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Source separation and anaerobic co-digestion of blackwater and food waste for biogas production and nutrient recovery

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ABSTRACT

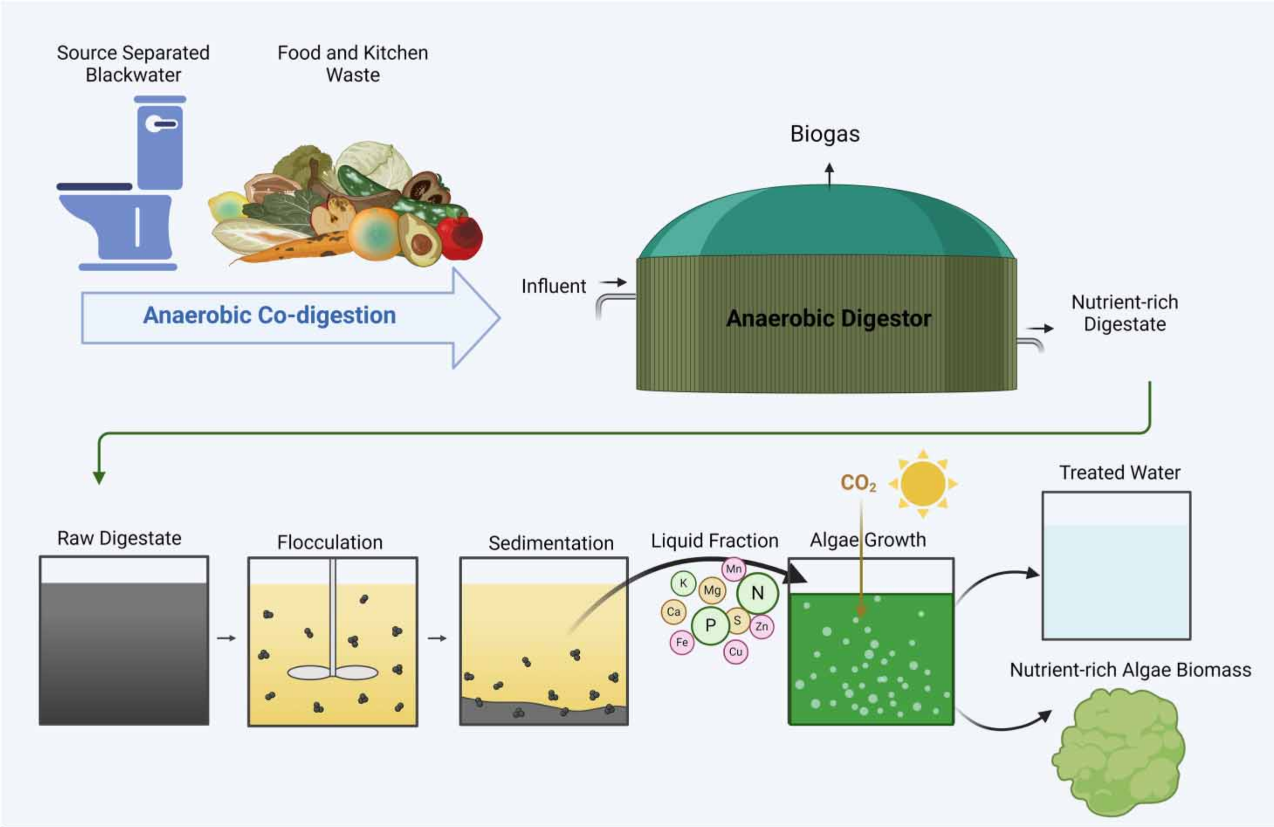
Anaerobic co-digestion of source-separated blackwater (BW) and food and kitchen waste (FW) offers decentralized circular economy solutions by enabling local production of biogas and nutrient-rich byproducts. In this study, a 2 m^3 pilot-scale continuously stirred tank reactor (CSTR) operated under mesophilic conditions was utilized for co-digestion of BW and FW. The process obtained a CH_4 yield of 0.7 $\pm$ 0.2 m^3/kg influent-volatile solid (VS), reaching a maximum yield of 1.1 $\pm$ 0.1 m^3/kg influent-VS, with an average organic loading rate of 0.6 $\pm$ 0.1 kg-VS/m^3/d and HRT of 25 days. The CH_4 production rate averaged 0.4 $\pm$ 0.1 m^3/m^3/d, peaking at 0.6 $\pm$ 0.1 m^3/m^3/d. Treatment of digestate through flocculation followed by sedimentation recovered over 90% of ammonium nitrogen and potassium, and 80–85% of total phosphorus in the liquid fraction. This nutrient-rich liquid was used to cultivate *Chlorella vulgaris*, achieving a biomass concentration of 1.2 $\pm$ 0.1 g/L and 85 $\pm$ 3% and 78 $\pm$ 5% ammonium nitrogen and phosphorus removal efficiency, respectively. These findings not only highlight the feasibility of anaerobic co-digestion of source-separated BW and FW in local biogas production but also demonstrate the potential of microalgae cultivation as a sustainable approach to converting digestate into nutrient-rich algae biomass.

Key words: anaerobic co-digestion, blackwater, food waste, microalgae, nutrient recovery, source separation

HIGHLIGHTS

- Anaerobic co-digestion of blackwater and food waste in a 2 m^3 CSTR.
- The maximum CH_4 production rate was 0.6 $\pm$ 0.1 m^3 per m^3 reactor volume per day.
- CH_4 productivity was 50 $\pm$ 1% higher on the pilot-scale than in laboratory-scale BMPs.
- Flocculation followed by sedimentation preserved >90% of macronutrients in digestate.
- *Chlorella vulgaris* treatment reduced total and dissolved COD by 50 $\pm$ 10%.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Every year, over one-third of the food produced for human consumption is wasted (Boliko 2019), amounting to approximately 1.3 billion tons globally, with an equivalent CO_2 emission of 30–40 billion tons (Katajajuuri *et al.* 2014; Counts 2024). Food and kitchen waste (FW) is predominantly disposed of in landfills in many regions of the world, leading to uncontrolled degradation, odor, significant nutrient and energy loss, and substantial greenhouse gas emission (Xue *et al.* 2024). Besides landfilling, biowaste, including food waste, is often processed through anaerobic digestion (AD) producing biogas and a nutrient-rich digestate that can be used directly or after preprocessing as a fertilizer. Source-separated blackwater (BW), consisting of urine and feces and containing the majority of the nutrients present in municipal wastewater (Wang *et al.* 2023), is currently diluted with other wastewater streams, and treated at centralized wastewater treatment plants. These plants consume considerable amounts of energy for water pumping and aeration of the activated sludge process (Eshetu Moges *et al.* 2018).

In the context of a circular economy, one approach involves source separation of BW from other municipal wastewater using minimal transport water followed by anaerobic co-digestion with FW, resulting in a nutrient-rich digestate (Kujawa-Roeleveld & Zeeman 2006). To enable nutrient recovery from municipal wastewater, the collection of BW with water-conserving toilets (e.g., vacuum toilets), followed by local and decentralized treatment, has been proposed and has the potential to facilitate 80–95% nutrient recovery (Gao *et al.* 2019). In BW, the nutrients are in a concentrated form and are separated from various heavy metals, microplastics, and chemicals present in municipal wastewater collected at centralized wastewater treatment plants (Eshetu Moges *et al.* 2018). Source-separated BW typically has a high chemical oxygen demand (COD) of $>$10 g/L and ammonium content of $>$1 g/L, resulting in a low carbon-to-nitrogen (C:N) ratio, making the AD process challenging due to potential ammonia inhibition (Gao *et al.* 2019; Zhang *et al.* 2019). The anaerobic co-digestion of FW and BW improves the buffering capacity and C:N ratio of the process, providing macro and micronutrients for the microbes (Lim *et al.* 2014; Wang *et al.* 2020).

Several studies have explored the co-digestion of BW and FW for biogas production (Kjerstadius *et al.* 2015; Zhang *et al.* 2019; Wang *et al.* 2020; Fendel *et al.* 2022), yet only a few have examined the nutrient fate and recovery (Kjerstadius *et al.* 2015; Giwa *et al.* 2022) and mostly in laboratory-scale reactors (Rajagopal *et al.* 2013; Minale & Worku 2014; Gao *et al.* 2019; Zhang *et al.* 2019; Gao *et al.* 2020; Wang *et al.* 2020; Zhang *et al.* 2021; Giwa *et al.* 2022). To date, the only pilot-scale study reported in continuously stirred tank reactors (CSTRs) has employed a 0.63 m^3 reactor for co-digestion of BW and FW, attaining a CH_4 yield of 222 to 332 L per kg-COD removed and a COD removal efficiency of 70–78% (Wasielewski *et al.* 2016). However, there have been no additional studies on the co-digestion of FW and BW in a pilot-scale in a CSTR system, which is typically used in the industry.

Depending on the feedstock used for AD, digestate can be preprocessed to separate it into liquid and solid fractions using energy-intensive industrial-scale techniques such as screw press, centrifugation, evaporation, or membrane technologies (Fuchs & Drosg 2013). In wastewater treatment, other separation techniques include flocculation followed by sedimentation. Several studies have reported the effectiveness of digestate flocculation (Bauer *et al.* 2021; Chini *et al.* 2021; Zuo *et al.* 2021), with some specifically applying both flocculation and sedimentation (Zhou *et al.* 2019; Bauer *et al.* 2021). The resultant solid digestate can be composted, utilized in hydrothermal processes for generating heat and biochar (Fuchs & Drosg 2013; Xia & Murphy 2016), or transformed into fertilizer granules (Czekała *et al.* 2022). However, there are relatively few studies on the use of liquid digestate. Liquid digestate from various sources has been employed for cultivating microalgal biomass (Erkelens *et al.* 2014; Parichehreh *et al.* 2019; Tan *et al.* 2022), yet no studies have been reported on microalgae cultivation using digestate derived from the co-digestion of BW and FW.

