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Modelling and optimisation of the biogas yield after hybrid alkaline-ultrasonic pre-treatment in the early stages of anaerobic digestion of pot ale to shorten the processing time

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ABSTRACT

Whiskey distillery wastewater (pot ale) is classified as a high organic content wastewater and its year-long large discharge volume makes it a suitable substrate for anaerobic digestion from environmental and economical perspectives. Prior to anaerobic digestion, a hybrid alkaline-ultrasonic pre-treatment was performed in order to alter the lignocellulosic structure of the pot ale. Effects of alkaline dose (0–3 M NaOH), amplitude ratio (40–100%) and exposure time (1–3 h) of ultrasonic pre-treatment on CH_4, CO_2 and H_2S generation within the first 2 days of anaerobic digestion were investigated at a lab scale batch reactor. Response surface methodology (RSM) was adopted as a process modelling and optimisation tool. Significant enhancements in the hydrolysis rate constant and methane yield were achieved in the early stages of digestion. The highest methane yield of the first 2 days digestion was 333 ± 5 mL/g VS after implementation of ultrasonic pre-treatment at 70 % amplitude for 3 h in isolation leading to 48 ± 4.4 and 56 ± 3.5 % reduction in chemical and biological oxygen demand respectively. The optimum pre-treatment conditions according to combined numerical and graphical optimisation, to maximise CH_4 yield while minimising the H_2S generation was identified as 40 % amplitude ratio, 1–2.5 h exposure time and 0 – 0.6 M NaOH.

1. Introduction

Biomethane generation through anaerobic digestion (AD) of lignocellulosic biomass has attracted global attention in the last decade as it not only addresses the climate change crisis but also valorises organic waste in a sustainable way (Xu et al., 2020). The whiskey manufacturing industry discharges 8–15 L of organic and lignocellulosic wastewater, called pot ale, per litre of malt whiskey produced, and this high strength and acidic wastewater must be treated either on-site or in a municipal wastewater treatment plant prior to discharge to the environment. Due to its high fibre content pot ale is traditionally used as an ingredient for animal feed. However the copper content of pot ale is a big limitation on the widespread adoption of feed production as it poisonous for sheep (Graham et al., 2012). Therefore it is most commonly used to produce pot ale syrup by recycling the waste heat of the manufacturing to evaporate the excess water content of pot ale before sending it to the landfill. The landfill applications however can potentially impair seed germination, reduce the soil pH and eventually can lead to inhibition of agricultural crops. Therefore, sustainable management of pot ale is one of the major environmental concerns of countries like Ireland and the UK due to its highly polluting strength explained in detail by (Gunes et al., 2019). The high organic content of pot ale and the shortcomings of the current waste disposal method renders it a suitable substrate for AD as the European Union targets a 80–95% reduction by 2050 in the total greenhouse gas emission of 1990 levels (Bryngelsson et al., 2016; European Commission, 2018) while European Council Directive 999/31/EC aims to minimise the landfilling of organic waste (Cesaro and Belgiorno, 2014). On the other hand, the resistance of lignocellulosic material to biodegradation due to the interlinks between the cellulose, hemicellulose layers and the surrounding lignin as well as presence of crystalline structure of cellulose (Taherzadeh and Karimi, 2008), long digestion time and generation of highly corrosive by-product (H_2S) in large volumes are the major challenges of commercialisation of the AD technology. The long digestion time is

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commonly associated with the hydrolysis step which is the first and rate limiting step of the process (Massé and Saady, 2015; Zhen et al., 2017). Increasing hydrolysis kinetics by introducing pre-treatment steps prior to digestion can enhance digester performance which shortens the digestion time and increases process intensification thus resulting in decreased retention time (Carrère et al., 2010). Furthermore, the structural alterations of the substrate prior to AD results in a greater process stability in terms of shifting the bacterial competition between the methanogenesis and the sulphidogenesis steps in favour of methanogens which eventually lowers highly toxic and corrosive H_2S generation. Therefore a pre-treatment step to modify the lignocellulosic structure prior to AD is often required to enhance methane production for 2nd generation biofuel generation (Moraes et al., 2015).

Chemical and physical pre-treatment methods are well accepted and commonly applied on varying substrates prior to AD. Alkaline pre-treatment is considered to be a more suitable chemical pre-treatment for pot ale in order to balance its acidic nature (Cesaro and Belgiorno, 2014; Mohana et al., 2009) in addition to its capability of releasing cellular substances though swelling particles as well as providing hemicellulose solubilisation (Gunes et al., 2019; Carrère et al., 2010; Li et al., 2012; Hendriks and Zeeman, 2009). Ultrasonic pre-treatment as a well-studied, environmentally friendly physical pre-treatment method achieves disintegration of structural integrity though cavitation mechanism (Pilli et al., 2011). The acoustic cavitation phenomenon results in excessively high local temperature and pressure which leads to interparticle collision and cell wall lysis (Ma et al., 2012). Combining ultrasonication with alkaline pre-treatment can bring the advantages of both methods to achieve a higher biogas quality and quantity in a shorter reaction time (Ma et al., 2012).

In this research alkaline-ultrasonic hybrid pre-treatment of pot ale was introduced to the literature for the first time. In addition, novelty of this research includes, (i) investigation of minimising long digestion times to improve applicability of AD technology at full scale, (ii) evaluation of the link between alkaline-ultrasonic hybrid pre-treatment and the lignocellulosic composition of pot ale, (iii) evaluation of pot ale digestate for land applications as biofertiliser. To the best of author's knowledge these topics are not addressed in the literature before. Enhancements in the biodegradability of pot ale arising from the applied pre-treatment was assessed at lab scale in a batch reactor and the efficiency of the reactions were determined by organic matter removal in terms of chemical oxygen demand (COD), biological oxygen demand (BOD) and sulphate. As such reductions in COD and BOD levels are the indicator of organic matter degradation. On the other hand reduction in sulphate content shows the process instability in terms of the bacterial competition between the methanogenesis and sulphidogenesis steps. Mathematical models were developed to assess the impact of the pre-treatment conditions on the first 2 days of anaerobic digestion yield to minimise the retention time as well as to elucidate interactions between the pre-treatment conditions. Following the validation of the models, they were used for process optimisation by using combined numerical and graphical optimisation to identify the optimum alkaline-ultrasonic hybrid pre-treatment conditions (Pandian et al., 2011).

2. Materials and methods

2.1. Feedstock and inoculum

The feedstock, pot ale, was collected from a small-scale Whiskey Distillery in Dublin, Ireland, while the inoculum (seed sludge) was supplied by a mesophilic anaerobic digestion plant processing industrial food waste in Ireland.

