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Biodegradation Kinetics of 2-Chlorophenol With Starch Water As Co-Substrate using Anaerobic Batch Reactor

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Abstract:- **Biological degradation of 2-chlorophenol (2-CP) was investigated in an anaerobic batch reactor using starch water as co-substrate. Experiments were carried out with various mixing ratios of synthetic starch water and 2-CP viz. 90:10, 80:20, 70:30, 60:40, 50:50 and the extent of degradation were quantified. High COD removal efficiency of 97.1% and 97.5% of 2-CP removal was achieved at mixing ratio of 80:20. The bio-kinetic parameters such as maximum specific growth rate (µmax), half velocity constant (Ks) and inhibition constant (Ki) were determined for a mixed anaerobic consortia by both Monod and Haldane models. The growth kinetics represented by Haldane model is found to be very suitable than the Monod model for degrading the combined wastewater of 2-CP and starch The maximum specific growth rate (µmax, h-1), substrate half saturation coefficient (Ks, mg/L) and the substrate inhibitory coefficient (Ki, mg/L) for Haldane model are in the range of 0.00005 - 0.02, 51.3 - 774 and 146.6 - 2272.7 .The correlation coefficient R2 was found to be 0.99 for Haldane model and 0.89 for Monod model.**

Keywords: 2 - Chlorophenol; anaerobic; batch reactor; starch; mixing ratio; kinetics

I. INTRODUCTION

Chlorinated phenols constitute an important class of pollutants because of their wide use in the production of wood preservers, pesticides and biocides. Due to their high toxicity, strong odor emission and persistence in environment and suspected carcinogen and mutagen to the living, chlorophenols pose a serious ecological problem as environmental pollutants [1,2]. These compounds are found to be toxic and recalcitrant and hence their discharge into the environment must be regulated. Development of more effective processes for chlorophenol removal is needed for controlled discharge of such toxic compounds. Different physical, chemical and biological methods such as activated carbon adsorption, chemical oxidation and aerobic/anaerobic biological treatment were used for removal of chlorophenols from industrial wastewater [2,3]. Physico-chemical methods are usually used to concentrate the chlorophenols and do not result in complete mineralization. Chemical or biological oxidation methods are much more effective for complete mineralization of chlorophenols usually in combination. Slow and partial degradation of chlorophenols under aerobic and anaerobic natural environment has been observed. Biodegradation of chlorophenols are more specific, relatively inexpensive and can be realized under aerobic and anaerobic conditions [2,4,5]. The position and the number of chlorine groups on the aromatic ring have a profound effect on biodegradability of chlorophenols. Usually biodegradability decreases and toxicity level increases with increasing number of chlorine groups. High concentrations of chlorophenols are inhibitory to microorganisms. However adaptation of microorganisms to chlorophenols was found to improve the biodegradative ability of the organisms and alleviate inhibitory effects to some extent [6]. Addition of growth substrates to the medium has shown to improve the extent of biodegradation of chlorinated aromatic compounds [7,8]. Modelling of the anaerobic processes is an interesting exercise for design, prediction and control purposes [9,10,11,12]. They are used to determine the importance of relationships between the design data and experimental results. They are also used to control and predict the plant design performance for optimizing the plant design. Gu and Knaebel studied the degradation kinetics of pentachlorophenol (PCP) [13,14]. Ning et al. [15] investigated the degradation of 2,4 DCP using a modified Haldane inhibition kinetic model to estimate the performance of anaerobic sludge granules in an anaerobic digester.

This study provides an alternative solution for the enhancement of bioremediation of persistent organic pollutants, especially in the case where microorganisms cannot use them as only carbon source. In order to achieve this, a cost effective and easily assimilated substrate (starch) is supplemented in the medium. According to this, we report on the biodegradation of 2-chlorophenol in the presence of starch water as the carbon source in a batch reactor at different mixing ratios and also the kinetic constants were estimated using Monod and Haldane model.

