BIODEGRADATION OF O-XYLENE BY *AZOTOBACTER CHROOCOCCUM*

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ABSTRACT:

The capability of *A. chroococcum* to degrade o-xylene was investigated. The bacterium was found to have best removal at pH of 7 and temperature of 30 °C. No removal was observed for pH less than 4 or greater than 9. The bacterium was able to degrade entire o-xylene at an initial concentration of 50, 100, and 150 mg/L in 60, 72 and 84 hours respectively. However only 72% and 50% removal was observed for the initial concentration of 200 and 250 mg/L. The Andrew-Haldane model was fitted to the experimental data and was found to have high correlation. Growth associated parameters viz. μ_{max} , K_S and K_i were found to be 0.33 (h^{-1}), 1.5(mg/L) and 239(mg/L) respectively.

Keywords : Biodegradation, Batch, o-Xylene, Chroococcum, Optimization

1. INTRODUCTION

Volatile Organic Compounds (VOCs) are hazardous for human health and environment. Among these VOCs, xylenes is one of the hazardous chemical that is found in many consumer products such as paints, adhesives, rubber, clothing, plastic bottles, cements, inks, dyes [1]. Xylene is released from various sources like vehicular exhaust, fuel filling station, commercial combustion units and industrial units like refinery, petrochemicals, medium scale chemical industries, etc. [2]. Central Pollution Control Board classifies o-xylene as a medium priority pollutant based on its photochemical ozone creation potential [3]. In India, xylene concentration in air has been reported as high as 124 $\mu g/m^3$ [4]. Acute xylene exposure causes dizziness, headache, nausea, vomiting, breathing difficulty and loss of coordination. Severe exposure to xylene causes visual blurring, tremors, heart beat irregularities, paralysis and loss of consciousness [2]. To abide by the government regulations for the protection of

environment, various control technologies are used by industries to eliminate or reduce their content. Several physical and chemical gas cleaning technologies like incineration, ozonation, combustion and adsorption are available but are either expensive or generate secondary pollutants that further require treatment [5]. Biological treatment is an established technology for air pollution control which is cost effective and environmentally safe method [1].

Some researchers have studied the biodegradation of xylene using *Pseudomonas putida* and *Pseudomonas fluorescens* [6], *Rhodococcus* sp. [7], *Paecilomyces variotii* [8]. According to the knowledge of the authors no work has been reported on biodegradation of o-xylene by *Azotobacter chroococcum***.** To use *A. chroococcum* for biofilteration of waste air stream, it is necessary to find the optimum conditions for biofilteration. In the present work, a batch study on biodegradation of o-xylene to determine optimum removal conditions and

maximum removal at the optimum condition is studied. Rather than using free cells, immobilized cells are being used in this study since they are known to provide many advantages over suspended cell systems like easier separation, greater operational flexibility and higher cell density which results in higher biodegradation rate per reactor unit volume [9]. Immobilized cells are also protected from harsh environmental conditions and are therefore more tolerant to high concentrations of toxic compounds. In this study corn cob has been used for immobilization of *A. chroococcum* since it is an important agricultural by product and is a low cost material as well as has high carbon content which may provide extra nutrition to immobilized bacteria.

2. MATERIALS AND METHODS

2.1 Microorganism, media and chemicals

The carbon free growth medium formulation included the following components in water : 8.8 g/L Na_2HPO_4, 1.2 g/L KH_2PO_4, 5 g/L NaCl, 1 g/L NH_4Cl, 0.25 g/L $MgSO_4.7H_2O$. All the components were sterilized at 15lbs for 15 min. To avoid precipitation during autoclaving $MgSO_4.7H_2O$ and o-xylene were sterilized separately. Various amounts of o-xylene, as the sole carbon source for cell growth, were aseptically added to nutrient media using a pipette to give the desired final concentrations. All the chemicals were obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Microbial strains of *A. Chroococcum* (MTCC 446) were obtained from MTCC, Chandigrah, India. The obtained microorganisms were initially cultured in nutrient broth and then plated on nutrient agar and preserved. The microbial culture was cultivated in 500 mL flask containing 100 mL of nutrient media with o-xylene as the sole carbon source. The cultures were acclimatized to o-xylene by exposing the culture to increasing concentration (10-250 mg/l) of o-xylene in a series of flasks. The startup of acclimatization was obtained by inoculating 100 mL of nutrient media containing 10 mg/L of o-xylene with cultures from nutrient agar slants under sterile conditions. After a good growth was observed, 5 mL of this culture was added to fresh nutrient media containing o-xylene as innoculum. The concentration of o-xylene in fresh nutrient media was increased sequentially to 250 mg/L.

