

**INVESTIGATION OF ACTIVATED SLUDGE BIOFLOCCULATION:  
INFLUENCE OF MAGNESIUM IONS**

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## **ABSTRACT**

# **INVESTIGATION OF ACTIVATED SLUDGE BIOFLOCCULATION: INFLUENCE OF MAGNESIUM IONS**

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Activated sludge systems are the most widely used biological wastewater treatment processes all over the world. The main working principles of an activated sludge system are the oxidation of biologically degradable wastes by microorganisms and the subsequent separation of the newly formed biomass from the treated effluent. Separation by settling is the most troublesome stage of an activated sludge process. A decrease in the efficiency of the separation of microbial biomass from the

treated effluent causes a decrease in the overall efficiency of the treatment plant. The efficiency of the separation process is related to the bioflocculation, which can be briefly defined as the aggregation of the bacteria into flocs through flocculation. Bioflocculation depends on the extracellular polymers (EPS) that are produced by microorganisms. The operating conditions of the activated sludge system is a key determinant of the synthesis of EPS and bioflocculation. The main objective of this study is to find out the effect of magnesium ions on the bioflocculation process under phosphorus deficient and sufficient conditions. In order to achieve this aim, the effects of magnesium ion in 4 different concentrations (0.9, 5, 10 and 20 meq/L) are investigated in semi continuous reactors. The reactors are operated at a mean cell residence time of 8 days and 20° C temperature. When reactors are confirmed to be at steady state, several sets of analysis are conducted. In particular, the surface chemical parameters including EPS and its components, electrical charge, and hydrophobicity as well as physical properties such as settlability, filterability, viscosity, floc strength, and turbidity are examined.

It has been understood that phosphorus deficiency causes severe filamentous bulking under magnesium rich conditions. Increasing the phosphorus concentration in the influent can cure this problem. After the sludges are cured some granular structures were observed in the microscopic investigations and they are thought to be polyphosphate granules in which microorganisms tend to accumulate phosphorus when they find the adequate source after a starvation period.

To consider the reactors operated at phosphate present conditions, it has been found that EPS increases with increasing influent magnesium concentration. However, protein type EPS (EPS<sub>P</sub>) exhibits a sharper increase when compared to the carbohydrate type EPS (EPS<sub>C</sub>) indicating the selective attitude of magnesium ions to protein type of polymers. It has been understood that the increase in the influent magnesium concentration results in an increase in dewaterability and zone settling velocity, and a decrease in the viscosity. Hydrophobicity was found to exhibit a maximum value at 10 meq/L magnesium fed sludge and then it dropped back. Surface charge values also made a minimum at 10 meq/L reactor and then no change occurred at the increase of the magnesium concentration to 20 meq/L. Finally, COD values were found to increase with the increasing magnesium concentration due to the increasing EPS.

Key words: Activated sludge, bioflocculation, extracellular polymeric substances, magnesium, phosphorus, sludge bulking.

## ÖZ

# AKTİF ÇAMUR YUMAKLAŞMASINDA MAGNEZYUM İYONLARININ ETKİSİNİN ARAŞTIRILMASI

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Aktif çamur sistemleri günümüzde en yaygın olarak kullanılan biyolojik atıksu arıtma sistemleridir. Aktif çamur sistemlerinin temel çalışma prensibi, atıksudaki organik kirliliğin mikroorganizmalar tarafından oksidasyonu ve bunu takiben oluşan yeni biyokütlenin çöktürülerek arıtılmış sudan ayrılması olarak özetlenebilir. Oluşan çamurun arıtılmış sudan çöktürme ile ayrılması aşaması, aktif çamur sistemlerinin en çok sorunla karşılaşılan aşamasıdır. Çamuru sudan ayırma işlemi esnasında oluşan bir problem yalnız aktif çamur sisteminin değil tüm arıtma tesisinin veriminin düşmesine yol açmaktadır. Çamurun çöktürülmesi

işleminin verimliliği ise yumaklaşmaya bağlıdır. Yumaklaşma kısaca bakterilerin bir araya gelerek yumaklar oluşturması olarak tanımlanabilir. Yumaklaşma işlemi ise bakteriler tarafından üretilen hücre dışı polimerler (HDP) ile yakından ilgilidir. Aktif çamur sistemlerinin işletim koşulları, HDP sentezi ve yumaklaşma üzerindeki temel belirleyici etkindir. Bu çalışmanın amacı besi ortamında fosforun varlığı ya da yokluğunda magnezyum iyonunun yumaklaşma üzerindeki etkisini saptamaktır. Bu doğrultuda, araştırma 4 ayrı magnezyum konsantrasyonunda (0.9, 5, 10 ve 20 meq/L) çalıştırılmış yarı sürekli reaktörlerle yürütülmüştür. Reaktörler 20° C sıcaklıkta çalıştırılmış ve çamur yaşı 8 gün olarak ayarlanmıştır. Reaktörler kararlı hale ulaştığında çeşitli deneyler yapılmıştır. Bu deneylerde, HDP ve bileşenleri, çamur elektriksel yükü ve çamur hidrofobisitesi gibi kimyasal parametrelerin yanı sıra çamurun çökelme, filtrelenebilme, viskozite, yumak mukavemeti ve bulanıklığı gibi fiziksel özellikleri de incelenmiştir.

Yapılan deneyler sonucunda, magnezyumca zengin ortamda, fosfor eksikliğinin ipliksi çamur şişmesine yol açtığı anlaşılmıştır. Giriş suyundaki fosfor miktarı arttırılarak bu problem ortadan kaldırılabilmektedir. İpliksi çamur şişmesi probleminin ortadan kaldırılmasını takiben yapılan mikrobik incelemelerde yumak yapısında bazı granüler yapılar gözlemlenmiştir. Bu granüler yapıların polifosfat granülleri olduğu ve bir kere fosfor kıtlığı çeken mikroorganizmaların yeterli fosfor kaynağına kavuştuklarında fosforu bu granüllerde depolama eğiliminde oldukları düşünülmüştür.



Fodforca yeterli kořullarda gerekleřtirilen deneylerin sonuları ele alındığında, HDP miktarının besi ortamındaki magnezyum konsantrasyonundaki artışa baėlı olarak arttıėı bulunmuřtur. Ancak, magnezyum iyonlarının protein tipi HDP'ekarřı olan seiciliėinin bir gostergesi olarak protein tipi HDP miktarı karbonhidrat tipi HDP miktarına kıyasla daha keskin bir artış gostemiřtir. Giriř suyundaki magnezyum iyonu konsantrasyonundaki artış, amurun susuzlařtırılabilirliėini ve okelme hızını arttırmakta ve viskozitesini azaltmaktadır. amurun hidrofobisite deėeri, 10 meq/L magnezyum ile beslenen reaktrde en yksek deėerine ulařmıř ve magnezyum konsantrasyonu arttırılınca tekrardan dřmřtr. amur elektriksel yk de 10 meq/L reaktr iin bir minimum deėere ulařmıř ve daha sonra 20 meq/L deėerine ykselen magnezyum konsantrasyonuyla bir deėiřime uėramamıřtır. Son olarak, KOİ deėerleri, artan magnezyum konsantrasyonuyla birlikte HDPnin de artmasına baėlı olarak ykselmektedir.

Anahtar kelimeler: Aktif amur, amur řiřmesi, fosfor, hcre dıřı polimerler, magnezyum, yumaklařma.

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## ABBREVIATIONS

|                         |  |
|-------------------------|--|
| <b>EPS</b>              | : Extracellular(or Exocellular) Polymeric Substances             |
| <b>EPS<sub>c</sub></b>  | : Carbohydrate constituent of Extracellular Polymeric Substances |
| <b>EPS<sub>p</sub></b>  | : Protein constituent of Extracellular Polymeric Substances      |
| <b>CER</b>              | : Cation Exchange Resin  |
| <b>MLSS</b>             | : Mixed Liquor Suspended Solids                                  |
| <b>MLVSS</b>            | : Mixed Liquor Volatile Suspended Solids                         |
| <b>VS</b>               | : Volatile Solids  |
| <b>COD</b>              | : Chemical Oxygen Demand   |
| <b>C/N</b>              | : Carbon to Nitrogen Ratio                                       |
| <b>θ<sub>c</sub></b>    | : Sludge Age (Mean Cell Residence Time)                          |
| <b>SRT</b>              | : Sludge Retention Time  |
| <b>SVI</b>              | : Sludge Volume Index  |
| <b>ZSV</b>              | : Zone Settling Velocity   |
| <b>SRF</b>              | : Specific Resistance to Filtration                              |
| <b>CST</b>              | : Capillary Suction Time   |
| <b>TKN</b>              | : Total Kjeldahl Nitrogen  |
| <b>PO<sub>4</sub>-P</b> | : Phosphate Phosphorus   |
| <b>COD:N:P</b>          | : Chemical Oxygen Demand to Nitrogen to Phosphorus ratio         |
| <b>DNA</b>              | : Deoxyribo nucleic acid   |
| <b>RNA</b>              | : Ribo nucleic acid  |

## CHAPTER 1

### INTRODUCTION

Activated sludge systems represent the most popular biological wastewater treatment processes. The primary objectives of such process are the oxidation of the biodegradable organic matter into carbon dioxide and new biomass, and incorporation of colloidal matter into settleable solids. Separation results from the aggregation of bacteria and other microorganisms into flocs through flocculation. By flocculation and settling, the system achieves a turbidity-free effluent and a return sludge to the head of the aeration tank. Unless effective flocculation takes place bacterial mass remains dispersed, making the whole treatment process ineffective (Durmaz and Sanin, 2003).

A key aspect in the operation of activated sludge systems is the separation of the biological solids from the liquid phase and the subsequent dewatering of these biosolids. Despite its obvious importance, the mechanisms involved in floc formation and the factors

influencing floc strength are not well understood. Researchers have shown that cations and exocellular biopolymers produced by the microbes are involved in this process, however, the mechanisms for their involvement have been disputed (Higgins and Novak, 1997a).

Among the investigations into the role of salts in bioflocculation only a limited number of studies have examined the effect of cations in the growth media and these studies have provided conflicting results. In addition to that, these studies were conducted on several different monocultures, grown in batch mode. Although the bacteria used in these studies were cultured from activated sludge systems, the results cannot be applied to the wide variety of organisms in these systems, especially since the studies reported conflicting results. Moreover, Higgins and Novak (1997a) stated that, although the settling and dewatering characteristics can be improved by batch addition of calcium and magnesium, a greater improvement occurred when the same amount of the cation was added to the feed, and the reactor was allowed to reach steady state. When cations are present in the feed, they can become incorporated into flocs as they form.

However, much of the work by Higgins and Novak (1997a, b) was performed using equal molar concentrations of calcium and magnesium does not fully explain the mechanisms behind the cation induced flocculation. In addition to that, the COD/N ratio in this study was around 6 which is much lower compared to the typical activated sludge treating domestic wastewater. Moreover, no steady-state laboratory

reactor studies comparing the effects of calcium versus magnesium addition on floc properties have been reported (Sobeck and Higgins, 2002).

In addition to that many researchers (Bruus et.al,1992; Eriksson and Alm,1991; Higgins and Novak, 1997 a,b; Novak and Haugan, 1981; and Tezuka, 1969)have suggested that cations interact with the negatively charged biopolymers (mostly proteins and polysaccharides) in activated sludge to change the structure of the floc. Through these studies, it has been observed that divalent cations tend to improve settling and dewatering characteristics (Murthy and Novak, 2001). One of these divalent cations is magnesium ion. Higgins and Novak (1997b) and Murthy et al. (1998) have shown that magnesium ions improve settling properties. While investigating the effects of cations, Higgins and Novak (1997a,b,1998) and Murthy and Novak (2001) employed continuous and SBR systems started up with a seed obtained from the activated sludge units of industrial and domestic wastewater treatment plants. Then they have introduced their cation-rich feed media to this already grown sludge and observe the effects of cations to sludge properties.

The aim of this study is to grow activated sludge at the presence of magnesium ions by taking seed from the primary clarifier effluent of a domestic wastewater treatment plant (Ankara Central Wastewater Treatment Plant). Therefore, the unknown and uncontrollable effects of other parameters that may have influenced the sludge properties during the growth of microorganisms are eliminated. Moreover, taking the seed

from the primary clarifier unit rather than the activated sludge unit makes it possible to obtain and investigate a sludge floc matrix and its properties which are developed under magnesium rich conditions. Furthermore, the batch addition of the magnesium rich feed media to a formerly grown activated sludge (Novak et al., 1998) may exhibit very different results from an activated sludge sample grown in magnesium rich feed media.

This study is conducted to investigate the effect of magnesium addition with feed on bioflocculation by looking at settling, dewatering, hydrophobicity, surface charge, rheology, extracellular polymer (EPS) content, and the ratio of protein type EPS to carbohydrate type EPS of the sludge.

Besides the objective of this study it is decided to investigate the nature of a bulking problem encountered during the study owing to the fact that in activated sludge systems bulking is a common problem that about 25% of the plants encountered (Urbain et al., 1993). For this purpose microscopic investigations, carbohydrate and protein analyses, PO<sub>4</sub>-P analyses, and SVI measurements were conducted. The phosphorus amount in the feed medium was increased step by step and each of the above measurements was repeated at each step just after the reactors reached steady state. The results of these investigations are also stated in this thesis although they do not contribute to the main aim of this study.



Consequently, the overall goal of this study is to investigate bioflocculation in activated sludge systems in relation to extracellular polymeric substances (EPS) and magnesium ion.

## **CHAPTER 2**

### **THEORETICAL BACKGROUND**

#### **2.1. History of Activated Sludge and Bioflocculation**

The activated sludge process was developed in England in 1914 and was so named because it involved the production of an activated mass of microorganisms capable of stabilizing a waste aerobically. Many versions of the original process are in use today, but fundamentally they are all similar. Organic waste is introduced into a reactor where an aerobic bacterial culture is maintained in suspension. The reactor contents are referred to as “mixed liquor”. In the reactor, the bacterial culture carries out the conversion of organic matter, nutrients, and oxygen into carbon dioxide, ammonia, new bacterial cells and some other end products. The aerobic environment in the reactor is maintained by continuous aeration. After a specified period of time, the mixture of new cells and old cells is passed into a settling tank, where the cells are separated from the treated wastewater. A portion of the

settled cells is recycled to maintain the desired concentration of organisms in the reactor, and a portion is wasted (Metcalf and Eddy, 1991).

Since the conception of the activated sludge process in 1914, researchers have sought to better understand the process of bioflocculation. Bioflocculation, the process of microbial aggregation, is critical to solid liquid separation, and poor bioflocculation leads to poor settling and dewatering properties. As a result, deterioration in effluent quality can occur along with increased difficulty in dewatering and increased costs for dewatering (Sobeck and Higgins, 2002).

Biological flocculation is not observed to occur until the microorganisms have entered into a restricted state of growth (endogenous growth phase). A direct correlation exists between extracellular polymer (EPS) accumulation and microbial agglutination. The mechanism of biological flocculation is interpreted as resulting from the interaction of high molecular-weight EPS, which have sufficiently accumulated at the microbial surface during endogenous growth. These polymers bond electrostatically or physically, and subsequently bridge the cells of the dispersion into a three-dimensional matrix of sufficient magnitude to subside under quiescent conditions (Pavoni et al., 1972).

Extracellular polymeric substances (EPS) are products of active secretion, cell surface material shredding, cell lysis, and sorption from the environment. Some of their functions are forming a protective layer for

the cells against the harmful external environment, such as biocides and sudden changes of pH, sorbing exogenous nutrients and organic molecules, and aggregating bacterial cells in flocs. Consequently, EPS play a crucial role in the flocculation, settling, and dewatering of activated sludge. Observations showed that microbial cells inside the flocs were cross-linked by EPS, forming a polymeric network with pores and channels. Such as polymeric surface had a vast surface area, capable of adsorbing pollutants, nutrients, and minerals (Harris and Mitchell, 1973; Costerton et al., 1987; Morgan et al., 1990; Urbain et al., 1993; Higgins and Novak, 1997 b, c; Finlayson et al., 1998; Jorand et al., 1998; Wingender et al., 1999; Liao et al., 2001; Liu et al., 2001a; Liu and Fang, 2003).

EPS are composed of a variety of organic substances namely carbohydrates, proteins, humus-like substances, uronic acids and deoxyribonucleic acids (DNA). However, only limited information is available about the concentrations about humus-like substances, uronic acids and DNA. Although many studies have been performed about EPS in the last 40 years, there are still some conflicting results in the literature due to the following reasons: (1) Activated sludge is a complex process. The nature of the sludge and EPS are dependent on a large number of process variables such as type of wastewater, nutrient level, sludge retention time (SRT), dissolved oxygen, and reactor configuration; (2) The amount and composition of EPS for a given sludge are strongly dependent on the extraction methods. The lack of a standardized EPS extraction procedure makes comparison and interpretation of published

results difficult. However, it appears that EPS extracted from sludges treating municipal wastewater are likely to contain more EPS than those treating industrial and synthetic wastewaters (Liu and Fang, 2003).

## **2.2. Mechanisms of Bioflocculation**

Researches have been demonstrated that microorganisms produce biopolymers that are released to the exocellular environment, either by cell lysis, or by active transport. These extracellular biopolymers or extracellular polymeric substances (EPS) then form a matrix which encapsulates the microbes and aids in the aggregation of the microorganism, and floc formation. The mass of total EPS in a floc has been found to represent up to 80 % of the mass of activated sludge. Since bioflocs are primarily made up of EPS, it can be inferred that the interactions between the EPS will be important to bioflocculation along with the characteristics of the EPS (Sobeck and Higgins, 2002).

In general, bioflocs have been found to have a net negative charge, which is a result of functional groups present on the EPS. As a result of these negative charges in bioflocs, the role of cations in bioflocculation has been shown to be important (Sobeck and Higgins, 2002).

Cations are an integral part of the floc structure, linking separate biopolymer components together into a floc structure. The cation content of wastewater, especially the ratio of monovalent to divalent cations, has been shown to influence the properties of activated sludge flocs. A high

concentration of monovalent cations is usually associated with a weak floc structure and a large amount of biocolloids in the solution (Novak et al., 2003).

From all the cations, calcium and magnesium have been studied more often since they are more commonly found in the natural waters. There were contradicting reports on the effect of magnesium on the flocculation of several pure cultures isolated from activated sludge. Tezuka (1969) found that flocculation of *Flavobacterium* was dependent on the presence of both calcium and magnesium. On the other hand, Shimizu and Odawara (1985) found that flocculation of *Agrobacterium* was dependent only on magnesium, but not on calcium. Angelbeck and Kirsch (1969) observed that flocculation of *Zoogloea ramigera* was adversely affected by the presence of calcium and magnesium; the culture tended to grow in dispersion in the presence of cations, but to grow in flocs when both calcium and magnesium were removed by the addition of chelating agents (Liu and Fang, 2003).

Urbain et al. (1992) found that the amount of EPS<sub>P</sub> (protein component of EPS) tended to increase with concentrations of calcium or magnesium, probably due to the formation of EPS-cation complexes. Dignac et al. (1998) compared the amount of EPS extracted by two processes: sonication alone and sonication plus cation exchange resin adsorption. They found that the latter process extracted nearly 100% more EPS<sub>P</sub>, but only 20% more of EPS<sub>C</sub> (carbohydrate component of EPS), than the

former process. This implies that the cations tend to bind more proteins than carbohydrates (Liu and Fang, 2003).

Higgins and Novak (1997a) indicated that the relative ratios and concentrations of cations played a role in determining settling and dewatering characteristics. When present in the feed, cations became incorporated within the microbe-biopolymer network, creating a dense floc that was more resistant to shear. This resulted in an improvement in settling and dewatering properties and reduced conditioning requirements. Higgins and Novak (1997a) also expressed results parallel to those of Dignac (1998) by reporting that divalent cations appear to bind mainly protein within the floc matrix.

Sobeck and Higgins (2002) explained the contradictory results about the effects of cations on activated sludge floc structure by saying that short duration batch experiments did not provide useful data in determining the effect of changes in the cation concentration on floc properties, effluent quality and settling and dewatering properties. Instead, the most accurate method to ascertain these effects are through laboratory, pilot, or full-scale, continuous flow systems that are allowed to operate for at least 2-3 SRTs under the adjusted influent cation concentrations.

The studies of Sobeck and Higgins (2002) carried out with bench-scale, continuous reactors, indicates that the addition of magnesium to the influent results in improvements over control values in floc properties as

measured by SVI, CST, SRF, cake solids and floc strength. Further increasing the cation concentration resulted in further improvements.

