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A Critical Review on The Effect of Feed to Inoculum Ratio on Biogas Digestion

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ABSTRACT

This paper primarily focuses on the effect of feed to inoculum ratio on biogas digestion; and outlines the various feeds, inoculums, and synergistic effects of the combination of inoculums by referring to the literature. The Start-up of an anaerobic digestion system is highly critical and pivotal for the successful operation of an anaerobic digester. For this purpose, a certain amount of inoculum is added to the digester along with the substrate to provide the necessary microorganisms to initiate the digestion process. The ratio and the type of inoculum used substantially affect the rate of biodegradation and the lag time. The degradation of substrate depends on the concentration of microorganisms. When food waste was considered as the feed it was found that factors such as waste oil content, the addition of alkaline buffer, particle size, organic loading rate had a considerable effect on the feed to inoculum ratio. Studies considering some other feeds such as animal by products from piggery slaughterhouses, poultry slaughterhouse wastes, agro-industrial waste, anaerobically digested sludge obtained from municipal wastewater, and dewatered digestate cake were also compared. Hence, the objective of this study is to offer an integrated view of the appropriate feed and the inoculum under the effect of various other essential parameters. The major performance indicators from this study were found to be particle size of inoculum, organic loading rate, the addition of alkaline buffer, F/I ratio, and structure of inoculum

Keywords: biogas; inoculum; feed for biogas; feed to inoculum ratio

INTRODUCTION

In the recent past, anaerobic digestion of waste is a subject undergoing intense study in the field of biofuel production from biomass waste. Contemporary research is focused on efficiently improving the methane yield of biomass waste using anaerobic digestion. The motivation for the development of anaerobic digestion is also derived from serious a shortage of fossil fuels and a dire need for renewable and sustainable alternative fuels. According to BP Statistical Review of World Energy 2020. 2019 global in oil and coal consumption reached 5159 and 3749 million

tons oil equivalent, respectively, and their share of total primary energy consumption reached 63.8%. While renewables (wind, geothermal, solar, biomass and waste) consumption was just 716 million tons oil equivalent, accounting for only 12% of global primary energy consumption [1]. It is known that the burning of coal and oil releases too many gaseous pollutants, particulate matters and greenhouse gases into the environment and causes serious air pollution and global warming. These factors kindled research in the field of renewable, sustainable and cleaner energy sources, especially biofuel from biomass waste.

The start-up of an anaerobic digestion system is highly critical and pivotal for the successful operation of an anaerobic digester. For this purpose, a certain amount of inoculum is added to the digester along with the substrate to provide necessary microorganisms to initiate the digestion process. Rate of biodegradation, the lag time and the possible degradation of substrate depends on the concentration of microorganisms. Therefore, the substrate to inoculum ratio is an important parameter in anaerobic digestion [2],[3]. The choice of inoculum and proper maintenance of various other parameters controls the yield of biogas. The inoculum initially provides the biomass system with the microbial population, which will then participate in the reactions resulting the degradation of organic matter. It also contains several macronutrients which can positively affect enzyme activity and biogas production. The inoculum also modifies the degradation rate, digestion time, biogas composition and reactor stability [3],[4]. A wide variety of samples has been used for such purposes in other researches, including sludge from anaerobic digesters treating municipal or agro-industrial wastewater, animal manures, landfill leachate, etc.

Inoculum is a key parameter for effective biogas digestion, this inoculum provides microbes, and acts as a nutrient source for improving microbial activity. The wide varieties of inoculum referred in this paper are granular sludge, suspended sludge, seed sludge, activated sludge, digestate cake and many more.

Although theoretically, the F/I ratio has an effect only on the kinetics, and not on the ultimate methane yield, which only depends on the organic matter content [5],[6], it is reported that too high F/I may be toxic while too low F/I may prevent induction of the enzyme necessary for biodegradation [7]. Each substrate has its optimum F/I ratio, considering the potential amount of volatile fatty acids (VFAs) produced and its capacity to act as a buffer for the medium due to the ammonium production by the hydrolysis of proteins [8]. A small amount of inoculum is preferred for biogas production [8]. Another point to be kept in mind is that the inoculum concentration should always be high compared to that of the substrate (in term of volatile solids) and the F/I should be recognised as one of the major parameters affecting the results of anaerobic assays [9].

