# EVALUATION OF EXTRACELLULAR BIOPOLYMER AND ITS IMPACT ON BIOFLOCCULATION IN ACTIVATED SLUDGE BIORECTORS

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## ABSTRACT

The influence and dynamics of bacterial extracellular polysaccharide (EPS) polymer production and its impact on bioflocculation in activated sludge (AS) bench-scale reactors were investigated. The impact of food to microbe ratio (F/M), reactor configuration and easily biodegradable carbohydrates in influent streams on biological processes that support or weaken good floc formation and the link with EPS quantity was studied. Bioreactors were run as either sequencing batch or continuous systems using wastewater media with glucose or acetate as C source in different F/M ratios. EPS levels were quantified using mid-infrared spectroscopy which provided a rapid technique for monitoring biological processes within AS WWTP. The analysis revealed an interdependent link between EPS production, sludge settling characteristics and mode of reactor operation. An inverse relationship between F/M ratios and EPS quantities was seen but a positive link between EPS and aggregation %, a measure of the efficiency of inter cell attachment and which indicates good settling properties, was also seen. This indicates that during high F/M conditions in AS reactors, low levels of EPS may be produced which could have a negative impact on settling of the biomass. Floc architecture was examined under the microscope. Transient growth of filamentous bacteria was seen in the reactors.

## KEYWORDS

**Activated sludge, extracellular polysaccharide biopolymer, sequencing batch, flocculation, bacterial aggregation**

# 1 INTRODUCTION

The efficiency of Activated Sludge (AS) Wastewater Treatment Plants (WWTP) firstly depends on the establishment of a metabolically active community and, secondly, on effective separation of the biosolids from the treated effluent in the secondary clarifier (McSwain et al., 2005). The process of bioflocculation is required for solid-liquid separation. Floc formation is largely mediated by the bacterial community, in particular, the production of EPS polymer and cell to cell adhesion or bacterial aggregation. Flocculation is critical for optimal operation of AS systems. There is still some degree of uncertainty as to the role of EPS in floc formation and the regulation of aggregation processes.

Activated sludge flocs are made up of complex aggregates which primarily consist of a network of extracellular polymeric substances and microorganisms embedded within the network (Wilen et al., 2008). These substances are a complex network which consists of polysaccharides, proteins, nucleic acids and humic substances (Heijstra et al., 2009). The role of EPS substances in flocculation, granulation and settling properties has previously been investigated (e.g. Li et al., 2008; McSwain et al., 2005; Sutherland, 2001). These studies have indicated that bacterial aggregation and EPS production are negatively as well as positively correlated. Further insight into the inter-cellular attachment processes that mediate aggregation and the role of EPS in this process is crucial for solid-liquid separation and to minimize periodic AS failure. Extracellular polysaccharide polymers also influence floc architecture and sludge dewaterability.

Reactor configuration and F/M ratio are two aspects of WWTP operation that fundamentally impact on the biological processes that underpin flocculation and promotion of good sludge characteristics. Sequencing batch reactor (SBR) technology is becoming increasingly popular as reactor configuration for nutrient removal in domestic wastewater treatment (Freitas et al., 2009). Studies have shown SBR operation to be superior to continuous systems at promoting aggregation (Beun et al., 1999). It has been postulated that wastewater composition, in particular the presence of readily biodegradable carbohydrates, varying F/M ratios and reactor configuration may promote excessive filament growth (Liu & Liu, 2006; Martins et al., 2004). The overgrowth of filamentous bacteria leads to the bulking of sludge. Organic loading rates have also been implicated in changing microbial population numbers and species diversity which impacts on F/M ratios in bioreactors (Li et

al., 2008). Varying F/M ratios influence nutrient conditions in bioreactors and within flocs that may result in instability of the biological processes in AS and a reduction in effluent quality, i.e. increasing filament numbers and reducing aggregation efficiency (increasing SVI).

The present study aimed to examine the impact of reactor configuration, readily biodegradable organics and F/M ratio on floc and sludge properties, and to determine the influence of EPS production on flocculation characteristics in bench-scale AS reactors.

# 2 MATERIALS & METHODS

## 2.1 ACTIVATED SLUDGE BIOREACTOR OPERATION

Three reactors (working volume 1500 ml) were operated as SBR or in continuous mode over a period of 10 weeks. Two SBR runs were completed with a total cycle time of between 7.3 and 13.3 h consisting of settling (15 min), static draw (28 min), feed (35 min) and reaction (6 or 12 h) phases with a 50 % volumetric exchange ratio. One to two cycles were performed daily. The hydraulic retention time (HRT) was 1.4 d and the sludge retention time (SRT) varied between 3.6 and 6.7 d. When operated in continuous mode, the HRT was 1.1 d and SRT between 0.7 and 1.4 d. Reactors were fed with a synthetic wastewater medium with either glucose or acetate as carbon (C) source. Glucose medium was administered to one SBR and the continuous reactor while the acetate-supplemented medium was administered to the other SBR reactor. The levels of glucose and acetate in the influent feeds were changed over a reactor run to facilitate varying F/M ratios. Samples were removed periodically for analysis after steady state conditions were attained (min three culture volume changes).

