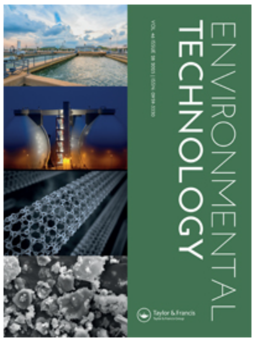

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Biomethanation of alkaline waste sludge in haloalkaline conditions: combined proof of concept experiments and technical economic evaluation

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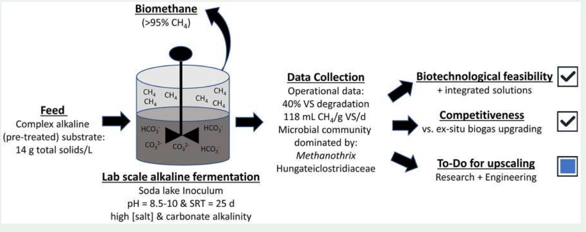
ABSTRACT

A highly pure biomethane stream (≈97% CH_4) was produced continuously under halo-alkaline conditions (pH > 9, 0.6 M Na^+) from complex alkaline organic waste residue originating from biopolymer extraction from sewage sludge. During the proof-of-concept operation, the substrate was degraded with similar efficiency (40% of the volatile solids, VS) compared to neutral conditions (36% of the VS). Operational data was utilised in a technical evaluation to identify bottlenecks for full-scale implementation at an early stage of process development and for comparison to conventional biogas upgrading using pressure swing and membranes. Initially identified bottlenecks for alkaline fermentation were related to overcautious assumptions, while others could be technically solved. Alkaline fermentation offers an attractive method for supplying increasingly needed high-purity biomethane using various recalcitrant substrates that have undergone alkaline pre-treatment. This is more feasible than the conventional ex-situ biogas upgrading. Next, upscaling steps for alkaline fermentation should be pursued. Strategies for integrated CO_2 sequestration and nutrient recovery are outlined, which will offer additional benefits in the future.



Highlights

- First successful semi-continuous biomethane production under alkaline conditions from complex substrate
- Techno economic evaluation: Scaling-up and scaling-down analyses identified bottlenecks that are relatively easy to resolve
- Next research steps and operational modifications to overcome bottlenecks were identified
- Alkaline fermentation is an attractive in-situ method for biomethane production with eventual CO_2 sequestration and nutrient recovery, instead of ex-situ biogas upgrading technologies.

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KEYWORDS
Alkaline fermentation; biomethane; techno-economic evaluation; semi-continuous; proof of principle

1. Introduction

Society is driving towards sustainability and circularity [1]. In this approach, it is essential to valorise waste streams. Granular and activated sludge wastewater treatment plants (WWTPs) will play an important role in the circular economy [2]. Resources that can be reclaimed in these WWTPs are, besides thermal and chemical energy, nitrogen, phosphorus, organic molecules like volatile fatty acids (VFAs), and biopolymers [3].

Biogas can be produced from the anaerobic digestion of organic waste streams, including WWTP sludge, which

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is an established practice worldwide. Biogas saves non-renewable resources and reduces geopolitical risks by decreasing the dependency on imported fossil fuels [4,5]. Sludge reduction associated with methanation also reduces the costs of sludge handling and disposal. Furthermore, anaerobic digestion invites the recovery of phosphorus and nitrogen through struvite, vivianite, or ammonia stripping [6–8].

Anaerobic digestion is conventionally performed under either mesophilic or moderately thermophilic conditions, and a pH range of 7-8. The biogas obtained from this process primarily consists of CH_4 (55-75%) and CO_2 (25-45%) [9]. Water vapour, hydrogen sulphide (H_2S), siloxanes, nitrogen (N_2), hydrogen (H_2), and ammonia (NH_3) are present at much lower concentrations [10,11]. Produced biogas is often used in a combined heat and power (CHP) unit to supply energy to the WWTP. Biogas is being increasingly upgraded to biomethane to allow direct injection into the natural gas grid [12]. Biogas upgrading involves the removal and capture of CO_2, resulting in streams that are highly concentrated in CO_2. This CO_2 can then be sequestered, either biologically or geologically, and stored over long-term, thus enabling a net negative CO_2 [13,14].

Hydrogen sulphide and siloxanes are commonly removed by activated carbon adsorption prior to biogas upgrading [10]. Recently, a biotechnology for H_2S removal under haloalkaline conditions (Thiopaq) has successfully been introduced into biogas purification practices for gas streams with high H_2S concentrations [15,16]. However, Thiopaq is not aimed at CO_2 removal. Owing to the short contact time and preferential uptake of H_2S into the alkaline stream there is little CO_2 absorption [16]. Second, the CH_4 percentage in the biogas must be increased before injection into the existing gas grid. This percentage differs across countries. In Europe, it ranges usually between 84 and 97% CH_4 [17–19].

Various techniques are used to increase the methane concentration in the biogas by eliminating CO_2. Currently, ex-situ technologies are used at full-scale plants to upgrade biogas outside of the anaerobic digester. Ex-situ technologies include absorption, adsorption, membrane separation, and cryogenic separation [20]. However, ex-situ technologies require additional equipment and space, making these only economically and energetically feasible for larger plants with biogas production higher than 2400 m^3/day [21].

In-situ technologies upgrade biogas within the anaerobic digester. In-situ technologies will not take up any additional footprint, while the CAPEX will remain in the same order of magnitude as ex-situ technologies [20]. They have decreased maintenance costs, can be easily implemented and are suitable for small installations. These technologies are currently not implemented at full-scale installations and are not as well explored as ex-situ technologies. Current in-situ strategies include high-pressure digestion, (bio)electrochemical systems, and the addition of H_2 for biomethanation of CO_2 [21]. These technologies are not yet feasible, and new opportunities need to be explored [20].

This paper focuses on halo-alkaline fermentation (pH > 9, 0.6 M Na^+) as an alternative in-situ technology. Under such conditions, acidic waste gases including CO_2 and H_2S will mostly remain in solution as bicarbonate/carbonate and hydrosulphide (HS^-), respectively. Thus, the alkaline digester acts as a scrubber, resulting in a high purity CH_4 gas stream. To produce biogas under these conditions, a specialised microbial community consisting of haloalkaliphilic anaerobic microorganisms enriched from anoxic sediments of saline soda lakes was utilised [22–24]. Previous studies on alkaline anaerobic digestion by such communities have used cyanobacterial biomass [25,26] or industrial waste [27] as substrates. Alkaline biomethanation is possible in a semi-continuous lab-scale reactor with trace-amounts of H_2S gas in the produced biogas [26]. Due to process inhibition by NH_3-N and VFAs only about 10% of the initial biomass was converted into methane [28]. No information is available on the efficiency of degradation/CH_4 production under haloalkaline conditions using more complex organic wastes, such as from WWTPs.

Recently, the concept for aerobic granular sludge (AGS) WWTPs as resource factories was introduced [29], based on extraction of extracellular polymeric substances (EPS) from AGS. This process produces an alkaline waste stream [29,30]. This study focuses on investigating and evaluating biomethane production from alkaline extraction residuals under alkaline conditions with the potential for net negative CO_2 emissions and resource recovery in future and circular WWTPs. Alkaline waste sludge with high pH and high salt content (due to base addition) and depleted in highly valuable EPS is optimally suited for alkaline fermentation.

Alkaline pre-treatment of sewage sludge, followed by neutrophilic anaerobic digestion after the neutralisation step, has been shown to increase the methane yield [31,32]. Alkaline fermentation is not restricted to the tested substrate but has a much broader potential [31]. The significance of our study is highlighted by the recent statement that the lack of organisms capable of operating at a pH above 8.5 is a major bottleneck for in-situ biogas upgrading [20].

