Lignocellulose digestate from the solid-state reactor -methanogen colonization and 'end-of-life' use as inocula source

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Abstract: Solid-state digestion is the preferred route for the anaerobic digestion (AD) of a solid substrate. Spent bio-digester liquid (BDL)/ cow dung remains the popular choice as the inocula source for these reactors. Alternative of spent biomass as a source of inocula would reduce the dependency on water/ liquid as a medium for harboring the bacterial population. It would increase the "end-of-life" use of spent biomass, increase the economy of the transportation required for inocula procurement at the reactor site, and reduce reactor start-up and operational costs. Bacterial colonization on the spent biomass/lignocellulose is known, but literature has focussed on microscopic imaging and diversity studies, efforts at quantification and potential use have been very limited. This study dwells deeper into the methane production potential of the digested lignocellulose. Six lignocellulosic species (4 agro residues, 2 dicots) were digested for a period of 94-95ds in a solid-state, leach bed reactor. The time profile of methanogen colonization on digesting lignocellulose was computed. High colonization with the activity of up to 53 l methane/kg digestate TS/d was obtained in *Broussonetia papyrifera* (dicot) and up to 46 for *Oryza sativa* (agro residues). Results indicate the potential of spent lignocellulose for use as a source of inocula in the treatment of wastewater in a packed bed reactor, and AD of the solid substrate. Based on the results, the study proposed the use of SMA to quantitate the methane production potential of the available source of spent biomass, to further calculate the achievable OLR, and/or inoculation needs of SSAD operated with spent biomass as an inocula source. To calculate the required quanta of the spent biomass, the study proposed the use of 1) S/I analog -kg feed TS/kg digestate TS, 2) SMA/S- TSMA required/kg feed TS. High activity colonization/biofilm formation on spent lignocellulose also renders it useful as natural/renewable alternative for support media and application in bioreactors fed with gas-based substrates. In this light, when lignocellulose is specifically used for the growth of biofilm, the representation of colonization as "l of methane/ kg TS fed/d" has more relevance. Data in the study suggests, *Zea mays, Oryza sativa, and Sorghum* with 17-20 l methane/kg TS fed/d, should be preferred for use as a natural support media for biofilm growth. The study also used the obtained results to compare the total VS production flux from the digesting feedstock in the reactor with the total VS handling potential of the colonized methanogens (computed from TSMA). This was studied to evaluate the implications of the methanogen colonization on the inoculation needs of the fed-batch reactor. The data indicated, VS flux handling potential from the colonized methanogens matched or surpassed the required VS flux handling potential from the digesting feedstock in SSBR in 18-39ds. The SRT at which this ratio becomes greater than or equal to one was coined as the "equalization point". In theory, methanogenic activity from the colonized methanogens should render inocula redundant beyond this equalization point. This opens a new direction to optimize SSAD operation, where the methanogenic colonization on the digesting feedstock, reduces the inoculation needs of the reactor in time. This implies, beyond the inoculation point, methanogens in the reactor should be in a state of starvation, this opens the possibility of a higher feeding rate or increased reactor feeding volume (by reducing the inocula occupied volume).

Introduction

Solid-state Anaerobic Digestion (SSAD) has recently gained popularity because of the smaller reactor volume required for a unit of waste stabilized. Further optimization of the SSAD mandates optimization of the inoculation needs of the reactor. Spent bio-digester liquid/cow dung remain the popular source of inocula. Commercial SSAD- KOMPOGAS, DRANCO, VALORGA, use the digested residue, premixed with feed for inoculation (Six & de Baere, 1992; Wellinger et al., 1993). However, a scientific basis/rationale for the selected digested residue to fresh feed ratio is often not presented in the literature. To move forward in the degradation of a solid substrate via SSAD, 2 things become important. First, push the frontier of achievable quanta of feed per unit reactor volume (either through higher OLR or through lower inocula occupied space). Secondly, a defined basis (S/I analog for SSAD) for OLR and inocula calculation.

