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Biodegradation of Waste Gas containing Mixture of BTEX by B. Sphaericus

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Abstract

In the present study, a biofilter reactor was effectively applied to remove BTEX from polluted air streams. A biological study was performed by isolation in solid agar slant media and contained cell shapes was identified by using an electron microscope. It was identified for seven different isolates that this mixed culture was gram positive. These strains were cultivated on substrates with BTEX as a carbon and energy sources. From seven isolates, Bacillus sphaericus was identified for biodegration of BTEX. All isolates cultivated in a pH range from 3-11 with an optimum range of 6-8, which was applicable for the temperature range of 15-45^{o}C giving a optimum range of 25-30^{o}C. The batch studies were carried out at five different initial BTEX concentrations ranging from 25-500 mg L^{-1}. B. sphaericus was able to give 100% degradation of BTEX at 200 mg L^{-1} after 72 hr. but for xylene it was around 90%. Finally the results signify that the B. sphaericus degrades BTEX at a faster rate and this strain can be used proficiently in biofilter for treating highly polluted air streams.

Key words: Biodgradation, BTEX, Bacillus sphaericus, growth rate.

Introduction

High emancipation of toxic volatile organic compounds (VOCs) into the environment owing to industrialization has crafted a huge global distress. Volatile organic compounds (VOCs) are regular pollutants produced by a variety of industries and their emissions are facing increasingly stringent environmental regulations[1]. Among these industries, refinery and petrochemical units, coating facilities, adhesives, pulp and paper and printing industries are the main sources of these pollutants.

Biofiltration is known as one of the most applicable technologies in biological treatment methods[2]. The processes involves passing of contaminated streams through a porous media on which microorganisms have been immobilized in the biofilter[3]. The microorganisms are capable of biodegrading the contaminants. Degradation mechanisms are different depending on various microorganisms. However, an oxidation process takes place subsequently, and alongside the microbial growth, CO_2 is produced[4, 5]. Generally, biofiltration has proven to be an applicable method for gas treatment since it is considered to be an economical technology compared with other techniques with high contaminant removal efficiency (RE) and also the minimum CO_2 is produced in this technology[6].

BTEX (benzene, toluene, ethyl benzene and o-, m- and p-xylenes) are the major components of gasoline and aviation fuels and are extensively used in industrial syntheses. It was confirmed by several studies that biofilters are able to successfully degrade several compounds like benzene, toluene and p-xylene[7-10]. Oxidation of benzene, toluene, ethylbenzene and xylene isomers (BTEX) by T. Versicolor was performed[11]. Since many bacterial strains metabolize BTEX, their biodegradation has been extensively investigated[12,13].

Various physico-chemical methods for the treatment of VOCs (BTEX, MTBX, and Pyridine) have been investigated. These include adsorption[14-20], sorption in zeolites [21], biodegradation[21,22,23]. A new bacterial strain, namely *B. sphaericus* was used in this study. Studies developed with *B. sphaericus* have shown that the bacterial strain has the ability to efficiently degrade BTEX. *B. sphaericus* was isolated from the biofilter unit which was used for the treatment of the mixture of BTEX. The isolated bacterial strain was identified from MTCC and IMTECH, Chandigarh, India, as *B. sphaericus* and was assigned a number 8103. The objective of this study was to investigate BTEX degradation by *B. sphaericus* in batch and isolation and characterization of bacterial strain proficiency of degrading high strength of BTEX and to identify the optimum conditions under which this strain can most efficiently breakdown BTEX.

Material and Methods

Chemicals and Media: In the bioreactor, nutrient solution with about 10 mL min^{-1} was continuously sprayed two times in a day for 30 min on the top of the packing media through the nutrient distribution system to insure satisfactory conditions of moisture and nutrients for microorganism activities using peristaltic pump. The composition of inorganic mineral salts solution (nutrients) is given in table 1. All the chemicals used will AR grade with more than 99% purity. The chemicals were from M/s S.D. fine Pvt. Ltd. India and M/s Ranbaxy Laboratories

Ltd., India. Other chemicals of biological grade were procured from Himedia, Mumbai and Genei, Bangalore.

