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BIODEGRADATION OF USED ENGINE OIL USING PSEUDOMONAS PUTIDA AND AZOTOBACTER CHROCOOCUM AS BIOSURFACTANT

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ABSTRACT

Present situation of the environment depicts an imperative need to treat our natural resources which have been become the garbage bins of our technology. By making use of crude oil extracted from the earth, numerous fuels, gases are obtained of which Engine oil is one component. While this oil is utilized the carbon soot particles get accumulated changing the oil property and making it unfit for use. This used engine oil is drained frequently and is either recycled or disposed in a manner harmful to the environment. Biodegradation is a cost effective, eco-friendly method to follow while dealing with such recalcitrant compounds. Azotobacter chrocoocum is selected for this study as it has an ability to assist in biodegradation due to its ability to produce natural biosurfactant. Pseudomonas putida is selected to study its individual biodegradation effect and in the form of a consortium. The degradation was studied before and after optimization, with the post optimization results being 1.97 folds higher than the prior results. Individually A. chrocoocum has shown degradation of 25.6% and P. putida 47.515% and the maximum degradation was seen in the mixture of A. chrocoocum and P. putida which degraded to an extent of 73.7%.

KEYWORDS: Biodegradation, biosurfactant, oil.

INTRODUCTION

Biosurfactant or bioemulsifier compounds can increase the solubility of crude-oil hydrocarbon. Individual microbial populations can usually metabolize only a limited range of substrate, therefore the performance of individual and mixed culture of *Azotobacter chrocoocum* and *Pseudomonas putida* were studied. *A. chrocoocum* is a Gram-negative bacteria found in neutral and alkaline soils, which play an important role in the nitrogen cycle in nature, binding atmospheric nitrogen and releasing it in the form of ammonium ions into the soil. *P. putida* is a Gram-negative, rod-shaped, saprotrophic soil bacterium. The diverse metabolism of wild-type strains of *P. putida* may be exploited for its bioremediation capabilities.

This study researches the oil degrading capabilities of *A. chrocoocum* and *P. putida* separately and together as mixed consortia.

MATERIALS AND METHODS

The used engine oil is taken in required quantities and heated in a microwave oven prior to addition into the media flask to dissipate volatile components and expel moisture present in the oil, if any. Pure cultures of the desired microorganisms were obtained from MTCC *A. chrocoocum* MTCC 7724 and *P. putida* MTCC 9220 and were inoculated in pre-sterilized 250 ml Erlenmeyer flasks containing 100 ml of Mineral medium (pH 7.0), at 30°C overnight in an orbital shaker at 140 rpm. Each set of flasks is inoculated with *A.chrocoocum*, *P. putida* and their mixture. After the overnight incubation used engine oil was then added in the concentrations 0.5%, 1%, 1.5% (V/V) respectively to each set of flasks. Optical densities were determined using UV-VIS spectrophotometer for all three sets at regular intervals i.e. on alternative days 15 days till the readings declined and showed no upscale. After the said incubation time based on the remaining oil content, the best degraded oil concentration which

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showed highest tolerance and degradation potential was fixed as constant and different physical parameters were varied to check their effect on the rate of growth and degradation.

Optimization methodology

Effects of various physical parameters such as pH, temperature, agitation speed on used engine oil degradation were studied. The parameters were standardized using one factor at a time and keeping the others constant.

Optimal time for maximum growth of microbes at 30°C, 150 rpm and pH 7 was studied by harvesting the growth media, determining the time for optimum growth of microbes and biodegradation of oil by measuring Optical density.

To determine the optimal incubation temperature for maximum culture growth and oil degradation, media was incubated at various temperatures 25°C- 40°C with an interval of 5°C for 15 days keeping other conditions at their predetermined level. Optical density was measured at regular intervals to determine the optimum temperature for growth and oil degradation.

To determine the effect of media pH on growth of culture, the media pH was varied from 5.0 to 8.0 adjusted by adding 1N HCl or 1N NaOH if and when required. The growth was carried out at conditions according to the new optimized time. Optical density was measured at regular intervals to determine the optimum temperature for growth and oil degradation.

