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Utilization of carbohydrates content of paper tube residuals for ethanol production

Farid Talebnia[a,b,*], Mohammad J. Taherzadeh[a]

[a] School of Engineering, University of Borås, 501 90 Borås, Sweden
[b] Faculty of Chemical Engineering, Noshirvani University of Technology, Babol, Iran

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ABSTRACT

Paper tube residual was utilized as a raw material for ethanol production. The effects of two pretreatment methods namely dilute acid steam explosion (DASE) and concentrate phosphoric acid (CPA) on enzymatic hydrolysis and SSF were studied. Cellulose, lignin, glue (PVA), and xylan were the main components of paper tube accounting for 52%, 20%, 9% and 7% of dry matter, respectively. Presence of PVA delayed the growth of yeast cells but showed no effect on ultimate yield of ethanol. Higher cellulase concentration as well as pretreatments increased hydrolysis rate and ultimate yield of ethanol. Enzymatic hydrolysis of native paper tube for 72 h resulted in 49% of theoretical glucose conversion while pretreatments by DASE and CPA increased this value to 67% and 93%, respectively. The best result of SSF process was from the CPA-pretreated paper tubes with an ethanol yield of 0.42 g/g after 48 h. Under optimal condition, 308 ml ethanol per kg paper tube could be produced.

1. Introduction

Utilization of fossil fuels such as oil, coal and natural gas in transportation and industrial sectors has drastically aggravated the greenhouse gases effect, such that in 2030, world CO_2 emissions are expected to be more than twice the level of 1990 (Ballesteros et al., 2006). On the other hand, volume of wastes from various origins is increasing worldwide due to increasing population size, wealth, and rate of production. Disposal of such waste is cost-intensive and usually problematic to the environment, in which long term solution in the future lies on reusing or recycling of such waste with the aid of advanced or newly developed technologies. Therefore, developing alternative renewable energy resources produced from waste materials is an urgent necessity and can contribute to alleviate the greenhouse effect.

Lignocellulosic waste materials obtained from energy crops, wood industries and agricultural residues are abundant globally and represent excellent resources of renewable energy (Lin & Tanaka, 2006). The carbohydrates in lignocellulosic residues can be utilized in various ways for energy purposes, among which ethanol production is one of the most investigated processes (Cheng & Timilsina, 2011; Talebnia & Taherzadeh, 2006). One of the main cost contributive factors in ethanol process is the cost of raw material which can be substantially reduced by utilization of industrial lignocellulosic wastes. However, due to structural complexity, pretreatment is required to disrupt the recalcitrant structure of lignocellulosic materials and to increase the accessibility of hydrolytic enzymes to the carbohydrates polymers (Geddes, Nieves, & Ingram, 2011). The waste from paper tube industry is a cellulose-rich material and therefore it has theoretically a great potential for ethanol production. Paper tube is produced from recycled paper (>90%) and adhesives (∼8–10%) and consequently, it is mainly composed of cellulose, lignin and adhesives. The adhesives are usually sodium silicate (water glass) and polyvinyl alcohol (PVA). No major problem is expected from water glass during pretreatment and hydrolysis, but PVA must be further investigated to address its toxicity on fermentation.

Ethanol production from lignocellulose has been the focus of research for several decades, however, to our knowledge no research work on production of ethanol from paper tube residuals is found in the literature. The objective of the current work was to investigate the potential and feasibility of paper tube residual as an alternative raw material for ethanol production. The main focus was to develop a suitable pretreatment method for efficient enzymatic hydrolysis and ethanol fermentation. The pretreated materials using different methods were then used in simultaneous

Abbreviations: DM, dry matter; FPU, Filter Paper Units; DASE, dilute acid steam explosion; CPA, concentrate phosphoric acid; IU, international units; PVA, polyvinyl alcohol; SSF, simultaneous saccharification and fermentation; AIR, acid insoluble residue; AIL, acid insoluble lignin.

* Corresponding author at: Faculty of Chemical Engineering, Noshirvani University of Technology, Babol, Iran. Tel.: +98 111 3234204; fax: +98 111 3234204.
E-mail address: f.talebnia@nit.ac.ir (F. Talebnia).

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saccharification and fermentation (SSF) process for optimal production of ethanol. The effects of potential inhibitory compounds originated from paper tube constituents such as adhesives and dyes, and from the pretreatments were also addressed.

