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Research article

Effects of electrokinetic and ultrasonication pre-treatment and two-step anaerobic digestion of biowastes on the nitrogen fertiliser value by injection or surface banding to cereal crops

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ABSTRACT

Biogas production from anaerobic digestion (AD) of biowastes is restricted by the recalcitrant nature of many substrates, and this may also reduce the fertiliser value of the produced digestate. The degradability of substrates can potentially be enhanced by physico-chemical pre-treatments before AD, and/or the degradation can be increased by a longer digestion time. In this study, we evaluated the effects of electrokinetic (high voltage) and ultrasonication pre-treatments of biowastes in a two-step AD process on nitrogen fertiliser replacement value (NFRV) of digestates obtained from two biogas plants with contrasting hydraulic retention time (HRT) in the primary AD step. The fertiliser value was tested by direct injection to spring barley and surface-banding to winter wheat, and the ammonium N was ^{15}N-labelled to evaluate ammonia losses. The electrokinetic pre-treatment step significantly ($p < 0.05$) increased the NH_4^+-N/total N in the digestates before the second AD step but had an insignificant effect on the fertiliser value in winter wheat and spring barley. Ultrasonication pre-treatment had also no significant effect on the fertiliser value. The two-step AD significantly ($p < 0.001$) increased ^{15}N recoveries and mineral fertiliser equivalence of labelled ammonium-N in winter wheat and reduced ammonia losses, with a significant effect ($p < 0.001$) observed in digestates sourced from a shorter HRT biogas reactor. The fertiliser equivalence of labelled ammonium-N in the digestates was 80–88% after injection, indicating relatively low N immobilisation with all the digestates. NFRV in the crops was mainly explained by the NH_4^+-N/total N ratio, C/N ratio and dry matter content of the digestates. The findings suggest that electrokinetic and ultrasonication pre-treatments combined with a second AD step have no considerable impact on the fertiliser value of digestates, whereas a second AD step significantly reduced ammonia losses after application by surface-banding in winter wheat.

1. Introduction

To increase agricultural productivity while simultaneously minimising the environmental impacts of mineral fertilisers and reducing dependency on fertiliser imports, there is a need to increase nutrient substitution in synthetic fertilisers with waste-derived nutrients originating from bio-based sources (Vico et al., 2020). Anaerobic digestion (AD) is a bioenergy technique that can effectively recover nutrients from bio-based wastes. During the AD process, biowastes are degraded in an oxygen-free environment to produce biogas and digestate as a by-product. The resultant digestate is characterised by a high NH_4^+-N/total N ratio due to the mineralisation of organically bound N. This increases the plant-available nitrogen in the digestate, making it an alternative to substitute mineral fertilisers (Moller and Muller, 2012).

The degradation efficiency during AD depends on the nature of the biomass used as inputs in addition to the digestion conditions of the digesters, with the recalcitrance of biomass making it inefficient and uneconomical for the biogas plants. During the hydrolysis phase of AD, microorganisms produce several hydrolytic enzymes that might not be sufficient to break complex lignocellulosic compounds (Atelge et al., 2020). Consequently, digestates can have relatively high dry matter contents (>5%), which is problematic during field applications and limits infiltration during surface banding applications. The reduced infiltration enhances ammonia volatilisation, thus reduces the fertiliser

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value of the digestate and, at the same time, it causes adverse environmental impacts such as acidification and eutrophication (Webb et al., 2013). Moreover, the high C/N ratio and decomposable C in resultant digestates, associated with the lignocellulosic nature of substrates, may cause N immobilisation in the soil after amendment with digestates affecting the fertiliser value (Sørensen et al., 2003). Manure contributes greatly to the ammonia and greenhouse gas emissions in Europe, and appropriate manure treatment technologies to reduce losses during storage and field application need to be increasingly employed (Hou et al., 2017). As a result, there is a need for research on novel processing and innovative technologies to improve the AD process to enhance nutrient recovery and increase biogas yields from bio-based sources.

Integration of pre-treatment steps of biowastes in AD systems and the extension of the AD process by two-step AD could be some of the approaches to improve the fertiliser value of digestates. Pre-treatment of the biowastes enhances changes in their physical and chemical properties to increase biomass accessibility to enzymes and microbes and the potential bio-methane production (Sarker et al., 2019). The techniques can improve the operation stability, reducing solids and the hydraulic retention time (HRT) required to degrade recalcitrant biowastes (Meegoda et al., 2018). Pre-treatment techniques are categorised as physical, chemical, biological or a combination of the three methods. Their effects on the biowastes after pre-treatment and resultant digestates depend on the treatment mechanism and input substrate composition. Furthermore, optimisation of the biogas reactors' operating parameters, i.e. extending HRT by including two-step digestion, could also improve the fertiliser value of the digestates. This practice is being adopted in many biogas plants, especially in Denmark, to increase biogas yield (Møller and Nielsen, 2016). The two-step AD increases the residence time of the substrates in the reactors, allowing more time for the degradation of substrates than in one-step AD with a short HRT (Nyang'au et al., 2022). In short HRT reactors, a fraction of organic matter in the substrates remains in the digester for a very short period (the time spent between adding fresh material and the following emptying of a small fraction in the digester).

Several pre-treatment techniques, such as electrokinetic and ultrasonication pre-treatments, have been extensively evaluated for the pre-treatment of biowastes at a lab-scale level. Many have shown promising results in the biogas yield compared to untreated after AD (Bundhoo and Mohee, 2018; Mirmohamadsadeghi et al., 2021; Volschan et al., 2021). These techniques have varying effects on the biogas yield and changes in biochemical properties of the digestate, depending on the biowastes used and the operation mechanism. For instance, the electrokinetic pre-treatment technique, which treats biowastes through exposure to a high-voltage electric field, has been reported to increase biogas yield and soluble chemical oxygen demand in waste-activated sludge (Lee and Rittmann, 2011; Veluchamy et al., 2017). The positive and negative charges induce sudden disruption of the cellular membrane of the substrates, break ionic bonds, break the flocs apart, reduce particle size, and increase their surface area for hydrolysis bacteria, which enhances the AD process (Tyagi and Lo, 2011). Likewise, ultrasonication treatment at the lab-scale or pilot-scale level has been reported to increase biogas yields (Azman et al., 2020; Somers et al., 2018). Ultrasonication enhances substrate biodegradability by causing high-energy microbubbles' formation and vigorous collapse, which create hydro-mechanical shear forces in cavitation that disrupt the substrate structure resulting in reduced particle size, higher organic compound solubilisation and enzyme release (Zhen et al., 2017). Electrokinetic and ultrasonication techniques were considered for this study as they emerge as promising and cost-effective techniques compared to traditional mechanical techniques for pre-treating biowastes, such as chopping and grinding, which are energy- and capital-intensive techniques (Romio et al., 2022).

Despite the electrokinetic and ultrasonication pre-treatments widely studied on the laboratory and pilot scales and in the treatment of waste-activated sludge, there is limited information on their full-scale applications on livestock manures and other crop wastes co-digested with cattle manure. Moreover, it is unclear how they affect plant N availability in the digestates when combined with or without a secondary AD step. Therefore, this study aimed to evaluate the effects of electrokinetic and ultrasonication pre-treatment and the effects of two-step AD on nitrogen fertiliser replacement value (NFRV) of digestates applied to spring barley by injection and winter wheat by surface banding. Additionally, the study aimed to estimate ammonia losses through volatilisation and identify the relationship between the digestates biochemical properties and NFRV in winter wheat and spring barley crops. We hypothesised that the electrokinetic and ultrasonication pre-treatment techniques and two-step AD would modify digestate's physical-chemical properties, such as dry matter, viscosity and nutrient solubilisation, hence improving the fertiliser value of the digestates.

2. Material and methods

2.1. Substrate materials

Pre-digestates were obtained from Aarhus University, Foulum biogas plant (Tjele, Denmark) and Ausumgaard biogas plant (Hjerm, Denmark) running at different hydraulic retention times. The Foulum biogas reactor was managed at a short HRT (14 days), while the Ausumgaard biogas reactor was managed at a longer HRT (60 days). Both reactors were operated at thermophilic conditions (51 °C). Before sampling, the Foulum biogas reactor was fed 75% cattle slurry and 25% mixed grass-clover silage (solid biomass). The inputs for Ausumgaard biogas consisted of 55% slurry, a mixture of cattle manure and pig slurry in a wet weight ratio of 7:3 and 45% solid biomass, including deep litter, straw, chicken manure and grass.

2.2. Pre-treatment of substrates

Portions of digestates from Foulum (AD1) and Ausumgaard (AD2) biogas plants were treated separately by full-scale electrokinetic and ultrasonication devices with a treatment capacity of approximately 1.5 m^3/h of biowastes before the secondary AD step (+AD30), as illustrated in Fig. 1. Electrokinetic pre-treatment was done by BioCrack® electrokinetic disintegration equipment (Vogelsang GmbH & Co. KG, Germany) at an intensity of 4.37 kWh/ton. The equipment consisted of two modules, each operating at a power requirement of 35 W. Samples were pumped through a treatment chamber where a high-voltage field was generated between the internal and external electrodes. As the sample flows through the treatment chamber in high electric fields, the electrical forces break the flocculant structures and clumps, such as aggregates and colloids.

BioPush ultrasonic reactor (Weber Entec GmbH & Co. KG, Waldbronn, Germany) was utilised for ultrasonication pre-treatment at a frequency of 20 kHz. The substrate was pumped through the inlet, and the flow was adjusted to reach a treatment intensity of 2.88 kWh/ton. Material disintegration occurs by converting electrical oscillations into mechanical vibrations transmitted to the medium by a sonotrode, which results in an increased surface area and enhanced degradation of the organic matter. Both treatment techniques used substrates from the primary digestion step to protect the equipment, as they cannot disintegrate large solid biomass. After pumping the AD1 and AD2 through the treatment devices for 10 min to achieve stability, samples were collected using 20 L containers through the devices' modified valve outlets.

Each biogas reactor's pre-treated and non-treated substrates were divided into two portions. The first portion was stored at −18 °C until the field experiments were set up, while the second portion was further anaerobically digested in a secondary digestion step (Table 1). Additionally, the liquid inlet (cattle slurry) and solid biomass (grass-clover silage) inputs in the Foulum biogas plant were sampled and stored at −18 °C for field experiments. Samples of substrates used at the Ausumgaard biogas plant were not analysed as they were unavailable.

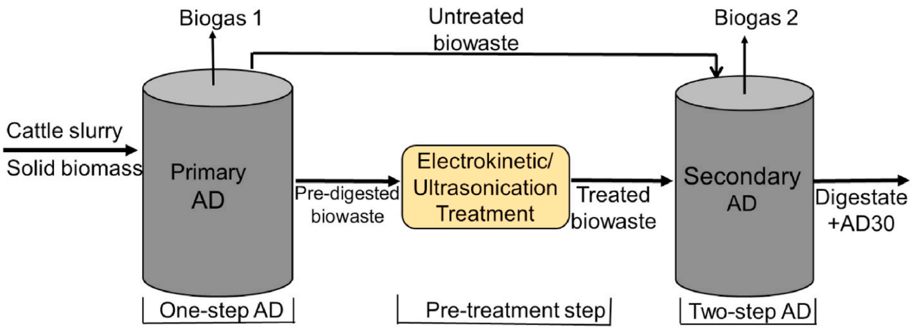

Fig. 1. Schematic representation of the two anaerobic digestion steps (primary and secondary) and pre-treatment step of digestates sourced from the primary AD step. The two digestates utilised had a contrasting HRT in the primary AD step. The primary AD step was in a flow reactor, while the secondary AD step (+AD30) was in a batch reactor.

Table 1
Overview of the substrates and digestates pre-treated either electrokinetically (EK) or ultrasonically (US) followed by a secondary digestion step (+AD30), which were applied to winter wheat and spring barley in field experiments.

Treatments Abbreviations	Biogas plant (AD1 = Foulum, AD2 = Ausumgaard)	Pre-treatment EK = Electrokinetic US = Ultrasonication	Secondary AD step + = 30days	Total HRT (Days)	Pre-treatment energy (kWh/ton)
Solid biomass	Input to AD1	–	–	–	–
Cattle slurry	Input to AD1	–	–	–	–
AD1	AD1	–	–	14	–
AD1 + EK	AD1	+EK	–	14	4.37
AD1 + US	AD1	+US	–	14	2.88
AD1^{+30AD}	AD1	–	+	44	–
AD1 + EK^{+30AD}	AD1	+EK	+	44	4.37
AD1 + US^{+30AD}	AD1	+US	+	44	2.88
AD2	AD2	–	–	60	–
AD2 + EK	AD2	+EK	–	60	4.37
AD2 + US	AD2	+US	–	60	2.88
AD2^{+30AD}	AD2	–	+	90	–
AD2 + EK^{+30AD}	AD2	+EK	+	90	4.37
AD2 + US^{+30AD}	AD2	+US	+	90	2.88

HRT=Hydraulic retention time.

2.3. Anaerobic digestion

The secondary AD step of the pre-treated and untreated pre-digested biowastes was done using six continuously stirred tank reactors (CSTR) with a working volume of 15 L in the batch reactors. Before start-up, all the reactors were filled with 1.5 L of inoculum. Three reactors were fed with untreated (AD1), Ultrasonicated pre-treated (AD1+US) or electrokinetically pre-treated (AD1+EK) digestates from the Foulum digester and digested in a second step at 51 °C for 30 days, resulting in digestates hereby referred to as AD1^{+AD30}, AD1+US $^{+\ AD30}$ and AD1+EK $^{+\ AD30}$ (Table 1). Three other reactors were fed with untreated (AD2), Ultrasonicated pre-treated (AD2+US) and electrokinetically pre-treated (AD2+EK) digestates from the Ausumgaard digester and digested at 51 °C for 30 days (secondary digestion step), resulting in digestates hereby referred to as AD2^{+AD30}, AD2+US $^{+\ AD30}$ and AD2+EK $^{+\ AD30}$ (Table 1).

The batch reactors were heated by electric blankets from the sides, and the temperature was monitored and controlled using temperature probes. Weekly sub-samples were taken from the reactors to analyse volatile fatty acids, ammonium nitrogen and pH to monitor the stability and possibility of inhibition in the AD process. Before collecting samples for analysis, the digestates were thoroughly homogenised using an electric-powered laboratory stirrer. Additionally, biogas yields were monitored weekly. After 30 days, the digestates were emptied manually and stored at −18 °C until spring 2021.

2.4. Field experiment

Field experiments were established in September 2020 and April 2021 at Aarhus University, Research Centre Foulum (56°30′N, 09°35′E). The soil in the site is loamy sand containing 83 g clay kg^{-1}, 284 g silt kg^{-1}, 610 g sand kg^{-1}, 31 g organic matter kg^{-1}, 15.6 g total C kg^{-1}, 1.3 g total N kg^{-1} and pH (1:2.5 H_2O) was 6.51. Winter wheat (*Triticum aestivum* L. CV KWS Extase) was sown in September 2020 with a 0.12 m distance between the rows. In mid-December 2020, 84 micro-plots were established after sprouting of wheat by inserting polyvinyl chloride (PVC) cylinders (diameter, 0.3 m; length 0.3 m, area = 0.0707 m^2) into the soil to a depth of 0.25 m. The micro-plots were 0.5 m from each other at a distance of 0.5 m within the rows, covering two winter wheat rows (Fig. S1). In April 2021, after spring ploughing and land harrowing, another set of 84 PVC cylinders were inserted in a nearby field in bare soil to be hand-seeded with spring barley after fertilisation.

