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CO-DIGESTION OF WASTEWATER SLUDGE WITH FOOD WASTE AND GREEN CUTTINGS: OPTIMIZATION OF METHANE PRODUCTION

KODIGESTIJA BLATA IZ ČISTILNE NAPRAVE Z ODPADNO HRANO IN ZELENIM ODREZOM: OPTIMIZACIJA PROIZVODNJE METANA

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Abstract

This article outlines our investigation into the methane production of wastewater sludge (WWS) from a wastewater treatment plant (WWTP) and a mixture of WWS with food waste (FW) and green cutting (GC). To determine the optimal mixture, two methane potential experiments were performed using the Automatic Methane Potential Test System (AMPTS II). In the first experiment, WWS and FW were used. The highest methane potential was measured in FW, and the lowest in WWS. The combination of both substrates did not approach the methane potential of FW (241.5 ± 15.7 mL CH4/g VS). In second experiment, we combined WWS and GC. The highest methane potential was obtained at 5.1% addition of GC to the WWS (relative to the organic load). This methane potential was 7.5% higher than the methane potential of WWS, which was 470 ± 17 mL CH4/g VS. We calculated the optimal mixture of both experiments using the simplex lattice design method. In experiment one, the model had relatively good fit to the measured values, however in the second experiment the differences were significant.

Keywords: anaerobic digestion, methane potential, wastewater sludge, green cutting, food waste.

Izvleček

V članku smo raziskovali proizvodnjo metana blata iz čistilne naprave (ČN) in mešanice blata z odpadno hrano in zelenim odrezom. Da bi določili optimalno mešanico, smo izvedli dva eksperimenta metanskih potencialov z uporabo Automatic Methane Potential Test System (AMPTS II). V prvem eksperimentu, kjer smo uporabili blato iz ČN ter odpadno hrano, smo največji metanski potencial izmerili pri odpadni hrani, najmanjšega pa pri blatu iz ČN. Kombinacija obeh substratov metanskega potenciala ni poslabšala, ampak se je približala metanskemu potencialu odpadne hrane (241,5 ± 15,7 mL CH_4/g VS). V drugem eksperimentu smo kombinirali blato in zeleni odrez. Največji metanski potencial smo dobili pri 5,1% dodajanju zelenega odreza k blatu (glede na organsko obremenitev). Ta metanski potencial je bil večji za 7,5% od metanskega potenciala blata iz ČN, ki je znašal 470 ± 17 mL CH_4/g VS. Z metodo simplex lattice design smo preverili optimalno mešanico obeh

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eksperimentov. Pri prvem eksperimentu se je model približal izmerjenim vrednostim, pri drugem pa so bile razlike velike.

Ključne besede: anaerobna presnova, metanski potencial, blato, zeleni odrez, odpadna hrana.

1. Introduction

During wastewater treatment, the process properties of primary and activated sludge differ from one another, just as wastewater properties differ from one municipality to another (Turovskiy and Mathai, 2005). The amount of wastewater sludge (WWS) that is produced onsite at wastewater treatment plants and is sent for incineration poses a significant financial burden on wastewater operators and the environment (Bougrier et al., 2006). The costs of sludge disposal can reach up to 60% of total wastewater treatment plant operation costs (Parawira, 2012; Xu et al., 2011). WWS is produced in the mechanical stage of treatment and in the biological processes of wastewater treatment, where microorganisms are needed to degrade the soluble components.

Anaerobic digestion is one of the possibilities for stabilizing and reducing the amount of wastewater sludge through biochemical process, where microorganisms transform and degrade organic compounds into smaller and simpler organic compounds and biogas. Biogas is a mixture of methane, carbon dioxide, water vapor, and trace gases such as hydrogen sulfide, free ammonia, and nitrogen. The organic matter is transformed in four main steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis, where hydrolysis is the rate-limiting step. The substrate degradation rate and hydrolysis are dependent on various environmental factors, such as pH, temperature, and the substrate's chemical composition. Further complex organic substrates can contain anaerobically non-biodegradable or poorly degradable compounds, such as lignin and keratin. Further larger substrates will degrade more slowly, because of the smaller surface area compared to the volume of substrate (Carlsson et al., 2012). The rate of substrate conversion to methane is given by the degradation extent (f_d) which indicates the degradability of the complex feed and hydrolysis rate coefficient (k_{hyd}), which estimates the rate of conversion (Jensen et al., 2014, 2011)

In anaerobic digestion, substrates are classified into five categories (i) OFMSW (organic fraction of municipal solid wastes); (ii) organic waste from the food industry; (iii) agricultural energy crops; (iv) manure, and (v) wastewater sludge (Carlsson et al., 2012; Murovec et al., 2015).

The use of energy crops and arable land for renewable energy has met a negative response in most EU countries due to food security and poor food self-sufficiency. It is thus important to replace energy crops and find other resources for substrates to lower greenhouse gas emissions and focus on waste resources (Voinov et al., 2015). Grass and green cuttings from roadsides are a potentially energetically unused source of organic material. Currently they are mulching instead of being harvested and energetically reused (Piepenschneider et al., 2015), which would lead to an additional substrate for biogas production and help reduce dependence on natural gas. However roadside grass and green cuttings can be polluted by traffic hazardous emissions, heavy metals such as iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), lead (Pb), chromium (Cr), nickel (Ni), cadmium (Cd), and cobalt (Co), and other negative impacts that interfere with normal plant growth (Voinov et al., 2015). Due to the high variability in the metal concentrations present in various plants, as well as differences in sampling time and soil, it is difficult to interpret the measured hazardous substances from roadside grasses (Piepenschneider et al., 2015).

