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Degradation of mixture of phenolic compounds by activated sludge processes using mixed consortia

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Abstract
This study describes the feasibility of aerobic treatment of wastewater having mixed Phenolic compounds (phenol, 2-4dinitrophenol, 2-4dichlorophenol, 4-chlorophenol, 4-nitrophenol) by using 9L lab scale Activated Sludge Process (ASP) at HRTs (Hydraulic Retention Time) varying between 3.0 ,2.5, 2.0, 1.5 and 1 day. Continuous monitoring of parameters like pH, Oxidation Reduction Potential (ORP), Chemical Oxidation Demand (COD) , compound reduction is used to asses the treatment efficiency of ASP. The highest percentage COD removal and percentage compound reduction of 98% and 99.3% of phenol was observed at 3.0 d HRT respectively. After treatment pollutants are removed in the order of phenol > 4chlorophenol (4CP) > 4nitrophenol (4NP) > 2-4dichlorophenol (2-4DCP) > 2-4 dinitrophenol (2,4DNP). The dissolved oxygen concentration and pH in the activated sludge reactor was found to be 1-3 mg/L and 7-8 respectively. The optimum biomass concentration was 2500-3000 mg/L, whereas the corresponding SVI was found to be around 70mL/g. The morphological characterization of aerobic granules was carried out by using SEM. Thus the results obtained indicate that ASP could be used efficiently for the treatment of wastewater containing mixed phenols.

Keywords: Activated sludge process, Aerobic treatment, 2-4DCP, 2-4DNP, Phenol.

1. Introduction
Phenolic derivatives are among the most important contaminants present in the environment. The toxic and hazardous nature of phenol and their associate derivatives and their amounts in the wastewater leads attention to the most of the researchers. Due to their characteristics of toxicity for humans and aquatic life they are included in the USEPA list of priority pollutants [1]. Moreover, effluents from industries rarely contain a single pollutant as the manufacture of chemicals involves multiple unit operations and processes. The multisubstrate nature of industrial effluent is characterized by the presence of a variety of compounds in varying concentrations [2]. Removal of phenols can be attained by physical-chemical (i.e. solvent extraction, activated carbon adsorption, Advanced Oxidation Processes (AOPs) [3] and biological processes [4-5]. However, the physico-chemical removal or treatment technologies have been found to be inherent drawback owing to the tendency to form secondary toxic intermediates and also proven to be costly [6-7]. Moreover, biological processes are less expensive if compared to AOPs that can also give the complete mineralization of the compound. Thus biological removal especially ASP has turned out to be a favorable alternative because it produces no toxic end products and it is of low cost [8-9]. An activated sludge process was employed due to its environmentally friendly approach and its

ability to completely mineralize toxic organic compounds. The main advantage resulting from the microbial consortium formed by acclimated activated sludge is the interaction between all the species present in flocs [10]. Most of the studies are carried out by using ASP for the treatment of phenol and cyanide containing wastewater [11], Pharmaceutical wastewater [12], Molasses wastewater [13], treatment of organic pollutants [14], and biomass activity test in ASP [15]. In the scientific literature single target compounds are estimated and only few information is obtained on the mixture of different phenolic compounds. A few studies were carried out on the degradation of mixed phenolic compounds by using aerobic batch reactor [16]. No study was carried out on the degradation of mixed phenolic compounds by using ASP. So in the present study an attempt has been made to evaluate the performance of lab scale activated sludge processes for the treatment of phenolic compound mixture.

The specific objectives include

1) Biodegradation of mixture of persistent compounds: phenol, 2-4 dichlorophenol, 4-chlorophenol, 4-nitrophenol and 2,4dinitrophenol by mixed consortia in activated sludge process.
2) To investigate the effect of HRT (3.0, 2.5, 2.0, 1.5 and 1.0) on the reactor performance.
3) Optimization of parameters like pH, SVI, MLSS, Dissolved oxygen for the degradation of mixture of phenolic compounds.
4) After the treatment the morphological characterization of aerobic granules using SEM analysis was carried out.
5) Cost estimation analysis has been carried out for the degradation of phenolic mixtures.
6) Microbial analysis of sludge at different HRTs are studied.
7) Cost estimation for the biodegradation of phenolic mixtures are studied.

