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Sustainable approach to produce bioethanol from Karanja (*Pongamia pinnata*) oilseed residue

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Abstract: Agricultural biomass is the world's largest, most sustainable, and most promising renewable energy source. This study investigated the potential of Karanja (*Pongamia pinnata*) oilseed residual waste (defatted kernel, hull, and their mixture) as a source of ethanol. The pretreatment of the finely ground samples was carried out using dilute sulfuric acid (0.5%) hydrolysis at a higher temperature of 121 °C and 15 psi pressure for 90 min. The pretreatment usually removes the lignin and facilitates the hydrolysis of cellulose and hemicelluloses. They were further hydrolyzed using 5% H_2SO_4 at 50 °C for 70 h. The analyses of hydrolyzed products have shown the presence of 66.06–67.12 mg g^{-1} of total reducing sugars, 0.41–0.47 mg g^{-1} of glucose, and 0.36–0.45 mg g^{-1} of xylose, from which the rate of hydrolysis and conversion percentage of cellulose to glucose (44.44%–55.16%) and hemicelluloses to xylose (53.76%–67.17%) was obtained. The hydrolyzed product of complex polysaccharide was further converted into ethanol by fermentation at 32 °C on an orbital shaker using commercial yeast. The results showed ethanol yield of about 10.3%, 8.3%, and 3.3%, respectively in fermentation broths of kernel, mixture, and hull samples, thus indicating Karanja oilseed wastes as potential sources for ethanol production.

Key words: Acid hydrolysis, fermentation, Karanja oilseed residues, pretreatment, renewable waste

1. Introduction

Energy is considered a prime agent in the generation of wealth and a significant factor in economic development. Energy resources have been split into 3 categories: fossil fuels, renewable resources, and nuclear resources. As limitations of fossil fuels, their contribution to greenhouse gas emissions, rise in crude oil prices in the international market, resource development, and waste management, especially in fast-developing countries, all favor liquid biofuel production across the globe. Biodiesel and bioethanol are 2 types of biofuels currently available. Biodiesel can be blended with diesel and bioethanol is mainly blended with petrol. Ethanol is an attractive alternative fuel because it is a renewable, biobased resource and it is oxygenated, which has a potential to reduce particulate emission in compression ignition engines. In addition to that, bioethanol has a higher octane number, higher range of flammability limits, higher flame speeds, and higher heats of vaporization than gasoline.

Since global climate change will have negative impacts on feedstock development for biofuel production, it is a challenge to meet the projected increases in biofuel's share in the fuel market (Fink and Medved 2011). Lignocellulosic raw material is emerging as a very attractive source of fuel bioethanol. Production of ethanol from lignocellulosic biomass contains 3 major processes, including pretreatment, hydrolysis, and fermentation (Chang and Holtzapple 2005). The macroscopic and microscopic size of biomass and crystalline structure of lignin is altered by pretreatment in such a way that it facilitates the hydrolysis of hemicellulose and cellulose to fermentable sugars. The obtained sugars are converted into ethanol by using suitable microorganisms. In general, pretreatment methods can be classified into 3 categories including physical, chemical, and biological pretreatment (Zheng et al. 2009). The degradation of hemicellulose by commonly used chemical processes yields xylose as the major fraction and arabinose, mannose, galactose, and glucose in smaller fractions in addition to potential microbial inhibitors. Ethanol-tolerant yeast has the ability to degrade some of the inhibitors; however, all compound aggregates may determine the toxicity of hydrolysate (Mussatto and Roberto 2004).

Hydrolysis is the breakdown of cellulose into cellobiose and glucose and hemicellulose to xylose, with the help of either enzymes or acid. The enzymatic hydrolysis involves mixing pretreated material with enzymes such as cellulase and β-glucanase, xylanase, or a mixture of

