Anaerobic digestion of gelatinous water at laboratory and pilot scale and nitrogen inhibition

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ABSTRACT

The anaerobic digestion of the liquid residue (gelatinous water) coming from the production of fat from animal residue, was studied at laboratory and pilot scale. Biodegradability (>98%) and biogas potential (675 mL of biogas/g of $COD_{applied}$) of this wastewater are very high. However, due to the high content on nitrogen, an inhibition of the anaerobic activity was observed for quite low concentrations of $N\text{-}NH_3$. Dilution of the wastewater and pH regulation in the reactor around 7.3 are the 2 solutions which were investigated to overcome the nitrogen inhibition at industrial scale. These two solutions were validated at laboratory scale in an anaerobic SBR and then onsite at pilot scale in a continuous reactor. A stable anaerobic digestion was observed in both reactors showing that no nitrogen inhibition was obtained when $N\text{-}NH_3$ concentration in the reactor was kept low.

Key words | ammonia inhibition, anaerobic digestion, gelatinous water, nitrogen

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INTRODUCTION

The gelatinous water is the liquid residue obtained from the process of production of animal fat called "humid melting". This water is made-up of the liquid content of the animal tissues, used as raw material and source of fatty matter, and of the water coming from the steam, used to extract the fatty matter from the animal tissues. The gelatinous water is a brownish, highly scented, slightly viscous liquid with high concentration in COD, fats and nitrogen. In France, gelatinous water represents around 150,000 tons/year produced by 14 plants. This water is generally concentrated before reuse in the production process or incineration at a cost around 100 €/ton. The aim of this work was to study the feasibility of anaerobic digestion as a new solution for the elimination of gelatinous water.

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MATERIALS AND METHODS

The substrates used

The gelatinous water used in this study was withdrawn from the gelatinous water storage tank of a plant producing animal fat from animal wastes. Two different batches of gelatinous water were used for the 2 experiments at laboratory scale but each experiment was entirely done with the same gelatinous water. For the pilot-scale experiment on-site, the gelatinous water was stored in a 1 m^3 tank and renewed once or twice a week.

Experimental device

Laboratory scale reactors

The experiments at laboratory scale were carried out in double-walled reactors of 5 L effective volume maintained at 35°C by a regulated water bath. Mixing in the reactors was done by a system of magnetic stirring. The rate of production of biogas was measured on-line by an Aalborg mass flow meter 0–50 mL/min fitted with a 4–20 mA output. The software Modular SPC©, developed at the INRA-Narbonne laboratory, was used for acquiring and treating the data (gas output, pH).

Pilot-scale reactor

The experiments at laboratory scale were carried out in 2 m^3 double-walled pilot maintained at 35°C. Mixing in the reactor was done by liquid recirculation from top to bottom at 12 m^3/h.

Seeding of the reactors

The laboratory scale reactors were inoculated with anaerobic sludge treating distillery vinasses. At the beginning of the experiment, the activity of the sludge was checked by addition of ethanol as sole source of carbon and energy. The pilot scale reactor was seeded with anaerobic sludge treating winery effluents (Ruíz *et al.* 2002).

Operation of the reactors

Laboratory scale reactors

The 5 L reactors were fed discontinuously 5 times a week with gelatinous water which was added manually once a day. After addition of the gelatinous water, the reactors were agitated continuously up to the next feed. There was then 4 batches of 24 hours and 1 batch of 3 days per week.

In the experiment with the raw gelatinous water, the reactor was operated in fed-batch mode during the four 24 h batches and in SBR mode for the last 3 day batch, as the volume of substrate added everyday was very small (60 mL). Withdrawal of the reactor was done once a week, at the end of the 3 day cycle. At the end of this batch, the agitation was stopped for 1 hour to let the sludge settle. After 1 hour of settling, the volume of supernatant corresponding to the volume of 5 feeds was removed from the reactor. For the experiment with diluted gelatinous water, decantation and withdrawal was done everyday as the volume exchange ratio was higher 6%.

