Effect of Substrate Concentration on Biodegradation of Phenol Using Continuous Reactor

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Abstract

Phenol is one of the major organic pollutants found in the industrial wastewater if disposed untreated causes greater chaos for environment. In the present study biodegradation of phenol using mixed culture bacteria was studied where the substrate was fed along with easily biodegradable substrate, glucose as one of the constituents until the microorganisms were specially acclamatised to the extent that the phenol alone serves as a sole carbon source supporting the metabolic activities. A continuous reactor was used for the biodegradation studies. The toxic substrate removal studies were carried out at various hydraulic retention times in the organic loading rates ranging from 0.00416 g m^{-3} d^{-1} to 0.0416 g m^{-3} d^{-1}. A correlation plot between phenol removal and organic loading rate is presented.

List of symbols:

L = g .m^{-3} .d^{-1}	Influent Organic Loading Rate
S = mg.l^{-1}	Influent Phenol concentration
θ = d^{-1}	Hydraulic Retention Time
T = °C	Wastewater Temperature

1. Introduction

The three-fold increase in the population along with the geometric growth in the necessities of the homosapiens created many industries which generate a wide variety of highly toxic wastes. The effluents of these industries often contain aromatic compounds that are resistant to natural degradation and therefore persist in the environment. One of the major organic pollutants found in wastewater is phenol which occurs naturally in the environment but is most commonly produced artificially from the industries thus its origin is both anthropogenic as well as xenobio[1]. Phenol besides being toxic, it is also a potential carcinogenic and mutagenic compound. It causes considerable threat to the eco-systems in water bodies and human health. The removal of persistent organic pollutant from wastewater effluent shall minimize the risk of pollution problems and enable it to be reused. Consequently, considerable efforts have been devoted to develop a suitable purification method that can easily destroy this bio-recalcitrant organic contaminant**.** Even at relatively low concentrations of 5–25 mg/L Phenol affects aquatic life, causing ecological imbalance and imparts objectionable tastes to drinking water, hence, the removal of phenol from wastewater is essential before discharge (Lathasree et al., 2004).

According to the U.S. EPA, phenol represent a group of organics frequently found in various industrial effluents and reported in hazardous waste sites and is considered to be a toxic compound by the Agency for Toxic Substances and Disease Registry (ATSDR, 2003). As per Hazardous Wastes (Management and Handling) amendment rules, 2000, phenol and phenolic compounds are classified under category of Class B (B-19) of schedule-II in the hazardous wastes list. Moreover according to the environmental protection rules of the Central Pollution Control Board (CPCB) and as per IS: 2490-1974 the discharge limit of phenols in inland water is 1 mg/L and in public sewers as per IS: 3306-1974 is 5.0mg/l.

2. Materials and Methods

The reactor used in this study was a laboratory model. It comprises of an aeration tank accompanied by a settling tank of same volume. The volume of the reactor was 2 liters. The reactor was a continuously operated as a completely stirred tank reactor. No recycling of solids was done. There were provisions for sludge ports at the bottom of the reactor. The reactor was made in perspex sheet and it was continuously aerated. The schematic diagram is shown in Figure. 1.

To understand the biodegradation of phenol by mixed culture aerobic bacteria, batch studies were conducted using shake flask method. The seed was brought from N.I.T. oxidation pond, aerated in shake flasks until biomass was developed and this was cultured on Nutrient Agar medium which was stored at 4° ±1°C. Stock solution was prepared by mixing the constituents indicated in Table 1 after autoclaving at 150°C for 30min and cooling for 30mins. Grown up mixed culture was taken in 500ml volumetric flasks and the stock solutions were added and this was incubated at

37°C in BOD Incubator for 48 hours. A sample from this was taken and again transferred to a 500mlvoumetric flask and 100ml of 25mg/l phenol solution was added and this was incubated and the process was continued until the phenol concentration reached upto 100mg/l. This culture was grown in 4 flasks of 1 liter capacity. After the development of biomass, the culture was transferred to a reactor which was operated in batch mode. For Continuous mode reactor the bacterial culture was grown in 2 four liter beakers. The culture was acclamatised to accept phenol as single limiting substrate. The concentration of the phenol was determined using the 4-Aminoantipyerene procedure as given in APHA, 1971.