The aims of this paper were to (A) set up and operate a semi-continuous halo-alkaline anaerobic biomethanation reactor using complex alkaline waste as a substrate to gain operational data in ‘steady-state’ and (B) use this data for scaling up and scaling down analyses to identify bottlenecks with respect to upscaling the alkaline fermentation technology at an early stage of development. This approach allows the investigation of the competitiveness of alkaline fermentation in comparison to conventional neutral pH digestion followed by biogas upgrading and helps to identify the major cost contributors to alkaline fermentation. This combined approach aims to guide further research and upscaling activities for the alkaline fermentation technology.

2. Material and methods

2.1. Materials

2.1.1. Media and buffer composition

A bicarbonate/carbonate buffered nutrient medium (pH = 9.6) was prepared by first dissolving 15 g/L Na_2CO_3 and 20 g/L $NaHCO_3$ in MilliQ. To this solution NaCl, K_2HPO_4, $MgCl_2$ were added (Table 1). Additionally, 1 mM Se/W and acidic trace metal (ATM) solutions [33] were added (Table 1; details of the solution preparation are given in SI – 1). The final medium was sparged with argon gas for 30 min and reduced by the addition of 0.1 mM Na_2S solution from a 75 mM anoxic stock. Dithionite was added to a final concentration of 0.1 mM to ensure fully anaerobic conditions. The medium was stored in gas-tight glass bottles, sealed with butyl rubber stoppers, at 4 °C and used within one week of preparation.

2.1.2. Substrate

The alkaline waste sludge was obtained from a demonstration installation designed to extract extracellular polymeric substances (EPS) from aerobic granular sludge at the WWTP Epe, The Netherlands [29]. During the EPS extraction process, the pH of the sludge was increased using KOH (ca. 9-10, measured at 80°C), and the temperature was increased to approximately 80 °C. It took about 2 h to solubilise the EPS. After the extraction step, the sludge was centrifuged using a decanter centrifuge. The centrate contains the solubilised EPS and was further processed for recovery. The solid material obtained from the centrifugation step is the alkaline waste sludge used as a substrate in this study. The characteristics of the substrate is are listed in Table 2. Total solids (TS) and volatile solids (VS) measurements on samples mixed with buffered media were performed on washed samples to eliminate the effect of the high carbonate concentrations (see section 2.2.3).

Table 1. Media composition and acidic trace metal media composition.

Compound		Concentration
NaCl	[g/L]	3
K_2HPO_4	[g/L]	1
$MgCl_2$	[g/L]	0.1
Se/W (1 mM)	[mL/L]	0.1
Acidic Trace Metals	[mL/L]	1
EDTA (trilon B)	[g/L]	5
$FeSO_4{\cdot}7H_2O$	[g/L]	2
$Zn{\cdot}7H_2O$	[g/L]	0.1
$MnSO_4$	[g/L]	0.04
H_3BO_3	[g/L]	0.3
$CoCl_2{\cdot}6H_2O$	[g/L]	0.2
$CuCl_2{\cdot}2H_2O$	[g/L]	0.0013
$NiCl_2{\cdot}6H_2O$	[g/L]	0.0029
$Na_2MoO_4{\cdot}2H_2O$	[g/L]	0.02

2.1.3. Inoculum

The inoculum consisted of anoxic sediments obtained from four hypersaline soda lakes of the Kundula steppe (Altai Region, Russia), which brines salinity and pH ranged from 100 to 300 g/L and 9.5 to 11.0, respectively [24]. The samples were taken from sediment depths between 5 and 20 cm, at 20–25°C water temperature, mixed in equal proportions, and homogenised with an equally mixed near-bottom brine sample at 1:1 to get a slurry. Ten milliliters of the slurry were added to a Falcon tube and mixed vigorously with 20 mL of anoxic medium. The slurry was centrifuged for 10 min at 10,000 rpm and 4̊C. The supernatant was removed and the pellet was resuspended in 20 ml anoxic medium. Next, the slurry was centrifuged at 500 rpm for 1 min to remove the coarse sandy fraction low in

Table 2. Alkaline waste pellet characteristics.

Parameter	Units	Concentration or ratio	St. Dev.	Method/Source
tCOD	[g O_2/kg waste sludge]	119	±4.6	Photometric (cuvette)
Total Nitrogen (TN)	[g/kg waste sludge]	5.4	±0.14	Photometric (cuvette)
Total Phosphorus (TP)	[g/kg waste sludge]	1.8	±0.04	Photometric (cuvette)
Total Sulfur (TSul)[1]	[g/kg waste sludge]	0.5	±0.04	CHNS Analyzer
Total solids (TS)	[g/kg waste sludge]	116	±0.29	[34]
Volatile solids (VS)	[g/kg waste sludge]	76	±0.18	[34]
pH	[-]	11		Potentiometric (@RT)
Ratio C/N[1]	[-]	12	±0.7	CHNS analyser

[1]see Table S1 in supplementary for additional information.

biomass, while the remaining fine particle fraction enriched in cells was used as the inoculum.

2.2. Methods

2.2.1. Batch incubations

Multiple steps were taken to start up and scale the alkaline fermentation process and establish the operation of a semi-continuous alkaline fermenter. Successive 50-, 900 – and 1,800-mL batch incubations were prepared under oxygen-free conditions under a continuous argon stream using argon flushed bottles. The methods and results of these batch experiments are described in detail in the supplementary information (section SI-3).

2.2.2. Semi-continuous operation

The transition towards semi-continuous operation was performed using inoculum from the third and largest batch set (1,800 mL batch). After gravity settling and decanting the liquid off, roughly 200 mL of the settled material was transferred under an argon stream into a 2 L argon flushed glass reactor. The substrate and oxygen free buffered nutrient media were added to a final TS of 8 g/L.

The feed bottle was equipped with a magnetic stirring bar and was placed on a weight scale to validate the accuracy of the feed pumps. The feed bottle was continuously mixed using a magnetic stirring bar. This bottle was open to avoid a vacuum.

Based on insights from the batch experiments, the solid retention time, SRT (corresponding to the hydraulic retention time, HRT) for semi-continuous operation was initially set to 35 days. Feeding of the substrate (40 g/d, pH = 9.5, TS = 8 g/L) of the reactor was done in 12-hourly cycles. The effluent removal took 10 min and was done daily while mixing (300 rpm). Influent wasfed, in roughly the same time at the end of the feeding cycle, while still mixing. The pH was not actively controlled, but was continuously measured and ranged from 9 to 9.2. Within this pH range, the methane content of the produced biogas was approximately 94%.

The temperature was maintained at 35 °C using a water bath and water jacket. The reactor feed and effluent were analysed weekly for tCOD, sCOD, NH_4^+-N, TS, VS, and VFAs. The gas was continuously measured on a mass spectrometer (CO_2, O_2, H_2O, N_2, Ar, H_2, CH_4). A thermal mass flow controller calibrated with nitrogen was used; however, argon was used as carrier gas instead. Therefore, the flow measurement was corrected using the molar heat capacities of argon and nitrogen. The carrier gas was added to the gas line (thus after the anaerobic digester). A gas hold-up container was introduced after the gas effluent, with a sufficiently large gas hold-up (0.5 L) to reduce the noise due to intermittent gas production from the bioreactor. The purpose of the argon is to maintain a consistent flow to the mass spectrometer, enabling the quantification of the total gas flow and gas composition, without altering the gas composition in the bioreactor.