Food waste is another co-substrate, which is increasingly used in biogas production. In the EU, around 89 million tons of food waste is produced annually, which is equal to 179 kg of food waste per capita (Bräutigam et al., 2014). Several authors report benefits from the co-digestion of several substrates, with the goal of improving anaerobic digestion and biomethane yield, diluting potential toxic compounds, and yielding synergetic effects to improve the environment for microorganisms, the nutrient balance, and the reduction of solids (Sosnowski et al., 2003).

To meet the future energy needs, we assessed the potential use of sewage sludge with green cutting at an agricultural biogas plant due to the substrate deficit and easy transition from crop usage to the other morally acceptable substrates. Second, food waste co-digestion with WW sludge was explored on laboratory batch bench scale reactors for the improvement of anaerobic digestion at municipal wastewater treatment plants. Third, the optimal ratio of feeding substrates was determined in laboratory batch reactors and was tested using a mixture design of WWS, GC, and FW and WWS.

2 Materials and methods

2.1 Inocula and substrates

Two batches of inocula for the experiments were collected, first from the 50,000 PE municipal wastewater treatment plant (WWTP) in Šaleška Valley and second from the agricultural biogas plant in Šijanec. Wastewater treatment at WWTP includes primary and secondary activated sludge treatment. Excess sludge has an average total solids content between 2% and 4%. There are 2 anaerobic digesters with a volume of 960 m^3 and operating under mesophilic temperature conditions at a temperature of 35 to 37 °C. The biogas produced is burned by a 150 kW biogas engine, with the generated heat used to heat anaerobic digesters.

For the first experiment, the WWTP inoculum was taken from first anaerobic digester, while wastewater samples were taken from the sludge thickener just before the mixture of primary and secondary sludge entered the anaerobic digester. Afterwards, the samples were transported to the Keterlab laboratory, located at the biogas plant in Vučja Vas, and were immediately analyzed and stored at 4 °C. For the second experiment, the inoculum from the Vučja Vas agricultural biogas plant was collected through the plumbing loop system as described before (Kolbl et al., 2014). The characteristics of input inocula are presented in Table 1.

Food waste was separately collected from nearby households. The roadside grass was collected from RCERO Puconci (Slovenia) and was previously processed with a Mashmaster mixer (Komptech); the particle size of the green cut treated in this way was less than 25 mm. Immediately after collection, the green cuttings were placed in plastic bags and brought to the laboratory for further analysis.

2.2 Batch experiments

The differences in WWS and the inoculum affects the methane production rate and methane potentials. The number of BMP reactors that were available in the laboratory was limited. As the duration of BMP takes between 20 to 45 days (depending on the daily methane production), it is not good practice to store the same WWS batch. The WWS in that case would undergo degradation and transformation of its biochemical/rheological properties, losing its VS. Further, the properties of WWS at WWTP are constantly changing/fluctuating, depending on the wastewater properties (wastewater also from industry), which influences the anaerobic digestion process. This made the sampling and measuring of two different batches of WWS the best available option. Freezing WWS changes its properties by breaking the WWS flocs matrix and the cell walls of the microbes present, thus releasing the intracellular matter and act as a pretreatment. This is also not recommended/good practice. Therefore, two experiments were carried out.

Alternative substrates to crops for use in biogas production were used to determine the methane potentials, using the same organic loading rates as in the WWTP Šaleška Valley reactors (2 to 3 g VS/L): WWS and food waste (experiment 1), and as at the Šijanec biogas plant (6 to 8 g VS/L): roadside grass and WWS (experiment 2). Positive (glucose) and negative controls (only inoculum) were included in both experiments. In both experiments, 5 L reactors operated under operating conditions as in biogas reactors in

WWTP. Pure substrates and their combinations are given in tables 2 and 3. A total of 30 reactors of 5 L were used. Combinations were based on the VS of each substrate, relative to the total organic load of 2.35 g VS/L for experiment 1 and 7.05 g VS/L for experiment 2.

The TS and VS of the substrates and the inoculum were determined according to the APHA (APHA, 2005) method. N_{total} and NH_4^+-N were determined by a SANplus Continuous Flow Analyzer (Skalar Analytical B.V., Netherlands) as described before (Kolbl et al., 2017).

The biogas titrator TitraLab AT1000 Series (Hach) was used to determine the volatile organic acids (VOA), total inorganic carbon (TIC), and VOA/TIC ratio. A HQ40D (Hach Lange) multimeter was used to measure pH.

