Journal of Toxicology and Environmental Health Sciences Vol. 1 (1) pp. 001-007, June, 2009
Available online at http://www.academicjournals.org/JTEHS
Academic Journals

Full Length Research Paper

Characterization of industrial waste and identification of potential micro-organism degrading tributyl phosphate

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Accepted 6 May, 2009

The present research study has been carried out in the waste disposal site for characterization of physical, chemical and biological parameters to assess the microbial consortium present in the contaminated site and to isolate the potential micro-organism for biodegradation of Tributyl Phosphate. The ambient conditions present in the contaminated site shows the values: pH (6.61), Temperature (35.6), Moisture (50.72%), Nutrients; Nitrogen (0.41%), Phosphorus (27.87 mg/l), and Sulphur (993.5 mg/l) respectively. The biological parameters studied indicate Dissolved Oxygen (4.58 mg/l), Biological Oxygen Demand (4.62 mg/l), Chemical Oxygen Demand (146.1mg/l).The microbial consortium identified was found to survive and multiply in the present environment conditions. Microbial consortium was sequenced and compared using BLAST, ClustalW and PHYLIP. In order to identify potential microorganism, microbial consortium was exposed to increasing concentration of Tributyl Phosphate viz. 10, 25, 50, 75 and 100 mg/l in MSM, the potential microorganism was found to survive at higher concentration and utilized it as a sole source of carbon. This organism was identified as *Pseudomonas pseudoalcaligenes* strain DSM 50018T using 16S rRNA sequencing. This organism was found to have high potential for degradation of Tributyl Phosphate present in Low Level Nuclear Waste.

Key words: Tributyl phosphate (TBP), low level nuclear waste, 16S rRNA sequencing, industrial effluent, biodegradation.

INTRODUCTION

Rapid industrialization and urbanization have enhanced the levels of organic and inorganic contaminants in the environment. Nuclear wastes generated through chemical processing in nuclear industry or nuclear weapons program have also enhanced the level of organic contaminants. The waste generated from nuclear industry generally contains radio nuclides, heavy metals along with myriads of toxic organics. Several physico-chemical methods to decontaminate the nuclear waste have been established and employed.

However, in Low-Level Nuclear Waste, concentrations involved are low and volumes are large. Hence physical and chemical methods cannot be practiced to decon-taminate the Low-level nuclear waste. The organics as well as inorganic chemicals present in the nuclear waste find their ways in soil-water causing environmental pollution. Most of these compounds can be inactivated or degraded by microorganisms (Kumar et al., 1996).Among them, Tri butyl phosphate (TBP) has been poorly investigated because of its low toxicity in mammals. (Healy et. al., 1995 McDonald et al., 2002).Nevertheless, its wide utilization in defoamers, plasticizers, herbicides, hydraulic fluids and as a solvent for conventional nuclear fuel processing generates large amount of wastes. This compound is very stable in the natural environment and is hardly affected by natural photolysis and hydrolysis (Environment Protec-

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tion Agency 1992). Recent studies have shown that nuclear waste contaminants can have both lethal and a sublethal effect on a variety of organisms. TBP is a known carcinogen and remain in the environment for a very long period of time. In spite of its low solubility in water (4 mM at 30˚C), TBP presents an acute toxicity hazard to freshwater living organisms. The acute toxicity values for fish (96hr LC50) range from 4.2 to 18 mg/l. Toxicity values for six species of algae ranged from 1.1 mg/l (*Scenedesmus subspicatus)* to 5-10 mg/l (*Chlorella emersonii*).(SIDS Initial Assessment Report, 2001) These hazardous waste generated by nuclear industries have become a treatment and disposal problem causing environmental concern for organic contaminant such as TBP. The recent advancement in bioremediation will be beneficial to treat the organic contaminants in the Low-Level Nuclear Waste. Bioremediation refers to site restoration through the removal of organic contaminants by micro-organisms. In order to explore the identification of potential micro-organisms the physico-chemical and biological characterization of the disposal site is important (Fulekar, 2005b). In the present study the microbial consortium at the industrial waste disposal have been assessed which are surviving and growing in the presence of the organic/inorganic contaminant including chemical parameters like P, N, S which provide the nutrient along with contaminant as a carbon source. The microbial consortium found was exposed to increasing concentration of TBP to identify the potential microorganisms which are capable to degrade this compound. The 16SrRNA method have been employed to identify the potential organism (Fulekar and Jaya, 2008) (Fulekar, 2008) for bioremediation of organic compound with special reference to TBP which can be useful to treat Low Level Nuclear Waste containing TBP as contaminant present in Low Level Nuclear Waste (EPA, 1992; Raushel, 2002).