After a month of incubation, stable gas production was achieved. The retention time was decreased to 25 days and the TS loading was increased to 14 g/L TS. This was decided based on the assumption that NH_4^+ concentration is the crucial operational parameter for process inhibition. The observed NH_4^+ values were far below the toxicity thresholds of 30 mM or 420 mgN/L total ammonia established for adapted pure cultures of dominant soda lake hydrogenotrophic methanogens from the genus *Methanocalculus* (D.Y. Sorokin, unpublished data). Additionally, no VFA accumulation – potentially inhibiting methanogenic activity – was observed [35]. The reactor was eventually operated with 14 g/L TS, a retention time of 25 days, and a 12-hour feeding cycle in which 28 mL substrate was fed. The final operating scheme is shown in Figure 1.

2.2.3. Analytical methods

Gas production during batch experiments was monitored using water displacement or syringes. Gas samples were stored in glass vials filled with concentrated NaCl brine and closed with butyl rubber stoppers. The gas sample was injected into the vials, displacing part of the brine. The vials were stored upside down. The brine prevents gas diffusion out of the vial since no gases dissolve in it. The gas composition was analysed by gas chromatography (Agilent Technologies 7890A GC system) using Agilent 19095P-MS6 + 19095P (60 mx 530 µm x µm) column and He as the carrier gas. The gas production and gas composition (CO_2, CH_4, O_2, H_2 and Ar) during semi-continuous operation were monitored using a ThermoFisher Scientific Prima BT bench top mass spectrometer using a Farady/SEM dual detector and the flow of argon was controlled by a separate thermal mass flow meter. Ammonium, sCOD, VFA, and pH were determined bi-weekly during batch experiments. Samples of 1.5 mL were taken from the supernatant using a syringe pre-washed with argon. Directly after the sample was taken, it was centrifuged and filtered through a 0.2 µm filter and stored in the freezer until measurements were taken.

Liquid samples (40 mL) were taken from the reactor once per week for VFA, sCOD, tCOD, NH_4-N, alkalinity, pH, and TS/VS analyses. First pH was measured. Then two times 15 mL sample was filled into pre-weighed falcon tubes, and the remaining sample was put on

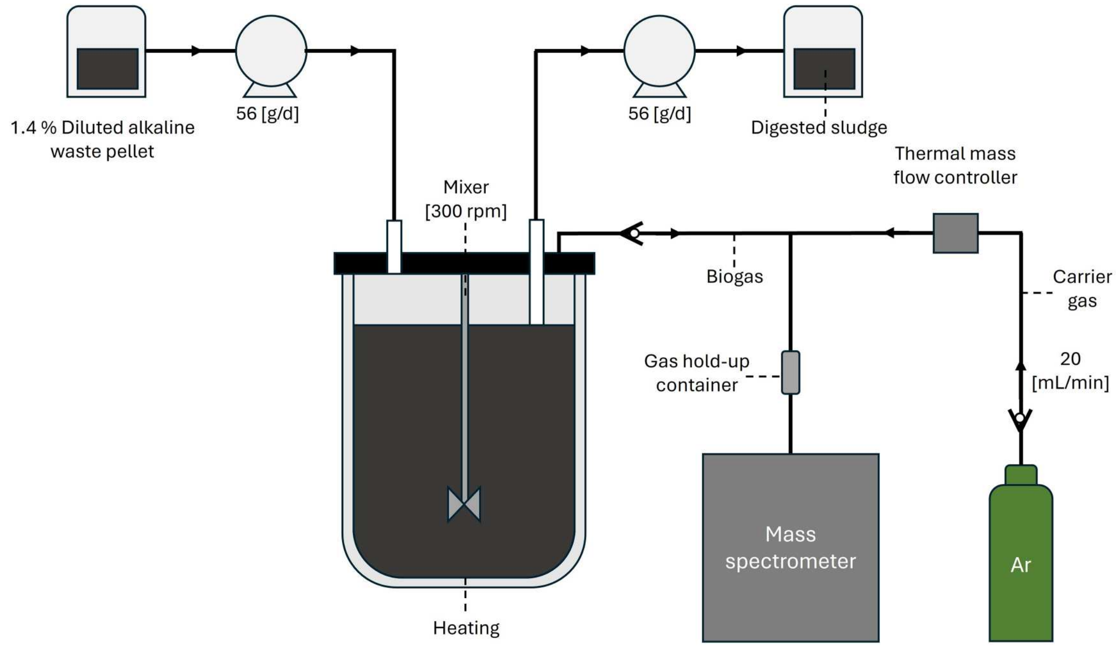

Figure 1. Schematic overview of the semi-continuous set-up. While mixing, 56 g/d of digested alkaline waste pellet is removed and subsequently 56 g/d of diluted alkaline waste pellet (14 g/L TS) is fed into the reactor per day. Heating is done by introducing hot water in the digester walls and mixing is achieved by using a mechanical mixer. Gas from the reactor and argon are flowing through a gas mixing container (0.5L) to allow proper quantification of intermittent gas production using the MS. Additionally, argon gas is supplied to maintain sufficient flow to the mass spectrometer for measurement without affecting the gas composition.

ice. The falcon tubes were centrifuged for 30 min at 18,500 RCF at 4 °C. The centrifuged samples were filtered through a 0.45 µm and subsequently through a 0.2 µm syringe filter.

The total and carbonate alkalinity were measured via titration in duplicate samples after diluting 5 mL of filtrate with 10 mL of demineralised water. A 0.5 M HCl solution was used to titrate the samples to pH 8 and 4.5, respectively, while continuously stirring the sample. The VFA composition was measured in 0.2 µm filtered samples on an HPLC (Thermo scientific Vanquish Detector, Autosampler and Pump and ERC RefractoMax520) using an Aminex® (R) HPX-87H column (300 × 7.8 mm). Finally, sCOD and NH_4-N were measured in 0.2 µm filtered samples using the LCK 614 and LCK 304 HACH LANGE kits, respectively. Measurements were done in a HACH LANGE DR 3900. For the tCOD measurements, unfiltered influent and effluent samples were used. The samples were diluted by at least a factor of 10 to dilute the salt concentration to below 0.3 g/L.

For the determination of TS and VS, the samples were washed to eliminate the influence of salts and carbonates on these measurements. For washing, 30 mL of sample was centrifuged at 18,500 RCF for 30 min at 4 °C. The clear supernatant was discarded. The pellet was resuspended in 10-20 mL demineralised water and again centrifuged at 18,500 RCF for 30 min at 0 °C. The demineralised water was removed, and the pellet was again resuspended in an additional 10-20 mL demineralised water. The TS and VS contents of the pellet were determined according to standard methods [34]. The CH_4 production was calculated based on the degradation of VS and COD.

2.2.4. Scaling-up and scaling down analyses

To identify potential processes and economic bottlenecks of the alkaline fermentation technology, a techno-economic comparison was carried out. For this evaluation, an alkaline digester with direct biomethane production and a conventional digester operated at near-neutral pH and subsequent biogas purification to produce biomethane using the same alkaline waste sludge as substrate were compared. The operational results obtained from the semi-continuous reactor in this study were linearly extrapolated to compile a scenario for a full-scale alkaline digester in terms of mass balances and degradation efficiency of the COD/VS. The TS concentration in the alkaline digestion system was assumed to be limited by ammonia toxicity, which was derived from ammonia release per mass substrate during reactor operation. A guideline of 30 mM or 420 mg N /L total ammonia was used as the threshold, obtained for adapted pure cultures of methanogens (unpublished data, D.Y. Sorokin). The neutral digestion scenario was compiled using experiences from common anaerobic digestion systems for WWTPs. The

% VS degradation in the neutral digester was obtained from batch experiments with alkaline AGS waste sludge (WWTP Epe) as substrate and digested sludge from a conventional digester as inoculum at a pH of 8.1 (WWTP Harnaschpolder); the results were corrected for the methane production by the inoculum (see SI). The %VS degradation in the reactor was determined by direct VS measurements in the influent and effluent. The methane production in both scenarios was based on mass spectrometer data obtained during the operation of the alkaline reactor. The biogas purification unit of the neutral digester comprises a membrane pressure system with an activated carbon filter for H_2S removal before. A representative three-stage polymeric membrane system was used as reference for biogas upgrading. It consists of hollow fiber membranes with high selectivity for CO_2 over CH_4. The NaOH dosing requirements to keep the pH in the alkaline fermenter stable at pH = 9.5 were calculated based on the assumption that CH_4 and CO_2 are produced in a ratio 60:40 and that for the neutralisation of 1 mol of CO_2 1 mol of − OH are required and adjusted for the produced NH_4^+. Full-scale installations, with the goal to produce biomethane (at least 95% CH_4) were compared (Figure 2).

