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PROCESS DEVELOPMENT ON SIMULTANEOUS SACCHARIFICATION AND FERMENTATION (SSF) OF *Jatropha curcas* SEED CAKE FOR BIOETHANOL PRODUCTION

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Lignocellulosic biomass or plant biomass is a highly latent source of agricultural waste, which can be utilised for the commercial production of bioethanol. Among the lignocellulosic biomass, *Jatropha curcas* has, therefore, been an attractive choice for a biofuel plant as a result of its unique characteristics. Nonetheless, lignocellulosic biomass has a rigid carbohydrate polymer matrix, which is structurally cross-linked and bonded to lignin. Hence, a pre-treatment is necessary to break the bond and increase the enzymes' accessibility to cellulose. However, pre-treatment is one of the most expensive procedures across the production of bioethanol. Therefore, the present study has attempted to eliminate the pre-treatment process and generate ethanol directly from the seed cake, which is obtained via post-ultrasonic assisted reactive biodiesel extraction. Additionally, the Van Soest characterisation method along with SEM and XRD analyses were utilised to evaluate the probability of an untreated *J. curcas* seed cake as bioethanol feedstock. Results depicted that the delignification process had occurred (+ 15% lignin reduction), and the total number of carbohydrate residual (12.13 % cellulose and 14.45% hemicellulose) was comparable to other studies. The delignification was further supported by the SEM and XRD analyses. Next, the objective focused on the production of bioethanol from the seed cake via SSF with three microbial fermenter species: *Saccharomyces cerevisiae, Candida glabrata*, and *Escherichia coli* K011. Then, the HPLC analysis was used to determine

the number of sugars and ethanol in the system. All three microbes showed potential in the production of bioethanol, and *S. cerevisiae* had specifically highest yield of ethanol (58.45%). As the production of ethanol was relatively low, Tween 20 was used to increase the rate of hydrolysis in an attempt to achieve a higher yield of ethanol. Nonetheless, the experiment revealed an exciting outcome when Tween 20 reacted differently with different microbial strains. Only *E. coli* showed an increment in ethanol yield (from 18.96% to 28.58% of ethanol yield) after the surfactant was added into the system. Other than that, the potential formation effects of the by-products and lignin inhibitory were also evaluated in this study. Five compounds such as acetic acid, lactic acid, levulinic acid, xylobiose, and xylotriose were detected in SSF with the presence of all three microbes. Xylobiose was the highest by-product concentration found in all three microbes with a concentration value of 3.37 g/L (*S. cerevisiae*), followed by *C. glabrata* with 4.47 g/L, and *E. coli* with 5.00 g/L. On the contrary, the lignin inhibitory study showed that when a higher concentration of *J. curcas* was added into the SSF system, it eventually slowed down the SSF rate of the microbes' commercial glucose and ethanol production. A 5% commercial glucose with an addition of 0% *J. curcas* concentration, recorded an ethanol value of 22.93 g/L for *S. cerevisiae*, 32.63 g/L for *C. glabrata*, and 5.71 g/L for *E. coli*. However, when 5% of *J. curcas* were added into the 5% of commercial glucose, the concentration reduced to 4.29 g/L, 29.09 g/L, and 0.84 g/L respectively. All the objectives were successfully achieved and it was proven that *J. curcas* seed cake can produce ethanol without the process of pre-treatment.

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PENAMBAHBAIKAN PROSES HIDROLISIS DAN PENAPAIAN SECARA SERENTAK (SSF) TERHADAP SISA *Jatropha curcas* BAGI PENGHASILAN BIOETANOL

