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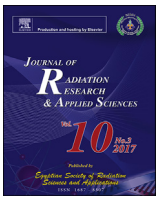

Bio-degradation of Bisphenol A by *Pseudomonas aeruginosa* PAb1 isolated from effluent of thermal paper industry: Kinetic modeling and process optimization

V. Vijayalakshmi [a, *], P. Senthilkumar [a], K. Mophin-Kani [b], S. Sivamani [c], N. Sivarajasekar [d], S. Vasantharaj [a]

[a] PG & Research Dept. of Biotechnology, Hindusthan College of Arts & Science, Coimbatore 641028, TN, India
[b] PG Department of Civil Engineering, UKF College of Engineering & Technology, Parippally, Kollam 691302, Kerala, India
[c] Chemical and Petrochemical Engineering Section, Engineering Department, Salalah College of Technology, Thumrait Road, Salalah 211, Sultanate of Oman
[d] Department of Biotechnology, Kumaraguru College of Technology, Coimbatore 641049, TN, India

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ABSTRACT

A bacterium isolated from effluent of thermal paper industry and identified as *Pseudomonas aeruginosa* (PAb1) based on 16SrRNA gene sequence analysis which could grow on basal mineral salt medium upon bisphenol A, which functions as an exclusive carbon source. Physicochemical variables of thermal paper industry effluent noted were significantly greater than the typical limit due to pollution of the acquiring water systems. The mathematic kinetic models like Monod, Moser and Tesier models were applied for batch fermentation of bisphenol A degradation in basal salt medium and the half saturation coefficient (K_S) and the regression coefficient R^2 using Monad, Moser and Tesier kinetic models registered as 9.947 g/L, 12.46 g/L and 14.14 g/L and 0.91, 0.94 and 0.84 respectively. Besides, the utmost specific growth rate μmax was witnessed as 0.841 h^{-1} for the *P. aeruginosa* (PAb1) regarding BPA degradation. Metabolic intermediates like phenol, acetophenone, and hydroquinone and *p*-hydroxybenzoic acid were also determined through the degradation process by GC-MS. The metabolic pathway of BPA degradation by the bacterial isolates was also designed in today's analysis. A probabilistic statistical model originated using Box-Behnken response surface methodology and process variables were optimized by nonlinear optimization.

1. Introduction

Bisphenol A (BPA) is an organic chemical compound, during polymerization process they produced from phenol and acetone by an acid or alkaline catalyzed condensation reaction hence the suffix name A. BPA is one of the harmful chemical, stated internationally (European Union Risk Assessment Report, 2003 and Nakanishi, Miyamoto & Kawaski, 2005) it is industrially important chemicals key raw materials for the production of polycarbonates, epoxy resins, food products, thermal paper industry and many other products (spivack, Leib, & Lobus, 1994; Kolvenbach et al., 2007; Inoue et al., 2008). Their wide spread consumption of this chemical substance, an significant amount of BPA is directly discharged into terrestrial and aquatic surroundings and becomes an severe toxic to aquatic microorganisms (Alexander, Dill, Smith, Guiney, & Dorn, 1988).

BPA is recognized as one of endocrine disruptors (Krishnan, Sathis, Permuth, & Tokes, 1993) it mimic human feminine sexual hormone estrogen. In addition, it is also reported as mutagenic and carcinogenic chemicals (Chai et al., 2005; Kang and Kondo, 2003; Kang, Ri, & Kondo, 2004; Kang, Yshikikatayama, & Kondo, 2006). Bisphenol A can be employed in paper mill industry as a graphic, color programmer and used as antioxidant in the color developing layer of thermal paper documents. Based on the wide application of Bisphenol A, it might be released directly into nearby aquatic systems through manufacturing process and recycling procedure of thermal paper sectors. Microbial biodegradations is a significant

* Corresponding author.
E-mail address: researchviji@rediffmail.com (V. Vijayalakshmi).
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mechanism for reduction of varied environmental pollutants. The significant amount of bacteria's have been isolated from different environmental resources like enrichments of sludge from waste materials treatment plant (Lobos, Leib, & Su, 1992;; Ike, Jin, & Fujita, 2000) aquatic conditions (Miho Sasaki, Maki, Oshiman, Mastumura, & Tsuchido, 2005), soil (Roben & Abeliovich, 2000; danzyl., Sei, Suda, Michihikoike, & Fujita, 2009) and compost leachate (Zhang et al., 2007). There are few number of bisphenol A degrading bacteria system was developed in lab conditions. However, the efficiency of bacterial degradation is low at high concentrations (Tropel & Van der Meer, 2004; Lee, Koo, Choi, Chai, & Jeung, 2005). Hence, the present study was designed to identify the bacterium from thermal paper effluents discharging points of nearby aquatic environments for BPA degradation. In addition an attempt was also made for kinetic analysis of biodegradation using Monod's, Moser and Tesier models, identification of possible metabolic possible metabolic pathway along with intermediates by using Gas chromatography Mass spectroscopic examination, optimization of degradation process variables such as initial concentration of BPA, pH and temperatures by using Box-Behnken response surface strategy and an empirical statistical model.

