ABSTRACT

Very high gravity (VHG) ethanolic fermentation is a high potential technology used in bioethanol production. However, the technology is often linked with the excessive amount of glucose that is entirely supplied at the beginning of the culture, causing the fermentation process to be slow. The high concentration of glucose in the fermentation medium also intensifies the osmotic pressure, which has a detrimental effect on yeast cells. The production of sago *hampas* was estimated to be 10 tons per day in Sarawak. Due to no proper treatment of this waste product, the disposal of sago *hampas* is polluting the environment. Sago *hampas* hydrolysate (SHH) was proven to be an excellent bioethanol substrate in normal gravity fermentation, but the feasibility of sago *hampas* in VHG fermentation is not yet been discovered. Hence, this study was carried out to elevate bioethanol production from SHH under VHG conditions by employing multiple modes of fermentations which are batch, fed-batch and repeated batch modes. The basic fermentation media consisted of 250 g/L SHH and commercial glucose (SHH+CG) supplemented with 5 g/L yeast extract. Fermentations were performed in a 2-L stirred tank bioreactor by using *Saccharomyces cerevisiae*. Our results showed that the maximum yeast cell concentration achieved in fed-batch mode was significantly improved by 1.5-fold compared to batch mode. The ethanol yield attained in the fed-batch culture represents an increment of 22% over that achieved in the batch culture. Additionally, the bioethanol productivity achieved in the fed-batch culture was enhanced by 1.8 times compared to the productivity attained in the batch culture. Nonetheless, the residual glucose in the fed-batch fermentation was 30 g/L which contributed to wastage in the cost of raw materials. Consequently, additional supplementation of the fermentation medium is necessary to increase yeast tolerance towards inhibitors, glucose consumption, and bioethanol

production. The effect of supplementing the basic media with various nutrients in bioethanol fermentation under VHG conditions was investigated. The nutrients added were magnesium sulphate (0.12 /L), urea (3 g/L), glutamic acid (5 g/L), and peptone (5 g/L). Our results showed that culture supplemented with peptone has significantly improved yeast growth by 0.9-fold, glucose consumption efficiency by 10%, and bioethanol production by 13% compared to the control culture with the production of 126.20 ± 3.0 g/L. An ample amount of active yeast cells were produced from VHG fed-batch bioethanol fermentation using SHH+CG supplemented with yeast extract and peptone. The cells were recycled to start a new cycle of bioethanol fermentation in a repeated batch system, which reduced the time and the operational cost of the bioethanol fermentation. The yeast was allowed to naturally sediment at the bottom of the fermenter for 6 h. After sedimentation, the concentration of viable cells recorded was 1.21 x10^6 ± 0.135 cells/mL. The fermentation broth was harvested from the bioreactor, fresh media was added to the bioreactor, and repeated batch fermentation was carried out. The results of the repeated batch had attained similar bioethanol fermentability to the fed-batch experiment. The ethanol yield ($Y_{E/s}$) was 0.475 ± 0.02, and the bioethanol concentration (P_E) recorded was 135.86 ± 2.19 g/L. Meanwhile, the results showed glucose consumption efficiency improved by 26% and 67% enhancement in P_E compared to the single batch fermentation. The results of this study demonstrated that SHH is a feasible substrate for bioethanol fermentation under VHG conditions. The employment of fed-batch and repeated batch systems have significantly improved yeast growth and bioethanol productivity in VHG fermentation than in batch mode.

Keywords: Bioethanol, sago *hampas* hydrolysate, very high gravity (VHG) fermentation, fed-batch system, repeated batch

Penggunaan Hidrolisat Hampas Sagu untuk Fermentasi Bioetanol 'Very High Gravity' (VHG) dalam Bioreaktor Tangki Berpengaduk

