INVESTIGATION OF PROTEIN AND CARBOHYDRATE COMPONENTS OF EXTRACELLULAR POLYMERIC SUBSTANCES IN THE SLUDGES HAVING PIN FLOC CHARACTERISTICS AND THEIR ROLE IN DEWATERABILITY

by

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INVESTIGATION OF PROTEIN AND CARBOHYDRATE COMPONENTS OF EXTRACELLULAR POLYMERIC SUBSTANCES IN THE SLUDGES HAVING PIN FLOC CHARACTERISTICS AND THEIR ROLE IN DEWATERABILITY

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ABSTRACT

In this research, protein and carbohydrate components of extracellular polymeric substances of the non settling sludges having pin floc characteristics and the role of EPS components were investigated. The results of pin floc sludges compared with waste activated sludges. Five different sludge samples were analyzed. The three pin floc sludge samples were obtained from sequencing batch raector, aerobic pond and facultative ponds of Bursa Hamitler Leachate Traetment Plant respectively. One of the samples was grown in the bench-scale batch reactor in laboratory and the other sample was obtained from a raw pharmaceutical industry wastewater treatment plant. Extracellular polymeric substances of sludge samples were extracted by a cation exchange resin at a dosage of 75 g/g VSS (volatile suspended solids). The dewatering characteristics of the sludge samples were determined by measuring the filterability in terms of capillary suction time and specific resistance time to filtration and compactibility in terms of cake solids concentration. The floc structures of sludge samples were also observed microscopically.

The result of observations showed sludges having microflocs (pin flocs) have very high EPS_p values compared to lab-grown sludge and typical waste activated sludges. Their EPS_c/EPS_p values were also low leading to very poor settling and dewatering properties. It seems that protein component of EPS may have an effect on the pinfloc (microfloc) formation and it deteriorates the sludge dewaterability and settleability.

Compactibility, in terms of cake solids concentration, increased by decreasing concentrations of $EPS_{protein}$. Filterability, in terms of specific resistance to filtration, increased considerably by increasing concentrations of $EPS_{protein}$.

Sludge samples having pin floc characteristics had very high SRF values compared to the samples of typical waste activated sludge. High SRF values indicate the difficulty of the sludge to be filtered. During the SRF experiments, filtration time for pin floc sludges were too long. On the other hand samples of typical WAS were filtered easily.

ÖZET

Bu çalışmada çökmeyen iğne başı yumak (pin-floc) çamur yapısına sahip çamurların hücredışı polimerik maddelerinin protein ve karbonhidrat bileşenleri ve bunların susuzlaştırma üzerindeki rolleri araştırılmıştır ve sonuçlar tipik atık aktif çamurlarla karşılaştırılmıştır. Araştırmada 5 farklı çamur numunesi analiz edilmiştir. Üç adet iğne başı yumak özelliği gösteren çamur numunesi sırasıyla Bursa Hamitler Sızıntı Suyu arıtma tesisinin ardışık kesikli reaktöründen, aerobik ve fakültatif havuzlarından alınmıştır. Atık aktif çamur numulerinin biri laboratuarda üretilmiştir ve diğeri ilaç hammadde endüstrisi atıksu arıtma tesisinden alınmıştır. EPS, 75 g/g uçucu askıda katı madde (UAKM) dozajında, katyon değiştirici reçine kullanılarak çamurdan ayrılmıştır. Çamur numulerinin susuzlaştırma karakteristikleri kapiler emme zamanı (CST) ve spesifik filtrasyon direnci (SRF) ölçülmesiyle filtrasyon cinsinden, kek katı konsantrasyonu cinsinden kompakt edilebilirlik olarak belirlenmiştir.

Çalışma sonuçları, mikroflok yapısına sahip çamur numulerinin $EPS_{protein}$ değerlerinin laboratuar ortamında yetiştirilmiş çamura ve ilaç endüstrisinden alınan tipik atık aktif çamura gore çok yüksek olduğunu göstermiştir. Mikroflok çamur yapısına sahip çamurların EPS_c/EPS_p değerlerinin de düşük olduğu ve zayıf çökelmeye ve susuzlaştırmaya yol açtığı gözlemlenmiştir.

$EPS_{protein}$'in azalmasıyla kek katı konsantrasyonu olarak kompakt edilebilirlik artmıştır ve $EPS_{protein}$'in artmasıyla özgül direnç cinsinden filtre edilebilirliği artmıştır.

Tipik aktif çamur numulerine kıyasla pin flok yapısına sahip çamur numunelerinin özgül dirençleri çok yüksektir. Spesifik filtrasyon direnci deneyleri sırasında pin flok çamur yapısına sahip çamurların süzülmeleri çok uzun zaman almıştır. Öte yandan tipik aktif çamur numuneleri kolayca süzülmüştür.

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Symbol	Explanation	Units used
A	Area on the bottom of the collar	m^2
b	Slope of the graphic of t/V versus t	s/m^6
BSA	Bovine Serum Albumin	
C	Sludge Total Solids	kg/m^3
C.A.	Centrifugal Acceleration	
CDM	Cake Dry Matter	
CER	Cation Exchange Resin	
C_k	Cake solids concentration of sludge sample after centrifugation	%
C_o	Suspended solids concentration of sludge sample before centrifugation	%
COD	Chemical Oxygen Demand	mg/L
CST	Capillary Suction Time	sec
CSTs	Capillary suction time corrected for different solids contents	
d	Filter paper depth	m
D_1, D_2	Diameters of the locations of electrical sensors	m
DNA	Deoxyribonucleic acid	
EDTA	Ethylenediaminetetraacetic acid	
EPS	Extracellular Polymeric Substances	
$EPS_{carbohydrate}$	Carbohydrate part of EPS	
$EPS_{protein}$	Protein part of EPS	
F/M	Food to Microorganism ratio	
g	Gravitational constant	m/s^2
H_0	Initial pellet height before centrifugation	mL
H_k	Final pellet height after centrifugation	mL
KHP	Potassium hydrogen phtalate	
L	Thickness	m

MLSS	Mixed Liquor Suspended Solids	mg/L
MLVSS	Mixed Liquor Volatile Suspended Solids	mg/L
ø	The constant for CST apparatus	
P	Capillary section	m/s
P	Pressure	N/m^2
PBS	Phosphate Buffer Solution	
r	Specific Resistance	m/kg
r_1	Radius from the centerline to the bottom of the tube	m
r_2	Radius from the centerline to the top of the sludge in the tube	m
SRF	Specific Resistance to Filtration	
SS	Suspended Solids	
SVI	Sludge Volume Index	
T	Temperature	C^o
t	Capillary suction time	sec
TDS	Total Dissolved Solids	
TKN	Total Kjeldahl Nitrogen	
TS	Total Solids	mg/L
w	Solids deposited per volume of filtrate in kg/m^3	kg/m^3
X	Filterability Constant	kg^2/m^4s^2
μ	Viscosity	$N.s/m^2$
ω	Rotational speed in radians per second	

1. INTRODUCTION

Microbial Extracellular Polymeric Substances, being the third major component of sludge flocs, are organic polymers produced by bacterial cell and can be expected to have an influence on sludge dewaterability through the high level of hydration of the polymer surrounding bacterial cell and its role in flocculation (Houghton et al., 2001).

Flemming and Windenger (2001) defined extracellular polymeric substances as organic polymers that are produced by the bacterial cell wall surrounding the cell wall as a hydrated capsule. They also stated that this component of sludge is considered to be important in determining the floc structure, flocculation process, floc charge, sludge settling properties and dewaterability. EPS are the construction materials for microbial aggregates such as biofilms, flocs, and sludge. EPS can be considered as the "house" of the microorganisms.

Bioflocculation of activated sludge, which is thought to be highly affected by extracellular polymers, is very important in determining the sludge dewatering and settling. The need for the understanding of these physical properties of activated sludges lead to the research of EPS and bioflocculation mechanisms together. Generally, it is claimed that sludge dewaterability decreases with the increase of EPS in sludge by deflocculation.

Bacterial flocculation is very important as floc characteristics directly effect the effluent quality and overall efficiency of the activated sludge process. Flocculation occurs naturally under normal operating conditions. Although many researchers investigated bioflocculation phenomenon, there still is not exact explanation of why flocculation occur naturally or why sometimes does not. While the certain amounts of filamentous microorganisms enhance bioflocculation, the overgrowth of these microorganisms causes to sludge bulking problem in activated sludge systems. On the other hand the absence of filamentous organisms causes to occurance of remarkably smaller flocs which are called pin flocs (pin point floc). Pin-floc consists of small visible floc particles, settle poorly and remain in the supernatant after the sludge has settled.

Although the causes of pin-floc formation has not been understood yet, Pipes (1979) observed that pin-floc occurs with low organic loading (F/M< 0.2 /d) and low SVI (<100). Pin floc consists only of floc forming bacteria without a filament backbone and usually are <50μm in diameter.

Dewatering is a physical process to remove the liquid portion of sludges. Only a small part of the sludge is solid matter. Therefore, the sludge volume reduction is a necessity in order to prevent disposing huge amounts of wastes. Sludge dewatering operation causes approximately 50 per cent of the annual operating costs of a treatment plant. Hence dewaterability is an important parameter by environmental and economical points of view.

This study investigates the protein and carbohydrate components of extracellular polymeric substances of non settling sludges having pin-floc characteristics. Pin-floc sludges and typcical waste activated sludges were analyzed in order to compare the effect of EPS on settleability and dewaterability of theses sludges. Five different sludge samples were analyzed. Sludges 1, 2 and 3 were obtained from sequencing batch raector , aerobic pond and facultative ponds of Bursa Hamitler Leachate Traetment Plant respectively. Sludge 4 was grown in the bench-scale batch reactor in laboratory and Sludge 5 was taken from FAKO pharmaceutical industry wastewater treatment plant, located in Kağıthane-İstanbul. Filterabiliy of sludge samples was determined in terms of CST and SRF. Compactibility was measured in terms of cake solids concentration. Settleability of the sludge samples was determined by measuring sludge volume index.

2. THEORETICAL BACKGROUND

2.1. Extracellular Polymeric Substances

2.1.1. Definition and General Characteristics

Microbial EPS, being the third major component of sludge flocs, are organic polymers produced by bacterial cell and can be expected to have an influence on sludge dewaterability through the high level of hydration of the polymer surrounding bacterial cell and its role in flocculation (Houghton et al., 2001)

Flemming and Wingender (2001) also defined extracellular polymeric substances as organic polymers that are produced by the bacterial cell wall surrounding the cell wall as a hydrated capsule. They also stated that this component of sludge is considered to be important in determining the floc structure, flocculation process, floc charge, sludge settling properties and dewaterability. EPS are the construction materials for microbial aggregates such as biofilms, flocs, and sludge. EPS can be considered as the “house” of the microorganisms.

Urbain et al. (1993) suggests that the overall floc structure of activated sludge is negatively charged and is result of physico-chemical interactions between microorganisms (mainly bacteria), inorganic particles (silicates, calcium phosphate and iron oxides), extracellular polymers and multivalent cations. A significant fraction of sludge mass consists of EPS, which are negatively charged, leading to the presence of large concentrations of counter-ions within the flocs. The basic components of an activated sludge floc is represented in Figure 2.1.

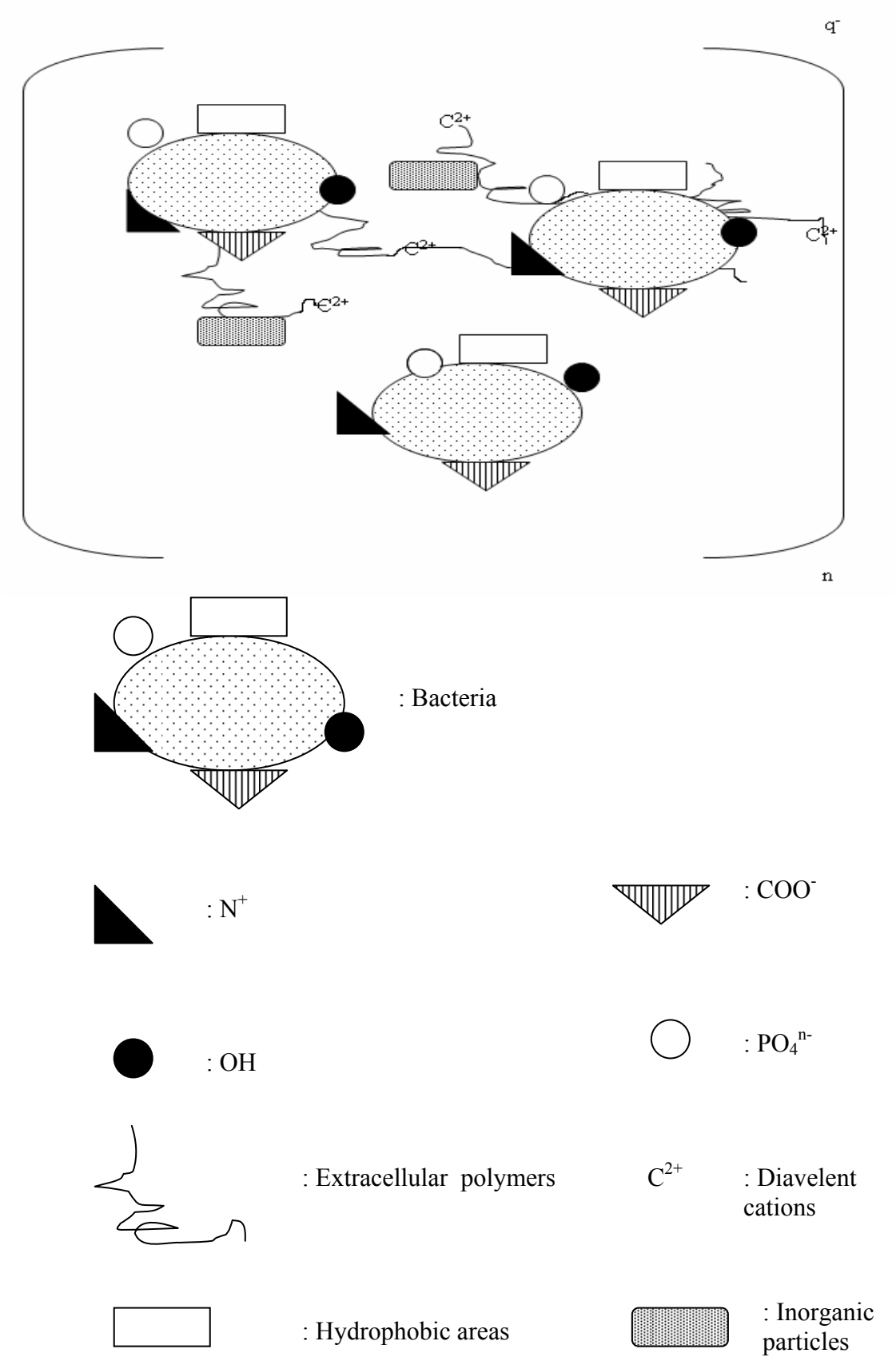

Figure 2.1. Basic components of an activated sludge floc (Urbain et al., 1993)

2.1.2. EPS Composition

Extracellular polymeric substances have a very heterogenous chemical composition mainly containing protein, carbohydrate, lipids, humic acids, and nucleic acids. Protein and carbohydrate are usually found as the major components of EPS, but there is not a certain opinion on which of these components has a higher portion (Çetin and Erdinçler, 2004).

