Anaerobic Fermentation of Substrate with High Nitrogen Content

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This work focuses on anaerobic fermentation of synthetic substrate with high nitrogen content. An anaerobic continuously stirred tank reactor was gradually loaded with synthetic substrate, and the first inhibition was observed on day 110 when the SBP decreased by 20 %. Another significant change was observed on day 135, when SBP dropped to 122 L kg^{-1} COD. At the same time, a gas washing bottle with hydrochloric acid was connected to capture ammonia from recirculated biogas. With this arrangement, a slight increase in the SBP production to 150 L kg^{-1} COD was observed. On day 164, the gas washing bottle was changed to two gas washing bottles with fritted discs. After ten days, a significant increase in SBP, to 320 L kg^{-1} COD, was observed, indicating that the system began to overcome inhibition. From these results, it can be concluded that this method is effective in mitigating ammonia inhibition.

Keywords:
absorption, ammonia inhibition, anaerobic digestion, CSTR reactor

Introduction

Anaerobic fermentation (AD) consists of the decomposition and stabilization of organic substances by microorganisms under anoxic conditions, leading to the formation of biogas and fermentation residue. This microbiological process is widely used, e.g., for the treatment of agricultural waste, municipal and industrial organic waste.[1,2] These substrates often contain significant amount of nitrogen, which can inhibit microorganisms.[3] Fermentation of these materials releases ammonia nitrogen predominantly in a less toxic ionized form (NH_4^+) at acidic to neutral pH values. Production of the toxic non-ionized form (NH_3) increases with the increasing pH. Significant differences in the inhibitory concentrations reported for ammonia in literature can be attributed to differences in substrates, inoculum, and environmental conditions such as temperature and pH.[4,5] Hobson[6] found that TAN concentration of 2 500 mg NH_4–N L^{-1} resulted particularly in methane production inhibition, while a concentration of 3 300 mg NH_4–N L^{-1} completely inhibited the methanogenesis process. In an adapted process, Angelidaki[4] state that the tolerance of ammonia nitrogen is up to 3 000–4 000 mg NH_4–N L^{-1}. However, Sawayama[7] and Lauterböck[8] observed inhibition only when the TAN concentration exceeded 6 000 mg NH_4–N L^{-1}.

Hansen[9] studied the effect of different concentrations and temperatures of ammonia on AD of pig manure. Temperature changes from 37 to 60 °C and total ammonia concentrations from 5.9±0.1 to 6.1±0.1 g N L^{-1} were studied in the experiment. At higher temperatures (55 and 60 °C) and total ammonia concentrations of 6.0±0.1 g N L^{-1} and 6.1±0.1 g N L^{-1}, increased concentrations of free ammonia (1.6 and 2.6 g N L^{-1}) and volatile fatty acids (11.5 and 15.8 g Ac L^{-1}) were observed along with methane yield reduction to 67 and 22 mL CH_4 g^{-1} VS, respectively. At mesophilic temperatures (37 and 45 °C), methane yields (188 and 141 mL CH_4 g^{-1} VS) were comparatively higher than at thermophilic temperatures, and under reduced free ammonia (0.75 and 1.4 g N L^{-1}) and volatile fatty acids concentrations (4.8 and 5.6 g Ac L^{-1}).

Gallert[10] analyzed the effect of ammonia on methanogenesis under mesophilic and thermophilic conditions. They used peptone (4 g L^{-1}) as the substrate in the presence of various amounts of ammonia ranging from 0 to 7 000 mg L^{-1}. Under mesophilic conditions, ammonia concentration increased from 0 to 7 000 mg L^{-1} and the biogas production decreased from 400 mL L^{-1} to 40 mL L^{-1}. Under thermophilic conditions, biogas production was reduced from ≈120 mL L^{-1} to ≈40 mL L^{-1}. Concentrations of TAN causing 50 % inhibition of methane production under mesophilic and thermophilic conditions were 2 900 and 1 830 mg TAN L^{-1}, respectively.

