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Hydrolytic pre-treatment methods for enhanced biobutanol production from agro-industrial wastes

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Abstract

Brewery industry liquid waste (BLW), brewery spent grain (BSG), apple pomace solid wastes (APS), apple pomace ultrafiltration sludge (APUS) and starch industry wastewater (SIW) have been considered as substrates to produce biobutanol. Efficiency of hydrolysis techniques tested to produce fermentable sugars depended on nature of agro-industrial wastes and process conditions. Acid-catalysed hydrolysis of BLW and BSG gave a total reducing sugar yield of 0.433g/g and 0.468 g/g respectively. Reducing sugar yield from microwave assisted hydrothermal method was 0.404 g/g from APS and 0.631 g/g from APUS, and, 0.359 g/g from microwave assisted acid-catalysed SIW dry mass. Parameter optimization (time, pH and substrate concentration) for acid-catalysed BLW hydrolysate utilization using central composite model technique produced 307.9 g/kg glucose with generation of inhibitors (5-hydroxymethyl furfural (20 g/kg), furfural (1.6 g/kg), levulinic acid (9.3g/kg) and total phenolic compound (0.567 g/kg)). 10.62 g/L of acetone-butanol-ethanol was produced by subsequent clostridial fermentation of the substrate.

Keywords: Agro-industrial wastes; pre-treatment; microbial inhibitors; central composite design; kinetic modelling; ABE fermentation.

1. Introduction

Increasing global energy demand, unstable and expensive petroleum resources, and concern over global climate changes have boosted the development of renewable energy sources, which, in turn, have driven scientific research towards the utilization of lignocellulosic biomass resources as a renewable feedstock for the production of energy and fuels. The demand for renewable resources to replace substantial amounts of non-renewable fossil fuels and minimize greenhouse gas (GHG) emissions largely rests on most abundant renewable biomass (He & Zhang, 2011). However, fermentation substrate has proven itself as one of the most important parameters influencing the final cost of produced biofuels. In this context, liquid biofuel produced from lignocellulosic waste biomasses could be a promising renewable energy source for a country with abundant biomass resources, such as Canada.

Agro-based industries, especially apple processing industries, brewery industries, and starch processing industries are experiencing a surge in their growth around the globe (Dhillon et al., 2013). About 60–70 % of food and beverage processing industry residues are discharged in the environment without any treatment and the reminder's potential is only tapped by means of anaerobic digestion (Maiti et al., 2016a). North America, one of the largest agro-industrial waste producers (Canada is indeed the second overall supplier of wood lignocellulosic biomass), retrieves only 20 % of the agro-industrial food wastes for animal feed. The rest is used for landfilling, incineration or composting, which contributes to about 10 % of the country's greenhouse gas emissions (Nigam & Pandey, 2009).

The efficient reuse of the residues generated from such activities a major logistical, financial and environmental issue. Due to their chemical properties, agro-industrial biomass wastes have the potential to become an innovative carbon source, which could be fermented to alcoholic compounds through environment friendly biochemical methods. Amongst the possible biochemically produced alcohols, biobutanol has been defined as a promising alternative due to its superior fuel properties as compared to ethanol (Naik et al., 2010).

The conversion of complex biomass into biobutanol requires effective utilization of C5 and C6 sugars present in hemicellulose, cellulose and starch, by processing these fractions either together or individually after separation (Gürbüz et al., 2012). Most of the naturally abundant clostridia are still not able to hydrolyse lignocellulosic based agro-industrial waste efficiently. The use of genetically modified strains has been suggested as a possible alternative to the use of the clostridia, however, it would greatly increase the final cost of biobutanol production, and has still not been implemented successfully in the large scale production of biofuels. Since acetone-butanol-ethanol (ABE) fermentation is naturally carried out by clostridial strains, an alternative solution to work with these strains is based on the partial hydrolysis of recalcitrant lignocellulosic material present in agro-industrial waste biomass to simple sugars in order to facilitate and increase the efficiency of clostridial fermentation.

Hydrolysis can be achieved enzymatically or via physicochemical methods. Enzymatic hydrolysis is considered an environmentally friendly process with broad prospects in the conversion of lignocellulose to biofuel. However, information on the optimal conditions of enzymatic hydrolysis in literature is limited. Commonly, product

inhibition, estimated running time etc. lead to addition of large amounts of expensive commercial enzymes, which increases the biobutanol production cost and hinders its commercialization (Wang & Chen, 2011). It has been reported that a highly selective and efficient enzymatic hydrolysis can contribute up to 16-20 % of the total production cost of butanol from lignocellulosic biomass (Montano, 2009). Additionally, enzymatic hydrolysis at high total solids concentrations (an unavoidable prerequisite for many feedstocks to achieve a large-scale production of biofuels, such as ethanol or butanol) could lead to a decrease in substrate conversion, referred to as the "solids effect" (Puri et al., 2013).

Physicochemical pre-treatment methods are less selective, and microbial inhibitors, such as furan derivatives, weak acids, and phenolic compounds are also produced in addition to the desired monosaccharaides (Maiti et al., 2016a). It has been reported that these inhibitory compounds could have a significant detrimental effect on microbial performance even at very low concentrations due to the synergistic inhibition effects (Baral & Shah, 2014).

Since substrate cost has the highest influence on butanol price (Qureshi & Blaschek, 2000), this work focused on the use of inexpensive, renewable agro-industrial wastes for the fermentative production of butanol using *Clostridium beijerinckii*. The potential use of apple processing industry wastes, brewery industry wastes and starch industry wastewater as the substrates for growth and butanol production by *C. beijerinckii* was systematically investigated. Hence, the objectives of these studies were: (1) to investigate the efficiency of different hydrolysis methods (namely chemical treatment, microwave assisted treatment, nano spray-dryer particles catalysed treatment, mechanical treatment

and hydrothermolysis) to enhance fermentable sugar production; (2) to identify inhibitors of butanol fermentation produced in agro-industrial wastes hydrolysates upon each hydrolysis method; (3) to optimize different process parameters of acid hydrolysis for using brewery industry liquid waste (BLW) as feedstock in order to increase the RSC (reducing sugar compound) concentration minimizing the presence of any microbial inhibitor; (4) to determine a kinetic model for the hydrolysis process; and (5) to ascertain the ability of *C. beijerinckii* NRRL B-466 to utilize BLW hydrolysate as substrate for ABE fermentation.

2. Materials and methods

2.1. Substrate selection and characterization

Brewery industry wastes (BLW and brewery spent grain (BSG)) used in the present investigation were obtained from La Barberie (Quebec, Canada). Starch industry wastewater (SIW) was obtained from ADM Ogilvic (Candiac, Quebec, Canada) and the apple industry wastes (apple pomace sludge (APS), and apple pomace ultrafiltration sludge (APUS)) were obtained from Lassonde Inc. (Rougemont, Montreal, Canada).

2.1.1. Brewery industry wastes

BSG is the waste resulting after the lautering process (separation of wort or mash filtration). BLW mainly consist of residual substances from production (a complex mixture of surplus yeast and plant residues, remaining fine spent grains and hops, etc.) and leachates from the cleaning of fermentation and storage tanks, as well as vat and bottle rinsing (Olajire, 2012). BLW has a high concentration of free RSCs, polysaccharide plant residues and yeast proteins.

2.1.2. Apple industry wastes

Both APS and APUS are rich sources of carbohydrates, minerals, vitamins and dietary fibres. Unlike the wastes from the brewery industry, apple pomace wastes had a higher concentration of fructose. Cellulose was the main polysaccharide in APS, which implied a higher concentration of aldohexoses in comparison with aldopentoses. No hemicellulose was found in APUS sample. The pH of the waste suspension before hydrolysis was lower (pH < 3.5) in comparison with other samples due to presence of organic acids (Kosseva & Webb, 2013).

2.1.3. Starch processing wastes

The extraction of starch is accompanied many liquid and solid carbon-rich wastes. Starch, an α 1-4 glycosidic linkage polysaccharide, is the energy reserve in plants, and it is widely present in waste residues produced from the processing of plant raw materials. Unlike cellulose (a β 1-4 glycosidic linkage polysaccharide), starch has been reported to be more susceptible to partial and total hydrolysis (Martin, 2012).

Three of the feedstock (BLW, SIW and APUS) were received as suspension in water, while BSG and APS were in solid state. The dry weight of all biomass has been considered to compare the efficiency of the hydrolysis techniques under study. The biomass was dried at 65 ± 1 °C for 72 h.

The presence of residual ethanol, which is reported as a microbial inhibitor (at a concentration above 40 g/L) (Lee et al., 2008), is an additional challenge in concentrated BLW samples for biobutanol production. The heating process carried out to dry the samples allowed the reduction of original ethanol content below the inhibitory level. Following this step, the dried BSG and APS was ground to particles of a smaller size (< 20 mm). The physicochemical characterization of the five agro-industrial wastes was

performed as given in Table 1. The measured values agree with values typically found in literature (Dhillon et al., 2011; Olajire, 2012; Verma et al., 2007). The initial concentration of free reducing sugars in APUS, APS and BLW was higher than that of BSG and SIW.

2.2. Pre-treatment of waste biomass

The choice of hydrolysis treatment for pre-treatment of biomass and its severity differed based on the heterogeneity and complexity of the substrate. In this study, the following hydrolysis techniques were applied to carry out the required pre-treatment of selected dried waste biomasses:

- **Chemical treatment**

I. Brønsted acid catalysed hydrolysis (0.1 M H_2SO_4, pH~1.2 ± 0.2) at 121 ± 1 °C for 40 min in autoclave (16 ± 0.2 psi).

II. H_2O_2 (30 v/v, 0.05 mL) catalysed acid hydrolysis (pH~3) at 121 ± 1 °C for 40 min in autoclave (16 ± 0.2 psi).

III. Alkali catalysed hydrolysis (1M NaOH, pH~10) at 121 ± 1ºC for 40 min in autoclave (16 ± 0.2 psi).

- **Microwave assisted treatment**

IV. Microwave-assisted (1000 W) Brønsted acid catalysed hydrolysis (0.1 M H_2SO_4, pH~1.2 ± 0.2) at 121 ± 1 °C for 25 min.