Frolound et al. (1996) found carbohydrate and protein as the major EPS components having a protein ratio between 0.2 and 0.5 (w/w). They also reported lipid, nucleic acid, and humic compounds. In addition to this Liu and Fang collected (2003) collected 75 sets of data in the literature on individual EPS constituents extracted from activated sludge treating different types of wastewater under specified extraction conditions. Based on data compiled $EPS_{protein}$:$EPSc_{arbohydrate}$ ratio in activated sludge varied over a wide range from 0.5 to 21.2. About two-thirds of the collected activated sludge data had the $EPS_{protein}$:$EPS_{carbohydrate}$ ratio between 2.0 and 10.0, and only 8% had the ratio below 1.0. This shows that EPS of activated sludge are predominantly composed of $EPS_{protein}$.

Due to the differences in sludge treatment methods, bacterial activities, feed compositions and extraction processes the EPS components in the different sludges show variations. Many researchers (Frolound et al., 1996; Durmaz and Sanin, 2001) discuss accuracy of the analytical measurement methods used in determining EPS components.

Table 2.1. shows the the concentrations of the major components of EPS according to Flemming and Wingender (2001).

Table 2.1. The composition of EPS (Flemming and Wingender, 2001)

Component	Content in EPS
Polysaccharides	40-95%
Protein	<1-60%
Nucleic acids	<1-10%
Lipids	<1-40%

2.1.3. EPS Extraction Methods

There are several techniques used for the extraction of EPS from sludge. Some of the methods that are commonly used are :

- extraction with mechanical homogenization
- centrifugal stripping
- thermal extraction/solvent precipitation process
- cation exchange resin (CER)
- EDTA
- sodium hydroxide (alkali stripping)

Some researchers extracted EPS from activated sludge only by centrifugation while others applied physical or chemical methods prior to centrifuging the sludge in order to threat the sludge. The aim of these treatments is to release EPS bound to the cell surface into the mixed liquor for extraction.

Higgins and Novak (1997a,b) classified EPS into two categories which are dissolved and bound. The dissolved EPS can be extracted by centrifugation alone while the bound

EPS requires additional treatments. These treatment methods may involve heating (Morgan et al., 1990), ultrasonication (Dignac et al., 1998), homogenization (Wuertz et al., 2001) and the additions of cation exchange resin (Durmaz and Sanin, 2001), caustic (Frølound et al., 1996), and sulfuric acid (Chen et al., 2001).

Many researchers used thermal extraction/solvent precipitation method for extraction of EPS. This method is based on centrifugation of a sludge sample at a low speed (washing step, including the removal of loosely bound biopolymers) and resuspending in Ringers solution for removing the loose slime polymers before heating at 80°C for 1 hour. After that, samples are centrifuged and the supernatant is precipitated using a solvent mixture (acetone and ethanol). Centrifugation was again used for separation of the biopolymers from the solvent. (Goodwin and Forster, 1985; Horan and Eccles, 1986; Morgan et al., 1990; Houghton et al., 2001; Houghton and Stephenson, 2002)

Frølound et al., (1996) used a CER that removes cations from sludge marix leading to breakup of the flocs and subsequent release of EPS in order to extract of EPS from activated sludge. The efficiency of this method was also compared to thermal extraction method and extraction with sodium hydroxide at pH 11 by the authors. They found CER method superior to other commonly used methods in terms of minimal disruption and yield of EPS. Total cell lysis was not considered to be significant for the composition of EPS as total cell biomass was less than 10 – 15 per cent of the total organic content in the investigated sludge. Many researcher (Frølound et al., 1995; Frølound et al., 1996; Nielsen et al., 1996; Dignac et al., 1998; Bura et al., 1998; Durmaz and Sanin, 2001; Mikkelsen and Keiding, 2002).

Dignac et al. (1998) stated that CER method should be used instead of using a chemical compound (e.g. EDTA) due to these reasons:

i) contamination risk of the samples by organic compounds is limited
ii) CER is easily removed from the cells by settling.

A new method was developed by Wuertz et al. (2001) for the extraction of EPS from

sludge with dicyclohexyl-18-crown-6 ether. They compared homogenization, extraction with EDTA, and extraction by using a CER methods with this new method. For mechanical homogenization method, sludge was homogenized for a period at a low rotation speed and than centrifugation was used for the separation of EPS. They added EDTA solution to sludge in an ice bath for a few hours and centrifuged the sample after this process for extraction with EDTA. Extraction with CER was done similar to the description of Frølound et al., (1996). The effectiveness of this new method was found to be two times higher than the other two methods.

2.1.4. Effect of EPS on Sludge Bioflocculation and Dewaterability

Bioflocculation of activated sludge, which is thought to be highly affected by extracellular polymers, is very important in determining the sludge dewatering and settling. The need for the understanding of these physical properties of activated sludges lead to the research of EPS and bioflocculation mechanisms together. Generally, it is claimed that sludge dewaterability decreases with the increase of EPS in sludge by deflocculation.

Bruus et al. (1992) reported that extraction of calcium ions either by an ion exhange process, or by a chelating reagent caused deflocculation of sludge and increase in the number of small particles and release of EPS. They observed an increase in turbidity and specific resistance to filtration (SRF) indicating poor filterability.

The effect of EPS components on sludge settleability properties in terms of sludge volume index (SVI) was searched by Goodwin and Forster (1985). They reported that, no recognizable trend or relationship appeared to exist between the settlement properties and polysaccharide fraction. The proportion of lipid was found to be related to the settlement characteristics, increasing as sludge volume index increased.

The effect of pretreating activated sludge with sulfuric acid and surfactant on its EPS, centrifugal dewaterability (compaction) and settleability was studied by Chen et al. (2001). They found that, treating activated sludge with sulfuric acid ad pH 2.5 or combined with surfactant was an effective method to remove the polymers from sludge surface and this

method included the decrease of EPS, which resulted in the improvement of dewaterability and settleability.

The relationship between sludge dewaterability in terms of CST_s (CST corrected for different solids contents) and the level of EPS present in raw, waste activated and anaerobically digested sludges was investigated by Houghton et al. (2001). They stated that for each type of sludge there was an optimal EPS level at which the sludge exhibits maximum dewaterability. According to the authors, waste activated sludge had the highest optimal EPS level and lowest CST_s. Increasing EPS were thought to aid sludge dewaterability by improving the level of sludge flocculation and this decreases the number of small particles present in the sludge, increase of which are one of the reasons of poor sludge dewaterability. They found that there is a certain level of sludge flocculation and further increases of EPS become detrimental to sludge dewaterability. On the other hand, raw sludge samples were found to be harder to dewater but they showed the strongest relationship between EPS yield and sludge dewaterability. The digested sludge having the lowest EPS level did not show a good relationship between these properties. The lowest yield of EPS was attributed to the alterations in the bacterial population as conditions within sludge changed from an aerobic to an anaerobic environment. They stated that, EPS of bacteria in the aerobic phase might be used as substrate by different bacterial populations in the anaerobic phase.

2.1.5. Effect of the Composition of EPS on Sludge Dewaterability

Although many researchers investigated the effect of EPS composition on biological sludge dewaterability, there is not a precise conclusion on this topic in the literature. A strong relationship was found between the components of EPS and dewaterability of sludges especially indicating the effect of protein fraction on bioflocculation and dewaterability by some authors. On the other hand, some of the studies do not involve any effect of EPS constituents and the mechanism is still not well known. There is still a need for the investigation of this topic as it can be important to clarify the main effect of EPS on this process.

The role of extracellular protein in bioflocculation and dewaterability properties was studied by Higgins and Novak (1997). They defined the biopolymer after extraction with sodium hydroxide as “bound” biopolymer fraction. An increase in divalent cation (calcium and magnesium ions) concentration was observed in the feed to the reactors was associated with an increase in bound extracellular protein concentration, but had little effect on the bound polysaccharide concentration, indicating the important role of extracellular protein in bioflocculation. The authors observed an improvement in sludge settling properties with the increase of divalent cations. Addition of monovalent cations, displacing the divalent cations, caused a decrease in bound protein content that was explained by ion-exchange reactions and settling properties deteriorated. They stated that divalent cations proteins including lectins within the floc matrix, and lectins with their multiple binding sites might act to bind polysaccharides within the biopolymer network. The cross-linking of polysacchariders to adjacent proteins and cation bridges act to stabilize the EPS network. Higgins and Novak (1997) added protein and polysaccharide degrading enzymes and they observed a significant deflocculation and poor dewatering (in terms of CST) by the degradation of biopolymer protein and no appreciable change was seen by the degradation of polysaccharide.

Wu et al. (1982) stated that the accumulation of biopolymeric substances such as polysaccharides and polyaminoacids on the surface of sludge microorganisms determines the performance of sludge filtration besides influencing the results of cell adsorption, bridging, and flocculation. The influence of influent wastewater compositions and process operating variables on sludge dewaterability was investigated and found that the sludge filterability is a function of the ratio of carbon to nitrogen in wastewater, the concentration ratio of chemical conditioner to sludge solids and the process controlling variables such as liquid aeration time, sludge age, and food to microorganism ratio. Wastewater with either carbon or nitrogen limitation with COD/N ratios of 5.3:1 and 106:1 were used respectively for this purpose. Specific resistance to filtration (SRF) was measured in order to determine the filterability. SRF of nitrogen-rich activated sludge was found to be consistently lower than that of the nitrogen deficient activated sludge. Nitrogen-rich sludge was high in protein content and low in carbohydrate content when compared to the nitrogen-limited sludge. They also indicated that sludge with large and strong flocs had excellent filtering properties. On the other hand, when activated sludge consists of a great deal of filamentous

growth or dispersed pinpoint flocs, the sludge behaved poorly in floc formation as well as dewatering. The authors also observed that, the EPS themselves bevahed like a sludge thickening agent and great amounts of EPS minimized the conditioner demand for the minimization of SRF.

Erdinçler and Vesilind (2000) studied the effect of cell disruption on compactibility of biological sludges the increase of protein concentration in the supernatant was used as an indicator of cell disruption. The study involved desorption of extracellular polymers and release of proteinaceous materials. Compactibility of sludges were improved while there was not a strong relationship between protein concentration and CST in all disintegration methods including alkali treatment, sodium chloride treatment, sonication and heat treatment. The improvement in compactibility and decrease in filterability of samples were attributed to the hydrolization of exocellular and intracellular materials especially EPS.

Changes in the composition of EPS and bulk water in activated sludge during anaerobic sludge was investigated by Nielsen et al. (1996) in order to relate the subject to the detoriation of dewaterability of sludges which are usually stored anaerobically before the dewatering process. A fast decrease in protein and carbohydrate parts of total sludge took place within three days of anaerobic storage as a result of degradation process, which accounted for approximately 20 per cent of the organic fraction. In the EPS matrix a similar initial degradation of proteins and carbohydrate were observed, whereas the DNA and uronic acid content showed minor changes. EPS protein was also found to be difficult to extract and no changes were observed in the extractability of EPS carbohydrate. Humic acids were the only analyzed EPS constituents that were released to the bulk water in significant amounts. They concluded that, the great reduction in proteins and some reduction in carbohydrate content after a few days should have affected the floc strength, leading to deflocculation and reduced dewaterability. And assuming that the EPS protein is important in gluing the aggregated microcolonies of activated sludge flocs, they stated that the removal of protein caused a weakening of entire floc leading to deflocculation, which was also promoted by ion exchange of Ca^{2+}.

Poxon and Darby (1997) investigated the relationship of the anaerobically digested sludge samples and dewaterability and quantified the EPS using an in situ dye-adsorption

method extracellular polymeric substances. CST was used for the determination of filterability index and corrected for solids concentration and temperature. Results showed that there was not a relationship between total EPS content and sludge filterability of sludges. The clear dependence of the biopolymer-dewaterability relationship on digester feed composition suggested that specific biochemical properties of the EPS, which would be expected to vary with feed conditions, are of greater importance than the total EPS concentrations in their influence on dewaterability.

The impact of anaerobic digester retention time on EPS and sludge dewaterability in terms of CST was investigated by Houghton et al. (2000). Results demonstrated a strong relationship between EPS yield and sludge dewaterability, and dewaterability decreased with the increase of EPS in sludges. No correlation between digester retention time and dewaterability was observed. However, digester efficiency appeared to effect dewaterability, with more effective digester operation making the produced sludge easier to dewater. The carbohydrate level of EPS decreased with the increasing digester efficiency was another observation but no definite relationship was found between EPS composition and sludge dewaterability. An increase in monovalent cations in the sludge also effected extracted EPS inorganic content and gave higher CST results.

Mikkelsen and Keiding (2002) suggested that, sludges had lower shear sensitivity and lower degree of dispersion with high EPS contents and this led to better filterability in terms of SRF. A correlation was observed between filterability and cake dry matter (CDM) content in an inverse manner in activated sludge i.e. high filtration resistance led to high dry matter content. While EPS has good effect on floc stability and filterability, the cake dry matter decreased with high EPS contents. In this study, a negative correlation was also observed between EPS protein fraction and CDM. This result was attributed to the water-holding capacity of protein fraction of EPS. Besides, high degree of sludge dispersion led to an increase the CDM.

The bound water content in activated sludge increases with a change of COD:N:P ratio from 100:1:1 to 100:5:1, meaning that the bound water content increased with increasing protein portion (Bura et al., 1988).

The effect of influent organic content on anaerobically digested primary sludge EPS content and dewaterability was investigated by Houghton and Stephenson (2002). They measured sludge filterability by using CST test and determined drainability by using a gravity drainage test. The authors compared 100 % primary sludge, primary sludge fed with glucose and primary sludge fed with propionic acid. Digestion reduced the dewaterability of the organic content of digester feed had an impact on the extracted EPS yield and composition. Digestion of primary sludge and primary sludge with propionic acid caused a reduction in the EPS yield. The EPS yield from digested primary sludge with glucose increased. Hence, digestion with glucose had the greatest impact on EPS composition of primary sludge by increasing the organic fraction where digester feed of propionic acid decreased the organic content. In all cases, the protein content of the extracted EPS increased and carbohydrate content decreased after digestion. Besides, digestion with glucose led to an overall increase in the size of sludge particles.The aut, decreased sluors stated that, sludge filterability (increased CST values) was related to higher levels of EPS and an increase in the mean particle size of the sludge, with EPS composition having no effect.

