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Improving enzymatic hydrolysis of brewer spent grain with nonthermal plasma

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Abstract

In this study, a new pre-treatment method based on novel non-thermal plasma technology was developed to improve the enzymatic hydrolysis of brewer's spent grain (BSG) and subsequent bioethanol production. A submerged dielectric barrier discharge plasma reactor system was applied for this purpose. Pre-treatments were performed by taking into account variables including; voltages (22 kV, 25 kV and 28 kV), solvent (acid, alkali and water) and time (5, 10, 15 min). The resulting treated biomass was subjected to enzymatic hydrolysis. A 2.14-fold increase in yield of the reducing sugar was achieved post hydrolysis when the biomass was treated in water for 10 min at a voltage setting of 28 kV (162.90 mg/g of BSG) compared to control (75.94 mg/g of BSG). This research suggests that subjecting lignocellulose to plasma discharges can enhance the efficiency of enzymatic hydrolysis. A high ethanol titre was also obtained upon fermentation of the hydrolysate (25.062 g/l).

Keywords: Lignocellulose; biomass pretreatment; brewer's spent grain; atmospheric plasma, bioethanol production

1 Introduction

Brewers spent grain (BSG) is a by-product of the brewing industry that is high in polysaccharide, protein and lipid content (Mussatto et al., 2006). The global average production of brewers spent grain in 2016 was 39 million tonnes with the European union alone contributing 3.4 million tonnes (Lynch et al., 2016). Most of the spent grain generated in breweries end up being utilised as animal feed, compost, fertiliser or sent to landfill. BSG is rich in cellulose and hemicellulose content which can be exploited to extract 5-C and 6-C fermentable sugars. Due to the highly recalcitrant nature of the biomass, BSG needs to be subjected to pre-treatment measures for the efficient extraction of sugars (Ravindran et al., 2018).

Pre-treatments are disruptive techniques that aim to expose the polysaccharide fraction of lignocellulose for enzymatic or microbial digestion (Hassan et al., 2018). Pre-treatment using atmospheric plasma potentially offers a non-toxic and sustainable method for the effective removal of recalcitrance from lignocellulose and other by-products. Several studies have investigated the effectiveness of plasma as a lignocellulose pre-treatment strategy for subsequent valorisation. For example, Schultz-Jensen et al. (2011) investigated the effectiveness of plasma for subsequent bioethanol production from wheat straw. They reported the extent of lignin removal by plasma with respect to time. Baltazar-y-Jimenez et al., (2008) studied the changes in cellulose fibres exposed to atmospheric air plasma treatment.

This study focusses on the effectiveness of atmospheric air plasma as a pre-treatment strategy for BSG. The experiment was designed to include a liquid-based treatment strategy involving acid, alkali and water. The pre-treated biomass was then subjected to enzymatic hydrolysis followed by bioethanol production.

2 Materials and methods

2.1 Feedstock

Brewers' spent grain (BSG) was supplied by a local brewery (Dublin, Ireland). The material was weighed and then dried in a hot air oven at 60°C for 48h. The dried BSG was then ground and sieved through a 350 µm mesh to obtain a relatively uniform particle size. The size-reduced biomass was stored in a cool and dry place for further experiments. All chemicals such as NaOH, sulphuric acid and saccharifying enzymes such cellulase and hemicellulase were purchased from Sigma Aldrich.

2.2 Component analysis

The compositional variations in BSG before and after pre-treatment was analysed by using the NREL protocol. As a control measure the composition of native BSG was also determined. Biomass was hydrolysed by mixing it with 72% H_2SO_4 for 1h at 30°C. The acid concentration was then diluted to 4% with deionised water and autoclaved at 121°C for 1h. The solid fractions were then separated from the liquids and dried at 80°C for 48h following which the acid insoluble lignin was determined. The liquid obtained was used to measure the acid soluble lignin by measuring the absorbance at 205nm. The individual sugars present in the hydrolysate were identified and quantified by HPLC (Rezex ROA H^+, Waters e2695 Separation module, RI) (Sluiter et al., 2008).

