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# Exocellular Polymeric Substances, Bioflocculation and Sludge Settling Properties in a Combined Anaerobic/Activated Sludge Process

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# EXOCELLULAR POLYMERIC SUBSTANCES, BIOFLOCCULATION AND SLUDGE SETTLING PROPERTIES IN A COMBINED ANAEROBIC/ ACTIVATED SLUDGE PROCESS

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# Table of Contents

# List of Figures

# List of Tables

# Acronyms

ABR: Aerobic Biofilm Reactor

AFBR: Anaerobic Fluidized Bed Reactor

AFB: Aerobic Fixed Bed

BOD: Biological Oxygen Demand

CER: Cation Exchange resin

COD: Chemical Oxygen Demand

DO: Dissolved Oxygen Levels

EPS: Exocellular Polymer Subtance

FCOD: Filtrate Chemical Oxygen Demand

GAC: Granular Activated Carbon

HRT: Hydraulic Retention Time

MLSS: Mixed-Liquor Suspended Solids

MLVSS: Mixed-Liquor Volatile Suspended Solids

RAS: Return Activated Sludge

SBR: Requential Batch Reactor

SCC: Solid Contact Chamber

SRT: Solid Retention Time

SVI: Sludge Volume Index

TCOD: Total Chemical Oxygen Demand

TKN: Total Kjeldahl Nitrogen

TS: Total Solids

TSS: Total Suspended Solids

UASB:  Upflow Anaerobic Sludge Blanket

UBAF: Upflow Biological Aerobic Filter

USSB:  Upflow Staged Sludge Bed

ZSV: Zone Settling Velocity

# Abstract

Combined anaerobic/aerobic processes for municipal wastewater treatment is quite recent; the studies developed have shown these processes are feasible for the removal of organic, nutrient substances and reduction of sludge produced.

Previous studies developed at the Marrero Wastewater pilot plant (fully aerobic system) revealed that the minimum solids contact chamber hydraulic residence (HRT) time in which bioflocculation occurs satisfactorily (effluent SS concentrations < 20 mg/L) is 15 min; however, in the combined anaerobic/aerobic system HRT< 100 minutes resulted in poor floc settling properties and turbid supernatants.

Exocellular polymeric substances (EPS) have been found to be the key factor for bioflocculation to occur. Past studies in fully aerobic pilot plant demonstrated that the concentration of EPS increased with mixed liquor concentration and solids retention time.

The main purpose of this research is to determine the effect of mixed volatile suspended solids(MLVSS), solids retention time(SRT), and dissolved oxygen(DO) in the production of EPS in the combined anaerobic/ solids contact chamber and its relationship with settling parameters.

To carry out the objectives of this investigation three experimental phases were developed : 1) The MLVSS concentration was varied between 1000-4000 mg/l, keeping the SRT and DO as constant as possible 2) The SRT was changed between 2-8 days, keeping the MLVSS concentration between 1500-3500 mg/l and DO between 2-3 mg/l 3) the DO concentration was varied between 0-5mg/l. For a DO of zero, EPS were extracted from the sludge produced in the anaerobic reactor.

Analysis of the data showed that the combined system proved to be unstable producing unexpected results such as no clear relationship between MLVSS and EPS. For a DO of zero, no EPS are produced and no flocculation takes place; therefore, effluents with poor quality can be expected from anaerobic treatment units. To meet secondary effluent standards in aeration chamber, capable of promoting the transformation from anaerobic to aerobic biota, and the generation of EPS, high SRT and HRT is required. Under these conditions the system anaerobic/solids contact chamber has an excellent potential for providing secondary treatment for municipal wastewater; nevertheless, the system is not as stable as the conventional aerobic one and bulking problems are common and difficult to control.

# 1. Introduction

## *1.1 Background*

Considerable interest is being shown in the field of wastewater treatment to use combined anaerobic and aerobic technologies for secondary municipal wastewater treatment. The advantages of these systems, compared to the traditional aerobic ones, are low energy consumption and the reduced production of surplus sludge. The majority of the full-scale anaerobic treatment plants that have been constructed are for the treatment of industrial wastewater. The full-scale applications of anaerobic techniques for the treatment of domestic wastewater are limited, and are mainly found in countries with a tropical climate (*Metcalf and Eddy*, 2003).

Several research projects dealing with anaerobic/aerobic wastewater treatment have been developed at the University of New Orleans at Marrero Wastewater Treatment Pilot Plant, New Orleans, LA. *Corzo* (2001) reported that the combined system Anaerobic Fluidized Bed Reactor (AFBR)/solids contact process, using activated carbon as the contact media for the anaerobic unit, has an excellent potential for providing secondary treatment for municipal wastewaters. She reported low operation and maintenance costs, and no costs associated with sludge stabilization since the waste sludge is sent to the anaerobic unit. *Bustillos* (2002) continued Corzo's research using the same combined system but with zeolite as the contact media for the anaerobic reactor. She also concluded that the combined system is highly efficient with 64% total COD, 45% filtered COD, and 92% TSS removal, and that the system reduces the amount of sludge produced.

In the solids contact chamber (SCC) the AFBR effluent is mixed and aerated with the recycled sludge, and a stable flocculent suspension is formed. The mixed liquor flows from the aeration basin to a secondary clarifier where the activated sludge is settled. The settled biomass is returned to the aeration tank to maintain the proper food-to-microorganism ratio. A portion of the thickened sludge is removed daily or periodically, from the aeration basin or from the returned sludge line, as the process produces excess biomass that would accumulate along with the nonbiodegradable solids contained in the influent wastewater.

Biological aggregation of particles involves the bonding of colloidal and suspended material into a settleable mass that can be separated through sedimentation. Under normal operating conditions, activated sludge flocculates naturally. This process, called bioflocculation, is thought to occur as a result of biopolymers secreted by microorganisms present in the mixed liquors.

The most commonly acknowledged theory, the polymer bridging model, was extended by *Parker,* et al., (1970, 1971, and 1972) who postulated the existence of two levels of structure in activated sludge flocs: the microstructure, consisting of polymer bridges between primary particles, and the macrostructure, consisting of a filament network which provides a "backbone" for the buildup of primary particle "flesh" *(Das et al., 1993).*

Jimenez (2000) concluded that bioflocculation plays a very important role in defining the quality of the final effluent, not only regarding the suspended solids concentration, but also with regard to both total COD and filtered COD.

Activated sludge flocs are made up of biological and nonbiological components. The biological components consist of a wide variety of bacteria, fungi, protozoa, and some metazoa.

The nonbiological component is made up of inorganic and organic particulates. Fungi, rotifers, and protozoa are also residents of activated sludge.

The most important parameters that are known to affect the size, structure and settleability of activated sludge flocs are: solid retention time (SRT), turbulence, dissolved oxygen concentration (DO), and mixed liquor suspended solids concentration (MLSS). According to *Parker et al*. (1970) SRT does not affect the flocculation state of the biological sludge. However, some authors (*Bisogni and Lawrence,* 1971; *Pitman,* 1975) have stated that at higher SRT the flocculation efficiency increases.

Turbulence in the SCC has been reported in terms of velocity gradient values (G). Numerous researchers have reported that excessive G values in the solids aerated solids contact chamber (ASCC) can lead to an excessive floc breakup in the system (*Parker et al*., 1970; *Das et al*., 1993).

Normally, the MLSS levels have been considered as an important factor in secondary sedimentation design only. *Tuntoolavest et al*. (1980) found that the mixed liquor suspended solids concentration to be the most important single factor, of those investigated, affecting suspended solids concentration in the effluent of an activated sludge pilot-plant.

Using an aerobic pilot plant, Jimenez (2002) found that the concentration of EPS increases as MLVSS increases. For commonly used MLVSS concentration in biological suspended-growth reactors (2000- 4000 mg/L) EPS concentration of 250 and 300 mg/L were reported. This range precisely corresponded to the lowest concentrations of supernatant suspended solids, thus demonstrating that the EPS concentration needs not to be at the maximum attainable to achieve the best effluent quality.

Another interesting point reported by Jimenez is that the EPS production approaches a maximum value at MLVSS concentrations exceeding 3,500 mg/L. Therefore, no beneficial effect could be expected on biological flocculation if the MLVSS concentration is kept higher than 3,500 mg/L. This behavior could be the result of the saturation of the available colloidal surfaces with the attached EPS chemical bridges.

Bulking is a situation affecting activated sludge solids separations. In the ideal "healthy" system, filamentous organisms grow within a floc (a large aggregate of adherent, or floc-forming, microorganisms, such as bacteria) and give it strength, with few filaments protruding out into the surrounding bulk solution. In such a system, there is no interference with the compaction and settling rates of the activated sludge prior to its recycling (*Jenkins*, 2003).

*LaMotta et al.* (2004) studied the effect of hydraulic retention time (HRT) on flocculation properties and settleability of the sludge in a combined trickling filter/solids contact (TF/SC) process. The HRT was varied between 5 and 30 min, while the other variables that could affect the bioflocculation performance (MLSS, influent COD, temperature, velocity gradient) were kept as constant as possible. For this experiment, they reported that the minimum solids contact chamber (SCC) hydraulic residence time in which bioflocculation occurs satisfactorily to produce final effluent SS concentrations of less than 20 mg/L is 15 min. However, in order to have a more stable operation the minimum hydraulic detention time they recommended is 20 min. These researchers reported a good relationship between MLVSS / EPS and MLVSS / Supernatant Concentrations (CCOD/TSS) and concluded that biological EPS excreted by microorganisms are the key to successful floc-particle aggregation.

*Bustillos* (2002) studied the effect of varying the hydraulic retention time (HRT) in the SCC of a combined AFBR/SCC pilot plant. The results showed no clear relationship between supernatant TCOD, FCOD, TSS and HRT, but the best results were obtained with a HRT of 100 minutes.

An important observation, made by *Corzo* (2001) and *Bustillos* (2002) is that the SCC receiving an anaerobic influent (effluent from the anaerobic process such as the AFBR), behaves differently from a SCC receiving an aerobic influent. While flocculation in the latter is highly successful using short HRTs, flocculation in the former requires longer contact times and is more sensitive to operating parameters such as the DO level and the SRT.

## *1.2Objectives and Scope*

Considering that EPS generation is the single most important parameter affecting flocculation efficiency, this research was focused on studying:

- The effect of operational parameters (MLVSS, SRT, DO) on EPS production and its relationship to the effluent quality.
- The possible relationship between MLSS and the sludge settling properties.

This study was conducted using a combined anaerobic/aerobic wastewater treatment pilot plant consisting of an anaerobic reactor (AFBR or UASB), an aeration chamber, and a settling tank with sludge recycle.

# 2. Literature Review

## *2.1 Combined Anaerobic/Aerobic Treatment Systems*

Traditionally, the treatment of industrial effluents with high organic matter content has been pursued in anaerobic biological reactors due to the significant economic and technical advantages that can be archived. Anaerobic treatment has been proved to have a high efficiency for suspended and soluble organic removal; however, it is inadequate for nutrient removal.

*Lacalle et al.* (2001) demonstrated that effective treatment of industrial wastewater with a high chemical oxygen demand (COD) and organic nitrogen content can be achieved with a combined system consisting of by an Upflow Anaerobic Sludge Blanket (UASB) and an Upflow Biological Aerobic Filter (UBAF).

The experiment was carried out using two biological reactors, a 10-L UASB and a 3-L UBAF. Both reactors were operated at a constant temperature of 33°C and connected in series with a recycling line from the exit of the aerobic reactor to the entrance of the UASB for the denitrification of the aerobic effluent. The recycling line had a buffer tank of 0.5 L from which the aerobic effluent was pumped to the UASB entrance. A liquid displacement device measured the biogas that was generated in the anaerobic unit. The effluent of the UASB flowed into the UBAF by gravity, where it was mixed with the air supplied by means of a diaphragm pump and the nitrification of the ammonia and aerobic oxidation of the organic matter that goes out of the UASB took place (Figure 2.1). After seven months of operation Lacalle reported 98% removal for organic matter and 91% for the total nitrogen entering the system.

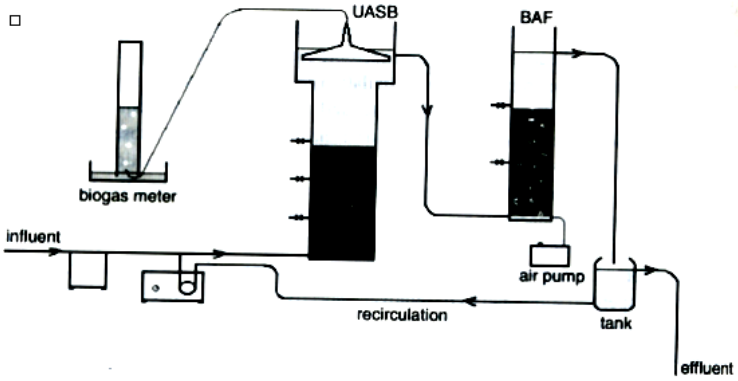



**Figure 2.1** Experimental set up UASB-UBAF (*Lacalle et al.* 2001).

Acording to *Lacalle et al.* (2001) the potential and economical advantages of the combined UASB- UBAF system are:

- It produces an effluent of very high quality that meets discharge limits for nitrogen.
- The UBAF technology allows for a better usage and control of the oxygen, and for the recirculation of a nitrified effluent to the UASB with a low oxygen content.
- The UBAF technology offers some advantages for nitrification such as very high biomass concentration (up to 30 g/L), high biomass retention time operation with low hydraulic retention time and an excellent behavior manner overcoming volumetric or substrate overloads.

*Callado et al*. (2001*)* studied the performance of an anaerobic/aerobic system composed of two sequential batch reactors (SBR) in series treating a synthetic substrate simulating domestic sewage. Both reactors have a capacity of 12.5 L and were operated for batch cycles of 12 hours receiving 8.0 L of substrate in each cycle. The first reactor (anaerobic) was fed with the synthetic substrate and was meant to remove the largest fraction of carbonic matter and promote substrate ammonification. The second reactor was operated alternating aerobic and anoxic conditions in order to achive conditions for nitrification, denitrification and biological phosphate removal in the same batch cycle. Sodium acetate was used as an external carbon source for phosphate removal. The system was operated for 41 days with 84 cycles of 12 hours, at a temperature of 28°C. The results for the 84 cycles of operation showed global COD removal of 94%, 90% nitrogen, and 90% phosphorus.

*Torres et al*. (2001) studied the operation of a pilot plant composed of a UASB reactor followed by an aerobic SBR treating domestic sewage with an intermediate tank to store the anaerobic effluent (Figure 2.2). Adopting the UASB reactor as the first biological treatment unit, the SBR was evaluated based on its performance in the supplemental removal of organic matter, nitrogen and phosphate. The pilot plant was operated at ambient temperature of 21° C ± 2°C for 6 months under different operational conditions. The UASB reactor (150 L) was operated at a constant hydraulic retention time (HRT) of 6 h while the SBR (90 L) was monitored in four different duration cycles (4, 6, 12, and 24 h) with aeration times of 2, 4, 10 and 22 h respectively.

The solid retention time (SRT) for all the HRT cycles of the SBR was approximately 30 d and the dissolve oxygen (DO) was always higher than 3.5 mg/l. After 6 months of monitoring the UASB reactor produced total COD, total suspended solid (TSS) and volatile suspended solid (VSS) removals of 65%, 66% and 62% respectively. With relation to the nutrient removal, the reactor did not remove nitrogen and phosphorus efficiently but the total Kjeldahl nitrogen (TKN) present in the influent (organic 56% and ammonium nitrogen 44%) was almost completely converted to $NH_4^+$ (97%). The combined UASB-SBR process reported high removal efficiency of COD, TSS, VSS, TKN for aeration times greater than 10 h. Complete nitrification for aeration time greater than 4h and phosphate for aeration times less than 2 h.

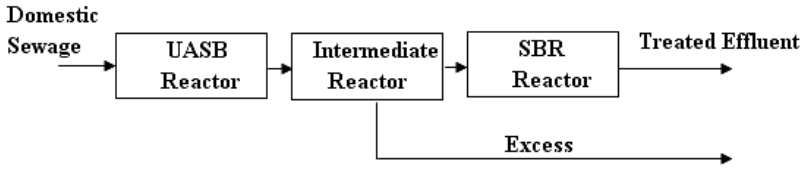



**Figure 2.2** Schematic layout of the experiment system UASB- SBR (*Torres, et al*. 2001).

*Collovignarelli et al*. (1990) also developed a research project using an anaerobic/aerobic pilot plant treating municipal sewage wastewater. The most important aims of their research were to reduce energy consumption, environmental impact, quantity of stabilized sludge produced, and area necessary for plant construction. The pilot plant consisted of a UASB reactor, with a capacity of 336 $m^3$. Part of the effluent was conveyed to an Anoxic Biological Fluidized Bed (ABFB), 8 $m^3$ capacity with 3 $m^3$ of quartzite sand, for pre-nitrification and finally to an Aerobic Biofilm Reactor (AFB), with random plastic media and a capacity of 8 $m^3$, for nitrification.

The results of the research conducted using these processes are encouraging both with regard to the removal of organic and nutrient substances, and to the reduction of well- stabilized sludge production. *Torres et al*., (2001) showed that the combined system is more attractive than the use of only anaerobic or aerobic units, because it is more flexible, efficient and economical to treat domestic wastewater.

*Jeníček et al*. (1999) demonstrated that the combined anaerobic treatment of the wastewater with the excess aerobic sludge could be carried out using the combined anaerobic-aerobic processes. In their study, these researchers treated artificial glucose based wastewater using a lab scale 4.0- L upflow staged sludge bed (USSB) divided into five compartments followed by a 0.5- L aerobic ABR. The USSB was operated in the mesophilic temperature range (35°C). Three different alternatives were carried out to optimize the performance of the USSB with respect to wastewater treatment and sludge stabilization. The difference between alternatives was basically the compartment into the USSB for the sludge, nitrified effluent from the aerobic unit and the influent wastewater.

Through this research, *Jeníček et al*. (1999) concluded that the combination of anaerobic treatment and biological sludge could have economic and ecological benefit. They reported organic and denitrification removals of 99.1% and 92.8% respectively.

*Corzo* (2001) reported that the combined system Anaerobic Fluidized Bed Reactor (AFBR)/solids contact process has excellent potential for providing secondary treatment for municipal wastewaters. *Bustillos* (2002) studied the effect of HRT on the effluent quality reporting that the efficiency of the anaerobic/aerobic process was higher at 100 minutes with 64% TCOD, 45% FCOD, and 92% TSS removal.

Even though the combined anaerobic/ SCC system was reported as feasible for the removal of organic matter, flocculation difficulties were apparent in the transition from anaerobic to aerobic conditions when compared to the studies developed by Jimenez (2002) and Rojas (2004) in a fully aerobic system. The fully aerobic system was reported to be efficient at HRT as low 30 min whereas Bustillos reported that low hydraulic retention times (60, 40, 20 min.), caused diminished settling properties and turbid supernatants were highly turbid.

## *2.2 Anaerobic Treatment*

Anaerobic treatment has been the technology traditionally selected for the stabilization of municipal sludge. The application of this technology for the treatment of industrial wastewater has been possible due to advances in new reactors design, in cluding the upflow anaerobic sludge bed (UASB) and the anaerobic fluidized bed reactor (AFBR). However, additional additional treatment, either physical or chemical or aerobic biological treatment is usually needed if the plant effluent is discharged directly to a receiving stream.

Anaerobic treatment involves the decomposition of organic and inorganic matter in the absence of oxygen. In a closed system, organic matter is converted by bacteria to a variety of end-products, including methane and carbon dioxide.

The early digester designs consisted of continuous stirred tank reactors in which effective contact between the waste requiring treatment and the active microbial is achieved by a long retention time within the reactor. Therefore, a large digester volume is required.

Recently, industrial wastewater treatment has been facilitated by the development of high-rate anaerobic reactors that achieve significantly high solids retention time (SRT) while using low HRT. Such differentiation allows the slowly growing microorganisms to remain within the reactor independently of the wastewater flow, thus allowing application of higher volumetric loading rates.

According to *Iza et al*. (1991) the concept of high-rate anaerobic reactors is based on three fundamental aspects:

- Accumulation, within the reactor, of biomass by means of settling, attached to solids (fixed or mobile), or by recirculation. This type of system allows the retention of slowly growing microorganisms by ensuring that the SRT is longer than the HRT.
- Improved contact between biomass and wastewater, overcoming problems of diffusion of substrates and products from the bulk liquid to biofilms or granules.
- Enhanced activity of the biomass, due to adaptation and growth.

*Iza, et al*. (1991) described different types of reactors, among them the UASB and the anaerobic fluidized bed reactor (AFBR).

### *2.2.1 Anaerobic Fluidized Bed Reactors (AFBR)*

The AFBR is a fixed-film reactor that fosters the growth of microorganisms on a hydraulically fluidized bed of media, usually sand or activated carbon. This process is similar to the packed-bed reactor in many respects, but the packaging medium is expanded by the upward movement of fluid (water) through the bed. The fluidized flow is generally produced by a combination of the influent and recirculation flow-rates (*Iza*, 1991).

The fluidized media provides an extremely large surface area on which a film of microorganisms can grow thus providing high concentrations of biomass. Because a large biomass can be maintained, the expanded-bed process can also be used for the treatment of municipal wastewater at very short hydraulic detention times of 5 – 10 hours, with BOD removals of 85 percent *(Robinson et al., 1997).*

Depending on the type of media used, the particles are fluidized by high upflow liquid velocities, around 20 m/h, to provide about 100 percent bed expansion (*Metcalf and Eddy*, 2003).

The fluidization process starts when an increasing flow of liquid passes through a bed of particles causing the bed to expand and the particles get suspended allowing them to freely move with respect to each other. After passing the threshold, which causes the fluidization the distance between particles increases because the bed is expanded and excess flow passes through the bed.

According to *Fan et al.* (1984) smooth fluidization, with homogeneous expansion, occurs if particles are uniform, and fluidization with a tendency to segregation, if particles are heterogeneous. The size distribution of particles plays an important role in the fluidization of the bed. If broader ranges are used, the smaller particles are highly fluidized, even washed out from the reactor, whereas the bigger remain non-fluidized, forming a fixed bed (*Shie et al.,* 1984).

Some of the design considerations for AFBR are: (*Iza,* 1990)

- AFBR usually operates with moderate to high recycle ratios: the effluent is recycled to achieve the upflow liquid velocity needed. Since the effluent has a relatively high alkalinity, when mixed with the incoming influent, it eases the pH control of the process.

- Since the recycle flow rate is higher than the influent flow rate, it is recommended to introduce the influent stream just before the recycle pump.
- AFBRs operate in meshophillic and submesophilic conditions. If the temperature decreases, so does the efficiency.

The selection of bed material for film support should consider several important factors that affect equipment size, the biological process, and process operation. The heavier the media the more expensive it is to fluidize it, thus influencing the economy of the system.

Granulated activate carbon (GAC) has been used in many AFBR for treating industrial and hazardous waste streams. The mean diameter of the GAC particle is 0.6 to 0.8 mm and the upflow velocity is 20-24 m/h. The main limitation of GAC is the higher initial cost. (*Metcalf and Eddy*, 2003)

Some of the benefits of using this type of media are:

- Higher biomass concentration maintained due to the porous structure of GAC.
- Adsorption properties help prevent toxic and inhibitory substances from decreasing biological treatment performance.
- Adsorption properties may minimize shock loads by sorption of increased organics.
- Adsorption properties may help acclimate and enhance biomass degradation of toxic compounds by providing more time of exposure.

