Organic matter capture by a high-rate inoculum-chemostat and MBBR system
Hadi Abbasi, Charles Élysée, Marc-André Labelle, Edith Laflamme, Alain Gadbois, Antoine Laporte, Peter L. Dold and Yves Comeau

ABSTRACT
The main objective of this study was to develop an innovative process to maximize the biotransformation of colloidal and soluble biodegradable matter (CSB) into particulate matter (XB) for energy recovery via methane production. Two configurations were studied: (1) high-rate moving bed bioreactor (HR-MBBR) and (2) inoculum-chemostat (IC) system consisting of a very HR-MBBR inoculating a continuous flow stirred-tank reactor. The effect of hydraulic retention time (HRT), specific organic loading rate (SOLR), and dissolved oxygen (DO) level were determined using real wastewater at pilot scale. Results showed that in the HR-MBBR process, a very high CSB biotransformation efficiency (90%) was obtained in a wide range of SOLRs (2.0 to 5.5 g CSB m⁻² d⁻¹) corresponding to an optimum HRT of 36 minutes. The IC process reached a maximum CSB biotransformation efficiency of 77%, at SOLRs ranging from 22 to 30 g CSB m⁻² d⁻¹ at an HRT of 3.7 hours. The DO concentration in the HR-MBBR influenced the CSB bio-transformation ratio, while the HRT and the SOLR were the dominant factors influencing this ratio in the IC process. Based on these results, the IC process could be an interesting alternative to high-rate systems towards obtaining energy positive/efficient from water resource recovery facilities.

Key words | chemostat, COD oxidation, high-rate MBBR, organic matter capture

INTRODUCTION
Environmental protection requirements and energy demand are major factors driving the energy efficiency of water resource recovery facilities (WRRFs). Conventional processes, like activated sludge (AS), are widely used for wastewater treatment, but they require a significant amount of energy (Jimenez et al. 2015). Therefore, process optimization and innovative treatment strategies are required to improve the energy balance and obtain cost-effective WRRFs (Metcalf & Eddy-Aecom 2014; Meerburg et al. 2013).

A central approach to obtain energy-positive WRRFs is to maximize the capture of organic matter for energy production via methanogenesis. The biodegradable organic matter consists of readily (RBCOD) and slowly biodegradable...
(SBCOD) fractions (Henze et al. 2000; Melcer 2003). Readily biodegradable matter is composed of soluble ($S_B$) and colloidal ($C_B$) matter that can be oxidized or stored directly by heterotrophic bacteria under aerobic conditions and used for the growth of new heterotrophic biomass $X_{OHO}$ via bio-transformation processes. Slowly biodegradable matter (mostly particulate matter, $X_B$ and ordinary heterotrophic organisms, $X_{OHO}$) requires conversion into a readily biodegradable form by hydrolysis prior to absorption and utilization. Thus, optimizing aeration, minimizing hydrolysis, minimizing oxidation of particulate matter, and capturing biodegradable organic matter to be sent to anaerobic digestion can improve the energy efficiency of WRRFs (Ødegaard 2000; Jimenez et al. 2015).

High-rate biological treatment profits from the high bacterial activity under high food-to-microorganism ratios and low solid retention times (SRTs) with relatively short hydraulic retention times (HRTs) resulting in the maximization of bio-transformation and capture of organic matter from wastewater (Grady et al. 2011; Jimenez et al. 2015). The high-rate moving bed bioreactor (HR-MBBR) is a promising process which is successfully used for organic matter recovery at low HRTs (30–90 min) while still maintaining a high COD removal efficiency (80–85%) (Ødegaard 2000). Biomass is grown in such HR processes, transforms $C_B$ and $S_B$ (CSB) into $X_{OHO}$, minimizing the oxidation of $X_B$ while increasing the production of $X_{OHO}$, thus maximizing the energy generation potential (Jimenez et al. 2015; Brosseau 2015).

The main objective of this study was to develop an innovative process combining an HR-MBBR and an AS process to maximize the bio-transformation of CSB into XB for energy recovery via methane production. For this purpose, two pilot-scale treatment configurations, including a high-rate MBBR (HR-MBBR) in parallel with a very HR-MBBR acting as an inoculum and an AS chemostat (IC) system, were tested to address the following specific objectives:

1. determine the operational parameters (HRT, specific organic loading rate (SOLR), dissolved oxygen (DO)) to maximize the performance of each treatment process;
2. maximize the bio-transformation of CSB into XB to allow the capture of $X_B$ to maximize methane production.

