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Agro-industrial wastes as feedstock for sustainable bio-production of butanol by *Clostridium beijerinckii*

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ABSTRACT

Three different inexpensive, abundant agro-industrial wastes: suspended brewery liquid waste (BLW), starch industry wastewater (SIW) and apple pomace ultra-filtration sludge (APUS) have been explored for biobutanol production. Physicochemical analysis of biomass for carbohydrate and nutrient pool, acid hydrolysis, fermentation of raw extracts and hydrolysates by *Clostridium beijerinckii* NRRL B-466 for ABE production and glucose supplementation to increase butanol production have been studied. The efficiency of the microorganism to produce butanol and other metabolites was explored via carbon balance. Inhibitory effects were minimized by dilution and detoxification method. Pretreated diluted extracts having reducing sugar content of 30 g/L gave optimum butanol production of 4.68 g/L, 1.4 g/L and 1.8 g/L from SIWH, APUSH and BLWH, respectively, as compared to 5.1 g/L obtained from control. Reducing sugar concentration in each diluted extract was increased to 60 g/L by 3% (w/v) glucose supplement and the butanol production was increased to 11.04 g/L (0.27 g/g), 9.3 g/L (0.24 g/g) and 8.06 g/L (0.25), respectively. For SIWH, the maximum yield of butanol (0.27 g/g) and acetone–butanol–ethanol (ABE) (0.46 g/g) was obtained. Thus, production of butanol, with 3% glucose supplement demonstrated that these inexpensive agro-industrial wastes could have important implications in stimulating energy-economics.

1. Introduction

Increasing global energy demand, unstable and expensive petroleum resources, and concern over global climate changes has led to the development of renewable energy sources that have driven research towards the utilization of biomass resources for the production of energy and fuels (Hu and Ragauskas, 2012). The demand for renewable resources largely rests on abundant waste biomass (Perlack et al., 2005). Also, fermentation substrate is an important factor influencing the cost of butanol production (Ezeji et al., 2007a). Thus, waste biomass based biofuel production could be a promising renewable energy source for a country with abundant biomass resources such as, Canada and others as shown in Table 1.

Biochemical production of butanol is promising due to its superior fuel properties as compared to ethanol (Cascone, 2008). Butanol, a four carbon containing aliphatic saturated alcohol ($C_4H_{10}O$, M_W 74.12) is considered as a superior biofuel due to its higher energy density (29.2 MJ/L), higher air fuel ratio (11.2), octane number (96), lower heat of evaporation

Abbreviations: APUS, apple pomace ultrafiltration sludge; BLW, brewery liquid waste; SIW, starch industry wastewater; APUSH, apple pomace ultrafiltration sludge hydrolysate; BLWH, brewery liquid waste hydrolysate; SIWH, starch industry wastewater hydrolysate.

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Table 1 – Statistical data on agro-industrial waste production in different parts of the world.

Year	Country/continent	Agro-industrial wastes produced ($t \times 1000$)	References
2007	Newzeland	150	Ajila et al. (2012)
2007	Belgium	1150	
2007	Czech Republic	460	
2007	Finland	860	
2007	Iceland	50	
2007	Italy	60, 107	
2007	Netherland	2390	
2007	Norway	160	
2007	Slovakia	4490	
2003	Bulgaria	50	
1999	United kingdom	84, 000	
1990	Austria	880	
2007	France	400	
2013	Brazil	324, 788	Virmond et al. (2013)
2013	Ukraine	48, 226	Waste of Agro-Industrial Complex—IFC (2015)
2003	Denmark	369	McPhail et al. (2012)
2009	Australia	112, 000	Nigam and Pandey (2009)
2009	China	1120, 000	
2009	India	672, 000	
2007	Japan	90, 430	
2009	North America	1456, 000	

(0.43 MJ/kg), and its ability to blend with gasoline in higher percentage (80–85%) without any modification of conventional Otto-cycle engine compared to bioethanol, which has energy density (19.6 MJ/L), higher air fuel ratio (9), octane number (126) and lower heat of evaporation (0.92 MJ/kg) (Cascone, 2008; Lee et al., 2008).