Table 1: *Characteristics of input substrates and inocula.*
Preglednica 1: *Izmerjeni vhodni parametri substratov in inokulumov.*

Parameter	Experiment 1			Experiment 2		
	WW sludge	Food wastes	Inoculum WWTP	Green cuttings	WW sludge	inoculum BGP Šijanec
TS (%)	2..44 ± 0.015	32.74 ± 0.60	2.21 ± 0.01	25.74	3.53 ± 0.04	8.42 ± 0.11
VS (%)	71.20 ± 0.02	79.18 ± 0.98	55.08 ± 0.32	82.04	73.02 ± 0.27	67.82 ± 0.27
VS (% FM)	1.74 ± 0.12	25.92 ± 3.81	1.22 ± 0.13	21.12	2.58 ± 0.04	5.77 ± 0.10
pH	6.81 ± 0.01	-	7.53 ± 0.02	-	6.78 ± 0.01	8.01 ± 0.01
VOA (mg /L)	3553 ± 214	-	2777 ± 123	-	4243 ± 32	7030 ± 46
TIC (mg/L)	564 ± 41	-	5671 ± 48	-	252 ± 10	30202 ± 630
VOA/TIC	6.343 ± 0.8	-	0.490 ± 0.026	-	16.837 ± 3.2	0.233 ± 0.073
N_{total} (mg/L)	-	-	5671 ± 38	-	6629 ± 65	6710 ± 250
NH_4^+-N (mg/L)	-	-	2277 ± 61	-	756 ± 24	-

Table 2: *Dosing of substrates to the anaerobic reactors.*
Preglednica 2: *Doziranje substratov v anaerobne reaktorje.*

Substrate	**Organic loading (g VS/L)**	**Substrate dosing (mL or g)**	**Water (mL)**
	Experiment 1- Batch 1		
Inoculum (batch 1)	0	3000 mL	0
Glucose (Sigma Aldricht)	2.35	7.1 g	150
WWS	2.35	405 mL	0
Food waste (FW)	2.35	27.2 g	170
WWS + FW	1.175 + 1.175	13.6 g FW and 202 mL WWS	85
	Experiment 2 - Batch 2		
Inoculum (batch 2)	0	3000 mL	0
GC	7.05	29.6 g	188
WWS + GC	3.525 + 3.525	135.7 mL + 16.7 g	95
WWS + GC	5.28 + 1.77	203.6 mL + 7.4 g	32
WWS + GC	5.99 + 1.06	230.7 mL + 4.4 g	11
WWS + GC	6.35 + 0.70	244.3 mL + 3 g	0
WWS + GC	6.69 + 0.36	257.9 mL + 1.5 g	0

Table 3a: *Experimental design tested in the simplex centroid mixture design of experiment one.*
Preglednica 3a: *Eksperimentalna zasnova mešanice za simpleksno centroidno zasnovo mešanice prvega eksperimenta.*

Independent variable, x_{ij}		Dependent variable, Y
FW (X_1)	WWS (X_2)	BMP (mL CH_4/g VS) - Y
1	0	292.0
1	0	308.9
1	0	355.1
0	1	239.6
0	1	248.2
0	1	256.7
0.5	0.5	229.7
0.5	0.5	244.7
0.5	0.5	259.7

Table 3b: *Experimental design tested in the simplex centroid mixture design of experiment two.*
Preglednica 3a: *Eksperimentalna zasnova mešanice za simpleksno centroidno zasnovo mešanice drugega eksperimenta.*

Independent variable, x_{ij}		Dependent variable, Y
WWS (X_1)	GC (X_2)	BMP (mL CH_4/g VS) - Y
1	0	453
1	0	470
1	0	487
0	1	136
0	1	154
0	1	172
0.5	0.5	282
0.5	0.5	299
0.5	0.5	316
0.75	0.25	237
0.75	0.25	280
0.75	0.25	323
0.85	0.15	247
0.85	0.15	275
0.85	0.15	303
0.9	0.1	297
0.9	0.1	321
0.9	0.1	345
0.95	0.05	480
0.95	0.05	507
0.95	0.05	528

2.3 Mixture design and analysis

A simplex lattice design was applied to test the mixtures. Simplex designs are usually used to study the effects of mixture components on the response variable. One set of two different mixture pairs (experiment 1 and experiment 2) was used in the two-separate analysis. Two independent variables and one response were used in calculating the linear and quadratic model for both experiments (Table 3a and 3b). The JMP statistical software was used. The standard forms of equations used are as follows in equations (1) and (2) (Song et al., 2021; Wang et al., 2013):

Linear:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 \quad (1)$$

Quadratic:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 \quad (2)$$

where Y represents the predicted response of biomethane potential, X_1 is the proportion of organic loading of substrate 1, and X_2 is the proportion of the organic loading of substrate 2. β_0 is a constant, β_1 and β_2 are linear coefficients, and β_{12} is the interaction coefficient.

The models' adequacy was assessed by the JMP statistical software. The comparison was based on the lack of fit p-values and regression coefficient R^2.

Tables 3a and 3b show the design used in the simplex centroid mixture in experiments 1 and 2.

