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Performance of Batch Stirred Tank Bioreactor and Internal Loop Airlift Bioreactor in Degrading Phenol using *Pseudomonas* spp.-A Comparative Study

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Abstract

Performance of two bioreactors namely batch stirred tank reactor (BSTR) and internal loop airlift reactor (ILALR) was evaluated in degrading phenol using *Pseudomonas* spp. and compared. A mixed culture of microorganisms, isolated from a sewage treatment plant, has been used to study the phenol degradation and culture growth kinetics. The BSTR took a maximum of 8 days to degrade the phenol completely with maximum initial concentration of 400 mgl^{-1}; where as ILALR took only 47 h to degrade phenol with a maximum initial concentration 600 mgl^{-1}. The rate of culture growth and phenol degradation expressed in terms of specific growth rate μ and specific degradation rate *q* respectively and calculated at various concentrations of phenol, demonstrated substrate inhibition behaviour. The biokinetic parameters were evaluated by fitting the experimental data to Haldane deterministic model. Based on the findings obtained in the study, the mixed microbial culture showed a better potential in degrading phenol in an ILALR than BSTR.

Keywords: Batch stirred tank reactor; internal loop airlift reactor; biodegradation; phenol; substrate inhibition

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Introduction

Synthetic organic chemicals like phenols and their derivatives lead to serious environmental contamination because of their toxicity towards aquatic biota. Various industries such as pulp and paper mills, herbicides and fungicides production etc, contribute phenols in their aqueous effluents [1,2,3]. The growing concern for aquatic contamination in the environment has led to quest for better wastewater treatment methods. Among the available treatment methods for phenolics in wastewater, microbe-based degradation of phenols appears to be more promising [4,5,6]. Aerobic degradation of phenol using pure microbial cultures has been studied extensively in batch shake flask [3,4,5,6]. However, aerobic degradation with a mixed culture consortium degrades phenol completely and does not leave any hazardous residues in the process. However, the successful applications of biological method for wastewater treatment often rely on the type of reactor system employed. For example, biological treatment of phenolics in conventional activated sludge seldom fails to achieve high efficiency in removing recalcitrant due to their toxic nature. In late 70s, batch stirred tank bioreactors were employed for such treatment application owing to their simplicity in construction and operation [7]. But in recent years, airlift bioreactors (generally employed for fermentation) have become popular for same purpose. The major advantage of airlift bioreactors is that they do not need any mechanical agitation. It requires only aeration which serves both as oxygen supply as well as mixing with low energy input for its operation [7]. In general, an internal loop airlift reactor is essentially a bubble column with a baffle or a draught tube separating the rising fluid from the sinking fluid. It is

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simple in construction and operation, where fluid circulation occurs in a defined cyclic pattern, *i.e.*, through a loop of conduits [8,9,10].

In recent years, attempts were made to evaluate the performance of ILALR towards adoptability for wastewater treatment; Loh and Ranganathan performed the biotransformation of 4-chlorophenol (4-cp) in presence of phenol in an External-loop fluidized bed airlift bioreactor (EFBAB). They tested the bioreactor for batch co-metabolic biotransformation of 4-cp in presence of phenol at various concentration ratios of phenol and 4-cp (ranging from 600 mgl^{-1}phenol and 200 mgl^{-1} 4-cp to 1600 mgl^{1} phenol and 200 mgl^{-1}4-cp) [11]. Viggiani *et al.* evaluated biodegradation of phenol with *Pseudomonas stutzeri* OX1 in an airlift biofilm reactor of 150 ml capacity. They observed that the culture took a long period of 7 days for completely degrading phenol with a maximum feed concentration of 450 mgl-1 [12]. They also studied substrate inhibition kinetics by fitting the experimental findings to Haldane model. Similarly Jajuee *et al*. studied the kinetics of p-xylene and naphthalene as single and also as mixed substrate in batch airlift immobilized bioreactor. They varied the *p*-xylene concentration between 15.4 mgl^{-1} and 75 mgl^{-1} and naphthalene concentration between 4 mgl-1 to 16.5 mgl-1 respectively and correlated the experimental results with Monod kinetic model [13]. Feng *et al*. studied the phenol degradation in an internal loop airlift bioreactor with yeast *Candida tropicalis*. Their studies mainly focused on the modeling of local dynamic behavior of the reactor and its effect on the phenol biodegradation [14]. Sahinkaya and Dilek studied the biodegradation kinetics of 2,4-dichlorophenol (2,4-DCP) by (a) culture (Culture M) acclimatized to mixture of 4-chlorophenol (4-CP) and 2,4-DCP and (b) culture (Culture 4) acclimated to 4-CP only. The study was carried out in aerobic batch reactors. It was also observed that the Haldane equation can be used to predict specific degradation rate (R2 > 0.99) as a function of initial 2,4-DCP concentrations and it adequately describes 2,4-DCP concentration profiles [15].

In spite of the fact that numerous research citation gathered so far the performance study on either BSTR or ILALR; a comparative study of these two reactors is still not reported anywhere. Focus of the present study is to evaluate the comparative performance of such bioreactors.

Materials and Methods

Chemicals and reagents

Phenol used in the study was of analytical grade and inorganic salts used in preparing microbial growth media were of reagent grade. All these chemicals were purchased from Merck®, India.

Microorganism and culture conditions

The microorganism used in this study is a mixed culture capable of degrading phenol. It has been isolated and enriched from a sewage treatment plant located in Guwahati, India. The obtained culture is identified as a mixed culture with predominantly *Pseudomonas* spp. according to the biochemical tests and their results. The culture was cultivated in a 250 ml flask containing 100 ml of Mineral Salt Medium (MSM) in an orbital shaker at 150 rpm and 27ºC. The MSM is composed of (in mgl^{-1}) $(NH_4)_2{\cdot}SO_4$ 230, $CaCl_2$ 8.0, $FeCl_3$ 1.0, $MnSO_4{\cdot}H_2O$ 100, $MgSO_4{\cdot}7H_2O$ 100, K_2HPO_4 500, KH_2PO_4 250 at pH 7.0 under agitation condition (150 rpm). The culture was then acclimatized over a period of one month to grow in MSM containing phenol and m-cresol as the sole carbon source up to a concentration of 800 mgl^{-1}. The detailed acclimatized phase of the culture was shown in Figure 1.

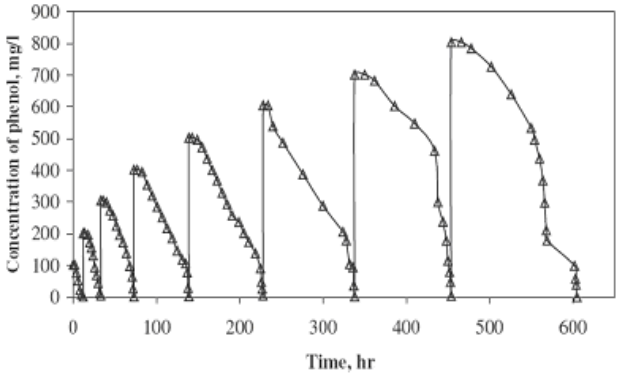

Figure 1. Acclimatized phase of the mixed microbial culture with phenol up to a maximum concentration of 800 mg l^{-1}.

Batch stirred tank reactor

The experimental setup used in this phenol biodegradation study consisted of a 5-litre glass vessel fitted with an impeller driven by a DC motor. Other parts of the setup include ports for sampling, provision for liquid inlet and outlet using peristaltic pump, (Miclins, India; Model no. PP 20) thermometer for monitoring the temperature inside the vessel. A schematic of this set up is shown in Figure 2.

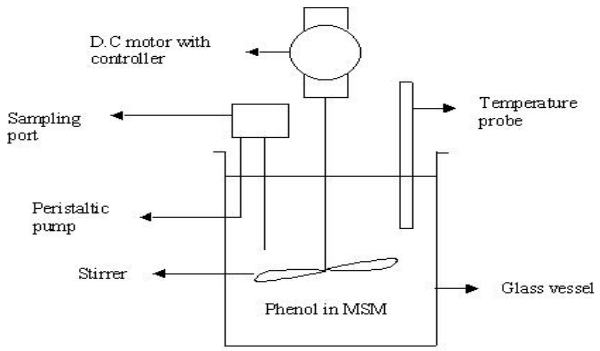

Figure 2. Schematic of the simple batch stirred tank reactor.

Internal loop airlift bioreactor

An internal loop airlift reactor made of perspex acrylic material with a working volume of 2.5-L was used throughout the study. The reactor consists of two concentric tubes, where the inner tube is removable draft tube (40 × 5 cm), the external tube has dimensions of (60× 8 cm). The top and bottom of the reactor was sealed with a flange made of stainless steel. Compressed air from a compressor was fed from the bottom of the reactor via a nozzle of diameter 0.8 cm. Sterile air was fed in the reactor by filtering it through an air filter. The nozzle was placed inside the inner tube and the superficial gas flow was measured with a rotameter. The schematic of the ILALR is shown in Figure 3. The superficial gas flow was maintained at 2 lmin-1 throughout all the experiments. This is an optimal gas flow rate as determined from the hydrodynamic study. No attempts were made to control the temperature inside the reactor however it was monitored to be 26º C ±1º C.

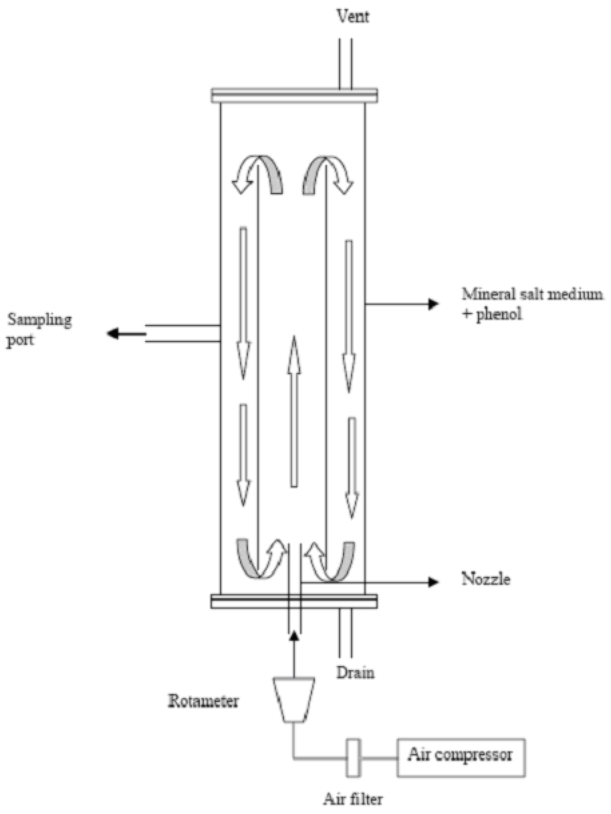

Figure 3. Schematic of the ILALR used in biodegradation of the phenol.

Batch biodegradation study in BSTR

Four batches of experiments were conducted with initial phenol concentration varying from 100 to 400 mgl^{-1} and a working volume 4-L of MSM. These concentration levels were chosen based on the extent of total time taken by the culture for complete removal/degradation of phenol in the media. The experiments were carried out under batch mode at nearly constant temperature of 29º C ± 2º C with continuous stirring at 150 rpm. Each experiment was performed until the residual phenol concentration in the media was found to be reduced to nearly 0 mgl^{-1}.

Batch biodegradation operation of ILALR

The biodegradation experiments were carried out in batch mode. The reactor (2.5-L capacity) was operated in batch to study the scale up effect from a shake flask of capacity 100 ml. The batch was initially run for single substrate degradation study with a phenol and m-cresol of concentrations 100 mgl^{-1} each. The study was repeated with various concentrations of substrates up to a maximum phenol and m-cresol feed concentration of 600 mgl^{-1} and 400 mgl^{-1}, respectively.

Analytical methods

Cell density in the samples was estimated with diode array spectrophotometer (Spekol 1200, Analytik Jena, Germany) by measuring its absorbance (OD) at 600 nm wavelength. OD_{600} was then converted to dry cell weight by a calibration curve. The curve is obtained by plotting dry weight of biomass per milliliter vs OD_{600}. High performance liquid chromatograph (Model UV 200 series: Perkin Elmer, U.S.A) was employed to quantify phenol and m-cresol concentrations in the biomass free samples. Samples were centrifuged 10,000 × g for 3 min (Biofuge Pico, Rota No.3328, Heraeus) and analyzed for residual phenol concentration The analysis was performed with C18 column (150 mm × 4.6 mm × 5 mm; Chromotopak) with acetonitrile/water (60/40) as the mobile phase at a flow rate of 1 $mlmin^{-1}$. The detection was done with a UV detector set at 275 nm. The retention period of phenol is 2.75 min.

Results and Discussions

In our earlier batch shake flask study [16], phenol degradation by the mixed microbial culture showed 100% degradation efficiency of phenol up to a maximum initial concentration of 800 mgl^{-1} in MSM within 69 h. However, experiments in that study were performed in batch shake flasks of 250 ml capacity with a working volume of only 100 ml. In order to establish treatment of phenol in higher scale bioreactors the present study was undertaken with an objective of investigating the kinetics of phenol degradation and growth of the culture in a simple BSTR and ILALR of higher volume.

Biomass growth and phenol degradation at different initial phenol concentrations

Figures 4a and 4b shows time profile of phenol degradation and of biomass growth cell concentration mgl^{-1} of the culture in BSTR. It is quite evident that the amount of time taken by the culture to degrade phenol was dependent on the initial phenol concentration in the media.

The culture could, however, degrade well up to a maximum concentration of 400 mgl^{-1} phenol in the media, which took a maximum time of about 194 h (8 days).

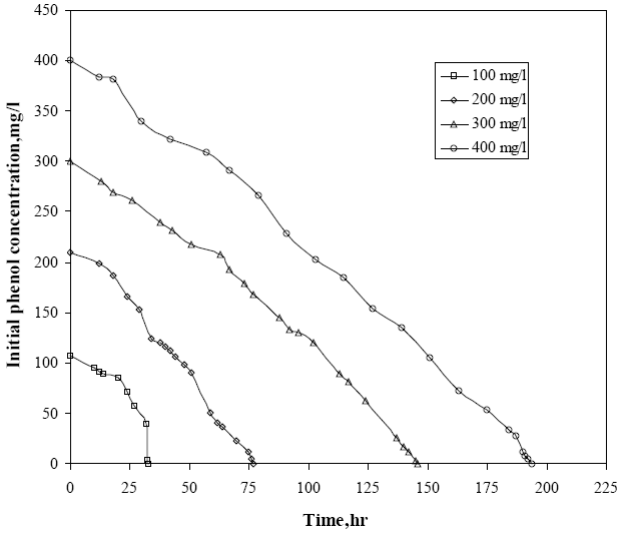

Figure 4a. Time profiles of phenol degradation at different initial phenol concentrations in BSTR.

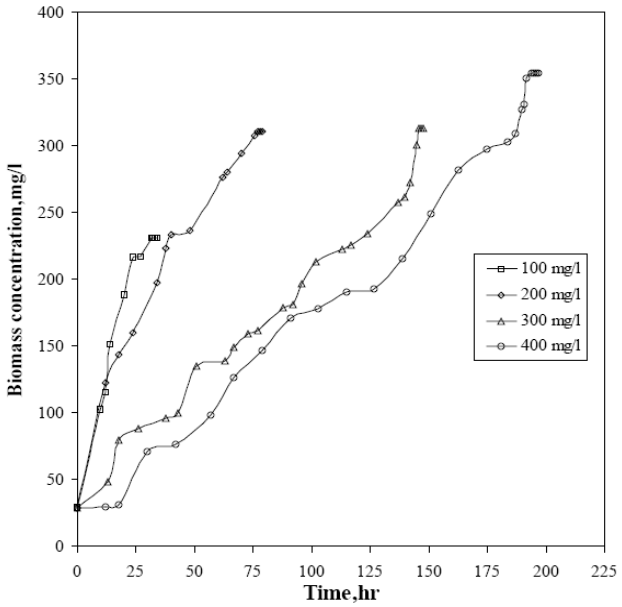

Figure 4b. Time profiles of biomass output at different initial phenol concentrations in BSTR.

Comparing this result with ILALR it is observed from the Figure 5a that phenol at concentrations 100, 200, 300, 400 and 600 mgl^{-1} was completely degraded by the culture within 10, 15.3, 17, 27.3 and 47 h, respectively. The results clearly showed good ability of the reactor in degrading phenol faster even at fairly higher concentration as opposed to the BSTR study that took 8 days. Use of mixed strain of *Pseudomonas* spp. has been proved to be advantageous as opposed to the study by Viggiani *et al.* who required nearly 7 days to degrade phenol at a maximum feed concentration 450 mgl-1 in an airlift reactor using *Pseudomonas stutzeri* OX1 [12]. An increase in phenol feed concentration up to 300 mgl^{-1} enhanced the specific growth rate of the culture, however the concentration above 300 mgl^{-1} shows a declining value of the growth rate. Thus the substrate inhibition sets in above 300 mgl^{-1}. Figure 5b show the biomass output profile as a function of phenol feed concentration in ILALR. Increase of culture growth at 400 mgl^{-1} of phenol the inhibition was found to be distinct and the culture took much longer time for its growth (maximum 194 h), where else the same culture in ILALR took only 27.3 h. However, the amount of biomass produced at this concentration was higher in ILALR. This indicates that the ILALR is highly efficient in degrading phenol.

