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Evaluation of alternative strategies for generating fermentable sugars from high-solids alkali pretreated sugarcane bagasse and successive valorization to L (+) lactic acid

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ABSTRACT

Economical bioprocessing of lignocellulosic biomass essentially demands production of fermentable sugars in the concentrated form before their valorization. The present study aimed towards hydrolysis of alkali pretreated sugarcane bagasse at high solid loading and its successive valorization to L (+) lactic acid (LA). Two strategies were evaluated, wherein pretreatment, 12.5% substrate loading, Cellic CTec2 enzyme complex and thermophilic *Bacillus coagulans* NCIM 5648 were common to the processes. In Process A, when Cellic CTec2 was dosed at 30 FPU g^{-1} dry biomass, it hydrolyzed 75.8 ± 1.7% cellulose and 88.6 ± 2.1% xylan in 24 h. However, when its loading was changed to 25 mg protein g^{-1} glucan in Process B, Cellic CTec2 hydrolyzed 72.3 ± 0.3% and 68 ± 0.8% cellulose and xylan respectively. Valorization of glucose-rich filtrates obtained from Process A and B using two different media resulted in 50.4 ± 1.2 g L^{-1} and 51.24 ± 1.31 g L^{-1} of LA production from 54.7 to 62.7 g L^{-1} of glucose respectively. Attaining 1.75–2.4 g L^{-1} h^{-1} LA productivity with two scenarios of separate hydrolysis and fermentation is highly encouraging. It opens newer avenues for bio-based LA production using a greener approach.

1. Introduction

Lignocellulosic biomass (LCB) is one of the most remunerative renewable sources of energy. Versatile products ranging from biopower to biofuels, bio-based bulk chemicals, and speciality products can be generated from LCB [1]. Two structural polysaccharides, namely cellulose and hemicellulose, together represent a significant fraction of LCB. Upon their successful depolymerization, fermentable sugars such as glucose and xylose are released predominantly. These sugars can be valorized to diverse and commercially important platform and commodity chemicals other than biofuels using thermal, chemical, biological route or their combination [1,2]. However, in a sustainable LCB based biorefinery, the high-titers of these products is inevitable, especially besides cellulosic ethanol for achieving an economically profitable and environmental trade-off [3,4]. Attaining high titres of desired products can only be accomplished, when enzymatic liquefaction is performed at high-solids of pretreated lignocellulosic biomass. Moreover, hydrolysis should be such that the sugar yields are not only concentrated but also uncompromised, thereby ascertaining the techno-commercial viability of the process.

However, several aspects govern the high solid loading enzymatic saccharification of any lignocellulosic biomass (Fig. 1). Feedstock selection and type of pretreatment have a direct impact on high solid loading hydrolysis. Besides this, biomass hydrolysis also depends on the choice of cellulase cocktail, its reaction towards water availability/constraint, effective mass transfer and feeding strategies for substrates [5–8]. However, a comprehensive review by Putro et al. highlights “pretreatment” as the primary decisive force that guides the successful commercial production of various bio-based fuels and chemicals [9].

Selection of right pretreatment technique remarkably alters the third vital component of LCB popularly known as lignin which acts as a barrier during depolymerization of cellulose. The unique

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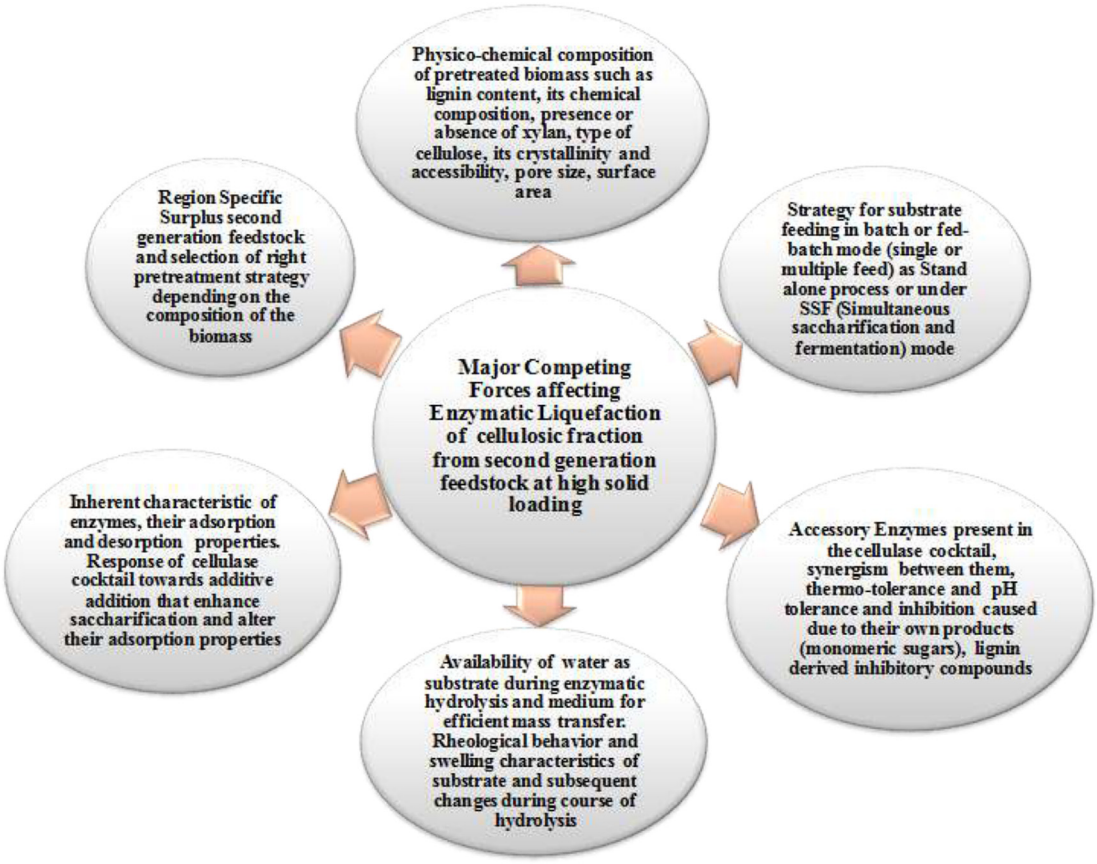