V. Microwave-assisted (1000 W) alkali catalysed hydrolysis (1 M NaOH, pH~10) at 121 ± 1 °C for 25 min.

- **Nano- spray dryer particle (NSPs) catalysed treatment**

Ca and Fe NPs (Nanoparticles) were prepared by using a nanospray dryer B-90 (Buchi, Switzerland). Solutions of 10 g/L $CaCO_3$ and 100 g/L $Fe(OAc)_2$ were prepared using distilled water and fed to the nanospray dryer at the liquid flow level of 3 (nearly 20 mL/h) with a constant air flow rate of 120 L/min at 120 °C (Sarma et al., 2014). The mesh hole size of the operating spray cap was about 4.0 mm. Nanoparticles were collected from the internal surface of the collecting electrode using the manual particle scraper and preserved in airtight glass container.

For the NPs catalyzed hydrolysis, the liquid was composed of water and (15±2.5) % of NPs per gram of dry biomass sample was used (Zhang et al., 2011) under the following conditions:

VI. Fe NPs catalysed hydrolysis (pH~3) at 121 ± 1 ºC for 40 min in autoclave (16 ± 0.2 psi).

VII. Ca NPs catalysed hydrolysis (pH~10) at 121 ± 1 ºC for 40 min in autoclave (16 ± 0.2 psi).

VIII. Both Ca and Fe NPs catalysed hydrolysis at 121±1°C for 40 min in autoclave (16 ± 0.2 psi).

Before fermentation, Fe NPs were removed by magnetic filtration and Ca NPs were removed by $Ca_3(PO_4)_2$ precipitation (Zhang et al., 2011; Lee et al. 2014).

- **Mechanical treatment**

IX. Ultra-sound assisted hydrolysis was carried out using an ultrasonication bath (Elma Hans Schmidhauer GmbH & Co. KG, Singen, Germany) for 24 h (20–400 kHz) without any pH adjustment.

- **Hydrothermolysis**

X. H_2O (pH~7) in autoclave (16 ± 0.2 psi).

XI. Microwave-assisted (1000 W) hydrolysis (H_2O, pH~7) in autoclave (16 ± 0.2 psi).

H_2SO_4 was selected as Brønsted acid as it is less volatile, less corrosive to the equipment and is economically more feasible (García Martín et al., 2013). The combination of the substrate and pre-treatment method achieving the most promising results during the pre-screening process was subsequently optimized by means of response surface methodology (RSM) for hyper-production of reducing sugar compounds (RSCs) and minimisation of inhibitory compounds (section 2.3). RSCs encompass total reducing sugar (TRS), glucose, fructose, galactose and xylose, while inhibitors group comprises furfural, 5-HMF (5-hydroxymethyl furfural), levulinic acid and total phenolic compounds (TPCs). All these chemicals (vanillin, vanillic acid, feluric acid, furfural, 5-HMF, acetic acid, levulinic acid, syringaldehyde, glucose, xylose, galactose and fructose were purchased from Sigma Aldrich (USA). All standards were of analytical grade.

2.2.3. Experimental design and RSCs production optimization through RSM

Central composite design was applied to optimize the production of RSCs and minimize the release of inhibitory compounds (dependent variable) for a BLW sample hydrolysed via Brønsted acid catalysis (selected combination), as a function of three independent variables: reaction time, pH and feedstock concentration. Experimental design construction made with the aid of Design-Expert®-7 software (Stat-Ease Inc., Minneapolis, USA) resulted in a set of 20 experiments, comprising 3 central points and three different code levels (low (-1), middle (0) and high (+1)) (Table 2).

A quadratic polynomial equation (Eq. 1) was proposed to interlink the effects of the three independent variables on reducing sugars as well as different inhibitors production as follows:

$$(Reducing\ sugar / Inhibitor)_{production} = X_0 + \sum_{i=1}^{n} X_i Y_i + \sum_{i=1}^{n} X_{ii} Y_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} X_{ij} Y_i Y_j \quad \text{(Eq.1)}$$

where (Reducing sugar/Inhibitor) $_{production}$ is the dependent variable; Y_i and Y_j are the independent variables (n = 3 (time, pH and feedstock concentration)); X_0 is the intercept constant and X_i, X_{ii} and X_{ij} correspond to the regression coefficients. The same software (Design-Expert®-7) employed for test-matrix design was used to check the experimental responses obtained. An analysis of variance (ANOVA report) was performed to fit the quadratic polynomial equations for the selected LA hyper-producing substrate(s). Final values of code factors (time, pH, feedstock concentration, time·pH, time·feedstock concentration, pH·feedstock concentration, time^2, pH^2, feedstock concentration^2) were considered to be statistically significant at $p < 0.05$. The quality of the model fit was evaluated by the coefficient of determination (R^2) and the adjusted coefficient of determination (R^2_{Adj}).

2.3. Biobutanol production

2.3.1. Detoxification of agro-industrial waste hydrolysate

Detoxification of previously selected agro-industrial waste hydrolysate (BLW) was carried out using a modified over-liming method (Martinez et al., 2001). The pH of the hydrolysate was adjusted to 10 with $Ca(OH)_2$ and kept at 30 °C for overnight. The hydrolysate was mixed with 1 g/L Na_2SO_3 and the mixture was heated at 90 ± 1°C for 1 h. Subsequently, the precipitate of metal hydroxides was separated by centrifugation at *7650 x g* (30 ± 1°C) for 30 min. Precipitate formed was discarded. The supernatant was

neutralized to pH 6.7 ± 0.1 with 1 M H_2SO_4 and centrifuged at 30 ± 1°C for 30 min at *7650 x g* in order to separate the precipitate. The clear supernatant was used as a carbon source to carry out the fermentation studies.

2.3.2. Microorganism and culture media

C. beijerinckii NRRL B-466, the microorganism selected for biobutanol production purposes, was obtained from the Agricultural Research Station, USDA (USA). The microorganism was grown and maintained in peptone-yeast extract-glucose (PYG) media under anaerobic condition for 17 h at 35 ± 1 °C and 150 rpm (Maiti et al., 2016b). The medium (g/L) comprised: glucose (10); yeast extract (10); peptone (5); tryptone (5); cysteine-HCl (0.5); K_2HPO_4 (2.04); KH_2PO_4 (0.04); $FeSO_4{\cdot}7H_2O$ ($1.1{\cdot}10^{-3}$); $CaCl_2$ ($8{\times}10^{-3}$); $MgSO_4{\cdot}7H_2O$ (0.0192); NaCl (0.08); and $NaHCO_3$ (0.4). Serum bottles of 125 mL with working volume of 50 mL were used as anaerobic batch reactors for both the inoculum development and the butanol production (Maiti et al., 2016b). Anaerobic conditions were maintained by sparging N_2 for 10 minutes and the bottles were immediately sealed by aluminium crimp seal containing silicone septum (Fisher scientific Canada) by using a hand-operated crimping tool (E-Z Crimper™, VWR, Ontario, Canada). Prior to culture development, the medium was sterilized for 20 min at 121 ± 1 °C. About 10 % (v/v) (dry cell weight 35 mg/mL) of microbial culture in its exponential phase of growth (OD_{600nm} = 1.3-1.5) was used as inoculum for all the experiments conducted in this investigation.

Chemicals such as glucose, urea, $MgSO_4{\cdot}7H_2O$, NaOH, $FeSO_4{\cdot}7H_2O$, $CaCl_2$, cysteine-HCl, NaCl, $NaHCO_3$, $Ca(OH)_2$, Na_2SO_3, H_2SO_4HCl, *n*-butanol, acetone, acetic acid, butyric acid, ethanol were purchased from Fisher Scientific (Ontario, Canada). Casein

peptone, tryptone, K_2HPO_4 and KH_2PO_4 were purchased from VWR (Ontario, Canada) and the yeast extract was a kind gift from Lallemand, Canada.

2.3.3 Batch fermentation

Batch fermentation was performed in 125 mL serum vials (working volume 50 mL) at pH 6.7 ± 0.1. As already described for inoculum development, the medium was sterilized and anaerobic conditions were established. The fermentation was started by inoculating *C. beijerinckii* 10% (v/v) (17 h in vegetative growth). All experiments were performed at 37 ± 1°C with shaking at 150 rpm for 72 h in triplicates (Maiti et al., 2015). Fermentation experiments were carried out separately using modified P2 medium taking following solutions: (1) 52 g/L of glucose as control sample; (2) Brønsted acid pre-treated BLW hydrolysate to check the simultaneous effect of total reducing sugars and inhibitory compounds in butanol production; and (3) detoxified BLW hydrolysate to attain the highest conversion efficiency of the BLW biomass to biobutanol.

Modified P2 media used in the present investigation was composed of different ingredients (buffer: KH_2PO_4 50 g/L, K_2HPO_4 50 g/L, $NH_4CH_3CO_2$ 220 g/L; minerals: $MgSO_4 \cdot 7H_2O$ 20 g/L, $MnSO_4 \cdot H_2O$ 1 g/L, $FeSO_4 \cdot 7H_2O$ 1 g/L, NaCl 1 g/L; vitamins: thiamin 0.1 g/L, biotin 0.001 g/L). Since brewery industry liquid wastes are already enriched with yeast protein, neither peptone nor yeast protein were added to P2 media. From each batch of fermentation, 1 mL of culture broth was harvested at definite intervals (12h) and used for metabolite analysis. Data presented herein are average value from duplicate runs for triplicate samples.

2.4. Analytical procedure

2.4.1 Reducing sugars and inhibitors analysis by LC/MS-MS method

Along with several reducing sugars (i.e., glucose, fructose, galactose and xylose), a complex mixture of microbial inhibitors (i.e., furfural, 5-HMF, acetic acid, levulinic acid, vanillin, vanillic acid, feluric acid and syringaldehyde) were also produced as a result of the pre-treatment step carried out in section 2.1.2 to break down hemicellulose, cellulose and starch present in agro-industrial wastes. To analyse different reducing sugars, hydrolysate samples were collected at 10-15-minute intervals and analysed using Liquid Chromatography-Mass Spectrometry (LC-MS) equipped with a 5µm, 150 mm ID, 4.6 mm df column where D_6 glucose was used as internal standard. Likewise, previously mentioned inhibitors produced during hydrolysis were analysed by Liquid-Chromatography-Tandem Mass Spectrometry (LC-TMS) (ZORBAX Carbohydrate, Agilent Technologies, USA) equipped with a biobasic-18 column (5µm, 250 mm ID, 4.6 mm df) of Agilent Technologies (USA). Phenylethanol-D_5 was used as internal standard using samples collected in each 10-15 min interval.