Digestate from AD of FW can be directly applied to the land as fertilizer without preprocessing. However, co-digestion with BW would limit the application of digestate on agricultural land, as in many countries, the use of digestate originating from municipal wastewater treatment and sewage sludge is restricted, for instance, due to its heavy metal content and potential presence of pharmaceuticals, microplastics, etc. The challenges of digestate treatment raise questions about the circularity of the biogas industry when treating municipal wastewater or sewage sludge. Besides direct use as fertilizer, digestate can serve as a nutrient source in other biological processes, such as the biological hydrogen methanation process (Kamravamanesh *et al.* 2023) or for cultivation of microalgae like *Chlorella vulgaris* (*C. vulgaris*) (Parichehreh *et al.* 2019) and *Scenedesmus acuminatus* (*S. acuminatus*) (Alves *et al.* 2019), for the production of biodiesel and/or bioenergy. Microalgae can utilize the nitrogen, phosphorus, and trace elements from the liquid fraction of digestate as well as CO_2 from the biogas to grow biomass. Utilizing a liquid fraction of the digestate for microalgae cultivation enhances process sustainability by reducing reliance on artificial synthetic nutrients and freshwater (Navarro-López *et al.* 2020; Ranglová *et al.* 2021). Nonetheless, the application of the liquid fraction of the digestate is limited by its physicochemical properties, such as opacity, which prevents light penetration, the presence of inhibitors, including bacteria (Bauer *et al.* 2021) and high ammonium content.

In this study, we demonstrate the pilot-scale co-digestion of source-separated BW and FW for biogas production under mesophilic conditions. The resulting digestate was treated through flocculation and sedimentation to separate the solid and liquid fractions, and the fate of nutrients was analyzed. In addition, we explored the potential of using liquid digestate for the cultivation of two microalgal strains, *S. acuminatus* and *C. vulgaris*. The findings presented here may encourage stakeholders to adopt source separation of organic and wastewater streams and may aid in the development of digestate treatment techniques to produce nutrient-rich algal biomass in the future.

2. MATERIAL AND METHODS

2.1. Waste material and inoculum

BW was obtained from vacuum toilets installed in Hiedanranta, Tampere, Finland, utilizing 1.5 L of water per flush. It was collected into 1,000 L tanks and stored on-site for a minimum of 30 days before usage. The FW was obtained from two local restaurants, from a student restaurant at the university campus (Juvenes Oy, Tampere) and from a lunch restaurant located in Hiedanranta (Zipatta, Tampere) and subsequently minced using a blender (Biltema, Denmark). The preprocessed FW was stored at 2–5 °C and utilized as feedstock. The contents of the two FWs collected varied and the FW from the student restaurant consisted mainly vegetable waste, while the FW from the lunch restaurant also contained food waste from the customers. Digested sewage sludge, serving as the inoculum, was obtained from the Viinikanlahti wastewater treatment facility in Tampere, Finland. Before using it as inoculum in laboratory assays, it was stored at 4 °C, while in the pilot reactor, it was used as such.

2.2. Biomethane potential tests

The biomethane production potential (BMP) of BW and FW was evaluated separately and in combination using triplicate batch assays. The assays were conducted in 1 L batch reactors with a working volume of 0.7 L operating under mesophilic conditions at 35 °C. Each reactor was equipped with a 5-L Supel™ Inert Foil gas sampling bag (Merck, Germany) to collect the produced biogas. The volatile solid (VS) ratio of BW to FW was 0.41, and the VS ratio of the substrate to inoculum was 1.0 g-VS/g-VS. A total of 80 mL of inoculum as well as $NaHCO_3$ (with the final concentration of 4 g/L) was added to each batch reactor and the initial pH ranged from 7.2 to 7.8. Before sealing, reactors were purged with nitrogen gas to establish an anaerobic environment. Alongside the primary reactors, the BMP of inoculum was assessed in a separate reactor. The cumulative methane production from the inoculum was subsequently deducted from the cumulative methane production in each reactor. The characteristics of BW, FW, and inoculum used in BMP assays are detailed in Table 1.

2.3. Pilot-scale reactor operation

A pilot-scale CSTR with a liquid volume of 2 m^3 (Metener Ltd, Finland) was operated in a semi-continuous mode for 153 days for co-digestion of BW and FW at a controlled temperature of 35 °C that was established by recirculating the reactor content through a heat exchanger situated outside the reactor. No pH control was used in the reactor. The reactor was fed once a day and each feeding cycle began by removing a specified volume of digestate, after which the substrate was thoroughly mixed in the feed tank using a submersible pump before being transferred to the reactor (despite utilizing mixing, stratification of the feedstock in the feed tank was occasionally observed). This process maintained a hydraulic retention time (HRT) of 25 days and an organic loading rate (OLR) between 0.42 and 0.91 kg-VS/m^3/d.

The characteristics of FW and BW used in the reactor studies are presented in Table 2. The feed was prepared in 1 m^3 scale weekly and was used over a period of 7 days, and despite the storage temperature of 1–10 °C, the biodegradation of organic matter in the feed was observed from time to time leading to an increase in volatile fatty acid (VFA) concentration in the feed tank and reducing the pH. This, however, had no significant impact on the process performance, but the influent COD and OLR were fluctuating. The co-digestion process commenced with an initial filling of the reactor with 1 m^3 of inoculum.

Table 1 | The characteristics of inoculum and substrates used for the BMP batch assays

	TS (%)	VS (%)	COD (g/L)	CODs (g/L)
Inoculum	3.3 ± 0.0	1.8 ± 0.0	N.D.	N.D.
Blackwater	0.33 ± 0.0	0.18 ± 0.0	5.4 ± 0.4	3.1 ± 0.2
Food waste	17.6 ± 0.0	16.3 ± 0.1	400 ± 45	92 ± 10

N.D. stands for not determined.

Table 2 | Characteristics of BW, FW, and a combination of BW and FW used as feed in pilot-scale studies are represented in average ± standard deviation

Parameter	Blackwater	Food waste	Blackwater and food waste
TS (%)	0.5 ± 0.1	17.4 ± 0.2	2.5 ± 0.5
VS (%)	0.3 ± 0.1	16.3 ± 0.1	2.2 ± 0.5
VS/TS	0.63 ± 0.05	0.94 ± 0.0	0.86 ± 0.05
COD (g/L)	11.0 ± 1	400 ± 45	21 ± 2.8
CODs (g/L)	3.1 ± 0.5	92 ± 10	13 ± 2.6
VFA (g-COD/L)	N.D.	N.D.	1–2.5
NH_4-N (g/L)	1.4 ± 0.0	0.11 ± 0.1	1.3 ± 0.1
Total phosphorous (mg/L)	170 ± 60	N. D.	146 ± 23
pH	8.7 ± 0.0	5 ± 0.1	4.2–7.0

N.D. stands for not determined.

Feeding began after a 14-day stabilization period, initially scheduled once every 7 days for the first 3 weeks. Upon achieving stable reactor performance, the feeding frequency was increased to once a day (the start of this feeding frequency, around day 30, was considered as the start of the process).

The volume of the produced biogas was measured using an Itro G4 RF1 (Itron, UK) gas flow meter. The collected gas was stored in a gas bag (IC2 Feeniks Oy, Finland), and its composition was analyzed using a Dräger X-am 8000 (SENSOREX, Finland). Feed and digestate were sampled three times per week, using at least duplicate samples. Before analysis, samples were centrifuged at 4,500 rpm for 30 min (Rotina 420, Hettlich, USA) and filtered through a 0.45 μm pore size filter (Chromafil, Macherey-Nagel, Germany).

The organic matter content in the reactor feed was high, as indicated by the elevated average ratio of VS to total solids (TS) and volatile suspended solids to total suspended solids (VSS/TSS) at 0.92 and 0.94, respectively. The COD of the feed was 21 ± 6 g/L with a soluble COD (CODs) of 14 ± 4 g/L, of which 1.2 ± 0.4 g/L constituted VFAs. The VFAs primarily included acetate, butyrate, propionate, and isobutyrate. The pH of the feed varied significantly, ranging from 4.2 to 7.0. The ammonium nitrogen (NH_4-N) concentration in the feed was high, averaging 1.16 ± 0.26 g/L. The variability in the concentration of organic matter, particularly noted during one feeding cycle, was likely due to hydrolysis and acidification reactions occurring in the feed tank, as evidenced by the decreasing trends in TS and VS values over time.

2.4. Digestate pretreatment before microalgal cultivation

Flocculation was conducted to remove organic matter and the solid fraction of the digestate using various Flopam cationic polyacrylamide polymers from SNF Finland Oy, including FO-4240 SH, FO-4290 SH, FO-4440 SH, and FO-4350 SH. These polymers, which vary in charge density and molecular weight, were tested at different concentrations with agitation at 300 rpm. The characteristics of the flocculants are detailed in Table 3. The flocculation process was carried out at room temperature (20–24 °C) without pH adjustment. Subsequently, the separation of the solid fraction was achieved through overnight sedimentation at room temperature.

2.5. Microalgae cultivations

Axenic cultures of *C. vulgaris* (SAG 211-12) and *S. acuminatus* (SAG 38.81) were obtained from the George-August Universität culture collection in Göttingen, Germany. Initial cultivation of both *S. acuminatus* and *C. vulgaris* was conducted in the N8 mineral media composed of the following: KNO_3 (0.5 g/L), KH_2PO_4 (0.74 g/L), Na_2HPO_4 (0.26 g/L), $MgSO_4{\cdot}7H_2O$ (50 mg/L), $CaCl_2{\cdot}2H_2O$ (17 mg/L), FeNaEDTA·$3H_2O$ (11 mg/L), $ZnSO_4{\cdot}7H_2O$ (3.2 mg/L), $MnCl_2{\cdot}4H_2O$ (13 mg/L), $CuSO_4{\cdot}5H_2O$ (18.3 mg/L), and $Al_2(SO_4)_3{\cdot}18H_2O$ (7 mg/L). This was done in shake flasks at room temperature (25–28 °C) under continuous illumination of 50 ± 5 μmol photon/m^2/s in photosynthetically active radiation, with agitation at 150 rpm using a shaking plate (Infors, Switzerland).

