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Increased biogas production from lignocellulosic biomass by soaking in water

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Abstract. Due to its large production worldwide, lignocellulosic biomass represents a substrate with great potential to produce biogas. However, this type of biomass is characterized by a complex and solid structure, which is difficult to decompose by anaerobic microorganisms. Applying the correct pre-treatment method can increase its biodegradability. Lignocellulosic substrate was pre-treated by soaking in water for one day at room temperature to increase biogas production and monitoring of long-term operation of laboratory models of anaerobic reactors for anaerobic digestion of such pre-treated maize waste was employed. Monitoring results in two reactors, R1 with biogas produced from a substrate soaked in water for one day and R0 with the production of biogas from a substrate mixed with water just before dosing into the reactor, were compared showing positive effect of the pre-treatment method. This was expressed by higher values of biogas production and higher methane content in biogas from the substrate soaked in water for one day. The achieved specific biogas productions during four different phases of reactor operation in reactor R1 were in the range of 190–335 mL g^{-1} of VS (volatile solids) and 101–221 mL g^{-1} of VS in reactor R0. Methane content of biogas during reactor operation was 49.3–55.2% in reactor R1 and 42.5–45.5% in reactor R0. During long-term operation of another reactor, pre-treated maize waste was used as a co-substrate for maize silage, in the ratio of 1:1 based on VS of the substrates proving as a suitable co-substrate for maize silage, as the achieved average value of specific biogas production during reactor operation at OLR (organic loading rate) = 1.75 kg VS m^{-3} d^{-1} was 510 mL g^{-1} of VS and during first 67 days at OLR = 2 kg VS m^{-3} d^{-1} it was 454 mL g^{-1} of VS.

Key words: anaerobic digestion, bioenergy, maize waste, methane, pre-treatment.

INTRODUCTION

The use of lignocellulosic biomass as a substrate for biogas production is important for the development of sustainable energy sources due to its large worldwide production. Lignocellulosic biomass includes energy crops, agriculture and forest residues, sewage sludge, animal and food waste, municipal solid waste etc (Abraham et al., 2020). However, due to its complex structure formed mainly by cellulose, hemicellulose, and lignin, it is difficult to produce biogas (Martínez-Gutiérrez, 2018). Factors affecting the recalcitrance of this biomass to the action of anaerobic microorganisms include structural and compositional properties of lignocellulosic biomass, accessible surface area of cellulose, cellulose polymerization and crystallinity, as well as cross-linkages of hemicellulose and lignin (Xu et al., 2019; Yoo et al., 2020).

Application of a pre-treatment method is the key step in the disintegration of its main components and their subsequent conversion to bioenergy (Mankar et al., 2021). Studied pre-treatment methods include various biological, chemical, and physical methods. Biological pre-treatment methods include pre-treatment by fungi (Mustafa et al., 2016; Singh, 2021), bacterial systems (Xu et al., 2018; Shah et al., 2019), and enzymes (Wang et al., 2018a; Abraham et al., 2020).

Chemical pre-treatment methods, characterized by high efficiency, include the use of lime, acid, ionic liquid, steam explosion, sulfur dioxide explosion, ammonia fiber explosion and others (Norrrahim et al., 2021). Mancini et al. (2018) used alkaline pre-treatment of wheat straw with 1.6% (w/w) NaOH at 30 °C for 24 hours, cumulative biomethane production yield was enhanced by 15% compared to the untreated sample. In Sarto et al. (2019), the use of 5% H_2SO_4 for the pre-treatment of water hyacinth for 60 minutes increased biogas production by 132% compared to the untreated substrate. However, when choosing a suitable chemical method, possible high economic costs associated with the price of the chemical agent, and dangerous properties of some chemical substances such as toxicity, flammability or corrosivity have to be considered together with the production of inhibitors of anaerobic processes and toxic waste, the disposal of which can further increase the economic costs of the used pre-treatment method. In the work of Jankovičová et al. (2022a), significant increases in biogas production were achieved even when using chemical agents at lower concentrations, 0.5% NaOH and 0.5% H_2SO_4. Pre-treatment by 0.5% NaOH increased the biogas production by 159% for rapeseed straw, 240% for wheat straw and by 59% for maize waste. However, the use of reagents even at low concentrations increases the costs and as well as the salinity of the digestate.

Physical processes can salso be used to improve the biodegradability of lignocellulosic materials, including mechanical pre-treatment (Dahunsi, 2019), ultrasound (Wang et al., 2012), and microwave radiation (Kaur & Phutela, 2016). In Dell'Omo & La Froscia (2018), an average yield gain of 50% compared to the untreated material was achieved when the particle size of wheat straw was reduced from a fraction of 30 mm to 300 μm and to 1,200 μm. However, energy requirements and high costs make physical methods disadvantageous. The effect of other pre-treatment methods enhancing solubilization and anaerobic biodegradability of lignocellulosic biomass as wheat straw are described in Rahmani et al. (2022).