Fig. 1. Critical factors affecting performance of enzyme and release of fermentable sugars during saccharification at high-solid loading.

structural and functional composition of this heterogeneous polymer and its interactions with the hemicellulosic fraction is accountable for biomass recalcitrance [9]. In the last 30 years, several pretreatment strategies such as physical, chemical, physico-chemical and biological have been evaluated by various researchers [9,10]. The first three strategies generally include size reduction techniques such as milling, extrusion, ultrasonication, microwave method, chemicals ranging from acids, alkali, ionic liquids to deep eutectic solvents, and organic solvents or combining mechanical disruption (explosion) with steam, hot water and chemicals such as ammonia and carbon dioxide [10].

In the present investigation, we evaluated alternative strategies for generating sugarcane bagasse (SCB) hydrolyzates at high solid loading. The glucose-rich hydrolyzates were successively valorized to L (+) lactic acid (LA) using two independent approaches (Fig. 2). SCB was the choice of the substrate since it was one of the surplus agro-industrial wastes in the year 2018–19, due to excessive sugarcane production (400.37 million tonnes) in India as per the press release by Department of Agriculture, Cooperation and Farmers Welfare [11]. The recent study by Ong et al. also confirms that India is the second-largest producer of sugarcane bagasse after Brazil [12].

Alkali pretreatment was the preferred method, based on the extensive research by several workers, who highlighted its importance and cited several advantages associated with this process. Some visible features that make this pretreatment attractive include high removal of lignin, partial removal of xylan, reduction in particle size of the biomass and alteration in morphology of cellulose (degree of polymerization, porosity & surface area) [13–15]. These researchers have found that the alkali pretreatment not only reduced the overall biomass recalcitrance but also contributed towards improved cellulose accessibility and enhanced hydrolysis.

In the present study, we targeted the valorization of sugar-rich hydrolyzate directly to LA owing to its high market demand, which is expected to be 8.77 billion USD by the year 2025 and its current global production being dominated via microbial fermentation [16,17]. Chemically known as 2-hydroxypropanoic acid, LA has dual functional groups and is one of the 12 most sought platform chemicals listed by National Renewable Energy Laboratory (NREL), US. It finds numerous applications in food, pharmaceutical and cosmetic industries, synthesis of biodegradable polymers, oxygenated chemicals and production of industrial solvents [18].

2. Materials and methods

2.1. Raw material and enzyme

Raw SCB was generously supplied by Dhampur Sugar Mills and Baramati Agro Ltd, Pune to CSIR-Indian Institute of Petroleum (CSIR-IIP) Dehradun and Vasant Dada Sugar Institute (VSI), Pune respectively.

"Cellic® CTec2" samples were kindly provided by Novozymes A/S (Bagsvaerd, Denmark) to CSIR-IIP, Dehradun and VSI, Pune with Lot No being VCSI0009 and VCSI0018 respectively. The protein content of the enzyme preparation with Lot no VCSI0009 was found to be 93.89 ± 0.99 mg protein g^{-1}, as per the Bradford assay performed with BSA Fraction V as the reference standard [19]. The FPU activity of the commercial enzyme (Lot No VCSI0018) was found to be 148 IU mL^{-1} as per the IUPAC protocol [20]. Cellic®

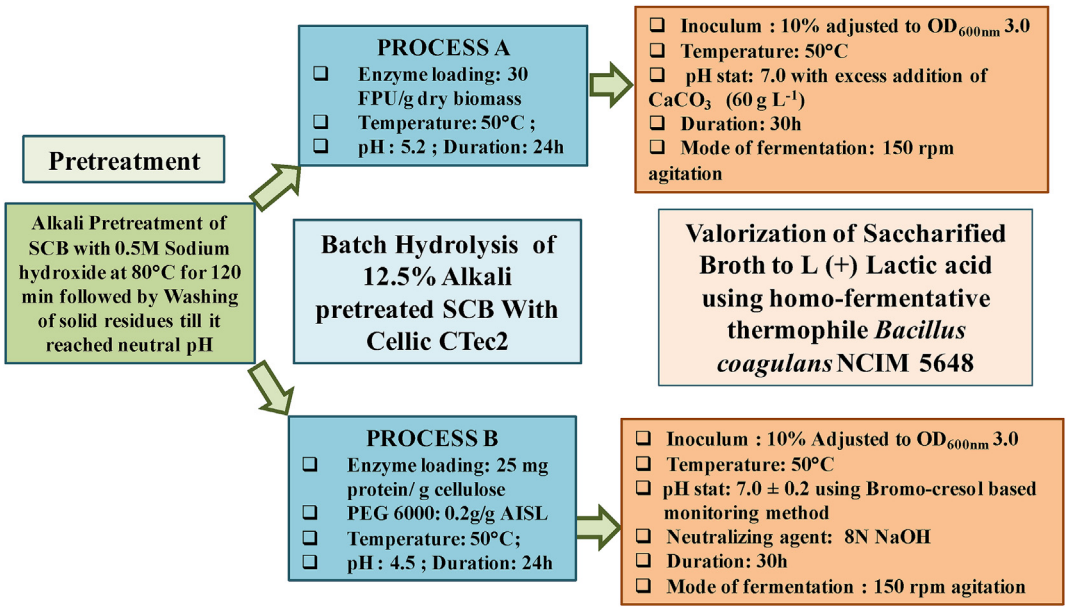


Fig. 2. Overall schematics for alternative strategies towards high-solids enzymatic saccharification of alkali pretreated SCB and its valorization to L (+) lactic acid.

CTec2 was stored at 4 °C until needed for hydrolysis of the pretreated biomasses.