All reactors were operated aerobically at a DO of 30% of $O_2$ saturation. This was controlled through an agitation-aeration programmable cascade system monitored using an Ingold Mettler Toledo DO probe. The temperature was regulated to 22 $^{o}$C, the mean summer temperature measured in AS reactors at the North Shore City Rosedale WWTP in Auckland. pH management with a 1 M KOH solution was implemented in all AS reactors to minimize excessive fungal growth in the reactors. The reactors were inoculated with 2 ml samples collected from the aerobic zones of the AS reactors at Rosedale and Mangere WWTP (mean sample MLSS 44.2 g $L^{-1}$).

## 2.2 GROWTH MEDIA

Synthetic wastewater media was made according to Tay et al. (2001) and Yoo et al. (1999). An acetic acid wastewater media (per L distilled water) consisting of sodium acetate (256.41 mg), ammonium acetate (240.88 mg), $KH_2PO_4$ (43 mg), $NaHCO_3$ (125.0 mg), $CaCl_2$ (10.0 mg), $MnSO_4$ (0.038 mg), $ZnSO_4$ (0.035 mg), $MgSO_4$ (25.0 mg) and yeast extract (50.0 mg) was fed into one of the SBR (total COD 0.63 g $L^{-1}$). Influent COD concentrations varied between 0.06 and 0.63 g $L^{-1}$ by changing the level of acetate in the influent feed. Organic loading rates (OLR) were 11.3, 22.8, 45.7 and 126.9 g COD $m^{-3}$ $d^{-1}$ during the acetic acid SBR run. Two COD levels of 0.2 and 2 g $L^{-1}$ of a glucose synthetic wastewater medium containing (per L) glucose (1400 mg), peptone (400 mg), $NH_4Cl$ (200 mg), $K_2HPO_4$ (45 mg), $CaCl_2.2H_2O$ (30 mg), $MgSO_4.7H_2O$ (25 mg), $FeSO_4.7H_2O$ (20 mg) were administered (total COD 2 g $L^{-1}$ ). Organic loading rates were 40.4 and 404.1 g COD $m^{-3}$ $d^{-1}$ in the glucose SBR while loading rates in the glucose continuous reactor were 42.1 and 420.7 g COD $m^{-3}$ $d^{-1}$ respectively for the two influent COD concentrations. One ml $L^{-1}$ of a microelement solution was added to the growth media containing (g $L^{-1}$) $H_3BO_3$ (0.05), $ZnCl_2$ (0.05), $CuCl_2$ (0.03), $MnSO_4.H_2O$ (0.05), $(NH_4)6Mo7O_2.4H_2O$ (0.05), $AlCl_3$ (0.05), $CoCl_2.6H_2O$ (0.05) and $NiCl_2$ (0.05).

## 2.3 ANALYTICAL METHODS

Samples were periodically removed from the reactors for MLSS and SVI determination according to Standard Methods (APHA-AWWA-WEF, 1998). Samples were also collected to determine the extent of microbial aggregation efficiency (%) adopted from Burdman et al. (1998). Sludge structure was assessed under light

microscopy using wet mount and staining (Gram and methylene blue) techniques. The presence of stalked and free-swimming ciliate protozoa and filamentous bacteria was monitored.

Reactor samples were also analyzed by Fourier-transform Infrared (FTIR) Spectroscopy to quantify EPS. The method for total polysaccharide production quantification was adapted from Marcotte et al. (2007) and Bramhachari & Dubey (2006). The method determines the total polysaccharide content in bioreactor samples by employing FTIR spectroscopy and is used as an indicator of EPS quantity. Method validation was previously reported by Marcotte et al. (2007). The amount of polysaccharide was estimated using the area under the peaks between 950 and 1201 $cm^{-1}$ in the FTIR spectra, associated with C-O stretching modes of the alcohol and ether functional groups ($A_{polysaccharide}$) (Nivens et al., 1993; Sylverstein et al., 1991). Normalisation of the polysaccharide amount to the quantity of the bacterial biomass was required and for this purpose the amide peak intensities were applied. The amide I (1650 – 1660 $cm^{-1}$) and amide II (1530 -1560 $cm^{-1}$) peaks can be used as probes for functional groups in proteins and, accordingly, used to quantify biomass. The area of the amide II peak was used to estimate the quantities of proteins in samples in this study since this spectral region falls within a component where interference associated with other bacterial components is minimal (Sylverstein et al., 1991).

