
Hüseyin KAHRAMAN1* and H. GECKIL2

1Inonu University, Art and Science Faculty, Department of Biology 44280-Malatya/ Turkey
2Inonu University, Art and Science Faculty, Department of Molecular Biology and Genetic 44280-Malatya/ Turkey

*husyin.kahraman@inonu.edu.tr

(Received: 11.04.2016; Accepted: 27.07.2016)

Abstract

This study is concerned with the potential use of *Pseudomonas aeruginosa* and its recombinant strain carrying *Vitreoscilla* hemoglobin gene (vhb) for degradation of important harmful aromatic compounds as toluene, xylene, benzene and 1-Cl -2,4-Dinitrobenzene (CDNB). *P. aeruginosa* and recombinant strain were grown in 10 ml LB overnight at 37°C in a shaking water-bath at 200 rpm. High aeration/high agitation cultures were incubated in a gyrator water-bath at 200 rpm in flasks containing 20 ml medium. *P. aeruginosa* and its various strains are known for their ease of use characteristics allowing gene transfer, expression and capabilities of degrading the heterocyclic aromatic compounds which are mostly harmful to all organisms. In this study, degradation of these single ring aromatic compounds by the recombinant strain that bears the vhb and its parental wild-type strain *P. aeruginosa* was carried out.

Keywords: Pseudomonas Aeruginosa, Vitreoscilla Hemoglobin, Organic Solvents.

1. Introduction

Pollution arising as a result of modern life's been focused on solutions today, and at least one of the issues that can be. One of these kinds of impurities and the like contained in the oil, known as BTEX briefly dramatically in its refined products and its alkylated derivatives with toluene, ethyl benzene and xylene is caused. These substances are the most widely known examples of typical aromatic ring compound. Essentially all the aromatic hydrocarbon is a benzene derivative. These compounds as they show similarities although alkenes annular structures also are more stable and do not undergo the reactions of alkenes suffered.

Therefore, they are seen as more dangerous environmental pollutants. With these substances are not made by living creatures just carry toxic to all living things. These substances are applied to the contaminated environment with physical and chemical decontamination methods are far from being a complete and effective solution. Micro molar concentrations of the residue from treatment with these types of technology are even show toxic to animals. Potential availability
of biochemical methods for such transactions in recent years due to have been investigated, and in particular substance has been determined to find alternative uses of *Pseudomonas* bacteria species equipped with the necessary gene systems to break down into less harmful compounds.

The hemoglobin (VHb) of the bacterium *Vitreoscilla* is the first discovered and probably best characterized of the microbial hemoglobin’s [1]. The *in vivo* role of VHb in its native host is suggested oxygen trapping/transfer, allowing this bacterium to survive and grow in micro aerobic environments [2]. Priority pollutants such as benzene, nitrobenzene, toluene and xylene (BTX) are common hydrocarbon constituents produced by petrochemical companies in vast amounts each year. BTX are also common ground water contaminants often found in oxygen-limited aquifers are classified as priority pollutants by the U.S. Environmental Protection Agency (EPA). *Pseudomonas aeruginosa* strains are typically active denitifiers and produce biosurfactants which are very beneficial for bioremediation, as they solubilize and mobilize hydrocarbons.

Having these versatile and unique metabolic characteristics, *P. aeruginosa* is one of the most suitable bacteria in initial studies of sequential hydrocarbon metabolism [3]. Given that *Pseudomonas* sp. are able to degrade a wide variety of organic chemicals, engineering them to produce VHb for better oxygen uptake for growth and metabolism may be of particular usefulness in the bioremediation of toxic wastes in situ where oxygen is limiting [4]. Attempt has been made to overcome the oxygen limitation problem by genetically engineering microorganisms to enhance their survival, growth and ability to degrade aromatic compounds under oxygen limiting conditions [5, 6].

2. Materials and Methods

2.1. Solvents

1-Cl-2,4-Dinitrobenzene (CDNB) (Aldrich Chemicals Co.), Benzene (Riedel-de Haën), Xylene and Toluene (Merck Chemicals Co.) and all other chemicals were of analytical grade.

![Figure 1. Structure of solvents](image)

2.2. Bacterial strains and growth media

Both *P. aeruginosa* (NRRL B-77) and its transposon-mediated *vhb* transferred recombinant strain named PaJC [6]. The Cells were maintained on LB agar plates at 4 °C with transfers at monthly intervals. The liquid media used throughout the study were Luria-Bertani (LB) broth. The final pH’s of both media were adjusted to 7.0. The concentrations of these compounds, based on our preliminary experiments, in LB were 9 mM, 0.05 mM, 15 mM and 3 mM for benzene, CDNB, toluene, and xylene, respectively.