MATERIALS AND METHODS

Sampling site

The industrial effluent treated by the various groups of industry such as Fertilizer, Petrochemical, Power plants and other chemical industries are discharged at this site through unlined channels. The industrial effluent was collected from the waste disposal site located in an industrial belt at Chembur 60km away from Bhabha Atomic Research Centre (BARC), Chembur, Mumbai, India. The effluent/ sediment samples were collected in pre-cleaned polythene bottles / bags for the characterization of physico-chemical and microbial assays.

Physico-chemical analysis of sample

Soil was air dried ground and passed through a 2 mm pore size sieve and was stored in sealed containers at room temperature. The samples were analyzed for the various physico-chemical parameters like pH, Temperature, Electrical Conductivity (EC), Total Solids (TS), Total Suspended Solids(TSS), Total Alkalinity(TA), Phosphate (P), Total Hardness(TH), Total dissolved solids(TDS), Sodium (Na), Potassium (K), including biological characterization like Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and Dissolved Oxygen (DO). The parameters were analyzed as per the "APHA, Standard Methods for Water and Waste Water Analysis" Volume 2, 1989 (APHA, 1989, 1979).

Microbial Characterization

Microbial characterization of the samples was done within 24 h from the time of sample collection. The samples from the effluent and sediment were prepared for the identification of microbes. Total bacterial count was carried out using serial dilution method in duplicates under aseptic conditions.1ml of the saline sediment suspendsion was serially diluted to 10^{-1} to 10^{-4}. 0.1 ml of serially diluted sample was spread plated on sterile nutrient media plates.(Kumar, 2004) Nutrient agar medium was used as it supports growth of all kinds of micro-organisms in a particular sample. For the effluent sample, 1 ml of the effluent was diluted to 10 ml followed by serial dilution and spread plating on sterile nutrient agar plates. The plates were incubated for 24 h at 37˚C.Isolated colonies were further analyzed using 16S rRNA sequencing.

Identification of potential micro-organism for bioremediation

Compound used: Commercial-grade Tri-n-butyl phosphate was obtained from Otto Kemi, India. The nutrient agar used for the isolation of bacteria was obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India and prepared according to manufacturer's instructions. All the solvents used for analysis were of HPLC grade while other chemicals were of AR grade.

Spiking of the compound: Erlenmeyer flask (250 ml) and nutrient culture media were autoclaved for 20 min at 121℃. Aliquots of 500 µl acetone containing the pesticide were aseptically added to the autoclaved and dried Erlenmeyer flasks allowing the acetone to evaporate. After complete evaporation of acetone, 100ml culture media was added. (Fulekarand Geetha, 2008)

Media used

Mineral salts medium (MSM) enriched with TBP was used for isolation and characterization of TBP degrading bacteria. The MSM has the following composition in (g/l): $CaCl_2$, 0.025; $MgSO_4.7H_2O$, 0.2; NaCl, 0.1; $(NH_4)_2SO_4$, 5.0; $FeSO_4.7H_2O$, 0.015; $ZnSO_4.7H_2O$, 0.00171; $FeSO_4$, $7H_2O$, 0.0015; CoCl2.6H2O, 0.000483; CuSO4.5H2O, 0.000471; $NaMoO_4.2H_2O$, 0.000453.The carbon source in MSM was replaced with Tributyl Phosphate. (Thomas and Macaskie, 2006) The pH was adjusted to 7.0 by the addition of 3.0 ml of 1 M NaOH. Controls were TBP-free culture media. The MSM was autoclaved (121℃, 15 min). TBP was self sterile and was spiked in the flask.