2.2. Alkali pretreatment and compositional analysis

The pretreatment protocol was done according to the method described by Liu et al. and experiments were performed at two independent locations, namely VSI and CSIR-IIP [21]. The raw bagasse was subjected to 0.5 M sodium hydroxide treatment at 80 °C for 120 min. The solid loading for the said pretreatment was 5% (w/v). After pre-treatment, the solids were separated by filtering and then washed until a neutral pH was obtained. The biomass obtained after alkali pretreatment was weighed and its moisture content was determined by moisture analyser to find out the total dry weight (DW) retained after alkali pretreatment as the solid fraction.

The cellulose and xylan rich solid residue obtained after pretreatment was stored at 4 °C until use. Ash content, structural carbohydrates and lignin (acid-soluble and insoluble) were determined before, and after alkali pretreatment of SCB as per the Laboratory Analytical Procedure (LAP) developed at National Renewable Energy Laboratory (NREL), United States [22,23].

2.3. Enzymatic saccharification of alkali pretreated SCB

Two independent approaches were undertaken for production of fermentable sugar from alkali pretreated sugarcane bagasse. A substrate loading of 12.5% (w/v) was chosen based on the solid loading experiments of Cellic CTec2 with alkali pretreated sugarcane (Fig. S1).

In the first approach or process A, the batch hydrolysis was carried out by loading the enzyme at 30FPU g^{-1} dry biomass, with pH being 5.2 ± 0.2 and 50 °C as described previously [24]. Whereas in the second approach or process B, alkali pretreated SCB was subjected to enzymatic saccharification using Cellic CTec2 with protein loading of 25 mg BSA equivalents g^{-1} cellulose content at 50 °C as described earlier [25]. The initial pH of the reaction mixture was adjusted to 4.5 ± 0.3 by the addition of 0.5N sulfuric acid.

Irrespective of the strategy adopted, the solid fraction was separated from the saccharified broth after 24 h. It was accomplished by subjecting the hydrolyzed slurry to centrifugation at 5752*g* for 10 min at 4 °C. After removal of the concentrated sugar-rich solution (filtrate), the residual biomass was repeatedly washed with water to remove the adhered sugars and enzyme from the residual biomass. Sugars and organic acids in the filtrate and the wash were estimated using HPLC. All the experiments were performed in triplicates.

2.4. Selection of lactic acid (LA) fermenting bacterium

Selection of the best LA fermenting bacterium was made by carrying out 72 h static fermentation in a synthetic media containing glucose 100 g L^{-1}, yeast extract 10 g L^{-1} and $CaCO_3$ 60 g L^{-1} with initial pH 7.0 incubated at 50 °C as described previously [26]. All the bacteria for screening were procured from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL) Pune, India and were routinely maintained on nutrient agar (NA).

Bacillus coagulans NCIM 5648 equivalent to *B. coagulans* DSM 1 and ATCC 7050 was shortlisted for the LA fermentation based on initial screening experiments as described in Table S1.

2.5. Inoculum development for high-density LA fermentation

Several drawbacks are associated with batch fermentation of LA, such as low cell biomass and low productivity. Therefore, high-density cell fermentation was the preferred choice, where the saccharified broths obtained from Process A and B were directly used [27]. This mode of fermentation also helped us in understanding the threshold capacity of *Bacillus coagulans* NCIM 5648 for efficiently producing LA.

The “wash” obtained during saccharification was diluted to 20 g L^{-1} glucose, which served as a sole carbon source for seed development.

Two different media’s were evaluated for both seed development and LA production. The composition of the medium used for Process A was the same as described in subsection 2.4.

However, the alternate medium (modified CM5) was phosphate-buffered medium containing "wash" obtained from Process B as sole carbon source and fortified with 0.2 g L^{-1} $MgSO_4$. $7H_2O$ and 0.25 g L^{-1} $CaCl_2$. $2H_2O$ [28]. Ammonium sulphate and yeast extract were added in the ratio of 1:10 based on glucose content present in the wash. Since the nitrogen source and necessary vitamins supplemented as yeast extract could be limit or adversely affect the metabolic performance of *B. coagulans* NCIM 5648, therefore their addition was proportionately increased with increasing glucose concentration in the medium.

Irrespective of the type of medium, 5 ml glycerol stock of *B. coagulans* NCIM 5648 was transferred to 50 ml of seed medium and incubated at 50 °C with 150 rpm. After 8h of incubation, the culture was centrifuged at 5752 *g* to remove the residual sugars and lactic acid from the actively growing cells of *Bacillus coagulans*. The pelleted cells were resuspended in physiological saline, and optical density (OD) was measured at 600 nm. 10% inoculum with optical density OD_{600nm} of 3.0 served as inoculum for the production medium.

2.6. High-density LA fermentation with real-time "filtrates" obtained from process A and B

The filtrates obtained after enzymatic saccharification served as the base medium for LA production. The production medium for Process A was identical to medium described in subsection 2.4. Since calcium carbonate was added in excess (60 g L^{-1}), the process does not require pH monitoring during the entire fermentation.

However, in the case of Process B, for monitoring the steady production of LA under pH-stat conditions, "Bromo-cresol purple" dye was added at a concentration of 50 mg L^{-1} in the production medium. The present experiment exploited the colour change of this pH indicator dye, which shifted from violet to yellow when the pH of the medium dropped from 7.0 to 5.2 due to LA production as described earlier [29]. The pH-stat conditions near neutrality (7.0 ± 0.2) were maintained during the entire course of fermentation using 8N sodium hydroxide. All the experiments were performed in triplicates.

2.7. HPLC determination of fermentable sugars during saccharification and LA during fermentation

Hydrolyzed sugars obtained after enzymatic saccharification and all the metabolites produced during fermentation were analyzed by high-performance liquid chromatography (HPLC). Use of Bio-Rad Column Aminex HPX-87H (300 mm × 7.8 mm) with 9 μm particle size was common to both the locations. Operating conditions and make of the HPLC system were slightly different, as shown in Table S2.