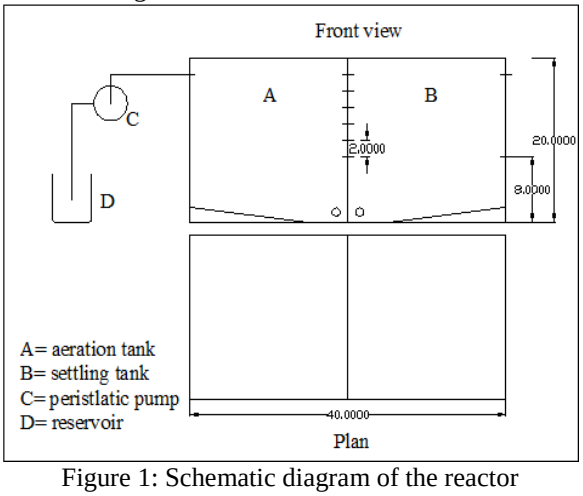

Figure 1: Schematic diagram of the reactor
(All Dimensions are in cm)

Table 1.Composition of synthetic waste water
(Swaminathan et al., 2000)

Constituents	Quantity mg/l
Glucose	1000
Di Potassium hydrogen phosphate	1079
Potassium di hydrogen phosphate	527
Urea	227
Magnesium Sulphate	100
Calcium chloride	0.75
Ferric Chloride	0.08

3. Results and Discussion

3.1 Batch studies

The batch reactor experiments were carried out using phenol as a single substrate at various concentrations ranging from 100mg/l to 200mg/l at a step interval of 50 mg/l. The flasks contacting samples were agitated at 150rpm in an orbital shaker. Samples were taken at every 6 hour interval and phenol and Biomass concentrations were determined. The growth curve of biomass and degradation curve of phenol at various residence times were shown in the figures 2 to 4 for various initial phenol concentrations the Residence time for complete degradation of phenol and Biomass concentrations were shown in Table 2.

Table 2

Initial Phenol Concentration	Residence time for complete degradation of phenol in hours	Biomass Concentration in mg/l
100	96	58
150	120	127
200	156	206

Literature was available for biodegradation of phenol using pure cultures. But very few reports were available for biological degradation of toxic phenolic waste wasters using mixed cultures. The advantages of mixed culture was that, easy to adapt for different toxic wastes, simplicity of operation and maintenance. Acclimatised mixed culture had better advantage over pure culture, where in the substrate had to be sterile and more factors influence/retards its growth and performance. It was observed that the rate of degradation of substrate decreased with the increase in the initial phenol concentration which indicates that there was inhibitory effect of substrate on the micro-organisms inturn which increased the time of the lag phase which had a proportional increase in the time for the substrate degradation

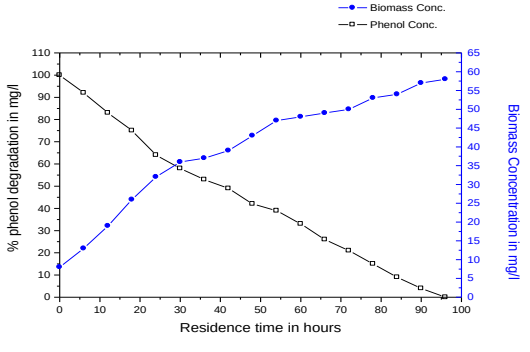

Figure 2:

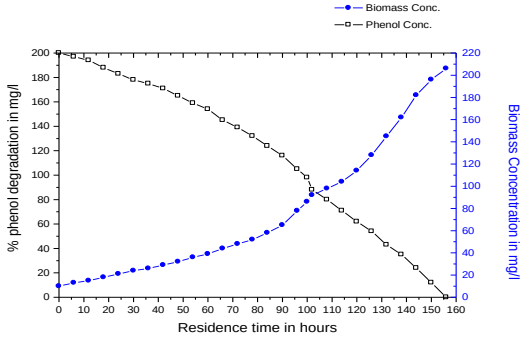

Figure 3:

