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A study on bioethanol production from cashew apple pulp and coffee pulp waste

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ABSTRACT

Bioethanol production from dry cashew apple pulp and coffee pulp was investigated. The pulp was digested with 2% sulfuric acid and subjected to high pressure (15 psi) cooking at 120 °C for 10 min followed by further 1 and a half hour pressure cooking at 90 °C to solubilize the pulp. Solubilized pulp was filtered and the debris on the filter paper was washed with minimum quantity of distilled water and then oven dried to find the weight of the insoluble lignin mass. Total sugar content in squeezed and dried cashew apple pulp (CAP), dry coffee pulp (DCP) and wet coffee pulp (WCP) was found to be 2.12, 1.62 and 0.62 g/100 ml of hydrolyzate. Reducing sugar content in squeezed CAP, DCP and WCP was found to be 0.14, 0.71and 0.23g/100 ml of hydrolyzate. Filtrate was neutralized with thick suspension of calcium hydroxide slurry until the pH reaches to 6.0. Neutralized slurry was kept at lab temperature overnight and the supernatant was decanted through filter paper. To 150 ml of filtrate yeast (*Saccharomyces creviciae*) was added at a concentration of 5.0 g/l concentration and subjected to fermentation for 48 h at 30 °C in a shaker incubator at 120 rpm. Ethanol content in the fermented broth was estimated by titrimetric and gas chromatographic method. Ethanol yield in the fermented broth was found to be 0.5, 0.46 and 0.46 g/g of sugar in squeezed CAP, DCP and WCP. Theoretical ethanol yield (Y_{max}%) of squeezed CAP, DCP and WCP was found to be 46, 9.35 and 40% respectively.

1. Introduction

Biomass utilization to produce ethanol offers environmental benefits in terms of nonrenewable energy consumption and global warming impact. Since ethanol is produced almost entirely from renewable resources, it looks like a viable alternative to conventional fuels [1]. Cellulosic biomass is one of the rich resources available in plenty and its environmental attributes can augment ethanol production on a scale which will have a major impact on fossil fuel [2]. At present ethanol has emerged as a potentially important alternative transportation fuel, because of its easy adaptability to the existing engines and it is a cleaner fuel with higher octane value than gasoline [3]. It is currently produced from sugars and starchy materials [4,5], but lignocellulosic biomass are most likely the alternative sources for the second generation ethanol production in the future [6,7]. The polymer of cellulose and hemicellulose should be first broken down for an effective hydrolysis and give rise to sugars [8–11]. Dilute sulfuric acid-based chemical pretreatment [12–16] is the most popular pretreatment method for lignocellulosic bioethanol production via enzymatic hydrolysis.

The objective of the present study is to produce bioethanol from waste biomass, such as cashew pulp after the extraction

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of liquid, dried and wet coffee pulp. Coffee pulp waste is generated in large quantities when coffee cherry processed by wet pulping method [17,20,21], which is known to contain 23–27% fermentable sugars on dry weight basis [17,20]. Consequently, most of the coffee pulp remains unutilized in many countries and a need exists for its treatment by appropriate biological waste treatment processes to overcome severe environmental pollution [19,21]. Being cost-intensive in nature, the treatment of the waste adversely affects the cost of production of coffee and hence it is generally dumped as a waste [17]. India is one of the largest producers of coffee in the world and also one of the major coffee pulp waste generators [18]. Annual coffee production in India is 295,000 tones [19] of which 75% is processed by wet pulping method for export as well as for domestic consumption and the remaining is sun dried and processed by dry pulping to produce cherry. In India, about 60% of the coffee produced annually is wet pulped and the remaining 40% is sun dried and processed by dry pulping and the husk obtained is dumped in the pits for natural degradation [19]. We were able to produce bioethanol by adopting conventional method of dilute acid pretreatment, neutralization, filtration, and fermentation with minor modifications in dilute acid pretreatment method from the agricultural biomass such as dry cashew apple pulp, dry and wet coffee pulp. The method adopted in this study yields good amount of ethanol. The lignocellulosic biomass used in this study has not been explored so far for the production of ethanol.

2. Methods

2.1. *Chemical pretreatment for digestion of cellulosic matter [12–16]*

45 g each of dried cashew apple pulp, dry and wet coffee pulp were treated with 300 ml of 2% (w/v) sulfuric acid and subjected to pressure cooking at 120 °C for 10 min at 15 psi in the autoclave. The partly digested mass was further subjected for 1.5 h for digestion at 90 °C, at low pressure.

2.2. *Filtration and neutralization [22]*

The slurry was filtered and neutralized with calcium hydroxide until the pH of the hydrolysate reached to 6.0. Calcium sulfate was allowed to precipitate out by keeping the sample overnight at lab temperature.

