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# Bioethanol production from bamboo (*Dendrocalamus* sp.) process waste

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ARTICLE INFO

*Article history:*
Received 29 July 2012
Received in revised form
10 July 2013
Accepted 11 October 2013
Available online 5 November 2013

*Keywords:*
Bamboo
*Dendrocalamus*
Pretreatment
Saccharification
Bioethanol
Biomass conversion

ABSTRACT

Bamboo as a feed stock for bioethanol production is interesting due to the relatively higher growth rate of these plants and their abundant and sustainable availability in the tropics. *Dendrocalamus* are bamboo varieties common in India, of which large amounts of biomass is generated annually as byproducts of bamboo processing industries. In the current study, process waste from bamboo industry was evaluated as a feedstock for bioethanol production by enzymatic saccharification. Dilute alkali pretreatment of the biomass resulted in efficient removal of lignin, effectively increasing the concentration of cellulose to 63.1% from 46.7%. Enzymatic saccharification of pretreated biomass was optimized following a response surface methodology and the optimal set of parameters for maximal saccharification was derived. Pretreatment method could recover 64.31% of the total sugar polymers and a hydrolysis efficiency of 82.36% was achieved. Direct fermentation of the enzymatic hydrolysate was efficient with ethanol production being 71.34% of theoretical maximum (3.08% v/v ethanol yield). Material balances were calculated for the entire process from raw biomass to ethanol and the overall process efficiency was found to be ~43%. The process has the potential to generate 143 L of ethanol per dry ton of bamboo process waste.



## 1. Introduction

Lignocellulosic biomass is the primary and most abundant organic material on the earth which makes it the most promising resource for the alternative energy [1]. Among the available lignocellulosic feedstock, bamboos are receiving a renewed interest due to their high growth rate and better reduction of carbon footprint compared to an equivalent area of woody plants [2]. Bamboos are a group of perennial evergreens belonging to the true grass family and enjoying wide distribution in India, especially in the north eastern region where it is an important resource with multiple applications [3]. *Dendrocalamus* sp. occupies more than 50% of the total area under bamboo growth in India [4]. India is the second largest producer of bamboo in the world with an annual production of about 32 million tons [5]. About 5.4 million tons of bamboo residues are generated in the country every year by the bamboo processing industries of which about 3.3 million tons remains as surplus [6,7]. Compared to other feedstock, bamboo biomass has a relatively high cellulose and low lignin content which makes it suitable for bioethanol production.

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0961-9534/$ – see front matter 
http://dx.doi.org/10.1016/j.biombioe.2013.10.015

Conversion of biomass into fermentable sugars depends on 1) composition of biomass i.e. – the amount of polysaccharides that can be converted in to fermentable sugars 2) method of pretreatment, which determines the structural changes in biomass 3) the efficiency of enzymatic hydrolysis. Plant biomass is made up primarily of the polymers cellulose, hemicellulose and lignin. Cellulose is the main structural polymer made up of glucose units and the strength of this polymer depends on the degree of polymerization that can vary between plant varieties [8]. Hemicelluloses and lignin are the other polymers, which bind the cellulose and make it harder for hydrolysis. The major hemicellulosic sugars found in the biomass are xylose, arabinose, mannose and galactose among which xylose constitutes the major portion [9]. Lignin is a polymer of aromatic compounds formed from the dehydration of three different monomeric alcohols namely (lignols) trans-p-coumarylalcohol, trans-p-coniferyl alcohol and trans-p-sinapyl alcohol, derived from p-cinnamic acid [8]. Removal of either lignin or the hemicellulose fractions from lignocellulose makes the cellulose polymer easily accessible by the hydrolyzing enzymes and the goal of all pretreatment strategies is to accomplish this. It is important to understand the composition of the biomass and devise appropriate strategies suitable for its pretreatment and saccharification for efficient conversion to bioethanol.

In comparison with chemical hydrolysis of biomass (performed mostly by acid), enzymatic hydrolysis is advantageous due to its specificity, mild operational conditions, and lesser or no byproduct formation [10]. The technical challenges in this process are related to the physico-chemical and structural properties of lignocellulosic biomass, which has to be modified for enzyme access to the polysaccharides for hydrolysis [11]. Altering the structural properties of lignocellulosic biomass will enhance the enzymatic digestibility, and this can be achieved by appropriate pretreatment of biomass. Some of the most common and efficient methods of pretreatment involve high temperature treatment of the biomass with dilute acid or alkali. Though several different pretreatment methods are in practice for biomass processing [12], alkali pretreatment remains one of the most promising, since it can remove a major portion of lignin while retaining the cellulose and hemicellulose in the solid. Dilute alkali pretreatment is known to remove the acetyl and various uronic acid groups in hemicelluloses [13]. The mechanism involved in the removal of lignin is based on the saponification of intermolecular ester bonds in the hemicelluloses and lignin Ref. [10].

Not many studies have been done on Indian bamboo varieties for their suitability as a feedstock for bioethanol production, despite the abundant availability of bamboo process waste and residues in a centralized fashion (near bamboo based industries). The objective of the current study was therefore to evaluate the potential of *Dendrocalamus* sp, the major bamboo variety which enjoys more than 50% distribution in areas of bamboo growth in the country. Compositional and structural analyses of the bamboo biomass were performed before and after pretreatment, which was performed using hot dilute alkali. Conditions of pretreatment and enzymatic hydrolysis were optimized for enhancing sugar yield. The biomass hydrolysate was finally evaluated for alcohol production, which was achieved with more than 70% efficiency indicating the potential for use of this feedstock in bioethanol production.

