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Degradation of Mono Ethylene Glycol by Few Selected Microorganisms and Developed Microbial Consortium

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Abstract: Mono-Ethylene Glycol (MEG), a major pollutant present in the effluents released from polyfilm manufacturing industry, petroleum industry, pharmaceutical industry, runway deicing, paints etc. The environmental protection agency has set a drinking water limit for MEG of 7 mg/L for adults. Toxicity and irritation of MEG was assessed in rabbit eyes. Ocular toxicity above 0.4% concentration consisted of conjunctival redness, chemosis, flare and iritis. In the previous studies, 15 bacterial, 3 fungal and 4 actinomycetes cultures were isolated from various ecosystems and were identified. They all showed ability to use MEG as sole source of carbon and energy. In the present investigation 5 best isolates were screened from all the isolates by checking their ability to grow at varying concentrations of MEG. The best 5 isolates screened were tested individually and in consortium for MEG degradation quantitatively using GC-FID. *Oliptrichum macrosporum* showed maximum 70.21% MEG degradation followed by *B. niacini* (67.87%), *Streptomyces* sp-1 (65.82%) and *A. terreus* (64.36%) at 30°C under shaking conditions (120 rpm) at an inoculum level of 5% (v/v). Minimum percentage (54.50%) of MEG degradation was shown by *A. faecalis*. On the contrary, the microbial consortium showed 75.49% of MEG degradation, which is approximately 5% more MEG degradation by the best degrader O. macrosporum, when tested individually. These studies can provide cost-effective, efficient ready-to-use microbial inoculum for the treatment of effluents containing MEG by the industries.

Key words: Monoethylene glycol · Biodegradation · Turbidometric analysis · GC-FID · Consortium

INTRODUCTION

Mono-Ethylene Glycol (MEG) is a clear colorless liquid with no odour. It has low volatility; it is miscible with water [1-3]. In 1993 estimated world production capacity of MEG was 9.4 million tones [4]. On a world wide basis approximately two third of MEG is used as a chemical intermediate in the manufacture of polyesters for fibers, polyfilm manufacturing industries, bottles, etc. with a further one quarter used as antifreeze in engine coolants. MEG is also used for runway deicing (the main source of high local concentration in the environment) as plasticizer in paints. MEG has been identified as a toxic substance for human consumption with effects such as comma, respiratory failure, gastro-intestinal upset, cardio-pulmonary effects and renal damage [2]. The environmental protection agency has set a drinking water limit for MEG of 7 mg/L for adults. Toxicity and irritation of MEG was assessed in rabbit eyes. Ocular toxicity above 0.4% concentration consisted of conjunctival redness, chemosis, flare and iritis [5]. Besides toxicity some MEG ethers and their metabolites are found to be responsible for mutations in *Salmonella typhimurium* his^- [6]. Chronic toxicity of MEG in rats showed 1% to 4% concentration of MEG in diet is responsible for increase in the mortality of the rats [7]. Studies on metabolic pathways showed that the degradation of MEG in *Flavobacterium sp*. proceeds via glyoxylate, glycolate and pyruvate. It also suggests that microbial degradation of MEG under auxic conditions occurred without accumulation of toxic and /or persistent organic intermediates [8, 9].

It is noteworthy that MEG and Di-Ethylene Glycol (DEG) are not utilized by microbial population capable of degrading higher molecular weight MEGs. Hence, there is possibility of MEG and DEG accumulation [10]. Aerobic

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and anaerobic biodegradation of Poly ethylene Glycol (PEG) 600, 6000 and 20,000 was investigated and it was found that aerobic degradation is more efficient than that of anaerobic [11].

Amongst most of the industries Polyfilm industry mainly uses MEG as a key ingredient for the production of Polyfilms. Thus industries tend to have MEG as a constituent of their effluent. MEG containing industrial waste waters, if discharged without treatment can lead be hazardous to flora and fauna consuming such contaminated water. Presence of such organic compound in the industrial effluents leads to the higher BOD and COD levels making it unfit for disposal in nature.