Before injection for product analysis, samples were centrifuged for 5 minutes at 7650 *x g* and the supernatant was filtered by 0.45 µm syringe filter. Methanol:water (8:2, v/v) and acetonitrile:water (8.5:1.5, v/v) were used to dilute the samples before analysing inhibitors and carbohydrate (Maiti et al., 2016b). All data presented are average values from duplicate runs for duplicate samples. Standard deviation for each data has been calculated with respect to the average (mean) value from duplicate runs for duplicate samples.

2.4.2 Carbohydrate analysis by standard DNSA (dinitrosalicylic acid) method

TRS concentration during the fermentation was determined by DNSA method using glucose as the standard (Miller, 1959). The amount of TRS extracted from hydrolysed

samples was determined by UV-visible spectrophotometer (Cary-50, Varian) using 3,5-dinitrosalicylic acid as the reagent (DNS method) at 540 nm.

2.4.3 Metabolite measurements

ABE and additional metabolites (e.g., butyric acid, acetic acid) produced during fermentation process were monitored by means of gas chromatography (GC7890B, Agilent Technologies, USA) equipped with FID detector, along with a HP-INNOWax column (30m, 0.25mm ID and 0.25µm df). The GC conditions comprised: helium carrier gas at a flow rate of 1 mL/min with a temperature cycle (initial temperature 50 °C; 10 °C/min increase up to 150 °C; 20 °C/min increase up to 250 °C) for a total method run time of 16 min. Before injection, the liquid samples were centrifuged for 5 min at 7650 x g and the supernatant was filtered by 0.45 µm syringe filter. Isobutanol was used as the internal standard (Maiti et al., 2015). Reducing sugar yield based on the dry weight of raw material was calculated as (Eq. 2):

$$Yield\ of\ reducing\ sugar\ (\%) = 100\ x \frac{Reducing\ sugar\ produced\ (g)}{Amount\ of\ substrate\ (g)} \quad \text{(Eq. 2)}$$

3. Results and Discussion

3.1 Comparison of different hydrolysis techniques to produce fermentable sugar compounds from agro-industrial wastes

3.1.1 Brewery industry wastes

Due to the lignocellulosic composition of the wastes, (in which outer lignin entirely covers and bounds the inner polysaccharide content), Brønsted acid catalysed hydrolysis was expected to be more promising over other employed techniques to significantly reduce recalcitrant nature of brewery industry wastes. Solubilisation of hemicellulose is favoured under low pH, as acidic conditions facilitate the breakdown of glycosidic bonds.

To enhance fermentable sugars, different hydrolysis techniques, such as chemical, hydrothermal, mechanical and nanoparticle application have been employed. In the current study, acid (0.1 M H_2SO_4) catalysed hydrolysis of BSG and BLW achieved a conversion of 0.468 g_{TRS}/g and 0.433 g_{TRS} /g of dry substrate, respectively (Table 4, Figure 1). Moreover, higher abundance of aldohexoses in BLW hydrolysate (i.e., glucose content), made it more promising for biofuel production compared to BSG. Other hydrolysis treatments tested showed poor performance (Table 4, Figure 1). In comparison to acid catalysed hydrolysis using autoclave, the microwave assisted acid hydrolysis promoted the emergence of undesired side products (Figure 1).

Physical or chemical pre-treatment methods in combination with enzymatic hydrolysis have been reported in literature. For example, Ravindran et al. reported a TRS yield of 0.228 g/g for BSG treated with microwave assisted alkali treatment followed by enzymatic hydrolysis (Ravindran et al., 2017), and White et al. reported glucose yield of 0.278 g/g from BSG treated with dilute acid and enzyme (White et al., 2008). Though the yield in these cases is more than that obtained in the current work (0.176 g/g), albeit the use of enzymes would increase the cost of the pre-treatment process. As already mentioned, authors preferred the utilization of acidic conditions rather than alkali utilization.

3.1.2 Apple industry wastes

The degradation of cellulose (main polysaccharide in apple pomace wastes) is described as an acid-catalysed and thermally accelerated chain scission mechanism. The process consists of two steps, an initial fast hydrolysis of the more solvent accessible amorphous region of cellulose, and a later, much slower hydrolytic attack on the

crystalline portion of cellulose (Hu & Ragauskas, 2012). The susceptibility of cellulose over different pH ranges has been reported to be dissimilar and more efficient at lower pH, when sugar monomer release from biomass is more effective over further conversion of different side products (Hu & Ragauskas, 2012). Bearing this in mind, dilute acid catalysed hydrolysis was expected to be more promising over other methods.

However, microwave assisted hydrothermal method proved to be more efficient, and 0.404 g $_{TRS}$/g and 0.631 g $_{TRS}$/g of dry substrate were obtained from APS and APUS samples respectively, which resulted in a slight improvement of 3–7 % over acid catalysed hydrolysis in autoclave (Table 4, Figure 1). Hydrothermolysis results reported in the literature have been lower than those obtained with dilute acid or alkali catalysed hydrolysis (Liu et al., 2015), but its use has been recommended based on the absence of a catalyst (acid or base) and easier reactor maintenance due to low corrosion potential (Alvira et al., 2010). NSPs catalysed hydrolysis, which was previously reported to be successful in crystalline cellulose hydrolysis (Feng & Fang, 2013), was not effective in this case (Table 4, Figure 1). Alkaline hydrolysis, a pre-treatment method typically used in delignification processes to enhance the accessibility of cellulose to hydrolytic enzymes (enzymatic hydrolysis) (Arreola-Vargas et al., 2015) also rendered good results in APS and APUS (the samples with highest lignin content), resulting in 0.244 g $_{TRS}$/g and 0.628 g $_{TRS}$/g, respectively.

3.1.3 Starch industry wastes

The assistance of microwave radiation improved the performance of Brønsted acid catalysed hydrolysis by 9–24 % (0.359 g $_{TRS}$/g and 0.246 g $_{glucose}$/g *vs* 0.330 g $_{TRS}$/g and 0.197 g $_{glucose}$/g). The results obtained were more satisfying in comparison with previous

works. Srinorakutara et al. (2006) reported that acid (0.6 M H_2SO_4) catalysed hydrolysis of starch industry waste (cassava waste) only produced about 0.122 g_{TRS}/g (Srinorakutara et al., 2006), while the TRS yield by acid hydrolysis was 0.122g/g for Sarchamo and Rehmann, and 0.678 g/g for Hernoux-Villière et al (Sarchami & Rehmann, 2015; Hernoux-Villière et al., 2013).

The production of fermentable sugars, and more specifically glucose, was observed to be higher in starch and brewery industry wastes (Table 4).

3.2 Comparison of different hydrolysis techniques to produce microbial inhibitors from five different agro-industrial wastes

Several substances are often formed during lignocellulosic feedstock pre-treatment which inhibit microbial fermentation. Thus, prior to fermentation, a thorough investigation on the capacity of the hydrolysis techniques under study to produce these inhibitors is compulsory as its influence was reported to be very significant (Baral & Shah, 2014). Inhibitors production pathway is based on cellulose and hemicellulose hydrolysis to carbohydrates and subsequent selective dehydration and rehydration to various organic compounds, such as furfural, 5-hydroxy methyl furfural, levulinic acid and formic acid, among others (Assary et al., 2012). Table 5 summarizes the undesired microbial inhibitors produced alongside with fermentable sugars for the different hydrolysis methods tested.

3.2.1. 5-Hydroxy methyl furfural and furfural

Furfural and 5-HMF are predominantly obtained from acid catalysed hydrolysis and have been reported to adversely affect metabolite promoting enzymes, inhibit DNA and protein synthesis and decrease cell permeability (Baral & Shah, 2014; Ezeji et al., 2007; Zhang et al., 2012b).

Fructose is the most effective carbohydrate for 5-HMF synthesis, since it presents higher reactivity (presence of five membered rings) in comparison to the naturally abundant glucose (Palmqvist & Hahn-Hägerdal, 2000). Thus, 5-HMF production by means of dilute acid catalysis was higher in APS and APUS samples (45.5 g/kg and 37.5 g/kg, respectively) compared to BLW, SIW and BSG (21.3 g/kg, 3.17 g/kg and 2.68 g/kg) due to higher abundance of fructose (Table 5). Microwave assisted treatment was detrimental in all cases (e.g., an increase in 5-HMF concentration of ~15 % for apple pomace substrates was observed) under the current experimental condition (Rosa et al., 2014).

Furfural production was favoured in hemicellulose rich biomass samples, such as BSG, resulting in a maximum inhibitor production of 48.65 g/kg in microwave assisted acid catalysed conditions. The lower activation energy for conversion of aldopentose monosaccharides to furfural compared to aldohexoses led to more abundance of furfural in waste hydrolysates (Enslow & Bell, 2012).

3.2.2 Organic acids

Presence of organic acids (e.g., acetic acid, formic acid and levulinic acid) in the hydrolysate reduces the pH of the medium, causing plasma disruption, cell rupture and termination of ABE fermentation (Wang & Chen, 2011). In the case of levulinic acid, the inhibitory effects on microbial glucose consumption, cell growth and biofuel production were not observed in the presence of less than 5 g/L (Lee et al., 2015).

Further transformation of furan derivatives and, to a lesser extent, reducing sugars led to the formation of organic acids in acid catalysed hydrolysis. Therefore, the methods producing higher amounts of 5-HMF and its sister chemical furfural, were also the main generators of levulinic acid. Dilute acid catalysed thermal hydrolysis (with or without

assistance of microwave radiation) method was the main responsible of levulinic acid production (76–92 % of the total inhibitor concentration) in all cases.

3.2.3 Phenolic compounds

In contrast to furan derivatives and organic acids, very low concentrations of phenolic compounds have been associated with disruption of ABE fermentation. Mechanism of inhibition of phenolic compounds has been based on their partitioning into biological membranes with the subsequent loss of membrane integrity (Maiti et al., 2016a). Hydrolysis of lignin can produce phenolic compounds. Since lignin is more susceptible to low pH values, alkaline treatments were discarded as they could be a source of phenolic compounds (Table 5). Again, dilute acid catalysed thermal hydrolysis produced the highest concentrations of inhibitors, vanillin being the most abundant compound in all feedstocks. Vanillin has been considered the strongest inhibitor amongst typical phenolic compounds, such as syringaldehyde or hydroxybenzoic acid (Li et al., 2014), so its elimination or detoxification for efficient fermentation was compulsory, as it is demonstrated in section 3.5. Unlike other wastes, phenolic inhibitors were not detected in SIW, due to its structural composition (Table 5).

