PRODUCTION OF XYLONIC ACID FROM XYLOSE USING RECOMBINANT *E. coli* BL21 (DE3): EFFECT OF MEDIUM REQUIREMENT

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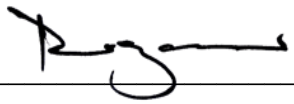

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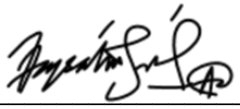

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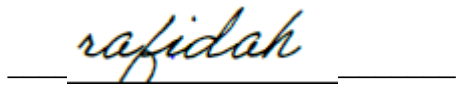

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ABSTRAK

Asid xilonik (XA) ialah asid organik yang bernilai tinggi dan mempunyai pelbagai aplikasi sama dengan asid gula lain seperti asid glukonik. Asid xilonik boleh digunakan dalam industri makanan, farmaseutikal dan kimia kerana ia mempunyai ciri-ciri yang sama seperti asid gula. Beberapa aplikasi termasuk berfungsi sebagai agen pengkompleks atau pengkelat, dalam penyebaran konkrit dan sebagai pelopor bagi sebatian seperti bersama poliamida, hidrogel dan 1,2,4-butanetriol. Dalam kajian ini, *E. coli* BL21 (DE3) rekombinan digunakan untuk menghasilkan asid xilonik dengan memanipulasi beberapa parameter seperti medium pertumbuhan; Luria Bertani (LB), Super Optimal Broth (SOB), Terrific Broth (TB), M9 Minimal Medium (M9), and 2×Yeast-Tryptone (2×YT), kepekatan nitrogen (kepekatan yis dan tripton), kepekatan pengaruh IPTG (0.01, 0.05, 0.1, 0.5 and 1.0 mM), masa pengaruhan (2, 3, 4, 5 and 6 jam), kepekatan substrat serta perbandingan substrat dari sumber yang berbeza. Sampel fermentasi telah dianalisis menggunakan kaedah *hydroxamate* dan DNS bagi mengenal pasti kepekatan XA dan xilosa yang terhasil setelah melalui proses fermentasi selama 24 jam, 200 rpm dan 37 °C. *E. coli* BL21 (DE3) rekombinan digunakan sebagai alternatif kepada pengeluar yang sedia ada, dengan keupayaan untuk meningkatkan penukaran xilosa kepada asid xilonik, pertumbuhan yang cepat, pengendalian yang mudah dan mantap. Dari kajian tersebut, keadaan kultur terbaik untuk penghasilan XA dalam *E. coli* BL21 (DE3) rekombinan adalah dalam medium Super Optimal Broth (SOB), kepekatan tripton 20 g/L, ekstrak yis 5 g/L, 0.5 mM IPTG dengan masa induksi 2 jam dan kepekatan D-xilosa 10 g/L. Kepekatan XA setinggi 8.69 g/L dari 10 g/L xilosa diperoleh ketika fermentasi dijalankan dalam keadaan kultur terbaik berbanding menggunakan hidrolisat pelepah kelapa sawit (OPF). Hasil semasa fermentasi menggunakan hidrolisat OPF menunjukkan kepekatan XA yang rendah. Ini disebabkan oleh keterbatasan metabolisme kecekapan xilosa yang mempengaruhi kadar pertumbuhan rekombinan *E. coli* BL21 (DE3). Semua keputusan yang diperoleh dari kajian ini menunjukkan kemungkinan memberangsangkan untuk penghasilan XA dari *E. coli* BL21 (DE3) rekombinan pada skala industri.

ABSTRACT

Xylonic acid (XA) is a valuable organic acid that has various applications similar to other sugar acids such as gluconic acid (GA). Xylonic acid can be used in food, pharmaceutical and chemical industries as it has similar characteristics as sugar acids. Some of the applications include the function as complexing agent or chelator, in dispersal of concrete and as precursor for compounds such as co-polyamides, hydrogels and 1,2,4-butanetriol. In this study, recombinant *Escherichia coli* (*E.coli*) BL21 (DE3) was used to produce xylonic acid by manipulating a few parameters such as growth medium; Luria Bertani (LB), Super Optimal Broth (SOB),Terrific Broth (TB), M9 Minimal Medium (M9), and 2×Yeast-Tryptone (2×YT), nitrogen sources (yeast and tryptone concentration), inducer of isopropyl ß-D-1-thiogalactopyranoside (IPTG) concentration (0.01, 0.05, 0.1, 0.5 and 1.0 mM), induction time (2, 3, 4, 5 and 6 h), substrate concentration as well as comparison of substrate from difference sources. Fermentation samples were analysed using hydroxamate method and DNS method to determine XA and xylose concentration from fermentation process at 24 h, 200 rpm and 37 °C. The recombinant *E.coli* BL21 (DE3) was exploited as an alternative to the existing producers, with the ability to enhance the conversion of xylose to xylonic acid, fast growth, easy to handle and robust. From the study, the best culture conditions for XA production in recombinant *E.coli* BL21 (DE3) was achieved in Super Optimal Broth (SOB) medium, 20 g/L of tryptone, 5 g/L of yeast extract, 0.5 mM of IPTG with 2 h induction time and 10 g/L of D-xylose as substrate. Concentration of XA as high as 8.69 g/L from 10 g/L xylose was obtained when fermentation was governed under the best culture conditions when comparing with oil palm frond (OPF) hydrolysate. The result during the fermentation of OPF hydrolysate shows low concentration of XA. This is due to the limitation of xylose efficiency metabolism which affected the growth rate of recombinant *E. coli* BL21 (DE3). These results suggest a promising industrial-scale production of XA from recombinant *E. coli* BL21 (DE3).

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