Advances in Bioresearch
Adv. Biores., Vol 6 (4) July 2015: 88-92
©2015 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
ICV 7.20 [Poland]

Advances in Bioresearch

ORIGINAL ARTICLE

Bioremediation of Basic Red 46 by *Pseudomonas* sp.

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ABSTRACT
Pollution resulting from industries has various effects on the organisms and environment. These pollutants are often form human industrial operations. Dyes are the most significant water pollution standards andcan harmwater resources. Some dyes are also toxic, carcinogenic and mutagenic. Dyes affect on the solubility of gases in water and prevent oxygen transfer to the varied part of water resources and perish organisms of water. Thus, removing dyes from wastewaters has become a major problem which illustrates the need for new water treatment methods. In this study, the ability of Pseudomonas sp.isolated from the soil of the East Azerbaijan, Iran in bioremediation of Basic Red 46 (BR46) was investigated. These isolate is more compatible with the weather of northwest region of Iran because they are indigenous. Isolate able to remove BR46 from contaminated water up to 32.76%. Reusability experiments confirmed the biodegradation process and four intermediate compounds produced during. This process was identified by GC-MS technique. It seems that the products of BR46 biodegradation are:2-Methyl Butanal, Benzenol, 2-Methyl-5-(1-methylethyl)phenol and Hexadecanoic acid.
Keywords: *Basic Red46, Pseudomonas, Biodegradation, Dye, Bioremediation*

Received 06/04/2015 Accepted 13/06/2015

How to cite this article:
Najibeh S, Ali F, Nader H. Bioremediation of Basic Red 46 by *Pseudomonas* sp. Adv. Biores. Vol 6 [4] July 2015: 88-92**.** DOI: 10.15515/abr.0976-4585.6.4.8892

INTRODUCTION

World population growing and increasing needs to various industries have led to the accumulation of a wide variety of pollutants in the environment. There are more than 3000 different varieties of azo dyes that are used extensively for textile, food and pharmaceutical industries. Because of this, industrial effluents often contain residual dyes, which affect water quality and may become a threat to organism's health, since certain azo dyes or their intermediates may be highly toxic. Some azo dyes have been recognized as the most problematic compounds in textile effluents as they are difficult to remove due to their low exhaustion and high water solubility [1]. So bioremediation of azo dyes waste water has become a matter of great concern [2]. Toxicity of reactive dyes has been describe at concentrations as low as 5.2mg/L [3]. Since these synthetic dyes have been widely used in different industries such as tannery, textile, food, paper, and pharmaceutical, the effluent discharge from these industries has destructive effects on the human health and environment, decrease sunlight penetration and oxygen solubility in water, toxic and mutagenic effects in living organisms [1].Recently, diverse methods dealing with treatment of textile wastewater like convention all methods including physico-chemical treatment, biological oxidation, adsorption and advanced oxidation processes, photolysis etc., have been examined[3]. Advanced processes, such as color adsorption by activated carbon, have been suggested, but not widely applied because of the high cost [4]. So these methods available for remediation have limited use and are not cost effective [5].One category of the most frequent dyes present in the wastewater that poses a serious health problem is azo dyes group. Basic Red 46 (BR46) as a mono azo dye is used commonly in acrylic, wool, and polyester textile printing and usually exists in their effluents. Therefore, it has always been meaningful to find effective and economic methods for treatment of dye contaminated waters. Biological methods which use bacteria, fungi and algae as cleanup agents are eco-friendly and mineralize dyes at low cost [1].Biological degradation is a viable method for bioremediation of organic

compounds. This technology applies metabolic variations of microorganisms to degrade hazardous pollutants. It's while biodegradation strategies do not suffer from these restrictions [5].Biodegradation of dyes has been widely studied. In 2003 ValliNachiyar and Suseela Rajkumar in studied degradation of a tannery and textile dye, Navitan Fast Blue S5R by *Pseudomonasaeruginosa*. In this project the degradation of Navitan Fast Blue S5R, a very important commercial dye in the textile and tannery industries was investigated. *P.aeruginosa* decolourized this dye at concentrations up to 1200 mg per liter and the microorganism was also able to decolorize various other tannery dyes at different levels. Decolourization of this dye started when the microorganism reached late exponential growth phase and after 24 h of incubation nearly 90% of the dye was degrade. HPLC analysis verified the formation of metanilic acid from the dye, which on further incubation was completely metabolized under shaken culture [6]. In 2008 Azeem Khalid *et al.,* examined decolorization of azo dyes by *Shewanella* sp. under saline conditions. This examination was follow out with 4azo dyes in the presence of varying concentrations of salt with *Shewanella putrefaciens* strain AS96. They indicated that *Shewanella* could be effective for the treatment of dye-containing industrial effluents containing high concentrations of NaCl [7]. The present work was aimed to assess bioremediation potential of BR46 by *Pseudomonas* sp. Experiments clarified biodegradation process and intermediate compounds produced during removal process were distinguished by gas chromatography mass spectrometry (GC-MS) analysis.

