Hindawi Publishing Corporation
Journal of Renewable Energy
Volume 2014, Article ID 208718, 10 pages
http://dx.doi.org/10.1155/2014/208718

Research Article

Magnetic Biofilm Carriers: The Use of Novel Magnetic Foam Glass Particles in Anaerobic Digestion of Sugar Beet Silage

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Received 30 September 2013; Revised 10 January 2014; Accepted 13 January 2014; Published 24 February 2014

Academic Editor: Yoon Y. Lee

The use of recently developed magnetic foam glass particles for immobilization of microbial biomass was tested. The effect of the particles was illustrated at the production of biogas from sugar beet silage as the sole substrate. Lab-scale fermentation experiments were conducted using a mesophilic completely stirred tank reactor and a magnetic separator. Microscopic analysis revealed biofilm coverage of 50–60% on the surface of the particles within 110 days. It was possible to recover 76.3% of the particles from fermentation effluent by means of a separation procedure based on magnetic forces. Comparing a particle charged reactor with a control reactor showed a small performance gain. The methane rate was increased from 1.18 ± 0.09 to 1.25 ± 0.06 L L^{-1} d^{-1} and the methane yield was increased from 0.302 ± 0.029 to 0.318 ± 0.022 L g^{-1} (volatile solids) at an organic loading rate of 3.93 ± 0.22 g L^{-1} d^{-1} (volatile solids). Maximum methane rates of 1.42 L L^{-1} d^{-1} at an organic loading rate of 4.60 g (volatile solids) L^{-1} d^{-1} (reactor including magnetic particles) and 1.34 L L^{-1} d^{-1} at 3.73 g L^{-1} d^{-1} (control reactor) were achieved. Based on the results, it can be concluded that the use of magnetic particles could be an attractive option for the optimization of biogas production.

1. Introduction

Bioenergy, including the production of biogas, is currently the largest renewable energy source. In 2008, biomass provided about 10% (50.3 EJ/yr) of the global primary energy supply. The potential deployment levels of biomass for energy by 2050 could be in the range of 100 to 300 EJ and 80 to 90% reduction of greenhouse gas compared to the use of fossil energy is possible [1].

Research and development including more sustainable feedstock and conversion technologies, increased conversion efficiency, and overall chain optimization are necessary to exploit this potential [1].

In the field of biogas production, a lot of different technologies were developed, depending on the characteristics of the input materials. For the anaerobic digestion of slurries, energy crops, or the organic fraction of municipal solid waste, the completely stirred tank reactor (CSTR) represents a proven and widely spread technology [2].

One limiting factor in anaerobic digestion is the concentration of microbial biomass inside the reactor. This is in particular relevant for low-solids substrates such as sugar beet silage which lead to short retention time [3]. Because of slow growth [4], especially the concentration of Archaea responsible for methane production is small [3].

In order to increase the microbial biomass, different immobilization techniques can be applied [5]. These techniques can consist of additional technical installations outside the reactor, for example, sedimentation tanks or membrane filtration, or the use of special types of reactors, for example, upflow anaerobic sludge blanket (UASB) reactors and expanded granular sludge blanket (EGSB) reactors, anaerobic filters (AF), or fluidized-bed-reactors (FBR). These techniques often show certain disadvantages, or they are limited to a small range of substrates. The membrane filtration method shows the risk of blockage due to biofouling [6], besides the effect that anaerobic microorganisms are exposed to higher stress levels during the process of filtration [7].

A sedimentation tank has specific space requirements [8]. Fixed biofilm techniques, AF or FBR, are used commonly for substrates showing very low solids contents, for example, waste water [9]. The range of substrates treatable in EGSB and UASB reactors is limited, because the formation of cell aggregates inside these reactors is connected to certain conditions such as hydrodynamics [5].

A relatively new approach for the immobilization of biomass in bioreactors is the application of magnetic particles serving as carriers for biofilm. This technique could provide some advantages to other techniques, as it is expected to be compatible with a broad range of substrates and applicable to common reactors such as the proven and versatile CSTR. The application of this technique is possible if the fermentation liquor's viscosity is not too high; further, the liquor may not contain long fibers or similar disturbing solids.

Hellman et al. [10] investigated the magnetic immobilization of biomass. Among three different materials, that is, iron, magnetite, and polystyrene, magnetite particles (approximate size 50 μm) were found to be the most promising carrier. The magnetite particles showed an extensive colonization, with preferential adhesion of hydrogenotrophic *Methanobacteriales*. However, an increased methanogenic performance of wastewater sludge digestion was not observed. The authors assume that the magnetic particles had improved the conditions for syntrophic acetogenesis and methanogenesis. These, however, were not the rate-limiting steps in the anaerobic digestion of wastewater sludge, but hydrolysis.

Similar studies with faster hydrolysable substrates are not known.

Aim of this study was to determine the feasibility of novel magnetic foam glass particles (MFGPs) for the immobilization of microbial biomass in a CSTR. Sugar beet silage was chosen as an easily hydrolysable substrate for anaerobic digestion by means of the CSTR.

The individual objectives were (1) to investigate the technical feasibility of a CSTR charged with MFGPs, (2) to observe the formation of biofilm on the surfaces of the MFGPs, (3) to determine the effects of MFGPs on the process behavior, and (4) to evaluate performance parameters of a MFGP-charged CSTR fed with sugar beet silage as the sole substrate.

2. Materials and Methods

2.1. Magnetic Foam Glass Particles (MFGPs). Several studies have shown that porous glass is a good carrier for biofilms [11–13].

The particles used in this study were recently developed by the companies Clariant Produkte (Deutschland) GmbH (Moosburg, Germany) and Dennert Poraver GmbH (Postbauer-Heng, Germany) in collaboration with the Leibniz Institute for Agricultural Engineering Potsdam-Bornim e.V. (Potsdam, Germany). The particles were made of foamed soda-lime silicate glass with a porous surface. Magnetic iron powder, Bayoxide E AB 21 (LANXESS Deutschland GmbH, Krefeld, Germany), was melt into the foam glass during the manufacturing process to attain magnetisable particles. Bayoxide E AB 21 is a pigment and coloring agent and it includes 97.5% Fe_2O_3 and 50 ppm heavy metals (Cu, Pb, Hg, and Cd). In addition to the iron powder, recycled glass, binding and expansion agents, and water were added to the production process. An expansion of the particles during the drying process leads to a fine-pored surface. Table 1 contains data describing the MFGPs used.