Measurements were undertaken on a Thermo Electron Nicolet 8700 FTIR spectrometer using attenuated total reflectance. Liquid samples (20 µl) collected from the bioreactors at the predetermined intervals were deposited in the centre of a ZnSe window and carefully spread to cover the surface of the window. The samples were dried under a constant gentle stream of $N_2$ to give a solid film. A total of 64 scans were performed with a resolution of 2 $cm^{-1}$ to obtain each spectrum in the region of 4000-400 $cm^{-1}$. The angle of incidence of the IR beam was 45º. The spectra were ATR corrected and baseline corrected using the Omnic spectroscopic software. The areas of the amide II ($A_{amideII}$) and polysaccharide ($A_{polysaccharide}$) peaks were determined by resolving the spectra through fitting individual peaks to the spectra using the array basic curve-fitting application in GRAMS 32 software (vers. 5). Twelve to 20 bands of mixed Lorentzian and Gaussian shapes were fitted in the region between 1660 and 950 $cm^{-1}$. The ratio of absorbance in the polysaccharide spectral region, $A_{polysaccharide,}$ to the area of the amide II peak, $A_{amideII,}$ was calculated to quantify total polysaccharide in bioreactor samples.

# 3 RESULTS AND DISCUSSION

## 3.1 IMPACT OF REACTOR CONFIGURATION AND F/M RATIO ON SLUDGE SETTLING

Three bioreactors were operated in the laboratory, two as SBR and a third as a continuous system. One SBR and the continuous reactor were fed with a synthetic wastewater medium with glucose as C source and the other SBR with acetate as C source. The COD in the wastewater feed was altered during the reactor runs to effect varying F/M ratios. The analysis revealed an interdependent link between EPS production, F/M ratio and sludge settling characteristics. The time series plots of all analyses for the three reactors are presented in Figure 1.

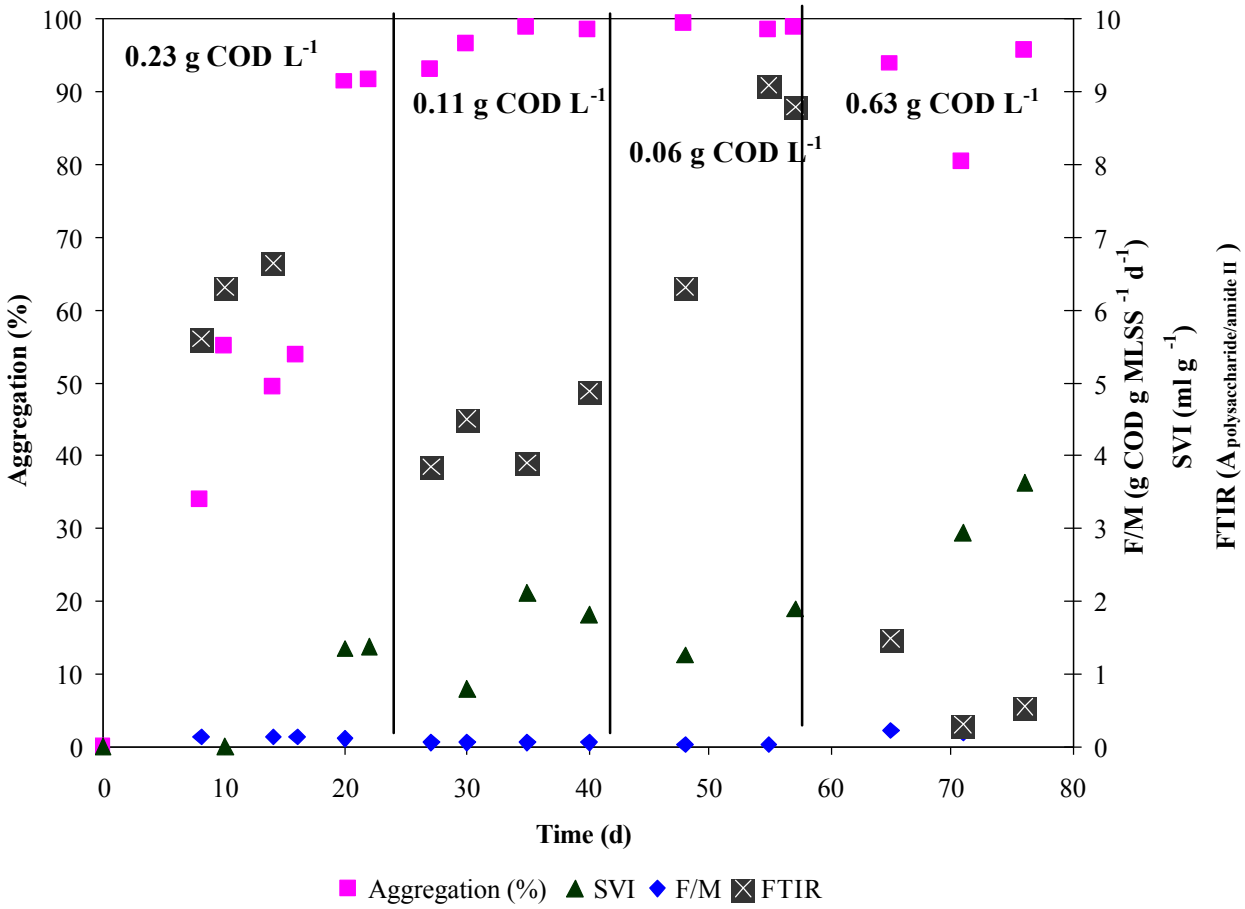
### 3.1.1 F/M vs EPS PRODUCTION AND AGGREGATION EFFICIENCY (%)

