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RESEARCH PAPER

Biochemical Methane Potential (BMP) Test for Thickened Sludge Using Anaerobic Granular Sludge at Different Inoculum/Substrate Ratios

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Abstract The biochemical methane potential (BMP) test for thickened sludge was evaluated at three different inoculum/substrate (I:S) ratios. The cumulative methane yield was 51.4 mL CH_4/g VS_{added} at an I:S ratio of 1:1, 76.3 mL CH_4/g VS_{added} at an I:S ratio of 1:3, and 21.9 mL CH_4/g VS_{added} at an I:S ratio of 1:8. The greatest ultimate methane yield and methane production rate constant were achieved at an I:S ratio of 1:3, whereas the least was obtained at an I:S ratio of 1:8. The maximum methane production rate constant was 0.38/day and the minimum methane production rate constant was 0.0016/day. For the case of a lower I:S ratio, the biomass activity may be affected due to the low substrate concentration. On the other hand, for the case of higher I:S ratios, anaerobic digestion of thickened sludge was inhibited by higher concentrations of volatile fatty acids and lower pH.

Keywords: biochemical methane potential, methane yield, inoculum/substrate ratio, thickened sludge

1. Introduction

After primary and activated sludge is thickened at a thickener, the thickened sludge is usually digested at an anaerobic digester in a municipal wastewater plant (WWTP). Even though several investigators reported the alternatives of anaerobic digestion for thickened sludge, the treatment of thickened sludge using anaerobic digestion has been predominately performed due to the many design and operation experiences [1-5]. In an anaerobic digestion process of thickened sludge, the conversion of biomass to methane requires sufficient inoculum since biomass consists primarily of complex organic polymers [6]. Thickened sludge usually consists of primary sludge and wasted secondary sludge. Activated sludge is a microbial matrix composed of microbial and extracellular polymeric substances (EPS). EPS consisted of polysaccharides, proteins, nucleic acids, uronic acids, humic substances, and lipids are known to be recalcitrant in anaerobic/aerobic digestion [7-11]. The typical concentration of solids is 0.5 ~ 1.5% in unthickened sludge and 4 ~ 6% in thickened sludge. In addition, occasional chlorine and polymer addition are accomplished in a gravity thickener in order to maintain the constant loading in a WWTP. These additions are the main reasons for variations in thickened sludge characteristics [12].

The biochemical methane potential (BMP) test is used to quickly estimate methane yield potential of target substrates. However, even though investigators showed several BMP results of thickened sludge a defined test method has not been developed for evaluating the inoculum for anaerobic digestion of thickened sludge. Inoculum volume percentages used in BMP tests have ranged from 2 to 72% and the importance of inoculum/substrate (I:S) ratio as a parameter for experimental design has not been clearly defined [13-15]. In addition, inoculum used in BMP tests was limited by anaerobic sludge. On the other hand, many investigators have evaluated the I:S ratio to estimate methane yield of individual substrates. Hashimoto [16] determined that ball-milled straw fermentation was severely affected by the I:S

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Table 1. Characteristics of granular sludge and thickened sludge

Parameter	Granular sludge	Thickened sludge
pH	7.5 ± 0.2	7.6 ± 0.1
ORP (mV)	-215 ± 32.0	-333 ± 25.5
Alkalinity (mg/L as $CaCO_3$)	5,000 ± 216.5	6,015 ± 450.6
COD (mg/L)	n.a.*	44,800 ± 280.5
TS (mg/L)	60,618 ± 235.5	30,300 ± 215.6
VS (mg/L)	40,707 ± 115.0	20,050 ± 145.0
VS/TS (%)	67.2	66.2

*Granular sludge (inoculum) was not fed for one week before the study of each condition.