In both the cases, standards, hydrolyzed, and fermentation samples were filtered through a 0.2 μm membrane filter before injecting into the column for the analysis. A calibration curve was drawn using all sugars (glucose, xylose, cellobiose, arabinose, fructose, sucrose) and possible metabolites (lactic acid, succinic acid, acetic acid, 1,3 propanediol, 2,3 butanediol, ethanol, xylitol, glycerol) as standards in the range of 0.2–1.0 mg mL^{-1}.

The optical purity of lactic acid was validated at VSI, Pune using a chiral column (Mitsubishi Chemical Holdings Corporation, MCI GEL CRS10 W(3μ) 4.6 ID x 50 mm. The operating conditions were: mobile phase 2 mM $CuSO_4$, flow rate 0.5 mL min^{-1}, injection volume 20 μL, UV detector, detection wavelength 254 nm, temperature 25 °C.

Saccharification Efficiency (%) was calculated using the following formula:

$$\text{Cellulose Saccharification Efficiency (\%)} = \frac{(\text{Total glucose released in g}) * 100}{\text{Glucan content in the substrate (g)} * 1.11}$$

$$\text{Xylan Saccharification Efficiency (\%)} = \frac{(\text{Total xylose released in g}) * 100}{\text{Xylan content in the substrate (g)} * 1.13}$$

where 1.11 and 1.13 is the polymerization factor for the cellulose and xylan respectively.

The productivity of L (+) lactic acid was calculated as the concentration of lactic acid produced per litre divided by the fermentation time (h) and is expressed as g L^{-1} h^{-1}. The lactic acid yield was calculated as actual lactic acid produced divided by the consumed glucose.

3. Results

3.1. Pretreatment of biomass and compositional analysis

The compositional analysis of SCB before and after alkaline pretreatment carried out independently at VSI and CSIR-IIP is depicted in Table 1. In both cases, the solid recovery was 61–64%, and the pretreated biomass showed 42–55% increase in cellulose content. Alkaline pretreatment led to significant delignification of SCB and 60–77% overall lignin content was removed in the form of black liquor. However, xylan fraction was also partially removed (41–44%) during the said process with 7–9% loss of cellulosic fraction during delignification.

3.2. Enzymatic saccharification of alkali pretreated SCB

When Cellic CTec2 was loaded at 30 FPU g^{-1} dry substrate with reaction conditions being 50 °C and pH 5.0 ± 0.2, it resulted in 75.8 ± 1.7% and 88.6 ± 2.1% of cellulose and xylan hydrolysis respectively from alkali pretreated SCB within 24 h, as shown in Fig. 3. The saccharification resulted in 56.1 and 18.0 g L^{-1} of glucose and xylose in the filtrate fraction, respectively.

However, when Cellic CTec2 was dosed at 25 mg protein g^{-1} cellulose content with 12.5% alkali pretreated SCB incubated at 50 °C and pH 4.5 ± 0.3, 72.3 ± 0.3% and 68.0 ± 0.8% of cellulose and xylan hydrolysis was achieved, respectively within 24h (Fig. 3). Besides glucose (63.8 ± 0.07 g L^{-1}) and xylose (16.18 ± 0.12 g L^{-1}) as main fermentable sugars in the filtrate fraction, sugars such as cellobiose and arabinose were also detected in qualitatively via HPLC.

3.3. High-density LA fermentation with real-time "filtrates" obtained from process A and B

When the high cell density fermentation was carried out in the medium containing an excess of calcium carbonate as neutralizing agent (Process A), the glucose was almost exhausted in 21 h, as shown in Fig. 4A. The lactic acid concentration in the production medium was found to be 50.4 ± 1.2 g L^{-1}, with productivity and product yield being 2.4 g L^{-1} h^{-1} and 0.92 g g^{-1} glucose consumed, respectively.

However, when the same inoculum was evaluated in an alternate medium (Process B), within 30 h, *B. coagulans* NCIM 5648 was able to ferment 62.7 g L^{-1} of glucose to 51.24 ± 1.31 g L^{-1} lactic acid (Fig. 4B). The LA yield was found to be 0.807 g g^{-1} glucose consumed with productivity being 1.75 g L^{-1} h^{-1}. This result was slightly inferior to Process A in terms of both yields and

Table 1
Solid Recovery and compositional analysis of sugarcane bagasse pretreated with alkali at two independent locations.

Biomass Components (%)	SCB from Baramati Agro Limited and processing at VSI, Pune		SCB from Dhampur Sugar mills and processing at CSIR-IIP, Dehradun	
	Raw (50 g dry weight)	Alkali pretreated (30.5 g dry weight)	Raw (250 g dry weight)	Alkali pretreated (160 g dry weight)
Cellulose	36.60 ± 0.67	57.01 ± 1.47	45.51 ± 0.65	64.62 ± 0.31
Xylan	16.02 ± 0.38	15.59 ± 0.93	20.81 ± 0.28	17.72 ± 0.19
Acid soluble lignin	5.27 ± 0.111	4.61 ± 0.05	2.35 ± 0.047	1.36 ± 0.15
Acid Insoluble lignin	26.83 ± 0.82	10.12 ± 0.72	19.7 ± 0.40	12.36 ± 0.37
Ash Content	2.45 ± 0.23	3.66 ± 0.18	3.47 ± 0.10	2.93 ± 0.11

Note: The values are the average of triplicates ± standard deviation.

