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Batch anaerobic digestion of deproteinated malt whisky pot ale using different source inocula

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ABSTRACT

A novel process has been developed for the selective removal of protein from pot ale with recovered protein holding potential as a value-added by-product for the whisky industry. The purpose of this work was to assess the effect of deproteination on pot ale physicochemical characterisation and anaerobic digestion (AD) treatment. Pot ales were taken from five malt whisky distilleries and tested untreated, after centrifugation/filtration and after deproteination at laboratory or pilot scale. At laboratory scale, the deproteination process removed around 20 % of total chemical oxygen demand (tCOD) from untreated pot ale and at least 30 % dissolved copper from centrifuged pot ale. Biochemical methane potential of untreated, filtered and deproteinated pot ale obtained at pilot scale has been determined using two types of inocula from different source. Average methane yield values of 554 ± 67 , 586 ± 24 and 501 ± 23 NI CH₄ kg⁻¹ VS were obtained for untreated, filtered and deproteinated pot ale respectively. A significant difference in methane yield was only observed for untreated pot ale using the two types of inocula. Specifically, when using a non-adapted inoculum untreated pot ale biogas yield was significant lower suggesting inhibition of the AD process. As no significant differences were found for treated pot ale (filtered and deproteinated) with the two inocula it suggests, deproteination may have a positive effect on AD start-up. The results present a clear case for continuation of this work and evaluating the effect on continuous AD.

KEYWORDS: anaerobic digestion; biochemical methane potential; pot ale; malt whisky; deproteination

1. INTRODUCTION

The Scotch whisky industry represents a quarter of UK food and drink export, currently valued at around £4 billion (Scotch Whisky Association, 2015). However, uses for the ~7 million tonnes of by-products generated annually are becoming increasingly costly and restricted due to tighter legislation.

Anaerobic digestion (AD) is a potentially promising treatment process for the various by-products generated including pot ale and spent lees. Pot ale has received particular interest given its comparative high chemical oxygen demand (COD) with previously published work demonstrating at laboratory scale AD for biogas and methane generation (Goodwin & Stuart, 1994; Tokuda et al., 1998; Kida et al., 1999; Akunna & Clark, 2005). Despite the potential benefit of AD, there has to date been limited successful uptake of the technology by the whisky industry. This has been in part due to problems associated with the COD, yeast cells (which sink to the bottom of reactors) (Goodwin & Stuart, 1994) and protein content of pot ale, all of which may make stable long term digestion difficult to achieve. In further support of this, protein breakdown in AD has been widely reported to lead to the build-up of ammonia which above certain concentration thresholds can have an inhibitory effect on methanogenic populations (Ariunbaatar et al., 2015; Vidal et al., 2000).

As a way to overcome the problems associated with whisky by-products for AD, various research groups have looked at feedstock enhancement (or pre-treatment) processes. Early work focused on enzymatic and microbial hydrolysis pre-treatment (Tokuda et al., 1998; Kida et al., 1999). More recently, there has again been an interest in enzymatic pre-treatment of spent wash (Mallick et al., 2010). Others have used pH amendment coupled with solid-liquid phase separation (Dionisi et al., 2014). In this latter case, the focus has been as a standalone wastewater treatment process rather than pre-treatment for AD.

In contrast to these studies, here the potential of a deproteination process capable of selectively removing protein from pot ale is assessed. As well as addressing potential issues with pot ale for AD (previously stated), the recovered protein rich material has potential resale value in the feed and food industries and has been demonstrated elsewhere as an economically viable option (Traub, 2015). A protein recovery process has been developed, patented and is now being commercialised (White et al., 2016). The process has been tested at a fully operational malt whisky distillery generating sufficient material for subsequent fish feeding trials (White et al., 2014).

The specific aims of this research were to assess the effects of centrifugation, filtration and the novel deproteination process on; 1) pot ale physicochemical characterisation and, 2) biogas and methane yield under batch AD conditions. To our knowledge, no previous biochemical methane potential (BMP) of pot ale has been reported previously. It has previously been reported that methane yields are similar at $496 \text{ NI CH}_4 \text{ kg VS}^{-1}$ and $415 \text{ NI CH}_4 \text{ kg VS}^{-1}$ for protein and carbohydrate respectively (Angelidaki & Sanders, 2004) so similar results between treated and untreated pot ale may be predicted given that these are the predominant substrates. The work also afforded the opportunity to assess the effect of different inocula on methane yield, which has previously been demonstrated to be significant for other feedstocks (De Vrieze et al., 2015). Assuming positive outcomes this work would support the case for longer term continuous AD experiments. The technology is also likely to be applicable to materials from other major fermentation processes. For example, Tequila vinasses, which are comparable in terms of COD, biological oxygen demand (BOD), pH and copper content (Méndez-Acosta, 2010).

2. MATERIALS AND METHODS

2.1 Malt whisky distillation and novel deproteination process

A schematic of the malt whisky distillation process illustrates the source and quantities of pot ale from a medium sized distillery with the basic steps in the patented deproteination process also shown (Figure 1).