ratio. Gunaseelan [17] evaluated the importance of I:S ratio to obtain methane using *parthenium* plants as feedstock. Raposo *et al.* [18] used the BMP test with maize at I:S ratios of 1:1, 1:1.5, 1:2, and 1:3. They stated that the greatest cumulative methane yield could be obtained at an I:S ratio of 1:1. However, although several researches on I:S ratios for industrial wastes were performed, little attention has been given to thickened sludge. In this study, three I:S ratios were used to estimate the methane yield of thickened sludge using anaerobic granular sludge. Qiao *et al*. [19] performed a BMP test with supernatant of hydrothermally treated municipal sludge at I:S ratio of 1:1.5. Apul and Sanin [20] set a BMP test of waste activated sludge at I:S ratio of 1:2. On the other hand, Girault *et al*. [21] achieved a BMP test of waste activated sludge at I:S ratio of 1:1. In this study, three I:S ratios were used to estimate the methane yield of thickened sludge using anaerobic granular sludge because this investigation was a first report to digest thickened sludge using anaerobic granules. In addition, the effects of high loading on treating thickened sludge were investigated in order to evaluate the capacity of anaerobic granular sludge processes. Generally, thickened sludge was digested using anaerobic sludge in a continuous stirred tank reactor because of the rate-limiting step of hydrolysis or the clogging of sludge between anaerobic granules in a high-rate anaerobic reactor such as an upflow anaerobic sludge blanket (UASB). This investigation assumed that anaerobic granules completely mixed with substrate (thickened sludge) and thickened sludge was not clogged in an anaerobic reactor. Consequently, the results of BMP test can represent one of alternatives to treat thickened sludge using a high-rate anaerobic reactor such as an UASB or an expanded granular sludge bed (EGSB). The effects of I:S ratio on cumulative methane yield, ultimate methane yield, and the methane production rate constant were evaluated.

Table 2. Experimental conditions for BMP test of thickened sludge

	Thickened sludge			Blank
Inoculum:substrate (I:S)	1:1	1:3	1:8	
Seed granule (mL)	15	15	15	15
Sample volume (mL)	15	45	120	0

2. Materials and Methods

2.1. Reducing media

In order to perform the BMP test with thickened sludge, the reducing media that Owen *et al.* [15] proposed was used. The concentrated stock media was prepared and stored at 4°C and remade whenever the stock was oxidized as evidenced by the resazurin indicator.

2.2. Inoculum and substrate

The characteristics of the inoculum and substrate used in this study are shown in Table 1. Anaerobic granular sludge was obtained from an UASB treating industrial waste. Thickened primary and secondary sludge was obtained from a municipal activated sludge facility. Granular sludge was used as inoculum and thickened sludge was used as substrate in the BMP tests.

2.3. Procedures

The BMP test was performed by a modification of the method used by Owen *et al.* [15]. Biomass activity and methane yield were measured using 250 mL serum bottles. The volume of granular sludge used in the test was 10% (liquid vol.) in a serum bottle with a total liquid volume of 150 mL. After purging with nitrogen gas for 30 min, the BMP tests were sealed and initiated.

Serum bottles were sealed with butyl rubber stoppers and maintained on a stirring table at 35 ± 1°C. The experimental conditions for the BMP testing of thickened sludge are shown in Table 2. The remaining volume used in each test was reducing media to bring the total volume to 150 mL. Each test was performed in triplicate.

2.4. Analytical methods

The pH, oxidation reduction potential (ORP), alkalinity, chemical oxidation demand (COD), and solids were measured according to Standard Methods for the Examination of Water and Wastewater [22]. After the BMP test was completed, pH, total volatile fatty acids (VFAs), and the volatile solids (VS) were measured. Gas volume was measured using the syringe method and equilibrating the pressure with atmospheric conditions. The gas collected in a gas cylinder was sampled to a Teflon bag and a gas syringe was used for measuring gas composition in biogas. Methane and carbon dioxide were analyzed by gas chromatography (Yanaco, model GC-180; thermal conductivity detector). The column was 2 m × 3.2 mm packed with 80/180 mesh Porapak Q (Supelco, Bellefonte, PA). The injector temperature, oven temperature, and detector temperature were 65, 55, and 75°C, respectively. Helium was used for carrier gas and the flow rate was 30 mL/min.

