THE EFFECTS OF BACTERIAL ENZYMES AND BIOSURFACTANTS ON HYDROCARBON DEGRADATION IN A CRUDE OIL POLLUTED FRESH WATER.

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ABSTRACT

The present work was designed to identify extracellular enzymes and biosurfactants from five bacterial isolates and investigate their actions on crude oil degradation as a means of bioremediation of hydrocarbon pollution.

The Five bacterial isolates including *Proteus vulgaris, Bacillus subtilis, Pseudomonas aeruginosa, Sarcinae litoralis,* and *Alcaligenes viscolatis* were selected for the study based on their relatively high efficiency for crude oil utilisation and their wide occurrence in the Niger Delta basin of Nigeria. The types of enzymes produced by the selected isolates were determined using standard API ZYM kit they were produced in spent media. The activity of the enzymes on crude oil biodegradation was investigated using the enzyme inhibition method. Biosurfactants were extracted from cultures grown in a neutral medium (pH 7.0) containing 2% glucose, shaken at 150 rpm at 37°C for 7 days. Qualitative drop-collapse, blue agar plate, haemolytic, emulsification activity (E_{24}), oil spreading techniques as well as the swirling beaker test were used to confirm the production of biosurfactant. The effect of pH, temperature and salinity concentration on biosurfactant production was evaluated using standard instrumental methods. Biosurfactants were evaluated using acid-precipitation followed by extraction using chloroform-methanol (2:1). The anthrone positive fraction of the biosurfactants was identified by measuring extract absorption in an iodine-polysaccharide complex using a spectrophotometer NOVASPEC II, (Pharmacia Biotech) at wavelengths range of 380 to 700 µm.

Enzyme systems detected with the API ZYM kit varied depending on the growth substrate used. Enzyme inhibition slowed down degradation of crude oil and the enzyme activity behaved similarly over time whereas the effect of

catalase, cytochrome c oxidase and lipase increased the biodegradation of crude oil in the enzyme cultures medium. All the microorganisms investigated produced highest biosurfactant in glycerol medium (1.26-1.64) mg/1 during a 48 h of growth. Biosurfactants production was optimum at pH 6.2 and 7.2 and temperature of 37°C and 2% NaCl. All biosurfactants emulsified oil to varying degrees with varying emulsification index (E_{24}) with over 60% emulsification activity. They were all stable with temperature between 15 and 90°C and pH range of 4.2-10.2. Only three isolates haemolysed blood agar and formed dark blue halos on agar plates indicating the production of glycolipids. The complex formed between the reaction of iodine with the polysaccharide of the biosurfactants had a maximum absorption wavelength between 380 to 420 µm.

In conclusion, the enzymes and biosurfactants produced by the investigated bacterial isolates were effective in degrading crude oil.