Previous research using activated carbon (*Hanaki et al.,* 1997) has demonstrated that this media not only functions as media for bacterial attachment, but it also works as adsorbent. It has also been reported that GAC could stabilize the impact of influent fluctuation more rapidly and effectively than bacteria.

*Padron,* (2004) investigated the rates of accumulation and removal of suspended solids in the AFBR using GAC as media, in a combined anaerobic fluidized bed reactor / solid contact chamber pilot system in which the sludge generated during the process is recycled to the anaerobic unit. He reported TSS removal efficiency of about 32%. Of the solids removed by the unit, 15.8% were degraded by the action of microorganisms, and the remaining 16.3% built up in the unit. At the applied solids load (1.09 kg SS/$m^3$.d) an accumulation rate of 76.78 g SS/d and degradation rate of 74.50 g SS/d was obtained in the unit. Therefore, at the applied solids load of 1.09 kg SS/$m^3$.d, 0.173 kg SS/$m^3$.d were consumed, and 0.173 kg SS/$m^3$.d accumulated in the bed and eventually would need to be removed.

*Iza, et al.* (1988) stated some of the advantages of the AFBR:

- Bigger surface area over which the adhesion takes place
- Higher biomass concentration than in suspended biomass systems, allowing operation with high organic loading rates resulting in smaller reactor volume
- Clogging problems minimized
- Low strength organism substrates can be treated because turbulence around the particles increases substrate transfer
- The pressure loss in the bed is low; therefore, there is low energy consumption
- The concentration of VSS in the effluent is low.

Some of the disadvantages of this process are the following : (*Metcalf and Eddy,* 2003)

- Care must be taken in the inlet and outlet design to assure good flow distribution
- Pumping power is required to operate the fluidize bed
- Cost of reactor packing
- One of the main problems of AFBR is the control of the biomass growth. Excessive biomass growth leads to the formation of thicker sludges that are not well attached to the support material. The collision due to the high upflow velocity can cause detachment of big part of the biofilm which can be washed away from the reactor. Another negative effect is that bioparticles with different thickness have different physical properties and thus different fluidization properties.
- To prevent the formation of thicker biofilms, the expansion of the bed should be kept constant, by wasting the excess of thicker sludge biofilm particles.
- Long of startup times are required

### *2.2.2 Upflow Anaerobic Sludge Blanket (UASB)*

One of the most notable developments in anaerobic treatment process technology was the UASB reactor developed by Dr. Gatze Lettinga in the late 1970s (*Metcalf and Eddy*, 2003). The key feature of the UASB process that allows the use of high volumetric COD loadings compared to other anaerobic processes is the development of a dense granulated sludge. The wastewater passes upwards through an anaerobic sludge bed where the microorganisms in the sludge come into contact with wastewater-substrates. (*Metcalf and Eddy,* 2003).

The resulting anaerobic degradation process typically is responsible for the production of gas (e.g. biogas containing $CH_4$ and $CO_2$). The upward motion of released gas bubbles causes hydraulic turbulence that provides reactor mixing without any mechanical parts.

The UASB concept was born out of the recognition that inert support material for biomass attachment was not necessary to retain high levels of active sludge in the reactor. Instead, the UASB concept relies on high levels of biomass retention through the formation of sludge granules. When the UASB concept was developed, Lettinga took into account the need to encourage the accumulation of granular sludge and discourage the accumulation of disperse sludge in the reactor.

First proposed for the treatment of high strength industrial wastewater at mesophilic temperatures, the UASB configuration satisfies the main characteristics required for biological treatment systems to be simple and efficient, (*Foresti,* 2002)

- High biomass concentration inside the reactor propitiating high SRT
- Development of structured multi-cellular aggregates in form of granules or dense sludge, composed of different species of microorganism groups responsible for the conversion of organic matter into methane and carbon dioxide
- Low requirement of nutrients and low excess sludge production.
- High stability in response to normal fluctuation of influent composition and concentration
- Capacity of accommodating high organic loading rate (OLR)
- Lower cost of construction, installation and operation than the conventional aerobic units, because the reactor does not require equipment for process maintenance and control.

The performance of the UASB configuration treating industrial wastewater, at the mesophilic temperature range and high OLR, induced researchers to apply it to domestic sewage treatment at the beginning of the 1980s ( *Foresti,* 2002). Nowadays, hundreds of UASB reactors are used in domestic sewage treatment systems, particularly in developing countries. At the beginning, the results of this process were not as satisfactory as expected due to the differences between industrial and domestic sewage, specially high fraction of particulate COD, presence of fatty compound, proteins, etc. These characteristics impose limitations on the anaerobic process with respect to COD removal efficiency and in terms of organic load rate and HRT applied. For these reasons the need of post-treatment in many situations is required.

*Florencio, et al*. (2001) developed a research with a full scale UASB plant in Brazil operated at ambient temperature. The main idea of the research was to monitor the performance of the reactor to determine if the technology was feasible. After 30 months of study, the reactor showed to be stable, regardless of the fluctuation in the influent characteristics. For organic load ranges of 0.5-2.5 kg COD /$m^3$d, removal efficiencies for COD were from 60%- 90 %.

*Lettinga, et al*. (1983) reported removal of 65-85% for COD using a UASB process treating domestic sewage using a granular seed sludge cultivated using sugar beet waste. Regarding the results obtained, anaerobic treatment of raw sewage not only looks attractive for tropical areas but also for moderate climate areas.

The start-up period has been considered a crucial step for the stable operation of anaerobic reactors. One of the main points frequently stressed is the need for the reactors to be inoculated with high quality methanogenic sludge. Recent studies on full-scale anaerobic reactors treating domestic sewage have shown that inoculation can be neglected (*Passig et al.,* 2000). Even very poor anaerobic sludge can be used as inoculum (*Rodriguez, et al*. 2001). However, it has been verified that the start- up period can last up to six months if no inoculum is added.

In *Rodriguez et al*. (2001) research, the reactor was seeded with a deficient quality inoculum. First the sludge (inoculum) was sequentially washed at different upflow velocities (selective pressure method). After being washed, the reactor was started with a initial HRT of 24.9 h, which was reduced to 6.7 h at the final stage. Along the starting- up phase, there was a positive evolution in terms of quantity, quality and spatial distribution of the sludge. Therefore, a positive evolution of organic matter removal mechanism was achieved. For HRT above 14 h the removal was mainly physical and for HRT below 9 h the removal mechanism was mostly biological. After this study *Rodriguez, et al*. (2001) concluded that the start-up of an UASB reactor for domestic sewage treatment seeded with low quality inoculums could be done with HRT as low as 15 or 12 hr. In this way it was possible to reduce the starting-up period of these reactors down to 4-6 weeks, provided that the starting methodology is properly applied.

Further studies related to the effect of temperature on the performance of UASB treating municipal wastewater reveal that that COD removals efficiencies in the ranges of 70-90% can be achieved up to HRT of 6h and 11°C (*Singh, et al.* 2003). The performance of UASB reactor proved not to be too efficient at 6 °C (COD removal 40%). This study revealed that UASB systems could be applied successfully for pre-treatment/treatment of municipal wastewaters under low-temperatures conditions.

## *2.3 Aerobic Biological Flocculation*

The primary purpose of biological wastewater treatment has been to covert the particulate organic matter in wastewater into flocculant settleable biological and inorganic solids that can be removed in sedimentation tanks (*Metcalf and Eddy,* 2003)

The conversion of readily biodegradable matter is carried out by bacterial cultures that hydrolyze and oxidize the incoming readily biodegradable organic matter, producing new growth while consuming dissolved oxygen and generating inert particulate organic material (*Henze, et al*. 2000).

In the aeration chamber, contact time is provided for mixing and aerating the influent wastewater with the microbial suspension (MLSS). The mixed liquor flows from the aeration basin to a secondary clarifier where the activated sludge is settled. A portion of the thickened sludge is removed daily or periodically, from the aeration basin or from the returned sludge line, as the process produces excess biomass that would accumulate along with the nonbiodegradable solids contained in the influent wastewater. *(Qasim, 1985)*.

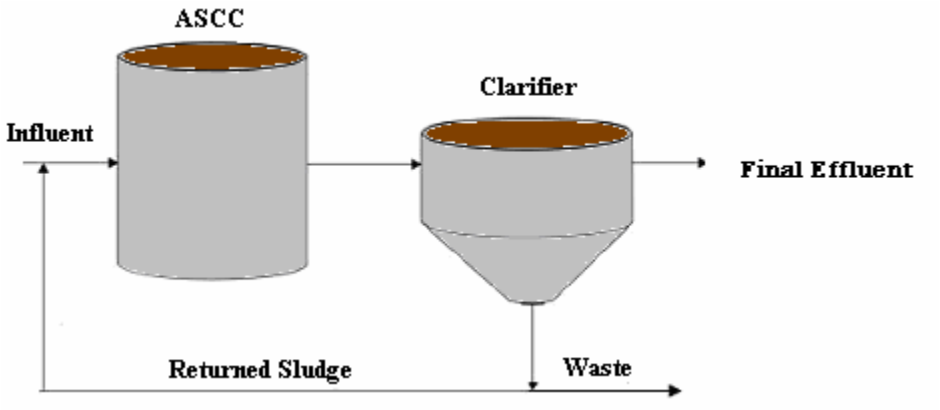



**Figure 2.3** Complete mix activated sludge process.

The aeration chamber must supply sufficient air to reverse the anaerobic condition of the particles and prepare the solids for bioflocculation (*Parker et al*. 1993). The flocculation process is initiated in the solid contact basin, and may continue in the secondary clarifier

Different aeration methods have been used in the SCC to provide dissolved oxygen, which is transferred to the bacteria culture. The most common types are the air diffusion and mechanical aeration systems. *Ridenour and Henderson* (1936, 1937) showed that diffused air systems lead to smaller effluent suspended solid (ESS) concentrations than mechanically aerated systems. *Parker,* (1983) demonstrated that the oxygen transfer efficiency of fine-bubble diffuser system is greater than that of coarse-bubble aeration systems, at the same air flow rate.

*Metcalf and Eddy*, (2003) provides the typical design parameters for commonly used complete mixed activated sludge process

**Table 2.1** Complete mixed activated sludge process parameters.

| Parameter | Design Criteria Range |
|---|---|
| Hydraulic Residence Time, h | 3-5 |
| Volumetric Loading<br>kg BOD/$m^3$d | 0.3-1.6 |
| Solids Retention Time (SRT), d | 1-4 |
| Food/ Microorganism Ratio<br>kg BOD/kg MLVSS d | 0.2-0.6 |
| MLSS, mg/L | 1400-4000 |
| RAS % of Influent | 25-100 |
| Oxygen Levels (DO), mg/L | 1.5-2.0 |
| **Minimum Mixing** | |
| Diffused Air, scf/ min/ Mgal | 2000- 4000 |
| Mechanical, hp/ mil. Gal | 60- 130 |

The flocculation of microbes is an essential part of any bio-oxidation system. In the activated sludge process the flocs remove both colloidal organic matter and soluble BOD (Steiner, *et al*. 1976). Their sedimentation characteristics must be such that the discharge standards of the final effluent are met with a high degree of consistency.

Activated sludge flocs are made up of biological and nonbiological components. The biological components consist of wide variety of bacteria, fungi, protozoa, and some metazoa. The nonbiological component is made up of inorganic and organic particulates. The basis of the floc appears to be heterotrophic bacteria including genera such as Pseudomonas, Achromobacter, Flavoracterium, Alcaligenes, Arthrobacter, Citromonas and Zooglea which have been suggested as floc forming bacteria. (*Dias and Bhat*, 1964)

Early suggestions that a single floc-forming organism, Zoogloea ramigera, was the sole basis of activated sludge floc have been discounted, although zoogleas are observe often in activated sludge. (*Williams and Unz,* 1983)

Besides microorganisms, activated sludge flocs contain organic and inorganic particles, fibers from the incoming wastewater, and exocellular polymeric substances that play a role in bioflocculation. These polymers are composed mostly of proteins and carbohydrates. (*Higging, et al.* 1997).

According to *Pavoni et al.* (1972) bioflocculation can be described as the result of the interaction of naturally produced, high-molecular-weight, long-chain polyelectrolytes with bacterial cells in such a way that the polyelectrolytes bridge the otherwise individual cells into an aggregate that will settle under quiescent conditions. These authors concluded that this process is not observed to occur until the microorganisms have entered into a restricted state of growth.

During the endogeneous stage, microorganisms consume readily available dissolved substrate, generating at the same time exocellular polymers that constitute the glue that binds particles into flocs. In addition, the bioflocculation kinetics suggests that during the initiation of the process, exocellular polysaccharides are responsible for bridging the distance between electrostatic cells to form a weak, elongated floc. Up to a certain level, further polysaccharide synthesis produces stronger flocs by binding cells more firmly (*Eriksson and Hardin*, 1984).

Several different concepts have been advanced explaining this phenomenon, but the most commonly acknowledged theory, the polymer bridging model, was extended by *Parker, et al., (1970, 1971, and 1972)* who postulated the existence of two levels of structure in activated sludge flocs: the microstructure, consisting of polymer bridges between primary particles, and the macrostructure, consisting of a filament network which provides a "backbone" for the buildup of primary particle "flesh" (*Das, D., et al.,* 1993).

Activated sludge composed primarily of microstructures results in small and relatively weak flocs (pinpoint floc) that can be sheared into small particles in high turbulent zones (Figure 2.4). On the other hand, macrostructures are formed by webs of filamentous organisms (*Sezgin*, 1980) that serve as a skeleton on which floc-forming organisms can grow (Figure 2.5). These types of flocs are characterized by the predominance of filamentous organism, strong and large flocs, and clear supernatants. However, *Urbain et al.* (1993) observed that the overgrowth of filamentous organisms is always associated with settling problems.

The presence of both filament and floc- forming organisms (microstructure and macrostructure) in the activated sludge composition develops a large (100- 200 µm) irregularly shaped and strong floc that will not be easily sheared in zones of high turbulence. When the content of filamentous and floc formation organism is balanced, an ideal sludge is formed (*Ekama et al.,* 1997). The ideal sludge has good settleability and good flocculation characteristics, and leaves a low suspended solid concentration in the supernatant (Figure 2.6).

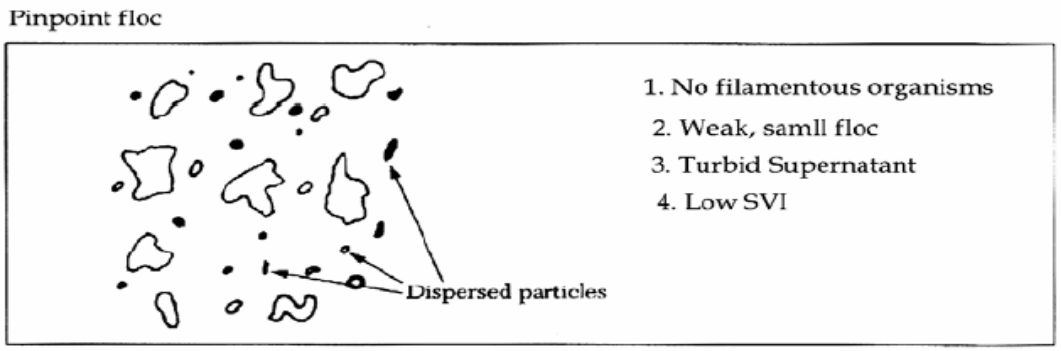



**Figure 2.4** Pinpoint Floc (Jenkins *et al.*, 1993).

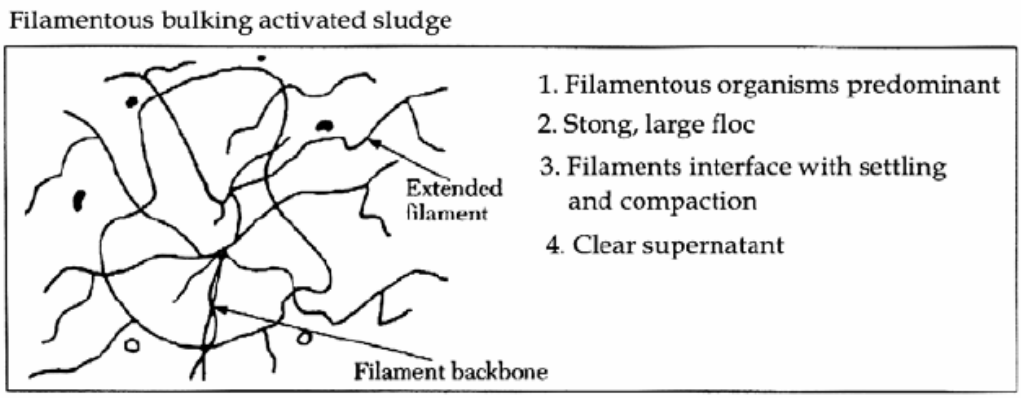



**Figure 2.5** Filament Organism (Jenkins *et al.*, 1993).

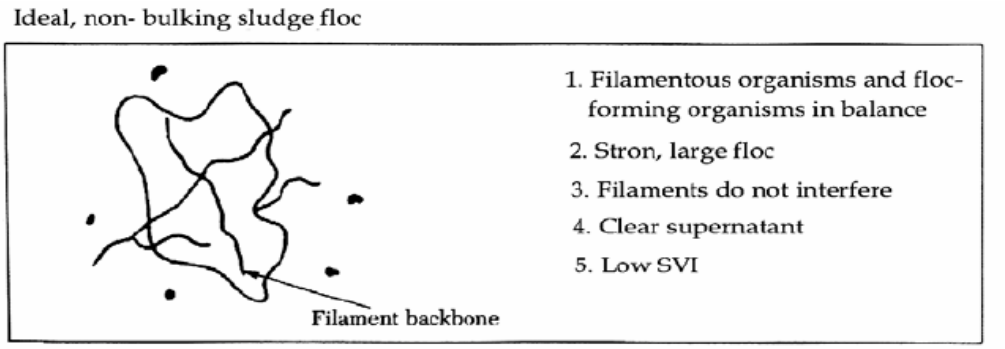



**Figure 2.6** Ideal Floc (Jenkins *et al.*, 1993).

## *2.4 Exocellular Polymeric Substances*

Exocellular polymeric substances (EPS) have been reported as a major sludge floc component. (*Li and Ganzarezyk,* 1990). The EPS are considered important for the physico-chemical properties of activated sludge flocs and have been implicated in determining the floc structure, floc charge, flocculation process, dewatering properties and settling properties.

Researchers have shown that interaction among micro-organism, exocellular biopolymers, and cations are important for flocculation in activated sludge systems. (*Forster* 1985; *Bruus, et al.* 1992; *Higgins and Novak* 1997).

Exocellular polymers are produced by bacteria and typically can be attached to the cell as a capsule, or excreted into the surrounding medium as slime. According to *Urban, et al.* 1993 EPS have two different origins: One from the activated sludge bacterial cells due to metabolism and cell autolysis, and one from compounds in the incoming wastewater.

The chemical composition of EPS matrix is reported to be very heterogeneous. According to *Goodwin and Forster* (1985), carbohydrates and protein are usually found as the major EPS components, with a protein to carbohydrates ratio of 0.2. In addition lipid ( *Goodwin and Forster*, 1985), nucleic acids (*Nishikawa and Kariyuma,* 1968; *Vallom and McLoughlin*, 1984; *Urbain, et al.* 1993) and humic compounds are also reported ( *Peter and Wuhrman*, 1970).

*Gehr and Henry (*1983) defined the polymeric matrix as materials that can be removed from microorganisms without disrupting the cells, and without which the microorganisms are still viable. The exocellular matrix is often term biopolymers or polysaccharides. In fact although polysaccharides predominate and represent the 65% of the exocellular material( *Horan and Eccles*, 1986) other substances are also present such as proteins, nuclei acids and lipids.

The biopolymers are thought to be the glue that hold bioflocs together. According to *Higgins, et al.* (1997) the biopolymers have a number of functional groups such as hydroxyls and negatively charged carboxyl groups. Therefore, biopolymers could bind through specific protein-polysaccharide interactions, hydrophobic, hydrogen bonding, and ionic interactions. *Tezuka* (1969) suggested that divalent cations form bridges between negatively charges sites on the biopolymers, which binds the biopolymers to microbial cells, and to other biopolymers.

Some researchers have suggested that polysaccharides play a major role in flocculation. For example *Bruus, et al.* (1992) suggested that divalent cations bridge negatively charged groups like polysaccharides within bioflocs. *Forster and Dallas-Newton* (1980) also suggested that cations might bridge among negatively charged carboxyl groups on uronic acids.

Most research on the characterization of exocellular biopolymer from activated sludge has focused on exocellular polysaccharides; however other studies have reported that exocellular protein concentration in activated sludge systems was greater than exocellular polysaccharides concentration ( *Teney and Verhoff,* 1973; *Brown and Lester,* 1980; *Barber and Veenstra,* 1986)

The variation in the EPS composition can be attributed to several factors. First, activated sludge from plants with different process design can give different EPS extracts (*Erickson and Alm*, 1991; *Urbain et al., 1993*; *Frolund et al.,* 1994). Second, different extraction procedures are used, which strongly affects the yield (*Brown and Lester*, 1980; *Gehr and Henry*, 1983); and finally, different analytical tools are used for analyzing the chemical composition of the extracted EPS, which can cause further variability in the results.

## *2.5 EPS Extraction*

Exocellular polymers are known to play a key role in wastewater treatment: they are important for the removal of pollution from wastewater, and for sludge settling (*Erickson and Alm 1991*; *Bruus et al.,* 1992; *Urbain et al.,* 1993). They have a great influence on activated sludge floc structure and they are important on the sludge treatment: dewatering (*Sanin and Vesislind*, 1994) and sludge biodegradation in anaerobic digestion (*Novak, et al.* 1977).

Because of EPS importance on coagulation and dewatering of activated sludge, investigators have attempted to extract and quantify these materials and to identify their properties and chemical composition.

A key point in determining the amount and composition of EPS in activated sludge is the extraction step. According to *Gehr and Henry* (1983), a good extraction procedure is effective when it causes minimal cell lysis and does not disrupt the exopolymers. Several methods have been investigated (mechanical and /or chemical) in comparative studies, but general method has yet been established for use by researchers dealing with activated sludge.

*Brown and Lester* (1979) have reviewed the techniques that have been used to extract polymers from activated sludge. These techniques may be grouped into three general classes:

1. High- speed centrifugal stripping followed by precipitation in either alcohol or acetone
2. Hydroxide addition, centrifugation, and precipitation; and
3. Boiling followed by precipitation.

In the work that has contributed mostly to an understanding of the role of biopolymers in bioflocculation, centrifugal stripping has been the method used for polymers collection. *Pavoni, et al.* (1972) assumed that at a force of 32000 G for 15 minutes, polymers are quantitatively extracted from activated sludge.

On the contrary, *Novak and Haugan* (1981), and Brown *and Lester* (1980) reported that no polymer stripping occurs as a result of centrifugation at high speeds. The apparent maximum polymer concentration value that occurred at the centrifugal force of 1000G was thought to be caused by the presence in the centrate of small floc that perhaps resulted from floc breakup associated with centrifugal turbulence. Novak and Haugan concluded that the centrifugal stripping used by *Pavoni, et al.* (1972) and other researchers to obtain both quantitative and qualitative measures of floc polymers does not extract material from flocs.