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There are several possible methods to mitigate ammonia inhibition in the AD process, such as e.g., struvite precipitation,[11] anammox,[12] acclimatization of methanogenic microorganisms,[13,14] co-digestion,[15] or ammonia stripping.[16–18]

De la Rubi[19] investigated the removal of ammonia in sulfuric acid in a recirculated biogas stream at different temperatures (35, 55 and 70 °C) and biogas flow rate (0.125; 0.250 and 0.375 L_{biogas} $L_{digestate}^{-1}$ min^{-1}). It was found that the removal of ammonia from the digestate, where the food waste was processed, was possible with biogas recirculation. An increase in ammonia removal rate (1.8–8.3 % d^{-1}) was observed at 35 °C as the flow rate increased from 0.125 to 0.375 L_{biogas} $L_{digestate}^{-1}$ min^{-1}. Higher ammonia removal (3.46 and 9.38 %) was achieved at 55 °C at flow rate 0.250 and 0.375 L_{biogas} $L_{digestate}^{-1}$ min^{-1}. The highest ammonia removal was achieved at 70 °C, 18.4 and 10.4 % d^{-1}, for 0.250 and 0.375 L_{biogas} $L_{digestate}^{-1}$ min^{-1}. However, the authors did not discuss the effects on anaerobic processes and biogas production, only on the efficiency of ammonia removal in recirculated biogas.

Abouelenien[16] demonstrated that they successfully captured ammonia in sulfuric acid in a recirculated biogas stream during the processing of treated and raw chicken manure. Chicken manure was anaerobically treated for 4 days at 55 °C with an initial pH of 8–9. They were able to capture 82 % of ammonia with this method. They compared the specific methane production of treated chicken manure (195 mL g^{-1} VS) and mixture (1:1) of treated and raw chicken manure (157 mL g^{-1} VS), however, they do not report methane production in the case where no biogas recirculation is involved. They also did not monitor long-term operation after inhibition and subsequent removal of ammonia in the recirculated biogas stream to suppress inhibition.

Jiang[20] processed the protein-rich substrate. Ammonia was removed from the recirculated biogas by absorption into water. They compared systems R1 and R2, where they recirculated biogas to the biogas headspace at R1 and R2 to the sludge bed. The absorption rate was found to be higher for the R2 system (14.2 mmol L^{-1} d^{-1}) than for the R1 system (6.8 mmol L^{-1} d^{-1}). These results showed that the R2 system could be processed at a higher organic load (4 g VTS L^{-1} d^{-1}) than the R1 system (3 g VTS L^{-1} d^{-1}), and could capture more ammonia. However, the authors did not achieve inhibition in the work, so they did not clearly demonstrate the suppression of inhibition by removing ammonia in the recirculated biogas.

As the literature suggests, many studies deal with the removal of ammonia from recirculated biogas, but none clearly discuss the effects on biogas production and suppression of ammonia inhibition. The novelty of this work may be that the removal of ammonia from the recirculated biogas by absorption into hydrochloric acid can effectively remove ammonia from the biogas, it can also affect the production of biogas and suppress ammonia inhibition. Therefore, the aim of this work was to process a synthetic substrate with a high nitrogen content in an anaerobic reactor, and subsequently mitigate ammonia inhibition.

Material and methods

Kinetic tests

Kinetic tests were performed according to the standard protocol introduced by Angelidaki.[21] The tests were performed in borosilicate glass SIMAX with a total volume of 310 mL in quadruple at three different inoculum to substrate ratios (based on COD) – ISR (ISR2, ISR4, ISR6), and at different concentrations of NH_4–N (0, 2 000, 6 000, 8 000 mg L^{-1}). The tests were performed under mesophilic conditions (37±0.5 °C). In total, 52 bottles were used for the kinetic tests. To each bottle, 200 g of inoculum (anaerobically stabilized sludge) was dosed with total dry matter of 16.9 g kg^{-1} and with volatile solids of 10.5 g kg^{-1}, the appropriate amount of substrate, according to individual ISR ratios, and ammonium chloride were added to increase the nitrogen concentration. A synthetic substrate (same as for long-term reactor operation), consisting of non-fat dry milk powder and peptone, was used in the tests. In the substrate itself, the concentration of total nitrogen was already 7.6 g L^{-1} (Tab. 1), which means that at sign 0 mg L^{-1} NH_4–N there was a certain amount of nitrogen in the system. NH_4–N was added in the tests because in a batch test, nitrogen present in the substrate may not cause inhibition due to the dilution.

Biogas production was monitored at regular intervals in all tests. Biogas production of each sample was determined volumetrically,[22] providing constant atmospheric pressure conditions. The biogas volume was measured by replacing water in the measurement device. For each measurement time, a needle was inserted into the rubber stopper of the sample bottle. This way, the headspace of each sample was connected to the top of a 50-mL glass burette filled with water. The opening at the bottom of the burette was linked with a rubber tube to a glass cylindrical flask containing water as well. The biogas produced flowed from the headspace of each bottle up into the burette and replaced the water that flowed from the burette to the cylindrical flask. The volume of biogas was taken as the volume of released water, readable from a graduated scale (in mL). Each bottle was stirred manually before the

gas volume measurement, to favor the release of biogas into the headspace. Kinetic tests were evaluated using first order kinetic reactions.

