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Bioremediation of Nuclear Waste Compound-TCE Using Microbial Consortium/Potential Organism in Different Designed & Developed Reactors

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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Original Research Article

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ABSTRACT

Nuclear waste dumping site found to contain the traces of organic compounds and heavy metals; in spite of the present physicochemical and biological treatment. The organic waste was commonly found to contain traces of Trichloroethylene. The remediation technology has been developed by using the bioresources of adapted microbial consortium and potential microorganisms at varying concentration in designed and developed reactors such as Shake Flask reactor (SFB), Packed Bed Reactor (PBR) and Hybrid Reactor (HR). The effectiveness of the remediation of TCE in different reactors studied. Remediation of TCE by microbial consortium and potential organism has been studied. The strain improvement method like immobilization, adaptation and mutation were found effective for developing cultures to increase biodegradation potential. In the present study, the reactors and the culture developed used for remediation of Nuclear Waste Compound- TCE has proved for developing bioremediation technology.

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1. INTRODUCTION

Nuclear wastes, also called as radioactive wastes mainly originate from nuclear-power generation (87%), radionuclide production for chemotherapy in the medical industry (1%), radioactive probe production in genetic studies (2%), and production of sterilization agents in agricultural and several other industrial applications. Nuclear wastes are also produced during uranium mining, milling, preparation of fuel for reactors, and nuclear weapon-production. Nuclear wastes may be in the form of gases, liquids, or solids, may be soluble or insoluble, and may give off various types of radiation at many energy levels. Nuclear waste contain a variety of organic compounds like trichloroethylene (TCE), tributyl phosphate (TBP), dibutylphosphate (DBP) etc. present as a result of different activities like spent ion exchange resins, decontamination solutions, organic solvents, scintillation liquids etc. The direct release and persistence of these contaminants in soil and water bodies possess lethal and sub-lethal effects on a variety of organisms.

Considering the wide range of utility and potential hazards of these organics a suitable method needs to be developed for transforming them into environment friendly compounds. The various remediation technologies used also includes in situ vitrification and soil incineration, excavation and land filling, soil washing, soil flushing, solidification, stabilization by electro kinetic systems and are colloquially termed as *"pump and treat"* and "*dig and dump"* techniques [1,2].

In the present study, the waste dumping site has been characterized for physico-chemical and microbial assays. The organic contaminant commonly found at the waste disposal site such as TCE was selected for developing the remediation technology using the indigenous microorganisms. The microbial consortium isolated from contaminated site was cultured and enriched for TCE degradation where, TCE served as the sole carbon and energy source. Further, this potential microbial culture was adapted to inhibitory concentrations of each of these compound in minimal salt medium (MSM) in a scale-up process technique. The culture was adapted to TCE, from 100 mg/l to 1000 mg/l. Biodegradation studies were then carried out using the indigenous as well as the adapted culture. Studies using indigenous cultures were conducted at the selected (low) concentrations of the compounds while the adapted culture was used for inhibitory concentrations of the compounds. The use of specialized cultures like immobilized cells (in ca-alginate gel), mutated stains and mixed cultures were also employed to enhance the degradation rate and substrate tolerance of the bacterium with respect to TCE. The efficacy of the specialized cultures so obtained was then compared with the indigenous cultures. The continuous degradation studies for TCE was also carried out using the specialized cultures. GAC immobilized cells and the use of mixed culture was employed during TCE bioremediation studies. A hybrid reactor and a packed bed reactor were used for continuous TCE degradation. The reactors were operated at various organic loadings and/or flow rates and the performance of the culture with respect to the remediation of the selected compounds was monitored. The microorganism, *P. pseudoalcaligenes* MHF ENV was identified as the versatile organism for remediation of TCE.

2. MATERIALS AND METHODS

2.1 Sampling Site

The chemical processes/operations of various small and large scale industries generate effluents which contain organic and inorganic wastes. These wastes are treated by various physico-chemical methods to comply with the standards prescribed under the Environmental Protection Act, 1986. However, a considerable amount of organics still persist in the effluents. The common waste disposal site contains effluents from various industries. Due to presence of various toxic pollutants, waste disposal site is a rich source of versatile microbes harboring various genes encoding enzymes that degrade these contaminants. Therefore, in the present study the common industrial waste disposal site located in an industrial belt at Chembur 60 km away from the Bhabha Atomic Research Centre (BARC), Chembur, Mumbai, India was selected as the sampling site. The industrial effluent treated by various groups of industries like Fertilizers, Petrochemical, Power plants and other chemical

industries are discharged at this site though unlined channels. The waste disposal site has been characterized for physico-chemical and microbial properties so as to assess the present status of the environmental conditions leading to water soil pollution in and around area.

2.2 Sample Collection

Contaminated soil and water samples were collected from thee different sites (I-III) around this industrial waste disposal site by following the stratified random sampling method. Soil samples were collected in clear plastic bags whereas the effluent samples were collected in pre-cleaned and dried plastic cans which were free from toxic substances. Sampling bottles were washed with detergent, rinsed with running water, and finally rinsed with double distilled water. For further physico-chemical and microbial characterization, samples were preserved at a low temperature (4℃) in the laboratory to avoid any deviation and contamination of samples.

2.3 Characterization of Water Sample

Water samples collected from thee different sites of waste disposal area were examined for various physicochemical parameters like pH, Temperature, Electrical Conductivity (EC), Total Solids (TS), Total Suspended Solids (TSS), Acidity (A), Total Alkalinity (TA), Phosphate (P), Hardness (H), Total dissolved solids (TDS), Sodium (Na), Potassium (K), including biological characterization like Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and Dissolved Oxygen (DO). Analysis were performed as per the procedures described in "Standard methods for the examination of water and waste water", 17th edition, [3,4].

2.4 Characterization of Soil Sample

Contaminated soil samples collected were air dried ground and passed through a 2 mm pore size sieve and were stored in sealed containers at 4℃ until use. The samples were analyzed for the above mentioned physico-chemical parameters as per the "APHA, [3,4].

2.5 Bioremediation Studies: Organic Contaminants

In this study, an organochlorine compound, TCE were selected as model compounds for the investigation on biodegradability of toxic organics commonly present in Low Level Nuclear Waste (LLNW). These organic contaminants were selected as they comprise a significant component of both soluble and solid LLNW. These organics if untreated; persist in soil-water and may cause severe environmental pollution.

2.6 Identification and Characterization of Potential Microorganism for Bioremediation of TCE

Microbial consortium was isolated from effluent and soil samples of the common industrial waste disposal site, which was further used for isolation of potential microorganism for bioremediation.

2.6.1 Enrichment & Isolation of potential microorganism for bioremediation of Trichloroethylene (TCE)

2.6.1.1 Spiking of the compound

TCE was added to the Bifidus Selective Medium (BSM) using micro syringe.

2.6.1.2 Enrichment and isolation of TCE degrader

The bacteria were obtained from the contaminated sites were screened for their ability to utilize TCE as the sole source of carbon. The cultures were grown individually in nutrient broth and after a period of 24 h incubation, ten milliliters of the broth was centrifuged, washed in phosphate buffer (25 mM, pH 7.0) and transferred to a 160 ml serum bottle containing 100 ml of BSM with the final OD of 0.1. TCE was added directly to get the final concentration of 50 mg/l. The bottle was tightly sealed with teflon coated rubber septa and screw cap to prevent the release of TCE by volatilization. The bottle was then incubated at 30℃ in a rotatory shaker operating at 150 rpm. After a period of 96 h samples were withdrawn to analyze the residual TCE concentration. The bacterium that exhibited highest degradation rate was selected as TCE degrader. Blank flasks consisted of sterile controls to monitor the abiotic loss of TCE.

2.6.2 Enrichment & Isolation of microbial consortium for bioremediation of Trichloroethylene (TCE)

Bacterial pure cultures were procured from culture banks and tested for their ability to degrade TCE. The cultures procured viz. *Pseudomonas resinovorans, P. fuoroscens,*

P. putida, P. aeruginosa were grown individually in nutrient broth and after a period of 24 h incubation, ten milliliters of the broth was centrifuged, washed in phosphate buffer (25 mM, pH 7.0) and transferred to a 160 ml serum bottle containing 100 ml of BSM with the final OD of 0.1. TCE was added directly to get the final concentration of 100 mg/l. The bottle was tightly sealed with teflon coated rubber septa and screw cap to prevent the release of TCE by volatilization. The bottle was then incubated at 30℃ in a rotatory shaker operating at 150 rpm. The cultures that depicted the ability to utilize 100 mg/l of TCE for its growth were pooled together. 1ml of each of these potential cultures was transferred to a 160 ml serum bottle containing 100 ml of BSM with the final OD of 0.1 and 50 mg/l of TCE. The bottle was then incubated at 30℃ in a rotatory shaker operating at 150 rpm for a period of 7 days. After the period of 7 days, the microbial consortium capable of degrading TCE was obtained. This microbial consortium was used further for adaptation studies at higher TCE concentrations.

