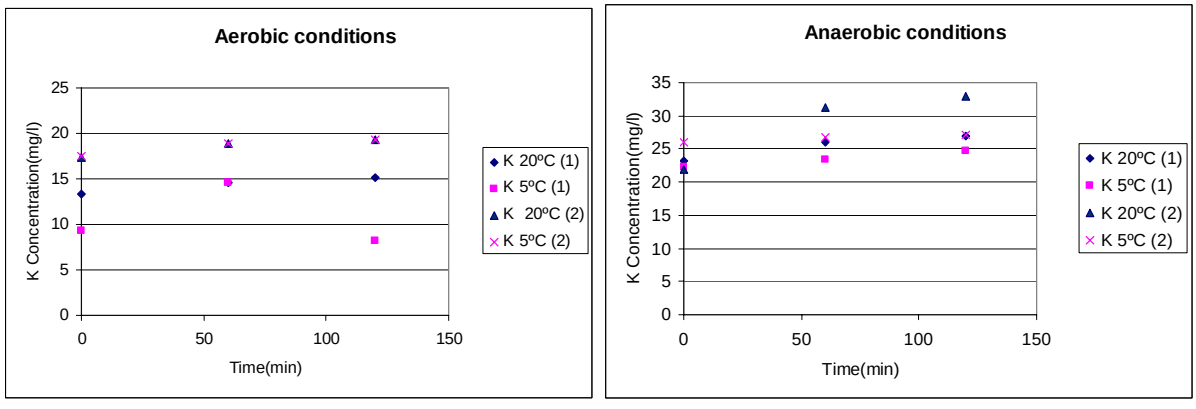


f)

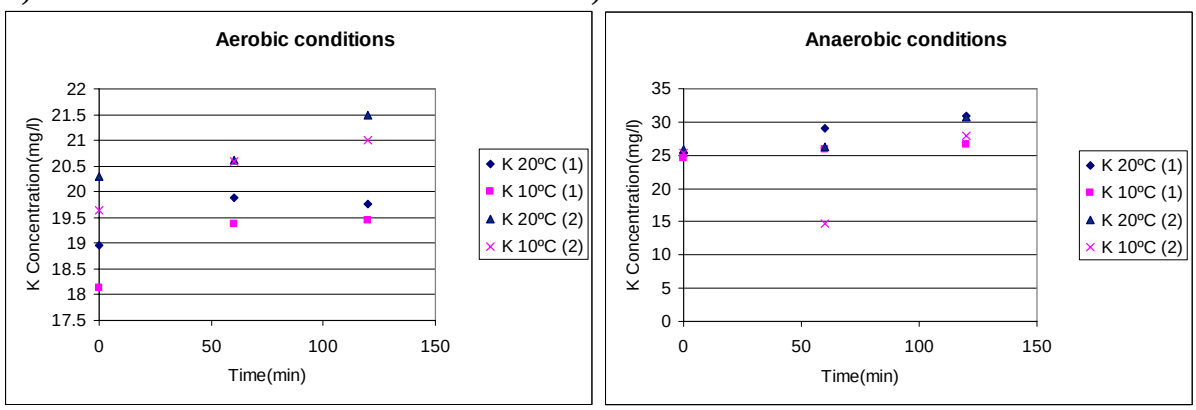


g) h)

Figure 15 Concentration of Al ions, in mg/l, that eroded from activated sludge flocs into the supernatant during the deflocculation at 700 rpm shear strength (a) aerobic conditions and 5 °C; (b) anaerobic conditions and 5°C; (c) aerobic conditions and 10°C; (d) anaerobic conditions and 10°C; (e) aerobic conditions and 15°C; (f) anaerobic conditions and 15°C; (g) aerobic conditions and 30°C; and (h) anaerobic conditions and 30°C.



a) b)



c) d)

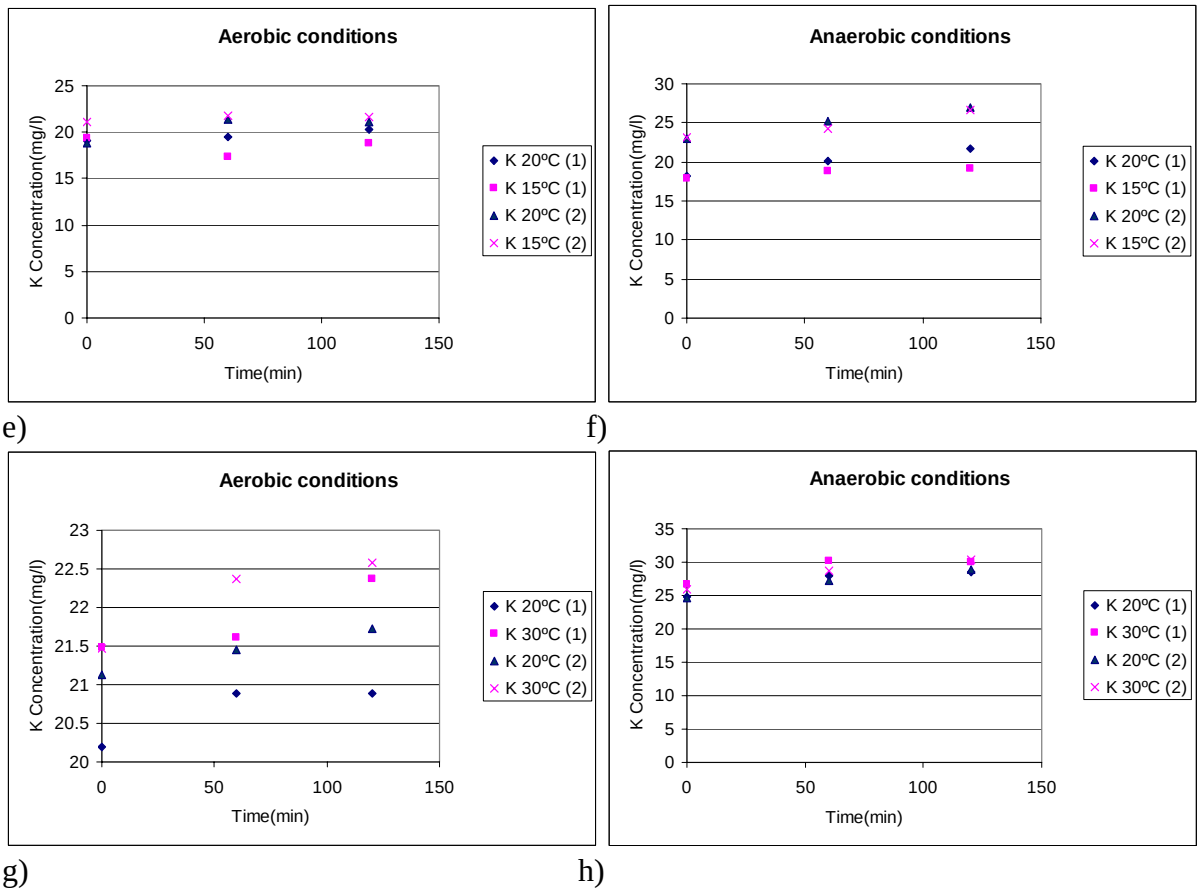
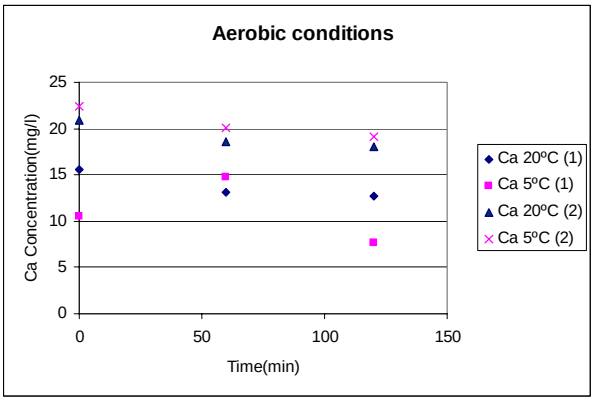
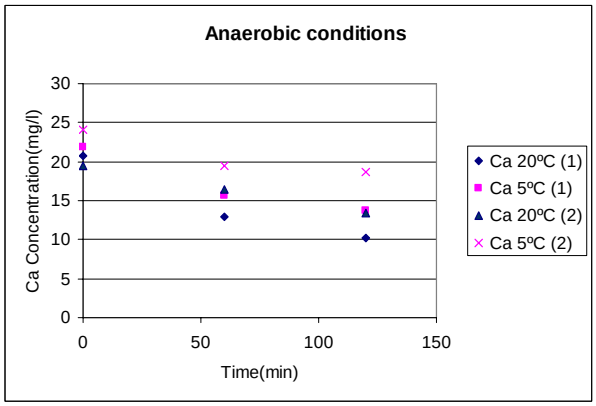


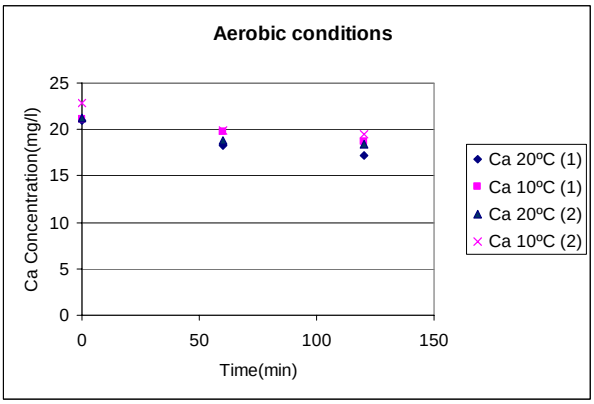
Figure 16 Concentration of K ions, in mg/l, that eroded from activated sludge flocs into the supernatant during the deflocculation at 700 rpm shear strength (a) aerobic conditions and 5 °C; (b) anaerobic conditions and 5°C; (c) aerobic conditions and 10°C; (d) anaerobic conditions and 10°C; (e) aerobic conditions and 15°C; (f) anaerobic conditions and 15°C; (g) aerobic conditions and 30°C; and (h) anaerobic conditions and 30°C.


