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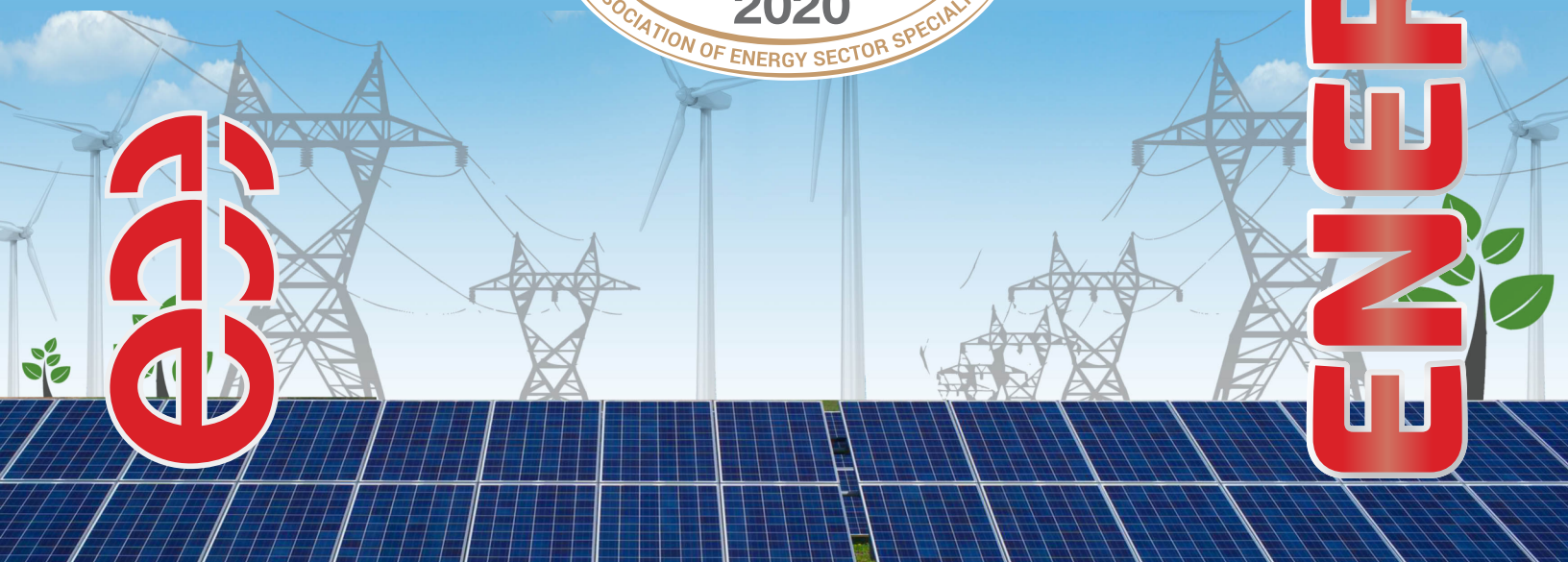

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Odnos inokulum/supstrat: proračunske metode

Inoculum to substrate ratio: Calculating methods

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Apstrakt **-** Anaerobna digestija je dokazana opcija za značajno povećanje proizvodnje biogasa uz korišćenje postojećih digestora i infrastrukture. Ko-digestiranje prehrambenog otpada sa kanalizacionim muljem iz otpadnih voda sve se više upotrebljava kako bi se povećala proizvodnja energije i anaerobna digestija učinila pristupačnijom; međutim, nedostaju saznanja o odgovarajućim omerima kodigestivnog supstrata i inokuluma (mikrobiološkog zasoja). Cilj ovog istraživanja bio je izvršiti iscrpnu studiju u pogledu količine mešovitog mulja iz otpadnih voda i organske frakcije komunalnog čvrstog otpada ili prehrambenog otpada. Uticaj odnosa inokulum/supstrat na stopu proizvodnje biogasa/metana i krajnji prinos procenjen je za analizu biohemijskog metanskog potencijala u anaerobnom reaktoru. Uporedne performanse napravljene su za različito izračunate koncentracije supstrata i inokuluma bazirane na osnovu prisustva isparljive čvrste supstance supstrata i inokuluma u gramima po gramu. Istraženi su različiti pristupi izračunavanja i kao rezultat istraživanja date su opšte i zajedničke formule.