*Brown and Lester* (1980) compared several polymer extraction techniques and found that treatment of activated sludge flocs with harsh extraction procedures, such as boiling or sodium hydroxide addition, may result in up to 100-fold increase in both the hexose sugar content and protein content of the supernatant liquor. However, because these treatments may also cause cell lysis or may hydrolyze polymeric molecules, interpretation of the data may be confusing or imprecise.

In a researched developed by *Azeredo, et al.* (1998) three extraction methods were applied: extraction with glutaraldehyde, extraction with vapor, and extraction by sonification and Dowex resin. From this research it was concluded that vapor extraction was not suitable because a great amount of intracellular material was extracted; sonification promoted the excretion of large quantities of protein, indicating cellular lysis or breakage of the cell membrane; therefore, estraction by glutaraldehyde was deemed the most suitable because it produced the highest TOC/protein ratio, and did not have a disruptive effect on the biomass.

The extraction method applied for this research was investigated by *Frølund, et al.* (1996) using cation exchange resin (CER). The CER removes cations from the sludge matrix leading to breakup of flocs and subsequent release of EPS. This extraction procedure was previously investigated by *Rudd, et al.* (1983), who compared it to other extraction procedures and found it successful.

*Frølund, et al.* (1994) used cation exchange resin to demonstrate that the composition of extracted EPS is different between treatment plants. Furthermore, using the CER method it has been demonstrated that a major part of the exoenzymes in activated sludge is located in the sludge EPS matrix and that they are very accessible for extraction. ( *Frølund, et al.* 1995)

The research of *Frølund, et al.* (1996) focused on the most important factors determining a good EPS extraction: extraction efficiency, cell lysis, and exopolymers disruption. The CER extraction procedure was more efficient for release of EPS than two other commonly used procedures. Heating the sludge to 80°C for 1h, or extraction with sodium hydroxide by increasing the pH to 11 gave approximately the same results. The yield using CER for 17 hr was twice as high than the previously mentioned procedures. Although induced lysis due to the extraction procedure is possible at high stirring intensities and/or high amount of CER, the results showed that no or only very little lysis occurred within the first 2 hr extraction irrespectively of the stirring intensity and the amount of CER, but the yield of EPS does depend on the amount of CER added, the stirring intensity, and extraction time. *Frølund, et al.* (1996) reported that for mild extraction with minimum risk of lysis, the extraction time was 0.5-1 h with 600 rpm intensity and approximately 70g CER/gVS. For effective extraction, long extraction time (minimum. 12 h), high stirring intensity (900 rpm) and approximatelyy 70 g CER/g Vs were used.

## *2.6 Biomass Settleability*

The effectiveness of the activated sludge process is primarily related to the sludge settling characteristics during secondary clarification. Such the settling characteristics are useful for both the proper design and operation of the clarifiers

The sludge volume index (SVI) introduced by Mohlman in 1934, has become the standard measure of the physical characteristics of activated sludge systems. It is defined as “the volume in ml occupied by 1g activated sludge after settling the aerated liquor for 30 min”.

The general acceptance of this arbitrary parameter as a basic measure of the physical properties of activated sludge solids is indicated by its wide-spread use both in operation of waste treatment facilities and in research in wastewater. The SVI has been used as a means for establishing the required sludge recirculation rate or for calculating the mixed liquor suspended solid concentration that can be maintained in the aeration tank. The most common use of this parameter has been in monitoring waste treatment plant operation and in comparing the settling characteristics of various sludge.

It has been demonstrated that the volume occupied by the sludge after 30 minutes depends on both the initial settling rate and the subsidence characteristics at the higher sludge concentration. The initial sludge interface velocity obtained in a batch settling test is used widely as an indicator of sludge settling characteristics.

*Dick and Vesilind* (1969) demonstrated that two different sludges having the same initial suspended solids concentration and identical 30 min sediments values can have identical SVI, but different settling properties.

The same researchers also demonstrated the effect of suspended solid concentration, interface velocity, cylinder diameter, initial depth of the cylinder, stirring and temperature on the SVI values. The results showed that:

- The rapid increase of the SVI with increasing concentration was due to failure in the sludge agglomeration into a coarse lattice to permit settling.
- There is not consistent meaningful relationship between the initial settling velocity and the SVI.
- The results of SVI experiments using various sized cylinders indicated that SVI values can be obtained that are appreciably greater or less than the value associated with the standard cylinder, and that the results from one liter cylinder may not be at all indicative of the true sludge settling characteristics.
- The lower settling velocities in short columns are thought to be caused by the increasing support provided by underlying solids. Depth affects different sludge differently; therefore, when comparing the settling characteristics of several sludges, the SVI test should be conducted in relatively tall cylinders.
- The SVI is influenced by the temperature at which the test is being conducted, as would be expected because of viscosity changes.
- Stirring of the sludge is thought to aid in agglomeration of the sludge and destroys the bridging within a sludge bed in small cylinders. Therefore the values are more realistic.

Because of all these arguments, *Dick and Vesilind* (1969) concluded that results of SVI test cannot be used with certainty to predict settling behavior in full scale plants. They suggested that alternate, more meaningful measurements of the physical characteristics of the activated sludge be used where possible. One basic measure, which could be determined with about the same ease as the SVI is the initial settling velocity associated with various concentrations of activated solids. This is determined by finding the slope of the interface subsidence curve of activated sludge solids in a comparatively large stirred settling column.

Figure 2.7 shows a typical curve of the interface level as a function of settling time. The zone settling velocity (ZSV) is defined as the displacement rate of the interface at the linear section of the curve. The zone settling velocity is influenced by several factors, but the most important is the initial sludge concentration. Several research workers have investigated the relationship between ZSV and the initial sludge concentration. The best known models to describe the relationship are those by *Vesilind* (1968) and by *Dick* (1972) which can be expressed as (*Vesilind,* 1968)

$$V = V_0 e^{-nx}$$

where V and X are the interface velocity and concentrations respectively, e is the base for natural log, and Vo and n are constants.

The use of these constants may afford a method by which the settling characteristics of different sludges may be compared. ( *Catunda and Haandel*, 1992)

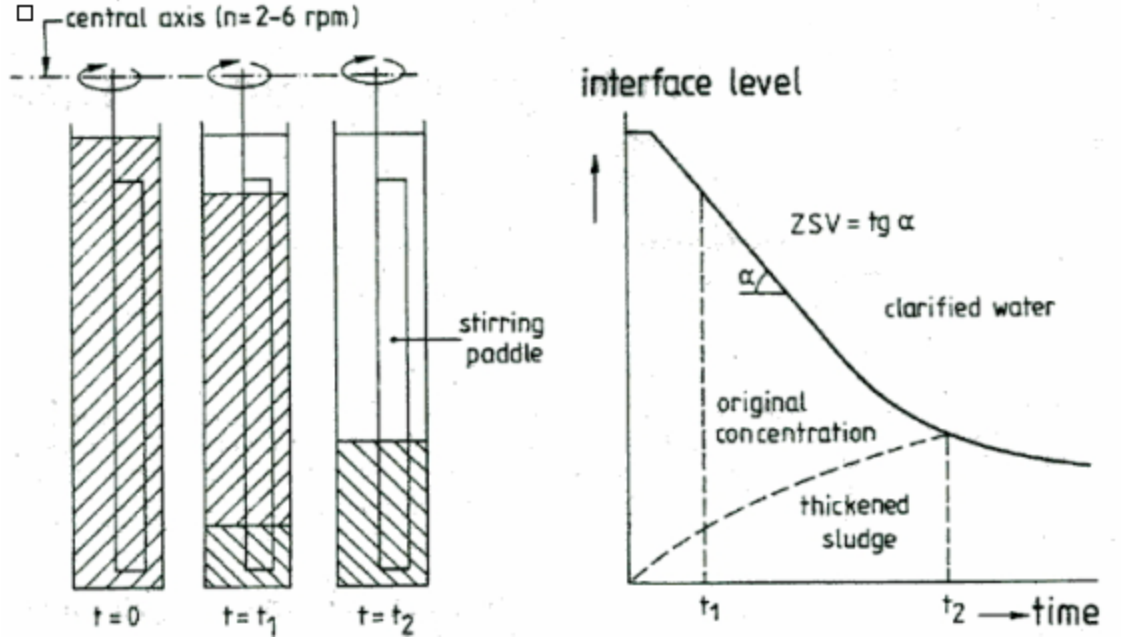



**Figure 2.7** Schematic presentation of the zone settling velocity test and typical curve of the interface level in a batch of settling activated (*Catunda and Haandel*, 1992).

## *2.7 Factors Affecting Biofflocculation and Sludge Settleability*

### *2.7.1 Effect of SRT*

Among the many factors affecting aeration basin performance, the spolids retention time (SRT) is especially important relative to the characteristics of the sludge produced. ( *Stansel & Shell*, 1974).

*Chao & Keinath* (1979) studied how SRT influenced sludge thickening and clarification characteristics of a laboratory activated sludge system using glucose as the only substrate

The results of this research showed that normal sludge characterized by low SVI levels occurred at SRT ranges above 5 days and near 2 days, while zoogleal bulking sludge were noted at 2-5 days. It also indicated the occurrence of filamentous bulking sludge at low SRT levels below 1.9 days. Other authors such as *Bisogni and Lawrence* (1971), and *Pitman* (1975) have stated that the flocculation efficiency increases at higher SRT, but other authors have concluded the opposite; *Parker et al.* (1970) stated that SRT does not affect the flocculation state of the biological sludge.

*B.Q. Liao et al. (2001)* studied the influence of SRT on the extracellular polymeric substances and physicochemical properties of sludge (hydrophobicity and surface charge) using laboratory scale sequencing batch reactors. The results demonstrated that EPS concentration was independent of the SRT. Sludge surfaces were reported to be more hydrophobic (large contact angle) and less negatively charged at high SRT( 16 and 20 days ) than at short SRT ( 4 and 9days)

*Cashion and Keinath* (1983) found that superior bioflocculation occurs at high mean cell residence times (i.e., SRT> 8 days) and low hydraulic residence times (e.g., HRT between 4 to 8 h).

### *2.7.2 Effect of DO*

Others parameter have showed to influence the biofflocculation and settleability of the sludge. High dissolved oxygen (DO) concentration in the activated sludge enhance exocellular bio-polymer production by the microorganisms and, therefore, enhances bioflocculation. According to some authors (*Starkey and Karr*, 1984; *Wilen and Balmer*, 1999), low dissolved oxygen levels (< 2.0 mg/ l) in the mixed liquor lead to a poor flocculated activated sludge and more turbid effluents.

*Sürücü and Dilek* ( 1989) reported that at DO concentration < 2.0 mg/l turbid effluent were obtained. They reported the cause to be due to the inhibition of Eucaryotes population. Another cause was that low DO concentration inhibits the production of exocellular polymers, which function during bio-flocculation. They also reported that zone settling velocities could not be determined at low DO levels (0.5, 1.0, 1.5 mg/l) because the flocculation of microorganism was not good. The mixed liquor was blackish and interface fall with respect to time could not be recorded. On the other hand, at high DO concentration (2.0 and 5.0 mg/l), microorganisms were well flocculated; cultures were lighter in color and the sludge settled without leaving high effluent turbidity with a distinct interface. They reported zone-settling velocities of 9.63 m/hr and 9.36 m/hr at DO levels of 2.0 mg/l and 5.0 mg/l respectively for MLSS of 3000 mg/l. Therefore, the settleability of activated sludge is not improved by the increasing the DO concentration from 2.0 to 5.0 mg/l. At higher DO concentration the flocs were bigger and non-dispersed, turbidity was low and the settlement was observed. Sludge volume indices were measured and it was observed that SVI values at 2.0 and 5.0 mg/l of DO concentration did not differ from each other appreciably.

*Wilen and Balmer* (1999) concluded that at high DO concentration flocs have higher compactness than at low DO concentration. Also, at low DO concentration, activated sludge has poor settling properties mainly due to excessive growth of filamentous bacteria and the formation of porous flocs.

The mixed liquor suspended solids concentration appears to have an effect on the effluent suspended solids concentration. Normally, a concentration between 2000 and 3000 mg/L is used in biological systems. In addition, *Wahlberg et al*. (1994) observed deterioration in the effluent suspended solids when MLSS concentrations rise.

### *2.7.3 Effect of MLSS concentration*

*Tuntoolavest et al*. (1980) found the mixed liquor suspended solids concentration to be the most important single factor, of those investigated, affecting the suspended solids concentration in the effluent of an activated sludge pilot-plant. Using multiple regression analysis, they found that 90% of the variability observed in the secondary clarifier effluent suspended solids concentrations could be explained in terms of the mixed liquor suspended solids concentration and aeration basin turbulence level.

*Chapman* (1983) reported effluent suspended solids discharged from a large-scale pilot plant to be adversely affected by increases in the MLSS concentration. *Wahlberg et al*. ($1994_b$) observed a direct relationship between effluent suspended solids concentration and MLSS; however, the deleterious impact of high MLSS concentration on clarifier performance was found for the cases where secondary clarifiers did not have ideal hydraulic characteristics. Special testing showed that regardless of MLSS level, the potential supernant quality was constant over a broad range.

*Jimenez* 2002 reported that the concentration of EPS increases as MLVSS increases. For MLVSS concentration between 2000 and 4000 mg/L, EPS TOC concentration of 250 and 300 mg/L were found to generate the lowest concentrations of supernatant SS. This demonstrated that the EPS concentration needs not to be at the maximum attainable level to achieve the best effluent quality.

## *2.8 Wastewater Composition, Operating Condition and Bulking Problems*

The factors that affect the clarification, settling, and thickening characteristics of the sludge can be grouped in the influent wastewater composition and the conditions in the biological reactors. Details of these two groups of factors are listed in Table 2.2

**Table 2.2** Factors affecting the clarification, settling and thickening characteristics of activated sludge ( *Ekama,* 1988).

| | |
|---|---|
| Water composition | Industrial Contribution<br>nutrients<br>temperature<br>pH<br>septicity |
| Biological system | configuration<br>temperature<br>mixing<br>pH<br>aeration<br>presence of anoxic and /or anaerobic zones<br>sludge age<br>reactors concentration |

Simplistically, activated sludge can be viewed as comprising two types of organisms, floc formers and filaments. (*Jenkins et al.,1994*). When the content of filamentous and floc forming organisms is balanced, an ideal sludge results. In an ideal sludge, la filaments grow largely within the floc, providing the flocs with strength and structure. A few filaments may protrude from the floc but only to a minor degree and the filaments do not interfere with the settling and thickening of the sludge. *Ekama (1988)* reported that an ideal sludge will have a good settleability (SVI of 80-120 ml/g) and good clarification characteristics leaving a low turbidity and suspended solids concentration in the supernatant.

When the filament and the floc formers content is not balanced, pin-point floc and bulking results. With the pin-point floc (Figure.2.4) there are too few filaments with the result that the flocs have no strength and structure. The flocs are small and weak and can readily be sheared and broken up at relatively low turbulence.

With filamentous bulking, (figure 2.5) there are too may filaments. They grow in large quantities outside the flocs and extended far out in the bulk liquid. This causes the flocs to be very diffused or causes bridging between the flocs , therefore interfering with the closeness with which the flocs can approach one another. The diffuseness of the flocs and/or the bridging between them causes the sludge to settle and compact very poorly, but the net-like structure of the filaments sweeps all the flocs together. Consequently such sludge will have poor settleability but leave an extremely clear supernatant virtually clear of suspended solids ( *Ekama* 1988).

Bulking and scum are common phenomena in activated sludge plants. Several authors (*Eikelboom*, 1977; *Wagner,* 1983) report that a considerable percentage of wastewater treatment plants suffer from bulking due to excessive growth of filamentous microorganism.

*Kappeler and Gujer* (1994) divided filamentous problems four main functional groups:

- Aerobic Bulking
- Scumming due to Actinomycetes
- Low F/M bulking and scumming
- Bulking due to sulphide oxiding bacteria

According to *Wanner and Grau* (1989) the microorganisms responsible for aerobic bulking are Sphaerotilus natans, Type 021N and Type 0961. They are assumed to be aerobic. Other authors mentioned that readily biodegradable substrate favors aerobic bulking. They stated that it can be assumed that slowly biodegradable substrate rather favors floc forming microorganisms than aerobic bulking filaments. (*Eikelboom*, 1977; J*enkins,* 1994)

Other causes of aerobic bulking are insufficient DO concentrations (Palm*, et al.* 1980) and temperature. Temperature significantly affects the type of predominant filamentous microorganism, but hardly the settling properties themselves.( *Kappeler and Gujer*, 1994).

In an emergency situation or while the factor causing bulking are being investigated, chlorine and hydrogen peroxide may be used to provide temporary help. Chlorination of the returned activated sludge (RAS) has been practiced quite extensively as a mean of controlling bulking. A typical design for a low (5 to10 hr SRT) system uses 0.002 to 0.008 kg of chlorine per kg MLSSd ( *Jenkins et al.,* 1994)

The use of selectors for bulking control in full-scale activated plants are becoming much more common now that laboratory research and some full-scale trials have provided a technical basis fro the design of such systems ( e.g. Jenkins et al., 1994).

According to *Parker et al* (2004) the action of selectors is to remove readily biodegradable BOD from solution, thereby reducing the immediate oxygen demand in the aeration zone immediately following the selector. The results is that substrate is available for filamentous organisms in the aerobic zone and higher dissolved oxygen levels can more easily be maintained.

*Parker et al* (2004) reported that immediate improvements on SVI were found by installing a selector in existing activated sludge plants ( Figure 2.9). This study was developed comparing the results from activated sludge plant with anoxic and anaerobic selectors to characterize the performance. Of the eleven plants with anaerobic selectors, ten plants reported values of SVI less than 150ml/g.

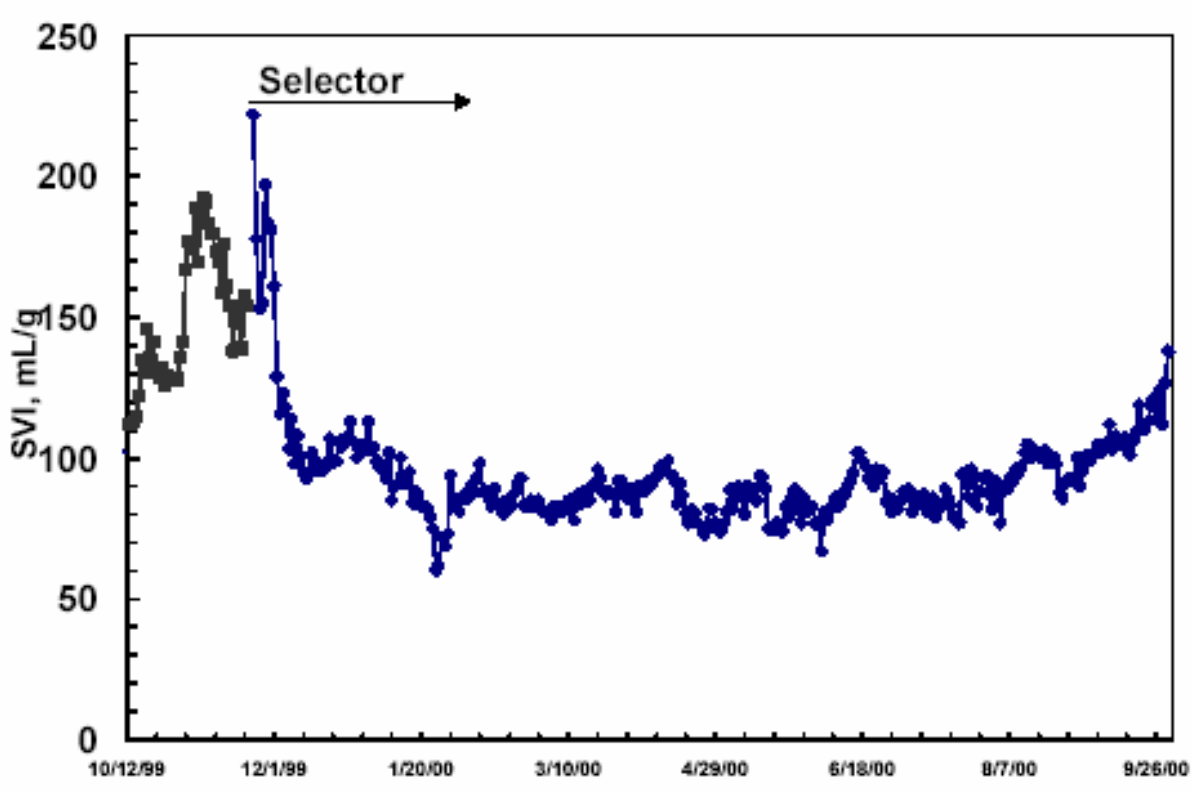



**Figure 2.8** Impact of Anaerobic selector at King County activated sludge System (*Parker et al* 2004)

In the same study, *Parker et al* (2004) pointed out the importance of dissolved oxygen concentration in selectors just as in conventional activated sludge plants. He reported results found by *Bratsy et al.* (2001) in Colorado Springs plant. This plant reported that for declining DO vales, SVI were high. (Figure 9.10)

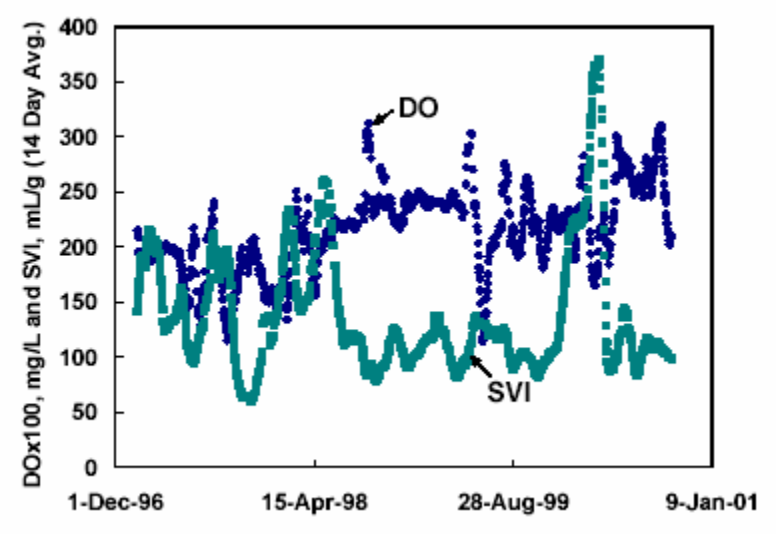



**Figure 2.9** Impact of DO level on anoxic selector performance at Colorado Springs (*Parker et al* 2004).

### *2.8.1 Floc and Microorganism Characterization*

Microscopic examination of activated sludge is useful for determining the physical nature of the activated sludge floc and the types and abundance of filamentous organisms. It generally gives information related to activated sludge behavior in solids separation processes because the physical properties of the activated sludge revealed during microscopic examination determine the settling and compaction characteristics.

The gram stain is a used routinely in floc and filamentous organism characterization. Examining wet mounts under phase contrast illumination at 100X magnification can also be used: to characterize floc size, floc characteristics (round, irregular, compact, diffuse); to determine the presence and types of protozoa and other microorganisms (e.g. rotifers, nematodes); determine the effect of filamentous organism on floc structure; determine filament organism abundance. Changes in these characteristics can provide identification of changes in the wastewater characteristics or of an operational problem. Early detection of filamentous or any changes that may induce problems will allow time for correcting action to be taken and minimize potential problems.