For subsequent cultivation in digestate, once *S. acuminatus* and *C. vulgaris* reached an optical density (OD_{750}) of 2, the cells were centrifuged at 4,500 rpm for 5 min, washed, and re-suspended either in pure digestate or digestate diluted with tap water having the following final digestate concentrations of 10, 13, 17, 20, 25, and 50% (v/v). A 15% (v/v) of cell suspension was used to inoculate 1 L batch reactors with a working volume of 0.7 L. Gas flow was regulated by mass flow meters,

Table 3 | Characteristics and performance of various polymers used for flocculation of digestate from anaerobic co-digestion of FW and BW

Polymer	Concentration range tested	Optimal concentration	Polymer properties	Results
FO-4240 SH	20–300 mg/L	20 mg/L	Cationic, medium charge density, medium MW	Particle agglomeration and sedimentation
FO-4290 SH	20–200 mg/L	None	Cationic, medium charge density, high MW	No agglomeration
FO-4440 SH	20–500 mg/L	100 mg/L	Cationic, high charge density, very high MW	Partial agglomeration
FO-4350 SH	20–250 mg/L	250 mg/L	Cationic, medium charge density, high MW	Liquid fraction remained turbid

MW, molecular weight.

maintaining an air flow rate of 10 mL/min. The system operated without pH (at pH 7–9) and temperature (20–35 °C) control. Continuous illumination was done using LEDs at an intensity of 40 μmol/m^2/s.

2.6. Analytical methods and calculations

TSS, VSS, TS, and VS were determined gravimetrically following the standard method (APHA 2018a). pH was measured with SenTix 41 electrode and WTW pH 3210 meters. Total COD (CODt) and soluble COD (CODs) were determined using the dichromate method (APHA 2018b). The concentration of VFAs was assessed using Shimadzu Ordior GC-2010 plus gas chromatograph, as described by Kokko *et al.* (2018), and was calculated in terms of CODs per L. For BMP experiments, CH_4 concentration was measured using a Perkin Elmer 500 GC-FID with a Mol-Sieve 5A PLOT column according to Kokko *et al.* (2018), while the volume in the gas bags was measured with the water displacement method (Owen *et al.* 1979).

Cation concentrations were analyzed using a Dionex DX-120 ion chromatograph (ThermoFisher Scientific, USA) and a Dionex Ionpac™ CS12A column (ThermoFisher Scientific, USA). The anions were measured using Dionex AS-DV and an Ionpac™ AS22 column (ThermoFisher Scientific, USA) according to the method described by Jermakka *et al.* (2021).

Microwave acid digestion of feed and digestate was performed using the MARS6 system (CEM, USA) and concentrated HNO_3 and HCl at 165 °C and 800 psi for 30 min. The elemental concentrations of the digested samples were subsequently measured via inductively coupled plasma mass spectrometry using Thermo Scientific iCAP™ RQ equipment. All measurements were carried out in kinetic energy discrimination mode, employing He as the collision gas in the collision/reaction cell and Ar as the carrier gas.

The CHNS/O analysis of algal biomass was conducted by flash combustion using a FlashSmart organic elemental analyzer (ThermoFisher, USA), with cysteine and methionine serving as calibration standards.

Due to significant variations in the feeding cycles during the reactor experiments, methane yields and OLRs were calculated as averages for each feeding cycle to ensure consistency and reliability in the data interpretation (feeding cycle implies each batch of fresh feed prepared).

3. RESULTS

3.1. Methane production from FW and/or BW in batch bottles

To assess the methane production potential, batch tests were conducted. Methane production from FW occurred more rapidly than from a combination of FW and BW. The highest methane yield was recorded from FW alone, achieving 584 ± 70 L/kg-VS (Figure 1). When BW was co-digested with FW, the methane yield was 492 ± 21 L/kg-VS. In contrast, the lowest methane yield of 304 ± 44 L/kg-VS was observed for BW alone (Figure 1). At the end of the BMP tests, the pH

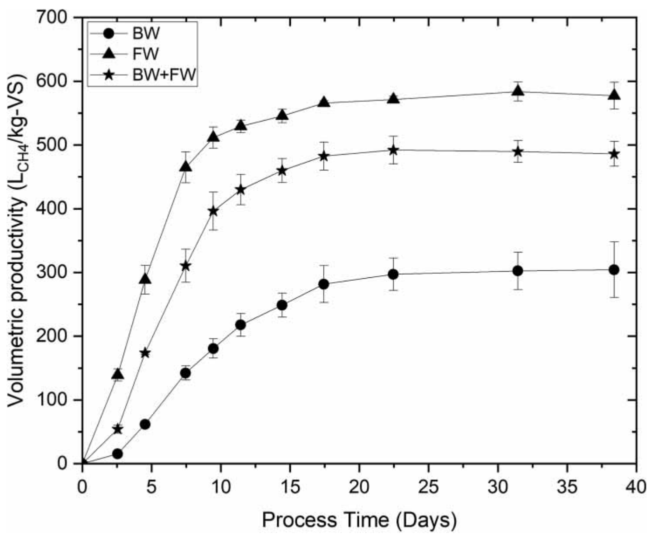


Figure 1 | BMP of BW, FW, and BW co-digested with food and kitchen waste (BW + FW). The cumulative methane production in the inoculum was subtracted from the cumulative methane production in the samples. Results are represented as the average of triplicate ± standard deviation.

stabilized at 7.8 ± 0.2, and no VFAs were detected. The effluent ammonium concentrations at the end of batch assays were 0.81 ± 0.3 g/L and 0.54 ± 0.5 g/L for BW and BW co-digested with FW, respectively. The highest methane production rates observed were 0.03 ± 0.0 L/L/d for BW, 0.14 ± 0.01 L/L/d for FW, and 0.06 ± 0.0 L/L/d for FW co-digested with BW.

3.2. Co-digestion of BW and FW on a pilot-scale and the fate of nutrients

Anaerobic co-digestion of FW with BW was conducted at a pilot-scale. The process startup phase lasted 3 weeks, after which the CH_4 content in the effluent gas was on average 68 ± 2%. The average OLR and HRT varied across each feeding cycle (Figure 2(c)), primarily due to inconsistent mixing and biodegradation of FW in the feed tank. The high methane yield observed during the first 20 days could be due to the additional CH_4 produced from inoculum. After day 30, the HRT was maintained at 27 ± 2 days and the OLR of the feed fluctuated within 0.59 ± 0.14 kg-VS/m^3/d, except around day 60 when it peaked at 1.1 ± 0.14 kg-VS/m^3/d (Figure 2(c)). The influent CODt showed fluctuations and was in the range of 14–33 kg/m^3 (Figure 2(a)). During this period, the total VFAs in the feed ranged from 1.0 to 2.5 g-COD/L (Table 2). From day 30 to day 90, the CH_4 content in the produced biogas was on an average 65 ± 3% (Figure 2(b)) and the average CH_4 production rate was 0.4 ± 0.1 m^3/m^3/d (Figure 2(c)). In addition, CH_4 yield was on averaged 0.7 ± 0.2 m^3/kg-VS with a maximum yield of 1.1 ± 0.1 m^3/kg-VS (Figure 2(d)).

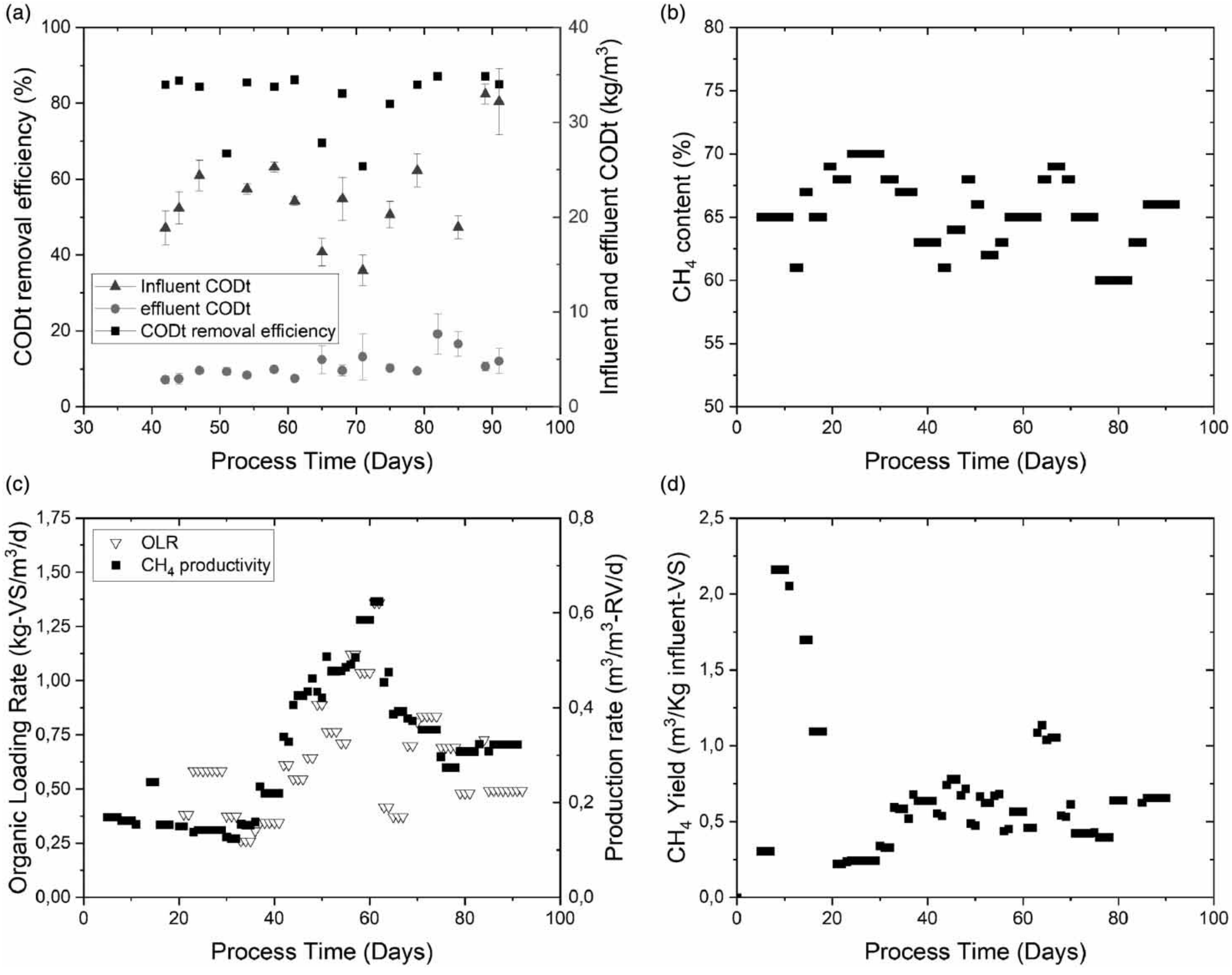


Figure 2 | The co-digestion of FW with BW for methane production in a 2 m^3 pilot-scale CSTR (a) varying influent CODt, effluent CODt, and CODt removal efficiency (b) the CH_4 content in the produced biogas, (c) the OLR and CH_4 production rate, and (d) the CH_4 yield based on influent-VS. The data points presented in (a) represent weekly average values and the data are represented for a period when the reactor started to stabilize, and feeding was done once a day.

