BIOSURFACTANT-MEDIATED DEGRADATION OF CASHEW KERNEL OIL BY MICROBES

Project Reference No.: 46S_BE_5378

COLLEGE : N.M.A.M. INSTITUTE OF TECHNOLOGY, NITTE

GUIDE (S) : Dr. SHYAMA PRASAD SAJANKILA
Dr. SANDESH K

STUDENT (S) : Ms. SNEHA R
Mr. MOHAMMED AFFAN
Ms. R S SHIVARANJANI
Ms. VAIBHAVI RAO

Keywords

Cashew oil, Biodegradation, fungi, bacteria, RSM, Biosurfactants

Introduction

Oil spills have become a threat to the environment. Numerous studies have revealed that microbial-based degradation is a better approach to deal with such oil spills, than physical and chemical approaches which has certain limitations. In recent studies, it has been observed that the microbes dwelling in oil rich soil have better degradation ability. Cashew oil is a versatile by-product of the cashew industry and is a viscous liquid that is obtained from the cashew nut by steam distillation or extraction with solvents. It contains a high proportion of phenolic compounds and is used as raw material for brake lining compounds, paints, varnishes, epoxy resins, etc. The pH of the oil ranges from 4 to 5.5 and it was found that cashew oil is toxic and corrosive to skin. Microbes that are capable of degrading cashew oil are important in the management of cashew processing wastes and environmental pollution control. Cashew processing generates large amounts of waste, including cashew apple residue and nut shells, as well as wastewater from cleaning and processing operations. Microorganisms that are capable of degrading cashew oil include bacteria, fungi, and yeast. Some of the commonly reported microorganisms that can degrade cashew oil include *Pseudomonas aeruginosa, Bacillus subtilis, Candida albicans,* and *Aspergillus niger.* These microorganisms are found to produce enzymes such as lipases, esterases, and proteases that are capable of breaking down the complex organic compounds in cashew oil into simpler compounds, which are less toxic and corrosive that can be assimilated by the microorganisms.

Objectives

- Identification and characterization of microbes from cashew kernel oil spilled area with the ability to degrade the oil.
- Optimize the factors (Varied media concentration, pH, Temperature, Nitrogen source and Inoculum density) affecting the oil degradation by the microbes.
- Preliminary characterization of biosurfactants being produced by the microbes.

Methodology

From collected soil sample, microbial isolation was performed by spread plating with three different media composition: Nutrient agar, Nutrient agar layered with oil, Agar-Agar layered with oil. In the presence of cashew oil, growth of *Rhizopus* and *Bacillus* species were observed. To check the level of degradation, the *Rhizopus s*pecies was grown in Potato Dextrose Broth (PDB) considering the following factors: Varied media concentration, pH, Temperature, Nitrogen source and Inoculum density. Different levels in these factors were considered. For example, for varied media composition three different PDB concentration such as $1/4^{th}$, $1/10^{th}$, and $1/20^{th}$. The optimum temperature was determined by growing the fungus in PDB (same as varied media composition with 1000µL) and incubated at different temperatures of 15°C, 20°C, 30°C, and 40°C. The fungus was grown in PDB media (same as varied media composition with 1000µL) containing different pH ranges including 4, 5, 6, 7 and 8. The pH of the media was adjusted by adding 0.1N NaOH and dil. HCl. Different nitrogen sources such as Ammonium oxalate, Ammonium acetate, Ammonium chloride, Ammonium sulphate, Potassium Nitrate, Sodium Nitrate, DL- Threonine and L-Glutamic acid were tested for the growth of fungi and degradation of oil. Fungi grown in the media containing Ammonium oxalate had better degradation. The effect of various concentrations of Ammonium oxalate (0.0325gm, 0.065gm, 0.130gm, 0.260gm and 0.520gm) were assessed on the degradation ability of *Rhizopus*. The effect of inoculum concentration on oil degradation was tested by seeding fixed number of spores to the media containing cashew nut oil. The range of spores (in lakhs) added were: 5×10^4, 1×10^5, 1.5×10^5, 2×10^5, 2.5×10^5, and 3×10^5. To standardize the above conditions, OFAT analysis was carried out and factors were optimized with the help of RSM analysis. The culture supernatant of *Rhizopus* species was checked for biosurfactant activity by

Drop collapsing method, Emulsification Index (E24), Oil spreading test.