Ključne reči: odnos inokulum\supstrat, anaerobna kodigestija, kanalizacioni mulj, biohemijski metanski potencijal

Abstract- Anaerobic codigestion (AcoD) is a proven option to significantly boost biogas production while utilizing existing digesters and infrastructure. Co-digestion of food waste with sewage sludge (SS) is increasingly exploited to raise energy production and make anaerobic digestion more affordable; however, there is a lack of information on appropriate codigestion ratios of substrates and inoculum. The aim of the present research was to conduct an exhaustive study regarding amounts of mixed sewage sludge and organic fraction of municipal solid waste (OFMSW) or food waste (FW). The effect of inoculum to substrate ratio (ISR) on biogas/methane production rates and ultimate yields was evaluated for biochemical methane potential (BMP) assay in an anaerobic reactor. Comparative performance was made for differently calculated concentrations of substrate and inoculum based on terms of g volatile solids (VS) substrate/g VS inoculum. Different calculating approaches were investigated and general and common formulas are given as a result of research.

Keywords - inoculum to substrate ratio, anaerobic codigestion, sewage sludge, biochemical methane potential assay

I. Introduction

The generation of heat and electricity through fossil fuels is acknowledged for most of the greenhouse gases (GHG) released into the atmosphere. Their negative impact could be reduced through better usage of the renewable energy sources. The energetic usage of biomass and organic residues performs a crucial role in the mitigation of GHG. The anaerobic digestion (AD) of organic wastes such as manure, sewage sludge, the organic fraction of municipal solid waste (OFMSW) and food waste (FW) presents a double advantage, as it produces biogas and simultaneously treats the residues, reducing their disposal in sanitary landfills.

MSW generation is influenced by economic conditions and living standards along with urbanization of population. In 2016, 1.3 billion metric tons of municipal solid waste (MSW) is generated in the world, and this amount is expected to rise to about 2.2 billion tons by 2025, although MSW generation in Organisation for Economic Co-operation and Development (OECD) member countries has been decreasing (Kosuke Kawai and Tomohiro Tasaki, 2016). Using OFMSW as a substrate for AD is an environmentally correct option for the management of MSW.

Every year, one third (about 1.3 billion tons) of the world food production is lost or wasted through the food supply chain, generating tremendous social, environmental, and economic problems. In the European Union, around 98 million tons of food waste is generated every year, which is expected to rise to about 139 million tons by 2020 without additional prevention policy or activities (European Communities, 2010). Considering the negative environmental impacts of landfilling, incineration or composting of food waste, anaerobic digestion (AD) has been proposed as a relatively cost-effective technology for renewable energy production and waste treatment of this high-moisture and energy-rich material (Xu et al., 2015). During AD, anaerobic microbes convert various types of biomass and organic wastes into biogas (60%- 70% methane, 30%-40% carbon dioxide, and traces of other gases such as hydrogen and hydrogen sulfide), leaving a nutrient-rich residue that can be used for land application. Compared with many other bioenergy technologies, AD can accommodate a much wider range of substrates, even

those with high moisture contents and impurities, and can be conducted in both large and small scale digesters and at all geographical locations (Xu et al., 2018). Moreover, FW with high concentrations of organic matter (volatile solids/total solids [VS/TS]: 0.8–0.9), high energy content and good biodegradability with wide availability have been regarded as the most promising anaerobic substrates.

Anaerobic digestion (AD) is a complex process that involves a diverse assemblage of bacteria and methanogenic archaea. The decomposition process of organic matter can be divided into four stages. Macromolecule organic matter in solids is firstly broken into easily dissolved monomers including the transformation from carbohydrates, protein and fat to sugar, amino acid and long-chain fatty acid, and this process is called hydrolysis. The hydrolysis step is generally considered as the rate-limiting step for complex organic substrates degradation reported by most researchers, resulting from the formation of toxic by-products (complex heterocyclic compounds) or non-desirable volatile fatty acids (VFAs) during the hydrolysis step. To enhance the hydrolysis, pretreatments could be carried out to improve the digestive efficiency of waste by solubilizing decomposable organic substances and also reduce the overall process time for AD. In the second stage, called acidogenesis, monomers further decompose into short-chain fatty acid including VFA; lactic acid, pyruvic acid, acetic acid, formic acid. And then, in the process of acetogenesis, acids like lactic acid and pyruvic acid start to be digested into acetic acid and hydrogen. In the last stage, called methanogenesis, hydrogen and acetic acids are transformed into methane by methanogenic archaea (Ren et al., 2018).

