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Biodegradation of crude oil in soil slurry phase by *Nocardia* sp.

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ABSTRACT

Biodegradation potential of crude oil has been studied in liquid and soil slurry culture. Studies were performed to optimize the factors affecting metabolic activity. Arabian Light(sulfur content 1%) was used as a representative crude oil and *Nocardia* sp. was selected as an oil degrading microorganism based on its ability to degrade and emulsify. Effects of various nutritional and environmental conditions as well as emulsification and surface tension were observed. Tentative optimization of environmental and nutritional condition were as follow; pH 8, sodium nitrate as inorganic nitrogen source, yeast extract 0.05%, phosphate concentration 0.25% and glucose addition of 1.0% (w/v basis), extent of degradation to 78 %.

Introduction

For relatively easy to degrade products, such as crude oil or light hydrocarbons, availability of adopted bacteria, aeration and application of nutrients are becoming prevalent techniques of decontamination[2,3] In this study, effective and reliable optimization condition for crude oil degradation that was later to be require to implement bioremediation in soil slurry condition is considered.

Materials and Methods

Microorganism *Nocardia* H17 was selected as hydrocarbon degrading microorganism. Herein it will be referred to as H17.

Media Arabian Light with 1% sulfur content was selected as crude oil. Various nutrients, yeast extract, $MgSO_4.7H_2O$, inorganic nitrogen source(NH_4NO_3, $NaNO_3$ or $(NH_4)_2SO_4$), phosphate source(K_2HPO_4 and KH_2PO_4) and trace metals were supplied in varying degree to find optimal condition and pH was adjusted with N NaOH and N HCl to be 8. Otherwise stated, media was cultivated at 30 ℃, 250 rpm and 1% v/v of crude oil is added and in the seed medium corresponding glucose was used.

Soil Soil was collected from local stream, Gab-chun near Yusung, Teajon. The soil was sampled from a depth less than 20 cm, and was composed of 85% sand, 11% silt and 4% clay, and identified as loamy sand.. The soil sample have 0.4% organic matter and 15% moisture content. The pH in distilled water was 7.3.

Determination of oil conversion Oil conversion is defined as the process by which crude oil is converted to a form which is no longer extractable by organic solvent such as toluene.[2] Each flask was taken for sampling at two point, at 4th day and 8th day and its contents and toluene was used to extract crude oil and optical density was measured at 520 nm to determine oil conversion. Oil conversion was determined with the previously prepared standard curve(Fig.1).[1] HP 8452A Diode array spectrometer was used for optical density.

Results and Discussion

Of three oil degrading strains(initially named H17, 24p, 80p respectively), H17 was selected based on its emulsification activity and oil conversion. (Fig 2) and subsequently identified as *Nocardia* sp..

Time profile of oil degradation by H17, before attaining an optimal oil degrading condition, was shown in Fig 3 over 240 hour period. Rapid degradation follows until 48 hours after inoculation and the degradation was almost completed before 120 hours(5 days) with overall oil conversion around 60%.

In general, H17 showed faster degradation rate in basic range but in rather extreme basic condition at pH 9, degradation was stoped around 40 % oil conversion. pH 8 was best suited for H17 for oil degradation.(Fig. 4)

Nitrogen is the most important nutrient in the microbial degradation of oil and its effect is studied. The effect of different inorganic nitrogen source on the degradation of crude oil by H17 is presented on Table 1 and sodium nitrate showed both the greatest rate and extent of biodegradation. The effect of concentration of sodium nitrate, which increased the dispersion effect on crude oil, were rather significant. It showed the greatest rate and extent of degradation at 0.1%(w/v) but high concentration of sodium nitrate decrease the rate and extent of oil conversion. (Fig. 5)

