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Assessment of Microbial Degradation of Volatile Organic Compounds from Industrial effluents

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Absratct: Volatile organic compounds are carbon-based chemicals that easily evaporate into the air at room temperature it is an important outdoor air pollutant. In the present study VOC degrading microorganisms were isolated and identified from industrial effluents. Biodegradation of VOC was measured by weight loss in the polymer and GCMS. Thus, the duration of the microbial colonization is an important factor that effect period. It was observed that isolate V2 is potential isolate for the degradation of VOC, so it was selected for the further screening VOC degradation by V2 isolates by GCMS. The removal efficiencies of VOC of different microbes were compared. The major characteristics of fungi and bacteria that govern the biodegradation were discussed. The efficiency of fungi was found to be greater than that of bacteria. On the other hand, mixed culture was possibly found to have higher efficiency which was a result of the cumulative efficiency conferred by the fungal presence and the moisture content that obliges to attain higher efficiency. The efficiency of degradation is also influenced by the volatile compounds present in the sample.

Key words: Volatile Organic Compounds, Microbial Degradation, GC-MS

INTRODUCTION

Environmental contamination by volatile organic compounds (VOCs) from petrochemical and energy-producing industries is continually increasing. Among VOCs, aromatic compounds such as benzene,

toluene and xylenes are the most severe contaminants because of their increasing use as gasoline, aircraft fuel and solvent. Due to their low water solubility, acute toxicity and genotoxicity, these compounds are classified as priority pollutants by European Environment Agency. VOCs are an important outdoor air pollutant[1]. Emissions of VOCs have primary as well as secondary harmful impacts on ground air quality and human health. Major VOC emission sources are automobiles (cars and trucks) exhaust and various industrial sites, such as plants manufacturing organic chemicals, polymers or synthetic fibers and smaller units such as painting and coating operations etc[2]. The acute effects of VOCs on health are eye irritation, nose irritation, throat irritation, headache, nausea/vomiting, dizziness, asthma exacerbation etc. The chronic effects of VOCs on health are cancer, liver damage, kidney damage, central nervous system damage etc[3]. Volatile Organic Compounds (VOCs) are man-made and/or naturally occurring highly reactive hydrocarbons. Volatile Organic Compounds (VOCs), which are released into the air mostly through the use of everyday products and materials, are present in both indoor and outdoor environments[4-6].

There are several microorganisms able to grow and transform volatile organic compounds. Screening activities performed have allowed the selection of three microbial strains, such as *Pseudomonas putida*, *Candida membranes* and *Penicilliums*p[7]. The organic chemical compounds that have significant vapor pressure that can affect environment and human health are called volatile organic compounds or VOCs. There are many volatile compounds and they are found almost everywhere. VOCs consist typically of light organic substances such as: propane, ether, benzene, methanol, ethanol, carbon tetrachloride and vinyl chloride; however the VOCs which come from crude oil may include many individual VOCs that cannot be determined[8-9].

Our previous studies have shown that among our microbial collection there are microorganisms able to grow and transform volatile organic compounds. Screening activities performed have allowed the selection of three microbial strains, such as *Pseudomonas putida*, *Candida membranes* and *Penicillium sp*[7,10]. In present study, the above mentioned strains were cultivated in medium containing as carbon source, individual, bi- and tertiary mixtures of volatile organic compounds. In order to evaluate the microbial degradability of organic compounds containing aromatic ring and substituted benzene derivates, the experiments were performed with benzene, toluene and o-xylene.

MATERIALS AND METHODS

Sample collection: The industrial effluents were collected from a biofilter of various industries from the Shendra MIDC, Waluj MIDC, Chikhalthana MIDC from the Aurangabad region. The effluents were free from larger inert materials (glass, stones, metals, etc.) as much as possible. These items are removed manually as much as possible to produce a homogenous compost inoculum. The Industrial effluents sample had the following basic properties: total solids (%TS) 81%; volatile solids at 550°C (%VS) 18%; pH 7.2; C/N ratio 15.3. It was used for isolation of polymer degrading microorganisms.

Isolation of VOC bacteria from industrial effluents: The samples were extracted using saline solution (0.85% NaCl), and the extracts were serial diluted. The diluted samples was distributed onto an LB (Lauria-Bertani) and incubated at temperatures 30°C and 37°C. The microbes were isolate and identified based

on morphology, color, and surface type of the colonies, and they were cultivated until a pure culture was obtained

Identification of isolates: The morphological characteristics of the isolates were identified by Gram staining and biochemical reactions. The biochemical reactions included glucose fermentation, catalase and oxidase production, egg yolk reaction and reaction in tryptosesoyabroth.

