

## Effects of agitation and exogenous H<sub>2</sub> on bioconversion of sugarcane bagasse into ethanol by *Clostridium thermocellum* ATCC 27405

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### Abstract

**Background:** The production of ethanol by a Consolidated Bioprocessing (CBP) strategy, which simultaneously combines cellulase production, lignocellulosic biomass hydrolysis and fermentation of released sugars to ethanol in one bioreactor, is a promising technology for cost reduction in the biological processing of biomass, specially using agroindustrial residues. *Clostridium thermocellum* is an anaerobic, thermophilic, strictly fermentative gram positive bacterium that meets all the requirements for CBP.

**Results:** Ethanol concentration obtained in the non-stirred fermentation process in flasks with raw bagasse was two times greater than that in the stirred system. The results observed using a pretreated sugarcane bagasse in non-stirred flasks regarding ethanol concentration, were slightly lower than with raw bagasse. The sparging of exogenous H<sub>2</sub> into the medium at atmospheric pressure inside the bioreactor showed to be unfavourable to achieve higher ethanol yields.

**Conclusions:** The strain investigated is a promising candidate for thermophilic fermentative ethanol production from dried ground raw sugarcane bagasse in a CBP strategy, although the alcohol concentrations need to be further improved. In future studies, it is recommended to investigate different modes of operation of the fermentation process, including pressurized conditions, as well as to use wet raw sugarcane bagasse aiming to achieve additional improvement in ethanol production and to reduce the costs of the process.

**Keywords:** consolidated bioprocessing; pretreated sugarcane bagasse; raw sugarcane bagasse; thermophilic.

### INTRODUCTION

Ethanol is an important alternative energy source in Brazil to reduce the dependency on fossil fuels. Conventional processes to produce ethanol are based on fermentation of sugars and starch, which can be used as a food supply. To avoid the competition between food and energy, recent biotechnological developments have led to an increased interest to utilize cellulosic residues as a resource to produce ethanol (Buaban et al. 2010), the so-called second generation ethanol (ethanol 2G). In the Brazilian case, the utilization of residues from sugarcane crop could increase the ethanol production in the range of 30 to 100%, depending on the process strategy (Pereira Jr et al. 2008).

However, the major technological impediment to utilize this important resource is the absence of low-cost technology for overcoming the recalcitrance of cellulosic biomass. A promising strategy to overcome this is the Consolidated Bioprocessing (CBP). This kind of process assumes the use of mono- or co-cultures of microorganisms capable to produce the cellulolytic enzymes, hydrolyse the

biomass and ferment the resulting sugars to desired products in a single step (Chen et al. 2011; Svetlitchnyi et al. 2013).

In this sense, anaerobic cellulolytic bacteria, specially the anaerobic genus *Clostridium*, have received much interest in recent years since they can utilize cellulosic biomass and generate ethanol (Demain, 2009; O-Thong et al. 2011).

The most special characteristic of some clostridia is the cellulosome - multi-enzymatic complex, discovered accidentally in the early 1980s (Bayer and Lamed, 2006). During the past 30 years, the cellulosome from *C. thermocellum*, a thermophilic bacterium, and from other species has been studied by many researchers. The cellulosome complex contains many different types of glycosyl hydrolases, including cellulases, hemicellulases and even other enzymes like  $\alpha$ -arabinofuranosidases and mannanases (Shoham et al. 1999).

Oligosaccharides derived from cellulose hydrolysis are actively transported via ATP-dependent cello-oligosaccharide ABC transporters, and cleaved to glucose by periplasmic  $\beta$ -glucosidases ( $G_n \leftrightarrow G_{n-1} + G$ ) or directly to glucose-1-phosphate ( $G_n + P_i \leftrightarrow G_{n-1} + G1P$ ) by cellodextrin and cellobiose phosphorylases (Zhang and Lynd, 2004; Rydzak et al. 2011; Gefen et al. 2012).