### MATERIALS AND METHODS

#### Pilot plant setup and configurations

The pilot plant, comprising (a) an HR-MBBR (1.6 m$^3$) and (b) a very HR-MBBR inoculum (0.4 m$^3$) followed by a chemostat (4.7 m$^3$) (Figure 1), was installed at the Repentigny municipal WRRF, Quebec. The raw wastewater influent containing about 10% industrial loading was subjected to 6 mm screening, fat and grease removal and grit removal prior to being fed to the pilot plant trains. The wastewater characteristics and operating conditions (OC) of the HR-MBBR and IC are presented in Table 1.

Additional screening was provided by another 6 mm punched hole strainer which was connected at the inlet of both systems to remove trash and which was cleaned manually every 2 days. The HR-MBBR and inoculum were filled with the carrier type K5 from AnoxKaldnes™ with a specific surface area of 800 m$^2$/$m^3$. All reactors were completely mixed and were equipped with fine and coarse bubble aeration systems, and a mechanical mixer. Probes (Hach) connected to the automation system (PLC), were used for real-time monitoring of DO, pH, and temperature.

### Aeration

The aeration system in the HR-MBBR process provided $16.3 \pm 1.2$ m$^3$/h provided through coarse (1/3) and fine (2/3) bubbles to ensure proper aeration and media mixing. The aeration system in the IC system provided $1.5 \pm 0.2$ and $2.5 \pm 1.3$ m$^3$/h in the inoculum and chemostat processes, respectively, via fine bubble diffusers.

### Sampling and analytical methods

The influent to each process was sampled two to five times per week. Multiple grab samples (taking into account the HRT) from the influent were mixed together to obtain a homogeneous composite sub-sample. Total and soluble COD, total and volatile suspended solids were analyzed at each sampling point according to Standard Methods 5220D (APHA 2012). Filtered COD was determined using both 1.2 μm glass microfiber filters (Whatman® 934-AH™, GE Healthcare Life Sciences).
Sciences, GBR) and 0.45 μm cellulose membranes (MF-Millipore™, EMD Millipore). Flocculated-filtered COD (ffCOD) was measured using the method developed by Mamais & Jenkins (1996). The COD fractions characterized were thus particulate COD (XCOD > 1.2 μm), colloidal and soluble COD (CSCOD < 1.2 μm), and soluble COD (ffCOD = SCOD < 0.45 μm). Colloidal COD (CCOD) fraction was calculated from the difference between CSCOD and SCOD. The colloidal and soluble unbiodegradable fraction (SU) was considered to be the typical 5% of the total COD (EnviroSim Associates Ltd 2016). The following formula was used to calculate the CU, CB, and SB, according to S, C, CS, and SU (given above) values:

\[
C_U = S_U \times (C/S - 1) \tag{1}
\]

\[
C_B = C_{COD} - C_U \tag{2}
\]

\[
S_B = S_{COD} - S_U \tag{3}
\]

The DO was measured with a portable DO-meter (HQ40d, Hach Company) and an LDO® probe (Hach Company).

The biofilm mass was measured every week by collecting carriers (20 carriers per sampling event) dried at 105°C overnight and weighed. The carriers were then soaked in 6% NaOH for 30 min to recover the biofilm from the carrier surface, after which the carriers were scraped clean and dried again at 105°C overnight. The difference between the dry weight of the carriers before and after cleaning represented the mass of biofilm on the carriers. The amount of biofilm per square meter of protected surface area of carriers (g TSS/m²) was determined by dividing the obtained total solids (TS) of the detached biofilm over the protected surface area of the number of carriers sampled (Andreottola et al. 2000, 2003). Considering a protected surface area of 23 cm²/carrier allowed determination of the specific biofilm concentration in g/m².
**RESULTS AND DISCUSSION**

The effect of HRT, SOLR, media fill volume fraction, and oxygen uptake rate (OUR) was considered in the following sections for maximizing the production of biodegradable sludge, based on the maximization of the removal efficiency of CSB (bio-transformation of CSB into XCOD) as well as the minimization of biodegradable particulate matter (XB) hydrolysis.

