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*Original Research Article*

# Degradation of Phenol in Nutrient Broth by Pseudomonas and *Bacillus* species using Biosimulator

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## ABSTRACT

**Phenol is a cognizant industrial contaminant in wastewaters from various industries, in this bioremedial study three isolated cultures *IES.S, IES.Ps* and *IES.B* separately and all in consortia (10%) from industrial wastes were grown in 1500 ml nutrient broth in sterile condition with 600 mg/l Phenol in the Biotron fermenter vessel, with specific conditions of rpm, DO. pH, temperature, COD and phenol was determined at specific interval of time. The results of this study showed that the pH of the system mostly remained acidic. The DO concentration was sustained at 5.0 mg/l. Phenol removal by *IES.S* was 15.50% (42 hours) and 18.83% (90 hours). Phenol removal percentage was much higher with *IES.Ps* as compared to that of *IES.S*. After 42 hours the phenol percentage removal efficiency was 32.10%. While at the end of the experimental run about 40% phenol was removed. While using *IES.B* Phenol removal efficiency was 32.16 (42 hours) and 39.53 % (90 hours). Phenol removal efficiency was relatively higher than *IES.S* and relatively lower than *IES.Ps*. Phenol removal was 31.57% (42 hours) and 39.38 % (90 hours) by using consortia of all three cultures. The degradation rates by using three different isolates were in descending order Consortium>*IES.Ps*>*IES.B*>*IES.S*.**

**Keywords: Biological treatment, Phenol removal, Industrial contaminant, Consortium, Bioremedial study, Removal efficiency**

## INTRODUCTION

Studies on phenol biodegradation have been conducted since several years by using activated sludge systems. However, the higher and variable phenol concentrations have indicated to cause the collapse of the system (Watanabe *et al.* 1996, 1999; Kibret *et al.* 2000).

Phenolic wastewater is hard to treat due to substrate inhibition. While bacterial growth and degradation of phenol are hampered by the toxic effect of augmented concentrations of the substrate itself and generally this is because of damage of cytoplasmic membrane (Keweloh *et al.* 1989). Phenol biodegradation is thus, a crucial criterion if varied pollutants have to be treated (Jiang, *et al.* 2002). Biological treatment technologies are likely process for phenol degradation from wastewater because of its economical and prevent formation of secondary contaminants, in comparison to physicochemical methods (Jiang *et al.* 2010, Husain *et al.* 2015). Physical and chemical elimination methods produce these noxious byproducts that enter the aquatic bodies causing aquatic Eco toxicity.

The potential of microorganisms to endure and degrade high phenolic concentrations is thus needed in biological treatment of wastewater plants as it can expedite the treatment of highly polluted industrial discharges. In this study, aerobically grown bacterial cultures were effectively utilized in a biosimulator for phenol degradation.

According to the Sind Environmental Protection Act 2014, industries releasing toxic discharges including priority pollutants like phenol and phenolic compounds should be controlled and managed before final disposal. Hence, in the present situation the effective attainment of such system would be accommodating for the industries that are discharging their waste containing phenol and intend to develop such treatment process that are efficient and economically achievable.

The current study was steered to assess the continuous biodegradation of phenol using all three isolates *IES.Ps, IES.S, IES.B* individually and then in consortium. These experimental studies were conducted in sterile condition. In that 1500 ml, nutrient broth (NB) (Merck) was taken in the fermenter vessel (Biosimulator, Model Bio-tron). It was autoclaved supplemented with 600 mg/l phenol and 10 % of 24 hours grown culture was inoculated. Two samples were drawn in a day while rpm was adjusted at 250.

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In the current scenario of liquid waste treatment, the main target of the procedure is to reduce chemical oxygen demand (COD), which is the amount of oxygen essential for the chemical oxidation of the pollutants in wastewater (Nair 2006).

## MATERIALS AND METHODS

This study was designed to work on the performance of biosimulator viz a viz three isolated bacterial strains using nutrient broth supplemented with phenol.

### Design and Operation of Biosimulator

The bench scale biosimulator (Model Bio-tron) consists of stainless steel reactor with a thick glass jar of borosilicate glass. It is fully equipped for observing and regulating temperature, rate of agitation and aeration. The sample can be strongly agitated by impeller with flat stirring paddles and by four vertical baffles. The sample treatment was done with preset DO concentration. Agitation was observed on a calibrated electrical tachometer, which gave a specific speed sign.

