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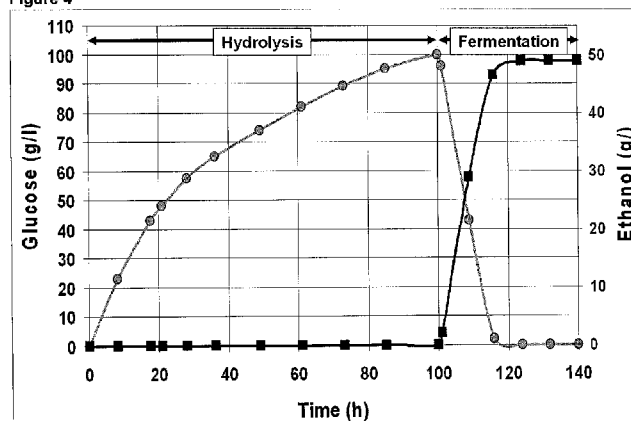
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(54) Title: FED BATCH PROCESS FOR BIOCHEMICAL CONVERSION OF LIGNOCELLULOSIC BIOMASS TO
ETHANOL

Figure 4



(57) Abstract: A method for optimization of a fed-batch lignocellulosic biomass hydrolysis process wherein the hydrolysis time is minimized by controlling the feed addition volume and / or batch addition frequency of prehydrolysed lignocellulosic feed and optionally the enzyme feed. The increase over time in hydrolysate consistency and volume and / or concentration of sugars released in the reactor, so that the enzymatic hydrolysis is controlled, significantly reduces the impact of cellulase feedback inhibition, especially for enzyme contents lower than 0.5%. The overall time to reach conversion of the total prehydrolysate feed is reduced significantly where the batch addition frequency is equal to one batch each time 70% to 90% of a theoretical cellulose to glucose conversion is reached, preferably when 80% conversion is reached. At an enzyme load of 0.3% in the reaction mixture, the optimum frequency each time 80% conversion was reached was found to be one batch every 105 minutes.

Fed Batch Process for Biochemical Conversion of Lignocellulosic Biomass to Ethanol

FIELD OF THE INVENTION

5 The present invention generally relates to the production of ethanol from biomass and in particular to a fed batch process for enzymatic hydrolysis of lignocellulosic biomass.

BACKGROUND OF THE INVENTION

10 The importance of ethanol as a clean transportation fuel has increased with the anticipated shortage of fossil fuel reserves and with increased air pollution.

 Ethanol is regarded as a more environmentally friendly fuel than gasoline because it adds less net carbon dioxide to the atmosphere. This is the main reason for significant research into economically viable ways of producing ethanol from renewable raw
15 materials.

 Fuel ethanol is distilled and dehydrated to create a high-octane, water-free alcohol. Ethanol is blended with gasoline to produce a fuel which has environmental advantages when compared to gasoline alone, and can be used in gasoline-powered vehicles manufactured since the 1980's. Most gasoline-powered vehicles can run on a
20 blend consisting of gasoline and up to 10 percent ethanol, known as "E-10".

 Ethanol can be produced in several different ways. For example, ethanol can be synthesized from gasified carbon-containing feedstock. More commonly it is produced by the fermentation of sugar from starchy plants such as corn or wheat or sugar or from sugar cane or sugar beets.

25 In North America the feedstock is primarily corn, while in Brazil sugar cane is used. The use of potential food or feed plants to produce ethanol is considered as disadvantageous due to the limited availability of such feedstock and the limited area of suitable agricultural land.

 An alternative to food or feed plants is lignocellulosic biomass. Biomass is widely
30 available and contains a high proportion of cellulose, hemicellulose and lignin. The four main categories of biomass are: (1) wood residues (including sawmill and paper mill discards), (2) municipal paper waste, (3) agricultural residues (including corn stover and corn cobs and sugarcane bagasse), and (4) dedicated energy crops (which are mostly composed of fast growing tall, woody grasses such as switch grass and Miscanthus).

Lignocellulosic biomass is composed of three primary polymers that make up plant cell walls: Cellulose, a polymer of D-glucose; hemicellulose that contains two different polymers i.e. xylan, a polymer of xylose and glucomannan, a polymer of glucose and mannose; and lignin, a polymer of guaiacylpropane- and syringylpropane units. Of these components cellulose is the most desirable since it can be broken down into monomer glucose that can be fermented to ethanol.

However it is not easy to convert lignocellulosic material into sugar. Cellulose fibers are locked into a rigid structure of hemicellulose and lignin. Lignin and hemicelluloses form chemically linked complexes that bind water soluble hemicelluloses into a three dimensional array, cemented together by lignin, that covers cellulose microfibrils and protect them from enzymatic and chemical degradation. These polymers provide plant cell walls with strength and resistance to degradation. This makes lignocellulosic materials a challenge to use as substrates for biofuel production.

A promising route for the conversion of lignocellulose to ethanol is called the enzymatic conversion process. This process consists of five main steps. The first step is the collection and transportation of the biomass to a central process plant. The second step is to pretreat the biomass (prehydrolysis) usually with a unit operation called steam explosion. However, prehydrolysis can be chemical, physical or biological. Diverse techniques have been explored and described for the pretreatment of size-reduced biomass material with the aim of producing substrate that can be more rapidly and efficiently hydrolysed to yield mixtures of fermentable sugars.