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several enzymes, namely endoglucanases (attacks the low-crystallinity region in the cellulose fiber, creating free chain ends), exoglucanase or cellobiohydrolase (causes removal of the cellobiose unit from free chain ends), and β-glucosidase (hydrolyzes cellobiose to produce glucose), as described Duff and Murray (1996), or glucuronidase, acetylesterase, xylanase, β-xylosidase, galactomannanase, and glucomannanase (degrade hemicellulose) (Bisaria 1991). A few microbial genera such as *Neurospora*, *Monilia*, *Paecilomyces*, and *Fusarium* have been used extensively to ferment cellulose directly to ethanol by a process called simultaneous saccharification and fermentation (SSF) (Lynd et al. 2005). Both the hydrolyzing enzyme and the fermenting organism have to operate under the same conditions, which results in a decrease in sugars, and ethanol yields the consolidated bioprocessing, featuring cellulase and xylanase production, cellulose and hemicellulose hydrolysis, and fermentation in a single step, it is an alternative approach that has good potential (Saxena et al. 1992). The other method of hydrolysis is known as direct microbial conversion (DMC), where all cellulosic biomass is converted to ethanol, in which both ethanol and all required enzymes are produced by a single microorganism. However, DMC is not considered the leading alternative process today because of the unavailability of organisms that produce cellulase, other enzymes, and ethanol at the required high concentrations and yields (Mishra et al. 2011). Hydrolysis by either concentrated acid or dilute acid is commonly used. Both processes are associated with certain advantages and disadvantages. The concentrated acid hydrolysis method uses concentrated (40%–70%) sulfuric acid as compared to 1%–2% sulfuric acid concentration at a high temperature (Nutawan et al. 2010). About 90% of both hemicellulose and cellulose sugars are recovered via the concentrated acid hydrolysis process, but it requires the separation of sugars and acid from the mixture. Additives such as lime or dilute sodium hydroxide may be used to decrease the acidity. The separation process also adds unwanted cost. Diluted acid hydrolysis occurs in less time than concentrated acid hydrolysis and is additionally advantageous in a continuous process. The disadvantages of this method are that only about a half portion of the sugars is efficiently converted to ethanol and a large portion of the sugars is degraded into infermentable sugars under high temperature and pressure. The hydrolyzed material is finally fermented with the help of suitable fermenting microorganisms to produce bioethanol. Depending on the raw material and the microorganisms used, different workers adopt different methods to produce ethanol. Generally, modified strains of *Saccharomyces cerevisiae*, *Aspergillus niger*, and *Zymomonas mobilis* are some of the widely used microorganisms for ethanol fermentation. Overall, it is evident that ethanol made from biomass has the greatest advantage in terms of environment, economics, and infrastructure.

The first-generation bioethanol made from starch and sugar appears unsustainable because of the potential stress on food. Introduction of second-generation biofuels, produced from cheap and abundant plant biomass, is seen as the most attractive solution to this problem (Gomez et al. 2008). Recently, Jatropha oilseed cake obtained as a byproduct from the oil extraction press (Parawira, 2010) was reported to be rich in carbohydrates, fibers, water, and carbon content, along with low contents of hydrogen and oxygen, and it was utilized for the production of ethanol using acid hydrolysis treatment (Visser and Adriaans 2007). Another such material available in abundance is Karanja (*Pongamia pinnata*), widely distributed throughout tropical Asia and the Seychelles Islands, South East Asia, Australia, and India (Prajapati et al. 2003). In many parts of the world, production of Karanja for industrial (biodiesel) and medicinal purposes is gaining popularity (Nagalakshmi et al. 2011). *Pongamia pinnata*, belonging to the family Leguminosae, is a prominent species having nonedible oilseed that can be grown easily in available wastelands. In India, about 0.93×10^6 ha of wastelands are covered with *Pongamia* trees in 8 states (Kumar 2010). The yield of oilseed per tree is between 8 kg and 24 kg. The typical oilseed has 30%–33% oil (Padhi and Singh 2011). From the above discussion of *Pongamia pinnata* in India, it can be estimated that the production of biodiesel from this oilseed is going to increase in the near future and the residual waste will remain in large quantities. Disposal of defatted cake left over after expelling oil from the seed will be emerging as a major problem in upcoming decades (Subbarao 2006). Thus, in the present study, efforts have been made not only to produce ethanol from Karanja oilseed cake, but aslo to treat other residual waste such as its defatted hull and a mixture of kernel and hull (50:50) by using dilute acid hydrolysis for the conversion of cellulose and hemicellulose into reducing sugars and finally fermenting the sugars into ethanol. Since the method for utilization of the Karanja oilseed residual waste for ethanol production is still a rare but sustainable approach, work in this area offers hope of new renewable raw material for ethanol-based industries.

2. Materials and methods

2.1. Sample preparation

The dried *Pongamia pinnata* whole seeds were obtained from the Indian Biodiesel Corporation, Baramati, Pune, Maharashtra, India. The whole seeds were cleaned and separated manually to obtain kernel and hull. Defatted kernel, hull, and the mixture of hull and kernel (50:50) were used as residual waste for ethanol production. For defatting,

samples and mixtures were first ground separately using a mechanical grinder followed by oil extraction using hexane as the solvent on a Soxhlet apparatus. The leftover defatted residues were dried in an oven at 70 °C to 80 °C overnight for removal of residual solvent. These dried samples were ground and passed through a 30-mesh-size sieve and stored in air-tight containers until further use.

2.2. Chemical analysis of defatted residues

Chemical analysis of defatted oilseed residues, dried kernel, hull, and mixture was carried out by using various biochemical methods. Neutral detergent fiber as described by the official methods of analysis of the AOAC (1975) and Goering and Van Soest (1975) was used for lignin determination. Total nitrogen was estimated by the Kjeldahl method and multiplied by a factor of 6.25 to get the crude protein content. The phenol sulfuric acid method (Dubosis et al. 1956; Krishnaveni et al. 1984) was used for total carbohydrate estimation. The amount of total carbohydrate was expressed as glucose equivalent by using standard glucose calibration curves having concentrations of 10–100 µg mL^{-1} ($r^2 = 0.994$). Cellulose was analyzed by the anthrone method (Updegroff, 1969) and expressed as mg g^{-1} of sample using standard cellulose calibration curves having concentrations of 40–200 mg mL^{-1} ($r^2 = 0.997$). The content of hemicellulose was estimated by analyzing neutral detergent fiber and acid detergent fiber (Goering and Van Soest 1975; Sadasivam and Manickam 1996).

2.3. Processing of residual biomass

The residual samples were further processed for ethanol production involving 3 steps: pretreatment of the residues, acid hydrolysis of the pretreated residues, and finally fermentation of the hydrolysate by commercial yeast to produce ethanol, described in detail as follows.

2.3.1 Pretreatment

Pretreatment of kernel, hull, and mixture was done to make all residues porous and to facilitate hydrolysis. Five grams of defatted residues were pretreated using an equal volume of 0.5% sulfuric acid. These samples were heated at a temperature of 121 °C and 15 psi pressure for 90 min. The pretreated samples were qualitatively analyzed using Molisch's test and Benedict's test (Sadasivam and Balasubramanian 1985), respectively, for the presence of total carbohydrate and reducing sugars. The samples were extensively washed with distilled water (Dowe and McMillan 2001), followed by centrifugation at 2200 rpm for 10 min, to remove inhibitors of yeast metabolism and the residual glucose until 0.1 g L^{-1} as in the supernatant estimated with the glucose oxidase method (Malik and Singh 1980; Krishnaveni et al. 1984). Bial's test was done for the presence of pentose sugar in washing (Sadasivam and Balasubramanian 1985). The residual pretreated samples were dried and analyzed for cellulose and hemicelluloses as per the above-mentioned procedure.

2.3.2 Hydrolysis

The pretreated residues were hydrolyzed by using an equal volume of dilute acid (5% H_2SO_4) hydrolysis at 50 °C for 70 h in glass bottles sealed with paraffin film tightly to restrict acid vapor formed due to heat. Intermittent shaking was given to prevent precipitation. Aliquots of samples were removed from the hydrolysate for analysis of total reducing sugars (Somogyi 1952) and expressed as glucose equivalent using standard glucose calibration curves (10–100 µg mL^{-1}, $r^2 = 1$). Glucose content was as estimated by the glucose oxidase method and expressed as glucose equivalent using standard glucose calibration curves (10–100 µg mL^{-1}, $r^2 = 0.998$), and xylose was estimated by taking the difference of total reducing sugar and glucose (Salvachua et al. 2011). The rates of hydrolysis of complex sugars, cellulose, and hemicellulose and the degree of conversion of these polymeric forms into simpler form were calculated by using the following formulae (Arthe et al. 2008).

2.3.2.1 Rate of hydrolysis (V)

$V = ds / dt = \text{reducing sugar}_{t} - \text{reducing sugar}_{t0} / t - t_0$,

where reducing sugar $_t$ is the concentration of sugar after time t, reducing sugar $_{t0}$ is the concentration of reducing sugar before hydrolysis, and t and t_0 are the final and the initial time in hours, respectively.

2.3.2.2. Cellulose conversion percentage (CC%)

$CC\% = (\text{glucose}_{t} - \text{glucose}_{t0}) \times 100 / C$,

where glucose $_t$ is the concentration of glucose after hydrolysis time t and glucose $_{t0}$ is the concentration of glucose before hydrolysis. C is the concentration of cellulose before hydrolysis.

2.3.2.3. Hemicellulose conversion percentage (HC%)

$HC\% = (\text{xylose}_{t} - \text{xylose}_{t0}) \times 100 / H$,

where xylose $_t$ is the concentration of xylose after hydrolysis time t and xylose $_{t0}$ is the concentration of xylose before hydrolysis. H is the concentration of hemicellulose before hydrolysis.

2.4. Fermentation

Yeast fermentation of hydrolyzed residual samples was carried out anaerobically for up to 9 days, separately in triplicate sets of 100 mL of broth each, to produce bioethanol from released sugars during hydrolysis. The fermenting yeast was obtained from commercially available active dried yeast (Suprim foods product, Pune, India). The dried yeast was activated by rehydrating (0.3 mg 50 mL^{-1} distilled water) at 37 °C on a shaker at 90 rpm for 2 h in 250-mL Erlenmeyer flasks. Yeast suspension of optical density 2.0 at 625 nm was used as the inoculum in the fermentation process. Prior to fermentation, the pH of the broth was adjusted to 4.2 by adding the required amount of NaOH pellet to allow yeast growth, and the volume of the broth

was brought to 100 mL using distilled water. The broth was sterilized by autoclaving (120 °C, 15 psi pressure, and 20 min) before inoculating the yeast. The fermentation was carried out in a moist heat-sterilized closed conical flask at 32 °C and an agitation rate of 60 rpm on an orbital shaker. The fermentation assembly was prepared by using a pair of flasks with outlet tubing connected to one flask and its mouth was tightly sealed with a rubber cork to maintain anaerobic condition. The CO_2 released through the outlet tubing was collected in a second flask containing lime water and turned the lime water milky. The aliquots of samples from the first flask were collected after every 24 h throughout the fermentation and were analyzed for total reducing sugar and glucose content. The amount of ethanol produced was determined by using the potassium dichromate method using the standard ethanol calibration curves (20–100 mg mL^{-1}, $r^2 = 0.995$) (Caputi et al. 1968) and was further processed for distillation.

3. Results

The chemical compositions of oil, cellulose, hemicelluloses, lignin, protein, and total carbohydrate obtained in defatted kernel, hull, and their mixture are given in Figure 1. The Karanja hull sample had the least oil content at 3.5%,

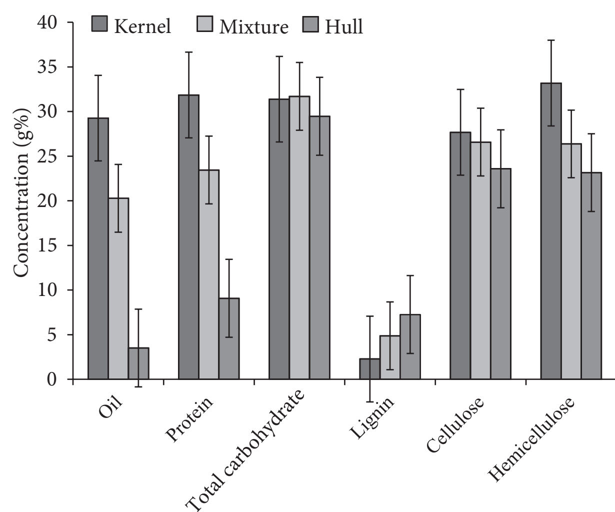

Figure 1. Chemical composition of Karanja defatted kernel, hull, and mixture. The values are means ± standard deviations (n = 3).

while the kernel sample had the maximum oil content at 29.36%. The oil content in the mixture sample was 20.28%. Similarly, the protein percentage was higher in the kernel sample (31.85%), followed by the mixture (23.44%) and hull (9.07%). The lignin content was the maximum in the hull sample (7.25%), followed by the mixture (4.87%) and kernel (2.27%). The total carbohydrate content was obtained in the range of 31.38%–33.18%, cellulose at 26.57%–31.69%, and hemicellulose at 23.15%–29.46% in all the residues.

Pretreatment is an unavoidable necessity for all cellulosic biomass, which has been used extensively by many workers until now (Moiser et al. 2005). The results of pretreatment were as represented in Table 1. The comparison of polymeric sugars before and after pretreatment showed about 30%–42% losses in cellulose content and 48%–51% losses in hemicellulose content. The acid hydrolysis of the lignocellulosic substrate is a cost-effective and sustainable approach instead of enzymatic hydrolysis. The pretreated residues were carried forward for acid hydrolysis using 5% sulfuric acid, after which the amounts of total reducing sugar, glucose, and xylose liberated in residues were observed, as shown in Table 2. These data were used for determining the rate of hydrolysis in terms of glucose, as well as total reducing sugars released. The complex sugar conversion percentage, i.e. cellulose to glucose and hemicelluloses to xylose, was also obtained. It was evident that cellulose to glucose conversion was lowest in the hull sample (44.44%) and maximum in the kernel sample (55.16%). The hemicellulose to xylose conversion was 67.17% and 53.76%, respectively, in the mixture and the kernel sample.

Different types of genetically modified microorganisms have been used to utilize glucose, xylose, mannose, galactose, and arabinose, which are 5 major sugars from biomass during fermentation (Mishra et al. 2011). In the present study of fermentation using commercial yeast, it was observed that in the kernel and mixture broth, initially there was a slow rate of consumption of total reducing sugar (Figure 2) and glucose (Figure 3) during the first 2 days of fermentation. However, the rapid rate of utilization of total reducing sugar including glucose in next 2–6 days of fermentation, again followed by the slow rate of depletion

Table 1. The comparison of amounts of cellulose and hemicellulose before and after pretreatment. The values are means ± standard deviations (n = 3).

Composition (mg g^{-1})	Kernel		Hull		Mixture	
	Before	After	Before	After	Before	After
Cellulose	94.03 ± 0.67	60.13 ± 0.99	112.24 ± 1.07	65.04 ± 1.04	104.52 ± 0.93	72.22 ± 0.89
Hemicellulose	88.83 ± 1.48	45.48 ± 0.64	97.99 ± 1.05	47.89 ± 1.00	93.87 ± 0.29	45.99 ± 1.36

Table 2. Acid hydrolysis studies in terms of estimation of reducing sugar released from cellulose and hemicellulose, conversion of cellulose to glucose and hemicellulose to xylose, and rate of hydrolysis in terms of total reducing sugar, glucose, and xylose. The values are means ± standard deviations (n = 3).

Oil seed residue	Reducing sugar released ($mg\ g^{-1}$)			Sugar conversion percentage ($mg\ g^{-1}$ %)		Rate of hydrolysis ($mg\ g^{-1}\ h^{-1}$)		
	Total reducing sugar	Glucose	Xylose	Cellulose to glucose	Hemicellulose to xylose	Total reducing sugar	Glucose	Xylose
Kernel	66.06 ± 1.34	36.25 ± 1.2	29.71 ± 0.6	55.16 ± 0.5	53.76 ± 0.7	0.82 ± 0.0	0.47 ± 0.0	0.36 ± 0.0
Mixture	72.88 ± 1.16	34.12 ± 1.4	38.76 ± 2.5	45.14 ± 1.2	67.17 ± 1.3	0.91 ± 0.01	0.47 ± 0.02	0.45 ± 0.01
Hull	67.12 ± 1.84	29.71 ± 1.1	37.41 ± 1.0	44.44 ± 0.6	60.46 ± 1.6	0.83 ± 0.02	0.41 ± 0.02	0.42 ± 0.00