An inverse relationship between F/M and total polysaccharide content ($A_{polysaccharide/amide\ II}$) which is used as indicator of EPS quantity was found in all reactor runs (Figure 2). Varying the influent COD concentration altered F/M ratios in the reactors. The results suggest that elevated F/M ratios contributed to reduced EPS production. The F/M ratio in the acetate SBR bioreactor increased to 0.22 g COD g $MLSS^{-1}$ $d^{-1}$ when the COD in the influent stream increased to 0.63 g $L^{-1}$ form day 57 (Figure 1a). This coincided with a reduction of $A_{polysaccharide/amide\ II}$ to < 1.49 from 8.78 measured during the influent COD concentration of 0.06 g $L^{-1}$ (average F/M 0.03 g COD g $MLSS^{-1}$ $d^{-1}$) between days 40 and 57. A similar trend was observed in the glucose SBR (Figure 1b). Reducing the COD in the influent feed by 10 times after day 39 effected an average F/M of 0.06 down from an average of 0.78 g COD g $MLSS^{-1}$ $d^{-1}$ measured during the 2 g COD $L^{-1}$ feed. At the same time, mean EPS quantities increased from an average 2.02 (max 2.89, min 1.56, < 39 days) to 4.1 (max 6.57, min 2.84) between days 46 and 65.

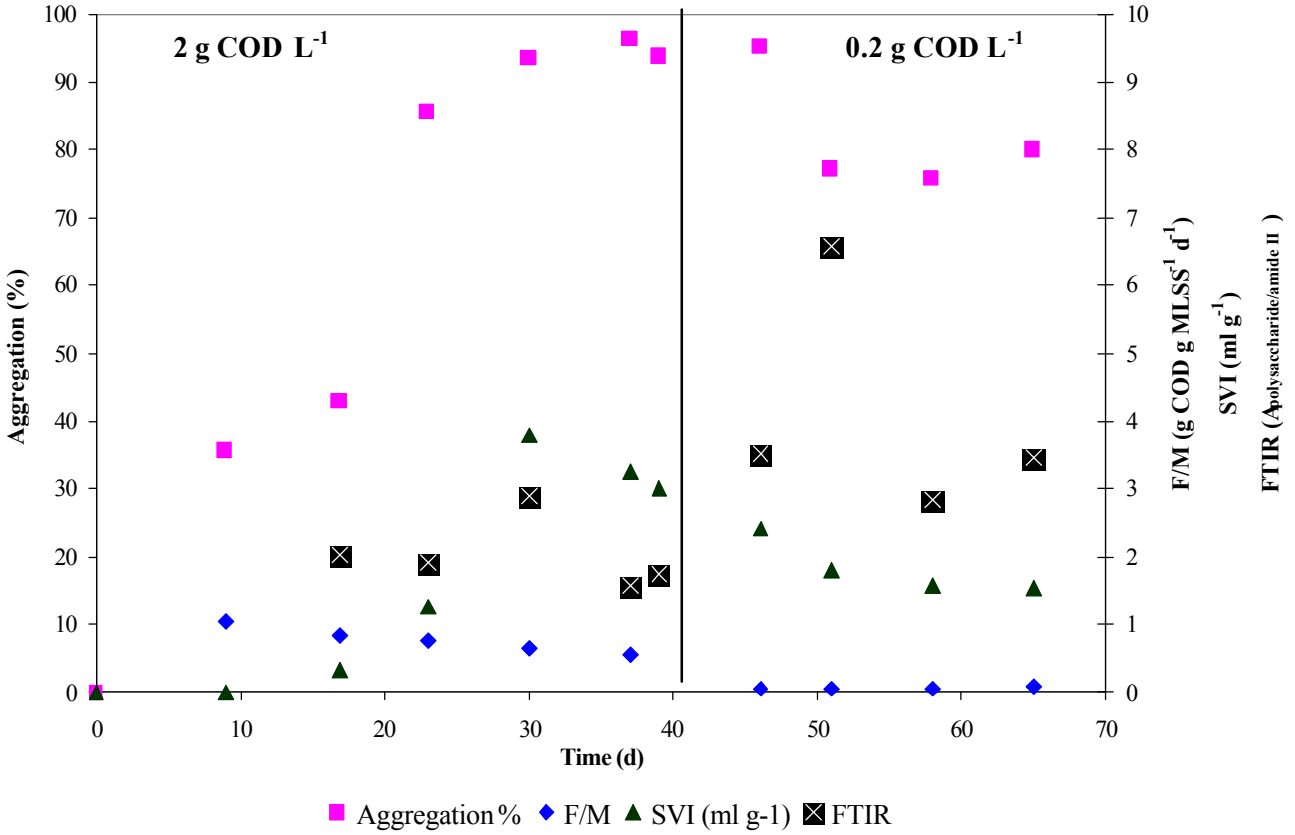
A strong increase in EPS quantity was measured in the continuous reactor after the COD in the feed was reduced to 0.2 g $L^{-1}$ after day 32 which coincided with reduced F/M ratios (~0.004 g COD g $MLSS^{-1}$ $d^{-1}$, Figure 1c). Sludge volumes measured throughout the study period were low. An increase in SVI was seen towards the end of the reactor run (elevated OLR) in the acetate SBR (Figure 1a) which corresponded to reduced EPS quantities and aggregation efficiency (%). Aggregation efficiency indices were generally > 80% in the acetate SBR. The glucose SBR showed increases in SVI values with lower EPS levels when the reactor was fed with the higher COD concentration feed (Figure 1b). Aggregation % also showed increases indicating efficient cell to cell attachment and good settling properties. Similarly, higher SVI numbers in the continuous reactor measured during the elevated COD concentration load showed lower EPS quantities (Figure 1c). Aggregation efficacy was low in the continuous reactor and reduced to < 3% during the 0.2 g $L^{-1}$ COD feed. Minimal sludge volumes were measured during this stage of the run. Washout of the bacteria proficient at aggregation may have occurred in the reduced COD conditions. The relationship between aggregation indices and EPS quantities across the three reactors indicated that there is a positive link between EPS levels and inter-cell attachment (Figure 3).

