



Research article

Different particle size study of castor deoiled cake for biofuel production with an environmental sustainability perspective

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ABSTRACT

Agro-industrial waste material such as non-edible deoiled Castor bean cake (CBC) is one of the most abundant sources for bioethanol demonstrating the feasibility of utilizing bioethanol as commercial biofuel. This is an alternative to mitigate fossil fuel dependence and carbon dioxide accumulation in the atmosphere. The CBC was pretreated with the help of thionyl chloride at a temperature of 35 °C for residence time 25 min. Subsequently, CBC substrate obtained from pretreatment was subjected to enzymatic hydrolysis with *T. viride* concentration varying from 0.5 to 5 g L⁻¹ at 35 °C, pH 6 for 48 h. Under optimized conditions the process integrating pretreatment followed by enzymatic hydrolysis for 48 h at 35 °C with pH 7 resulted in 76 g L⁻¹ of reducing sugars from 100 g CBC. The obtained sugar was further fermented at 30 °C for 72 h with *saccharomyces cerevisiae* as a fermenting media which yields 37.5 g L⁻¹ of bioethanol. A study of different particle sizes of CBC with BSS-5, BSS-10, BSS-20 was done for efficient enzymatic hydrolysis and fermentation into bioethanol. On a pilot-scale 375 g L⁻¹ of bioethanol was obtained from 1 kg of CBC with the same reaction conditions. The present study demonstrates optimized solid: liquid ratio 1:2 for hydrolysis, fermentation process, and the production cost for bioethanol per L. Figure S1 represents graphical abstract for the production of bioethanol from CBC in supplementary information.

1. Introduction

Global climate change, environmental deterioration, and rise in pollution causes health problems among populations which have been attributed because of the consumption of fossil fuels [1,2]. Biomass is currently the most widespread form of renewable energy with huge potential in the production of biofuels for transportation, electricity, and heat [3,4]. The most important biomass sources are lignocellulosic feedstocks such as agriculture, forest, and industrial residues to provide biofuel materials and chemicals [5, 6, 7, 8]. Most of those sustainable bio refinery products are often produced from plant polysaccharide (glucans, hemicelluloses, starch, and pectic materials) and lignin [10]. Bioethanol is a source of renewable and biodegradable energy due to its eco-friendly manufacturing process [11,12]. Liquid fuels like bioethanol, biodiesel, and biogas are the types of biofuel.

Many Scientists have considered non-edible oilseed and food waste as feedstock for the production of biofuel. The fuels produced from them reduce fuel import costs, greenhouse phenomena, and air pollution [13, 14, 15, 16, 17, 18].

Bioethanol is industrially produced from sugar and starch-based materials from inexpensive and abundant lignocellulosic substrates such as agricultural residue, forest residue, and municipal solid wastes [4,19,20]. Comparative table for the production of bioethanol from different biomass given in Table 1.

In addition to this for the enzymatic transformation of cellulose to sugars, fungal enzymes were used. From the study, it is concluded that fungi can also be a cost-effective production of enzymes for bioethanol production by using barley straw [32]. As per the reported literature agro-industrial waste such as castor bean cake (CBC) can be utilized for the production of bioethanol as shown in Table 2 [13,14,18,33]. The novelty of the present work is compared with recent literature as mentioned in Table 2.

By pressing castor beans during the production of oil, deoiled castor cake was obtained as a waste. It was found that pressing 1 ton of castor bean produces about 550 kg of deoiled cake, which varies depending on how the oil is extracted and the oil content of the seeds. After detoxification CBC can be used as an organic manure insecticide [35]. Due to the large starch content, this CBC can be transformed into bioethanol

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Table 1. Comparative table for different biomass into bioethanol production.

Feedstock	Glucose	Bioethanol	References
Banana waste	100 g L ⁻¹	42 g L ⁻¹	[21]
Corn cob	51 g L ⁻¹	21.9 g L ⁻¹	[22]
Agave salmiana	50 g L ⁻¹	24 g L ⁻¹	[23]
Agave bagasse	110.5 g L ⁻¹	36 g L ⁻¹	[24]
Corn straw	-	17 g L ⁻¹	[25]
Vetiver grass	21 g L ⁻¹	6 g L ⁻¹	[26]
Potato peel waste	69 g L ⁻¹	21 g L ⁻¹	[27]
Cassava peel waste	11.026 g L ⁻¹	3.76 %	[28]
Rice straw	90 %	62.6 %	[29]
Wheat straw	65.2 g L ⁻¹	24.4 g L ⁻¹	[30]
Pomegranate peels	36 g L ⁻¹	14.3 g L ⁻¹	[31]

