Available at www.sciencedirect.com

SCIENCE DIRECT®

journal homepage: www.elsevier.com/locate/watres

Performance of an aerobic/anaerobic hybrid bioreactor under the nitrogen deficient and low F/M conditions

Christopher M. Sales[a], *Wen K. Shieh*[b,*]

[a]Department of Civil and Environmental Engineering, University of California, Berkeley, CA 94720, USA
[b]Department of Chemical & Biomolecular Engineering University of Pennsylvania Philadelphia, PA 19104-6393, USA

ARTICLE INFO

Article history:
Received 16 May 2005
Received in revised form
29 December 2005
Accepted 21 January 2006

Keywords:
Bioreactor
Biomass zone
Bulking sludge
Aerobic/anaerobic
Biomass holdup

ABSTRACT

A bioreactor system without a biomass-liquid separation unit is evaluated for its chemical oxygen demand (COD) removal and biomass retention capabilities under the nitrogen deficient and low F/M conditions that are known to produce bulking biomass. A fully oxygenated stream recycled from an external oxygenator delivers the oxygen to an upflow bioreactor in which a biomass zone is formed and maintained in the absence of gas effervescence. COD is removed with up to 90% efficiency by means of aerobic and anaerobic bacterial activities occurring in the biomass zone. The biomass is bulking which is brought about by the extensive filamentous growth caused by the nitrogen deficient and low F/M conditions adopted. However, the biomass zone is undisturbed at superficial upflow velocities as high as 0.66 cm/min, because it has a porous, mat-like matrix that is augmented by the entanglement of filamentous bacteria with the cell clusters. A low-VSS effluent (*i.e.*, <10 mg/L) is produced directly from the bioreactor.

1. Introduction

The activated sludge process, which is most commonly used in biological wastewater treatment, combines a biochemical stage (aeration tank) that supports the microbial metabolism of organic compounds with a physical stage (secondary clarifier) that provides biomass-liquids separation to achieve the required treatment efficiency. Although, the activated sludge process is highly effective at the biochemical stage, the performance of the secondary clarifier is often plagued by the formation of activated sludge particles/flocs that have poor settling characteristics as a result of poor bioflocculation and/or unsatisfactory balance between floc-forming and filamentous bacteria (Albertson, 1991; Chiesa, 1998; Eikelboom, 1975; Eikelboom and Geurkink, 2002; Grady et al., 1999; Martins et al., 2004a; Metcalf and Eddy, Inc., 2003; Seviour et al., 1994; Strom and Jenkins, 1984; Wanner, 1994).

A number of techniques have been developed to control the sludge bulking problems depending on the groups of filamentous bacterial involved that have high affinities for different limiting substrates/nutrients (Chambers, 1982; Eikelboom, 1975; Eikelboom and Geurkink, 2002; Gabb et al., 1991; Grady et al., 1999; Linne and Chiesa, 1987; Marten and Daigger, 1997; Pujol and Boutin, 1989; Shao and Jenkins, 1989; Wanner, 1994). The kinetic-based approach is often used to control the filamentous bacteria that have a high affinity for readily biodegradable carbonaceous substrates by reconfigurating the bioreactor to create a high substrate concentration region near the inlet to stimulate the growth of floc-forming bacteria (Gabb et al., 1991; Grady et al., 1999; Martins et al., 2004a; Metcalf and Eddy, Inc., 2003). On the other hand, the metabolic-based approach attempts to use alternative terminal electron acceptors other than oxygen to curb the filamentous growth (Chambers, 1982; Gabb et al., 1991; Grady et al., 1999; Linne and Chiesa, 1987; Marten and Daigger, 1997; Pujol and Boutin, 1989; Shao and Jenkins, 1989; Sheker et al., 1993). In many cases, however, the problems of bulking sludge may still persist because of shifts

*Corresponding author.
E-mail address: shieh@seas.upenn.edu (W.K. Shieh).
0043-1354/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.watres.2006.01.043

in operating conditions and/or predominant bacterial populations, although the frequency of occurrences can significantly be reduced.