II. MATERIALS AND METHOD

A. Biomass

The methanogenic granular sludge with unknown microorganisms used in this study was procured from the anaerobic digester treating tapioca starch effluent of M/s Srimannarayana sago factory, Rasipuram, Tamilnadu, India. Before loading the reactor the granular sludge was clearly washed, filtered through fine mesh to avoid all inorganic mineral contents. The volatile suspended solids content of the sludge was then estimated as 45000mg/L [16].

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B. Preparation of synthetic 2-CP and starch waters

The stock synthetic 2-CP water was prepared by dissolving 1 g of 2-CP in one litre of warm distilled water, from this stock solution the required concentration of 2-CP were taken for the experiments. The synthetic starch water was prepared by dissolving 1 g of starch powder in one litre of water to give a COD concentration of about 2200 mg/L. It was pre-acidified before being fed to avoid the settling of starch particles. Since the simulated 2-CP and starch water were deficient in available nutrients, ammonium chloride, Ferric chloride, calcium chloride and potassium di-hydrogen orthophosphate were added to adjust the C : N : P ratio to 100 : 5 : 1. This is a proprietary nutrient mixture available for biological treatment systems (McDougall, 1994).

C. Analytical procedure

All analyses were carried out in accordance with Standard Methods [16]. COD was measured using the open reflux method, gas production by water displacement method and 2-CP was determined using 4-amino anti-pyrine method, the colour intensity was measured by a spectrophotometer (Hitachi U-2001) and 2-CP removal were measured in terms of absorbance at a wavelength of 500 nm, in which the colour intensity absorbed maximally, (λmax) and the absorbance values were proportional to 2-CP concentration.

D. Experimental setup

Batch studies were carried out in the laboratory using pet bottles of 5 L capacity each. To maintain anaerobic conditions, the bottle mouth was tightly closed with rubber cork. Two ports were provided for sampling and gas collection. The gas was measured by water displacement method. The reactor was seeded with 0.5 L of seed sludge and 3.5 L of synthetic 2-CP-starch water (various mixing ratio) the space above the liquid level acts as a gas holder. In order to obtain anaerobic conditions the bottles were purged with nitrogen. The experiment was carried at an initial pH of 7 for 20 days at room temperature (32±10C). Decrease in pH in the reactor was adjusted by NaHCO3.The values of pH, COD, 2-CP, Biomass and Biogas were monitored daily.

III. RESULTS AND DISCUSSION

A. pH

The initial pH of all the mixing ratios was maintained at 6.8 by adjusting with sodium bicarbonate. At the end of the process, the pH reduces sharply to 6.3 for the mixing ratio of 90:10 (starch water and 2-CP). Whereas, for 80:20, 70:30, 60:40, and 50:50 mixing ratios the pH reduced from 6.8 to 6.2, 6.8 to 6.2, 6.8 to 6.3, and 6.8 to 6.3 respectively. The gradual reduction of pH in the reactor was due to the presence of higher concentration of fatty acids formed during the degradation. Phoolphundh [17] reported the optimum pH for anaerobic treatment of 2-CP is 7.8. The optimum reactor operation conditions were achieved in anaerobic system at pH values greater than 6.5 [18].

