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Kinetics Of Batch Microbial Degradation Of Phenols By Indigenous *Pseudomonas Aeruginosa*

S.E.Agarry[1]*, E.Betiku[2], B.O.Solomon[3]

[1]Biochemical Engineering Research Unit, Department of Chemical Engineering, Ladoke Akintola University of Technology, Ogbomoso, (NIGERIA)

[2]Biochemical Engineering Research Unit, Department of Chemical Engineering, Obafemi Awolowo University, Ile-ife, (NIGERIA)

[3]National Biotechnology Development Agency, Abuja, (NIGERIA)

Tel : +2348055529705

E-mail: sam_enagarry@yahoo.co.uk

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ABSTRACT

The potential of various organisms to metabolize organic compounds has been observed to be a potentially effective means in disposing of hazardous and toxic wastes. Phenolic compounds have long been recognized as one of the most recalcitrant and persistent organic chemicals in the environment. The bioremediation potential of an indigenous *Pseudomonas aeruginosa* was studied in batch culture using synthetic phenol in water in the concentration range of (100-500)mg/L as a model limiting substrate. The effect of initial phenol concentration on the degradation process was investigated. Phenol was completely degraded at different cultivation times for the different initial phenol concentrations. Increasing the initial phenol concentration from 100mg/L to 500mg/L increased the lag phase from 0 to 24hrs and correspondingly prolonged the degradation process from 54hrs to 168hrs.There was decrease in biodegradation rate as initial phenol concentration increased. Fitting data into three different kinetic models (Monod, Haldane, and Yano and Koga) showed that the difference in fit between the models was very small and thus statistically insignificant. Thus, the Yano and Koga model has been used to interpret the free cell data on phenol biodegradation. The kinetic parameters have been estimated up to initial phenol concentration of 500mg/L. The r_{smax} decreased, K_s and K_i increased with higher concentration of phenol. The r_{smax} has been found to be a strong function of initial phenol concentration.

KEYWORDS

Pseudomonas aeuginosa;
Phenol;
Biodegradation;
Kinetic model;
Batch cultivation;
Bioreactor.

INTRODUCTION

Phenol and its derivatives is the basic structural unit in a wide variety of synthetic organic compounds[4]. It is an organic, aromatic compound that occurs naturally in the environment [29], but is more commonly produced artificially from industrial activities such as petroleum processing, plastic manufacturing, resin production, pesticide production, steel manufacturing and the production of paints and varnish[6,23]. This aromatic compound is water-soluble and highly mobile[10] and as such waste waters generated from these industrial activities contain high concentrations of phenolic compounds[9] which eventually may reach down to streams, rivers, lakes, and soil, which represent a serious ecological problem due to their widespread use and occurrence throughout the environment[13].

Phenol is a listed priority pollutant by the U.S. Environmental Protection Agency[12] and is considered to be a toxic compound by the Agency for Toxic Substances and Disease Registry[5]. The adverse effects of phenol on health are well documented[8] and death among adults has been reported with ingestion of phenol ranging from 1 to 32g[29]. The low volatility of phenol and its affinity for water make oral consumption of contaminated water the greatest risk to humans[29].

A variety of techniques have been used for the removal of phenol from industrial effluents and contaminated waters with bioremediation receiving the most attention due to its environmentally friendly, its, ability to completely mineralize toxic organic compounds and of low-cost[19,29]. Microbial degradation of phenol have been actively studied and these studies have shown that phenol can be aerobically degraded by wide variety of fungi and bacteria cultures such as *Candida tropicalis* [9,31,32]; *Acinetobacter calcoaceticus*[27]; *Alcaligenes eutrophus*[18,22]; *Pseudomonas putida*[16,20,25]; and *Burkholderia cepacia G4*[14,36].

A variety of kinetic substrate utilization and inhibition models have been used to describe the dynamics of microbial growth on phenol. Of these various models, the Monod and Andrew (Haldane) equations has been extensively used to describe phenol biodegradation[6,26,30]. The Monod and Andrew (Haldane) equations are based on the specific growth rate[6,30], but may also be related to the specific substrate consumption rate[11,36]. Other kinetic models have been propagated. Sokol[35] has reported a better fit for a modified Monod-Haldane equation while Schroeder et al.[34] have shown a better fit for Yano and Koga equation amongst the tested inhibition models. Inspite of the rather extensive use of phenol biodegradation processes, surprisingly, little work has been published on phenol microbial degradation kinetics based on specific substrate consumption rate using pure or mixed culture systems. The present study investigated the effect of initial phenol (substrate) concentration on the degradation potential of an indigenous (local strains) *Pseudomonas aeruginosa* isolated from an oil-polluted swampy area of Warri in niger-delta region of Nigeria.