Isolation and taxonomic characterization of TBP degrading bacteria

The potential bacteria capable of degrading TBP were isolated from the effluent sediments. The sediment (1 gm) was suspended in 10 ml of nutrient broth. 1 ml of the nutrient broth containing 24 h old mixed culture was then inoculated in 250 ml Erlenmeyer flasks containing 100 ml of mineral salts medium supplemented with TBP (10 mg/l).(Singh D and Fulekar, 2007). The flasks were incubated on a rotary shaker at 150 rpm for 10 days at 30℃. (Fulekar and Geetha, 2008) At periodic intervals, a loop full of bacterial growth from the flasks was streaked onto mineral agar supplemented with TBP (10 mg/l) and the plates were incubated at 30℃ for 5 - 6 days (Thomas and Macaskie, 1996). The number of bacteria that survived was as-

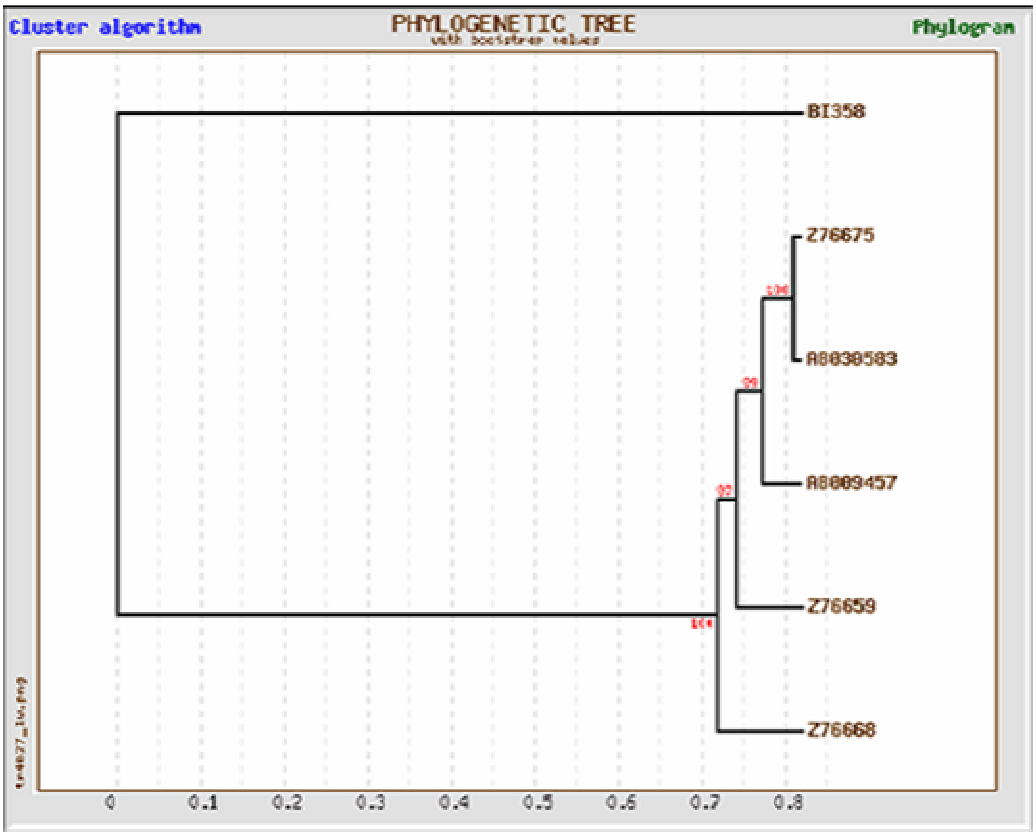

Figure 1. Phylogenetic tree of the most potential bacteria representing the evolutionary relationship with different bacteria.

sessed. After 10 days, 1 ml of this Nutrient culture media was transferred to MSM containing 25 mg/l of TBP. Similarly, 1 ml of the culture media was transferred to MSM containing 50, 75 and 100 mg/l. Bacterial isolate grown on TBP (100 mg/l) containing agar thereby depicting the ability to utilize TBP as the sole carbon source was isolated and was subjected to morphological, cultural and biochemical studies. The 16S rRNA gene of this bacterial strain that displayed maximum compound utilization potential was partially sequenced.