2.6.3 Adaptation of the potential bacterium to TCE using laboratory scale-up process

The bacterium *P. pseudoalcaligenes* MHF ENV was acclimatized to higher concentrations of TCE (100 mg/ l) using scale-up process. A 5 ml aliquot of the pre- grown culture in 50 mg/l of TCE was sub cultured after 96 h into fresh BSM with 100 mg/l of TCE. The bottle was kept on orbital shaker incubator at 150 rpm, 30℃ for 96 h. After 96 h significant bacterial growth was observed; the BSM had turned turbid. Thereafter the culture was sub-cultured to new BSM with increments in TCE concentrations (250, 500, 750 and 1000 mg/l) till the culture was sub-cultured to BSM containing 1000 mg/l. The total adaptation period lasted for sixty two days. No sample was taken from these flasks till this time so as to avoid contamination and save time. This adapted culture was further used for batch TCE degradation studies.

2.6.4 Adaptation of the microbial consortium to TCE using laboratory scale-up process

The microbial consortium was adapted to TCE in a scale-up process. The consortium was adapted to higher TCE concentration from 100-500 mg/l. The total adaptation period lasted for sixty two days. No sample was taken from these flasks till this time so as to avoid contamination and save time. This adapted culture was further used for batch TCE degradation studies.

2.6.5 Bioremediation of TCE in bioreactor with potential/adapted microorganism

Bioremediation of the organic contaminants was carried out using the adapted potential bacterium in a batch and (/or) continuous bioreactor. The organic solvents used for stock preparation and extraction obtained from Merck were of highest purity. The components used for bacteriological medium, mineral salt medium and immobilization were purchased from Hi-Media, India.

2.6.6 Bioremediation of TCE in bioreactor using the potential / adapted microorganism

Bioremediation studies for TCE degradation were carried out using indigenous (potential) and adapted culture of *P. pseudoalcaligenes* MHF ENV. Degradation studies were carried out in various bioreactors like shake flask bioreactor (SFB), packed bed bioreactor (PBR) and two-stage bioreactor.

2.6.7 Bioremediation of TCE in a SFB using potential microorganism

2.6.7.1 Design and development of SFB for TCE degradation

The SFB designed for TCE degradation consisted of a serum bottle made up of glass with a capacity of 250 ml. The bottle was provided with a teflon coated rubber septa and screw cap to prevent the release of TCE by volatilization. The mixing and aeration was provided by the use of an orbital shaker. The orbital shaker incubator also had a temperature control provision. The nutrient medium consisted of BSM as described in section with TCE as the sole carbon source.

2.6.7.2 Development of inoculum for SFB

Indigenous culture: The culture was grown in nutrient broth and after a period of 24 h incubation, 10 ml of the broth was centrifuged, washed in phosphate buffer (25 mM, pH 7.0) and used as inoculum.

Adapted culture: The adapted biomass was obtained used as inoculum in SFB.

2.6.7.3 TCE degradation in SFB using indigenous culture

The experimental set up for TCE degradation in consisted of 5 SFB with sterile BSM and TCE as sole carbon source. The inoculum was added to each bottle (final $OD_{600\ nm}$ of 0.1) followed by addition of TCE to get the final concentration of 25, 50 and 100 mg/l. Each bottle was then sealed and kept in an orbital environ-shaker operated at 30℃ and 150 rpm for a period of 120 h. Every 24 h, one bottle was removed for analysis of residual TCE and change in OD at 600 nm and COD was monitored. The chloride ion release was checked using a chloride ion selective electrode. The COD was measured using open reflux method [5] Parallel control bottles were utilized to assess abiotic losses (volatilization and adsorption losses). Controls contained the same aqueous volume and compound concentration but were not inoculated. Biodegradation was assessed by comparing the disappearance of TCE in experimental bottle compared to the controls over time. A second control set containing autoclaved (dead) cells and TCE was used to assess non-active biodegradation (i.e., binding to the cell wall). All experiments were conducted under aseptic conditions and in triplicates.

2.6.7.4 TCE degradation in SFB using adapted cells

Batch TCE degradation studies were also carried out using adapted cells of *Pseudomonas pseudoalcaligenes* MHF ENV. Batch TCE degradation experiments were conducted in similar way as described for the indigenous cells. TCE concentrations of 100, 250, 500, 750 and 1000 mg/l were selected for degradation study. Samples were withdrawn and monitored for change in CFU and TCE degradation. Other parameters like BOD, COD and chloride release were also monitored. The rate of TCE degradation by adapted and indigenous bacterium was compared and adaptation of the culture was confirmed.

2.6.8 Bioremediation of TCE in designed and developed PBR

2.6.8.1 Design and development of PBR for TCE degradation

The PBR used for TBP degradation was a tubular, jacketed column made of glass, with dimensions of 40 x 6 cm from Pharmacia Ltd. The bed volume was 75 ml. The reactor was provided with an inlet for entry of the feed solution in the reactor and a outlet for removal of spent medium from the reactor. The feed solution was sterile Minimal Salts Medium (MSM) with TCE as sole carbon source. The feed was kept on a magnetic stirrer for constant mixing of the solution. The stirring of the medium also served for aeration of the medium. The medium was pumped into the reactor with the help of a peristaltic pump in an upward flow. The reactor was operated at different flow rate by increasing or decreasing the rpm of the peristaltic pump. All components in contact areas of the reactor were made of glass. The PBR performance was evaluated on the basis of the void volume (30 ml) in the reactor.

2.6.8.2 Development of inoculum – GAC immobilized culture for PBR

The inoculum used in the PBR was GAC immobilized culture of *P. pseudoalcaligenes.* GAC (Calgon type MRX-P, Calgon Carbon Corp., Pittsburgh, PA, USA) with a mesh size of 10 x 30 was used as the biomass support for immobilization. The GAC (30 g) was washed several times with deionized water to remove free residues, dried in oven at 105℃ and autoclaved at 121℃, 15 min. The reactor was inoculated by recirculating 2.0 liters of bacterial inoculum (pregrown in 500 mg/l TCE) contained in BSM at the dilution rate of ml 0.5/h. The medium was fed with 500 mg/l TCE after every two days to avoid starvation of the bacterial culture. After a period of seven days, colonization of the support material with biofilms formation was observed. To check biomass immobilization, GAC (1.0 g) was withdrawn from the reactor, dispersed in 50 ml of saline and vortexed for 30 s. The resulting bacterial suspension was centrifuged and the pellet was dried at 60℃ in an oven and checked for the dry weight.

2.6.8.3 PBR working for TCE degradation

After bacterial colonization, the PBR was started with passage of sterile feed solution of BSM with 500 mg/l of TCE. The reactor was then operated at various dilution rates like 15, 30, 60, 120 and 180 ml/h each for a period of fifteen days. The reactor was started with the lowest, flow rate of 15 ml/h and was then increased to the next flow rate after a period of 15 days. Likewise after a period of 15 days, the flow rate was increased till 180 minutes/hour (m/h). After the PBR was

operated at all the flow rates at 500 mg/l, the organic loading was increased by increasing the TCE concentration in the feed solution to 750 mg/l and then to 1000 mg/l. The reactor was operated at all the flow rates at each of the concentration for a period of 15 days. The treatment efficiency was monitored by measuring outlet TCE concentration (abiotic losses were subtracted from the outlet values).

2.6.9 Bioremediation of TCE in designed and developed hybrid reactor

2.6.9.1 Design and development of two-stage bioreactor

A TSR was developed by connecting a plug flow reactor (PFR) to the PBR used in section. The PFR was made of glass with dimensions of PBR 25 x 2.5 cm; and working volume of 85 ml. The PFR was connected in series to the PBR such that, the effluent from PBR was channeled into the PFR. Since, the outlet samples of PBR were channeled to the PFR, the flow rate of PBR and PFR was same over the particular period of time under study. Sterile air was passed into the PFR at the flow rate of 3 liter/hr

2.6.10 Development of inoculum

2.6.10.1 PFR working for TCE degradation

The outflow samples from PFR were analyzed for residual TCE concentration and biomass concentration. The residual biomass concentration was measured using infra-red moisture determination instrument (Kett, Japan). The effluent TCE concentration was corrected for the volatilization losses as measured in the condenser outlet.

2.6.10.2 Stability studies in hybrid reactor

The hybrid reactor was further operated at the dilution rate of 60 ml/h and inlet TCE concentration of 750 mg/l for a period of sixty days and its performance with respect to the effluent TCE concentration and biomass generated were monitored. After a period of sixty days, the reactor was inoculated with contaminating bacteria (consortium of bacterial cultures tested during screening process) at the dilution rate of 120 ml/h for a period of 8 h. The reactor was then again operated at dilution rate, 60 ml/h; inlet TCE, 750 mg/l for a period of thirty days. The effluent TCE concentration was monitored and the contaminating bacteria were monitored by spread plate technique. The identity of *P. pseudoalcaligenes* MHF ENV was easy due to its green pigmentation and was reconfirmed by biochemical tests.

