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Effective delignification of cabbage stem by organosolvent pre-treatment and subsequent enzymatic hydrolysis of pre-treated substrate

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Abstract: Non-edible portions of brassica vegetables are discarded in huge quantities, which is a great concern for causing environmental pollution. In the present study, cabbage stem was utilised for fermentable sugars production through enzymatic hydrolysis. Proximate composition of cabbage stem showed presence of lignocellulosic components (cellulose, hemicellulose and lignin) in appreciable amounts. Different pre-treatment approaches (acid, alkali and organosolvent) have been investigated for the effective delignification of cabbage stem and subsequent enzymatic hydrolysis of pre-treated substrate for better release of fermentable sugars. Organosolvent pre-treatment released highest quantity of cellulose content (69.28 ± 0.58^{a}%) in comparison to acid and alkali pre-treatment. Optimisation of different process conditions for enzymatic saccharification of pre-treated cabbage stem with cellulase enzyme was carried out and maximum yield of reducing sugar content of 782 ± 1.14^{b} mg/g was achieved at 1.5% (w/v) substrate concentration, 50°C temperature, pH 4, usage of 15 U/g enzyme concentration, and incubation time of 240 minutes. Valorisation of cabbage stem as the novel lignocellulosic waste sources into bio-products through enzymatic saccharification process for the mitigation of environmental pollution caused by the accumulation of these household or domestic organic residues has an added advantage.

Keywords: cabbage stem; cellulase; organosolvent pre-treatment; reducing sugars.

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1 Introduction

Lignocellulosic biomass (LCB) has been considered as one of the most promising sustainable and renewable feedstock to produce fermentable sugars via fermentation for production of value added bio-based materials. Valorisation of LCBs from food wastes for the production of food ingredients in formulation of novel food products has been the subject of intensive research over the past decades. Cabbage is attractive to be used as raw material for the conversion of LCB into fermentable sugars through enzymatic saccharification since it is one of the most cheap, abundant renewable sources. *Brassicaceae* family provides highest percentage of waste index but is rich source of nutritionally important components (Anwar et al., 2014). Cabbage (*Brassica oleracea* var. *capitata*) is the member of *Brassicaceae* family contains large amount of carbohydrate and thus is a good source of dietary fibre (Rekha and Ranu, 2018). Cabbage is enriched in phytochemicals such as sulphoraphane and glucosinolates which exhibit effective anti-carcinogenic properties (Rekha and Ranu, 2018). Due to the presence of antioxidants, polyphenols, dietary fibres, vitamins (Vitamin C, K, B complex and folic acid), minerals (iron and sodium) and low calorie content, cabbage exhibit beneficial effects on human health (Hanif et al., 2006). LCB is composed of cellulose, hemicellulose and lignin but due to the highly complex intertwined structure and heterogenous characteristics contribute to lignocellulosic recalcitrance. Thus transition of LCB into fermentable sugars faces many challenges and LCB must be pre-treated to overcome the recalcitrance. Pre-treatment is an important upstream process which is necessary to overcome the natural recalcitrance by disrupting the lignin to expose cellulose and hemicelluloses, allowing an increased saccharification for the bioconversion of LCB into fermentable sugars. Many methods have been developed for pre-treating LCB which include physical, chemical, thermochemical and biological processes. Among several pre-treatment strategies, chemical pre-treatment are being extensively used (Sun and Cheng, 2002).

Acid, alkali and organosolvent pre-treatment methods are commonly used reagents with or without addition of catalysts. Acids namely, H_2SO_4, HCl, phosphoric acid are used for pre-treatment methods for cellulose hydrolysis and achieves higher reaction rates (Singh et al., 2015). However, acid pre-treatment has many disadvantages, which are mainly, corrosive, hazardous and formation of inhibitory products namely, 2-furfuraldehyde, hydroxymethyl furfural, acetic acid, etc. (Sun and Cheng, 2002). Alkaline hydrolysis with some commonly used bases such as lime, $Ca(OH)_2$, NaOH, liquid ammonia, etc. are generally considered for carrying out the reaction. The mechanism of alkali based pre-treatment is based on removal of cross linkage between ether and C-C linkages to the lignin polymer between lignin and hemicelluloses (Maurya et al., 2015). Dilute NaOH treatment causes swelling, increases surface area and decreases crystallinity of cellulose. It mainly disrupts lignin carbohydrate complex thus, improving structural integrity of lignocellulosic fraction (Li et al., 2012). Organosolvent pre-treatment method with low boiling point solvents (methanol and ethanol) is more efficient for delignification of feedstock. The treatment of feedstock with organic solvents (with or without catalysts) yields reducing sugars in highest amount by breaking lignin bonds (sinapyl and guaiacyl) but the recycling of solvents are necessary as it adds to the cost of reaction for production of reducing sugars (Sindhu et al., 2012). An advantage of organosolvent pre-treatment than other pre-treatments is the creation of sustainable renewable market through extraction of economically viable pure lignin, an

important by-product (Borand and Karaosmanoğlu, 2018). It also reduces the incidence of non-productive binding on lignin surface (Huijgen et al., 2011). Therefore, pre-treatments are the central processing technology for conversion of waste to energy as well as other by-products and in revenue generation. Malwe and Hebbar (2020) highlighted the financial feasibility of a waste to energy plant in Raipur City of India.

Liquefaction of waste feedstock was achieved by employing enzymatic systems for release of reducing sugars in significant quantity. Enzymatic digestibility of pre-treated substrates is an important phenomenon for production of reducing sugars, which are further utilised for production of products of higher value. Enzymes of glycosyl hydrolase group are employed for enzymatic saccharification of waste substrates. Cellulase and xylanase synergistically act upon feedstock breaking intermolecular bonds for release of glucose, xylose, arabinose and other important reducing sugars (Verardi et al., 2012).

Although few studies have demonstrated processing of cabbage wastes for the development of value added products (food formulations and animal feed). There is no information on the pre-treatment of cabbage waste to improve enzymatic hydrolysis of cabbage waste for better release of fermentable sugars.

The present study deals with the comparative evaluation of different pre-treatment methods on cabbage stem in order to facilitate the release of fermentable sugars through enzymatic hydrolysis. The objective of this research work is to optimise process parameters for the most effective pre-treatment technology of cabbage stem and evaluate the effects of various parameters for enzymatic hydrolysis of pre-treated cabbage stem.

2 Material and methods

2.1 Collection of substrate

The cabbage (*Brassica oleraceae* var. *capitata*) waste (stem) was collected from the local market of Shibpur, West Bengal, India. The stem was washed thoroughly with distilled water to remove the adhered dust and air dried for 24 hours. Waste material was cut, milled and sieved to particle size of 0.25 mm. The obtained sample was stored in airtight plastic bags for the following experiments.

2.2 Chemicals

All the reagents and solvents used in this study were of analytical grade and obtained from MERCK, India. The commercial cellulase Celluclast® (Novozyme) 1.5 L from *Trichoderma reesei* procured from Sigma-Aldrich Corp. was administered for enzymatic saccharification process. Commercial xylanase of recombinant origin expressed in *Aspergillus oryzae* was procured from Sigma-Aldrich Corp. (St. Louis, Missouri, USA) for enzymatic saccharification process.