2.2. Analytical methods

Pot ale was initially characterised in terms of its solid content; total solids (TS), volatile solids (VS), moisture (MC), organic content; chemical oxygen demand (COD), biological oxygen demand (BOD), volatile fatty acids (VFAs) namely acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid and valeric acid, lignocellulosic content; hemicellulose, cellulose and lignin fractions as well as inorganic content; phosphorous ($P\text{-}PO_4^{3-}$), nitrogen in forms of nitrate ($N\text{-}NO_3$-), nitrite ($N\text{-}NO_2$-) and ammonia ($N\text{-}NH_3$), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn) and zinc (Zn). The efficiency of AD was assessed by measuring COD and BOD levels in the supernatant of the pot ale sludge mixture after centrifuging at 10 000 rpm for 30 min prior to and after AD. In addition, sulphate concentration in the supernatant was monitored before and after the reactions to evaluate potential bacterial competition between the methanogenic and sulphate reducing bacteria. The influence of ultrasonic pre-treatment on biomass (pot ale) was associated with the degree of substrate disintegration which was assessed by taking 2 mL sample at 15 min intervals to monitor the changes in chemical properties by means of increase in soluble COD level (Pilli et al., 2011) over the treatment time according to Eq. 1.

$$\mathbf{DD_{COD}},\% = \left[\frac{\mathbf{COD_{us}} - \mathbf{COD_0}}{\mathbf{COD_{NaOH}} - \mathbf{COD_0}}\right] \times 100 \tag{1}$$

Where; COD_{us} [mg/L] is the COD in the supernatant of the ultrasonicated sample, COD_0 [mg/L] is the COD in the supernatant of the untreated sample, and COD_{NaOH} [mg/L] is the maximum COD release in the supernatant after NaOH disintegration by treating the sample with 1 M NaOH, at a ratio of 1:2 for 10 min at 90 °C (Pilli et al., 2011).

The energy applied during the disintegration process is expressed by Eq. 2.

$$E_s = \frac{P \times t}{V \times VS} \tag{2}$$

Where; E_s [kJ/kg VS] is specific energy input, P [kW] is power input, t [s] is sonication time, V [L] is volume of the feedstock and VS [kg/L] is the volatile solids concentration.

2.3. Pre-treatment and anaerobic biodegradability

The effects of ultrasonic pre-treatment prior to anaerobic digestion of pot ale, which was performed using a Hielscher Ultrasonics UP 400S (maximum power of 400 W, 24 kHz) with a H22L2D horn, was assessed in isolation as well as in combination with alkaline pre-treatment at different concentrations of NaOH (1.5 and 3 M). The ultrasonic pre-treatment was applied to pot ale in 400 mL batches and the temperature was controlled at room temperature by employing a water bath during the pre-treatment, because increased operation temperature reduces the intensity of the ultrasonication (Sharma et al., 2013). The amplitude ratio (40, 70 and 100 %) and exposure time (1, 2 and 3 h) were the investigated parameters of ultrasonic pre-treatment with the indicated levels. Alkaline pre-treatment was performed by dropwise addition of 1.5 and 3 M NaOH solution into 400 mL pot ale until pH 10 was reached, it was then stirred for 6 min at 600 rpm. The experimental design is outlined in Table 1.

Following the pre-treatments, anaerobic digestion potential was evaluated at lab scale in a batch reactor with an inoculum:substrate ratio of 5:1 on a dry weight basis. The total AD time was 21 days. The quality and the quantity of the produced biogas was analysed using BIOGASS 5000 gas analyser provided by CSL Ltd. The schematic diagram of the experimental set up is presented in Fig. 1.

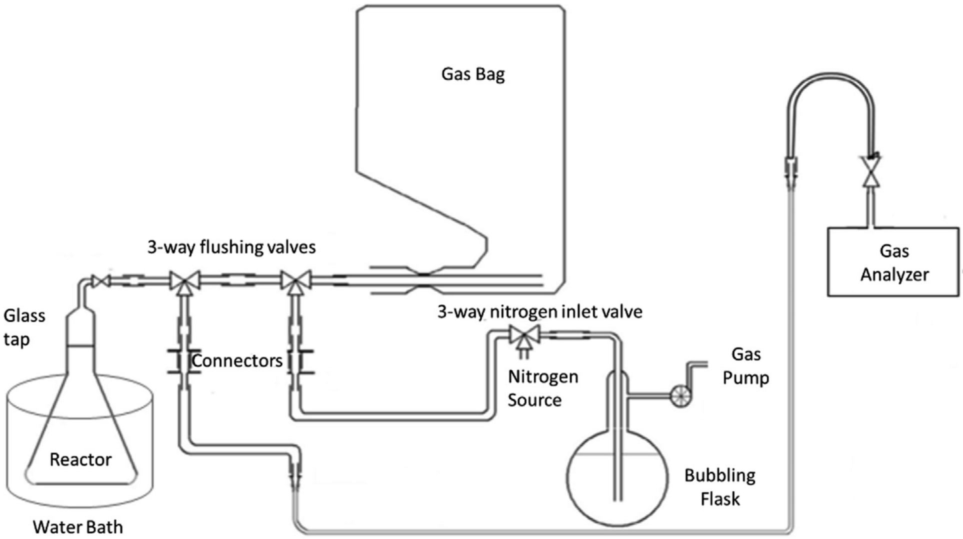

Fig. 1. Bioreactor set-up.

Table 1
Summary of the design factors and the responses.

Design Factors	Levels	Response of Interest
	40	Methane Yield in 2 days (ml/g VS)
Amplitude Ratio (%)	70	CO_2 Yield in 2 days (ml/g VS)
	100	
	1	
Exposure Time (h)	2	
	3	H_2S generation in 2 days (ppm)
	0	
NaOH Dose (M)	1.5	
	3	

The effects of design factors on the CH_4 and CO_2 yields in addition to H_2S generation within the first 2 days of AD were investigated at three levels according to Box Behnken Design within the RSM. In addition, hydrolysis rate constants for different pretreatment conditions were calculated according to the first order kinetic model given in Eq. 3 (Xu et al., 2020; Vazifehkhoran et al., 2018). A two tailed *t*-test was used for statistical analysis of the hydrolysis constant.

$$B_t = B_0\,[\,1 - e^{(-k_h t)}] \tag{3}$$

Where; B_t [ml CH_4/g VS] is the CH_4 yield at time t, B_0 [ml CH_4/g VS] is the ultimate CH_4 yield, k_h [d^{-1}] is the first-order hydrolysis constant, and t is the time of digestion (days).

