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Polycyclic aromatic hydrocarbons removal by immobilized bacterial cells using annonaceous acetogenins for biofilm formation stimulation on polyurethane foam

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ABSTRACT

Wastewaters containing polycyclic aromatic hydrocarbons (PAH) must be treated before discharge in water bodies to avoid environmental pollution and comply with environmental protection regulations. The development of novel PAH removal technologies from wastewaters is thus of great importance. The aim of this work was to use immobilized bacteria on polyurethane foam (PUF) for acenaphthene, fluoranthene and pyrene removal using annonaceous acetogenins (ACG) to stimulate biofilm and possibly enhance PAH removal activity. Different ACG were tested for their capacity to stimulate biofilm formation on *Pseudomonas monteilii* P26, a known naphthalene degrader. Itrabin, jetein and an ethanolic extract of *Annona cherimola* pulp were selected for showing a high stimulation level at low concentration in microplate biofilm formation assay. On PUF, the biofilm formation was strongly stimulated by itrabin and the ethanolic extract. However, there was no difference between the PAH removal percentages of the different systems (sterile PUF, cell immobilized in presence of ACG and cells immobilized without ACG). In average, 99% acenaphthene, 98% fluoranthene and 92.5% pyrene were removed in 7 days mostly by sorption (initial PAH concentration was 50 ppm). A 15–22% removal was attributed to biodegradation or bioaccumulation in the systems with immobilized cells. In this case, stimulating biofilm formation did not enhance PAH removal by immobilized bacteria on PUF.

1. Introduction

Polycyclic aromatic hydrocarbons (PAH) are found in domestic, industrial wastewater and urban storm water [1–3]. They originate from incomplete combustion of fossil fuels and are characterized for presenting two or more benzene fused rings. PAH are persistant pollutants and have carcinogenicity and mutagenicity properties [2]. Since these compounds are classified as priority pollutants by the majority of the environmental protection agencies of the world, the discharge of water that contains them into receptor water bodies is regulated. Therefore, maximum concentration limits of PAH in wastewater must be observed. To comply with regulations, economic and efficient wastewater treatment methods have to be developed as it is reflected in the extense literature that has been published in recent years about the subject [4–7]. The use of bacteria immobilized in biofilm for PAH removal from a liquid phase could be a promising technology. It is well known that biofilms exhibit advantages over planktonic cells regarding its resilience against a harmful environment as could be a wastewater [8]. Besides, since the biofilm is attached to a surface, it could be easily removed from the liquid without using costly separation techniques. In addition, the biofilm support surface could contribute to the removal of contaminants if it possesses sorbent capacity. The addition of stressing factors during the cell immobilization process can stimulate biofilm formation increasing cell density and subsequent contaminant removal [9,10]. Annonaceuos acetogenins (ACG) are natural stressors isolated from

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plants of the Annonaceae family [11,12]. They have cytotoxic activity and have been studied as antitumoral, antiparasitic and pesticidal compounds [13,14]. They have also been proposed as biofilm formation stimulants for a *Pseudomonas plecoglossicida* strain [11,15,16]. The aim of this work is to use ACG to produce high density biofilms of *Pseudomonas monteilii* P26 to be used in the removal of acenaphthene, fluoranthene and pyrene from water. These pollutants are, respectively, the numbers 001, 039 and 084 in the US EPA priority pollutant list [17]. This means that these pollutants are regulated by this agency and therefore their disposal limited.

The ACG itrabin, jetein, laherradurin and squamocin, as well as an ethanolic crude extract of *Annona cherimola* seeds, were tested for biofilm stimulation. Itrabin and jetein are two saturated γ-lactone which present 35 carbon atoms with two or one tetrahydrofuran (THF) rings respectively [18]. Laherraduin and squamocin are, respectively, saturated and unsaturated γ-lactone with 37 carbon atoms and two THF rings [16] (Fig. 1). Polyurethane foam (PUF) was used as support for biofilm formation because of its low cost and known sorbent capacity of hydrophobic compounds [19]. Corn steep liquor was used as culture medium since it is a low cost industrial waste.

2. Materials and methods

2.1. Microorganisms and culture conditions

The strain *Pseudomonas monteilii* P26 (Genbank Acc. Num. HE798531) was isolated from sediments contaminated with petroleum oil originated from Patagonian coast, in Caleta Cordova, Chubut, Argentina [20]. Pure cultures were routinely maintained in JPP broth (% m/v: NaCl, 2; yeast extract, 0.1; meat peptone, 0.2; pH = 7.0) [21] with 20% v/v glycerol at −20 °C. Cell propagation was carried out in corn steep liquor (10% v/v, pH 7 and sterilized by heat at 121 °C for 15 min) at 30 °C and 180 rpm for 48 h. The inocula were obtained from frozen stock cultures and its final concentration was 5% v/v.