3 Results and discussion

Th anaerobic digestion assay of FW, WWS, and their combinations (experiment 1) was run for 32 days. In all cases, the majority of methane was produced in the first 3 days, after which the methane production rate was slowly lowered. The highest methane yield was achieved by FW and reached 241.5 ± 15.7 mL CH_4/g VS (Figure 1). WWS methane yield was lower compared to the FW (165.9 ± 1 mL CH_4/g VS). In the second experiment, the highest methane production was in the reactors, where we had 95% of the organic matter of the WWS and 5% of the organic matter GC. In this case, the methane potential of WWS was 470 ± 17 mL CH_4/g VS (Figure 2). The best co-digestion combination in experiment 2 was 6.6975 g VS of WWS + 0.3525 g VS of GC and it achieved a methane yield of 505 ± 24 mL CH_4/g VS, which was the highest methane yield in experiment 2. All other co-digestion combinations had significantly lower biomethane potential, ranging between 264 ± 27 and 309 ± 43 mL CH_4/g VS. The lowest BMP of 148 ± 30 mL CH_4/g VS was measured for GC. GC contains a larger proportion of lignin, which is anaerobically non-degradable, which is why the BMP is also lower. In all cases, the most methane was produced during the first 8 to 10 days, after which the daily methane production began to decline and level off. This generation of methane is slightly more gradual compared to the first experiment, where most of the methane was generated already in the first five days. This could be attributed to the different source of inocula, organic loading rate and different batches of WWS, since the structure and characteristics of wastewater sludge may vary (Kolbl et al., 2014; Murovec et al., 2015). WWS is a complex substrate, consisting of particles, microorganisms, and extracellular polymeric substances (EPS), which are being produced and excreted by microorganisms (Parawira, 2012). The main forces that glue the components of the WWS flocs into a 3D matrix are van der Waals forces, hydrophobic interactions, and polymer bridging via various cations due to electrostatic bonding with bivalent cations. The main components of EPS are carbohydrates and proteins, but in smaller quantities lipids, nucleic acids, and humic compounds are also found there. Different organic macromolecules in the EPS matrix have different potentials to bind each other into the floc's matrices (Nielsen et al., 1996). The operation of anaerobic digestion is usually evaluated as the reduction of organic matter. The amount of organic matter that will be decomposed and converted into biogas depends on the sludge characteristics, temperature and organic load (Braguglia et al., 2011).

The lower BMP of the GC was expected due to the structure of this substrate, since it is the lignin that slows down the process of anaerobic metabolism. Lignin acts to prevent the degradable part of the substrate from being hydrolyzed (Hendriks and Zeeman, 2009).

The contents of dry matter, organic matter, and pH in experiment 1 after 34 days did not differ significantly between the individual reactors (Figure 3). The pH was in the range of 7.5, the content of TS around 4.2%, VS (% TS) around 65% and VS (% fresh matter (FM)) around 2.8. No significant differences were found when measuring VOA, TIC, and VOA/TIC (experiment 1). The lowest concentration of volatile organic acids (VOA) was in reactors with food waste. The larger standard deviation most likely occurred due to the very nature of the food waste, which contains various forms of accessible food for microorganisms and contains larger particles compared to liquid sewage sludge. A drawback of FW is that it can also contain unwanted inorganic substances, such as plastic, wood, and metals. These unwanted substances can damage pumps, clog pipes, release toxic substances in anaerobic reactors, etc. Therefore, before using FW, it is necessary to remove such substances. A higher VOA/TIC ratio in all cases suggests that, on a realistic full scale, the organic loading should be slightly reduced to avoid oversaturation. As a result, the balance between the microorganisms could be disrupted, the hydrolysis process would proceed more slowly, and acids would begin to accumulate. That would lead to a drop in pH and thus the death of methanogenic microorganisms. As a result, methane formation would worsen or even stop.

The lowest concentration of nitrogen and ammonium nitrogen after 32 days was in the biomass (WWTP inoculum), a little increased in the other anaerobic reactors, which is shown in Figure 3. There were no significant differences in nitrogen and ammonium nitrogen concentrations between the reactors. The highest concentration of ammonium nitrogen was measured in WWS (607 mg/l). The concentrations of NH_4^+-N were below the 1500 mg/l, which is toxic to microorganisms.

In experiment 2, the concentrations of ammonia, nitrogen, and pH were higher than in experiment 1 (Figure 4). Consequently, an increased concentration of free ammonia NH_3 was observed. This can hinder the activity of methanogenic archaea and thereby reduces methane production. Also, hydrolysis coefficients are lower than in optimal operating conditions. The latter was seen in the first days of methane production – most of the methane was produced after 10 days.

The contents of TS, VS, VOA, TIC, and pH did not differ significantly between the reactors (Figure 4). Reactors with glucose had the highest content of TS (7.77 ± 0.25%), and the lowest was measured for GC (6.84 ± 0.56%), which also had the lowest content of VS (65.13% and 4.46%). The concentration of VOA was between 6096 mg/L and 6569 mg/L, the concentration of TIC between 32596 mg/L and 35937 mg/L, and their ratio between 0.177 and 0.195.

When using GC as a substrate for biogas production, we must pay attention to the age of the GC, and consequently the proportion of lignin in the GC (lignin is not anaerobically degradable). Usually the size of GC is too large for immediate dosing into the anaerobic reactor, wherefore particle size must be mechanically reduced and the dosing system must be modified.