These approaches have in common the use of conditions and procedures which greatly increase the surface area to which aqueous reactants and enzymes have access. In particular, the percentage of the major cellulosic materials that are opened up In steam explosion, the biomass is fiberized and the cellulose is fractured making it more susceptible to the third step called enzymatic hydrolysis. Highly specialized enzymes catalyse the depolymerization of the cellulose into glucose. The final two steps are fermentation of the glucose to ethanol and the separation of the ethanol from the aqueous fermentation broth. Ultimately the separation step removes the last remaining water making a water free ethanol suitable for blending with gasoline.

Pretreatments of lignocellulosic biomass, such as steam explosion based pretreatments, generally result in extensive hemicellulose breakdown and, to a certain extent, to the degradation of hemicellulose. This results in the production of soluble and insoluble xylooligosaccharides, acetic acid and furfural. These pretreatment methods

may employ hydrolytic techniques using acids (hemicellulose hydrolysis) and alkalis (lignin removal).

A useful form of biomass for the production of ethanol is the agricultural residue, corncobs. It is relatively high in cellulose (35-40%) and it is also high in hemicellulose and low in lignin content. The hemicellulose content of corncobs makes up almost 30% of the total dry matter (DM). Moreover, much of the hemicellulose is acetylated which means that breakdown and liquefaction of the hemicellulose leads to the formation of acetic acid. This is a problem, since the acid is a powerful inhibitor of the ethanol fermentation process, remains in the pretreated biomass and carries through to the hydrolysis and fermentation steps. On the other hand the low pH of acetic acid helps in the prehydrolysis process. Hemicellulose is a heteropolymer or matrix polysaccharide present in almost all plant cell walls along with cellulose. While cellulose is crystalline, strong, and resistant to hydrolysis, hemicellulose has a random, amorphous structure with little strength. Hydrolysis of hemicellulose can be relatively easily achieved with acids or enzymes. Hemicellulose contains many different sugar monomers. For instance, besides glucose, hemicellulose can include xylose, mannose, galactose, rhamnose, and arabinose. Xylose is the monomer present in the largest amount.

While cellulose is highly desirable as a starting material for enzymatic ethanol production, high concentrations of the products of enzymatic cellulose and hemicellulose hydrolysis interfere with the performance of cellulose and hemicellulose degrading enzymes. Especially toxic are glucose, cellobiose and xylose, all of which are products of the enzymatic hydrolysis of hemicellulose, and are inhibitors of cellulase enzymes.

A typical cellulose hydrolysis pattern in a batch mode enzymatic process is characterized by a two phase curve, with an initial logarithmic phase followed by an asymptotic phase. During the first phase, cellulose is mainly depolymerised and hydrolyzed into soluble gluco-oligosaccharides then cellobiose. Subsequent conversion of cellobiose to glucose is carried out by cellobiases during the second phase of hydrolysis. A rapid release of glucose is normally observed in the initial phase with about half of the cellulose hydrolysed. Hydrolysis of the second half of the cellulose requires days to complete.

Several mechanisms have been proposed for this insufficient hydrolysis phenomenon. However, end-product inhibition of cellulases has been shown to play a major role in hindering continuously fast cellulose to glucose conversion rate.

Several cellulolytic enzymes are involved in the first phase of hydrolysis. The cellobiases are the predominant group of enzymes that carry out the latter step of conversion. As a final product, glucose has a direct inhibitory effect on cellobiase activity.

There is also evidence that glucose has a significant inhibitory impact on exoglucanase and endoglucanase. It has also been shown that cellobiose exhibits a greater inhibitory effect than glucose on cellulase activity during cellulose hydrolysis. It is hypothesized that a high glucose content in the hydrolysate leads to the accumulation of cellobiose which then acts as a secondary inhibitor.

This is a problem since a medium to high-solids operation of the enzymatic hydrolysis of lignocellulose is required to reduce capital costs and increase product concentration to reduce ethanol separation costs.

Enzymatic hydrolysis of lignocellulosic biomass can be carried out in batch or continuous reactors. In a batch process, all components, including pH-controlling substances, are placed in the reactor at the beginning of the hydrolysis. During the hydrolysis process there is no input into or output from the reactor. In a continuous process, there are both input and output flows, but the reaction volume is kept constant.

In an alternative batch process configuration, a fed-batch process, nothing is removed from the reactor during the process, but one substrate component is progressively added in order to control the reaction rate by substrate concentration. The substrate is fed continuously into the reactor over the hydrolysis period without withdrawing any hydrolysate. This type of feeding of the substrates has been found to overcome effects such as substrate inhibition on the product yield.

Of course, substrate inhibition can also be counteracted by increasing the amount of enzyme used in the reaction mixture. However, due to the high cost of enzyme, that approach is uneconomical and the process is normally operated at the lowest enzyme concentration possible.

The main advantages of the fed-batch operation are the possibilities to control the reaction rate by the substrate feed rate. Because practical models for model-based control are rare, fed batch processes are usually run with a predetermined feed profile. Still, it remains a challenge of the enzymatic hydrolysis process to operate the process at the optimal conditions, since the lower the enzyme concentration in the reaction mixture, the higher the danger of substrate or product inhibition of the enzyme.

Usual industrial practice is to develop a reference profile for the substrate feed rate based on operational experience and to implement it in the plant with suitable adjustments to account for the actual conditions in the reactor.