Therefore, even if achieving the maximum production of easily fermentable sugars is the goal, in fermentative butanol synthesis, the formation of unintended by-products (microbial inhibitors) in an unavoidable outcome during these pre-treatments that must not be neglected. Efficiency of each pre-treatment is marked by both factors. A brief summary of efficiencies of different pre-treatment process has been illustrated in Figure 1 (TRS vs By-products). It has been observed that the best process condition for the release of higher concentration of easily fermentable sugars and lower by-products were different

for each industrial waste biomass. Thus, release of easily fermentable sugars as well as by-products typically depended on both nature of the substrate and pre-treatment process.

Based on the initial characterization of the for agro-industrial wastes, BLW was selected as an optimal substrate due to its high initial concentration of RSCs. This, coupled with the greater availability of BLW over other feedstocks, converted this waste in the most promising substrate for biobutanol production purposes. In addition, BLW was already enriched with yeast protein and other essential micronutrients, and presented higher total solids concentration in comparison with other valid options, such as SIW (Table 1). In this case, dilute acid hydrolysis gave the highest conversion of TRS/g dry substrate as compared to the other methods. However, acid hydrolysis pre-treatment was also shown to produce higher concentrations of microbial inhibitors (such as phenolics), and therefore, RSM was used to optimize parameters to enhance TRS concentration and reduce inhibitor concentration for acid hydrolysis of BLW.

3.3 Optimization of process parameters to enhance fermentable sugar production from BLW using RSM

The ranges of the variables investigated and responses of the previously selected substrate samples in terms of TRS and inhibitors production are given in Table 3. Results of central composite design which consists of experimental data for studying the effect of three independent variables (reaction time, pH, feedstock concentration) on fermentable sugar production and inhibitory compounds generation when dilute acid hydrolysis was applied to a BLW sample have been presented. The data was fitted in a quadratic polynomial equation for all the desired and undesired compounds and the analysis of variance described in Table 6 indicated that the model was significant ($p < 0.005$) in all

cases. Corresponding equations to predict fermentable sugars production and inhibitors production using BLW (Eq. 3-10) in terms of code factors were as follows:

Fermentable sugars:

- $TSR = (+89.74 + 7.81 \cdot Con + 5.95 \cdot Time + 306.24 \cdot pH - 9.73 \cdot 10 - 3 \cdot Con \cdot Time + 0.35 \cdot Con \cdot pH - 0.04 \cdot Time \cdot pH - 0.08 \cdot Con^2 - 0.07 \cdot Time^2 - 312.05 \cdot pH^2)$ (Eq. 3)
- $Glu\cos e = (-85.98 + 9.28 \cdot Con + 6.88 \cdot Time + 230.33 \cdot pH - 0.02 \cdot Con \cdot Time + 0.63 \cdot Con \cdot pH + 0.27 \cdot Time \cdot pH - 0.11 \cdot Con^2 - 0.08 \cdot Time^2 - 197.92 \cdot pH^2)$ (Eq. 4)
- $Galactose = (-4.84 + 0.69 \cdot Con + 0.49 \cdot Time + 21.22 \cdot pH - 2.49 \cdot 10 - 4 \cdot Con \cdot Time + 0.06 \cdot Con \cdot pH - 0.03 \cdot Time \cdot pH - 9.11 \cdot 10 - 3 \cdot Con^2 - 5.54 \cdot 10 - 3 \cdot Time^2 - 20.05 \cdot pH^2)$ (Eq. 5)
- $Xylose = (-13.55 + 0.75 \cdot Con + 1.10 \cdot Time + 20.18 \cdot pH - 1.02 \cdot 10 - 3 \cdot Con \cdot Time + 0.20 \cdot Con \cdot pH - 0.01 \cdot Time \cdot pH - 0.011 \cdot Con^2 - 0.01 \cdot Time^2 - 24.88 \cdot pH^2)$ (Eq. 6)

Inhibitors:

- $5 - HMF = (+64.82 - 0.86 \cdot Con - 0.38 \cdot Time - 24.69 \cdot pH + 6.09 \cdot 10 - 3 \cdot Con \cdot Time + 0.41 \cdot Con \cdot pH + 0.25 \cdot Time \cdot pH + 1.19 \cdot 10 - 3 \cdot Con^2 - 1.90 \cdot 10 - 3 \cdot Time^2 - 8.90 \cdot pH^2)$ (Eq. 7)
- $Furfural = (+3.20 - 0.05 \cdot Con + 0.29 \cdot Time - 5.11 \cdot pH + 1.19 \cdot 10 - 4 \cdot Con \cdot Time + 0.03 \cdot Con \cdot pH + 0.01 \cdot Time \cdot pH + 5.14 \cdot 10 - 5 \cdot Con^2 - 6.03 \cdot 10 - 4 \cdot Time^2 - 1.54 \cdot pH^2)$ (Eq. 8)
- $Levulinic\ acid = (+20.13 - 0.57 \cdot Con + 0.25 \cdot Time + 4.86 \cdot pH - 1.26 \cdot 10 - 3 \cdot Con \cdot Time + 0.06 \cdot Con \cdot pH - 0.15 \cdot Time \cdot pH + 5.98 \cdot 10 - 3 \cdot Con^2 - 9.58 \cdot 10 - 5 \cdot Time^2 - 10.25 \cdot pH^2)$ (Eq. 9)
- $TPC = (+3822.05 - 106.66 \cdot Con + 22.99 \cdot Time - 2112.98 \cdot pH - 0.15 \cdot Con \cdot Time + 34.93 \cdot Con \cdot pH - 4.56 \cdot Time \cdot pH + 0.75 \cdot Con^2 - 0.08 \cdot Time^2 - 117.68 \cdot pH^2)$ (Eq.10)

The goodness of the model adjusted for the range of variables posed was checked by the determination coefficient (R^2). In both models, R^2 values higher than 0.85 indicated

that 85% variations in fermentable sugars production can be well explained by the model (Table 6).

BLW hydrolysis using 40 g $_{substrate}$/L, pH of 0.76 and 40 min produced the maximum amount of reducing sugar compounds (TRS, glucose, galactose and xylose), a parameter-combination quite interesting compared to literature reports, since an elevated substrate concentration has been linked to a decreasing reaction rate due to inhibition processes by other compounds, such as sugar-derived inhibitors, insufficient mixing (mass transfer limitations) or other effects related to the increased content of insoluble solids (Kristensen et al., 2009). Even if maintaining high substrate concentrations throughout the hydrolysis and subsequent conversion process from biomass to biobutanol is important for the energy balance and economic viability of biobutanol production, most of the hydrolysis processes of different feedstocks utilize comparatively lower substrate concentrations as shown in Table 5. In this case, a lower substrate concentration favoured the conversion of reducing sugar compounds into inhibitors. This effect could be reinforced by the accumulation of free reducing sugar compounds already present in the untreated (not hydrolysed) biomass (Table 1). Glucose represented the 84% of the total BLW-derived carbohydrates - a promising result considering that glucose is the preferred carbon source for clostridia cultures (Sarchami & Rehmann, 2015).

Besides, an increase in time from 40 to 60 min or more led to lowering of RSC concentration as the hydrolysis process had enough time to reach the activation energy to produce further unwanted by-products, especially TPC and levulinic acid. The synthesis of latter inhibitor also benefited from precursors, such as 5-HMF and furfural (Morone et

al., 2015), which suffered a drop in their concentration above 20 and 40 min, respectively.

3.4 Kinetic modelling of acid catalysed hydrolysis of BLW

The models proposed in literature to explain dilute acid hydrolysis are generally based on pseudo homogeneous irreversible first-order reactions, such as Seaman's model and two-fraction model (Aguilar et al., 2002). These models can be generalized as in Eq. 11:

$$Substrate\ (S) \xrightarrow{k_1} Product\ (P) \xrightarrow{k_2} Decomposition\ byproducts \qquad \text{(Eq. 11)}$$

where k_1 is the rate of the generation reaction and k_2 is the rate of the decomposition reaction (min^{-1}). Solving the differential equations, TRS concentration can be predicted through Eq. 12:

$$P = P_0\ x\ e^{-k_2 t} + S_0 \frac{k_1}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) \quad \text{(Seaman's model)} \qquad \text{(Eq. 12)}$$

where S and P are concentrations of substrate (BLW) and product (TRS) expressed in g/L, t is time (min) and subscript 0 indicates initial conditions. It can be inferred that larger the value of k_1, the higher the rate of TRS formed and the lower the process time required for maximizing production yield. On the contrary, the higher the value of k_2, the greater the rate of inhibitor production. Two-fraction model goes forward one more step and distinguishes between readily reacting lignocellulosic biomass fraction and not reaction susceptible fraction. The ratio between them is the parameter α (g/g) and Eq.12 is modified as follows (Eq. 13):

$$P = P_0\ x\ e^{-k_2 t} + \alpha S_0 \frac{k_1}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) \quad \text{(Two-fraction model)} \qquad \text{(Eq. 13)}$$

Once optimal conditions have been established in this study to obtain the maximum amount of RSC with the lowest concentration of inhibitors, it is interesting to compare

the results obtained with commonly applied kinetic methods useful for economical estimations in order to check the validity of the results (Lenihan et al., 2011). In our case, Eq. 13 was used to fit the data by means of non-linear regression analyses and values of 0.031 min^{-1}, 0.014 min^{-1} and 0.60 g/g were achieved for k_1, k_2 and α, respectively. The most desirable operating conditions will result in a high value of k_1 and a low value of k_2. Comparing the values of k_1 and k_2, it can be observed that the kinetic coefficient of the TRS formation is 2.2-fold higher than that of the degradation reaction. The value of k_1 is in accordance with the previously reported values (Aguilar et al., 2002; Jiang et al., 2012; Sarkar & Aikat, 2012). Nevertheless, the relatively high k_2 value confirms the significant influence of the time factor, which confirms that beyond the optimized value (40 min), accumulation of degradation by-products could happen. About 60% of the substrate was susceptible to dilute acid hydrolysis, which is in the common range for lignocellulosic feedstock (Aguilar et al., 2002).

