EFFECTS OF ALKALINE AND BEATING PRETREATMENT ON ANAEROBIC DIGESTION OF DISTILLERY CO-PRODUCTS

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ABSTRACT: Whiskey distillery co-products are a high strength organic waste, typically consisting of pot ale (liquid waste) and draff (solid waste). Treatment and disposal of these waste streams is important from an environmental and economic point of view. Anaerobic digestion of pot ale and spent lees mixture (with 5:1 the ratio) has been performed after application of alkaline and beating pretreatments in order to improve the digestibility of the substrate. Using a Response Surface Methodology (RSM) approach, different digestion temperatures (32-35-38°C), beating times (0-7.5-15 minutes) and sludge percentages (10-30-50 %) were assessed in batch mode, focusing on maximizing the yield of biogas. Alkaline pretreatment was applied first by using 1M NaOH solution up to pH 10, based on significant results have been obtained from the preliminary experiments. The optimum beating time was determined as 7.5 minutes, correspondingly the maximum biogas yields was achieved by 7.5 minutes beaten samples under the 50 % sludge and 38 °C digestion temperatures with 5966.87 mL biogas per gram total solids.
Keywords: Anaerobic digestion, lignocellulosic sources, pretreatment, wastewater treatment

1 INTRODUCTION

The popularity of craft Whiskey and the number of small scale and craft distilleries have seen a significant rise worldwide, with a 3-fold increase in production facilities in the US between 2010-2016, and increase from 4-19 operating distilleries in Ireland between 2013-2016 [1]. The whiskey distillation process is quite polluting due to the characteristics of the waste streams as well as the amount of waste generated – typically 8-15 L of pot ale per litre of malt whiskey generated, where pot ale is a highly turbid, concentrated liquid waste from the distillation process [2]. Moreover, approximately 3.4 million tonnes of solid waste (spent barley and yeast) originate from the mashing and fermenting steps respectively [3,5]. The characteristics of distillery waste streams require treatment prior to discharge to the environment. Pot Ale contains 30,000 – 50,000 mg/l chemical oxygen demand (COD) and 25,000 – 35,000 mg/l biological oxygen demand (BOD) [6,7]. In addition to high COD and BOD levels of pot ale, it also has low pH (around 4), and a rich content of lignins, hemicelluloses, phosphate, sulphate, nitrite, nitrate, ammonia as well as heavy metals such as copper, iron and magnesium [2,8,10]. Due to the high organic content and acidic nature, pot ale is one of the major concerns of whiskey distilleries, whereas solid waste can potentially be used as animal nutrition after processing [5]. Uncontrolled land disposal of pot ale causes a sharp drop in soil alkalinity and it potentially impairs seed germination, while release to water bodies would likely result in eutrophication and fish kill due to its turbidity and high COD, being directly linked to the level of dissolved oxygen in the water [11,12]. In addition, European Council Directive 999/31/EC requires reduction in landfilling of organic waste [13].

As such, a biological process such as Anaerobic Digestion (AD) is attractive for the treatment of distillery waste, with advantages over other biological wastewater treatment processes such as less sludge production, low energy consumption, destruction of pathogens, odour limitation and higher ability to cope with high strength distillery and brewery wastes [14,15].

AD is not only an environmentally friendly waste treatment process, it also provides a renewable energy source in the form of biomethane. The first anaerobic digestion plant in Europe was established in England in 1895 [16] with much recent research focusing on improvements in the production of biomethane by using different feedstocks such as municipal organic waste [17], brewery wastes [18] wheat straw and sugarcane bagasse [19], maple, oak and corn leaves and stalks [20], maize straw [21], lignocellulosic biomass [22], corn stover [23], palm waste [24] and algal biomass [25].

The AD process is typically described as consisting of multiple parallel reactions carried out by different types of bacteria in four stages, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis. The early stages of AD, hydrolysis and acidogenesis, are generally considered as rate-limiting steps since both mass transfer and feed availability have a critical effect the reaction kinetics [26,27]. Pretreatments are generally required in order to reduce this rate limiting effect, especially when lignocellulosic material with low degradability is used as feedstock. Many different types of pretreatment methods have been recently applied to lignocellulosic material in order to break down its three dimensional crystal structure to render cellulose accessible to bacterial attack [13,28].