In our previous studies, 15 bacterial, 3 fungal and 4 actinomycetes cultures were isolated from various ecosystems and were identified. These cultures showed ability to use MEG as a sole source of carbon and energy [12]. In the present investigation, 5 efficient isolates were screened from a total of 22 isolates on the basis of their ability to grow at varying concentration of MEG. Later these five efficient microbial isolates were tested individually and in combination for MEG degradation. The percent degradation of MEG was determined quantitatively using GC-FID.

MATERIAL AND METHODS

Effect of Varying Concentrations of Meg on the Growth of Isolated Microorganisms

Material: Mono-Ethylene Glycol, Merck (Darmstadt, Germany), Minimal salt medium (Inorganic medium) gm/l: (K_2HPO_4 0.625, KH_2PO_4 0.375, $MgSO_4.7H_2O$ 0.25, NH_4Cl 0.5, NaCl 1.0 and trace element solution 1 ml/l).

Trace Element Solution Mg/l: ($CaCl_2$ 40, $FeSO_4.7H_2O$ 40, $MnSO_4.4H_2O$ 40, $ZnSO_4.7H_2O$ 20, $CuSO_4.5H_2O$ 5, $CoCl_2.6H_2O$ 5, $Na_2MoO_4.2H_2O$ [13].
Physiological saline (0.85% (w/v) NaCl).

Microorganisms: Bacterial cultures (15), Fungal cultures (3) and Actinomycetes (4) which were isolated and identified in our previous studies.

Method: Bacterial suspensions were prepared in sterile physiological saline and their optical density was adjusted to 0.1 at 600 nm in order to have uniform cell density. A set of screw cap tubes containing 10 ml sterile mineral salt medium with varying concentration of MEG ranging from 0.25, 0.50, 0.75 and 1.0% (v/v) were inoculated with 1 ml of bacterial suspension. Spore suspensions of fungi and actinomycetes were prepared in sterile saline containing 10^6spore/ml and 1ml of this was inoculated into screw cap tubes containing the medium and MEG concentrations as mentioned above.

All the tubes were incubated on a rotary shaker (120rpm) at 30°C for 7 days. After incubation the bacterial growth was measured by taking the O.D. at 600 nm. The growth of fungal and actinomycetes cultures was estimated by filtering the growth contents through Whatman filter paper number 1. The biomass obtained was dried in an oven at 65°C till constant dry weight obtained.

Quantitative Degradation of Meg

Material

Microorganisms: Two bacteria, *Alcaligens faecalis* strain CK221*, Bacillus niacini* Pm1. Two fungi: *Oliptrichum macrosporum, Aspergillus terreus* and a *Streptomyces* sp-1.

Gas Chromatography (Chemito-7610) with Flame Ionization Detector (GC-FID), GC column: Stainless steel packed column of SE-30 (Liquid phase: - 5% SE-30; Solid support CHW (HP); Mesh size: - 80/100; Sr. no: - TOB-3682; Length: - 1mt). Oxygen gas, Hydrogen gas and Nitrogen gas as a carrier gas

Method: Alcaligens faecalis CK221, *Bacillus niacini* Pm1, *Oliptrichum macrosporum, Aspergillus terreus* and *Streptomyces* sp-1 showing maximum efficiency to use MEG as the sole source of carbon and energy from the previous experiment. A consortium of the above 5 cultures was prepared and tested for its ability in MEG degradation. Bacterial, fungal and actinomycete suspension were prepared according to the procedure mentioned above. Microbial consortium of all five cultures was prepared and inoculated at 1% (v/v) inoculum level. Flasks were incubated at 30°C on a shaker (120rpm) at 30°C for 12 days. A 100 ml un-inoculated mineral salt broth (0.2% (v/v) MEG) was kept as control. Reduction in MEG concentration by selected individual cultures and the developed microbial consortium was measured by removing samples periodically till 12 days of incubation and the residual concentration of MEG was analyzed by Gas Chromatography with Flame Ionization Detector.

