PRODUCTION OF XYLOOLIGOSACCHARIDES FROM LIGNOCELLULOSIC BIOMASS USING ENZYMATIC HYDROLYSIS

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SUPERVISOR'S DECLARATION

We hereby declare that We have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Doctor of Philosophy.

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I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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ABSTRAK

Xylooligosaccharides (XOS) menjadi perhatian di kalangan penyelidik baru-baru ini kerana sifatnya yang mengandungi prebiotik. Pemprosesan XOS untuk tujuan penjualan bergantung kepada kanji sebagai sumber bahan mentah. Walaubagaimanapun, pengeluaran XOS daripada sumber tanaman komoditi telah dipertikaikan kerana ia meningkatkan harga pasaran XOS disebabkan sumber bahan mentah yang terhad. Sehubungan itu, biomas lignoselulosa dilihat sebagai sumber terbaik untuk menggantikan tanaman komoditi di dalam pengeluaran XOS disebabkan ia telah terbukti mengandungi kandungan xylan yang tinggi di mana boleh menghasilkan bahan kimia yang lain. Di dalam kajian ini, tiga jenis biomas lignoselulosa digunakan yang terdiri daripada hampas pelepah kelapa sawit (OPFB), tandan buah kosong kelapa sawit (OPEFB) dan jerami padi (RS) telah digunakan untuk penghasilan XOS melalui hidrolisa enzimatik dengan menggunakan Cellic Htec2 enzim. Pada awal kajian, biomas lignoselulosa ini disaring dari segi komposisi bagi memilih bahan mentah yang terbaik untuk penghasilan XOS. Bagi tujuan ini, kondisi tetap di dalam pra-rawatan telah digunakan di mana 10% bahan mentah direndam selama 12 jam di dalam 0.1% asid nitrik (NHO_3) yang dilakukan di dalam kukusan air dengan suhu ditetapkan pada 60 ℃. Dari data yang diperolehi, OPFB dikenalpasti sebagai bahan terbaik untuk digunakan dalam pemprosesan XOS bedasarkan kepada komposisi pra-rawatan OPFB yang memberikan peratusan pemulihan xylan dan pembuangan lignin yang paling tinggi berbanding dengan OPEFB dan RS. Selepas itu, kondisi pra-rawatan untuk OPFB telah dikaji melalui gerak balas permukaaan (RSM) dimana suhu, masa, kepekatan asid, kocakan dan kepekatan substrat dinilai semasa proses penyaringan, sementara suhu dan kepekatan asid telah menjadi factor terpilih untuk proses pengoptimuman. Hasil penemuan yang telah diperolehi menunjukkan bahawa perlaksanaan pra-rawatan sederhana iaitu dijalankan pada 42 °C dengan 0.02% kepekatan asid telah meningkatkan kandungan xylan sebanyak lebih kurang 4% (daripada 27.63% semasa penyaringan kepada 28.74% dalam pengoptimuman). Walaubagaimanapun, kandungan lignin lebih tinggi semasa proses pengoptimuman (17.94%) dibandingkan dengan kandungan lignin semasa proses penyaringan (15.03%). RSM juga telah digunakan dalam proses dilaksanakan untuk rawatan enzim bagi mencapai penghasilan XOS yang optima dimana lima factor telah dinilai semasa penyaringan (kepekatan substrat, dos enzim, suhu, masa dan kelajuan kocakan) dan dua factor yang memberi kesan paling tinggi terhadap rawatan enzim (kepekatan substrat dan dos enzim) dianalisis dengan lebih lanjut dalam kajian pengoptimuman. Untuk mencapai pemulihan xylan yang tinggi dalam OPFB, kondisi bagi pra-rawatan dikaji dengan menggunakan kaedah gerak balas permukaan (RSM). Hasil penemuan yang telah diperolehi menunjukkan bahawa perlaksanaan pra-rawatan sederhana iaitu dijalankan pada 42 °C dengan 0.02% kepekatan asid telah memaksimakan kandungan xylan sehingga 28.74% sementara meminimakan kandungan lignin menjadi 17.94%. RSM sekali lagi telah dilaksanakan tetapi untuk rawatan enzim untuk mencapai penghasilan XOS yang optima. Setelah RSM dijalankan, penghasilan XOS telah meningkat daripada 4.43 mg/mL di dalam kajian saringan, kepada 5.63 mg/mL di dalam kajian pengoptimuman. Hasil XOS tertinggi telah diperolehi apabila kajian dijalankan menggunakan 5% bahan mentah dengan dos enzim sebanyak 420 U/mL. Proses rawatan enzim telah disiasat dengan menggunakan persamaan Prout-Tompkins yang telah diubahsuai. Model ini boleh menggambarkan bagaimana proses rawatan enzim berlaku berdasarkan kepada nilai faktor pra-eksponen yang mewakili ciri topokimia. Nilai ini menurun apabila penukaran xylan kepada XOS adalah pada tahap maksima. Ini menunjukkan bahawa kebolehaksesan tapak aktif juga menurun pada ketika itu. Daripada hasil kajian ini, penggunaan kondisi yang sederhana di dalam pra-rawatan asid nitrik boleh meningkatkan penghasilan XOS dengan penglibatan komersial enzim Cellic Htec2 yang mana di samping itu dapat mengurangkan kos pengeluaran dengan menggunakan enzim ini. Kajian ini penting bagi mengenalpasti sisa pertanian kos rendah yang mempunyai potensi bagi menghasilkan produk kimia terutama XOS yang mana boleh diaplikasikan dalam industry pemakanan dan farmasi.