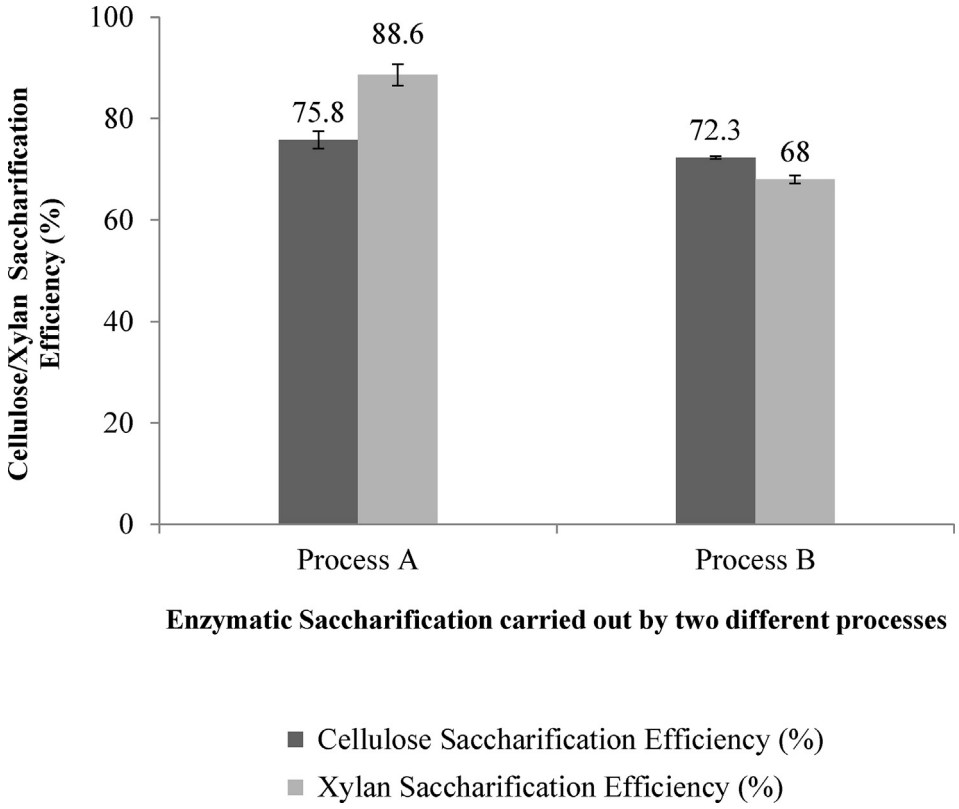


Fig. 3. Cellulose and xylan saccharification efficiency of Cellic CTec2 with alkali pretreated SCB as substrate after 24h hydrolysis when loaded on basis of 30 FPU g^{-1} biomass (Process A; pH 5.2) and 25 mg protein g^{-1} glucan content (Process B; pH 4.5) conducted at 50 °C.

productivity of LA. The lower LA yields may be attributed to intermittent pH restoration. During the addition of 8N NaOH, the flasks were frequently opened, thereby switching the anaerobic to microaerobic condition. As a result, some glucose may be diverted towards biomass generation resulting in an overall reduction in LA yields and productivity.

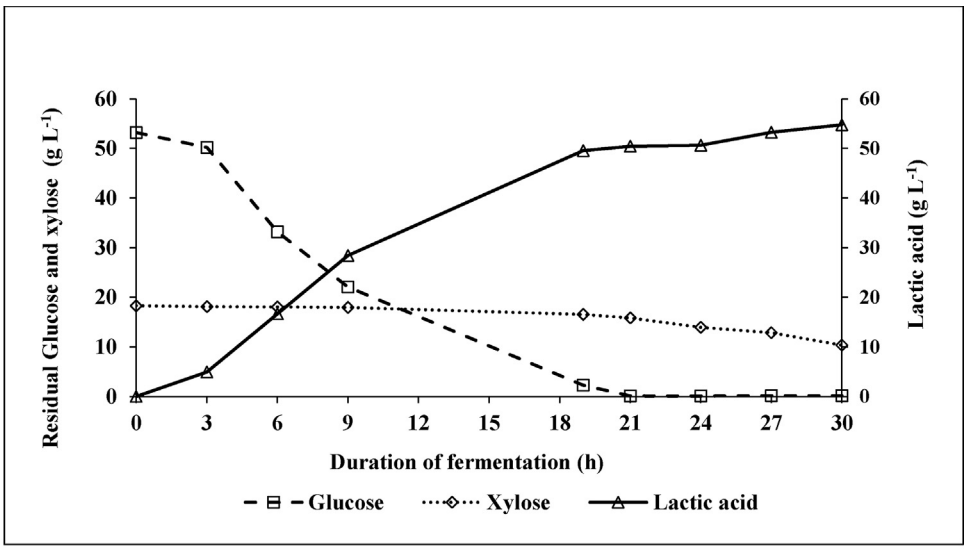
Thus during the entire course of LA fermentation, despite two different media composition, *B. coagulans* NCIM 5648 did not assimilate xylose, and its concentration almost remained constant. However, the entire process scheme of separate hydrolysis and fermentation (SHF) developed independently at two locations was comparable and was able to produce ~ 52.5 ± 2.7 g L^{-1} of an optically pure product within a short incubation time of 21–30 h.

4. Discussion

4.1. Pretreatment of biomass and compositional analysis

During alkaline pretreatment, the hydroxyl group of the sodium hydroxide chemically interacts with the various lignin moieties. More particularly, NaOH interacts with the phenol type α-aryl and alkyl ethers, phenolic and non-phenolic β-aryl ethers leading to their cleavage, dissolution and subsequent removal from biomass fraction, as described in an elaborative manner by Xu et al. [15]. In the present study also, the treatment with 2% NaOH at ambient pressure and temperature facilitated prominent delignification (60–77% lignin removal). These results are in concurrence with the work of several researchers in state of the art, wherein prominent delignification was observed after NaOH pretreatment, as highlighted in Table 2.

A solid recovery of 61–64% after alkaline pretreatment is in agreement with the findings of Nakanishi et al., where they reported 64.0 ± 0.7% mass yields when 1.5% sodium hydroxide pretreatment was conducted for 6.6% SCB at 130 °C for 30 min [33]. However, Chang et al. have reported a solid yield of 73.2% under identical conditions with 10% SCB [32].