*Figure 1: Time-series showing aggregation capacity (%), SVI (ml $g^{-1}$), F/M (g COD g $MLSS^{-1}$ $d^{-1}$) and EPS (FTIR, $A_{polysaccharide/amid\ II}$) quantities measured during different influent COD concentrations in the three reactor studies .Vertical lines indicate transition between different COD feeds.*

*(a) Acetate-fed SBR*



*(b) Glucose-fed SBR*




*(c) Glucose-fed continuous reactor*

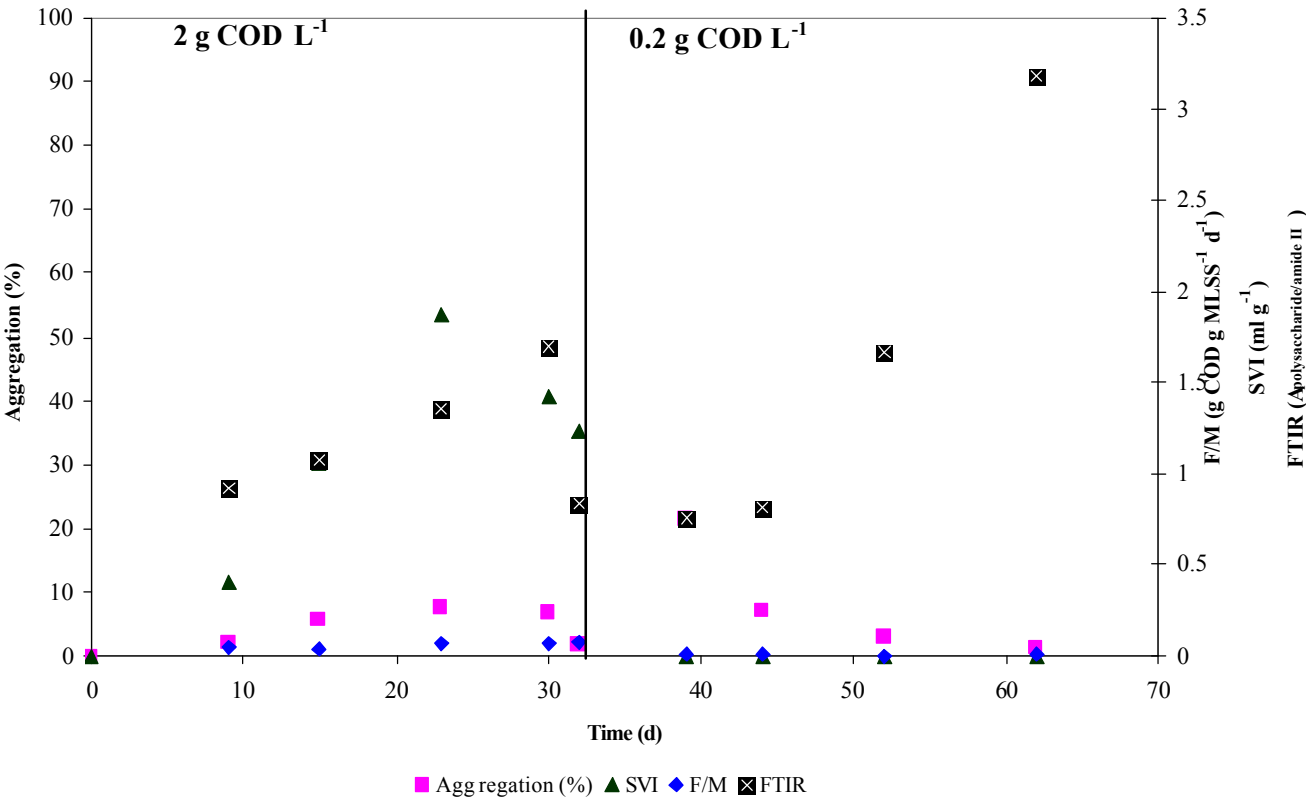


*Figure 2: Relationship between EPS* (FTIR, $A_{polysaccharide/amidII}$) *quantities and F/M ratios (g COD g MLSS$^{-1}$ d$^{-1}$) across the three reactors.*

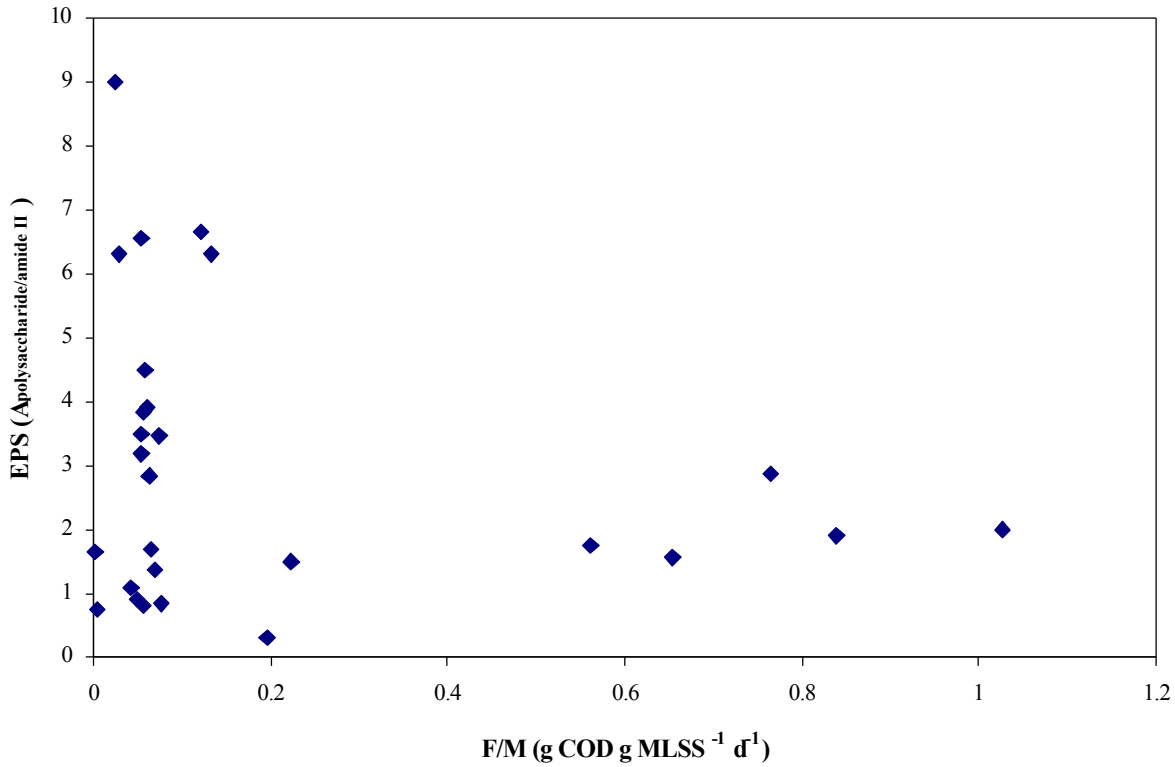
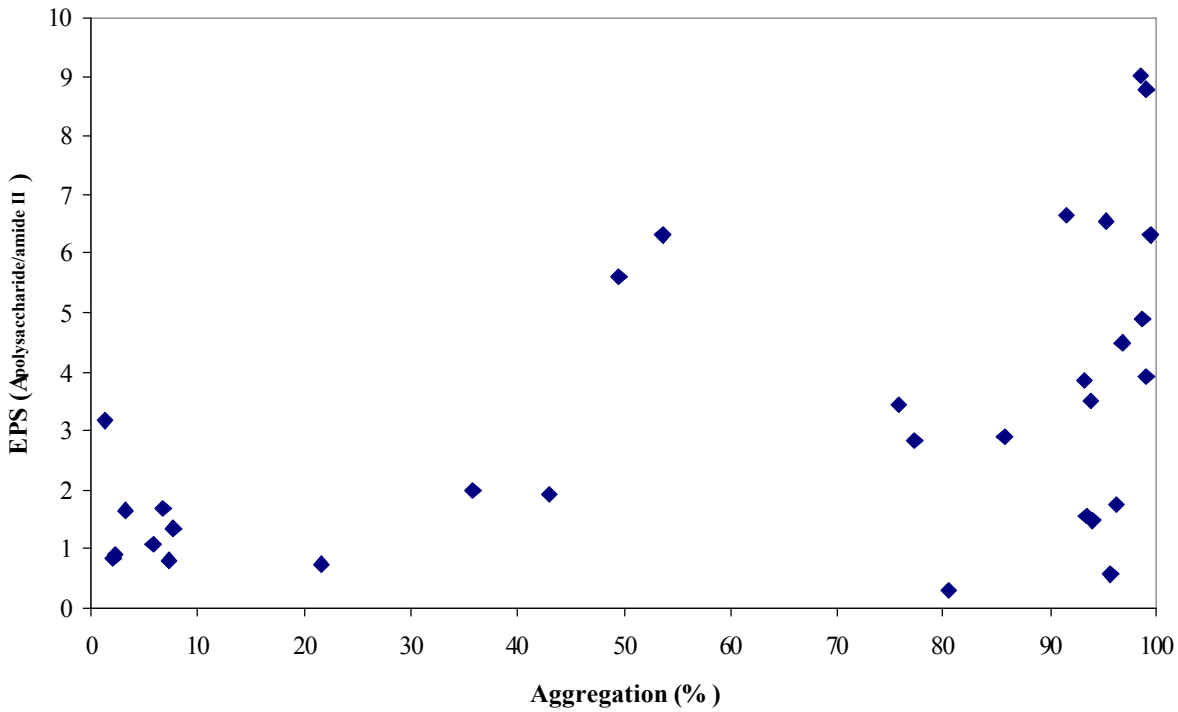



