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BIOREMEDIATION OF METHANOL BY HALO-ALKALIPHILIC METHYLOTROPHS FROM LONAR CRATER

Tambekar DH* and Rajgire AV

PG Dept. of Microbiology, SGB Amravati University, Amravati – 444604, Maharashtra, India.

ABSTRACT

Methanol is the toxic pollutant and hazardous to human health. Methylotrophic bacteria use methanol as a sole source of carbon and energy and detoxify it, therefore attempt was made to isolate efficient methanol utilizing microbe from alkaline Lonar Lake (situated in Buldhana district of Maharashtra state, India). Lonar Lake has a unique ecosystem, harbors various unidentified, unique haloalkaliphilic bacterial species which have potential to degrade or remove chemical toxic pollutant from environment. In these studies, two bacterial strains *Pseudomonas aeruginosa*(DHT 2) and *Enterobacter cloacae* (DHT 8)were isolated and identified from sediment samples by using minimal salt medium containing 2% methanol. These *P. aeruginosa*and*E. cloacae* utilized methanol 78% and 75% in 96 h respectively. It is therefore recommended that these bacterial strains are best choice for bioremediation of methanol on contaminated sites.

Keywords: Lonar Lake, Methanol, Methylotrophs, *P. aeruginosa*, *E. cloacae*.

INTRODUCTION

Methanol is primarily used as an industrial solvent for inks, resins, adhesives and dyes and also used as a popular organic solvent in the manufacture of cholesterol, streptomycin, vitamins, hormones and other pharmaceuticals industries. It is well absorbed by inhalation, oral and topical exposure and hazardous to human health [1-3].Methylotrophs are a unique group of methylotrophic bacteria, which utilize methanol as sole carbon as an energy source and can also grow on a number of different one carbon compounds including methane, methylated amines and methylated compounds containing sulphur [4, 5]. Methylotrophic bacteria, phylogenetically distributed across diverse phyla, contribute significantly towards the biogeochemical cycling of carbon by facilitating the incorporation of C1 compound-derived carbon into biomass [6]. Methanol dehydrogenase (MDH) catalyzes the conversion of methanol to formaldehyde in methylotrophs [7].

Lonar crater is a simple, bowl-shaped, near-circular formed by meteor impact around 52 000 years ago and situated in Buldhana district of Maharashtra state, India has a unique ecosystem and harbors various unidentified, unique haloalkaliphilic bacterial species which have potential to degrade or remove chemical toxic pollutant from environment [8, 9]. Lonar Lake water harbors dense cyanobacterial (Spirulina) blooms and its decomposition in soda lakes likely to produce high quantities of methane, methanol, methylamine and dimethylsulfidefavouring the surveillance of methylotrophs in this lake [10]. Therefore attempt was made to isolate efficient methanol degrading microbial strain from alkaline Lonar Lake.

MATERIALS AND METHODS

Isolation, Enrichmentand characterization of Methanol-Degrading Bacteria:

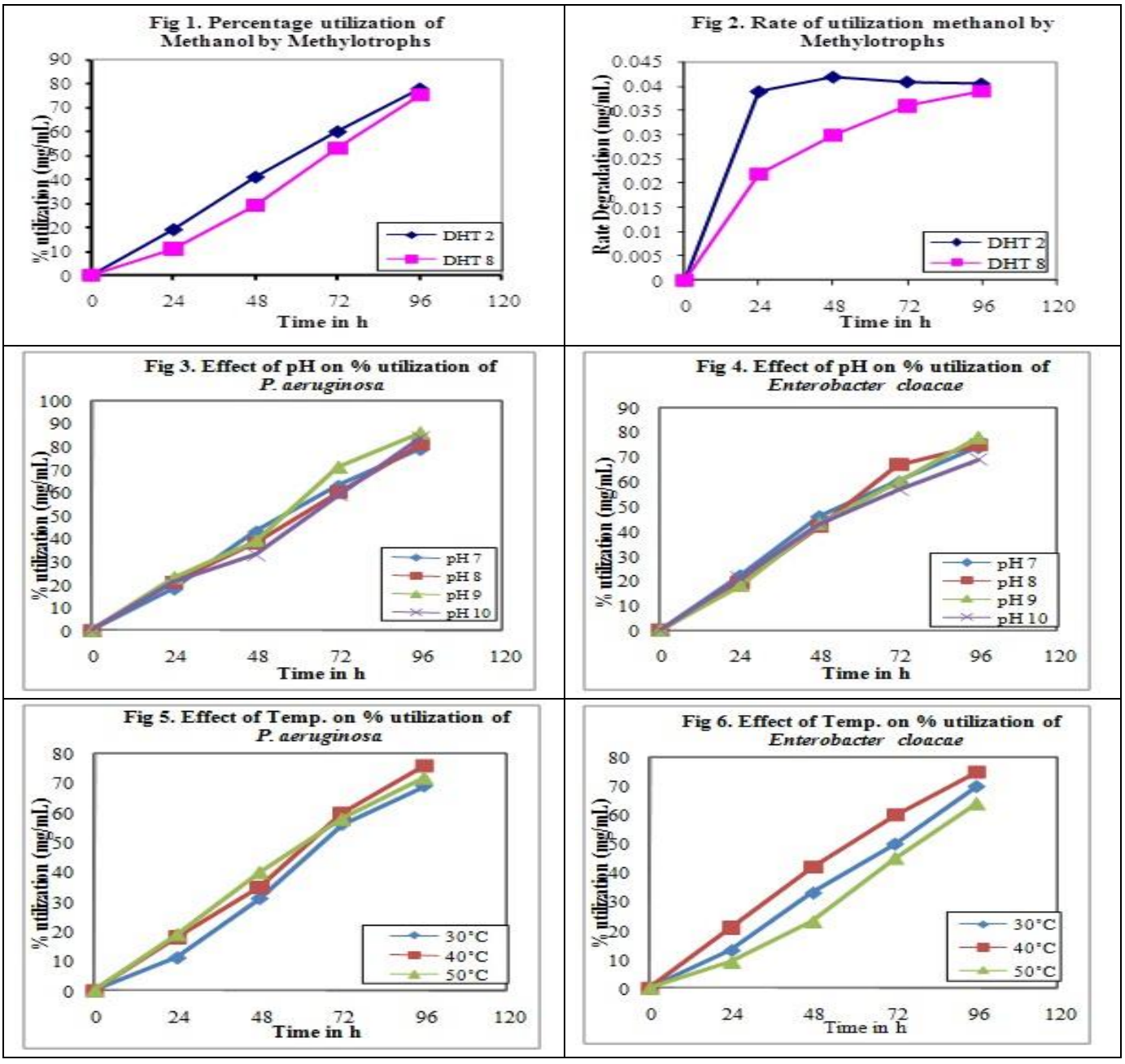
A total of twelve, four each, sediment, matt and water samples were collected from four different location of alkaline Lonar Lake during monsoon season 2014. All samples were labeled and kept in sterile plastic bottle (water sample) and zip lock bag (sediment and matt sample) at 4^0C until analysis. The samples were inoculated in minimal salt media (MSM) containing 2% methanol as

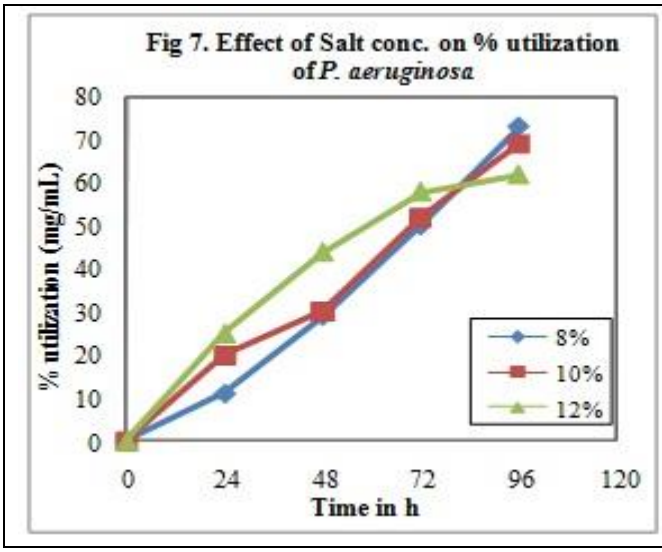
Corresponding Author:-**Tambekar DH** Email: diliptambekar@rediffmail.com

