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Studies on growth kinetics of predominantly *Pseudomonas* sp. in internal loop airlift bioreactor using phenol and *m*-cresol

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Abstract−Growth profile of predominantly *Pseudomonas* species was studied using wastewater containing phenol and *m*-cresol, as single and multi component systems in an internal loop airlift bioreactor (ILALR). The species utilized for the study was isolated from a wastewater treatment plant. The reactor was operated at both lower and higher hydraulic retention time (HRTs), 4.1 h and 8.3 h, respectively. The inlet phenol and concentration was varied between 100 and 800 mg/L with 800 mg/L as shock loading concentration for an HRT of 8.3 h. For 4.1 h HRT, the concentration was varied 100 and 500 mg/L using 500 mg/L as a shock loading concentration. The study showed complete degradation of both phenol and *m*-cresol, when present individually at an HRT of 8.3 h with an enriched biomass output. The specific growth rate of the culture at various phenol and *m*-cresol concentrations was fitted to a Monod model. The biokinetics value showed good potential of *Pseudomonas* species employing the internal loop air lift bioreactor in utilizing high strength phenolics containing wastewater. Culture growth profile with both phenol and *m*-cresol as mixtures also showed decreased lag times with complete utilization of the phenolics.

Key words: Biomass, Internal Loop Airlift Bioreactor, Monod Model, Phenolics, *Pseudomonas* sp., Wastewater

INTRODUCTION

In the last few decades, various bioreactor designs have been proposed for wastewater treatment with an aim to ensure increased oxygen transfer rate and minimal power consumption. One of the most promising bioreactors is the airlift bioreactor, which was first patented by Lefrancois *et al.* in 1955 [1]. They are pneumatically agitated reactors where fluid circulation is carried out in a defined cyclic pattern through a loop of conduits. They do not need any mechanical agitation and little energy is sufficient for required aeration in the process. Compared to conventional reactors such as the stirred tanks or bubble columns, shear stress in ILALR is relatively constant and mild throughout the reactor, which is favorable for microbial growth [2]. All these advantages explain why so many researchers have focused their attention on ALRs [3-7].

The biodegradation of phenolics has been studied extensively in batch reactors [8-12] but not in airlift reactors. Some studies are as follows: Quan et al. [4] performed a study using an internal loop airlift reactor immobilized with *Achromobacter* sp. to degrade phenol and 2,4 dichlorophenol. They obtained a removal efficiency of about 99.6% at a hydraulic retention time (HRT) of 8 h at a maximum phenol concentration of 200 mg/L. Vinod and Reddy [13] studied the biodegradation of phenolic wastewater using microorganisms in a fluidized-bed bioreactor (FBR). Experiments were conducted at wastewater flow rate of 510 mL/h and with a feed concentration of phenol as high as 1,254 mg/L. Although they obtained better treatment efficiency, a fluidized bed bioreactor has a complicated operational protocol and is thus difficult to adapt to real wastewater treatment. Jajuee et al. [6] studied the kinetics of biodegradation of *p*-xylene and naphthalene and oxygen transfer in an airlift immobilized bioreactor. The reactor was operated under continuous mode. The biodegradation rates were 81 mg/L and 40 mg/L of *p*-xylene and naphthalene, respectively. The maximum specific growth rate, μ_{max}, and the value of the limiting nutrient concentration, K_s, which results in a growth rate of half the maximum value, were found to be 0.005 h^{-1} and 2.21 mg/L, respectively. Zhao et al. [14] in their integrated anaerobic/aerobic biodegradation study in an internal airlift loop reactor, observed that the reactor could degrade the influent COD from 3,700 mg/L to 400 mg/L (phenol removal rate was over 99%) with a residence time of 24 h.

The main objective of this work was to evaluate the growth kinetics of the predominantly *Pseudomonas* sp. in a lab-scale internal loop airlift reactor for biodegradation of dissolved phenol and *m*-cresol, respectively, under continuous operation. The growth kinetics of the microbe on the utilization of phenolics was also evaluated with Monod model.

MATERIAL AND METHODS

1. Chemicals and Reagents

Phenolic compounds, *viz*., phenol and *m*-cresol, used in the study, were of analytical grade; glucose and inorganic salts, used in preparing the microbial growth media, were of reagent grade. All the chemicals and reagents were purchased from Merck®, India.