2.3. *Estimation of total and reducing sugar*

Total sugar in the neutralized hydrolysate was estimated by phenol sulphuric acid method using maltose as standard [23] and the reducing sugar in the hydrolysate was estimated by DNSA method using glucose as standard [24].

2.4. *Estimation of cellulose, hemicellulose and lignin content*

Cellulose, hemicellulose and lignin content in the digested raw material were estimated by the method of McMillan [25].

2.4.1. *Hydrolysis yield of cellulose [26]*

The hydrolysis yield of cellulose was calculated by using the formula

$$\text{Cellulose (\%)} = \frac{\text{Cellulose (Sg)} - \text{Cellulose (Gg)}}{\text{Cellulose (Sg)}} \times 100$$

where, Cellulose (Sg) is the amount of cellulose in the substrate, cellulose (Gg) is amount of lignin in the residual solid after acid hydrolysis.

2.5. *Fermentation*

Fermentation of the neutralized liquor was carried out using Chung and Lee [27], Chen et al. [28], method with minor modifications. The total quantity of hydrolyzate was measured and divided into equal portions of 300 ml each and taken in a 500 ml Erlenmeyer flask. The hydrolyzate was inoculated with bakers yeast at a concentration of 5 g/L. The flask was kept in a shaker incubator maintained at 30 °C for 48 h at 120 rpm.

2.6. *Estimation of alcohol in the fermented broth*

Alcohol content in the fermented sample was estimated by titrimetric method using potassium dichromate, potassium iodide against sodium thiosulfate and using starch as indicator [29,30]. The alcohol content was also estimated by gas chromatographic analysis. The two values were found to be nearly equal.

2.7. *Lignin residue after acid pretreatment [31]*

For each sample of raw materials used to extract cellulosic sugars after dilute acid pretreatment, the residue left as insoluble mass was weighed to find the lignin.

2.8. *Ethanol yield*

From the equation,

$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$

According to the equation, bioethanol yield per molecule of glucose is 0.5111 [32]. Similarly, the percent yield of alcohol was calculated corresponding to the reducing sugar values of various biomass samples by using the following equation [33].

$$\text{Ethanol (\%)} = \frac{\text{Ethanol produced (g)} \times 100}{\text{Cellulose (Sg)} - \text{Cellulose (Gg)} \times 0.5668 - \text{Glucose (Gg)} \times 0.5111}$$

The factor of 0.5668 is the theoretical conversion factor for ethanol from cellulose by *Saccharomyces cerevisiae* [33].

3. Results and discussion

The Lignocellulosic biomass is an abundant, renewable and underutilized global carbon source [34]. Thus, agricultural residues, such as cashew apple pulp and coffee pulp produced in large quantities in India, hitherto being unexplored so far for the production of bioethanol were considered for the present study. The potential yield of bioethanol from lignocellulosic sources varies significantly among agricultural residues [35]. Therefore, an attempt made to produce bioethanol from dry cashew apple pulp, dry and wet coffee pulp is being discussed here.

Percentage lignin content in cashew apple pulp (CAP) and wet coffee pulp (WCP) was found to be significantly lower than dry coffee pulp DCP (Fig. 1). Percentage cellulose content was found to be significantly higher in CAP and WCP when compared with DCP (Fig. 1). Probably the reason for disparity in the results of cellulose and lignin content of DCP and WCP is due to the moisture content of the sample when it was processed for study on total weight basis. WCP is known to contain 80–85% moisture [17,20]. Dry coffee pulp on weight basis is much more in volume than wet coffee pulp when it is processed for acid digestion. It is also evident from the present study that 2% sulphuric acid pre-treatment of CAP and WCP pulp significantly solubilizes the cellulose and hemi-cellulose.

It has been reported that there is increase in lignin content of barley hay, pearl millet hay and sweet sorghum hay on dilute acid pretreatment [28]. In the present investigation also the DCP treated with dilute acid showed increased lignin content. These results are consistent with other experimental findings that the effect of sulphuric acid pretreatment on lignin degradation is minimal and it is not uniform for all biological materials [25,35]. The acid–insoluble lignin content is expected to form a complex of lignin and condensed protein, which became insoluble on treatment with acid, contributes to the lignin mass [28].

Cereal straws from both Europe and North America are characterized by cellulose contents between 35% and 40%, lignin content between 15% and 20% and hemicellulose content at 26% [36]. Softwood cellulose content tends to be around 40% of total dry weight, while hardwood cellulose content is slightly higher at 42% [37]. Reported hemicellulose content can range between 18% and 28% for soft woods, and between 24% and 33% in hardwoods. Lignin contents range between 27% and 34% for soft woods, and 23%–30% in hardwoods [37]. Compared to these reports, lignin content of DCP is within the range suggested for hardwood and softwood, whereas cellulose content in CAP and WCP seems to be comparatively higher than the existing reports [36,37].

