

**INVESTIGATION OF SODIUM AND POTASSIUM IONS
IN RELATION TO BIOFLOCCULATION OF MIXED CULTURE
MICROORGANISMS**

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ABSTRACT

INVESTIGATION OF SODIUM AND POTASSIUM IONS IN RELATION TO BIOFLOCCULATION OF MIXED CULTURE MICROORGANISMS

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Bioflocculation happens naturally and microorganisms aggregate into flocs during wastewater treatment. It is critical to understand the mechanisms of bioflocculation and its impact on the following solid/liquid separation process since separation by settling is one of the key aspects that determine the efficiency and the overall economy of activated sludge systems. Bioflocculation occurs via extracellular polymeric substances (EPS) and cations by creating a matrix to hold various floc components together so the cations become an important part of the floc structure.

The main objective of this study is to investigate the effects of monovalent cations specifically potassium and sodium (K and Na) on the bioflocculation, settleability and dewaterability of activated sludge. The particular aim is to grow the mixed culture microorganisms in the presence of specific cation so that the

effect of cation on the stimulation of EPS production can be seen. In order to achieve this aim, semi-continuous reactors were separately operated at concentrations of 5, 10, and 20 meq/L of each cation with mixed culture bacteria and fed with synthetic feed medium representing influent to the activated sludge systems. Also, a control reactor at low cation dose was operated for each reactor set. The effective volume of the reactors was 2 L with 8 days of sludge residence time (SRT) and pH was kept at 7.7 ± 0.3 . The activated sludge reactors were operated until the reactors reached steady state and then related analyses were conducted.

It was found that addition of potassium and sodium ions at increasing concentrations resulted in increase in total polymer concentration. However, potassium ions promoted the synthesis of both polysaccharide and protein type polymers whereas sodium ions tended to stimulate production of protein type polymers and had an affinity to bind more protein within the floc structure. Sodium sludges had lower hydrophobicity and higher surface charges, so sodium ions led to deterioration in flocculation of sludges. Addition of both these ions decreased the dewaterability, sodium ions had more detrimental effect on dewaterability of sludges compared to potassium ions. The examination of data related to settleability showed that potassium ions led to no drastic deterioration in settling characteristics of the activated sludge but the addition of sodium ions deteriorated the settleability. In addition, it was seen that while the addition of potassium ions to the feed led to a decrease in viscosity, increase in sodium concentration correlated with an increase in viscosity. Finally, the comparison of chemical oxygen demand (COD) removal efficiency of these cations showed that sodium is more efficient in COD removal.

Key words: Activated sludge, bioflocculation, dewaterability, extracellular polymeric substances, potassium, settleability, sodium, viscosity.

ÖZ

SODYUM VE POTASYUM İYONLARININ KARIŞIK KÜLTÜR MİKROORGANİZMALARININ YUMAKLAŞMASINA ETKİLERİNİN İNCELENMESİ

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Biyoflokülasyon, mikroorganizmaların atıksu arıtımı sırasında doğal olarak yumaklar oluşturmasıdır. Yumaklaşma ve bunu takiben oluşan çamurun arıtılmış sudan çöktürülerek uzaklaştırılmasını etkileyen mekanizmaların anlaşılması, aktif çamur sistemlerinin verimini ve toplam arıtım maliyetini belirleme de önemli rol oynamaktadır. Yumaklaşma farklı yumak bileşenlerinin bir arada tutulmasını sağlayan hücre dışı polimerler (HDP) ve katyonların meydana getirdiği bir matriks ile sağlanmaktadır ve dolayısıyla katyonlar yumak yapısının önemli bir parçası haline gelmektedirler.

Bu çalışmanın amacı tek değerlikli özellikle potasyum ve sodyum (K ve Na) iyonlarının aktif çamurun yumaklaşma, çökme ve susuzlaştırma özelliklerine olan etkilerini araştırmaktır. Çalışma özellikle karışık kültür mikroorganizmalarını belirli bir katyon varlığında büyütürken katyonun HDP

sentezine olan etkilerini de görmeyi hedeflemektedir. Bu amaçla her set için 5, 10 ve 20 meq/L katyon konsantrasyonlarında, aktif çamur sistemine benzer koşulları sağlamak amacıyla karışık kültür mikroorganizmaları ve sentetik besi ortamı kullanılarak yarı-sürekli reaktörler işletilmiştir. Aynı zamanda her set için ilgili katyonu düşük dozda içeren kontrol reaktörü çalıştırılmıştır. 2 L'lik hacime sahip reaktörlerin çamur yaşı 8 gün olarak ayarlanmıştır ve pH'ları 7.7 ± 0.3 civarında tutulmuştur. Aktif çamur reaktörleri kararlı hale ulaşmaya kadar çalıştırılmış ve sonra ilgili analizler gerçekleştirilmiştir.

Sodyum ve potasyum iyonlarının artan konsantrasyonlarda eklenmesi toplam polimer miktarında artışa neden olmuştur. Ancak, potasyum iyonları polisakkarit ve protein tipi polimer olmak üzere her iki tip polimerin sentezlenmesini teşvik ederken, sodyum iyonları protein tipi polimerleri bağlama eğiliminde olup bu tip polimerlerin üretimini teşvik ettiği ortaya çıkmıştır ve dolayısıyla yumak yapısında da bu tip polimerlerin daha baskın olarak yer aldığı görülmüştür. Sodyum çamurlarının daha düşük hidrofobisiteye ve yüzey yüküne sahip oldukları belirlenmiştir. Aynı zamanda, sodyum ve potasyum iyonlarının eklenmesi çamurun filtrelenebilme yeteneğini azaltmıştır ve sodyum iyonlarının filtrelenebilme özelliği üzerinde daha fazla kötüleştirici etkisinin olduğu ortaya çıkmıştır. Çamurun çökelebilirliği ile ilgili testlere ait sonuçlar incelendiğinde potasyum iyonları çamurun çökme özelliğinde herhangi bir kötüleşmeye neden olmazken, sodyum iyonlarının eklenmesi çamurun çökme özelliğini bozmuştur. Ayrıca potasyum iyonlarının eklenmesi çamurun viskozitesini azaltırken, sodyum iyonu konsantrasyonundaki artış çamur örneklerinin viskozite değerlerindeki artışla kolerasyon göstermiştir. Son olarak, potasyum ve sodyum iyonlarının kimyasal oksijen ihtiyacı (KOİ) giderim verimleri karşılaştırıldığında sodyum iyonlarının KOİ gideriminde daha etkili oldukları ortaya çıkmıştır.

Anahtar kelimeler: Aktif çamur, yumaklaşma, filtrelenebilme, hücre dışı polimerler, potasyum, çökme, sodyum, viskozite.

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LIST OF ABBREVIATIONS

BOD	: Biochemical oxygen demand, mg/L
C/N	: Carbon to Nitrogen ratio
CER	: Cation exchange resin
COD	: Chemical oxygen demand, mg/L
COD:N:P	: Chemical Oxygen Demand to Nitrogen to Phosphorus ratio
CST	: Capillary suction time, sec
DCB	: Divalent cation bridging theory
DLVO	: Derjaguin, Landau, Verwey and Overbeek theory
DNA	: Deoxyribo nucleic acid
DO	: Dissolved oxygen, mg/L
EDTA	: Ethylenediaminetetraacetic acid
EGTA	: Ethyleneglycoltetraacetic acid
EPS	: Extracellular polymeric substances, mg/L
EPS_c	: Carbohydrate constituent of EPS, mg/L
EPS_p	: Protein constituent of EPS, mg/L
LPS	: Lipopolysaccharide
MATH	: Microbial adhesion to hydrocarbons
MLSS	: Mixed liquor suspended solids, mg/L
MLVSS	: Mixed liquor volatile suspended solids concentration, mg/L
OD	: Optical density
PBS	: Phosphate buffer saline
RNA	: Ribo nucleic acid
SRF	: Specific resistance to filtration, m/kg
SRT	: Sludge residence time
SVI	: Sludge volume index, mL/g
TKN	: Total Kjeldahl Nitrogen

VSS : Volatile suspended solids, mg/L
ZSV : Zone settling velocity, cm/sec

CHAPTER 1

INTRODUCTION

Activated sludge process is the most commonly used biological process for secondary wastewater treatment. In this process, microorganisms in wastewater are grown in flocculated form, converting organic matter into carbon dioxide, ammonia-N, and new biomass to remove organic matter from the influent wastewater. The quality of the effluent from activated sludge treatment plants is highly dependent on the efficiency of the solid-liquid separation process. The separation of solid biomass from sludge liquid in a clarifier can be achieved by good bioflocculation.

Activated sludge flocs are composed of microorganisms, debris, extracellular polymeric substances (EPS) and inorganic cations (Eriksson and Alm, 1991; Bruus *et al.*, 1992; Higgins and Novak, 1997a,b). Bacteria are the most effective and predominant organisms in activated sludge, and protozoa, fungi, filamentous microorganisms and viruses are present. Specific filamentous microorganisms form a skeleton-like structure for the flocs as hypothesized by the backbone theory (Parker *et al.*, 1972; Sezgin *et al.*, 1978) but the predominance of filamentous bacteria over other organisms eventually leads to sludge bulking and foam formation contributing to the activated sludge settling problems (Soddell and Seviour, 1990). Some studies performed to monitor the population dynamics under stable environmental conditions (Fernandez *et al.*, 1999; Kaewpipat and Grady, 2002) but many characteristics of the biological treatment system remain poorly understood due to the high sensitivity of the microorganisms to environmental changes.

The extracellular polymeric substances (EPS) are the major constituent of the floc structure and can originate from two possible sources: microbial activity such as metabolism and cell lysis and wastewater influent (Urbain *et al.*, 1993; Dignac *et al.*, 1998). The EPS which is made up of protein, polysaccharide, lipids and nucleic acids provide a biopolymer matrix for enmeshment of the microbes and cations. The biopolymers have functional groups such as phosphate and carboxyl contributing negative charges to the floc structure to enhance bioflocculation. A number of studies have focused on understanding of the composition and amount of EPS in order to reveal the influence of EPS on settling, dewatering and effluent quality. However, variation in extraction procedures and use of different activated sludge sources lead to conflicting results in literature (Brown and Lester, 1979; Morgan *et al.*, 1990; Frolund *et al.*, 1996; Higgins and Novak, 1997a,c; Shin *et al.*, 2001).

Although multivalent cations become an important component of floc structure by providing bridging of the negative sites on the biopolymer network (Bruus *et al.*, 1992; Urbain *et al.*, 1993; Higgins and Novak, 1997a), monovalent cations should be taken into consideration because previous studies revealed that excess concentration of monovalent cations leads to deterioration in settleability, dewaterability of sludges and effluent quality of the system (Higgins and Novak, 1997a; Novak *et al.*, 1998; Sobeck and Higgins, 2002). Also, Higgins and Novak (1997b) suggested that the ratio of monovalent to divalent cations on a charge equivalent basis could be served as a rapid indicator of sludge characteristics.

Among the monovalent cations, especially sodium (Na) and potassium (K) have been studied to determine influence of monovalent cations on activated sludge system (Bruus *et al.*, 1992; Zita and Hermansson, 1994; Higgins and Novak, 1997a, b; Murthy and Novak, 1998, 2001; Sobeck and Higgins, 2002). Zita and Hermansson (1994) demonstrated that ionic strength of the solution should be considered as an important factor determining the floc stability and potassium and kalsiyum produced similar effects on floc stability, explained by the double

layer theory (DLVO). Likewise, Cousin and Ganczarczyk (1998) reported that addition of monovalent cations resulted in improvement in floc properties. On the other hand, Murthy and Novak (1998) showed that potassium addition improved the floc strength and settleability of sludge while it caused deterioration in dewatering property and effluent quality. Higgins and Novak (1997a,b) reported that sludge settling and dewatering properties deteriorated with increasing sodium addition so it is not supported by DLVO theory since the addition of sodium ions should improve floc properties according to this theory. Researchers explained the deterioration with divalent cation bridging theory (DCB) proposing the addition of monovalent cations displace the divalent cations within the floc structure by ion exchange, making flocs weak and fragile.

In majority of the studies reported (Higgins and Novak, 1997a,b), the role of cations in bioflocculation were conducted as short duration batch experiments but batch tests generally do not reveal the ultimate effect of cation additions on systems. Also, in many studies mono culture bacteria were used to illustrate the effect of monovalent cations on activated sludge. Actually, use of monoculture bacteria does not represent the situation in activated sludge systems because different types of microorganisms participate in systems so each of them contributes discrepant properties to the floc structure. Although a little number of studies used the mixed culture bacteria grown in sodium and potassium rich feed media separately, the variety in operational conditions and given feed media resulted in no consensus in results. In addition, activated sludge characteristics in relation to monovalent cation type and concentration have not been thoroughly understood yet.

Therefore, the objective of this study is to investigate the effects of sodium and potassium ions and their concentrations on flocculation, settleability and dewaterability of activated sludge in the highlight of their atomic properties and special functions in cell by operating semi-continuous activated sludge reactors. The mixed culture bacteria taken as a seed from the primary clarifier effluent of

Ankara Central Wastewater Treatment Plant were grown with rich synthetic feed media of each cation. After the reactors reached steady state, chemical analyses (EPS extraction, cation content of sludge, conductivity); surface chemical analyses (hydrophobicity, surface charge); and physical analyses related with settleability, dewaterability and rheology were conducted to examine the effect of sodium and potassium ions on bioflocculation.

CHAPTER 2

LITERATURE REVIEW

2.1. History and Principles of Activated Sludge Systems

Activated sludge systems represent the most widely used biological wastewater treatment processes. The activated sludge process is an aerobic (oxygen-rich), continuous-flow biological method for the removal of soluble (dissolved) organic materials which are absorbed through the cell walls of the microorganisms and into the cells and conversion of these materials into more microorganisms, carbon dioxide, water, and energy. Also, in this process, insoluble particles are adsorbed on the cell walls, transformed to a soluble form by enzymes (biological catalysts) secreted by the microorganisms, and absorbed through the cell wall, where they are also digested and used by the microorganisms in their life-sustaining processes (Junkins *et al.*, 1983; Corbitt, 1990).

The invention of the activated sludge process is connected with the efforts of British and American engineers at the beginning of the last century to intensify biological purification in fixed-film systems. Sewage treatment using the activated-sludge process evolved from the fill-and draw system which was developed by Arden and Lockett in 1914. They introduced a recycle of suspension formed during the aeration period (so called return sludge). The suspension, known as activated sludge, was in fact an active biomass responsible for the improvement of purification performance (Wanner, 1994).

The conventional activated sludge process (Figure 2.1) consists of an aeration basin in which air as an oxygen source is introduced to the system to provide

surviving of aerobic organisms which are responsible for the degradation of the organic materials and mixing of the activated sludge in suspension form. As the microorganisms are mixed, they collide with other microorganisms and stick together to form larger particles called flocs. The large flocs that are formed settle more readily than individual cells. These flocs also collide with suspended and colloidal materials (insoluble organic materials), which stick to the flocs and cause the flocs to grow even larger. The microorganisms digest these adsorbed materials, thereby re-opening sites for more materials to stick. The aeration basin is followed by a secondary (final) clarifier (settling tank) in which aggregated and suspended biomass are separated from the surrounding wastewater by bioflocculation and gravity settling. A portion of the settled microorganisms, referred to as return sludge, is recycled to the aeration basin to maintain an active population of microorganisms and an adequate supply of biological solids for the adsorption of organic materials. Excess sludge is wasted by being piped to separate sludge-handling processes. The liquids from the clarifier (effluent) are transported to facilities for disinfection and final discharge to receiving waters, or to tertiary treatment units for further treatment (Junkins *et al.*, 1983; Corbitt, 1990).

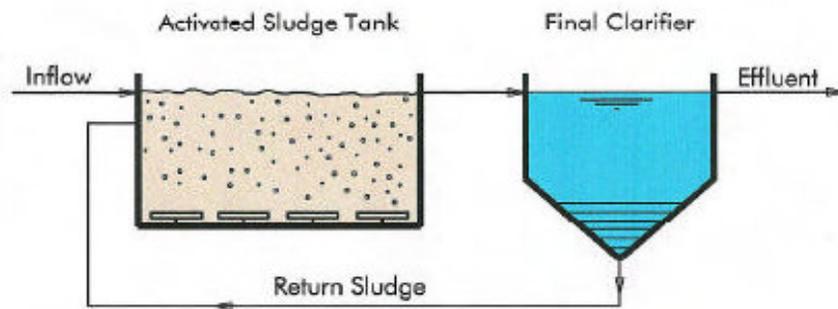


Figure 2.1. General representation of activated sludge process

2.2. Microbiology of Activated Sludge Systems

Bioflocculation is the natural tendency of microorganisms to aggregate as flocs and the success of biological wastewater treatment is dependent on the flocculation ability of microorganisms. The typical microbiology of activated sludge consists of approximately 95% bacteria and 5% other organisms (protozoa, rotifers, fungi, filamentous organisms, viruses etc.). The majority of the bacterial genera in activated sludge are Gram-negative. Although organisms present in activated sludge systems range from viruses to multicellular organisms, the predominant and most active are heterotrophic bacteria that include genera such as *Pseudomonas*, *Achromobacter*, *Flavobacterium*, *Bacillus*, *Alcaligenes*, *Artrobacter*, *Micrococcus*, *Citromonas* and *Zooglea*. These organisms utilize the organic substances present in wastewater as a carbon source for cell synthesis and as an energy source for reproduction, digestion, movement etc. (Ganczarzyk, 1983).

The majority of bacteria in activated sludge belong to gram negative genera. The cell wall structure of Gram-negative bacteria is composed of three distinct layers: Cytoplasmic membrane, peptidoglycan and outer membrane. The outer membrane is composed of phospholipids and lipopolysaccharides which face the external environment. The outer membrane is impermeable to macromolecules and allows only limited diffusion of hydrophobic substances through its lipopolysaccharide (LPS)-covered surface. The molecular basis of integrity of the outer membrane lies in its LPS. LPS binds cations, since it is polyanionic because of a number of negative charges in its lipid A and inner-core parts. The highly charged nature of lipopolysaccharides confers an overall negative charge to the Gram negative cell wall (Vaara, 1992).

Apart from the heterotrophic bacteria, autotrophic bacteria in activated sludge reduce oxidized carbon compounds such as carbon dioxide for cell growth. These bacteria obtain their energy by oxidizing ammonia nitrogen to nitrate

nitrogen in a two-stage conversion process known as nitrification. Due to the fact that very little energy is derived from these oxidization reactions, and because energy is required to convert carbon dioxide to cellular carbon, nitrifying bacteria represent a small percentage of the total population of microorganisms in activated sludge. In addition, autotrophic nitrifying bacteria have a slower rate of reproduction than heterotrophic, carbon-removing bacteria. Two genera of bacteria are responsible for the conversion of ammonia to nitrate in activated sludge, *Nitrobacter* and *Nitrosomonas* (UCLA College of Letters and Science, 2007).

Filamentous organisms are also part of the floc structure and form a backbone onto which EPS producing bacteria can attach (Parker *et al.* 1972; Sezgin *et al.*, 1978). When an activated sludge culture contains filamentous organisms, large floc sizes are possible because the filamentous organism backbone provides the floc with strength (Jenkins *et al.*, 1993). However, it was observed that a small increase in the number of filamentous organisms such as *Sphaerotilus*, *Geotrichum*, *Beggiatoa* and *Thiothrix* could cause activated sludge bulking and foam formation (Palm *et al.*, 1980; Soddell and Seviour, 1990). The observations suggest that a balance between the floc-forming and filamentous bacteria is necessary to obtain a good settling performance (Govoreanu *et al.*, 2003).

Protozoa are unicellular organisms that are important to public health and process microbiology in water and wastewater treatment plants. In these systems, many species of protozoa such as flagellates, motile ciliates, stalked ciliates, amoebae, rotifers and nematodes are most commonly observed. Protozoa are heterotrophic organisms that can absorb soluble food, which is transported across the cytoplasmic membrane. Also, the holozoic protozoa are capable of engulfing particles such as bacteria and colloidal matter (Ganczarczyk, 1983; Bitton, 1994). Protozoa are a useful biological indicator of the condition of the activated sludge. Being strict aerobes, these microorganisms prove to be excellent indicators of an aerobic environment (though some protozoa are capable of surviving up to 12

hours in the absence of oxygen). Protozoa also act as indicators of a toxic environment, as they exhibit a greater sensitivity to toxicity than bacteria. A clue that toxicity may be a problem in a system is the absence of or a lack of mobility of these organisms in activated sludge (UCLA College of Letters and Science, 2007).

Fungi are also a constituent of activated sludge. These eucaryotic organisms that produce long filaments called hyphae, which form a mass called mycelium metabolize organic compounds and can successfully compete with bacteria under certain environmental conditions in a mixed culture. They grow well under acidic conditions (pH=5) (Bitton, 1994). The most common sewage fungus organisms are *Sphaerotilus natans* and *Zoogloea* sp. (Curtis, 1969).

Additionally, viruses of human origin that are excreted in large quantities in feces (adenovirus, coxsackievirus, echovirus, infectious hepatitis, poliovirus, and reovirus) may be found in raw sewage influent, but a large percentage appear to be removed by the activated-sludge process (Grabow, 1968).

2.3. History of Bioflocculation

Since the beginning of research into biological waste treatment, bacterial flocculation has received a great deal of attention. Butterfield (1935) has firstly introduced the floc-forming bacteria, *Zooglea ramigera* which are extremely prevalent in activated sludge and it has been considered to contribute significantly to the development of a well flocculated sludge mass. Later studies by McKinney and Horwood (1952) and McKinney and Weichlein (1953) revealed that different species of bacteria are present in sludges and they are able to form flocs as well as floc-forming bacteria.

McKinkey (1952) proposed a theory of floc formation in which capsulated bacteria are flocculated by different chemical interactions between adjacent cells.

The interactions between cations and negatively charged bacterial surfaces provide reduction in net surface charge, which allows to cells to approach one another closely. Later on, while Pavoni *et al.* (1972) rejected this theory because non-capsulated bacteria which are capable of forming flocs have been investigated, Tezuka (1969) supported this approach. Although being studied with non-capsulated bacteria, *Flavobacterium*, Tezuka (1969) theorized that metallic cations could be bound to the negatively charged surfaces of microbial cells to form a bridge between them and thus cause flocculation to occur.

Bacterial flocculation has been explained as a colloid coagulation, irrespective of cell's inorganic or organic nature and dependent only on the neutralization of the negative charge on the cell surface (Atkinson and Daoud, 1976). Although bacterial surface charge plays a significant role in agglomeration of microorganisms, it can not be considered as the predominant mechanism in bioflocculation. Hence, in activated sludge processes, cell-cell attachment or bioflocculation has generally been considered to occur via secreted extracellular polymers that act either as bridges or via charge neutralization (Busch and Stumm 1968; Harris and Mitchell 1973; Treweek and Morgan 1977).

It is now widely accepted that microorganisms, extracellular polymeric substances (EPS) and cations are major components of activated sludge flocs (Bruus *et al.*, 1992; Higgins and Novak, 1997a) and the EPS form a matrix which encapsulates the microbes and aids in the aggregation of the microorganisms and floc formation (Urbain *et al.*, 1993; Dignac *et al.*, 1998; Sobeck and Higgins, 2002).

2.4. Extracellular Polymeric Substances (EPS)

Microbial extracellular polymeric substances (EPS) are the key components for the aggregation of microorganisms in flocs. Activated sludge EPS can be

contributed from two sources: One is from bacterial cells (active secretion, cell surface material shedding, cell lysis) and the other one is from wastewater (biosorption) (Harris and Mitchell, 1973; Urbain *et al.*, 1993; Dignac *et al.*, 1998; Wingender *et al.*, 1999). The metabolic products produced by bacteria are largely identified as proteins and polysaccharides, the lysed products are mainly proteins, polysaccharides, lipids and nucleic acids, and the influent wastewater polymers are humic acids and other introduced synthetic or organic polymers (Murthy, 1998).

EPS produced by the microorganisms exist as a tightly bound (capsular) EPS and soluble EPS (loosely adhered slimes and free dissolved matter). The bound EPS has a more organized polymeric structure, densely packed, less diffusible and tightly bound to the cell than slimes (Gehr and Henry, 1983; Hsieh *et al.*, 1994; Nielsen *et al.*, 1997; Bhaskar and Bhosle, 2005). Capsular EPS (or bound EPS) are held to the cell wall either by linkages between the carboxyl groups of EPS and hydroxyl groups of lipopolysaccharides (Sutherland, 1977) or by a covalent bonding through phospholipids (Roberts, 1996) and glycoproteins (Chester and Murray, 1978). Activated sludges contain capsular polymers attached to flocs at concentrations generally higher than those of the slime polymers so capsular EPS plays an important role in the sludge flocculation. The slime polymers are not involved in flocculation process because they remain in the dissolved or colloidal phases of an effluent (Brown and Lester, 1979; Gehr and Henry, 1983).

EPSs were identified as the third major components of the activated sludge floc matrix following microbial cells and water (Li and Ganczarczyk, 1990). The EPS form a three dimensional, gel-like, highly hydrated and often charged biofilm matrix. In general, the proportion of EPS in biofilm varies from 50 % to 90 % of the total organic matter (Flemming and Wingender, 2001). Costerton *et al.* (1978) and Fletcher and Marshall (1982) revealed that EPS excreted by microorganisms can enhance the survival and propagation of microorganisms in

natural environments. Most notably, they are involved in the attachment of cells to surfaces and their presence stabilizes the environment immediately around the cell by localizing extracellular enzymes and buffering the cell against quick ionic changes in the surrounding water (Decho, 1990). EPS also form a protective layer for the cells against the biocides, phagocytosis, amoebic attack, bacteriophage, desiccation and sudden changes of pH. They play a crucial role in sorbing exogenous nutrients, cations and dispersals (Wilkinson, 1958; Wingender *et al.*, 1999; Liu and Fang, 2003).

2.4.1. Composition of EPS

Characterization of extracted EPS has shown that EPS is composed of polysaccharides (neutral and acidic polysaccharides, lipopolysaccharides), proteins, lipids, nucleic acids (DNA, RNA) and humic compounds (Nishikawa and Kariyuma, 1968; Peter and Wuhrman, 1971; Riffaldi *et al.*, 1982; Vallom and McLouglin, 1984; Goodwin and Forster, 1985; Eriksson and Alm, 1991; Urbain *et al.*, 1993; Frolund *et al.*, 1996). Also, some exopolymers contain glycoproteins, glucosides and glucophosphates (Ford *et al.*, 1991).

It is widely accepted that polysaccharides, proteins and nucleic acids are the major components of EPS. There is no consensus about the predominant constituent of EPS. Most research on the characterization of extracellular biopolymers from activated sludge has focused on extracellular polysaccharides (Forster, 1971; Horan and Eccles, 1986; Morgan *et al.*, 1990; Bejar *et al.*, 1998) whereas many studies have reported that the extracellular protein concentration in activated sludge systems was greater than the exocellular polysaccharide concentration (Tenney and Verhoff, 1973; Brown and Lester, 1980; Barber and Veenstra, 1986; Eriksson and Alm, 1991; Urbain *et al.*, 1993; Higgins and Novak, 1997a,b; Jorand *et al.*, 1998).

It was suggested that a large number of exopolysaccharides are polyanionic due to presence of uronic acids (Forster, 1971; Brown and Lester, 1980; Horan and Eccles, 1986; Frolund *et al.*, 1996). Uronic acids, that are specific components of extracellular and cell wall material, have a carboxyl group substituted at the C-5 location. The carboxyl groups will be unprotonated at typical pH ranges found in activated sludge systems and therefore contribute to the negative charge of the flocs (Sobeck and Higgins, 2002).

Horan and Eccles (1986) purified exopolysaccharide fractions from five different effluent treatment works and revealed many similarities both in terms of monomer composition and molecular weight distribution. They were detected only five monomers, namely: glucose, galactose, mannose, glucuronic acid and galacturonic acid, and all the polysaccharide fractions were of high molecular weight, ranging from 300,000 to 2,000,000. They reported that the extracted extracellular polymers contained 66 % carbohydrate, 24 % nucleic acids and 10 % protein.

Hejzlar and Chudoca (1986) isolated polymers containing sugars, amino sugars, uronic acids and amino acids. These polymers obtained under three different growth conditions: during growth, starvation and decomposition of activated sludge microorganisms. The polymers are a mixture of heteropolysaccharides from the outer layers of microbial cells and composed of 28.7 % carbohydrate, 10.3 % amino sugars, 3.2 % uronic acids and 25.9 % amino acids.

It was found that only mucopolysaccharide composed of glucosamine, glucose, mannose, galactose, and rhamnose, which was only 10 % of the total polysaccharides isolated from the floc contributed to floc formation, whereas the other 90 % of the polysaccharides could not be shown to play a role in floc formation (Tago and Aida, 1977). Morgan *et al.* (1991) found that activated sludge EPS contain high concentrations of ribose and rhamnose. Rhamnose is a

basic constituent of bacterial cell wall lipopolysaccharides (Fox *et al.*, 1990) and ribose is released by RNA hydrolysis (Dignac *et al.*, 1998).

Bejar *et al.* (1998) studied with *H. eurihalina* strains and they found that EPS of *H. eurihalina* strains have the same types of neutral sugars but different composition in uronic acid, hexamines, acetyl and sulphate content. The dominant constituent of extracellular polymers is carbohydrate with the range of 31-44.1 % of total dry weight of EPS.

Glucose, galactose and rhamnose were found to be major constituents of EPS and the percentage of neutral sugars in EPS extracted by the combination of sonication with CER treatment was reported as 34.9 % glucose, % 16.9 galactose, 15.3 % rhamnose, 14 % mannose, % 6.1 fucose, 4.8 % ribose, 4.7 % arabinose and 3.3 % xylose (Dignac *et al.*, 1998).

The presence of large quantities of proteins surrounding the bacterial cells has already been pointed out in several studies concerning activated sludge (Urbain *et al.*, 1993; Frolund *et al.*, 1996; Higgins and Novak, 1997c). Several classes of proteins exist in the exocellular environment of the bacteria. These include extracellular enzymes, proteinaceous S-layers, lectins, intracellular protein from cell lysis or cell wall turnover, or polypeptide capsular material. Higgins and Novak (1997c) carried out amino acid sequence analyses in order to determine protein present in activated sludge samples. They revealed that the protein found in the exocellular biopolymer extract was a lectinlike protein. The binding site inhibition studies showed that the protein had lectinlike activity. Lectins are one of the most plausible types that could be involved in bioflocculation. The lectins produced by bacteria are typically located on appendages such as the pili and fimbriae of bacteria. The binding site of lectins is specific for certain sugar residues and binding is non-covalent and reversible. Many lectins produced by bacteria are specific for galactose, mannose, glucose, and fucose (or derivatives of these sugars) (Higgins and Novak, 1997c).

Frolund *et al.* (1996) indicated that activated sludge mainly consisted of protein (46-52 % of VSS), humic compounds (18-23 % of VSS) and carbohydrate (17 % of VSS). DNA and uronic acids were also found in the EPS.

Dignac *et al.* (1998) found that proteins were the major constituent of EPS, which was confirmed by pyrolysis/GC/MS analysis of freeze-dried samples. The protein predominance in EPS was confirmed since the main fragments obtained were characteristic of proteins: pyridine, methylpyridine, styrene, pyrrole, methylpyrrole, benzonitrile, indole and methylindole. P-cresol and phenol, characteristic of para-phenolic tyrosine, were found in EPS structure. The presence of furaldehyde and methylfurfural was attributed to neutral sugars and the presence of nucleic acids in EPS was confirmed by the large furfuryl alcohol peak. Amino sugars known as important constituents of bacterial cell walls also detected in EPS by the acetamide peak.

Bura *et al.* (1998) reported that protein was found to be the dominant component in both the CER-extracted and steam-extracted EPS. The protein and carbohydrate concentrations were identified as 162 and 12.7 mg/g VSS, respectively. Acidic polysaccharides (containing uronic acids) represented a small portion (4.5 mg/g VSS) of the polysaccharides found in the EPS. It was also found that α -D-mannosyl and α -D-glucosyl residues were predominant in the floc matrix of municipal activated sludge.

Wilen *et al.* (2003) determined EPS composition of seven wastewater treatment plants. For most sludges, protein was found as a major EPS component (19-45 %) followed by carbohydrates (7-32 %). The uronic acids made up 1-3 % of the EPS or 8-26 % of the carbohydrates.

Higgins and Novak (1997c) indicated that the extracted biopolymer protein samples are rich in the carboxyl containing groups such as aspartate (asx) and glutamate (glx). Also, a high proportion of amino acids with hydrophobic groups

such as glycine (gly) and alanine (ala) are present in the protein. The diacid amino acids, aspartic and glutamic acid were important constituents of EPS, as already reported by Morgan *et al.* (1991). The extracellular negatively charged functional groups are known to play an important role in floc structure. The dissociated carboxyl groups of diacid amino acids give a negative charge to proteins, and can be involved in electrostatic bonds with multivalent cations (Eriksson and Alm, 1991; Bruus *et al.*, 1992).

Dignac *et al.* (1998) measured amino acids of protein in activated sludges and found that carboxyl containing groups such as aspartic and glutamic acids accounted for a large portion in their protein samples. Alanine, leucine and glycine were other important amino acids in extracellular proteins. Their hydrophobic properties suggested that they are likely to be involved in hydrophobic bonds. Electrostatic and hydrophobic bonds are closely combined in flocs, as pointed out by Urbain *et al.* (1993).

As can be understood from the results of studies, there is no consensus about EPS quantity and composition.

2.4.2. Factors Affecting the Production of Extracellular Polymeric Substances

The EPS production and composition were found to vary between different full scale treatment systems as well as in different lab-grown cultures reflecting the importance of wastewater&feedwater composition and operation conditions for microbial communities and their response to environmental conditions. Several factors are known as affecting the quality and quantity of EPS.

One of the factors is the type of microorganism. Complex microbial communities are directly responsible for the effectiveness and success of the wastewater

treatment process (organic matter reduction, nitrogen and phosphorus removal). While some researchers studied with single type microorganism in order to investigate EPS production and composition, the others studied with mixed culture bacteria. According to type of microorganism, variation was observed in the composition of extracted EPS. For example, Brown and Lester (1980) indicated that concentration of hexose sugars was much higher in the *K. aerogenes* culture than in the activated sludges. Also, Bejar *et al.* (1998) revealed that EPS of *H. eurihalina* strains studied have the different composition in uronic acid and hexosamines.

The production of EPS is also dependent on the growth phase of the bacteria. Microorganisms secrete extracellular polymers during the endogenous or stationary phases of growth. (Ganczarzyk, 1983). Bhaskar and Bhosle (2005) also indicated that EPS production began in the late exponential phase and continued during the stationary phase. Tenney and Verhoff (1973) theorized that the ratio of extracellular polymer to microbial surface was not great enough to induce flocculation until the endogenous phase. The maximum amount of extracellular polymer was accumulated after cell multiplication had ceased; this was reflected in the increased diameter of the capsules during the endogenous phase of growth (Brown and Lester, 1979).

Sludge and EPS are usually characterized through measurements of sugars, proteins, nucleic acids and lipids by colorimetric methods (Dignac *et al.*, 1998). These methods, when applied on such complex high molecular weight organic compounds, are subject to interferences that can vary from one method to another, giving questionable results (Brown and Lester, 1980). For example, Bradford or Phenol-Sulphuric Acid (Lowry) method is widely used for protein analysis. Several studies showed that the Bradford method tended to underestimate the EPS_p (protein of EPS) content in activated sludge (Frolund *et al.*, 1996; Durmaz and Sanin, 2001) and biofilm (Nielsen *et al.*, 1997). Frolund *et*

al. (1996) reported that the Lowry method was more accurate for the EPSp analysis of activated sludge.

Another limitation of colorimetric methods is that a standard is used, for instance glucose for sugar measurement or bovine serum albumin for protein measurement. The results of the assays are dependent on a reference that could not be representative of the complexity of the organic matrix (Horan and Eccles, 1986; Dignac *et al.*, 1998). In addition, analysis considers only homopolymers, whereas organic matter is also composed of heteropolymers and complex structures (Dignac *et al.*, 1998).

A number of studies have focused on understanding the composition and quantity of EPS. A variety of extraction procedures and variation in sludges investigated have often brought controversy in defining its characteristics. EPS attached to the outer membrane (e.g. lipopolysaccharides in gram negative species) or to the peptidoglycan (e.g. teichoic acids in gram positive species) are too close to ensure that only non-covalently bound exocellular structures are removed by a defined extraction procedure (Urbain *et al.*, 1993). Studies using extraction methods such as heating (Morgan *et al.*, 1990), sonication (Urbain *et al.*, 1993), ultrasonication (Dignac *et al.*, 1998), homogenization (Wuertz *et al.*, 2001), the addition of cation exchange resin (CER) (Frolund *et al.*, 1996; Nielsen *et al.*, 1996), ethylenediaminetetraacetic acid (EDTA) (Liu and Fang, 2003), caustic (Frolund *et al.*, 1996), sulfuric acid (Chen *et al.*, 2001), and a combination of sonication and CER (Dignac *et al.*, 1998) have yielded differences in quantity of EPS constituents. Therefore, there is still no unified method for EPS extraction.