16SrRNA analysis was done using predetermined universal primers of 16S rRNA. The nucleotide sequences were used for BLAST analysis against the NCBI data base to obtain related sequences of related organisms. These sequences were aligned using CLUSTALW and a phylogenetic tree was constructed using the PHYLIP analysis programme.(Fulekar and Jaya 2008) Using the bootstrap values, the hit list for the 16S rRNA sequence homology identified the micro-organism as *Pseudomonas pseudoalcaligenes* strain DSM 50018T as shown in Figure 1.

RESULTS AND DISCUSSIONS

The waste generated by various industries are being treated to comply with the standards prescribed under the EPA, 1986.The treated effluents are being discharged through the open channel at the disposal site. The present study has been carried out to study the physico-chemical and biological characteristics of the industrial waste disposal site. The microbial consortium which could survive in such contaminated site has also been assessed to identify the potential micro-organism degrading TBP.

The physical parameters (Table 1) studied shows that, the pH was in the range of 6.2 - 7.0 with an average of 6.61.The temperature was found to be 42℃.Besides, the moisture content and the alkalinity was found to be 50.72% and 585.52 mg/l respectively. The chemical parameters (Table 1) highlighted DO (4.58 mg/l), BOD (4.62 mg/l), and COD (146.1 mg/l) which provided the ambient conditions at the contaminated site in which the adapted microorganisms survive and multiply. The chemical parameters - P (27.87 mg/l), N (0.41%), S (993.5 mg/l), Na (4695.5 mg/l), K (391.3 mg/l) also served as a nutrient to microorganism whereas the carbon source was obtained from the breakdown products of various organic contaminants The microbial consortium adapted at the contaminated site under these conditions was assessed. TBP was taken as one of the compound under study for the degradation by the microbial consortium /microorganism. The microbial consortium present at the site was analyzed by 16SrRNA sequencing and based on their sequencing they were identified as *Bacillus cereus* strain EK15, *Pseudomonas* sp. *N9-5, Pseudomonas pseudoalcaligenes* DSM 50018T, *Pseudomonas* sp. LM8, Shewanella NH21 (Table 2).

The microbial consortium assessed was further expos-

Table 1. Physico-chemical parameters of industrial effluents/sediments.

S/No.	Parameters	Effluent			Sediment			Average values
		Sample I	Sample II	Sample III	Site I	Site II	Site III	
1	Color	Pale Yellow	Pale Yellow	Blackish	Black	Black	Black	Black
2	Odor	Pungent	Pungent	Pungent	Pungent	Pungent	Pungent	Pungent
3	pH	6.8	6.3	7.0	6.5	6.2	6.9	6.61
4	Temperature (ºC)	40	42	39	30	28	35	35.6
5	Conductivity (mS)	16.8	17.2	16.8	17.0	16.1	15.9	16.63
6	Total Solids (mg/l)	26.13	29.5	35.13	—	—	—	30.2
7	Total Dissolved Solids (mg/l)	15.23	15.40	15.22	—	—	—	15.28
8	Total Soluble Solids (mg/l)	8.43	6.30	6.2	—	—	—	6.97
9	Moisture Content (%)	NA	NA	NA	62.66	52.31	37.19	50.72
10	Bulk Density (Cm^3)	—	—	—	0.68	0.63	0.67	1.98
9	Alkalinity (mg/l)	566.6	483.3	816.6	780	866.67	733.34	585.52
10	Dissolved Oxygen (mg/l)	3.16	4.83	2.4	5.36	6.13	5.63	4.58
11	Biological Oxygen Demand (mg/l)	3.0	4.80	2.36	6.83	5.44	5.32	4.62
12	Chemical Oxygen Demand (mg/l)	160	102.67	176	160	102	176	146.1
13	Phosphate(mg/l)	30.6	30.0	33.3	28.7	35.3	9.34	27.87
14	Total Organic carbon (%)	1.06	1.53	0.7	4.26	3.8	3.83	2.53
15	Total Organic Matter (%)	1.93	2.96	1.33	7.27	6.6	7.16	4.54
16	Sulfate (mg/l)	1100	1430	2560	380	420	71	993.5
17	Nitrogen (%)	0.035	0.0525	0.0175	1.32	0.42	0.65	0.41
18	Sodium (mg/l)	8820	8890	8522	752	650	539	4695.5
19	Potassium (mg/l)	550	542.0	535.0	96	102.0	94.0	319.3

exposed to TBP at increasing concentration varying from 10, 25, 50, 75 and 100 mg/l in MSM. The growth was observed as turbidity and optical density (OD) was measured daily at 600nm to assess the survival and growth of microorganisms at the respective TBP concentration. The microorganisms surviving at various concentration of TBP was assessed by spread plate method on miminal agar containing TBP (Table 3).