2. Microorganism and Culture Conditions

The microorganism used in this study was a mixed culture with *Pseudomonas* group as dominant species, which is capable of degrad-

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ing both phenol and *m*-cresol. It was isolated and enriched from a sewage treatment plant located in Guwahati, India. The culture was identified to be predominantly *Pseudomonas* species as per the routine biochemical and morphological tests like light microscope and scanning electron microscopy [15]. The culture was initially cultivated in 250 mL flasks containing 100 mL of mineral salt medium (MSM) in an orbital shaker set at 150 rpm and 27 °C. The MSM was composed of (in mg/L): $(NH_4)_2 \cdot SO_4$, 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; with a pH of 7.0 under agitation condition (150 rpm). The culture was then acclimatized for about one month in MSM containing either phenol or *m*-cresol as the sole carbon source up to a concentration of 800 and 1,000 mg/L, respectively. An inoculum size of 10% of the working volume of the reactor was used. The inoculum was prepared from the acclimatized culture of both phenol and *m*-cresol separately and used for the single pollutant study. For mixed substrate study a similar inoculum size was prepared from *m*-cresol acclimatized culture.

3. Experimental Setup of the Internal Loop Airlift Bioreactor

Utilization of phenolics was carried out in an ILALR and its schematic is presented in Fig. 1. The reactor was made of acrylic material with an external tube dimension of 60 cm height and 8 cm diameter. The height and diameter of internal tube positioned inside was 40 and 5 cm, respectively. The reactor had a working volume of 2.5 L. Through a stainless steel nozzle of diameter 0.8 cm, air was distributed into the reactor from an air compressor; the airflow into the reactor in turn was measured and controlled with a rotameter (Telelin Instruments India) with a needle valve. All the components used in the reactor were resistant to embrittlement, corrosion and swelling due to the phenolics.

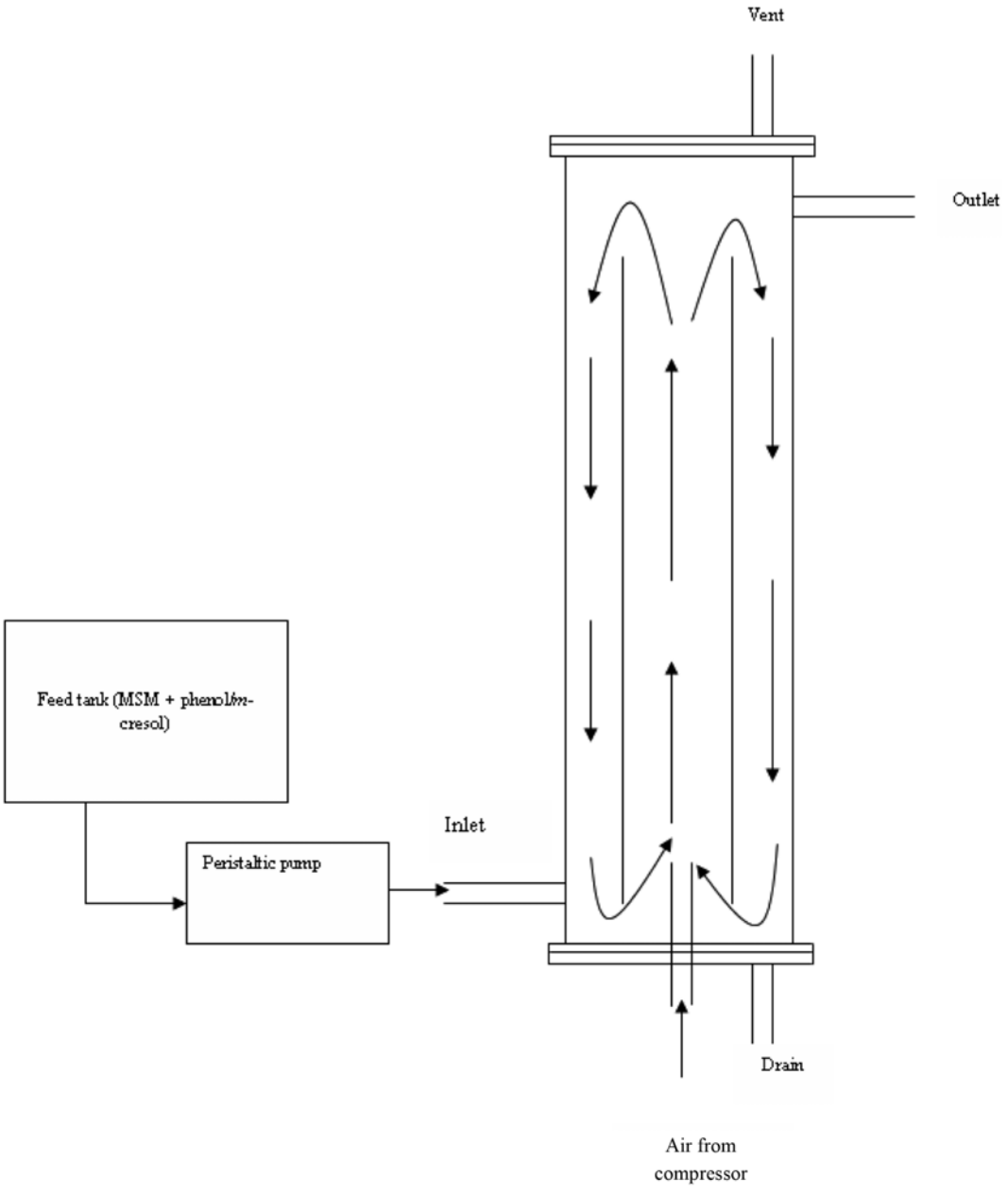

Fig. 1. Schematic of the ILALR used in the study.

4. Biodegradation Experiments in ILALR

Based on our previous hydrodynamic study [16], the superficial gas flow into the reactor was fixed at 2 $l \cdot min^{-1}$ throughout the experiments. The reactor was operated at an optimum temperature necessary for the culture to grow and degrade the phenolics, 26± 1 °C. Free cell culture was adopted in the present study. The reactor was initially operated under batch mode with an initial phenol concentration of 50 mg/L in MSM and continued until an enriched culture was obtained. Once the batch operation was found to be successful in treating the phenol containing synthetic wastewater, the reactor was switched to continuous mode. Similar procedure was followed for *m*-cresol degradation in the study. There was a washout of negligible amount of biomass.

For continuous utilization of phenol and *m*-cresol in MSM as synthetic wastewater, the ILALR was operated at two different feed flow rates of 5 and 10 mL/min, respectively, so as to give corresponding HRT values of 8.3 and 4.1 h. These HRTs were achieved by employing a peristaltic pump (Miclins, India; Model no. PP 20). Samples were collected at regular intervals and subsequently analyzed for biomass and residual phenol and *m*-cresol concentrations. The various stages involving inlet concentration of phenol/*m*-cresol, flow rate (HRT) and hours of operation are detailed in Table 1.

A continuous reactor study was also performed with phenol and *m*-cresol as mixtures in the synthetic wastewater. Based on the above experimental results of the single phenolic continuous degradation study, a concentration range of 100-300 mg/L each of phenol and *m*-cresol was chosen in this present mixture degradation study. For choosing a combination of concentration levels of these two compounds, a 2^2 factorial design of experiments with the two compounds as the factors at two different levels was applied. Tables 2 and 3 show the design matrix employed along with the operating conditions (HRT, stages and treatment time) in the ILALR. In these tables, '−1' indicates low level (100 mg/L) and '+1' indicates high level (300 mg/L) of the two factors and '0' indicates center point or middle level (200 mg/L) of the two factors.

Shock loading experiments were also performed for both the compounds. The details of the shock loading concentration are shown in Table 1.

Table 2. 2^2 full factorial design matrix along with the stages and time of operation for phenol/*m*-cresol as mixture at 8.3 h HRT

Stage of operation	Factors and their levels		Hours of operation (h)
	Phenol	*m*-Cresol	
I	−1	−1	26
II	0	0	30
III	−1	+1	24
IV	0	0	26
V	+1	−1	30
VI	0	0	24
VII	+1	+1	28
VIII	−1	−1	28

Table 3. 2^2 full factorial design matrix along with the stages and time of operation for phenol/*m*-cresol as mixture at 4.1 h HRT

Stage of operation	Factors and their levels		Hours of operation (h)
	Phenol	*m*-Cresol	
I	−1	−1	26
II	0	0	16
III	−1	−1	14
IV	−1	+1	16
V	−1	−1	18
VI	0	0	12
VII	−1	−1	16
VIII	+1	−1	14
IX	−1	−1	16
X	+1	+1	4
XI	−1	−1	24

5. Analytical Methods

Cell density in the samples was estimated with a diode array spectrophotometer (Spekol 1200, Analytik Jena, Germany) by measuring its absorbance (optical density-OD) at a wavelength of 600 nm.