To study the effect of agitation speeds, flasks with inoculated Mineral media broth were incubated at 0, 50, 100, 150, 200, 250 and 300 rpm maintaining the predetermined conditions and optimized temperature and pH. Optical density was measured at regular intervals to determine the optimum temperature for growth and oil degradation.

Measurement of remaining oil:

The broth culture is then centrifuged in a macrocentrifuge at 3000rpm to remove the culture. Remaining residual oil was extracted via liquid-liquid extraction by adding 5ml of benzene to broth culture in flask and shaking thoroughly as described by Adebusoye et al. After removing the aqueous phase with separating funnel, the residual oil is kept in an oven to evaporate hexane at 40°C and the residual oil is measured in a pre-weighed flask on an electronic weigh balance.

The beaker with the residual diesel oil was allowed to cool to room temperature and weighed to determine the quantity of residual diesel oil by difference. Similarly, oil from control flasks was extracted and the amount of degraded oil is determined.

Determination of Oil degradation:

The residual oil from the microbe degraded flasks was compared with the oil content from the control flask. Results were expressed as percentages of respective controls using the following equation and depicted in a tabular form.

$$\% \text{ of Residual Oil} = \frac{Wt.of\ remnant\ oil}{Wt.of\ initial\ oil} \times 100$$

RESULTS AND DISCUSSIONS

Growth in 0.5% Oil

The chosen strains were inoculated in a MM broth media containing 0.5% V/V used engine oil. Growth of *A. chrocoocum* was seen to increase in terms of OD with the highest value measured on the 9th day and then decline. Growth of *P.putida* was seen to increase steadily and reach maximum growth on 11th day and then gently decline. The mixed consortia showed a steady increase in OD and reached maximum on 9th day and decreased sharply (Fig.1).

The decrease may be due to depletion of carbon with time. The growth rate of the mixed consortia was seen to be greater than the single cultures. *A. chroococcum* co-inoculants have the capability of increasing biodegradation efficiency of crude oil hydrocarbon. **(**Suryatmana.P et.al, 2006)

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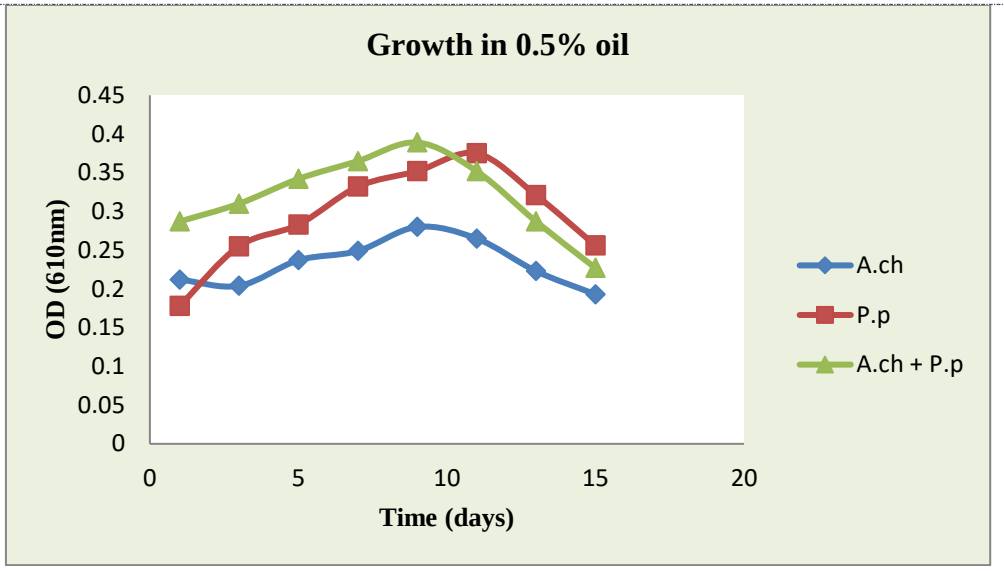

Fig 1: Comparative graph of growth of A.ch, P.p and A.ch + P.p in 0.5% oil.

