

Fermentation of Oil Palm Frond Juice for Solvent production Using *Clostridium acetobutylicum* (ATCC4259)

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ABSTRACT: Abundant sources of palm oil based biomass provide an impetus for the sustainable generation of biofuel. Biomass such as palm oil agro-plantation waste are emerging as a promising sources of renewable liquid fuels, hence, in order to investigate the potentials of using oil palm frond juice (OPF) as feedstock for solvent production, nutrient and sugar contents analysis was examined. A total solvent of 8.41g/L and 8.61g/L and acids of 3.76g/L and 3.41g/L at pH 6.0 and 6.3 were obtained at initial glucose concentration of 42 g/L. In general, *Clostridium acetobutylicum* (ATCC4259) strain was capable of converting sugar in OPF Juice to solvent and acids. The result showed that direct utilization of OPF Juice provide advantages over traditional pretreatment and enzymatic hydrolysis. Thus, OPF juice has a great potential for solvent production.

Keywords: *Clostridium acetobutylicum*; Feed stock; Fermentation; Oil palm frond juice; Solvent;

I. INTRODUCTION

The high rate of fossil fuel depletion over the decade, coupled with the environmental deterioration resulting from consumptions of the products as transportation fuels, the recognition of the fact that fossil fuel reserve is finite and its depletion is occurring at faster than predicted have necessitated the search into alternative and sustainable resource like bio butanol and bioethanol in large capacity using low cost and readily available substrate [1]. Thus, the concept of waste to weight were focused particularly on the agro waste which can be transformed into value added products thereby reducing waste generation and enhancing eco-efficiency [2]. The agricultural waste product generated from plantation can thus, be utilize resourcefully and efficiently to other value added products [4]. Oil Palm Frond is the major biomass in the form of solid generated in the plantation as a result of harvesting and pruning which is left to decay in order to ensure nutrient conservation in the soil [5-8]. Therefore to ensure the viability of OPF feed stock as fermentative substrate for biofuel production, there are some criteria that need to be satisfied. These includes practically cost effective, impurities free, can produce high yield of product desired, has to be substantially available locally and be handle at minimum risk of health and safety [8, 9].

Oil palm frond juice have the potential to be converted to fermentable sugar for the production of value added products such as bioethanol, bio butanol and bioplastic [10]. Research on sugar production from oil palm frond by Kosugi et al [11] revealed that high amount of readily available sugar were contain in the oil palm frond (OPF). Furthermore, the production of sugars from dried oil palm frond has recently been reported [2, 12]. Recent studies have indicated that renewable sugar can be obtained from oil palm frond by the application of simple extraction technology using conventional sugar cane pressing machine [8]. Attempts have been made by researchers to produce bioethanol fuel from OPF juice [1, 15]. In search for viable alternatives for biofuels, OPF juice is used as an alternative raw materials for solvent production. In this study, the production of solvent via fermentation of OPF juice using *Clostridium acetobutylicum* ATCC4259 strain with addition of organic and inorganic nitrogen supplement was attempted.

II. MATERIALS AND METHODS

2.1 Raw Materials and Inoculum Preparation

The OPF juice was obtained by pressing the oil palm petiole using a sugarcane pressing machine. The total sugar content in OPF juice used in this study is shown in Table 1. *Clostridium acetobutylicum* (ATCC4259) was obtain from (American Tissue Collection Centre). The inoculums were grown for 48 hours at 37°C in Modified Reinforced *Clostridium* Medium (RCM2107).

2.2: Fermentation

The batch fermentation of OPF juice was carried out in 130ml serum bottle with 100ml working volume. The preparation of anaerobic medium was carried out by degassing the medium with oxygen-free nitrogen for 15minutes using Hungate Technique (Miller and Wolin1976). The experiment was conducted in glucose based at the optimum fermentation parameters indicated in Table2

Table2: Fermentation parameters for C. acetobutylicum ATCC4259.

Parameter	Value
Fermentation Temperature (°C)	37
Fermentation time,(hours)	48
Initial sugar, (g/l)	30.2
Culture inoculum age, (hours)	18-20
Inoculum (vol %)	10
Acidity of fermentation broth	6.2-4.0
Type of sugar	Glucose

2.3: Analytical procedure:

Cell concentration was analyzed by oven dry method.The sample drawn after every 4 hours were centrifuged at 4800rev/min for 10minutes and the supernatant decanted and kept at -80°C prior to reducing sugar, ABE and acid concentration analysis. The cells were than dried at 60°C for 48 hours. At 4 hour intervals, samples of were drawn from serum bottle for total reducing sugars and ABE analysis. Acetone-Butanol-Ethanol (ABE), Acetic acid and butyric acid were determined by a gas chromatography(Model 6890N, Agilent Technologies,USA) equipped with a flame ionization detector (FID). The separation of ABE and organic acids was achieved by using a capillary polyethylene glycol (PEG) column (HP-INNO wax, Agilent Technologies) and helium as a carrier gas. The column temperature was initially held at 35°C, programmed with the following

