NUTRIENT CONDITIONS INFLUENCE SIZE DISTRIBUTION AND SETTLING PROPERTIES OF ACTIVATED SLUDGE FLOCS

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ABSTRACT

The study aimed to investigate the relationship between activated sludge (AS) particle (floc) size distribution and nutrient conditions in different bioreactor configurations. Size distribution profiles of flocs that formed in continuous (B1), continuous with clarifier and return sludge (B2) and SBR (B3) reactors were investigated in parallel under identical nutrient conditions. An eight-fold dilution of the influent COD of a synthetic dairy processing wastewater resulted in a "feast and fast" regime that triggered significant effects on the biomass and flocculation characteristics. Floc size analysis of reactor MLSS revealed a shift in floc sizes when reactors were fed with the minimum COD wastewater feed (0.61 g L^{-1}). Increasing floc size distributions were detected for all reactors during the low COD feed though different size patterns were observed for the different reactor configurations. These increases corresponded with variations in aggregation indices and EPS quantities. The SBR yielded comparatively larger flocs when operated under both maximum and minimum COD feeds as indicated by $d(0.9)$ values (90% of particles $\leq d$ in size). Overall the results indicated that floc formation and floc size are mediated by nutrient concentrations and represents an important step towards improved process control.

KEYWORDS

Activated sludge, aggregation, bioreactor, dairy processing wastewater, extracellular polysaccharide polymer, flocculation, particle size distribution

1 INTRODUCTION

Dairy processing wastewater primarily originates from the washing and rinsing waters produced during the reception and packaging of milk, from tanks and pumps or spillage during the processing steps. The water also comes from the wash down of plant and apparatus. Dairy processing wastewaters generally have a high concentration of biodegradable organic matter (Kotoupas et al., 2007). The main component is whey which is the residue that is obtained when casein and fat are separated from milk. Whey constitutes approximately 85-95 % of the milk volume and retains ~55 % of milk nutrients, the most abundant of which are lactose (4.5 – 5% w/v), soluble proteins (0.6-0.8% w/v) and lipids (0.4-0.5% w/v). Whey can be captured and utilised in, for example, the manufacturing of animal feed. However, it is mostly in small-scale milk farming and cheese production where the whey is not collected and is discharged into on-site ponds and other treatment facilities or to municipal sewerage systems.

The high BOD content of dairy processing effluents may influence biological processes in small-scale on-site wastewater treatment systems and disposal of these effluents to land over longer periods of time may have a detrimental impact on receiving environmental bodies (Ghaly et al, 1988). Accordingly, various biological treatment processes have been assessed for their suitability to treat

wastewaters generated from dairy operations, including single and two-stage anaerobic digestion (Yan et al., 1990) and coupled anaerobic-aerobic treatment (Frigon et al., 2007).

The most abundant municipal wastewater treatment technology in operation today is the activated sludge (AS) process. Recent studies have shown that food to microorganism (F/M) ratios and nutrient concentrations in AS systems may be important cues that drive bacterial aggregation and extracellular polysaccharide (EPS) polymer production (Ehlers & Turner, 2009). Both factors contribute to the efficiency of flocculation and settling of biomass which are crucial to the performance of AS systems. It is therefore expected that a high-strength wastewater, such as dairy processing waste, will have significant effects on the biological processes that underpin effective treatment using the AS process. A better understanding of the relationship between waste strength, flocculation and settling is an important first step towards design of more effective and reliable treatment systems. The aim of this study was to investigate the influence of various treatment system parameters on flocculation and settling of AS in lab scale reactors treating synthetic dairy processing effluents.