Estimation of VOC degradation: VOC degradation is defined as the process by which VOC is converted to a form that is no longer extractable by benzene. Oil conversion was determined by extracting 100ml of incubation mixture with 10ml of benzene, the aqueous phase was extracted a second time with 10ml of benzene and the combined benzene fractions were filtered through Whatman no.1 filter in order to clarify the extract. The benzene was evaporated at 37^{o}c to constant weight in a tarred large Petri dish. The rate of oil degradation was expressed in milligrams as well as in percentage.

Conversion of milligrams to %

Rate of oil degradation (z) = x/y * 100

Rate of oil degradation (%) = z 100

x= periodical Benzene extractable oil (mg)

y= Control Benzene extractable oil (mg)

Physico-chemical characteristics of industrial water: Physico-chemical characterization of the steel industry effluent was performed, according to standard protocol of American Public Health Association (APHA). The test includes the parameters such as:pH, BOD, COD, DO, Alkalinity, Chloride, Phosphate, Total hardness (calcium, magnesium), Total solids, Total dissolved solids, Total suspended solids, Sodium chloride,

Isolation of VOC-Tolerant Strains and Growth Conditions: The samples were extracted using saline solution (0.85% NaCl), and the extracts will be serial diluted. The diluted samples will distributed onto an LB (Lau- ria-Bertani) and incubated at temperatures 30°C and 37°C. The microbes were sorted based on morphology, color, and surface type of the colonies, and they will be cultivated until a pure culture will obtained.

Cultivation of microorganisms in VOCs containing medium: To select the VOC-tolerant strains, the strains were grown in an LB agar plate covered with 150 ml of Xylene (99.9%, Sigma-Aldrich), petroleum ether (99.7%, Sigma-Aldrich), Napthalene (99.9%, Sig- ma-Aldirich), Benzene and Chloroform (local store) at 30°C. For the growth experiment, subcultures of VOC strains will be grown in an LB medium containing 1% (v/v) of the VOC (Xylene, petroleum ether, Napthalene, benzene, Chloroform) at 30°C, placed overnight on a shaking incubator (200 rpm). The main cultures will be inoculated with 1% of subculture (v/v) in a modified BH medium (Bushnell-Hass agar) containing 1% (v/v) of VOCs (Xylene, petroleum ether, Napthalene, benzene, Chloroform), and incubated at 30°C with shaking at 200 rpm for 7 days. The growth of 4 VOC strains on the VOCs will indicated in terms of ΔOD600 and Ysub (grams of cell dry weight per mole). The substrate molar yield (Ysub) will expressed as the cell dry weight per mole of the carbon source.

Biodegradation of VOCs by microorganisms: For the VOC degradation experiment, the strains were grown aerobically in a BH liquid medium with 1% (v/v) of Xylene, Petroleum ether, Napthalene, benzene and Chloroform at 30°C on a shaking incubator (180 rpm) up to OD600 > 1. After removal of the old medium by centrifugation, the cells of the strains were resuspended in a fresh BH medium to an OD600 of 0.5. The 50 ml resuspension was transferred with toluene or cyclohexane to 500 ml serum bottles. The bottles was plugged with rubber stoppers and completely sealed with aluminum caps. While the cells was cultivated on the shaking incubator at 30°C, the gas in the headspace of the se- rum bottles was sampled using a gas-tight syringe.,

Screening of VOC degrading bacteria by UV-visible spectrophotometer: he studies on the possibility of using UV-VIS spectrophotometer as analysis method for BTX were carried out on aqueous solution of various concentrations (20, 40 and 60 µ L/L) of each organic compound: Xylene, petroleum ether, Napthalene, benzene, Chloroform. The UV spectra of the solution ns were registered in the wave length range 190-350 nm, using a Varian Cary 50 spectrophotometer. The studies on the BTX removal were carried out on synthetic waste water containing a mixture of Xylene, petroleum ether, Napthalene, benzene, Chloroform, each in a concentration of 20 µ L/L. At the BTX removal by air-stripping various air flow rates were used (20, 40 and 100 L/h); the other conditions were identical: a volume of waste water of 600 mL and the temperature of 18°C. The studies were carried out in batch system, using equal volumes of synthetic waste water. The studies regarding the BTX removal by adsorptionon active charcoal were carried out on a column containing 4 g of granular active charcoal. The efficiency of the adsorption process was studied for three various water flow rates: 1 L at a flow rate of 0.26 L/h, 5 L at 1.66 L/h and 5 L at 3.65 L/h.

Determination of degradation of VOCs by GCMS: VOCs in the gas samples will be analyzed by Gas Chromatography (GC6850N, Agilent Technologies Inc.) with a wax column (30 m × 0.32 mm × 0.25 µm, Supelco, USA) and an FID detector. The oven temperature will be maintained at 100°C for 5 min and then increased to 320°C at 5°C/min. The inlet temperature was 230°C. The column was diluted with N2 gas at a flow rate of 35 ml/min. VOCs were identified via a comparison of the retention times with the standard. Degradation experiments will be performed in triplicate.