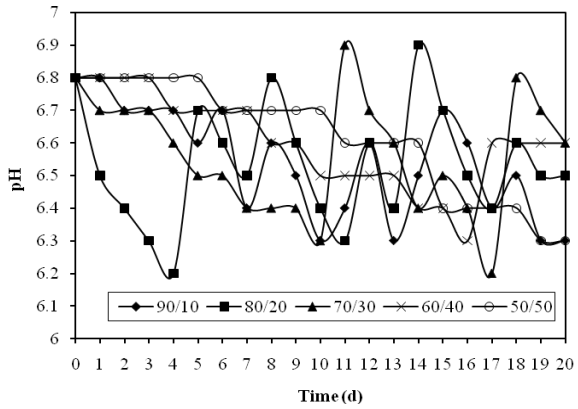

Fig.1 pH at different mixing ratios

B. Effect of COD removal efficiency

The COD of synthetic 2-CP and starch water were 1600 and 2600 mg/L, respectively. From the stock solution the starch and 2-CP water were mixed at different ratios viz. 90:10, 80:20, 70:30, 60:40, 50:50. The COD of the feed decreases as the 2-CP concentration increases, due to the dilution effect of wastewater. The percentage removal of COD versus time at different mixing ratios is shown in Fig. 2. The COD reduction starts after a long lag phase of 48 h may be due slow acclimatization of the organisms to the new environmental conditions. The COD removal was linearly increased from 6th to 16th day for 80:20 mixing ratio and after that it attains the stationary phase. The maximum COD removal efficiencies of 94.2, 97.1, 83.0, 72.6, and 46.9 % were obtained for different mixing ratios 90:10, 80:20, 70:30, 60:40 and 50:50, respectively. The COD removal efficiency was found to be comparatively low for mixing ratios 70:30, 60:40 and 50:50 may be due to inhibitory effect of excess 2-CP in the wastewater. The high COD removal efficiency of 97.1% was achieved at 80:20 mixing ratio on 18th day. Chen et al. [19] studied the response of activated sludge in the presence of 2,4-dichlorophenol using a batch culture system with a maximum COD removal of 90%.

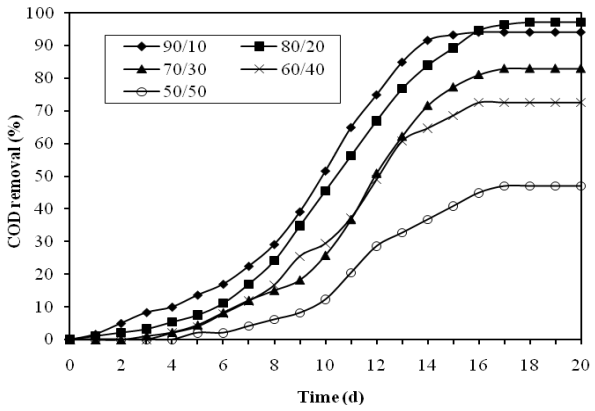

Fig.2 COD removal (%) at different mixing ratios

C. Effect of 2-CP removal efficiency

Initially, 2-CP removal efficiency was found to be low for all mixing ratios may be due to the new environment for the microorganisms. The duration of lag phase in 2-CP

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removal process increased with increase of 2-CP concentration may be due to slow acclimatization of microorganisms to high concentration. The removal efficiency gets decreased as the percentage of 2-CP concentration increases due to inhibitory effect, whereas in the mixing ratio of 90:10, even though there was low concentration of 2-CP the removal efficiency of 2-CP was low, due to the effect of high organic loading rate. Maximum 2-CP removal efficiency of 97.5% was achieved at mixing ratio of 80:20. Figure 3 shows the percentage removal of 2-CP at various mixing ratios of starch water. Maximum removal of chlorophenol (92%) in a simulated high strength industrial wastewater using anaerobic batch reactor was reported [20].