efficiently [36]. *Ricinus Communis L.* (castor plant) also known as castor oil bean is a non-edible oil crop that is an individual from the family Euphorbiaceae. Castor is a significant non-edible seed that can endure various states of climate [37]. The plantation cost of castor is fundamentally not exactly like different plants for example soybean, rapeseed, and jatropha [38]. Castor oil seeds contain 40 % oil and around 5 % ricin, which is a highly toxic protein [39,40]. Castor oil is utilized for an assortment of industrial, cosmetic, and medical applications [41] More-over it is utilized for Biodiesel production [42]. Some natural issues like ozone-depleting substance outflow and its outcomes show the significance of utilizing biofuel [39,43].

According to Food and Agriculture Organization Corporate Statistical India is the world's single biggest maker of castor seed representing over 85% followed by China with around 7% and Brazil with around 5% of world castor seed yield. There has been a steady expansion in yield basically because of ascending levels in India. At present bioethanol is being produced in India simply by changing over sugar molasses to ethanol at the modern level, this usually leads to food-fuel conflict [44] Simultaneously enormous amounts of nonedible oil seeds like castor, jatropha, Karanja are handled to fabricate biodiesel and other substance subsidiaries leaving behind the deoiled cake as waste which is having genuine disposal issue. CBC contains large quantities of starch which is responsible for the production of ethanol. The solid residue obtained after ethanol separation can be used as organic manure by testing its C: N ratio

[13,45] that residue can also be used as biogas which can be produced through anaerobic digestion [11]. Hence treating castor bean cake for the production of bioethanol can serve as a waste minimization technique.

As per the National policy of India, a base degree of biofuel became promptly accessible in the market to satisfy the need at some random time. A demonstrative objective of 20% blending of biofuel for both biodiesel and bioethanol by 2017 is proposed. The blending level of bioethanol has now been made obligatorily viable from October 2008 and will keep on being compulsory driving up to the demonstrative objective [46].

The present study aims at the development of an enzymatic hydrolysis and fermentation process with different particle sizes to detoxify the leftover cake. The novelty of this work is discussed below.

- > Significance of different particle sizes on the production of bioethanol.
- > Minimum dilution ratio as compared to reported literature.
- > Higher production of glucose and bioethanol by enzymatic hydrolysis and fermentation process respectively.
- > Analysis of residue obtained after bioethanol collection by testing its C: N ratio and NPK content.
- > Costing for the production of bioethanol per L.

2. Materials and methods

2.1. Raw materials

Castor deoiled cake was obtained from the Jayant agro-organics ltd and dried at 100 °C for 24 h. Castor seed cake screened to achieve different particle sizes BSS-5 (3.35 mm), BSS-10 (1.70 mm), and BSS-20 (0.780 mm). Thionyl chloride was procured from Loba Chemie, a Commercial source of *Trichoderma viride* (T. viride) 1% W.P. Antagonistic fungus, wettable powder (1 × 10⁸ CFU/g. Min) was obtained from Vijay seed company and *Saccharomyces cerevisiae* (yeast) were obtained from the local grocery shop.

2.2. Pretreatment

The 100g of castor seed cake of different particle sizes were pre-treated by thionyl chloride with 8.175g (wt. basis) at 35 °C for 25 min.

Table 2. Comparative table for production of bioethanol by using Castor bean cake.