Further costs for mowing and transporting GC must be considered when calculating cost/ benefit. Even more, regarding the small improvement of methane potential, the usage of the GC is economically questionable. The quantity of GC depends on the time of year, meaning that it is not always available.

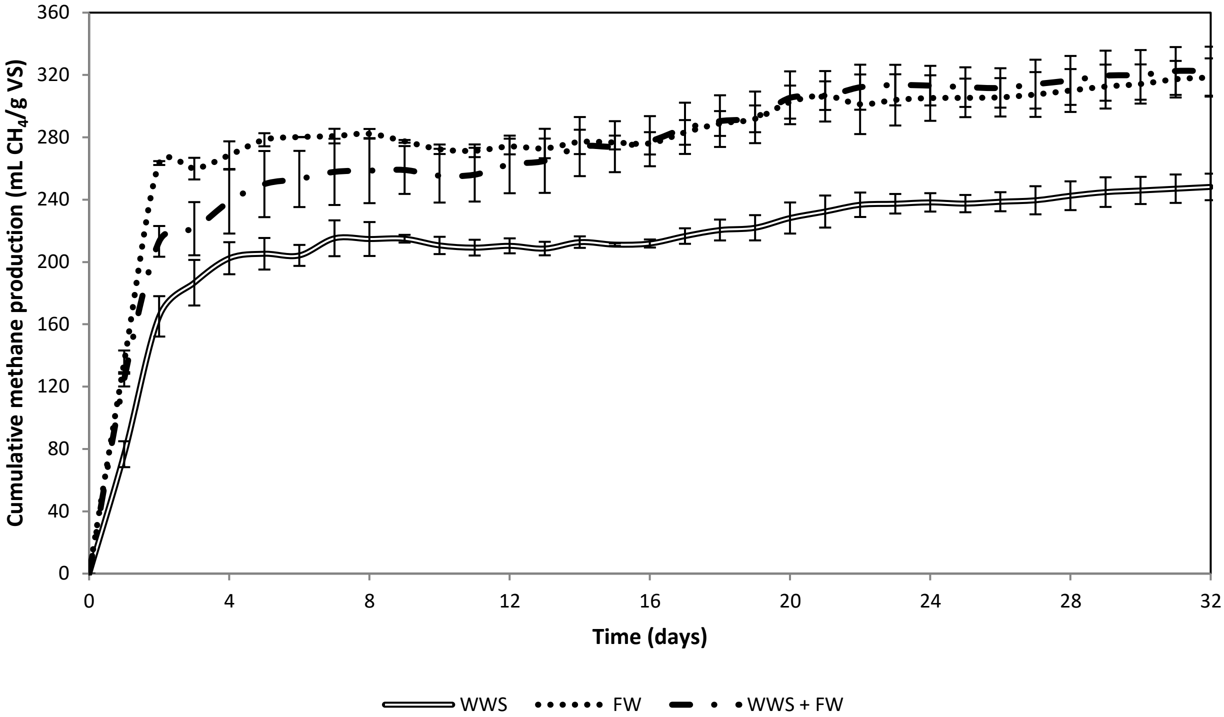

***Figure 1:** The time course of cumulative methane yield of mono-digestion of WWS, FW and co-digestion of WWS and FW.*
***Slika 1:** Časovni razvoj kumulative metanskih potencialov monopresnove blata iz ČN (WWS) in bioloških odpadkov (FW) in ko-digestija blata iz ČN (WWS) in bioloških odpadkov (FW) pri različnih kombinacijah.*