Morphological and Biochemical Analysis: The morphological properties of four isolated VOC strains were examined using light microscopy. The color and surface texture of the four VOC strains on the LB agar plate and the BH agar plate with 1% (v/v) VOCs were investigated. An assessment of the biochemical pro- perties was performed.

RESULTS AND DISCUSSION

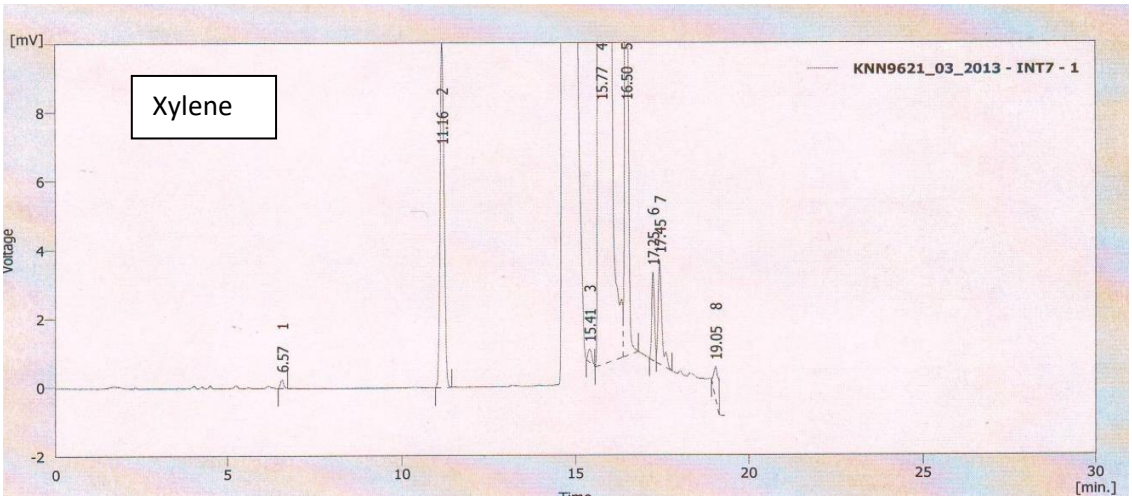
The present study deals with the isolation, identification, media and ability of VOC degrading microorganisms from industrial effluents. Different types of changes are produced by the microorganism during biochemical analysis. Synthetic plastic sample collected from the college campus was used in when the total biodegradation process of any organic substrate is considered the formation of microbial colony is critical to the initiation of biodegradation. These bacterial strains were isolated and characterized through macroscopic and microscopic studies. Biodegradation of VOC was measured by

weight loss in the polymer and GCMS. Thus, the duration of the microbial colonization is an important factor that effect period.

From the above table it was observed that isolate V2 is potential isolate for the degradation of VOC, so it was selected for the further screening VOC degradation by V2 isolates by GCMS. The UV spectra of Xylene, Petroleum ether, Naphthalene, Benzene and Chloroform solutions of various concentrations are presented. From the registered spectra the parts within significant absorption were removed. The spectra were also registered 24 hours after the preparation of solutions; the results confirmed the stability of solutions in the hermetically closed flask

Studies on the dependence of absorbance on the wave length for Xylene, petroleum ether Napthalene, Benzene and Chloroform water solutions, at the beginning of the study it was investigated the possibility of using UV-VIS spectrophotometry as analysis method for Xylene, petroleum ether Napthalene, Benzene and Chloroform. This method is faster, less expensive and more available than other consecrated methods for the analysis of volatile organic compounds, such as gas chromatography. From the registered spectra the parts with insignificant absorption were removed.

Gas chromatography analysis of VOC degradation by Microorganism: In evaluating VOC degradation, GC was applied in the estimation of the VOC potential of effluents samples. Gas chromatography (GC) methods do provide some information about the product type. Most methods involve a sample preparation procedure followed by analysis using GC techniques. GC determination is based on selected components or the sum of all components detected within a given range. In that study, GC was used for analysis of VOC degradation. In general, GC was used for analysis of VOC and biomarkers to evaluate the successfulness of applied VOC degradation treatments. To see the degradation of VOC in addition to using the method of analysis was also performed Gravimetric extracted Industrial effluents samples by using gas chromatography (GC). GC results of the analysis done by comparing the GC profile of the curve results from the control Industrial effluents extract samples (before treatment) to extract the ground after being given treatment.