Despite fluctuations in the pH of the feed (ranging from 4.2 to 7.0), the effluent pH was in the range of 7.4 ± 0.2 throughout the process without pH control (Figure 3(a)). The total VFAs in the digestate were maintained below 0.4 g-COD/L and after day 70, VFAs were completely consumed (Figure 3(b)). Throughout the process, the CODt removal efficiency was 83 ± 4% (Figure 2(a)) with a total VS removal efficiency of 82 ± 6% indicating effective organic matter degradation and stability of the AD process over time.

To assess the fate of nutrients in the AD process, a detailed analysis of both the feed and digestate was conducted. The analyses focused on macronutrients present in the combined feed of BW and FW, including total phosphorus (P), NH_4-N, sodium (Na), potassium (K), magnesium (Mg), sulfate (SO_4), phosphate (PO_4), and nitrate (NO_3). The reduction in nutrient concentration in the digestate compared with feed can be attributed to the potential stratification of some solids either in the feed tank, in the digester, or the digestate tank, leading to generally lower concentrations in the digestate compared with the initial feed (Table 4). In contrast, the concentration of NH_4-N was higher in the digestate, averaging 1.5 ± 0.15 g/L, compared with 1.1 ± 0.05 g/L in the feed. This could be attributed to ammonification of organic matter during AD. Furthermore, the analysis revealed that the average concentration of several micronutrients, including iron (Fe), nickel (Ni), cobalt (Co), copper (Cu), chromium (Cr), and lead (Pb), exhibited slight increases in the digestate relative to their levels in the feed (Table 4). This could be attributed to the breakdown and transformation of these elements into more soluble forms during

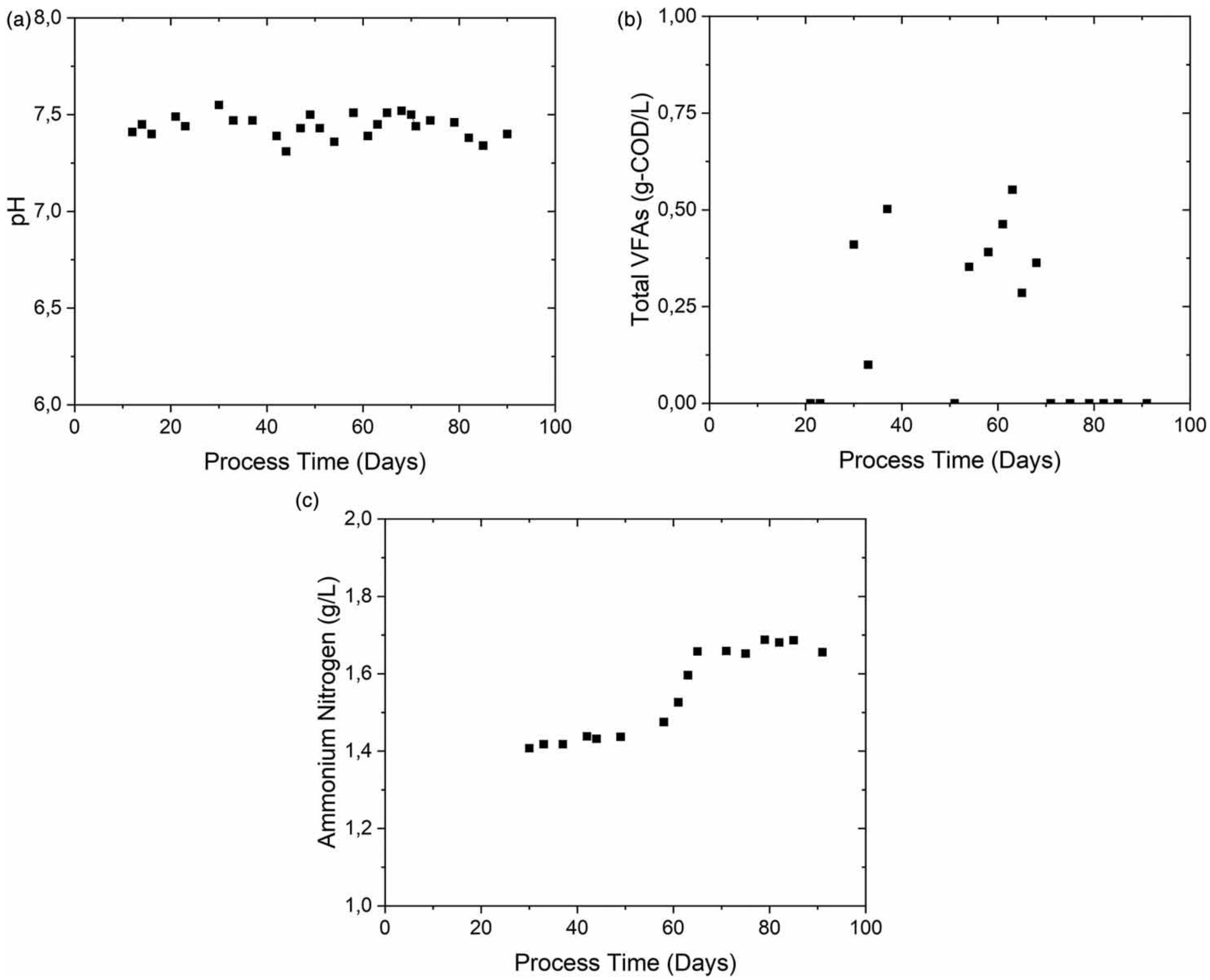


Figure 3 | Characteristics of the digestate from anaerobic co-digestion of FW and BW in a 2 m^3 pilot-scale reactor in terms of (a) pH, (b) total VFAs represented as CODs, and (c) effluent ammonium nitrogen content. The data points represent weekly average values and the data are represented for a period when the reactor started to stabilize, and feeding was done once a day.

Table 4 | Carbon, macronutrients, and selected micronutrients in feedstock, digestate, and liquid (LF) and solid (SF) fractions of the digestate from a pilot-scale anaerobic co-digestion of BW and FW

	CODs (g/L)	CODt (g/L)	Total P (mg/L)	PO_4-P (mg/L)	NH_4-N (g/L)	NO_3 (mg/L)	SO_4 (mg/L)	K (g/L)	Na (g/L)	Mg (mg/L)	Ca (mg/L)	Fe (mg/L)	Ni (mg/L)	Zn (mg/L)	Co (mg/L)	Cu (mg/L)
Feed	12.8 ± 2.6	21 ± 3	146 ± 23	131 ± 28	1.1 ± 0.05	134 ± 11	174 ± 17	0.56 ± 0.06	0.46 ± 0.06	69 ± 12	182 ± 74	100 ± 4	5 ± 0.0	1.3 ± 0.4	3 ± 0.8	4 ± 0.0
Digestate	3.2 ± 0.7	4.4 ± 1	72 ± 23	64 ± 4	1.6 ± 0.2	126 ± 1	116 ± 7	0.47 ± 0.06	0.42 ± 0.05	38 ± 14	158 ± 15	105 ± 10	4.3 ± 1	1 ± 0.3	0.3 ± 0.0	4.3 ± 0.1
LF	2.0 ± 0.1	2.5 ± 0.1	53 ± 8	50 ± 2	1.4 ± 0.1	118 ± 1	117 ± 5	0.4 ± 0.05	0.4 ± 0.05	32 ± 2	151 ± 12	812 ± 7	3.3 ± 0.0	1.2 ± 0.0	0.1 ± 0.0	3.3 ± 0.05
SF	N.D.	N.D.	12 ± 4	N.D.	0.2 ± 0.1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	12 ± 0.2	0.5 ± 0.0	0.07 ± 0.0	0.01 ± 0.0	0.0

The liquid and solid fractions were separated with an optimized flocculation process with FO-4240 (see Section 3.3).
N.D. stands for not determined.

the AD process, emphasizing the complex interactions and transformation that organic substrates undergo during AD (Fuchs & Drosg 2013).

3.3. Separation of the digestate into solid and liquid fractions

The digestate resulting from anaerobic co-digestion of BW and FW exhibited an average TS content of 0.8 $\pm$ 0.1% and VS content of 0.4 $\pm$ 0.1%. The ammonium concentration in the digestate varied initially, ranging from 1.4 $\pm$ 0.1 g/L between days 29–60. This concentration increased slightly to 1.6 $\pm$ 0.1 g/L by day 63 and maintained this level until the end of the monitoring period (Figure 3(c)). To effectively separate the liquid and solid fractions of the digestate, a flocculation process was optimized using four different polymers (Table 3). Among these, FO-4240 and FO-4350 achieved complete flocculation of solid matter, forming agglomerates that led to efficient sedimentation of the solid particles and a distinct separation of solid and liquid fractions. In contrast, FO-4440 resulted in only partial agglomeration with more than a 50% volumetric solid fraction and no efficient sedimentation, while FO-4290 showed no agglomeration at all.