2. Materials and methods

2.1. Substrate, enzymes, and yeast strain

The paper tubes used in the current work were the rejected products of Nordens Pappersindustri AB (Sandared, Sweden) in a volume of about 300 tonnes/year. The samples were shredded and then knife-milled with a food grinder (DeLonghi S.p.A., 31100 Treviso, Italy) to less than 1 mm in average diameter. Total dry content of paper tube was determined by drying at 110 °C for 48 h. The carbohydrates content and structural components of paper tubes were determined based on the method provided by National Renewable Energy Laboratory (Sluiter et al., 2010). Two enzymes, cellulase from *Trichoderma reesei* ATCC 26921 (SIGMA, C2730) and β-glucosidase from Almonds (SIGMA, G0395), were used for enzymatic hydrolyses and SSF experiments. Cellulase activity was determined according to the standard method provided by NREL (Decker, Adney, Jennings, Vinzant, & Himmel, 2003). The activity of cellulase was measured as 97 FPU/ml of enzyme solution. The activity of β-glucosidase was reported as 5.2 IU/mg solid by the supplier. The yeast *Saccharomyces cerevisiae* CBS 8066, obtained from Centraalbureau voor Schimmelcultures (Delft, the Netherlands), was used in all experiments. The strain was maintained on agar plates made from yeast extract 10 g/l, soy peptone 20 g/l, and agar 20 g/l with D-glucose 20 g/l as an additional carbon source. All experiments in the current work were performed in duplicates, and the data reported are the average of the two replications.

2.2. Toxicity test of polyvinyl alcohol (PVA)

Since PVA is a component used as glue in production of paper tubes, an experiment was conducted to examine the toxicity or inhibition effects of this component on the growth and ethanol yield of *S. cerevisiae*. Therefore, the yeast *S. cerevisiae* was anaerobically cultivated in a defined medium containing 10 g/l PVA and the result was compared to that from PVA-free medium.

2.3. Pretreatments of paper tubes

The effects of two pretreatment methods *i.e.* dilute acid steam explosion (DASE) and concentrate phosphoric acid (CPA) were studied in this work. In DASE pretreatment, samples of ground paper tube were pretreated with steam explosion unit as described elsewhere (Talebnia, Pour Bafrani, Lundin, & Taherzadeh, 2008). In brief, the steam was directly injected to the reactor previously loaded with 1.0 l paper/water slurry containing 10% solid fraction. The reaction variables *i.e.* temperature, duration time as well as sulfuric acid (H_2SO_4) concentration were selected as 180 °C, 10 min and 0.5%, respectively. Concentrate phosphoric acid (H_3PO_4) pretreatment was performed according to Zhang et al. (2007) with minor modifications. In short, 5 g paper tube samples were treated with 50 ml of H_3PO_4 (85%) at 50 °C for 1 h on a rotary shaker bath with the speed of 130 rpm. The resultant pretreated samples after washing were used for further processing including enzymatic hydrolyses and SSF experiments.

2.4. Enzymatic hydrolyses

Various samples of paper tubes both treated and untreated along with pure cellulose Avicel (Fluka 11365) as a reference were added into 250 ml conical flasks containing 50 mM sodium citrate buffer at pH 4.8 to obtain 100 ml of milled-paper/water slurry with solid fractions of 5%. Cellulase and β-glucosidase enzymes were added as 20 FPU and 10 IU/g of dry matter (g DM), respectively. The slurries were then hydrolyzed by the enzymes at 45 °C and 130 rpm for 72 h in a shaker bath.

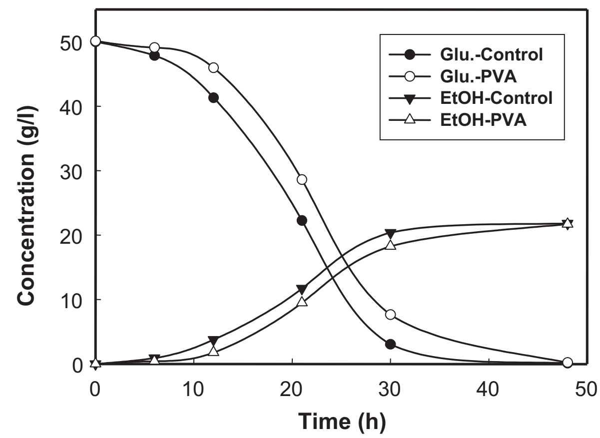

Fig. 1. Time course of ethanol production and glucose consumption in anaerobic batch cultivation of *S. cerevisiae* using synthetic defined medium with or without the presence of polyvinyl alcohol (PVA).

