INFLUENCE OF MULTIPLE SUBSTRATES ON ANAEROBIC PROTEIN DEGRADATION IN A PACKED-BED BIOREACTOR

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Abstract. This work reports on the anaerobic degradation of bovine serum albumin (BSA) from the standpoint of substrate consumption kinetics. The substrates used consisted of BSA as the only carbon source and BSA mixed with carbohydrates and lipids. A bench-scale horizontal-flow anaerobic immobilized biomass (HAIB) reactor was operated with a 4-hour hydraulic detention time. The reactor’s performance was evaluated based on physicochemical and chromatographic analyses and on microscopy techniques. The first-order kinetic model fitted the experimental data well and the apparent kinetic parameter was estimated for each experimental condition. The initial protein degradation rates were negatively affected by the presence of other organic compounds such as carbohydrates and lipids.

Keywords: anaerobic degradation, proteins, HAIB reactor, kinetics, metabolic pathways

Introduction
Wastewaters contain a wide variety of organic compounds that are biodegraded simultaneously, but there are relatively few studies about how the use of multiple substrates affects the kinetics of degradation of a specific compound, such as protein, in a mixed anaerobic community. Some authors (Torres, 1992 and Gadelha, 2000) concluded that proteins were the main compounds responsible for the remanent COD in the effluent of anaerobic reactors treating domestic sewage. Breure et al. (1986) and Martins et al. (1991) also studied the anaerobic degradation of protein molecules, stating that the efficiency of the anaerobic protein degradation process is closely linked with the separation of the protein from the other organic compounds present in wastewater, such as carbohydrates and lipids. However, it is known that this separation process can be very expensive or even impossible to achieve.

In this context, experiments were performed in a packed-bed reactor and the biochemical reaction kinetics was used as a tool to evaluate the anaerobic process of protein degradation. The influence of the carbohydrates and lipids on the protein degradation kinetics was evaluated. In addition, the microbial stratification was observed throughout the reactor using microscopy techniques.

Material and Methods
A 1m long, 5cm diameter HAIB reactor with a total volume of 2 l was used in this study. Cubic polyurethane foam matrices (5-mm sides) were used as the support for biomass immobilization. The bed porosity was 40%, resulting in a reaction volume of 800 ml. The reactor consisted of 11 equidistant sampling ports along its length and a gas collector at its surface. The inoculum was taken from an UASB reactor treating poultry slaughterhouse wastewater. The granules were macerated and immobilized in the matrices, according to the procedure described by Zaiat et al. (1994).

The reactor was operated with a 4-hour hydraulic detention time and subjected to four successive operating conditions using different carbon sources. Initially, the reactor was fed with synthetic wastewater containing only BSA as carbon source (COD ~ 400 mg/l).

The second carbon source employed was BSA plus starch and glucose (COD ~ 550 mg/l), the third experiment was carried out with synthetic substrate containing all the carbon sources previously used plus lipids (COD ~ 600 mg/l), and finally, the reactor was again fed exclusively with BSA, but with the same COD as in the third wastewater combination. A solution of inorganic nutrients was used to nutritionally balance the substrates.
The substrate reservoir was maintained at a temperature below 5°C to minimize the occurrence of biochemical reactions outside the reactor and the substrate was heated to 30 ± 1°C before entering the reactor.

The reactor’s performance and stability were evaluated by analyzing the influent and effluent bicarbonate alkalinity and chemical oxygen demand (COD) (Standard Methods for Examination of Water and Wastewater, 1998).

Spatial profiles of protein concentration (Lowry modified by Petterson, 1979), volatile acids concentration by gas chromatography (Moraes et al. 2000) and ammonium-nitrogen concentration (Standard Methods, 1998) were taken under each experimental condition to assess the behavior of the protein degradation kinetics. Upon conclusion of each experimental step, three matrices with biomass were collected from each intermediate sampling port and immediately replaced with clean matrices to maintain the flow pattern. One particle from each port was subjected to the technique described by Nation (1983) and modified by Araújo (1995) for observation in a scanning electronic microscope to verify the biofilm. The biomass from the other matrices was detached and analyzed by optic microscopy (phase contrast and fluorescence), allowing for an evaluation of biomass stratification throughout the reactor.

Results and Discussion

The acclimatization period lasted for 26 days of operation, after which the spatial profiles were taken at 10-day intervals. After the acclimatization period, the reactor was considered to have reached a steady state and stable rates of organic matter removal efficiency (around 90% as COD) and bicarbonate alkalinity (around 600 mg CaCO$_3$.l$^{-1}$) were observed.

Figure 1 presents the spatial profiles obtained under each experimental condition. The first-order kinetic model was found to adequately represent the experimental data under each experimental condition, showing the following correlation values: 0.99, 0.98, 0.97 and 0.99 for operational conditions 1 to 4. The kinetic parameters of anaerobic protein degradation, shown in Table 1, were estimated by non-linear regression (Levenberg-Marquardt method – Microcal Origin 6.0®).