Changes in FW management policy are increasingly promoting recycling and energy recovery, creating a demand for AD treatment of FW. Because of this demand a great number of FW anaerobic digesters have been built over the past decade. A small number of wastewater treatment plants (WWTPs) have also begun treating FW by simultaneously digesting FW with sewage sludge (SS) known as anaerobic co-digestion (AcoD). AcoD has been shown consistently in these cases to have a synergistic effect; making AD more efficient, boosting biogas production, stabilising digestate, and increasing the amount of key nutrients in biosolids (Joel Edwards et al., 2017). Since WWTP are the largest users of AD, AcoD provides a unique opportunity for WWTPs as a practical mean to optimise nutrient and energy recovery from sludge. Additionally, AcoD offers a new and localised treatment alternative for FW managers.

II. INOCULUM, SUBSTRATE AND CO-SUBSTRATES RATIOS

Inoculation is the medical term for the introduction (implantation) of germs, infectious material, serum, or other bodies into the tissues of animals, plants, and the rootstocks for the development of germs; inoculation of healthy individuals with weakened germs or their poisons is also performed in order to induce a mild form of the disease, which is subsequently followed by resistance (immunity) to the disease. No standard method has been defined for this procedure, however, and even for the study of simpler soluble substrates the conclusions of an international working party are still awaited.

The bioconversion of complex organic substrates such as biomass into methane requires that sufficient inoculum be present to complete the process. Since methanogenesis requires at least three distinct groups of bacteria, it is apparent that care must be taken to assure that the needed bacteria are present in sufficient numbers to degrade the primary and intermediate products of fermentation. In discussing the microbial basis and methods of measuring biodegradation, the importance of having sufficient concentrations of necessary microorganisms, enzymes and growth factors to degrade complex compounds is emphasized (Hashimoto, 1989). Hashimoto's study evaluated the effect of inoculum/substrate ratio on methane yield and production rate in small-batch fermentors. Four trials were conducted with substrate concentrations of 10, 20, 30 or 40 g ball-milled straw diluted with tap water to a total weight of 1 kg. Inoculum concentrations were: 100 (control), 90, 70, 50, 30 and 20 or 10% by volume. Results showed that the ultimate methane yield was drastically lower at inoculum/substrate ratios (on a volatile solids basis) below 0.25. Methane production rate increased at a decreasing rate up to an inoculum/substrate ratio of two, after which it remained relatively constant.

For the optimization of the anaerobic digestion process, the selection of inoculum source and the inoculum to substrate (I/S) ratio are the important operational parameters for the assessment of anaerobic biodegradability of solid wastes. It is more appropriate to use active anaerobic inoculum or inoculum from animal waste such as bovine manure to reduce digestion period and digester volume. Only limited information is available on total solids content and the proportion of the inoculum required during start-up period of anaerobic digestion of solid wastes. For the anaerobic solid-state fermentation, the inoculum with high methanogenic activity and low biodegradability are necessary, and the findings were validated using a mathematical model (Kalyuzhnyi et al. 2000).

In Sri Bala Kameswari et al. study increase in biogas generation for I/S ratio of 0.25–2.30 is reported. When I/S ratio gained from 0.50 to 1.00 a substantial enhancement in biogas generation was observed whereas for upgrowth of I/S ratio from 1.00 to 2.30 an increment of biogas generation of 1.5–2.12% was recorded; however, it is marginal with respect to amount of inoculum added. The use of more inoculum amount in codigestion process has no significant influence on biogas generation beyond certain point. However, excessive use of inoculum leads to an increase in digester volume unnecessarily required for the co-digestion. Hence, it was observed that I/S ratio of 1.0 was optimal wherein maximum conversion rate of substrate is noticed beyond which the increase was negligible.

