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Kinetic study for aerobic treatment of phenolic wastewater

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ABSTRACT

Conventional physico-chemical treatment of industrial wastewater containing compounds such as phenol encounters difficulties due to low substrate level, additional use of chemicals, and generation of hazardous by products along with increased process cost. Biological treatment appears to be a solution for treatment of such industrial wastewater. In the present study an aerobic sequential batch reactor (SBR) has been used for treatment of synthetic wastewater containing phenol. The effects of increasing phenol concentrations on the sludge characteristic have been also investigated. It was observed that, activity of activated sludge for acclimatization of phenol decreases at concentrations above 2000 mg L^{-1}. It may be attributed to toxicity of phenol to active biomass at higher concentrations. Kinetics of phenol degradation has also been studied using Haldane model.

1. Introduction

Water is basic need of life on earth. Although 70% of the earth is water, but only one percent is accessible in form of surface freshwater. This one percent surface water is regularly renewed by

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rainfall and thus is made available for human use. Water is the biggest crisis faced by world today. Toxic compounds are naturally not present in water. When these compounds enter the stream, they show adverse effect towards living organisms. The major agents creating toxic pollution are herbicides and pesticides originating from agriculture and industrial effluents. Phenol and its compounds are toxic to environment. Although Phenol is reported as toxic and inhibitory substrate, however it is also carbon source for an acclimated biomass [1]. Phenol is a known human carcinogen [2]. Molecule of phenol has a hydroxyl group attached to the benzene ring structure. Phenol is an organic compound which is translucent, crystalline white powder, hygroscopic and changes to red color when comes in contact with air. It is soluble in water, petroleum glycerol and alcohol. Phenol is weakly acidic as the molecule has little tendency to lose H^+ ion from the hydroxyl group to form water-soluble phenoxide anion $C_6H_5O^-$. Since phenol is soluble in water, its degradation to reach safety levels of 0.1–1 mg L^{-1} is hard.

Phenol is extensively used in various kinds of industries such as petroleum refineries, gas and coke oven industries, pharmaceuticals, explosive manufacture, phenol–formaldehyde resin manufacture, plastic and varnish industries. Phenol enters water during the manufacturing and processing steps in these industries. Phenol in a concentration ranging from 50 to 2000 mg L^{-1} has been reported by many researchers in industrial wastes [3,4]. Proper treatment of wastewater containing phenol is required before it is discharged to external environment because of its toxicity to living organisms. The permissible limit of phenol is 1 mg L^{-1} for industrial effluents to be discharged into inland surface waters (IS: 2490-1974) and 5 mg L^{-1} for discharge into public sewers (IS: 3306-1974).

2. Treatment of phenolic wastewater

There are two common methods used to eliminate the phenol contents in wastewater, i.e., physico-chemical and biological methods. However, most of the physico-chemical methods cause secondary problems in the effluents. Also, the physico-chemical method to degrade phenol usually involves high capital. Phenol is usually separated from wastewater through separation processes such as steam distillation, extraction, adsorption and membrane based solvent extraction. Phenol may also be abated in water solution through oxidation and bio-filtration [5].

Physical and chemical removal methods generate secondary by-products which eventually enter the environment as toxic aquatic pollutants. Chloro-phenols are generated if chlorination is used in the phenol degradation [6]. Physico-chemical methods used for treatment of phenol containing wastewater are ionization, adsorption, reverse osmosis, electrolytic oxidation, H_2O_2 and photo catalysis. The reason of using chemical processes along with oxidizing agents is to efficiently reduce the content of phenolic compounds in the wastewater. During the oxidation process, oxidizing agents transform these toxic substances to less harmful elements which are safe to be discharged to the environment.

Ozone is one of the strongest oxidants used for phenol degradation because of its availability, solubility and generation of less-toxic substances. Molecules of ozone react with electron-rich sites of the organic pollutant. Also hydroxyl radicals are generated from the reaction of ozone which acts as oxidants for pollutant molecule.

Adsorptive process using activated carbon is widely applied for removal of contaminants in wastewater [7]. Adsorption involves activities such as saturation, adsorption, desorption and regeneration. It has been reported that some of the phenols and its derivatives may get adsorb on activated carbon irreversibly, where the irreversibly adsorbed phenol cannot be desorbed in water [8]. This problem results in difficulty in the regeneration of adsorbent.

