

**INTERNATIONAL JOURNAL OF ADVANCES IN
PHARMACY, BIOLOGY AND CHEMISTRY****Research Article****Bioremediation of C₁ Compounds from
Methylotrophic Bacteria isolated from Lonar lake****Tambekar DH, Rajgire AV, Gaikwad JN.**

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ABSTRACT

Alkaline Lonar Lake situated in the Buldhana district of Maharashtra state, India have a unique ecosystem and harbors various unidentified, unique haloalkaliphilic bacterial species which have potential to degrade or remove chemical toxic pollutant from environment. Methanol is the toxic pollutant and hazardous to health therefore attempt was made to isolate methanol degrading bacteria from alkaline Lonar Lake. Methylotrophic bacteria have ability to utilize methanol as a source of carbon and energy. In these studies, water, matt and sediment samples were collected from Lonar Lake and inoculated in minimal salt medium containing 2% methanol as a only source of carbon and energy. Methylotrophic Bacterial isolate was characterized by cultural, morphological, biochemical tests and identified by 16S rRNA gene sequencing as *Ochrobactrum oryzae*. This *Ochrobactrum oryzae* bacterium was further screened for its ability to utilize methanol by spectrophotometric method. The study suggested that the *Ochrobactrum oryzae* possesses a distinct capability for methanol utilization which could be harnessed for the detoxification of methanol on polluted sites and might be useful to control global warming and other C₁ compounds pollution.

Keywords: Lonar Lake, Alkaliphilic bacteria, Methylotrophs, C₁ compounds and *Ochrobactrum oryzae*.

INTRODUCTION

Lonar crater (centered at 191590 N and 761310 E) is a simple, bowl-shaped, near-circular crater formed by meteor impact around 53 000 years ago, being the only well-preserved terrestrial crater in Maharashtra, India.^{1,2,3,4} Environmental pollution is an important consequence of industrial processes and human activity. Different industries, including mining and electroplating, releases aqueous effluents containing a variety of toxic chemicals; wastewater from these industries have permanent toxic effects to human and the environment. Methanol is one of the popular organic solvents and finds extensive applications in industries and household uses. It is rapidly and well absorbed by inhalation and by oral and topical exposure. Although poisonings that are resulted by absorption through the skin and inhalation and most disastrous methanol intoxications are related to ingestion of methanol itself or methanol containing beverages^{5,6}. Methylotrophs are a unique group of methylotrophic bacteria, which utilize methanol as

sole carbon as an energy source and can grow on a number of different one carbon compounds^{7,8,9}. Methane oxidizing bacteria (MOB) include species in the Alpha proteobacteria (type II MOB) and in the Gamma proteobacteria (type I MOB). They are a subset of the methylotrophic bacteria which can grow on a number of different one carbon compounds including methane, methanol, methylated amines and methylated compounds containing sulphur^{10, 11, 12}. These microbes adapt quite rapidly and grow at extreme condition using hazardous compounds as carbon and energy sources, microbes can adapt rapidly to extreme conditions in waste streams¹³. The alkaline Lonar Lake harbours the many industrially important microbes which might be degrade methanol like toxic industrial effluent. The methanol degrading bacteria present in the Lonar Lake has not been studied in detailed so far. Therefore attempt was made to apply culture dependent strategy to explore the diversity of

methanol degrading bacteria from Lonar Lake and identification of this degrader based on cultural, morphological biochemical characters and 16S rRNA gene sequence analysis. In the present study, we have isolated and characterized the wide variety of halophilic microorganisms which are capable to degrade methanol.

MATERIALS AND METHODS

Collection of Samples: A total of twelve sediment, matt and water samples were collected from four different location of alkaline Lonar Lake during monsoon season 2013 using sterilized spatula. All samples were labeled and kept in sterile plastic bottle (water sample) and zip lock bag (sediment and matt sample) at 4°C until analysis.

Isolation and biochemical characterization of methylotrophic Bacteria: The samples were inoculated in minimal salt media containing 2% methanol as carbon source. All flasks were incubated at 37°C in rotary shaker (100 rpm) for three days each and five times repeated sub culturing were made in same medium for enrichment. After enrichment the broth were inoculated on solid nutrient agar plate and after incubation well isolated and morphologically distinct colonies were selected and stored as a stock culture. The bacterial isolate was characterized by morphological and cultural characteristics. While rapid detection kit (Hi-media) was used to perform biochemical tests. The kit involves indole, methyl red, voges prauskar, citrate, lactose, xylose, maltose, fructose, dextrose, galactose, raffinose, trehalose, melibiose, sucrose, L-arabinose, mannose, inulin, sodium gluconate, glycerol, salicin, glucosamine, dulcitol, inositol, sorbitol, rhamnose, cellobiose, melizitiouse, α -methyl mannose, xylitol, arabinose, citrate, malonate, sorbase, nitrate reduction, urease and starch hydrolysis tests and identified by 16S rRNA sequencing from NCCS, Pune.