Batch fermentation experiments using wheat straw showed that the methane yield was drastically reduced at an inoculum to substrate ratio below 0.25 on a VS basis. The specific methane production rate was also found to increase, but at a decreasing rate, as I/S was raised stepwise to 2. At ratios above this, the rate was found to remain relatively constant (Raposo et al., 2006). In order to improve the rate limiting step of hydrolysis during anaerobic digestion process, the enzyme application is an option to enhance the digestion process. The I/S proposed by Owen et al. as a standard was approximately 1, but Chynoweth et al. state

that increasing the I/S may be necessary for some type of substrates and suggested a I/S of 2. Neves et al. used a range of I/S (2, 1, 0.74 and 0.43) and two sludge inoculum types (granular and suspended) when assaying the BMP of kitchen wastes. The use of a granular inoculum prevented acidification of the reactors over the range of I/S used, but the suspended sludge only avoided acidification at the highest I/S.

Optimization of I/S ratio is not only an important operational parameter for assessment of anaerobic biodegradability of solid wastes but it also helps in arriving the reactor volume required for co-digestion of solid wastes. The results obtained from batch reactor studies are useful in approximation of reactor size required to avoid under design or over design of the digester volume. Hence it is appropriate to consider I/S ratio and to conduct the studies in batch and pilot scale reactors before implementing the co-digestion process in full-scale plants especially for continuous mode of operation. Though the science of anaerobic digestion is well established, but the process efficiency in terms of biogas generation purely depends on parameters like I/S ratio, activity of the inoculum and biodegradability of the waste.

Tendency observed in Rodriguez-Chiang and Dahl paper was that the lower the I/S ratio, the longer time span over which methane was being produced. Also, the higher the I/S, the earlier the maximum production rate peaks were observed. This may be due to the higher microbial activity present in the larger amount of inoculum used for higher ratios. Increasing the I/S increases the amount of active methanogens in the inoculum, which moreover decreases the time required for the growth of sufficient methanogenic population to initiate methanogenic activity.

Moreover, the optimal I/S ratio depends on the tested substrate. For instance, the optimal I/S (based on VS) ratio of microalgae BMP is 2 in order to avoid volatile fatty acids (VFAs) accumulation (Alzate et al., 2012). In the case of the BMP of maize, the authors noticed only slight variations of methane yield with different I/S (based on VS) ratios (1, 1.5, 2 and 3) and the optimal ratio is 1 (Raposo et al., 2006). In Y. Zhou et al. study on mesophilic digestion of bean refuse, S/I (based on VS) ratios in the range of 0.1 to 3 are tested. The authors highlighted that the methane yield reaches its maximum at S/I values of 0.6 to 0.9, and inhibitions occur when the ratio exceeds 1, due to VFAs accumulations. However, in the latest study it is mentioned that for easily degradable substrates where rapid accumulation of fermentation intermediates such as VFAs could lead to inhibition of anaerobic digestion, a ratio greater than or equal to 4 should be applied; and for less degradable substrates, such as lignocellulosic organic matter, a ratio less than or equal to 1 can be applied (Caillet et al., 2019).

In addition to ISR, there are other important factors to ensure an optimum bio-methane conversion. First, inoculum should be taken fresh from an active anaerobic reactor, preferably fed with a similar type of substrate to secure adapted microbial species. Furthermore, macro- and micronutrients/vitamins are needed for anaerobic microorganism to perform at their optimum. Finally, the substrate should contain minimum amount of inhibitors, such as heavy metals, ammonia, and sulphide.

Previous studies analyzed the effect of ISR on methane yield with different substrates, and some contradictory results were obtained. During another research carried out on sunflower oil cake with ISRs of 3, 2, 1.5, 1, and 0.8 on VS basis, methane yield coefficients increased with an increase in ISRs. The amount of inoculum added did not only determine the ultimate methane yield, but also changed the rate of methane production in relation to inhibition potential of substrate (Raposo et al., 2009). Different methane potential tests conducted on swine slurry with substrate/inoculum ratios of 1, 2, and 3 yielded similar ultimate methane yields at all ratios. However, the methane production rate at a substrate to inoculum ratio of 1 was faster than those at ratios of 2 and 3 (Gonzalez-Fernandez and Garcia-Encina, 2009).

In Eskicioglu and Ghorbani research for ISR range of 3.67–0.46 g/g, first order biodegradation rate constant of whole corn stillage decreased significantly with decreasing ISR, indicating an initial reactor overloading or substrate inhibition process. However, ISRs did not affect the ultimate biodegradability of corn whole stillage with similar specific methane yields at the end of 22 days of mesophilic digestion. The BMP assays indicated that whole corn stillage is a suitable substrate for anaerobic digestion due to high organic removals achieved. However follow-up studies should pursue the continuous-flow anaerobic digesters at larger scales to verify the potential of this technology for the full-scale applications.