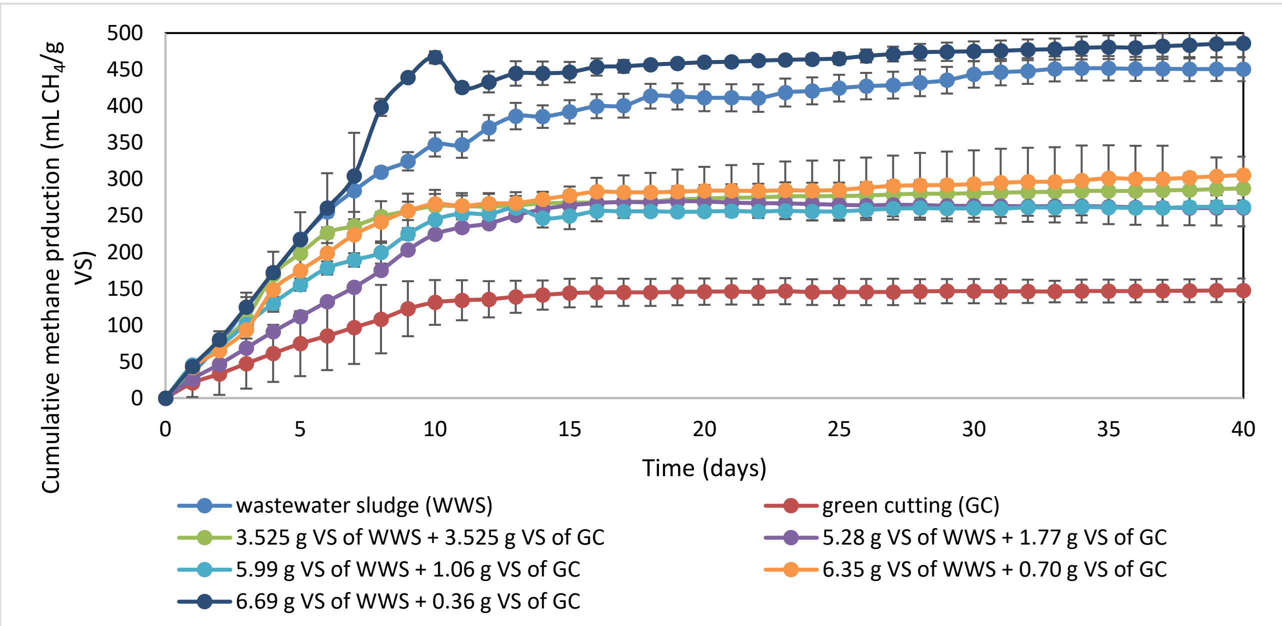

***Figure 2:** The time course of cumulative methane yield of mono-digestion of WWS, GC and co-digestion of WWS and GC combinations.*
***Slika 2:** Časovni razvoj kumulative metanskih potencialov monopresnove blata iz ČN (WWS) in zelenega odreza (GC) in kodigestija blata iz ČN (WWS) in zelenega odreza (GC) pri različnih kombinacijah.*