3. Results and Discussion

3.1. Effects of inoculum/substrate ratio on cumulative methane ratio

Ultimate methane yield and the methane production rate constant were calculated using the following equation [16]:

$$B = B_o(1 - e^{-kt}) \quad (1)$$

where,

B: methane yield at time t (mL CH_4/g VS_{added})
B_o: ultimate methane yield (mL CH_4/g VS_{added})
k: methane production rate constant (1/day)

The effect of the I:S ratio on cumulative methane yield at 35°C is shown in Fig. 1. The production of methane gas in the blank sample was subtracted from all data to correct for methane produced by the inoculum alone. Methane produced in this study began after a 15 day acclimation period. The acclimation period was not dependent upon the I:S ratio. This suggests that an acclimation period of 15 days could be necessary when granular sludge inoculum is used to ferment thickened sludge, irrespective of the I:S ratio. The inoculum had treated soluble waste and had to develop enzymes for hydrolyzing solids in the thickened sludge [23-26]. In other words, this suggests that the rate-limiting step of hydrolysis of thickened sludge determines the digestion period in an anaerobic reactor. Also, acclimation periods should be considered when test results are used for inoculation of full-scale anaerobic digester systems [27].

The cumulative methane yield sharply increased between about 15 days and approximately 21 days, irrespective of the I:S ratio. After 21 days, methane production ceased as either the substrate became exhausted or inhibitory conditions developed. The greatest amount of cumulative methane yield (76.27 mL CH_4/g VS_{added}) was achieved at an I:S ratio of 1:3. The cumulative methane yield at an I:S ratio of 1:1 and at an I:S ratio of 1:8 were 51.39 mL CH_4/g VS_{added} and 21.93 mL CH_4/g VS_{added}, respectively. This suggests that the optimal digestion condition of thickened sludge is not a linear function of the I:S ratio. Ultimate methane yield is a function of several parameters such as an I:S ratio, the particle size of substrate, and the composition of substrate. Sharma *et al.* [28] reported a significant increase of methane yield when the particle size of substrate decreased from 30 to 0.1 mm. Torres-Castillo *et al.* [29] stated that ultimate methane yield of barley straw ranged 240 ~ 370 mL CH_4/g VS_{added}. Thickened sludge from a municipal WWTP is usually composed of organic, inorganic, and unknown matter [30-33]. It seems that lower organic fraction in thickened sludge compared to activated sludge makes the actual I:S ratio to increase. Methane yield, however, would be deteriorated due to the increase of particle size and the heterogeneity of substrate. Consequently, ultimate methane yield from a municipal wastewater would decrease less than the theoretical methane yield production. The theoretical methane production can be calculated from Bushwell's equation [34]:

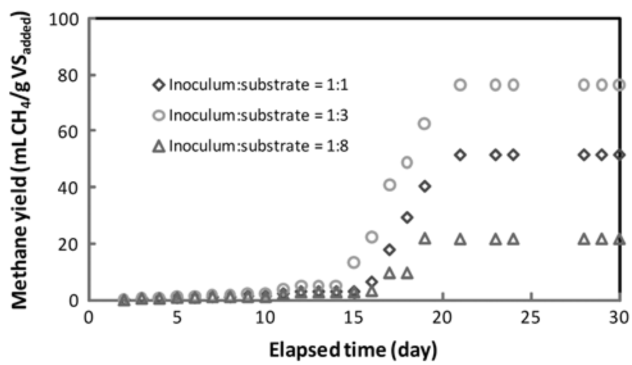

Fig. 1. Effects of inoculum/substrate ratio on cumulative methane yield.

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right)H_2O \rightarrow \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right)CO_2 + \left(\frac{n}{2} + \frac{a}{8} + \frac{b}{4}\right)CH_4 \quad (2)$$

$$B_u = \frac{\left(\frac{n}{2} + \frac{a}{s} - \frac{b}{4}\right)22.4}{12n + a + 16b} \text{ mL } CH_4/\text{g } VS_{added} \quad (3)$$

where,

B_u: theoretical methane production

On the basis of VS_{VFA} ($C_2H_4O_2$), $VS_{carbohydrate}$ ($C_6H_{10}O_5$), $VS_{protein}$ ($C_5H_7O_2N$), and VS_{lipid} ($C_{57}H_{104}O_6$), the theoretical methane production is 370, 415, 496, and 1,014 mL CH_4/g VS_{added}, respectively. When the formula of biomass is simply assumed to be $C_5H_7O_2N$ (empirical formula), 496 mL CH_4/g VS_{added} is theoretically produced. Given that the greatest amount of cumulative methane yield was 76.27 mL CH_4/g VS_{added}, the ratio of the methane production at optimal condition to the theoretical methane production was 0.1. Ramdani *et al.* [32] reported that the fraction of polysaccharides, proteins, and unknown matter in activated sludge was 0.65 : 0.28 : 0.07 in the unit alternating aerated and non-aerated conditions. Wilén *et al.* [33] showed that the fraction of polysaccharides and proteins in floc (EPS) was approximately 0.7 : 0.3. This shows that activated sludge is mainly composed of polysaccharides and proteins, especially polysaccharides. Even though thickened sludge is commonly mixed with inorganic, polymers, and unknown matter, methane production at optimal condition (I:S ratio of 1:3) was an extraordinary result. This might be toxic effects of heavy metals or antibiotics that existed in thickened sludge used in this study.

