Solid–liquid separation of an effluent produced by a fixed media biofilm reactor

Bernard Patry, Étienne Boutet, Serge Baillargeon and Paul Lessard

ABSTRACT

An experimental study dedicated to the characterization of the settleability of solids produced in immersed fixed media biofilm reactors has been carried out. The influence of operating temperature (0.1 to 16 °C) and surface organic loading rate (OLR) (0.4 to 10 g of soluble carbonaceous BOD₅ per m² of media per day) on settleable solids quantities, particle size distributions (PSD) as well as flocs morphology was evaluated. Results have shown that the OLR has no statistically significant influence on the settleability of the suspended solids. However, the operating temperature was identified as a factor that significantly influences the settling potential. The highest operating temperatures (14–16 °C) were related to the worst settling performances. On the other hand, the best settling performances were observed at intermediate operating temperatures (around 10 °C). The latter conditions were also associated with the largest fractions of large particles (>100 μm) in the effluent. Differences in PSD were found to be well correlated with settling performances. Part of the performance results variability which cannot be explained by differences in PSD can potentially be attributed to differences in flocs morphology (compactness).

Key words | cold temperature, fixed media biofilms, particle size distributions, settling potential, solid–liquid separation

INTRODUCTION

Solid–liquid separation represents a crucial step in any biological wastewater treatment processes, as it has a large impact on the facility’s effluent quality. Only a few studies focusing on the separation of solids produced in fixed-film processes were conducted. The relatively low solids concentrations produced by fixed-film systems (Ødegaard et al. 2010; Ivanovic & Leiknes 2012) do not foster a bioflocculation as efficient as in activated sludge secondary settlers where hindered settling occurs. The solids produced in fixed-film biological reactors are mostly fragments of biofilm detached by means of sloughing, erosion, abrasion or predator grazing, depending on the process (Wuertz et al. 2003). The settling potential of that type of particulate matter is quite different from the separation potential of activated sludge (Melin et al. 2005; Karizmeh et al. 2014). Several studies have highlighted the weak settling potential of biofilm reactor effluent suspended solids (SS) (Vanhooren 2002; Marquet et al. 2007; Ivanovic & Leiknes 2012; Karizmeh et al. 2014). Knowing this, optimization of settling performance after biofilm reactors is even more important.

Biofilm reactors operating conditions such as temperature and surface organic loading rate (OLR) have an influence on many system properties related to settling potential (Ødegaard 2000; Hoang et al. 2014; Karizmeh et al. 2014). Indeed, operating temperature can influence the characteristics of the biofilm (Hoang et al. 2014) and, subsequently, the characteristics of biologically produced solids found in the reactors’ effluent. More, previous studies focusing mainly on moving bed biofilm reactors (MBBR) have shown negative correlations between surface OLR applied and settling performance for the range of OLR studied (from less than 10 gCOD/m² d up to more than 60 gCOD/m² d) (Ødegaard 2000; Karizmeh et al. 2014). Differences in solids properties such as size distributions were identified as key factors by many authors to explain settling performance variations after MBBR (Melin et al. 2005; Aehl et al. 2006). The latter findings were also made when considering non-submerged systems such as trickling filters (TF) (Zahid & Ganczarczyk 1990; Schubert & Günthert 2001; Marquet et al. 2007).
Most published data concerning the settling potential of solids produced in biofilm reactors, which are rather scarce, are mainly related to MBBR and non-submerged media systems. Biofilm growth conditions are different for these two technologies. In the case of MBBR, biofilm grows on immersed fluidized carriers exposed to important shear stresses (Ødegaard 2006) while in the case of non-submerged systems, biofilm grows in discontinuous wet conditions (Henze et al. 2008). A new technology has been developed using an inert immersed fixed (i.e. non-fluidized and self-supported) media (BIONEST®) which has originally been used in biofilm reactors for onsite decentralized domestic wastewater treatment. Growth conditions associated to this technology are in between the ones of MBBR and TF. Typically, in these applications, the OLR is of 0.5 gCOD/m² d and the reactor configuration have a hydraulic retention time (HRT) of 2.3 days (Québec 2012). Organic matter removal and nitrification performance data for the new and more intensive (high OLR) application can be found in Boutet et al. (2017). However, no literature regarding the settling potential of solids from the biofilm produced in such process can be found.