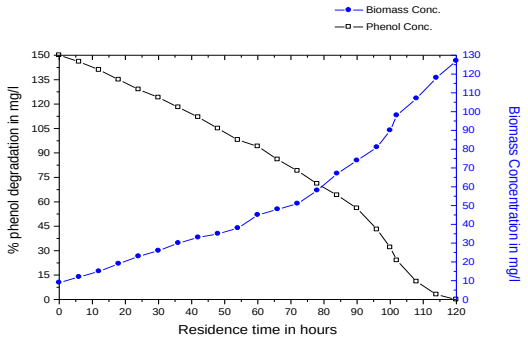

Figure 4:

3.2. Acclimatization of micro organisms with phenol as the substrate

Biomass formed should be acclimatized in such a way that phenol alone serves as a sole source of carbon for their metabolic activities. In order to acclamatise, the glucose that's been fed regularly had to be withdrawn slowly and proportionately phenol should be spiked slowly along with glucose. The spiking should be such that there was a gradual increase in the phenol concentration tolerable by the biomass as phenol inhibits microbial growth, with gradual decrease in the glucose concentration proportionately. As indicated in the literature the mixed culture took a very long time for acclimatization and it was around three months. The process was started with initial phenol concentration of 0.01g i.e. for a volume of 1000ml the nutrient medium comprised of 1% phenol and remaining 99% of glucose as carbon source. Day by day glucose along with nutrients was gradually withdrawn and phenol was spiked in, at the end acclimatised culture started growing with 100% phenol as sole source thus replacing glucose. The pH of the MLSS was measured often especially before and after feeding every day. Sudden drop if any in pH value was adjusted using sodium bicarbonate solution. Such sudden pH drop was an indication of putrefaction also occurs if the aeration was insufficient.

The micro organisms were acclimatized to utilize the phenol as the sole source of carbon to support their metabolic activities. This has helped to maintain the system at a higher organic loading and the removal efficiency was almost 95%.

3.3 Effect of organic loading on organic removal

For the biodegradation study, initial phenol concentrations ranging from 100 mg/L to 500 mg/L at a step interval of 10mg/l from 100mg/l to 150 mg/l, at a step interval of 50mg/l from 150mg/l to 500 mg/l were used. The extent of phenol degradation using these different initial phenol concentrations was investigated for several residence times by intermittent sampling.

Figure.2 shows the results of organic removal under different organic loading rates (OLR). Since the micro organisms have been acclimatized to accept phenol as the source for their metabolic activities, at a Hydraulic Retention Time (HRT) of 24 hours the treatment efficiency for organic loading rate of 0.0041 g m^{-3} d^{-1} was found to be 93.07% and for the highest loading of 0.0166 g m^{-3} d^{-1}, the treatment efficiency dropped down only to 80.95%. At a HRT of 24 hours the treatment efficiency for highest organic loading rate of 0.0104 g m^{-3} d^{-1} was found to be 86.05% where as for the highest loading of 0.0416 g m^{-3} d^{-1} at 6 hour HRT, the treatment efficiency dropped down only to 65.32%. It was observed that the removal efficiency was proportional with the HRT.

In the HRT range of 12h to 18h, for organic loading rate of 0.015-0.020 g m^{-3} d^{-1} range the removal efficiency was nearly 75-80% which indicates that the treatment process reached steady state.

Considering HRT of 6 hours the OLR was increasing from 0.0167g.m^{-3}.d^{-1} to 0.0416g.m^{-3}.d^{-1} the removal efficiencies were found to be 80.95% and 65.32% which implies that even there was a threefold increase in the OLR the variation in the removal efficiency was about 19%. The decrease in the removal efficiency with the increase in the OLR was dependent factor on the residence time as the residence time decreases the removal efficiency drops down while on extended aeration the organic removal was found significant.