3.5 Biobutanol and ABE production using BLW as substrate

Once operational variables of pre-treatment stage were evaluated and optimized by means of RSM, this section focused on the production of biobutanol by *C. beijerinckii* NRRL B-466 using BLW as raw material. Butanol fermentation by clostridia is characterized by synthesis of butanol along with by-products acetone and ethanol in the ratio 6:3:1. In control batch fermentation, using modified P2 medium containing 52 g/L glucose solution, *C. beijerinckii* NRRL B-466 produced 14.46 g/L of ABE in 72 h (Figure 2A). In this run, ABE yield reached 0.41 $g_{ABE}/g_{glucose}$ (74% of the glucose was efficiently utilized) and productivity was 0.15 g/L.

In the second batch experiment, the efficacy of the strain to exploit the nutrient and free RSC content (52 g/L) present in the raw BLW hydrolysate was studied. Batch fermentation of undetoxified BLW performed in P2 media resulted in no ABE production. Although the residual sugar concentration was high enough for solventogenic phase development, the presence of different microbial inhibitors within the hydrolysate solution (furfural 0.64 g/L, 5-HMF 1.12 g/L, levulinic acid 0.24 g/L, acetic acid 1.56 g/L and total phenolic compounds 0.31 g/L) prevented the transformation of intermediate products in butanol.

Clostridial strains have the ability to metabolize low concentration of inhibitors, such as furfural or 5-HMF, improving ABE fermentation in terms of cell concentration and solvents production (Gao & Rehmann, 2016). However, synergistic detrimental effect of weak acids, furan derivatives and phenolic compounds have been reported to either halt or slow down reaction rates of the fermentation (Jönsson et al., 2013). Thus, an extra detoxification step was required to achieve successful fermentation.

Detoxification by the modified over-liming method gave a sugar reduction of less than 10%. Using detoxified BLW hydrolysate for ABE fermentation (Figure 2B), an ABE yield of 0.30 $g_{ABE}/g_{glucose}$ was produced by *C. beijerinckii* NRRL B-466, resulting in a total ABE concentration of 10.6 g/L, which means that the culture performed much better than it did in the previous test. The TRS concentration reduced from 52.9 ± 0.8 g/kg to 15.4 ± 0.1 g/kg. The results obtained are consistent with those reported by other authors. Zhang et al. (2012) observed that sugar utilization ratio increased by 27 % when whether corncob residue hydrolysate was detoxified with $Ca(OH)_2$ (Zhang et al., 2012a). Similarly, Liu et al. (2015) increased butanol formation from 0.4 g/L to 5.5 g/L when the

pH of switchgrass hydrolysate was adjusted to 6 and 4 g/L of $CaCO_3$ were added prior to fermentation stage (Liu et al., 2015).

4. Conclusion

This study demonstrated that hydrolytic pre-treatment enhanced production of fermentable sugars from complex biomass. However, increased production of microbial inhibitors counter balanced biobutanol production potential. Hydrolysis pre-treatment step makes detoxification process another unavoidable necessity to enhance biobutanol production, increasing biofuel production cost. In any case, the ability to produce high value industrial solvents, such as ABE, from the inexpensive agro-industrial wastes could have positive effects on bioenergy production as well as on waste management, uplifting the agribusiness and employment in agro-industrial sector.

E-supplementary data of this work can be found in online version of the paper

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References

1. Aguilar, R., Ramırez, J., Garrote, G., Vázquez, M. 2002. Kinetic study of the acid hydrolysis of sugar cane bagasse. *Journal of Food Engineering*, **55**(4), 309-318.
2. Alvira, P., Tomás-Pejó, E., Ballesteros, M., Negro, M. 2010. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. *Bioresource technology*, **101**(13), 4851-4861.
3. Arreola-Vargas, J., Razo-Flores, E., Celis, L.B., Alatriste-Mondragón, F. 2015. Sequential hydrolysis of oat straw and hydrogen production from hydrolysates: role of hydrolysates constituents. *International Journal of Hydrogen Energy*, **40**(34), 10756-10765.

4. Assary, R.S., Kim, T., Low, J.J., Greeley, J., Curtiss, L.A. 2012. Glucose and fructose to platform chemicals: understanding the thermodynamic landscapes of acid-catalysed reactions using high-level ab initio methods. *Physical Chemistry Chemical Physics*, **14**(48), 16603-16611.
5. Baral, N.R., Shah, A. 2014. Microbial inhibitors: formation and effects on acetone-butanol-ethanol fermentation of lignocellulosic biomass. *Applied microbiology and biotechnology*, **98**(22), 9151-9172.
6. Dhillon, G.S., Brar, S.K., Verma, M., Tyagi, R.D. 2011. Utilization of different agro-industrial wastes for sustainable bioproduction of citric acid by Aspergillus niger. *Biochemical Engineering Journal*, **54**(2), 83-92.
7. Dhillon, G.S., Kaur, S., Brar, S.K. 2013. Perspective of apple processing wastes as low-cost substrates for bioproduction of high value products: A review. *Renewable and Sustainable Energy Reviews*, **27**, 789-805.
8. Enslow, K.R., Bell, A.T. 2012. The kinetics of Brønsted acid-catalyzed hydrolysis of hemicellulose dissolved in 1-ethyl-3-methylimidazolium chloride. *RSC Advances*, **2**(26), 10028-10036.
9. Ezeji, T., Qureshi, N., Blaschek, H.P. 2007. Butanol production from agricultural residues: impact of degradation products on Clostridium beijerinckii growth and butanol fermentation. *Biotechnology and bioengineering*, **97**(6), 1460-1469.
10. Feng, G., Fang, Z. 2013. Solid-and nano-catalysts pretreatment and hydrolysis techniques. in: *Pretreatment Techniques for Biofuels and Biorefineries*, Springer, pp. 339-366.
11. Gao, K., Rehmann, L. 2016. Combined Detoxification and In-situ Product Removal by a Single Resin During Lignocellulosic Butanol Production. *Scientific Reports*, **6**.
12. García Martín, J.F., Sánchez, S., Cuevas, M. 2013. Evaluation of the effect of the dilute acid hydrolysis on sugars release from olive prunings. *Renewable Energy*, **51**, 382-387.
13. Gürbüz, E.I., Wettstein, S.G., Dumesic, J.A. 2012. Conversion of hemicellulose to furfural and levulinic acid using biphasic reactors with alkylphenol solvents. *ChemSusChem*, **5**(2), 383-387.
14. He, J., Zhang, W. 2011. Techno-economic evaluation of thermo-chemical biomass-to-ethanol. *Applied Energy*, **88**(4), 1224-1232.
15. Hernoux-Villière, A., Lassi, U., Hu, T., Paquet, A., Rinaldi, L., Cravotto, G., Molina-Boisseau, S., Marais, M.-F., Lévêque, J.-M. 2013. Simultaneous microwave/ultrasound-assisted hydrolysis of starch-based industrial waste into reducing sugars. *ACS Sustainable Chemistry & Engineering*, **1**(8), 995-1002.
16. Hu, F., Ragauskas, A. 2012. Pretreatment and lignocellulosic chemistry. *Bioenergy Research*, **5**(4), 1043-1066.
17. Jiang, C.W., Bai, C.C., Xiao, H. 2012. Hydrolysis Kinetics of Straw Biomass Catalyzed by Diluted Sulphuric Acid in the Present of Fe2+. *Advanced Materials Research*. Trans Tech Publ. pp. 484-487.
18. Jönsson, L.J., Alriksson, B., Nilvebrant, N.-O. 2013. Bioconversion of lignocellulose: inhibitors and detoxification. *Biotechnology for Biofuels*, **6**(1), 1.

19. Kosseva, M., Webb, C. 2013. *Food industry wastes: assessment and recuperation of commodities*. Academic Press.
20. Kristensen, J.B., Felby, C., Jørgensen, H. 2009. Yield-determining factors in high-solids enzymatic hydrolysis of lignocellulose. *Biotechnology for Biofuels*, **2**(1), 1.
21. Lee, H., Hamid, S., Zain, S. 2014. Conversion of lignocellulosic biomass to nanocellulose: structure and chemical process. The Scientific World Journal, 2014.
22. Lee, S.J., Lee, J.H., Yang, X., Kim, S.B., Lee, J.H., Yoo, H.Y., Park, C., Kim, S.W. 2015. Phenolic compounds: Strong inhibitors derived from lignocellulosic hydrolysate for 2, 3-butanediol production by Enterobacter aerogenes. *Biotechnology journal*, **10**(12), 1920-1928.
23. Lee, S.Y., Park, J.H., Jang, S.H., Nielsen, L.K., Kim, J., Jung, K.S. 2008. Fermentative butanol production by Clostridia. *Biotechnology and bioengineering*, **101**(2), 209-228.
24. Lenihan, P., Orozco, A., Mangwandi, C., Rooney, D., O'Neill, E., Walker, G., Ahmad, M. 2011. *Kinetic modelling of dilute acid hydrolysis of lignocellulosic biomass*. INTECH Open Access Publisher.
25. Li, Y., Qi, B., Wan, Y. 2014. Inhibitory effect of vanillin on cellulase activity in hydrolysis of cellulosic biomass. *Bioresource technology*, **167**, 324-330.
26. Liu, K., Atiyeh, H.K., Pardo-Planas, O., Ezeji, T.C., Ujor, V., Overton, J.C., Berning, K., Wilkins, M.R., Tanner, R.S. 2015. Butanol production from hydrothermolysis-pretreated switchgrass: Quantification of inhibitors and detoxification of hydrolyzate. *Bioresource technology*, **189**, 292-301.
27. Maiti, S., Gallastegui, G., Sarma, S.J., Brar, S.K., Le Bihan, Y., Drogui, P., Buelna, G., Verma, M. 2016a. A re-look at the biochemical strategies to enhance butanol production. *Biomass and Bioenergy*, **94**, 187-200.
28. Maiti, S., Sarma, S.J., Brar, S.K., Le Bihan, Y., Drogui, P., Buelna, G., Verma, M. 2016b. Agro-industrial wastes as feedstock for sustainable bio-production of butanol by Clostridium beijerinckii. *Food and Bioproducts Processing*, **98**, 217-226.
29. Maiti, S., Sarma, S.J., Brar, S.K., Le Bihan, Y., Drogui, P., Buelna, G., Verma, M., Soccol, C.R. 2015. Novel spectrophotometric method for detection and estimation of butanol in acetone–butanol–ethanol fermenter. *Talanta*, **141**, 116-121.
30. Martin, A.M. 2012. *Bioconversion of waste materials to industrial products*. Springer Science & Business Media.
31. Martinez, A., Rodriguez, M.E., Wells, M.L., York, S.W., Preston, J.F., Ingram, L.O. 2001. Detoxification of dilute acid hydrolysates of lignocellulose with lime. *Biotechnology progress*, **17**(2), 287-293.
32. Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical chemistry*, **31**(3), 426-428.
33. Montano, D. 2009. Process design and evaluation of butanol production from lignocellulosic biomass. Energy research Centre of the Netherlands ECN.
34. Morone, A., Apte, M., Pandey, R. 2015. Levulinic acid production from renewable waste resources: Bottlenecks, potential remedies, advancements and applications. *Renewable and Sustainable Energy Reviews*, **51**, 548-565.