*EPA( 1977)* present a chart to evaluate the predominance of microorganism versus F/M ratio and SRT. Fig.2.10

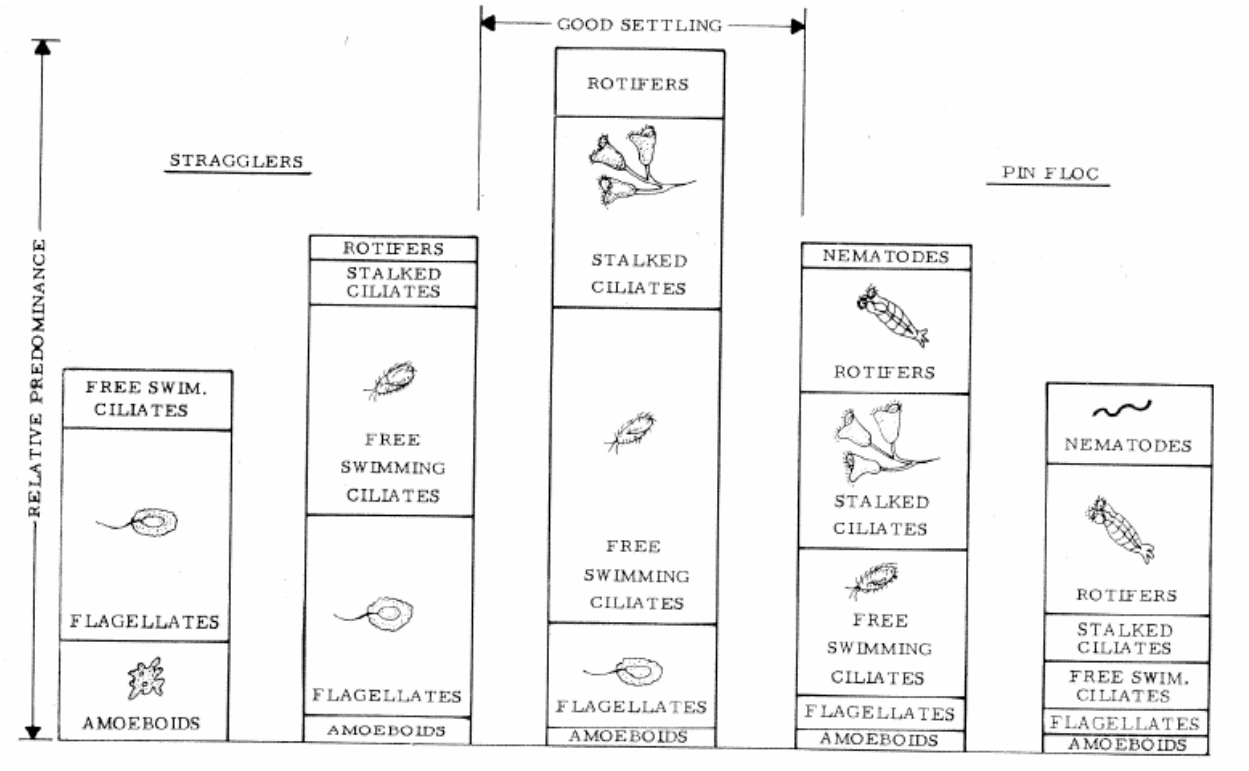



**Figure 2.10** Relative predominance of microorganism versus sludge quality (EPA, 1977).

# 3. Experimental Phase

## *3.1 Introduction*

This research project was developed using the combined anaerobic/aerobic pilot plant system located at the pilot plant at the Marrero Wastewater Treatment Plant in Marrero, Louisiana. The components of the anaerobic/aerobic system are: a rotating screen, AFBR or UASB, aerated solid contact chamber, and a secondary clarifier (Fig 3.1)

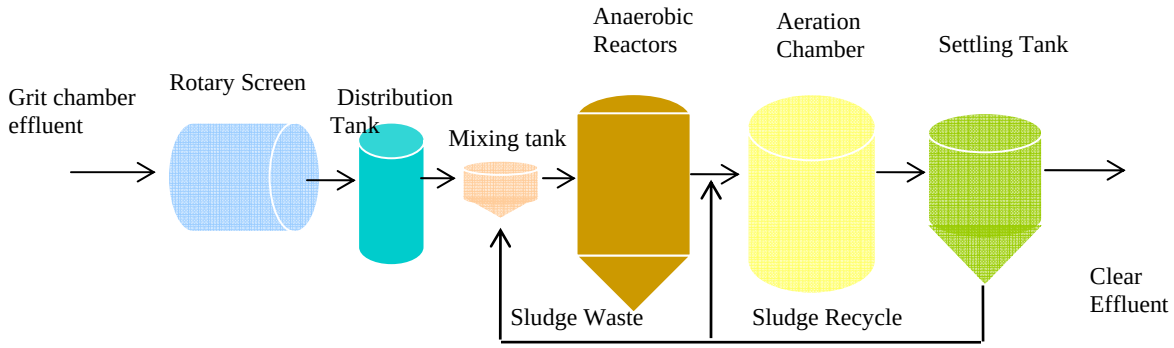



**Figure 3.1** Pilot plant diagram.

## *3.2 Pilot plant Description*

### *3.2.1 Feeding System*

The pilot plant is fed with municipal wastewater from the Marrero Wastewater Treatment Plant grit chamber splitter box by a 37 W Teel self priming pump, Model 3P551, 259 $m^3$/d at 3 m of head (Appendix A.1). The suction system consists of a perforated pipe (102 mm) covered with a metal screen with 9.5 mm. pore size. To prevent large solids from clogging the suction system, the perforated pipe is cased with a 203 mm PVC pipe.

From the source, the wastewater flows to a rotating-drum screen (Hycor Rotostrainer) model RSA2512UBCR, with 0.5 mm clear spacings and a 25 W drive motor system. As the wastewater enters the rotating cylindrical screen, the solids larger than the screen openings ride over the top of the screen, are removed by a blade assembly located in the front part of the unit, and are then collected in an external basin (Appendix A.2). Table 1 presents the average values of the different parameters of the effluent from the rotating screen.

**Table 1.** Average values of the rotating screen effluent

| Parameter | Effluent |
|---|---|
| TSS, mg/L | 152 |
| TCOD, mg/L | 293 |

The effluent from the rotatory screen is pumped out from effluent holding tank by a 75 watt Teel submersible centrifugal pump, Model 1P809, 81 $m^3$/d at 0.3 m of head to a 0.12 $m^3$ distribution tank. This distribution tank has an electric 186.4 W drum mixer installed to keep a homogenous wastewater and prevent the sedimentation of solids (Appendix A.3). This flow is discharged by gravity into a 0.53 $m^3$ polyethylene tank, which serves as a holding container for the AFBR influent wastewater and returned sludge from the clarification stage. This tank is furnished with a 14.9 W submersible pump, Model 1P808, 36 $m^3$/d at 0.3 ft of head that keeps the tank hydraulically mixed to avoid settling of solids, and a float valve that controls the flow into it. ( Appendix A.4 )

The water from the distribution tank is fed into the anaerobic reactor using a diaphragm pump Model 07135-05. The pump flow rates are controlled adjusting the pump settings. The flow rate fed either to the AFBR or UASB was maintained as close as possible to at 2.88 $m^3$/d throughout the experimental phase. It is important to highlight that while one of the anaerobic units was used for the experimental phase the other anaerobic unit effluent was sent to the pilot plant final effluent discharge line.

### *3.2.2 Anaerobic Reactors*

The anaerobic reactors used for this research correspond to two different treatment processes. The AFBR is an attached growth process that uses activated carbon as supporting media, whereas the UASB is a sludge blanket process with no media, but pure sludge. The characteristics of the media for the AFBR are in Table 3.2

**Table 3.2** Characteristics of the supporting media (Corzo, 2001).

| **Characteristic** | AFBR |
|---|---|
| Density ($g/cm^3$) | 0.48 |
| Mesh | 40/80 |
| Surface area ($m^2/g$) | 1150 |

The reactors are 400 L cylindrical polyethylene tanks with a 60 degree conical bottom. The tanks have a diameter of 0.86 m and a height of 1.16 m The flow was fed into the reactors from the bottom to the top using a diaphragm pump Model 07135-05. Part of the treated wastewater leaves the reactors as effluent to the aerobic unit and the rest flows through the internal recirculation lines to increase the upflow velocity to fluidize the bed. The recirculation is achieved utilizing a 372.5 W centrifugal pump with 207 $m^3$/d capacity at 3 m of head for the AFBR and 29.2 W magnetic centrifugal pump with 39 $m^3$/d capacity at 2m of head for the UASB reactor. (Figure 3.2)

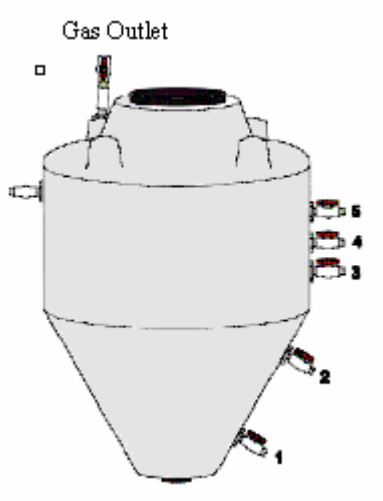



**Figure 3.2** Schematic representation of the Anaerobic Reactors ( *Padron,* 2004).

The characteristics of the influent fed to both reactors, which was composed of raw wastewater and the recycled sludge, are shown in Table 3.3.

**Table 3.3** Characteristics of the Reactors Influent

| Parameter | AFBR | UASB |
|---|---|---|
| Total COD, mg/L | 301 | 306 |
| Total Suspended Solids, mg/L | 144 | 170 |
| Volatile Suspended Solids, mg/L | 126 | 152 |

### *3.2.3 Aerobic Solid Contact Chamber (ASCC)*

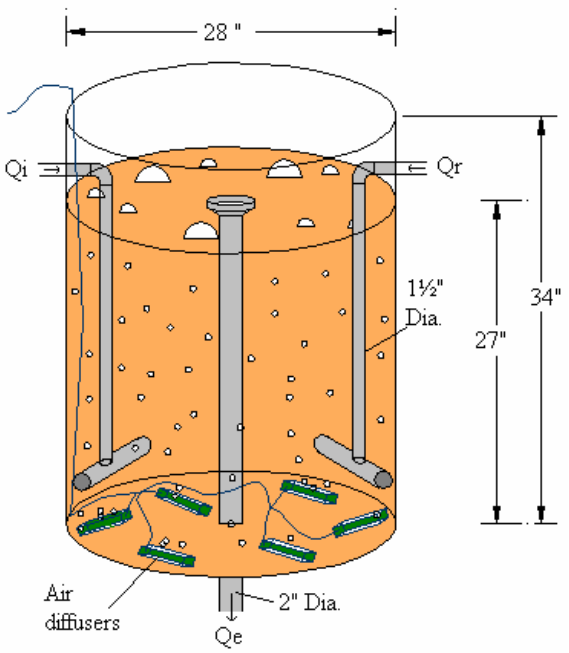
As part of this research, different hydraulic retention times (HRT) were used and a constant influent flow 2.88 $m^3$/d; therefore, two different contact chambers were used. For the HRT of 120 min, a 0.32 $m^3$ polyethylene tank and for the HRT of 180 min a 0.57 $m^3$ tank. (Appendix A.6)

The tanks were equipped with 6 heat-bonded silica fine-pore diffusers, with a length of 15 cm and a width of 4 cm, a suggested maximum air flow rate of 0.852 ($m^3$/h) and maximum pore size of 80 microns that, according to the manufacturer, generates bubble size 0.5 – 20 mm ( *Bustillos*, 2001). The air was injected into the system by a GAST compressor, Model 4F742, with 559.3 w and a flow meter was placed at the inlet of the solid contact chamber to regulate the amount of air. The compressor provides air to maintain the desired dissolved oxygen levels in the aeration chamber and the velocity gradient for uniform mixing.

The solid particles coming from the anaerobic unit are in the SCC mixed with the recycle solids from the clarifier. These solids come into the ASCC from the bottom and get mixed with the aid of the ascending flow and the turbulence produced by the air diffusers. The air injected into the ASCC creates what is called an aerobic biological treatment. Table 3.4 shows the operational parameters of the aeration basin

**Table 3.4.** Operational Parameters of the Aerated Solids Contact Chamber.

| Parameter | Values | Units |
|---|---|---|
| HRT | 120,180 | min |
| SRT | 2-9 | days |
| MLVSS | 1700-4000 | mg/l |
| DO | 0-5 | mg/l |




**240 L ASCC**

**Figure 3.3.** Diagram of the Aerated Solids Contact Chamber.

### *3.2.4 Secondary Clarifier*

After leaving the aeration tank, the mixed liquor enters the secondary clarifiers at the top of a center well and discharges at 2/3 the depth of the clarifier. The tank provides a location where the activated sludge solids can be separated from the liquid in the mixed liquor coming from the aeration tanks.

This effluent enters tangentially into the clarifier through a 203-mm diameter center well designed to distribute the flow equally in all directions, the destroy the inflow energy, and to provide improved conditions for flocculation to occur. A rotatory arm was installed at the bottom of the clarifier to avoid the formation of solids clumps and to prevent sludge bridging at the sludge withdrawal point. This arm was moved by a 1-rpm gear motor installed at the top of the clarifier. The effluent from the clarifier leaves the unit through a 38 mm PVC pipe located at the top of the tank ( Appendix A.7).

Activated sludge solids from the secondary clarifiers are recycled into the ASCC and wasted into the mixing tank located before the anaerobic units using 14.9 W TEEL / 1P808 submersible pumps controlled by timers. (Figure 3.4)

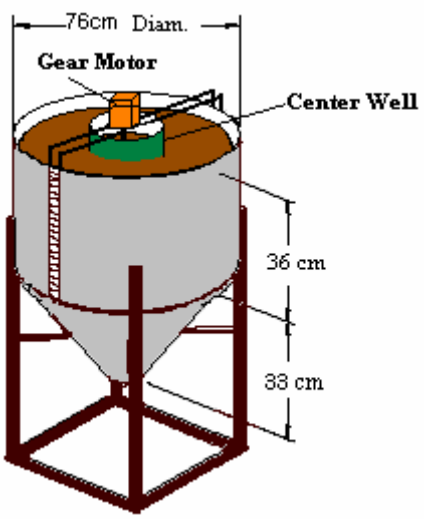



**Figure 3.4.** Diagram of the Secondary Clarifier.

## *3.3 Sampling and Laboratory Analysis*

### *3.3.1 Sampling Phase*

The sampling phase was initiated in May 2003 and finished in December 2004. Samples were usually collected in the morning and taken to the SUESC laboratory in the CERM building for analysis.

Samples were collected from the effluent of the rotary screen, the effluent of the anaerobic units, the aerobic solid contact chamber, and included supernatant and MLSS; return sludge pumping system; and secondary effluent.

Sampling and analysis followed the recommendations and procedures published in Standard Methods (APHA, 1999).

### *3.3.2 Field Data*

#### *3.3.2.1 Plant flowrRate*

The flow rate to the anaerobic units is equal to the flow to the ASCC. This flow was measured daily to ensure a constant HRT and was compared with the final effluent.

#### *3.3.2.2 Recycle flow rate*

The recycled sludge flow rate was measured daily in the clarifier-ASCC line by quantifying the time required to fill a 1liter cylinder. The sludge collected for the measurement was returned to the system to maintain steady state conditions.

#### *3.3.2.3 Supernatant*

Supernatants from the ASCC were collected every time that samples were collected to observe the unit's performance. At the same time, they were compared with the final effluent to determine if the clarifier was working under ideal conditions. A one- or two-liter mixed liquor sample was collected and left to settle for 30 minutes. Then, a representative volume was carefully collected with a siphon to avoid alteration of the sludge blanket.

#### *3.3.2.4 Hindered (Zone)settling velocity as a function of sludge concentration*

The settling parameters Vo and n were measured every time samples were collected.

To determine these parameters of the activated sludge, a plot of the interfacial settling rate versus sludge concentration is necessary. To get each interfacial settling rate, sampling and analysis followed the recommendations and procedures published in Method 2710E of the Standard Methods 1998.

A well-mixed sample from the ASCC was removed and the TSS concentration was measured. The stirring mechanism of the graduated cylinder was activated, 2 liters of MLSS were taken, and the height of solids-liquid interface was recorded at intervals of one-half or one minute.

A plot of the interface height in cm vs. time in minutes was developed. A straight line was drawn through the data points, ignoring the initial shoulder or reflocculation period and compression shoulder. The interface settling rate was calculated as the slope of the line in cm/min.

To determine zone settling rate parameters Vo and n a best fit was applied to the plot of MLSS concentration versus. The procedure is as follows:

- The original sample was taken from SCC and MLSS concentration and the interface settling rate were recorded
- Concentration and/ or dilution of the MLSS was used to get at least 5 points
- Run settling test and get curve

*3.3.2.5 Stirred sludge volume index (SVI)*

The stirred SVI was determined by placing a mixed-liquor sample in a 2L cylinder, activating the stirring mechanism, and measuring the settled volume after 30 min and the corresponding sample MLSS concentration. The SVI is computed using:

$$SVI = \frac{(settled\ volume\ of\ sludge, mL/L)(10^3 mg/g)}{(suspended\ solids, mg/L)} = \frac{mL}{g}$$

*3.3.2.6 pH*

This parameter was measured daily with a WTW pH meter, Model 330. An electrode was introduced into the container with the water desired to be sampled. Previously, the equipment was calibrated according to the instructions given in the manual.

*3.3.2.7 Dissolved oxygen and temperature*

These two parameters were measured daily using an YSI, Model 550A, handheld dissolved oxygen and temperature meter. Then the probe was introduced into the ASCC until the screen indicated stable values. The equipment was calibrated previously according to the instructions indicated.

***3.3.3 Laboratory Analyses***

Five parameters were measured, total COD (TCOD), dissolved COD (DCOD), total and volatile suspended solids (TSS and VSS) and exocellular polymer concentration as total organic carbon(TOC). Table 3.5 shows the parameters analyzed in the samples collected at each location.

**Table 3.5** Water Quality Parameters Analyzed at Each Sampling Point.

| **Sampling Point** | **Parameter Analyzed** | | | | |
|---|---|---|---|---|---|
| | **TSS** | **VSS** | **TCOD** | **DCOD** | **EPS (TOC)** |
| Effluent Screen | x | _ | x | _ | _ |
| Effluent Anaerobic Unit | x | | x | x | _ |
| MLSS | x | x | _ | _ | x |
| Aeration Basin Supernatant | x | x | x | x | _ |
| Recirculation Sludge | x | x | _ | _ | _ |
| Final Effluent | x | x | x | x | _ |

*3.3.3.1 Total suspended solids*

TSS tests were performed using Method 2540D of Standard Methods (APHA, 1998) and Hach No. 30 glass-fiber filter paper was used for filtration of samples. This test represents the amount of solids (organic and inorganic) suspended in a specific wastewater sample.

*3.3.3.2 Volatile Suspended Solids (VSS)*

Volatile suspended solids are those solids lost on ignition (heating to 500 degrees C.) They give a rough approximation of the amount of organic matter present in the solid fraction of wastewater, activated sludge and industrial wastes. VSS tests were performed using Method 2540E of Standard Methods (APHA, 1998).

*3.3.3.3 Total chemical oxygen demand*

The chemical oxygen demand (COD) is used as a measurement of oxygen consumed to completely oxidize the organic and inorganic compounds in wastewater. The samples were homogenized by mixing using magnetic stirrers and analyzed according to the method 5220D of the Standard Methods (APHA, 1998).

*3.3.3.4 Dissolved chemical oxygen demand*

100 ml of samples were flocculated by adding 1mL of a 100-g/L of zinc sulfate solution and vigorously mixed for one minute using a magnetic stirrer. The pH of the mixed sample was then adjusted to approximately 10.5 with a 6-M sodium hydroxide solution. Then, the sample was allowed to settle quiescently for a few minutes (Standard Methods, Section 417B, 1998). Clear supernatant (25 ml) was withdrawn with a pipette and then passed through a Hach No. 30 glass qualitative filter paper with a pore size of 0.45 µm. The COD of the supernatant filtrate was defined to be the truly dissolved COD of the sample.

*3.3.3.5 Grain Stain, Modified Hucker method*

The Grain Stain, Modified Hucker method is used for the routinely floc and filamentous organism characterization. Three drops of equal volume of the sample are placed in the microscope slide and air-dried. Crystal violet is added to stain for 1 min, rinse with dionized water. Then the slide is immersed in iodine for 1 min and air-dried. The slide is held in angle and decolorized with 95% ethanol and then air-dried. The final step is to stain it with Safranin for 1 min then air-dried. All solutions are prepared according to *Jenkins et al* (2004).

*3.3.3.6 Exocellular polymers measurement as total organic carbon (EPS TOC)*

The EPS were extracted by using the extraction method developed by Frølund *et al.*, (1996). Activated sludge (6 L) was collected from the aeration tank of the pilot plant and transported to the UNO Environmental laboratory within 30 minutes. The sludge was settled for 1.5 h at 4$^{o}$C and the supernatant decanted.

To remove any EPS from bulk water, a washing step was performed on the sludge, adding distilled water (to its original volume) to the previously decanted sludge and letting it settle for 1.5 h at 4$^{o}$C and the supernatant decanted. Thickened sludge (500 ml) was centrifuged at 2,000 g for 15 minutes. The sludge pellets were resuspended to their original volume using a buffer consisting of 2mM $Na_3PO_4$, 4mM $NaH_2PO_4$, 9mM NaCl, and 1mM KCl at pH 7.

The following procedure was performed for the EPS extraction: 300 ml of sludge were transferred to an extraction beaker and the CER (Cation Exchange Resin, Dowex 16-40 mesh in sodium form) was added (60g CER/g VS). The suspension was stirred for 2 hours at 9000 rpm. The extracted EPS were harvested by centrifugation of a sample of the CER-sludge suspension for 1 minute at 12,000g to remove the CER. Then, the supernatant was centrifuged twice for 15 minutes at 12,000g in order to remove remaining floc components.

The amount of the EPS was quantified by measuring the total organic carbon content of the sample by using an Apollo 9000 TOC Combustion Analyzer fabricated by Tekmar-Dohrmann.

# 4. Results and Discussion

The results and analyses of the various experiments conducted along the course of this investigation are presented in this chapter. The results and discussion of each set of experiments carried out in this research are presented separately;

## *4.1 Wastewater Characterization*

*Anaerobic Reactors Influent*

The characteristics of the influent fed to both anaerobic reactors, which was composed of raw wastewater and the recycled sludge, are shown in Table 4.1

**Table 4.1** Average characteristics of the Anaerobic Reactors Influent (Padron 2004, Silva 2004).

| Parameter | AFBR | UASB |
|---|---|---|
| Total COD, mg/L | 301 | 306 |
| Total Suspended Solids, mg/L | 144 | 170 |
| Volatile Suspended Solids, mg/L | 126 | 152 |

*Anaerobic Reactors Effluent*

The characteristics of the wastewater entering the ASCC, represented by the effluent of the anaerobic units are presented in Table 4.1.