The purpose of the current research is to look for accounting methods of inoculum to substrate ratio, as one of the most important factors likely to influence the result of a BMP test carried out using a batch assay, and to monitor the progress of the biodegradation by standardized procedures. In this case, any biodegradable material could be chosen as substrate because of its appropriateness for anaerobic biodigestion, with sewage sludge as the inoculum because of its widespread availability and relatively uniform properties.

III. CALCULATION METHODS

Biomethane potential (BMP) tests were carried out in 160 mL glass serum bottles at mesophilic temperature (37 ± 1 °C) based on the procedure described by Angelidaki et al., 2009. All tests contained 70 mL inoculum, different quantities of sewage sludge (SS) and/or glycerol (GLY) and deionized water. The later was added to adjust the total test volume to 85 ml. Five mixtures between SS and GLY were tested and contained 0.25, 0.5, 1, 2 and 4% of GLY wet-basis (w/w) concentration. All tests had an inoculum to substrate ratio (ISR) of 2 on a VS basis (Jensen et al., 2011).

If inoculum volume is denoted with V_I and overall volume of digester content by ALL, equations can be applied:

$$V_I = 0.07l,$$

$$ALL = V_I + (V_{SS}) + (V_{GLY}) + V_W = 0.085ml,$$

where V_{SS}, V_{GLY}, V_W are volumes of sewage sludge, glycerol and water respectively and brackets represent optionality of substrate. Inoculum to substrate ratio of 2 (VS) can be written as:

$$ISR = \frac{I}{S} = 2(\frac{g_{VS}}{g_{VS}}).$$

In most common case of Jensen's study, former equation has form:

$$V_I \cdot I_{VS} = ISR \cdot (V_{SS} \cdot S_{SS_{(VS)}} + V_{GLY} \cdot S_{GLY_{(VS)}}),$$

where $I_{VS}, S_{SS_{(VS)}}, S_{GLY_{(VS)}}$ are volatile solid concentration of inoculum, sewage sludge and glycerol respectively, in this very case density of VS ($\frac{g}{l}$). When referred to any type of substrate, former equation has been recorded as:

$$V_I \cdot I_{VS} = ISR \cdot \sum_i V_{S_i} \cdot S_{S_{(VS)_i}}.$$

Amounts of volatile solids, in these case masses (g), are expressed within next features:

$$I = V_I \cdot I_{VS},$$

$$S = \sum_i S_{S_i} = \sum_i V_{S_i} \cdot S_{S_{(VS)_i}}.$$

For example, when only sewage sludge is used as substrate without glycerol as co-substrate, maximum SS volume is 0.015l (with no water used). So, if $V_{SS_{\max}} = 0.015l$ max content of volatile solids in inoculum will be:

$$I_{VS_{\max}} = \frac{V_{SS_{\max}} \cdot S_{SS_{(VS)}}}{V_I} \cdot ISR = 14.7\frac{g}{l}$$

When mixtures between SS and GLY were tested and glycerol in different mass percent concentration of $\omega(\%) = (0.25), (0.5), (1), (2), (4)$ as co-substrate is used, first it is necessary to convert percent composition into mass fraction $\frac{\omega(\%)}{100\%} = \omega$. Mass fraction, by using m_{GLY} and m_{SS} for masses of glycerol and sewage sludge respectively, can be written down as:

$$\frac{m_{GLY}}{m_{GLY} + m_{SS}} = \omega,$$

$$m_{GLY} = m_{SS} \cdot \frac{\omega}{1-\omega}.$$

Further, from overall volatile solid equation:

$$V_I \cdot I_{VS} = (\frac{m_{SS}}{\rho_{SS}} \cdot S_{SS_{(VS)}} + \frac{m_{SS}}{\rho_{GLY}} \cdot \frac{\omega}{1-\omega} \cdot S_{GLY_{(VS)}}) \cdot ISR,$$

$$I = m_{SS} \cdot (\frac{S_{SS_{(VS)}}}{\rho_{SS}} + \frac{S_{GLY_{(VS)}}}{\rho_{GLY}} \cdot \frac{\omega}{1-\omega}) \cdot ISR,$$

$$m_{SS} = \frac{I}{\frac{S_{SS_{(VS)}}}{\rho_{SS}} + \frac{S_{GLY_{(VS)}}}{\rho_{GLY}} \cdot \frac{\omega}{1-\omega}} \cdot \frac{1}{ISR},$$

where ρ_{SS} and ρ_{GLY} are densities of sewage sludge and glycerol.

