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Performance of a horizontal-flow anaerobic immobilized biomass (HAIB) reactor and dynamics of the microbial community during degradation of pentachlorophenol (PCP)

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ABSTRACT

The anaerobic biological treatment of pentachlorophenol (PCP) and methanol as the main carbon source was investigated in a horizontal-flow anaerobic immobilized biomass (HAIB) reactor at 30 ± 1 °C, during a 220-day trial period. The reactor biomass was developed as an attached biofilm on polyurethane foam particles, with 24 h of hydraulic retention time. The PCP concentrations, which ranged from 2.0 to 13.0 mg/L, were controlled by adding synthetic substrate. The HAIB reactor reduced 97% of COD and removed 99% of PCP. The microbial biofilm communities of the HAIB reactor amended with PCP, without previous acclimatization, were characterized by polymerase chain reaction (PCR) and amplified ribosomal DNA restriction analysis (ARDRA) with specific *Archaea* oligonucleotide primers. The ARDRA technique provided an adequate analysis of the community, revealing the profile of the selected population along the reactor. The biomass activities in the HAIB reactor at the end of the experiments indicated the development of PCP degraders and the maintenance of the population of methanogenic *Archaea*, ensuring the high efficiency of the system treating PCP with added methanol as the cosubstrate. The use of the simplified ARDRA method enabled us to monitor the microbial population with the addition of high concentrations of toxic compounds and highlighting a selection of microorganisms in the biofilm.

1. Introduction

Pentachlorophenol (PCP) was the most widely distributed and commonly used of all chlorinated aromatic pesticides in most industrialized countries, and it is still used in some tropical countries. The literature indicates that 80% of PCP production was destined for the preservation of wood [1]. PCP was frequently used as a solvent, similar to mineral spirits or diesel fuel. Over the years the continued use of PCP led to its accumulation in the food chain [2], causing public health problems involving increased cancer diseases. Despite Brazil's regulations on environmental preservation and recovery, some chlorinated aromatic compounds are still present in different areas as a "stock of hazardous chemicals", and more seriously, in areas of the southeast that underwent uncontrolled industrialization in the early 1960s [3].

Today, the correlation between pollution and health is much more widely acknowledged in Brazil, leading to the need for the development of more research and the application of innovative technologies to treat hazardous wastes.

PCP degrades in the environment by chemical and biological processes, and prokaryote and eukaryote species are active degraders of this compound in soils and sediments. These biological discoveries claim bioremediation is the best solution for the removal of toxic compounds.

The reaction of reductive dehalogenation is the most well-known biochemical pathway to dechlorinate compounds such as PCP [4,5] under anaerobic conditions. Since 1990, the anaerobic process has proved to be an appropriate method for degrading PCP under methanogenic conditions. A good example of this is the fact that anaerobic treatment has already demonstrated a consistent capacity to treat chlorinated compounds such as PCP using UASB reactors [6–8], hybrid anaerobic systems and fixed film reactors [9–12], or even expanded systems using activated granular carbon as the support medium [13]. PCP removal efficiencies in the range

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Nomenclature and units

HAIB	horizontal-flow anaerobic immobilized biomass
PCP	pentachlorophenol
PCR	polymerase chain reaction
ARDRA	amplified ribosomal DNA restriction analysis
UASB	upflow anaerobic sludge blanket
MBR	membrane bioreactors
VS	volatile solids, g/L
HRT	hydraulic retention time, h
bp	base pairs
AF	*Archaea* forward
RA	reverse *Archaea*
COD	chemical oxygen demand, mg/L
VFA	volatile fatty acids, mg/L
FISH	fluorescence in situ hybridization
MBR	membrane bioreactors
dNTP	deoxyribonucleotide triphosphates
TVS	total volatile solids, mg/L
VSS	volatile suspended solids, mg/L
BTEX	benzene, toluene, ethylbenzene and xylene
TCP	trichlorophenol
DCP	dichlorophenol
SEM	scanning electron microscopy
DGGE	denaturing gradient gel electrophoresis

of 90 –99% have been reported in research on these anaerobic reactor configurations. The highest PCP removal rate that has been reported in these UASB reactors was 140.7 mg/L per day [8] and COD was completely removed. Juteau et al. [9] achieved a maximum PCP removal of 15 mg/L per day, similar to Montenegro et al. [10] and Saia et al. [12] in fixed film reactors. COD removal was higher than 90% [10] and 80% [12].