Aerobic conditions
Ca Concentration(mg/l)
Time(min)
Ca 20ºC (1)
Ca 5ºC (1)
Ca 20ºC (2)
Ca 5ºC (2)

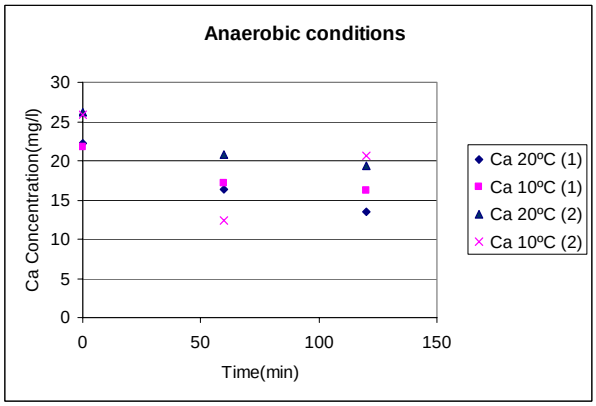
a)


Anaerobic conditions
Ca Concentration(mg/l)
Time(min)
Ca 20ºC (1)
Ca 5ºC (1)
Ca 20ºC (2)
Ca 5ºC (2)

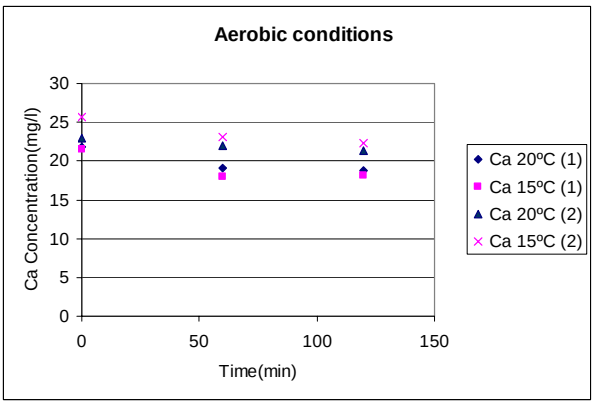
b)


Aerobic conditions
Ca Concentration(mg/l)
Time(min)
Ca 20ºC (1)
Ca 10ºC (1)
Ca 20ºC (2)
Ca 10ºC (2)

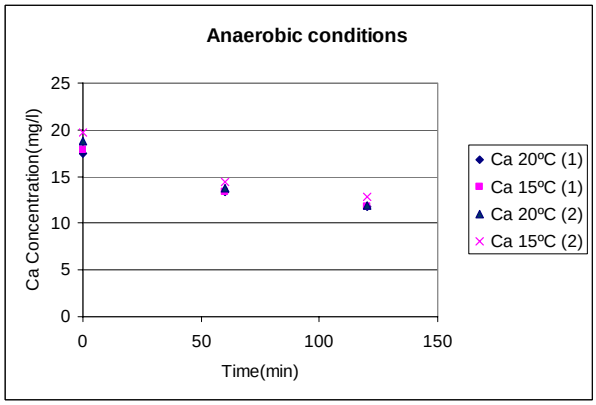
c)


Anaerobic conditions
Ca Concentration(mg/l)
Time(min)
Ca 20ºC (1)
Ca 10ºC (1)
Ca 20ºC (2)
Ca 10ºC (2)

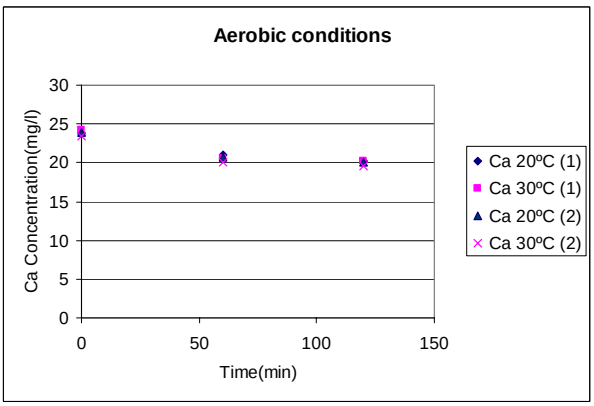
d)


Aerobic conditions
Ca Concentration(mg/l)
Time(min)
Ca 20ºC (1)
Ca 15ºC (1)
Ca 20ºC (2)
Ca 15ºC (2)

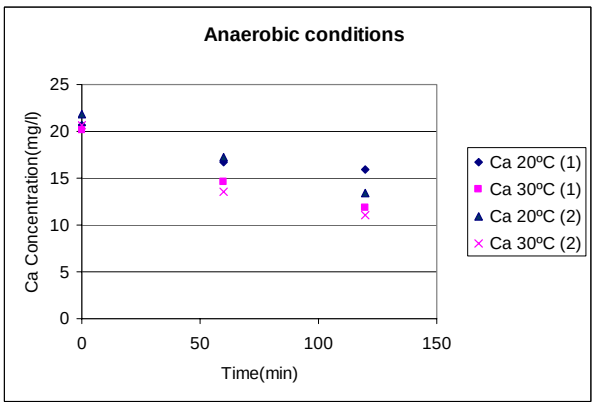
e)


Anaerobic conditions
Ca Concentration(mg/l)
Time(min)
Ca 20ºC (1)
Ca 15ºC (1)
Ca 20ºC (2)
Ca 15ºC (2)

f)

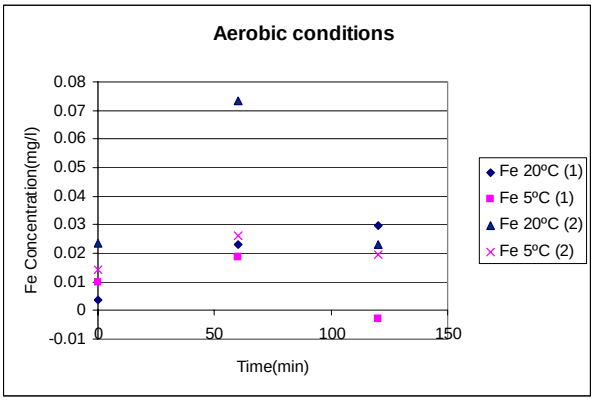

Aerobic conditions
Ca Concentration(mg/l)
Time(min)
Ca 20ºC (1)
Ca 30ºC (1)
Ca 20ºC (2)
Ca 30ºC (2)

g)

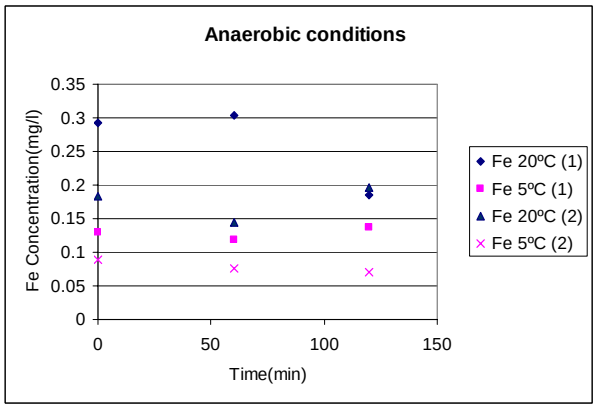

Anaerobic conditions
Ca Concentration(mg/l)
Time(min)
Ca 20ºC (1)
Ca 30ºC (1)
Ca 20ºC (2)
Ca 30ºC (2)

h)

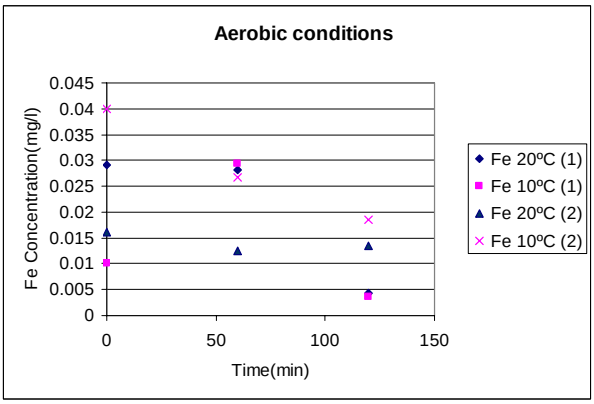
Figure 17 Concentration of Ca ions, in mg/l, that eroded from activated sludge flocs into the supernatant during the deflocculation at 700 rpm shear strength (a) aerobic conditions and 5 °C; (b) anaerobic conditions and 5°C; (c) aerobic conditions and 10°C; (d) anaerobic conditions and 10°C; (e) aerobic conditions and 15°C; (f) anaerobic conditions and 15°C; (g) aerobic conditions and 30°C; and (h) anaerobic conditions and 30°C.