Substrate	Reaction parameters	Pretreatment	Sugar	Ethanol	References
Castor bean cake	castor cake quantity: 100 g, Dilution ratio: 1:6, Temperature: 30 °C, pH = 6.6, Enzymes used: α-amylase, pullulanase, glucoamylase, Yeast used: <i>Saccharomyces cerevisiae</i>	-	63.7 g L ⁻¹	34.5 g L ⁻¹	[18]
Castor bean cake	castor cake quantity: 1 kg of castor plant, Dilution ratio: 1:5, Temperature: 37 °C, Time: 72h, pH = 7, Enzyme used: carboxymethylcellulase, Yeast used: <i>Saccharomyces cerevisiae</i>	8% (w/v) sodium hydroxide at 0 and 100 °C for 30 and 60 min	-	63 g	[13]
Castor bean cake	Castor cake quantity: 1 kg, Dilution ratio: NIL, pH = 5, Temperature: 37 °C, Time: 96 h, Enzyme used: 15 FPU cellulase and 30 IU β-glucosidase, Yeast used: <i>Saccharomyces cerevisiae</i>	8% w/v sodium hydroxide at 100 °C for 60 min	-	30.1 g	[34]
Castor bean cake	Castor cake quantity: 100 g, Dilution ratio: 1:6, pH = 5, Temperature = 30 °C, Time = 72 h, Enzyme used: carboxymethylcellulase, Yeast used: <i>Saccharomyces cerevisiae</i>	-	5220 mg L ⁻¹	35 g L ⁻¹	[14]
Castor bean cake	Castor cake quantity: 200 g, Dilution ratio: 1:6, pH = 5.5, Temperature = 35 °C, Time = 24 h, Enzyme used: Cellic CTec3, Yeast used: <i>Saccharomyces cerevisiae</i>	Autohydrolysis pretreatment	99.9 g L ⁻¹	50.5 g L ⁻¹	[33]
Castor bean cake	Castor cake quantity: 100 g (with optimized particle size BSS-10), Dilution ratio: 1:2, pH = 6, Temperature: 30 °C, Time: 72 h, Enzyme used: T. viride 1%, Yeast used: <i>saccharomyces cerevisiae</i>	thionyl chloride 8.175g (w/w) at 35 °C for 25 min.	76 g L ⁻¹	37.5 g L ⁻¹	Present study

Table 3. Pre-analysis of Castor seed cake.

Sr. No.	Nutrients	Result
1.	Total solid (%)	98.0
2.	Crude fibre (%)	4.43
3.	Crude protein (%)	35.43
4.	Crude fat (%)	25.10
5.	Total ash (%)	7.14
5.	NFE (%)	24.88
6.	Oil content (%)	2

2.3. Enzymatic hydrolysis (Saccharification)

The pretreated cake was hydrolyzed by using *T. viride* to break the complex cellulosic material of castor seed cake. The optimized hydrolysis period obtained was 48 h. The hydrolysis was done for 100 g of pretreated castor cake. The solid-liquid ratio for the hydrolysis was 1:2 with different concentrations of *T. viride*. During the reaction, *T. viride* produces cellulase enzyme and further, it produces glucose at 35 °C with pH 6. The hydrolysates were collected for the estimation of sugar by the standard DNSA method [47,48]. UV-660 double beam Spectrophotometer was used to analyze the samples. The sugar yield was analyzed for different concentrations of *T. viride* (0.5–5 g L⁻¹).

2.4. Bioethanol production

After the hydrolysis stage fermentation was carried out in a 5L autoclavable glass fermenter in the presence of *Saccharomyces Cerevisiae*. The reaction was maintained at 30 °C under stirring of 300 rpm for 72 h. *Saccharomyces Cerevisiae* was used as a microorganism for fermentation with a 10% (w/w) concentration. The bioethanol was separated from the fermented substrate by vacuum distillation at 75 psi, 65 °C for 2 h. The distillate samples were taken for the analytical measurements of bioethanol.

2.5. Detection of bioethanol by using gas chromatography (GC)

Different concentrations of ethanol absolute (GC grade) were prepared and then detected in GC (Chembioteck 2010) with an FID detector. Column flow was 2 ml/min. The sample volume was 1 µL, oven and detector temperatures were 60 °C and 200 °C respectively.

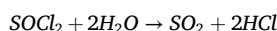
3. Results and discussion

3.1. Compositional analysis of castor deoiled cake

Before proceeding for hydrolysis & fermentation, pre-analysis of the cake was completed from Sheetal laboratory, Pune to check the contents in castor deoiled cake which is given in Table 3. Compositional analysis performs through estimation of moisture content by utilizing standard methods based on the moisture content of deoiled cakes has been found. After calculating the initial moisture, the remaining amount of de-oiled cake was considered as total solids.

