MICROBIOLOGICAL STUDY OF THE DEVELOPMENT OF BIOFILM IN AN ANAEROBIC FIXED-BED REACTOR

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Abstract: The treatment capacity in anaerobic systems is basically determined for the active population concentration of microorganisms retained in the reactor. Anaerobic filter is a treatment system developed to favor the immobilization and biomass adherence, reaching good performance in the removal of organic matter. However, several factors interfere in the biomass adherence on supports in fixed bed reactors, such as: form, size, porosity, specific surface and the nature of the solid supports, besides their electrostatics charges. Such factors may influence the performance of the anaerobic fixed bed reactor (Characklis & Trulear, 1982; Vijayalakshimi et al., 1990). According to Ince (1999), anaerobic filter with supports of high porosity and specific surface, presents better efficiencies in both start up and steady state of the system than the reactors with conventional support.

This study was carried out to verify the influence of the support materials on the growth and retention of the biomass, using resources of optical, fluorescence and scanning electron microscopy (SEM) to examine the structure and biological composition of cell aggregates in biofilms and the possible influence of those support materials on the colonization pattern.

KEYWORDS: Anaerobic process, Fixed-bed reactor, Biofilm, Innert support

Materials and methods

Experimental Apparatus
An anaerobic reactor was used with a total volume of 34.5 L. It was built in PVC, with diameter of 200 mm and 1.20 m height, with 28.8 L useful volume and 5.7 L for gas separation (16.5% of the total volume). It operated with hydraulic detention time (TDH) of 24 h (Figure 1) in an acclimatized camera (30°C ± 1°C). The gas generated in the reactor was directed to a measurement system composed of a Mariotte’s bottle.

The experiment was performed in three steps, during which the reactor was fed with synthetic domestic sewage. In a first step the reactor was operated for 122 days with influent chemical oxygen demand (COD) of 466 ± 142 mg/L. In the second step, which occurred from the 122nd to the 244th day, the influent COD was 739 ± 169mg/L and in the third one the reactor was operated for 149 days with influent COD of 1267 mg/L ± 400 mg/L.

Synthetic Domestic Sewage
The synthetic domestic sewage was prepared in agreement with the composition and procedures described by Torres (1992). Besides the organic components, a mineral salt solution and a metal solution were added into the synthetic wastewater. Table 1 presents the composition of the synthetic domestic sewage.

<table>
<thead>
<tr>
<th>Organic fraction</th>
<th>Percentage of COD</th>
<th>Composed Organic</th>
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<tbody>
<tr>
<td>Proteins</td>
<td>50%</td>
<td>Meat Extract</td>
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Support Media

In stages I and II, 18 vertical modules were used as material supports, each one composed of with 5 pieces of PVC of 20 mm, connected to each other by PVC tubes with diameter of 25 mm and 10-cm height.

In stage III, 16 metallic stems (stainless steel) were fixed inside the reactor. In each stem, 4 different supports were attached (PVC, polyurethane foam, refractory brick and special ceramic), 3.5 cm distant from each other.

Those modules were important to provide support removal from the reactor without damage in the biofilm structure.

<table>
<thead>
<tr>
<th>Carbohydrates</th>
<th>40%</th>
<th>Sucrose 20%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial starch</td>
<td>60%</td>
<td>Cellulose 20%</td>
</tr>
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</table>

| Lipids | 10% | soybean oil |

Characterization of Attached Biomass and Granules

In the steps I and II, 7 biofilm samples were taken at three different heights (0.28, 0.54 and 0.79 m from the bottom of the reactor), for both the attached biomass quantification and microscopic examination. In the third step, the supports were removed from two different heights of the metallic stems (0.3 and 0.37 m from the bottom of the reactor) in order to evaluate the effect of the support position inside the reactor on the biomass quantification.

The immobilized biomass was indirectly quantified through measures of total volatile solids (TVS), expressed per unit of support, since the supports present the same dimensions.

A microscope (Olympus BHT 2) equipped with phase contrast and fluorescence attachment was used for the observation and photomicroscopy of biofilm. The fluorescence was verified by using a UV light source attached to the microscope (this technique was used to distinguish the methanogenic cells). The cells were scraped from their supports and rinsed with distilled water and drops of the resulting liquid were fixed in gel agar (2%).