Growth in 1% Oil

The chosen strains were inoculated in a MM broth media containing 1% V/V used engine oil. The optimum growth and hence degradation, for *A. chrocoocum* was seen on 7th day at an OD of 0.279 and that of *P.putida* on 9th day of incubation at an OD of 0.313.It was observed that the maximum degradation and growth in the mixed consortium of bacteria i.e. *A. chroococum* and *P.putida* on 7th day of incubation at an OD of 0.369 and represented in Fig 2.

An increase in oil degradation is correlated to an increase in cell number indicating that the bacterial isolates were responsible for the oil degradation. (R.Thenmozhi et al, 2011).

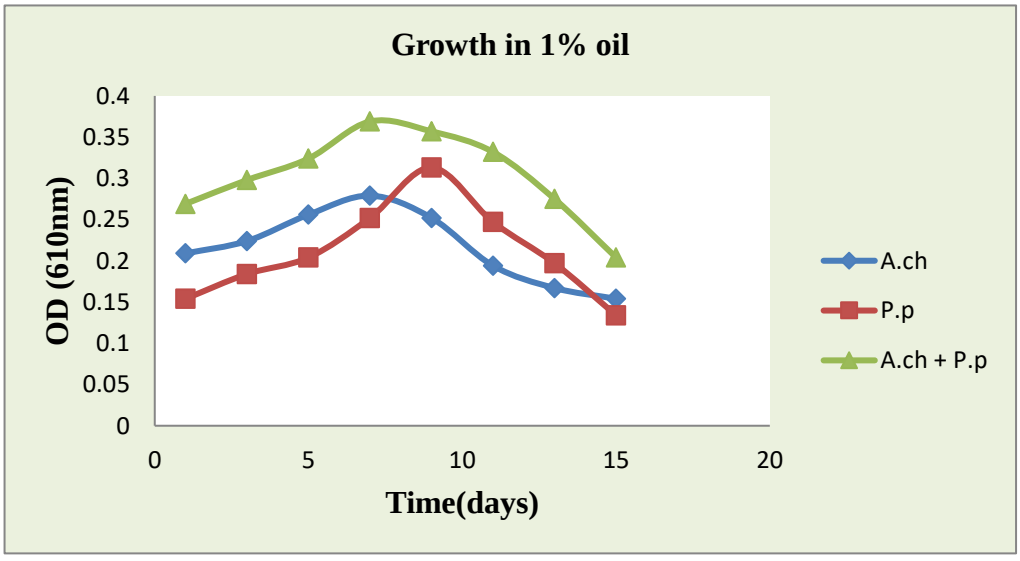

Fig 2: Comparative graph of growth of A.ch, P.p and A.ch + P.p in 1% oil.

Growth in 1.5% of Oil:

The chosen strains were inoculated in a MM broth media containing 1.5% V/V used engine oil. *A. chrocoocum* and *P.putida* showed a declining trend in their growth from the 1st day. Due to the high oil concentration, the growth rates have been retarded. This may be due to minor toxic ingredients in the engine oil which are inhibiting the microorganism's growth and /or oil degradation.

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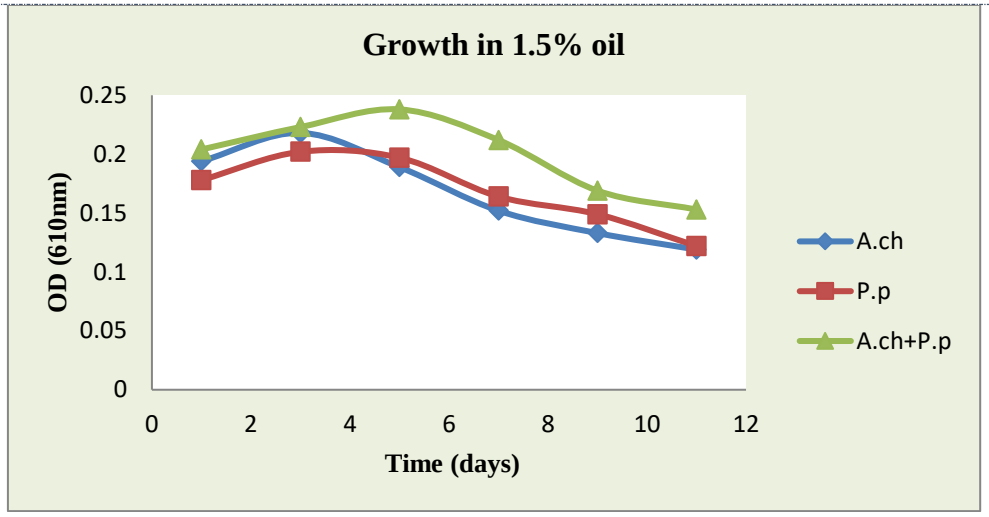