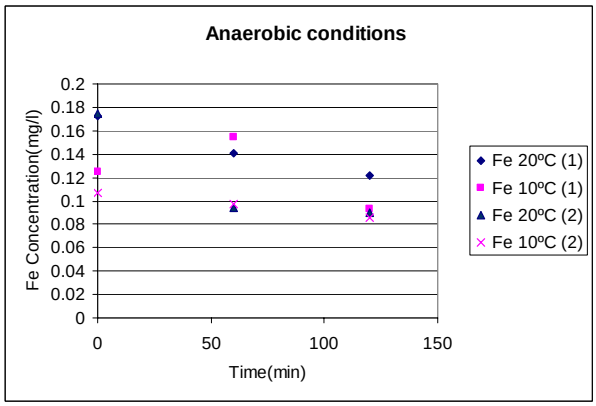
a)



b)



c)



d)

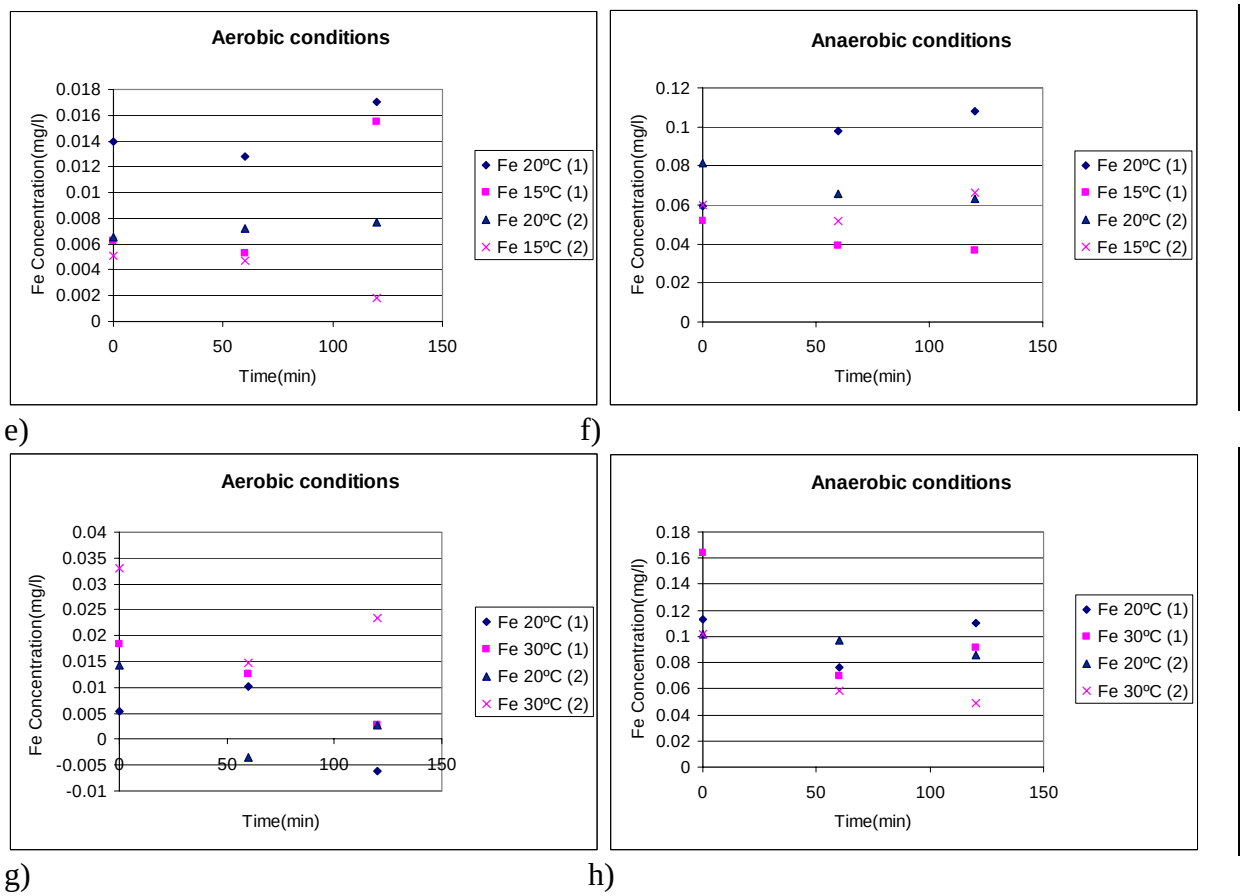
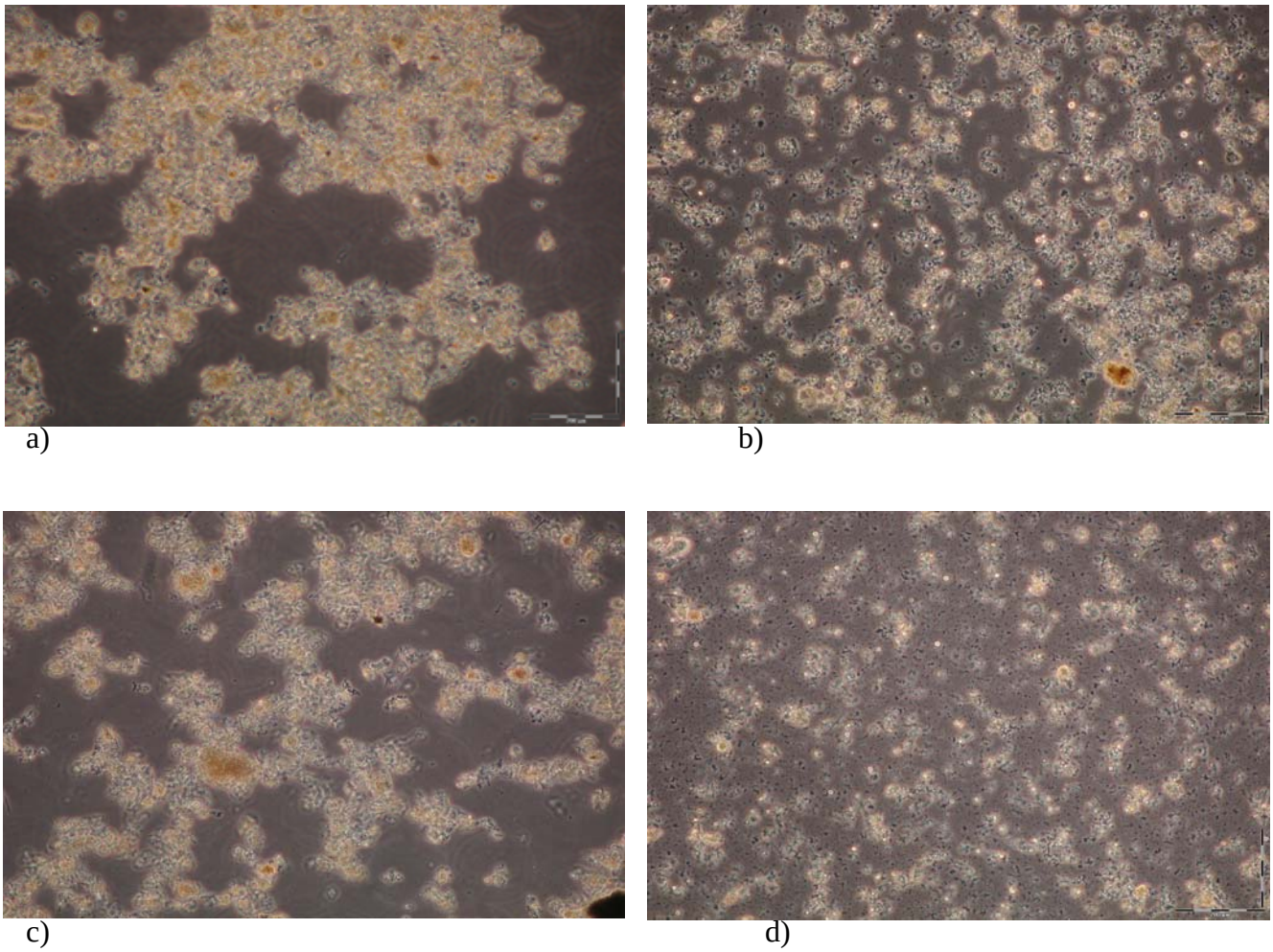


Figure 18 Concentration of Fe ions, in mg/l, that eroded from activated sludge flocs into the supernatant during the deflocculation at 700 rpm shear strength (a) aerobic conditions and 5 °C; (b) anaerobic conditions and 5°C; (c) aerobic conditions and 10°C; (d) anaerobic conditions and 10°C; (e) aerobic conditions and 15°C; (f) anaerobic conditions and 15°C; (g) aerobic conditions and 30°C; and (h) anaerobic conditions and 30°C.

Activated sludge flocs images

The flocs include microorganisms, organic and inorganic particles. They are glued together by polymers produced by bacteria. The images showed that the flocs are more open and branched under anaerobic conditions(figure 19c). Pinpoint and less number of dense large microbial colonies were formed and free cells were released at the end of each shear test, it could be due to shearing effects more than temperature effects.



a) b)

c) d)

Figure 19: activated sludge flocs magnified 100 times at ambient temperature 20°C (a) after one hour of shearing at 100 rpm and under aerobic conditions (b) after two hour of shearing at 700 rpm and under aerobic conditions (c) after one hour of shearing at 100 rpm and under anaerobic conditions (d) after two hour of shearing at 700 rpm and under anaerobic conditions.

5.0. Chapter five: Conclusion

- The activated sludge flocs deflocculated significantly more at different low temperatures (5ºC, 10ºC and 15ºC) compared to at 20ºC under aerobic conditions. On the contrary, it deflocculated more at 20ºC than at 5ºC, 10ºC and 15ºC under anaerobic conditions.