Despite the effective flocculation observed with FO-4350, the liquid fraction post-sedimentation appeared turbid, suggesting incomplete separation or the presence of residual colloidal particles. Consequently, polymer FO-4240, at an optimal concentration of 20 mg/L, was selected for the subsequent digestate treatment process due to its superior performance in clarifying the liquid fraction and the low concentration required to achieve high-efficiency flocculation.

After flocculation and sedimentation with FO-4240, the digestate's volumetric composition was 88% liquid and 12% solid. After treatment, the liquid digestate displayed a reduced TS content of 0.3 $\pm$ 0.0% and a VS content of 0.09 $\pm$ 0.0%. In addition, the CODt of the liquid fraction was measured at 2.5 $\pm$ 0.1 g/L, with the CODs of 2 $\pm$ 0.1 g/L (Table 4). This indicates a substantial reduction in organic matter, reflecting the effectiveness of the treatment process in managing and reducing the solid content of the digestate.

During the flocculation and sedimentation process, the pH of the liquid fraction of the digestate increased from 7.4 $\pm$ 0.2 to 8.4 $\pm$ 0.2. The detailed characteristics of both liquid and solid fractions of the digestate are provided in Table 4.

A significant portion of the nutrients was retained in the liquid fraction, with more than 90% of NH_4-N and over 70% of total phosphorus preserved (Table 4). Similarly, high recovery efficiencies were observed for other essential macronutrients, with more than 90% of sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), NO_3, PO_4, and SO_4 remaining in the liquid fraction. This effective nutrient conservation during the flocculation process suggests that these nutrients can potentially be recycled or repurposed from liquid fraction.

A smaller proportion of metals, including iron (Fe), nickel (Ni), zinc (Zn), and cobalt (Co), were found in the solid fraction of the digestate (Table 4). This separation is advantageous as the liquid fraction remains rich in nutrients, making it suitable as feedstock in biological processes where microorganisms require both macronutrients and trace metal salts for growth and production.

3.4. Feasibility of using the digestate for nutrient recovery

In this study, the growth and nutrient utilization of two lipid-producing algal strains, *S. acuminatus* and *C.vulgaris*, were investigated using the liquid fraction of digestate as a growth medium. Photoautotrophic growth experiments were performed in 1 L batch reactors with various dilutions of the liquid digestate mixed with tap water to assess the feasibility of using digestate as a nutrient source for algal cultivation.

C. vulgaris exhibited initial growth with a 7–8-day lag phase in dilutions containing 20, 17, and 13% liquid digestate. However, following this period, growth accelerated rapidly, culminating in a biomass concentration of 1 $\pm$ 0.1 g/L by day 20 of the process (Figure 4(a)). Interestingly, in a 10% digestate dilution, *C. vulgaris* showed immediate growth without a lag phase, achieving a biomass concentration of 1.2 $\pm$ 0.1 g/L by day 19. This surpasses the growth observed in the control group cultivated in synthetic N8 mineral media, which recorded 1.0 $\pm$ 0.1 g/L (Figure 4(a)).

In contrast, no growth was observed with *S. acuminatus* cultivated in either the undiluted liquid digestate or its various dilutions (Figure 4(b)), indicating a potential inhibitory effect of the nutrient profile of the liquid digestate for this algal strain.

The CHNS analysis of the dried biomass of *C. vulgaris* cultivated on the liquid fraction of digestate showed the following composition: 8.6 $\pm$ 0.2% nitrogen (N), 47.2 $\pm$ 1% carbon (C), 4.7% $\pm$ 0.5% hydrogen (H), and 1 $\pm$ 0.2% sulfur (S), and the remainder comprising other elements. After the growth of *C. vulgaris*, the liquid digestate showed an average reduction of 85 $\pm$ 3% in NH_4-N and 78 $\pm$ 5% in PO_4-P content on day 20 of the cultivation. While no nitrate was detected in the liquid digestate post-cultivation, the composition of Na, K, Mg, and Ca remained unaffected.

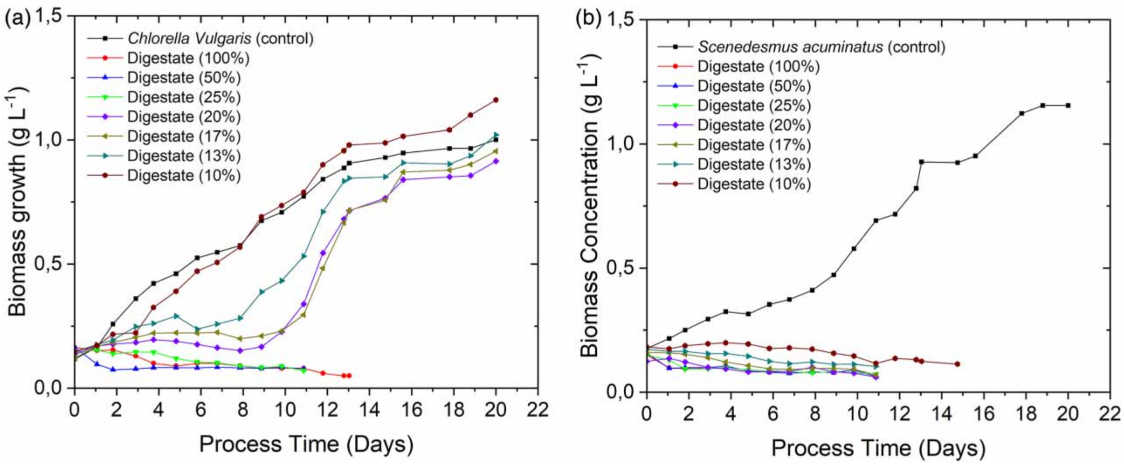


Figure 4 | Growth of (a) *C. vulgaris* and (b) *S. acuminatus* on the liquid fraction of digestate derived from anaerobic co-digestion of BW and FW and its various dilutions with tap water.

The nutrient-rich profile of the liquid digestate underscores its potential as a medium for cultivating nutrient-rich algae, which could be further exploited as biofertilizer or in other biotechnological applications.

4. DISCUSSION

4.1. Biomethane production potential of BW co-digested with FW in laboratory and pilot-scale

The characteristics of the BW and FW analyzed in this study, including TS (2.5 ± 0.5%), and VS (2.2 ± 0.5%) content, pH (7.4 ± 0.2), CODt (22 ± 6 kg/m^3), and CODs (13 ± 3 kg/m^3), align with values reported previously (Rajagopal *et al.* 2013; Lavagnolo *et al.* 2017; Gao *et al.* 2019). The synergy of BW and FW for co-digestion was evident in the current study. BW, characterized by a very low TS and VS content (approximately 0.5%), contributed minimal organic matter to the digestion process but had a high NH_4-N concentration of 1.4 ± 0.1 kg/m^3 and pH of 8.7 ± 0.1. In contrast, FW had a high TS and VS content of 16–18%, serving as the major source of organic matter and thus resulting in C:N ratio ranging from 8.8:1 to 9.5:1 of the combined feedstock.

Despite previous reports of high concentrations of free ammonium (greater than 205 mg/L) inhibiting methanogenesis in AD of BW collected from vacuum toilets (Gao *et al.* 2019, 2020), no such inhibitions were observed in the current study. This is likely due to the co-digestion with FW, which balanced the concentration of free ammonium in the digestor. The operational OLR of 0.6 ± 0.1 kg-VS/m^3/d resulted in an average NH_4-N concentration of 1.1 ± 0.05 kg/m^3 in the feed, which was found favorable to biogas production (Figure 3(c)).

In the laboratory trials, the methane production potential, as assessed by BMP tests, revealed a methane productivity of 583.7 ± 70 L/kg-VS for FW, surpassing previously reported yields of 400–496 L/kg-VS (Rajagopal *et al.* 2013; Ebner *et al.* 2016). This higher productivity could be attributed to the composition of FW obtained in the current study containing more meat waste, as well as the low OLR used in the batch tests. For BW, the methane yield of 304 ± 44 L/kg-VS (Figure 1) was in the range reported in previous studies (220–400 L/kg-VS) (Lavagnolo *et al.* 2017; Zuo *et al.* 2021). When co-digested with FW, a maximum methane yield of 492 ± 21 L/kg-VS, closely aligning with the previously documented range of 520–590 L/kg-VS (Lavagnolo *et al.* 2017) was attained.

At the pilot-scale, the CH_4 yield of FW co-digested with BW was 0.73 ± 0.1 m^3/kg-VS at an OLR of 0.59 ± 0.14 kg-VS/m^3/d, which was on average 67% higher than in laboratory-scale BMP studies, which showed 0.49 ± 0.02 m^3/kg-VS. The CH_4 production rate in pilot studies ranged from 0.6 to 2.3 m^3/m^3/d, comparable to the highest CH_4 production rates reported by Gao *et al.* (2020) of 2.4 ± 0.15 m^3/m^3/d for co-digestion of BW and FW, despite using a digestor of only 3.5 L size. During the pilot study, the CH_4 content in the biogas was 65 ± 3%, like that observed in the batch assay (64 ± 3%) in this study. The CODt removal efficiency achieved during the pilot study averaged 83 ± 4% (Figure 3(d)), which was at the upper end of the range (52–84%) reported in previous studies (Rajagopal *et al.* 2013; Gao *et al.* 2020). In addition, the VS removal efficiency of 82 ± 6% in this study is in a higher range than previously reported similar studies (Gao *et al.* 2020).

Furthermore, both the average (0.7 ± 0.1 m^3/kg-VS) and the maximum (1.1 ± 0.1 m^3/kg-VS) methane yields observed in the pilot trials were significantly higher than those previously reported for laboratory-scale digesters (0.21–0.37 m^3/kg-VS) (Rajagopal *et al.* 2013; Gao *et al.* 2020). In the current study, conducted using a 2 m^3 pilot-scale CSTR, the HRT ranged from 20 to 27 days, aligning with the 10–30 days reported for CSTRs with an OLR of 0.59 ± 0.14 kg-VS/m^3/d, resulting in an average methane yield of 0.7 ± 0.1 m^3/kg-VS. The HRTs for co-digestion of BW and FW vary significantly depending on the reactor system employed. In accumulation systems, HRTs are reported to range from 105 to 280 days, although these studies do not specify the corresponding OLR. Notably, Gao *et al.* (2019) reported an HRT of 2.6 days in a laboratory-scale 3.5 L UASB, although the scalability of such a system remains unexplored.