Various sequencing batch reactor (SBR) and continous reactor configurations are currently used in full-scale AS systems. The different reactor modes of operation will potentially select for the establishment of different activated sludge microbial populations and, as a result, may exhibit different flocculation characteristics. It has been indicated that there is a clear link between settleability and particular sludge populations in, for example, a SBR (Govoreanu et al., 2003). This raises the question of whether there is a particular reactor configuration or operational mode that is more suitable for treatment of high-strength wastes. To address this, the operation of an SBR, a continuous reactor and a continuous reactor with a clarifer and return activated sludge (RAS) were employed to investigate the link between reactor configuration, AS population dynamics and flocculation characteristics. Further objectives of this study were (i) to investigate the relationship between extracellular polymer (EPS) production, bacterial aggregation and COD removal efficiency when the reactors are operated under a "feast and fast" nutrient cycle regime; and (ii) to assess the aerobic digestibility of a dairy processing wastewater.

2 METHODS

2.1 BIOREACTOR OPERATION

Three lab-scale bioreactors (two New Brunswick BioFlo 3000, and a Labfors 4, InforsHT, each with a working volume of 1.5 L) were operated for a period of 60 d as a continuous reactor (bioreactor 1 – B1), continuous reactor and clarifier with RAS (bioreactor 2 – B2) and SBR (bioreactor 3 – B3) respectively. Bioreactors 1 and 2 were operated with a hydraulic retention time (HRT) of 1.2 d and solids retention times (SRT) of between 1.1 and 1.3 d (B1) and 1.2 and 1.4 d (B2). Settled biomass from the clarifer was recycled to B2 once every 48 h (RAS at 6.7% of the reactor working volume). Sequencing batch operation (B3) was managed through a programmable control sequence in Iris vers. 5 software (InforsHT). Bioreactor 3 was operated with a total cycle time of 12 h consisting of settling (30 min), static draw (28 min), feed (35 min) and reaction (10.4 h) phases. The HRT was 2.6 d (daily volumetric exchange volume of 39%) and the SRT varied between 6.7 and 8.2 d.

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°C, the mean summer temperature measured in AS reactors at the North Shore City Rosedale Rd WWTP in Auckland. pH management was implemented in all AS reactors with a 1 M KOH solution to minimize excessive fungal growth in the bioreactors. The reactors were inoculated with 2 ml samples collected from the aerobic zones of the

AS reactors at Rosedale Rd WWTP. Samples were collected periodically for analyses (MLSS, aggregation indices, COD discharge concentrations, EPS levels and particle size distribution) and microscopic evaluation of the microbial communities after and during steady state conditions were attained in the three bioreactors as confirmed by stable MLSS levels. COD measurements were undertaken by WaterCare Services Ltd (Mangere).

2.2 SYNTHETIC WASTEWATER MEDIUM

The bioreactors were fed with a wastewater medium typically produced in the dairy processing industry (Yang et al., 2007). Dry whey powder was sourced from Fonterra (NZMP™ Whey powder 621) and comprised (in g 100 g^{-1}): protein, 14.1; moisture, 4.5; fat, 1.0; total carbohydrate, 72.5; and ash, 7.9 (pH, 6.7). The concentration of the whey in the feed was altered to produce influent COD concentrations of 4.74 g L^{-1} (maximum) and 0.61 g L^{-1} (minimum). These levels were based on characteristic COD concentrations in wastewater streams from the dairy industry (Gutierrez et al., 1991). The eight-fold dilution in influent COD represents the occurrence of feast and fast conditions in AS reactors. The synthetic wastewater feed consisted of the following constituents: ammonium chloride (1553 mg L^{-1}), potassium phosphate (225 mg L^{-1}), sodium carbonate (1200 mg L^{-1}), magnesiumsulphate (75 mg L^{-1}), zinc chloride (15 mg L^{-1}), ferrous sulphate (55 mg L^{-1}), manganese chloride (10 mg L^{-1}) and ammonium molybdate (15 mg L^{-1}).