Fig. 6 shows yeast extract, which provide organic nitrogen source as well as growth factors, was required for the growth of H17. At low concentration, concentration of yeast extract and oil conversion was strongly related but leveled off above 0.05%(w/v). At higher concentration(over 0.1%), the yeast extract affected little in oil conversion. (Fig. 7) The effect of phosphate concentration was considered(Fig. 8). The rate of degradation was strongly related to phosphate concentration but at the highest level of experiment, 0.3%(w/v), the extent of degradation actually decreased. Thus demonstrated phosphate limitation is not a factor this experimental condition in short period. Glucose was added as alternative carbon source for H17 but even with additional carbon source, the consumption of crude oil was increased, as shown in Fig. 9. This is presumed to be an effect of increased number of cells available to utilize crude oil soon after depleting glucose as carbon source and enhanced dispersion of crude oil which suspected to be cause by the hydrophobization of the cell.

Oil degradation was performed under varying degree of contamination by crude oil(Fig. 10). At concentrations over 1%, the rate and extent of degradation decreased. This is strongly suggestive of toxic effect of crude oil at higher concentration.

References

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2. Reisfeld,A., Rosenberg,E., Gutnick,D. Applied Microbiol. 24(3):363-368(1972)
3. Venkateswaran,K.,et al Can. J. Microbiol. 41:418- 425(1995)

Tab. 1 Effect of different inorganic nitrogen source

Inorganic nitrogen source	Oil conversion in 4 days(%)	Oil conversion in 8 days(%)
Ammonium Sulfate ($(NH_4)_2SO_4$)	24.1	59.3
Ammonium Nitrate (NH_4NO_3)	16.9	39.6
Sodium Nitrate ($NaNO_3$)	26.5	63.4

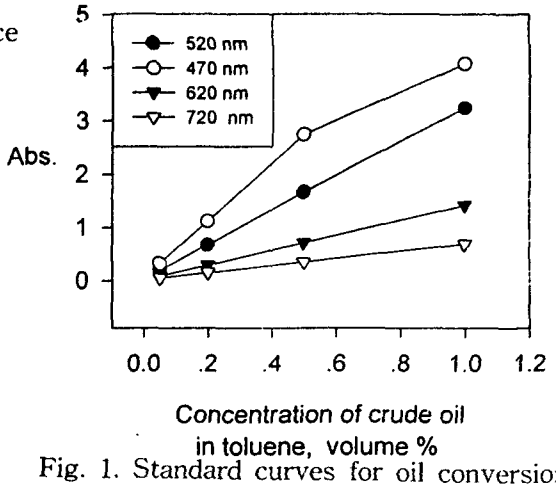

Fig. 1. Standard curves for oil conversion at differnet wavelength

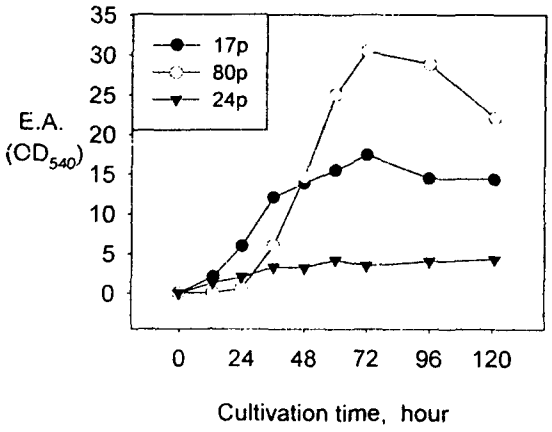

Fig. 2. Emulsfication activity of three oil degrading strains(H17, 24p, 80p)