The aim of this study was thus to extend the knowledge concerning settleability of solids produced in biofilm reactors by studying reactors using an inert immersed fixed media. More specifically, the study aimed at providing, on one hand, outcomes regarding the influence of operating OLR and temperature on solids settleability and, on the other hand, to provide explanations for performance variations observed as a function of operating conditions.

**METHODS**

**Experimental setup and studied variables**

Cells containing BIONEST® inert fixed media (Figure 1) were incorporated in 12 pilot reactors (0.115 m³ each) fed with domestic wastewater from the municipality of Grandes-Piles (QC, Canada). Before being pumped into the pilot reactors, raw wastewater was stored in a basin (HRT = 25 h) where primary settling treatment occurred. Peristaltic pumps allowed continuous feeding of the 12 pilot reactors with the supernatant of the settling basin. Each reactor was equipped with three cells. The cells were filled with media so that the obtained specific volumetric surface area was 160 m² per m³ of cell. Continuous aeration in the reactors was provided by compressors forcing air through perforated pipes creating fine bubbles from the bottom of the reactors. The latter system allowed to maintain a non-limiting dissolved oxygen (DO) concentration oscillating between 8 mg/L (for highest operating temperatures) and 14 mg/L (for lowest operating temperatures) along the experimental campaign. Diffusors were also installed to periodically carry out coarse bubbles injection cycles (4 min every 68 min). These cycles were carried out to promote biofilm detachment and, at the same time, to control biofilm thickness.

The experimental setup was designed to allow the evaluation of the influence of operating conditions on settleability of produced solids. Controlled variables were the reactor temperature and the OLR. The temperature was controlled.
using thermostats coupled with glycol cooling systems while the OLR was controlled by setting the wastewater feeding flow rate. The OLR was therefore lumped with the reactors’ HRT which is also dependent on the flow rate. Nine combinations of operating conditions (three different OLR and three different temperatures) were maintained in the 12 pilot reactors. Intermediate OLR conditions were duplicated for every temperature level. A summary of the observed operating conditions is presented in Table 1.

**Sampling and operating conditions**

Effluent samples were taken directly downstream of the pilot reactors to measure the influence of the operating conditions on the solids’ settling potential. Samples were taken weekly during two different sampling periods spread over a total period of 7 months to get a representative portrait of the reactors’ effluent. Sampling was done over periods of 72 min to systematically include complete coarse bubbles cycles. Thus, samples SS concentration were as representative as possible of the average effluent SS concentrations. Analyses concerning the particulate fraction were carried out within 3 days after sampling to minimize the alteration of flocs (Jenkins et al. 2004). Carbonaceous biochemical oxygen demand – 5 days’ (CBOD5) analyses were conducted within 2 days after sampling (APHA/ AWWA/WEF 1998).

The soluble CBOD₅ was considered as the organic loading index during the study. Measurements of soluble CBOD₅ were carried out according to Standard Methods (APHA/ AWWA/WEF 1998). Pilot reactors temperature was monitored using probes equipped with thermometers. DO, pH and conductivity were also monitored to validate that the reactors were operating under appropriate conditions for bacterial growth.

Since variations were observed over time for the key monitored variables (OLR and temperature), they were not considered as fixed categoric variables during the analysis of results. They were rather considered as continuous variables.

**Settling performance**

To quantify the settleability of the produced solids, a settleable solids test was performed on each sample collected during the two sampling periods. The settling tests were performed at room temperature according to Standard Methods (APHA/ AWWA/WEF 1998) (method 2540F). Quantities of settleable and non-settleable solids as well as SS removals were deduced from these settling tests. SS removal is defined as the ratio of settleable solids concentration to total SS concentration.

**Measurements performed to explain variations in settling performance**

Protocols were also selected to relate variations in settleability to variations in solids properties. Particle size distributions (PSD) were systematically measured on the samples collected during the first sampling campaign. The focused beam reflectance measurement technique (FBRM®, Mettler-Toledo) was used. The latter consists of the measurement of strings, a distance corresponding to the difference between two opposite edges of a particle, with a probe equipped with a rotating laser allowing a high-speed scan of the samples. The measurement of a very large number of strings during the scan makes it

