شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

بسم الله الرحمن الرحيم

HANAA ALY

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جامعة عين شمس

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قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات

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HANAA ALY

Butanol production by *Clostridium* species from macroalgal substrate

Presented by

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Summery

- Our study focused on the importance of macroalgal feedstock especially *Sargassum latifolium* for biofuels production especially biobutanol production.
- Cellulose, hemicellulose, lignin, and minerals contents for *Sargassum latifolium* were determined. The results revealed that S. *latifolium* has high carbohydrates content (40.84%) compared with lignin content (8.95%) which makes it easy for pretreatment, hydrolysis, and production of high total reducing sugars. In addition to, *S. latifolium* was rich with minerals (P, Na, K, Mg, Cd, Zn, Cu, Ni, Fe, and Pb).
- Our substrate (S. *trifolium)* was cut and milled before hydrolysis process.
- Different hydrolysis processes (chemical and biological) were carried out. The chemical hydrolysis process by sulfuric acid which gave the greatest TRS (7.68 g/L) compared to hydrochloric acid (5.48 g/L). Our results showed that the chemical hydrolysis process was more effective for the small size biomass compared to the big one.
- The combination between the base treatment (KOH) and the acid hydrolysis (HCl) was more effective than combination between NaOH treatment and H_2SO_4 hydrolysis.
- The biological hydrolysis process was carried out by using different fungal strains (*Trichoderma viride* F94; *T. reesei* F68; *Aspergillus terreus* F98, *A. niger* F66). *Trichoderma viride* F94 was the most promising fungal strains for total reducing sugars production than other fungal strains.

- The combination between base treatment (KOH) and fungal hydrolysis (*Trichoderma viride* F94) yield a high TRS than fungal (*Trichoderma viride* F94) hydrolysis without base treatment.
- Finally, the fungal pretreatment followed by acid hydrolysis was evaluated in our study. We found that fungal pretreatment with *Trichoderma viride F94* followed by HCl hydrolysis produce ahigh TRS.
- In this study, different sugars (glucose, arabinose, mannitol, and xylose) released from different hydrolysis process were determined by using the HPLC analysis. Our results showed that the maximum total fermentable sugars were produced from H_2SO_4 only (12.65 g/L) and the combination between fungal treatment (*T. viride F94*) and HCl hydrolysis (11.41 g/L). Moreover, The HPLC analysis for H_2SO_4 hydrolysate demonstrated that this hydrolysate contains a high glucose concentration than mannitol concentration and small concentration of xylose and arabinose. On the other hand, the combination between fungal treatment (*T. viride F94*) and HCl hydrolysis produced ahigh mannitol concentration than glucose concentration followed by small concentration of xylose and arabinose.
- These hydrolysates were rich with different fermentable sugars especially glucose and mannitol which were suitable for fermentation process. So that we used these hydrolysates (H_2SO_4 only and the combination between fungal treatment (*T. viride-F94*) with HCL hydrolysis) for further TRS optimization and ABE fermentation process.
- Different parameters (acid concentration, substrate concentration, temperature, and reaction time) were studied to enhance the

hydrolysis process by using OFAT method, the optimum obtained conditions for maximum TRS production were 6% (v/v) acid concentration, 90 g/L S. *latifolium* at 120^{o} C for 20 min.

- Thirty- three anaerobic mesophilic isolates were isolated from five cultivated crop soil. Only thirteen isolates gave gas production, acetone positive test, gram and spore positive test.
- Evaluation the ability of the thirteen *Clostridium* isolates for biobutanol and ABE production from different fermentation medium (the standard medium (T6) and two selected hydrolysate media (sulfuric acid and the combination between *T. viride* F-94 + HCl hydrolysate).
- The maximum biobutanol and ABE solvents from the fermentation of T6 Medium and two selected hydrolysates (sulfuric acid and the combination between *T. viride* F94 + HCl hydrolysate) by HG1 strain were 4.471&8.604; 3.743&6.175; 2.233&3.265 g/L, respectively.
- The most promising strain (HG1) for biobutanol and ABE production was identified as *Clostridium acetobutylicum* MN871409 by using Butanol dehydrogenase (bdhA) specific gene as molecular marker gene.
- Different fermentation parameters affecting on biobutanol and ABE production of sulfuric acid hydrolysate as inoculum size, incubation period and pH was determined by using CCD design of RSM, ANOVA test demonstrated that inoculum size and incubation period were effective on biobutanol and ABE production than pH. The maximum biobutanol and ABE produced from the fermentation of sulfuric algal hydrolysate by *Clostridium*

acetobutylicum MN871409 at 8.63 (v/v) inoculum size, after 7.0 days at 6.5 pH were 8.60 and 10.80 g/L.

ABSTRACT

Macroalgae with several species are an abundant, carbon-neutral renewable resource and rich in carbohydrates which makes it suitable for biobutanol production. Recently, due to advantages of biobutanol as a liquid biofuel, it can be used as a substitute for gasoline and diesel. Many researches mentioned that, different *Clostridium* species are able to use fermentable sugars for production of biobutanol from different biomasses through ABE (Acetone- Butanol- Ethanol) fermentation process. In this study the brown macroalga (*Sargassum latifolium*) was used as carbon source for biobutanol production. Thirty-three anaerobic mesophilic isolates were isolated on RCM medium from five soil cultivated with different crops. Only thirteen spores forming, mesophilic, anaerobic *Clostridium* isolates used for the fermentation process. 90 g/L from *S. latifolium* were hydrolyzed by sulfuric acid (6 % (v/v)) followed by thermal pretreatment at 120°C for 20 minutes. The maximum total sugars produced from sulfuric acid hydrolysis of *Sargassum latifolium* at optimum conditions were 24.10 g/L. These fermentable sugars were fermented by *Clostridium* isolates. The most promising isolate (HG1) which produce the highest butanol and ABE level (8.60 and 10.80 g/l, respectively) at optimum fermentation conditions (inoculum size 8.63 %, incubation period 7.08 days, and pH 6.47) was identified according to Butanol dehydrogenases (bdhA) gene analysis as *Clostridium acetobutylicum* MN871409. This thesis emphasizes the importance of macroalgae (*S. latifolium)* as a renewable feedstock for the production of fermentable sugars which are suitable for biobutanol production.

Key words: Macroalgae; *Sargassum latifolium*; hydrolysis; *Clostridium* Isolation; ABE fermentation; Biobutanol.

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