2.3 Pre-treatment of BSG using atmospheric air pressure plasma

Pre-treatment of BSG was performed in a submerged dielectric barrier discharge (DBD) plasma reactor. Fig. 1 shows the schematics of the submerged DBD plasma reactor. The DBD plasma source employs a conventionally coaxial electrode configuration. The high voltage electrode is sealed in a quartz tube. The DBD tube and the ground electrode are submerged into the treated liquids so that the liquid serves as both an additional dielectric

barrier layer and the coolant for the discharge. Consequently, the plasma column length is determined by the depth of the DBD tube within the liquid. The thickness of both inner and outer quartz barrier was 2 mm and the discharge gap between the two coaxial quartz tubes was also 2mm. In this study, the liquid suspension was treated in a cylindrical acrylic tank with a total volume of 300ml. Air was used as the plasma working gas and its flow rate controlled by a mass flow controller (KOFLOC DF300C). In this study, the air flow rate was fixed at 1SLM. The post-discharge afterglow plasma effluent is bubbled into the liquid through four gas diffusers which are attached at the end of the tube. The plasma discharge is driven by a high voltage (HV) AC power supply (PVM500, Information Unlimited). The pre-treatment experiment was designed to determine the voltage and reaction time that best suited recalcitrance removal from BSG while collaborating with the nature of the solvent. Therefore, three voltage (peak-to-peak value) settings viz. 22 kV, 25 kV and 28 kV were tested for different duration settings (5, 10 and 15 min).

2.4 Enzymatic hydrolysis

The enzymatic hydrolysis of BSG was performed based on previous work conducted in our lab (Ravindran et al., 2018). The hydrolysates were then collected by centrifugation of the reaction mixture at 10000 rpm for 15 min followed by reducing sugar content estimation following dinitrosalicylic acid assay. Enzymatic digestibility was measured by calculating the hexoses yield (% cellulose) released from hydrolysis after pretreatment. The enzymatic digestibility was calculated by methods described by Zhang et al., 2007.

2.5 Individual sugar, inhibitor and organic acid analysis

Detection and quantification of monosaccharides and any organic acids formed was performed using an Allaince HPLC (Waters, e2695 Separation module) with a Rezex ROA-Organic acid H+ (8%) column, (350 x 7.8 mm; Phenomenex, UK) equipped with RI detector.

The HPLC system was also equipped with an autosampler, degasser and isocratic pump. The HPLC was operated in isocratic mode using H_2SO_4 (0.005 M) as the mobile phase. Samples were analysed at 65°C maintaining a flow rate of 0.6 ml/min. An isocratic mobile phase of 0.01M sulphuric acid was used to detect and estimate the number of inhibitors such as furfural and hydroxymethyl furfural in the hydrolysate.

2.6 Bioethanol production

The sugar rich hydrolysate obtained after enzymatic hydrolysis was filtered to remove any particulate matter and supplemented with 0.1% yeast extract and 0.1% peptone. The reaction mixture was autoclaved and then subjected to fermentation to produce bioethanol. Fermentation was carried out in 250 ml conical flasks with a total reaction volume of 100 ml. 1 ml of *Saccharomyces cerevisiae* inoculum (2.7×10^8 cells/ml) was added to the reaction mixture. The fermentation reaction was conducted at 30°C for 72h. After fermentation the reaction mixture was centrifuged and filtered using 0.4 µ filters and the ethanol produced was estimated by using Gas Chromatography. The injector temperature was set at 220°C while the column oven was set at an initial temperature of 80°C and ramped up to 160°C at a rate of 40°C per min and held for 7 minutes. A flame ionisation detector was used for analysing the ethanol content and was set at a temperature of 200°C.

2.7 Characterisation of pre-treated BSG

2.7.1 FTIR analysis

Any induced chemical changes of the functional groups of the different components in BSG by plasma treatment was studied using FTIR spectroscopy. Untreated BSG was used as the control to compare the differences. A Perkin Elmer Spectrum GX FT-IR (UATR) microscope (USA) was employed for this study. The FTIR spectra for the BSG samples were recorded from 4000 to 400 cm^{-1} with 16 scans at a resolution of 0.3 cm^{-1} in transmission mode.