To take this forward, this study investigated the alternative of spent lignocellulose as a source of inocula in SSAD. Colonization of the bacteria on lignocellulose has been observed in the literature (Brethauer et al., 2020; Chanakya & Khuntia, 2014; Dumitrache et al., 2013), however efforts for quantification were not undertaken. Studies were focussed on microscopic imaging and tapping the diversity of the colonized bacteria. Chanakya et al., 1997, made efforts to quantitate the colonization by studying the methanogenic activity, but the study was in

its preliminary stages. The designed protocol has been used as the basis of this study. This study builds upon the work of Chanakya et al., 1997, and takes the frontier forward, by 1) quantitating the TSMA not only per unit digestate TS but also on expected methane production potential per unit of TS fed, 2) studying the dynamics of the VS production flux and VS flux handling potential of the colonized methanogens as a function of time, 3) proposing a S/I analog for inoculation with spent biomass in SSAD.

Materials and methods

A solid-state stratified bed reactor (SSBR) design detailed by Chanakya et al., 1999, was operated in a fed-batch mode for a period of 94-95ds. Total solids (TS) and volatile solids (VS) of the feedstocks were quantitated using the standard protocol detailed in APHA (1999), standard manual. To study the methanogenic potential of the colonized methanogens, a specific methanogenic assay (SMA) protocol detailed by Chanakya et al., 1997, was employed (Table 1) Gas production was quantitated using downward displacement of acidified water, and gas quantification was done using GC.

Table 1 SMA experimental conditions

Test	Incubation conditions	Incubation period (hrs)
Acetoclastic SMA (ASMA)	2g of digested feedstock (sample) + 40ml anoxic water + 20mg sodium acetate.	48
Hydrogenotrophic (HSMA)	2g. sample + 2ml of anoxic water + 20ml injected H_2 and 5ml CO_2. Sample spread into a thin layer and packed in a nylon mesh to enable the maximum surface area of contact for colonized methanogens to H_2.	6
TSMA	HSMA+ASMA	
Blank	2g sample + 40ml of anoxic water.	48

Results and discussion

<u>*Methanogen colonization, use as inocula and natural support for biofilm growth*</u>: Table 2, represents the results summary of the data obtained from the computed time profile (d 4-95) of the SMA on digested lignocellulose. TSMA results have been represented on basis of the per-unit digestate TS and per unit feed TS. Representation on per unit digestate TS is relevant for 'end-of-life' use of the digestate as inocula (source of methanogen). Agro residues in the study recorded 36-46 l methane production potential /kg digestate TS/d except for *S. officinarum*, where the rates were lower (18 l methane/kg digestate TS/d). In dicots, *T. stans* recorded TSMA of 20 while *B. papyrifera*, which recorded the highest TSMA at 53. Based on the results, agro residues-*Z. mays*, *Sorghum*, and *O. sativa* seem to be better support media for the colonization of the methanogens. Further, slow degradation (i.e., higher half-life), retention of bed porosity and tissue integrity of the spent agro residues for longer periods makes them a preferable source of inocula compared to high compacting *T. stans* and *B. papyrifera*. The study proposes the use of 1) "S/I analog"–kg TS fed/kg digestate TS, 2) "SMA/S" –(l TSMA required/d)/(kg TS fed), for reactor design calculations- operating OLR, inoculation needs. Considering lignocellulose for the specific purpose of growing biofilms, TSMA representation on a per unit TS fed basis is more relevant. Agro residues (*Z. mays, Sorghum, O. sativa*) with TSMA of 17-20 l methane production potential/kg TS fed/d are better suited for use as natural support alternative for biofilm growth.

<u>*Implications of the colonization on the inoculation needs of the reactor*</u>: The SRT beyond which relative VS flux handling potential of total colonized methanogens in the fed-batch reactor equals or surpasses the required VS flux handling potential from the total digesting feedstock in the SSBR was coined as the 'equalization point'. In theory, inocula should be rendered obsolete beyond this point. The equalization point was obtained between 18-39ds for the studied feedstocks. The concept of equalization point opens a new direction of increasing OLR beyond equalization point or increasing the available reactor space for feeding by reducing the inocula occupied space.