In late March 2021, all winter wheat plots received an early mineral N application of 40 kg N ha^{-1} in ammonium nitrate. In April, the digestates from the primary AD step (untreated and pre-treated), digestates from the secondary AD step (untreated and pre-treated), cattle slurry and solid biomass from the Foulum biogas plant (Table 1) and five rates of liquid ammonium nitrate (0, 50, 100, 150 and 200 kg N ha^{-1}) were applied in the winter wheat micro-plots. The treatments were mixed with a small amount of highly enriched $(NH_4)_2SO_4$ (60 atom % ^{15}N) to achieve around 1 atom % excess ^{15}N in the ammonium-N immediately before application to the field. Next, the digestates and substrates were applied by surface banding at the rate of 150 kg total N ha^{-1}. The bands were placed between the two rows of winter wheat. Finally, the area surrounding the micro-plots was fertilised with granular NPK (21:4:10) at 147 kg N ha^{-1}. On 23rd April (digestate application day), the weather was partly cloudy and windy (6 m s^{-1}), and the air temperature was 5.1 °C. All treatments were applied in four replicates in a complete randomised block design.

In the spring barley micro-plots, the same treatments as in winter wheat (Table 2) were applied at the rate of 150 kg total N ha^{-1} before sowing. To simulate direct injection, the top 5 cm soil layer was removed to a bucket, a 5 cm slit made in the centre of each cylinder, then the ^{15}N-labelled treatments and five rates of liquid ammonium nitrate (0, 50, 100, 150 and 200 kg N ha^{-1}) were applied. The mineral N treatments were used to establish an N fertiliser response curve. The soil was then returned to the cylinders and compacted slightly by hand. The compaction was followed by hand seeding 26 kernels of spring barley (*Hordeum vulgare* L. CV RGT Planet) per micro-plot in two rows 0.12 m apart. The area around the cylinders was also seeded by hand using the same variety of spring barley and fertilised with granulated NPK (21:4:10) fertiliser at the rate of 126 kg N ha^{-1}. The spring barley treatments were organised in a complete randomised block design of four replicates arranged in two rows.

After applying the organic treatments, all the micro-plots were applied with essential nutrients in solutions. The nutrients were applied at rates equivalent to 25 kg P ha^{-1}, 99 kg K ha^{-1}, 15 kg S ha^{-1}, 100 Kg Ca ha^{-1}, 49 kg Mg ha^{-1}, 2.3 Kg Mn ha^{-1}, 0.26 Kg Zn ha^{-1}, 0.15 kg B ha^{-1}, 0.14 Kg Cu ha^{-1}, 0.06 Kg Mo ha^{-1} and 0.1 Kg Co ha^{-1}. The nutrients were added to ensure it was only nitrogen limiting crop growth in the experiment. The plots were treated with herbicides as the surrounding field, but hand-weeding was also performed in the micro-plots twice in May and June 2021. Early in July, all the micro-plots were irrigated manually once with 2.5 L of water, each equivalent to 35.4 mm of rainfall due to low rainfall. The total above-ground biomass (grain + straw) of the spring barley and winter wheat was harvested by hand in the second week of August 2021, leaving a stubble of 5 cm.

2.5. Weather and climate

The total rainfall received between October 2020 and July 2021 was 569 mm. The minimum rainfall was in February 2021, while the highest was in May 2021 (Fig. 2). Shortly before the onset of rainfall in early July, the crops were irrigated. The average temperature and soil

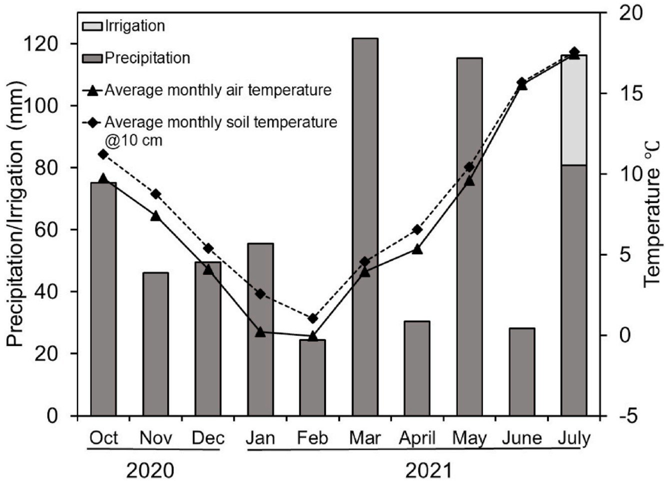

Fig. 2. Average monthly air temperature (fully line), average monthly soil temperature at 10 cm depth (dotted line) and monthly precipitation in mm during the experiment. The grey part indicates the amount of water irrigated by hand.

Table 2
Chemical composition of the substrates and digestates used in the field experiment. Values correspond to means and standard deviation in brackets (n = 3).

Treatments	Dry matter (% of FM)	Volatile solids (% of DM)	pH	Total N (% of FM)	NH_4^+-N (g 100 g^{-1} total N)	Total C (g 100 g^{-1} FM)	P (g kg^{-1} FM)	K (g kg^{-1} FM)	C/N ratio	Hemi-c (% of DM)	Cellulose (% of DM)	Lignin (% of DM)	NDSF (% of DM)	*Viscosity (Pa.s)
Solid biomass	32.1 (0.22)	93.9 (0.43)	–	0.66 (0.021)	7	16.54 (0.21)	0.76	3.63	25.1	23.9 (0.80)	30.8 (0.98)	8.6 (0.41)	36.9 (0.32)	–
Cattle slurry	5.0 (0.02)	76.9 (0.97)	7.09	0.24 (0.003)	52	2.26 (0.01)	0.46	2.51	9.4	14.0 (0.68)	16.3 (0.19)	8.6 (0.09)	60.2 (1.82)	–
AD1	7.6 (0.12)	80.3 (0.14)	7.52	0.37 (0.002)	46	2.35 (0.01)	0.49	2.96	6.4	13.8 (0.64)	24.6 (0.88)	15.5 (0.62)	46.4 (0.75)	10.0
AD1 + EK	7.7 (0.21)	80.4 (0.23)	7.54	0.34 (0.008)	49	2.35 (0.08)	0.53	2.96	6.9	11.2 (0.30)	23.9 (0.53)	15.7 (0.12)	48.9 (0.42)	6.5
AD1 + US	7.7 (0.02)	80.9 (0.08)	7.41	0.35 (0.004)	46	2.18 (0.09)	0.48	2.93	6.2	10.2 (0.12)	23.8 (1.24)	14.6 (0.73)	50.5 (0.15)	5.0
AD1^{+30AD}	6.3 (0.27)	79.1 (1.07)	7.55	0.33 (0.009)	62	1.94 (0.05)	0.37	2.95	5.9	7.9 (0.25)	22.4 (1.22)	17.3 (0.21)	52.3 (0.70)	–
AD1 + EK^{+30AD}	6.5 (0.06)	79.7 (0.25)	7.53	0.34 (0.004)	60	1.83 (0.15)	0.50	2.98	5.4	8.9 (0.30)	24.0 (0.31)	17.6 (0.30)	49.1 (0.70)	–
AD1 + US^{+30AD}	6.5 (0.03)	77.9 (0.43)	7.67	0.34 (0.001)	61	2.17 (0.17)	0.50	3.03	6.4	6.5 (0.30)	23.4 (0.80)	17.1 (0.30)	53.1 (0.16)	–
AD2	9.2 (0.09)	74.6 (0.18)	8.01	0.53 (0.001)	52	3.49 (0.13)	0.83	4.81	6.6	4.5 (0.05)	18.2 (0.24)	15.7 (0.60)	61.5 (0.33)	1.0
AD2 + EK	9.3 (0.19)	73.4 (0.83)	8.10	0.50 (0.011)	55	3.23 (0.23)	0.90	4.90	6.5	3.2 (0.05)	17.1 (0.76)	14.9 (0.54)	64.5 (0.52)	0.6
AD2 + US	9.3 (0.13)	74.8 (0.07)	8.07	0.51 (0.007)	54	3.72 (0.04)	0.89	4.90	7.3	3.4 (0.03)	17.1 (0.43)	14.4 (0.29)	64.6 (0.65)	0.8
AD2^{+30AD}	8.9 (0.02)	74.8 (0.43)	8.49	0.50 (0.004)	60	3.17 (0.10)	0.94	5.04	6.3	2.5 (0.15)	18.2 (0.70)	15.3 (0.20)	64.1 (0.51)	–
AD2 + EK^{+30AD}	9.3 (0.06)	74.0 (0.46)	8.41	0.53 (0.008)	57	3.43 (0.07)	0.89	5.41	6.5	2.6 (0.97)	16.8 (0.95)	14.6 (0.02)	64.9 (1.00)	–
AD2 + US^{+30AD}	8.6 (0.19)	73.3 (0.88)	8.36	0.51 (0.007)	60	3.38 (0.17)	0.84	5.14	6.6	2.8 (0.10)	17.0 (0.079	14.9 (0.26)	65.1 (0.16)	–

