Advances in Environmental Biology, 7(9): 2081-2090, 2013
ISSN 1995-0756

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Role Of Nutrients On Biodegradation Of 1,4 Dioxane By A Bacterial Consortium Enriched From Industrial Sludge

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Arulazhagan. P, Yeom I.T, Sivaraman C, Srikanth M and Rajesh Banu J: Role Of Nutrients On Biodegradation Of 1,4 Dioxane By A Bacterial Consortium Enriched From Industrial Sludge

ABSTRACT

The cyclic ether 1,4 dioxane is a carcinogenic, emerging micropollutant present in water and wastewater. The treatment methods are ineffective due to its high solubility (low Henrys law constant 5 x 10^{-6} atm m^3/ mol) and also its heterocyclic structure (with two linear ethers). The present study was focused on biodegradation of 1,4 dioxane by a bacterial consortium enriched from 1,4 dioxane contaminated industrial sludge. The Bacterial consortium degraded 74% of 1,4 dioxane in 72 h. To enhance the biodegradation process, additional carbon substrates such as glucose, yeast extract and tetrahydrofuran (THF) were used. Among those carbon substrate, THF acts as a best substrate in accelerating the biodegradation process for complete degradation of 1,4 dioxane and also reducing the time taken for biodegradation. Yeast extract as additional substrate showed 92% of 1,4 dioxane degradation in 72 h whereas glucose showed 59% degradation in 72 h and also extended the time taken for complete degradation of 1,4 dioxane which was at 168 h. Further, the bacterial strains present in the consortium were identified as: bacterium enrichment culture clone strain AYS1 (JQ419749), *Runella sp.* AYS2 (JQ419750), *Achromobacter sp.* AYS3 (JQ419751), *Marinobacter sp.* AYS4 (JQ419752) and *Rhodanobacter sp.* AYS5 (JQ419753) using molecular techniques.

Key words: biodegradation, 1,4 dioxane, micropollutants, tetrahydrofuran

Introduction

The cyclic ether 1,4-dioxane, an organic solvent is one of the major emerging pollutant in water and wastewater. The 1,4 dioxane is a colourless, flammable liquid with faint odour and belongs to heterocyclic organic compound with two oxygen atoms, each with free electrons which make them hydrophilic and miscible in water (Fig. 1). In addition, its low Henry's law constant prevents from volatilization (Table 1). The 1,4 dioxane is short-lived in atmosphere with a half life of 6 to 10 h. The breakdown products of 1,4 dioxane consist of aldehydes and ketones [21]. Dioxane is used as a stabilizer in chlorinated solvents, mainly for 1,1,1-trichloroethane (TCA), which stabilize the solvents by behaving as a lewis base and inhibiting the reactions of solvent with acids and metal salts that may degrade the properties of solvents. The major outfall of dioxane is from cosmetic and pharmaceutical industries. United States Environmental Protection Agency (USEPA) and International Agency for Research on Cancer (IARC) classify dioxane as group B2 (probable human) carcinogen. Accordingly, 1,4-dioxane has been nominated for inclusion in the guidelines of drinking water produced by World Health Organisation (WHO) in 2003.The compound is found to be carcinogenic and detected in groundwater and landfill sites [16,33,32]. The carcinogenic oral slope factor for 1,4 dioxane is 1.1 x 10^{-2} mg/kg/d, with a lifetime cancer risk of 1 in 10^{-4} for a drinking water concentration of 0.3 mg/L [2,11]. The treatment of 1,4 dioxane in wastewater is the major challenge ahead for the scientists and engineers. There are many treatment technologies available, but bioremediation plays vital role among the technologies as it is nature's way to clean the contaminated wastewater. The use of micro organisms for the removal of pollutants termed as Bioremediation which was proven as relatively cost-effective and efficient treatment technology [20,5].

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Zenker *et al* [35] reported that 1,4 dioxane is used as a solvent stabilizer in industries to prevent the breakdown of chlorinated solvents during manufacturing processes. Initially 1,4 dioxane was used as a stabilizer for 1,1,1-trichloroethane which was banned in 1996 by the montreal protocol. The 1,4 dioxane is typically found at solvent release sites and polyethylene terephthalate (PET) manufacturing sites [21]. The 1,4 dioxane is produced as a by-product from dimmerization of ethylene oxide during polyethoxylated alcohol formation which is used as a sulphate surface active agent [35]. Therefore, 1,4 dioxane is used in shampoos, liquid soaps, hair lotions and other cosmetic products. As an industrial solvent, 1,4 dioxane is used for different purpose such as degreasing, electronics, metal finishing, fabric cleaning, pharmaceuticals, herbicides and pesticides, antifreeze, paper manufacturing process and also in various applications. Hence 1,4 dioxane act as one of the emerging major toxic micropollutants in groundwater, industrial and domestic wastewater. In South Korea, due to high amount of 1,4 dioxane released into the rivers, the government fixed 5mg/L as its discharge limit for 1,4 dioxane in wastewater.