2.3 ANALYTICAL METHODS

2.3.1 AGGREGATION INDEX

Samples were collected to determine an aggregation index (%), a factor which reveals the degree of biomass settleability adopted from Burdman et al. (1998). A higher percentage is indicative of good settling. An inverse relationship between aggregation indices and SVI is expected. Known aliquots of bioreactor samples (10 ml) were transferred to conical tubes and allowed to stand for 30 min. Turbidity of the supernatant was measured at 550nm (OD_s). Afterwards, the samples were vigorously homogenised using a vortex for 1 min and the total turbidity (OD_t) measured. The percentage aggregation was estimated as follows:

$$\% \text{ Aggregation} = [(OD_t - OD_s) \times 100]/OD_t \quad (1)$$

2.3.2 PARTICLE SIZE DISTRIBUTION

Particle size analysis was carried out immediately upon collection of bioreactor MLSS to determine floc characteristics. The samples (250 mL) were diluted with tap water until obscuration was within range using laser diffraction in a Malvern Mastersizer 2000 (stirrer speed, 440 rpm; pump speed, 900 rpm). Five consecutive measurements were made of each sample to determine the stability of the flocs within the instrument and under the operational conditions.

2.3.3 EXTRACELLULAR POLYSACCHARIDE (EPS) POLYMER ANALYSIS

Reactor samples were also analyzed by Fourier-transform infared (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 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. MLSS samples (20 µl) collected from the bioreactors 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 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.

2.3.4 MICROSCOPIC EVALUATION OF MLSS SAMPLES

Sludge structure was assessed under light microscopy using wet mount and Gram staining. The appearance of flocs and presence of filamentous bacteria were monitored.

3 RESULTS AND DISCUSSION

3.1 PARTICLE (FLOC) SIZE DISTRIBUTION

Off-line particle size analysis was conducted on bioreactor MLSS samples to determine the floc size distribution of the biomass. Houghton et al. (2002) utilised laser diffraction for measurement of digested sludge samples and observed stable floc sizes when a Mastersizer was operated under different conditions. In the current experiment the $d(0.1)$, $d(0.5)$ and $d(0.9)$ values (10, 50 and 90% of particles $\leq d$ in size) remained stable across the replicates which indicate minimal floc disruption during measurements.

Particle size distribution patterns differed between the three bioreactor configurations. The SBR (B3) showed comparatively larger fractions when it was operated under both maximum and minimum COD influent concentrations (Table 1). The SBR exhibited the highest $d(0.9)$ values exemplified by the major peak in the 45 to 104 µm range (46.8 % v/v; 4.74 g COD L^{-1} feed) and minor peak between 954 and 1905 µm (14.3 % v/v; 0.61 g COD L^{-1} feed) (Figures 1e and f). Out of the continuous systems, B1 exhibited larger flocs compared with B2 as can be seen from its d values in Table 1 and in Figure 1 during both maximum and minimum COD feeds.

Table 1: Analytical parameters for the three bioreactors operated under maximum and minimum COD influent conditions.

Maximum COD feed (4.74 g L^{-1})					Particle size analysis			
	COD g L^{-1} (% removal)	COD g L^{-1} Bioreactor 2: total removal after clarifier (%)	Maximum EPS (mean EPS)	Maximum aggregation % (mean aggregation)	*d*(0.1) n=5	*d*(0.5)	*d*(0.9)	Specific surface area (m^2 g^{-1})
Bioreactor 1 (B1)	1.54 (67.5)		12.0 (7.2)	39.9 (32.6)	10.786	35.630	104.264	0.378
Bioreactor 2 (B2)	1.29 (72.8)	0.92 (80.6)	5.7 (3.3)	41.1 (35.7)	9.119	25.415	59.719	0.387
Bioreactor 3 (B3)	0.96 (79.7)		11.8 (6.4)	95.8 (77.5)	21.833	64.016	141.595	0.194
Minimum COD feed (0.61 g L^{-1})								
B1	0.41 (32.8)		104.7 (50.9)	47.8 (43.7)	6.439	200.203	694.647	0.344
B2	0.59 (3.3)	0.37 (39.3)	20.4 (14.9)	70.6 (64.4)	12.3336	32.086	113.787	0.125
B3	0.32 (47.5)		19.8 (11.8)	98.0 (92.4)	23.957	136.316	1110.416	0.124

Figure 1: Particle (floc) size distribution for Bioreactor 1 (a, b); 2 (c, d) and 3 (e, f) during the maximum (4.74 g L^{-1}) and minimum (0.61 g L^{-1}) COD influent feed (error bars represent standard deviations across five consecutive measurements).