In Pavi et al., paper mass of VS added to the reactors was the same for every stage of the experiment, whereas the ratios of OFMSW/FWS were changed. Four different OFMSW/FVW ratios were tested in triplicates: 1/0, 1/1, 1/3, and 0/1. The mixtures were made based on the VS. The substrate/inoculum (S/I) ratio used was 1/1, based on the VS. This S/I proportion was selected according to previous experiments.

Mass of VS added to the reactors considering mentioned constraints can be noted as:

$$m_{VS} = const = S + I.$$

The relations of volatile solid masses are arranged as:

$$\frac{S_{OFMSW}}{S_{FVW}} = \frac{1}{0}; \frac{1}{1}; \frac{1}{3}; \frac{0}{1} = \frac{a}{b}.$$

Inoculum to substrate ratio of 1 (VS) was defined by its reciprocal value in Pavi's paper, so:

$$SIR = 1 = \frac{S}{I} = \frac{1}{ISR}.$$

Further it can be written as follows, since VS fractions of inoculum and substrates are given in percent's:

$$m_{OFMSW} \cdot S_{OFMSW_{(VS)}} + m_{FVW} \cdot S_{FVW_{(VS)}} = SIR \cdot m_I \cdot I_{VS},$$

$$S_{OFMSW} + S_{FVW} = SIR \cdot I,$$

$$S = SIR \cdot I.$$

When previous equality is included in the mass equation for volatile solids, expression is made:

$$m_{VS} = SIR \cdot I + I = I \cdot (SIR + 1),$$

$I = \frac{m_{VS}}{SIR+1}$, and in this very case $I = \frac{m_{VS}}{2} = S$.

$$\frac{m_{VS}}{SIR+1} = m_I \cdot I_{VS}$$

$$m_I = \frac{m_{VS}}{SIR+1} \cdot \frac{1}{I_{VS}}$$

Since $I = \frac{S}{SIR}$, next follows:

$$S + \frac{S}{SIR} = m_{VS},$$

$$S = \frac{m_{VS}}{1 + \frac{1}{SIR}} = \frac{SIR \cdot m_{VS}}{SIR+1}.$$

Using formula for the mass of volatile solids from substrates, expressions are obtained:

$$m_{OFMSW} \cdot S_{OFMSW_{(VS)}} + m_{FVW} \cdot S_{FVW_{(VS)}} = \frac{SIR \cdot m_{VS}}{SIR+1},$$

$$S_{OFMSW} + \frac{b}{a} \cdot S_{OFMSW} = \frac{SIR \cdot m_{VS}}{SIR+1},$$

$$m_{OFMSW} \cdot S_{OFMSW_{(VS)}} \cdot (1+\frac{b}{a}) = \frac{SIR \cdot m_{VS}}{SIR+1},$$

$$m_{OFMSW} = \frac{SIR \cdot m_{VS}}{SIR+1} \cdot \frac{a}{a+b} \cdot \frac{1}{S_{OFMSW_{(VS)}}}.$$

Finally, considering that $S_{FVW} = \frac{b}{a} S_{OFMSW}$, mass of fruit and vegetable waste substrate is as follows:

$$m_{FVW} \cdot S_{FVW_{(VS)}} = \frac{b}{a} \cdot m_{OFMSW} \cdot S_{OFMSW_{(VS)}},$$

$$m_{FVW} = \frac{b}{a} \cdot \frac{S_{OFMSW_{(VS)}}}{S_{FVW_{(VS)}}} \cdot m_{OFMSW}.$$

Eskicioglu and Ghorbani in their experiment used serum bottles (125 mL) with butyl rubber stoppers as batch digesters. Whole corn stillage was diluted to achieve different percent VS (w/w) concentrations. Then 40 mL diluted stillage and 40 mL mesophilic inoculum was added into the serum bottles to achieve ISRs of 3.67, 1.83, 0.92, and 0.46 g/g on VS basis.