Frolund *et al.* (1998) extracted EPS from activated sludge using cation exchange resin (CER). CER extraction procedure is partly chemical (removal of divalence cations such as calcium) and partly mechanical due to the applied shear. The result showed that CER was more efficient for releasing EPS than two other

commonly methods (thermal heating and sodium hydroxide extraction) in terms of yield and minimal disruption of the exopolymers (Hoa, 2002). Sodium hydroxide treatment cause large disruption in all cultures. Ultrasonication released low concentrations of EPS but cause no significant cell disruption. Thus it could be used as primary treatment in conjunction with other extraction method. Thus, there is a need to establish a standardized EPS measurement technique to evaluate the effect of EPS components.

The nutrients of the feed wastewater have been identified as one of the factors affecting EPS. The nature and concentration of nutrients affect the biodegradation of organic waste (Bura *et al.*, 1998). Nutrients are necessary components for the growth of bacteria as well as to stimulate the production of surface biopolymers. Pavoni *et al.* (1972) reported that the production and ratio of compositions of exocellular polymers produced is different for different carbon source in activated sludge. The effect of easily biodegradable carbon source, glucose, on EPS production and sludge settleability was studied by Jorand *et al.* (1994). They found that the addition to the activated sludge of easily degradable nutrients such as glucose increased both exopolymer production and the sludge volume index. By optimising (COD:N:P) ratio, EPS can be controlled. Durmaz and Sanin (2003) indicated that with the increase of the C/N ratio, microorganism concentration and the total EPS produced increased significantly. Microorganisms produced dominantly higher quantities of carbohydrate type of polymers as opposed to proteins at high C/N ratios.

The type of wastewater has an effect on EPS production. When Liu and Fang (2003) compared the results of studies which investigated the EPS production, they found that EPS extracted from sludges treating municipal wastewater contains more EPS than those treating industrial and synthetic wastewaters. Also, under oxygen limitation or depletion conditions, the suppression of EPS production occurred (Starkey and Karr, 1984). An evaluation of EPS compositions revealed that DO level has a more profound effect on carbohydrate

as opposed to protein. The high airflow rate increased carbohydrate levels whilst protein level remains fairly constant (Hoa, 2002). The ratio of carbohydrate to protein increased with the increasing of DO so poor settling occurs.

Çetin and Sürücü (1990) reported that the relationship between temperature and settlement of sludge was mainly due to the changes in the structure of bacterial growth at different temperatures. On the other hand, temperature also had effect on protein and lipid structure. Therefore, this led to the changes in cell membrane, EPS structure and functioning. It also made changes on EPS charges, which resulted in low flocculation abilities and hence low settleability at high temperatures. In addition, at high temperature the viscosity of exocellular materials also decrease and may also slow down efficient bioflocculation (Hoa, 2002).

Many researchers studied about the effects of sludge retention time (SRT), the age of sludge biomass in a bioreactor, on EPS and sludge characteristics. At low SRTs, bacterial cells produce low molecular weight polymers, which are not effectively capable of agglutination, so it causes pin-point floc formation. When the SRT of systems is increased, bacteria generate high molecular weight polymers that are able to provide effective floc formation. Eriksson *et al.* (1992) observed that at higher sludge ages the bacterial cells are surrounded by EPS in more aggregates. Murthy (1998) indicated that soluble polysaccharide and colloidal protein increased in the effluent as the SRT increased to above 10 days. It was also found that an increase in solids retention time (SRT) resulted in an increase in polysaccharide in the solution and in the effluent. On the other hand, Liao *et al.* (2001) and Sesay and Sanin (2004) found that total amount of EPS was independent on SRT.

Bacterial cells can be regarded as charged particles having an overall net charge at pH values encountered in natural environments (Ward and Berkeley, 1980). Heterotrophic bacteria typically grow best at a pH near 7, with a minimum pH of

approximately 5.5 and a maximum between 8.5 and 9.5 (Brock and Madigan, 1991). The pH level affects enzymatic activity as well as growth rate. The pH level has effects on EPS that are anionic and non-ionic in nature at most pH values. The increase in pH introduces more negative charges, which causes an increase in the number of available reactive sites on non-ionic exocellular polymers (Hoa, 2002). Hydrophobic and cationic bridging effects between humic substance and activated sludge EPS were the mechanisms responsible for biosorption under the presence of divalent cations; however, the former was most significant at low pH, whereas the latter was predominant near neutral pH (Esparza-Soto and Westerhoff, 2003).

2.5. Mechanisms of Bioflocculation

The building blocks of a floc are colonies, which are created by two or more individual microorganisms “sticking” together. In “this sticking reaction”, extracellular polymers are necessary. Most bacterial surfaces are covered with a slime or capsule extracellular polysaccharide layer, and this layer mediates the formation of colonies. Depending on the surface composition of microorganisms, different flocculation mechanisms become important (Sanin and Vesilind, 1996).

Various mechanisms have been proposed by researchers to explain floc formation. These mechanisms are *Zoogloea ramigera* theory (Butterfield, 1935; Heukelekian and Littman, 1939; Wattie, 1943), filament backbone theory (Parker *et al.*, 1971; Sezgin *et al.*, 1978), polymer bridging model (Tenney and Stumm, 1965; Busch and Stumm, 1968), double layer compression theory (DLVO) (Zita and Hermansson, 1994), metal ion bridging theory (McKinney, 1952; Tezuka, 1969; Kakii *et al.*, 1985; Eriksson and Alm, 1991; Bruus *et al.*, 1992), gel formation theory (Bruus, *et al.*, 1992; Sanin and Vesilind, 1996) and hydrophobic interactions (Urbain *et al.*, 1993; Jorand *et al.*, 1994).

Zoogloea ramigera is known as floc-forming bacteria. According to this theory, flocculation was only dependent on the production of a highly gelatinous matrix by *Zoogloea ramigera* (Butterfield, 1935; Dias and Bhat, 1964). Later studies by McKinney and Horwood (1952) and McKinney and Weichlein (1953) demonstrated that many other bacteria, isolated from sewage sludges, were capable of forming flocs as well. Crabtree and McCoy (1967) concluded that *Zoogloea ramigera*, as reported in waste treatment and other literature, is not true species, but only one of many kinds of closely related floc forming bacteria.

The filamentous backbone theory introduced by Parker *et al.* (1971) suggested that filamentous bacteria form a backbone onto which EPS producing bacteria can attach. Sezgin *et al.* (1978) proposed that there are two levels of structure in activated sludge floc. These have been termed as “microstructure” and “macrostructure”. Macrostructure is formed by filamentous microorganisms that form a network or backbone within the floc-forming bacteria cling (Jenkins *et al.*, 1993). It is recognized that filamentous microorganisms play an important role in floc structure in activated sludge by forming the backbone of floc, surrounded by an extensive network of extracellular polymers carrying with it many microbial colonies. Smaller flocs can also be connected by filamentous microorganisms to form larger flocs (Sanin and Vesilind, 1996).

According to polymer bridging model (Tenney and Stumm, 1965; Ries and Meyers, 1968), electrostatic surface charge reduction to zero is required for flocculation to occur. Microscopic observations showed that microbial cells inside the flocs were cross-linked by EPS (Leppard, 1992; Jorand *et al.*, 1995), forming a polymeric network with pores and channels (Li and Ganczarczyk, 1990). Such a polymeric network had a vast surface area, capable of adsorbing colloids by the help of negatively charged functional groups. Collisions between these particles lead to the formation of floc particles (Liu and Fang, 2003). Naturally occurring anionic biopolymers coagulate bacteria via the polymer-surface and polymer-polymer interactions. The nature of these interactions may

be hydrophobic, hydrogen bonding, physical enmeshment or electrostatic (Eriksson and Alm, 1991).

DLVO (Derjaguin, Landau, Verwey and Overbeek) theory is known as the double layer model used to visualize the ionic environment in the vicinity of a charged colloid and explains how electrical repulsive forces occur. An illustration of DLVO model is shown in Figure 2.2. It is easier to understand this model as a sequence of steps that would take place around a single negative colloid if its neutralizing ions were suddenly stripped away. Effect of the colloid on the positive ions (often called *counter-ions*) in solution is important. Initially, attraction from the negative colloid causes some of the positive ions to form a firmly attached layer around the surface of the colloid; this layer of counter-ions is known as the *Stern layer*. Additional positive ions are still attracted by the negative colloid, but now they are repelled by the Stern layer as well as by other positive ions that are also trying to approach the colloid. This dynamic equilibrium results in the formation of a *diffuse layer* of counterions. They have a high concentration near the surface that gradually decreases with distance, until it reaches equilibrium with the counter-ion concentration in the solution (Zeta-meter Inc., 2007).

The diffuse layer can be visualized as a charged atmosphere surrounding the colloid. The charge density at any distance from the surface is equal to the difference in concentration of positive and negative ions at that point. Charge density is greatest near the colloid and gradually diminishes toward zero as the concentration of positive and negative ions merge together. The attached counter ions in the Stern layer and the charged atmosphere in the diffuse layer refer to as the *double layer*. Electrostatic repulsion becomes significant when two colloids approach each other and their double layers begin to interfere. In addition to this repulsive force, all particles possess an attractive force, van der Waals force that is a function of the molecular structure of the particle. The combination of these forces results in a net repulsive force, an energy barrier that prevents the particles

from coming together (Zeta-meter Incorporation, 2007). The reduction of energy barrier to zero is necessary to provide coalescing of particles. As the ionic strength increases by the addition of cations, the size of double layer decreases, which decreases the repulsion between particles, allowing short range attractive forces to promote bioflocculation (Sobeck and Higgins, 2002). Zita and Hermansson (1994) indicated that the addition of any ions such as potassium and calcium increased the solution ionic strength and compressed the double layer, thereby improving bioflocculation, settling and dewatering properties.

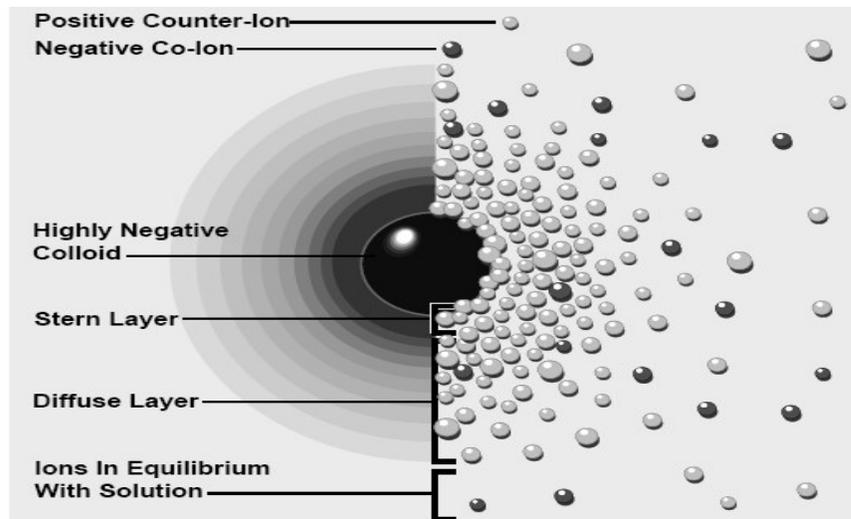


Figure 2.2. Depiction of double layer theory (Zeta-meter Inc., 2007).

Microorganisms, extracellular polymeric substances (EPS), and cations are major components of activated sludge flocs (Bruus *et al.*, 1992; Higgins and Novak, 1997a). It is thought that biopolymers originated from microbial activity and wastewater influent provides a matrix in which microorganisms can be aggregated to form flocs (Urbain *et al.*, 1993; Dignac *et al.*, 1998). Because a majority of exocellular biopolymers are negatively charged, multivalent cations become an important component of the floc structure by providing bridging to the negative sites within the biopolymer network (Bruus *et al.*, 1992; Urbain *et*

al., 1993; Higgins and Novak, 1997a). Therefore, without the proper cations, flocs would be weak and dispersed and this would result in poor settling, dewatering and effluent quality.

Historically, divalent cations such as calcium and magnesium have been of great interest in understanding bioflocculation mechanisms. The first researchers to propose the salt or divalent cation bridging (DCB) theory included McKinney (1952) and Tezuka (1969). Tezuka (1969) demonstrated the role of divalent cations in experiments that examined floc formation during the growth of monocultures, and found that calcium and magnesium were important to the bioflocculation process.

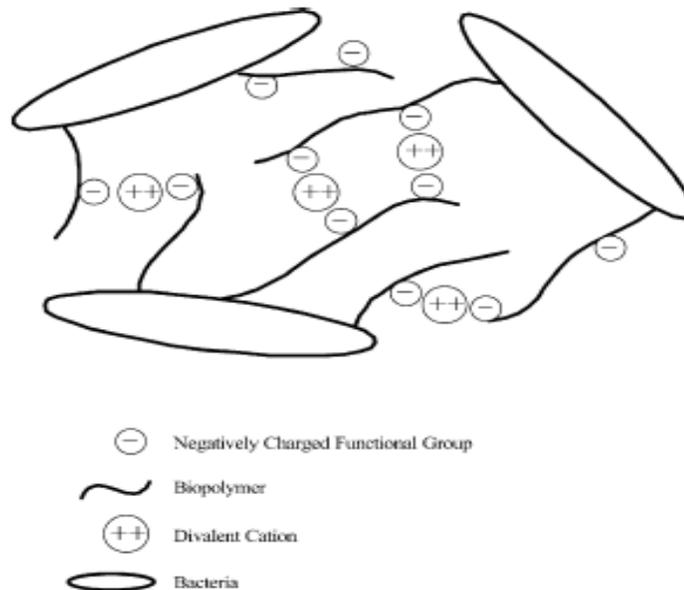


Figure 2.3. Schematic representation of divalent cation bridging theory in floc structure (Sobeck and Higgins, 2002)

According to the DCB theory, divalent cations bridge negatively charged functional groups within the EPS and this bridging helps to aggregate and

stabilize the matrix of biopolymer and microbes and therefore promote bioflocculation (Sobeck and Higgins, 2002) as demonstrated in Figure 2.3. Calcium removal from flocs using a cation exchange resin (Keiding and Nielsen, 1997), EDTA (Kakii *et al.*, 1985), or EGTA (Bruus *et al.*, 1992) resulted in the desorption of organic matter and poor settling and dewatering properties. While these studies stressed the effect of calcium on the stability of floc, other studies have demonstrated that magnesium as well as calcium is important for floc stability.

Alginate is a polysaccharide produced by bacteria such as *Azotobacter* sp., *Pseudomonas aeruginosa* and is typically made up of repeating mannuronic and guluronic acids. The unique composition of this polysaccharide results in the formation of alginate gels in the presence of calcium ions. This gel is referred as the egg-box model. Bruus *et al.* (1992) suggested that polysaccharides in biopolymers are alginates and these biopolymers bind to calcium ion to form a gel-like floc structure. There is an electrostatic repulsion between particles and particles and between particles and negatively charged alginate molecules. The addition of calcium ions reduces this repulsive barrier, and, in addition to being attracted to particle surfaces, calcium ions can form metal ion bridges between particles and particles and alginate molecules. The alginate theory could be considered a subset of DCB theory, but the DCB theory suggests a non-specific binding of divalent cations rather than the specific interaction and gel formation between calcium and alginate (Sobeck and Higgins, 2002).

Hydrophilic and hydrophobic properties of EPS are another important factors affecting the flocculation mechanism. Hydrophobicity is of specific importance since activated sludges are highly hydrated structures. Cell surfaces are known to exhibit hydrophobic areas and hydrophobic molecules such as lipids or proteins can be trapped into the flocs (Urbain *et al.*, 1993). A high proportion of amino acids with hydrophobic groups such as glycine (gly) and alanine (ala) are present in the protein (Higgins and Novak, 1997c). It was claimed that more

hydrophobic molecules that are important for the flocculation process are firmly bound to floc (Guellil *et al.*, 1998).

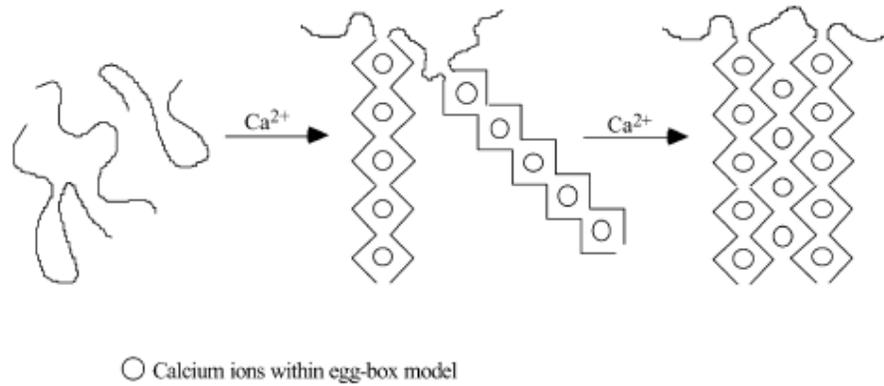


Figure 2.4. Schematic representation of gel formation between calcium and alginate in egg-box model (Sobeck and Higgins,2002)

Jorand *et al.* (1998) suggested that EPS may be involved in floc cohesion in two ways: i) through the hydrophilic chains, represented by polysaccharides creating a matrix in which bacteria are embedded and ii) through a glue creating bridges or reticular points between polysaccharides represented by hydrophobic heteropolymers. Higgins and Novak (1997c) also showed that hydrophobic amino acids of protein were significant constituents in activated sludge and suggested that biopolymers can also bind through hydrophobic interactions. Therefore, the hydrophilic and hydrophobic balance should be considered for good flocculation.

2.6. Cations

While carbon, oxygen, hydrogen, nitrogen, phosphorus, and sulfur are needed for the synthesis of macromolecules of the cell, ions are required for three basic functions: i) as enzyme activators called either coenzymes or metal cofactors; ii)

to transfer electrons in oxidation-reduction reactions; and iii) to serve as regulators of osmotic pressure (Gerardi *et al.*, 1994). Besides, many researchers have revealed that polyvalent cations provide flocculation by bridging negative sites on extracellular polymers (Tezuka, 1969; Pavoni *et al.*, 1972; Bruus *et al.*, 1992; Higgins and Novak, 1997a, b; Dignac *et al.*, 1998). Sodium, potassium, ammonium, calcium, magnesium, iron and aluminum are the major cations found in activated sludge. Although there is some debate about the specific role of these cations, it has been generally accepted that divalent and trivalent cations are better flocculants than monovalent cations. The significance of cations has been dealt in many studies using a variety of experimental approaches or with different types of sludge and this has led to several different models of floc structure (Park, 2002).

The tendency of different cations to form cation bridges depends on the ionic charge and the size of the hydration shell of the cations. Each metal ion has a number of water molecules associated with it that surrounds the ion. This is called as hydration shell and as the size of ion increases, the hydration shell radius decreases so cations with high valence and size and thin hydration shell can easily approach charged surfaces and form bonds with multiple sites of negatively charged polymers (Piirtola *et al.*, 1999). As can be seen from Figure 2.5 and Table 2.1, for calcium and magnesium, calcium has higher ionic and lower hydration shell radius so calcium loses its hydration shell easily when it approaches the surface of polymeric substances. It establishes a stronger bond with the negatively charged functional groups of EPS than magnesium whose close approach to the surface is prevented by the water molecules around it. For monovalent cations, the comparison of hydrated radius of sodium and potassium reveals that potassium with hydrated radius of 0.53 nm will exchange for sodium, hydration radius of 0.79 nm on the exchange sites. It means that sodium is a smaller ion compared to the ionic size of potassium, so sodium holds the water so strongly that it does not lose its hydration shell near the charged groups of EPS so flocculation cannot effectively occur. Also, when the relative flocculating

powers of these cations are compared calculated by Rengasamy and Naiudu (1998), calcium is the good flocculator among them while sodium is the poorest one so cations with a single charge, small size and large hydrated radii are the poorest flocculators.

Forster and Lewin (1972) have shown a higher binding of calcium rather than magnesium ions on EPS recovered from activated sludge and Forster (1985b) hypothesized that ionic size of cations may influence their binding ability to charged (carboxyl) and uncharged groups (hydroxyl) in the EPS; thus as calcium ions larger than magnesium ions, their binding may be favoured.

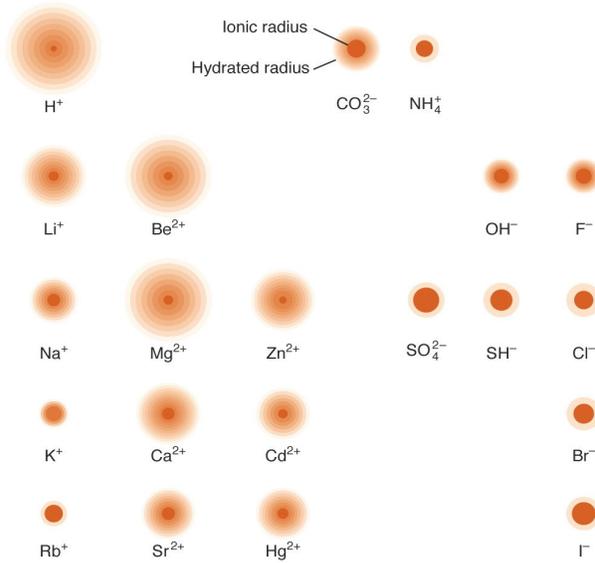


Figure 2.5. Schematic representation of some ions with ionic and hydrated radii

Table 2.1. Cations with their radii and relative flocculation power

Cation	Valence	Ionic radius (nm)	Hydrated radius (nm)	Relative flocculation power
Sodium	1	0.095	0.79	1.0
Potassium	1	0.133	0.53	1.7
Magnesium	2	0.065	1.08	27.0
Calcium	2	0.099	0.96	43.0

According to cation-bridging model, removal of divalent cations from the floc would lead to deterioration in the settling and dewatering properties due to a weakening of the floc structure (Tezuka, 1969; Novak and Haugan, 1979; Bruus *et al.*, 1992). Floc structure is weakened because divalent cations bridge negatively charged sites on biopolymers, binding them to microbial surfaces and to other biopolymers. The binding of biopolymers stabilized the biopolymer network, which improved floc formation as well as settling and clarification (Higgins and Novak, 1997a).

Trivalent cations have higher charge valence and lower solubility than divalent cations. According to Kakii *et al.* (1985), iron and aluminum were not affected by the acid treatment of activated sludge at pH 3 while most of calcium and magnesium were extracted, indicating that iron and aluminum are more strongly associated with sludge matrix than divalent cations. It was found that iron has an affinity to biopolymers in the sludge, and the presence of iron will result in a better dewatered product (Murthy, 1998). The presence of iron may be important to biopolymer retention in the activated sludge floc.

Cousin and Ganczarzyk (1998) reported that the addition of sodium to a biological suspension increased floc size and improved floc porosity. Zita and Hermansson (1994) reported that floc stability was affected by ionic strength of a

solution and these effects could be explained by DLVO theory. They also reported that potassium and calcium produced similar effects on particle stability. On the other hand, Bruus *et al.* (1992) found that the addition of sodium and potassium to a thickened sludge resulted in the release of calcium and the increase of turbidity. In addition, they also found the addition of monovalent cations resulted in deterioration of sludge dewaterability.

It was reported that WWTPs treating industrial wastewater where sodium hydroxide is used for pH control generally produce sludges with poor settling and dewatering properties (Murthy *et al.*, 1998). It is theorized that monovalent cations displace divalent cations within the cation bridged floc structure by ion exchange, making flocs weak and sensitive to any physiochemical changes on the floc structure.

It was found that sodium ions in influent wastewater caused an increase in proteins and polysaccharides in solution, thereby increasing the effluent COD concentration of the treated municipal wastewater (Murthy and Novak, 2001). Researchers observed an improvement in settling properties with an increase in potassium ion concentration, associated with an increase in turbidity and dissolved organic carbon (Higgins and Novak, 1997b; Murthy and Novak, 1998). Microbial reactions related to potassium and sodium cations might be different and therefore produce different results.

Higgins and Novak (1997a) reported that when the sum of the monovalent cation concentrations (divided by the sum of the divalent cation concentrations) was greater than 2, then this could cause floc property deterioration. From this, they proposed using the M/D ratio as an indicator to determine when high concentrations of monovalent cations such as sodium could cause problems with floc properties.

Although previous studies indicated that both divalent and trivalent cations are extremely important in well-flocculated activated sludge, monovalent cations have not been studied as much. Especially, their effect in the feed media as the culture grows has not been studied and role of each specific monovalent cation is not clearly understood. In this study, the role of potassium and sodium cations on settling, dewatering and effluent quality is examined and the results obtained are discussed in following parts.

2.7.Activated Sludge Properties Related to Bioflocculation

2.7.1.Dewaterability

Much of the excess sludge produced from wastewater treatment plants has a high percentage of water content, and the difficulty involved in the dewatering process has been revealed. Dewatering is normally required before the incineration of the sludge, because the removal of excess water content leads to a decrease in the energy cost for incineration. However, it is often noted that the dewatering of activated sludge presents difficulties because of the water closely associated with the solid phase (Kang *et. al*, 1989).

Vesilind (1994) identified the water distribution in sludge in four different physical states: bulk or free water, interstitial water, vicinal water and water of hydration. Free water is not associated with suspended particles. The easiest water to remove from a wet sludge is bulk water, which can be eliminated by drainage, thickening, or mechanical dewatering. Interstitial water trapped in the interstitial spaces of the flocs and organisms by the capillary force and can be released by either destruction or compression of floc structures using sufficient mechanical energy. Vicinal water is the multiple layers of water molecules held tightly to the particle surface by hydrogen bonding. Some of the vicinal water is within the cell structure of microbial organisms. Vicinal water cannot be mechanically removed unless some prior conditioning is affected. Water of

hydration is the water chemically bound to the particles and can be removed only by applying thermal energy.

Dick and Ball (1980) summarized the factors influencing dewaterability into three categories: i) fluids properties: bound water content, viscosity, ionic strength, density; ii) particle properties: size and shape distribution, surface potential and area, density; iii) sludge properties: solids concentration, permeability, yield strength and electrokinetic properties. Among these, particular attention has been paid to particle size, water distribution and rheological properties (Lotito *et. al*, 1993). Knocke and Zentkovich (1986) reported that dissolved oxygen (DO) concentration, pH, mean cell residence time, waste characteristics and exocellular polymer content define the activated sludge dewatering characteristics.

The dewaterability of activated sludge can be analyzed by Specific Resistance to Filtration (SRF), Capillary Suction Time (CST) and floc strength.

Specific Resistance to Filtration is based on Darcy's law that governs flow through porous media and describes the relationship among flow rate, pressure drop and resistance. Specific resistance denotes the resistance of unit weight of cake per unit area at a given pressure. SRF test is generally conducted by using Buchnel funnel apparatus. A known volume of sample is filtered under vacuum and volume of filtrate withdrawn from the sludge sample is recorded at predetermined time intervals. Specific resistance to filtration is calculated from the slope of t/V versus V by using the following equation:

$$\frac{t}{V} = \frac{\mu r_w}{2PA^2}V + \frac{\mu R_f}{PA} \dots\dots\dots 2.1$$

where:

t = time, s

V = volume of filtrate, m^3

μ = viscosity of filtrate, N/m^2

r = specific resistance to filtration, m/kg

w = weight of dry cake solids per unit volume of filtrate, kg/m^3

P = pressure difference, N/m^2

A = filtration area, m^2

R_f = specific resistance of filter medium

The range of the specific resistance for activated sludge was reported by some researchers. SRF value was found between 2.6×10^7 and 1.6×10^8 cm/g by Coakley and Jones (1956); 10^9 and 10^{13} cm/g by Heukelekian and Weisberg (1956) ; 10^{10} and 10^{14} m/kg by Li and Ganczarczyk (1986) depending on sludge conditioning, vacuum pressure, solids content, and other factors.

Capillary Suction Time (CST) is a very useful test for quickly determining the ability of the sludge to release water in the dewatering processes. The rate at which water permeates through the filter paper varies depending on the condition of the sludge and the filterability of the cake formed on the filter paper. The details of CST apparatus used in the experiments are given in Section 3.2.3.4.

Floc strength and stability of the floc plays an important and perhaps critical role in determining sludge dewatering properties. A review of the literature shows there to be no established standardised floc strength test, although a number of techniques have been evaluated. Floc strength may be broadly considered in terms of the energy required to break flocs under tension, compression or shear (Zhang *et al.*, 1999; Jarvis *et. al.*, 2005). The floc strength of sludges are generally measured by conducting shear tests and evaluating the changes in CST over time. Floc strength is not an independent property; both the floc structure

and particle bond strength are interrelated with floc strength (Hermawan *et al.*, 2004).

Karr and Keinath (1978) reported the 'supracolloidal' particles in the size range of 1-100 μm had the greatest effect on the dewaterability of sludges, and as the concentration of the particles in this size range increased, dewaterability decreased. The shift in particle size distribution was likely the cause of the deterioration in dewatering properties since smaller particle sizes are associated with poor dewatering (Higgins and Novak, 1997c).

Particle size distribution appears to affect dewatering properties, where smaller particles (colloidal and supracolloidal) cause blinding of filters and sludge cakes (Novak *et al.*, 1988; Sorensen *et al.*, 1997) and deter the release of water in the sludge cake (Murthy, 1998). As the floc density approaches the density of the water, the sludge particle tends to possess poorer settling and dewatering properties (Murthy and Novak, 2001). When the particle size increases, the density decreases, and the porosity increases (Clauss *et al.*, 1998).

Liu and Fang (2003) concluded that the increase of EPS in sludge would lower the sludge dewaterability, based on measurements of SRF (Shioyama and Toriyama, 1985; Kang *et al.*, 1989; Pere *et al.*, 1993), CST (Eriksson and Hardin, 1984; Eriksson and Eriksson, 1988; Eriksson and Alm, 1991, 1993; Pere *et al.*, 1993) and floc strength (Eriksson and Alm, 1991, 1993). The increase in EPS content above 35 mg-EPS/g-SS tended to increase the amount of surface water bound by EPS, and thus lowered the sludge dewaterability (Houghton *et al.*, 2000, 2001).

While some researchers found that the amounts of EPSp have positive (Higgins and Novak, 1997a,b,c) effect on dewaterability of the sludge, some revealed that the release of protein into solution was accompanied by the deterioration in dewatering properties as indicated by the increase in the CST (Murthy and

Novak, 1998,1999; Houghton *et al.*, 2000, 2001; Novak *et al.*, 2001). However, the effect of the hydrophilic EPS_c on sludge dewaterability was mostly negative (Wu *et al.*, 1982; Murthy and Novak, 1999).

Bruus *et al.* (1992) compared the dewaterability of the three sludges and found that the sludge rich in copper had the highest dewaterability, followed by the calcium-rich sludge, and followed by the magnesium-rich sludge. Bruus *et al.* (1992) also reported that the addition of sodium and potassium to a thickened sludge resulted in the release of calcium and the increase of turbidity.

While the increase in the concentrations of calcium and magnesium caused a decrease in the bound water content of the sludge, it caused an increase in the bound biopolymer concentration, floc strength, cake solids and floc density. Floc strength measurements indicated flocs were more resistant to shear at higher cation concentrations. The divalent cations created a tighter bound network of biopolymer that was more resistance to shear. Briefly, a higher divalent cation content yielded good dewatering properties, low conditioning chemical requirements and low solution polysaccharides. The removal of divalent cations from the floc by monovalent cations would lead to deterioration in the dewatering properties measured by SRF or CST (Bruus *et. al*, 1992; Higgins and Novak, 1997a; Higgins and Sobeck, 2002). Floc structure is weakened because divalent cations are believed to act as a bridge between negatively charged sites within the biopolymer network.

Weak flocs are characterized by increasing CST due to floc breakup during mixing, whereas stable flocs show a much smaller increase in CST with mixing. The weakening of floc structure was also demonstrated by floc strength measurements in which resistance to shear decreased at the higher sodium concentrations (Higgins and Novak, 1997a). According to Novak (2001), the ammonium ion also replaces divalent cations in a same manner as the sodium, deteriorating dewaterability of the activated sludge.

Murthy and Novak (1998) studied the effect of potassium on activated sludge characteristics and found that high potassium improved the floc strength while it caused deterioration in dewatering property and effluent quality. It was revealed that an increase in the monovalent to divalent ratio greater than approximately 2:1 will result in a decrease in the dewatering rate as measured by SRF (Higgins and Novak, 1997c).

2.7.2. Settleability

Sludge flocculation and settling are crucial to the effective operation of an activated sludge treatment process since the overall effectiveness of the process depends on the efficiency of the solids separation step (Verstraete and Vaerenbergh, 1986; Andreadakis, 1993). Settling is a process used for various stages within a wastewater treatment plant to reduce the concentration of settleable and suspended matter. The settling properties of activated sludge are determined by Zone Settling Velocity (ZSV) and Sludge Volume Index (SVI).

Zone settling occurs when particle aggregate, forming a mass which settles as a blanket with a distinct interface between the settling sludge and clarified effluent (Weber, 1972). Zone settling has been widely utilized in research circles, especially in the settler models based on flux theory. The solids flux theory is a generally accepted approach for describing the thickening function of activated sludge and applied in most of the clarifier models (Renko, 1998). Since the solids flux theory was developed by Kynch (1952), a number of velocity functions for the zone settling have been reported (Vesilind, 1968; Dick and Young, 1972; Vaerenbergh, 1980; Cho *et al.*, 1993). From these, the Vesilind (1968) function is widely accepted and used (Zhang *et. al*, 2006). Therefore, the settling characteristics of sludge can be determined by zone settling velocity which is a series of settling tests at various solids concentrations and recording the sludge-water interface height with time. The velocity of the settling is a function of solids concentration (Vesilind, 1994).

Sludge Volume Index (SVI) is the most commonly used test by researchers and plant operators for determining the settleability of activated sludge. It is defined as volume in milliliters occupied by 1 g of a suspension after 30 min settling. It is not a good tool for research purposes. It shows a single point on the settling curve and this tells us not much about the way the sludge settles. It is designed for the treatment plant operators to quickly measure sludge settleability to gain an idea. SVI values below 100 are usually considered acceptable; SVI >150 is a badly bulking sludge.

Settleability is a very variable characteristic, strongly related to the structure and nature of the activated sludge floc which in turn depend on the sludge age, F/M ratio, level of nutrients, type of microorganisms and organic loading (Chao and Keinath, 1979; Barahona and Eckenfelder, 1984; Andreadakis, 1993). Hilligardt and Hoffman (1997) also revealed that type and concentration of EPS, cations, density, particle shape, porosity and particle size distribution affect the settling characteristics of the sludge.

For example, the nitrogen and phosphorus levels of wastewater affect the settleability of activated sludge. N and P deficiency encourages the growth of filamentous organisms such as *Thiothrix ssp.*, type 021N, type 0041, type 0675 (Jenkins *et al.*, 1993) so bulking conditions occur. In nitrogen deficient activated sludge systems, not only filamentous bulking but also non-filamentous bulking was observed (Peng *et al.*, 2003). They also found that when BOD/N value of 100/4, the optimum ratio for brewing sludge settling, is changed to 100/3, although filamentous bulking was observed early period of the process, excessive growth of viscous zoogaea caused the viscous bulking rest of the process period. Durmaz and Sanin (2003) reported that a feed medium having a C/N ratio of 43 also led to viscous bulking which is related to the morphological characteristics of the floc and the presence of large amounts of exocellular slime (Andreadakis, 1993).

EPS provide an extensive surface area per unit volume for microorganism binding and have a significant influence on activated sludge floc settling. Several studies have focused on the correlation between polymer concentration and settleability (Forster, 1971; Kiff, 1978; Urbain *et al.*, 1993; Eriksson and Alm, 1991, Liao *et al.*, 2001) and found a positive correlation between SVI and EPS concentration. High concentrations of EPS resulted in deterioration in settleability of the sludge with high SVI values. This can be partially explained by steric forces arising from the EPS. The EPS molecules extended out from cell surfaces physically prevent the cells from forming close contact. The EPS may also form a dense gel that resists the expression of water from gel pores (Liao *et al.*, 2001). Moreover, some researchers found a negative correlation between SVI and EPS (Goodwin and Forster, 1985; Yun *et al.*, 2000), while in some cases, no correlation was observed between these parameters (Chao and Keinath, 1979; Bura *et al.*, 1998; Jorand *et al.*, 1998).

Settleability of sludge was affected more by the composition and properties (e.g. hydrophobicity and surface charge) of EPS rather than the amount of EPS produced. Sponza (2004) studied with four different industrial wastewaters (chemical, dye, leather and winery) and revealed that high SVI values are associated with low protein content in EPS, indicating low settleability of activated sludge samples. On the other hand, some studies confirmed the positive correlation between protein and SVI (Barber and Veenstra, 1986; Urbain *et al.*, 1993; Liao *et al.*, 2001). Also, Higgins and Novak (1997a,c) indicated an increase in bound protein provide an improvement in settling of the activated sludge samples by decrease in SVI. Also, it was found that the release of protein into solution led to formation of poorly settled sludges and weak flocs. On the other hand, Goodwin and Forster (1985) reported no reasonable trend between protein content of EPS and settlement of sludge.