2. Materials and methods

2.1 Chemicals

Phenol, 2-4DCP, 2, 4DNP, 4CP, 4NP was in granular form (purity>98%) and supplied by Merck., India.

2.2 Experimental apparatus

ASP reactor (Figure 1) is of 11L capacity with two chambers of which one is for aeration (9L) and other for settling (2L). Aeration was achieved by stirrer and submerged aeration was achieved by air compressor which is arranged at bottom of the aeration tank to facilitate uniform and effective mixing. Peristaltic pumps were used to supply the feed to the reactors and were calibrated to determine the required flow rate. The excess suspended solids (SS) in the reactor are wasted periodically and hence a constant SS values were maintained in the reactor.

Figure 1. Activated sludge reactor used for the degradation phenolic compounds

2.3 Start up of the reactor

The activated sludge required to start the reactor was obtained from Jeedimetla Effluent Treatment Plant, Hyderabad. This sludge has a concentration of TSS of between 30 and 35 g/L, 90% being VSS, and its SVI was 70 ml/g. The reactor was filled with this sludge up to 1/3 of its volume and was fed with a synthetic water as carbon source and adding the necessary nutrients for bacterial development. The pH in the reactor ranged between 7.0 to 8.0. The reactor was stabilized and the oxygen concentration in the reactor was maintained around 1-3mg/l. The characteristics of the synthetic wastewater is given in Table 1. Reactors are operated in continuous mode. COD: N: P ratio was maintained around100:5:1. During start-up period phenols were not added to the reactor. HRT of the reactors was maintained at 1day. Initially the MLSS concentration was 800mg/L and the concentration increased to 20 days and after stabilized at around 3000mg/L (Figure 2). The COD% reduction was low initially but latter on proper acclimation to microorganisms the COD% reduction was increased to 80% where steady state of the system was acquired (Figure 3).

Table 1. Characteristics of sewage used in the study

S.No	Parameter	Value
1.	$_pH$	6.9-7.6
2.	Electric conductivity(umho/cm)	1200
3.	Total hardness as $caco_3$	800
4.	Sulphates	180
5.	Nitrates	120
6.	COD	1100-5200
7.	BOD	525-2800
8.	Phosphates	53
9.	Total solids	440
10.	Total suspended solids	160
11.	Total dissolved solids	280
12.	Total alkalinity as $caco_3$	1300

