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Full Length Research Paper

Influence of pH, temperature and glucose on biodegradation of 4-aminophenol by a novel bacterial strain, *Pseudomonas* sp. ST-4

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Aromatic compounds such as 4-aminophenol are toxic to the environment and thus should be eliminated effectively. Biodegradation of aromatic compounds is an efficient and environment friendly technique as addition of selected microbes does not add any kind of pollutants and actively remove even the most recalcitrant pollutants. We investigated the effectiveness of *Pseudomonas* sp. strain ST-4 in the biodegradation of 4-aminophenol under variable pH, temperature and glucose regimes and sorted out optimum conditions for maximum biodegradation of 4-aminophenol. Maximum biodegradation of 4-aminophenol by *Pseudomonas* sp. strain ST- 4 was observed at pH 8, temperature 30°C and glucose concentration of 15 mM at 72 h, respectively.

Key words: Biodegradation, 4-aminophenol, *Pseudomonas* sp. ST-4, pH, temperature, glucose.

INTRODUCTION

Aromatic amines including anilines and their derivatives have been reported to be widely occurring due to their industrial importance as synthetic chemicals. Being hazardous to environment and health, considerable attention has been paid to their elimination (Takenaka et al., 2003). 4-Aminophenol (Figure 1) is synthesized from phenyl hydroxylamine (Sone et al., 1981) and is formed by microbial transformation of hydroxylaminobenzene (Schenzle et al., 1997), 4-nitrophenol and aniline (Cerniglia et al., 1981; O'Connor et al., 2006). 4-aminophenol is highly toxic and carcinogenic, irritating to eyes, brain and respiratory systems (Wang et al., 1997). 4-Aminophenol is an intermediate in the degradation of hydroxyacetanilide (Hart and Orr, 1975) and azo dyes (Tan et al., 1999). From medical point of view, it is required to produce clofibrates, paracetamol and other medicines. It is also used as photograph developer, rubber autoxidation agent and petroleum additives (Wang et al., 1997).

Prevalence of these compounds in the environment has stimulated investigations into the biodegradation of these hazardous substances in water or contaminated soil as biological treatment of the polluted ecosystems can be the most effective mean to eradicate such contaminants. However, this effort is often limited by the antimicrobial action of the pollutants. High concentrations of toxic chemicals usually reduce the capabilities of microorganisms to remove these compounds (Dean-Ross and Rahimi, 1995). The members of genus *Pseudomonas* are known for biodegradation of aniline, phenols and substituted benzene aerobically (Zhao et al., 2000; Annadurai et al., 2007).

The environmental fate of the organic pollutants largely depends upon physical and biochemical aspects of the pollutant, its environment and on the availability of the predominant electron acceptor (Boyd et al., 1983). Suitable pH, temperature and glucose concentration has been observed to enhance the biodegradable ability of microorganism. During present research, emphasis has

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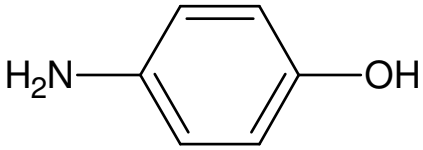

Figure 1. 4 -Aminophenol.

been paid to investigate the influence of pH, temperature and glucose level of the medium on biodegradation of 4 -aminophenol by *Pseudomonas* sp. strain ST- 4 and to search favorable levels of these 3 factors, in order to maximize the metabolic functions and biodegradation potential of this bacterial strain.

MATERIALS AND METHODS

Analytical methods

The experiment was conducted using orbital type shake flask incubator at 100 rpm. Viable cell count using serial dilution and plating technique was used to check the growth of the strain ST-4. UV 240 spectrophotometer (Shimadzu model) at λ 230 nm was used to analyze degradation analysis of 4-aminophenol. The concentration of 4-aminophenol was calculated against calibration curve of standard 4-aminophenol, for which the samples were taken at regular intervals during biotransformation experiment and centrifuged in micro-centrifuge at 16,000 rpm for 5 min. The supernatant was thus analyzed after making required dilutions.