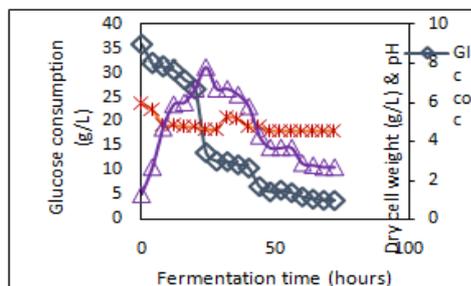
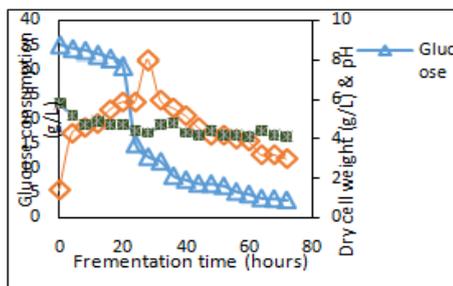


Figure 1: Growth and sugar consumption profile at pH 6.0 Figure 2: Growth and sugar consumption profile at pH 6.3

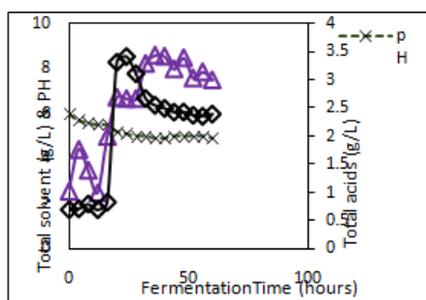
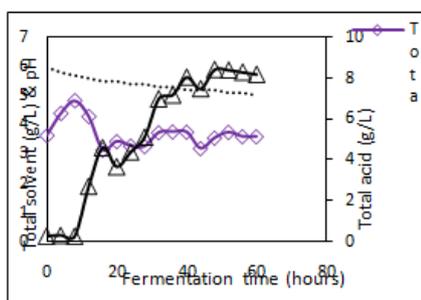


Figure 3: Total Solvents and acid concentration at pH6.0 Figure 4: Total Solvents and acid concentration at pH6.3

Increments; 100C /min to 1100C, 200C /min to 155°C, 40⁰C /min to 200°C held for minute, and the final temperature of 250°C was held for 2 minutes. The temperature of the detector and injector were maintained at 300°C and 250°C respectively. The peak area of the respective compounds was quantified based on 1% standard prepared.

III. RESULTS AND DISCUSSION

Figures 1 and 2depicts the biomass concentration and sugar consumption profile for solvent and acid production by clostridium acetobutylicum ATCC4259 at pH 6.0 and 6.3respectively.The sugar consumption and the growth of C. acetobutylicum ATCC4259 on OPF which act as substrate in the fermentation was monitored

every 4 hours for 72 hours. The highest biomass concentration of 8.0g/L and 7.8g/L were observed at 28 hrs fermentation for pH 6 and 6.3 respectively. The exponential growth was as a result of sugar consumption by the bacterial strain. Thus, the *C. acetobutylicum* was able to grow in oil palm juice media. The pH of the medium plays a vital role in solvent fermentation. In acidogenesis, rapid formation of acetic and butyric acids causes a decrease in pH. In Figures 3 and 4, acidogenic phase was observed at 24 hrs of fermentation where acetic and butyric acid are produced accompanied by total solvents and acid production of 8.41g/L, 8.61g/L and 3.76g/L, 3.41g/L respectively. This is in agreement with Linggang et al [16] who reported similar observation of ABE fermentation by *C. acetobutylicum* ATCC824 using sago pit residues hydrolysate. However, Monot et al [17] argued that the presence of organic acids at high concentration during fermentation causes decreased sugar uptake capability of the microorganism, resulting in low ABE production. Examining the pH from beginning at 0 hr to the end of fermentation at 72 hrs, shows that pH started at 6.0 in Figures 1, 2, 3 and 4, then decreases in almost linear fashion to pH 4.5 due to extensive formation of acids (acetic and butyric acids) mainly during vegetative phase. This extreme acidity may explain the limitation in sugar uptake as well as in butyric acid uptake for strain toward solvent production [18]. Furthermore, synthesis of autolysins usually present at high concentrations at the end of exponential growth phase of solventogenic clostridia causes drop in biomass, as increasing the amount of butanol induced the release of cell-free autolysin [13, 19-21]. OPF juice can thus, be used as substrate for solvent production.

IV. CONCLUSION

This study revealed the possibility of Solvent production using *C. acetobutylicum* (ATCC4259) with addition of organic nitrogen supplement. It further demonstrated the potential of utilizing the inexpensive readily available oil palm frond biomass as raw materials for solvent production by fermentation. Although some further process enhancement are needed.