Table 3 summarises the numbers used to determine the CAPEX and OPEX for both technologies. A replacement time of 20 years was assumed for CAPEX. For the membrane system, slightly higher maintenance costs were assumed (4%) in the membrane-based biogas upgrading compared to the alkaline fermentation system (3%) because of the higher complexity of the ex-situ technology. Inside the system boundaries are digesters, sludge dewatering facilities and gas purification including core equipment (pumps, agitators and piping), chemical consumption (polyelectrolyte, PE to facilitate sludge dewatering and NaOH for maintaining the pH) and power supply. All costs were estimated based on unit equipment costs from earlier projects at West-European price levels in 2021. The additional costs (extra costs, unforeseen, incompleteness) were calculated based on a percentage of the investment costs common for engineering projects. Note bene, the technical evaluation was part of scaling-up and scaling down. Thus, the relative and not absolute costs were of interest in the evaluation to identify potential bottlenecks that could be addressed in the lab work at an early stage (scaling-up and scaling down). Polyelectrolyte (PE) consumptions during dewatering were estimated. It was assumed that alkaline fermented sludge required more PE owing to elevated pH and higher charge respectively. It was assumed that the final TS of the sludge cake was identical in both scenarios.

1. Results

3.1. Semi-continuous reactor operation

Results are presented from the operation of the alkaline reactor at a pH = 9.6, with 14 g/L TS substrate feeding, solid and hydraulic retention times of 25 days, and 12 h feeding cycles (28 mL substrate was fed every

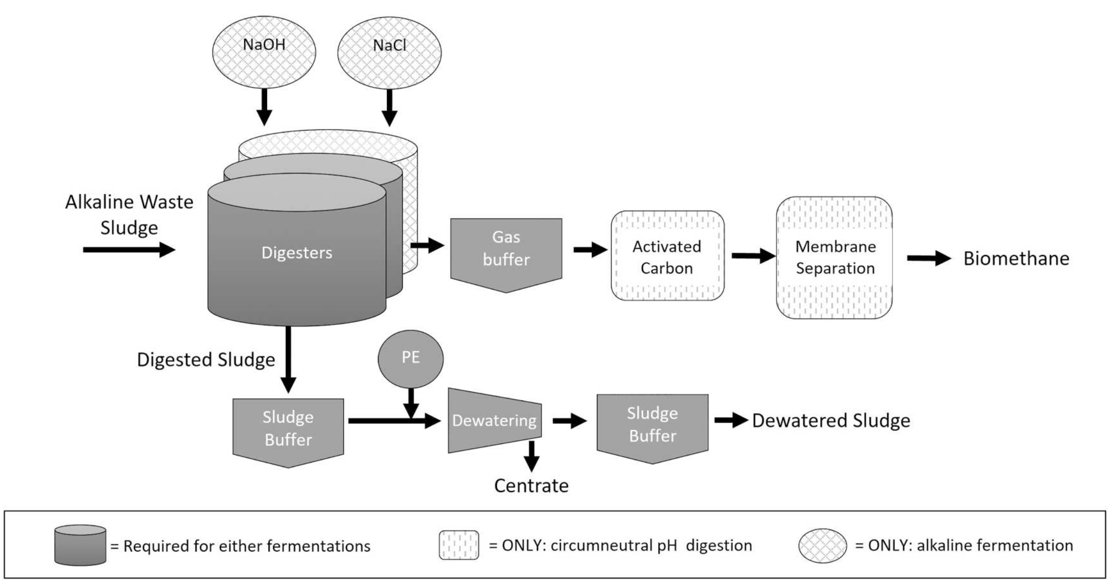

Figure 2. Scenarios for the techno-economic comparison. Grey boxes are present in both scenarios. Additional steps of the alkaline process are defined by the cross pattern, which includes an additional digester and dosing of alkalinity (NaOH) and salt (NaCl). The circumneutral pH digestion is defined by the vertical lines, where this includes biogas upgrading using activated carbon and membrane separation. Digested sludge management is identical for both scenarios.

Table 3. Assumption for the technological evaluation.

Parameter	UoM	Neutral anaerobic digestion	Alkaline anaerobic digestion
Total solids load	[kg/day]	21.000	21.000
Ingoing TS concentration	[g/L]	60	39
SRT	[Days]	23	25
Temperature	[°C]	35	35
Temperature outside	[°C]	9	9
Methane production[1]	[mL CH_4/g VS/d]	118	118
VS removal[2]	[%]	36	40
Max. volume/ digester[3]	[m^3]	5000	5000
Energy biomethane	[MJ/Nm^3]	36	36
NaOH (33%)[4]	[m^3/d]	-	7
NaCl	[kg/d]	-	808
Biogas upgrading efficiency	[%]	99.5	-
Active carbon consumption[5] VOC	[g/Nm^3 biogas]	0.15	-
Active carbon consumption[5] H_2S	[g/Nm^3 biogas]	0.45	-
PE consumption	[g PE_{active}/ kg TS]	15	20

[1]Obtained from mass spectrometer measurements during the operation of the alkaline digestion reactor.
[2]VS removal is based on experimental batch data of the alkaline waste sludge.
[3]If the maximum volume is surpassed, additional digester(s) is/are required.
[4]Linearly extrapolated from the 14 g/L alkalinity requirement.
[5]Refers to activated carbon that needs to be refilled/regenerate.

12 h, Figure 1). The reactor was successfully operated, and data for the technological evaluation were obtained after stable operation was achieved (Figure 3).

COD measurements were conducted to determine the COD/VS ratio of the alkaline waste sludge as the basis for the COD mass balance. The undiluted substrate gave a COD:VS ratio of 1.56, which is equal to the theoretical methane production of 219.5 mL CH_4/g VS_{added}/d, 1.9x higher compared to the value determined with the MS (123 mL CH_4/g VS_{added}/d). For substrate mixed with the alkaline nutrient media, an even higher COD/VS ratio of 1.93 was determined resulting in a methane production of 271.6 mL CH_4/g VS_{added}/d. The reason for this large deviation remains unclear. It could be related to matrix effects on measurements in the media with high carbonate and salt concentrations, non-representative samples or analytical errors. Under a steady state, approximately 40% of the VS and 43% of the tCOD were degraded during the operation. The MS detected on average 123 mL CH_4/g VS_{added}/d after flow correction. Methane production rates, as calculated from VS and COD degradation, were significantly higher. In a later study, the measurements from the MS were confirmed using a pressure meter and by collecting and quantifying the gas produced in a gas bag. Thus,