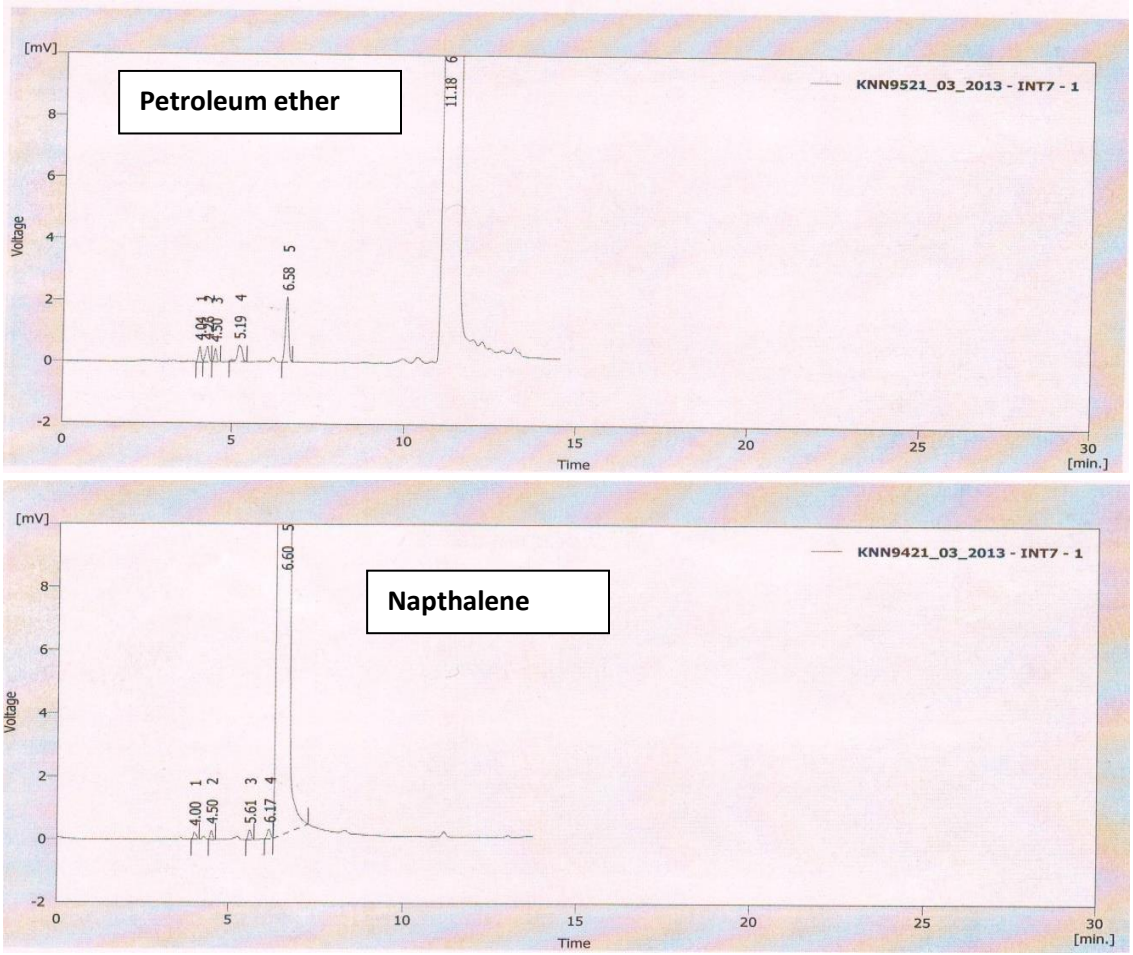
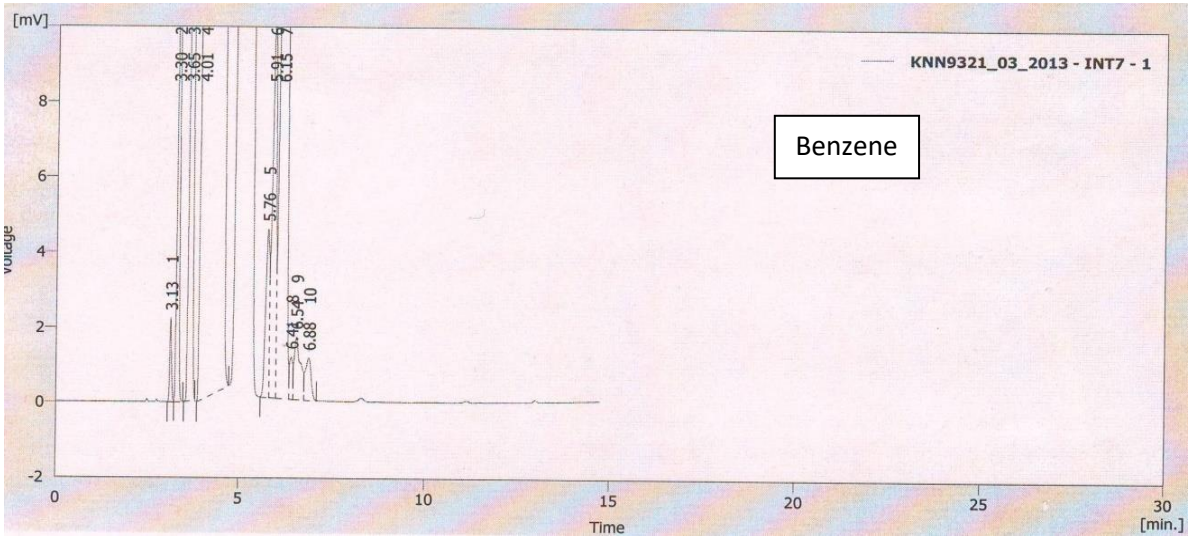