Chemicals such as hydrogen peroxide alone or hydrogen peroxide coupling with iron (II) salt (Fenton reaction) also have been used for phenol oxidation. Hydrogen peroxide (H_2O_2) is rich in oxygen content, low cost, acts as a strong oxidant. Researchers have reported alternative oxidation methods for the removal of phenol through oxidation by chlorine, chlorine dioxide and potassium permanganate [9]. However, these processes result in formation of secondary chlorinated organic compounds. Thus, the biological method such as activated sludge process has been used extensively in

phenol removal to overcome the weakness of secondary pollution and high capital cost involved in the physico-chemical method.

In biological treatment, the microorganisms degrade phenol into other non-toxic compounds. Aerobic biodegradation of phenol is most common. The ability of active biomass to degrade pollutants is affected by the presence of naturally occurring carbon sources. In general, adaptation to variations in the concentration of nutrients such as glucose, yeast extract, and $(NH_4)_2SO_4$ enhances the ability to degrade phenols. Biodegradation of phenols increases at higher concentrations of inorganic nutrients [10].

Activated sludge generally consists of microorganisms, non-living organic materials, and inorganic compounds. Activated sludge process is defined as a system in which active biomass is re-circulated and oxidation of organic contents occurs in the presence of oxygen [11]. In the past time, researchers have used single microbial species for phenol biodegradation which limits its field applications as variety of contaminants may be present in waste [5]. Biological removal of the phenolic compounds is difficult at either low concentration (lower than 200 mg L^{-1}), or at sufficiently high phenol concentration, as it inhibits growth rate of the microorganisms [6]. The presence of toxic pollutants such as phenol may also result in the deflocculation, which results in settling problems in clarifier. Thus, to achieve satisfactory phenol removal efficiency, phenol concentration needs to be maintained below the threshold limits and acclimatization of the microorganisms to the toxic wastewater is a must. It has been reported that acclimatized activated biomass degrades phenolic compounds more effectively than the pure strains by one or more than two orders of magnitude faster [11]. Activated sludge has been successfully applied for phenol degradation with the help of batch reactor up to 1500 mg L^{-1} at a pH of 6 [12]. Moving bed bio-film reactor has been used for treating phenol containing wastewater with high TDS. Maximum COD removal was found 97.44% at HRT of 44 h and initial phenol concentration of 1400 mg L^{-1} [13]. Phenol and COD removal efficiency above 99% have been reported at inlet phenol concentration of 800 mg L^{-1} and HRT of 24 h [14]. Maximal removal rate of 2.92 g phenol L^{-1} d^{-1} at a hydraulic retention time (HRT) of 0.95 days and a total organic loading rate (OLR) of 15.3 g COD/(L^{-1} d^{-1}) with a phenol concentration of 4.9 g L^{-1} has been observed using fixed bed bio film reactor [15]. Efficient removal of COD and Phenol removal was reported at organic and phenol loading rate of 5 kg COD m^{-3} d^{-1} and 400–1200 mg phenol L^{-1} wastewater using expended granular sludge bed anaerobic filter bioreactor [16]. Biological treatment has been reported to effectively degrades phenol up to the concentration of 420 mg L^{-1} [17].

3. Material and methods

3.1. Phenol volatilization test

Volatilization test has been performed before acclimatization to determine the potential of phenol loss under aerated condition [1]. Phenol (Product code – 5170 B; Grade-AR; molecular weight – 94.11) was purchased from Laba Chemie, India and same was used in all the experiments. Therefore, during the test, any loss in phenol concentration can be attributed to air stripping from aeration. The test was carried out in a 5 L reactor with phenol containing wastewater at various concentrations of 500, 1000, 1500, 2000, 2500 and 3000 mg L^{-1}. Aeration along with mixing was provided using air stone diffusers. 20 mL of the sample was collected from reactor at the beginning of the test and then after every one hour for phenol analysis. The total duration of the test was six hours. The phenol content of the samples collected was determined to examine loss of phenol by volatilization by UV–vis spectrophotometer using 4-aminoantipyrene method [18].

3.2. Acclimatization of activated sludge towards phenol

Researchers reported that acclimatization gives rise to new biomass population adapted to the toxic compounds by consuming it as a substrate [6,19]. Acclimatization of activated sludge towards increasing phenol concentration (from 500 mg L^{-1} to 3000 mg L^{-1}) was carried out using batch reactor (20 L). The seed sludge for acclimatization of the SBR was brought from a real scale sewage

Table 1
Composition of nutrient water used for acclimatization.