It can be considered that there was an optimum dose of EPS for good settling. Below optimum level, with an increasing of EPS, flocculation process improved progressively. On the other hand, above the optimum value, sludge settling worsens gradually. The diverse correspondences between sludge settling and EPS which above optimum dose could be interpreted by likening EPS behavior to that of a polyelectrolyte in the flocculation process of particles suspension. While the destabilization of suspension and agglomeration of the particles were particularly satisfactory only with optimum polyelectrolyte doses (Beccari *et al.*, 1980).

Bura *et al.* (1998) showed correlation only between DNA and SVI. The highest SVI values corresponded to the highest concentration of DNA. High DNA level in EPS is associated with high bound water, low negative charges and high SVI values, indicating poor settleability (Sponza, 2002).

The effect of carbohydrate fraction on sludge settleability was conflicting in different reports. The positive correlation between carbohydrate and SVI was reported by Forster (1971). Goodwin and Forster (1985) examined the relationship between composition of activated sludge surface and their settlement characteristics, the results showed that the carbohydrate fraction increased as settlement improved (decrease SVI). Reversibly, Andreadakis (1993) and Bura *et al.* (1998) reported that SVI was not correlated to carbohydrate content of EPS. Another study found that SVI increased with the EPS_p, but no correlation was found with EPS_c or lipids in EPS (Martinez *et al.*, 2000). On the other hand, Goodwin and Forster (1985) found the increase in the lipids was correlated to increase in SVI, indicating poor settling. Also, the increasing of ratio of the carbohydrate to protein in EPS worsened the sludge settling. The significant increase of this ratio and SVI caused the bulking of sludge (Shin *et al.*, 2001).

In the floc structure there appeared to be water channels, gaps and reservoirs. This finding indicates that the water flow through the flocs is possible. Logan

and Hunt (1988) reported that microbial and inorganic flocs are highly porous; listing porosities ranging from 0.66 to 0.999 for a 100 μm radius aggregate.

Some studies have revealed the specific relationship between floc size and the settling properties of biological suspensions (Andreadakis, 1993) and pointed out a correlation between the SVI and the mean particle size. Also, an increase in floc density or floc particle size has been shown to be necessary for improvements in settling properties (Higgins and Novak 1997a). The size of the flocs ranges from 20 to 200 μm according to Mueller *et al.* (1967). Hillgardt and Hoffmann (1997) indicated that particle size distribution of the measured sludge showed an increase of particle size with increasing SVIs. Sludge with a SVI exceeding 100 had particles greater than 100 μm . It was found that an increase of the floc size corresponded to an increase of the SVI due to the presence of filamentous microorganisms so the correlation between floc size and settling properties were highly dependent of the type of microorganisms present in the floc structure (Wilen and Balmer, 1999; Govoreanu, 2003).

Some researchers have demonstrated that the settling properties of the activated sludge were dependent on both the concentrations and ratios of cations in the feed (Forster, 1985b; Bruus *et al.*, 1992; Higgins and Novak, 1997 a,b,c; Murthy *et al.*, 1998; Murthy and Novak, 1998; 2001). These studies revealed that sodium and ammonium ions are positively correlated with SVI. Poor settling that occurred in the reactors with monovalent cations such as sodium and ammonium was caused by ion-exchange processes in which divalent cations were displaced from within the floc by the sodium or ammonium. According to cation-bridging model, removal of divalent cations from the floc would lead to deterioration in the settling (Higgins and Novak, 1997a). Potassium addition to the feed had significantly better settling properties in terms of SVI so potassium ions play different role in settling of the activated sludge (Murthy and Novak, 1998).

Settling began to improve at calcium to magnesium ratios of 0.7-2 based on equivalent basis by measurement of SVI and a positive correlation was found between bound protein concentration and SVI (Higgins and Novak, 1997 a,b,c). Higgins and Novak (1997) reported that when the sum of the monovalent cation concentrations (Na^+ , NH_4^+ , and K^+) divided by the sum of the divalent cation concentrations (Ca^{2+} and Mg^{2+}) was greater than 2, this could cause deterioration in floc properties and settling.

According to Kakii *et al.* (1985) iron and aluminum are more strongly associated with sludge matrix than divalent cations. It has been reported that ferric iron has high affinity for protein (Novak *et al.*, 2001; Muller, 2001). The presence of iron that binds with the protein content of biopolymer and this metal-protein complex is both biologically resistant and physically stable, enhancing the compactability of sludge. Both divalent and trivalent cations positively affect the floc stability and settling and that each has a unique role in floc formation, sludge properties and effluent quality.

2.7.3. Rheology

Rheology provides information that is of major importance in determining the design requirements for a pumping scheme. Moreover, fluidodynamic parameters have been found to be correlated to other material characteristics, such as particle size and distribution, surface charge, degree of hydration, cohesion of flocs in suspension, and to the treatability of sludges, i.e. their suitability for thickening, flocculation, and centrifugation. Rheological measurements may be also used as an operational guide or control technique (Dick and Ewing, 1967; Lotito *et al.*, 1997).

One common rheological measurement is viscosity defined as the resistance of a liquid to flow under shear stress. The viscosity of the sludge, equal to the local slope of the curve describing the shear stress at different shear rates, is also a

function of the rate of shear (Schramm, 1994). Most fluids can be classified as Newtonian fluids, which the shear stress is linearly related to the shear rate.

$$\tau = \mu (dv/dy) \dots \dots \dots 2.2$$

where: τ = shear stress, μ = viscosity, dv/dy = shear rate (velocity gradient).

In Newtonian liquids, the viscosity in the above equation is considered constant for a specific temperature and solids concentration in the suspension. It means that, at a given temperature and pressure, the viscosity is independent of the flow rate and there is no initial stress to overcome before movement occurs when force is applied at rest (Lotito *et al.*, 1997).

The sludge is microbial slurry where the particles constantly interact with each other and concentrations are not very dilute. Also, activated sludge has a broad particle size and shape distribution so it would be unrealistic to expect that this theory would be applied for wastewater (Sanin, 2002).

Several authors have published works on the rheological properties of sludges (Dick and Ewing, 1967; Hendo and Kanari, 1980; Mulbarger, 1981; Frost, 1983). It was established that wastewater sludges are non-Newtonian fluids, because the shear rate or the velocity gradient is not linearly proportional to the shear stress. Moreover, the rheological behaviour of sludges has been shown to be thixotropic, which means that the viscosity of such a fluid does not remain constant for a given shear rate, it also becomes a function of time. This property is often encountered with flocculated suspensions such as activated sludge and parameters resulting from this property can be used as a field control index for characterization and control of sludge conditioning with polyelectrolytes (Tixier *et al.*, 2003).

Several mathematical models have been developed to describe the relation between shear stress and shear rate. The most common time independent rheological models used to model the laminar flow of non-Newtonian sludges are Bingham plastic (Frost and Ovens, 1982), Ostwald pseudoplastic (Valioulis, 1980) and dilatant with power-law behaviour (Graf, 1971).

The presence of initial yield stress in the Bingham plastic is due to the resistance of the sludge solids to deformation until sufficient stress is applied to exceed the yield strength of the solid phase (Dentel, 1997). Once this stress is exceeded, the sludge will flow and the relationship between the shear stress and shear rate will become linear. Bingham plastic model is represented by the following equation:

$$\tau = \tau_y + \eta (dv/dy) \dots\dots\dots 2.3$$

where τ_y is the yield stress and η is the plastic or Bingham viscosity. This behavior is shown schematically in Figure 2.6. The yield strength of sludges has been exponentially correlated to the suspended solids concentration (Forster, 1981), while differences in the yield strength can be interpreted in terms of more general nature of the sludge.

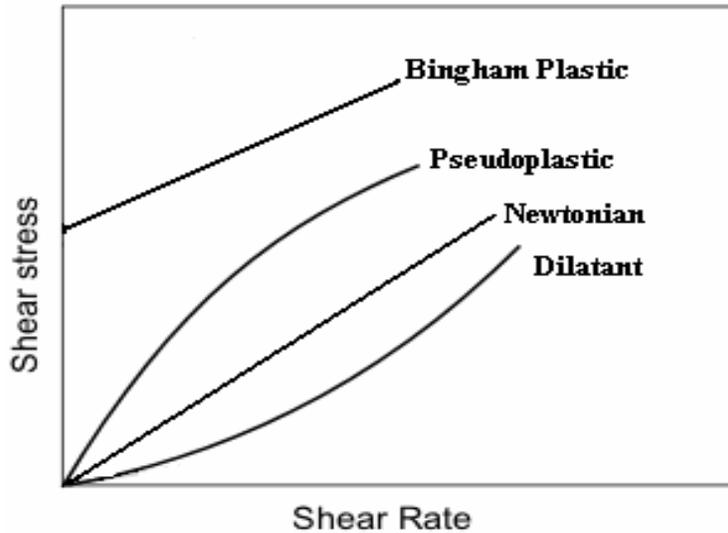


Figure 2.6. Rheogram models for Newtonian and non-Newtonian suspensions

Most wastewater sludges are neither Newtonian nor plastic but fall somewhere in between. These fluids are called pseudo-plastic fluids and represented by an equation known as the power law,

$$\tau = K (dv/dy)^n \dots\dots\dots 2.4$$

where K is fluid consistency index, a term equivalent to viscosity, and n is the flow behaviour index. When the shear stress increases, the flocs are broken down into smaller particles and viscosity decreases. In other words, the apparent viscosity gets less as mixing increases, so such fluids are also known as shear thinning. This behavior is given in Figure 2.6. The value of n is smaller than 1 for this type fluids and when n gets smaller and smaller than 1, the flow character deviates more from Newtonian. Also, increase in solids concentration is accompanied by increase of apparent viscosity (Sanin, 2002).

Dilatancy is frequently observed in fluids containing high levels of deflocculated solids and when the rate of shear decreases more in proportion to the shear stress,

the fluid is known as a dilatant or shear thickening. Increasing viscosity with an increase in shear rate characterizes the dilatant fluid. Above equation for pseudoplastic fluids also defines flow of dilatant type fluids, with one exception, n is greater than 1.

Main complications leading to the non-Newtonian behavior arise from the presence of particle in the system. To take this into account, Einstein considered a fluid in laminar flow through dilute, spherical, non-interacting and rigid particles and stated the relationship between viscosity and solids concentration as follows:

$$\eta / \eta_0 = 1 + 2.5 \phi \dots\dots\dots 2.5$$

where;

η = viscosity of the dispersion

η_0 = viscosity of the dispersion medium

ϕ = volume fraction occupied by the particles

Viscosity was shown to depend on factors such as particle size, shape, surface charge, pH, ionic strength, solids concentration and flocculation. With increasing solids concentration, pseudoplastic viscosity increased and the flow behaviour index decreased indicating that the flow tends to be stronger non-Newtonian. Also, presence of extracellular polymers affects the viscosity of sludge. Removal of polymers by multiple centrifugations runs correlates well with the decrease in viscosity (Sanin and Vesilind, 1994). Tixier *et al.* (2003) revealed that sludge suspension equilibrium viscosity increased with increasing pH and decreased with cation addition. The surface charge presented by sludge flocs was shown to be a factor that greatly affects viscosity, as demonstrated by the linear correlation with zeta-potential. A decrease in electrostatic charge was supposed to decrease repulsive effect between particles that facilitate flow and to promote a compaction of flocs that lower viscosity. Cation addition was shown to generate

a viscosity decrease by decreasing bound water content of sludge, in the same proportion, independent of the valency of the cation. The decrease in the particles double layer thickness coupled to the decrease in surface charge was supposed to induce a decrease in interparticle interactions, which lead to lower viscosity values (Forster, 1983; Tixier *et al.*, 2003).

2.7.4. Hydrophobicity

Hydrophobicity is of specific importance since activated sludges are highly hydrated structures (Urbain *et al.*, 1993). Hydrophobicity interactions involve in the mechanisms of floc forming based on bacteria aggregation and adhesion. Hydrophobic interactions result from the behaviour of colloids incapable of interacting electrostatically or establishing hydrogen bonds with water and are therefore drawn together when plunged in an aqueous phase (Magnusson, 1980).

Some studies have addressed the roles of hydrophobic interactions in the flocculation process and in the adhesion of bacteria to flocs in wastewater (Valin and Sutherland, 1982; Urbain *et al.*, 1993; Jorand *et al.* 1998). These investigations have shown that flocculation and sedimentation of flocs depend on the internal and external hydrophobicities of the flocs and of produced exocellular polymer in the flocs (Zita and Hermansson, 1997). It was pointed out the hydrophobic-hydrophilic balance should be considered as an important factor for determination of flocculation process (Eriksson and Axberg, 1981).

Cell surfaces are known to exhibit hydrophobic areas (Magnusson, 1980) and hydrophobic molecules such as lipids or proteins from the cells can be trapped into the flocs. Cell surface hydrophobicity may be promoted by specific proteins with specific amino acid sequences. Higgins and Novak (1997c) reported that a high proportion of amino acids with hydrophobic groups such as glycine and alanine are present in the protein. Bacterial glycoproteins, which are known to have a relatively low isoelectric point, exhibit hydrophobic behaviour. Also, the

presence of certain fimbriae has been shown to impart hydrophobic surface properties to various microorganisms (Doyle and Rosenberg, 1990). Extracellular lipids obtained from *Mycobacterium* and *Corynebacterium* influence the hydrophobicity of bacterial surfaces. The hydrophobicity of the surfaces of a wide range of *Staphylococci* has been attributed to proteins attached to the cell wall (Mozes *et al.*, 1991).

In 1980, Rosenberg *et al.* showed that various bacterial strains thought to possess hydrophobic surface characteristics adhered to liquid hydrocarbons and proposed Microbial Adhesion to Hydrocarbons (MATH) method which is a general technique for studying cell surface hydrophobicity. The hydrophobicity measurement of the activated sludge reflects the hydrophobicity of the sludge floc surfaces so relative hydrophobicity is measured by MATH not absolute hydrophobicity (Doyle and Rosenberg, 1990). Jorand *et al.* (1998) reported that at least 7 % of the dissolved carbon and 12 % of the proteins obtained with the XAD-8 resin, a non-ionic macroporous sorbent used for extraction of hydrophobic organics, may be considered as hydrophobic.

Liao *et al.* (2001) found that hydrophobicity was not influenced by the total EPS, but by the individual EPS constituents. EPS_c had a negative effect on the hydrophobicity and surface charge of the sludge, due to the hydrophilic nature of carbohydrates. EPS_p, on the other hand, had a weak but positive influence on the surface charge. Jorand *et al.* (1998) also reported that the EPS_p contributed to the sludge hydrophobicity, but not the EPS_c. It was suggested that the proportion of EPS components (protein/carbohydrates) is more important than the quantities of individual EPS components in flocculation. Wilen *et al.* (2003) revealed that the EPS showed positive correlation to negative surface charge and a negative correlation to relative hydrophobicity and flocculation ability.

Sludge hydrophobicity may be affected by a number of operating parameters, including substrate, bacterial growth phase and conditions, oxygen, temperature,

pH, ionic strength (Mozes and Rouxhet, 1987), and the presence of multivalent cations or phosphate (Liu and Fang, 2003). Cells grown at the stationary phase were reported to be more hydrophobic than those at the exponential phase (Hazen *et al.*, 1986; Hazen and Hazen, 1988). Allison *et al.* (1990) found that cells grown in stationary phase were more hydrophobic than those grown in exponential phase; this implies that sludge hydrophobicity increases with SRT. Liao *et al.* (2001) found that sludge surfaces at high SRTs (16 and 20 d) were less negatively charged and more hydrophobic (larger contact angle) than those at lower SRTs (4 and 9 d).

Wrangstadh *et al.* (1986) studied with *Pseudomonas sp.* and found in a batch study that cells in starvation had a lower hydrophobicity due to the release of hydrophilic EPS_c; the hydrophobicity increased 10 h later when the EPS_c were consumed by endogenous respiration. The reduction of sludge hydrophobicity resulting from oxygen limitation or depletion may lead to the disintegration of sludge flocs (Liu and Fang, 2003). Palmgren *et al.* (1998) studied the influence of oxygen on the cell surface hydrophobicity of four bacteria isolated from activated sludge. The bacteria were grown in batch cultures with and without oxygen limitation. It was found that oxygen limitation generally caused a lowering of the cell surface hydrophobicity. Researchers also showed that oxygen limitation generally caused a lowering of the cell surface hydrophobicity especially in the stationary phase of growth.

Urbain *et al.* (1993) obtained empirical results that sludge settleability was improved at a low concentration of EPS when internal hydrophobicity of flocs increased. Jorand *et al.* (1994) have also confirmed that improvements in bioflocculation and settling are correlated to an increase in floc hydrophobicity. Dignac *et al.* (1998) found that hydrophobic amino acids were also better extracted by CER, indicating that not only negatively charged hydrophilic proteins but also hydrophobic proteins are associated with cation binding in the floc structure.

2.7.5. Surface Charge

Microbial cells, EPS and sludge flocs carry negative charges due to the ionization of the anionic functional groups, such as carboxylic and phosphate. These properties may be characterized by zeta potential or surface charge (Liu and Fang, 2003). Flocs and EPS of all activated sludge carried negative charge, mostly within the range of -0.2 to -0.6 meq/g-VSS with a zeta potential of -20 to -30 mV. A positive correlation was found between surface charge and SVI (Forster, 1968; Goodwin and Forster, 1985). Magara *et al.* (1976) found the correlation negative, and yet Barber and Veenstra (1986) found no correlation at all. Urbain *et al.* (1993) reported that sludge flocs with high negative surface charge did not settle well.

Each species of bacteria displays different surface electrical potential due to the specific chemical structures on its cell surface. The zeta potential of activated sludge is always very high, therefore, extracellular polymers must be very important in the flocculation. Since EPS are polymer accumulated on the surface of microorganisms, it is most likely that the surface charge is due to the EPS's functional groups, which carry either positive or negative charge depending on the nature of groups and pH. At neutral pH, functional groups such as carboxylic and phosphate carry negative charge, while those like amino groups carry positive charge. Therefore, surface charge of sludge was strongly dependent on the EPS's chemical group composition and concentration (Jia *et al.*, 1996).

Negative surface charge decreased with the increase of protein:carbohydrate ratio. Therefore, it supported that high concentration of anionic surface biopolymers (carbohydrate) can consequently be correlated with deteriorating sludge settling characteristics because of the influence of the floc-repulsion (Hoa, 2002).

CHAPTER 3

MATERIALS AND METHODS

3.1. Reactor Operation

Experiments were conducted with 2 L semi-continuous reactors that were seeded with mixed culture bacteria obtained from the primary settling tank effluent of Ankara Central Wastewater Treatment Plant. The activated sludge reactors were operated until the reactors reached steady state and then required analyses were conducted.

Two reactor sets were operated in which potassium and sodium were added separately to the synthetic feed of reactors at concentrations of 0.5, 5, 10, and 20 meq/L. The 0.5 meq/L reactor was used as a control for each reactor set. Eight reactors were run in each set which are shown schematically in Figure 3.1 and were fed with synthetic medium which contains the essential amount of nutrients and minerals for the microbial community. The composition of the synthetic feed medium supplied for control reactor is given in Table 3.1.

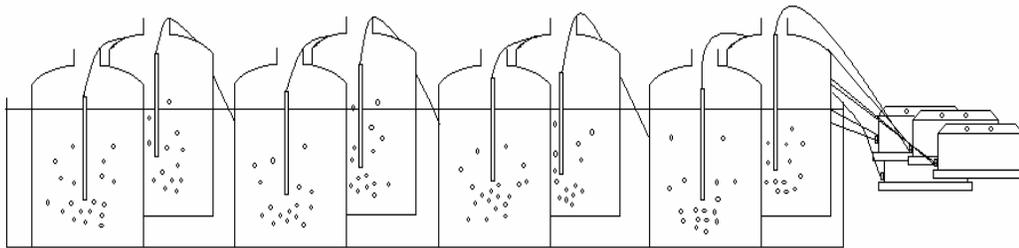


Figure 3.1. Schematic representation of reactor setup

Table 3.1. The composition of the synthetic feed medium for control reactor

Constituent	Concentration (mg/L)
Glucose	163.54
Peptone	942.5
NH ₄ Cl	225
MgSO ₄ .7H ₂ O	112.5
FeSO ₄ .7H ₂ O	3.75
ZnSO ₄ .7H ₂ O	3.75
MnSO ₄ .7H ₂ O	3.75
NaHCO ₃	42
CaCl ₂	15
KCl	37.25

The reactors were completely mixed by supplying oxygen using air pumps which maintained the dissolved oxygen (DO) concentration as minimum 3 mg/L and the pH was maintained at 7.7 ± 0.3 . The C/N ratio of the feed to the reactors 17 in terms of the ratio COD toTKN. Phosphate buffer was not used to adjust the pH in reactors to eliminate the formation of salt precipitation as Ca₃(PO₄)₂ and to avoid concentration effect of KH₂PO₄ and K₂HPO₄ on the potassium reactors and sodium counterpart of buffers in sodium reactors. Peptone was used as the sole phosphorus source for the reactors, because it was a special type phosphorus rich peptone. The activated sludge systems were operated with 8-days mean cell residence time. This was provided by wasting 250 mL sludge from each reactor daily. The reactors were kept in water bath at 25°C to maintain the temperature of the reactors constant.

The alimentation of reactors was performed daily as the same following way. After the reactors were mixed completely, 250 mL of sludge was wasted from each reactor. Then the reactors were left for settling for 2 hours. At the end of 2h-settling period, the supernatant of each reactor was siphoned out. Then the feed solutions were added to the reactors and the volume of the reactor was

completed to 2 L using distilled water to prevent any ion originating from tap water.

3.1.1. Steady State Determination

The semi-continuous activated sludge reactors were operated until the reactors reach the steady state which is necessary step to be conducted analyses. The reactors were considered to be at steady state dependent on the Mixed Liquor Suspended Solids (MLSS) and Mixed Liquor Volatile Suspended Solids (MLVSS) concentrations (Appendix A). Obtaining the similar results for these conducted parameters following 4-6 measurements indicated the achievement of the steady state.

3.2. Analyses Conducted Under Steady State Conditions

Once the reactors reached steady state, chemical, surface chemical and physical analyses of sludge samples obtained from operating reactors were conducted to determine the effect of sodium and potassium ions on the activated sludge characteristics obtained from reactors.

3.2.1. Chemical Analyses Conducted at Steady State

The conducted chemical analyses are extracellular polymer extraction from sludge, measurement of ion concentration in sludge and determination of the conductivity. EPS were extracted by using cation exchange resin (CER) from the sludge of the reactors. The extraction procedure was followed by carbohydrate and protein analyses.

Potassium and sodium ion concentration measurements in sludge were conducted at steady state conditions. After digestion procedure, obtained samples were

diluted and measurements were carried out. Also, conductivity of each reactor was measured at steady state.

3.2.1.1. Polymer Extraction and Analysis Techniques

The extraction step is the key point in determining the amount and composition of EPS in activated sludge. It is suggested that the effective and good extraction procedure should result in less cell lysis and minimal disruption of the exocellular polymers. The method carrying these properties is the cation exchange resin (CER) method and it was recommended first by Frolund *et al.* (1996). CER extraction process is widely used in the extraction of floc-bound biopolymers so in this study, CER extraction technique developed by Durmaz and Sanin (2001) which was similar to a method used by Frolund *et al.* (1996) was employed to the sludge samples.

DOWEX 50X8, 20-50 mesh in the sodium form strongly acidic cation exchange resin supplied from Fluka was used during the application of the CER extraction method. Prior to extraction procedure, MLVSS value of each sludge sample was determined and in the highlight of the previous study conducted by Durmaz and Sanin (2001), 100 g CER/ g VSS dose was accepted and employed during the extractions. The required CERs based on the dose were weighed for each reactor and were put into jar test beakers to wash with phosphate buffer saline (PBS) by stirring for 1h at 120 rpm. The composition of PBS is given in Table 3.2. The aim of the washing is to avoid leaching of possible contaminants, if any, from the CER and to prevent interferences during the chemical analysis of extracellular polymers. Then the CER was filtered through 0.45 μm Millipore filter and dried at room temperature until the next day.

Table 3.2. The composition of the phosphate buffer saline (PBS) solution

Constituent	Concentration (mg/L)
NaCl	4
KCl	0.1
KH ₂ PO ₄	0.06
Na ₂ HPO ₄	0.455

Extracellular polymer extraction procedure was conducted on 250 mL sludge of each reactor. For each reactor, 50 mL sludge was separated in order to perform MLVSS measurement and the remaining 200 mL sludge was centrifuged at 3500 rpm for 15 minutes. The centrate was discarded and the pellet was resuspended using PBS to 200 mL in a jar test beaker. Then the washed and dried CER weighed for that sludge sample was added to the beaker. In addition, two control samples were employed. The first control was only sludge sample with no CER addition and was named as “sludge control”. It was used to determine the contribution by stirring during the polymer extraction procedure. The second control was CER without the addition of sludge and was named as a “CER control” which was prepared to understand the effect of CER itself. Although each reactor had its own “sludge control”, only one “CER control” was used for all the reactors due to minimum contribution originated from CER. The control samples had volumes of 200 mL and the suspensions were in PBS parallel to the test sludges. The same extraction procedure was applied to all reactors. After the beakers were placed at a standard jar test apparatus, they were operated for 5 hours at a constant stirring speed of 120 rpm. The extraction time and stirring speed was selected relied on the previous study (Durmaz and Sanin, 2003).

After 5 hour-stirring, the beakers were allowed to settle for half an hour. The liquid fraction at the top was removed and centrifuged for 15 minutes at 3500 rpm. These samples were analyzed to measure the protein and carbohydrate concentration of each sludge sample.

3.2.1.1.1. Carbohydrate Analysis

Polymer extraction procedure was followed by the determination of contents of the EPS. Carbohydrate content of the EPS was measured using phenol-sulphuric acid method developed by Dubois *et al.* (1956) and alginate was used as a standard.

In order to measure carbohydrate concentration, 2 mL samples were taken and put into test tubes which were set in triplicate order for each reactor. 50 μ L phenol which was prepared as 80% (w/w) and 5 mL sulphuric acid were added into each tube. The samples were allowed to stand for 10 minutes at room conditions. Then, the tubes were vortexed and placed into an incubator at 30°C for 15 minutes. The formation of yellow-orange color is the characteristic for this method and then the absorbance of each sample was measured at 480 nm using Pharmacia LKB Novaspec II Spectrophotometer. The calculation of the each carbohydrate concentration was achieved by using a standard calibration curve which was prepared prior to experiment by using alginate. The calibration curve is depicted in Appendix B.

3.2.1.1.2. Protein Analysis

The protein content of the EPS was measured using folin-ciocalteu phenol reagent method developed by Lowry *et al.* (1951) and bovine serum albumin was used as a standard.

Prior to the application of method, four reagents were prepared in order to use in the analysis. The reagents were named as reagent A, B, C and D. Reagent A included 2% w/v sodium carbonate in 0.1 N NaOH. Reagent B was prepared by dissolving 1% w/v sodium potassium tartarate in 0.5 % w/v cupric sulphate. Reagent C was composed of 1mL of Reagent B and 49 mL of Reagent A. Lastly,

Reagent D contained the Folin-Ciocalteu's phenol reagent which was diluted with deionized water by the ratio of 10:10.

After CER extraction procedure, 600 μ L sample taken from the supernatant of each sample was added into triplicate-ordered test tubes. Each tube containing sample was mixed with 3 mL Reagent C and then they were allowed to stand for 10 minutes at room temperature. At the end of this period, 300 μ L Reagent D was added and the tubes were vortexed well and quickly as soon as possible. Then the samples were kept at room temperature for half an hour. The formation of the blue color is characteristic for this spectrophotometric measurement. The absorbance based on the intensity of the blue color was measured at 750 nm by using Pharmacia LKB Novaspec II Spectrophotometer. The calculation of the protein concentrations was carried out by using a standard calibration curve by using Bovine Serum Albumin as a standard. The protein calibration curve was prepared prior to each experiment. One of them is presented in Appendix B.

3.2.1.2. Ion Concentration Measurement in Sludge

Ion concentration was measured in each sludge sample by using microwave assisted digestion process. Since the ions associated with the floc structure was impossible to measure without extraction, this type of a procedure was used. The procedure described by Özsoy (2006) was applied to the sludge samples. In order to handle enough amount of sludge, approximately 400 mL wasted sludge was collected for each reactor and centrifuged for 5 minutes at 3500 rpm to separate water part of the sludge. After removing of the supernatant part, pellet containing sludge was dried at 103°C for 24 hours. 0.25 g dried and powdered sludge was weighed for each reactor and then added into teflon vessels of Berghof speedwave MWS-2 microwave digester. Triplicate vessels were used for each reactor in order to take average and find out the standard deviation. To achieve microwave digestion of sludge samples, 5 mL nitric acid (65% w/v) and 5 mL

hydrofluoric acid (40% w/v) was put into teflon vessels containing dried sludge. A blank was used containing no sludge sample to obtain contribution derived from the used HNO₃ and HF. The vessels were placed into table of digester and a program was run for digestion. The program was composed of three stages each of which was operated at different time, temperature and power presented in Table 3.3. Following the digestion process in microwave, the sludge samples and supernatants were put into teflon beakers and then they were boiled until 3-5 mL sample was remained in the beakers. The volume of the obtained samples were completed to 25 mL with distilled water and filtered through Millipore filter. After the required dilutions were conducted, the concentration of sodium and potassium ions in sludge was determined using Jenway PFP7 Model Flame Photometer.

Table 3.3. Stages of the program run for microwave digestion process

Program Stage	Time (min)	Temperature (°C)	Power(W)
Stage 1	40	200	800
Stage 2	25	100	400
Stage 3	1	20	400

3.2.1.3. Conductivity

Conductivity in water is affected by the presence of inorganic dissolved solids. In order to see the effect of an increasing addition of ions on activated sludge reactors, the conductivity measurements were conducted using CyberScan PC 510 pH/conductivity meter. After the reactors were mixed well, they were allowed to settle for 2 minutes and then the conductivity probe was placed into the reactors at 25°C to measure conductivity expressed as microsiemens (or milisiemens) per centimeter.

3.2.2. Surface Chemical Analyses Conducted at Steady State

The surface chemical properties of the activated sludge play a crucial role in determining dewatering and settling characteristics of sludge and also contribute to formation of the floc structure. In order to understand surface properties of the sludge samples obtained from operated reactors, hydrophobicity and zeta potential analyses were conducted.

3.2.2.1. Hydrophobicity

Relative hydrophobicity can be measured by different methods such as contact angle, microbial adhesion to hydrocarbons (MATH), salt aggregation and acid precipitation followed by adsorption by hydrophobic resins (Liu and Fang, 2003). MATH (Rosenberg *et al.*, 1980) was selected in order to use in the experiments since it is the most commonly used and simplest method to conduct. In this test, the selection of an organic and aqueous phase was important for accurate measurement of hydrophobicity. In this study, n-Hexadecane and phosphate buffer saline was used as organic and aqueous phase, respectively.

The MATH procedure was applied as described in (Durmaz, 2001; Vatansever, 2003 and Turtin, 2003). Sludge samples taken from each reactor were washed twice with PBS (Table 3.2) by centrifuging at 3500 rpm for 5 minutes. 5 mL bacterial suspension was taken and diluted with PBS to adjust absorbance or optical density (OD) 0.4 at 600 nm using the Milton Roy Company Spectronic 20D Spectrophotometer. After each dilution, the bacterial suspensions were vortexed to provide homogenization during spectrophotometric measurements. Once the adjustment of OD to 0.4 was achieved, this value was accepted as initial optical density. Then, 10 mL bacterial suspension was taken into a test tube and 0.5 mL n-Hexadecane was added to it. The test tube was vortexed for 2 minutes for homogenization and stand for 15 minutes at room temperature to

provide separation of organic phase into the upper part of the test tube. The liquid remaining at the bottom of the test tube was taken by the help of a Pasteur pipette and the optical density of the liquid was measured to obtain the final optical density. The hydrophobicity as percent was calculated as:

$$\text{Hydrophobicity (\%)} = \left(1 - \frac{OD_{final}}{OD_{initial}} \right) * 100 \dots\dots\dots 3.1$$

3.2.2.2. Zeta Potential

Microbial cells, EPS and sludge flocs carry negative charges due to the ionization of the anionic functional groups, such as carboxylic and phosphate. These properties may be characterized by zeta potential or surface charge (Liu and Fang, 2003). 10 mL wasted sludge was taken from each reactor and diluted to 250 mL with PBS (Table 3.2). Next, the sludge samples were sonicated for 1 minute at 25°C for homogenization. Then, the measurement of zeta potential was conducted using Zeta Sizer Nano series ZS90.

3.2.3. Physical Analyses Conducted at Steady State

Physical properties affecting the settling and dewatering characteristics of the activated sludge were analyzed to reveal the effect of ion type and concentration at steady state conditions. The performed tests are particle size, viscosity, specific resistance to filtration (SRF), capillary suction time (CST), floc strength, sludge volume index (SVI), zone settling velocity (ZSV), and turbidity.

3.2.3.1. Particle Size

Particle size measurements were conducted since the particle size distribution affects settling and dewatering characteristics of the sludge. Prior to the

measurement of floc size, 10 mL wasted sludge was taken from each reactor and diluted to 250 mL with PBS (Table 3.2). Dilution was required for prevention of multiple scattering caused by concentrated sludge samples and to obtain particle counts within the limits of the analyzer. Then, the sludge samples were sonicated for 1 minute at 25°C to provide homogenization. Floc size measurements were carried out by using a Malvern Mastersizer 2000 with particle size detection range of 0.02-2000 μ . Mie theory describing the scattering of light by particles was used by Mastersizer to measure floc size. The Mastersizer software generates a volume-weighted floc size distribution. In order to compare the mean particle size of sludge samples, the volume-weighted average diameter, which is also known as the mass mean diameter, $D[4,3]$ was used.

3.2.3.2. Viscosity

The rheological properties of the sludge samples were investigated by using a rotational viscometer called Brookfield LVDVII+ with ultra low viscosity adapter.

Viscosity measurements were conducted at 6 different shear rates; 1.83, 3.67, 7.34, 14.7, 36.7, and 73.4 sec^{-1} due to non-Newtonian behavior of the sludge. The apparent viscosities of the sludge samples were determined by calculating the ratio of shear stress to shear rate at a constant shear rate. At the same time in order to reveal the relationship between the apparent viscosity and suspended solids concentration (MLSS), viscosity measurements were performed at 5 different suspended solids concentration prepared by diluting the sludge samples of each reactor. The time lasted for measurement was maintained constant at 1 min. Shear stress (dyne/sq.cm) versus shear rate (sec^{-1}) graphs were plotted to determine the flow characteristic of the sludge samples. Apparent viscosity (cP) versus MLSS (mg/L) graphs was drawn and a constant MLSS value was selected in order to compare viscosity values of sludge samples with each other.

3.2.3.3. Specific Resistance to Filtration

The dewaterability of the sludge samples was determined by applying specific resistance to filtration (SRF) test described by Vesilind (1979). A 50 mL sample was filtered using Whatman 40 filter paper under 21 inch-Hg vacuum pressure into graduated cylinder. The time was recorded every 5 mL volume interval withdrawn from the sludge. The filtration procedure was followed by the determination of solids concentration in the cake by the gravimetric analysis. Time/volume (t/V) versus volume (V) graph was plotted for each reactor by using recorded data and graphs are depicted in Appendix C. SRF was calculated for each reactor using the slope of plotted graphs from the following formula:

$$r = \frac{2PA^2b}{\mu w} \dots\dots\dots 3.2$$

where:

r : Specific resistance to filtration, m/kg

P : Pressure difference, N/m²

A : Filtration area, m²

b : Slope of the t/V versus V plot, s²/m⁶

μ : Viscosity of filtrate, N.s/m²

w : Weight of dry cake solids per unit volume of filtrate, kg/m³

3.2.3.4. Capillary Suction Time

The dewatering property was also measured with capillary suction time (CST) applying Method 2710G (APHA 2000). As can be seen from Figure 3.2, Type 304 M Triton Electronics Capillary Suction Timer composed of a test block and

sludge reservoir was used for the measurement of CST. A sheet of Whatman 17 chromatographic paper which had 7x9 cm size was placed into the test block on which two electrical contact points were present. A small cylinder called as a sludge reservoir was insert into the block and the sludge sample was added in the sludge reservoir. The time started when the filtrate of sludge flowing in the paper reached inner electrical contact and the time ended when the filtrate reached the outer contact. The CST measured as seconds was read from digital display.