2.2 Feedstocks and inocula

Pot ales from four independently owned malt whisky distilleries were sampled and stored at $4 \text{ }^\circ\text{C}$ until further processing at laboratory scale. Pot ale from a fifth malt whisky distillery was processed through a pilot scale rig directly at the distillery before transporting and storing at $4 \text{ }^\circ\text{C}$ prior to analysis. Two anaerobic inocula sludges from different sources were used for BMP testing. The first inoculum was a whole digestate 'sludge' (hereafter referred to as WD sludge) obtained from a commercial scale mesophilic anaerobic digester plant treating a variety of food waste materials located in Scotland, UK. The second consisted of

granular sludge obtained from the upflow anaerobic sludge blanket (UASB) digester wastewater treatment plant of a local whisky distillery.

2.3 Physicochemical characterisation

Physicochemical characterisation of the different materials used in this work is shown in Table 1 and Table 2. Total solids (TS) and volatile solids (VS) were determined in triplicate for each sample and inoculum as per standard methods (APHA, 2005). Sample and inoculum pH was determined using a portable probe (HI 8424; Hanna Instruments Ltd, UK) directly in the sample or as a 1:5 (w/v) suspension for the granular sludge inoculum. Total COD (tCOD) was determined in triplicate using LCK514 test kits measured using a DR1900 spectrophotometer (Hach Lange Ltd, Salford, UK). Total N was determined by standard Kjeldahl method except site 5 which was determined using a Hach LCK338 kit and DR2800 spectrophotometer (Hach Lange Ltd, Salford, UK). Crude protein content was determined by multiplying the nitrogen content by a factor of 6.25 (FAO, 2003).

Samples processed at laboratory scale were subjected to additional characterisation including protein, carbohydrate, inorganic and heavy metals analysis. Soluble protein was determined on pre-treated samples (see section 2.4) using the dye binding method (Bradford, 1976) with bovine serum albumin as calibration standard. Total carbohydrate was determined using the phenol-sulphuric acid assay (Fournier, 2001). Inorganic and heavy metals characterisation was carried out by an external United Kingdom accreditation scheme (UKAS) registered laboratory. Specifically, metals were determined by inductively coupled plasma mass spectroscopy (ICP-MS); chloride, nitrate, phosphate (ortho) and sulphate by ion chromatography; phosphate (total), nitrite and sulphide colorimetrically using a Konelab60i autoanalyser; and ammoniacal nitrogen by the indophenol blue reaction and spectrophotometry at 655 nm.

2.4 Laboratory scale feedstock enhancement

Samples of pot ale from sites 1-4 were pre-treated by centrifugation for 1 hour at 4500 rpm using a Beckman Coulter AVANTI j-265 p centrifuge fitted with a JLA 8.1 rotor. The supernatant material was then syphoned into a 2L bottle ensuring no solids were collected

and subjected to the Horizon Proteins patented (GB 1411943.2) deproteination process. Untreated (UN), centrifuged (C) and deproteinated (D) pot ale from the four sites were analysed using methods described in section 2.3.

2.5 Pilot scale feedstock enhancement trial

Pilot plant tests took place at a malt whisky distillery in the Spey area in Scotland (site 5). Pot ale was stored in a tank, the solids decanted overnight and purged. The remaining solids in the liquid were pumped through a 1-micron filter bag (Pall) and then process through the pilot plant for subsequent protein recovery and purification tests. The flow-through or deproteinated material was collected for partial physicochemical characterisation (section 2.3 methods) and BMP testing. Pilot tests were repeated three times under the same conditions.

2.6 Biochemical methane potential (BMP) tests

Untreated (UN), filtered (F) and deproteinated (D) pot ale from the fifth site were compared in batch digestion experiments. Biogas production of untreated and treated pot ale was compared in triplicate using the two types of inocula. An inoculum to substrate ratio (ISR) of 2:1 based on volatile solid (VS) content (Table 2) was used in granular and whole digestate sludge inocula (Raposo et al., 2011a). Additionally, 4:1 ISR was tested for the WD sludge inoculum. Specifically, BMP tests were performed in 165 ml glass serum bottles containing the pot ale + inoculum and the volume of liquid adjusted with deionised water to give the same effective volume for the two ISR ratio. pH was adjusted to approximate 7.0 with NaOH 1 M. NaHCO_3 (5 g l^{-1}) was added to increase the buffer capacity of the system. The bottles were incubated at 37 °C in an orbital incubator with continuous mixing of the content of the bottles at 110 rpm. The headspace of each 'reactor' was purged with nitrogen gas (100 %) for one minute to ensure anaerobic conditions. In each experiment, biogas production from triplicate blank reactors containing the same amount of inoculum, and water were measured. The biogas production of blanks was subtracted from sample assays. Cellulose positive controls were run to verify the health of the inoculum for each experiment. Biogas

production was quantified by measurement of pressure increase in constant volume using a digital manometer (Model SMC ZSE30, Japan).