2.3 Compositional analysis of cabbage stem

Protein content of cabbage stem was carried out by Lowry et al. (1951). Neutral detergent and acid detergent fibre contents were ascertained by methods described by Goering and Van Soest (1970). The subtraction of respective neutral detergent and acid detergent fibre

content furnishes the hemicellulose content of cabbage stem. Cellulose content of waste material was determined. Moisture and ash content were carried out using the standard methods of AOAC (2005). Acid insoluble lignin content of raw cabbage stem and pre-treated cabbage stem was determined according to TAPPI (2006) method. Waste sample was hydrolysed with 72% sulphuric acid for three hours, and then the solution was diluted with distilled water to 3% of sulphuric acid concentration, and further hydrolysed at 100°C for four hours. The resulting mixture was filtered under vacuum with Whatman No. 1 filter paper. The residue obtained was washed with distilled water to remove the final traces of acid. Acid soluble lignin was estimated spectrophotometrically (JASCO V 630 UV Vis Spectrophotometer, Maryland, USA) at 208 nm by NREL method (Sluiter et al., 2008).

Acid insoluble residue was dried until a constant weight was achieved for calculating acid-insoluble lignin or Klason lignin content, which is a percent weight of the residue to weight of sample.

For each determination, the acid insoluble or Klason lignin content in the test specimen was calculated according to the following equation

$$Klason\ lignin\ (\%) = \frac{A}{W} \times 100 \tag{1}$$

where A = weight of lignin, (g) and W = oven-dried weight of test specimen, (g).

All the assays were done in triplicate. All compositional percentages of raw and pre-treated waste materials were calculated on dry mass basis of waste substrates.

2.4 Pre-treatment of cabbage stem

Cabbage stem was pre-treated with three different pre-treatment methods including sodium hydroxide (NaOH) pre-treatment, hydrochloric acid (HCl) pre-treatment and organosolvent pre-treatment with methanol and sodium acetate as catalyst.

2.4.1 Acid and alkali pre-treatment

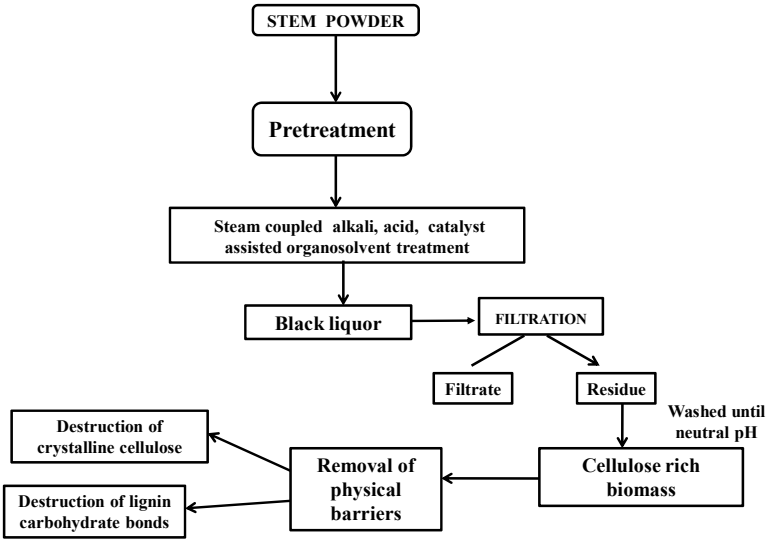
Pre-treatment with alkali and acid were done according to the method described by Olanbiwoninu and Odunfa (2012). 1 gram of cabbage stem was suspended separately in 100 ml of different concentrations of pre-treatment liquids (0.01 M to 0.25 M) in 250 ml Erlenmeyer flasks. Pre-treatments were carried out in autoclave at 121°C, 15 kPa pressure for 30 minutes. Each sample was immediately cooled and neutralised using 0.1 M HCl or NaOH. The neutralised pre-treated samples were centrifuged (R-24 Research Centrifuge REMI, Mumbai, Maharashtra) at 7,000 Xg for 15 minutes. The residues were collected and washed with deionised water, followed by drying at 65°C and were stored at 4°C prior to enzymatic hydrolysis. A portion of washed pre-treated solid residue was used for compositional analysis and characterisation. After pre-treatment, the liquid fractions (prehydrolyzates) were collected to determine the total reducing sugar content by DNS method (Miller, 1959).

2.4.2 Organosolvent pre-treatment

Organosolvent pre-treatment was done according to process developed by Sindhu et al. (2012). 1 gram of cabbage stem was separately mixed with methanol 100% (v/v) with

addition of 0.1 M sodium acetate as catalyst in 250 ml Erlenmeyer flasks. The solution was autoclaved at 121°C, 15 kPa pressure for 30 minutes. The resulting solution was cooled and centrifuged (R-24 Research Centrifuge REMI, Mumbai, Maharashtra) (6,000 Xg for 10 minutes) to remove the supernatants. The pellet was washed with distilled water until neutral pH was achieved followed by drying at 65°C and were stored at 4°C prior to enzymatic hydrolysis. The methanol was evaporated from the solution by rotary vacuum evaporation method and recycled. A portion of washed pre-treated solid residue was used for compositional analysis and characterisation. After pre-treatment, the liquid fraction (prehydrolyzate) was collected to determine the total reducing sugar content by DNS method (Miller, 1959).

Figure 1 Schematic outline of different pre-treatment approaches for delignification of cabbage stem



2.5 Changes in chemical composition

Screening of different pre-treatment methods employed for establishment of substantial pre-treatment efficiency was studied. Differences in lignocellulosic composition (cellulose, hemicelluloses, acid soluble and acid insoluble lignin) were calculated based on wt% of dried substrate cabbage stem by the methods described in the previous section. The pre-treatment process which achieved significant delignification, was further optimised.

2.6 Determination of pre-treatment efficiency of pre-treated cabbage stem

Pre-treatment efficiency was established based on partial removal or dissolution of physical barrier hemicellulose and improved cellulose conversion to fermentable sugars. The calculations were done according to the NREL LAP method (Hames et al., 2008).

2.6.1 Solid fraction yield of pre-treated cabbage stem

The loss in weight of solid residue obtained after pre-treatment process was measured gravimetrically in relation to the dried cabbage stem according to the following equation

$$Y\,\% = \frac{M_f}{M_i} \times 100 \tag{2}$$

where Y = yield of solid residue (% dry weight basis), M_f = weight (g) of pre-treated cabbage stem (dry basis), M_i = weight (g) of raw cabbage stem (dry basis).