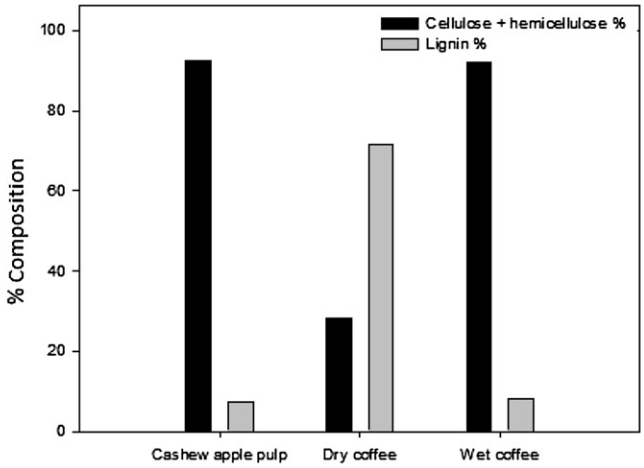

Fig. 1 – Percentage of cellulose and lignin content in cashew apple pulp (CAP), dry coffee pulp (DCP), wet coffee pulp (WCP). Values are mean ± SD, $p \leq 0.05$ of samples in triplicate.

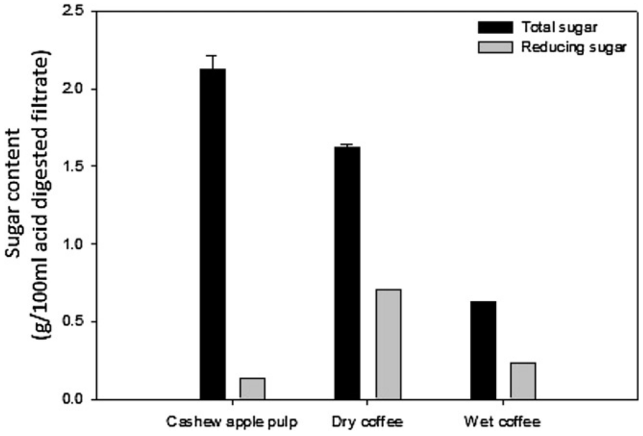

Fig. 2 – Total and reducing sugar content in cashew apple pulp (CAP), dry coffee pulp (DCP), wet coffee pulp (WCP). Values are mean ± SD, $p \leq 0.05$ of samples in triplicate.

It was observed that total sugar content in CAP and DCP was significantly higher than WCP, whereas reducing sugar content in DCP was comparatively higher than CAP and WCP (Fig. 2). Probably the reason for lesser amount of total and reducing sugars in the WCP is due to the moisture content of the sample when it was processed for study on total weight basis. WCP is known to contain 80–85% moisture [17,20], which adds to the weight of the sample when processed with acid digestion. Therefore, sugar yield from the WCP will be

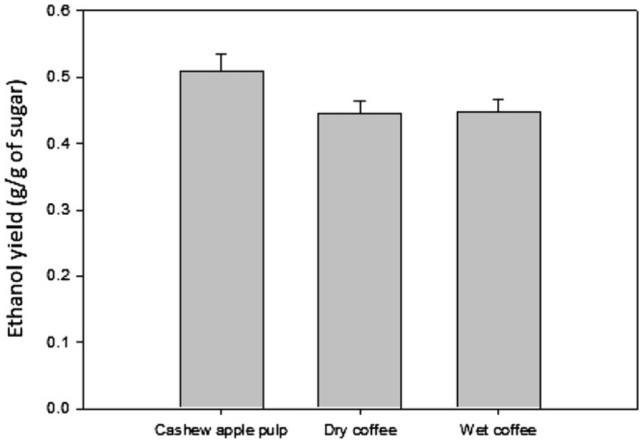

Fig. 3 – Ethanol yield (g/g of sugar) in cashew apple pulp (CAP), dry coffee pulp (DCP), wet coffee pulp (WCP). Values are mean ± SD, $p \leq 0.05$ of samples in triplicate.

