Role of biomass adaptation in the removal of formic acid in sequencing batch reactors

D. Dionisi, M. Majone, A. Bellani, C. Cruz Viggi and M. Beccari

ABSTRACT

This study deals with formic acid removal in activated sludge processes, in particular in the processes carried out in sequencing batch reactors (SBRs). Formic acid removal has been investigated in a SBR fed with acetic and formic acids at equimolar concentrations. Biomass performance in the reactor has been investigated both by the analysis of the removal of the two substrates and by batch tests. Regarding SBR process, the obtained results show that a relevant difference occurred between formic and acetic acid profiles. Acetic acid was never found in the effluent and was always completely removed during the reaction phase. On the other hand, formic acid removal was determined by biomass acclimation, which is in turn determined by sludge age imposed to the system. Batch tests confirmed that formic acid removal occurs only if biomass is acclimated. It has been shown that the minimal sludge age to obtain complete formic acid removal is much higher than those predictable with the classical models of microbial growth in wastewater treatment processes. The advantages of SBRs over continuous-flow systems in the removal of formic acid have also been highlighted.

Key words | activated sludge, formic acid, sequencing batch reactors

INTRODUCTION

The activated sludge process is one of the most widespread treatments for wastewaters of both industrial and municipal origin (Beccari et al. 2002). Only few studies about the removal of formic acid by mixed cultures have been published, whereas formic acid is present in many industrial wastewaters (e.g. from the process for 1,2-dichloroethane production, or for dimethyterephthalate production, or from the Gas-To-Liquids process). Biological degradation of formic acid is not as easy as that of acetate or propionate, due to the need of producing metabolic intermediates from a substance with only one carbon atom (van Dien & Lidstrom 2002).

Therefore, a better knowledge of the removal’s mechanism of formic acid is therefore required, as it may allow a better design and operation of activated sludge plants, with positive consequences in the whole industrial production processes.

In this study, the operation of a sequencing batch reactor (SBR) fed with acetic and formic acids at equimolar concentrations is reported.

The study was carried out also through batch tests with acclimated biomass, performed with acetic acid as well, and with mixtures of the two substrates. Acetic acid removal was studied for comparison purposes and because it is often simultaneously present with formic acid in industrial wastewater. The removal mechanisms of the two substrates was studied by measuring the removed substrate and the associated oxygen uptake rate (OUR). The removal of formic acid in the system was investigated with particular focus on the role of biomass adaptation.
METHODS

SBR operation

The sequencing batch reactor was operated under fully aerobic conditions with the following phase sequence (4 cycles/day): feed (10 min); reaction (220–310 min); settling phase (30–120 min); effluent withdrawal (10 min). No sludge withdrawal was carried out. Solid Retention Time (SRT) was therefore only dependent on solid losses from the effluent. Initially, the length of the settling phase was 30 min and the length of the reaction phase 310 min; then, due to the poor settleability of the biomass the length of the settling phase was increased to 120 min, with a consequent decrease of the reaction phase (220 min). The volume of the filled reactor was 1.2 L and the volume fed per cycle was 250 mL. The feed of the reactor was formic acid and acetic acid at equimolar concentrations (404 mg/L for formic acid and 527 mg/L for acetic acid), with an organic load of 0.6 gCOD/L/d. The temperature of the reactor was 25°C and the pH was 7.5.

SBR was regularly sampled usually 3 times per week for analytical determinations of formic and acetic acids and for biomass concentration, as volatile suspended solids (VSS), at the end of the reaction phase in the effluent and in the mixed liquor, respectively. Solid Retention Time (SRT) was calculated as week average (usually corresponding to 3 following samples).

Batch tests

The activated sludge was withdrawn from the SBR at the end of the cycle, put in an aerated batch reactor (200 mL), and diluted to the chosen concentration (approximately 700 mg VSS L⁻¹) with a mineral medium with the following composition (mg/L): (NH₄)₂SO₄ (40), K₂HPO₄ (5), KH₂PO₄ (4), CaCl₂-2H₂O (10), MgSO₄·7H₂O (10), FeCl₃·6H₂O (2), Na₂EDTA (5), ZnSO₄·7H₂O (0.1), MnCl₂·4H₂O (0.03), H₃BO₃ (0.3), CoCl₂·6H₂O (0.2), NiCl₂·6H₂O (0.02), CuCl₂·2H₂O (0.01), NaMoO₄·2H₂O (0.03). The reactor was aerated along all the test. At regular time intervals, aeration was interrupted in order to measure the Oxygen Uptake rate (OUR) as the slope of dissolved concentration vs. time. The substrates spiked in the test were the following: formic acid alone, acetic acid alone or formic/acetic acids mixtures in the ratios (% mol/mol) 25/75, 50/50, 75/25. The overall initial concentration was 5 mmol/L. The substrates were then spiked at the chosen concentration. During each test, the sludge was sampled at regular intervals and filtered on a 0.45 µm cellulose acetate filter (for substrates analysis in the filtrate). The length of the test was 5 h.