For reasons of simplicity, the particles are called magnetic particles. Indeed, the particles are not permanently magnetic, as the process cannot work with particles which aggregate to permanent lumps. Magnetisable particles is the more appropriate designation for the particles. Only in a strong magnetic field, the used particles become magnetic. As soon as the magnetic field is removed, the particles lose their magnetism.

As the used particles are still at the development stage, reasonable statements according to the costs of the used magnetic particles are not possible at this point.

2.2. Feedstock and Nutrient Supplements. The sole substrate used in these experiments was sugar beet silage (*Beta vulgaris* subsp. *vulgaris*). The beets were harvested in the region of Barsinghausen/Groß Munzel, Germany, in 2009, chopped, mashed, and ensiled.

Two different charges of this sugar beet silage were obtained and stored immediately before starting the periods of the experiment, start-up, and regular operation. Charge 1 was ensilaged for 22 weeks and then stored in a 1000 L intermediate bulk container without cooling. As the sugar beet silage of charge 1 showed signs of degradation, it was discarded and charge 2 was obtained before starting the regular operation period. Charge 2 was ensilaged for 8 weeks and then stored at 4°C in airtight 60 L plastic barrels to avoid premature aging of the substrate. The storage method applied to charge 2 caused a much higher stability of the silage. Specific data of the sugar beet silage is shown in Table 2. The presented values were averaged over the whole corresponding experimental period, 125 days of start-up and 93 days of regular operation.

The potential biogas and methane yields of the sugar beet silage were determined by conducting a fermentation test according to the guideline "VDI-Richtlinie 4630" [14]. Based on two repetitions, the biogas and methane potentials were $0.831 \pm 0.029\ m^3$ biogas per kg volatile solids (VS), respectively $0.407 \pm 0.022\ m^3$ methane per kg VS at charge 1 and $0.763 \pm 0.001\ m^3$ biogas per kg VS, respectively $0.327 \pm 0.003\ m^3$ methane per kg VS at charge 2. Weiland [2] reported sugar beet biogas yields of 0.730 to $0.770\ m^3\ (kg\ VS)^{-1}$. In the guide to biogas [15] values of 0.580 to $0.676\ m^3$ biogas per kg VS and 0.314 to $0.367\ m^3$ methane per kg VS are stated as biogas and methane potentials for sugar beet. According to that, the determined yield is in the range of expectations.

Different sugar beet silage can affect the methanogenic performance significantly [16]. Further, the different method of storage of the used substrate charges caused differences in quality, as shown in Table 2. To avoid interference between the influence of the MFGPs and the effects caused by

Table 1: Specification of the magnetic foam glass particles.

Parameter	Value	Analytical system
Particle diameter range (mm)	0.1–0.3	Mastersizer 2000 and Scirocco 2000 (Malvern Instruments, Worcestershire, UK)
Mean pore diameter (nm)	72.37	Pascal 440 (Thermo Electron Corporation, Waltham, USA)
Total porosity (—)	0.57	
Bulk density (kg m^{-3})	0.88×10^3	
Apparent density (kg m^{-3})	2.05×10^3	
Total cumulative volume (m^3 kg^{-1})	6.50×10^{-4}	
Total specific surface area (m^2 kg^{-1})	3.51×10^4	
BET surface area (m^2 kg^{-1})	9.64×10^2	ASAP 2020 (Micromeritics, Aachen, Germany)
Magnetic volume susceptibility (—)	5.25×10^{-3}	FMA 5000 (FORGENTA, Berlin, Germany)
Zeta potential (mV) in 1 mM TRIS-buffer solution	−60.3	Zetasizer 3000 HSA (Malvern Instruments, Worcestershire, UK)

Table 2: Chemical analysis of the sugar beet silage.

Parameter	Charge 1	Charge 2
pH (—)	3.41 ± 0.10	3.36 ± 0.06
Conductivity (mS cm^{-1})	3.14 ± 3.10	1.35 ± 0.97
$TS_{105°C}$ (% FM)	18.09 ± 0.92	19.05 ± 0.40
$VS_{550°C}$ (% TS)	69.77 ± 4.72	79.16 ± 0.57
Acetic acid (g (kg FM)$^{-1}$)	10.77 ± 4.60	8.16 ± 0.58
Propionic acid (g (kg FM)$^{-1}$)	0.62 ± 0.47	0.15 ± 0.04
Sum of VFA C_2–C_6 (g (kg FM)$^{-1}$)	11.89 ± 5.41	8.40 ± 0.69
Ethanol (g (kg FM)$^{-1}$)	19.30 ± 8.78	8.40 ± 0.59
Propanol (g (kg FM)$^{-1}$)	1.14 ± 0.34	1.20 ± 0.35
Lactic acid (g (kg FM)$^{-1}$)	24.95 ± 6.08	20.61 ± 1.42
NH_4-N (g (kg FM)$^{-1}$)	0.067 ± 0.006	0.075 ± 0.012
N_{Kjel} (g (kg FM)$^{-1}$)	1.359 ± 0.028	1.442 ± 0.125
Sugar content (g (kg FM)$^{-1}$)	37.95 ± 9.07	44.91 ± 4.05
COD (g (kg FM)$^{-1}$)	136.52 ± 15.91	167.51 ± 3.61

the change of substrate on the methanogenic performance, the reactors were shut down before regular operation.

The higher stability reached charge 2 while storing should minimize the possibility that variance in the methanogenic performance of the reactors is caused by the quality of the substrate. As both fermenters were always fed with sugar beet silage of the same charge and the same age, an influence on the performance of the fermenters is unlikely, too. In addition, only the values of the regular operation period with its optimized setup are used for evaluation.