### **2.2.1. Theories of Bioflocculation Related to the Cations Present**

Mineral cations tend to complex with EPS, affecting bioflocculation, settling, and dewaterability of the sludge. There are three main bioflocculation models: double-layer compression model, alginate theory and cation and polymer bridging models(Liu and Fang, 2003; Sobeck and Higgins, 2002).

The *double-layer compression model* was derived from the theory of DLVO (Derjaguin-Landau-Verwey-Overbeek), which was originally developed for colloidal particles. The double-layer theory describes charged particles as having a double layer of counter ions surrounding the particle. The first layer is referred to as the Stern layer which is comprised of a tightly associated layer of counter ions, and the second layer is referred to as the diffuse layer which is made up of less tightly associated counter ions. The concentration of ions in the diffuse layer decreases with distance from the particle surface until the concentration of ions equals that of the bulk solution. The result is an electric potential that develops around the particle. This double layer or cloud of ions surrounding the particle results in repulsion of adjacent particles and inhibits aggregation. As the ionic strength increases, the size of the double layer decreases, which decreases the repulsion between particles,



allowing short-range attractive forces to promote aggregation. The addition of cations to a solution would therefore result in an improvement in bioflocculation due to a decrease in the size of the double layer and the repulsive forces between particles (Liu and Fang, 2003; Sobek and Higgins, 2002).

*Alginate theory* is based on alginate, which is a polysaccharide produced by algae and bacteria and is typically made up of repeating mannuronic and guluronic acid residues. The unique composition of this polysaccharide results in the formation of alginate gels in the presence of calcium ions. This gel is typically referred to as the egg-box model, and is depicted in Figure 1. Several bacteria that are known to produce alginate such as *Azotobacter* sp. and *Pseudomonas aeruginosa* have been identified in activated sludge. This suggests alginate may be present in activated sludge. This model was proposed as a result of experiments that examined the role of calcium in microbial aggregation. Bruus et al., (1992) demonstrated that when high concentrations of sodium were added to activated sludge, this resulted in ion-exchange which displaced calcium from within the floc. This displacement resulted in deterioration in floc properties measured by supernatant turbidity and SRF. Since alginate aggregation is specific for calcium, the researchers inferred that calcium induced aggregation of alginate was important to the bioflocculation process. They also reported that the addition of magnesium caused a similar exchange of calcium within the floc and deterioration in floc properties. As a result, they concluded the biopolymer had greater affinity for calcium than magnesium which

supports the role of alginate in bioflocculation (Sobeck and Higgins, 2002).

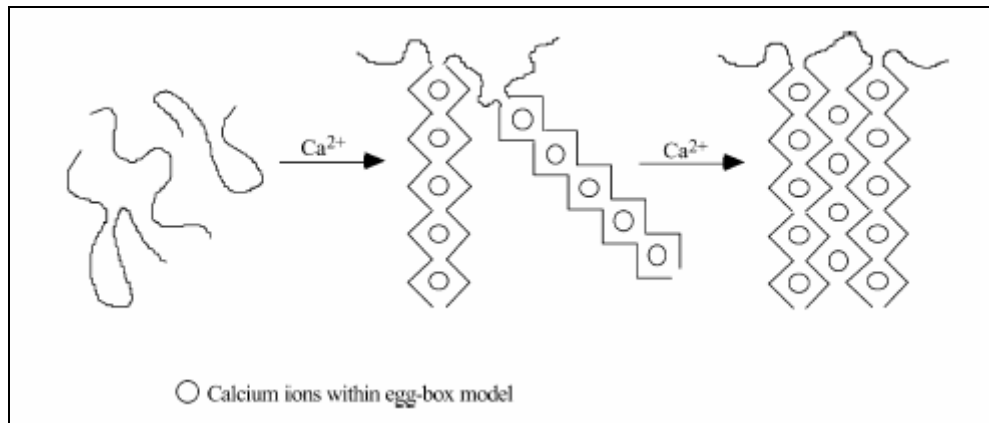


Figure 2.1. The egg-box model described by the alginate theory (Sobeck and Higgins, 2002)

There are several suggested models emphasizing the role of cations and polymers on the bridging mechanisms within the floc structure: cation bridging model, divalent cation bridging theory, and polymer bridging model. Although they have different names the suggested mechanism is the same.

In the *cation bridging model*, cations serve as a bridge between negatively charged EPS of neighboring microbial cells. The bridging stabilizes the floc network and thus improves sludge bioflocculation, settling, and dewaterability (Liu and Fang, 2003).

The role of divalent cations was demonstrated in experiments that examined floc formation during the growth of monocultures, and found that calcium and magnesium were important to the bioflocculation process. According to the Divalent Cation Bridging (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. A depiction of the divalent cation bridging model is shown in Figure 2. The alginate theory could be considered a subset of DCB theory, but divalent cations rather than the specific interaction and gel formation between calcium and alginate. The DCB theory has been supported by a work that demonstrated the addition of sodium caused a deterioration in the floc properties due to displacement of divalent cations from binding sites within the floc (Sobeck and Higgins, 2002).

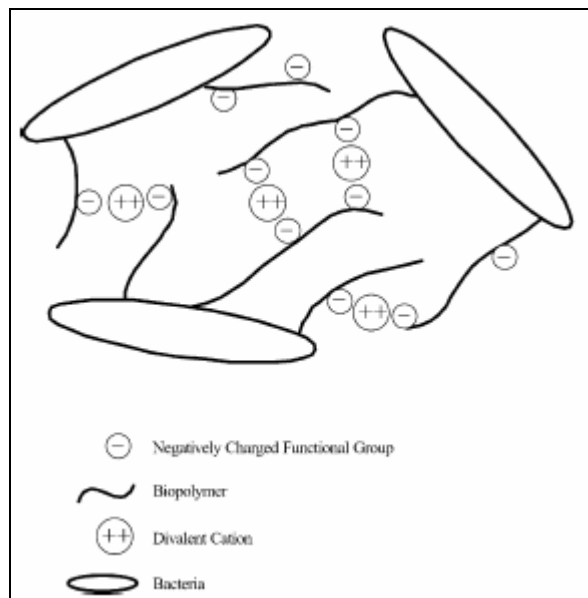


Figure 2.2. A depiction of the divalent cation bridging model (Sobeck and Higgins, 2002).

The polymer bridging model of coagulation developed by LaMer et.al (1957), Healy and LaMer (1962), and LaMer and Healy (1963). According to this theory, electrostatic surface charge reduction to zero takes place for flocculation. In order to achieve this, in the polymer bridging model, the EPS which contain different negatively charged groups, are bound together by means of divalent and/or trivalent cations. Therefore, large polymeric networks in which the different floc constituents such as single bacteria and bacterial colonies are embedded can be formed. This theory is supported by several studies where it has been shown that the removal of divalent and trivalent cations from the sludge flocs leads to deflocculation and desorption of macromolecules (Bruus et al., 1992; Higgins and Novak, 1997c; Wilén et al., 2003).

### **2.2.2. Other Mechanisms of Bioflocculation**

Besides the above theories Urbain et al. (1993) emphasizes the effect of *hydrophobicity* in the processes of bioflocculation and settling. Urbain et al.(1993) indicates that in such a highly hydrated systems as biological sludges, internal hydrophobic interactions are involved in flocculation mechanisms and their balance with hydrophilic interactions determine the sludge settling properties. Urbain et al. (1993) assumes that if the famous EPS are really needed in the floc structure, hydrophobic areas in between the cells act as essential adhesives. Generally, hydrophilic molecules are polar or charged while hydrophobic molecules are non-polar. Since non-polar molecules less readily mix in water compared to polar molecules, they should contribute to the binding together of the

sludge flocs (Jin et al., 2003). Significant correlations were observed by Wilén et al. (2003) between the flocculating ability and the hydrophobicity, indicating that the surface properties of the sludge flocs take a major role in the bioflocculation process. Detailed studies carried on the activated flocs by confocal laser scanning microscopy showed adhesion of hydrophobic, but not hydrophilic bacteria, inside flocs (Olofsson et al., 1998; Hermansson, 1999). Also, free-living bacteria in the effluent water that had not attached to the sedimenting flocs were shown to be predominately hydrophilic in situ, in wastewater (Zita and Hermansson, 1997; Hermansson, 1999).

Parker et al., (1972) has developed another theory to explain the mechanism of bioflocculation. The *filament backbone theory* is well-established theory suggesting that when flocs are built up, the bacteria and other floc constituents attach to each other by means of polymer bridging bonds. These aggregated particles then attach to the filaments, acting as a backbone in the floc, by polymer bridging bonds and enmeshment. The filaments are believed to provide the floc structure with an extra tensile strength. In the absence of filaments, the flocs may become weak and when exposed to shear they may easily break up which will lead to the formation of smaller flocs (Wilén et al., 2005).

Sezgin, et al. (1978) described activated sludge in two parts: macrostructure and microstructure. The microstructure is defined by process of microbial adhesion, aggregation, and bioflocculation. It is the basis for floc formation because, without the ability of one

microorganism to stick to another large aggregates of microorganisms such that exist in activated sludge would never form. The macrostructure of activated sludge consists of filamentous organisms that form the network within a floc onto which floc-forming bacteria cling. This network of filamentous organisms provides activated-sludge flocs with strength and the attainment of large size. As a consequence, their integrity is preserved in the aeration basin, where conditions of increasing shear occur in a turbulent environment (Jenkins et al., 1993). Urbain et al. (1993) stated that filamentous microorganism were present in all the activated sludge samples taken from different wastewater but they are not always associated with poor settling.

### **2.3. Activated Sludge Properties Related to Bioflocculation**

#### **2.3.1. Dewaterability**

Sludge dewatering is a paramount process in wastewater treatment systems as it reduces the volume of sludge and, consequently, the costs for transporting the sludge to its ultimate disposal site. Moreover, dewatered sludge is generally much easier to handle and transport. In addition, removal of moisture from sludge can increase the energy content for incineration, reduce the requirement for supplemental bulking agents during composting, and decrease the amount of leachate production in landfills (Lo et al., 2001).

However, one of the least-understood processes in wastewater treatment is sludge dewatering. The lack of understanding is largely due to the physical, chemical and microbial complexity of the sludge. Sludge dewatering could be affected by a number of EPS-related parameters, such as floc size, structure, and bound water content. It has been widely reported that the increase of EPS in sludge would lower the sludge dewaterability, based on the measurements of specific resistance to filtration (SRF), capillary suction time (CST), and floc strength (Liu and Fang, 2003)

Activated sludge is comprised of a microbial consortium and organic and inorganic matter held together in a matrix formed by extracellular biopolymer and cations. Although most of the biopolymer is incorporated into the activated sludge floc matrix, a portion remains unattached in solution as biocolloids. Studies have shown that these biocolloids are responsible for most of the polymer conditioning demand and deterioration of sludge properties is usually accompanied by the release of biopolymer from the floc (Novak et.al, 2003).

Recently, Houghton et al. (2001) found that dewaterability of activated sludge as is, increased with the EPS content until reaching the maximum level at 35 magnesium-EPS/g-SS, beyond which the dewaterability decreased. They concluded that the increase of dewaterability with EPS at low concentration was due to the enhancement of flocculation at low EPS levels. Flocculation resulted in increasing floc sizes, thereby improving the sludge dewaterability. The increase in EPS content above

35mg-EPS/g-SS tended to increase the amount of surface water bound by EPS, and thus lowered the sludge dewaterability (Liu and Fang, 2003).

In a study conducted by Durmaz and Sanin (2003), it was found out that up to a certain amount, EPS production improves the filtering property of the sludge leading to the conclusion that a certain concentration of EPS is needed for healthy flocculation. However, these results show that as important as the total quantity, the  $EPS_F/EPS_C$  ratio determines the dewaterability. As the  $EPS_F/EPS_C$  ratio decreases filterability was improved, up to a certain point. However, with a further increase in the production of carbohydrate type polymers (further decrease of  $EPS_F/EPS_C$ ) filterability deteriorated significantly.

Dewaterability is a property that is also affected by mineral cations present in the sludge. Many studies found that sludge containing high concentrations of calcium and magnesium in general had good settling and dewatering properties. Replacing divalent cations with monovalent cations in batch studies resulted in the deterioration of sludge settling and dewatering properties (Liu and Fang, 2003).

### **2.3.2. Rheology**

All fluids can be classified in terms of their flow properties, or their rheology. One common rheological measurement is viscosity, classically defined as the rate of displacement of a fluid with a given shear force. Consider a fluid between a fixed lower boundary and the upper



boundary which moves at a velocity  $u$ . This velocity is the result of a force  $\tau$  (shear stress) imposed upon the upper boundary. The velocity profile thus formed is linear for many fluids, with a slope of  $du/dy$ . This slope relates the shear force and displacement as:

$$\tau \propto \frac{du}{dy} \text{ or } \tau = \mu \frac{du}{dy} \quad (2.1.)$$

where  $\mu$  is the proportionality constant called the viscosity,  $\tau$  is the shear stress and  $du/dy$  is the shear rate.

Most fluids can be classified as Newtonian fluids which behave like the fluid above – that is, the shear stress is proportional to the displacement or the shear rate. However, many fluids do not behave like this. These fluids are called as non-Newtonian fluids. For example, the *plastic* fluid, which is a well-known type of non-Newtonian fluid, requires a certain finite shear force before it can be displaced. This shear, called *yield stress*, is another rheological property of that particular type of fluid. The shear rate- shear stress relationship for a plastic fluid may be expressed as:

$$\tau = \tau_y + \eta \frac{du}{dy} \quad (2.2.)$$

where  $\tau_y$  = yield stress

$\eta$  = plastic viscosity

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

$$\tau = K \left( \frac{du}{dy} \right)^n \quad (2.3.)$$

where K = fluid consistency index

n = a constant, known as the flow behavior index

Fluid consistency index, K, can be considered as the analogous term to viscosity whereas, flow behavior index, n, is less than 1 and shows the degree of deviation from Newtonian behavior.

Note that the Newtonian flow can be considered merely a special case of the power law, where n=1 and  $\eta=\mu$  (Vesilind, 1979).

A third category of non-Newtonian fluids is the dilatant fluids, which are encountered with less frequency. These fluids can be describes by the same equation as 2.3. the only difference is that the n value is greater than 1 for dilatant fluids. Newtonian as well as non-Newtonian fluids are shown in Figure 2.3. with respect to their shear stress-shear rate relationship.

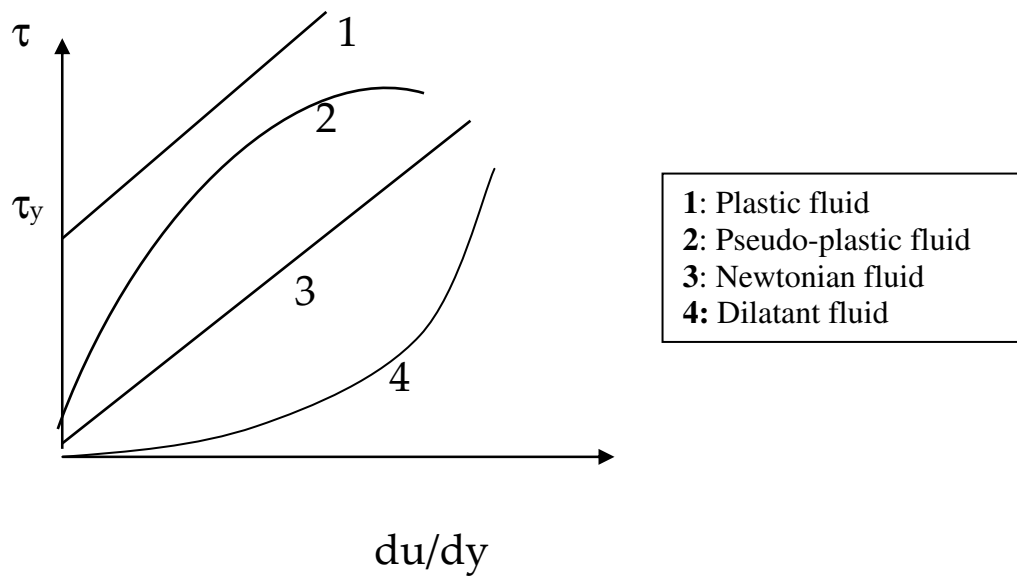


Figure 2.3. The rheograms for various fluids (Vesilind, 1979).

For non-Newtonian fluids, when the viscosity is expressed, the term apparent viscosity is used. Apparent viscosity is defined as, the ratio of shear stress to rate of shear of a non-Newtonian fluid (the viscosity) measured in poises. The apparent viscosity changes with changing rates of shear and temperature and must therefore, be reported as the value at a given shear rate and temperature (ChevronTexaco, 2005; Schlumberger, 2005).

Apparent viscosity was found to decrease with increasing conductivity of sludge. This was believed to be due to the compression of the electrical double layer around the particles that results in a more compact floc structure and coiled polymer conformation in and out of the flocs. Presence of extracellular polymers affected the viscosity of sludge as it was also suggested in earlier researchers. It was observed

that apparent equilibrium viscosity of activated sludge is reduced by the action of cellulose, indicating that the surface polysaccharides have a significant role in determining the rheological characteristics of activated sludge (Forster, 1983; Forster 1983). Removal of polymers by multiple centrifugation runs correlated well with the decrease in viscosity (Sanin, 2002). It was also reported by Forster (1983) that metal ions cause a decrease in bound water content and thus the sludge viscosity.

### **2.3.3. Settling**

Sedimentation process quickly became recognized as the weak link in the activated sludge treatment scheme when larger scale, continuous flow systems were brought on line. This is still the case today in that most effluent problems are associated with poor settling (Sobeck and Higgins, 2002).

The bulk research on sludge settlement has been predominantly based on the quantitative characteristics of the mixed liquor (e.g. flows and solids concentration), and has been mostly empirical. However, settleability is a very variable characteristic, strongly related to the structure and nature of the activated sludge floc which in turn depend on the conditions prevailing in the aeration basin. (Andreadakis, 1993).

Due to the adhesive nature of EPS (Fang et.al, 2000), flocs of activated sludge are stronger in adhesion than the mineral flocs (Li and Ganczarczyk, 1986). The adhesive nature could be due to several types of

interactions and mechanisms, including reversible adhesion, long range forces as described by DLVO theory, EPS interwinding (Pavoni et al.,1972), bridging by multivalent cations (Bruus et al. ,1992), and hydrophobic interactions (Urbain et al., 1993; Liu and Fang, 2003).

Most studies showed that sludge settled better with lower EPS. In most cases SVI increased linearly with the EPS content of the sludge (Urbain et al.,1993; Eriksson and Alm, 1991). However, in some cases there were either lack of correlation between SVI and EPS or that the former actually increased with the decrease of the latter (Liao et al. 2001; Jorand et al., 1998; Goodwin and Forster, 1985). Another study found that SVI increased with the EPS<sub>p</sub>, but had no correlation with EPS<sub>c</sub> (Martinez et al., 2000; Liu and Fang, 2003).

Mineral cations tend to complex with EPS, affecting bioflocculation, settling, and dewaterability of the sludge. There were contradicting reports on the effects of magnesium on the bioflocculation and settling properties of the sludge. Tezuka (1969) found that flocculation of *Flavobacterium* was dependent on magnesium only if it is accompanied by calcium. Endo et al. (1976) found that magnesium does not effect the flocculation of *Flavobacterium*. Yet, Shimizu and Odawara (1985) found that flocculation of *Agrobacterium* was dependent only on magnesium. Angelbeck and Kirsch (1969) observed that flocculation of *Zoogloea ramigera* was adversely affected by the presence of magnesium.

There are several types of settlement problems which are more or less directly related to the morphological and surface characteristics of the floc. These settlement problems are explained in Section 2.5. in detail.

#### **2.3.4. Hydrophobicity**

Hydrophobic interaction among microbial cells is crucial to the floc formation and sludge settling. Cell surfaces are known to have hydrophobic areas, and hydrophobic lipids or proteins could be trapped into the flocs. Bacteria in activated sludge may be either hydrophobic or hydrophilic. Hydrophobic bacteria produced lesser amounts of EPS than the hydrophilic ones (Jorand et al. 1994; Liu and Fang, 2003).

Sludge flocculation generally increases with the hydrophobicity of EPS, cells, and flocs. Sludge hydrophobicity may be affected by a number of operating parameters, including substrate, bacterial growth phase and conditions, oxygen, temperature, pH, ionic strength, and the presence of multivalent cations or phosphate (Mozes and Rouxhet, 1987; Liu and Fang, 2003).

EPS may influence surface charge and hydrophobicity of cells, and it consequently influences the bioflocculation and settling (Harris and Mitchell, 1973, 1975). Overall speaking, sludge hydrophobicity increases with the sludge EPS<sub>P</sub> content (Durmaz and Sanin, 2003; Sesay and Sanin, 2004) and an increased hydrophobicity generally leads to a better flocculation (Liu and Fang, 2003).