The purpose of this study was to evaluate the influence of parameters such as the feed to inoculum ratios and inoculum type, on the methane yield. The influence of the structure of inoculum and incorporation of one inoculum into another, have also been studied with the help of literature.

LITERATURE REVIEW

Feed or Waste	Inoculum	Feed/ Inoculum ratio's	Optimum: Feed/Inoculum ratio	Parameters	Yield	Reference Paper
Food waste: FW was collected from school canteen after which oil was extracted and it was used for adjusting the waste cooking oil ratio in FW, and the ratio was characterized by the concentration of the EE (Ether Extract) in the VS of the FW	Seed sludge obtained from the waste water treatment plant	F/I: 0.5- 1.2; EE/VS: 33%-53%	The optimum EE/VS and F/I ratios for the AD of FW are 43% and 0.70, respectively	F/I ratio's, EE/VS	The highest yield was achieved from FW with an EE/VS ratio of 43% and an F/I ratio of 0.70 and FW with an EE/VS ratio of 46% and a F/I ratio of 0.60, ranging from 1015 to 1035 mL/g VS.	[10]
Food waste	Incorporation of granular sludge (inoculum B) into flocculant sludge (inoculum A)	A:B mixtures were 100:00, 75:25, 60:40, 50:50, 40:60, 25:75, 00:100	An optimum inoculum mixture for the AD is 75:25 or 50:50 FW	Inoculum ratio's	Specific Methanogenic Activity (0.047- 0.19 g CODCH4 g VS-1 d-1)	[14]
Food waste: The composition of FW includes rice, vegetable, meat and oil, which were all easily biodegradable and perishable	Sewage sludge	S/I ratios of 1/2, 1/1 and 2/1	Optimum ratio: ¹ / ₂ , it exhibited better performance	Organic loading, S/I ratio and pH. Organic loads (5, 10, 20 and 40 g VS/L), S/I ratios (1/2, 1/1 and 2/1) and initial pH values (6.5, 7.5, 8.0 and 8.5).	Digester with lowest OL (5 g VS/L) and S/I ratio of ¹ / ₂ with pH of 7.5 resulted in highest methane yield of (551.4 mL/g VS)	[16]
Food waste: The first	Two inoculum types	Three ISR were	With larger PS of 5 mm, an ISR	PS and ISR	Inoculum-to- substrate ratios of	[19]

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and five NaHCO3 concentrati		reactor	$VS_{feedstock}/g$			-	
NaHCO3 concentrati						VS, respectively.	
concentrati							
ons (i.e., 0,							
			ons (i.e., 0,				

Kitchen Waste: Waste produced in the restaurant	Granular sludge and Suspended sludge	300, 500, 600, and 900 mg/g VS _{added}) 0.5–2.3 g VS/g VS	When a 2 mg NaHCO3/g COD and for waste/inoculum ratios beyond 0.5 g VS/g VS acidification occurred	Alkalinity/CO D ratios of 2 and 37 mg NaHCO3/g COD, inoculum, F/I ratio's	Specific methanogenic activity of granular sludge in the presence of H2/CO2 was observed as 1029±14 (ml CH4 (STP)/g VS waste per day)	[9]
Three species of seaweed such as Laminaria digitata, Fucus serratus, and Saccharina latissimi and on a non-marine cellulose biomass seeded with uncultivated and unadapted anoxic marine sediments	The first seed inoculum was anaerobic digested sludge obtained from municipal wastewater treatment plant, the second inoculum was anoxic surface sediments	Each culture composed of substrates to inoculum ratio (VS:VS) of 1:1	Theculturesseededwithdigestedsludgeshowedgreatresultswhencomparedwithculturesseededwithanoxicsurfacesediments	Inoculum	The specific methane yield for cultures seeded with digested sludge were respectively 256, 230, 103 and 270 dm ³ kg ⁻¹ VS _{added} .	[12]
Thickened Waste Activated Sludge (TWAS), Primary Sludge (PS), Source Separated Organics (SSO), and Cattle Manure	Digestate cake, liquid digestate	F/M ratio from 0.25 to 0.5	The specific methanogenic activity of the digestate cake $(5.0 \pm 0.5 \text{ mL-}$ CH4/g-VSS.d) was higher than that of the liquid digestate $(3.4 \pm$ 0.2 mL-CH4/g- VSS.d) for the food to microorganism ratio of 0.5	Inoculum	Maximum methane yield of 277 mL/g- TCOD _{added} was achieved for SSO with digestate cake as inoculum	[13]
A coarse-cut fodder maize: 2	Digester sludge from a	four different	$(r_{I/S} = 1)$ gave a higher maximum	Inoculum to	$\begin{array}{cccc} 23 & ml & CH_4 & g \\ VSS^{-1} & day^{-1} & for & a \end{array}$	[15]