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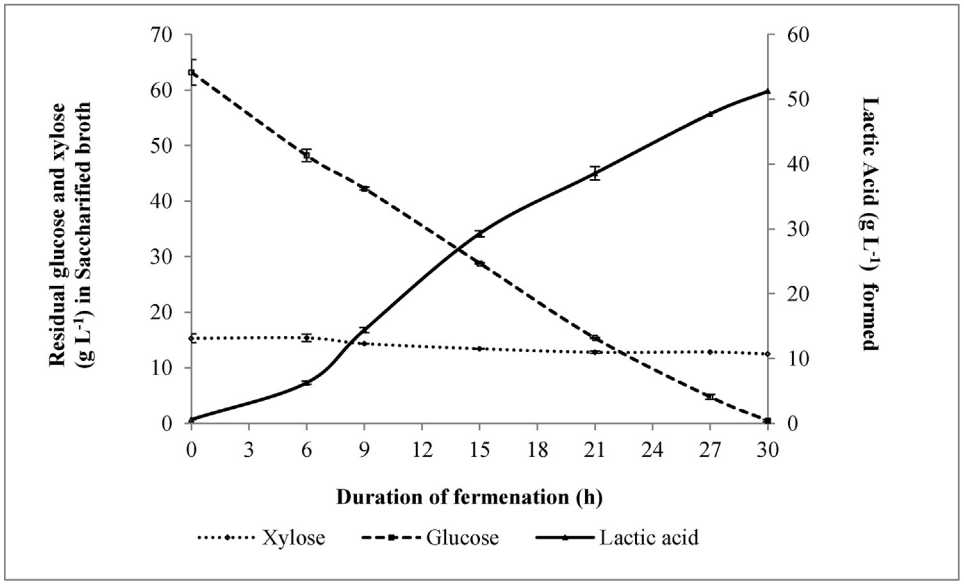

b

Fig. 4. High cell density and pH-controlled fermentation by *Bacillus coagulans* NCIM 5648 of saccharified broth under agitated conditions (a) From Process A (b) From Process B.

4.2. Enzymatic saccharification of alkali pretreated SCB

When Cellic CTec2 was loaded on FPU basis, 75.8 ± 1.7% glucan hydrolysis was achieved within 24 h only from 12.5% alkali pretreated SCB. These results are far better than the digestibility of 2% alkali pretreated sugarcane bagasse reported by Zhang et al. [36]. They attained ~51% glucose yield after 72 h incubation when the same enzyme was loaded at 20 FPU g^{-1} dry pretreated biomass. Tsai et al. got saccharification yield of 75.4 ± 2.9% for cellulose with 10% alkali pretreated Napier grass. However, the cellulose conversion took 96 h due to low enzyme loading (12 FPU Cellic CTec2 g^{-1} dry biomass) [37].

In the alternative strategy, more than 60 g L^{-1} of glucose and 15 g L^{-1} xylose was released from pretreated biomass within 24 h by loading Cellic CTec2 at 25 mg g^{-1} glucan content. This result is also highly encouraging. The present results are far superior to Mokomele et al. especially considering the "incubation period". They reported 75% carbohydrate conversion of ammonia fibre explosion (AFEX) pretreated SCB after 96 h of incubation at 50 °C and pH 5.0 when incubated with 20 mg Cellic CTec3 g^{-1} glucan content [38].

However, when Chen et al. carried out hydrolysis of 10% alkali pretreated corn stover for a prolonged period of 120 h, they obtained maximum hydrolysis of 82.63% and 76.62% for cellulose and xylan respectively when Cellic CTec2 was loaded at 4 mg protein g^{-1} glucan content at 50 °C and pH 5.0 [39]. Similar observations were made by Mukasekuru et al. wherein they adopted a fed-batch approach for enzymatic hydrolysis of high solids SCB (20%). Where, approximately 160 g L^{-1} fermentable sugars have been achieved with 83% cellulose and 90% xylan hydrolysis at 5.26 FPU g^{-1} glucan content after 72 h using a combination of accessory enzymes and

Table 2
Prior art related to sodium hydroxide assisted pretreatment with sugarcane bagasse.

Compositional analysis after alkali pretreatment	Pretreatment conditions	Reference
Glucan: 63.86 ± 0.88% Xylan: 21.92 ± 0.29% Klason Lignin: 8.0 ± 0.02%	NaOH- 3%; Duration:145 min Temperature: 80 °C; Solid loading: 5%	[30]
Glucan: 56.33 ± 0.79% Xylan: 20.86 ± 0.90% Klason Lignin: 9.93 ± 0.57%	NaOH- 8%; Duration: 40 min Temperature: 100 °C; Solid loading: 8.3%	[31]
Glucan: 63.5 ± 0.46% Xylan: 29.0 ± 0.81% Klason Lignin: 9.7 ± 0.1%	NaOH- 2%; Duration:120 min Temperature: 80 °C; Solid loading: 10%	[32]
Glucan: 58.6 ± 1.2% Xylan: 22.1 ± 1.4% Klason Lignin: 8.8 ± 0.9%	NaOH- 1.5%; Duration:30 min Temperature: 130 °C; Solid loading: 6.6%	[33]
Glucan: 61.99 ± 1.07% Xylan: 25.04 ± 0.99% Klason Lignin: 8.15 ± 0.16%	NaOH- 2%; Duration:120 min Temperature: 80 °C; Solid loading: 5%	[34]
Glucan: 72.2 ± 3.1% Xylan: 10.2 ± 1.7% Klason Lignin: 7.8 ± 0.4%	NaOH- 4%; Duration:30 min Temperature: 121 °C; Solid loading: 10%	[35]

additives [40].

Thus with new generation enzyme formulations that have LPMO activity, longer incubation time is beneficial only if the enzyme loading is less whether in terms of protein or FPU activity. With higher enzyme loadings at high-solids, shorter incubation time is more advantageous as these enzymes are high performing and reach product saturation within 48 h [data yet not published].