MATERIALS AND METODS

Basic Red 46 ($C_{18}H_{21}N_6Cl$) was purchased from Merck. Figure 1 represents the chemical structure of BR46 [1]. *Pseudomonas* sp. isolated from soil of East Azerbaijan, Iran, by the enrichment technique. Isolate was first sub-cultured from stock cultures on Mueller Hinton Agar containing (per liter): 2g Beef Extract, 17.5 g Acid Hydrolysate of Casein, 1.5 g Starch, and 17g agar. After incubation at 30°C for 1 day, the isolate *Psudomonas* sp. was transferred to Mueller Hinton Broth Medium, containing (per liter): 2 g Beef Extract, 17.5 g Acid Hydrolysate of Casein and 1.5 g Starch. Once the culture reached to proper turbidity, 300 µl of the culture with 0.5 McFarland turbidity was used for biodegradation process in mineral medium (MM), containing (per liter): KH_2PO_4 (3g), K_2HPO_4 (12 g), NaCl (0.5 g), $MgSO_4.7H_2O$ (0.246 g), NH_4Cl (1 g) and, $CaCl_2$ (0.147 g). The desired pH was adjusted using 0.1 mol/L H_2SO_4 or 0.1mol/LKOH by pHmeter (654 pH meter Metrohm, Switzerland). The experiments were performed in 250 mL Erlenmeyer flasks containing 200mL MM supplemented with 40 ppm filter-sterilized BR46 as sole source of carbon. Two cultures including: (1) MM+BR46+bacterial isolate and (2) MM+BR46 were used as controls. To evaluate the BR46 biodegradation potential of isolate *Psudomonas* sp., the samples were incubated on 80 rpm shaking incubator at 30 ºC for 12 days. After 12 days, 20ml aliquots of samples were transferred to sterile micotubes and centrifuged at 13000 rpm for 15 minutes to remove the bacterial cells. 10ml of the supernatants were used for UV-VIS spectrophotometery scanning (UV Shimadzu-1700) at wave lengths 450-650 nm. The spectrophotograms of the samples before and after the inoculation were plotted by Excel 2007 and compared. All experiments were done in triplicates. [1, 5, 8].

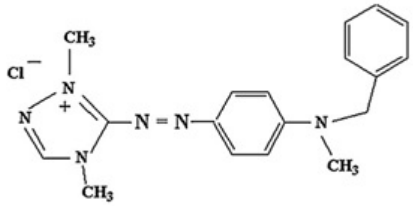


Figure 1- chemical structure of BR46

Metabolites produced during biodegradation of BR46 were identified using GC-MS. GC-MS analysis was conducted by GC-MS Agilent 6890 (USA), equipped with a 30 m×0.25 mm×25µm HP-5MS capillary column coupled with an Agilent 5973 mass spectrometer (Agilent Technologies, Palo Alto, CA) operating in EI mode at 70 eV with the following features: a quadropole filter and Helium as the carrier gas with a pressure of 34 psi at injection port. The initial oven temperature was 50°C, post-run temperature was 300°C and injection port temperature was 280°C[5, 8]. At regular time intervals during decolorization process, samples of dye solution were taken and dye removal percentage was calculated as follows:% decolorization/adsorption= (Initial absorbance - Observed absorbance/ Initial absorbance)×100 [9,10].

RESULTS AND DISCUSSION

Azo dyes are exceedingly used in industries. Dyeing because of their resistance to degradation, producing free aromatic amines that are potentially toxic and mutagenic. Release of azo dyes into the environment

from the wastewater of dye-utilizing industries has become a major worry in effluent treatment. Among the various types of biomass, *Pseudomonas* was able to degrade some dyes, mostly found in textiles industries [11]. Spectroscopy measurement was conducted to verify the biodegradation ability of isolate *Pseudomonas* sp. to degrade BR46. For this, the spectroscopic curves were plotted for the samples with and without inoculated *Pseudomonas* sp.(Fig.2).

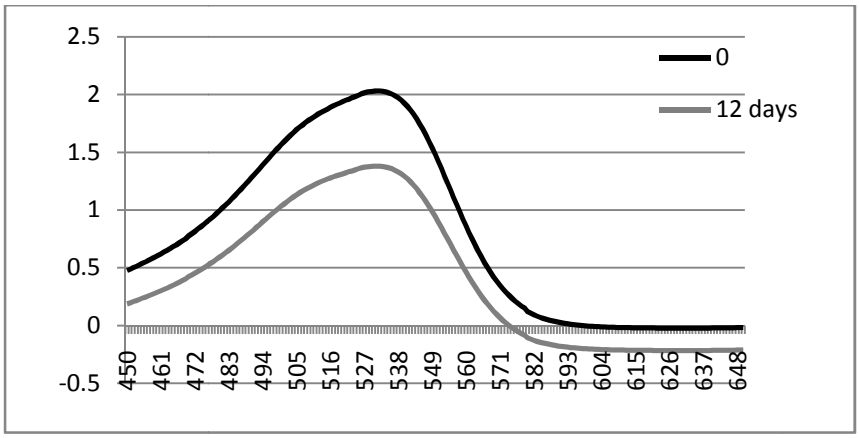
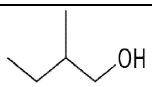
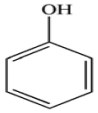
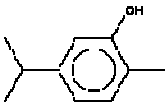


Figure 2.Spectrophotometeric curves of BR46 before and after the inoculation.