At 100 ppm only one bacterial colony was observed which was further assessed by biochemical tests, followed by its 16S rRNA sequencing including its phylogenetic tree (Table 4 and Figure 1). Based on the sequencing and phylogenetic analysis the microorganism was identified as *Pseudomonas pseudoalcaligenes* strain DSM 50018T. This microorganism, adapted in the contaminated site could degrade the higher concentration of TBP, utilizing it as a sole source of carbon. Earlier studies shows that *Pseudomonas pseudoalcaligenes* JS45 utilizes nitrobenzene as the sole source of nitrogen, carbon, and energy. (He and Chain, 1998).Another study by on Pseudomonas pseudoalcaligenes KF707 depicts its ability to utilize 2- and 4-fluorobiphenyl as sole carbon and energy sources.(Murphy et al., 2008).The organophosphorus hydrolyse gene was reported in *Pseudomonas pseudoalcaligenes* C2-1.This organo-phosphorus hydrolase gene was expressed in *Pichia pastoris plants.* The protein expressed by this gene is able to hydrolyze phosphoester bonds and reduce the toxicity of organophosphorus compounds. (Xiao-Yu Chu et al., 2008). In previous studies, TBP degrading mixed culture of Pseudomonas spp. were isolated that utilize TBP as a sole source of carbon and phosphorus. (Thomas and Macaskie, 1996) In the present study it was found that the isolated microorganism *Pseudomonas pseudoalcaligenes* DSM 50018T could utilize TBP as a sole source of carbon. The microorganism *Pseudomonas pseudoalcaligenes* DSM 50018T identified in the present research study could serve as a potential microorganism for the bioremediation of low level nuclear waste containing organic contaminants with special reference to TBP. This microorganism will be submitted to the gene bank for its beneficial use to biodegrade the hazardous compound s such as TBP.

Table 2. Microbial characteristics of industrial effluent and sediments.

S/No.	Bacterial Identification Method	Description	16S rRNA Gene Sequence
1	16S rRNA Gene sequencing	Shewanella sp. NH21	TAAGCGCACGCAGGGGCTTGTTAAGTTAGATGTGATATTTAACCT GGGAATTGCATTTAAGACTGGCTAAGGTGGAGAGGGGGGTGGAA TTTCCGGTGTAGCGGTGAAATGCGTAGAGATCGGAAGGAACATC AGTGGCGAAGGCGACCCCCTGGCCAAAGACTGACGCTCAGGTG CGAAAGCGTGGGGAGCAACAGGATTAGATACCCTGGTAGTCCAC GCCGTAAACGATGTCAACTTGGAGTTTGTGTTCTTGAAACGTGGA CTCCGGAGCTAACGCGTTAAGTTGACCGCCTGGGGAGTACGGCC GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCG GTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCT ACTCTTGACATCCAGAGAATTTGCTAGAGATAGCTTASTGCCTTC GGGAACTCTAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTT GTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCT TTGTTGCCAGCACGTAATGGTGGGAACTCAAAGGAGACTG
2	16S rRNA gene	Bacillus cereus strain EK-15	GTAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGCTAGTTCG GGCTCAACCGTGGAGGGTCATTGGAAACTGGGTTGAGGCAGAAG AGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATAT GGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGA CACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCC TGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGT TTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTG GGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGG GCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGC GAAGAACCTTACCAGGTCTTGACATCCTCTGAAAACCCTAGAGAT AGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTTG TCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA GCGCAACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTA AGGTGACTGCGGTGACAAACCGGAGGAAGGTGGGGATGACGTC AAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTAC
3	16S rRNA gene	*Pseudomonas* sp. N9-5	TAAGCGCGCGTAGGTGGTTTGATAAGTTGGATGTGAAAGCCCCG GGCTCAACCTGGGAATTGCATCCAAAACTGTCTGACTAGAGTATG GCAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGA TATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGGCTAAT ACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGA TACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGG GATCCTTGAGATCTTAGTGGCGCAGCTAACGCATTAAGTCGACCG CCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACG GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAA CGCGAAGAACCTTACCAGGCCTTGACATGCAGAGAACTTTCCAG AGATGGATTGGTGCCTTCGGGAACTCTGACACAGGTGCTGCATG GCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTA ACGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTTAAGGTGGG CACTCTAAGGAGACTGCCGGTGACAA
4	16S rRNA gene	*Pseudomonas* sp. LM8	TAAGCGCGCGTTGTGAAAGCCCCGGGCTCAACCTGGGAATTGCA TCCAAAACTGTCTGACTAGAGTATGGCAGAGGGTGGTGGAATTTC CTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTG GCGAAGGCGACCACCTGGGCTAATACTGACACTGAGGTGCGAAA GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCG TAAACGATGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGC GCAGCTAACGCATTAAGTCGACCGCCTGGGGAGTACGGCCGCAA GGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGG AGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGCC TTGACATGCAGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGA ACTCTGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTG AGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAG TTACCAGCACGTTAAGGTGGG