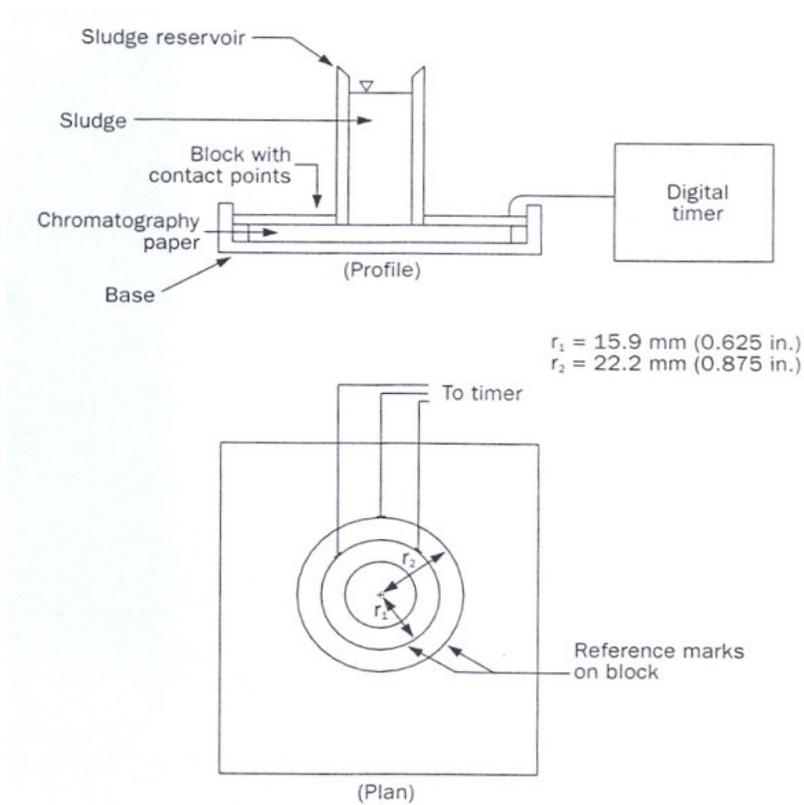


Figure 3.2. Illustration of CST apparatus from APHA (2000)

3.2.3.5. Floc Strength

Floc strength measurements were conducted to determine the resistance of the sludge samples to shear. The floc strength was analyzed by applying a constant shear, 1000 rpm, to the sludge samples at different time intervals: 0, 0.5, 2, 4, 8 minutes and measuring CST over time. Zero means that CST of sludge sample was measured prior to shear application in order to see shear effect. 120 mL sludge sample was taken into a beaker and 1000 rpm shear was applied by using Velp Scientifica Stirrer Type PW for each time interval. Then, triplicate CST measurements were performed and CST versus time graphs were plotted for each reactor. The comment of floc strength of the sludge samples was made by examining the slope values of the graphs.

3.2.3.6. Sludge Volume Index

Sludge volume index (SVI) is one of the used analyses determining the settleability of the sludge. Prior to SVI measurement, suspended solids concentration was measured as MLSS. The mixed sludge added to 1 L graduated cylinder was allowed to settle for 30 minutes. At the end of the 30 minutes, the settled volume was recorded and SVI value of the sludge was calculated by using following formula:

$$SVI = \frac{30 \text{ minute settled sludge volume (mL / L)} * 1000}{\text{Suspended Solids Concentration (mg / L)}} \dots\dots\dots 3.3$$

3.2.3.7. Zone Settling Velocity

Zone settling velocity (ZSV) is the common method used for determining the settleability of the sludge. Since it is greatly dependent of solids concentration, ZSV measurements were conducted at three different MLSS concentrations. After suspended solids concentration was determined, a well-mixed sludge sample was poured into 1L graduated cylinder and was allowed to settle for

about 1 hour. The settlement of solid-liquid interface was followed with respect to time. Time was recorded at every 20 mL fall of the solid-liquid interface (corresponding to 0.5 cm height) during settlement. Measurements were continued until the sludge settlement velocity decreased remarkably. This procedure was repeated for each MLSS concentration. After all data was collected, height of the settling interface versus time graphs was plotted. A straight line was drawn on the graph by ignoring reflocculation period to obtain ZSV as a slope. Then, ZSV (cm/s) versus MLSS (mg/L) graphs were plotted in order to show the effect of solid concentration on sludge settling velocity as depicted in Appendix D.

3.2.3.8. Turbidity

The sludge sample was poured into a 1 L graduated cylinder and allowed to settle for 1 hour. The supernatant taken after 1-hour-settlement was put into turbidity measurement cell and analyzed by using Hach Turbidimeter 2100N. The unit of the turbidity was expressed as Nephelometric Turbidity Units (NTU).

3.2.4. Other Measurements

MLSS and MLVSS: One of the most important parameters used in making control decisions in the activated sludge process of wastewater treatment systems is mixed liquor suspended solids (MLSS) and/or mixed liquor volatile suspended solids (MLVSS). In this study, MLSS and MLVSS concentrations were followed in order to determine steady state period. MLSS is a measure of dry solids concentration in mg/L in mixed liquor in an aeration tank. MLVSS is generally defined as the microbiological suspension in the aeration tank of an activated-sludge biological wastewater treatment plant.

MLSS and MLVSS were measured approximately every three days in order to monitor whether the steady state was achieved or not. The sludge samples were

analyzed using Method 2540D and 2540E (APHA, 2000) for MLSS and MLVSS measurement, respectively.

Chemical Oxygen Demand (COD): COD is the milligrams of oxygen required to chemically oxidize the organics in one liter of wastewater. The COD measurement was conducted by the closed reflux colorimetric method using Hach DR2000 spectrophotometer.

pH: pH was measured by CyberScan PC 510 pH meter/conductivity meter.

Dissolved oxygen (DO): DO was measured by Hach Sension 378 pH/conductivity/DO meter.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Effect of Potassium Ion Concentration on Sludge Characteristics

The aim of this part is to investigate the effects of three different concentrations of potassium ions on chemical, surface chemical and physical characteristics of activated sludge which was obtained from lab-scale semi-continuous activated sludge reactors. Steady state was achieved prior to analyses.

4.1.1. Reactors and Achievement of Steady State Conditions

Potassium ions were separately added to the feed of laboratory scale semi-continuous reactors in four different concentrations as given in Table 4.1 and each concentration was operated with replica reactors. The reactors with 0.5 meq/L were used as control reactors for this reactor set since control reactors were used as basis for observing the effects of increasing cation concentration. The sodium concentration of all these reactors was 0.5 meq/L, calcium was 0.3 meq/L and magnesium was 0.9 meq/L. Higher concentrations of potassium were achieved with the addition of the precalculated doses of KCl into the feed given in Table 4.2.

Table 4.1. Potassium ion concentration in potassium reactors

Concentration in Reactors	Potassium Reactors
K = 0.5 meq/L	C1, C2
K = 5 meq/L	R1, R2
K = 10 meq/L	R3, R4
K = 20 meq/L	R5, R6

Table 4.2. Composition of the synthetic feed medium for control reactor

Constituent	Concentration (mg/L)
Glucose	163.54
Biosate Peptone	942.5
NH ₄ Cl	225
MgSO ₄ .7H ₂ O	112.5
FeSO ₄ .7H ₂ O	3.75
ZnSO ₄ .7H ₂ O	3.75
MnSO ₄ .7H ₂ O	3.75
CaCl ₂	15
NaHCO ₃	42
KCl	37.25

Reactors were brought to steady state, which is necessary step to be conducted analyses. In the literature, it was reported that the analyses should be performed after the systems are operated for at least 2–3 SRTs for the adaptation and response of microorganisms found in reactors to the new conditions (Forster and Dallas-Newton, 1980). For this, the steady state determination was achieved by measuring the mixed liquor suspended solids (MLSS), and mixed liquor volatile

suspended solids (MLVSS) concentrations. The reactors were considered to be at steady state when the variability in the MLSS and MLVSS was less than 10% between following conducted measurements

4.1.2. Effect of Potassium Ion on Chemical Characteristics of Activated Sludge

The analyzed chemical characteristics are determination of effluent soluble COD, composition of EPS in terms of protein and carbohydrate, measurement of potassium ion concentration in sludge and of conductivity in the liquid. During the whole study, 4 control reactors were operated: two with potassium, other two with sodium. Since the variation was always less than about 25 %, the control data was obtained by taking the average of potassium and sodium replica control reactors (a total of 4). The steady state values of each parameter analyzed were calculated by taking the average values of replica reactors during the steady state period and reported here in this section. The replication between the replica reactors was extremely good. In the following sections, each analysis will be discussed.

4.1.2.1. Effluent Soluble Chemical Oxygen Demand (COD)

The effluent soluble COD values were measured for the determination of effect of potassium ions at rising concentrations on effluent quality. The original COD of the feed to the reactors was 1395 mg/L. It can be seen from Figure 4.1, the increase in potassium concentration was correlated well with the increase in COD. It is obvious that greater effluent COD was observed at greater potassium ion concentrations. Also, the examining of COD removal values of reactors indicated that the COD removal efficiency reduced with the addition of potassium ions at increasing concentrations, however, the comparison of potassium reactors with control reactor revealed that no significant decrease was

observed in COD removal efficiency up to 5 meq/L of potassium. This result is parallel to effluent COD values since an important increase was observed when concentration shifted from 5 meq/L to 10 and then to 20 meq/L. Therefore, it can be concluded that 10 meq/L and further addition of potassium ions to the reactors cause an increase in proteins and polysaccharides in solution, thereby increasing the effluent COD concentration. This can be originated from the removal of divalent cations from the floc structure by potassium ions. This is believed to originate from the displacement of divalent cations that weakens the floc structure and results in the release of protein and polysaccharides to the solution, contributing to soluble COD. This conclusion is also supported by the findings of Murthy and Novak (1998). They found that a high concentration of sodium or potassium ions led to a greater concentration of solution proteins and polysaccharides.

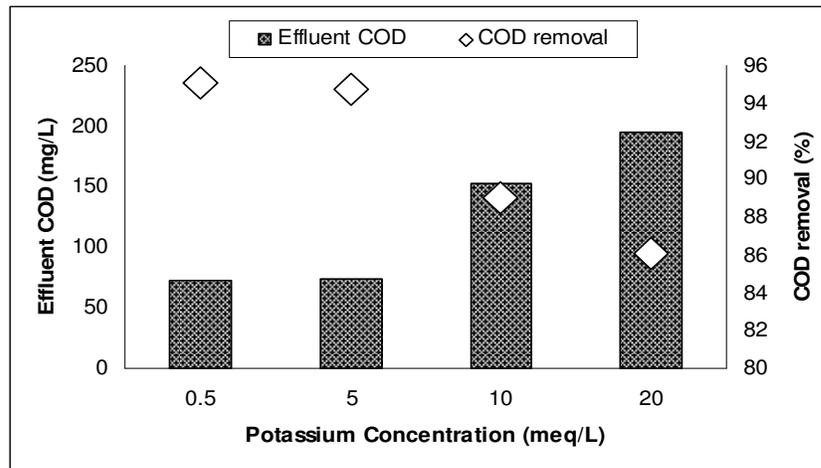


Figure 4.1. The effect of potassium ions on COD removal efficiency of reactors

4.1.2.2. Extracellular Polymer Production and Composition

Extracellular polymeric substances (EPS) are a major component of activated sludge floc. The main components of EPS are protein, polysaccharides, lipids,

nucleic acids and humic substances (Tenney and Verhoff, 1973; Brown and Lester 1980; Barber and Veenstra 1986; Eriksson and Alm 1991; Urbain *et al.* 1993, Frolund *et al.*, 1996). Frolund *et al.* (1996) stated that the EPS are considered important for the physico-chemical properties of activated sludge flocs and have been implicated in determining the floc structure (Eriksson and Hardin, 1984); the floc charge (Horan and Eccles, 1986); the flocculation process (Ryssov Nielsen, 1975; Brown and Lester, 1980; Barber and Veenstra, 1986; Eriksson and Alm, 1991); the settling properties (Forster, 1985; Goodwin and Forster, 1985; Urbain *et al.*, 1993) and the dewatering properties (Kang *et al.*, 1989).

Cation Exchange Resin (CER) method was used to extract the EPS. CER removes cations from the sludge matrix leading to breakup of the flocs and a subsequent release of EPS attached to the sludge matrix by metal ions (Frolund *et al.*, 1996). The CER extraction method was found superior to other commonly used methods in terms of yield and minimal disruption of exopolymers.

In reference to these findings, extraction and quantification of EPS were conducted from potassium fed reactors. According to obtained results, quality and quantity of EPS composition changed at different concentrations of potassium ion. The relationship between total EPS, the sum of the protein and carbohydrates, and potassium concentration was presented in Figure 4.2 as the average of two replica reactors.

As seen from Figure 4.2, the rising addition of potassium ion led to increase in total amount of EPS. It means that potassium addition increases biopolymer production or improves binding of biopolymer to the flocs. Wilkinson (1958) revealed that potassium, calcium and magnesium ions all stimulated EPS synthesis; a deficiency of any of these ions caused a decrease in extracellular polymer production. At the same Figure 4.2; the mass of protein and carbohydrate is given separately as a function of potassium concentration added

to the feed. It is revealed that the increase in potassium concentration is positively correlated with the increase in the carbohydrate concentration. The protein content decreased when shifted from 0.5 meq/L to 5 meq/L but then, addition of potassium ions at rising concentrations provided an increase in the concentration of protein. Therefore, it can be said that potassium ions promote both the synthesis of protein and polysaccharide type polymers. Murthy and Novak (1998) found that potassium was the only ion that was positively correlated with slime protein and polysaccharide.

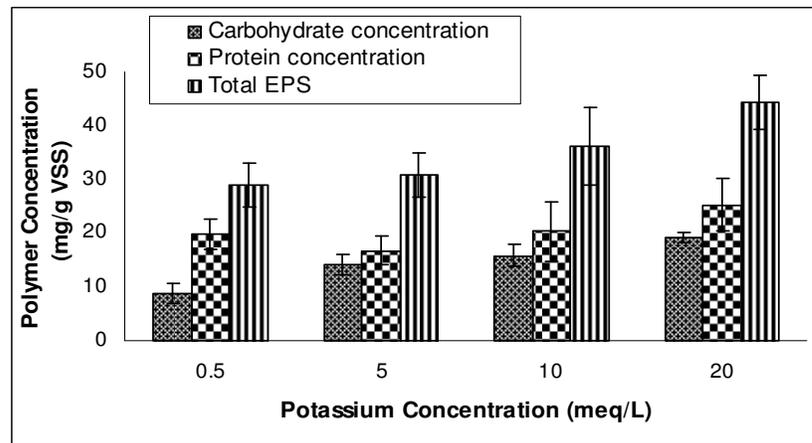


Figure 4.2. Effect of potassium concentration on the production and composition of EPS

Also Figure 4.2 shows that the protein content of EPS is greater when compared to carbohydrate content for all of the studied potassium concentrations. There is a lack of information about either accumulation of ions in the extracellular structure of the activated sludge and affinity of these ions to the specific constituents of the EPS. According to these results, it can be said that although addition of potassium ions contributes to increase in the carbohydrate content, potassium ions tend to stimulate the synthesis of proteinaceous polymers and are able to bind more protein than carbohydrate within the floc structure. It was

revealed that cellular potassium activates various cytoplasmic enzymes and is required for protein synthesis (Epstein *et al.*, 1993) since the peptidyl transferase reaction during the protein synthesis is potassium dependent. Also, it was reported that potassium starvation impairs the protein synthesis reaction in some microorganisms (Alahari and Apte, 2004).

Apart from their stimulation effects on polymer production, cations act as binding agents of the flocs. The tendency of different cations to form cation bridges depends on the ionic charge and the size of the hydration shell of the cations. Each metal ion has a number of water molecules associated with it due to dipole character of the water molecule. This is called as a hydration shell and it was reported an inverse correlation between ion size hydration shell radius (Piirtola *et al.*, 1999).

As it is given in Table 2.1 in Chapter 2 and explained in that section, although potassium and sodium ions have same charge, potassium has smaller hydrated radius compared to that of sodium, so it loses its hydration shell easier near the charged groups of EPS and establishes a stronger bond with charged sites on EPS than sodium whose approach to the surface is prevented by water molecules around it. Brown and Lester (1979) indicated that different metal adsorption sites exist on anionic and neutral polysaccharides. Metal ions with different charges may also bind at different sites of extracellular polymers. Also, Forster (1985b) hypothesized that ionic size of cations may influence their binding ability to charged (carboxyl) and uncharged groups (hydroxyl) in the EPS, so each cation with different hydration shell size might have affinity to bind certain constitute of EPS. Our findings reveal that potassium has affinity to bind both of polymers in floc structure.

It is evident from data that protein and carbohydrate are important biopolymer components in the flocculation process and potassium ions at increasing concentrations stimulate the synthesis of more extracellular biopolymers.

Although protein was the dominant constituent of the EPS of potassium reactors, potassium ions promote the synthesis of both the protein and polysaccharide type biopolymers.

4.1.2.3. Potassium Concentration in Floc Structure

Potassium concentration incorporated into the floc structure was determined based on a microwave digestion and extraction procedure of sludge when reactors reached the steady state. These results are illustrated in Figure 4.3.

As can be seen from Figure 4.3, the increase in cation concentration incorporated into the floc matrix was correlated positively to the increase in cation concentrations incorporated in the feed. However, a one to one parallel increase was not observed with respect to cation dose in feed medium and floc matrix. For example, when concentration shifted from control reactor (0.5 meq/L) to 5 meq/L reactor, although potassium concentration added to the feed was increased 10 times, only a two times increase was observed in potassium concentration incorporated into the floc structure.

The increase in potassium concentration in floc matrix is parallel to the already discussed increase in EPS content of the sludge with the increase in ion concentrations. This indicates that potassium ions stimulate the synthesis of more protein type polymers compared to carbohydrate type polymers since one of the steps of protein synthesis is potassium dependent (Alahari and Apte, 2004) as mentioned earlier. It is seen that the highest amount of ions in the floc matrix is found at the highest potassium concentrations with the greatest amount of EPS.

Potassium is a major cytoplasmic cation in growing bacterial cells, composing 0.8-1.5 % of the dry mass of the cell and plays important roles in the cell physiology. It is required in activating enzymes, including those involved in protein synthesis, and in the maintenance of osmotic pressure and regulation of

pH (Gerardi *et al.*, 1994; Kawano *et al.*, 1999). When the osmotic pressure of the medium increases, potassium ions are transferred into the cell for the adjustment of pressure. This might explain the results given in Figure 4.3. With the increase of osmotic pressure due to increasing potassium ions in the medium, more of it is transferred into the cell. Due to its osmotic function, potassium ions incorporated into the flocs is believed to be mostly inside the cells rather than being in the EPS matrix. Therefore, potassium can be considered as an intracellular cation.

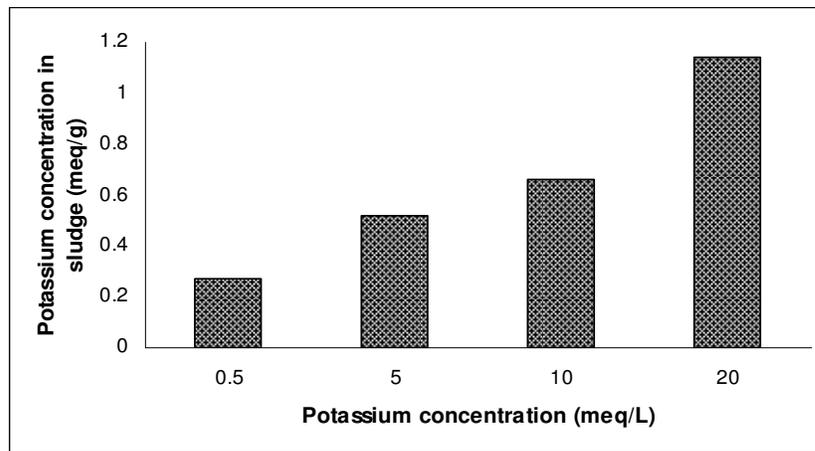


Figure 4.3. Potassium concentration incorporated into floc structure with respect to potassium concentration in feed medium

4.1.1.4. Electrical Conductivity

After potassium reactors reached the steady state, conductivity of the liquid fraction of reactors was measured as the last chemical characteristic of sludge.

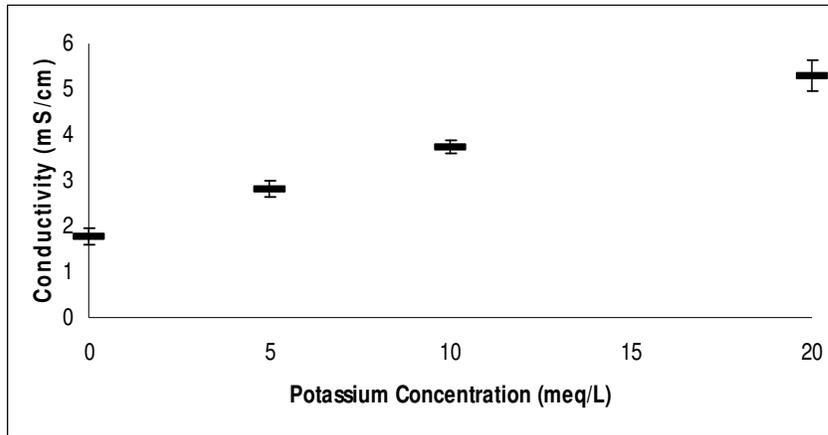


Figure 4.4. Conductivity values of potassium containing sludges with respect to potassium concentration

Conductivity is the measurement of the total amount of dissolved ions in the water. Therefore, the rising addition of the potassium as a salt to the system resulted in the increase in dissolved ion concentration and this was accompanied by the increase in the electrical conductivity as shown in Figure 4.4. As expected, the utmost increment was obtained shifting from 10 meq/L reactor to 20 meq/L reactor.

4.1.3. Effect of Potassium Ion on Surface Chemical Characteristics of Activated Sludge

4.1.3.1. Hydrophobicity

The outer surface of microbial cells contains a variety of chemical compounds that may be involved in the attachment of cells to surfaces. Hydrophobic/hydrophilic interactions play a large role in attachment, leading to the development of the concept of cell surface hydrophobicity as a measure of the tendency of a cell to attach to a surface. There is no absolute measurement of

hydrophobicity so relative hydrophobicity is measured by the Microbial Adhesion to Hydrocarbons (MATH) test.

As can be seen in Figure 4.5, a decrease was observed in the hydrophobicity when potassium sludges were compared to control reactor. There was no appreciable change in hydrophobic character of the sludge when potassium concentration increased from 0.5 to 5 meq/L. A further increase of potassium concentration to 10 meq/L led to a noteworthy decline in the value of hydrophobicity. Despite the fact that a small increase was observed shifting from 10 meq/L reactor to 20 meq/L, hydrophobicity of this reactor decreased when control reactor value was taken into consideration. When the obtained data related with hydrophobicity was examined, it was seen that the values change in the range of 51-59 % so it can be considered that although the rising addition of potassium to the feed led to a small decline in the hydrophobicity value, no drastic change in the hydrophobic character of the sludge was observed.

When the relation between hydrophobicity and EPS content of the sludge is considered, Liao *et al.* (2001) proposed that the proportions of EPS components were more important than the quantities of individual EPS components in controlling hydrophobicity. Therefore, in order to evaluate the hydrophobic character of sludge, EPS_p/EPS_c ratio should be taken into consideration since hydrophobicity of sludge depends on the interactions between hydrophilic and hydrophobic properties of the polymers. EPS_p/EPS_c ratio was determined for each reactor as demonstrated in Figure 4.5. As understood, when potassium concentration was increased from 0.5 to 5 meq/L, a significant decrease was observed in the EPS_p/EPS_c ratio from 2.2 to 1.2 and further addition of potassium to the feed led to no obvious increase in the ratio.

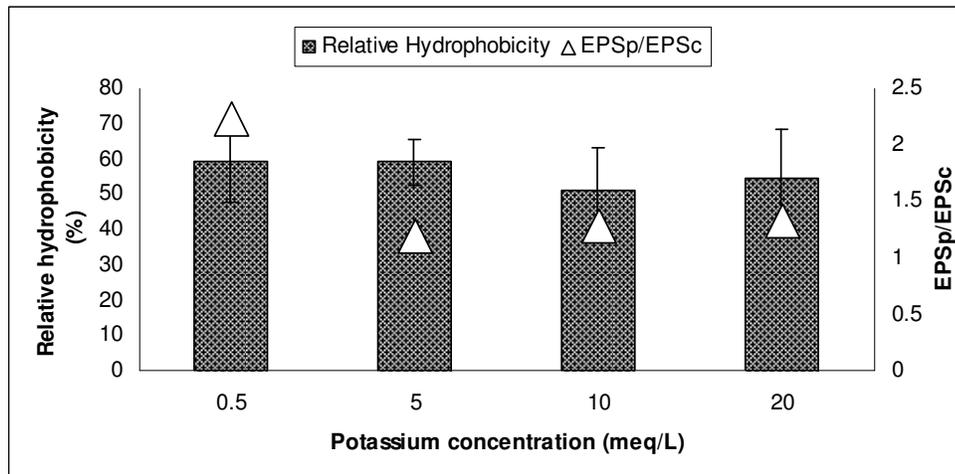


Figure 4.5. Relative hydrophobicity value and EPSp/EPSc ratio of each reactor with respect to potassium concentration

Hydrophobic molecules such as lipids or proteins from the cells can be trapped into the flocs and cell surface hydrophobicity may be promoted by specific proteins with specific amino acid sequences. Higgins and Novak (1997a) showed that hydrophobic amino acids of protein were significant constituents in activated sludge and suggested that biopolymers can also bind through hydrophobic interactions. The increase in the potassium concentration promotes the production of protein type polymers but it can be said that these produced protein type polymers may not contribute hydrophobicity of the sludge since they may not contain the specific hydrophobic amino acids such as alanine, leucine and valine. Morgan *et al.* (1991) revealed that diacidic amino acids, such as aspartic acid and glutamic acid are found largely in EPS and are negatively charged polar hydrophilic amino acids so the produced protein or bound protein in potassium-containing sludges can be substantially in hydrophilic character and the increase in the protein and carbohydrate introduced the hydrophilicity rather than hydrophobicity to the sludge. Jorand *et al.* (1998) reported that the EPSp contributed to the sludge hydrophobicity, but not the EPSc. According to our results, it can be concluded that hydrophobicities of the sludge not only depend

on the EPSp but also EPSc and cation type. Also, the amino acids forming the structure of the protein should be analyzed whether hydrophilic or hydrophobic since the structure of them determines the sludge hydrophobicity.

4.1.3.2. Zeta Potential

Zeta potential is the one of the surface chemical properties investigated in this study. Microbial cells, EPS and sludge flocs can be regarded as charged particles having an overall net negative charge at pH values encountered in natural environments (Harden and Harris, 1953; Burns, 1979) due to the ionization of the anionic functional groups, such as carboxylic, hydroxyl and phosphate. At neutral pH, while functional groups such as carboxylic and phosphate carry negative charge, amino groups carry positive charge. Therefore, surface charge of sludge was strongly dependent on the EPS's chemical group composition and concentration (Jia *et al.*, 1996). Flocs and EPS of all activated sludge carried negative charge, mostly within the range of -0.2 to -0.6 meq/g-VSS with a zeta potential of -20 to -30 mV (Liu and Fang, 2003). The obtained results demonstrated in Figure 4.6 are found in this range.

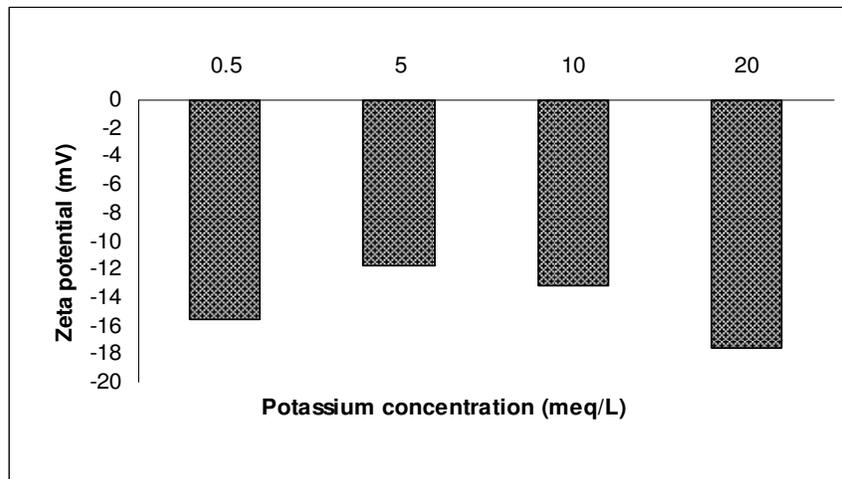


Figure 4.6. Effect of potassium concentration on the zeta potential of sludge

In this study, addition of potassium ions at different doses introduces different doses of positive ions into the system. In addition to this, potassium ions added are influencing the polymer production by stimulating different composition of polymers produced. For this reason, the effect of cation on charge of sludge is another complex issue to evaluate.

The zeta potential of control reactor was measured as -15.6 mV and a slight decrease was observed with the rising of potassium concentration to 5 meq/L. This situation can be attributed to the neutralization of the negative surface charges by the introduction of positively charged potassium ions. When potassium concentration increased to 10 and then 20 meq/L, an increasing trend was obtained in the zeta potential. This can be explained by the relationship between surface charge and EPS. The effect of EPS constituents on surface charge have been shown in literature (Magara *et al.*, 1976; Horan and Eccles, 1986, Morgan *et al.*, 1990, Mikkelsen and Keiding, 2002). The addition of more potassium to the feed was not sufficient to neutralize the negative charges since increase in the concentration of especially carbohydrate type polymers introduces more negative charge to the floc surfaces, causing an increase in zeta potential. According to results, as demonstrated in Figure 4.2, the increase in the total EPS was correlated to increase in surface charge so it can be concluded that there was a positive correlation between zeta potential and total EPS as stated in previous studies (Mikkelsen and Keiding, 2002; Wilen *et al.*, 2003). In addition, Morgan *et al.* (1990) and Liao *et al.* (2001) indicated that the correlation can be considered between zeta potential and EPS_p/EPS_c ratio rather than total EPS produced but such a correlation was not observed in our study. This is believed to originate due to the presence of different concentration of potassium ion in different reactors. When the obtained zeta potential data of potassium-containing sludges are considered (5, 10 and 20 meq/L), it can be suggested that carbohydrate and hydrophilic protein type polymers have a strong effect on surface charge by introducing extra negative charge to the floc surface. The addition of increasing

potassium ion concentration is not enough to neutralize the overall surface charge.

Liao *et al.* (2001) stated that introduction of more charge to the sludge surfaces increases the polar interactions of EPS with water molecules so an inverse correlation is revealed between the surface charge and hydrophobicity of sludge. Therefore, the introduction of more negative charges to the surface lowers the hydrophobicity. In this research, a general trend showed that an inverse correlation was present between these parameters parallel to that was suggested by Liao *et al.* (2001).

4.1.4. Effect of Potassium Ion on Physical Characteristics of Activated Sludge

4.1.4.1. Particle Size

Particle size is an important parameter since it affects the sludge settling, dewatering and rheological properties. In order to compare the mean particle size of sludge samples, the volume-weighted mean diameter values generated by Mastersizer software were used.

The examination of the particle size measurements of reactors from Figure 4.7 showed that a significant increase was observed from 119.49 to 179.41 in floc size when the potassium concentration shifted from 0.5 to 5 meq/L. The further addition of potassium from 5 meq/L to 10 meq/L and then 20 meq/L resulted in decrease in floc size as 131.92 and 101.91, respectively.

The increasing addition of potassium ions is believed to cause the displacement of divalent cations from within the floc structure. This displacement results in deterioration in flocculability and dissociation of flocs. This causes an increase in small particles. This is the general trend observed in this study. This finding in fact proves the divalent cation displacement and floc breakage when high quantities of monovalent ions are present.

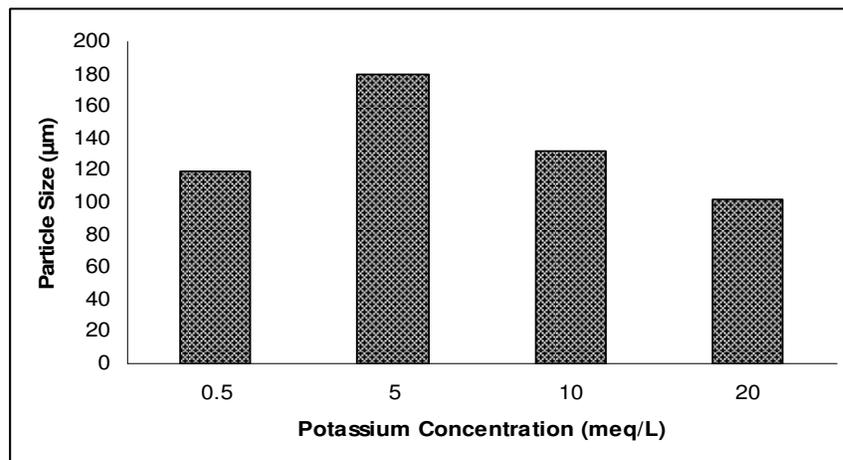


Figure 4.7. Particle size measurements with respect to potassium ion concentration

4.1.4.2. Dewaterability

Sludge dewaterability was determined by measurements of specific resistance to filtration (SRF), capillary suction time (CST) and floc strength.

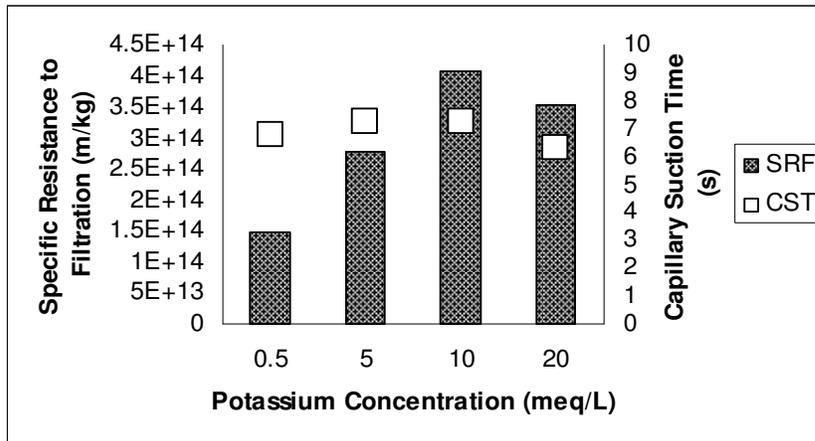


Figure 4.8. Effect of potassium concentration on the dewaterability of activated sludge

Specific resistance to filtration is one of the tests which is used to investigate the effect of potassium ion concentration on dewaterability of sludge and the related data is illustrated in Figure 4.8.

It can be seen from Figure 4.8 that an increase in the potassium was correlated with the increase in SRF although a small decrease was observed when concentration was increased from 10 to 20 meq/L reactor. As potassium ion concentration increased from control level to 5 meq/L, a sharp increase was observed in SRF. This increase continued with a further addition of potassium ions from 5 to 10 meq/L but then the increase of potassium ions to 20 meq/L led to a slight decrease in SRF value but when it was compared to control reactor value, an appreciable increase was still observed. As a whole, it can be said that the dewatering property deteriorated with the increase in SRF. Capillary suction time measurements of reactors are also demonstrated in Figure 4.8 to evaluate the effect of potassium addition to the dewaterability of the activated sludge. When all data was examined, the addition of potassium to the system led to an increase in CST except for 20 meq/L reactor data. As a general observation, it can be said that the increase in potassium concentration was associated with the

increase in CST and SRF showing the deterioration in the dewatering properties of sludge. Parallel results obtained with CST and SRF strengthened the observation about the deterioration of dewater property of activated sludge at increasing concentration of potassium ion.

The findings of this study are in agreement with findings of Murthy and Novak (1998). They studied the effect of potassium ion on sludge dewatering and revealed that the increase in concentration of potassium was associated with deterioration in dewatering properties (increase in CST).

According to cation-bridging model, removal of divalent cations from the floc leads to deterioration in the settling and dewatering properties due to a weakening of the floc structure (Tezuka, 1969; Novak and Haugan, 1978; Bruus *et al.*, 1992, Higgins and Novak, 1997a). Floc structure is weakened because divalent cations are believed to act as a bridge between negatively charged sites within the biopolymer network, not monovalent cations. Potassium is a monovalent cation and in the highlight of the findings of literature, it does not take part in the floc structure as a bridging cation. The addition of potassium at higher concentrations had a negative effect on filterability of sludge since by replacing divalent cations, especially calcium in wastewater with potassium cations weakened the floc structure, resulting in sludge disintegration and consequently in deterioration of sludge dewatering properties. Besides, the large amount of added potassium were accumulated in the cells as mentioned earlier so the produced EPS can not be kept firmly in floc matrix, causing weak floc formation. The mentioned inferences are confirmed by the relative flocculation power of cations. As can be shown in Table 2.1, potassium is not a better flocculator as divalent cations, calcium or magnesium, so flocculation cannot effectively occur. The dispersed flocs due to weakening of floc structure by addition of potassium clog the filter pores, causing increase in SRF and CST.

The strength and stability of floc also plays an important role in determining sludge dewatering properties. The weakening of floc structure was demonstrated by floc strength measurements in Figure 4.9. The comment of floc strength of the sludge samples was achieved by inverting the slope values of the CST-mixing time graphs.