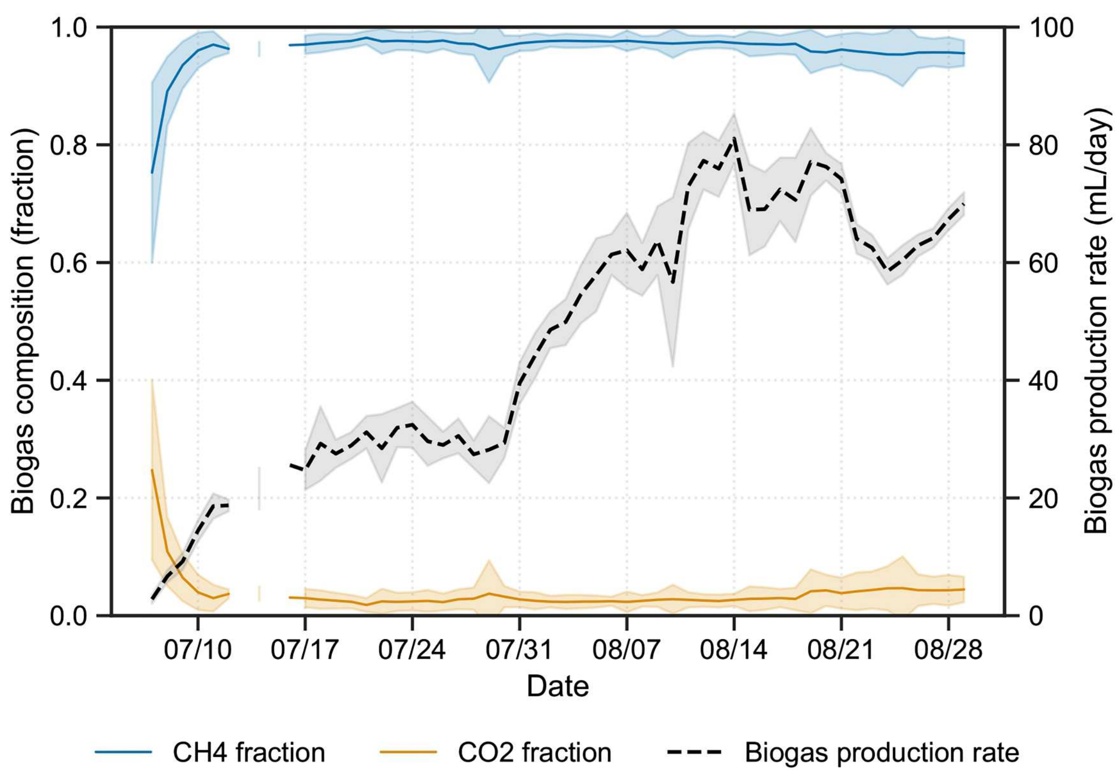

Figure 3. Results from mass spectrometer measurement, methane fraction (blue), carbon dioxide fraction (red), and biogas production rate in black. Shaded areas are the 95% confidence intervals. Data from 05.08. until the end of measurements were considered in the evaluation. The methane content in the produced gas was consistently above 95%.

Table 4. Influent and effluent composition of the alkaline fermentation reactor.

Parameter	Feed	Effluent
pH	9.6	9.0
TS (g/L)	14.3 (± 0.92)	9.9 (±0.06)
VS (g/L)	9.9 (±0.65)	6.0 (±0.01)
tCOD (g/L)	19.1 (±0.80)	10.8 (±0.38)
sCOD (g/L)	3 (±0.7)	0.7 (±0.1)
N-NH_4^+ (mg/L)	55.9 (±2.69)	150 (±3.14)

See Figure S5 for additional information.

it was decided to use the data obtained with the MS for all further analyses and discussions. The gas composition throughout the semi-continuous operation was 97% (±1%) methane, and 3% (±1%) carbon dioxide. The composition of the feed and effluent during stable operation is summarised in Table 4.

3.2. Scaling-up and scaling down analyses

Mass balance results of the alkaline digestion are shown in Figure S 6. Full-scale systems were dimensioned for both alkaline fermentation and conventional digester systems. Subsequently, costs for both systems were priced (Figures 4 and 5). The costs per year summed up to 3.2 mio. Euro/y for the alkaline fermentation system and 2.3 mio. Euro/y for the conventional digester. For the neutral digestion scenario, CAPEX contributed with 608,000 Euro/y and OPEX with 1.7 mio. Euro to the total cost. For the alkaline fermentation system, CAPEX summed up to 719,000 Euro/y and OPEX to 2.5 mio. Euro/y. Thus, the most significant difference between the annual cost is attributed to the ca. 42%

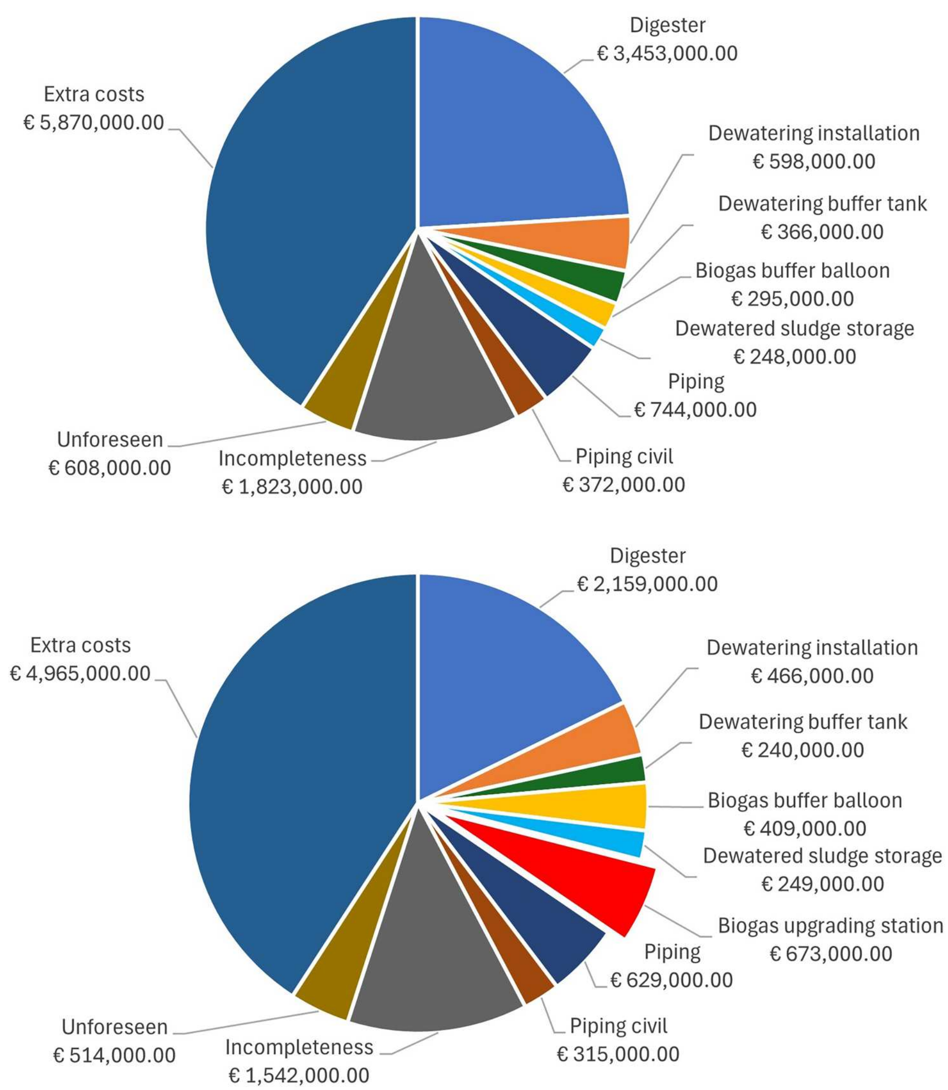

Figure 4. Total CAPEX broken down for alkaline (top) and neutral (bottom) pH fermentation systems. The major difference is shown in digester cost as this is 1.6x higher for the alkaline scenario. Additionally, the dewatering unit is 1.3x times higher and sludge buffer is 1.5x higher in the alkaline digestion scenario. Due to CO_2 still in the biogas of the neutral digestion system, the biogas buffer balloon is 1.4x times higher and due to the need for a biogas upgrading system, an additional €673,000 is required. Eventually, the overall CAPEX costs are ∼18% higher for the alkaline scenario.