This approach is far from optimal, since it is empirical in nature and operator dependent, which invariably leads to undesired fluctuations in the product yield. Alternatively, mathematical models of the hydrolysis process are used to calculate an

optimum substrate flow rate profile off-line and to implement it in the actual fermentation unit to maximize product yield.

A number of different optimization methods and strategies for maximization of the product yield of fed-batch processes were reported. Most of the optimization methods
5 rely on complex mathematical models for computing an optimal feed profile.

Optimal control techniques rely upon an accurate model of the process and for many years mechanistic models have been used to develop optimal control strategies for fed-batch processes. However, mechanistic models of fed-batch processes are usually very difficult to develop due to the complexity and nonlinear nature of the processes.
10

SUMMARY OF THE INVENTION

It is now an object of the present invention to provide a process which overcomes at least one of the above disadvantages.

15 It is a further object to provide a method for the optimization of a fed batch hydrolysis process wherein the process operating parameters are adjusted by means of controlling the feed of the prehydrolysate, preferably the batch volume and/or batch addition frequency of the prehydrolysate and optionally also the enzyme feed, the increase over time in hydrolysate consistency and volume and/or the concentration of
20 sugars released in the reactor, so that the enzymatic hydrolysis is controlled to significantly reduce the impact of cellulase feedback inhibition, especially for low enzyme contents in the reaction mixture, for example enzyme contents lower than 0.5%.

The inventors have now surprisingly discovered that the phenomenon of cellulase product inhibition in the hydrolysate can be reduced, even at very low enzyme loads, by
25 adding the prehydrolysate feed in multiple small batches while closely controlling the batch addition frequency and batch volume, and possibly also the amount of cellulase enzymes, added in each step. In particular, the conditions are chosen such that a high glucose concentration is achieved in the reaction mixture, while the impact of cellulase product and/or substrate inhibition is limited at the same time.

30 The inventors have discovered that hydrolysis rates in the reaction mixture slow down dramatically as the conversion rate surpasses 70% of the theoretical cellulose to glucose conversion. The inventors have further discovered that the overall time to reach conversion of the total prehydrolysate feed is reduced significantly if the batch addition frequency is equal to one batch each time 70% to 90% conversion of the previous batch
35 is reached in the reaction mixture. The optimum frequency was found to be one batch

each time 80% conversion is reached. At an enzyme load of 0.3% in the reaction mixture, the optimum frequency each time 80% conversion was reached was found to be one batch every 105 minutes (min).

In one aspect, the invention provides a process for the hydrolysis of lignocellulosic biomass, such as corncobs, which process includes the steps of filling the reactor with water, adding cellulose enzyme(s) and then carrying out sequential additions of lignocellulosic prehydrolysate feed batches at a preselected batch volume and at a preselected batch addition frequency over a total feed time. Hemicellulolytic enzymes can also be added in steps, either separately or together with the prehydrolysate feed. As the feed is added, the consistency and solids concentration rise until the total desired dry matter content is achieved. The frequency of lignocellulosic prehydrolysate addition is preferably maintained constant over the entire feed time. The batch volume, which means the portion of the total added feed which is added at each feed step, is preferably held constant over the total feed time.

In one aspect, a process for the hydrolysis of lignocellulosic biomass, comprises: filling a reactor vessel with water; adding a cellulase enzyme; and sequentially adding a lignocellulosic prehydrolysate feed into the reactor vessel to produce a reaction mixture, whereby the prehydrolysate feed is added in batches at a preselected batch volume and a batch addition frequency over a total feed time to achieve a preselected final consistency and a preselected dry matter content in a final reaction mixture, the batch addition frequency being equal to one batch each time 70% to 90% of a theoretical cellulose to glucose conversion is reached in the reaction mixture.

In one case, the batch addition frequency is one batch every 80 to 105 min.

In another case, the batch addition frequency is one batch each time 80% of the theoretical cellulose to glucose conversion is reached in the reaction mixture.

In another case, the preselected batch volume and the batch addition frequency are maintained constant throughout the total feed time.

In another case, the preselected batch volume and/or the batch addition frequency are decreased towards an end of the total feed time.

In another case, the batch addition frequency is one batch every 105 min, the preselected consistency is 17% and the preselected addition period is 12 to 35 hours.

In another case, the total feed time is one batch every 17 to 25 hours.

In another case, the total feed time is 20 hours.

In another case, the batch addition frequency is one batch every 105min, the preselected consistency is 24% and the total feed time is 80 to 120 hours.

In another case, the total feed time is 90 to 110 hours.

In another case, the total feed time is 95 hours.

In another case, the cellulase enzyme is added at an enzyme load of 0.3% in the reaction mixture and the batch frequency is one batch each time 80% conversion is reached.

In another case, the maximum batch addition frequency is one batch every 105 minutes.

In another case, the batch volume is progressively decreased in a second half of the total feed time.

In another case, the batch volume is progressively decreased in a last quarter of the total feed time.

In another case, the enzyme is added in an amount lower than 1% of the final reaction mixture.

BRIEF DESCRIPTION OF THE DRAWINGS

Other objects and advantages of the invention will become apparent upon reading of the detailed description and upon referring to the drawings in which:

Figure-1 A and B show feed time profiles used to reach a consistency of 17 % DM.

Figure 1B shows additions of prehydrolysates which were carried out at a frequency of one every 105min. The lines are not completely straight due to the moisture content of the prehydrolysate.

Figure 2 shows the change in the conversion time of cellulose to glucose as a function of the feed time of the substrate required to reach 17 % consistency.