---

**Table 1** Average and standard deviation of pilot reactors operating conditions

<table>
<thead>
<tr>
<th>Operating conditions levels</th>
<th>DO (g/m³)</th>
<th>pH</th>
<th>Conductivity (µS/cm)</th>
<th>OLR (g soluble CBOD₅/m² d)ᵃ</th>
<th>HRT (h)</th>
<th>Temperature (°C)</th>
<th>Effluent SS (g/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>OLR</td>
<td>Avg</td>
<td>SD</td>
<td>Avg</td>
<td>SD</td>
<td>Avg</td>
<td>SD</td>
</tr>
<tr>
<td>Low</td>
<td>Low</td>
<td>13.4</td>
<td>1.4</td>
<td>8.3</td>
<td>0.5</td>
<td>926</td>
<td>682</td>
</tr>
<tr>
<td>Intermediate</td>
<td>13.3</td>
<td>1.5</td>
<td>8.4</td>
<td>0.6</td>
<td>962</td>
<td>667</td>
<td>1.4</td>
</tr>
<tr>
<td>High</td>
<td>12.6</td>
<td>1.7</td>
<td>8.3</td>
<td>0.5</td>
<td>883</td>
<td>554</td>
<td>3.5</td>
</tr>
<tr>
<td>Intermediate</td>
<td>Low</td>
<td>11.0</td>
<td>0.9</td>
<td>8.0</td>
<td>0.6</td>
<td>937</td>
<td>789</td>
</tr>
<tr>
<td>Intermediate</td>
<td>10.5</td>
<td>1.1</td>
<td>8.0</td>
<td>0.7</td>
<td>867</td>
<td>653</td>
<td>1.4</td>
</tr>
<tr>
<td>High</td>
<td>8.8</td>
<td>2.0</td>
<td>8.0</td>
<td>0.6</td>
<td>831</td>
<td>553</td>
<td>3.5</td>
</tr>
<tr>
<td>High</td>
<td>Low</td>
<td>9.8</td>
<td>0.2</td>
<td>8.0</td>
<td>0.6</td>
<td>881</td>
<td>611</td>
</tr>
<tr>
<td>Intermediate</td>
<td>9.8</td>
<td>0.2</td>
<td>8.0</td>
<td>0.5</td>
<td>839</td>
<td>638</td>
<td>1.3</td>
</tr>
<tr>
<td>High</td>
<td>8.5</td>
<td>0.8</td>
<td>7.7</td>
<td>0.5</td>
<td>804</td>
<td>523</td>
<td>3.5</td>
</tr>
</tbody>
</table>

ᵃCalculation is made considering the specific media surface of each reactor (5 m²). Avg, average; SD, standard deviation.
possible to plot a representative portrait of the sample’s PSD. For the purpose of the study, proportions of particle size classes corresponding, by definition, to settleable (>100 μm) and non-settleable solids (<100 μm) (Levine et al. 1985) were derived from the PSD results and used as key variables for the results analysis. These classes are referred to as small and large particles hereinafter.

Microscopic observations were also carried out in parallel to qualitatively evaluate morphological differences between flocs found in the effluent of the 12 pilot reactors. From each raw sample, two sub-samples were examined using a phase contrast optical microscope (Olympus® BHT). Samples were examined fresh at a magnification of 100X.

Statistical analysis

Statistical analysis of the data was performed using SAS 9.4 (SAS Institute Inc.). Mixed regression with repeated measurements was achieved to perform hypothesis tests allowing to conclude on the significance of the studied effects. Final structure of regression models used for results analysis were based on minimizing the Akaike information criterion (AIC).

RESULTS AND DISCUSSION

Operating conditions

The influent composition during the two sampling periods was similar, with average concentrations of 118 g/m³ (SD = 79 g/m³) for SS, 89 g/m³ (SD = 78 g/m³) for soluble CBOD₅, an average pH of 7.4 (SD = 0.5) and an average conductivity of 833 μS/cm (SD = 276 μS/cm). The low average influent SS concentration is attributable to the settling stage prior to the pilot reactors.

Despite the relatively constant HRT but due to the fluctuating nature of municipal wastewater, applied OLR varied during the monitored periods. Differences between the three controlled feed rates have resulted in OLR ranging from 0.2 to 7.4 g soluble CBOD₅/m² d. A summary of the average operating conditions and standard deviations is presented in Table 1. The control systems allowed the temperature to be maintained relatively constant in reactors operated at intermediate (approximately 10 °C) and high (approximately 15 °C) temperatures. However, temperature in pilots operating at low temperature (approximately 0.5 °C) increased during the second campaign (carried out during summer).