The goal is to find an effective pre-treatment method employing eco-friendly reagents, low energy consumption and cost-effective operation. Therefore, the aim of this study was to investigate the effect of simple pre-treatment methods, such as soaking the lignocellulosic biomass in water, on the biodegradability and biogas yield. Efficiency of the chosen pre-treatment method for biogas production was monitored during long-term operation of the reactors, providing good insight into the ongoing anaerobic degradation of the pre-treated lignocellulosic substrate. Since long-term monitoring of the process is applied, adaptation of the biomass to the dosed substrate is considered and various parameters of the digestate are monitored to indicate the inhibition of biogas production.

The studied pre-treated lignocellulosic material was one of the most common materials produced in agriculture, i.e. maize waste (also known as maize straw, corn stover). This material is a valuable source of energy because maize production constantly increases and the total mass of these residual parts of the plant (leaves, stalks, husk) is approximately 1.01 kg of dry matter per 1 kg of harvested maize grains (expressed in dry matter) (Mazurkiewicz et al., 2019). The use of such pre-treated maize waste as a co-substrate for maize silage was also investigated, whether the pre-treated maize waste can replace a significant part of the used maize silage in practice, since maize silage is still used as the main substrate for biogas plants in the European Union. However, this is not a long-term sustainable substrate (Hutňan, 2016).

MATERIALS AND METHODS

Substrate and inoculum

The used lignocellulosic substrate was maize waste, which contains various parts of the plant as stalks, leaves, and husks (except grains), as well as post-harvest residues. The plant was harvested at the stage of full physiological maturity and dry, i.e. with high dry matter content, from agricultural fields in Slovakia. It involved harvesting grain maize, and the residues of the plant (maize waste) was used as material in experiments. Furthermore, the material was stored at room temperature with no special drying in laboratory conditions.

The dry material was disintegrated into two different fractions. One of the fractions was ground with the particle size of 2 mm and the other was cut with the particle size of 1–3 cm. During the long-term operation of one reactor, maize waste was used as a co-substrate for maize silage, which is one of the most used substrates in biogas plants.

Table 1. Characteristics of the used substrates

	TS (g g^{-1})	VS (g g^{-1})
Maize waste	0.94	0.86
Maize silage	0.41	0.39

During the reactor long-term operation, digestate from biogas plant in Hurbanovo (Slovakia) was used as inoculum. This biogas plant uses maize silage as a substrate. As an inoculum during the long-term operation of the reactors, where only pre-treated maize waste was processed, anaerobic sludge from the wastewater treatment plant Devínska Nová Ves (Slovakia) was used. Characteristics of the used substrates are given in Table 1 and those of the used inoculum are given in Table 2.

Table 2. Characteristics of the used inoculum

	TS (g L^{-1})	VS (g L^{-1})
Digestate from biogas plant	30.54	20.37
Sludge from wastewater treatment plant	15.32	9.50

Degradation test

Lignocellulosic substrate, maize waste, was pre-treated using two different methods: boiling maize waste in water, and soaking maize waste in water at room temperature. The ratio of solid to liquid was 1 g of dry maize waste: 50 mL of water in both cases. Particle size of the used substrate was 1 cm. COD (chemical oxygen demand) of initial material was 1.108 g g^{-1}.

During pre-treatment by boiling the substrate in water (95 ± 5 °C), a sample of the liquid portion was taken every 15 minutes for 1 hour and its COD was determined. During pre-treatment by soaking the substrate in water (22 ± 2 °C), a sample of the liquid portion was taken at certain time intervals for 10 days to determine the value of COD of the liquid phase.

To determine the solubility of the material, the parameter degree of solubilization was used according to Penaud et al. (1999). The measurements were performed three times and the results show the average values of the degrees of solubilization obtained from these measurements. The degree of solubilization was calculated according to equation:

$$degree\ of\ solubilization = \frac{COD_{released}}{COD_{initial}}.100\ (\%) \qquad (1)$$

where $COD_{released}$ is COD released from 1 g of maize waste in the liquid phase sample taken at certain time intervals during pre-treatment; $COD_{initial}$ is COD of 1 g of untreated maize waste.

Monitoring of long-term operation of model of anaerobic reactor

The device for monitoring long-term operation of the reactor consists of a closed reactor vessel with an opening for dosing the substrate and with a small opening with a tube connected to a device for measuring the produced biogas, a heating device, and a stirrer with adjustable speed.

Two reactors (R1, R0) for anaerobic digestion of pre-treated maize waste were performed under mesophilic conditions (37 °C) in semi-continuous reactors with a working volume of 1.4 L. As a daily dosed substrate to reactor R1, maize waste pre-treated by soaking in water for one day was used. And as daily dosed substrate to reactor R0, maize waste mixed with water just before dosing into the reactor was used.

Reactors were operated in four different phases, during which the organic loading rates (OLR) of the reactors and the particle size of used substrate changed. Characteristics of the operating phases of the reactors, i.e. the duration of the phases, changing OLR, and the particle size of the used substrate are shown in Table 3. Thus, in phases I and II, the ground fraction of the substrate was used and in phases III and IV, cut fraction of substrate was used.