Floc strength measurements indicated that flocs were more sensitive to shear with the increase in potassium concentration although a small increase was observed in shifting from 10 to 20 meq/L. When the data belonging to 20 meq/L reactor was compared to that of control reactor, it was clear that a significant decrease still occurred. Resistance to shear decreased with the addition of potassium ions due to floc break-up during mixing. Flocs becoming more fragile and weak led to an increase in CST with mixing. The increase in CST was correlated with the decrease in floc strength. It can be concluded that the rising addition of potassium ions to the feed decreases floc resistance to shear. Previous studies (Higgins and Novak, 1997b, and Murthy *et al.*, 1998) indicated that monovalent cations reduce the strength of the bonds, and this leads to a loose structure, often decreasing floc density and floc strength. Murthy and Novak (2001) showed that for lower divalent cation and greater potassium ion concentration, the flocs seem to be loosely bound with some biocolloids in solution. As a result, it can be said that the inability of potassium ions to form bridges between EPS and floc surfaces and replacement of divalent cations within the floc structure by potassium ions results in formation of weak floc structure, causing deterioration in dewaterability of sludge.

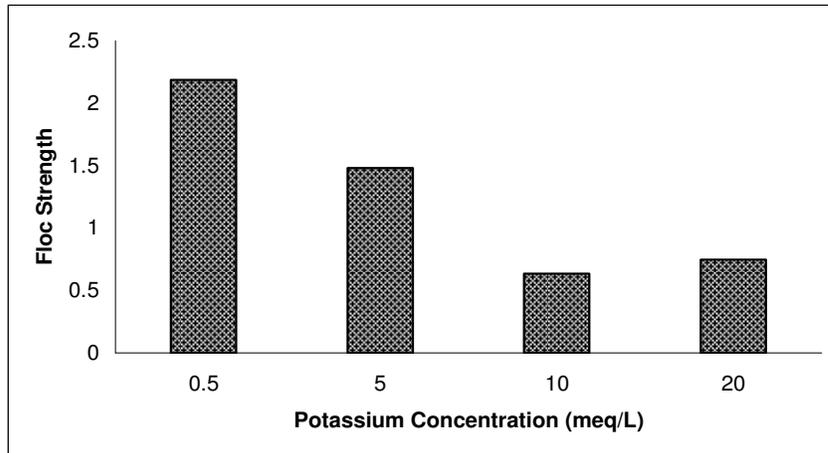


Figure 4.9. Effect of potassium concentration on resistance of floc structure to the shear

It was widely reported that the presence of high EPS concentration in the activated sludge led to poor dewaterability (Shioyama and Toriyama, 1985; Eriksson and Alm, 1991, 1993; Pere *et al.*, 1993 and Houghton *et al.*, 2000, 2001). Also, Murthy and Novak (1998) studied with potassium rich sludges reported that an increase in soluble polymers was associated with deterioration in dewatering properties (increase in CST). From the results of our study, it can be said that SRF, CST and floc strength measurements were consistent with each other and the increase in EPS lower the dewaterability. Houghton *et al.* (2000, 2001) found that dewaterability of activated sludge was source dependent and dewaterability increased with a decrease of EPS. They concluded that the increase of dewaterability with EPS at low concentration was due to enhancement of flocculation at low EPS levels. The further increase in the amount of EPS beyond the certain level which is required for flocculation results in poor dewaterability. As the level of EPS in the sludge increased, the sludge became harder to dewater. The reason why the increase in EPS led to deterioration in dewaterability can be that the increase in EPS content raised the amount of surface water bound by EPS or the EPS molecules extending out from

cell surfaces physically preventing the cells from forming close contact (Liao *et al.*, 2001).

Many researchers reported that the amounts of EPSp and EPSc in sludge were of significance to the sludge dewaterability. Some researchers found that protein type polymers affect dewaterability of sludge positively (Higgins and Novak, 1997a, b, c). On the other hand, Murthy and Novak (1998) indicated that an increase in potassium was correlated to an increase in slime and soluble protein in the activated sludge, causing a deterioration in dewatering properties. It can be seen from our data, a negative correlation was obtained between protein concentration and dewaterability. Also, it has been revealed that there was mostly a negative correlation between EPSc and filterability (Wu *et al.*, 1982; Murthy and Novak, 1999) and in this study, the negative relationship also was observed between carbohydrate and dewaterability. The dewatering properties (SRF and CST) deteriorated with the increase in the concentration of both carbohydrate and protein. As can be seen from Figure 4.5, only a significant decrease was observed in EPSp/EPSc when concentration shifted from 0.5 to 5 meq/L and EPSp/EPSc ratio remained nearly constant in potassium rich reactors due to similar increment in both carbohydrate and protein type polymers. Therefore, it can be said that the decrease in EPSp/EPSc ratio was correlated with deterioration in dewaterability due to presence of more carbohydrate than protein in 5 meq/L reactor and the deterioration was followed in other reactors compared to control reactor due to the remaining of EPSp/EPSc almost same level and to the increase in polymers.

As mentioned earlier, the introduction of more negative charge contributed by especially carbohydrate content of EPS to the sludge surface led to decrease in hydrophobicity. Therefore, it can be concluded that one of the reasons of deterioration in dewaterability can be a decline in hydrophobicity. It was stated that increased hydrophobicity generally leads to a better flocculation and dewaterability (Durmaz and Sanin, 2003; Sesay and Sanin, 2004; Liu and Fang,

2003). The deterioration in flocculation ability of sludges due to decrease in hydrophobic character corresponded to poor dewaterability. Also, particle size distribution appears to affect dewatering properties, where smaller particles cause clogging of filters and sludge cakes Novak *et al.* (1988).

4.1.4.3. Settleability

Sludge flocculation and settling are crucial to the effective operation of an activated sludge treatment process (Liu and Fang, 2003). Settleability generally depends on sludge concentration and floc characteristics. In this study, ZSV (Zone Settling Velocity) and SVI (Sludge Volume Index) are analyzed parameters to evaluate the effect of potassium concentration on sludge settleability.

Zone settling velocity (ZSV) is the common method used for determining the settleability of sludge and is a strong function of sludge solids concentration (Vesilind, 1994). Therefore, solids concentration (MLSS) versus ZSV graph was plotted for each reactor as given in Appendix D. Then, a fixed solids concentration (3500 mg/L) was selected to determine the ZSV at different concentrations. This is plotted as Figure 4.10.

Figure 4.10 shows that the increase of potassium concentration from control level to 5 meq/L led to a decrease of ZSV from 0.0412 to 0.0228 cm/s. The decrease in settling velocity of sludge at 5 meq/L concentration was approximately 50% compared to control value. When the potassium concentration was increased from 5 meq/L to 10 and then 20 meq/L, the settling velocity slightly increased to 0.0287 and then decreased to 0.0258 cm/s, respectively. As a whole, it can be said that the most significant change in settling velocity was observed with the addition of 5 meq/L potassium. The further addition of potassium ions led to no significant change in ZSV.

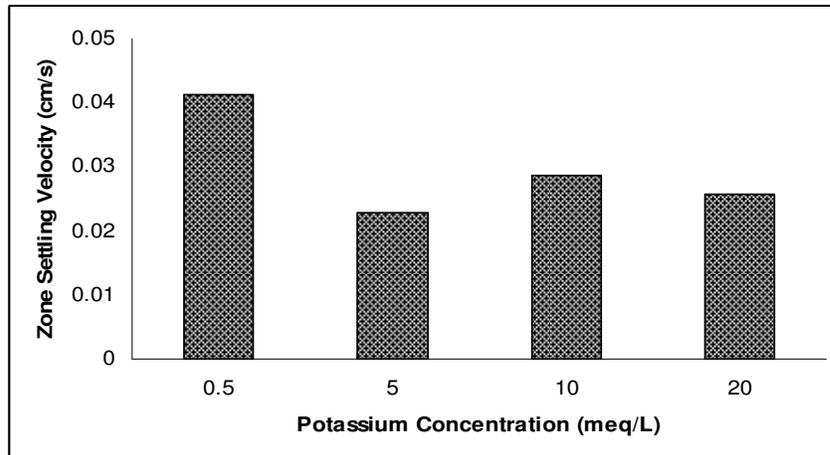


Figure 4.10. Zone settling velocities at different potassium concentrations at a fixed MLSS concentration of 3500 mg/L

Sludge volume index (SVI) is another key parameter to define sludge settleability. SVI values less than 120 indicate well settling sludge and if this value is exceeded, settling problems occur (Jenkins *et al.*, 1993). In this study, in the highlight of the SVI values obtained for the reactors (Figure 4.11), it can be said that the obtained data can be considered in acceptable levels for good settling (<120) as stated in literature. The increase of potassium concentration from control level to 5 meq/L led to observable increase in SVI, which contributed to difficulty in settling. As stated in literature, there was an inverse correlation between SVI and ZSV, in other words, the highest SVI values corresponded to the lowest values of ZSV. Therefore, the increase in SVI with the shift in 0.5 to 5 meq/L concentration was in parallel to decrease in settling velocity (ZSV). The increase in SVI can be resulted from displacement of divalent cations from the floc structure with the addition of potassium ions in monovalent character but 112.63 mL/g which was the SVI of the reactor fed by 5 meq/L potassium, even though a bit high, can still be considered to be within the acceptable range for good settling. The further addition of potassium to the reactors caused decrease in SVI values. When the reactors fed by different concentrations of potassium were compared, a slight decrease was observed in

SVI with the rising addition of potassium, indicating improvement in settling. Murthy and Novak (1998) reported that an increase in concentration of potassium ion associated with improvement in the settling properties of sludge. In this study, although the obtained SVI values were greater than the SVI of control reactor, the values were found in acceptable levels as mentioned earlier. Also, although there is no one to one correlation with respect to cation dose, SVI values found in accordance with ZSV measured. Therefore, the findings of this study are in conformity with literature.

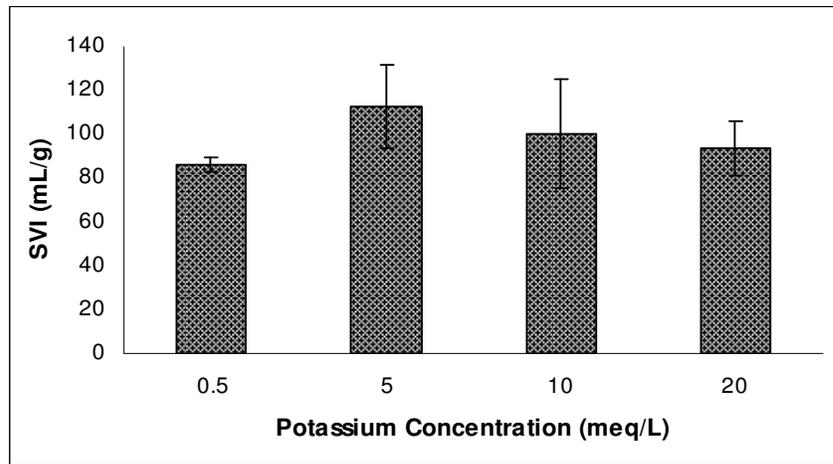


Figure 4.11. SVI values with respect to potassium concentration

When the relation between total EPS and settleability was considered, the increase in total EPS from control level to 5 meq/L led to a deterioration in settleability of sludge but further increase of EPS concentration (Figure 4.2) resulted in improvement in sludge settling compared to the reactor with 5 meq/L concentration as can be seen in Figure 4.10 and 4.11. In literature, it was widely accepted that settleability of sludge was affected more by the composition of EPS rather than the amount of EPS produced (Bura *et al.*, 1998). According to our results, it can be concluded that settleability was positively correlated with protein concentration. When SVI values of reactors were taken into

consideration, the addition of 5 meq/L potassium to the reactor led to increase in SVI. This increase can be explained by the decrease in protein concentration. The addition of 10 and then 20 meq/L potassium to the reactors was correlated with the decrease in SVI and increase in protein. Therefore, it can be said that protein content of EPS has a greater influence on settleability than carbohydrate content of EPS.

When the EPSP/EPSC ratios of reactors were examined (Figure 4.5), the decrease of this ratio worsened the sludge settling. Also, Durmaz and Sanin (2003) indicated that the reduction in EPSP/EPSC caused problems in settleability of sludge. Therefore, it can be stated that settleability of sludge depends on some sort of balance between protein and carbohydrate fraction of EPS. The excessive production of either type of EPS component (protein and carbohydrate) may cause a deterioration of settleability. In the highlight of these findings, potassium ions stimulate both the production of protein and carbohydrate type polymers and provide a balance between these polymers indicating good settling. It can be stated earlier, potassium has lower relative flocculation power compared to those of divalent cations.

Jorand *et al.* (1994) reported that an improvement was observed in settling because of an increase in floc hydrophobicity. In this study, the increase in SVI and decrease in ZSV compared to control reactor, can be attributed to decrease in hydrophobicity (Figure 4.5). Also, the introduction of more negative to the floc surface indicated by an increase in zeta potential (Figure 4.6) resulted in difficulty in settling of sludges although SVI values were found in optimum range for good settlement of sludge.

Some studies have revealed the specific relationship between floc size and the settleability (Andreadakis, 1993). An increase in particle size has been shown to be necessary for improvements in settling properties (Higgins and Novak 1997a and Murthy and Novak 1997). The size of the flocs ranges from 20 to 200 μ m

according to Mueller *et al.* (1967). Hillgardt and Hoffmann (1997) indicated that particle size distribution of the measured sludge showed an increase of particle size with increasing SVIs. According to the results of our study, a positive correlation was observed between the SVI and the mean particle size. The increase in SVI was correlated to increase in the size of the flocs. When the potassium concentration was shifted from 5 meq/L to 10 and then to 20 meq/L, the decrease in SVI values was associated with the decrease in floc particle size. It can be seen that potassium reactors with particle size greater than 120 had high SVI values. In the highlight of the findings, it can be concluded that the optimum floc size for good settling changes between 100 and 120 μm . The increase or decrease in floc size beyond the optimum value leads to increase in the small particles, causing deterioration in settling. In the literature, Hillgardt and Hoffmann (1997) indicated that particle size distribution of the measured sludge showed an increase of particle size with increasing SVIs. Sludge with a SVI exceeding 100 had particles greater than 100 μm .

4.1.4.4. Rheology

Viscosity is a measure of the resistance of a fluid to deform under shear stress and shear stress vs. shear rate graphs were plotted for each potassium concentration to determine the flow model that the the sludge samples follow. As presented in Figure 4.12, the curves exhibited the highest conformity to power law equation of non-Newtonian pseudoplastic flow that is demonstrated in equation.

$$\tau = K (dv/dy)^n$$

where K is fluid consistency index, a term equivalent to viscosity, n is the flow behaviour index and dv/dy is the shear rate. When the shear stress increases, the flocs are broken down into smaller particles and viscosity decreases. The value of n is smaller than 1 for this type fluids and when n gets smaller than 1, the flow

character deviates more from Newtonian. When the n values were examined in Figure 4.12, an increase in potassium concentration was associated with the increase in n values except for reactor with 20 meq/L potassium. Also, it can be seen from Figure 4.12 the viscosity of the sludge (K) decreased significantly with increasing of potassium concentration except for reactor with 20 meq/L potassium. As a result, it can be said that the rising addition of potassium to the feed leads to increase in flow behavior index, decreasing viscosity so sludge becomes less viscous and easier for pumpability.

In order to determine the effect of solids concentration on viscosity, apparent viscosities were plotted as a function of 5 different solids concentrations at a fixed shear rate of 73.4. Once apparent viscosity (cP) versus MLSS (mg/L) graphs were plotted as in Figure 4.13, a constant solids concentration as 5000 mg/L was selected to compare apparent viscosity values of reactors each other. It can be seen from Figure 4.13, the increase in solids concentration was correlated to increase in apparent viscosity for each potassium containing sludge reactor. This result was confirmed by the findings of Sanin (2002).

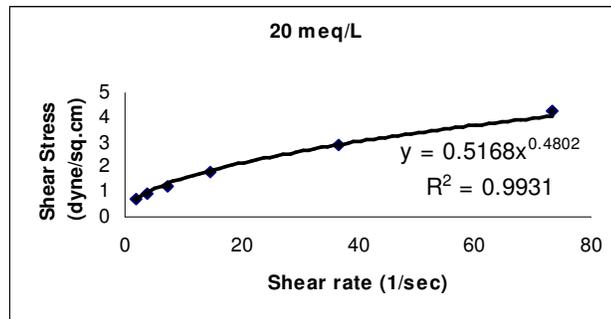
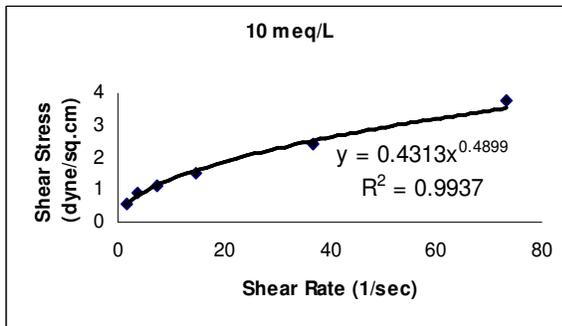
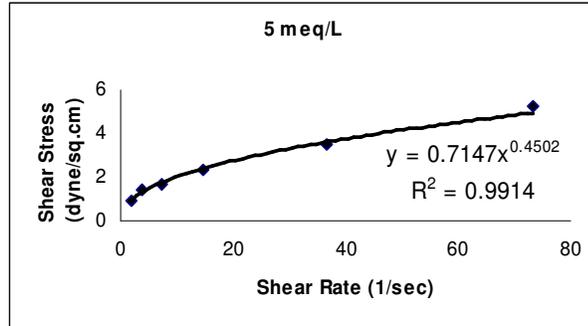
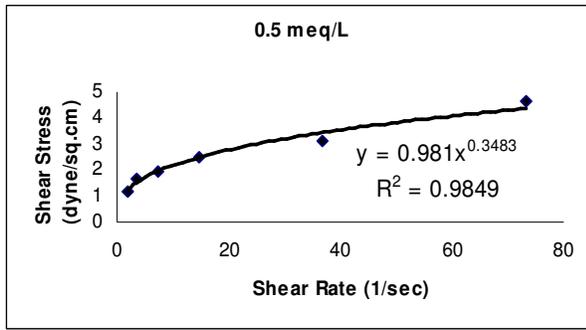


Figure 4.12. Shear rate versus shear stress graphs of reactors at different potassium concentration

Also, the examining of the results of viscosity measurements in Figure 4.14 revealed that the increasing addition of potassium led to decrease in viscosity of sludges expect for reactor with 20 meq/L potassium concentration. A small decrease was observed when potassium concentration shifted from control level to 5 meq/L. This situation can be explained by the significant increase of particle size of flocs from 119.49 to 179.41 shifting from 0.5 to 5 meq/L reactor. The further increasing of potassium concentration from 5 meq/L to 10 meq/L resulted in decrease in viscosity, and then addition of 20 meq/L sodium led to a small increase in viscosity. Forster (1983) indicated that the presence of metal ions caused a decrease in bound water content, which affects the viscosity of sludge. Therefore, it can be concluded that the addition of potassium ions to the feed leads to decrease in bound water content of sludge and repulsion between flocs, so this facilitates the pumpability of the sludge.

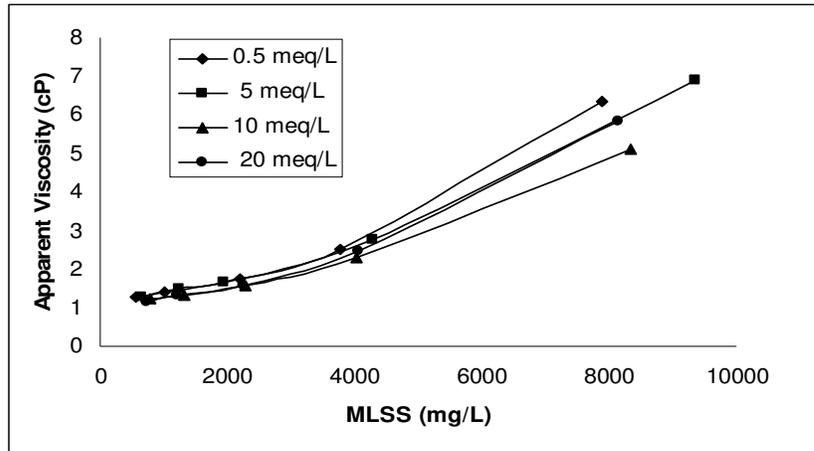


Figure 4.13. Apparent viscosity values with respect to potassium concentration at a shear rate of 73.4 sec^{-1}

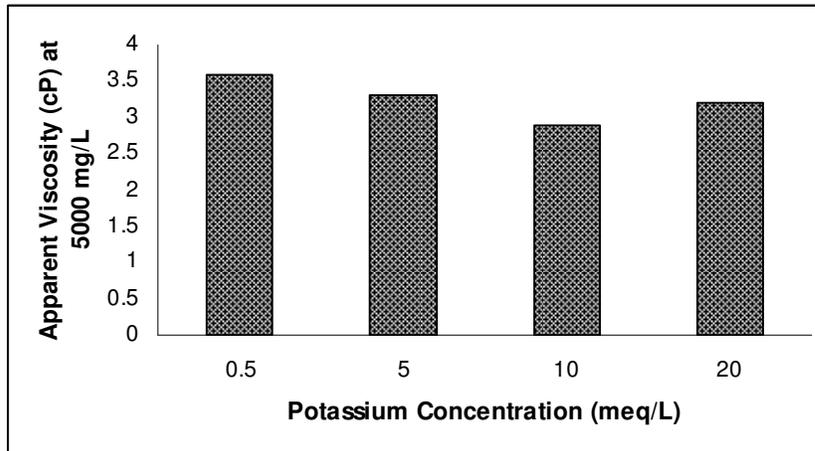


Figure 4.14. Apparent viscosity versus potassium ion concentration at 5000 mg/L

Earlier works revealed that extracellular polymers affect the viscosity of sludge (Forster, 1982; Forster, 1983). It can be said from the results of this study that the increase in protein and carbohydrate concentrations by the addition of potassium ion was correlated to the decrease in viscosity, so pumpability of the sludge was positively correlated with both fractions of EPS. Also, when the potassium concentration was increased from 0.5 to 5 meq/L, a decrease was observed in protein concentration from 19.9 to 16.8 mg/L and this decrease led to also decrease in viscosity of sludge. Therefore, protein fraction has a larger effect on viscosity rather than carbohydrate fraction of EPS.

Sanin (2002) indicated that the increase in the conductivity is accompanied by a decrease in viscosity. The low ionic strength is expected to create a higher viscosity since this type of a system creates a higher resistance during flow. In this study, the rising addition of potassium ions contributed to increase in conductivity of the system (Figure 4.3), so this creates a lower resistance and viscosity.

4.1.4.5. Turbidity

The turbidity measurements were conducted following 1 hr settlement of sludge samples and the measured turbidity values are illustrated as Figure 4.15. Although a small decline was observed at 5 meq/L, the addition of potassium ions at rising concentrations led to increase in effluent turbidity. Murthy and Novak (1998) indicated a deterioration in effluent total suspended solids with an increase in potassium. The increase in effluent turbidity is similar to the trend they observed except for 5 meq/L reactor.

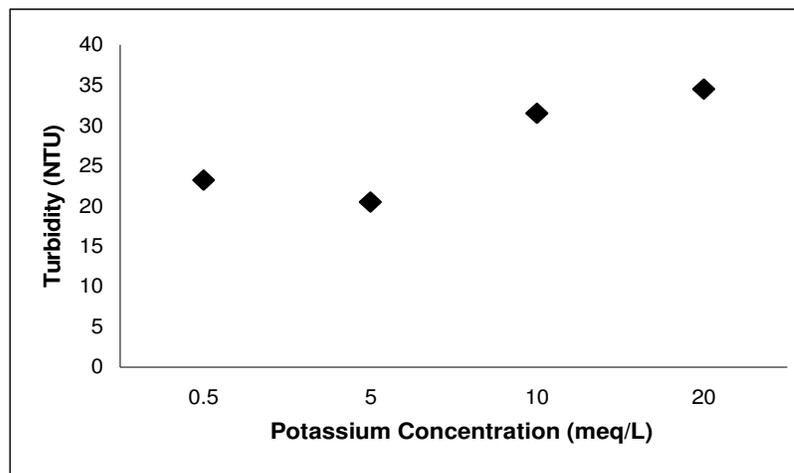


Figure 4.15. Turbidity measurements with respect to potassium concentration

Also, the findings of this study is in agreement with the findings of some researchers who observed an improvement in settling properties and deterioration in dewaterability with an increase in potassium ion concentration, associated with an increase in turbidity and dissolved organic carbon (Higgins and Novak, 1997b; Murthy and Novak, 1998).

4.2. Effect of Sodium Ion Concentration on Sludge Characteristics

The purpose of this part is to evaluate the effect of sodium ions on sludge characteristics at three different (5, 10 and 20 meq/L) concentrations. For this aim, the same analyses conducted for potassium reactors were carried out after the sodium reactors reached the steady state.

4.2.1. Sodium Reactors and Achievement of Steady State Conditions

Eight lab-scale semi-continuous activated sludge reactors were set up using mixed culture bacteria obtained from the primary settling tank effluent of Ankara Central Wastewater Treatment Plant. Sodium was added to the synthetic feed whose constituents are given in Table 4.2 of reactors at concentrations of 0.5, 5, 10, 20 meq/L. The rising concentrations of sodium (5, 10 and 20 meq/L) were achieved with the addition of the precalculated doses of NaCl into the feed. These reactors were operated with their replicas for each cation concentration as given in Table 4.3 and the 0.5 meq/L reactor was used as a control reactor. The same conditions (SRT, pH, DO, water bath temperature and COD/TKN) were provided for the sodium reactors as stated in section 4.1.1 for potassium reactors.

Table 4.3. Sodium ion concentration added to sodium reactors

Concentration in Reactors	Sodium Reactors
Na = 0.5 meq/L	C1, C2
Na = 5 meq/L	R1, R2
Na = 10 meq/L	R3, R4
Na = 20 meq/L	R5, R6

As mentioned earlier, reactors were operated at least 4 times the mean cell residence time for achievement of steady state conditions by monitoring MLSS and MLVSS values of reactors. Similar to potassium reactors, all of the conditions as well as the feeding regime were kept constant until steady-state was achieved and during the subsequent experiments.

4.2.2. Effect of Sodium Ion on Chemical Characteristics of Activated Sludge

Effluent soluble COD and COD removal as percentage, EPS production and composition, sodium concentration in floc structure and conductivity analyses were conducted to determine the chemical characteristics of sodium containing sludges. Since the variation was always less than about 25 %, the control data is obtained by taking the average of potassium and sodium replica control reactors (a total of 4). Also, the data presented in figures for each cation concentration is determined by taking average of two replica reactors.

4.2.2.1. Effluent Soluble Chemical Oxygen Demand (COD)

Soluble effluent COD measurements and removal rates are demonstrated in Figure 4.16. The influent COD added to the reactors was 1395 mg/L. Effluent COD decreased when concentration shifted from 0.5 to 5 meq/L. Then, the addition of sodium ions at rising concentrations led to increase in COD. The calculation of COD removal rates as a percentage indicated that although an increase was observed with the addition of 5 meq/L sodium to the feed, COD removal efficiency decreased with the further addition of sodium. Murthy and Novak (2001) reported that sodium ions in influent wastewater caused an increase in proteins and polysaccharides in solution, thereby increasing the effluent COD concentration of the treated municipal wastewater. In a similar manner, the comparison of COD values of sodium sludges revealed an increase in COD, so obtained results are in accordance with literature.

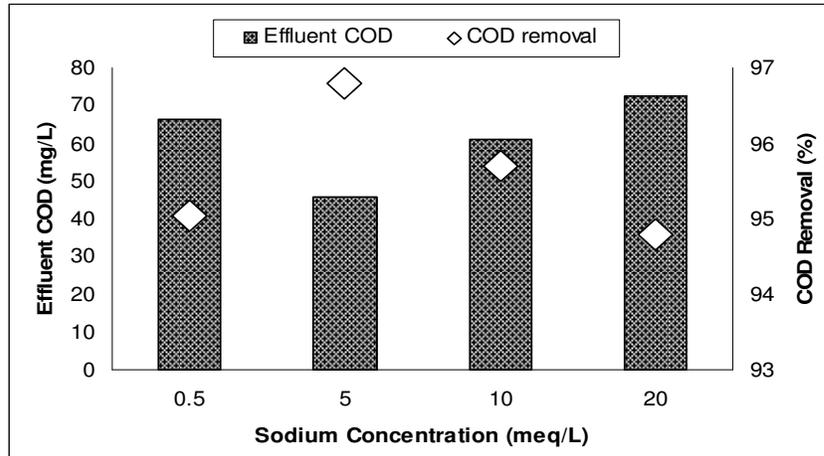


Figure 4.16. The effect of sodium ions on COD removal efficiency of reactors

4.2.2.2. Extracellular Polymer Production and Composition

As mentioned in previous sections, the major constituents of EPS, protein and polysaccharides, were determined by using CER extraction method in this study.

According to Figure 4.17, quality and quantity of EPS composition changed at different concentrations of sodium ion. Since the variation is less than approximately 25 % between the values, the control data was obtained by taking the average of potassium and sodium replica control reactors except for Control 2 (C 2) sodium reactor due to bulking like conditions. Also, the data indicated in Figure 4.17 for each sodium concentration were determined by taking average of two replica reactors.

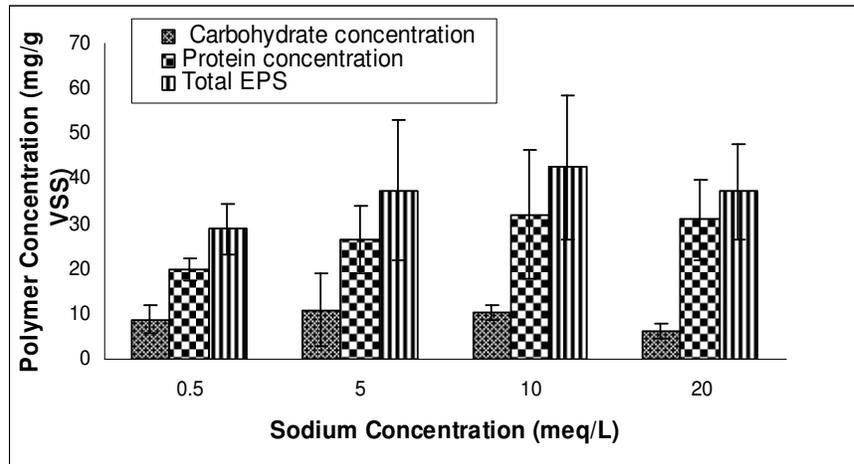


Figure 4.17. Effect of sodium concentration on the production and composition of EPS

As can be seen Figure 4.17, total polymer concentration increased with the rising addition of sodium ions except for reactor with 20 meq/L sodium concentration. The shift in sodium concentration from 0.5 to 5 meq/L led to increase in polymer concentration from 28.8 to 37.4 mg/g VSS. The further addition of sodium as 10 meq/L increased total polymer concentration to 42.5 mg/g VSS, but the rise in sodium concentration from 10 meq/L to 20 meq/L resulted in decrease in polymer concentration to 37.1 mg/g VSS. In the highlight of results, it can be said that sodium addition up to certain concentration increases biopolymer production.

To comment on the effect of EPS on sludge characteristics, EPS constituents and their amount should be taken into consideration instead of total EPS because each type of EPS influence sludge structure in a different manner. As illustrated in Figure 4.17, a positive correlation was obtained between protein content of EPS and sodium concentration except for 20 meq/L reactor. However, the decrease in protein concentration from 32.1 to 30.9 with the increase in sodium concentration from 10 to 20 meq/L is not a significant decline so protein can be considered remaining constant in 20 meq/L reactor. Therefore, the further

addition of sodium beyond 10 meq/L did not lead to obvious change in protein concentration. On the other hand, sodium ions were correlated negatively with polysaccharide type polymers. Although a small increase was observed shifting from control reactor to 5 meq/L reactor, carbohydrate concentration decreased from 10.9 to 10.4, and then 6.2 mg/g VSS for reactors with 10 and 20 meq/L sodium concentration, respectively.

The interaction of cations with activated sludge is not completely explained by simple charge competition (M/D ratio) in the divalent charge-bridging model. Murthy and Novak (1998) stated that sodium and potassium ions interact with activated sludge flocs in a different manner in terms of physico-chemical characteristics. In addition, there are differences between sodium and potassium in their cellular functions and characteristics such as ionic size, valency and hydrated shell size and these are expected to influence settling, dewatering and flocculation ability of activated sludge. Sodium is involved in various transport processes provide cell wall stability, and enzyme activity, so sodium ions may promote the enzyme activity related to protein synthesis (Gerardi *et al.*, 1994). According to obtained results, sodium ions tend to stimulate production of protein type polymers and have an affinity to bind more protein within the floc structure, so our findings are in line with the enzymatic activity of sodium ions related to protein synthesis.

As stated earlier, the ionic charge, size and radius of the hydration shell affect the binding ability of cations. When sodium is taken into account, sodium is a monovalent cation and its ionic radius smaller than potassium as demonstrated in Table 2.1 Sodium holds more water molecules around it very strongly due to its small size so its hydrated radius becomes larger compared to potassium ion. As a result, the water molecules around it prevent the approach of sodium ions to the charged surfaces, so it does not establish bond with charged polymers easily and flocculation cannot effectively occur. Also, when the relative flocculation powers of cations calculated by Rengasamy and Naiudu (1998) are examined given in

Table 2.1, it is revealed that sodium is the poorest flocculator due to its single charge, small size and large hydrated radius. It means that the tendency of sodium ions to form covalent bonding and complexes with negatively charged EPS is the lowest one in stated cations.

As a result, it can be suggested that binding sites on EPS, which are available for divalent cations, are not suitable for monovalent cations. Therefore, monovalent cations especially sodium ions may be responsible for release of more polymers into solution with the increase in its concentration since they are not capable of forming strong bonds with EPS as divalent cations due to its atomic structure. Also, it can be said that each cation with different cellular function and atomic characteristic might have different affinity to bind certain constituent of EPS. Our findings reveal that while potassium ions have affinity to bind both types of polymers, sodium ions promote the binding of protein within the floc structure.

4.2.2.3. Sodium Concentration in Floc Structure

For the measurement of sodium concentration in floc structure, a microwave digestion procedure of sludge as described by Özsoy (2006) was applied.

Along with the changes in quality of polymer, the amount of sodium ions incorporated into the sludge floc matrix changed with dose of sodium added. As can be demonstrated in Figure 4.18, the increase in sodium concentration added to feed medium is correlated with the increase in sodium concentration incorporated into the sludge floc. This increasing trend can be originated from cellular function of sodium ions.

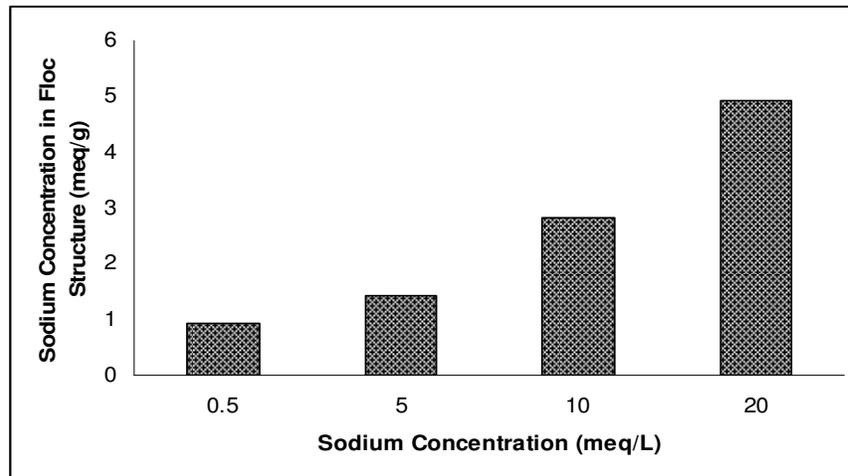


Figure 4.18. Sodium concentration incorporated into floc structure with respect to sodium concentration in feed medium

Sodium ion can be used as a motive force, for nutrient uptake (Wilson and Ding, 2001) and as a buffer for pH homeostasis in microorganisms (Krulwich *et al.*, 2001). Also, the transport of small molecules such as sugars, amino acids and vitamins into the cell is achieved by symport transport with sodium ions. Therefore, the mentioned functions of Na ions explain the reason why sodium is found in excess amount in floc structure. It is revealed that the movement of sodium ions into the cell during symport transport is coupled with the movement of calcium ions outside the cell. Therefore, it can be said that sodium ions mostly reside and function within the cells rather than in the EPS matrix. So, the addition of sodium ions at rising concentrations leads to the removal of divalent cations from within floc structure, causing release of polymers to the effluent due to being as intracellular cation rather than extracellular cation.

When the relationship between EPS and cation concentration incorporated into floc structure is considered, it was revealed that total EPS of the sodium-containing sludges increased up to 10 meq/L, which was consistent with the increase in sodium concentration incorporated into the floc. Then, the further

addition of sodium ions led to the decrease in total EPS although an increase was observed in sodium ions in the floc structure. As mentioned earlier, sodium ions are mostly retained in the cell rather than EPS matrix so the rising addition of sodium results in weakening of the floc structure and the release of polymers into the solution. The decrease in the total EPS can be originated from release of biopolymers.

4.2.2.4. Electrical Conductivity

Examining the results of conductivity measurements in Figure 4.19 shows that the increase in sodium concentration was accompanied by the increase in conductivity. The feed constituents were kept constant in reactors except for NaCl. Therefore, the increase in conductivity is originated from the dissolution of NaCl. The more NaCl is dissolved in the water, the higher is the value of the electrical conductivity.