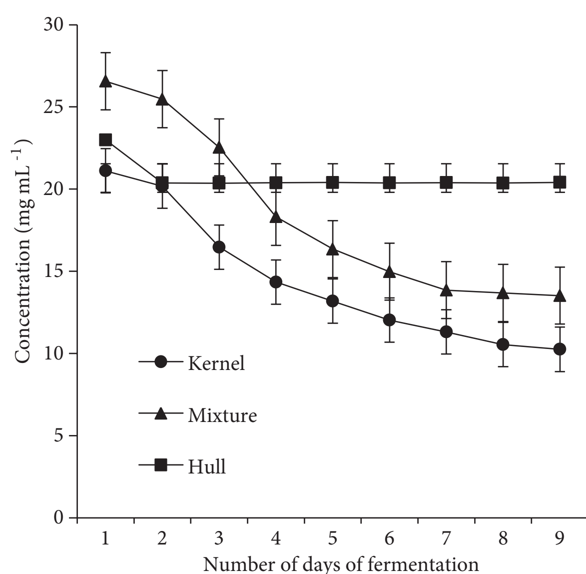

Figure 2. Day-wise estimation of total deducing sugar in kernel, mixture, and hull samples during fermentation. The values are means ± standard deviations (n = 3).

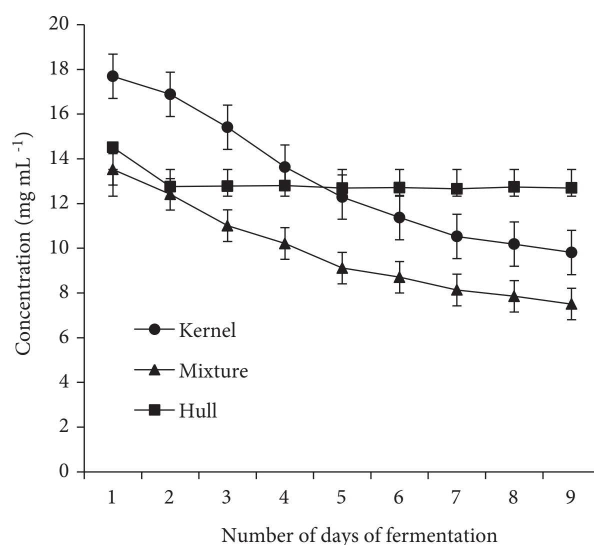

Figure 3. Day-wise estimation of glucose in kernel, mixture, and hull samples during fermentation. The values are means ± standard deviations (n = 3).

of sugars until the ninth day, was observed. The amount of total reducing sugar, glucose, and xylose estimated after the first day of fermentation in the broth was: 21.11 mg mL^{-1}, 17.69 mg mL^{-1}, and 3.42 mg mL^{-1}, respectively, in kernel; 22.99 mg mL^{-1}, 14.51 mg mL^{-1}, and 8.48 mg mL^{-1}, respectively, in hull; and 26.56 mg mL^{-1}, 13.53 mg mL^{-1}, and 13.03 mg mL^{-1}, respectively, in mixture sample. The amount of total reducing sugar, glucose, and xylose estimated on the last day of fermentation, meanwhile, was: 10.25 mg mL^{-1}, 9.81 mg mL^{-1}, and 0.44 mg mL^{-1}, respectively, in kernel broth; 20.37 mg mL^{-1}, 12.75 mg mL^{-1}, and 7.62 mg mL^{-1}, respectively, in hull broth; and 13.51 mg mL^{-1}, 07.50 mg mL^{-1}, and 6.01 mg mL^{-1}, respectively in mixture sample. The increase in ethanol production (Figure 4) was in accordance with the decrease in the total reducing sugars, glucose, and xylose in all the broths. The ethanol content was increased in the kernel sample from 0.013 mL mL^{-1} to 0.105 mL mL^{-1} of broth, in hull sample from 0.025 to 0.033 mL mL^{-1} of broth, and in the mixture sample from 0.011 to 0.083 mL mL^{-1} of broth after 9 days of fermentation.