Results and conclusion

The microbes from the cashew oil contaminated soil were isolated by spread plate method. Two types of microbes were observed and were identified as *Bacillus* and *Rhizopus* species. *Bacillus* species were identified by performing gram staining method and *Rhizopus* species were identified by performing lactophenol cotton blue staining method.

Figure 1: Control sample without fungal spores. It can be observed that the oil has been sticking to the flask surface.

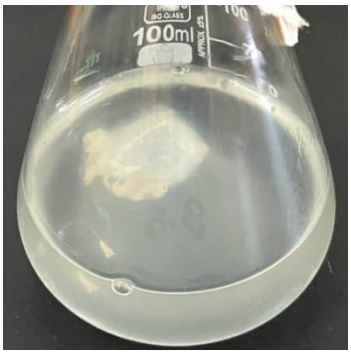

Figure 2: This image depicts the oil degradation and the fungal growth. It can be observed that the oil has been clumped together by the fungi and it had grown surrounding the clumped oil.

After sub-culturing of the microbes, the oil degradation and growth of the bacteria was observed and was found that the *Bacillus* species that was isolated from the cashew oil-contaminated soil was not a good degrader of the cashew oil. Therefore, further studies were carried out in the *Rhizopus* species. Further OFAT analysis was performed for the *Rhizopus* species. The best degradation was found in the media containing 0.06gm of PDB granules (1/20th PDB conc.), Ammonium Oxalate of 0.13gm as nitrogen source and 2.5×10^5 spores inoculated, kept in 20°C incubator shaker at 50rpm for one week. The fungi that could degrade the cashew oil could also produce some extracellular chemicals that could be used as biosurfactants. So, biosurfactant activity was checked for the fungal media using three methods: Drop collapsing method, Emulsification Index (E24), and Oil spreading test. All the three methods showed a positive result. In drop collapsing method the drop of culture supernatant had collapsed indicating the presence of biosurfactants. Oil spreading test had a clear zone indicating a positive result. Emulsion layer was formed with an %E24 value of 57.14. The factors were optimized using Central Composite Design (CCD) using RSM approach. The interaction between the factors were present as per the surface plot.

The optimum growth was found to be 1/18.72th for varied media composition, 0.124gm for ammonium oxalate concentration and 2.67×10^5 spores for spore count.

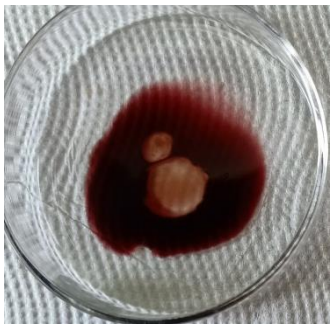

Figure 3: Drop Collapsing test; the culture supernatant collapsed in a minute resulting a flat surface indicating presence of biosurfactants.

Figure 4: Oil spreading test; Appearance of clear zone on the surface of water.

Figure 5: Emulsification Index: Emulsion layer was formed with an %E24 value of 57.14

Innovation in the project

The fungus isolated from oil rich soil was found to be the best degrader as well as capable of producing biosurfactant under oil stress.

Scope for Future work

1. The isolated *Rhizopus* can be used as a bio-degrader for oil spills (A detailed study with other types of oil degradation is required along with studying the mechanism/pathway of degradation).
2. The biosurfactant produced can be used to aggregate the oil for physical removal from aquatic environment (Both fresh water and marine water)