Glycolysis proceeds by the Embden-Meyerhof-Parnas pathway and because  $NAD^+$ -producing and  $NAD^+$ -consuming reactions must be balanced, factors that affect this cycle can also affect product formation. In *C. thermocellum* the primary enzymatic mechanisms of  $NAD^+$  production are lactate dehydrogenase, acetaldehyde-ethanol dehydrogenase, and ferredoxin-dependent hydrogenases (Rydzak et al. 2012). Hydrogen production is favourable to produce  $NAD^+$  (Kim et al. 2006), but when  $H_2$  accumulates, the production is inhibited, and lactate or ethanol must be produced to recover  $NAD^+$  at the expense of acetate and ATP (Li et al. 2012). Therefore, recognition of the effect of  $H_2$  hypersaturation on metabolites distribution may have an important impact on anaerobic fermentation technology (Lamed et al. 1988).

Several studies have shown changes in end-product yields not only in response to changes in  $[pH_2]$  but caused by the agitation rates (Ng et al. 1977; Doremus et al. 1985; Freier et al. 1988; Lamed et al. 1988; Bothun et al. 2004). Agitation can facilitate  $H_2$  transfer to the head space, which relieves the inhibition of acetate formation caused by the high  $H_2$  concentration in the medium (Lamed et al. 1988). This, in turns, may lead to reduced ethanol concentrations. Another effect of agitation concerns on cellulose degradation. According with Freier et al. (1988) the activity of cellulosomal system can be negatively affected depending on the rates applied (Freier et al. 1988; Lamed et al. 1988).

Hence, the aim of this research was to evaluate the ability of *C. thermocellum* to produce ethanol from sugarcane bagasse (SCB), an agroindustrial lignocellulosic residue very abundant in Brazil and in other tropical countries, and the effects of agitation and exogenous  $H_2$  on the ethanol production. To the best of our knowledge, this is the first work to report a Consolidated Bioprocessing (CBP) strategy for the production of ethanol by *C. thermocellum* using raw and pretreated SCB.

## MATERIALS AND METHODS

### Organism and maintenance medium

*Clostridium thermocellum* ATCC 27405 was employed for all growth experiments. Fresh cultures were maintained by routinely transferring 10% (v/v) mid-exponential phase inoculum into modified DSMZ M122 medium containing (g/L):  $(NH_4)_2SO_4$ , 1.3;  $KH_2PO_4$ , 1.3;  $K_2HPO_4$ , 4.3;  $MgCl_2 \times 6H_2O$ , 0.5;  $CaCl_2 \times 2H_2O$ , 0.1; MOPS, 10.0; Yeast extract, 5.0;  $FeSO_4 \times 7H_2O$ , 0.00125; cellobiose, 6.0;  $C_2H_3NaO_2S$ , 0.124; resazurin, 0.001. Cultures were grown at 60°C, pH 7.2 and stored anaerobically at 4°C.

## Sugarcane bagasse (SCB)

Raw SCB containing 40% cellulose, 22% hemicellulose and 23% total lignin, according to internal analysis (unpublished data) based on NREL/TP-510-42618 (Sluiter et al. 2012) was used directly and after diluted acid pretreatment, as previously described (Petrobras, 2005). The pretreatment conditions were: 1% of H<sub>2</sub>SO<sub>4</sub> (v/v), 30 min, 121°C, with agitation, using a conical-shaped vessel with a screw mounted on an orbital arm. Using the same methodology, the composition of the pretreated bagasse was determined as 53% cellulose, 8% hemicellulose and 32% total lignin, (unpublished data). Pretreated SCB was exhaustively washed to remove free carbohydrates and furanic compounds resulted from dehydration reactions during pretreatment. The efficiency of washes was verified by HPLC analysis. Both materials were dried and grounded (sieve size 0.178 mm) before use.