4. Discussion

With the ever-increasing demand for energy and the fast depletion of petroleum resources globally, there is an increased interest in alternative fuels, especially liquid transportation fuels (Wyman 2007; Lynd et al. 2008). India has shown positive signs towards renewable energy technologies and committed to the use of renewable sources to supplement its energy requirements. In 2003, the Planning Commission of the Government of India presented an extensive report on the development of

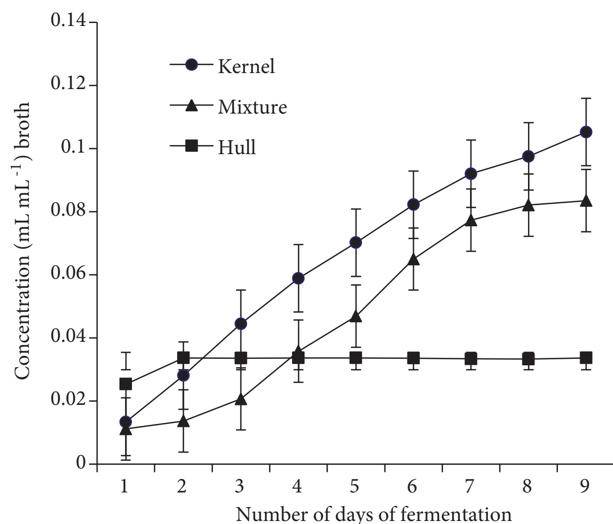

Figure 4. Day-wise estimation of ethanol produced in kernel, mixture, and hull broth during fermentation. The values are means ± standard deviations (n = 3).

biofuels (Planning Commission 2003), and bioethanol and biodiesel were identified as the principle biofuels to be developed for the nation. The blending targets for ethanol and biodiesel in gasoline or petroleum and diesel were proposed as 10% and 20% by 2011–2012, respectively (Planning Commission 2003), and a 5% ethanol blend in gasoline was made mandatory in 11 states and 3 union territories of the nation. Indian distilleries use molasses as the feedstock for ethanol production and the annual supply of molasses is sufficient only for producing approximately 2.7×10^9 L of ethanol (Sukumaran et al. 2010). Hence, in this regard, ethanol production from lignocellulosic material is needed. The availability of Karanja oilseed and defatted oilseed cake in India was estimated to be around 0.20 and 0.145×10^6 t yearly, respectively, before the introduction of the Biodiesel Program (Radhakrishna 2003). The total ethanol demand for all sectors combined was approximately 3020×10^6 L in 2011–2012 (Ray et al. 2011). The available Karanja oil seed cake (0.145×10^6 t) in India can roughly fulfill up to 10% of the ethanol requirement.

The chemical composition has shown that all the residues have a sufficient quantity of cellulose and hemicelluloses and the lowest amounts of lignin. Lignin is a very complex molecule constructed of phenylpropane units linked in a 3-dimensional structure, which is particularly difficult to biodegrade. Lignin is the most recalcitrant component of the plant cell wall, and the higher the proportion of lignin is, the higher the resistance to chemical and enzymatic degradation (Taherzadeh and Karimi 2008). Since there was a smaller amount of lignin obtained in the kernel as compared to the hull and mixture samples, the kernel sample has a relatively higher potential to undergo chemical degradation.

The aim of pretreatment was to alter recalcitrant properties of lignocellulosic material in order to prepare the material for chemical degradation. Since cellulose and hemicellulose are cemented together by lignin (Mohammad and Keikhosro 2008), the loss of both cellulose and hemicellulose fraction after pretreatment suggests loss of the recalcitrance of lignocellulosic material. As reported by Dowe and McMillan (2002), pretreated samples contain acetic acid and furfural, other inhibitors of yeast metabolism. Hence, after pretreatment all samples were washed for removal of 3 major classes of inhibitor: organic acids (acetic, formic, and levulinic acids), furan derivatives (furfural and 5-hydroxymethylfurfural), and phenolic compounds (Chandel et al. 2011). The acid hydrolysis indicated that in addition to glucose, xylose also contributed to total reducing sugars, which support the results of xylose content calculated in the present work. This was also supported by the reported literature on agriculture residues showing that the dominant sugar in hemicellulose is xylose (Lavarack et al. 2002). Thus, the pretreatment and acid hydrolysis performed on various residual biomasses of Karanja oilseed to convert the cellulose into glucose and hemicellulose into pentose sugar xylose has shown positive results.