Fig. 1 : GC chromatogram of Xylene, Petroleum ether, Napthalene,



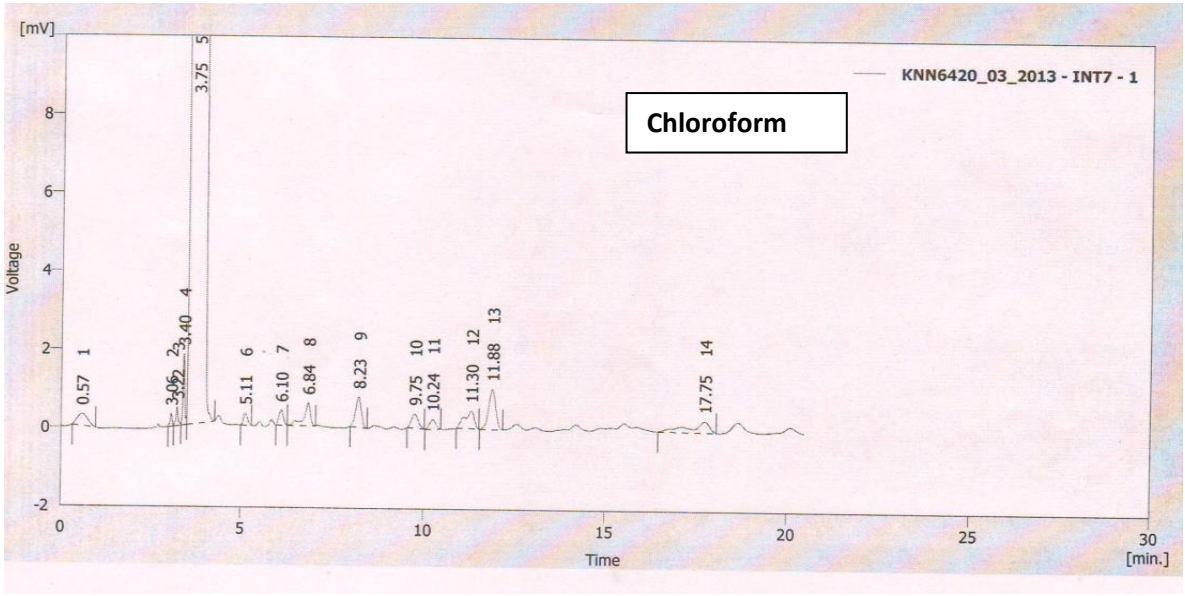


Fig. 2 : GC chromatogram Benzene and Chloroform

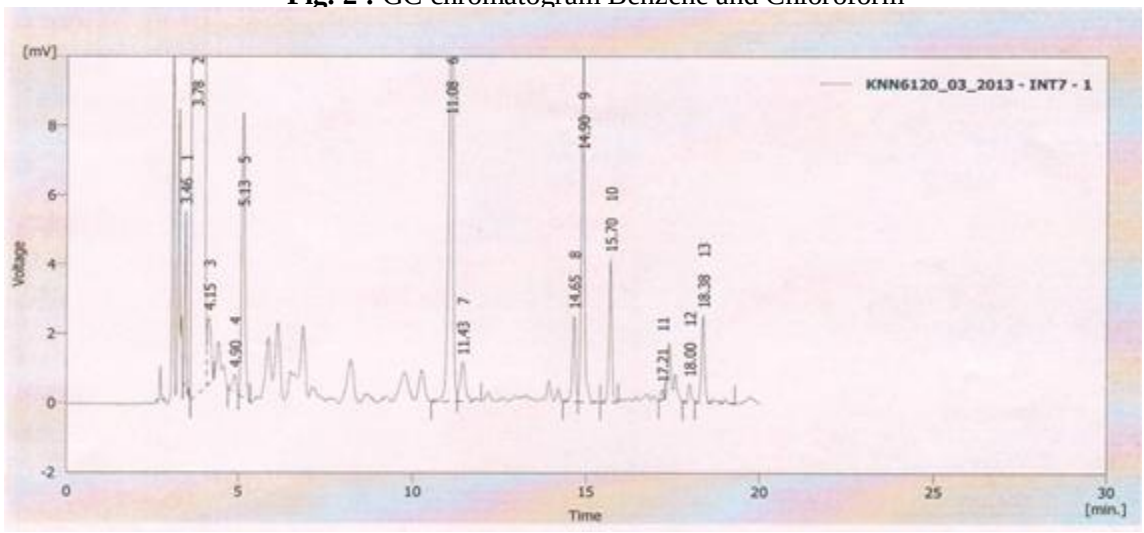


Figure 3. Profile curves of Gas chromatography of degradation of Xylene by *Pseudomonas putida*