Sponza (2002) studied the EPS and physico-chemical properties of flocs in steady and unsteady-state activated sludge systems, indicating that the 70-80 per cent of EPS organic material could be attributed to proteins. Low protein and high DNA levels of EPS were associated with high bound water and low negative charges in activated sludge samples, and these results did not correlate with the results of Bura et al. (1998). This was also related to high sludge volume index (SVI) value, indicating poor settleability and contradicted the report of Goodwin and Forster (1985). Besides, nitrogen deficiency increased the protein level in the EPS and was associated with good settling and high negatively charged floc surfaces in activated sludge.

2.2. Floc Formation in Activated Sludge

Activated sludge process is the most common process to treat domestic wastewater. The size of activated sludge particles varies from a few microns or less to several thousand microns. Activated sludge flocs occurs naturally in an activated sludge process

which is called bioflocculation. Bacterial flocculation has a great importance as floc characteristics directly effects the effluent quality and overall efficiency of the activated sludge process. Under normal conditions activated sludge flocculates naturally. Although many researchers investigated bioflocculation phenomenon, there still is not exact explanation of why flocculation occur naturally or why sometimes does not.

The formation of an activated sludge floc with a well-developed macrostructure can only occur when conditions are suitable for proper microstructure formation- aggregates can only form when the bacterial-sized primary particles flocculate. Additionally, filamentous and zoogleal microorganisms are both essential to the integrity of the macrostructure of the activated sludge floc. Filaments form a rigid backbone for the floc, to which flocculent zoogleal microorganisms attach like flesh on a bone (Parker, 1970).

According to Sezgin et al. (1978) if there are not sufficient filaments, the floc will be weak and subject to breakup into smaller aggregates. Such a condition is commonly referred to as pin-floc or pin-point floc. This type of sludge is characterized by quite high zone settling velocities and poor clarification above the sludge interface during settling. When there are too many filametous microorganisms and they grow out of the general confines of the floc into the bulk medium, their bridging interferes with the close approach of the flocs to produce a slowly settling, poorly compacting, bulking sludge. This is commonly referred to as filamentous bulking. The supernatant above the settling sludge interface is typically quite clear because this type of floc, with a well-developed filament backbone, is not very sensitive to floc breakup. When the filaments are present in sufficient numbers to produce an adequate floc backbone without significant outgrowth into the bulk solution, both strong and large flocs and a sludge that settles and compacts well results.

2.2.1. Bulking Sludges

While the certain amounts of filametous microorganisms enhance bioflocculation, the overgrowth of these microorganisms causes to sludge bulking problem in activated sludge systems. When there are too many filametous microorganisms and they grow out of the general confines of the floc into the bulk medium, their bridging interferes with the close approach of the flocs to produce a slowly settling, poorly compacting, bulking sludge. This

is commonly referred to as filamentous bulking. The supernatant above the settling sludge interface is typically quite clear because this type of floc, with a well-developed filament backbone, is not very sensitive to floc breakup.

2.2.2. Pin-floc Sludges

When the growth rate is too fast, floc forming species may grow in a dispersed and non-settleable form. Small, weak flocs can be formed in activated sludge that lead to a turbid effluent in the final clarifier. These small flocs termed pin floc, consist only of floc forming bacteria without a filament backbone and usually are <50μm in diameter. Pin floc occurs most commonly at starvation conditions- a very low F/M and long sludge age. Chronic toxity can also cause a pin point condition. On the other hand the absence of filamentous organisms causes to occurance of remarkably smaller flocs which are called pin flocs (pin point floc). Pin floc consists of small visible floc particles, settle poorly and remain in the supernatant after the sludge has settled.

Comas et al. (2003) stated that if only small flocs or microflocs are formed, the so-called pin-point problem is diagnosed; otherwise, if microorganisms do not form consistent biological flocs, but are dispersed, forming only small clumps (smaller than 30 mm) or growing as single cells causing uniformturbidity and hindering settleability, then dispersed growth is diagnosed. Although these two types of deflocculation are similar, their origin and microscopic observations are different. Dispersed growth is caused by a lack of production of extracellular polymeric substances (EPS), which prevents the bioflocculation. Pin-point is caused by a total absence of filamentous bacteria, which prevents the growth of activated sludge flocs; only small, compact and weak flocs are produced (smaller than 75 mm). Usually pin-point flocs are accompanied by a fraction of large flocs with a high density giving an activated sludge with good sedimentation but very poor flocculation.

Pin floc or pin-point floc is due to a macrostructure failure that usually occurs at vet low organic loading but may, in some industrial wastewater activated sludge systems, be caused by chemical interference in floc formation (i.e., dispersion). Basically, there is no macrostructure cause there are no (or very few) fialmentous organisms present. The floc

relies solely on the microstructure (glycolax) to maintain its integrity and, if this is lacking, the flocs can break up easily. The flocs are small, roughly spherical, weak and readily broken up and sheared in the aeration basin. (Jenkins et al., 1993)

Gerardi and Berger (1987) classified types of flocs that can be found in the secondary clarifier of an activated sludge plant based on the changes in the activated sludge into nine groups which are shown in the following:

- Ideal floc: Contains floc forming bacteria and filamentous bacteria that grow in balance.
- Inter-floc bridging: A filamentous floc in which the predominant filametous bacteria cause inter-floc bridging.
- Diffused floc: A filamentous floc in which the predominant filamentous bacteria produce a diffused floc.
- Viscous Floc: Contains excessive amounts of exocellular slime that is produced by the floc forming bacteria during the periods of nutrient defficiency.
- Floating Floc: Produced through the release of non-degredable surfactants and lipids by Nocardia.
- Gas Entrained Floc: Results from the entrapment of the insoluble nitogen gas released during denitrification.
- Curdled Floc: Formed by the interaction of floc-forming bacteria and relatively high concentrations of toxic substances such as multivalent cations.
- Pin Point Floc: Floc in which filamentous bacteria are absent.
- Dispersed Floc: Contains small clusters of bacterial cells, but lacking true floc particles.

2.3. Dewaterability of Biological Sludges

Dewatering is a physical process to remove the liquid portion of sludges. Huge part of the sludge is in the form of a liquid or semisolid liquid that contains from 0.25 to 12 per cent solids by weight. (Tchobanoglous and Burton, 1991). Therefore, the sludge volume reduction is a necessity in order to prevent disposing huge amounts of wastes. Sludge dewatering operation causes approximately 50 per cent of the annual operating costs of a

treatment plant. Hence dewaterability is an important parameter by environmental and economical points of view.

Mikkelsen and Keiding (2002) defined optimum sludge handling as follows:

- A low sludge mass for disposal, by means of a low solids mass and a high dry matter content of the dewatered sludge cake.
- High dewatering rate
- Low conditioner dose.

Dick and Ball (1980) summarized the factors which effects the dewatering of sludges into three categories:

- Fluid properties: bound water content, viscosity, ionic strength, and density,
- Particle properties: size and shape distribution, surface potential, surface area, and particle density,
- -Sludge properties: solids concentration, permeability, yield strength, and electrokinetic properties.

Chen et al., (1996) stated that the dewaterability of sludge can be characterized by two ways: the residue moisture amount in the sludge cake after a dewatering process and the easiness of the filtration of the sludge.

Bruus et al. (1992) stated that one of the most expensive and least understood processes in wastewater treatment is the seperation of liquids from solids from liquids. The dewatering of activated sludge is seldom ecomically and technically succesful. The absence of a scientific understanding of the dewatering process is due to the complexibility and the dynamics of the sludge matrix. This makes it difficult to find general characteristics to describe the specific sludge in terms of dewatering, for instance: particle size distribution, floc structure and composition considering the extracellular polymeric substances and presence of filamentous bacteria, bound water content, added chemicals, viscosity etc.

Vesilind (1994) classified the water in sludge into four categories:

- Free water: water that is not associated with and not influenced by the suspended solid particles.
- Interstitial water: water that is trapped in crevices and interstitial spaces of the flocs and microorganisms.
- Vicinal water: water that is associated with the multiple layers of water molecules held tightly to the particle surface. This water can be within the cells as well, as long as it is associated with a solid surface.
- Water of hydration: water that is chemically bound to the particles and can only be removed by thermal destruction of the particles.

Figure 2.2. represents of a sludge floc and the classification of water in sludge.

Kopp and Dichtl (2000) made a similar definition of the water in sludge. Their classification is as follows:

- free water, which is not bound to the particles
- interstitial water, which is bound by capillary forces between the sludge flocs
- surface water, which is bound by adhesive forces
- intracellular water

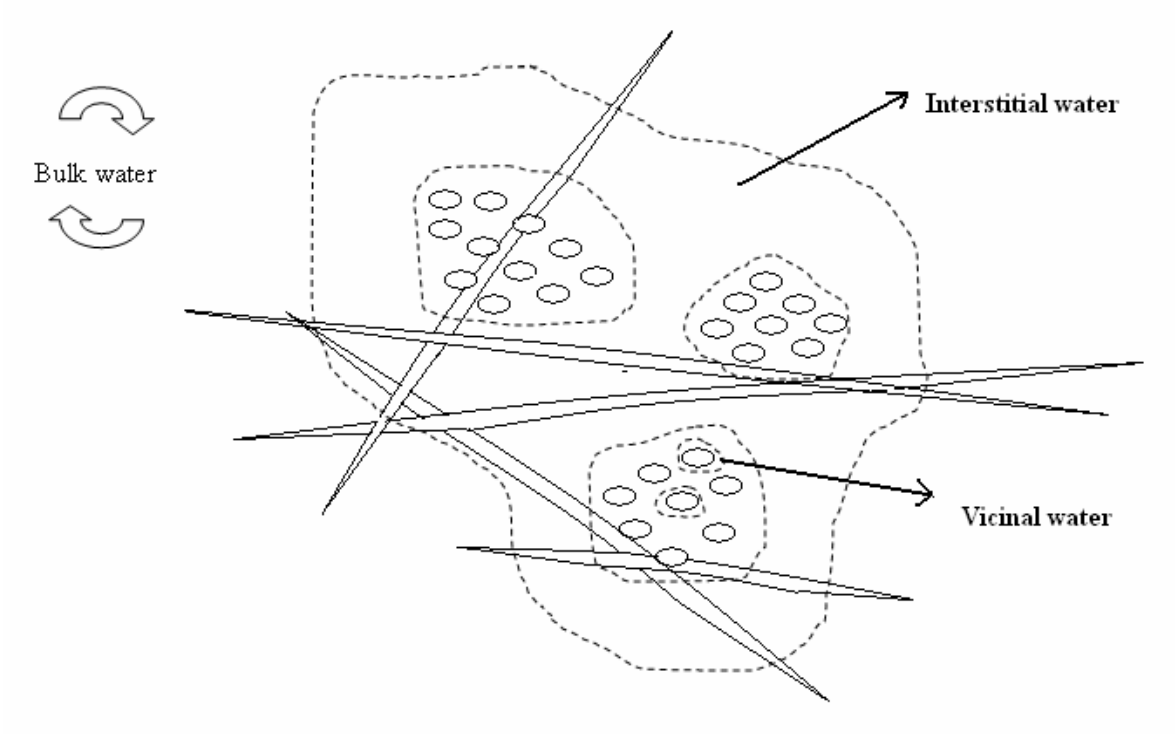

Figure 2.2. Classification of water in sludge (Vesilind, 1994)

2.4. Dewaterability Measures

Liquid-solid seperation of sludges or in other words dewaterability are indicated by three methods. The tests of spesific resistance to filtration (SRF) and capillary suction time (CST) measurement are two-well known parameters that are used for explaining the filterability of a sludge sample. Besides, compaction test is useful in measuring the compactibility of the sludge sample.

2.4.1. Specific Resistance to Filtration

Vesilind (1974) defined specific resistance to filtration (SRF) as the resistance to filtrate flow caused by a cake unit weight of dry solids per unit filter area.

Specific Resistance to Filtration (SRF) test is based on an analysis of modified form of Darcy's law for the flow of a liquid through a porous medium.

Using Darcy's law;

$$\frac{dV}{d\theta} = \frac{P}{\mu}\frac{AK}{L} \tag{2.1.}$$

V = volume of filtrate
θ = time of filtration
P = pressure difference
A = area
μ = viscosity
K = permeability
L = thickness

resistance can be defined as $R = {}^{1}\!/_{K}$ then

$$\frac{dV}{d\theta} = \frac{P}{\mu}\frac{A}{LR} \tag{2.2.}$$

in a filter, resistance is contributed by both the filter medium and cake;

$$\frac{dV}{d\theta} = \frac{P}{\mu}\frac{A}{(LR + Rf)} \tag{2.3.}$$

where Rf is resistance of filter medium (Vesilind, 1974).

The volume of the cake can be expressed as :

$$LA = vV \tag{2.4.}$$

Where v is the volume of cake deposited per unit volume of filtrate. Substituting,

$$\frac{dV}{d\theta} = \frac{PA^2}{\mu\left(RvV + R_f A\right)} \tag{2.5.}$$

When the cake is expressed as dry weight/volume instead of cake/volume of filtrate,

$$\frac{dV}{d\theta} = \frac{PA^2}{\mu\left(wrV + R_f A\right)} \tag{2.6.}$$

where

w = weight of dry cake solids per unit volume of filtrate
r = specific resistance by unit weight

Assuming constant pressure over time,

$$\int_0^\theta d\theta = \int_0^V \left(\frac{\mu wrV}{PA^2} + \frac{R_f \mu}{PA} \right) dV \tag{2.7.}$$

$$\frac{\theta}{V} = \frac{\mu rv}{2PA^2} V + \frac{\mu R_f}{PA} \tag{2.8.}$$

The plot of θ/V versus V will usually generate a linear relationship. If the slope of the line is defined as “b”, then

$$b = \frac{\mu rw}{2PA^2} \tag{2.9.}$$

and the specific persistance is (Vesilind, 1974) :

$$r = \frac{2PA^2 b}{\mu w} \tag{2.10.}$$

2.4.2. Capillary Suction Time

CST test is a more practical method when compared to the SRF test. The digital timer on the CST apparatus measures the flow of the sludge sample through a filter paper in terms of seconds. High CST values indicate hardness of the sludge to release its water and in contrast to this, sludges that allow thier water easily give low CST test results. CST apparatus is presented in Figure 3.1. and the method is described in Section 3.2.10

In order to use CST test as a fundamental measure of sludge dewaterability, Vesilind (1988) developed a mathematical model for CST.

$$X = \left[\left(D_2^2 - D_1^2 \right) \left(\frac{\pi d}{AP} \right) \left(\frac{\mu C}{t} \right) \right] \qquad (2.11.)$$

where,

X = filterability constant (kg^2/m^4s^2)
D_1, D_2 = diameters of the locations of electrical sensors (m)
d = filter paper depth (m)
A = area on the bottom of the collar (m^2)
P = capillary section, analogous to the head term in Darcy equations (m/s)
μ = viscosity of water at the temperature of the sludge sample used in the CST test (kg/sm)
C = sludge total solids (kg/m^3)
t = capillary suction time (s)

In this research, viscosity was assumed as that of water, although supertanant viscosity is known to be about 10 per cent higher than water viscosity.

