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Research paper

Integration of broth extraction and recycling scheme to bio-ethanol production from water hyacinth

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Abstract

Bioethanol production from water hyacinth (Eichhornia crassipes) has been progressively studied these recent years. However, the technology was still inapplicable because of low final ethanol concentration which leads to uneconomic process. Since acquiring broth with high ethanol concentration by common processing scheme is quite unlikely for this type of biomass, other approaches must be taken. Combining several strategies ever studied, this study employed salt enhanced extraction and direct broth recycling as the main strategies to improve the final concentration. The aim of this study, thereafter, was to evaluate the compatibility and implications of these processes integration. In this research, pre-blended and dried water hyacinth was subjected to dilute acid pretreatment followed by enzymatic hydrolysis, fermentation, and extraction. Broth recycling was done by directly replacing a portion of enzyme with filtered broth. Results showed that extraction enhances ethanol production, while recycling trades ethanol yield for increase in level.

Keywords: *Bioethanol; Economic; Extraction; On-Site Enzyme; Recycling; Water Hyacinth.*

1. Introduction

Bioethanol has been successfully produced from water hyacinth. However, the technologies have yet to be applied due to economic issue. Generally, ethanol production from water hyacinth requires four steps: pretreatment, hydrolysis, fermentation, and purification. Much research has been focused on evaluating and modifying the first three steps, however till the time this paper was written, none has included modification of the last step into their study [1-18].

In many studies on ethanol production from water hyacinth, the final ethanol concentration spanned from 1.01 g/L to 16.9 g/L with most reaching below 4.5 g/L and those with higher end results usually employed pre-concentration or high biomass loading [3], [7], [10-12], [14-19]. Despite the success in producing the ethanol, the concentrations were still considered unfavorable for large scale production. This is because distillation of ethanol is only economical if the ethanol concentration is above 40 g/L [20]. However, arriving at such high concentration will require that the fermentation is carried out at high biomass suspension which has negative impacts on hydrolysis and also leads to high energy input for efficient mixing [21]. Another way to reach such concentration is by pre-concentration of the broth as conducted by Takagi et al. [16], but this method also leads to higher energy input.

Another approach, which is adopted in this study, is to replace the distillation with extraction and integrate recycling into the process line. Despite possible problems occurring from these changes, a number of studies around these subjects have produced encouraging results. Among them were studies on the solvent selection [22-24], effects of extraction [25], [26], salt effects on equilibrium [27], [28], inhibition [29], non-sterile fermentation [30], and recycling of various portion of fermentation broth [31-33]. Combining the results of these studies, we acquired salt enhanced extraction and direct broth recycling as two new and potential strategies for better ethanol production. Nevertheless, directly implementing these processes without proper assessment is too risky.

Therefore, this paper focused on evaluating the compatibility of the aforementioned modifications on bioethanol production from water hyacinth and the implications that follow along.

2. Materials and experimental procedure

2.1. Material collection

Water hyacinth was collected from local ponds in University of Sumatera Utara, Medan, Indonesia. Saccharomyces cerevisiae (SC) and Ganoderma boninense (GB) were purchased from University of Sumatera Utara, Medan, Indonesia. Trichoderma reesei (TR), Aspergillus niger (AN) and Candida utilis (CU) were purchased from Bandung Institute of Technology, Bandung, Indonesia. Palm biodiesel was of industrial grade and all chemicals used were of analytical grade.

2.2. Preparation and storage of water hyacinth

Water hyacinth was chopped to pieces and the root was removed then, it was blended to slurry and filter-pressed to reduce water content. Afterwards, the filtered water hyacinth was dried further and stored in closed, separated container at 4 – 6°C.

2.3. Storage and inoculation of microorganisms

All microorganisms, except SC which was kept in granular form in closed container at 8°C, were grown in potato dextrose agar (PDA) at 20°C. Prior to usage, SC was warmed to 20°C for 30 mins, whilst TR, AN, and CU were inoculated at 20°C for 2 days (1 day for CU)

in liquid media containing 22% sucrose, 1% KH_2PO_4, and 1% $(NH_4)_2SO_4$ [34]. All procedures were done aseptically.