## Fermentation experiments

**Experiments in flask.** Batch experiments were carried out anaerobically in 50 mL sealed serum bottles filled with 30 mL of medium (in g/L): (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1.3; KH<sub>2</sub>PO<sub>4</sub>, 1.3; K<sub>2</sub>HPO<sub>4</sub>, 4.3; MgCl<sub>2</sub> x 6H<sub>2</sub>O, 0.5; CaCl<sub>2</sub> x 2H<sub>2</sub>O, 0.1; MOPS, 10.0; Yeast extract, 5.0; C<sub>2</sub>H<sub>3</sub>NaO<sub>2</sub>S (sodium thioglycolate), 0.124; resazurin, 0.001; and 10 ml/l of a mineral solution. This solution contained (mg/L): disodium EDTA, 400; MnSO<sub>4</sub>, 258; (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub> x 4H<sub>2</sub>O, 400; CoCl<sub>2</sub> x 6H<sub>2</sub>O, 40; NiCl<sub>2</sub> x 6H<sub>2</sub>O, 20; ZnSO<sub>4</sub> x 7H<sub>2</sub>O, 20; CuSO<sub>4</sub> x 5H<sub>2</sub>O, 3.2; FeSO<sub>4</sub> x 7H<sub>2</sub>O, 400. Dried ground raw and pretreated SCB were added to a concentration of 10 g/L (1% w/v). All batches were inoculated with freshly growing cultures at exponential phase. The volume of inoculum used was 20% (v/v). The initial pH was adjusted to pH 7.2 by using NaOH and measured at the end of the experiments, and the temperature was maintained at 60°C. All batches were incubated for 3 days, with daily sample withdrawal. All experiments were carried out in duplicates. First batch, using raw bagasse, was performed to evaluate the effect of agitation (without agitation and 80 rpm) on the ethanol production. Second batch, using the agitation condition selected, compared the results between raw and pretreated bagasse.

**Experiments in bioreactor.** Two anaerobic batches were carried out in 1.3L bioreactor (New Brunswick BioFlo 110) filled with 500 mL of the same medium above mentioned. Dried ground raw SCB was added to a concentration of 10 g/L (1% w/v). Batches were inoculated with freshly growing cultures at exponential phase. The volume of inoculum used was 10% (v/v). The pH was maintained at 7.2 ± 0.1 by using NaOH 1M and the temperature at 60°C. The agitation was kept to a minimum allowed by the equipment (50rpm) to make possible the pH control. Batches were incubated for 3 days, with daily sample withdrawal, in duplicate. In the first batch N<sub>2</sub> was continuously sparged into the medium inside the bioreactor. In the second batch N<sub>2</sub> was sparged only until the medium reaches anaerobic conditions, then the medium was inoculated and subsequently sparged with H<sub>2</sub> until the end of the experiment.

## Analytical methods

Cell growth was determined by measuring the absorbance (OD<sub>600</sub>) of inoculum samples at wavelength of 600 nm using Hach spectrophotometer (model DR5000).

Preparation of liquid samples was performed by filtration through a 0.2 µm nylon membrane prior to analysis for ethanol, acetic acid, lactic acid, cellobiose, glucose and xylose concentration, which was done by high performance liquid chromatography (HPLC) (Agilent 1260 Infinity) equipped with RI detector and Bio-Rad Aminex HPX-87H column. The operational conditions were: mobile phase, 5 mM H<sub>2</sub>SO<sub>4</sub>; flow, 0.7 mL/min; injection volume, 20 µL; column temperature, 65.0°C.

## Calculations

The initial mass of cellulose contained in the substrate (40% in raw bagasse and 53% in pretreated bagasse) was converted in glucose equivalents by multiplying this amount by the factor 1.111 (corresponding to the molecule of water incorporated to each glucose unit during hydrolysis). All components analyzed were converted in glucose equivalents, with the exception of xylose, using theoretical conversion factors. Ethanol yield (EY<sub>P/S</sub> g/g; mol/mol) was obtained by dividing the mass of ethanol in terms of glucose equivalents by the total of cellulose mass (in glucose equivalents)

consumed during the process. Note: the end-products carried over with the inoculum were subtracted from the initial amount observed at time zero.

## RESULTS AND DISCUSSION

The agitation is an important variable since its variation can to control the concentration of dissolved gas and, consequently, to change the proportion between end-products (Bothun et al. 2004). In this sense, the first experiment in serum bottles was carried out either at 80rpm or without mechanical stirring, using raw bagasse as substrate (Table 1).