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>$K_{i}^{app}$ (h$^{-1}$)</th>
<th>Initial reaction rate (mg S.A.B.$l^{-1}$.min)</th>
<th>Initial reaction rate (mg NH$_4^{+}$.l$^{-1}$.min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA (COD = 400mg l$^{-1}$)</td>
<td>0.541 ± 0.008</td>
<td>3.11</td>
<td>0.59</td>
</tr>
<tr>
<td>BSA + Carbohydrates</td>
<td>0.430 ± 0.014</td>
<td>2.47</td>
<td>0.36</td>
</tr>
<tr>
<td>BSA + Carbohydrates+Lipids</td>
<td>0.292 ± 0.008</td>
<td>1.53</td>
<td>0.28</td>
</tr>
<tr>
<td>BSA (COD = 600mg l$^{-1}$)</td>
<td>0.400 ± 0.007</td>
<td>2.94</td>
<td>0.72</td>
</tr>
</tbody>
</table>

The initial protein degradation rate decreased when another carbon source was added to the synthetic wastewater. Martins et al. (1991) came up with a similar finding in their study of the anaerobic degradation of albumin and casein, as did Breure et al. (1986) in their study of gelatin anaerobic degradation. The lipid-containing system presented the lowest kinetic constant and, thus, the lowest reaction rate. The kinetic constant estimated for the fourth condition differed from that of the first operating condition, which was unexpected. This fact may have been related to a possible change in the biomass, and will be discussed with the microbiology analyses.
The degradation efficiencies of organic matter, calculated from the influent and effluent COD values, were 91% under the first condition (protein - COD ~ 400 mg.l⁻¹), 93% under the second (protein plus carbohydrates), 76% under the third (protein, carbohydrates and lipids) and 83% under fourth (protein - COD ~ 600 mg.l⁻¹). These results indicate that the presence of lipids in wastewater with high protein concentrations decreases the degradation efficiency of organic matter. Although the presence of carbohydrates reduced the initial protein degradation rate, the overall efficiency was similar in both experiments, indicating that the 4-hour hydraulic detention time sufficed to provide suitable organic matter degradation even in the system with carbohydrates, in which the kinetic constant was about 26% lower than that observed in the system containing only protein. In fact, the similarity of the efficiency rates demonstrates that, under our operating conditions, these organic fractions do not have to be separated to maintain good anaerobic protein degradation. COD removal efficiencies of 87%, 90%, 70% and 79% were observed under the first, second, third and fourth conditions, respectively.

A chromatographic analysis detected only a considerable amount of acetic and propionic acids in samples taken along the reactor’s length. The highest residual fatty acid concentrations were observed in the substrate whose composition contained lipids. The presence of lipid molecules can retard protein degradation and can also reduce the degradation rate of organic matter. This fact can be ascribed to the low availability of lipids due to their low solubility.

Vidal et al. (2000) reported that the presence of lipids in wastewater whose composition included carbohydrates and protein caused the organic matter degradation rate to decrease, preventing the accumulation of fatty acids. In contrast, in our study, the highest concentrations of volatile fatty acids were found in the synthetic substrate containing lipids.

The ammonium-nitrogen concentrations found in the effluent under the first, second and third operating conditions were very similar. These results were congruent with the protein degradation efficiencies, since the three operating conditions had the same initial protein concentration value. It is worth noting that ammonium production in the presence of lipids occurred at a lower rate compared to the rate under the other conditions, as shown in Table 1. Because ammonium is a by-product of protein hydrolysis and amino acid consumption, this confirms that the presence of lipids
has a negative effect on these processes. The higher ammonium concentrations found in the effluent under the fourth operating condition were attributed to the higher initial protein concentrations.

The gas composition was analyzed throughout the operating phase using gas chromatography techniques. However, methane and carbon dioxide volumes could not be measured due to problems in the configuration of HAIB reactor, which did not expel a constant amount of biogas. High percentages of methane gas (up to 72%) were found after the start-up period, which were a good indication of the system’s operational stability and the rapid evolution of the anaerobic degradation process.

The SEM analysis revealed that the immobilization patterns were similar to those previously described by Varesche et al. (1997) in polyurethane foam matrices taken from a fixed-film reactor treating glucose-based substrate.

The optical microscopy analyses showed several major morphologies, which varied according to the operating condition and the position along the length of the HAIB reactor. The morphologies predominating throughout the operation time were Methanosaeta-like morphologies, hydrogenothrophic rods, filaments and non-fluorescent rods. Fluorescent cocci, vibrios, Methanosarcina-like spirochaete and non-fluorescent cocci were also present.

In the first operating condition, Methanosaeta-like morphologies were found to predominate from the middle to the back of the HAIB reactor, while hydrogenotrophic archaea rods were prevalent in the front and non-fluorescent rods from the front to the middle of the reactor. In the second condition, the Methanosaeta-like morphologies had shifted to the front of the reactor. Hydrogenotrophic archaea rods were more numerous in the middle of the reactor and a predominance of filaments was observed from the middle to the back. A predominance of non-fluorescent cocci, sometimes in chains, was also observed at the back of the reactor. The introduction of lipids caused the Methanosaeta-like morphologies to return to the reactor’s second half. Under this operating condition, the predominance of filaments was very evident. A predominance of vibrios resembling Desulfovibrio sp, which can ferment this substrate in the presence of lipids and the absence of sulfate, was also found (Voordouw, 1995). Filaments were still present throughout the HAIB reactor’s length under the last operating condition, although a predominance of non-fluorescent rods was also found at the front of the reactor.

References