According to Weiland [2], the C/N ratio should be in the range between 15 and 30. Since the used sugar beet silage had a C/N ratio of 44.4 ± 6.2 (determined only at charge 2), the addition of N was necessary. The optimum growth conditions for *Methanosaeta concilii*, which is the most ammonia-sensitive methanogen, are in the range of 250–1100 mg ammonium nitrogen (NH_4-N) per liter fermentation liquor [16]. Accordingly, the concentration of NH_4-N inside the reactors was adjusted to 500 mg NH_4-N L^{-1}. Therefore, a 1 : 1 mixture of ammonium bicarbonate (NH_4HCO_3) and ammonium carbamate (NH_4COONH_2) (Roth, Karlsruhe, Germany) dissolved in tap water was used. The given volume and the NH_4-N concentration of the solution depended on the corresponding NH_4-N values of the sugar beet silage. The total solids (TS) content of the total feeding was set to 5% fresh mass (FM) by adjusting the volume of the ammonium carbonate solution, total feeding being defined as the sum of sugar beet silage and ammonium carbonate solution. The concentration of NH_4-N of the total feeding was set to 1 g L^{-1} by varying the concentration of NH_4-N of the ammonium carbonate solution.

To ensure a sufficient supply of trace elements, a trace element solution was prepared according to the recipe DSMZ 144 provided by Deutsche Sammlung von Mikroorganismen und Zellkulturen but in a 5-fold concentration. The solution was fed in a ratio of 0.01 mL per g VS (fed sugar beet silage) as recommended by Abdoun and Weiland [17].

2.3. Experimental Setup. For testing the magnetic immobilization technique, corresponding setup was used consisting of two identical CSTRs, one of which was used for reference purposes. The setup of the used CSTR system is shown in Figure 1.

The reactors were made of acrylic glass, had a working volume of 50 L, and were equipped with a heating jacket and a stirrer for homogenization of the fermentation liquor. Three outlets in different heights of 2.3 cm, 15.8 cm, and 32.5 cm above the reactor bottom were used to remove fermentation liquor.

Feeding and stirring were automatically operated by an electronic control program. This program facilitated continuous feeding. Different algorithms of stirring were tested. Effluent was released continuously from the midlevel outlet by means of an overflow pipe.

The organic loading rate (OLR) was related to the corrected VS content VS_{corr}. VS_{corr} was defined as the sum of VS, volatile fatty acids (VFA), lactic acid, and alcohols.

The magnetic immobilization reactor (MR) was charged with the magnetic particles and equipped with a magnetic

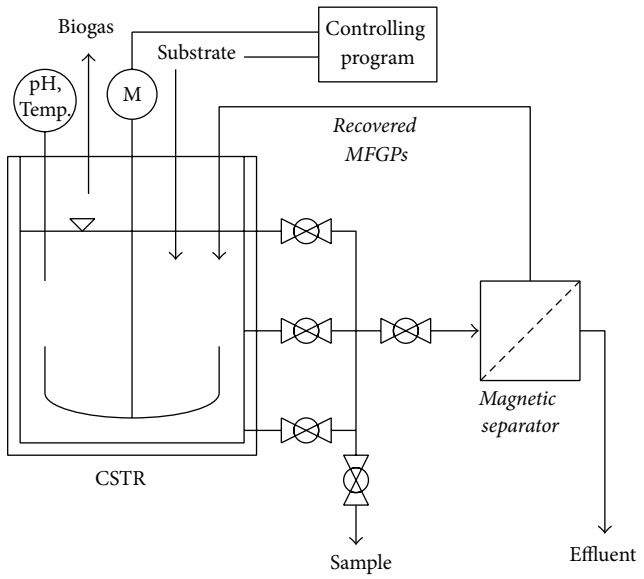


Figure 1: CSTR reactor system; components in cursive lettering were only used at the reactor including magnetic immobilization (MR).

separator (Figure 1). The initial concentration of MFGPs inside the reactor was set to 1% w/w. The magnetic separator was a common Liquimag LM9-E-050-7 by S + S Separation and Sorting Technology GmbH (Schönberg, Germany), a tool widely used in food processing to protect products from metallic contamination. The second reactor was employed as control reactor (CR). The CR served as reference, did not contain any MFGPs, and was not equipped with a magnetic separator.

2.4. Reactor Start-Up and Operation. Besides the magnetic technique, both reactors were operated identically. The substrate sugar beet silage (charge 1 while start-up and charge 2 in regular operation) was digested continuously under anaerobic and mesophilic conditions. The temperature was kept at 41°C since Demirel and Scherer achieved successful digestion of sugar beet silage at a temperature between 41 and 42°C [16].

For the start-up, both reactors were inoculated with collected fermentation effluent from other experimental reactors operated under mesophilic conditions at the Leibniz Institute for Agricultural Engineering Potsdam-Bornim. During the first 35 days of operation, the organic loading rate was increased from 1.0 to 4.0 g VS_{corr} L^{-1} d^{-1}. The level of 4.0 was then kept for 40 days. Consecutively, from days 75 to 125, the OLR was lowered to 1.0 g VS_{corr} L^{-1} d^{-1} using steps of 1.0 g VS_{corr} L^{-1} d^{-1}. During the start-up, only trace elements were added to the feed. The MFGPs were introduced into the MR on day 15. A stable biofilm should develop on the surfaces of the MFGPs during the start-up.

After the start-up, the OLR of the reactors was increased in steps of approximately 0.5 from 1.0 to 4.6 g VS_{corr} L^{-1} d^{-1} until the digestion process reached its limit of capacity at the control reactor. During regular operation, both the trace element solution and the ammonium carbonate solution were fed to CSTRs. The trace element solution was mixed into the beet silage, whereas the ammonium solution was fed directly to the reactors. Adding the ammonium carbonate solution at the beginning of the regular operation caused a reduction of the hydraulic retention time (HRT) from 79 to 31 days (OLR 1.5 g VS_{corr} L^{-1} d^{-1}). The added volume of the ammonium carbonate solution depended on the OLR and was raised from 1000 to 2800 g d^{-1} during the experiment. In the end of the experiment, a HRT of 11 days was reached (OLR 4.6 g VS_{corr} L^{-1} d^{-1}).