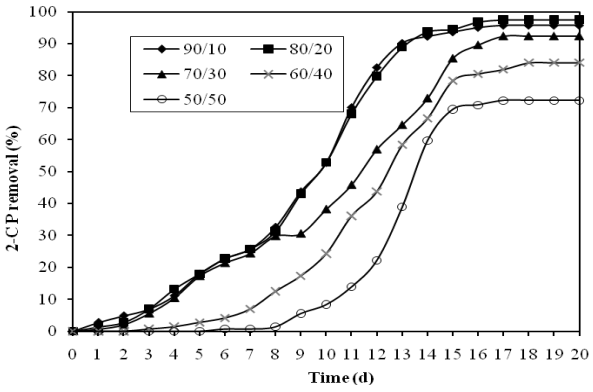

Fig.3 2-CP removal (%) at different mixing ratios

D. Biomass production

Biomass was measured as Mixed Liquid Volatile Suspended Solids (MLVSS) for various mixing proportions. The growth of biomass gradually increased with the reduction of COD concentration. The degradation started with an initial biomass concentration of 45000 mg/L for all mixing ratios. The growth rates of biomass were low for the first two days this may be due to the new environmental conditions and substrate. After 48 h the organisms were able to acclimatize with the new environment and the growth rate of biomass was increased and finally it reached a steady state. Figure 4 shows the biomass concentration decreases with the increase in the mixing ratio of the 2-CP wastewater. The maximum growth of biomass achieved at different mixing ratios of 90:10, 80:20, 70:30, 60:40 and 50:50 were 51000, 52000, 49650, 46250 and 46000 mg/L. Optimum biomass growth (45000-52000 mg/L) was found at 80 and 20% (starch and 2-CP) of mixing, respectively. The available carbon source in the reactor increases with increase in the concentration of starch leading to high biomass content. Keharia et al. [21] also reported that in batch process using anaerobic methanogenic sludge the biomass concentration increased from 2200 to 9000 mg/L. Somasiri et al. [22] reported that the growth of biomass in terms of total volatile suspended solids was 82300 mg/L in an UASB reactor.

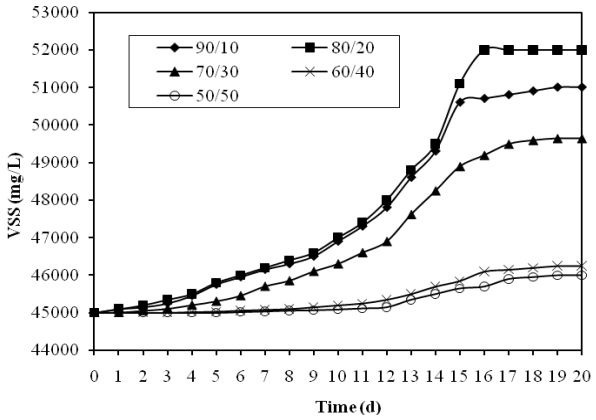

Fig. 4 Biomass at different mixing ratios

D. Biogas production

Figure 5 shows the amount of gas produced at various mixing ratios of substrate and co-substrate. The maximum biogas production was about 10.1 L/d at 80% of synthetic starch and 20 % of synthetic 2-CP water mixing when compared to other mixing ratios. The biogas production for other mixing ratios 90:10, 70:30, 60:40, and 50:50 was 9.7, 7.3, 5.5 and 3.3 L/d, respectively. The inhibitory effect of the 2-CP at higher mixing ratios reduced the biogas generation. Isik and Sponza [23] reported the inhibition of dye concentration on biogas production which has accomplished with the inhibition of glucose degradation using anaerobic batch reactor.

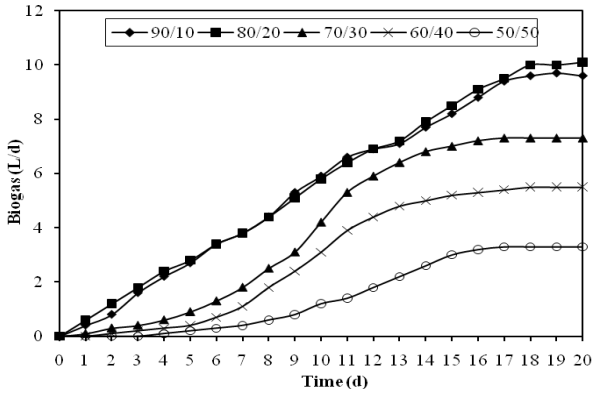

Fig. 5 Biogas production at different mixing ratios

E. Growth kinetics

The bio-kinetic parameters such as maximum specific growth rate (μmax), half velocity constant (Ks) and inhibition constant (Ki) were determined by the plot of specific growth rate against the combined 2-CP and starch water for a mixed anaerobic consortia. Table 1 shows the bio-kinetic constants obtained by plotting Monod and Haldane equations. It is evident that the growth kinetics represented by Haldane model is very suitable than the Monod model for degrading the combined wastewater of 2-CP and starch. The correlation coefficient R2 was found to be 0.999 for Haldane model and 0.978 for Monod model. The maximum specific growth rate (μmax, h-1), substrate half saturation coefficient (Ks, mg/L) and the substrate inhibitory coefficient (Ki, mg/L) for Haldane model are in the range of 0.00005-0.02, 51.3-774 and 146.6-2272.7

