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Evaluation of the microbial diversity in an UASB reactor treating wastewater from an unbleached pulp plant

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Abstract

The microbial community in the sludge blanket of a bench scale upflow anaerobic sludge blanket (UASB) reactor with partial recirculation of the effluent, treating wastewater from an unbleached pulp plant, was evaluated by means of the small subunit (SSU) rRNA gene amplified by polymerase chain reaction (PCR) and separated using the denaturing gradient gel electrophoresis (DGGE) technique. The average COD removal efficiency was 80–86% without recirculation and 75–78% with recirculation. Partial recirculation of the effluent did not alter the predominant population in the reactor under the conditions studied. A microscopic analysis revealed the presence of a variety of cellular morphologies. The DGGE results revealed that the structure of the microbial community was found to be complex and most of the population was similar, persisting throughout the experimental period. The microbial community of the Domain *Bacteria* was more sensitive to operational changes than the Domain *Archaea*.

Keywords: Anaerobic process; Black liquor; Denaturing gradient gel electrophoresis; Microbial community; Pulp and paper wastewater; Upflow anaerobic sludge blanket reactor

1. Introduction

Most pulp and paper mills produce large amounts of effluents with highly polluting loads. Discharged into the environment, these effluents cause considerable environmental impact for they contain biodegradable organic material and compounds toxic to aquatic organisms. Moreover, these effluents are strongly colored owing to the presence of lignins, resins, tannins and chlorophenolic compounds that are resistant to biodegradation [1–3].

However, recent advances in the identification of toxic substances and a growing body of information on the biodegradation and tolerance to toxicity of anaerobic microorganisms are contributing to change this situation and it has been demonstrated that the anaerobic treatment of effluents heretofore considered toxic is technically feasible.

Many researchers are developing techniques for the biological treatment of pulp and paper effluents. These studies include the work of Thakur [4], who successfully removed color from Kraft pulp bleaching effluent using eight fungi and three bacteria lineages, that of Smith et al. [5], who demonstrated the stability of the bacterial community in activated sludge treating pulp mill wastewater, and Buzzini and Pires [6], who used an upflow anaerobic sludge blanket reactor (UASB) to treat the effluent from a Kraft pulp plant. However, there is still a paucity of information on the dynamics and structural diversity of microbial communities.

The UASB is recognized as an effective technology for the treatment of high-strength industrial wastewater because of its high biomass concentration and microbial diversity [7–9]. The use of UASB reactors in the treatment of complex effluents often requires long hydraulic retention times [10]. In this case, the upward velocity is very low, hindering the mass transfer processes. Campos and Anderson [11] found that the upward velocity exerts a considerable effect on the

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development of sludge in UASB reactors, acting as a selection process in the development of the biomass.

Variations in the composition of trophic levels due to changes in one or more operating conditions (e.g., substrate composition, temperature, solid retention time and other operating parameters) influence the entire structure of the microbial community, affecting bioreactor performance [12].

Over the last decade, several attempts have been made to analyze the microbial community structure using more advanced methods [13]. Some of these methods include techniques common to molecular biology, which favor strong development in several fields of biology, particularly in environmental microbiology. Today, the detection and characterization of bacteria in natural environments and in reactors treating residues is carried out with greater assurance. Microbial communities have been characterized using the PCR/DGGE technique to estimate the bacterial diversity, including the non-culturable groups [14,15], in order to evaluate the structure of the microbial community along time [16,17] and explain the events of succession and disturbance of the microbial community [18].

In this work, it was decided to use the PCR/DGGE technique to monitor and characterize the structure of the microbial community on a temporal scale in a UASB reactor treating Kraft pulp black liquor with partial effluent recirculation, aiming to add to the body of knowledge and information on the diversity and dynamics of the microorganisms that are commonly present in these environments.

2. Materials and methods

2.1. Reactor design

The UASB reactor used in this study had a total volume of 15.0 l; its body was made of Plexiglas and the gas–solid–liquid separator of ANSI 316 stainless steel. The sludge blanket was developed in the reactor's 57 cm high body, whose circular section was 15 cm in diameter. The gas–solid–liquid separator was located 60 cm from the reactor's entrance.

The reactor was kept in an acclimatized chamber at $30 \pm 3\ ^{\circ}C$ to prevent any possible interference caused by variations in temperature and was fed using a dosing pump (prominent, model Gamma G/4b) with a maximum outflow of $2.3\ l\ h^{-1}$. A similar pump was used for recirculation.

2.2. Inoculum

The reactor was inoculated with granulated sludge from a UASB reactor treating effluents from a poultry slaughterhouse (Avícola Dacar S.A., Tietê, state of São Paulo, Brazil). The amount of sludge added to the reactor was 4.5 l, i.e., about one third of the total volume.

Table 1
Main characteristics of the wastewater

Parameter	Value
pH	6.8–7.2
COD ($mg\ l^{-1}$)	1400
BOD ($mg\ l^{-1}$)	660
N_{total} ($mg\ l^{-1}$)	24.0
Sulfide ($mg\ l^{-1}$)	7.0
P_{total} ($mg\ l^{-1}$)	4.0

2.3. Wastewater characteristics

Black liquor has already been used by other researchers as the basis for the composition of Kraft pulp mill effluent and has proved suitable for bench scale studies on the treatment of effluents from integrated pulp and paper plants or simply from cellulose pulp [1,6,19]. Because it contains the chemical reagents used in the process and the by-products generated in the decomposition of the constituents of wood, black liquor can satisfactorily simulate this type of effluent. Yeast extract ($120\ mg\ l^{-1}$), ammonium chloride ($70\ mg\ l^{-1}$), monobasic sodium phosphate ($35\ mg\ l^{-1}$), and ethanol (0.025%) were added to the diluted black liquor. These compounds were used to supply the amounts of nitrogen and phosphorus recommended for the process. Ethanol was used as an additional source of carbon. Table 1 lists the main characteristics of the wastewater used in this study.

It should be noted that not all the micronutrients specifically required for the growth of anaerobic microorganisms were added because this study aimed to keep the synthetic effluent as similar as possible to the wastewater from the Kraft cellulose pulping plant. In a full scale wastewater treatment plant the micronutrients would be added only if these compounds were essential to reach the needed efficiency. Acetic acid (0.01% final concentration) was added to the diluted black liquor (pH of around 13.0) to keep the pH within the 6.5–7.5 range, which is suitable for the anaerobic treatment.

2.4. Reactor operation

Operation of the reactor began with the effluent described in Table 1, with a mean chemical oxygen demand (COD) of $800\ mg\ l^{-1}$. The reactor was batch-operated from day 1 to day 4, after which it was continually operated with a 36 h hydraulic retention time. The changes in organic loading and hydraulic retention time were made according to the reactor's performance in response to the COD removal efficiencies and the other monitored parameters (Table 2). The partial recirculation of the effluent began on the 90th day of operation. Table 2 lists the recirculation ratios used along with the operational parameters that were changed during the experiment.