### **2.3.5. 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. Zeta potential, which represents the potential drop in the diffuse double layer on the surface, is measured based on the electrophoretic mobility in an electric field using a potentiometer. The results in the literature show that flocs and EPS of all activated sludge carried a negative charge mostly within the range of -0.2 to 0.6 meq/g-VSS with a zeta potential of -20 to -30mV (Liu and Fang, 2003; Liu et al., 2001b; Weemaes et al., 2000; Daffonchio et al., 1995).

## **2.4. Operational Conditions of Activated Sludge and Sludge Settlement Problems**

### **2.4.1. History of Activated Sludge and Sludge Bulking Problem**

In activated sludge, degradation of the quality of effluent discharged is most often due to problems in separating the biomass from the treated water in the final clarifier. Poor separation results most frequently from sludge bulking (Pernellé et al., 2001). Sludge bulking is a state when the sedimentation rate of the activated sludge is low and the compaction of activated sludge flocs is poor (Novak et.al, 1993).

The negative influence of filamentous microorganisms on sludge settling is well-known. Their control is still very difficult to achieve because of the diversity of species and thereby of environmental parameters which influence their (over)growth. This biological problem hides the fact that there are few information on the physicochemical structure of activated sludge floc and its relationships to the sludge settling capacity (Urbain et al.,1993)

### **2.4.2. Types of Activated Sludge Bulking**

Mainly two types of sludge bulking have been described in the literature. First one is the more common filamentous bulking, the second one is the viscous or non-filamentous bulking.

#### **2.4.2.1. Filamentous Bulking**

Filamentous microorganisms are an essential part of the floc population in the activated sludge process. The filaments form the backbone to which floc-forming bacteria adhere (Sezgin et al., 1978; Madoni et al., 2000).The most common sludge bulking problem is caused by an excessive growth of filamentous microorganisms. The effect of filamentous organism on floc structure is as follows: filaments stretch out the flocs (diffused open structure) or form a bridging structure. In both cases the floc structure is expanded and filaments mechanically prevent activated sludge flocs from well compaction (Novak et al., 1993).



Filamentous microorganisms are slow-growing organisms that can be characterized as having maximum growth rates ( $\mu_{\max}$ ) and affinity constants ( $K_s$ ) lower than non-filamentous organisms (Martins et al., 2003). However, as Chudoba et al.(1973) hypothesized, when the substrate concentration is low (typically  $C_s < K_s$ ) filamentous bacteria have a higher substrate uptake rate than floc formers, and thereby, win the competition for substrate. This theory by several studies point out that the feeding pattern had a strong influence on the kinetics, and on the population dynamics of activated sludge.

However, until now no one has unequivocally shown that the filamentous bacteria have in general a lower maximal growth rate than other bacteria present in the sludge. Moreover, there is no theoretical explanation why a filamentous morphology would lead to a lower growth rate. Another theory proposed to explain the filamentous sludge bulking mentions that morphology of filamentous bacteria aid in substrate uptake under low nutrients or oxygen concentrations. Till the early 1970s, the competition between filamentous and non-filamentous bacteria was based on the fact that the surface-to-volume ( $A/V$ ) ratio is higher for filamentous bacteria (Pipes. 1967). Especially, at low substrate concentration this high  $A/V$  ratio gives advantages to the organisms since the mass transfer to the cells with a high  $A/V$  ratio is more facilitated. At lower substrate concentrations this would lead to a relatively higher growth rate (Martins et.al, 2004).

Martins et.al (2003), hypothesized that filamentous bacterial structures originate from the presence of substrate gradients in sludge flocs. At low bulk liquid substrate concentration filamentous bacteria or filamentous bacterial structures give easier access to the substrate at the outside of the flocs and thereby, proliferate. At high bulk liquid substrate concentration there is no substrate advantage for filamentous organisms and smooth bacterial structures predominate.

Surveys of plants with this type of operational problem have made it possible to list several factors which encourage bulking: oxygen deficiencies, nutrients deficiencies, presence of reduced sulphur compounds, decrease in food/micro-organisms ratio (F/M), and wastewater composition (Duchéne, 1994; Jenkins et al., 1993; Gaval and Pernelle, 2003).

#### **2.4.2.2. Non-filamentous (Viscous) Bulking**

Another type of activated sludge bulking is called non-filamentous bulking. It is also called viscous bulking or zooglear bulking and it is predominantly caused by an excessive growth of *Zooglea*-like microorganisms. In some cases other microorganisms producing slime substances can cause similar irregularities (poly-P bacteria). If the zooglear microorganisms are present in large amounts, the exocellular slime causes the sludge flocs to have a voluminous character and polymeric compounds produced by microorganisms which form the exocellular slime usually contain a lot of water. Horan and Shanmugan

(1986) also reported that viscous bulking can be linked to the extracellular polymers with plentiful hydration water and it is considered as the result of the excessive production of microbial polymers due to certain stimulating environmental conditions. In fact, the nature and concentration of nutrients affect the biodegradation of organic waste as well as the composition of EPS (Bura et al., 1998; Durmaz and Sanin, 2003).

## **2.5. Effect of Nutrient Deficiency on Activated Sludge Bulking**

Deficiency of nutrients has been shown to cause both filamentous and viscous bulking. In one of those studies with sufficient phosphorus source, the effects of nitrogen deficiency on activated sludge bulking were studied in an SBR fed with brewing process wastewater (Peng et al., 2003). The experimental results show that the sludge settled properly at an influent BOD/N value of 100/4. When the value of BOD/N was 100/3, filaments had an excessive growth at one time during the reaction process. Afterwards, the number of filamentous bacteria began to reduce and simultaneously an excessive growth of viscous zoogloea with high percentage of moisture was observed and non-filamentous activated sludge bulking occurred. When the influent BOD/N value was 100/2, the excessive growth of filamentous microorganisms was not observed at all and the sludge characterization was similar to the case in which BOD/N value was 100/3. When the value of influent BOD/N was 100/0.94, a more serious non-filamentous bulking occurred (Orhon and Artan, 1994). Parallel to these findings, Durmaz and Sanin (2003) found that

C/N ratio measured as COD/TKN greatly affects the EPS production such that as the C/N ratio increases, carbohydrate fraction in EPS increases. This situation caused viscous bulking at C/N ratio of 43, which is the highest C/N ratio studied in this work.

These findings indicate that under certain nutrient limitation (in the presence of excessive carbon source) overproduction of extracellular polymers was observed. This situation leads to the non-filamentous type of bulking.

In conventional biological treatment processes, organisms use phosphorus during cell synthesis, maintenance, and energy transport. As a result, 10 to 30% of the influent phosphorus is consumed by organisms for their metabolic processes depending upon the sludge age, the COD/P ratio in the influent and the sludge handling and treatment methods (Orhon and Artan, 1994; Gerardi, 1994)

The experimental studies for optimization of chemical pre-precipitation by Ericsson and Eriksson (1986) included as an important part, the influence of low soluble phosphorus concentrations on the activated sludge. During extreme pre-precipitation conditions the sedimentation characteristics of the activated sludge deteriorated with a very marked increase of sludge volume index and growth of filamentous organisms. Specialized laboratory studies showed that wide variations of pre-precipitation dosages used in the plant trial operation apparently caused shifts in microbial population. This was indicated by variations in the

amount of filamentous bacteria. Ericsson and Eriksson (1986), concluded that easily assimilated phosphate depletion favors filamentous organisms' growth.

## CHAPTER 3

### MATERIALS AND METHODS

#### 3.1. Experimental Setup and Reactor Operation

Semi-continuous lab-scale activated sludge reactors were operated during this study. The microbial seed for the start up of the reactors was obtained from the effluent of primary settling tank of Ankara Central Wastewater Treatment Plant. The reactor setup for both of the bulking experiments in the phosphorus deficient conditions and sludge characteristics investigations under the influence of magnesium ions is as shown in Figure 3.1. For both parts of the study, reactors with a volume of 2 liters were used. A continuous supply of oxygen was maintained by the use of air pumps which made sure that the dissolved oxygen concentration in the reactor medium has never dropped under 3 mg/L. The air pumps have also maintained the mixing in the reactor. The sludge age for the reactors was 8 days. This was maintained by wasting 250 mL of sludge from each reactor everyday. Distilled water was used

to prepare the reactor medium and also the synthetic feed solutions. The temperatures of the reactors were kept around 20°C.

Reactors were fed daily following the procedure explained here. After wasting 250 mL of sludge from the reactors, they were left for settling for 2 hours. Then their supernatants were siphoned off and new feed solutions were added on to the settled sludge. The remaining volume was filled with distilled water to 2- liter level. The air pumps were installed again and the reactors were left in these circumstances until the next day.

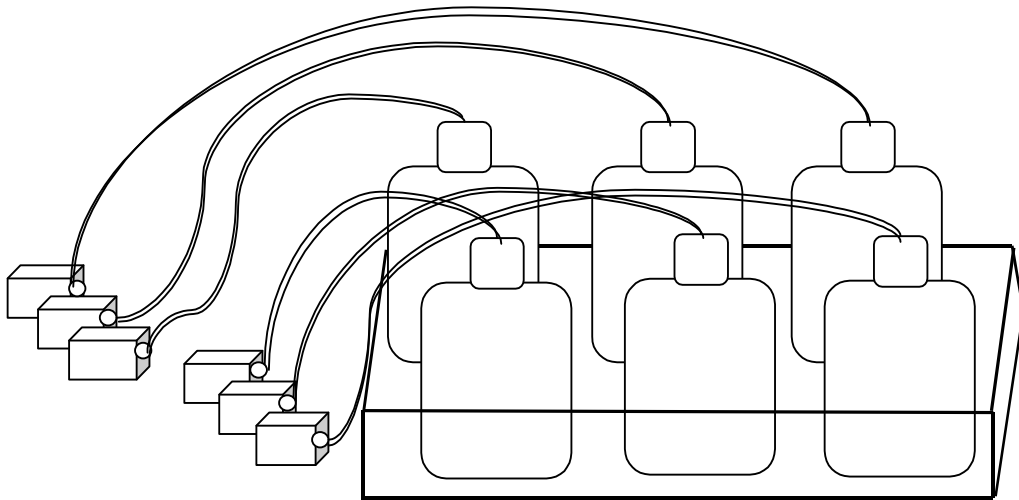


Figure 3.1. A schematic representation of the reactor setup

### 3.1.1. Phosphorus Deficient Conditions

As the laboratory studies proceed, a problem, which contributed to this study as a side topic of investigation, had occurred. Inorganic phosphate salts that are in the original feed medium formulation were omitted from the feed to prevent precipitation due to high magnesium concentrations studied. Considering peptone which is in the feed contains some phosphorus, reactors were started with no inorganic phosphorus salt addition. However, the phosphorus content of the peptone was not enough and the deficiency of the phosphorus in the feed media caused a serious sludge bulking problem in the reactors. Owing to this fact it is decided to investigate the nature of this sludge bulking problem.

Semi-continuous lab-scale activated sludge reactors were fed with the composition of synthetic wastewater adapted from AEEP(1972) and shown in Table 3.1.

Three semi-continuous reactors were operated at different magnesium ion concentrations: 5, 10 and 20 meq/L of magnesium ion concentration. The C/N ratio of the feed to the reactors was 21 in terms of the ratio of COD to TKN.



Table 3.1. The composition of synthetic feed added to the reactors for phosphorus deficient conditions (AEEP, 1972)

| Constituent                          | Concentration (mg/L) |
|--------------------------------------|----------------------|
| Glucose                              | 935                  |
| NH <sub>4</sub> Cl                   | 225                  |
| FeSO <sub>4</sub> .7H <sub>2</sub> O | 3.8                  |
| ZnSO <sub>4</sub> .7H <sub>2</sub> O | 3.8                  |
| MnSO <sub>4</sub> .H <sub>2</sub> O  | 2.3                  |
| NaHCO <sub>3</sub>                   | 180                  |
| CaCl <sub>2</sub>                    | 15                   |
| Peptone                              | 200                  |
| Tris buffer                          | 18.2                 |
| HCl                                  | 27.4                 |

Since the reactors are operated to investigate (the effect of phosphorus deficiency and) the influence of magnesium ions, the phosphate buffer had been eliminated from the feed medium initially. Instead, Tris buffer is added to keep the reactors at around pH=7. Tris buffer is hardly a biodegradable substance having a BOD<sub>5</sub> value of 0.8 mg/L. The only phosphorus source in the medium is the peptone used. The analysis of peptone showed that the reactors received 0.6 mg/L of phosphorus, which showed that the reactors are operated at a severe phosphorus limited situation.

The reactors were brought to steady state and the analyses of the reactors were conducted. To investigate the effects of nutrient deficiency on sludge bulking, sludge volume index (SVI), total suspended solid (MLSS), and volatile suspended solid (MLVSS) concentrations were determined. For the chemical investigation of the sludges, experiments were run to determine chemical oxygen demand (COD), carbohydrate content, and nitrogen, and phosphorus concentrations. Sludge flocs were examined under microscope and the photomicrographs were taken.

After the initial assessments and demonstration of bulking situation, it is decided to continue the investigation with only 10 meq/L reactor because of the similar results that were exhibited by all reactors and the 5 and 20 meq/L reactors were discarded. The bulking was attempted to be cured by increasing the concentration of phosphorus in the feed medium first to 6 mg/L and then to 12 mg/L in two steps. The phosphorus was added as  $\text{KH}_2\text{PO}_4$  to the reactor. To summarize; the study was conducted as 3 series of experiments as shown in Table 3.2.

The chemical precipitation because of the reactions of phosphate salt with the cations thought to be insignificant since the MLVSS/MLSS ratio has shown values quite close to 1 and exhibit a slight drop in the first series to 0.9 at the end of the second series of experiments. Following each increase of phosphorus, reactors were run for at least 3-4 times of  $\theta_c$  to achieve steady state conditions. After the second increase, the wastewater had sufficient phosphorus to supply the stoichiometrically required amount (C:N:P ratio of 100:5:1).

Table 3.2. A summary of experimental pathway

|   | <b>PO<sub>4</sub>-P concentration<br/>in the feed medium<br/>(mg/L)</b> | <b>Corresponding<br/>COD:N:P ratio</b> |
|---|---|--|
| <b>1<sup>st</sup> Series of Experiments</b> | 0.6   | 100:5:0.05                             |
| <b>2<sup>nd</sup> Series of Experiments</b> | 6   | 100:5:0.5                              |
| <b>3<sup>rd</sup> Series of Experiments</b> | 12  | 100:5:1                                |

It is also important to mention that in the 1<sup>st</sup> series of experiments of the phosphorus deficient operation, because of the bulking situation, there may have been some fluctuation (decrease) in the mean cell residence time ( $\theta_c$ ) value at which the reactors were operated. This must have been occurred due to the unintentional wastage of sludge during the siphoning process of supernatant. Unfortunately, it is not possible to estimate the exact change in the  $\theta_c$  value. It can only be said that the reactors were handled with extreme care in order to avoid great fluctuations in the  $\theta_c$  of the system.

### **3.1.2. Phosphorus Sufficient Conditions**

Four different concentrations of Magnesium (0.9, 5, 10, 20 meq/L) were examined in semi-continuous activated sludge reactors having 8 days of sludge residence time. The control reactor was containing necessary minimum amount of Magnesium (0.9 meq/L). The reactors had a working volume of 2 liters and fed with a synthetic feed medium, composition of which Table 3.3 and adapted from AEEP (1972).

Table 3.3. The composition of synthetic feed added to the reactors for phosphorus present conditions (AEEP, 1972)

| <b>Constituent</b>                   | <b>Concentration (mg/L)</b> |
|--------------------------------------|-----------------------------|
| Glucose                              | 163.5                       |
| Biosate Peptone                      | 942.5                       |
| NH <sub>4</sub> Cl                   | 225                         |
| FeSO <sub>4</sub> .7H <sub>2</sub> O | 3.8                         |
| ZnSO <sub>4</sub> .7H <sub>2</sub> O | 3.8                         |
| MnSO <sub>4</sub> .H <sub>2</sub> O  | 2.3                         |
| CaCl <sub>2</sub>                    | 15                          |
| NaHCO <sub>3</sub>                   | 180                         |

The required amount of MgCl<sub>2</sub>.6H<sub>2</sub>O addition in order to obtain 5,10, and 20 meq/L magnesium ion concentrations are 507.5, 1015, and 2030 mg/L, respectively. By this addition we obtain a magnesium concentrations of 60 mg/L, 120 mg/L, and 240 mg/L for 5, 10, and 20 meq/L reactors, respectively.

The required phosphorus was supplied from biosate peptone. No constituent was added to adjust the pH since the pH values were in the required range of  $7.7 \pm 0.3$  without the addition of a buffer solution. The system temperature was kept at 20°C. The dissolved oxygen (DO) concentration in the reactors adjusted to be minimum at 3 mg/L at all times. The reactors were first brought to steady state which was followed by measuring the mixed liquor suspended solids (MLSS), and mixed

liquor volatile suspended solids (MLVSS) concentrations. Obtaining the same results for these parameters during 4-6 successive days indicated that the steady state had been reached and the necessary analyses were conducted.

### 3.2. Analytical Methods Used

#### 3.2.1. Phosphorus Deficient Reactors

Physical and chemical analyses that are conducted are given in Table 4 below. All the analyses, except carbohydrates, are conducted according to Standard Methods (1995). Carbohydrate analysis was done with phenol sulphuric acid method (Dubois, 1956).

Table 3.4. Experimental analysis matrix for phosphorus deficient conditions

| Parameter           | <i>Measured in feed<br/>wastewater</i> | <i>Measured in sludge<br/>solids</i> |
|---------------------|--|--------------------------------------|
| SVI                 |  | X                                    |
| MLSS                |  | X                                    |
| MLVSS               |  | X                                    |
| Total carbohydrates |  | X                                    |
| Phosphate           | X                                      | X                                    |

### 3.2.1.1. Carbohydrate Analysis

A pretreatment procedure developed by Yurteri (1982) was applied to the bacterial culture from the phosphorus deficient reactors in order to burst the cells and extract their carbohydrate contents before carbohydrate analysis. The purpose of this analysis is to break all the floc structure and burst the cells in order to make sure all the carbohydrate content was carried to the bulk solution. After this the carbohydrate content carried into the solution was detected by using the method proposed by Dubois (1956). Although it is verified for protein analysis (Durmaz, 2001), this method was not verified for carbohydrate analysis and it is not known what percent of the carbohydrate in the cell structure is brought to bulk solution by using this method. It is employed in this section in order to obtain a general idea about the carbohydrate content of the sludge.

For this purpose, 5 mL sludge which is taken from the reactor medium was centrifuged at 3500 rpm for 15 minutes and the centrates were discarded. The 25 mL 1N NaOH was added into the tube containing the bacterial cells and vortexed for complete mixing. This mixture was boiled until the cells were burst and then centrifuged again. The centrates were used for carbohydrate analysis. Carbohydrate analysis was conducted according to the phenol sulfuric acid method which is described in detail in Section 3.2.2.1.1.

### **3.2.1.2. Nitrogen and Phosphorus Analyses**

The phosphorus analyses were carried out according to the ascorbic acid method given in APHA Standard Methods. Prior to this method, 50 mL of sludge sample were taken and centrifuged at 3500 rpm for 10 minutes. Then, the centrates were discarded and distilled water was added on the sludge sample to obtain a 50 mL sample to apply the ascorbic acid method in the APHA Standard Methods.

Nitrogen measurements were also done according to APHA Standard Method 4500.

### **3.2.1.3. Microscopic Analyses, MLSS, MLVSS, COD, DO and pH Analyses**

Microscopic analyses were carried out by taking photomicrographs by a Leica DFC 280 device.

MLSS, MLVSS and COD measurements were performed as described by Standard Methods. DO concentration was measured using a YSI model 51B DO meter and pH was measured by Jenway 3010 pH meter.

## **3.2.2. Phosphorus Sufficient Reactors**

### **3.2.2.1. Polymer Extraction and Detection Techniques**

According to some researchers EPS may be classified into two categories: dissolved and bound. The dissolved EPS are those that can be extracted by centrifugation alone, whereas the bound EPS are those required additional treatments. Such treatment processes may include heating, ultrasonication, homogenization, and the additions of cation exchange resin (CER), ethylenediaminetetraacetic acid, caustic, and sulfuric acid (Liu and Fang, 2003).

In this study CER extraction process was employed in order to obtain comparable results with previous studies (Durmaz and Sanin, 2001; Vatansever, 2005) in which CER extraction technique had also been used.