Biogas production was measured using a manometric method and calculated according to the ideal gas law from the pressure measured in the bottle and considering the headspace volume. The biogas produced was purged after every measurement; daily during the most active part of the assay and approximately every two or three days in the last part. Biogas compositional analysis was carried out once per week. The biogas was analysed for CH₄ and CO₂ content using a gas chromatography thermal conductivity detector instrument (200 series GC; Ellutia UK, Cambridge). The packed column (PorapakTM) was fed with helium as carrier gas at a constant flow rate of 25 mg l⁻¹. Temperature of oven, injector port and detector were 50, 99 and 120 °C respectively. An external standard (60 % CH₄ and 40 % CO₂ (v/v)) was used for quantification of CH₄ content. The CH₄ content was corrected using equation 1 as previously reported (Kafle et al., 2013).

$$CH_4_{corrected} = \frac{CH_4_{measured}}{CH_4_{measured} + CO_2_{measured}} \times 100 \quad (1)$$

First-order rate constant (K) has been estimated by means of equation (2):

$$B(t) = B_0 (1 - e^{-kt}) \quad (2)$$

Where B (L CH₄ kg⁻¹ SV) is the cumulative methane production yield at a given time, B₀ (L CH₄ kg⁻¹ SV) is the ultimate methane yield, K (days⁻¹) is the first-order rate constant and t (days) is the time (Angelidaki et al., 2009).

Theoretical methane potential was calculated on a COD (BMP_{THCOD}) basis applying equation 3 (Raposo et al., 2011a).

$$BMP_{THCOD} = VS_{ADDED} \left(\frac{g \text{ COD}}{g \text{ VS}} \right) * 350 \quad (3)$$

2.6 Statistical analysis

One way ANOVA was used to compare the biogas yield means followed by Tukey's post hoc test to assign difference between groups at significance level of 0.05. The analysis was performed using statistic software Minitab, Inc. release 17.

3. RESULTS AND DISCUSSION

3.1 Deproteination process efficiency at laboratory scale

Here deproteination process efficiency is defined as the difference between the total protein content in the untreated pot ale (stream UN in Figure 1) and the protein content in the deproteinated pot ale (stream D in Figure 1) over the protein content in the untreated pot ale as described in the Equation below (Eq 4). The equation assumes that the volume of untreated and deproteinated pot ale are the same.

$$\% \text{ Deproteination efficiency} = \frac{UN \text{ protein} - D \text{ protein}}{UN \text{ protein}} \quad (4)$$

The deproteination process has been shown to remove up to 90 % of the soluble proteins (Traub, unpublished). In the laboratory scale process used here at least 60 % of soluble protein was removed from the tested pot ales (62 % Site 1, 64 % Site 2 and 71 % Site 3). This compares with a loss of only 10 and 1 % soluble carbohydrate in sites 1 and 2, and an increase of 17 % in site 3 (Table 1). When samples were analysed with the Kjeldahl method (which assumes that all N is protein), deproteination from untreated pot ale was 63 % (site 1), 42 % (site 2) and 47 % (site 3). The discrepancy between the Kjeldahl and the Bradford results (Table 1), is that the latter responds better to proteins greater than 3 kDa and to peptides comprised of aromatic (phenylalanine, tyrosine and tryptophan) and basic (arginine, histidine and lysine) amino acids (Evans et al., 1999). Additionally, the Kjeldahl method takes into account the protein content from the yeast particles which are made of approximately 50 % protein on a dry matter basis (Kida et al., 1999). Therefore, for the site 4 laboratory and site 5 pilot tests, total N (Kjeldahl) method was used to quantify protein

removal. The site 4 test showed a 47 % deproteination, a result in agreement with the experiments described in the previous paragraph. The pilot test achieved 71 % protein removal. In the mass balance schematic in Figure 1, 83 % deproteination was calculated, which is based on the assumption that yeast (suspended solids component) is made of 50 % protein. This value is closer to the values achieved during the pilot tests, but subsequent tests have reached deproteination efficiencies of ~90% (these results are however not included in this work) and suggest that the example in Figure 1 is realistic and attainable for a potential large scale process.

3.2 Deproteination and pot ale wastewater treatment

Mirroring the protein removal, both VS and tCOD content decreased from untreated > centrifuged > deproteinated pot ale for samples from sites 1-4. The laboratory scale centrifugation process removed around 5 % tCOD from all four pot ales. As such this was less efficient than a laboratory solid-liquid separation using Whatman GF/C filter paper which was able to remove 14 % tCOD (Dionisi et al., 2014) and is assumed to be more effective as removing fine suspended solids although data on this parameter is not reported. For the pilot scale experiment at site 5, a centrifuge was unavailable and so this was substituted for filtration. Interestingly, 15 % tCOD was removed by the filtration process at pilot scale making it directly comparable with that achieved by Dionisi et al., (2014). With the deproteination process included, tCOD removal was around 20 % for sites 1-4 using the laboratory scale process. This is comparable with a previous study using a higher speed centrifuge (6000 rpm and 3100 g) and pot ale with reasonably similar tCOD of 62 g l⁻¹ (Tokuda et al., 1999). The pilot scale experiment at site 5 achieved greater tCOD removal (35 %) than laboratory scale deproteination experiments due to improvements introduced during the field trials; specifically shorter loading cycles and a higher operating temperature that are thought to have increased protein solubility.