First order kinetic model

First order kinetic model is the simplest model used to describe the exponential biogas production rate of the AD process, assuming that hydrolysis is the rate-limiting step, according to the equation:[23]

$$G(t) = G_0\left(1 - e^{-k \cdot t}\right)$$

where:

$G(t)$ – cumulative biogas (or methane) yield at digestion time t (mL g^{-1} COD),

G_0 – biogas (or methane) potential of substrate (mL g^{-1} COD), also called ultimate biogas or methane potential

k – biogas (or methane) production rate constant or first order disintegration rate constant (L d^{-1})

t – digestion time (d).

A nonlinear least-square regression analysis was performed using the solver tool in MATLAB 2019 to fit the kinetic equation of the first order to the average cumulative specific biogas production (SBP) curves. This method searches for the main kinetic parameters for model with the primary aim of minimizing the sum of the squares of the differences between the predicted and the measured values.

Long-term operation of CSTR reactor

The experiment was performed in a CSTR reactor (Fig. 1) made of stainless steel by ASIO – SR s.r.o., with an operating volume of 6.5 L. The diameter and height of the reactor were 20 cm and 25 cm, respectively. At the top of the reactor was a feeding hole, a thermometer for monitoring and regulating the temperature, and a pH probe (GP HU014MP, Greisinger) for continuous monitoring of the pH in the reactor. Data were recorded at five-minute intervals in the AMiT control program with unlimited archiving time. Stirring of the reactor was ensured by a paddle stirrer with adjustable rpm (Heidolph RZP 2020). The rpm was maintained at a frequency of 30. Anaerobically stabilized sludge from the wastewater treatment plant (WWTP) Devínska Nová Ves with an initial total solid (TS) concentration of 25.86 g kg^{-1} and volatile solid (VS) concentration of 14.98 g kg^{-1} (57.8 %) was used as inoculum. The reactor was operated under mesophilic

Table 1 – *Basic characteristics of substrate*

	COD (g L^{-1})	TS (g kg^{-1})	VS (g kg^{-1})	N_{TOT} (g L^{-1})
Substrate	96	66.4	59.5	7.6

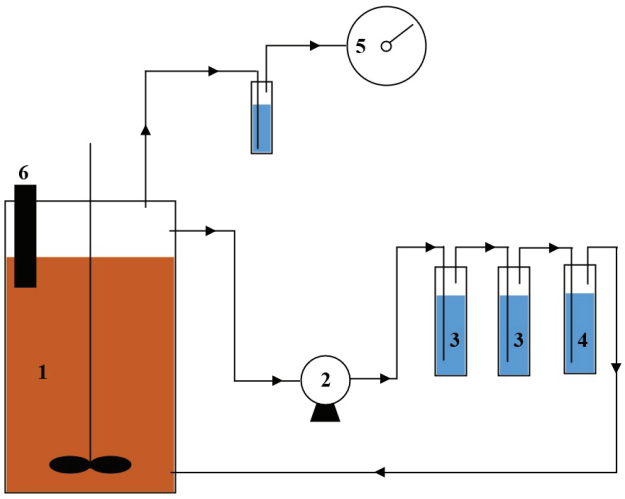

Fig. 1 – *Scheme of CSTR reactor with external ammonia removal: 1 – CSTR reactor, 2 – peristaltic pump, 3 – gas washing bottle with 10 % HCl solution, 4 – washing bottle with distilled water, 5 – drum gas meter, 6 – feeding hole*

conditions (37±0.5 °C). Reactor start-up started at an organic loading rate (OLR) of 0.25 g COD L^{-1} d^{-1}. After reaching the OLR of 2 g COD L^{-1} d^{-1} and achieving ammonia inhibition, a recirculation device for biogas purification was installed. A distribution element (on day 135) was installed at the outlet to pass a part of the biogas through a gas washing bottle with hydrochloric acid (HCl) and distilled water, and the excess biogas was discharged through a water seal and a drum gas meter. Biogas was pumped from the reactor headspace to an external gas washing bottle using a peristaltic pump (Heidolph Pumpdrive 5001), with a flow rate of 5 L d^{-1}, and from day 150, the flow rate was increased to 15 L d^{-1}. The change occurred on day 164, when the gas washing bottle was replaced with two gas washing bottles with fritted discs to ensure an increase in surface area for the phase transfer of ammonia from biogas to hydrochloric acid. Subsequently, the biogas was returned through the lower side valve directly into the sludge bed. Biogas recirculation ensured stripping of ammonia from the sludge bed. Synthetic substrate consisting of non-fat dry milk powder and peptone in the ratio of 3:1 based on chemical oxygen demand (COD) was used as the substrate. Basic characteristics of the substrate are shown in Table 1.