The CER extraction technique is different from the carbohydrate measurement technique of Yurteri (1982) which is described in Section 3.2.1.1. The aim of CER extraction technique is to extract the EPS bound only in the floc structure without bursting out the microbial cells.

Dowex 50X8 (20-50 mesh) strongly acidic cation exchange resin (CER) in Na-form supplied by Fluka was used during the application of a method similar to the one optimized and used by Durmaz and Sanin (2001). Prior to the extraction, in order to avoid leaching from the CER and subsequent interference in the chemical analysis of the extracellular



polymers, CER was washed by adding 1 kg of CER into 2 L phosphate buffer saline (PBS) solution and stirred for at least 1 hour. Afterwards, CER was dried at room temperature. The composition of PBS can be seen in Table 3.5.

Table 3.5. Composition of the Phosphate Buffer Saline (PBS) Solution

| <b>Constituents</b>              | <b>Concentration (mg/L)</b> |
|----------------------------------|-----------------------------|
| NaCl                             | 4000                        |
| KCl                              | 100                         |
| KH <sub>2</sub> PO <sub>4</sub>  | 60                          |
| Na <sub>2</sub> HPO <sub>4</sub> | 455                         |

In carrying out the extractions, for each reactor 200 mL of the daily wasted sludge was taken, divided into two, and centrifuged at 3500 rpm for 15 minutes. The centrates were discarded and the pellets were resuspended to 100 mL each by the addition of PBS solution within the jar test beakers. Into one of the beakers, previously washed CER was added at a dose of 100gCER/gVS, which is a dose determined to be optimum by Durmaz and Sanin (2001). The other beaker, which was containing sludge only, was used as a control so that there was no CER addition into this reactor. A second control for each reactor is a CER sample mixed with PBS up to 100 mL without the addition of sludge. Therefore, for each reactor there were 3 jar test beakers two of which were controls.

On these 3 jars belonging to the same sludge, polymer extraction process was initiated immediately, by using a jar test apparatus operating at a constant stirring speed of 120 rpm for 5 hours.

Following the polymer extraction process protein and carbohydrate components of the EPS were measured. For this purpose, the liquid fractions remaining after the CER extraction process were collected from the extraction beakers and they were centrifuged at 3500 rpm for 15 minutes. The carbohydrate and protein measurements were realized by using samples taken from the centrates according to the Dubois and Lowry methods which are described in detail in Section 3.2.2.1.1. and Section 3.2.2.1.2.

#### **3.2.2.1.1. Carbohydrate Measurement (by Dubois Method)**

Phenol sulfuric acid method developed by Dubois et al. (1956) was applied in order to measure the carbohydrate content of the EPS. The principle of the method is the spectrophotometric measurement of color formed upon the addition of phenol (50  $\mu$ L) and sulphuric acid (5 mL) into a sample (2 mL) containing sugar in the test tubes. The absorbance of the characteristic yellow-orange color was then measured at 480 nm by using Milton Roy Company Spectronic 20D.

In accordance with the Dubois method, the carbohydrate concentrations were measured by taking triplicate 2 mL samples for each reactor into

test tubes. After the addition of sulfuric acid and phenol, the samples were allowed to stand for 10 minutes. Then they were vortexed and put into 30°C incubator for 15 minutes and finally the absorbance of the samples were measured. A previously prepared calibration curve by using alginate as the standard was then used to calculate the concentration of carbohydrate in each sample. This calibration curve is presented in Appendix A.

#### **3.2.2.1.2. Protein Measurement (by Lowry Method)**

The protein content of the EPS was also detected using folin phenol reagent method developed by Lowry et al. (1951). The main principle of this method is the spectrophotometric detection of the blue color which is produced due to the reaction of proteins with copper ion in alkaline solution and the reduction of the phosphomolybdate-phosphotungstic acid in the Folin reagent by the aromatic amino acids in the treated proteins (Liu and Fang, 2003).

For the application of the Lowry method, four different reagents were prepared at first:

- Reagent A was obtained by dissolving 2 % w/v sodium carbonate in 0.1 N NaOH
- Reagent B was obtained by dissolving 1 % w/v sodium potassium tartarate in 0.5 % w/v cupric sulphate

- Reagent C was obtained by mixing reagent B and A with a volumetric ratio of 1 to 49, respectively
- Reagent D was obtained by diluting Folin-Ciocalteu's phenol reagent with distilled water by the ratio of 10:9.

3 mL of reagent C is added onto 0.6 mL samples which were taken in triplicate for each reactor. Then, the samples left to stand at room temperature for 10 minutes. Reagent D is added afterwards, and it was made sure to mix well with the samples by vortexing. The samples were left to stand for 30 minutes at room temperature prior to the spectrophotometric analysis of the intensity of their blue color with a Milton Roy Company Spectronic 20D at 750nm. The concentrations were calculated based on the calibration curve prepared using Bovine Albumin as the standard reagent on the triplicate samples extracted from all reactors. The protein calibration curve is presented in Appendix A.

#### **3.2.2.2. Specific Resistance to Filtration**

The dewatering characteristics of the sludge samples were determined by using specific resistance to filtration (SRF) test. The SRF test was applied as described by Vesilind (1979). A Buchnel funnel apparatus as shown in Figure 3.2. was used for SRF test. A 50 mL sample was filtrated under vacuum and volume of filtrate withdrawn from the sludge sample was recorded at predetermined time intervals. Tests were conducted under a vacuum pressure of 21 in. Hg and a Whatman 40 filter paper were used during the filtrations. Following the filtration, the solids

concentration in the cake deposited on the filter paper was determined by gravimetric analyses. Time/volume versus volume graphs were plotted for each reactor. Specific resistance to filtration was calculated using the slope of this plot using the below formula:

$$r = \frac{2PA^2b}{\mu w} \quad (3.1)$$

where: P: Pressure difference, N/m<sup>2</sup>  
A: Filtration area, m<sup>2</sup>  
b: Slope of the t/V versus V plots, s<sup>2</sup>/m<sup>6</sup>  
 $\mu$ : Viscosity of filtrate, N/m<sup>2</sup>  
w: Weight of dry cake solids per unit volume of filtrate, kg/m<sup>3</sup>

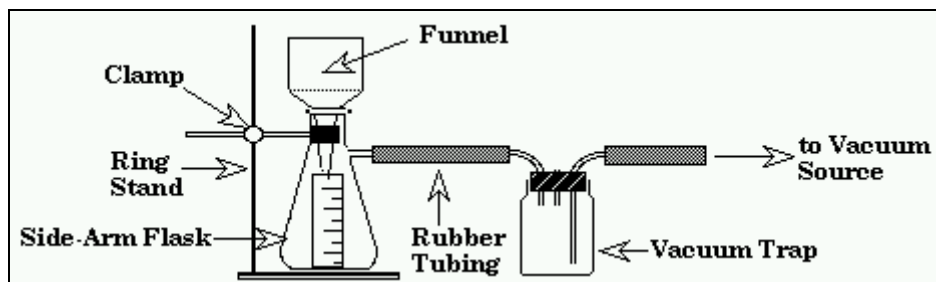


Figure 3.2. Buchner Funnel Apparatus (University of Maryland, 2005)

### **3.2.2.3. Zone Settling Velocity**

Sludges often can be characterized by how well they settle. Settling tests are commonly conducted in 1-liter cylinders. First the sludge is mixed to distribute the solids evenly. As the solids settle, a clear interface will emerge between the solids and the liquid, and the height of this interface can be recorded over time. (Vesilind, 1979).

After plotting sludge blanket height versus time graphs for each reactor's sludge the zone settling velocities were calculated from the slopes according to the method described by Metcalf&Eddy (1991).

The zone settling velocity experiments were carried out for 3 different MLSS concentrations for each reactor. The MLSS concentrations were determined to be the MLSS, 1.5\*MLSS, and 2\*MLSS for each reactor. After obtaining the zone settling velocities for 3 different concentrations, ZSV versus MLSS concentration graphs were plotted. These graphs are presented in Appendix C. Afterwards, the ZSV values at 3000mg/L were read from the ZSV versus MLSS concentration graphs and ZSV versus magnesium ion concentration graphs were plotted.

### **3.2.2.4. Sludge Volume Index**

The effectiveness of bioflocculation and settling of activated sludge is often characterized by the sludge volume index (SVI), which is defined as the volume (in mL) occupied by 1 g of sludge after 30 minutes of

settling in a 1-L cylinder. The lower the SVI, the denser the settled sludge and thus the better the settleability of the sludge. An activated sludge with a SVI between 40 and 120 is considered satisfactory, and over 150 is considered bulking (Liu and Fang, 2003).

In order to conduct an SVI analysis, a well-mixed 1L portion of the reactor contents were poured into a 1L graduated cylinder and left to settle down for 30 minutes. After that, the volume of the settled sludge is recorded. Also, a MLSS measurement is conducted parallel to SVI measurement. Finally, the SVI value is determined according to the below equation:

$$SVI = \frac{30 \text{ minutes settled sludge volume (mL/L)} \times 1000}{\text{suspended solids concentration (mg/L)}} \dots\dots(3.2.)$$

#### **3.2.2.5. Turbidity**

Supernatants at the end of 60 minutes settling were analyzed for their turbidity in NTU units by using Hach 2100A Turbidimeter which was previously calibrated with standard turbidity solutions.

#### **3.2.2.6. Viscosity**

Rheological properties of sludge including apparent viscosity were determined by using a rotational viscometer named Brookfield LVDVII+ with ultra low viscosity adapter. Due to non-Newtonian flow property of sludge, viscosity measurements were conducted at 6 different shear

rates – 1.83, 3.67, 7.34, 14.7, 36.7, and 73.4 sec<sup>-1</sup>. In addition to that, since the sludge viscosity is a function of solids concentration, measurements were conducted at 5 different solids concentration for each reactor. For each one of the 5 samples measurements were done at these 6 different shear rates. At the end, one fixed suspended solids concentration was chosen and the viscosity values of each reactor at this suspended solids concentration were compared.

### 3.2.2.7. Hydrophobicity

Among all methods for hydrophobicity measurements, Bacterial Adhesion to Hydrocarbons (BATH) is the simplest to conduct and thus most commonly used. In BATH method, a diluted sludge sample is mixed with a hydrophobic organic solution. The hydrophobicity is measured from direct optical density (OD, normally measured at 600 nm) between the mixed solution and the control. However, measurements of BATH are dependent on the choices of organic and aqueous phases. The common choices of organic phase are *n*-octane and *n*-hexadecane, and those of the aqueous phase are deionized water and the phosphate-urea-magnesium buffer solution (Liu and Fang, 2003).

In this study BATH method was applied by using *n*-hexadecane as the organic phase. The spectrophotometric analyses were conducted using Milton Rot Company Spectronic 20D spectrophotometer. Sludge samples from each reactors were washed with phosphate buffer saline solution (Table 3.5.) before the BATH test to minimize the electrostatic effects.



Absorbance of a 5 mL bacteria suspension (optical density) was adjusted to 0.4 as the initial OD at 600nm. Next 0.5 mL of *n*-hexadecane was added to the test tube containing the suspension and vortexed for 2 minutes. Following the 15 minutes of settlement, optical density was measured again at 600nm as a final optical density (Durmaz and Sanin, 2003). Hydrophobicity was calculated as:

$$\text{Hydrophobicity}(\%) = \left(1 - \frac{OD_{final}}{OD_{initial}}\right) \times 100 \dots\dots\dots(3.3.)$$

### 3.2.2.8. Surface Charge

The surface charges of the sludge samples were measured by using a Zeta Sizer Nano Serves ZS90 device.

### 3.2.2.9. Ion Measurement

Magnesium ion measurements were carried out both on the sludge samples. For this purpose, the sludge samples were passed through a microwave assisted digestion process. Sludge samples were dried in the furnace at 103 °C for 24 hours. A sample of 0.25 grams of each of dried sludge weighed in cleaned Teflon vessels. For the microwave digestion of sludge samples, 5 mL of hydrofluoric acid (40% w/v) and 5mL of nitric acid (65% w/v) were added into vessels. Then the microwave digestion process which consists of the 3 steps below, took place:

- Step 1: 40 minutes at 200 °C under 800 Watt power

- Step 2: 25 minutes at 100 °C under 400 Watt power
- Step 3: 1 minutes at 20 °C under 40 Watt power

After the completion of the digestion procedure in microwave, both the final solutions of sludge and supernatant samples were boiled till the final volume was reduced to near dryness and then diluted to a total of 25 mL. Then the determination of the metal ion was carried out by ATI Unicam 929 Atomic Absorption Spectrometer with electrode discharge lamps.

#### **3.2.2.10. Other Measurements**

MLSS, MLVSS, COD, DO, and pH measurements were conducted as described in Section 3.2.1.2.

## CHAPTER 4

### RESULTS AND DISCUSSION

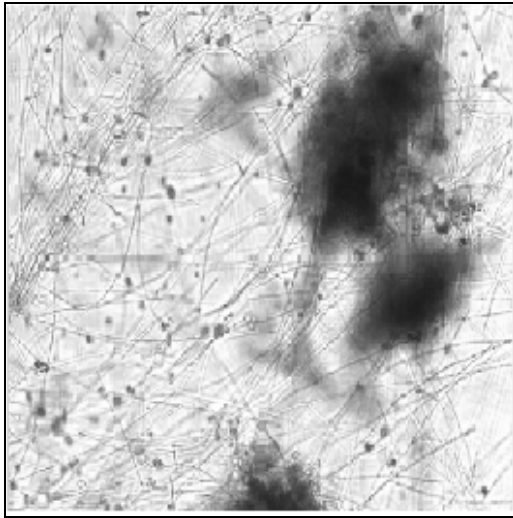
#### 4.1. Results from the Reactors Operated at limited Phosphorus Concentration

##### 4.1.1. Results of 1<sup>st</sup> Series of Experiments

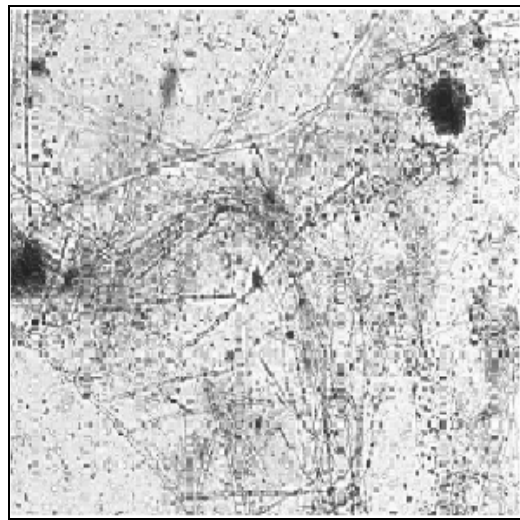
The initial results indicated that avoiding phosphorus in the feed to the reactors led to a serious sludge bulking problem in 5,10, and 20 meq/L reactors and this was attributed to the insufficient amount of phosphorus in the medium. This became obvious even in the first two weeks of the reactor operation. SVI values were extremely high for all the reactors.

An examination of sludges under microscope indicated that the sludges grown under magnesium rich conditions exhibit the same properties independent of the cation concentration studied. Figure 4.1. a, b and c shows the photomicrographs of 5, 10, and 20 meq/L magnesium fed sludges, respectively. As given in the photomicrographs, sludges grown under magnesium rich conditions show filamentous bulking with plenty

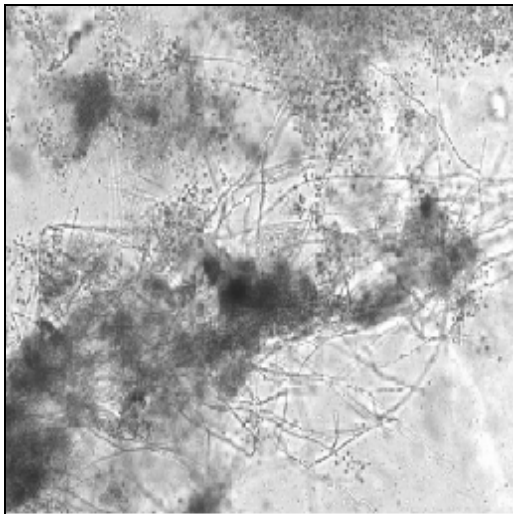
of filamentous microorganisms present. Because of this 5 and 10 meq/L reactors were discarded and for the rest of the study, 10 meq/L magnesium reactor was the only reactor run for the magnesium effect set under phosphorus deficient conditions and the related sludge analyses were done with the sludge from this reactor only. Therefore, from these three magnesium reactors which were operated at 5, 10, and 20 meq/L, Table 4.1. has only the results of 10 meq/L of magnesium.



a.



b.



c.

Figure 4.1. Sample picture a. from 10 meq/L Magnesium fed sludge in Phosphorus deficiency, b. from 5 meq/L Magnesium fed sludge in phosphorus deficiency, c. from 20 meq/L Magnesium fed sludge in phosphorus deficiency

Results of other parameters measured during the phosphorus deficient operation of the reactors when the sludge bulking was experienced are all given in Table 4.1.

Table 4.1. The results for the phosphorus deficiency study at the 1<sup>st</sup> stage

| <b>Parameter</b>                                   | <b>Stages of the Study</b> |
|--|----------------------------|
|  | <b>1<sup>st</sup></b>      |
| Calcium in the feed (meq/L)                        | 0.3                        |
| Magnesium in the feed (meq/L)                      | 10                         |
| PO <sub>4</sub> -P(mg/L)                           | 0.412                      |
| MLSS(mg/L)   | 675                        |
| CARBOHYDRATE(mg/L)                                 | 57.94                      |
| SVI (mL/g)   | 1036                       |
| CARBOHYDRATE (as the % of overall cell mass)       | 8.6                        |
| PO <sub>4</sub> -P (as the % of overall cell mass) | 0.061                      |

As presented in Table 4.1., SVI value of the reactor at the end of the 1<sup>st</sup> series of experiments is 1036 mL/g, indicating that a severe sludge bulking problem is encountered. This sludge bulking problem was attributed the phosphorus deficient conditions in the reactor medium. Since the phosphate buffer was not added to the medium, the phosphorus present in peptone alone could not supply the required amount according to the required C:N:P ratio of the system. So it was obvious that a severe phosphorus limitation caused activated sludge bulking.

In the phosphorus deficient conditions, results in Table 4.1. indicate that the system have a very low MLSS concentration. This is believed to be due to nutrient deficient growth (P-limited) in the reactor and bulking.

The results of the  $\text{PO}_4\text{-P}$  analyses supports the considerations that the settling problems and the insufficient MLSS concentrations are a result of the lack of phosphorus in the floc structure.

Jenkins et al. (1993) indicated that if the type of the bulking is viscous, then the carbohydrates are expected to be at least as 20% in sludge composition. However, according to the carbohydrate measurement results in Table 4.1., the carbohydrate content of the sludge is 8.6%, which is a far lower value than 20%. Therefore, the carbohydrate results also support the findings about the type of the bulking encountered in this study is not viscous but filamentous bulking.

#### **4.1.2. Results of 2<sup>nd</sup> Series of Experiments**

The 2<sup>nd</sup> series of experiments were conducted following the physical/chemical analysis of the sludges in the first part. Phosphorus containing feed medium consisting  $\text{KH}_2\text{PO}_4$  was added to the reactor to provide the C:N:P ratio of 100:5:0.5. Continuous recording of SVI and MLSS were done. Table 4.2. represents the results of the analyses made at this second stage of study following the addition of phosphorus rich medium.

The SVI results of the 2<sup>nd</sup> series of experiments indicate that the sludge's settling characteristics improved as soon as the phosphorus concentration in the feed is increased (Table 4.2.). Figure 4.2. shows that the amount of filamentous microorganisms decreased significantly. As a result of this decrease flocs can easily attach to each other to form bigger flocs and settle out easily. Therefore, as represented by increase in the MLSS values, the microorganisms did not experience a wash out because of the poor settleability. Parallel to the increase in the amount of phosphorus in the reactor feed medium, the percentage of PO<sub>4</sub>-P in the total sludge mass also increased as presented in Table 4.2.