2.6.10.3 Bioremediation of TCE in a bioreactor using microbial consortium

The same procedure was followed as described above. TCE concentration of 100-500 mg/l was selected for bioremediation studies.

2.6.10.4 Bioremediation of TCE in designed and developed hybrid reactor using microbial consortium

The same procedure was followed as mentioned above. The organic loading varied between 500-2500 mg/l.

3. RESULTS AND DISCUSSION

In the present research study, common industrial waste disposal site located at Chembur, Mumbai was characterized for physico-chemical and microbial characteristics. The microbial consortium present at the waste disposal sites was characterized. This microbial consortium was used further for enrichment and isolation of potential bacterium capable of degrading the selected organic contaminant –Trichloroethylene (TCE). The microbial consortium/individual isolates were exposed to the selected contaminant in an incubator shaker for enrichment and isolation of the potential microorganism. The monoclony of the microorganism that survived at the highest concentration of each compound was further adapted to inhibitory compound concentration using adaptation in a scale up process technique. Microbial consortium as well as potential microorganism was identified by using biochemical assays and 16SrDNA sequencing technique.

After the isolation and identification of the potential bacterium capable of degrading the compound TCE, the study further aimed at enhancing the performance of the bacterium with respect to the compound degradation rate, degradation efficiency and substrate tolerance. Thus, various methods like immobilization and use of mixed microbial cultures were employed to enhance the bioremediation rate. The degradation rate and substrate tolerance of these modified or the specialized culture so obtained was then compared with that of the

indigenous culture. Further, bioremediation of organic contaminant (TCE) was carried out using these specialized cultures in a shake flask bioreactor under controlled environmental conditions. Continuous degradation studies were also conducted in various bioreactors like packed bed reactor and hybrid reactor with immobilized/ free cells as catalysts. The reactor system was optimized with respect to flow rate and the stability of the performance was also monitored.

3.1 Identification of Potential Consortium for TCE Bioremediation

Various *Pseudomonas* cultures were procured from National Collection of Industrial Microorganisms (NCIM) and Institute of Microbial Technology (IMTECH). These cultures were cultured in nutrient broth containing 100 mg/l of TCE and incubated individually for two weeks. The cultures were then tested for their ability to utilize 100 mg/l of TCE as sole carbon source in MSM. The cultures capable of utilizing TCE as sole carbon source namely *Pseudomonas putida, Pseudomonas resinovorans and Pseudomonas pseudoalcaligenes* were then pooled together and exposed again to TCE concentration of 100 mg/l. After a period of 7 days, the culture was plated on nutrient medium. It was observed that, all the three cultures were compatible with each other and all the three cultures dominated equally in the medium. This microbial consortium comprising of various species of *Pseudomonas* cultures developed was used further for bioremediation studies. The potential and versatility of *Pseudomonas* species in the field of bioremediation has been reported in many research studies. The *Pseudomonas* species have been found to be versatile for their ability to degrade the different type of pollutants including; PCBs, textile dye direct orange 102 [6] and crude oil components [7].

3.2 Adaptation of Isolated Microorganism to the Selected Compound (TCE) in Scale-up Process

The microorganism, *P. pseudoalcaligenes* MHF ENV isolated and identified as a potential degrader for the organic contaminant (TCE) was adapted in minimal salt medium (MSM) from lower to higher concentrations using scale up process technique in incubator shaker under controlled environmental conditions. The scale-up process has been carried out with subsequent repeated sub-cultures from low concentration of organic contaminants to higher concentration upto 14 days under controlled environmental conditions. After, scale up process microorganism was found adapted to different concentrations of selected organic contaminants.

3.3 Adaptation of Potential Bacterium to High TCE Concentration Using Scale-up Process

P. pseudoalcaligenes MHF ENV was found adapted to TCE concentrations of 100 mg/l up to 500 mg/l using scale-up process technique in shake-flask bioreactor under controlled environmental conditions. During scale up process, microbial growth was observed in terms of OD at 600 nm using UV-Visible spectrophotometer. The growth pattern of potential microbial consortium during adaptation process on increasing TCE concentrations indicates that maximum OD_{600} observed was 0.25 after 6 days in 100 mg/l from initial OD_{600} of 0.071, followed by 0.4 for 250 mg/l from initial OD_{600} of 0.065 on day 14. Whereas in case of 500 mg/l maximum growth observed at 14^{th} day i.e. 0.289. At 100 mg/l, the cells exhibited exponential growth till 6^{th} day, after which the OD was found decreasing due to the depletion of TCE in the medium. In case of 250 and 500 mg/l, the culture exhibited exponential growth throughout the period of adaptation i.e. till 14^{th} day. At 500 mg/l maximum growth was observed after 14^{th} day with the OD of 0.289 (Fig. 1). After adaptation of the culture using scale-up process the culture was found to grow and survive at inhibitory concentrations of TCE of up to 500 mg/l. This adapted culture was used further for bioremediation studies.

3.4 Adaptation of Microbial Consortium to High TCE Concentration Using Scale-up Process

The microbial consortium isolated for TCE degradation was adapted to TCE concentrations of 100 mg/l up to 500 mg/l using scale-up process technique in shake-flask bioreactor under controlled environmental conditions. During scale up process, microbial growth was observed in terms of OD at 600 nm using UV-Visible spectrophotometer. The growth pattern of potential microorganism *P. pseudoalcaligenes MHF ENV* during adaptation process to increasing concentrations of TCE indicates that maximum OD_{600} observed was 0.42 after 6 days in 100 mg/l from initial OD_{600} of 0.05, followed by

.36 for 250 mg/l from initial OD_{600} of 0.725 on day 8. Whereas in case of 500 mg/l maximum growth observed at 10^{th} day i.e. 0.689. A small lag phase was observed in case of 500 mg/l of TCE. At the end of the experiment, in all concentrations microorganisms reached at stationary phase due to death of microorganism after a period of 7 days. There was no significant increase in the OD_{600} that of control. After adaptation of the culture using scale-up process the culture was found to grow and survive at inhibitory concentrations of TCE of up to 500 mg/l. This adapted culture was used further for bioremediation studies.

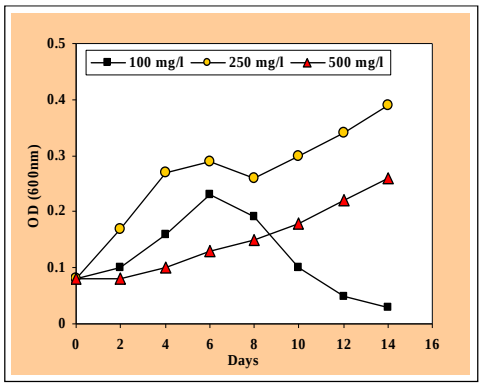


Fig. 1. Change in optical density during adaptation of the culture to inhibitory concentrations of TCE

3.5 Biodegradation of TCE in bioreactor with *P. pseudoalcaligenes* MHF ENV (adapted/indigenous microorganism.)

Trichloroethylene (TCE), a chlorinated aliphatic compound is one of the most predominant groundwater contaminants. The entry of TCE into the environment is a significant public health and environmental concern due to its carcinogenicity, toxicity, and flammability. The U.S. Environmental Protection Agency (U.S. EPA) has reported TCE as an environment priority pollutant [8]. TCE removal by dumping and air stripping is restricted by the legislation and thus attention is now being paid to its biological degradation in soil and water. In the present study, bioremediation of TCE has been carried out using potential microorganism *P. pseudoalcaligenes* MHF ENV in different bioreactors under controlled conditions.

3.6 TCE degradation in Shake-Flask Bioreactor (SFB)

A SFB was developed or TCE degradation that consisted of a serum bottle with BSM and TCE as sole carbon source. The serum bottle was housed in temperature controlled cabinet. The inoculum used in this SFB was culture of *P. pseudoalcaligenes*.

i) *Using indigenous culture:* Batch TCE degradation studies were carried out in shake-flask bioreactor using *Pseudomonas pseudoalcaligenes* MHF ENV at selected TCE concentrations viz. 25 mg/l, 50 mg/l and 100 mg/l. Analysis of experimental medium when compared with that of the control showed that, *P. pseudoalcaligenes* MHF ENV depicted the ability to utilize TCE as the sole carbon source. This finding is in contrast to earlier reported isolates that required the presence of a primary substrate like methane, propane, isoprene, phenol etc. for TCE degradation [9-11]. Analysis of biodegradation samples results showed complete degradation of 25 mg/l and about 86% degradation at an initial TCE concentration of 50 mg/l in a time span of 72 h and 96 h (Table 1) and (Fig. 2). At higher TCE concentration (100 mg/l) 34% degradation was achieved at after the period of 120 h. The slower degradation at 100 mg/l shows that this concentration of TCE was toxic to *Pseudomonas pseudoalcaligenes* MHF ENV. Thus, bioremediation of TCE was found effective by at the concentrations of 25 and 50 mg/l.

ii) *Using adapted culture:* Biodegradation studies were carried out at 100 mg/l using adapted culture in shake flask bioreactor. It was observed that, the adapted culture could completely degrade 100 mg/l of TCE in 72 h as compared to 34% for the non-adapted culture in 120 h (Table 1) and (Fig. 2).