FM=Fresh matter, DM = dry matter, Hemi-c = hemicellulose, NDSF=Neutral detergent soluble fibre. For treatment abbreviations and explanations, refer to Table 1.
*1 Pa s (Pa.s) = 1000 cP (cp) = N*s/m^2 = kg/m/s.

temperature at 10 cm during the growing season were 7.4 °C and 8.5 °C, respectively. The lowest monthly average temperatures were recorded in January and February, while the highest average temperatures of up to 18 °C were recorded in July 2021 (Fig. 2).

2.6. Analytical methods

Total N in the substrates and digestates was analysed according to APHA (2005) by the Kjeldahl digestion method (Kjeltec™ 8400, Foss Analytical CO. LTD, Denmark) and ammonium-nitrogen by an automated distillation-titration method (Sommer et al., 1992) using Gerhardt Vapodest 10s distillation apparatus (Bonn, Germany). Acid detergent fibre (ADF), acid detergent lignin (ADL) and neutral detergent fibre (NDF) in the substrates and digestates were analysed to determine the hemicellulose, cellulose and lignin contents (Van Soest et al., 1991) using a Foss Fibertec 2010 System (Foss Analytical CO. LTD, Denmark). Total carbon content was analysed by elemental analyser (Vario MAX Cube, Elementar Analysensteme GmbH). Phosphorous and potassium in substrates and digestates were analysed by inductively coupled plasma-optical emission spectrometry. The apparent viscosity of the digestates was measured using Brookfield rotational viscometer model DV-II+ (Brookfield AMETEK GmbH, Germany). Approximately 600 g of the digestate was transferred to a beaker in water bath heating at 51 °C for 1 h, then mixed gently before submerging a spindle (disc spindle model LV#63), and the viscosity was measured at 5 rpm.

The harvested wheat and spring barley samples were oven-dried at 60 °C for 48 h for dry matter determination and then finely milled for total N and ^{15}N analysis using an elemental analyser (PDZ Europa ANCA-GSL) coupled to an isotope ratio mass spectrometer (PDZ Europa 20–20, Sercon Ltd., Cheshire, UK) at the Stable Isotope Facility, UC, Davis, USA (Table S1). For organic treatments, 1 g sample was immediately extracted with 100 ml 1 M KCL (in triplicate) and then frozen. Samples were then concentrated for ^{15}N analysis using a diffusion method described by Sørensen and Jensen (1991). Adding magnesium oxide into the flasks makes the N in the KCL extracts diffuse as NH_3 to acidified glass filter traps enclosed in Teflon tape. The samples were shaken gently daily on a horizontal shaker. After 72 h, the trap was taken out with a tweezer and dried in a desiccator. The dried trap was then transferred into a tin capsule for analysis (Sørensen and Jensen, 1991).

2.7. Calculations

Response curves were established based on the DM yield and N uptake of the crops fertilised with varying rates of mineral N fertiliser. From the response curves, the agronomic efficiency (AE) and N uptake efficiency (NUE) were calculated according to Jensen (2013), using equations (1) and (2):

$$AE = (DM_2 - DM_1) / (N_2 - N_1) \times 100\% \tag{1}$$

$$NUE = \left(N_{Uptake2} - N_{Uptake1}\right) / \left(N_2 - N_1\right) \times 100\% \tag{2}$$

where DM_1 is the total crop yield in the reference crops, DM_2 is the total crop yield in the fertilised crops, $N_{Uptake1}$ is the plant N uptake in reference plants, and $N_{Uptake2}$ is the N uptake in fertilised plants. N_1 and N_2 are the total N added by the treatments. Using equations (1) and (2), the nitrogen fertiliser replacement value (NFRV) of the treatments was calculated according to Jensen (2013) using equation (3) as follows:

$$NFRV = \left(AE_{Trt}/AE_{Min}\right) \times 100 \quad \text{Or} \quad NFRV = \left(NUE_{Trt}/NUE_{Min}\right) \times 100 \tag{3}$$

where AE_{Trt} is the agronomic efficiency in the crops fertilised with an experimental treatment, AE_{min} is agronomic efficiency in the crops fertilised with mineral fertiliser, NUE_{Trt} is the NUE in the experimental treatments, while $NUE_{min}$is the NUE in crops fertilised with mineral fertiliser. This method of NFRV estimation relies on the assumption that there is no interaction between the soil N pools. Using ^{15}N recoveries in the above-ground biomass (straw + grain), the NUE of the treatments was estimated, and a comparison was made using equations (4) and (5):

$$Ndf = \left(\frac{^{15}N\ excess\ \ in\ plant}{^{15}N\ excess\ \ of\ fertilizer}\right) \times Npu\ \left(Kg\ N\ ha^{-1}\right) \tag{4}$$

$$\%\ labelled\ ^{15}N\ Recovery = \frac{Ndf}{Nf} \times 100 \tag{5}$$

Where *Ndf* is the total N derived from ^{15}N-labelled fertiliser assimilated by crops based on the isotopic ratios, Npu is the plant total nitrogen uptake, whereas *Nf* is the total NH_4^+-N applied in Kg N ha^{-1}. The Mineral fertiliser equivalence (MFE) of labelled NH_4^+-N pool in digestates was calculated based on the ^{15}N recoveries from digestates in winter wheat and spring barley above-ground biomass relative to the average recoveries from labelled mineral fertilisers (Sørensen and Thomsen, 2005). The ammonia losses through volatilisation were estimated by calculating the differences in MFE between winter wheat and spring barley, assuming that other gaseous losses of labelled N were similar in the two crops.

2.8. Statistical analysis

The statistical software R version 4.0.3 (R Development Core Team, 2021) was used to analyse the data. Effects of pre-treatment techniques and two-step AD on DM yield, N uptake, labelled ^{15}N, and NFRV were assessed using the analysis of variance (ANOVA) test separately for winter wheat and spring barley. A three-way ANOVA was done to test the effects of the pre-treatment technique, manure type and digestion step on the NFRV and ^{15}N recoveries. Post-hoc comparisons were made using the Tukey HSD test to identify treatments significantly different from each other. Shapiro-Wilk tests and a visual examination of the residuals against the fitted values were performed to check normality and homoscedasticity assumptions. Linear regression models were used to calculate the relationship between the NFRV based on N uptake and biochemical characteristics of the organic treatment. The models were compared using Akaike's information criteria (AIC), which compares models and describes the kind of information lost. The lower the AIC, the better the model. The mineral reference curves were established using linear regression with N uptake as the response variable and N application as an explanatory variable. For all statistical tests, a significance level of 0.05 was used.