Table 2 Summary table					
Category	Feedstock	Max. TSMA		Ratio of relative VS flux handling potential from quantitated TSMA to VS flux production rate*	
		l methane/kg digestate TS/d	l methane/kg TS fed/d	SRT (ds) (Equalization point)	TSMA/VS flux*
Agro residues	*Oryza sativa*	46	20	25	2.7
	Saccharum officinarum	18	11	18	1.1
	Sorghum	42	20	24	3.6
	Zea mays	36	17	38	1.0
Dicots	*Broussonetia papyrifera*	53	13	39	1.0
	Tecoma stans	20	9	18	1.0
*Working assumption =Entire VS flux produced is converted to biogas, with methane content of 70%. Therefore,70% of produced VS flux is used for calculations for comparison with TSMA.					

Conclusion

In this study, methanogenic colonization on the lignocellulosic digestate was studied using the methane production potential of the digestate. Six lignocellulosic feedstocks (4 agro residues and 2 dicots) were considered for the study and the potential 'end-of-life' use of the digestate as an inocula source was investigated. High activity of up to 53l methane/kg digestate TS/d was recorded for *B. papyrifera* (dicot) and up to 46 for *O. sativa* (agro residues). In this light, the use of SMA (a 48hrs short experiment) was proposed to be employed for evaluating the methane production potential of the spent biomass resource available for inoculation. Further, the study proposed two concepts that can be employed to calculate the reactor's feeding rate and inoculation needs with spent biomass as an inoculation source- 1) "S/I analog" for solid-state reactor – kg feed TS/kg digestate TS, 2) "SMA/S"- (TSMA(l/d) of the spent biomass)/(kg feed TS). Slow degrading agro residues (*O. sativa, Z. mays*, and *Sorghum*) with higher relative shelf-life/ half-life, were recommended as a potential natural/ sustainable alternative for biofilm growth. These natural biofilm carriers can also be employed in the gas-fed reactor- like bio-methanation reactor for carbon dioxide capture and conversion to methane. Based on the results of colonization, the relative VS flux production from the digesting feedstock was computed to the VS handling potential of the methanogens colonized on the digestate in the reactor. Between 18-39 ds, activity of the colonized methanogens on the studied lignocellulose equalized or surpassed the required VS handling potential for the reactor (conversion to biogas, with 70% methane content). This SRT required for equalization of the VS flux was coined as the 'equalization point'. This indicates, in theory, beyond the equalization point, methanogens are in a state of starvation and should be able to support higher operating OLR. Alternatively, inocula occupied volume can be diverted for feeding to increase the quanta of introduced feed. The proposed direction shows promise as the next step in SSAD optimization and should be explored in detail.

Reference

- Brethauer, S., Shahab, R. L., & Studer, M. H. (2020). Impacts of biofilms on the conversion of cellulose. *Applied Microbiology and Biotechnology*, *104*(12), 5201–5212. https://doi.org/10.1007/S00253-020-10595-Y
- Chanakya, H. N., & Khuntia, H. K. (2014). Treatment of gray water using anaerobic biofilms created on synthetic and natural fibers. *Process Safety and Environmental Protection*, *92*(2), 186–192. https://doi.org/10.1016/J.PSEP.2012.12.004
- Chanakya, H. N., Srikumar, K. G., Anand, V., Modak, J., & Jagadish, K. S. (1999). Fermentation properties of agro-residues, leaf biomass and urban market garbage in a solid phase biogas fermenter. *Biomass and Bioenergy*, *16*(6), 417–429. https://doi.org/10.1016/S0961-9534(99)00015-X
- Chanakya, H. N., Venkatsubramaniyam, R., & Modak, J. (1997). Fermentation and methanogenic characteristics of leafy biomass feedstocks in a solid phase biogas fermentor. *Bioresource Technology*, *62*(3), 71–78. https://doi.org/10.1016/S0960-8524(97)00139-9
- Dumitrache, A. D., Wolfaardt, G., Allen, G., Liss, S. N., & Lynd, L. R. (2013). Form and function of Clostridium thermocellum biofilms. *Applied and Environmental Microbiology*, *79*(1), 231–239. https://doi.org/10.1128/AEM.02563-12/SUPPL_FILE/ZAM999103975SO1.PDF