During long-term operation of the laboratory model, parameters such as pH (Hach HQ11d), chemical oxygen demand (COD), total ammonia nitrogen (TAN), total nitrogen (N_{TOT}), and volatile fatty acids (VFA) were monitored according to APHA, AWWA, WEF (2017).[21] Analyzes were performed from filtered sludge water taken as excess sludge. The concentration of free ammonia nitrogen (FAN) was calculated according to the following equation:[24]

$$FAN = TAN \cdot \left(1 + \frac{10^{-pH}}{10^{-\left(0.09018 + \frac{2729.92}{T\,(K)}\right)}} \right)^{-1}$$

Where:

FAN – free ammonia nitrogen concentration (mg L^{-1})

TAN – total ammonia nitrogen concentration (mg L^{-1})

T – temperature (K).

In addition, the amount of biogas produced (at laboratory temperature) was measured using a drum gas meter (type AMS Spectrum TCM 143/10 – 4726), and biogas composition was measured using a portable gas analyzer GA 2000 Plus (Geotechnical Instruments, UK). This analyzer is able to measure the content of the following compound: CH_4, CO_2, O_2, H_2 and H_2S. CH_4 and CO_2 contents were measured by infrared cell, while O_2, H_2 and H_2S contents by electrochemical cell. The biogas for determination of the composition was collected in the sampling bag.

Results and discussion

Kinetic tests

The pH values measured at the beginning of the test ranged from 6.83 to 7.64. At the end of the test, the lowest pH was 5.42 and the highest was 7.15. In Fig. 2, ISR2_2000 represents a test at the ratio of ISR = 2, and the dose of ammonia nitrogen of 2 000 mg L^{-1}. Lower pH values from 5.42 to 6.16 were recorded at all ISR ratios where the concentration of ammonia nitrogen was increased, suggesting inhibition of anaerobic processes due to the high ammonia concentration. The decrease in pH was due to successive phenomena as the high concentration of ammonia inhibits methanogenesis so that VFA does not decompose in the system, but the first phases of anaerobic decomposition continue, so VFA begins to accumulate in the system, which in turn leads to lower pH. At the other ratios, namely ISR2, 4 and 6, the pH values were in the neutral range after the end of the test (6.83–7.15).

Based on the measured biogas production at individual ISR ratios, curves of specific cumulative biogas production (SBP) were constructed, where SBP was expressed as the volume of biogas produced from the mass of COD in the sample. Fig. 2 shows SBP for all three ISR ratios used in the experiment. This biogas production is only indicative as it is influenced by the conditions of a disposable kinetic test. The concentration of ammonia in the substrate was sufficient to achieve inhibition, but the substrate was diluted in single tests and the inhibitory effect may not have occurred, therefore these doses of ammonia nitrogen were used in the tests.

Table 2 – *Maximum volumes of biogas produced at different ISR ratios and different ammonia nitrogen concentrations, and theoretical biogas production*

NH_4–N (mg L^{-1})	Biogas production (mL)		
	ISR=2	ISR=4	ISR=6
0	401	545	656
2 000	111	140	236
6 000	26	59	70
8 000	33	34	37
Theoretical biogas production	1 357	828	552

Table 2 shows the maximum volumes of biogas produced at different ISR ratios and different ammonia nitrogen concentrations, and theoretical biogas production considering the theoretical specific production of methane of 0.35 m^3 kg^{-1} COD and the concentration of methane in the produced biogas of 50 %.

A comparison of theoretical and real biogas production showed approximately 75 % inhibition at ISR = 2, and almost no inhibition at ISR = 6. From the course of the experimental curves, it can be seen that the specific biogas production decreased with the increasing concentration of ammonia nitrogen, already at a dose of 2 000 mg L^{-1} of ammonia nitrogen, 93 % inhibition occurred. With further increase in ammonia nitrogen to 6 000 mg L^{-1} and 8 000 mg L^{-1}, inhibition of approximately 100 % could be observed.

Table 3 shows kinetic parameters (experimental and calculated) for the first order kinetics. In Fig. 2, the courses of experimental values and calculated values of biogas production according to first order kinetics at different ratios of ISR and ammonia nitrogen concentration are presented.

Such biogas production is only indicative as it is influenced by the conditions of a single kinetic test. Actual biogas production may be higher because the anaerobic biomass adapts to the substrate during continuous processing of the substrate in an anaerobic reactor. Actual production may also be lower as the continuous processing leads to gradual concentration of inhibitors, which may not be apparent when diluted in a single test.