*Figure 3: Relationship between EPS* (FTIR, $A_{polysaccharide/amidII}$) *quantities and aggregation efficiency (%) across the three reactors.*



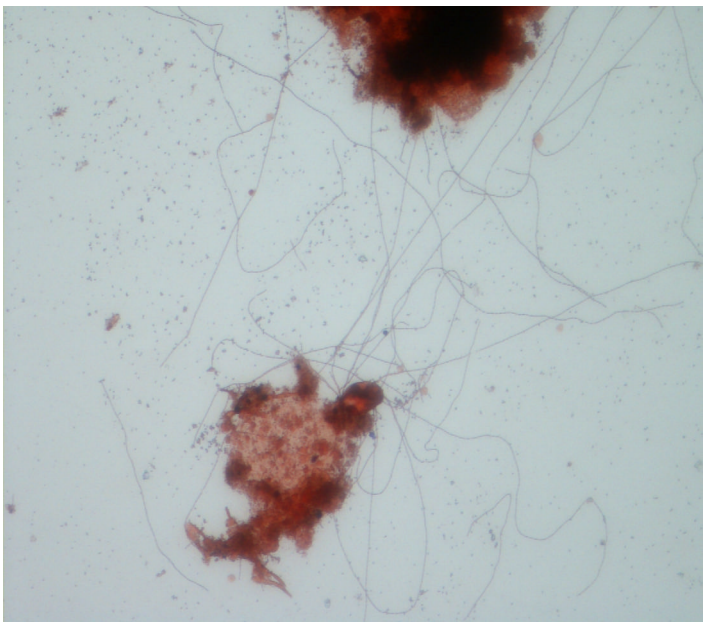
### 3.1.2 REACTOR CONFIGURATION AND EPS PRODUCTION

The establishment of aggregating populations was evident in the SBR when aggregation capacities are compared to index percentages measured in the continuous reactor. This supports previous studies that showed that the use of SBR instead of continuous reactors could lead to self aggregation of biomass under aerobic conditions (De Kreuk, 2009). The continuous reactor did not form an efficient aggregating population and during the low COD influent feed, aggregating microorganisms may not have been able to compete for limiting nutrients and washout occurred. Levels of EPS quantities were similar in the glucose and acetate-fed SBR. Lower EPS levels were detected in the continuous reactor. Li and Yang (2007) however found higher EPS concentrations in glucose-fed AS reactors than in acetate-fed systems in addition to varying flocculation characteristics.