A summary of the pilot-scale HR-MBBR and IC effluent characteristics of the five OC is presented in Table 2.

No significant nitrification occurred, as expected under such high-rate conditions, as shown by the very low concentration of nitrate (0.1 mg N/L) in the effluent of the HR-MBBR and IC processes.

### Effect of HRT, SOLR, and DO on bio-transformation of CSB and hydrolysis XCOD

The effect of HRT on the bio-transformation of the CSB and SB, and the hydrolysis of XCOD in the HR-MBBR at OC OC1, OC2, and OC5 based on the HRT are shown in Figure 2(a) and Table 2. CSB and SB bio-transformation increased from 75 ± 6% to 83 ± 6% by increasing HRT from 25 min to 54 min. The bio-transformation of CSB and SB into XB showed no significant difference at HRTs longer than 36 min and reached a plateau at 85 ± 6% (below an SOLR 36 ± 6 kg COD m⁻² d⁻¹).

The same tendency also has been observed between HRT and CSB bio-transformation by Brosseau et al. (2016) and Aygun et al. (2008); however, the bio-transformation efficiencies were systematically different due to influent COD concentration, available surface area for biofilm growth, and HRT in their experiments.

A lower value of XCOD and COD removal efficiency was observed at higher HRT (56 min and 54 min), probably due...
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Units</th>
<th>HR-MBBR OC1</th>
<th>HR-MBBR OC2</th>
<th>HR-MBBR OC3</th>
<th>HR-MBBR OC4</th>
<th>HR-MBBR OC5</th>
<th>IC OC1</th>
<th>IC OC2</th>
<th>IC OC3</th>
<th>IC OC4</th>
<th>IC OC5</th>
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<tr>
<td>COD loading</td>
<td>–</td>
<td>kg COD/d</td>
<td>17 ± 2</td>
<td>29 ± 5</td>
<td>39 ± 6</td>
<td>14 ± 4</td>
<td>38 ± 9</td>
<td>21 ± 2</td>
<td>15 ± 1</td>
<td>11 ± 1</td>
<td>18 ± 2</td>
<td>18 ± 7</td>
</tr>
<tr>
<td>HRT</td>
<td>–</td>
<td>min</td>
<td>54</td>
<td>36</td>
<td>25</td>
<td>54</td>
<td>25</td>
<td>154</td>
<td>225</td>
<td>304</td>
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<td>154</td>
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<tr>
<td>Solids retention time</td>
<td>SRT</td>
<td>d</td>
<td>1.4</td>
<td>1.4</td>
<td>1.6</td>
<td>–</td>
<td>1.6</td>
<td>0.6</td>
<td>0.8</td>
<td>0.7</td>
<td>–</td>
<td>0.6</td>
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<td>Biofilm</td>
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<td></td>
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<tr>
<td>Total suspended solids</td>
<td>TSS</td>
<td>mg/L</td>
<td>–</td>
<td>641 ± 5</td>
<td>868 ± 122</td>
<td>500 ± 64</td>
<td>1,168 ± 260</td>
<td>351 ± 68</td>
<td>357 ± 62</td>
<td>340 ± 60</td>
<td>119 ± 13</td>
<td>–</td>
</tr>
<tr>
<td>VSS/TSS ratio</td>
<td>fVT</td>
<td>g VSS/g TSS</td>
<td>–</td>
<td>0.63 ± 0.02</td>
<td>0.77 ± 0.02</td>
<td>0.64 ± 0.03</td>
<td>0.63 ± 0.02</td>
<td>0.74 ± 0.03</td>
<td>0.70 ± 0.00</td>
<td>0.70 ± 0.02</td>
<td>0.69 ± 0.02</td>
<td>–</td>
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<td></td>
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</tr>