2.7.2 Thermal Behaviour

Any induced differences in the thermal behaviour of BSG after pre-treatment was studied using differential scanning calorimetry (DSC). 55 mg of the pretreated BSG was placed in an aluminium pan with an empty pan used as a reference. All measurements were carried out between 25°C and 500°C with a linear increase of 10°C in a Shimadzu DSC-60 installed with TA-60WS software.

3 Results and discussion

3.1 Effect of pre-treatment on the composition of brewer's spent grain

Untreated BSG was subjected to composition analysis to determine the nature of the polysaccharides as well as recalcitrant materials present. Glucose was found to be the predominant sugar in the BSG samples followed by xylose. This makes BSG a good substrate for biofuel production following saccharification strategies. The glucose content arising from the cellulose fraction of native BSG was found to be 19.21±0.3g/100g of BSG. Xylose was found to be the second most abundant sugar in BSG contributing to ~63% of the total hemicellulose content (26.94±0.6g/100g). BSG was also rich in lignin with the total lignin content amounting to 30.48±2.1g/100g. These findings were in agreement with the composition of BSG reported by studies reported elsewhere (Ikram et al., 2017). Several studies have reported the conversion of glucose and xylose into fuel alternatives such as ethanol and butanol (Das et al., 2013).

Atmospheric pressure plasma are sources of highly reactive species such as ozone, hydroxyl ions, hydronium ions etc. These species react with chemical structures in their vicinity to effectively disintegrate the constituent chemicals. The ozone molecule, typically generated in abundance in air plasmas, has the capacity to specifically attack lignin while leaving the cellulose and hemicellulose intact. Characterisation of the pretreated BSG revealed that the

168 total lignin content was reduced to 19.50±0.5 g/100g of BSG. An increase in cellulose
169 registering of 28.13±0.9g/100g of BSG was found while only a slight increase in
170 hemicellulose was observed (27.55±0.3g/100g of BSG). This might be due to the inherent
171 crystalline nature of cellulose to resist chemical attacks. Meanwhile, hemicellulose is
172 amorphous in nature can deteriorate more readily in the presence of reactive species
173 (Chundawat et al., 2010).

174 Several studies have been published focusing on the utilisation of BSG for the production of
175 value-added products. For example, in a recent study Liguori et al., 2018 achieved 75g/l of
176 reducing sugar on subjecting BSG to enzymatic hydrolysis. This study involved the use of
177 concentrated alkali to digest the lignin fibres. The alkaline treatment was preceded by
178 hemicellulose removal using sulfuric acid. A thermomechanical pretreatment was devised for
179 BSG by Pierre et al., (2011) where a D.I.C (in French: Détente Instantanée Contrôlée) reactor
180 was used. Employing a range of processing pressures the researchers was able to obtain a
181 maximum glucose yield of 24g per 100g of BSG. An earlier study performed in our
182 laboratory examined the effect of various pretreatments on the composition as well as
183 enzymatic hydrolysis of BSG. The reducing sugar yielded from novel dielectric barrier
184 discharge plasma was comparable with the figures obtained for different pretreatment
185 strategies (Ravindran et al 2018).

186 **3.2 Effect of different solvents on plasma pre-treatment of BSG**

187 Table1 presents the results obtained in the study. All the pre-treatment parameters tested were
188 effective in yielding higher reducing sugar compared to control. The results show that plasma
189 pre-treatment of lignocellulosic biomass in either acid, neutral or alkaline conditions can
190 result in considerable removal of recalcitrance. However, the application under alkali
191 conditions resulted in excessive foaming and spilling of the liquor especially in the case of