Table 4.2. The results for the phosphorus deficiency study at the 1<sup>st</sup> and 2<sup>nd</sup> stages

| Parameter  | Stages of the Study |                 |
|--|---------------------|-----------------|
|  | 1 <sup>st</sup>     | 2 <sup>nd</sup> |
| Calcium in the feed (meq/L)                        | 0.3                 | 0.3             |
| Magnesium in the feed (meq/L)                      | 10                  | 10              |
| PO <sub>4</sub> -P(mg/L)                           | 0.412               | 4.1             |
| MLSS(mg/L)   | 675                 | 2000            |
| CARBOHYDRATE(mg/L)                                 | 57.94               | 228.7           |
| SVI  | 1036                | 72              |
| CARBOHYDRATE<br>(as the % of overall cell mass)    | 9                   | 11              |
| PO <sub>4</sub> -P (as the % of overall cell mass) | 0.061               | 0.205           |



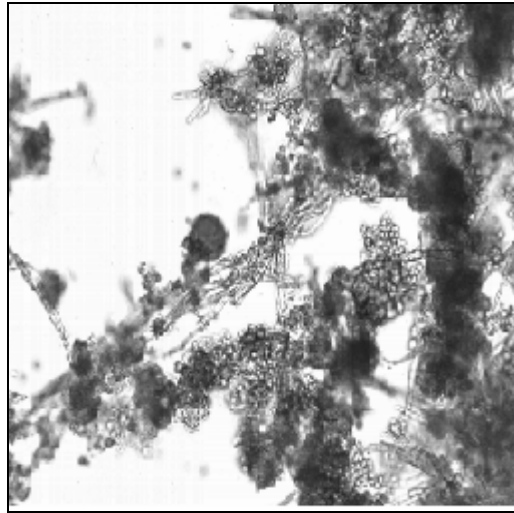


Figure 4.2. Sample picture from 10 meq/L Magnesium fed sludge when C:N:P is 100:5:0.5

While considering the results of 2<sup>nd</sup> series of experiments, a significant reason for the increase of sludge carbohydrate concentration can not be identified because the amount of carbohydrate in the feed medium remains the same as the first step. However, the increase in the amount of carbohydrate is smaller than 20% which indicates that viscous bulking situation is not of concern. In addition to that, it can be said depending on the SVI data that, this increase in carbohydrate does not have a negative impact on the settling characteristics of the sludge. To conclude, at this step of the study we could not find a correlation between settling properties and carbohydrate concentration of the activated sludge. In a study conducted by Andreadakis (1993), although a different carbohydrate measurement method was employed (anthrone method), it is also concluded that the carbohydrate content of the activated sludge flocs was not correlated to sludge settleability.

### 4.1.3. Results of 3<sup>rd</sup> Series of Experiments

The 3<sup>rd</sup> series of experiments were conducted after the phosphorus in the feed medium is increased to 12 mg/L, which is the stoichiometrically required value to obtain the C:N:P ratio of 100:5:1. The results can be observed from Table 4.3. The carbohydrate values decreased in all reactors compared to the results of first series of experiments. Also the amount and percentage values for PO<sub>4</sub>-P increased significantly. Considering the SVI values it can clearly be seen that there is no bulking problem anymore. In addition to that, when the photomicrographs are investigated an excessive growth of filamentous microorganisms is not observed at full phosphorus dose (Figure 4.3.).

Table 4.3. The results for the phosphorus deficiency study at the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> stages

| Parameter  | Stages of the Study |                 |                 |
|--|---------------------|-----------------|-----------------|
|  | 1 <sup>st</sup>     | 2 <sup>nd</sup> | 3 <sup>rd</sup> |
| Calcium in the feed (meq/L)                        | 0.3                 | 0.3             | 0.3             |
| Magnesium in the feed (meq/L)                      | 10                  | 10              | 10              |
| PO <sub>4</sub> -P(mg/L)                           | 0.412               | 4.1             | 8.2             |
| MLSS(mg/L)   | 675                 | 2000            | 2710            |
| CARBOHYDRATE(mg/L)                                 | 57.94               | 228.7           | 85.2            |
| SVI  | 1036                | 72              | 90              |
| CARBOHYDRATE (as the % of overall cell mass)       | 8.6                 | 11.4            | 3.1             |
| PO <sub>4</sub> -P (as the % of overall cell mass) | 0.061               | 0.205           | 0.303           |

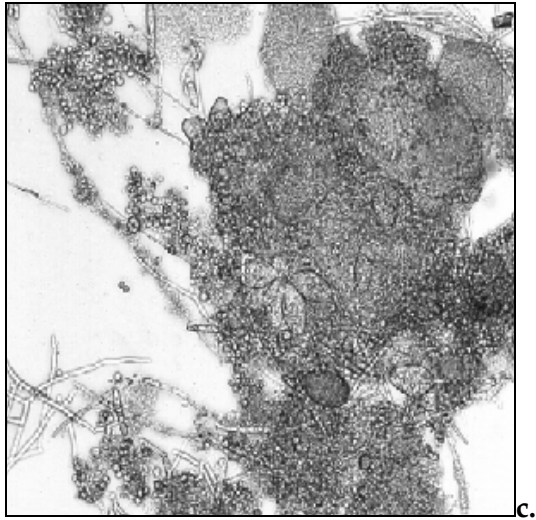


Figure 4.3. Sample picture from 10 meq/L Magnesium fed sludge when C:N:P is 100:5:1

At the end of the 3<sup>rd</sup> series of experiments, apart from the recovery in the settling properties of sludge, some granular structures can be seen in the photomicrographs. It is thought that these structures are the polyphosphate granules mentioned by many other authors in the literature. It is known from the literature that as a result of X-ray analysis, researchers found that metal ions are incorporated in the structure of volutin granules. Besides phosphorus, the metals calcium, magnesium and potassium were the most frequently detected components of polyphosphate granules. Schönborn et al (2001) indicated that the addition of extra calcium and magnesium ions into the influent wastewater of an enhanced biological phosphorus removal pilot plant improved P-elimination. This enhancement resulted in the formation of polyphosphate granules, which contains metals calcium, magnesium

and potassium besides phosphorus and the quantitative ratios of calcium, magnesium and potassium varied in dependence on the influent concentration of these metals. Polyphosphate granules are said to appear as spherical, dark, electron-dense bodies under electron microscope. In addition to that, Khoshmanesh et al.(2002) reported that under aerobic conditions when only acetate was available, Poly-P accumulating organisms take up phosphorus as polyphosphate granules inside the cell. In fact, in some cells Poly-P granules occupied almost the total cell volume. The primary purpose of the stored polyphosphate in most bacteria is that it serves as a phosphorus source for periods of phosphorus starvation (Wanner, 1994). Therefore, in this study, it is concluded that, once the microorganisms experience a starvation for phosphorus, they tend to store it as polyphosphate granules as soon as an adequate phosphorus source is provided.

When the changes in the  $\text{PO}_4\text{-P}$  amounts in the sludge is considered it can be seen that the  $\text{PO}_4\text{-P}$  in the sludge, as expressed in terms of mg/L, exhibits a parallel increase to the increase of phosphorus in the feed media. As the phosphorus in the feed increased 10 times during the 2<sup>nd</sup> stage the  $\text{PO}_4\text{-P}$  amount in the sludge also increased 10 times. The same is valid for the 3<sup>rd</sup> stage in which the  $\text{PO}_4\text{-P}$  in the sludge doubled following the increase in the phosphorus in the influent. However, the  $\text{PO}_4\text{-P}$  concentrations as expressed as the percentage of the overall cell mass do not exhibit a similar trend. The increases in the  $\text{PO}_4\text{-P}$  percentage in the cell mass are 3.4 and 1.5 times for the 2<sup>nd</sup> and 3<sup>rd</sup> stages, respectively. The reason for percentage values exhibiting a smaller

increase is the parallel increase in the MLSS values. Since the microorganisms found the adequate amount of nutrient and since the settling characteristics improved to prevent their washout with the effluent the MLSS values increased. This resulted in a slighter increase in the PO<sub>4</sub>-P values as % of the overall cell mass.

Another important point, which also explains why the research is completed by using only 10 meq/L Magnesium reactor, is that, there is no difference in the sludge properties observed under microscope at the first step between the reactors receiving different concentrations of magnesium ion as their feed water. Furthermore, according to the results of a similar topic studied with calcium ions, 5, 10, and 20 meq<sup>-1</sup> calcium reactors have shown the same behavior by means of sludge bulking and settling problems at each 3 steps of the research (Turtin et al., 2005) and so seemed the magnesium reactors from microscopic investigations at the end of the 1<sup>st</sup> series of experiments. This summarizes to that, above 5 meq/L cation concentration in the medium, sludge bulking and settling characteristics related to phosphorus deficiency do not differ depending on the influent cation concentration for Ca ions. A similar argument can be expected for magnesium ions.

## **4.2. Results from the Reactors Operated ad Phosphorus Present Conditions**

There were four sets of reactors operated under four different influent magnesium ion concentrations (0.9, 5, 10, 20 meq/L). The reactor with an influent magnesium concentration of 0.9 meq/L was the control reactor. After the reactors reached steady state, various physical and chemical analyses including SRF, ZSV, SVI, turbidity, hydrophobicity, viscosity, ion measurements and  $EPS_P$  and  $EPS_C$  were conducted on the sludge samples.

### **4.2.1. Effect of Magnesium Ions on Extracellular Polymer Composition**

When the effects of magnesium on protein and carbohydrate constituents of EPS ( $EPS_P$  and  $EPS_C$ ) are considered it can be said that both  $EPS_P$  and  $EPS_C$  increases as the magnesium ion concentration increases. This can clearly be observed from Figure 4.4. The data presented in Figure 4.4. are obtained by taking the average of two replicate reactors for each magnesium dose except for the 10 meq/L reactor which had no replicate.

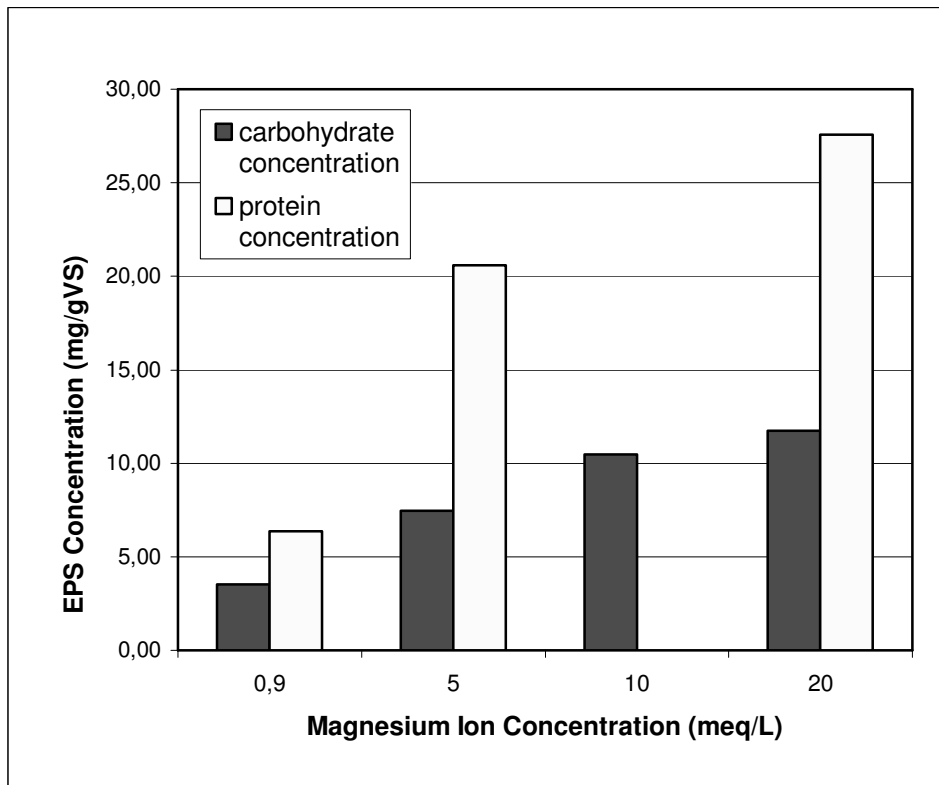


Figure 4.4. EPS<sub>P</sub> and EPS<sub>C</sub> results versus Magnesium ion concentration

Unfortunately no data could be obtained for protein concentration in Magnesium reactors at 10 meq/L ion concentration. A deterioration had occurred in that sludge due to experimental error for that part of analyses. Although there is a missing data for the EPS<sub>P</sub> value of the reactor being fed with 10 meq/L magnesium, it is still possible to observe an increasing trend for the EPS<sub>P</sub> data.

It is also obvious from the steeper increase of protein data in Figure 4.4. that EPS<sub>P</sub> is present in higher amounts in the total EPS when compared with EPS<sub>C</sub>. This result supports the findings of Dignac (1998) and Higgins and Novak (1997a), reporting divalent cations appear to bind

mainly protein within the floc matrix. The difference is that the studies by Dignac (1998) and Higgins and Novak (1997a) had been carried out using calcium and magnesium ions together and therefore to decide the effects of distinct ions is not possible from those results. On the other hand, now, it can confidently be said that magnesium ions definitely tend to bind more protein than carbohydrate within the floc structure. In a parallel study, calcium ions were found to bind selectively the carbohydrate fraction of EPS (Vatansever, 2005).

#### **4.2.2. Cation Measurements**

The aim of the cation measurements was to determine the magnesium ion concentrations incorporated into the floc structure. For this purpose microwave digestion and atomic absorption spectroscopy are employed. The results are presented in Figure 4.5. As the magnesium ion concentrations in the feed medium increase the magnesium ion concentrations incorporated into the floc structure also increase as can be observed from Figure 4.5. However, the increase of magnesium in the floc structure does not show a one to one parallel increase to the magnesium in the feed medium. For instance, from control reactor to the 5 meq/L reactor the magnesium concentration in the feed media increased 5 times but the magnesium concentration in the floc structure increased only by 1.5 times.



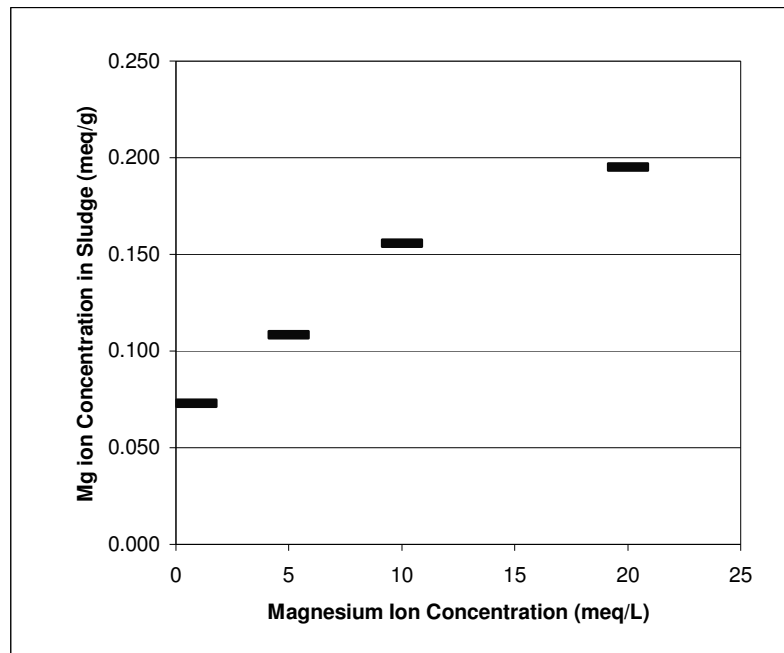


Figure 4.5 Magnesium ion concentration detected in the floc structure

On the other hand, a constant increase in the magnesium ion concentration within the floc structure can be observed. The EPS content of the sludge exhibits a parallel increase to the increasing magnesium ion concentration within the floc structure. However, a steeper increase in the  $EPS_P$  can be seen when compared to  $EPS_C$ . This concludes to that the affinity of magnesium ions to the protein type of polymers are higher than their affinity to carbohydrate type polymers.

### **4.2.3. Effect of Magnesium Ions on Physical and Chemical Properties of Activated Sludge**

#### **4.2.3.1. Dewaterability**

The dewaterability of activated sludge samples was determined by the SRF test. The SRF values were calculated according to Equation 3.1. and the slope values obtained from the  $t/v$  vs.  $v$  graphs that are presented in Appendix B. Figure 4.6. exhibits the results of these tests. A significant decrease in the SRF values can be seen as the magnesium concentration is shifted from 0.9 meq/L to 5 meq/L. Although not that significant, the SRF values continue to decrease as the influent magnesium concentrations increase. The results found by Higgins and Novak (1997b) in batch test experiments are parallel to our findings.

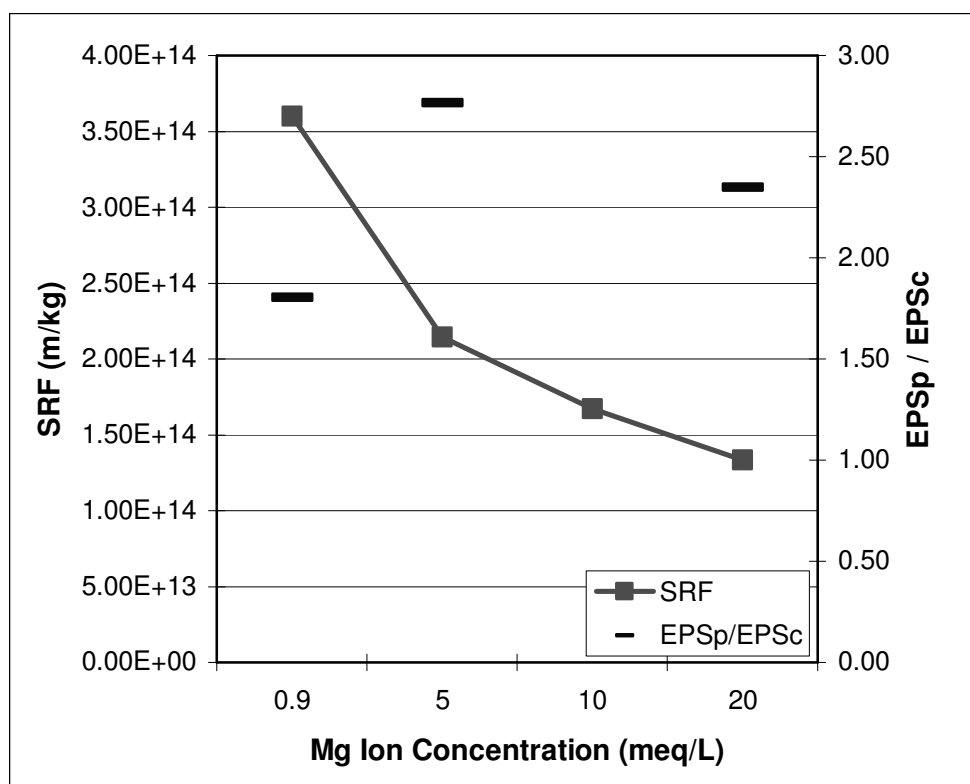


Figure 4.6. SRF and ESP<sub>P</sub>/ESP<sub>C</sub> values versus Magnesium ion concentration

Wu et al. (1982) stated that sludge dewaterability is closely related to sludge floc-forming ability. When activated sludge flocs are large and strong, its filtering property is excellent. It was also reported by Higgins and Novak (1997a), Keiding and Nielsen (1997) and Jin et al. (2004) that high concentrations of divalent and trivalent metal ions were related to low values of bound water and so improved dewaterability. The findings of this study reveals parallel results indicating that as the magnesium ion concentration in the feed media increases, the sludge becomes more easily dewaterable.

Novak et al. (2003) says that biocolloids, the biopolymers unattached to the activated sludge floc, are responsible for most of the polymer conditioning demand. From the results of this investigation, it can be concluded that, magnesium ions helps biocolloids to incorporate into the activated sludge floc and therefore, improve the dewaterability of the sludge by acting as a conditioner. As the magnesium concentration is shifted to higher values, more and more biocolloids become incorporated in the floc structure maintaining a more easily dewaterable sludge. The change in the SRF values with increasing magnesium ion concentration in the floc structure can be seen from Figure 4.7. It is obvious from Figure 4.7. that as the magnesium ion concentration incorporated into the sludge floc increases, the dewatering of the sludge becomes an easier process.