Defined mineral salt media (PNR) with composition, 13.6% KH_2PO_4, 2.4% $(NH_4)_2SO_4$ and 2.5% NaOH for PN salt (20x) and 8.0% $MgSO_4$, 0.2% Fe_2SO_4 and 4% HCl for R salt, have been used throughout the experimental work to observe degradation and optimization processes (De-Frank and Ribbons, 1976). 2% (w/v) agar (Difco Laboratories and Detroit) was used in solid media and glucose was added in 1 mM concentration as source of energy. 50 ppm of 4-aminophenol was used during this study for degradative results. For each experiment, induction of the strain with 50 ppm 4-aminophenol was carried out as induction was found to give better results (Khan et al., 2006).

Effect of pH on 4-aminophenol biodegradation

In order to determine the effect of pH on biodegrading potential *Pseudomonas* sp. strain ST-4 on 4-aminophenol, the nutrient broth cultures were first prepared. These were used as inoculum for PNR-G media after 24 h. The medium was induced with 50 ppm 4-aminophenol and was grown for 48 h at 30°C and 100 rpm. The culture medium was centrifuged at 8,000 rpm for 30 m at 4°C and the pellet was washed twice with 50 mM phosphate buffer and re-suspended in different flasks with PNRG media at different pH levels; 3, 4, 5, 5.5, 6, 6.5, 7, 7.5, 8, 9, 10, 11 and 12. 50 ppm of 4-aminophenol was then added to these flasks. CFU (colony forming unit) and degradation results were tested by taking out samples at 0, 24, 48 and 72 h intervals.

Effect of temperature on 4-aminophenol biodegradation

Experiment was carried out at various temperatures in order to determine the effect of temperature on biodegradation of 4-aminophenol by *Pseudomonas* sp. strain ST- 4. Inoculum in nutrient broth was incubated for 24 h and then added to PNR-G media flasks having 50 ppm 4 -aminophenol for induction. After 48 h, culture media was centrifuged at 8,000 rpm and 4°C for 30 min. The pellet was washed twice with 50 mM phosphate buffer and added to PNR-G (50 ppm 4-aminophenol) flasks and incubated in shaking incubator at 20, 30, 35 and 37°C. For degradation and CFU analysis, samples were drawn at regular intervals of time, that is, 0, 24, 48 and 72 h.

Effect of glucose concentration on 4-aminophenol biodegradation

Glucose effect the biodegrading potential of *Pseudomonas* sp. strain ST-4 as it acts as source of energy and carbon for bacterial strain. To find the optimum glucose amount, different concentration of glucose, that is, 0, 2.5, 5, 7.5, 10, 15 and 20 mM were used. First, a 24 h incubated culture in nutrient broth was induced with 50 ppm 4-aminophenol for 48 h and harvested at 8,000 rpm for 30 min at 4°C. The pellet was washed twice with 50 mM phosphate buffer and then dispensed in separate flasks, each containing different ranges of glucose as well as 50 ppm 4-aminophenol. Samples at 0, 24, 48 and 72 h were analyzed for CFU and biodegradation.

RESULTS

In order to study the effect of pH on 4-aminophenol biodegradation, a wide range of pH levels (3 - 12) were used. At pH 3 and 4, no bacterial growth was recorded but reduction of 23.0 and 26.0% in 4-aminophenol concentration were observed, which indicated autoxidation of the compound at acidic environment. The pH levels from 5 to 10 supported the bacterial count as shown by CFU results taken at 10^7 dilution factor. The reduction in 4-aminophenol at these pH level was due to the biodegradation of *Pseudomonas* sp. strain ST-4. There was a gradual increase in rate of reduction of the compound till pH 8 (82.6%), after which it declined till it reaches to the point when there was no more reduction at pH 12. Degradation at pH 11 was the result of auto oxidation as no bacterial growth was observed at this pH level. The pH 12 values showed no effect on degradation of the compound (Figure 2).

All microbes exhibit affinity towards some specific temperature. Since *Pseudomonas* belongs to mesophyllic group of bacteria, the temperature range selected for the degradation of 4-aminophenol was 25, 30, 35 and 37°C. Maximum biodegradation of 4-aminophenol (53.4%) was observed at 30°C, as compared to 15.6, 36.1 and 25.5% biodegradation at 25, 35 and 37°C respectively. All the temperatures supported nearly equal growth of cells as indicated by CFU at dilution factor 10^7 (Figure 3).