2.6.2 Hemicellulose solubilisation of pre-treated cabbage stem

Hemicellulose fraction solubilisation of the solid residue obtained after pre-treatment process was determined by the following equation

$$\%\ Hs = 100 - \frac{H_f}{H_i} \cdot Y \tag{3}$$

where % Hs = hemicellulose solubilisation (% dry weight basis), H_i = hemicelluloses content in raw cabbage stem (% dry weight basis), H_f = hemicelluloses in pre-treated cabbage stem (% dry weight basis), Y = yield of solid residue (% dry weight basis)

2.6.3 Cellulose conversion determination of pre-treated cabbage stem

Cellulose conversion after pre-treatment (acid, alkali and organosolvent) process was ascertained by measurement of reducing sugar released after each pre-treatment process. The calculation was done according to following formula:

$$\%\ cellulose\ conversion = \frac{reducing\ sugar\ released\ (g)}{1.11 \times cellulose\ content\ (g)} \times 100 \tag{4}$$

where 1.11 is the correction factor for conversion of cellulose to reducing sugar.

2.7 Optimisation of organosolvent pre-treatment

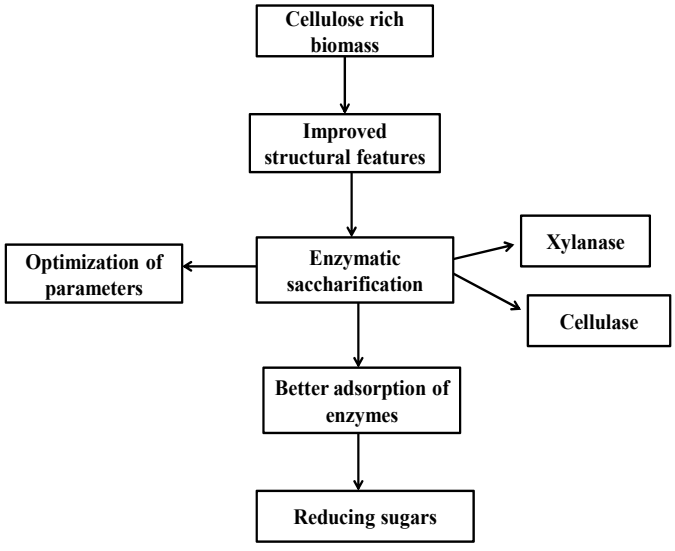
Optimisation of various process parameters involved in pre-treatment of cabbage stem was carried out in a stepwise manner. Pre-treatment was performed at different substrate concentrations (5% to 25% w/v), at different incubation temperatures (50°C to 121°C) and at different residence times (30 minutes to 120 minutes) to find out the optimum conditions for maximum release of reducing sugar. Different concentrations of methanol (30% to 90% v/v) were added along with Na acetate (0.01 M to 0.25 M) during pre-treatment to find the effects of solvent and catalyst concentrations. The reducing sugars were expressed as mg per gm of dried substrate.

2.8 Enzymatic saccharification of pre-treated cabbage stem

Enzymatic hydrolysis of organosolvent pre-treated cabbage stem was carried out according to Leustean et al. (2011). In 150 ml Erlenmeyer flasks 1 gm of pre-treated waste biomass was incubated separately with 1 ml of commercial cellulase from

Celluclast® (700 EGU/g) and 1 ml of cellobiase (≥ 250 units/g) and 1 ml of xylanase from Sigma (≥2,500 U/g). The total volume of reaction mixtures were made up to 30 ml with 50 mM citrate buffer (pH 4.8) for cellulase and 50 mM sodium citrate buffer (pH 5.4) for xylanase respectively. In addition, 1 ml of 2% (w/v) sodium azide was also added to the solution to prevent microbial contamination. The waste substrate was incubated at 50°C for 6 hours in a shaking water bath (RS 12 REMI Research Centrifuge, Mumbai, Maharashtra) (150 rpm) and then rapidly cooled on ice to stop the reaction. The sample was then centrifuged (R-24 REMI Research Centrifuge, Mumbai, Maharashtra) to remove the unhydrolysed residue. The supernatant (hydrolysates) was collected and analysed for reducing sugar content by DNS method (Miller, 1959).

Figure 2 Schematic outline of enzymatic saccharification of pre-treated cabbage stem



2.9 Optimisation of parameters affecting enzymatic saccharification

Different process parameters such as substrate loading (5% to 30% w/v), enzyme loading (5 to 25 U/g), incubation temperature (30°C to 90°C), reaction time (60 minutes to 300 minutes) and pH values (2 to 6) during enzymatic saccharification by cellulase.

2.10 Statistical analysis

All experiments were carried out in triplicate and standard deviation was determined. To determine the significance, the data were analysed by one-way ANOVA using Origin 2018 software. Tukey test was performed for p-value determination. Values of $p < 0.05$ were considered as significant value.

3 Results and discussion

3.1 Proximate composition of cabbage stem

Lignocellulosic components (cellulose, hemicelluloses and lignin) are the main primary attributing factors for categorising any residues or unused portions under waste biomass (Anwar et al., 2014). Table 1 presented the proximate composition of cabbage stem and it had been found that it was a good source of cellulose (15.20 ± 0.51^{a}%) and reducing sugars (2.21 ± 0.77^{a}%) which are primarily utilised for the production of fermentable sugars and ethanol, the widely used biofuel. Rekha and Ranu (2018) determined the proximate composition of dehydrated cabbage stem powder obtained from domestic waste and found that the cabbage stem contained carbohydrate content in appreciable amount (44.28 g/100 g), similar to the findings reported in the present study (Table 1). Wadhwa and Bakshi (2013) reported the lignocellulosic composition of brassica vegetable wastes and demonstrated that the holocellulose (cellulose and hemicelluloses) contents of most of the brassica vegetable wastes ranges from 20–50%. In the present study holocellulose (22.80%) content of cabbage stem was found within this reported range. Other non-carbohydrate lignocellulosic component such as lignin was also present in significant amount (8.63 ± 1.21^{a}%) which is the principal physical barrier associated with recalcitrant nature of waste biomass indicating the requirement for pre-treatment of cabbage stem. In the present study, other compositional aspects such as total protein and crude fibre were found to be present in higher amounts (Table 1) than those reported by Rekha and Ranu (2018) (22.3 g/100 g for dietray fibre and 19.2 g/100 g for protein). The difference in composition of cabbage stem might be due to several contributing factors such as species or variety, soil types and growth environment (Kazemi et al., 2016).

3.2 Effect of different pre-treatments on chemical composition of cabbage stem

Pre-treatment is the central process technology for the removal of in-built structural resistance that prohibits enzyme access to cellulose and thus, impedes enzymatic saccharification of waste biomasses. The removal of lignin and hemicellulose before enzymatic hydrolysis of lignocellulosic waste was highly necessary because it increased the conversion of cellulose to reducing sugars for production of high value products.