Figure 3 shows the change in the conversion time of cellulose to glucose as a function of the feed time of the substrate required to reach 24 % consistency. Hydrolysis experiments were carried out at 50 °C, pH 5.0. pH adjustment chemical used was liquid ammonia (30 %). Commercially available lignocellulolytic enzyme was used at a load of 0.3 %. Similar results were obtained at Laboratory (1 kg beaker) and pilot scale (300 kg tank).

Figure 4 shows an example of 2.5 tonne fed batch hydrolysis of corncobs at 17 % followed by a batch ethanologenic fermentation of the resulting hydrolyzate. Hydrolysis was carried out at 50 °C, pH 5.0, 0.5 % enzyme load. Fermentation was carried out at 33 °C, pH 5.3 using an industrial grade C6-fermenting yeast. Hydrolysis and

fermentation pH adjustment was carried out using liquid ammonia (30 %). Grey circles indicate glucose concentration. Black squares indicate Ethanol concentration.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

5 Before explaining the present invention in detail, it is to be understood that the invention is not limited to the preferred embodiments contained herein. The invention is capable of other embodiments and of being practiced or carried out in a variety of ways. It is to be understood that the phraseology and terminology employed herein are for the purpose of description and not of limitation.

10 The invention is directed to ethanol from biomass processes and especially to enzymatic hydrolysis processes. In particular, the invention is directed to processes intended to limit the negative impact of product inhibition in the cellulose containing hydrolysate when lignocellulosic biomass is used as the starting material.

15 A preferred aspect of the invention is a process for the enzymatic hydrolysis of lignocellulosic biomass for generating a cellulose hydrolysate with reduced feed back inhibition compared to standard fed batch processes. The preferred process of the invention includes the steps of filling the reactor with water and then carrying out sequential additions of lignocellulosic prehydrolysate and enzymes at a constant ratio over a predetermined time. As the prehydrolysate feed and fresh enzymes are added,
20 the consistency and solids concentration rise until the total desired dry matter content is achieved.

25 A series of enzymatic hydrolysis reactions of a feedstock such as corncobs were conducted at medium and high consistencies that ranged from 17 % to 32 % to determine optimum process conditions. The effectiveness of each set of hydrolysis conditions was determined by monitoring the time to reach percentages of the theoretical maximum cellulose to glucose conversion in order to evaluate overall cellulose digestibility e.g. $t_{90\%}$ the time to reach 90 % conversion. The prehydrolysate feedstock was prepared in a batch or continuous steam explosion pretreatment.

30 Composition analysis was carried out at the analytical laboratory of Paprican (Montreal, Canada), using the TAPPI methods T249 cm-85 and Dairy one (wet chemistry analysis). Total feed times assayed for prehydrolysate and enzyme feeds ranged from 2 hours to 140 hours (h)

 The hydrolysis process operating conditions were screened with respect to high cellulose to glucose conversion rates obtained at low enzyme loading. The hydrolysis

conditions were chosen to ensure a high glucose concentration was achieved, while the impact of product inhibition of the cellulases was limited at the same time.

Hydrolysis time of the corncobs prehydrolysate at 17 % consistency was generally less than 100 hours.

5 Quantification of soluble products from pretreatment and enzymatic hydrolysis was carried out by HPLC analysis. Target molecules were monitored to determine the relative contents of cellulose and downstream inhibitors in the prehydrolysate obtained. The target molecules were sugar monomers such as glucose and xylose. The summary results of the test treatment series are plotted in Figures 1 and 2.

10 As shown in Figure 1 A and B, in a fed batch hydrolysis the feed and enzymes can be added in different ways. We have previously found that small sequential additions of new feed and enzymes carried out on a regular basis gave much faster hydrolysis than adding the total mass of feed and enzymes in one addition. In each case, a predetermined amount of water was added to a beaker and then the feed and enzymes
15 were added over different feed time periods that ranged from 2 h to 68 h. As mentioned above, the enzyme feed can be correlated with the prehydrolysate feed, or carried out completely independently. The complete enzyme charge can also be added in one single feed step at the beginning of the feed time. All hydrolysates were continuously maintained at the same enzyme load using sequential additions of enzyme.

20 Figure 2 shows that adding the prehydrolysate and enzyme over a period of 18 hours to reach 17% consistency led to the shortest conversion time to reach up to 90 % or 95% conversion was. To achieve 100% conversion, the feed time should be extended to about 40 h. The overall hydrolysis time almost doubles between 90% and 100% conversion. Similar results were obtained in the lab (1 kg beaker) and at pilot scale (300
25 kg tank) using 18 h feed time. Additions of prehydrolysate were carried out each 105min. This number was chosen based on our experience that it requires about 105min for liquefaction of the cellulose to occur. Acceptable feed frequencies would be one every 80 min to one every 105 min. In each case, substrate was added at intervals of 105 min. The batch volume, which means the quantity of substrate added at each additional step
30 was varied to give the desired consistency in the desired total feed time.

Figure 3 shows the change in the conversion time of cellulose to glucose as a function of the total feed time of the substrate to reach 24% consistency.

The optimum total feed time to reach 80 %, 85 % or 90 % conversion of 24 % consistency hydrolysate was 80 h, 90 h and 100 h respectively. At 24% consistency 150
35 grams per liter (g/L) glucose were detected after 180 h. Similar results were obtained in

the lab (1 kg beaker) and at pilot scale (300 kg tank) using 140 h total feed time. In each case the substrate was added at intervals of 105 min. The batch volume, which means the quantity of substrate added was varied to given the desired consistency in the desired total feed time.