<tr>
<td>Total COD</td>
<td>COD</td>
<td>mg COD/L</td>
<td>313 ± 39</td>
<td>357 ± 58</td>
<td>335 ± 71</td>
<td>338 ± 92</td>
<td>307 ± 89</td>
<td>414 ± 55</td>
<td>371 ± 12</td>
<td>460 ± 95</td>
<td>389 ± 31</td>
<td>341 ± 56</td>
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<tr>
<td>Colloidal COD</td>
<td>C_{COD}</td>
<td>mg COD/L</td>
<td>12 ± 3</td>
<td>8 ± 4</td>
<td>17 ± 5</td>
<td>22 ± 6</td>
<td>6 ± 3</td>
<td>14 ± 8</td>
<td>8 ± 4</td>
<td>19 ± 11</td>
<td>10 ± 6</td>
<td>21 ± 7</td>
</tr>
<tr>
<td>Soluble COD</td>
<td>S_{COD}</td>
<td>mg COD/L</td>
<td>24 ± 4</td>
<td>29 ± 8</td>
<td>38 ± 8</td>
<td>33 ± 5</td>
<td>29 ± 9</td>
<td>40 ± 10</td>
<td>40 ± 10</td>
<td>38 ± 3</td>
<td>41 ± 5</td>
<td>40 ± 4</td>
</tr>
<tr>
<td>TSS</td>
<td>X_{TSS}</td>
<td>mg TSS/L</td>
<td>293 ± 67</td>
<td>327 ± 67</td>
<td>245 ± 80</td>
<td>316 ± 114</td>
<td>320 ± 141</td>
<td>364 ± 51</td>
<td>503 ± 63</td>
<td>256 ± 66</td>
<td>302 ± 54</td>
<td>319 ± 54</td>
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<tr>
<td>VSS</td>
<td>X_{VSS}</td>
<td>mg VSS/L</td>
<td>166 ± 25</td>
<td>206 ± 33</td>
<td>182 ± 47</td>
<td>184 ± 48</td>
<td>170 ± 52</td>
<td>218 ± 31</td>
<td>171 ± 25</td>
<td>185 ± 29</td>
<td>185 ± 27</td>
<td>170 ± 31</td>
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<tr>
<td>VSS/TSS ratio</td>
<td>fVT</td>
<td>g VSS/g TSS</td>
<td>0.57 ± 0.07</td>
<td>0.63 ± 0.04</td>
<td>0.76 ± 0.07</td>
<td>0.60 ± 0.09</td>
<td>0.57 ± 0.13</td>
<td>0.60 ± 0.06</td>
<td>0.57 ± 0.07</td>
<td>0.74 ± 0.10</td>
<td>0.62 ± 0.04</td>
<td>0.59 ± 0.10</td>
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<tr>
<td>X_{COD}/VSS ratio</td>
<td>fCV</td>
<td>g X_{COD}/g VSS</td>
<td>1.7 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>1.8 ± 0.1</td>
<td>1.7 ± 0.2</td>
<td>1.7 ± 0.2</td>
<td>1.9 ± 0.4</td>
<td>1.8 ± 0.1</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>S_{Alk}</td>
<td>mg CaCO_3/L</td>
<td>–</td>
<td>–</td>
<td>156 ± 10</td>
<td>–</td>
<td>162 ± 11</td>
<td>184 ± 15</td>
<td>–</td>
<td>193 ± 18</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>pH</td>
<td>–</td>
<td></td>
<td>7.3 ± 0.2</td>
<td>7.5 ± 0.2</td>
<td>7.4 ± 0.2</td>
<td>6.7 ± 0.2</td>
<td>7.3 ± 0.2</td>
<td>7.9 ± 1.0</td>
<td>7.5 ± 0.1</td>
<td>7.7 ± 0.4</td>
<td>8.1 ± 0.4</td>
<td>6.7 ± 0.1</td>
</tr>
</tbody>
</table>

### Process performance

- **CSB biotransform efficiency**
  - R_{CSB}: %
    - HR-MBBR: 85 ± 6, 86 ± 9
    - IC: 78 ± 13, 78 ± 13
- **CSB specific removal rate**
  - S_{RCSB}: g CS_{COD} m^{-2} d^{-1}
    - HR-MBBR: 3 ± 1, 6 ± 1, 10 ± 2, 3 ± 1, 7 ± 2
    - IC: 39 ± 10, 35 ± 4, 28 ± 4, 19 ± 7
- **X_{COD,eff.} / (CSB + X_{OHO})_{net}**
  - HR-MBBR: 0.83 ± 0.15, 0.86 ± 0.06
  - IC: 0.88 ± 0.12, 1.01 ± 0.18, 0.91 ± 0.12, 0.95 ± 0.33
to the partial release of particulate matter from biofilms caused by abrasion at a long HRT (Hoang 2013). Minimal hydrolysis of particulate organic matter can be achieved at a low HRT, as can be achieved in a HR-MBBR process (Schubert et al. 2013).