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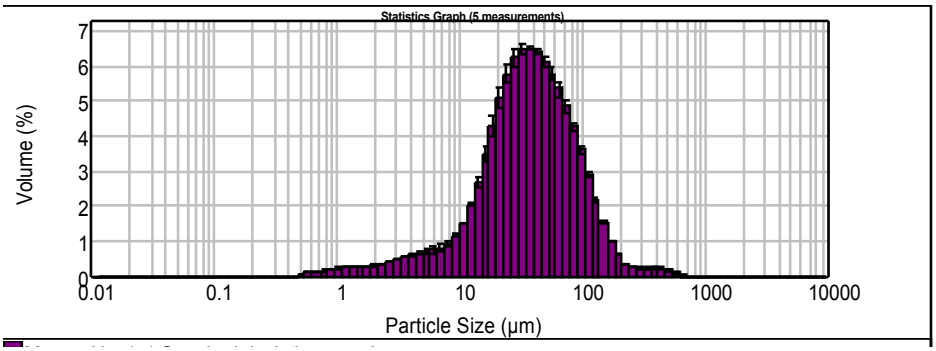
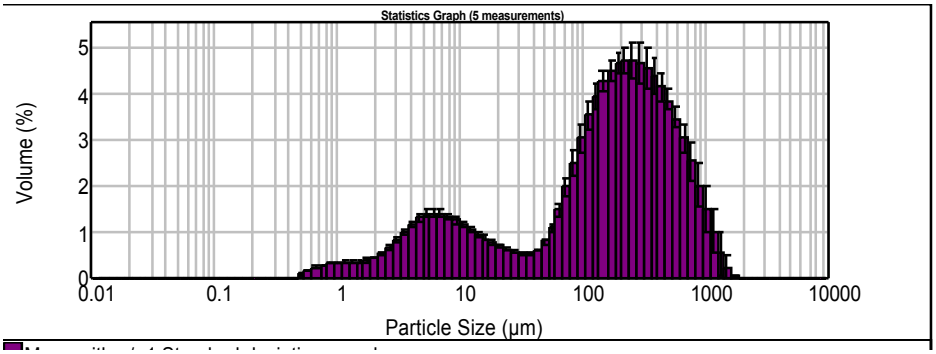
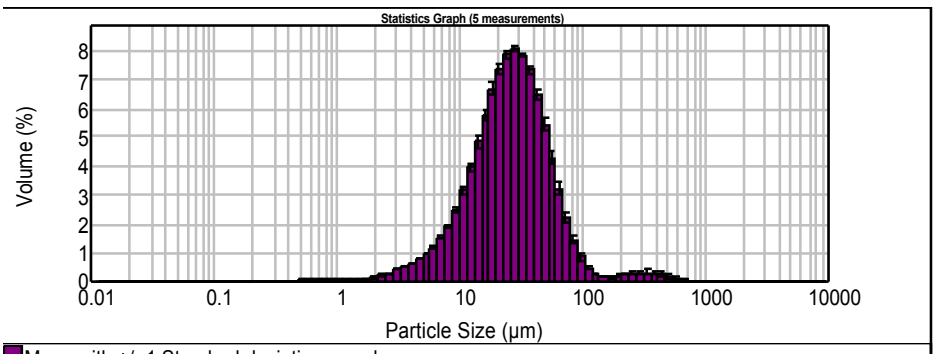
(a) Bioreator 1: 4.74 g COD L^{-1}