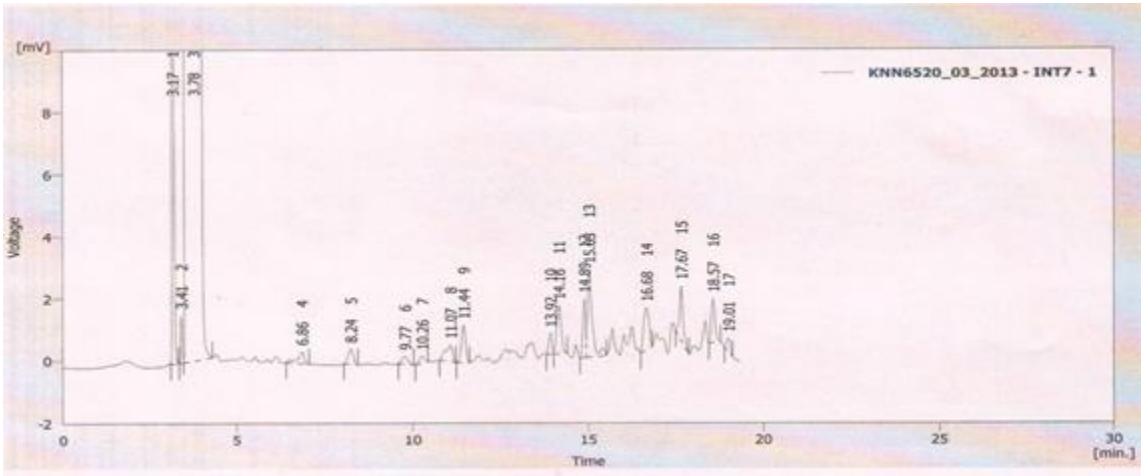


Figure 4. Profile curves of Gas chromatography of degradation of Petroleum ether by *Pseudomonas putida*

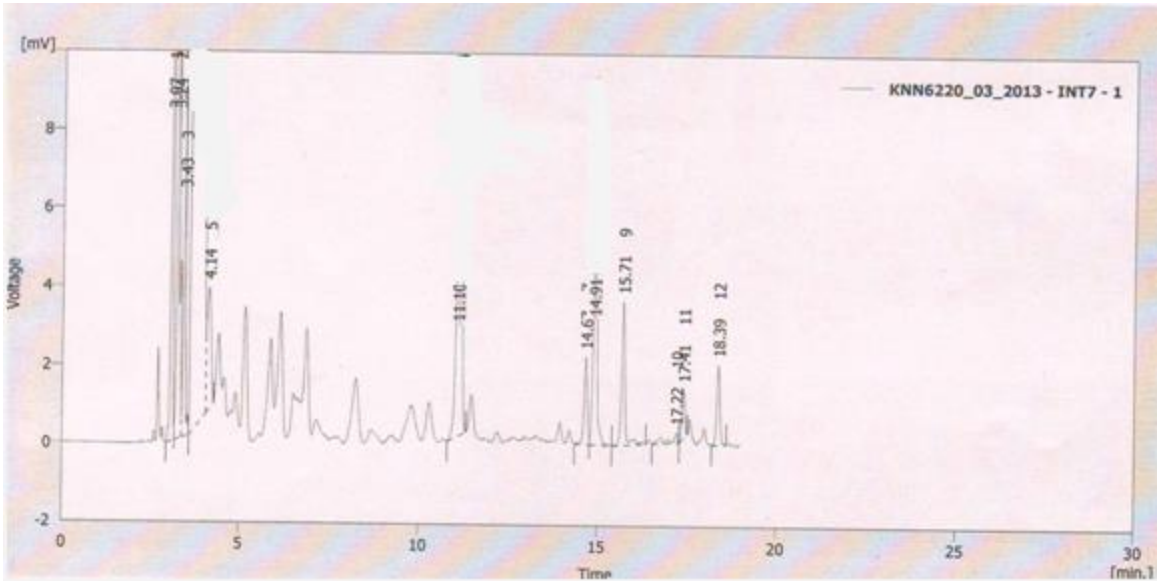


Figure 5. Profile curves of Gas chromatography of degradation of Naphthalene by *Pseudomonas putida*