Table 2. Continued.

5	16S rRNA gene	*Pseudomonas pseudoalcaligenes* 50018T	DSM	CGGTCGAAGTTCACACATGCAAGTCGAGCGGTGAAGGGAGCTTG CTCCTGGATTCAGCGGCGGACGGGTGAGTAATG CCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGG GCGCTAATACCGCATACGTCCTGAGGGAGAAAGT GGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGGA TTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGC GACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAA CTGAGACACGGTCCAGACTCCTACGGGAGGCAGC AGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATG CCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAG CACTTTAAGTTGGGAGGAAGAGCAGTAAGTTAATACCTTGCTGTT TTGACGTTACCAACAGAATAAGCACCGGCTAACT TCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTAATC GGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTC AGCAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCA TCCAAAACTACTGAGCTAGAGTACGGTAGAGGGTG GTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGG AACACCAGTGGCGAAGGCGACCAC

Table 3. Comparative chart of potentiality of microorganisms from effluent and sediments against different concentration of TBP.

S/No	Concentration of TBP	Growth observed on TBP	No. of colonies found after spread plate (Effluent- Sediment)	Colony identification	No. of colonies found after spread plate (Effluent)	Colony Identification
1	10 ppm	+++	Three colonies	*Pseudomnas* sp. LM8, *Pseudomonas pseudoalcaligenes* DSM 50018T,*Bacillus cereus strain* EK-15	Two colonies	*Bacillus cereus* strain EK-15, *Pseudomonas* sp.N9-5
2	25 ppm	+++	Two colonies	*Pseudomonas* sp. LM 8, *Pseudomonas pseudoalcaligenes* DSM 50018T	One colony	*Pseudomonas* sp. N9-5
3	50 ppm	++	Two colonies	*Pseudomonas* sp. LM8 *Pseudomonas pseudoalcaligenes* DSM 50018T	One colony	*Pseudomonas* sp. N9-5
4	75 ppm	++	One colony	*Pseudomonas pseudoalcaligenes* DSM 50018T	Nil	-
5	100 ppm	+	One colony	*Pseudomonas pseudoalcaligenes* DSM 50018T	Nil	-

Conclusion

The present research study has identified the microbial consortium adaptability in industrial effluent disposal site. The potential micro-organism Pseudomonas *pseudoalcaligenes DSM 50018T* identified from microbial consortium by 16srRNA methods and confirmed on the basis of phylogenetic tree and sequencing. This organism has high potential to biodegrade the organic pollutant such as TBP present in low level nuclear waste.

Table 4. Biochemical characteristics shown by the most potential micro-organism isolated at 100ppm of TBP.