2.2 Analytical methods

The concentration of o-xylene in liquid was calculated using Henry's partition coefficient based on headspace gas concentration. The value of Henry's partition coefficient for o-xylene at 30 °C is 0.19 [10]. Samples of the headspace gas were withdrawn from the bottles using a Hamilton 1001 gas-tight syringe (Hamilton Co.) and analyzed using Michro 9100 gas chromatography (Netel India Ltd.) equipped with a flame ionization detector and a 30 m HP 5 Capillary column. The oven, injector and detector temperature were fixed at 60, 210 and 230°C respectively. Nitrogen was used as carrier gas. The dry weight was determined by filtering the biomass through the Whatman filter paper and drying the filter for 12 h at 105°C.

2.3 Biodegradation studies

To understand the effect of various parameters like pH, temperature, substrate concentration on the growth of *A. chroococcum*, batch studies were performed. In first set of experiments, initial concentration of o-xylene was fixed at 50 mg/l and pH was varied from 1 to 12 and temperature was varied from 15 °C to 45 °C. The above biodegradation study was allowed for 48 hours. In second set of experiments, the removal of o-xylene with respect to time was studied at initial concentration of 50, 100, 150, 200 and 250 mg/L o-xylene for 96 hours. All the experiments were conducted in 500 mL bottles having a sampling port sealed with Teflon coated silicone septum to collect samples at regular intervals. Only 100 mL working volume of liquid was taken in 500 mL bottles to avoid deficit of oxygen. All the batch runs were operated at an agitation rate of 150

rpm. For immobilization of *A. chroococcum* on corn cob, preweighted amount of sterilized corn cob was added to 24 hour old culture of *A. chroococcum* and left to agitate for 48 hours. After 48 hours, immobilized cells were filtered out and added to fresh medium containing o-xylene. All the experiments were conducted in triplicate and their average value were reported.

3. RESULTS AND DISCUSSIONS
3.1 SEM studies
The SEM provides crucial information for studying topographic and structural features of o-xylene degrading microorganisms. Figure 1(a) and 1(b) shows the image of *A. chroococcum* immobilized on the surface of corn cobs before and after biodegradation of o-xylene. The following image clearly shows extensive bacterial adhesion and utilization of o-xylene for its growth.

3.2 Effect of pH

The pH value is a key factor in microbial metabolic processes. It influences the redox potential and enzymatic activity [11]. The pH of the medium was adjusted by 1M NaOH and 1M HCl. A control was also run without microbial cells to measure the loss of o-xylene due to any volatilisation. The effect of pH on removal of o-xylene was studied at 50 mg/L for pH ranging from 1-12 after allowing the biodegradation for 2 days. Figure 2 shows the effect of pH on percentage removal of o-xylene by bacterial cells. It was seen that percentage removal of o-xylene increased from 14% to 79 % on increasing pH from 4 to 7. On further increase in pH to 9, the removal efficiency decreased to 58.7%. It was also observed that at pH less than 4 or greater than 9, there was no removal due to biodegradation, which was due to the inhibitory effect of superacidity or superalkalinity on the activity of intracellular enzyme of bacteria. The optimum pH was found to be 7.0.

3.3 Effect of temperature
The biodegradation of o-xylene by immobilized cells was carried out at temperatures ranging from 15 °C to 45 °C for an initial concentration of 50 mg/L of o-xylene at pH=7. The results are shown in figure 3. The results reveal that removal efficiency increases on increasing temperature from 15°C to 30°C.