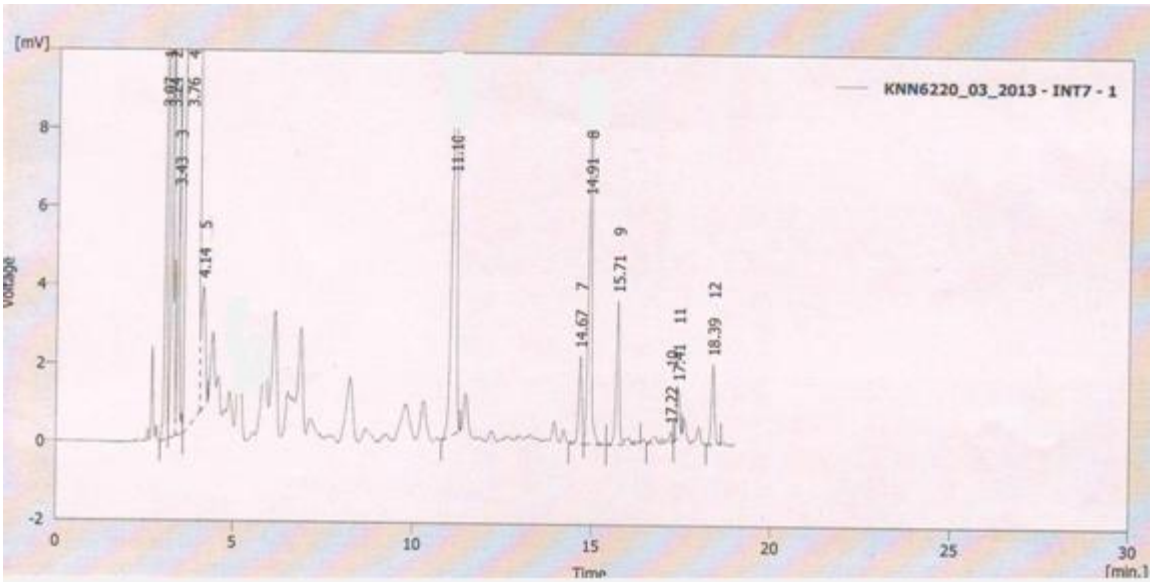


Figure 6. Profile curves of Gas chromatography of degradation of Benzene by *Pseudomonas putida*

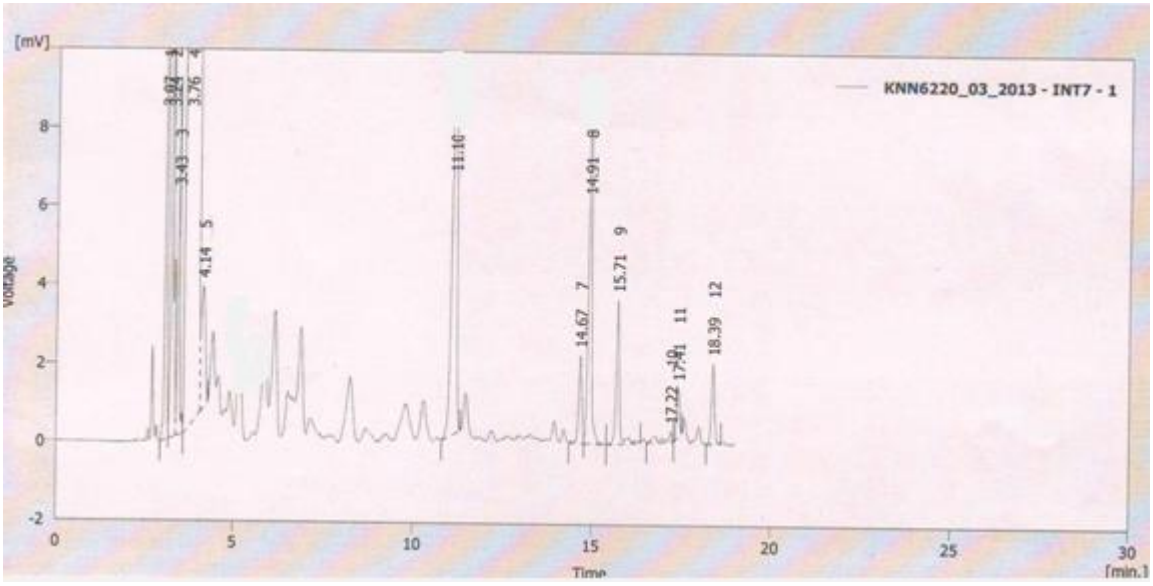


Figure 7. Profile curves of Gas chromatography of degradation of Chloroform by *Pseudomonas putida*

