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Energy and Carbon Footprint Analysis of a 2,3-Butanediol integrated Oil Palm Empty Fruit Bunches Circular Biorefinery

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ABSTRACT

This study highlights the bio-based production of 2,3-butanediol (2,3-BDO) using a newly isolated strain for the bioconversion of organosolv-pretreated empty fruit bunches (EFB) of oil palm. The microbially produced 2,3-BDO has been considered similar in functionality and a more sustainable chemical than fossil-based chemical (1,4-BDO). As diol pretreated lignocellulosic biomass is hemicellulose-free and rapidly hydrolyzable, EFB was successfully converted into 40 g/L 2,3-BDO with 0.48 g/g-glucan (96% of the maximum theoretical yield). In addition, a comparative energy assessment revealed that 2,3-BDO bio-production consumed >50 MJ/kg energy from the biomass, which was equivalent to the fossil energy consumption of 1,4-BDO industrial production but 39% reduced GHG emissions than the conventional processes. This finding demonstrates the cost-effectiveness and eco-friendly features for 2,3-BDO production using waste biomass derived from the exiting industry. Principally, by recycling the produced 2,3-BDO for replacing the used pretreatment reagent (organosolv), the biorefinery can eventually become self-sustainable without the need of additional energy and chemicals.

Keywords: 2,3-Butanediol, organosolv-pretreatment, oil palm empty fruit bunches, simultaneous saccharification and fermentation, energy and carbon footprint.

NONMENCLATURE

Abbreviations	
1,4-BDO	1,4-Butanediol
2,3-BDO	2,3-Butanediol
GHG	Greenhouse gas emissions
FEC	Fossil energy consumption
EFB	Empty fruit bunches
SSF	Simultaneous saccharification and fermentation
SED	Substrate enzyme digestibility
HPLC	High performance liquid chromatography

1. INTRODUCTION

The increased population density, rapid industrialization, growing energy demand, fossil fuels depletion, and high GHG emissions, altogether have increased the attention to reduce the petroleum-based compounds via utilization of renewable energy. Butanediol is one of the commodity petrochemicals with diverse applications especially in polymer (butadiene monomer) and aviation industries (methyl ethyl ketone; MEK derivative) [1,2]. Two major isomers are commonly used in chemical processing i.e. 1,4-butanediol (1,4-BDO) and 2,3-butanediol (2,3-BDO). The schematic process flow of the petroleum and bio-based production processes have been demonstrated in **Fig. 1**. 1,4-BDO has a higher market value but the production process of this solvent is entirely dependent on fossil-based compounds [3]. In contrast, 2,3-BDO is a natural metabolite produced by many microorganisms during their carbon metabolic pathways. It is generally considered as a green product due to its biodegradability, hence promotes environmental sustainability.

For green BDO production, lignocellulosic biomass has been successfully utilized as a biorefinery feedstock. Oil palm empty fruit bunches (EFB) is one of the potential feedstocks due to its abundance and high fraction of lignocellulosic sugars (cellulose 24-35%, and hemicellulose 18-24%) [4]. However, the recalcitrant nature of EFB is quite high due to its 14-31% of lignin in the plant cell wall structure [4], which hinders the subsequent enzymatic saccharification. Pretreatment is essential to fractionate the lignin from the biomass and many different physical, chemical, biological, and enzymatic methods have been introduced to achieve various biorefinery targets, i.e. saccharification, fermentation, and lignin valorization [4,5]. Organosolv pretreatment is a promising method for biomass valorization. The organic solvents tend to extract lignin from lignocellulosic sugars and result in high-quality cellulose for subsequent biorefinery process