- The concentration of the total biopolymers protein, carbohydrate and humic substances, was higher at 5ºC, 10ºC and 15ºC than at 20ºC under aerobic conditions, while it was higher at 20ºC than at 5ºC, 10ºC and 15ºC under anaerobic conditions (they followed the turbidity curve).

- The dissolved biopolymers (protein, carbohydrate and humic substances) were released more at 20°C than at 5°C, 10°C and 15°C under aerobic conditions. The concentrations of dissolved polymers were also higher at 30°C than at 20°C. Under anaerobic conditions, the concentration of dissolved biopolymers followed the turbidity, i.e. the concentrations were higher at 20 °C and 30 °C than at 5°C, 10°C and 15°C.

- The concentrations of Na were rather similar under aerobic and anaerobic conditions.

- The concentration of K were slightly higher under anaerobic conditions than under aerobic conditions .

- Very low concentrations of Fe were found in the supernatant at aerobic conditions, whereas slightly higher concentrations were observed for anaerobic conditions

- The concentration of Ca, Mg and in some cases Fe ions decreased with time of the experiment in both test and reference reactors under both aerobic and anaerobic conditions.

6.0. Chapter six: References

Balmér, P., Ekfjorden, L., Lumley, D. and Mattsson, A. (1998). Upgrading for nitrogen removal under severe site restrictions. Water Science and Technology 37, 185-1998.

Nielsen P.H., Frølund B. and Keiding K. (1996) changes in exopolymers composition by anaerobic storage of activated sludge. Applied Microbiol. Biotechnology, 44, 823-830.

Tsuneda S., Aikawa H., Hayashi H., Yuasa A. and Hirata A. (2003) Extracellular polymeric substances responsible for bacterial adhesion into solid surface, FEMS Microbiology Letters 223, 2287-292.

Garnier C., Görner T. and Lartiges B.S., (2005), Characterization of activated sludge exopolymers from various origins: A combined size exclusion chromatography and infrared microscopy study, Water Research, Volume 39, issue 13, pages 3044-3054.

Morgan-Sagastume F., and Allen D.G., (2005) Physicochemical properties and stability of activated sludge flocs under temperature up shifts from 30 to 45ºC, Journal of colloid and interface Science, Volume 281, issue 1, 136-145.

Morgan-Sagastume F., and Allen D.G. (2003) Effect of temperature transient conditions on aerobic biological treatment of wastewater, Water Research Volume 37, issue 15, pages 3590-3601.

Mullar E., Kriebitzsch K., wilderer P.A and Wuertz S., (2002), Community structure of micro and macroflocs in pinpoint sludge and the influence of sludge age and potassium addition on microfloc formation, Water Science and Technology, Volume 46, issue ½, pages 405-412.

Murthy S.N., Novak J.T. and Haas R.D (1999) Monitoring cations to predict and improve activated sludge settling properties of industrial wastewaters, Water Science and Technology, Volume 38, issue 3, pages 119-126.

Wilen B.M., Keiding K and Nielsen P.H, (2000) Anaerobic deflocculation and aerobic reflocculation of activated sludge, Water Research, Volume 34, issue 16, pages 3933-3942.

Wilen B.M., Nielsen P.H., Keiding K. and Nielsen P.H. (2000) Influence of microbial activity on the stability of activated sludge flocs. Colloids and surfaces B: Biointerfaces Volume 18, pages 145-156.

Wilen, B.M., Keiding K. Nielsen P.H., (2004) Flocculation of activated sludge flocs by stimulation of the aerobic biological activity, Water Research, Volume 38, issue 18, pages 3909-3919.

Jarvis P., Jefferson B., Gregory J. and Parsons S.A. (2005) A review of floc strength and breakage, Water Research, Volume 39, issue 14, pages 3121-3137.

Liu Q.S., Liu Y., Tay J.H. and Show K.Y., (2005) Responses of sludge to shear strength, Process Biochemistry, Volume 40, issue 10, pages 3213-3217.

Liu Y. and Tay JH. (2002) The essential rule of hydrodynamic shear forces in the formation of biofilms and granular sludge. Water Research, Volume 36, issue 7, pages 1653-1665.

Neyens E., Baeyens J. and Baeyens Creemers C. (2003) Alkaline thermal sludge hydrolysis, J Hazard. Mater. 97B, pages 295-314.

Appendices

Appendix (I): the analytical methods used for measurement of carbohydrates, protein and humic substances

(1) Carbohydrates

The anthrone method was used for measuring carbohydrates. It is a colorimetric method; the carbohydrates are hydrolysed with concentrated acid and thereafter complex binding with anthrone. The intensity of colour is proportional to the concentration of carbohydrates.
The standard curve is prepared according to the following method and modified to give a lower concentration level of 5 ppm.

Standard curve

A stock solution (1000 ppm) of glucose was made (2ml aliquots were used in each experiment). The stock solution was diluted 10 times to 100ppm. The standard curve then made accordingly:

Table:

Conc. [ppm]	Amount of stock solution [µl]	Water [µl]
0	0	5000
2.5	250	9750
5	250	4750
10	500	4500
25	1000	3000
50	2000	2000

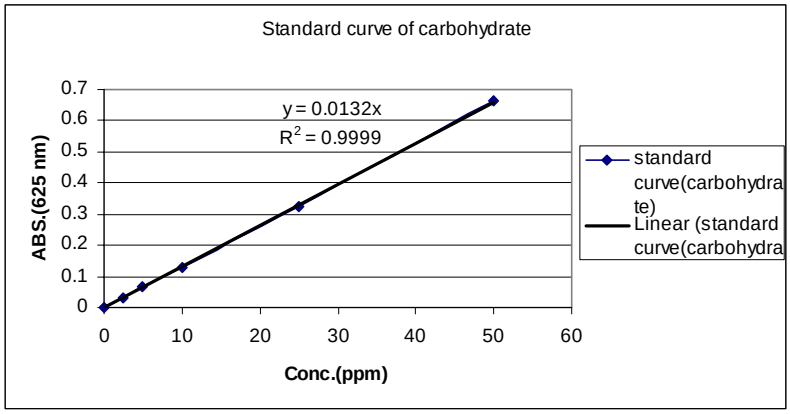


Figure x: standard curve of carbohydrate

Reagent solutions

27.5 ml distilled water is mixed with 472.5 ml concentrated H_2SO_4; 0.625 g anthrone was added while the solution was still warm.
27.5 ml distilled water was added to 472.5 ml concentrated H_2SO_4.

The reagent solutions were stored at 4ºC for 2 hours, and new reagents were made each time.

Procedure
0.1 ml sample or standard was pipeted to glass tube. To each sample or standard, three tubes were used; 2 for duplicate analysis and 1 for a blind sample.
2 ml of reagent A were added to each tube and 2 ml of reagent B to each blind test and they were mixed.
The samples were heated up to 100ºC (hatch method) for 14 min, and then the tubes were cooled rapidly on cooled water bath for 5 min.
The samples were measured on a spectrophotometer at 625 nm with the blind reagent as a reference.

(2) Protein and humic substances

Standard curve
A stock solution (1000 mg/l bovin serum albumin (BSA) was prepared and kept in the freezer. From this a 100-ppm solution was made and the standard curve was made accordingly:

Conc. [ppm]	Amount of stock solution [µl]	Water [µl]
0	0	5000
2.5	250	9750
5	250	4750
10	500	4500
25	1000	3000
50	2000	2000
100	4000	0

The standard curve of the humic substances was made in the same way. The stock solution of humic substances was made at a concentration of 1000 mg/l Janssen humic solution; from this a 100-ppm solution was made each time.

Reagents

5.71 g NaOH and 28.571 g Na2CO3 were dissolved in distilled water to a volume of 1000ml.
0.7143 g CuSO4.5H2O dissolved in distilled water to 50 ml.
1.4286 g Na-tartrate was dissolved in distilled water to 50 ml.
Reagent A, B and C were mixed in a ratio 100:1:1.
Reagent A, distilled water and reagent C were mixed in a ratio 100:1:1 (same of the reagent D but with distilled water instead of Cu).
5ml Folin-Ciocalteus was added to 6 ml distilled water (a fresh solution was made each time)
* Reagent A, B and C can be kept in a fridge for one month, while reagent D and E were made each time.

Procedure:
2 reagent tubes were prepared for each measurement with Cu and 2 for each without Cu for duplicate determination. The frozen samples were homogenized.

500 µl Homogenized sample or standard was pipeted to each tube. 700 Ml of reagent D were added to 2 of the four tubes and reagent E to the two others and they were mixed (Humic acid standards were only prepared with reagent E (without Cu)), protein standards were analysed with reagent D and E.
The samples were stand for 10 min. 100 µl of reagent F was added.
All samples were measured, in the same order as the Reagent F was added, at 750 nm on a spectrophotometer.
The zero sample (blind sample with and without Cu) was set to Zero with distilled water as a reference.