Table-1
Target concentration of nutrients used in bioreactor

Components	Concentration of the constituents (g L^{-1})	Essential nutrient	Concentration of the essential nutrients (g L^{-1})
Macro nutrients			
KH_2PO_4	0.91	P	0.207
K_2HPO_4	0.4	P	0.071
$Na_2HPO_4 \cdot 2H_2O$	2.39	P	0.207
KNO_3	2.96	N	0.41
$(NH_4)_2SO_4$	1.97	NH_4^+	0.534
$MgSO_4.7H_2O$	2.0	Mg^{++}	0.364
$FeSO_4.7H_2O$	0.2	Fe	0.04
$NaHCO_3$	0.5	Na^+	0.136
Micro nutrients			
$MnSO_4.7H_2O$	0.88	Mn	0.175
$ZnSO_4.7H_2O$	0.04	Zn	0.009
$CaCl_2.2H_2O$	3.0	Ca^{++}	0.816
$Na_2MoO_4.2H_2O$	1.0	Mo	0.394
$CoCl_2.6H_2O$	0.04	Co	0.007

Isolation of Strains: Bioreactor was operated for six months for the biodegradation of BTEX. Samples were taken from bioreactor and purified through serial dilution technique. Plates were made by spreading purified samples on the nutrient agar plate. Serially diluted sample was then spread aseptically on the solid nutrient agar plate and the plates were incubated at 30°C for 48 hr. Several different colonies from bioreactor sample were obtained after incubation. Morphologically different colonies were subculture on different nutrient agar plates for further study. By this type of streaking, one isolate from chosen among seven isolates from bioreactor were found to have grown profusely. Maintenance of isolated strain was done by periodical transfer onto nutrient agar slant and storing at 4°C for further study, as well glycerol stocks of the cultures were prepared and stored at - 80°C.

Screening of isolated strains: The pure culture thus obtained from bioreactor treating BTEX was subculture in basal salt medium and the most efficient isolate were selected for the study. Seven pure isolated were patterned for their capability to grow in BSM with BTEX by vaccinating them into separate 500 mL bottles. So as to comprehend their degradation capability, biodegradation experiments of BTEX conducted, in replica, by using a volume of 100mL of BSM in 500 mL gas-tight bottle containing 5µL of each component. Design of the batch process set up is shown in figure 1. Teflon-coated silicone septum, sealed with brass cap, was used for collection of gas samples at regular periods. So as to preclude a deficiency of oxygen experiments were performed with only 100 mL of working volume in 500 mL bottles, in order to provide adequate amount of oxygen remained within the bottles. To avoid the possibility of volatilization of the BTEX compounds, the bottles were constricted in the arrangement. The samples of single colony from each plate were vaccinated discretely in the bottles. The operating conditions are 120 rpm for the shaking and at 30 °C for 48 hr. Consequently, 1 mL of culture from bottle was transported into 100 mL of fresh BSM media and 10 µL of each component were incubated for 48 hr. The cultures are then striped on solid nutrient agar plate. To enhance the utilization of the BTEX, the colonies were revaccinated into 100 mL BSM with 10 µL of each component and then the bottles are incubated and examined to select the most efficient isolates.

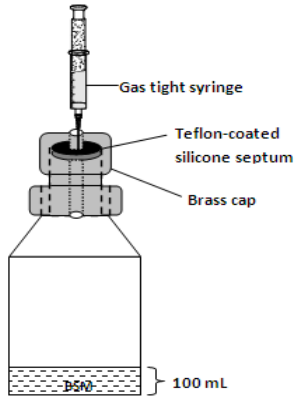

Figure-1
Schematic diagram for batch experiments

Strain Identification for Bioreactor: Based on the detailed of biochemical tests and gram straining results, the sample 01 was identified as a gram positive having an effective growth between 10 to 40°C and pH tolerance to a wide range of 3.0 - 11.0. The identification procedure of isolated bacteria was performed according to Buchanan and Gibbons, Bergey's Manual[24]. Based on the tests (table 2 and table 3), with reference to the Bergey's manual, the sample 01 was tentatively identified as genera *Bacillus*. This strain was also re-identified at Institute of Microbial Technology (IMTECH), Chandigarh, India, as *B. Sphaericus* and was assigned a number MTCC 8103.