Changes in chemical composition observed after the three different steam coupled chemical pre-treatments of cabbage stem were presented in Table 2 indicating that compared to untreated waste biomass, the higher cellulose content in the thermochemically pre-treated waste might be due to the removal of physical barriers such as hemicellulose and lignin. The results presented in Table 2 confirmed that recovery of hemicellulose and lignin in cabbage stem obtained after pre-treatments were low. The fractionation of individual lignocellulosic components (cellulose, hemicelluloses and lignin) in the present study had been achieved more effectively with steam coupled methanol pre-treatment with Na acetate as catalyst than alkaline or acid pre-treatment methods. Data presented in Table 2 exhibited that cellulose content in organosolvent pre-treated biomass was maximum (69.28 ± 0.58^{a}%) followed by alkali and acid pre-treatments. The better yield of cellulose can be attributed to the proper defibrillation or removal of β1-4 aryl ether linkages associated with lignin units (Singh et al., 2015). Chen et al. (2015) studied four organosolv pre-treatment processes for effective pre-treatment and enzymatic digestibility of wheat straw. The chemical composition

differed in solid recovery, xylan and lignin solubilisation and maximum glucan recovery (72.2%) was obtained with formiline pre-treatment.

Table 1 Proximate composition of cabbage stems (% dried matter)

Components	*Cabbage stem*
Moisture	9.18 ± 0.25^{a}
Cellulose	15.20 ± 0.51^{a}
Neutral detergent fibre	23.61 ± 0.92^{a}
Acid detergent fibre	16.01 ± 0.28^{a}
Hemicellulose	7.60 ± 0.42^{a}
Total lignin	8.63 ± 1.21^{a}
Total protein	20.60 ± 0.53^{a}
Total fat	0.10 ± 0.09^{a}
Reducing sugar	2.21 ± 0.77^{a}
Ash	7.60 ± 0.01^{a}

Notes: Sample evaluation was done in triplicate. Values were calculated as mean ± SD ($n = 3$). Lowercase letters indicate significant differences ($p \leq 0.05$).

Table 2 revealed that pre-treatment of cabbage stem with methanol-Na acetate removed a large proportion of hemicellulose (3.71 ± 0.70^{b}%) when compared to the untreated biomass (7.60 ± 0.42^{b}%), indicating that organosolvent pre-treatment effectively removed hemicellulose. Hemicellulose a heteropolysaccahride with acetyl bonds hindered the efficient enzymatic digestibility by creating a barrier through shielding the cellulose fibres (Zhang et al., 2016). In the present study maximum hemicellulose solubilisation percentage in catalyst assisted organosolvent treatment (76.11 ± 1.36%) indicated proper dissolution of hemicellulose and furthermore, improved the porosity and surface area which subsequently improved the enzyme adsorption on the substrate surface (Bensah and Mensah, 2013).

Table 2 Chemical composition of untrated and pre-treated cabbage stem

Substrate	*Pre-treatment methods*	*Cellulose yield (% DM)*	*Hemicellulose yield (% DM)*	*ASL (% DM)*	*AIL (% DM)*	*Solid fraction yield (% DM)*	*Hemicellulose solubilisation (%)*
Cabbage stem	Untreated	15.20 ± 0.50^{a}	7.60 ± 0.42^{b}	Negligible	$8.63 \pm 1.20^{a,b}$	100.00	0
	Alkali (NaOH)	50.10 ± 0.40^{a}	4.54 ± 0.80^{b}	5.93 ± 0.59^{c}	$2.70 \pm 0.50^{a,b}$	56.19 ± 1.42	$66.43 \pm 2.31^{c,d}$
	Acid (HCl)	39.82 ± 0.27^{a}	5.36 ± 0.62^{b}	4.54 ± 0.40	$4.09 \pm 0.16^{a,b}$	61.28 ± 1.21	$56.78 \pm 2.36^{c,d}$
	Organosolvent (methanol)	69.28 ± 0.58^{a}	3.71 ± 0.70^{b}	7.00 ± 0.69^{c}	$1.63 \pm 0.56^{a,b}$	48.92 ± 1.39	$76.11 \pm 1.36^{c,d}$

Notes: DM: dried matter sample evaluation was done in triplicate. Values were calculated as mean ± SD ($n = 3$). Lowercase letters indicate significant differences ($p \leq 0.05$).

According to Matsakas et al. (2018), lignin removal is the prerequisite for improving the enzymatic hydrolysis process as lignin creates a shielding effect upon cellulose which negatively affects the outcome of the enzymatic saccharification step. Low lignin content of pre-treated cabbage stem (Table 2) indicated that organosolvent pre-treatment efficiently removed lignin by disrupting ester or β-O-4, β-5 and 5-5 ether bonds thereby increasing the porosity of biomass. However, Salapa et al. (2017) noted insignificant pre-treatment efficiency by methanol pre-treatment of wheat straw under mildest conditions (160°C, 20 minutes). Total lignin removal by alkali, acid and orgnosolvent pre-treatments were 8.17%, 8.32% and 8.78% respectively for cabbage stem. For each pre-treatment of cabbage stem, ASL was found to be higher than AIL (Table 2). Similar results were demonstrated in our previous experiment where more ASL than AIL was obtained in both dilute phosphoric acid (4% v/v) and organosolvent pre-treatments of cauliflower stalk and leaf.

According to Harmsen et al. (2010), organosolvent pre-treatment increased the digestibility of waste biomasses by increasing the porosity of feedstock by achieving remarkable delignification by employing organosolvent method. Low boiling point organic solvent (methanol) is advantageous for pre-treatment method as they produced less inhibitor and can be recycled (Li et al., 2017). Martin et al. (2011) studied the effect of different glycerol concentrations and reaction times on pre-treatment of sugarcane bagasse and exhibited that solvent concentration has correlation in increased xylan solubilisation and lignin removal. Although numerous potential pre-treatment methods such as acid, hydrothermal, ozone treatment, and pH controlled water pre-treatments have been extensively studied, however, organosolvent pre-treatment has some advantages over other methods. Organic solvents are advantageous than other pre-treatments because it improved reaction mechanisms by increased catalytic efficiency (lower activation energy) and selective product formation (Zhang et al., 2012). Organosolvent pre-treatment combined with steam is imperative for unlocking polysaccharides structure and delignification. Steam treatment was effective in the disruption of lignin and carbohydrate covalent (LCC) bond and degradation of lignin into small molecular fragments (Li et al., 2012). In addition to these aforementioned effects, the addition of mild acid catalyst (sodium acetate) minimised the cellulose degradation and thus, proper delignification of waste biomass was achieved (Sindhu et al., 2012; Matsakas et al., 2018). Ravindran et al. (2017) reported the pre-treatment of coffee waste pulp with 50% (v/v) ethanol at 120°C for 30 minutes with H_2SO_4 as catalyst and maximum reducing sugar of 283.12 mg/g was obtained after the pre-treatment. Conventional lower boiling point solvents, mainly, methanol and ethanol are employed for subsequent organosolvent pre-treatment but methanol is more advantageous as it is cheap and can be recycled faster in comparison, to ethanol, which is expensive to procure (Sun and Cheng, 2002). However, no literature on methanol pre-treatment of cabbage stem for enzymatic hydrolysis was found. Matsakas et al. (2018) observed that the cellulose content increased promisingly up to 77.9% w/w, while the yields of lignin and hemicellulose were lowered drastically (7.0% w/w and 8.9% w/w for lignin and hemicellulose respectively) after ethanol pre-treatment of birchwood biomass. Another study (Li et al., 2012) reported the chemical pre-treatment of meso bamboo using organosolvent method with 75% ethanol as solvent with 2% (w/w) sulphuric acid as catalyst and achieved a marked solubilisation of lignin (87.2%) at 160°C for 30 minutes. Li et al. (2017) reported the formiline pre-treatment of wheat straw and achieved the highest degree of delignification (84%), glucan recovery (75.5%) and a great fraction of xylan dissolution

(89.2%). Pascal et al. (2019) reported the acid catalysed atmospheric glycerol (ac-AGO) pre-treatment under optimised condition of 200°C for 15 minutes with 0.006% H_2SO_4 lowers the hemicelluloses and lignin removal rates (82 and 52% respectively). Another study (Mondylaksita et al., 2020) reported the recovery of glucan rich residue of oil palm fruit empty bunch (OPFEB) under optimised conditions of 0.07% H_2SO_4 as catalyst at 210°C for 90 minutes at solid to liquid (S/L) ratio of 1:10.