Analytical methods

Formic acid and acetic acid were determined by liquid chromatography (HPLC, WATER 996, Photodiode Array Detector, with UV-Visible detector at variable wavelength, column SUPELCOGEL C-610H, 50 cm × 7.8 mm ID, at 210 nm, eluent H₃PO₄ 0.1% in distilled water 1 ml min⁻¹).

RESULTS AND DISCUSSION

SBR performance

Figure 1A shows effluent concentration of formic and acetic acid during the SBR run. It is evident that a relevant difference occurred between formic and acetic acid profiles. Acetic acid was never found in the effluent because it was always completely removed during the reaction phase. On the other hand, formic acid was often present in the effluent of the reactor during initial phase of reactor operation (with settling time 30 min) at concentrations up to 350 mg/L, which indicates poor removal (feed concentration: 404 mg/L). In the second phase of reactor operation (with a settling time of 120 min) formic acid removal was complete and it was no longer detected in the effluent.

Effluent formic acid concentration corresponds with the profile of SRT during reactor run well (Figure 1B). Indeed, in the first phase of reactor operation (settling phase of 30 min), the SRT was low, below 5 days. This was due to the poor settling properties of the biomass, with high solid losses in the effluent; more than 10% of VSS in the completely mixed system (MLVSS) were lost in the effluent (Figure 1B). The increase of the settling time, and the consequent decrease of solid losses in the effluent, caused a relevant increase in SRT, up to 20 days. Correspondingly to the increase in SRT, formic acid removal also increased.
In the second phase of reactor operation (settling time 120 min) SRT presented a maximum, then decreased to values similar to the ones in the first phase. This decrease in SRT was due to a slight increase in effluent VSS and to increased sludge withdrawals for carrying out batch tests. It can be observed that, while SRT values at the end of reactor run were similar to initial values, formic acid removal continued to be complete. This indicates that, once biomass adapted to formic acid, formic acid removal could be maintained even at low SRT.

In summary, Figure 2 shows effluent concentration of formic acid versus Solid Retention Time (SRT). It can be observed that formic acid was totally removed only for SRT higher than 5–7 days.

Table 1 shows a summary of the operating conditions and the performances of the SBR.

**Table 1** | Summary of the operating conditions and the performances of the SBR (average values of last period; days 110–220)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic load rate (OLR) (gCOD/l/d)</td>
<td>0.58</td>
</tr>
<tr>
<td>Mixed liquor (mg/L) VSS</td>
<td>1,700</td>
</tr>
<tr>
<td>Effluent (mg/L) VSS</td>
<td>135</td>
</tr>
<tr>
<td>SVI (ml/gTSS)</td>
<td>47</td>
</tr>
<tr>
<td>Substrates at the end of the aerobic phase (mg/L)</td>
<td>≤10</td>
</tr>
<tr>
<td>Formic acid</td>
<td>≤10</td>
</tr>
<tr>
<td>Solid retention time δc (d)</td>
<td>8.2</td>
</tr>
</tbody>
</table>

SRT) and during the second phase (long settling phase and high SRT). A comparison of typical batch tests with acetic acid during the two phases (low SRT and high SRT) of reactor operation is shown in Figure 3. For both phases, after substrate addition, acetic acid started to be removed. OUR immediately increased with respect to the endogenous value. During the test OUR increased, so indicating that autocatalytic growth occurred. When acetic acid was depleted from the medium, OUR sharply decreased.

The comparison of the tests with formic acid during the two phases in Figure 4 shows a very different behaviour. In the first phase, at low SRT, formic acid (Figure 4A) was removed at a slow rate (7 mgCOD/gCOD/h). Immediately after formic acid addition, OUR (Figure 4B) increased with respect to the endogenous value, then slowly decreased. This suggests that in the first phase of reactor operation biomass was not capable of autocatalytic growth on formic acid, i.e. was not completely adapted to formic acid. Indeed...
autocatalytic growth would have caused an increase in OUR profile during growth on formic acid. The behaviour of biomass from SBR in the first phase (low SRT) was the same of unacclimated activated sludge, so confirming the absence of growth (data not shown).