Reactions:
Cu^{+2} is reduced under complex formation with peptides to Cu^{+}. In an alkaline environment, the peptides bonds are hydrolysed which will further reduce the Cu^{+2}. the Cu^{+} is thereafter reduced by folin-Ciocalteus-phenol reagent. The reduction gives a blue colour, which can be measured spectrophotometrically at 750. Reduction of other compounds like trypsine, cystin. This means that the obtained colour is dependent on the total protein composition. Therefore, different concentrations of protein will be obtained by different standards of proteins. Humic substances interfere with the protein assay. This can be avoided by doing the following calculations (ABS=absorbance)

$$ABS_{total} = ABS_{protein} + ABS_{humus}$$

$$ABS_{blind} = 0.4 \times ABS_{protein} + ABS_{humus}$$

ABS_{total} = total absorbance with $CuSO_4$
ABS_{blind} = total absorbance without $CuSO_4$
ABS_{humus} = Absorbance coming from humic substances (calculated)
$ABS_{protein}$ = Absorbance coming from protein (calculated)

Calculations:
Standard curve for protein was mad by plotting the concentration in ppm against ABStotal and adjusted to pass through (0.0) (Figure x). The concentration was calculated according to:
$$ABS_{protein} = 1.25 \times (ABS_{total} - ABS_{blind})$$

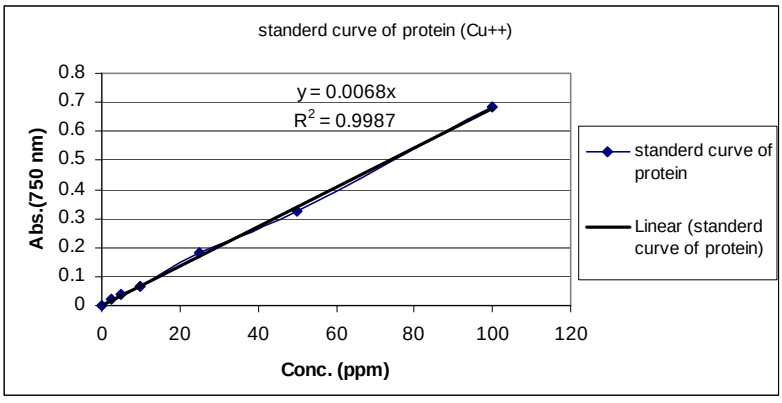


Figure x: standard curve of protein

The humic substances standard curve was made with plotting the concentrations in ppm against the ABS_{blind} (Figure x). The concentration of humic substances was calculated according to the following equation.

$ABS_{humus} = AB_{Stotal} - ABS_{protein}$

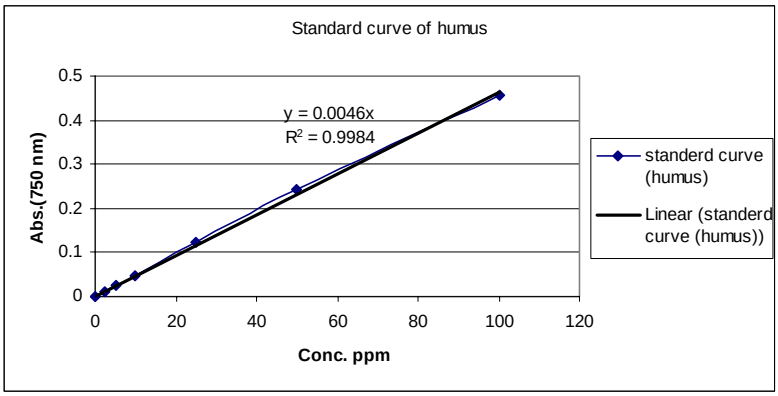


Figure x: standard curve of humus

Appendix (II): The concentration of total biopolymers (average concentration of the duplicate experiments) in the total sludge (mg/g MLSS)

	Average concentration (aerobic)			Average concentration (anaerobic)		
Temperature	Total protein	Total humus	Total Carbohydrate	Total protein	Total humus	Total Carbohydrate
5°C	192.4525	68.74989	46.81994	229.6556	73.01605	50.32695
10°C	149.0046	51.76181	35.54456	258.414	78.6817	59.81313
15°C	265.3499	59.58659	51.99932	241.7429	82.74232	60.77522
30°C	249.824	84.62525	51.33049	316.7242	67.85451	53.98

Appendix (III): The ratio between the total biopolymers in the total sludge

	Ratio under aerobic conditions			Ratio under anaerobic conditions		
Temperature	Total protein	Total humus	Total Carbohydrate	Total protein	Total humus	Total Carbohydrate
5ºC	4.110481	1.468389	1	4.563271	1.450834	1
10ºC	4.19205	1.456251	1	4.320355	1.315459	1
15ºC	5.10295	1.145911	1	3.977655	1.361448	1
30ºC	4.866971	1.648635	1	5.867436	1.257031	1

Appendix (IV):The average concentration of total and dissolved biopolymers (mg/g MLSS) and the absorbance/ MLSS values, in the supernatant for the experiments performed under aerobic conditions

20ºC (Reference reactor of 5ºC)							
Time (min)	Absorbance/MLSS	Total Protein	Total Humus	Total Carbohydrate	Dissolved Protein	Dissolved Humus	Dissolved Carbohydrate
0	0.003084	1.853792	1.441034	0.715796	0.727534	1.557002	0.575068
20	0.010416	2.662785	1.889931	1.145854	0.761929	1.771731	0.686263
40	0.013846	3.837161	2.60462	1.504995	0.808771	1.797663	0.945394
60	0.017936	4.997367	2.673145	1.693531	0.833713	1.948737	0.960297
80	0.019447	5.613187	2.995517	1.805209	0.84312	1.984102	0.948118
100	0.021902	5.864745	3.058825	1.97331	0.858655	2.04316	1.079563
120	0.024609	6.4261	3.443736	2.139126	1.061561	1.922734	1.110814
5ºC (Test reactor)							
Time (min)	Absorbance/MLSS	T protein	T Humus	T Carbohydrate	D protein	D Humus	D Carbohydrate
0	0.004532	2.372904	1.399183	0.763885	0.789959	1.238909	0.675549
20	0.014193	4.255791	1.973954	0.987186	0.718175	1.464736	0.645661
40	0.018881	4.801102	2.526931	1.09782	0.746205	1.322246	0.598206
60	0.023286	5.074889	2.912688	1.599857	0.836754	1.264804	0.623926
80	0.025364	6.048495	2.743169	1.787151	0.658932	1.300277	0.676829
100	0.029896	6.974113	3.079463	1.982997	0.796183	1.482813	0.630716
120	0.033199	7.564271	3.306149	2.259211	0.861743	1.604831	0.907557
20ºC (Reference reactor of 10ºC)							
Time (min)	Absorbance/MLSS	T protein	T Humus	T Carbohydrate	D protein	D Humus	D Carbohydrate
0	0.003602	1.628084	2.281195	0.797445	1.175311	1.883103	0.889336
20	0.009949	3.465362	2.546379	1.377498	1.046631	2.245889	1.003312
40	0.013012	4.342434	2.541299	1.485557	1.170835	2.075364	1.06548
60	0.014384	4.323946	3.168557	1.708493	1.269536	2.26934	1.188086
80	0.01676	6.448231	2.542189	1.879374	1.195511	2.313702	1.191828
100	0.019985	6.105284	3.719839	2.16131	1.108733	2.483138	1.232768
120	0.021477	6.013604	3.939751	2.229993	1.176138	2.0156	1.284215
10ºC (Test reactor)							
Time (min)	Absorbance/MLSS	T protein	T Humus	T Carbohydrate	D protein	D Humus	D Carbohydrate
0	0.0041	2.258196	2.251977	1.633383	0.951578	1.601122	0.817099
20	0.012316	4.399386	2.79346	1.71952	1.109561	1.487866	1.237735
40	0.014505	5.179143	2.639819	1.904352	0.984529	1.77561	0.963451
60	0.018887	6.042698	3.135309	1.897724	1.163387	2.0063	0.939345
80	0.021581	6.189778	3.310217	2.111166	0.938489	1.872181	1.053893
100	0.023923	6.983053	3.699697	2.314478	0.83565	2.208248	1.208662
120	0.025689	7.361551	3.977449	2.537284	0.575655	2.276269	0.942728
20ºC (Reference reactor of 15ºC)							
Time (min)	Absorbance/MLSS	T protein	T Humus	T Carbohydrate	D protein	D Humus	D Carbohydrate
0	0.002918	2.181539	2.550182	1.245575	1.039265	1.707319	0.791303
20	0.009387	3.850136	2.652794	1.595575	1.377061	1.60797	0.927366
40	0.013109	5.083885	2.660284	1.858755	1.36574	1.817673	1.160063
60	0.016075	4.92853	2.918169	2.115454	1.110998	1.677096	1.090614
80	0.016896	5.995594	3.199221	2.223863	1.258586	1.952687	1.053311
100	0.019042	6.717504	3.348694	2.535078	1.32591	2.125086	1.091128
120	0.021106	7.108398	3.257583	2.630813	1.402495	2.083859	1.122565
15ºC (Test reactor)							
Time (min)	Absorbance/MLSS	T protein	T Humus	T Carbohydrate	D protein	D Humus	D Carbohydrate

0	0.003864	2.552931	2.289976	1.600844	1.189644	1.451162	0.877889
20	0.012304	3.773143	2.794549	1.65219	1.393235	1.297023	0.841684
40	0.0159	5.17027	3.038559	2.005884	1.355307	1.379783	0.828606
60	0.018664	5.98453	3.241312	2.162391	1.22551	1.596966	0.948561
80	0.021313	6.345009	3.368044	2.320634	1.588295	1.203564	0.902433
100	0.02417	7.005853	3.541084	2.480962	1.153333	1.923942	1.039719
120	0.025052	7.431275	3.708096	2.81082	1.343257	1.501795	1.12276
20ºC (Reference reactor of 30ºC)							
Time (min)	Absorbance/MLSS	T protein	T Humus	T Carbohydrate	D protein	D Humus	D Carbohydrate
0	0.003727	1.847476	2.64985	1.020367	1.010561	2.04883	0.861861
20	0.010157	3.696063	3.296859	1.716668	1.211152	1.848122	0.945086
40	0.014404	4.597257	3.300816	2.029525	1.314989	1.695137	0.974725
60	0.017388	5.419071	3.495378	2.244234	1.377305	1.604056	1.084225
80	0.019787	6.299109	3.591955	2.559441	1.457397	1.430144	1.150547
100	0.020583	6.522819	3.791599	2.570418	1.236668	1.786019	1.129733
120	0.022652	7.017178	3.994268	2.719963	1.402821	1.807558	1.051453
30ºC (Test reactor)							
Time (min)	Absorbance/MLSS	T protein	T Humus	T Carbohydrate	D protein	D Humus	D Carbohydrate
0	0.003864	2.370886	2.584644	1.208913	1.24736	1.690029	0.906334
20	0.01298	4.798578	3.558104	1.967847	1.536964	1.392175	0.95992
40	0.01877	6.25507	3.517395	2.376268	1.009381	1.296615	1.119873
60	0.022515	6.390517	3.909877	2.691939	1.328633	1.261059	1.139881
80	0.025069	7.7688	4.051771	2.97326	1.401641	1.132571	1.190334
100	0.028036	8.54164	4.04173	3.255742	1.524501	1.175797	1.407236
120	0.030883	9.134192	4.459834	3.588951	1.418892	1.45036	1.538327

Appendix (V): The average concentration of total and dissolved biopolymers (mg/g MLSS) and the absorbance/ MLSS values, in the supernatant for the experiments performed under aerobic conditions

20ºC (Reference reactor of 5ºC)							
Time (min)	Absorbance/MLSS	Total Protein	Total Humus	Total Carbohydrate	Dissolved Protein	Dissolved Humus	Dissolved carbohydrate
0	0.012845	4.859329	3.482894	1.573773	0.973293	2.980618	1.413852
20	0.033154	8.878318	4.74788	2.498282	0.921618	3.635547	1.525126
40	0.045618	10.32702	5.685503	3.240893	1.228246	4.136581	1.464697
60	0.060258	13.07324	7.068985	3.935524	1.09746	5.389741	1.589969
80	0.070467	14.73677	8.38793	4.468699	1.38577	5.626977	1.860196
100	0.085002	15.63949	9.732684	4.958145	2.126676	5.65246	2.02715
120	0.092105	17.7233	11.17033	5.699752	2.718271	5.674047	2.4563
5ºC (Test reactor)							
Time (min)	Absorbance/MLSS	T protein	T Humus	T Carbohydrate	D protein	D Humus	D Carbohydrate
0	0.014033	4.920468	3.043831	1.698216	0.830252	2.582919	1.035858
20	0.031283	8.359305	3.878642	2.397901	0.847105	2.764618	1.429432
40	0.040442	9.747275	4.485129	2.823632	1.046484	2.920418	1.595171
60	0.047453	10.94394	4.634357	3.375789	1.250113	2.947625	1.39745
80	0.051714	12.90286	5.605166	3.705989	1.164873	3.005025	1.614429
100	0.05887	14.33707	6.822639	4.130915	1.354433	3.232181	1.324691
120	0.065747	14.69141	7.448032	4.736162	1.363138	3.562383	1.493681
20ºC (Reference reactor of 10ºC)							
Time (min)	Absorbance/MLSS	T protein	T Humus	T Carbohydrate	D protein	D Humus	D Carbohydrate
0	0.009562	4.690419	3.764621	1.600151	1.136243	2.302602	0.843195
20	0.023329	8.286487	4.432069	2.210502	1.028345	2.76143	0.979088
40	0.032787	9.804564	5.532904	2.654338	1.258415	2.620457	1.028632
60	0.043383	12.34575	6.511045	3.368248	1.135041	3.325358	1.068323
80	0.05461	14.5154	7.645797	3.837032	1.190254	3.39874	1.164508
100	0.063825	15.77956	9.02414	4.407112	1.099676	4.361101	1.168375
120	0.07697	17.16329	10.8491	5.135613	1.290092	4.440596	1.273579
10ºC (Test reactor)							
Time (min)	Absorbance/MLSS	T protein	T Humus	T Carbohydrate	D protein	D Humus	D Carbohydrate
0	0.009464	5.215854	3.509607	1.591828	0.935322	2.084297	0.684115
20	0.02553	9.559691	4.54958	2.610043	1.012869	2.031723	0.83161
40	0.035427	10.6034	5.256579	2.946071	1.006052	2.164698	0.957434
60	0.042376	12.12421	5.799117	3.327942	1.120166	2.391013	0.849147
80	0.046469	13.09648	6.525908	3.730007	1.166677	2.446528	0.935264
100	0.048945	11.9754	7.238013	3.885938	1.193983	2.39994	1.061087
120	0.055017	13.1749	7.926169	4.075889	1.229949	2.885678	1.071673
20ºC (Reference reactor of 15ºC)							
Time (min)	Absorbance/MLSS	T protein	T Humus	T Carbohydrate	D protein	D Humus	D Carbohydrate
0	0.009373	4.694956	4.878973	1.504938	1.773231	2.266634	1.078586
20	0.026582	8.552797	8.005378	2.534344	1.4405	2.739409	1.406201
40	0.038755	13.27125	7.288008	3.300653	1.925687	3.051586	1.57622
60	0.050476	14.6152	9.004624	3.901379	1.870051	3.55854	1.624272
80	0.061065	15.82629	10.13975	4.432297	1.828462	4.338524	1.770539
100	0.074002	19.47168	11.73824	5.176348	2.02247	5.214579	1.656802
120	0.084595	22.91282	13.34434	5.289231	1.870088	5.419929	2.026349

15ºC (Test reactor)							
Time (min)	Absorbance/MLSS	T protein	T Humus	T Carbohydrate	D protein	D Humus	D Carbohydrate
0	0.010268	7.159787	4.275303	2.150231	1.371369	2.959265	1.29374
20	0.02453	11.36259	5.412685	2.745014	1.35758	3.420157	1.24824
40	0.03627	14.09817	6.907047	3.106445	1.496155	3.722366	1.377057
60	0.045044	15.55816	7.654848	3.633981	1.634693	4.024801	1.382995
80	0.054275	17.74673	9.059779	4.199716	1.842454	4.339119	1.48285
100	0.061916	17.11366	10.37803	4.510711	1.953284	4.796964	1.56325
120	0.066035	19.09839	10.16026	4.70263	1.509889	5.222921	1.439277
20ºC (Reference reactor of 30ºC)							
Time (min)	Absorbance/MLSS	T protein	T Humus	T Carbohydrate	D protein	D Humus	D Carbohydrate
0	0.012053	5.915846	4.828804	2.194107	1.240459	4.306875	1.086867
20	0.031696	10.50227	6.46757	3.008443	1.572732	4.549497	1.309205
40	0.048269	13.54886	7.518432	3.530952	1.441675	5.409512	1.272794
60	0.060949	15.83052	9.339427	4.158233	1.385656	6.109075	1.400004
80	0.077854	18.86697	10.85798	4.809961	2.060007	6.708477	1.581375
100	0.088504	19.44089	12.64798	5.150071	2.344981	7.219766	1.592983
120	0.099616	21.90695	13.89685	5.794703	2.463524	7.638147	1.714048
30ºC (Test reactor)							
Time (min)	Absorbance/MLSS	T protein	T Humus	T Carbohydrate	D protein	D Humus	D Carbohydrate
0	0.019478	7.918795	5.90168	2.658114	1.473751	4.985869	1.242074
20	0.044252	14.0812	7.840875	3.74894	1.356788	6.125357	1.501503
40	0.072925	19.93114	10.52171	5.075992	2.166534	7.513049	1.63531
60	0.091977	24.2158	12.37071	5.668127	3.014905	8.59286	1.80462
80	0.108881	24.4539	16.19488	6.481798	3.386301	9.414328	1.949128
100	0.119094	26.00097	17.89603	6.975167	3.741389	10.4477	2.19831
120	0.129093	27.7654	19.51831	7.377771	5.031407	10.4888	2.288428

Appendix (VI): The ratio between the biopolymers in the supernatant after 2hr in the reference and test reactors under both aerobic and anaerobic conditions

Ratio between biopolymers under Aerobic conditions						
	Ratio between total biopolymers			Ratio between dissolved biopolymers		
Temperature	Total protein	Total Humus	Total Carbohydrate	Dissolved protein	Dissolved humus	Dissolved Carbohydrate
Ref. 5°C	3.004077	1.60988	1	0.955661	1.730924	1
5°C	3.348191	1.463408	1	0.94952	1.768298	1
Ref.10°C	2.696692	1.76671	1	0.915842	1.569519	1
10°C	2.90135	1.567601	1	0.610627	2.414555	1
Ref.15°C	2.701977	1.238242	1	1.249366	1.856337	1
15°C	2.64381	1.319222	1	1.196388	1.337592	1
Ref.30°C	2.57988	1.468501	1	1.334174	1.719105	1
30°C	2.545087	1.242657	1	0.922361	0.942817	1
Ratio between biopolymers under anaerobic conditions						
	Ratio between total biopolymers			**Ratio between dissolved biopolymers**		
Temperature	Total protein	Total Humus	Total Carbohydrate	Dissolved protein	Dissolved humus	Dissolved carbohydrate
Ref. 5°C	3.109486	1.959793	1	1.106653	2.309997	1
5°C	3.101965	1.572588	1	0.912604	2.384969	1
Ref. 10°C	3.342014	2.112523	1	1.012966	3.486707	1
10°C	3.2324	1.944648	1	1.14769	2.692685	1
Ref. 15°C	4.331976	2.522927	1	0.922885	2.674726	1
15°C	4.061215	2.160548	1	1.04906	3.628849	1
20°C(Ref.30°C)	3.780513	2.398199	1	1.437255	4.456203	1
30°C	3.763385	2.645556	1	2.19863	4.583406	1

Appendix (VII): Values of dissolved oxygen, pH and conductivity at the end of each experiment

Aerobic conditions	1st Experiment				2nd Experiment		
	Temperature (ºC)	DO(mg/L)	pH	Conductivity (µS/cm)	DO (mg/L)	pH	Conductivity (µS/cm)
Reference reactor of 5 ºC	20	5	7.05	632	4.5	7.21	928
Test reactor	5	10.9	7.25	651	10.6	7.52	959
Reference reactor of 10 ºC	20	6	7.33	899	2.7	7.32	969
Test reactor	10	9.4	7.49	910	8.2	7.47	977
Reference reactor of 15 ºC	20	3.2	7.12	778	5.2	7.15	933
Test reactor	15	6.4	7.26	784	7.8	7.39	930
Reference reactor	20	4.2	7.21	1068	4.2	7.2	965
Test reactor	30	2.5	7.29	1070	3.5	7.25	970
Anaerobic conditions	1st Experiment			2nd Experiment			
	Temperature (ºC)	DO (mg/L)	pH	Conductivity (µS/cm)	DO (mg/L)	pH	Conductivity (µS/cm)
Reference reactor	20	0.2	8.22	850	0.2	8.1	959
Test reactor	5	0.1	8.33	867	0.4	8	976
Reference reactor	20	0.02	8.1	988	0.02	8.11	1691
Test reactor	10	0.03	8.07	994	0.004	8	1709
Reference reactor	20	0.02	7.5	790	0.03	8.1	929
Test reactor	15	0.02	7.94	773	0.02	8.28	926
Reference reactor	20	0.03	8.18	996	0.03	8.15	1019
Test reactor	30	0.03	8.26	986	0.03	8.26	1011

Appendix (VIII): Average concentration of ions (mg/g MLSS) in the total sludge

	Aerobic conditions						**Anaerobic conditions**					
Temp.	**Na**	**Mg**	**Al**	**K**	**Ca**	**Fe**	**Na**	**Mg**	**Al**	**K**	**Ca**	**Fe**
5ºC	19.034	3.6528	12.25	18.69	15.267	124.66	29.217	4.272819	11.77	25.12743	12.385	135.17
10ºC	26.676	3.7419	11.11	23.62	8.1905	119.24	20.588	5.006719	12.34	18.76746	11.294	132.97
15ºC	27.564	4.3444	13.85	26.89	11.695	125.11	20.826	4.407822	13.99	24.22449	9.1169	141.1
30ºC	27.033	4.656	13.52	24.28	12.219	121.49	31.724	4.831177	14.75	33.07583	14.914	144.58

Appendix (IX): The concentration of ions (mg/L) in the supernatant of the duplicated experiments performed under aerobic conditions

	I	II	I	II	I	II	I	II	I	II	I	II
20ºC(Reference Reactor of 5ºC)												
Time (min)	Na	Na	Mg	Mg	Al	Al	K	K	Ca	Ca	Fe	Fe
0	95.81602	140.5187	5.30882	8.245455	0.042068	0.03706	13.39891	17.29353	15.59087	20.95616	0.003603	0.023428
60	100.8107	149.626	4.633318	7.51867	0.103793	0.07748	14.52096	18.92495	13.10803	18.55554	0.023308	0.07328
120	103.2666	151.9535	4.449973	7.300265	0.079538	0.074283	15.13066	19.3217	12.76263	18.00282	0.029575	0.023025
5ºC (Test Reactor)												
Time (min)	Na	Na	Mg	Mg	Al	Al	K	K	Ca	Ca	Fe	Fe
0	61.82792	147.4577	3.725353	9.1925	0.038115	0.05814	9.330705	17.54132	10.53988	22.43709	0.009895	0.01431
60	101.6073	152.1109	5.396055	8.54476	0.07148	0.068845	14.57466	18.93513	14.71398	20.11061	0.018805	0.026235
120	53.74614	152.6612	2.89656	8.283953	0.047105	0.067448	8.25054	19.28942	7.68018	19.17841	-0.00293	0.01958
20ºC (Reference Reactor of 10ºC)												
Time (min)	Na	Na	Mg	Mg	Al	Al	K	K	Ca	Ca	Fe	Fe
0	146.483	158.8645	8.898203	8.60195	0.050695	0.043603	18.95454	20.30403	20.90342	21.23464	0.02909	0.01617
60	148.1646	159.8153	8.044778	7.588975	0.060318	0.08455	19.88705	20.62129	18.2889	18.87195	0.0281	0.012623
120	147.8918	163.2553	7.600393	7.399165	0.062768	0.095305	19.75231	21.49717	17.17869	18.35699	0.004293	0.013588
10ºC (Test Reactor)												
Time (min)	Na	Na	Mg	Mg	Al	Al	K	K	Ca	Ca	Fe	Fe
0	142.7957	157.6621	9.129945	9.007455	0.05529	0.065503	18.13441	19.64585	21.12486	22.81311	0.010043	0.039863
60	146.8164	161.2961	8.708535	8.404983	0.072198	0.076656	19.36539	20.59541	19.78882	19.88564	0.029308	0.026635
120	147.6172	144.0445	8.489345	7.513273	0.0586	0.086668	19.43543	21.00268	18.74089	19.42608	0.003575	0.01847
20ºC (Reference Reactor of 15ºC)												
Time (min)	Na	Na	Mg	Mg	Al	Al	K	K	Ca	Ca	Fe	Fe
0	123.0454	139.0896	7.169945	8.517748	0.053155	0.06115	19.09261	18.8601	21.86609	22.93229	0.013928	0.006548
60	122.2354	155.0819	6.339055	8.446485	0.108313	0.114703	19.46353	21.42005	19.143	21.95651	0.012775	0.007255
120	124.7254	152.0793	6.201618	8.26885	0.115638	0.099705	20.32668	21.06605	18.74355	21.30061	0.017058	0.007728
15ºC (Test Reactor)												

Time (min)	Na	Na	Mg	Mg	Al	Al	K	K	Ca	Ca	Fe	Fe
0	120.8962	156.0155	7.125395	9.583635	0.06897	0.045855	19.40616	21.13809	21.4364	25.672	0.00622	0.005083
60	111.8428	157.5448	6.112463	8.933263	0.08764	0.101328	17.28393	21.75677	17.95326	23.11977	0.00527	0.004752
120	117.4768	157.2959	6.215695	8.687915	0.104033	0.106498	18.78674	21.68335	18.20816	22.22483	0.015505	0.00184
20ºC (Reference Reactor of 30ºC)												
Time (min)	Na	Na	Mg	Mg	Al	Al	K	K	Ca	Ca	Fe	Fe
0	181.4167	164.7379	10.04073	9.138575	0.07555	0.043145	20.19553	21.13243	23.66542	23.94417	0.00532	0.014185
60	185.0601	163.9059	9.274263	8.157	0.095723	0.093218	20.8872	21.44869	21.09238	20.70882	0.010283	-0.00361
120	183.5736	166.2722	8.917035	7.954463	0.101093	0.099435	20.88263	21.73157	20.25243	20.11162	-0.00627	0.002688
30ºC (Test Reactor)												
Time (min)	Na	Na	Mg	Mg	Al	Al	K	K	Ca	Ca	Fe	Fe
0	186.2797	162.0016	10.01507	8.702725	0.15087	0.064893	21.48066	21.47508	24.20285	23.45413	0.018295	0.033113
60	185.8998	166.3112	8.844483	7.8002	0.104615	0.097788	21.61152	22.36579	20.58831	20.01477	0.01269	0.014725
120	188.2155	168.5651	8.758293	7.698358	0.105285	0.09551	22.36695	22.5826	20.21064	19.62192	0.002623	0.02337

Appendix (X): The concentration of ions (mg/l) in the supernatant of the duplicated experiments performed under anaerobic conditions

20ºC (Reference Reactor of 5ºC)												
	I	II	I	II	I	II	I	II	I	II	I	II
Time (min)	Na	Na	Mg	Mg	A	Al	K	K	Ca	Ca	Fe	Fe
0	132.9732	116.9981	7.892848	7.148373	0.061075	0.036435	23.22232	21.96067	20.67676	19.40639	0.293128	0.182845
60	132.2204	148.6423	6.090355	7.44514	0.121265	0.173595	26.01813	31.18653	12.98433	16.39211	0.303763	0.14495
120	130.6425	150.178	5.328633	6.978888	0.197245	0.110378	26.98947	32.93566	10.2494	13.406	0.185343	0.195803
5ºC (Test Reactor)												
Time (min)	Na	Na	Mg	Mg	Al	Al	K	K	Ca	Ca	Fe	Fe
0	130.3854	145.7639	8.295648	8.934608	0.049515	0.041558	22.23593	26.07868	21.87552	24.0755	0.129003	0.089183
60	131.6635	148.7036	6.739755	7.589293	0.069178	0.05855	23.30815	26.77984	15.71234	19.43705	0.11916	0.075035
120	133.6614	150.3327	6.234018	7.397765	0.136838	0.06487	24.7043	27.20157	13.67108	18.69881	0.13771	0.070188
20ºC (Reference Reactor of 10ºC)												