Table 1. Various stages of continuous operation in the ILALR treating synthetic wastewater containing either phenol/*m*-cresol (* Shock loading concentration)

Stage of operation	HRT (h)	Phenol concentration (mg/L)	Hours of operation in each stage (h)	*m*-Cresol concentration (mg/L)	Hours of operation in each stage (h)
I	8.3	100	30	100	25
II		200	48	200	24
III		300	40	300	26
IV		600*	36	400	30
V		800*	32	500*	28
VI		-	-	800*	7
VII		-	-	100	30
I	4.1	100	30	100	24
II		200	38	200	24
III		300	30	300	26
IV		500*	24	500*	5
V		100	46	100	27

OD_{600} was then converted to dry cell weight by a calibration curve, which was obtained by plotting dry weight of biomass per milliliter of sample vs OD. Samples were then centrifuged (Biofuge Pico, Rota No.3328, Heraeus) at 10,000 ×*g* for 3 min and analyzed for phenol and *m*-cresol concentration using a high performance liquid chromatograph (HPLC-Model UV 200 series: Perkin Elmer, U.S.A.) to quantify phenol and *m*-cresol concentrations in the biomass free samples. The analysis was performed with C18 column (150 mm× 4.6 mm×5 mm; Chromotopak) with Acetonitrile/Water (60/40) as a mobile phase at a flow rate of 1 mL/min, and the detection was with a UV detector set at 275 nm. The retention period for phenol was 2.75 min, and for *m*-cresol, it was 3.25 min.

RESULTS AND DISCUSSION

1. Culture Growth in ILALR Using Wastewater Containing Phenol or *m*-Cresol as a Single Pollutant

To prototype a real wastewater operation system, continuous treatment of phenol was carried out in ILALR by employing *Pseudomonas* spp. under two different HRTs, namely, 4.1 and 8.3 h, as indicated in Table 1. The time profile of culture growth in ILALR is illustrated in Fig. 2. A complete utilization of the compound by the culture was observed well up to a maximum concentration of 800 mg/L phenol in the reactor. The reactor was continuously operated for 170 h at 8.3 h HRT by changing the concentration from 100 to 800 mg/L in five steps. A complete utilization of phenol by the culture was achieved at its all concentrations with an HRT of 8.3 h, with a final biomass yield of 750 mg/L. Furthermore, no lag was observed in the culture growth, which confirmed the potential of the culture and the reactor in utilizing phenol even at higher concentration.

However, for *m*-cresol, 100% efficiency in the reactor could be observed only up to 500 mg/L concentration, above which the efficiency was found to decrease, but not significantly. The operation yielded a final biomass output of 550 mg/L, which was lower that the previous one. From the growth profile (Fig. 2) it was evident that no lag phase was present. This could be because *m*-cresol is more difficult than the phenol for the culture to utilize completely in the continuous operation with a maximum inlet concentration of 800 mg/L. Due to a fall in efficiency of the reactor in treating *m*-cresol, its initial concentration in the feed was lowered to 100 mg/L to get a stable performance thereafter, and a smooth growth profile was observed.

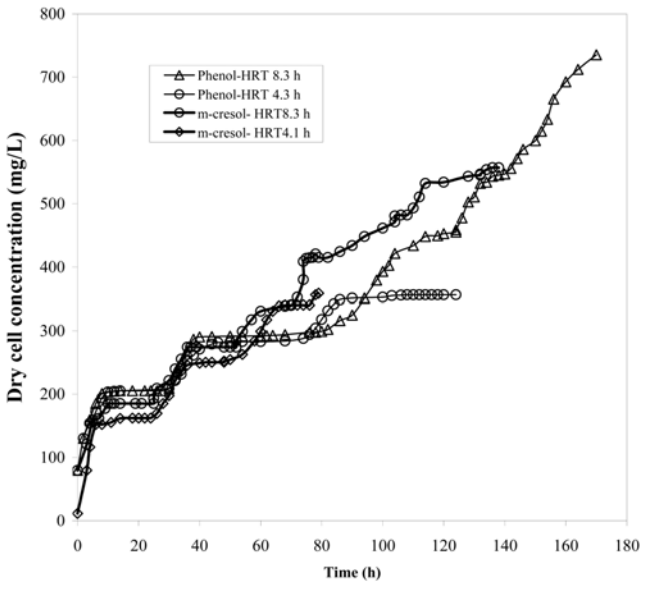

Fig. 2. Biomass output as a function of time for phenol and *m*-cresol as single pollutant in the continuous opeartion of ILALR at different HRTs.