Table 3. Characteristics of operating phases of reactors R1 and R0

Phase	Duration (days)	Particle size of substrate	organic loading rate (kg VS m^{-3} d^{-1})
I	0–31	2 mm	0.62
II	32–80	2 mm	1.24
III	81–145	1–3 cm	1.24
IV	146–189	1–3 cm	1.24

In the first three phases, the substrate was dosed to reactors R1 and R0 together with water in which it was soaked. In phase IV, only the substrate itself was dosed into reactor R1, i.e. without soaking water, to achieve a similar water content of maize waste as that of maize silage, so that the pre-treated maize waste could be used in practice as a suitable co-substrate for maize silage.

To evaluate the suitability of maize waste pre-treated by soaking in water for one day as a co-substrate for maize silage, long-term operation of the anaerobic reactor was monitored, where anaerobic digestion of pre-treated maize waste as a co-substrate for maize silage was performed under mesophilic conditions (37 °C) in a semi-continuous reactor with the working volume of 15 L. Particle size of the pre-treated maize waste was 1–3 cm and the substrate soaked in water for one day was dosed into the reactor without the soaking water. The reactor was started-up only by dosing maize silage and gradually increasing the OLR up to 1.75 kg VS m^{-3} d^{-1}, and it was operated at two different values of organic loading rate: 1.75 kg VS m^{-3} d^{-1} for 154 days and 2 kg VS m^{-3} d^{-1} for 84 days. These conditions bring the operation of the model closer to the OLR at real biogas plants. Maize waste pre-treated by soaking in water for one day was used as a co-substrate for maize silage in the ratio of 1:1 based on VS of the substrates.

During long-term operations of the anaerobic reactors, substrates were dosed daily and biogas production was monitored. Once a week, parameters of excess digestate such as pH, total solids (TS), and volatile solids (VS) were determined, together with parameters such as chemical oxygen demand (COD), concentration of ammonia nitrogen ($N\text{-}NH_4$), phosphate phosphorus ($P\text{-}PO_4$), and volatile fatty acids (VFA), which were determined from the filtered digestate sample. Methane content of biogas was determined also in each monitored phase.

Analytical methods

Parameters such as chemical oxygen demand (COD), ammonia nitrogen ($N\text{-}NH_4$), phosphate phosphorus ($P\text{-}PO_4$), volatile fatty acids (VFA), total solids (TS) and volatile solids (VS) were determined according to APHA, AWWA, WEF (2017); pH values were determined using pH meter Hach HQ11d. Biogas composition was measured by gas analyzer GA 2000 Plus (Geotechnical Instruments, UK).

RESULTS AND DISCUSSION

Degradation test

Regarding the economic difficulty of pre-treatment by boiling in water (heat consumption), the possibility of soaking in water at room temperature was tested. In the degradability test, both pre-treatment methods of lignocellulosic biomass were compared based on their degree of solubilization.

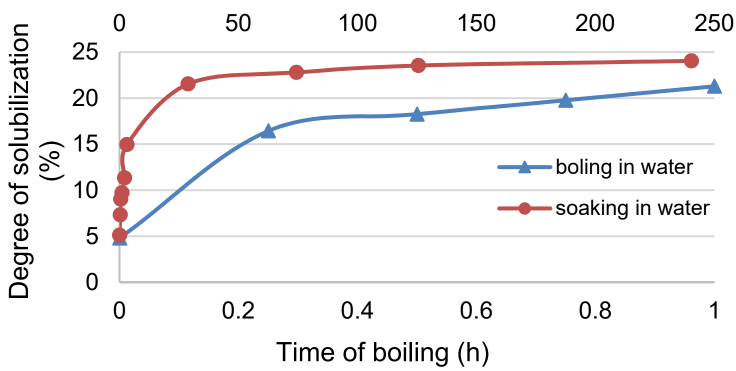

Figure 1. Course of the degree of solubilization during maize waste pre-treatment.

From the courses of the degree of solubilization during the pre-treatment shown in Fig. 1, a comparable course of the degree of solubilization in the selected methods is evident. It is obvious that degradability of the material increased with the increasing pre-treatment time for both chosen methods, as the values of the degree of solubilization increased with pre-treatment time of both methods.

The fastest increase in the solubilization degree in case of boiling in water was observed in the first 15 min, when the achieved value was 16.5%. After boiling maize waste in water for 1 hour, the degree of solubilization was 21.3%.

Of course, soaking maize waste in water at room temperature requires much longer pre-treatment time. The most significant solubilization of the material was observed in the first days of soaking. After approximately 1 day (29 hours) of soaking, the degree of solubilization in water was 21.5%. With longer time, the material decomposed gradually at much lower speed. After 10 days of soaking, the degree of solubilization was measured to be 24.1%.

