

COMMENTARY



Bioremediation of petroleum wastewater by hyper-phenol tolerant *Bacillus cereus*: Preliminary studies with laboratory-scale batch process

Aditi Banerjee^a and Alope K. Ghoshal^b

^aCentre for the Environment, Indian Institute of Technology Guwahati, Guwahati, Assam, India; ^bDepartment of Chemical Engineering, Indian Institute of Technology Guwahati, Guwahati, Assam, India

ABSTRACT

Petroleum wastewater samples from oil refinery and oil exploration site were treated by hyper phenol-tolerant *Bacillus cereus* (AKG1 and AKG2) in laboratory-scale batch process to assess their bioremediation efficacy. Quality of the treated wastewater samples were analyzed in terms of removal of chemical oxygen demand (COD), total organic carbon (TOC) and ammonium nitrogen content, and improvement of biological oxygen demand (BOD). Adaptation of these bacteria to the toxic environment through structural changes in their cell membranes was also highlighted. Among different combinations, the co-culture of AKG1 and AKG2 showed the best performance in degrading the wastewater samples.

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Fossil fuel, is a major source of energy and will remain so in coming decades. However, environmental pollution due to production and transportation of refinery products poses critical challenges. Wastewater from petroleum refineries usually contains high concentrations of aliphatic and aromatic petroleum hydrocarbons which can easily contaminate sources of drinking water, downstream from discharges.¹⁻³ Bioremediation of petroleum wastewater offers a potential option to address the environmental issues. Biodegradation behavior of individual components of petroleum wastewater such as phenol, benzene, hexadecane, xylene and toluene is well documented.³⁻⁵ However, bioremediation of real petroleum wastewater is complicated because it consists of numerous components with varying chemical structure and toxicity.

In this regard, we have reported, the isolation of *Bacillus cereus* (AKG1 MTCC9817 and AKG2 MTCC9818) showing high phenol tolerance and also studied their degradation pathways.^{6,7} Capabilities of these strains for degrading phenol, following their immobilization in calcium-alginate beads, have also been reported.^{8,9} Herein, we try to evaluate the capabilities of these bacteria (either as free

cells or immobilized in calcium-alginate beads) in bioremediation of petroleum wastewater samples collected from oil refinery site (Sample I) and oil exploration site (Sample II). The bacterial strains were used in 5 different ways – free cells of AKG1, free cells of AKG2, free cells of co-cultured AKG1 and AKG2, immobilized AKG1 and immobilized AKG2 – for the biodegradation of wastewater samples over a period of 20 d.

Chemical oxygen demand or COD indirectly indicates the amount of organic pollutants present in a wastewater sample. Thus, it is an important parameter for any wastewater treatment process. The removal of COD (Table 1), when all combinations of free bacteria without external N₂ source were considered, was found to be in the range of 34%–83%, using external N₂ source resulted in slight increase in COD removal. Overall, co-culture system showed the highest reduction in COD level irrespective of N₂ source. Interestingly, COD removal by immobilized cells were lower than that of the free cells possibly due to mass transfer limitations.⁹ Phenolics are major organic compounds found in refinery wastewater and have a direct relation with COD. The biodegradation of wastewater samples in present studies was also observed to reduce the

CONTACT Aditi Banerjee  aditi@iitg.ernet.in  Centre for Environment, Indian Institute of Technology Guwahati, Assam, India-781039.

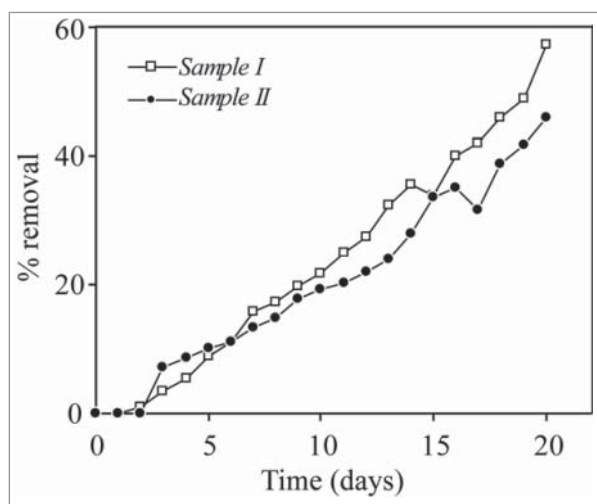
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Table 1. Removal of COD and TOC from wastewater.