2.4. Enzyme production

Enzyme production was carried out in a 100-ml vial in which 10 g of water hyacinth (moisture adjusted to 70%) was mixed with Mendel Weber solution at a ratio of 3 ml to 1 g biomass. The mixture was autoclaved (121°C, 15 lb_f) for 15 minutes, and subsequently cooled down to 20°C. Inoculums of TR and AN (1.5 ml per 10 g biomass) were grown separately in the mixture and incubated at 20°C for 1 week. Enzymes were extracted by distilled water at a ratio of 4 – 5 ml per g biomass. The liquor was separated by centrifugation at 2,500 rpm and 4°C for 15 minutes. Supernatant from both cultures was then mixed at a ratio of 1:1 and stored in dark glass bottle at 4°C.

2.5. Pretreatment of water hyacinth

Water hyacinth was pretreated by dilute acid pretreatment (DAP), in which 1 g of dried water hyacinth was mixed with 20 ml of 2% (v/v) sulfuric acid, and autoclaved (121°C, 15 lb_f) for 1 hour, followed by neutralization with concentrated NaOH (5 – 10 M) to pH of 4 – 5 [35]. After pretreatment, samples were cooled down to 20°C.

2.6. Hydrolysis, fermentation and extraction of water hyacinth

The hydrolysis, fermentation and extraction were carried out simultaneously. After pretreatment, 30 ml enzymes, 0.3075 g (25 mM) $MgSO_4$, 0.5 g (1% w/v) granulated SC, 1 ml (2% v/v) CU inoculums, and 0 – 50 ml (ratio of 0 – 1) palm oil biodiesel was added. The mixture was fermented at 20°C for 24 hours. Afterwards, fermentation broth was separated from the solvent (palm oil biodiesel) and filtered. Both the solvent and the broth were stored in separate dark glass bottles and frozen.

2.7. Recycling of fermentation broth

Broth recycling was done by replacing a portion of enzymes with 0 – 30 ml of fresh fermentation broth which was prepared as previously described. The solvent ratio was fixed at 0.4 (equivalent of 20 ml palm oil biodiesel).

2.8. Characterization of enzyme

Crude enzyme was analyzed for its cellulase activity by CMC assay and the activity was expressed as FPU/ml [36].

2.9. Analysis of fermentation broth and solvent

Fermentation broth was analyzed for its sugar and ethanol content, pH, density, and viscosity. The solvent was analyzed for its ethanol content, density, and viscosity. Concentration of reducing sugar was analyzed by spectrophotometer UV-Visible (SHIMADZU 1800) using DNS method [37] and was expressed as equivalent glucose concentration against calibration curve. Ethanol concentration was analyzed by GC using static head space analysis [38] at adjusted salt concentration of 0.1 mM $MgSO_4$ against calibration curve. Iso-propanol was used as an internal standard. Density, viscosity, and pH were measured by using pycnometer, Oswald viscometer, and pH meter.

3. Results and discussion

3.1. Initial analysis

Two mixtures of enzymes were produced during experiment. The first mixture was found to have cellulase activity of 0.12 FPU/ml, while the second mixture had cellulase activity of 0.25 FPU/ml.

3.2. Effect of solvent ratio

Simultaneous ethanol extraction can increase ethanol production because mass transfer of ethanol out of the broth reduces ethanol inhibition [25], [26]. For these experiments, enzyme used had cellulase activity of 0.12 FPU/ml. Results of effect of solvent ratio on ethanol production are shown in fig.1. Results showed that solvent usage improved ethanol production. Rising solvent ratio also caused slight increase in the production, although it caused ethanol level of both the solvent and the broth to fall. The decrease was possibly due to dilution by increased solvent volume. On sugar level, no significant change was detected.

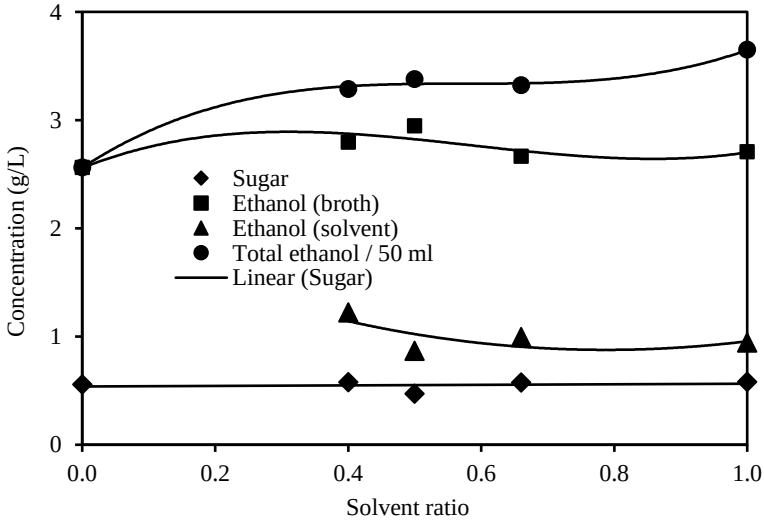

Fig. 1: Effect of Solvent Ratio on Ethanol Production.