Since the *Pseudomonas* sp. strain ST-4 was unable to utilize 4-aminophenol in the absence of glucose, it indicated that the glucose acts as an facilitator as it provide additional energy supplement to bacteria for 4-aminophenol biodegradation. In order to determine the appropriate level of glucose to enhance the degradation process, various concentration of glucose were tested. Different ranges of glucose concentration used were 0, 2.5, 5, 7.5,

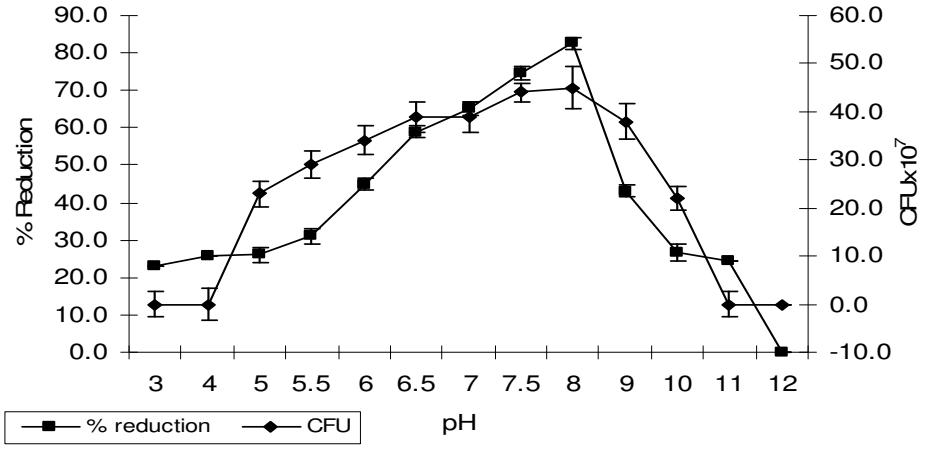

Figure 2. Effect of different pH levels on degradation of 4-aminophenol by *Pseudomonas* sp. ST- 4 after 72 h.

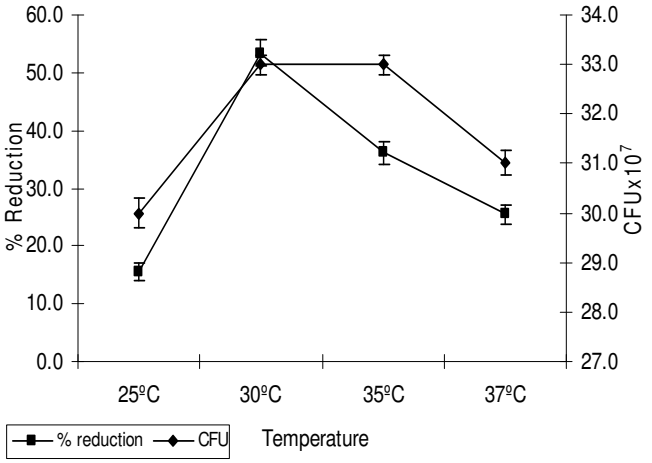

Figure 3. Effect of different temperatures on 4-aminophenol biodegradation by *Pseudomonas* sp. ST-4 after 72 h.

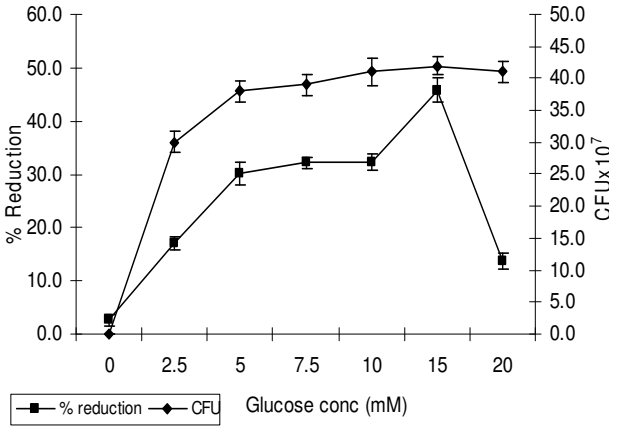

Figure 4. Effect of different glucose concentration on 4-aminophenol biodegradation by *Pseudomonas* sp. strain ST-4 after 72 h.