	COD removal (%)					
	Without supplement		Organic nitrogen supplement (Yeast)		Inorganic nitrogen supplement (Ammonium Sulfate)	
	Sample I	Sample II	Sample I	Sample II	Sample I	Sample II
Free AKG1	61.11	34.14	69.44	51.21	66.66	41.46
Free AKG2	41.66	61.53	55.55	69.23	47.22	64.10
Co-culture	83.78	70	89.18	79.5	86.48	72.5
Immobilized AKG1	24.32	10.25	24.12	10.02	24.40	10.25
Immobilized AKG2	10.81	10.25	10.50	10.33	10.10	10.75
	TOC removal (%)					
Free AKG1	57.12	30.00	65.45	48.00	63.51	35.00
Free AKG2	34.68	41.59	45.52	55.99	39.77	45.00
Co-culture	83.45	54.00	84.54	75.95	77.28	70.00
Immobilized AKG1	18.75	13.30	—	—	—	—
Immobilized AKG2	8.29	11.80	—	—	—	—

concentration of phenolics by 57% (Fig. 1) and 45% in Sample I and Sample II, respectively. The reduction in total organic carbon (TOC) present in wastewater samples (Table 1) also followed similar trend as that of COD. Without any external N₂ source, Removal of TOC was found to be in the range of 30%–83%. The TOC level was reduced by 45–84% and 35–77% in presence of organic and inorganic N₂ sources, respectively.

During microbial treatment of petroleum wastewater samples, in addition to COD removal, prominent increase in BOD was also observed (Figs. 2C and 2D). After 20 d of bioremediation, initial BOD values of 180 mg/L (sample I) and 420 mg/L (sample II) increased to as high as 861 mg/L and 1532 mg/L,

**Figure 1.** Removal of phenolics (%) from wastewater samples by co-culture system.

respectively. The ratio of BOD to COD is an index of biodegradability performance. The low BOD/COD ratio (0.030~0.065) of wastewater samples before bioremediation indicated difficulty of treating them by biological methods. However, after treatment, the ratio was increased to 0.89 and 0.79 for sample I and sample II, indicating significant improvement of biodegradability of the samples. BOD/COD ratio of 0.9~1.0 is generally considered as the cut-off point for the biodegradable wastewater.^{10,11} Excess N₂ of wastewater should be removed as it may lead to eutrophication and toxicity.¹² During bioremediation of wastewater, this is achieved by biological nitrification. A satisfactory level of NH₄⁺-N removal from wastewater samples (Table 2) was achieved by co-culture system (Sample I: 68.12% and Sample II: 69.45%) followed by strain AKG1 (54%) for sample I and AKG2 (82.46%) for sample II. In case of the immobilized system, the reduction varied in the range of 31.5%~42.46% by the various strains.

Microorganisms are able to tolerate several toxic organic compounds by means of different kinds of adaptive mechanisms including changes in the fatty acid composition of membrane lipids.^{13–16} Fourier transform infrared (FTIR) spectra of cell membranes isolated from control bacteria and those employed in bioremediation of wastewater samples showed several absorption peaks indicating various functional groups (Fig. 3). Close inspection of the absorption peaks of wastewater exposed bacteria showed shift of bands relative to those observed in case of phenol used as substrate (Fig. 3B and 3D). These shifts in FTIR suggested that polysaccharides and proteins (major components