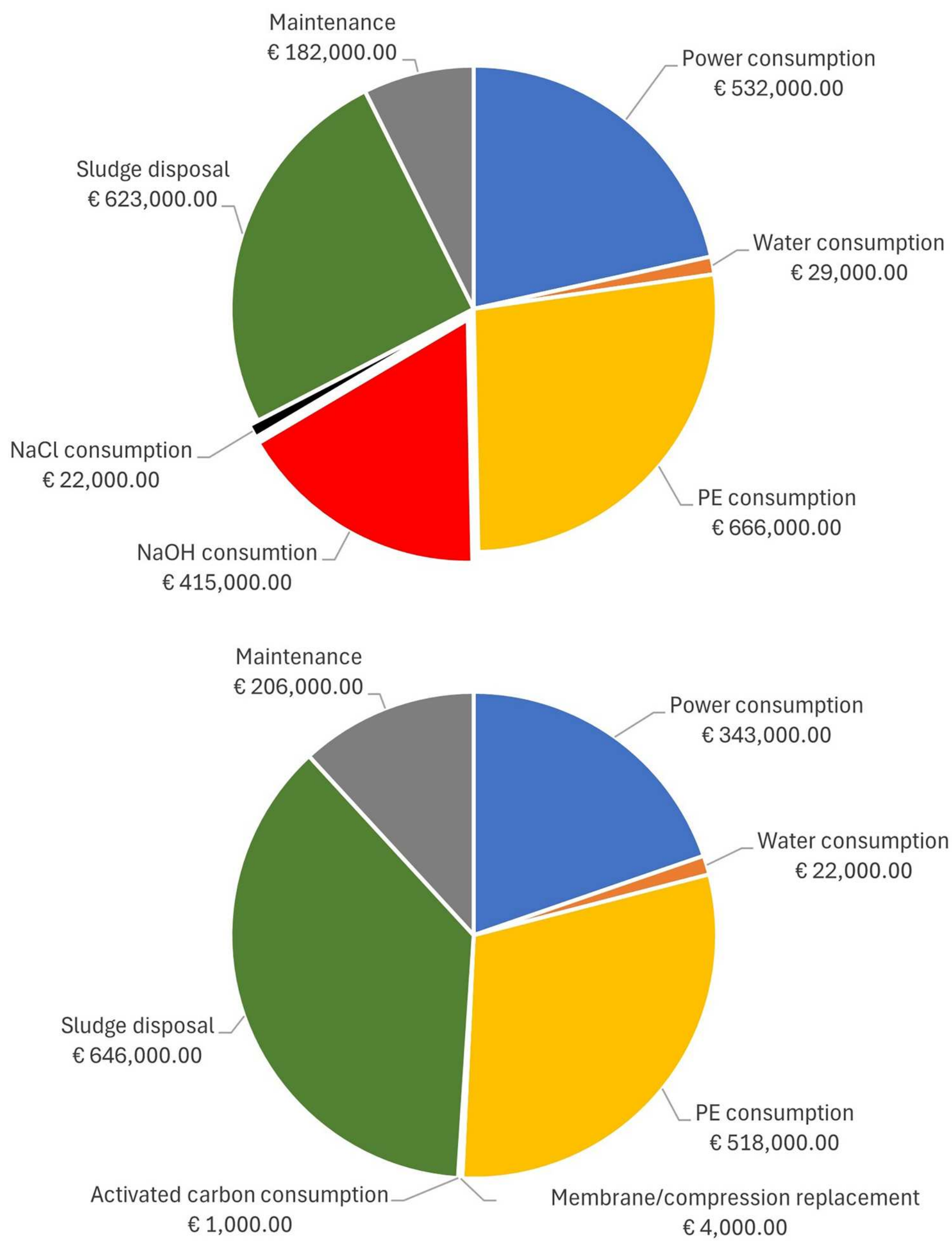

Figure 5. Total OPEX broken down for the alkaline (top) and neutral (bottom) fermentation systems. The OPEX cost for alkaline digestion is significantly higher compared to alkaline digestion (~42%). This is due to the addition of NaOH dosing, increased PE consumption & higher power consumption. To account for the higher maintenance using a biogas upgrading installation, a 1% increase is used for maintenance on the circumneutral pH digestion. For neutral digestion, no addition of acid (HCl) to neutralise the substrate was assumed.

higher OPEX for the alkaline fermentation (Δ729,000 €/y), whereas the difference of 18% higher CAPEX was less significant (Δ111,000 €/y).

The assumption that a maximum N-NH_4^+ concentration of 30 mM during alkaline fermentation can be allowed before inhibition of the methanogenic community occurs limits the loading of the alkaline fermentation system to 39 g/L TS. Thus, for the same TS load, larger hydraulic loads must be handled during alkaline fermentation. For the design of the digesters, this effect was amplified by the longer SRT chosen during alkaline versus neutral digestion (23 versus 25 days). For the alkaline fermentation system, the investment costs for the digester (x1.6), dewatering unit (x1.3) and sludge buffer (x1.5) are higher. Because of the CO_2 in the emitted gas from the neutral digester, higher costs for the gas balloon (x1.4) were calculated. Furthermore, a biogas purification unit of about 673,000 Euro is required to produce biomethane.

Overall, alkaline fermentation has about 729,000 euros higher OPEX per year, which is explained by the NaOH needed for pH adjustment (415,000 €/y), higher power consumption for larger equipment to handle higher volumes (1.6x higher) and higher costs for PE dosing during sludge dewatering (1.3). Due to more complex treatment chain for the biogas upgrading, the maintenance costs are 1.1x higher for neutral compared to the alkaline fermentation installation. In the neutral pH scenario, no acid was added for substrate neutralisation. Biogas production revenue was not shown but was considered in OPEX. The production equalled 738,000 €/y and 658,000 €/y for alkaline digestion and neutral digestion, respectively.

4. Discussion

4.1. Biomethane production using alkaline fermentation process

4.1.1. Batch experiments

The main goal of the batch experiments was to obtain inoculum for the reactor operation. However, the observed rates are briefly discussed because of their novelty and because they contain valuable information for the large scientific community that uses batch tests to evaluate fermentation performances. Specific methane yields ranged from 120 (50 mL batches), in the first incubation, to 249 mL CH_4/g VS_{added}, in the final 1,800 mL batch test, indicating that the community gradually adapted to the substrate. These rates were compared to non-published research from our group on alkaline fermentation using similar substrate [36,37]. Sels [36] used granules from the WWTP Vroomshoop, NL and extracted EPS from it. Both granules and extraction residuals, were fermented and showed yields of 130 and 200 mL CH_4/g VS_{added}, respectively. Equal to 24% and 35% of the theoretical maximum CH_4 yield, respectively, indicating that EPS extraction enhances methane production. Sels [36] observed a methane yield of 240 mL CH_4/g VS_{added} when EPS extraction residuals came from laboratory grown granules. This equals 42% of the theoretical CH_4 yield. Dragone [37] observed a specific methane yield of 378 mL CH_4/g VS_{added}, using residuals from EPS extraction of laboratory AGS, equal to a VS degradation of 64%. The high methane production is surprising as the substrate is similar to that in the study by Sels [36]. The similarity of the substrates is underlined by the substrate composition TN (81 mg N/g VS vs. 63–84 mg N/g VS) and tCOD (1.5 g/g VS vs. 1.34-1.45 g/g VS) in the work of Dragone [37] and Sels [36] respectively. Differences between studies include a higher inoculum-to-substrate ratio of 2.5 and the usage of sodium carbonate instead of KOH for EPS extraction by Dragone [37]. Endogenous methane production of the added inoculum can explain the higher methane yields [38].

