OPTIMIZATION OF XYLONIC ACID PRODUCTION USING RECOMBINANT *E. coli* BL21 (DE3) WITH INSERTED GENE FROM *Ralstonia pickettii*

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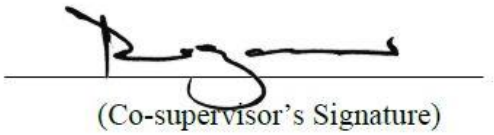

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ABSTRAK

Asid ᴅ-Xylonik (XA) adalah kompaun asid gula lima karbon yang telah tersenarai dalam senarai teratas 30 bahan kimia dari biomas dengan nilai tambahan yang mempunyai pelbagai potensi. XA mempunyai ciri-ciri seakan sama dengan asid glukonik (GA) yang digunakan dalam pelbagai industri seperti industri makanan, pembinaan dan tekstil. Walaubagaimanapun, penghasilan GA menggunakan glukos sebagai substrat menjadikan penghasilannya bersaing dengan penghasilan makanan. Harga glukos yang semakin meningkat di pasaran telah menjadikan XA yang dihasilkan meggunakan karbohidrat bukan makanan sebagai pengganti yang lebih bernilai dan lebih murah berbanding GA. Dewasa ini, XA dihasilkan melalui penukaran mikrob yang telah diolah secara genetik berbanding hanya pengekstrakan XA dari proses oksidasi xylos yang terjadi secara semulajadi. Namun begitu, penghasilan XA sehingga kini belum dihasilkan pada skala besar oleh kerana beberapa limitasi yang dihadapi oleh industri berasaskan bio seperti kadar penghasilan XA yang rendah dan sesetengah spesis bakteria yang menghasilkan XA memerlukan medium yang rumit. Dalam kajian ini, kaedah satu-faktor-pada-satu-masa (OFAT) dan reka bentuk komposit pusat (CCD) menggunakan perisian Design Expert telah diadaptasikan bagi mengkaji dan mengoptimakan kesan suhu, pH awal medium dan kadar pengocakan terhadap penghasilan XA daripada *E. coli* BL21 (DE3) rekombinan, diikuti dengan kajian kinetik menggunakan persamaan Leudeking-Piret untuk membanding dan menilai penghasilan XA dari proses penapaian di dalam kelalang goncang dan bioreaktor 2 L. Dalam kajian ini, suhu, pH awal medium dan kadar pengocakan telah dipelbagaikan dari 25°C hingga 40°C, pH 5.5 hingga pH 8.5 dan 50 rpm hingga 250 rpm. Manakala bagi proses pengoptimuman, parameter ditetapkan pada 35°C hingga 39°C, pH 6.5 hingga pH 7.5 dan 150 rpm hingga 250 rpm, yang menjana sebanyak 17 eksperimen termasuk tiga titik tengah. Sampel penapaian telah dianalisis menggunakan kaedah Hydroxamate dan DNS bagi mengenal pasti kepekatan XA dan xylos yang terhasil. Proses OFAT mencatatkan kepekatan XA tertinggi (9.82 ± 0.22 g/L) pada 37°C, pH 7 dan 200 rpm. Proses optimasi pula memberikan model kuadratik yang bersesuaian dengan data eksperimen dengan nilai R dan R^2 masing-masing ialah 0.9661 dan 0.9333. Parameter optimum bagi penghasilan XA adalah 36.8°C, pH awal medium 6.8 dan kadar pengocakan 208 rpm. Kepekatan XA sebanyak 11.15 ± 0.80 g/L telah diperolehi apabila proses penapaian dijalankan menggunakan parameter optimum. Semasa proses penapaian di dalam bioreaktor menggunakan parameter optimum, kepekatan XA hanya mencapai 6.89 g/L XA daripada 10 g/L xylos. Ini menunjukkan penurunan kadar XA sebanyak 24% yang dihasilkan di dalam bioreaktor berbanding di dalam kelalang goncang. Kadar pertumbuhan sel *E. coli* BL21 (DE3) rekombinan dan penghasilan XA yang lebih tinggi telah diperolehi daripada penapaian di dalam kelalang goncang iaitu $0.273h^{-1}$ kadar pertumbuhan sel dan 9.06 g L^{-1} kepekatan XA. Kajian kinetik menggunakan persamaan Leudeking-Piret menunjukkan bahawa XA adalah produk yang berkait dengan kadar pertumbuhan sel. Oleh itu, untuk meningkatan kadar kepekatan XA yang terhasil daripada penapaian di dalam bioreaktor, kadar pertumbuhan sel *E. coli* BL21 (DE3) rekombinan juga harus ditingkatkan. Secara keseluruhannya, proses optimasi telah meningkatkan 13.5% kepekatan XA yang terhasil dari *E. coli* BL21 (DE3) rekombinan. Semua keputusan yang diperolehi dari kajian menunjukkan kemungkinan memberangsangkan untuk penghasilan XA dari *E. coli* BL21 (DE3) rekombinan dengan gene dari *Ralstoniaa pickettii* pada skala industri.