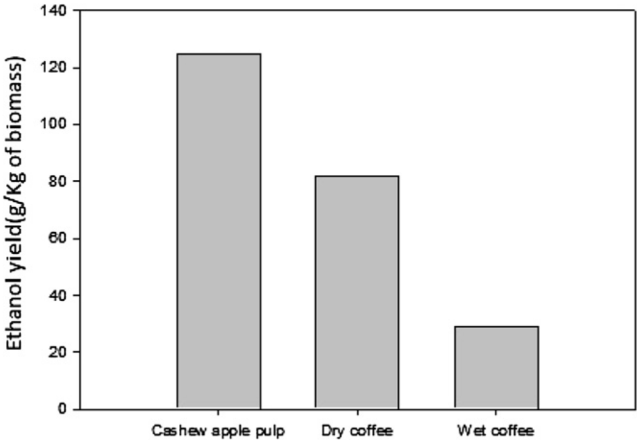

Fig. 4 – Ethanol yield (g/Kg of biomass) in cashew apple pulp (CAP), dry coffee pulp (DCP), wet coffee pulp (WCP). Values are mean ± SD, $p \leq 0.05$ of samples in triplicate.

comparatively lower than DCP. The total sugar content in the cashew apple is reported to be 25% of dry mass and in the juice 1.5–5 g/L [38]. In the present study also, significantly high amount of total sugar content was found in CAP as compared to DCP and WCP.

Ethanol yield (g/g of sugar) was significantly high in CAP followed by DCP and WCP (Fig. 3). These results correlate well with the total sugar content in CAP (Fig. 2) hence the ethanol yield in CAP is comparatively higher. Fermentable reducing sugar plays a major role in ethanol yield and it is obvious that fermentable sugars in CAP, DCP and WCP were available in good quantity hence the ethanol yield in these three samples is quite good. The ethanol yield in g/g of sugar from CAP, DCP and WCP was comparatively higher than the values reported for barley hay and straw, pearl millet hay, sweet sorghum hay, triticale hay and straw, and wheat straw by Chen et al. [28]. This could be due to the difference in techniques adopted for hydrolysis and estimation.

Fig. 4 gives the ethanol yield (g/Kg of biomass) in CAP, DCP and WCP, which was significantly high in CAP followed by DCP and WCP. Once again the disparity in the results of DCP and WCP is because of difference in moisture content between the two and the volume of the sample processed. The ethanol yield obtained from CAP and DCP were comparatively higher than the value reported for barley, triticale, and wheat straw and cotton stalk but was comparatively lower than the value reported for trticale hay [28]. This could be probably due to the difference in technique adopted.

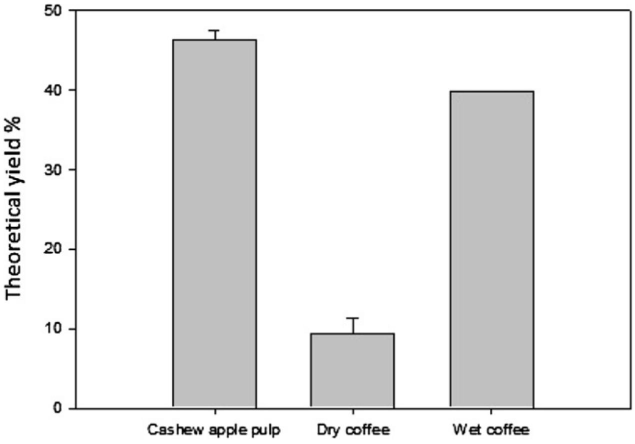

Fig. 5 – Theoretical ethanol yield (Y_{max} %) in cashew apple pulp (CAP), dry coffee pulp (DCP), wet coffee pulp (WCP). Values are mean ± SD, $p \leq 0.05$ of samples in triplicate.

Theoretical ethanol yield was significantly higher in CAP and WCP as compared to the value obtained for DCP (Fig. 5). Theoretical ethanol yield values of CAP and WCP were found to be less than the values reported for agricultural residues such as barley hay and straw, pearl millet hay, sweet sorghum hay, triticale hay and straw, wheat straw by Chen et al. [28]. USDE [39] reported, theoretical ethanol yield of 416L/tonne for rice straw, 432L/tonne for Wheat straw and 428L/tonne for bagasse respectively. Compared to USDE data, theoretical ethanol yield from cashew apple pulp and coffee pulp agricultural residues is low. At this juncture, there is no information available in the literature with respect to the dry cashew apple pulp and coffee pulp agricultural residues derived bioethanol. Therefore, it is difficult to compare our results.

4. Conclusion

It can be concluded from this study that the dry cashew apple pulp, dry and wet coffee pulp are the potential source of ethanol production and have remained unexploited till date. If these agricultural residues are put to good use, such as production of bioethanol, contamination and fouling of the environment can be avoided. More over, it will add to the carbon credit of the country by using it for bioethanol production as a renewable resource for energy and provide value addition to the farm sector.

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