## 2. Materials and methods

### 2.1. Raw material and preprocessing

Bamboo process residue samples (including bamboo stalk and internodal regions) procured from northeastern provinces of India and were kindly provided by TIFAC, DST-Govt. of India. The samples were air-dried and were cut into ~5–7 cm long pieces using a motorized disc saw. The small pieces of bamboo were then milled using a knife mill and the milled material (size 200 μm –8 mm) was stored in airtight containers until used. Moisture content was estimated prior to use using an infrared moisture analysis balance (A&D, USA).

### 2.2. Composition analysis

Chemical composition of biomass was carried out based on the NREL protocol [14]. Initially the sample was dried to constant weight at 105 °C to measure the total solids in the biomass. The water and ethanol soluble material in the biomass was analyzed by extracting 10 g of moisture corrected biomass in a Soxhlet apparatus [15]. Three hundred milligrams of accurately weighed and moisture corrected, extractive free sample was mixed with 10 ml of 64% (v/v) $H_2SO_4$ and it was hydrolyzed for 4 h at 30 ± 2 °C. The hydrolyzed material was diluted to 100 ml with de-ionized water and subjected to a second hydrolysis by autoclaving the sample for 1 h at 121 °C. The supernatants were collected and neutralized. Total carbohydrates in the biomass were analyzed by HPLC (Shimadzu, Japan) coupled with a carbohydrate analysis column (Biorad Aminex HP 87P). The mobile phase was de-ionized water at a flow rate of 0.6 ml/min and the injection volume was 20 μl. Acid soluble lignin was measured by UV spectroscopy at 205 nm. The acid insoluble lignin was estimated by oxidation method where the sample was heated up to 575 °C in a muffle furnace (14). Soluble protein was measured by Bradford's method [16].

### 2.3. Pretreatment

Pretreatment of the milled *Dendrocalamus* sp. biomass was done using dilute NaOH at elevated temperature in a high-pressure reactor (Amar Equipments, India). Conditions for pretreatment were optimized following a Taguchi design [17]. The parameters studied were -mixing rate (between 50 and 200), residence time (between15 and 60 min) and temperature (between 120 °C and 180 °C), the levels of which were varied according to the Taguchi design matrix (Table 1). The biomass loading was 5% w/w and NaOH concentration was 2% w/w. The samples after pretreatment were neutralized using 1 N $H_2SO_4$ to a final pH of 5.5–6, washed with tap water and was then air-dried (30 °C) to remove excess moisture. The air-dried samples corrected for moisture content were used for hydrolysis using a commercial cellulase preparation (Zytex & Group Co., Mumbai, India). The conditions for hydrolysis were biomass loading of 10% (w/w), enzyme loading 30 Filter Paper Units (FPUs)/g biomass, Surfactant concentration –0.2% (w/w)

**Table 1 – Taguchi design matrix for optimization of alkali pretreatment of bamboo biomass with response (reducing sugar yield).**

| Run# | Temperature °C | Mixing rate (rpm) | Time (min) | Reducing sugar yield (g/g) |
|---|---|---|---|---|
| 1 | 120 | 50 | 15 | 0.349 |
| 2 | 120 | 100 | 30 | 0.308 |
| 3 | 120 | 150 | 45 | 0.393 |
| 4 | 120 | 200 | 60 | 0.440 |
| 5 | 140 | 50 | 30 | 0.410 |
| 6 | 140 | 100 | 15 | 0.475 |
| 7 | 140 | 150 | 60 | 0.504 |
| 8 | 140 | 200 | 45 | 0.401 |
| 9 | 160 | 50 | 45 | 0.538 |
| 10 | 160 | 100 | 60 | 0.576 |
| 11 | 160 | 150 | 15 | 0.453 |
| 12 | 160 | 200 | 30 | 0.578 |
| 13 | 180 | 50 | 60 | 0.670 |
| 14 | 180 | 100 | 45 | 0.682 |
| 15 | 180 | 150 | 30 | 0.764 |
| 16 | 180 | 200 | 15 | 0.730 |

and an incubation time of 48 h. The hydrolysate generated through enzymatic saccharification was analyzed for reducing sugars using the DNS method [18]. Pretreatment efficiency was monitored in terms of the susceptibility for enzymatic hydrolysis, measured as sugar yield from the biomass. The results were analyzed using Design Expert® software (Stat Ease Corp, USA). Conditions that gave the best reducing sugar yield were considered optimal for pretreatment and were adopted for further experiments. Validation of the design model was performed by analyzing the correlation between predicted and experimental values.

### 2.4. *Optimization of enzymatic saccharification*

Optimization of the enzymatic saccharification of pretreated bamboo biomass was carried following a response surface Box Behnken Design matrix [19] generated using Design Expert® software (Stat Ease Corp, USA). The hydrolysis was carried out in 150 ml screw caped conical flasks with a working volume of 20 ml using a commercial cellulase (Zytex & Group Co., Mumbai, India). The parameters optimized were – biomass loading (7.5–15% w/w), enzyme loading (20–80 FPU/g), Tween80 concentration (0.05–0.2% w/w), and incubation time (24–60 h). Each parameter was tested at 3 levels following the design matrix as outlined in Table 2. The hydrolysis experiments were performed in 50 mM citrate buffer at 50 °C in a shaking water bath (150 rpm). After hydrolysis, the supernatants were collected and the total reducing sugar content was analyzed by DNS method [18]. Optimization of parameters for enhancing the response (ie sugar yield) was performed using the numerical optimization function built into Design Expert Software. Three dimensional response surfaces were constructed using the software for analyzing interaction effects.