Composition	Concentration (mg/L)
Bactopeptone	188
Glucose	586
Ammonium chloride	172
Magnesium sulfate	49
Dipotassium hydrogen phosphate	250
Sodium bicarbonate	14.7

treatment plant located in Noida, NCR region; India. Aactivated sludge was first cultured for one month with nutrient water (base mix) having composition given in Table 1. After the completion of culturing phase, phenol was introduced slowly to replace the glucose as a carbon source.

3.2.1. Batch reactor

The batch reactor was operated in four steps consisting of Fill, React, Settling and Idle periods comprising the ratio of 2:8:2:0 for a total react time of 8 h and cycle time of 12 h. Settling time was chosen depending upon settling characteristics of sludge while idle period was eliminated as sludge wasting was not carried during acclimatization study. Sample was fed to the reactor during the Fill mode at a rate of 150 mL min^{-1}. Aeration was provided throughout the Fill and React periods using stone diffusers connected with aeration pump. Sample was degraded in the reactor during the total 8 h React mode. The samples of treated effluent were withdrawn from reactor for analysis after settling period of two hour duration.

During acclimatization in first phase, batch reactor was operated on base mix (nutrient water with glucose as carbon source, having composition as given in Table 1) till the reactor attained pseudo steady state in terms of effluent COD. In second phase, synthetic sample with phenol concentration of 500 mg L^{-1} was introduced to reactor and reactor was operated till the effluent COD got stabilized. In third, fourth, fifth, sixth and seventh phase reactor was operated with a increasing phenol concentration (thereby decreasing glucose concentration) of 1000, 1500, 2000, 2500 and 3000 mg L^{-1} (phenol) respectively till the reactor attained pseudo steady state in terms of effluent COD. During acclimatization period, the phenol removal efficiency was checked regularly by effluent COD analysis along with sludge characteristics. Fig. 1 shows batch reactor used during acclimatization period.

3.2.2. MLSS monitoring

During the acclimatization period sludge monitoring was also conducted to determine the impact of change in phenol concentrations on the activated sludge consortia. This change was closely observed and examined at every variation of phenol concentration. Mixed liquor suspended solids (MLSS) was monitored as per recommended methods [18].

4. Results and discussions

4.1. Phenol volatilization test

Based on the phenol volatilization tests (Fig. 2) it was observed that there was no loss of phenol at studied phenol concentrations. The results are in accordance with the results reported [2].

4.2. Phenol acclimatization

Batch reactor has been used in the study to acclimatize the activated sludge at increasing phenol concentration. Acclimatization was carried to produce the enzymatic material which helps in biodegradation of toxic material like phenol. Also the sludge concentration indicates new biomass

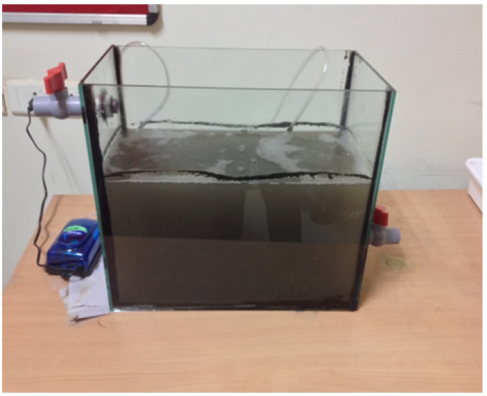

Fig. 1. Experimental set up of batch reactor.

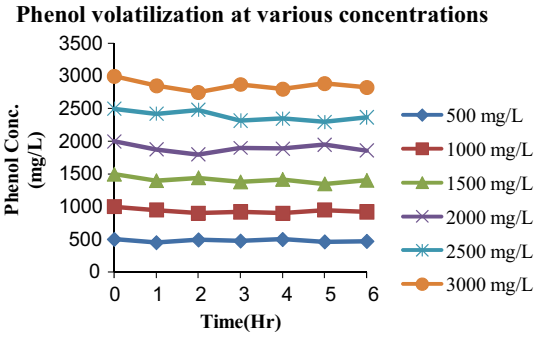

Fig. 2. Phenol volatilization Test.