Fig 3: Comparative graph of growth of A.ch, P.p and A.ch + P.p in 1.5% oil.

Effect of physical parameters

Effect of agitation speed:

After determining the optimum time period of each strain and the mixture the incubation were carried out only till that time i.e. 7th day for *A. chroococum*, 9th day for *P. putida* and 7th day for the mixture.

- At static state the growth rate is comparatively less than the growth at other speed, which seems to be necessary for optimum growth.
- The optimum speed for both *A. chrocoocum* and *P.putida* was found to be at 200 rpm as the growth of microbes is higher than at any other speed. For the mixture 150 rpm it was found to be the optimum condition. (Fig. 4)
- With increase in the speed than the optimum, may result in the coagulation of microbes.

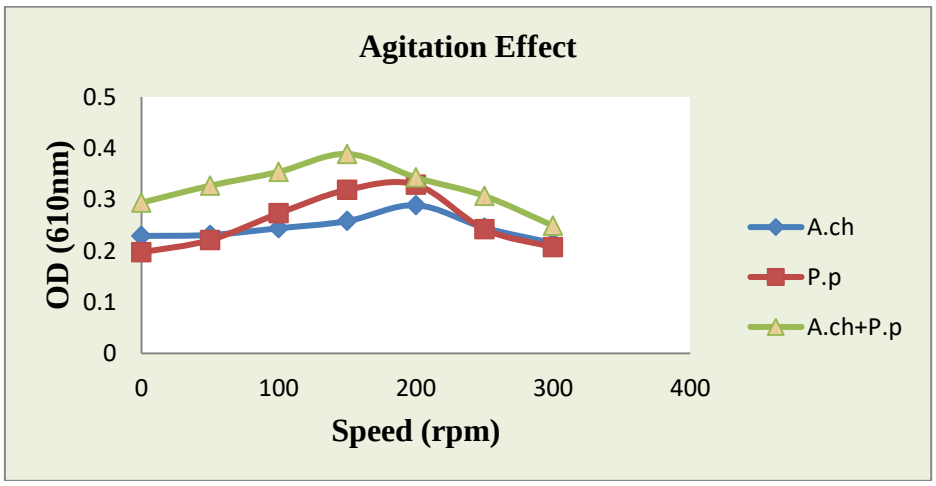

Fig 4: Comparative graph showing effect of agitation speed on growth of A.ch, P.p and A.ch + P.p in 0.5% oil.

Effect of pH on oil degradation:

After determining the optimum time period of each strain and the mixture, the incubation was carried out only till that time i.e. 7th day for *A. chroococum*, 9th day for *P. putida* and 7th day for the mixture. Also the optimum agitation speed maintained for *A.chroococum* and *P. putida* was 200 rpm and 150 rpm for the mixture of *A. chroococum* and *P. putida.*

There was a sharp decline in growth and nitrogen fixation above pH 7.0 (A.S. Ninawe et.al. 1995). The effect of pH on the growth of *A. chroococum* and *P. putida* is quiet clear from the readings that the growth increased along with the pH and that neutral pH 7 is more advantageous to the maximum growth of organisms as shown in Fig.5.