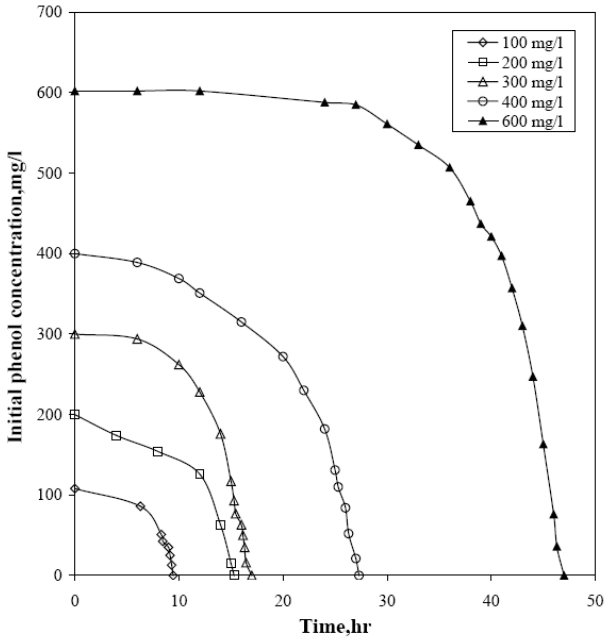

Figure 5a. Batch biodegradation patterns of phenol shown by the culture in ILALR.

In order to relate the pattern of phenol degradation with the culture growth in the system, kinetics of these two phenomena were analyzed. This was achieved by calculating the specific growth rate μ, h^{-1} and specific substrate degradation rate q, h^{-1} from the biomass output and phenol degradation profiles respectively. The following relationships were used for the above purpose:

$$\mu = \frac{1}{X}\frac{dX}{dt} \qquad (1)$$

$$q = -\frac{1}{X}\frac{dS}{dt} \qquad (2)$$

Where X = biomass concentration (mgl^{-1}), S = substrate concentration (mgl^{-1}) and t = time taken by the culture for completely degrading the substrate (h).

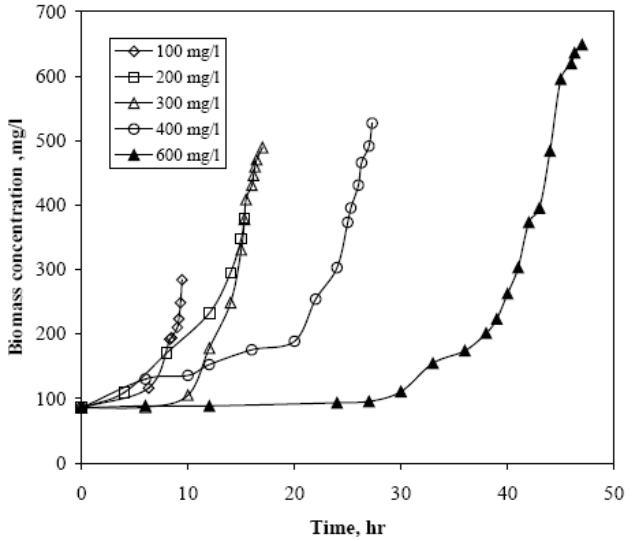

Figure 5b. Biomass profile of the culture at various initial phenol concentrations in the ILALR.

Figures 6 and 7 illustrate the above two rates calculated at different initial phenol concentrations in the media for both BSTR and ILALR. These clearly indicate that both these rates correspond well with each other. It is also observed that the substrate inhibits at a phenol feed concentration of 200 $mgl^{-1}$and 300 $mgl^{-1}$for BSTR and ILALR respectively. Viggiani et al. too observed that substrate inhibition commences at phenol concentration 200 mgl^{-1} [12].

Modeling the kinetics of the culture growth and phenol degradation

Since the growth rate (μ) and phenol degradation rate (q) demonstrated substrate inhibition characteristics a suitable deterministic model based on substrate inhibition was used to model these rates with respect to phenol concentration. Haldane proposed the first and most popular model for substrate inhibition kinetics (equation 3). This model was utilized by most of the researchers for growth inhibiting substrates like phenols and phenolics (3,6). The mathematical form of the Haldane model is as follows:

$$\mu = \frac{\mu_{max} S}{K_s + S + \frac{S^2}{K_i}} \qquad (3)$$

where S = limiting substrate concentration (mgl^{-1}), μmax = maximum specific growth rate (h^{-1}), K_i = inhibition coefficient (mgl^{-1}) and K_s = half saturation coefficient (mgl^{-1}).