3. Results

3.1. Effect of pre-treatment and two-step digestion on biochemical characteristics of the digestates

The biochemical properties of the substrates and digestates are summarised in Table 2. The electrokinetic pre-treatment significantly ($p < 0.05$) increased the NH_4^+-N/total N ratio in AD1+EK and AD2+EK treated digestates; however, this increase in NH_4^+-N/total N ratio was offset after the secondary AD step (Fig. 3). The secondary digestion step (two-step AD) significantly ($p < 0.001$) increased the NH_4^+-N/total N ratio in the digestates, with a considerable effect observed in AD1 digestates sourced from the Foulum biogas plant running at a shorter HRT. The increase in NH_4^+-N/total N in both AD1 digestates ($AD1^{+AD30}$, AD1+US $^{+\ AD30}$ and AD1+EK $^{+\ AD30}$) ranged between 22 and 35% after the secondary AD step, whereas in AD2 digestates($AD2^{+AD30}$, AD2+US $^{+\ AD30}$ and AD2+EK $^{+\ AD30}$), it ranged between 4 and 15% (Fig. 3, Table 2). The two-step AD resulted in an average of 17% decrease in the DM content in AD1 digestates, whereas it resulted in a 4% decrease in AD2 digestates. The decrease in DM content was in line with the decrease in C content in the digestates, which ultimately reduced the C/N ratio in the digestates after the two-step AD (Table 2). Electrokinetic

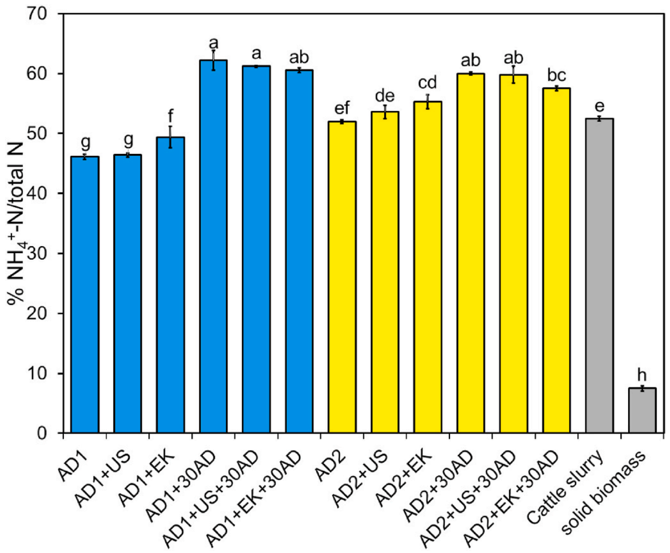

Fig. 3. Effect of pre-treatment techniques, electrokinetic (EK) and ultrasonication (US), and two-step-AD (+30AD) on percentage NH_4^+-N/total N in two digestates (AD1 and AD2) sourced from two biogas plants with contrasting HRT in a primary AD step. Letters indicate significant differences among the treatments estimated with Tukey's test ($p < 0.05$). Error bars indicate standard deviation ($n = 3$).

and ultrasonication pre-treatments reduced the viscosities of the digestates before the secondary AD step (Table 2).

The pre-treatments and two-step AD effects positively influenced fibre degradation in the organic treatments. Electrokinetic pre-treatment decreased the hemicellulose content by 11% and 29% in AD1+EK and AD2+EK, respectively. The combined effects of the pre-treatments and two-step AD reduced the hemicellulose by 20% and 42% in AD1+EK^{+AD30} and AD2+EK$^{+AD30,}$ respectively (Table 2). The hemicellulose reduced due to ultrasonication pre-treatment was 15% and 24% in AD1+US and AD2+US, respectively, whereas the reduction after two-step AD was 30% and 38% in AD1+US^{+AD30} and AD2+US^{+AD30} respectively. Additionally, the pre-treatment techniques positively increased the NDSF in the digestates, with the highest increase occurring after the secondary AD step. On average, the pH of both the digestates slightly increased after the two-step AD.

3.2. Dry matter yield and crop N uptake

The pre-treatments (electrokinetic and ultrasonication) and two-step AD tended to positively influence the winter wheat dry matter yields and N uptake, but the effects were statistically insignificant. The above-ground biomass DM yield ranged from 10 to 13 Mg DM ha^{-1}, with the lowest being in the solid biomass and the highest in AD2+US^{+30AD} digestate. The crop N uptake ranged from 105 to 145 kg N ha^{-1}; the highest uptake was in AD1+US^{+30AD}. The combined effects of ultrasonication pre-treatment and two-step AD in AD2+US^{+30AD} and AD1+US^{+30AD} digestates tended to increase plant DM yield and N uptake (Table 3). Electrokinetic pre-treatment before the second AD step tended to increase winter wheat DM yield and N uptake by 3–9%. The secondary AD step tended to increase the winter wheat N uptake, but the effect was insignificant (Table 3).

Table 3
Dry matter and crop N uptake of above-ground biomass of winter wheat measured after surface banding application and spring barley measured after induced direct injection of the digestates and manures. Values correspond to means and ±standard deviation ($n = 4$).

	DM yields (Mg ha^{-1})		Crop N uptake (Kg ha^{-1})	
Treatment	Winter wheat	Spring Barley	Winter wheat	Spring Barley
Solid biomass	10.0 ± 0.8^{a}	2.9 ± 0.5^{a}	105 ± 6.7^{a}	36 ± 3.4^{a}
Cattle slurry	10.3 ± 1.0^{ab}	8.3 ± 1.0^{b}	126 ± 19.0^{ab}	105 ± 14.2^{b}
AD1	12.5 ± 1.2^{ab}	8.8 ± 1.7^{b}	133 ± 14.0^{b}	107 ± 17.7^{b}
AD1 + EK	12.8 ± 1.8^{b}	9.9 ± 0.9^{b}	140 ± 10.6^{b}	106 ± 26.2^{b}
AD1 + US	11.7 ± 1.1^{ab}	8.6 ± 1.3^{b}	129 ± 4.2^{ab}	106 ± 21.8^{b}
AD1^{+30AD}	12.1 ± 0.9^{ab}	8.9 ± 0.6^{b}	142 ± 17.1^{b}	112 ± 7.8^{b}
AD1 + EK^{+30AD}	12.6 ± 0.6^{ab}	8.9 ± 0.7^{b}	142 ± 3.9^{b}	117 ± 24.2^{b}
AD1 + US^{+30AD}	12.9 ± 0.7^{b}	8.8 ± 0.4^{b}	145 ± 17.9^{b}	111 ± 22.7^{b}
AD2	10.9 ± 1.2^{ab}	7.8 ± 1.1^{b}	121 ± 6.0^{ab}	93 ± 7.3^{b}
AD2 + EK	11.9 ± 0.8^{ab}	8.2 ± 1.3^{b}	130 ± 10.7^{ab}	111 ± 16.5^{b}
AD2 + US	11.2 ± 1.1^{ab}	8.4 ± 1.7^{b}	128 ± 9.0^{ab}	106 ± 22.1^{b}
AD2^{+30AD}	11.9 ± 1.2^{ab}	8.0 ± 0.5^{b}	130 ± 13.2^{ab}	104 ± 2.8^{b}
AD2 + EK^{+30AD}	11.3 ± 1.6^{ab}	8.7 ± 0.8^{b}	127 ± 14.2^{ab}	112 ± 15.1^{b}
AD2 + US^{+30AD}	13.1 ± 1.2^{b}	8.0 ± 1.2^{b}	138 ± 9.5^{b}	109 ± 18.8^{b}

Means followed by different letters within each column are significantly different ($p < 0.05$).

Except for solid biomass, the spring barley DM yields and N uptakes were not significantly different between the differently treated manures ($p > 0.05$). The DM yield ranged between 3 and 10 Mg DM ha^{-1}, while the N uptake ranged from 36 to 117 kg N ha^{-1}. The highest DM yield was in AD1+EK, while the lowest was in solid biomass; for N uptake, the highest was in AD1+EK^{+30AD}. The second AD step tended to positively affect the spring barley N uptake, but the effect was insignificant. The pre-treatment techniques had no significant effects on the spring barley DM yield and N uptake (Table 3).