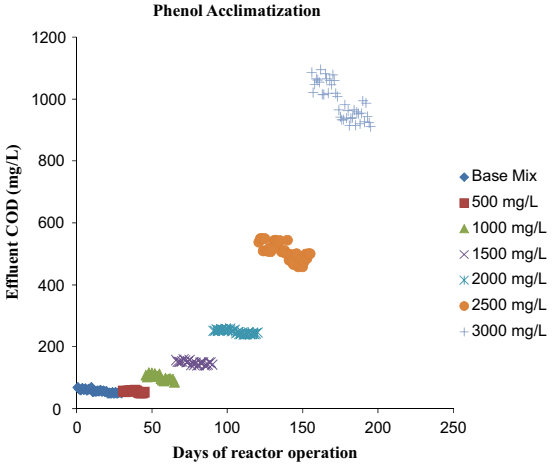

Fig. 3. COD during acclimatization period.

which can adapt to phenol as carbon source [6]. The length of the acclimatization was varied depending on the phenol concentration and the bioactivity of biomass. It has been reported that that the period of acclimatization is long for anaerobic environments [20].

During first phase of study the change of COD was monitored every day after addition of nutrient water (Base mix) for a period of 30 days. During second phase (Day 31–45), third phase (Day 46–65) and fourth phase (Day 66–90) synthetic sample with increasing phenol concentration i.e., 500 mg L^{-1}, 1000 mg L^{-1} and 1500 mg L^{-1} respectively, were fed to reactor and activated sludge exhibited a COD removal efficiency of 95% during all the phases with effluent COD of 50 mg L^{-1}, 100 mg L^{-1} and 150 mg L^{-1}. During fifth phase of study (Day 91–120) sample with phenol concentration of 2000 mg L^{-1} was fed and the removal efficiency decreased to 93% with effluent COD of 250 mg L^{-1} which is still within permissible limits as prescribed by Indian standards (as per IS:10500). However during sixth phase (Day 121–155) and seventh phase (Day 156–195) on feeding sample with phenol concentration of 2500 mg L^{-1} and 3000 mg L^{-1} respectively, removal efficiency decreased to 90% and further to 83% with effluent COD concentrations of 500 mg L^{-1} and 1000 mg L^{-1} respectively. It was observed that effluent COD was under prescribed limit of 250 mg L^{-1} (as per IS:10500) for influent phenol concentrations up to 2000 mg L^{-1}. However, accumulation of phenol was observed when concentration of phenol was increased to 2500 mg L^{-1} in the influent. The effluent COD fluctuated between 450 mg L^{-1} and 550 mg L^{-1}. These effluent quality levels did not comply with Central Pollution Control Board (CPCB) discharge requirements where the effluent discharge into inland waters for the purpose of human consumption shall contain COD less than 250 mg L^{-1}. It is clear that phenol removal efficiency is maximum up to medium phenol concentration (500–1500 mg L^{-1}), beyond which it starts to decrease and finally accumulation of substrate occurs showing substrate inhibition. The ability of activated sludge acclimatized to 2500 mg L^{-1} of influent phenol concentration declined drastically, may be attributed to the phenol toxicity which retarded the activity of activated sludge towards biodegradation. COD during the acclimatization period has been shown in Fig. 3.

4.2.1. MLSS during acclimatization

The sludge concentrations measured as mixed liquor suspended solids (MLSS), with varying influent phenol concentration and the data for same has been analyzed and represented through Fig. 4. From the Fig. 4 it is clear that the sludge concentration varies from 2000 to 12000 mg L^{-1} when the feeding composition was base-mix. It is inferred from the figure that growth of biomass (MLSS) remains around 6000–13000 mg L^{-1} for influent phenol concentration from 500 mg L^{-1} to 2000 mg L^{-1}. Further on increasing the phenol concentration beyond 2000 mg L^{-1}, MLSS decreases rapidly to 4000 mg L^{-1}. It shows that at medium phenol concentration (500–2000 mg L^{-1}) maximum efficiency for phenol removal and new cell formation is observed. However on increasing influent phenol concentration to 2500 mg L^{-1} phenol, removal efficiency is decreased, resulting in decrease of biomass synthesis. At influent phenol concentration of 3000 mg L^{-1}, MLSS concentration was observed as low as 2800 mg L^{-1}. It shows the toxicity of phenol to microbes at increasing concentration which tends to limit their growth.