MATERIALS AND METHODS

Microorganism

The microorganism *Pseudomonas areuginosa* being an indigenous strain isolated from an oil-polluted area in niger-delta region of Nigeria was procured from the department of microbiology, Obafemi Awolowo University, Ile-ife, Nigeria. The microorganism was maintained on nutrient agar slant and stored at $4^0C \pm 1^0C$ for further use.

Culture medium and inoculum preparation

The mineral salt medium used was modified from the one suggested by Bettman and Rehm[7]. The medium had the following composition per litre: 700ml deionized water, 100ml buffer solution A, 100ml trace elements solution B, 50ml solution C and 50ml solution D.Compositions of each solution were as follows: Buffer solution a composition K_2HPO_4 1.0g, KH_2PO_4 0.5g, $(NH_4)_2SO_4$ 0.5g, deionized water 100ml. Trace element solution B composition NaCl 0.5g, $Cacl_2$ 0.02g, $MnSO_4$ 0.02g, $CuSO_4.5H_2O$ 0.02g, H_3BO_3 0.01g, deionized water 50ml. Solution C composition $MgSO_4.7H_2O$ 0.5g, deionized water 50ml, Solution D composition $FeSO_4$ 0.02g, molybdenum powder 0.02g, deionized water 50ml. To prevent the precipitation of $CaSO_4$ and $MgSO_4$ in storage, the water, buffer solution A, trace elements solution B, solution C and solution D were autoclaved at 121^0C for 15minutes. After cooling, all the solutions were then mixed together and kept as stock solution from which known quantities were

FULL PAPER

taken for the cultivation of the microorganisms

A primary culture was prepared by transferring two loops full of microorganisms from an agar slant culture into 100ml of feed medium containing 20ml of mineral salt medium and 80ml of 50mg phenol solution in a 250ml Erlenmeyer conical flask. This was then incubated in a NewBrunwick gyratory shaker (G25-R model, N.J. U.S.A) for 48hrs at a temperature of 30^{0}C and agitated with a speed of 120rpm. Thereafter, 10ml of the primary culture was transferred into another 100ml of feed medium in a 250ml Erlenmeyer conical flask and the incubation process was repeated. This was the secondary culture that was used as the inoculum for the degradation studies as this ensures that the organisms had fully adapted to growth on the phenol as sole source of carbon and energy.

Experimental design to study the free suspended cell system

The experimental studies were carried out in a NewBrunswick Microferm Twin Bioreactor (PH-22 model, N.J., U.S.A) with 4litres working volume. 800ml of the autoclaved mineral salt medium and 3 litres of phenol solution(100mg/l) were measured into the bioreactor vessel and 200ml of the inoculum was introduced aseptically to make up 4litres of working volume. The bioreactor was operated for several hours at a temperature of 30^{0}C, aeration rate of 3.0vvm and agitation of 300rpm. Culture broth was withdrawn at every 6hrs for biomass and phenol determination.

Estimation of phenol concentration

The undegraded phenol was estimated quantitatively by the spectrophotometric method using 4-amino antipyrene as colour indicator[37] at an absorbance of 510nm.

Estimation of biomass concentration

The biomass concentration was estimated using the dry weight method. 50ml sample of culture broth was withdrawn from the bioreactor and centrifuged (Gllenkamp centrifuge) at 4000rpm for 20minutes in plastic centrifuge tubes. The supernatant was decanted into small bottles and stored at 4^{0}C for subsequent phenol estimation. The pellets was re-suspended in de-ionized water and re-centrifuged. The supernatant was decanted and pellets rinsed off from the tube into a pre-

