

Pentachlorophenol Removal via Adsorption and Biodegradation

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Abstract—Removal of PCP by a system combining biodegradation by biofilm and adsorption was investigated here. Three studies were conducted employing batch tests, sequencing batch reactor (SBR) and continuous biofilm activated carbon column reactor (BACCOR). The combination of biofilm-GAC batch process removed about 30% more PCP than GAC adsorption alone. For the SBR processes, both the suspended and attached biomass could remove more than 90% of the PCP after acclimatisation. BACCOR was able to remove more than 98% of PCP-Na at concentrations ranging from 10 to 100 mg/L, at empty bed contact time (EBCT) ranging from 0.75 to 4 hours. Pure and mixed cultures from BACCOR were tested for use of PCP as sole carbon and energy source under aerobic conditions. The isolates were able to degrade up to 42% of PCP under aerobic conditions in pure cultures. However, mixed cultures were found able to degrade more than 99% PCP indicating interdependence of species.

Keywords—Adsorption, biodegradation, identification, isolated bacteria, pentachlorophenol.

I. INTRODUCTION

BIOFILM systems are increasingly found to be more efficient in removing trace toxic compounds in wastewater. Besides being less sensitive to the presence of toxic and inhibitory than dispersed growth systems, and therefore are more resistant to shock loading. These advantages render biofilm systems more suitable for use in treatment of industrial wastewaters containing adsorbable organohalides (AOX). In this study pentachlorophenol was chosen as a representative AOX compound because it is quite commonly found in industrial effluents, especially those from pulp and paper mills and wood preservation industries. Many chlorophenols have recently been shown to be anaerobically and aerobically biodegradable [6], [14], [3], [12], [9]. It has been shown that some gram negative bacteria, especially facultative bacteria, may be particularly important in dechlorination reaction. Reductive dechlorination, or direct removal of chlorine atom from the aromatic ring as a first step is a significant process, because the dechlorinated products are usually less toxic and are more readily degraded either anaerobically or aerobically [8].

Granular activated carbon (GAC) is a good bacterial immobilization matrix as it is very adsorptive and has a very high surface-to-volume ratio, due to its large number of

internal pores and rough surface texture. The biofilm on activated carbon performs a combination of physical and biological removal mechanisms; adsorption onto GAC and biological degradation by microorganisms grown on GAC. The adsorptive function of GAC contributes towards the reduction of the aqueous phase concentration of the inhibitory organic compound to below threshold level under which the inhibitory effect on the biological degradation decreases [15].

Removal of PCP via biofilm and adsorption was studied here. PCP removal studies were conducted using sequencing batch reactor (SBR) and continuous biofilm GAC column reactor (BACCOR). Isolates were characterised and biodegradation studies were carried out using pure and mixed cultures.

II. METHODOLOGY

A. Batch Tests

Batch tests were carried out in the 500ml glass flasks. Each flask contained 100 ml nutrient solution with 100mg/L of PCP as sole carbon source. Weighted GAC (0.01-0.1g) were kept in a mixed culture reactor to form biofilm on activated carbon (BAC). After 2 days, BAC were filtered out with the filter papers and placed inside the glass flasks with nutrient solution. The flasks were shaken in an incubator shaker at 25°C and 120rpm. The PCP concentrations were monitored at interval times till equilibrium (around 10 days) was reached. Removal of the PCP by the BAC was then compared with that from the GAC adsorption studies (without the biofilm).

B. SBR Study

Two 1L sequencing batch reactors (SBRs) were set up to investigate the removal efficiency of chlorophenol by biodegradation (suspended growth system) and biodegradation-adsorption (attached growth system). The sludge originated from a pulp and paper mill wastewater treatment plant was transferred to the SBRs. These SBRs were fed with simulated wastewater contained PCP 100mg/L and nutrient salt. In the attached growth SBR, 200g/L of 2-3mm granular activated carbon (GAC) was used as a medium for attached growth of bacteria. Both reactors were operated at a hydraulic retention time (HRT) of 3 days for 3 month to acclimatise the biomass.