### 2.5. *Validation of saccharification conditions and time course study*

Validation of the optimized conditions of saccharification was performed by hydrolyzing the biomass in larger working volume employing the optimized conditions. Hydrolysis

**Table 2 – Box Behnken Design matrix for optimization of bamboo process waste saccharification.**

| Run# | Biomass loading (% w/w) | Enzyme loading (FPU/g) | Surfactant (% w/w) | Incubation time (h) | Reducing sugar (g/g) |
|---|---|---|---|---|---|
| 1 | 15 | 50 | 0.125 | 24 | 0.695 |
| 2 | 15 | 50 | 0.125 | 60 | 0.647 |
| 3 | 7.5 | 50 | 0.2 | 42 | 0.152 |
| 4 | 7.5 | 50 | 0.125 | 24 | 0.229 |
| 5 | 15 | 20 | 0.125 | 42 | 0.540 |
| 6 | 11.25 | 50 | 0.125 | 42 | 0.636 |
| 7 | 11.25 | 50 | 0.2 | 60 | 0.847 |
| 8 | 11.25 | 50 | 0.2 | 24 | 0.298 |
| 9 | 7.5 | 20 | 0.125 | 42 | 0.183 |
| 10 | 7.5 | 50 | 0.05 | 42 | 0.246 |
| 11 | 11.25 | 80 | 0.05 | 42 | 0.557 |
| 12 | 15 | 80 | 0.125 | 42 | 0.500 |
| 13 | 11.25 | 20 | 0.05 | 42 | 0.593 |
| 14 | 11.25 | 50 | 0.125 | 42 | 0.508 |
| 15 | 7.5 | 50 | 0.125 | 60 | 0.183 |
| 16 | 11.25 | 50 | 0.125 | 42 | 0.491 |
| 17 | 11.25 | 80 | 0.125 | 24 | 0.343 |
| 18 | 15 | 50 | 0.2 | 42 | 0.706 |
| 19 | 15 | 50 | 0.05 | 42 | 0.352 |
| 20 | 11.25 | 80 | 0.2 | 42 | 0.526 |
| 21 | 11.25 | 50 | 0.05 | 24 | 0.715 |
| 22 | 11.25 | 80 | 0.125 | 60 | 0.471 |
| 23 | 11.25 | 20 | 0.125 | 60 | 0.518 |
| 24 | 11.25 | 50 | 0.05 | 60 | 0.400 |
| 25 | 11.25 | 20 | 0.125 | 24 | 0.469 |
| 26 | 11.25 | 20 | 0.2 | 42 | 0.419 |
| 27 | 7.5 | 80 | 0.125 | 42 | 0.331 |

experiments were conducted in a Parallel Bioreactor (Infors HT, Switzerland) equipped with Ruston turbine impellers and with a working volume of 600 ml. Saccharification was performed in a total volume of 200 ml following one of the predicted optimal combinations of parameters, which used only 47 FPUs/g enzyme loading. The optimum levels of other parameters selected were: biomass loading – 11.27%, mixing rate – 200 rpm and incubation temperature – 50 °C, Tween 80 concentration – 0.05%. One-milliliter samples were taken at 2 h intervals and were analyzed for fermentable sugars using HPLC.

### 2.6. Ethanol production

Ethanol production was studied using the enzymatic hydrolysate of alkali pretreated bamboo. The pretreated biomass was saccharified under the optimized conditions when a hydrolysate containing 6.68% glucose and 1.32% xylose was obtained. A nutrient supplement was added to the hydrolysate so that it contained finally 0.3% Yeast extract, 0.025 M $(NH_4)_2SO_4$, 0.01 M $MnCl_2$, 0.01 M $MgSO_4 \cdot 7H_2O$, 0.05 M $K_2HPO_4$ and 0.05 M $NaH_2PO_4$. Fifty milliliter of the hydrolysate in 100 ml screw capped bottles was inoculated with 5% v/v of a 12 h old seed culture of *Saccharomyces cerevisiae*. Incubation was carried out at 28 ± 2 °C without agitation for 48 h. Fermentation broth was centrifuged at 13,000 rpm for 10 min at 4 °C, and the supernatant was then filtered through 0.22 μm nylon membrane. The filtrate was analyzed for ethanol content by HPLC following a modified method of Soto et al. [20].

### 2.7. Structural characterization of native and pretreated biomass

#### 2.7.1. Scanning electron microscopy (SEM)

In order to study the surface properties, SEM images were taken using JOEL JSM-5600 scanning electron microscope. The samples were first coated with gold and platinum in a JOEL JFC-1200 fine coater and the sample was observed under a voltage of 10–15 kV. Images were acquired at a magnification of 1500×.