4.2.2. Kinetics of biodegradation of phenol

Kinetic study of biodegradation of phenol has been performed using Haldane equation [21].

$$\mu = \frac{\mu_{max} S}{K_s + S + \frac{S^2}{K_I}}$$

Where μ and μ_{max} are specific growth rate and maximum specific growth rate (h^{-1}).

S is substrate concentration (mg L^{-1}),
K_s is half-saturation constant (mg L^{-1})
K_I is inhibition constant.(mg L^{-1})

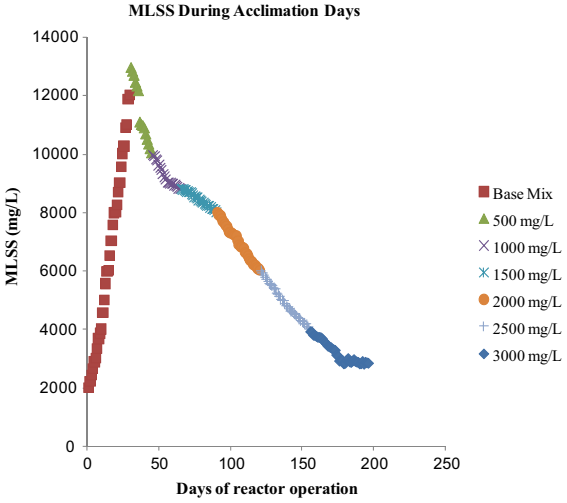

Fig. 4. MLSS during acclimatization.

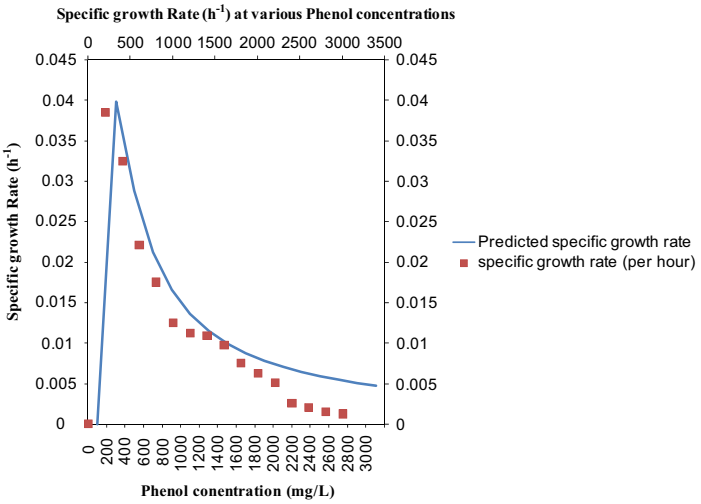

Fig. 5. Specific growth rate (h^{-1}) at various initial phenol concentrations.

For assessment of experimental specific growth rate (μ), the hourly biomass growth data was measured at various phenol concentrations (200–3000 mg L^{-1}) as dry cell weight as reported in literature [22]. In this method, A 50 mL sample was taken from the reactor and centrifuged at 4000 rpm for 20 min. The supernatant was transferred to small viols and used for estimation of phenol. The pellets were re-suspended in water and re-centrifuged. The supernatant was wasted and pellets rinsed off from the tube into a pre conditioned and pre-weighed 1.2 μ filter paper (Whatmann). This filter paper was then dried in an oven at 105 °C for 24 h and weighed until at constant weight obtained. The difference between initial weight of filter paper and final weight was used to estimate biomass concentration.

Table 2
Kinetic constants for aerobic phenol biodegradation using mixed culture.

S.No	Authors	Conc. range (mg/L)	μ_{max} (h^{-1})	K_s (mg L^{-1})	K_I (mg L^{-1})
1.	Pawlowsky and Howell (1973)	0–900	0.260	25.4	173
2.	Pawlowsky and Howel (1973)	0–1000	0.223	5.86	934.5
3.	Tang and Fan (1987)	–	0.326	19.2	229.3
4.	Lallai et al.(1998)	–	0.365	10.95	113
5.	Kumaran, and Paruchuri (1997)	60–500	0.542	36.2	145
6.	Buitron et al. (1998)	40	0.258	3.9	121.7
7.	Marrot et al., (2006)	2500	0.438	29.5	72.4
8.	Saravanan et al.(2008)	100–800	0.3085	44.92	525.00
9.	Bajaj et al.(2009)	23.5–659	0.3095	74.65	648.13
10.	Dey and Mukherjee et. al. (2010)	100–700	0.150	51.8	404.04
11.	Szetela et al (1981)		0.326	19.2	229.3
12.	Duan Z. (2011)	0–1500	0.4695	603.9869	28.4860
13.	Nuhoglu and Yalcin (2005)	1450	0.143	87.44	107.06
14.	Adamo et al.(1984)	–	0.131	5.0	142
15.	This study	500–3000	0.355	603.803	40