Similar degrees of solubilization on carbon basis (approximately 25%) were achieved by Rogalinski et al. (2008), where the liquid hot water method (100 bar, 190 °C, 12 min) in combination with other methods was used for the pre-treatment of rye straw. However, the yield of unwanted degradation products, as HMF and furfural, increased with the treatment severity.

As soaking the material at room temperature for one day resulted in the same solubilization degree as when boiling the material for 1 hour, the method of soaking lignocellulosic biomass in water at room temperature was used in further experiments. This method does not have high energy demand and therefore it represents a simpler and more advantageous method in practice.

Long-term operation of anaerobic reactors for anaerobic digestion of pre-treated maize waste

The effect of the selected pre-treatment method, soaking maize waste in water for one day, was observed by monitoring biogas production in reactor R1 during long-term operation. To assess whether this chosen method has a positive effect on biogas production, the long-term operation of reactor R0 was also monitored, where the dosed maize waste was mixed with water just before its dosing into the reactor. Monitoring results are presented as cumulative biogas production in Figs 2–5, which are divided into individual graphs for each phase of operation for better representation of the differences in biogas production for reactors R1 and R0. Average value of cumulative biogas production, daily biogas production, specific biogas production and methane content of produced biogas are reported in Table 4.

Table 4. Results of long-term operation of reactors R1 and R0

Phase	Cumulative biogas production (L)		Daily biogas production (L d^{-1})		Specific biogas production (L g^{-1} VS)		Methane content (%)	
	R1	R0	R1	R0	R1	R0	R1	R0
I	6.73	2.81	0.166	0.086	0.192	0.101	49.3	42.5
II	41.29	19.21	0.597	0.308	0.335	0.173	49.3	42.5
III	61.24	23.07	0.515	0.261	0.296	0.150	55.2	43.7
IV	17.90	22.36	0.503	0.383	0.289	0.221	51.1	45.5

The initial phase of operation of the reactors (at OLR = 0.62 kg VS m^{-3} d^{-1}) lasted 32 days. During the first days, the inoculum in the reactors adapted to the used substrates, which can be seen in the almost zero biogas production in Fig. 2. It is clear from the previous results that the pre-treatment by soaking maize waste in water causes solubilization of the material and the release of the organic part of the solid material into the solution thus simplifying the availability of substances necessary for anaerobic digestion. A significantly positive effect was observed when using the substrate soaked in water for one day (R1) compared to the substrate mixed with water immediately before its dosing into the reactor (R0). Biogas production from the substrate soaked for one day was by about 140% higher compared to the substrate in reactor R0.

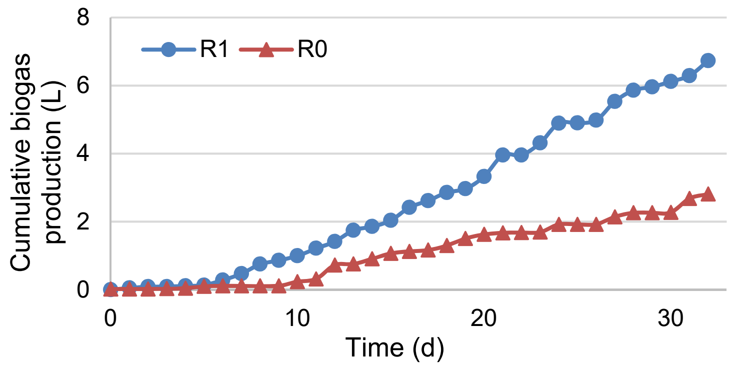

Figure 2. Cumulative biogas production during phase I of long-term operation of reactors R1 and R0.

After 32 days of operation, OLR in the reactors was doubled (1.24 kg VS m^{-3} d^{-1}). Pre-treatment conditions remained unchanged. Similar to the lower OLR, an almost 120% increase in biogas production was observed for the one-day-soaked substrate. When comparing the values of specific biogas production, 0.335 L g^{-1} VS for R1 and 0.173 L g^{-1} VS for R0, an increase of 94% was observed.

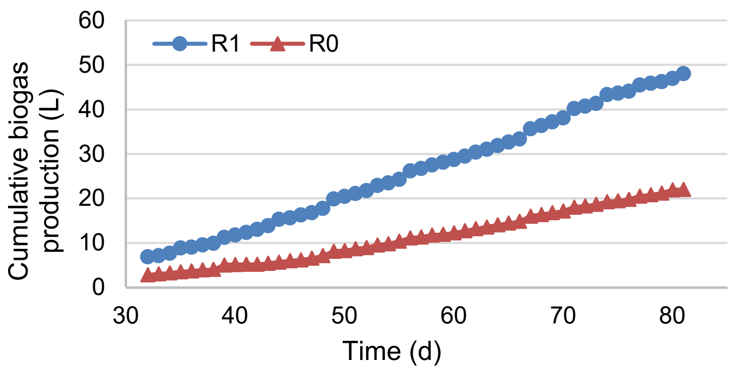

Figure 3. Cumulative biogas production during phase II of long-term operation of reactors R1 and R0.