#### 2.7.2. XRD

Crystallinity of native and alkali (NaOH) pretreated samples were measured using an X-pert pro diffractometer (PANalytical, The Netherlands) set at 40 kV, 30 mA. Radiation was CuKα ($\lambda = 1.54$ A°), and grade ranges between 10° and 30° with a step size of 0.03°. Crystallinity was determined as percentage of crystalline material in the biomass and was expressed as Crystallinity Index (CrI). Crystallinity of cellulose was calculated according to the empirical method proposed by Refs. [21], for native cellulose. CrI (%) = $[(I_{002} - I_{am})/I_{002}]^* 100$; where $I_{002}$ is the intensity of 002 peak at $2\theta = 22.4$ and $I_{am}$ is the intensity of background scatter at $2\theta = 18.0°$.

## 3. Results and discussion

### 3.1. Biochemical characterization of bamboo biomass

Proportion of chemical components in the biomass varies based on the environmental factors, growing conditions, and maturity of the plant. Quantification of structural components in the biomass can be achieved by eliminating the extractable materials from the biomass by water, followed by ethanol extraction [22]. The extractable materials include non-structural sugars, tannins, chromogenic substances (eg. chlorophylls) etc. Based on the HPLC analysis, it was estimated that the bamboo biomass has a holocellulose content of 63.11% of its dry weight. Glucans were the major fraction, which contributed 46.68% while hemicellulose was 16.43% (Table 3). The acid insoluble fraction, which was 17.66% of total dry biomass, included the insoluble proteins and other inorganic matters in addition to the major fraction – lignin.

### 3.2. Pretreatment

One of the major bottlenecks in biomass to bioethanol conversion is the pretreatment of biomass, since this is one of the most expensive and energy consuming steps in the whole conversion process. Objectives of several research efforts have been directed towards developing energy efficient processes where lesser amount of chemicals, lesser temperature and shorter residence times would be employed [23]. Hot dilute NaOH causes breakage of the ester and glycosidic bonds which causes structural alterations in lignin, partial decrystallization of cellulose and to an extent breakage of hemicellulose [24]. The efficacy of the pretreatment strategy is largely dependent on the type of feedstock itself and regardless of the mode of pretreatment, the final objective is to increase the susceptibility to enzymatic hydrolysis. It therefore becomes imperative that the pretreatment methods need to be evaluated not only with respect to the removal of lignin or breakage of hemicellulose but also on the actual impact of the process on the next step of enzymatic hydrolysis. So in this study, pretreatment efficacy was evaluated in terms of the susceptibility of the pretreated material for enzymatic hydrolysis which was monitored as sugar yield. Table 2 shows the yield of reducing sugars obtained for the different combinations of pretreatment process variables tested following a Box Behnken design.

It was observed that in general, all tested parameters influenced the pretreatment efficiency positively. However, the effect of temperature was highest and this was the only parameter which influenced the process significantly (Fig 1). Though the effect of temperature was dominating, the increase in rate of mixing and residence time could also improve the pretreatment efficiencies. Graphs plotted for the pretreatment efficiency (reducing sugar yields against two of the tested parameters while keeping the others at middle level),

**Table 3 – Biochemical composition of native and pretreated bamboo biomass.**

| Parameters | Native biomass | Alkali pretreated biomass |
|---|---|---|
| Cellulose (%) | 46.68 ± 0.03 | 63.11 |
| Hemicellulose (%) | 16.43 ± 0.29 | 14.19 |
| Lignin (%) | 17.66 ± 0.39 | 5.25 |
| Water & ethanol extractives and others (%) | 19.17 ± 1.17 | 16.75 |

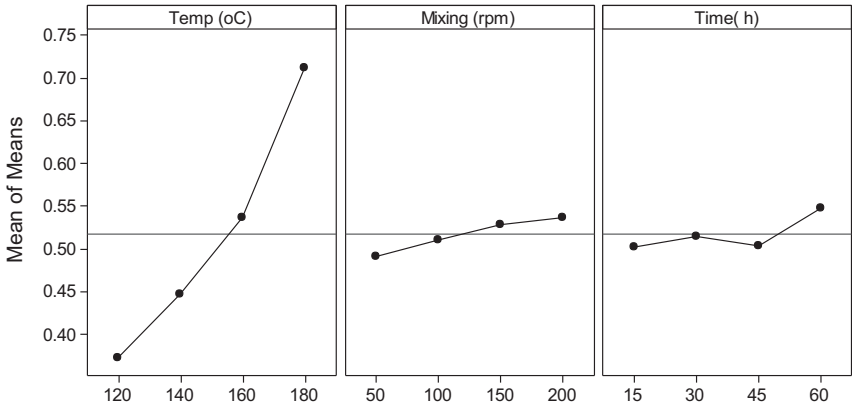


**Fig. 1 – Effect of process conditions on pretreatment efficiency.**

indicated that a reduction in pretreatment temperature can be brought about by using a higher residence time and vice versa without affecting sugar yield (Fig. 2A). Similarly, an increase in mixing rate can bring down the residence time without compromising the pretreatment efficiency (Fig. 2B).