The results also seem to follow an exponential trend in this range. On further increasing temperature, the removal efficiency decreases. *A. chroococcum* was able to degrade 79.6% of o-xylene at 30°C. Therefore *A. chroococcum* can grow from 15°C to 45 °C but maximum removal of o-xylene occurs at temperature of 30°C.

3.4 Effect of substrate concentration
The effect of initial substrate concentration on the removal of o-xylene was studied at 50, 100, 150, 200 and 250 g/L. Figure 4 shows the percentage of o-xylene remaining after the biodegradation of *A. chroococcum* with respect to time. *A. chroococcum* was able to degrade around 100% of o-xylene at an initial concentration of 50, 100, 150 mg/L in 60, 72 and 84 hours respectively. However it was able to degrade only 72% and 50% for the initial concentration of 200 and 250 mg/L. The time required for biodegradation was found to increase on increasing initial concentration of o-xylene. A region of relatively less rate of substrate removal was observed towards the end of substrate consumption curve for high concentrations. The degradation rate was high at low concentration whereas it was low at high concentration. It could be due to the deficit of oxygen since no constant supply of oxygen was available as stated by Morgen et al., 1993. They found oxygen supply found to be a limiting factor in the biodegradation of benzene, toluene, ethyl benzene and xylenes in groundwater [12]. Since the experiments were conducted in 500 mL bottle with 100 mL working volume, therefore only a limited amount of air was available for the growth of bacteria.

Another reason could be the fall in pH of the solution over time. Fakhruddin and Quilty (2005) found out that the removal of 2-chlorophenol was inhibited due to significant fall in pH [13]. Figure 5 shows the increase in biomass concentration due to different initial concentrations of 50, 100, 150, 200 and 250 mg/L over a period of 60 hours. The study was stopped at 60 hours since the bacteria attained stationary phase in all cases. The cell growth followed the pattern of a lag phase in which no appreciable change in concentration of biomass was observed. It was followed by a log phase in which biomass concentration increased exponentially. At the end of log phase, the biomass concentration became constant indicating the stationary phase. The lag phase increased from 6 hours to 20 hours on increasing the initial concentration of o-xylene from 50 mg/L to 250 mg/L. The maximum biomass concentration increased on increasing the initial concentration from 50 mg/L to 150 mg/L. On further increment in initial concentration to 250 mg/L, the maximum biomass concentration decreased. A possible explanation that could be given for this behavior is due to self inhibition by o-xylene [14].

3.5 Growth kinetics

The specific growth rate in exponential phase was calculated using the following equation [15].

$$\mu = \frac{\ln(X_2 / X_1)}{(t_2 - t_1)} \qquad (1)$$

Here X_1 and X_2 represents the biomass concentration at start and end of log phase respectively. t_1 and t_2 represents time of start and end of log phase respectively. The maximum value of specific growth rate was 0.25497 h^{-1} at initial concentration for 50 mg/L o-xylene. The specific growth rate was observed to decrease with an increase in initial concentration. Amir et al. also observed the decrease in specific growth rate of consortium of bacterial culture used for biodegradation of o-xylene on increasing initial concentration of o-xylene [16].

The Haldane-Andrews model was used to describe the specific growth rate for a single substrate as given by following equation [17]:

$$\mu = \mu_{max} \frac{S}{K_s + S + \dfrac{S^2}{K_i}} \qquad (2)$$

μ_{max} represents maximum specific cell growth rate (1/h), S is the substrate i.e o-xylene concentration (mg/L), K_S is the saturation constant (mg/L) and K_i is the self inhibition constant. The Andrew-Haldane model was fitted to the experimental data and was found to have high correlation (figure 6).

From the graph it has been observed that maximum specific growth rate occurred at initial concentration of 20 mg/L. Table 1 compares the kinetic parameters obtained in this work with other works on biodegradation of o-xylene. *A. chroococcum* was found to have higher maximum specific growth rate than *P. Putida* and *P. aeruginosa.*. A higher K_i value of 238.6 indicates that the culture is less sensitive to substrate inhibition.

