PURE CELLULOSE CONVERSION TO GLUCOSE WITH INSTANT PRESSURE DROP (DIC) TECHNOLOGY

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ABSTRACT

New technology and processes for conversion of cellulosic materials into glucose utilising Instant Pressure Drop technology (in French Détente Instantanée Contrôlée or DIC) are currently being developed at the Université de La Rochelle (ULR), France. DIC Technology is based on a thermomechanical effect induced by an abrupt transition from high steam pressure to a vacuum to instigate changes in biomaterials. Parameters studied include steam temperature and pressure, treatment time, vacuum level, acid concentration and initial moisture content. The resistance of some part of cellulosic material to biological conversion has led us to emphasize our work for the development of a viable process for optimum conversion of cellulose to glucose and to optimise the kinetics of cellulose-glucose conversion. Minimum formation of undesirable by-products of autohydrolysis which may inhibit biological conversion of glucose to ethanol is an utmost concern. Current work is leading to a new DIC system design that will enable the study of pretreatment and in-situ catalytic cellulosic material conversion to glucose through several process steps for an effective recovery and material balance of solid, liquid and gaseous residuals. DIC technology can be developed as a stand alone process or integrated into existing bio-ethanol process after appropriate process integration steps. This work is a small part of biofuel programs in ULR which utilise the DIC technology.

Keywords: *DIC, autohydrolysis, cellulose, glucose, pretreatment, biofuel*

INTRODUCTION

Various processes and configurations have been studied either through conversion of cellulosic materials into ethanol via fermentation or through acid and enzymatic hydrolysis of cellulose into glucose followed by fermentation of glucose into ethanol. Many physicochemical structural and compositional factors hinder the hydrolysis of cellulose present in biomass to sugars and other organic compounds that can later be converted to fuels. Pretreatment has been identified as an important step to increase amorphous portion in cellulosic materials and to increase its availability for more rapid hydrolysis and higher monomeric sugars yields [1]. However, it is well known that the hydrolysis of cellulosic materials at high temperature treatment always generate inhibitors that will interfere fermentation activities. A group of researchers in Université de La Rochelle (ULR) is currently working on an Instant Pressure Drop technology (in French Détente Instantanée Contrôlée, DIC) for biomass conversion. Current DIC system set-up in ULR consists of 4 types of DIC systems utilising steam or microwave system to supply heat and pressure for treatment. Our initial work in this area was related to the conversion of pure cellulosic material into glucose for utilisation in existing bioethanol industry.

DIC Technology

The DIC technology developed in 1988, was a process based on thermomechanical effect induced by an abrupt transition from high steam pressure to vacuum (with absolute pressure change of greater than 5 bar/s), the process leads to the expansion of products [2, 3]. This process utilised vacuum, temperature and material properties to achieve its objective. DIC technology application was successfully demonstrated in raw food material sterilization, drying of fruits and seaweed, removal of certain inhibitors in legumes, mechanical-structure modification to assist in the extraction of certain oligosaccharide for food and pharmaceutical application, coffee extraction, preserving water-logged wood, extraction of active ingredients and preservation of rice [2-5]. It was reported that DIC treatment was able to create more porous and expanded material within short treatment time [2, 3]. Outcome of this work will be used to developed new system known as microDIC (μDIC) with the aim for better control on all process parameters such as temperature which was not directly studied before. In an earlier work, glass transition temperature for cellulose was determined to be at about 180-240°C, which is exceeding the maximum temperature at 0.7 MPa steam pressure can be supplied by system.

Other primary factors such as pressure, time and material properties will be monitored or changed according to the experimental needs. Design of the new system also taking into consideration on the recovery of liquid and gaseous products generated during the process. New μDIC design was fully programmable and automatically controlled through electrical and pneumatic valve system through human machine interface (HMI) AGP 3000 series. HMI is programmed with GP-Pro Ex software and can be regularly updated for new and better processing sequences specific for process being studied.

