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IMPROVEMENT OF THE METHANE GENERATION POTENTIAL OF THE ANAEROBIC DIGESTION SOLID RESIDUE BY IMPLEMENTING A BIOLOGICAL POSTTREATMENT WITH THE BIOMYCETE *IRPEX LACTEUS* AT VARIOUS CONDITIONS

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ABSTRACT

Biological pretreatment of different digestates with *Irpex lacteus* was employed to investigate the potential of the fungal strain to exploit any digestible organic matter in the spent anaerobic digestion effluent in methane recovery. In all cases, pretreatment of sludge with the non-selective *I. lacteus* promoted methane generation. Incubation of *I. lacteus* in digestate for 20 days at 28°C increased the methane generation potential of the sludge by 1.67-fold. Prolonging incubation time to 30 days led to an increment in methane yields (1.24-fold), however, the increase was lower than that accomplished at a shorter pretreatment period (20 days) due to a substantial loss of fermentable organics, especially cellulose. Regardless the digestate type, inoculation with *I. lacteus* enhanced the digestibility of sludge by at least 1.6-fold. The degree of improvement was depended on the amount of lipids and proteins supplied by the fungus when the modified sludge contained a small carbohydrate proportion and on the availability for digestion of cellulose when the pretreated digestate was rich in carbohydrates.

1. INTRODUCTION

Anaerobic digestion (AD) is the biological process in which microorganisms decompose an organic substance in an oxygen free environment to produce biogas and a solid effluent called digestate. Recently, AD was implemented for exploitation of a host of organic material streams ending up in a large number of biogas plants worldwide and global production of enormous quantities of digestate. Depending on process parameters, the AD by-product is generally composed of 80-97%wt. of liquid and 3-20%wt. of solid fraction, both of which are organic fertilizers with slight differences in their fertilization capacity. Storage expenses accompanied with additional costs of collection, handling and transportation obstruct its extensive valorisation in soil amelioration. Storage in open tanks may also contain concerns because of the existence of uncontrolled biogas release risks (Sambusiti et al., 2015). Depending on the source of feedstock, digestate may encloses heavy metals like Cu, Cr, Zn and As and pathogenic microorganisms such as *Escherichia coli, Salmonella spp.* etc. that can accumulate in the environment and due to their toxicity hazard human health (Swain et al., 2016, Adamse et al., 2017). Despite the several studies conducted on exploitation of digestate in soil remediation and energy sector, the management of the AD effluent still comprises challenges that need to be addressed (Guilayn et al., 2020).

Digestate contains organic substances recalcitrant to AD of bacteria and non-degradable during the process. Lignin is non-degradable whereas by being chemically bonded to cellulose and hemicellulose it prevents the plant cell wall polymers degradation (Zanellati et al., 2020). In addition to lignin, cellulose degradation is limited by its crystallinity (Musatti et al., 2017). Therefore, cellulose and lignin accumulate in digestate while hemicellulose is the substance mostly removed from the material during AD (Sambusiti et al., 2015). In total, a 40-70%wt of biomass is converted to produce biogas throughout AD process whereas 35% of the organic matter remains in the final sludge (Uludag-Demirer and Demirer, 2022). White rot fungi (WRF) are considered to be the most effective lignin-degrading microorganisms in nature (Wan and Li, 2010) and their activity could destruct the complex structure of lignin and recover monomeric sugars existing in the

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carbohydrate fraction of a digestate that could be subsequently implemented in biogas production. Even though fungi grow at a slower rate in a digestate than in a raw biomass, methane generation potential of digestates has been observed to be altered with WRF treatment (López et al., 2013). It is indicative that a pretreatment of a digestate with *Trichoderma reesei, Myceliophthora thermophila and Phanerochaete chrysosporium* can rise methane yields by 35 to 50% (Götze at al., 2022), whereas an increment of 71.5% has also been reported for a solid fraction of digestate pretreated by *Cephalotrichum stemonitis* (Zanellati et al., 2021). In contrast, in a number of instances, inoculation of digestate with WRF such as *Pleurotus ostreatus* was reported to have an opposite impact reducing the capability of AD effluent to produce methane, whereas in other cases methane generation capability of a digestate was not influenced by the operation of fungi like *Phanerochaete flavido-alba* (Brémond et al., 2020). It is apparent, therefore, that the manner that a pretreatment with fungal species will impact the methane generation capacity of a digestate could not be easily predicted.