Analytical Method: Samples withdrawn periodically were subjected to determine residual MEG concentration. Direct injection method was used where 2µl of water

sample was injected into the injector port with the help of air tight, glass micro-syringe. Residual MEG concentration was analyzed after standardizing the equipment by injecting standard MEG reagent 4 to 5 times till it has given constant results i.e. same Retention Time and Percent Area in the chromatograms. After stabilization of the GC, analysis was carried out for the samples. Analysis was carried out at 220°C injector and detector temperature. Analysis was carried out isothermally at constant column temperature 220° C. Nitrogen gas was used as a carrier gas with the flow rate adjusted at 40mL/min.

- Percent degradation of MEG was calculated in the following way:

$$\text{Residual concentraion \%} = \frac{\text{Area of sample peak} \times 100}{\text{Area of control Peak}}$$

Percent degradation = 100 - % Residual Concentration.

RESULTS AND DISCUSSIONS

Effect of Varying Concentrations of Meg on the Growth of Microbial Isolates: The results obtained by using different concentrations MEG as a sole source of carbon and energy on the growth of the selected bacterial, fungal and Streptomyces cultures are recorded in Tables 1, 2 and 3

The results showed that at lower concentration of MEG i.e. 0.25% (v/v) *B. niacini* Pm1 showed maximum growth (O.D. = 2.04) followed by *Alcaligenes faecalis* CK221 (O.D. = 1.83), therefore both these cultures were selected for the further quantitative studies as well as for development of the consortium. Least growth was showed by *L. fusiformis* (O.D. = 0.05) followed by *P. galaciei* (O.D. = 0.06) at the same MEG concentration. *A. faecalis* zjs03 showed maximum growth (O.D. = 1.40) followed by *A. faecalis* CK221 (O.D. = 1.36) at 1% (v/v) MEG concentration and least growth was observed for *L. fusiformis* (O.D. = 0.03) followed by *P. Galaciei* (O.D. = 0.04).

Results also confirmed that some of the selected bacterial cultures showed increase in the growth with subsequent increase in the concentrations of MEG. On the contrary, for few isolated cultures, the growth in terms of turbidity decreased with increase in concentration of MEG. The declined in the growth of these cultures may be due to the toxic effect of MEG or their low metabolic ability towards MEG as a substrate. The results obtained for the fungal cultures are shown in Table 2.

Table 1: Effect of varying concentrations of MEG on the growth of different Bacterial isolates

	O.D at 600nm at varying MEG concentrations %(v/v)			
Bacterial Isolates	0.25	0.5	0.75	1.00
A. faecalis CK221	1.83	1.71	1.48	1.36
B. subtilis	0.94	0.82	0.71	0.63
P. galaciei	0.06	0.05	0.04	0.04
B. cereus.	0.22	0.17	0.16	0.15
S. maltophilia	0.22	0.20	0.16	0.15
A. faecalis zjs03	1.50	1.50	1.41	1.40
K. turfanensis	0.26	0.26	0.21	0.18
A. faecalis KZJ01	1.26	1.24	1.20	1.20
L. fusiformis	0.05	0.05	0.04	0.03
B. niacini Pm1	2.04	2.04	1.87	1.32
B. cereus SBD1-8	0.09	0.08	0.07	0.07
A. faecalis	0.66	0.60	0.60	0.51
B. fusiformis LLP	0.28	0.39	0.38	0.19
K. pneumonia	0.63	0.71	0.75	0.41
M. luteus	0.45	0.49	0.31	0.28

Table 2: Effect of varying concentrations of MEG on the growth of different Fungal isolates