Dionisi et al. (2014) report that the other main pollutants in pot ale are ammoniacal N, phosphorus and copper. For ammoniacal N, the concentration in untreated pot ales from sites 1-3 was found to be below a typical discharge limit of 10 mg l⁻¹ (Table 1) and, therefore, more likely to be a concern post AD. The make-up of nitrogen in site 4 was

different to the other three sites with higher ammoniacal N and lower nitrate. The reason for this difference is unconfirmed and requires further investigation..

For phosphorus (here assessed as total and ortho phosphate) the deproteination process showed no consistent removal pattern (Table 1) for the three pot ales tested and as such the process is not considered to impact on this pollutant. Analysis of two further pot ales supports this observation (data not shown). Assuming a UK discharge limit for ortho phosphate of around 10 mg l⁻¹; treatment downstream of the AD process such as that used by Tokuda et al., (1999) is likely to be required.

Finally, total Cu is typically reported in pot ale as between 2-5 mg l⁻¹ (Graham et al., 2012), with removal of 50 % of this (insoluble fraction) achievable by filtration (Dionisi et al., 2014). Therefore, the removal of additional (dissolved) Cu through a process such as deproteination is potentially advantageous from both wastewater treatment and AD pre-treatment perspectives. Here it was found that the deproteination process removed between 30-35 % dissolved Cu when compared to centrifuged only samples (sites 1-3) and considerably more for site 4. The process may therefore be important in achieving discharge consent limits for sensitive water bodies with or without AD downstream processing.

3.3 Pot ale batch digestion and effect of deproteination on methane yield

After the pilot-scale experiment, samples (untreated, filtered and deproteinated pot ale) were subsequently used in batch AD experiments to compare methane yields.

3.3.1 Batch test using granular sludge as an inoculum

Firstly, anaerobic assays were performed in batch mode using the granular sludge as inoculum at 2:1 ISR. Specific methane production yields for treated and untreated pot ale using the granular sludge are shown in Figure 2A. Methane production started immediately for all pot ale samples (treated and untreated) with highest biogas yield in the first few days of the process (Figure 2A). As described, this inoculum was collected from a local whisky distillery and these results showed the strong affinity of the inoculum microbial community for the pot ale substrate.

Data fitting to the equation 1 allowed the estimation of the first-order rate constant (K). Kinetic constants of methane production were 0.38 ± 0.01 , 0.40 ± 0.04 and $0.57 \pm 0.04 \text{ d}^{-1}$ for untreated, filtered and deproteinated respectively. No differences were observed between untreated and filtered pot ale, but a significantly higher K was obtained for the deproteination process. The higher rate constant suggests an increase in the hydrolysis step; hence the deproteination process could act as a pre-treatment of pot ale anaerobic digestion.

Deproteinated pot ale represents a loss of 20.6 % of biogas yield compared with untreated pot ale. This data is in accordance with a high loss (35 %) of total chemical oxygen demand (tCOD) after the deproteination process. The theoretical potential based on total chemical oxygen demand (tCOD) determined by equation 3 were 709, 664 and 593 $\text{NI CH}_4 \text{ kg}^{-1} \text{ VS}$ respectively for untreated, filtered and deproteinated pot ale. The BMP obtained in batch assay represents 89, 91 and 84 % of the respectively theoretical values. However, it has to be taking into account the limitation of accurate COD measurement of 'complex' substrates such as pot ale with mixed phase composition.

3.1.2 Batch test using food waste fed whole digestate (WD sludge) as an inoculum

In order to better understanding of the effect of deproteination on pot ale anaerobic digestion, a non-adapted inoculum was used in subsequently batch assays. Whole digestate sludge from mesophilic anaerobic digester treating food waste was selected. Food waste is a heterogeneous substrate composed mainly of carbohydrates and rich in proteins (15-25%) (Capson-Tojo et al., 2016). Hence, it was expected to find microbial diversity in this sludge, and an initial microbial community with relative affinity to pot ale composition.

Methane yields for treated and untreated pot ale using WD sludge inoculum at 2:1 ISR are shown in Figure 2B. As observed, methane production profile in function of time was completely different that observed in Figure 2A. Methane production was slower, indicating low methanogenic activity, and the plateau phase was not reached after 40 days. It was not possible to determine kinetic constants for 2:1 ISR because experimental data did not fit with model equation 1. As the rate constant is affected by ISR, a high ISR (4:1) was also

tested using WD sludge as inoculum. Methane production for untreated and treated pot ale is represented in Figure 2B.

The K value using 4:1 ISR ratios were 0.18, 0.17 and 0.19 day⁻¹ for untreated, filtered and deproteinated pot ale respectively. These values are lower than obtained using the granular sludge suggesting that hydrolysis rate could be limited. Ultimate methane productions yields are shown in Table 3 and will be discussed later.

Final pH values were around 8 for all the samples without showing any difference between the different treatments.

3.3.2 Effect of deproteination on methane yield

For every substrate analysed (untreated, filtered and deproteinated pot ale) using the two type of inocula only significant differences in methane yield were observed for untreated pot ale (Table 3). However, non-significant differences were found for filtered and deproteinated pot ale.

