Anaerobic Treatment of Pharmaceutical Waste Fermentation Broth

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Original scientific paper
Received: March 9, 2009
Accepted: September 18, 2009

Dedicated to the memory of Professor Dr. Valentin Koloini

The feasibility of anaerobic co-digestion of pharmaceutical waste fermentation broth (spent mycelia) mixed with pig slurry and corn-grass silage was studied. The waste broth proved very toxic to luminescent bacteria *Vibrio fischeri* (30 min EC_{50} = 1.19–3.35 vol. %) while it expressed lower toxicity to activated sludge (180 min EC_{50} = 4.11–15.32 vol. %). Initially, stabilization studies in aerobic and anaerobic conditions were performed to confirm feasibility of anaerobic degradation. A 30 L conventional mesophilic reactor was used for further digestion experiments. The control experiment (pig slurry and corn-grass silage only) with OLR of 1.5 kg m^{-3} d^{-1} achieved 70 % COD removal and methane production of 25.4 L d^{-1}. The first experiment with added fermentation broth (OLR of 1.8 kg m^{-3} d^{-1}) achieved 79 % COD removal and good methane production (30.9 L d^{-1}). The second experiment with more fermentation broth (OLR of 2.2 kg m^{-3} d^{-1}) failed after 20 days, but the system recovered when the OLR was reduced to 2.0 kg m^{-3} d^{-1}. In the third experiment only pharmaceutical broth was used as a substrate. The process failed after 10 days of operation due to toxic shock. It has been concluded that the toxic impact could be avoided with an OLR not higher than 2.0 kg m^{-3} d^{-1}, but at the same time pharmaceutical broth must not contribute more than 25 % to the total OLR.

Key words:

Anaerobic digestion, biogas production, pharmaceutical fermentation broth, spent mycelia, toxicity

Introduction

The pharmaceutical industry produces a wide range of products, which are used in human and veterinary medicine. All pharmaceutical manufacturing processes may produce considerable amounts of wastewaters and wastes; however fermentation and synthesis usually generate the largest volumes of waste with high organic load.[1] The anaerobic process is very suitable for the treatment of such high strength wastewaters and broths. Preliminary research papers[2] have already shown that pharmaceutical wastes mostly exhibit toxic effects to aerobic as well as anaerobic processes, but anaerobic process can be adapted to a certain level of toxicity. When properly conducted, the anaerobic process can produce considerable amounts of biogas, contributing to of the increase in renewable energy thus reducing the environmental impacts of pharmaceutical wastes with beneficial energy production. Recently, many studies have shown that anaerobic treatment of pharmaceutical wastewaters can be successfully accomplished,[3,4,5] although research on anaerobic treatment of high strength pharmaceutical wastes, such as broth in our study, is scarce. However, the pharmaceuticals remaining in the wastewaters after separation procedures and introduced as influent to anaerobic treatment plant, are toxic to bacteria and therefore may significantly decrease the efficiency of COD removal and biogas production.[6,7,8] Therefore, we can assume that a similar effect may appear conducting anaerobic digestion of waste pharmaceutical broth.

The aim of our research was to determine the feasibility of co-digestion of waste pharmaceutical broth with conventional substrates such as pig slurry and silage in a pilot plant reactor to be later used in a full-scale device in a local biogas production plant currently under construction. Our goal was to determine the properties of waste pharmaceutical fermentation broth, to define the conditions of the digestion process, the composition of the mixture of substrates, and to avoid inhibition of the anaerobic digestion process.

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Materials and methods

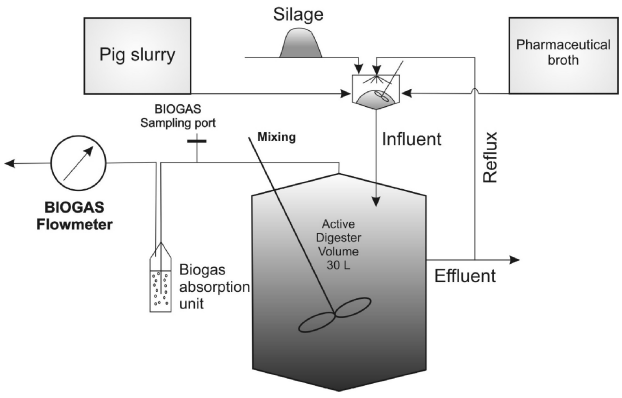
Characterization of the waste fermentation broth