As shown, isolate *Pseudomonas* sp. can use BR46 as the sole source of carbon during the incubation time. As a basic method, spectroscopy was applied to determine the qualitative degradation potential. Then a more detailed method like GC-MS can complement biodegradation examination both quantitatively and qualitatively [1,8].GC-MS method was used to recognize the metabolites produced during biodegradation of BR46 by the isolate *Pseudomonas* sp..According to these data, it seems that the products of BR46 biodegradation are:2-Methyl Butanal, Benzenol, 2-methyl 5-(1-methyl ethyl) phenol and Hexadecanoic acid. The consequences of GC-MS analysis are summarized in Table 1.

Table 1- Identified metabolites during biodegradation of BR46.

No	Compound name	Structure	Retention time (min)	Main fragments
1	2-Methyl Butanal		1.933	57.10, 41.10, 43.10, 56.10, 42.10
2	Benzenol		26.11	28.1, 32.00, 56/10, 69/10
3	2-Methyl-5-(1-methylethyl)phenol		26.389	28.10, 135.10, 32.00, 91.10
4	Hexadecanoic acid		34.048	28.10, 18.10, 32.00, 55.10, 43.00

In some studies, GC-MS has been used to check metabolites produced during biodegradation of aromatic compounds. For example, Mulla et al., (2011) distinguisheddegradation metabolites of 2-nitrotoluene by a *Micrococcus* Strain using GC-MS [12]. Khataee et al., (2011) have also used GC-MS method for identify of metabolites produced during the degradation of Basic Red46 [13]. Vafaei et al., (2012) also determined degradation metabolites of Baric Red46 by *Azolla filiculoides* using GC-MS. Their GC result is similar to our result [2]. Hosseini Abari et al., (2013) have used GC to identify toluene biodegradation rate wastewater by bacteria and in another study by Moghadam et al., (2013) used the

same method to show that *Roseovarius* sp. isolated from coastal sediments of Nayband Bay in Iran are able to degrade phenanthrene [14,15]. In some of these studies, biodegradation capability is the only case being investigated, but in this project, as well as revealing BR46degradation capability, biodegradation metabolites were also recognized. Sun-Young et al., in 2002 studied degradation of triphenylmethane by *Citrobacter* sp. A *Citrobacter* sp., isolated from soil at an effluent treatment plant of a dyeing and textile industry, biodegrade several recalcitrant dyes except Bromophenol Blue [16]. Biodegradation progress was quantitatively estimated during 12 successive days. The results obtained at this step, are briefed in Fig. 3. As can be seen, BR46 concentration decreased during 12 consecutive days of incubation and therefore, this dye had been used by the bacterial isolate as the sole source of carbon.

There is a notable decrease in BR46 concentration in culture medium during the 12 days of incubation, while this consumption rate becomes slower in the following days. This change is maybe because of some factors such as reduction of carbon source and production of inhibitory metabolites in the medium. In conclusion, our findings revealed that *Pseudomonas* sp. degrade BR46. This isolate were able to degrade 32.72% of BR46.

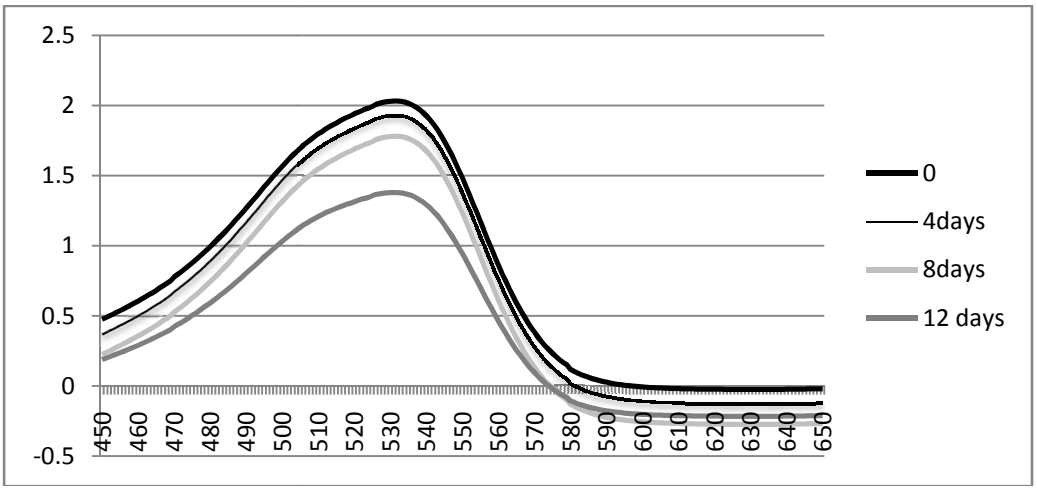


Figure3. Progress of biodegradation during 12 days.

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