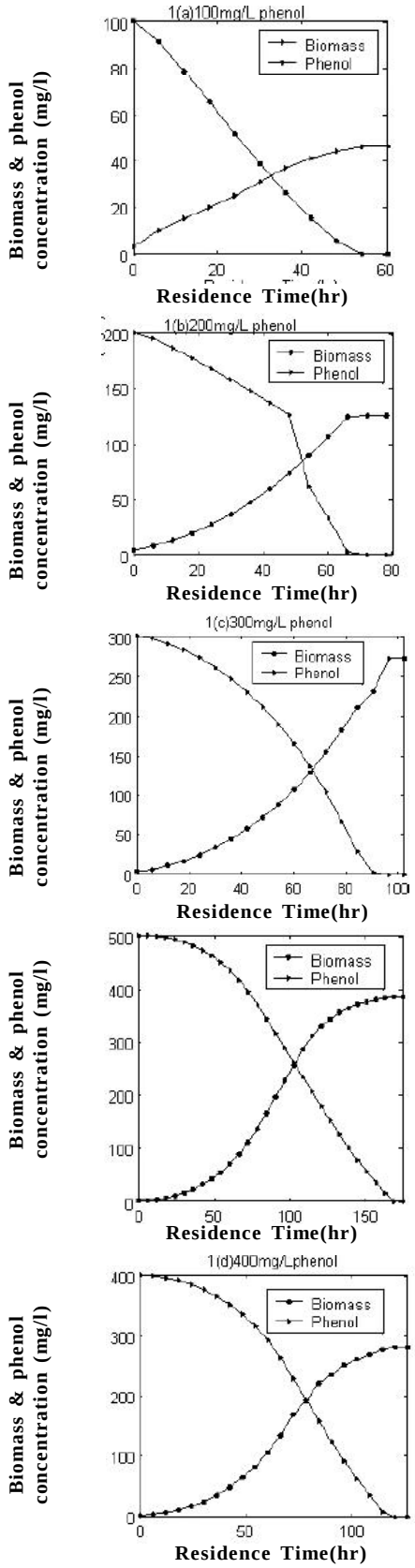

Figure 1(a-e) : A plot of phenol and biomass concentration as a function of time

weighed 1.2μm pore filter paper (Whatman GF/C). The filter paper was then dried in an oven at 105^0C for between 12-24hrs, cooled in a dessicator at room temperature and re-weighed until a constant dry weight was obtained. The difference between the pre-weighed filter paper and the second weight was used to estimate the dry weight of the biomass.

RESULTS AND DISCUSSION

Five batch cultivation experiments were carried out using phenol as single limiting substrate for *Pseudomonas aeruginosa.* Different initial phenol concentrations of 100mg/L to 500mg/L were used. The extent of phenol degradation using these different initial phenol concentrations was investigated for several batch residence times by intermittent sampling.

Figure 1(a-e) shows the biodegradation potential of the indigenous *P.aeruginosa* in degrading synthetic phenol waste in the concentration range of 100mg/L to 500mg/L. Since the degradation proceeds with biomass(cell mass) growth, the figure also depicts the typical cell growth curve. The cell growth curve has typical exponential and stationary phases with increasing lag phase. It could be seen from figure 1(a-e) that the different initial phenol concentrations ranging from (100-500) mg/L were completely degraded (consumed) at different residence time of 54hr, 72hr, 96hr, 120hr, 168hr, and during these times the biomass correspondingly increased to a maximum of 46mg/L, 125mg/L, 232mg/L, 280mg/L, and 385mg/L respectively. No lag phase was observed for initial phenol concentration of 100 and 200mg/L as shown in figure 1(a-b). However, for initial phenol concentration of(300, 400, and 500) mg/L corresponding lag phase of 6hr, 12hr, and 24hr was observed respectively as shown in figure 1(c-e) Moreover, as shown in figure 1(a-e) which indicates the comparison of the time course for phenol substrate consumption of all the five batches, it is evident that the rate of degradation decreased with increase in the initial phenol concentration. Bandyopadhyay et al.[6] and Ruiz-ordaz et al[32] reported a similar observation on *Pseudomonas putida* and *Candida tropicalis* grown on phenol respectively. Thus, it is observed that as the initial phenol concentration increases the duration of the lag phase increases and thereby prolonging the biodegradation time as a result of decrease in the rate of degradation. This observation is supported by the earlier works of Andrews[3], Hill and Robinson[16], Collins and Daugulis[10], and Oboirien et al.[26]

Evaluation of biokinetic parameters

Batch phenol degradation was carried out with free suspended cells of indigenous *P.aeruginosa* under different initial phenol concentrations as stated above. In this work, phenol well known as an inhibitory substrate under different concentrations(100-500)mg/L was completely degraded by the *P.aeruginosa* as shown in figure1(a-e). According to Prpich and Daugulis[29], the rate of substrate consumption was suggested to be the most important measure of microbe performance. Zilli et al.[39] gave a similar report. Relatively very few data exists in the literature on this parameter. Most of the data available concerns specific growth rate. It was on this basis that the specific phenol (substrate) consumption rate was calculated and plotted against phenol concentration as shown in figure 2. As seen from this figure 2, the specific phenol consumption rate (r_s) decreases as the phenol concentration (S) decreases for each of the different initial phenol concentrations. Therefore, it seems that there is also an influence of the initial phenol concentration on the specific phenol consumption rate. Hinteregger et al.[17] and Abd-El Hameidshalaby[1] reported a similar observation.