The pharmaceutical broth originated from clavulanic acid production using the mycelium organism *Streptomyces clavulagerus*. During its production a considerable amount of waste broth (spent mycelia) is produced, which has to be disposed of with minimum environmental impact. Characterization of the broth was accomplished using two different batches of waste broth named Batch 1 and Batch 2, sampled at three weeks interval. The aim of multiple sampling was to obtain average values of the measured parameters and to assess the impact of changes in broth quality on its treatment characteristics. Until the experiments, waste broth was kept in a cool dark storage (4 ± 1 °C). Prior to the anaerobic digestion experiments, the waste broth was analyzed and characterized in terms of its biodegradability and toxicity. Its toxicity towards a mixed culture of aerobic microorganisms by measurements of the inhibition of oxygen consumption[9] was determined, and *Vibrio fischeri* bioluminescence inhibition[10] was measured. In both cases the EC_{50} values were calculated as the vol. % of the sample which affects 50 % of the tested organisms. The biodegradability of the diluted broth (0.15 vol. %, initial COD was 100 mg L^{-1}) was assessed by measurement of oxygen consumption in a closed respirometer.[11] The contribution of abiotic elimination was also evaluated.

To compare biodegradability of the broth in aerobic and anaerobic conditions, two stabilization studies were carried out to measure changes in toxicity during its biodegradation, as well as changes of other parameters (COD, DOC, NH_4^+-N, NO_2^--N, Cl^-, Etc.) versus time.[12] The stabilization study represented a link between biodegradation and changes in toxicity of the sample. Stabilization (ageing) allowed us to assess the toxic fraction as permanent or biodegradable. At the same time, we could also differentiate among toxicity originating from raw wastewater and that formed during degradation processes as a consequence of stable metabolites. These studies were performed in a 10 L batch reactor, where the broth was diluted 14 times (7 vol. %). In the first experiment, the reactor was aerated (400 rpm) to maintain more than 6 mg L^{-1} of O_2 (28 days, aerobic conditions), while in the second, the system was sealed to assure less than 0.1 mg L^{-1} of O_2 (28 days, anaerobic conditions). Samples were redrawn during both experiments periodically to determine COD, BOD_5, DOC, NH_4-N, NO_2^--N, Cl^- and toxicity as for the raw broth.

Reactor design and conditions

For digestion experiments, a 30 L pilot plant anaerobic digester was used under mesophilic conditions. This reactor was designed to handle semi-solid substrates with semi-continuous feed of substrate daily (setup shown in Fig. 1). The temperature of the reactor was set to 37 ± 1°C, to simulate the conditions of the full-scale device, which is going to utilize the pharmaceutical broth for biogas production. The initial experiment was a control trial, where only pig slurry and corn-grass silage were used as substrates as in the full-scale rector. The designed Organic Loading Rate (OLR) was 1.5 kg m^{-3} d^{-1}. To handle the semi-solid input substrate like corn-grass silage, a part of the reactor effluent (reflux) was used in order to achieve appropriate hydraulic properties for liquid handling of all substrates in the influent. In the control experiment is applied 1515 mL (125 mL of pig slurry, 100 g (250 mL strewed) of corn-grass silage and 1250 mL of reactor effluent. In further experiments where pharmaceutical broth was introduced in the influent of the digestion reactor, the mixture of 50 vol. % of Batch 1 and 50 vol. % of Batch 2 was used.



F i g . 1 – *Setup of the digestion reactor*

In the first experiment with pharmaceutical waste broth, the addition of pharmaceutical broth matches the conditions that would occur in the full-scale model. The OLR was 1.8 kg m^{-3} d^{-1}. 1465 mL of mixture was used as a feed; a part of the reactor effluent was substituted with pharmaceutical broth (125 mL pig slurry, 100 g of corn-grass silage, 1000 mL of reactor effluent and 200 mL of pharmaceutical broth). The second experiment was planned to gradually increase the amount of pharmaceutical broth followed by increased organic load to the point where the anaerobic process would be inhibited. The amount of pharmaceutical broth was increased by 50 %, because the organic load was still relatively low. With the 1565 mL of the substrate feed (125 mL of pig slurry, 100 g of