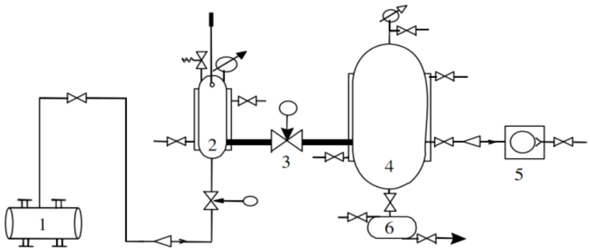

Figure 1: Typical DIC system layout (1: boiler; 2: DIC reactor; 3: decompression valve; 4: vacuum tank; 5: vacuum pump; 6: condensation tank).

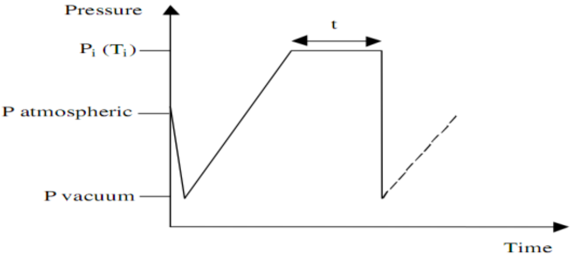

Figure 2: Typical DIC process cycles for pressure against time, with preparation at atmospheric pressure, starting of vacuum, treatment at maximum steam pressure followed by release to vacuum level and atmosphere.

Strategy and Objectives

In this initial cellulose-glucose hydrolysis work, we will explore possibilities to develop various processes and combinations while maintaining glucose quality. Tools and process control procedures will be developed at this stage. Due to the nature of this project complexity, it will be divided into three stages (DIC treatment, hydrolysis and ion-exchange) and results obtained will be analysed with response surface methodology (RSM) to establish and estimate optimal value in each higher-order model for further optimisation. The present work will concentrate only on the understanding of pure cellulose materials autohydrolysis to undisclose the processing effect of actual biomass containing lignin, hemicellulose and cellulose at later stages. The success of this preliminary work is hoped to contribute to an improvement in high solid loading, dilute-acid hydrolysis technology making it competitive against existing enzymatic hydrolysis process [6]. DIC pretreatment with short processing time at low acid concentration and low steam pressure is capable to be integrated into existing bioethanol facilities. Enzymatic processes are reported to have a better process yield and quality, however most of current enzymatic hydrolysis is slow and very much rely on pretreatment steps such as thermochemical pretreatment prior to enzymatic process itself [7], to enhance the hydrolysis rate.

MATERIALS AND METHODS

The classical experimental setup was previously described [2-5] with the basis unit of either through horizontal or vertical connection to the vacuum chamber as illustrated in Figure 1. Both systems were upgraded several times from simple mechanical system to fully electrical and pneumatic control systems.

Experimental Design

Experiment was done according to central composite rotatable design with 3 variables. The design is with eight (2^3) factorial points and six star points for central composite design, eight replications were done for each central point. Twenty two experimental trials for each design were done in random order to minimise unexpected variability in selected response from outside factor. All variables and factors used in this design were decided based upon earlier preliminary trials result. Response obtained was analysed with Statgraphic 5.1. Two sets of experimental design and its parameters as listed in the following table were studied for this report.

Cellulose samples used in this work were approximately 800mg each in the form of thin strips of filter paper, diameter 110 x 1mm, it was cut to size approximately 2 x 2mm for kinetic analysis. Crystallinity index of the same material was 89.7 by x-ray diffraction (XRD) [8]. Material used was as is during DIC treatment.

