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ABSTRACT

The sugarcane bagasse was hydrolysed by 3% (w/v) H₂SO₄ solution in an autoclave at 1.5 kg/cm² pressure gauge (126.7°C) for 60 min reaction time. After concentration of hydrolysate, 2.4% (w/v) of activated charcoal pellet was added to remove inhibitors. The detoxified hydrolysate from sugarcane bagasse was then used as the fermentation medium for the tested yeasts. The xylose-fermenting yeasts namely; *C. guilliermondii* 5068, *Kluyveromyces marxianus* 5057 and *Hansenula anomala* 5302 were employed for this experiment based on the previously reported for efficient xylitol production in sugarcane bagasse hydrolysate.

Isolation of xylose reductase (XR) gene from *K. marxianus* 5057 was conducted and determined for gene expression. The 4.5 kb XR fragment was characterised. The XR gene of *K. marxianus* 5057 at 4.5 kb was then transformed into the wild type of *K. marxianus* 5057. The 6 favoured recombinants of *K. marxianus* 5057 (rKm1, rKm2, rKm3, rKm4, rKm5, rKm6) were chosen and used to compare the growth and their ability of maximal xylitol production in sugarcane bagasse hydrolysate. Meanwhile, the gene fragment was expressed in *Pichia pastoris* and sequenced for nucleotide characteristics. The apparent molecular weight of XR protein was 37.5 kDa and capable for encoding a polypeptide of 329 amino acids.

Comparisons of growth and xylitol production from sugarcane bagasse hydrolysate by both wild types (*C. guilliermondii* 5068, *K. marxianus* 5057) and recombinant cultures (rKm1, rKm2, rKm3, rKm4, rKm5, rKm6) were investigated in

shake-flask cultivation at 30°C and 200 rpm for 120 h. The highest yield of xylitol (15.64 g/l) and 0.13 g/l/h productivity was obtained in the batch of rKm6 tested culture, resulting in the biomass concentration of 5.30 g/l.

Finally, fermentation characteristics of recombinant *K. marxianus* 5057 (rKm6) containing the XR gene from *K. marxianus* 5057 were investigated in an attempt to improve the maximum xylitol production from sugarcane bagasse hydrolysate. The batch processes were carried out in the 2.5 l stirred tank reactor with 1.2 l working volume. Xylitol was produced in bagasse hydrolysate medium under aeration rate of 1.5 vvm and agitation rate of 200 rpm after 168 h fermentation with a maximum yield of 45.38 g/l or 0.27 g/l/h productivity, which is about two times that of shake flask culture.