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A comparative study between a pure strain and a mixed consortium for utilization of carbon–sources in perchlorate biodegradation

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Abstract— *A comparative study between pure strain and a mixed culture isolated from wastewater treatment plant was done for carbon-source utilization during perchlorate degradation in batch system. Wide varieties of carbon-source which are commonly utilized by microbes were tested to serve the purpose. A pure strain Dechlorosoma sp. KJ which is reported for perchlorate degradation was compared with a mixed microbial culture. The mixed consortium has shown greater adaptability to utilize carbon sources for perchlorate degradation than the pure strain. Amongst all the carbon-sources utilized, acetic acid showed best result for perchlorate degradation by the pure strain but succinic acid for the mixed culture. Three microbial strains capable of degrading perchlorate were isolated from the mixed population and preliminary morphological and biochemical characterization were done for all the individual strains. The 16SrDNA has shown that the three strain belonged to three different genera and all of them belong to same family proteobacteria.*

Keywords— ***Perchlorate, mixed culture, carbon-source, Dechlorosoma sp. KJ, proteobacteria.***

I. INTRODUCTION

Perchlorate contamination is mostly associated with military activities or defense contractors (**Gullick et.al, 2001**) Perchlorate is used as the primary ingredient of solid rocket propellant. Wastes from the manufacture and improper disposal of perchlorate-containing chemicals are increasingly being discovered in soil and water. Perchlorate has been added to the U.S Environmental protection Agency's drinking water Candidate Contaminant List (**Urbansky, 2000**). There are various technologies available for perchlorate removal from contaminated water and wastewater. However, physicochemical process such as adsorption and membrane technology suffers from several drawbacks (**Logan, 2001**). Bioremediation of perchlorate-contaminated waters is promising. Bacteria capable of perchlorate degradation appear to be widely distributed in nature. Perchlorate is used as an electron acceptor by some bacteria for cellular respiration and is degraded completely to chloride ion (**Logan, 2001**). Perchlorate is degraded via a three-step process.

$$ClO_4^- \xrightarrow{\text{(Perchlorate reductase)}} ClO_3^- \xrightarrow{\text{(Perchlorate reductase)}} ClO_2^- \xrightarrow{\text{(Chlorate reductase)}} Cl^- + O_2$$

Perchlorate reductase reacts with both perchlorate and chlorate (ClO_3^-). In the first step perchlorate gets reduced to chlorate and then in the second step chlorate is transformed to chlorite (ClO_2^-). The final reduction of chlorite to chloride and oxygen is catalyzed by a separate non-respiratory enzyme chlorite dismutase. (**Xu et al., 2004**). Oxygen produced during perchlorate reduction is rapidly used by bacteria (**Logan, 2001**). Several perchlorate reducing bacteria has been isolated and identified till date. Bioreactors have been developed to remove perchlorate from drinking water sources and

wastewaters using the various microorganisms (**Kim et al., 2001**).

However utilization of carbon-sources for perchlorate degradation has not yet studied extensively. Acetate has been commonly found to be preferred by the PRB (perchlorate reducing bacteria) as electron donors during perchlorate reduction. In the present study, a wide range of compounds which are commonly used for microbial culture has been selected to analyze their utility as electron donor for perchlorate reduction by a pure strain and an enriched mixed consortium. Acetic acid has been found to be preferred by the pure culture but succinic acid was preferred by the mixed cultures. Phenol and other aromatic pollutants like benzene, xylene, bromophenol, chlorophenol and nitrophenol were also accepted as sole C-sources by the mixed consortium. The individual microbial strains present in the mixed microbial population were also identified by biochemical and 16SrDNA analysis and were found to belong under same family.

II. MATERIAL AND METHODS

2.1. Materials

Chemicals and reagents used in the study was of analytical grade, inorganic salts used in preparing microbial growth media were of reagent grade. Sodium perchlorate ($NaClO_4.H_2O$), procured from Merck, India was used as the source of ClO_4^- in all the experiments. All the other chemicals used in this study were purchased from Merck, India.

2.2. Analytical Procedure

Measurement of chemicals was done by electronic balance (Make; Simadzu). pH was measured by electronic pH machine (Make: Systronics). Perchlorate was measured by ionchromatography with Dual3 column and RP guard column (Metrohm, Switzerland).