Equation above was rearranged by Vesilind for a given series of tests, using a common instrument and paper and the modified equation is :

$$X = \left[\phi \left(\frac{\mu C}{t} \right) \right] \qquad (2.12.)$$

where,

ϕ = a dimensionless constant characteristics of the CST apparatus and paper used and should not vary with the sludge sample used.

2.4.3. Compactibility

One of the indicators of the efficiency of a dewatering operation is the cake solids concentration after centrifugation.

Emir (2002) stated that the compactibility is an important analysis for the determination of sludge dewaterability.

It is more feasible to use filterability and compactibility together for the measurement of sludge dewaterability and determination of optimum dewatering process (Emir, 2002).

Figure 3.2. shows the compactibility method and the method was described in Section 3.2.11 in a detailed form.

Chu and Lee (2001) stated that centrifugation of a suspension would include the following dewatering steps:

1. Centrifugal sedimentation: Since the centrifugal acceleration is generally much greater than the gravitional acceleration, sedimentation is significant in a centrifuge cell. Large particles would quickly settle towards the filter medium and a clear supernatant hence appears with an interface.

2. Centrifugal filtration: Filtrate would flow through th efilter medium owing to the pressure gradient generated by centrifugal field. Accompanied with the particle settling, the particles would form a saturated cake. The moisture inside the cake is in capillary state.

3. Centrifugal dewatering: the centrifugal pressure ovrecomes the capillary suction force to an extent and removes away to an extent the moisture existing in the cake interstices. The residual moisture is in funicular state. Collapse of void structure would also occur as well which yields the decrease of cake thickness and cake porosity.

4. Air drying: After most free water in the cake interstice has been removed, air could flow pass the void pores in the cake and carry away some residual moisture. The moisture in the cake is pendular state.

3. MATERIALS AND METHODS

3.1. Experimental Set-up

In this study, the sludge samples used for the experiments are given in the following:

Sludge 1; taken from the sequencing batch reactor of Bursa Hamitler Leachate Traetment Plant. (Pin-floc sludge)

Sludge 2; obtained from the aerobic pond of Bursa Hamitler Leachate Treatment Plant. (Pin-floc sludge)

Sludge 3; obtained from the facultative pond of Bursa Hamitler Leachate Treatment Plant. (Pin-floc sludge)

Sludge 4; waste activated sludge produced in the bench-scale batch reactor.

Sludge 5; obtained from Faco pharmaceutical industry wastewater treatment plant, located in Kağıthane-İstanbul.

Sludge 4 was generated in a laboratory scaled batch reactor. The reactor was fed synthetically. Table 3.1 shows the synthetic media that is used to feed the reactors once a day. The mean cell residence time was adjusted to 10 days. 10 per cent of the reactor content was wasted daily in order to maintain the mean cell residence time. The environmental parameters of syntatic wastewater is indicated in Table 3.2.

The typical waste activated sludge obtained from Faco pharmaceutical raw material industry was used to compare the dewatering charactersitics of sludges. The sludge sample was collected from aeration tank prior to sludge treatment units. Faco pharmaceutical industry wastewater treatment plant, located in Kağıthane-İstanbul, has both biological and chemical units.

Table 3.1. Composition of the synthetic feed solution

Nutrient	Amount (mg/L)
Glucose	350
Peptone	78
K_2HPO_4	600
KH_2PO_4	300
NH_4Cl	225
$MgSO_4.7H_2O$	112.5
$FeSO_4.7H_2O$	3.75
$ZnSO_4.7H_2O$	3.75
$MnSO_4.H_2O$	2.29
$CaCl_2.2H_2O$	19.86
$NaHCO_3$	180

Table 3.2. The quality of the wastewater in terms of selected environmental parameters

Parameter	Amount (mg/L)
COD	430
TKN-N	78
NH_3-N	57

3.2. Analytical Methods

Analytical methods used in this study is shown in Table 3.3.

Table 3.3. Analytical methods used in the study.

Analysis	Method	Instrument	Reference
COD (mg/L)	Dichromate Closed Reflux Method	HACH OD Digester HACH DR/2010 Spectrophotometer	APHA-AWWA-WPCF, Standard Methods for the Examination of Water and Wastewater, 1975.
NH_3-N (mg/L)	Preliminary Distillation Method	Gerhardt Vapodest 12 Distillation Apparatus	APHA-AWWA-WPCF, Standard Methods for the Examination of Water and Wastewater, 1975.
pH	4500-HB Method Electrometric	WTW Inolab pH meter Level 2	APHA-AWWA-WPCF, Standard Methods for the Examination of Water and Wastewater, 1975.
TS /mg/L)	Gravimetric Method	Julabo Ecotemp TW12 Evaporator, oven (103^{o}C), desiccator	APHA-AWWA-WPCF, Standard Methods for the Examination of Water and Wastewater, 1975.
MLSS (mg/L)	Gravimetric Method	Oven (103^{o}C), desiccator	APHA-AWWA-WPCF, Standard Methods for the Examination of Water and Wastewater, 1975.
MLVSS (mg/L)	Gravimetric Method	Furnace (600 ^{o}C), desiccator	APHA-AWWA-WPCF, Standard Methods for the Examination of Water and Wastewater, 1975.
EPS Extraction	Extraction with DOWEX cation exchange resin	Jar Test Apparatus F.615, Hettich Universal 16A Centrifuge	Frølound et al. , 1996.
Microscopic Observation	By Microscope	Olympus BX-50 epifluorescence microscope	
Protein (mg/L)	Lowry Method with Pierce Modified Lowry Protein Assay Kit	Shimadzu UV-120-01 Spectrophotometer	Lowry et al. , 1951.
Carbohydrate (mg/L)	Phenol-sulfuric Acid Method	HACH DR/2010 Spectrophotometer	Rao and Pattabiraman, 1989.
CST as filterability constant	Instrumental Method	CST Instrument	Vesilind, 1988.
SVI	Settling Colones	Imhoff Cones	APHA-AWWA-WPCF, Standard Methods for the Examination of Water and Wastewater, 1975.
SRF	Filtration	Mililpore Filtration Apparatus	Vesilind, 1978
Compactibility as Cake Solids Concentration	By centrifugation	Hettich Universal 16A Centrifuge	Erdinçler and Vesilind, 2000, Vesilind, 1978.

3.2.1. Chemical Oxygen Demand Analysis

Chemical Oxygen Demand (COD) Analysis was performed by using dichromate closed reflux method according to the Standard Methods for the Examination of Water and Wastewater. Samples were refluxed with potassium dichromate ($K_2Cr_2O_7$) and sulfuric acid (H_2SO_4) for two hours at 150ºC in HACH COD Digester. Interference from chloride was prevented with the addition of mercury sulfate ($HgSO_4$). Silver sulfate (Ag_2SO_4) was used as catalyst. Absorbance values was measured colorimetrically at 600 nm by using HACH DR/2010 Portable Data Logging Spectrophotometer potassium hydrogen phthalate (KHP) solutions were used for preparing calibration curves.

3.2.2. NH_3-N Analysis

NH_3-N was determined by Preliminary Distillation Method by using Gerhardt Vapodest 12 Distillation Apparatus. NaOH solution was used to adjust pH of the samples to 9.5 and samples were distilled into boric acid solution for 15 minutes. In order to measure the concentration of NH_3-N, standard sulfuric acid solution was used in titration of distillate.

3.2.3. pH Analysis

WTW Inolab pH meter Level 2 was used for pH Analysis.

3.2.4. Total Solids Analysis

Total Solids (TS) Analysis was performed according to Standart Methods (APHA-AWWA-WPCF, 1975). 20 mL of well-mixed sludge sample was evaporated on the steam bath (Julabo Ecotemp TW 12) and then dried for one hour at 103^{o}C in oven. The dish was cooled in a desiccator and weighed. TS content of the sludge sample was found by measuring the difference between the weight of (dry residue + dish) and the weight of dish divided into the volume of the sludge sample.

3.2.5. Mixed Liquor Suspended Solids Analysis

The sludge sample was filtered through a preweighed glass-fiber filter paper (Whatman GF/C) and dried for one hour at 103^{o}C in an oven. The filter paper was cooled in a desiccator and weighed. In order to find the concentration of MLSS in the sludge sample the difference between the dry weight of (sample residue + filter paper) and the weight of filter paper was divided by the volume of sample.

3.2.6. Mixed Liquor Volatile Suspended Solids Analysis

Mixed Liquor Volatile Suspended Solids Analysis (MLVSS) was carried out by igniting the residue on the filter paper used in MLSS analysis in a crucible for 30 minutes at 550±50^{o}C in furnace. The crucible with filter paper and inorganic residue was cooled in a desiccator and weighed. In order to find the concentration of MLVSS in the sludge sample, the difference between the weight of (sample residue + filter paper + crucible) before and after ignition was divided by the volume of the sample.

3.2.7. EPS Extraction Procedure

The method which was used for extraction of EPS using a CER is similar to the description of Frølund *et al*. (1996). A strongly acidic CER, DOWEX 50 * 8, 20-50 mesh in the sodium form, was obtained from Fluka.

Before the extraction process, a washing was performed to remove any EPS from bulk water of sludge. 300 mL of sludge samples was centrifuged at 2000 g for 20 minutes using centrifuge instrument (Hettich Universal 16 A). By using phosphate buffer solution, supernatants were discarded and sludge pellets were resuspended to their original volume (300 mL). Phosphate buffer solution consisted of 2 mM Na_3PO_4, 4mM NaH_2PO_4, 9 mM NaCl, and 1 mM KCl at pH 7. These samples were transferred to extraction beakers and CER was added to each sample at a dosage of 75 g/g VSS. Then, samples were stirred at 150 rpm for seven hours. In this extraction method, tightly bound EPSs were shifted from sludges to the liquid phase (PBS). Protein and carbohydrate concentrations of sludge

samples were measured continuously by taking the samples hourly from one of the two reactors of each set to determine the optimal extraction time.

After the extraction operation, sludge samples were first settled by gravity and then the supernatant was centrifuged at 3650 g for 5 minutes at 4^{o}C, in order to separate EPS/sludge suspension from CER. The supernatants were again centrifuged twice at the same speed for 10 minutes to remove remaining flocs.

3.2.8. Protein Analysis

Protein concentrations of all the sludge samples were determined by the use of Pierce Modified Lowry Protein Assay Kit, (Product No. 23240). BSA was used as standard. Test tube procedure was carried out. 0.2 ml of sample was placed in a test tube. At 15-second intervals 1.0 ml of Modified Lowry Reagent was added to each test tube. Each tube was mixed and incubated at room temperature for exactly 10 minutes. Exactly at the end of each tube's incubation period, 100 µl of prepared 1 N Folin-Ciocalteeu Raegent was added and mixed. All the tubes were covered and incubated at room temperature for 30 minutes. Shimadzu UV-120-01 Spectrophotometer was set to 750 nm and absorbance values was measured and compared to calibration curve. BSA was used as standard for preparing calibration curve.

3.2.9. Carbohydrate Analysis

Carbohydrate Analysis was carried out by a modification of phenol-sulfuric acid method described by Rao and Pattabiraman (1989). Three milliliters of concentrated sulfuric acid was added into one milliliter of sample in a glass test tube. The tubes for differnt sludge samples were left to cool and fifty microliters of 90% phenol was added into the tubes. The mixture was mixed and allowed to stand for 30 minutes at room temperature for the formation of yellow-orange color before taking the absorbance values. Absorbance values were measured at 480 nm on the HACH DR/2010 spectrophotometer and compared to calibration curve. Glucose was used for preparing calbration curve.

3.2.10. Capillary Suction Time Analysis

As described by Vesilind (1988), the filterability of sludges was measured by Capillary Suction Time Analysis (CST). To measure the sludge filterability, high and low CST values are compared. High CST values indicate the slow releasing of the liquid part of sludges and low CST values show the easy separation of sludge water. In Figure 3.1, the CST Instrument (Venture Innovations, Inc.) is shown.

The CST apparatus consists of two plastic books, a stainless-steel collar, three electrical sensors fixed on the upper plastic block, a piece of filter paper and an electrical timer. CST filter paper was obtained from Venture Innovations, Inc. (Part No: IFP-9052). The paper was placed between these plastic blocks. Two milliliters of sludge sample is poured into the collar and flowed through the paper circularly forming a wet blot in the filter paper. The diameters of first circle, which had two sensors on it, and second circle on which one sensor was present, were 3.2 cm and 4.6 cm, respectively. When the sample reached the inner circle, the sensors perceived the water and the electrical timer started to count with a signal. After a period, the sensor of outer circle perceived the liquid part of the sludge sample and the timer stopped. The period of reaching from the inner circle to the outer one was called as “Capillary Suction Time (CST)” in seconds. The CST test depends on the sludge solids concentration and the instrument being used. Temperature also affects the test due to its effect on viscosty (Vesilind, 1988).

Temperature, T, in the experiments was 25^{o}C and viscosity of water, $\mu_{water,}$ was $0.890.10^{-3}$ kg/s.m at this temperature. The constant for CST apparatus and CST paper used in this study (ø) was obtained from the manufacturer instructions and was 0.118. These values of viscosity and ø were used in the calculation of filterability constant. Filterability constant (X) was calculated by Equation 2.1. according to the CST test results in order to normalize the sludge solids of sludge samples from different reactors. The results are given in Chapter 4.

