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Simultaneous biotreatment of Polycyclic Aromatic Hydrocarbons and dyes in a one-step bioreaction by an acclimated *Pseudomonas* strain

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HIGHLIGHTS

- Acclimated *Pseudomonas stutzeri* efficiently remediates PAH and dye-polluted effluents.
- Viable biotreatment medium and optimum operating conditions were determined.
- Kinetics of the biotreatment and its economic advantages were ascertained.

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ABSTRACT

A *Pseudomonas stutzeri* strain acclimated to the presence of neoteric contaminants has been proposed for simultaneously remediating an effluent polluted with Polycyclic Aromatic Hydrocarbons and a diazo dye. The pollutants chemical nature imposed a strict control of both the medium composition and the operating conditions. pH, temperature and agitation rates of 7.0, 37.5 and 146 rpm, respectively, led to optimum levels of contaminant removal (higher than 60%) after RSM optimization. The validity of these conditions was checked at flask and bioreactor scale and the kinetics of the biotreatment was elucidated. The simulation of this one-step process applied at larger scale for the remediation of a 200,000 m^3/year-effluent from a leather factory was compared with a conventional two-steps option. Great reductions in treatment times and in investment and manufacturing costs were concluded, proving the promising potential of the proposed process.

1. Introduction

Globally, during the last decades economic development has marched hand in hand with an environmental collapse due to the thoughtless introduction of polluted-industrial effluents. More and more regulations prompt the academic and industrial community to come forward with competitive and environmentally friendly solutions. One of the sectors causing great environmental concerns is the leather and textile industry, since they generate a variety of pollutants ranging from surfactants, heavy metals, sulfides, acids, alkalis, and dyes to Polycyclic Aromatic Hydrocarbons (PAHs) (Li et al., 2010). The importance of the latter two kinds of contaminants has been underscored by current international environmental legislation (USEPA, 2008; EU-EEB, 2005). The health and environmental risk of these aromatic compounds has been well documented, as they involve carcinogenic, mutagenic and toxic effects, and are considered to bear a great recalcitrance (Simarro et al., 2011; Haritash and Kaushik, 2009; Bae and Freeman, 2007; Zaharia and Suteu, 2013). These concerns have urged the search of treatment technologies to remove them from the environment, and a number of physico-chemical alternatives have been successfully proposed like adsorption, ozonation, electrochemistry and flocculation (Vecino et al., 2013; Sancar and Balci, 2013; Iglesias et al., 2013; Devesa-Rey et al., 2012). However, economic and operational inconveniences have favored the application of biotechnological tools to remediate PAH- and dye-polluted effluents, since they usually involve lower cost and improved social perception (Deive et al., 2010; Moscoso et al., 2012a, 2013a).

Hitherto, research works have mainly focused on the treatment of a mixture of PAHs or dyes independently (Moscoso et al., 2012b; Álvarez et al., 2013), while a lack of knowledge is detected in the finding of suitable strategies to remediate all the contaminants when present together in the same effluent. A successful outcome should satisfy three main requirements: (i) the chemical structure of the contaminants, (ii) the selected microbial agent, and (iii) the operating conditions of the process (Haritash and Kaushik, 2009; Moscoso et al., 2012a).

Attending to these demands, the aspect related to the chemical nature should be firstly addressed to ensure that the contaminant

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is susceptible to be bioremediated in the aqueous effluent. In this sense, PAHs are thermodynamically stable molecules, with elevated hydrophobicity, so they should be solubilized by adding surfactants in order to make them bioavailable (Yang et al., 2015). On the other hand, dyes are usually hydrophilic and possess complex aromatic molecular structures that are classified on the basis of the chromophore group (Robinson et al., 2001). In this sense, azo dyes make up the most common group of direct dyes, since about 60–70% of the produced dyes belong to this category (Bae and Freeman, 2007).

In relation to the bioremediation agent, different microbial strains have been proposed as suitable candidates to yield high levels of PAHs (Ghosh et al., 2014; Peng et al., 2013) or dye removal (Liu et al., 2014; Manenti et al., 2014). However, a lack of studies is detected on the finding of microorganisms able to concomitantly biotreat both kinds of contaminants. In previous investigations, we have underscored the potential of a *Pseudomonas stutzeri* strain for the degradation of PAHs (Moscoso et al., 2012a,b,c, 2013a, 2015), metal working fluids (Moscoso et al., 2012d), or pesticides (Moscoso et al., 2013b), and its capacity to be adapted to neoteric solvents like ionic liquids (Álvarez et al., 2015). This fact was explained in terms of a genetic alteration, as the acclimated strain throve under pollutant concentrations up to 10 times higher by means of the synthesis of an exopolysaccharide (Álvarez et al., 2015). Therefore, this flexible nature has encouraged us to apply it for the combined bioremediation of both kinds of pollutants, which is the main aim of this work.