Similarly, the effect of HRT on removal and bio-transformation of COD, $X_{\text{COD}}$, $C_B$, and $S_B$ were evaluated through the IC process at OC OC1, OC2, and OC3 in Figure 2(b) and Table 2.

A positive correlation was observed between $S_B$ bio-transformation and HRT in the IC system due to the prolonged contribution of inoculum by transferring and establishing active biomass in the chemostat at higher HRT. The $C_S_B$ bio-transformation efficiencies were 56% ± 5%, 62% ± 5%, and 74 ± 6% at HRTs of 154 min, 225 min, and 304 min, respectively, across the IC process. The concentration of COD and $X_{\text{COD}}$ did not effectively change in the IC process at HRTs 154 and 304 min based on removal efficiency compared to HR-MBBR, due to the minimum effect of hydrolysis on particulate matter.

This phenomenon supported the results of Confer & Logan (1998), which found that hydrolysis rate is much more on the biofilm surface than at the surface of sloughed biofilm.

The overall efficiency of biotransformation of influent biodegradable organic matter into particulate matter across each process was also characterized by the ratio of effluent particulate COD to influent total biodegradable COD. Results are presented in Table 2 as $X_{\text{COD}}_{\text{eff}}/(C_B + X_{\text{OHO}})_\text{inf}$. This fraction was lower across the IC process ($0.96 \pm 0.22 \text{ g } X_{\text{COD}}/\text{g } BCOD$) than the HR-MBBR process ($0.86 \pm 0.11 \text{ g } X_{\text{COD}}/\text{g } BCOD$), suggesting that less hydrolysis of particulate organic matter took place in the first one.

The effect of the SOLR on the removal of $C_S_B$ was also assessed in HR-MBBR and IC processes (Figure 3). The higher specific removal rates were attained as the SOLR was increased in both HR-MBBR and IC processes, whereas HRT and SOLR were identified as an important constraint on the bio-transformation (especially for IC process).

A maximum $C_S_B$ bio-transformation rate (90 ± 3%) in the HR-MBBR process was achieved at SOLR from 2.0 to 5.5 $\text{ g } C_S_B \text{ m}^{-2} \text{ d}^{-1}$, corresponding to an optimum HRT of 36 min. These values for the IC process reached in maximum the specific removal of 80 ± 3% corresponding to SOLR of 22 to 40 $\text{ g } C_S_B \text{ COD m}^{-2} \text{ d}^{-1}$ at an optimum HRT of 225 min.

The observed linear pattern between SOLR and $C_S_B$ removal efficiency was observed with the studies of Ødegaard (2000), Brosseau (2015), and Helness et al. (2005) in lab- and pilot-scale experiments with HR-MBBRs. Aygun et al. (2008) also demonstrated that by increasing the SOLR from 6 to 96 $\text{ g } \text{ COD m}^2 \text{ d}^{-1}$, the organic removal efficiency decreased from 95% to 45%.

In this context, Orantes & Gonzalez-Martinez (2005) established an asymptotic relationship between the mass of attached biofilm and SOLR, in which no further biomass is attached at high SOLR. Hence, at high SOLR, less biofilm
can be established through the inoculum process and limited by short HRT, less contribution of inoculum could reasonably be expected to transfer active biomass into the chemostat.

Variation of DO during aerated and non-aerated periods in the HR-MBBR and chemostat was monitored, based on the OC of OC2 and OC1, respectively. The oxygen concentration dropped more rapidly in the HR-MBBR than in the chemostat when the aeration system was switched off for 3 minutes. Calculation of the OUR in two reactors indicated over five-fold higher OUR in the MBBR (50 ± 2 mg O₂ L⁻¹ h⁻¹) than in the chemostat (10 ± 1.5 mg O₂ L⁻¹ h⁻¹). In this context, the SOUR value across HR-MBBR and chemostat process was 55 ± 1 mg O₂ g⁻¹ VSS h⁻¹ and 53 ± 6 mg O₂ g⁻¹ VSS h⁻¹, respectively.

The highest OUR in the HR-MBBR can be correlated to the oxidation of more readily biodegradable matter produced by hydrolysis of biofilm surface (Confer & Logan 1998) and the slowly biodegradable matter that results from lysis of decayed biomass in the HR-MBBR system, whereas the source of active biomass in the chemostat process was provided from inoculum continually with lower SRT (SRT_{IC:OC1}: 0.6 d and SRT_{HR-MBBR:OC2}: 1.4 d) and no further accumulation.

