Bioresource Technology 102 (2011) 2242–2247

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Degradation of Tectilon Yellow 2G by hybrid technique: Combination of sonolysis and biodegradation using mutant *Pseudomonas putida*

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ARTICLE INFO

Article history:
Received 5 July 2010
Received in revised form 2 October 2010
Accepted 6 October 2010
Available online 12 October 2010

Keywords:
Hybrid technique
Tectilon Yellow 2G
Dye degradation
Mutagenesis
Growth kinetics

ABSTRACT

Degradation of Tectilon Yellow 2G (TY2G), an azo dye has been studied by hybrid technique involving pretreatment by sonochemical method and further biological treatment by *Pseudomonas putida* mutant. Pretreatment experiments were carried out by sonolysis of the dye solution at different concentrations (100–1000 mg/L). Wild type Gram-negative *P. putida* species isolated from the textile effluent contaminated soil, which was found to be effective towards dye degradation, has been acclimatized so as to consume TY2G as the sole source of nutrition. Mutant strain was obtained from the acclimatized species by random mutagenesis using the chemical mutagen ethidium bromide for various time intervals (6–30 min). The optimum mutagenesis exposure time for obtaining the most efficient species for dye degradation was found to be 18 min. An efficient mutant strain *P. putida* ACT 1 has been isolated and was used for growth experiments. The mutant strain showed a better growth compared to the wild strain. The substrate utilization kinetics has been modeled using Monod and Haldane model equations of which the Haldane model provided a better fit. The enzyme kinetics of the mutant and wild species was obtained using Michaelis–Menten equation. The mutated species showed better enzyme kinetics towards the degradation of TY2G.

1. Introduction

The pollution of water resources and soil due to colored effluents has grabbed the attention of scientific investigators around the world. The environmental damage created by the discarded and untreated coloring materials from the textile and dyeing, food additives, cosmetics and printing industries is more pronounced in the areas nearby. This alarming situation has attracted the interest of scientists and technologists towards the introduction of novel technologies towards wastewater management and effluent treatment. Azo dyes are widely used and dangerous class of colored compounds that have been found to be both carcinogenic and mutagenic (García-Montaño et al., 2007; Pinheiro et al., 2004). Hence, stringent standards have been imposed over the treatment of effluents containing azo colorants in both developed and developing countries. Azo dyes contain —N=N— (azo) group which upon reduction produces highly toxic aromatic amines. Therefore, the hybrid technique approach for the degradation of azo dyes becomes essential.

Sonolysis is an efficient method for the degradation of textile effluents and this method is quick and reliable since it is a physico-chemical method (Gopinath et al., 2010). In sonolysis, pressure waves generated are directed into the dye solution, producing cavitation or micro bubbles. These are subjected to expansion and compression cycles that lead to the violent collapse of the bubbles thus generating higher temperatures of the range 2000–5000 K. These localized high temperatures lead to the pyrolytic cleavage of complex molecules into hydroxyl, hydrogen and organic radicals. Sonochemical degradation of various azo dyes such as Basic Blue 41 (Abbasi and Asl, 2008), C.I. Direct Red 23 (Song et al., 2007), Methyl Orange (Okitsu et al., 2008), C.I. Reactive Black 5 (He et al., 2007a) and C.I. Reactive Yellow 84 (He et al., 2007b) were reported in the literature earlier. The main disadvantages of sonochemical degradation are the expensive nature of the process and high energy consumption.

Biodegradation of dyes are highly economical yet time consuming process (Santos dos et al., 2007). Bafana et al. (2007) studied the decolorization of azo dye Direct black 38 by acclimatized textile sludge and reported 76% decolorization. It has been reported that mutagenesis of bacterial species enhances the dye degrading and consuming capability of the bacterium. Random mutagenesis can help in producing distinct strains of the microorganisms which are improved towards several applications (Stanbury et al., 1995; Lotfy et al., 2007; Meleigy and Khalaf, 2008; Kamath et al., 2008). Several ways of inducing mutation exist such as, UV irradiation, ethyl methyl sulfonate (EMS) and ethidium bromide (EtBr) (Chandra et al., 2008). Gopinath et al. (2009) reported that chemical method of mutagene-

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doi:10.1016/j.biortech.2010.10.022

sis produces effective strains which were more effective in degrading the azo dyes. Acuner and Dilek (2004) studied the treatment of TY2G by *Chlorella vulgaris* and reported around 88% degradation of TY2G using acclimatized microorganisms.

TY2G is an azo dye containing various functional groups such as sulphonic acid, chlorine and heterocyclic nitrogen atom. Thus, the degradation of TY2G by microorganisms requires the enhanced ability of the microbes to get acclimatized to break the azo bonds and also to survive the chemical species constituting the dye (Acuner and Dilek, 2004). The knowledge on the biodegradation of TY2G is limited. This motivates the study of enhanced biodegradation of TY2G using hybrid technique and mutagenesis. Development of microorganisms with enhanced capacities for biodegradation towards various azo dye compounds improves the spectrum of colored effluents that can be treated biologically. The treatment of a mixture of azo dye compounds as a combination also simulates the typical industrial effluent which can be treated effectively, which demands the requirement of knowledge on biodegradation of various azo dyes. In this work, the degradation of azo dye, TY2G was achieved by the combination of sonochemical degradation followed by the biodegradation of mutated *Pseudomonas putida*. Different growth and degradation models were tested with the experimental results and best fits were found out.