1500 ml of nutrient broth (Merck Germany) was taken in a biosimulator glass vessel, wrapped in a brown paper bag and autoclaved at $121^0$C, 15 psi for 20 minutes. 600 mg/l phenol and 24 hours grown respective culture (10%) was inoculated in the sterilized vessel and fermenter was turned on, with different conditions of rpm, DO. The DO maintained at 5.0 mg/l.

Temperature was checked using mercury thermometer by submerging it into sample. The thermometer was marked with Celcius grade, its calibration was performed periodically when used.

The pH of the sample was noted by using HACH Session 156 Multiparameter pH probe. The probe was immersed in the medium and the reading was noted after one minute of constant reading. DO was determined by using HACH Session 156 Multiparameter dissolved oxygen meter. COD was determined by dichromate reflux method using HACH COD reactor as per APHA (2005). Ammonia was estimated by nesslerization method (APHA 2005). Nitrate was also determined by the method depicted in APHA (2005).

Phenol was determined by using HPLC (Shimadzu) using a Novapak C18 column (250 mm X 4.6 mm, 5 mm particle size). 100% methanol was used for the mobile phase. An injection volume was 20 ml. The flow rate was 0.8 ml/min and detection at 254 nm was with a UV-Vis absorbance detector. The sample was centrifuged (Micro TTR) for 10 minutes at 5◦C before injection.

### Culture used

The bacterial cultures utilized in the present study were isolated from the activated sludge system of Pakistan Refinery Limited. Culture *IES.Ps* used in the study that was already present in the Institute of Environmental Studies, University of Karachi. It is a leading degrader as it has successfully degraded malathion (organophosphate pesticide) and cypermethrin (carbamate pesticide) in other research studies conducted in the Institute as mentioned in the previous research work (Hasan and Jabeen 2015, Sirajuddin *et al.* 2010 and Jabeen *et al.* 2013).

## RESULTS AND DISCUSSION

### Degradation of Phenol in Biosimulator containing Nutrient Broth (NB) using *IES.S*

Isolated culture of *IES.S* was grown in biosimulator containing 1500 ml nutrient broth in sterile condition along with 600 mg/l Phenol. The results of the studies were shown in Table 1. The pH of the system swayed in a range between 6.9 to 8.07. From the Table 1 it can be seen that the pH of the medium was near the alkaline side. This was likely due to the release of ammonia and ammonium in the system. The pH results support the findings of Hashmi (2007) who also reported alkaline pH of the system. The DO concentration was sustained at 5.0 mg/l. The change in the DO concentration was negligible.

**Table 1.** Performance efficiency of biosimulator containing nutrient broth supplemented with 600 mg/l phenol and 24 hours grown culture of *IES.S*.

| Serial No. | Time (hours) | Temp (C) | pH | DO (mg/l) | COD (mg/l) | COD removal percentage | Phenol concentration mg/l | Phenol removal percentage |
|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 32.5 | 6.9 | 4.92 | 3,000 | - | 599 | |
| 2 | 6 | 34.5 | 6.8 | 5.01 | 2600 | 13.33 | | |
| 3 | 18 | 34.5 | 8.07 | 5.02 | 2070 | 31.00 | | |
| 4 | 24 | 34 | 8.07 | 4.91 | 1980 | 34.00 | | |
| 5 | 42 | 34 | 8.07 | 5.01 | 1500 | 50.00 | 507 | 15.50 |
| 6 | 48 | 34 | 8.07 | 4.95 | 1160 | 61.33 | | |
| 7 | 66 | 34.5 | 8.27 | 5.02 | 970 | 67.66 | | |
| 8 | 72 | 34 | 8.27 | 4.96 | 580 | 80.66 | | |
| 9 | 90 | 34 | 8.07 | 5.01 | 460 | 84.66 | 487 | 18.83 |

COD was progressively removed from initial hours till the end of the experiment. The percentage removal efficiency of COD was from 13.33(6 hours) to 84.66 (90 hours). The COD removal was in direct relationship with retention time.

The percentage removal efficiency with regards to phenol was 15.50 (42 hours) and 18.83 (90 hours). This would mean that the culture shown its preference towards the nutrient broth rather the phenol itself. It was due to the fact that organism at first utilized the readily available carbon source. However, the culture showed its acclimatization in phenol containing nutrient broth (Fig. 1 - 3).