Analytical methods: Concentrations of BTEX in the gases mixture were analyzed by using a Netel India Limited (model- MICHRO 9100) gas chromatograph equipped with a capillary column type HP5 (30m×0.249mm×0.25µm film thickness) and with a flame ionization detector. The injector, oven and detector temperature was maintained at 210°C, 60°C, and 230°C, respectively. The hydrogen gas was used as the fuel and nitrogen, as the carrier gas at a flow rate of 20 mL min^{-1}. The calibration curve was prepared by injecting known amounts of the BTEX into a

sealed bottle equipped with a Teflon septum according to the standard procedure [25]. The injected amount of BTEX was allowed for evaporating in the air space within the bottle at experimental temperature (30°C). For the calibration, air samples were drawn from the bottle by a 1 mL gas tight syringe (Hamilton-Bonaduz-Schweiz) and analyzed by gas chromatograph. The air samples were drawn from the various sampling ports by using a gas tight syringe and analyzed.

Spectrophotometer (Model UV 210 Shimadzu, Japan) was used for the measurement of biomass concentration and optical density of the culture at 600 nm.

Table-2

The Biochemical Tests of Seven BTEX Degrading Strains Isolated from an Active Bioreactor

Tests	**01**	**02**	**03**	**04**	**05**	**06**	**07**
Gram straining	-	-	-	-	-	-	-
Klingler Iron Agar Slant	**Butt → Red** **Slant → Red**	**Butt → Red** **Slant → Red**	**Butt → Red** **Slant → Red** **Bottom → Black**	**Butt → yellow** **Slant → Red** **Bottom → black**	**Butt → Red** **Slant → Red** **Bottom → black**	**Butt → Red** **Slant → Red** **Bottom → black**	**Butt → Red** **Slant → Red**
Catalase	+	-	+	+	+	-	-
Indole	-	-	-	+	-	+	-
Methyl red	-	-	-	+	+	+	+
Voges-Proskauer	-	-	-	-	-	-	-
Citrate	+	-	+	+	+	+	-
H_2S production	-	+	+	+	+	-	-
Glucose	-	-	+	-	-	+	-
Maltose	+	-	+	+	+	+	+
Sucrose	+	-	+	+	+	+	-
Lactose	+	-	+	+	+	+	-
Urease	+	+	+	+	+	+	-
Oxidase	*	+	*	*	*	*	*
Ornithine decarboxylase	*	+	*	*	*	*	*
DNase	*	+	*	*	*	*	*
L-arabinose	*	+	*	*	*	*	*

Table-3

The Characterization of Seven BTEX Degrading Strains Isolated from an Active Bioreactor

Isolated bacteria	**01**	**02**	**03**	**04**	**05**	**06**	**07**
Gram straining	-	-	-	-	-	-	-
Morphology under microscope							
Cell type (shape)	Short rod	Rods	Rods	Short rods	Rods	Rods	Rods
Color	Yellowish white	Yellowish white	White	White	Yellowish white	Yellowish white	White
Size	0.5x1.6µm	0.5-0.6 x 1.6-2.8 µm	0.5-0.6 x 1.6-2.8 µm	0.5x1.6µm	0.5-0.6 x 1.6-2.8 µm	0.5-0.6 x 1.6-2.8 µm	0.5-0.6 x 1.6-2.8 µm
Surface	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Arrangement	Groups	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated
Density	Opaque	Translucent	Opaque	Opaque	Opaque	Opaque	Opaque
Elevation	Convex	Convex	Convex	Convex	Convex	Convex	Convex
Motility	-	+	-	-	-	-	-
Physiological characteristics							
Ph							
3	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+
6	++	++	++	++	++	++	++
7	+++	+++	+++	+++	+++	+++	+++
8	++	++	++	++	++	++	++
9	+	+	+	+	+	+	+
11	+	+	+	+	+	+	+
RPM	125	125	125	125	125	125	125

Results and Discussion

Biodegradation of BTEX: Biological removal of BTEX using the isolated strains *Bacillus sphaericus* has been discussed in this section. In order to understand the growth pattern of the isolates, the effects of various operating parameters like pH, temperature and growth study were performed in basal salts medium with various concentrations of BTEX. For each batch experiment, one of the following parameters was varied while the others were kept constant: pH, temperature, BTEX concentration, and reaction time (table 4).