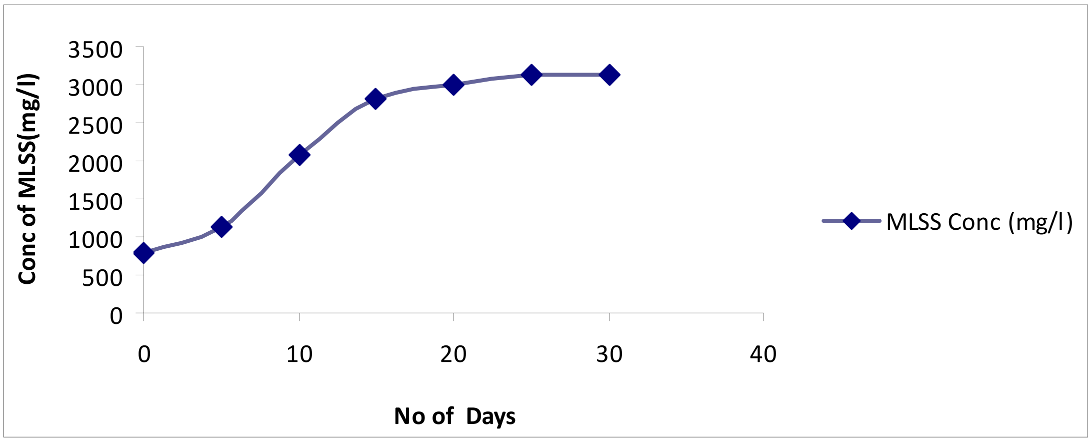

Figure 2. No. of days Vs MLSS conc during startup of the reactor

2.4 Acclimation of activated sludge reactor with phenol, 2-4DNP, 2-4DCP, 4-CP, 4NP

After the start up of the reactor, the mixture of phenol (10mg/L), 2-4DCP (10mg/L), 2-4DNP (10mg/L), 4-CP (10mg/L), 4-NP (10mg/L) was introduced in to the reactor at an total influent concentration of 50mg/L. The wastewater containing phenolic mixtures was then increased gradually from 50 to 100, 150, 200, 250 and 300mg/L. At each increment reactor was operated for 30 days and the reactor took 30 days for acclimation with 50 mg/L of phenolic compounds. The total study period was around 180 days upto 300mg/L. During the acclimation period HRT of the reactor was maintained at 1day HRT. After acclimation, the reactor was operated at 5 different HRTs 3.0, 2.5, 2.0, 1.5 and 1.0 days for degradation of phenolic mixtures in wastewater.

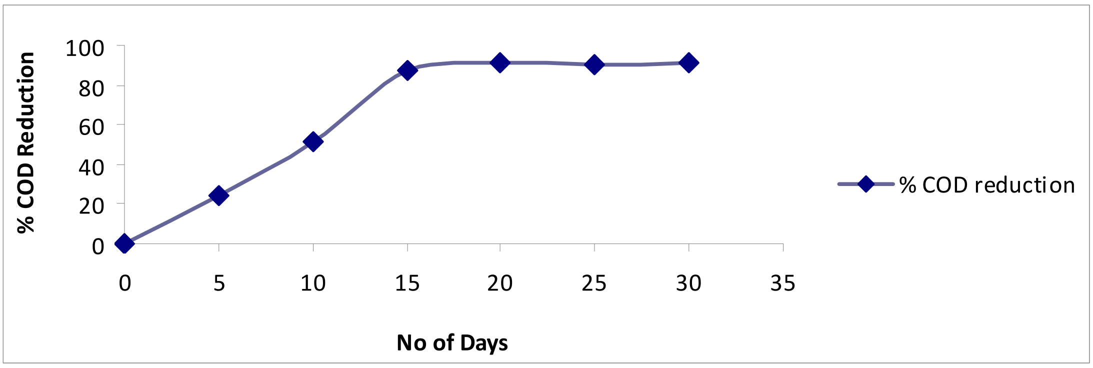

Figure 3. No of days Vs % COD reduction during staetup of the reactor

2.5 Analytical methods

Analysis of alkalinity, chlorides, nitrates, total suspended solids and COD were conducted in accordance with Standard Method [17]. Phenol, 2-4DNP, 2-4DCP, 4-CP and 4NP concentration was measured by Systronics-1272 UV-visible Spectrophotometer at 236nm and further confirmed by reverse phase high-performance liquid chromatography. Agilent Technologies HPLC was used with Chemosoft software. Column used was C-18 with mobile phase having a mixture of methanol and water in the ratio of 80:20. The flow rate was 1ml/min with retention time of 5min.

2.6 Toxicity test

The acute toxicity test was performed to evaluate the detoxification using Daphnia magna. The toxicity was expressed as effective concentration 50 (EC_{50}) concentration/dilution, which produces the immobility of 50% of the organisms of the population tested after a period of exposure of 24 to 48 h. The organisms sensitivity was evaluated before the bioassays are performed. Potassium dichromate was used as a reference toxic compound. General conditions of exposure are showed in the Table 2.
Biodegradation assays and toxicity was evaluated in samples taken both at the beginning and also at the end of the reaction in the influent and effluent of the activated sludge reactor.