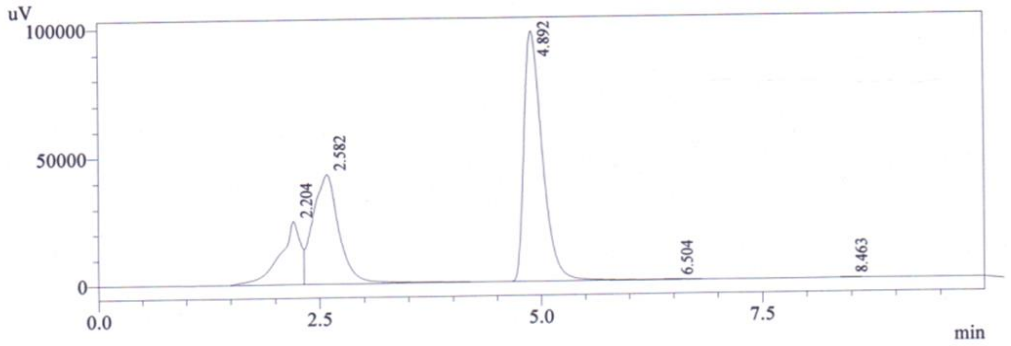


**Figure 1.** Phenol degradation by *IES.S* in NB at 0 hour.

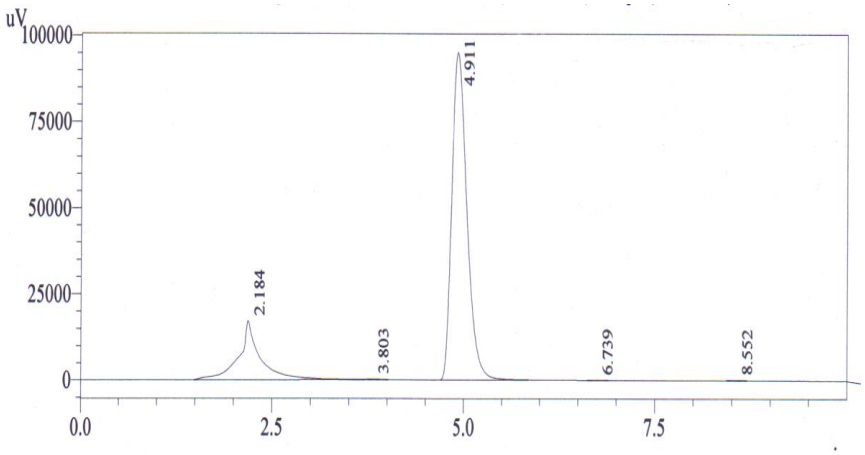


**Figure 2.** Phenol degradation by *IES.S* in NB at 42 hour.

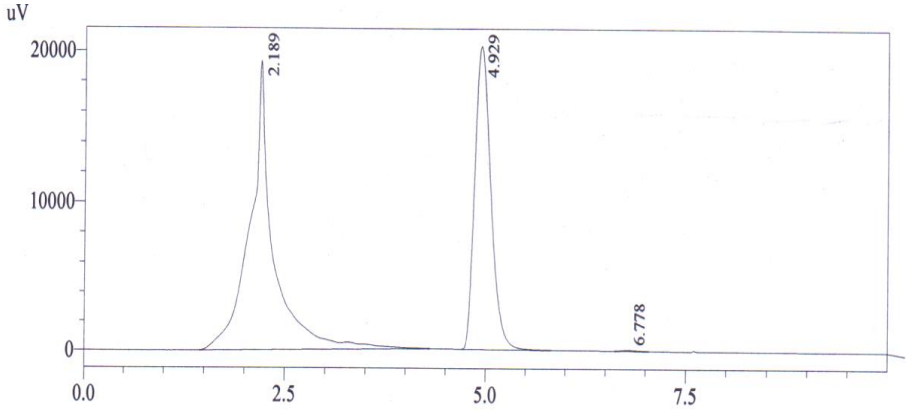


**Figure 3.** Phenol degradation by IES.S in NB at 90 hour.

**Degradation of Phenol in Biosimulator containing Nutrient Broth using *IES.Ps***

The results of the study are shown in Table 2 and Fig. 4 to 6. The trend of alteration in the pH with *IES.Ps* was analogous to that of *IES.S*. The pH of the medium was in the range of 6.86 to 7.27. However, the pH was not stimulated much towards the alkaline side as was detected in case of *IES. S*. From the Table 2, it can be seen that the pH was neutral at the end of the experiment. The DO concentration was kept at 5.0 mg/l.