	Fungal biomass at varying MEG concentrations % (v/v)			
	0.25	0.5	0.75	1.0
Fungal cultures	----------Dry weight gm/100ml----------			
Oliptrichum macrosporum	0.58	0.75	0.87	0.40
Aspergillus terreus	0.59	0.59	0.78	0.62
Torula herbarum	0.52	0.52	0.57	0.53

Table 3: Effect of varying concentrations of MEG on the growth of different Streptomyces isolates

	Actinomycete biomass at varying MEG concentration % (v/v)			
	0.25	0.5	0.75	1.0
Actinomycete cultures	----------Dry weight in gm/100ml----------			
Streptomyces sp-1	0.86	0.83	0.78	0.62
Streptomyces sp-2	0.53	0.47	0.42	0.39
Streptomyces sp-3	0.21	0.17	0.20	0.16
Streptomyces sp-4	0.26	0.19	0.14	0.09

The results recorded in table 2 showed that out of 3 fungal cultures checked for their ability to utilize MEG as a source of carbon and energy. *O. macrosporum* was found to yield more growth (0.87gs/100ml) followed by *A. terreus* (0.78g/100ml) and minimum growth of *T. herbarum* (0.57gs/100ml) in terms of dry weight at 0.75% (v/v) MEG. Increase in growth was seen with increase in concentration of MEG up to 0.75% (v/v) and declined significantly at 1% (v/v) confirming that the growth of this fungal culture was inhibited by high concentration of MEG. Fungal isolates *O. macrosporum* and *A. terreus*

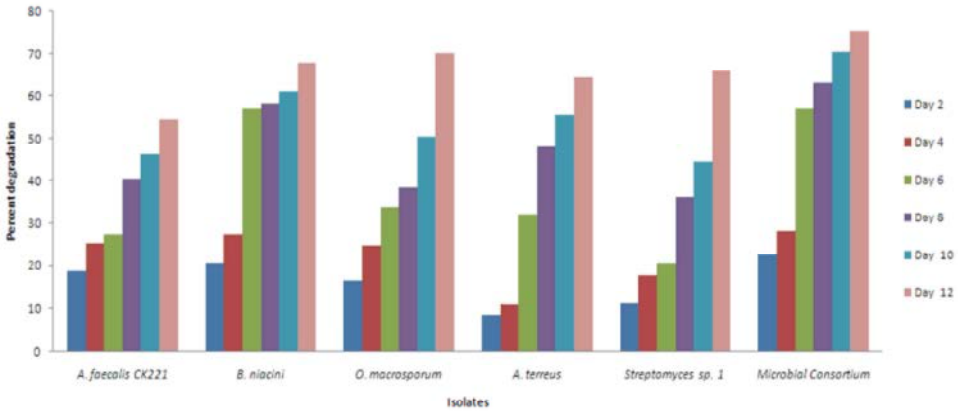

Fig 1: Percent degradation data of MEG by individual microbial cultures and developed consortiu

were selected for the further quantitative studies as well as for the consortium development. The results obtained for the Actinomycete cultures are shown in Table 3.

The results recorded in Table 3 confirmed that unlikely to bacterial and fungal cultures tested, the growth of the selected 4 cultures of *Streptomyces* species, decreased with increase in the concentration of MEG i.e. from 0.25 to 1.0% (v/v) of MEG. *Streptomyces* sp-1 showed maximum growth in terms of dry weight 0.86gm/100ml at 0.25% (v/v) of MEG. At the same concentration, *Streptomyces* sp-3 showed minimum growth i.e. 0.21 gm/100ml at 0.25% (v/v) of MEG. Out of the 4 actinomycetes tested, *Streptomyces* sp-1 was found to be the potential utillisers of MEG as a source of carbon and energy so selected for the further quantitative studies as well as for the consortium development.

Quantitative Degradation of MEG: Gas chromatographic analysis data for individual cultures as well as for the consortium is shown in Fig 1.