2.2. Extraction, purification and characterization of annonaceous acetogenins

Itrabin, laherradurin, jetein and squamocin (ACG) were extracted by maceration in ethanol of dried and grinded *Annona cherimola* seeds (1000 g). Vacuum evaporation of the solvent yielded an ethanolic extract. The ethanolic extract was first

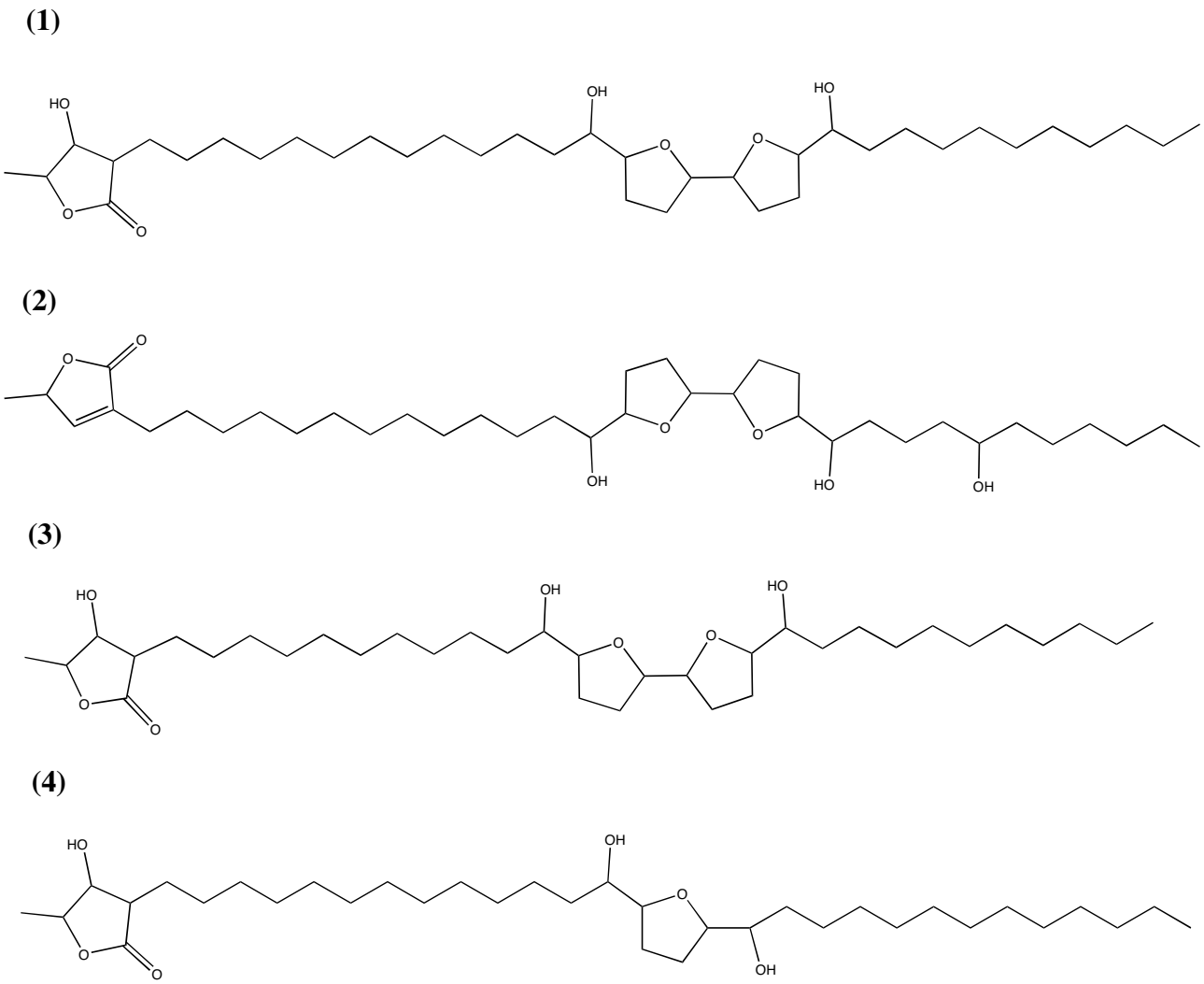

Fig. 1. Annonaceous acetogenins used in this study. (1) Laherradurin. (2) Squamocin. (3) Itrabin. (4) Jetein.