Time (min)	Na	Na	Mg	Mg	Al	Al	K	K	Ca	Ca	Fe	Fe
0	151.1813	281.6633	9.007833	24.24361	0.04336	0.069218	25.31195	25.87046	22.25649	26.12693	0.172043	0.174138
60	155.0306	276.4796	7.995055	21.07758	0.110075	0.101393	29.06015	26.17269	16.28746	20.84543	0.140795	0.093838
120	155.5739	292.712	7.456093	21.69738	0.127365	0.142525	30.82038	30.75798	13.49395	19.37442	0.12125	0.08955
10ºC (Test Reactor)												
Time (min)	Na	Na	Mg	Mg	Al	Al	K	K	Ca	Ca	Fe	Fe
0	151.496	282.5775	8.913575	24.42277	0.04885	0.099738	24.57217	25.298	21.80167	25.91379	0.125193	0.106765
60	152.905	225.2842	7.94658	12.38617	0.101408	0.059233	25.96808	14.73739	17.11733	12.40875	0.153975	0.097295
120	156.6839	289.6421	7.6551	22.38791	0.100883	0.07865	26.65666	27.89735	16.12719	20.59781	0.093583	0.085633
20ºC (Reference Reactor of 15ºC)												
Time (min)	Na	Na	Mg	Mg	Al	Al	K	K	Ca	Ca	Fe	Fe
0	121.6507	148.2102	7.648833	8.424398	0.06037	0.105148	18.1175	22.91427	17.48648	18.76153	0.059433	0.081408
60	123.9406	150.3306	7.027953	7.271108	0.157535	0.21163	20.1636	25.19374	13.38728	13.78743	0.09763	0.065588
120	123.8745	150.7956	6.813863	6.951015	0.205118	0.253158	21.67079	26.97784	11.79539	11.91678	0.107785	0.06345
15ºC (Test Reactor)												
Time (min)	Na	Na	Mg	Mg	Al	Al	K	K	Ca	Ca	Fe	Fe
0	119.9635	151.8578	7.766175	8.765628	0.083103	0.09077	17.8366	23.14286	17.83105	19.76307	0.051983	0.060123
60	123.759	149.6862	6.648453	7.363963	0.101955	0.150588	18.81982	24.23043	13.41702	14.45912	0.039203	0.05204
120	124.717	154.4003	5.965265	7.296373	0.101723	0.162575	19.1383	26.57633	11.7448	12.7762	0.036753	0.066265
Reference Reactor of 30ºC(A)												
Time (min)	Na	Na	Mg	Mg	Al	Al	K	K	Ca	Ca	Fe	Fe
0	161.3775	172.335	8.90062	8.70459	0.186985	0.0994	24.89475	24.69442	20.71667	21.88572	0.113053	0.101733
60	167.2616	175.044	8.225063	7.847215	0.404308	0.310593	27.96121	27.13008	16.67774	17.20068	0.07647	0.097465
120	160.715	173.2609	7.99239	7.044005	0.591425	0.484333	28.50536	28.84171	15.9727	13.43259	0.110248	0.0853
Test Reactor 30ºC(B)												
Time (min)	Na	Na	Mg	Mg	Al	Al	K	K	Ca	Ca	Fe	Fe
0	164.1148	172.678	8.86932	8.48461	0.16089	0.098	26.73193	25.84329	20.14777	20.66592	0.164063	0.10215
60	167.3063	171.6506	7.695123	6.89318	0.522935	0.453743	30.10954	28.75508	14.55497	13.55135	0.069853	0.058153
120	160.4228	171.3466	6.847178	6.288013	0.634085	0.616323	29.92011	30.28081	11.81108	11.07396	0.091578	0.049225

Appendix (XI): The average concentration of ions (mg/L) in the supernatant of experiments performed under aerobic conditions

20ºC (Reference reactor of 5ºC)						
Time (min)	Na	Mg	Al	K	Ca	Fe
0	118.1673	6.777138	0.039564	15.34622	18.27352	0.013515
60	125.2183	6.075994	0.090636	16.72295	15.83178	0.048294
120	127.6101	5.875119	0.07691	17.22618	15.38272	0.0263
5ºC (Test reactor)						
Time (min)	Na	Mg	Al	K	Ca	Fe
0	104.6428	6.458926	0.048128	13.43601	16.48848	0.012103
60	126.8591	6.970408	0.070163	16.7549	17.4123	0.02252
120	103.2037	5.590256	0.057276	13.76998	13.42929	0.008324
20ºC (Reference reactor of 10ºC)						
Time (min)	Na	Mg	Al	K	Ca	Fe
0	152.6737	8.750076	0.047149	19.62928	21.06903	0.02263
60	153.9899	7.816876	0.072434	20.25417	18.58043	0.020361
120	155.5736	7.499779	0.079036	20.62474	17.76784	0.00894
10ºC (Test reactor)						
Time (min)	Na	Mg	Al	K	Ca	Fe
0	150.2289	9.0687	0.060396	18.89013	21.96899	0.024953
60	154.0562	8.556759	0.154378	19.9804	19.83723	0.194858
120	145.8308	8.001309	0.072634	18.96406	19.08348	0.011023
20ºC (Reference reactor of 15ºC)						
Time (min)	Na	Mg	Al	K	Ca	Fe
0	131.0675	7.843846	0.057153	18.97635	22.39919	0.010238
60	138.6587	7.39277	0.111508	20.44179	20.54976	0.010015
120	138.4024	7.235234	0.107671	20.69636	20.02208	0.012393
15ºC (Test reactor)						
Time (min)	Na	Mg	Al	K	Ca	Fe
0	138.4559	8.354515	0.057413	20.27212	23.5542	0.013525
60	134.6938	7.522863	0.094484	19.52035	20.53652	0.021395
120	137.3864	7.451805	0.105265	20.23504	20.21649	0.008673
20ºC (Reference reactor of 30ºC)						
Time (min)	Na	Mg	Al	K	Ca	Fe
0	173.0773	9.589654	0.059348	20.66398	23.8048	0.009753
60	174.483	8.715631	0.09447	21.16795	20.9006	0.003339
120	174.9229	8.435749	0.100264	21.3071	20.18203	-0.00179
30ºC (Test reactor)						
Time (min)	Na	Mg	Al	K	Ca	Fe
0	174.1407	9.358898	0.107881	21.47787	23.82849	0.025704
60	176.1055	8.322341	0.101201	21.98866	20.30154	0.013708
120	178.3903	8.228325	0.100398	22.47477	19.91628	0.012996

Appendix (XII): The average concentration of ions (mg/l) in the supernatant of experiments performed under anaerobic conditions

20ºC (Reference reactor of 5ºC)						
Time (min)	Na	Mg	Al	K	Ca	Fe
0	124.9856	7.52061	0.048755	22.59149	20.04158	0.237986
60	140.4313	6.767748	0.14743	28.60233	14.68822	0.224356
120	140.4102	6.15376	0.153811	29.96256	11.8277	0.190573
5ºC (Test reactor)						
Time (min)	Na	Mg	Al	K	Ca	Fe
0	138.0747	8.615128	0.045536	24.15731	22.97551	0.109093
60	140.1835	7.164524	0.063864	25.04399	17.57469	0.097098
120	141.9971	6.815891	0.100854	25.95294	16.18495	0.103949
20ºC (Reference reactor of 10ºC)						
Time (min)	Na	Mg	Al	K	Ca	Fe
0	216.4223	16.62572	0.056289	25.5912	24.19171	0.17309
60	215.7551	14.53632	0.105734	27.61642	18.56645	0.117316
120	224.143	14.57674	0.134945	30.78918	16.43418	0.1054
10ºC (Test reactor)						
Time (min)	Na	Mg	Al	K	Ca	Fe
0	217.0367	16.66817	0.074294	24.93509	23.85773	0.115979
60	155.0946	10.16638	0.08032	20.35273	14.76304	0.146346
120	223.163	15.0215	0.139766	27.277	18.3625	0.089608
20ºC (Reference reactor of 15ºC)						
Time (min)	Na	Mg	Al	K	Ca	Fe
0	134.9304	8.036615	0.082759	20.51589	18.124	0.07042
60	137.1356	7.14953	0.184583	22.67867	13.58735	0.081609
120	137.3351	6.882439	0.229138	24.32431	11.85608	0.085618
15ºC(Test reactor)						
Time (min)	Na	Mg	Al	K	Ca	Fe
0	135.9107	8.265901	0.086936	20.48973	18.79706	0.056053
60	136.7226	7.006208	0.126271	21.52513	13.93807	0.045621
120	139.5586	6.630819	0.132149	22.85731	12.2605	0.051509
20ºC (Reference reactor of 30ºC)						
Time (min)	Na	Mg	Al	K	Ca	Fe
0	166.8562	8.802605	0.143193	24.79459	21.3012	0.107393
60	172.1528	8.036139	0.35745	27.54564	16.93921	0.086968
120	166.9879	7.518198	1.137879	28.67354	14.70264	4.527774
30ºC (Test reactor)						
Time (min)	Na	Mg	Al	K	Ca	Fe
0	168.3964	8.676965	0.129445	26.28761	20.40684	0.183106
60	169.4784	7.294151	0.463339	29.43231	14.05316	0.064003
120	165.8847	6.567595	0.625204	30.10046	11.44252	0.070401