Result shows that, adaptation of the culture enhanced the TCE degradation rate by 66% as compared to the indigenous culture. This finding is in accordance with earlier research studies reporting that, adaptation acclimatizes the culture to the compound and thereby exhibits enhanced degradation rates [12]. Spain et al. [13] have also reported that, an increase in the rate at which microorganism transforms the contaminant provides evidence that adaptation has occurred and bioremediation is working. Studies on cell growth pattern showed that, the lag period of 3 h required by the indigenous culture to start TCE degradation was overcomed by the adapted culture. Moreover, the adapted

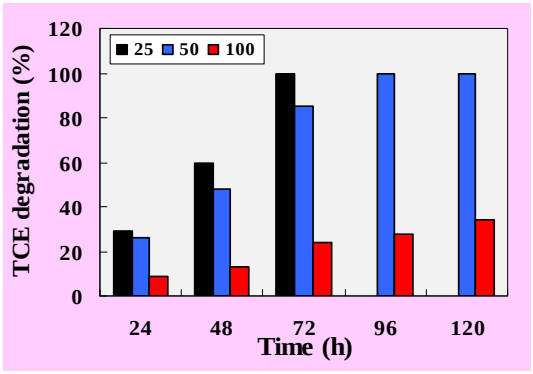
culture depicted higher cell growth than the indigenous culture. In other words, adaptation of the culture was effective to enhance the growth and TCE degradation rate of the culture. Biodegradation studies using adapted culture at higher TCE concentrations showed complete TCE degradation at the concentration of 250 mg/l in a time span of 96 h whereas; about 80%, 33% and 20% degradation was achieved at TCE concentrations of 500, 750 and 1000 mg/l respectively after a period of 120 h (Fig. 3a). Thus, the adapted culture of *Pseudomonas pseudoalcaligenes* MHF ENV depicted the ability to utilize high TCE concentration of up to 1000 mg/l although, degradation at TCE concentrations beyond 500 mg/l was relatively slower. The maximal degradation rate of 3.33 ± 1.1 mg/l/ h was achieved at the TCE concentration of 500 mg/l. Meza et al. [14] have reported TCE degradation rates of 23–50 mg/l/h at TCE concentrations of 228–900 mg l^{-1} of TCE. These rates are much lower in comparison to the initial rates of 1.6-2.9 mg/l/h depicted by the isolate in this study at TCE concentrations of 500-1000 mg/l. Recently, [8] have reported the ability of a *Bacillus cereus* strain 2479 to utilize TCE as the sole carbon source up to 2000 mg l^{-1} with a degradation rate of 33.3 mg l^{-1} h^{-1}. However there is no evidence of TCE degradation below 1000 mg l^{-1}. In contrast to this, the bacterium in this study degraded TCE to environmental safe levels (2.5 mg l^{-1}). Complete mineralization of a compound is often a desirable property in degradation studies [15]. The bacterial stain used in this study depicted complete mineralization of TCE with release in chloride ions thus proving to be an efficient TCE degradation process. This shows that *P. pseudoalcaligenes* MHF ENV can be used to remediate TCE to decontaminate the environment.

The environmental parameters have also been studied under which TCE was remediated using flask shake method under controlled environmental conditions in an incubator shaker. pH was monitored throughout the experiment which was found to be decreasing from 7 to 6.4, 6.3 and 6.5, 6.7 after 5 days of the experiment in case of 250 mg/l, 500 mg/l, 750 mg/l and 100 mg/l respectively. This variation in pH is due to the interaction of microorganism with TCE and decreasing the pH due to the various intermediates formed during TCE degradation. Therefore, as the concentration of TCE increases (up to 500 mg/l), the intermediates formed increases and results into decrease in the pH. Say et al. [16] have also correlated degradation of TCE with pH values. Temperature was found to be optimum for the growth and proliferation of microorganism for bioremediation of TCE. It was found to stable at 33℃ throughout the experiment. Biological parameters were also studied as a parameter responsible for microbial growth and proliferation. Microbial growth, measured in terms of cfu/ml was found highest at a concentration of 500 mg/l as compared to 250 mg/l whereas in case of 750 and 100 mg/l growth was slower. Growth was very slow in 1000 mg/l of TCE concentration. Maximum growth was observed on 4^{th} day in case of 250 mg/l and at initial concentration of 500 mg/l an exponential growth was found till the 5^{th} day. In case of 750 and 1000 mg/l a small lag phase was observed followed by exponential growth till 5^{th} day. Reduction in total cfu count shows the death of microorganism and lack of nutrients available after a certain period (Fig. 3b).

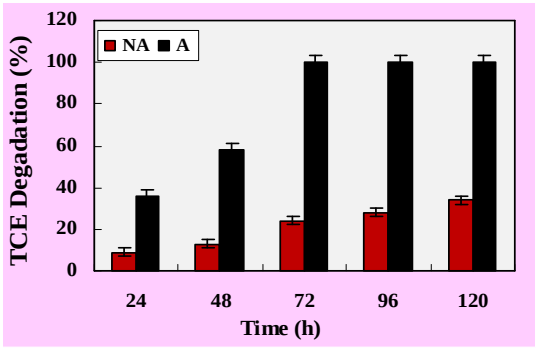
BOD was found to increase from 3.5 mg/l to 4.0 mg/l in 250 mg/l, from 3.9 mg/l to 4.9 mg/l in 500 mg/l from 1.6 mg/l to 2.8 mg/l in 750 mg/l and from in 2.8 to 3.4 mg/l for 1000 mg/l of TCE concentration after a period of 120 h (Fig. 3c). The observed increase in BOD is primarily due to the consumption of oxygen by the microbial

Table 1. Variation in residual TCE concentration, TCE removal efficiency and cell count during TCE degradation in shake flask bioreactor using adapted (A) and non-adapted culture (NA)

TCE (100 mg/l)	Shake flask bioreactor studies									
	Day I (24 h)		Day II (48 h)		Day III (72 h)		Day IV (96 h)		Day V (120 h)	
	NA	A	NA	A	NA	A	NA	A	NA	A
Residual TCE (mg/l)	81.0	64.0	87.0	42.0	76.0	Nil	72.0	Nil	66.0	Nil
Removal efficiency (%)	6.0	36.0	13.0	58.0	24.0	100	28.0	100	34.0	100
Cell count (Log c.f.u.)	7.76	8.32	6.88	7.53	8.97	10.05	8.07	10.69	8.13	10.47



(a)



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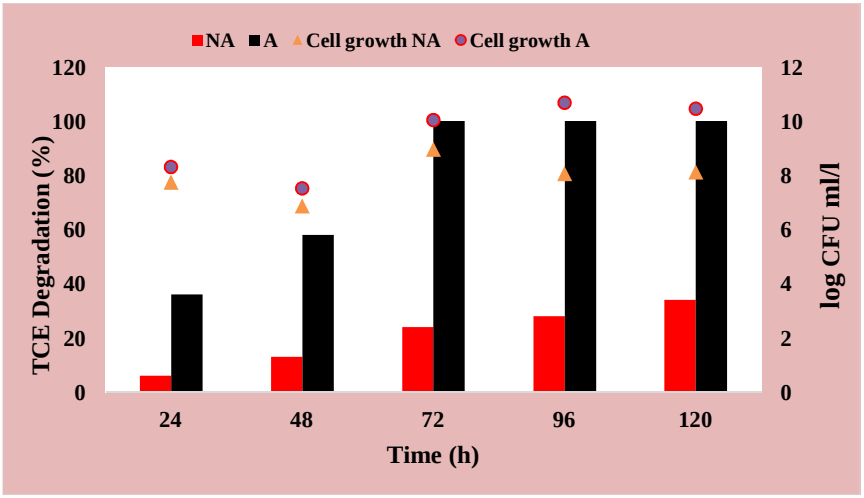


(c)

Fig. 2. TCE degradation by *P. pseudoalcaligenes* MHF ENV at various concentrations (a). Comparison of TCE degradation and cell count (b,c) using adapted (A) and non-adapted culture (NA) culture of *P. pseudoalcaligenes* MHF ENV at 100 mg/l of TCE

biomass for their growth and proliferation. These results are supported by findings of Medjor et al. [17] who reported increase in the BOD valued during bioremediation of the contaminant. In case of control, BOD was found to decrease after a period of 5 days up to non-detectable amount which shows there was no growth of microorganisms. Therefore, less growth of microorganisms results into less increase in BOD values.