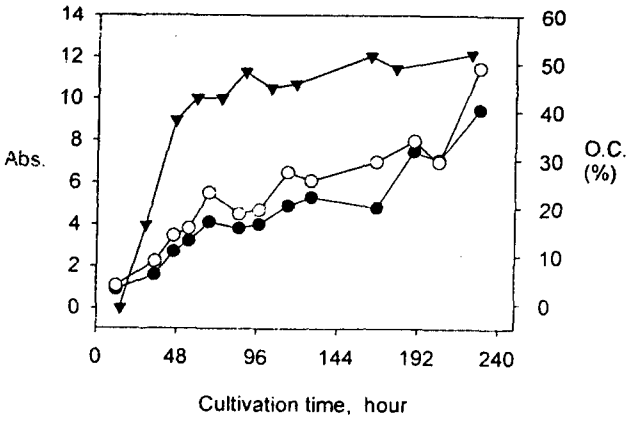

Fig. 3. Time profile of cell growth, emulsification activity and oil conversion

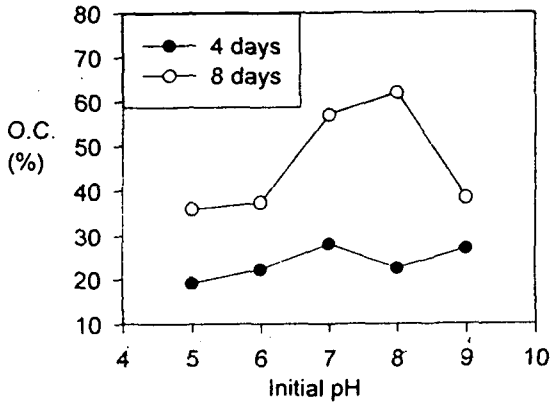

Fig. 4. Effect of initial pH on oil conversion. pH was adjusted with N NaOH and HCl solution

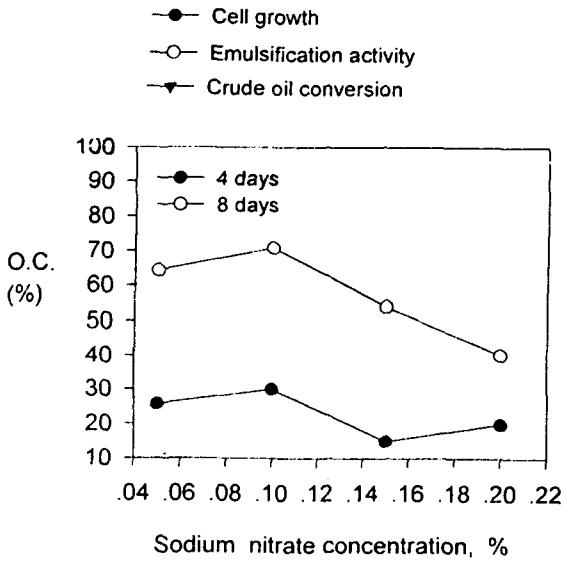

Fig. 5 Effect of sodium nitrate concentration on oil conversion

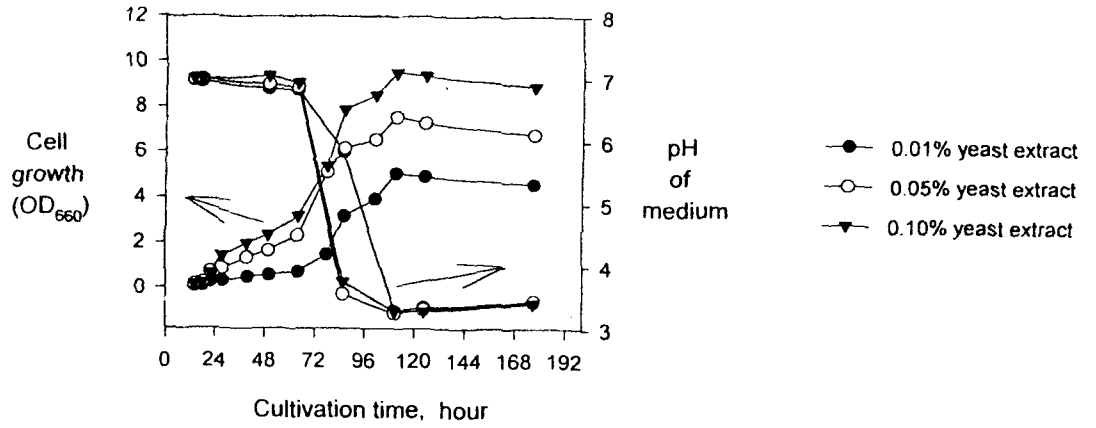

Fig. 6. Effect of yeast extrat concentration on H17 growth. Incubated in glucose medium(1% w/v) and initial pH 7