## 3.2 MICROSCOPIC EVALUATION OF SLUDGE SAMPLES

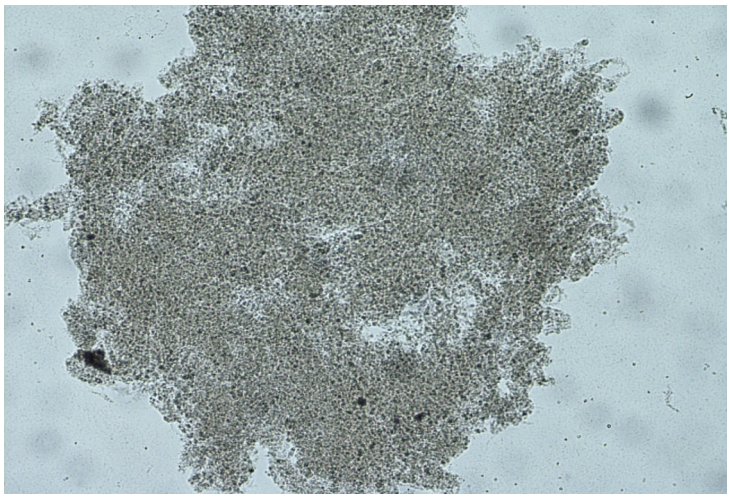
Microscopic evaluation of reactor samples showed different morphologies during the runs. Filamentous bacteria were seen at different stages over the course of the study but over-proliferation never occurred and impacts on sludge settleability (bulking) were not observed. Photograph 1 shows the appearance of filaments in the acetate SBR. This suggests that, in this case, neither reactor configuration nor the availability of easily biodegradable C sources supported filamentous growth in the reactors as was reported by Martins et al. (2004) and Liu and Liu (2006). Good floc formations were observed under wet-mount light microscopy examination. Floc formation was seen during the early stage of elevated COD feed in the continuous reactor (Photograph 2a). Floc formers were absent after day 32 when the COD concentration in the influent feed was lowered. Comparable floc morphologies were observed between SBR reactors. The establishment of free-swimming and stalked ciliates was visible under the microscope (Photograph 2b) and their presence can be regarded as indicative of the quality of the final effluent in AS plants (Curds, 1982).

*Photograph 1: Gram stain of sludge sample from the acetate SBR showing appearance of filaments after 35 d of operation (COD influent 0.11 g $L^{-1}$) visualized under light microscopy (400x).*

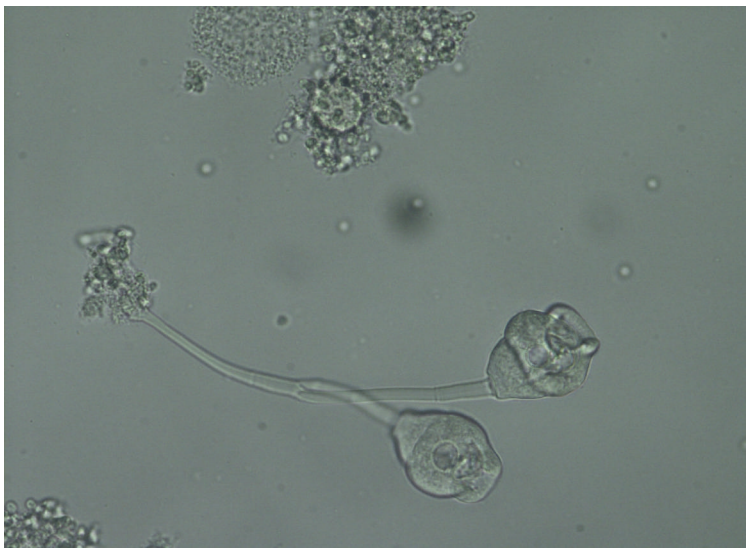


*Photograph 2: Wet mount examination of reactor samples showing (a) floc formation in the glucose-fed continuous reactor after 9 d of operations during the 2 g $L^{-1}$ COD feed (200x) and (b) the presence of stalked ciliates in the glucose SBR after 30 d (influent COD 2 g $L^{-1}$)(400x).*

(a)



(b)



## 4. CONCLUSIONS

An inverse relationship between F/M ratio and EPS levels was seen in the reactors. A positive trend between aggregation efficiency and EPS levels in reactors were observed. This indicates that during low F/M conditions in AS reactors, high levels of EPS may be produced which could lead to increasing aggregation of the biomass. Therefore, EPS at the concentrations measured in this study may be important for good flocculation since it has been argued that excessive EPS levels may impair sludge settling (Li & Yang, 2007). FTIR spectroscopy provided an effective technique to rapidly assess EPS accumulation in the AS bioreactors circumventing

laborious EPS extraction protocols. Monitoring of biological processes within treatment plants using rapid techniques such as FTIR spectroscopy could assist in improved management of AS. Future bioreactor studies will focus on the incorporation of a clarifying vessel into the continuous reactor configuration and assessing the impact of a return activated sludge (RAS) stream on the floc-forming bacterial community. DNA-based community profile studies will also be undertaken to determine if populations present in the lab-scale AS reactors are similar to communities found in WWTP.

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