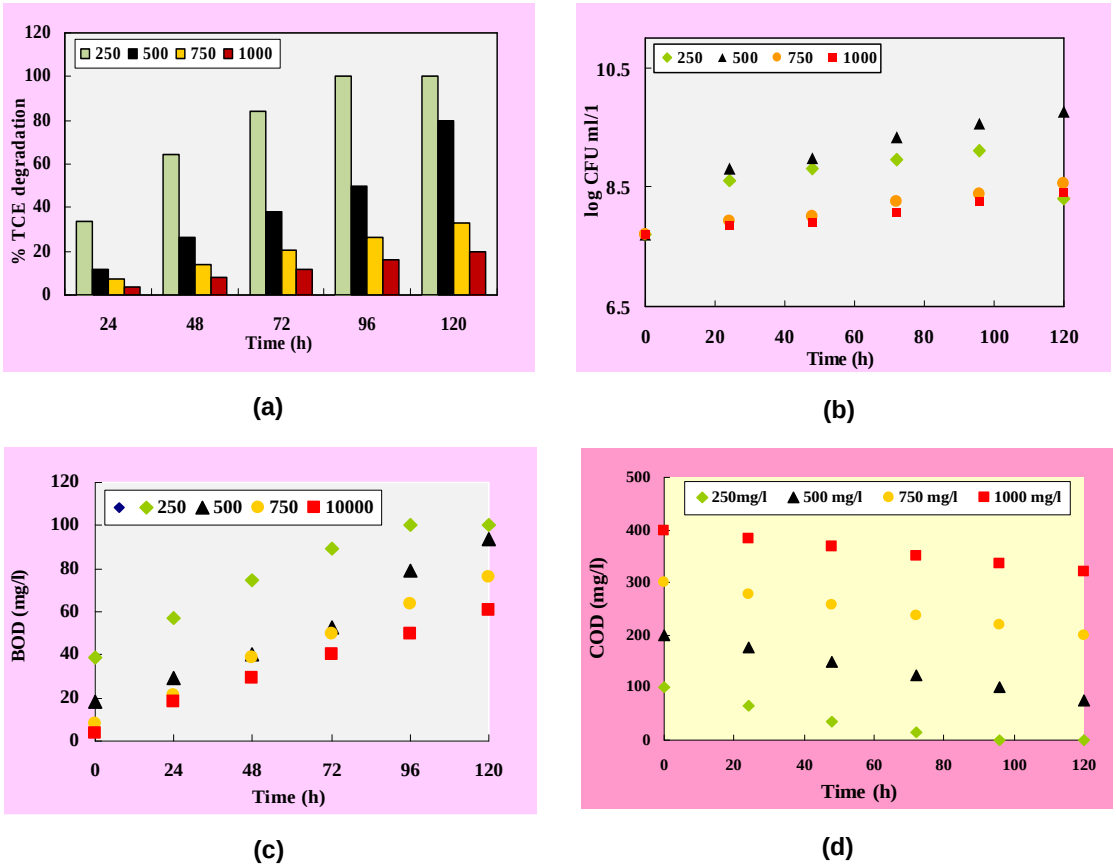


Fig. 3. Change in TCE concentration (a), cell count (b), BOD (c) and COD (d) during TCE bioremediation over a period of 120 h

Chemical oxygen demand (COD) was also monitored which shows the decrease in COD levels over a period of bioremediation, which indicates the degradation of organic compounds by potential microorganism present in the minimal salt medium. Significant decrease in COD values was observed at all the selected concentrations as the bioremediation experiment proceeded. COD decreased up to 99.1% in 250 mg/l, 75.8% in 500 mg/l, 34% in 750 mg/l and 27% in 1000 mg/l concentration of TCE containing medium after a period of 5 days of experiment. (Fig. 3d)

3.7 TCE Degradation in PBR Using Potential Bacterium

Continuous TCE degradation has been reported using biological means like use of bacterial cultures [18,19] as well as by physical methods like photocatalytic degradation. In the present study, continuous TCE degradation studies was carried out in a PBR using *P. pseudoalcaligenes* MHF ENV. The PBR was operated at various flow rates and the effect of flow rate on degradation rate, efficiency and effluent TCE concentration was monitored.

Results show that, at the lowest flow rate, analysis of the effluent samples showed that the reactor required a period of five days for achieving steady–state as observed from the effluent TCE concentration. An increase in the flow rate till 60 ml h^{-1} increased the TCE degradation rate up to 510 ± 1.6 mg $l^{-1}h^{-1}$. A proportional increase in biomass was observed with increase in the flow rate. At higher flow rates beyond 60 ml h^{-1} TCE degradation rate decreased possibly due to less contact between the cells and the substrate. The reactor was also operated with higher organic loading of 750 and 1000 mg l^{-1} at flow rates as above.

From the various parameters studied, the optimal condition for PBR operation with respect

to TCE degradation efficiency were; the flow rate of 60 ml h^{-1} and the organic loading of 500 mg/l that achieved complete degradation of 500 mg l^{-1} with the retention time of 0.5 h. However, maximal TCE degradation rate of 596 ± 1.7 was achieved at the TCE loading of 750 mg/l with flow rate of 30 ml/h (Table 2). The optimum degradation rates obtained in our studies were the highest rates achieved till date.

3.8 TCE Degradation in Hybrid Bioreactor (HR) Using Potential Bacterium

Although significant amount of TCE removal was obtained in the PBR, the effluent TCE concentration was noteworthy. To overcome this limitation, a PFR in conjugation with PBR was used that served an additional treatment to minimize the effluent TCE concentration to negligible amounts. Although the use of hybrid reactors combining trickling filter and activated sludge process [20], coupled anaerobic-aerobic bioreactor [21] has been described in previous studies, we report the novel combination of PBR and PFR for TCE aerobic degradation. The PBR served as a chamber for maximal TCE removal and biomass generation, while this generated biomass was used for further reduction of TCE to negligible levels in the PFR. The effect of flow rate on TCE degradation rate and effluent TCE concentration in hybrid bioreactor was monitored each for a period of 15 days.

Results show that, the rate of TCE degradation in PFR was dependent on the biomass concentration in the PBR outlet. With the increase in biomass concentration in the PBR effluent, the TCE degradation rate increased. The maximal TCE degradation rate in PFR was found to be 66.5 ± 2.0 mg $l^{-1}h^{-1}$ at the flow rate of 60 ml h^{-1} and inlet TCE of 500 mg l^{-1}. However, considering the objective of achieving negligible effluent concentrations for which the hybrid reactor was designed the TCE degradation rate of 53.9 ± 1.4 mg $l^{-1}h^{-1}$ achieved at the flow rate of 30 ml h^{-1} and inlet TCE being 750 mg l^{-1} in PFR was considered to be optimum with zero TCE discharge in the effluent. While, the hybrid reactor depicted complete TCE removal efficiency of 500 mg l^{-1} and 750 mg l^{-1} at flow rate 30 mg $l^{-1}h^{-1.}$ From the various parameters studied, it was observed that the use of PFR in conjugation with PBR can enhanced the TCE degradation efficiency of up to 30.4 ± 2.1% in 500 mg/l inlet and 20.54 ± 1.2% in the case of 750 mg/ l inlet (Table 3). In other words, the biomass obtained from PBR effectively served as a source of inoculum in PFR for enhancing the degradation rate and efficiency of TCE degradation.

In order to check the stability of bacterium used in this study, the hybrid reactor was operated at the flow rate 30 ml h^{-1}; inlet TCE, 750 mg l^{-1} for a period of 100 days to monitor the stability of the culture with respect to TCE degradation and thereby check the reproducibility of the prescribed optimum conditions in the hybrid reactor. Results show that, the culture depicted constant TCE removal efficiency in the PBR and PFR over a period of two months. The reactor was then inoculated with contaminating mixed consortium and operated in a non-sterile mode for a period of thirty days to check the stability of the culture in the presence of other cultures. Analysis of the effluent samples after contaminating the reactor depicted some variations in the effluent biomass and effluent TCE concentration for initial seven days. However after a period of seven days, a steady state was achieved after which constant TCE removal efficiency with constant values of biomass was achieved in the effluent samples of PBR and PFR. Results show the dominance of the culture of *P. pseudoalcaligenes* MHF ENV with the non-existence of the contaminating bacteria after seven days. Thus, in contrast to earlier indings, the bacterium used in our study depicted constant effluent TCE values for over a period of 100 days of stability study in hybrid reactor (Fig. 4). Moreover, when operated in non-sterile mode the culture depicted dominance over the contaminated bacteria within a period of seven days. From the effluent TCE and biomass values it can be inferred that the bacterium maintained its TCE degradation potential and depicted sustained degradation. In any degradation process, whether chromosomal or plasmid encoded, the probability of losing the useful degradative properties of bacterial cultures appears to be high. The loss of TCE degradation ability has also been reported in both whole cells and purified enzymes of TCE degraders including soluble methane monooxygenases from *Methylosinus trichosporium* OB3b [22] and *Nitrosomonas europaea* [23], toluene dioxygenase (TDO) from *Pseudomonas putida* F1, and butane-oxidizing bacteria, i.e., *Pseudomonas butanovora*, *Mycobacterium vaccae*, and *Nocardioides* sp. CF8 [24]. The unsustainable TCE degradation could be attributed to various reasons like

cytotoxicity, inhibition, or inactivation of TCE-degrading enzymes [25].