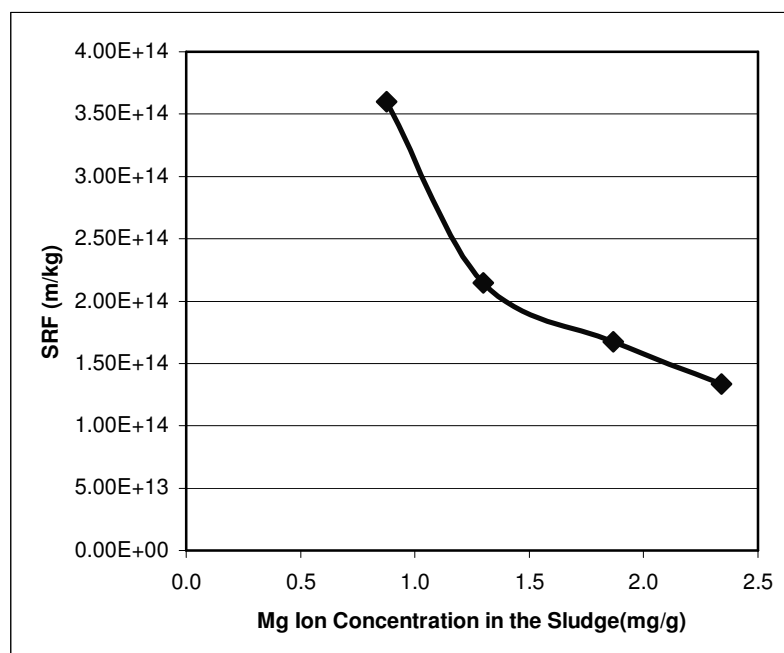


Figure 4.7. SRF values versus Magnesium ion incorporated into sludge floc

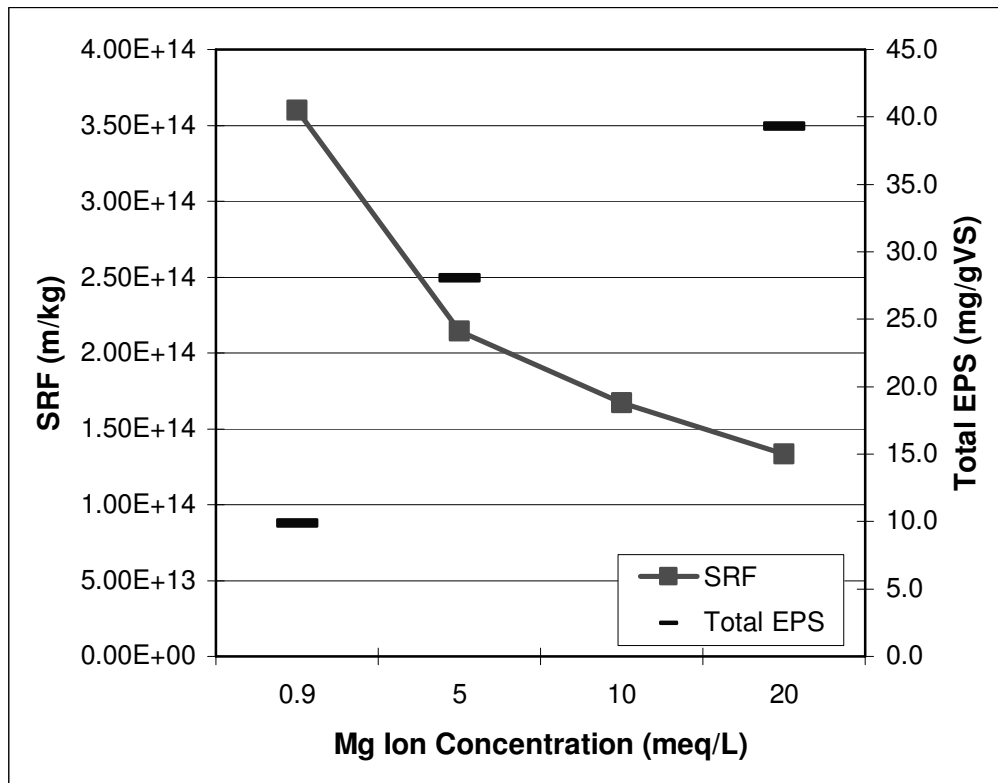


Figure 4.8. SRF and Total EPS values versus Magnesium ion concentration

Liu and Fang (2003) mentioned that it has been widely reported that as EPS content of the sludge increases the dewaterability decreases ( Figure 4.8.). However, in this study, although the total EPS values increases the SRF values continue to decrease. When considered together, this parallel relationship between SRF and total EPS and the reporting of Liu and Fang (2003) brings out the suggestion that EPS may not be the only and most important parameter controlling the dewaterability. The reason of why the EPS is increasing is also important. And the reason is the presence of magnesium ions in this case. Magnesium ions seem to have a

more significant effect on the dewaterability of sludge when compared to EPS for this specific case.

It is also essential to check, apart from the total EPS, the effect of  $EPS_P/EPS_C$  ratio while considering the relationship between EPS and dewaterability. Many researchers pointed out the effect of  $EPS_P/EPS_C$  ratio on the dewaterability. Some found the increase in the  $EPS_P/EPS_C$  ratio have a positive effect (Higgins and Novak, 1997 a, b, c) and some found a negative effect (Murthy and Novak, 1998, 1999; Houghton et al., 2001, 2001; Novak et al., 2001) on dewaterability. However, the increase in the  $EPS_C$  have a negative effect on sludge dewaterability mostly (Wu et al., 1982; Murthy and Novak, 1999). Higgins and Novak (1997a,b,c) concluded that bound  $EPS_P$  improved sludge dewaterability and  $EPS_P$  was of more significance than  $EPS_C$  to sludge dewaterability. It can be observed from Figure 4.6. that as  $EPS_P/EPS_C$  ratio increases, specific resistance to filtration decreases initially. However, after the second data point, although the  $EPS_P/EPS_C$  ratio decreases again, the specific resistance to filtration continues to decrease. This shows that, at least for this specific case, magnesium ions have a greater influence on the dewaterability of the sludge.

In order to get rid of any doubts, it is also important to mention that Houghton et. al (2001) reported that the dewaterability improves until a total EPS concentration of 35 mg/gSS. The total EPS values in this study reaches to a maximum value of only 40 mg/gVS in terms MLVSS and 32

mg/gSS in terms of MLSS. Therefore, it is not possible to compare our finding to the results of Houghton et al. (2001).

#### 4.2.3.2. Settling

Settling characteristics was one of the physical properties of sludge investigated during this study. To monitor the settling characteristics both zone settling velocity (ZSV) and sludge volume index (SVI) analyses were carried out. Jin et al. (2003) mentions that, for a specific sludge, the ZSV is a function of suspended solids concentration. The graphs presenting the relationship between MLSS concentration and ZSV were plotted and can be seen in Appendix C. From these graphs, ZSV versus magnesium concentration graphs were plotted at a fixed solids concentration of 3000 mg/L MLSS (Figure 4.9.). As can be seen clearly from Figure 4.9., a very sharp increase was observed in ZSV values when the magnesium ion concentration in the feed shifted to 5 meq/L from the control reactor levels (0.9 meq/L). This indicates that sludge settles much faster when the magnesium ion concentration is increased. Not an improvement in ZSV is observed with further magnesium ion concentration increases to 10 and 20 meq/L. On the contrary, even a decrease in ZSV was observed. This decrease in the ZSV can be attributed to the increased resistance to settlement as a result of increasing EPS concentrations. As the EPS network within the flocs developed further, it caused a higher friction with the water molecules resulting in a decrease in ZSV.

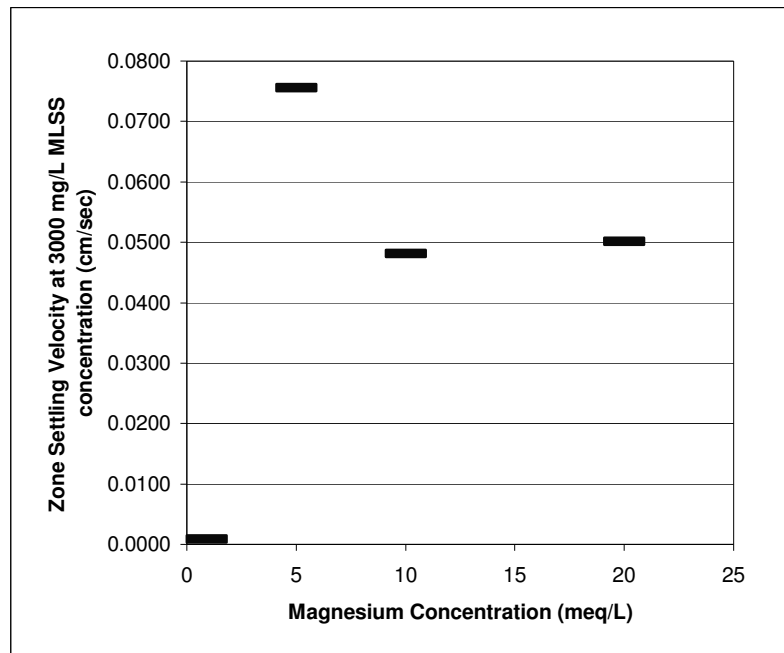


Figure 4.9. ZSV data versus Magnesium ion concentration

SVI values less than 150 indicate well settling sludge and if this value is exceeded settling problems occur such as bulking (Goodwin and Forster, 1985). SVI value was considerably high in control reactor (213 mL/g) as given in Figure 4.10. However, when magnesium ion concentration was increased, SVI values of all the reactors decreased significantly. SVI values for 5, 10, and 20 meq/L magnesium fed reactors change between 66 and 79 mL/g indicating good settleability. Increasing magnesium ion concentration from 5 meq/L to 20 meq/L did not cause an improvement; in fact, SVI values increased slightly when ion concentration is shifted from 10 meq/L to 20 meq/L.



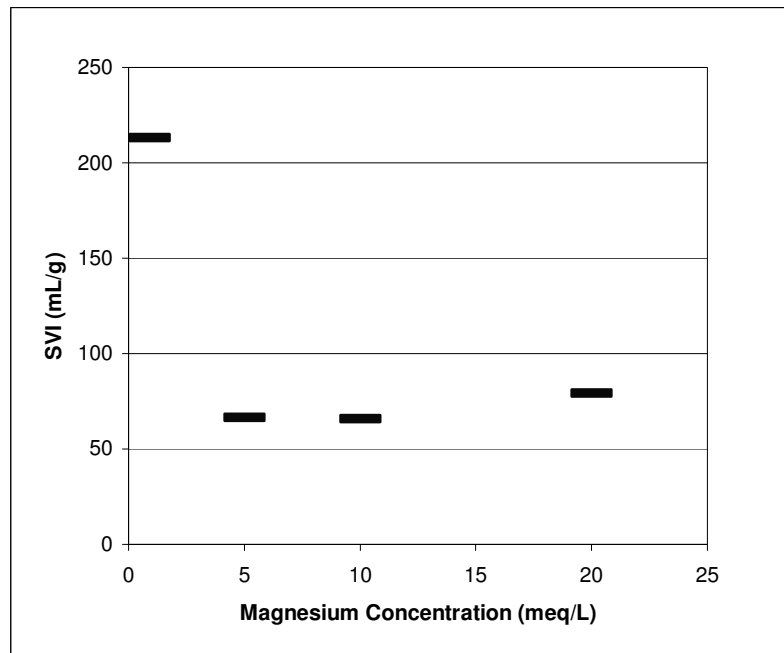


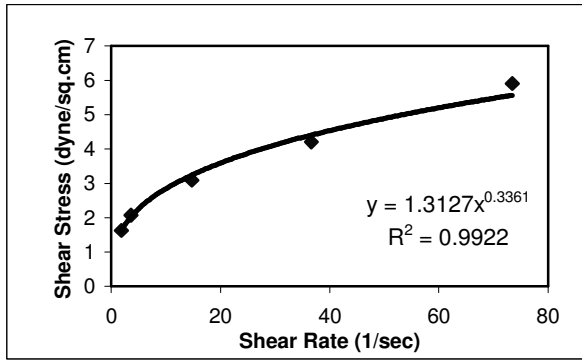
Figure 4.10. SVI values versus Magnesium ion concentration

Higgins and Novak (1997b) indicate that settling characteristics of sludge was improved when cations were added to the feed. In addition to that, Sobeck and Higgins (2002) report that magnesium ion addition to the influent results in improvements in settling measured by SVI. Jin et al. (2003) also reported that cations like calcium, magnesium, aluminum, and iron in the sludge improved the sludge compressibility and settleability. However, high concentrations of extracted EPS was concluded to have poor compressibility and settleability. The results of this study support the above mentioned findings with the contribution that further increasing the magnesium concentration in the influent over 5 meq/L does not further improve the SVI, in fact; after 10 meq/L the SVI begins to increase slowly, but not to a problematic level. This slight increase can be attributed to greater amount of polymers.

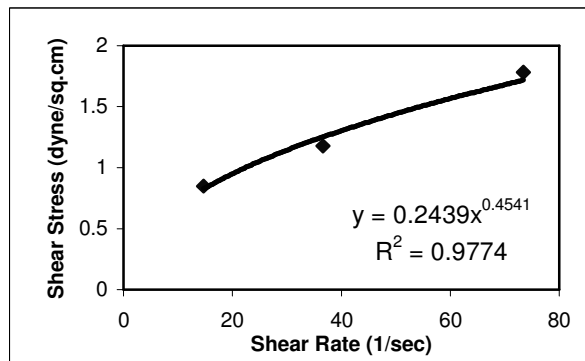
#### 4.2.3.3. Rheology

As one of the physical analyses, viscosity experiments were conducted. Graphs exhibiting the relationship between shear stress and shear rate were plotted (Figure 4.11) and their conformity to different sludge flow models were analyzed. The curves gave the highest fit to the power law equation of the pseudoplastic fluids which is represented in Equation 2.3. In this equation,  $n$  is the flow behavior index and as  $n$  deviates from 1 more, the fluid deviates from the Newtonian behavior more. When the  $n$  values in Figure 4.11. are examined, after an initial increase following the increase in magnesium concentration from 0.9 to 5 meq/L, the  $n$  value begins to drop. The more the increase in the magnesium concentration in the feed, the more the deviation of the fluid from the Newtonian behavior.

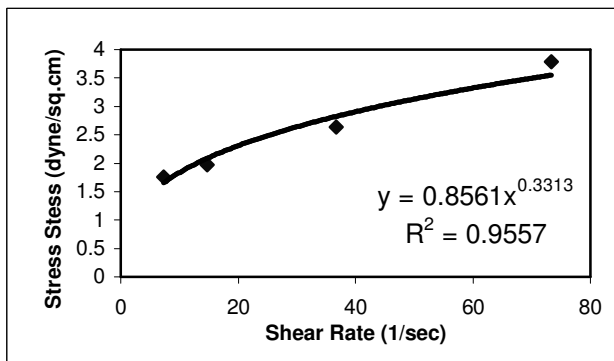
Viscosity data obtained from the experiments are presented in Figure 4.12. for both shear rates of  $36.7 \text{ s}^{-1}$  and  $73.4 \text{ s}^{-1}$ . Apparent viscosity values were decreased significantly when the cation concentrations increased from 0.9 meq/L to 5 meq/L. However, further increasing the cation concentration to 10 meq/L and then to 20 meq/L does not exhibit a similar decrease as above. This means that further increasing the magnesium concentration from 5 meq/L to 10 meq/L and then to 20 meq/L does not result in an improvement in viscosity and pumpability of the sludge.



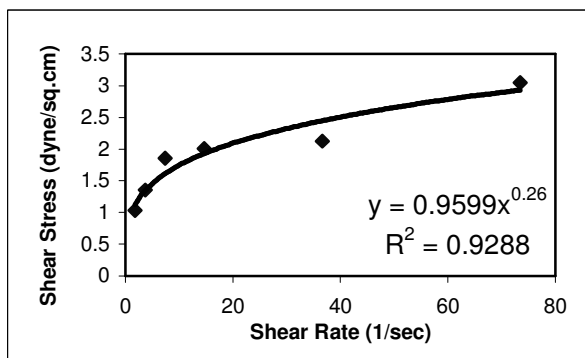
a.



b.



c.



d.

Figure 4.11. Shear Rate versus Shear Stress graphs for a. 0.9 meq/L, b. 5 meq/L, c. 10 meq/L, d. 20 meq/L magnesium reactors

When the relationship between EPS and apparent viscosity is considered it can be seen from Figure 4.12. that as  $EPS_p$  concentration increases the apparent viscosity decreases considerably between first and second data points. This is also valid for the  $EPS_c$ . However, after the second data point, although the EPS levels continue to increase for both carbohydrate and protein, the viscosity values stays stable. Forster (1982) indicates that polysaccharide components have a significant importance determining the viscosity of activated sludge suspensions. However, it can be concluded from the results of this study that the decrease in viscosity depends not only on the carbohydrate or on the protein content of EPS. Because, although the EPS concentrations and  $EPS_p/EPS_c$  ratio increase constantly with the increasing magnesium concentration, the viscosity values do not exhibit a parallel change to EPS. This can be observed more obvious at the last two data points of Figure 4.12.

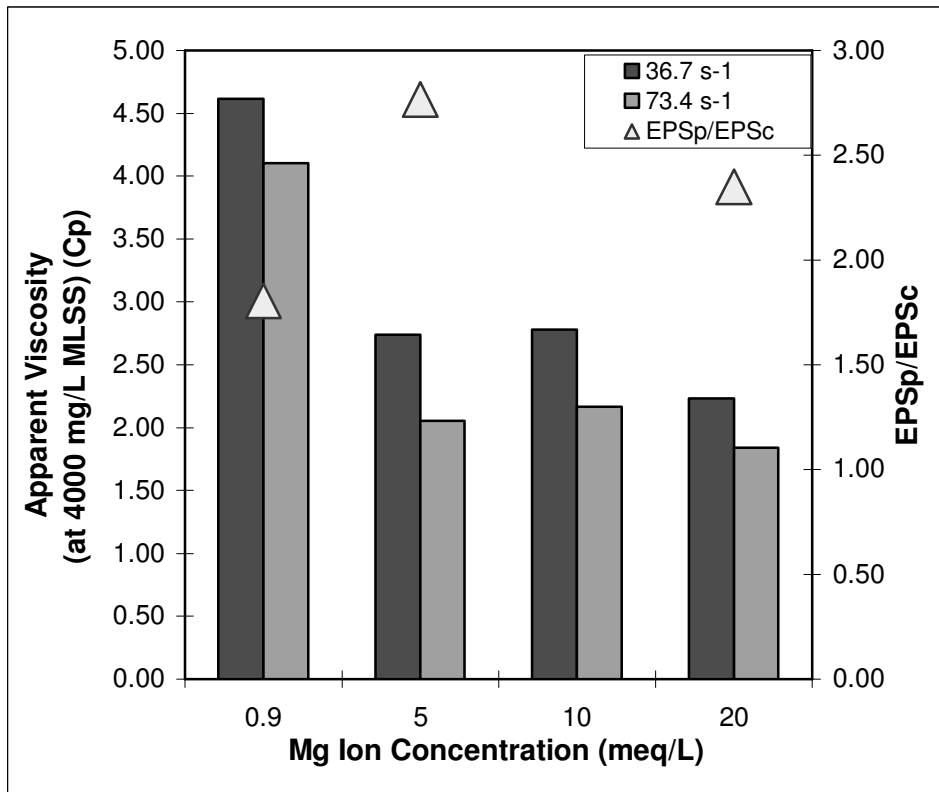


Figure 4.12. Viscosity vs. Magnesium ion Concentration at 4000 mg/L MLSS

The relationship between the apparent viscosities of sludges and the MLSS concentration were also analyzed at a fixed shear rate of  $36.7 \text{ s}^{-1}$  and presented in Figure 4.13. The curves fit to exponential law equation. It can be seen from Figure 4.13. that as the microorganism concentration in the system increases the apparent viscosity also increases. It can also be seen that as the magnesium ion concentration in the influent decreases the viscosity increases. The dependence of the viscosity on the solids concentration is in the form of an exponential function. As the magnesium concentration decreases, the increase of viscosity with the solids concentration becomes much sharper.