Beginning at day 197, silicone antifoaming emulsion (Roth, Karlsruhe) with a concentration of 0.06 g (L reactor volume)$^{-1}$ was added to both reactors in intervals of 3 to 9 days.

The MFGP-containing effluent liquor of the MR was continuously flushed through the magnetic separator. The used separator had to be operated manually. In order to recover the separated MFGPs, the insert of the separator was removed. The magnetic rods were removed from the insert and the MFGPs were flushed with MFGP-cleared effluent liquor. Finally, the separated particles were returned to the MR. This recovery procedure was executed once every day. The addition of the ammonium solution during regular operation caused an increase of the effluent liquor that had to be separated from MFGPs. It was not possible to reduce the interval of recovering due to the time demanding process of manual operation. Instead, the effluent leaving the separator was stored for posttreatment every day during the regular operation period. The posttreatment was conducted by moving the magnetic insert of the separator through the stored effluent several times until no more recovery of MFGPs was visible.

2.5. Analytical Methods. The performance and stability of the reactors was monitored by daily detection of pH (pH combined electrode Xerolyt HA 405-DXK-58/425, Mettler Toledo) and biogas production (multi-chamber rotor gas meter TG 05, Ritter Apparatebau GmbH & Co. KG). The compounds of the produced biogas were analyzed daily by gas analyzer SSM 6000 (Pronova Analysentechnik GmbH & Co. KG). To obtain the volumetric methane rate, the daily methane production was related to the effective reactor volume. The methane yield was related to the total amount of fed volatile solids VS_{corr} (the sum of VS, VFA, lactic acid, and alcohols) in accordance with the calculation of the OLR.

The fermentation liquor of both reactors was sampled once a week. Concentrations for NH_4-N, nitrogen (N_{Kjel}), C_2–C_6 VFA, and lactic acid were determined, employing the following methods:

NH_4-N: conversion into NH_3 by steam distillation and back-titration according to VDLUFA Methodenbuch volume 3, chapter 4.8.2 [18];

N_{Kjel}: method by Kjeldahl according to DIN EN 25663: 1933-11;

C_2–C_6 VFA: gas chromatographic analysis, GC 8360 Carlo Erba (Fisons Instruments), column DB-FFAP

(length 30 m, diameter 0.53 mm, film thickness 0.25 μm), and flame ionization detector;

lactic acid: high-performance liquid chromatography, DIONEX ULTIMATE 3000 (Thermo Scientific, Sunnyvale, USA), column Eurokat H (length 300 mm, diameter 8 mm, film thickness 10 μm) and detector SHODEX RI-101 (Showa Denko, Tokyo, Japan).

The ratio of volatile organic acids to total inorganic carbon (VOA/TIC) of the fermentation liquor was determined by titration with 0.05 molar sulphuric acid according to the Nordmann method [19].

With respect to the MR, the magnetic volume susceptibility (X_V) of the reactor effluent was determined in intervals of 2 to 21 days using a Forgenta magnetic analyser FMA 5000 (FORGENTA Forschungstechnik—und Geräte-Entwicklung, Berlin, Germany). In order to determine the magnetic separation efficiency, the reactor effluent was measured before and after passing the magnetic separator. Relating the values of magnetic susceptibility to the concentration of MFGPs inside the reactor effluent (c_{MFGP}) was facilitated by means of a calibration function

$$X_V = 2.1539 * 10^{-7} * c_{\mathrm{MFGP}} - 2.725 * 10^{-9}, \quad R^2 = 0.9965. \tag{1}$$

The calibration function was determined by adding defined amounts of MFGPs to MFGP-free effluent taken out of the CR and measuring the magnetic susceptibility.

The development of biofilm formation on the MFGPs and the structure of these biofilms were monitored by means of phase-contrast and fluorescence microscopy. Microscopic analysis was conducted in intervals of approximately 4 weeks beginning at day 40 of the experimental period.

Fluorescence of the present microorganisms was obtained by staining nucleic acids with the green fluorescent dye SYTO 13 (excitation 450–490 nm) and stimulating the autofluorescence of methanogenic Archaea by UV-light at an excitation of 359–371 nm. Autofluorescence appears, if coenzyme F_{420}, a coenzyme involved in redox reactions in methanogenic microorganisms [20], is present and points to the activity of these organisms.

The microscopic analyses were conducted under exclusion of air by means of the gas Protectan (Tetenal, Norderstedt, Germany). For each microscopic analysis, the MFGPs included in 4 mL of sampled fermentation liquor were separated manually by means of small magnet and washed 7 times in 15 mL of 0.01 M Na-Hepes buffer with pH 7.5 (Sigma-Aldrich, St. Louis, USA). After washing, the MFGPs were stained with SYTO 13 (Molecular Probes, Oregon, USA) and incubated for 10 minutes. A 500 μM stock solution of SYTO 13 and dimethylsulfoxide (Sigma-Aldrich, St. Louis, USA) was used in final concentrations of 1 to 10 μM, depending on the intensity of the biofilm formation. After staining, the MFGPs were transferred into a MicroLife-slide (Glaswarenfabrik Karl Hecht, Sondheim in der Rhön, Germany). The used slides had chamber depths of 0.25–2.0 mm. Finally the microscopic analysis was conducted by using a Zeiss Axiostar plus FL, the objectives Zeiss EC "Plan-Neofluar" 5x/0.16 Ph1 (a = 18.5 mm), Zeiss EC "Plan-Neofluar" 20x/0.5 Ph2 (a = 2.0 mm) and Zeiss LD "Plan-Neofluoar" 63x 0.75 Korr Ph2 with extra long operating distance (D = 0–1.5, a = 2.2 mm at D = 0 and a = 1.2 mm at D = 1.5), Zeiss AxioCam MRc5, and the program Carl Zeiss AxioVision 4.8 (Carl Zeiss, Jena, Germany).