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Bio-jisim berlignosellulos seperti sisa daripada hasil pertanian ialah salah satu sumber berpotensi tinggi untuk menghasilkan bio-ethanol. Di antara sisa pertanian tersebut, *Jatropha curcas* telah menerima perhatian yang luar biasa kerana ciri-cirinya yang unik dan sesuai digunakan dalam penghasilan bio-ethanol. Namun, disebabkan bio-jisim ini mempunyai struktur polimer karbohidrat yang berselirat dan berantai dengan lignin, proses pra-rawatan diperlukan bagi memutuskan rantaian tersebut lantas meningkatkan kebolehcapaian enzim kepada struktur sellulos. Pun begitu, proses pra-rawatan ini adalah salah satu proses yang termahal di dalam penghasilan bio-ethanol. Maka, kajian ini dijalankan bertujuan untuk membuang proses pra-rawatan tersebut dengan cara menggunakan terus sisa *Jatropha curcas* yang terhasil selepas daripada proses pengekstrakan biodiesel yang menggunakan kaedah ultrasonik. Potensi sisa *Jatropha curcas* yang tidak dirawat sebagai sumber bio-ethanol dikaji dengan menggunakan kaedah pencirian van Soest dan disokong dengan analisis SEM dan XRD. Hasil kajian menunjukkan telah berlakunya proses penyahlignin sebanyak $\pm$ 15% dan masih terdapat baki sisa karbohidrat sebanyak 12.15% sellulos, 14.45% hemisellulos. Jumlah sisa karbohidrat ini selari dengan beberapa dapatan kajian lain. Kejadian penyahlignin juga disokong dengan analisis SEM dan XRD. Seterusnya, objektif kedua dijalankan untuk mengkaji penghasilan bio-ethanol daripada sisa Jatropha dengan menggunakan tiga spesis mikrob iaitu: *Saccharomyces cerevisiae,*

Candida glabrata dan *Escherichia coli* K011. Analisis HPLC digunakan untuk mengesan jumlah penghasilan ethanol dan gula selepas proses hidrolisis dan penapaian sisa *J. curcas*. Ketiga-tiga mikrob menunjukkan kebolehan menghasilkan etanol daripada sisa tersebut dan *S. cerevisiae* mencatatkan jumlah penghasilan yang tertinggi (58.45%). Disebabkan penghasilan ethanol yang agak rendah, Tween 20 digunakan di dalam kajian ini untuk menigkatkan kadar hidrolisis seterusnya mencapai penghasilan etanol yang lebih tinggi. Menariknya, setiap jenis mikrob memberikan tindak balas yang berbeza apabila ditambah Tween 20 ke dalam system SSF dan hanya *E. coli* menunjukkan peningkatan dalam penghasilan etanol (dari 18.96% kepada 28.58%). Penghasilan produk sampingan dan kesan penghalangan daripada lignin turut dikaji keatas ketiga-tiga mikrob. 5 produk sampingan teriri daripada asid asetik, asid laktik, asid levulinic, "xylobiose" dan "xylotriose" dikesan wujud sewaktu proses SSF untuk menghasilkan bio-etanol bagi ketiga-tiga mikrob. Xylobiose adalah produk sampingan yang mencatatkan bacaan tertinggi dalam ketiga-tiga mikrob dengan kepekatan sebanyak 3.37 g/L (*S. cerevisiae*), 4.47 g/L (*C. glabrata*) dan 5.00 g/L (*E.coli*). Manakala, kajian kesan penghalangan lignin menunjukkan semakin tinggi kandungan *J. curcas* yang digunakan, semakin perlahan kadar penggunaan glukosa komersial bagi ketiga-tiga mikrob. Pada set eksperimen 5% glukosa dicampur dengan 0% sisa *J. curcas*, bacaan ethanol berada di paras 22.93 g/L, 32.63 g/L dan 5.71 g/L bagi *S. cerevisiae, C. glabrata* dan *E. coli*. Walau bagaimanapun, dalam set eksperimen yang lain, apabila 5% *J. curcas* ditambah ke dalam 5% glukosa, bacaan ethanol menunjukkan pengurangan kepada 4.29 g/L, 29.09 g/L and 0.84 g/L bagi mikrob-mikrob tersebut. Tuntasnya, kesemua objektif telah berjaya dijalankan dan terbukti bahawa sisa *J. curcas* boleh menghasilkan etanol tanpa proses pra-rawatan.