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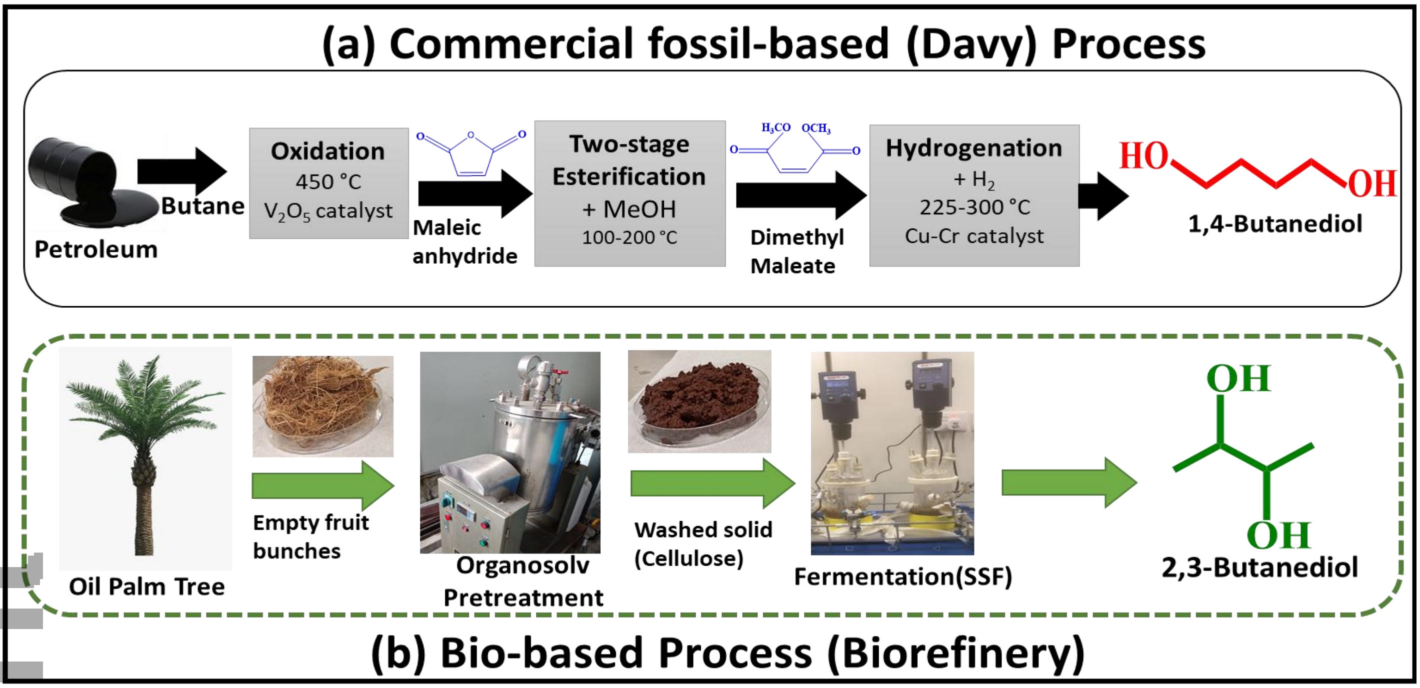

Fig.1. Schematic diagram of production of (a) commercial fossil-based 1,4-BDO and (b) bio-based 2,3-BDO.

[6,7]. For instance, ethanol was reported to recover >88% sugars from woody biomass [8]. Nonetheless, glycerol delignified only <30% of lignin due to its lipophilic compounds [9]. Whereas polyhydric alcohol i.e. butanediol (both 1,4-BDO and 2,3-BDO) fractionated lignocellulose into reactive lignin and cellulose with high structural integrity [10]. In addition, organosolv method produces less inhibitory compounds and provides the advantage of solvent recovery and recyclability in the pretreatment process [11].

In this study, diol-pretreatment was investigated to valorize oil palm EFB for subsequent use as a cellulosic substrate in 2,3-BDO fermentation. The main purpose was to obtain high EFB sugars through enzymatic hydrolysis for maximizing bioproduction of 2,3-BDO via SSF process. Furthermore, energy and carbon footprint analysis of the biological and conventional processes were critically evaluated. To our knowledge, this is the first study to compare the energy and carbon footprint of solely bio-based with commercial fossil-based BDO production.

2. MATERIAL AND METHODS

2.1 *Materials and chemicals*

Oil palm EFB was collected from the palm oil farms in Thailand. The commercial cellulase (Cellic® CTec2) enzyme was generously provided by Novozyme China. All other chemicals were procured from Sigma Aldrich (USA) and J&K Acros Organics (China).

2.2 *Diol pretreatment of EFB*

A modified organosolv pretreatment was performed using a customized rotating digester (Xian Yang Tong Da Light Industry Equipment Co. Ltd, China) according to our previous study [10]. Briefly, 65% of 1,4-BDO was added with an acid catalyst (15 mM H_2SO_4) to 1 kg EFB biomass at 1:7 solid/liquid ratio. The reaction conditions were 170°C for 20 min. After pretreatment the reactor was allowed to cool to 50°C and then the pretreated substrate was filtered to separate the solid and spent liquid. The solid fraction was washed three times to remove the residual solvents. The chemical composition of the substrate was determined according to the NREL protocol [12]. To compare, a dilute acid (DA) pretreatment was performed under similar conditions. The pretreatment efficiency was evaluated via enzymatic hydrolysis by adding 2% (w/v) washed solids, 50 mM acetate buffer, 7.5 FPU/g enzyme loading in a 500 mL flask, and incubated at 50 °C, 150 rpm for 72 h. Samples were subsequently collected after 6, 12, 24, 48 and 72 h for analyzing the released sugar in the mixture.