10, 15 and 20 mM. Our results showed that 15 mM concentration was optimum, as 45.8% reduction in 4-aminophenol was observed after 72 h. Without glucose, that is, at 0 mM, bacterial cells number was zero after 24 h from initial 38 x 10^7 cells/ml used as inoculum while 2.7% of reduction in 50 ppm 4-aminophenol was the possible indication of either autoxidation or some degradative activity by the inoculum at initial stage of the experiment. With glucose the cell number increased from 30 x 10^7 at 2.5 mM level contents to 42 x 10^7 at 15 mM glucose level (Figure 4).

DISCUSSION

Biodegradation is an important mean to eliminate toxic wastes from the environment (Ogbo and Okhuoya, 2008). Our study is based on biodegradation of 4-aminophenol by *Pseudomonas* sp. strain ST-4 at different pH, temperature and glucose concentrations. This bacterial strain is generally present in the soil and it has been reported previously by various researchers that soil inhabiting bacteria possess greater ability to degrade aromatic amines and to utilize them as source of carbon and energy (Berthe-Corti and Fetzner, 2002; Olukayode et al., 2006; Slaoui et al., 2007).

pH plays a vital role in biodegradation and gives an insight to degradation process of 4-aminophenol as itbiodegraded at pH ranges of 5 to 10 at different rates. A reduction in 4-aminophenol biodegradation in more acidic and alkaline media was due to cell lyses, as confirmed by conducting CFU (pH 5, 5.5 and 10) that showed a decrease in cell number as compared to the number of viable cells in the inoculum used. High acidic (pH 3 and 4) and alkaline (pH 11 and 12) medium yielded zero result for CFU, indicating that the bacterial cell were not viable at extreme pH levels and the reduction in

4-aminophenol, could be the result of autoxidation of the 4-aminophenol.

Similarly, microbial population was found to be able to biodegrade a number of aromatic compounds and their substituted compounds, though they proposed that a relatively neutral pH was beneficial for phenolic degradation (Zeyer et al., 1985; Wang and Barlaz, 1997). The high sensitivity of *Pseudomonas* for pH and optimum pH for the degradation of 4-aminophenol by *Pseudomonas putida* F1 and *P. putida* RE204 was reported to be pH 8 (Zeyer et al., 1985; Rabia, 1998) that was similar to our current findings.

Temperature exerts a strong selective pressure on microbial communities and can affect the degradation of organic compounds through direct effects on enzyme activity (Pettersson and Baath, 2003). Effect of temperature was observed on *P. sp. strain* ST-4 and 30°C was found to be the most effective temperature for biodegradation. A similar study on the degradation of nitrobenzene by *Pseudomonas pseudoalcaligenes* observed that 30°C was the optimum temperature for biodegradation (Nishino and Spain, 1993). It was demonstrated that growth and metabolic activity of *Pseudomonas* sp. Ap-3, was maximum at 30°C (Takenaka et al., 2000).

The biodegradation of synthetic chemicals in the presence of an alternative carbon source enhance the ability of the bacteria to metabolize the target chemical effectively. Our analysis of 4-aminophenol biodegradation in the presence of glucose gives promising results with increasing glucose amounts till 15 mM. Above this concentration, glucose was found to resist 4-aminophenol degradation while its absence resulted in halting of biodegradation process. It was reported that some microbes carried out the degradative mechanism effectively in the presence of high levels of glucose (Konopca et al., 1989). Similarly, it was observed that the utilization of one type of organic substrate effects the utilization of another (Zissi and Lyberators, 1999). It is because the presence of glucose provides a source of carbon and energy for the microbes, thus enhancing their activity to utilize the resistant aromatic amines.

Conclusion

Bacterial strain belonging to genus *Pseudomonas,* capable of using 4- aminophenol as growth substrate was investigated for its biodegradative abilities under different pH values, temperatures and glucose concentrations. Due to positive results, *Pseudomonas* strain ST-4 may prove to be important in bioremediation and wastewater treatment. Further studies on 4-aminophenol metabolic pathways involved in biodegradation, identification and isolation of genes responsible for producing degradative enzymes and sequencing and cloning of such genes may increase further knowledge about the abilities of this bacterial strain towards biodegradation process.

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