A higher concentration of protein in untreated pot ale is the most significant difference between untreated and treated pot ale samples. Protein rich substrates are widely reported to potentially cause inhibition of the AD process through ammonia generation (Vidal et al., 2000; Chen et al. 2008; Chen et al., 2014). Despite kinetics were very different for the three batch assays, there are no significant differences on the extents of anaerobic biodegradation for treated pot ale (filtered and deproteinated) reaching comparable experimental ultimate methane production (Table 3). A lower protein in the influent would avoid the inhibition caused by accumulating of intermediate compounds during the protein degradation.

Granular sludge presented the higher methane yield for untreated pot ale, close to the theoretical value. Using WD sludge as an inoculum, the methane yield decrease 39.5 % for 2:1 ISR and 12.5% for 4:1 ISR. The ISR is considered a critical factor for the BMP test because it influences in on the kinetics of the process, affecting even the methane yield (Raposo et al., 2011a). This study also reflects the influence of inocula in the BMP of a complex substrate as pot ale, with high COD and high nitrogen content. Various authors have found that inoculum source influenced biogas yield using a variety of substrates (De Vrieze et al.,

2015; Neves et al., 2008). De Vrieze et al. (2015) attributed the enhanced results using a granular inoculum to the higher abundance of methanogens in those compared with another sludge types. In addition, using the granular inoculum, hydrolysis was not the rate-limiting step.

The use of two types of inocula in this work has facilitated the comparison of untreated and treated pot ale. Although the results using granular sludge were satisfactory, the inhibition observed using the WD sludge suggests that protein reduction in pot ale should have a positive impact in AD performance. In this sense, to work at higher organic loading rates (OLR) would be an option for treated pot ale that should be explored in continuous AD experiments.

Deproteination process also implies a reduction of soluble copper in the effluent. Copper has been reported to inhibitory for AD process (Romero-Güiza et al., 2016). A lower concentration of copper should avoid the possible inhibitory effects of pot ale on AD. In this sense, an anaerobic continuous experiment would be necessary to determine the maximum operational ORL and the possible effects in the process for copper inhibition.

Biochemical methane potential of pot ale (untreated and treated) has been determined in this study. To our knowledge, this is the first reported value for biogas yield of whisky industry by-product. The results of three batches of anaerobic test are summarised in a boxplot graphic (Figure 3). An average methane yield values of 554 ± 67 , 586 ± 24 and 501 ± 23 $\text{NI CH}_4 \text{ kg}^{-1} \text{ VS}$ were obtained for untreated, filtered and deproteinated pot ale respectively. For untreated pot ale, a greater distribution in the boxplot was observed. As there was a significant difference between batch tests using these inocula, the mean value is likely to have been affected by when inhibition, particularly using the WD sludge 2:1 ISR ratio. BMP values are higher than those reported for other common substrates such sludge or manures but in accordance with other agro-industrial waste (Raposo et al., 2011b). The results suggest that deproteinated pot ale can be an excellent substrate for AD. Although the methane production per litre of pot ale results higher for untreated pot ale (Table 3), with deproteinated pot ale would be possible to work at higher OLR. Continuous experiments will be required to determine the maximum OLR.

4. CONCLUSIONS

Conclusions drawn from this work are:

- The selective deproteination process is reproducible using pot ale samples from different sources and consistently achieving removal of > 60 % protein.
- Pilot plant trials of the deproteination process at a distillery suggest that the process is scalable, but there are however, areas for improvements, including deproteination efficiencies and better integration between the deproteination and distillery processes. This should encourage more research and innovation about the potential utilisation of pot ale and its components.
- As well as protein removal, the process described in this work, is also a beneficial wastewater treatment process. It reduces up to 35 % tCOD and 30-35 % soluble copper, which may also have positive impact for continuous AD.
- The work highlighted that the pot ale deproteination process is not only a water treatment solution. This process allows the recovery of valuable materials, such as proteins, that could be reintroduced into the human food chain, thus not only improving sustainability of the whisky industry, but of other industries that would require to source raw materials utilising non-renewable resources. There are interesting synergies that should be exploited between food and drink industries.
- A significant difference in methane yield was only observed for untreated pot ale using the two types of inocula. A lower yield was obtained when a non-adapted inoculum was tested indicating the inhibition of the AD process. Non-significant differences were found for treated pot ale (filtered and deproteinated) with the two inocula suggesting that deproteination may have a positive effect on AD start-up.
- These results present a clear case for continuation of this work through continuous culture studies.

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Figure 1. Schematic showing a malt whisky distillation process, traditional by-products (draff, pot ale and spent lees), novel by-products and relevant quantities assuming a medium size malt whisky distillery.

