RESEARCH PAPER | Microbiology | Volume : 4 | Issue : 10 | October 2014 | ISSN - 2249-555X

Optimization of Growth Conditions of Native Hydrocarbon Utilizing Bacterial Consortium "HUBC" Obtained From Petroleum Pollutant Contaminated Sites

KEYWORDS: Bioremediation, HUBC, Optimization, Petroleum pollutants

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ABSTRACT *Studies on optimizing methodologies for bioremediation of petroleum pollutants are growing rapidly worldwide. This investigation is focused on optimizing growth conditions of hydrocarbon utilizing bacterial consortium (HUBC). It was found to be an efficient consortium for degradation of crude oil (K#X) among five consortia obtained from crude oil polluted sites of ONGC. Statistical analysis for HUBC growth parameters was performed using SPSS 16.0. All analysis were performed in triplicates and results are represented as mean ± standard deviation. Optimization studies for HUBC revealed that 3% v/v crude oil or 1% w/v glucose, pH 7.2, incubation at 37°C at 180 rpm with 2% v/v inoculum as optimum conditions for its growth. To date 2% v/v crude oil concentration is reported for growth of hydrocarbon degraders. It has been concluded that "HUBC" is mesophilic, halo-tolerant and aerobic crude oil utilizer which can be useful for cleanup of hydrocarbon contaminated environments.*

I. Introduction

Increasing public awareness regarding impact of environmental pollution has led to the search and development of technologies that helps in cleanup of organic and inorganic contaminants such as hydrocarbons and metals. Microbial bioremediation is widely used technique for treating hydrocarbon pollution in both terrestrial and aquatic ecosystems. Indigenous hydrocarbon degrading microorganisms particularly bacteria play a significant role in this process (Farag and Soliman, 2011). Atlas, 1981 focused on examination of factors such as nutrients, physical state of the oil, oxygen, temperature, salinity and pressure influencing petroleum biodegradation rates, with a view to develope practical environmental applications. Optimization of growth conditions for isolate/consortium for pollutant removal prior to the field trials is growing rapidly worldwide. According to Yakimov et al. (2007), petroleum-degrading microorganisms are widely distributed in soil, water and sediments. However they may not be present in sufficient numbers to achieve contaminant remediation. Contaminant removal can be achieved by inoculating polluted area with highly effective hydrocarbon-degrading microbial strains/ consortium to augment/stimulate existing ones. Research for standardizing in situ degradation process is required for successful full-scale operation (Bidoia et al., 2010). Statistical analysis methods such as factorial design, regression analysis, t-test, mean, mean ± standarad deviation (s.d.) are used for analysis of results (Farag and Soleman, 2011)

The research work was undertaken with a mandate for optimization of native hyrocarbon utilizing bacterial consortium (HUBC) for application in bioremediation of hydrocarbon pollutants which is a thrust area of research in petroleum industry. Statistical analysis for the HUBC growth parameters were performed using SPSS 16.0. All analysis were performed in triplicates and results are represented as mean ± standard deviation (s.d.).

II.Materials and methods

2.1.Source of HUBC

Six isolates used to prepare HUBC for this study were isolated from various ONGC oil fields located in Ankleshwar and Ahmedabad assets. Enrichment, isolation and qualitative crude oil utilization is described elsewhere (Varjani et al., 2013; Varjani and Upasani 2013).

2.2.Inoculum preparation of HUBC

Inoculum preparation as well as growth optimization of HUBC was carried out in BHM using 1% v/v crude oil from well K#X. Activated consortium was prepared in BHM supplemented with 1% v/v crude oil, incubated for 24 h at 37 ± 1°C at 180 rpm.

2.3.Optimization of growth parameters for HUBC

The parameters studied included static and shaking (180 rpm) conditions, varying temperatures (°C) (30, 37, 45 and 50), varying pH values (6, 7.2, 8, 9, 10 and 11), NaCl concentration (% w/v) (0, 1, 2, 3, 5, 7, 10 and 12), petroleum (1% v/v) namely crude oil, nonane, decane, dodecane, n-paraffins, kerosene, diesel and xylene; and non-petroleum carbon source (1% w/v or v/v) namely glucose and glycerol; crude oil concentration (% v/v) (1, 2, 3, 4, 5, 7 and 10), nitrogen source (1% w/v) (ammonium nitrate, potassium nitrate, peptone, tryptone, no additional nitrogen-source and inoculum ratio (% v/v) (1, 2, 3, 5, 7 and 10) as followed by Bayoumi and Abul-Hamd, (2010). These experiments were performed in triplicates for 10 days utilizing the resultant optimum parameters. For all the experiments growth was measured as biomass (Bordoloi and Konwar, 2008).