**Table 4.2**. Characteristics effluent from the Anaerobic Reactors.

| Parameter | Value |
|---|---|
| Total Suspended Solids, mg/L | 130.5 |
| Total COD, mg/L | 249.1 |
| Total DOD, mg/L | 69.8 |
| Total POD, mg/L | 190.3 |

Figure 4.1 shows a linear relationship between the TCOD and PCOD entering the SCC with $R^2$ of 0.93. It also noticeable that for TCOD values less than 50mg/l the total organic matter in is dissolved form.

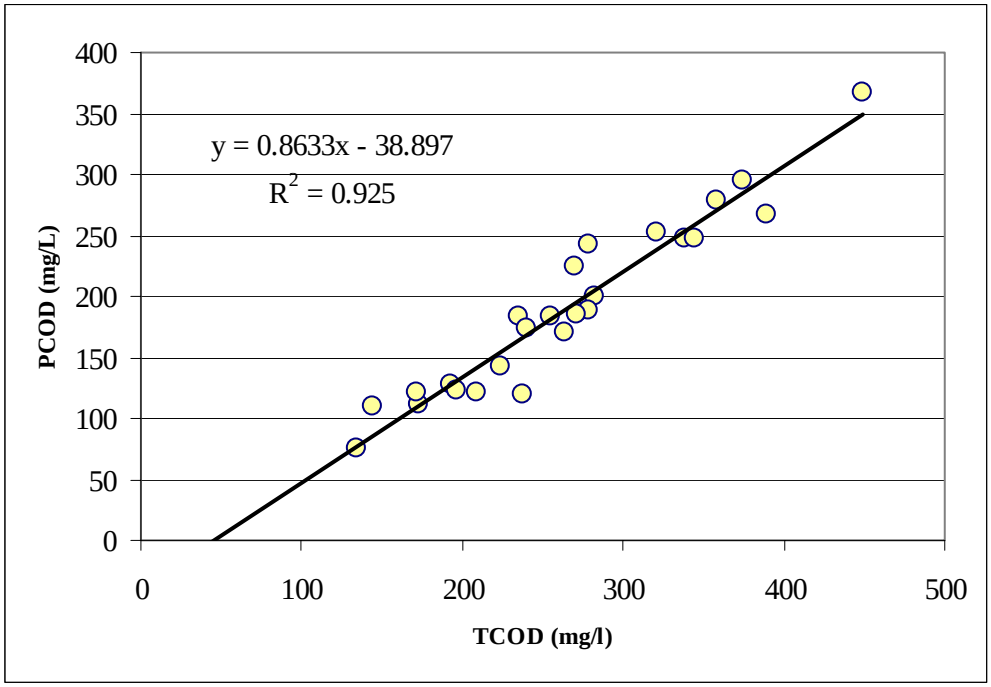



**Figure 4.1** Relationship between TCOD and PCOD entering the SCC.

Combining the data of this research with data from other researchers who used an aerobic suspended growth pilot plant (Jimenez, 2002 and Rojas, 2004) the following relationship was found: PCOD= 0.889 TCOD -22.85 with $R^2$=0.98.

About 89 % of the organic matter in wastewater is in particulate form independently of the type of system ( fully aerobic or combined). These results are shown in figure 4.2

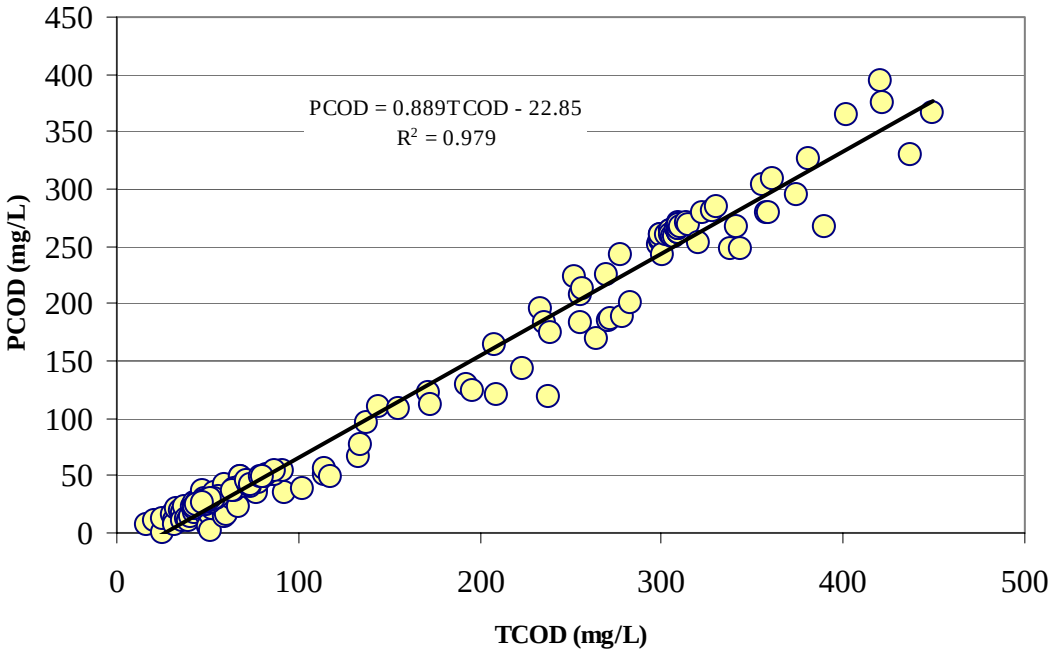



**Figure 4.2** Relationship between TCOD and PCOD in wastewater (data from Jimenez, 2002; Rojas, 2004 and this research).

## *4.2 General Operation Problems*

During the experimental phase of this research it was a challenge to keep the dissolved oxygen and the solids retention time constant. Figure 4.2 shows that the SRT changes from 2 to 4 days within a week. DO decrease from approximately 5.5 mg/l to 4mg/l in 2 days.

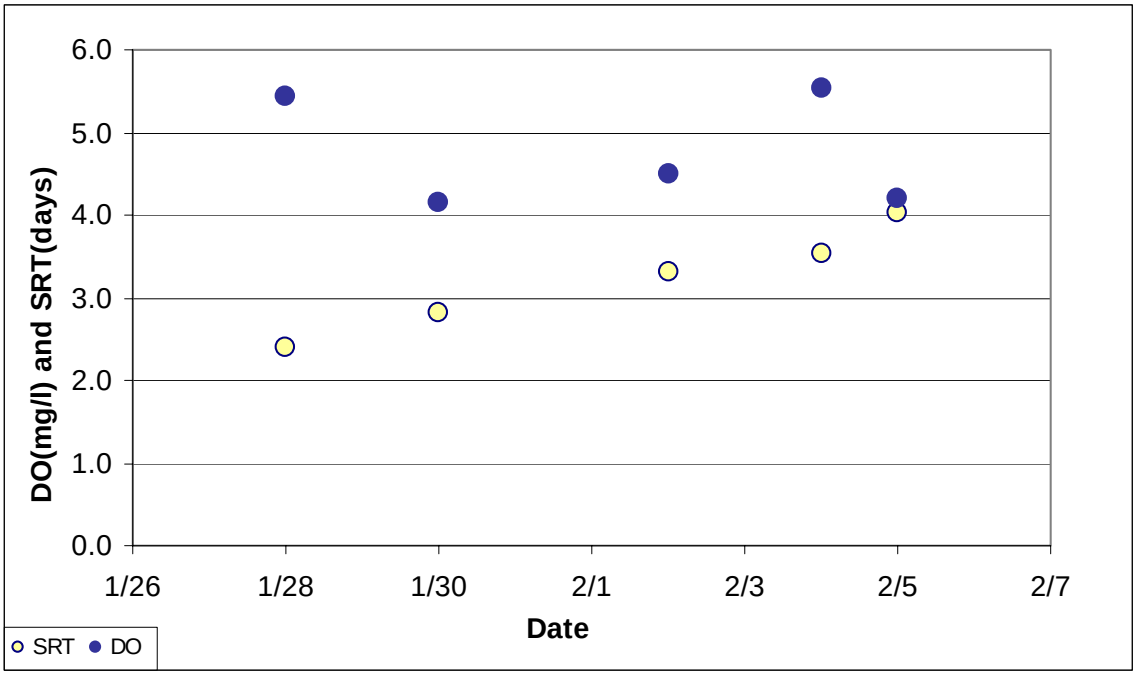



**Figure 4.3** Variability of DO and SRT within a week

Increasing the MLSS concentration above 4000 mg/l was not possible because the size of the clarifier was not appropriate compared to the size of the SCC. Also the clarifier was not operating well; therefore solids were lost in the effluent.

## *4.3 MLSS and EPS*

To determine the existence of a relationship between the solids concentration in the SCC and the exocellular polymeric substances, two different type of experiment were set up.

*4.2.1 MLSS and EPS (using dilution)*

This experiment was developed diluting the SCC MLVSS to different concentrations, assuming the SRT and DO were constant for each MLVSS concentration.

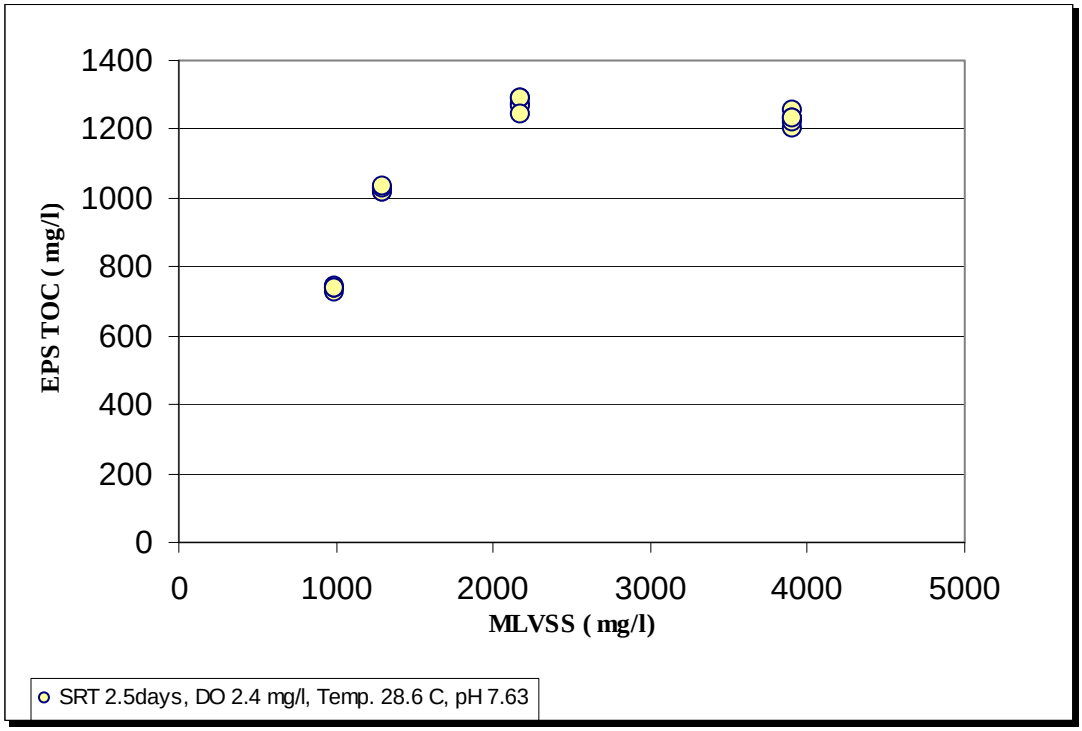



**Figure 4.4** Relationship between MLVSS and EPS for a mixed liquor sample diluted 3 times.

Figure 4.3 shows that there is no straight line relationship between MLVSS and EPS. This behavior could be the result of dilution or the possibility that MLVSS is not directly related to EPS concentration. The first dilution (from 4000 to 2500) shows that dilution alone extracts additional polymers from the floc, to the extent that the EPS concentration remains practically constant.

*4.3.2 MLSS and EPS (no dilution)*

In order to eliminate the possible effect of dilution, the samples for the EPS extraction were taken on a daily basis maintaining the other operating parameter as constant as possible. First, the MLVSS was varied from a low concentration (1700 mg/L) to a high concentration (3300 mg/L). The desired solids concentration and SRT were selected by adjusting the solids contact chamber recycled and wastage flow-rates of the system. The SRT was kept between 2-4 days throughout the experiment and DO between 2-3 mg/l. Samples at different MLVSS concentrations were collected and the EPS were extracted as described in the previous chapter. The data corresponding to this experiment (Appendix B.4) correspond to different days within one year because the pilot plant could not be kept operating at stable conditions for the reason mentioned in section 2.9.

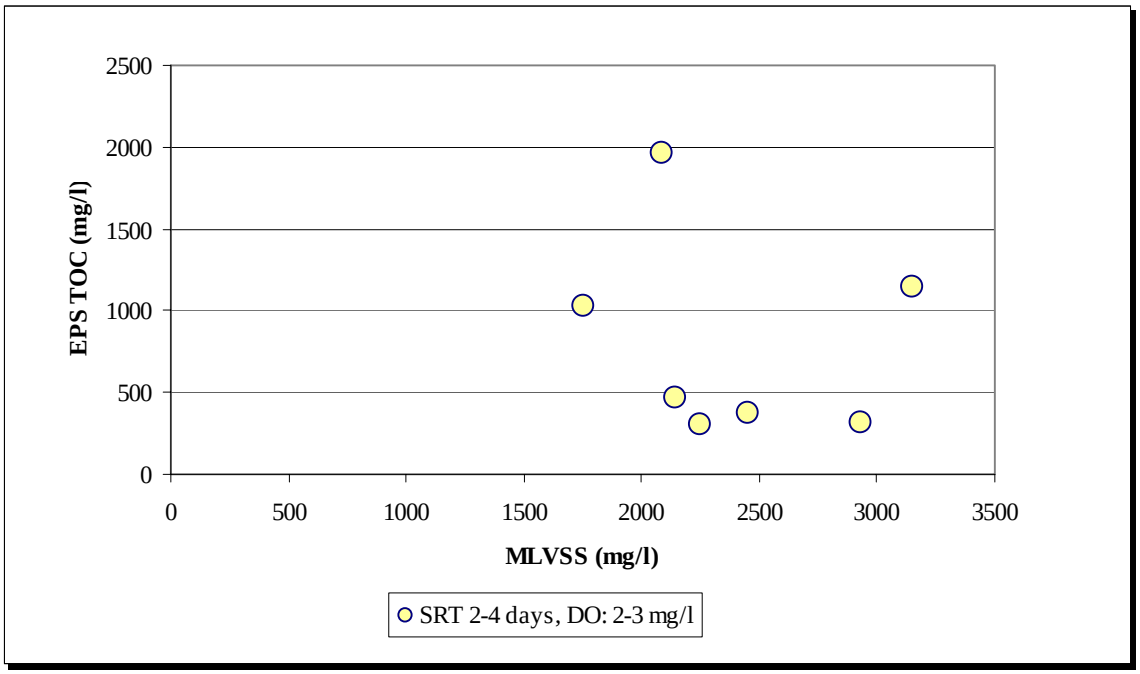



**Figure 4.5** Effect of MLVSS on EPS concentration.

The data presented in Figure 4.4 show two interesting points: First, the EPS TOC concentration is significantly higher than the values reported by Jimenez (2002). Second, there is not a clear relationship between the solids concentration and the polymeric substances. The corresponding values of effluent quality shown in figure 4.5 demonstrate that the effluent quality does not vary linearly with MLVSS; rather, the points are scattered. It is important to point out that the results are a result of the plant operating at unsteady state.

A statistical analysis based on the null hypothesis Ho: slope = 0 was performed by examining the range of values containing the estimate of the slope at the 95% confidence interval. If the value zero is contained within the range determined with a confidence of 95%, then the null hypothesis cannot be rejected. On the other hand, if zero is not contained in the interval, there is a linear dependency of MLVSS on the EPS.

Based on the results on appendix C.4 the null hypothesis can not be rejected.

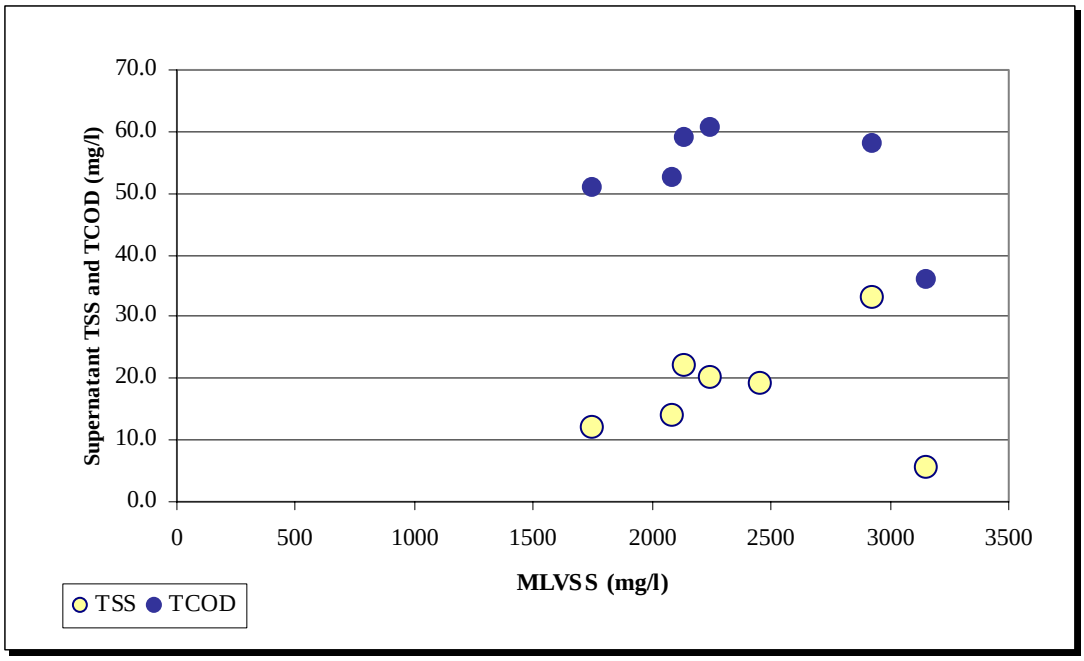



**Figure 4.6** Effect of MLVSS on supernatant quality.

Previous investigations have demonstrated EPS play an important role in biofflocculation. To prove this with the results from this research, a relationship between EPS/MLVSS and effluent quality was developed. (Figure 4.6)

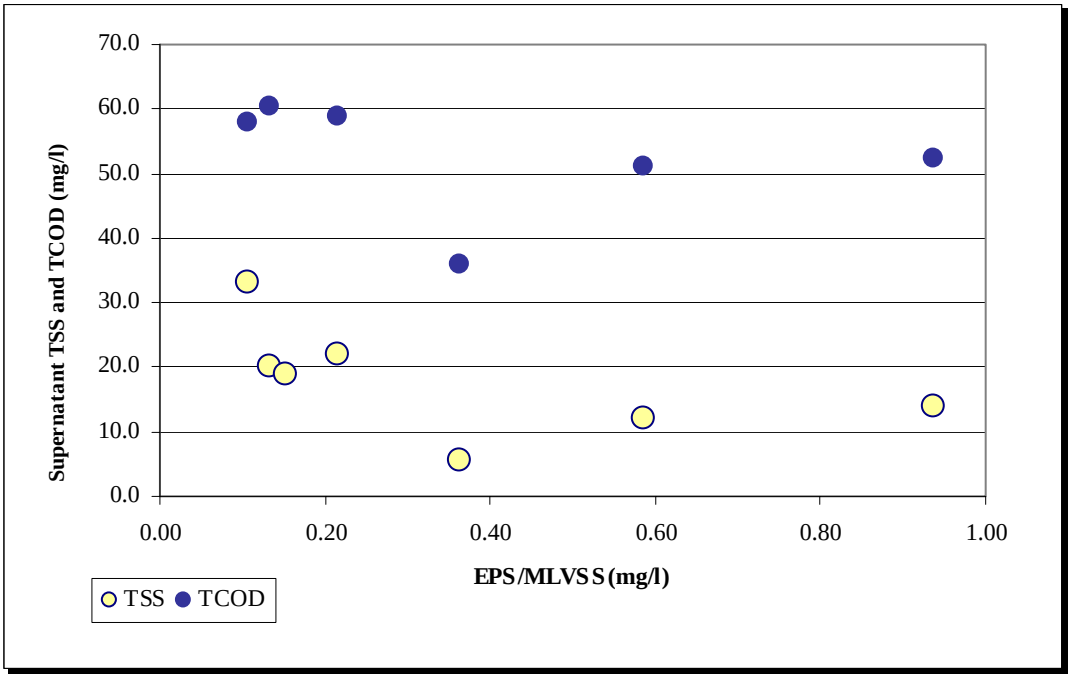



**Figure 4.7** Effect of EPS/MLVSS on supernatant quality.

It can noticed that as the EPS/MLVSS increases, TSS concentration decreases, but if levels off for values of EPS/MLVSS >0.35 this is not so clear for TCOD. Table 4.3 summarizes the results of the statistical analyses performed on the data presented above, and shows that there is no correlation between the tested variables..

**Table 4.3** Summary of Regression Analysis for MLVSS.

| **Relationship** | **Null Hypothesis (Ho): slope=0** | | **Coefficient of Determination ($R^2$)** |
|---|---|---|---|
| MLVSS vs. EPS (dilution) | Rejected | | 0.5309 |
| MLVSS vs. EPS | | Not Rejected | 0.0174 |
| MLVSS vs. Supernatant SS | | Not Rejected | 0.0451 |
| MLVSS vs. Supernatant TCOD | | Not Rejected | 0.4294 |
| EPS/MLVSS vs. Supernatant SS | | Not Rejected | 0.2396 |
| EPS/MLVSS vs. Supernatant TCOD | | Not Rejected | 0.0787 |

## *4.4 SRT and EPS*

To determine if the SRT had a direct effect on the EPS production, this value was varied between 2-9 days adjusting the wastage flows of the system. At each SRT, mixed liquor samples were collected from the contact tank and the EPS were extracted. The MLVSS concentration was kept between 1700-2500 mg/L and DO between 2-3mg/l.