Effects of temperature on the biodegradation of BTEX by *B. sphaericus*: The percentage removal of BTEX by *B. sphaericus* was shown in figure 2 a and 2 b. The effect of temperature on the biodegradation of BTEX were observed at low (50 mg L^{-1}) and high (100 mg L^{-1}) concentration at the fixed pH of 7.0 for 24 hr. An Increasing behaviour of removal efficiency were found when temperature increases gradually. The results disclosed that the strains are mesophilic bacteria. It was observed that the percentage removal of BTEX by *B. sphaericus* increases gradually with the increase in temperature. It was concluded from the figure that the percentage removal of BTEX increases with increase in temperature from 15 to 25^{o}C following an exponential trend in both the low and high concentration. Then the curve follows a saturation phase from 25 to 35^{o}C which signifies that the optimum temperature would lie in this region. The removal efficiency decreased drastically on further increase in temperature for both low and higher concentrations of BTEX. From the batch study it is observed that the growth of *B. sphaericus* is possible in between 10 to 35^{o}C giving a maximum removal percentage at the temperature 30^{o}C.

Effects of pH on the biodegradation of BTEX by *B. sphaericus:* pH has a significant effect on the degradation efficiency of the strain. The removal of BTEX of 50 and 100 mg L^{-1} by *B. sphaericus* at various initial pH values are shown in fig. 3(a) and 3(b) for the time period of 24 hr. at 30 ^{o}C. Low concentration of BTEX was removed when the initial pH values are 3,4,5,6 and 7. In comparison to low concentration, high concentration of BTEX is less degradable. The percentage removal of BTEX reached maximum at pH 7.0, specifically between the optimum pH of 6.0 and 8.0. A comparative study was done for the pH value from 3 to 11 and we concluded that the remaining concentration was minimum at pH 7 especially between the optimum pH of 6.0 and 8.0. Concentration is comparatively high outside the optimum range due to the fact that the hindrance effect of super acidity and super alkalinity is effective on the activity of intracellular enzyme of *B. sphaericus*.

Table-4
Set of batch experiments of BTEX used to test optimum degradation conditions

B. Test	Parameters Varied	Temp. ^{o}C	pH	Cont. mg L^{-1}	Reaction Time hr
1	pH	30	3, 4, 5, 6, 7, 8, 9, 10, 11,	100	72
2	Temperature	10, 15, 20, 25, 30, 35, 40, 45	7	50, 100	24
3	Reaction time	30	7	100	12, 24,36, 48, 60, 72
4.	Contaminate concentration	30	7	50, 100, 200, 500, 1000	150

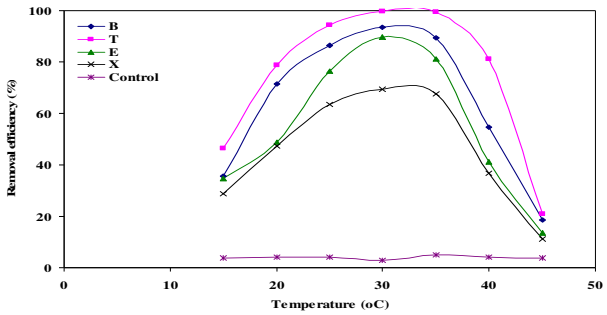

Fig 2 a

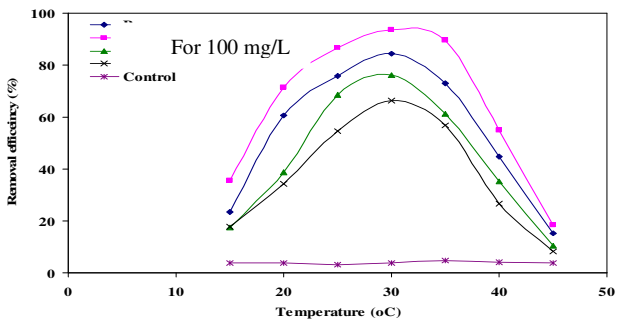

Fig 2 b

Figure-2
Effects of temperature on the biodegradation of BTEX by *B. Sphaericus* of concentration of (a) 50 mgL^{-1} (b) 100 mg L^{-1}