Long-term operation of anaerobic reactor

Start-up of the anaerobic reactor took 97 days, when OLR of 2 g COD L^{-1} d^{-1} was achieved. The average specific biogas production was 536 L kg^{-1} COD

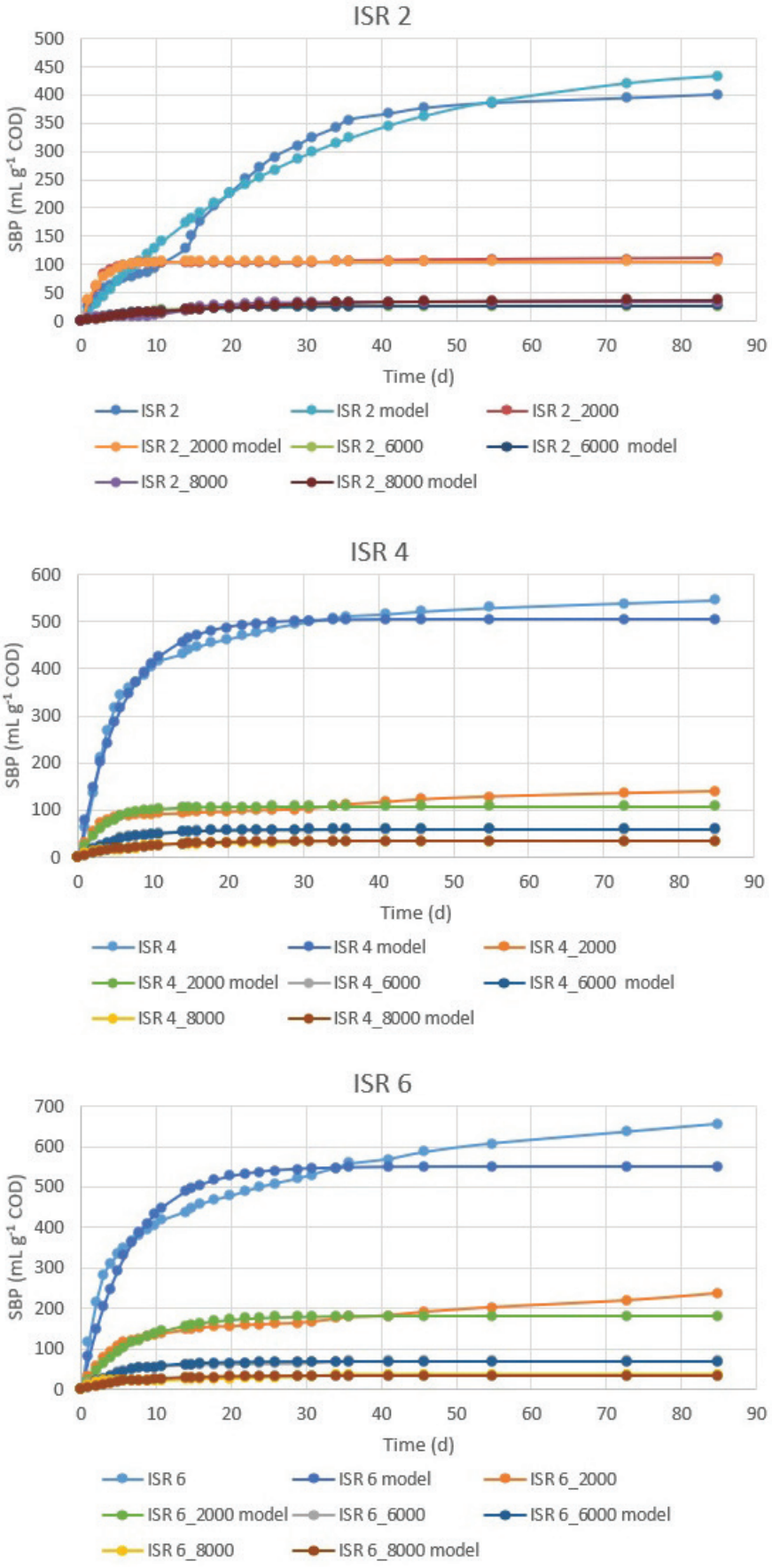

Fig. 2 – *Experimental values and calculated values (model) of biogas production according to first order kinetics at different ISR ratios and ammonia nitrogen concentrations*

Table 3 – *Experimental and calculated kinetic parameters for first order kinetics*