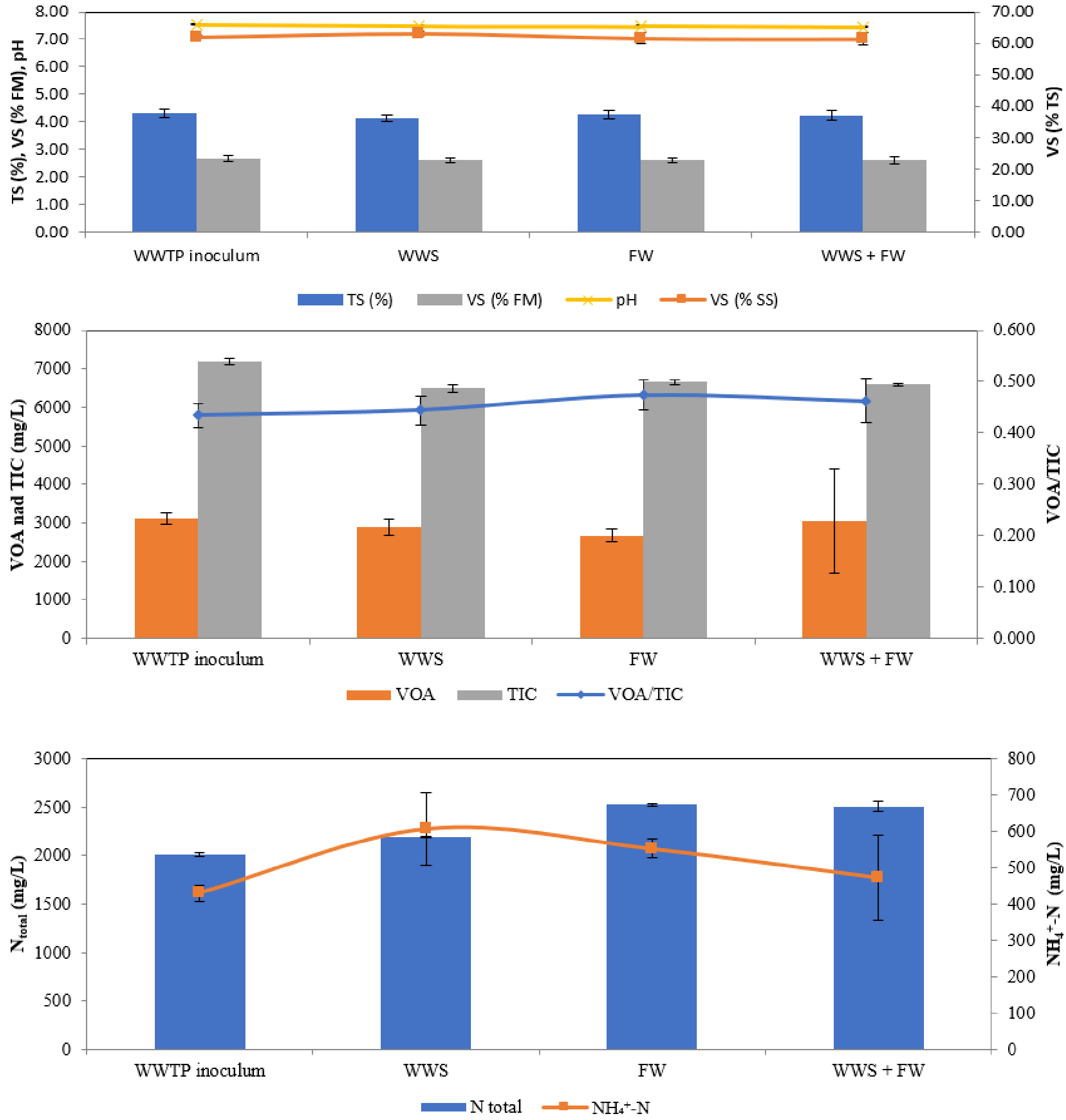

***Figure 3:** Measured values of total solids (TS), volatile solids (VS), pH, volatile organic acids (VOA), total inorganic carbon (TIC), ratio VOA/TIC, total nitrogen (Ntotal), and ammonium nitrogen (NH_4^+-N) in anaerobic reactors after 34 days of anaerobic digestion process in experiment 1. Error bars represent one standard deviation.*
***Slika 3**: Izmerjene vrednosti suhe snovi (SS), organske snovi (OS), pH, hlapnih organskih kislin (VOA), celotnega anorganskega ogljika (TIC), razmerje VOA/TIC, celotnega dušika (Ntotal) in amonijevega dušika (NH_4^+-N) v anaerobnih reaktorjih po 34 dneh anaerobne presnove pri eksperimentu 1. Napake predstavljajo eno standardno deviacijo.*

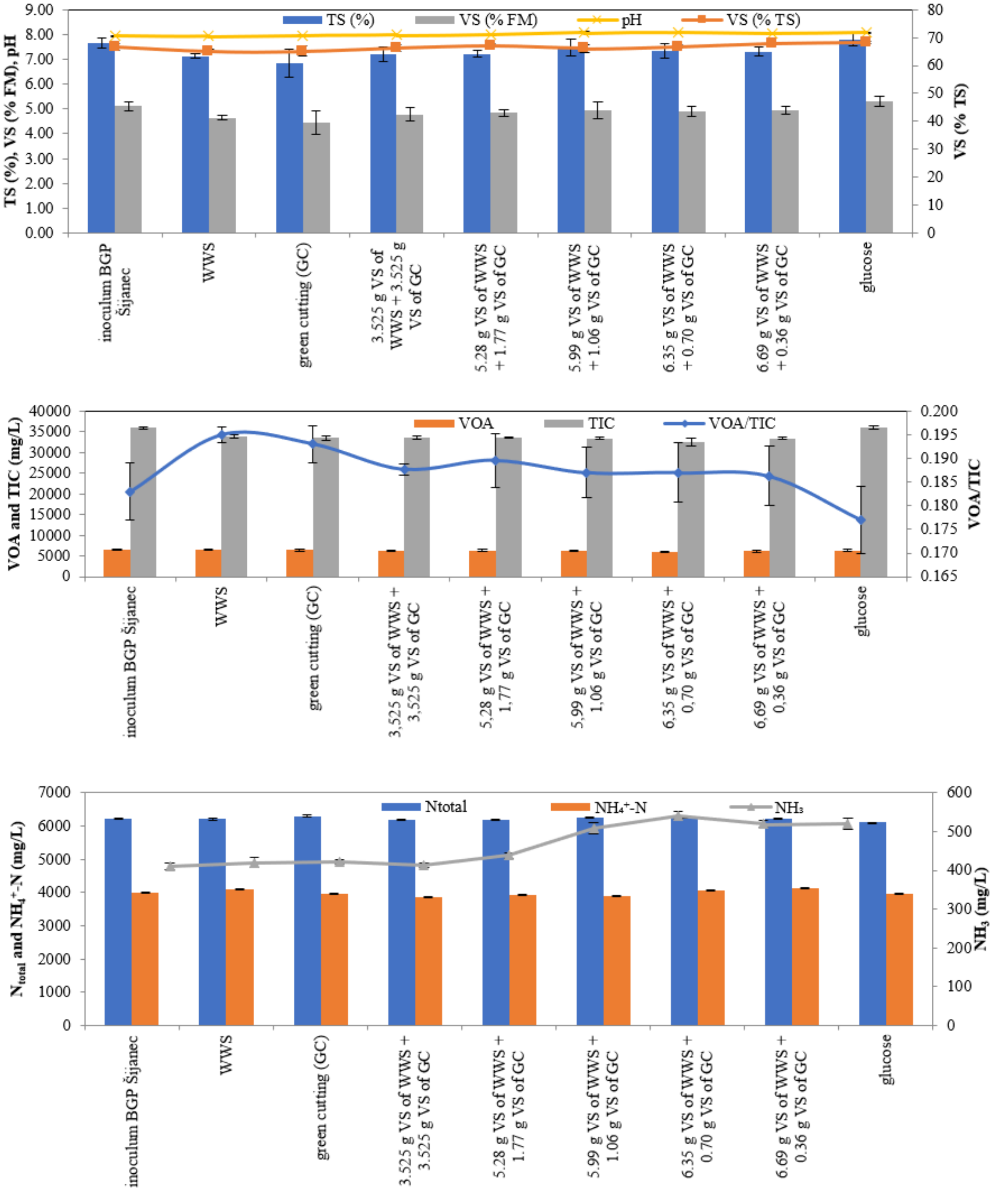

Figure 4: *Measured values of total solids (TS), volatile solids (VS), pH, volatile organic acids (VOA), total inorganic carbon (TIC), ratio VOA/TIC, total nitrogen (Ntotal), and ammonium nitrogen (NH_4^+-N) in anaerobic reactors after 40 days of anaerobic digestion process in experiment 2. Error bars represent one standard deviation.*
Slika 4: *Izmerjene vrednosti suhe snovi (SS), organske snovi (OS), pH, hlapnih organskih kislin (VOA), celotnega anorganskega ogljika (TIC), razmerje VOA/TIC, celotnega dušika (Ntotal) in amonijevega dušika (NH_4^+-N) v anaerobnih reaktorjih po 40 dneh anaerobne presnove eksperimenta 2. Napake predstavljajo eno standardno deviacijo.*

