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Fermentative and sulphate-reducing bacteria associated with treatment of an industrial dye effluent in an up-flow anaerobic fixed bed bioreactor

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ABSTRACT

This work aims to study effects of increasing wastewater loading rates (WLRs) on the performance of an up-flow anaerobic fixed bed bioreactor and on the dynamics of the bacterial community of the sludge using polymerase chain reaction–single stranded conformation polymorphism (PCR–SSCP) methods. The analysis showed that WLRs variations influence the bacterial community structure and affect the bioreactor performance. For WLRs of 0.34–0.85 g l^{-1} d^{-1}, the bioreactor showed a high performance and maintained highest colour and chemical oxygen demand (COD) removal yields with average values of 95% and 90%, respectively. The molecular fingerprint revealed a positive correlation between the diversity and the bioreactor performance. Increasing the WLR to 1.7 g l^{-1} d^{-1} affected significantly the bioreactor performance, the colour and COD removal efficiencies dropped to average values of 75% and 70%, respectively and bacterial and archaeal communities' profiles changed.

The gas production rates increased when WLR increased. The highest value of 0.68 l l^{-1} d^{-1} was obtained at an hydraulic retention time (HRT) of 1 day. The use of molecular and microbiological methods to recover bacterial populations involved in this anaerobic process showed that fermentative (*Clostridium* spp.) and sulphate-reducing bacteria (SRB) (*Desulfovibrio* spp.) were the prominent members of the bioreactor bacterial community.

1. Introduction

Textile industry wastewater is rated as the most polluting among all industrial sectors. Important pollutants in textile effluents are mainly recalcitrant organics, colours, toxicants and inhibitory compounds, surfactants, chlorinated compounds and salts [1]. It has been proven that some of the dyes and/or products are carcinogens and mutagens and also cause harm to the flora and fauna in the natural environment [2–4]. Therefore, textile wastewater containing dyes must be treated before their discharge into the environment [5,6]. The impact and toxicity of dyes that are released into the environment have been extensively studied [7]. Colour can be removed from wastewater by chemical and physical methods including absorption, coagulation–flocculation, oxidation and electrochemical methods. These methods are quite expensive, have operational problems [8], and generate huge quantities of sludge [9]. Among low cost, viable alternatives, available for effluent treatment and decolourization, biological processes are recognised, by their capacity to reduce biochemical oxygen demand (BOD) and chemical oxygen demand (COD) [10].

Anaerobic digestion techniques are becoming increasingly important and intensively studied since they are cost-effective and environmentally safe. Several mechanisms have been proposed for the decolourization of dyes under anaerobic conditions [6]. One of these is the reductive cleavage of the azo bond by unspecific cytoplasmic azoreductases with flavoproteins (FMNH and FADH2) as cofactors [11,12]. Most microorganisms reported for the presence of azoreductases are facultative anaerobic bacteria [7]. A second proposed mechanism is an intracellular, non-enzymatic reaction consisting of a simple chemical reduction of the azo bond by reduced flavin nucleotides. More recently, it has been suggested that the dye decolourization can be achieved through a biomediated chemical reduction by extracellular redox mediators [13]. Various bacteria can therefore participate in the dye decolourization. Among the anaerobes, sulphate-reducing bacteria (SRB) can degrade the dye, but competition for this substrate may exist

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with other anaerobic bacteria, notably the fermentative acidogens involved in this anaerobic process. However, the latter microorganisms may not be the only microorganisms responsible for the decolourization [14].

The failure of many anaerobic bioreactors to operate reliably and with constant performance has underlined the need for more basic information on the biological aspects of the anaerobic digestion ecosystem [15]. In order to better understand the functions of the microbial community, a full description of the microbial ecosystem is required.

Classically, this has been addressed by enumerating members of certain microbial groups by using various culture media, followed by identification of a number of dominant isolates by phenotypic tests or molecular techniques such as ribotyping, randomly amplified polymorphic DNA analyses, and sequencing. However, cultivation-dependent approaches do not necessarily provide reliable information on the composition of entire microbial communities. It is therefore difficult to assess the significance of cultured microorganisms in microbial ecosystems because of the disparity between culturable microorganisms and the real existing biodiversity in the ecosystem. Indeed, microbial communities may contain viable non-culturable bacteria that might become culturable depending of the availability of an appropriate medium to sustain their growth [16].

Acquisition of DNA sequences is a fundamental component of most phylogenetic, phylogeographic, and molecular ecological studies. Single-stranded conformation polymorphism (SSCP) offers a simple, inexpensive and sensitive method for detecting whether or not DNA fragments are identical in sequence, and so can greatly reduce the amount of sequencing necessary [17]. SSCP can be applied without any a priori information on the species and then can give a more objective view of the microbial community. From this microbial community, the result is a pattern in which each peak can be correlated with the V3 16S rRNA sequence of one microorganism. This method SSCP has been applied to study microbial communities, e.g. in water, in the compost of organic agricultural substrate, and in anaerobic digesters [16].