**Table 1. Effect of agitation on final end-products concentrations and sugars accumulation (mM - 72 hrs) of *C. thermocellum* growing on 1% dried ground raw sugarcane bagasse (10 g/L).**

Agitation	End-products and sugars concentrations (mM)						EY <sub>P/S</sub> (g/g; mol/mol)	E/A ratio
	Cellobiose	Glucose	Xylose	Acetate	Lactate	Ethanol		
80 rpm	0.5 ± 0.1	1.0 ± 0.0 <sup>a</sup>	1.4 ± 0.0 <sup>a</sup>	9.0 ± 0.9	2.7 ± 0.0 <sup>a</sup>	10.7 ± 0.7	0.22; 0.86	1.4
Without	n.d.	n.d.	0.7 ± 0.0 <sup>a</sup>	12.1 ± 0.5	3.1 ± 0.0 <sup>a</sup>	21.9 ± 0.6	0.33; 1.29	1.8

n.d.: not detected; a Standard deviation below detection limit; E - ethanol; A - acetate. Note: for the purposes of calculating, we considered a total of 40% of cellulose in the biomass, *i.e.*, 10g/L of raw SCB correspond to 4 g/L of cellulose.

The results show an increase in the ethanol production by 2-fold and similar concentrations for acetate and lactate in non-stirred system. Taking into account the increasing in ethanol/acetate ratio, it is likely that the lack of agitation caused a shift in the metabolism from acetate to ethanol. It is worth to remember that static conditions are closer to the conditions in a natural environment of anaerobic cellulolytic bacteria (Freier et al. 1988). The final pH averages were very similar in both experiments, 6.7 (80 rpm) and 6.5 (non-stirring).

According to Lamed et al. (1988), during unstirred processes the medium becomes supersaturated with H<sub>2</sub> and this supersaturation leads to a higher ethanol/acetate ratio, which was also observed in this work (Table 1). The experiments carried out by Freier et al. (1988) demonstrated that in static cultures, cellulose with a high concentration of adsorbed cells settles down, and the H<sub>2</sub> produced is to a great extent entrapped in the sediment, influencing the fermentation, whereas in agitated cultures, the H<sub>2</sub> formed can escape for the gas phase.

Hydrogen (H<sub>2</sub>) production is favorable because NAD<sup>+</sup> is produced without the utilization of pyruvate for lactate or ethanol production. When reducing equivalents are recovered via the hydrogenases, more carbon is available for ATP production and biosynthesis. However, when H<sub>2</sub> accumulates, the production is inhibited, and lactate or ethanol must be produced to recover NAD<sup>+</sup> at the expense of acetate and ATP (Lamed et al. 1988; Bothun et al. 2004; Li et al. 2012).

Freier et al. (1988) also reported that to shake a culture utilizing cellulose, at certain levels (50-100 strokes/min), enhances cellulose degradation. However, to shake a culture at 200 strokes/min caused a slight decrease in the release of reducing sugars.

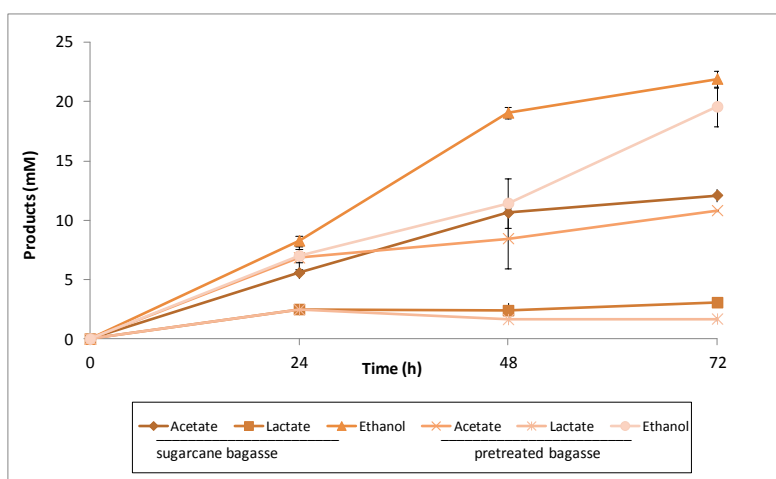
With regard to acetate concentrations, it is worth remembering it is possible that the acetate detected in the medium may be derived from the cleavage of hemicellulose-containing acetyl groups by the action of acetyl xylan esterase existing on the cellulosomes (Kosugi et al. 2002) or resulting from the bacterium metabolism.