corn-grass silage, 1000 mL of reactor effluent and 300 mL of pharmaceutical broth) the OLR was raised to 2.2 kg m^{-3} d^{-1}. Other authors reported effective biogas production at OLRs larger than 2.2 kg m^{-3} d^{-1},[3,5,6] while some reports even exceeded 10 kg m^{-3} d^{-1};[4,8,13] therefore, OLR of 2.2 kg m^{-3} d^{-1} was considered low enough not to pose a problem. After the occurrence of inhibition in the second experiment, the OLR was reduced to 2.0 kg m^{-3} d^{-1} by reducing the amount of pharmaceutical broth in the entering substrate. 1515 mL of substrate (125 mL of pig slurry, 100 g of corn-grass silage, 1000 mL of reactor effluent and 250 mL of pharmaceutical broth) was introduced into the reactor daily. In the third experiment, only pharmaceutical broth alone was used as a substrate (600 mL per daily feed, OLR was 1.6 kg m^{-3} d^{-1}).

Analytical methods

Waste broth (Batch 1 and Batch 2) was analyzed prior to the anaerobic digestion experiments. Analytical control of the waste broth included pH, BOD_5,[14] COD,[15] DOC (Shimadzu TOC 5000A Analyzer, 1998)[16] and nitrogen as ammonium[17] and nitrate nitrogen[18] determination. Chloride was measured by a titrimetric method.[18] The same parameters were also followed during the stabilization study. In Batch 2, concentrations of AOX,[19] metals,[20,21] PAHs[22] and PCB[23] were also determined in accordance with Slovenian legislation for pharmaceutical wastewaters.[24]

During operation of the pilot reactor, the characteristics of the influent and effluent were measured on a daily basis for COD, total suspended solids (TSS), volatile suspended solids (VSS) and pH according to Standard Methods.[18] In addition, the biogas flow was measured online using an Agilent ADM 2000 device and later its composition was determined by GC/MS method.

Results and discussion

Characterization of the waste fermentation broth

Physical-chemical and toxicological analyses of the raw sample are presented in Table 1. The average COD of pharmaceutical broth was 75 g L^{-1} with a high solids content of 60 g L^{-1}; 94 % of solids were volatile, indicating a large proportion of organic material that can be usefully transformed to biogas. BOD_5 was in the range of 9–30 g L^{-1}, DOC reached up to 18.5 g L^{-1} and the concentration of NH_4-N was 54 to 75 mg L^{-1}. The waste broth did not contain AOX, BTX or PCB, while concentrations of metals were low, except for Zn, which was added to the broth at the beginning of the fermentation process as an essential growth element.

Table 1 – *Physical-chemical analysis of the waste fermentation broth*

Parameter	Batch 1	Batch 2
pH	5.4 ± 0.1	6.1 ± 0.1
COD/mg L^{-1}	73000 ± 5000	75900 ± 5000
DOC/mg L^{-1}	18500 ± 370	17900 ± 350
IC/mg L^{-1}	2000 ± 40	30 ± 0.6
BOD_5 /mg L^{-1}	9200 ± 1000	39000 ± 5000
$N\text{-}NH_4^+$/mg L^{-1}	75.9 ± 5.0	64.1 ± 5.0
$N\text{-}NO_2^-$ /mg L^{-1}	25.5 ± 2.0	892.1 ± 10
Cl^- /mg L^{-1}	600 ± 50	781 ± 50
AOX/μg L^{-1}	/	< 2500
BTX/μg L^{-1}	/	< 0.5
PCB/μg L^{-1}	/	< 5
Total Cr/μg L^{-1}	/	44.9
Co/μg L^{-1}	/	2.9
Ni/μg L^{-1}	/	26.0
Cu/μg L^{-1}	/	270
Zn/μg L^{-1}	/	7642
Cd/μg L^{-1}	/	0.8
Pb/μg L^{-1}	/	1.1
As/μg L^{-1}	/	2.5
Se/μg L^{-1}	/	< 10.0

/… Not determined.

