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BIOREMEDIATION OF PETROLEUM PRODUCTS THROUGH BACTERIAL ISOLATES

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Abstract:-Bioremediation is the use of biological treatment to destroy or reduce the concentration of hazardous wastes from contaminated sites. such system involves the uses of biological organisms such as bacteria fungi and plants, to reduce or eliminate toxic pollutants from contaminated sites by degradation assimilation or transpiration in the atmosphere is called bioremediation degradation is the mode of elimination mostly in case of organic compounds while heavy metals are assimilated.

These microbes and higher plants can also be variously modified by genetic engineering to become efficient and suitable for bioremediation.

Key Words: Bioremediation, Hazardous wastes, Oil spills, Hydrocarbon.

I. INTRODUCTION

Oil spills causes several damage to the ecosystems and pose threats of fire, ground water pollution due to percolation and air pollution due to evaporation. In addition oil refineries generate huge quantities of oily sludge a hydrocarbon waste. The US environmental protection agency and the Exxon Company used microorganism to clean up Alaskan beaches contaminated by the voldez oil spill. In India a consortiums of bacterial species have been developed to combat oil spills and oily sludge the inoculant is called Oil zapper. Inoculation with oil zapper reduced oily sludge contamination in soil to merely 0.5% in 360 days from the initial 13.41%. In contrast declined to only 11.35 in uncontaminated land. Oil zapper has been effective in relatively large scale field trails as well.

To study the utilization of hydrocarbons (bioremediation) by the bacterial isolates of the present study mere incubated at 30^{0C} for 10 days after incubation period the total introgen content of the soil of control as well as due experimental was analyse by the kjeldhal method.

To study the role of bacterial isolates in bioremediation of petroleum products an experiment was set up. In this experiment the N_2 contents of the soil samples spilled with petrol, diesel / kerosene was determined in comparison with the control in which only petroleum products were added. The increase in the N_2 content of the soil is taken as utilization of the hydrocarbon by the bacterial isolates.

II. MATERIALS AND METHODS

N_2 estimation from soil

Principle - During digestion ammonia is released by disintegration of complex N_2 compounds, is trapped in H_2SO_4 to from ammonium sulphate when this is made alkaline by adding of NaOH during distillitation, ammonia is liberated, which combines with

boric acid to form ammonium borate, boric acid colour changes to blueish green to raise in pH. When this, mixture is titrated against H_2SO_4 / HCl boric acid is reformed and the pH of the solution is lowered to 4.7 when the indicator turns pink.

Digestion - Soil sample -10g, 300ml KJ Flask + add 20ml, distilled water and keep for 1 hr. later add 20g $NaSO_4$, 1 g clalyst mixer ; pinch of granulated Zn and 35ml conc. H_2SO_4 Swir the flask gently then heat gently after 30minutes i.e. when frothing stops raise the heat till acid boils and condense approximately 1/3 way up the neck of the flask continue digestion at this heat and rotate the flask at interval until organic matter is destroyed.

Digestion to be continued for 1hr. after the digest became apple green. Add 20ml distilled water mix well allow to stand 3 to 4 min, then transfer supernatant liquid to 50ml volumetric flask, make up the volume.

Distillation - 20ml / 10 ml aliquot of digest / water into the dist. chamber through the funnel a wash twice with 1 ml dist. water each time and then add 20ml (10 ml) NaOH (40%). Take 5ml (40% Boric acid with mixed indicator) in conical flask and dip the other end of silver rod in to the solution.

Continue the distillation till 25ml of distillated is collected and titrate it against standard H_2SO_4 (N/14) colour of distillate green, when titrated turns pink.

Reagents

1. **N/14 H_2SO_4** - Normality of conc. H_2SO_4 = 36N
   Preparing by dissolving 1.98ml conc H_2SO_4 in H_2O and making volume to one litre.
2. **Catalyst mixer** - 1mg $CuSO_4$, 8g. Potassium sulphate, 1 gram selenium dioxide are powered separately and then mixed thoroughly together.
3. **Mixed Indicator** :- Mix 6ml methyl red solution (0.16% in 95% alcohol) and 12ml bromocresol green (0.04% in H_2O) add 0.6ml of 95% alcohol to the mixture.
4. **Conc. H_2SO_4** (N_2 free)
5. **Boric Acid** : Dissolve 40g of boric acid in water and dilute to 100ml.
6. **Sodium hydroxide** :- Dissolve 500g in 1 litre of water and allow to stand for a few days sephon the clear. Supernatant in another bottle and label as 40% NaOH.