2.4. Box-Behnken Design (BBD)

BBD offered by Design Expert Software Version 11 combines statistical mathematical techniques for accurate process modelling as well as data interpretation and prediction. Moreover, it is an efficient tool for assessment of multiple parameter influences on multiple responses of interest with a minimum number of experiments. The impact of the 3 design factors (A: Amplitude Ratio, B: Exposure Time, C: NaOH Dose) at 3 levels on the biogas quality and quantity produced in the first 2 days of AD was identified by performing experiments under 13 different conditions from which 5 are the centre points. A quadratic model given in Eq. 4 achieved the best fit to the experimental data according to the stepwise regression analysis conducted.

$$y = b_0 + \sum b_i X_i + \sum b_{ii} X^2{}_{ii} + \sum b_{ij} X_i X_j \tag{4}$$

The adequacy of the developed models was then tested by the regression analysis which applied to the actual data and residuals (the differences between the actual and the predicted data) to compute R^2, Adjusted R^2 and Adequacy Precision ratio as well as the analysis of variance (ANOVA). The models and all terms in the model were considered to be significant only if the corresponding p value was smaller than the significance level ($\alpha = 0.05$ and 0.01) indicating sufficient adequacy within the confidence interval ($1-\alpha$) (Benyounis et al., 2005, Eltawahni et al., 2011, Tedesco et al., 2013). In case of having the quadratic effect of the design factors as significant model terms, their first order was included to the estimated models in order to support the model hierarchy as suggested by the software (Guo et al., 2016; Gunes et al., 2020; Ockuly et al., 2017). In addition, models were validated by performing independent experiments under varying conditions which were not introduced to the model previously. The results of the validation experiments and the predicted data was statistically analysed by applying a paired *t*-test. After passing all of the significance tests, the developed quadratic models in terms of actual and coded factors were used for data interpretation, prediction and process optimisation. For the optimisation study, a combination of numer-

Table 2
Characteristics of pot ale as received.

Compound	Value
TS (g/g sample)	0.089 ± 0.0004
VS (g/g sample)	0.077 ± 0.008
Moisture %	91.13 ± 0.042
COD (mg/L)	38,867 ± 115
BOD (mg/L)	30,965 ± 666
$SO_4{}^{2-}$ (mg/L)	190 ± 31
*VFAs (mM)	134.89
Hemicellulose (%)	11.5 ± 0.3
Cellulose (%)	10.6 ± 1.8
Lignin (%)	26.9 ± 1.6
P-$PO_4{}^{3-}$ (mg/L)	778 ± 7
N-$NO_3{}^{-}$ (mg/L)	111 ± 20
N-NH_3 (mg/L)	45 ± 7
N-$NO_2{}^{-}$ (mg/L)	33 ± 4
Cu (mg/L)	53.04 ± 3.21
Fe (mg/L)	2.55 ± 0.21
Mg (mg/L)	107.28 ± 0.44
Mn (mg/L)	0.30 ± 0.01
Zn (mg/L)	0.49 ± 0.01

ical and graphical optimisation tools were utilised which relies on the desirability function adopted (Pandian et al., 2011).

3. Results and discussion

3.1. Feedstock and inoculum characteristics

A detailed characteristics of pot ale is given in Table 2. Seed sludge used as inoculum had 5.6 % (v/v) dry matter in addition to a FOS/TAC ratio of 0.25 ± 0.05 (Winde et al., 2018).

3.2. Biogas yield and organic degradation

The BBD matrix for modelling the effects of alkaline and ultrasonic pre-treatment on biogas yield is given in Table 3 along with the cumulative CH_4 and H_2S production results while the first 2 days CH_4, CO_2 yields and H_2S production is presented in Fig. 2. The cumulative CH_4 yield was observed to be within 605 ± 70–894 ± 55 mL/g VS for all design points, which was significantly higher than the control.

Significantly enhanced CH_4 yields with a maximum of 333 ± 5 mL/g VS (std 10 in Fig. 2) were observed with the standard (std) no 5 – 17 within the first 2 days of AD in comparison to control. Although the cumulative CH_4 yield of the std no 1 – 4 was not significantly different than the other design points, the first 2 days yield was found to be significantly lower, indicating a lag phase in the microbial growth. For instance the hydrolysis constant of std 1 within the first 2 days and 2nd – 6th days of AD was found to be 0.0312 ± 0.002 and 0.1483 ± 0.005 day^{-1} respectively. Only the std no 1 – 4 had significantly ($p < 0.05$) higher hydrolysis rate constants in the 2nd – 6th days of AD than the first 2 days, indicating that implementation of the ultrasonic pre-treatment at an amplitude ratio of 40 and 100 % following the 1.5 M NaOH pre-treatment triggered formation of inhibitory compounds in the reactor which resulted in a lag phage in the reactor. This was explained by the formation of potential inhibitory compounds commonly associated with the pre-treatments of lignocellulosic biomass such as hydroxymethylfurfural (HMF), furfurals and soluble phenolic compounds during the pre-treatment step (Zhen et al., 2017; Hendriks and Zeeman, 2009; Ariunbaatar et al., 2014; Chen et al., 2014). Biogas yield of lignocellulosic biomass (wheat straw) was recently reported to be inhibited by 30 % due the presence of furfural with the concentration of 98.5 ± 7.0 mg/g TS (Wang et al., 2018). A range of 100–120 mg/L HMF concentration was also reported to be inhibitory resulting in a lag phase in the AD process (Horn et al., 2011). Another potential reason for the lag phase might be presence of sulphur in forms of sulphur and sulphate as it directly causes toxicity to methanogenic microbes. Sulphur concentration of 280 mg/L (in form of sulphate) was reported to cause a lag phase in the AD of high strength wastewater (Vazifehkhoran et al., 2018). In addition, presence of high concentration of chloride is known to be toxic to anaerobic bacteria. Cl- concentration of 5 g/L stresses methanogenic activity under mesophilic condition (Sánchez-Olguín and Forster, 2010), at 8 g/L it starts to inhibit CH_4 production (Jørgensen, 2009) and at 10 g/L causes an irreversible inhibition to the AD process (Sánchez-Olguín and Forster, 2010). On the other hand, pre-treatment conditions of the rest the samples prevented the lag phase occurring with regards to faster reaction kinetics within the first 2 days of AD by potentially releasing more readily digestible soluble matter or/and mitigating the lignocellulosic resistance to biodegradation. This results show that the lag phase did not occur due to the inoculum being of inadequate quality or being used without an acclamation time.

The influence of ultrasonic and alkaline pre-treatments on the feedstock was also evaluated in terms of the degree of disintegration based on the specific energy delivered during the ultrasonic pre-treatment as well as the modifications in the lignocellulosic structure of the pot ale. The specific energy delivered during the ultrasonic pre-treatment was calculated according to Eq. 2 and the results are given in the supplementary data (S1). Standards 10 and 12 were selected to monitor the disintegration levels during application of the ultrasonic pre-treatment on non-treated and alkaline pre-treated pot ale at the highest specific energy delivered and the results are given in Fig. 3.

