



Exploring Extracellular Hydrolytic Enzyme and Bio Flocculant-Producing Bacterial Isolates for Bioremediation of Vegetable Oil Refinery Effluent.

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ABSTRACT:

The process of vegetable oil refining generates an enormous amount of wastewater. This study focused on characterization of vegetable oil refinery effluent (VORE) sample untreated and treated by bacteria for various physicochemical parameters. The sample has high COD and BOD; 12.32g/L and 1.7g/L respectively, this highlights the need for proper treatment. The VORE samples were treated by bacteria producing extracellular hydrolytic enzymes and bioflocculant, identified as *B. licheniformis*, *S. stutzeri*, *B. amiloliquificans* and *S. mutabilis*. The bacterial treatment was performed in a separate 250ml flask containing 100ml effluent and inoculated with 2% of respective 24h grown culture. The bacterial treatment achieved a significant decrease in BOD and COD. The treated effluent showed significant increase in seed germination representing a reduction in toxicity in comparison to untreated effluent. This highlights the suitability of present study isolates for VORE treatment with high BOD and COD.

1. Introduction

The major source of vegetable oil is from seeds such as; soybean, sunflower, sesame, coconut, palm, rice bran, and groundnut. India is amongst the largest producers of oilseeds in the world, accounting for 36.56 MT production of nine different oilseeds in the year 2020-21 [1]. The oil production i.e., extraction of oil from seeds is done by physical or chemical extraction using a solvent. Crude oil extracted from seeds must process before human consumption [2]. This process includes degumming, alkali neutralization, bleaching, heating, and deodorization [3], which generates a higher amount of wastewater [4]. Generally, different processes involved in vegetable oil refining used a huge amount of water and generate an equal amount of wastewater, but chemical refining produces 20-30 times higher wastewater than water used in this process [5,6]. A substantial amount of waste is generated including wastewater, organic solid waste, and inorganic residues [7]. In the degumming step, some phosphoric acid is added to separate phospholipids, increasing the phosphorus level in the effluent. In the neutralization step sodium salt of free fatty acid ("soap stocks") were generated and split using H₂SO₄ in a stoichiometric amount which makes wastewater highly acidic [8]. A typical effluent from vegetable oil contains a higher amount of COD, BOD, TSS,

TDS, nickel, oil & grease, fats, soap and sludges which cause deterioration of the environment and human health [6,9,10]. Wastewater generated both quantitatively and qualitatively has a profound impact on ecology, resulting in increased pollution load and concentration. Treatment of this type of effluent is more difficult because of its complex molecular structure [3].

There are numerous physicochemical (skimming of oil, flocculation, coagulation, air floatation) methods available to remove colloidal pollutants [11]. Chemical flocculating agents are the choice of many to remove the pollutant, but flocculating agents like inorganic and organic polymers may be toxic to the ecosystem. In comparison with this, bioflocculant produced by microorganisms are non-toxic, harmless, efficient, and biodegradable [12]. The biological methods are more suitable in terms of generation of secondary pollutant, partial treatment, investment costs. Further use of bacteria to remove pollutants is safe, eco-friendly and inexpensive, five to twenty times less costly than chemical treatments and more economical than physical-chemical treatment methods.

Bacteria are suitable for the degradation of pollutants because of their diverse carbon utilization ability [13]. The bioremediation process mainly depends on microorganisms that attack pollutants enzymatically and convert it to