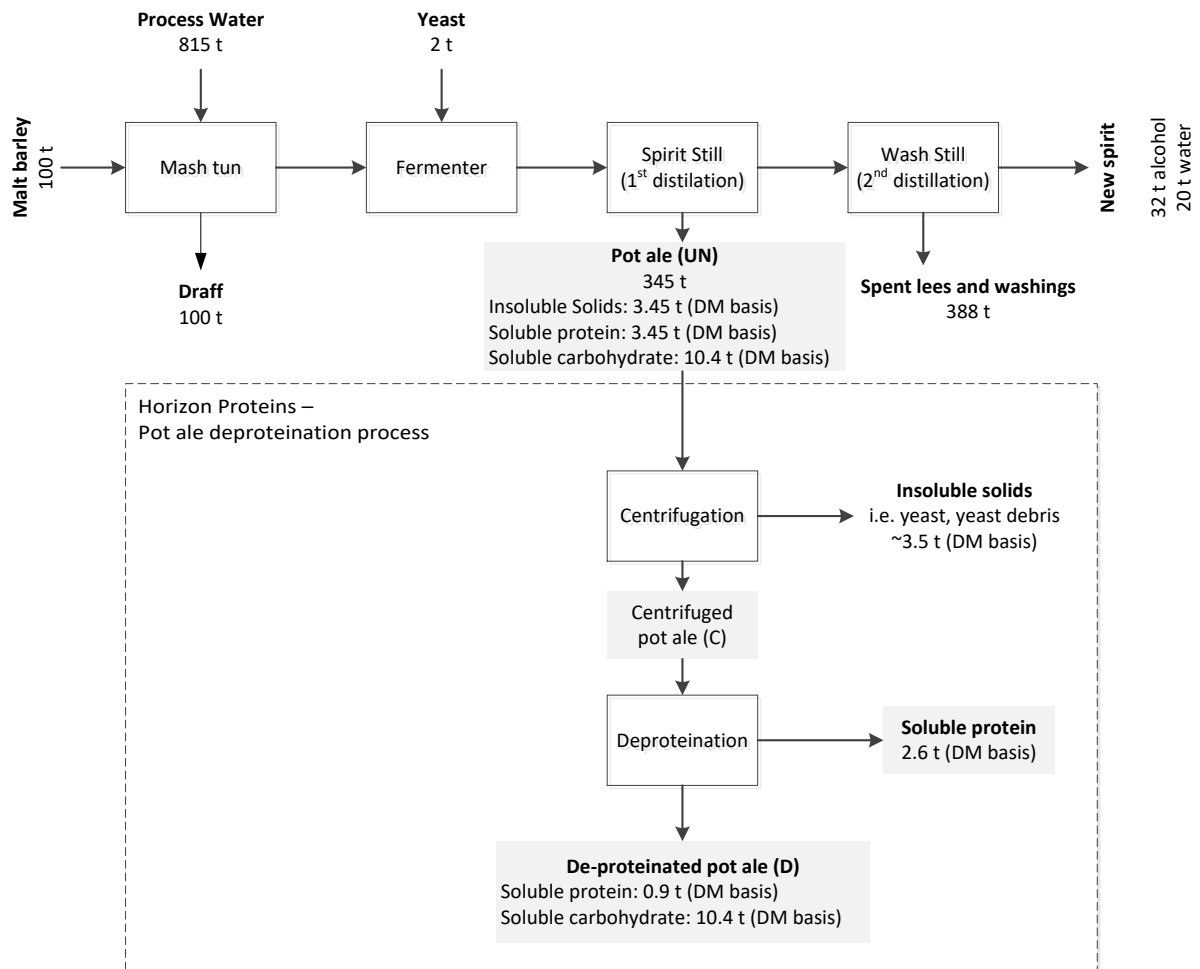


Figure 2. Cumulative methane production of untreated (UN), filtered (F) and deproteinated (D) pot ales from pilot study (site 5) A) 2:1 ISR granular sludge; B) 2:1 ISR whole digestate sludge; C) 4:1 ISR whole digestate sludge.