Chemical pretreatment is a widely accepted method in AD of various substrates, and would seem to be eminently suitable here due to the naturally acidic nature of whiskey distillery wastes [3,13], with NaOH suggested in the literature as being effective for sludge as an alkaline pretreatment [3]. Alkaline pretreatments are generally applied at ambient temperature, providing a reduction in the degree of polymerisation crystallinity of cellulose and partial hydrolysis and solubilisation of hemicellulose and lignin [15, 29–31].

Mechanical pretreatment, by means of disintegrating and/or grinding, increases the specific surface area of the substrate, making the feedstock more accessible for the microbes, and resulting in the release of cell compounds. AD of substrates with a smaller particle radius has been shown to result in higher COD degradation as well as a higher methane production rate [32]. Moreover, mechanical pretreatment offers a better final residue dewaterability, no odour generation and an easy implementation [33].

In this study beating and alkaline pretreatment are introduced as a hybrid chemical/mechanical pretreatment method. In addition, the impact of beating time is assessed by observing the relation between the time of beating and VFAs in sample. With beating, an increased surface area provides accelerated hydrolysis and acidogenesis steps, and consequently the risk of overloading and sharp pH drops due to accumulation of VFAs up to the inhibitory levels is possible [27,34,35]. The most common types of VFAs in AD are acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid and valeric acid [35,36]. Although smaller particle size allow microbes to reach a greater specific surface area and increase the yield of AD, excessive reduction in particle size causes VFA accumulation during AD and decrease in both methane production and solubilisation. The effects of particle size on AD performance has been studied in detail by [27]. Therefore in order to achieve a balanced microbial growth for each stage of AD, optimised reduction of the particle size of the feedstock must be identified to enhance the methane yield. In order to prevent hydrolysis/acetogenesis steps inhibition concentration of VFAs should be kept in the range of 6.7–9.0 mM [37]; initial VFA concentration minimisation is, as such, desirable. To the best of the authors' knowledge combination of alkaline and beating pretreatment prior to anaerobic digestion has not been addressed in the literature for any substrate. This work investigates the enhancements achieved by the combination of alkaline and beating pretreatments prior to anaerobic digestion of pot ale with co-digestion technique in batch mode.

2 MATERIALS AND METHODS

Distillery co-products (pot ale and spent lees) and digested sludge were supplied by a small scale distillery and a large scale wastewater treatment plant in Dublin, Ireland. The ratio of pot ale to spent lees in the distillery co-product samples was approximately 5:1 by volume. All samples were characterized in terms of total suspended solids (TSS), volatile solids content (VSC), moisture content, sulphate, phosphate, nitrite, nitrate, chemical oxygen demand (COD), biological oxygen demand (BOD) and volatile fatty acids (VFA) prior to and after AD. TSS, VSC and moisture content analysis was performed according to the standard methods. Dissolved Cu was analysed by atomic absorption spectroscopy. VFAs were analysed using an Agilent 7890 gas chromatograph. The concentration of sulphate, phosphate, nitrite, nitrate and COD were analysed by Hach DR 2000 spectrophotometer, whereas BOD was measured using a BD 600 Tintometer. The total solids content of each sample was calculated from the moisture content of each sample (TS = 1 – Moisture content) by drying at 105 °C until a constant sample weight was achieved.

In order to minimise the risk of sludge masking on organic content removals, the supernatant of all samples were analysed as a mixture after centrifuging at 10 000 rpm for 12 minutes using a Sorvall RC 5B Plus centrifuge. The chemical composition is given in Table I. Application of the pretreatments and establishment of anaerobic digesters were completed within the collection day. Excess amount of materials were stored at -18°C for further use.