The VFAs in the feed ranged from 1.0 to 2.5 g-COD/L and were predominantly consumed during the digestion process. From days 21 to 69, the VFA levels fluctuated but ultimately remained below 0.5 g-COD/L. By day 70, no VFAs were detected (Figure 3(b)), indicating a more efficient VFA degradation compared with previous studies, which reported total VFA concentrations of 0.76–8.89 g/L (Elmitwalli *et al.* 2006; Kujawa-Roeleveld & Zeeman 2006; Rajagopal *et al.* 2013; Zhang *et al.* 2019; Gao *et al.* 2020; Wang *et al.* 2020).

Throughout the pilot study, the effluent NH_4-N concentration increased from an initial average of 1.1 ± 0.05 g/L in the feed to 1.6 ± 0.1 g/L in the digestate (Figure 3(c)). This increase is likely due to the ammonification process, which involves the degradation of proteins, nucleic acids, and other nitrogen compounds present in the feed, such as meats and plant material. Solubilization makes the organic nitrogen available for ammonification, resulting in increased NH_4-N concentration in the effluent. Ammonification has been reported in AD of FW where it can inhibit the digestion process due to toxicity to hydrogenotrophic methanogens (Serna-Maza *et al.* 2014; Chen *et al.* 2016). Therefore, controlling and understanding ammonification is crucial in managing AD processes using FW as feedstock. Notably, no inhibitory impact for ammonium concentration was detected in this study, aligning with the absence of VFA accumulation toward the end of the pilot trial (Figure 3(b)).

The negligible denitrification observed in the system under anaerobic conditions may indicate insufficient denitrifying bacteria or competing scenarios between denitrifying bacteria and methanogens for carbon sources. Methanogens may have outcompeted denitrifies, leaving the nitrate concentration in the feed unaffected (Deng & Shi 2020). Only very high nitrate concentrations (exceeding 1.5 g/L in AD) can inhibit methanogenesis (Sheng *et al.* 2013) by creating a competitive environment, reducing the availability of the carbon source for methanogens, and favoring the dominance of bacteria converting nitrate to nitrite, which is reported toxic to methanogens (Klüber & Conrad 1998).

While the 2 m^3 CSTR pilot reactor used in this study demonstrated high organics removal efficiency, and CH_4 content a limitation was observed in maintaining a long steady state during the process, a common challenge in large-scale biogas facilities (Leitão 2004; Owusu-Agyeman *et al.* 2019). In this study, fluctuations in influent CODt and VS content were primarily due to stratification from inefficient mixing and the biodegradation of FW in the feed tank. This issue could be mitigated by real-time monitoring and adaptation of OLR, automated daily feed preparation, or using feed storage tanks and pumps that allow for better mixing of the feedstock, thereby buffering against short-term fluctuations in VS.

4.2. Potential of digestate as a feedstock and nutrient source for microalgae cultivation

Besides biogas, digestate is a valuable byproduct of the AD process (Xia & Murphy 2016). For digestate to be utilized in various applications, some preprocessing is essential. The choice of downstream processing methods depends on the digestate composition, specifically to remove solids and adjust nutrient levels (Bauer *et al.* 2021). In this study, the aim was to remove suspended organics, including colloids and solid matter, from the digestate while retaining most of the nutrients in the liquid phase to reduce turbidity and facilitate light penetration into the liquid digestate, making it suitable as feedstock for microalgal cultivation. Therefore, various cationic polyacrylamide flocculants differing in charge density and molecular weight were evaluated for the downstream processing of digestate obtained from the co-digestion of BW and FW.

The flocculant FO-4240 SH, characterized by medium charge density and molecular weight, demonstrated the highest efficiency in solid removal from the digestate. Following sedimentation, the resulting liquid fraction contained, on an average, 62 ± 1% less TS and 77.5 ± 2% less VS compared with the whole digestate. In addition, CODt and CODs were reduced by 43 ± 2% and 48 ± 1%, respectively, in the liquid fraction.

Over 90% of macronutrients, including NH_4-N, K, and NO_3, were present in the liquid fraction, whereas only 15–20% of the total phosphorus and some fractions of metals such as Fe, Ni, Zn, and Co remained in the solid fraction. This distribution contrasts with other studies, which reported 20–30% of NH_4-N, 55–65% phosphorus, and 20–30% potassium remaining in the solid fraction post-flocculation of digestate obtained from AD of agricultural and food waste (Bauer *et al.* 2021).

Chini *et al.* (2021) demonstrated that chemical flocculants, such as polyphenolic organic polymers and polyacrylamide, exhibit considerably lower nutrient removal efficiencies (20–65%) compared with centrifugation for digestate from the AD of swine manure. Similarly, Chuda & Ziemiński (2021) found that the combination of centrifugation with high-density cationic polyacrylamide flocculants effectively retained only 61% of nitrogen, 75% of potassium, and 33% of phosphorus in the liquid fraction of digestate originating from the AD of sugar beet pulp.

The use of flocculation followed by sedimentation for digestate treatment holds potential for large-scale application due to low investment cost and low energy input in comparison to filtration, centrifugation, and other dewatering techniques commonly done for digestate treatment.

One effective strategy for recovering nutrients from the liquid fraction of digestate is through microalgae cultivation. In this study, to ensure adequate light penetration for algal growth and balance nutrient concentrations, the liquid fraction of digestate was diluted with tap water. Our results demonstrated distinct growth patterns between the two algal strains. No growth was observed for *S. acuminatus* on the digestate or its dilutions. A possible explanation for this could be the presence of competitive microorganisms in the digestate, which might consume or outcompete the microalgae (Bauer *et al.* 2021). Previous studies have demonstrated successful growth of *S. acuminatus* on other types of digestate, where sterilization of liquid digestate through autoclaving was performed before cultivation (Park *et al.* 2010; Dickinson *et al.* 2015). Another explanation could be the nutrient profile of the liquid digestate, which may have an inhibitory effect on *S. acuminatus*. Potential factors contributing to this inhibition could include high concentrations of ammonium, heavy metals, or other compounds present in the digestate that *S. acuminatus* is less capable of tolerating or metabolizing compared with *C. vulgaris*.

Similar findings have been reported where specific algal strains are inhibited by ammonia (Cho *et al.* 2013), organic constituents, COD (Franchino *et al.* 2016; Tigini *et al.* 2016), and heavy metals (Wong *et al.* 1994) in digestates or wastewaters. An ammonium concentration of 0.3 mM at pH 6 has been reported as inhibitory to the growth of *Scenedesmus obliquus* (Lu *et al.* 2018).

C. vulgaris grown in 20, 17, and 13% liquid digestate revealed a lag phase of 7–8 days, likely representing the adaptation period required for the cells to acclimate to the new environment. This phenomenon has been reported with other digestates (Cai *et al.* 2013; Prajapati *et al.* 2014; Zhu 2015) and wastewaters (Åkerström *et al.* 2014; Dickinson *et al.* 2015). The immediate growth and higher final biomass concentration for *C. vulgaris* at 10% dilution suggest that this concentration of digestate provides an optimal balance of nutrients, minimizing inhibitory effects while supplying adequate resources for rapid growth. This observation aligns with previous studies that have shown *C. vulgaris* can adapt and thrive in nutrient-rich wastewater, including digestates (Wang *et al.* 2020).

Notably, the growth of *C. vulgaris* on 10% liquid digestate was comparable to its growth in control conditions using N8 mineral media, achieving a higher biomass concentration of 1.2 ± 0.1 g/L at the end of the experimental period. The biomass concentrations observed in this study are within the range of 0.1–1.7 g/L reported for various microalgal species grown on different liquid digestates (Xia & Murphy 2016). Some studies have achieved higher biomass concentrations up to 4.0 g/L by employing sterilization methods for digestate, such as autoclaving, or by making dilutions using synthetic mineral media or other wastewater (Åkerström *et al.* 2014; Cheng *et al.* 2015). However, sterilization by autoclaving is an energy-intensive and costly process, and not feasible for large-scale applications. In addition, using mineral salts to dilute digestate is neither sustainable nor practical.

Furthermore, growth inhibition at higher concentrations of digestate (greater than 20%), primarily due to photoinhibition and/or nutrient overload, was observed in this study and has been similarly reported in other research (Hollinshead *et al.* 2014; Fernandes *et al.* 2020). This highlights the importance of optimizing digestate concentration to balance nutrient availability and prevent inhibitory effects.

In this study, carbon constituted 47.2 ± 1% of the *C. vulgaris* biomass cultivated on liquid digestate derived from anaerobic co-digestion of BW and FW. Following the removal of *C. vulgaris* biomass via sedimentation or filtration, the digestate supernatant exhibited an average reduction of 50 ± 10% in both CODs and CODt. Notably, *C. vulgaris* is known for its mixotrophic growth capabilities (Xia & Murphy 2016), suggesting that the carbon for biomass growth could have originated from both the organic matter in the digestate as well as from CO_2 in the air supplied to the culture.

Biomass analysis indicated that the primary nutrients utilized from the digestate for biomass production were sulfur, nitrogen, phosphorus, and carbon. The liquid fraction of digestate after *C. vulgaris* growth contained on average 85 ± 3% and

78 ± 5% lower ammonium nitrogen and phosphorus content. This composition highlights the potential of utilizing liquid digestate as a medium for cultivating nutrient-rich algae. The significant nitrogen content is particularly noteworthy, as it is a critical nutrient for algal growth and protein synthesis. The effective uptake of nitrogen by *C. vulgaris* demonstrates the potential of using digestate as a nutrient source in a circular economy framework. By recycling nutrients from digestate, the need for synthetic fertilizers can be reduced, thereby lowering the environmental footprint of agricultural practices, and promoting sustainable nutrient management.