The removal efficiency of filterable biodegradable organic matter (CS₈) by the IC and HR-MBBR processes was about 75% and 85%, respectively, which corresponded to HRT of 141 min and 36 min.

Further tests were conducted to assess the effect of oxygen concentration on the bio-transformation rate in the HR-MBBR process. For this purpose, the DO concentration was changed from 1–2 mg O₂/L to 2–4 mg O₂/L during OC4 and OC1, respectively, for a duration of 1 week each.

The role of the DO concentration as an effective and sensitive parameter controlling the removal of S₈ and C₈ fractions, but not that of particulate COD, is illustrated in Figure 4 and Table 2. The maximum S₈ and C₈ removal efficiency, 86 ± 7% and 77 ± 17%, respectively, was obtained during OC1. The S₈ and C₈ removal efficiency was significantly decreased to 67 ± 20% and 53 ± 12%, respectively, as DO concentration was less than 2 mg O₂/L, indicating that the DO concentration (below 2 mg O₂/L) was a limiting factor in the HR-MBBR system. For an optimal COD removal, DO should be maintained higher than 2 mg O₂/L, as indicated by a 13% decline in COD removal when the
DO level was decreased from 2 to 1 mg/L while only a 6% increase in COD removal was observed with an increase in DO level from 2 to 6 mg/L (Wang et al. 2005).

**Effect of media fill volume fraction on bio-transformation of CS$_B$**

The effect of the two media fill volume fraction (OC3: 35% v/v, OC5: 50% v/v) on HR-MBBR treatment efficiency was assessed at HRT of 36 min. As the media fill volume fraction increased from 35% to 50% in the HR-MBBR, both S$_B$ and C$_B$ bio-transformation efficiency was increased from 76 ± 4 and 84 ± 6 to 89 ± 10, 90 ± 8, respectively (Figure 5(a) and Table 2). Similarly, Azizi et al. (2015) reported an effective treatment can be obtained by increasing media fill volume fraction up to 40% v/v, due to higher available surface area for biofilm growth. The removal of particulate and total COD decreased slightly by increasing the media fill volume fraction. Collision and attrition in the HR-MBBR reactor could lead to biofilm detachment from the outer surface and increase the total and particulate COD in the effluent due to the high volume of media and shear forces (Ødegaard 2000).

The effect of media fill volume fraction in the inoculum based on OC1, OC4, and OC5, aimed at transferring active biomass to the chemostat, demonstrated the opposite effect on bio-transformation of S$_B$ and C$_B$ in the IC process. Increasing the media fill volume fraction from 15% v/v to 45% v/v in the inoculum decreased the bio-transformation of S$_B$ and C$_B$ from 58 ± 4% to 50 ± 6% and 69 ± 14% to 45 ± 10%, respectively, in the IC process (Figure 5(b)). The media fill volume fraction ranging from 15% to 22% in the inoculum did not significantly affect the removal of particulate and total COD, while increasing the media fill volume fraction up to 45% significantly influenced the removal of total and particulate COD.

Higher media fill volume fraction (up to 45% v/v) may increase the development of active biomass in the inoculum (less biomass to be sloughed off the media) and may lead to less transferring of active biomass from inoculum to chemostat due to HRT constraints, therefore, less removal of S$_B$ and C$_B$ occurred in the chemostat.

**Effect of OC on attached biofilm concentration**

The attached biofilm growth concentration in the HR-MBBR was directly correlated to the SOLR (Figure 6).

Biofilm growth concentration in the HR-MBBR reactor reached a plateau of 18.0 ± 1.6 g TSS/m$^2$ at SOLR more than 8.0 ± 2.7 g CS$_B$ m$^{-2}$ d$^{-1}$. In this context, a two-parameter exponential equation ($R^2$: 0.95) showed the best fit to the biofilm concentration data. The concentration of attached biofilm during OC1 to OC5 was increased from 4.5 ± 2.6 g TSS/m$^2$ to 18.5 ± 1.2 g TSS/m$^2$ by increasing the SOLR from 3.1 ± 0.9 g CS$_B$ m$^{-2}$ d$^{-1}$ to 15.6 ± 2.8 g CS$_B$ m$^{-2}$ d$^{-1}$, respectively.