The high SDs in Table 1 for low level temperature were caused by this seasonal fluctuation.

Averages for pH, conductivity and DO concentration are also presented in Table 1. Usual and similar orders of magnitude were observed for pH and conductivity in all pilot reactors. DO was maintained at high concentrations to ensure unlimited conditions for oxidation and adequate mixing. Finally, raw effluent SS concentrations, which were highly fluctuant for all the reactors, are also presented in Table 1.

Settleability

Results obtained from settling tests are considered as indexes of solids settleability. Statistical analysis of non-settleable SS concentrations (Figures 2 and 3) showed that, of all fixed effects included in the mixed regression model (OLR, HRT, temperature and effluent SS concentration), only temperature has a significant influence on settling performances (P-value < 0.01). Average SS removal is significantly higher (25% higher) for pilots operated at low temperatures, from approximately 0.2 to 14 °C. Lower settling performances were observed during tests carried out on samples coming from pilots operated at more than 14 °C. Consequently, non-settleable SS concentrations are lower for pilots operated at low temperatures (Figure 2). The settling performance seems to be maximized at intermediate operating temperatures. The observed quadratic tendency appears to be significant in
the regression model. A lower AIC is obtained when it is integrated in the model.

OLR does not appear as a statistically significant fixed effect in the regression model (P-value > 0.05). However, it can be seen graphically that the large variability in performance, which is not conclusive on the influence of OLR, is not of the same magnitude over the entire range of applied OLR. At OLR greater than 3 g soluble CBOD₅/m²·d and independently of the operating temperature, settling performance is increased (Figure 3). Below this OLR, the performance range widens significantly. Non-settleable SS concentrations range from 1 to 65 g/m³.

Parallel quantification of particle size classes was made to associate the results described above with variations in the physical properties of solids. Statistical modelling showed a significant correlation between the size of the solids and operating temperature (P-value < 0.01) which also showed a significant correlation with settling performance. Graphically, it is possible to observe that the temperature has an influence on the average proportions of the large (>100 μm) and small (<100 μm) particle classes. Figure 4(a) shows that the average proportion of large solids decrease with increasing operating temperatures. As in the case of settling performance, a slight decrease in the average proportion of large solids is seen for the pilots operated at temperatures below the intermediate range: 9 to 10 °C. The quadratic effect is significant (P-value < 0.01). Among the considered model structures, the one including this effect presents the lowest AIC. As shown in Figure 4(b), OLR has no significant influence on average proportions

![Figure 3](image1.png) | Non-settleable SS as a function of applied OLR.

![Figure 4](image2.png) | Average proportions over total particulate matter of large (100–1,000 μm) and small (1–100 μm) particles as a function of operating temperature (a) and applied OLR (b).
of large and small particles. Average proportions remain unchanged over the entire applied OLR range. It is, however, important to note that only few PSD measurements were made when high OLR (>2.5 g soluble CBOD₅/m² d) were applied since high OLR were observed mostly during the period when no PSD measurement was done.

Combination of settling tests results and measurements of PSD makes it possible to link the settling performance to solids physical properties. Figure 5 shows that a significant portion of the settling performance variation can be explained by the variations observed in the proportions of large and small particles classes derived from the PSD ($R^2 = 0.50$). This correlation is consistent with the laws of fluid mechanics which stipulate that an increase in particle size has a positive influence on the sedimentation rate (Newton’s law) and consequently, on the settling potential. Given the significant influence of operating temperature on proportions of particle size classes (Figure 4), the relation between these proportions and the solids settleability (Figure 5) can partially explain the observed correlation between the settling performance and operating temperature (Figure 2). Thus, it is consistent to state that the best settling performances associated with effluent samples taken from pilot reactors operated at temperatures around 10 °C are attributable to an increase in the proportion of large solids in the effluent.

Based on previous studies, hypotheses can be made to explain observed correlations. Hoang et al. (2014) have suggested that long-term exposure to cold temperatures (1 °C) is related to an increase of biofilm thickness caused by eased penetration of DO in biofilm when DO saturation is increased at these temperatures. Knowing that the detachment mechanism has an important influence on effluent particle sizes (Wuertz et al. 2003), one can suppose that temperature has an influence on the predominant detachment mechanism. It is, indeed, an interesting hypothesis since similar biofilm thicknesses have qualitatively been observed during the sampling periods in all the highly loaded pilot reactors. From another perspective, a modification of the extracellular polymeric substances matrix which is an important component of biofilm structure and a determining factor of the flocculation potential of flocs (Cetin & Surucu 1989; Liao et al. 2001) could be related to the variations in PSD and consequent settling performances. A deeper investigation of the influence of temperature on both biofilm and floc properties as well as detachment mechanisms is, however, required to verify these assumptions.