higher voltage levels and longer duration treatments. This restricted the inclusion of higher voltages and longer treatment times. On the other hand, a higher reducing sugar yield was obtained for all settings of voltage level and treatment times when water was used as the pre-treatment solvent. This can be attributed to the increases in the concentration of reactive species generated. The initial pH of the solution exposed to plasma plays an important role in the degradation process. In addition to the aforementioned species, UV light and shock waves may also cause breakdown of the lignocellulose matrix (Bruggeman et al., 2007). A low pH environment leads to reduced efficiency of the plasma induced degradation (Su et al., 2002). At a pH range of 6.5-8.5, O_3 and OH radicals predominate the disintegration process for the lignocellulosic matrix. The oxidative capacity of O_3 disrupts the —C=C— bonds present in the aromatic rings (Grabowski et al., 2007). The abundance of this kind of bond may be cited as the reason to why pre-treatment techniques specifically decompose this type of polymer. However, at a higher pH of >8.5 the presence of OH_2^- can scavenge $^{\bullet}OH$ radicals, decreasing the efficiency of the pre-treatment process. The findings of an extensive study on the effect of pH on the efficiency of atmospheric air plasma treatment performed in our lab can be found here (Sarangapani et al., 2018). The maximum reducing sugar yield was obtained when water was used as the pre-treatment solvent and treatment was performed maintaining a voltage of 28kV for 10 min (162.9±0.5 mg/g of BSG). Additionally, the enzymatic digestibility was determined to be 86.8%. This was possibly because of the extensive damage incurred by the polysaccharide fraction along with lignin which left less cellulose and hemicellulose for enzymatic degradation.

3.3 Inhibitors in pre-treatment liquor

The pre-treatment liquor was subjected to HPLC analysis and was found to be have trace amounts of individual sugars such as glucose, galactose and mannose. Interestingly, small amounts of organic acids *viz.* citric acid and oxalic acids were also found. Schultz-Jensen et

al. (2011), in an extensive study involving plasma treatment of wheat straw reported the formation of a spectrum of organic acids. However, no furfural or HMF formation was observed.

3.4 FTIR and DSC profiles of untreated and pre-treated brewer's spent grain

Fourier Transform Infrared Spectroscopy is a simple qualitative analytical technique that indicates compositional changes in the biomass based on prominence, presence or absence of functional groups pertaining to each individual component (cellulose, hemicellulose and lignin). Diminishing of respective peaks indicates change in the inherent structure due to bond breakage. For example, the glycosidic linkages between cellulose and hemicellulose is represented by a peak at 895 cm^{-1}. The intensity of this peak was lower in the plasma treated BSG compared to the native counterpart indicating bond breakage. The peak at 1247 cm^{-1} which represents aryl-alkyl ether bonds (C-O-C) was also less prominent in the pretreated BSG. There was stark reduction of the peak at 1526 cm^{-1} which represents C=C bonds in the aromatic ring of lignin for the plasma treated biomass. This agreed with the changes in the composition of pretreated BSG. The breakage of ester and carboxylic bonds in the hemicellulose was recognizable in the FTIR spectrum with a diminished band at 1739 cm^{-1} for the BSG exposed to plasma. The pre-treatment however did not result in high degrees of demethylation of lignocellulose which was observable by bands at 2920 cm^{-1} for both native and treated BSG. Furthermore, the absence of bands in the 3000-3500 cm^{-1} range is indicative of stretching of –OH groups in the pretreated BSG (Santos et al., 2015).

Differential Scanning Calorimetry (DSC) was performed to study any changes brought about by plasma in the physical properties of BSG. The thermogram for native BSG was devoid of any glass transition. The crystallisation event occurred between 26°C and 309.9°C. Vaporisation of water and changes in the crystalline nature of the sample may have

contributed to this event. This was followed by melting. The melting temperature was found to be 361.05°C. The effect of plasma on the physical properties of BSG was evident as there were stark differences between the thermograms of the pretreated and native BSG. The DSC thermogram for plasma treated BSG showed distinct peaks that suggested an event for crystallisation from 28.49°C to 279.11°C. This was followed by a short melting and then ending transient. The melting temperature was recorded at 296°C.