**Table 2.** Performance efficiency of biosimulator containing nutrient broth supplemented with 600 mg/l phenol and 24 hours grown culture of *IES.Ps*.

| Serial No. | Time (hours) | Temp (C) | pH | DO (mg/l) | COD (mg/l) | COD removal percentage | Phenol concentration mg/l | Phenol removal percentage |
|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 32.0 | 6.86 | 4.95 | 2570 | - | 598 | - |
| 2 | 6 | 32.5 | 6.76 | 5.0 | 2440 | 5.0 | | |
| 3 | 18 | 32.5 | 6.77 | 5.01 | 2260 | 12.06 | | |
| 4 | 24 | 32 | 6.75 | 4.96 | 1990 | 22.56 | | |
| 5 | 42 | 33 | 7.07 | 5.01 | 1640 | 36.18 | 406 | 32.10 |
| 6 | 48 | 33 | 7.07 | 4.98 | 1180 | 54.08 | | |
| 7 | 66 | 33.5 | 7.27 | 5.0 | 980 | 61.86 | | |
| 8 | 72 | 33 | 7.07 | 4.97 | 550 | 78.59 | | |
| 9 | 90 | 32 | 7.0 | 5.02 | 430 | 83.26 | 359 | 39.96 |

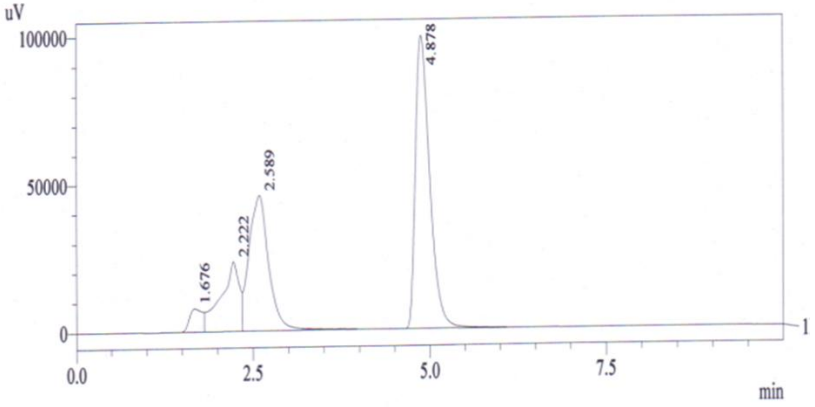


**Figure 4.** Phenol degradation by *IES.Ps* in NB at 0 hour.

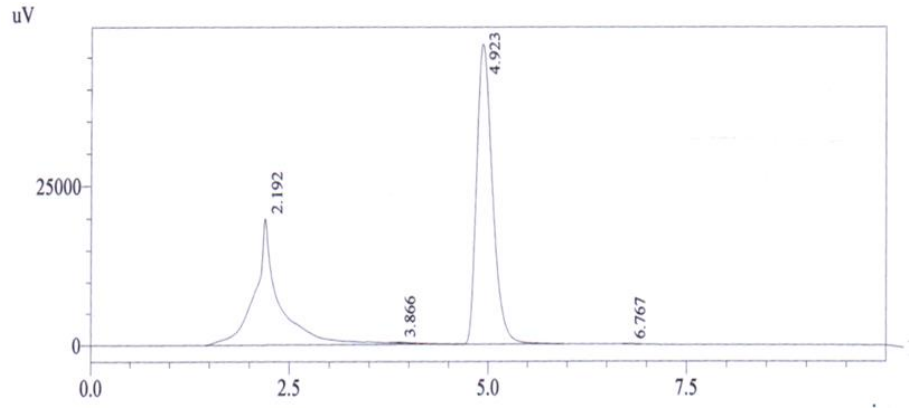


**Figure 5.** Phenol degradation by *IES.Ps* in NB at 42 hour.

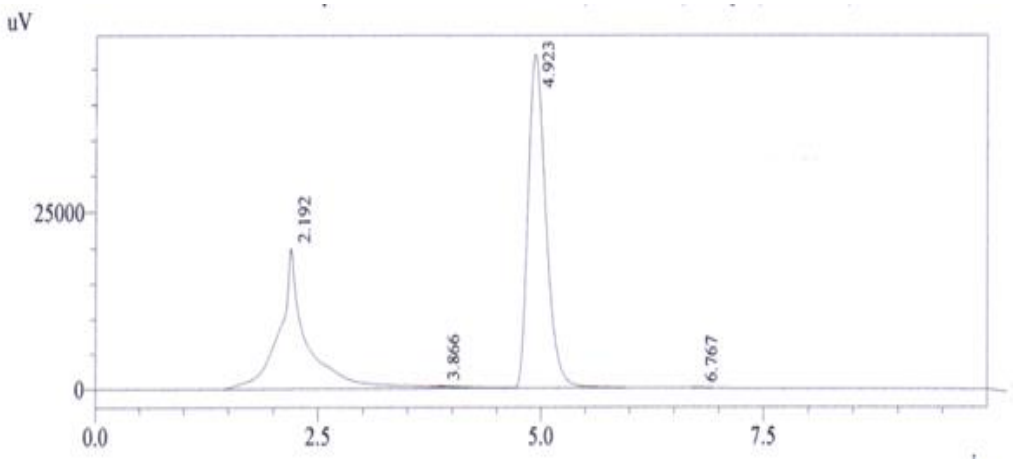


**Figure 6.** Phenol degradation by IES.Ps in NB at 90 hour.

COD removal efficiency was from 5.0% (6 hours) to 83.26 % (90 hours) that seems to be little lesser as compared to that of *IES.S*. This would confirm the organism adaptation in phenol containing nutrient broth.