2.5. Simultaneous saccarification and fermentation (SSF)

SSF was performed under anaerobic condition in citrate buffer (50 mM and pH 4.8) with solid concentration of 5%. All flasks were autoclaved and then the required enzymes and yeast *S. cerevisiae* were aseptically added to each flask to have a final volume of 100 ml. All the SSF experiments were performed at 37 °C. The cellulase enzyme was added at 10 or 20 FPU/g DM, while β-glucosidase concentration was kept constant at 10 IU/g DM in all experiments. Pure nitrogen gas was sparged into the media at the beginning of the fermentation and during the sampling to keep the anaerobic condition.

2.6. Analytical methods

An HPLC was used for analyses of the metabolites. A lead-based ion-exchange column (Aminex HPX-87P, Bio-Rad, USA) was used at 85 °C for measuring glucose and xylose concentrations. Ultra-pure water was used as eluent at a flow rate of 0.6 ml/min. Concentration of ethanol, furfural and HMF was determined using a hydrogen-based ion-exchange column (Aminex HPX-87H, Bio-Rad) at 60 °C using 5 mM H_2SO_4 at a flow rate of 0.6 ml/min. A refractive index (RI) detector (Waters 2414, Milipore, Milford, USA) and UV absorbance detector at 210 nm (Waters 2487) were used in series. Furfural and HMF concentrations were analyzed from UV chromatograms, whereas the rest of the chemicals were quantified with the refractive index (RI) detector.

3. Results

3.1. Effect of PVA

The inhibitory effect of PVA on the applied yeast strain was evaluated, and Fig. 1 shows the time course of glucose consumption and ethanol production in fermentation of defined medium with or without the presence of PVA. The fermentation of glucose by applied *S. cerevisiae* was not influenced with the presence of PVA in respect with ultimate yield of ethanol produced. The yeast cells were able to assimilate the sugar in both media and produce

Table 1
Composition of paper tubes used in this work.

Components	% of dry solid
Cellulose	52% (±2)
Xylan	6.2% (±0.5)
AIR	24% (±2)
AIL	20% (±1.7)
Adhesive	9% (±1)

ethanol with the same yield of 0.43 (±0.02) g/g. In addition, the production of other metabolites such as glycerol and acetic acid was not substantially influenced by the addition of PVA. For instance, glycerol was produced by the yields of 0.071 (±0.004) and 0.073 (±0.003) g/g in PVA-free and PVA-contained media, respectively. The effect of PVA was only to prolong the lag phase in the growth of yeast cells where glucose was completely consumed within 50 h of fermentation (Fig. 1). This fact suggests that the effect of PVA might be overcome by adaptation of cells to this medium or by application of higher cell concentration in the medium.

3.2. *Enzymatic hydrolysis of pretreated paper tubes*

The effects of two different pretreatment methods were compared for effective enzymatic saccharification and SSF process. First, the chemical composition of paper tube was determined and the results are presented in Table 1. The results showed that the glucose is the main product of hydrolyzed samples along with minor amount of xylose. Glucose and xylose account for 58% (±2) and 7% (±0.5) of paper tube dry matter, respectively. This means that the cellulose content of paper tubes is around 52% (Table 1). Besides, the acid insoluble residue (AIR) and acid insoluble lignin (AIL) contents of raw paper tube were 24% (±2) and 20% (±1.7), respectively (Table 1). The lignin content of pretreated samples was also determined in order to estimate the effect of pretreatments on lignin removal. While DASE pretreatment showed only a little effect on the removal of lignin, CPA substantially decreased the lignin content of paper tube. Analyses showed that about 64% (±2.5) of original lignin was dissolved during CPA pretreatment compared to 8% (±0.5) in DASE pretreatment.