While reduction in pretreatment temperatures is advantageous considering the significant reductions possible in energy requirement; reduction in residence time also serves the reduction in the amount of sugar degradation products

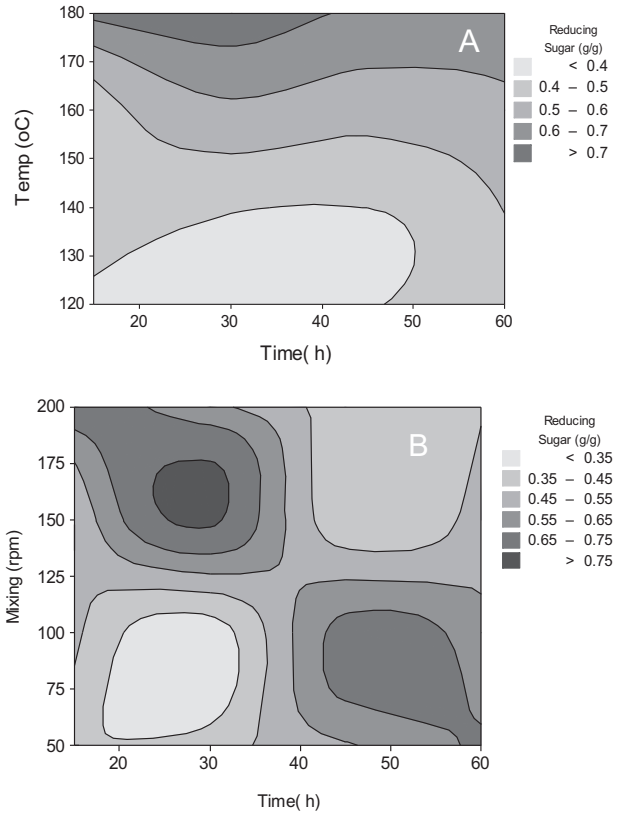


**Fig. 2 – Interaction effects of parameters affecting pretreatment efficiency A) Effect of pretreatment temperature and residence time B) Effect of mixing rate and residence time.**

that are inhibitory to the later fermentation process for generating alcohol [25]. Strategies which will help to achieve both reductions in temperature and residence time are required for an efficient biomass to alcohol conversion process. There are multiple strategies from post pretreatment particle size reduction, to use of gaseous ammonia proposed for achieving this [26,27]. Nevertheless, the importance of process optimizations in obtaining the best-suited conditions that will reduce the pretreatment temperature as well as residence time cannot be overlooked, especially since the parameters and conditions can vary largely depending on the type of biomass feedstock. In the present study, it was observed that the conditions that resulted in maximal sugar yield were a temperature of 180 °C, 150 rpm agitation and a residence time of 30 min when a reducing sugar yield of 0.764 g/g was obtained (Table 1). The biomass loading and NaOH concentrations were 5% and 2% respectively. However, under the same temperature, but with only half the residence time (15 min) and a higher mixing rate (200 rpm), the sugar yield was 0.730 g/g, which is only marginally lower than the best yield obtained under the optimized conditions. It was therefore decided to conduct the bamboo biomass pretreatment under the latter set of conditions which required only 15 min of residence time, for rest of the experiments.

Compositional analyses of the pretreated bamboo indicated that there is an almost 70% reduction in lignin compared to untreated sample and an increase in cellulose content to 63.11% from 46.68%. However, there was also a 10.3% reduction in the hemicellulose content on alkali pretreatment (Table 3). Structural changes in the biomass during pretreatment were analyzed using SEM and XRD. A comparison of the surface morphology of native and pretreated bamboo biomass indicated that alkali pretreatment results in a swelling of the fibers and an increase in surface area. The gaps between fibers were increased on pretreatment, which possibly aids in better enzyme binding and hydrolysis (Fig. 3A and B). Changes in structural properties were also confirmed by XRD analyses of the crystallinity of biomass. The CrI of pretreated biomass had increased to 69.3 from 60.8. Alkali pretreatment of biomass effectively removes lignin without significantly affecting the hemicellulose structure at least in several biomass types, leaving mostly cellulose in the pretreated material. Since the

