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Biodegradation of resorcinol by *Pseudomonas* sp.

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ABSTRACT

Objective: To investigate the ability of *Pseudomonas* sp. isolated from East Azarbaijan, Iran in bioremediation of resorcinol.
Methods: Resorcinol biodegradation was evaluated using spectrophotometry and confirmed by gas chromatography-mass spectroscopy.
Results: This isolate was able to remove up to 37.12% of resorcinol from contaminated water. Reusability experiments had confirmed the biodegradation process which produced seven intermediate compounds. These intermediates were characterized by gas chromatography-mass spectroscopy technique. The products of resorcinol biodegradation were apparently 1, 4-cyclohexadiene, nonadecene, 2-heptadecanone, 1-isopropyl-2-methoxy-4-methylbenzene, hexadecanoic acid, 9-octadecenoic acid, phenol and 5-methyl-2-(1-methylethyl).
Conclusions: The findings revealed that *Pseudomonas* sp. is able to degrade resorcinol. Because of being an indigenous organism, this isolate is more compatible with the climate of the northwest region of Iran and possibly will be used for degradation of other similar aromatic compounds.

1. Introduction

Phenolic compounds have highly toxic effects on aquatic organisms and most of them have been recognized as carcinogens and genotoxicants. Phenol has already been described as a major toxic pollutant. Yet, very little is known about the toxicity of other phenolic compounds such as resorcinol. Because of the position of benzene ring, resorcinol has been used as a raw material for chemical industries and also as a solvent. It has also been employed in textile, as a disinfectant in cosmetic products, exfoliating agent and therapeutically in the treatment of human acne[1]. Even small amounts of dye in the water *e.g.* 5–20 mg/L are highly visible and additionally, the water transparency and the gas solubility of water bodies are influenced[2]. It has been proved by several studies that multiple routine applications of resorcinol on skin have adverse effects on thyroid gland in animals and humans. Acute toxication with resorcinol is generally originated from oral intake, being characterized by several symptoms such as vomiting, diarrhea, nausea, methemoglobinemia, hepatic dysfunctions, pulmonary edema and despair of the central nervous system[3].

Resorcinol is currently eliminated from waste streams by conventional techniques such as physical, biological or chemical processes. Examples include activated carbon adsorption, anoxic biodegradation, anaerobic biodegradation, aerobic biodegradation and abiotic degradation catalyzed by birnessite. Complex processes have been suggested such as color adsorption by activated carbon. However, they are not widely applied because of their high costs[1,3]. Consequently, these remediation methods have restricted usage and have no financially savvy[4].

Therefore, finding effective and economic methods for the treatment of dye-contaminated waters is always meaningful. Biological methods utilizing bacteria, fungi and algae as cleanup agents are eco-friendly and decompose dyes at a low cost[5]. Biodegradation is a biological method for bioremediation of organic pollutants. This innovation employs metabolic varieties of microbes to remove dangerous pollutants. While, biological degradation

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methodologies do not suffer from some restrictions of conventional methods[4]. Literature review shows that there are a limited number of microorganisms, especially bacteria, with the capability of aerobic degradation of resorcinol[6].

Biodegradation of resorcinol has been extensively investigated. Latkar *et al.*[7] studied kinetics of anaerobic biodegradation of resorcinol, catechol and hydroquinone in upflow-fixed, film–fixed bed reactors. Catechol can inhibit resorcinol degradation in this study. This inhibition is of the uncompetitive type and V_{max} for resorcinol reduced by catechol[7].

Yao *et al.*[8] in 2006 applied a combination of hydrogen peroxide as an oxidizer and an enzyme was derived from *Serratia marcescens* AB90027 for the decomposition of phenolic compounds. Phenol and resorcinol degradation was limited. Nevertheless, some phenolic compounds were completely degraded by this process[8]. In 2007, Rodriguez *et al.*[9] studied the influence of resorcinol oxidation on the removal of resultant organic carbon by activated carbon adsorption. The effect of resorcinol and activated carbon concentrations on the removal rates has been investigated[9].