Sampling and analysis

Samples were taken regularly at feed, in the reactor and in the reactor outflow. The samples were centrifuged at 15,000 rpm during 15 min. Volatile fatty acids (VFA) were analysed using a gas chromatograph fitted with a flame ionization detector (Chrompac CP9000) and coupled with an integrator (Shimadzu CR 3A). Gas composition was measured using a chromatograph Shimadzu GC 8ª associated with an integrator Shimadzu GC 3A. The vector gas was argon. Other parameters were measured following *Standard Methods* (APHA 1992).

Calculation of biomass inhibition

The percentage of inhibition of the anaerobic biomass for a given cycle t was calculated using the maximum biogas flow rate (Q_{max}) measured at the beginning of the cycle. It was expressed as the difference between the average Q_{max} without inhibition (first 30 days for the raw gelatinous water) and Q_{max} at t divided by Q_{max} from the cycles without inhibition.

Pilot scale reactor

The feed of the reactor was made-up of gelatinous wastewater diluted 2 to 2.5 times and stored in a 1 m^3 tank. During the first 25 days corresponding to the start-up period, the reactor was fed discontinuously with raw gelatinous water and three batches were done (days 1, 8 and 16) with the addition of 1 kg COD/m^3 at each feed. From day 26 to day 49, the reactor was fed continuously with raw gelatinous water and then with diluted wastewater until the end of the experiment; pH regulation at 7.3 started on day 72.

RESULTS AND DISCUSSION

Anaerobic treatment of the raw gelatinous water at laboratory scale

The anaerobic digestion of gelatinous water was studied first in an anaerobic SBR (anSBR) to estimate the biodegradability of its organic matter (methane potential and purification efficiency) and to determine the maximum loading rate which can be applied to an anSBR with suspended biomass.

Characterization of the gelatinous water used and working conditions

The average composition of the gelatinous water used was: 164 g total COD/L, 93 g of total solids (TS)/L, 88 g of total volatile solids (TVS)/L, 6.6 g of N-TKN/L, 2.2 g of N-NH_4^+/L (33% of the N-TKN content) and 28 g of fat/L. The organic fraction of the gelatinous water was very high with TVS representing 95% of the TS. The fat fraction was mainly made up of free fatty acids (88%) indicating a high level of hydrolysis. The 14:0, 16:0, 18:0, 18:1 and 18:2 fatty acids represented 75% of the total fatty acids.

At each feeding, 60 mL of gelatinous water were added to the reactor. The hydraulic retention time (HRT) was then 83 days and the organic loading rate (OLR) was 2 g of COD/L.d or 1.08 g of total volatile solids (TVS)/L.d.

Biogas production rate during a cycle

The biogas production rate was monitored online for each treatment cycle and an example is presented in Figure 1. At the beginning of the reaction phase the ratio substrate to micro-organisms ratio (S/X) was high and the biogas production rate was almost constant and at its maximum (around 10 mL/min in the 5 L reactor) for about 4 hours showing that the degradation reactions occurred at their highest rates. After 4 hours, the biogas production rate decreased rather rapidly to reach 3 mL/min 9.5 h after the feed indicating the exhaustion of the more rapidly biodegradable substrates and of the substrates present at low concentration. 9.5 hours after the feed, 75% of the total biogas produced during a cycle had already been produced, and after that time, the biogas production rate remained low and decreased slowly to reach values close to 1 mL/min after 24 hours, showing the presence of slowly biodegradable organic matter. At the end of the cycle, the biogas production rate was around 10% of the maximum value reached at the beginning of the reaction phase and the organic matter added was considered as eliminated. Furthermore, at the end of the reaction phase, the production of biogas was very low allowing the settling of the biomass and its separation from the supernatant.