2.3 *Simultaneous saccharification and fermentation*

A newly isolated wild-type strain *Klebsiella pneumoniae* PM2 (MT422266) was employed in this study. Diol pretreated EFB (20%; dry wt. basis) was used as a substrate with 100 mL reaction volume in a fermentation vessel. For pre-hydrolysis, 15 FPU/g Ctec2 enzyme and acetate buffer pH-4.8 were added and incubated for 12 h at 50°C, 200 rpm. Afterwards, 10% (v/v) PM2 inoculum was seeded to the fermenter with 1% yeast extract and peptone and cultivated at 30 °C, initial pH 7, and 150 rpm agitation. Similar setup was run for glucose fermentation to compare the production performance with that of crude sugars (EFB biomass). During fermentations, small aliquots were periodically drawn for metabolites analysis via High performance liquid chromatography (HPLC).

2.4 Energy and carbon assessment

Overall process energy was assessed by determining the related energy/water footprints of all intermediate processes. For bio-based 2,3-BDO, consumption energy was the summation of energy input by pretreatment, milling, and product separation. For water footprint analysis, pretreatment process was the most consumable step which required around 7 L of water as a mixture of organosolvent for treating 1 Kg of EFB biomass [13]. For conventional 1,4-BDO production, input energies of oxidation, esterification, and hydrogenation units were calculated according to the commercial Davy process, described in [14,15]. Whereas the sole energy output was the purified butanediol. Carbon footprint analysis was calculated based upon the data sources available in literature (journal articles and patents) [14,16,17]. The functional unit was set at 1 kg of bio/fossil- based BDO.

3. RESULTS AND DISCUSSIONS

3.1 Effect of diol pretreatment

The physical and chemical changes of raw and pretreated EFB biomass are displayed in Fig.2. After diol pretreatment, hemicellulose (~98%), lignin (~60%) solubilized in the solvent and insoluble cellulose pulp (87%) was recovered in the solid fraction (**Fig.2a**). The high hemicellulose and lignin removal were the result of optimal acid charge (15 mM) with diol solvent which impeded lignin condensation [10]. Comparatively, DA pretreatment resulted in complete hemicellulose removal but lignin solubilization was constrained due to limited depolymerization [10]. Our data demonstrated that acid catalyzed-diol solvent was quite effective in obtaining high-quality cellulose by reducing the pretreatment complexity in the biorefinery process.

3.2 Enzymatic hydrolysis

To further assess the pretreatment efficiency on cellulose digestion, substrate enzyme digestibility (S.E.D) was calculated. **Fig. 2b** shows that almost 94% of the cellulose was converted into monomeric sugars within first 12 hours of the reaction using diol- pretreated EFB. The cellulose conversion was reached up to 98% till 72 h. Results revealed that cellulose accessibility was quite higher due to almost complete hemicellulose removal and 60% delignification. Higher S.E.D also reflected the low affinity of organosolv lignin adsorption at the active sites of enzymes, leading to improved lignocellulosic saccharification [18].