The profile obtained for phenol/*m*-cresol degradation in the reactor operated at HRT of 4.1 h, presented in Fig. 2, showed that complete utilization of phenol/*m*-cresol could be achieved only up to a maximum concentration of 300 mg/L of either of the compounds; above this concentration of the individual compound, the culture in the reactor failed to take up the compounds completely.

2. Biodegradation Kinetics

Based on the Monod kinetic model [17], the specific growth rate of the culture at various phenol and *m*-cresol concentrations was fitted:

$$\mu = \frac{\mu_{max} S}{K_s + S} \quad (1)$$

Where S=Limiting substrate concentration (mg/L), μ=Specific substrate utilization rate (h^{-1}), K_s=Half saturation coefficient (mg/L) and μ_{max}=Maximum specific growth rate (h^{-1}). It was observed from the estimated biokinetic parameters that the value of maximum specic growth rate (μ_{max}) is 0.008 h^{-1} and K_s was found to be 58 mg/L, respectively, for phenol. The same was calculated for *m*-cresol value of maximum specic growth rate (μ_{max}) of 0.005 h^{-1} and K_s was found to be 43 mg/L, respectively. Jajuee et al. [6] obtained a μ_{max} value of concentration of 0.005 h^{-1} in biodegradation of *p*-xylene and naphthalene in an airlift immobilized bioreactor. The obtained μ_{max} value was found to be higher in the present study. Hence the obtained biokinetic value study revealed the potential of culture in utilizing phenol and *m*-cresol efficiently under ILALR.

3. Performance of the Culture Growth in ILALR to Shock Loading Conditions of Either Phenol/*m*-Cresol

The present study aimed to prototype the industrial wastewater treatment plant operation. The intermittent organic loading conditions when concentrations of pollutants change abruptly were also evaluated. Initially, the reactor was operated at low phenol/*m*-cresol concentration of 100 mg/L for a specific period of time and the concentration of phenol was increased suddenly to 600 mg/L and then to 800 mg/L at 8.3 HRT; whereas, for *m*-cresol at the same HRT the concentration was increased from an initial 100 mg/L concentration to 500 mg/L and then to 800 mg/L. AT 4.1 h HRT in the reactor, the shock loading conditions of phenol/*m*-cresol were set to a maximum at 500 mg/L. Fig. 2 shows the performance of the biomass out profile of the ILALR operated at 8.3 and 4.1 h HRT, respectively, under these shock loading conditions of phenol/*m*-cresol.

From Fig. 2 it is clear that for phenol at 8.3 HRT when the loading concentration was increased to 800 mg/L, the growth rate of the culture fell sharply, but in due course of operation the reactor regained its stability (12 h) with a maximum biomass output. Since the reactor was operated as free cell culture mode escape of biomass occurred continuously. For *m*-cresol at a maximum shock loading concentration of 800 mg/L, the stability of the reactor was highly affected

and the degradation efficiency fell drastically, which in turn influenced the growth of the culture. Longer lag was observed in the *m*-cresol profile, which shows that the stability of the culture in the reactor was affected. This lag and fall in culture utilization efficiency could be supposed due to more toxicity of *m*-cresol as compared to phenol.

Similarly, the biomass profile was for 4.1 h HRT too with both phenol/*m*-cresol concentration of 500 mg/L and the finding of same is illustrated in Fig. 2, continuous growth of the culture. From the figure it is clear that at this HRT, a maximum shock loading phenol/ *m*-cresol concentration of 500 mg/L with phenol/ *m*-cresol as a single component system in an ILALR affected the stability of the reactor. However, the reactor showed a better performance with a steady growth of culture for phenol than that of *m*-cresol. Hence, it is evident that lower HRT (4.1 h) with higher pollutant concentration affects the stability of the reactor. The findings showed that the culture had a high potential to uptake the phenol even at a maximum of 800 mg/L but at an HRT of 4.1 h.

