Soluble microbial product formation during biological treatment of fermentation industry effluent

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Abstract The most important factor that affects the quality of effluents and overall organic matter removal in biological treatment processes is the presence of soluble microbial products (SMP) that are produced during biological treatment and remain in effluent. High strength wastewater from fermentation industry (FIE) was used as a slowly degradable substrate to investigate SMP formation in aerobic and anaerobic treatment. For SMP determination which forms a major part of residual soluble COD of the wastewater, the initial inert soluble COD (SI) was determined. It was found that SI/So is 0.122 and 0.164 for aerobic and anaerobic processes, respectively. During aerobic treatment of FIE for initial COD (So) values varying between 1540–6645 mg COD/L, SMP are expected to vary within the range of 12.5–8.6% of So, respectively. In anaerobic treatment of the FIE, for values of 2670–6585 mg COD/L, SMP are expected to vary within the range of 9.8–6.2% of So, respectively. Molecular weight distribution analyses of raw wastewater, aerobic and anaerobic treatment effluent indicate that in both systems the effluent of FIE presents similar molecular weight fractions and are very high compared to raw wastewater.

Keywords Aerobic treatment; anaerobic treatment; fermentation industry effluent; molecular weight distribution; residual COD; soluble microbial product

Introduction Effluents from biological treatment processes contain a variety of soluble organic matter, including residual influent substrate, Soluble Microbial Products, and non- or slowly biodegradable organic materials. Many experimental results have shown that only a small fraction of the effluent was original influent substrate in terms of biodegradable organic matter, while the majority of the soluble organic matter was of microbial origin (Grady et al, 1972; Grady and Williams, 1975). Therefore, an important factor affecting the quality of effluent and overall organic matter removal in biological treatment processes is the presence of SMP that are produced during biological treatment (Ritmann et al., 1987; Gaudy and Blachy, 1985; Parkin and McCarty, 1981b). Effluents from aerobic and anaerobic treatment processes contain complex organic compounds that must frequently be removed in tertiary treatment systems. The physical and chemical characteristics of these organic compounds affect both the selection and performance of the subsequent treatment processes and the ultimate environmental impact of the treated wastewater.

There is an increasing concern that effluent toxicity may actually be created in the biological treatment process itself. In other words, SMP may be more toxic than the original organic compounds present in the wastewater (Eckenfelder, 1988). Even though the significance of SMP formation has been emphasized in the literature, very limited information is given on the quantity and the structure of SMP produced during biological treatment and on the factors affecting the production of SMP.

Most of the studies mentioned in the literature describe SMP generation from aerobic processes using synthetic substrate, whereas limited information is available on SMP generation from anaerobic processes treating industrial wastewater.

This study was conducted using an industrial wastewater, fermentation industry effluent, for aerobic and anaerobic processes.
Materials and method

High strength wastewater from the fermentation industry (baker’s yeast industry) was used as a slowly degradable substrate for the aerobic and anaerobic experiments to investigate SMP formation. More information on FIE is given by Oztürk and Ciftci (1995).

The wastewater has a COD of 15,000 mg/L. The bacterial culture used to seed the aerobic and anaerobic batch reactors were taken from a full-scale, two-stage treatment plant that was part of the baker’s yeast industry and acclimated to wastewater under lab conditions. Solutions A and B were added to the reactors to meet the nutrient requirement of the microorganisms and to act as a pH buffer. Nutrient solutions are given in Table 1 and Table 2, respectively.

Nutrient solutions were added to the reactors with respect to nutrient capacity of the wastewater. All chemicals used in the study were of MERCK quality.

Experimental set-up. Cylindrical glass batch reactors of 5 L were used in the aerobic experiments. Excess aeration was supplied to obtain complete mixing and to meet the oxygen requirement in batch reactors. Air was supplied via air pumps (aquarium pumps) and applied to reactors by means of fine bubble porous diffusers. In the anaerobic part of the study, several batch reactors were used to perform the experiments. Anaerobic reactors of 4 L volumetric capacity were maintained at a constant temperature in a water bath containing 30 L of water at a temperature of 35ºC. Reactors were mixed with the magnetic stirrers. All anaerobic reactors were washed with nitrogen gas to prevent air entering to the reactor after sampling. After setting up the reactors, soluble COD was monitored. A sample was taken for soluble COD analysis every day or once in two days until the reactors reached the plateau. Each experiment was conducted twice.

Gel permeation chromatography was used to determine the molecular weight distribution of biologically treated effluents. The samples were filtered through 0.45 µm membrane filters before the analysis was done.