The above model equation was solved using nonlinear regression technique in MATLAB® 7.0 and the biokinetic parameters were evaluated. It was observed from the estimated biokinetic parameters that the value of maximum specific growth rate (μ_{max}) is 0.48 h^{-1} and other parameters like K_s and K_i were found to be 171.2 $mgl^{-1}$and 294.7 mgl^{-1}, respectively. In comparison to BSTR study (μ_{max}= 0.0324 h^{-1}; K_s =40.57 mgl^{-1}; K_i = 140.65 mgl^{-1}) the estimated values are found to be very high. Among these three parameters, μmax is having highest significance, since it represents the culture growth for the given concentration of substrate. In comparison to our previous batch shake flask study μ_{max}= 0.3085 h^{-1}; K_s =44.92 mgl^{-1}; K_i = 525 mgl^{-1} [16] was found to be higher than that of BSTR, but less than that of ILALR expect K_i. In other words, it is the potential of the system in degrading the substrate effectively. Moreover the present study mainly focused on the wastewater treatment where growth parameter defines the efficiency of plant. Higher growth rate denotes the potential of the ILALR system in degrading phenol using *Pseudomonas* spp.

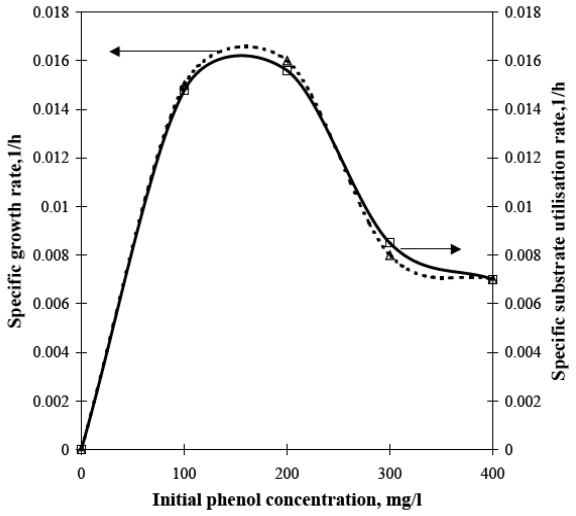

Figure 6. Comparison of specific growth and specific substrate utilization rates at different initial phenol concentrations for BSTR.

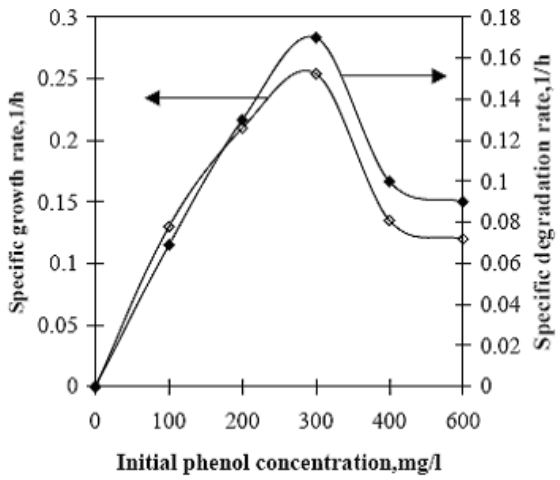

Figure 7. Comparison of specific growth and specific substrate degradation rates at different initial phenol concentrations for ILALR.

Conclusions

Performance of phenol degradation was studied using a mixed consortium of *Pseudomonas* spp. strain, isolated from a sewage treatment plant, in a simple stirred tank bioreactor and internal loop airlift bioreactor operated under batch mode and compared. The culture specific growth and phenol specific degradation rates correlated well with each other at all initial phenol concentrations. In the concentration range studied, phenol was found to exhibit substrate inhibition characteristics on these two rates, at 200 $mgl^{-1}$and 300 $mgl^{-1}$for BSTR and ILALR, respectively. Suitable substrate inhibition model found in the literature were fitted to explain the behaviour of the system. The values of biokinetic constants estimated from the Haldane model showed a good potential of the culture in treating phenol-containing wastewaters using internal loop airlift reactors operated under batch mode.

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