Table 2. Conditions of exposure of the acute toxicity test carried out with Daphnia magna

Exposure type	Static without water exchange
Temperature	20
Light	No illumination
Dilution water	Reconstituted distilled water
pH	7.8+0.2
Hardness	250+25mg CaCo3 L-1
Dissolved oxygen	80% of saturation
Conductivity	170
Size of the container	20 ml test tubes
Volume of the sample	10 ml
Age of the organisms	6-24 h
Number of organisms/test	5
Number of triplicates	4
Total number of test organisms	20
Feeding	No feeding
Aeration	No aeration
Test duration	24-48 h
Measure effect	Immobilization
End point	EC50 24/48 h, Spearman-Karber method

2.7 Aerobic granules
Aerobic granules were cultivated using different HRTs ranging from 3 to 1 d, and the results showed that HRT played a role in shaping granule characteristics and reactor performance. Reactors were inoculated with aerobic granules cultivated from activated sludge. These seed granules have a specific gravity (SG) of 1.64 g cm^3, a mean diameter of 0.88 mm and an IC of 99.5%. The aim of this study is to evaluate the effect of HRT on the development of aerobically grown microbial granules.

2.8 Microbial analysis of activated sludge
The activated sludge used in the present work was subjected to microbial analysis to know the probable microorganisms involved in the biodegradation of waste. Various media and stains used in the analysis were procured from M/s Hi-Media, Bombay. Various dilutions of activated sludge were used to determine the total plate count and a loop full of this diluted culture was subjected to microbial analysis using standard procedures. A loop of culture was streaked on Nutrient Agar (NA) for bacteria and Potato Dextrose Agar (PDA) for fungi. These plates were incubated at room temperature (30+20C) for 24-48 hours and observed for growth.

3. Results and discussions
It was observed that after 60 days stepwise increase in phenolic mixture concentration up to 150mg/L caused inhibition to the performance of the reactor and the removal efficiencies are 56%. But the removal efficiencies further increased to 99% from 90^{th} day onwards having low effluent concentration of 1.4 and 0.8mg/L (Figure 4). The reason may be due to the sufficient acclimatization provided for the successful degradation of phenolic compounds. The COD removal percentage gradually decreased with decrease in HRT. This may be due to the inhibitory effect of higher concentrations on the treatment efficiency. The COD removal efficiency dropped to 74%. Initially, this is due to the inhibitory effect of phenolic mixtures on the reactor performance. But later on the COD removal efficiency recovered to 99.7% from 89.2% (Figure 5). It can be concluded from the study that COD% reduction decreased by providing the sufficient time to microorganisms to adjust to new feed composition. The degradation of each phenolic compound decreased with increase in pollutant loading rate. However the pollutants were removed in the following order phenol > 4CP > 4NP > 2,4DCP > 2,4 DNP (Figure 6). This is may be due to the following reason that, greater the number of substituents in structure the more toxic recalcitrant and less degradable [18]. The release of chlorides in the degradation of 4CP, 2-4DCP was observed and analysed. The similar results was obtained by Shu-Guang Wang et al in the biodegradation of 2,4DCP in sequencing batch reactor [19].