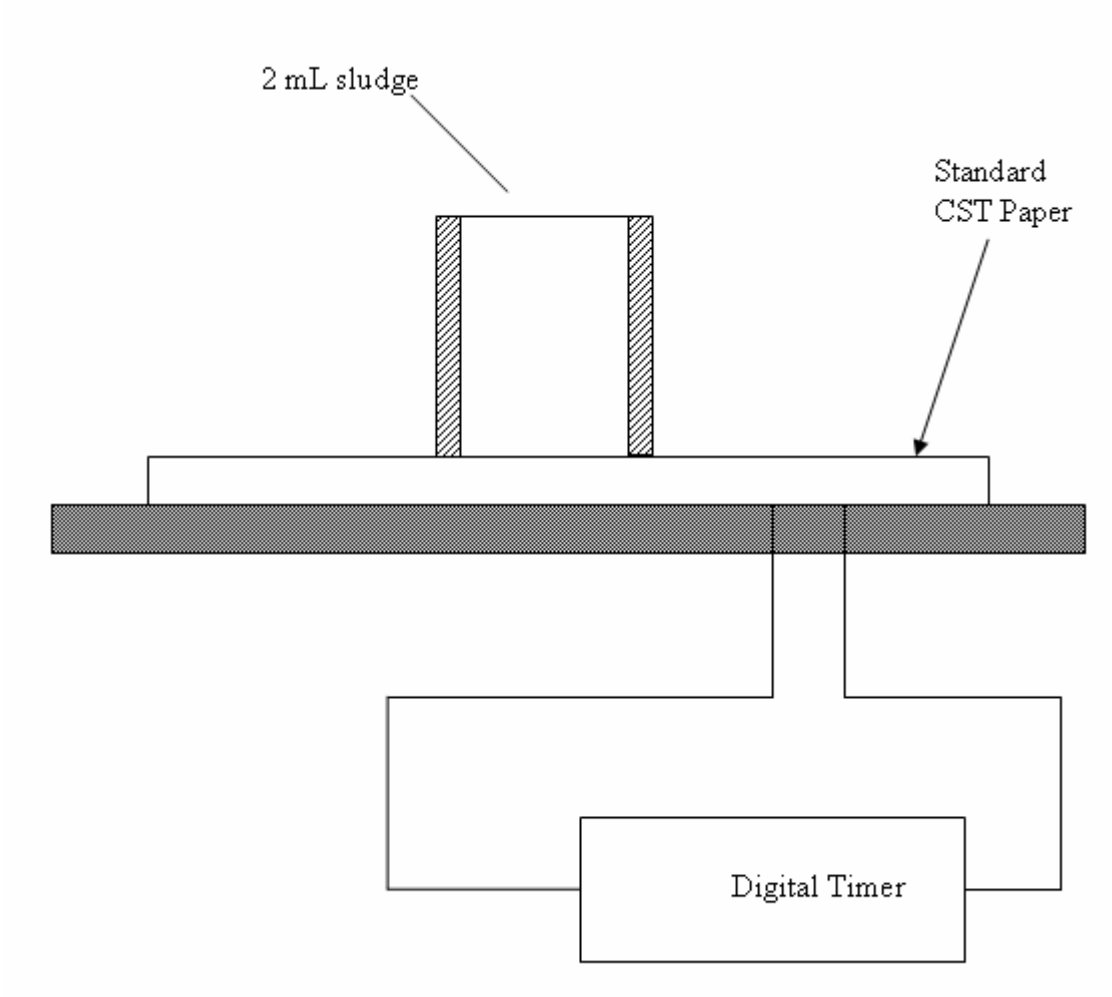

Figure 3.1. Capillary suction time (CST) apparatus.

3.2.11. Sludge Compaction Analysis

Compactibility of sludge is also considered as a dewaterability parameter together with CST. Compaction tests were carried out by using a centrifuge (Hettich Universal 16A). 10 mL samples were centrifuged at 2800 g (4850 rpm) for 30 minutes. The heights of compacted sludge layer (pellet) were measured (Erdinçler, 1996; Erdinçler and Vesilind, 2000).

The centrifugal acceleration (C.A.) calculation is given in Equation 3.1.

$$C.A.(gravities) = \left[\left(\frac{\omega^2}{g}\right).\left(\frac{r_1 + r_2}{2}\right)\right] \qquad (3.1.)$$

where,

ω = rotational speed in radians per second

g = gravitational constant (9.81 m/s^2)

r_1 = radius from centerline to the bottom of the tube (r_1=0.08 m for the above mentioned device)

r_2 = radius from centerline to the top of the sludge tube (r_2=0.135 m).

The method used for the determination of sludge compaction degree was based on the calculation of cake solids concentration as described by Vesilind (1978). The compactibility of sludges, in terms of cake solids concentration, was calculated as :

$$C_k = C_0 x\left(\frac{H_0}{H_k}\right) \qquad (3.2.)$$

C_k = cake solids concentration of sludge sample after centfrifugation (%)

C_0 = suspended solids concentration of sludge sample before centrifugation (%)

H_0 = initial pellet height before centrifugation (in this study, H_0=10 mL)

H_k = final pellet height after centrifugation (mL) (Vesilind, 1978).

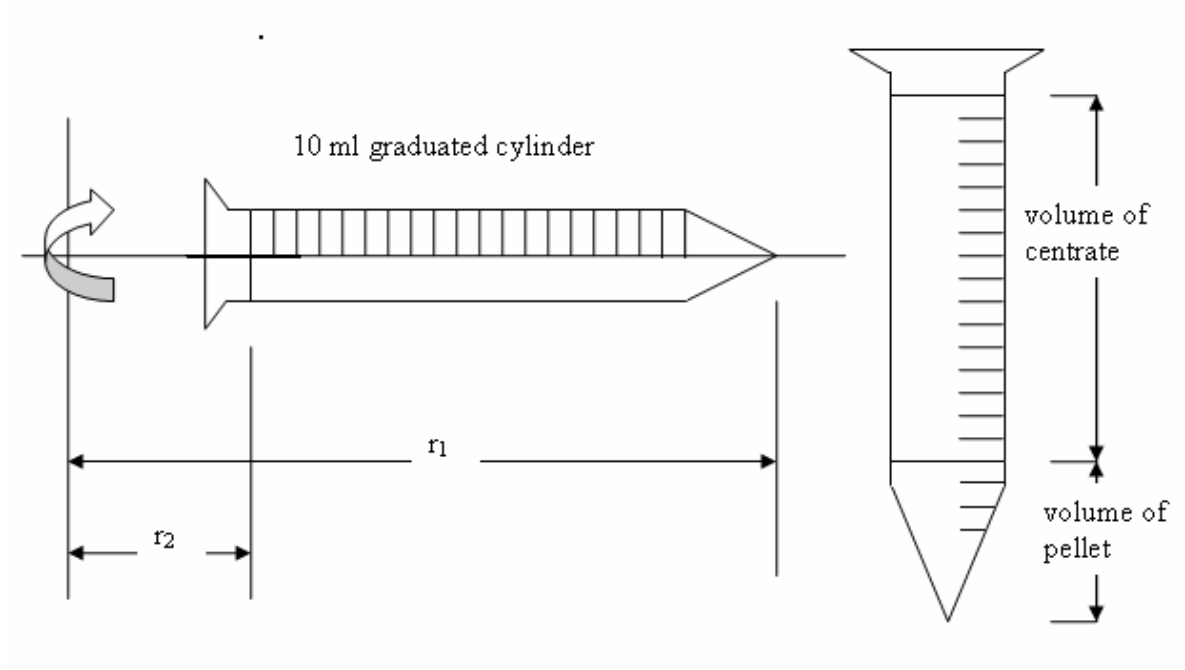

Figure 3.2. Compaction Test (Emir, 2002)

3.2.12. Specific Resistance to Filtration Analysis

In this study, Milipore Filtration Apparatus and Whatman No 1 filter paper were used to measure specific resistance. The pressure was adjusted to 800 mbar by a vacuum pump and controlled throughout the experiment by a gauge. After drainage via gravity for two minutes, the vacuum was turned on. The filtrate volume was recorded as a function of time. The test was maintained until vacuum breaks or until test has been conducted for 30 minutes. Specific resistance was calculated by the formula of:

$$r = \frac{2PA^2b}{\mu w} \qquad (3.3.)$$

where;

r	=	specific resistance (m/kg)
P	=	pressure in N/m^2 [80,000 N/m^2 (=800 mbar = 11.6 psi)]
A	=	area of filter in m^2 (1.73×10^{-3} m^2)
μ	=	dynamic viscosity (1.002×10^{-3} $N.s/m^2$ at 20^oC)
b	=	slope of the graphic of t/V versus t, s/m^6
w	=	solids deposited per volume of filtrate in kg/m^3

The "b" value was gathered from the Figure 3.3. To convert it from s/mL^2 to s/m^6, "b" value was multiplied with 10^{12} . Similarly, a solid deposited per volume of filtrate was converted from per cent to kg/m^3 by multiplying 10^4 (10,000 mg/L = 1 %).

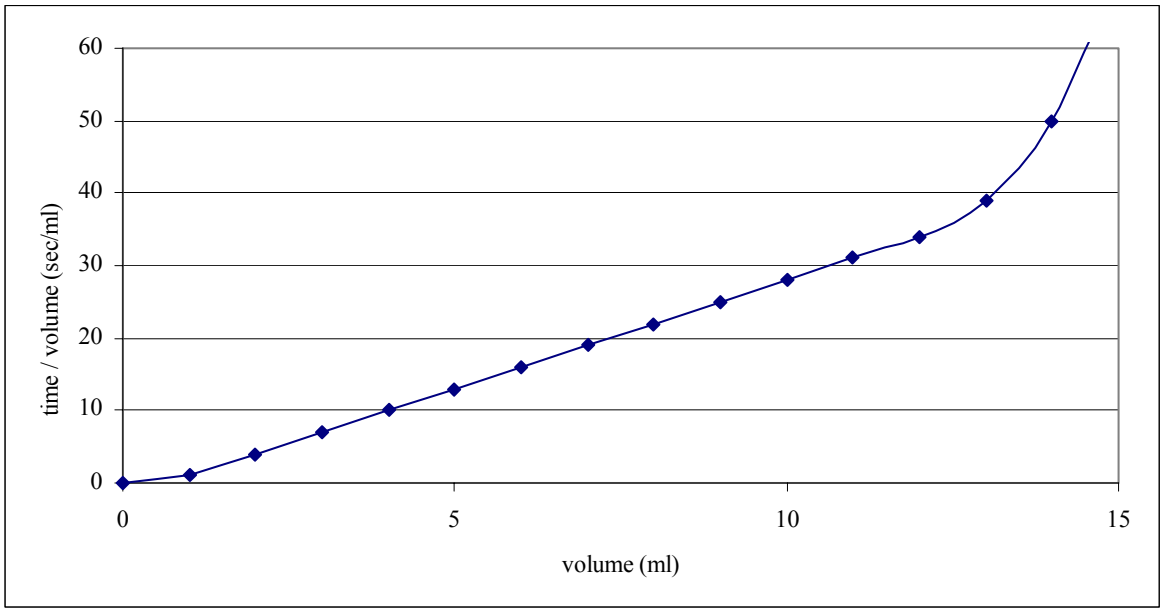

Figure 3.3. The graphics of time/volume versus volume

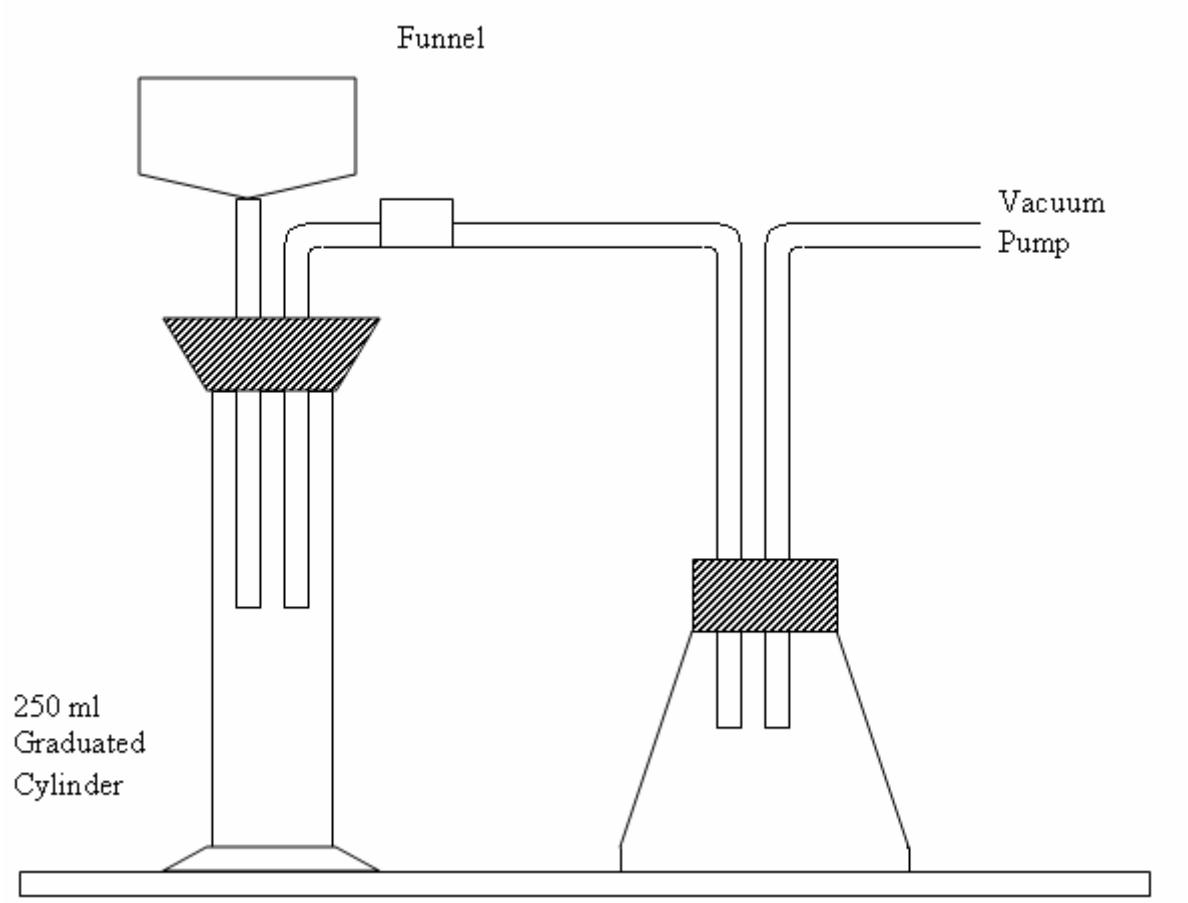

Figure 3.4. The filtration apparatus used for SRF test

Three stages of filtration, whose definitions are given below, can be seen in Figure 3.2.

i) The cake formation stage : the resistance is related to the medium and thus supercedes the resistance of the cake.

ii) The filtration step : there appears to be a linear relation between filtrate volume and the inverse flow rate.

iii) The consolidation/expression step : the flow rate ceases rapidly because the driving force is diminished strongly by the osmotic pressure of the cake (Keiding and Rasmussen, 2001).

"w" is calculated using the following equation:

$$w = \frac{c_0 c_k}{100(\ c_k - c_0\ )} \qquad (3.4.)$$

where c_0 is the percent solids in the feed sludge and c_k is the concentration of cake solids. The per cent solids in the feed sludge were determined by the total solids test (APHA/AWWA, 1989) and the steps given below was used to estimate the concentration of cake solids (Vesilind, 1978).

1) put a reasonable amount of sludge into the preweighed dish and weigh
2) dry in 105 ^{o}C oven. Cool in desiccator and weigh
3) calculate per cent solids as :

Per cent Solids = (weight of dish & dry sludge – weight of dish) / (weight of dish & wet sludge – weight of dish) x 100 (3.5.)

3.2.13. Sludge Volume Index Analysis

The sludge volume index (SVI) is the volume in milliliters occupied by one gram of activated sludge after the mixed liquor has settled for 30 minutes. 1 liter of mixed liquor was allowed to settle in an Imhoff cone for 30 min and sludge volume index was calculated by using following equation:

SVI= settled sludge volume (ml/L) × 1000/suspended solids (mg/L) (3.6.)