Although the fraction with smaller particle size has increased accessible surface area and porosity of the particles, the process of mechanical disintegration of the material is energetically and economically demanding (Hernández-Beltrán et al., 2019). Due to the difficulty of maize waste pre-treatment by disintegration and to compare the effect of material disintegration on biogas production, the cut fraction of maize waste (1–3 cm) was used in phase III. A slight decrease in daily biogas production was observed compared to phase II. The use of a larger fraction of the lignocellulosic substrate decreased the value of specific biogas production in phase III by only 13% for R1 and by 15% for R0 compared to phase II, where a smaller fraction was used. However, the increase in specific biogas production in R1 (0.296 L g^{-1} VS) compared to R0 (0.150 L g^{-1} VS) is still quite high, up to 98%.

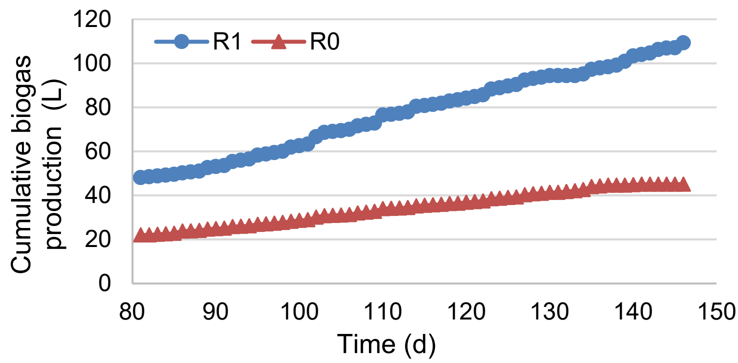

Figure 4. Cumulative biogas production during phase III of long-term operation of reactors R1 and R0.

In the last phase, when only the solid phase of the substrate soaked in water for one day was dosed into reactor R1, the water content of the substrate (80–85%) was comparable to that of maize silage (about 70%) (Şenol et al., 2020). The use of solid substrate without soaking water in phase IV was done to achieve water content of maize waste similar to that of maize silage to use pre-treated maize waste as a suitable co-substrate for maize silage. Thus, in phase IV, the closest approach to real conditions was used. Also, when dosing substrate with soaking water into the reactor, HRT (hydraulic retention time) decreased, which is a disadvantage. Even when processing maize silage at biogas plants, which has even higher TS than soaked maize waste without soaking water, no technological problems were observed. From the achieved value of SBP, 0.289 L g^{-1} VS, in phase IV for reactor R1, it is clear that part of well degradable organic matter available for biogas production was released into the soaking water; however, this amount is small. Also, in full scale conditions, soaking water would not be disposed of but reused for soaking another batch of substrate (with the necessary addition of fresh water). Organic substances released into the soaking water would therefore be present when soaking the following new batch of substrate the next days.

Gradual dosing of only the solid phase into reactor R1 had an inhibitory effect. The substrate was more difficult to decompose, which can also be seen in the qualitative parameters of digestate and liquid phase of digestate. Slowdown in the biogas production rate, as seen in Fig. 5, led to the shut-down of the operation of R1.

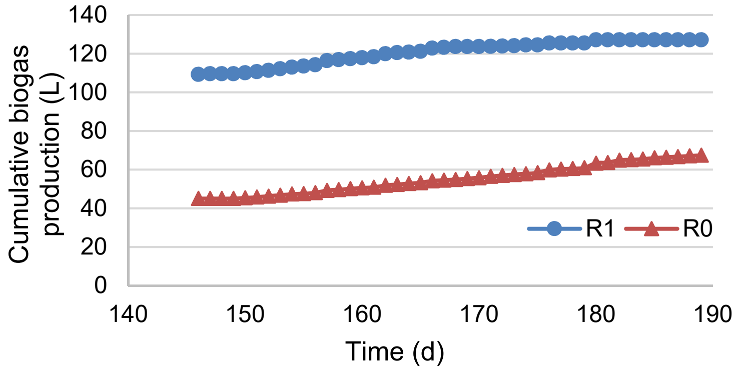

Figure 5. Cumulative biogas production during phase IV of long-term operation of reactors R1 and R0.

As it can be seen in Table 4, higher methane content of biogas was achieved in reactor R1 compared to reactor R0 in each phase of operation. The table shows average values of methane content during the phases; however, at the end of phase IV, the methane content in reactor R1 was only 45.2%.

Monitoring of digestate and liquid phase of digestate water parameters showed that the reactors were operated at a neutral pH value, i.e. at pH values of 6.7–7.5. Concentrations of N and P were sufficient for the COD:N:P ratio to be considered suitable for anaerobic digestion, COD:N:P = 500:7:1 (Gil et al., 2019). When maize waste is used as a mono-substrate for biogas production, deficiency of selected micronutrients cannot be excluded. As stated in the work of Lebuhn et al. (2014), synergistic detrimental effects on biocenosis cannot be excluded if several trace elements concomitantly approach limiting levels at anaerobic treatment of maize silage. However, as sludge from the municipal wastewater treatment plant was used here as inoculum in reactors R1 and R0, it is assumed that no micronutrient deficiency occurred during the experiments and no additional addition was done during the experiment. However, this can be a problem in long-term use in practice.