5 Acceptable conditions for fed batch hydrolysis of corncobs were found to be a 12 h to 35 h total feed time for 17 % consistency hydrolysis or 80 h to 120 h total feed time for 24 % consistency hydrolysis. Improved results were achieved using a total feed time of 17 h to 25 h at 17 % consistency or 90 h to 110 h total feed time at 24 % consistency. Optimal results were achieved using 25 h or 95 h total feed time at 17 % or 24 %
10 consistency, respectively.

The governing factors for the effectiveness of fed batch hydrolysis were found to be total feed time and batch addition frequency.

EXAMPLE

15 Ground corncobs of 0.5 to 1 cm³ particle size were pretreated by autohydrolysis steam explosion pretreatment at 205 °C, i.e. cooking pressure of 235 psig for a residence time of 8 min.

Prehydrolysed corncobs were shredded in a garden shredder and then diluted with fresh water to the desired consistency for hydrolysis and fermentation.

20 A 2.5 ton hydrolysis and fermentation trial was carried out at 17 % consistency. Enzymatic hydrolysis was carried out at 50 °C, pH 5.0. Fermentation was carried out at 33 °C, pH 5.3. Aqueous ammonia at 30 % concentration was used to adjust pH. Commercially available lignocellulosic enzyme product and industrial grade ethanologenic yeast were used.

25 Pilot scale hydrolysis and fermentation was carried out in a heat traced, jacketed 6000 liter tank equipped with a recirculation pump, a high speed mixer and a wiper.

Co-addition of corncobs prehydrolysate at 35 % DM and liquid enzyme was made over a period of 16 h. Ten additions were carried out with a gap of 105 min in-between each addition such as described in Figure 1A and 1B (dotted line). The first addition of
30 prehydrolysate and enzyme was carried out at time zero of the hydrolysis feed time. This feeding procedure was determined as being in the range of optimum feed time to reach 90 % to 95 % of the maximum theoretical cellulose to glucose conversion of 17 % consistency pretreated corncobs hydrolyzate at laboratory and smaller pilot scale (Figure 2).

Results of the pilot scale trial showed that a concentration of 100 g/L glucose was reached at $t_{90\%}$ i.e. 100 h hydrolysis (Figure 4). Hydrolysis time of the 2.5 tonnes trial was in accordance with above discussed results obtained at laboratory and 300 kg pilot scale.

5 In this example a titer of 5 % alcohol was reached by 20 hours fermentation.

CLAIMS:

1. A process for the hydrolysis of lignocellulosic biomass, comprising:
filling a reactor vessel with water;
5 adding a cellulase enzyme; and
sequentially adding a lignocellulosic prehydrolysate feed into the reactor vessel to
produce a reaction mixture, whereby the prehydrolysate feed is added in batches at a
preselected batch volume and a batch addition frequency over a total feed time to
achieve a preselected final consistency and a preselected dry matter content in a final
10 reaction mixture, the batch addition frequency being equal to one batch each time 70% to
90% of a theoretical cellulose to glucose conversion is reached in the reaction mixture.
2. The process of claim 1, wherein the batch addition frequency is one batch every 80 to
105 min.
- 15 3. The process of claim 1, wherein the batch addition frequency is one batch each time
80% of the theoretical cellulose to glucose conversion is reached in the reaction mixture.
4. The process of claim 2, wherein the preselected batch volume and the batch addition
20 frequency are maintained constant throughout the total feed time.
5. The process of claim 2, wherein the preselected batch volume and/or the batch
addition frequency are decreased towards an end of the total feed time.
- 25 6. The process of claim 1, wherein the batch addition frequency is one batch every 105
min, the preselected consistency is 17% and the preselected addition period is 12 to 35
hours.
7. The process of claim 6, wherein the total feed time is one batch every 17 to 25 hours.
- 30 8. The process of claim 7, wherein the total feed time is 20 hours.
9. The process of claim 1, wherein the batch addition frequency is one batch every
105min, the preselected consistency is 24% and the total feed time is 80 to 120 hours.

35

10. The process of claim 9, wherein the total feed time is 90 to 110 hours.
11. The process of claim 10, wherein the total feed time is 95 hours.
- 5 12. The process of claim 1, wherein the cellulase enzyme is added at an enzyme load of 0.3% in the reaction mixture and the batch frequency is one batch each time 80% conversion is reached.
- 10 13. The process of claim 12, wherein the maximum batch addition frequency is one batch every 105 minutes.
14. The process of claim 13, wherein the batch volume is progressively decreased in a second half of the total feed time.
- 15 15. The process of claim 14, wherein the batch volume is progressively decreased in a last quarter of the total feed time.
16. The process of claim 1, wherein the enzyme is added in an amount lower than 1% of the final reaction mixture.