2.3 Biomass determination

Sample (10.0ml) was withdrawn from culture flasks, and centrifuged at 10,000 rpm for 20 min. The cell pellet was dried overnight in an oven at 70°C and dry weight of cell-mass was measured gravimetrically until constant weight was obtained. Biomass was calculated as g/l (Bordoloi and Konwar, 2008).

2..4. Statistical analysis

SPSS for Windows, version 16.0 was used for statistical evaluation for various growth parameters used to optimize results of HUBC growth. All analysis were performed in triplicates and represented as mean ± standard deviation (s.d.).

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III. RESULTS AND DISCUSSIONS

The mutagenic, carcinogenic and immunotoxic effects caused by petroleum pollutants pose serious threat to ecosystem and living beings. Green process through microbes especially bacteria and their consortia have caught the attention for cleanup of pollutants by bioremediation of petroleum contaminated sites. It was necessary to determine the optimum growth conditions for hydrocarbon utilizing bacterial consortium (HUBC) from ONGC sites in Gujarat with possible applications in bioremediation of petroleum pollutants.

The effect of environmental parameters viz. agitation, temperature and pH on growth of HUBC. Shaking at 180 rpm was optimal for HUBC growth. HUBC grew optimally at temperature 37°C and pH 7.2 (Data not shown). Highest growth was observed in three and ten days with an inoculum size (% v/v) of 10 and 2 percent, repectively (Fig. 1). For all graphs in this paper data represents mean ± s.d., n=3; error bars indicate s.d. Results for different carbon sources on HUBC growth are represented in Fig. 2. Maximum biomass (3.124 ± 0.30 g/l) was obtained with crude oil (Fig. 2). HUBC could also utilize N-paraffins, therefore it has the potential to solve well clogging problems during pumping out of the crude as well as in bioremediation of hydrocarbon pollution. The optimum concentration of crude oil as sole carbon source for growth of consortium/individual isolate (s) is 2% v/v, above which it is reported to be toxic. However, the HUBC used in this study grew optimally at 3% v/v crude oil concentration, first times report (Fig.3).

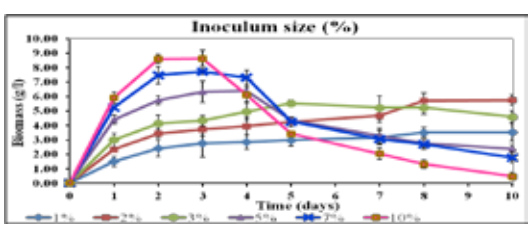

Fig. 1: Effect of Inoculum concentration on crude oil (K#X) degradation by HUBC

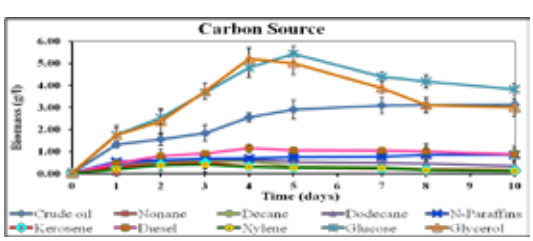

Fig. 2: Effect of C - substrate on crude oil (K#X) degradation by HUBC

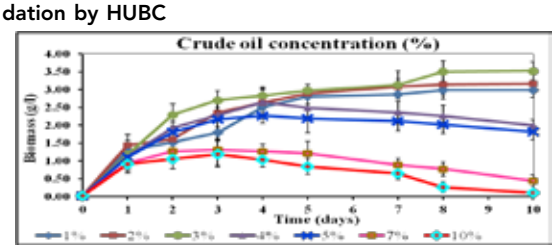

Fig. 3: Effect of C-substrate concentration on crude oil (K#X) degradation by HUBC

The growth of HUBC declined with increasing NaCl concentration, however considerable growth was observed upto 5% (w/v) indicating its halo-tolerant nature (Fig. 4). There was no profound effect of nitrogen source on its growth (Data not shown).