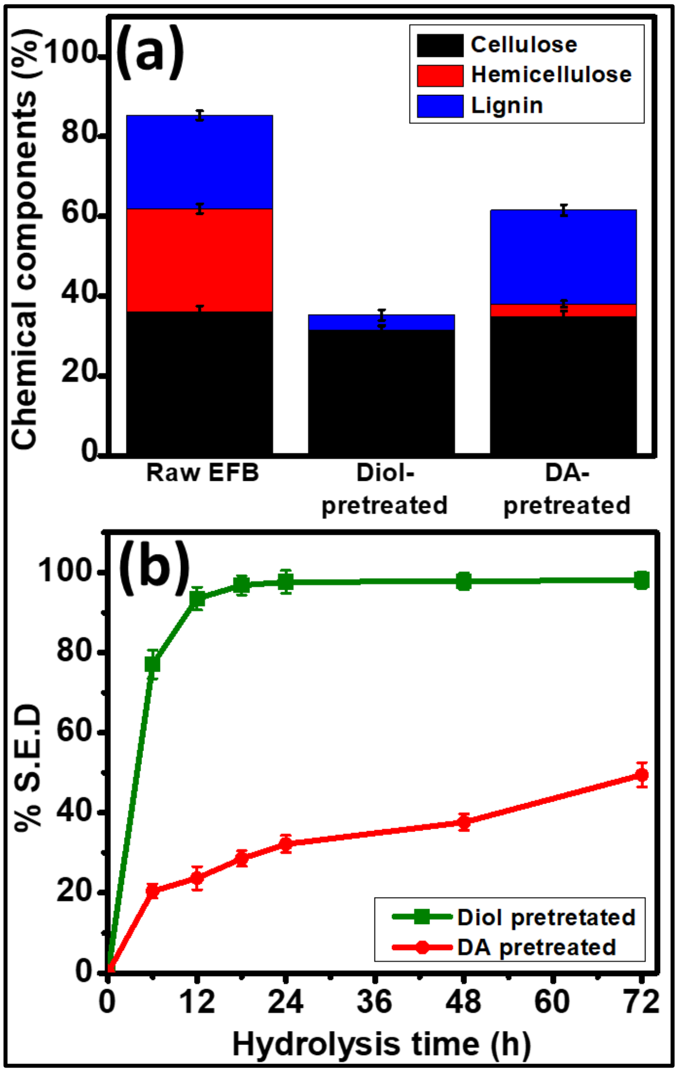

Fig. 2. Effects of diol pretreatment in comaprison with dilute acid (DA) pretreatmnet . (a) chemical composition of raw and pretreated EFBs (b) Substrate enzyme digestibility (S.E.D) of the diol and DA-pretreated EFB.

3.3 SSF for 2,3-BDO production

2,3-BDO production performance was evaluated by the newly isolated *K. pneumoniae* PM2 strain under two scenarios. Scenario-I depicted the utilization of pure sugars via batch fermentation, whereas scenario-II was conducted using diol-pretreated EFB via simultaneous saccharification and fermentation (SSF). Results in **Fig. 3a** display that the cells rapidly consumed >70% of glucose in just 24 h of cultivation, yielding 45g/L of 2,3-BDO, which lowered down along with the declining growth