2. Experimental design

2.1. Reagents, sample collection and media

The pure nature of BPA, all microbiological media was purchased from Himedia, Bangalore, India. The reagents and solvents used for GCMS analysis is HPLC grade. The effluent samples were collected from the flow region at 5–10 m depth of effluent discharge point in sterile screw capped bottles, brought to laboratory immediately and stored at 4 °C.The Nutrient agar media and Luria Bertani (LB) broth was used for isolation and purification of bacterial species from effluent mixing zone. Basal Salt Mineral (BSM) medium was used for bisphenol A degradation study (Zhang et al., 2007).

2.2. Analysis of physicochemical parameters

The Physicochemical parameters like Electrical Conductivity (EC), pH, Temperature (Temp) and Turbidity (TDY) of water samples were analyzed in-situ and expressed by Nephlo-Turbidity Unit (NTU). The other parameters like Dissolved Oxygen, Total Dissolved Solids, Total Suspended Solids, Total Hardness, Total Alkalinity, Ammoniacal nitrogen (NH_4N), Nitrite (NO_2), Nitrate (NO_3), Phosphate (PO_4), Chemical Oxygen Demand (COD) Biochemical Oxygen Demand (BOD), Total Kjeldhal nitrogen (TKN), Chloride (Cl-), Sulphate (SO_4), Magnesium (Mg), Sodium (Na) and Potassium (K^+) were analyzed in laboratory after sample preservation, the results were expressed in mg/L, except pH, EC and Temperatures (Khanna, Bhutita, ni, & Matta, 2011); American Public Health Association (APHA, 2005) guidelines.

2.3. Isolation of bisphenol A resistant bacteria

The bacterium present in water sample from the effluent disposal site of paper mill industry was enriched in the Luria-Bertani (LB) agar plates containing 5 mM of Bisphenol A. The LB agar plates were prepared by dissolving 1 g NaCl, 1 g tryptone, 0.5 g yeast extract, 1.5 g agar and the pH was adjusted to 7–7.2. The medium was sterilized at 121 °C for 15 min. The growth of the bacterial colonies was observed after 24 h incubation at 37 °C. The morphologically distinct bacterial strains were selected and isolated on nutrient agar medium by repeated streak plate method. The bacterial colonies were screened for their bisphenol A degradation in the LB medium and the best isolates were selected.

2.4. Identification of bacterial strains

The isolated strains were identified using the morphological features like gram staining endospore staining, motility test and 16S ribosomal RNA sequence (16SrRNA) analysis. Genomic DNA isolation was carried out using the standard protocol (Ausubel, 1992). The widespread universal bacterial 16SrRNA gene primers viz Pf: 5^{I}–AGAGTTTGACCTGGCTCAG-3^{I}, and Pr-5^{I}- ACGGCTAC-CTTGTTACCGACT- $3^{I,}$ were used for PCR amplification of 16SrRNA gene. Sequences were initially analyzed at National Centre for Biotechnology Information (NCBI) by using Basic Alignment Search Tool (BLAST), corresponding sequences were matched and the phylogenic tree was constructed using Neighbor-Joining method.

2.5. Determination of biomass and growth of Pseudomonas aeruginosa with BPA at different concentrations in basal salt medium

The bacterium, *Pseudomonas aeruginosa* (PAb1) was transferred to basal mineral salt medium (BSM) with varying concentrations (1 Mm–35 mM) of BPA. The chemical present in the medium acts as sole carbon and energy source. The bacterial cells were cultivated in Erlenmeyer 500 ml flask added with 100 ml medium, at a temperature of 35 °C and the pH was adjusted to 7. The flask was incubated in rotator shaking incubator at 120 rpm.

The bacterium *Pseudomonas aeruginosa* (PAb1) grown in a nutrient medium containing different concentration BPA ranging from 5 mM to 35 mM, after reaching 1 OD, 100 ml of sample withdrawn from the conical flask and centrifuged at 4000 rpm for 20 min. The supernatant was decanted and the pellet was re-suspended in to deionized water again re-centrifuged. The supernatant was decanted and pellet was rinsed off in to a pre-weighed 0.45 μm pore filter paper. The filter paper was dried in hot air oven, cooled in desiccators at room temperature, and reweighed until a constant weight is obtained. The difference between the pre-weighed filter paper and the final constant weight is the Cell Dry Weight (CDW) (Abuhameda, Bayraktar., Mehmetŏglu, & Mehmetŏglu, 2004).

2.6. Microbial growth kinetics of BPA degradation-Theoretical analysis

Direct monitoring of the cell morphology and biomass concentrations in culture medium is not easily possible. However, using mathematical model is a suitable way to describe the substrate uptake and cell growth behavior of the microbes (Ardestani, 2011). The values of biomass concentration derived from batch culture of *Pseudomonas aeruginosa* (PAb1) in nutrient medium was analyzed by application of mathematical models like Monod, Moser and Tesier type kinetic equations in order to determine the kinetic parameters. The specific growth rate (μmax) in exponential phase was calculated using the following equation:

$$\mu max = \ln \frac{CDW2 - CDW1}{t2 - t1} \quad (1)$$

where, μmax - maximum specific growth rate, and t = biomass at different time points (t_1 and t_2 respectively).

The microbial growth kinetics has been described by an empirical model equation (2) actually suggested by Monod, 1942. The Monod model unveiled the idea of a rise limiting substrate. The following equation is needed for the experimental data analysis of growth kinetics.

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