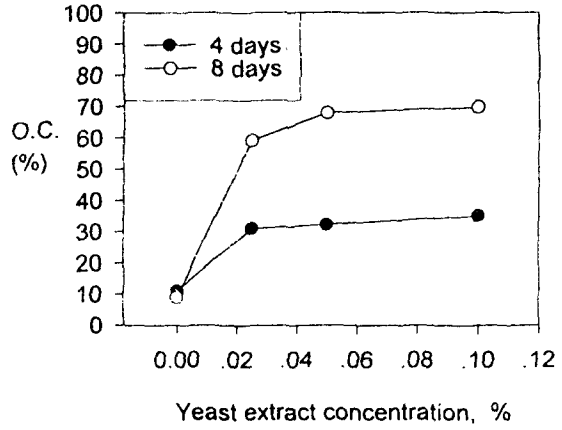

Fig. 7. Effect of yeast extract concentration on oil converion

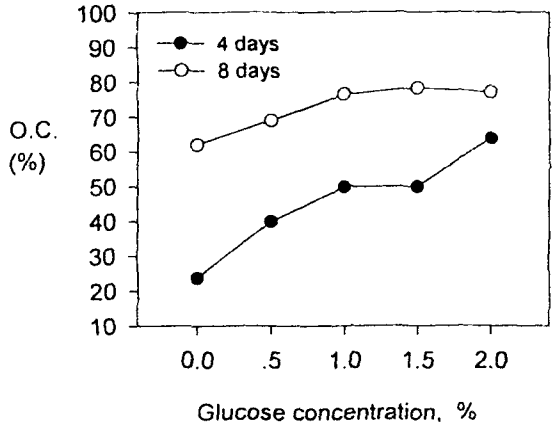

Fig. 9. Effect of glucose concentration on oil conversion

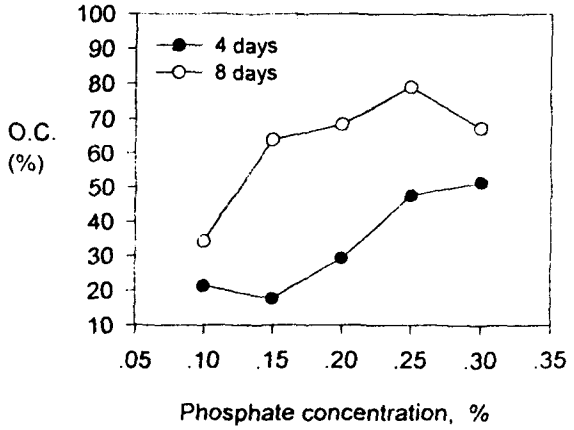

Fig. 8. Effect of phosphate concentration on oil conversion

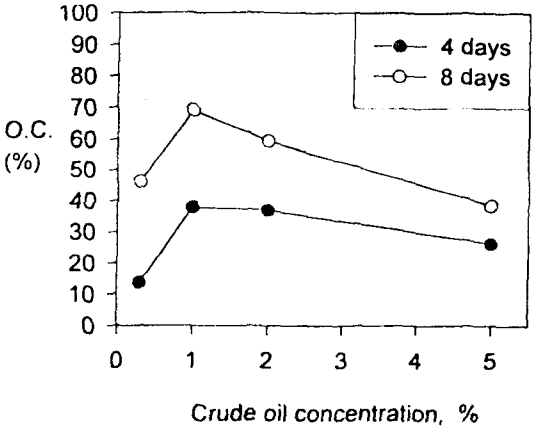

Fig. 10 Effect of crude oil concentration on oil conversion