ABSTRACT

D-Xylonic acid (XA) is a five-carbon sugar acid compound that has made the list of top 30 value-added chemicals from biomass with promising potentials. XA have similar properties as gluconic acid (GA) which is used in many different fields such as food, construction and textile industries. Production of GA however uses glucose as substrate therefore making its production competes with food production. With the increasing glucose price in the market, XA that is produced from non-food carbohydrate would be a valuable and cheaper substitute to GA. In recent years, XA is produced through microbial conversion of genetically engineered microorganism rather than extracting naturally oxidized XA from xylose. However, production of XA is yet to be produced at an industrial scale as the bio-based industry is still facing certain limitations such as low XA yield, slow production rate and certain bacterial species that produce XA requires complex growth medium. In this study, one-factor-at-a-time (OFAT) and central composite design (CCD) using Design Expert Software were employed to screen and optimize the effect of temperature, initial pH of medium and agitation rate on XA production from recombinant *E. coli* BL21 (DE3) fermentation in shake flask, followed by kinetic study using Leudeking-Piret equation to compare and evaluate XA production in shake flask and 2 L bioreactor fermentation. In screening, temperature, initial pH of medium and agitation rate were varied from 25°C to 40°C, pH 5.5 to pH 8.5 and 50 rpm to 250 rpm respectively. Meanwhile in optimization, process parameters were set at 35°C to 39°C, pH 6.5 to pH 7.5 and 150 rpm to 250 rpm which generated a total of 17 experiments with three centre points. Fermentation samples were analyse using Hydroxamate method and DNS method to determine XA and xylose concentration respectively. OFAT results show that the highest concentration of XA (9.82 ± 0.22 g/L) was obtained at 37°C, pH 7 and 200 rpm. Optimization results show that the developed quadratic model is fitted with the experimental data with R and R^2 value of 0.9661 and 0.9333 respectively. The optimize condition for XA production were 36.8°C, initial pH of 6.8 and the agitation rate of 208 rpm. Concentration of XA as high as 11.15 ± 0.80 g/L was obtained when fermentation was governed under the optimize culture conditions. During fermentation in bioreactor using optimized parameters, XA production reduces to 6.89 g/L XA from 10 g/L xylose. This shows a 24% reduction of XA produced from fermentation in bioreactor compared to shake flask. Higher specific growth rate of recombinant *E. coli* BL21 (DE3) and higher concentration of XA was obtained by fermentation in shake flask which is 0.273 h^{-1} and 9.06 g L^{-1} XA respectively. The kinetic study using Leudeking-Piret equation illustrates that XA is growth-associated product. Hence, to increase concentration of XA in bioreactor fermentation, recombinant *E. coli* BL21 (DE3)'s growth rate must also be increase. Overall, optimization process for temperature, pH and agitation rate had further increased XA from recombinant *E. coli* BL21 (DE3) by 13.5% higher compared to OFAT process. These results suggest a promising industrial-scale production of XA from recombinant *E. coli* BL21 (DE3) with inserted gene from *Ralstonia pickettii*.

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