C. Continuous Biofilm on GAC Column Reactor (BACCOR)

BACCOR was a GAC column of Plexi-Glass (1m height, 5.5cm internal diameter) and packed with 60 cm height of coconut-shell based 0.5-1.0 mm sized GAC (360g/L). Operating conditions for BACCOR are summarised in Table I. Concentrations of PCP-Na ranged from 10 and 100 mg/L and EBCT ranged from 45 minutes to 4 hours. In Run 1 to Run 4, BACCOR was fed with PCP in tap water, while in

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Run 5 to Run 6; it was fed with PCP in prefiltered river water.

TABLE I
OPERATIONAL CONDITIONS FOR BACCOR

Run number	Run 1	Run2	Run 3	Run 4	Run 5	Run6
Time (days)	1-161	162-204	205-265	266-389	390-428	429-501
EBCT (hour)	2	4	0.75	2	2	1
Influent PCP-Na (mg/L)	10-100	20-100	10-25	10-30	10	10
Background water	Tap water	Tap water	Tap water	Tap water	River water	River water

D. Isolation and Identification of Microorganisms from BACCOR

Screening of PCP-biodegradative Strains and acclimatisation

Sludge from the BACCOR fed with PCP-Na (10mg/L) was transferred to a 1 L batch reactor. The batch reactor was fed with PCP-Na (10mg/L) as carbon source (Table I). The acclimatisation was continued for 3 months. Performance analyses were not made routinely during acclimatisation but occasionally PCP concentrations were determined using UV-VIS spectrophotometer.

Media

Isolation agar and nutrient agar: Two types of nutrient medium were used in this experiment. Isolation agar with 10mg/L PCP as the sole carbon source and nutrient agar. The content of isolation agar was as shown in Table II. Nutrient agar was used to sub-cultured the isolated strains. The nutrient agar was prepared according to manufacturer's instructions. The content of growth medium was as shown in Table II. All components, except PCP solution and FeSO₄ solution were diluted in distilled water and made up to 1.0L. The solution was adjusted to pH 7.4 and autoclaved. FeSO₄ and PCP solutions were added into the solution aseptically via syringe with sterile 0.2µm syringe filter. The solutions were mixed thoroughly and distributed aseptically into sterile plates.

Inoculation and Growth medium.

The content of the inoculation medium was shown in Table II but with 3 g/L glucose instead of PCP. The medium was well mixed, pH adjusted to 7.0 and autoclaved. The autoclaved medium was then distributed into sterile flasks. Growth medium was prepared similarly with isolation agar except no bacterial agar was added. The autoclaved medium was distributed into 250 mL flasks stoppered with glass wool.

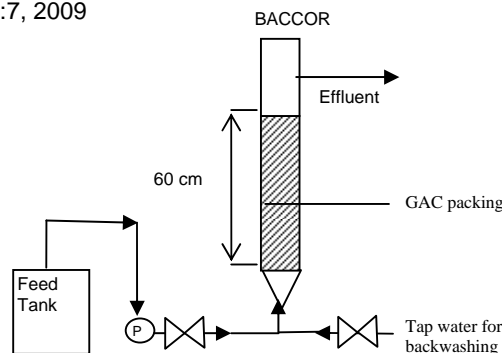


Fig. 1 Schematic diagram of BACCOR

Isolation of PCP-degrading culture from biofilm column reactor

Liquid samples from acclimation batch reactor were spread onto isolation agar plates which containing PCP and nutrient salts. These colonies grown on the agar were subsequently subcultured, resulting in isolated pure culture. Gram staining and API-20E (BioMérieux) was used to identify the bacterial isolates.

TABLE II
GROWTH MEDIUM

Compound	Quantity (mg/L)
KH ₂ PO ₄	1000
Na ₂ HPO ₄ ·12H ₂ O	1250
(NH ₄) ₂ SO ₄	1000
MgSO ₄ ·7H ₂ O	500
CaCl ₂ ·6H ₂ O	50
FeSO ₄ ·7H ₂ O	5
PCP	10
pH	6.8-7.0

TABLE III
CONDITIONS FOR HPLC ANALYSIS

Column	ZORBAX SB-C18, 4.6x150mm (Agilent)
Flowrate	1.000 mL/min
Temperature	35°C
Detection:	UV 254nm
Mobile phase	30% phosphate buffer 70% Acetonitrile