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Figure 1 A

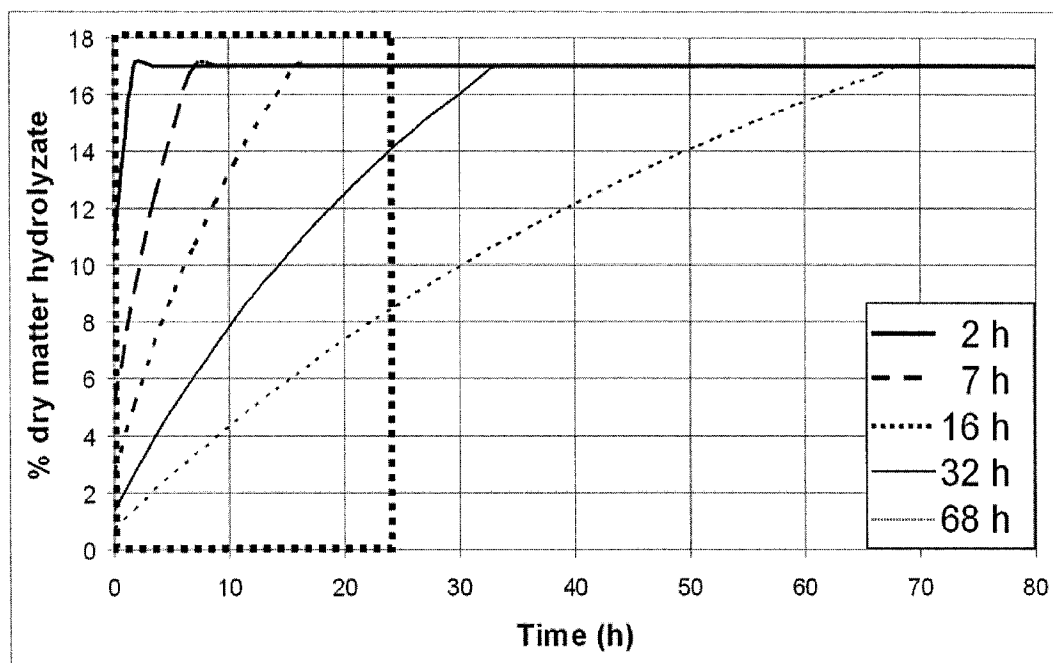
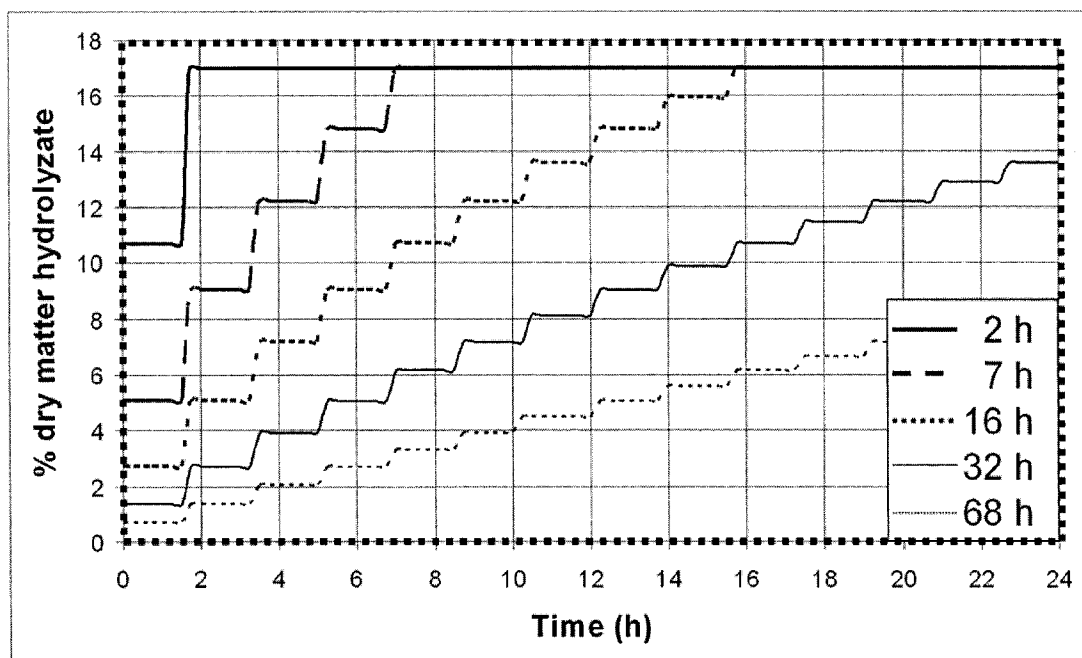


