

Full Length Research Paper

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

Sumera Afzal Khan<sup>1#</sup>, Muhammad Hamayun<sup>2#</sup>, Abdul Latif Khan<sup>2</sup>, Bashir Ahmad<sup>1</sup>, Safia Ahmed<sup>3</sup> and In-Jung Lee<sup>2\*</sup>

<sup>1</sup>Center of Biotechnology and Microbiology, University of Peshawar, Pakistan

<sup>2</sup>School of Applied Biosciences, Kyungpook National University, Korea

<sup>3</sup>Department of Microbiology, Quaid-i-Azam University, Pakistan

<sup>4</sup>Kohat University of Science & Technology, Kohat Pakistan

Accepted 17 April, 2009

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

\*Corresponding author. E- mail: [ijlee@knu.ac.kr](mailto:ijlee@knu.ac.kr). Tel.: +82-53-950-5708. Fax: +82-53-950-6880.

#These authors contributed equally to this paper

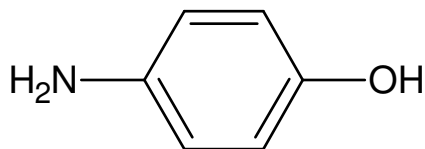


Figure 1. 4-Aminophenol.

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  $\lambda$  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%  $\text{KH}_2\text{PO}_4$ , 2.4%  $(\text{NH}_4)_2\text{SO}_4$  and 2.5% NaOH for PN salt (20x) and 8.0%  $\text{MgSO}_4$ , 0.2%  $\text{Fe}_2\text{SO}_4$  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 min 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

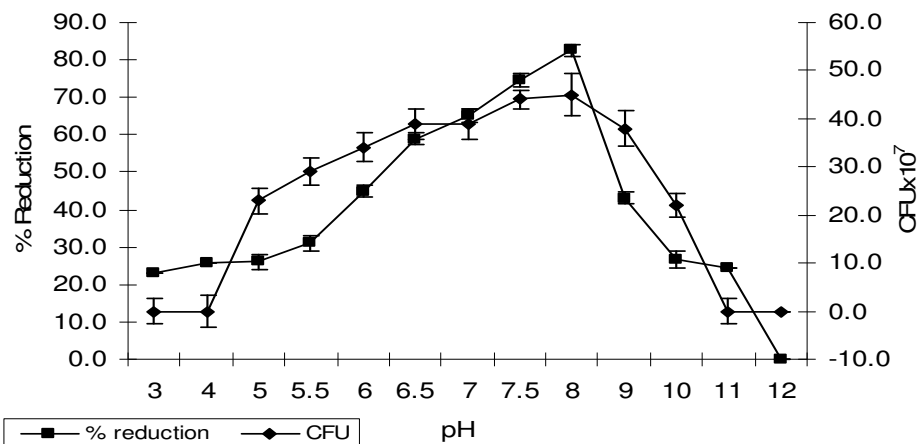
Glucose effect the biodegrading potential of *Pseudomonas* sp. strain ST-4 as it acts as source of energy and carbon for bacterial strain. To find the optimum glucose amount, different concentration of glucose, that is, 0, 2.5, 5, 7.5, 10, 15 and 20 mM were used. First, a 24 h incubated culture in nutrient 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

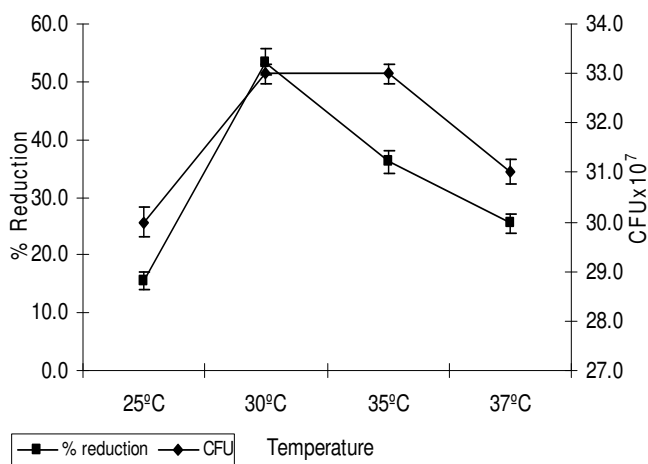
In order to study the effect of pH on 4-aminophenol biodegradation, a wide range of pH levels (3 - 12) were used. At pH 3 and 4, no bacterial growth was recorded but reduction of 23.0 and 26.0% in 4-aminophenol concentration were observed, which indicated autoxidation of the compound at acidic environment. 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).

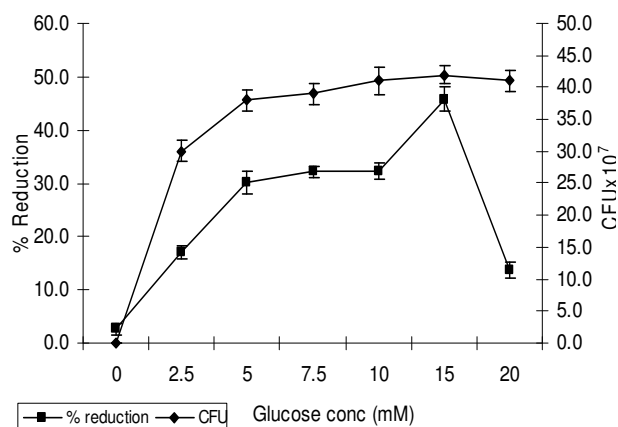
Since the *Pseudomonas* sp. strain ST-4 was unable to utilize 4-aminophenol in the absence of glucose, it indicated that the glucose acts as an facilitator as it provide additional energy supplement to bacteria for 4-aminophenol biodegradation. In order to determine the appropriate level of glucose to 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  $38 \times 10^7$  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  $30 \times 10^7$  at 2.5 mM level contents to  $42 \times 10^7$  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 it biodegraded 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.

## ACKNOWLEDGEMENT

This work was supported by the Korea Research Foundation Grant funded by the Korea Government (KRF-521-F00001).

## REFERENCES

- Annadurai G, Ling LY, Lee J (2007). Biodegradation of phenol by *Pseudomonas pictorum* on immobilized with chitin. *Afr. J. Biotechnol.* 6(3): 296-303.
- Berthe-Corti L, Fetzner S (2002). Bacterial metabolism of *n*-Alkanes and ammonia under oxic, suboxic and anoxic conditions. *Act. Biotechnol.* 22(3-4): 299-336.
- Boyd SA, Shelton DR, Berry D, Tiedje JM (1983). Anaerobic biodegradation of phenolic compounds in digested sludge. *Appl. Environ. Microbiol.* 46: 50-54.
- Cerniglia CE, Freeman JP, Baalen V (1981). Biotransformation and toxicity of aniline and aniline derivatives of cyanobacteria. *Arch. Microbiol.* 130: 272-275.
- Dean-Ross D, Rahimi M (1995). Toxicity of phenolic compounds to sediment bacteria. *Bull. Environ. Contam. Toxicol.* 55: 245-250.
- De-Frank JJ, Ribbons DW (1976). The p-cymene pathway in *Pseudomonas putida* PL Isolation of a dihydrodiol accumulated by a mutant. *Biochem. Biophys. Res. Commun.* 70: 1129-1135.
- Hart A, Orr OL (1975). The degradation of paracetamol (4-hydroxyacetanilide) and other substituted acetanilides by a *Penicillium* species. *Antonie Leeuwenhoek.* 41: 239-247.
- Khan SA, Hamayun M, Ahmad S (2006). Degradation of 4-aminophenol by newly isolated *Pseudomonas sp. strain* ST-4. *Enzym. Microb. Technol.* 38: 10-13.
- Konopca A, Knight D, Turco R (1989). Characterization of a *Pseudomonas sp.* capable of aniline degradation in the presence of secondary carbon source. *Appl. Environ. Microbiol.* 55: 385-389.
- Nishino SF, Spain JC (1993). Degradation of nitrobenzene by a *Pseudomonas pseudoalcaligenes*. *Appl. Environ. Microbiol.* 59: 2520-2525.
- O'Connor OA, Young LY (1996). Effects of six different functional groups and their position on the bacterial metabolism of monosubstituted phenols under anaerobic conditions. *Environ. Sci. Technol.* 30(5): 1419-1428.
- Ogbo EM, Okhuoya JA (2008). Biodegradation of aliphatic, aromatic, resinic and asphaltic fractions of crude oil contaminated soils by *Pleurotus tuber-regium* Fr. Singer - a white rot fungus. *Afr. J. Biotechnol.* 7(23): 4291-4297.
- Olukayode OA, Matthew OI, Sunday AA Musa KI (2006). Utilization of alicyclic compounds by soil bacteria. *Nat. Sci.* 4(3): 65-68.
- Pettersson M, Baath E (2003). Temperature-dependent changes in the soil bacterial community in limed and unlimed soil. *FEMS Microbiol. Ecol.* 45: 13-21.
- Rabia B (1998). Treatability studies of phenolic wastes. M.Phil. Dissertation. Department of Biological Sciences, Q.A.U. Islamabad, Pakistan.
- Schenzle A, Lenke H, Fischer P, Williams PA, Knackmuss HJ (1997). Catabolism of 3-nitrophenol by *Ralstonia eutropha* JMP134. *Appl. Environ. Microbiol.* 63: 1421-1427.
- Slaoui M, Ouhssine M, Berny E, Elyachioui M (2007). Biodegradation of the carbofuran by a fungus isolated from treated soil. *Afr. J. Biotechnol.* 6(4): 419-423.
- Sone T, Tokuda Y, Sakai T, Shinkai S, Manabe O (1981). Kinetics and mechanisms of the Bamberger rearrangement. Rearrangement of phenylhydroxylamines to p-aminophenols in aqueous sulphuric acid solutions. *J. Chem. Soc. Perkin. Trans. 2:* 298-302.
- Takenaka S, Murakami SJ, Kim Y, Aoki K (2000). Complete nucleotide sequence and functional analysis of the gene for 2-aminophenol metabolism from *Pseudomonas sp. AP-3*. *Arch. Microbiol.* 174: 265-272.
- Takenaka S, Okugawa S, Kadowaki M, Murakami S, Aoki K (2003). The metabolic pathway of 4-aminophenol in *Burkholderia sp.* strain

- AK-5 differs from that of aniline and aniline with C-4 substituents. *Appl. Environ. Microbiol.* 69 (9): 5410-5413.
- Tan NC, Prenafeta-Boldu FX, Opsteeg JL, Lettinga G, Field JA (1999). Biodegradation of azo dyes in cocultures of anaerobic granular sludge with aerobic aromatic amine degrading enrichment cultures. *Appl. Microbiol. Biotechnol.* 51: 865-871.
- Wang YS, Barlaz MA (1997). Anaerobic biodegradability of cellulose and hemicellulose in excavated refuse samples using a biochemical methane potential assay. *J. Ind. Microbiol.* 13: 147-153.
- Zeyer J, Wasserfallin A, Timmis KN (1985). Microbial mineralization of ring-substituted aniline through an ortho-cleavage pathway. *Appl. Environ. Microbiol.* 50: 447-453.
- Zhao JS, Singh A, Huang XD, Ward OP (2000). Biotransformation of Hydroxylaminobenzene and Aminophenol by *Pseudomonas putida* 2NP8 cells grown in the presence of 3-nitrophenol. *Appl. Environ. Microbiol.* 66(6): 2336-2342.
- Zissi US, Lyberators GC (1999). Kinetics of growth and aniline degradation by *Stenotrophomonas maltophilia*. *Water Environ. Res.* 71: 43-49.