Preparation of inocula and starter culture and confirmation test

To prepare a starter culture, isolated colony from an agar plate (2 days old) was inoculated into inoculation medium and incubated at 28°C, at 120 rpm in a shaker for 24 hours. The cells were harvested by centrifugation (5 min, at 13000 rpm) and the cell pellet was re-suspended in 5 mL potassium phosphate buffer. 1 mL of the cell suspension was added into flasks containing growth medium with 10 mg/L PCP as sole carbon source. Two control flasks (growth medium + 1 mL distilled water) were run simultaneously, incubated at 28°C, and shaken at 120 rpm. At certain time intervals, samples were aseptically withdrawn from the flasks and examined for PCP concentration and viable cells count

using plate count method. All experiments were done in triplicates.

Biodegradation using Mixed Cultures

2 mL of mixed culture from acclimatisation batch reactor was transferred to flasks containing growth medium with 10 mg/L PCP as sole carbon source. The experiment was conducted in triplicates with two controls. At certain time intervals, samples were aseptically withdrawn from flasks and examined for PCP concentration. Biomass was determined at the starting and the end of the experiment.

E. Analysis for PCP

PCP concentrations of influent and effluent were monitored via absorbance at 320nm using UV-Vis Spectrophotometer. The samples from the SBRs were also analyzed using high performance liquid chromatography (HPLC) with a ZORBAX SB-C18 column (4.6X150mm). The compositions of the eluting solvents were acetonitrile (70%) and 0.05M of Potassium phosphate (30%). The conditions for HPLC analysis are shown in Table III. At the end of each contact time interval, 4 mL samples were collected from each flask (with PCP-Na as sole carbon source) and centrifuged at 13000rpm for 5 minutes to separate the biomass. The supernatant was filtered through 0.2µm syringe filter for subsequent PCP-Na analysis. The filtrate obtained was injected into HPLC system. Samples were taken at inlet and outlet of BACCOR at least once a week, and analysed for PCP-Na. Initially (for 3 months), PCP was determined using UV-VIS spectrophotometer at 248nm, after filtration of sample through 0.45 µm membrane. Thereafter, PCP was analysed using 4-aminoantipyrene method with chloroform extraction. This method is more sensitive and the limit of detection is 10 µg/L of phenol.

Biomass determination

Biomass concentration in the BACCOR was measured via NaOH digestion. Samples from mixed culture degradation experiment were taken and filtered through 0.2µm nylon membrane filter, dried at 105°C (1 hour) and weighted.

III. RESULTS AND DISCUSSION

A. Batch Test

Results of the batch tests are shown in Fig. 2. The combination of biofilm-GAC (BAC) showed better PCP removal than GAC adsorption alone by 30%. After 10 days, 90% and 60% of PCP was removed by BAC and GAC respectively. Initially the adsorption rate by BAC was lower than adsorption by GAC alone but increased after 4 days. The biofilm covered the crevices and macropores of the activated carbon and therefore the diffusion of PCP was slower and causing subsequent reduction the adsorption rate. Reference [16] reported the same phenomenon in their studies. The excessive biological activity on the GAC surface was found impeding the adsorption process and reduces the treatment efficiency of the refractory compounds [7], [10], [16]. Comparison with other studies is shown in Table IV.

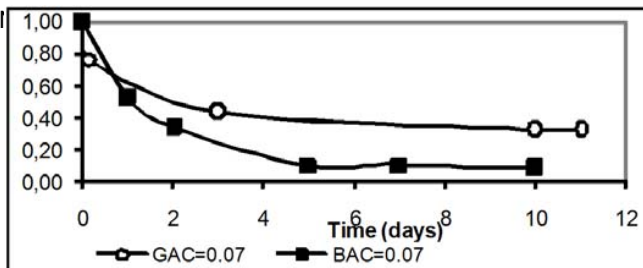


Fig. 2 PCP changes in Batch test with GAC and BAC

TABLE IV
COMPARISON WITH OTHER BIOFILM ACTIVATED CARBON STUDIES

Reference	Compounds	Percent removal (%)	
		BAC	GAC
This study	PCP 100mg/l	90	64
Walker and Weatherley, 1999	Acid dye 100mg/l	80-97	25-55
Rodman and Shunney, 1970	Acid dye	95	-

B. SBR Test

The PCP concentration changes in the SBRs are as shown in Fig. 4. Both the attached and suspended growths SBR gave good removals of PCP. The SBR system was able to remove 90% of the PCP within two days. Figs. 4-7 shows the HPLC chromatogram for the influent and effluent of SBRs showing significant reduction of PCP after treatment. The biomass concentration in the suspended growth SBR was 1000mg/L; and for the attached growth biofilm SBR, the biomass was 400mg/L. The attached growth SBR gave better in PCP removal, showing that the attached growth system having both biomass and GAC was more efficient in PCP removal.