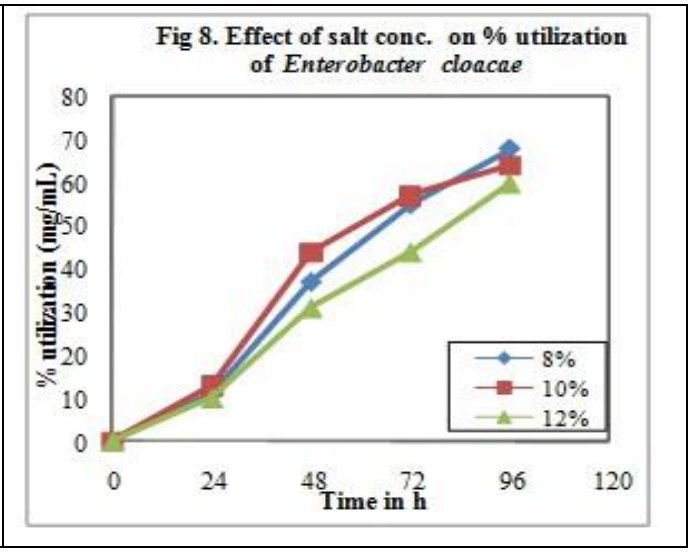
sole carbon source. All flasks were incubated at 37^0C in rotary shaker (100 rpm) for three days and five repeated sub culturing was made in MSM medium. After enrichment the broths were subculture on nutrient agar and after incubation well isolated and morphologicallydistinct colonies were selected and stored as a stock culture. Isolates were characterized culturally, morphologically and biochemically by commercially available Hi-media Rapid Detection kit KB003 and KB009. These bacteria were also identified on the basis of 16S rRNA gene sequencing at NCCS, Pune.

Estimation of Methanol utilizing potential

For estimation of methanol utilization, 5mL culture broth of isolates was inoculated in MS medium containing 5mg/mL methanol as a source of carbon. The methanol concentration was determined by analyzing samples at every interval of 24 h by using UV- Visible spectrophotometer at 481 nm. In this methodSodium nitroprusside (SNP) react with methanol to form colored product. Absorbance of product is linear with certain extent of the concentration of methanol [11]. The effect of environmental parameters such as pH, temperature and salt concentration on methanol utilization efficiency was also studied.







RESULTS AND DISCUSSION

Bioremediation, a biological treatment process which degrades or reduces or utilizes the hazardous pollutants in the environment and cleans the sites that have been subjected to pollution by microbes. Microorganisms have an ability to grow in polluted environment and are generally assumed to be tolerant to pollutant [12]. In the present study, attempt was made to isolate methanol utilizing organisms from halophilic environment such as Lonar Lake, having a unique ecosystem and harbors various unidentified, unique haloalkaliphilic bacterial species which have potential to degrade or remove chemical toxic pollutant from environment.

In the present investigation, two bacterial strains DHT 2 and DHT 8 were successfully isolated as prominent methanol utilizer by using enrichment technique. These isolated bacterial strains were characterized by cultural, morphological and biochemical test (Table 1). These DHT 2 and DHT 8 bacterial isolates were analyzed for the 16S rRNA gene sequencing and results suggested that DHT 2 was belongs to genus *Pseudomonas* of Pseudomonadaceae family having the nearest neighbor as *Pseudomonas aeruginosa*with 100% similarity and DHT 8 was belongs to the genus Enterobacter of Enterobacteriaceae family having the nearest neighbor as*Enterobacter cloacae* with 99% similarity. Daphne and Loka, [13] isolated methanol degrading *Flavobacterium*and *Pseudomonas*sp, from estuarine water and offshore sediment. Four methylotrophic strains including *Acinetobacterbaumani, Achromobactrumxylosoxidans, Ochromobactrumtritici*and *Pseudomonas aeruginosa*were isolated in the sediments of Lonar Lake by Tambekar*et al.,* [5, 14]. Thakkar and Ranade, [15] isolated alkaliphilic*Methanosarcina* from Lonar Lake. Tambekar and Pawar, [16] isolated six *Pseudomonas*strains fromLonar Lake having good potential to degrade methanol.