3.2. Effects of inoculum/substrate ratio on methanogenic potential

The effect of I:S ratio on the methanogenic potential of thickened sludge is shown in Fig. 2. The BMP test can help determine the optimal conditions for specific inocula and target substrates. Although it is a clear conclusion from the experiment that this is not a simple matter and batch parameters can only provide a very general indication of performance in semi-continuous or continuous systems, the ultimate methane yield and the methane production rate constant of each target substrate can be used to identify the optimal operation condition in terms of the I:S ratio for a target substrate in an anaerobic digester. As shown in Fig. 2, the optimal I:S ratio for thickened sludge was approximately 0.4 mL/mL. As the I:S ratio increased up to approximately 0.4 mL/mL, the ultimate methane yield and methane production rate constant sharply increased. This implies that the biomass could be inhibited due to the high concentration of VFAs (low pH). Commensurately, the ultimate methane yield and methane production rate constant decreased as the I:S ratio approached 1.0. Generally, when the concentration of substrate is low in a reactor, the biomass cannot properly induce enzymes. In addition, given that substrate level phospholylation occurs in methanogenesis, a high concentration of substrate is required to be complete methanogenesis. Hashimoto [16] reported that a significant increase in ultimate methane yield could be obtained using I/S ratios greater than 0.25.

The variation in the methane production rate constant during the study period is shown in Fig. 3. The methane production rate constant sharply increased after the acclimation period of approximately 15 days. After approximately 21 days, methane produced in each serum bottle decreased as either the substrate was exhausted or inhibitory conditions developed. The rate values can provide important information for inoculation of digester system. Each methane production rate constant can be recalculated during the methane producing period of a BMP test at each I:S ratio. The methane production rate constant is a measure of the activity of biomass in a given test condition and corresponding test period. Because this biomass activity is a function of several parameters, the BMP test should be done for different inocula and target substrates.

Maximum, minimum, quartiles, upper limit, lower limit, average, and standard deviation of the methane production rate constant under each test condition are shown in Table 3. As shown in Table 3, the distribution for methane production rate was left skewed, irrespective of test condition (I:S ratio). Median at an I:S ratio of 1:8 was approximately two times as great as those at other test conditions, while average methane production rate constant under each test condition was similar. This means that the slope for methane production rate constant at an I:S ratio of 1:8 sharply

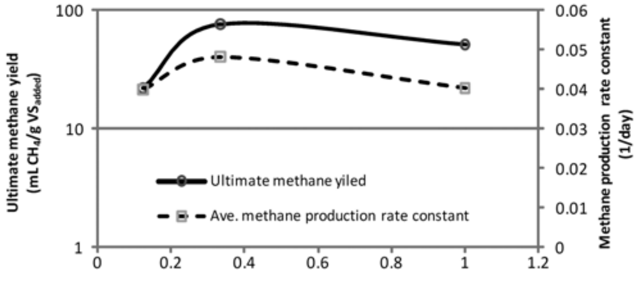

Fig. 2. Effects of inoculum/ultimate ratio on methane yield and methane production rate constant.

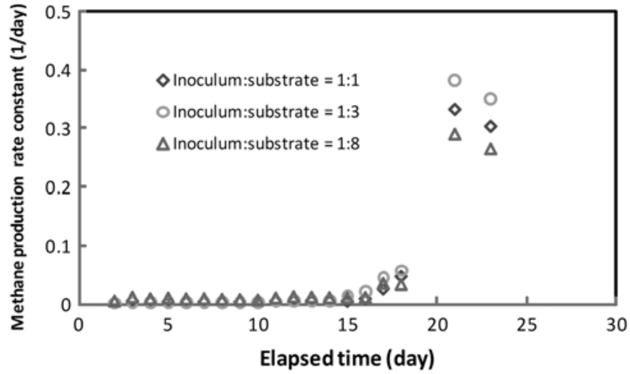

Fig. 3. Variation of methane production rate constant during the study period.