Table 1: Experimental Design

Parameter	Moisture (% db), W		Acid (M), [C]		Time (s), t	
Trial set no	1	2	1	2	1	2
Point max ($+\alpha$)	60%	80%	0.40	0.60	300	500
Point min ($-\alpha$)	12%	20%	0.00	0.20	30	30
Point central	36%	50%	0.20	0.40	165	265
Point 1:	14%	18%	0.12	0.12	80	140
Point (-1):	22%	32%	0.08	0.18	85	125
Point (+1):	50%	68%	0.32	0.52	245	405

Each DIC treatment combination was done on 2g cellulose materials at maximum 0.7MPa saturated steam pressure, temperature was not studied and only reflected as a function of pressure. Material was exposed to saturated salt environment in desiccators to get uniform startup moisture content of 7% (db). Different sulfuric acid concentration was added to material to achieve moisture and acid combination specified in experimental design. All treatments were conducted within 3 hours adjustment to limit potential material degradation due to exposure to acid. DIC treated material was dried at room temperature for 24 hour before further test. 500mg dried DIC treated material were mixed with 10ml water, homogenized for 1 minute at 5000rpm, filtered and injected into LCMS with electrospray ionization (ESI) method without column for qualitative analysis of selected response and its degradation products. Mobile phase was acetonirile and water (70:30) at flow rate of 0.2ml/min. ESI-MS was operated with 3.5kV potential, 35V fragmentation, ion sodiated glucose $(M+Na)^+$ with m/z 203 [9] and levulinic acid $(M+H)^+$ with m/z of 117 are selected for measurement with both total ion count (TIC) and selected ion monitoring (SIM) programs. Quantitative HPLC analysis was done on above preparation after appropriate dilution and filtration using HPLC-RID, ICE-COREGEL 87H3 (Transgenomic) and mobile phase of 0.1% sulfuric with glucose as main response. Qualitative determination for levulinic acid with DAD 210nm also done for comparison with ESI-MS. RSD for chromatogram peak area from same sample concentration from different injection was limit to below 5%.

Qualitative and quantitative procedure with ESI-MS and HPLC-RID/DAD respectively are developed in this stage. RID/DAD cycle need about 40 minutes to complete compare to ESI-MS that complete between 2 to 5 minutes for fast determination cycles. ESI-MS will later developed into quantitative procedure at later stage. Hydrolysis kinetic on selected sample in experimental design combination was done with 2% H_2SO_4 on 1g material in 10ml solution in a 122°C oil bath. 200μl hydrolysate was withdrawn at 30, 60, 120 and 300 minutes, 200μl fresh acid solution of same concentration was added back immediately. Hydrolysate was analysed quantitatively as above after dilution with mobile phase. Moisture content was determined with Sartorius MA 30 at 105°C, 4 minutes through-out the experiments.

RESULTS AND DISCUSSION

The selected response to develop RSM model was based on the immediate availability of glucose after DIC treatment. It was found that all samples possess sugar caramel smells. All hydrolysate was found containing glucose with some have traces of glucose degradation products such as levulinic acid, formic acid and acetic acid through ESI-MS response. Trace of hydroxymethyl furfural (HMF), a precursor to levulinic acid also found in both DIC hydrolysate and in some part of low acid concentration hydrolysis but was not analysed with RSM for it was not a direct response to this process. Based on results from both experimental design blocks it can be concluded that moisture content, W, acid concentration, [C] and treatment times, t; each were playing significant roles towards glucose formation as presented in Figure 3. Both pareto and trends graphs reveal that there are certain effects when parameter was changed and each will contribute to the autohydrolysis of glucose and fermentation inhibitors existence. It was found that treatment time did not contribute much to the autohydrolysis of cellulose to glucose, contrast to the kinetic study. Higher moisture content not only reducing glucose availability, but was found to generate more glucose degradation products, suggest the importance to control the initial moisture content of feed material either through pre-drying or utilizing DIC capability to reduce material moisture content to certain extend. For acid concentration, low acid was found to positively help cellulose autohydrolysis process, which is also good for equipment design.