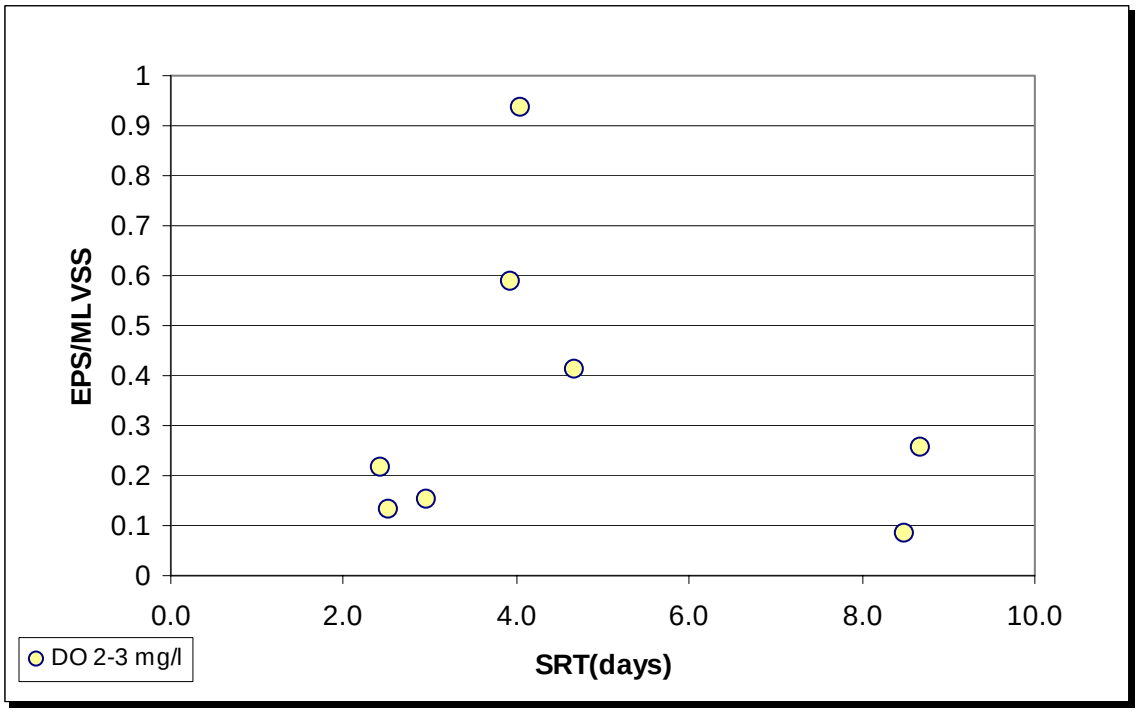



**Figure 4.8** Effect of SRT on EPS /MLVSS.

Figure 4.7 summarizes the results and shows that the concentration of the EPS, expressed as mg TOC/mg VSS, increases as the SRT increases up to a value of 4 days, but after that, there is a drop in the EPS concentration. Concentrations around 1.13 to 0.94 mg TOC/mg VSS are found in the SRT range of 2 to 4 days.

Figure 4.8 shows the corresponding supernatant quality, measured in terms of supernatant TSS and TCOD. This graph shows an effluent improvement as the SRT increases and coincides with the results by other researchers (*Metcalf and Eddy*, 2004) who have recommended using SRT values greater than between 1-3 days for the development of flocculent biomass for treating domestic wastewater.

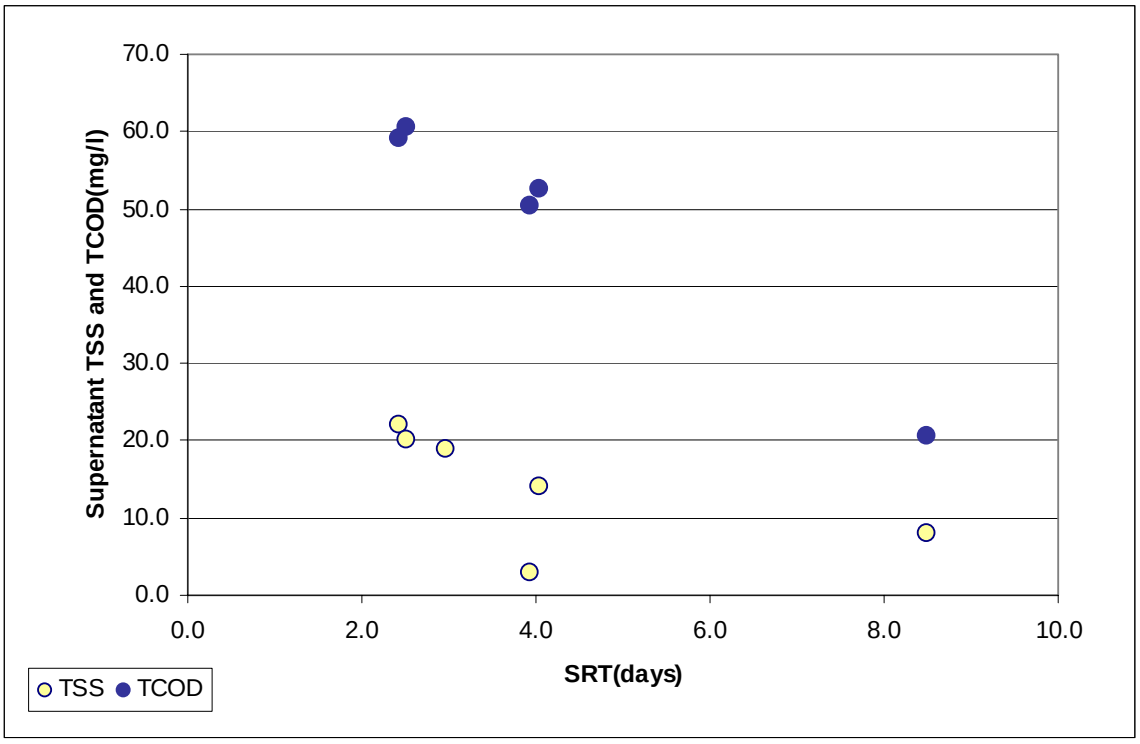



**Figure 4.9** Effect of SRT on supernatant quality.

Table 4.4 shows that there is no linear relationship between the SRT and the EP/MLVV, but it is clear that as the SRT increases the supernatant TCOD decreases linearly.

**Table 4.4** Summary Regression Analysis for SRT.

| **Relationship** | **Null Hypothesis (Ho): slope=0** | | **Coefficient of Determination ($R^2$)** |
|---|---|---|---|
| SRT vs. EPS/MLVSS | | Not Rejected | 0.0016 |
| SRT vs. Supernatan SS | | Not Rejected | 0.2915 |
| SRT vs. Supernatan TCOD | Rejected | | 0.8692 |

## *4.5 DO and EPS*

To prove that DO is an important parameter in activated sludge process in relation to bioflocculation, the EPS were extracted from the UASB for DO of zero, and for DO>0, the air injected to the SCC was varied using the control valve of the flow meter. For the SCC, DO values less than 1.5mg/l were not evaluated due to bulking problems that were experienced.

**Table 4.5**. EPS concentration from the UASB Reactor

| **Parameter** | **Concentration, mg/l** |
|---|---|
| MLSS | 21850 |
| MLVSS | 13385 |
| DO | 0 |
| EPS | 269 |
| EPS/mg/l MLVSS | 0.02 |

Despite the large concentration of biomass in the UASB sample, there were no visible floc particles, and the sludge settleability was poor. The results in Table 4.3 show that there is no flocculation taking place in the fully anaerobic sludge. The EPS concentration per MLVSS is practically zero.

Figures 4.9 and Table 4.5 show that there is no linear correlation between the EPS concentration per unit biomass and DO when all the SRTs are considered, but for SRT>4 days, the EPS/MLVSS is directly proportional to DO. At the same time this graphs demonstrate that even for a DO concentration as low as 1.7 mg/L, the EPS concentration is much higher than the amount when no oxygen is present.

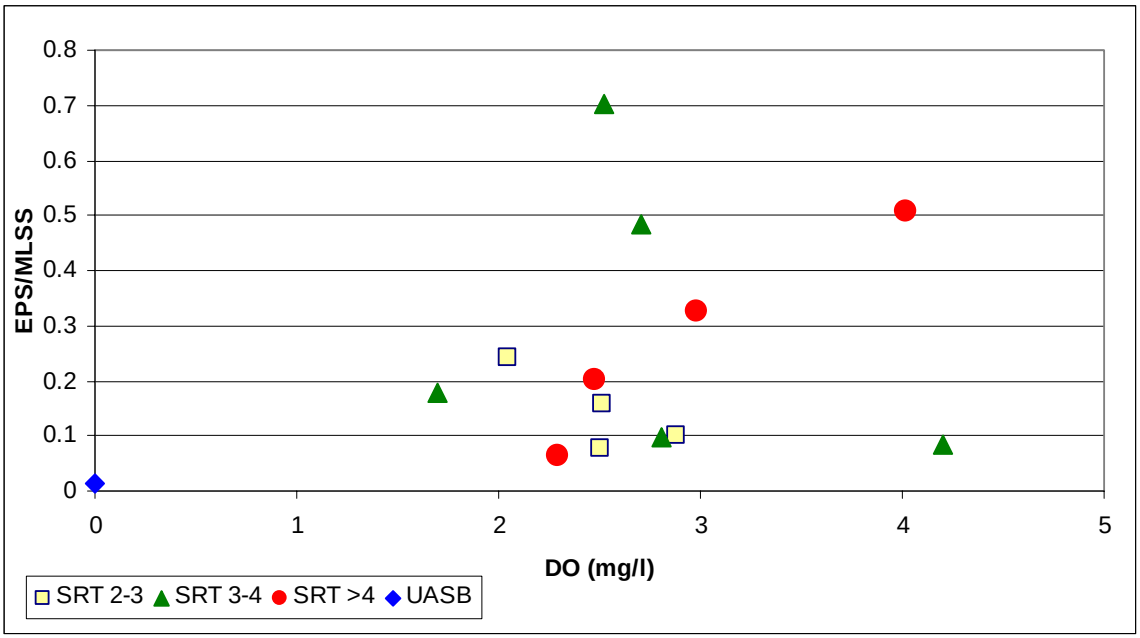



**Figure 4.10** Effect of DO on EPS /MLVSS.

Figures 4.10 and 4.11 show that the quality of the supernatant does not depend on SRT; but is clear that in aerobic condition the TCOD decreases significantly in comparison to the anaerobic condition.

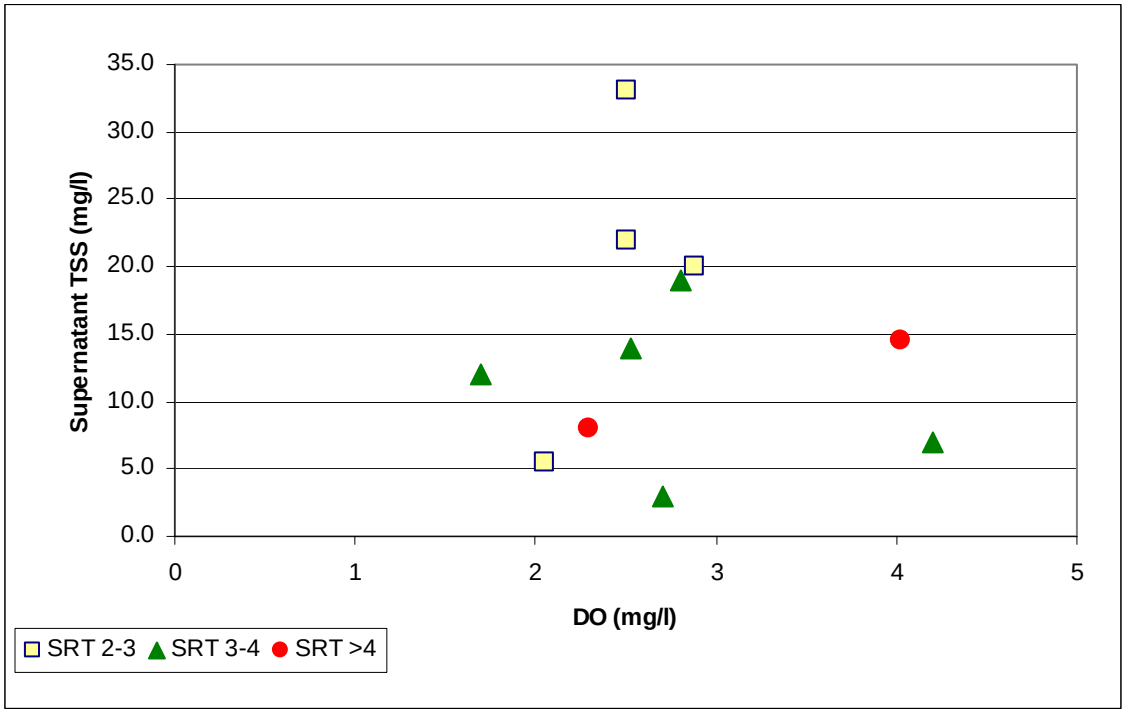



**Figure 4.11** Effect of DO on Supernatant SS.

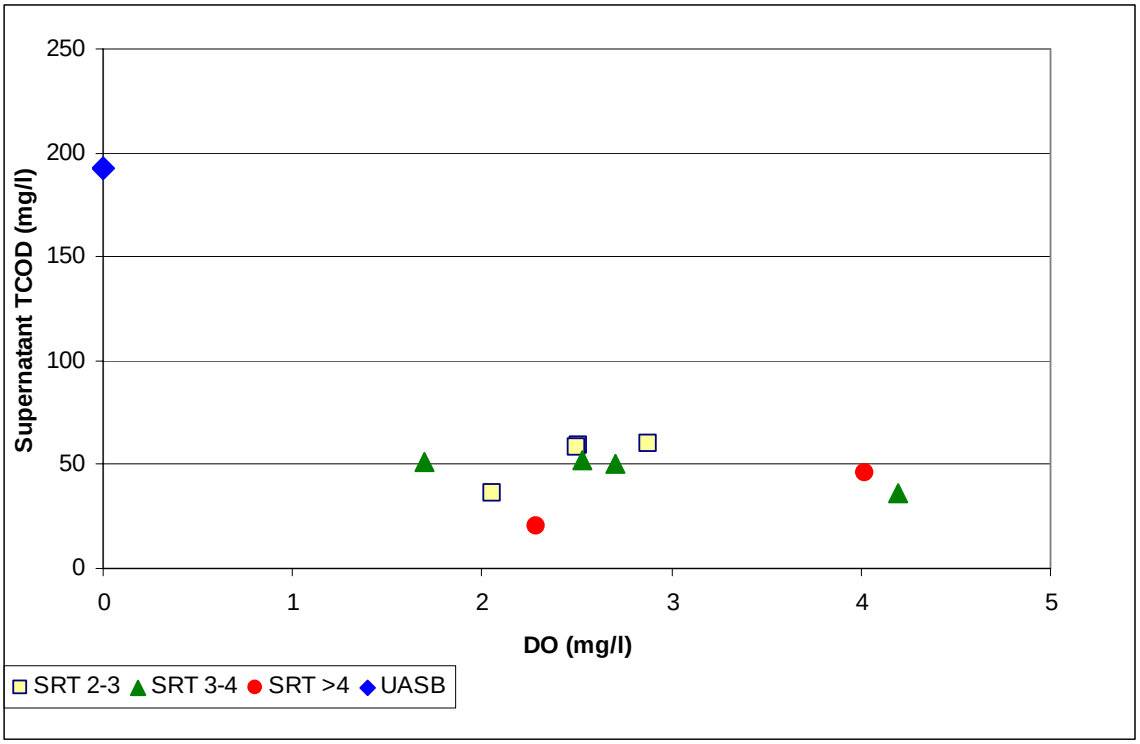



**Figure 4.12** Effect of DO on supernatant TCOD.

**Table 4.6** Summary Regression Analysis for DO.

| **Relationship** | **Null Hypothesis (Ho): slope =0** | | **Coefficient of Determination ($R^2$)** |
|---|---|---|---|
| DO vs. EPS/MLVSS | | Not Rejected | 0.0282 |
| DO vs. Supernatan SS | | Not Rejected | 0.0086 |
| DO vs. Supernatan TCOD | | Not Rejected | 0.0003 |

## *4.6 MLSS and Settling Parameters (Vo and n)*

To determine the relationship between the biomass concentration in the SCC and the Vesilind's settling parameter ( Vo and n), the zone settling test was carried out in the pilot plant. DO was kept between 2-3 mg/l, SRT 2-4 mg/l and MLSS between 2000-3000 mg/l.

Figures 4.10 and 4.11show the results for the fully aerobic system (Rojas, 2004) and the results of this research. These figures show that as the concentration of biomass increases the parameter Vo decreases and n increases. Figure 4.11 presents a linear relationship between Vo and MLSS for the aerobic system with a correlation of $R^2$= 0.74;while the combined anerobic/aerobic system showed a low $R^2$ of 0.14. This graph demonstrates that the transition from anaerobic to aerobic conditions and unstable conditions in the system had a noticeable effect on the settling properties of the sludge.

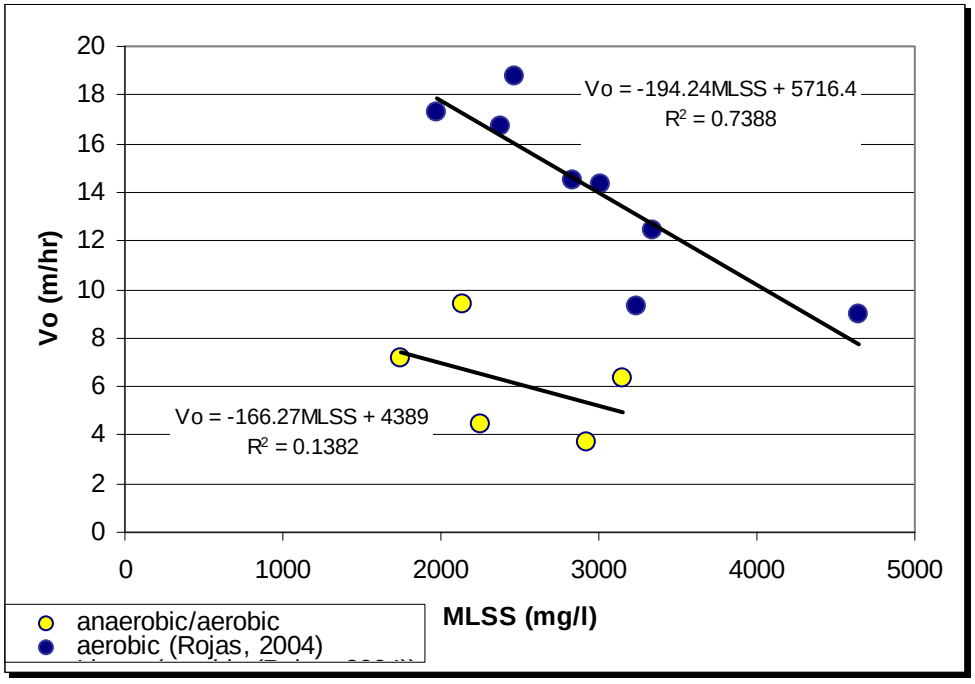



**Figure 4.13** Relationship between Vo and MLSS in the combined and fully aerobic system.

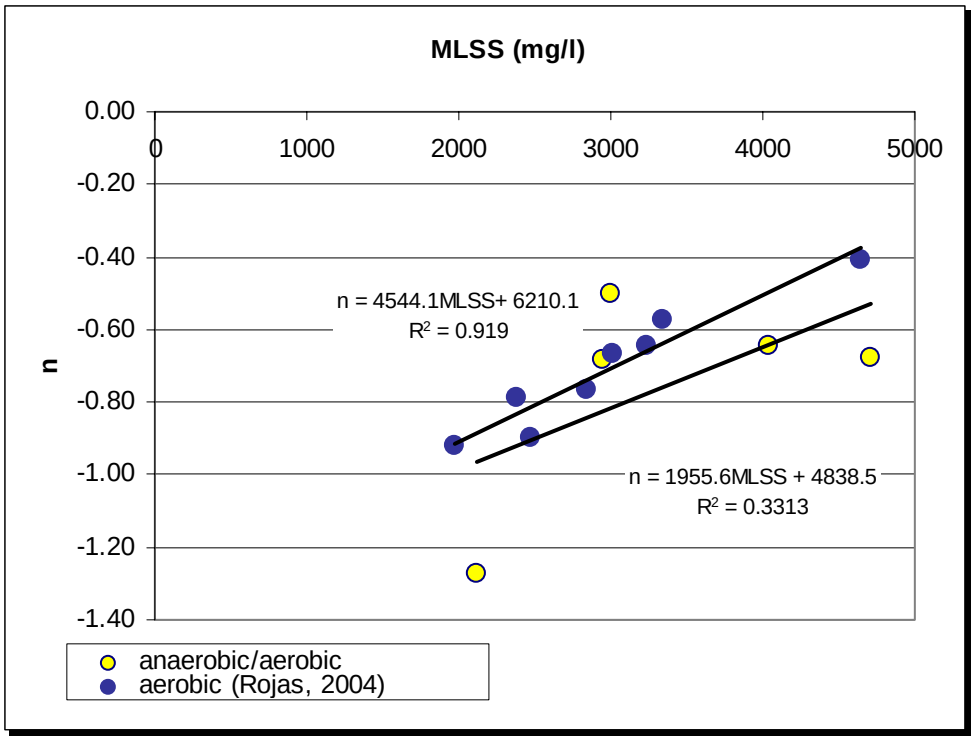



**Figure 4.14** Relationship between n and MLSS for the combined and fully aerobic system.

## *4.7 Clarifier performance*

All the analyses presented above were based on the supernatant concentrations. Figure 4.17 shows a graph relating the final effluent quality to the supernatant quality regarding TSS and TCOD.

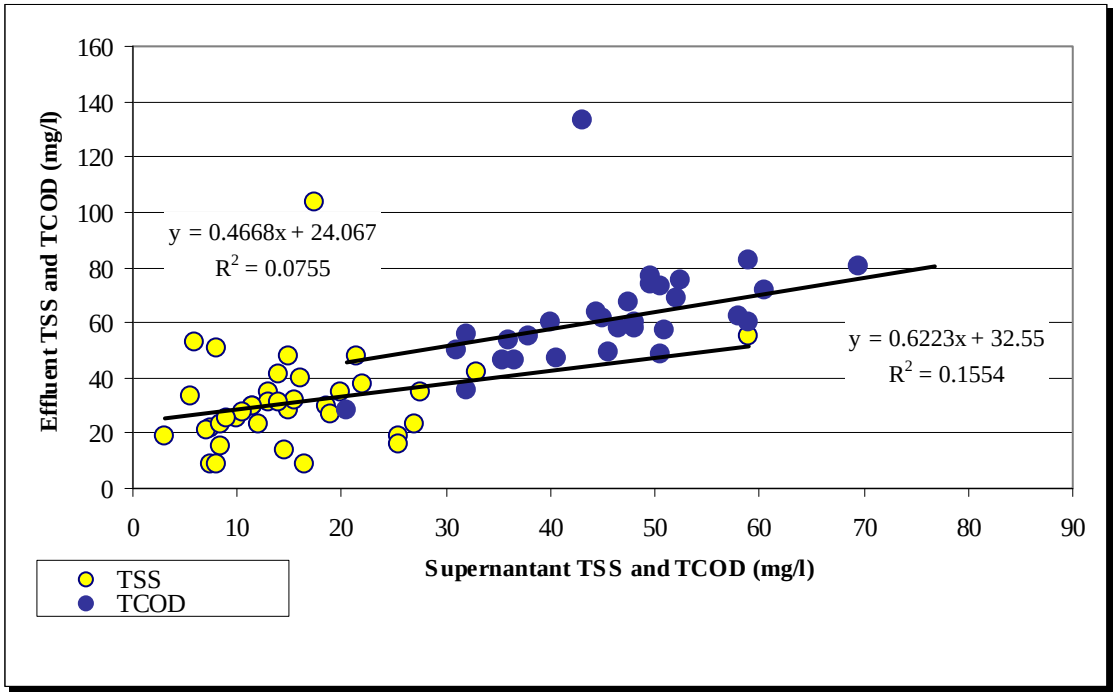



**Figure 4.15** Clarifier performance.

Figure 4.17 shows that there is not a statistically significant correlation between supernatant SS and the final effluent SS because the coefficient of determination $R^2$ is 0.075. The correlation for TCOD is a little better, but not significant at $R^2 = 0.155$. The hypothesis of the slope being equal to zero could not be rejected at the 95% confidence interval. These results suggest that the sedimentation unit was not working properly, and, consequently, there are significant differences between the TSS and TCOD concentrations obtained from the supernatant samples compared than those obtained from the final effluent samples.