The present work aims to assess removal potential of resorcinol by *Pseudomonas* sp. Reusability experiments displayed the biodegradation process and the chemical intermediates produced during removal process were characterized by gas chromatography-mass spectrometry (GC-MS) analysis.

2. Materials and methods

Resorcinol was purchased from Merck. The bacteria were isolated from soil samples of East Azarbaijan, Iran. The bacterial isolate was identified as a member of genus *Pseudomonas* by the analysis of 16S rRNA gene sequence. First of all, bacteria were cultured on Mueller–Hinton agar containing 2.0 g beef extract, 17.5 g acid hydrolysate of casein, 1.5 g starch and 17.0 g agar (per liter). After incubation at 30 ℃ for one day, *Pseudomonas* sp. colonies were transferred to Mueller–Hinton broth medium containing 2.0 g beef extract, 17.5 g acid hydrolysate of casein and 1.5 g starch (per liter). Once the culture reached the proper turbidity, 300 μL of 0.5 McFarland standard was utilized for microbial degradation procedure in mineral medium: KH_2PO_4 (3.0 g), K_2HPO_4 (12.0 g), NaCl (0.5 g), $MgSO_4 \cdot 7H_2O$ (0.246 g), NH_4Cl (1.0 g) and $CaCl_2$ (0.147 g) (per liter). The optimum pH was adjusted using 0.1 mol/L of H_2SO_4 or 0.1 mol/L of potassium hydroxide by pH meter (654 pH meter, Metrohm, Herisau, Switzerland). The experiments were performed in 250 mL Erlenmeyer flasks containing 200 mL mineral medium reinforced with 40 mg/L sterilized resorcinol as only source of carbon. The controls included mineral medium + resorcinol + bacterial isolate and mineral medium + resorcinol. To evaluate the resorcinol biodegradation by *Pseudomonas* sp., the falcon tubes were incubated on incubator with 80 r/min at 30 ℃ for 12 days. After 12 days, 20 mL of samples were handed over to falcon tubes and centrifuged at 13 000 r/min for 15 min to remove the bacterial debris. The supernatants (10 mL) was utilized for ultraviolet-visible spectroscopic scanning (UV Shimadzu-1700, Shimadzu, Kyoto, Japan) at wavelengths 230–310 nm. The results of the spectroscopy before and after the treatment were depicted by Excel 2007 software and matched. All tests were done at least for three times[4,5,10].

Biodegradation metabolites of resorcinol were characterized utilizing GC-MS Agilent 6890 (USA) equipped with a 30 m × 0.25 mm × 25 μm HP-5MS capillary column, paired with an Agilent 5973 mass spectrometer (Agilent Technologies, Palo Alto, CA) operating in electron ionization mode at 70 eV with the features: helium as the transporter gas with 0.234 26 Pa[Please confirm our conversion] pressure at injection port and a quadrupole filter. The start oven, injection port and post run temperatures were 50, 280 and 300 ℃, respectively[4,5,10]. Samples of resorcinol enriched mineral medium were taken at regular time intervals to calculate dye removal percentage as follows[11,12]:

% Removal = [(Initial absorbance – Observed absorbance)/Initial absorbance)] × 100

3. Results

The spectroscopy curves were represented for the tests with and without treated *Pseudomonas* sp. (Figure 1).

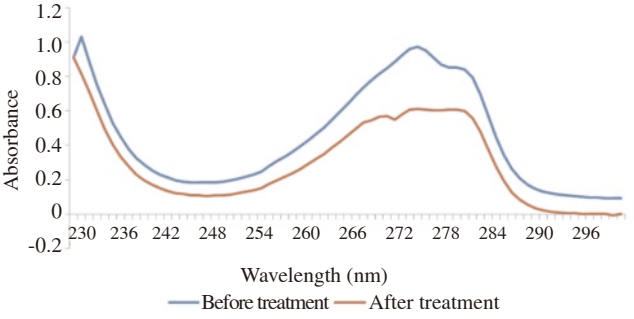

Figure 1. Spectrophotometric curves of resorcinol before and after the inoculation.