At the end of the non-stirred process no sugar accumulation was detected (Table 1), with exception of xylose, since it cannot be fermented by *C. thermocellum*, although it is known that this genus can produce xylanases (Kosugi et al. 2002). It seems that the sugars released by the action of enzymes (cellobiose and glucose) were consumed immediately. On the other hand, in the agitated process there were still available sugars for the microorganism. As mentioned by Freier et al. (1988) it is possible that

agitation (50 rpm) has improved the action of cellulases and that cells have not been able to assimilate the sugars at the same rate from which they were released. In this sense, Islam et al. (2009) and Otajevwo and Aluyi (2011) assert that the hydrolysis rate by the cellosomal enzymes exceeded the rates of carbon utilization by the cells. They consider that the accumulation of sugars is a clear indication of the high activity of the bacterial enzymes.

Despite of the best results reached in the non-stirred system, the concentrations are still too low, if compared with the ethanol concentrations obtained with yeasts. It is possible that the substrate drying process has influenced these results. According to Weise (1998), when the substrate is submitted to drying process a phenomenon known as hornification can occur. This is when the fibers pores collapses, reducing dramatically the volume of pores and, consequently, the access of enzymes to the cellulose. Even when the fibers are resuspended in water, the original water-swollen state is not regained.

The second experiment was carried out with dried ground pretreated bagasse, in the same conditions (non-stirred), in order to compare with the products concentrations obtained with dried ground raw bagasse. Figure 1 shows the comparison between these experiments.



**Fig. 1** Concentrations of end-products (mM) over the time (72 hrs) in batch fermentation (flasks) of *C. thermocellum* with 10 g/L of dried ground raw sugarcane bagasse and pretreated bagasse.

Analyzing the total ethanol concentration achieved, it can be observed a slightly higher value using raw bagasse. We could say that, despite of the total original lignin presence in the raw bagasse, the microorganism was able to hydrolyze the cellulosic substrate. It is important to note that total cellulose available in the pretreated bagasse is higher (53%) than in the raw bagasse (~40%). The presence of inhibitors, especially furfural and 5-hydroxymethylfurfural, could not explain the concentration obtained because the SCB was exhaustively washed after pretreatment.

Table 2 presents the accumulation of sugars,  $Y_{P/S}$  and ethanol/acetate ratio obtained with pretreated bagasse over the experiments (72 hrs). Ethanol yield was slightly lower (0.29 g/g or 1.13 mol/mol) than that obtained with raw bagasse (0.33 g/g or 1.29 mol/mol) and the ethanol/acetate ratio was exactly the same of that observed with raw bagasse (1.8), at the end of the process (72 hrs).

According to Lynd et al. (2002), *C. thermocellum* is able to grow on pretreated lignocellulose substrates which contain all or most of lignin present in the substrate before the pretreatment. The authors obtained similar results using Avicel and pretreated wood. Therefore, the presence of insoluble lignin *per se* does not reduce hydrolysis rates.

**Table 2. Sugars accumulation of *C. thermocellum* growing on 1% of dried ground pretreated sugarcane bagasse (10 g/L).**

Time (hrs)	Sugars concentrations (mM)			EY <sub>P/S</sub> (g/g; mol/mol)	E/A ratio
	Cellobiose	Glucose	Xylose		
24	1.9 ± 0.0 <sup>a</sup>	1.9 ± 0.1	0.6 ± 0.0 <sup>a</sup>	0.15; 0.59	1.0
48	1.1 ± 0.5	1.1 ± 0.3	0.3 ± 0.0 <sup>a</sup>	0.23; 0.90	1.4
72	1.2 ± 0.0 <sup>a</sup>	1.1 ± 0.0 <sup>a</sup>	0.5 ± 0.0 <sup>a</sup>	0.29; 1.13	1.8

<sup>a</sup> Standard deviation below detection limit; E - ethanol; A - acetate. Note: for the purposes of calculating, we considered a total of 53% of cellulose in the biomass, i.e., 10 g/L of pretreated SCB correspond to 5.3 g/L of cellulose.

In comparison with the results of ethanol production using pretreated bagasse (19.6 mM), 18.8 mM of ethanol were obtained in an experiment with Avicel, in the same conditions (unpublished data), confirming the statements above.

Considering that using a natural substrate, without prior pretreatment, is much more attractive for developing a new bioprocess, in terms of economy, raw bagasse was selected for the bioreactor trials.