Determination of Methanol degradation potential: For determination of methanol utilization, the bacterial isolate was grown in nutrient broth and incubated overnight at 37°C. This culture was then inoculated in minimal salt medium containing 2% methanol as sole source of carbon and energy. The methanol concentration was determined by analyzing samples at each 24 h by using UV- Visible spectrophotometer at 481 nm. In these method Sodium nitroprusside (SNP) can react with methanol to form colored product. Absorbance of product is linear with certain extent of the concentration of methanol¹⁴.

RESULTS AND DISCUSSION

Lonar Lake is unique in the world due to extremely high carbonate alkalinity, high pH 9 to 11 and high salinity^{15,16}. Lonar Lake water is green throughout the year because of dense cyanobacterial bloom dominated by *Arthrospira*¹⁷. Methylotrophic bacteria are unique organisms with the ability to use C₁ compounds as only sources of carbon and energy, and play a important role in global carbon cycling. While studying the methylotrophic bacteria from Lonar Lake, a total of twelve samples comprising of sediment, matt and water samples were collected and processed on minimal salt media containing 2% methanol as carbon source. After five times subculturing in minimal salt medium containing 2% methanol, the broth was then inoculated on Nutrient agar plate for isolation of methylotrophs. Then well isolated colonies are transfer on Nutrient agar slant for further characterization. The bacterial strain was analyzed for standard biochemical test and further confirmed by 16S rRNA sequencing. The isolate was gram negative, short rod, aerobic and motile. The isolate was characterized cultural, morphological and biochemical. The biochemical test was done by Hi-media Rapid Detection Kit KB003 (Table 1). Isolate was also identified by 16S rRNA sequencing from NCCS, Pune and identified as *Ochrobactrum oryzae*. Four methylotrophic strains including *Acinetobacter baumani*, *Achromobacterum xylooxidans*, *Ochromobacterum tritici* and *Pseudomonas aeruginosa* in the sediments of Lonar Lake were isolated by Tambekar *et al.*, (2011) using minimal salt medium containing all required salts and trace elements with 2% methanol as the single source of carbon and energy. Gainutdinova *et al.*,¹⁵, isolated methanotrophs from the surface layers of sediment samples and from dipper horizons. Tambekar *et al.*¹², isolated Methylotrophic bacteria and reported as *Pseudomonas aeruginosa*.

In the present study, the isolate was studied for methanol degradation or its utilization. The study showed that the percent utilization was maximum in 96 h (78%) while the rate of degradation was maximum upto 48 h ((0.045) and then decline to 0.038) in 96 h (Fig. 1 & 2). The rate of degradation was also affected by pH of the environment and it was found to be 78% in 96h at pH 7 as compared to 60% at pH 9 (Fig. 3). The methanol degradation was recorded highest (0.042mg/h) at pH 7 and at pH 8 within 72h and then declined (Fig. 4). The effect of temperature on methanol utilization was studied and it was found that the optimum temperature for organism was 40°C, while at 30°C and 50°C, the percent utilization and rate of degradation was found

to be 0.038 and 74% mg/mL after 96 hrs (fig. 5 and 6).

Temperature was also found to affect both percent utilization and rate of degradation. The optimum temperature for percent utilization and rate of degradation was found to be 40°C. At 40°C there was gradual decrease in percent utilization and rate of degradation (Fig 5 and 6). Tambekar *et al.*,¹² (2013) studied percent utilization and rate of degradation of methanol from *Pseudomonas aeruginosa* and results was found to be 70% and 0.036mg/mL.

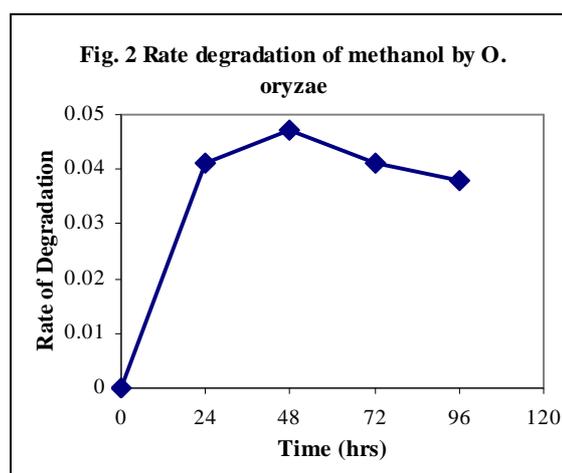
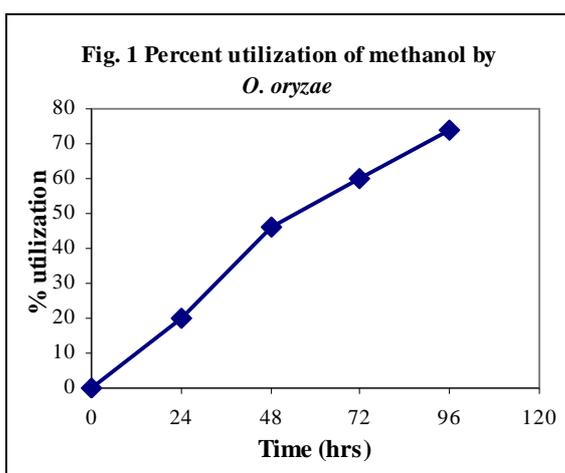
CONCLUSION