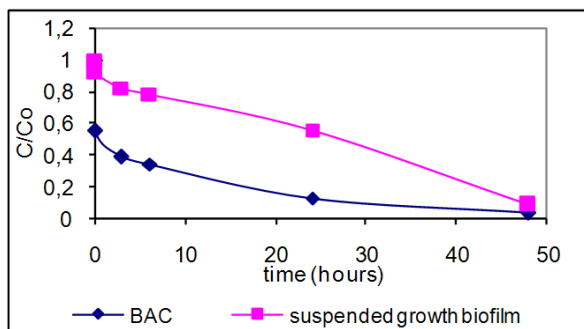


Fig. 3 PCP concentration changes in SBRs

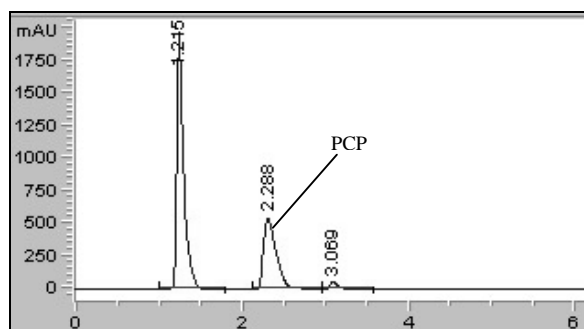


Fig. 4 HPLC chromatogram for SBR influent

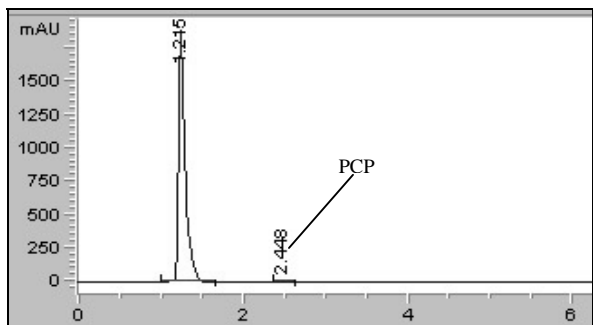


Fig. 5 HPLC chromatogram for effluent from suspended growth SBR

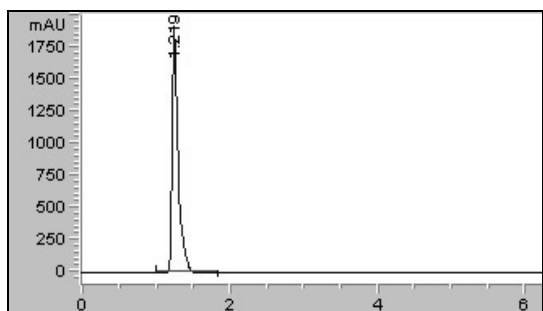


Fig. 6 HPLC chromatogram for effluent from attached growth SBR

C. BACCOR

Removal performance during the experiment is shown in Fig. 7 and Table V. Phenols concentration (as measured by 4-aminoantipyrine method) in effluent was maintained under 1.0mg/L during the experiment where the EBCT ranging from 45 minutes to 4 hours. PCP was not detected with this method even though at the start up of the reactor, where 100 mg/L of PCP was used. Throughout the experiment (500 days), the phenols in effluent was maintained below 0.52 mg/L and average more than 98% removal of PCP was achieved. It was very interesting to find the removal rate of PCP Run 1 to Run 4 was not varying with different EBCT. This has suggested that the removal of PCP was not caused by GAC adsorption only, but a second removal mechanism had also taken place. The second mechanism that may explain these results was biodegradation by microorganisms that grow on the GAC.