The intracellular compound release was found to be significantly higher for combined chemical and ultrasonic pre-treatment than the ultrasonic pre-treatment in isolation for the specific energy range of 55 000–80 000 kJ/kg VS according to the degree of disintegration percentages. The significant enhancement in the degree of disintegration within this range was associated reduction in

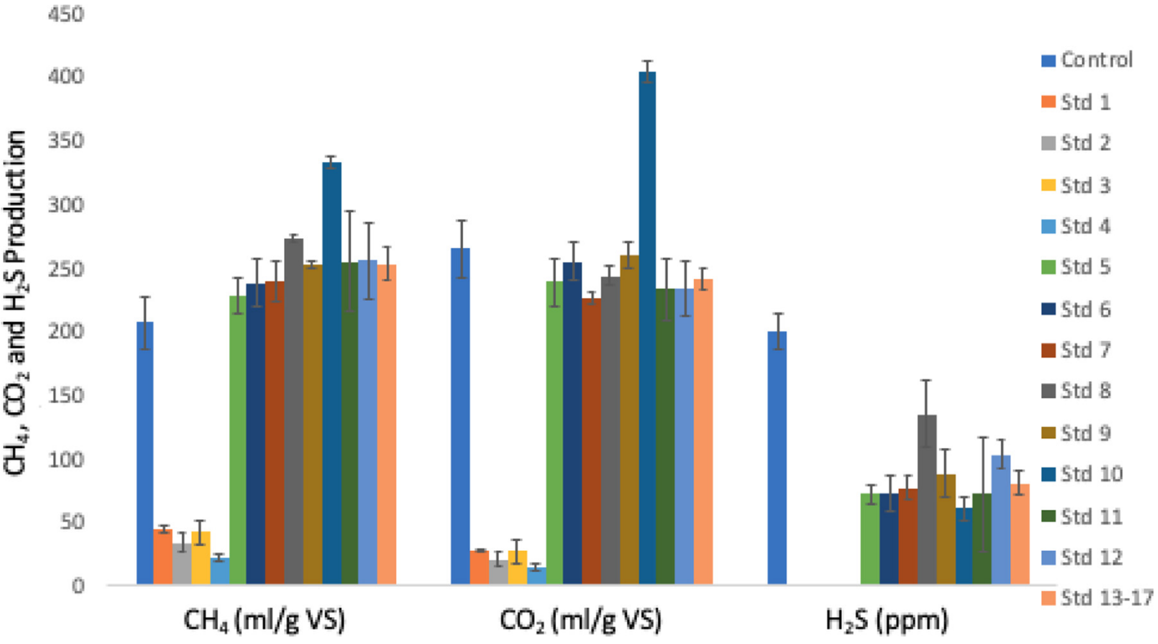

Fig. 2. Bar diagram of CH_4, CO_2 yields and H_2S production in the first 2 days of AD.

Table 3
Design matrix with recorded responses after 21 days of digestion.

	Factor A	Factor B	Factor C		
std	Amplitude Ratio –	Exposure Time h	Alkaline Dose M	Total CH_4 yield ml/g VS	Total H_2S production ppm
Control	N/A	N/A	N/A	625 ± 2	738 ± 161
1	0.4	1	1.5	722 ± 22	189 ± 47
2	1	1	1.5	705 ± 45	228 ± 86
3	0.4	3	1.5	691 ± 78	195 ± 94
4	1	3	1.5	642 ± 12	241 ± 64
5	0.4	2	0	567 ± 60	307 ± 97
6	1	2	0	590 ± 126	289 ± 116
7	0.4	2	3	816 ± 4	268 ± 99
8	1	2	3	810 ± 21	404 ± 132
9	0.7	1	0	639 ± 7	326 ± 63
10	0.7	3	0	876 ± 26	315 ± 68
11	0.7	1	3	630 ± 157	212 ± 160
12	0.7	3	3	605 ± 70	265 ± 33
13	0.7	2	1.5	801 ± 104	282 ± 118
14	0.7	2	1.5	894 ± 55	386 ± 24
15	0.7	2	1.5	627 ± 74	204 ± 34
16	0.7	2	1.5	818 ± 14	134 ± 17
17	0.7	2	1.5	847 ± 94	412 ± 21

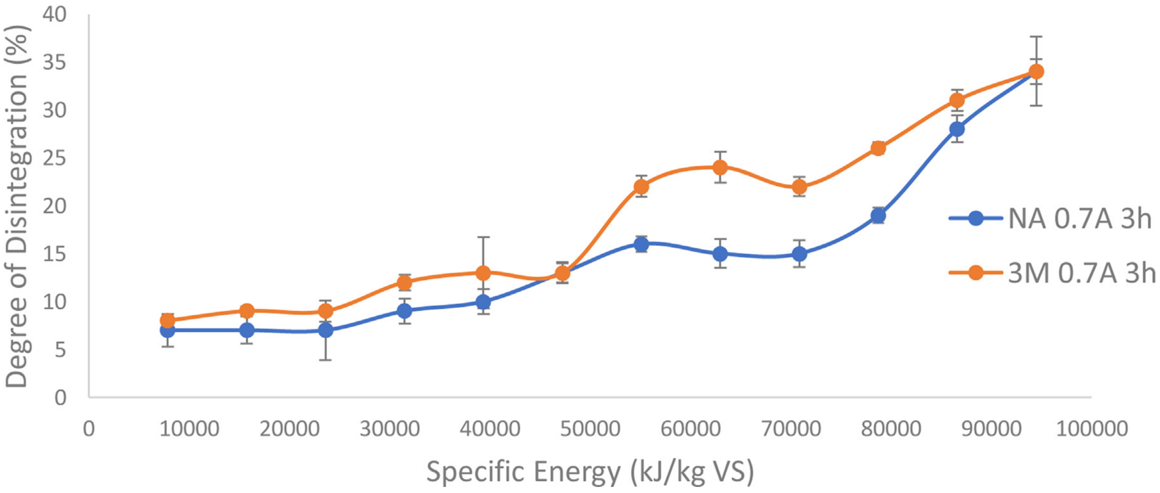

Fig. 3. Degree of disintegration based on specific energy delivered during ultrasonic pre-treatment.

the polymerization degree of the lignocellulosic structure as well as hemicellulose hydrolysis achieved by 3 M NaOH pre-treatment applied prior to ultrasonic pre-treatment which rendered biological flocs more accessible (Hendriks and Zeeman, 2009; Modenbach and Nokes, 2012; Gáspár et al., 2007). No significant difference was observed in the degree of disintegration arising from alkaline pre-treatment prior to ultrasonic pre-treatment outside the range identified. In addition, no further significant increase was seen in the degree of disintegration beyond specific energy level of 94 500 kJ/kg VS indicating de-agglomeration of the biological flocs (Zhang et al., 2015) when the 94 500 kJ/kg VS specific energy was reached.

The lignocellulose fractions of pot ale as received (control) after ultrasonic pre-treatment at 70 % amplitude for 3 h (0.7A 3 h) and 3 M alkaline (3 M) pre-treatment alone as well as in combination (3 M + 0.7A 3 h) is given in Fig. 4.