All these strains belonged to the genus *Bacillus*. Some other strains of genera *Alcaligensxyloxidans, Pseudomonas putida, Pseudomonas fluroscence, Paecilomycesvariotii, Cladosporiumsphaerrospermum*were tolerant to VOCs on the LB plate, but they could not grow on VOCs on the BH minimal agar plate. The five strains exhibited a differentiated morphology in the color and form of the colony: white, ivory, pale pink, beige, or yellow in colony color; rough, smooth, rigid, soft, sticky, moisture-less or wrinkled on the colony surface (**Table 1**). The growth of strains on the VOCs on

minimal agar (**Table 1**) provided a clue that the strains could use Xylene, petroleum ether Napthalene, Benzene and Chloroform as a carbon source. The degradation of Benzene and Chloroform by the VOC strains was directly determined by GC, and the culture was grown in sealed bottles.

TABLE 1: The identified bacterial strain

Sr. No.	Isolates	Code No.	Identified isolate
1	Isolate 1	V1	Alcaligensxyloxidans
2	Isolate 2	V2	Pseudomonas putida
3	Isolate 3	V3	Pseudomonas fluroscence
4	Isolate 4	V4	Paecilomycesvariotii
5	Isolate 5	V5	Cladosporiumsphaerrospermum,

SN.	Isolates code	Xylene		Petroleum ether		Naphthalene		Benzene		Chloroform	
		OD Control	OD (Exp.)	OD Control	OD (Exp.)	OD Control	OD (Exp.)	OD Control	OD (Exp.)	OD Control	OD (Exp.)
1	V1	0.18	0.12	0.22	0.15	0.17	0.10	0.19	0.16	0.16	0.10
2	V2		0.06		0.07		0.09		0.09		0.04
3	V3		0.11		0.10		0.10		0.10		0.10
4	V4		0.10		0.14		0.14		0.12		0.06
5	V5		0.16		0.13		0.12		0.16		0.08

TABLE 2: Analysis of VOC Degradation of UV-spectrophotometer

TABLE 3: Peak area of Gas chromatography of VOC industrial effluents after degradation by *Pseudomonas putida*

Isolates	Xylene	Petroleum ether	Napthalene	Benzene	Chloroform
V2	0.02	0.07	0.05	0.07	0.08

Table 3 lists the 14 strains that were tolerant to 1% (v/v) of Xylene, petroleum ether Napthalene, Benzene and Chloroform on an LB agar plate, and that could simultaneously grow on the 1% (v/v) of the same VOC as the sole C source on the BH minimal agar plate.

For this measurement, we selected four strains— V1, V2, V3, V4, and V5—that grew dominant, overall, on Xylene, petroleum ether, Napthalene, Benzene and Chloroform, simultaneously, and that were morphologically obviously different from each other. Xylene, petroleum ether was degraded similarly.

The VOC strain degraded toluene best, which decreased by 29.6% (59.8 ppm of the total 202.3 ppm) after seven days of incubation. The V3 and V1 strains degraded 21.1% and 11.6% of the toluene, respectively. In the case of cyclohexane, the V4 and V5 strains degraded similarly, with a 25.9% (54.8 ppm of the total 211.3 ppm) and a 25.6% (57.6 ppm of the total 225.5 ppm) reduction of cyclohexane, respectively; the V3 strain resulted in a 21.2% reduction of cyclohexane (44.9 ppm of the total 211.6 ppm).

The isolated strains were growing on VOCs were identified according to Bergey's manual of systematic bacteriology and by considering the physiological and biochemical characteristics. The strains were characterized by differentiated utilization patterns of sugar, sugar alcohol, acids, and sugar derivatives mainly using API 50 CHB. Among the monosaccharides, all of the four tested strains utilized glucose, fructose, and ribose. In addition to those three common sugars, strain V3 can use L-arabinose, D-xylose, galactose, and D-raffinose, and strain VOC18 can use D-mannose. V1, V4, and V3cannot metabolize any of the tested sugar alcohols. On the other hand, V3 can use glycerol, mannitol, and inositol. All four strains can use two disaccharides, namely, maltose and trehalose. In addition, strain V4 degrades cellobiose and sucrose, and V3 degrades sucrose.

CONCLUSION

The present study focuses on some habitually used solvents that are impending pollutants are listed based on their level of toxicity. Various organisms that are adeptly liable for the degradation of volatile organic compounds are discussed. The parameters that influence the efficiency of organic degradation were found to include moisture content as a foremost key factor. The removal efficiencies of VOC of different microbes were compared. The major characteristics of fungi and bacteria that govern the biodegradation were discussed. The efficiency of fungi was found to be greater than that of bacteria. On the other hand, mixed culture was possibly found to have higher efficiency which was a result of the cumulative efficiency conferred by the fungal presence and the moisture content that obliges to attain higher efficiency. The efficiency of degradation is also influenced by the volatile compounds present in the sample.

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