4. CONCLUSION

In the present work the ability of *A. chroococcum* to degrade o-xylene was evaluated in batch reactor and optimum conditions were found out for its use in continuous reactor. The SEM analysis showed extensive bacterial adhesion and utilization of o-xylene for its growth. The optimum pH was found to be 7.0. There was no removal due to biodegradation at pH less than 4 or greater than 9 due to highly acidic and basic conditions which had inhibitory effect on the activity of intracellular enzyme of bacteria. *A. chroococcum* was found to grow from 15°C to 45 °C but maximum removal of o-xylene occurred at temperature of 30°C. *A. chroococcum* was able to degrade almost 100% of o-xylene at an initial concentration of 50, 100, 150 mg/L in 60, 72 and 84 hours respectively. However it was able to degrade only 72% and 50% of o-xylene for the initial concentration of 200 and 250 mg/L. The Andrew-Haldane model was fitted to the

experimental data and was found to have high correlation. μ_{max}, K_S, K_i, was found to be 0.33 (h^{-1}), 1.5(mg/L) and 239(mg/L) respectively. It has also been observed that maximum specific growth rate occurred at initial concentration of 20 mg/L.

ACKNOWLEDGEMENT

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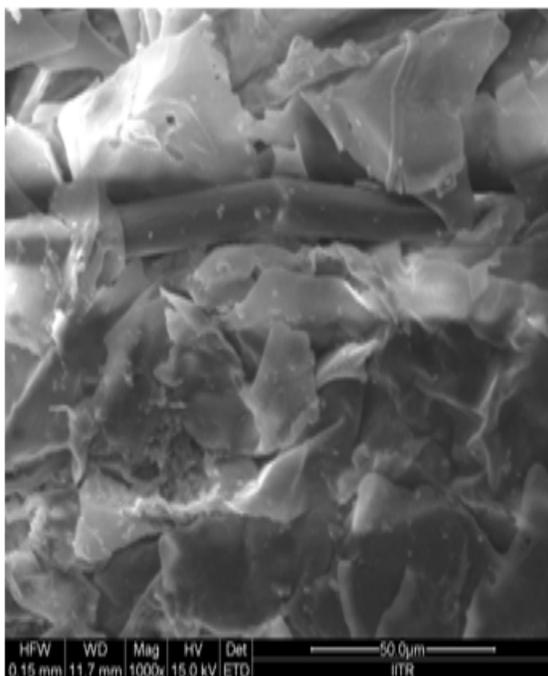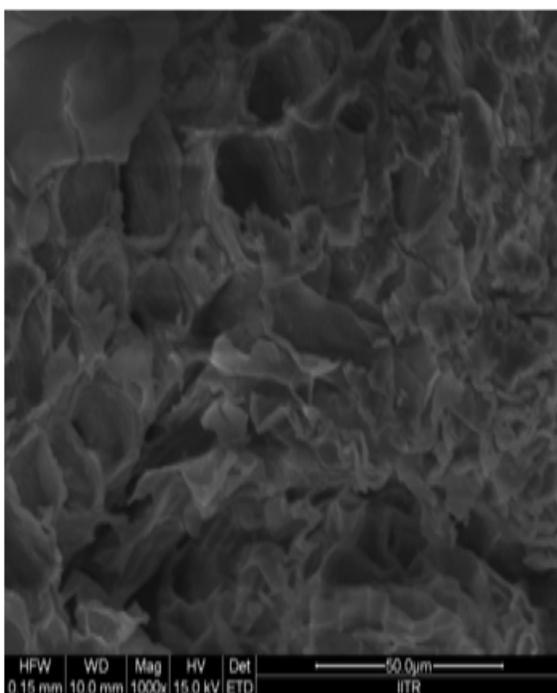
Figures and Tables:

Figure 1(a): *A. Chroococcum* immobilized on the surface of com cob before biodegradation of o-xylene
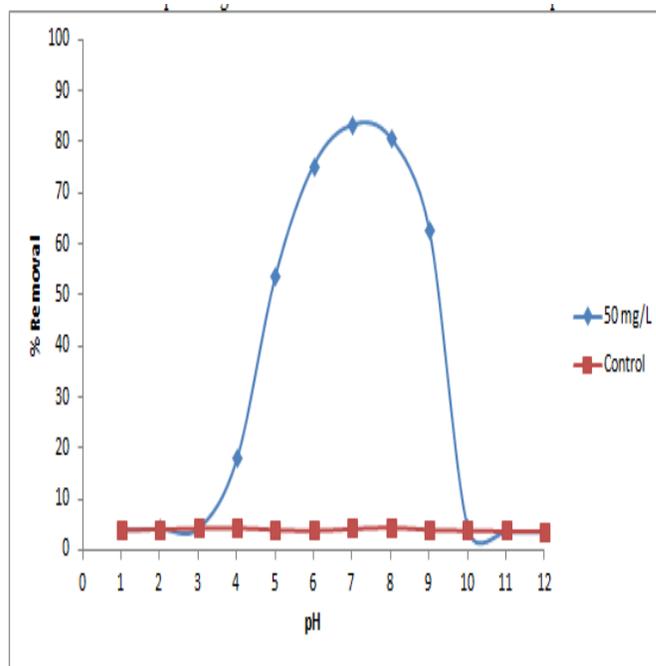

Figure 2: Effect of pH on percentage removal of o-xylene (Temperature = 30°C, Initial concentration = 50 mg/L, agitation rate = 150 rpm, contact time = 48 hours)
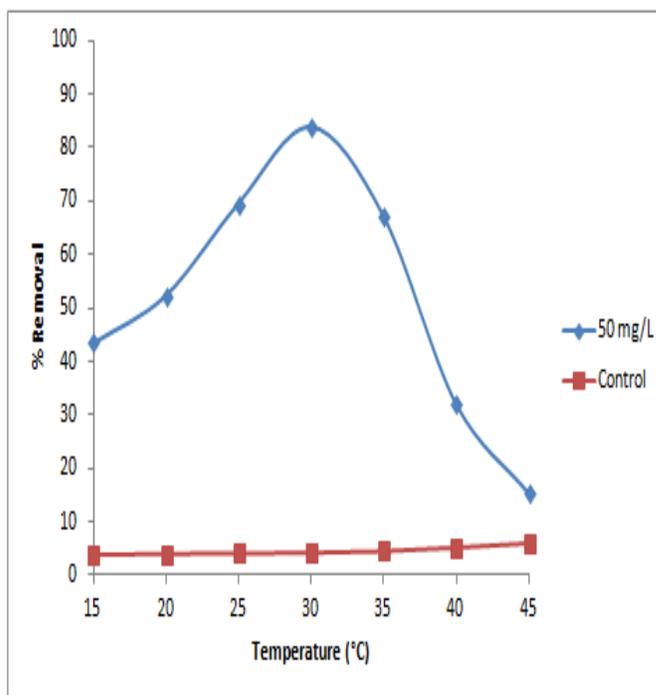

Figure 1(b): *A. Chroococcum* immobilized on the surface of com cob after biodegradation of o-xylene