Although no significant lignin removal was seen arising from the applied pre-treatments, 3 M alkaline pre-treatment alone resulted in a significant reduction in cellulose ($p < 0.05$) and a significant increase in the hemicellulose content ($p < 0.05$) in comparison with the control. Lignin is an heterogeneous, amorphous biopolymer consists of phenyl propane (p-coumaryl, coniferyl and sinapyl alcohol) networks held together by different bonds which makes the lignocellulosic matter water insoluble and resistant to biological degradation (Hendriks and Zeeman, 2009; Chen et al., 2010; Kratky and Jirout, 2011).

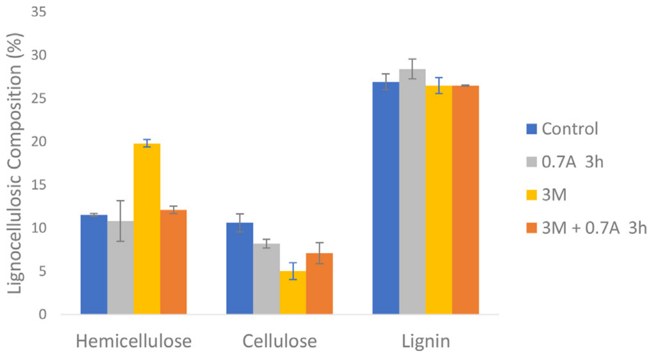

Fig. 4. Lignocellulosic structure of non-treated (NT), US treated (70 % amplitude for 3 h), alkaline treated (3 M) and combined alkaline US treated pot ale (3 M + 0.7 3 h).

In addition, no significant difference was seen in the lignin and cellulose content of pot ale due to the hybrid pre-treatment in comparison to 3 M NaOH pre-treatment stands alone. Although applied pre-treatment does not achieve a significant delignification, the lignin structure may be modified due to alternation in the chemical properties of lignin which eventually render the biomass more digestible in comparison to non-pre-treated biomass with approximately the same lignin fraction (Agbor et al., 2011).

The efficiency of AD was assessed by measuring the organic matter concentration in the supernatant of the inoculum and substrate mixture prior to and after digestion. The overall organic matter removals in terms of COD, BOD and SO_4 are given in Table 4.

The reductions in the COD and BOD concentrations were attributed to the organic degradation while reduction in SO_4 concentration was associated with bacterial competition between the methanogenesis and sulphidogenesis stages. Increased initial organic matter concentrations (COD_0 and BOD_0) were seen with pre-treated samples in comparison to the control rendering the substrate more suitable for anaerobic digestion. A significant increase in the initial COD value (by 29.5 % with regard to control) was seen due to implementation of combined ultrasonic pre-treatment at 100 % amplitude for 2 h with 3 M NaOH pre-treatment which led to the highest $(COD)_0$ value of 26,663 mg/L (std no 8 in Table 4). Similarly the initial BOD value was increased from 12,667 to 13,490 mg/L ($p < 0.05$) as a result of applied ultrasonic pre-treatment at 70 % for 1 h (std no 9 in (Table 4). Increased COD values of pot ale was also reported by (Mallick et al., 2009) as a result of the pre-treatment step.

Among the only ultrasonic pre-treated samples (std no 5, 6, 9, 10), std no 10 had the highest COD and BOD removals as 48 ± 4.4 and 56 ± 3.5 % respectively. The std no 10 showed significantly higher COD and BOD ($p < 0.05$) removals in comparison to the control. On the other hand, std no 5, 6, 9 and 10 had significantly lower H_2S degradations than the control with p values of 0.0027, 0.019, 0.0069 and 0.0034 respectively, indicating the lignocellulosic modifications and increased disintegration levels altered the bacterial competition in favour of the methanogens. Further significant enhancements in the COD and BOD removals was seen by combining ultrasonic pre-treatment with alkaline pre-treatment. The highest COD and BOD removals were determined as 57 ± 4.1 and 60 ± 3.3 % with std no 8 (Table 4) when the ultrasonic pre-treatment was combined with 3 M NaOH. Moreover, the std no 8 showed significantly lower SO_4 removal than the control. In addition, the total VFA concentration of the supernatant of pot ale-sludge mixture was determined to be in the range of 21.79–38.64 mM prior to AD with no significant differences were seen due to varying pre-treatment conditions. The results of VFA analysis prior to AD is given in the supplementary data (S2). No VFA was detected in the supernatant of pot ale-sludge mixture after the digestion indicating that the organic matter was fully broken down.

3.3. Mathematical model estimations and validations

Box-Behnken design under Response Surface Methodology involving 3 numerical factors at 3 levels was adopted as modelling tool due to its ability to produce predictive models with the minimum number of experiments. The coding format of the design factors is given in Table 5. The ANOVA results for CH_4, CO_2 and H_2S generation within the first 2 days of AD are given in Tables 6–8.

The estimated models for CH_4 and H_2S generation were evaluated by the p-value within 95 % confidence interval (α: 0.05), while the confidence level for the model developed for CO_2 was 99 % with an α value of 0.01. The data fit to the estimated quadratic models was tested by stepwise regression analysis and R^2 values were found to be close to 1 for all cases indicating that the models passed the required adequacy measures. The significance of the regression analysis was also tested by a lack of fit test applied on residuals of each model (the differences between the predicted and experimental data) and no significant lack of fit in data was seen within the identified confidence level for each response of interest. In addition adequate precision, which compares the predicted values of the standards to the average prediction error, values for all models were determined to be greater than 4 indicating the models achieved adequate discrimination.

For the modelling of CH_4 yield, the second order effects of amplitude ratio (A^2), exposure time (B^2) and alkaline dose (C^2) were found to be significant model terms according to the p values lower than 0.0001 (Table 6). In the case of CO_2 production, first and second order effects of alkaline dose (C, C^2), second order effects of amplitude ratio (A^2), exposure time (B^2) and interaction between exposure time and alkaline dose (BC) were found to be significant model terms according to the p values (Table 7). Finally significant model terms for H_2S generation were determined (Table 8) as the first and the second order effects of alkaline dose (C, C^2), second order effect of exposure time (B^2) and amplitude ratio (A^2), the interactions between amplitude ratio and alkaline dose (AC) and exposure time and alkaline dose (BC). Subsequently, lower order (first order) impact of the significant parameters were added to the models as part of stepwise regression in order to increase accuracy by supporting model hierarchy. These model terms were A, B, C and BC for CH_4 model, and A and B for CO_2 and H_2S models.

The mathematical models developed for CH_4, CO_2 and H_2S generation within the first 2 days of AD are given in Eqs. 5–7 and Eqs. 8–10 in terms of coded and actual factors respectively.

Table 4
Organic matter removal with alkaline and ultrasonic pre-treatment.