The depletion of sugar contents was observed during fermentation and remained constant on the ninth day in the kernel and mixture broth. However, the reduction of sugars was limited only up to the second day of fermentation in the hull broth. This ceasing of fermentation in hull broth might be due to the presence of uninvestigated chemical components in the hull sample. As the summation of chemical composition, combined oil, protein, lignin, and total carbohydrate content in the kernel, mixture, and hull was 94.86%, 80.28%, and 49.28%, respectively. This indicates that in a hull, about 50% of the organic and inorganic matter remains uninvestigated, which might include inhibitors of yeast metabolism.

The commercial yeast used for fermentation in current studies has a capacity to utilize pentose sugar in a form of xylose, in addition to glucose, which is a hexose sugar, for production of ethanol. To confirm our findings, we tried inoculating the commercial yeast on MXYP broth having composition of malt extract at 0.3 g, xylose at 1.0 g, yeast extract at 0.3 g, peptone at 0.5 g, and distilled water at 100 mL, which was a modified form of the MGYP broth routinely used for yeast culture (malt extract at 0.3 g; glucose at 1.0 g; yeast extract at 0.3 g; peptone at 0.5 g, and distilled water at 100 mL). The production of ethanol was observed as 0.3 mL mL^{-1} in 100 mL of broth (results not presented here) as estimated by the method discussed above. Thus, it can be said that ethanol production from commercially available yeast was a cost-effective approach

to produce ethanol from both glucose and xylose obtained after hydrolysis of oilseed residues. Recently, studies on production of ethanol from Jatropha oilseed cake (Mishra et al. 2011) and textile cotton waste (Chandrashekhar et al. 2011) under the same laboratory conditions reported an ethanol concentration of 0.016 mL mL^{-1} of broth after the first day, which gradually increased to 0.079 mL mL^{-1} after 9 days of fermentation, in the case of Jatropha oilseed cake, while in the case of textile cotton waste, the ethanol obtained was 0.015 mL mL^{-1} broth on the first day, which increased to 0.080 mL mL^{-1} ethanol at the end of the ninth day of fermentation. The result of ethanol production correlates well with the previous results related to sugar change during fermentation, as there was a slow rate of ethanol production during the first 2 days of fermentation and it accelerated the next 3 days in kernel and mixture broth. Similarly, no further increase in the ethanol content was observed in the hull broth after 2 days of fermentation, which correlates well with the constant sugar content in the hull broth after 2 days of fermentation. These results once again suggest the presence of an inhibitor of yeast metabolism, which needs to be further investigated. This can also be a reason for the lower ethanol yield in the mixture broth as compared to kernel broth, even at the higher content of total reducing sugar.

Furthermore, from the above results it can be correlated that 100 g of Karanja kernel will produce 0.21 L of ethanol and 100 g of Karanja mixture will produce 0.16 L of ethanol, which is much higher than the ethanol production from other reported crop residues, such as barley straw (0.031 L 100 g^{-1}), corn stover (0.029 L 100 g^{-1}), oat straw (0.026 L 100 g^{-1}), rice straw (0.028 L 100 g^{-1}), sorghum straw (0.027 L 100 g^{-1}), wheat straw (0.029 L 100 g^{-1}), and sugarcane bagasse (0.028 L 100 g^{-1}) (Kim and Dale 2004).

In conclusion, Karanja oilseed residual waste such as the kernel and the mixture of the hull and kernel are good raw materials for lignocellulosic ethanol production. Karanja hull alone is an unsuitable substrate for commercial yeast to produce bioethanol. Kernel and mixture samples have shown the capability to undergo acid hydrolysis and fermentation for production of ethanol. Fermentation using commercial yeast has shown less time of lag phase, rapid rates of utilization of fermentable sugars (glucose and xylose), and higher amounts of ethanol yield, which suggest this as a sustainable approach for production of ethanol from Karanja oilseed residual biomass. Further research is required to improve the method of pretreatment to reduce the loss of cellulose and hemicellulose to make the process more economic. Additionally, to utilize sugars released from the hydrolysis of the hull would be a rare approach to utilize hull material for ethanol production. Still no pilot scale studies are available in this regard. Further consideration of Karanja plantations for the development of the biodiesel industry may yield 2 important biofuels: biodiesel and bioethanol, which can be produced at the cost of only a single material.

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