## *4.8 Bulking Problems*

During the experimental phase of this research, operational problem related to bulking sludge were encountered, especially for DO concentrations below 1.5 mg/l. The sludge had a dark brown color and sludge did not settle. As a control mechanism, chloride was poured in the clarifier for consecutives day and microscopic monitoring was done on in a daily basis to determine any change.

The following picture corresponds to a sample taken during bulking problems. As is shown in the pictures the filaments are extending outside the floc forming a web that affect sludge settling.

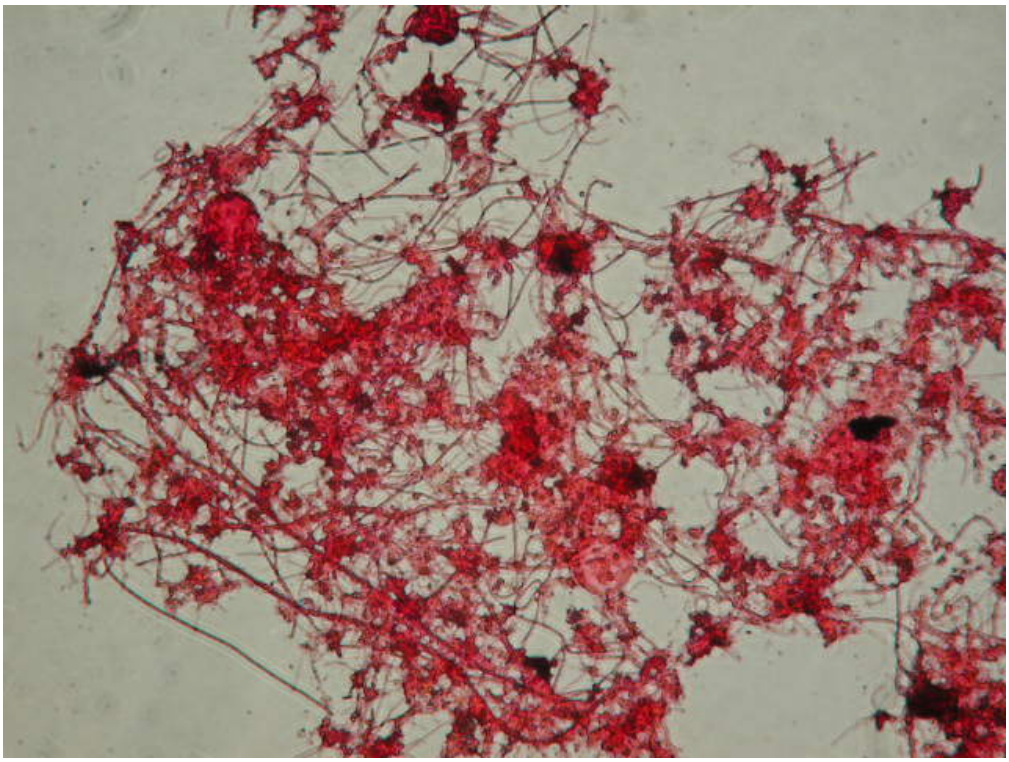


**Figure 4.16 Filamentous Bulking**

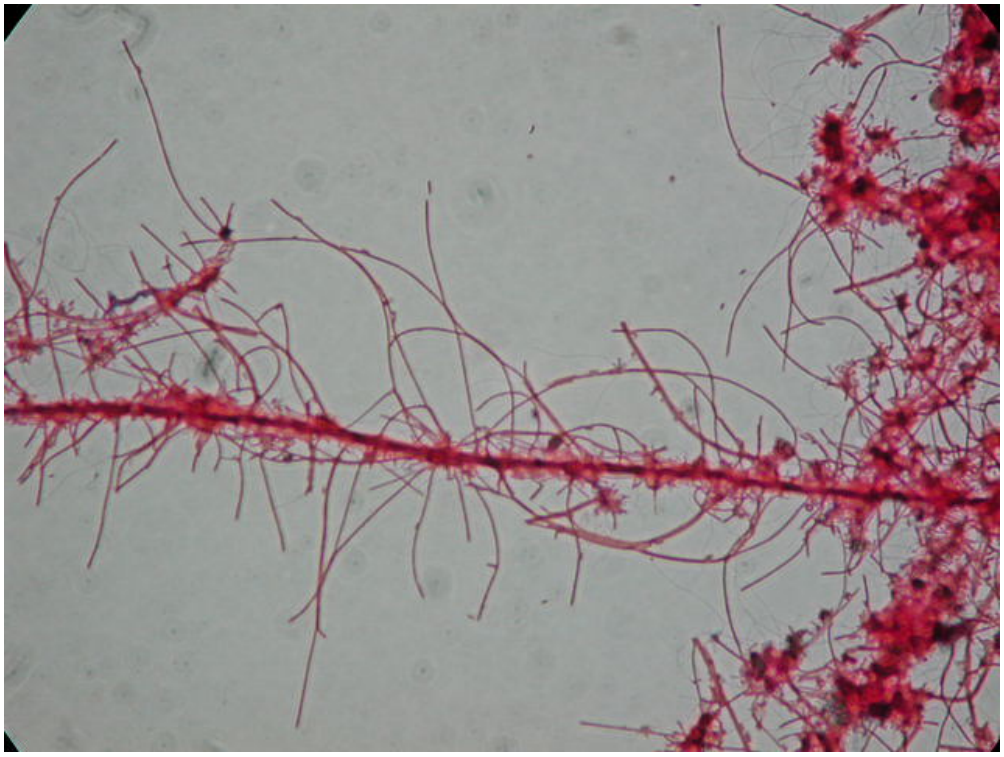


**Figure 4.17.** Filament extending out of the floc.

Besides bulking problems it is important to mention that the pilot plant presented unstable condition due to:

- Operational problems such as: loss of flow due to electrical break down causing changes in the HRT, MLVSS concentration and SRT, anaerobic condition in the SCC.
- Loss of sludge during heavy rainfall.

Intrusion of toxic substances causing bloom of crawlers and turbidity in the effluent.

# 5. Conclusions and Recommendations

The following conclusions can be drawn from this research project:

- The anaerobic/aerobic process is effective for providing secondary wastewater treatment, and enables sludge stabilization with no costs associated for construction and operation of digesters. HRT higher than 120 minutes may be required in the aeration chamber to get more stable conditions.
- The majority of the total organic material from municipal wastewater is in the form of organic particulate material.
- There was no clear correlation between mixed liquor volatile suspended solids and the concentration of exocellular polymeric substances in the SCC.
- As the ratio of EPS/MLVSS increases effluent improvement are seen as SRT increases.
- If SRT>4 days, EPS/MLVSS is directly proportional to DO.
- Best effluent quality was observed at SRT = 8 days
- There is almost no polymer production under anaerobic conditions.
- Exocellular polymeric substance per mg of biomass in anaerobic conditions is near zero and this would explain the poor flocculation observed in anaerobic reactors.
- Dissolved oxygen plays an important role in sludge settling parameters Vo and n.
- Bulking problems were encountered for DO concentration less than 1.5 mg/l.
- The combined anaerobic/aerobic system was demonstrated to be highly efficient, with 86% TCOD, 66% PCOD, and 90% TSS removal.

Based on the experience of this research project, the following items are suggested for further investigation:

- Investigate the effect of HRT on EPS production.
- Improve the performance of the final clarifier unit.
- Study the effect of incorporating a selector to improve compaction characteristics.

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# APPENDIX A

**Picture A.1**. Splitter Box

**Picture A.2**. Rotary Screen

**Picture A.3**. Distribution Tank

**Picture A.4**. Mixing Tank

**Picture A.5**. Anaerobic reactor

**Picture A.6**. Aeration Chamber

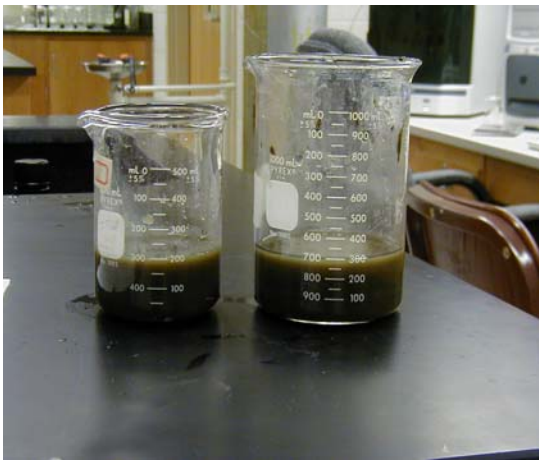
**Picture A.7**. Secondary Clarifier

## EPS Extraction Procedure

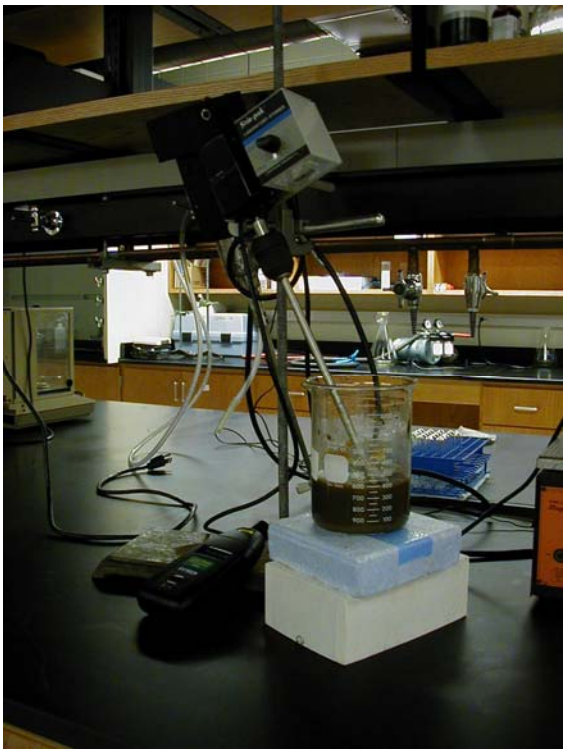
**Picture A.8**. Sludge after 2hr at 4ºC

**Picture A.9** Decant supernatant

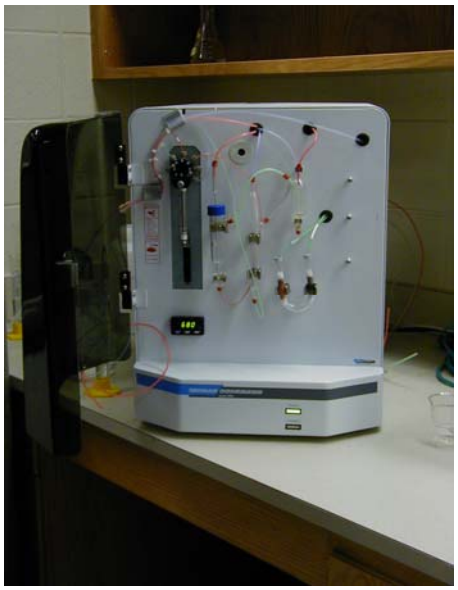
**Picture A.10** Centrifuge at 2000g for 15 min and discard supernatant



**Picture A.11** Re-suspend sludge pellets to original volume (500ml) with buffer solution and take 300ml

**Picture A.11** Add CER ( 60g CER per kg MLVSS) and mixed for 2hrs at 900rpm



**Picture A.12** Centrifuge supernatant (twice) for 15 min at 12000 rpm and read EPS as TOC using Apollo 8000

# APPENDIX B

**Table B.1** Relationship between TCOD and PCOD entering the SCC

| AFBR | | | UASB | | |
|---|---|---|---|---|---|
| TCOD | DCOD | PCOD | TCOD | DCOD | PCOD |
| mg/l | mg/l | mg/l | mg/l | mg/l | mg/l |
| 145 | 35 | 110 | 134 | 58 | 76 |
| 173 | 61 | 112 | 172 | 50 | 122 |
| 193 | 64 | 129 | 196 | 73 | 123 |
| 209 | 88 | 121 | 238 | 119 | 119 |
| 224 | 81 | 143 | 264 | 94 | 170 |
| 236 | 53 | 183 | 270 | 45 | 225 |
| 240 | 66 | 174 | 278 | 36 | 243 |
| 256 | 73 | 183 | 321 | 69 | 253 |
| 271 | 86 | 186 | 344 | 97 | 247 |
| 272 | 85 | 187 | 390 | 123 | 267 |
| 279 | 90 | 189 | 449 | 82 | 367 |
| 283 | 83 | 200 | | | |
| 338 | 90 | 248 | | | |
| 358 | 79 | 280 | | | |
| 375 | 80 | 295 | | | |

**Table B.2** Relationship between TCOD and PCOD in Wastewater

| TCOD mg/l | PCOD mg/l | TCOD mg/l | PCOD mg/l | TCOD mg/l | PCOD mg/l | TCOD mg/l | PCOD mg/l |
|---|---|---|---|---|---|---|---|
| 17 | 7 | 52 | 29 | 115 | 57 | 155 | 108 |
| 21 | 11 | 52 | 15 | 118 | 50 | 172 | 122 |
| 26 | 1 | 52 | 3 | 134 | 67 | 173 | 112 |
| 26 | 12 | 52 | 27 | 134 | 76 | 193 | 129 |
| 31 | 15 | 52 | 30 | 138 | 96 | 196 | 123 |
| 32 | 11 | 53 | 21 | 145 | 110 | 208 | 164 |
| 32 | 6 | 54 | 29 | 209 | 121 | 309 | 261 |
| 34 | 22 | 55 | 35 | 224 | 143 | 309 | 266 |
| 36 | 20 | 55 | 30 | 233 | 196 | 310 | 262 |
| 36 | 17 | 57 | 31 | 236 | 183 | 310 | 263 |
| 37 | 11 | 58 | 22 | 238 | 119 | 310 | 266 |
| 37 | 24 | 59 | 14 | 240 | 174 | 310 | 270 |
| 38 | 13 | 59 | 43 | 252 | 224 | 310 | 269 |
| 40 | 11 | 61 | 16 | 255 | 208 | 310 | 265 |
| 41 | 15 | 64 | 37 | 256 | 183 | 310 | 265 |
| 42 | 23 | 64 | 30 | 257 | 212 | 310 | 268 |
| 43 | 23 | 65 | 39 | 264 | 170 | 310 | 269 |
| 43 | 18 | 65 | 37 | 270 | 225 | 311 | 267 |
| 43 | 26 | 68 | 22 | 271 | 186 | 314 | 270 |
| 44 | 21 | 69 | 48 | 272 | 187 | 315 | 268 |
| 44 | 22 | 69 | 48 | 278 | 243 | 321 | 253 |
| 44 | 24 | 69 | 39 | 279 | 189 | 323 | 278 |
| 45 | 24 | 71 | 45 | 283 | 200 | 328 | 281 |
| 45 | 28 | 73 | 42 | 299 | 252 | 330 | 285 |
| 45 | 24 | 74 | 41 | 300 | 255 | 338 | 248 |
| 46 | 23 | 74 | 43 | 300 | 261 | 341 | 267 |
| 47 | 23 | 77 | 38 | 300 | 257 | 344 | 247 |
| 47 | 27 | 77 | 38 | 300 | 256 | 356 | 304 |
| 47 | 26 | 78 | 35 | 300 | 256 | 358 | 280 |
| 48 | 31 | 79 | 44 | 300 | 260 | 359 | 279 |
| 48 | 38 | 79 | 49 | 301 | 243 | 361 | 309 |
| 48 | 25 | 80 | 48 | 303 | 260 | 375 | 295 |
| 48 | 29 | 82 | 48 | 305 | 264 | 381 | 326 |
| 48 | 29 | 83 | 50 | 305 | 264 | 390 | 267 |
| 49 | 26 | 84 | 51 | 305 | 260 | 403 | 364 |
| 50 | 15 | 86 | 50 | 306 | 258 | 421 | 395 |
| 50 | 25 | 87 | 50 | 307 | 259 | 422 | 376 |
| 50 | 25 | 87 | 53 | 308 | 265 | 438 | 330 |
| 50 | 29 | 91 | 54 | 308 | 266 | 449 | 367 |
| 51 | 6 | 93 | 35 | 309 | 267 | | |
| 51 | 25 | 102 | 38 | 309 | 267 | | |
| 51 | 29 | 115 | 51 | 309 | 261 | | |

**Table B.3** Relationship between MLVSS and EPS (dilution)

| Sample | MLSS mg/l | MLVSS mg/l | EPS mg/l | EPS mg/l |
|---|---|---|---|---|
| 1 | 6072 | 3910 | 250.70 | 1253.48 |
| | 6072 | 3910 | 246.76 | 1233.82 |
| | 6072 | 3910 | 240.75 | 1203.77 |
| | 6072 | 3910 | 243.87 | 1219.34 |
| | 6072 | 3910 | 246.09 | 1230.45 |
| 2 | 3380 | 2180 | 253.96 | 1269.78 |
| | 3380 | 2180 | 253.22 | 1266.10 |
| | 3380 | 2180 | 257.73 | 1288.65 |
| | 3380 | 2180 | 258.44 | 1292.18 |
| | 3380 | 2180 | 248.67 | 1243.37 |
| 3 | 2038 | 1296 | 205.68 | 1028.39 |
| | 2038 | 1296 | 203.04 | 1015.19 |
| | 2038 | 1296 | 203.85 | 1019.23 |
| | 2038 | 1296 | 205.26 | 1026.28 |
| | 2038 | 1296 | 207.12 | 1035.61 |
| 4 | 1522 | 996 | 145.29 | 726.47 |
| | 1522 | 996 | 147.25 | 736.25 |
| | 1522 | 996 | 148.54 | 742.70 |
| | 1522 | 996 | 147.78 | 738.92 |
| | 1522 | 996 | 147.76 | 738.81 |

**Table B.4** Relationship between MLVSS and EPS

| HRT | SRT | DO | Temperature | pH | EPS | MLSS | | SUPERNATANT | | | | EPS/MLVSS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | TSS | VSS | TSS | TCOD | DCOD | PCOD | |
| min | days | mg/l | °c | | mg/l | mg/l | mg/l | mg/l | mg/l | mg/l | mg/l | |
| 120 | 3.9 | 2.70 | 23.9 | NA | 1028 | 2120 | 1752 | 12.0 | 51 | 45 | 6 | 0.59 |
| 124 | 4.0 | 2.53 | 29.1 | NA | 1958 | 2784 | 2088 | 14.0 | 53 | 32 | 21 | 0.94 |
| 120 | 2.4 | 2.51 | 26.3 | 6.94 | 462 | 2942 | 2140 | 22.0 | 59 | 45 | 14 | 0.22 |
| 123 | 2.5 | 2.88 | 29.0 | 7.46 | 298 | 2994 | 2248 | 20.0 | 61 | 45 | 16 | 0.13 |
| 120 | 3.0 | 2.81 | 29.4 | 7.55 | 376 | 3790 | 2454 | 19.0 | NA | NA | NA | 0.15 |
| 120 | 2.7 | 2.50 | 29.9 | 7.46 | 314 | 4036 | 2930 | 33.0 | 58 | 36 | 22 | 0.11 |
| 112 | 2.2 | 2.05 | 28.6 | NA | 1143 | 4705 | 3152 | 5.5 | 36 | 19 | 17 | 0.36 |

**Table B.5** Relationship between SRT and EPS/MLVSS

| HRT | SRT | DO | Temperature | pH | EPS | EPS/MLVSS | MLSS | | SUPERNATANT | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | TSS | VSS | TSS | TCOD |
| min | days | mg/l | °c | | mg/l | | mg/l | mg/l | mg/l | mg/l |
| 112 | 2.2 | 2.05 | 28.60 | | 1143.29 | 0.36 | 4705 | 3152 | 5 | 36 |
| 120 | 2.4 | 2.51 | 26.30 | 6.94 | 462.38 | 0.22 | 2942 | 2140 | 22 | 59 |
| 123 | 2.5 | 2.88 | 29.00 | 7.46 | 298.29 | 0.13 | 2994 | 2248 | 20 | 61 |
| 120 | 2.7 | 2.50 | 29.90 | 7.46 | 313.79 | 0.11 | 4036 | 2930 | 33 | 58 |
| 120 | 3.0 | 2.81 | 29.40 | 7.55 | 375.64 | 0.15 | 3790 | 2454 | 19 | NA |
| 121 | 3.5 | 1.70 | 27.80 | 7.33 | 436.19 | 0.23 | 2432 | 1897 | 12 | 51 |
| 120 | 3.9 | 2.70 | 23.90 | | 1027.71 | 0.59 | 2120 | 1752 | 3 | 51 |
| 120 | 4.0 | 4.20 | 20.60 | 7.45 | 285.98 | 0.11 | 3374 | 2552 | 7 | 37 |
| 124 | 4.0 | 2.53 | 29.10 | | 1957.91 | 0.94 | 2784 | 2088 | 14 | 53 |
| 127 | 4.4 | 4.02 | 23.30 | 7.33 | 2015.37 | 0.78 | 3980 | 2587 | 15 | 46 |
| 120 | 4.7 | 2.98 | 23.30 | | 736.79 | 0.41 | 2264 | 1780 | NA | NA |
| 116 | 8.5 | 2.29 | 25.20 | | 146.65 | 0.08 | 2254 | 1760 | 8 | 21 |
| 120 | 8.7 | 2.48 | 23.50 | | 458.91 | 0.26 | 2278 | 1792 | NA | NA |

**Table B.6** Relationship between DO and EPS/MLVSS

| HRT | SRT | DO | Temperature | pH | EPS | EPS/MLVSS | MLSS | | SUPERNATANT | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | TSS | VSS | TSS | TCOD | DCOD | PCOD |
| min | days | mg/l | °c | | mg/l | | mg/l | mg/l | mg/l | mg/l | mg/l | mg/l |
| UASB | NA | 0 | 29.8 | 6.78 | 268.55 | 0.02 | 21850 | 13385 | NA | NA | NA | NA |
| 121 | 3.5 | 1.70 | 27.80 | 7.33 | 436.19 | 0.23 | 2432 | 1897 | 12 | 51 | 45 | 6 |
| 112 | 2.2 | 2.05 | 28.60 | NA | 1143.29 | 0.36 | 4705 | 3152 | 5 | 36 | 19 | 17 |
| 116 | 8.5 | 2.29 | 25.20 | NA | 146.65 | 0.08 | 2254 | 1760 | 8 | 21 | 10 | 11 |
| 120 | 8.7 | 2.48 | 23.50 | NA | 458.91 | 0.26 | 2278 | 1792 | NA | NA | 72 | NA |
| 120 | 2.7 | 2.50 | 29.90 | 7.46 | 313.79 | 0.11 | 4036 | 2930 | 33 | 58 | 36 | 22 |
| 120 | 2.4 | 2.51 | 26.30 | 6.94 | 462.38 | 0.22 | 2942 | 2140 | 22 | 59 | 45 | 14 |
| 124 | 4.0 | 2.53 | 29.10 | NA | 1957.91 | 0.94 | 2784 | 2088 | 14 | 53 | 32 | 21 |
| 120 | 3.9 | 2.70 | 23.90 | NA | 1027.71 | 0.59 | 2120 | 1752 | 3 | 51 | NA | NA |
| 120 | 3.0 | 2.81 | 29.40 | 7.55 | 375.64 | 0.15 | 3790 | 2454 | 19 | NA | NA | NA |
| 123 | 2.5 | 2.88 | 29.00 | 7.46 | 298.29 | 0.13 | 2994 | 2248 | 20 | 61 | 45 | 16 |
| 120 | 4.7 | 2.98 | 23.30 | NA | 736.79 | 0.41 | 2264 | 1780 | NA | NA | NA | NA |
| 127 | 4.4 | 4.02 | 23.30 | 7.33 | 2015.37 | 0.78 | 3980 | 2587 | 15 | 46 | 23 | 23 |
| 120 | 4.0 | 4.20 | 20.60 | 7.45 | 285.98 | 0.11 | 3374 | 2552 | 7 | 37 | 26 | 11 |