3.2.14. Microscopic Analysis

Type of floc structures in the sludge samples were observed by an epifluoroscence microscope (Olympus BX-50 with Olympus 40 water immersion lenses with a×10 eyepiece). The micrographs were transferred to a computer using a Spot Insight color cooled digital camera with PC archieve and measurement software.

4. RESULTS AND DISCUSSION

4.1. Determination of EPS Components

This study investigates the relationship among extracellular polymeric substances, dewaterability and sludges having different settling properties. In this study five different sludge samples were analyzed. Sludges 1, 2 and 3 were obtained from sequencing batch raector, aerobic pond and facultative ponds of Bursa Hamitler Leachate Traetment Plant respectively. Sludge 4 was produced in the bench-scale batch reactor in laboratory and Sludge 5 was taken from FAKO pharmaceutical industry wastewater treatment plant, located in Kağıthane-İstanbul. Table 4.1. shows the characteristics of the sludge samples.

Table 4.1. Average characteristics of sludges.

Sludge No.	1	2	3	4	5
pH	8.59	8.61	8.64	6.55	8.33
TS (mg/L)	16315	17588	15903	4950	12330
MLSS(mg/L)	1615	1990	1375	3130	9065
MLVSS(mg/L)	1040	1380	1280	2585	4480
TDS(mg/L)	13210	13500	13500	2990	4600
COD(mg/L)	3777	6841	4530	3156	5410
NH_3-N(mg/L)	291	684	755	20	62
COD/MLVSS	3.6	4.6	3.5	1.2	1.2
C/N	13	10	6	158	87

Protein and carbohydrate parts of EPS were measured analytically. Dewaterability was determined by measuring filterability and compactibility. Filterability was measured in terms of specific resistance to filtration. The sludge was centrifuged and the volume and

the solid contents of the cakes were measured to determine the compactibility. C/N ratios of sludge samples was measured in terms of COD/ NH_3-N.

Cation exchange resin (CER) at a dosage of 75g CER/g VSS was used for extraction of EPS of sludges. In Table 4.2 CER dosages for the sludge samples are given.

Table 4.2. Cation exchange resin dosages for the sludges.

Sludge No.	MLVSS(mg/L)	g DOWEX/L	g DOWEX/300mL
1	1040	78	23.4
2	1380	103.5	31.1
3	1280	96	28.8
4	2585	193.9	58.2
5	4480	336	100.8

After the extraction process, in order to determine the amounts of extracted $EPS_{protein}$ and $EPS_{carbohydrate}$ in the supernatants of sludges, Lowry and Phenol-Sulfuric Acid Methods were used respectively. The results of these measurements are given in Table 4.3, Table 4.4, Table 4.5 and Table 4.6. The increase in concentrations of $EPS_{protein}$ and $EPS_{carbohydrate}$ are shown in Figures 4.1 to 4.5.

Table 4.3. Increase in the protein concentrations of sludge supernatants by time in terms of mg/L.

Sludge No.	C/N Ratio	$EPS_{protein}$ (mg/L)						
		Time (h)						
		0	1	2	3	4	5	6
1	13	0	60.33	142.4	409.0	593.6	614.0	621.0
2	10	0	-	101.3	354.3	511.6	573.1	607.3
3	6	0	-	53.5	101.3	347.5	456.9	429.5
4	158	0	-	101.3	135.5	183.4	251.8	272.2
5	87	0	-	135.5	265.4	299.6	320.1	347.4

Table 4.4. Increase in the carbohydrate concentrations of sludge supernatants by time in terms of mg/L.

Sludge No.	C/N Ratio	$EPS_{carbohydrate}$ (mg/L)						
		Time (h)						
		0	1	2	3	4	5	6
1	13	0	47.6	63.55	65.98	71.18	82.92	83.02
2	10	0	13.21	16.04	17.92	23.27	36.84	36.20
3	6	0	7.12	14.95	17.42	17.52	19.11	19.60
4	158	0	13.11	29.36	45.22	58.05	84.70	85.70
5	87	0	48.14	80.59	94.31	101.70	106.35	106.65

Table 4.5. Increase in the extracted $EPS_{protein}$ concentrations of sludge supernatants by time in terms of mg/g VSS.

Sludge No.	C/N Ratio	$EPS_{protein}$ (mg/g VSS)						
		Time (h)						
		0	1	2	3	4	5	6
1	13	0	58.0	136.9	393.3	570.8	590.4	597.1
2	10	0	-	73.4	256.7	370.7	415.3	440.1
3	6	0	-	41.8	79.1	271.5	356.9	335.5
4	158	0	-	39.2	52.4	70.9	97.4	105.3
5	87	0	-	52.4	102.6	115.9	123.8	134.4

Table 4.6. Increase in the extracted $EPS_{carbohydrate}$ concentrations of sludge supernatants by time in terms of mg/gVSS.

Sludge No	C/N Ratio	$EPS_{carbohydrate}$ (mg/gVSS)						
		Time (h)						
		0	1	2	3	4	5	6
1	13	0	45.77	61.11	63.44	68.44	79.73	79.83
2	10	0	9.6	11.62	12.99	16.86	26.70	26.23
3	6	0	5.6	11.68	13.61	13.69	14.93	15.91
4	158	0	5.1	11.36	17.49	22.46	32.77	33.15
5	87	0	10.8	17.99	21.05	22.70	23.74	23.81

The optimal extraction time was determined by evaluating the rate of increase in the concentrations of extracted protein and carbohydrate parts of EPS. Optimal extraction time was selected as 5 hours due to the fact that no significant change was observed in the concentrations of EPS components. Figure 4.1., Figure 4.2., Figure 4.3., Figure 4.4 and Fig. 4.5 show changes in the concentrations of $EPS_{protein}$ and $EPS_{carbohydrate}$ with time.

Due to the release of carbohydrate and protein parts of EPS, carbohydrate and protein concentrations in the supernatants of all the sludge samples increased by time during the extraction.

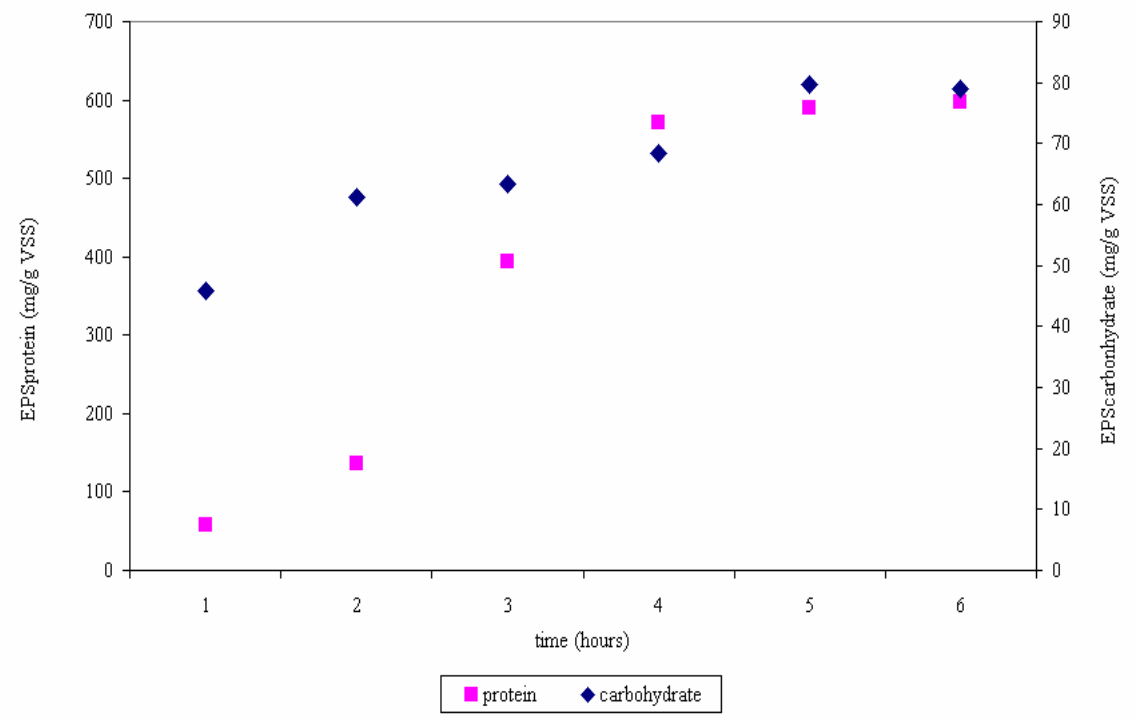

Figure 4.1.Change in the extracted EPS protein and carbohydrate in the sludge sample of Reactor 1.

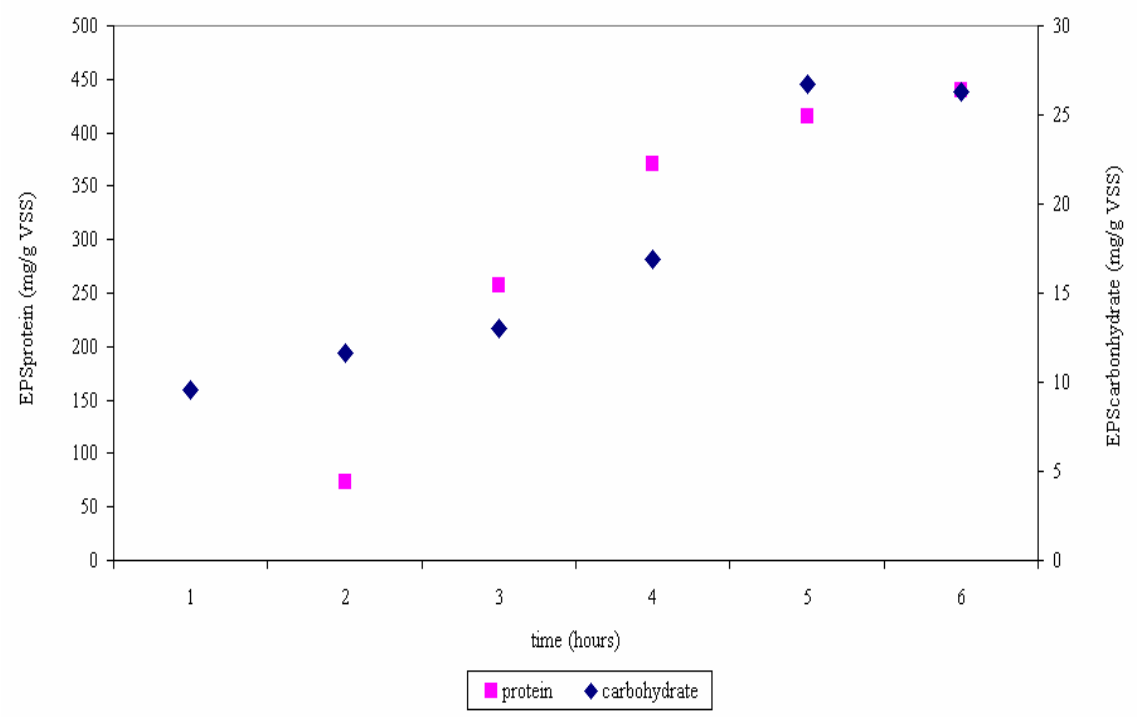

Figure 4.2. Change in the extracted EPS protein and carbohydrate in the sludge sample of Reactor 2.

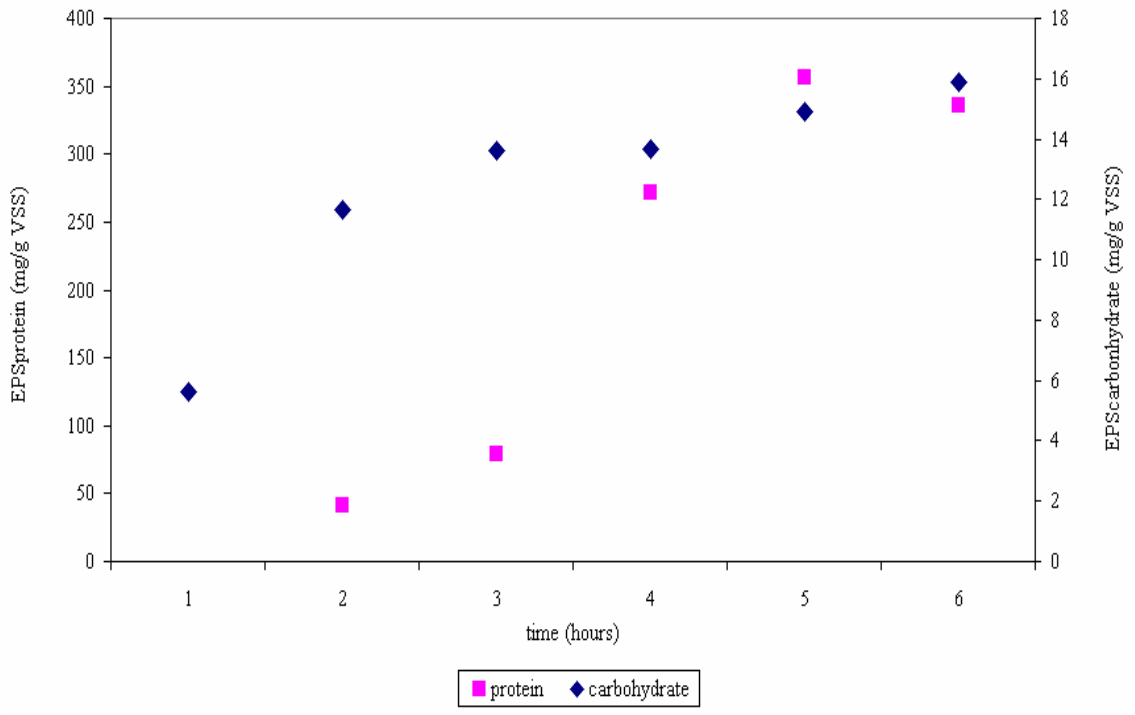

Figure 4.3. Change in the extracted EPS protein and carbohydrate in the sludge sample of Facultative Reactor 3.