Since desired volatile solid concentrations are $S_{S_{(VS)}} = (0.29); (0.58); (1.15); (2.3)(\%)$, dilution of corn stillage was performed by adding tap water into origin substrate of 11.5 (%, w/w) VS using next formula:

$$\omega_{VS} = \frac{m_{VS}}{m_S} = \frac{S_{S_{(VS)}}}{100\%},$$

$$m_{CS} + m_W = \frac{m_{VS}}{\omega_{VS}},$$

$$m_W = m_{CS} \cdot (\frac{S_{CS_{(VS)}}}{100\% \cdot \omega_{VS}} - 1).$$

When it comes up to inoculum to substrate ratio, already defined volatile solid proportion should be multiply with amounts of inoculum and substrate, volumes V_I and V_S in this very case:

$$V_S \cdot S_{S_{(VS)}1,2,3,4} = S_{1,2,3,4},$$

$$V_I \cdot I_{VS} = I,$$

$$ISR = \frac{I}{S} = ISR_{1,2,3,4} = \frac{I}{S_{1,2,3,4}}.$$

Since in this study $V_S = V_I$, final equation for inoculum to substrate ratio could have even simpler form:

$$ISR = \frac{I_{VS}}{S_{S_{(VS)}1,2,3,4}}.$$

Substrate to inoculum ratios (S/I ratios) for BMP assay cultures in Yoon et al. study were prepared based on the VS content of substrate and inoculum. The four different S/I ratios tested (0.1; 0.5; 1.0 and 1.5) were prepared while maintaining a constant VS concentration (10.56 g-VS/L) and volume (60.0 mL) at inoculum input, varying the amounts of solid substrate added to BMP assay vials over the ranges of 0.372 to 5.584 g for blood, 0.247 to 3.706 g for intestine residue, and 0.250 to 3.748 g for digestive tract content. BMP assays were carried out in a working volume of about 60 mL in a 160 mL serum bottle.

Taking into account that inoculum volume V_I is already determined and volatile solids density $I_{VS}(\rho)$ given; mass of volatile solids in inoculum I is well known. Four substrate to inoculum ratios which were tested and I known from the very beginning give familiar equation:

$$SIR_{1,2,3,4} = \frac{S_{S_{1,2,3,4}}}{I} = (0.1); (0.5); (1.0); (1.5)$$

Substrates were added in ranges of masses and amounts of their VS are expressed in g_{VS}/kg which correspond to per mills (‰), so mass of substrate solids is being calculated by formula:

$$S_{S_{1,2,3,4}} = \frac{S_{S_{(VS)}}}{1000} \cdot m_{S_{1,2,3,4}}.$$

This gives us on very simple way the stated substrate masses added into serum bottles expressed in grams:

$$m_{S_{1,2,3,4}} = \frac{SIR_{1,2,3,4} \cdot I \cdot 1000}{S_{S_{(VS)}}}.$$

It is also worth to mention that concentration of volatile solids expressed as (percent, per mile) mass fraction multiply with density of substrate (or inoculum) gives density of VS in appropriate medium.

$$I_{VS} \cdot \rho_I = \rho_{I_{VS}}$$

$$S_{S_{(VS)}} \cdot \rho_S = \rho_{S_{(VS)}}$$

In Zahan et al., research BMP of single substrates was assessed in a batch assay conducted under the same AD conditions (same inoculum, mesophilic temperature and time). The C/N ratio was maintained constant and a substrate: Inoculum (S:I) ratio of 1:2 (g/g VS) was applied. The BMP tests were undertaken with 2%, 3%, and 4% VS in the reactors. Biogas and methane composition were analysed regularly, whereas the digestate was characterised at the end of the experiment.

Two initial equations for volume calculations are:

$$V_I \cdot I_{VS} = ISR \cdot V_S \cdot S_{S_{(VS)}}$$

$$V_I + V_S = V$$

where V is overall volume of digester.