WRF can be classified as selective and non-selective, with the former mineralizing lignin and partly hemicellulose and cellulose and with the latter arbitrarily consuming all components of lignocellulose (Basinas et al., 2024; Wan and Li, 2010). Selectivity of a fungal strain is interconnected with the incubation time, both of which largely determine the influence of a fungus on the digestibility of a material (Albornoz et al., 2018). For non-selective species, a prolonged incubation time may lead to an excessive loss of digestible organics and eventually lessen the biodegradability of a substrate (Zanellati et al., 2020). Mustafa et al. (2016) pretreated rice straw with *T. reesei* ascertaining that methane yield rose by 45% with the increment of incubation time from 10 to 20 days, whilst methane output reduced with the extension of time to 30 days. Likewise, the non-selective basidiomycete *I. lacteus* showed a better potential to improve the biogas generation capacity of a biomass when pretreatment conducted for short periods and negatively affected the methane production potential of corn silage when pretreatment lasted between 10-30 days (Basinas et al., 2022; 2024). Digestate constitutes a spent AD effluent with a high proportion of lignin and a low fraction of available for anaerobic conversion soluble components. A pretreatment with *I. lacteus* at relatively short periods could provide a depolymerization of lignin and a recovery of other heteropolymers such as cellulose and hemicellulose that were bounded in the material. Nevertheless, the application of *I. lacteus* for the pretreatment and improvement of the AD performance of a digestate has attracted limited attention.

A parameter modifying the efficiency of a fungus to delignify a biomass material and improve its digestibility is related to the physicochemical properties of the parent lignocellulose (Xu et al., 2020). Individual AD effluents practically possess diverse physical and chemical characteristics due to the varying feedstocks and AD systems implemented for their production. Alike biomass, therefore, a treatment of two individual digestates with the same fungus would affect their methane generation capabilities at a different extent. The level that the pretreatment of two differently derived digestates by *I. lacteus* methodology is influenced by the parent substrate used for the generation of digestates has not been investigated to any great extent.

In the current work, two AD effluents were treated with *I. lacteus* to determine the potential of biological treatment to eliminate the recalcitrance of a sludge in AD and improve the methane generation capacity of the spent AD effluent. One of the main objectives was to determine the pretreatment time required for the non-selective basidiomycete *I. lacteus* to bring an advancement in the digestibility of a digestate. Another aim was to clarify the impact of the pristine feedstock used for the production of a digestate on the efficiency of *I. lacteus* to improve the methane yields from the sludge. The proposed digestate treatment followed by recirculation of the modified digestate into a biogas reactor is expected to provide a significant advancement in anerobic digestion efficiency.

2. MATERIALS AND METHODS

2.1 Fungus and digestate

The non-selective basidiomycete *Irpex lacteus* 617/93 used for the modification of digestates was provided by the Czech Collection of Basidiomycetes (Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague) whereas cultivated on malt extract/glucose (MEG) agar plates (5 $g.L^{-1}$ malt extract, 10 $g.L^{-1}$ glucose, 20 $g.L^{-1}$ agar).

The reference digestate referred to as the mixed (industrial) digestate (MD) was a residue obtained from an agricultural biogas plant (Pustějov II - Zemspol Studénka Ltd). The plant was operated at mesophilic conditions (40°C) with a mixture of corn silage and cattle slurry and with an organic loading rate (OLR) at 3.30 $kg_{VS}m^{-3}d^{-1}$ and hydraulic retention time (HRT) at 36 days. A second digestate (CSD) was prepared from mono-digestion of corn silage in a laboratory 60 L reactor at similar conditions to the biogas plant, i.e. mesophilic temperature (40°C), OLR at 3.37 $kg_{VS}m^{-3}d^{-1}$ and HRT at 36 days.

2.2 Fungus cultivation

20 g of digestate particles were placed in 250-mL Erlenmeyer flasks, moisturized and sterilized at 121°C for 60 min (Systec VX-5, Germany). Digestates were inoculated with 1 mL of fungal inoculum, preparation of which was described in an earlier study (Basinas et al., 2022). Following a method described by Rouches et al. (2016), 25 mg of glucose and 2.5 mg of diammonium tartrate per g of total solids (TS) were added in flasks which sealed with cotton plugs. Solid state fermentation (SSF) cultures were incubated statically for 20 and 30 days at a temperature of 28°C.