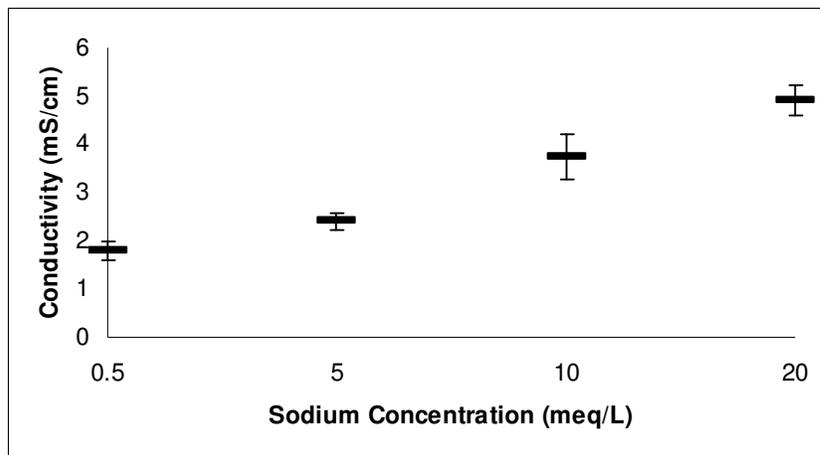


Figure 4.19. Conductivity values of sodium containing sludges with respect to sodium concentration

4.2.3. Effect of Sodium Ion on Surface Chemical Characteristics of Activated Sludge

4.2.3.1. Hydrophobicity

Microbial adhesion to hydrocarbons (MATH) was used to measure relative hydrophobicity of microbial flocs and values are presented in Figure 4.20.

As the hydrophobicity values are examined, it can be seen that the increase in sodium concentration was accompanied by a decrease in hydrophobicity values of sludge samples except for reactor with 20 meq/L. When sodium concentration was increased from control level to 5 meq/L, hydrophobicity decreased from 59.1% to 42.4%. The decrease in hydrophobicity continued for 10 meq/L sodium concentration and its hydrophobicity was 29.7%. It is seen that hydrophobicity dropped to the half of the control reactor level. Then, the addition of 20 meq/L sodium to the feed led to a small increase in hydrophobicity compared to 10 meq/L reactor. However, this increase is not appreciable because the comparison of hydrophobicities of 20 meq/L and control reactor reveals that hydrophobicity decreases approximately half of the control reactor value. Therefore, as a general finding, it can be said that addition of sodium at rising concentrations to the feed results in decrease in hydrophobic character of sludge.

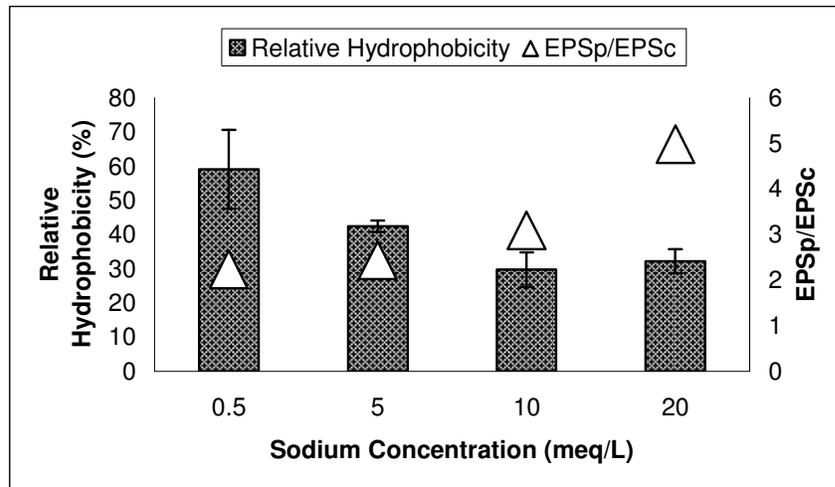


Figure 4.20. Relative hydrophobicity value and EPSp/EPSc ratio of each reactor with respect to sodium concentration

When the relation between hydrophobicity and EPS content of the sludge is considered, Liao *et al.*, (2001) proposed that the proportions of EPS components were more important than the quantities of individual EPS components in controlling hydrophobicity. Therefore, EPSp/EPSc ratio was determined for each reactor as demonstrated in Figure 4.20. As can be seen that increase in sodium concentration is correlated to increase in EPSp/EPSc ratio. Although in literature, it was reported that the EPSp contributed to the sludge hydrophobicity (Jorand *et al.*, 1998), our results indicate that the increase in protein concentration leads to decrease in hydrophobicity of sludge. Similarly, Wilen *et al.* (2003) indicated that protein in EPS was negatively correlated to hydrophobicity. The increase in the sodium concentration promotes the production of protein type polymers but it can be speculated that these produced protein type polymers may not contribute to hydrophobicity of the sludge since they do not contain the specific hydrophobic amino acids. Due to increase in presence of hydrophilic side chains contributed by protein and carbohydrate type polymers with the addition of sodium ions at rising concentrations, a decrease in hydrophobicity of sludge was observed. It can be concluded that in order to show the effect of EPSp on

hydrophobicity, the types of amino acids in structure should be determined because only hydrophobic side chains introduce hydrophobicity to sludge. Therefore, an increase in EPS_p cannot be interpreted as increase in hydrophobicity. Also, EPS_c and the cation type should be taken into consideration to determine the sludge hydrophobicity.

4.2.3.2. Zeta Potential

Activated sludge carries negative charge at neutral pH due to ionization of the functional groups of EPS such as carboxyl and phosphate. Also, amino groups of EPS introduce positive charge to the surface of flocs. Therefore, zeta potential of sludge depends on the EPS's chemical group composition and concentration (Jia *et al.*, 1996).

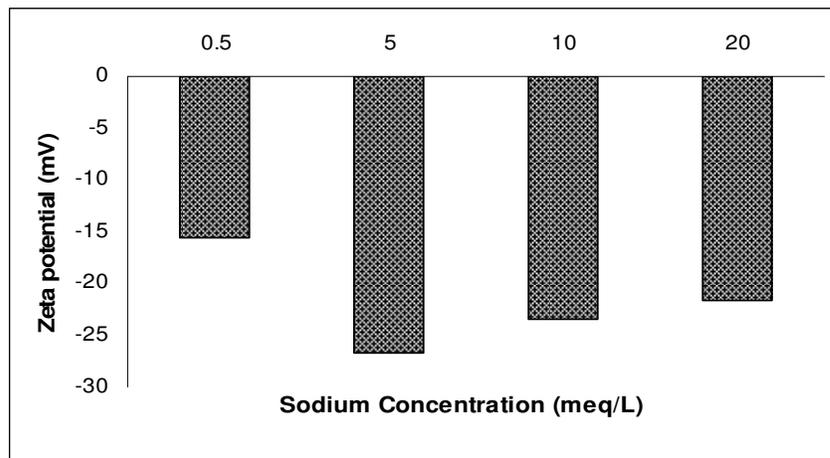


Figure 4.21. Effect of sodium concentration on the zeta potential of sludge

The measured zeta potential values of sodium reactors changed between -15.6 and -26.7 as given in Figure 4.21 and these values were found in acceptable levels as stated in literature (Liu and Fang, 2003). When sodium concentration increased from 0.5 to 5 meq/L, zeta potential increased from -15.6 to -26.7 mV.

Although the increase in sodium concentration introduces positive charge to the sludge surface, the increase in total polymer concentration introduces more charges that are mainly negative so sodium ions become insufficient to neutralize them. Also, examining of the constituents of EPS revealed that an increase in protein and carbohydrate type polymers contribute negative charge to the surface of flocs. Especially hydrophilic negatively charged side chains of protein may lead to contribution of extra negative charge. The addition of 10 meq/L sodium to the feed resulted in decrease in zeta potential from -26.7 to -23.4. This decrease is not a considerable decrease when the values are compared to each other. The increase in total EPS is not correlated to the zeta potential.

As stated by Liao *et al.* (2001), surface charge is not influenced by total EPS, but by EPS components. As can be seen from Figure 4.17, while no observable change occurred in the concentration of carbohydrate type polymers, an increase was observed in protein concentration shifting from 5 meq/L to 10 meq/L reactor. Therefore, only protein introduces extra charge to the surface and this can be neutralized to a certain point with the addition of sodium ions or some of produced protein type polymers introducing positive charge rather than negative charge. The further addition of sodium as 20 meq/L was accompanied by the decrease in zeta potential as -21.7. The approach mentioned above is also valid for this situation. As a whole, when the zeta potential values of sodium-containing sludges are compared to that of control reactor, an increase is observed but the comparison of sodium-containing reactors indicate a decrease attributed to production of some hydrophilic positively charged proteins rather than negatively charged ones in reactors.

Inverse correlation between zeta potential and hydrophobicity was reported in literature (Liao *et al.*, 2001; Pere *et al.*, 1993). It means that the increase in negative surface charge is accompanied by a decrease in hydrophobicity. As a general trend, it can be said that sodium sludges have higher zeta potential values compared to control reactor so this increase is correlated to decrease in

hydrophobicity. Therefore, it can be concluded that the shift in the balance between hydrophilic and hydrophobic groups affects the surface charge and hydrophobic character of sludge.

4.2.4. Effect of Sodium Ion on Physical Characteristics of Activated Sludge

4.2.4.1. Particle Size

The floc size distribution is one of the most important physical parameters governing the solid-liquid separation properties of activated sludges (Wilén *et al.*, 2003). It is for this reason that particle size measurements were carried out and results are illustrated in Figure 4.22.

As can be seen from Figure 4.22, particle size distribution is between 103.01 and 169.50 μm . It indicates that floc size of sodium sludges are in 20-200 μm range stated by Mueller (1967) for activated sludge. A small decrease was observed in floc size from 119.49 to 103.01 μm with the addition of sodium from 0.5 to 5 meq/L. The further addition of sodium from 5 meq/L to 10 meq/L and then 20 meq/L resulted in increase in floc size as 129.08 and 169.5 μm , respectively. As a general, it can be said that an increasing trend was observed in particle size with the increase in addition of sodium to the feed.

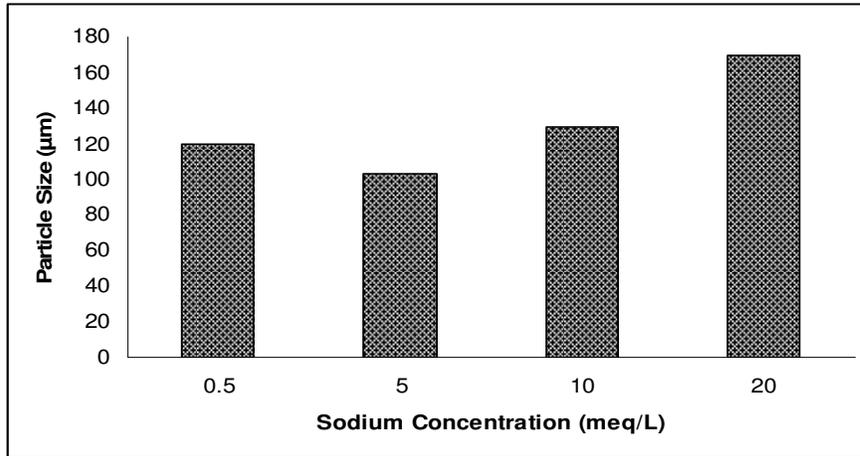


Figure 4.22. Particle size measurements with respect to sodium ion concentration

4.2.4.2. Dewaterability

Dewaterability of sludge samples was determined by specific resistance to filtration (SRF), capillary suction time (CST) and floc strength tests. SRF values with respect to sodium concentration are illustrated in Figure 4.23. Examining the obtained values revealed that an increase in addition of sodium ions was correlated to increase in SRF except for 20 meq/L reactor. When sodium ions increased to 5 meq/L from control level, SRF raised to 1.15×10^{15} m/kg from 1.48×10^{14} m/kg.. SRF continued to increase to 2.15×10^{15} m/kg with the addition of 10 meq/L sodium, but then, the increase in sodium concentration led to decrease in SRF. However, the comparison of SRF of reactor with 20 meq/L sodium concentration and control reactor shows the deterioration in dewaterability of sludge fed with 20 meq/L sodium. Therefore, it can be said that high values of SRF compared to control reactor corresponded to poor dewaterability.

CST values are also demonstrated in Figure 4.23 and a similar trend was observed in CST measurements with respect to sodium concentration. As it is seen from the figure, CST value of control reactor was 6.8 s and it increased to

19 s and 24.2 s for 5 and 10 meq/L sodium concentrations, respectively, and then decreased to 11.1 s. A similar decrease was observed for 20 meq/L reactor in SRF test and when it was compared to control reactor value, an appreciable increase was observed in CST and especially in SRF. Our results are in parallel to literature (Bruus *et. al*, 1992; Higgins and Novak, 1997a; Higgins and Sobek, 2002). They reported that addition of monovalent cations to the system (removal of divalent cations from the floc) would lead to a deterioration in the dewatering properties measured by increase in SRF or CST.

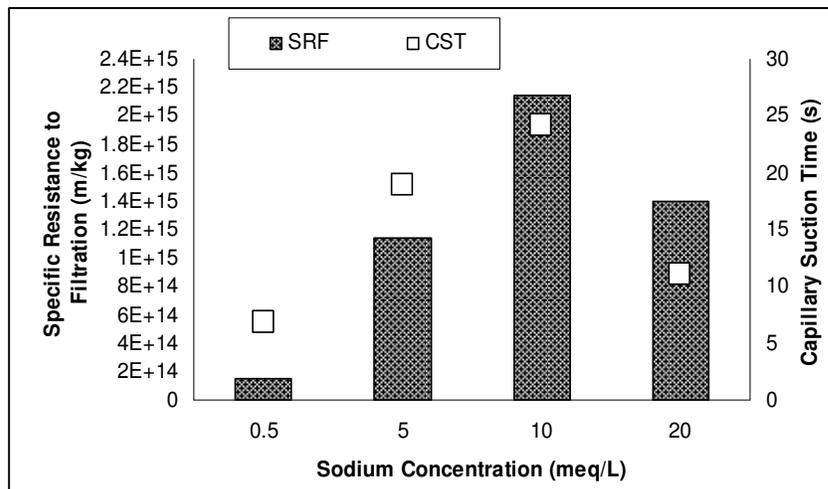


Figure 4.23. Effect of sodium concentration on the dewaterability of activated sludge

The floc strength measurements given in Figure 4.24 indicate that although the rising addition of sodium to the feed led to a small increase in the floc resistance of sludge samples, the comparison of floc strength measurements of sodium reactors with control reactor revealed that flocs became much weaker and less resistant to shear with the addition of sodium. It means that greater addition of sodium ions to the feed leads to reduction in the strength of the bonds by the displacement of divalent cations from the floc structure and this decreases the floc resistance to shear. Higgins and Novak (1997a) reported that high sodium

concentrations decreased the resistance of sludge to shear, weakening the floc structure.

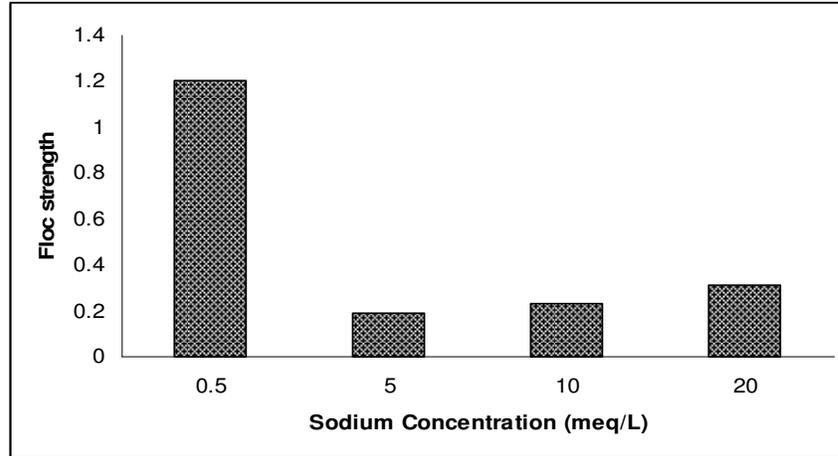


Figure 4.24. Effect of sodium concentration on resistance of floc structure to the shear

Examining of the relative flocculation powers of cations demonstrated in Table 2.1, it is revealed that sodium is the poorest flocculator compared to other ions given in table, so bioflocculation is hindered by sodium ions. The addition of sodium at rising concentrations to the feed leads to deterioration in floc stability and the formation of dispersed loose flocs. These flocs block filter pores, causing an increase in SRF, CST and decrease in floc strength values.

In the highlight of the results conducted for dewaterability by measuring SRF, CST and floc strength, in general, it is seen that measurements are consistent with each other. Results indicate that an inverse correlation is valid between dewaterability and total EPS depending on the sodium concentration. It means that dewaterability of sludge decreased as total EPS increased. This finding is supported by many studies reported in literature (Shioyama and Toriyama, 1985; Eriksson and Alm, 1991, 1993; Pere *et al.*, 1993 and Houghton *et al.*, 2000, 2001). As stated earlier, the deterioration in dewaterability by the increase in

EPS can be caused due to the increase in amount of surface water bound by EPS or the increase in the EPS molecules extending out from cell surfaces (Liao *et al.*, 2001).

When the effect of EPS_p/EPS_c ratio on dewaterability is considered, it is seen that the increase in EPS_p/EPS_c ratio was correlated to deterioration in dewaterability. This can be caused by greater increase in protein concentration with the addition of sodium ions compared to carbohydrate concentration. Although Higgins and Novak (1997a, b, c) indicated that protein type polymers affect dewaterability of sludge positively, our results revealed a general negative correlation between dewaterability and EPS_p. It can be suggested that the greater increase in any of EPS components compared to others leads to shift in balance between them, which cause deterioration in sludge properties.

It can be seen from the obtained data that a positive correlation was observed between hydrophobicity and dewaterability. This type of correlations have also been shown earlier (Durmaz and Sanin, 2003; Sesay and Sanin, 2004). On the other hand, an inverse correlation is observed between zeta potential and dewaterability. Therefore, in general, it can be said that sodium containing sludges with higher zeta potential and lower hydrophobicity values lead to deterioration in dewaterability.

Also, the shift in particle size distribution causes deterioration in dewatering properties of activated sludge since small flocs are associated with poor dewatering by increase in SRF and CST. Higgins and Novak (1997a) have shown that the increase in addition of calcium and magnesium ions is correlated to improvement in settling and dewatering properties of activated sludge while the increase in sodium ions added to the feed lead to deterioration in both properties.

4.2.4.3. Settleability

The settlement of activated sludge is crucial for efficiency of solids separation step of operation. Therefore, settleability of activated sludge was determined by sludge volume index (SVI) and zone settling velocity (ZSV) tests.

ZSV graphs were plotted against sludge concentration (MLSS) for each reactor as given in Appendix. In the highlight of examination of graphes, a fixed solids concentration (3500 mg/L) was determined and then, ZSV versus sodium concentration graph was plotted as demonstrated in Figure 4.25. The increase in sodium concentration results in decrease in settling velocity of sludge except for 20 meq/L reactor. The ZSV value of control reactor was 0.041 cm/s. When sodium concentration is increased to 5 meq/L, a drastic decrease is observed in settling velocity. A sharp decrease in velocity occurs from 0.026 to 0.009 cm/s shifting from 5 meq/L to 10 meq/L sodium concentration. Although addition of 20 meq/L sodium leads to increase in settling velocity to 0.014 cm/s, ZSV of its is approximately half of the value of control reactor. Therefore, it can be said that replacement of divalent cations with sodium ions based on ion-exchange mechanism might have happened and caused deterioration in floc integrity and led to the formation of dispersed flocs. It is for this reason that settleability of sludge becomes harder.

As can be seen from Figure 4.26, a positive correlation is observed between SVI and sodium concentration. SVI value of control reactor was 85.98 mL/g and this value indicates well settling sludge in the highlight of literature because Jenkins *et al.* (1993) revealed that SVI values less than 120 mL/g indicate well settling sludge. An increase in SVI value occurred from 85.98 to 105.45 mL/g when concentration was shifted to 5 meq/L. This value also takes part in acceptable levels for well settling. The increasing of sodium concentration to 10 meq/L led to a sharp increase in SVI value as 150.11 mL/g and this value indicates a problem in sludge settling because Jenkins *et al.* (1993) reported that if SVI

value is exceeded 120 mL/g, settling problems occur. Then, although the addition of 20 meq/L sodium to the feed resulted in a decrease in SVI, obtained value 132.64 mL/g is higher compared to control SVI value. Sobeck and Higgins (2002) reported that the addition of sodium resulted in SVI values greater than control reactor values indicating that increasing sodium adversely affected settling properties. Therefore, our findings in this study are in parallel with literature.

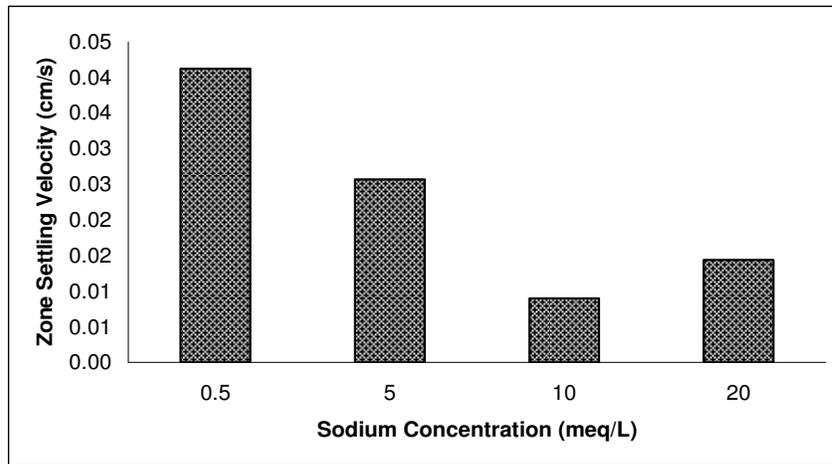


Figure 4.25. Zone settling velocities at different sodium concentrations at a fixed MLSS concentration of 3500 mg/L

It is known that there is an inverse relationship between SVI and ZSV. The increase in SVI is correlated to decrease in ZSV. Examining of obtained results indicates that SVI and ZSV measurements are consistent with each other. Therefore, it can be said that sodium ions at rising concentrations lead to poor settleability due to monovalent character. As stated earlier, sodium is the poorest flocculator due to its small ionic size, and large hydrated radius (Table 2.1). Also, the increase in the concentration of sodium ions has negative effect on floc integrity due to displacement of divalent cations within floc structure (Higgins and Novak, 1997 a,b,c; Murthy *et al.*, 1998; Murthy and Novak, 1998; 2001).

Therefore, it can be concluded that the gaps forming by removal of divalent cations may not be filled with sodium ions due to its atomic structure, resulting in sludge disintegration and consequently in deteriorated sludge settling properties.

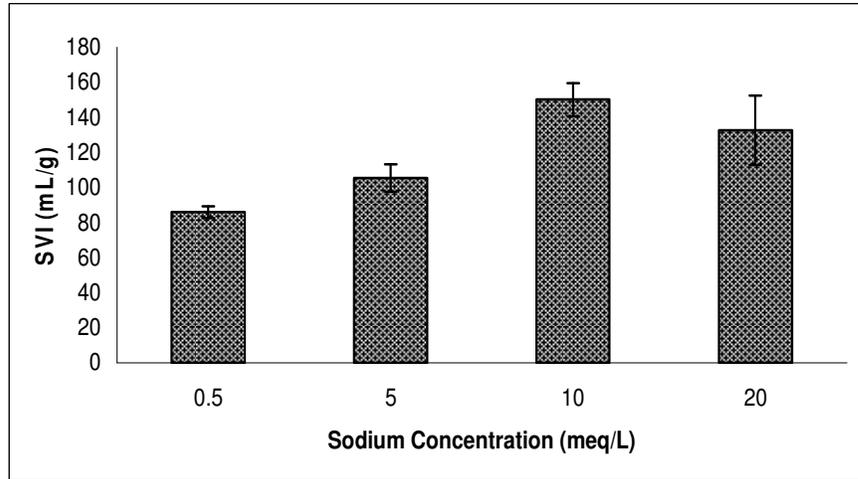


Figure 4.26. SVI values with respect to sodium concentrations

The obtained results indicate that sludge settleability decreases linearly with the increase of total EPS. The decrease in ZSV can be attributed to the increased resistance to settlement as a result of increasing EPS concentrations since higher concentration of EPS results in more friction with water molecules. Our findings indicate an inverse correlation between EPS_p and settleability. Sponza (2004) also observed such an inverse relation between EPS_p and SVI. It was reported that retention of protein within the EPS structure allows a more rigid structure to develop such that sludges with lower protein content are more settleable. Therefore, it can be said that the increase in EPS_p with the addition of more sodium ions results in formation of more rigid floc structure, causing difficulty in settlement by increase in SVI or decrease in ZSV. Also, a negative correlation was observed between carbohydrate content and settleability.

Jorand *et al.* (1994) confirmed that improvements in bioflocculation and settling are correlated to an increase in floc hydrophobicity. In other words, decrease in hydrophobicity results in deterioration in settleability. In accordance with literature, our findings from this study show that decrease in hydrophobic character of sludge leads to increase in SVI or decrease in ZSV, causing deteriorated settling properties. Also, sodium-containing sludges with higher zeta potential values compared to control reactor exhibit difficulty in settlement so introduction of more negative charge to surface prevent sludge from settling.

It was reported that an increase in particle size is necessary for improvements in settling properties (Higgins and Novak 1997a and Murthy and Novak 1997). Opposed to these findings, examining of the relation between mean particle size and settling revealed a negative correlation. Hilligart and Hoffmann (1997) indicated that an increase in particle size is correlated to increase in SVIs. As a similar manner, the increase in floc size of sodium sludges was accompanied by increase in SVI and decrease in ZSV. As mentioned earlier, in the highlight of our findings it has been concluded that the optimum floc size for good settling changes between 100 and 120 μm . The shift in particle size beyond this value leads to deterioration in settleability due to increase in number of small flocs.

4.2.4.4. Rheology

For the determination of flow model of sodium-containing sludges, shear stress vs shear rate graph was plotted for each sodium concentration. As can be seen from Figure 4.27, the curves give highest fit to the equation of non-Newtonian Bingham plastic fluids. Bingham plastic model is represented by the following equation:

$$\tau = \tau_y + \eta (dv/dy) \dots\dots\dots 4.1$$

where τ_y is the yield stress and η is the plastic or Bingham viscosity. The presence of initial yield stress in the Bingham plastic is due to the resistance of the sludge solids to deformation until sufficient stress is applied to exceed the yield strength of the solid phase (Dentel, 1997). Once this stress is exceeded, the sludge starts flow and the relationship between the shear stress and shear rate becomes linear.

When the τ_y values were examined in Figure 4.27, an increase in sodium concentration was correlated to the increase in yield stress except for reactor with 20 meq/L sodium. It means that addition of sodium ions up to 10 meq/L leads to increase in the resistance to deformation and then at 20 meq/L sodium concentration, a decrease occurs in yield stress. Also, it can be seen from Figure 4.27, the Bingham viscosity increased significantly with increase in sodium concentration except for reactor fed with 20 meq/L sodium. The reactor with 20 meq/L sodium concentration has higher viscosity value compared to control reactor. As a result, it can be said that the rising addition of sodium to the feed leads to increase not only in yield stress, but also in viscosity. It is for this reason that pumpability of sludge becomes more difficult.

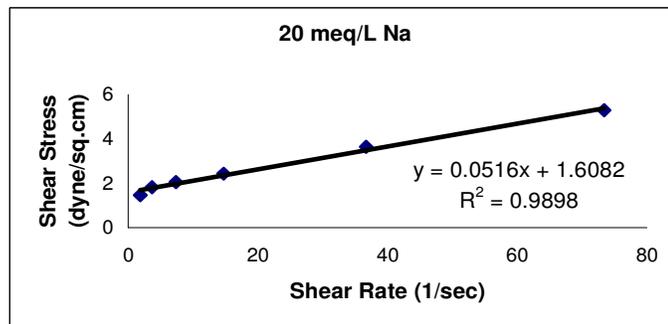
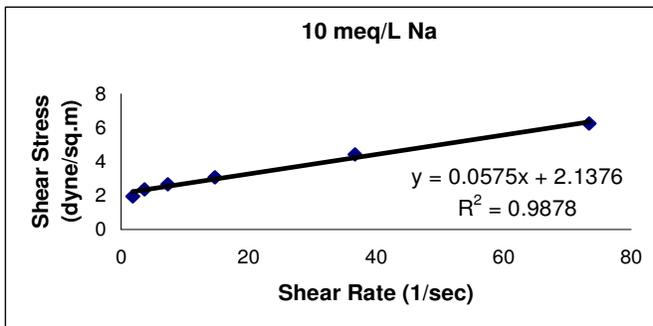
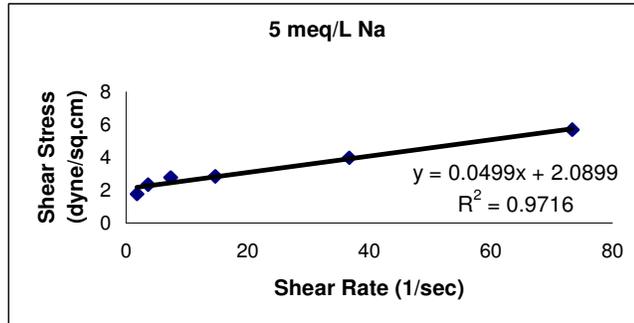
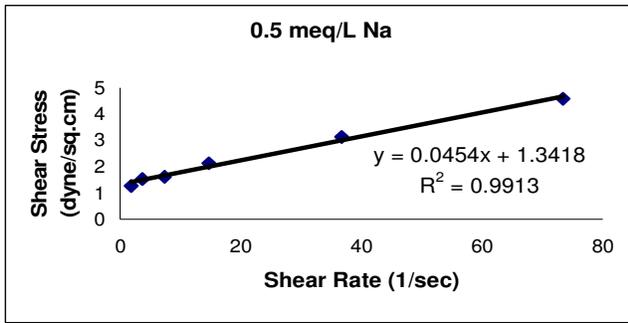


Figure 4.27. Shear rate versus shear stress graphs of reactors at different sodium concentrations

In order to reveal the correlation between solids concentration and viscosity, apparent viscosities of sodium sludges were plotted as a function of five different solids concentrations at a fixed shear rate of 73.4 sec^{-1} . As can be observed from Figure 4.28, the increase in solids concentration was accompanied by an increase in apparent viscosity for each reactor. This result was in accordance with the findings of Sanin (2002).

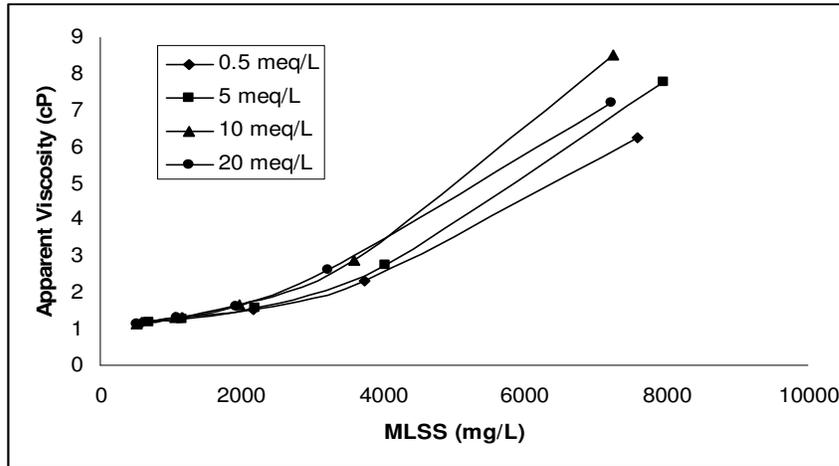


Figure 4.28. Apparent viscosity values with respect to sodium concentration at a shear rate of 73.4 sec^{-1}

To investigate the correlation between apparent viscosity and sodium concentration, a fixed solid concentration was selected as 5000 mg/L . For each sodium concentration viscosity value was determined at 5000 mg/L by using the graph demonstrated in Figure 4.28. The viscosity vs sodium concentration was plotted as illustrated in Figure 4.29. Examining of the results of viscosity measurements in Figure 4.29 shows a positive correlation between viscosity and sodium concentration. An increase was observed when sodium concentration shifted from control level to 5 meq/L . Then the further increase of sodium concentration from 5 meq/L to 10 meq/L resulted in a sharp increase in viscosity

and then, a small decrease occurred at reactor with 20 meq/L sodium concentration. As a general trend, it can be said that the increase in sodium concentration is correlated to increase in viscosity, making sludge more difficult to pump.

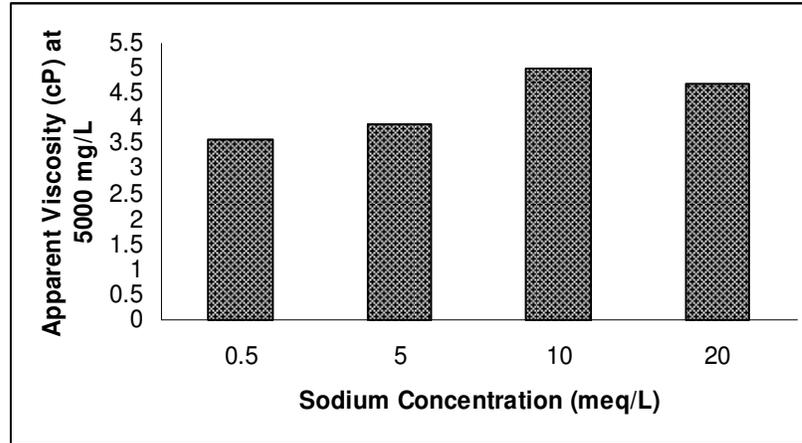


Figure 4.29. Apparent viscosity versus sodium ion concentration at 5000 mg/L

Forster (1982;1983) reported that the amount and composition of EPS influence sludge viscosity. When the obtained results are taken into account, it can be said that the increase in total EPS and constituents, especially protein, was correlated to increase in viscosity. As stated in Sanin (2002), biopolymers are high-molecular mass and high viscosity substances, so in the highlight of our findings and literature, it can be concluded that increase in polymer concentration leads to increase in viscosity and difficulty in pumpability. Also, Sanin (2002) reported that the increase in the conductivity is accomplished by a decrease in viscosity. Similarly, Forster (1982) indicated that the addition of NaCl to activated sludge samples, the viscosity of the system is lowered. As opposed to these findings, examining of our findings showed that increase in conductivity is correlated to increase in viscosity. As mentioned earlier, an increase in sodium concentration leads to increase not only in production of polymers, but also in conductivity.

Also a bulking sludge is produced at high sodium concentrations. So, introduction of more viscous polymers and an extended floc network to the sludge is accompanied by increase in viscosity.

4.2.4.5. Turbidity

The turbidity measurements were conducted following 1 hour settlement and measurements are presented in Table 4.6. Although an increase was observed when sodium concentration was shifted to 0.5 to 5 meq/L, further addition of sodium to the feed led to a decrease in turbidity compared to control reactor.

Table 4.4. Turbidity and SVI values of sodium reactors with respect to sodium concentration

Sodium Concentration (meq/L)	0.5	5	10	20
Turbidity (NTU)	23.20	28.73	15.05	19.69
SVI (mL/g)	85.98	105.45	149.14	128.05

Bruus *et. al* (1992) demonstrated that when high concentrations of sodium were added to activated sludge, this resulted in ion-exchange which displaced divalent cations from within the floc. This displacement resulted in deterioration in floc properties measured by supernatant turbidity and SRF. In the highlight of our results, addition of sodium ions led to poor dewaterability by increase in SRF and CST. On the other hand, a decrease in turbidity is in contradiction with literature. The decrease in the turbidity values of sodium reactors with 10 and 20 meq/L compared with control reactor can be explained by the increase in SVI values of these reactors. High SVI values cause low turbidity supernatant due to bulking-like conditions. It is for reason that formation of clear effluent due to bulking like conditions led to decrease in turbidity.

4.3. Comparison of Results of Potassium and Sodium Sludges

In the highlight of the obtained results; although both of them are monovalent cations, the effects of these ions on chemical, surface chemical and physical characteristics of activated sludge are different. In following sections, the differences between sodium and potassium sludges will be discussed.

4.3.1. Comparison of Chemical Properties

4.3.1.1. Effluent Soluble Chemical Oxygen Demand (COD)

The comparison of COD removal efficiencies of potassium and sodium reactors indicated a similar manner with respect to COD removal. The examining of COD removal values of reactors indicated that the COD removal efficiency decreased with the addition of potassium and sodium ions at increasing concentrations (Figure 4.30). However, when the values are taken into account, it was observed that potassium reactors less effective in the removal of COD compared to sodium reactors.

Potassium reactors especially 10 and 20 meq/L reactors showed lower than 90 % removal efficiency for COD indicating the presence of organic material in the effluent.