Few treatment methods were proved to be successful and economically feasible for removing 1,4 dioxane from wastewater. Traditional remediation technologies such as carbon adsorption and air stripping are inefficient and require high cost for the removal of 1,4 dioxane. Only few ex-situ technologies including chemical oxidation with combined addition of ozone and hydrogen peroxide or hydrogen peroxide with UV light [26] were used commercially to destroy 1,4 dioxane, but the cost of applying these technologies for higher concentration of waste stream was very high. Ultrasonic frequencies were used for treating 1,4 dioxane in aqueous solutions. Beckett and Hua [6] were used four ultra sonic frequencies (205, 358, 618 and 1071 kHz) for treating 1,4 dioxane. Among various treatment technologies, eco-friendly biological treatment of 1,4 dioxane was one of the developing methods. A very limited study on biodegradation of 1,4 dioxane was performed. Co-metabolism using THF sufficiently enhanced the biodegradation of 1,4 dioxane [35,31]. Also conventional biological treatment of 1,4 dioxane is found to be the area of interest for the environmental treatment technologist. On the other hand, combined treatment technologies are also given equal importance to arrive complete treatment of 1,4 dioxane. The 1,4 dioxane can be degraded by both pure and mixed cultures. The bacterial strains such as *Rhodococcus* sp, *Mycobacterium vaccae, Pseudonocardia dioxanivorans* CB1190, *Pseudonocardia* sp ENV478 and *Mycobacterium* sp PH-06 were able to utilize 1,4 dioxane as sole carbon source [7,9,25,31,15]. Thus, the treatment of 1,4 dioxane in water and wastewater is a great challenge ahead. This article details about the biodegradation of 1,4 dioxane by a bacterial consortium enriched from industrial sludge and role of additional carbon sources on biodegradation of 1,4 dioxane.

Material and Methods

Enrichment of bacterial consortium:

The bacterial consortium was enriched from 1,4 dioxane contaminated industrial sludge, South Korea. The consortium was enriched on basal salt medium with 1,4 dioxane and Tetrahydrofuran (THF). After three weeks, the consortium was grown only on 1,4 dioxane as sole carbon source. During enrichment, the initial concentration of 1,4 dioxane was 20 mg/L and after four to five identical transfers with different concentrations, finally the consortium was maintained at the concentration 100 mg/L of 1,4 dioxane as sole carbon source.

Chemicals :

All the chemicals used in the study are analar grade. The 1,4 dioxane, 1,4 dioxane-d8 (Gas Chromatography standard) and THF were purchased from Sigma Aldrich chem., USA (purity 99.8%).

Basal Salt Medium (BSM):

The carbon free basal salts medium (BSM) contained NH_4Cl-2.0g, K_2HPO_4-3.24g, NaH_2PO_4-1.0g, $MgSO_4$-0.20g, $FeSO_4$-0.012, $MnSO_4$-0.003, $ZnSO_4$-0.003, $CoCl_2$-0.001 and Distilled water-1L. The final pH of the medium was adjusted to 7.4 with 0.1N NaOH. Stock solutions of 1,4 dioxane (1000 mg/L) and THF (1000 mg/L) were prepared and stored.

Studies on 1,4 dioxane degradation by bacterial consortium:

For the degradation study, the bacterial consortium was inoculated in basal salt medium containing 1,4 dioxane as sole carbon source. Different compositions used in the degradation of 1,4 dioxane (1,4 D) were i) BSM + 1,4 D + bacterial consortium (BC); ii) BSM + 1,4 D and iii) BSM + bacterial consortium (BC) where ii) and iii) served as controls. The culture prepared in duplicates were kept in shaker at 150 rpm and extracted at every 24 h time interval for 5 days. The culture samples were extracted twice with methylene chloride (v/v) in which 1-2g of sodium chloride was added to increase the extraction efficiency. The extracts were filtered through anhydrous sodium sulphate and condensed to 1mL and analysed in Gas Chromatography (GC). The 1,4 dioxane-d8 was used as internal standard and also to check the extraction efficiency. The extraction efficiency of 1,4 dioxane using methylene

chloride was 90 to 95%. Protein estimation was done by Bradford protein estimation kit purchased from sigma (Bradford 1976). COD and MLVSS concentration were measured as per APHA standard methods [2]. Additional substrates such as THF, yeast extract and glucose were used at the concentration of 20 mg/L in the medium along with 100 mg/L of 1,4 dioxane.