REFERENCES

- [1]. Lim, S.H., I. Darah, and C.O. Ibrahim, Oil Palm Frond for the Production of Bioethanol. *International Journal of Biochemistry and Biotechnology*, (2012). 1(1): p. 7-11.
- [2]. Goh, C.S., et al., Bio-ethanol from lignocelluloses: status, perspective and challenges in Malaysia. *Bioresource Technology*, 2010. 101: p. 4834-4841.
- [3]. MPOB, M.P.O.B.-. Overview of the Malaysian oil palm industry 2010. 2011, Ministry of Plantation Industries and Commodities Malaysia
- [4]. Shuit, S.H., et al., Oil palm biomass as a sustainable energy source: A Malaysian case study. *Energy*, 2009. 34: p. 1225-1235.
- [5]. Hassan, O.A., et al., Oil palm fronds as a roughage feed source for ruminants in Malaysia oil- palm fronds as a roughage, 1995, ASPAC Food & Fertilizer Technology Center. p. 1-8.
- [6]. Wan Zahari, M., et al., Utilization of oil palm frond—based diets for beef and dairy production in Malaysia. *Asian-Australasian Journal of Animal Sciences*, 2003. 16(4): p. 625-634.
- [7]. Zahari, M.W., A.R. Alimon, and H.K. Wong, Utilization of oil palm co-products as feeds for livestock in Malaysia. *Industrial Crops and Products*, 2009: p. 243-302.
- [8]. CheMaail, C.M.H., et al., Oil Palm Frond Juice as Future Fermentation Substrate: A Feasibility Study *Biomed Research International*, 2014. 2014: p. 1-8.
- [9]. Ray, A., Second-generation hydrocarbon fuels from oil palm by-products. *Journal of oil palm & Environment*, 2013. 4: p. 22-28.
- [10]. Zahari, M.W., A.R. Alimon, and H.K. Wong, Utilization of oil palm co-products as feeds for livestock in Malaysia, in *Biofuel co-products as livestock feed*, P.S.M. Harinder, Editor. 2012, Food and Cultural Organization United Nation. Rome. p. 243– 302.
- [11]. Kosugi, A., et al., Ethanol and lactic acid production using sap squeezed from old oil palm trunks felled for replanting. *Journal of Bioscience and Bioengineering*, 2010. 110: p. 322-325.
- [12]. Fazilah, A., et al., Physicochemical properties of hydrothermally treated hemicellulose from oil palm frond. *J. Agric. Food Chem.*, 2009. 57: p. 1527-1531.
- [13]. Madihah, M.S., et al., Anaerobic fermentation of gelatinized sago starch-derived sugars to acetone-1-butanol-ethanol solvent by *Clostridium acetobutylicum*. *Folia Microbiologica*, 2001. 46: p. 197-204.
- [14]. Ennis, B.M., N.A. Gutierrez, and I.S. Maddox, The Acetone-Butanol-Ethanol Fermentation: A Current Assessment. *Process Biochem*, 1986. 21: p. 131-145.
- [15]. Abdullah, S.S.S., Y. Shirai, and E.K. Bahrin, Fresh oil palm frond juice as a renewable, non-food, non-cellulosic and complete medium for direct bioethanol production. *Industrial crops and products*, 2014.
- [16]. Linggang, S., Acetone-Butanol-Ethanol Production by *Clostridium acetobutylicum* ATCC824 using Sago Pith Residues Hydrolysate. *Bioenergy Research*, 2013. 6(1): p. 321-328.
- [17]. Monot, F., et al., Acetone and Butanol Production by *Clostridium* in a Synthetic Medium. *Applied Environmental Microbiology*, 1982. 44(6): p. 1318-1324.
- [18]. Hung, H., H. Liu, and Y.R. Gan, Generic Modification of Critical Enzymes and Involved Genes in Butanol Biosynthesis from Biomass. *Biotechnology advances*, 2010. 28(5): p. 651-657.
- [19]. Ezeji, T.C., et al., Achievements and Perspectives to Overcome the Poor Solvent Resistance in Acetone and Butanol-Producing Microorganisms. *Appl. Microbiol. Biotechnol*, 2010. 85: p. 1697-1612.
- [20]. Jones, D.T. and D.R. Woods, Acetone-Butanol Fermentation Revisited. *Micribiol Rev.*, 1986. 50(4): p. 484-524.
- [21]. Mukhopadhyay, A., N.J. Hillson, and J.D. Keasling, Control of stress Tolerance in Bacterial Host for Bioproduction of Fuels, in *Microbial Stress Tolerance for Biofuels*. 2012. p. 209-238.
- [22]. Tomovska, J., S. Presilski., N. Gjorgievski., N. Tomovska., M.S. Qureshi and N.P. Bozinovska., 2013. Development of a spectrophotometric method for monitoring angiotensin-converting enzyme in dairy products. *Pak Vet J*, 33(1): 14-18.