The change in operating parameters of the reactors such as OLR and the size of the substrate particles affect mainly the change of parameters such as COD (Fig. 6) and VFA (Fig. 7). The OLR increase in phase II led to an almost double increase in COD. In R1, the average value of COD in phase I was 540 mg L^{-1} and in phase II it increased to 1,139 mg L^{-1}. A similar increase in COD also occurred in R0, from 566 mg L^{-1} to 915 mg L^{-1}. VFA concentration varied during phases I and II in similar values for both reactors, i.e. between 100-200 mg L^{-1}. By changing the size of the substrate particles in phase III, the material decomposed more slowly and accumulated in the reactors, which was also reflected in the COD values, which increased by 80% in R1 and 110% in R0. Average VFA concentration after changing the size of the substrate particles also increased to 426 mg L^{-1} (R1) and 433 mg L^{-1} (R0), which represents an increase of 156% and 168%. During phase IV, concentrations of COD and VFA continued to increase but a more visible increase was observed in R1 when only the solid phase of the one-day-soaked substrate was used. Gradual densification of the reactor volume of R1 led to problems with operation and its subsequent termination.

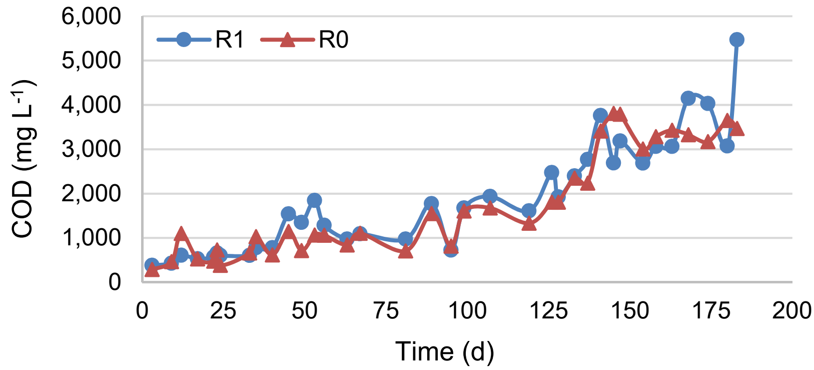

Figure 6. Variation of COD concentration during operation of reactors R1 and R0.

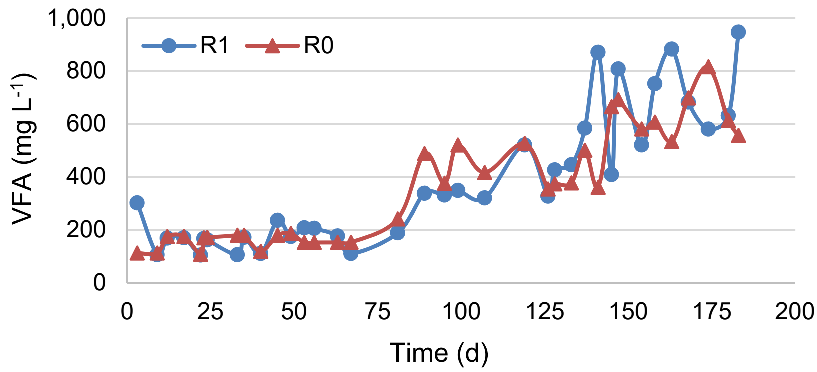

Figure 7. Variation of VFA concentration during operation of reactors R1 and R0.

Average values of digestate parameters as TS and VS did not change significantly during individual phases (Table 5). By increasing OLR and the particle size of the substrate, the substrate was more difficult to decompose and a part of the material remained undecomposed in the reactor, which was reflected in the change in the composition of the collected digestate, which contained more rigid undissolved parts. The solid undissolved material apparently settled at the bottom of the reactor, which also causes occasional jamming of the stirrer. Towards the end of phase IV in reactor R1, TS values reached up to 23–24 g L^{-1}.

Table 5. Average values of TS a VS of digestate during operation of reactors R1 and R0

Phase	TS (g L^{-1})		VS (g L^{-1})	
	R1	R0	R1	R0
I	14.1	12.7	9.1	8.3
II	17.4	15.2	12.3	10.1
III	16.4	18.2	10.9	12.1
IV	19.5	18.6	15.1	14.9

One of the reasons for substrate accumulation in the reactor was the technical limitation of the reactor model, where the only possible method of digestate removal from the reactor - from the reactor surface, contributed to the constant increase in the digestate density since the more liquid part of the digestate was removed.