Figure 1 B



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Figure 2

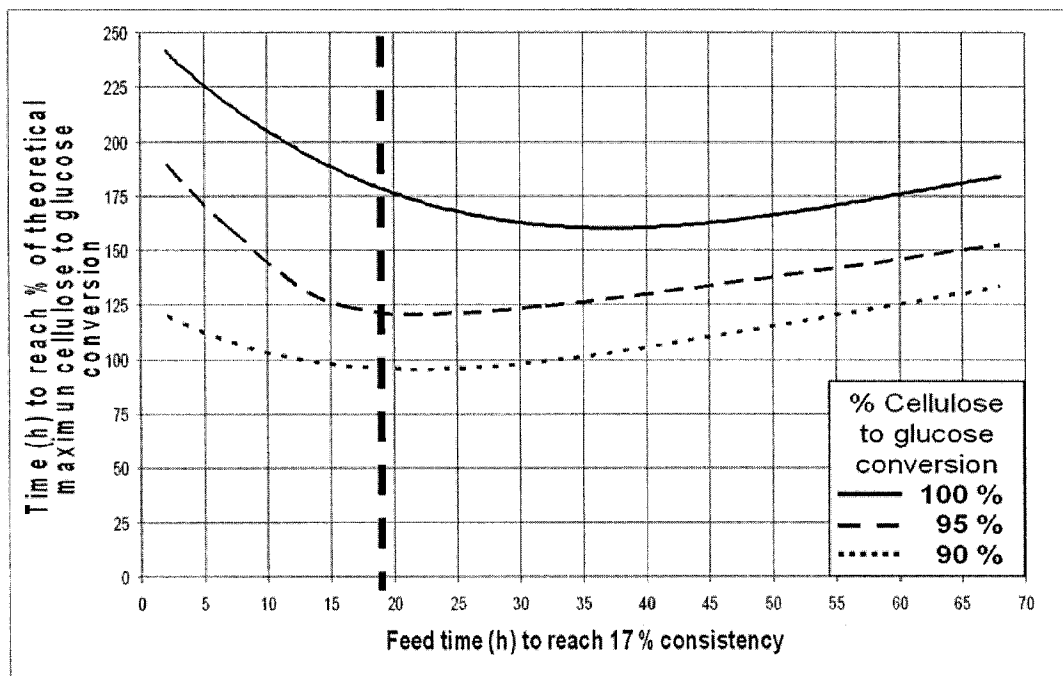
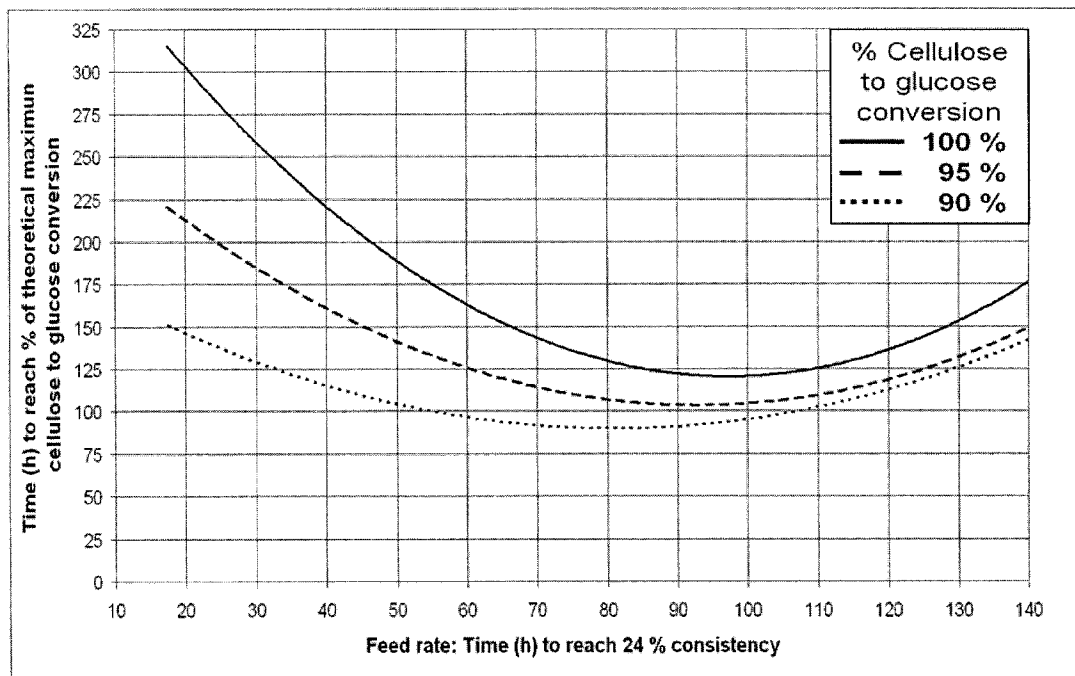
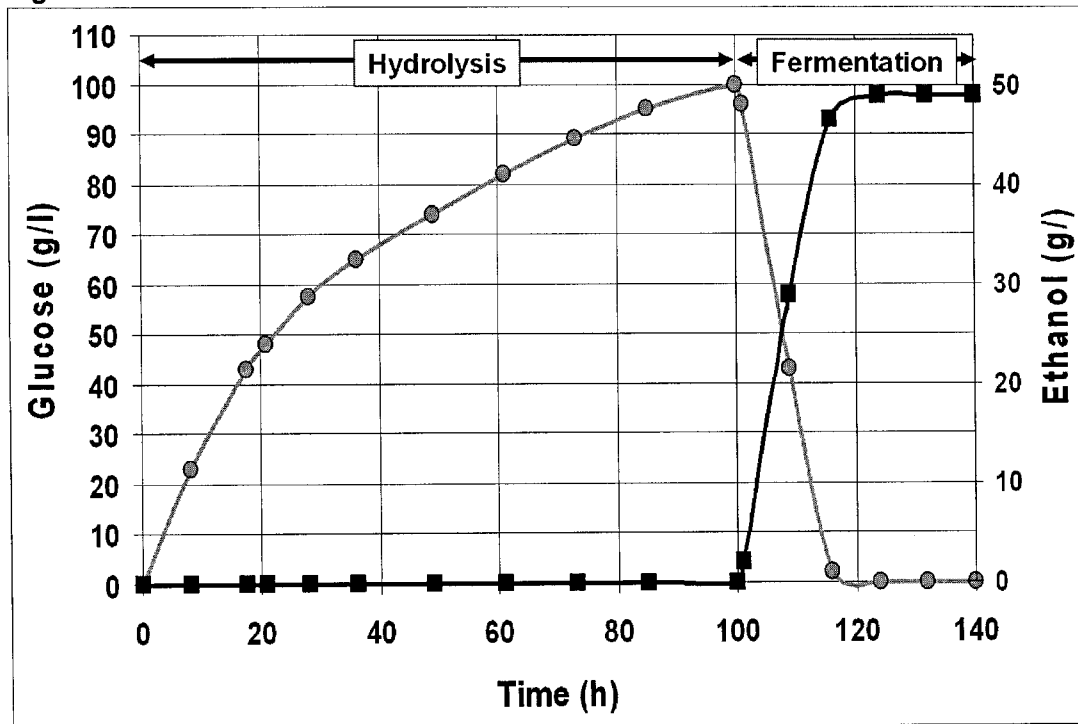