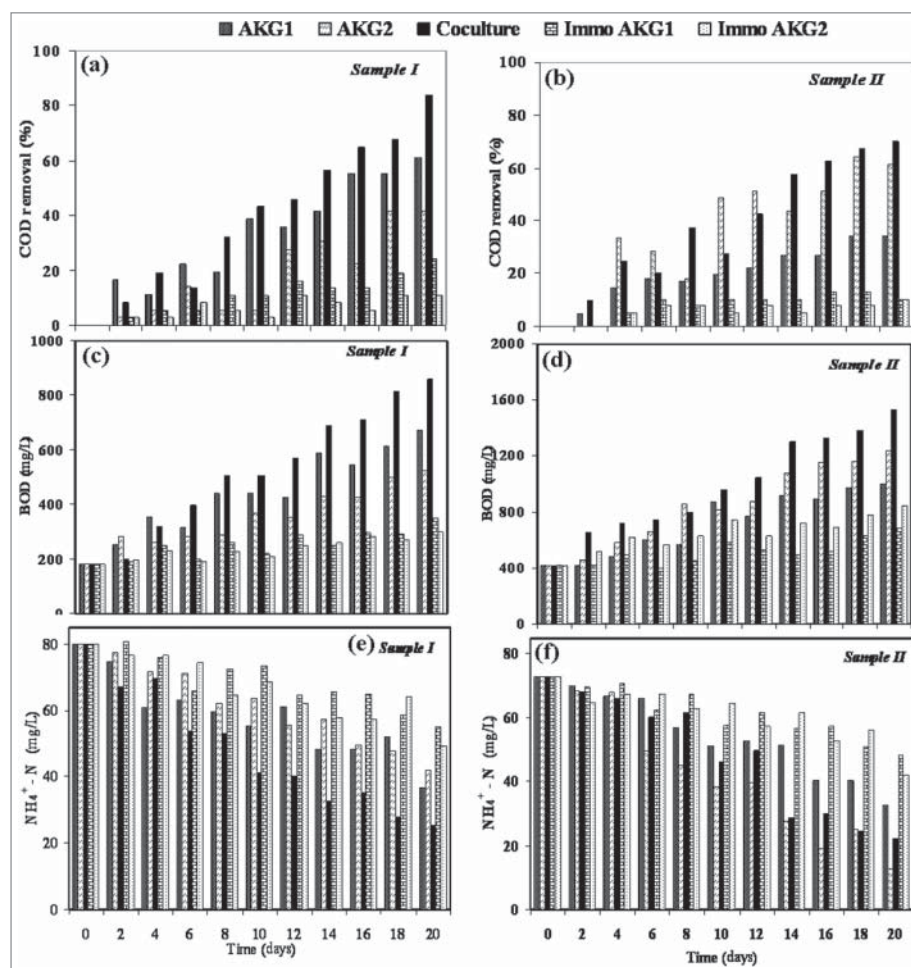


Figure 2. Effect of bioremediation on COD removal (A and B), BOD increment (C and D) and ammonia-nitrogen removal (E and F).

of membrane) were affected and evolved their structure in the presence of wastewater.¹⁷ Overall, co-culture of the free cells of *B. cereus* AKG1 and AKG2 showed the best performance in bioremediation of petroleum wastewater samples in the present study.

Experimental

Strains AKG1 and AKG2⁶ were grown in 1 g/L phenol-MSM medium as reported earlier.⁶⁻⁹ 100 mL of sterilized wastewater samples were taken into conical flasks and then incubated with specific strain (OD =

0.8) at their optimum growth condition (37°C at 120 rpm with definite pH condition) for desired period of time. Immobilized bacteria employed for bioremediation were prepared by previous method.^{8,9} During the experiment, samples (2 mL) were withdrawn time-to-time to measure COD and NH₄⁺-N by previous method.¹⁶ TOC content of the samples was determined in a TOC analyzer (O.I. analytical Aurora, Model 1030). Concentrations of phenolic compounds were analyzed by high performance liquid chromatography (HPLC, ProSTAR, Varian) equipped with a UV-visible detector and C₁₈ column.

In order to study bacterial membrane adaptation, cells were grown in MSM medium with either phenol (control group) or wastewater as the carbon source. Grown bacteria were harvested by centrifugation (8000 rpm, 10 min) and bacterial membrane was isolated by previously reported method.¹⁸ Isolated membranes were lyophilized for further FT-IR analysis in Perkin-Elmer, PE-RXI.

Table 2. Removal of Ammonium- nitrogen from wastewater.

Sample	% of Ammonia-Nitrogen reduction after 20 days				
	AKG1	AKG2	Co-culture	Immobilized AKG1	Immobilized AKG2
Sample I	54	47.5	68.12	31.5	38.5
Sample II	55.34	82.46	69.45	33.83	42.46

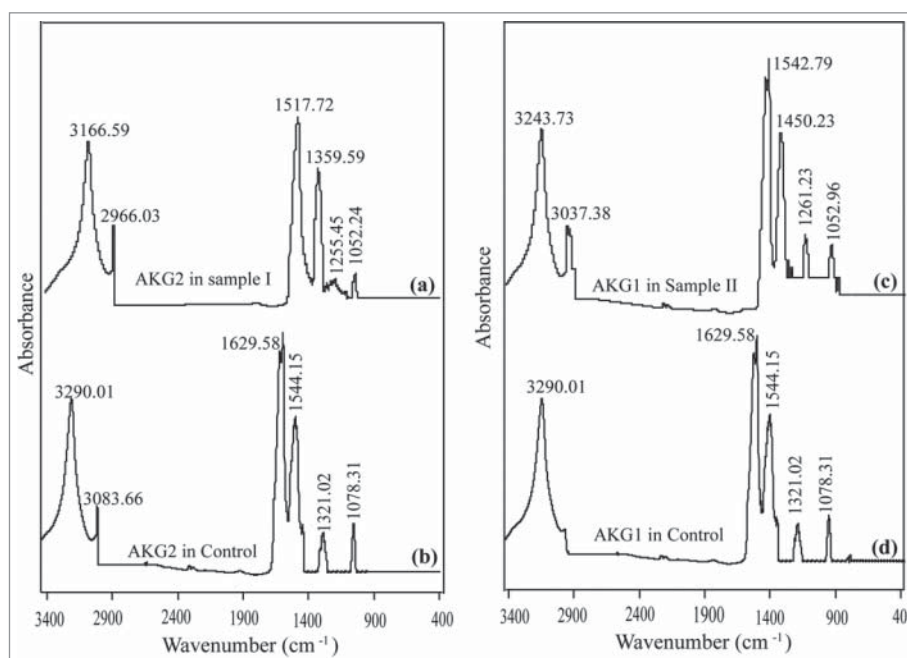


Figure 3. FTIR of bacterial membrane isolated from bacteria grown in control (phenol) and wastewater samples.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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References