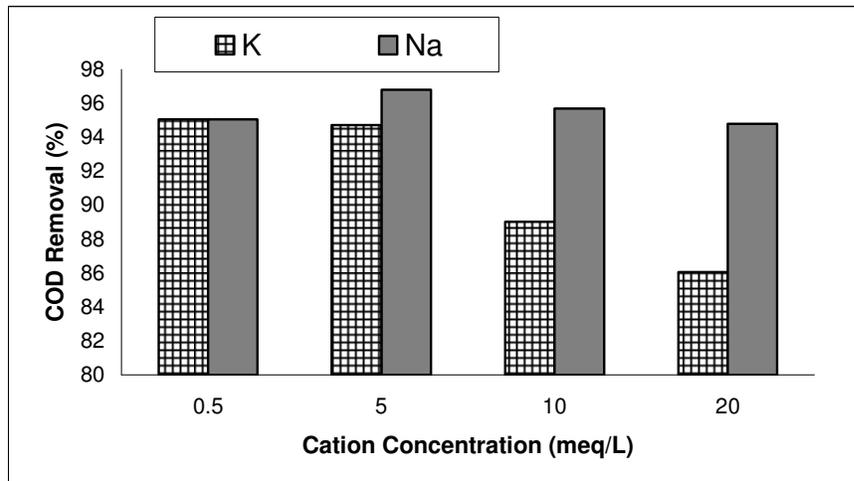


Figure 4.30. COD removal with respect to cation type and concentration

4.3.1.2. Extracellular Polymer Production and Composition

According to the results obtained, quality and quantity of EPS changed at different concentrations of potassium and sodium ions. The relationship between total EPS and cation concentration is presented in Table 4.5. As it is seen from Table 4.5, the rising addition of potassium and sodium ions led to increase in total amount of EPS except for 20 meq/L sodium reactor.

The mass of protein and carbohydrate is given separately in Table 4.5 as a function of cation concentration added to the feed. It can be seen that the increase in potassium concentration was positively correlated with the increase in the carbohydrate concentration. On the other hand, sodium addition at rising concentrations up to 10 meq/L led to no significant change in carbohydrate concentration and further addition caused decline in the level of carbohydrate. The protein content decreased when shifted from control to 5 meq/L but then, addition of potassium ions at rising concentrations provided an increase in the concentration of protein. For sodium reactors, the increase in sodium concentration was accompanied by an increase in protein. Although protein was

the dominant constituent of the EPS of sodium and potassium reactors, sodium ions tend to stimulate production of protein type polymers and have an affinity to bind more protein within the floc structure. On the other hand, potassium ions promote the synthesis of both the protein and polysaccharide type polymers. This is confirmed by Murthy and Novak (1998) found that potassium was the only ion that was positively correlated with slime protein and polysaccharide.

Table 4.5. Effect of cation concentration on the production and composition of EPS

EPS (mg/gVSS)	Cation Concentration (meq/L)			
	Control	5	10	20
K-carbohydrate	8.9	14.1	15.8	19.1
K-protein	19.9	16.8	20.3	25.3
K-total EPS	28.8	30.9	36.1	44.4
EPSp/EPSc	2.24	1.19	1.28	1.32
Na-carbohydrate	9.0	10.9	10.4	6.2
Na-protein	19.9	26.5	32.1	30.9
Na-total EPS	28.8	37.4	42.5	37.1
EPSp/EPSc	2.24	2.43	3.09	4.98

The difference between the affinities of monovalent cations to the different constituents of EPS can be related to the function of each cation in the cell as stated earlier. Besides their stimulation effects on polymer production, cations act as binding agents of the flocs. The ionic charge, ion size and radius of the hydration shell affect the binding ability of cations. As the size of ion increases, the hydration shell radius decreases so cations with high valency, size and thin hydration shell can easily move close to the charged sites of surfaces and establish bonds with negatively charged sites of EPS (Piirtola *et al.*, 1999). The hydration shell radius of cations follow the order $Mg^{2+} > Ca^{2+} > Na^{+} > K^{+}$. Although K and Na ions have the same charge, potassium has smaller hydrated radius (0.53 nm) compared to sodium (0.79 nm), so it loses its hydration shell

easier near the charged groups of EPS and establishes a stronger bond with charged sites on EPS than sodium. On the other hand sodium's approach to the surface is prevented by water molecules around it. Rengasamy and Naiudu (1998) calculated the relative flocculation power of cations and they obtained the following order: $\text{Ca}^{2+} > \text{Mg}^{2+} > \text{K}^+ > \text{Na}^+$. It means that the tendency of cations to form covalent bonding and complexes increases as stated order. It can be said that sodium is the poorest flocculator due to its single charge, small size and large hydrated radius.

Results presented here show that these ions stimulate the production of polymers and influence the charge distribution around an activated sludge particle. The more important issue is that whether they are able to cause flocculation of these particles. It can be suggested that binding sites on EPS which are available for divalent cations, are not suitable for monovalent cations. Our findings reveal that while potassium ions have affinity to bind both type of polymers, sodium ions promote the binding of protein within the floc structure.

4.3.1.3. Cation Concentration in Floc Structure

Along with the changes in quality of polymer, the amount of cations incorporated into the sludge floc matrix changed with the type and dose of cations added (Figure 4.31). The greatest amounts of cations in the sludge are seen for sodium reactors followed by potassium. Sodium ion can be used as a motive force, for nutrient uptake (Wilson and Ding, 2001) and as a buffer for pH homeostasis in microorganisms (Krulwich *et al.*, 2001). The transport of small molecules such as sugars, amino acids and vitamins into the cell is achieved by symport transport with sodium ions. Therefore, the mentioned functions of sodium ions explain the reason why sodium is found in excess amount in floc structure. Potassium and magnesium ions are cofactors for certain enzymes (Brdjavonic *et al.*, 1996) and it was revealed that cellular potassium is required for protein synthesis since one of the steps of protein synthesis is potassium dependent (Alahari and Apte, 2004).

Also, when the osmotic pressure of the medium increases, potassium ions are transferred into the cell for the adjustment of pressure. Sanin *et al.* (2006) reported the concentrations of divalent cations in floc structure with monovalent cations and it was revealed that potassium is the second highest cation incorporated into the floc structure following sodium. It is known that the movement of sodium ions into the cell during symport transport is coupled with the movement of calcium ions outside the cell. Therefore, it can be concluded that whereas monovalent cations and partly Mg ions mostly reside and function within the cells rather than in the EPS matrix, especially calcium ions exist outside the cell, in the EPS structure (Sanin *et al.*, 2006).

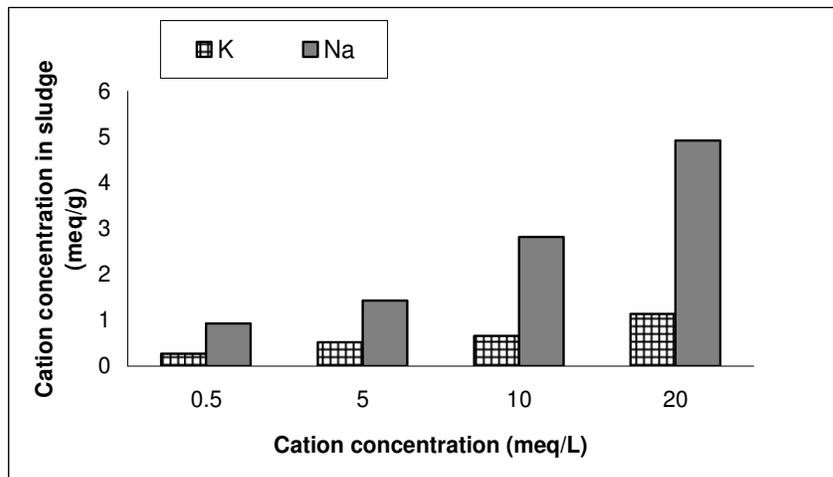


Figure 4.31. Cation concentration incorporated in floc structure with respect to cation type and concentration

4.3.1.4. Electrical Conductivity

Conductivity is the measurement of the total amount of dissolved ions in the water. It is expected that the addition of salts to the feed should lead to increase in conductivity due to dissolution of salts. As can be expected, an increase was

observed in conductivity values of both potassium and sodium sludges as illustrated in Table 4.6.

Table 4.6. Conductivity measurements with respect to cation type and concentration

Conductivity (mS)	Cation Concentration (meq/L)			
	Control	5	10	20
Potassium	1.78	2.81	3.74	5.30
Sodium	1.78	2.41	3.74	4.92

4.3.2. Comparison of Surface Chemical Properties

4.3.2.1. Hydrophobicity

Relative hydrophobicity of the sludges were measured to investigate the effect of sodium and potassium on surface characteristics of the sludge samples. As the hydrophobicity values are examined in Figure 4.32, it can be seen that the increase in cation concentrations is accompanied by a decrease in hydrophobicity values of sludge samples for both reactors (except for 20 meq/L). It is seen that lower hydrophobicity values are obtained especially for sodium ions such that hydrophobicity dropped to the half of the control reactor levels. On the other hand, a much less decrease occurred for potassium reactors.

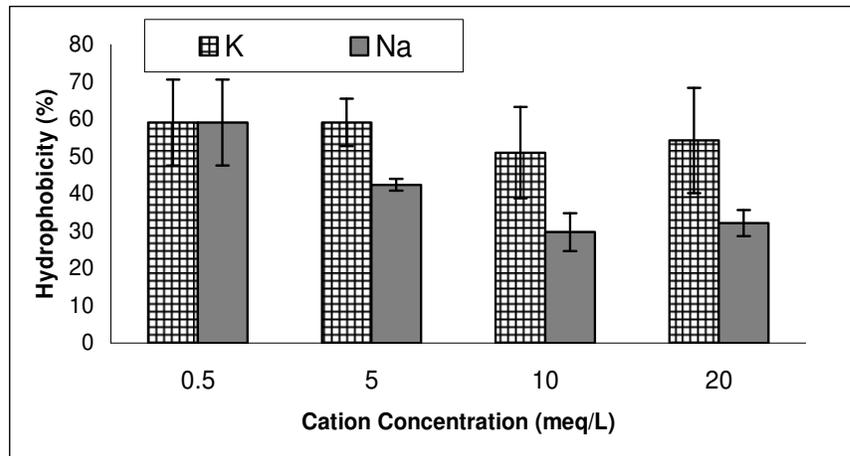


Figure 4.32. Relative hydrophobicity values with respect to cation type and concentration

Hydrophobic molecules such as lipids or proteins from the cells can be trapped into the flocs and cell surface hydrophobicity may be promoted by specific proteins with specific amino acid sequences. It is seen that the increase in addition of potassium to the feed led to a small decline in the hydrophobicity value, no drastic change in the hydrophobic character of sludge was observed. It can be said that the produced polymers with the rising addition of potassium did not affect the hydrophobicity much. On the other hand, the increase in the sodium concentration promotes the production of protein type polymers but it can be speculated that these produced protein type polymers may not contribute to hydrophobicity of the sludge since they do not contain the specific hydrophobic amino acids. Also, it can be said that the increase in sodium concentration was accompanied by an increase in protein level with hydrophilic side chains, causing decrease in hydrophobicity. In general, it can be concluded that whereas addition of potassium ions to the feed at increasing concentrations results in no significant change in hydrophobic character of sludge, increase in addition of sodium ions causes decrease in hydrophobicity of sludge due to contribution of hydrophilic protein type polymers to system.

4.3.2.2. Zeta potential

The zeta potential of potassium control reactor was measured as -15.6 mV and a slight decrease in negativity was observed with the increase of potassium concentration to 5 meq/L (Table 4.7). This situation can be attributed to the neutralization of the negative surface charges by the introduction of positively charged potassium ions. When potassium concentration increased to 10 and then 20 meq/L, an increase in the negativity was obtained. The addition of more potassium to the feed seems to be not sufficient to neutralize the negative charges since increase in the concentration of especially carbohydrate type polymers introduces more negative charge to the floc surfaces, causing an increase in zeta potential. In the opposite way, the higher amount of protein type polymers normally contributing to positive surface charge of sodium sludge samples is expected to lead to a decrease in zeta potential values. However, this could only be observed at high sodium concentrations here. The examination of Table 4.7 reveals that even though there is a decrease in zeta potential value with the addition of sodium ions at rising concentrations, sodium reactors have higher zeta potential values compared to potassium sludges. The reason of this may be the production of hydrophilic positively charged proteins rather than negatively charged ones in sodium reactors. Therefore sodium sludges with higher surface charges have a negative effect on floc structure due to the prevention of strong floc formation.

Table 4.7. Zeta potential values with respect to cation type and concentration

Zeta potential (mV)	Cation Concentration (meq/L)			
	Control	5	10	20
Potassium	-15.6	-11.7	-13.2	-17.6
Sodium	-15.6	-26.7	-23.4	-21.7

4.3.3. Comparison of Physical Properties

4.3.3.1. Particle Size

In order to compare the mean particle size of potassium and sodium sludges, the volume-weighted mean diameter values were taken into account as illustrated in Figure 4.33.

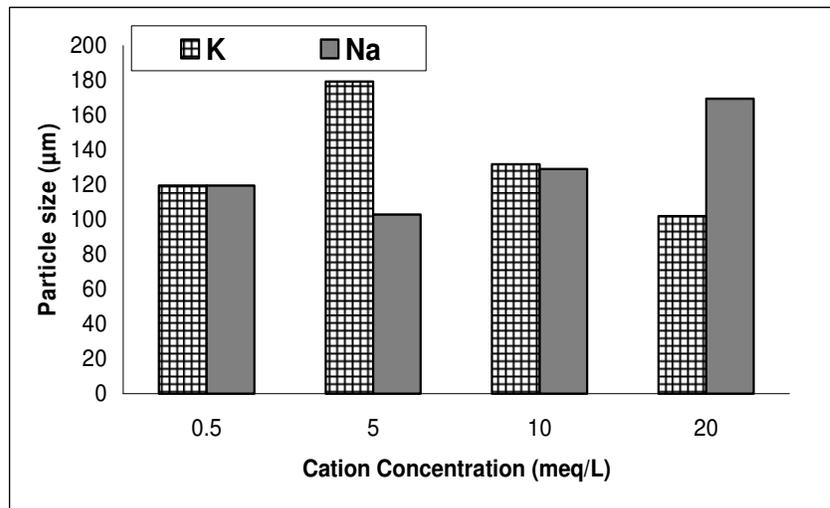


Figure 4.33. Particle size measurements with respect to cation type and concentration

The examination of the particle size measurements of both reactors from Figure 4.33 showed that while the increase in the addition of potassium ions is accompanied by a decrease in particle size, the rising addition of sodium ions is correlated to increase in size of flocs. It was reported that an increase in particle size is necessary for improvements in settling properties (Higgins and Novak 1997a and Murthy and Novak 1997). Opposed to these findings, our results indicated that increase in SVI or decrease in ZSV was correlated to increase in particle size. In other words, examining of the relation between mean particle

size and settling revealed a negative correlation for sodium and potassium sludges. As mentioned earlier, in the highlight of our findings it can be suggested that 100-120 μm is optimum floc size for good flocculation. Increase or decrease beyond this range led to dispersion of flocs, contributing to deterioration in settleability and dewaterability due to formation of small flocs.

4.3.3.2. Dewaterability

When all the data in Table 4.8 are examined, it is seen that the increase in cation concentration is correlated with increase in SRF and CST values of both sodium and potassium reactors (except for 20 meq/L). The comparison of SRF values of potassium and sodium reactors revealed that SRF values of sodium reactors are greater than that of potassium reactors. In a similar manner, even though all the CST values are small, CST of sodium reactors are much greater than that of potassium reactors. Therefore, it can be said that the dewaterability of sodium reactors are much worse than that of potassium reactors.

Table 4.8. Parameters related to dewaterability with respect to cation type and concentration

Ion Type	Ion Conc. (meq/L)	SRF (m/kg)	CST (s)	Floc Strength
Potassium	Control	1.48×10^{14}	6.80	1.20
	5	2.78×10^{14}	7.22	1.48
	10	4.08×10^{14}	7.28	0.63
	20	3.52×10^{14}	6.29	0.75
Sodium	Control	1.48×10^{14}	6.80	1.20
	5	1.15×10^{15}	18.95	0.19
	10	2.15×10^{15}	24.20	0.23
	20	1.40×10^{15}	11.10	0.31

The floc strength measurements given in Table 4.8 indicate that sodium sludges have lower floc strength values compared to that of potassium sludges, so it can

be said that addition of sodium ions leads to reduction in the strength of the bonds and flocs became much weaker and less resistant to shear. As a result, addition of sodium ions results in poorer dewaterability compared to potassium sludges by measurements of SRF and CST

4.3.3.3. Settleability

The comparison of ZSV values of sodium and potassium reactors (Table 4.9) revealed that although addition of 5 meq/L sodium and potassium to the feed led to drop of settling velocity nearly half of the control reactor value, further addition of these ions exhibited different settleability. The further addition of potassium ions beyond 5 meq/L resulted in no significant change in ZSV whereas sodium addition was correlated to decrease in settling velocity, causing poor settleability. In a similar manner with regard to ZSV, SVI measurements given in Table 4.9 indicated that although the obtained SVI values greater than the SVI of control reactor, the values are take part in acceptable levels based on literature (Jenkins *et al.*, 1993). On the other hand, the increasing of sodium concentration beyond 5 meq/L led to a sharp increase in SVI values of reactors with 10 and 20 meq/L sodium concentration and these values indicate a problem in sludge settling because Jenkins *et al.* (1993) reported that if SVI value is exceeded 120 mL/g, settling problems occur. Sodium sludges had been shown to be bulking in earlier researchers also (Higgins and Novak, 1997a).

Table 4.9. Settleability of activated sludge with respect to cation type and concentration

Ion Type	Ion Conc. (meq/L)	ZSV (cm/s)	SVI (mL/g)
Potassium	Control	0.041	85.98
	5	0.023	112.63
	10	0.029	100.22
	20	0.026	93.63
Sodium	Control	0.041	85.98
	5	0.026	105.45
	10	0.009	150.11
	20	0.014	132.64

4.3.4.4. Rheology

The findings of this study showed that potassium sludges exhibited the highest sodium sludges gave highest fit to Bingham plastic flow. Therefore, it can be said that although both of them are monovalent cations, they affect sludge characteristics in a different manner depending on the cation type.

The viscosity value of potassium reactors (Figure 4.34) was more or less constant at average of 3 cP. Whereas the apparent viscosity of sodium reactors showed an increasing trend with concentration and increased to a value of straightly higher than 4 cP. The viscosity of sodium reactors was thought to originate from the extended floc network of sodium sludges (which were bulking) and this would cause difficulty in pumping of sludge.

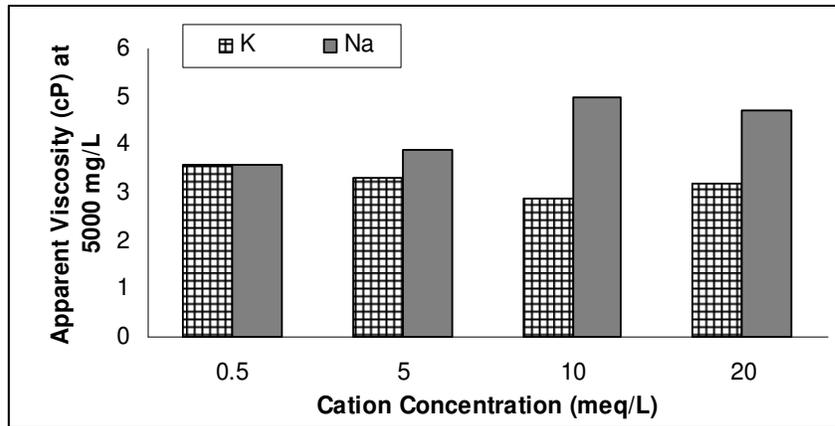


Figure 4.34. Apparent viscosity values with respect to cation type and concentration at 5000 mg/L

4.3.4.5. Turbidity

According to Table 4.10, although the turbidity values did not change parallel to the cation concentrations added for both cation types, an improvement was observed for sodium ion above 5 meq/L whereas the situation was just the opposite for potassium ion showing the highest turbidity value at 20 meq/L. Researchers observed an improvement in settling properties with an increase in potassium ion concentration, associated with an increase in turbidity (Higgins and Novak, 1997b; Murthy and Novak, 1998). The decrease in the turbidity values of sodium reactors with 10 and 20 meq/L compared with control reactor can be explained by the increase in SVI values of these reactors. High SVI values cause low turbidity supernatant due to bulking-like conditions. Nevertheless, all the turbidities measured are low and well within the acceptable levels.

Table 4.10. Turbidity values with respect to cation type and concentration

Turbidity (NTU)	Cation Concentration (meq/L)			
	Control	5	10	20
Potassium	23.2	20.5	31.5	34.5
Sodium	23.2	28.7	15.1	19.7

CHAPTER 5

CONCLUSIONS

This research investigated the effects of monovalent cations, potassium and sodium, on chemical, surface chemical and physical characteristics of activated sludge by operating semi-continuous activated sludge reactors.

The conclusions drawn from the whole experimental results are presented below.

- The results from this study indicated that the quality and quantity of EPS composition changed at different concentrations of potassium and sodium ions. It is evident from the data that potassium and sodium ions at increasing concentrations stimulated the synthesis of more extracellular biopolymers. Although protein was the dominant constituent of EPS in both of the reactors, potassium ions promoted the synthesis of polysaccharide type polymers as well. On the other hand, sodium ions tended to stimulate production of protein type polymers and have an affinity to bind more protein within the floc structure. This could result from differences in their cellular functions and characteristics such as ionic size, valency and hydrated shell size.
- Surface characteristics of sludge samples were determined by measurements of hydrophobicity and zeta potential. It can be concluded that whereas addition of potassium ions to the feed at increasing concentrations resulted in no significant change in hydrophobic character of sludge, increase in addition of sodium ions caused decrease in

hydrophobicity of sludge due to contribution of hydrophilic protein type polymers to system. Also, zeta potential measurements indicated that sodium containing sludges have higher negative surface charges compared to potassium reactors. However, the addition of sodium ions at rising concentrations resulted in increase in protein constituent of EPS, contributing to extra positive charge to the surface so a decrease occurred in zeta potential values. It can be concluded that sodium-containing sludges with lower hydrophobicity and higher surface charges exhibits poor flocculation.

- The examination of particle size measurements of reactors indicated that while the increase in the addition of potassium ions was accompanied by a decrease in particle size, the rising addition of sodium ions was correlated to increase in size of flocs. In the highlight of findings, it can be concluded that 100-120 μm is optimum floc size for good flocculation. Increase or decrease beyond this range led to increase in amount of small flocs, contributing to deterioration in dewaterability. This conclusion was confirmed by the measurements of SRF, CST and floc strength. The reduction in floc strength values implied that addition of monovalent cations to the feed led to displacement of divalent cations within the floc structure by weakening the floc structure. Especially sodium ions, poorer flocculator, caused the formation of weak and dispersed flocs. In the same way, higher SRF and CST values were obtained with the addition of sodium ions except for 20 meq/L reactor. As a general statement, it can be said that addition of sodium ions to the feed led to much greater deterioration in floc stability and dewaterability of activated sludge when compared to potassium ions.

- Results of this study demonstrated that the cation type and concentration have an important effect on flocculation and settling of activated sludge. Sludge settlement measured by ZSV and SVI revealed that whereas

addition of potassium ions to the feed resulted in no observable change in settleability of activated sludge, the increase in addition of sodium ions led to detrimental effect on settling. This detrimental effect can be explained by displacement of divalent cations from within the floc structure by sodium ions. Inability of sodium ions to fit in the gaps remaining from the removal of divalent cations found in the EPS matrix and to bind them due to their smaller ionic size, larger hydrated radius and monovalent charge made the sludge dissociated. Besides sodium ions tended to stay within the cells due to their vital functions for cells intracellularly.

- In this study, it was observed that potassium sludges exhibited pseudoplastic flow whereas sodium sludges showed Bingham plastic flow. Also, it was revealed that the addition of potassium ions to the feed led to a slight decrease in viscosity. This could be resulted from a decline in bound water content of sludge and repulsion between flocs. On the other hand, increasing sodium concentration above control reactor levels caused the sludge to be more viscous. This could be resulted from contribution of more viscous polymers to the system and the presence of an extended floc network with the increase in total EPS.
- From the results of turbidity measurements, it was seen that an improvement was observed for sodium ion above 5 meq/L whereas increasing potassium concentration above control level led to an increase in turbidity. The improvement in turbidity values of sodium sludges could have resulted from bulking-like conditions. This situation led to formation of clear effluent and low turbidity values.
- The increase in potassium and sodium concentration was correlated to increase in effluent COD due to increase in EPS. Also, examination of COD removal rates of reactors indicated that the COD removal

efficiency decreased with the increase in addition of potassium and sodium ions. On the overall though, the COD removal efficiency of all the reactors were considered acceptable being higher than 90%. The only two reactors which did not fit this generalization was 10 and 20 meq/L potassium reactors, which showed about 85% COD removal efficiency.

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APPENDIX A

SOLIDS CONCENTRATIONS OF REACTORS WITH RESPECT TO TIME TO DETERMINE STEADY STATE CONDITIONS

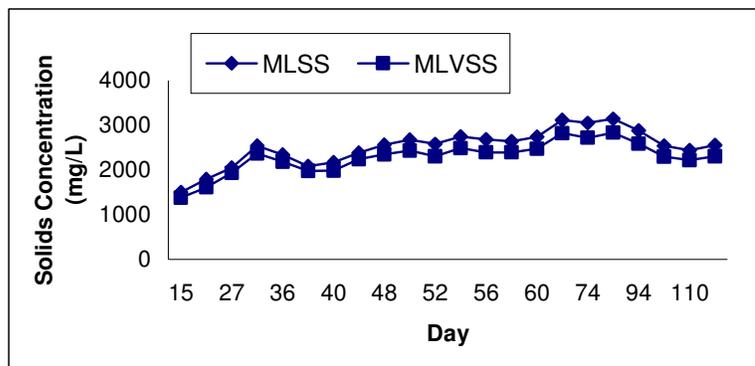


Figure A.1. The change in solids concentration with respect to time for control reactor 1 ($K = 0.5$ meq/L)

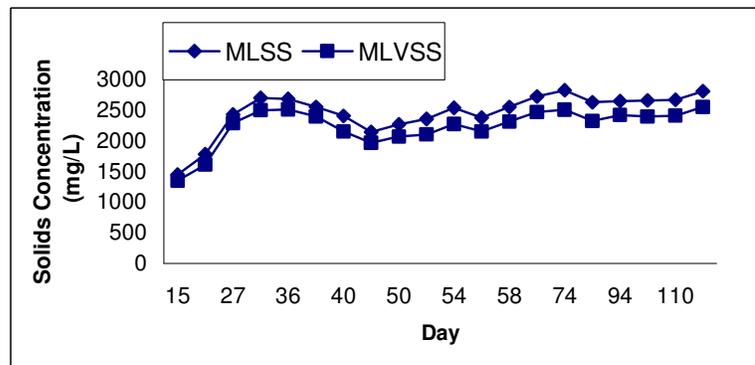


Figure A.2. The change in solids concentration with respect to time for control reactor 2 ($K = 0.5$ meq/L)

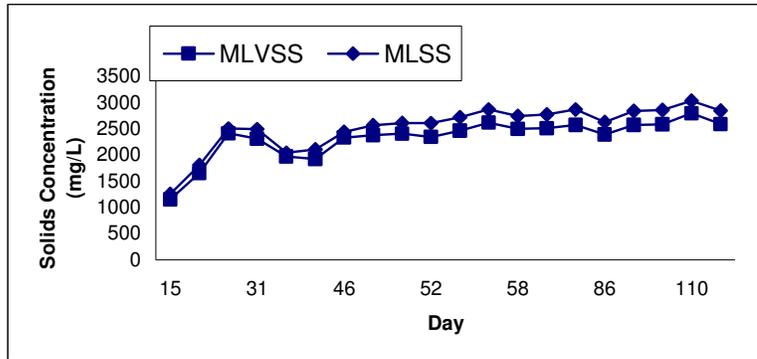


Figure A.3. The change in solids concentration with respect to time for reactor 1 (K = 5 meq/L)

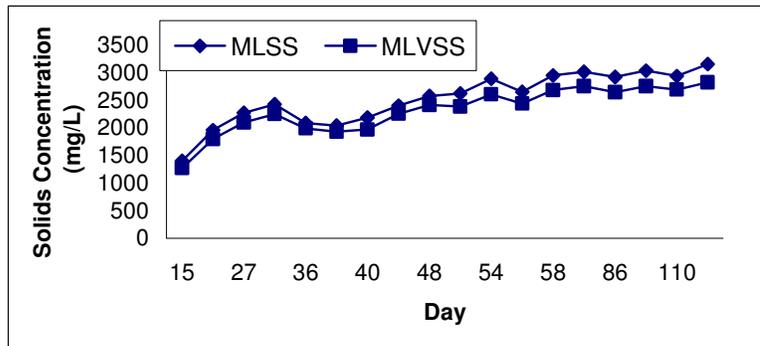


Figure A.4. The change in solids concentration with respect to time for reactor 2 (K = 5 meq/L)

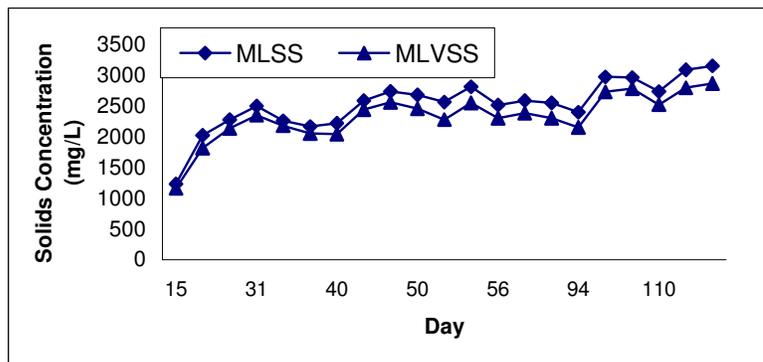


Figure A.5. The change in solids concentration with respect to time for reactor 3 (K = 10 meq/L)

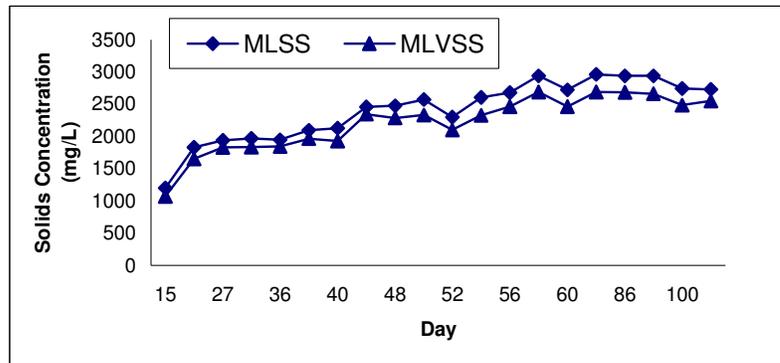


Figure A.6. The change in solids concentration with respect to time for reactor 4 (K = 10 meq/L)

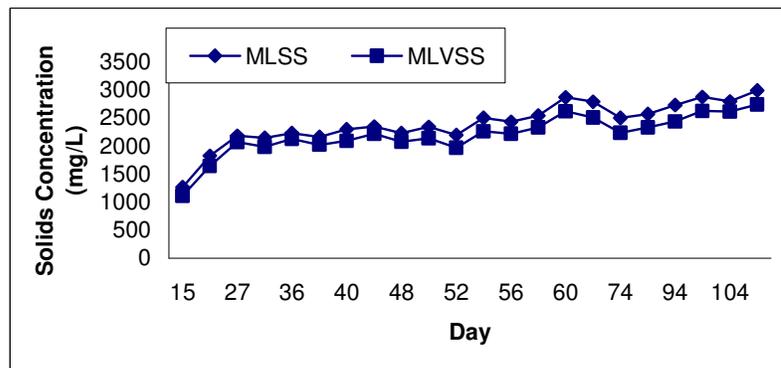


Figure A.7. The change in solids concentration with respect to time for reactor 5 (K = 20 meq/L)

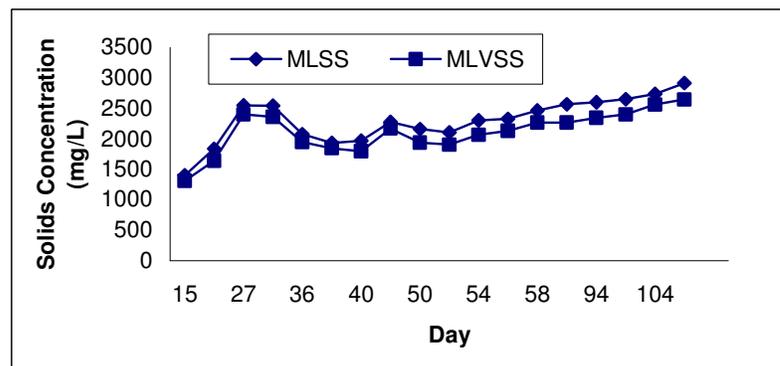


Figure A.8. The change in solids concentration with respect to time for reactor 6 (K = 20 meq/L)

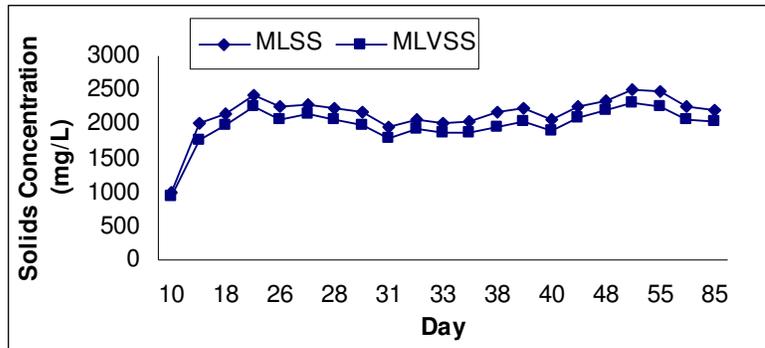


Figure A.9. The change in solids concentration with respect to time for control reactor 1 ($N_a = 0.5$ meq/L)

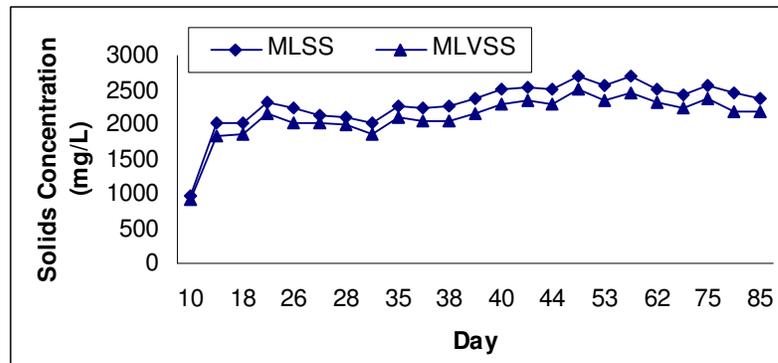


Figure A.10. The change in solids concentration with respect to time for control reactor 2 ($N_a = 0.5$ meq/L)

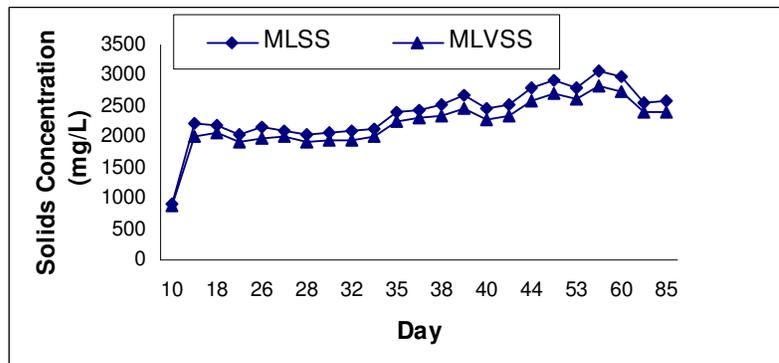


Figure A.11. The change in solids concentration with respect to time for reactor 1 ($N_a = 5$ meq/L)

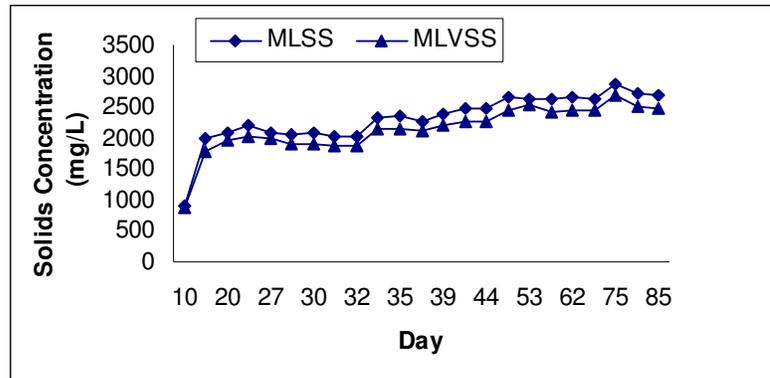


Figure A.12. The change in solids concentration with respect to time for reactor 2 (Na = 5 meq/L)