![Figure 1. Experimental set up of the fixed-bed reactor with the longitudinal shafts in which supports were fixed.](image)
For scanning electron microscopy (SEM) a Zeiss DSM-960 was used. The samples were fixed with 2.5% glutaraldehyde. Afterwards, the cells were washed with phosphate buffer solution, dehydrated in ethanol solutions of increasing concentration (50%, 70%, 80%, 90%, 95% and 100%) and fixed in hexamethydisilazane for 30 seconds. The particles were then coated with gold powder and attached to the microscope supports with silver glue. The sample preparation for SEM was described in Nation (1983) and adapted by Araújo (1995).

Results and Discussion

The reactor operated with COD removal efficiencies of 86±7% in the first and second steps and 69±15% in the third step.

The supports for biological microscopic exam and scanning electron microscopy (SEM), in steps I and II, were removed from the bottom of the reactor (0.28 m from the base), from the middle (0.54 m from the base) and from the highest part (0.79 m from the base of the reactor). However, there was no significant change in the predominant morphologies, which was proven by the hydrodynamic tests of the reactor accomplished by Passig & Blundi (1996). The tests showed that the reactor behaved as 3 continuous stirred tank reactors in series and with longitudinal dispersion coefficient of 0.17.

It was observed an increase from 97g of attached biomass / support m² in the 62nd day of operation to 202g of attached biomass / support m² in the 253rd day of operation for the PVC support. During this period, a thin biofilm attached to the support material could be observed. According Verrier et al. (1988), PVC presented a slow colonization and with small microbial diversity.

In the steps I and II, samples of granules, that grew inside the reactor, were analyzed and the following predominant morphologies were verified: cocci, rods, vibrio; and Desulfomaculum sp-like cell, besides microorganisms whose morphology resembled Methanosaeta sp type.

In steps I, II and III, the following predominant morphologies were observed attached to the PVC support: short rods; fluorescent rods; curved rods; vibrios; cocci; microorganisms whose morphology resembled Methanosarcina sp cells, and methanogenic Methanothrix sp-like cells. So, the biomass attached to the PVC presented high diversity, thus contracting the findings of Verrier et al. (1988). Probably, the complex synthetic substrate had contributed for such diversity.

In steps I, II and III, samples of suspended sludge presented the following predominant morphologies: free rods, fluorescent rods, curved rods; vibrio, cocci; spore forming bacteria; bacteria whose morphology resembled the genus Desulfomaculum sp. and the metanogenic Methanosaeta sp.

In step III, the main objective was to verify the predominant morphotypes in different support material (polyurethane foam, PVC, porous ceramic and refractory brick). No differences could be observed in the cellular morphologies in the biofilms developed on the supports. The occurrence of rods, cocci, diplococcs-shaped cells and methanogenic Methanosaeta sp and Methanosarcina sp -like cells was verified. The scanning electron microscopy aimed at the observation of the biofilm attached to the materials supports, and then the occurrence and predominance of some morphotypes was verified. Methanogenic archea, Methanosarcina sp -like cells prevailed in the polymeric materials (polyurethane foam and PVC), fastened to the wall of the supports. In the ceramic materials (porous ceramic and the refractory brick), there was a predominance of Methanosaeta sp -like cells. In comparison to other supports, the special porous ceramic presented a more uniform colonization, practically uniting its pores. The refractory brick also presented that distribution, although with less evidence.

Ince et al. (1999) developed a study of resistance, mean porosity influence and biomass adherence to supports of an anaerobic filter treating domestic sewage. Microscopic observations showed that the number of methanogenic autofluorescent microorganisms varied from 15-28% of the total number of microorganisms along the anaerobic filter. Methanogenic archea Methanococcus sp -like prevailed,
followed by short and long rods, filamentous and Methanosarcina sp -like. Murray & Van den Berg (1981); Pérez et al. (1992); Urrutia et al. (1998) reported on the discharge capacity of clay in sticking anaerobic microorganisms, particularly the filamentous morphology, possibly due to the characteristics of the material. Its rough surface, with a great amount of cracks and rifts favored the adherence and accumulation of Methanoseta sp cells.

Conclusions
In steps I and II, the granular sludge, the biofilm fixed on the PVC support material and the suspended material, presented, in a general way, basically the same predominant morphologies. It was observed the prevalence of Methanoseta sp -like archaea. Methanosarcina sp -like archaea were also observed, but in a few amounts, which can be due to the operational condition of the reactor.

Through the microscopic observations of the biomass attached to the supports, a difference among the adhesion of methanogenic arcaheas was verified. In the polymeric supports Methanosarcina-like cells prevailed, stuck on an isolated and punctual way, and close to the wall of the support. In the ceramics, Methanoseta-like cells prevailed forming bunches between the pores and cracks of the material.

References