Agro-based industries are experiencing a surge in their growth around the globe (Dhillon et al., 2013). Statistical data on agro-industrial wastes production by different parts of the world have been reported in Table 1. According to Federation of Indian Chambers of Commerce and Industry report (2011) about (60–70%) of these agro industrial waste (food and beverage processing industry) are discharged in the environment without any treatment and rest was utilized for anaerobic digestion in Africa, Eastern Europe, Latin America and Asia (except Japan) (Initiative, 2011). These by-products, if not managed properly, could have adverse effects. Hence, these inexpensive, abundant, micronutrient rich fermentation substrates could be potentially used as alternate carbon sources for bioprocessing.

Bio-butanol can be produced from diverse biomass ranging from starchy food materials to renewable wastewater biomass using acetone butanol ethanol (ABE) fermentation (Maiti et al., 2015a; Ezeji et al., 2003, 2007b; Qureshi et al., 2010a,b). Although research on upstream and downstream processing has significantly progressed, bio-butanol production has several challenges, of which cost of the feedstock is the major economic constraint (60% of the total cost of biobutanol production) followed by the cost of butanol separation from the dilute broth (Thang et al., 2010). In this context, bio-butanol production from inexpensive, abundant, lignocellulose and starch based waste biomass could be a promising renewable energy source for countries with abundant biomass resources as shown in Table 1. Canada is one of the higher agro-industrial waste producers in North America. Annual production of apple industry solid waste ($10.327–12.393 \times 10^3$) tons, apple industry ultrafiltration sludge ($206.54–413.09 \times 10^4$) hectolitre, brewery industry liquid waste ($219–657 \times 10^5$) hectoliter and brewery industry spent grain (301.75×10^5) tons were produced in Canada (Dhillon et al., 2013; Olajire, 2012). About 20% of agro-industrial food wastes have been utilized for animal feed, while the rest is used for landfilling, incineration or composting, which contributes about 10% of the country's GHG emissions (Nigam and Pandey, 2009). Since substrate cost has the most influence on butanol price (about 60% of the production cost) (Qureshi and Blaschek, 2000), this research has focused on the potential of inexpensive agro-industrial wastes, such as apple pomace ultra filtration sludge (APUS), brewery industry liquid waste (BLW) and wheat based starch industry wastewater (SIW) as the substrates for butanol production by *Clostridium beijerinckii* NRRL B-466.

Beer is the fifth most consumed beverage and Canada produces a large amount of beer (with an estimated 21.9 million hectoliters being produced annually) that leads to abundance of brewery wastes (effluent and solid wastes) (Brewers Association of Canada website: www.brewers.ca, Olajire, 2012). Previously solid waste from brewery industry, known as brewery industry spent gain had been utilized as a substrate for ABE production (Wang et al., 2009). In the present study, suspended brewery liquid waste (BLW), produced after second fermentation during beer production, has been explored for production of biobutanol. Production of bio-butanol using starch from different sources had been reported in the literature (Li et al., 2013, http://www.dissertationtopic.net/doc/110338). We have investigated the utilization of carbohydrate pool of starch industry waste water (SIW) for bio-butanol production. Apple pomace solid waste (APS) was previously utilized by (Voget et al., 1985) for ABE fermentation by *Clostridium acetobutylicum* and *Clostridium butylicum* (Voget et al., 1985). However, nutrient rich apple pomace ultrafiltration sludge APUS, another waste produced at the stage of ultra-filtration of apple juice remained almost unexplored (Dhillon et al., 2013). Potential of these biomass wastes for ABE production using *C. beijerinckii* NRRL B-466 has also been studied in this work. The main objectives of these studies are: (1) to ascertain the ability of *C. beijerinckii* NRRL B-466 to utilize the organic pool of these inexpensive agricultural wastes to produce biobutanol; (2) to increase sugar pool utilization, hence butanol production, by optimal glucose supplementation and (3) to investigate the efficiency of