The optimum condition for the highest glucose immediate availability from both experimental design was calculated with Statgraphic 5.1, to be W at 20%, [C] at 0.20M and t at 173s. Maximum glucose yield possible for this DIC combination was estimated to be 200ppm. Hence this was very low yield but the effects of treatment were very significant to increase total glucose formation as discussed below. Maximum accumulated glucose (Figure 4) from kinetic analysis with 2% acid concentration of non DIC treated materials was approximately 316ppm after 300 minutes, while glucose with DIC treated material was between 1000 to 1250ppm and still increasing. For selected DIC treated materials, maximum glucose was found to exceed more than 3 to 4 times of the non DIC treated materials. High severity DIC treatment was found to give higher accumulated glucose during low acid concentration hydrolysis process. Total glucose was 30-70 times higher in hydrolysate of DIC treated against non-DIC treated material during first 30 to 60 minutes hydrolysis, but it was leveled to about 3-4 times after about 5 hour hydrolysis. This phenomenon is believed to be corresponding with the availability of amorphous portion of cellulose generated during DIC treatment for the hydrolysis process. Further work with XRD will help to explain the changes and relationship in the amorphous-crystalline portion of the materials.

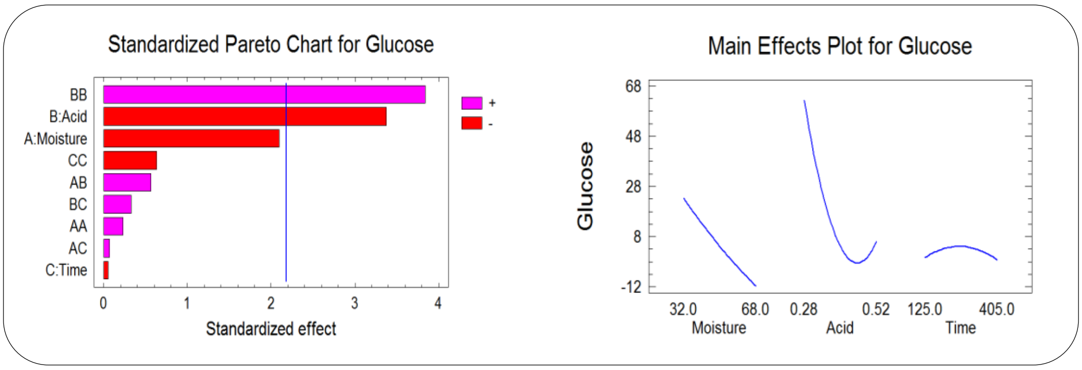

Figure 3: Pareto and trends charts for main parameters effect (W, [C] and t) towards glucose availability.

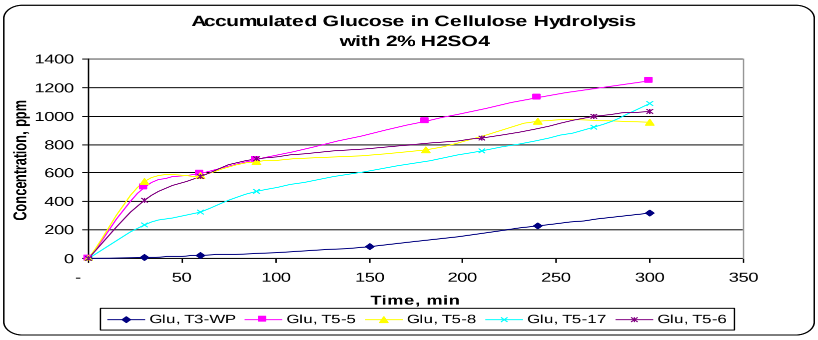

Figure 4: Glucose concentration in hydrolysate during hydrolysis kinetic of DIC and non DIC treated samples. T3-WP: filter paper; T5-6: 32%, 0.52M, 125s; T5-8: 32%, 0.28M, 125s; T5-17: 50%, 0.40M, 265s; T5-6: 32%, 0.52M, 125s.