Exp no		*COD_0 (mg/L)	COD Removal (%)	*BOD_0 (mg/L)	BOD Removal (%)	*$(SO_4)_0$ (mg/L)	SO_4 Removal (%)
Ultrasonic pre-treatment only							
Control		20,583	38 ± 2.5	12,667	43 ± 2.3	745	52 ± 2.6
5	0.4A 2h	20,900	37 ± 5.0	13,173	52 ± 4.6	686	25 ± 2.1
6	1.0A 2h	25,460	44 ± 2.5	12,223	34 ± 2.3	662	35 ± 3.0
9	0.7A 1h	24,953	46 ± 4.3	13,490	48 ± 1.0	870	47 ± 2.1
10	0.7A 3 h	25,080	48 ± 4.4	12,920	56 ± 3.5	886	38 ± 0.7
Combined ultrasonic alkaline pre-treatment							
1.5 M NaOH							
1	0.4A 1h	26,080	51 ± 2.5	11,780	55 ± 2.2	744	43 ± 4.6
2	1.0A 1h	25,587	43 ± 5.0	13,300	47 ± 4.0	841	28 ± 2.6
3	0.4A 3 h	23,940	39 ± 3.3	17,100	44 ± 2.9	612	35 ± 3.7
4	1.0A 3 h	25,143	50 ± 2.7	12,413	54 ± 3.7	699	35 ± 1.8
13−17	0.7A 1.5h	21,090	34 ± 4.4	13,933	50 ± 4.0	799	36 ± 1.9
3 M NaOH							
7	0.4A 2h	22,673	44 ± 4.1	12,793	48 ± 3.7	891	40 ± 3.1
8	1.0A 2h	26,663	57 ± 6.1	13,463	60 ± 3.3	757	31 ± 4.8
11	0.7A 1h	25,650	49 ± 4.2	13,047	53 ± 3.9	861	41 ± 3.1
12	0.7A 3 h	26,600	51 ± 4.3	13,173	55 ± 4.4	693	37 ± 2.1

* Average of triplicates.

Table 5
Coding format and abbreviation of the variables.

Variable	Abbreviation	Coded Factors		
		−1	0	1
A: Amplitude Ratio (%)	Amp%	0.4	0.7	1
B: Exposure Time (h)	ET	1	2	3
C: NaOH Dose (M)	A Dose	0	1.5	3

Table 6
ANOVA results for CH_4 yield α: 0.05.

Source	Sum of Squares	df	Mean Square	F Value	p-valueProb > F	
Model	1.54E+05	7	22041.18	40.41	< 0.0001	significant
A-Amplitude (%)	21.13	1	21.13	0.039	0.8484	
B-Exposure Time (h)	578	1	578	1.06	0.3301	
C-Alkaline Dose (M)	91.13	1	91.13	0.17	0.6923	
BC	1600	1	1600	2.93	0.1209	
A^2	64298.02	1	64298.02	117.89	< 0.0001	
B^2	37461.92	1	37461.92	68.69	< 0.0001	
C^2	55611.6	1	55611.6	101.96	< 0.0001	
Residual	4908.7	9	545.41			
Lack of Fit	3805.5	5	761.1	2.76	0.1735	not significant
Pure Error	1103.2	4	275.8			
Cor Total	1.59E+05	16				

R^2: 0.9692, Adj R^2: 0.9452, Pred R^2: 0.7812, Adeq Precision: 17.509.

Table 7
ANOVA results for CO_2 yield α: 0.01.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	181254.7	7	25893.52	31.16	< 0.0001	significant
A-Amplitude (%)	21.13	1	21.13	0.03	0.8768	
B-Exposure Time (h)	2346.13	1	2346.13	2.82	0.1271	
C-Alkaline Dose (M)	6050	1	6050	7.28	0.0244	
BC	5112.25	1	5112.25	6.15	0.0349	
A^2	69958.78	1	69958.78	84.20	< 0.0001	
B^2	32163.2	1	32163.2	38.71	0.0002	
C^2	72643.46	1	72643.46	87.43	< 0.0001	
Residual	7477.8	9	830.87			
Lack of Fit	7071	5	1414.2	13.91	0.0122	not significant
Pure Error	406.8	4	101.7			
Cor Total	188732.5	16				

R^2: 0.9604, Adj R^2: 0.9296, Pred R^2: 0.6334, Adeq Precision: 18.1745.

Table 8
ANOVA results for H_2S production α: 0.05.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	24672.42	8	3084.05	26.87	< 0.0001	significant
A-Amplitude (%)	435.13	1	435.13	3.79	0.0874	
B-Exposure Time (h)	2	1	2	0.02	0.8982	
C-Alkaline Dose (M)	1081.13	1	1081.13	9.42	0.0153	
AC	812.25	1	812.25	7.08	0.0287	
BC	841	1	841	7.33	0.0268	
A^2	5540.53	1	5540.53	48.28	0.0001	
B^2	8347.27	1	8347.27	72.74	< 0.0001	
C^2	8422.42	1	8422.43	73.39	< 0.0001	
Residual	918.05	8	114.76			
Lack of Fit	647.25	4	161.81	2.39	0.2097	not significant
Pure Error	270.8	4	67.7			
Cor Total	25590.47	16				

R^2: 0.9641, Adj R^2: 0.9283, Pred R^2: 0.6813, Adeq Precision: 16.7266.

As the mathematical models in coded factors brings the parameters to a dimensionless area by reducing their magnitude to a range of -1 to 1, they can be used to evaluate the influence strength of parameters with different units on each response of interest. As such by considering the coefficients each of the model terms, the quadratic effect of the alkaline dose (C^2) has the highest impact on all models. This was followed by the second order effect of the amplitude ratio (A^2) for the developed models for CH_4 and CO_2 generation while the quadratic effect of exposure time (B^2) was found as the second most powerful parameter for H_2S generation.

Final Equation in terms of coded factors:

$$CH_4\left(\frac{ml}{gVS}\right) = 253.4 + 1.6\,A + 8.5B - 3.4C - 20BC - 123.6A^2 - 94.3B^2 + 119.9C^2 \quad (5)$$

$$CO_2\left(\frac{ml}{gVS}\right) = 238.8 + 1.6A + 17.1B - 27.5C - 35.8BC - 128.9A^2 - 87.4B^2 + 131.4C^2 \quad (6)$$

$$H_2S\ (ppm) = 80.8 + 7.36A + 0.5B + 11.63C + 14.25AC + 14.5BC - 36.28A^2 - 44.53B^2 + 44.73C^2 \quad (7)$$

The developed models in terms of actual factors Eqs. 8–10 for CH_4, CO_2 and H_2S production respectively were used to validate the estimated models by means of data prediction and performing further experiments to carry out statistical analysis.