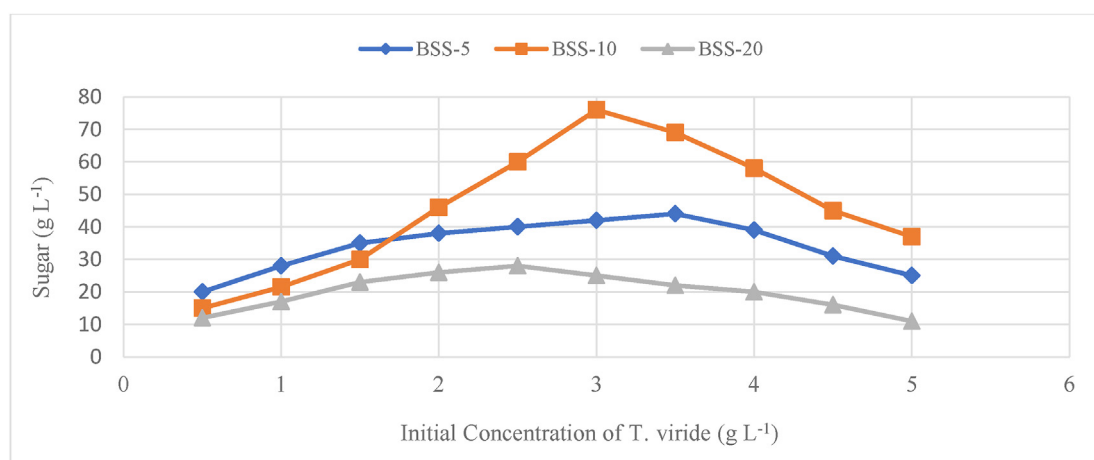
3.2. Enzymatic hydrolysis of CBC by using *T. viride* with different particle sizes

As per the reported literature, one of the important factors which influence enzymatic hydrolysis is the accessible surface area. Another part that influences hydrolysis is pore size or pore volume. An increase in pore size will increase the accessible surface area. Accessible surface area is hard to determine but measuring how much surface is available to the enzymes for the reaction is possible. For smaller particle sizes higher surface area is a consequence [49, 50, 51, 52]. Also bigger pore sizes in substrates provide higher conversion yield as reported [53]. H. Bateni et al stated that the pretreated substrates had more open and accessible structures compared to untreated substrates because of their compact structure and low porosity [13]. Hence from reported literature to make CBC more digestible by enzymes hydrolysis is very important before fermentation. CBC with different particle sizes was pretreated with thionyl chloride to break the compact structure. Analysis of sugar was done by standard DNSA method by using UV-660 double beam spectrophotometer. "When thionyl chloride was added in cake in presence water it generates hydrogen chloride which is responsible to break an open and accessible structure" [54].



The pretreated castor cake with different particle sizes viz. BSS-5, BSS-10, and BSS-20 were treated at 35 °C with an optimum solid-liquid ratio of 1:2 and along with the different concentrations of *T. viride* for further hydrolysis to see the effect of particle size on the yield of sugar. Among all sizes, BSS-10 is optimum with 76 % sugar formation. Instead of *T. viride* the commercial enzyme Cellulase (ASP Niger sigma) can also be used but the cost is very high as compared to *T. viride* which secret large amounts of cellulolytic enzymes when added to the pretreated cake.

The hydrochloric acid released in the above reaction and *T. viride* addition was responsible for the hydrolysis of cellulose. As the main