	G_0 [mL g^{-1} COD]	k [L d^{-1}]	SBP (exp.) [mL g^{-1} COD]	SBP (model) [mL g^{-1} COD]	R^2
Blank	10.69	0.0369	10	10	0.9959
ISR 2	458.30	0.0342	401	433	0.9853
ISR 2_2000	104.75	0.4750	111	105	0.9939
ISR 2_6000	27.29	0.1055	26	27	0.9924
ISR 2_8000	36.77	0.0583	33	37	0.9601
ISR 4	504.22	0.1728	545	504	0.9899
ISR 4_2000	108.30	0.2824	140	108	0.9065
ISR 4_6000	59.66	0.1797	59	60	0.9951
ISR 4_8000	34.04	0.1371	34	34	0.9850
ISR 6	549.72	0.1581	656	550	0.9561
ISR 6_2000	182.47	0.1444	236	182	0.9488
ISR 6_6000	67.29	0.1821	70	67	0.9940
ISR 6_8000	32.87	0.1558	37	33	0.9009

with methane content of approximately 56 %. Fig. 3 shows the specific biogas production during the experiment.

Fig. 4 describes the course of nitrogen compounds concentration in the reactor. During the start-up, an increase in the concentration of ammonia and total nitrogen can be seen. This phenomenon can be explained by an excess of nutrients in the substrate. The concentration of free ammonia nitrogen (FAN) was at the level of 60 mg L^{-1}. A significant increase occurred on day 63 when the FAN concentration doubled, probably due to an increase in pH to 7.6 (Fig. 5).

At day 110 of reactor operation, a decrease (20 %) in biogas production was recorded for the first time, which can be seen in Fig. 3. This decrease was caused by an increase in the TAN concentration to 4 733 mg L^{-1} and FAN concentration to 402 mg L^{-1}. Also, from this day, continuous increase in the concentration of COD to 12 380 mg L^{-1} and VFA to 7 480 mg L^{-1} was observed (Fig. 5). Similar results were achieved by Sung.[25] At a TAN concentration of 4.92 g L^{-1}, they observed a 39 % decrease in biogas production and an accumulation of VFA in the reactor.

At day 135 of reactor operation, the specific biogas production decreased to 122 L kg^{-1} COD (Fig. 3), representing an approximately 77 % decrease in biogas production. In kinetic tests, inhibition was already observed at 2 000 mg L^{-1} NH_4–N, but in the reactor operation, it was achieved only at a TAN concentration of about 4 000 mg L^{-1}. This may be due to the acclimatization of anaerobic biomass during long-term operation. The quality of biogas also deteriorated with the methane content falling to 47.3 %. At the same time, external removal of ammonia from the system was started. The