Final Equation in terms of actual factors:

$$CH_4\ (ml/VS) = -431 + 1927.7\,Amp\% + 425\,ET - 423.1\,A\,Dose - 20\,ET\,A\,Dose - 1373.1\,Amp\%^2 - 94.3\,ET^2 + 114.9\,A\,Dose^2 \quad (8)$$

$$CO_2\ (ml/gVS) = -763.3 + 2010.5\,Amp\% + 402.5\,ET - 145.8\,A\,Dose - 23.8\,ET\,A\,Dose - 1432.2\,Amp\%^2 - 87.4\,ET^2 + 58.4\,A\,Dose^2 \quad (9)$$

$$H_2S\,(ppm) = -32.9 + 493.9 \times Amp\% + 149.6\,ET - 229.5\,A\,Dose + 47.5\,Amp\%\,A\,Dose + 14.5\,ET\,A\,Dose - 403.1\,Amp\%^2 - 44.5\,ET^2 + 44.7\,A\,Dose^2 \quad (10)$$

The design expert software provides validation of estimated models as post analysis step by testing normal distribution of residuals as well as comparison of predicted and actual values of all interests of responses. The validation results for CH_4 yield are given in the supplementary data (S3). Achieving normal distribution of residuals proves that ANOVA was a suitable statistical tool for analysis of the estimated models while having design points close to diagonal line indicates that developed model was significant. Similar patterns were observed for CO_2 and H_2S production.

The models were then further challenged by performing validation experiments involving combination of varying parameters which had not been introduced during model development. Results of the validation experiments are given in Table 9.

According to applied two-tailed *t*-test on the validation experiments using Eqs. 8–10 (actual factors to predict CH_4 yield, CO_2 yield and H_2S production), no significant difference was observed between predicted and observed parameters, indicating a successful validation of all models within the 95 % confidence interval.

The perturbation graphs showing the effects of the significant design factors on all responses of interest in dimensionless area are given in the supplementary data (S4). All design factors showed a similar pattern for all responses as the amplitude ratio (A) and the exposure time (B) of the ultrasonic pre-treatment resulted in the highest values in the centre point while the lowest value was observed with the centre point of the alkaline dose (C).

The significant interaction stated in ANOVA analysis between the exposure time (B) of the ultrasonic pre-treatment and alkaline dose (C) in CO_2 yield (Table 7) and H_2S production (Table 8) is detailed in Fig. 5. No significant difference was found in CO_2 and H_2S yields arising from the implementation of 3 M NaOH pre-treatment prior to AD in comparison with non-alkalised pot ale when the time of the ultrasonic pre-treatment was 1 h. In contrast, as the exposure time was increased, 3 M NaOH pre-treatment resulted in significantly lower CO_2 yield (Fig. 5 (1)) while it caused a significant increase in H_2S production (Fig. 5 (2)) with regard to AD of non-alkalised pot ale.

3.4. Optimisation

Anaerobic digestion yield within the first 2 days was optimised by using a combination of numerical and graphical optimisation methods provided by Design Expert Software. The optimisation was performed by predicting CH_4, CO_2 and H_2S generation by using the validated mathematical models in terms of actual factors Eqs. 8–10 respectively. Firstly, numerical optimisation was performed on the data set to identify the optimum levels of the design factors (A: Amplitude ratio, B: Exposure time and C: Alkaline Dose) with the aim of minimisation in order to reduce the cost of pre-treatment. CH_4 yield, on the other hand, was aimed to maximise while CO_2 and H_2S generations were aimed to minimise. In the numerical optimisation, the highest importance (level 5) was assigned to maximising CH_4 yield and minimising H_2S production due its high corrosivity. The details of numerical optimisation criteria are given in the supplementary data (S5) The solutions of the numerical optimisation, based on this criteria, were computed by the Design Expert Software based on the desirability function. The suggested numerical optimisation solution are given in S5. Subsequently, a graphical optimisation was performed on the suggested solutions by numerical optimisations to identify an optimum area presenting the target yields of the individual responses which were associated with the factor levels in the numerical optimisation solutions. The graphical optimisation result is given in Fig. 6. The optimum area (marked in yellow) was determined by overlapping the boundaries of interest of the responses predicted by the numerical optimisation according to the optimisation criteria stated above.

As such, upper and lower limits of the optimum area are as follows 211 – 131 mL/g VS for CH_4, 223 – 119 for CO_2 and 70 – 51 ppm for H_2S. These correspond to significantly lower CO_2 and H_2S generation than the control. The operation conditions correspond to the areas marked in yellow results in the same yields, however lower alkaline dose (0 – 0.6 M) and shorter exposure time (1–2.5 h) are more preferable from economical perspective.

3.5. Digestate quality as Biofertiliser

A pot ale digestate sample was taken from std no 12 (Table 3) after 21 days and analysed for micro (P, K, Ca, Mg) and macro (Fe, Mn, Zn, Cu) mineral content as well as for heavy metal (As, Cd, Co and Mo) content to evaluate its potential for agricultural use. As the inorganic content of the digestate is directly linked to raw material source, results of one digestate sample are given in Table 10 along with mineral analysis of inoculum and an industrial digestate sample which is used as biofertiliser in Ireland.

No major differences were observed between the composition of pot ale digestate and the industrial digestate except for Cu concentration. High Cu concentration in pot ale digestate is arising from its presence in pot ale (reported to be 53.04 ± 3.21 mg/L (Gunes et al., 2020)) as the raw material of the process. The presence of Cu in pot ale, on the other hand, is attributed to potential mass transfer during the distillation step of the whiskey manufacturing by using copper stills (Graham et al., 2012). Although presence of copper is considered to enriched the soil quality, high concentrations is reported to be lethal for sheep (Graham et al., 2012) however no negative impacts are seen on cattle. Slight differences between the industrial digestate and pot ale digestate in the concentration of P, K, Mg, Zn, Mn, Cu and Fe are attributed to use of different material as feedstock as well as different inoculum substrate ratios in industry.

Table 9
Validation experiment results.