Phenol removal percentage was much higher with *IES.Ps* as compared to that of *IES.S*. After 42 hours the phenol percentage removal efficiency was 32.10%. While at the end of the experimental run about 40% phenol was removed. This could be possibly due to the fact that nutrients in the nutrient broth was the first choice of the culture and during such period the organism adapted itself to the phenol containing medium and once the phase of the adaption was over *IES.Ps* speedily utilizing phenol.

**Degradation of Phenol in Biosimulator containing Nutrient Broth using *IES.B***

The results of this study were given in Table 3 and Fig. 7 to 9. *IES.B* growing in nutrient broth with 600 mg/l phenol presented alkaline pH values after 18 hours. pH of the system fluctuated between 6.47 to 8.47. Change in the pH depicted the rapid conversion of nutrients in to ammonium ions. The DO concentration was maintained at 5.0 mg/l.

**Table 3.** Performance efficiency of biosimulator containing nutrient broth supplemented with 600 mg/l phenol and 24 hours grown culture of *IES.B*.

| Serial No. | Time (hours) | Temp (C) | pH | DO (mg/l) | COD (mg/l) | COD removal percentage | Phenol concentration (mg/l) | Phenol removal percentage |
|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 32 | 6.47 | 5.02 | 3798 | - | 597 | - |
| 2 | 6 | 35 | 6.67 | 5.01 | 3560 | 6.26 | | |
| 3 | 18 | 35 | 8.57 | 5.01 | 3321 | 12.55 | | |
| 4 | 24 | 34 | 8.67 | 4.96 | 2654 | 30.12 | | |
| 5 | 42 | 34.5 | 8.37 | 4.97 | 2312 | 39.12 | 405 | 32.16 |
| 6 | 48 | 33 | 8.57 | 5.01 | 1987 | 47.68 | | |
| 7 | 66 | 33 | 8.37 | 4.98 | 1823 | 52.0 | | |
| 8 | 72 | 32.5 | 8.37 | 5.02 | 1772 | 53.34 | | |
| 9 | 90 | 33 | 8.47 | 4.95 | 981 | 74.17 | 361 | 39.53 |