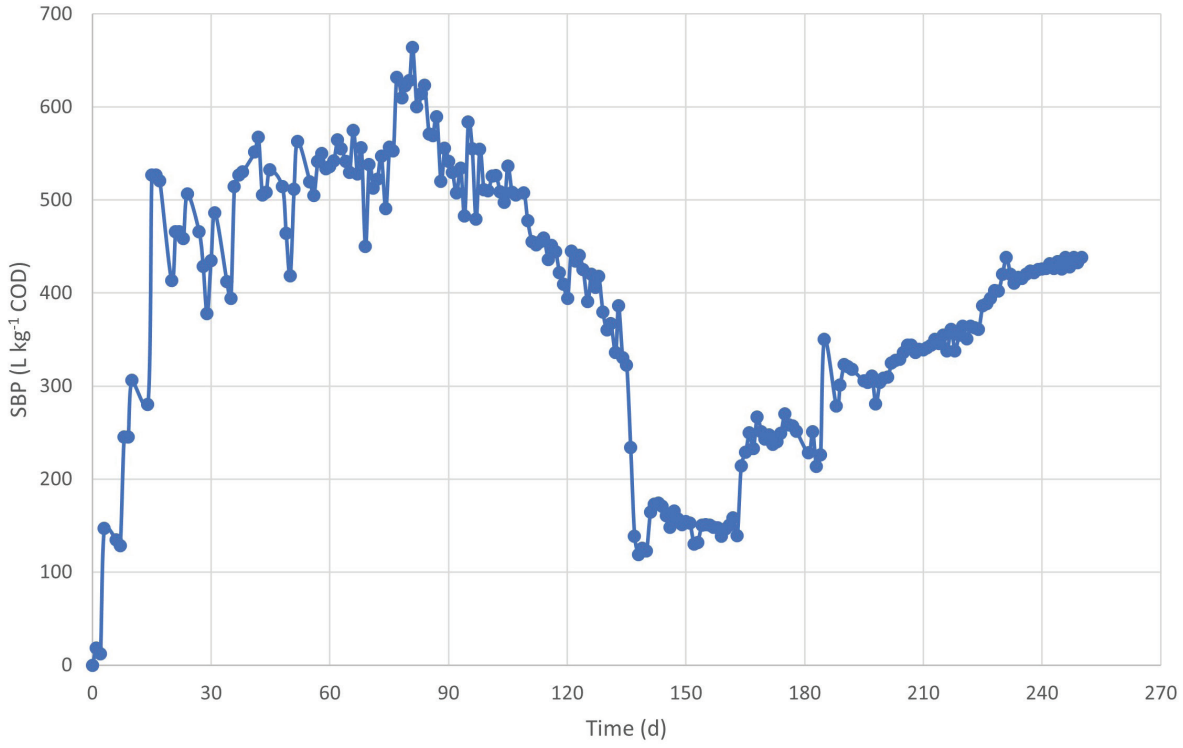

Fig. 3 – *Specific biogas production (SBP)*

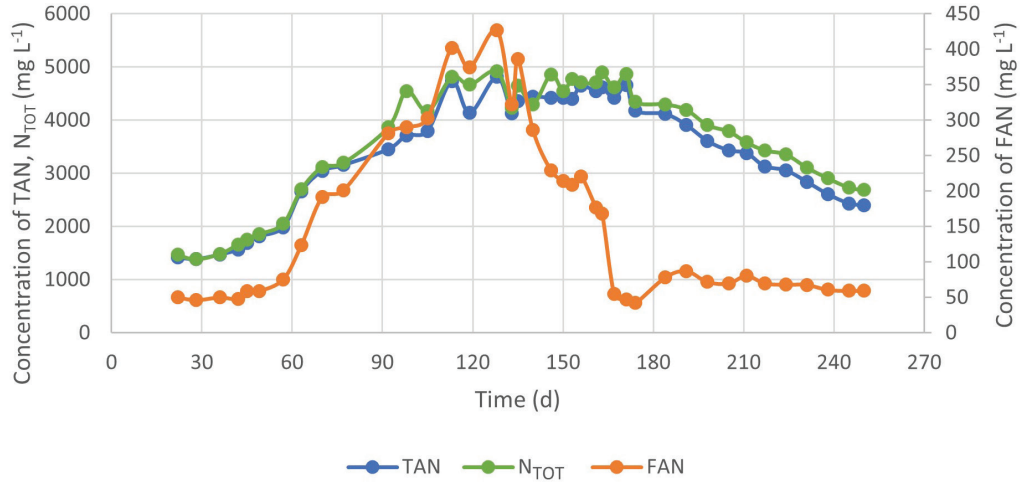

Fig. 4 – *Concentration of total nitrogen (N_{TOT}), total ammonia nitrogen (TAN), and free ammonia nitrogen (FAN)*

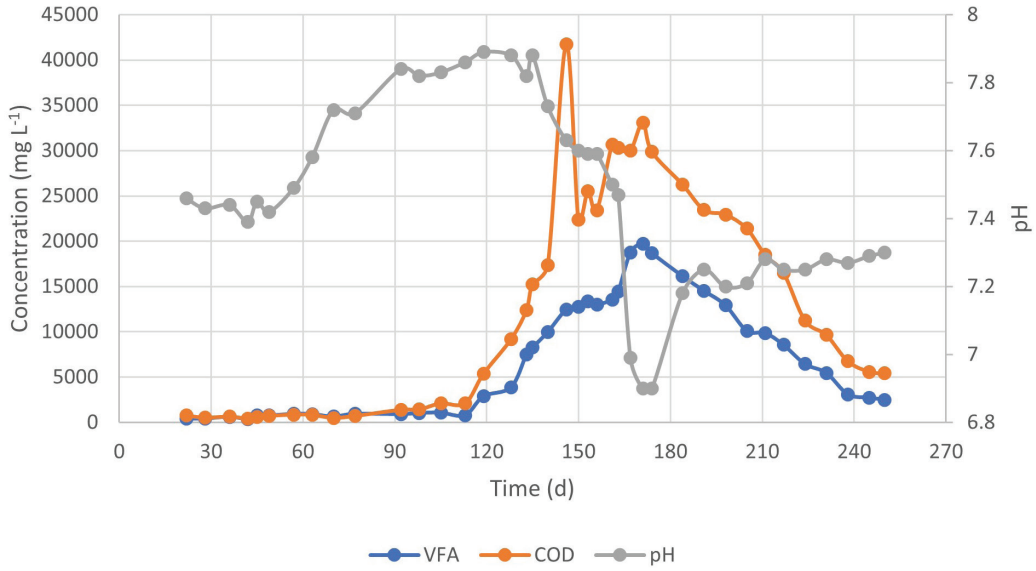

Fig. 5 – *Volatile fatty acids (VFA) and chemical oxygen demand (COD) concentration and pH profile*