Validation Points	CH_4 Yield (ml/g VS)		CO_2 Yield (ml/g VS)		H_2S production (ppm)	
	Experimental	Predicted	Experimental	Predicted	Experimental	Predicted
NA 0.7A 1h	489 ± 48 *p:* 0.0736	577	282 ± 40 *p:* 0.5447	257	194 ± 16 *p:* 0.2529	220
1 M 0.7A 1h	194 ± 10 *p:* 0.0824	248	194 ± 10 *p:* 0.0943	146	201 ± 25 *p:* 0.2088	149
2.5 M 0.7A 1h	193 ± 7 *p:* 0.4582	187	193 ± 7 *p:* 0.4721	198	186 ± 33 *p:* 0.1376	111

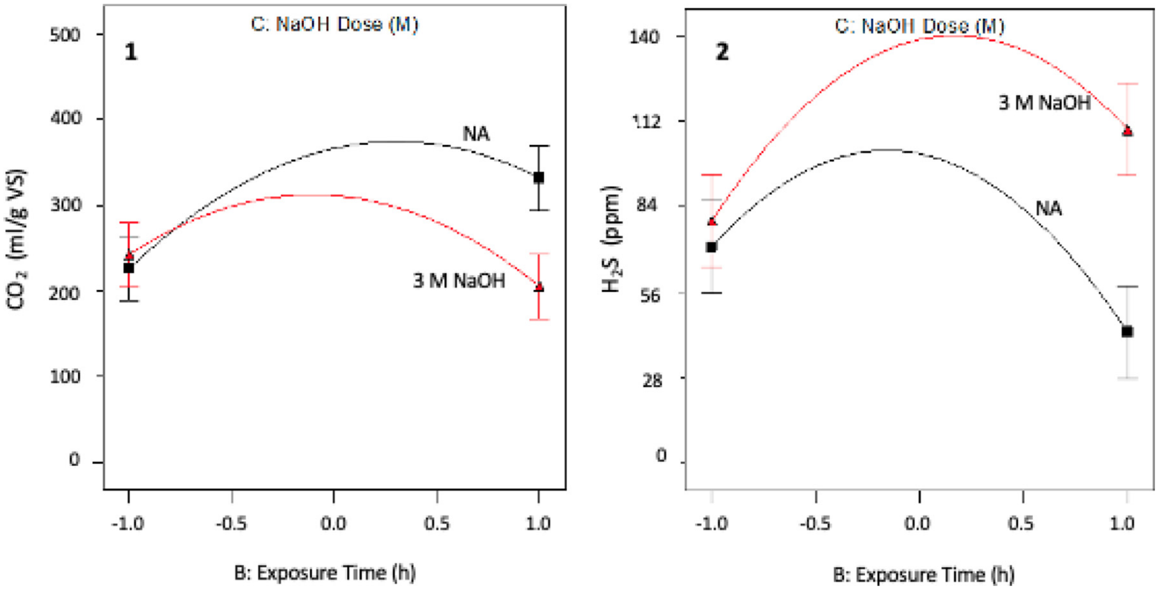

Fig. 5. Interaction graphs of 1. CO_2 yields and 2. H_2S generation.

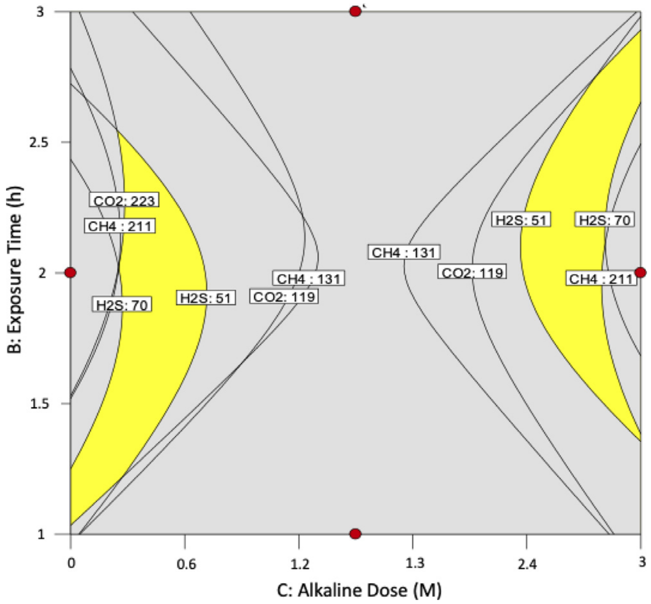

Fig. 6. Optimum area at 40 % amplitude ratio.

Agricultural use of pot ale digestate is considered to be a sustainable and valuable biofertiliser which eliminates the risk of pathogenic bacteria (mainly *Salmonella* and *Klebsiella spp*) in the animal by-product based fertilisers. Salmonella are reported to be transmittable to humans as a result of consuming contaminated food or water as well as through the food chain. Similarly, *Klebsiella* were previously associated with the human infections (Owamah et al., 2014). Digestate originating from pig slurry (Kuusik et al., 2017) cow dung and chicken droppings (Alfa et al., 2014), food waste and human excreta (Owamah et al., 2014) have been shown to contain these pathogens. Therefore the EU agricultural regulation No 1774/ 2002 dictates a pasteurisation step prior to the application of animal and/or human co-product based digestate to farm land (EUROPEAN COMMUNITIES, 2002). The pot ale digestate might potentially eliminate the pasteurisation step due to its origin. However, microbial characterisation and certification are required before approval for use, to ensure public safety.

4. Conclusions

In the first 2 days of AD, a maximum CH_4 yield of 333 ± 5 mL/g VS was achieved as a result of 70 % amplitude ultrasonic pre-treatment for 3 h (std 10) which corresponds a 60 % increase with regards to control. Moreover, the pre-treated samples had significantly lower H_2S production than the related control. This work demonstrated that ultrasonic pre-treatment resulted in significant improvements in biogas quality and quantity by increasing the soluble organic matter of pot ale.

Design Expert Software was employed for mathematical modelling as well as process optimisations. The optimum pre-treatment conditions were identified as ultrasonic pre-treatment at 40 % amplitude ratio for 1–2 h following 0 – 0.6 M NaOH pre-treatment. Operation under these conditions leads to a CH_4 yield of 211 mL/g VS and 51–70 ppm H_2S generation within the first 2 days of AD. The H_2S production showed a 2.5 fold drop in comparison to the non-treated sample, indicating achievement of a more stable process.

Table 10
Total mineral analyses of inoculum and digestate samples.

Compound	Inoculum	Industrial digestate	Pot ale digestate
P	1620.5 ± 18.2	1082.6 ± 6.8	1347.0 ± 66.1
K	1225.3 ± 3.7	1090.0 ± 13.4	1140.8 ± 8.6
Mg	209.6 ± 11.4	104.8 ± 2.3	124.6 ± 34.2
Zn	16.87 ± 0.4	12.52 ± 0.3	13.07 ± 0.33
Mn	18.47 ± 0.1	12.54 ± 0.2	15.31 ± 0.50
Cu	3.40 ± 0.1	2.70 ± 0.4	62.76 ± 0.4
Fe	349.0 ± 3.8	224.3 ± 3.5	255.8 ± 17.3

All units are in mg/L.

Producing lower H_2S reduces the cost of biogas upgrading as well as enhancement of combined heat and power unit (CHP) yield for heat and electricity production, which can be used in the distillery manufacturing process (Appels et al., 2008). Therefore it is concluded that introducing a hybrid alkaline ultrasonic pre-treatment prior to AD can potentially reduce the cost of post treatment in CHP and biogas upgrading. Finally, the mineral quality of pot ale was found to be sufficient for valorisation as a biofertiliser due to similarities in composition to an industrial digestate that is used in farmland applications.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psep.2020.08.013.

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