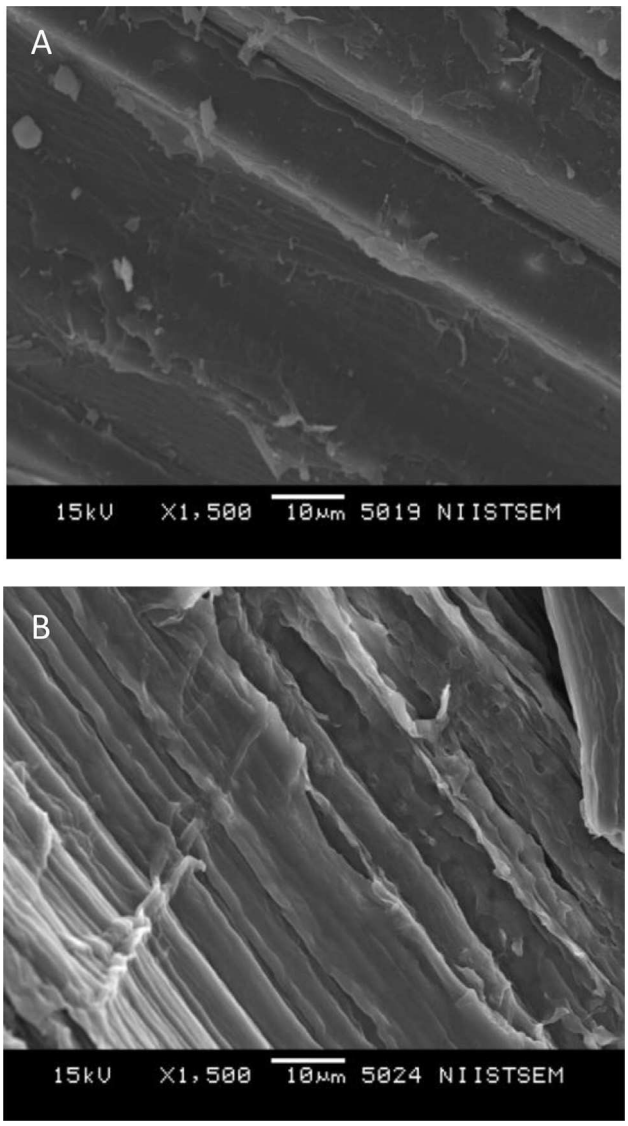


**Fig. 3 – SEM images showing structure modifications resulting from alkali pretreatment of bamboo biomass A) Untreated bamboo process waste showing intact structure B) Alkali pretreated bamboo process waste showing disrupted structure and increased surface area.**

carbohydrate polymers are left undisturbed to a large extent, the CrI can increase considerably as the material after pretreatment contains crystalline cellulose as the major component [27]. However, it may also be noted that a reduction in CrI is observed for the sample in several pretreatment scenarios, either due to the removal of hemicellulose or due to the opening up of cellulose structure induced by the pretreatment resulting in amorphous regions in the polymer [28]. In either case, better hydrolysis efficiencies are reported to be associated with reduced crystallinity [25].

### 3.3. *Optimization of enzymatic hydrolysis*

Enzymatic hydrolysis is believed to be the step which contributes most to the cost of biomass to ethanol conversion due mainly to the cost of enzymes; and several studies have stressed the importance of reducing the cost of this step [29,30]. Designing of proper enzyme blends (cocktails) suitable for a feedstock and fine tuning of the conditions of saccharification are important in reducing the usage of enzyme and the time needed for saccharification and to enhance the yield of fermentable sugars [31]. In the current study, important parameters like the solid liquid ratio (biomass loading), enzyme loading, surfactant concentration and incubation time was optimized so as to obtain maximal sugar yield within the shortest time using minimal amount of enzymes. The yield of reducing sugars obtained for the different experimental combinations of parameters ranged from 0.152 g/g to 0.847 g/g which represented 17.5 and 98.6% of theoretical maximum respectively (Table 2). A second order polynomial function was fitted so as to represent sugar yield as a function of the tested parameters.

$$Y = 0.55 + 0.18A + 0.0001B + 0.007C + 0.026D - 0.047AB + 0.11AC - 0.0005AD + 0.036BC + 0.02BD + 0.22CD - 0.13A^2 - 0.046B^2 - 0.0011C^2 - 0.00063D^2$$

Where $Y$ = Sugar yield and $A$, $B$, $C$ and $D$ are the coded values for biomass loading, enzyme loading, surfactant concentration and incubation time respectively. The Fischer's test for analysis of variance performed on the experimental data indicated that the model was significant with an F value of 6.58 and a $p$-value of 0.006. The model terms A, AC and CD had $p$-values less than 0.05 indicating that increase biomass loading influenced the sugar yield linearly and there was significant interaction between the parameter combinations of biomass loading and surfactant concentration, and between surfactant concentration and incubation time. Within the tested levels, an increase in biomass loading resulted in an increased sugar yield. Lower biomass loadings allow for higher amount of free water resulting in a higher availability of enzyme per unit biomass and a resulting increase in efficiency. However the total yield of sugars tends to be lesser in this case, since the amount of sugar polymers available will be lesser [32]. A higher biomass loading is better for achieving higher sugar concentrations, which is eventually good for the following fermentation step [33]. The observed increase in yield of sugars could be consequence of higher availability of cellulose and hemicellulose due to increased biomass loading. Nevertheless, the observation should be interpreted only in context, since the relationship between biomass loading and sugar yield would be linear only in a range defined by the type of biomass, pretreatment method and the enzymes and conditions used for hydrolysis. It can be easily gauged that biomass loadings beyond the levels defined by above factors would result in a reduced efficiency due to non-specific binding of the enzyme as well as the lesser availability of enzyme. Reducing the non-specific binding to lignin and better availability of enzymes may be ensured by the use of surfactants [34]. The potential advantage of using surfactant is visible in the observed interactions of this parameter with biomass loading and incubation time. Whereas, an increase in surfactant concentrations enable to use higher biomass loadings without affecting the sugar yields (Fig 4A), it also

