

Title : Evaluating the bioethanol production potential of *Spathaspora passalidarum* and *Kluyveromyces marxianus* using spent mushroom substrate

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ABSTRACT

Spent mushroom substrate (SMS) is a byproduct of the mushroom cultivation industry and poses a significant disposal challenge. This study aimed to evaluate the potential of xylose-fermenting yeasts, *Spathaspora passalidarum* and *Kluyveromyces marxianus*, for bioethanol production from SMS. The proximate composition of SMS was analyzed, revealing cellulose, hemicellulose, and lignin contents of $37.29\% \pm 0.21$, $20.07\% \pm 0.72$, and $27.12\% \pm 0.87$, respectively. SMS pretreated with 2% (w/v) NaOH and further hydrolyzed with 0.1% (v/v) H_2SO_4 yielded total reducing sugar concentrations of 19.95 ± 0.25 mg/mL and 38.80 ± 0.76 mg/mL, respectively. To assess yeast tolerance, hydrolysates obtained from NaOH pretreatment and NaOH followed by H_2SO_4 hydrolysis were tested at concentrations of 25%, 50%, 75%, and 100% (v/v) using a spot test. The results showed that *S. passalidarum* and *K. marxianus* could thrive at 25% (v/v) and 100% (v/v) hydrolysate concentrations after NaOH pretreatment and NaOH followed by H_2SO_4 hydrolysis, respectively. To further evaluate their ethanol production potential, fermentation of the SMS hydrolysate at each selected concentration is currently in progress.

Keywords: Bioethanol, *Kluyveromyces marxianus*, *Spathaspora passalidarum*, Spent mushroom substrate

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