According to Layokun et al.[21] the growth of microorganisms corresponds to the degradation (consumption) of the substrate. Hence, the growth of microorganisms on phenol can be described by the most

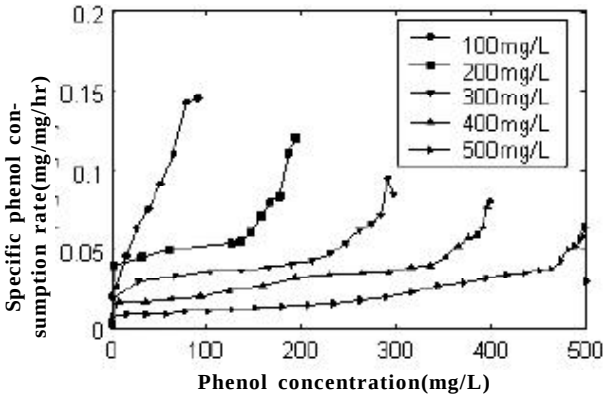

Figure 2 : Specific phenol consumption rate as a function of phenol concentration

FULL PAPER

commonly used kinetic models that can be based on specific substrate consumption rate as proposed by Posten[36] and which have been used by Zilli et al.[39] and Schroder et al.[34]. In this work, phenol was completely degraded and kinetic models of Monod[24], Haldane Andrews[3], and Yano and Koga[38] based on specific substrate consumption rate were fitted to the experimental batch data obtained for the indigenous *P. aeruginosa*. The classical method of obtaining kinetic parameters(constants) is to linearize kinetic models. Recently, non-linear least squares computer fitting of data to model equations has been used[34]. The non-linear least square fitting routine of Matlab 6.5 software package(with trust-region algorithm) was used to fit the kinetic models to the different batch experimental data. The parameters of Monod(K_s and r_{smax}), Haldane (K_s, r_{smax}, K_i), and Yano and Koga(K_s, $r_{smax,}$ K_i) were fitted to the experimental calculated specific phenol consumption rate and the corresponding phenol concentration under the constraint that r_s never exceeds the maximum obtainable specific consumption rate(r_{smax}) and the results are presented in TABLE 1. As seen from the TABLE, the differences in fit(based on the coefficient of correlation, R^2) between the three different models examined at the different initial phenol concentrations is very small and thus statistically insignificant as indicated by their SSE(sum of square errors) values. Yang and Humphrey [37] made similar observations with Andrew's equation and two other models in describing phenol degradation by *Pseudomonas putida* and *Trichosporon cutaneum*. However, it could be seen from the TABLE that the inhibition constant(Ki) values seems very large. The reason for this may be due to the fact that the phenol (substrate) was not in close proximity to the inhibition constant. Hill and Robinson[16] reported a similar observation. Since phenol has been used widely as a model inhibitory substrate, its biodegradation kinetics has been determined for many microorganisms and substrate inhibition was observed for all previously reported studies. Therefore, the Yano and Koga model was chosen as the kinetic model to evaluate the degradation of phenol by the indigenous *P.aeruginosa*. As observed from the TABLE, the Yano and Koga model parameters(r_{smax}, K_s and K_i) show a definite trend of variation. The r_{smax} decreased while K_s and K_i increased as the initial phenol concentration increased. Considering the fact that K_s is inversely related to the affinity of the microbial system for the substrate[28], this increase of K_s corresponds to a decrease in affinity of the bacteria (*P.aeruginosa*) for phenol. It is therefore evident that inhibition becomes prominent as initial phenol concentration increases. Thus, the R_{smax} is a strong function of initial phenol concentration(S_o) The variation of r_{smax} with S_o has been indicated in figure 3 and also fitted by the fourth order polynomial fit from which r_{smax} at any value of S_o within the range of 500mg/L of phenol concentration may be predicted.