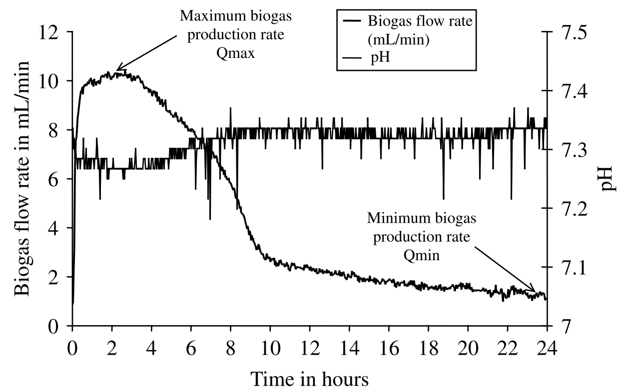

Figure 1 | Biogas production rate and pH with time during a cycle with gelatinous water.

Methane potential and biodegradability

The biogas potential of the gelatinous water was calculated from the volume of biogas produced during a cycle and the quantity of organic matter added. Biogas potential was very high with 675 mL of biogas/g of COD and 1,260 mL of biogas/g of TVS indicating a very high biodegradability. Soluble COD at the outlet of the reactor was always less than 2 g/L and purification efficiency was then very high with more than 98% of COD removal.

Inhibition of the anaerobic activity

The daily biogas production curves were characterized by 2 parameters (Figure 1): (i) the maximum biogas production rate measured in the first hours following the feeding of the reactor which shows the maximum activity of the biomass; (ii) the final biogas production rate at the end of the cycle, 24 hours after the feeding of the reactor, which shows the remaining activity of the biomass at the end of the cycle. From the monitoring with time of the biogas production rate curves during each treatment cycle, it was then possible to follow with accuracy the changes in the activity of the biomass with time (Figure 2).

Figure 2 clearly shows that during the first month of operation, the activity of the biomass did not change much and the shape of the biogas curves was rather close with a maximum between 9 and 11.4 mL/min and a minimum between 1 and 2 mL/min. In that period, soluble COD at outlet was always less than 1.3 g/L with no VFAs and suspended solid and volatile suspended solid concentrations were almost constant with average values of respectively, 16.5 g/L and 12.3 g/L. These results show that there was no organic matter accumulation and that COD removal was very high.

After one month, biogas production rate profiles started to deteriorate with a decrease in the maximum biogas production rates indicating a decrease in the maximum activity of the anaerobic microorganism and an increase of the biogas production rates after 24 h indicating that more and more organic matter was not eliminated at the end of the cycles.

The OLR was constant throughout the entire experiment but the decrease of the activity of the biomass led to an acidification of the reactor after less than 2 months of operation with a rapid increase of both soluble COD and volatile fatty acid concentration which reached respectively 14.5 g/L and 11 g/L on day 60. At that time, the biogas production rate was very low and almost constant between two feeds. The volume of biogas produced in 24 hours was then low indicating a high decrease in the activity of the anaerobic biomass and a failure of the reactor.

As nitrogen concentration of the gelatinous water was high, an inhibition by nitrogen and more particularly by the free ammonia form was suspected. Indeed, free ammonia has been suggested to be the active component causing ammonia inhibition (Braun *et al.* 1981). Figure 3 shows that N-TKN and N-NH_4^+ concentrations increased rapidly with the addition of the gelatinous water to reach more than 3 g/L after two month of operation. N-NH_3 concentration increased also over time to reach more than 50 mg/L at the end of the experiment.

Figure 4 shows that the inhibition of the anaerobic microorganisms by NH_3 started at concentrations of more than 20 mg/L and increased very rapidly between 40 and 50 mg/L to reach 60% of inhibition at the end of the experiment. Considering a non-competitive inhibition as proposed by Siegrist & Batstone (2001), the K_I value of 18 mg/L obtained here is of the same order of magnitude as the 6 mg/L obtained by these authors. Moreover, Aspé *et al.* (2001) reported ammonia inhibitory effect on anaerobic digestion from different studies and some of them showed 50% inhibition at N-NH_3 concentrations between 20 and 95 mg/L. At 60% of inhibition, the reactor underwent an overloading and the reactor was stopped.