2.3. Experimental method

Dechlorosoma sp. KJ and the mixed culture both were cultured in 100ml culture media in 250 ml Erlenmeyer flasks. Per Liter of liquid media contained the following compounds, Sodium perchlorate and each carbon-source, 0.5 g, $K_2HPO_4.3H_2O$, 1.55 g $NaH_2PO_4.H_2O$, 0.85 g, $NH_4H_2PO_4$, 0.50 g. and 10.0 ml of Trace Mineral Supplement , composed of $MgSO_4.7H_2O$, 3.0 g/L ;$MnSO_4.H_2O$, 0.5 g/L; NaCl, 1.0 g/L; $FeSO_4.7H_2O$, 0.1 g/L; $Co(NO_3)_2.6H_2O$, 0.1 g/L; $CaCl_2$ (anhydrous), 0.1 g/L; $ZnSO_4.7H_2O$, 0.1 g/L ;$CuSO_4.5H_2O$, 0.01 g/L; $AlK(SO_4)_2$ (anhydrous), 0.01 g/L; H_3BO_3, 0.01 g/L ;$Na_2MoO_4.2H_2O$, 0.01 g/L; Na_2SeO_3 (anhydrous), 0.001 g/L; $Na_2WO_4.2H_2O$, 0.01 g/L; $NiCl_2.6H_2O$, 0.02 g/L. two replica for each carbon-source were prepared.

The pH of the media was adjusted to 7.0 using NaOH. Medium was sterilized by autoclaving and degassed by purging gaseous nitrogen. Anaerobic condition was maintained by sealing the flasks with rubber corks. Culture flasks were kept in constant temperature at 28°C.

All flasks were kept static condition for almost ten days (234 hrs.). 5 ml culture media were taken out from each flask and centrifuged in 8000 rpm for 10minutes to pellet down the cell debris. The clear supernatants were used to measure the effluent perchlorate concentration.

The strains were isolated by serial dilution followed by plating. Cultures were plated on a solid medium and incubated in an anaerobic jar containing the media with 1.5 g L^{-1} agar. Microscopic examination of the mixed culture was done under scanning electron microscope. The biochemical analysis was done following Burgey's manual.

III. RESULT AND DISCUSSION

Microbial growth and degradation of perchlorate were measured for both pure strain and the mixed microbial consortium. However, *Dechlorosoma* sp. KJ failed to accept amino acids as carbon source. The mixed culture has been found to be capable of utilizing amino acids as the sole carbon-source for perchlorate degradation (Table1). The average % perchlorate degradation by both the cultures shows that acetic acid is the most favored carbon-source for pure strain and succinic acid for the mixed culture (Fig.1). For each of the carbon-sources used, the mixed culture has shown to degrade perchlorate more compared to the pure strain. The difference between the strains was clear by the morphological and biochemical characterization (Table 2 and Fig.2).

Table 1: Growth and perchlorate degradation by pure and mixed culture using different carbon-sources.

C-source added (300mg/L) in the synthetic media	Pure strain (*Dechlorosoma* sp. KJ)		Mixed culture	
	Growth	Perchlorate degradation	Growth	Perchlorate degradation
Acetate	+	+	+	+
Oxalate	+	+	+	+
Citrate	+	+	+	+
Pyruvate	+	+	+	+
Aspartate	+	-	+	+
Fumerate	+	+	+	+
Malate	+	+	+	+
Succinate	+	+	+	+
Propionate	+	-	+	+
Alanine	-	-	+	+
Leucine	-	-	+	+
Glycine	-	-	+	+
Proline	-	-	+	+
Valine	-	-	+	+
Phenylalanine	-	-	+	+
Glucose	-	-	+	-
Fructose	-	-	+	-
Sucrose	-	-	+	-
Lactose	-	-	+	+
Arabinose	-	-	+	-
Mannose	-	-	+	-
Raffinose	-	-	+	-
Sorbose	-	-	+	-
Mannitol	-	-	+	-
Inositol	-	-	+	-
Salicin	-	-	+	-
Starch	+	+	+	+
Peptone	+	+	+	+
Yeast extract	+	+	+	+
Brewr's yeast	+	+	+	+
Cotton seed protien				