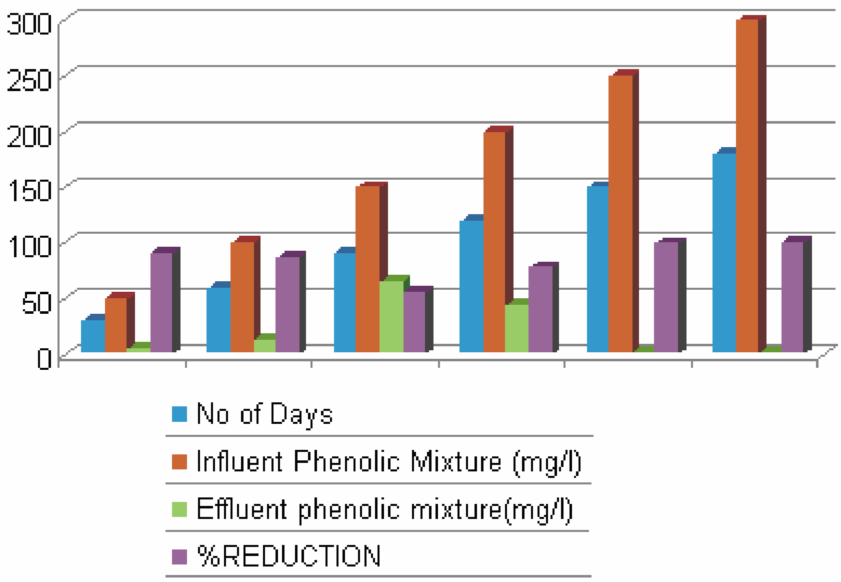

Figure 4. Percentage compound reduction of mixed phenolic compounds

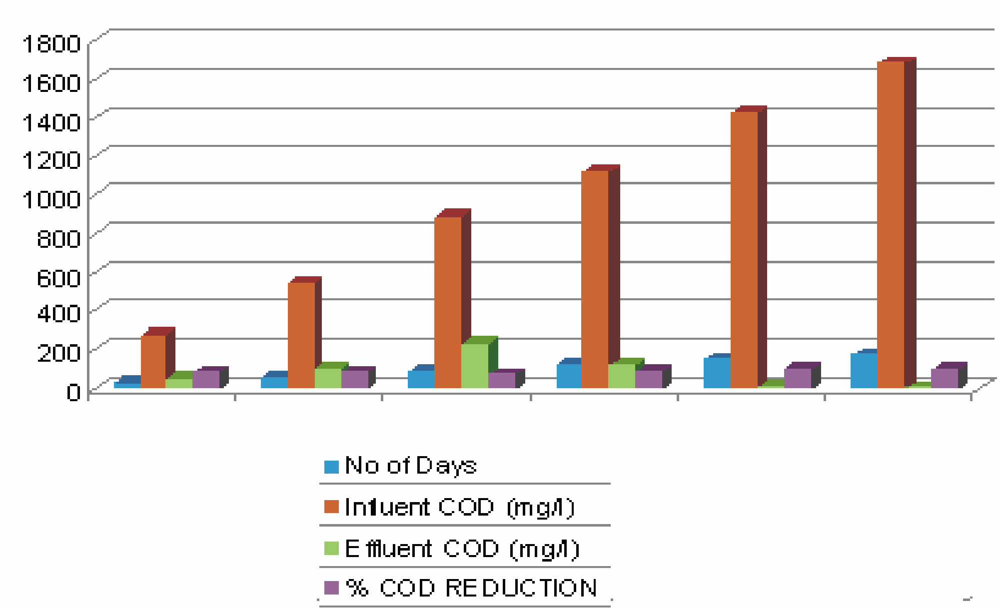

Figure 5. Percentage of COD reduction during the treatment of mixed phenolic compounds