Since in phase IV, only the solid part of the substrate was loaded to reactor R1 and the solid and liquid part was dosed to rector R0, more digestate was taken from reactor R0 than from R1. This increased OLR in R1. The undecomposed maize waste was also removed from R0, but this undecomposed material remained in R1, which significantly increased the retention time so that it could decompose more deeply. Thus, the collapse of R1 could be caused by a substantial extension of the retention time, when the accumulated substrate could begin to decompose. However, mainly the phases where hydrolysis and acidogenesis of anaerobic decomposition took place, which was manifested by an increase in VFA.

Positive effect of soaking lignocellulosic biomass in water at room temperature for one day was demonstrated as higher biogas production in reactor R1 in each phase of operation. Wang et al. (2018b) pre-treated rice straw hydrothermally at different temperatures (90 °C, 150 °C, 180 °C and 210 °C) achieving biogas yield increase of only 3% compared to untreated rice straw. Even a 30% decrease in biogas yield at 210 °C was observed as severe inhibitions appeared at higher pre-treatment temperatures.

Long-term operation of anaerobic reactor for anaerobic digestion of pre-treated maize waste as co-substrate for maize silage

Results of long-term operation of the reactor for anaerobic digestion of such pre-treated maize waste as co-substrate for maize silage are shown as the course of SBP (specific biogas production) (Fig. 8) during the operation of this reactor at two different values of OLR.

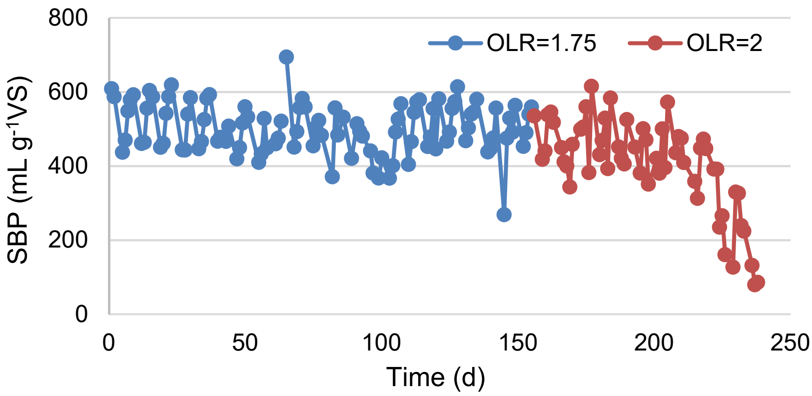

Figure 8. Specific biogas production during long-term operation of the reactor.

At OLR of 1.75 kg VS m^{-3} d^{-1}, anaerobic digestion of these co-substrates was characterized by stable operation. Average value of daily biogas production was 13.4 L d^{-1} and that of specific biogas production was 510 mL g^{-1} VS. Methane content of biogas was 54%. Specific biogas production has proven the suitability of using such pre-treated maize waste as co-substrate for maize silage. Specific biogas production during long-term operation of the reactor for mono-digestion of maize silage given in literature ranges from 479 mL g^{-1} VS (Jankovičová et al., 2022b) up to the maximum of 655 mL g^{-1} VS in Hutňan et al. (2010).

At OLR of 2 kg VS m^{-3} d^{-1}, the reactor operation was relatively stable for the first 67 days, then operational problems occurred, which further led to the termination of monitoring due to reactor overload, as it can be seen in Fig. 8. During the first 67 days, average value of daily biogas production was 13.6 L d^{-1} and that of specific biogas production was 454 mL g^{-1} VS. Methane content of biogas was also lower in comparison with that obtained at a lower OLR, namely 51%.

Performance of the anaerobic digestion process was also monitored via the changes in digestate liquid phase (filtered sample) parameters. At OLR of 1.75 kg VS m^{-3} d^{-1}, COD values ranged between 2,000–4,000 mg L^{-1}, $N\text{-}NH_4$ between 200–400 mg L^{-1}, and $P\text{-}PO_4$ was in the range of 20–50 mg L^{-1}. At OLR of 2 kg VS m^{-3} d^{-1}, COD values ranged between 3,000–5,000 mg L^{-1}, $N\text{-}NH_4$ was in the range of 300–600 mg L^{-1}, and $P\text{-}PO_4$ in the range of 25–70 mg L^{-1}. Therefore, N and P concentrations were sufficient for the COD:N:P ratio to be suitable for anaerobic digestion. Even in this case, micronutrients were not supplied since the digestate from the biogas plant, used as the inoculum in this reactor, supplies these micronutrients during the maize silage processing.

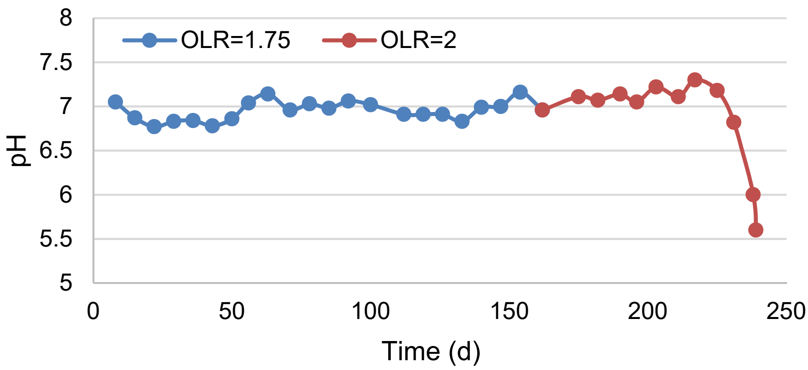

Figure 9. Variation of pH during long-term reactor operation.