The enzymatic hydrolysis of both pretreated and untreated paper tubes was carried out under conditions formerly described and the profile of glucose production for different samples are shown in Fig. 2. The rate of reaction for untreated paper tubes was low, and after 72 h, only 49% (±1) theoretical yield of glucose conversion was achieved. Pretreatment of sample in steam-explosion reactor enhanced the rate of enzymatic hydrolysis and led to 67% (±1) theoretical yield for glucose production at the same time (Table 2). The best result for enzymatic hydrolysis was obtained from CPA pretreated sample, where the initial rate of reaction was significantly higher than other samples (Fig. 2). After 21 h of hydrolysis, the respective glucose concentration for untreated, DASE and CPA pretreated samples was 8.67, 11.31 and 21.97 g/l equal to 30, 39 and 76% of theoretical value (Table 2). At the end of reaction period *i.e.*72 h, CPA pretreated samples yielded 26.87 g/l glucose equal to 93% of theoretical conversion yield. The corresponding value for pure cellulose was 53% (Table 2). The rate of glucose production of untreated paper tube sample was slightly lower than that of pure cellulose. Pretreatments especially with CPA, greatly enhanced the rate of glucose production such that after 21 h of hydrolysis, an initial rate of 1.05 g/l/h was achieved compared to 0.41 g/l/h for untreated sample showing an increase by 2.5-fold (Table 2). This result indicates that CPA pretreated paper tube is highly susceptible for enzymatic attack and therefore, pretreatment with H_3PO_4 is the most efficient method in making the paper tube sample accessible to the enzymes attack.

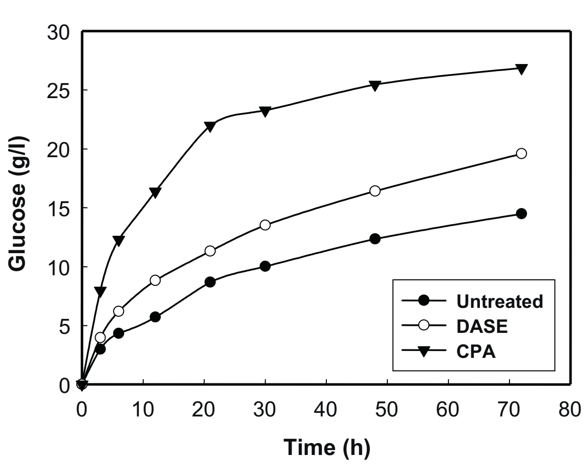

Fig. 2. Enzymatic hydrolysis of untreated and pretreated paper tube samples (cellulase: 20 FPU/g DM and β-glucosidase: 10 IU/g DM).

3.3. *SSF experiments using S. cerevisiae*

Simultaneous saccharification and fermentation (SSF) of pretreated and untreated paper tubes was investigated and the results were compared with those of pure cellulose (Avicel) as a reference. The anaerobic experiments were carried out under conditions described earlier and the most important results including the profiles of glucose consumption and ethanol production are summarized in Fig. 3. The effect of cellulase enzyme loading was investigated by performing two sets of experiments, where 10 and 20 FPU/g DM were added into the medium. In the early stage of experiments (within the first 3–6 h), the concentration of glucose in the medium in all cases increased due to the action of hydrolytic enzymes and then start to decline due to assimilation of glucose by the yeast cells. In overall, the higher cellulase enzyme loading increased the maximum ethanol concentration and enhanced the rate of ethanol production. However, this effect was much more pronounced for CPA pretreated paper tube samples especially at the higher enzyme loading (20 FPU/g DM). Two-fold increase in loading of cellulase enzyme resulted in 28, 20 and 25% higher ethanol concentration for untreated, DASE and CPA pretreated materials, respectively (Fig. 3). When the loading of cellulase enzyme was increased, the production rate and concentration of glucose, cellobiose and ethanol for CPA pretreated paper tubes was substantially higher than the other samples (Fig. 3). The concentration profiles of cellobiose with cellulase loading at 20 FPU/g DM for various samples were highly resembled to the profiles of glucose (data not shown). Both of these sugars are intermediate chemicals in the sequential reactions from cellulose to ethanol. Inspection the profile of these sugars indicates that except in the early stage of SSF experiments, the rate of enzymatic hydrolysis controls the overall rate of the reaction.

At 20 FPU enzymes loading, the ethanol production rates calculated after 21 h of SSF for Avicel, untreated and DASE-pretreated paper tubes were almost the same and remained constant at 0.30 (±0.01) g/l/h. The corresponding value for CPA pretreated materials, however, was 0.50 g/l/h representing 67% increase in ethanol productivity (Fig. 3). The concentration of ethanol after 48 h for CPA pretreated paper tubes with 20 FPU cellulase loading reached to 11.94 g/l and then slightly declined indicating that the fermentation was practically completed at this time period. This ethanol concentration can be interpreted to an ethanol yield of 0.42 g/g glucose, which is similar to what was obtained with applied yeast in synthetic medium. These results confirm that almost all cellulose content of the paper tube could be converted to ethanol with satisfactory yield and conversion rate using appropriate

Table 2
Cellulose conversion and glucose production rate in enzymatic hydrolysis of Avicel and paper tube samples with or without pretreatments.