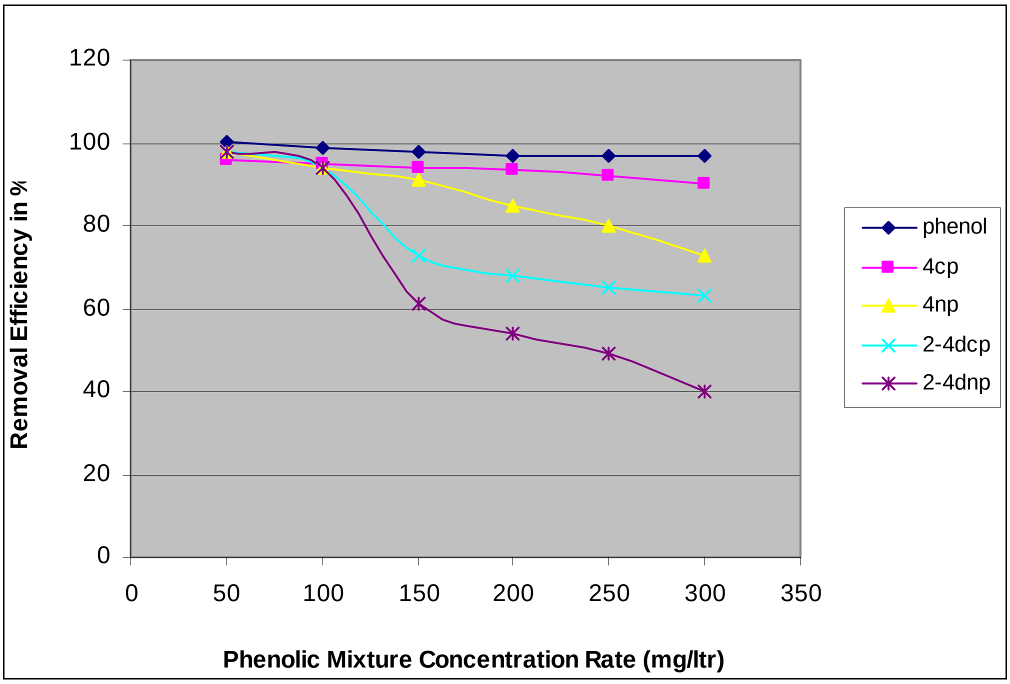

Figure 6. Phenolic compounds removal order

3.1 Hydraulic retention time (HRT)

After acclimation of sludge, study of HRT was carried out to know the effect of degradation of phenolic compounds. The performance of the activated sludge reactor at different HRTs are given in the Table 3. The reactor performance is unstable initially between 1-6 days and later gradually stabilized when changed over to new HRT. The reactor was operated at steady state for 30 days at each HRT . The COD percentage decreased with decrease in HRT. The COD removal percentage was decreased to 93% from 98% with lowering of HRT from 3.5 to 1.0. This may be due to inhibitory effect of the higher concentrations of phenolic compounds to the efficient treatment. The reactor feeding with phenol, 2,4DCP, 2-4DNP, 4CP and 4NPs the removal efficiencies are at the range of 90-99% and there is no effect on percentage compound removal with lowering of HRT from 3.5 to 1.0. The complete dechlorination of 4CP and 2-4DCP resulted in the range of 7-10 mg/L.

Table 3. Percentage removal for phenolic mixture at various HRTs

HRT (days)	COD Removal %	Phenolic Mixture Concentration (mg/ltr)		Chlorine release efficiency (mg/ltr)
		Inffluent	Effluent	
3.0	98	300	2.0	8.6
2.5	97.3	300	2.1	7.5
2	94.5	303	2.0	6.6
1.5	94	301	2.4	8.4
1.0	93	300	2.5	9.2

3.2 Operational parameters

Sludge volume index is one of the important parameter to asses the stability of the sludge in suspended growth systems like ASP with increase in pollutant loading rate, SV decreased. This may be attributed to the fact that increase in pollutant loading rate increased the toxic load in the effluent, there by causing inhibition to the native biomass growth and its metabolic activities. The SS was in the range of 2500-3000mg/L. With increasing the loading rates the MLSS decreased rapidly indicating the toxic nature of the influent feed. The F/M ratio was in the range of 0.2 to 0.4 which is optimum for good systems. However the F/M ratio was quite high after increasing the loading rate which indicates the inhibition due to toxicity.

3.3 Oxidation reduction potential

The oxidation and reduction potential system depends on the activity of the microbial consortia. It is useful in the evaluation of magnitude, characteristics and process change. When the ORP is in between +400mv and +20mv aerobic system prevails. In the present study ORP was continuously monitored and observed to be between 50mv-50mv throughout the study, confirming the optimum aerobic conditions prevailing in the reactors.

3.4 Effect of pH

Biological systems are dependent on the pH change, which are bought about by the oxidation and reduction of various substrates in the process. Activities of microorganisms are more in a specific pH value. Aerobic systems work in pH range of 6.5-8.5. The pH of the effluent from the process was continuously monitored throughout the study. It was observed to be between 8.0-8.25.

3.5 Toxicity test

The high average levels of toxicity are expressed by EC_{50} h of 0.7% v/v were found in the feeding of the activated sludge reactor. Due to high percentages of removal of mixed phenolic compounds, the samples taken from the effluent does not contain any toxicity. The absence of toxicity may be explained by the presence of heterogeneity of the organisms such as algae, rotifers and ciliates. These results are significant because according to Argentine National Act on hazardous Wastes (Act 24051/92) and its regulating Decree, (Decree 830/93) toxicity is an essential characteristic which identifies hazardous waste. So these findings may help in the treatment of industrial effluents and bioremediation of environments polluted by phenolic compounds. These values are similar to that of A.Gallego et al.,2003 findings for the treatment of phenolic compounds [20].