The attached biofilm concentration in inoculum after an increase from 6.0 ± 0.5 to 13.1 ± 0.8 g TSS/m$^2$ reached a
plateau with an average concentration of 11.7 ± 1.1 g TSS/m², while the SOLR ranged over 39.7 ± 13.8 g CSB m⁻² d⁻¹. Moreover, under high SOLR in the inoculum, sloughing phenomenon was observed frequently and elevated effluent (chemostat influent) suspended solids concentrations. Downing et al. (2013) and Bassin et al. (2016) also indicated that high SOLRs potentially enhanced biofilm detachment rates. In this context, Aygun et al. (2018) also reported a plateau occurred in biomass production level after SOLR reached 50 g COD m⁻² d⁻¹.

The trend of VSS/TSS ratio in attached biofilm across the HR-MBBR and inoculum range was 0.67 ± 0.06 and 0.71 ± 0.02 mg VSS/mg TSS, respectively (Table 2). The VSS/TSS ratio obtained by De Oliveira et al. (2014) based on pilot scale average values was 0.69 mg VSS/mg TSS; however, this value reported by Jahren et al. (2002) operated at lab scale, equal to 0.91, was much higher. This may be due to the fibrous materials with low VSS/TSS ratio (almost 0.55 mg VSS/mg TSS) carried by raw wastewater and despite biomass adhering to the carriers.

HR-MBBR and IC effluent

The $f_{VT}$ (VSS/TSS) and $f_{CV}$ (XCOD/VSS) ratios in the effluent of HR-MBBR and IC processes are shown in Figure 7. The $f_{VT}$ value in the HR-MBBR increased from 0.5 to 0.8 g VSS/g TSS with an increasing SOLR from 2 to 16 g CSB m⁻² d⁻¹. In the IC process, the $f_{VT}$ value only increased from 0.60 to 0.7 g VSS/g TSS as the SOLR increased from 20 to 90 g CSB m⁻² d⁻¹ despite some fluctuations that may have resulted from detached biofilm. The values of $f_{CV}$ in the effluent was 1.7 ± 0.2 g XCOD/g for all OC.

The $f_{VT}$ value in the effluent reported by Broseau et al. (2016) was 0.81 to 91 g VSS/g TSS, while $f_{CV}$ varied between 1.24 and 1.6 g XCOD/g for all OC based on HRT and SOLR; the same ratio was also reported by Karizmeh (2012).

The effect of the HRT on the effluent COD fractions was evaluated for the HR-MBBR and IC processes (Figure 8). The particulate matter fraction increased after the HR-MBBR or the IC processes, with the largest proportion observed with an HRT of 36 min in the HR-MBBR and of 225 min in the IC process. The fractionation of COD showed that particulate and soluble COD were predominant in the influent and effluent of HR-MBBR and IC processes, whereas the COD contained a small portion of colloidal matter.

Particle agglomeration occurred with increasing HRT up to 36 and 225 min across the HR-MBBR and IC processes.
processes; however, with increasing HRT from 36 min to 54 min in HR-MBBR, a movement from particulate toward smaller particle (colloidal) matter was observed. The same characteristics in the MBBR effluent were observed by Brosseau et al. (2014) between 37 and 40 min HRT.

Particle agglomeration also resulted from increasing the HRT from 0.75 to 4 hours (Melin et al. 2015; Åhl et al. 2015; Ødegaard et al. 2015; Karizmeh 2015), but Karizmeh et al. (2015) later demonstrated that by independently decreasing HRT and SOLR, a shift toward smaller particle size was observed. Moreover, during degradation of particulate matter and formation of smaller particles, more surface area of substrate is available for hydrolysis (Dimock & Morgenroth 2015).

The average value of SVI on different operating condition was measured to evaluate the sludge settleability of each process.

Slightly better settling sludge was obtained in the IC process (SVI of 70 ± 11 mL/g) than in the HR-MBBR (94 ± 10 mL/g). Better floculating solids may have resulted from the IC process on which configuration most favors the proper maintenance of SVI in a higher SOLR even at lower SRT (SRT\text{IC}: 0.6 ± 0.1 d and SRT\text{HR-MBBR}: 1.5 ± 0.1 d). These results are supported by Liu et al. (2004) who demonstrated that low organic loading rate resulted in irregular shape with poor settling characteristics and high SVI value. According to the theory, low substrate concentrations favor the growth of filamentous over floc-forming bacteria (Chudoba et al. 1975; Chudoba 1985).