The toxicity and biodegradability of the waste broth is presented in Table 2 and Table 3. Batch 2 was less toxic to luminescent bacteria and activated sludge than Batch 1. The mixed culture of aerobic

Table 2 – *Toxicity of the waste fermentation broth*

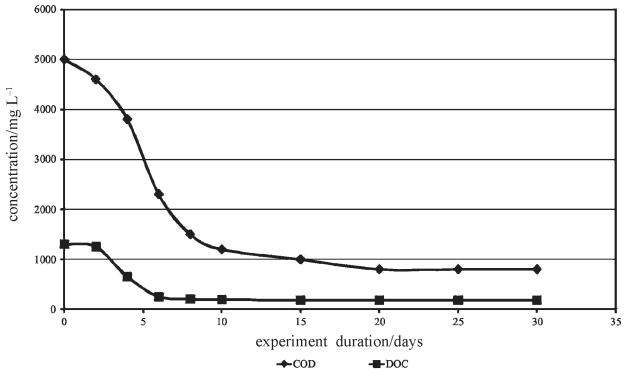
Toxicity test	Batch	Parameter	Value
Bioluminescence inhibition of *Vibrio fischeri*	1	30 min EC_{50} (vol. %)	1.19
	2	30 min EC_{50} (vol. %)	3.35
Inhibition of oxygen consumption	1	180 min EC_{50} (vol. %)	4.11
	2	180 min EC_{50} (vol. %)	15.32

Table 3 – *Aerobic biodegradability of diluted waste fermentation broth*

Batch	Biodegradation/%	Abiotic removal/%
1	74 ± 3	9 ± 2
2	66 ± 5	30 ± 7

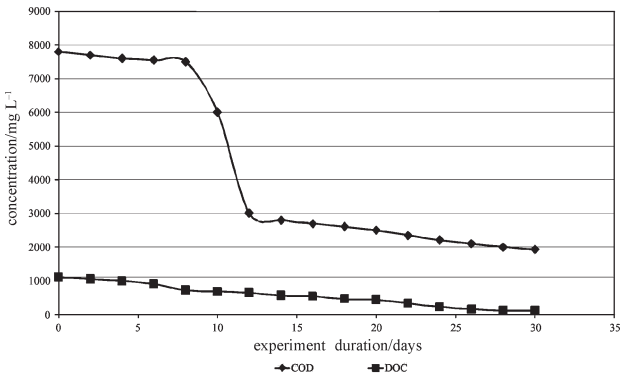
activated sludge was less sensitive to toxic components in the waste broth than the *Vibrio fischeri* culture. The toxicity of the broth could not be correlated with its composition. Diluted broth (0.15 vol. %, COD = 100 mg L^{-1}) also degraded readily (66–74 %). In the case of Batch 2, the abiotic control was not sterilized reliably, so the high level of abiotic removal (Table 3, 30 %) could be attributed to the presence of native active and resistant microorganisms in the broth, which were able to degrade the diluted broth even after sterilization by addition of $HgCl_2$ (10 g L^{-1}, as recommended by standard procedure[10]), because many bacterial cells were detected by microscopic examination in the abiotic control after the biodegradation experiment confirming non-sterile conditions. The initial toxicity and biodegradability testing confirmed that with dilution of the broth its toxicity could be avoided and its biodegradability in aerobic conditions could be increased. To determine the feasibility of anaerobic treatment of such a waste broth and to assess its performance relative to aerobic treatment, stabilization studies in aerobic and anaerobic conditions were performed.

The results of the aerobic stabilization study with Batch 1, presented in Fig. 2, indicated 80 % COD removal and 86 % DOC removal. 88% of BOD_5 was also removed (397/47 mg L^{-1}) in 28 days. The concentration of NH_4-N decreased by 73 % (351.5/95.5 mg L^{-1}), while the concentration of NO_2^--N remained below 2 mg L^{-1}. The concentration of chloride increased by 192 % (233/681 mg L^{-1}) due to the degradation processes. However, the remaining degraded broth was much more toxic to *Vibrio fischeri* (30 min EC_{50} = 0.50 vol. %) and activated sludge (180 min EC_{50} = 1.28 vol. %) after 28 days of degradation in aerobic conditions than the raw waste pharmaceutical fermentation broth (Table 2). This could be explained by the formation of toxic and harmful by-products/metabolites during aerobic biodegradation. The residue was also poorly biodegradable according to measurement of oxygen consumption. Its biodegradability reached 22 ± 3 %, while abiotic elimination was 12 ± 4 %.