Table 3. Statistical analysis of methane production rate constant under each test condition

I:S ratio (mL/mL)		1:1	1:3	1:8
Methane production rate constant (1/day)	Upper limit	0.0122	0.0387	0.0167
	Max.	0.3311	0.3839	0.2905
	Q3[c]	0.0069	0.0173	0.0117
	Q2[b]	0.0044	0.0045	0.0098
	Q1[a]	0.0033	0.0029	0.0084
	Min.	0.0019	0.0016	0.0045
	Lower limit	-0.0020	-0.0186	0.0035
	Ave.	0.0404	0.0482	0.0400
	Std.	0.0981	0.113	0.0843

[a]First quartile (the median of the part of the entire data set that lies at or below the median of the entire data set).
[b]Second quartile (the median of the entire data set).
[c]Third quartile (the median of the part of the entire data set that lies at or above the median of the entire data set).

increased (Fig. 2). In other words, it is evidently shown that ultimate methane yield at an I:S ratio of 1:8 was sharply decreased as compared to that at an I:S ratio of 1:3 because of inhibitory conditions. On the other hand, as shown in Fig. 2, the change of ultimate methane yield at an I:S ratio of 1:8 to that at an I:S ratio of 1:1 was not significant.

The characteristics of the thickened sludge after the BMP test are shown in Table 4. As shown in Figs. 1 and 3, the greatest methane yield was achieved at an I:S ratio of 1:3. At an I:S ratio of 1:8, the total methane production was the lowest in terms of the percentage of the theoretical methane production of all three tests. After the BMP test, the characteristics of the substrate (thickened sludge) were consistent with Figs. 1 and 3. As shown in Table 4, the BMP test with an I:S ratio of 1:8 resulted in an acidic pH, a relatively high VFA concentration, a low percentage of VS reduction, and low ultimate methane yield. The production of VFAs resulted in a lowering of the pH that inhibited methanogenesis at an I:S ratio of 1:8 as compared to the other test conditions. Several investigators showed the detrimental effects of low pH on anaerobic granules. Lopes *et al.* [35] investigated the influence of low pH on nutrient dynamics and characteristics of granular sludge. According to their results, the necessary nutrient for growth such as cobalt, nickel, iron, and copper were solubilized from granules at low pH. Sallis and Uyanik [36] reported that a high loading late in anaerobic baffled reactor can lead to instability, accumulation of VFA, and low pH. The percentage of autofluorescent methanogens in granules under these conditions decreased from 16 to 2.9%. Yang *et al.* [37] stated that pH is a key parameter to determine in the construction of a stable for sludge granulation because the matrix of granules are highly dependent upon the total EPS contents in granules, which are controlled by pH. According to their results, the EPS contents in granules increased with pH and the density and size of granules increased at pH of 8.1 compared to lower pH. Raposo *et al.* [18] reported that longer chain fatty acids were accumulated as an I:S ratio decreased. Lim *et al*. [38] showed the effect of the concentration of substrate on the partitioning of VFAs in an aerobic reactor. According to their research, the fraction of longer chain fatty acids increased as the concentration of substrate increased. Speece [39] stated that the elevated VFAs are due to the toxicity, overload of substrate, mass transfer limitation, and nutrients limitation. This suggests that methane yield was significantly inhibited at a low I:S ratio and shows why the optimal I:S ratio is indespensible to proper anaerobic digestion.