3.3. Fertiliser value of the manures in winter wheat and spring barley

The NFRV shows the amount of mineral fertiliser N that can be replaced by a given quantity of N in the digestates. The NFRV varied according to the choice of the reference based on either the DM or N use efficiency, but the trend was consistent. In winter wheat, based on the N uptake in the above-ground biomass, the NFRV varied from 14% in solid biomass to 51% in AD1+US^{+30AD}, while based on DM, it ranged from 16% (solid biomass) to 65% (AD2+US^{+30AD}). The electrokinetic pre-treatment tended to increase NFRV in digestates just before secondary AD compared to untreated digestates (AD1 and AD2) and ultrasonicated pre-treated digestates. However, these effects were offset after the secondary AD step (Table 4). On the other hand, the ultrasonication pre-treatment effect on digestates tended to increase NFRV in the digestates after the two-step AD (Table 4). The second digestion step tended to greatly influence the NFRV in the digestates, with the most significant

Table 4
Nitrogen fertiliser replacement value (NFRV, based on N uptake and DM yield) of the digestates and manures after surface banding to winter wheat and induced direct injection to spring barley. The fertiliser value is expressed as a percentage of total manure N applied. Values correspond to means ± standard errors ($n = 4$).

	% NFRV in winter wheat		% NFRV in spring barley	
Treatment	N uptake	DM yield	N uptake	DM yield
Solid biomass	14 ± 3^{a}	16 ± 4^{a}	10 ± 2^{a}	13 ± 4^{a}
Cattle slurry	38 ± 10^{ab}	21 ± 8^{ab}	79 ± 8^{b}	90 ± 8^{b}
AD1	44 ± 7^{b}	55 ± 11^{bc}	77 ± 4^{b}	80 ± 1^{b}
AD1 + EK	46 ± 6^{b}	61 ± 16^{c}	86 ± 14^{b}	90 ± 15^{b}
AD1 + US	35 ± 2^{ab}	57 ± 0^{bc}	74 ± 7^{b}	83 ± 2^{b}
AD1^{+30AD}	48 ± 8^{b}	55 ± 7^{bc}	89 ± 2^{b}	100 ± 4^{b}
AD1 + EK^{+30AD}	48 ± 2^{b}	57 ± 6^{bc}	85 ± 13^{b}	103 ± 6^{b}
AD1 + US^{+30AD}	51 ± 6^{b}	63 ± 6^{c}	84 ± 9^{b}	102 ± 4^{b}
AD2	33 ± 1^{ab}	36 ± 4^{ac}	72 ± 2^{b}	75 ± 3^{b}
AD2 + EK	40 ± 5^{ab}	43 ± 7^{ac}	82 ± 9^{b}	85 ± 11^{b}
AD2 + US	38 ± 1^{ab}	40 ± 9^{ac}	74 ± 4^{b}	78 ± 5^{b}
AD2^{+30AD}	36 ± 6^{ab}	49 ± 13^{ac}	83 ± 2^{b}	90 ± 4^{b}
AD2 + EK^{+30AD}	37 ± 7^{ab}	58 ± 2^{bc}	84 ± 8^{b}	91 ± 11^{b}
AD2 + US^{+30AD}	43 ± 5^{b}	65 ± 11^{c}	88 ± 3^{b}	91 ± 9^{b}

Means followed by different letters within each column are significantly different ($p < 0.05$).

effect on average being in AD1 digestates, where there was an increase of 18% in NFRV versus 5% in AD2 digestates (Table 4). Electrokinetic and ultrasonication effects had no significant effect ($P > 0.05$) on the winter wheat NFRV. Whether based on DM yield or N uptake, the NFRV variations among each treatment were relatively high (Table 4, Fig. S2, Fig. S3).

On average, the NFRV of digestates in spring barley was two-fold higher than in winter wheat. Based on the N uptake, the NFRV ranged from 10% in solid biomass to 89% in AD1^{+AD30} treatment, while it ranged from 13 to 103% when based on the DM yield of the barley (Table 4). The NFRV was not significantly different among digestates and only significantly lower ($p < 0.05$) for the solid biomass. Among the digestates, before the two-step AD, the NFRV trend was AD < AD + US < AD + EK, while after the secondary AD step of the digestates, the trend of NFRV was AD < AD + EK < AD + US. Like in winter wheat, the positive electrokinetic pre-treatment effect on NFRV was offset after the secondary AD step. Both the pre-treatments and two-step digestion had an insignificant effect ($p > 0.05$) on the NFRV in spring barley (Table S2).

3.4. *The fate of ^{15}N applied to winter wheat and spring barley*

The second digestion step and source of the digestates significantly ($P < 0.0001$) influenced the ^{15}N recoveries and % MFE in winter wheat (Table 5, Table S2). A post hoc comparison among the treatments revealed that ^{15}N recoveries and % MFE were higher for AD1 digestates than for AD2 digestates. Both the ^{15}N recoveries and % MFE of the digestates followed the same trend with the calculated NFRV based on the traditional non-isotopic method from the mineral N response curves, with the isotopic method having less variability among the treatments. The electrokinetic pre-treatment tended to improve the ^{15}N recoveries from the digestates before two-step AD. In contrast, the ultrasonication pre-treatment tended to improve the ^{15}N recoveries after the second digestion step (Table 5). In spring barley, ^{15}N recoveries and % MFE were not significantly different among the treatments (Table 5).

The second digestion step significantly ($p = 0.0219$) reduced ammonia losses after surface banding of digestates in winter wheat. A post hoc test indicated a more significant effect ($P < 0.0001$) in digestates sourced from the shorter HRT reactor (Table 5, Table S2). The two-step AD reduced the NH_3 losses by 40% in AD1 digestates, whereas the NH_3 losses were insignificantly reduced in AD2 digestates (Table 5). The estimated negative N losses from solid biomass indicate possible higher N immobilisation after simulated injection in spring barley compared to surface-banding. There was a significant interaction between the second digestion step and the source of the digestates ($p = 0.0047$) and their effects on the ammonia losses (Table S2).

3.5. *Relationship between NFRV (based on crop N uptake) and biochemical properties of the experimental treatments*

The relationship between the NFRV (based on the N uptake) in winter wheat and spring barley was compared with the biochemical properties of the organic treatments, as shown in Table 6, with linear regression models and Akaike's information criterion (AIC). There were significant positive correlations between NFRV and NH_4^+-N/total N in winter wheat and spring barley ($p < 0.001$). The C/N ratio and DM content negatively and significantly correlated with the NFRV in winter wheat and spring barley (Table 6). The NFRV in spring barley and winter wheat showed a negative correlation with both hemicellulose and cellulose in digestates (Table 6).

4. Discussion

4.1. *Effect of pre-treatments (electrokinetic and ultrasonication) and two-step AD on biochemical properties of substrates*

The solubilisation of the organic matter in biowastes during AD depends on how the lignin protects the hemicellulose and cellulose, which hinders microbial and enzymatic attack (ŞEnol, 2020). Additionally, biodegradation and solubilisation of organic matter in the biowastes are influenced by the operation mechanism of the pre-treatment technique, application time, densities and nominal specific energy values (Bundhoo and Mohee, 2018; Mirmohamadsadeghi et al., 2021). The observed significant effects of electrokinetic pre-treatment on NH_4^+-N/total N ratio compared to ultrasonication pre-treatment could be attributed to its operation mechanism. The charges created by the high voltage field cause shock waves and induce sudden rupture of substrates' cell walls and microbial cell membranes, making nutrients readily available in the digestates (Volschan et al., 2021). Therefore, we presume that most of the NH_4^+-N released after the treatment are derived from microbes in the manure. The increase in NH_4^+-N/total N ratio after electrokinetic pre-treatment is consistent with the findings of Westerholm et al. (2016) and Rittmann et al. (2008), who found an increase in NH_4^+-N by 25–53% and 15%, respectively, after full-scale treatment of sludge at varying

Table 5

Recovery of labelled ammonium-N, mineral fertiliser equivalence (MFE) of ammonium-N in digestates calculated based on ^{15}N uptake in winter wheat and spring barley in above-ground biomass relative to ^{15}N uptake in mineral fertiliser and estimated ammonia losses in winter wheat after surface banding application of organic fertilisers. The ammonia loss is estimated from the difference in MFE between spring barley and winter wheat, assuming no ammonia loss by injection to spring barley.