4. Culture Growth in ILALR Using Wastewater Containing Both Phenol and *m*-Cresol Using the ILALR

Fig. 3 shows the biomass output profile for phenol/*m*-cresol as mixture, for both 8.3 and 4.1 h HRTs. It is evident that the reactor manifests a high performance in treating the pollutants completely at an HRT of 8.33 h for all the concentration combinations except 300 mg/L of phenol and *m*-cresol. For most of the component combinations, the reactor treated with 100% efficiency without any fall in its degradation efficiency, with a maximum biomass output. Moreover, for 8.3 h HRT the biomass profile was steady without any lag phase till 700 mg/L of biomass. A sharp fall could be observed at higher combinations, i.e., 300 mg/L of phenol and *m*-cresol. But in due course of operation the sharp fall was overlooked. From the figure it is evident that there was only a decline in biomass output after 175 h of operation for different combinations. The decline observed for 300 and 300 mg/L of both phenol and *m*-cresol, which is due to higher concentration of *m*-cresol, was noted due to its higher toxicity in combination. Being a free cell culture growth the experiment showed a maximum biomass yield of 750 mg/L, which is very high. It also showed the potential of culture and reactor in utilizing the phenolics.

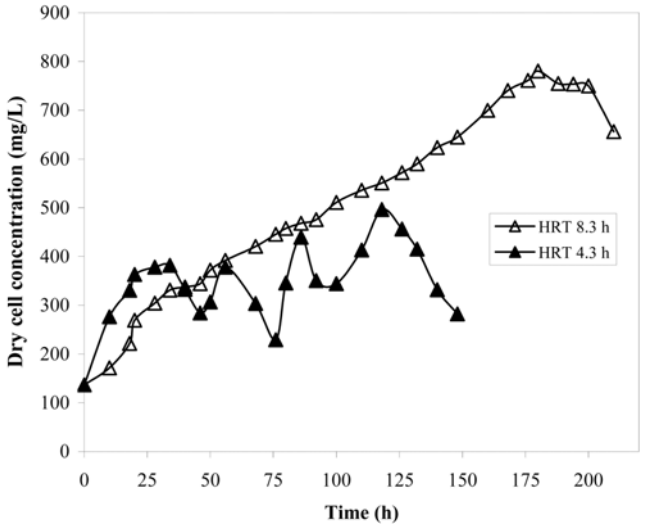

Fig. 3. Biomass output as a function of time for phenol and *m*-cresol as mixed substrate in the continuous opeartion of ILALR at different HRTs.

Similarly, the results of HRT 4.1 h (Fig. 3) revealed that a complete utilization was achieved when phenol/*m*-cresol concentration was 100 mg/L. The major fall in the growth rate was reflected in reduced degradation efficiency. The experiment showed a maximum biomass output of 450 mg/L only. A sinusoidal profile was observed when the reactor was operated at 4.1 h HRT. Such profile was obtained because the recalcitrant component concentration was higher and at the operational HRT the biomass could not completely take them up, which affected the system stability. Hence, it is clear from the study that an HRT of 8.33 h favored the growth of the culture with a maximum biomass output and with a minimum lag in growth profile. When comparing the growth profile at both the HRTs, when the reactor was operated at 4.1 HRT the culture could not utilize the phenolics, so such hanging or lag in growth was observed. Moreover, the adopted HRT is less with higher phenolic concentration, resulting in such lag.

CONCLUSIONS

The results of the present study substantiated the growth kinetics and potential of a continuously operated ILALR along with the culture of *Pseudomonas* sp. in treating phenolic wastewater. The growth profile of the culture had a slight lag phase only at higher concentration of phenol/*m*-cresol at an HRT of 4.1 h. Overall, at 8.3 h HRT a good growth profile with a maximum biomass out was observed. Moreover, continuous treatment of the phenol and *m*-cresol as individual components showed a complete utilization by the culture at HRT values of 8.3 h and 4.1 h. Shock loading of pollutants in the reactor revealed better stability of the system at higher value of HRT (8.3 h) than at a lower one (4.1 h). The obtained biokinetc value showed the potential of the reactor along with the culture in utilizing high strength phenolics effectively.

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