Effects of substrate concentration on the biodegradation of BTEX by *B. sphaericus:* Performance of batch studied for BTEX degradation in basal salt medium by *B. sphaericus* is shown in figure 4(a), (b), (c) and 4(d). The *B. sphaericus* degrades benzene with an initial concentration of 25, 50, 100, 200 and 500 mg L^{-1} in 12, 18, 28, 56 and 72 hr., respectively. In the case of toluene, it was found as 10, 14, 22, 44 and 72 hr., respectively. For ethyl benzene it was 18, 26, 34, 68 and 72 hr., respectively. A similar trend was also found for xylene. The *B. sphaericus* degrades xylene in 21, 30, 40, 72 and 72 hr., respectively. At higher concentration we perceived that towards the end of the substrate concentration curve, a reduced rate of substrate removal region exists. Results show that the degradation time of BTEX was low at low substrate concentration since degradation rate is high at low substrate concentration. We also conclude that at higher concentration of BTEX degradation rate is low and degradation time is high. This can be clarified by 3 ways, firstly there is the slight (<10%) fall in pH of the solution over time, since BTEX is a heterocyclic aromatic compound and is a weak organic base. Secondly, there is the deficit of oxygen since the experiments were performed in bottles of 500mL with 100mL working volume which shows that culture was not able to degrade efficiently in high concentration of BTEX under hypoxic condition in bottles. Thirdly, in an exponential phase, a drop in oxygen concentration can hinder the growth rate. Low values of both oxygen and pH may affect the kinetics of substrate consumption adversely.

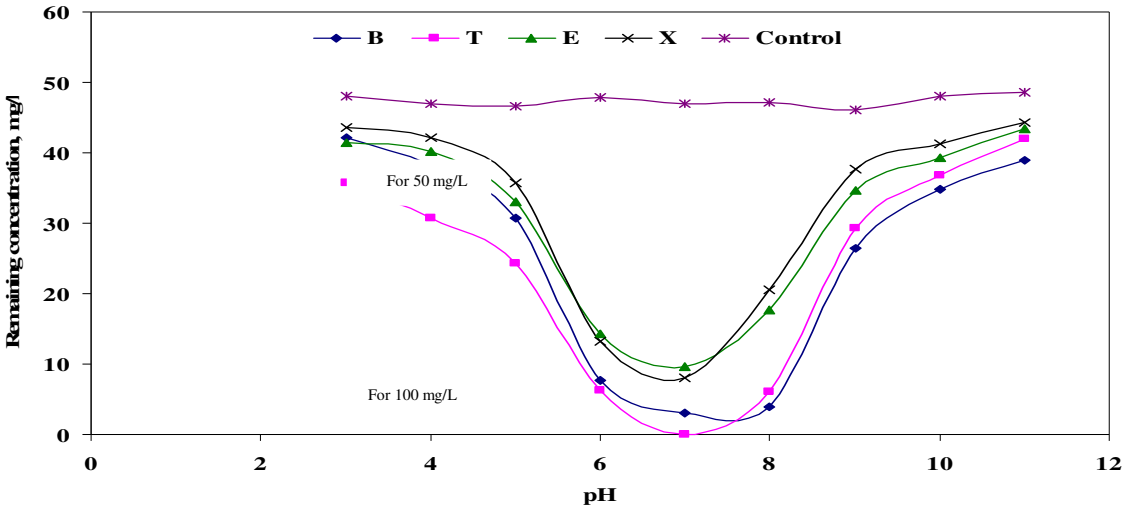

Figure-3 (a)