35. Naik, S.N., Goud, V.V., Rout, P.K., Dalai, A.K. 2010. Production of first and second generation biofuels: a comprehensive review. *Renewable and Sustainable Energy Reviews*, **14**(2), 578-597.
36. Nigam, P.S.-N., Pandey, A. 2009. *Biotechnology for agro-industrial residues utilisation: utilisation of agro-residues*. Springer Science & Business Media.
37. Olajire, A.A. 2012. The brewing industry and environmental challenges. *Journal of cleaner production*.
38. Palmqvist, E., Hahn-Hägerdal, B. 2000. Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition. *Bioresource Technology*, **74**(1), 25-33.
39. Puri, D.J., Heaven, S., Banks, C.J. 2013. Improving the performance of enzymes in hydrolysis of high solids paper pulp derived from MSW. *Biotechnology for biofuels*, **6**(1), 1.
40. Qureshi, N., Blaschek, H. 2000. Economics of butanol fermentation using hyper-butanol producing Clostridium beijerinckii BA101. *Food and bioproducts processing*, **78**(3), 139-144.
41. Ravindran, R., Jaiswal, S., Abu-Ghannam, N., Jaiswal, A.K. 2017. A comparative analysis of pretreatment strategies on the properties and hydrolysis of brewers' spent grain. Bioresource Technology.
42. Rosa, R., Ponzoni, C., Leonelli, C. 2014. Direct energy supply to the reaction mixture during microwave-assisted hydrothermal and combustion synthesis of inorganic materials. *Inorganics*, **2**(2), 191-210.
43. Sarchami, T., Rehmann, L. 2015. Optimizing Acid Hydrolysis of Jerusalem Artichoke-Derived Inulin for Fermentative Butanol Production. *BioEnergy Research*, **8**(3), 1148-1157.
44. Sarkar, N., Aikat, K. 2012. Kinetic study of acid hydrolysis of rice straw. *ISRN biotechnology*, **2013**.
45. Sarma, S.J., Das, R.K., Brar, S.K., Le Bihan, Y., Buelna, G., Verma, M., Soccol, C.R. 2014. Application of magnesium sulfate and its nanoparticles for enhanced lipid production by mixotrophic cultivation of algae using biodiesel waste. Energy, 78, 16-22.
46. Srinorakutara, T., Kaewvimol, L., Saengow, L.-a. 2006. Approach of cassava waste pretreatments for fuel ethanol production in Thailand. *J. Sci. Res. Chula. Univ*, **31**(1), 77-84.
47. Verma, M., Brar, S.K., Tyagi, R., Surampalli, R., Valéro, J. 2007. Starch industry wastewater as a substrate for antagonist, Trichoderma viride production. *Bioresource technology*, **98**(11), 2154-2162.
48. Wang, L., Chen, H. 2011. Increased fermentability of enzymatically hydrolyzed steam-exploded corn stover for butanol production by removal of fermentation inhibitors. *Process biochemistry*, **46**(2), 604-607.
49. White, J.S., Yohannan, B.K., Walker, G.M. 2008. Bioconversion of brewer's spent grains to bioethanol. FEMS Yeast Research, 8(7), 1175-1184.
50. Zhang, F., Deng, X., Fang, Z., Zeng, H., Tian, X., Kozinski, J. 2011. Hydrolysis of microcrystalline cellulose over Zn-Ca-Fe oxide catalyst. Petrochemical Technology, 40(1), 43-48.

51. Zhang, W., Liu, Z., Liu, Z., Li, F. 2012a. Butanol production from corncob residue using Clostridium beijerinckii NCIMB 8052. *Letters in applied microbiology*, **55**(3), 240-246.

52. Zhang, Y., Han, B., Ezeji, T.C. 2012b. Biotransformation of furfural and 5-hydroxymethyl furfural (HMF) by Clostridium acetobutylicum ATCC 824 during butanol fermentation. *New biotechnology*, **29**(3), 345-351.

Figure captions

Figure 1. Effect of different pre-treatments on total reducing sugars (TRS) and by-products formation from different agro industrial wastes such as BLW: brewery industry liquid waste, BSG: brewery spent grain, APS: apple pomace solid waste, APUS: apple pomace ultrafiltration sludge, SIW: starch industry wastewater where I : H_2SO_4/autoclave, II : H_2O_2/autoclave, III : NaOH/autoclave, IV: H_2SO_4/microwave, V : NaOH/ microwave, VI : Ca NSPs, VII : (Fe+ Ca) NSPs, VIII : Fe NSPs, IX : Ultra-sonication, X : H_2O/autoclave, XI : H_2O/microwave.

Figure 2 ABE production in P2 media with 52 g/L of glucose (control sample) (A), and a detoxified BLW hydrolysate sample (B) by means of *C. beijerinckii* NRRL B-466. No ABE production was observed in the raw (undetoxified) BLW hydrolysate sample.

Tables

Table 1. Physicochemical characterization of agro-industrial wastes.

Components	Brewery Industry waste			Apple Industry waste		
	BSG	**Surplus yeast**	**Spent hops**	**APS**	**APUS**	**SIW**
pH	5.2 ± 0.1	5.4 ± 0.1	5.1 ± 0.1	3.2 ± 0.1	3.4 ± 0.1	3.3 ± 0.2
Total solid (g/L)	-	229.4 ± 1.5	-		384.5 ± 2.4	16.4 ± 0.2

Ash content (%)	7.79 ± 0.65	8.9 ± 1.3	-	4.71 ± 0.53	2.55 ± 0.78	3.55 ± 0.94
Extractive (%)	3.53 ± 0.42	5.7 ± 0.6	-	3.12 ± 0.78	2.85 ± 0.23	1.24 ± 0.74
Carbohydrates (dry weight) (%)	-	36.4 ± 1.5	40.0 ± 0.5	66.0 ± 1.7	56.2 ± 1.3	-
Crude fiber (%)	-	3.0 ± 1.5	26.5 ± 2.4	33.45 ± 3.4	-	-
Cellulose (dry weight) (%)	17.1 ± 1.0	-		13.2 ± 1.3	11.8 ± 1.8	-
Hemicellulose (dry weight) (%)	32.5 ± 1.5	-		0.8 ± 0.1	-	-
Lignin (dry weight) (%)	13.4 ± 1.9	-		23.5 ± 2.1	20.6 ± 2.6	-
Free reducing sugar (g/kg)	22.7 ± 5.3	102.8 ± 4.7	-	155.1 ± 2.1	175.4 ± 5.9	21.6 ± 1.0
Glucose (g/kg)	1.6 ± 0.1	55.8 ± 1.3	-	35.5 ± 1.0	40.4 ± 1.8	1.25 ± 0.1
Fructose (g/kg)	-	-	-	32.7 ± 1.7	30.7 ± 2.7	-
Galactose (g/kg)	-	5.9 ± 0.9	-	3.9 ± 0.7	-	-
Xylose (g/kg)	-	5.7 ± 0.9	-	3.1 ± 1.0	-	-

Table 2. Central composite design ranges of the three variables considered for present investigation.

Serial number	Variable	Coded levels				
		-α	Low	Middle	High	+α
1	Concentration (g/L)	6.36	20	40	60	73.64

2	Time (min)	16.3	20	40	60	73.64
3	pH	0.02	0.32	0.76	1.20	1.80

Table 3. Experimental design and the responses obtained for parameter optimisation for dilute acid hydrolysis for BLW substrate

Run	Variables			Response							
	Concentration (g/L)	Time (min)	pH	TRS (g/kg)	Glucose (g/kg)	Galactose (g/kg)	Xylose (g/kg)	5-HMF (g/kg)	Furfural (g/kg)	Levulinic acid (g/kg)	TPR (g/kg)
1	20.0	60.0	1.20	203.5	164.5	8.3	6.7	10.6	0.3	0.9	256.8
2	40.0	40.0	0.76	433.3	307.9	23.9	26.9	20.0	1.7	9.5	560.3
3	40.0	40.0	0.76	433.3	307.9	23.9	26.9	20.0	1.7	9.5	560.3
4	60.0	60.0	1.20	239.7	185.4	9.3	6.5	14.9	0.6	<0.1	46.9
5	20.0	20.0	1.20	209.3	167.7	8.9	7.6	12.5	0.5	<0.1	124.6
6	6.4	40.0	0.76	332.8	218.5	17.1	15.8	33.1	2.9	25.2	2767.6
7	40.0	73.6	0.76	367.6	240.7	22.1	17.4	16.1	1.0	14.6	1062.8
8	40.0	40.0	0.76	433.3	307.9	23.9	26.9	20.0	1.7	9.5	560.3
9	40.0	40.0	1.80	168.5	156.0	8.4	6.9	7.9	0.3	<0.1	124.9
10	20.0	60.0	0.32	345.1	175.9	16.2	19.0	20.7	1.8	21.9	2150.3
11	60.0	60.0	0.32	364.5	161.6	15.8	9.6	13.9	0.9	17.8	598.8
12	40.0	40.0	0.76	433.3	307.9	23.9	26.8	20.0	1.7	9.5	560.3
13	40.0	40.0	0.76	433.3	307.9	23.9	26.8	20.0	1.7	9.5	560.3
14	60.0	20.0	0.32	384.5	198.9	15.6	11.7	15.0	1.3	13.7	539.9
15	40.0	16.4	0.76	387.8	226.8	18.0	12.5	24.1	1.6	8.5	264.0
16	73.6	40.0	0.76	395.1	175.9	14.9	17.4	14.1	1.2	11.7	456.6
17	20.0	20.0	0.32	345.1	175.9	16.2	17.4	35.1	2.2	14.5	1745.3
18	60.0	20.0	1.20	256.8	200.2	10.8	7.1	10.7	0.4	<0.1	36.8
19	40.0	40.0	0.02	409.6	281.5	22.3	24.9	26.9	2.0	12.0	1257.7
20	40.0	40.0	0.76	433.3	307.9	23.9	26.9	20.0	1.7	9.5	560.3