	Winter wheat		Spring Barley		Estimated % NH_4^+-N losses
	% Recovery	% MFE	% Recovery	% MFE	
Solid biomass	30.2^{acd}	50.3(1.7)	4.5^{a}	10.4(0.7)	-39.9^{a}
Cattle slurry	34.5^{def}	57.3(3.4)	34.1^{b}	77.9(1.8)	20.6^{bcd}
AD1	31.1^{bcd}	51.7(1.4)	36.0^{b}	82.4(1.0)	30.7^{de}
AD1 + EK	32.2^{cf}	53.6(0.7)	36.4^{b}	83.2(5.9)	29.7^{cde}
AD1 + US	31.6^{bce}	52.5(2.4)	37.2^{b}	85.1(4.7)	32.6^{e}
AD1^{+AD30}	37.7^{ef}	62.6(1.8)	36.4^{b}	88.3(1.3)	20.7^{bcd}
AD1 + EK$^{+ AD30}$	37.6^{f}	62.6(1.3)	35.7^{b}	81.5(3.1)	19.0^{bc}
AD1 + US$^{+ AD30}$	38.5^{ef}	63.9(0.1)	35.1^{b}	80.2(3.1)	16.3^{b}
AD2	25.4^{a}	42.3(0.9)	34.9^{b}	79.7(6.5)	37.4^{e}
AD2 + EK	26.4^{ac}	43.9(1.3)	35.5^{b}	81.1(4.1)	37.3^{e}
AD2 + US	26.3^{ab}	43.7(1.3)	37.5^{b}	83.7(1.7)	40.0^{e}
AD2^{+AD30}	26.1^{ab}	43.4(1.9)	36.3^{b}	83.0(0.6)	39.6^{e}
AD2 + EK$^{+ AD30}$	25.7^{ab}	42.8(1.0)	36.2^{b}	82.8(4.6)	40.0^{e}
AD2 + US$^{+ AD30}$	27.6^{ac}	45.9(1.5)	37.3^{b}	85.3(6.1)	39.4^{e}
Mineral N	60.1(2.7)	–	43.8(2.6)	–	–

Means followed by different letters within each column are significantly different ($p < 0.05$). For mineral N and %, MFE values correspond to averages and standard errors in brackets ($n = 4$). The reference treatment with mineral N was not included in the statistical analysis.

Table 6
Relationship between NFRV (y) based on the N uptake in winter wheat and spring barley and biochemical properties (x) of manures and digestates.

Parameter (x)	Winter wheat				Spring barley			
	Linear regression	p-value	R^2	AIC	Linear regression	p-value	R^2	AIC
NH_4^+-N/total N	y = 11 + 0.54x	<0.001	0.83	93	y = 6 + 1.36x	<0.001	0.96	96
C/N ratio	y = 51–1.50x	<0.001	0.82	93	y = 106–3.79x	<0.001	0.96	95
DM (%)	y = 51–1.17x	<0.001	0.85	91	y = 104–2.86x	<0.001	0.96	96
pH	y = 76–4.40x	0.260	0.34	88	y = 68 + 1.75x	0.655	0.14	89
N (% of DM)	y = 9 + 6.07x	0.014	0.64	102	y = −12 + 17.73x	<0.001	0.87	111
Hemicellulose (% of DM)	y = 45–0.72x	0.084	0.48	105	y = 95.92–2.39x	0.003	0.74	120
Cellulose (% of DM)	y = 50–0.52x	0.031	0.25	108	y = 134–2.77x	0.022	0.61	124
Lignin (% of DM)	y = 3.4 + 2.46x	0.003	0.74	98	y = 5 + 4.86x	0.008	0.68	122

AIC = Akaike's information criterion.

energy intensities using electrokinetic devices.

The levelling-off and reduction in the NH_4^+-N/total N ratio in the electrokinetically treated digestates after the two-step AD could be linked to instability in the AD process or microbial utilisation of the released C and N. The increase in pH after electrokinetic pre-treatment could be attributed to the formation of ammonium carbonate and removal of CO_2 as a result of the transformation of carbonates and hydrogen atoms to carbon dioxide and water (Moller and Muller, 2012). However, the results contradict the findings of Touch et al. (2017), who reported a decrease in pH after electrokinetic pre-treatment of sediments, which they attributed to the release of protons due to oxidation of the organic compounds at the anode.

The second AD step significantly increased the NH_4^+-N/total N ratio in the digestates, which is explained by the enhanced mineralisation of organically bound N (Moller and Muller, 2012) due to the prolonged residence time in the biogas reactors. The shift, for instance in Denmark, towards the co-digestion of manure with agricultural wastes such as straw, grass and deep litter with relatively low biodegradability combined with short HRT (15–30 days) results in digestate characteristics with higher dry matter content, which ultimately influences its NUE and management during storage and field applications. In this study, a two-step AD process was exploited to prolong the retention time of the digestates in the reactor. The extended retention time improves the AD process's stability, resulting in more enhanced nutrient solubilisation and mineralisation in digestates, as found in this study. The findings align with those of Feng et al. (2017), who reported an improved AD process with increased biomethane yield using a serial continuous stirred tank reactors AD process in a co-digestion set up with meadow grass. The enhanced degradation of the organic matter by an extended AD stabilises the digestates in addition to the improved fertiliser value, reducing possible residual methane potential during storage and application (Nyang'au et al., 2022; Zilio et al., 2022).

The differences in the effect of the second AD step on AD1 and AD2 digestates is attributed to the fact that AD1 digestates were sourced from a reactor with shorter HRT. The prolonged digestion of AD2 in the primary step would have resulted in the complete degradation of decomposable matter, resulting in a negligible effect after the secondary AD step. The 20–50% decrease in viscosity just after pre-treatment of digestates could be attributed to a decrease in DM content due to physical disruption and the effect on the particle size distribution. The improvement of rheological properties of the digestates, such as viscosity, would improve mixing during AD and allow higher organic loading rates, reducing the cost of operation of the biogas plants and improving digestate infiltration into the soil after application, reducing ammonia volatilisation (Romio et al., 2022).

4.2. *Effects of the pre-treatments and two-step AD on NFRV and ^{15}N recoveries in winter wheat and spring barley*

The significant effect of the treatments on the ^{15}N recoveries and MFE in winter wheat could be attributed to differences in ammonia loss from the labelled NH_4^+-N in the manures. The tended higher ^{15}N recoveries in winter wheat from ultrasonicated treated digestates after two-step digestion may be linked to the enhanced AD process and reduced digestates' viscosities. The reduction of DM content during the two-step AD process makes the digestate less viscous, making it infiltrate faster into the soil, decreasing the proportion of ammonia exposed to the atmosphere (Webb et al., 2013). The enhanced infiltration minimises ammonia volatilisation and consequently improves the fertiliser value of the digestate. The improved digestate properties of ultrasonicated treated digestates are consistent with Gong et al. (2015). They observed disruption of the sludge morphology and increased soluble total N and protein concentration after ultrasonication treatment for 20 min.

The relatively low NFRV and ^{15}N recoveries in winter wheat after application of AD2 digestates compared to AD1 digestates after two-step AD, despite having a similar NH_4^+-N/total N ratio, could be linked to a relatively higher DM content, which could reduce soil infiltration after application, increasing ammonia volatilisation (Pedersen et al., 2021). This is exacerbated by relatively higher digestate pH and sunny-windy days immediately after digestate application (Sommer and Hutchings, 2001), as experienced in this study. The volatilisation reduces the available N for plant uptake and, consequently, the NFRV and ^{15}N recoveries. This is consistent with the observed significant negative correlation between DM content and NFRV of the digestates in winter wheat. Similarly, De Notaris et al. (2018) reported unexpected reduced NFRV following surface banding of some green manures in winter wheat, which they attributed to ammonia losses due to a higher DM content.