Figure 3 : Effect of temperature on percentage removal of o-xylene (pH = 7 , Initial concentration = 50 mg/L, agitation rate = 150 rpm, contact time = 48 hours)
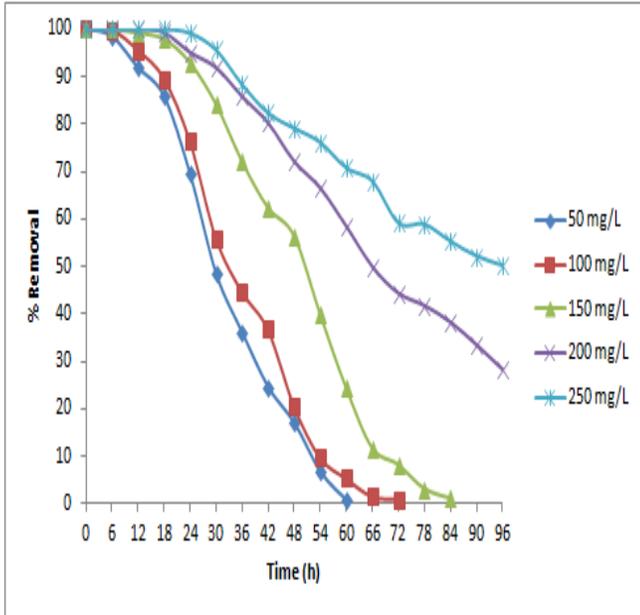
Figure 4: Effect of initial substrate concentration on removal of o-xylene (pH = 7 , Temperature =30°C, agitation rate = 150 rpm)

Table 1: Kinetic parameters for degradation of o-xylene

Bacterial species	μ_{max} (h^{-1})	K_S (mg/L)	K_i (mg/L)	Reference
P. putida F1	0.19	2.55	5	Trigueros et al., [18]
P. aeruginosa	0.006	1.427	153.55	Lin et al., [19]
A. chroococcum	0.32834	1.50538	238.631	This work

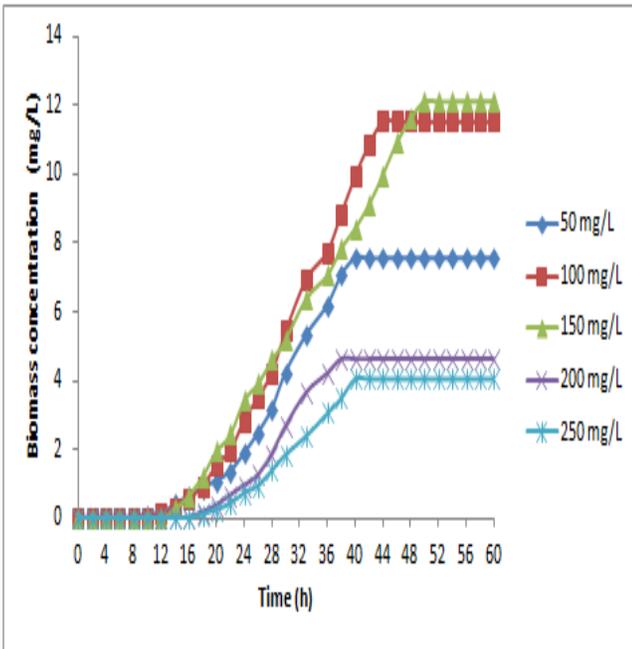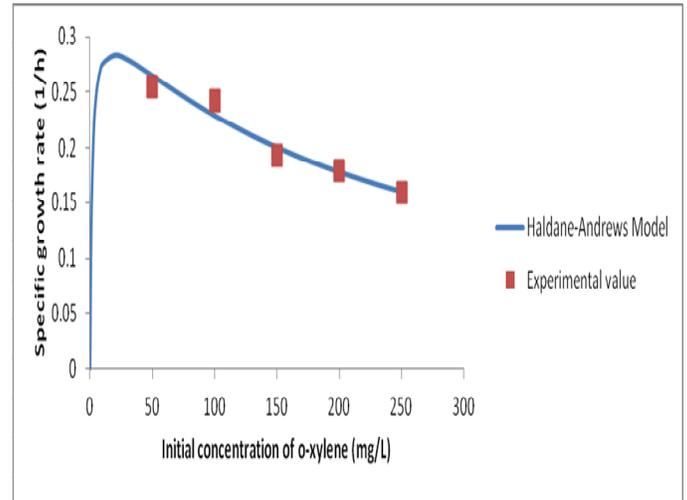**Figure 6 :** Variation of specific growth rate with initial concentration of o-xylene (pH = 7 , Temperature =30°C, agitation rate = 150 rpm)

Figure 5: Effect of initial substrate concentration on biomass concentration (pH = 7 , Temperature =30°C, agitation rate = 150 rpm)