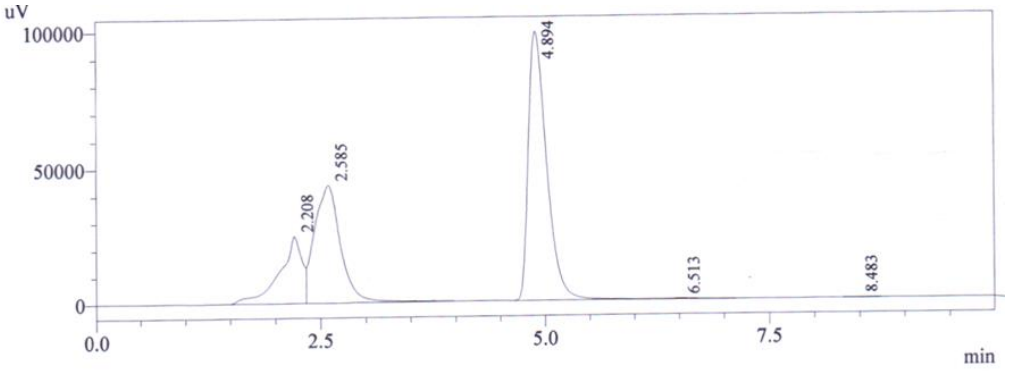


**Figure 7.** Phenol degradation by *IES.B* in NB at 0 hour.

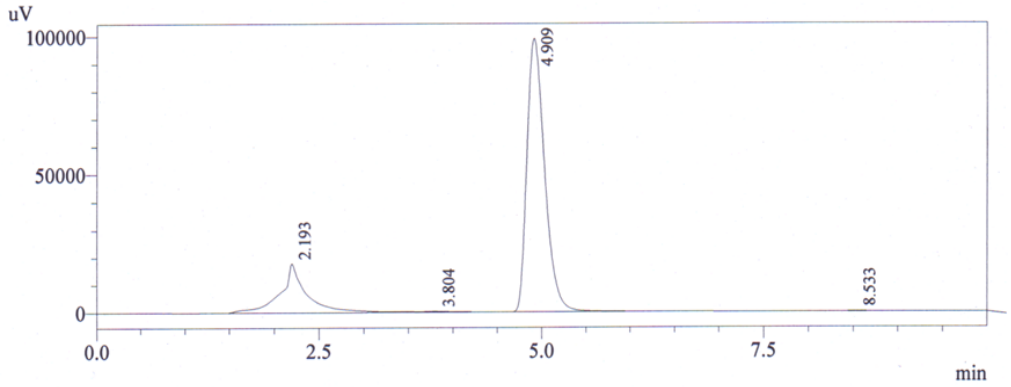


**Figure 8.** Phenol degradation by *IES.B* in NB at 42 hour.

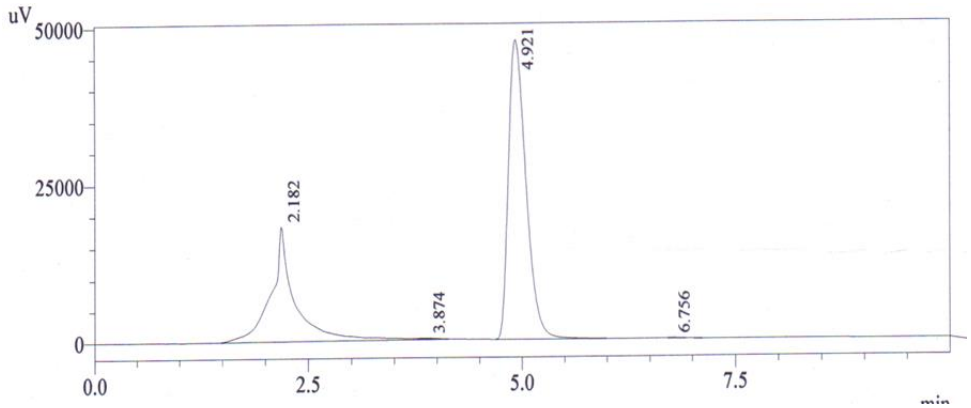


**Figure 9.** Phenol degradation by *IES.B* in NB at 90 hour.

With *IES.B* the COD removal percentage was little less as compared to that *IES.S* and *IES.Ps*. COD removal was in the range between 6.26 to 74.17 %. Phenol removal efficacy was 32.16 (42 hours) and 39.53 % (90 hours). Phenol removal efficacy was comparatively higher than *IES.S* and relatively lower than *IES.Ps*.

**Degradation of Phenol in Biosimulator containing Nutrient Broth using bacterial consortia of *IES.Ps, IES.S* and *IES.B***

In this experiment *IES.S, IES.Ps* and *IES.B* were grown in 1500 ml nutrient broth in sterile condition with 600 mg/l Phenol. The results of this study are shown in Table 4 and Fig. 10 - 12. Interestingly, it can be seen that in this experimental run, pH of the system mostly remained acidic. However, at 66 hours pH was 7.27. Acidic pH represents the carbon di oxide production and carbonic acid formation. Phenol biodegradation in an aqueous solution significantly depends on pH of the medium, which affects the degree of ionization and surface charge of the absorbent (Annadurai *et al.* 2000). Analogous results were accomplished by Karigar *et al.* (2006) for *Arthrobacter citreus*. The DO concentration was constant at 5.0 mg/l.