GC analysis:

A Hewlett-Packard 6890 gas chromatograph equipped with 5973 mass spectrometer with HP-5MS (30m x 0.25mm I.D x 0.25µm) fuse-silica capillary column was used for analysis. The column temperature program was set at 100 ºC hold for 1 min, 15 ºC/min to 160 ºC and 5 ºC/min to 300 ºC hold for 7 min. The GC injector was held isothermally at 280 ºC with a splitless period of 3 min. Helium was used as carrier gas, at a flow rate of 1 mL/min by using electronic pressure control.

Mineralisation of 1,4 dioxane by the Bacterial Consortium:

The study was conducted in basal salts medium with both 1,4 dioxane (100 mg/L) and the bacterial consortium, while 1,4 dioxane + BSM served as control. The serum bottles were sealed completely (airtight) with an aluminium stopper. The culture was kept shaking at 150 rpm at 37°C. Samples were collected at 24h intervals and analysed for CO_2 evolution in a gas chromatograph. Carbon dioxide content was measured with a thermal conductivity detector using a Porapak Q column (80/100 mesh, 2 m) with an external standard. The carrier gas was helium and the column temperature was 50°C. The temperature of the injector and the detector was 100°C. Samples (250µL) of the headspace gas from the culture flasks were withdrawn with a gas-tight syringe and injected into the gas chromatograph for CO_2 determination.

Extraction of bacterial DNA:

DNA from the bacterial cells were extracted using Qiagen (QIAamp® DNA stool Mini kit Cat.No.51504) DNA isolation kit. Using the protocol from manufacturer, DNA was eluted in 50µL of AE buffer and stored at 4ºC for further use.

PCR for Denaturing Gradient Gel Electrophoresis (DGGE):

PCR amplifications of DNA extracted from enrichment culture was conducted in 50µL volume containing 1X PCR buffer, 1.5mM $MgCl_2$, 0.5 µM of each primer, 200µM of each dNTP, 0.6Uof Taq polymerase and sterile water. 1µL of template DNA was used in each PCR reaction. Negative controls with 1µL of sterile water instead of target DNA were included in each PCR amplification. The primers used were 27F and 1492R. These are universal eubacterial primers binding to phylogenetically highly conserved regions of the 16S rRNA gene. The PCR program was used with an initial 95°C denaturation step for 5 min, followed by 32 amplification cycles of denaturation for 30 s at 94°C, 30 s at 52°C, and 1 min at 72°C and the final elongation step at 72°C for 10 min. After successful amplification, 5 µL aliquot of the above PCR products were taken for a second PCR utilizing the 968 F primer with a GC clamp and the 1492R primers [28].

Denaturing Gradient Gel Electrophoresis (DGGE):

Denaturing Gradient Gel Electrophoresis (DGGE) was conducted using the CBS DGGE Scientific system (CBS scientific company, USA). Between 10 – 15 µL of PCR products were loaded onto 8% (w/v) polyacrylamide gels (40% acrylamide stock solution, 2% bis solution 37.5:1). The 30-60% gradient of denaturant (7M urea and 40% formamide v/v as 100% denaturant, Nikolausz *et al*. 2008) was used to prepare the gel and the gel was run for 16 hours in 1X TAE buffer at 60 volts. The bands in the gel were finally visualized using silver staining.

Identification of bacterial strains by phylogenetic analysis:

Bands from DGGE gel were excised with a sterile razor blade and soaked in 50µL of sterile water overnight. A portion (5µL) was used as the template for PCR as described above to confirm the band for amplification. The PCR products were purified using gel extraction kit (Chromous Biotech, India). The purified PCR fragments were cloned into pGEM-T Easy vector using pGEM-T Easy Vector systems II (Promega Corp., Madison, USA) according to manufacturer's instructions. DNA sequencing was performed by Chromous Biotech (Bangalore, India). The sequence identification was performed by the use of BLASTN facility of NCBI. Phylogenetic and molecular evolutionary analyses were carried out using MEGA (Molecular Evolutionary Genetic Analysis) version 5. The partial 16S rRNA sequences were deposited in GenBank under accession numbers JQ149749 to JQ149753.