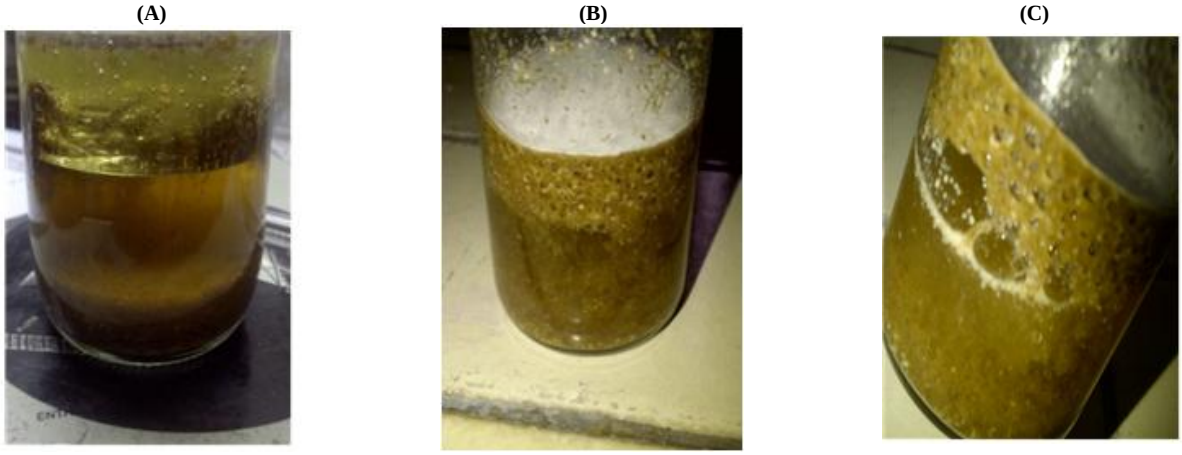

Fig. 2: Gathering of Substrate above Broth, with Solvent (A) and Without Solvent (B) and (C).

As palm biodiesel was used as solvent, problems might arise concerning the compatibility of the fermentation. Yet, the increase in ethanol production during these experiments verifies that palm biodiesel is non-toxic and non-inhibitory for the microorganisms in these fermentations. Direct observation also revealed that palm biodiesel did not form emulsions in fermentation broth. Among all components of palm biodiesel, methyl laurate is the only component with potential toxicity on yeasts [22-24]. However, the overall toxicity might be small because methyl laurate was present at low concentration in the palm biodiesel and addition of magnesium sulfate into the fermentation broth had reduced the solubility of all compounds in the biodiesel.

At solvent ratio of 0.5, broth ethanol level fluctuated higher than expectation while solvent ethanol level was lower than expectation. This phenomenon can be explained by looking at the broth-solvent interface in which substrate gathered and floated in-betweens (as pictured in Fig. 2), leading to disruption on the equilibrium. The floating of the solids might be due to gas discharge during fermentation.

3.3. Effect of recycling on ethanol production

In these experiments, enzyme used had cellulase activity of 0.25 FPU/ml. Results are shown in Fig. 3.