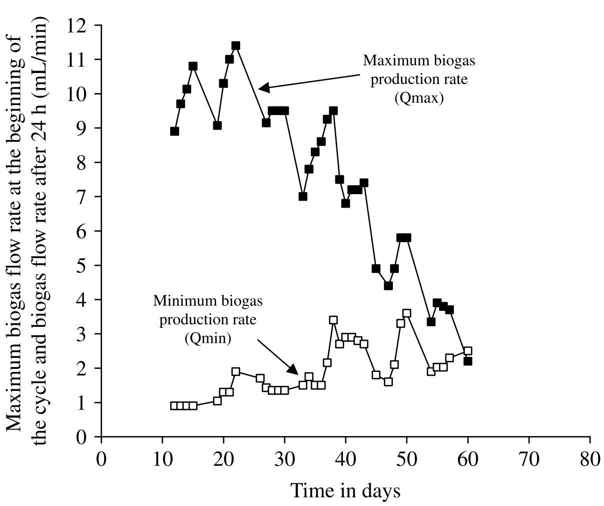

Figure 2 | Maximum and minimum biogas production rates during a cycle.

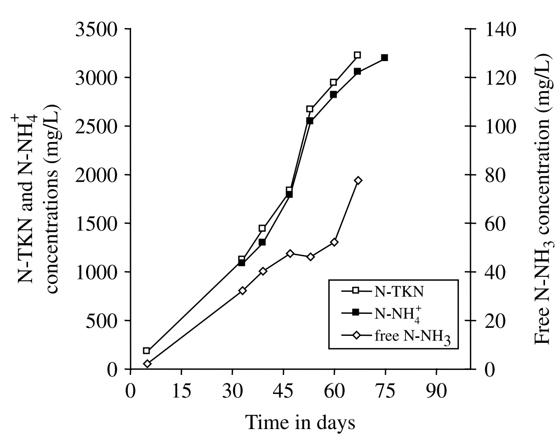

Figure 3 | Evolution of N-TKN, N-NH_4^+ and free N-NH_3 concentrations over time.

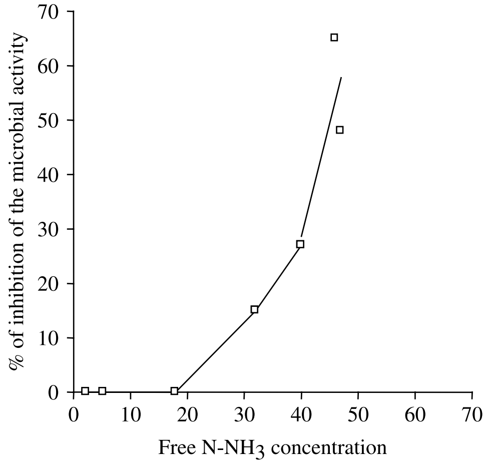

Figure 4 | Inhibition of the microbial activity over free N-NH_3 concentration (mg/L).

Two solutions were proposed to remove the inhibition by free ammonia: dilution of the raw gelatinous water by 2 or 3 and pH control at 7.3 by addition of phosphoric acid. Dilution is conceivable because diluted wastewater is available at the plants which produce the gelatinous water and a dilution by a factor 2 to 3 is possible. These solutions were first validated at laboratory scale and then, onsite at pilot scale.

Validation of the solutions proposed to overcome biomass inhibition

Validation at laboratory scale

A new batch of gelatinous water was used for this experiment with the following composition: 108 g total COD/L, 26 g soluble COD/L, 4.1 g of N-TKN/L, 1.9 g of N-NH_4^+/L. At each feeding, 300 mL of gelatinous water diluted 3 times (41 g of total COD/L and 9.6 of soluble COD/L) were added to the reactor. An AnSBR was operated for 67 days at an hydraulic retention time (HRT) of 17 days, organic loading rates of 2.4 $\pm$ 0.3 g of COD/L.d and 0.2 g of COD/g of VS.d and pH regulation at 7.3 by addition of phosphoric acid.