ABSTRACT

Xylooligosaccharides (XOS) has become the center of attention among researchers recently due to its properties that possess the prebiotic effect. The industrial XOS production mainly relies on starch as source of xylan. Nevertheless, the sustainability production of XOS from commodity crop has been disputed since it triggers the increment of XOS market price due to the scarcity of raw material supply which mainly came from corncob. Corresponding to this, lignocellulosic biomass is notified as the best candidate to substitute commodity crop as raw material in XOS production since this material has been proven having high xylan content that can be converted into other value-added chemicals. This present study used three types of lignocellulosic biomass namely oil palm frond bagasse (OPFB), oil palm empty fruit bunch (OPEFB) and rice straw (RS) for XOS production via enzymatic hydrolysis using commercial Cellic Htec2 enzyme. At the beginning of this study, all raw materials were screened in terms of compositions to select the best material for XOS production. For this purpose, fixed pretreatment condition was employed where the solid was soaked in 0.1% nitric acid (NHO_3) with 10% solid loading in water bath with temperature maintained at 60 ℃ for 12 h and revealed that OPFB was the best candidate for XOS production due to the highest enhancement of xylan yield and lignin removal after pretreatment. The pretreatment conditions of OPFB then studied via response surface methodology (RSM) where temperature, time, acid concentration, agitation and substrate loading were evaluated during screening, whilst temperature and acid concentration being the selected factors for optimization. The findings indicated that the implementation of nitric acid pretreatment at 42 °C with 0.02% acid has increased the xylan content by about 4% (from 27.63% during screening to 28.74% in optimization). However, lignin content was identified higher at this condition (17.94%) compared to amount of lignin from the screening study (15.03%). RSM was also employed in the enzymatic hydrolysis to achieve the optimum XOS production in which five factors were assess in screening (substrate loading, enzyme dosage, temperature, time and agitation speed) and two most contributed factors from screening study (substrate loading and enzyme dosage) were further analysed in optimization study. From the results, XOS production was increased from 4.43 mg/mL in screening study, to 5.63 mg/mL in optimization study. The maximum XOS acquired during optimization when the sample was reacted with 420 U/mL enzyme dosage using 5% (w/v) solid loading. The performance of enzymatic hydrolysis process in XOS production was investigated using Modified Prout-Tompkins equation. The model reflects the heterogenous behavior of the process through pre-exponential factors that represents the topochemical characteristic which decreased at maximum xylan conversion. This showed that accessibility of active site also decreased at this point. Overall, the finding of this study suggested that application of mild nitric acid pretreatment can improve production of XOS with the involvement of commercial Cellic Htec2 enzyme which consequently reduce the production cost with the usage of this enzyme. This study is important to discover the potential of low-cost agricultural waste for production of value-added product especially XOS that can be useful in food and pharmaceutical industries.

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