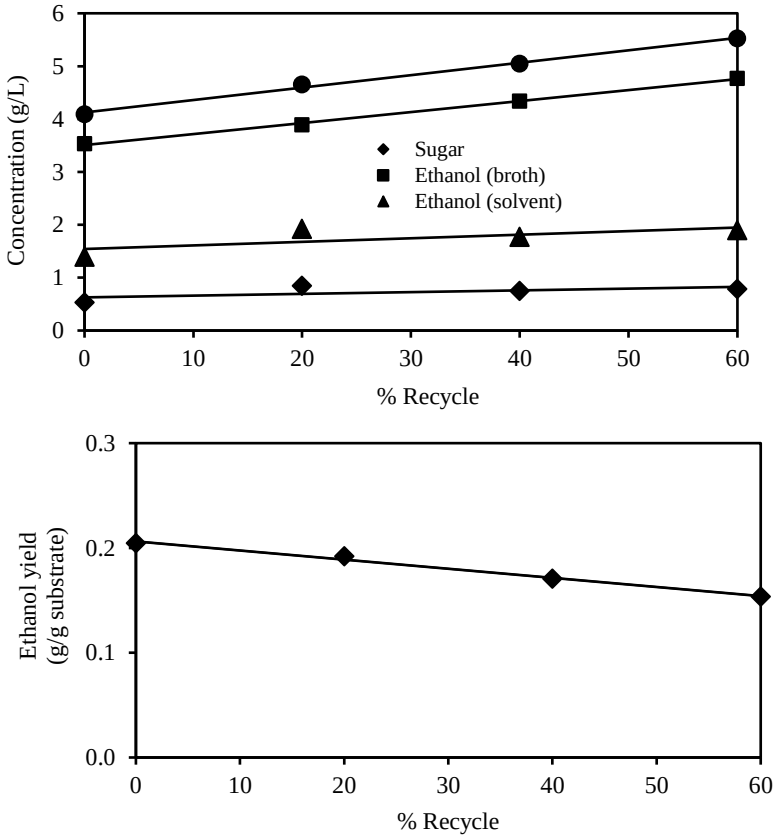

Fig. 3: Effect of Recycling on Ethanol Production.

Higher recycle volume raised ethanol concentration of both solvent and broth but lessened ethanol yield. This rise of ethanol concentration is expected since ethanol in fresh fermentation broth will accumulate with each recycling. On the other hand, the decrease in ethanol yield might be caused by cumulative inhibitory effects of accumulated substrates, products, and other inhibitors. This standpoint was supported by many reports which claimed that certain pretreatments, such as DAP, could generate various inhibitors [39]. Furthermore, yeasts are also inhibited by ethanol [40,41] and cellulase is inhibited by glucose [1,40]. The success in direct recycling of freshly fermentation broth without the need of additional sterilization procedure in this study also implies that non-sterile fermentation can be

applied to this method of ethanol production, although there might be some effects of such procedure as reported by Zaafouri et al. [42].

3.4. Rheological data of broth and solvent

Rheological data is important for design of bioreactor. In this study, the density and viscosity of both fermentation broth and solvent were measured. The data is displayed in Table 1. As outlined in Table 1, usage of solvent seems to cause subtle decline in broth density and viscosity. In contrast, recycling leads to marginal increase in density and viscosity of both broth and solvent. This was probably due to impurities accumulation.

Table 1: Density and Viscosity of Fermentation Broth and Solvent

Solvent ratio	% Recycle	Density (kg/m^3)		Viscosity (mPa·s)	
		Broth	Solvent	Broth	Solvent
0.00	0	1033.39	-	0.98490	-
0.40	0	1024.82	866.296	0.93696	5.03296
0.50	0	1021.68	864.322	0.92858	5.06891
0.66	0	1022.91	864.552	0.93805	4.96261
1.00	0	1024.00	864.785	0.92133	4.96038
0.40	0	1024.82	865.621	0.95101	4.97084
0.40	20	1029.58	873.082	0.94916	5.07647
0.40	40	1033.25	866.457	0.96384	5.10125
0.40	60	1036.79	866.800	0.97164	5.26084

To properly address the effect of solvent usage on broth rheological properties, additional rheological data of fermentation broth in previous study (a total of 13 data points with variations on $MgSO_4$ concentration, microbial choices, and fermentation duration; extraction and recycling procedures were not involved), was imported as in [35]. The fermentation broth in reference had average density of 1032.02 ± 0.75 kg/m^3 and average viscosity of 0.9849 ± 0.0093 mPa s (unpublished results). Meanwhile, when solvent was used with no recycling involved, fermentation broth had average density of 1023.35 ± 1.36 kg/m^3 and average viscosity of 0.9312 ± 0.0078 mPa s.

4. Conclusions

Results suggested that simultaneous extraction and direct broth recycling can be incorporated into ethanol production from water hyacinth without much technical difficulties. For extraction by palm biodiesel, this solvent did not pose serious treat on the process despite the low ethanol distribution coefficient. For direct broth recycling, this operation demonstrated high potency for manipulating the maximum obtainable ethanol concentration in exchange of the yield, and vice versa. Both procedures did not affect rheological properties of the broth. However, additional researches on the interrelated impacts of solvent ratio, $MgSO_4$ concentration, and recycle ratio on ethanol production are required to harvest the full benefits of this combination.

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