The variations in the removal efficiencies at different OLR's at HRT 6h when compared to other HRT's can be due to the pseudo shock on the system. But it is to be mentioned that actual shock loading test has not been carried out.

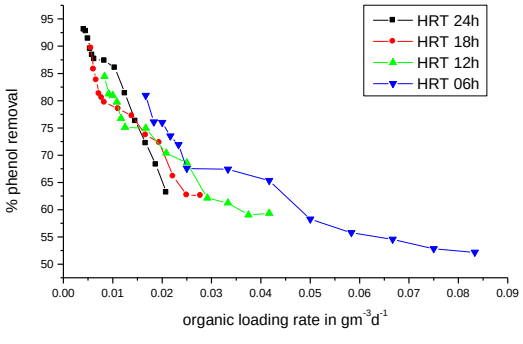

Figure 5: Removal efficiency Vs. Organic loading of phenol g.m^{-3}.d^{-1}

. Determination of Bio-Kinetic parameters

From the experiment, the effluent concentrations and MLVSS concentrations were estimated at the end of the HRT's and the data was fitted to Monod kinetic model for the determination of yield coefficient (Y), endogenous or decay coefficient (k_d),maximum substrate utilization rate (k) and Half saturation constant (K_s).

The yield coefficient and endogenous or decay coefficient were found by plotting (So-S)/θX as abscissa and 1/θ as ordinate. It follows the following equation:

$$\frac{1}{\theta} = Y\left\{\frac{So-S}{\theta X}\right\} - k_d \ldots\ldots\ldots\ldots\ldots (1)$$

From the graph shown in Fig. 3 the slope of the plot gives the Y value and the 'y' intercept gives the k_d value

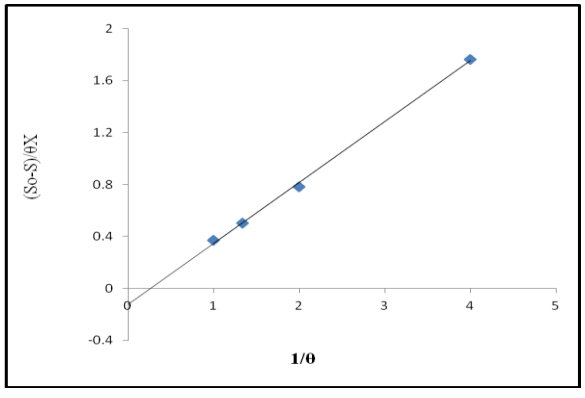

Figure 6: (So-S)/θX Vs. 1/θ

The maximum substrate utilization rate constant and Half saturation constant can be computed from the 1/S Vs. θX/(So-S) and the graph was shown in fig. 4. It follows the following equation:

$$\frac{\theta X}{So - S} = \left(\frac{Ks}{k}\right)\frac{1}{S} + \frac{1}{k} \ldots\ldots\ldots\ldots\ldots (2)$$

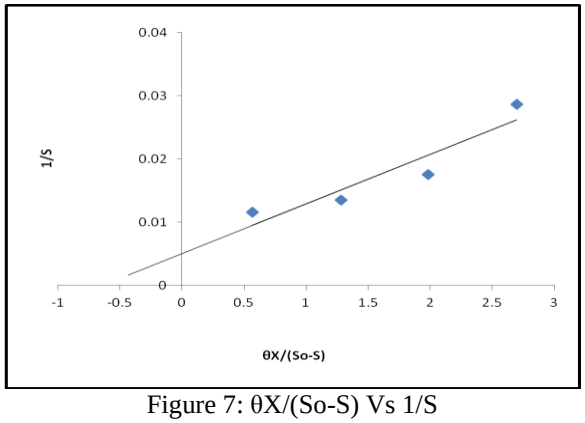

Figure 7: θX/(So-S) Vs 1/S

Table 3

Bio-kinetic coefficient	Mixed culture bacteria
Yield coefficient	0.468
Endogenous decay coefficient	0.120 d^{-1}
Half saturation coefficient	301.5 mg/l
Maximum substrate utilization rate constant	2.77 d^{-1}

Yield coefficient of 0.468, endogenous decay coefficient of 0.120 d^{-1}, half saturation coefficient of 301.5 mg/l and maximum substrate utilization rate constant 2.77 d^{-1} were found and these coefficients determined may serve as basis for the design and simulation of continuous bioreactors treating phenolic wastewaters.