Special heed must be paid to the operating conditions selected to develop the bioprocess once the biotreatment medium was designed. Factors like pH, temperature, and agitation should be optimized prior to sketch the bioremediation process at real scale. Valuable means to reach this target are computational tools like simulation software (SuperPro Designer v8.5) and experimental designs (Design Expert 7.0), saving time and money to reach the optimum process.

In summary, considering the pollutant charge of textile and leather waste effluents, three model PAHs of low (phenanthrene, PHE) and high molecular weight (pyrene, PYR, and benzo[a]anthracene, BaA) and a common azo dye (Reactive Black 5) have been selected. This scenario raises problems related to the different nature of the pollutants such as the degree of hydrophobicity and the carbon source, which will compel us to optimize the biotreatment medium and propound the ideal range of operation. Additionally, the bioprocess will be kinetically characterized both at flask and bioreactor scale by fitting to known models and these data will be employed to simulate the process and will pay off in a one-step biotreatment strategy.

2. Methods

2.1. Chemicals

The pollutants Reactive Black 5 (RB5), phenanthrene (PHE), pyrene (PYR) and benzo[a]anthracene (BaA) (structures shown in Fig. S1) were acquired from Sigma–Aldrich, with purities higher than 99%. The same supplier provided the non-ionic surfactant Tween 80, benzyl benzoate, salts of the medium and chloroform. Glucose was purchased from Scharlau, and HCl and hexane were supplied by Prolabo.

2.2. Microorganism

The bacterium *P. stutzeri* CECT 930 was acquired from the Spanish Type Culture Collection (ATCC 17588). This bacterium was acclimatized for two months in a lab-scale bioreactor in the presence of $C_2C_1imC_2SO_4$ (0.2 mM) under controlled agitation, aeration and temperature as previously reported (Álvarez et al., 2015).

2.3. Bioremediation medium

Minimal medium (MM) was used, composed of (g/L in distilled water): $Na_2HPO_4{\cdot}2H_2O$ 8.5, KH_2PO_4 3.0, NaCl 0.5, NH_4Cl 1.0, $MgSO_4{\cdot}7H_2O$ 0.5, $CaCl_2$ 0.0147. MM also contained trace elements as follows (mg/L in distilled water): $CuSO_4$ 0.4, KI 1.0, $MnSO_4{\cdot}H_2O$ 4.0, $ZnSO_4{\cdot}7H_2O$ 4.0, H_3BO_3 5.0, $FeCl_3{\cdot}6H_2O$ 2.0. Different concentrations of glucose and Tween 80 were also included in the culture medium as carbon source and solubilizing agent, respectively.

2.4. Biotreatment at flask scale

It was carried out in 250 mL-Erlenmeyer flasks containing 50 mL of MM. The pH was initially adjusted to 7.0 and the MM was autoclaved at 120 °C for 20 min. The dye (0.04 g/L) and PAHs (100 μM each) were sterilized by filtration through a 20 μm filter prior to the addition to the autoclaved medium in order to avoid any possible alteration of the chemical structure of the pollutants. The flasks were inoculated (3% v/v) with previously obtained cell pellets, which were them incubated in an orbital shaker (Thermo Fisher Scientific 496) at 37 °C and 146 rpm.

2.5. Biotreatment at bioreactor scale

The scaling up of the process was carried out by operating in a 2-L bioreactor (model BIOSTAT®B-MO), filled with 1.5 L of medium. The temperature and initial pH was fixed at the optimum operating conditions. It was inoculated with actively growing cells (3% v/v) and air was sparged at a continuous rate of 0.17 vvm (volumes per minute, which involves the use of an air flowrate of 0.25 L/min).

2.6. Analytical methods

2.6.1. Biomass determination

Cells were harvested by centrifugation (10 min, 9300 g, and 4 °C), and the supernatant was reserved for pollutants analysis. Biomass concentration was measured by turbidimetry at 600 nm in a UV–vis spectrophotometer (UV-630 Jasco), and the values were converted to grams of cell dry weight per liter using a calibration curve. (Biomass (g/L) = 0.5663·Absorbance – 0.0401, R^2 = 0.996).

2.6.2. Adsorption test

PAHs biosorption over the biomass was determined as follows. 50 mL of culture medium were taken and centrifuged for 10 min at 5.900 g and 4 °C. The supernatant was withdrawn and biomass was freeze-dried during 4 h at −40 °C and $7.9 \cdot 10^{-5}$ atm using a TelStarCryodes. Afterwards, 10 mL of hexane were added and ultrasounds were applied (Bransonic 3510) for 30 min. Again, the sample was centrifuged for 10 min and 100 μL of supernatant were taken into a vial, where 10 μL of Internal Standard (IS) were added. Samples were analyzed by GC–MS as explained later on.

2.6.3. Dye decolourisation

Dye concentration in the culture media was analyzed by UV–vis spectrophotometry taking into account the maxima wavelength recorded for RB5 dye (597 nm). Each decolourisation value was the mean of two parallel experiments. Abiotic controls (without microorganisms) were always included. Decolourisation (D) was expressed in terms of percentage units by using the expression: