Biodegradation Modeling of Nitrophenolic Pollutant in a Slurry Bubble Reactor

Zeinab Salehi[*1], Shohreh Fatemi[1] and Farzaneh Vahabzadeh[2]
[1]School of Chemical Engineering, College of Engineering, University of Tehran, Tehran, Iran
[2]Chemical Engineering Department, Amirkabir University of Technology, Tehran, Iran
(Received 4 March 2014, Accepted 9 September 2014)

Abstract

Biodegradation kinetics of 4-nitrophenol (PNP) in aqueous solution by a gram negative soil bacterium, *Ralstoniaeutropha* was firstly studied in a small scale batch reactor. The degradation of PNP was evaluated at initial PNP concentrations ranging from 3 mg/L to 14 mg/L. The rate of PNP consumption by the bacterium culture was modeled using Monod and Contois kinetics in batch condition. PNP degradation by adapted *R. eutropha* was well fitted to the Monod equation for the pollutant with initial concentration of 3-8 mg/L, whereas for PNP with initial concentration of 14 mg/L the experimental data were better fitted to the Contois kinetic model. The process of degradation of PNP was scaled up in a slurry bubble reactor with 250 ml working volume in presence of 0.75 L/min air flow rate. The derived kinetic model was validated by comparison the outlet time dependent experimental data of PNP concentration with the model in the slurry bioreactor and the results showed good agreement between experiments and the model.

Keywords:Biodegradation, p-Nitrophenol, Ralstoniaeutropha, Kinetic modeling, Slurry bubble reactor

Introduction

Chemical and petroleum industries produce a wide variety of highly toxic organic wastes. These wastes often contain aromatic compounds that are resistant to natural degradation and therefore persist in the environment. One of the organic pollutants found in these wastewaters is nitrophenols. Biodegradation of phenolic wastewater has advantages over other methods of treatment. Phenolic wastewater treatment is done by using methods like freely suspended cell systems, trickling filters, rotating disc, activated sludge, biological fixed film methods and fluidized bed bioreactors. Slurry bubble column bioreactors have a number of advantages in terms of in design and operation as compared to other reactors. They have excellent heat and mass transfer characteristics. Little maintenance and low operating costs are required due to lack of moving parts. Due to the spread use of the three-phase reactors and the economic advantages resulted from an optimum operation, the modeling of these reactors plays an important role in the continuous improvement of the multiphase technology [1-5].

Nitrophenols are widely used in the production of pesticides, herbicides, explosives, dyes and so on. P-nitrophenol (PNP) is a toxic nitroaromatic compound and a potential environmental contaminant of water due to its high solubility into water [6-8]. *Ralstoniaeutropha*(formerly named *Alcaligeneseutrophus*) is a Gram-negative bacterium recognized for its efficiency in degrading a wide variety of aromatic pollutants, especially nitroaromatics (NAs) [9].

Several numbers of microbes are found to be capable of degrading PNP via aerobic as well as anaerobic pathways. Although numerous publications have focused on the metabolic pathways of PNP degradation, some studies have focused on the kinetics of PNP biodegradation and very little information is available on the kinetics of PNP degradation in a slurry bubble reactor [10-13]. Removal kinetics of 4-nitrophenol

* Corresponding author: Tel: +98-21-61112257 Fax: +98-21-66957784 Email: zsalehy@ut.ac.ir

biodegradation in a sequencing batch reactor has been studied and was well described by the by the Haldane equation. High removal rates, short acclimation times and good settling characteristics of produced sludge confirmed the suitability of periodic systems in enhancing the bacterial potentialities for biodegradation of xenobiotic compounds [2, 3].

The objective of this study is kinetic modeling of biodegradation of PNP in the slurry reactor by the free cells of *Ralstoniaeutropha*. Results obtained herein provide useful information concerning the design criteria and conditions of slurry bioreactor for the industrial operations.

2. Material and methods

2.1. Microorganism and cultivation medium

*R. eutropha*was supplied by Japan Collection of Microorganisms (JCM) (accession number of strain: JCM 20644 in 2007). The growth medium (liquid culture 'LC' medium) was prepared as described elsewhere [14].

2.2. Chemicals

P-Nitrophenol (PNP) was purchased from Kanto Chemical Co., Inc. (Japan). All chemicals used in this study were of analytical grade with the highest purities.

2.3. Analytical methods

HPLC analysis was used to determine PNP concentration (Shimadzu HPLC system equipped with an Inertsil ODS-3V reverse phase column and a UV detector at 280 nm; acetonitrile and $NaClO_4$ (pH adjusted to 2.5) were the system solvents for this measurement). Analysis was done on the cell-free supernatants obtained after centrifugation at 12000 rpm for 20 min. Cell growth measurement was performed spectrophotometrically at wave length of 546 nm.

2.4. Kinetic experiments

For the kinetic study of biodegradation, 5 ml of the adapted *R. eutropha* culture was transferred to freshly prepared the liquid culture medium (LC)(50 ml) containing PNP at 3, 5, 8 and 14 mg/L concentrations for different experiments. Yeast extracted at 0.25 g/L was added to the LC medium (pH 7, incubation conditions: shaking at the reciprocal mode, temperature of 30°C for 20 h). The inoculated culture grew and it was centrifuged (2700 rpm, 20 min), and the sediment was used to prepare a *R. eutropha* cell suspension. The cell suspension was adjusted to an initial optical density of 0.1 at 546 nm. Fifty milliliters of sterile medium consisting of LC medium and PNP was inoculated in a 250 ml Erlenmeyer flask and incubated in a reciprocal shaker at 30°C. Sampling carried out in time intervals.

2.5. Slurry reactor

A schematic illustration of the bioreactor used for the biodegradation of PNP and validation of the kinetic models is shown in Figure 1. The bioreactor was constructed by B.E.MarubishiCo.Ltd/ Model MDS-U50 (Japan) and had a working volume of 250 ml (temperature of 30°C, pH7). In the bioreactor agitation of phases carried out by air bubbles produced by air sparger. Sterile bioreactor consisting the LC medium (250 ml) plus PNP (3 mg/L) was inoculated. The cell suspension was adjusted to an initial optical density of 0.1 at 546 nm. The airflow rate was measured by a calibrated rotary meter and the volumetric airflow rates in this study were 0.75 L/min. Aeration was required to disperse the cells uniformly throughout the bioreactor besides to supply oxygen to the microorganisms.

The mass balances of PNP and cell were written at unsteady state conditions to model PNP biodegradation in the slurry bioreactor, with the following assumptions:

1-All the experiments were carried out at constant temperature of 30°C and pressure of 1 atm.
2-Free cells of the microorganisms were homogeneously dispersed in bioreactor.
3-The species' concentration was assumed uniform inside the liquid.

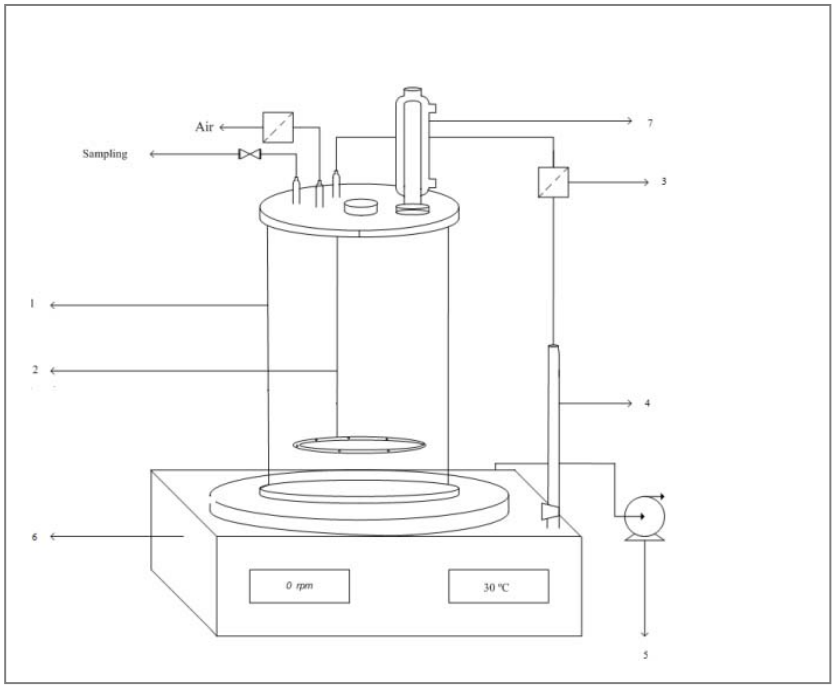

Figure 1: Schematic layout of a slurry bioreactor: 1.Bioreactor, 2.Air sparger, 3.Air filter, 4.Air flowmeter, 5.Pump, 6.Heater, 7. Condenser

4-Air was bubbled throughout the reactor and a homogeneous mixture was prepared in the reactor.
5- System was working at batch condition with no liquid inlet and no outlet of PNP and free cells.
6- The oxygen content was in extra ratio and liquid was assumed to be saturated from oxygen.
7- The death kinetic for microorganisms was considered besides kinetic growth.

Initial and operation conditions are listed in Table 1.

Table 1: Initial, and operation conditions

Parameter (Unit)	Value
S_0 (mg/l)	2.797
X_0 (mg/l)	71.394
V_R(cm^3)	250
Q (l/min)	0.75

The model included two ordinary differential equations to predict the dynamic of batch biomass of *R.eutropha* (X) with the simultaneous consumption of PNP (S). These two dynamic ordinary differential equations are:

$$\frac{dS}{dt} = \frac{1}{Y_{X/S}}\mu X \quad (1)$$

$$\frac{dX}{dt} = \mu X - \alpha X \quad (2)$$

Where μ is the microbial growth rate constant, $Y_{X/S}$ is the biomass yield and $\alpha$is the average decay ratio. The kinetic model was studied in three different PNP initial concentrations of 5.645, 8.321 and 13.841 mg /L, respectively.Whereas the initial cell suspension was adjusted to an initial optical density of 0.1 at 546 nm.The Matlab programming software was used for the kinetic modeling. Two different rate equations were tried. The growth rate constants (μ) and unknown parameters were determined by regression of the models on the experimental concentration-time results.

3. Results and discussion

The experimental results of concentration-time at different initial PNP concentrations are presented in Figure 2. It is observed that the lower PNP concentration is readily degraded. PNP at initial concentration of 3.14 mg/L after 6 h

was completely degraded as shown in Figure 2a. When level of PNP increased to 5.645, 8.322 and 13.841 mg/L, the PNP biodegradation was completed after about 17, 38 and 134 h, respectively. As shown in figure 2 b,c and d, it was observed that the cell concentration of *R.eutropha* was reduced in all experiments. PNP in the absence ofa growth substrate provided a poor medium for bacteria and transformed by co-metabolism. It can be inferred that adapted bacterium of *R.eutropha* consumed consumes PNP as a carbon, nitrogen and energy source. Hill et al studied on co-metabolic degradation of 4-chlorophenol by *Alcaligeneseutrophus* using either phenol as sole substrate or mixtures of phenol and 4-chlorophenol [15]. Phenol was found to be the sole source of carbon and energywhile 4- chlorophenol was utilized only as a co-metabolism. They observed no growth or 4-chlorophenol consumption without presence of Phenol (primary growth substrate). They found as 4-chlorophenol is a co-metabolite, it does not contribute to production of biomass but it is also clear that cells are unable to utilize the growth substrate (phenol) to produce nearly as much biomass. The loss of microbial biomass or enzyme activity caused by autooxidation (endogenous decay), proteolysis, depletion of cofactors (such as NADH), product toxicity and suicide inactivation [15,16].

A summary of the model and the parameters of this study as well as sum of square of deviation (SSD) is provided in Table 2. Table 3 shows the bio-kinetic parameters of *R.eutropha* growing on PNP at initial concentrations of 5.645 and 8.321.

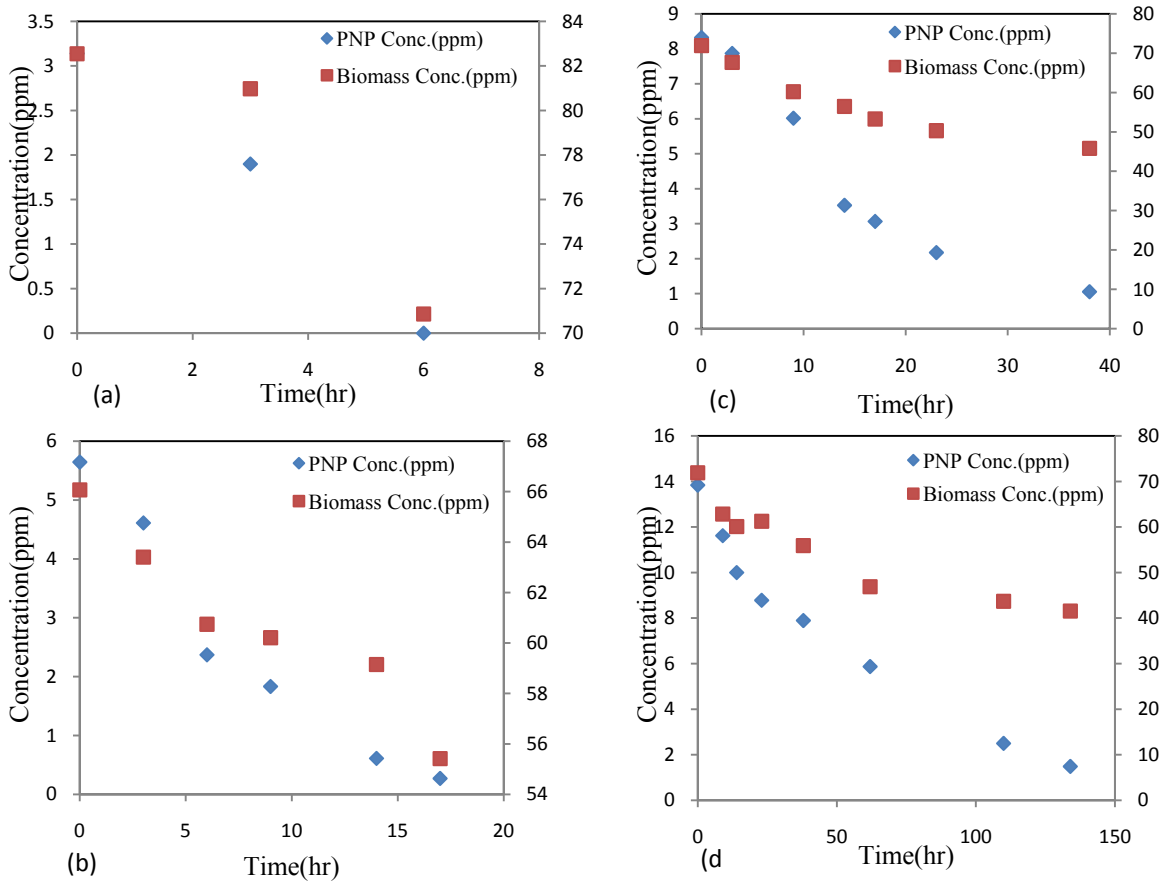

Figure 2: Progress curve of PNP biodegradation and time course of biomass at PNP initial concentration of (a): 3 mg/L, (b). 6 mg/L, (c) 8 mg/L,(d) 14 mg/L

Table 2: Summary of the kinetic models and SSD

Model	Equation	SSD	PNP Concentration (mg/l)
Monod	$\mu = \frac{\mu_m S}{K_s + S}$	0.55	0-8
Contois	$\mu = \frac{\mu_m S}{K_s X + S}$	0.68	8-14

Table 3: Kinetic parameters of R.eutropha variation at PNP initial concentrations of 5.645 and 8.321 mg/l.

Kinetic parameters*				
$Y_{X/S}$	α	K_s	μ_m	PNP initial con.(mg/l)
$(mg/L)_{cell}$ /$(mg/L)_{substrate}$	h^{-1}	mg/L	h^{-1}	
4.085	0.033	0.227	0.028	5.645
5.91	0.046	1.78	0.037	8.321

* Monod model

Table 4: Kinetic parameters of PNP degradation (PNP initial concentration: 13.841)

Kinetic model	Kinetic parameters						
	μ_m	K_s	$Y_{X/S}$	α	K_i	n	S_m
Monod	0.03	1	15.376	0.035	-	-	-
Contois	0.105	61	0.107	0.05	-	-	-

The bio-kinetic parameters of two models for the PNP initial concentration of 13.841 mg/L are given in Table 4. Figure 3 shows degradation data by *R.eutropha* at three PNP initial concentrations. Figure 4 indicates biomass data.The models describing the biodegradation of organic compounds that are not supporting growth when the responsible populations are growing logistically, logarithmically, or linearly or are not increasing in numbers was presented in the literature[17].Phenol at a concentration of 1 ng/ml did not affect the growth of *P. acidovorans*. These data were best fit by the model that incorporated the equation for logarithmic growth and assumed a concentration of test substrate well below itsK_m value [17].

Validation of the kinetic models was conducted using PNP degradation data in the scaledup slurry bioreactor by free cells of *R.eutropha*. As Monod equation was confirmed for the lower level of initial PNP concentration, the slurry bubble reactor was modeled by Monod equation and the results can be observed in Figure 5 with good consistency between experiment and model for PNP initial concentration of 2.797 mg/L. The sum of square of deviation between the experiment and model was calculated as 0.072.

4. Conclusions

In this study the performance of slurry bioreactor filled with free cells of *Ralstoniaeutropha* was examined and modelled for PNP degradation in relatively

low level of concentrations. Experimental results obtained herein can provide useful information concerning the design criteria and operation of slurry bioreactor for the pilot and industrial scales. The biodegradation of PNP and variation of the cell was modelled by Monod and Contios kinetic equations for initial PNP concentrations ranging from 3 mg/L to 14 mg/L. PNP degradation by adapted *R. eutropha* was shown to fit the Monod equation when the pollutant added at initial concentration of 3-8 mg/L. Implementation of the bubble slurry bioreactor at larger scale showed appropriate PNP biodegradation in presence of free cells with consistent results of the model and experiments.

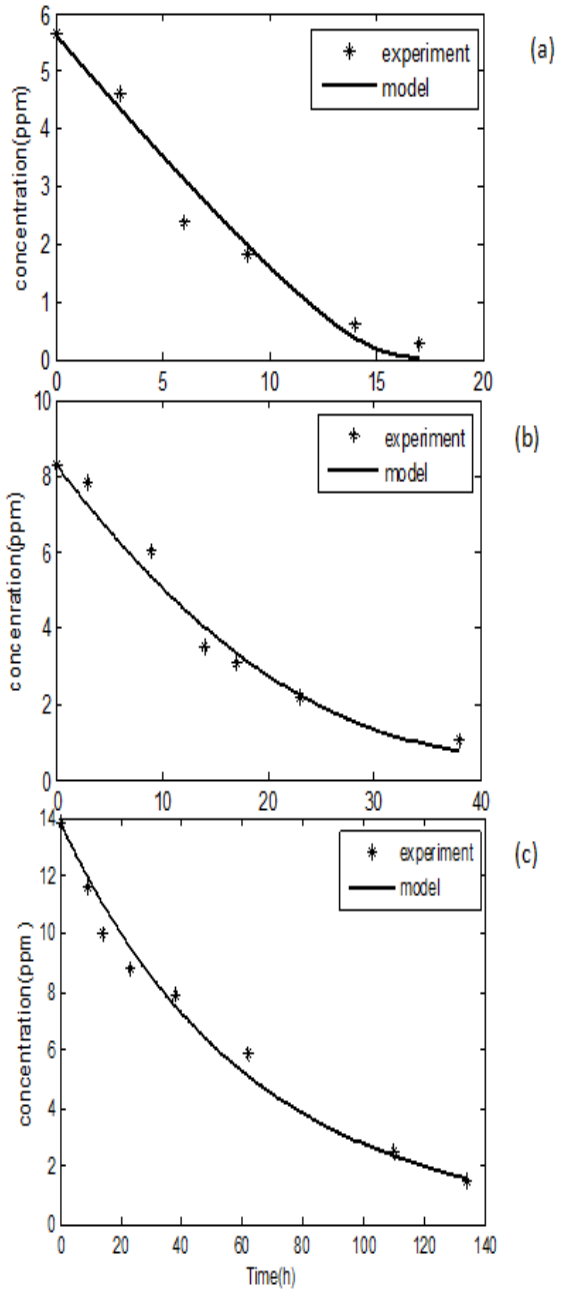

Figure 3: Comparison between experimental data and model calculations for the degradation of PNP by R. eutropha. Monod model for PNP initial concentrations of :(a): 6 mg/L, (b). 8 mg/L, , and Contois Model for (c) 14 mg/L.

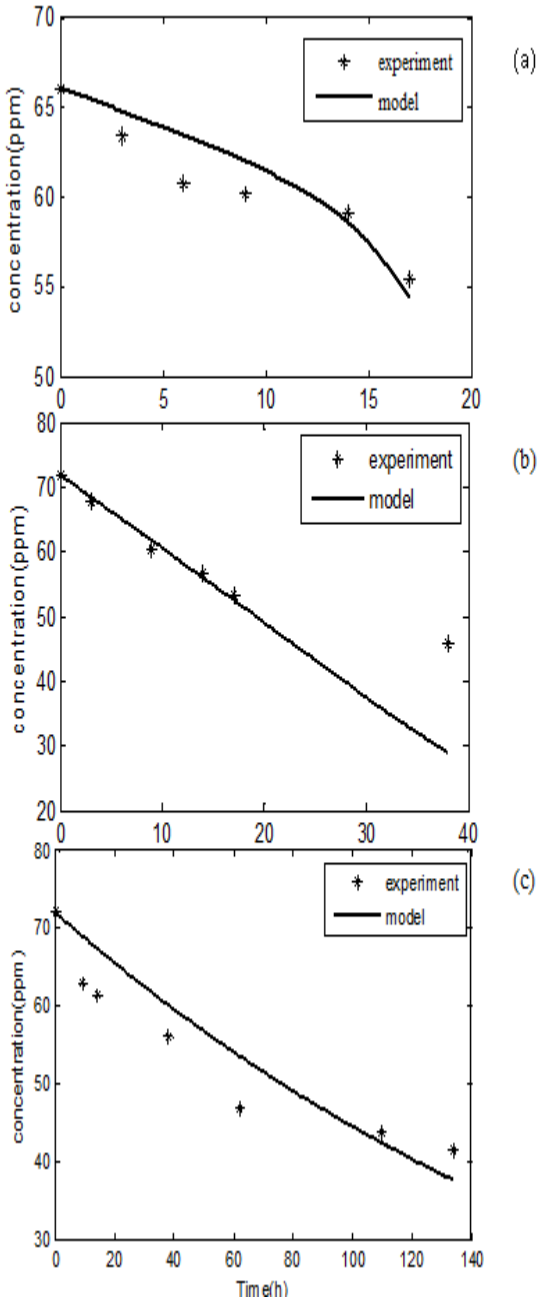

Figure 4: Comparison between the experimental data and model calculations for biomass Monod model for PNP initial concentration of (a): 3 mg/L, (b). 6 mg/L, (c) 8 mg/L,(d) and Contois model for 14 mg/L.

Acknowledgment

The authors would like to thank Iran National Sciences Foundation (INSF) for their financial support of this project.

Nomenclature

S_0 Initial PNP concentration (mg/L)
X_0 Initial cells concentration(mg/L)
V_R Liquid volume (cm^3)
Q Input air flow rate (L/h)
A Area of bioreactor (cm^2)
u_g Air superficial velocity (cm/s)
ρ_l Liquid density (g/cm^3)
μ_l Liquid viscosity (poise)

References:

1- Chaudhari, R. V. and Ramachandran, P. A. (1983). "Three-phase catalytic reactors." *AlChE J.*, Vol. 26, pp. 177-201.

2- Tomei, M.C., Annesini, M.C. and Bussoletti, S. (2004). "4-Nitrophenol biodegradation in a sequencing batch reactor: kinetic study and effect of filling time." *Water Res.*, Vol. 38, pp.375–384.

3- Tomei, M.C. and Annesini, M.C. (2005). "4-Nitrophenol biodegradation in a sequencing batch reactor operating with aerobic–anoxic cycles." *Environ Sci Technol.*, Vol. 39, pp. 5059–5065.

4- Yi, S., Zhuang W.Q., Wu, B., Stay, T.L. and Tay, J. H. (2006). "Biodegradation of p- nitrophenol by aerobic granules in a sequencing batch reactor."*Environ. Sci. Technol.*, 40, pp. 2396–2401.

5- Martin-Hernandez, M., Carrera, J., Perez, J. and Suarez-Ojeda, M.E. (2009). "Enrichment of a K-strategist microbial population able to biodegrade p-nitrophenol in a sequencing batch reactor." *Water Res.*, Vol. 43, pp. 3871–3883.

6- Salehi, Z., Sohrabi, M., Vahabzadeh, F., Fatemi, S. and Kawase, Y. (2010). "Modeling of p-nitrophenol biodegradation by *Ralstoniaeutropha* via application of the substrate inhibition concept." *J Hazard. Mater.*, Vol. 177, pp. 582–585.

7- She, Z., GaoJin, M., C., Chen, Y. and Yu, J. (2005). 'Toxicity and biodegradation of 2,4-dinitrophenol and 3-nitrophenol in anaerobic systems." *Process Biochem.*, Vol. 40, pp. 3017–24.

8- Qiu, X., Zhong, Q., Li, M., Bai, W. and Li, B. (2007). "Biodegradation of p-nitrophenol by methyl parathion-degrading *Ochrobactrum* sp. B2." *Int Biodeterior. Biodegrad.,* Vol. 59, pp. 297–301.

9- Muller, R. H. and Babel, W. (1996). "Growth rate-dependent expression of phenol-assimilation pathwaysin *Alcaligeneseutrophus* JMP 134 – the influence of formate as an auxiliary energy source on phenol conversion characteristics."*Appl. Microbiol. Biotechnol.,*Vol. 46, pp.156-162.

10- Salehi,Z., Yoshikawa, H., Mineta, R. and Kawase, Y. (2011). "Aerobic biodegradation of p-nitrophenol by acclimated waste activated sludge in a slurry bubble column." *Process. Biochem.,* Vol. 46, pp. 284–289.

11- Qiu, X., Wu, P., Zhang, H., Li, M. and Yan, Z. (2009). "Isolation and characterization of *Arthrobacter* sp. HY2 capable of degrading a high concentration of p-nitrophenol." *BioresourTechnol.*, Vol. 100, pp. 5243–5248.

12- Gemini, V.L., Gallego, A., de Oliveira, V.M., Gomez, C.E., Manfio, G.P., SKorol. E.(2005)."Biodegradation and detoxification of p-nitrophenol by *Rhodococcuswratislaviensis*." *Int Biodeterior. Biodegrad.*, Vol. 55, pp.103–108.

13- Rezouga, F., Hamdi, M. and Sperandio, M. (2009)."Variability of kinetic parameters due to biomass acclimation: case of para-nitrophenol biodegradation." *Bioresour. Technol.*, Vol. 100, pp. 5021–5029.

14- Muller, S., Bley, T. and Babel, W. (1999). "Adaptive responses of *Ralstoniaeutropha* to feast and famine conditions analysed by flow cytometry." *J. Biotechnol.*, Vol. 75, pp.81–97.

15- Hill, A., Milne, B. J. and Nawrocki, P. A. (1996). "Cometabolic degradation of 4-chlorophenol by *Alcaligeneseutrophus*." *Appl. Microbiol. Biotechnol.*, Vol. 46, pp.163-168.

16- Craig Criddle, S. (1993). "The kinetics of cometabolism."*Biotechnol. Bioeng.*, Vol. 41, pp. 1048–1056.

17- Schmidt, S. K., Simkins, S. and Alexander, M. (1985)."Models for the Kinetics of Biodegradation of Organic Compounds Not Supporting Growth." *Appl. Env. Mic.,* pp. 323-331.