This experiment was prolonged until nitrogen concentration inside the reactor had stabilized (Figure 5) at values close to 1 g/L for both N-TKN and N-NH_4^+. With these concentrations and a maximum pH of 7.3, free ammonia concentration was always less than 25 mg/L.

During the full experiment with the diluted gelatinous water, the anSBR had stable behavior and no inhibition of

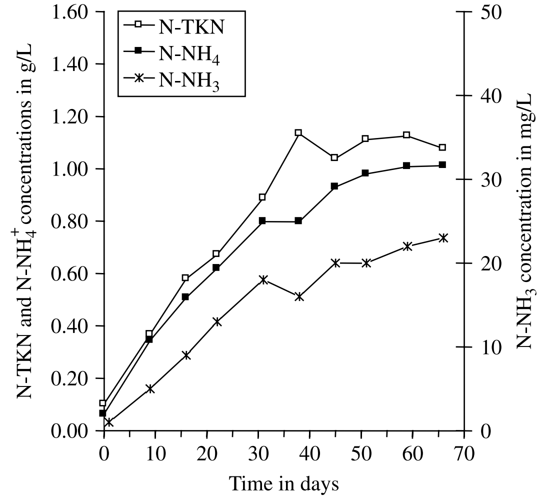

Figure 5 | Evolution of N-TKN, N-NH_4^+ and free N-NH_3 concentrations over time.

the anaerobic biomass was observed indeed: (i) biogas production rate profiles were close one to the other for the entire experiment with an average maximum between 1 and 2 h after the feed of 8 mL/min and an average minimum at the end of the cycle of 1.2 ml/min; (ii) soluble COD removal was very high and soluble COD at outlet was always less than 0.65 g/L (Figure 6) and removal efficiency for this parameter was 95% on average; (iii) VSS concentration in the reactor was almost constant with an average value of 12 g/L indicating that there was no accumulation of non-degraded solids in the reactor. Suspended solids in the effluent

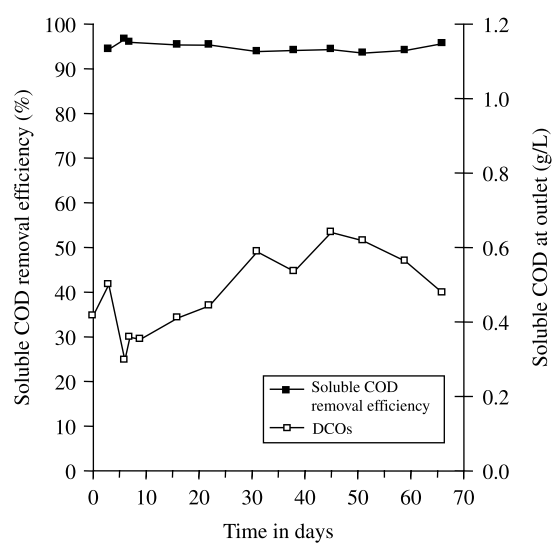

Figure 6 | Soluble COD at outlet and soluble COD removal efficiency over time.

withdrawn from the SBR was 1.8 ± 0.19 g/L indicating that the settling of the anaerobic sludge was quite good.