A bioreactor system without a biomass-liquid separation unit has been developed as an alternative to conventional activated sludge processes. The oxygenated stream recycled from an external oxygenator is used to deliver the oxygen influx to the upflow bioreactor. The absence of gas effervescence in the bioreactor facilitates the gravitational separation of the biomass from the axial liquid flow and therefore, a reactive biomass zone is formed and maintained. This paper reports and discusses a laboratory study in which the bioreactor systems was evaluated for its COD removal and biomass retention capabilities under the nitrogen deficient and low F/M conditions that are known to produce bulking biomass (Grady et al., 1999; Martins et al., 2004a; Metcalf and Eddy, Inc., 2003).

2. Bioreactor system design

The bioreactor flow schematic illustrated in Fig. 1 was adopted. A glass column (I.D.: 6.3 cm, length: 40 cm, volume: 1247 cm^3) with a bottom cone (altitude: 3.8 cm, volume: 40 cm^3) and an enlarged top section (I.D.: 9.0 cm, volume: 723 cm^3) was used as the bioreactor. The bioreactor effluent was discharged from the port in the top section that maintained the liquid volume in the bioreactor at 1680 cm^3. A glass beaker (I.D.: 9 cm, height: 18 cm, volume: 1145 cm^3) with two ports was used as the oxygenator. The top port was used to discharge the system effluent stream and maintain the liquid volume in the oxygenator at 550 cm^3. Diffusive aeration was provided using an air diffusion stone and an aquarium aerator. The oxygenator was covered with a Plexiglas plate with three holes that accommodated for the air line, the feed line, and the bioreactor effluent line. The bioreactor effluent stream and the feed stream were mixed and aerated in the oxygenator. The oxygenated stream drawn from the bottom port of the beaker was introduced downward into the bottom cone via a glass elbow connector that was fused to the reactor wall directly above the bottom cone. The bioreactor was covered at the top with a plastic Petri dish to prevent the entrapment of ambient air.

3. Experimental design

Glucose was used as the sole carbonaceous substrate and two feed C:N ratios at 50:1 and 100:1 were tested. The biomass harvested from a packed-bed bioreactor treating an aquaculture water (4 g volatile suspended solids, VSS) was used to inoculate the bioreactor. The bioreactor was fed with a synthetic feed containing glucose (carbon source), NH_4Cl (nitrogen source), $NaHCO_3$ (total alkalinity source), KH_2PO_4 (buffer), and minerals (i.e., Ca, Fe, Mg, and Co). The bioreactor startup conditions were: C:N ratio, 50:1; feed rate, 1.0 mL/min, and oxygenated stream rate, 2.5 mL/min. The inoculating cells promptly formed a mat-like biomass zone in the lower portion of the bioreactor, yielding an initial biomass zone height and volume of 25 cm (measured from the top of the bottom cone) and 780 cm^3, respectively. The mean feed chemical oxygen demand (COD) concentration was maintained at 250 mg/L (236 to 265 mg/L) which yielded a mean COD influx of 15 mg/h (14.2–15.9 mg/h). pH and temperature in the bioreactor were maintained at 7.3+0.2 and 22+1 °C, respectively. The feed solution was refrigerated at 2 °C to preserve its quality. The empty bed hydraulic retention time (HRT) based on the biomass zone volume was approximately 13 h. The bioreactor achieved stable COD removal 2 weeks after startup. The preliminary data on biomass holdup and bioreactor effluent VSS (VSS: volatile suspended solids) suggested that the bioreactor was operated at a mean cell residence time (MCRT) > 100 days.

The flow rate of the oxygenated stream, which varied from 2.5 to 20.5 mL/min, was chosen as the sole experimental variable to control the oxygen influx to the biomass zone. Once stable COD removal was achieved at a given oxygen influx, grab samples taken from the feed container,

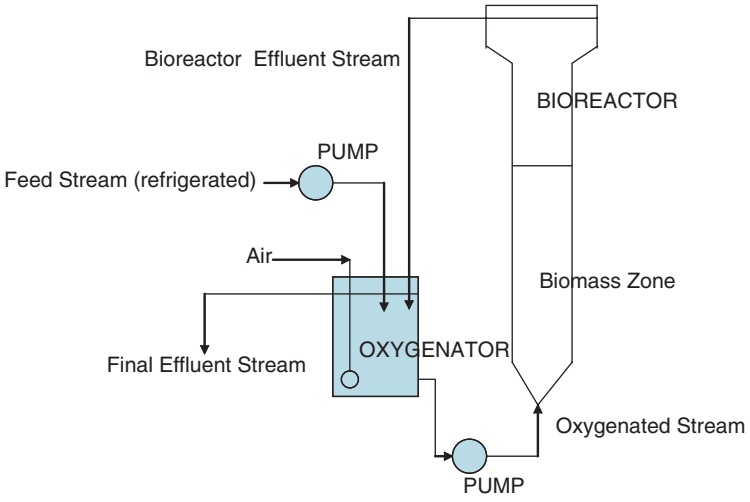

Fig. 1 – Bioreactor flow schematic (not prepared to the exact scale).