Moreover, to obtain industrially relevant sugars levels (more than 100 g L^{-1}) researchers tend to feed pretreated biomass at concentrations of 25–40% which is futile because this target can easily be achieved even with 15–20% solids. It should be known that any efficient pretreatment strategy leads to cellulose-rich biomass wherein its content is 55% or more. Therefore for maximum sugar extraction from cellulosic fraction, research with these new generation enzymes should be targeted towards 80% saccharification or above rather than inefficient hydrolysis at 25–40% solids. Use of additives and accessory enzymes or fine-tuning with pretreatment strategies can be prospective approaches which can lead to attaining high efficiency of cellulose hydrolysis [40,41].

4.3. High-density LA fermentation with real-time "filtrates" obtained from process A and B

The LA productivity (2.4 g L^{-1} h^{-1} -Process A; 1.75 g L^{-1} h^{-1} -Process B) by *Bacillus coagulans* NCIM 5648 exceeded the performance of *Bacillus coagulans* LA1507. The LA productivity of this organism was 1.59 g L^{-1} h^{-1} which was attained in fed-batch simultaneous saccharification and fermentation done with 17.5% alkali pretreated sweet sorghum bagasse and cellulase loading being 25 FPU g^{-1} pretreated biomass [42].

Notably, the duration of fermentation in the present experiments carried out using two different strategies superseded the results of separate hydrolysis and fermentation (SHF) conducted with *Bacillus* sp. NL01 where it could produce 56.37 g L^{-1} LA within 42 h from enzymatic hydrolyzate of corn stover containing glucose, cellobiose and xylose at a concentration of 85.64, 5.46 and 2.91 g L^{-1} [43].

The present results are far superior to the results obtained by Adsul et al., wherein the mutant of *L. delbrueckii* (UC-3) was able to produce 67 g L^{-1} lactic acid from 80 g L^{-1} sugarcane bagasse cellulose in 96 h when the reaction was carried out at pH 6.5 and temperature of 42 °C under simultaneous saccharification and fermentation (SSF) conditions [44]. The longer duration in the latter case was due to choosing SSF approach, which often compromises over the operating conditions, namely temperature and pH. These physicochemical parameters may be radically different for enzymatic hydrolysis (45–55 °C; pH 4.5–5.5) and the favourable environment required by fermenting bacterium (eg. *L. delbrueckii*; Temp 42 °C and pH 7.0) to produce the targeted product efficiently.

Table 3 depicts the LA fermentation studies reported in literature using different combinations of feedstocks and microbial strains, including the results of the present study. As observed in Table 3, the best LA productivity was reported when the feedstock was paper sludge as it is one of the easiest feedstocks to be hydrolyzed as, being lignin and hemicellulose free [46]. In the present study with SCB as feedstock, LA yield and productivity achieved using *Bacillus coagulans* NCIM 5648 was far better when compared

Table 3
Comparative performance of L(+)Lactic acid production conducted with different lignocellulosic feedstocks and bacterial strains.

Microorganism	Feedstock	Lactic acid (g L^{-1})	Lactic acid yield g g^{-1}	Lactic acid productivity (g L^{-1} h^{-1})	References
Lactobacillus pentosus CECT 4023T	What straw	17.76	0.84	0.37	[45]
Lactobcillus delbreuckii mutant UC-3	Sugarcane bagasse cellulose	67	0.83	0.93	[44]
L. rhamnosus ATCC 7469	Paper sludge	73.0	0.97	2.90	[46]
Lactobacillus lactis mutant RM2†24	Bagasse-derived cellulose	73.0	0.73	1.52	[47]
Lactobacillus brevis	Corncob	39.1	0.70	0.81	[48]
L. brevis and *L. rhamnosus*	Corn Stover	21.0	0.70	0.58	[49]
L. pentosus ATCC 8041	Sugar cane bagasse	64.8	0.93	1.01	[50]
Bacillus sp. 17C5	Sugar cane bagasse	55.5	0.89	0.29	[51]
Bacillus sp. NL01	Lignocellulosic hydrolyzates	75.0	0.75	1.04	[43]
Bacillus coagulans NCIM 5648	Sugarcane bagasse	50.4*	0.92*	2.4*	This study
Bacillus coagulans NCIM 5648	Sugarcane bagasse	51.2#	0.807#	1.75#	This study

Note: * represents Lactic acid obtained by Process A and #represents Lactic acid obtained from Process B.

to *Lactobacillus pentosus* ATCC 8041 [50] and *Bacillus* sp. 17C5 [51].

The authors are of the view that SHF should be more preferred over SSF approach, especially while using new generation enzymes like Cellic CTec2. Since these enzymes have high LPMO activity, the oxidoreductase enzymes present in the enzyme cocktail often compete with the fermenting microbe for molecular oxygen. As a result, either there is a severe impendence in saccharification or significant impairment in fermentation potential of the bacterium used.

Unlike most of the *Bacillus* strains reported earlier such as *B. coagulans* C 106 [52], *B. coagulans* GKN316 and *B. coagulans* NL01 [53], the bacterium used in the present study, namely *Bacillus coagulans* NCIM 5648 was unable to valorize xylose to L (+) lactic acid. Though there was a slight uptake of xylose in both the processes, but the xylose consumption is ruled out. This claim is strongly supported by the evidence of genetic analysis of *B. coagulans* NCIM 5648, wherein three xylose transporters have been identified. Still, the gene encoding for xylose isomerase lacked thereby disrupting xylose utilization [54,55].