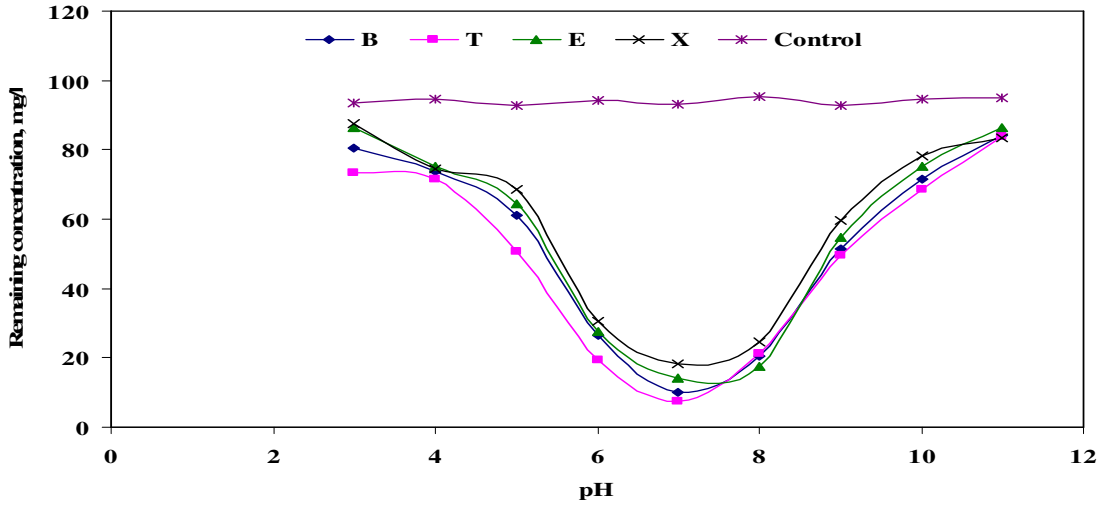

Figure-3 (b)
Figure -3
Effects of pH on the biodegradation of BTEX by *B. sphaericus* of concentration of (a) 50 mg L^{-1} (b) 100 mg L^{-1}

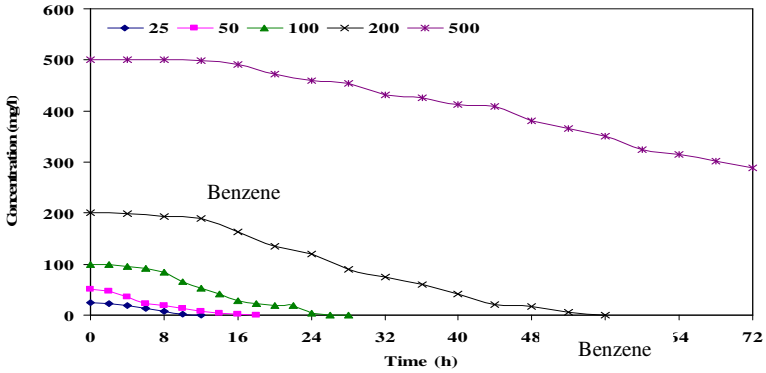

Figure-4 (a)

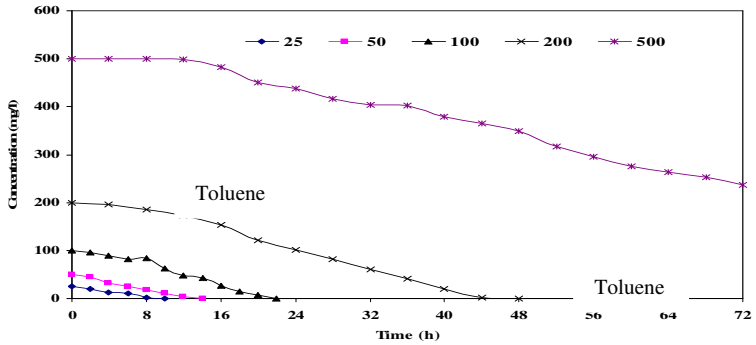

Figure-4 (b)

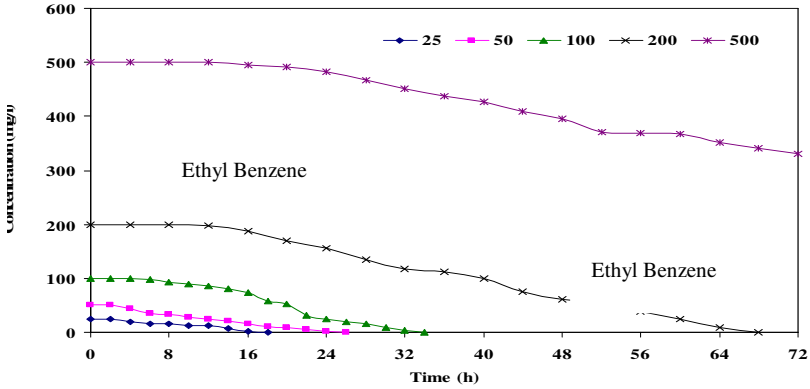

Figure- 4 (c)