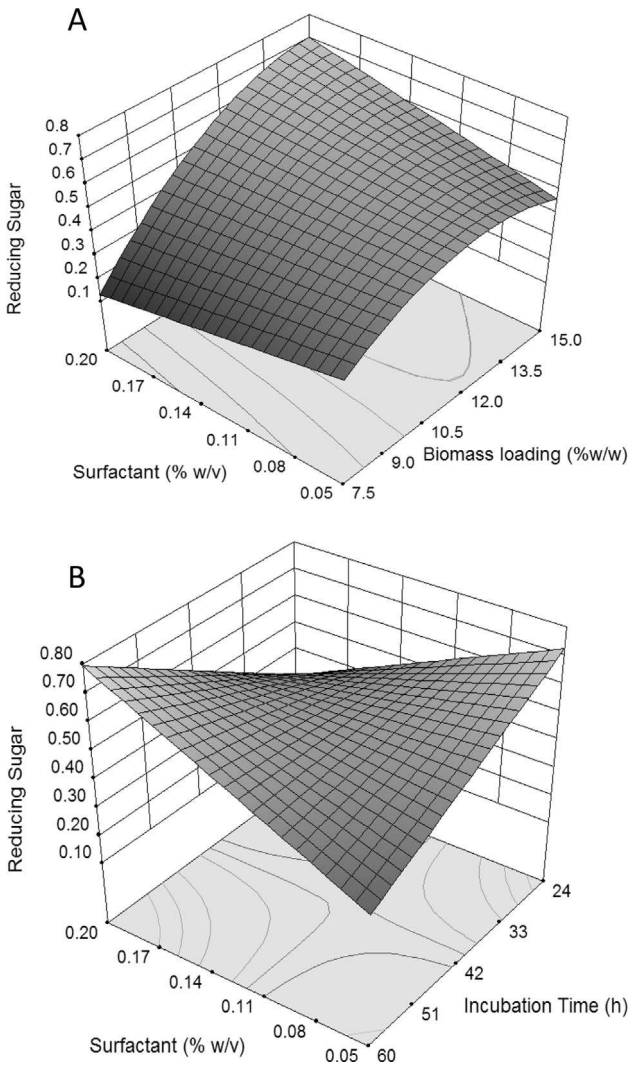


**Fig. 4 – Parameter Interactions in saccharification of bamboo process waste A) Interaction between biomass concentration and surfactant concentration B) Interaction between surfactant concentration and incubation time.**

allows for longer availability of enzyme in its active form allowing the hydrolysis for longer duration (Fig 4B). Though it might be expected that once the maximum sugar concentration is attained it stays constant; it was observed that in trials with this biomass as well as other feedstock, that the sugar concentration is getting reduced with longer incubations. It might be speculated that there is a non-specific binding of sugar on the unhydrolyzed material or transglycosylation by beta glucosidase might play a role. Further studies are warranted to test this.

Optimal conditions for obtaining maximal sugar yield were predicted using the numerical optimization function in Design Expert ® software where the goal was set as maximizing response. Among the multiple solutions provided, 5 were selected based on a high predicted yield between 0.7 g/g and the maximum (0.86 g/g). The conditions predicted for obtaining maximal yield of 0.86 g/g was 13.9% biomass loading, 41 FPUs/g enzyme loading, 0.188% surfactant and 59 h of incubation (Table 4). However, with a combination of parameters that used 11.3% biomass loading, 47 FPU/g enzymes loading, 0.05% surfactant and importantly with only 24 h of incubation, the predicted yield was 0.73 g/g which was not a major reduction from the highest predicted yield. It was therefore decided to validate this set of conditions for bamboo biomass saccharification by performing the reaction in a larger scale in reactor and also to do a time scale study to monitor the rate of sugar production as a function of time.

Data given in Fig. 5 indicated that at 26 h the total sugar yield was 0.744 g/g which was close to what was predicted (0.730 g/g for 24 h incubation). Sugar concentration reached a peak at around 36 h (0.787) and was maintained almost steady with a mean sugar yield of 0.787 ± 0.006 g/g. Considering that the pretreated bamboo contains 63.11 and 14.19% of cellulose and hemicellulose respectively (Table 3), the theoretical maximum of sugar that can be liberated from this feedstock is 0.862 g/g. The amount of sugars that was actually generated during enzymatic hydrolysis performed according to the predicted condition (No. 3, Table 4) was 91.33% of the theoretical maximum possible and it could be achieved with an incubation of 24–36 h, which is a significant reduction in time.

### 3.4. *Ethanol production from bamboo biomass and material flow during biomass to ethanol process*

Enzymatic hydrolysate containing 6.68% glucose and 1.32% xylose was fermented after supplementation with necessary nutrients for yeast growth. Alcohol production after 48 h incubation period was 3.08% v/v (2.43% w/v), which was 71.34% of the theoretical maximum amount possible from the hydrolysate. The flow of material was monitored through every operation from raw biomass to ethanol (Fig 6, Supplementary Sheet 1). Starting from a total of 176.26 g of moisture corrected raw biomass, the final yield of ethanol was 19.95 g (25.28 ml) which represented an overall raw biomass to ethanol conversion efficiency which was 42.82% of the theoretical maximum, implicating that there is a potential for generating 143 L of ethanol per dry ton of milled bamboo biomass using the process developed. The greatest loss of material was during the pretreatment; where 35.69% of biomass was lost in