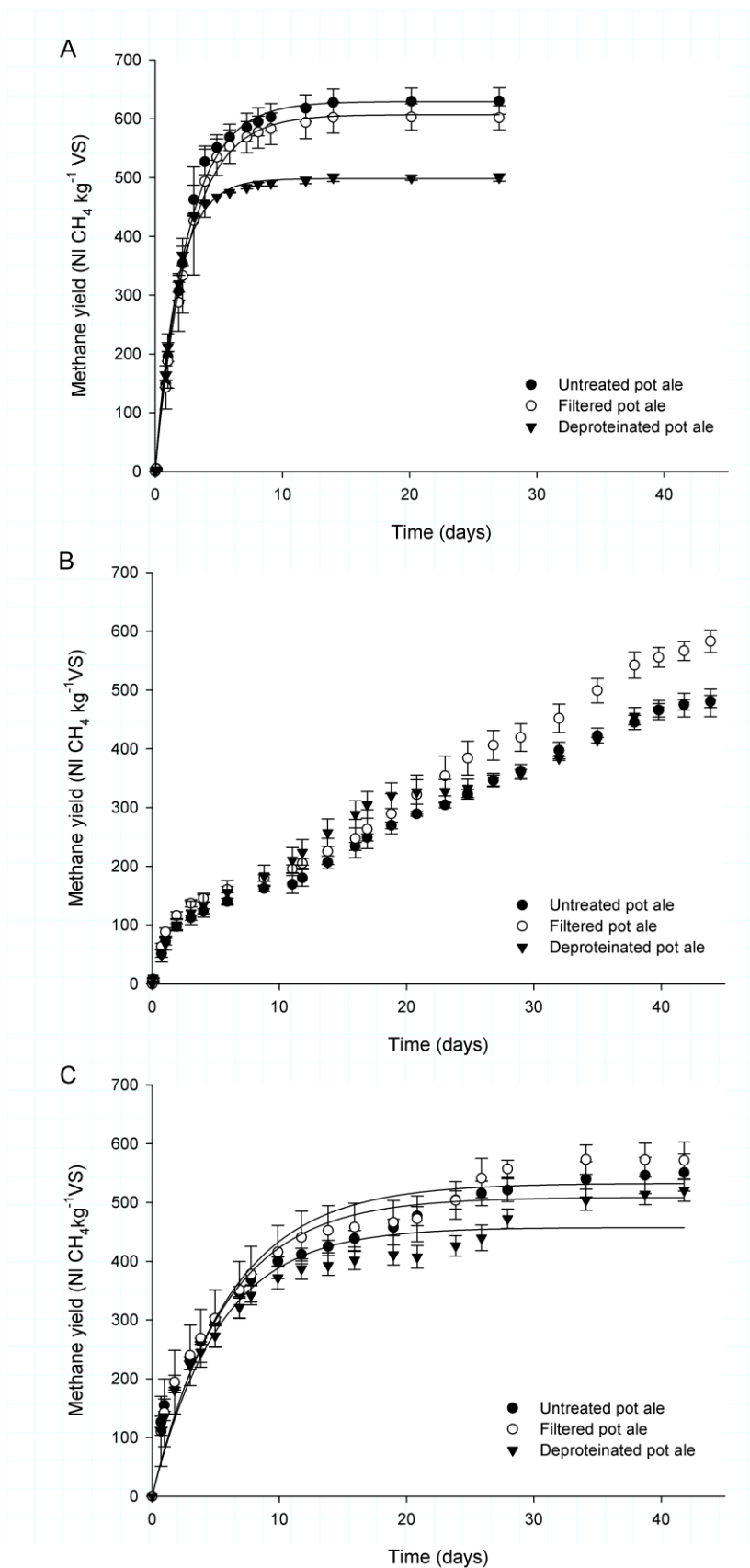


Figure 3. Biochemical methane production (BMP) boxplots for untreated (UN), filtered (F) and deproteinated (D) pot ale from pilot study (site 5)

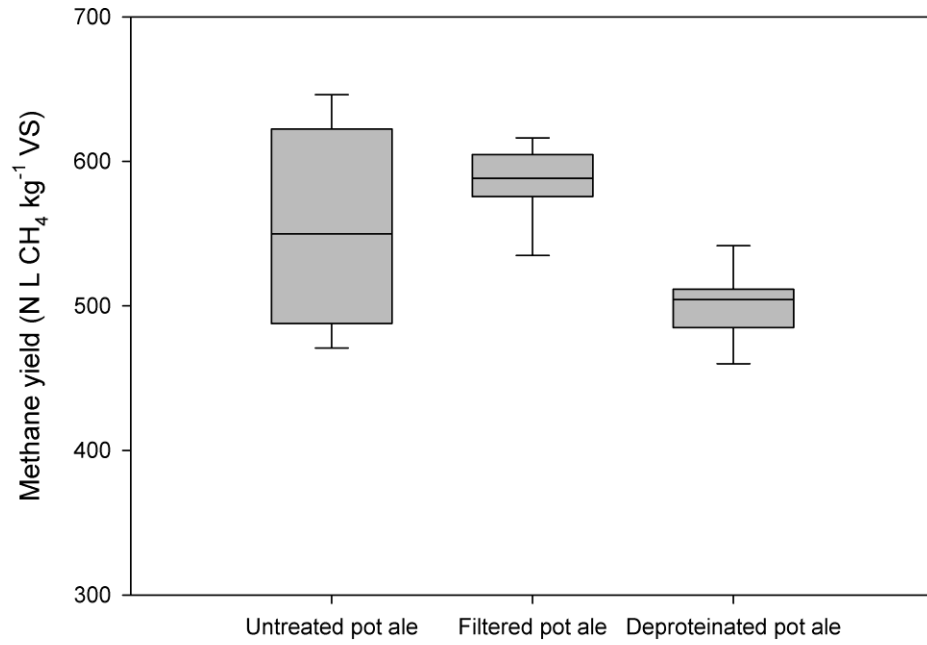


Table 1. Physicochemical characterisation of untreated (UN), centrifuged (C) and deproteinated (D) pot ales from laboratory scale trials (sites 1-4)