Results and Discussion

Biodegradation of 1,4 dioxane by bacterial consortium:

Biodegradation of 1,4 dioxane by the bacterial consortium enriched from 1,4 dioxane contaminated industrial sludge was studied. Initially, the bacterial consortium was enriched in mineral medium with 1,4

dioxane as sole carbon source and the growth study revealed the degradation of 1,4 dioxane. Further, degradation of 1,4 dioxane by bacterial consortium was studied, which showed 74 ±1.6% degradation in 72 h. After 72 h, degradation of 1,4 dioxane was absent and no change in protein concentration was observed. Biodegradation of 1,4 dioxane by gas chromatograph analysis showed the reduction in 1,4 dioxane concentration and increase in protein concentration in the inoculated flasks. The biodegradation of 1,4 dioxane is significant in the inoculated flasks relative to its controls. Loss of 1,4 dioxane in control flasks was measured to be 5.5 ± 2.5% at the end of the study (Fig. 2). The losses may be attributed to abiotic processes such as adsorption, evaporation and diffusion onto fine particles [22]. The percentage of residual 1,4 dioxane left over in the medium at 72 h was found to be 26 ± 1.6%. Proteins in the biomass was quantified as a measure to check the biodegradation of 1,4 dioxane. There was an increase in protein concentration throughout the course of this study i.e., 160 ± 10µg in 0 h was increased to 430 ± 8µg at 72 h. No increases in protein were found in the control flasks during the study which confirmed the sterile conditions. *Mycobacterium* sp. PH-06 was able to degrade 90% of 1,4 dioxane when the initial concentration supplied to medium was 1g/L [15]. The kinetic parameters for (of) 1,4 dioxane biodegradation by bacterial consortium were determined using Monod's equation. The bacterial consortium had a maximum specific growth rate (μ_{max}) of 0.38 day^{-1} and substrate half saturation constant (K_s) of 10.19 mg/L. These values were in agreement with previously reported values for 1,4 dioxane degradation by industrial activated sludge [19,12]. The yield of cell growing on 1,4 dioxane was very low and resulted in low growth rate [19].

Role of additional nutrients in the biodegradation of 1,4 dioxane:

The biodegradation of a pollutant was not only influenced by microorganisms but also by physical and chemical properties such as pH, temperature, moisture content, presence of alternate carbon source, nutrient content. To analyse the role of additional substrate in biodegradation of 1,4 dioxane, additional substrates were added in the form of nutrients to BSM. Glucose and yeast extract were selected as additional carbon and nitrogen substrates, since these compounds were reported to enhance the biodegradation of aromatic hydrocarbons [3]. Chemical Oxygen Demand (COD), Mixed Liquor Volatile Suspended Volatile Solids (MLVSS) and residual 1,4 dioxane were measured to quantify the extent of biodegradation. Addition of glucose decreased the biodegradation of 1,4 dioxane when compared with the flasks containing 1,4 dioxane and consortium (BSM + 1,4 D + BC) i.e., 38 ± 1.1% at 72 h against the flasks (BSM + 1,4 D ± BC) which had 26.1 ± 1.6% of residual 1,4 dioxane (Fig. 3). There was a significant increase in MLVSS in control (520 ± 17mg/L) at 72 h when compared to flasks added with glucose (430 ± 21mg/L). Reduction in COD was observed in both the flasks but removal was slightly higher in the flasks containing 1,4 dioxane and bacterial consortium (1,4 D + BSM + BC) (Fig. 4). The decrease in extent of biodegradation was attributed to availability of simple sugars in the medium which slow down the degradation activity of bacterial consortium. [27,25]. Addition of yeast extract significantly enhanced the biodegradation of dioxane which was evidenced from increase in MLVSS during the course of this study (Fig. 5). There was an apparent reduction in COD which was observed in the yeast extract added flasks relative to the flasks containing 1,4 dioxane and bacterial consortium (1,4 D + BSM + BC).