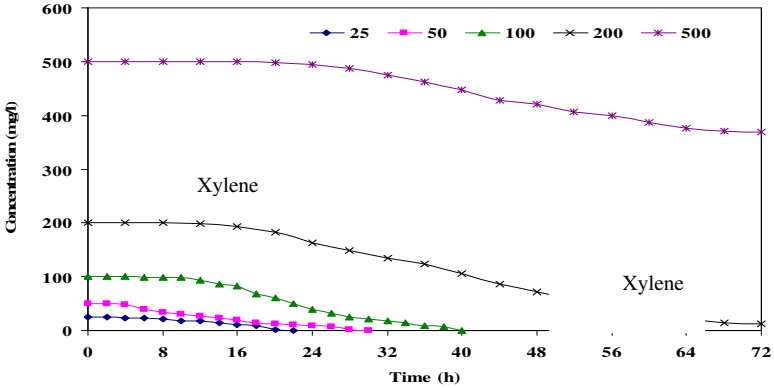

Figure-4 (d)

Figure-4
Effects of substrate concentration on the biodegradation of BTEX by *B. Sphaericus*

Growth of *B. sphaericus* (cell biomass) in BTEX: The growth of organisms increased with the increase in BTEX concentration. But the lag phase was extended at higher concentration of BTEX, which demonstrate positive correlation between cell biomass and BTEX degradation. In the batch studies, the biomass concentrations of *B. sphaericus* were initially low, but later on growth increases exponentially. Biodegradation rates were calculated as total degradation of substrate concentration per total degradation time and cell concentration obtained. It was cleared from figure 5(a), (b), (c) and (d) that the BTEX was maximum utilized by *B. sphaericus* effectively upto 200 mg L^{-1}.

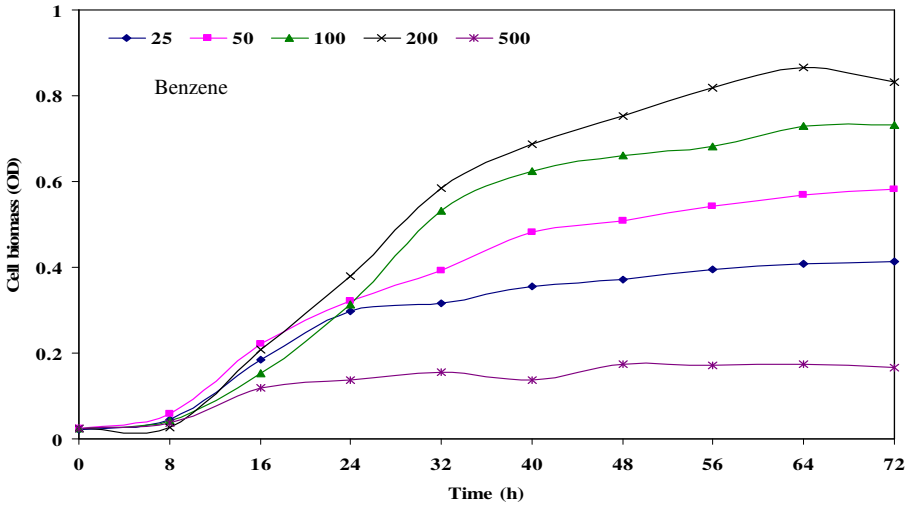

Fig 5 (a)

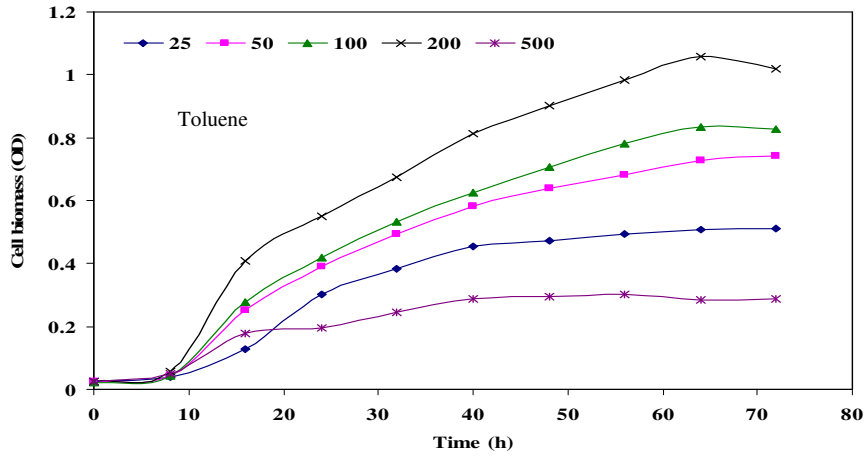

Fig 5 (b)