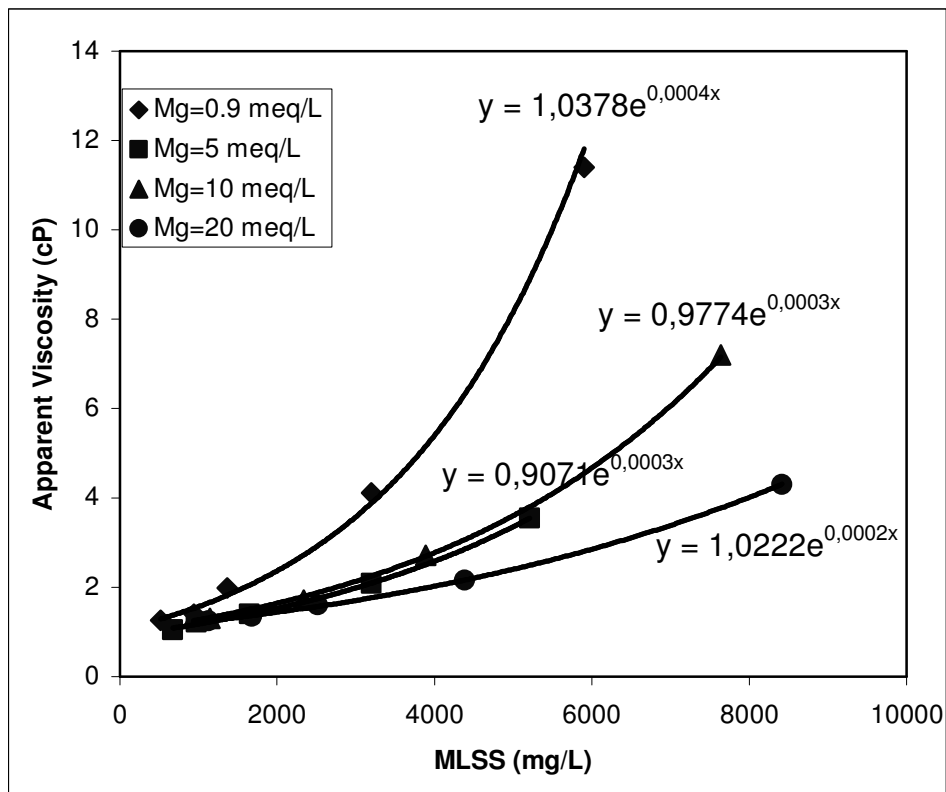


Figure 4.13. Apparent viscosity versus MLSS concentration at a shear rate of  $36.7 \text{ s}^{-1}$ .

#### 4.2.3.4. Hydrophobicity

One of the surface chemical properties investigated in this study is the hydrophobicity. The hydrophobicities were determined by BATH test. It should be emphasized that this test does not give exact hydrophobicity values but it gives relative hydrophobicity values, instead. Figure 4.14. shows the relation between magnesium ion concentration in the feed and hydrophobicity. As stated earlier by many researchers (Liu and Fang, 2003; Goldberg et al., 1990), sludge hydrophobicity was affected from the

presence of multivalent cations, specifically magnesium in this study. As magnesium ion concentration increased from 0.9 meq/L to 5 meq/L, a significant increase in the hydrophobicity of the sludge was observed. Although, not as drastic as in the case of 0.9 to 5 meq/L reactors, when magnesium ion concentration was increased further to 10 meq/L, hydrophobicity continued to increase. On the other hand, when the change in the hydrophobicity at the increase of magnesium ions from 10 to 20 meq/L is considered, it can be seen that the hydrophobicity values dropped back to the level of the hydrophobicity at 5 meq/L reactor. Therefore, it can be said that there is a peak value for hydrophobicity around 10 meq/L magnesium ion concentration and this value may be assumed as an upper limit for the sludge grown under the influence of high magnesium concentrations. This relationship is also valid for magnesium ions incorporated into the floc structure and hydrophobicity. Up to a value of magnesium concentration of 0.156 meq/L in the floc structure the hydrophobicity increase. However, a further increase in the magnesium ion incorporated in the sludge structure produces a decrease in the hydrophobicity.

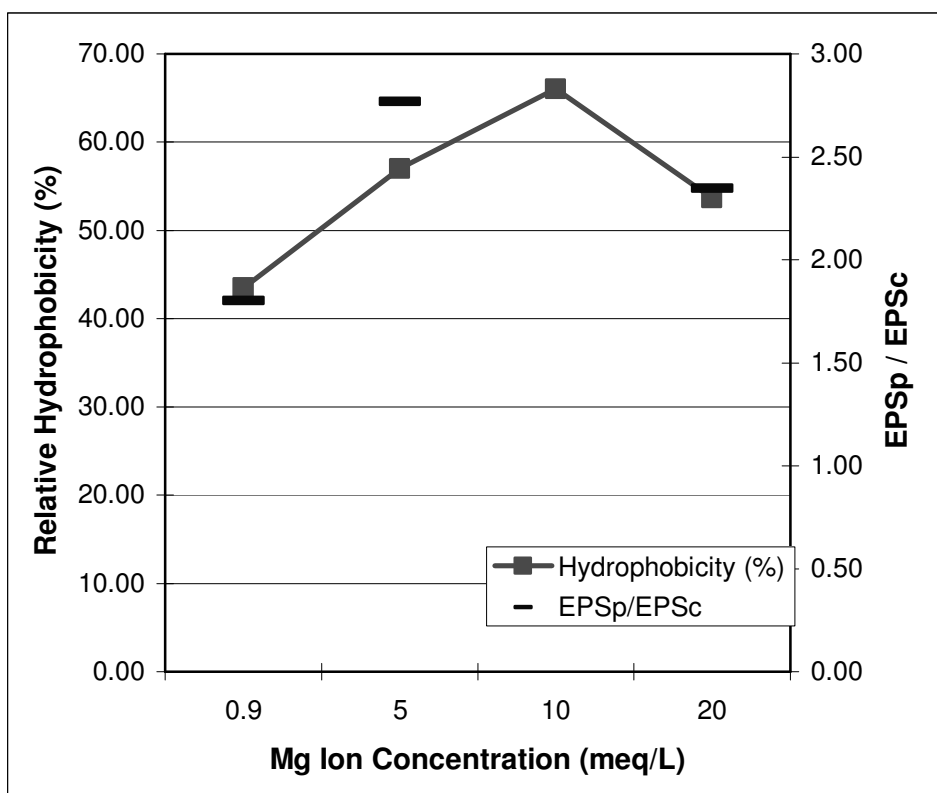


Figure 4.14. Hydrophobicity and EPS<sub>P</sub>/EPS<sub>C</sub> ratio versus Magnesium ion concentration

When the relation between hydrophobicity and EPS content of the sludge is considered, Liu and Fang (2003) and Liao et al. (2001) mentions that sludge hydrophobicity increases with the sludge EPS<sub>P</sub> content. The findings of this study support the results reported by Liu and Fang (2003) and Liao et al. (2001) to some extent. As can be seen from Figure 4.14., hydrophobicity values increase until the EPS<sub>P</sub>/EPS<sub>C</sub> value reaches to 2.77 (an EPS<sub>C</sub> value of 10.5 mg/gVS and EPS<sub>P</sub> value of 27.6 mg/gVS). However, after this point, since the EPS<sub>P</sub>/EPS<sub>C</sub> ratio decreases again, the hydrophobicity also drops back. Although the total EPS values increases



the hydrophobicity does not increase parallel to them indicating that it depend on the ratio of  $EPS_P/EPS_C$  rather than the total EPS values.

In addition to that, Jorand et al. (1994) and Liu and Fang (2003) also indicate that hydrophobic bacteria produces lesser amounts of EPS when compared to hydrophilic bacteria. The results obtained from this study contradicts with these reports to some extent. Because up to a certain point (10 meq/L magnesium fed sludge), hydrophobicity and EPS exhibits a parallel increase. However, after that point, although EPS continues to increase, hydrophobicity drops back. This can be explained by suggesting that magnesium addition makes it possible for hydrophobic bacteria to continue to produce EPS up to a certain point. But after that an inverse relationship between hydrophobicity and EPS can be observed again.

#### **4.2.3.5. Surface Charge**

Many researchers reported that flocs and EPS mostly have a negative charge changing between the zeta potential values of -20 to -30mV (Liu and Fang, 2003; Liu et al., 2001b; Weemaes et al., 2000; Daffonchio et al., 1995). The surface charge values obtained from the zeta potential measurements of magnesium reactors give, in general, parallel results to these widely accepted values. It can be seen from Figure 4.15. that most of the zeta potential values changes between -20 and -30mV. The only exception is the 5 meq/L magnesium fed reactor sludge with a zeta potential value of -35mV. A rapid increase in negativity of surface was

observed with respect to the control reactor, when the magnesium concentration was raised to 5 meq/L. As the magnesium concentration shifted to 10 meq/L, the zeta potential value decreased to -28mV and no change was observed after increasing the magnesium concentration to 20 meq/L.

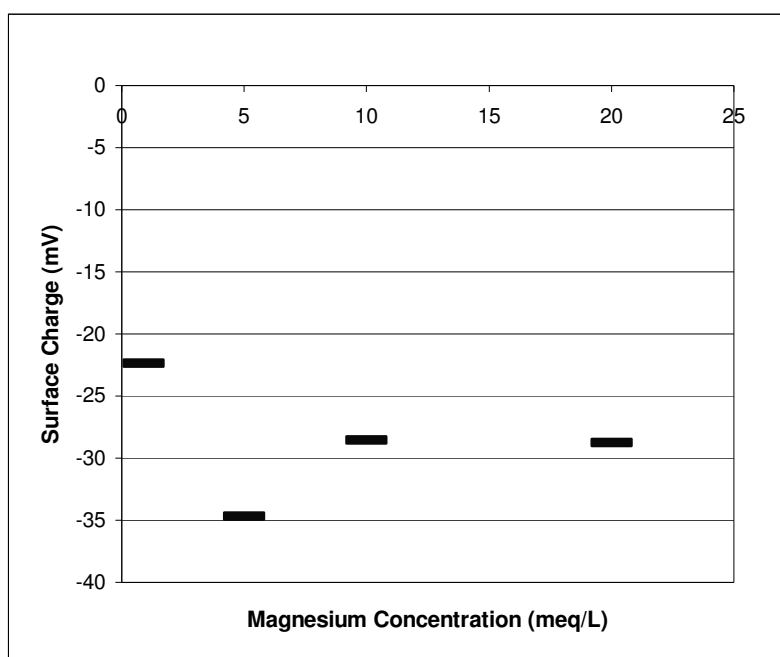


Figure 4.15. Zeta potential values versus Magnesium ion concentration

Moreover, although the EPS concentrations increase parallel to the magnesium concentrations, the surface negativity values does not increase parallel to them. As mentioned above, it is widely known that EPS has a negative charge. The magnesium cations must have neutralized the negative charge coming from the increasing EPS thus resulting in a decrease in the surface charge values. Another suggestion to the decrease in the surface charge can be  $EPS_P/EPS_C$  ratio. Liao et al.

(2001) suggested that the  $EPS_P/EPS_C$  ratio is important in determining the surface charge and this could be related to the unique charge properties of proteins. The amino groups in proteins carry positive charges, and can neutralize some of the negative charge from carboxyl and phosphate groups and therefore, decrease the net negative surface charge of sludge flocs. As the  $EPS_P/EPS_C$  ratio increases as the magnesium concentration in the influent increases this may be resulted in a decrease in the surface charge because of the charge neutralization.

It has been stated that there is an inverse correlation between hydrophobicity and surface charge (Pere et.al, 1993; Liao et.al, 2001). The results of this study, however, contradicts with this statement. When the general trends are considered the surface charge values increase with increasing hydrophobicity values.

#### **4.2.3.6. Other Parameters**

The turbidity values were measured after 60 minutes of settling and the results are shown in Figure 4.16. There is no obvious trend in turbidity values with respect to the increasing magnesium ion concentrations. Increasing the magnesium concentration over 5 meq/L improved the turbidity of the effluent slightly. However, all the turbidities measured are low and well within the acceptable levels.

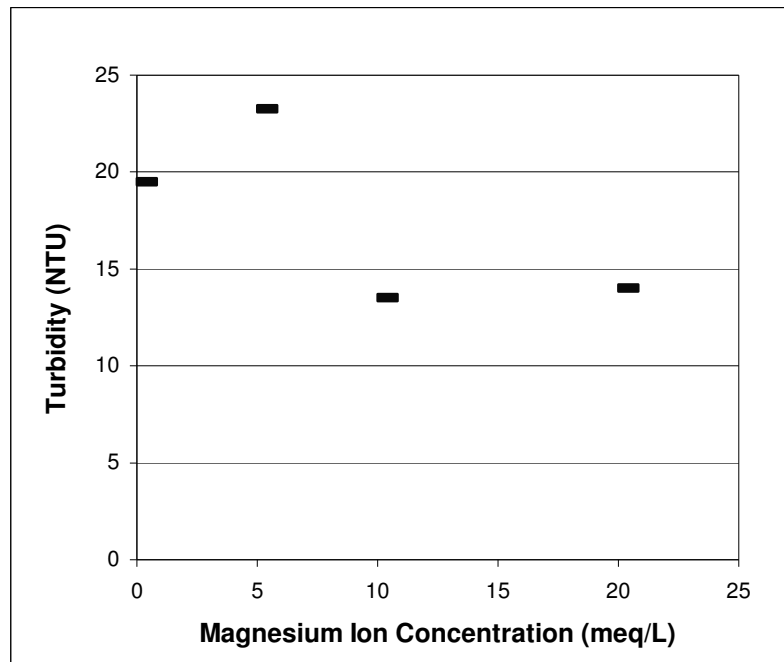


Figure 4.16. Effluent turbidity values versus Magnesium ion concentration

In addition to the turbidity, the COD values of the effluent were also measured. COD values of the effluent decreased as the magnesium ion concentration in the feed media increased from 0.9 meq/L to 5 meq/L as demonstrated in Figure 4.17. Afterwards, as the magnesium ion concentration is increased to 10 and then to 20 meq/L, the COD values exhibit a constant increase. In an earlier studies by Murthy and Novak (1998, 2001), it was stated that, for industrial wastewater treatment plants operating the activated sludge process with no proteins and polysaccharides in the influent stream, there is a substantial concentration of these polymers in the effluent. The presence of these polymers results in a contribution to effluent COD. Depending on this information the increase in the effluent COD can be explained by the

increase in the EPS content parallel to the magnesium ion concentration. 5 meq/L magnesium ion concentration seems to be the optimum dose for treatment in terms of COD reduction.

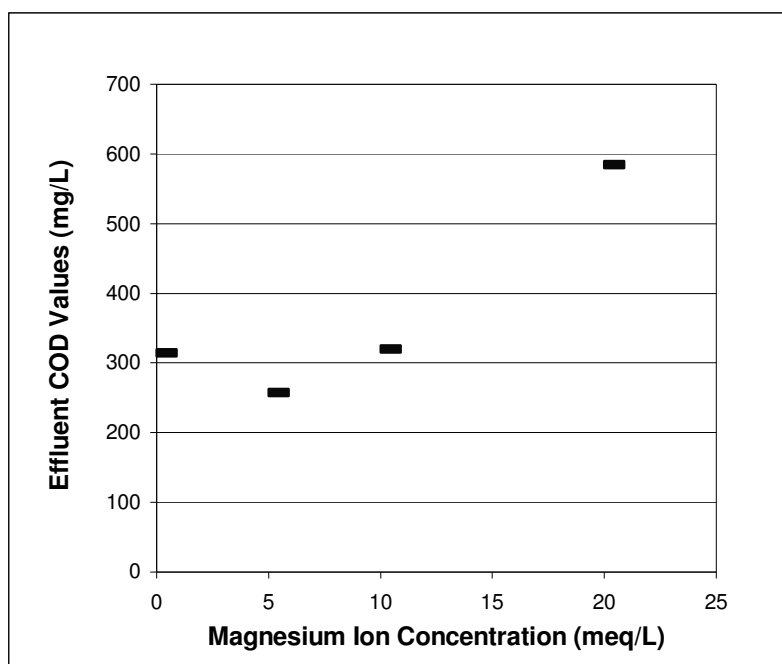


Figure 4.17. Effluent COD values versus Magnesium ion concentration

#### 4.2.4. Suggested Bioflocculation Model in Relation to Magnesium Ions

It is observed that as the magnesium ion in the feed medium increases the magnesium ion incorporated into the sludge floc also increases. The greater the amount of magnesium ions incorporated into the sludge floc, the stronger and more compact the floc structure. Therefore, the sludge becomes easier to dewater, pump and settle. Although the EPS content

of the sludge increases with the increasing magnesium ion concentration, magnesium ions are capable of binding these polymers into a more tight and compact floc structure. This decreases the water content within the floc structure resulting in an easily dewaterable sludge. Also this compactness improves the sludge's viscosity characteristics during pumping and reduces the frictional forces between the floc and water molecules resulting in a better settling.

It is also observed that as the amount of magnesium ion incorporated into the sludge increases the  $EPS_P/EPS_C$  ratio also increases. This may be because the magnesium stimulates the production of protein type of polymers or it may be because magnesium is more likely to bind protein type of polymers rather than carbohydrate type ones. Whatever the mechanism behind the affinity of magnesium ions to protein type polymers is, it is obvious that the increase in the  $EPS_P/EPS_C$  ratio improves the physical characteristics of the sludge.

Consequently, the mechanism of bioflocculation encountered in this study is a polymer-divalent cation bridging mechanism. However, it is important to mention that the polymers are mostly the protein type polymers.

## CHAPTER 5

### CONCLUSIONS

- It has been well understood that phosphorus deficiency causes severe bulking when magnesium is present. The nature of this bulking seems to be filamentous bulking which can easily be detected by an examination with light microscopy.
- Increasing the phosphorus concentration in the feed medium resulted in an improvement in sludge settling characteristics even in the first few weeks following the increase. In addition to that, by raising the phosphorus in the feed medium even to the half of the stoichiometrically required amount, the filamentous bulking was eliminated. By microscopic investigations, it is observed that the ratio of filamentous microorganisms within the whole microbial population decreased to exhibit a healthier sludge structure.
- It is also observed from the microscopic investigation that some granular structures occurred within the floc structure after increasing

the phosphorus source. These granular structures is thought to be polyphosphate granules and microorganisms that initially starved for phosphorus tend to accumulate phosphorus as polyphosphate granules once they are exposed to the adequate source for it.

- From the results obtained from phosphorus rich reactors, it was observed that as the magnesium ion concentration increases, both protein and carbohydrate fractions of EPS increases. Magnesium ions have definite positive effect to hold EPS within the floc structure. But this effect is more significant for protein content when compared to the carbohydrate content. In other words, magnesium ions are much more selective for proteins rather than carbohydrates.
- Although magnesium ion concentrations incorporated in the floc structure do not exhibit a one to one parallel increase to the increases in the influent concentrations of magnesium ions when measured in terms of mg/gSS, it can be understood from the results of magnesium measurement within the sludge floc structure that, as the magnesium concentration in the influent increases the percentage of magnesium ions incorporated into the floc structure also increases.
- The dewaterability results indicate that as the magnesium concentration increases the dewaterability improves. When the dewaterability is considered in relation to the EPS, there was no decrease in the dewaterability with increasing EPS as mentioned by various other authors in the literature. In fact, the situation is just the



opposite and dewaterability increases parallel to the EPS content. At this point it can be concluded that although EPS have a known negative effect on sludge dewaterability, since the magnesium ions have a more significant effect on the dewatering characteristics of the sludge, the dewaterability may increase depending on the magnesium ion addition even if the EPS content increases.

- Considering the settling characteristics of the sludges, 5 meq/L magnesium fed reactor exhibits the best result in terms of both SVI and ZSV. Although settling characteristics of the sludge deteriorates to some extent (but not to a problematic level) when 10 and 20 meq/L magnesium concentrations are reached, it is still obvious from the comparisons with control reactor that magnesium addition definitely enhances the settling characteristics.
- Viscosity data obtained from the analyses indicate that the lowest viscosity is observed at 20 meq/L magnesium fed reactor. However, the viscosity values of 5 and 10 meq/L magnesium fed reactors do not differ very much from the 20 meq/L reactor. On the other hand, magnesium addition produces a significant decrease (~ 50%) in viscosity when compared to control reactor. Moreover, the data in the literature indicated that the polysaccharide content is important in determining the viscosity. However, over a value of 7.45 mg/gSS for carbohydrate and 20.60 mg/gSS for protein, although the  $EPS_P$  and  $EPS_C$  continue to increase, there is no change in the viscosity values.

- While considering the hydrophobicity, it can be observed that 10 meq/L magnesium fed sludge exhibits the highest hydrophobicity values and the hydrophobicity results exhibit a parallel trend to the values of  $EPS_P/EPS_C$  ratio. The maximum hydrophobicity value reached is 66 %. While considering the relationship between hydrophobicity and EPS, the results found in this study are parallel to those in the literature to some extent because literature states that as EPS increases hydrophobicity also increases. However, according to the results, this is valid only up to a  $EPS_C$  value of 10.5 mg/gVS and  $EPS_P$  value of 27.6 mg/gVS. After these points, although the EPS levels continue to increase for both protein and carbohydrate, the hydrophobicity decreases parallel the  $EPS_P/EPS_C$ . this also indicates that the hydrophobicity values changes with  $EPS_P/EPS_C$  ratio rather than total EPS values.
- Surface charge values coincides with those given in the literature (-20 to -30 mV) with the only exception of 5 meq/L reactor (-35mV). Addition of positively charge magnesium ions decreased the surface charge in 10 and 20 meq/L reactors. Although it is known that EPS is mostly negatively charged and EPS is further increases in the 10 and 20 meq/L reactors, magnesium ions neutralized the negative charge originating from EPS and the surface charge values in 10 and 20 meq/L reactors. The surface charge values dropped even below the values of 5 meq/L reactor which is having lower EPS content when compared to 10 and 20 meq/L reactors. Another reason for the explanation for this decrease in 10 and 20 meq/L reactors is the

increase in the  $EPS_P/EPS_C$  ratio with the increasing magnesium concentration. Since most of the protein structures are positively charged the increase in the  $EPS_P/EPS_C$  ratio can cause a decrease in the surface charge.