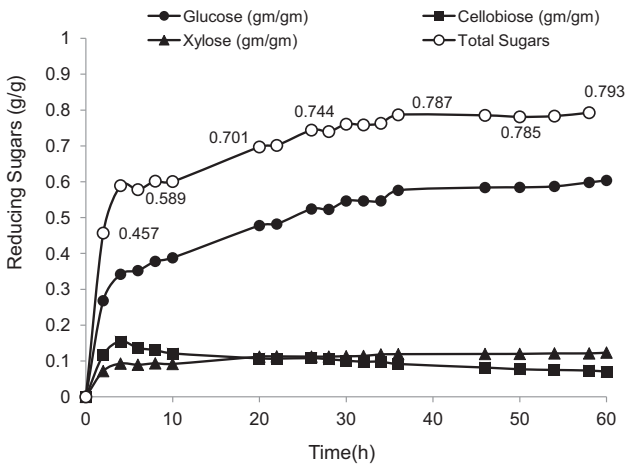


**Fig. 5 – Sugar generation during enzymatic hydrolysis of bamboo biomass.**

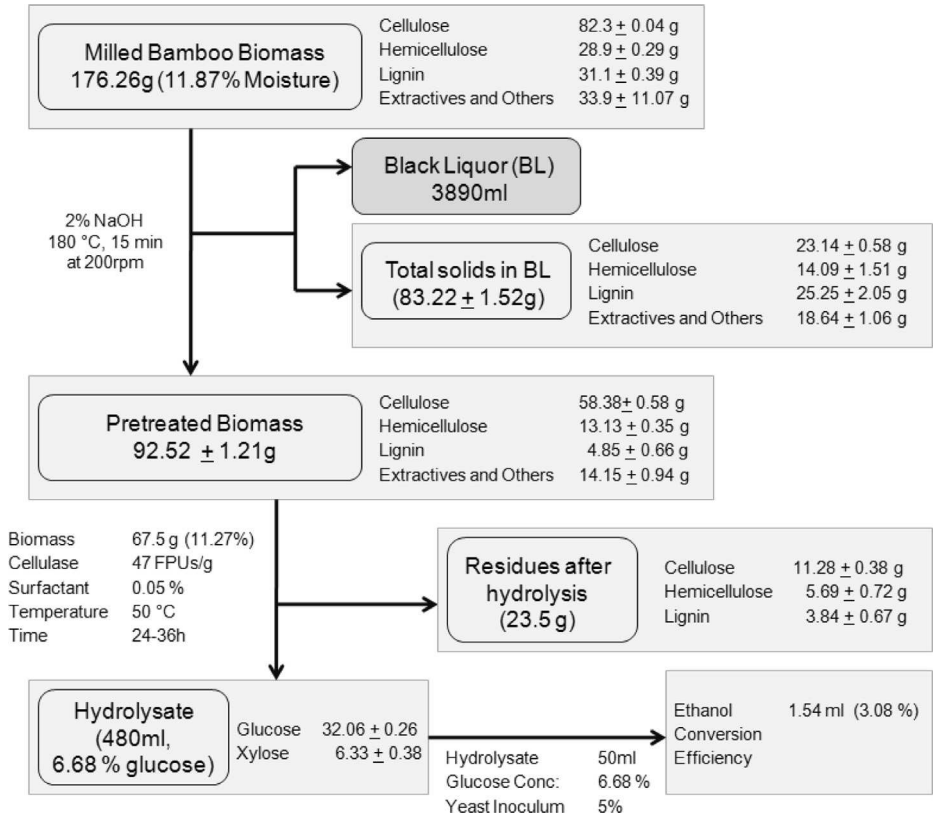


**Fig. 6 – Process Flow and material balance for bamboo to ethanol conversion.**

**Table 4 – Multiple combinations of parameters predicted for hydrolysis of pretreated bamboo.**

| No | Biomass loading (% w/w) | Enzyme loading (FPUs/g) | Surfactant (% w/v) | Incubation time (h) | Reducing sugar (g/g) |
|---|---|---|---|---|---|
| 1 | 14.6 | 29 | 0.190 | 58 | 0.85 |
| 2 | 13.9 | 41 | 0.188 | 59 | 0.86 |
| 3 | 11.3 | 47 | 0.050 | 24 | 0.73 |
| 4 | 11.4 | 58 | 0.050 | 24 | 0.71 |
| 5 | 12.7 | 45 | 0.199 | 60 | 0.87 |

the pretreatment liquor which indicates the need to refine the solid liquid separation step further to recover the fine particles. Also the analysis points out the need for further improvements in hydrolysis and fermentation efficiencies from the current values of 82.36% and 71.34% respectively. Nevertheless, it may be noted that the solid liquid separation done at lab scale with a smaller amount of material will amplify the losses because all steps are done manually with loss of material in sieving, washing etc and increase in scales itself will result in a significant reduction of the percentage of material loss.

## 4. Conclusion

The current study has demonstrated the potential for use of Indian bamboo (*Dendrocalamus* sp.) for bioethanol production. Process optimizations in pretreatment and saccharification could result in final efficiencies of 64.31% and 82.36% respectively for these unit operations. Without any optimization, alcohol fermentation using the bamboo hydrolysate achieved 71.34% efficiency. The overall efficiency for conversion of bamboo biomass to ethanol was 42.82% of the theoretical maximum with a projected yield of 143.45 L/dry ton of the biomass. These figures correspond to a projected potential of producing about 473 million liters of ethanol from the 3.3 million tons of surplus bamboo process waste available in the country; an un-ignorable quantity. With better strategies for solid liquid separation and further improvements in the efficiencies of saccharification and fermentation, the amount of ethanol that can be produced and its economics can possibly improve significantly.

## Acknowledgments

Authors would like to acknowledge Technology Information, Forecasting and Assessment Council (TIFAC), Department of

Science and Technology (DST), Government of India and Council of Scientific and Industrial Research (CSIR), New Delhi, for financial support to the Centre for Biofuels at NIIST. We are grateful to staff of Electron Microscopy Section, XRD facility and Chemical Sciences Division of NIIST for SEM, XRD and FTIR analyses respectively. We thank TIFAC for arranging bamboo samples.

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