F i g . 2 – *Concentrations of COD and DOC during the aerobic stabilization study*

On the other hand, degradation of Batch 2 in anaerobic conditions resulted in a non-toxic residue. COD removal reach 75 % while DOC removed 86 %. These removal rates in anaerobic conditions were identical to those aerobic (Fig. 3), but degradation started later and was much slower. BOD_5 dropped from an initial 2600 mg L^{-1} to 495 mg L^{-1} (81 %), while ammonium nitrogen increased by 268 % (68/250 mg L^{-1}) as a result of biodegradation of organic matter, which was not followed by nitrification. This was also confirmed by an increase in nitrite N concentration (17.8/39.6 mg L^{-1}). The concentration of the chloride went up by 31 %.



F i g . 3 – *Concentrations of COD and DOC during anaerobic stabilization study*

Since the anaerobic stabilization study revealed that the remaining broth was less toxic to *Vibrio fischeri* (30 min EC_{50} = 44.7 vol. %) and activated sludge (180 min EC_{50} = 68.9 vol. %) than the raw broth (Table 2), it was estimated that the majority of toxic compounds were effectively removed during anaerobic degradation and probably no toxic metabolites were formed. The residue of anaerobic stabilization was also poorly biodegradable later in aerobic conditions. It biodegraded less than 5 % in a closed respirometer, while its abiotic elimination reached a negligible 2 %.

It was confirmed that anaerobic digestion of the investigated waste pharmaceutical fermentation broth has certain advantages. Degradation is slower than in aerobic conditions, but according to COD, DOC and BOD_5 the degradation levels attained are comparable. On the other hand, nitrification is suppressed in anaerobic conditions. But the final anaerobic residue is stable and less toxic, and thus more suitable for final disposal. Because stabilization studies were performed using diluted broth, it was estimated that further experiments in the mesophilic anaerobic digester should also be performed with diluted pharmaceutical broth to avoid toxic shock and to allow possible adaptation of the biomass.

Start-up of the reactor and control experiment

To start up the reactor, 10 L of active anaerobic sludge (concentration 25 g L^{-1}) from the local sewage sludge digestion plant was used as inoculum (1/3 of the reactor). 125 mL of pig slurry, diluted with tap water (approx. 450 mL) and 100 g of corn-grass silage was fed into the reactor on a daily basis over the next 30 days, until the 30 L volume were filled. There were no problems regarding start-up of the reactor. A control experiment was then conducted over an additional 33 days with pig slurry and corn-grass silage only, the characteristics of influent and operation conditions matching the conditions in the local biogas plant. Average influent and effluent values are shown in Table 4; process efficiency is presented in Fig. 4. With the OLR of 1.5 kg m^{-3} d^{-1} the control experiment showed an expected COD removal of 70 % and TSS and VSS degradation of 77 % and 80 %, respectively. After 33 days, the control experiment was just prolonged into the first experiment with no adaptation period.

The first experiment

In the first experiment, the amount of pharmaceutical broth matching the conditions in the design of the full-scale model of the local biogas plant was added. The OLR was 1.8 kg m^{-3} d^{-1} which represents 20 % increase in comparison to the control experiment. Although the increased feed started with no adaptation period, the system displayed no problems and it was performing very well under the higher load of pharmaceutical broth (Fig. 5). The experiment was conducted for 52 days and there were no significant increases in effluent parameters in comparison to the control experiment (Table 4). This first experiment has shown that the pharmaceutical broth can be easily co-digested; the COD removal even increased; 79 % compared to 70 % in the control experiment (Fig. 4, Fig. 5), and reached comparable degradation efficiencies according to TSS (80 %) and VSS (83 %). Regarding the obtained results, it was estimated that the process had not yet reached its maximum load and performance, therefore an increased amount of pharmaceutical broth was added to the influent used in the second experiment.