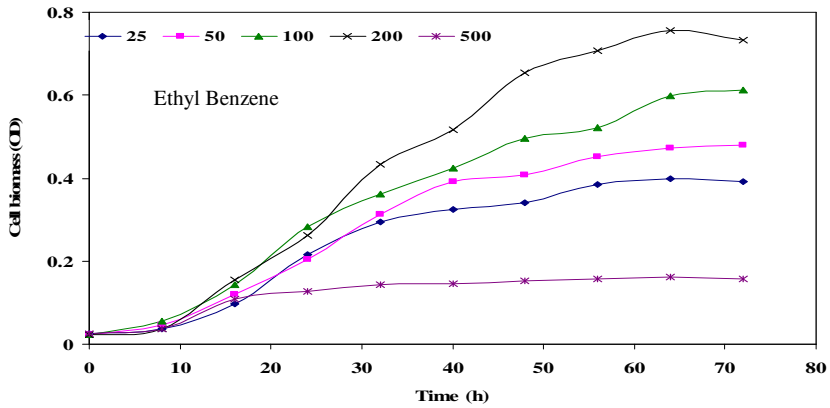

Fig 5 (c)

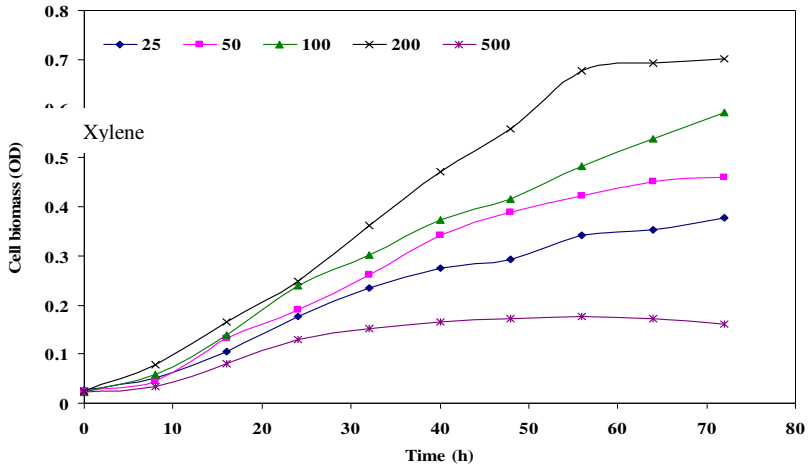

Fig 5 (d)

Figure-5
Growth of *B. sphaericus* (cell biomass) in BTEX

Conclusion

The objectives of this study were to quarantine the BTEX degrading bacteria from the biofilters and to examine its biological characteristics. The stains of *B. sphaericus* were isolated and identified. The results reveals that the pure *B. sphaericus* strain quarantined in this report, has a high capability for entirely degrading BTEX at concentration lower than 200 mg L^{-1}. But for xylene at a concentration of 200 mg L^{-1}, degradation was incomplete with around 90 % of the xylene degraded after 72 hr. These results intimate that *B. sphaericus* has potential for the use in biofilters for the anticipation of BTEX contaminated environments. The results exposed that *B. sphaericus* can cultivate at high concentration of BTEX from 15-45^{o}C, while it was less energetic when the temperature was higher than 40 ^{o}C. All isolates cultivated in a pH range from 3.0-11.0 with the optimum range of 6.0-8.0. Analysis of the results showed that the conditions of biofilter were most suited for toluene degradation followed by benzene, ethyl benzene and then xylene. Further, the method, adopted here for the isolation and identification of the strains by classification, biochemical at genetic level, will be very useful for the use of microbial culture in the operation of any bioreactor to treat gaseous pollutants like VOCs.

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