GC-MS helped us to recognize the metabolites produced during microbial degradation. It appeared that the results of resorcinol microbial degradation by *Pseudomonas* sp. were 1, 4-cyclohexadiene, nonadecene, 2-heptadecanone, 1-isopropyl-2-methoxy-4-methylbenzene, hexadecanoic acid, 9-octadecenoic acid, phenol and 5-methyl-2-(1-methylethyl) (Table 1).

Table 1
Identified metabolites during biodegradation of resorcinol.

No.	Compounds' name	Retention time (min)	Main fragments
1	1,4-Cyclohexadiene	19.615	28.10, 93.10, 91.10, 136.10, 121.10
2	Nonadecene	33.957	28.10, 18.10, 32.10, 43.10, 57.10
3	2-Heptadecanone	34.018	28.10, 18.10, 43.10, 59.10
4	1-Isopropyl-2-methoxy-4-methylbenzene	25.032	28.10, 149.10, 32.10, 164.10, 18.10
5	Hexadecanoic acid, methyl ester	34.247	74.10, 28.10, 87.00, 43.10, 18.10
6	9-Octadecenoic acid	32.736	28.10, 74.00, 87.10, 18.10, 43.10
7	Phenol, 5-methyl-2-(1-methylethyl)	26.084	28.10, 135.10, 32.00, 150.10, 18.10

4. Discussion

Spectroscopic measurement was carried out before and after treatment to confirm the potential of isolate *Pseudomonas* sp. to degrade resorcinol. *Pseudomonas* sp. isolate can make use of resorcinol as the only resource of carbon during the treatment. Spectroscopy was used as a screening procedure to check the qualitative degradation possibility. After that, a more comprehensive procedure such as GC-MS could complete degradation assessments quantitatively and qualitatively[5,10].

Some researchers have applied GC-MS to determine metabolic intermediates of aromatic compounds biodegradation. For example, Pardeshi *et al.*[13] studied photocatalytic degradation of resorcinol. photocatalytic degradation intermediates were identified using GC-MS[13]. *Pseudomonas putida* was able to degrade some dyes typically used in textile industries[14]. Mulla *et al.*[15] detected biodegradation metabolites of 2-nitrotoluene by *Micrococcus* exploiting GC-MS. Khataee *et al.*[16] have also used this technique to characterize metabolites consume amid the deterioration of basic red 46. Vafaei *et al.* also identified basic red 46 degradation metabolites by *Azolla filiculoides* using GC-MS and their results were similar to the present study[5,17]. Abari *et al.*[18] used GC for monitoring bacterial biodegradation rate of toluene in wastewater. In a study, Moghadam *et al.* in 2013 utilized the similar methodology to demonstrate that bacterial isolate from coastal sediments of Nayband Bay in south of Iran were capable to biodegrade phenanthrene[19]. In a large part of these researches, biological degradation capability is the only matter of investigation, but in this project, in addition to aromatic compounds degradation potential, bacterial degradation products were also distinguished. Resorcinol was diminished during 12 days of incubation. The dye was utilized as the only resource of carbon element. There was a notable consumption in resorcinol concentration during 12 days of incubation, while this depletion rate turned out to be slow in the next days. Change was perhaps due to a few causes, such as reduction of carbon source and production of inhibitory metabolites. Pollution resulting from industries has various effects on the environment and organisms. These pollutions are often form human industrial operations. Dyes are the most significant standards of water pollution and release of wastewater contaminated with artificial dyes can do harm to water resources. In this study, the ability of *Pseudomonas* sp. bacterial strains isolated from the soil of East Azarbaijan, Iran in biodegradation of resorcinol was investigated. And, the results confirmed the ability of *Pseudomonas* sp. in biodegradation of resorcinol dye which was evidenced by the reduction of absorbance at 230 and 298 nm in the UV-vis spectra. Seven compounds resulting from biodegradation were characterized by GC-MS. The results showed that bacteria could play an important role in the decomposition of paint and remove them from the environment.

In conclusion, the findings revealed that *Pseudomonas* sp. was able to degrade 37.12% of resorcinol.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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