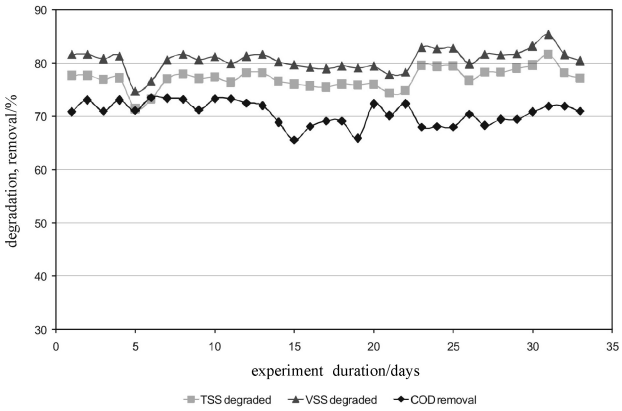


Fig. 4 – *Degradation of TSS, VSS and COD removal in the control experiment*

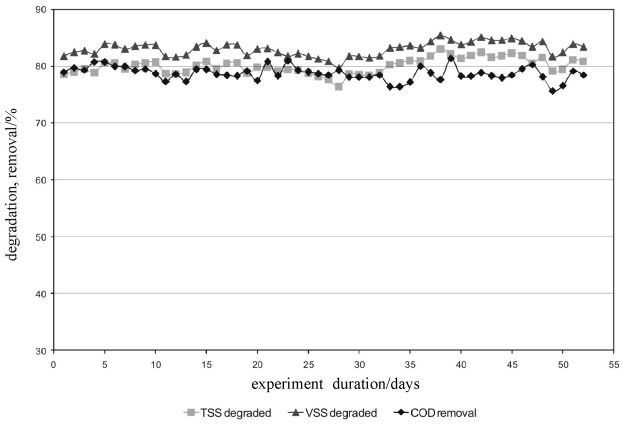


Fig. 5 – *Degradation of TSS, VSS and COD removal in the first experiment*

The second experiment

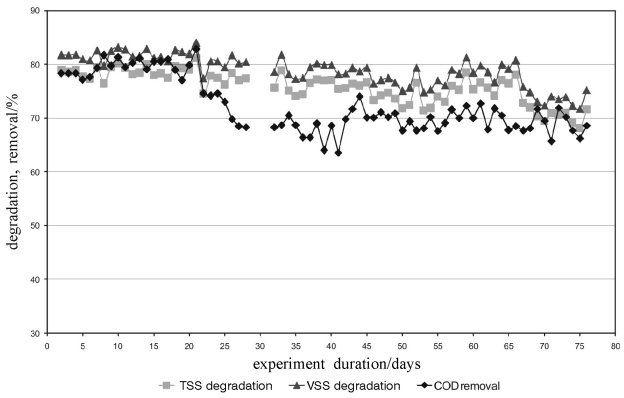
In the second experiment, the initial OLR was increased to 2.2 kg m^{-3} d^{-1}, representing a 22 % increase compared to the first experiment (1.8 kg m^{-3} d^{-1}) and a 47 % increase compared to the control experiment. From the experience in the first experiment and considering the fact that other authors reported similar or slightly higher OLR with pharmaceutical wastewaters,[3,5,6] no problems

Table 4 – *Average influent and effluent values of all experiments*

	Influent parameters				Effluent parameters			
	COD/g L^{-1}	TSS/g L^{-1}	VSS/g L^{-1}	pH	COD/g L^{-1}	TSS/g L^{-1}	VSS/g L^{-1}	pH
Control experiment	84.7	93.8	86.5	6.6	24.8	21.4	16.7	7.5
First experiment	112.8	109.4	103.1	6.2	24.0	21.7	17.5	7.5
Second experiment	117.4	101.4	95.3	6.0	36.2	26.1	21.7	7.6
Third experiment	82.0	61.6	57.6	5.2	*	21.4	18.0	*

* Due to the occurred inhibition, followed by increase or decrease of value, average values are not representative.