flow of recirculated biogas was approximately 5 L d^{-1}, and it was firstly passed through a 10 % HCl solution, and then through distilled water, and returned to the bottom of the reactor. As may be seen in Fig. 4 removal of ammonia from the system provided a slight decrease in FAN concentration to 229 mg L^{-1}. The same trend could be observed for pH changes (Fig. 5). However, this process design was ineffective as the concentration of COD and VFA continued to increase; COD concentration increased to 41 750 mg L^{-1} and VFA concentration to 12 460 mg L^{-1}. Ammonia removal also had a positive effect on the specific biogas production, which may be seen in Fig. 3.

At day 150, the recirculated biogas flow increased to 15 L d^{-1}; which, however, had no significant effect on the specific biogas production (Fig. 3) or on the TAN or FAN output parameter (Fig. 4), but the COD concentration dropped very significantly by about 50 % (Fig. 5). A slight increase could still be observed in the concentration of VFA.

At day 164, the gas washing bottle was changed with two gas washing bottles with fritted discs (extra coarse frit with pores size of 100–160 mm) in a row to remove ammonia from the recirculated biogas. This change increased the specific biogas production to 214 L kg^{-1} COD (Fig. 3). Favorable effect on output parameters was also observed. In Fig. 5, a sudden decrease in pH caused by the accumulation of VFA in the reactor may be seen. The decrease in pH in the reactor also ensured a decrease in FAN, which may be seen in Fig. 4.

At day 174, the system began to overcome the inhibition, which could be observed in the increase in specific biogas production (Fig. 3), and methane content increase in biogas back to 55.6 %. Also, Figs. 4 and 5 show that the concentration of the monitored parameters decreased.

The ammonia removal efficiency in HCl was monitored between 184 and 217 days of reactor operation. During this period, the HCl in gas washing bottles changed 4 times (every 8 days). The average concentration of NH_4–N in the gas washing bottles after 8 days was 3 620 mg L^{-1}, which represents an ammonia absorption rate 32.3 mmol L^{-1} d^{-1}. This is approximately 4–5 times higher than reported by Sun[26] (6.3–7.8 mmol L^{-1} d^{-1}). However, they captured ammonia from biogas in clean water. With a total volume of HCl solution in the gas washing bottles of 225 mL, the absolute amount of removed NH_4–N was 814.5 mg. After four changes of the contents of the gas washing bottles, the amount of removed NH_4–N was 3.26 g. At that time, the NH_4–N concentration decreased from 4 117 to 3 133.5 mg L^{-1}, which was 6.39 g of NH_4–N at a reactor volume of 6.5 L. We can therefore conclude that the removed amount of NH_4–N was approximately 51 % of the decrease in NH_4–N in the reactor. An overall nitrogen balance could not be performed because the system was in an unsteady state of ammonia inhibition reduction. The pH remained almost unchanged after 8 days and was less than 1.

Conclusion

Treatment of a synthetic substrate with high nitrogen content in an anaerobic reactor leads to inhibition of anaerobic microorganisms. Kinetic tests showed that the substrate itself could cause inhibition of the process due to low C:N ratio of 13. Therefore, the C:N ratio has to be adjusted, or the reactor can be operated at lower OLR or with long hydraulic retention time. The first inhibition was monitored during long-term operation of the reactor at day 110. From this day, biogas production gradually decreased, and reached 77 % inhibition on day 135. On the same day, the removal of ammonia from the recirculated biogas began. Removal of ammonia by means of a gas washing bottle was not as effective as that using gas washing bottles with fritted discs connected in series. This is because relatively large bubbles are formed in the gas washing bottle compared to the gas washing bottle with fritted discs, where the bubbles are much smaller, and thus, the surface and contact with the absorbent is much larger.

According to the currently measured values, it can be concluded that the capture of ammonia in hydrochloric acid is an effective method for mitigating the inhibitory effect of ammonia in the anaerobic reactor.

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Abbreviations

AD – Anaerobic digestion
COD – Chemical oxygen demand
CSTR – Continuous stirred-tank reactor
FAN – Free ammonia nitrogen
HCl – Hydrochloric acid
ISR – Inoculum to substrate ratio
N_{TOT} – Total nitrogen
OLR – Organic load rate
SBP – Specific biogas production
TAN – Total ammonia nitrogen
TS – Total solids
VFA – Volatile fatty acids
VS – Volatile solids
VTS – Volatile total solids
WWTP – Wastewater treatment plant

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