oxygenator, and above the biomass zone were filtered using GF/A glass fiber filters and then analyzed for COD and SS/VSS (suspended solids/volatile suspended solids) to obtain steady-state performance data. In addition, filtered feed NH_4^+–N concentrations were occasionally analyzed to ensure that the prescribed feed C:N ratio was maintained. The duration of each experimental run was approximately 3 weeks and five sets of samples were analyzed weekly.

A two-channel YSI biological oxygen monitor was used to measure the DO concentrations in the oxygenator and through the biomass zone. The biomass holdup was measured once in each experimental run. Three biomass samples (sample size: 3–4 mL) were, respectively, taken from the bottom, middle, and top sections of the biomass zone and then mixed to form a composite biomass zone sample (~10 mL). The Hach procedure was used for the measurements of COD. The procedures described in Standard Methods for the Examination of Water and wastewater (1998) were employed for the measurements of NH_4^+–N (4500-NH_3B and 4500-NH_3C), biomass holdup (2540D and 2540E), and SS/VSS (2540D and 2540E).

4. Results and discussion

4.1. Oxygen influx and oxygen utilization

According to the flow schematic illustrated in Fig. 1 the oxygen influx to the biomass zone and the oxygen utilization in the biomass zone can, respectively, be calculated by

$$R_{OI} = Q_1 C_{O1}, \quad (1)$$

$$R_{OU} = Q_1(C_{O1} - C_{O2}), \quad (2)$$

where R_{OI} is the oxygen influx (mg O_2/h), Q_1 is the flow rate of the oxygenated stream (L/h), C_{O1} the DO concentration in the oxygenated stream (mg/L), R_{OU} is the rate of oxygen utilization in the biomass zone (mg O_2/h), and C_{O2} is the DO concentration in the bioreactor effluent (mg/L).

Two input streams were delivered to the oxygenator for oxygenation: the feed (or influent) stream and the bioreactor effluent stream. The oxygenator was ~100% efficient at the combined flow rates from 2.2 to 20.5 mL/min. The corresponding oxygen influxes to and the superficial upflow velocities (u_p's) through the bioreactor ($u_p = Q_1/A$, A is the bioreactor cross-sectional area, cm^2) were 1.2–10.7 mg O_2/h and 0.08–0.66 cm/min, respectively.

Fig. 2 shows that the utilization of oxygen in the biomass zone was complete. The DO concentrations measured at the top of the biomass zone were < 0.2 mg/L which was the detection limit of the DO meter employed. Since the absence of gas effervescence in the bioreactor would reduce the magnitude of axial dispersion in the direction of flow (Fogler, 1992), the DO concentrations measured at various depths in the biomass zone would provide better insight into the pattern of oxygen utilization. Examples of DO concentration profiles through the biomass zone at three oxygen influxes are illustrated in Fig. 3. Similar profiles were also observed at the feed C:N ratio = 100:1. It is seen that the DO concentrations decreased continuously in the direction of flow, with

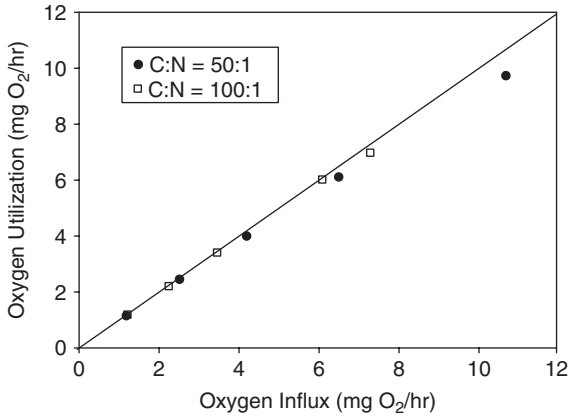

Fig. 2 – The oxygen influx to the biomass zone versus the oxygen utilization in the biomass zone.