Co-metabolic studies in the presence of Tetra Hydro Furan (THF):

After enriching 1,4 dioxane degrading bacterial consortium, co-metabolic studies were conducted with THF to check for enhanced biodegradation of 1,4 dioxane. The flasks were inoculated with bacterial consortium and biodegradation of 1,4 dioxane was monitored in the presence of THF at a concentration of 20 mg/L. THF was used because of its structural similarity with 1,4 dioxane [34]. Addition of THF significantly enhanced the biodegradation of 1,4 dioxane when compared to flasks containing 1,4 dioxane and bacterial consortium (1,4 D + BSM + BC) (Fig. 3). At the end of this study, 1,4 dioxane was completely degraded by bacterial consortium in the flasks supplied with THF as additional carbon source. Previous reports on the co-metabolic activity of dioxane degraders [34,25,27] were in agreement with the present study by bacterial consortium from the industrial sludge. Sun *et al* [27] reported that 1,4 dioxane degradation was enhanced with the increasing concentrations of THF. Increase in MLVSS and COD reduction was observed throughout the course of the study (Fig. 6). THF, as an additional carbon source, was also completely utilised by the bacterial consortium along with 1,4 dioxane at 72 h.

Mineralisation of 1,4 dioxane by the bacterial consortium:

The ability of bacterial consortium to mineralise 1,4 dioxane was studied by the respirometric experiments. The serum bottles added with BSM and 1,4 dioxane served as test bottles whereas the serum bottles devoid of bacterial consortium served as control bottles. Evolution of CO_2 through abiotic process such as hydrolysis, oxidation and photolysis could be evaluated from control bottles. The total

CO_2 evolution (%) was the difference between the final quantities of CO_2 in the test bottle and 1,4 dioxane free bottle (control bottle). Respirometric experiments showed that the bacterial consortium able to mineralise 1,4 dioxane as sole carbon source. The percentage of carbon dioxide evolved from test bottles was found to be 71.82 $\pm$ 1.8% at the end of the study (Fig. 7). In case of control bottles, the percentage of CO_2 evolution was found to be 6.22 $\pm$ 0.81%. Residual 1,4 dioxane present in the bottle was 26%$\pm$ 1.6. The removal of micropollutants through abiotic process became significant only when the Henry's law constant ranges between 10^{-2} to 10^{-3} atm m^3/ mol [10]. The results confirmed 1,4 dioxane was completely degraded into carbondioxide and water by the bacterial consortium [4].

Identification of strains in the bacterial consortium:

From DGGE analysis, DNA bands confirmed that the bacterial consortium consists of five bacterial strains which were designated as AYS1, AYS2, AYS3, AYS4 and AYS5. Based on BLAST search, the strain AYS1 showed 97% similarity to uncultured marine bacterium and was closely related to *Sphingomonas paucimobilis* through phylogenetic analysis (Fig. 8). *Sphingomonas* was known to degrade a wide variety of aromatic hydrocarbons [17,18]. The 16S rRNA gene analysis of the strain AYS2 showed 100% similarity with *Runella sp.* Strain AYS3 showed 100% similarity with *Achromobacter xylosoxidans* and found to be closely related to *Achromobater denitrificans* by phylogenetic analysis. The strain AYS4 showed 100% similarity with *Marinobacter sp.* which was known to degrade a range of nitroaromatic and phenolic compounds. The strain AYS5 showed 100% similarity with *Rhodanobacter sp.* which was closely related to *Rhodanobacter lindaniclasticus* through phylogenetic analysis. The strain was capable of degrading the recalcitrant chlorinated aromatic compounds such as lindane and wide variety of aromatic compounds [14,23]. Thus, the research concludes that 1,4 dioxane degrading bacterial consortium consists of five different bacterial strains. Among them, AYS1 was capable of degrading 90% of 1,4 dioxane (100 mg/L) with THF as an additional substrate in 72 h (data not shown).

The 1,4 dioxane is reported as a probable human carcinogen (IARC 1999) and has been detected as pollutant in surface and groundwater. Therefore, dioxane was included in the Final Third Drinking Water Contaminant Candidate List (CCL3) by U.S. EPA in September 2009 (U.S. EPA 2009). Natural attenuation mechanisms such as sorption and volatilisation are not significant due to 1,4 dioxane properties viz., hydrophilic and low Henry's constant which reflects its complete miscibility with water and low volatility. Although, physical and chemical treatments of 1,4 dioxane found to be effective but expensive on economical considerations. Bioremediation is perhaps an attractive option and in this study, biodegradation of 1,4 dioxane by bacteria is known to be occurring via metabolic and co-metabolic reactions. Limited number of research works were done on the biological treatment of emerging micropollutant 1,4 dioxane in wastewater. However, the role of nutrients in 1,4 dioxane degradation has not been well documented. In the present study, bacterial consortium enriched from 1,4 dioxane contaminated sludge was studied for its capability to degrade 1,4 dioxane as sole carbon source. The bacterial consortium was able to degrade 1,4 dioxane without addition of extra carbon and the degradation profile was supported by increase in protein concentration in the biomass. To further enhance the 1,4 dioxane biodegradation, nutrients were added and studied the degradation. Addition of glucose lowered the biodegradation rates whereas yeast extract greatly favoured the removal of 1,4 dioxane by the bacterial consortium. There was an apparent increase in MLVSS and reduction in COD in flasks added with yeast extract and 1,4 dioxane. The effect of THF on 1,4 dioxane biodegradation resulted in complete removal of 1,4 dioxane at the end of the study.