The results presented in Table 2 indicated that among the different pre-treatment methods, organosolvent treatment coupled with steam was found to be the most effective in terms of lignocellulosic components fractionation and β-aryl ether bond breakage between lignin and carbohydrate which increased the reducing sugar release (Li et al., 2012; Singh et al., 2015). Hence, methanol along with Na acetate as catalyst was selected as pre-treatment reagent for further studies.

3.3 Optimisation of various process conditions for organosolvent pre-treatment of cabbage stem

The effects of different process parameters on organosolvent pre-treatment were investigated and presented in Figure 3. The sugar yield in the prehydrolyzates was reported in mg based on 100 gm of dry cabbage stem (mg/g).

Optimisation of different concentrations of methanol from 30% to 90% (v/v) in presence of Na-acetate as catalyst showed that 70% (v/v) was optimum with reducing sugar yield of 723 ± 0.721^{c} mg/g respectively.

Pre-treatment with different concentrations of Na-acetate (0.01 M to 0.25 M) revealed that 0.15 M catalyst produced maximum reducing sugar content (415 ± 0.49^{c} mg/g). Addition of catalyst in the reaction mixture lowered the incubation temperature needed for reaction to proceed (Sindhu et al., 2012).

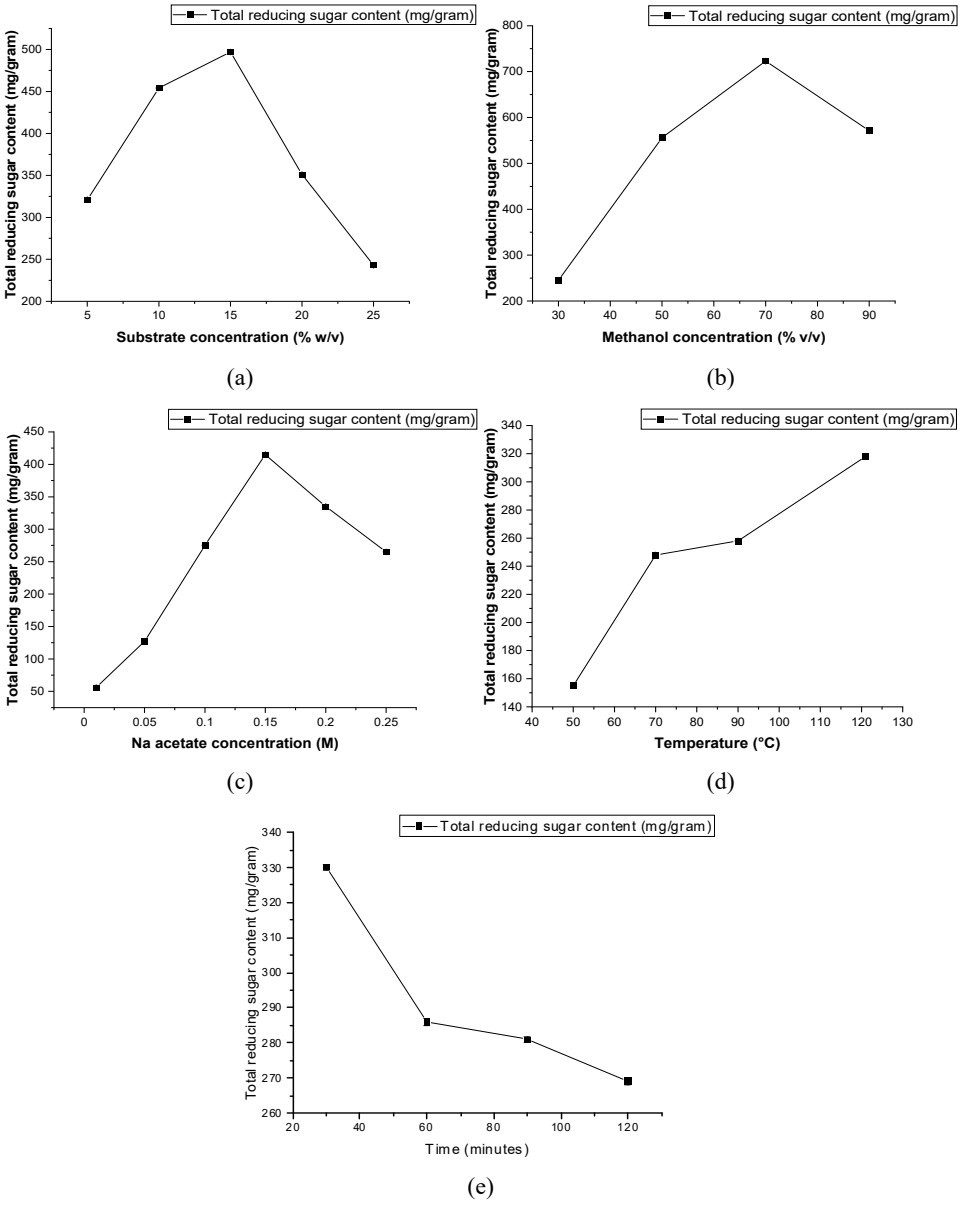
Substrate concentration was an important parameter for achieving higher yield of reducing sugar content. Significant amount of substrate concentration was needed for proper accessibility of pre-treatment reagent to feedstock. 15% (w/v) of cabbage stem yielded maximum amount of reducing sugar content of $651 \pm 0.81^{a,b}$ mg/g. Substrate concentration beyond 15% (w/v) reduced the yield of reducing sugar which decreased the proper accessibility of subtrate to pre-treatment reagent and caused improper removal of lignin (Sindhu et al., 2012).

Incubation temperature and residence time also significantly affected the yield of reducing sugar content. Moderate reaction temperature of 121°C and reaction time of 30 minutes achieved maximum reducing sugar yield of $318 \pm 0.21^{a,d}$ mg/g and $330 \pm 0.72^{a,d}$ mg/g respectively. At moderate temperature and time, hydrolysis of hemicellulose takes place which results in acetyl bond breakage and dissolution of hemicellulosic fraction (Idrees et al., 2014; Sindhu et al., 2012). Wildschut et al. (2013) reported the ethanol organosolv pre-treatment of wheat straw and established the effect of different parameters (reaction time, temperature, acid catalyst dose, solvent concentration and particle size) on delignification, xylan solubilisation and cellulose enzymatic digestibility. They exhibited that ethanol concentration improves lignin solubility by breaking down hemicellulsoe lignin linkage and hydrolysis of ether linkages in lignin polymer.