Parameter	Unit	Site 1			Site 2			Site 3			Site 4		
		UN	C	D	UN	C	D	UN	C	D	UN	C	D
pH*	n/a	3.5	3.5	3.9	3.8	3.8	4.0	3.9	3.9	4.0	4.2	4.2	4.0
Total solids (TS)*	% w/w	4.64 (0.03)	4.63 (0.04)	3.73 (0.03)	4.69 (0.01)	4.55 (0.01)	4.04 (<0.01)	3.38 (0.01)	3.16 (0.01)	2.73 (0.05)	4.17 (0.02)	3.96 (0.01)	3.40 (0.01)
Volatile solids (VS)*	% TS	88.8 (<0.1)	85.2 (0.1)	89.2 (0.1)	91.2 (0.1)	91.0 (0.1)	89.8 (0.1)	93.5 (<0.1)	92.9 (<0.1)	89.2 (0.1)	90.1 (<0.1)	89.9 (0.1)	86.9 (<0.1)
Total COD*	g l ⁻¹	57.1 (1.1)	54.3 (1.8)	47.8 (0.8)	61.4 (0.5)	58.7 (0.2)	50.7 (0.1)	48.7 (0.3)	46.2 (0.4)	38.6 (0.1)	52.0	51.0	42.0
Carbohydrate*	g l ⁻¹		8.2 (0.1)	7.4 (0.1)		19.3 (0.4)	19.1 (0.1)		8.7 (0.4)	10.2 (0.1)		22.0	17.4
Soluble protein*	mg l ⁻¹		496(81)	189(88)		608(92)	218(144)		484 (90)	143 (126)		n.d.	n.d.
Suspended solids	mg l ⁻¹	2700	190	240	1700	8	1100	3500	1300	1300	3800	150	240
Ca, total	mg l ⁻¹		120	500		51	220		28	66		20	4.2
Cu, total	µg l ⁻¹		129	79		710	510		1500	790		3027	1218
Mg, total	mg l ⁻¹		270	330		240	230		120	97		140	130
K, total	mg l ⁻¹		1100	33		1200	150		990	140		1100	19
Na, total	mg l ⁻¹		750	2400		20	570		15	450		38	1400
Zn, total	µg l ⁻¹		1100	1040		775	747		368	512		1100	580
Ammoniacal N	mg l ⁻¹	<0.015	<0.015	0.2	0.12	0.12	0.22	<0.015	<0.015	<0.015	7.9	8.4	210
Chloride	mg l ⁻¹	320	320	280	440	400	400	360	350	350	330	300	620
Nitrate	mg l ⁻¹	53	53	50	33	32	30	8.3	8.2	7.4	0.19	0.19	0.22
Nitrite	mg l ⁻¹	9.5	16	14	<0.035	<0.035	<0.035	<0.035	<0.035	<0.035	n/d	n/d	n/d
Total N[#]	mg l ⁻¹	1200	780	440	1900	1700	1100	1400	1200	740	1480	1355	790
Crude protein	g l ⁻¹	7.5	4.9	2.8	11.9	10.6	6.9	8.8	7.5	4.6	9.3	8.5	4.9
Phosphate (ortho)	mg l ⁻¹	180	190	190	250	250	250	180	180	140	450	430	390
Phosphate (total)	mg l ⁻¹	210	290	370	560	550	490	220	210	220	n/d	n/d	n/d
Sulphate	mg l ⁻¹	180	140	130	120	100	140	120	120	120	110	99	58
Sulphide	µg l ⁻¹	66	84	81	100	69	42	27	27	21	0.07	0.04	0.02

*Values are expressed as means (n = 3) with standard deviation in parentheses. [#]Site 1-3 by Kjeldahl and site 5 using Hach kit LCK338. n/d – not determined

Table 2. Physicochemical characterisation of untreated (UN), filtered (F) and deproteinated (D) pot ales from pilot study (site 5) and inocula used in batch AD experiments.

Parameter	Unit	Site 5			Granular inoculum	WD sludge inoculum
		UN	F	D		
pH	n/a	3.2	3.1	3.2	8.3	7.8
Total solids (TS)*	%	3.62 (0.03)	2.91 (0.04)	2.63 (0.02)	3.30 (0.01)	10.1 (0.1)
Volatile solids (VS)*	% TS	89.38 (0.12)	86.9 (0.2)	85.25 (0.04)	70.14 (0.04)	91.9 (0.4)
Total COD	g l ⁻¹	63.2 (1.4)	53.7 (0.3)	41.2 (0.2)	n/d	n/d
Total N[#]	mg l ⁻¹	2525	1716	721	n/d	n/d

[#] Hach kit LCK338. WD – whole digestate: n/d – not determined

Table 3. Experimental ultimate methane production of untreated (UN), filtered (F) and deproteinated (D) pot ale from pilot study (site 5).

Batch test	Inoculum	NI CH ₄ kg ⁻¹ VS	NI CH ₄ L ⁻¹ pot ale
UN	2:1 ISR granular sludge	630 ± 22 ^A	19.7 ± 0.7 ^A
	2:1 ISR WD sludge	482 ± 10 ^C	15.0 ± 0.3 ^C
	4:1 ISR WD sludge	551 ± 31 ^B	17.2 ± 1.0 ^B
F	2:1 ISR granular sludge	602 ± 21 ^A	15.1 ± 0.5 ^A
	2:1 ISR WD sludge	586 ± 19 ^A	14.7 ± 0.5 ^A
	4:1 ISR WD sludge	571 ± 32 ^A	14.4 ± 0.8 ^A
D	2:1 ISR granular sludge	500 ± 6 ^A	10.6 ± 0.1 ^A
	2:1 ISR WD sludge	483 ± 27 ^A	10.2 ± 0.6 ^A
	4:1 ISR WD sludge	520 ± 38 ^A	11.0 ± 0.4 ^A

Superscript letters indicate statistically different means at p<0.05