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Table 4. Influence of hydrolysis technique on fermentable RS production from agro-industrial wastes

Hydrolysis technique	Treatment	BLW (g/kg)			BSG (g/kg)			APS (g/kg)			APUS (g/kg)		SIW (g/kg)	
		Total RS	Glucose	Xylose	Total RS	Glucose	Xylose	Total RS	Glucose	Xylose	Total RS	Glucose	Total RS	Glucose
Chemical	H_2SO_4/autoclave	433.1	307.2	14.0	468.2	175.6	128.1	375.1	104.5	19.5	611.0	275.2	329.9	197.0
	H_2O_2/autoclave	75.3	41.1	5.7	53.9	32.3	22.5	290.9	85.6	1.0	378.5	190.9	56.0	20.7
	NaOH/autoclave	68.0	30.0	3.8	37.5	26.2	18.4	244.4	78.3	0.3	628.1	202.8	152.5	88.4
	H_2SO_4/microwave	302.1	239.9	8.3	413.4	146.9	97.5	360.7	122.2	10.6	336.8	143.9	359.3	246.2
	NaOH/microwave	132.7	119.1	4.9	59.9	34.8	12.7	199.7	84.6	1.1	299.6	78.1	62.0	39.0
NPs catalysed	Ca NSPs	153.1	134.1	5.7	122.2	64.4	38.6	255.4	106.0	0.5	335.2	56.2	71.8	43.3
	(Fe+ Ca) NSPs	98.8	150.9	ND	57.9	28.7	19.6	295.2	4.2	1.7	353.7	63.5	64.6	37.7
	Fe NSPs	67.1	15.9	ND	36.1	16.8	15.7	132.0	7.8	0.2	256.6	43.7	38.3	22.5
Mechanical	Ultra- sonication	80.6	22.5	ND	180.6	82.5	56.8	333.8	100.5	5.7	520.1	197.1	50.6	35.5
Hydrothermal	H_2O/autoclave	32.5	16.6	ND	32.5	22.6	1.2	230.0	71.3	0.4	597.9	199.6	32.5	16.6
	H_2O/microwave	48.2	33.2	ND	88.2	33.2	5.7	404.5	162.5	21.3	631.3	286.8	28.0	13.2

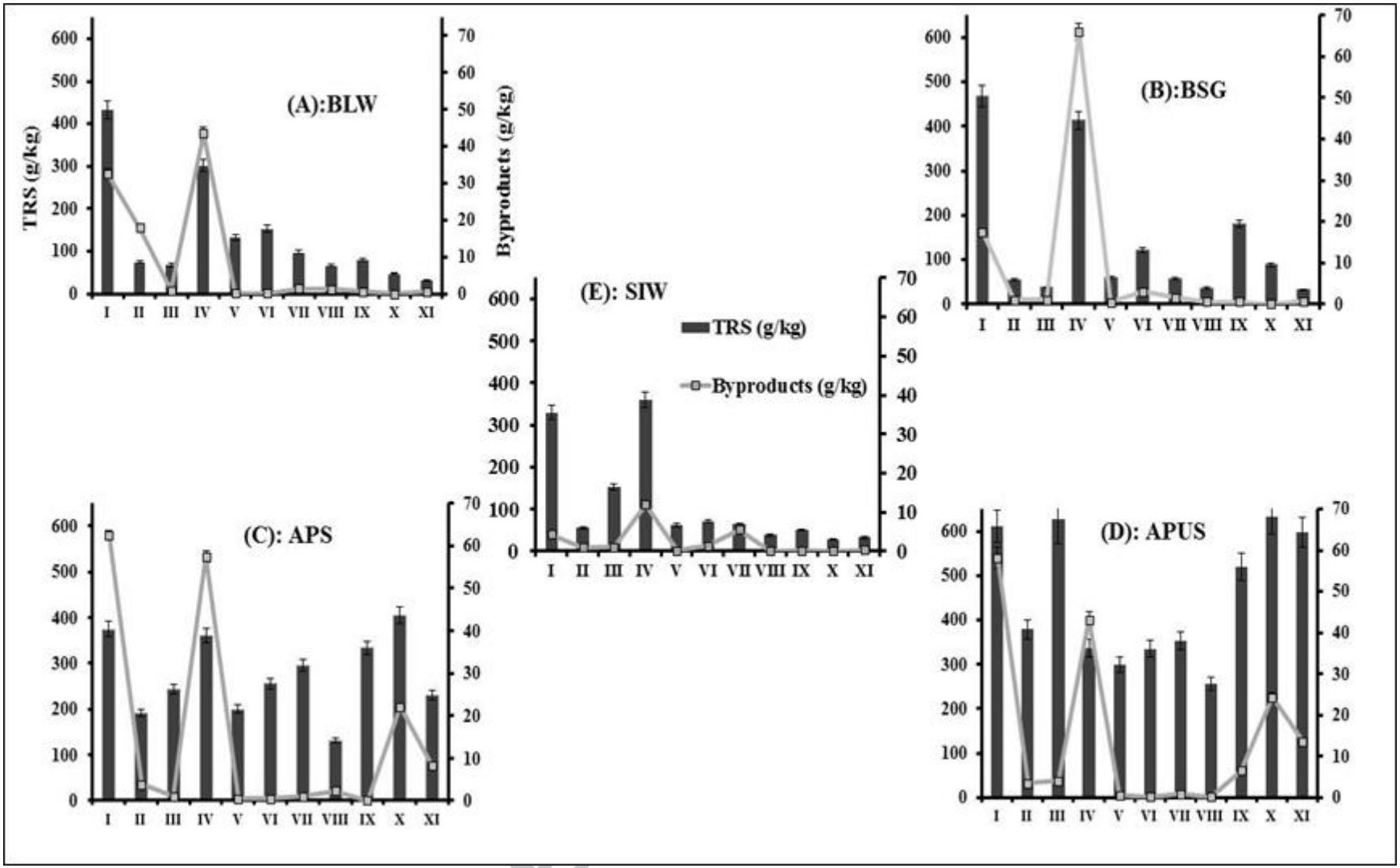
Table 5. Influence of hydrolysis techniques on production of inhibitory compounds from agro-industrial wastes

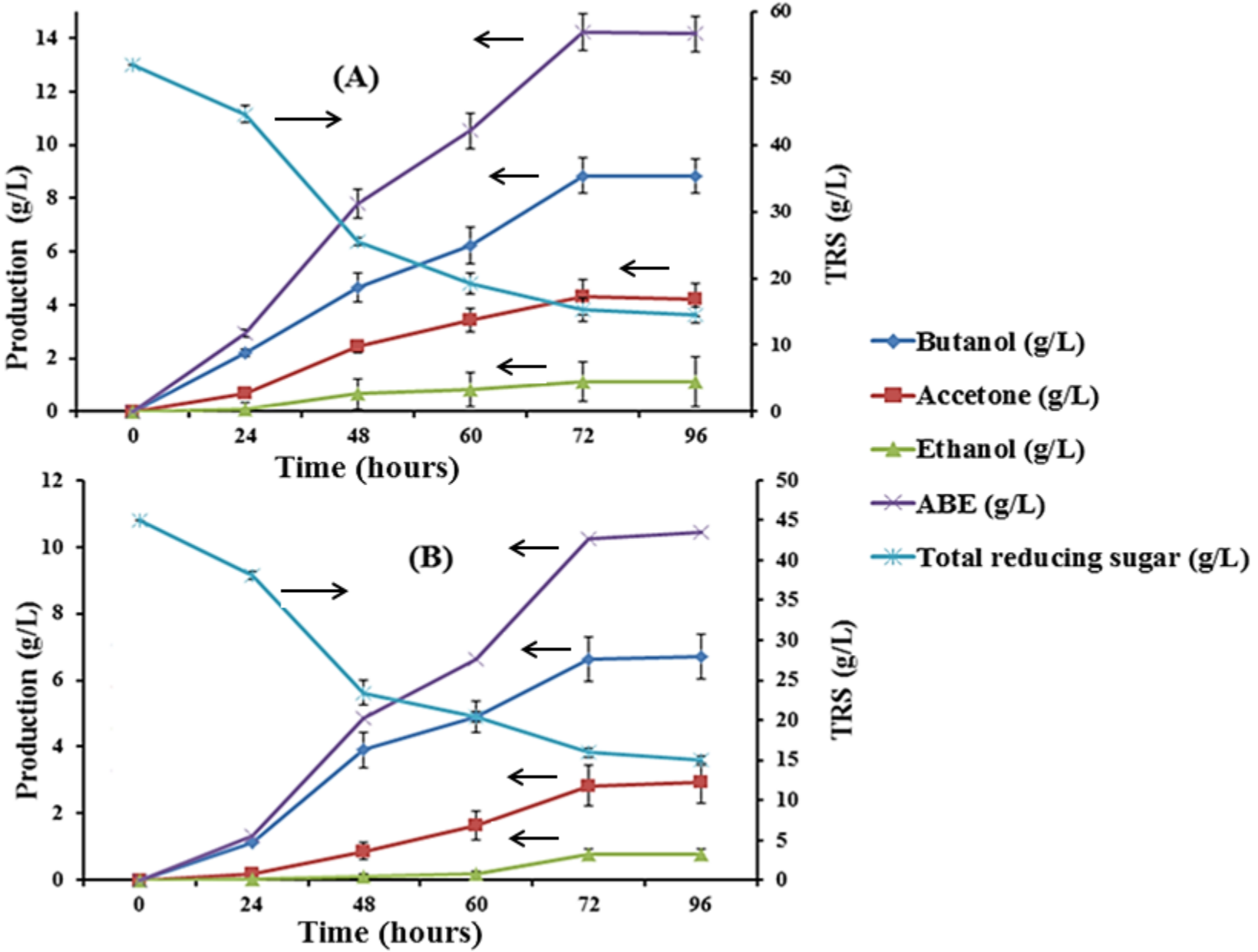
	Inhibitor concentration	Hydrolysis techniques used										
		Chemical					Catalyzed NPs			Mechanical	Hydrothermal	
		H2SO4/ autoclave	H2O2/ autoclave	NaOH/ autoclave	H2SO4/ microwave	NaOH/ microwave	Ca NSP	(Fe+ Ca) NSP	Fe NSP	Ultra-sonication	H2O/ autoclave	H2O/ microwave
BLW (g/kg)	5-HMF	21.3	16.9	0.2	29.7	ND	ND	0.8	0.5	ND	ND	ND
	Furfural	1.7	0.7	0.4	1.8	0.1	0.1	0.7	0.4	0.2	ND	0.3
	Levulinic acid	9.5	0.4	0.4	12.0	0.1	ND	ND	0.4	0.4	ND	0.4
	Syringaldehyde	47.5	ND	ND	62.4	ND	ND	ND	ND	3.4	12.4	10.8
	Ferulic acid	57.3	ND	ND	80.1	ND	ND	ND	ND	8.7	26.1	12.5
	Vanillin	280	ND	ND	322	ND	ND	ND	ND	40.5	18.3	13.7
	Vanillic acid	101	ND	ND	132	ND	ND	ND	ND	6.4	ND	ND
BSG(g/kg)	5-HMF	3.2	0.2	0.2	13.6	0.3	0.6	0.7	0.4	ND	ND	ND
	Furfural	11.5	0.4	0.4	48.6	0.2	2.4	0.5	0.1	0.2	ND	0.3
	Levulinic acid	2.8	0.4	0.4	3.9	<0.1	0.1	0.4	0.1	0.4	ND	0.4
	Syringaldehyde	70.8	ND	ND	102	ND	ND	ND	ND	9.2	2.4	2.6
	Ferulic acid	97.9	ND	ND	147	ND	ND	ND	ND	14.8	9.1	15.1
	Vanillin	357	ND	ND	319	ND	ND	ND	ND	18.0	8.3	12.3