mm sieve	municipal	inoculum	specific methane	substrate ratio	r _{I/S} of 1.	
	wastewater	to	production rate			
	treatment		F			
	plant	substrate				
	plan	ratios (3, 2,				
		1.5 and 1)				
		1.5 and 1)				
Four solid agro-	Three	Four SIR,	The optimum	Feed,	WW and JW,	[17]
industrial waste,	Three	i.e. 0.25,	SIR is 0.5 for	Inoculum, SIR	yielded 446.23	[1/]
namely	different	0.5, 1 & 2	WW and JW,	ratio's	and 445.97	
namery	inoculum,		and 0.25 for		anu 443.97	
winery waste	namely	(on a			NmLCH ₄ /gVS _{subs}	
(WW), cotton	anaerobic	volatile	CGW and OP		trate, respectively	
		solids (VS)			and CGW and	
gin waste	sludge (AS),	basis)				
(CGW), olive	landfill				OP, yielded	
pomace (OP)	leachate (LL)				267.96 and	
and juice	and thickened				258.65	
industry waste	anaerobic				Neel CIL / NG	
	sludge (TAS)				NmLCH ₄ /gVS _{subs}	
(JW)					trate, respectively	
	T 1 1 1					1101
Sugarcane	The sludge	Substrate	Optimum ratio is	Inoculum	The methane	[18]
distillery	come from the	to	ratio D	ratio's	potential of the	
wastewater	active	inoculum		_	vinasse (r <mark>atio D)</mark>	
(Vinasse)	mesophilic	ratios			is 109.58	
	biogas plant of	(gCOD/gV		ſ	Nl _{CH4} .kgCOD ⁻¹	
	the sugarcane	S) are 0.5			after 16 days	
	distillery	(ratio A),			with a	
	(Vinasse	0.75 (ratio			biodegradability	
	sludge)	B), 1 (ratio			of 0.31.	
		C) and 2				
		(ratio D).			For less	
					degradable	
				1	substrates, thus	
					the maximum	
					production was	
					obtained with the	
					ratio 1 for	
					I(VS)/S(VS).	
Different	Digested	S/I ratios	Optimum S/I	<mark>S/I rat</mark> io's	At the lowest S/I	[21]
piggery	piggery slurry	of 0.1, 0.5,	ratio is 0.1		ratio of 0.1 ,	
slaughterhouse		1.0, and			BMPs of piggery	
wastes, such as		1.5			blood, intestine	
piggery blood,					residue, and	
intestine					digestive tract	
residue, and					content were	
digestive tract					determined to be	
content					0.799, 0.848, and	
content					$1.076 \text{ Nm}^3 \text{ kg}^{-1}$ -	
					-	
					VS _{added} ,	
					respectively	
	1					L

