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**Development of Efficient Microbial Consortia for the Production of Bioethanol Using Rice Straw as the Low-Cost Substrate; A Step into the Circular Bio Economy**

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**Abstract**

Energy crisis is one of the major concerns in the globe, which is driven by urbanization and industrialization-related anthropogenic activities. Therefore, the world is looking for alternative energy sources as a green and sustainable strategy in the circular bio-economy. Since second-generation bioethanol has a comparatively smaller environmental effect, it is seen as a possible alternative energy source. Therefore, the present study focuses on developing a microbial consortium under various chemical pretreatment and fermentation conditions to create an efficient saccharification technique for rice straw, a lignocellulosic agricultural by-product. For the study, cellulase-positive bacteria were isolated from compost, soil, and cow dung samples. The primary screening of bacteria was carried out using the Congo red-CMC agar assay, and the DNS method was used for the secondary screening of cellulose-positive bacteria. After evaluating their antagonistic effects, the best potential isolates were combined to create four microbial consortia, and the effectiveness of their saccharification was determined using the DNS. Moreover, the Rice straw was subjected to alkali and acid pre-treatments, including 4% NaOH (66.7%), 2% NaOH (80.0%), 0.5% H<sub>2</sub>SO<sub>4</sub> (54.3%), 1% H<sub>2</sub>SO<sub>4</sub> (81.2%), and 2% H<sub>2</sub>SO<sub>4</sub> (44.7%) to facilitate the accessibility of cellulose for enzymatic saccharification under nutrient rich media and 50 mM sodium citrate buffer. The starved commercial *Saccharomyces cerevisiae* was used to ferment hydrolysates in a medium that was rich in nutrients, and the final bioethanol yield was quantified by the GC-FID method. Based on the results, 29 morphologically different bacterial isolates were isolated from compost, cow dung, and agricultural soil, and out of those, six potential isolates were selected by secondary screening. The highest ethanol yield was recorded by the consortia D, in 2% H<sub>2</sub>SO<sub>4</sub> pre-treatment in nutrient media (3.96±0.5% v/v), followed by the consortia A in 2 % H<sub>2</sub>SO<sub>4</sub> pre-treatment in nutrient media (2.95±0.4%), and consortia C in 4% NaOH pre-treatment in buffer (2.43%). The biochemical identification confirms that the bacteria in the best consortia D belong to the genus *Bacillus* spp. and *Lactobacillus* spp. Further, the findings of the present study provide a valuable insight into the production of bioethanol from waste rice straw using native bacterial consortia as a sustainable approach.

**Keywords:** *Bioethanol production, Microbial consortia, Rice straw, Cellulolytic microorganism, Circular bioeconomy*