3. Results and Discussion

3.1. Technical Aspects of the Magnetic Immobilization Technique. Prior to the fermentation experiments, the separator's removal efficiency was tested using untreated MFGPs and xanthan in water solutions (mass ratio 1 : 400 and 1 : 1000) as artificial fermentation liquor. The viscosity of the used liquors was determined by means of a rheometer MC1/RM300 (Parr, Illinois, USA) and the cylinder system Z10. It was observed, that the 1 : 1000 solution had a similar viscosity as the fermentation liquor, whereas the viscosity of the 1 : 400 solution showed values twice as high than the viscosity of the fermentation liquor.

These experiments were presented in Thomas [21] and the major results are listed below. At a MFGP concentration of 1% and a flow rate through the magnetic separator of 1.8 $m^3\,h^{-1,}$ the recovery rates were 26.7% (1 : 400 solution), 50.6% (1 : 1000 solution), and 72.8% (tap water). By increasing the number of separation cycles from one to five and ten, the recovery improved to 44.1% and 53.9% (1 : 400 solution) and to 96.7% and 99.1% (tap water).

The viscosity of the liquor was found to be a strong influence on the recovery. To achieve a satisfactory performance of the magnetic separation of the used particles, the values of the liquor's viscosity should not exceed the values measured at the 1 : 1000 xanthan-water solution. As the used liquors were non-Newtonian fluids, their viscosity is characterized by values of the dynamic viscosity at different shear velocities. At the 1 : 1000 xanthan-water solution values of 0.024 Pa s (at 20 s^{-1}), 0.016 Pa s (at 50 s^{-1}), 0.019 Pa s (at 100 s^{-1}), 0.022 Pa s (at 200 s^{-1}), and 0.038 Pa s (at 300 s^{-1}) were measured for the dynamic viscosity.

Based on these results, the magnetic separation efficiency was regarded as being sufficient for the purpose of the present work.

Looking at the fermentation experiments, magnetic susceptibility measurement revealed that the reactor's effluent liquor, which was continuously fed to the magnetic separator by means of an overflow pipe, contained only 0.36 ± 0.24% w/w (start-up) and 0.08 ± 0.02% w/w (regular operation from day 126 to day 218) of MFGPs. This gap between the initial set point of 1% w/w was found to be mainly caused by strong sedimentation effects inside the reactor. Sedimentation likely interfered with the formation and metabolism of MFGP-bound biofilms. By the end of the experiment, effluent was sampled directly from the lower, the middle and the upper outlet (the outlets were placed 2.3, 15.8 and 32.5 cm above reactor bottom) while stirring at 10 rpm. The effluents showed MFGP concentrations of 0.15, 0.10 and 0.10% w/w (lower, middle and upper outlet; values from single measurement).

A period of 10 minutes without stirring caused a remarkable gradient. Effluent liquor from the lowest outlet showed a concentration of 0.80%, whereas liquor from the middle and upper outlet had only 0.25 and 0.17% of MFGPs.

MFGPs were lost during operation of the MR. Using the magnetic separator's feed concentration as basis, the single stage separation efficiency was 89.5 ± 11.3% (start-up) and 39.1 ± 10.7% (regular operation). By means of a manual second stage separation, the recovery was increased to 76.3% (single measurement value) during regular operation. The loss of MFGPs accumulated to 59% during the conducted experiment.

Formation of foam up to a height of 7.5 cm above the liquid level inside the CR required an adjustment of stirring at day 168; the MR showed no formation of foam. Removing the foam was possible by resetting stirring from intervals of 180 s at a rotational speed of 25 min^{-1} divided by breaks of 300 s to permanent stirring at a rotation speed of 10 min^{-1}. Stirring was reset at both reactors. Formation of foam increased during further operation up to heights of 15 cm (CR) and 2.5 cm (MR) above the liquid level despite the stirring. Using antifoaming emulsion beginning at day 198 successfully prevented the formation of foam.

Based on these observations, it can be concluded that both spatial distribution and magnetic separation of the MFGPs need further optimization.

The particles showed a strong tendency for sedimentation on the bottom of the reactor. Stirring caused accumulation of particles in the center of the reactor volume. A homogenous distribution of particles inside the reactor volume was hard to reach because of these effects. If a more homogenous distribution is aspired, the density of the MFGPs has to be adjusted. On the other hand, it was possible to keep the particles in certain areas of the reactor by using these effects. To keep particles at areas that are distant to the reactor outlet, could offer an interesting option to improve the MFGPs retention and to reduce the size of the magnetic separator.

The achieved recovery grades of magnetic separations using the magnetic separator Liquimag LM9-E-050-7 were not high enough to prevent high losses of MFGPs during the conducted experiment because the losses cumulate with every following separation. Reducing these losses is possible by increasing the efficiency of the magnetic separation procedure. The magnetic force that is acting on a magnetic particle inside a magnetic field is linearly correlated with the magnetic volume susceptibility and the volume of the particle [22]. According to that, the performance of the magnetic separation procedure could be improved by adapting the properties of the used MFGPs. The use of another magnetic separator that is better suited for the intended purpose than the used common separator could also increase the separation efficiency. In addition, lost particles should be substituted during the operation of the reactor.

3.2. Biofilm Formation. The MFGPs inside the MR promoted the growth of a distinctive biofilm. Microscopic analyses on day 110 (start-up phase) showed average biofilm coverage of the MFGP surface of 50–60%. Colonization was found to start at protected areas such as cracks and holes.

Later, on day 170, average coverage increased up to 90% and an intensely covered MFGP is shown in Figure 2. The microorganisms were embedded in a slime matrix with a thickness of up to 42 μm. Extensive colonization was verified by means of SYTO 13. Furthermore, UV excitation confirmed the presence of methanogenic cofactor F_{420} at multiple locations of each particle. This, consequently, provides evidence to the hypothesized growth of methanogenic biofilms on magnetic foam glass particles.

The duration of approximately 3 to 4 months required for effective coverage of the particles is within the range determined in other studies. According to a literature review by Escudié et al. [23] on anaerobic biofilm reactors, the start-up period for extensive biofilm formation usually varies between 2 and 9 months.

The general growth [24] and the microbial community [25] of biofilm on carriers are depending on physicochemical surface properties of the carriers.