Calculations :-

Mg/100g = (T - B) x N x 14/S x 100

T = Sample titration ml standard acid
B = Blank titration ml. standard acid
N = Normality of standard acid N/14
S = Sample weight in grams

III. OBSERVATION

N_2 estimation of the soil samples

After digestion and distillation of the control as well as experimental soil material the following observations were made.

The four isolates of the genus *Bacillus* and six isolates of the genus *Pseudomonas* utilized petrol preferentially in comparison to diesel and kerosene. The nitrogen content increased to 3.20mg/100g. in comparison to control which was in traces.

In another combination two isolates of the genus *Corynebacterium* and two isolates of the genus *Flavobacterium* were used. These siolates utilized diesel folowed by kerosene in contrast to the above forms. (Table).

In still other combination *Serriata*, *Arthrobacter* and *Xanthomonas, Cellulomonas* was used. These two combinations utilized petrol preferentially in comparison to diesel and kerosene. The nitrogen content was 14.4mg / 100g in case of *Serriata, Arthrobacter* and 16.6mg / 100g in case of *Xanthomonas, Cellulomonas* in comparison to control which was in traces.

The remaining bacterial isolates were unable to utilize petrol, diesel and kerosene as substrate.

Table : Showing ability of bacterial isolates in the utilization of various petroleum hydrocarbons.

S.No.	Combination of organisms used	Control	Utilization of hydrocarbons taken as increase in nitrogen content mg/100g of soil		
			Petrol	**Diesel**	**Kerosene**
1.	*Bacillus mycoides* *Bacillus megatherium* *Bacillus lecheniformis* *Bacillus subtilis*	Traces	3.20	1.60	1.60
2.	*Pseudomonas aeruginosa* *Pseudomonas putida* *Pseudomonas pseudomallei* *Pseudomonas cepacia* *Pseudomonas stutezeri* *Pseudomonas avenae*	Traces	3.20	1.60	1.60
3.	*Corynebacterium cyslitidis* *Corynebacterium* sp.1 *Flavobacterium ferrugineum* *Flavobacterium* sp.1	Traces	4.80	12.80	9.60
4.	*Serriata fonticola* *Serriata proteamaculans* *Arthrobacter flavoscens* *Arthrobacter globiformis*	Traces	14.40	1.60	1.60
5.	*Xanthomonas fragariae* *Xanthomonas campestris* *Cellulomonas* sp.1 *Cellulomonas* sp.2	Traces	16.00	1.60	1.60

RESULTS AND DISCUSSION

Experiment to demonstrate the utilization potential of three hydrocarbons (petrol, diesel and kerosene) by the various bacterial isolates of the present study was conducted. The bacterial isolates were used in combinations (table) Increase in the nitrogen content of the soil (sterile) which was previously soaked with the hydrocarbons and inoculated with test organisms and incubated, is taken as the utilization potential of that bacterial group. Results obtained indicate that out of the five combinations *Xanthomonas campestris, Xanthomonas fragariae, Cellulomonas flavigena and Cellulomonoas* sp.1. (Combinations No.5), appeared to be the best which utilized preferentially the petrol. The next in line was that of combination no. 4 (*Serriata fonticola, Serriata proteamaculans, Arthrobacter flavescens, Arthrobacer globiformis*) which also preferred petrol. For combination No. 3 (*Corynebacterium cyslitidis, Corynebacterium* sp.1, *Flavobacterium ferrugineum, Flavobacterium* sp.1) diesel followed by petrol was the choice.

Employing bacterial consortia for cleaning (bioremediation) of hydrocarbon spilled soils and water bodies is widely used indifferent part of the world (Roffey et al. (1994). Chaineau et al. and Lethbridge et al. (1995), Breghard et al. (1996). Because of the lack of necessary facilities and funding, and by using a simple method as stated above the utilization potentials of various bacterial isolates of the present study was done.

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