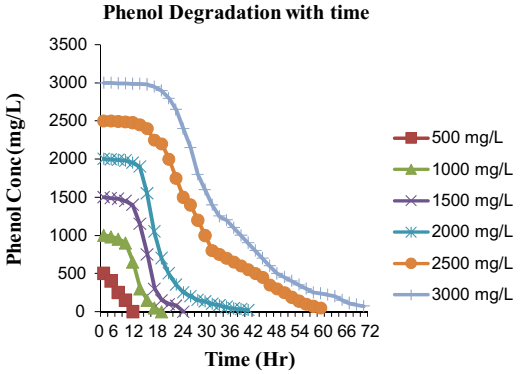

Fig. 6. Phenol biodegradation with time.

Specific growth rate values were calculated for experimented phenol concentration as reported by researchers using a graph between biomass (on logarithmic Y axis) and time (on X axis) using

$$\mu = \ln(N_2/N_1)/(T_2 - T_1)$$

Where N_1 and N_2 = biomass at time1 (T_1) and time2 (T_2) respectively.

The experimental specific growth rate data was plotted against various initial Phenol concentrations (Fig. 5). Fig. 5 shows that specific growth rate increases with increase of phenol concentration up to 200 mg L^{-1}, after which it start to decrease with increase in substrate concentration, suggesting inhibition behavior of phenol at higher concentrations. Table 2 shows values of kinetic constants of Haldane equation as obtained by other workers and used in this work. Maximum specific growth rate, half saturation constant and inhibition constants were determined by using non linear regression method. Following values of constants were obtained $\mu_{max} = 0.355\ h^{-1}$, $K_s = 603.803$ mg L^{-1}, $K_i = 40.603$ mg L^{-1}. Values of these constants obtained here are similar to the values obtained by researchers in 2011 [12] at phenol concentration of 1500 mg L^{-1}. In this work similar growth rate has been obtained at higher substrate concentration (2000–3000 mg L^{-1}) which shows improvement over previous works as phenol has been reported to be toxic to microbes even at lower concentration. This improvement may be attributed to increase in the period of acclimatization. Also the value of

inhibition constant has been increased to from 28 to 40 mg L^{-1}, which shows decrease in inhibitory effect of substrate on microbes, towards degradation.

4.2.3. Phenol degradation with time

This test was carried out after acclimatization of sludge. During this test, the aeration was provided continuously in the reactor and phenol concentration was observed at interval of every two hours. The sludge started degradation of phenol immediately at phenol concentration of 500 mg L^{-1} moreover at this concentration phenol could be degraded completely within 8 h. However at 1000, 1500, 2000, 2500 and 3000 mg L^{-1}, the time lag of 6,8,10,12 and 18 h was observed. At Phenol concentration of 2000, 2500 and 3000 mg L^{-1} it took 38, 60 and 72 h to degrade. It is observed from Fig. 6 that phenol biodegradation rates decreases upon increasing initial phenol concentrations which suggests the inhibition to bacteria by high phenol concentrations.

5. Conclusion

Conventional physico-chemical treatment of industrial wastewaters containing compounds such as phenol encounters difficulties due to low substrate level, additional use of chemicals, and generation of hazardous by products along with increased process cost. Biological treatment appears to be a solution for treatment of such industrial wastewaters. In present study it has been observed that, activity of activated sludge for acclimatization of phenol decreases at concentrations above 2000 mg L^{-1}. It may be attributed to toxicity of phenol to active biomass at higher concentrations. Also MLSS concentration follows the same trend which ensures that phenol beyond a concentration of 2000 mg L^{-1} presents toxicity for bacterial growth and stability.

Author disclosure statement

All authors of this paper declare that there is no commercial association with any institution of any country to carry out work reported in this paper.

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