were expected in process performance. For the first 20 days of operation performance was excellent; average COD removal was 80 %, average TSS and VSS degradation were 79 % and 82 %, respectively (Fig. 6). After day 20 the process efficiency started to deteriorate rapidly. In just 7 days COD removal decreased to 68 %, pH dropped from 7.6 to 7.2, and the biogas production decreased by nearly 60 % (Fig. 8). At this point, we decided to stop feeding the reactor for 3 days to induce recovery. After 3 days the experiment continued from day 31 to day 48 using the same influent as in the first experiment (OLR was 1.8 kg m^{-3} d^{-1}). After day 48, the amount of pharmaceutical broth was increased to achieve the OLR of 2.0 kg m^{-3} d^{-1}. Process performance was relatively constant, the pH was again 7.6 and the biogas production increased; however the average COD reduction was only 70 % whereas average TSS and VSS degradation were 74 % and 77 % respectively. The most likely reason for such abrupt process inhibition is a probable toxic effect originating from the pharmaceutical broth, because the organic overload at OLR 2.2 kg m^{-3} d^{-1} under these conditions is highly unlikely.[3,5,6] To test the possibility of the inhibitory effect of the pharmaceutical broth, we conducted the third experiment with pharmaceutical broth as a single substrate, resulting in lower ORL.

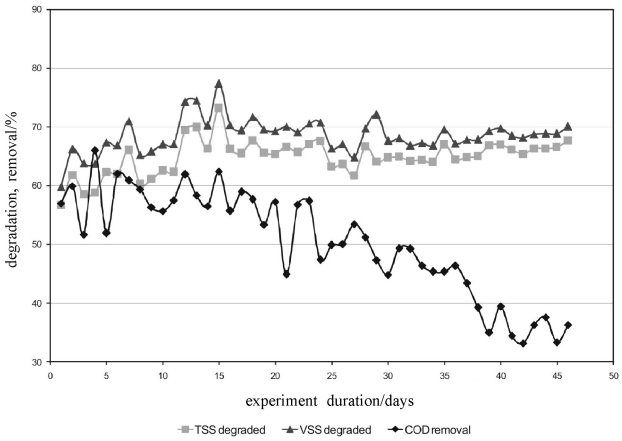


F i g . 6 – *Degradation of TSS, VSS and COD removal in the second experiment*

The third experiment

In the third experiment, only pharmaceutical broth with OLR of 1.6 kg m^{-3} d^{-1} was used as inflow substrate. After approx. 130 days of overall operation with pharmaceutical broth as a co-substrate, and process failure and recovery in the second experiment, it was assumed that the contact time of pharmaceutical broth and mesophilic sludge was sufficiently long (over 6 retention times) to attain possible adaptation that should enable introduction of pharmaceutical broth as a single substrate.

For a period of 10 days the process ran in the range of expected parameters (Fig. 7), pH was in the range of 7.5 to 7.6. In the next 15 days, pH remained between 7.4 and 7.5. During the first 25 days of the third experiment, average COD removal was 57 %, with no trend of decreasing. After day 25, COD removal started to decrease and after 20 days the process failed completely. The pH also decreased gradually from 7.4 on day 25 to 6.9 on day 46. Obviously, the waste pharmaceutical broth had a toxic effect on the anaerobic digestion, because the applied OLR of 1.6 kg m^{-3} d^{-1} was sufficiently low to exclude an organic overload. On the other hand, VSS and TSS removal showed no decrease during the process failure (Fig. 7). The average TSS and VSS removal was 65 % and 68 %, respectively. That could be explained with good performance of hydrolysis stage of anaerobic process which remained unaffected by the toxic impact of the waste broth.



F i g . 7 – *Degradation of TSS, VSS and COD removal in the third experiment*

Biogas production

Biogas production in the control experiment regarding methane was 25.4 L d^{-1}, specific methane production (SMP) was 646 L kg^{-1} of COD and 633 L kg^{-1} of VSS inserted. The biogas consisted in average of 65 % methane and 35 % of CO_2.

In the first experiment, methane production reached 30.9 L d^{-1}, which represents a 21 % increase according to 20 % increase of OLR. SMP was 590 L kg^{-1} of COD and 645 L kg^{-1} of VSS inserted, which is comparable to the production in the control experiment. The average composition of biogas was 70 % methane and 30 % CO_2. At the beginning of the second experiment, methane production was about 35 L d^{-1} (Fig. 8), with SMP 537 L kg^{-1} of COD and 628 L kg^{-1} of VSS inserted, before the process started to collapse. When the feeding of the reactor was stopped in order to induce recovery (in