7

Wastes of	Microbes as	ISR: 1, 2,	ISR: 4, TS: 5%	% of TS and	The biogas and	[11]
	primary	and 4		digester	methane yields of	
slaughter	inoculum and			volume	0.574 and 0.402	
industry, such	Activated				m3/kg-VS _{added}	
as sludge	Sludge as				respectively	
produced in	secondary					
wastewater	inoculum					
treatment						
plants as well as						
residues of						
livestock and						
poultry						
slaughterhouse						
-						
Two different	Granulated	Substrate	MA only and 2/3	Temperature	The highest	[23]
substrates from	sludge from	to	MA + 1/3 MPW	(35, 45, and	cumulative	
macroalgae	food industry	inoculum	as substrates	55 °C), and	biogas	
(MA) and		ratio (S/ X	have the highest	S/X ratio's	production (and	
market place		= 0.5, 2.0,	BMPs at 35° C		BMP) were	
waste (MPW).		4.0, and	and an S/X ratio		obtained for MA	
The substrates		6.0 as g	of 4.0 g		only at an S/X	
were used as		VS _{substrate} /g	VS _{substrate} /g		ratio of <mark>4.0 g</mark>	
MA only, MPW		VS _{inoculum}),	VS _{inoculum}		VS/g VS as 357	
only, MA-					L _{biogas} /kg VS	
MPW mixture,				1	(197 L CH4/kg	
pretreated MA,					VS) and 33	
and pretreated					L _{biogas} /kg VS (17	
MA-MPW					L CH ₄ /kg VS),	
mixture.					respectively, at	
					35 and 55 °C.	

A. Feed: Food Waste (FW)

Food waste is one of the common feeds used for biogas production due to its abundance and easy availability. When food waste is considered as feed the waste oil present in it must also be evaluated which may typically vary from 1% to 5% (wet basis) [24]. It has also been reported that waste cooking oil often results in higher biochemical methane production than carbohydrates and protein [25]. But, biodegradation processes of FW could be

inhibited by long-chain fatty acids (LCFAs), which are produced from waste cooking oil and can possibly cause toxicity to microorganisms [26]. Hence, when AD characteristics of FW containing different waste cooking oil and F/I ratios were investigated it was found that the optimum EE/VS and F/I ratios for the AD of FW are 43% and 0.70 respectively, as they resulted in the highest biogas yield and methane content [10].

The impact of different F/I ratios and adding external alkaline buffer on the biogas yield when vegetable waste was considered as feed have also been investigated. When an external alkalinity source was not added the results showed a negative relation between the biogas yield and the F/I ratio for F/I ratios of 0.5-2.0. At an F/I of 0.5, an optimum biogas and methane yields of about 851 and 306 mL/g VS, respectively were obtained. It was found that adding a buffer at this F/I ratio i.e 0.5 had no considerable effect on biogas and methane yields. Nevertheless, it was found that when the quantity of feedstock increases, a higher of concentration alkaline buffer i.e., NaHCO₃ must be added to maintain the stability of reactors. The optimum NaHCO₃ concentration was reported to be 300 mg/g VS feedstock at an F/I of 1.0. The highest biogas and methane yield was achieved when the reactors were operated at an F/I of 2.0 [22].

The particle size of the inoculum also has an effect on the methane yield. It has been suggested that particle size (PS) reduction improves the anaerobic degradability of food waste. For smaller PS of 1 mm and 2 mm, a combination with an ISR of 3:1 and 4:1 helped to stabilize the systems, while with a larger PS of 5 mm, an ISR of 2:1 was most suitable [19]. B.A. Parra-Orobio and team found out that incorporating granular sludge into flocculent sludges benefits the anaerobic digestion process of FW. Improvements were observed in aspects such as the contribution of the buffer capacity and trace elements, higher hydrolytic activity, lower specific acidogenic activity, and higher specific methanogenic activity [14]. The advantage of using a granular sludge was further studied in order to define the reasonable condition of waste/inoculum ratio and added alkalinity that could be applied in practice by L. Neves et al. [9] using kitchen waste as feed at waste/inoculum ratios between 0.5 and 2.3 g VS/g VS, for alkalinity/COD ratios of 2 and 37 mg NaHCO3/g COD. A granular sludge and a suspended sludge with a significantly higher methanogenic activity were compared. It was found that the use of granular inoculum prevented acidification during the anaerobic batch biodegradation for waste/inoculum ratios in the range of 0.5–2.3 g VS/g VS, when the alkalinity/COD ratio was 37 mg NaHCO₃/g COD. Whereas, in similar conditions using a suspended sludge, the methane production rates and biodegradability were found to be significantly lower. It was also established that when the added alkalinity was decreased to 2 mg NaHCO₃/g COD, the ratio of waste/inoculum was clearly more important than the inoculum activity, since, irrespective of the sludge used, acidification occurred at waste/inoculum ratios higher than 0.5 g VS/g VS.