The immediate and robust growth observed at lower digestate concentrations suggests that optimizing the dilution ratio is crucial for maximizing algal growth and productivity. In contrast, the lack of growth in *S. acuminatus* highlights the importance of strain selection based on the specific nutrient and inhibitory profiles of the growth medium. Future studies should focus on identifying and mitigating inhibitory components in the digestate, as well as exploring a broader range of algal strains to fully leverage the nutrient potential of the digestate. Overall, our findings demonstrate the potential for integrating algal cultivation with waste management practices, contributing to a circular bioeconomy by converting waste streams into valuable biomass.

In the context of a circular bioeconomy, nutrient-rich digestate derived from anaerobic co-digestion of BW and FW can be efficiently pretreated using a process of flocculation followed by sedimentation. The resulting liquid fraction of the digestate may then serve as a medium for cultivating microalgae biomass. *C. vulgaris*, in particular, has been widely studied for its lipid production capacity, which can be utilized in biofuel production (Moradi & Saidi 2022). Depending on its composition, the harvested microalgal biomass could be used in biorefinery for bioenergy, biopolymer production, and as a biofertilizer. However, for industrial-scale microalgae cultivation for biorefinery applications to be economically viable, outdoor cultivations in open ponds must be thoroughly investigated.

AD of organic matter typically produces biogas containing 30–45% CO_2, depending on the process's efficiency. Harnessing this biogenic CO_2 is crucial for enhancing the sustainability and efficiency of the process. It can be injected into the microalgae culture as a carbon source (Tripathi *et al.* 2023) or upgraded to methane via biological hydrogen methanation (Kamravamanesh *et al.* 2023). In addition, sludge treatment remains a persistent challenge in waste management. AD is commonly employed to treat sewage sludge and food waste, resulting in digestate that can be utilized in agriculture, soil amendments, or composting, provided contaminant levels are within regulatory limits. However, reject water separated from the solid fraction of digestate poses a significant issue due to its high nitrogen content. Directing this water to wastewater treatment plants for nitrogen removal is costly and increases energy and chemical consumption.

Treating reject water through microalgae cultivation offers several advantages: it can reduce nitrogen load and associated treatment costs, and microalgae can efficiently utilize the concentrated nutrients in the reject water. This approach maintains the existing treatments required for the solid digestate. Moreover, using BW instead of sewage sludge or municipal wastewater for microalgae cultivation is advantageous due to the higher concentration of nutrients in forms readily available for microalgal uptake.

5. CONCLUSIONS

This study demonstrated the potential of anaerobic co-digestion of source-separated blackwater and food waste in a 2 m^3 pilot-scale CSTR, attaining a CH_4 yield of 0.7 ± 0.1 m^3/kg-VS with VS removal efficiency of 82 ± 6%. Beyond biomethane production, the resulting nutrient-rich digestate holds significant value. Flocculation and subsequent sedimentation preserved over 90% of NH_4-N and other macronutrients, along with more than 75% of total phosphorus, in the liquid fraction of digestate. This pretreatment, combined with dilution, facilitated the growth of *C. vulgaris* on diluted digestate. Biomass analysis indicated that carbon, nitrogen, and sulfur were predominantly utilized by the algae for biomass production and this treatment reduced CODt and CODs of digestate by 50 ± 10%. The liquid fraction of digestate after *C. vulgaris* growth contained on average 85 ± 3% and 78 ± 5% lower NH_4-N and PO_4-P content. The successful cultivation of *C. vulgaris* on liquid digestate underscores the feasibility of utilizing waste-derived nutrients for algal biomass production, thereby presenting a sustainable approach to nutrient recycling.

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AUTHOR CONTRIBUTIONS

D.K. and M.K. conceptualized the study and planned the experiments. D.K. performed most of the experiments, the data analysis, as well as the data visualization, and wrote the original draft of the manuscript. M.K. performed the project administration. D.K. and M.K. reviewed and edited the manuscript and read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

REFERENCES

Åkerström, A. M., Mortensen, L. M., Rusten, B. & Gislerød, H. R. 2014 Biomass production and nutrient removal by *Chlorella* sp. as affected by sludge liquor concentration. *J. Environ. Manage.* **144**, 118–124.

Alves, J. L. F., Da Silva, J. C. G., Costa, R. L., F, S., Junior, D. S., da Silva Filho, V. F., Moreira, R. D. F. P. M. & José, H. J. 2019 Investigation of the bioenergy potential of microalgae *Scenedesmus acuminatus* by physicochemical characterization and kinetic analysis of pyrolysis. *J. Therm. Anal. Calorim.* **135** (6), 3269–3280.

APHA 2018a 2540 Solids. In: *Standard Methods For the Examination of Water and Wastewater*. (Lipps, W. C., Baxter, T. E. & Braun-Howland, E. eds.). APHA Press, Washington, DC, USA. DOI:10.2105/SMWW.2882.030.

APHA 2018b 5220 Chemical oxygen demand (COD). In: *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, APHA Press, Washington, DC, USA.

Bauer, L., Ranglová, K., Masojídek, J., Drosg, B. & Meixner, K. 2021 Digestate as sustainable nutrient source for microalgae – Challenges and prospects. *Appl. Sci.* **11** (3), 1056.

Boliko, M. C. 2019 FAO and the situation of food security and nutrition in the world. *J. Nutr. Sci. Vitaminol.* **65** (Supplement), S4–S8.

Cai, T., Park, S. Y., Racharaks, R. & Li, Y. 2013 Cultivation of *Nannochloropsis salina* using anaerobic digestion effluent as a nutrient source for biofuel production. *Appl. Energy* **108**, 486–492.

Chen, H., Wang, W., Xue, L., Chen, C., Liu, G. & Zhang, R. 2016 Effects of ammonia on anaerobic digestion of food waste: Process performance and microbial community. *Energy Fuels* **30** (7), 5749–5757.

Cheng, J., Xu, J., Huang, Y., Li, Y., Zhou, J. & Cen, K. 2015 Growth optimisation of microalga mutant at high CO_2 concentration to purify undiluted anaerobic digestion effluent of swine manure. *Bioresour. Technol.* **177**, 240–246.

Chini, A., Hollas, C. E., Bolsan, A. C., Antes, F. G., Treichel, H. & Kunz, A. 2021 Treatment of digestate from swine sludge continuous stirred tank reactor to reduce total carbon and total solids content. *Environ. Dev. Sustain.* **23** (8), 12326–12341.

Cho, S., Lee, N., Park, S., Yu, J., Luong, T. T., Oh, Y.-K. & Lee, T. 2013 Microalgae cultivation for bioenergy production using wastewaters from a municipal WWTP as nutritional sources. *Bioresour. Technol.* **131**, 515–520.

Chuda, A. & Ziemiński, K. 2021 Digestate mechanical separation in industrial conditions: Efficiency profiles and fertilising potential. *Waste Manage.* **128**, 167–178.

Counts, T. W. 2024 *Tons of Food Lost or Wasted*. The World Counts.

Czekała, W., Jasiński, T., Grzelak, M., Witaszek, K. & Dach, J. 2022 Biogas plant operation: Digestate as the valuable product. *Energies* **15** (21), 8275.

Deng, G. & Shi, X. 2020 The effects of carbon source and COD/N ratio on simultaneous denitrification and methanogenesis in an upflow anaerobic sludge blanket reactor. *Renew. Energy* **157**, 867–873.

Dickinson, K., Bjornsson, W., Garrison, L., Whitney, C., Park, K., Banskota, A. & McGinn, P. 2015 Simultaneous remediation of nutrients from liquid anaerobic digestate and municipal wastewater by the microalga *Scenedesmus* sp. AMDD grown in continuous chemostats. *J. Appl. Microbiol.* **118** (1), 75–83.

Ebner, J. H., Labatut, R. A., Lodge, J. S., Williamson, A. A. & Trabold, T. A. 2016 Anaerobic co-digestion of commercial food waste and dairy manure: Characterizing biochemical parameters and synergistic effects. *Waste Manage.* **52**, 286–294.

Elmitwalli, T., Van Leeuwen, M., Kujawa-Roeleveld, K., Sanders, W. & Zeeman, G. 2006 Anaerobic biodegradability and digestion in accumulation systems for concentrated black water and kitchen organic-wastes. *Water Sci. Technol.* **53** (8), 167–175.

Erkelens, M., Ward, A. J., Ball, A. S. & Lewis, D. M. 2014 Microalgae digestate effluent as a growth medium for *Tetraselmis* sp. in the production of biofuels. *Bioresour. Technol.* **167**, 81–86.

Eshetu Moges, M., Todt, D. & Heistad, A. 2018 Treatment of source-separated blackwater: A decentralized strategy for nutrient recovery towards a circular economy. *Water* **10** (4), 463.

Fendel, V., Kranert, M., Maurer, C., Garcés-Sánchez, G., Huang, J. & Ramakrishna, G. 2022 Stakeholder assessment on closing nutrient cycles through co-recycling of biodegradable household kitchen waste and black water between rural and urban areas in south India. *Recycling* **7** (4), 49.

Fernandes, F., Silkina, A., Fuentes-Grünewald, C., Wood, E. E., Ndovela, V. L., Oatley-Radcliffe, D. L., Lovitt, R. W. & Llewellyn, C. A. 2020 Valorising nutrient-rich digestate: Dilution, settlement and membrane filtration processing for optimisation as a waste-based media for microalgal cultivation. *Waste Manage.* **118**, 197–208.

Franchino, M., Tigini, V., Varese, G. C., Sartor, R. M. & Bona, F. 2016 Microalgae treatment removes nutrients and reduces ecotoxicity of diluted piggery digestate. *Sci. Total Environ.* **569**, 40–45.

Fuchs, W. & Drosg, B. 2013 Assessment of the state of the art of technologies for the processing of digestate residue from anaerobic digesters. *Water Sci. Technol.* **67** (9), 1984–1993.

Gao, M., Zhang, L., Florentino, A. P. & Liu, Y. 2019 Performance of anaerobic treatment of blackwater collected from different toilet flushing systems: Can we achieve both energy recovery and water conservation? *J. Hazard. Mater.* **365**, 44–52.