It is important to note that serum bottles experiments cannot provide pH control and this process variable is an important factor not only for the microorganism growth but also for cellulases production (Otajevwo and Aluyi, 2011). The improvement of the production or activities of enzymes can positively influence ethanol production.

Therefore, the subsequent step of this work was performed in an instrumented bioreactor with pH, temperature and agitation controls.

As reported by Islam et al. (2009) low pH is the prime factor for the stoppage of fermentation in the stationary phase of *C. thermocellum* and Dumitrache et al. (2013) assert that sporulation occurs in the late exponential phase as a response to decreased pH. During the experiments carried out by Freier et al. (1988), the growth of *C. thermocellum* ceased completely when the pH fell below 6.2.

On the other hand, pH control requires agitation. For this reason, in the following experiments we used the minimum agitation allowed by the equipment – 50 rpm, which was sufficient to keep the solids in suspension.

The first trial in bioreactor shows a considerable drop in ethanol production and a rise in lactate and acetate production in comparison with the trials in flasks (Table 3). The same tendency was verified in previous experiments using pure cellulose as substrate (unpublished data).

**Table 3. Final end-products concentrations and sugars accumulation of *C. thermocellum* growing on 1% of dried ground raw sugarcane bagasse (10 g/L) in bioreactor.**

Time (hrs)	End-products and sugars concentrations (mM)						E Y <sub>P/S</sub> (g/g; mol/mol)	E/A ratio
	Cellobiose	Glucose	Xylose	Acetate	Lactate	Ethanol		
24	n.d.	n.d.	n.d.	14.1 ± 0.0 <sup>a</sup>	5.8 ± 0.0 <sup>a</sup>	7.4 ± 0.0 <sup>a</sup>	0.15; 0.59	0.5
48	n.d.	n.d.	n.d.	18.5 ± 0.0 <sup>a</sup>	5.9 ± 0.0 <sup>a</sup>	8.2 ± 0.1	0.14; 0.55	0.4
72	0.2 ± 0.0 <sup>a</sup>	1.5 ± 0.0 <sup>a</sup>	1.0 ± 0.0 <sup>a</sup>	17.1 ± 0.0 <sup>a</sup>	5.3 ± 0.0 <sup>a</sup>	6.0 ± 0.2	0.10; 0.39	0.4

n.d.: not detected; <sup>a</sup> Standard deviation below detection limit; E - ethanol; A - acetate. Note: for the purposes of calculating, we considered a total of 40% of cellulose in the biomass, i.e., 10 g/L of raw SCB correspond to 4 g/L of cellulose.

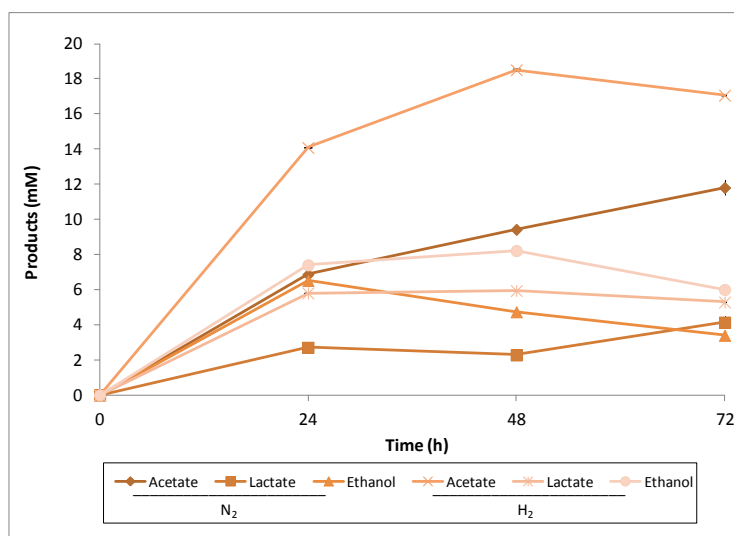
There are some hypotheses that can explain these results. Contrary to what happens in serum bottles, in bioreactor (1) it was not possible to maintain the  $[pH_2]$ , (2) ethanol can be dragged to the headspace, since no evidences about the consumption of ethanol by *C. thermocellum* could be found in the literature, and (3) gas injection ( $N_2$ ) and agitation may have facilitated the loss of ethanol and  $H_2$  produced to the headspace.