Fig. 8 shows the biomass concentration measured as MLSS in the reactor changes with operating time. The highest MLSS measured was 9400 mg/L although this high biomass concentration was causing blockage to the reactor and backwash was needed to clear the blockage. Backwash was conducted at average once every week. The MLSS was maintained at 2000 to 7000 mg/L during this experiment. In Run 3 where the EBCT was 45 minutes, high removal rate (99.6%) was probably caused by the average high MLSS (6000 to 7000 mg/L) and therefore, the results was not influenced by shortening the EBCT from 4 hours to 45 minutes. In Run 2, where the biomass was around 3000 mg/L, the 4 hours EBCT has helped to increase the removals rate from 99.6% to 99.7%. These results have shown the inter-relationship between EBCT with biomass concentration in PCP removals. The PCP removal was reduced to 98% when feeding with river water spiked with 10mg/L PCP (Run 5 and 6). River water is much complex and this would lead to competitive adsorption by GAC and

bi:3, No.7, 2009 influence the PCP removal. In Run 5 and Run 6, biomass also showed influence in PCP removals. In Run 5 where the biomass was around 3000 mg/L, removal rate (98.6%) was lower than Run 6 (98.9%). Here, the MLSS was double in Run 6 (6000 mg/L) compared with Run 5.

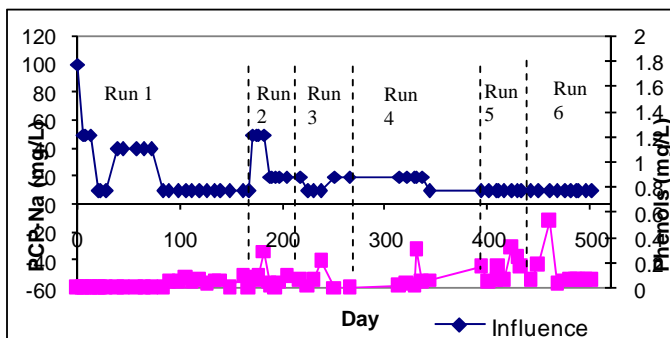


Fig. 7 Performance of BACCOR according to different residence time

TABLE V
PERFORMANCE OF BACCOR

Run	Ave. MLSS (mg/L)	Influent PCP (mg/L)	Min (µg/L)	Max (µg/L)	Ave. (µg/L)	Ave. % removal
1	2900	50*	0	0	0	99.6
		40*	0	0	0	
		10	0	97	51	
2	3000	50	62	297	133	99.7
		20	0	90	44	
		10	0	0	0	
3	6500	20	0	67	22	99.6
		10	0	210	79	
		20	15	304	107	
4	5250	20	15	304	107	99.6
5	3000	10	53	325	156	98.6
6	6000	10	32	528	125	98.9

* PCP was measured using UV-VIS spectrophotometer at 248 nm

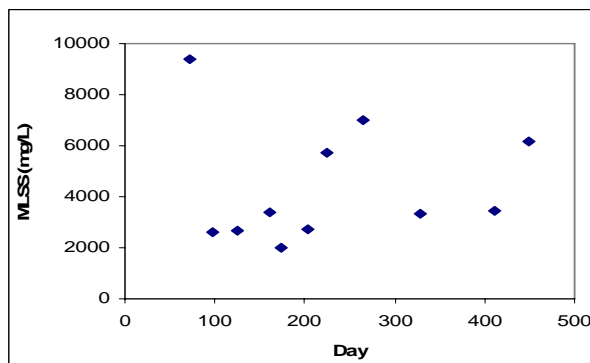


Fig. 8 Biomass changes in BACCOR

D. Isolation and Identification

After five days of incubation, pinpoint sized colonies surrounded by clear 'halo' were found on isolation agar indicating that these microbes were able to grow in the presence of PCP as the sole carbon source. Further sub-culturing found three gram-negative isolates of rod type bacteria. Their morphology is shown in Table VI. Tests with API-20E system have managed to identify two of them: Isolate 1 as *Klebsiella terrigena* and Isolate 2 as *Pseudomonas aeruginosa*. Isolate 3 was tentatively identified as *klebsiella sp.* Table VI lists some of the properties of these bacteria. All of them are classified as

aerobes but are also able to grow anaerobically, thus facultative bacteria.