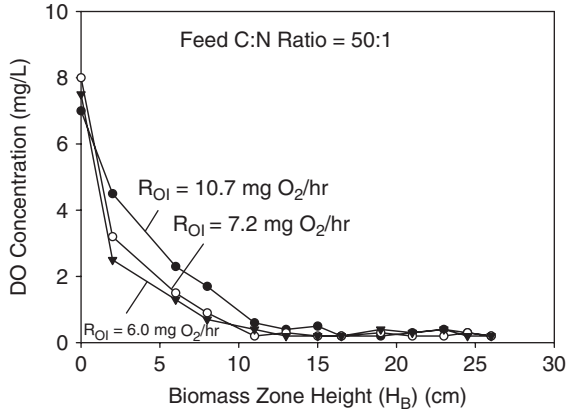

Fig. 3 – Examples of DO concentration profiles through the biomass zone.

sharp decreases taking place near the bioreactor entrance where oxygen was readily available to support the oxygen utilization activities. The pre-dissolution of oxygen in the oxygenated stream yielded better mass transfer of oxygen between the liquid and biomass phases but reduced the degree of longitudinal mixing (Fogler, 1992). The nitrogen deficient conditions imposed had no impacts on the bacterial oxygen utilization activities. The upper portion of the biomass zone was oxygen deficient although the penetration of oxygen in the direction of axial flow could be increased at higher Q_1's. Under the conditions tested, it was estimated that at least 60% of the biomass zone would be deprived of oxygen.

4.2. COD removal

Since the COD values measured in the oxygenated stream agreed well with those calculated from the mass balance equation written around the oxygenator, the biomass zone was the only reactive region in the bioreactor system. The steady-state COD removal rate in the biomass zone is calculated by

$$R_C = Q_1(C_1 - C_2), \quad (3)$$

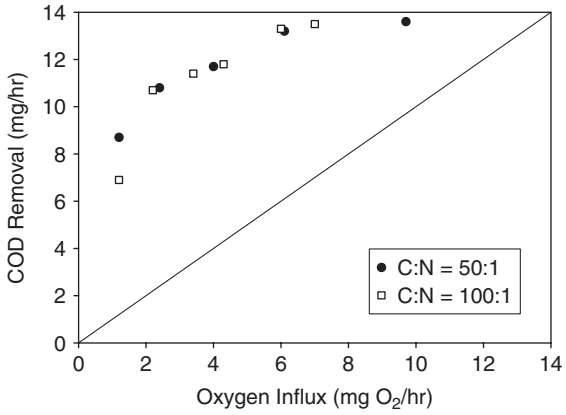

Fig. 4 – COD removal as a function of oxygen influx.

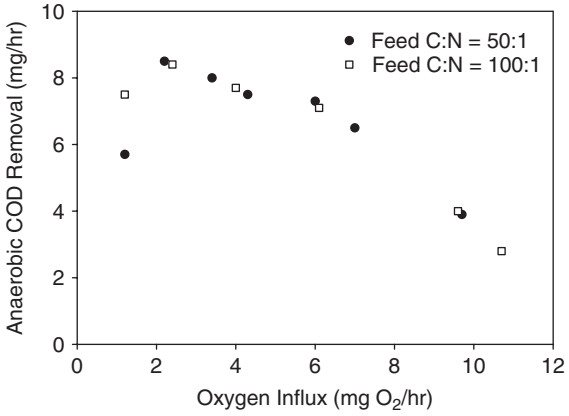

Fig. 5 – Anaerobic COD removal as a function of oxygen influx.

where R_C is the COD removal rate (mg COD/h), C_1 the COD concentration in the oxygenated stream (mg/L), and C_2 is the COD concentration in the bioreactor effluent stream (mg/L). Fig. 4 shows the rate data on COD removal as a function of oxygen influx. Since the bioreactor was operated under the low F/M conditions (i.e., ~0.144 g COD/g VSS), the COD measurements on both the oxygenated stream and bioreactor effluent stream would have included those from the cellular materials released from the decayed cells and the extracellular polymeric substances (EPS) produced. The observed trends of COD removal were similar for both feed C/N ratios, and they increased with increasing oxygen influx until leveling off at about 13.5 mg COD/h at the oxygen influx of about 6.0 mg O_2/h. As a result, COD was removed with up to 90% efficiency based on the system flow scheme and the experimental conditions adopted.