Samples	21 h		48 h		72 h	
	r_{Glu} (g/l/h)	Conversion (%)	r_{Glu} (g/l/h)	Conversion (%)	r_{Glu} (g/l/h)	Conversion (%)
Avicel	0.45	31	0.30	45	0.23	53
Untreated	0.41	30	0.26	43	0.20	49
DASE	0.54	39	0.34	57	0.27	67
CPA	1.05	76	0.53	88	0.37	93

pretreatment method. Based on the dry matter of paper tubes, within the time period applied in this work, the maximum attainable glucose from 1000 g paper tube sample was 540 g obtained after CPA pretreatment. Likewise, the maximum ethanol produced at the best condition was 308 ml.

4. Discussion

There are several types of paper tubes, depending on the type of recycled papers used, adhesives, processing conditions and the specifications of the final product. Nevertheless, the main constituents of residues from these paper tubes are mainly cellulose, lignin and adhesives. The waste paper tubes might be either disposed off imposing an increase in the cost of end product or might be incinerated for generation of heat and energy. However, a more viable process would be utilization of paper tube residuals in an advanced and integrated process where the high cellulose content of paper tube is used for production of value-added products such as fuel ethanol. The lignin content can be burnt to supply energy required for the process plant and left over solid residue might be used as an additive for the construction industry.

For production of lignocellulosic ethanol, pretreatment is an essential step that aims to improve the rate of production as well as the total yield of liberated sugars in hydrolysis step (Hendriks & Zeeman, 2009). A number of pretreatment methods have been developed and applied for various lignocellulosic materials. The overall efficiency of the pretreatment process is correlated to a good balance between low inhibitors formation and high substrate digestibility. Our results show that the inhibitory effect of adhesive (PVA) as one of the main structural components of paper tubes, on enzymatic hydrolysis as well as fermentation was negligible as the rate of hydrolysis and ethanol production yield of untreated sample were comparable with those from pure cellulose (Table 2). Furthermore, no formation of inhibitors such as furfural at substantial level was detected after both pretreatments. In overall, no significant difficulties with respect to inhibition were encountered in working with paper tube samples during enzymatic hydrolysis and SSF experiments indicating that chemicals used during manufacturing process such as adhesive and dyes are not toxic or their concentrations are below toxic level.

The enzymatic hydrolysis rate of untreated paper tubes was slightly lower than that of pure cellulose. Presence of residual lignin on the surface of cellulose chains may reduce the accessibility of