This paper aims to study the diversity of bacterial species present in the sludge of an up-flow anaerobic mesophilic fixed bed bioreactor treating textile wastewater containing indigo dye and to select and isolate the most dominant SRB and fermentative bacteria involved in this process.

2. Materials and methods

2.1. Bioreactor design and operational condition

The experiments were carried out in an up-flow anaerobic fixed bed bioreactor with a total liquid volume of 0.7 l. The schematic representation of the experimental set-up is shown in Fig. 1. The anaerobic sludge was cultured in glass cylinder filled with Flocor (Φ3L3, porosity 95%, specific surface area 230 $m^2\,m^{-3}$) as a support made in polyethylene for the growth of microorganisms [18]. The bioreactor was maintained at 37 °C by running water through its outer mantle. The anaerobic synthetic medium for bioreactor feeding contained (per liter) indigo: 100 mg, starch: 1 g and 10 ml trace metal solution. The trace metal solution was prepared according to the composition mentioned previously [2,19], but with an extra addition of $CaCl_2$ (5 g/l). It contained in g/l—$MgSO_4{\cdot}7H_2O$: 5, $FeCl_2{\cdot}4H_2O$: 6, $COCl_2$: 0.88, H_3BO_3: 0.1, $ZnSO_4{\cdot}7H_2O$: 0.1, $CuSO_4$: 0.05, $NiSO_4$: 1, $MnCl_2$: 5, $(NH_4)_6\,MO_7O_{24}{\cdot}4H_2O$: 0.64, $CaCl_2{\cdot}2H_2O$: 5. The pH influent was adjusted to 7.0–7.2 by adding HCl (2 M). The medium was sterilized by autoclaving for 20 min at 121 °C. Before and after inoculation, the bioreactor was flushed with N_2 [20]. The bioreactor was inoculated with mixed non-defined cultures obtained from an anaerobic sludge from a (i) digester treating sulphate effluent, (ii) the microflora from the cow rumen, and (iii) a mixed sludge obtained from an anaerobic reactor treating industrial effluents. First the system was operated batch-wise with circulation for one month until biofilm formation was established. The indigo concentration of the feed was 30 $mg\,l^{-1}$ and 2 mM ethanol was fed as the carbon and energy source for microorganisms. Continuous feeding with the synthetic medium (soluble COD: 1700 mg/l, $OD_{620\,nm}$: 0.45) was started at low wastewater loading rates (WLRs) of 0.34 $g\,l^{-1}\,d^{-1}$ and was increased with time. Bioreactor operating conditions are presented in Table 1.

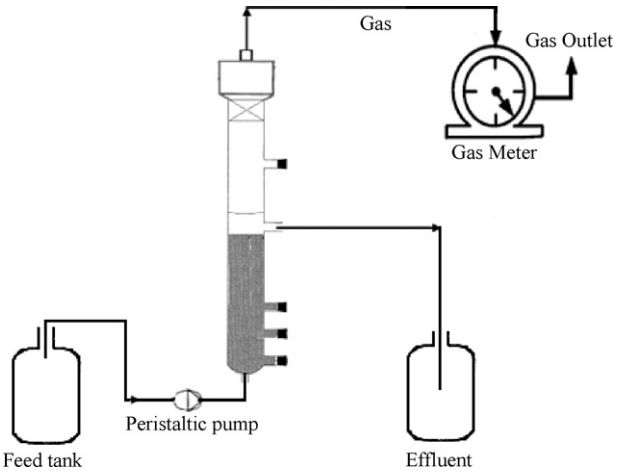

Fig. 1. Schematic representation of the experimental set-up used for the anaerobic textile wastewater treatment.

2.2. Physico-chemical analyses

The pH was measured using a digital calibrated pH-meter (HANNA pH 210). Chemical oxygen demand (COD) was measured following standard method [21]. Colour was measured by an UV–vis spectrophotometer (Jenway UV visible spectrophotometer) at a wavelength of 620 nm [10]. Measured COD and absorbance values were used for calculation of biodegradation and decolourization efficiencies. The decolourization was determined as presented in the following Eq. (1):

$$D = 100\frac{D_i - D_t}{D_i} \quad (1)$$

where D is the decolourization of dye (in %), D_i is the initial concentration of the dye, D_t is the dye concentration along the time. The COD removal was determined as presented in the following Eq. (2):

$$C = 100\frac{C_i - C_t}{C_i} \quad (2)$$

where C is the COD removal (in %), C_i is the initial COD, C_t is the COD along the time [22]. The produced gas was measured daily by gas meter, and the gas samples were analysed using an ORSAT apparatus [23]. Volatile fatty acid (VFA) content as acetic acid in effluents was determined by titration method recommended by Buchauer [24].

Bacterial growth was measured at 580 nm with an UV 160A Shimadzu spectrophotometer. Sulphide (H_2S) production was determined colorimetrically as described by Cord-Ruwisch [25]. Hydrogen production was determined with a GC-8A Shimadzu gas chromatograph. Electron microscopy observations were performed as described by Fardeau et al. [26].