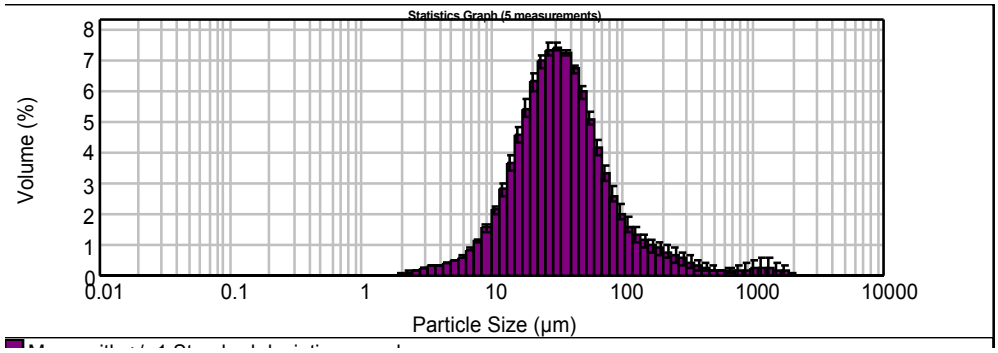
(b) Bioreactor 1: 0.61 g COD L^{-1}



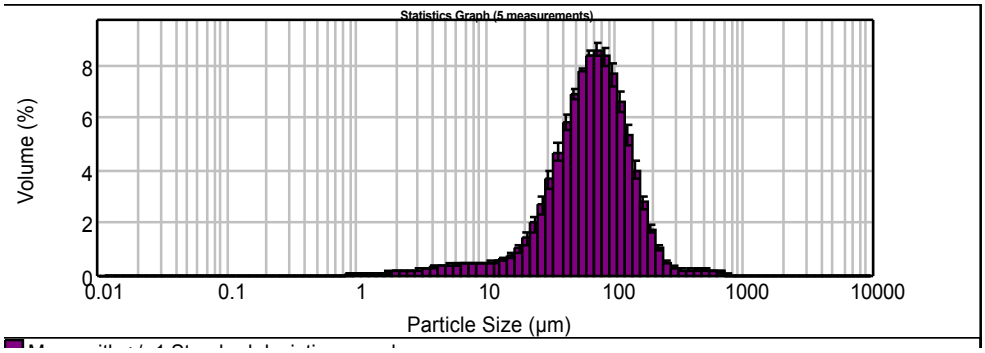
(c) Bioreactor 2: 4.74 g COD L^{-1}



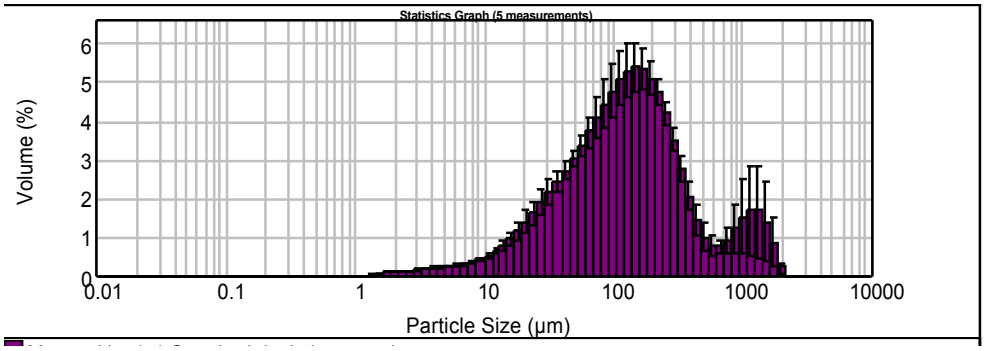
(d) Bioreactor 2: 0.61 g COD L^{-1}



(e) Bioreactor 3: 4.74 g COD L^{-1}



(f) Bioreactor 3: 0.61 g COD L^{-1}



The change in influent COD concentrations from 4.74 g L^{-1} to 0.61 g L^{-1} resulted in reduced F/M conditions in the reactors. Food to microorganism ratios declined from ~0.1 to ≤ 0.01 g COD g $MLSS^{-1}$ d^{-1} in B1 and B2 and from < 0.9 to ~0.1 g COD g $MLSS^{-1}$ d^{-1} in B3 over the experimental runs. This coincided with the formation of larger floc sizes in B1 and B3 and marginal increases in B2 (Table 1). The recycling of RAS may have contributed to the stabilisation of floc size fractions in B2 during the experimental period. This could have been brought about by digestion of biomass and EPS polymer in the clarifier, coupled with a reduction in EPS production when the aerobic AS community was exposed to anaerobic conditions in the clarifier (Wilen & Balmer, 1999). The larger floc size distribution in B3 is indicative of selection pressures administered in sequencing batch operation which drive flocculation processes (De Kreuk, 2009).

Analysis of particle size distributions provides information on floc formation and the underlying biological processes of bacterial aggregation consolidated by a matrix of extracellular polymeric material (McSwain et al., 2005). Bioreactor 1 showed a dramatic increase in EPS levels (Table 1) when the community entered the starvation cycle (min COD feed) coupled with a shift towards larger floc sizes: $d(0.9)$ values for the maximum and minimum feeds were 104.3 and 694.7 µm respectively. The particle size distribution for the 0.6 g COD L^{-1} medium is skewed towards larger floc sizes that made up a 49% per volume fraction of the size distribution of the flocs (Figures 1a and b). The largest particle fractions were measured in the 208 to 954 µm range for B1 after day 31 (during min COD feed) of the experimental run. In contrast, EPS levels in B3 showed only a smaller average increase upon entering the starvation cycle (Table 