Table I. Chemical composition of the pot ale and spent lees, as received, before AD

Compound	Value
TSS (g/g sample)	1.76
VSC (g/g sample)	0.08
COD (mg/L)	51813
BOD (mg/L)	43005
Nitrite(mg/L)	825
Phosphate (mg/L)	1946
Sulphate (mg/L)	1144
Nitrate (mg/L)	452
VFAs (mM)	8.4
Cu (mg/L)	17.4

2.1 Hollander Beater Specifications

The Reina model Hollander beater (Figure 1) consists of a beater tub equipped with a 24 bladed drum with max spin speed of 580 RPM. An adjustable bed plate with sharp channels is placed under the rotating and moveable wheel to decrease particle size, with the gap size between blades and bed-plate adjusted to 76 micrometre, as appropriate to the solid content of the sample.

Motor	1 HP (746 W)
	220 V
	6.9 A
	1 Phase
	1450 RPM
V- Belt drive	2.5: 1 Reduction
Drum Speed	580 rpm
Tub Volume	Maximum Capacity: 90 litres Working capacity: 40 litres
Drum diameter	200 mm
Drum paddles	24 paddles

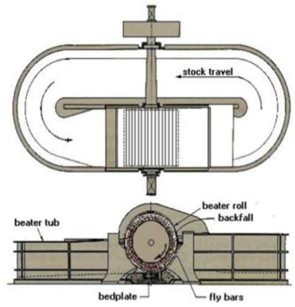

Figure 1. Technical Specification of the Hollander Beater

The bioreactor system constitutes two main parts, 500 ml conical flasks with 400 ml working volume and biogas compatible sampling bags. The connection in between them is provided by plastic tubing, quick release tubing connectors and 3-way valves. To ensure anaerobic conditions, nitrogen was flushed into each reactor twice in order to get rid of residual oxygen from within the flasks and tubing according to [38] A fixed mesophilic temperature was obtained by placing the conical flasks into temperature controlled water baths. A Dräger X-A 3000 model biogas analyser was used for qualitative analysis of biogas as well as verification of anaerobic conditions. AD experimental set up and methodologies match with reported standard VDI 4630 [38].

2.3 Experimental Methodologies

2.3.1 Assessment of Pre-treatments

For Alkaline pre-treatment, 0.1 M NaOH was added dropwise to the pot ale/spent lees sample until pH 10 was reached, and the pH 10 adjusted sample was stirred for 6 min. The Hollander beater was fed with 20 L of sample to ensure a complete rotation. Beating for 0, 5 and 7.5 minutes prior to, after and without pH 10 NaOH treatment of sample was applied. 400 ml of sludge and sample in the required ratio (10 – 50 % sludge) were placed into 500 ml conical flasks and AD experiments were performed under mesophilic conditions (35 ± 3 ˚C, as specified). All experiments were performed in triplicate or duplicate as indicated for each parameter. All flasks were shaken once per day in order to provide a better interaction between the bacteria and the biomass as well as to allow for degasification of the flasks. Once the biogas production rate reduced less than 1% of the most recent measured rate based on the cumulative biogas generation, samples were disconnected for chemical characterisation.

2.3.2 Beating Time and VFAs

The pot ale and spent lees sample was beaten for 30 min with total VFAs measured every 2.5 mins.

2.4 Design of Experiments (DOE)

RSM is a combination of mathematical and statistical techniques, which is used for modelling, interpreting, predicting and optimising a process of several input variables. The parameters for testing were examined as three factors at three levels according to Box Behnken design, which is one of RSM designs developed for optimisation. The recorded responses were analysed via Design expert software V7.