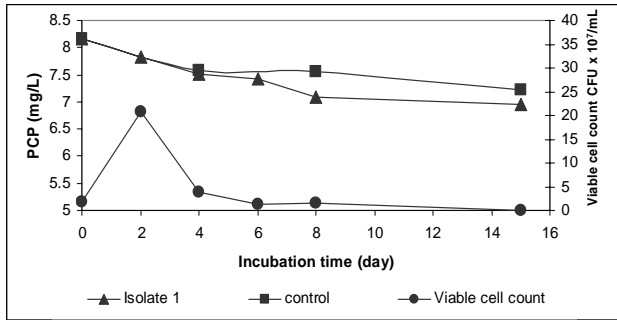


Fig. 9 Survival and growth of isolate 1 (*K. terrigena*) under aerobic conditions, in a medium with PCP as sole carbon source

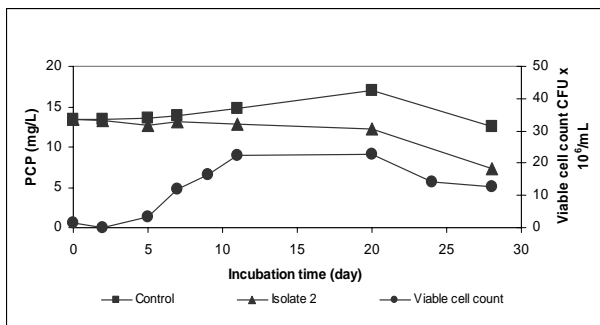


Fig. 10 Survival and growth of isolate 2 (*P. aeruginosa*) under aerobic conditions, in a medium with PCP as sole carbon source

Fig. 9 shows the response of isolate 1 (*K. terrigena*) to PCP as sole carbon source. Only about 13% of PCP had been reduced in 15 days. The concentration of isolate 1 (*K. terrigena*) increased in day 2 (20.7×10^7 /mL) and gradually declined to 1.1×10^6 /mL in 15 days. Isolate 2 (*P. aeruginosa*) showed more significant PCP reduction compared to the other two isolates (Fig. 10). About 42.5 % of PCP was reduced after 28 days of incubation. The growth of *P. aeruginosa* decreased after 2 days of incubation, showing that the isolate was adapting to the growth medium. The isolate concentrations then increased from 3.23×10^7 /mL (day 5) to 2.23×10^8 /mL (day11). During this period, the PCP reduction ranged from 0.8 to 12%. *P. aeruginosa* concentration decreased to 1.26×10^8 /mL during the 28 days test.

Isolate 3, which was tentatively identified as *Klebsiella spp.* did not show significant growth in which the culture was not able to survive after 2 days incubation with 11% of PCP reduction during the first 7 days in the first experiment (results not shown here). The isolate concentration decreased to below 10^3 /mL after 2 days incubation. In a second experiment carried out with longer incubation time, the same lag time was found at the beginning of the experiment (Fig. 11). The concentration of isolate decreased to below 10^3 /mL during the first 9 days but rapidly increased back to 10^7 /mL after that lag period. The PCP concentrations were not significantly decreased during the lag period. After the lag period, the PCP concentrations decreased to 22.7% within 28 days of incubation.

The mixed culture was found able to utilise and reduce PCP from 7 mg/L to 0.02 mg/L in 10 days incubation. The

concentration of PCP increased from 0.01 mg/L to 0.23 mg/L in that period of time showing the growth of the mixed culture in the experiments (Fig. 12) and a yield value of $Y=0.0315$ mg biomass/mg PCP. Chromatograms obtained by HPLC analysis of control and mixed culture sample (after 10 days incubation) are shown in Fig. 13. It was found that the PCP concentration was significantly reduced one of the most interesting results of this study is that the isolated bacteria was not able to degrade PCP for more than 50% when in monoculture, and required about 5 to 9 days lag period before starting to degrade PCP. In the mixed culture, there was no significant lag time and very effective degradation was observed (99% removal of PCP) in 10 days time showing that different microorganisms are responsible for different steps to synergistically complete the biodegradation.