Table 1: Physicochemical Properties of 1,4 Dioxane

Property	Value
Molecular formula	$C_4H_8O_2$
CAS No.	123-91-1
Molecular Weight	88.11
Density	1.033g/L at 20° C
Solubility	Miscible in water (log K_{ow} = - 0.27)
Henrys law constant	5 x 10^{-6} atm m^3/ mol
Physical state	Colourless, inflammable liquid
Melting point	11.8° C
Boiling point	101° C
Vapour Pressure	4.9 KPa at 25° C
Stability	Stable in light
Reactivity	Reacts with oxygen to form peroxide
Toxicity	Carcinogen (1.1E – 2 mg/kg/d)

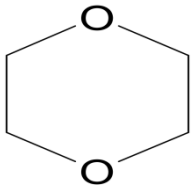

Fig. 1: Structure of 1,4 Dioxane

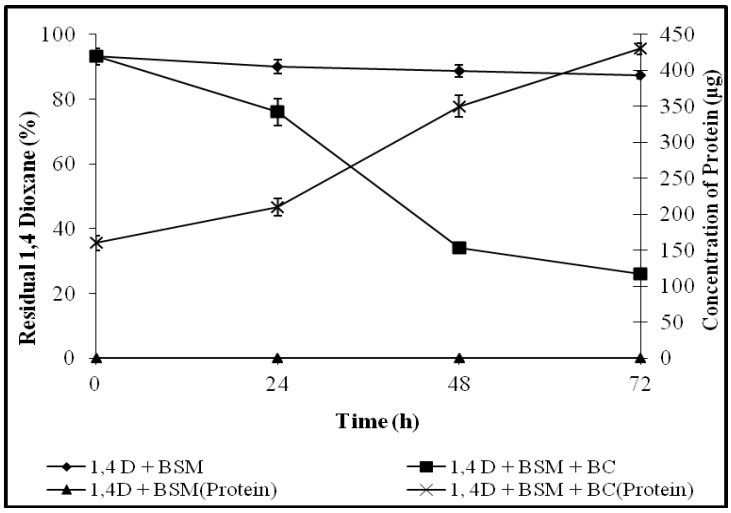

Fig. 2: Biodegradation of 1,4 dioxane by bacterial consortium

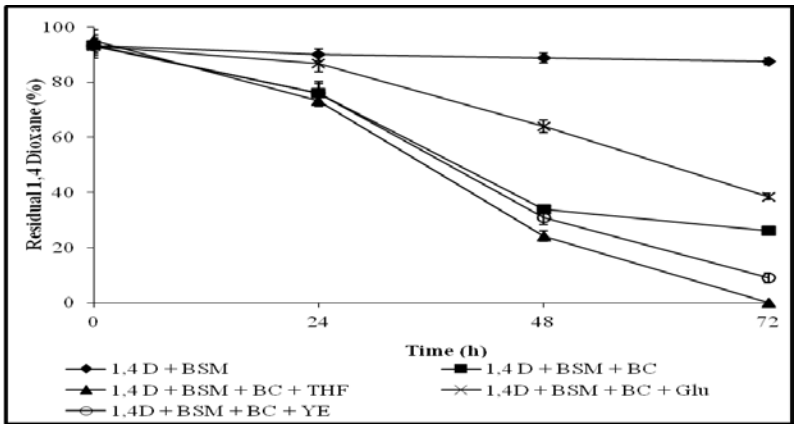

Fig. 3: Role of Nutrients and Co-metabolism in 1,4 dioxane biodegradation by bacterial consortium