3.5 Bioethanol production using plasma treated BSG

The hydrolysate obtained after enzymatic digestion of the plasma pretreated BSG was subjected to fermentation for the production of bioethanol. *S. cerevisiae* was employed as the ethanol producer. A maximum ethanol titre of 25.062 g/l of ethanol was obtained after fermentation for 72h. On the other hand, the bioethanol production using the control sample yielded 11.231 g/l of ethanol. The ethanol titre obtained in this study is comparable to ethanol yields reported elsewhere. For example, Srinorakutara et al. (2014) reported an ethanol titre of 30.40 g/l employing optimised fermentation methods using acid pretreated sugar cane trash. The ethanol yield obtained from the fermentation of lignocellulose-derived sugars can depend on process optimisation as well as the nature of ethanol producers used. On calculating the yield, 1g of reducing sugar yielded 0.16g of ethanol. A low theoretical yield may have been contributed by the abundance of xylose in the hydrolysate which remained unutilised due to the absence of a pentose-fermenting microbe. A synergistic combination of microbial species that can ferment 5-C and 6-C sugars can result in higher ethanol titre (Das et al., 2014).

4 Conclusion

In our study, atmospheric plasma in solvents was an effective pre-treatment strategy to reduce recalcitrance from BSG. There was 36% decrease in the total lignin content of pretreated

BSG using a water solvent. This resulted in a higher reducing sugar yield following enzymatic hydrolysis registering a 2.14-fold increase. There were no toxic inhibitors found following the treatment although trace amounts of organic acids were detected. This may have contributed to the higher ethanol titre following fermentation. This technology offers a promising method to achieve recalcitrance removal from lignocellulose.

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5 References

1. Baltazar-y-Jimenez, A., Bistritz, M., Schulz, E., Bismarck, A. 2008. Atmospheric air pressure plasma treatment of lignocellulosic fibres: Impact on mechanical properties and adhesion to cellulose acetate butyrate. Composites Sci. Technol. 68(1), 215-227.
2. Bruggeman, P., Graham, L., Degroote, J., Vierendeels, J., Leys, C. 2007. Water surface deformation in strong electrical fields and its influence on electrical breakdown in a metal pin–water electrode system. J. Phys. D: Appl. Phys. 40(16), 4779.
3. Chundawat, S.P.S., Vismeh, R., Sharma, L.N., Humpula, J.F., da Costa Sousa, L., Chambliss, C.K., Jones, A.D., Balan, V., Dale, B.E. 2010. Multifaceted

characterization of cell wall decomposition products formed during ammonia fiber expansion (AFEX) and dilute acid based pretreatments. Bioresour. Technol. 101(21), 8429-8438.

4. Das, S.P., Ravindran, R., Deka, D., Jawed, M., Das, D., Goyal, A. 2013. Bioethanol production from leafy biomass of mango (*Mangifera indica*) involving naturally isolated and recombinant enzymes. Prep. Biochem. Biotechnol. 43(7), 717-734.

5. Das, S.P., Ravindran, R., Ghosh, A., Deka, D., Das, D., Jawed, M., Fontes, C.M., Goyal, A. 2014. Efficient pretreatment for bioethanol production from water hyacinth (*Eichhornia crassipes*) involving naturally isolated and recombinant enzymes and its recovery. Environ. Prog. Sustain. Energy. 33(4), 1396-1404.

6. Grabowski, L., Van Veldhuizen, E., Pemen, A., Rutgers, W. 2007. Breakdown of methylene blue and methyl orange by pulsed corona discharge. Plasma Sources Sci. Technol. 16(2), 226.

7. Hassan, S. S., Williams, G. A., & Jaiswal, A. K., 2018. Emerging technologies for the pretreatment of lignocellulosic biomass. Bioresour. Technol. 262, 310-318

8. Ikram, S., Huang, L., Zhang, H., Wang, J., Yin, M. 2017. Composition and Nutrient Value Proposition of Brewers Spent Grain. J. Food Sci. 82(10), 2232-2242.

9. Kerby, C., Vriesekoop, F. 2017. An overview of the utilisation of brewery by-products as generated by British craft breweries. Beverages, 3(2), 24.

10. Liguori, R., Soccol, C., Porto de Souza Vandenberghe, L., Woiciechowski, A., & Faraco, V. 2015. Second generation ethanol production from brewers' spent grain. Energies, 8, 2575-2586.

11. Mussatto, S. I., & Roberto, I. C., 2006. Chemical characterization and liberation of pentose sugars from brewer's spent grain. J. Chem. Technol. Biotechnol.: Int. Res. Process, Environ. Clean Technol. 81(3), 268-274.