Isolates *P.aeruginosa* and *E. cloacae* were studied to check its ability to utilize or degrade methanol by analyzing residual methanol after each interval of 24h to 96 h by using UV- visible spectrophotometer at 481 nm.The effect of environmental parameters such as pH, temperature and salt concentration on methanol utilization efficiency was also studied. *P.aeruginosa* utilized 78% and *E. cloacae* 75% (rate of utilization 0.0406 mg/mL and 0.0390 mg/mL) methanol in 96 h respectively (Fig. 1 and 2). Similar result was recorded by Tambekar*et al.,* [17], who isolated two methanol utilizing haloalkaliphilic*Pseudomonas* sp. from Lonar Lake which utilizes 79% to 82% methanol at pH 10 and temperature 40˚C. The optimum methanol utilization 86% was observed at pH 9 by *P.aeruginosa*and 78% methanol utilized by *E. cloacae* at pH 9 (Fig. 3 and 4). Tambekar*et al.,* [5] reported 70% percent utilization and 0.036mg/mL rate of utilization of methanol by *Ps. aeruginosa*. Some bacteria are known for their bioremediation potential, including members of *Pseudomonas* sp., *Enterobacter-clostridium* species [12]. The optimum temperature 40˚C for both isolates was recorded for methanol utilization;76 % was recorded by*Ps.aeruginosa*and 75% by *E. cloacae* (Fig 5 and 6). Tambekar*et al.,* [18] isolated *Ochrobactrumoryzae* from Lonar Lake and observed that *O. oryzae* utilization 78% methanol at pH 7 and at 40˚C. These results are in concurrence with present study. In different salt concentration (8% to 10%) *Ps.aeruginosa*utilized 73% on 8% sail concentration and 68% methanol utilized on 8% salt concentration by *E. cloacae* (Fig 7 and 8). Tambekar*et al.,* [14] reported that *Pseudomonas aeruginosa* is new species, not previously recorded in Lonar Lake, which can utilized methanol as carbon source.

Table 1. Morphological and biochemical characteristics of bacteria isolated from Lonar Lake

Test	***Ps. aeruginosa* (DHT 2)**	***Enterobacter cloacae*(DHT 8)**	**Test**	***Ps. aeruginosa* (DHT 2)**	***Enterobacter cloacae*(DHT 8)**	**Test**	***Ps. aeruginosa* (DHT 2)**	***Enterobacter cloacae*(DHT 8)**
Shape	R	R	Catalase	+	+	Lactose	-	-
Color of colony	Green	Cream	Oxidase	+	-	Arginine	-	+
Gram staining	-ve	-ve	MR	-	-	Sucrose	-	+
Texture	Sm	Sm	VP	-	+	Maltose	-	+
Arrangement	S	S	Citrate	+	-	Fructose	-	+
Motility	+	+	Xylose	+	+	Dextrose	+	+
Growth at different temperature			Lysine Utilization	-	-	Nitrate reduction	+	+
30^0c	++	++	Arabinose	-	+	Mannose	-	+
40^0c	++	++	Glucose	+	+	Melibiose	-	+
50^0c	+	+	Galactose	+	+	Glycerol	-	+
Growth at different pH			Raffinose	-	+	Salicin	-	δ
pH 7	+	+	Trehalose	-	+	Dulcitol	-	-
pH 8	+	+	Mannitol	-	+	Inocitol	-	-
pH 9	+	+	Adonitol	-	-	Sorbitol	-	+
pH 10	+	+	Saccharose	-		Erythritol	-	-
pH 11	+	+	Esculin hydrolysis	+	+	Melezitose	-	-
Growth at different salt conc.			α- Methyl-D-Glucoside	-	-	Ornithine	+	+
1%	+	+	Rhamnose	-	+	Xylitol	+	+
2%	+	+	Cellibiose	-	+	Sorbose	-	-
3%	+	+	ONPG	-	+	L-Arabinose	-	+
4%	+	+	Esculin	+	+	Inulin	-	-
5%	+	+	Malonate	+	+	Sodium Gluconate	-	-
Note:- + = Positive; - = Negative; R= Rod; S= Single; Sm=Smooth;								

CONCLUSION

The aerobic methanol utilizing *P.aeruginosa* and *E. cloacae* appears to have greater potential for enhanced methanol degrade through utilization of methanol as sole source of carbon and energy. Resistance against a high concentration of methanol facilitates its use for biological treatment system of wastewater. Here we report these methylotrophs capable of grow at relatively at high pH, temperature and salt concentrations. The present technology could be efficient and beneficial to treat the waste generated by chemical industry.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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