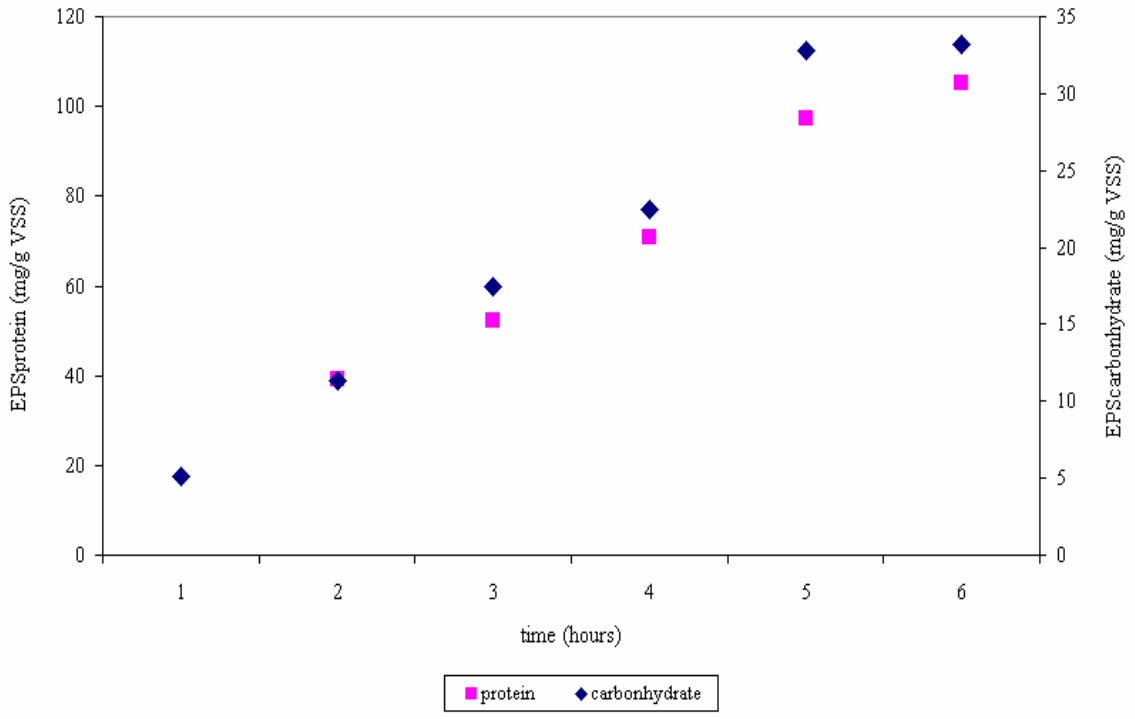

Figure 4.4. Change in the extracted EPS protein and carbohydrate in the sludge sample of Reactor 4.

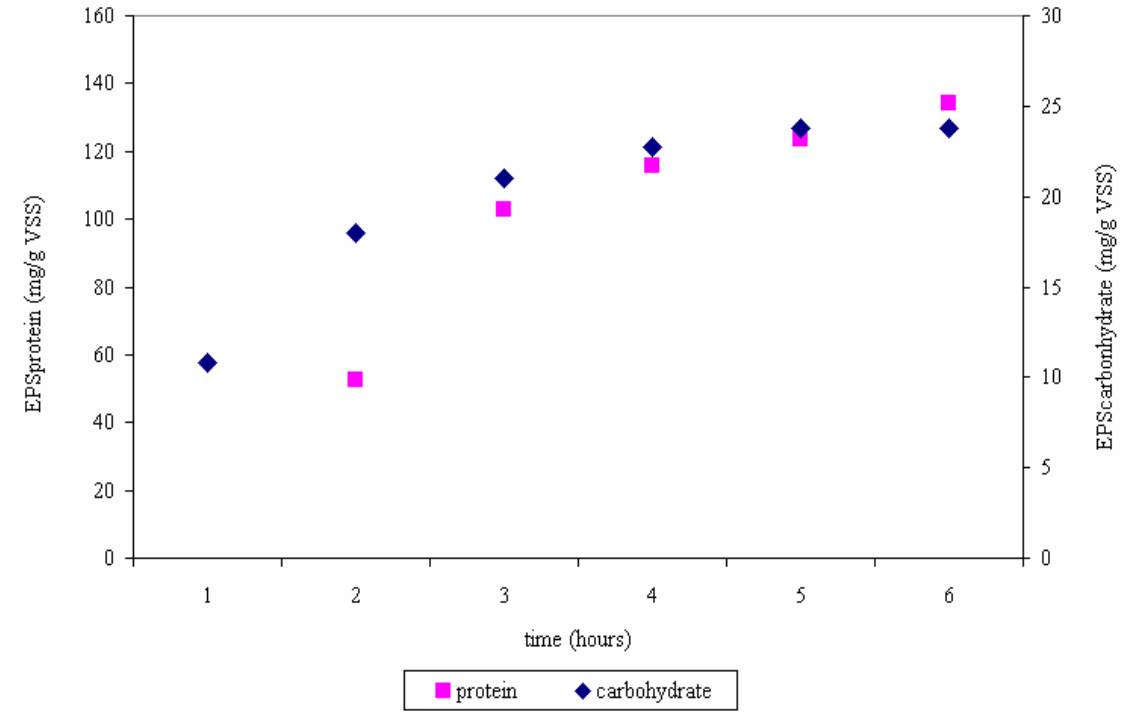

Figure 4.5. Change in the extracted EPS protein and carbohydrate in the sludge sample of Reactor 5.

It has been observed that in the pin floc sludge samples 1, 2 and 3 the proteinaceous part of EPS was much higher, 3-6 times, than that of typical waste activated sludge samples, 4 and 5.

4.2. EPS and Settleability

Sludge volume index (SVI) of the sludge samples 1, 2 and 3 were very low compared to sludge samples of 4 and 5 as a characteristics of pin-point sludge. A cloudy supernatant and visible microflocs were observed at these sludge samples. Sludge volume index values of the sludge samples 4 and 5 were found to be 71.8 and 89.3 respectively indicating good settling properties (Table 4.7). A clear supernatant and visible large flocs were observed at sludge samples 4 and 5.

Table 4.7. Sludge Volume Index (SVI) of sludge samples.

Sludge No.	mL settled sludge in 30 minutes	MLSS (mg/L)	SVI (mL/g)
1	20	1615	12.4
2	6.5	1990	3.3
3	1	1075	0.9
4	225	3130	71.8
5	790	8840	89.3

The results of sludge volume index and EPS components showed that, settleability improved considerably with decreasing concentrations of $EPS_{protein}$ (Table 4.8). Extremely low SVI values measured in sludge samples 1, 2 and 3 can be explained with the presence of pin-point flocs. Pin flocs remain suspended in the sludge without settling and led to turbidity. With decreasing concentrations of $EPS_{protein}$, sludge volume index increased to 71.8 and 89.3 indicating good settling properties for sludge 4 and 5 respectively. Analysis of EPS composition (Table 4.8) suggested that higher carbohydrate to protein ratio improved flocculation and settleability. No correlations were found between concentrations of $EPS_{carbohydrate}$ and settleability.

Table 4.8. Concentrations of EPS components and settleability.

Sludge No.	EPS_p (mg/gVSS)	EPS_c (mg/gVSS)	EPS_c/EPS_p	SVI (ml/g)	Type of flocs
1	597.1	79.83	0.13	12.4	visible micro flocs,turbid supernatant
2	440.1	26.23	0.06	3.3	visible micro flocs, turbid supernatant
3	335.5	15.91	0.05	0.9	visible micro flocs,turbid supernatant
4	105.3	33.15	0.3	71.8	large flocs, clear supernatant
5	134.4	23.81	0.2	89.3	large flocs, clear supernatant

4.3. EPS and Dewaterability

Dewaterability of sludge samples was determined by measuring their filterability and compactility. Compactibility of sludge samples was estimated by centrifuging the sludge samples at 2800g (4850 rpm) for 30 minutes and measuring the solid content and volume

of the sludge cakes after centrifugation. Filterability was determined by CST and SRF analysis.

Table 4.9. Compactibility of the sludge samples.

Sludge No.	Suspended Solids Concentration,(%)	Initial Pellet Height (H_0), (mL)	Final Pellet Height (H_K), (mL)	Compactability* Cake Solids Concentration, (%)
1	0.162	10	0.25	6.48
2	0.199	10	0.25	7.96
3	0.138	10	0.15	9.20
4	0.313	10	0.25	12.52
5	0.907	10	0.75	12.09

$$* C_k = C_0 x \left(\frac{H_0}{H_k} \right)$$

In this study, similar to Mikkelsen and Keiding (2002) stating that there was a negative correlation between EPS protein fraction and cake dry matter content, an increase was observed in EPS protein fraction with the decreasing cake solids concentration.

Sludge compactibility increased with the decreasing amounts of $EPS_{protein.}$ Cake solids concentration of sludge 4 was 12.52% while it had a $EPS_{protein}$ value of 105.3 mg/gVSS which is the lowest value of all sludge samples. Also, sludge 1 had a cake solids concentration of 6.48% while having the highest amount of $EPS_{protein}$ value which is 12.52%.

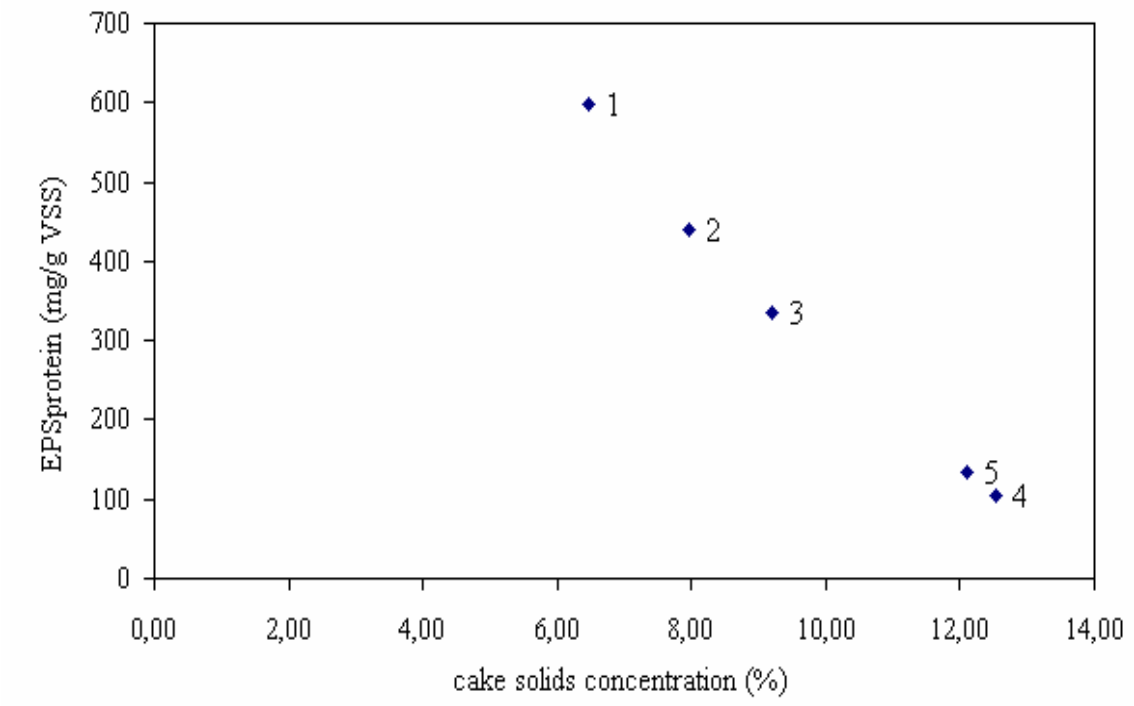

Figure 4.6. The relationship between cake solids concentration and $EPS_{protein}$.

Figure 4.7. demonstrated that specific resistance to filtration values increased with increasing amounts of $EPS_{protein}$. Sludge samples 1, 2 and 3 had very high SRF values compared to the sludges 4 and 5. High SRF values indicate the difficulty of the sludge to be filtered. During the SRF experiments, filtration time for sludges 1, 2 and 3 were too long. On the other hand sludges 4 and 5 were filtered easily. No correlations were found between $EPS_{carbohydrate}$ and dewaterability measurement methods. C/N ratio also showed no correlation with dewaterability measurement methods.

According to Table 4.10 $EPS_{protein}$ and $EPS_{carbohydrate}$ values showed no correlation with CST values. When the correlation of filterability measures was investigated, no similar tendency was observed in CST and SRF results. This can be attributed to hardness of obtaining homogenous samples especially in pin-floc sludge samples containing very small floc particles.

$EPS_{carbohydrate}$ values showed no correlation with dewaterability measurement methods. Table 4.11 shows the $EPS_{protein}$ and $EPS_{carbohydrate}$ values and dewaterability measurement methods.

Table 4.10. Protein and carbohydrate components of EPS and dewaterability measurement methods.

Sludge No.	EPS_p	EPS_c	EPS_c:EPS_p	CST ($kg^2/m^4.s^2$ x10^{-5})	Cake Solids Concentrations(%)	SRF(m/kg)×10^{11}
1	597.1	79.83	0.13	11.3	6.48	319
2	440.1	26.23	0.06	7.3	7.96	292
3	335.5	15.91	0.05	12.9	9.2	265
4	105.3	33.15	0.3	8.1	12.52	5.84
5	134.4	23.81	0.2	4.1	12.09	18.4

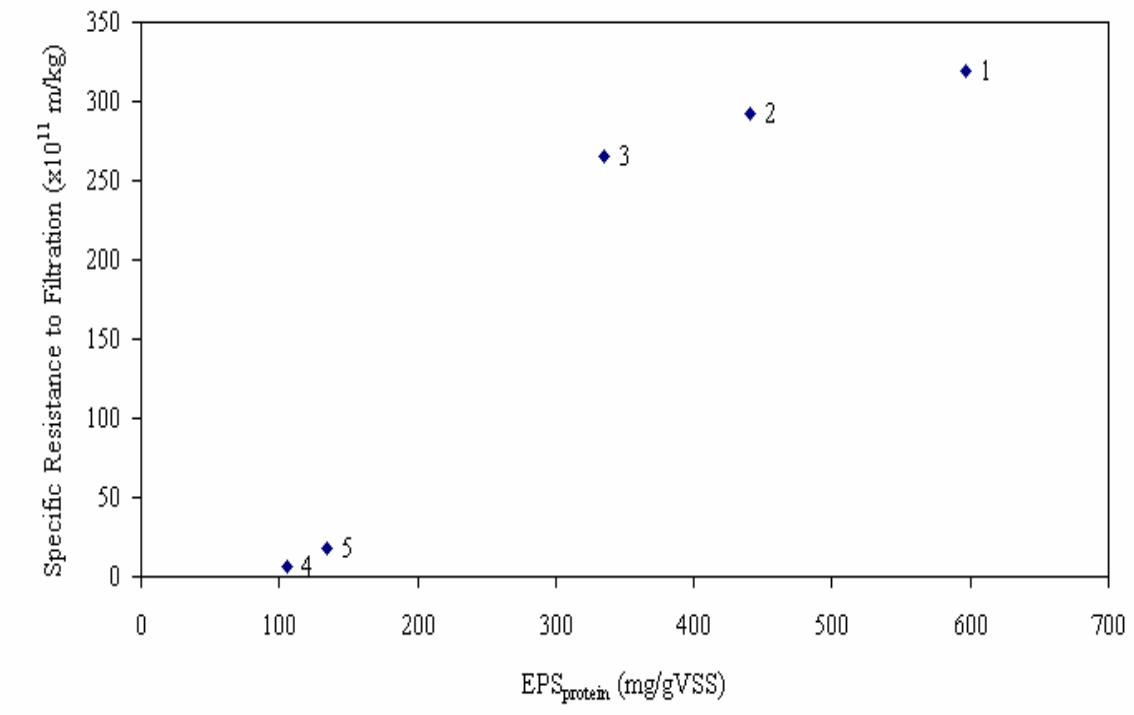

Figure 4.7. The relationship between EPSprotein and SRF of sludges.