2.3 Methane yields and biodegradability determination

2.3.1 Inoculum

Inoculum for the AD tests was a liquid effluent from the first stage of the same biogas plant that provided the MD. A centrifuge (BEHO CHC-61A) operated at 25°C and 1200 rpm was used to reduce the TS concentration in inoculum from 7.22 (as received) to 2.65%wt. Inoculum main properties were: volatile solid (VS) concentration at 74.31%wt_{TS}, C/N ratio at 11.42 and pH at 7.51. Prior to AD assays inoculum was stored at 4°C.

2.3.2 Biochemical methane potential (BMP) assays

BMP tests were conducted in 1-L glass bottles connected with burettes allowing for a volumetric measurement of the produced biogas (Figure 1). AD tests were performed at mesophilic conditions (40±0.5°C) for 40 days. Stirring was applied for all experiments. For the BMP assays, 800 g of inoculum were placed in a glass reactor and mixed with 30 g of substrate. MD and the same material pretreated with *I. lacteus*, at 28°C for 20 and 30 days were the substrates utilized for the investigation of the impact of the pretreatment time on the methane generation capacity of digestate. The impact of digestate type and origin was assessed in a glass reactor using a second AD effluent (CSD) as the substrate and an additional run using as the feedstock the same sludge after pretreatment with *I. lacteus* (28°C, 20 days). Biogas generation potential of inoculum was determined in a blank run with 800 g of inoculum in a reactor and was considered in the estimation of the amount of gas that actually was produced from each substrate. Biogas production potential of fugus was considered insignificant given the low growth and the limited quantity in reactors compared to the substrate and AD inoculum. To validate tests repeatability each experiment was triplicated. Deviations among recurring tests were minimal in all cases and the mean values were used in graphs. Before tests, reactors were purged with nitrogen for sufficient time to ensure that anaerobic conditions were established. A gas analyser (Geotechnical Instruments Biogas5000) equipped with dual-wavelength IR sensors was utilized for determination of biogas composition (CH_4, CO_2, O_2, H_2, and H_2S). Biogas volume and composition were monitored on a daily basis and the obtained data was normalized to 0°C, 1 atm and dry conditions.

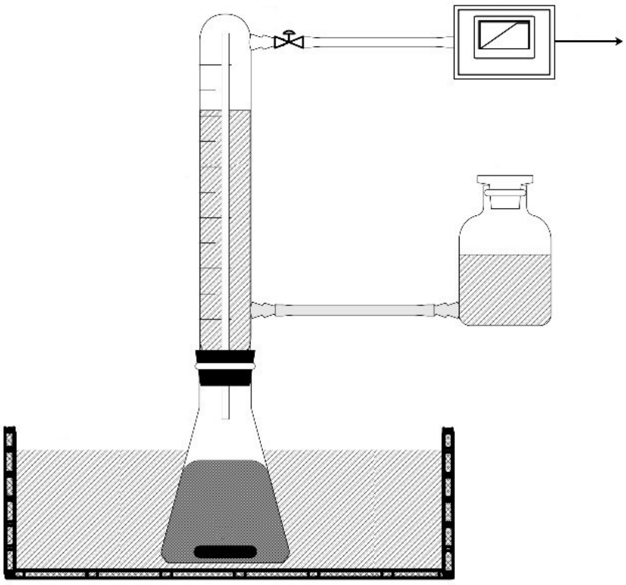

FIGURE 1: Representation of the experimental setup for BMP tests (Basinas et al., 2024).

2.4 Physico-chemical analyses

A laboratory mill (IKA Tube Mill Control) was used for the homogenization of digestates. The pH was measured in a WTW 340i pH-meter with SenTix 410 sensor. TS concentration was determined in a KERN DLB 160 3A moisture analyser (EN 15934:2012 standard). VS content was determined in a LECO TGA 701 thermogravimetric analyser by measuring the loss on ignition of the material at 550°C. Elemental analysis (C, H, N, S) for the determination of the C/N ratio was conducted in a LECO Truspec CHN and S628 elemental analyser (ASTM D5373-16 standard). Lipids were determined by the EN ISO 6492:1999 standard, carbohydrates according to the ASTM D5896-96, proteins by titration based on the EN ISO 5983:1997, starch content by applying the EN ISO 6493:2000 and fibers following the EN ISO 6865:2000 standard method. Cellulose, hemicellulose and lignin concentrations were assessed according to a method proposed by Van Soest (1991).