Aeration requirements

The maximum efficiency of HR-MBBR and IC processes bio-transformation were 90 ± 3% and 77 ± 3%, respectively, which corresponded to HRTs of 36 min and 3.7 hours, SRTs of 1.5 ± 0.1 d and 0.6 ± 0.1 d, and SOLR of 2.0 to 5.5 g CSB m\textsuperscript{-2} d\textsuperscript{-1} and 22 to 30 g COD m\textsuperscript{-2} d\textsuperscript{-1}.

The OUR in the HR-MBBR (50 mg O\textsubscript{2} L\textsuperscript{-1} h\textsuperscript{-1}) was determined to be two and a half times greater than in the IC process 20 mg O\textsubscript{2} L\textsuperscript{-1} h\textsuperscript{-1} (accounting for the inoculum process OUR of 10 mg O\textsubscript{2} L\textsuperscript{-1} h\textsuperscript{-1}). The total oxygen demand was calculated to be 0.54 ± 0.03 and 0.81 ± 0.07 kg O\textsubscript{2}/kg CS\textsubscript{B} added for the HR-MBBR and IC processes, respectively. The blower provided 16 ± 1 m\textsuperscript{3}/h and 3.5 ± 0.2 m\textsuperscript{3}/h of air in the HR-MBBR and IC reactors to maintain DO level 3–4 mg O\textsubscript{2}/L and 6–7 mg O\textsubscript{2}/L, respectively. It should be noted that oxygen transfer rates and efficiency (OTE) at full scale may differ due to the shallow depth of the pilot reactors.

In a high-rate AS process, Jimenez et al. (2015) reported that the maximum removal efficiency was obtained at an HRT ranging between 30 and 45 min, at an SRT of 0.6 ± 0.1 d. They observed that the optimal removal of S\textsubscript{B} (80%) required an oxygen concentration of 0.38 ± 0.12 kg O\textsubscript{2}/kg COD (based on the use of net oxygen consumption of biotransformation).

Poor oxygen transfer efficiencies (rapidly rising bubbles) related to the coarse diffuser in the HR-MBBR can also lead to less DO and excessive power requirement compared with the fine diffuser in the chemostat.

The DO concentration dropped below 2 mg/L in the HR-MBBR process within 5 min of the non-aeration period, while this value took almost 30 min in the
CONCLUSION

The objective of this study was to determine the potential of an innovative high-rate IC process compared with a typical HR-MBBR process for colloidal and soluble organic matter transformation into particulate matter for anaerobic digester methane production. The effect of SOLR, OUR, and HRT on the removal and bio-transformation of $C_B$ and $S_B$ fractions was studied using real wastewater in a pilot scale system operated under five OC. The SOLR in the HR-MBBR and IC processes were varied between 2 to 16 g m$^{-2}$ d$^{-1}$ and 20 to 90 g m$^{-2}$ d$^{-1}$ respectively, using different HRTs.

The following conclusions were drawn:

- $C_{SB}$ bio-transformation into $X_B$ in the HR-MBBR process increased with HRT (and SOLR) up to 36 min with a $C_{SB}$ capture efficiency as high as 90 ± 5%, while in the IC process, an HRT of 3.7 hours was required for a $C_{SB}$ capture of up to 77 ± 3% at SOLRs between 22 and 30 g $C_{SB}$ m$^{-2}$ d$^{-1}$.

- The SOUR value across the HR-MBBR and chemostat, to maintain a DO level above 2 mg O$_2$/L, was similar in both systems (55 ± 1 and 53 ± 6 mg O$_2$ g$^{-1}$ VSS h$^{-1}$, respectively).

- A slightly better settleability of produced particulate matter, based on SVI values, was obtained in the IC process (70 ± 11 mL/g) than in the HR-MBBR (94 ± 10 mL/g), possibly due to the fact that better flocculating solids may have resulted from higher SOLR and lower SRT values in the IC than the HR-MBBR process.

The innovative IC process can be a competitive alternative process to maximize the bio-transformation of $C_S$ to minimize $X_B$ and $X_{OHO}$ oxidation to improve the energy balance at WRRFs.

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