The column having 60 cm length with a diameter of 1 cm was mounted on a stable laboratory stand in a vertical position. Samples were introduced as carefully as possible to avoid disturbing of the bed.

Elution was carefully carried out under a constant hydrostatic pressure head to achieve a constant flow rate. One ml of sample was pipetted onto the drained surface of the gel bed. As soon as all of the sample entered the gel bed, the eluant (0.1 N LiOH-NaCl) was applied on

<table>
<thead>
<tr>
<th>Table 1 Composition of nutrient solution A</th>
<th>Table 2 Composition of nutrient solution B</th>
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<tr>
<td>Constituent</td>
<td>Concentration (g/L)</td>
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<tr>
<td>K₂HPO₄</td>
<td>320</td>
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<tr>
<td>KH₂PO₄</td>
<td>160</td>
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<tr>
<td>MgSO₄.7H₂O</td>
<td>15</td>
</tr>
<tr>
<td>FeSO₄.7H₂O</td>
<td>0.5</td>
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<tr>
<td>ZnSO₄.7H₂O</td>
<td>0.5</td>
</tr>
<tr>
<td>MnSO₄.7H₂O</td>
<td>0.5</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>2</td>
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the surface of the gel and the pump was reconnected. The outlet of the column was opened and 2 mL fractions were collected until a total of 100 mL had passed through the column. The collected fractions were read at 280 nm by SECOMAM UV-VIS Spectrophotometer and then chromatograms (absorbance versus elution volume) were obtained. The MWD of the samples was calculated by using corresponding chromatograms.

Analytical procedures

All analyses were performed according to the procedures described in Standard Methods (1992). Soluble COD was measured using the dichromate open reflux method and volatile acid was measured as acetic acid using the distillation method. Gel permeation chromatography (Sephadex G-50) was used for the determination of MW distributions. BOD₅ analyses were performed according to the titrimetric method.

Throughout this study, mostly soluble fraction of the wastewater was considered. Therefore, all samples were subjected to vacuum filtration before analyses. Soluble means anything that could pass through 0.45 µm membrane filters (S&SCHUELL, 47 mm).

Results and discussion

In order to determine SMP which forms a major part of the residual soluble COD of the wastewater, the initial inert soluble COD(SI) must be known. At the end of each experiment, SMP was calculated by subtracting the SI from residual soluble COD (SR).

The SI/S₀ ratio was found to be 0.117 by using the comparison method (Germirli, 1990) and as 0.122 by using the direct determination method (Orhon, 1992) for aerobic process while this ratio was found to be 0.164 by using the direct determination method for anaerobic process. In anaerobic application of the direct determination method, VFA must be considered while calculating SI (COD corresponding VFA should be subtracted).

SMP formation during aerobic and anaerobic treatment of FIE with various initial COD concentrations

Degradation of FIE was monitored aerobically for six various initial concentrations. The reactors were seeded with a small amount of sludge (30 mg VSS/L) acclimated to FIE for several months at a sludge age of five days. Solution A was used to meet the nutrient requirement of aerobic microorganisms. Similarly degradation of the wastewater was monitored anaerobically for four various initial concentrations. The reactors were seeded with an amount of sludge (100 mg VSS/L) acclimated to wastewater anaerobically at laboratory conditions. Solution B was used to meet the nutrient requirement of anaerobic microorganisms and to act as a pH buffer.

The threshold value as expressed by SCOD measurements in an anaerobic reactor is the sum of the SMP, SI and VFA. Therefore, SMP produced in each set was obtained by subtracting (SI+VFA) from the threshold values.

The results of the experiments are given in Tables 3 and 4.

Using the results given in Tables 3 and 4, a relationship can be developed between S₀ and SR and SMP for aerobic and anaerobic treatment as shown in Figures 1 and 2.

As it is seen in Figures 1 and 2, as S₀ increases SMP increases while SMP/S₀ decreases for both of the systems. The relationships between SR and S₀, SMP and S₀ can be expressed by the following equations;

For aerobic system:

\[ S_R = 0.196 \, S_0 + 89 \quad (r=0.998) \]  
\[ \text{SMP} = 0.076 \, S_0 + 89 \quad (r=0.987) \]
For anaerobic system:

\[
S_R = 0.198 S_o + 180 \quad (r=0.999) \quad (3)
\]

\[
SMP = 0.034 S_o + 180 \quad (r=0.993) \quad (4)
\]

The intercept value of the equations found to be 89 mg/L and 180 mg/L for aerobic and anaerobic processes, respectively. This phenomena was explained by Chudoba (1985b) as equilibrium concentration. It has been observed that microorganisms excrete some organic compounds in order to establish a concentration equilibrium across the cellular membrane. However, these organic compounds differ with individual types of activated sludge (Parkin and McCarty, 1981a).