Independent input factor levels and variables are decided as 0, 7.5, 15 min for the beating time (BT); 32, 35 and 38 °C for the temperature (T) and 10, 30, 50 % for the percentage of sludge (%S). Level 0 of factor BT refers to untreated sample. Second order polynomials were matched to the experimental data in order to obtain in the regression equations. Sequentially, F-test, lack of fit test and other adequacy measures were then used in selecting the best models. Stepwise regression method was applied onto the experimental data and it was fitted to the second order polynomial Eq (1) in order to identify the relevant mathematical model terms. Response plots were generated using the same statistical software [39–41].

$$Y= b_0 + \sum b_i X_i + \sum b_{ii} X^2_{ii} + \sum b_{ij} X_i X_j \qquad (1)$$

Both the value of the each term in the model and the probability > F (also called as p-value) of the model can be computed by analysis of variance (ANOVA). In case of obtaining a Prob. > F of the model and of each term in the model less than the level of significance (i.e. α=0.05), the model is then considered to be adequate within the interval of confidence (1-α). An adequate model suggests that the reduced model has successfully passed all the required statistical tests and can be used to predict process responses and optimisation [40]. Subsequently, results are used for optimising the process in order to maximise the biogas yield with the best factor levels under specific user defined criteria by using the numerical and graphical methods which are provided by the software.

3 RESULTS AND DISCUSSION

3.1 Biogas Production

Biogas yields from preliminary experiments are given in Table II. It was found that mechanical pre-treatment alone did not significantly improve biogas generation, while alkaline pre-treatment in isolation had a significant effect. The combination of alkaline pre-treatment followed by mechanical pre-treatment showed an improvement in the amount of biogas generated, in comparison with alkaline pre-treatment alone. Gas generation with combined alkaline/mechanical pretreatment was improved almost 3-fold over the untreated samples, with gas generation of >7 L after three weeks digestion. In order to be able to see a statistically significant effect in terms of beating time, using draff, which is the solid waste of whiskey distillery, as an additional compound is considered.

Table II. Preliminary experiment results of biogas yields of control and pretreated samples.

Sample	Produced Biogas ± STD [mL/gTS]
Untreated Sample	1545 ± 331
Alkaline	3111 ± 146
5 Min Beaten	1417 ± 11
7.5 Min Beaten	1312 ± 347
5 Min Beaten + Alkaline	2640 ± 86
Alkaline + 5 Min Beaten	3459 ± 75
7.5 Min Beaten + Alkaline	2877 ± 298
Alkaline + 7.5 Min Beaten	4091 ± 119

STD: Standard Deviation Values

The highest cumulative biogas production over the 39 days of AD was achieved with alkaline pre-treatment following by beating for 7.5 mins (285% higher than the control). A p-value of 0.00828 (<0.05) was found upon application of a one-way ANOVA, indicating that the order in which the pre-treatments were performed had a significant effect on total biogas generation. As such, alkaline pretreatment followed by beating pretreatment was selected as the baseline hybrid pre-treatment for further experiments.

The effect of beating time has been evaluated with the level of total VFAs in pot ale and spent lees sample as excessive small particle size of feedstock might lead to inhibition within the reactor. Figure 2 gives the trend of VFAs with the increased beating time. Based on the preliminary results, the beating range was decided as 0 to 15 minutes for further experiments.

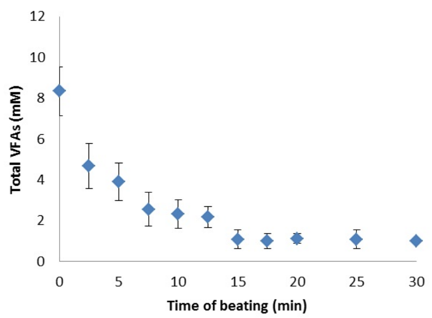

Figure 2. VFA levels according to increasing beating time.

The results of the DOE are presented in Table III in a standard order according to the Box-Behnken design matrix. A variety was seen in methane content of the biogas. The maximum biogas quality and quantity were achieved in the case of samples 11 and 12. Corresponding methane percentage of these samples were 54.7, 56.7 in respectively, indicating that 7.5 minutes beating time has the greatest effect on biogas quality.