The NFRV of the digestates in spring barley in our study, on average, were 30% and 80% higher than the average NFRV of the digestates (from co-digestion of cover-crops and straw) reported by De Notaris et al. (2018) and Fontaine et al. (2020) respectively. These differences could be attributed to input feedstock in the AD reactors. For instance, some of the digestates used by Fontaine et al. (2020) were from a co-digestion of cattle manure and wheat straw, which has relatively low N content, while the primary substrate for AD1 digestate used in this study was a grass-clover mixture with a higher N concentration. In general, both one-step and two-step AD of biowastes had a positive effect on the NFRV of the digestates, i.e. a 39% increase in NFRV in winter wheat through primary co-digestion of solid biomass and cattle slurry (inputs of AD1), and a further 9% increase in NFRV following a second digestion step of AD1digestates. This is attributed to the enhanced mineralisation of organically bound nitrogen by AD.

The recovery of ^{15}N from digestates in spring barley was lower than the recovery from mineral N fertiliser, indicating extra microbial immobilisation in the soil of ammonium-N from the digestates. The ratio between the crop ^{15}N recovery from cattle slurry and mineral N fertiliser, here called MFE, was 78% and similar to the 79% found after the injection of cattle slurry by Sørensen (2004) in the same soil type. The MFE of ammonium-N in digestates was slightly higher than for the cattle slurry at 80–88%, indicating that the digestates still caused significant N immobilisation due to the decomposition of organic matter.

The recovery of labelled mineral fertiliser N in spring barley, at 44%, was slightly lower than in a previous study by Sørensen and Thomsen (2005), who found recoveries of 48–56% in spring barley on a similar soil type. This difference could possibly be explained by different climatic conditions that might also influence apparent added nitrogen interactions caused by pool substitution explained by labelled N standing proxy for unlabelled soil N that would otherwise have been immobilised (Jenkinson et al., 1985). Soil microbes prefer ammonium-N to nitrate, and after application of ^{15}N-labelled ammonium, immobilisation-mineralisation reactions incorporate mostly ^{15}N into microbial biomass, causing increased release of unlabelled N into the inorganic N pool, which is ultimately utilised by plants (Jenkinson et al., 1985).

4.3. Estimated ammonium nitrogen losses and differences in NFRV between winter wheat and spring barley

The differences in the NFRV, MFE and recoveries in winter wheat and spring barley are linked to the method of application and timing. Direct injection of digestates minimises exposure to the atmosphere, mitigating ammonia volatilisation alongside triggering other beneficial benefits in the soil (Webb et al., 2013). This increases the fertiliser value of the digestates to meet the plant N demand throughout its growth period (De Notaris et al., 2018). In winter wheat, digestates were surface banded in the growing crop, and the application method increased the risks of ammonia volatilisation and consequently reduced the NFRV of the digestates (Webb et al., 2013). Despite the crop canopy being able to mitigate NH_3 volatilisation through interception by absorbing some NH_3 to the leaves (Sommer and Hutchings, 2001), during the time of application of digestates in this study (early spring), the winter wheat had only a small canopy which compounded the volatilisation risk. The reduction efficiencies of ammonia emissions by simulated injection in spring barley were consistent with those of Wagner et al. (2021).

The reduction of the ammonium-N losses by the secondary AD step is attributed to the reduction of DM content, which influences digestates' viscosity and, ultimately their infiltration into the soil. This reduction only occurred with AD1 digestates, and the high ammonium-N losses from AD2 digestates, both after the first and second AD step, could be linked to higher DM content and pH than in AD1 digestates. This could be explained by factors such as exposed surface area as a function of time after application, increasing the risk of volatilisation (Pedersen et al., 2021).

This study shows that using % MFE based on the ^{15}N recoveries in winter wheat and spring barley can estimate the ammonia losses from the digestates after surface banding application. However, caution should be taken as ammonia volatilisation maybe be overestimated in ^{15}N balance measurements as part of volatilised labelled N can be reabsorbed by plants in the field but not in the same plot where it was applied (Sørensen and Amato, 2002). The unaccounted ^{15}N in both winter wheat and spring barley could be linked to either ammonia volatilisation (for the case of wheat), leaching, denitrification, remains in soil, stubbles and roots (Sørensen and Thomsen, 2005).

4.4. Relationship between the digestates' biochemical properties and NFRV in winter wheat and spring barley crops

Correlations between the NFRV and biochemical properties of the digestates could be used to predict the plant-available N. For instance, the high coefficient of determination in the correlations between NH_4^+-N/total N and NFRV indicates that NH_4^+-N/total N could explain most of the variation in NFRV in both winter wheat and spring barley, and this is consistent with previous studies (De Notaris et al., 2018; Fontaine et al., 2020; Jensen, 2013). Moreover, the negative correlations between NFRV in the crops with C/N ratio and DM content demonstrate that they can equally be used to predict plant-available N in addition to NH_4^+-N/total N ratio, and this is consistent with other studies (Pedersen et al., 2020; Sørensen et al., 2003; Webb et al., 2013). The negative correlation between NFRV in winter wheat and DM content in digestates could be linked to reduced fertiliser value due to ammonia losses via volatilisation. However, relating NH_3 losses exclusively to dry matter may be too simple as other factors play a role, such as weather conditions, crop height, digestate pH, viscosity, total ammoniacal nitrogen and application method (Hafner et al., 2018), and also need to be considered. The high C/N ratio and DM content in solid biomass explain the low NFRV in winter wheat and spring barley. A high C/N ratio in digestates causes immobilisation of N, as the soil microbes need extra energy to decompose the organic matter (Cavalli et al., 2017).

4.5. Perspectives

The present study confirmed the poor utilisation of N in digestates by surface-banding in growing crops like winter wheat due to high ammonia losses. This is a problem in many areas where winter cereals are dominant crops, like in Denmark, especially because of the increased use of dry matter-rich biomasses on biogas plants increasing the dry matter content of digestates. We found insignificant effects of the pre-treatments used with or without a post-digestion on the N fertiliser value of digestates, and the use of these pre-treatments cannot be justified by an improved fertiliser value of the digestates. Post-treatment of digestates is probably a more effective measure, e.g. by solid-liquid separation where the liquid fraction can be used in growing crops while the solid fraction should be carefully handled to avoid ammonia losses and other gaseous emissions (Sørensen and Thomsen, 2005). However, considering the tendencies of the two pre-treatment techniques to increase the N fertiliser value, reduce digestate viscosities and increase methane production with a positive energy balance, as found by Romio et al. (2022), may make their integration into the biogas plants ultimately economically profitable. Our study also indicates that a secondary digestion step could reduce the dry matter content and potential ammonia loss after field application under conditions with a short HRT in the first digestion step.

5. Conclusions

This study shows that the secondary digestion step, which prolongs the retention time of digestates in the biogas reactors, could improve the nitrogen use efficiency in crops through increased nutrient solubilisation and a change in the digestate's biochemical properties. The secondary digestion step significantly reduced ammonia losses after surface banding of digestates in winter wheat, with a significant effect observed in digestates sourced from a shorter hydraulic retention time reactor. The electrokinetic pre-treatment step significantly increased the NH_4^+-N/total N ratio in the digestates before the second AD step but had no significant effect on the N fertiliser value in winter wheat and spring barley. The ultrasonification treatment prior to a secondary AD step also showed no significant effect on the N fertiliser value. The method of application of the digestates to winter wheat (surface banding) and spring barley (simulated injection) greatly influenced the NFRV in the crops. Lastly, the study reveals that the fertiliser value of the digestates could be predicted by the NH_4^+-N/total N ratio, C/N ratio or dry matter content of the digestates.

Credit authors statement

Jared Nyang'au: Conceptualization, Investigation, Methodology, Formal analysis, Visualization, Writing – original draft. Henrik B Møller: Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition. Peter Sørensen: Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2022.116699.

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