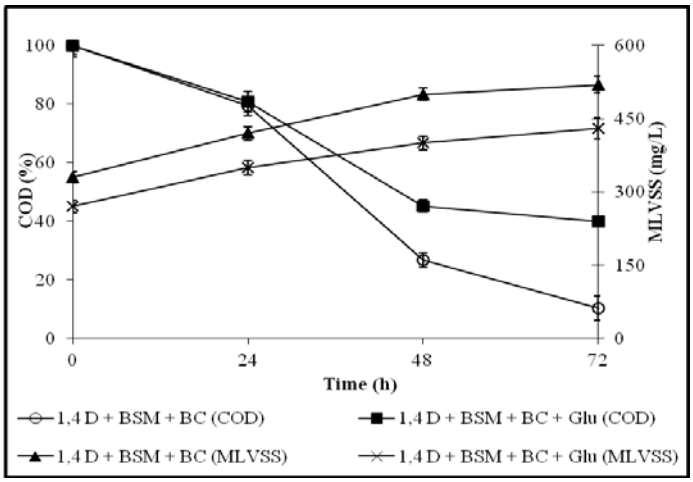

Fig. 4: COD and MLVSS profile during 1,4 dioxane biodegradation in the presence of Glucose

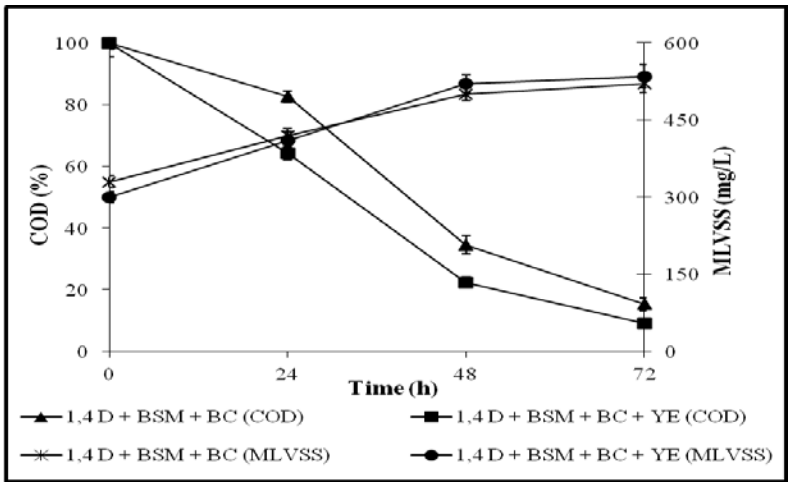

Fig. 5: COD and MLVSS profile during 1,4 dioxane biodegradation in the presence of Yeast Extract

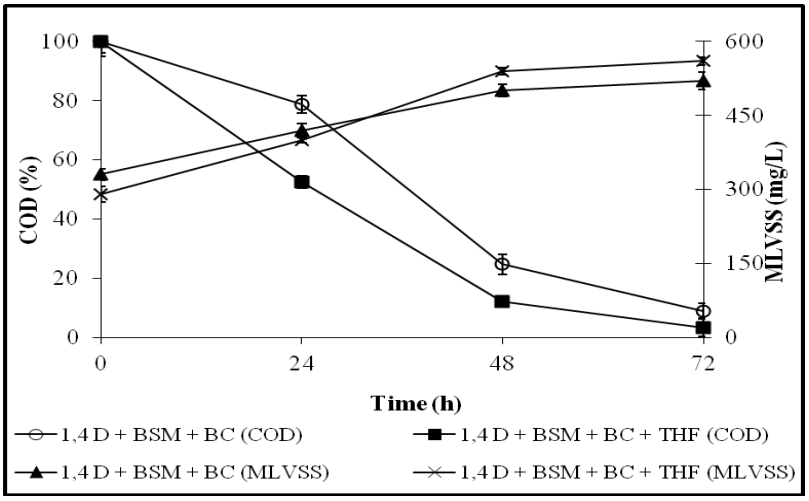

Fig. 6: COD and MLVSS profile during 1,4 dioxane biodegradation in the presence of THF