**Table B.7** Relationship between MLSS and Vo and n

Anaerobic/aerobic system

| MLSS | MLVSS | Vo | n | DO | SRT | SVI |
|---|---|---|---|---|---|---|
| mg/l | mg/l | m/hr | | mg/l | days | ml/g |
| 2120 | 1752 | 7.16 | -1.27 | 2.70 | 3.9 | 275.28 |
| 2942 | 2140 | 9.39 | -0.68 | 2.51 | 2.4 | 48.43 |
| 2994 | 2248 | 4.40 | -0.50 | 2.88 | 2.5 | 73.88 |
| 4036 | 2930 | 3.71 | -0.64 | 2.50 | 2.7 | 271.12 |
| 4705 | 3152 | 6.30 | -0.68 | 2.05 | 2.2 | NA |

Aerobic system (Rojas, 2004)

| MLSS | Vo | n | SRT |
|---|---|---|---|
| mg/l | m/hr | | days |
| 1,978 | 17.28 | -0.9192 | 0.93 |
| 2,376 | 16.68 | -0.7907 | 0.96 |
| 2,470 | 18.76 | -0.8998 | 1.15 |
| 2,836 | 14.52 | -0.7684 | 1.18 |
| 3,014 | 14.36 | -0.6692 | 1.19 |
| 3,242 | 9.34 | -0.6442 | 1.06 |
| 3,348 | 12.45 | -0.5721 | 1.16 |
| 4,645 | 8.95 | -0.4080 | 1.34 |

**Table B.8** Clarifier performance

| SUPERNATANT | | TOTAL EFFLUENT | |
|---|---|---|---|
| TSS | TCOD | TSS | TCOD |
| mg/l | mg/l | mg/l | mg/l |
| 3.0 | 51 | 18.50 | 49 |
| 5.5 | 36 | 33.50 | 54 |
| 6.0 | 50 | 52.50 | 74 |
| 7.0 | 37 | 21.00 | 46 |
| 7.5 | 36 | 22.00 | 46 |
| 7.5 | NA | 8.50 | NA |
| 8.0 | 50 | 51.00 | 77 |
| 8.0 | 21 | 8.50 | 28 |
| 8.5 | NA | 15.50 | NA |
| 8.5 | 41 | 23.50 | 47 |
| 9.0 | 40 | 25.00 | 60 |
| 10.0 | 47 | 25.50 | 58 |
| 10.5 | 32 | 27.50 | 56 |
| 11.5 | 59 | 29.50 | 60 |
| 11.5 | 31 | 30.00 | 50 |
| 12.0 | 51 | 23.00 | 58 |
| 13.0 | 48 | 35.00 | 67 |
| 13.0 | 38 | 31.00 | 55 |
| 14.0 | 52 | 31.00 | 69 |
| 14.0 | 53 | 41.50 | 75 |
| 14.5 | 46 | 13.50 | 50 |
| 15.0 | NA | 47.50 | NA |
| 15.0 | 48 | 28.00 | 58 |
| 15.5 | 48 | 32.00 | 60 |
| 16.0 | 45 | 40.00 | 64 |
| 16.5 | 32 | 9.00 | 35 |
| 17.5 | 43 | 103.50 | 134 |
| 18.5 | 45 | 29.50 | 62 |
| 19.0 | NA | 27.00 | NA |
| 20.0 | 61 | 34.50 | 72 |
| 21.5 | 51 | 48.00 | 73 |
| 22.0 | 59 | 38.00 | 83 |
| 25.5 | NA | 18.67 | NA |
| 25.5 | NA | 15.67 | NA |
| 27.0 | 77 | 23.00 | 51 |
| 27.5 | NA | 34.67 | NA |
| 33.0 | 58 | 42.00 | 63 |
| 59.0 | 70 | 55.00 | 81 |

# APPENDIX C

## Statistical Analysis

**C.1** TCOD and PCOD entering the SCC

| *Regression Statistics* | |
|---|---|
| Multiple R | 0.962163 |
| R Square | 0.925758 |
| Adjusted R Square | 0.92253 |
| Standard Error | 19.30582 |
| Observations | 25 |

ANOVA

| | *df* | *SS* | *MS* | *F* | *Significance F* |
|---|---|---|---|---|---|
| Regression | 1 | 106893.006 | 106893.0057 | 286.7959 | 1.75479E-14 |
| Residual | 23 | 8572.43431 | 372.7145353 | | |
| Total | 24 | 115465.44 | | | |

| | *Coefficients* | *Standard Error* | *t Stat* | *P-value* | *Lower 95%* | *Upper 95%* | *Lower 95.0%* | *Upper 95.0%* |
|---|---|---|---|---|---|---|---|---|
| Intercept | -45.8986 | 14.6643648 | -3.129941054 | 0.004701 | -76.23410617 | -15.5631 | -76.2341 | -15.5631 |
| 145 | 0.885622 | 0.05229524 | 16.93504986 | 1.75E-14 | 0.777441697 | 0.993803 | 0.777442 | 0.993803 |

**C.2** TCOD and PCOD in wastewater (data from Jimenez(2002), Rojas (2004) and this research)

| *Regression Statistics* | |
|---|---|
| Multiple R | 0.989381 |
| R Square | 0.978874 |
| Adjusted R Square | 0.978743 |
| Standard Error | 16.75191 |
| Observations | 163 |

ANOVA

| | *df* | *SS* | *MS* | *F* | *Significance F* |
|---|---|---|---|---|---|
| Regression | 1 | 2093461 | 2093461 | 7459.956 | 8.9E-137 |
| Residual | 161 | 45180.86 | 280.6265 | | |
| Total | 162 | 2138642 | | | |

| | *Coefficients* | *Standard Error* | *t Stat* | *P-value* | *Lower 95%* | *Upper 95%* | *Lower 95.0%* | *Upper 95.0%* |
|---|---|---|---|---|---|---|---|---|
| Intercept | -23.1043 | 2.1992 | -10.5058 | 5.46E-20 | -27.4473 | -18.7613 | -27.4473 | -18.7613 |
| 17 | 0.890159 | 0.010306 | 86.37104 | 8.9E-137 | 0.869806 | 0.910511 | 0.869806 | 0.910511 |

**C.3** MLVSS and EPS (dilution)

| *Regression Statistics* | |
|---|---|
| Multiple R | 0.728658447 |
| R Square | 0.530943133 |
| Adjusted R Square | 0.503351553 |
| Standard Error | 154.0985632 |
| Observations | 19 |

ANOVA

| | *df* | *SS* | *MS* | *F* | *Significance F* |
|---|---|---|---|---|---|
| Regression | 1 | 456949.9245 | 456949.9245 | 19.24294023 | 0.0004025 |
| Residual | 17 | 403688.2421 | 23746.36718 | | |
| Total | 18 | 860638.1666 | | | |

| | *Coefficients* | *Standard Error* | *t Stat* | *P-value* | *Lower 95%* | *Upper 95%* | *Lower 95.0%* | *Upper 95.0%* |
|---|---|---|---|---|---|---|---|---|
| Intercept | 769.4522071 | 74.18354853 | 10.37227556 | 9.03703E-09 | 612.93838 | 925.966 | 612.938 | 925.966 |
| 6072 | 0.092149557 | 0.021006686 | 4.386677585 | 0.000402531 | 0.0478293 | 0.13647 | 0.04783 | 0.13647 |

**C.4** MLVSS and EPS

| *Regression Statistics* | |
|---|---|
| Multiple R | 0.131728295 |
| R Square | 0.017352344 |
| Adjusted R Square | -0.22830957 |
| Standard Error | 740.2356223 |
| Observations | 6 |

ANOVA

| | *df* | *SS* | *MS* | *F* | *Significance F* |
|---|---|---|---|---|---|
| Regression | 1 | 38704.39381 | 38704.39 | 0.070635058 | 0.803550455 |
| Residual | 4 | 2191795.106 | 547948.8 | | |
| Total | 5 | 2230499.5 | | | |

| | | *Coefficients* | *Standard Error* | *t Stat* | *P-value* | *Lower 95%* | *Upper 95%* | *Lower 95.0%* | *Upper 95.0%* |
|---|---|---|---|---|---|---|---|---|---|
| Intercept | | 1257.014282 | 1899.905594 | 0.661619 | 0.544384143 | -4017.980231 | 6532.009 | -4017.98 | 6532.009 |
| | 1752 | -0.19924632 | 0.749687285 | -0.26577 | 0.803550455 | -2.280716221 | 1.882224 | -2.28072 | 1.882224 |

**C.5** MLVSS and supernatant SS

| *Regression Statistics* | |
|---|---|
| Multiple R | 0.212369366 |
| R Square | 0.045100748 |
| Adjusted R Square | -0.19362407 |
| Standard Error | 9.931922379 |
| Observations | 6 |

ANOVA

| | *df* | *SS* | *MS* | *F* | *Significance F* |
|---|---|---|---|---|---|
| Regression | 1 | 18.63600475 | 18.636 | 0.188923585 | 0.68623496 |
| Residual | 4 | 394.5723286 | 98.64308 | | |
| Total | 5 | 413.2083333 | | | |

| | *Coefficients* | *Standard Error* | *t Stat* | *P-value* | *Lower 95%* | *Upper 95%* | *Lower 95.0%* | *Upper 95.0%* |
|---|---|---|---|---|---|---|---|---|
| Intercept | 27.90609448 | 21.07554501 | 1.324098 | 0.256065089 | -30.6091205 | 86.42131 | -30.6091 | 86.42131 |
| 2120 | -0.00253807 | 0.005839301 | -0.43465 | 0.68623496 | -0.018750603 | 0.013674 | -0.01875 | 0.013674 |

**C.6** MLVSS and supernatant TCOD

| *Regression Statistics* | |
|---|---|
| Multiple R | 0.655265404 |
| R Square | 0.42937275 |
| Adjusted R Square | 0.239163666 |
| Standard Error | 8.865347895 |
| Observations | 5 |

ANOVA

| | *df* | *SS* | *MS* | *F* | *Significance F* |
|---|---|---|---|---|---|
| Regression | 1 | 177.4168201 | 177.4168 | 2.257372475 | 0.22999529 |
| Residual | 3 | 235.7831799 | 78.59439 | | |
| Total | 4 | 413.2 | | | |

| | *Coefficients* | *Standard Error* | *t Stat* | *P-value* | *Lower 95%* | *Upper 95%* | *Lower 95.0%* | *Upper 95.0%* |
|---|---|---|---|---|---|---|---|---|
| Intercept | 87.32928497 | 22.92794608 | 3.808858 | 0.031812657 | 14.36225922 | 160.2963 | 14.36226 | 160.2963 |
| 1752 | -0.01350903 | 0.008991303 | -1.50246 | 0.22999529 | -0.042123397 | 0.015105 | -0.04212 | 0.015105 |

**C.7** EPS/MLVSS and supernatant SS

| *Regression Statistics* | |
|---|---|
| Multiple R | 0.489445732 |
| R Square | 0.239557125 |
| Adjusted R Square | 0.049446406 |
| Standard Error | 0.309873774 |
| Observations | 6 |

ANOVA

| | *df* | *SS* | *MS* | *F* | *Significance F* |
|---|---|---|---|---|---|
| Regression | 1 | 0.120996311 | 0.120996 | 1.260092677 | 0.324456508 |
| Residual | 4 | 0.384087022 | 0.096022 | | |
| Total | 5 | 0.505083333 | | | |

| | | *Coefficients* | *Standard Error* | *t Stat* | *P-value* | *Lower 95%* | *Upper 95%* | *Lower 95.0%* | *Upper 95.0%* |
|---|---|---|---|---|---|---|---|---|---|
| Intercept | | 0.642035898 | 0.31489506 | 2.038888 | 0.111083902 | -0.232254761 | 1.516327 | -0.23225 | 1.516327 |
| | 12 | -0.01711203 | 0.015244047 | -1.12254 | 0.324456508 | -0.059436378 | 0.025212 | -0.05944 | 0.025212 |

**C.8** EPS/MLVSS and supernatant TCOD

| *Regression Statistics* | |
|---|---|
| Multiple R | 0.280539056 |
| R Square | 0.078702162 |
| Adjusted R Square | -0.22839712 |
| Standard Error | 11.26469805 |
| Observations | 5 |

ANOVA

| | *df* | *SS* | *MS* | *F* | *Significance F* |
|---|---|---|---|---|---|
| Regression | 1 | 32.51973338 | 32.51973 | 0.256275958 | 0.647548824 |
| Residual | 3 | 380.6802666 | 126.8934 | | |
| Total | 4 | 413.2 | | | |

| | | *Coefficients* | *Standard Error* | *t Stat* | *P-value* | *Lower 95%* | *Upper 95%* | *Lower 95.0%* | *Upper 95.0%* |
|---|---|---|---|---|---|---|---|---|---|
| Intercept | | 56.32461578 | 7.665137792 | 7.348154 | 0.0052085 | 31.93070345 | 80.71853 | 31.9307 | 80.71853 |
| | 0.59 | -8.30856755 | 16.41240487 | -0.50624 | 0.647548824 | -60.54021379 | 43.92308 | -60.5402 | 43.92308 |

**C.9** SRT and EPS/MLVSS

| *Regression Statistics* | |
|---|---|
| Multiple R | 0.0404 |
| R Square | 0.001632 |
| Adjusted R Square | -0.0982 |
| Standard Error | 0.300934 |
| Observations | 12 |

ANOVA

| | *df* | *SS* | *MS* | *F* | *Significance F* |
|---|---|---|---|---|---|
| Regression | 1 | 0.001481 | 0.001481 | 0.016348 | 0.900794 |
| Residual | 10 | 0.905611 | 0.090561 | | |
| Total | 11 | 0.907092 | | | |

| | | *Coefficients* | *Standard Error* | *t Stat* | *P-value* | *Lower 95%* | *Upper 95%* | *Lower 95.0%* | *Upper 95.0%* |
|---|---|---|---|---|---|---|---|---|---|
| Intercept | | 0.358073 | 0.206165 | 1.736826 | 0.113063 | -0.10129 | 0.817437 | -0.10129 | 0.817437 |
| | 2.2 | -0.00549 | 0.042899 | -0.12786 | 0.900794 | -0.10107 | 0.0901 | -0.10107 | 0.0901 |

**C.10** DO and EPS/MLVSS

| *Regression Statistics* | |
|---|---|
| Multiple R | 0.168063 |
| R Square | 0.028245 |
| Adjusted R Square | -0.0601 |
| Standard Error | 0.283175 |
| Observations | 13 |

ANOVA

| | *df* | *SS* | *MS* | *F* | *Significance F* |
|---|---|---|---|---|---|
| Regression | 1 | 0.025638 | 0.025638 | 0.319727 | 0.583121 |
| Residual | 11 | 0.882069 | 0.080188 | | |
| Total | 12 | 0.907708 | | | |

| | *Coefficients* | *Standard Error* | *t Stat* | *P-value* | *Lower 95%* | *Upper 95%* | *Lower 95.0%* | *Upper 95.0%* |
|---|---|---|---|---|---|---|---|---|
| Intercept | 0.154392 | 0.330905 | 0.466576 | 0.649908 | -0.57392 | 0.882709 | -0.57392 | 0.882709 |
| 0 | 0.066281 | 0.117219 | 0.565444 | 0.583121 | -0.19172 | 0.324277 | -0.19172 | 0.324277 |

**C.11** DO and supernatant ss

| *Regression Statistics* | |
|---|---|
| Multiple R | 0.092522 |
| R Square | 0.00856 |
| Adjusted R Square | -0.11537 |
| Standard Error | 9.771164 |
| Observations | 10 |

ANOVA

| | *df* | *SS* | *MS* | *F* | *Significance F* |
|---|---|---|---|---|---|
| Regression | 1 | 6.594875 | 6.594875 | 0.069074 | 0.799332 |
| Residual | 8 | 763.8051 | 95.47564 | | |
| Total | 9 | 770.4 | | | |

| | | *Coefficients* | *Standard Error* | *t Stat* | *P-value* | *Lower 95%* | *Upper 95%* | *Lower 95.0%* | *Upper 95.0%* |
|---|---|---|---|---|---|---|---|---|---|
| Intercept | | 18.04496 | 13.46698 | 1.33994 | 0.217075 | -13.01 | 49.0999 | -13.01 | 49.0999 |
| | 1.7 | -1.20918 | 4.600811 | -0.26282 | 0.799332 | -11.8187 | 9.400316 | -11.8187 | 9.400316 |

**C.12** DO and supernatant TCOD

| *Regression Statistics* | |
|---|---|
| Multiple R | 0.016759 |
| R Square | 0.000281 |
| Adjusted R Square | -0.14254 |
| Standard Error | 14.19503 |
| Observations | 9 |

ANOVA

| | *df* | *SS* | *MS* | *F* | *Significance F* |
|---|---|---|---|---|---|
| Regression | 1 | 0.396265 | 0.396265 | 0.001967 | 0.965867 |
| Residual | 7 | 1410.493 | 201.4989 | | |
| Total | 8 | 1410.889 | | | |

| | | *Coefficients* | *Standard Error* | *t Stat* | *P-value* | *Lower 95%* | *Upper 95%* | *Lower 95.0%* | *Upper 95.0%* |
|---|---|---|---|---|---|---|---|---|---|
| Intercept | | 47.73478 | 19.65284 | 2.4289 | 0.045494 | 1.263231 | 94.20633 | 1.263231 | 94.20633 |
| | 1.7 | -0.29646 | 6.685069 | -0.04435 | 0.965867 | -16.1041 | 15.51121 | -16.1041 | 15.51121 |

**C.13** MLSS and Vo

| *Regression Statistics* | |
|---|---|
| Multiple R | 0.2936964 |
| R Square | 0.0862576 |
| Adjusted R Square | -0.3706137 |
| Standard Error | 2.975303 |
| Observations | 4 |

ANOVA

| | *df* | *SS* | *MS* | *F* | *Significance F* |
|---|---|---|---|---|---|
| Regression | 1 | 1.67134361 | 1.67134 | 0.1888 | 0.706303637 |
| Residual | 2 | 17.70485639 | 8.85243 | | |
| Total | 3 | 19.3762 | | | |

| | *Coefficients* | *Standard Error* | *t Stat* | *P-value* | *Lower 95%* | *Upper 95%* | *Lower 95.0%* | *Upper 95.0%* |
|---|---|---|---|---|---|---|---|---|
| Intercept | 9.1538794 | 7.5220882 | 1.21693 | 0.34774 | -23.21107649 | 41.5188352 | -23.21107649 | 41.5188352 |
| 2120 | -0.0008732 | 0.002009542 | -0.4345 | 0.7063 | -0.009519539 | 0.0077732 | -0.009519539 | 0.0077732 |

**C.14** MLSS and n

| *Regression Statistics* | |
|---|---|
| Multiple R | 0.4877756 |
| R Square | 0.237925 |
| Adjusted R Square | -0.1431125 |
| Standard Error | 0.0913494 |
| Observations | 4 |

ANOVA(n)

| | *df* | *SS* | *MS* | *F* | *Significance F* |
|---|---|---|---|---|---|
| Regression | 1 | 0.005210558 | 0.00521 | 0.62441 | 0.512224424 |
| Residual | 2 | 0.016689442 | 0.00834 | | |
| Total | 3 | 0.0219 | | | |

| | *Coefficients* | *Standard Error* | *t Stat* | *P-value* | *Lower 95%* | *Upper 95%* | *Lower 95.0%* | *Upper 95.0%* |
|---|---|---|---|---|---|---|---|---|
| Intercept | -0.4461103 | 0.230947427 | -1.9317 | 0.19313 | -1.439797547 | 0.54757699 | -1.439797547 | 0.54757699 |
| 2120 | -4.875E-05 | 6.16981E-05 | -0.7902 | 0.51222 | -0.00031422 | 0.00021671 | -0.00031422 | 0.00021671 |

**C.15** Clarifier performance (TSS)

| *Regression Statistics* | |
|---|---|
| Multiple R | 0.255978 |
| R Square | 0.065524 |
| Adjusted R Square | 0.038825 |
| Standard Error | 16.7831 |
| Observations | 37 |

ANOVA

| | *df* | *SS* | *MS* | *F* | *Significance F* |
|---|---|---|---|---|---|
| Regression | 1 | 691.2704 | 691.2704 | 2.454165 | 0.1262113 |
| Residual | 35 | 9858.532 | 281.6723 | | |
| Total | 36 | 10549.8 | | | |

| | *Coefficients* | *Standard Error* | *t Stat* | *P-value* | *Lower 95%* | *Upper 95%* | *Lower 95.0%* | *Upper 95.0%* |
|---|---|---|---|---|---|---|---|---|
| Intercept | 24.6654 | 5.294043 | 4.659087 | 4.47E-05 | 13.91791342 | 35.4129 | 13.91791 | 35.4129 |
| 3 | 0.441264 | 0.281674 | 1.566577 | 0.126211 | -0.130565059 | 1.013093 | -0.13057 | 1.013093 |

**C.16** Clarifier performance (TCOD)

| *Regression Statistics* | |
|---|---|
| Multiple R | 0.405099 |
| R Square | 0.164105 |
| Adjusted R Square | 0.134252 |
| Standard Error | 17.3323 |
| Observations | 30 |

ANOVA

| | *df* | *SS* | *MS* | *F* | *Significance F* |
|---|---|---|---|---|---|
| Regression | 1 | 1651.36 | 1651.36 | 5.497047 | 0.026369845 |
| Residual | 28 | 8411.44 | 300.4086 | | |
| Total | 29 | 10062.8 | | | |

| | | *Coefficients* | *Standard Error* | *t Stat* | *P-value* | *Lower 95%* | *Upper 95%* | *Lower 95.0%* | *Upper 95.0%* |
|---|---|---|---|---|---|---|---|---|---|
| Intercept | | 32.3395 | 13.12322 | 2.464297 | 0.02013 | 5.457779121 | 59.22122 | 5.457779 | 59.22122 |
| | 51 | 0.637591 | 0.271943 | 2.344578 | 0.02637 | 0.080540932 | 1.194641 | 0.080541 | 1.194641 |

# VITA

Jackeline Luque was born in Tegucigalpa, Honduras on December 2, 1976. Prior starting graduate school at the University of New Orleans (UNO), she obtained a Bachelor's Degree of Science in Civil Engineering from Universidad Nacional Autonoma de Honduras (UNAH), in September 1999.

During June 2001 to December of 2002, Jackeline Luque worked as a Graduated Research Assistant at the University of New Orleans pursuing a Masters of Science in Environmental Engineering. In spring 2003, the author started pursing her Ph.D. at the same institution as a graduate assistant achieving her final academic goal on May 2005. The author graduated with an overall GPA of 4.0. Her academic emphasis has been in the areas of Water Resources, Drinking Water and Wastewater Treatment.