BIOTRANSFORMATION OF SOLUBLE STARCH TO CYCLODEXTRIN USING IMMOBILIZED RECOMBINANT *Escherichia coli* ON HOLLOW FIBER MEMBRANE

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ABSTRAK

Minat industri yang semakin meningkat terhadap siklodekstrin (β-CD) tidak dapat dinafikan disebabkan oleh struktur uniknya yang mampu membentuk agen inklusi. β-CD telah digunakan secara meluas dalam pelbagai industri seperti dalam farmaseutikal, kosmetik dan bioteknologi. β-CD terbentuk melalui tindak balas enzim antara cyclodextrin glukanotransferase (CGTase) dan kanji. CGTase boleh didapati secara semula jadi dalam *Bacillus* sp. Walau bagaimanapun, *Bacillus* sp. menghasilkan jumlah CGTase yang rendah. Oleh itu, *E. coli* yang diubah suai secara genetik boleh digunakan untuk menyelesaikan masalah dengan menghasilkan jumlah CGTase yang tinggi. Kelemahan pengeluaran CGTase apabila menggunakan *E. coli* sebagai perumah ialah berlakunya lisis sel. Oleh itu, imobilisasi sel adalah cara alternatif untuk mengurangkan lisis sel dengan pengeluaran CGTase yang tinggi. Penjerapan adalah teknik yang terkenal dalam imobilisasi sel. Walau bagaimanapun, kelemahan kaedah ini ialah detasmen sel daripada sokongan. Oleh itu, kajian kinetik boleh disiasat untuk meningkatkan hasil produk. Objektif kajian ini adalah untuk mengoptimumkan hasil imobilisasi dengan memanipulasi parameter imobilisasi sel E.coli pada membran gentian berongga melalui teknik penjerapan dan menilai prestasi CGTase tidak bergerak pada penghasilan β-CD. Rekombinan *E. coli* telah dialihkan pada membran gentian berongga. Kesan masa sentuhan, suhu, kadar pengadukan, pH dan jenis medium ke atas hasil imobilisasi sel (kepekatan sel) telah disiasat dengan menggunakan kaedah satu faktor pada satu masa (OFAT). Kepekatan sel oleh sel yang tidak bergerak menunjukkan 150 mg/ml di bawah keadaan 24 jam masa sentuhan, 200 rpm dan pH 7 pada 30 °C dengan menggunakan medium *Terrific*. Kepentingan parameter proses (suhu dan masa sentuhan) pada hasil imobilisasi dioptimumkan lagi dengan menggunakan metodologi permukaan tindak balas (RSM). Di bawah keadaan yang dioptimumkan (20 jam masa sentuhan dan 35°C), ±227 mg/ml kepekatan sel telah direkodkan. Kajian kinetik penjerapan sel pada membran gentian berongga juga dilakukan. Berdasarkan pekali korelasi (R^2), model yang paling sesuai ialah model kinetik urutan kedua Pseudo (R^2 = 0.9515). Selain itu, model itu juga dipasang pada isoterma Langmuir dengan R^2 sebanyak 0.989. Kesan parameter tindak balas seperti kepekatan substrat, pH dan kadar pergolakan ke atas penghasilan β-CD olehsel tidak bergerak telah ditentukan. Keputusan menunjukkan bahawa pada 6% kepekatan substrat, pH 9 dan 200 rpm kadar pengadukan, pengeluaran β- CD tertinggi sebanyak ±7.8 mg/ml dihasilkan. Kemudian, pada keadaan tindak balas yang dioptimumkan (200 rpm, pH 8.5 dan 5.5% kepekatan substrat), pengeluaran β-CD tertinggi (±11.6 mg/ml) direkodkan. Untuk kajian kinetik tindak balas, ia menunjukkan bahawa Vmax kedua-dua sel tidak bergerak (±2.18 mg/ml.hr) dan sel bebas (±2.25 mg/ml.hr) adalah hampir serupa. Nilai Km untuk sel tidak bergerak (±5.14 mg/ml) adalah hampir sama dengan sel bebas (±5.42 mg/ml). Sel yang tidak bergerak juga boleh mengekalkan 24.4% daripada aktiviti awalnya walaupun selepas 6 kitaran yang berjaya.

ABSTRACT

The growing interest of industries toward β-cyclodextrin (β-CD) is undeniably due to its unique structure that capable of forming inclusion agent. β-CD has been widely used in numerous industries such as in pharmaceutical, cosmetic and biotechnology. β-CD is form through the enzymatic reaction between cyclodextrin glucanotransferase (CGTase) and starch. The CGTase can be found naturally in *Bacillus* sp. However, *Bacillus* sp. produce low amount of CGTase. Thus, genetically modified *E. coli* can be used to solve the problem by producing high amount of CGTase. The drawbacks of CGTase production when using *E. coli* as a host is the occurrence of cell lysis. Hence, cell immobilization is an alternative way to reduce the cell lysis with high CGTase production. Adsorption is well known technique in cell immobilization. The advantage of adsorption technique is simple, direct contact between nutrients and matrix, and enhancing the cell stability with high yield of cell immobilization. Besides, kinetic study is also performed to understand the mechanism of adsorption reaction. The objectives of this study are to optimize the immobilization yield by manipulating the process parameters of the recombinant *E. coli* cells on the hollow fiber membrane via adsorption technique and to evaluate the performance of immobilized CGTase on the production of CD. The recombinant *E. coli* was immobilized on the hollow fiber membrane. The effect of contact time, temperature, agitation rate, pH and type of medium on the cell immobilization yield (cell concentration) was investigated by using one-factor-at-one-time (OFAT) method. The cell concentration by the immobilized cells showed 150 mg/ml under the conditions of 24 hr of contact time, 200 rpm and pH 7 at 30 °C by using Terrific broth. The significant of process parameters (temperature and contact time) on the immobilization yield was further optimized by using response surface methodology (RSM). Under the optimized conditions (20 hr of contact time and 35°C), ±227 mg/ml of cell concentration was recorded. The kinetic study of the adsorption of cell onto the hollow fiber membrane was also performed. Based on the correlation coefficient (R^2), the best-fitted model is the Pseudo second-order kinetics model (R^2 = 0.9515). Besides, the model was also fitted to Langmuir isotherm with the R^2 of 0.989. The effect of reaction parameters such as substrate concentration, pH and agitation rate on production of β-CD by the immobilized cells was determined. The result showed that at 6% of substrate concentration, pH 9 and 200 rpm of agitation rate, the highest CD production of ±7.8 mg/ml was produced. Then, at the optimized reaction conditions (200 rpm, pH 8.5 and 5.5% of substrate concentration), the highest CD production (±11.6 mg/ml) was recorded. For the reaction kinetic study, it showed that the V_{max} of both immobilized cells (±2.18 mg/ml.hr) and free cells (±2.25 mg/ml.hr) was almost similar. The K_m value for the immobilized cells (±5.14 mg/ml) was almost the same as the free cell (±5.42 mg/ml). The immobilized cells also could retain 24.4% from its initial activity even after 6 successful cycles. Thus, these findings showed that the immobilized recombinant *E. coli* on the hollow fiber membrane is a promising technique to produce high concentration of β-CD.

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