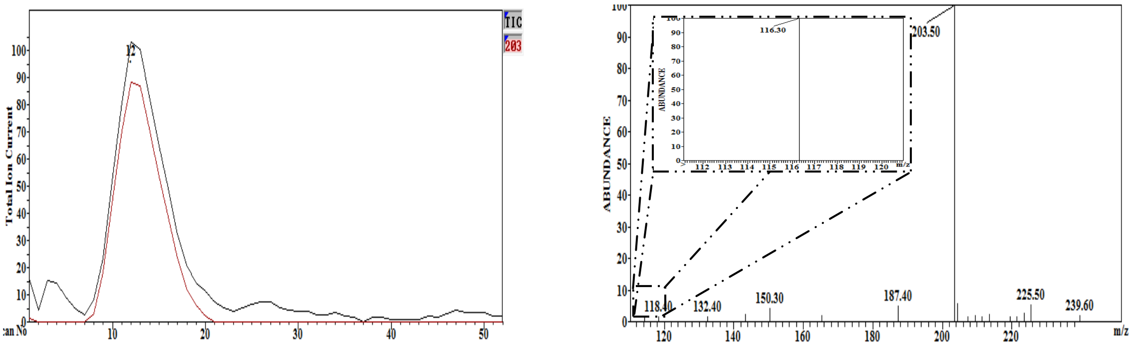

Figure 5: TIC spectrum for glucose (m/z 203.50) dominating the entire area and trace of levulinic acid (m/z 116.30).

In kinetic stage, LCMS was used as a tool to qualitatively check the existence of glucose degradation products in each DIC combination. Surprisingly, different responses of glucose degradation products against DIC treatment were observed and still being studied. In this preliminary work, SIM and TIC response of LCMS was successfully used as a tool to detect the availability of selected fermentation inhibitors that was very common in cellulose autohydrolysis. Figure 5 represent the TIC response for glucose. The selected inhibitors were found to set some availability trends in different parameters combination and nature of its formation during the process is still being studied.

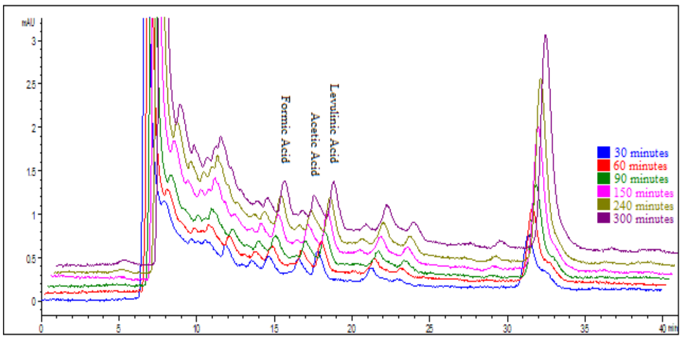

Figure 6: Impact of prolonged hydrolysis at 122^{o}C 2% H_2SO_4, between 30, 60, 90, 150, 240 and 300 minutes towards generation of fermentation inhibitors in DIC treated samples.

CONCLUSIONS

The objective to suppress the fermentation inhibitor factors for bioconversion to bioethanol needs further review due to various inhibitors formation at prolonged hydrolysis time as shown in Figure 6 through process combinations. It was suggested that short treatment time will favour the maximum glucose formation and inhibit the formation of potential inhibitors. Wet oxidation with alkali was expected to reduce the formation of furan and phenols [10]. Results and processes developed in this work are very useful for the design of new µDIC system. Tools that were used and developed in this work (experimental design, RSM and LCMS/DAD 210nm) were also found to be very selective and helpful in each of our decision making process.

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NOMENCLATURE

(db)	dry basis
[C]	sulfuric acid concentration
RSM	response surface methodology
LA	levulinic acid
HMF	hydroxyl methyl furfural
AA	acetic acid
RSD	relative standard deviation
TIC	total ion count
SIM	selected ion monitoring
ESI	electrospray ionization