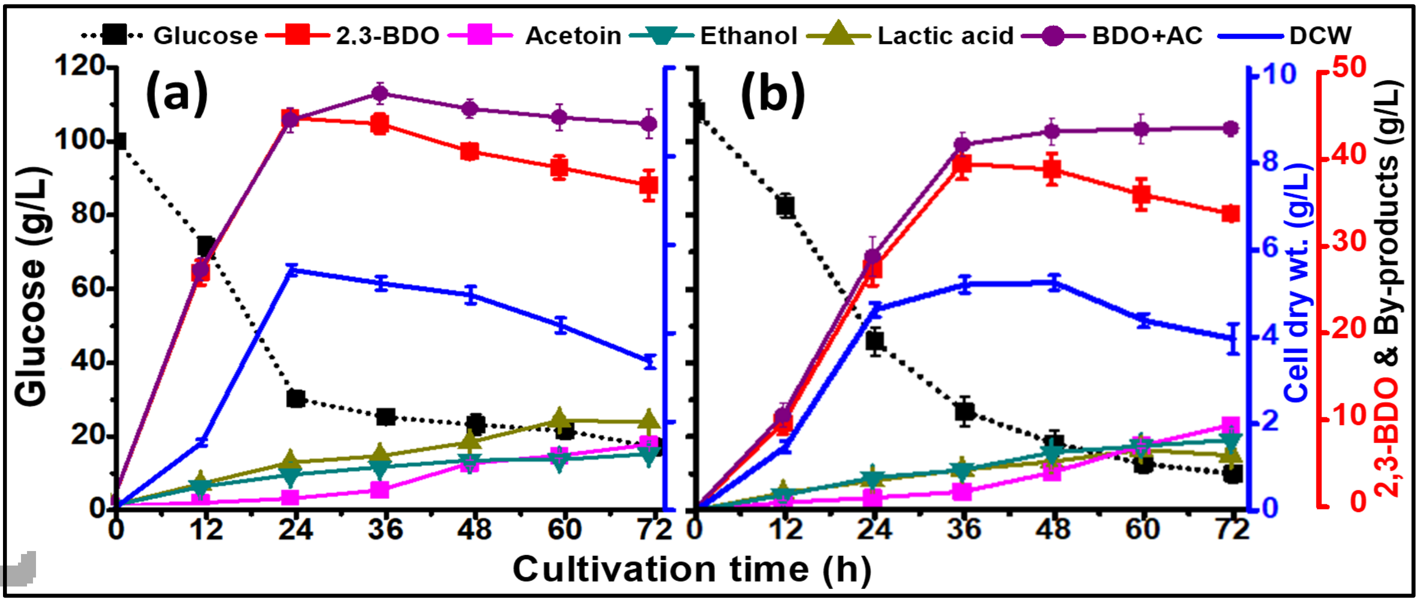

Fig. 3. Fermentation profile with time course of cell growth, 2,3-BDO, and by-products generation (a) pure glucose (b) diol-pretreated EFB substrate.

rate of strain PM2. In case of EFB biomass, 108 g/L glucose was released during first 12 h via enzymatic saccharification. It was quickly assimilated by PM2 at the rate of 1.7 g/L/h and cell growth was exponentially increased. **Fig. 3b** depicted that the highest 2,3-BDO production was observed at late-stationary phase (56 – 60 h) with ~40 g/L titer, 0.48 g/g yield and 1.08 g/L/h productivity. Since 2,3-BDO production is a growth-related phenomenon and the higher concentrations are observed during stationary phase of the cell growth [19]. Afterwards, the sugar consumption was reduced with a steady rate of 0.6 g/L and other by-products (i.e. acetoin, ethanol, lactic acid) were slightly increased till the end of cultivation, indicating the diversion of metabolic route towards mixed-acid fermentation [20].

Results obtained from both the scenarios revealed that utilization of diol-pretreated EFB sugars can be a suitable alternative for 2,3-BDO bioproduction. This in turn also favours the resource recovery and somewhat omiting the food-feed-fuel debate by managing the utilization of biomass in an economical and sustainable way.

3.4 Energy recovery and carbon conversion efficiency

The net energy consumption of the processes was determined based on the enthalpy of all system factors. As shown in **table 1**, the cumulative energy consumption of solely bio-based 2,3-BDO production was lower than the commercial 1,4-BDO production [14], based on the final BDO yield. The fossil energy consumption (FEC) of 2,3-BDO (53.57 MJ/kg) was almost equivalent to 1,4-BDO (53.97 MJ/kg), which implies that lignocellulosic pretreatment is the highly energy-intensive process, required for bioconversion. However, the biocatalyst (2,3-BDO producing PM2 strain) might have a potent capability to make the bioprocess a robust and analogous to the conventional production. Carbon footprint analysis revealed that GHG emissions from 1,4-BDO combustion was 39% higher than bio-2,3-BDO. This biorefinery product can also be counted as carbon-neutral since the related carbon is biogenic that may help in reducing the GHG emissions up to -7.9 kgCO$_2$-e/kg BDO (Table 1)[21].

Table 1. Comparative energy and carbon footprint analysis of butanediol production processes.

Process	Fossil-Energy Consumption (MJ/kg)	GHG emissions	GHG Reduction
		(kgCO$_2$-e/kg)	
Bio-based 2,3-BDO	53.57 ±0.07 [a]	3.6	-7.9
Fossil-based 1,4-BDO	53.97 ±0.07[b]	5.9	+5.9

[a] calculated based upon the methodology used in [13].

[b] Data obtained from [14].

Nonetheless, uncertainty of the bio-BDO output is highly linked to the feedstock cultivation, material and energy prices, input of any additional heat during lignocellulosic feedstock bioconversion (pretreatment/hydrolysis), and combustion of unconverted biomass solids in a biorefinery plant, which may affect the overall 2,3-BDO recovery efficiency via fermentation [15].

CONCLUSION

Diol pretreatment is demonstrated to be an effective strategy for EFB bioconversion, and this approach has become more sustainable through integrating the SSF product into the pretreatment process. The energy and carbon estimates from this study indicate that the integrated biorefinery process can create carbon benefits than the conventional process by reducing GHG emissions from +5.9 kg-CO2/kg-BDO to -7.9 kg-CO2/kg-BDO. Further improvements to reduce carbon footprint can be achieved by process optimization and recyclability of renewable materials. This approach may also offer a cost-effective, energy-efficient integrated biorefinery system, thereby strengthening the economic and environmental sustainability of the oil palm industries.

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