ABSTRAK

Proses fermentasi very high gravity (VHG) merupakan teknologi yang mempunyai potensi yang tinggi dalam pembuatan bioetanol. Akan tetapi, teknologi VHG sering dikaitkan dengan penggunaan glukosa berkepekatan sangat tinggi pada permulaan proses fermentasi yang menyebabkan proses metabolisma glukosa sepenuhnya oleh yis terhalang kerana tekanan osmotik yang tinggi. Penghasilan hampas sagu dianggarkan sebanyak 10 ton sehari di Sarawak. Ketiadaan cara rawatan sisa sagu yang betul menyebabkan pembuangan hampas sagu telah mencemar alam sekitar. Hidrolisat hampas sagu (SHH) telah terbukti sebagai substrat yang efektif untuk fermentasi bioetanol dalam normal graviti. Akan tetapi, perlaksanaan fermentasi bioetanol dalam keadaan VHG menggunakan SHH masih belum diketahui. Maka, kajian ini dijalankan untuk meningkatkan pembuatan bioetanol dalam fermentasi VHG daripada SHH menggunakan pelbagai jenis sistem fermentasi iatiu, secara kelompok, separa kelompok dan kelompok berulang. Media asas dalam uji kaji ini ialan 250 g/L SHH dan glukosa komersial (SHH+CG) yang ditambah dengan 5 g/L ekstrak yis. Semua proses fermentasi dijalankan di dalam 2-L bioreaktor tangki berpengaduk menggunakan Saccharomyces cerevisiae*. Data kultur separa kelompok menunjukkan kepekatan sel yis telah meningkat secara signifikan sebanyak 1.5-kali ganda dan hasil bioetanol diperoleh telah meningkat sebanyak 22% daripada kultur kelompok. Mahupun begitu, glukosa yang masih berbaki sebanyak 30 g/L dalam kultur separa kelompok boleh dikira membazir kerana melibatkan kos bahan mentah. Untuk mengurangkan pembaziran kos, penambahan suplemen dalam media fermentasi adalah penting untuk meningkatkan ketahanan yis terhadap perencatan*

serta menambahbaik metabolisma glukosa oleh yis. Suplemen yang ditambah ke dalam media fermentasi ialah magnesium sulfat (0.12 g/L), urea (3 g/L), asid glutamik (5 g/L) dan pepton (5 g/L). Keputusan eksperimen menunjukkan kultur yang diberi suplemen pepton telah meningkatkan pembiakan yis sebanyak 0.9-kali ganda dan kecekapan penggunaan glukosa sebanyak 10% apabila dibandingkan dengan kultur kawalan. Selain itu, kultur di dalam formulasi media itu juga telah menghasilkan 13% lebih banyak etanol iaitu sebanyak 126.20 ± 3.0 g/L. Sejumlah sel aktif yang mencukupi telah dihasilkan di dalam fermentasi secara separa kelompok dalam kondisi VHG mengunakan media asas mengandungi pepton. Sel-sel tersebut dikitar semula untuk digunapakai di dalam kitaran fermentasi bioetanol yang baru mengunakan mod kelompok berulang. Kesan penggunaan sel kitar semula di dalam fermentasi secara kelompok berulang dalam keadaan VHG telah dikaji. Yis dibiarkan mendap dalam bioreaktor selama 6 jam. Selepas proses pemendapan, kepekatan sel hidup yang telah dicatat ialah sebanyak 1.21 x 10^6 ± 0.135 sel/mL. Cecair fermentasi telah dikeluarkan daripada bioreaktor, media baru telah ditambah ke dalam bioreaktor dan fermentasi secara kelompok berulang telah dijalankan. Keputusan eksperimen didapati sama dengan keputusan fermentasi secara separa kelompok. Hasil ethanol ($Y_{E/s}$) ialah 0.475 ± 0.02 dan penghasilan bioetanol (P_E) ialah sebanyak 135.86 ± 2.19 g/L. Apabila dibandingkan dengan fermentasi secara kelompok, kecekapan penggunaan glukosa meningkat sebanyak 26% dan 67% peningkatan P_E diperoleh. Data menunjukkan SHH boleh digunapakai di dalam fermentasi berkeadaan VHG. Penggunaan sistem separa kelompok dan kelompok berulang telah meningkatkan pembiakan yis dan peghasilan bioetanol dalam fermentasi VHG.

***Kata kunci**: Bioetanol, hidrolisat hampas sagu, fermentasi very high gravity (VHG), sistem separa kelompok, fermentasi secara kelompok berulang*