From a comparison of aerobic and anaerobic test results it is seen that aerobic processes caused more SMP generation from anaerobic processes, as shown in Figure 2.

MWD of aerobic and anaerobic treatment effluent of FIE

In order to evaluate the variation of molecular weight distribution of FIE at the end of tests, the original and treatment effluent of FIE were subjected to gel filtration. The calculated MWD are shown in Table 5.

As seen in Table 4, the low MW fractions were converted to higher MW fractions during the aerobic and anaerobic treatment. While the original FIE has no fraction with MW above 25 K, at the end of the experiment, the fraction higher than 25 K is 17% and 13% for aerobic and anaerobic effluent, respectively. In other words, while 27% is higher than 1 K in the influent, 61% and 51% is higher than 1 K in the aerobic and anaerobic effluent, respectively. It proves that some compounds with high MW are formed during biological treatment which are called SMP. Comparative MWD analysis results are shown in Figure 3.

Figure 3 indicates that the residual components in aerobic and anaerobic treatment have similar MW fractions and are very high compared to influent.
SMP formation in two-stage treatment of the FIE

The behavior of residual COD in a two-stage treatment was investigated. For this purpose, aerobic degradation of residual COD produced in anaerobic system was investigated. The threshold values of anaerobic reactors were subjected to aerobic treatment to examine the degradability of the residual COD from anaerobic process.

When the anaerobic reactors had reached the plateau, the reactors were decanted and supernatants were filtered and then subjected to aerobic treatment. Reactors were seeded with a small amount of sludge (30 mg VSS/L) acclimated to anaerobic effluent under laboratory conditions. VFA values were included while considering the threshold values of anaerobic reactors which were also influent concentrations for the following aerobic treatment. The results of aerobic treatment of anaerobic treatment effluent are given in Table 6.

According to the values of Table 6, the residual SMP from anaerobic process was partially removed in the aerobic process. The total removal efficiency is found to be 32% (average of the four reactors). However, it should be considered that since the volatile acids are removed in aerobic treatment, they must not be taken as part of the residual COD from the anaerobic treatment. Therefore, in the following calculations COD equivalence of VFA values were subtracted from residual COD of anaerobic treatment.

Thus, the average removal efficiency of SMP is found to be 25%. The similar test was conducted to determine if the residuals from aerobic treatment were degradable anaerobically for only one reactor. It is seen that the residual COD from aerobic treatment was partially removed in anaerobic treatment. The removal efficiency is found to be 23%. When VFA is considered, the removal efficiency is found to be 26%. The main aim of this test was...
to evaluate the degradability of residual COD varying from one phase to another rather than to find the precise removal efficiency.

Conclusions
Conclusions attained in this study may be summarized as follows.

• The ratio of $S_1/S_o$ is found to be 0.122 and 0.117 for FIE by using direct determination and comparison method, respectively (under aerobic conditions). However, this ratio is determined to be 0.164 by using direct determination method under anaerobic conditions. It is seen that there is a difference of 4.2% between the ratios of $S_1/S_o$ obtained for aerobic and anaerobic processes. This difference is due to VFA remaining at the end of the anaerobic process which behaves as an inert material.

• The initial inert soluble COD of a wastewater should be determined aerobically if the wastewater is to be treated aerobically and should be determined anaerobically if the wastewater is to be treated anaerobically as some inert materials for one phase may not be inert for the other phase.

• During aerobic treatment of FIE for $S_o$ values varying between 1540–6645 mg/L, SMP, as predicted by Eq. (2), are expected to vary within the range 12.5–8.6% of $S_o$, respectively. During anaerobic treatment of the FIE, for values of 2670–6585 mg/L, SMP as predicted by Eq. (4) are expected to vary within the range 9.8–6.2% of $S_o$, respectively.

• The ratio of SMPA/SMPAN is about 1–1.5 for FIE and increases as $S_o$ increases.

• The results of MWD of raw, aerobic and anaerobic treatment effluent of FIE indicates that both the aerobic and anaerobic treatment effluent of FIE present similar molecular weight fractions and are very high compared to raw wastewater.

• In a two-stage treatment of FIE, it was found that the SMP formed during anaerobic treatment was subsequently removed in aerobic treatment with an efficiency of 25%. This shows that some inert materials for one phase may not be inert for the other phase.

References