3.3. Calculations for sludge handling costs and biogas avenues at each I:S ratio

Apul and Sanin [20] calculated sludge handling costs and biogas revenues from anaerobic digestion with several assumptions. Assumptions to calculate sludge handling costs and biogas revenues at each I:S ratio were to be done by Apul and Sanin [20]. The currency used in these calculations was Euro and all assumptions used for calculations are shown in Table 5. Here the simple calculations were aim to represent sludge handling costs and biogas revenues at each I:S ratio at a WWTP already serving for 75,000 population equivalent. In order to simplify the calculation for sludge handling costs, other costs such as polymer, the electricity of dewaterbility, and labor were excluded. The total costs for handling sludge were 156,810, 151,718, and 160,752 €/yr at each I:S ratio, respectively (Table 5). Biogas revenues of secondary sludge at each I:S ratio were calculated on the basis of ultimate methane yield. The potential of methane production for primary sludge was 1.2 times as high as waste activated sludge [40]. Apul and Sanin [20] assumed that the electrical energy potential of digester methane was 6.5 kWh/m3 and the cost of energy was 0.077 €/kWh (average institutional electrical

Table 4. Characteristics of thickened sludge after BMP test

I:S ratio (mL/mL)	pH*	Volatile fatty acids (mg/L)*	VS reduction (%)*	Ultimate methane yield (mL CH_4/g VS_{added})*
1:1	8.0	121	32.4	51.39
1:3	8.2	86	38.6	76.27
1:8	6.4	186	27.6	21.93

*Data presented as mean of determination resulted from replicate.

Table 5. Assumptions for calculating biogas revenue from anaerobic digestion and calculation for sludge handling and biogas revenue under different I:S ratio conditions[a]

Parameter		Primary sludge	Secondary sludge	Total
Assumptions				
Total mass entering digester (dry tons/day)		3	3	6
Organic content (%)		65	75	
VS reduction (%)				
I:S = 1:1		50	32.4[b]	
I:S = 1:3		50	38.6[b]	
I:S = 1:8		50	27.6[b]	
Ultimate methane yield (mL CH_4/g VS_{added})				
I:S = 1:1			51.39[b]	
I:S = 1:3			76.27[b]	
I:S = 1:8			21.93[b]	
Cost of disposal (€/ton)		50	50	
Cost of transport (€/ton)		50	50	
Sludge handling costs				
I:S = 1:1	Disposal cost (€/yr)	36,956	41,446	78,402
	Transportation cost (€/yr)	36,956	41,446	78,402
	Total cost (€/yr)	73,918	82,892	156,810
I:S = 1:3	Disposal cost (€/yr)	36,956	38,900	75,856
	Transportation cost (€/yr)	36,956	38,900	75,856
	Total cost (€/yr)	73,918	77,800	151,718
I:S = 1:8	Disposal cost (€/yr)	36,956	43,417	80,373
	Transportation cost (€/yr)	36,956	43,417	80,373
	Total cost (€/yr)	73,918	86,834	160,752
Biogas revenues				
I:S = 1:1 (€/yr)		11,266	9,388	20,654
I:S = 1:3 (€/yr)		16,720	13,933	30,653
I:S = 1:8 (€/yr)		4,807	4,006	8,813

[a]WWTP serving for 75,000 population equivalent.
[b]Data taken from Table 4.

cost in EU), respectively. In addition, it was assumed that fifty percent of methane produced was used for heating and/or maintaining the anaerobic digester. Consequently, biogas revenues were 20,654, 30,653, and 8,813 €/yr at each I:S ratio, respectively (Table 5). Vesilind [41] stated that sludge handling and operating cost causes most costs (approximately 50%) in a WWTP. As shown in Table 5, The ratios of sludge handling costs at I:S ratios of 1:1, 1:3, 1:8 were 1.03:1.00:1.06, respectively. On the other hand, The ratio of biogas avenues at I:S ratios of 1:1, 1:3, 1:8 were 0.67:1.00:0.29, respectively. When considering sludge handling costs and biogas avenues at each I:S ratio, it is evidently shown that thickened sludge can be more effectively digested at an I:S ratio of 1:3.

4. Conclusion

The BMP test of thickened sludge at different inoculum/substrate ratios was evaluated. The greatest methane yield was achieved at inoculum/substrate ratio of 1:3, whereas the least was obtained at inoculum/substrate ratio of 1:8. The optimal condition for ultimate methane yield and methane production rate constant was approximately 0.4 mL/mL. The optimal inoculum/substrate ratio for inoculating thickened sludge was 1:3. At inoculum/substrate ratios greater than 1:3, methane yield also decreased as VFA production reduced pH and inhibited methanogenesis. The BMP test can be a useful tool for determining optimal inoculum/substrate ratios for inoculating anaerobic systems.

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