Figure 3



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Figure 4



INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER IPC: <i>C12P 19/14</i> (2006.01) , <i>C12P 19/00</i> (2006.01) According to International Patent Classification (IPC) or to both national classification and IPC																										
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC: <i>C12P 19/14</i> (2006.01) , <i>C12P 19/00</i> (2006.01) Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used) Databases: Canadian Patent Database, EPODOC, medline, pubmed; Keywords: lignocellulose, lignocellulosic, cellulose, biomass, fermentation, fed-batch, cellulase, ethanol, saccharification, hydrolysis																										
C. DOCUMENTS CONSIDERED TO BE RELEVANT <table border="1"> <thead> <tr> <th>Category*</th> <th>Citation of document, with indication, where appropriate, of the relevant passages</th> <th>Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td>X</td> <td>LU Y et al., (2010), "Influence of high solid concentration of enzymatic hydrolysis and fermentation of steam-exploded corn stover biomass", Appl. Biochem. Biotechnol. Vol 160, pages 360-369; epub 15 July 2008 *whole document*</td> <td>1 and 16</td> </tr> <tr> <td>X</td> <td>ROSGAARD L et al., (2007), "Effects of substrate loading on enzymatic hydrolysis and viscosity of pretreated barley straw", Appl. Biochem. Biotechnol. Vol 143, pages 27-40; epub 17 April 2007 *whole document*</td> <td>1 and 16</td> </tr> <tr> <td>X</td> <td>HODGE DB et al., (2009), "Model-based fed-batch for high-solids enzymatic cellulose hydrolysis", Appl. Biochem Biotechnol. Vol 152, ages 88-107, epub 30 May 2008 *whole document*</td> <td>1, 3, 12 and 16</td> </tr> </tbody> </table>			Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	X	LU Y et al., (2010), "Influence of high solid concentration of enzymatic hydrolysis and fermentation of steam-exploded corn stover biomass", Appl. Biochem. Biotechnol. Vol 160, pages 360-369; epub 15 July 2008 *whole document*	1 and 16	X	ROSGAARD L et al., (2007), "Effects of substrate loading on enzymatic hydrolysis and viscosity of pretreated barley straw", Appl. Biochem. Biotechnol. Vol 143, pages 27-40; epub 17 April 2007 *whole document*	1 and 16	X	HODGE DB et al., (2009), "Model-based fed-batch for high-solids enzymatic cellulose hydrolysis", Appl. Biochem Biotechnol. Vol 152, ages 88-107, epub 30 May 2008 *whole document*	1, 3, 12 and 16												
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☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex. <table border="1"> <thead> <tr> <th>*</th> <th>Special categories of cited documents :</th> <th>"T"</th> <th>later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</th> </tr> </thead> <tbody> <tr> <td>"A"</td> <td>document defining the general state of the art which is not considered to be of particular relevance</td> <td>"X"</td> <td>document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"E"</td> <td>earlier application or patent but published on or after the international filing date</td> <td>"Y"</td> <td>document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"L"</td> <td>document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"&"</td> <td>document member of the same patent family</td> </tr> <tr> <td>"O"</td> <td>document referring to an oral disclosure, use, exhibition or other means</td> <td></td> <td></td> </tr> <tr> <td>"P"</td> <td>document published prior to the international filing date but later than the priority date claimed</td> <td></td> <td></td> </tr> </tbody> </table>			*	Special categories of cited documents :	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	"E"	earlier application or patent but published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family	"O"	document referring to an oral disclosure, use, exhibition or other means			"P"	document published prior to the international filing date but later than the priority date claimed		
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Date of the actual completion of the international search 16 June 2010 (16-06-2010)		Date of mailing of the international search report 15 July 2010 (15-07-2010)																								
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476		Authorized officer Nicole Harris (819) 997-4541																								

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2010/000456

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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A	OLOFSSON K et al. (1 May 2008), "A short review on SSF – an interesting process option for ethanol production from lignocellulosic feedstocks", Biotechnology for Biofuels, vol 1, no 7, pages 1-14 *whole document*	1-16
P, A	ALVIRA P et al., (2010), "Pretreatment technologies for an effective bioethanol production process based on enzymatic hydrolysis: a review", Biosource Technology vol 1010, pages 4851-4861; epub 29 December 2009 *whole document*	1-16
P, A	ANDRIC P et al., (2010), "Reactor design for minimizing product inhibition during enzymatic lignocellulose hydrolysis: 1. Significance and mechanism of cellobiose and glucose inhibition on cellulytic enzymes", Biotechnology Advances, vol 28, pages 308-324; epub 18 January 2010 *whole document*	1-16