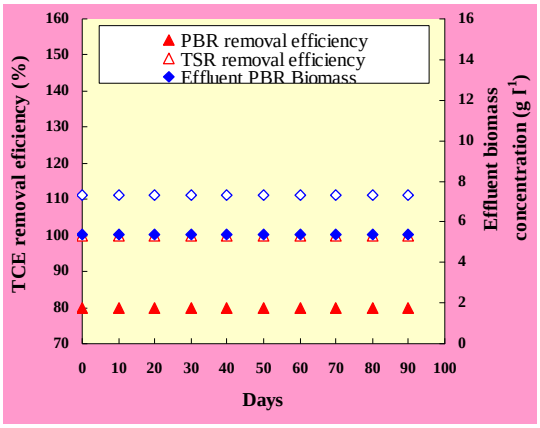


Fig. 4. Stability profile of the bacterium in PBR over a period of 100 days

3.9 Bioremediation of TCE by Microbial Consortium

In the past decade the use of microbial consortium in bioremediation has gained focus mainly due to the recalcitrant nature of the pollutants. Microbial consortium has been employed in degradation of many recalcitrant compounds like TCE, Benzene, Xylene, Toluene, Chlorpyrifos etc. [26-28]. The different genetic composition of the members in a consortium leads to faster contaminant removal altogether. In the present study, microbial consortium was developed using laboratory and pure cultures. Using this consortium batch degradation studies were carried out at various TCE concentrations viz. 250, 500, 750 and 500 mg/l. During the course of experiment, change in cell count was monitored daily, where the growth pattern of the culture at various TCE concentrations and degradation of different TCE concentration was studied (Fig. 5).

The environmental parameters have also been studied under which TCE was remediated using flask shake method under controlled environmental conditions in an incubator shaker. pH was monitored throughout the experiment which was found to be decreasing from 7 to 6.4, 6.3 and 6.5, 6.7 after 5 days of the experiment in case of 250 mg/l, 500 mg/l, 750 mg/l and 100 mg/l respectively. This variation in pH is due to the interaction of microorganism with TCE and decreasing the pH due to the various intermediates formed during TCE degradation. Therefore, as the concentration of TCE increases (up to 500 mg/l), the intermediates formed increases and results into decrease in the pH. Say et al. have also correlated degradation of TCE with pH values (Say 2001). Temperature was found to be optimum for the growth and proliferation of microorganism for bioremediation of TCE. It was found to stable at 33℃ throughout the experiment.

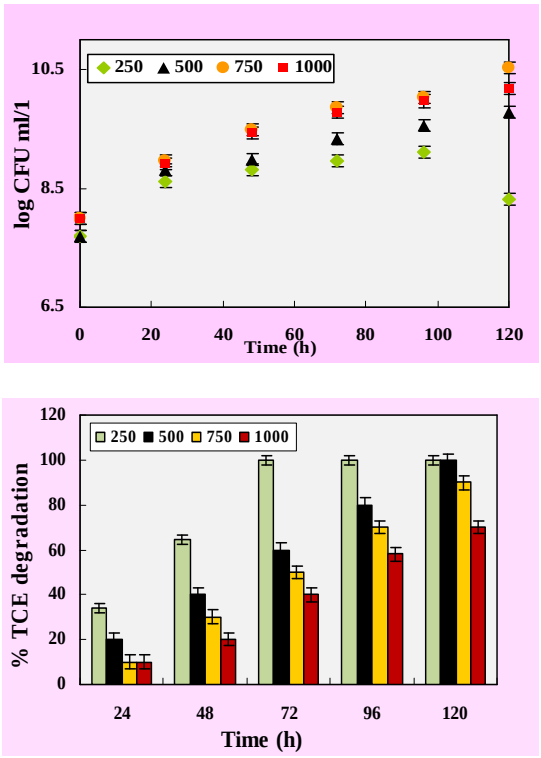


Fig. 5. TCE degradation profile by consortium of *Pseudomonad cultures* at 250, 500, 750 and 1000 mg/l over a period of 120 h

BOD was found to increase from 3.5 mg/l to 4.0 mg/l in 250 mg/l, from 3.9 mg/l to 4.9 mg/l in 500 mg/l from 1.6 mg/l to 2.8 mg/l in 750 mg/l and from in 2.8 to 3.4 mg/l for 1000 mg/l of TCE concentration after a period of 120 h. The observed increase in BOD is primarily due to the consumption of oxygen by the microbial biomass for their growth and proliferation. These results are supported by findings of Medjor et al., who reported increase in the BOD valued during bioremediation of the contaminant [17]. In case of control, BOD was found to decrease after a period of 5 days up to non-detectable amount which shows there was no growth of microorganisms. Therefore, less growth of microorganisms results into less increase in BOD values.

Table 2. Performance of *P. pseudoalcaligenes* culture in PBR with respect to degradation rate, COD, effluent TCE and biomass concentration at various dilution rates and organic loadings

PBR studies TCE concentration (mg l-1)			500				750				1000			
Dilution rate (h)	HRT (h)	Flow rate	Effluent TCE (mg l-1)	Degradation rate	Effluent biomass (g l-1)	COD (mg/ l)	Effluent TCE (mg /l)	Degradation rate	Effluent biomass concentration (g /l)	COD (mg l/1)	Effluent TCE (mg l/)	Degradation rate	Effluent biomass concentration (g /l)	COD (mg/ l)
0.5	2	15	0	250 ± 1.8	23 ± 1.9	10±2.1	100 ± 2.7	325 ± 1.9	30 ± 0.7	42 ± 1.2	950 ± 2.0	25 ± 1.5	3.0 ± 1. 0	375 ± 1.6
1	1	30	152 ± 1.7	348 ± 1.2	32 ± 2.1	60 ± 1.9	154 ± 1.9	596 ± 1.7	54 ± 2.1	63 ± 2.1	977 ± 2.1	23 ± 2.6	2.3 ± 1.5	387 ± 2.1
2	0.5	60	245 ± 1.4	510 ± 1.6	47 ± 1.7	102 ± 1.6	480 ± 2.8	540 ± 2.4	49.5 ± 1.9	190 ± 1.6	990 ± 1.7	20 ± 1.0	1.8 ± 1.6	392 ± 3.1
4	0.25	120	407 ± 2.3	372 ± 2.1	34 ± 1.3	170 ± 2.1	711 ± 0.9	156 ± 0.7	14.5 ± 1.9	281 ± 1.0	996 ± 1.4	16 ± 2.3	1.3 ± 1.0	395 ± 1.3
6	0.16	180	470± 1.4	180 ± 1.0	16±2.3	190 ± 1.9	740 ± 1.6	60 ± 1.9	5.3 ± 2.4	295 ± 1.6	1000 ± 3.0	0	1.0 ± 1.5	398 ± 1.6

± represents the standard deviation of three replicates

Table 3. Performance of *P. pseudoalcaligene*s MHF ENV in PFR at various organic loadings and dilution factors and comparative study of TCE efficiency in PBR, PFR and hybrid reactor

PFR studies			TCE concentration (mg/l)													
			500							750						
Dilution rate	Flow rate	HRT	Inlet TCE	Effluent TCE	Degradation rate		TCE removal efficiency (PBR)	TCE removal efficiency (HR)*	Enhanced degradation (HR)	Inlet TCE	Effluent TCE	Degradation rate		TCE removal efficiency (PBR)	TCE removal efficiency by (HR)	Enhanced TCE degradation (HR)
					PFR*	HR*						PFR*	HR*			
0.17	15	5.6	NA	NA	NA	NA	100	NA	NA	100	NG	17	342	86.6	100	13.4
0.35	30	2.8	152	NG	53.2	40.2	69.6	100	30.4	154	NG	53.9	649.9	79.46	100	20.54
0.70	60	1.4	245	150	66.5	57.5	51	70	19	480	410	49	589	36	45.33	9.33
1.41	120	0.70	407	372	49.35	42.3	18.6	25.6	7	711	680	43.7	199.7	5.2	9.3	4.1
2.11	180	0.47	470	460	21.1	20.1	6	10	4	740	730	21.1	81.1	1.3	2.6	1.3

** values lie within 1 standard deviation of mean*

Chemical oxygen demand (COD) was also monitored which shows the decrease in COD levels over a period of bioremediation, which indicates the degradation of organic compounds by potential microorganism present in the minimal salt medium. Significant decrease in COD values was observed at all the selected concentrations as the bioremediation experiment proceeded. COD decreased up to 99.1% in 250 mg/l, 75.8% in 500 mg/l, 34% in 750 mg/l and 27% in 100 mg/l concentration of TCE containing medium after a period of 5 days of experiment.