The rate data in Fig. 4 reveal that COD removal could not be completely accounted for by the aerobic activities, especially at low oxygen influxes. The oxygen utilization data suggest that aerobic COD removal would range from 1.2 to 10.7 mg/h (respiratory oxygen requirements were assumed to be negligible), which indicate the involvement of other reaction activities. To further delineate the phenomenon observed, the diagonal shown in Fig. 4 is used as the baseline to estimate the stoichiometric amount of COD that would have been removed aerobically. Since the recipe used to prepare the feed solution eliminated the possibilities that NO_X^- and SO_4^{-2} could be used as viable alternative terminal electron acceptors, the additional COD removal observed was attributed to the anaerobic activities. The anaerobic COD removal rate was estimated as: total COD removal rate—stoichiometric aerobic COD removal rate, and the results are illustrated in Fig. 5. A peak anaerobic COD removal rate of 8.6 mg COD/h was observed at the oxygen influx of about 2.2 mg O_2/h. Beyond that the percentage of aerobic COD removal increased with increasing oxygen influx. At the maximum oxygen influx of 10.7 mg O_2/h, aerobic COD removal accounted for about 67% of total COD removal (i.e., 9 mg/h). By reaction stoichiometry, COD removal could be totally sustained aerobically at the oxygen influxes > 12.5 mg O_2/h.

The rate data in Figs. 4 and 5 confirm that COD removal was carried out by both the aerobic and anaerobic activities in the biomass zone. At low oxygen influxes, the aerobic activities would likely be limited to the region near the bottom the biomass zone where oxygen would be readily available. Since the majority of the biomass zone was deprived of oxygen (Fig. 3), aerobic COD removal would be small as compared to its anaerobic counterpart. As the oxygen influx increased, oxygen would become available to the bacterial cells colonized in the region away from the bioreactor inlet and the aerobic activities would increase accordingly. The anaerobic COD removal activities decreased at an average rate of about 0.55 mg COD removed/mg O_2 added as the oxygen influx was increased to > 2.2 mg O_2/h (Fig. 5).

4.3. *Stability of biomass zone*

A biomass zone was promptly formed in the bioreactor after startup that could primarily be attributed to the absence of gas effervescence that facilitated the gravitational separation of the inoculating cells from the axial liquid flow. Since the flow rate of the oxygenated stream was varied to attain a range of oxygen influxes for oxygen delivery, it is critical that the biomass zone formed would remain undisturbed at high superficial upflow velocities. Two parameters were selected to assess the stability of the biomass zone: biomass zone height (H_B) and biomass holdup (M_X). The data in Fig. 6 indicate that, over the range of u_p's tested (i.e., 0.08–0.66 cm/min), the variations of H_B and M_X were small, i.e., 25–27 cm and 2.36–2.60 g VSS, respectively. Moreover, the nitrogen deficient conditions applied had no discernible effects on the values of H_B and M_X observed. To further ascertain that the biomass zone was not susceptible to the variations in u_p, the data in Fig. 6 were analyzed by the linear regression techniques. For instance, a simple linear regression equation was proposed that related M_X to u_p as $M_X = \beta_0 + \beta_1 u_p + \varepsilon$, where β_0 and β_1 are regression coefficients and ε is random error. β_0 and $\beta_1$were estimated as B_0 and B_1, respectively, from the M_X and u_p data using the least square technique (Montgomery et al., 2004). Then, the following hypothesis was tested at the level of significance $\alpha = 0.05$:

H_0 : $\beta_1 = 0$,
H_1 : $\beta_1 \neq 0$.