**Figure 1.** Sugar formation with BSS-5, BSS-10, and BSS-20 particle size.

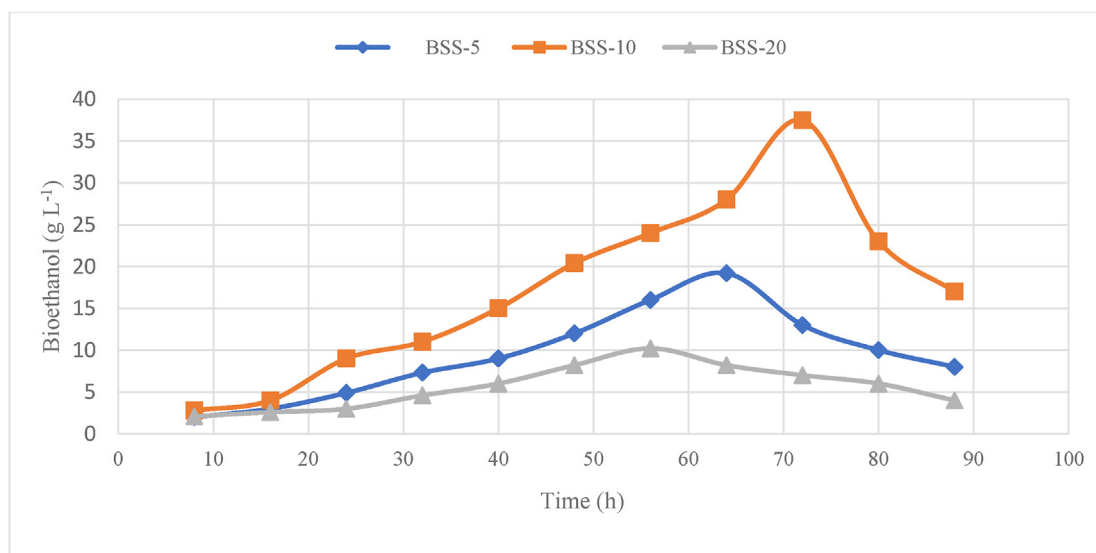


Figure 2. Study of different solid: liquid ratio.

component of cellulose is a biopolymer consisting of many glucose units connected through β -1,4-glycosidic bonds. Breakage of the β -1,4-glycosidic bonds by acids leads to the hydrolysis of cellulose polymers, resulting in the sugar molecule glucose or oligosaccharides. Hydrolysis of cellulose and starch yields glucose only [55] (see Figure 1).

3.3. Synthesis of bioethanol production

The hydrolysed cake was fermented with *Saccharomyces cerevisiae* yeast with 10 % (w/w) concentration at 30 °C temperature for 72 h with pH 6 as shown in Figure 2. After fermentation, the fermented cake was carried for vacuum distillation at temperature 60 °C and 15 psi pressure for separation of bioethanol. 100 g of CBC gives 37.5 g L⁻¹ of ethanol with an optimum particle size of BSS-10. The residue which was obtained after fermentation can be used as organic manure as per the tested C: N ratio. Analysis of bioethanol was done by GC 2010 Chembioteck. The

results for the production of bioethanol for a different interval of time (Figure 3). The maximum concentration of bioethanol in BSS-10 was dependent upon the four factors: external surface area, accessible internal surface area, enzyme adsorption, and enzyme accessible surface area. As per the reported literature, a decrease in the particle size will decrease external surface area, accessible internal surface area, enzyme adsorption, and enzyme accessible surface area [56] which is responsible for the decrease in bioethanol production in BSS-20 particle size. On large scale with the same reaction parameters by using 1 kg CBC 375 g L⁻¹ of bioethanol was produced. Yeast concentration was kept constant throughout all the fermentation reactions because by increasing the concentration of yeast there was no further increase in bioethanol yield. Hence 10 % (w/w) concentration is optimum. The residual solids left behind after ethanol separation in vacuum distillation can be used as organic manure after checking the C: N ratio, Nitrogen, Phosphorous, and Potassium content. Also, this residue can be used for the production of biogas by an