Figure 3 indicated that the optimum conditions for methanol pre-treatment of cabbage stem were 70% (v/v) concentration of methanol, 0.15 M concentration of Na acetate,

15% (w/v) substrate concentration, incubation temperature and time of 121°C and 30 minutes respectively.

Figure 3 Reducing sugar content under different parameters of optimisation of organosolvent pre-treated cabbage stem



3.4 Enzymatic saccharification of pre-treated cabbage stem

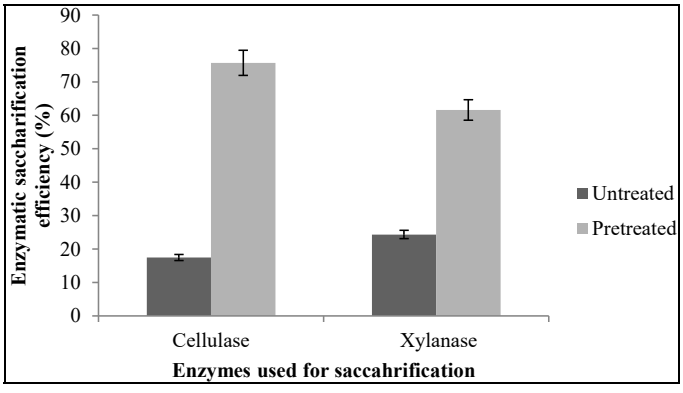
Enzymatic hydrolysis is the most important step for conversion of pre-treated substrates into products of higher value. In the present study, organosolvent pre-treated cabbage

stem was hydrolysed using commercial xylanase and cellulase enzymes and the subsequent production of reducing sugar was determined on the basis of mg/gm dried weight of pre-treated substrate.

Figure 4 showed that compared with untreated biomass, higher total reducing sugar yield was achieved when pre-treated cabbage stem was hydrolysed with cellulase and xylanase in Na acetate-based organosolvent pre-treatment. Results presented in Figure 4 also indicated that enzymatic saccharification efficiency for cellulase induced enzymatic hydrolysis of pre-treated cabbage stem after five hours of reaction time was higher than those for xylanase.

Achievement of effective enzymatic hydrolysis of pre-treated cabbage stem was due to the shortening of fibre length of cellulose and less rigid or amorphous cellulose recovery which enhanced the yield of reducing sugar content after enzymatic saccharification process (Kumar and Sharma, 2017).

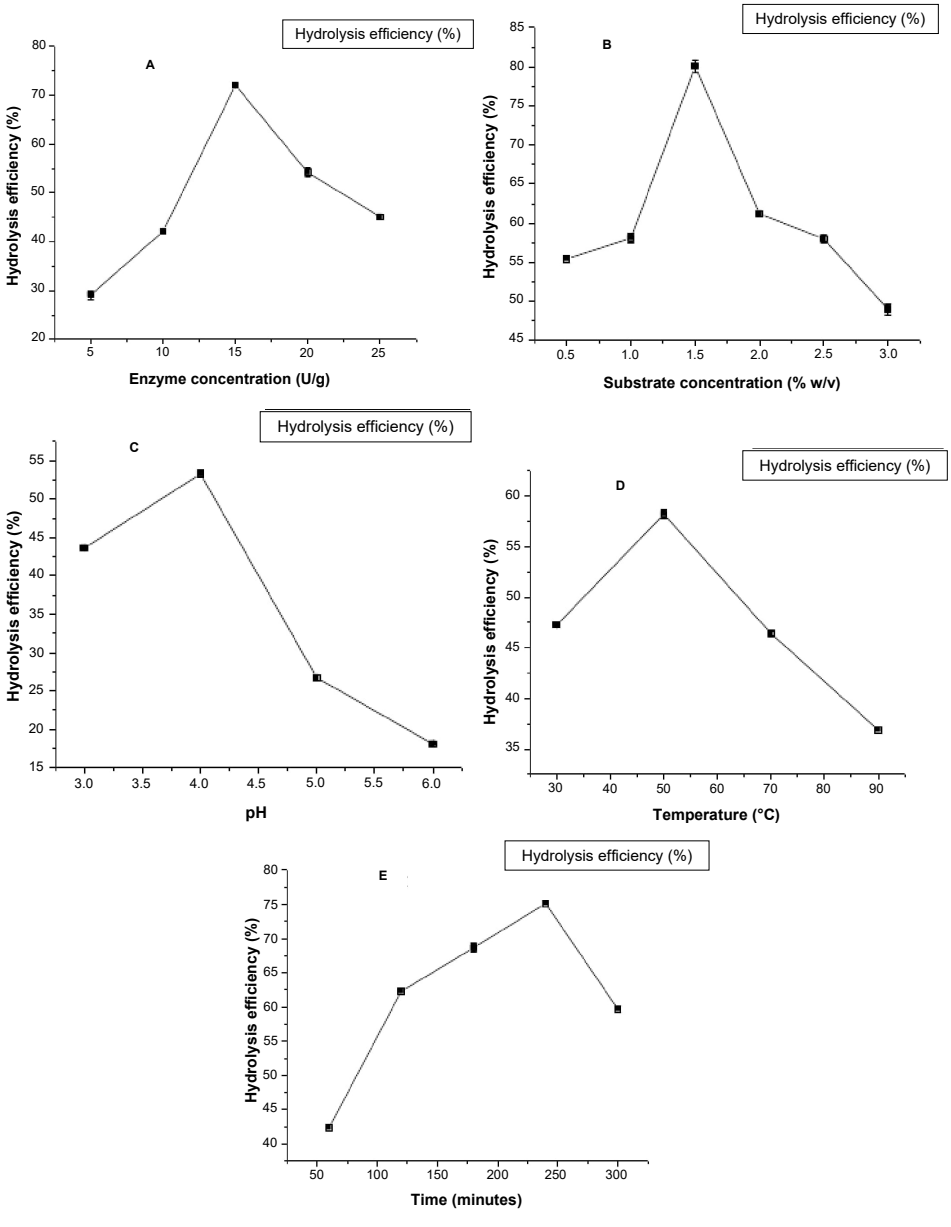
Figure 4 Total reducing sugar content of untreated and organosolvent pre-treated cabbage stem



3.5 Optimisation of different parameters of enzymatic saccharification of pre-treated cabbage stem with cellulase

Influence of different process parameters such as biomass loading, enzyme loading, temperature, pH and time to bioconversion of cellulose and the dynamic accumulation of reducing sugars over enzymatic hydrolysis (under optimal conditions) were studied. Figure 5 exhibited that the yield of enzymatic digestion of cellulose in pre-treated substrates was optimum using cabbage stem concentration of 1.5% w/v. The influence of enzyme loading in the bioconversion process was represented in Figure 5. Maximum enzymatic reaction yield was obtained using 15 U/g cellulase dosage for cabbage stem. Effect of temperature on enzymatic hydrolysis of cellulosic materials revealed that maximum reducing sugar yield of $627 \pm 0.97^{c,d}$ mg/g was obtained when saccharification of the waste biomass was carried out at 50°C. The pre-treatment beyond 50°C showed reduction in sugar yield at 90°C and the reducing sugar yield was $332 \pm 0.28^{c,d}$ mg/g. Optimisation of incubation time on enzymatic bioconversion of cabbage stem showed that maximum reducing sugar was produced at 240 minutes. The pre-treatment beyond 240 minutes showed constant sugar yield and at 240 minutes the reducing sugar yield was 701 ± 0.65^{d} mg/g.