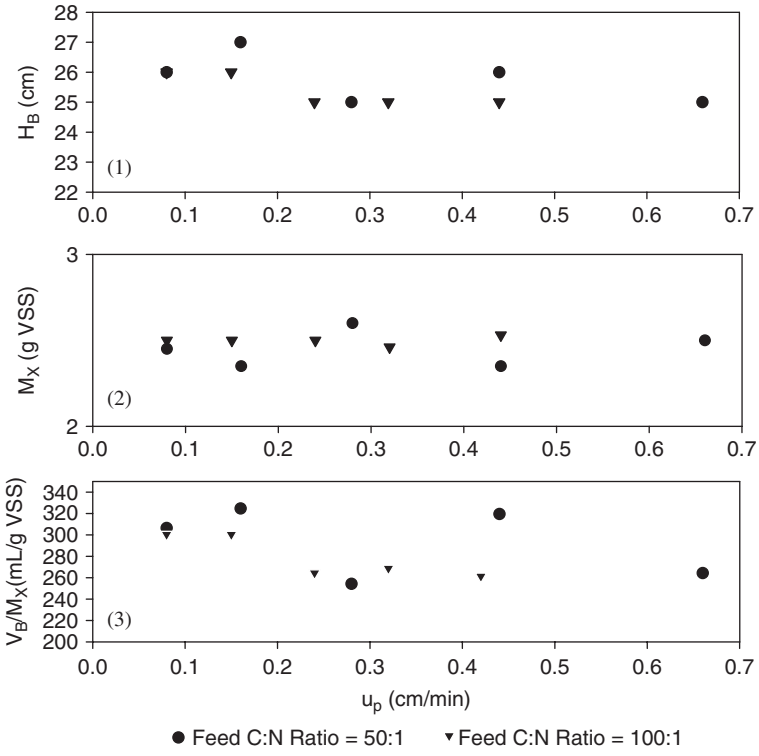

Fig. 6 – Mean Biomass zone height (H_B), mean biomass holdup (M_X), and specific biomass volume (V_B/M_X) as a function of superficial upflow velocity (u_p).

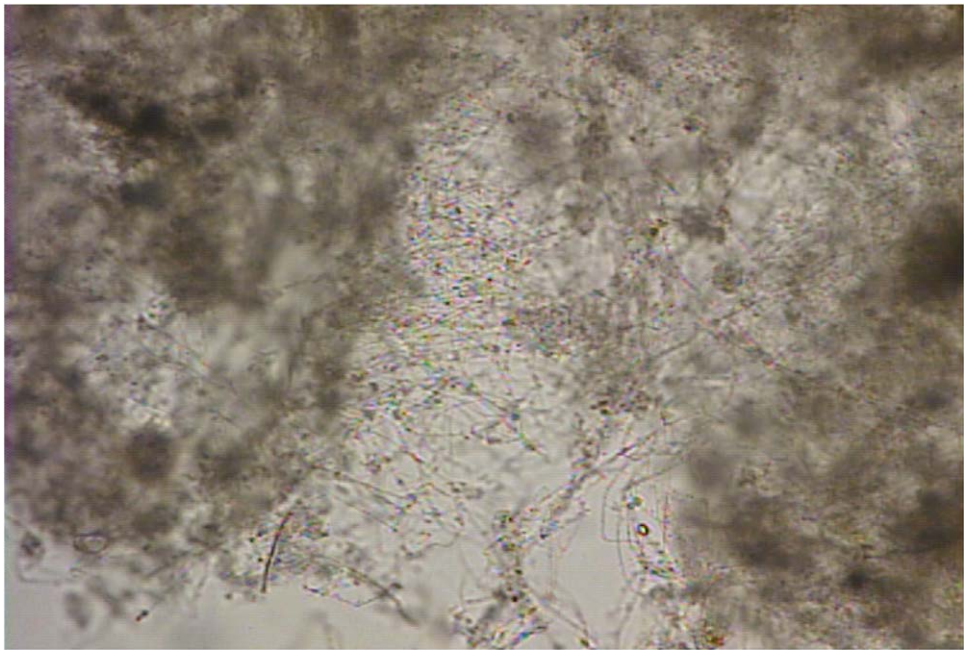

Fig. 7 – Photograph showing the entanglement of filamentous bacteria with the cell clusters in the biomass zone.

A student-t test statistic T_v, where $v = n - 2$ is the degree of freedom and n is the number of data points used in the test, was defined in terms of B_0 and B_1, and its value was calculated as 1.220, which is $< t_{0.025,9} = 1.833$ (a two-sided t-test) (Montgomery et al., 2004). Therefore, H_0 was accepted and M_X was deemed to be independent of the superficial upflow velocities as high as 0.66 cm/min. The same conclusion was also reached with regard to H_B.