Gao, M., Zhang, L. & Liu, Y. 2020 High-loading food waste and blackwater anaerobic co-digestion: Maximizing bioenergy recovery. *Chem. Eng. J.* **394**, 124911.

Giwa, A. S., Memon, A. G., Vakili, M., Ge, Y. & Wang, B. 2022 The resource recovery potential of blackwater and food waste: Anaerobic co-digestion in serial semi-continuous stirred tank reactors. *Int. J. Environ. Sci. Technol.* **19** (6), 5401–5408.

Hollinshead, W. D., Varman, A. M., You, L., Hembree, Z. & Tang, Y. J. 2014 Boosting D-lactate production in engineered cyanobacteria using sterilized anaerobic digestion effluents. *Bioresour. Technol.* **169**, 462–467.

Jermakka, J., Freguia, S., Kokko, M. & Ledezma, P. 2021 Electrochemical system for selective oxidation of organics over ammonia in urine. *Environ. Sci. Water Res. Technol.* **7** (5), 942–955.

Kamravamanesh, D., Rinta Kanto, J. M., Ali-Loytty, H., Myllärinen, A., Saalasti, M., Rintala, J. & Kokko, M. 2023 Ex-situ biological hydrogen methanation in trickle bed reactors: Integration into biogas production facilities. *Chem. Eng. Sci.* **269**, 118498.

Katajajuuri, J.-M., Silvennoinen, K., Hartikainen, H., Heikkilä, L. & Reinikainen, A. 2014 Food waste in the Finnish food chain. *J. Cleaner Prod.* **73**, 322–329.

Kjerstadius, H., Haghighatafshar, S. & Davidsson, Å. 2015 Potential for nutrient recovery and biogas production from blackwater, food waste and greywater in urban source control systems. *Environ. Technol.* **36** (13), 1707–1720.

Klüber, H. D. & Conrad, R. 1998 Effects of nitrate, nitrite, NO and N_2O on methanogenesis and other redox processes in anoxic rice field soil. *FEMS Microbiol. Ecol.* **25** (3), 301–318.

Kokko, M., Koskue, V. & Rintala, J. 2018 Anaerobic digestion of 30 – 100-year-old boreal lake sedimented fibre from the pulp industry: Extrapolating methane production potential to a practical scale. *Water Res.* **133**, 218–226.

Kujawa-Roeleveld, K. & Zeeman, G. 2006 Anaerobic treatment in decentralised and source-separation-based sanitation concepts. *Rev. Environ. Sci. Bio/Technol.* **5** (1), 115–139.

Lavagnolo, M. C., Girotto, F., Hirata, O. & Cossu, R. 2017 Lab-scale co-digestion of kitchen waste and brown water for a preliminary performance evaluation of a decentralized waste and wastewater management. *Waste Manage.* **66**, 155–160.

Leitão, R. C. 2004 *Robustness of UASB Reactors Treating Sewage Under Tropical Conditions*. Wageningen University, Wageningen, The Netherlands.

Lim, J. W., Chiam, J. A. & Wang, J.-Y. 2014 Microbial community structure reveals how microaeration improves fermentation during anaerobic co-digestion of brown water and food waste. *Bioresour. Technol.* **171**, 132–138.

Lu, Q., Chen, P., Addy, M., Zhang, R., Deng, X., Ma, Y., Cheng, Y., Hussain, F., Chen, C. & Liu, Y. 2018 Carbon-dependent alleviation of ammonia toxicity for algae cultivation and associated mechanisms exploration. *Bioresour. Technol.* **249**, 99–107.

Minale, M. & Worku, T. 2014 Anaerobic co-digestion of sanitary wastewater and kitchen solid waste for biogas and fertilizer production under ambient temperature: Waste generated from condominium house. *Int. J. Environ. Sci. Technol.* **11** (2), 509–516.

Moradi, P. & Saidi, M. 2022 Biodiesel production from *Chlorella vulgaris* microalgal-derived oil via electrochemical and thermal processes. *Fuel Process. Technol.* **228**, 107158.

Navarro-López, E., Ruíz-Nieto, A., Ferreira, A., Acién, F. G. & Gouveia, L. 2020 Biostimulant potential of *Scenedesmus obliquus* grown in brewery wastewater. *Molecules* **25** (3), 664.

Owen, W., Stuckey, D., Healy Jr., J., Young, L. & McCarty, P. 1979 Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Water Res.* **13** (6), 485–492.

Owusu-Agyeman, I., Eyice, Ö., Cetecioglu, Z. & Plaza, E. 2019 The study of structure of anaerobic granules and methane producing pathways of pilot-scale UASB reactors treating municipal wastewater under sub-mesophilic conditions. *Bioresour. Technol.* **290**, 121733.

Parichehreh, R., Gheshlaghi, R., Mahdavi, M. A. & Elkamel, A. 2019 Optimization of lipid production in *Chlorella vulgaris* for biodiesel production using flux balance analysis. *Biochem. Eng. J.* **141**, 131–145.

Park, J., Jin, H.-F., Lim, B.-R., Park, K.-Y. & Lee, K. 2010 Ammonia removal from anaerobic digestion effluent of livestock waste using green alga *Scenedesmus* sp. *Bioresour. Technol.* **101** (22), 8649–8657.

Prajapati, S. K., Kumar, P., Malik, A. & Vijay, V. K. 2014 Bioconversion of algae to methane and subsequent utilization of digestate for algae cultivation: A closed loop bioenergy generation process. *Bioresour. Technol.* **158**, 174–180.

Rajagopal, R., Lim, J. W., Mao, Y., Chen, C.-L. & Wang, J.-Y. 2013 Anaerobic co-digestion of source segregated brown water (feces-without-urine) and food waste: For Singapore context. *Sci. Total Environ.* **443**, 877–886.

Ranglová, K., Lakatos, G. E., Manoel, J. A. C., Grivalský, T., Estrella, F. S., Fernández, F. G. A., Molnar, Z., Ördög, V. & Masojídek, J. 2021 Growth, biostimulant and biopesticide activity of the MACC-1 *Chlorella* strain cultivated outdoors in inorganic medium and wastewater. *Algal Res.* **53**, 102136.

Serna-Maza, A., Heaven, S. & Banks, C. J. 2014 Ammonia removal in food waste anaerobic digestion using a side-stream stripping process. *Bioresour. Technol.* **152**, 307–315.

Sheng, K., Chen, X., Pan, J., Kloss, R., Wei, Y. & Ying, Y. 2013 Effect of ammonia and nitrate on biogas production from food waste via anaerobic digestion. *Biosyst. Eng.* **116** (2), 205–212.

Tan, X.-B., Zhang, Y.-L., Zhao, X.-C., Yang, L.-B., Yangwang, S.-C., Zou, Y. & Lu, J.-M. 2022 Anaerobic digestates grown oleaginous microalgae for pollutants removal and lipids production. *Chemosphere* **308**, 136177.

Tigini, V., Franchino, M., Bona, F. & Varese, G. C. 2016 Is digestate safe? A study on its ecotoxicity and environmental risk on a pig manure. *Sci. Total Environ* **551–552**, 127–132.

Tripathi, S., Choudhary, S., Meena, A. & Poluri, K. M. 2023 Carbon capture, storage, and usage with microalgae: A review. *Environ. Chem. Lett.* **21** (4), 2085–2128.

Wang, H., Li, Z., Zhou, X., Wang, X. & Zuo, S. 2020 Anaerobic co-digestion of kitchen waste and blackwater for different practical application scenarios in decentralized scale: From wastes to energy recovery. *Water* **12** (9), 2556.

Wang, X., Chen, J., Li, Z., Cheng, S., Mang, H.-P., Zheng, L., Jan, I. & Harada, H. 2023 Nutrient recovery technologies for management of blackwater: A review. *Front. Environ. Sci.* **10**, 1080536.

Wasielewski, S., Morandi, C., Mouarkech, K., Minke, R. & Steinmetz, H. 2016 Impacts of blackwater co-digestion upon biogas production in pilot-scale UASB and CSTR reactors. In: *13th IWA Specialized Conference on Small Water and Wastewater Systems & 5th IWA Specialized Conference on Resources-Oriented Sanitation*.

Wong, S., Nakamoto, L. & Wainwright, J. 1994 Identification of toxic metals in affected algal cells in assays of wastewaters. *J. Appl. Phycol.* **6**, 405–414.

Xia, A. & Murphy, J. D. 2016 Microalgal cultivation in treating liquid digestate from biogas systems. *Trends Biotechnol.* **34** (4), 264–275.

Xue, L., Song, G. & Liu, G. 2024 Wasted food, wasted resources? A critical review of environmental impact analysis of food loss and waste generation and treatment. *Environ. Sci. Technol.* **58** (17), 7240–7255.

Zhang, L., Guo, B., Zhang, Q., Florentino, A., Xu, R., Zhang, Y. & Liu, Y. 2019 Co-digestion of blackwater with kitchen organic waste: Effects of mixing ratios and insights into microbial community. *J. Cleaner Prod.* **236**, 117703.

Zhang, Q., Li, R., Guo, B., Zhang, L. & Liu, Y. 2021 Thermophilic co-digestion of blackwater and organic kitchen waste: Impacts of granular activated carbon and different mixing ratios. *Waste Manage.* **131**, 453–461.

Zhou, J., Wu, Y., Pan, J., Zhang, Y., Liu, Z., Lu, H. & Duan, N. 2019 Pretreatment of pig manure liquid digestate for microalgae cultivation via innovative flocculation-biological contact oxidation approach. *Sci. Total Environ.* **694**, 133720.

Zhu, L. 2015 Microalgal culture strategies for biofuel production: A review. *Biofuels Bioprod. Biorefin.* **9** (6), 801–814.

Zuo, S., Zhou, X., Li, Z., Wang, X. & Yu, L. 2021 Investigation on recycling dry toilet generated blackwater by anaerobic digestion: From energy recovery to sanitation. *Sustainability* **13** (8), 4090.

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