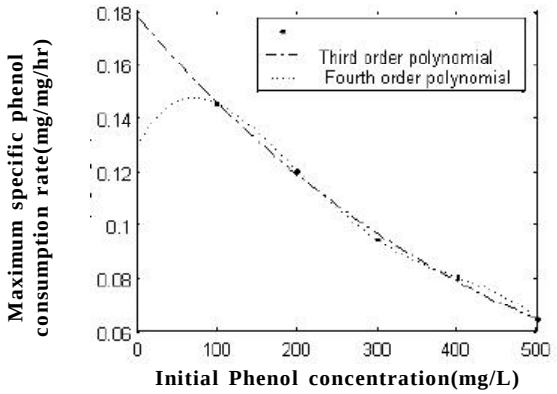

Figure 3 : Variation of maximum specific phenol consumption rate with intial phenol concentration

Assessment of performance

Phenol has been used widely as a model inhibitory substrate and its biodegradation kinetics has been determined for many microorganisms. The performance of this indigenous *P.aeruginosa* is being compared with well known effective degraders of phenol with emphasis on maximum specific substrate consumption rate. Reported values of the maximum specific substrate consumption rate(r_{smax}) varied from 0.001 to 2.6hr^{-1} [14,34,39]. Folsom et al.[14] and Schroder et al.[34] reported a r_{smax} value of 2.6hr^{-1} and 0.4hr^{-1} for *P.seudomonas cepacia* G4 respectively While, Zilli et al.[39] reported a value of 0.0016hr^{-1} for *Pseudomonas putida* NCIMB 10015. The r_{smax} value of 0.12mg/mg/hr for indigenous *P.aeruginosa* was lower than that reported for *Pseudomonas cepacia* G4, however greater than that of *P.putida* NCIMB 10015. For equivalent initial phenol concentrations, the phenol removal efficiencies of the indigenous *P.aeruginosa* were found to be higher than that of indigenous *P.fluorescence* as reported by Agarry[2].

CONCLUSIONS

The present study shows the potential of the isolated indigenous *P.aeruginosa* for phenol wastewater treatment. The performance of the indigenous strain in biodegradation of phenol in the nutrient medium is excellent. The parameter K_s and K_i increased with the higher values of initial phenol concentration, while the other parameter r_{smax} decreased with the corresponding increase in the initial phenol concentration, indicating inhibition effect of phenol.

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NOMENCLATURE

K_5 Half-saturation constant (mg/L)
K_i Inhibition constant (g/L)
K_5 Specific phenol(substrate) consumption rate(mg/mg/hr)
r_{5max} Maximum specific phenol (substrate) consumption rate (mg/mg/hr)

TABLE 1: Kinetic constants obtained from the fitting of batch experimental runs data from phenol degradation by *P. aeruginosa* to some kinetic models

Microorganism	Model	S_o (mg/l)	K_s (mg/l)	r_{smax} (hr^{-1})	K_i (g/l)	SSE	R^2
Pseudomonas Aeruginosa	Monod	100	23.8	0.145	-	0.003	0.83
	Yano & koga		23.8	0.145	5.3	0.003	0.81
	Haldane		45.8	0.145	21.2	0.007	0.62
	Monod	200	74.2	0.12	-	0.004	0.65
	Yano&koga		74.5	0.12	8.4	0.004	0.65
	Haldane		71.3	0.12	36.9	0.004	0.64
	Monod	300	139.8	0.094	-	0.003	0.68
	Yano & koga		134.5	0.094	9.5	0003	0.68
	Haldane		134.7	0.094	39.3	0.003	0.67
	Monod	400	201.5	0.08	-	0.002	0.68
	Yano & koga		204.6	0.08	10.8	0.002	0.70
	Haldane		199.9	0.08	41.7	0.002	0.69
	Monod	500	331.2	0.064	-	0.003	0.67
	Yano & koga		316.0	0.064	12.5	0.003	0.67
	Haldane		332.5	0.064	51.5	0.003	0.66

APPENDIX

Equs. for kinetic models according to TABLE 1

Monod(1949): $$r_s = \frac{r_{s,max}\, S}{K_s + S}$$

Haldane(Andrews,1968): $$r_s = \frac{r_{smax} S}{K_s + S + \frac{S^2}{K_i}}$$

Yano and Koga (1969): $$r_s = \frac{r_{smax} S}{K_s + S + \frac{S^3}{K_i^2}}$$

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