**Table 4.** Performance efficiency of biosimulator containing nutrient broth supplemented with 600 mg/l phenol and 24 hours grown culture of *IES.Ps, IES.S, IES.B*.

| Serial No. | Time (hours) | Temp (C) | pH | DO (mg/l) | COD (mg/l) | COD removal percentage | Phenol concentration mg/l | Phenol removal percentage |
|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 32 | 6.89 | 4.95 | 3234 | - | 589 | - |
| 2 | 6 | 32 | 6.77 | 5.01 | 2780 | 14.03 | | |
| 3 | 18 | 33 | 6.75 | 5.03 | 2270 | 29.80 | | |
| 4 | 24 | 33.5 | 6.05 | 4.98 | 1987 | 38.55 | | |
| 5 | 42 | 33 | 6.07 | 5.02 | 1554 | 51.94 | 403 | 31.57 |
| 6 | 48 | 34 | 6.08 | 4.99 | 1160 | 64.13 | | |
| 7 | 66 | 33.5 | 7.27 | 5.01 | 930 | 71.24 | | |
| 8 | 72 | 34 | 6.37 | 4.97 | 540 | 83.30 | | |
| 9 | 90 | 35 | 6.27 | 5.10 | 370 | 88.55 | 357 | 39.38 |

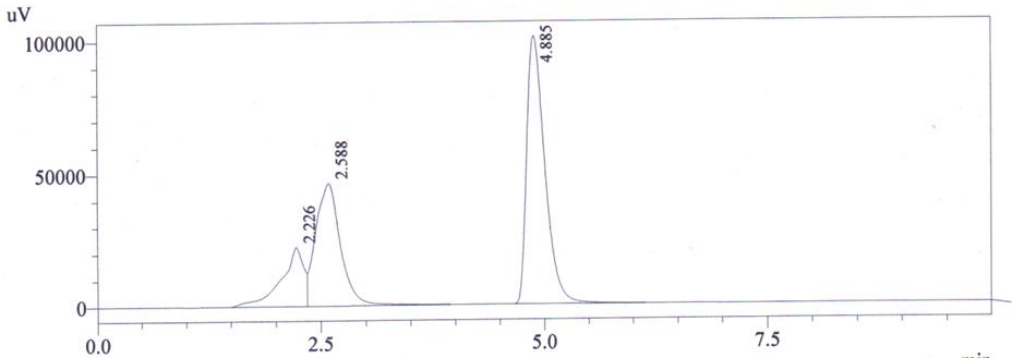


**Figure 10.** Phenol degradation by *IES.Ps, IES.S* and *IES.B* in NB at 0 hour.

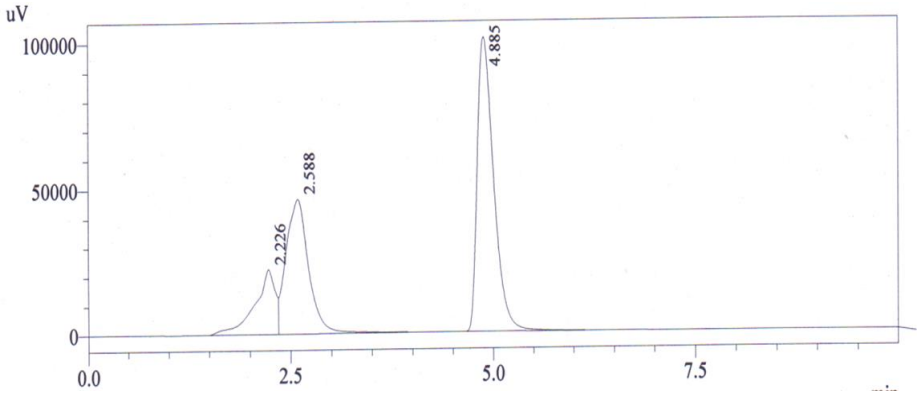


**Figure 11.** Phenol degradation by *IES.Ps, IES.S* and *IES.B* in NB at 42 hour.

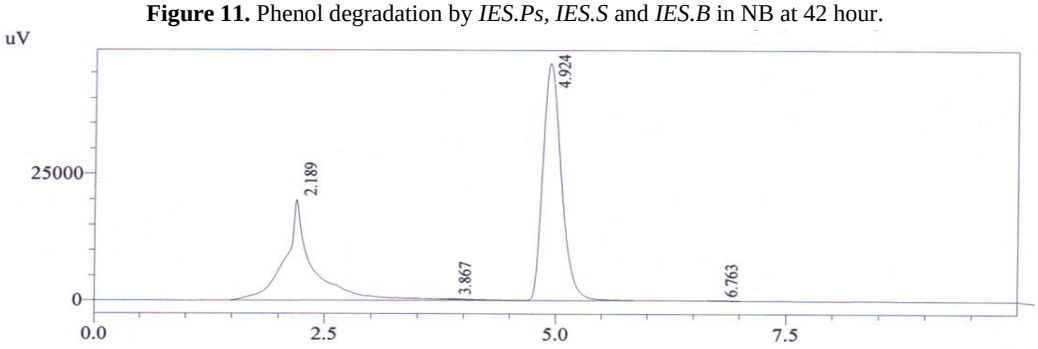


**Figure 12.** Phenol degradation by *IES.Ps, IES.S* and *IES.B* in NB at 90 hour.

It was found that COD removal percentage was maximum and reached to 88.55%. COD removal percentage ranged between 14.03 to 88.55%. According to Toprak (1995) COD removal was not only a function of the hydraulic retention time and allied organic loading rate but also subjected to the influent COD concentration and temperature. Ahmed *et al.* (1988) also reported that by increasing retention time the efficiency of COD removal also improved. The log phase of bacterial density appears to be proportional to the COD removal. Enhanced COD removal efficiency can be observed when the cells are growing in the log phase. Barker and Dold (1995) observed worthy COD and nitrogen equilibriums on numerous types of laboratory scale activated sludge system.

No significant change in phenol removal was noticed with respect to studies conducted with the individual cultures. Phenol removal was 31.57% (42 hours) and 39.38 % (90 hours). The decline in phenol concentration was observed to be linear with passage of time, indicating a constant biodegradation rate that was contingent profoundly on temperature. In the present study the temperature range was 32 to 35 ºC. As reported by El Naas *et al.* (2009), the optimized temperature for phenol degradation by *P. putida* was found about 30 ◦C. Higher temperatures negatively influence the bacterial activity and therefore obstruct its biodegradation abilities. It was seen in the previous studies that sudden rise in temperatures higher than 35 ◦C can detrimentally distress the bacterial enzymes which help in cleavage of the benzene ring that is the main phase in the biodegradation. While with lower temperatures exposure the bacterial activity becomes slow. Moreover, phenol toxicity also sinks the bacterial functions at low temperatures (Rozich and Colvin 1986). This is entirely