SCREENING OF LIPASE-PRODUCING MICROORGANISMS ISOLATED FROM PUEBLA SOILS FOR TRANSESTERIFICATION OF WASTE COOKING OIL

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ABSTRACT

Thirty two lipase producing strains were isolated from different soils from Puebla City by employing conditioning culture techniques with media that contain waste cooking oil. The pH conditions were 5, 7 and 9, hence given a total of 9 independent cultures. The first screening and isolation criteria was carried out with samples of the liquid media inoculated in agar plates with same media composition and conditions as described above and 0.001% (w/w) of fluorescent dye Rhodamine B. The microbial growth was presented in all plates, however not all microbial colonies presented lipase activity evaluated as a result of a characteristic dark halos (fluorescent lost) observed under UV light (355 nm) due an association of Rhodamine B with esters formed by lipase transesterification and/or esterification reactions. Microorganisms with remarkable lipase activity were selected for further purification. In media with pH values of 7 have been isolated 5, 9 and 9 strains from CN, MM and MMS respectively. Seven strains for media at pH value of 5, corresponding to 3 from CN and 4 from MM. Only two strains were isolated in MMS at pH value of 9. In subsequent studies, isolated microorganism will be identified and their lipase activity will be quantified by transesterification reactions with waste cooking oils and methanol for Biodiesel production.

INTRODUCTION.

Biodiesel has gained importance in the recent past for its ability to replace somehow the fossil fuels which are likely to run out with the century. The environmental issues concerned with the exhaust of gases emissions by the usage of fossil fuels also encourage the usage of biodiesel which has proved to be eco-friendly [Ranganathan et al. 2008]. Biodiesel is an alternative fuel, non-toxic, biodegradable and renewable source of energy [Lu et al. 2007]. Generally, biodiesel is often associated with fatty acids alkyl esters produced employing alkali-catalyzed transesterification of refined vegetable oils and fats with short chain alcohol. The general reaction is shown in (Fig. 1). The stoichiometry requires 3 moles of alcohol and 1 mol of triglyceride to give 3 moles of fatty acid esters and 1 mol of glycerine. The overall process is a sequence of three consecutive reversible reactions where diglyceride and monoglyceride are intermediate products [Pinto et al. 2005].

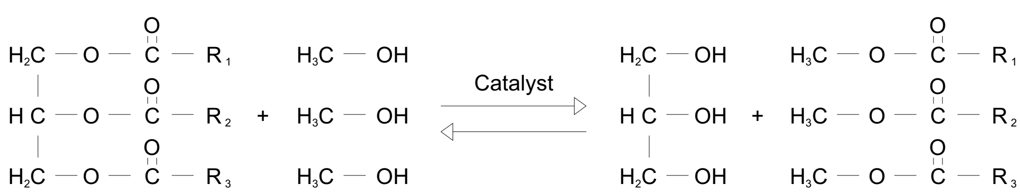

Figure 1. Transesterification reaction of triglyceride.

The transesterification is also catalized by acid and enzimes, using virgin vegetal oil [Pinto et al. 2005]. The production of biodiesel from unrefined feedstocks, e. g. waste cooking oils using alkaline catalyst, has encountered some difficulties as a result of the properties of the feedstocks and their free fatty acids (FFA) content. Enzymatic transesterification is a process eco-friendly and under mild conditions can overcome the problems of chemical processes as high temperatures, multistage in the downstream to get a high purity product and enables easy separation from the byproduct, glycerol [Ranganathan et al. 2008]. However, the cost of enzymes is expensive; also enzymatic inactivation could take place in transesterification process. Hence, the industrial demand for new sources of