When the  $H_2$  is accumulated in the medium its production is inhibited. Hence, more ethanol or lactate must be produced to recover  $NAD^+$  at the expenses of acetate and ATP (Lamed et al. 1988; Bothun et al. 2004; Li et al. 2012). That would explain higher concentrations of ethanol achieved during experiments in flasks. Thus, the opposite situation (no accumulation of  $H_2$  in bioreactors) would explain higher concentrations of acetate.

The explanation for this fact is that acetate production from acetylCoA is more energetically favourable than ethanol production. However, to produce one molecule of acetate, *C. thermocellum* produces two molecules of  $H_2$ , in order to maintain a redox balance. Increased  $[pH_2]$  changes the normal electrons flow from the reduced ferredoxin ( $FdH_2$ ) to the  $NAD^+$ , making the production of  $H_2$  thermodynamically unfavourable. For that reason, maintaining  $[pH_2]$  inhibit acetate production and increase  $NADH$  concentration, providing more reducing equivalents to produce ethanol (Bothun et al. 2004).

In the second bioreactor trial we explored the possibility to increase the ethanol concentration switching the sparging gas. In this case, after achieve the anaerobiosis and add the inoculum, the  $N_2$  was replaced by  $H_2$ . Li et al. (2012) carried out a similar experiment with the same strain, which shows that acetate production decreased by 35%, lactate production remained low, and ethanol production increased by 350%. However, the type of reactor used by the authors allowed the maintenance of  $[pH_2]$ , which was not possible in our bioreactor model.

Figure 2 shows the comparison between the experiments in bioreactors. It can be observed that ethanol concentration was even lower (43%) than the first trial in bioreactor, as well as lactate production (23%) and acetate production (31%). The ratio ethanol/lactate decreased from 1.1 (in the previous experiment) to 0.8 in this experiment and the ratio ethanol/acetate was almost the same (0.3).



**Fig. 2** Concentrations of end-products (mM) over the time (72 hrs) in batch fermentation (bioreactors) of *C. thermocellum* with 10 g/L of raw sugarcane bagasse sparged with  $N_2$  and with  $H_2$ .

We could say that H<sub>2</sub> injection caused alterations in the *C. thermocellum* metabolism, in particular, in regard to lactate production. It was possible to observe also that the effect of H<sub>2</sub> injection on ethanol production was negative. It is possible also that H<sub>2</sub> no longer had effect after achieve saturation point.

As mentioned above, several studies have shown changes in end-product yields in response to changes in [pH<sub>2</sub>], but in these studies pressurized systems were used and the gas was added in the headspace. In our case the fermentation process was carried out at atmospheric pressure and the H<sub>2</sub> was sparged in the medium.

It should be pointed out that, considering that sugarcane bagasse is an agroindustrial residue and that the results obtained in this work showed that *C. thermocellum* ATCC 27405 is able to use this kind of waste without any pretreatment, we can then consider this strain as a promising fermentative agent for a consolidated bioprocessing using a natural biomass.

## CONCLUDING REMARKS

This study investigated the possibility of using raw and pretreated sugarcane bagasse (SCB) as a substrate for ethanol production by *C. thermocellum* ATCC 27405, as well as the effects of agitation and addition of exogenous H<sub>2</sub> during the fermentation process. In non-stirred system it was possible to increase ethanol concentrations from 10.7 mM to 21.9 mM with raw SCB (non-pretreated). However, using diluted acid pretreated sugarcane bagasse, under the same conditions; ethanol concentration was reduced from 21.9 to 19.6 mM, stating that there is a possibility to develop a consolidated bioprocess using this strain without the need to pretreat the lignocellulosic biomass.

The lower ethanol concentrations observed in bioreactor scale, as compared to flasks, revealed the need to further develop the fermentation process, in order to manipulate fermentation conditions to redirect the metabolism for the desired end-product - the ethanol. One possible change is to use different reactor configurations to allow the fermentation process to be carried out under pressure. We indicate also the necessity to evaluate the use of wet sugarcane bagasse in order to avoid pores collapse and, consequently, improve ethanol yields.

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