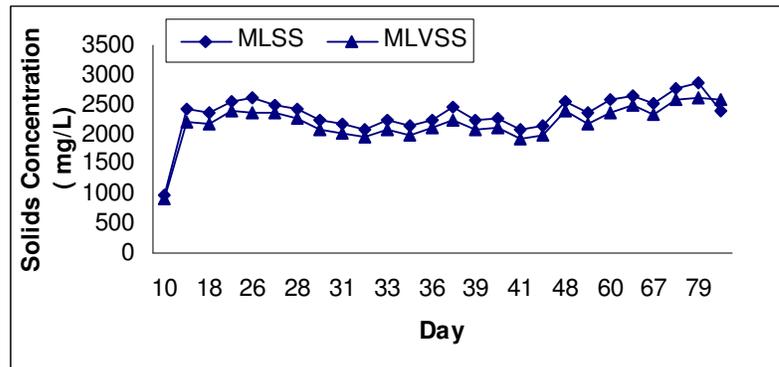


Figure A.13. The change in solids concentration with respect to time for reactor 3 (Na = 10 meq/L)

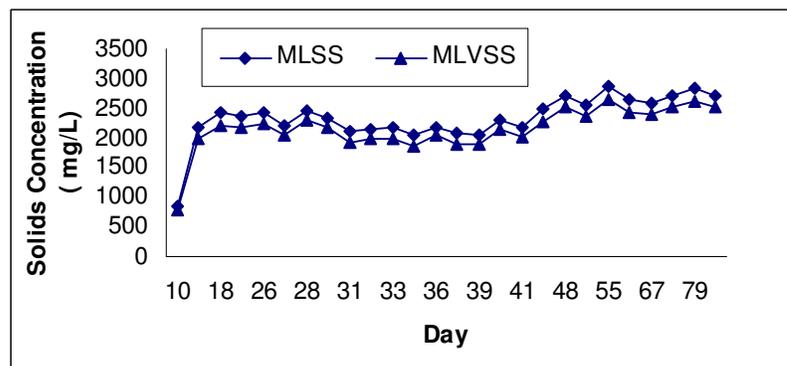


Figure A.14. The change in solids concentration with respect to time for reactor 4 (Na = 10 meq/L)

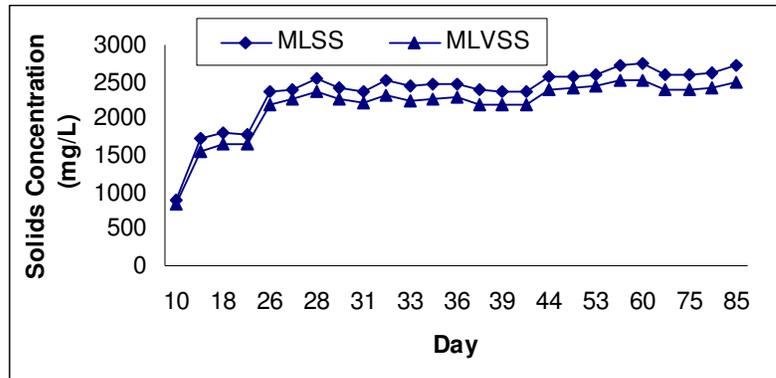


Figure A.15. The change in solids concentration with respect to time for reactor 5 (Na = 20 meq/L)

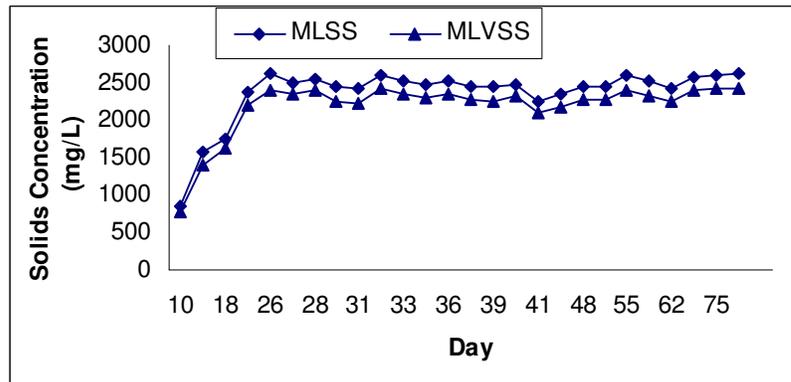


Figure A.16. The change in solids concentration with respect to time for reactor 6 (Na = 20 meq/L)

APPENDIX B

CALIBRATION CURVES

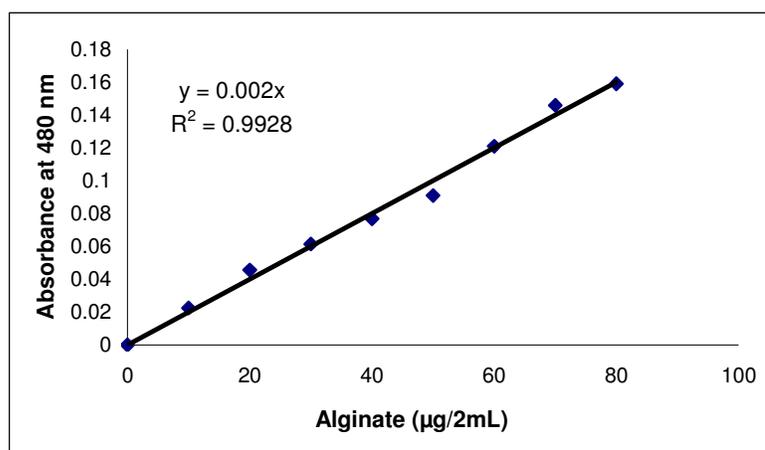


Figure B.1. Calibration curve carbohydrate measured by Dubois method for potassium reactors

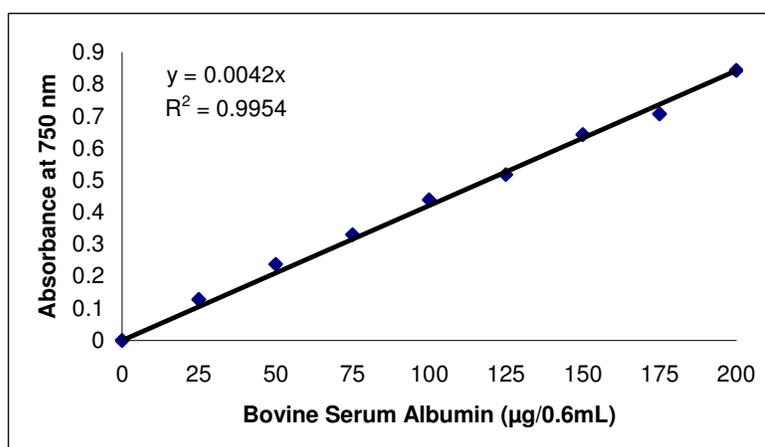


Figure B.2. Calibration curve for protein measured by Lowry method for potassium reactors

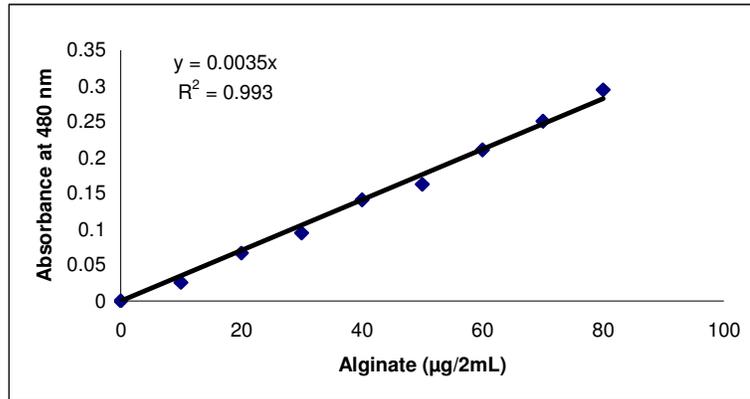


Figure B.3. Calibration curve carbohydrate measured by Dubois method for sodium reactors

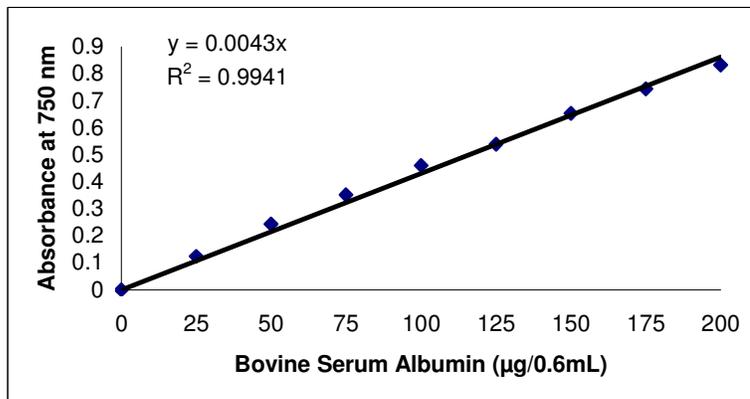


Figure B.4. Calibration curve for protein measured by Lowry method for sodium reactors

APPENDIX C

GRAPHS USED FOR THE DETERMINATION OF SPECIFIC RESISTANCE TO FILTRATION (SRF)

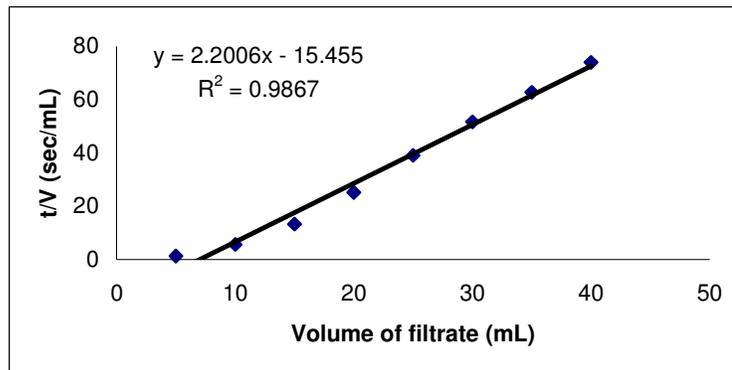


Figure C.1. t/V vs V graph for control reactor 1 (K= 0.5 meq/L)

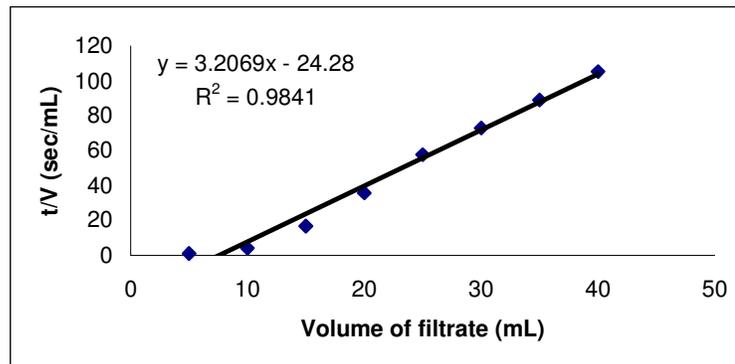


Figure C.2. t/V vs V graph for control reactor 2 (K= 0.5 meq/L)

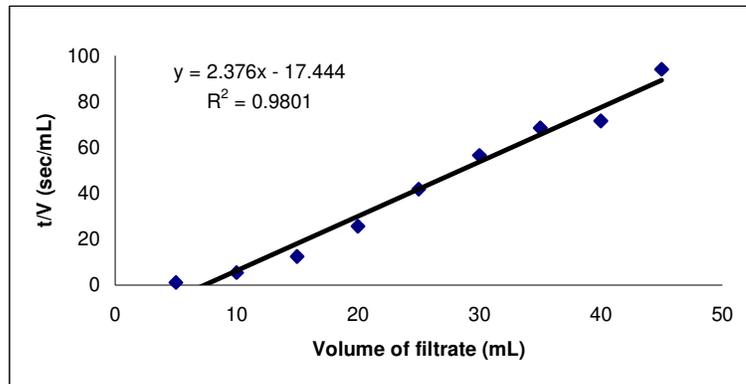


Figure C.3. t/V vs V graph for reactor 1 (K= 5 meq/L)

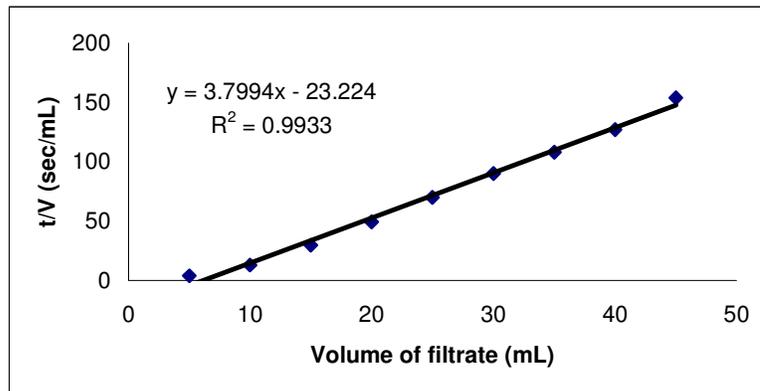


Figure C.4. t/V vs V graph for reactor 2 (K= 5 meq/L)

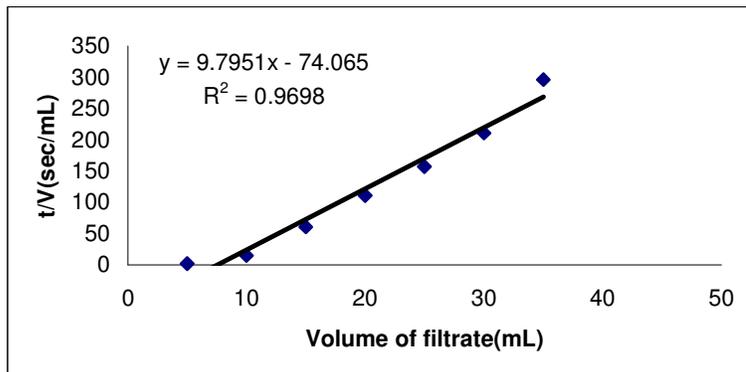


Figure C.5. t/V vs V graph for reactor 3 (K= 10 meq/L)

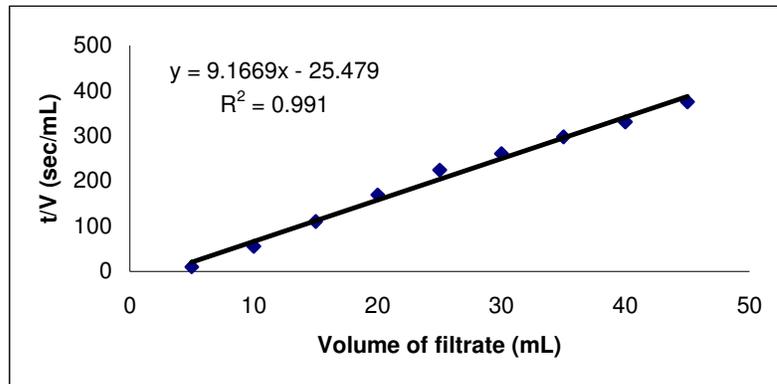


Figure C.6. t/V vs V graph for reactor 4 (K= 10 meq/L)

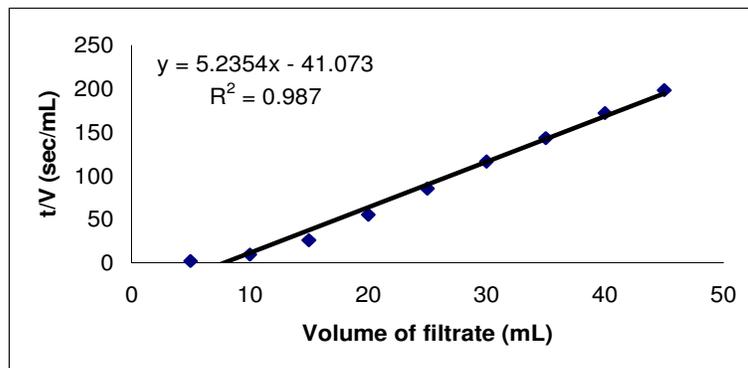


Figure C.7. t/V vs V graph for reactor 5 (K= 20 meq/L)

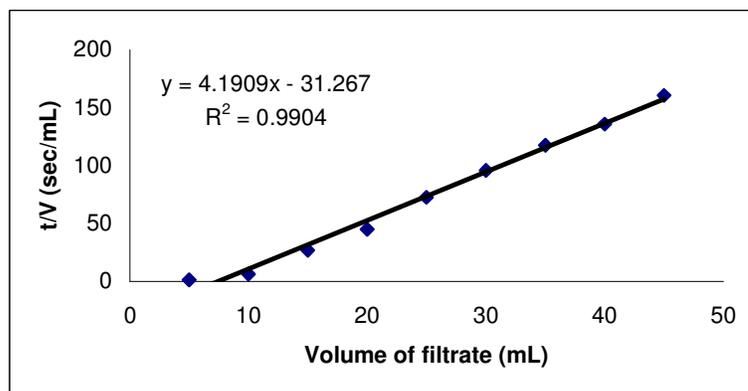


Figure C.8. t/V vs V graph for reactor 6 (K= 20 meq/L)

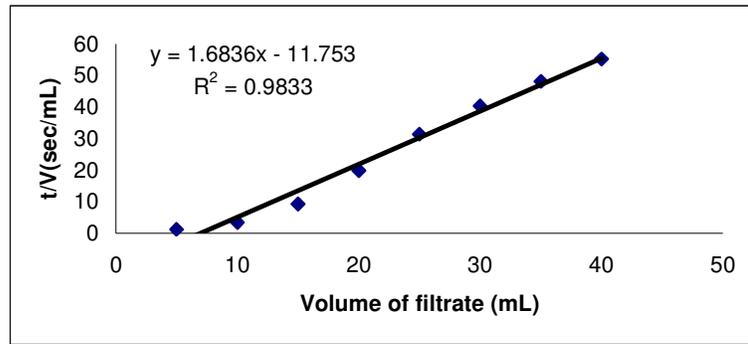


Figure C.9. t/V vs V graph for control reactor 1 ($Na = 0.5$ meq/L)

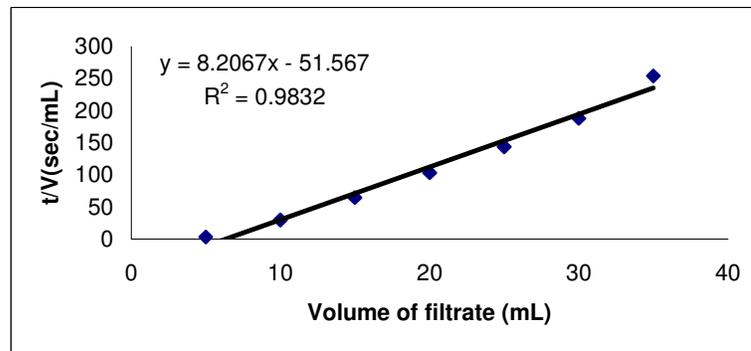


Figure C.10. t/V vs V graph for reactor 1 ($Na = 5$ meq/L)

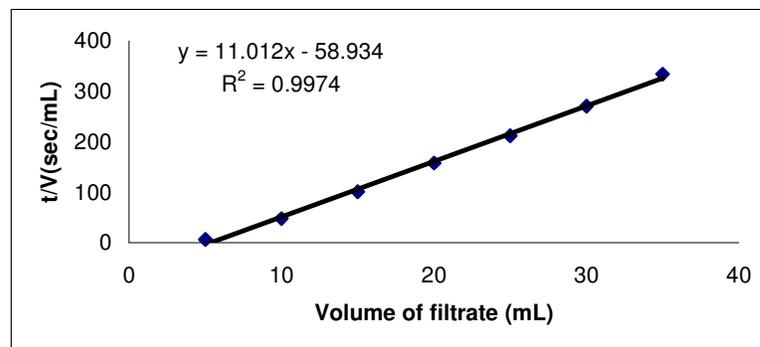


Figure C.11. t/V vs V graph for reactor 2 ($Na = 5$ meq/L)

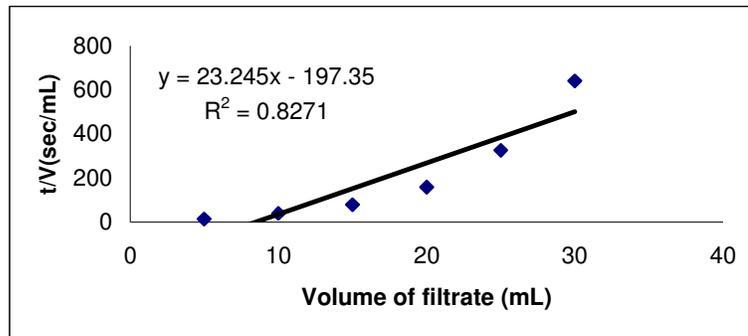


Figure C.12. t/V vs V graph for reactor 3 (Na = 10 meq/L)

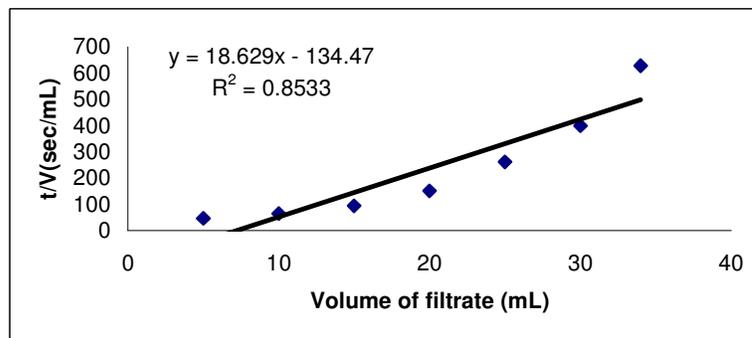


Figure C.13. t/V vs V graph for reactor 4 (Na = 10 meq/L)

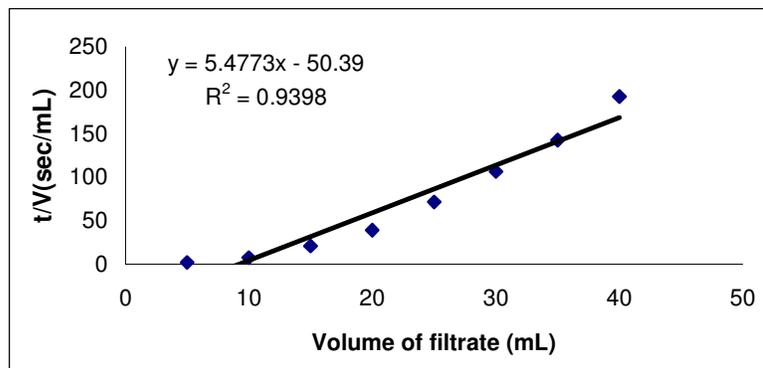


Figure C.14. t/V vs V graph for reactor 5 (Na = 20 meq/L)

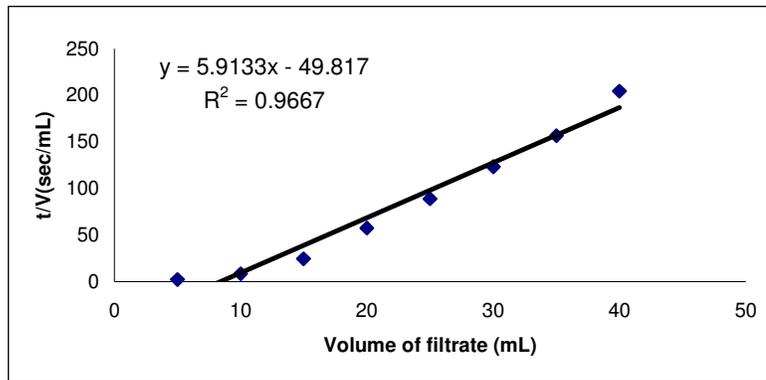


Figure C.15. t/V vs V graph for reactor 6 ($Na = 20$ meq/L)

APPENDIX D

ZONE SETTLING VELOCITIES OF THE REACTORS

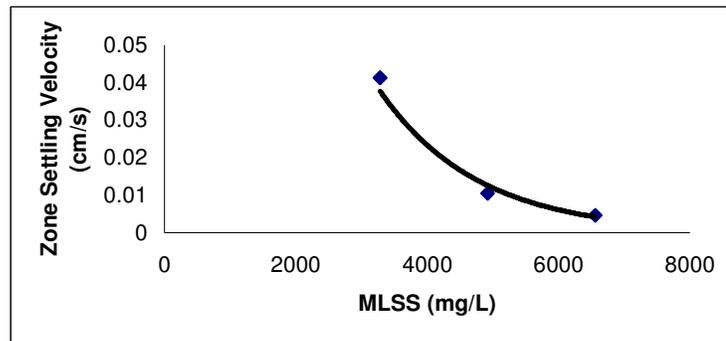


Figure D.1. Zone settling velocity versus MLSS concentration for control reactor 1 ($K= 0.5$ meq/L)

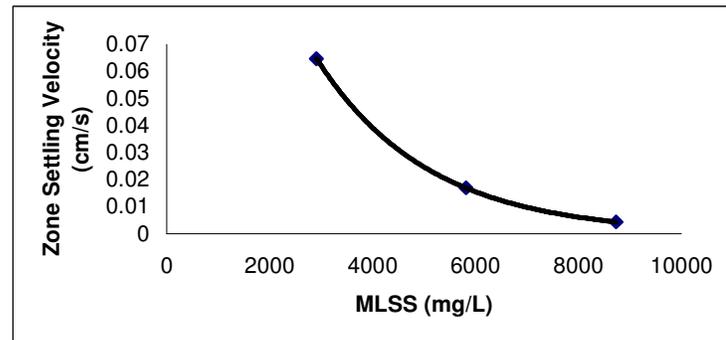


Figure D.2. Zone settling velocity versus MLSS concentration for control reactor 2 ($K= 0.5$ meq/L)

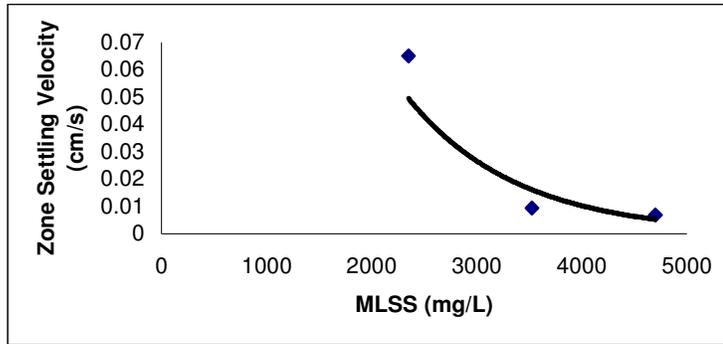


Figure D.3. Zone settling velocity versus MLSS concentration for reactor 1 (K= 5 meq/L)

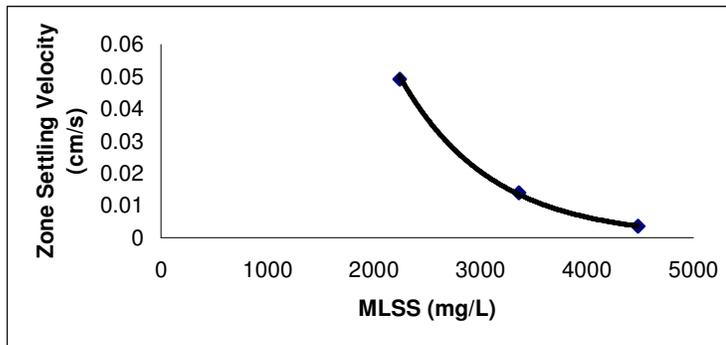


Figure D.4. Zone settling velocity versus MLSS concentration for reactor 2 (K= 5 meq/L)

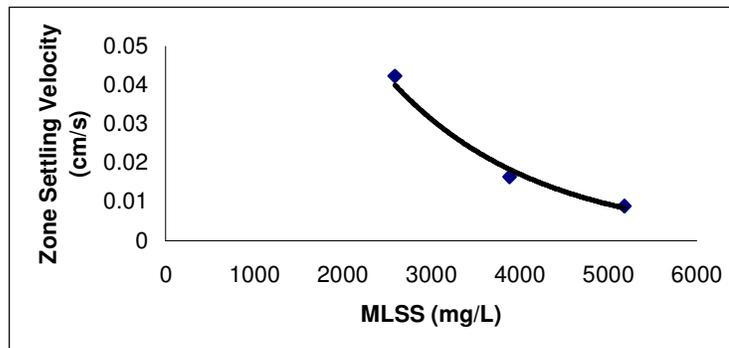


Figure D.5. Zone settling velocity versus MLSS concentration for reactor 3 (K= 10 meq/L)

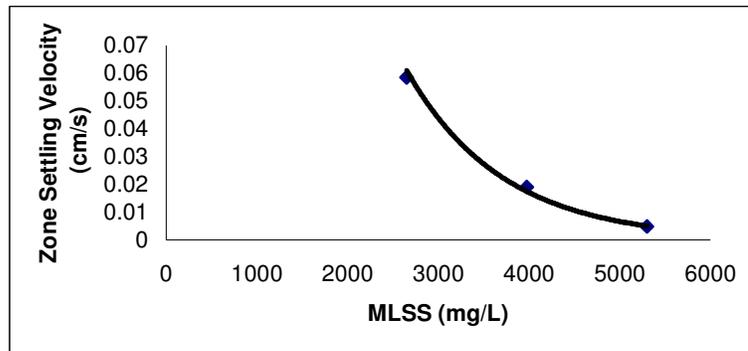


Figure D.6. Zone settling velocity versus MLSS concentration for reactor 4 (K= 10 meq/L)

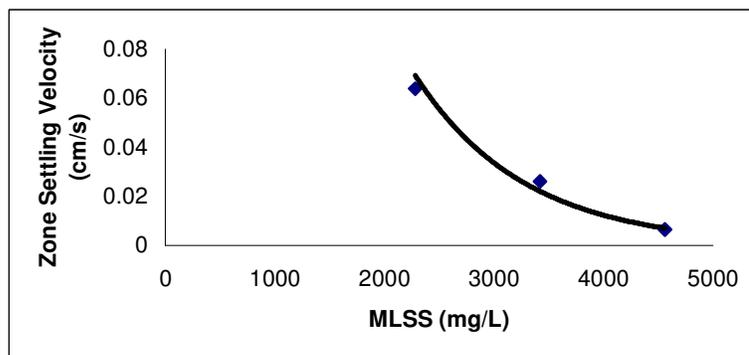


Figure D.7. Zone settling velocity versus MLSS concentration for reactor 5 (K= 20 meq/L)

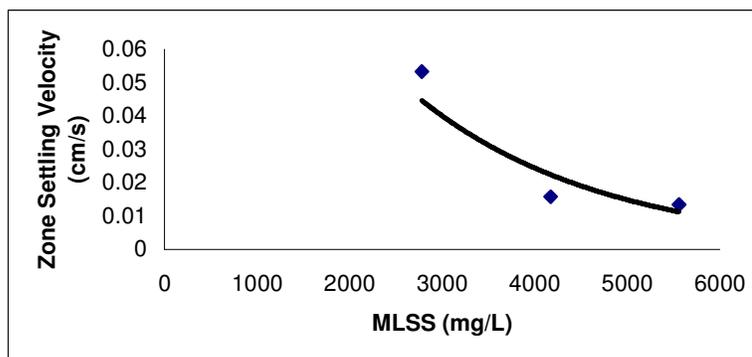


Figure D.8. Zone settling velocity versus MLSS concentration for reactor 6 (K= 20 meq/L)

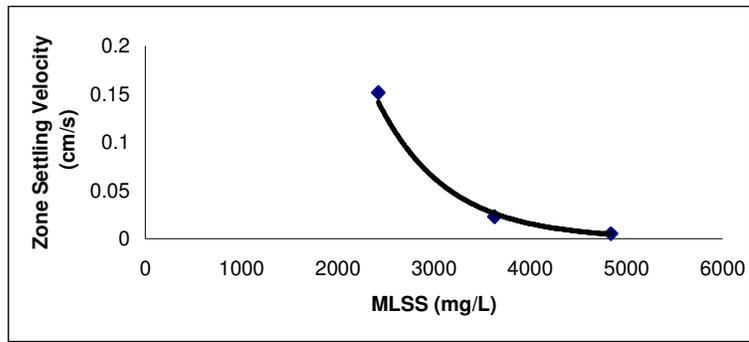


Figure D.9. Zone settling velocity versus MLSS concentration for control reactor 1 ($N_a = 0.5$ meq/L)

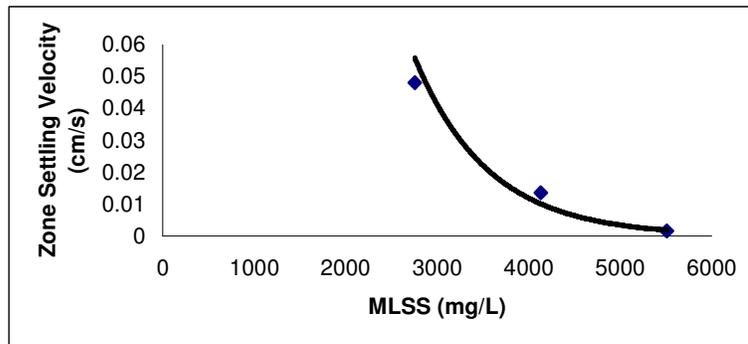


Figure D.10. Zone settling velocity versus MLSS concentration for reactor 2 ($N_a = 5$ meq/L)

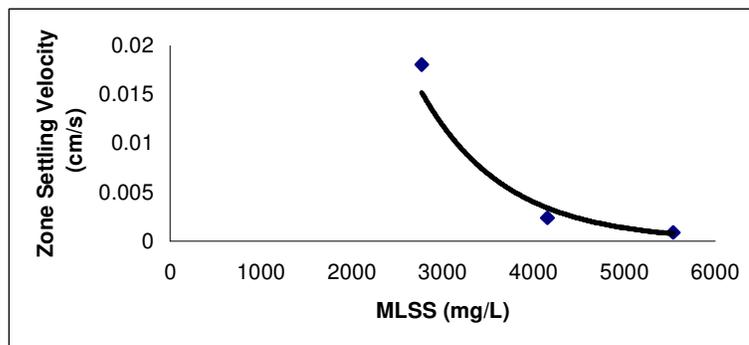


Figure D.11. Zone settling velocity versus MLSS concentration for reactor 3 ($N_a = 10$ meq/L)

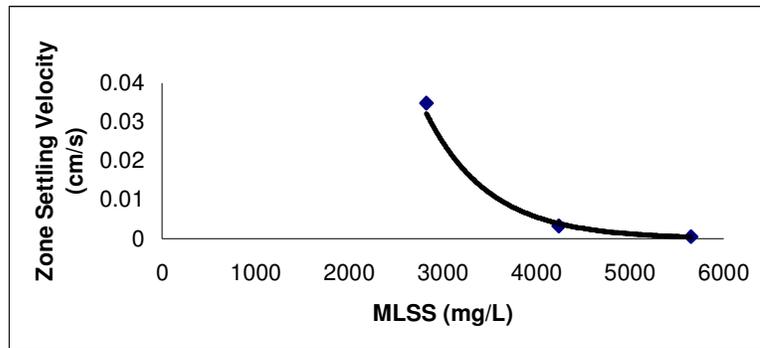


Figure D.12. Zone settling velocity versus MLSS concentration for reactor 4 (Na = 10 meq/L)

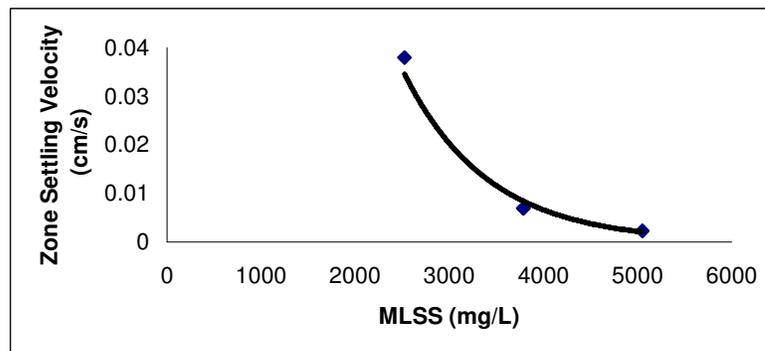


Figure D.13. Zone settling velocity versus MLSS concentration for reactor 5 (Na = 20 meq/L)

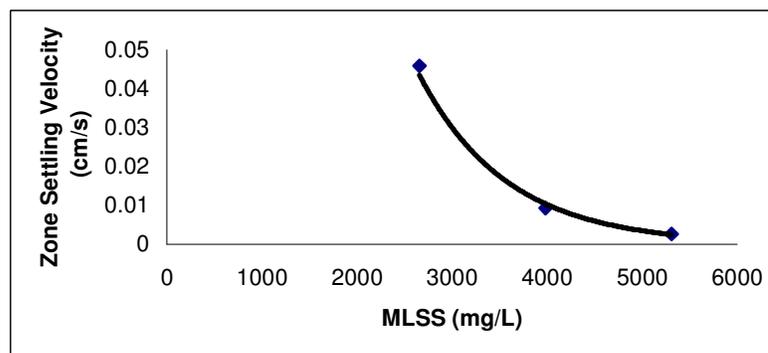


Figure D.14. Zone settling velocity versus MLSS concentration for reactor 6 (Na = 20 meq/L)