3.6 Effect of HRT on the aerobic granules

Differences in the characteristics of aerobic granules could be observed at different HRTs. Bioflocs with poor settleability were the major form of biomass at the HRT of 24 h. The biomass size varied with different HRTs. The mean diameters were 3.5, 1.2, 1.1 and 0.7 mm for HRTs of 3.0, 2.5, 2.0 and 1 d respectively. The long HRT encouraged the growth of suspended solids because of the low frequency of volumetric exchange. As a result, reactor biomass was eventually dominated by tiny bioflocs. HRTs of 2–2.5d resulted in stable aerobic granules. This optimum range of HRTs was found to be short enough to suppress the growth of suspended sludge in favour of granular sludge, and long enough for the reactor to avoid the extreme sludge loss as a result of hydraulic washout. It is clear from the results in this study that an optimum range of hydraulic selection pressures is required for the formation and retention of aerobic granules in the reactor.

3.7 Microbial analysis

The result of microbial analysis of the sludge total count (mean of 5 values) of the activated sludge at 4 different HRTs are presented in Tables 4 & 5. Quantitative analysis of microbial isolates yielded 10 ten major isolates. The isolates were mostly gram negative in nature with positive motility. The total plate count analysis carried out during the study revealed that highest cell density was observed at 3.0 HRT indicating the reduced microbial activity at higher concentrations. Further it was also observed that the counts on the nutrient agar (bacterial) was more than those of PDA (fungal) indicating good sludge characteristics for effective settleability.

Table 4. Quantitative analysis of the microbial isolates from activated sludge

S.no	Gram characteristics	Morphorlogical characteristics on nutrient agar	Motility	Type of growth on nutrient agar
1.	Negative	Rods, colony appears to be flat with entire margin were observed.	Positive	Uniform growth
2.	Negative	Rods in clusters and single,punctiform colonies with entire margin were observed.	Positive	Uniform growth
3.	Negative	Rods in clusters and single convex,irregular colonies with entire margin were observed.	Negative	Pellicle formation
4.	Negative	Rods in single and in pairs, convex, round white colored colonies with an entire margin.	Positive	Pellicle formation

Table 5. Total plate count (CFU/ml) of the activated sludge

S.no	HRT	dilution	Cell count on NA		Cell count on PDA	
			X2	S.D	X2	S.D
1.	Raw effluent	Undiluted	2	± 0.76	Nil	
2.	3.0	10	140	± 13.75	40	± 17.75
3.	2.5	10	116	± 20.8	27	± 25.40
4.	2	10	98	± 15.68	36	± 23.60
5.	1.5	10	76	± 28.47	24	± 20.85
6.	1.0	10	84	± 23.4	41	± 30.0

3.8 Cost estimation

Cost estimation was done for the present activated sludge reactor for treating the mixed phenolic compounds. The overall costs are represented by the sum of capital costs, the operating and maintenance costs. For the full scale system these costs strongly depend on the nature and the concentrations of the pollutants, the flow rate of the effluent and configuration of the reactor. An estimation costs has been made in this section regarding the operation costs for the treatment processes used for the degradation of phenol,2-4DCP, 2,4DNP,4CP and 4NP for 180days. The total estimated cost is 85.6$ for the degradation of phenolic compounds Table 6.

Table 6. Operational cost for the ASP reactor

S.No	Requirement	Cost US $
1	Electricity	13.5$
2	Phenols	0.1$
3	Nutrients	72.0$
4	Total Cost	85.6$

4. Conclusions

Based on the present study the following conclusions are obtained

1. The result of study showed that all five Phenolic compounds from synthetic wastewater could be treated effectively by activated sludge processes at different HRT s varying between 3.0, 2.5, 2.0, 1.5, and 1d.
2. The degradation order of phenol > 4CP > 4NP > 2,4DCP > 2,4 DNP was observed during the study.
3. The operational parameters SVI and MLSS decreased due to the toxicity of pollutant loading rate.
4. The total estimated cost for the degradation 300mg/L of phenolic compounds was found to be appx. 85.6$.
5. The highest percentage COD removal and percentage compound reduction of 98% and 99.3% of phenol was observed at 3.0 d HRT respectively.

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