During the second phase of SBR operation, on the other hand, biomass behaviour with respect to the removal of formic acid was completely different. Formic acid was removed at a much higher rate than in the previous phase (26 mgCOD/gCOD/h), as well as OUR profile continued to increase during formic acid removal. This suggests that biomass was able of autocatalytic growth on formic acid, differently from what observed during the first phase of reactor operation.

The comparison of biomass behaviour during the first and the second phase of reactor operation shows the relevance of adaptation to formic acid. A completely acclimated biomass, which is able to utilise formic acid as only source of carbon and energy, is obtained only in the second phase of reactor operation, when the SRT was high enough. It is likely that in the first phase of reactor operation, the SRT was too low to allow biomass complete adaptation to formic acid.

![Figure 3](https://iwaponline.com/wst/article-pdf/58/2/303/437073/303.pdf)

**Figure 3** | Comparison of batch tests with acetic acid during the first phase (low SRT) and during the second phase (high SRT) of SBR operation. Initial biomass concentration was 620 mgVSS/L in the test during the first phase and 700 mgVSS/L in the test during the second phase.

![Figure 4](https://iwaponline.com/wst/article-pdf/58/2/303/437073/303.pdf)

**Figure 4** | Comparison of batch tests with formic acid during the first phase (low SRT) and during the second phase (high SRT) of SBR operation. Initial biomass concentration was 620 mgVSS/L in the test during the first phase and 700 mgVSS/L in the test during the second phase.

![Figure 5](https://iwaponline.com/wst/article-pdf/58/2/303/437073/303.pdf)

**Figure 5** | Typical cycles of the SBR (second phase-high SRT).
In order to have a deeper insight into the removal of formic and acetic acid, the interactions between the two substrates were investigated in batch tests with the two substrates simultaneously added at different ratios (75/25, 50/50 and 25/75%) and compared to tests in the absence of the respective substrates.

In all cases the removal rate of acetic and formic acid was unaffected by the presence of the other substrate. This seems to indicate that different microbial groups were involved and confirms the role of sludge acclimation to increase formic acid removal by microorganism.

According to the classic theory of microbial growth on soluble substrates in bioreactors, under steady-state conditions, the solid retention time (SRT) is linked to substrate concentration in the effluent ($S$) by

$$\text{SRT} = \frac{1}{\mu_{\text{max}} - b}$$

where $\mu_{\text{max}}$ and $K_S$ are the parameters of Monod equation and $B$ takes into account biomass decay.

The $\mu_{\text{max}}$ on formic acid of the acclimated biomass ($5 \text{ d}^{-1}$) was calculated on the basis of the OUR increase shown in Figure 4B, according to the procedure described by Kappeler & Gujer (1992).

A specific rate of endogenous metabolism, $b$, of $0.4 \text{ d}^{-1}$ was also determined by respirometry.

As for $K_S$, from Figure 4A it is evident that an almost constant removal rate was obtained in the range $70–20 \text{ mg/L}$, so indicating a low $K_S$ value. Even by assuming a prudent value for $K_S$ of $10 \text{ mg/L}$, it can be calculated that 99% removal of formic acid should be obtained for SRT higher than $0.8 \text{ d}$. Based on above reported kinetic constant, a theoretical steady-state curve of effluent concentration can be calculated as function of SRT (Figure 2). It can be seen that theoretical steady-state curve would predict a better removal than experimentally observed at low SRT during first phase of acclimation, so confirming the high role of acclimation itself.

It is also important to underline the specific role of discontinuously fed reactors, such as SBRs, in determining biomass complete adaptation to formic acid. Indeed, comparison of removal rates of formic and acetic acids, as well as the analysis of typical cycles of the SBR (Figure 5), showed that acetic acid was removed much faster than formic acid; thus for most of the SBR cycle formic acid was the only substrate present in the system and biomass adaptation was stimulated. These substrate profiles are different from those of continuously fed systems, where substrates are always simultaneously present, even though at low concentration.

### CONCLUSIONS

Complete formic acid removal was obtained in an SBR at OLR 0.6 gCOD/L/d at SRT higher than approximately 5 days. However, complete adaptation to formic acid required a higher SRT in order to obtain a biomass able of autocatalytic growth on formic acid. Once adaptation has been obtained, stable performance was possible even at lower SRT. Directly starting at low SRT, on the other hand, biomass adaptation was not complete and autocatalytic growth was not obtained. It is important to underline that the minimum SRT to obtain the total formic acid removal was anyway higher than that predictable by the classic models of microbial growth applied in wastewater treatment.

### REFERENCES