1). However, particle sizes measured in the SBR (B3) exhibited similar shift towards larger floc sizes ($d(0.9)$, 1 110.4 µm) although these made up a smaller volume (14.3%) than the predominant peak (57.2% v/v) of between 79 and 479 µm (Figure 1f). The measured EPS levels on the day of the particle size analysis (day 46) were 104.7 and 19.8 for B1 and B3, respectively. This suggests that the concentration of EPS may have played a role in the increase in particle sizes to a specific degree since a 5.3-fold difference in EPS levels (a four-fold mean difference detected from day 37 during the minimum feed) between B1 and B3 could not have been the sole contributing factor for the shift to larger floc sizes in the two reactors. This signals that EPS levels as measured by FTIR spectroscopy may not have been a major contributing factor to the formation of flocs in the bioreactors. This could be due to the fact that the FTIR analysis measures total polysaccharides (normalized to bacterial numbers) and does not discriminate between bound and suspended EPS material. The presence of bound EPS may in fact play a significant role in cementing bacteria in flocs. The increase in EPS production in B1 could also have been to maintain the AS population in continuous operation during starvation. The results indicate that the nature of EPS polymer driven by mode of reactor operation and nutrient conditions influenced settling capacity of the flocs.

3.2 EXTRACELLULAR POLYMER (EPS) AND THE IMPACT ON SLUDGE SETTLEABILITY

Large quantities of EPS did not facilitate good flocculation properties as exemplified by the comparatively high EPS concentrations measured in B1 (mean EPS 7.2 ± 2.9 and 50.9 ± 39.3 during the maximum and minimum COD feed, respectively) but showing poor aggregation (settling) indices (mean 32.6% ± 5.6 and 43.7% ± 10.3). Similar EPS quantities were measured in B2 (3.3 ± 1.9 and 14.9 ± 5.2) and the SBR (B3; 6.4 ± 3.2 and 11.8 ± 4.1) while aggregation values were significantly higher in the SBR (77.5% ± 16.7 and 92.4% ± 3.6). This supports observations from other researchers that sequencing batch operation is superior at promoting aggregating populations (Beun et al., 1999).