Table III. Design matrix and measured biogas yield

Design matrix *				Response 1	Response 2
Exp No.	Beating Time (Min)	Temp (°C)	Sludge Percentage (S%)	BioGas Volume mL/gTS	Methane Percentage CH_4 %
1	0	32	30	611 ± 51	8.1
2	15	32	30	473 ± 5	9.4
3	0	38	30	676 ± 58	7.5
4	15	38	30	682 ± 35	9.6
5	0	35	10	230 ± 29	3.6
6	15	35	10	458 ± 162	4.2
7	0	35	50	5193 ± 20	33
8	15	35	50	4884 ± 116	43.7
9	7.5	32	10	1111 ± 83	5.1
10	7.5	38	10	917 ± 7.3	2.4
11	7.5	32	50	5496 ± 196	54.7
12	7.5	38	50	5966 ± 10	56.7
13	7.5	35	30	1015 ± 27.2	7.5
14	7.5	35	30	1102 ± 25	5.2
15	7.5	35	30	1098 ± 32.8	6.9
16	7.5	35	30	1116 ± 15.3	6.1
17	7.5	35	30	1081± 43	5.8

3.2 Organic Content Removal

The percentage reduction in COD, BOD, N-Nitrate, N-Nitrite and Sulphate is given in Table IV.

Table IV. Percentage removal of organic compounds in pot ale sludge mixture after AD

Sample No	COD	BOD	NO_3-N	NO_2-N	SO_4
10 % Sludge					
5	31.9	50.0	75.1	62.3	56.3
6	48.0	43.4	33.5	95.7	59.5
9	41.8	46.8	15.0	87.7	65.3
10	15.8	21.8	56.3	49.4	68.1
30 % Sludge					
1	43.1	43.5	91.8	68.5	79.4
2	44.6	48.7	53.3	97.2	79.4
3	39.2	36.8	72.9	56.2	75.6
4	23.0	41.0	54.0	65.1	81.3
13-17	28.3	59.7	9.1	90.5	70.5
50% Sludge					
7	53.9	55.4	42.1	17.7	28.8
8	78.4	60.8	43.5	41.9	25.4
11	63.7	48.6	22.5	96.6	23.6
12	59.2	64.7	44.6	90.6	35.6

It has been reported that copper ions have an impact on organic content removal [42], in particular COD, as it results in microbial inhibition. More efficient COD and BOD degradation has been obtained from the samples with high sludge percentage, due to the higher initial concentration of microorganisms in the seeding sludge and also potentially due to lower Cu amounts with higher sludge content. Although all reactions were started at pH7, the final pH values (after 29 days retention time) were measured as 5.6 and 5.3 for the reactors which contain 10 and 30% sludge. This pH drop potentially arises from the VFAs accumulation in acidogenesis stage and may impede methanogenic activity. Therefore, higher SO_4 removal was obtained with the samples with 10 and 30% of sludge, however, SO_4 was converted into H_2S gas (> 10000 ppm) by sulphate reducing bacteria as a by-product of the process [32,43,44].

3.3 Model Estimation

The developed quadratic model is significant in terms of biogas volume response according to the fit summary output. R^2, adjusted R^2 and predicted R^2 were computed as 0.991, 0.988 and 0.987 respectively, for the reduced quadric model. It is seen that R^2 and adjusted R^2 are very close to 1. This indicates that an adequate model has been developed.

It was found by ANOVA that the percentage of sludge (S%) and the second order effect of percentage of sludge are the most significant parameters on the biogas yield. The beating time was insignificant for this model this is attributed to the naturally low solid content of the sample. The final mathematical model was built in terms of actual factors (given in eqs 2) associated to the corresponding response by the software.

$$\text{Biogas volume} = 1141.063 + 38.75{*}BT - 120.588S\% - 201778BT^2 + 3.099375S\%^2 \quad (2)$$

Obtained surface response is given in Figure 3. Mainly a linear interaction was obtained as temperature does not affect the experiments with high sludge percentages significantly. However, this relationship acts in a way that the effect of temperature has a peak when the sludge percentage is 20 and the beating time is 7.5 minutes.