- When the relationship between hydrophobicity and surface charge is considered, the results of this study contradict with those reported in the literature. Although it is reported in the literature that there is an inverse relationship between hydrophobicity and surface charge, when the general trends in the findings of this study is considered, a direct relationship can be observed.
- The effluent COD values exhibit an increase with the increasing influent magnesium concentration. The reason for this increase is the increasing amount of EPS due to the increase in magnesium.
- From the results of the physical analyses it can be seen that dewaterability, settling, and rheological characteristics improved significantly when the magnesium ion concentration was increased from 0.9 meq/L to 5 meq/L. When the magnesium ion concentration increased further, there was no further improvement in settling and rheological characteristics of the sludge. Dewaterability, on the other hand, improved further as the magnesium ion concentration was increased to 10 and 20 meq/L. However, this increase is not a very sharp and significant one. Consequently, it can be said regarding the operation of an activated sludge plant that, 5 meq/L magnesium dose

is the optimum dose. On the other hand, if the dewaterability is the primary concern in the operation of the treatment plant, then magnesium concentrations higher than 5 meq/L are also suggested.

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# APPENDIX A

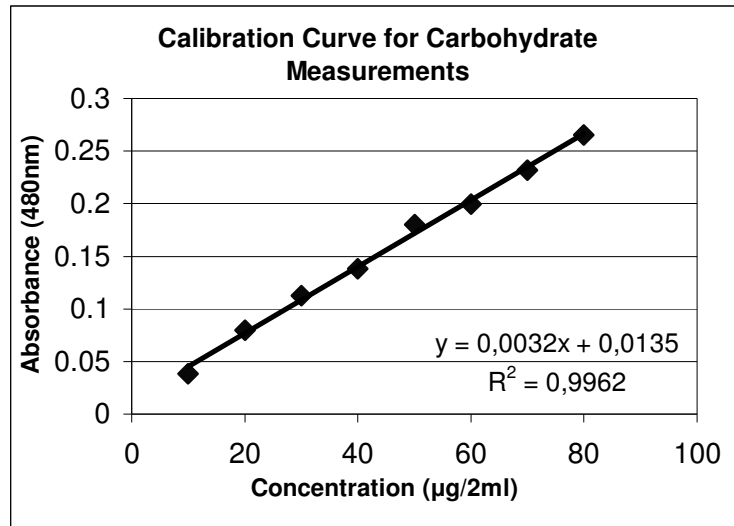


Figure A.1. Calibration curve for Dubois Method

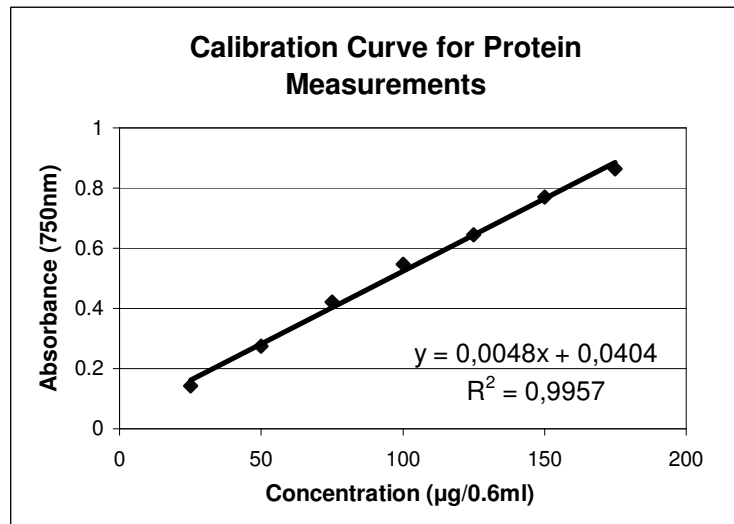


Figure A.2. Calibration curve for Lowry Method

# APPENDIX B

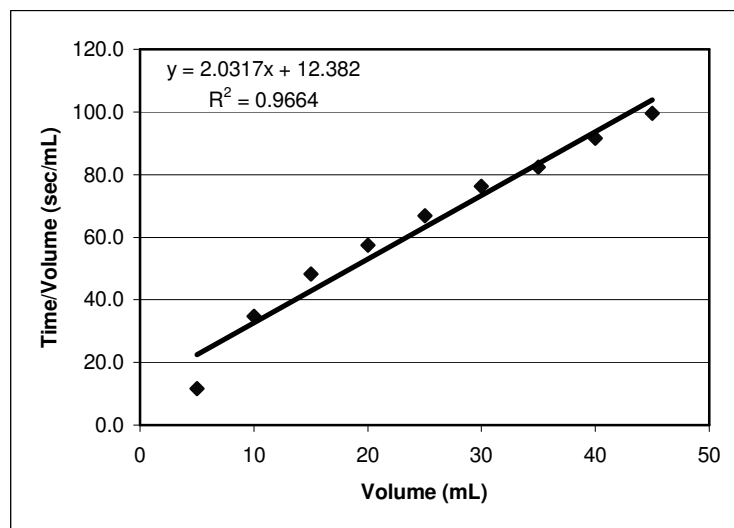
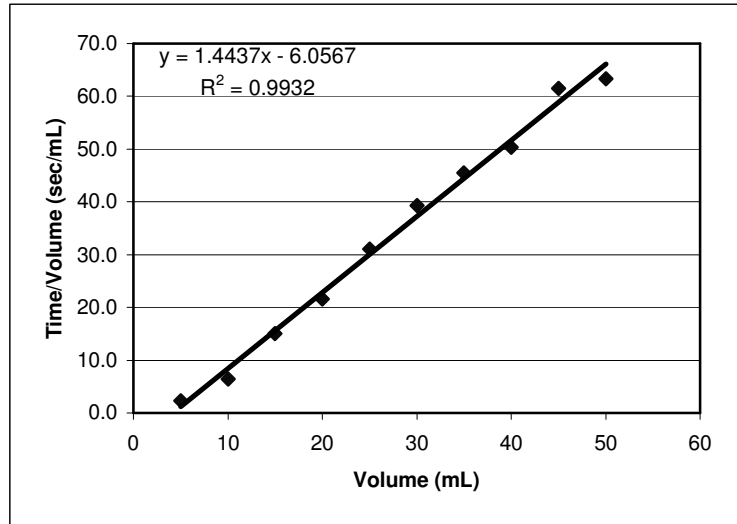
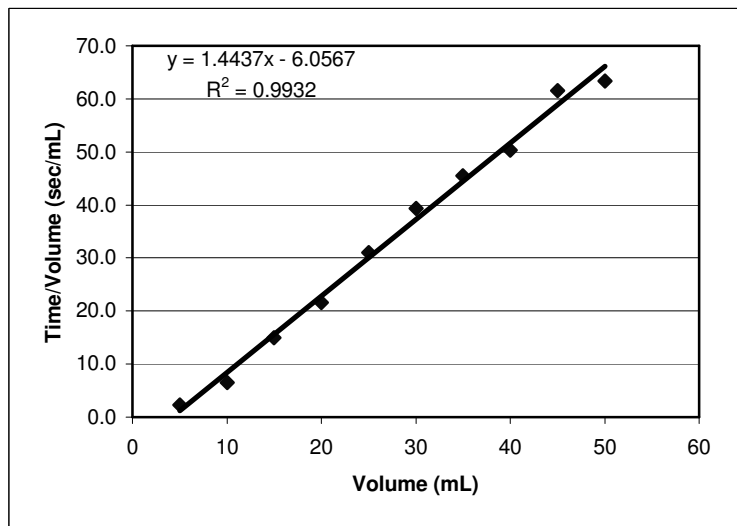


Figure B.1. Time/Volume versus Time graph for 0.9 meq/L magnesium reactor



a.



b.

Figure B.2.a. and b. Time/Volume versus Time graphs for 1<sup>st</sup> and 2<sup>nd</sup> replicas of 5 meq/L magnesium reactor

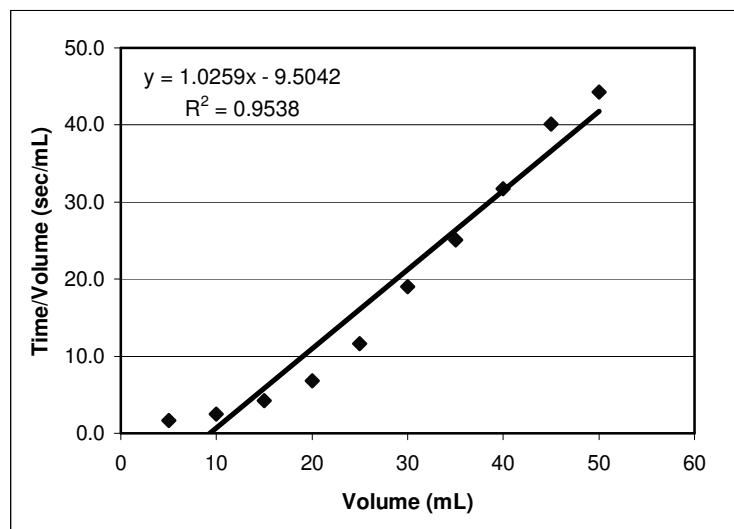
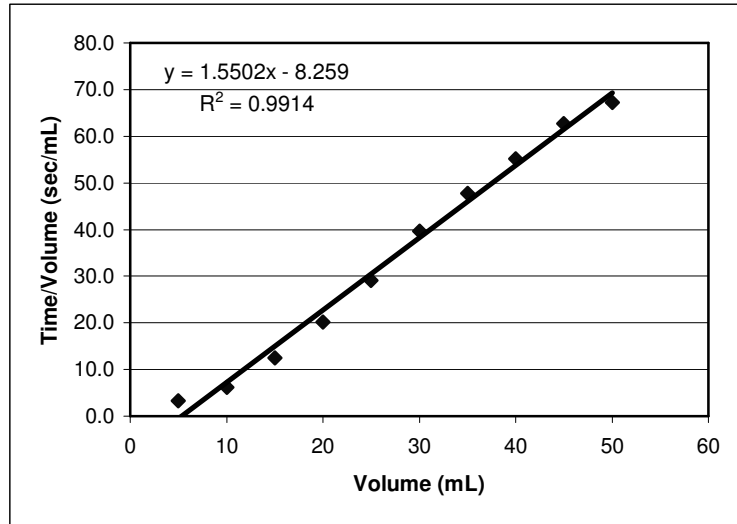
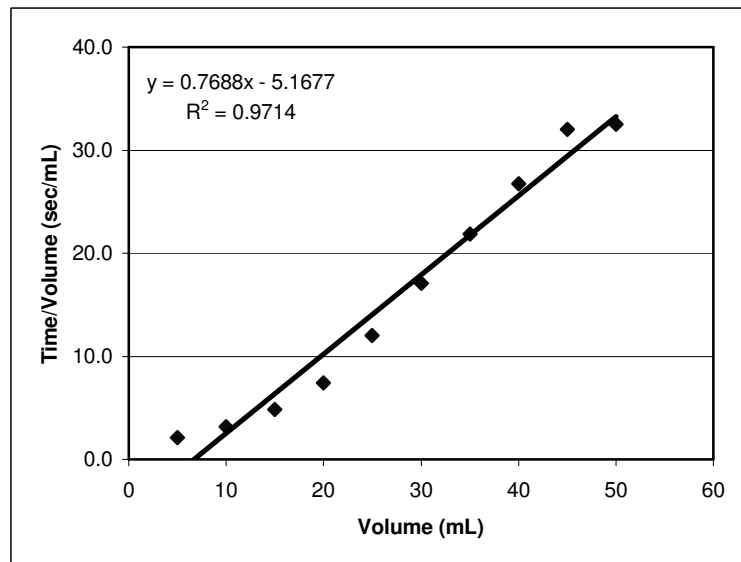


Figure B.3. Time/Volume versus Time graph for 10 meq/L magnesium reactor





a.



b.

Figure B.4.a. and b. Time/Volume versus Time graphs for 1<sup>st</sup> and 2<sup>nd</sup> replicas of 20 meq/L magnesium reactor

# APPENDIX C

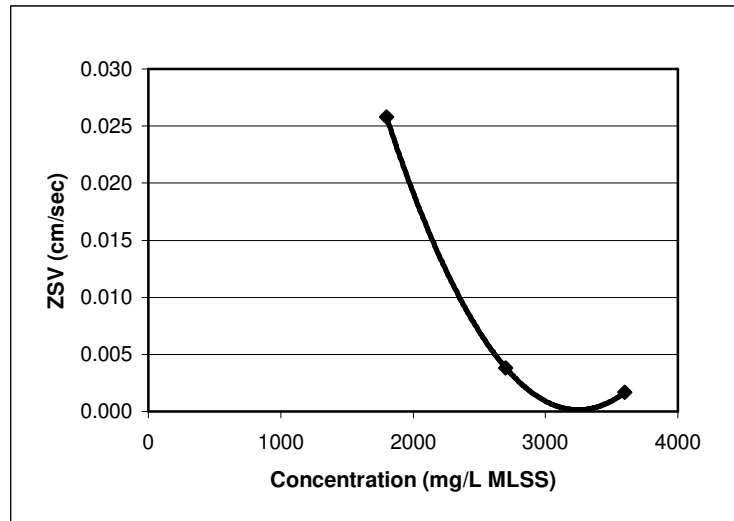
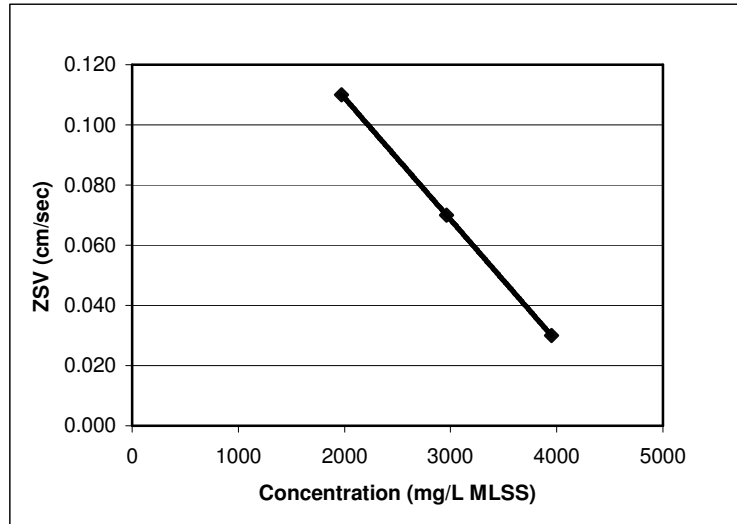
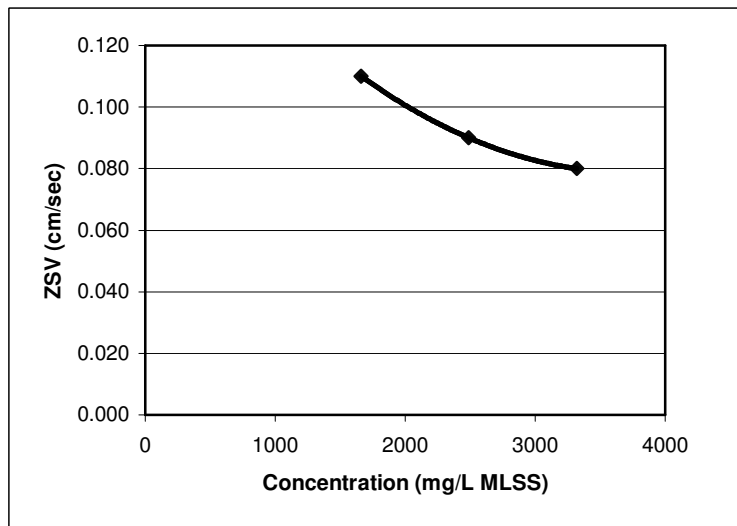


Figure C.1. ZSV versus MLSS concentration graph for 0.9 meq/L magnesium reactor



a.



b.

Figure C.2.a. and b. ZSV versus MLSS concentration graphs for 1<sup>st</sup> and 2<sup>nd</sup> replicas of 5 meq/L magnesium reactor

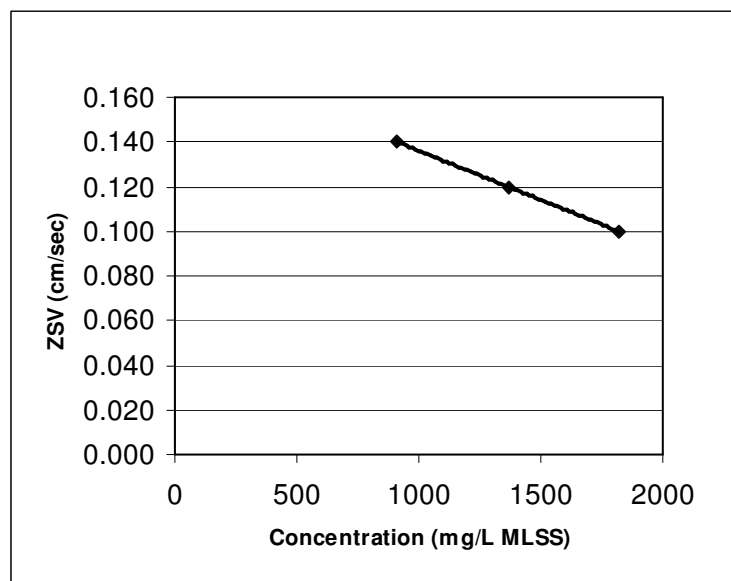
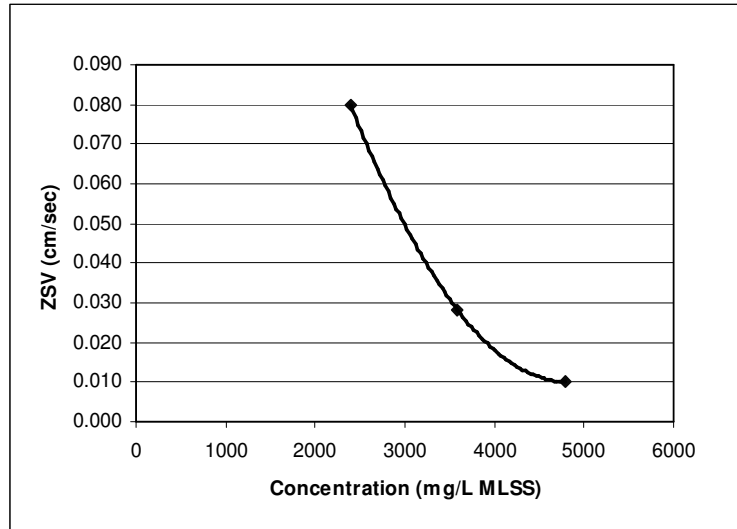
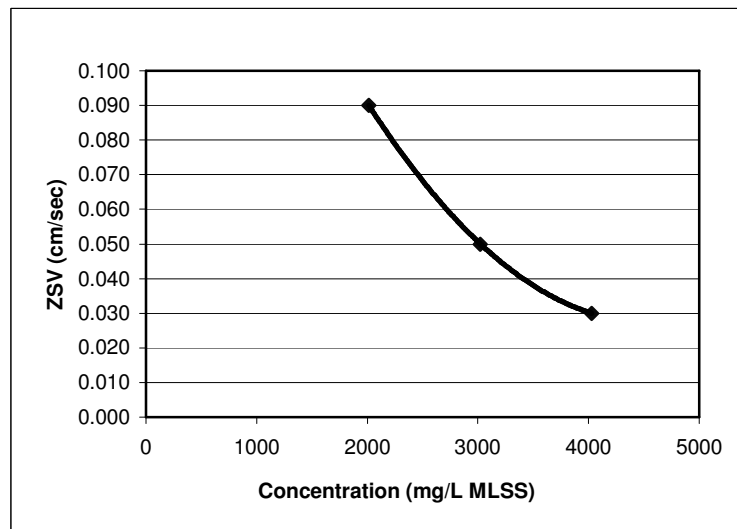


Figure C.3. ZSV versus MLSS concentration graph for 10 meq/L magnesium reactor



a.



b.

Figure C.4.a. and b. ZSV versus MLSS concentration graph for 1<sup>st</sup> and 2<sup>nd</sup> replicas of 20 meq/L magnesium reactor