316 12. Mussatto, S., Dragone, G., Roberto, I. 2006. Brewers' spent grain: generation,
317 characteristics and potential applications. J. Cereal Sci. 43(1), 1-14.
318 13. Ravindran, R., Jaiswal, S., Abu-Ghannam, N., Jaiswal, A.K. 2018. A comparative
319 analysis of pretreatment strategies on the properties and hydrolysis of brewers' spent
320 grain. Bioresour. Technol. 248, 272-279.
321 14. Santos, D.M.d., Bukzem, A.d.L., Ascheri, D.P.R., Signini, R., Aquino, G.L.B.d. 2015.
322 Microwave-assisted carboxymethylation of cellulose extracted from brewer's spent
323 grain. Carbohydr. Polymer. 131, 125-133.
324 15. Sarangapani, C., Lu, P., Behan, P., Bourke, P., Cullen, P.J. 2018. Humic acid and
325 trihalomethane breakdown with potential by-product formations for atmospheric air
326 plasma water treatment. J. Indust. Eng. Chem. 59, 350-361.
327 16. Schultz-Jensen, N., Kádár, Z., Thomsen, A.B., Bindslev, H., Leipold, F. 2011.
328 Plasma-assisted pretreatment of wheat straw for ethanol production. Appl. Biochem.
329 Biotechnol. 165(3-4), 1010-1023.
330 17. Srinorakutara, T., Suttikul, S., Butivate, E., Panphan, V., Boonvitthya, N. 2014.
331 Optimization on pretreatment and enzymatic hydrolysis of sugarcane trash for ethanol
332 production. J. Food Sci. Eng. 4(3), 148-154.
333 18. Su, Z.-Z., Ito, K., Takashima, K., Katsura, S., Onda, K., Mizuno, A. 2002. OH radical
334 generation by atmospheric pressure pulsed discharge plasma and its quantitative
335 analysis by monitoring CO oxidation. J. Phys. D, 35(24), 3192.
336 19. Zhang, Y. H. P., Schell, D. J., McMillan, J. D. 2000. Methodological analysis for
337 determination of enzymatic digestibility of cellulosic materials. Biotechnol. Bioeng, 96
338 1, 188-194.