Thus by choosing a right combination of pretreatment and saccharification in the present study irrespective of the type of strategy employed, at two independent locations, ~388 ± 38.25 g of monomeric sugars were produced per kg of initial raw sugarcane bagasse within the short time duration. Moreover, the recent reaction network flux analysis (RNFA) done by Marks et al. affirm that a minimum sugar yield of 400 g kg^{-1} biomass is necessarily

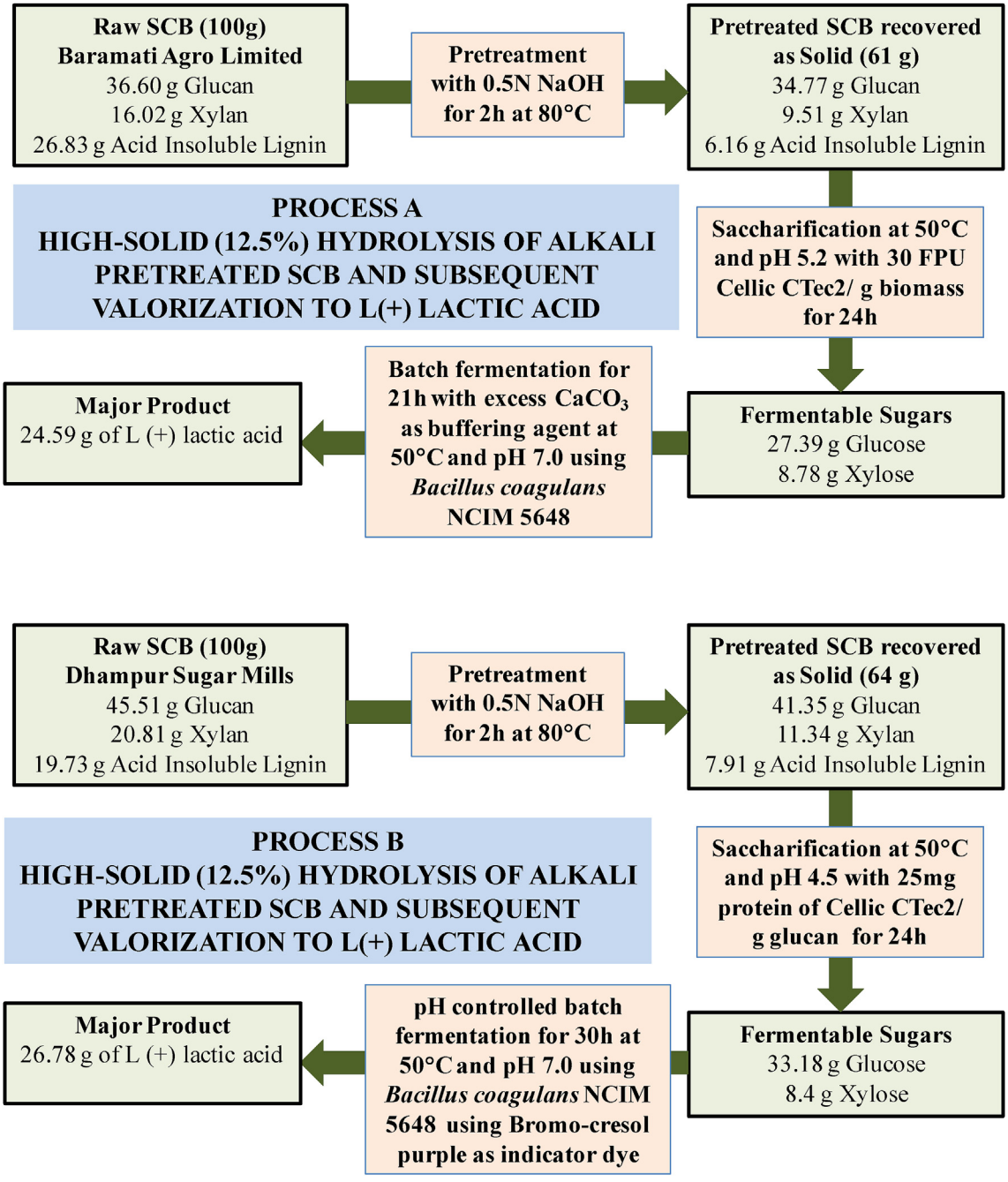

Fig. 5. Envisaged representation for efficient conversion of glucose to lactic acid from initial 100g raw sugarcane bagasse by Process A and Process B.

required for the economically feasible production of biofuels [56]. Thus, the sugar production by especially Process B from alkali pretreated sugarcane bagasse was able to meet the threshold viable sugar yield criterion (Fig. 5), considering this latest report as the benchmark [56].

The recent study by Mandegari et al. further increases the relevance and impact of the present study [50]. While evaluating four potential biorefinery scenarios with sugarcane as feedstock, LA and ethanol production from glucose and xylose respectively or production of solely lactic acid from glucose and xylose was the most rewarding combination in terms of life cycle assessment (LCA) and internal rate of return (IRR) respectively [57].

5. Conclusions

The present study concluded with the fact that choosing the right pretreatment process and integrating it with the correct strategy for enzymatic hydrolysis and fermentation can maximize production of any bio-based chemical within shorter incubation time. Despite opting for two alternative strategies during high-solids SHF, 52.5 ± 2.7 g L^{-1} of an optically pure LA production was achieved within a short duration of 45–54 h from sugar-rich filtrate containing ~58.7 g L^{-1} glucose. This study also emphasizes on the washing of the residual biomass after saccharification. Washing step not only ensures the recovery of all the entrapped hydrolyzed sugars and its utilization but simultaneously also helps in identifying the “true” saccharification potential of an enzyme cocktail.

CRediT authorship contribution statement

Ketaki Nalawade: Investigation, Methodology. **Pratibha Baral:** Investigation, Writing - original draft, Formal analysis. **Snehal Patil:** Investigation, Methodology. **Anushka Pundir:** Formal analysis, Investigation. **Akhilesh K. Kurmi:** Methodology, Investigation. **Kakasaheb Konde:** Funding acquisition, Conceptualization, Supervision, Validation, Data curation, Writing - review & editing. **Sanjay Patil:** Funding acquisition, Conceptualization, Supervision, Validation, Data curation, Writing - review & editing. **Deepti Agrawal:** Funding acquisition, Conceptualization, Supervision, Validation, Data curation, Writing - review & editing.

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Appendix A. Supplementary data

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