The methane yield during neutral digestion of EPS extraction residuals from Epe WWTP ranged from 230 to 260 mL CH_4/g VS [30]. A similar order of magnitude of specific methane production at pH = 8 was determined in biochemical methane potential batch tests for excess granular sludge (194 mL CH_4/g VS_{added}) and selection spill sludge (296 mL CH_4/g VS_{added}, 39). A mixture of these sludges is used as the raw material for EPS extraction. It cannot be excluded that the alkaline substrate (although diluted 1:3) had a negative effect on biogas production during the control incubation with sludge from a full-scale anaerobic digester. However, in thermo alkaline pre-treated sludge higher salt and pH of sludge are observed, which still allows biogas production. In the only other published study on alkaline batch fermentations, freeze-dried cyanobacterial (*Spirulina*) biomass was used as substrate. In these experiments 85 mL CH_4/g VS_{added} was produced [25,38]. *Spirulina* is a difficult substrate (observed biodegradability: 13%). High NH_4^+ concentrations (1,123 mg/L) might have inhibited methanogenesis. NH_4^+ inhibition was probably not an issue in our incubations, concentrations remained at 150 mg N-NH_4^+/L, far below the threshold of 420 mg N-NH_4^+/L. In contrast to other research, no significant accumulation of VFA was observed [39]. VFA can inhibit methanogens at high levels and indicates metabolic imbalances or incomplete CH_4 production [35].

4.1.2. Semi-continuous reactor operation

Inoculum from the batch experiments was used to operate a semi-continuous reactor with a substrate from a WWTP for the first time to obtain operational data. The reactor was operated with VS concentrations twice as high than the batch systems (9.9 g VS/L, SRT = 25 days). COD/VS degradation in the reactor was approximately 40%, 1.6 times higher compared to the initial batch experiments, indicating adaption of the microbial community. Acetate was the only detectable VFA. The effluent acetate concentration was low, with 0.5 mM, indicating efficient substrate conversion at the chosen SRT.

The pH dropped during operation from 9.6 to about 9, probably because of CO_2 production as VFA did not accumulate in significant concentration. The CH_4 content of the produced gas was 97%, indicating the possibility of operating at pH = 9 or lower while still allowing the production of biomethane suitable for grid injection [12]. Neither NH_3 nor H_2S was detected in the gas phase. Accordingly, at pH = 9, sulphide is mainly present as dissociated HS^-. Outgassing can be expected for NH_3 since under the operational conditions, ca. 70% of the total ammonium nitrogen should be present as aqueous NH_3 (VisualMinteq). Perhaps stirring was too low for stripping. The process conditions are beneficial for integrated NH_3 recovery as discussed below.

Data for the evaluations were collected after three solid retention times (SRT). Whether pseudo steady-state operation conditions were achieved remains unclear as the pH continuously decreased from 9.6 to 9.0 and a consistent increase of N-NH_4 from 90 to approximately 150 mg/L was observed after the three SRTs.

Potential inhibition of the process can be attributed to the accumulation of NH_4^+, VFA or phenolic compounds or metal limitations at high pH. VFA and NH_4^+ accumulation was not observed. The slurry turned brown after mixing the substrate and medium, indicating the release of phenolic compounds [40,41]. Potential inhibition was not investigated in this study. The risk of essential metal limitation (Mg, Cu etc.) due to high pH is considered low since soluble carbonate ions have moderate chelating properties [42]. In sulphide-rich soda lake sediments and anaerobic digesters, the formation of Me-sulphides may cause problems though for Cu, Ni, Co and Mo availabilities. In the next step, the metabolic routes should be identified to reveal mechanisms and pathways of fermentation at high pH: what are the hydrolytic enzymes, physiological adaption (towards high carbonate/NH4+), risk of (micro-) nutrients or metal limitations and how do thermodynamics look like for alkaline fermentation? The substrate composition and precise degradation routes need to be established to obtain insights to what extent the current work can be translated to other substrates.

4.2. Technical evaluation of the alkaline fermentation technology

Under the analysed scenario, biomethane production with an anaerobic digester and membranes for biogas upgrading performed better than an alkaline fermentation system. Per annum additional costs sum up to 760,000 € for a digestion installation with 21,000 kg TS/d (equals sludge production in a WWTP for 400,000 p.e., people equivalent). For an installation with EPS extraction, the capacity would be 520,000 p.e. (30% of TS is recovered as EPS). The analyses revealed that these additional costs are mainly related to (A) the lower TS loading in the alkaline system as restricted by NH_4^+ production and assumed toxicity at elevated pH = 9.5, and (B) by the NaOH dosing to fully compensate every mole of CO_2 produced to maintain the pH at 9.5. In the following section, it is elaborated on why these bottlenecks can be easily overcome for alkaline fermenters in the future and what the next research steps are.

The lower operational TS explains all extra CAPEX, including costs, which are calculated as a percentage of the main equipment contributing to more than 60% of the CAPEX (engineering costs, unforeseen …). The lower TS loading explains about 25% of the extra OPEX because larger equipment with higher power consumption is needed. Accordingly, the threshold for inhibiting N-NH_3 levels for alkaline digestion should be carefully evaluated by investigating methane production at the operational pH under different total ammonia concentrations: Can the community adapt to higher concentrations and at which free ammonia concentration kicks inhibition in? Our assumed inhibition threshold is too conservative as in future an operational pH < 9.5 is more realistic as at pH = 9 still 97% of the produced gas is methane. Operation at a lower pH drastically decreases the risk of ammonia inhibition. This bottleneck can additionally be eradicated by removing and recovering ammonium (section 4.4). Lastly, the alkaline residuals from the extraction of EPS have a C:N ratio of 12, which is in the range of sewage sludge, albeit with relatively high nitrogen content [43]. Thus, for most substrates, higher TS loading is feasible because NH_4^+ release is related to the substrate C:N ratio. Alternatively, co-feeding of substrates with high C:N ratios is an option. Such substrates (cellulose) are often recalcitrant and therefore ideally suited because alkaline conditions will increase substrate degradability, and alkaline pre-treatments are well suited for combination with alkaline fermentation.

The largest contribution to OPEX is costs for NaOH dosing to maintain a stable pH of 9.5. To determine the dosing, it was assumed that (I) pH is kept stable during operation and (II) for this 1 mol – OH has to be added per mol of CO_2 – assuming CH_4 and CO_2 are released in a 60:40 molar ratio [44]. Several routes to overcome this bottleneck. First, a pH of 9 can be operated and still achieves sufficient gas purity as indicated above. Only if alkaline fermentation can replace part of the alkaline pre-treatment to improve substrate degradability, a lower pH might be needed. This will require research in the future. Second, in this study, the substrate with a pH of ca. 11 and a TS of ca. 110 g/L came from EPS extraction. Dilution prior to fermentation might be needed. Here dilution can be done with centrate from alkaline sludge dewatering, for example after NH_4^+ stripping and recovery. Third, the pH of the used substrate was higher than the pH needed for the alkaline fermentation to produce a sufficiently pure gas stream. Thus, a pH buffer is available to diminish the pH drop induced by the CO_2 production. Depending on the pH of the substrate and minimal required CH_4 content of the biomethane dosing of base can be drastically reduced or even omitted. The latter, for example when the substrate is pre-treated under thermo-alkaline conditions for higher CH_4 yield. The reactor used in this study should be operated at higher substrate concentrations to reveal this effect. During neutral digestion pH adjustment is often needed for alkaline pre-treated sludge/substrates [45]. Such an acid addition step was not included in the neutral digestion scenario since the dilution of substrate, required to bring the sludge after pre-treatment to 60 g TS/L prior to pumping it in into the digester, might make this unnecessary. Additionally, VFA production and low base dosing can make this acid dosing step unnecessary [31]. Overall, an optimistic scenario for a neutral digestion system, especially for substrates which require pre-treatment but no dilution prior to digestion, was assumed. If the pH still has to be adjusted, the CH_4 to CO_2 ratio can be improved, e.g. by H_2 addition to convert CO_2 into CH_4 [46]. In case chemical dosing for pH adjustment is needed at the end needed, other less pricy chemicals can be used instead of NaOH. For instance (burnt) lime. This would, however, precipitate a large fraction of the carbonate as calcium carbonates. Carbonate and sodium are characteristic parameters in the natural habitat of the haloalkaliphylic anaerobes used in the current research and will influence the metabolism and physiological adaptions of this community [22,23,42]. Whether organisms can adapt to Ca or whether $Ca(OH)_2$ based alkaliphilic methanogens can be used remains to be investigated. To illustrate the significance for the choice of the base for the medium composition, the dosing of NaOH would add ca. 3 g Na/L (the buffered nutrient media contains ca. 12 g Na/L).