Figure 5 Different parameters are varieted for optimisation of enzymatic saccahrification of cabbage stem with cellulase enzyme



The results indicated that optimum conditions of enzymatic hydrolysis of cabbage stem pre-treated with catalyst assisted 70% (v/v) organosolvent with were biomass loading of 1.5% w/v, enzyme loading of 15 U/g, incubation time 240 minutes and incubation temperature 50°C. Maximum reducing sugar yield at optimum conditions were 782 ± 1.14^{b} mg/g and maximum enzymatic hydrolysis efficiency was 79.23 ± 0.93^{b} %. Many literatures have suggested optimisation of process parameters for obtaining significant amount of reducing sugar content for enzymatic saccharification. Amiri et al.

(2014) reported the 1% w/w sulphuric acid catalysed ethanol organosolv pre-treatment of rice for production acetone-butanol-ethanol (ABE). The enzymatic hydrolysis pre-treated substrate resulted in higher yield of glucose (44.2%) at 45°C for 72 hours at solid loading of 8% using 25 FPU cellulose and 40 IU β-glucosidase. Li et al. (2017) reported the enzymatic saccharification of formiline pre-treated wheat straw with cellulase enzyme and obtained maximum reducing sugar yield at optimised conditions of 5% solid loading, 3.5 FPU/g of enzyme loading, incubation time of 12 hour and incubation temperature of 50°C. Acetone pre-treated rice straw at 11.25% (w/w) solid loading, 60 FPU enzyme loading, surfectant concentration of 0.05% and incubation time of 60 hour yielded maximum reducing sugar content of 0.655 g/g (Sindhu et al., 2012). Pascal et al. (2019) reported the enzymatic digestibility of acid catalysed atmospheric glycerol organosolv pre-treated sugarcane bagasse exhibited better glucose (70%) yield after 72 hours of reaction at modest cellulose loading.

This study will contribute to understanding the role of raw and pre-treated LCB (cabbage stem) as novel substrate for production of fermentable sugar that could be converted to value added products. The results of this investigation for the first time shed light on a comprehensive approach of organosolvent with catalyst pre-treatment of cabbage stem which deconstructed the recalcitrant nature and released fermentable sugars. To the best of our knowledge, the present study for the first time provided information on the valorisation of cabbage stem to give them an added value and a solution to reduce large amount of organic wastes.

4 Conclusions

In the present work different thermochemical pre-treatment approaches were studied for its efficacy in ascertaining deliginification efficiency and retaining of highest cellulose content of cabbage stem. A mild and low energy intensive enzymatic saccharification process of pre-treated cabbage stem increased the release of reducing sugars under optimised condition of biomass loading of 1.5% w/v, enzyme loading of 15 U/g, incubation time 240 minutes and incubation temperature 50°C. There is very little information available on the valorisation of cabbage wastes to give them an added value and a solution for the removal of this large amount of organic solid wastes. The results of this investigation for the first time shed light on a comprehensive approach of catalyst assisted organosolvent pre-treatment of cabbage wastes which are considered both milder and greener process technology for deconstruction of recalcitrancy and release of fermentable sugars in quantifiable amount. Experimental studies carried out revealed that pre-treated cabbage wastes have a potential to be used as novel substrate for bioconversion into various value added biomaterials. Combination of effective pre-treatment technique and enzymatic saccharification can be applied for efficient production of fermentable sugars which further can be utilised for production of useful bio based materials.

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References

Amiri, H., Karimi, K. and Zilouei, H. (2014) 'Organosolv pretreatment of rice straw for efficient acetone, butanol, and ethanol production', *Bioresource Technology*, Vol. 152, pp.450–456.

Anwar, Z., Gulfraz, M. and Irshad, M. (2014) 'Agro-industrial lignocellulosic biomass a key to unlock the future bio-energy: a brief review', *Journal of Radiation Research and Applied Sciences*, Vol. 7, No. 2, pp.163–173.

AOAC (2005) *International Official Methods of Analysis of AOAC International*, 18th ed., AOAC International, Gaithersburg, USA.

Bensah, E.C. and Mensah, M. (2013) 'Chemical pretreatment methods for the production of cellulosic ethanol: technologies and innovations', *International Journal of Chemical Engineering*, Vol. 2013, pp.1–21.

Borand, M.N. and Karaosmanoğlu, F. (2018) 'Effects of organosolv pretreatment conditions for lignocellulosic biomass in biorefinery applications: a review', *Journal of Renewable and Sustainable Energy*, Vol. 10, No. 3, p.33104.

Chen, H., Zhao, J., Hu, T., Zhao, X. and Liu, D. (2015) 'A comparison of several organosolv pretreatments for improving the enzymatic hydrolysis of wheat straw: substrate digestibility, fermentability and structural features', *Applied Energy*, Vol. 150, pp.224–232.

Goering, H.U. and Van Soest, P.J. (1970) *Forage Fiber Analyses (Apparatus, Reagents, Procedures, and Some Applications)*, No. 379, US Agricultural Research Service.

Hames, B., Ruiz, R., Scarlata, C., Sluiter, A., Sluiter, J. and Templeton, D. (2008) 'Preparation of samples for compositional analysis', *Laboratory Analytical Procedure (LAP)*, Vol. 1617.

Hanif, R., Iqbal, Z., Iqbal, M., Hanif, S. and Rasheed, M. (2006) 'Use of vegetables as nutritional food: role in human health', *Journal of Agricultural and Biological Science*, Vol. 1, No. 1, pp.18–22.

Harmsen, P.F.H., Huijgen, W., Bermudez, L. and Bakker, R. (2010) *Literature Review of Physical and Chemical Pretreatment Processes for Lignocellulosic Biomass*, No. 1184, Wageningen UR-Food & Biobased Research.

Huijgen, W.J., Smit, A.T., Reith, J.H. and Uil, H.D. (2011) 'Catalytic organosolv fractionation of willow wood and wheat straw as pretreatment for enzymatic cellulose hydrolysis', *Journal of Chemical Technology & Biotechnology*, Vol. 86, No. 11, pp.1428–1438.

Idrees, M., Adnan, A., Bokhari, S.A. and Qureshi, F.A. (2014) 'Production of fermentable sugars by combined chemo-enzymatic hydrolysis of cellulosic material for bioethanol production', *Brazilian Journal of Chemical Engineering*, Vol. 31, No. 2, pp.355–363.