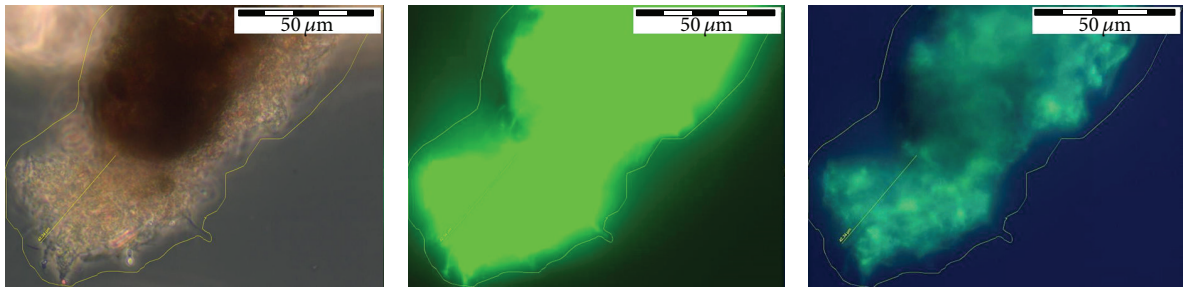
Total adhesion free energy between a microorganism and a surface [26], the surface charge [27], hydrophobicity [28], total surface energy, and surface roughness [25] were determined as important factors in the adhesion of microorganisms. Porosity was found to be one of the most important influencing factors of biofilm formation [24]. Silva et al. [29] showed that shape and mean pore diameter are important factors, impacting the adherence of biomass on different support materials (polyurethane foam, vegetal carbon, low-density polyethylene, and alumina-based ceramics). Increased biomass adherence was observed especially at high mean pore diameter (543 μm). An irregular shape (vegetal carbon pellet) seems to be advantageous.

The carriers used in this study showed a homogenous spherical shape, total porosity of 0.57, a negative surface charge (a zeta potential of −60.3 mV), and a mean pore diameter of 72 nm. There may be considerable potential for further improvement of the magnetic carriers used in this study. Variation of the physicochemical properties of the used MFGPs could accelerate the formation of biofilm on their surface. The evaluation of suitable modification of the used MFGPs will be the objective of further studies.

3.3. Effect on the Process Behavior. The course of both reactors' process parameters, that is, pH, OLR, methane yield, methane rate, VOA/TIC ratio, and concentration of VFA during regular operation, is presented in Figure 3. Values of methane rate, methane yield, and methane content of the reactors determined during start-up and regular operation are presented in Table 3.

The methane rate increased coherently to the OLR as expected. Methane rates of 1.18 ± 0.09 (CR) to 1.25 ± 0.06 L L^{-1} d^{-1} (MR) and methane yields of 0.302 ± 0.029 (CR) to 0.318 ± 0.022 L (g VS_{corr})$^{-1}$ (MR) were achieved at an OLR of 3.93 ± 0.22 g VS_{corr} L^{-1} d^{-1} (volatile solids). Methane contents of 52.6% ± 1.5 (CR) and 52.8% ± 1.6 (MR) were obtained during regular operation of the reactors.

Since the increase of the OLR from 4.06 to 4.62 g VS_{corr} L^{-1} d^{-1} at day 210, the pH and the methanogenic performance of the CR decreased sharply. Feeding of the CR



(a) (b) (c)

Figure 2: An intensely covered MFGP; sample taken at day 170-phase contrast (a) and fluorescence microscopy (SYTO 13 (b) and autofluorescence (c)), 63-fold objective magnification; (micrograph: Clariant).

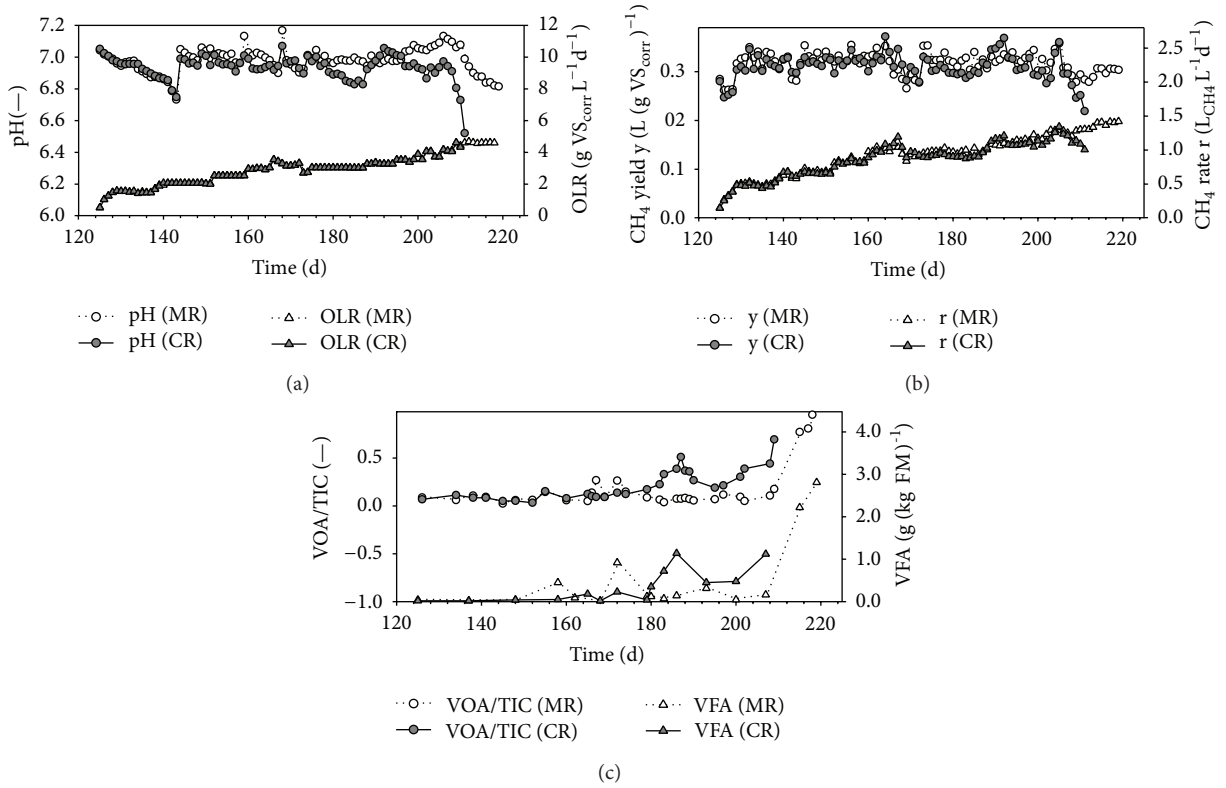


Figure 3: pH and organic loading rate (a), methane yield and methane production rate (b), VOA/TIC ratio and concentration of volatile fatty acids (c) of methanogenic reactors with (MR) and without (CR) magnetic foam glass particles.