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respectively for different mixing ratios For Monod model the maximum specific growth rate (µmax, h-1) and the substrate half saturation coefficient (Ks, mg/L) are in the range of 0.00002-0.0024 and 10.11-1600 respectively. The Ki values indicate that the inhibition effect is observed only at high concentrations of 2-CP. Bhunia and Ghangrekar (2008) reported the Ks and Ki values for Haldane model was 167.2 and 3636.36 and µmax and Ks values for Monod model were 0.048 and 226.1 respectively on treating textile dyeing wastewater by anaerobic process.

TABLE 1 Growth kinetic parameters of Monod and Haldane model for treatment of combined 2-CP and starch water at different mixing ratios using mixed culture (Temperature = 32±10C, pH= 7)

		Monod Model		Haldane Model		
Starch (%)	2-CP (%)	μ_{max} (h^{-1})	Ks (mg/L)	μ_{max} (h^{-1})	Ks (mg/L)	Ki (mg/L)
90	10	0.0024	202.59	0.007	567.4	370.4
80	20	0.0021	10.11	0.02	774.6	146.6
70	30	0.0022	131.2	0.005	94.6	373.1
60	40	0.0003	61.66	0.0007	51.3	1180.6
50	50	0.00002	1600	0.00005	500	2272

F. Endogenous or decay coefficient

Decline in cell population is shown in growth curve after the complete consumption of substrate. During the death phase some weaker cell population becomes food for the healthier one. This part of the growth curve in a batch reactor was modeled by the following equation:

$$dx/dt = -kd\ X \qquad (1)$$

where, kd is known as endogenous or decay coefficient, in order to determine the kd values the process was continued and the biomass concentrations was measured for few days even after the maximum removal of substrate. The selection of particular growth run was arbitrary, assuming that the kd is not dependent on initial concentration. The negative intercept in the graph plotted for the entire growth region as (S0-S)/θX versus 1/θ, were S0-initial substrate concentration mg/L, S-substrate concentration after 24 h mg/L, θ-time h, gives the decay rate coefficient for different mixing ratio of synthetic 2-CP and starch water. The decay rate coefficient for the mixing ratio of combined starch water and 2-CP of 90:10, 80:20, 70:30, 60:40 and 50:50 are 0.0064, 0.0027, 0.0027, 0.0022 and 0.0004 h-1 respectively. Bhunia and Ghangrekar [24] have reported the decay rate coefficient for treating textile dyeing wastewater by anaerobic process as 0.004 h-1. The values obtained in this study were comparable with the reported values. The decay rate coefficient obtained by Lee et al. [25] was 0.001-0.033 h-1 for degradation of textile dyeing effluent using Aeromonas caviae under anaerobic condition.

G. Yield coefficient

Yield coefficient for 2-CP combined with starch water at different mixing ratio was obtained from the slope of the graph plotted between 1/θ versus (So-S)/θX. The values of yield coefficient for different mixing ratios 90:10, 80:20, 70:30, 60:40 and 50:50 of 2-CP and starch water was 0.4, 0.76, 0.78, 0.13 and 0.04 respectively. The respective coefficient of correlations R2 was 0.979, 0.918, 0.964, 0.961, and 0.825. The R2 values are higher than the values obtained by Bhunia and Ghangrekar [24] this may due to addition of co-substrate.

IV. CONCLUSION

Biodegradation of 2-CP in the presence of starch water showed that the presence of conventional carbon sources enhances growth and xenobiotics consumption, which indicates that co metabolism was responsible for the breakdown of the above toxic compounds. Maximum 2-CP and COD removal occurred at 20:80 mixing ratio of 2-CP and starch water. The results of kinetic studies revealed that the growth kinetics represented by Haldane model is more suitable than the Monod model for degrading the combined wastewater of 2-CP and starch. The results obtained from kinetic studies can be used for designing bench scale, pilot scale and full scale continuous reactor with same operational condition for treating 2-CP and starch water.

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