Most studies conducted by researchers examined the effects of a single operating parameter on batch anaerobic digestion of while combined/compound food waste, effects of organic load, S/I ratio, and pH adjustment have been less studied. Zhang et al. [16] studied the dynamic behaviors of batch anaerobic digesters treating FW under different conditions comprehensively and systematically. There was more emphasis on the effects of three key operating parameters including organic load, S/I ratio, and initial on methane production, pН organics destruction, and process stability. Methane yield was proved to be inversely proportional to OL and S/I ratio. Digester with lowest OL (5 g VS/L) obtained the greatest methane yield (551.4 mL/g VS), highest organics removal (94.1%) and good stability. g VS/L Enhancing OL to 10 was recommended for satisfactory stability and higher volumetric methane productivity.

B. Other feeds and inoculums:

Obata, et al [12] probed into the intrinsic biodegradation potential of marine organic sediment for biogas production from various species of marine macroalgae and nonmarine biomass. Biogas production potential tests were carried out on three species of seaweed namely, Laminaria digitata, Fucus serratus, and Saccharina latissima, and on a non-marine cellulose biomass seeded with uncultivated and unadapted anoxic marine sediments. As a comparison, the same experiments were also carried out using the same substrates but seeded with active mesophilic anaerobically digested sewage sludge. The highest methane yield was observed in both L. digitata and S. latissima for the cultures seeded with anoxic marine sediments, cultures while F, serratus and cellulose performed relatively poorly. For those seeded with digested sludge, all cultures performed relatively well, except F. serratus. Marine biomass, such as seaweed, offers an attractive option for producing renewable energy in a more sustainable manner. Seaweeds have a number of advantages over terrestrial biomass as a source of renewable energy. These include the ability to efficiently fix CO2 faster than most terrestrial plants, lack of lignin, which makes up a bulk of terrestrial biomass, thereby making it a relatively easier material for bioconversion, and its cultivation does not require arable land or freshwater. Hence, it was concluded that marine sediments can be an effective inoculum for seaweed digestion.

E. Hosseini Koupaie et al. [13] compared, the application of liquid and dewatered digestate cake for the inoculation of the (AD) process. Four different types of municipal

and industrial waste streams namely, primary sludge, thickened waste activated sludge, source separated organics and cattle manure were compared. It was established that the specific methanogenic activity of the digestate cake was higher than that of the liquid digestate for the food to microorganism ratio of 0.5. The BMP results also revealed that regardless of the type of substrate used, the application of the digestate cake inoculum achieved as statistically significantly higher methane production rate compared to the utilization of liquid digestate, most likely due to the lower concentration of dissolved contents (i.e., ammonia, soluble organic matter, heavy metals, etc.) in the diluted digestate cake. The findings of this study suggest that the digestate cake can be used as an effective alternative to the liquid digestate for the inoculation of full-scale anaerobic digesters.

The influence of different substrate to inoculum ratios (SIR) and inoculum types on the methane potential of four solid agroindustrial waste, namely winery waste (WW), cotton gin waste (CGW), olive pomace (OP), and juice industry waste (JW) were investigated by F.-M. Pellera and E. Gidarakos. Four SIR, i.e. 0.25, 0.5, 1 and 2 (on a volatile solids (VS) basis) were tested and three different inocula, namely anaerobic sludge, landfill leachate, and thickened anaerobic sludge, were compared. All four materials were proved viable substrates for anaerobic digestion. Furthermore, anaerobic sludge was found the most adequate inoculum among tested samples, and due to its high availability, it may be considered a viable choice in real-scale applications. Contrarily, using landfill leachate and thickened anaerobic sludge for the same purpose showed lower efficiencies. The optimum SIR for determining the methane potential of the studied substrates were of 0.5 for WW and JW, yielding 446.23 and 445.97 NmLCH4/gVS substrate, respectively, and of 0.25 for CGW and OP, yielding 267.96 and 258.65 NmLCH4/gVS substrate, respectively. It was found that higher SIR delayed methane production, indicating process inhibition [17].