Table 4.11. CST results of sludge samples.

Sludge No.	Total Solids Concentration,(kg/m^3)	CST[1], (sec)	Filterability Constant, X, ($kg^2/m^4.s^2$) ($x10^{-5}$)
1	16.32	15.23	11.3
2	17.59	25.27	7.3
3	15.9	12.87	12.9
4	4.95	6.43	8.1
5	12.33	31.8	4.1

[1] measured with original CST papers (Venture Innovations, Inc.) and calculated by Equation 2.12.

Sludge flocs were observed by an epifluorescence microscope (Olympus BX-50 with Olympus 40 water immersion lenses with ×10 eyepiece). Microscopic observations of the mixed liquors revealed the floc structure of each sludge sample (Fig 4.8). In the sludge sample 5 large flocs were observed (Fig 4.8d), while sludge samples 1, 2 and 3 showed microflocs indicating pinpoint floc sludge. (Figures 4.8a, 4.8b and 4.8c)

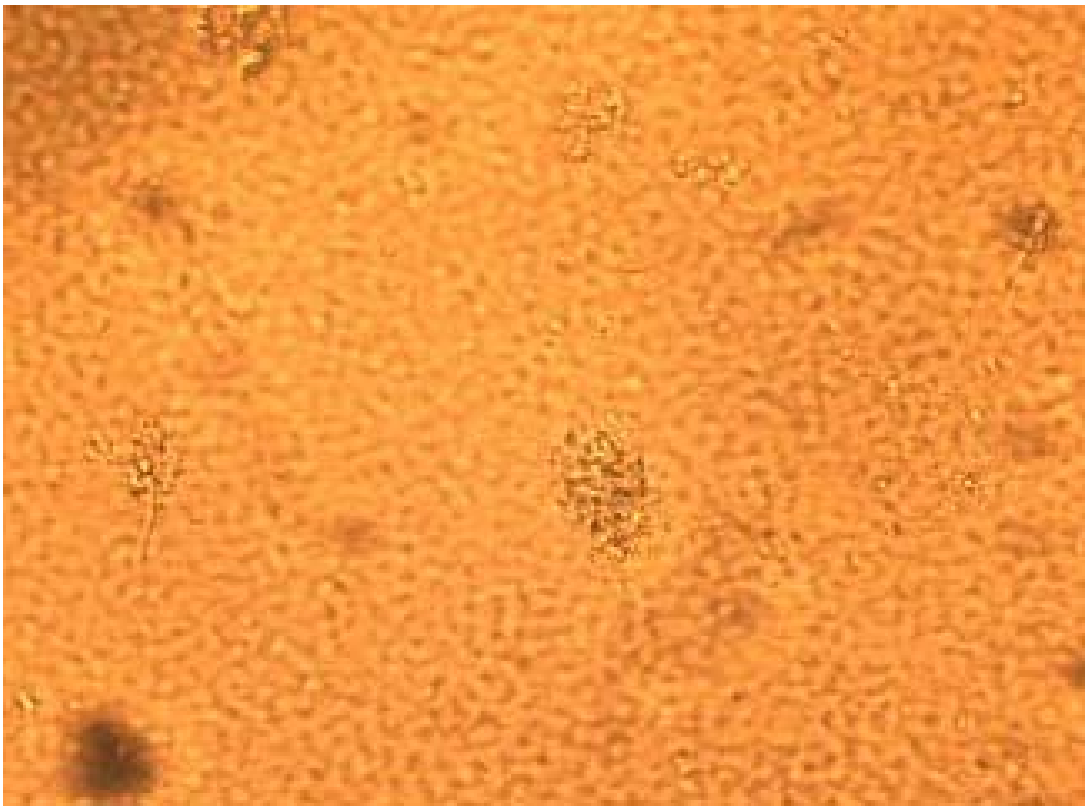

Figure 4.8a. Microscopic photograph of sludge sample 1. (×400)

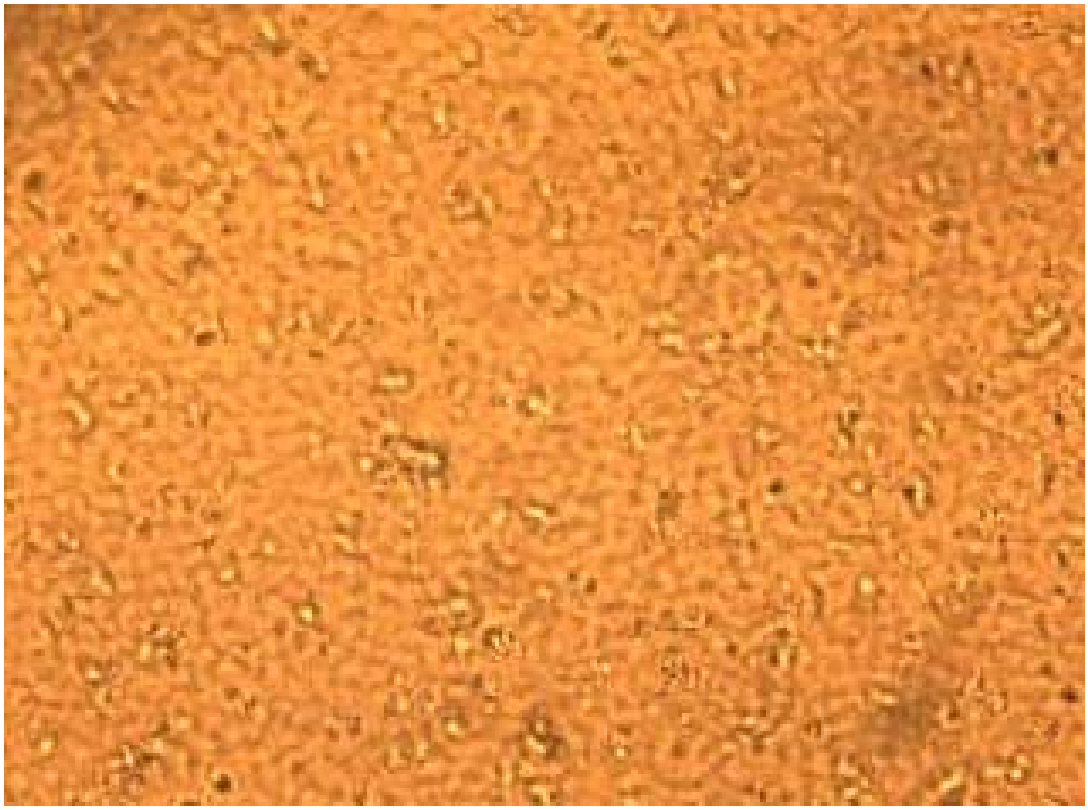

Figure 4.8b. Microscopic photograph of sludge sample 2. (×400)

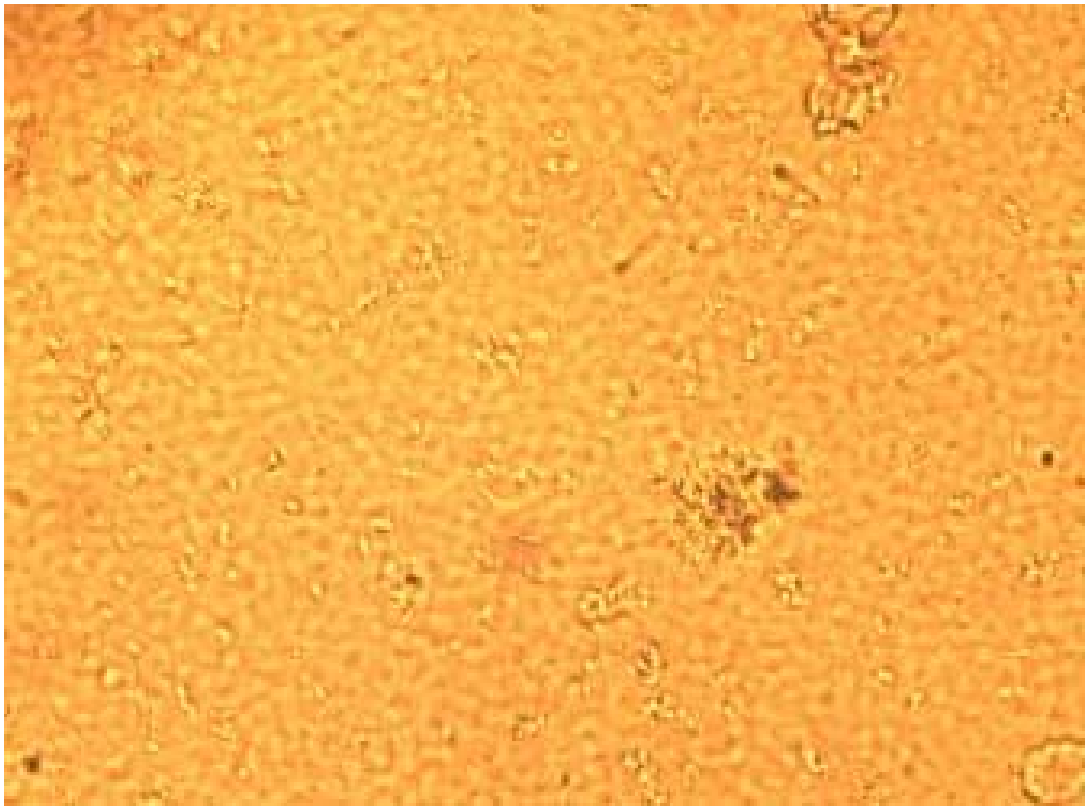

Figure 4.8c. Microscopic photograph of sludge sample 3. (×400)

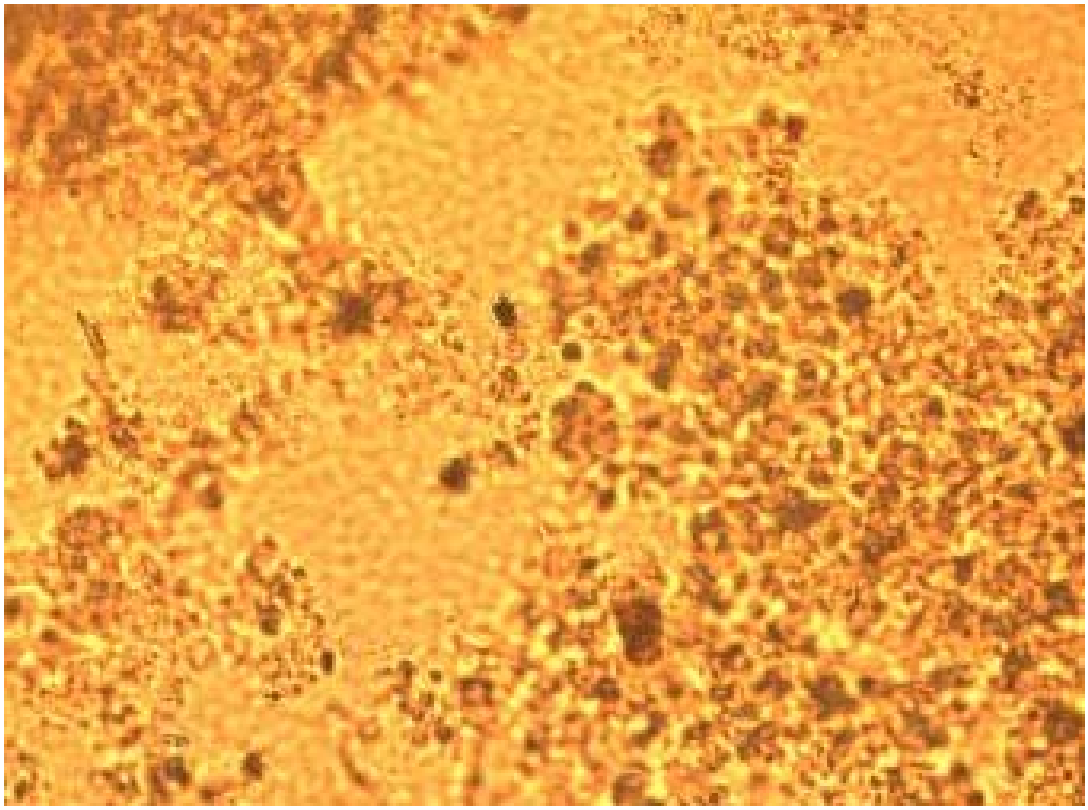

Figure 4.8d. Microscopic photograph of sludge sample 5. (×400)

In this study, it was observed that sludges 1, 2 and 3 having pin flocs had higher amounts of $EPS_{protein}$ when compared to typical WAS. Typical waste activated sludges had lower $EPS_{protein}$ concentrations and better settling and dewatering characteristics.

5. CONCLUSIONS

This study investigates the protein and carbohyrate comtonents of EPS of the non-settling sludges having pin floc characteristics and their role in dewaterability. Cation exchange resin at a dosage of 75g/gVSS was used in order to extract EPS from the sludge samples. Analytical methods were used to measure the $EPS_{protein}$ and $EPS_{carbohydrate}$. Dewaterability was determined by measuring filterability and compactibility. Filterability was estimated by measuring SRF and filterability constant, X, based on CST analysis. Compactibility was determined by measuring cake solids concentration.

The following results can be drawn based on the results of this study:

- The observations showed that flocs of sludge samples became smaller and weaker with the increasing amounts of the protein content of extracted EPS ($EPS_{protein}$).

- Filterability, in terms of specific resistance to filtration, increased by the increasing concentrations of $EPS_{protein}$. On the other hand, compactibility, in terms of cake solids concentration, increased by the decreasing concentrations of $EPS_{protein}$.

- No correlations were found between concentrations of $EPS_{protein}$ and CST.

- No similar tendency was observed in CST and SRF values. This can be explained with difficulty of obtaining homogenous samples especially in pin-floc sludge samples containing microflocs..

- Settleability improved with the decreasing concentrations of $EPS_{protein}$. Settleability of sludge samples having pin floc characteristics were very low compared to typical waste activated sludge samples having larger and stronger flocs.

- The optimal extraction time was selected as 5 hours based on the optimal time investigation tests.

- Filterability time was too long for the sludge samples having pin floc characteristics. However, typical waste activated sludge samples were filtered easily. This can be attributed to small particles of pin floc sludge samples that clog the filter paper.

- The protein content of extracted EPS, EPS_p, increased with decreasing C/N ratio of sludge. It seems that, under low carbon conditions, available carbon was used for biomass synthesis only while the nitrogen was used for the production of $EPS_{protein}$.

- The sludge cake solids concentration and the amount of $EPS_{protein}$ were inversely related to each other. This might be explained with the water-holding capacity of the proteinaceous part of EPS.

- SVI becomes meaningless to measure the settling properties of sludges having pin-floc characteristics.

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