2.3. Growth conditions

Growth of non-transformed and recombinant cells was compared in LB media. *P. aeruginosa* and recombinant strain were grown in 10 ml LB overnight at 37°C in a shaking water-bath at 200 rpm. A 1/100 inoculum of overnight cultures grown in a LB medium was made in 20 ml of the same medium.
in 125 ml Erlenmayer flasks and inocula in flasks were grown for different periods of time at 37°C. High aeration/high agitation cultures were incubated in a gyrator water-bath at 200 rpm in flasks containing 20 ml medium [7]. Growth was monitored by both OD 

2.4. Statistical analysis

The data represent the arithmetic averages of at least three replicates. The analyses were carried out using t-test (95% confidence limit). For clarity, no error bars are given, but they are mostly less than 10% of the respective data point.

3. Results and Discussion

Biomass values, about the total cell density information in order to have been considered. CDNB, toluene, xylene environment of the PaJC in accordance with wild type was shown to achieve higher biomass values. CDNB, toluene, xylene environment of the PaJC in accordance with wild type was shown to achieve higher biomass values. Significant differences were not observed in all other media. Pseudomonas aeruginosa and Pseudomonas putida with a similar study, there was no significant difference between the wild type and recombinant bacterial biomass values in the first 48 hours [8]. Statistical significance of the differences was in the times to be shown on the chart. Most statistically significant difference is observed in the presence of xylene (Fig 3). However, recombinant bacteria reached more biomass values in the presence of aromatics compared to wild bacteria. In this regard, the cleaning of the media may be contaminated with aromatics recombinant bacteria efficiently.

Microorganisms; aerobic, microaerobic, under hypoxic or anaerobic conditions BTX can destroy. BTX’s biological degradation has been studied widely and is presently being studied. It is usually faster than anaerobic degradation of the aerobic degradation was observed [9].

Effective as BTX’s known to break down all the components are not pure strains of any bacteria. Especially the xylenes, are known to be resistant to bacterial destruction. Pseudomonas stutzeri is one of the few organisms that can break down xylene. These compounds are destroyed by the slow destruction that can cause toxicity and microorganisms; they can be successful in the destruction of only low concentrations [10, 11].

The biodegradation of alkyl benzene such as toluene under aerobic conditions it is easier. In bacterial BTX degradation media has been reported to increase the inhibitory effect of coexistence of these substances. Again, the biological degradation of organic contaminants could be observed only during secondary metabolism was induced by nitrogen or carbon starvation [9, 12-14]. In the studies, some of Pseudomonas, under hypoxic conditions using nitrate as the terminal electron acceptor has been shown to break down the BTX. However,
compared to benzene, toluene has been shown in these studies faster destroyed [15,16]. However, destruction of the molecular oxygen is needed for the hydroxylation of aromatic rings [17].

*Pseudomonas* species have been performing the biological degradation of toxic chemicals show great diversity [8, 18-19]. Some of *Pseudomonas* species in the aerobic and facultative chemolithotrophs, chemoorganotrof is heterocyclic and aromatic substances are capable of destroying or using the cellular energy and materials [20].

*Pseudomonas* the SNF made in some studies to increase the bioremediation has been shown to have significant potential [21, 22].

One of the most important reasons is that they lead to higher uptake of oxygen species transferred VHb compared to wild-type [18].

PaJC was advantageous biomass production in the presence of CDNB. Because often limiting oxygen plays a role in this type of bioprocesses. We have done so far in the literature for similar studies could not find work.

**Figure 1.** Viable cell mass of *P. aeruginosa* (open symbols) and its recombinant PaJC (filled symbols) strain grown in LB medium + Benzene

**Figure 2.** Viable cell mass of *P. aeruginosa* (open symbols) and its recombinant PaJC (filled symbols) strain grown in LB medium + CDNB

**Figure 3.** Viable cell mass of *P. aeruginosa* (open symbols) and its recombinant PaJC (filled symbols) strain grown in LB medium + Xylene

**Figure 4.** Viable cell mass of *P. aeruginosa* (open symbols) and its recombinant PaJC (filled symbols) strain grown in LB medium + Toluene

**Figure 5.** Viable cell mass of *P. aeruginosa* (open symbols) and its recombinant PaJC (filled symbols) strain grown in LB medium

4. Acknowledgements

Authors are grateful to Research Fund Unit of Inonu University for financial support (APYB 2002/N05).
5. References