When activated sludges and sugarcane distillery wastewater (vinasse) were studied by Helene Caillet et al [18] it was found that the production of methane increases with the decrease of this ratio, thus the maximum production was obtained with a ratio 1.

In another study by Yoon et al [21] investigated animal byproducts from piggery slaughterhouses as plausible alternative sources for biogas production. The study primarily focused on the effects of S/I ratios on BMP tests and anaerobic biodegradability (D_{deg}). Different piggery slaughterhouse wastes, such as piggery blood, intestine

residue, and digestive tract content were considered. At the lowest S/I ratio of 0.1, methane yield of piggery blood, intestine residue, and digestive tract content were determined to be 0.799, 0.848, and 1.076 Nm3 kg-1 -VS added, respectively, which above the theoretical methane were potentials of 0.539, 0.644, and 0.517 Nm³ kg-1 –VS added for blood, intestine residue, and digestive tract content, respectively. But, methane yield at higher S/I ratios of 0.5, 1.0, and 1.5 was not significantly different for the different S/I ratios tested. These results imply that, for high methane yield, the S/I ratio of anaerobic reactor should be above 0.1 and the inoculum should be sufficiently stabilized. Anaerobic co-digestion of poultry slaughterhouse wastes with slaughterhouse sewage sludge was investigated by P. Latifi et al¹¹. Slaughterhouse wastes contain high amounts of organic matter and protein, which is a beneficial attribution however, anaerobic digestion of this waste alone results in the accumulation of fatty acids which inhibit the process of biogas production. Such negative effects on anaerobic digestion can be greatly reduced using co-digestion. The optimal condition for the co-digestion of slaughterhouse wastes with the sludge was found to be ISR of 4 and TS of 5%. The parameters considered and

material used were scaled up by 20 times and similar results were obtained. It was also found out that decreasing the ISR any further or increasing the TS could lead to the accumulation of fatty acids and ammonia, which would severely undermine the yields of biogas.

Hulya Civelek Yoruklu and team considered macroalgae (MA) and marketplace wastes (MPW) as substrates. Here combinations of these substrates namely MA only, MPW only, MA–MPW mixture, pretreated MA, and pretreated MA-MPW mixture was studied for the effects of parameters such as temperature (35, 45, and 55° C) and substrate to inoculum ratio (S/ X) = 0.5, 2.0, 4.0, and 6.0 as g VSsubstrate/gVSinoculum). The highest cumulative biogas production was observed for MA only at an S/X ratio of 4.0 g VS/g VS as 357 L_{biogas}/kg VS (197 L CH₄/kg VS) and 33 L_{biogas}/kg VS (17 L CH₄/kg VS), respectively, at 35° and 55° C. For pretreated substrates, the highest cumulative biogas production and BMP were observed as 287 L_{biogas/} kg VS and 146 L CH₄/kg VS using pretreated macroalgae at 35° C. It can be concluded from this study that for such types of substrates pretreatment negatively affects the biogas production [23].

RESULTS AND DISCUSSION



The highest methane yielding feed from the graph was found to be found to be digestive tract contents of piggery slaughter house among the various feeds considered, this is because of high organic content intact in this type of feed. After piggery slaughter house, food waste is the most promising feed in achieving good amount of biogas when essential parameters like co-digestion, incorporation, particle size are maintained properly.

CONCLUSION

Some of the plausible suggestions with regard to the feed and inoculum are listed as follows; firstly, if the feed was co-digested the yield was found to increase. A possible reason asserted by the authors was that it provided a more balanced provision of

feedstock needed by anaerobic microorganisms and it also diluted the toxic and inhibitory substances. It was also reported that using granulated sludge as inoculum resulted in much higher biogas yield as compared to the conventional sludge. Another choice in terms of the growth process would be between suspended and attached. Suspended growth process takes skill and demands continuous control to maintain good structure and efficiency. Whereas attached growth processes are easier to maintain but can only be used for small-scale treatments. Most studies conducted by researchers examined the effects of a single operating parameter on batch anaerobic digestion of food waste, while combined/compound effects of organic load, S/I ratio, and pH adjustment have been less studied so future research should be directed in this.

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