Lastly, elevated PE dosing contributed to higher OPEX in the alkaline fermentation scenario. The dosing was estimated, and experimental data are needed. Dewatering experiments showed that with increasing salinity sludge dewatering improved [47]. At a salinity of 10,000 ppm, a final TS of ca. 22% and at 20,000 ppm, a final TS of 27% in the sludge cake was reached, without the addition of PE. In the same study, addition of 270 mg/L $FeSO_4$ increased the final TS to ca. 32% at 20,000 ppm and had no effect on dewaterability at 10,000 ppm salinity. Lo et al. (2001) postulated that at a higher salt concentration the thickness of the double layer on the particles is compressed and thus their particle surface charge reduced [47]. Furthermore, salting out can facilitate the mobilising of floc bound water. Remmen [48] reported inconsistent results regarding the effect of adding 0–25 g NaCl/L to surplus sewage sludge. In some samples, the final TS in the sludge cake increased from 12 to more than 30% after adding 15−25 g NaCl/L. A characterisation of the alkaline sludge (charge and Z potential) including a tailored choice of PE with subsequent systematic testing of different PE doses should be performed to carefully evaluate the PE consumption for sludge digested at alkaline pH.

Scaling-up and scaling-down analyses identified three aspects of the alkaline fermentation technology that make it, in its current setting, less favourable compared to a biogas installation with subsequent biomethane upgrading. Based on these insights, suggestions were formulated to address these aspects through modelling and future reactor operation. Once addressed, the techno-economic analyses should be repeated. In the current evaluation, based on experimental results, an SRT of 25 days for the alkaline fermenter was assumed. This assumption should be further examined by investigating the reaction kinetics for the alkaline fermentation system. Minimising the retention time will significantly influence the economics of the alkaline fermentation technology.

The next evaluation must include CO_2 taxation. Alkaline fermentation invites CO_2 sequestration for net negative CO_2 emissions (section 4.3). Neutral digestors emit significant greenhouse gases during operation/dewatering/sludge disposal and membrane gas purification, which require further treatment. The quantity of these bleed gas emissions is lower at high pH conditions and also because no membrane is needed. Alkaline fermentation facilitates resource recovery. This can in the future also be considered. With the current knowledge it can be assumed that, eventually, alkaline fermentation will be a more favourable option as it is technically simpler, allows resource recovery and CO_2 sequestration and can be used for fermentation of recalcitrant substrates. Once this is confirmed, the community can be adapted to full-scale operational conditions, larger-scale tests and optimisations should follow.

Alkaline residuals from EPS extraction were utilised in the fermentation tests. This feedstock is likely to gain momentum in the future. However, in the short term and with greater significance, sewage sludge, manure and recalcitrant organic substrates from food and agricultural industries are more significant feedstocks. The global push for biomethane production increasingly employs (thermo-alkaline) pre-treatment to enhance the degradability of rather inert substrates. Alkaline fermentation integrates these trends, leveraging high pH and alkalinity, combined with solid retention times (SRT) of several weeks, to potentially replace conventional pre-treatment steps and boost methane yields from recalcitrant substrates. Under the experimental conditions employed in this work, partial protein hydrolysis – also observed in analytical protein determination [49,50] – and cleavage of ester and glycosidic bonds, facilitate structural modifications in, for example, lignocellulosic feedstocks [51].

Despite these extreme conditions, no process instability was observed, likely due to the microbial community's adaptation to high-pH environments. Nolla-Ardévol [25] reported significantly improved degradation of wheat straw under similar conditions, warranting further investigation through batch incubations. A key advantage is that the high substrate pH is essentially 'free,' derived from alkaline wastes (e.g. extraction residuals with pH ≈ 10–11) or alkaline-pretreated substrates (pH > 11). This eliminates the need for caustic dosing to maintain stable pH during operation, especially at higher substrate loadings, as the substrate's pH exceeds the operational pH [29]. While our study did not directly demonstrate the potential of alkaline fermentation to replace pre-treatment – due to the substrate undergoing thermo-alkaline pre-treatment during EPS extraction – the operational conditions present no significant challenges. Instead, they may enable new opportunities for integrated resource recovery.

High operational pH and elevated temperatures are beneficial for the implementation of ammonia stripping, for example, using gas mixing systems [52] or via ammonium sulphate recovery using technologies such as hollow fibre membrane contactor modules during the sludge digestion or recirculation processes [53]. An elevated pH is also beneficial for precipitating ammonium phosphates such as struvite. Sole phosphate recovery at high pH is feasible via calcium phosphates or vivianite formation. For these routes, the phosphorus speciation during the alkaline fermentation process will have to be determined coupled with geochemical modelling and trial experiments including the evaluation of the carbonate chemistry on crystallisation / precipitation and scaling reactions. The dissolved carbonate can be sequestered and utilised in different forms. Feasible options are direct metal carbonate precipitation [43,54], biomethanation with H_2 addition [46] or other direct CO_2 utilisation [55]. These routes are promising and require further investigation and integration in the techno-economic evaluation.

Environmental impacts, such as the high salt concentrations of the centrate, are outside the scope of this study but need to be addressed in LCA studies.

5. Conclusions

A semi-continuous alkaline fermenter successfully produced high purity biomethane from the complex organic residuals. Scaling-up and scaling down analyses and technical comparison with a conventional fermenter revealed that the alkaline options' lower total solids (TS) in the feed sludge and high NaOH dosing need to be addressed. These issues stem from initial assumptions that turned out to be over cautious and which require empirical evaluation, such as the operational pH and potential NH_4^+ inhibition from higher TS operation and the necessity of buffering every mol of CO_2. Once these limitations are addressed, alkaline fermentation shows promise for extremely simple energy-efficient biomethane production with net-negative CO_2 emissions and integrated nutrient recovery from diverse organic substrates. Further research will address substrate composition, turnover, and microbial community and dynamics, while exploring possibilities for CO_2 sequestration and updating the technical evaluations.

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Disclosure statement

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Authorship contribution statement

Ramon Zwaan was responsible for the original draft preparation, software development, methodology design, investigation, formal analysis, and data curation. Dimitry Y. Sorokin contributed to the conceptualisation, supervision, methodology, validation, and critical revision of the manuscript. Gerben R. Stouten contributed to methodology and critical revision of the manuscript. Mark C.M. van Loosdrecht was involved in the conceptualisation, supervision, resource provision, project administration, funding acquisition, and critical revision of the manuscript. Philipp Wilfert contributed to the conceptualisation, supervision, validation, resource provision, project administration, and critical revision of the manuscript. All authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Data availability statement

All data generated during the experimental work are available and can be shared without restriction. Part of the data used in the techno-economic assessment is based on internal company data and can only be shared in part, and upon reasonable request, due to confidentiality constraints.

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