Validation at pilot scale

A 2 m^3 pilot reactor was set-up at a plant, located in Brittany (North West France), producing animal fat from slaughterhouse residues to validate the treatment of gelatinous water by anaerobic digestion. The feed of the reactor was made-up of gelatinous wastewater diluted 2 to 2.5 times and stored in a 1 m^3 tank. The wastewater was renewed once to twice a week. The composition of the water changed quite a lot during this experiment with: 124 ± 36 g COD/L and 9.75 ± 4 g N-TKN/L. During the first 25 days corresponding to the start-up period, the reactor was fed discontinuously with raw gelatinous water and three batches were done (days 1, 8 and 16) with the addition of 1 kg COD/m^3 at each feed. From day 26 to day 49, the reactor was fed continuously with raw gelatinous water and then with diluted wastewater until the end of the experiment. The initial loading rate was low (0.26 kg COD/m^3.d) and it was then regularly increased with time (Figure 7), by an increase of the volume fed each day. The reactor behaved very well indeed, the OLR increase was quite quick and an OLR of 4 kg COD/m^3.d was reached in about 2 months. The removal efficiency was always excellent even for the highest OLR and soluble COD after day 45 was always less than 3 g/L (Figure 7).

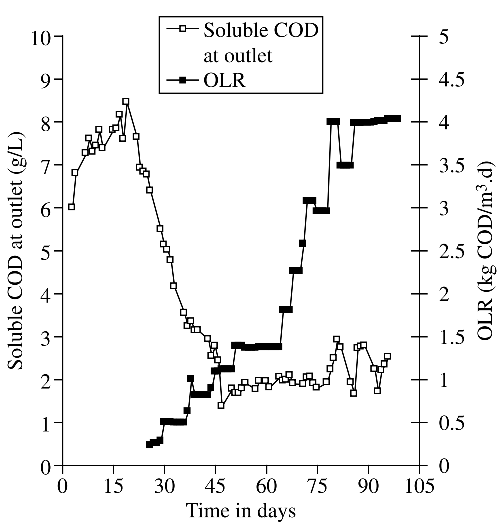

Figure 7 | Evolution of OLR and soluble COD at out let over time.

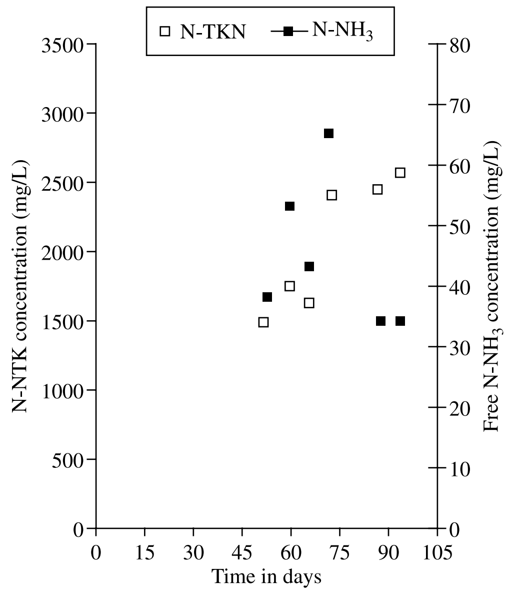

Figure 8 | Evolution of N—TKN concentration and free N—NH_3 concentration over time.

pH regulation started on day 72 to decrease the free ammonia concentration in the reactor (Figure 8) to around 35 mg/L for the highest nitrogen concentrations and no inhibition of the biological activity was observed in these operating conditions. Though a N-NH_3 concentration up to 65 mg/l was punctually reached, this had no irreparable effect on the bacterial activity.

CONCLUSION

The anaerobic biodegradability of gelatinous water, coming from the production of animal fat from animal residue, was very high with a biogas potential of 675 mL of biogas/g of $COD_{applied}$ and more than 98% COD removal efficiency. However, due to the high nitrogen content of this substrate, a rapid inhibition of the anaerobic biomass was observed for quite low concentrations of N-NH_3. Two solutions are possible at industrial scale to overcome this inhibition: dilution of the wastewater and pH regulation in the reactor around 7.3. These two solutions were validated at laboratory scale and then onsite at pilot scale. A stable anaerobic digestion was observed in both reactors showing that no inhibition was obtained when N-NH_3 concentration in the reactor was kept low.

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