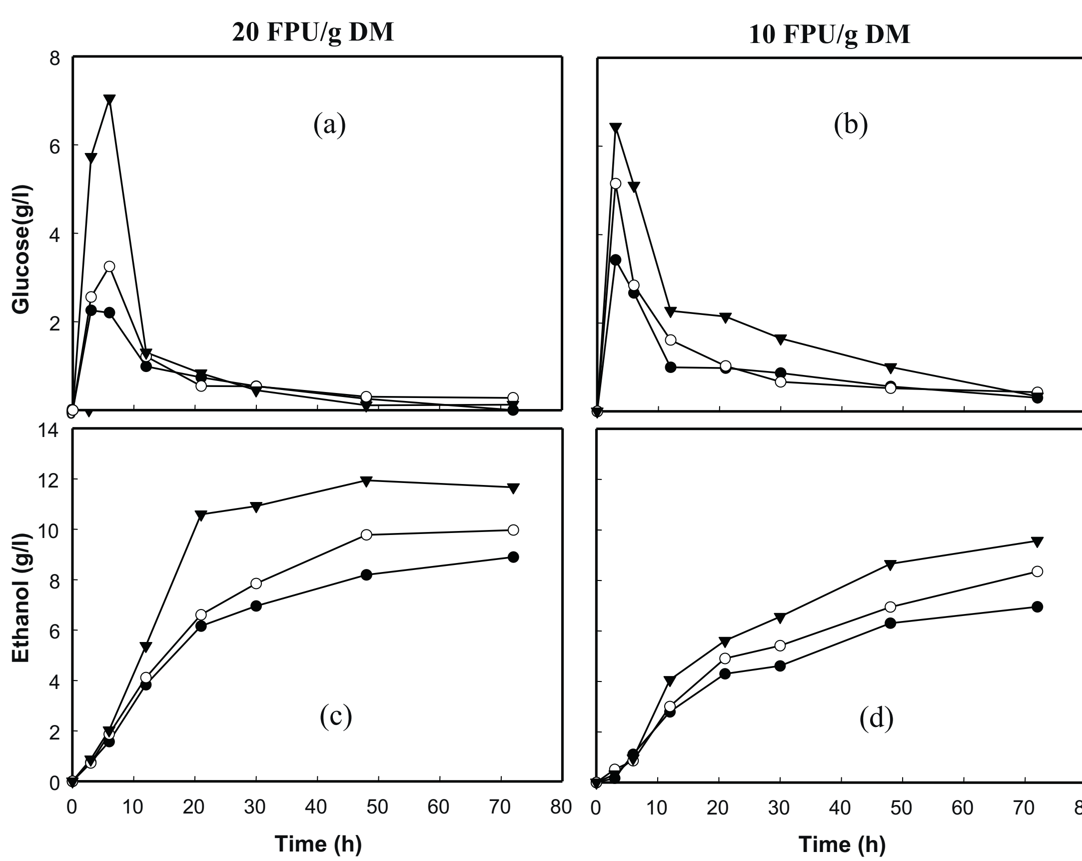

Fig. 3. Profiles of glucose (a and b) and ethanol (c and d) concentration during SSF process using *S. cerevisiae*: (●) Untreated, (○) DASE, and (▼) CPA.

enzymes and the rate of hydrolysis (Zhang et al., 2007). Lignin showed to adsorb irreversibly cellulytic enzymes that can ultimately lead to decreased activity due to unproductive binding of enzymes to lignin residues (Eriksson, Börjesson, & Tjerneld, 2002). Two groups of interactions namely hydrophobic and ionic-type interactions are thought to play an important role in adsorption of cellulytic enzymes to lignin (Berlin et al., 2006).

In lignocellulosic materials, hemicellulose is the most thermal-chemically sensitive fraction that starts to solubilize into the water at temperature higher than 150 °C and among its various components, xylan can be extracted the most easily (Bobleter, 1994; Lawther, Sun, & Banks, 1996). Physico-chemical pretreatments such as DASE are known to increase the accessibility of hydrolytic enzymes to the cellulose through dissolution of hemicellulose fraction of lignocellulosic materials (Alvira, Tomás-Pejó, Ballesteros, & Negro, 2010). In fact, DASE pretreatment is mostly efficient in hemicellulose solubilization rather than lignin removal (Galbe & Zacchi, 2002; Sánchez & Cardona, 2008; Tomas-Pejo, Oliva, & Ballesteros, 2008). The waste paper tube contains minor amount of xylan as the main hemicellulosic polymer. Therefore, minor effect of steam explosion pretreatment might be due to the specific structure of paper tube that contains small fraction of hemicellulose, which has been mainly removed during the former production processing steps.

Pretreatment of cotton-based textile using CPA greatly improved the rate of enzymatic hydrolysis and increased the yield of ethanol (Jeihanipour & Taherzadeh, 2009). However, the best results were obtained by alkaline pretreatment confirming that the efficiency of CPA pretreatment may vary based on the nature of material used. It seems that this method suits well for utilized paper tubes. The great improvement in enzymatic hydrolysis and subsequent fermentation rates of CPA pretreated samples compared to DASE pretreated and pure cellulose material (Table 2 and Fig. 2) could be attributed to binary effects of CPA as a cellulose solvent and cellulose hydrolysis catalyst, *i.e.* CPA not only disrupts linkages among components of lignocellulosic materials, but also reduces the recalcitrance of cellulose by breaking down the orderly H bonds among the cellulose chains (Zhang et al., 2007). Thus, a combination of chemical effect (cellulose decrystallization) and physical effect (increased accessible surface area) contributes to a substantial increase in digestibility of CPA pretreated paper tubes.

5. Conclusion

The feasibility of ethanol production from paper tube residuals was investigated. Paper tube was composed of approximately 65% glucose and xylose as two monomeric sugars after hydrolysis confirming that paper tube residuals could be considered as potential raw materials for production of ethanol. Enzymatic hydrolysis of both native and pretreated paper tubes was examined and then both materials were subjected to SSF process. Pretreatments as well as increase in cellulase loading improved the rate of hydrolysis and the yield of ethanol. The best result was obtained from CPA pretreated samples with cellulose loading at 20 FPU/g DM that yielded 0.42 g/g glucose ethanol after 48 h of SSF. The results confirm possibility of ethanol production about 308 ml/kg DM of paper tube residual under optimal condition.

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