2. Methods

2.1. Isolation and culture of microorganisms

The microorganisms employed in this study were isolated from the soil sample contaminated with textile effluents, collected from Thirupur, South India. The soil sample was inoculated in a nutrient broth (10% w/v) containing TY2G (50 mg/L) and incubated at 37 °C. After 48 h of incubation, 1 mL of the culture was serially diluted and aliquots of 0.1 mL were withdrawn from the 10^{-5} dilution. The aliquots of the sample were then inoculated by spread plate method in nutrient agar plates containing 200 mg/L TY2G and incubated at optimum temperature of 37 °C for 48 h. Control plates were also maintained. After 48 h of incubation, the colonies were screened for their capability to form a clear zone around them. Those colonies that showed a clear zone were considered to be effective towards degradation and these species were subcultured again in a nutrient broth containing 200 mg/L of TY2G. Morphological and biochemical tests were performed. The 16SrRNA sequence analysis of the isolate was performed as per the procedure described by Okeke et al. (2008). The 16SrRNA nucleotide sequence was initially analyzed at NCBI server using BLAST tool and the corresponding sequences were downloaded. The bacterial nucleotide sequences were aligned using the CLUSALX program. The phylogenetic tree was constructed by the neighbor-joining method using MEGA-4.1 (Beta) software. The results showed that the microorganism responsible for the degradation of the dye to be *P. putida* and this was used for further studies.

2.2. Acclimatization of P. putida

To acclimatize the isolated *P. putida* towards azo dye degradation, the isolated strain was initially inoculated in nutrient broth containing 200 mg/L TY2G dye as the growth medium. Then, the broth was incubated at 37 °C until the color disappeared in the growth medium. After 48 h of incubation, i.e., after complete color disappearance, the biomass produced was used as inoculum for the next step. In the next step, nutrient concentration in the broth was reduced by 10% and was incubated until the color removal occurs. The reduction of nutrient concentration in the broth was gradually increased and biomass produced in each step was used as inoculum of subsequent steps. Finally, after confirming the acclimatization of the bacterium in the dye added growth medium, the azo dye TY2G was used as a sole source of nutrition for its growth.

2.3. Ultrasound equipment

A tank type sonochemical reactor with an operating frequency of 30 kHz was used for the pretreatment process. The tank was made up of stainless steel and the bottom of the tank was fitted with ultrasonic transducer for the production of ultrasonic waves. The equipment was provided with timer control and temperature indicator. The %decolorization was determined at regular intervals using:

$$\%Decolorization = \frac{[dye]_i - [dye]_o}{[dye]_i} \times 100 \quad (1)$$

where $[dye]_i$ is the initial dye concentration (mg/L) and $[dye]_o$ is the observed dye concentration (mg/L) at time t (min).

2.4. Induction of mutagenesis

The cells were harvested from the culture media by centrifugation at 6000 rpm for 10 min. The cell pellet obtained was washed twice with distilled water and was resuspended in sterile deionised water. Colony counting method was employed to determine the bacterial count and the count was adjusted to 2.36×10^7 CFU/mL. To carry out chemical mutagenesis, the cell pellet was suspended in 20 mL of 20 μg/L ethidium bromide (EtBr) solution and was shaken thoroughly in an orbital shaker for 30 min. Aliquots were withdrawn at regular intervals (6 min) and serially diluted for viable cell counting by spread plate technique and screening. The %survival was calculated using:

$$S = \frac{N_i - N_d}{N_i} \times 100 \quad (2)$$

where S is the %survival, N_i is the initial viable cell count in CFU/mL and N_d is the viable cell count after mutation in CFU/mL.

2.5. Selection of mutants

To select the mutant colonies, 1 mL of the mutated sample was serially diluted to obtain a dilution of 10^{-5}. From the diluted sample, 0.1 mL was inoculated in an agar medium containing TY2G and was incubated at 37 °C for 24 h. The colonies formed were counted and each colony was inoculated in a test tube containing 10 mL of TY2G (300 mg/L), alongside a control broth of wild species. The %decolorization was estimated after 12 h and the strains that showed better decolorization compared to the wild species were considered as positive mutants and the rest were considered as negative. Further, the mutant that showed the best decolorization was isolated and named as *P. putida* ACT 1 and was used for further experiments.

2.6. Decolorization experiments

Batch experiments were conducted to determine the degradation capacity of the mutated strains. 100 mL of TY2G solution of concentrations in the range 100–1000 mg/L was taken and pretreated for 1 h by sonolysis. The pretreated solutions were taken in 250 mL Erlenmeyer flasks and inoculated with 1 mL of *P. putida* ACT 1 and incubated at 37 °C. Samples were withdrawn at 3 h time interval to determine the residual TY2G and biomass concentrations.