Table 3: Methane rate, methane yield, and methane content of methanogenic reactors with (MR) and without (CR) magnetic foam glass particles during periods of constant OLR and HRT.

Reactor days (—)	HRT (d)	OLR (g VS$_{corr}$ L^{-1} d^{-1})	Methane rate (L L^{-1} d^{-1})		Methane yield (L (g VS$_{corr}$)$^{-1}$)		Methane content (%)	
			MR	CR	MR	CR	MR	CR
35–75 (start-up)	40	4.15 ± 0.18	1.63 ± 0.16	1.60 ± 0.18	0.393 ± 0.037	0.385 ± 0.045	56.3 ± 1.0	55.9 ± 1.5
125–137	32	1.51 ± 0.04	0.49 ± 0.04	0.47 ± 0.04	0.324 ± 0.025	0.310 ± 0.024	53.5 ± 1.9	52.8 ± 1.2
138–151	23	2.02 ± 0.10	0.65 ± 0.05	0.64 ± 0.04	0.322 ± 0.020	0.316 ± 0.011	53.2 ± 2.1	53.1 ± 2.0
152–159	18	2.54 ± 0.00	0.83 ± 0.03	0.81 ± 0.03	0.328 ± 0.012	0.321 ± 0.013	52.8 ± 0.8	52.7 ± 0.9
160–187	15	3.07 ± 0.17	0.97 ± 0.05	0.95 ± 0.08	0.318 ± 0.023	0.310 ± 0.020	52.5 ± 1.6	52.2 ± 1.1
188–199	14	3.38 ± 0.12	1.11 ± 0.06	1.10 ± 0.05	0.328 ± 0.010	0.327 ± 0.023	52.9 ± 0.4	53.0 ± 1.0
200–208	12	3.93 ± 0.22	1.25 ± 0.06	1.18 ± 0.09	0.318 ± 0.022	0.302 ± 0.029	53.8 ± 0.4	53.0 ± 1.1
209–218/211	11	4.60 ± 0.07	1.35 ± 0.05	1.08 ± 0.07	0.295 ± 0.011	0.239 ± 0.017	51.3 ± 0.9	48.7 ± 1.2

was stopped at day 212, when values of pH below 6.5 were measured. While pH and consequently the methanogenic performance of the CR declined, the process remained stable at the MR. The pH of the reactor content from the MR asymptotically approached a lower limit, about 6.8.

The development of VOA/TIC ratio and concentration of VFA were different at CR and MR. Until the beginning overload of the CR at day 210, it showed an average VOA/TIC ratio of 0.20 ± 0.14; the average concentration of VFA was about 0.35 ± 0.40 g (L FM)$^{-1}$. The corresponding values of the MR, 0.10 ± 0.06 (VOA/TIC ratio) and 0.18 ± 0.24 g (L FM)$^{-1}$ (concentration of VFA), were approximately half as high. Since the raise of the OLR at day 210, the VOA/TIC ratio and the concentration of VFA increased at both reactors. By the end of the digestion experiment, VOA/TIC ratios and VFA concentrations of 0.70 and 1.12 g (L FM)$^{-1}$, respectively, 0.95 and 2.81 g (L FM)$^{-1}$ were reached at CR and MR.

The VOA/TIC ratio represents a fast control of the process stability [2]. TIC represents the alkalinity of the fermenter. For a stable anaerobic digestion of sugar beet, the value of TIC should be 4000 to 6000 mg $CaCO_3$ L^{-1} [16]. During regular operation, the TIC remained stable at both reactors, values of 4226.34 ± 501.36 and 4126.86 ± 547.69 mg $CaCO_3$ L^{-1} were measured at MR and CR, respectively.

The increase of the VOA/TIC ratio in the end of the digestion experiment was caused by the increasing concentration of VOA in the fermentation liquor. Values of VOA/TIC over 0.3 indicate that the process may be inhibited due to hyperacidity in the digester [30]. The observed stability of the methanogenic process at the MR in spite of high values of VOA/TIC does not follow this conclusion. This may be regarded as a successful protection of methanogenic microorganisms living inside the biofilm formed on the MFGPs.

The concentrations of NH_4-N and N_{Kjel} of the fermentation liquor were similar at both reactors. During the regular operation period of the reactors concentrations of 0.48 ± 0.18 and 0.46 ± 0.17 g L^{-1} were determined for NH_4-N at MR and CR, repectively. N_{Kjel} showed values of 1.32 ± 0.24 and 1.21 ± 0.19 g L^{-1} at MR and CR, repectively. The adjustment of the concentration of NH_4-N inside the reactors to the target value of 0.5 g L^{-1} could be realized successfully by adding ammonium carbonate.

Positive effects of the MFGPs on the process stability can be conducted from the presented data. Process performance and pH levels showed less fluctuation in comparison to the control reactor. The loading limit of the control reactor was reached at OLR of 4.62 g VS_{corr} L^{-1} d^{-1} (at day 210). The capability and pH of the control reactor heavily decreased at that point, as stabilization of the methanogenic process could be excluded virtually. In contrast, the fermenter containing MFGPs was able to recover after a short period of adaptation and further operation at OLR 4.62 g VS_{corr} L^{-1} d^{-1} was possible.