The inhibitory effect of the phenol will be observed on the cell growth during the degradation of the organic matter due to which there will be a lag and this can be determined by a high value of endogenous coefficient but in the present study as the biomass was acclamatised to take phenol as sole source of carbon the inhabitation of cells by the toxic compound was nullified. The endogenous coefficient determined cannot be compared with the limiting values as it was not determined when the biomass was acclamatised to toxic substrate.

Swaminathan et al., (1999) studied on the removal of 2,4-dichloro phenol with acclamatised mixed culture bacteria using modified rotator biological contractors which showed 100% removal of the substrate. Haijuan et al., (2010); Prasad et al., (2010); Aggary and Soloman, (2008) stuided on the removal of phenol using pure culture bacteria with out acclamatising the biomass to the influent substrate which showed inhibitory effect on the biomass growth

Hence it can be inferred that if the biomass was acclamatised to the toxic compound that was to be removed the inhibitory effect of the compound on the system decreases and noteworthy removal efficiencies can be achieved.

4. Mathematical model

A linear regression model with the parameters, influent organic loading (L), influent substrate concentration (S), retention time (θ), Liquid Temperature (T), is attempted. The removal efficiency expressed as fraction (F) is given by:

$$F = A_0 + A_1 * L + A_2 * S + A_3 * \theta + A_4 * T \ldots\ldots\ldots\ldots\ldots (3)$$

In the present case T is assumed constant. Hence we can write:

$$F = A_0 + A_1 * L + A_2 * S + A_3 * \theta \ldots\ldots\ldots\ldots (4)$$

The following correlation was obtained:

F = 0.85374 + 0.0000028*L – 0.00043*S + 0.004421*θ

(Number of data points = 32)

The observed and predicted data of removal efficiency for phenolic waste in continuous reactor is presented in Figure.

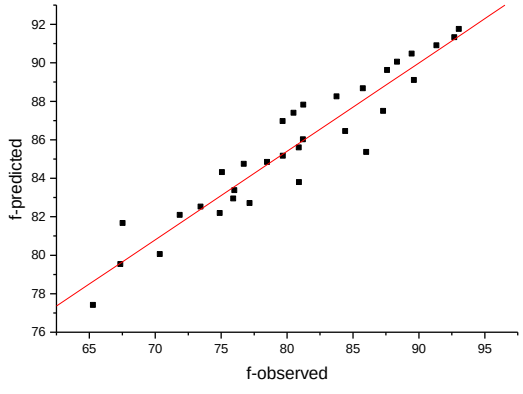

Figure 8: First order fit for the observed and predicted data of removal efficiency for phenolic waste

5. Conclusions

1. Micro-organisms can be acclimatised to accept phenol alone as the sole source of carbon for their metabolic activities.
2. The mixed culture bacteria even though takes a longer time for acclimitasion, once acclimatised, accepts phenol to support the metabolic activities.
3. The mixed culture bacteria were capable of degrading phenol even at higher organic loading rates with significant removal efficiencies.
4. There will be no inhibitory effect on biomass by toxic substrate as it was acclamatised to accept phenol as sole source of carbon.
5. The treatment efficiency for organic loading rate of 0.0041 g m^{-2} d^{-1} was found to be 93.07% and for the highest loading of 0.416 g m^{-2} d^{-1}, the treatment efficiency dropped down only to 65.32%. The results show that the removal efficiency was proportional with the HRT.
6. Mathematical model based on experimental data was derived and good agreement has been found.

6. References

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