Other than one composite biomass zone sample (sample size: ~10 mL) taken in each experimental run for the measurement of M_X, no additional biomass was removed intentionally during the entire experimental period. VSS

measurements using 100-mL bioreactor effluent samples often produced negative readings, which suggested that VSS present in the samples were negligible. Subsequent VSS data based on 200-mL sample sizes indicated a low-VSS bioreactor effluent (i.e., <10 mg/L), and suggested that the growth of new bacterial cells was virtually offset by the death of the old bacterial cells in the biomass zone. In addition, a number of composite biomass zone samples were centrifuged at $1300 \times g$ for 10 min to separate the water from the bacterial aggregates, and it was estimated that the porosity of the biomass zone was roughly 0.85. Therefore, the biomass zone was porous to permit unhindered axial fluid flow without excessive biomass detachment by fluid shear stresses.

Examinations performed using a video-equipped optical microscope revealed that filamentous bacteria were abundant in the biomass zone, and they were mostly entangled with the cell clusters (Fig. 7). The presence of extensive filamentous growth in the bioreactor was a direct result of the nitrogen deficient and low F/M conditions applied. The entanglement of filamentous bacteria increased the degree of bulking in the biomass zone, as indicated by the high biomass specific volume values plotted in Fig. 6 (i.e., 250–325 mL/g). The biomass specific volume, which is defined as V_B/M_X (V_B is the volume of the biomass zone, mL), was used because it is analogous to the conventional sludge volume index (SVI) but provides a better description of the biomass matrix characteristics observed. On the other hand, the entangled filamentous bacteria formed the backbone to which the cell clusters could attach and therefore, a stable, mat-like biomass matrix was formed that was separated from a distinct clear supernatant zone near the top section of the bioreactor. The observation was in line with this "backbone structure" provided by filamentous bacteria as suggested in a number of studies reported elsewhere (Martins et al., 2004b, Sezgin et al., 1978). In addition, the stability of the biomass zone could also be enhanced by the increased production of EPS, as reported elsewhere in bioreactors that were operated under the low F/M conditions (Tay et al., 2003; van Loosdrecht et al., 1995). The biomass characteristics observed in this study were quite different from those of sludge granules that were reported to be formed in bioreactors operated under the high DO and organic loading conditions (Mishima and Nakamura, 1991; Tay et al., 2003).

5. Conclusions

A bioreactor system, which utilized a fully oxygenated stream recycled from an external oxygenator for oxygen delivery, was evaluated for its COD removal and biomass retention capabilities under the nitrogen deficient and low F/M conditions known to cause biomass bulking. A reactive biomass zone was formed in the upflow bioreactor in the absence of gas effervescence without using a separate biomass-liquid separation unit for cell capture and recycle. The following conclusions can be made.

1. The oxygen utilization activities in the bottom region of the bioreactor were rapid and therefore, the upper portion of the biomass zone was deprived of oxygen. Over the ranges of the oxygen influxes tested (i.e., 1.2–12.3 mg O_2/h), COD was removed with up to 90% efficiency (based on the mean feed COD influx of 15 mg/h) by both the aerobic and anaerobic activities occurring in the biomass zone. COD was removed anaerobically at the low oxygen influxes (<6.0 mg O_2/h), beyond that aerobic COD removal became increasing predominant. Under the conditions tested, a complete aerobic COD removal was feasible at the oxygen influxes >12 mg O_2/h.
2. A stable biomass zone with a porous, mat-like matrix was formed and maintained in the bioreactor despite the fact that biomass bulking was brought about by the extensive filamentous growth that was encouraged by the nitrogen deficient and low F/M conditions adopted. The absence of gas effervescence facilitated gravitational separation of biomass from the axial fluid flow. The biomass matrix was further augmented by the entanglement of filamentous bacteria with the cell clusters. As a result, the biomass zone was undisturbed (as measured by the biomass zone height and biomass holdup) at superficial upflow velocities as high as 0.66 cm/min.
3. A low-VSS effluent (i.e., <10 mg/L) was produced directly from the bioreactor without using a biomass-liquid separation unit to capture and recycle the dislodged cells.

Acknowledgment

This research was supported partially by Grants from the NSF AMP (Alliance for Minority Participation) Program and the Nassau Fund at the University of Pennsylvania.

REFERENCES

Albertson, O.E., 1991. Bulking sludge control—progress, practice and problems. Water Sci. Technol. 23, 835–846.

Chambers, B., 1982. Effect of longitudinal mixing and anoxic zones on settleability of activated sludge. In: Chambers, B., Tomlinson, E.J. (Eds.), Bulking of Activated Sludge Preventive and Remedial Methods. Ellis Horwood Limited, Chichester, UK, pp. 166–186.