3.4. Maximum Process Performance. Maximum methane rates of 1.34 L L^{-1} d^{-1} at OLR 3.73 g VS_{corr} L^{-1} d^{-1} and 1.42 L L^{-1} d^{-1} at OLR 4.60 g VS_{corr} L^{-1} d^{-1} were achieved by CR and MR, respectively. The methane yields fluctuated closely around 0.300 L (g VS_{corr})$^{-1}$; maximum values of 0.372 L (g VS_{corr})$^{-1}$ at OLR 3.00 g VS_{corr} L^{-1} d^{-1} and 0.358 L (g VS_{corr})$^{-1}$ at OLR 3.73 g VS_{corr} L^{-1} d^{-1} were obtained at CR and MR, respectively.

The achieved maximum performance is comparable to the results of other studies using beet silage as sole substrate. Demirel and Scherer [16] achieved a methane rate of 1.80 L L^{-1} d^{-1}, a biogas rate of 2.86 L L^{-1} d^{-1}, a methane yield of 0.454 L (g VS)$^{-1}$, a biogas yield of 0.720 L (g VS)$^{-1}$ and a methane content of 63% at the digestion of sugar beet silage at OLR 3.968 g VS L^{-1} d^{-1} and HRT 25 d. The higher methanogenic performance in comparison to the results of this study may be attributed to the characteristics of the digested sugar beet silage. At Demirel and Scherer [16], the reactor process performance was affected significantly by the use of two different charges of sugar beet silage (both obtained from the same area in Soltau, Germany). Specific gas production rates of 0.490 L (g VS)$^{-1}$ d^{-1} (first charge) and 0.720 L (g VS)$^{-1}$ d^{-1} (second charge), and volumetric gas production rates of 1.74 L L^{-1} d^{-1} (first charge) and 2.86 L L^{-1} d^{-1} (second charge) were obtained at OLR 3.560 g VS L^{-1} d^{-1} and 3.968 g VS L^{-1} d^{-1}, respectively.

The use of different particles to enhance the performance of anaerobic digestion processes was investigated in several studies.

At particles serving as adsorbent substrate can accumulate, these areas of adsorption provide a more favorable growth environment for the bacterial substrate system [31]. Twofold enhancement in total gas production with 17% enriched methane content was achieved with the addition of 4 g L^{-1} of silica gel in anaerobic digestion of a mixture of cheese whey, poultry waste, and cattle dung [31]. The addition of 5% commercial charcoal powder to cow dung on a dry weight basis resulted in an increase of gas production by 17% and 34.7% in batch and semicontinuous fermenters, respectively [32]. The increase in methanogenic performance can be attributed to enhanced microbial activity in the digester, contributed by growth of bacteria on the surface of the particles [32]. Removing slowly biodegradable organic materials [32] and serving as source of nutrient [33] have to be considered as side effects when adsorbents are used.

Compared to these results, the effect of the particles tested in this study was small. The addition of 1% w/w magnetic carriers to a CSTR caused a 6% increase of the maximum methane rate.

The magnetic carriers and the magnetic separation technique used in this study still contain a high potential for development, as stated before in Sections 3.1 and 3.2. Hence it can be assumed that a further increase of the methanogenic performance by magnetic retention of biomass using MFGPs is possible. The improvement of the magnetic carriers and the magnetic separation technique is subject of further studies.

4. Conclusions

The porous surface of the tested magnetic foam glass particles turned out to be suitable for settlement of methanogenic

microorganisms. 50 to 60% of the surfaces were covered by a biofilm within 110 days. The magnetic properties of the tested particles enabled retention of the particles inside the reactor by means of magnetic forces. The attainable recovery grade of the used magnetic separation technique was about 76.3% during regular operation of the reactor. Within the total experimental period of 218 days, the losses of MFGPs accumulated to 59%.

The observed effect on the methanogenic performance of a CSTR was small. Stabilization of the methanogenic process caused an increase of the maximum methane rate from 1.34 to 1.42 $L\,L^{-1}\,d^{-1}$. The value of the organic loading rate when the maximum methane rate was reached was shifted from 3.73 to 4.60 $g\,L^{-1}\,d^{-1}$. During the experimental period, the reactor including particles always showed a slightly higher performance, but this difference was within the scatter of the experimental values.

According to the achieved retention of methanogenic microorganisms inside the reactor, the use of magnetic particles can be considered as a promising technique to enhance the methanogenic performance of common CSTRs. The small effect on the reactor performance is probably due to a low initial particle concentration of 1% w/w and the losses of particles during the operation of the reactor. Besides, the used magnetic particles include a high potential for optimization regarding surface properties, shape, size, and magnetizability. Utilization of this potential could cause a more considerable effect on the methanogenic performance.

Abbreviations

AF: Anaerobic filter
ATB: Leibniz Institute for Agricultural Engineering Potsdam-Bornim
C_2–C_6: Short-chain volatile fatty acids
c_{MFGP}: Concentration of MFGPs inside the reactor effluent
COD: Chemical oxygen demand
CR: Control reactor
CSTR: Completely stirred tank reactor
DSMZ: Deutsche Sammlung von Mikroorganismen und Zellkulturen
EGSB: Expanded granular sludge blanket reactor
FBR: Fluidized-bed-reactor
FM: Fresh mass
HRT: Hydraulic retention time
MFGP: Magnetic foam glass particle
MR: Magnetic immobilization reactor
N_{Kjel}: Nitrogen content determined according to the method by Kjeldahl (DIN EN 25663:1933-11)
NH_4COONH_2: Ammonium carbamate
NH_4HCO_3: Ammonium bicarbonate
NH_4-N: Ammonium nitrogen
OLR: Organic loading rate
r: Methane rate
TS: Total solids
$TS_{105°C}$: Content of total solids determined at 105°C
UASB: Upflow anaerobic sludge blanket reactor
VFA: Volatile fatty acids
VOA/TIC: Ratio of volatile organic acids to total inorganic carbon
VS: Volatile solids
$VS_{550°C}$: Content of volatile solids determined at 550°C
VS_{corr}: Corrected VS content
X_V: Magnetic volume susceptibility
y: Methane yield.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

The authors would like to thank Christof Thomas for his ambitious work.

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