Kazemi M., Torbaghan A.E., Abadi E.I.K., Tazik S.A. and Tohidi R. (2016) 'Red (*Brassica oleracea* var. *capitata*) and white (*Brassica oleracea* var. *botrytis*) cabbage leaves nutritional value as forage feed: comparison study of in vitro gas production and determination of chemical composition', *Direct Research Journal of Agriculture and Food Science*, Vol. 4, No. 7, pp.176–181.

Kumar, A.K. and Sharma, S., (2017) 'Recent updates on different methods of pretreatment of lignocellulosic feedstocks: a review', *Bioresources and Bioprocessing*, Vol. 4, No. 1, p.7.

Leustean, I., Georgescu, L. and Bahrim, G. (2011) 'Preliminary study for optimization of enzymatic hydrolysis of waste cellulosic materials', *The Annals of the University Dunarea de Jos of Galati Fascicle VI – Food Technology*, Vol. 35, No. 1, pp.27–33.

Li, T., Fang, Q., Chen, H., Qi, F., Ou, X., Zhao, X. and Liu, D. (2017) 'Solvent-based delignification and decrystallization of wheat straw for efficient enzymatic hydrolysis of cellulose and ethanol production with low cellulase loadings', *RSC Advances*, Vol. 7, No. 17, pp.10609–10617.

Li, Z., Jiang, Z., Fei, B., Pan, X., Cai, Z. and Yu, Y., (2012) 'Ethanol organosolv pretreatment of bamboo for efficient enzymatic saccharification', *BioResources*, Vol. 7, No. 3, pp.3452–3462.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) 'Protein measurement with the Folin phenol reagent', *Journal of Biological Chemistry*, Vol. 193, pp.265–275.

Malwe, A.A. and Hebbar, S. (2020) 'Financial feasibility of a waste to energy facility: a case study', *International Journal of Environment and Waste Management*, Vol. 26, No. 3, pp.269–295.

Martin, C., Puls, J., Saake, B. and Schreiber, A. (2011) 'Effect of glycerol pretreatment on component recovery and enzymatic hydrolysis of sugarcane bagasse', *Cellulose Chemistry and Technology*, Vol. 45, No. 7, pp.487–494.

Matsakas, L., Nitsos, C., Raghavendran, V., Yakimenko, O., Persson, G., Olsson, E., Rova, U., Olsson, L. and Christakopoulos, P. (2018) 'A novel hybrid organosolv: steam explosion method for the efficient fractionation and pretreatment of birch biomass', *Biotechnology for Biofuels*, Vol. 11, No. 1, p.160.

Maurya, D.P., Singla, A. and Negi, S. (2015) 'An overview of key pretreatment processes for biological conversion of lignocellulosic biomass to bioethanol', *3 Biotech*, Vol. 5, No. 5, pp.597–609.

Miller, G.L. (1959) 'Use of dinitrosalicylic acid reagent for determination of reducing sugar', *Analytical Chemistry*, Vol. 31, No. 3, pp.426–428.

Mondylaksita, K., Ferreira, J.A., Millati, R., Budhijanto, W., Niklasson, C. and Taherzadeh, M.J. (2020) 'Recovery of high purity lignin and digestible cellulose from oil palm empty fruit bunch using low acid-catalyzed organosolv pretreatment', *Agronomy*, Vol. 10, No. 5, p.674.

Olanbiwoninu, A.A. and Odunfa, S.A. (2012) 'Enhancing the production of reducing sugars from cassava peels by pretreatment methods', *International Journal of Science and Technology*, Vol. 2, No. 9, pp.650–652.

Pascal, K., Ren, H., Sun, F.F., Guo, S., Hu, J. and He, J. (2019) 'Mild acid-catalyzed atmospheric glycerol organosolv pretreatment effectively improves enzymatic hydrolyzability of lignocellulosic biomass', *ACS Omega*, Vol. 4, No. 22, pp.20015–20023.

Ravindran, R., Jaiswal, S., Abu-Ghannam, N. and Jaiswal, A.K. (2017) 'Two-step sequential pretreatment for the enhanced enzymatic hydrolysis of coffee spent waste', *Bioresource Technology*, Vol. 239, pp.276–284.

Rekha and Ranu, P. (2018) 'Nutritional and organoleptic evaluation of dehydrated cabbage powder incorporated in Sev', *International Journal of Agriculture Innovations and Research*, Vol. 6, No. 4, pp.8–9.

Salapa, I., Katsimpouras, C., Topakas, E. and Sidiras, D. (2017) 'Organosolv pretreatment of wheat straw for efficient ethanol production using various solvents', *Biomass and Bioenergy*, Vol. 100, pp.10–16.

Sindhu, R., Binod, P., Janu, K.U., Sukumaran, R.K. and Pandey, A. (2012) 'Organosolvent pretreatment and enzymatic hydrolysis of rice straw for the production of bioethanol', *World Journal of Microbiology and Biotechnology*, Vol. 28, No. 2, pp.473–483.

Singh, S., Cheng, G., Sathitsuksanoh, N., Wu, D., Varanasi, P., George, A., Balan, V., Gao, X., Kumar, R., Dale, B.E. and Wyman, C.E. (2015) 'Comparison of different biomass pretreatment techniques and their impact on chemistry and structure', *Frontiers in Energy Research*, Vol. 2, p.62.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D. and Crocker, D. (2008) 'Determination of structural carbohydrates and lignin in biomass', *Laboratory Analytical Procedure*, Vol. 1617, pp.1–16.

Sun, Y. and Cheng, J. (2002) 'Hydrolysis of lignocellulosic materials for ethanol production: a review', *Bioresource Technology*, Vol. 83, No. 1, pp.1–11.

TAPPI, T. (2006) 'Reaffirmation of T 222 om-02: acid-insoluble lignin in wood and pulp', *2005–2006 TAPPI Test Methods*.

Verardi, A., De Bari, I., Ricca, E. and Calabrò, V. (2012) 'Hydrolysis of lignocellulosic biomass: current status of processes and technologies and future perspectives', *Bioethanol*, pp.95–122.

Wadhwa, M. and Bakshi, M.P.S. (2013) 'Utilization of fruit and vegetable wastes as livestock feed and as substrates for generation of other value-added products', *Rap Publication*, Vol. 4.

Wildschut, J., Smit, A.T., Reith, J.H. and Huijgen, W.J. (2013) 'Ethanol-based organosolv fractionation of wheat straw for the production of lignin and enzymatically digestible cellulose', *Bioresource Technology*, Vol. 135, pp.58–66.

Zhang, K., Pei, Z. and Wang, D. (2016) 'Organic solvent pretreatment of lignocellulosic biomass for biofuels and biochemicals: a review', *Bioresource Technology*, Vol. 199, pp.21–33.

Zhang, Z., Harrison, M.D., Rackemann, D.W., Doherty, W.O. and O'Hara, I.M. (2016) 'Organosolv pretreatment of plant biomass for enhanced enzymatic saccharification', *Green Chemistry*, Vol. 18, No. 2, pp.360–381.