The experimental data of the response variable based on the independent variables and residuals are presented in Table 4 and Table 5. To determine the best model to fit the measured data, standard deviations and R^2 values were calculated. When modeling combinations of WWS and FW in experiment 1, the quadratic models fit was R^2 = 0.79297 and the overall quadratic model was statistically significant ($p < 0.05$). Equation (3) for biomethane potential response for quadratic model is:

$$Y = 318.637X_1 + 248.153X_2 - 154.78X_1X_2 \quad (3)$$

ANOVA results in experiment 1 indicate that the coefficients β_1, β_2, and β_{12} are statistically significant ($p<0.05$), meaning that the coefficients had a significant effect on the response. Negative coefficients for β_{12} suggest that the substrates were not completely complementary in their contribution to methane production (Wang et al., 2013).

When the modeling combinations of WWS and GC in experiment two, neither linear nor quadratic models were shown to be the best for predicting BMP response. The experimental data are not in the best agreement with predicted values (R^2 = 0.604707; F = 29.06563 and R^2 = 0.663468; F = 17.74335 for the linear and quadratic models, respectively). To achieve better results, the experiment mixture in pilot reactors should be more carefully chosen. Both models were statistically significant ($p < 0.05$). The regression equation (4) for the biomethane potential response for the quadratic model in experiment 2 is:

$$Y = 440.621X_1 + 167.981X_2 - 315.21X_1X_2 \quad (4)$$

As in experiment 1, the ANOVA results indicate that the coefficients β_1 and β_2 are statistically significant, except that of β_{12} ($p>0.05$). The differences between the measured data and the predicted BMP in both experiments are statistically insignificant ($p>0.05$).

Table 4*: The simplex centroid mixture design and corresponding predicted values and residuals of experiment 2 for the quadratic model.*
Preglednica 4: *Zasnova simpleks centroidov mešanice in pripadajoče napovedane vrednosti in reziduali eksperimenta 1 za kvadratni model.*

FW – X_1	WWS – X_2	measured BMP (mL CH_4/g VS)	predicted BMP (mL CH4/g VS)	residuals
1	0	239.6	248.2	-8.6
1	0	248.2	248.2	0
1	0	256.7	248.2	8.5
0	1	165.9	165.9	0
0	1	164.7	165.9	1.2
0	1	167.1	165.9	-12
0.5	0.5	229.7	244.7	-15.0
0.5	0.5	244.7	244.7	0.0
0.5	0.5	259.7	244.7	15.0

Table 5*: The simplex centroid mixture design and corresponding predicted values and residuals of experiment 2 for the quadratic model.*
Preglednica 5: *Zasnova simpleks centroidov mešanice in pripadajoče napovedane vrednosti in reziduali eksperimenta 2.*

WWS – X_1	GC – X_2	measured BMP (mL CH_4/g VS)	predicted BMP (mL CH4/g VS)	residuals
1	0	453	408.6	44.4
1	0	470	408.6	61.4
1	0	487	408.6	78.4
0	1	136	137.3	-1.3
0	1	154	137.3	16.7
0	1	172	137.3	34.7
0.5	0.5	282	273.0	9.0
0.5	0.5	299	273.0	26.0
0.5	0.5	316	273.0	43.0
0.75	0.25	237	340.8	-103.8
0.75	0.25	280	340.8	-60.8
0.75	0.25	323	340.8	-17.8
0.85	0.15	247	367.9	-120.9
0.85	0.15	275	367.9	-92.9
0.85	0.15	303	367.9	-64.9
0.9	0.1	297	381.5	-84.5
0.9	0.1	321	381.5	-60.5
0.9	0.1	345	381.5	-36.5
0.95	0.05	480	395.0	85.0
0.95	0.05	507	395.0	112.0
0.95	0.05	528	395.0	133.0

4 Conclusions

In a co-digestion experiment, the proportion of each component in the mixture plays a significant role. In this study, two BMP tests were carried out to optimize the methane yield by combining a mixture of WWS and FW in experiment one and a mixture of WWS and GC in experiment two. The mixture of WWS and FW had no negative effects on methane production; the combination turned out to be as good as mono-digestion of FW. The addition of WWS to food waste does not impair methane production.

In experiment two, the optimal combination to achieve the highest methane yield was in the reactors, where we had 95% of the organic matter of the WWS and 5% of the organic matter GC. This methane yield compared to the WWS and GC mono-digestion was 7.5% and 228% higher, respectively. The models can present a positive or negative interaction between the substrates used in the experiment. This is then reflected in the optimization of the mixture and can have a positive benefit on methane production, as well as on the process stability. The mixture design can be used as a first step in maintaining or ensuring the stable process, and as a starting point for semi-continuous experiments.

The above results confirmed that using the mixture design methodology could estimate the composition of feedstock mixture for maximum methane production. To achieve that, the experiments and mixture must be properly designed.

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