Reducing the influent COD content induced sudden starvation conditions in the bioreactors. An explanation for the increased aggregation in B1 was that starvation conditions triggered the bacteria to change their surface properties, either through or in concert with an increased production of EPS. Starvation has previously been noted as a trigger to drive bacterial aggregation processes (Bossier &

Verstraete, 1996). EPS levels were significantly higher in B1 when the influent COD concentration decreased after day 31. A possible explanation for this is that the AS community underwent surface property changes stimulated by nutrient starvation that would have the effect of promoting survival and avoiding washout. However, this resulted in a ~10% increase only in aggregation (Table 1). In a study by Yang & Li (2009), it was found that EPS is important for floc formation however excessive loosely-bound or diffused EPS production in AS reactors deteriorated the floc structure and sludge settleability. Mean MLSS levels in the continuous reactor were 27.9 g L^{-1} (0.6 g COD L^{-1}) down from 33.8 g L^{-1} (4.7 g COD L^{-1}). The reduction in biomass may have been due to the inability of members of the community to compete for available carbon in spite of elevated EPS concentrations in this reactor. This enforces the notion that the type of the EPS material in addition to the fact that the levels of biomass and selection pressures stimulated by reactor configuration may play a crucial role in consolidating bacterial aggregates.

3.3 COD REMOVAL EFFICIENCY

The degree of COD removal was shown to correlate with efficiently aggregating bacteria. Sequencing batch operation, which demonstrated the highest aggregation indices (Table 1), showed a 79.7% COD reduction when B3 was fed with 4.7 g COD L^{-1} compared to a 67.5 and 72.8% reduction in B1 and B2 respectively. A 47.5% COD reduction was seen in B3 (0.6 g L^{-1} feed) compared to 32.8% reduction in B1 and 3.1 % only in B3. This demonstrates that there is a measure of congruency between COD removal and good flocculation characteristics. Total COD removals in B2 were 80.6 and 39.3% for the 4.7 and 0.6 g L^{-1} feeds where samples were taken from the clarifier overflow. Digestion processes in the clarifier resulted in the further reductions in COD from B2.

4 CONCLUSIONS

Floc size distributions in the bioreactors shifted to larger particles when the influent COD feed was reduced to the minimum concentration albeit in various degrees depending on reactor configuration. The eight-fold reduction in influent COD in the synthetic dairy processing wastewater effected a strong impact on the AS communities in the reactors. The decrease in whey concentration in the feed exposed the community to conditions that are similar to starvation conditions akin to seasonal influent carbon concentration variations in primary influent in AS systems. The results show that in lab-scale reactors, changes in nutrient conditions in AS processes produce different flocculation characteristics that are linked with reactor mode of operation. This coincided with changes in floc sizes and, consequently, the settling properties of the biomass. Larger flocs showed improved settling in the reactors however selection pressures administered by sequencing batch operation may have also contributed to this observation. Changes in F/M ratios during the feast and starvation cycles have an effect on EPS levels and flocculation of biomass. Low F/M ratios (≤0.01 g COD g $MLSS^{-1}$ d^{-1} in B1 and B2, and 0.1 g COD g $MLSS^{-1}$ d^{-1} in B3) promoted the production of EPS, influenced floc sizes and sludge settling properties. This was essentially driven by nutrient concentration cues. SBR operation produced optimum COD removal and flocculation characteristics regardless of the influent COD levels. This supports the view that SBR reactors are superior at supporting favourable AS operation even in the presence of high strength effluents. The investigation into the interdependent link between EPS polymer, nutritional characteristics and settling properties is an important step towards enhanced understanding of AS microbial processes and will contribute to an improvement in process control in AS reactors.

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