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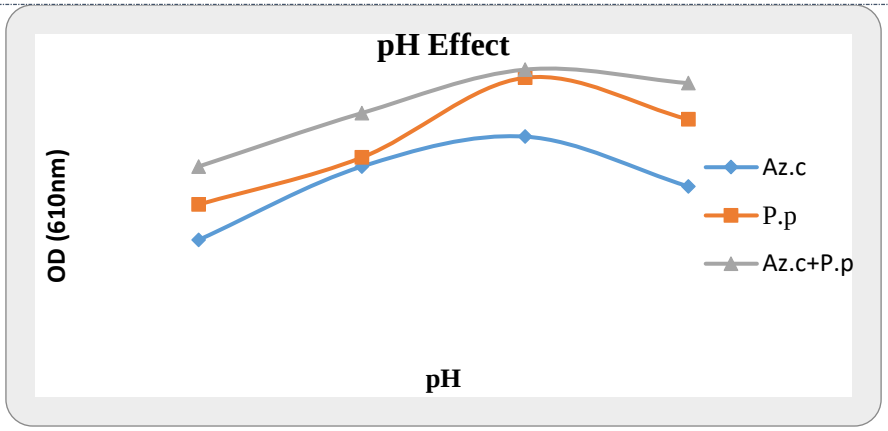

Fig 5: Comparative graph showing effect of pH on growth of A.ch, P.p and A.ch + P.p in 0.5% oil.

Effect of temperature on oil degradation:

After determining the optimum time period of each strain and the mixture, the incubation is carried out only till that time i.e. 7th day for *A. chroococum*, 9th day for *P. putida* and 7th day for the mixture also at an optimum pH of 7 and the optimum agitation speed maintained for *A. chroococum* and *P. putida* was 200 rpm and 150 rpm for the mixture of *A. chroococum* and *P. putida.*

As the degrading components are the enzymes released by the microbes during the stationary phase, they are susceptible to temperature due to their biological nature. Hence the growth levels are maintained at optimum only till 37°C and declined at 40°C due to temperature inactivation. Oxygenase enzyme involved in degradation is encoded by plasmids and other chromosomal genes (Rosenberg et al, 1996).

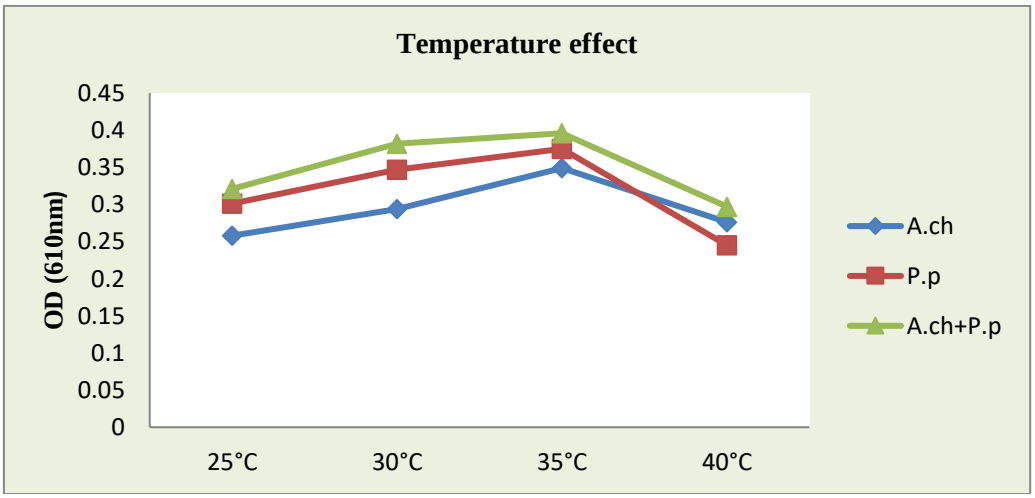

Fig 6: Comparative graph of effect of temperature on growth of A.ch, P.p and A.ch + P.p in 1% oil.

Analyzing the remnant oil

Pre-Optimization:

Oil quantities measured after growth in 1% oil before optimization i.e. at temperature 30°C, agitation speed 150 rpm and pH 6 was shown in the Fig.7.