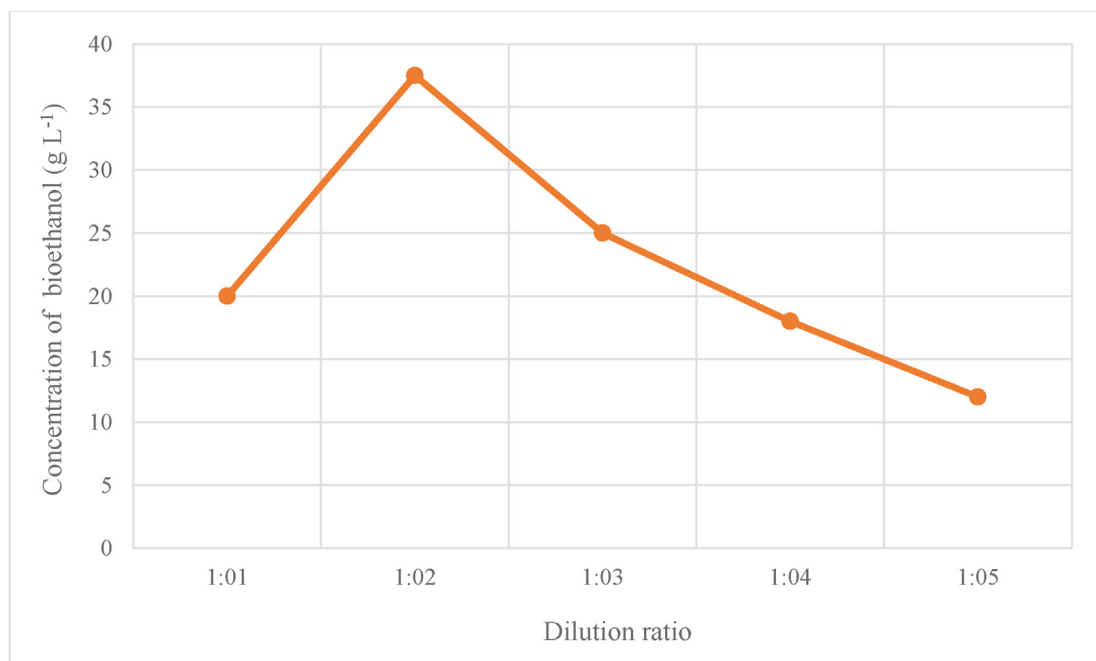


Figure 3. Reaction for production of bioethanol in 5L Autoclavable Glass fermenter.

anaerobic digester [13] which can be further utilized as an energy source for heating, cooking, lighting, and engine operation. Mass balance for the above bioethanol production can be given in figure S2 in supplementary information.

3.4. Study of solid-liquid ratio

From the above results, BSS-10 particle size gives maximum yield for sugar and bioethanol. Hence BSS-10 particle has been considered as the optimum size for the study of different solid-liquid ratios. The figure shows the study of different solid-liquid ratios from 1:1 to 1:5. With a 1:2 solid: liquid ratio 37.5 g L⁻¹ bioethanol was produced. By increasing the dilution ratio to 1:3, 1:4, 1:5, the concentration of bioethanol is decreasing due to increasing the dilution which further causes difficulty in the separation of the product during vacuum distillation. At 1:1 solid: liquid ratio concentration of bioethanol produced was less due to insufficient moisture content required for hydrolysis. Hence 1:2 solid: liquid ratio has been taken as optimum. As per the reported literature for hydrolysis of CBC solid-liquid ratio was 1:6 [18]. In this study, the optimized solid-liquid ratio was 1:2.

3.5. Analysis of residue

Analysis of residue of deoiled cakes was done at Chromein laboratory, Pune for C: N ratio to utilize the residue as organic manure. Organic manure analysis was done for optimized size only. After analysis the C: N ratio obtained was 23.56 with 2.31% Nitrogen, 1.48 % P₂O₅, and 0.96% K₂O. This type of organic manure boosts the proliferation of fungi in soil responsible for the plant taking advantage of nutrients. It improves soil's physical, chemical, and biological properties, as well as soil structure and texture. It increases the water retention capacity of the soil. It acts as much thereby minimizing the evaporation losses of moisture from the soil.

3.6. Environmental sustainability of the process

Per year production of CBC in India is 0.50 million metric tons which causes pollution. Due to ricin content, it cannot be used for animal feeding and we cannot use it as such in soil dumping this waste deoiled cake is used as a raw material in this process. This process includes hydrolysis and fermentation which are environment-friendly reactions. The residue generated after the separation of bioethanol was used as organic manure which improves the soil quality.

4. Costing for production of bioethanol

The final ethanol cost was roughly estimated according to the economic evaluations. Hence the production cost for bioethanol obtained was 32 Rs/L only by using CBC. It is one of the novelties of this work as per the literature available [14,18].

5. Conclusions

The novelty of this work is the production of bioethanol from CBC with optimized production cost, solid-liquid ratio, and particle size while the waste residue is tested as organic manure successfully. The acidic pretreatment and enzymatic hydrolysis process pointed out as a promising procedure providing optimum process conditions that is pH 7, temperature 35 °C and concentration of *T. viride* used was 3 g with optimized solid-liquid ratio 1:2. The present work exhibited a study for different particle sizes of CBC. The optimum particle size BSS-10 produces 76 g L⁻¹ reducing sugars by using *T. viride* enzyme which will further convert into bioethanol and produces 37.5 g L⁻¹ bioethanol by the fermentation process. *Saccharomyces cerevisiae* was used as a fermenting agent with a 10% (w/w) concentration for bioethanol production. Accordingly, we can industrially benefit from the CBC waste from

castor oil production and the biodiesel industry. As per the recent literature cost, reduction and scale-up are the two main parameters which need to be studied [18]. Based upon the optimum results of the present study showed that integrated ethanol production through the proposed technology cost around 32 Rs/L. However further detailed investigation is needed to determine a more accurate estimation about the feasibility of the technology and economic details [34].

Declarations

Author contribution statement

Minal Deshmukh: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Ashwini Pande: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Anant Marathe: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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