Further, expression for substrate volume is obtained:

$$(V - V_S) \cdot I_{VS} = ISR \cdot V_S \cdot S_{S_{(VS)}}$$

$$V_S = \frac{V \cdot I_{VS}}{ISR \cdot S_{S_{(VS)}} + I_{VS}}$$

The novelty of the previous methods is total percentage content of volatile solids in reactor $C(\%)$. It has constant value for given volumes and can be calculated by equation:

$$\frac{V_I \cdot I_{VS} + V_S \cdot S_{S_{(VS)}}}{V_I + V_S} = C$$

Adjustments of VS content in digester are being done by formula:

$$\frac{C \cdot V}{V + V_w} = C_{adj}$$

where C_{adj} is desired percentage content of volatile solids and V_w tap water volume.

When it comes to co-digestion of the different wastes, experiment was carried out for two substrates (CL:YW, CL:HG, CL:WS), three substrates (CL:YW:HG, CL:YW:WS), and four substrates (CL:YW:HG:WS) combined at different ratios. The BMP of these wastes was carried out under mesophilic conditions in 500 mL digesters with 1:2 g/g VS of substrate to inoculum for all the reactors. Substrate loading varied depending on the mixture of substrates.

In the most complex case $YW_{25}WS_{25}HG_{25}$, relations are applied:

$$CL : YW : WS : HG = 25 : 25 : 25 : 25$$

$$S_{CL} = S_{YW} = S_{WS} = S_{HG}$$

$$V_{CL} \cdot S_{CL_{(VS)}} = V_{YW} \cdot S_{YW_{(VS)}} = V_{WS} \cdot S_{WS_{(VS)}} = V_{HG} \cdot S_{HG_{(VS)}}$$

Since $V_I + V_S = V$ it can be written:

$$V_I + (V_{CL} + V_{YW} + V_{WS} + V_{HG}) = V$$

$$V_I + V_{CL} + V_{CL}\frac{S_{CL_{(VS)}}}{S_{YW_{(VS)}}} + V_{CL}\frac{S_{CL_{(VS)}}}{S_{WS_{(VS)}}} + V_{CL}\frac{S_{CL_{(VS)}}}{S_{HG_{(VS)}}} = V$$

$$V_I + V_{CL}\left(1 + \frac{S_{CL_{(VS)}}}{S_{YW_{(VS)}}} + \frac{S_{CL_{(VS)}}}{S_{WS_{(VS)}}} + \frac{S_{CL_{(VS)}}}{S_{HG_{(VS)}}}\right) = V$$

$$V_I + k \cdot V_{CL} = V$$

$$V_I = V - k \cdot V_{CL}$$

By equality of inoculum to substrate equation $V_I \cdot I_{VS} = ISR \cdot \sum S_{S_i}$ and given relations, further is applied:

$$V_I \cdot I_{VS} = ISR \cdot (S_{CL} + S_{YW} + S_{WS} + S_{HG})$$

$$V_I \cdot I_{VS} = ISR \cdot 4S_{CL}$$

$$V_I \cdot I_{VS} = 4ISR \cdot V_{CL} \cdot S_{CL_{(VS)}}$$

Using obtained equation for inoculum volume, final expression for substrate volume is acquired:

$$V_{CL} = \frac{V \cdot I_{VS}}{4ISR \cdot S_{CL_{(VS)}} + k \cdot I_{VS}}$$

Over the calculated substrate volume and using correlations, it is possible to determine all others volumes and ultimately obtain percentage VS content:

$$\frac{V_I \cdot I_{VS} + (V_{CL} \cdot S_{CL_{(VS)}} + V_{YW} \cdot S_{YW_{(VS)}} + V_{WS} \cdot S_{WS_{(VS)}} + V_{HG} \cdot S_{HG_{(VS)}})}{V_I + V_{CL} + V_{YW} + V_{WS} + V_{HG}} = C$$

IV. CONCLUSION

The main conclusion from the literature is that ultimate methane yields as well as the methane production rates are substrate and inoculum specific so that general statements of digestion performance cannot always be made. The purpose of this study was to determine the effect of inoculum to substrate ratio as well as the biodegradation rate from different types of substrates. Overall remark is non-uniformity in terms of VS content, whereas it is often expressed in various types of units such as g/kg, g/l, % etc. which cause difficulties in notation of method's equations requiring density or unalike conversions. Also, beside final values of ratios stated in materials and methods chapters, there are no standard procedures for obtaining, calculation and utilization of ISR. Paper was submitted with the aim of contributing the formulation of common calculating methods of inoculum to substrate ratios and expanding the knowledge catalog of anaerobic digestion.

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