Regardless of the type of anaerobic reactor employed, the key (design and operation) to an efficient system is the development of a protocol for the acclimation and maintenance of adequate biological activity [14]. This is especially truly for the nutritional equilibrium essential to the dehalogenation process. Dietrich and Winter [15] showed that some organic growth factors could improve chlorophenol degradation, such as yeast extract and peptone. Montenegro et al. [10] reported improved PCP degradation through the addition of methanol in effluents from semi-Kraft paper plants. Similar results were reported by Wu et al. [6].

The high efficiency of horizontal anaerobic immobilized biomass (HAIB) reactors treating PCP was demonstrated previously by Damianovic [16]. These reactors were inoculated with a mixture of sanitary and industrial sludge previously exposed to PCP using glucose, acetic acid and formic acid as the main carbon sources. Saia et al. [12] obtained similar results in a HAIB reactor inoculated with natural sediments from a polluted Brazilian estuarine area under the same operational conditions. The reactor was fed with synthetic wastewater with higher salinity and glucose as the sole carbon source.

Molecular techniques, with no previous cultivation, have been applied extensively to improve the efficiency of the analyses commonly used in the process of biological treatments [17]. Tartakovsky et al. [18] used a competitive PCR technique to observe and enumerate a strain of anaerobic pentachlorophenol (PCP) degrader, *Desulfitobacterium frappieri* PCP-1, which was added to anaerobic systems to augment a mixed bacterial community in a UASB reactor. Guiot et al. [19] and Montenegro et al. [20] reported good PCP degradation through bioaugmentation of the anaerobic biomass in a UASB system and from its enrichment in a hybrid anaerobic reactor, respectively, using the FISH (fluorescence in situ hybridization) technique. According to Guiot et al. [19], the FISH results showed that the PCP-1 strain was able to attach rapidly to the granule and colonize other layers on the granule surface.

The present strategy of applying polymerase chain reaction (PCR) and amplified ribosomal DNA restriction analysis (ARDRA) to characterize the microbial communities of the PCP-HAIB reactor biofilm was chosen due to the facility of the protocols of these two molecular techniques. ARDRA is a rapid technique for analyzing the dynamics of populations in different conditions or spatial positions. This method is based on the principle that the restriction sites on the RNA operon are maintained according to the phylogenetic patterns [21]. This technique was used previously by Zhang et al. [22] to shed light on the pioneer microorganisms responsible for the surface colonization that leads to the formation of biofilm in laboratory-scale membrane bioreactors (MBRs).

Fernandez et al. [23] used ARDRA and reported that the phylogenetic analysis revealed metabolic variations in the functionality of the community established in the methanogenic reactor through genetic changes and that an extremely dynamic community can be developed in a simple ecosystem. A huge population with little diversity contributed significantly to this dynamics. Thus, molecular analyses allowed for the detection of minor variations in the microbial diversity commonly associated with environmental factors that are rarely detected through other analyses.

In this report, we describe a PCP dehalogenating anaerobic system (HAIB reactor) added to a synthetic culture medium containing methanol as the main electron donor and carbon source, which was inoculated with biomass without previous exposure to toxic compounds. This study focuses on the behavior of the methanogenic population, an important group, for degradation of organic matter present in the substrate.

The microbial diversity of the biofilm is explained by the combination of controlled operational conditions of the anaerobic process and molecular methods, serving as the basis for a discussion of the HAIB reactor as a potential system for the large-scale bioremediation of areas contaminated with chlorinated compounds.

2. Materials and methods

The horizontal-flow anaerobic immobilized biomass (HAIB) reactor used in this study consisted of a 5.0 cm diameter, 99.5 cm

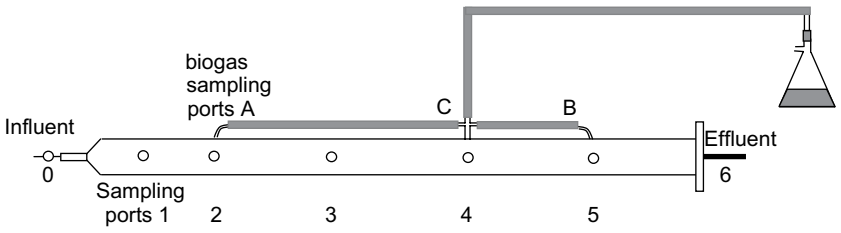

Fig. 1. Scheme of the HAIB reactor.