For proper process operation, the optimum operating pH range for maximum methanogens growth is above 6.8 (Björnsson et al., 2000; Azizan et al., 2021). Fig. 9 shows pH changes during the reactor operation, while at OLR = 1.75 kg VS m^{-3} d^{-1} there were no significant changes in pH, at OLR = 2 kg VS m^{-3} d^{-1}, pH began to decrease from approximately the 220th day of operation - i.e. similar to the observed decrease in biogas production. The achieved low pH values, up to 5.6 when the reactor operation stopped, thus indicated reactor overload. Similarly as in Braz et al. (2019), pH values below 6 were observed after organic overload. Drastic pH drop was caused by the accumulation of VFAs, acidogenic and acetogenic bacteria, which reflects kinetics imbalance between acid production and consumption rate. Over-acidification/souring problem is the most common issue in the anaerobic digestion process (Alavi-Borazjani et al., 2020). Rapid accumulation of VFAs is shown in Fig. 10.

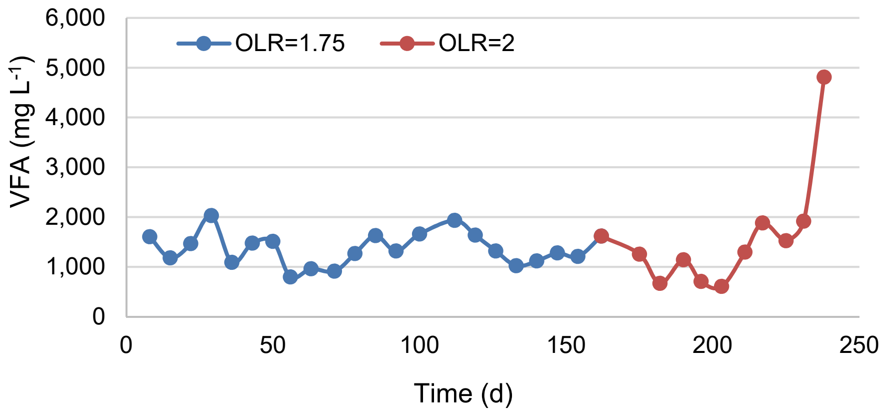

Figure 10. Variation of VFA concentration during long-term reactor operation.

Reactor overload can be caused by the accumulation of substrate at the bottom of the reactor. Accumulation of undecomposed material is also confirmed by the increase in TS and VS values of the digestate shown in Fig. 11.

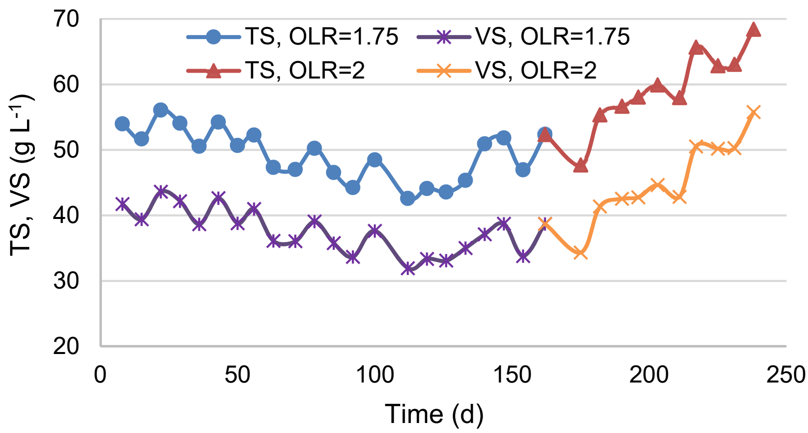

Figure 11. Variation of TS and VS concentration during long-term reactor operation.

CONCLUSIONS

The studied pre-treatment method, soaking lignocellulosic biomass at room temperature in water for one day, has been proven to have great potential in increasing biogas production from lignocellulosic materials such as maize waste. Higher values of cumulative biogas production, daily biogas production, specific biogas production, and higher methane content in biogas prove that pre-treatment of maize waste by soaking in water for one day has a positive effect on biogas production compared to maize waste mixed with water just before its dosing into the reactor. Based on the achieved average value of specific biogas production during the reactor operation at OLR of 1.75 kg VS m^{-3} d^{-1}, 510 mL g^{-1} VS, maize waste pre-treated by this method has been found to be suitable co-substrate for maize silage. Also during the first 67 days of reactor operation at OLR of 2 kg VS m^{-3} d^{-1}, sufficiently high average value of specific biogas production was achieved (454 mL g^{-1} VS), but at this higher OLR, technological problems associated with reactor overload occurred.

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