Chiesa, S.C., 1998. Growth and control of filamentous microbes in wastewater management systems. In: Sikdar, S.K., Trvine, R.L. (Eds.), Biodegradation Technology Developments, vol. 2. Technomic Publishing Cp. Inc., Lancaster, PA, pp. 1–34.

Eikelboom, D.H., 1975. Filamentous organisms observed in activated sludge. Water Res. 9, 365–388.

Eikelboom, D.H., Geurkink, B., 2002. Filamentous microorganisms observed in industrial activated sludge plants. Water Sci. Technol. 46, 535–542.

Fogler, H.S., 1992. Elements of Chemical Reaction Engineering, second ed. Prentice-Hall Inc., Englewood Cliffs, NJ.

Gabb, D.M.D., Still, D.A., Ekama, G.A., Jenkins, D., Marais, G.v.R., 1991. The selector effect on filamentous bulking in long sludge age activated sludge systems. Water Sci. Technol. 23, 867–877.

Grady Jr, C.P.L., Daigger, G.T., Lim, H.C., 1999. Biological Wastewater Treatment, second ed. Marcel Dekker, Inc., New York, NY.

Linne, S.R., Chiesa, S.C., 1987. Operational variables affecting performance of the selector-complete mix activated sludge process. J. Water Pollut. Control Fed. 59, 716–721.

Marten, W.L., Daigger, G.T., 1997. Full-scale evaluation of factors affecting the performance of anoxic selectors. Water Environ. Res. 69, 1272–1281.

Martins, A.M.P., Pagilla, K., Heijnen, J.J., van Loosdrecht, M.C.M., 2004a. Filamentous bulking sludge—a critical review. Water Res. 38, 793–817.

Martins, A.M.P., Picioreanu, C., Heijnen, J.J., van Loosdrecht, M.C.M., 2004b. Three-dimensional dual-morphotype species modeling of activated sludge. Environ. Sci. Technol. 38, 5632–5641.

Metcalf and Eddy, Inc., 2003. Wastewater Engineering, fourth ed. McGraw-Hill, New York, NY.

Mishima, K., Nakamura, M., 1991. Self-immobilization of aerobic activated sludge—a pilot study of the aerobic upflow sludge blanket process in municipal sewage treatment. Water Sci. Technol. 23, 981–990.

Montgomery, D.C., Runger, G.C., Hubele, N.F., 2004. Engineering Statistics, third ed. John Wiley & Sons, Inc., New York, NY.

Pujol, R., Boutin, P., 1989. Control of activated sludge bulking: from the lab to the plant. Water Sci. Technol. 21, 717–726.

Seviour, E.M., Williams, C., DeGrey, B., Soddell, J.A., Seviour, R.J., Lindrea, K.C., 1994. Studies on filamentous bacteria from Australian activated sludge plants. Water Res. 28, 2335–2342.

Sezgin, M., Jenkins, D., Paker, D.S., 1978. A unified theory of filamentous activated sludge bulking. J. Water Pollut. Control Fed. 50, 362–381.

Shao, Y.J., Jenkins, D., 1989. The use of anoxic selectors for the control of low F/M activated sludge bulking. Water Sci. Technol. 19, 609–619.

Sheker, R.E., Aris, R.M., Shieh, W.K., 1993. The effects of fill strategies on SBR performance under nitrogen deficiency and rich conditions. Water Sci. Technol. 28, 259–266.

Standard Methods for the Examination of Water and Wastewater, 1998. , 20th ed. APHA-AWWA-WEF, Washinbgton, DC.

Strom, P.F., Jenkins, D., 1984. Identification and significance of filamentous microorganisms in activated sludge. J. Water Pollut. Control Fed. 56, 449–459.

Tay, J.H., Pan, S., Tay, S.T.L., Ivanov, V., Liu, Y., 2003. The effect of organic loading rate on the aerobic granulation: the development of shear force theory. Water Sci. Technol. 47 (11), 235–240.

van Loosdrecht, M.C.M., Eikelboom, D., Gjaltema, A., Mulder, A., Tijhuis, L., Heijnen, J.J., 1995. Biofilm structures. Water Sci. Technol. 32 (8), 35–43.

Wanner, J., 1994. Activated Sludge Bulking and Foaming Control. Technomic Publishing Co. Inc., Lancaster, PA.