S/ No	Characteristics	Result	S/No.	Characteristics	Result
1.	ONPG	–	15.	Esculin	–
2.	Lysine decarboxylase	–	16.	Arabinose	–
3.	Ornithine decarboxylase	–	17.	Xylose	+
4.	Urease	–	18.	Adonitol	–
5.	Deamination	–	19.	Rhamnose	–
6.	Nitrate reduction	+	20.	Cellobiose	–
7.	H2S production	–	21.	Melibiose	–
8.	Citrate utilization	+	22.	Saccharose	–
9.	Voges Proskauer's	–	23.	Raffinose	–
10.	Methyl Red	–	24.	Trehalose	–
11.	Indole	–	25.	Glucose	+
12.	Malonate	+	26.	Lactose	–
13.	Gram Characteristic	–	27.	Oxidase	+
14.	Motility	Motile			

ACKNOWLEDGEMENTS

The present research work is a part of the Board of Research in Nuclear Sciences (BRNS) project entitled "Biotechnological approaches for the remediation of Low Level Nuclear Waste". The authors are grateful to the BRNS for giving the facility and financial grants to carry out the research work. The first author would like to extend thanks to the senior colleague Mrs. Anamika Singh for her technical assistance and support.

REFERENCES

APHA (1989). Standard methods for examination of water and waste water. American Public Health Association, New York.

APHA (1979). Standard methods for examination of water and waste water. American Public Health Association, New York.

EPA (1992). Chemical information collection and data development tributyl phosphate test results. www.epa.gov/oppt/chemtest/tributph.htm

Fulekar MH (2005a). Bioremediation technologies for environment. Ind. J. Environ. Prot. 25(4): 358-364.

Fulekar MH (2005b). Environmental Biotechnology, Oxford and IBH publishing house, New Delhi, India. pp. 52-68.

Fulekar MH (2008). Bioinformatics: Applications in Life and Environmental Sciences. Capital & Springer publication, Germany. pp. 172-192.

Fulekar MH, Geetha M (2008). Bioremediation of chlorpyrifos by *Pseudomonas aeruginosa* using scale up technique. J. Appl. Biosci. 12: 657-660.

Fulekar MH, Jaya S (2008). Bioinformatics Applied in Bioremediation. Innovative Romanian Food Biotechnology 2:28-36.

He Z, Spain JC (1998). A Novel 2-Aminomuconate Deaminase in the Nitrobenzene Degradation Pathway of *Pseudomonas pseudoalcaligenes* JS45. J. Bacteriol. 180: 2502-2506.

Healy CE, Beyrouty PC, Broxup BR (1995). Acute and subchronic neurotoxicity studies with tri-N-butyl phosphate in adult Sprague_Dawley rats. Am. Ind. Hyg. Assoc. J. 56: 349-355.

Kumar H (2004). Recent trends in Biotechnology. Agrobios, India. pp. 55-58.

Kumar S, Mukerji KG, Lal R (1996). Molecular aspects of pesticide degradation by microorganisms. Crit. Rev. Microbiol. 22: 1-26.

McDonald AR, Odin M, Choudhury H (2002). Chronic risk assessment for tributyl phosphate (CAS NO. 126-73-8). In: Society of Toxicology Meeting, Nashville, TN, March 18-21.

Murphy CD, Quirke S, Balogun O (2008). Degradation of fluorobiphenyl by *Pseudomonas pseudoalcaligenes KF707*.FEMS microbiology letters (FEMS Microbiol Lett). 286(1): 45-90.

Raushel FM (2002). Bacterial detoxification of organophosphate nerve agents. Curr. Opin. Microbiol. 5: 288-295.

SIDS (2001). Initial Assesment Report for 12th SIAM Paris, France. pp. 2-13.

Singh D, Fulekar MH (2007). Bioremediation of phenol using microbial consortium in bioreactor. Innovative Romanian food Biotechnology. 1(30): 31-36.

Thomas R, Macaskie LE (1996). Biodegradation of tributyl phosphate by natural occuring microbial isolates and coupling to the removal of uranium from aqueous solution. Environ. Sci. Technol. 30: 2371-2375.

Xiao-Yu Chu, Ning-Feng Wu, Min-Jie Deng, Jian Tian, Bin Yao and Yun-Liu Fan (2008). Expression of organophosphorus hydrolase OPHC2 in *Pichia pastoris*: Purification and characterization. 49(1): 9-14.