- [1] Hoffman DJ, Rattner BA, Burton GA Jr, Cairns J Jr. Handbook of ecotoxicology. CRC Press, 2002.
- [2] Hu G, Li J, Zeng G. Recent development in the treatment of oily sludge from petroleum industry: A review. *J Hazard Mater* 2013; 261:470-90; PMID:23978722; <https://doi.org/10.1016/j.jhazmat.2013.07.069>
- [3] Singleton I. Microbial metabolism of xenobiotics: fundamental and applied research. *J Chem Technol Biotechnol* 1994; 59:9-23; <https://doi.org/10.1002/jctb.280590104>
- [4] Organization WH. Phenol, environmental health criteria-EHC 161. WHO, Geneva 1994.
- [5] Mahajan S. Pollution control in process industries. Tata McGraw-Hill Education, 1985.
- [6] Banerjee A, Ghoshal AK. Isolation and characterization of hyper phenol tolerant *Bacillus* sp. from oil refinery and exploration sites. *J Hazard Mater* 2010; 176:85-91; <https://doi.org/10.1016/j.jhazmat.2009.11.002>
- [7] Banerjee A, Ghoshal AK. Phenol degradation by *Bacillus cereus*: Pathway and kinetic modeling. *Bioresource Technol* 2010; 101:5501-7; <https://doi.org/10.1016/j.biortech.2010.02.018>
- [8] Banerjee A, Ghoshal AK. Phenol degradation performance by isolated *Bacillus cereus* immobilized in alginate. *Inter Biodeterioration Biodegradation* 2011; 65:1052-60; <https://doi.org/10.1016/j.ibiod.2011.04.011>
- [9] Banerjee A, Ghoshal AK. Biodegradation of phenol by calcium-alginate immobilized *Bacillus cereus* in a packed bed reactor and determination of the mass transfer correlation. *J Environ Chem Engineer* 2016; 4:1523-9; <https://doi.org/10.1016/j.jece.2016.02.012>
- [10] Celenza G. Industrial Waste Treatment Process Engineering: Biological Processes. CRC Press, 1999.
- [11] Contreras S, Rodriguez M, Momani FA, Sans C, Esplugas S. Contribution of the ozonation pre-treatment to the biodegradation of aqueous solutions of 2, 4-dichlorophenol. *Water Res* 2003; 37:3164-71; PMID:14509703; [https://doi.org/10.1016/S0043-1354\(03\)00167-2](https://doi.org/10.1016/S0043-1354(03)00167-2)
- [12] Sarioglu M. Removal of ammonium from municipal wastewater using natural Turkish (Dogantepe) zeolite. *Separ Purific Technol* 2005; 41:1-11; <https://doi.org/10.1016/j.seppur.2004.03.008>
- [13] Ramos JL, Duque E, Rodríguez-Herva JJ, Godoy P, Haïdour A, Reyes F, et al. Mechanisms for solvent tolerance in bacteria. *J Biol Chem* 1997; 272:3887-90; PMID:9020089; <https://doi.org/10.1074/jbc.272.7.3887>
- [14] Gao B, Zhu X, Xu C, Yue Q, Li W, Wei J. Influence of extracellular polymeric substances on microbial activity and cell hydrophobicity in biofilms. *J Chem Technol Biotechnol* 2008; 83:227-32; <https://doi.org/10.1002/jctb.1792>
- [15] Yu GH, He PJ, Shao LM, Zhu YS. Extracellular proteins, polysaccharides and enzymes impact on sludge aerobic

- digestion after ultrasonic pretreatment. *Water Res* 2008; 42:1925-34; PMID:18082240; <https://doi.org/10.1016/j.watres.2007.11.022>
- [16] Liu Y, Yang CH, Li J. Adhesion and retention of a bacterial phytopathogen *Erwinia chrysanthemi* in biofilm-coated porous media. *Environ Sci Technol* 2007; 42:159-65; <https://doi.org/10.1021/es071698k>
- [17] Long G, Zhu P, Shen Y, Tong M. Influence of extracellular polymeric substances (EPS) on deposition kinetics of bacteria. *Environ Sci Technol* 2009; 43:2308-14; PMID:19452879; <https://doi.org/10.1021/es802464v>
- [18] Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Canadian J Biochem Physiol* 1959; 37:911-7; PMID:13671378; <https://doi.org/10.1139/o59-099>