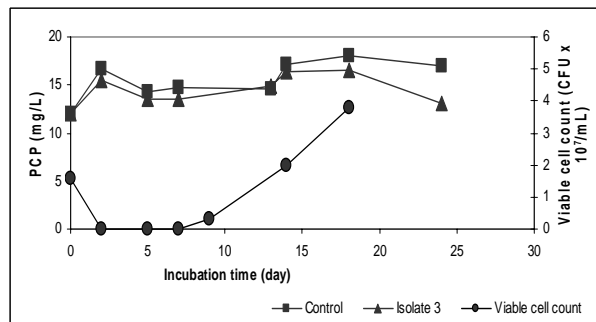


Fig. 11 Survival and growth of isolate 3 (*Klebsiella spp.*) under aerobic conditions, in a medium with PCP as sole carbon source

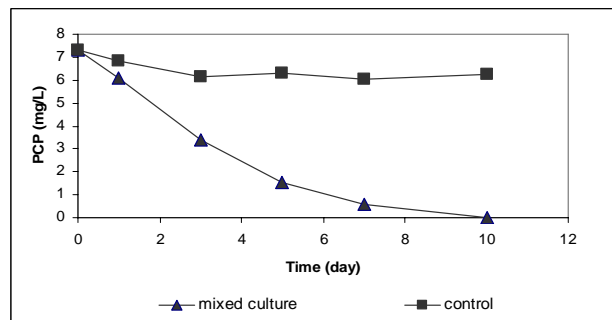


Fig. 12 PCP concentration changes with mixed culture in nutrient solution with 10 mg/L as sole carbon source

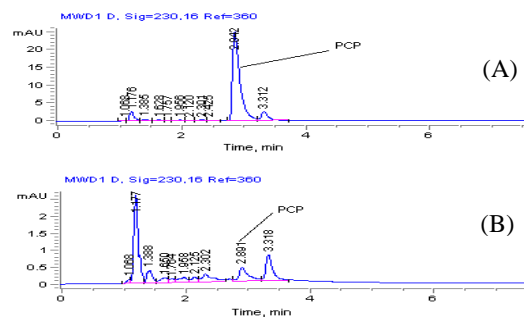


Fig. 13 Chromatograms of control (A) and sample for mixed culture after 10 days incubation (B) by HPLC analysis

TABLE VI
CULTURAL CHARACTERISTICS OF THE ISOLATED BACTERIA

	Isolate 1	Isolate 2	Isolate 3
Margin	Smooth	smooth	Smooth
Elevation	Raised	Convex	Raised
Configuration	Round with raised	Round with raised for small colony, irregular and spreading for big colony	Round
Color	Beige	Beige with blue-green pigment	beige

TABLE VII
SOME PROPERTIES* OF THE GRAM-NEGATIVE ISOLATES

Test	Reactions/Enzymes	Isolate 1	Isolate 2	Isolate 3
ONPG	Beta-galactosidase	+	-	+
CIT	Citrate utilisation	+	+	-
H ₂ S	H ₂ S production	-	-	-
UREA	urease	-	-	-
IND	Indole production	-	-	-
GEL	Gelatinase	-	+	+
GLU	Fermentation/oxidation	+	-	+
Nitrate reduction GLU tube	NO ₂ production	+	+	-
API-20E identification		<i>Klebsiella terrigena</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella spp.</i>

* Tests according to API-20E system: + positive result; - negative result.

IV. CONCLUSION

The results from this study have shown that the biofilm attached on the GAC are able to remove the pentachlorophenol (PCP) in simulated industrial wastewater. The combination of biofilm-GAC batch process gave higher reduction of PCP than GAC adsorption by 30%. For SBR process, it was found that both SBRs could remove more than 90% of the PCP after acclimatisation. However, the attached growth SBR was found more effective than suspended growth SBR without GAC. Results obtained showed that BACCOR was able to remove PCP-Na ranged from 10 to 100 mg/L with EBCT ranging from 45 minutes to 4 hours. More than 98% removal was achieved in this study. Biomass concentration as high as 9000 mg/L was measured, although this would cause blockage to the GAC bed. Regular backwash was performed, once in every week, and the biomass concentration was maintained in the range 3000 to 7000 mg/L. When individual bacterial isolate was used from BACCOR sludge very little (42 %) PCP has been utilised as sole carbon and energy source. While in mixed culture, more than 99% of PCP has been utilised. This shows that the species exist in the mixed culture are interdependence to utilise PCP.

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