National Institute of Technology Rourkela

Defence Seminar

Seminar Title	: Multistep improvement of Klebsiella sp. SWET4 strain to obtain higher ethanol yield from cellulosic fruit waste: single step for waste to energy conversion
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Venue	: BM Seminar Room
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Abstract : The pretreatment process involved in 2nd generation bioethanol production infers significant cost along with environmentally hazardous byproducts. Hence, its substitution with direct fermentation would significantly advance the process. Since raw substrates would be used during fermentation, its growth inhibitor content would be an important substrate selection criterion. This study revealed that the growth of *Klebsiella* sp. SWET4 was significantly reduced by phytate, phenolic acid, cyanide, and tannin at 3.09%, 0.22%, 0.38%, and 0.04% per µg/mL, respectively. Since banana peel contained the least amount of these growth inhibitors, it was predicted to be the best substrate for SWET4. Moreover, the potential of the banana peel as a probable substrate for ethanol production was evaluated with the help of logical prediction. The Whole Genome Sequencing of SWET4 (5665821 bases) revealed the presence of 5 major cellulose metabolizing (*bcsZ, bglC, bglA, celA, chbA*), besides 4 key xylan degrading (*xynB, xynT, xylA, xylB*) and 4 principal ethanol fermentation (*nifJ, adhE, acs, adh1*) genes. Expression study with qPCR confirmed the functionality of these genes. The lignolytic potential of SWET4 was evident in kinetic study and the presence *of yfeX/efeB, katG, katE*, etc. genes was confirmed. SWET4$^{adh1+adhE}$ recombinant strain exhibited a remarkable 7.76-fold increase in ethanol productivity from the banana peel in facultative anaerobic conditions. qPCR analysis confirmed 106.15- and 22.78-fold higher expression of *adh1* and *adhE* genes, respectively. Optimization using Artificial Neural Network modeling and Genetic Algorithm was found better than Response Surface Methodology (RSM) for predicting bioethanol production by SWET4$^{adh1+adhE}$. After optimization, the enhanced biomass productivity of 2.33 g/L was achieved along with ethanol production of 24.47 g/L as confirmed by HPLC. The process demonstrated an ethanol yield of 0.44 g/g from carbohydrates surpassing many 2nd generation bioethanol processes. Further, a minimum selling price of $2/kg of distillate was found to make the process economically feasible which is significantly low. The breakeven point of the process was found to be 30% of its total capacity. The techno-economic analysis highlighted feasibility, particularly emphasizing the economic advantages of eliminating pretreatment steps, highlighting the process&rsquos innovation and viability in the field of 2nd generation bioethanol production.

Keywords: Bioethanol Banana Peel Whole genome Metabolism Gene expression Strain improvement Techno-economic