3.10 TCE Degradation in PBR Using Microbial Consortium

Continuous TCE degradation has been reported using biological means like use of bacterial cultures [18,19] as well as by physical methods like photocatalytic degradation. In the present study, continuous TCE degradation studies were carried out in a PBR using microbial consortium. The PBR was operated at various flow rates and the effect of flow rate on degradation rate, efficiency and effluent TCE concentration was monitored.

Results show that, at the lowest flow rate, analysis of the effluent samples showed that the reactor required a period of five days for achieving steady state as observed from the effluent TCE concentration. An increase in the flow rate till 60 ml h^{-1} increased the TCE degradation rate up to 510 ± 1.6 mg $l^{-1}h^{-1}$. The biomass utilizes TCE for its growth and maintenance thereby increasing in number. In accordance to this, a proportional increase in biomass was observed with increase in the flow rate. At higher flow rates beyond 60 ml h^{-1} TCE degradation rate decreased possibly due to less contact between the cells and the substrate. The reactor was also operated with higher organic loading of 750 and 1000 mg l^{-1} at flow rates as above.

From the various parameters studied, the optimal condition for PBR operation with respect to TCE degradation efficiency were; the flow rate of 60 ml h^{-1} and the organic loading of 500 mg/l that achieved complete degradation of 500 mg l^{-1} with the retention time of 0.5 h. However, maximal TCE degradation rate of 596 ± 1.7 was achieved at the TCE loading of 750 mg/l with flow rate of 30 ml/h. (Table 4). The optima degradation rates obtained in our studies were the highest rates achieved till date.

3.11 TCE Degradation in Hybrid Bioreactor Using Microbial Consortium

Although the use of hybrid reactors combining trickling filter and activated sludge process [20], coupled anaerobic-aerobic bioreactor [21] has been described in previous studies, we report the novel combination of PBR and PFR for TCE aerobic degradation. The PBR served as a chamber for maximal TCE removal and biomass generation, while this generated biomass was used for further reduction of TCE to negligible levels in the PFR. The effect of flow rate on TCE degradation rate and effluent TCE concentration in hybrid bioreactor was monitored.

Results show that, the rate of TCE degradation in PFR was dependent on the biomass concentration in the PBR outlet. With the increase in biomass concentration in the PBR effluent, the TCE degradation rate increased. The maximal TCE degradation rate in PFR was found to be 86.5 ± 2.0 mg/l/h at the flow rate of 60 ml/h and inlet TCE of 500 mg/l. However, the TCE degradation rate of 83.9 ± 1.4 mg/l/h achieved at the flow rate of 30 ml/h and inlet TCE being 750 mg/l in PFR was considered to be optimum with zero TCE discharge in the effluent. The hybrid reactor depicted complete TCE degradation of 750 mg l^{-1} within 2.8 h with the degradation rate of 849 ± 2.3 mg1/h is the highest TCE degradation rate reported till date. From the various parameters studied, it was observed that the use of PFR in conjugation with PBR can enhanced the TCE degradation efficiency of up to 50.4 ± 2.1% with 500 mg/l inlet and 60.54 ± 1.4 in 750 mg/l inlet (Table 5). In other words, the biomass obtained from PBR effectively served as a source of inoculum in PFR for enhancing the degradation rate and efficiency of TCE degradation.

3.12 Analysis of TCE Degradation using GC-MS

Biodegradation was checked by monitoring the residual TCE concentration in the spent medium after every 24 h using GC-MS (Fig. 6). The adapted culture depicted TCE removal of 96, 80, 70, and 53% at 250, 500, 750 and 1000 mg/l of TCE respectively in a time span of 5 days. The

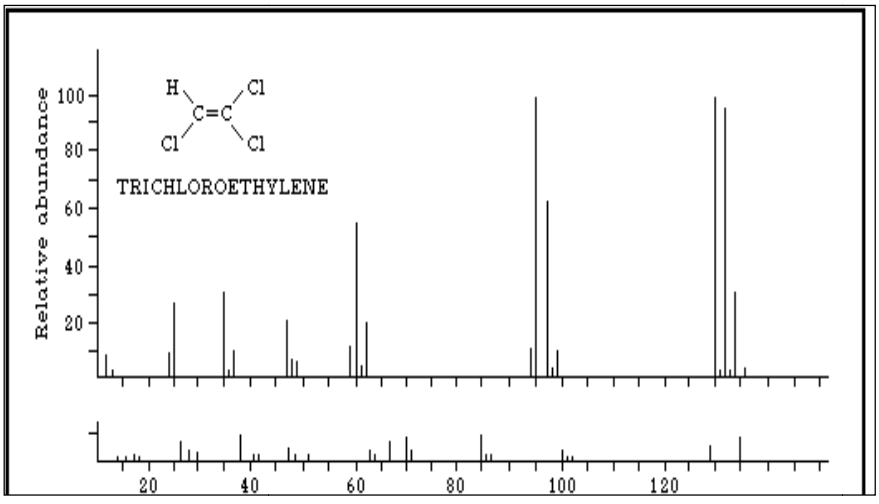
degradation rates at each of these increasing concentrations were 52.85, 42.28, 38.5 and 18.57 mg/l/h. An increase in cell count was observed up to day 2 for 250 and 500 mg/l of TCE followed by a gradual decline due to decrease in TCE concentration in the MSM. For 750 mg/l of TCE, maximum cell count was observed at day 4 followed by decline in total cfu in the MSM. At the highest concentration of 1000 mg/l the culture exhibited an extended lag phase for first two days suggesting that the culture requires some time probably for acclimatization or for enzyme synthesis at this concentration. This finding suggests that at higher concentration viz. 1000 mg/l is relatively slower and require longer degradation time.

3.13 Comparison of TCE Degradation Using Potential and Microbial Consortium

Bioremediation of TCE has been carried out using microbial consortium and potential culture of *P. pseudoalcaligenes* MHF ENV at 250, 500, 750 and 1000 mg/l independently by shake flask method under controlled environmental conditions. The degradation capacities of these cultures differ due to variation in their genetic composition and / or different potential.

The results obtained by microbial consortium and potential bacterial culture were compared with respect to cell growth and TCE degradation. It was observed that, the cell count of the microbial consortium culture at all the concentrations of TCE was higher than that of the potential bacterial culture. At 500 mg/l, the maximum cell count achieved by the microbial consortium culture was 9.2×10^5 as compared to 8.07×10^4 for the potential bacterial culture on the 4th day of the experiment (Table 6). An increase in cell count in the microbial consortium culture indicates that, the use of consortium alleviates substrate toxicity. The microbial consortium culture showed an increased TCE removal of 60%, 52%, 30% and 20 at 250, 500, 750 and 1000 mg/l of TCE as compared to potential microbial culture. The maximum degradation rate achieved by the microbial consortium was 15 mg/l/h was higher than 1.2 mg/l/h, for the potential microbial culture. Thus, microbial consortium depicted enhanced degradation by 12.5% as compared to potential bacterial culture at the optimal concentration of 500 mg/l. The cell growth and the degradation profile of the microbial consortium culture at high TCE concentrations shows that the microbial consortium culture depicted high substrate tolerance of up to 1000 mg/l as compared to 500 mg/l by the potential bacterial culture.

Thus the cell count, TCE degradation rate and substrate tolerance of the microbial consortium culture were significantly higher from those obtained with potential inoculums.