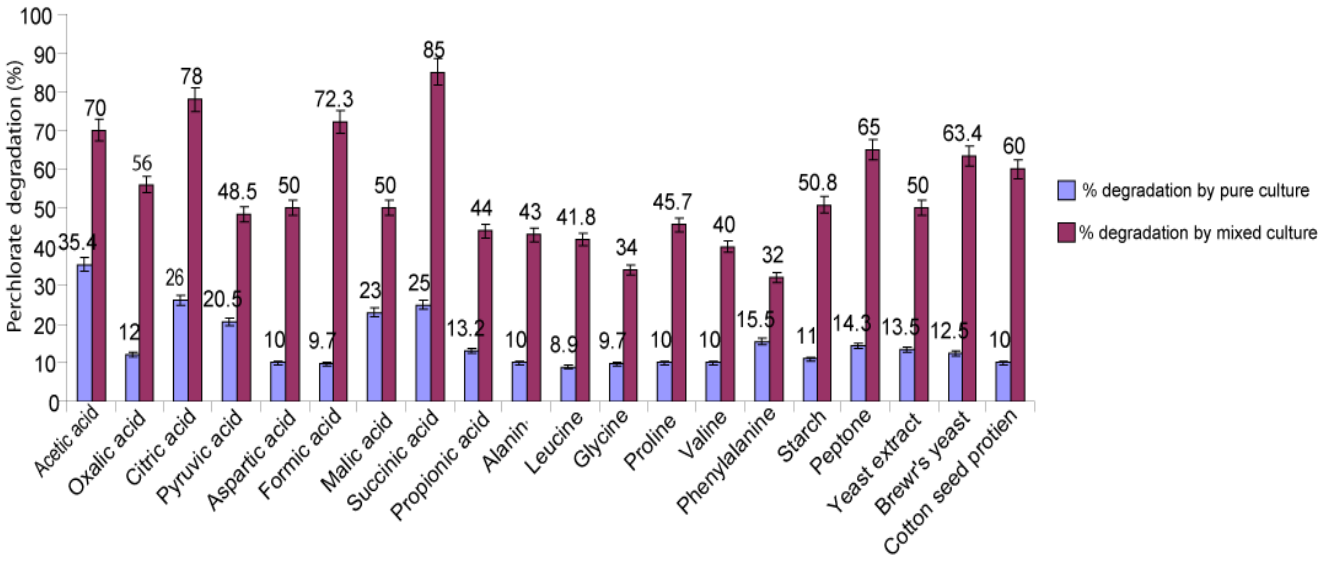

Fig.1: Perchlorate degradation (%) by pure and mixed culture using different carbon-sources.

Table 2: Morphological and biochemical characterization of the three isolates in mixed consortium

Tests	Strain A	Strain B	Strain C
Configuration	circular	circular	circular
Margin	entire	entire	entire
Elevation	convex	convex	convex
Surface	smooth	smooth	smooth
Pigment	cream	cream	cream
Opacity	transparent	opaque	opaque
Gram's reaction	Gram-ve	Gram-ve	Gram-ve
Cell shape	rod	Coccoid to small rod	Ovoid or rod shaped
Arrangement	pairs	Pairs or small chains	Singly or in pairs
Spore(s)	-	-	-
Motality	motile	Non-motile	Non-motile
Biochemical tests			
Growth on McConkey	-	+(NLF)	-
Indole test	-	-	-
Methyl red test	-	-	-
Voges Proskauer test	-	-	-

Citrate utilization	-	+	-
Gelatin Hydrolysis	+	-	+
Esculin hydrolysis	+	-	+
Starch hydrolysis	-	-	-
Urea hydrolysis	-	(+)	-
Nitrate reduction	+	-	+
Ornithine decarboxylase	-	-	-
Lysine decarboxylase	-	-	-
Arginine dihydrolase broth	-	-	-
Catalase test	-	+	-
Oxidase test	+	+	+
Tween 20 hydrolysis	-	+	-
Tween 40 hydrolysis	+	+	+

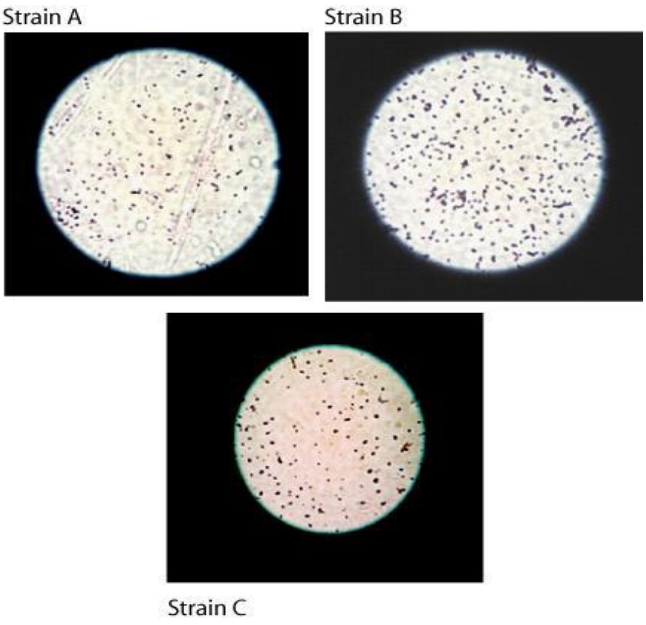

Fig.2: Photographs showing individual strains (A, B and C) after Gram staining.

IV. CONCLUSION

The mixed culture has shown greater adaptability of using carbon-sources than the pure strain *Dechlorosoma* sp. KJ for perchlorate degradation. Therefore, the mixed consortium holds a better potential for further research in perchlorate biodegradation using succinic acid as sole carbon-source. The degradation performance of the isolated strains also explores the possibility of promising research in the same aspect.

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