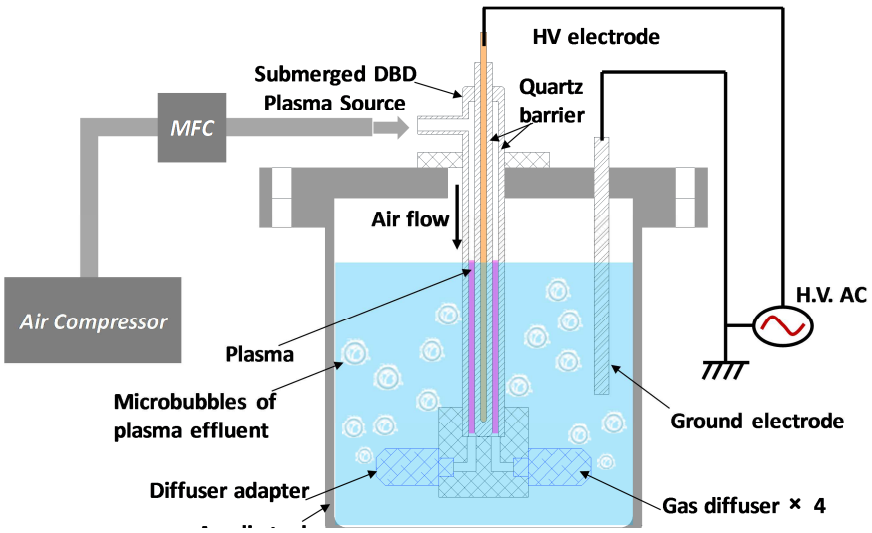
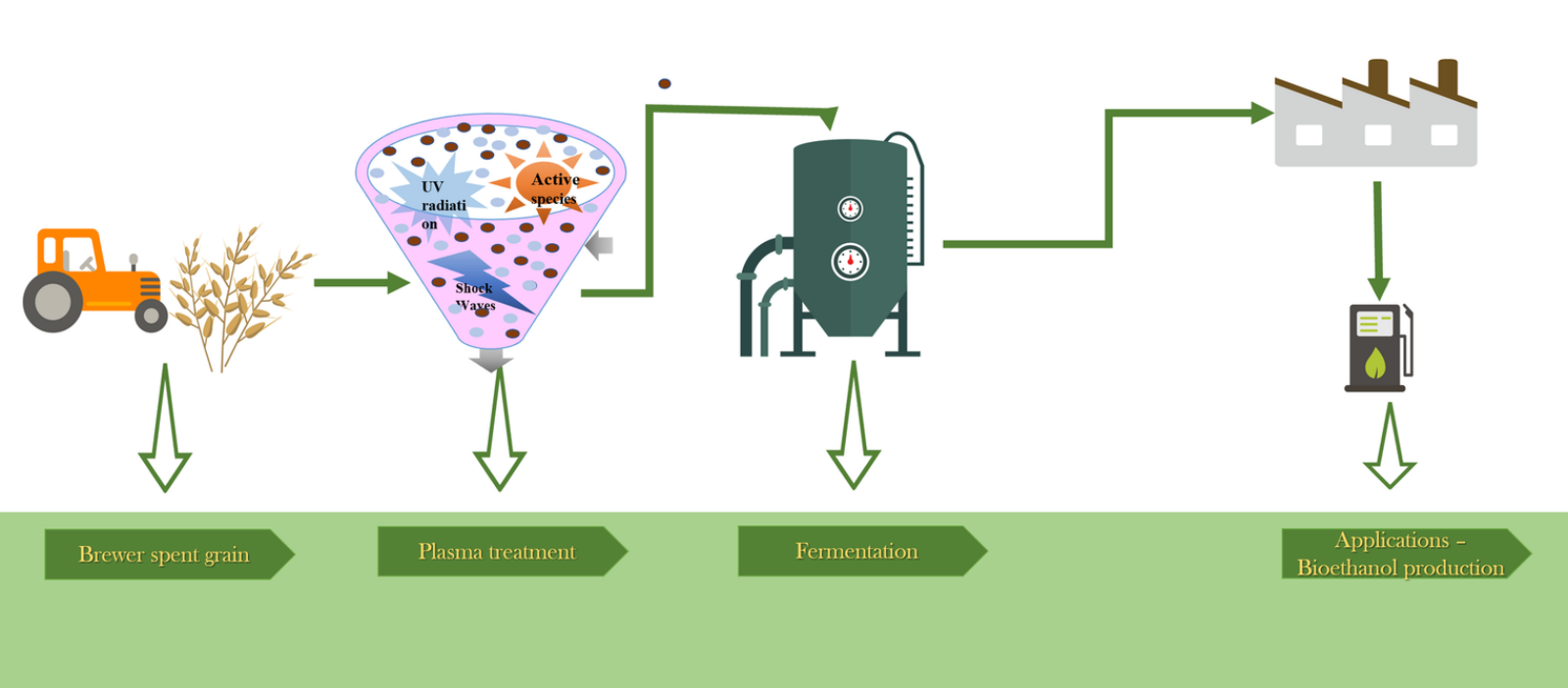


Fig. 1. Schematic of atmospheric pressure plasma reactor

Table 1. Reducing sugar yield obtained after enzymatic hydrolysis of each experimental trail

S. no	Voltage (kV)	Time (min)	Solvent	Reducing sugar (mg/ml)
1	22	5	Water	154.18±1.2
2	22	10	Acid	123.95±0.3
3	22	15	Alkali	130.18±2.1
4	25	5	Acid	129.25±0.7
5	25	10	Alkali	135.04±1.3
6	25	15	Water	148.55±0.4
7	28	5	Alkali	149.48±0.2
8	28	10	Water	162.9±0.5
9	28	15	Acid	137.66±1.9
10	Control	-	-	75.94±0.1


UV radiation
Active species
Shock Waves
Brewer spent grain
Plasma treatment
Fermentation
Applications – Bioethanol production