defined for high phenol concentrations. The literature reports that the degradation rate can be sensitive because of any deviation external to the optimum temperature range (Mordocco and Jenkins 1999), and this happens because of the higher production of metabolites at 30 ◦C (Sá,C.S.,and Boaventura, 2001). The decrease in phenol concentration is almost linear with time. The consortium of bacterial culture altogether degraded phenol up to 40.5% in comparison with all other three cultures individually.

In the past studies, research work has been done using single bacterial strain for biodegradation of phenol which restricts its uses as diverse nature of noxious waste might be exist in effluents (Guido *et al.* 2008). Phenolic biological elimination is problematic at small concentration (less than 200 mg/l), and at quite high

phenol concentration, as it impedes growth rate of the microbes (Marrot *et al.* 2006). The toxic contaminants such as phenol may cause the deflocculation, which triggers in settling complications in clarifier.

For adequate phenol degradation efficiency, the phenol concentration has to be sustained less than the threshold limits and adapted microorganisms should be introduced to the toxic wastewater. Lawrence *et al.* (2009) stated that adapted activated biomass reduces phenolic substances more commendable than the single microbial strain more rapidly twice or thrice. Activated sludge has been effectively employed for the biodegradation of phenol in the batch reactor upto 1500mg/l at a pH 6 (Duan 2011). Moving bed bio-film reactor has been utilized for treating phenolic wastewater with high Total Dissolved Solids. The COD elimination was 97.44% at hydraulic retention time (HRT) of 44 hours and initial phenol concentration of 1400 mg/l (Galgale *et al.* 2014). Phenol and COD reduction efficacy was reported more than 99% at initial phenol concentration of 800mg/l and Hydraulic Retention Time of 24hours (Nakhil *et al.* 2014). Highest elimination rate of 2.92 g phenol/l/ d at a hydraulic retention time (HRT) of 0.95 days and a total organic loading rate of 15.3 g COD/ (/L/ d) with a phenol concentration of 4.9 g/L has been noticed using fixed bed bio film reactor (Bajaj *et al.* 2008). Effective reduction of COD and Phenol removal was described at organic and phenol loading rate of 5 kg COD $m^3$ /d and 400–1200 mg phenol/L wastewater by exhausted granular sludge bed anaerobic filter bioreactor (Collins *et al.* 2005). According to Firozjaee *et al.* (2013) bioremedial treatment has been stated to successfully reduce phenol up to the concentration of 420mg/L.

## CONCLUSIONS

All three bacterial strains were grown in nutrient broth supplemented with 600 mg/l phenol separately and then in consortium in fermentor vessel, showing degradation rates in descending order Consortium>*IES.Ps*>*IES.B*>*IES.S*. This reveals that consortium can work better than individual cultures in terms of phenol removal.

## ACKNOWLEDGEMENTS

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