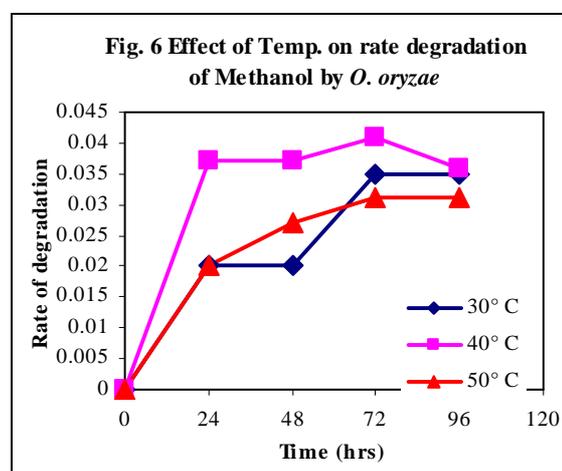
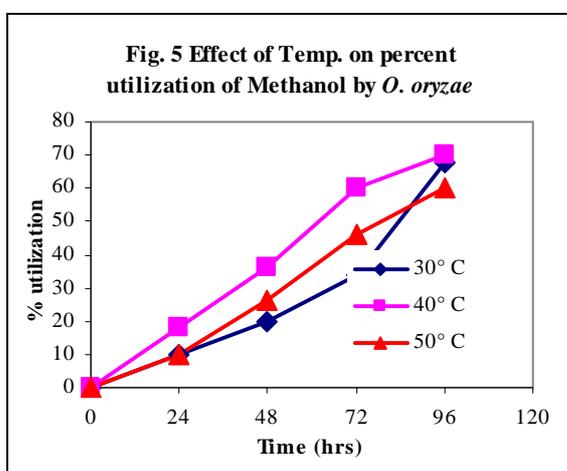
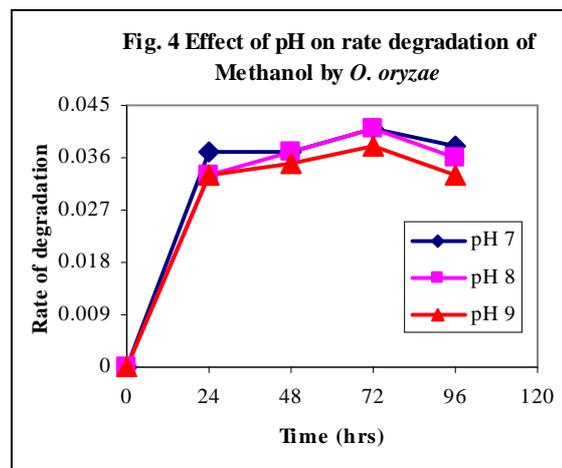
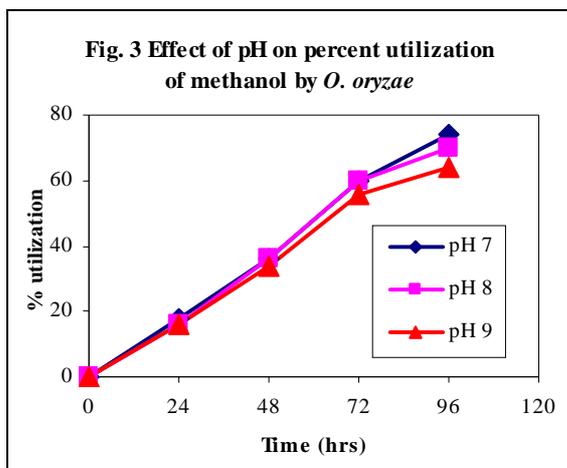
Present investigation successfully showed methanol removal by potent bacterial isolate *Ochrobactrum oryzae* and a very good methanol removal in shake flask condition. The result also concludes that bacterial isolates might be exploited for bioremediation of methanol from the industrial effluent and other polluted sites.

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

TEST	RESULT	TEST	RESULT	TEST	RESULT
Colony shape	Circular	Citrate	-	Adonitol	+
Colour of colony	Milky white	Nitrate reduction	+	Lysine Utilization	-
Gram staining	-	Lactose	-	Ornithine	+
Shape	Short Rod	Xylose	-	Mannitol	-
Arrangement	Single	Glucose	-	Rhamnose	-
Motility	+	Arabinose	-	Cellibiose	-
Catalase	+	Saccharose	-	ONPG	-
Oxidase	+	Galactose	-	Esculin	-
Indole	-	Raffinose	-	Malonate	-
Methyl Red	-	Trehalose	-	Urease	+
Voges Proskauer's	-	Mannitol	-	Phenylalanine	-

Note: + = Positive, - = Negative





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