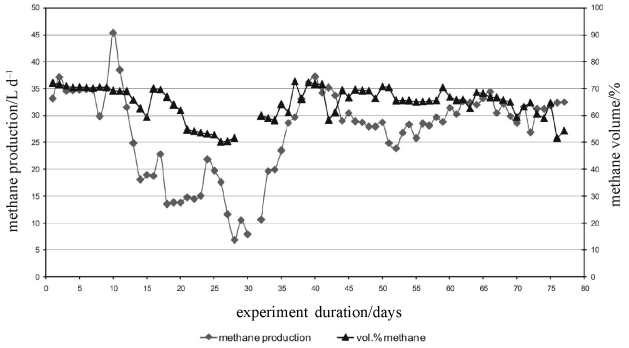


Fig. 8 – *Biogas production during the second experiment*

the second experiment), the methane production was only 8-10 L d^{-1}, with low SMP of 283 L kg^{-1} of COD and 356 L kg^{-1} of VSS inserted. After recovery of the system, the average methane production increased to 29.4 L d^{-1} with average SMP of 475 L kg^{-1} of COD and 585 L kg^{-1} of VSS inserted. Volume % of methane during the second experiment is shown in Fig. 8.

In the first 10 days of the third experiment average methane production was 22.5 L d^{-1} (Fig. 9). That was in the range of expected value, due to lower OLR in the third experiment. Average SMP was 484 L kg^{-1} of COD and 654 L kg^{-1} of VSS inserted, which is comparable to all other experiments. Average methane content in biogas was 56.3 % which is lower than in other experiments. After day 10, a rapid deterioration of the process efficiency began. Methane production declined to 2.6 L d^{-1}, the process was subsequently totally inhibited and stopped.

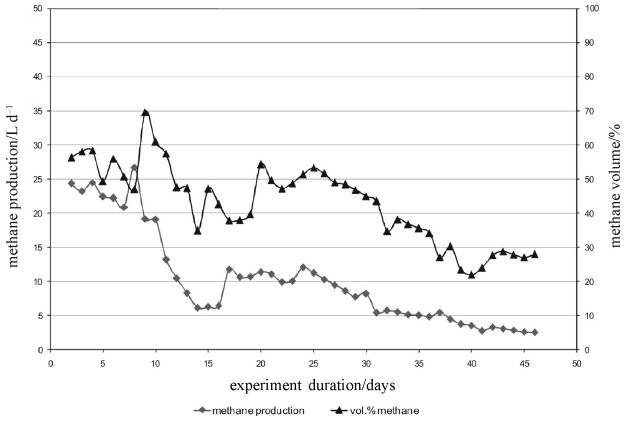


Fig. 9 – *Biogas production during the third experiment*

Conclusions

This study has demonstrated that anaerobic digestion of waste pharmaceutical broth as a single substrate is not possible due to toxic effect. However, anaerobic co-digestion of pig slurry, corn-grass silage and pharmaceutical broth with beneficial effects is possible. Our conclusion is that the co-digestion in full scale will proceed with no adverse effects providing two conditions are met. Firstly, the OLR of 2.0 kg m^{-3} d^{-1} must not be exceeded for longer periods of time (more than 7 days), and secondly, the pharmaceutical broth must not constitute more than 25 % of total OLR, to avoid toxic effects. It has been shown, that under strictly defined conditions it is possible to avoid the toxic effects of pharmaceutical waste broth on anaerobic biomass and conduct co-digestion successfully.

List of symbols

AOX – Absorbable Organic Halogens
BOD_5 – Biochemical Oxygen Demand, mg L^{-1}
BPR – Biogas Production Rate, m^3 of biogas per m^3 of reactor per day, m^3 m^{-3} d^{-1}
BTX – Benzene, Toluene, Xylene
COD – Chemical Oxygen Demand, mg L^{-1}
DOC – Dissolved Organic Carbon, mg L^{-1}
HRT – Hydraulic Retention Time, days
NH_4-N – Ammonium Nitrogen, mg L^{-1}
OLR – Organic Loading Rate of COD, kg of COD per m^3 of reactor per day, kg m^{-3} d^{-1}
PAH – Polycyclic Aromatic Hydrocarbons
PCB – Polychlorinated Biphenyls
SMP – Specific Methane Productivity, m^3 of methane per kg of COD/VSS inserted, m^3 kg^{-1}

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