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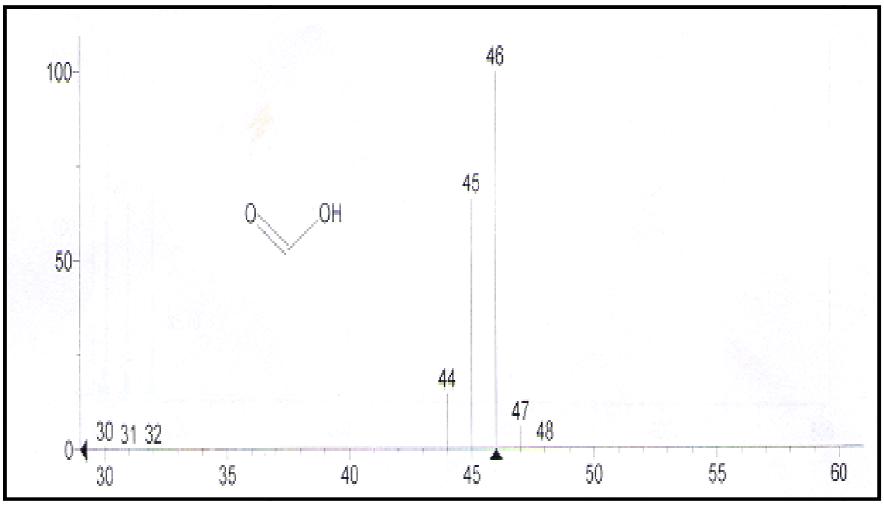

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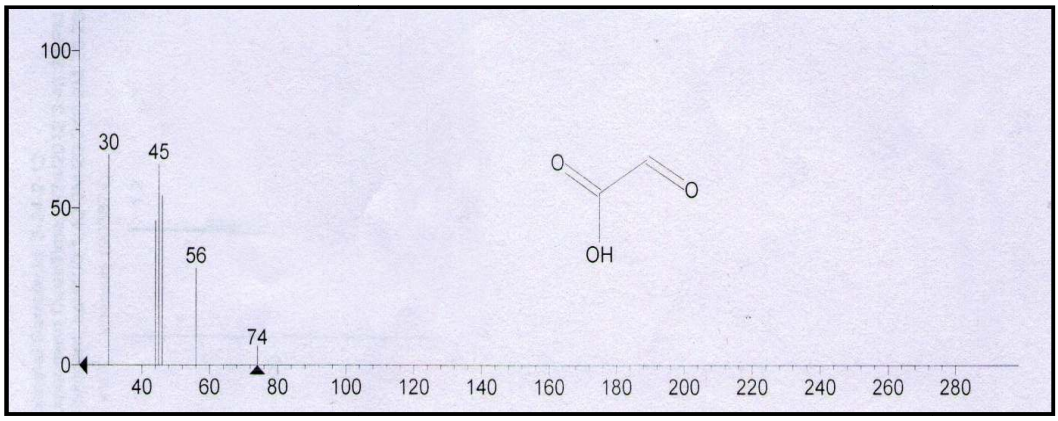

(c)

Fig. 6. Mass profile of (a) TCE (m/z identification ion- 95), (b) formate (m/z identification ion-45,46) and (c) glyoxylate (m/z identification ion-74). The spectrum represents the plot of m/z (x-axis) against relative intensity (y-axis)

3.14 Comparison of TCE Degradation in Hybrid Reactor Using Potential and Microbial Consortium

Continuous TCE degradation studies were conducted in TSR using potential and microbial consortium culture at the concentration ranging in 500-1000 mg/l and flow rates ranging between 15-180 ml/h.

With organic loading of 500 mg/l, complete degradation was observed at 15 ml/h in potential and consortium culture. At 30 ml/h flow rate, 87% degradation of TCE was observed by potential microorganism as compared to 100% degradation using microbial consortium. Above, 30 ml/h there was decrease in TCE removal varying between 50-20% for potential and 70-50% for consortium. With organic loading of 750 mg/l, the maximal degradation was achieved at 30 ml/h was 80% by potential microorganism as compared to 100% degradation using microbial consortium (Table 7). The free cells depicted comparatively lesser degradation of 32%. At 180 ml/h, there was negligible degradation using potential bacterium while the consortium depicted 68% degradation. While, at 1000 mg/l loading negligible TCE degradation was observed using potential as well as consortium. The studies show that consortium depicted superior TCE degradation in hybrid reactor as compared to potential bacterium.

Table 4. Performance of microbial consortium in PBR with respect to degradation rate, COD, effluent TCE and biomass concentration at various dilution rates and organic loadings

PBR studies TCE concentration (mg l-1)			500				750				1000			
Dilution rate (h)	HRT (h)	Flow rate	Effluent TCE (mg l-1)	Degradation rate	Effluent Biomass (g l-1)	COD (mg/ l)	Effluent TCE (mg /l)	Degradation rate	Effluent Biomass concentration (g /l)	COD (mg l/1)	Effluent TCE (mg l/)	Degradation rate	Effluent Biomass concentration (g /l)	COD (mg/ l)
0.5	2	15	0	250 ± 1.8	03 ± 1.9	10±2.1	100 ± 2.7	325 ± 1.9	30 ± 0.7	42 ± 1.2	95 ± 2.0	25 ± 1.5	3.0 ± 1.0	375 ± 1.6
1	1	30	15 ± 1.7	348 ± 1.2	13 ± 2.1	60 ± 1.9	154 ± 1 .9	596 ± 1.7	54 ± 2.1	63 ± 2.1	97 ± 2.1	23 ± 2.6	2 .3 ± 1.5	387 ± 2.1
2	0.5	60	24 ± 1.4	510 ± 1.6	37 ± 1.7	102 ± 1.6	480 ± 2.8	540 ± 2.4	49.5 ± 1.9	190 ± 1.6	99 ± 1.7	20 ± 1.0	1.8 ± 1.6	392 ± 3.1
4	0.25	120	40 ± 2.3	372 ± 2.1	44 ± 1.3	170 ± 2.1	71 1 ± 0.9	156 ± 0.7	14.5 ± 1.9	281 ± 1.0	99 ± 1.4	16 ± 2.3	1.3 ± 1.0	395 ± 1.3
6	0.16	180	47± 1.4	180 ± 1.0	16±2.3	190 ± 1.9	740 ± 1.6	60 ± 1.9	5.3 ± 2.4	295 ± 1.6	100 ± 3.0	0	1.0 ± 1.5	398 ± 1.6

Table 5. Performance of microbial consortium at various organic loadings and dilution factors and comparative study of TCE degradation efficiency in PFR and hybrid reactor

PFR studies			TCE concentration (mg/l)													
			500							750						
Dilution rate	Flow rate	HRT	Inlet TCE	Effluent TCE	Degradation rate		TCE removal efficiency (PBR)	TCE removal efficiency (HR)*	Enhanced degradation (HR)	Inlet TCE	Effluent TCE	Degradation rate		TCE removal efficiency (PBR)	TCE removal efficiency by (HR)	Enhanced TCE degradation (HR)
					PFR*	HR*						PFR*	HR*			
0.17	15	5.6	NA	NA	NA	NA	100	NA	NA	400	NG	17	342	86.6	100	73.4
0.35	30	2.8	82	NG	53.2	401.2	69.6	100	50.4	54	NG	83.9	849.9	79.46	100	60.54
0.70	60	1.4	65	50	86.5	576.5	51	70	19	480	41	49	589	36	45.33	53.3
1.41	120	0.70	47	72	49.35	421.3	18.6	25.6	7	71	680	43.7	199.7	5.2	9.3	41
2.11	180	0.47	40	60	21.1	201.1	6	10	4	74	73	21.1	81.1	1.3	2.6	13

** values lie within 1 standard deviation of mean*

Table 6. Comparison of TCE degradation at 500 mg/l using potential bacterium and microbial consortium

TCE (mg/l)	Shake flask bioreactor studies									
	Day I (24 h)		Day II (48 h)		Day III (72 h)		Day IV (96 h)		Day V (120 h)	
	P	C	P	C	P	C	P	C	P	C
Residual TCE (%)	91.0	64.0	87.0	42.0	76.0	Nil	72.0	Nil	66.0	Nil
Removal efficiency (%)	9.0	36.0	13.0	58.0	24.0	100	28.0	100	34.0	100
Cell count (Log c.f.u./ml)	7.76	8.02	7.88	8.53	7.97	9.05	8.07	9.2	8.13	9.47

Table 7. Comparison of various parameters during TCE bioremediation by potential microorganism and consortium in hybrid bioreactor

Sr. no.	Flow rate	Dilution rate	Degradation rate (mg/l/h)		Effluent TCE concentration (mg/l)		Removal efficiency (%)	
			Potential microorganism	Microbial consortium	Potential microorganism	Microbial consortium	Potential microorganism	Microbial consortium
1	15	0.17	6.52 ± 2.1	NA	Nil	NA	100 ± 1.0	NA
2	30	0.35	12.26 ± 1.3	13.04 ± 1.1	30 ± 1.1	Nil	80 ± 2.1	100 ± 0.2
3	60	0.70	15.65 ± 1.6	18.19 ± 1.2	110 ± 2.1	35.0 ± 1.2	73 ± 2.1	89 ± 1.7
4	120	1.41	12.04 ± 1.3	27.71 ± 1.6	300 ± 1.4	75 ± 1.1	54 ± 1.9	78 ± 1.6
5	180	2.11	NA	32.08 ± 1.5	NA	90 ± 1.2	NA	68 ± 1.2

4. CONCLUSION

The remediation technology has been established by using the bio resources of adapted microbial consortium and potential microorganisms at varying consortium in designed and developed reactors such as SFB, PBR and HR. The reactors were operated at various organic loadings and/or flow rates and the performance of the culture with respect to the remediation of the selected compounds was monitored. Results show that, the rate of TCE degradation in PFR was dependent on the biomass concentration in the PBR outlet. TCE degradation was observed using potential as well as consortium. The studies show that consortium depicted superior TCE degradation in hybrid reactor as compared to potential bacterium. The microorganism, *P. pseudoalcaligenes* MHF ENV was identified as the versatile organism for remediation of TCE.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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