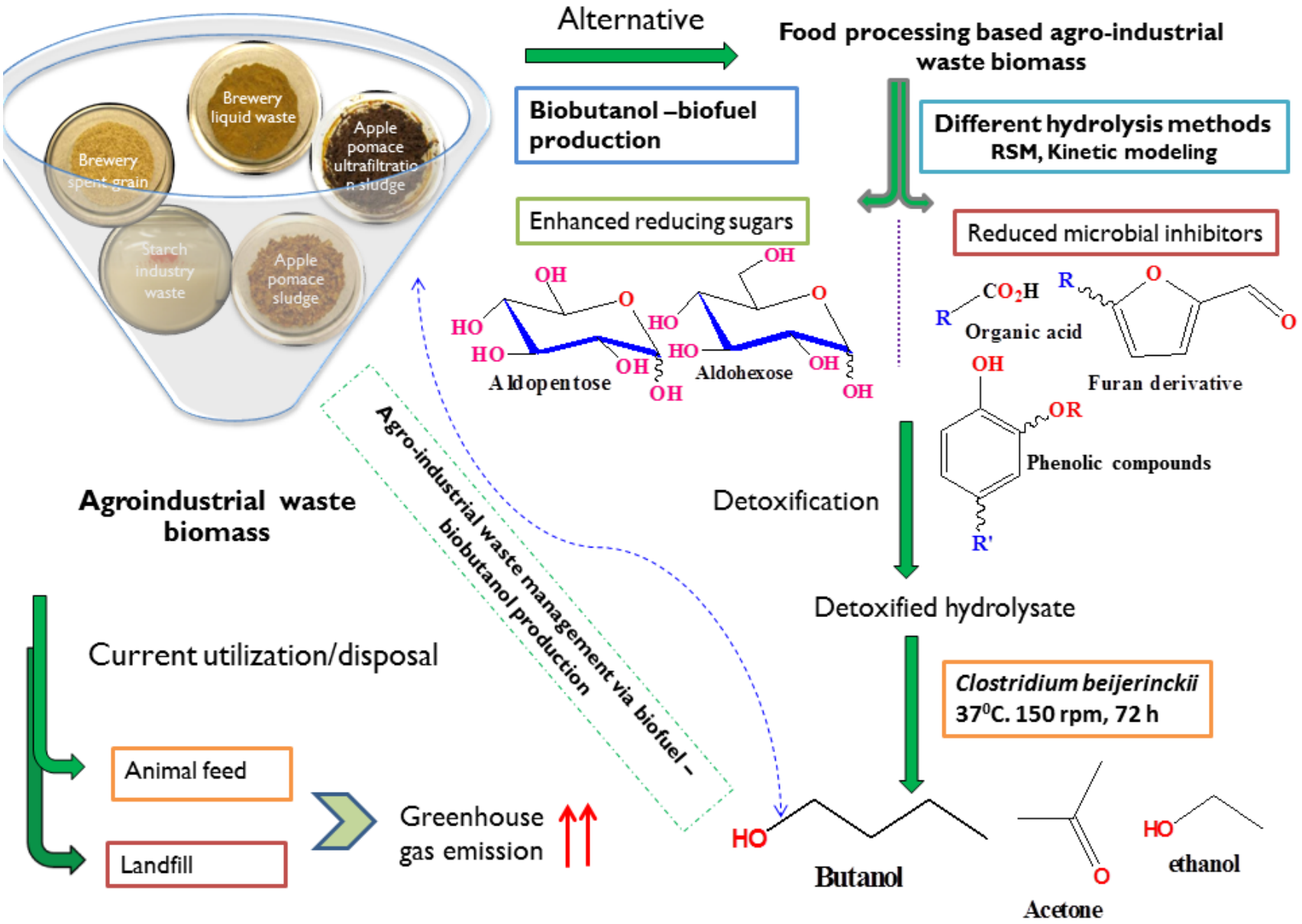
	Vanillic acid	134	ND	ND	190	ND	ND	ND	ND	ND	ND	ND
APS (g/kg)	5-HMF	45.5	2.9	0.3	52.4	ND	0.1	0.1	0.1	ND	0.3	1.7
	Furfural	3.1	0.6	0.4	4.6	0.4	<0.4	<0.4	<0.4	0.1	0.4	6.7
	Levulinic acid	13.9	0.4	0.4	0.4	0.1	0.4	1.0	2.2	ND	0.4	ND
	Syringaldehyde	34.3	ND	ND	47.6	ND	ND	ND	ND	11.6	30.6	43.4
	Ferulic acid	111	ND	ND	169	ND	ND	ND	ND	22.8	49.6	19.2
	Vanillin	123	ND	ND	168	ND	ND	ND	ND	24.8	57.4	22.3
	Vanillic acid	105	ND	ND	133	ND	ND	ND	ND	9.2	11.5	5.3
APUS (g/kg)	5-HMF	37.5	2.6	0.9	43.2	ND	0.2	0.4	<0.4	3.8	ND	12.4
	Furfural	1.3	0.4	2.8	0.1	0.4	<0.4	<0.4	<0.4	2.3	<0.1	1.2
	Levulinic acid	19.3	0.4	0.4	ND	0.1	<0.4	0.5	0.1	0.4	ND	ND
	Syringaldehyde	22.2	ND	ND	33.8	ND	ND	ND	ND	9.3	13.2	10.8
	Ferulic acid	87.8	ND	ND	94.5	ND	ND	ND	ND	36.5	34.7	54.2
	Vanillin	124	ND	ND	180	ND	ND	ND	ND	32.5	83.4	94.7
	Vanillic acid	133	ND	ND	134	ND	ND	ND	ND	4.7	4.4	7.9
SIW (g/kg)	5-HMF	2.7	ND	0.6	3.6	ND	ND	ND	ND	ND	ND	ND
	Furfural	1.6	0.7	0.4	6.1	0.2	1.1	5.6	0.1	0.2	ND	0.3
	Levulinic acid	<0.1	0.2	0.2	0.2	ND	0.3	ND	ND	<0.1	ND	ND

Table 6. Analysis of variance **(**ANOVA) for the fitted quadratic polynomial model for fermentable sugar compounds (glucose, galactose and xylose) and inhibitors (5-HMF, furfural, levulinic acid and TPC).

Sources	Sum of squares	Degrees of freedom	Mean squares	R-Squared	F value	P value
Glucose	60230.40	9	6692.27	0.8578	6.70	0.0032 (significant)
Galactose	592.42	9	65.82	0.9000	10.00	0.0006 (significant)
Xylose	1157.61	9	128.62	0.9179	12.42	0.0002 (significant)
5-HMF	873.74	9	97.08	0.9180	12.45	0.0002 (significant)
Furfural	8.45	9	0.94	0.8913	9.11	0.0009 (significant)
Levulinic acid	825.48	9	91.72	0.8637	6.00	0.0049 (significant)
TPC	$9.082 \cdot 10^6$	9	$1.009 \cdot 10^6$	0.9075	10.90	0.0004 (significant)






Alternative
Food processing based agro-industrial waste biomass
Biobutanol –biofuel production
Different hydrolysis methods
RSM, Kinetic modeling
Enhanced reducing sugars
Reduced microbial inhibitors
Brewery liquid waste
Brewery spent grain
Apple pomace ultrafiltration sludge
Starch industry waste
Apple pomace sludge
Aldopentose
Aldohexose
Organic acid
Furan derivative
Phenolic compounds
Agroindustrial waste biomass
Agro-industrial waste management via biofuel – biobutanol production
Detoxification
Detoxified hydrolysate
Current utilization/disposal
Clostridium beijerinckii
37⁰C. 150 rpm, 72 h
Animal feed
Landfill
Greenhouse gas emission
Butanol
Acetone
ethanol

Highlights

- Study of waste pre-treatments to enhance reducing sugars and reduce inhibitors
- Total reducing sugar yield of 0.433 g/g BLW with acid-catalysed hydrolysis
- Parameter optimization by RSM to enhance sugar and minimize inhibitors from BLW
- Development of kinetic modeling to enhance reducing sugar for scale-up
- Production of 10.2g/L ABE by clostridial fermentation of substrate