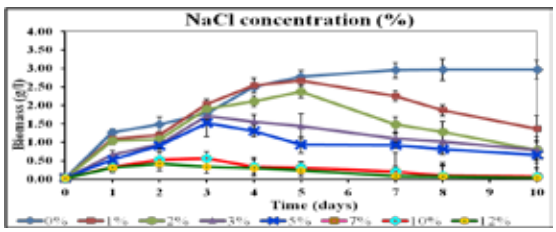

Fig. 4: Effect of NaCl concentration on crude oil (K#X) degradation by HUBC

Optimization studies

revealed that shaking condition (180 rpm); temperature 37°C; pH 7.2; NaCl concentration 0% w/v; 'C source' Hydrocarbon: Crude oil; Non-hydrocarbon: Glucose; concentration of crude oil 3% v/v; 'N source' Inorganic: NH_4NO_3, Organic: Tryptone; inoculum volume 2 (% v/v) resulted in optimum growth of "HUBC". The dry biomass under optimum conditions without aid of nitrogen source was 3.757 ± 0.31 g/l at 10 days of incubation. HUBC is thus a halotolerant, aerobic crude oil utilizing mesophile.

Bioremediation by biodegradation is considered as one of the primary mechanisms for elimination of hydrocarbon pollutants from the environment (Leahy and Colwell, 1990). Success of bioremediation process i.e. biodegradation of organic contaminants is directly or indirectly influenced by environmental conditions (pH, temperature; salinity; oxygen availability); pollutants properties (availability, type and length of hydrocarbons; dispersion into aqueous phase; volatilization); cell metabolic pathways and several structural changes from inclusions to complex extracellular polymeric spheres; microbial communities; physico-chemical properties of soil (density; water holding capacity; pH; moisture; texture) and medium composition (carbon source, nitrogen source and other nutrients), etc. (Beškoski et al., 2011). Soil contaminants change chemical and physical properties of soil structure such as the soil pH value, total organic matter and electricity conductivity, which affects response of microbial communities to pollutants (Adebusoye et al., 2008).

Optimizing environmental as well as nutritional parameters significantly affect microbial biodegradation rates of TPH. Numerous studies have identified the relationship between soil conditions and microbial activity. Temperature affects both physical state of hydrocarbons present in pollutant contaminated sites and microbes utilizing it. At low temperatures, viscosity of oil increases, volatilization of toxic short-chain alkanes and their water solubility decreases, delaying onset of biodegradation (Atlas, 1981; Leahy and Colwell, 1990). Extremes pH could hamper the ability of microbes to degrade hydrocarbons. In one study, adjustment of pH from 4.5 to 7.4, doubled the rate of gasoline biodegradation, but it dropped significantly when pH was further raised to 8.5 (Leahy and Colwell, 1990).

IV. CONCLUSION

Optimization of growth studies revealed that HUBC is a mesophilic, halo-tolerant, aerobic crude oil utilizer. Utilization of N-paraffin makes HUBC suitable as paraffin/wax degrader in oil wells during crude oil pumping, with possi-

Volume : 4 | Issue : 10 | October 2014 | ISSN - 2249-555X

bility of avoiding well clogging problems. HUBC being halotolerant (growing upto 5% w/v NaCl) and having optimal 3% v/v crude oil concentration can be very useful for sea surface oil spill management. Biaugmentation studies with HUBC will open up commercial utility of the consortium in bioremediation of oil spills. Further, studies on quantitative biodegradation capability of the consortium will provide an effective and eco-friendly technology for the degradation of hydrocarbons.

ACKNOWLEDGEMENT

We express our gratitude to Gujarat National Law University (GNLU), Gandhinagar, Gujarat for granting permission to Ms. Sunita to perform research work. We are grateful to Institute of Reservoir Studies (IRS), ONGC, Ahmedabad. Laboratory facility provided at M. G. Science Institute (DBT, "Star College Scheme Grants") is highly acknowledged.

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