Results depicted in Fig: 1 revealed that percent degradation of MEG increased with respect to incubation period up to 12 days by all the 5 microbial cultures tested. Minimum degradation of MEG after 2 days of incubation i.e. 8.33 % was showed by *A. terreus* and 20.47% by *Bacillus niacini*. After 2 days of incubation, the microbial consortium comprising of 5 different cultures showed 22.56%, which is more than the percent degradation showed by the best bacterial culture *Bacillus niacini* Pm1. After 12 days of incubation, the *Oliptrichum macrosporum* showed maximum 70.21% MEG degradation followed by *B. niacini* (67.87%), *Streptomyces* sp. 1 (65.82%) and *A. terreus* (64.36%) of MEG degradation. Minimum percentage (54.50%) of MEG degradation was shown by *A. faecalis*. On the contrary, the microbial consortium showed 75.49% of MEG degradation, which is approximately 5% more MEG degradation by the best degrader *O. macrosporum*, when tested individually. It was also observed that further incubation beyond 12 days showed no significant increase in the percent degradation of MEG and therefore, the experiment was terminated.

The capacity to use MEG as a carbon and energy source is wide spread among aerobic microorganisms. Under auxic conditions several pure cultures of different bacterial groups proved capable of degrading MEG [8, 14, 15]. LuAnn *et al.* [16] reported potential of *Pseudomonas*, *Aspergillus*, *Enterobacter*, *Acenetobacter* and *Agrobacterium* in degradation of MEG by a steady state model of MEG contaminated soil. Reports indicated till date has been more on high molecular weight Poly-Ethylene Glycol (PEG) biodegradation, including aerobic degradation of PEGs using soil microorganisms [17]. Isolates from soil sediments were also exploited for their use in PEG biodegradation [18]. Sewage bacteria have been used to degrade PEGs [19]. Symbiotic degradation of PEGs has been reported using *Flavobacterium* and *Pseudomonas sp*. [20-22]. Several studies have been carried out on biodegradation of certain glycolic compounds viz tri-propylene glycol [23], polyesters [24], ethylene glycol monomethyl ether (EGME) [25], polyalcohol ethoxylate [10], propylene glycol [16], acetone and ethylene glycol [29], PEG (20,000)-phtalate polyster [28], PEG and poly propylene glycols [29], ethylene and propylene glycol [9, 30, 31]. Cox [32]; Ogata *et al.* [33]; Pearce and Heydeman [34], have observed inability of PEG degrading microorganisms to degrade Di-ethylene glycol and Mono-ethylene glycol. Further some researchers hypothesized that accumulation of PEG byproducts resulted in phytotoxicity within a graywater recycling hydroponics system [35].

The degradation of many xenobiotics has been reportedly performed by mixed cultures, although most of the symbiotic mechanisms remain unclarified [13]. We isolated MEG utilizing cultures. It was also found that MEG was more efficiently degraded by the consortium than by the respective single cultures.

The symbiotic mechanisms of mixed cultures are classified as follows [36] i) supply of some nutrients or co-factors necessary for the growth of component bacteria or the metabolism of substrates, ii) improvement of environments (pH, redox potential, removal of toxic metabolites etc). iii) complementation of metabolizing steps which are deficient in single cultures and iv) other mechanisms. Mixed cultures seem to be a potent tool for the microbial degradation of recalcitrant materials. In many cases, it is so difficult that a single organism is furnished with all kinds of biochemical steps for xenobiotics. Therefore, the biodegradation of co-polymers or xenobiotics will possibly performed by mixed cultures, in which each microorganism plays one of the roles required for ultimate degradation. Mixed cultures seem to be a potent tool for the microbial degradation of recalcitrant materials [28].About 5 to 21% increase in MEG degradation was achieved with the developed microbial consortium at same inoculum concentration, compared to the individual cultures tested.

These studies can provide cost-effective, efficient ready-to-use microbial inoculum for the treatment of industrial effluents containing MEG by the end users in near future.

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