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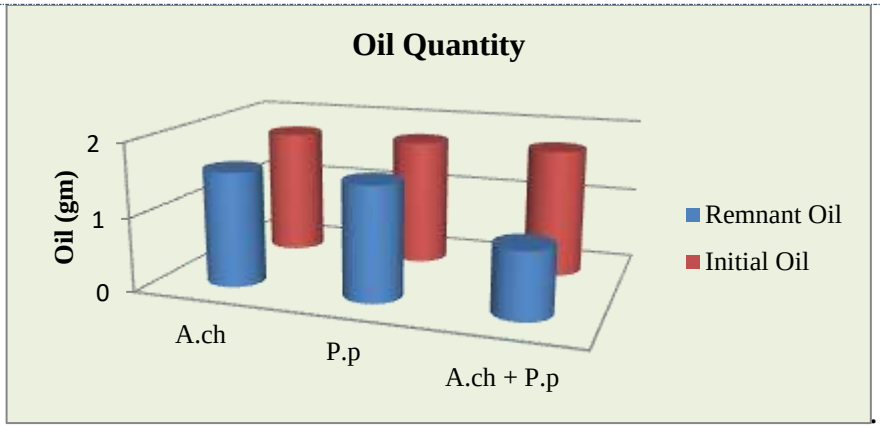

Fig 7: Comparative graph of oil degradation by A.ch, P.p and A.ch + P.p in 1% oil before optimization.

Post-Optimization:

The maximum degradation is observed after optimizing the temperature, agitation speed and pH. *A.chrocoocum* showed proportional increase in the degradation capacity before and after optimization. *P.putida* also showed comparative hike in degradation of 1% oil. The mixed strain showed highest improvement in degradation of oil than any one of the strains individually taken (Fig.8).

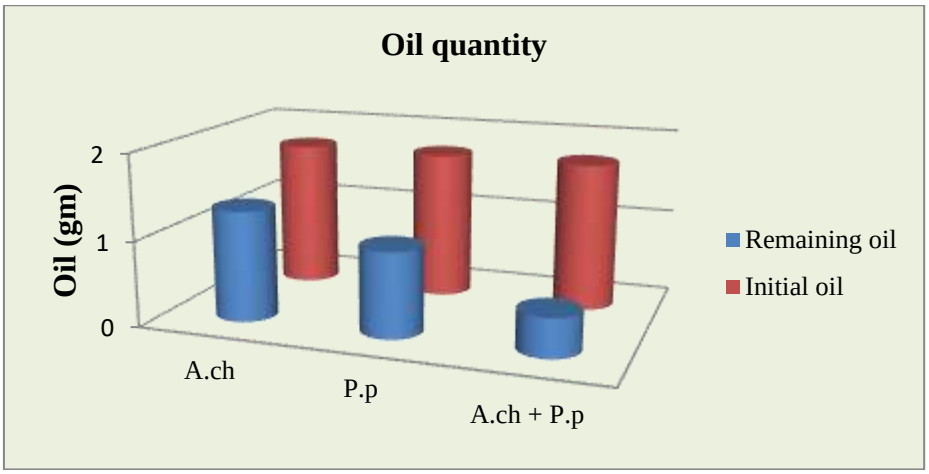

Fig 8: Comparative representation of difference of oil quantity before and after optimization.

CONCLUSION

When maintained under optimum conditions tested in this study, 1%(V/V) used engine oil was degraded using *A.chrocoocum* in 7days.*P.putida* showed maximum degradation in 9 days and the mixture of both *A.chrocoocum* and *P.putida* in 7 days. The mixture showed the highest growth and degradation of oil up to 73.7 %. The conditions which favored the degradation are *A.chrocoocum* whose agitation speed of 200 rpm, pH 8 and temperature of 35ºC *A.chrocoocum+P.putida* agitation speed of 150 rpm, pH 7 and temperature of 35ºC and *P.putida* agitation speed of 200 rpm, pH 7 and temperature of 35ºC. Present study confirmed that *A.chrocoocum* produces biosurfactant which is very helpful in used engine oil degradation which illustrates the fact that the mixture always had greater capacity than the individual organism used. This kind of synergistic interactions can be harnesses and a wide range of bacteria can be used to form variable consortia to benefit from their interactions.

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