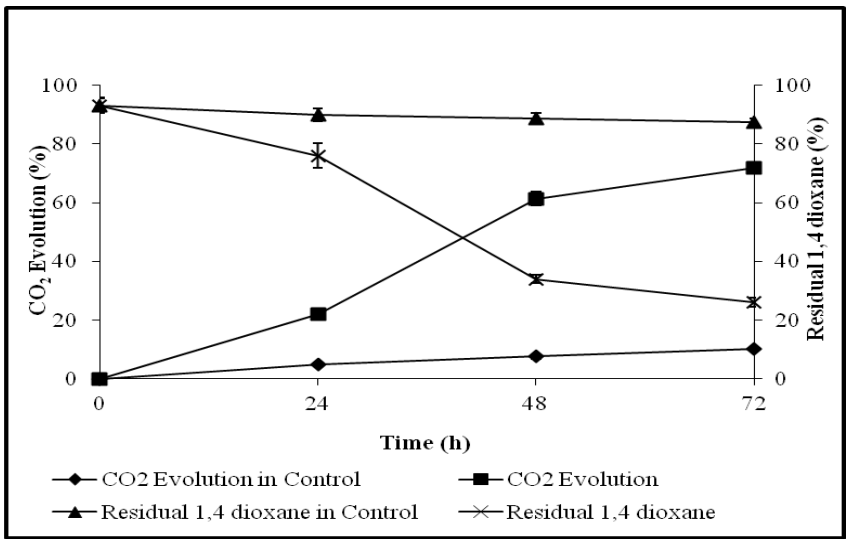

Fig. 7: Mineralisation of 1,4 dioxane by bacterial consortium

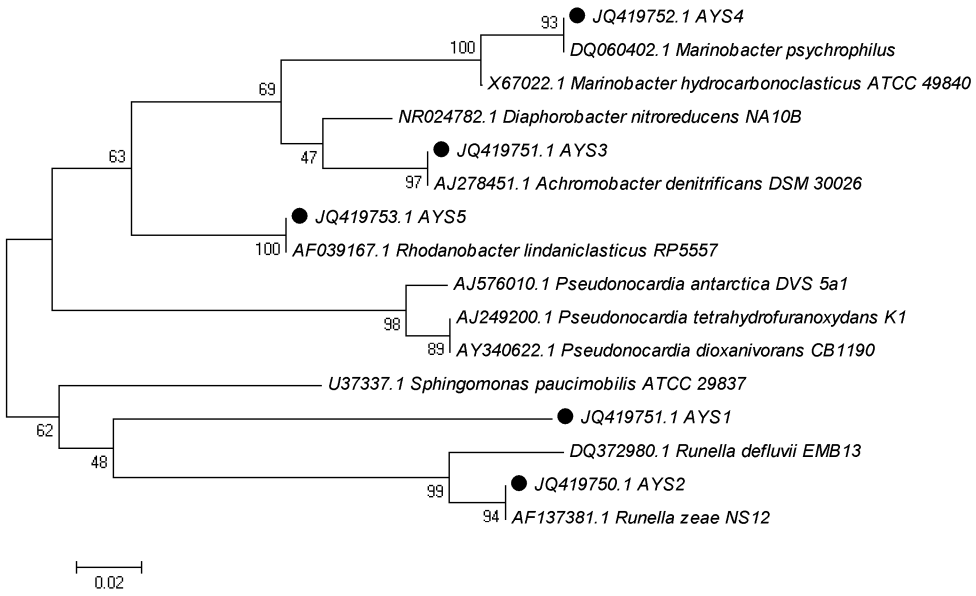

Fig. 8: Phylogenetic tree analysis of bacterial species present in the consortium and their closely related bacteria obtained from BLAST search by Neighbour Joining Method. Numbers at the nodes are the bootstrapping values. Scale bar represents 0.1 substitutions per nucleotide position

The present study showed that co-metabolism plays vital role in the degradation of 1,4 dioxane. The study also detailed, initially the consortium grow on THF and biodegradation of 1,4 dioxane begins when the concentration of THF is low. The cell yield reported is also the same for degradation of 1,4 dioxane with THF or with THF alone. Phylogenetic analysis showed that the species in the bacterial consortium is versatile in degrading variety of environmental pollutants. The bacterial consortium along with THF in industrial wastewater showed more than 90% COD reduction in lab scale reactor study.

Conclusions:

The 1,4 dioxane is one of the major emerging micropollutant and its treatment ability was less by the available physicochemical treatment technology. The present work on 1,4 dioxane adds more knowledge in the biodegradation and the role of additional nutrients in the biodegradation process. Thus, the present study concludes that the bacterial consortium enriched from industrial sludge was capable of degrading 1,4 dioxane effectively. Also states that the bacterial consortium achieved complete degradation of 1,4 dioxane in the presence of THF and yeast extract upto 90% in 72 h. The novel bacterial consortium could be employed in the treatment of 1,4 dioxane using activated sludge process.

Acknowledgement

The author would like to thank Brain Korea 21 (BK 21) Programme from Korean ministry of education, South Korea for financial support. Also would like to thank Centre of Excellence in Environmental Studies, King Abdulaziz University, Jeddah, Saudi Arabia supporting in identification of bacterial strains.

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