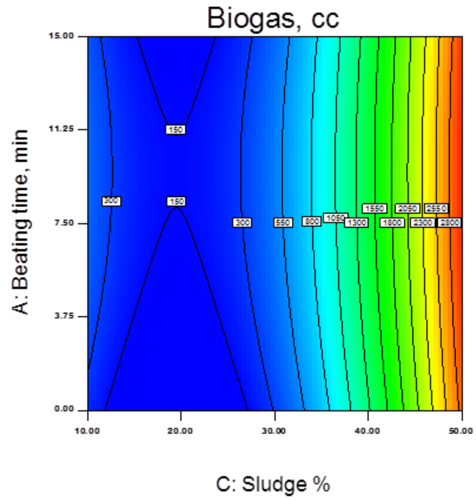

Figure 3. Combined effects of the parameters onto biogas yield

Also, three different parameters have been coded within the -1 to 1 range. 0 represents the centre point conditions which are 7.5 minutes beating, 35°C incubation temperature and 30% sludge. Similarly, -1 and 1 represents 0 minutes beating, 32°C incubation temperature, 10% sludge and 15 minutes beating, 38°C incubation temperature, 50% sludge. The perturbation plot is given in terms of coded units to be able to see the effect of each factor in Figure 4 where A is beating time, C is percentage of sludge. Perturbation plot is useful plot that shows the individual effect of each factor on the response of interest. When the software plots the effect of certain factor, by default the software will set the values of the other factors in their centre points.

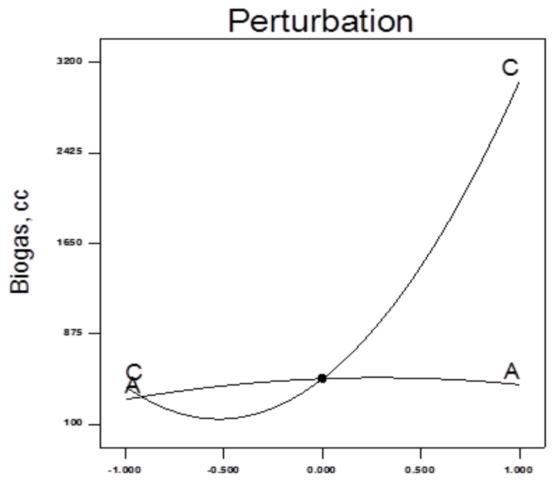

Figure 4. Deviation from the reference point in terms of coded units

According to Figure 4 it can be easily seen that the biogas yield is mainly affected by the sludge percentage (Factor C) and the beating time (Factor A). Factor B (temperature), is insignificant to response variation and thus does not exist in such figure, whereas increasing S% improves the response. Although the bacterial growth rate and the stability of an AD process are dependent on temperature, in this case ±3 °C variations do not significantly alter the response.

4 CONCLUSIONS

The biogas yields obtained from anaerobic digestion of whiskey distillery liquid waste streams (pot ale and spent lees) in batch lab-scale reactor are examined. A hybrid alkaline/beating pretreatment has been introduced. Chemical compounds were analyzed before and after anaerobic digestion to evaluate the efficiency of the treatment. COD and BOD removals up to 78.4 and 60.8 % respectively were achieved. The maximum biogas yield of 5966.87 mL (56.9 % CH_4 biogas per gram total solids) over 29 days was achieved for samples subjected to alkaline conditions and subsequently beaten for 7.5 minutes; co-digested in a 1:1 ratio with sludge at 38 °C digestion temperature.

Although the beating pretreatment has no significant effect on the biogas volume (according to the mathematical model developed), it increases the biogas quality by up to 10.7% (see Exp. No. 7 and 8 in Table III). On the other hand a low biogas generation and a low biomethane quality was obtained from the samples which contain low sludge percentage (10 and 30%) regardless of the beating time. These results are attributed to the lack of agitation and pH control within the reactors as well as the small temperature range chosen to perform the experiment, which do not strongly affect the methane content. In order to be able to see a statistically significant effect in terms of beating time, using draff, which is the solid waste of whiskey distillery, as an additional compound is considered for the further experiments.

6. ACKNOWLEDGEMENTS

This research is funded by Alltech Ireland LTD.

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