Even though the influence of OLR on settleability is not significant when considering the entire range studied, a tendency can be observed when comparing average results for pilots operated at low (<2.5 CBOD₅/m² d) and high OLR (>2.5 CBOD₅/m² d) (Figure 3). Lower non-settleable solids concentrations seem to be related with higher OLR. This tendency could not be explained by differences in proportions of particle size classes because almost no difference was seen (Figure 4) and because only few PSD measurements were done on samples collected when high OLR were applied. Nevertheless, based on previous studies (Wijeyekoon et al. 2004; Szilagyi et al. 2013), biofilm properties governing reactors’ effluent particle sizes and settleability are influenced by OLR. Indeed, biofilm properties...
attachment, as well as its growth rate, are positively influenced when increasing the applied OLR. Moreover, Wijeyekoon et al. (2004) reported that operation at high OLR is associated with the development of a compact biofilm. It is thus consistent to say that high OLR can potentially induce an increase in particle sizes and settleability. Many studies focusing on MBBR have, however, shown that an increase in OLR was related to poor settling performances (Ødegaard 2000; Melin et al. 2005; Aehl et al. 2006). An assumption made was that when OLR is increased, because of the need for further aeration for substrate degradation, higher shear forces are created and erosion detachment associated with small particles creation becomes predominant (Melin et al. 2005). Since aeration was constant for all the pilot reactors studied during the present study, a direct comparison with these results is not appropriate.

As pointed out before, a portion of settling performance variability which cannot be explained by differences in proportions of particle size classes is potentially attributable to variations in flocs morphology. Parameters theoretically determining solids settleability such as relative density and drag coefficient are directly linked to floc morphology (McGauhey 1956). Microscopic observations have thus been made to identify qualitatively morphological differences between flocs from the effluent of pilots operated at different conditions. Figure 6 presents flocs of similar sizes but coming out from pilots operated at different temperatures: intermediate and high. It is possible to see that the structures of these flocs of similar size differ non-negligibly with respect to compactness. The floc from the pilot operated at high temperature (Figure 6(a)) has a diffuse shape allowing light to pass through the sample while the floc from the pilot operated at intermediate temperature (Figure 6(b)) is more compact, thus limiting light transmission through the sample. Since theoretical settleability of solids depends on their physical properties, this morphological difference can potentially explain part of the observed differences in settling performances when operating conditions are changed.

**CONCLUSIONS**

Settling performance, as well as particles properties measurements, allowed the evaluation of the settleability of solids produced in immersed fixed media biofilm reactors. Operating temperature appeared to have a significant effect on settling potential of particles produced within the studied pilot reactors. Pilots operated at intermediate temperatures (around 10°C) produced the best solids in terms of settling potential. The latter decreased for pilots operated under and over that temperature. Proportions of key particle size classes were found to be correlated with settling performance. The lowest non-settleable SS concentrations, seen at intermediate operating temperatures, were related to the highest fractions of solids with size greater than 100 μm.

Unlike operating temperature, applied surface OLR had no significant influence on settleability and PSD. Nonetheless, trends suggesting that high-load operation (>2.5 g of soluble CBOD5/m² d) may be associated with improved settling performance could be drawn. Further experiments at constant high-load are needed to conclude on these trends.

The obtained results demonstrated the relevance of considering the influence of operating temperature in the design of solid–liquid separation processes following immersed fixed media biofilm reactors. Optimization of solid–liquid separation within processes subjected to large spatial or seasonal variations of temperature should therefore be done considering the influence of operating temperature on the settleability of biologically produced solids.

![Figure 6](image-url)
ACKNOWLEDGEMENTS

This research project was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC), the Fonds de recherche du Québec – Nature et technologies (FRQNT) and Bionest Technologies Inc. The authors would also like to thank the research and development team from Bionest Technologies for the technical and logistics support provided.

REFERENCES


First received 9 June 2017; accepted in revised form 30 October 2017. Available online 10 November 2017