3. RESULTS AND DISCUSSION

3.1 Digestate properties and degradation with fungal pretreatment

The results for the main physicochemical characteristics of untreated digestates appear in Table 1. VS and TS proportion in MD (71.59%wt_{TS} and 7.22%wt) and CSD (68.28%wt_{TS} and 8.24%wt) (as received) agreed with values appear in the literature for similar digestates (Sambusiti et al., 2015; Zanellati et al., 2020; 2021; Zhong et al., 2016). TS fraction increased to 94.08 and 93.69%wt. in digestate MD and CSD, respectively with the drying applied prior to the treatment with the fungus (Table 1). The pH values of MD (8.61) and CSD (8.14) (as received), were inside an alkaline range, which is typical for AD effluents (Brémond et al., 2020; Zanellati et al., 2020; 2021). The pH of MD and CSD dropped with drying to 6.63 and 6.97, respectively and sludges converted to neutral or slightly acidic before treatment with the fungus. According to earlier reports (Brémond et al., 2020), the pH intervals facilitating fungal growth encompass a neutral and a part of the alkaline range (6.5-8.7), hence the dried sludges would be foreseen to be suitably colonized by *I. lacteus*.

MD and CSD contained a great proportion of holocellulose (cellulose and hemicellulose) (23.22 and 26.22%wt_{TS}, respectively) indicating that a significant quantity of digestible organic substances remained unconverted in the precedent AD. Lignin and cellulose prevailed in digestates consistent with results for biomass- and waste- sludges (Zanellati et al., 2020; Zhong et al., 2016). Lignin fraction at 15.28 and 14.62%wt_{TS} in MD and CSD, respectively was also consistent with proportions reported for sludges generated from mono-digestion and co-processing of biomass with other substrates (Musatti et al., 2017; Rodriguez Correa et al., 2017; Zhong et al., 2016). Digestates contained a high concentration of lignin as a consequence of polymer's recalcitrance to AD microorganisms (Fang et al., 2020),

TABLE 1: Main characteristics of untreated and pretreated with *I. lacteus* digestates.

Parameter	MD			CSD	
	Raw	Treated		Raw	Treated
		20d	30d		20d
pH	6.63	8.94	8.20	6.97	6.40
TS (%wt.)	94.08	16.29	16.77	93.69	16.56
VS (%wt_{TS})	71.59	73.43	73.83	68.28	75.86
Elemental composition (%wt_{TS})					
H	5.15	4.91	4.90	4.86	5.12
N	3.05	3.25	3.19	2.45	3.03
S	0.38	0.36	0.36	0.22	0.31
O*	24.82	29.02	28.72	25.55	28.73
C/N ratio	15.41	13.70	13.93	18.01	15.56
Main components (%wt_{TS})					
Lipids	3.39	3.93	3.17	2.76	2.81
Carbohydrates	<0.05	2.98	2.26	<0.05	0.43
Proteins	14.20	16.60	13.10	11.90	12.70
Starch	<0.5	1.09	1.63	0.64	1.14
Fibers	11.54	4.54	3.30	15.35	7.40
Cellulose	16.22	12.28	11.08	16.80	13.92
Hemicellulose	7.00	<0.25	<0.25	9.42	<0.25
Lignin	15.28	10.94	12.40	14.62	9.42
Cellulose/Lignin ratio	1.06	1.12	0.89	1.15	1.48

** by subtraction, TS=Total solids (105°C), VS=Loss on ignition (550°C)*

which suggests that the AD performance of sludges could likely be advanced by a WRF pretreatment.

Incubation of WRF in digestates modified the composition of sludges (Table 1). Concentration of TS decreased 5.6 and 5.8-fold with SSF treatment likely due to a consumption of polymers, proteins, soluble sugars and volatile fatty acids (VFA) by the fungus (Zanellati et al., 2021). The water quantity contained in fungal inoculum may also affect the proportion of TS in an incubated material. Contrary to TS, the fraction of VS slightly increased (2.5 and 3.0%) with cultivation of *I. lacteus* in MD and 1.1-fold in CSD (20 days). The small amendment in VS concentration resembles to those reported for the treatment of agricultural digestates by the basidiomycete *C. stemonitis* (Zanellati et al., 2021). Cultivation of a fungal strain in a material would normally expected to introduce a reduction in VS concentration due to removal of polysaccharides. However, organic fraction is possible to maintain stable or increase due to the organic matter contained in fungal mass that is formed on substrate because of mycelia growth. The latter could counterbalance a part or even exceed the quantity of VS consumed by WRF (Zanellati et al., 2021).

I. lacteus amended the fraction of plant cell wall polymers in digestates (Table 1). Based on the theory (Sun et al., 2011), lignin cannot serve as carbon and energy source for fungal survival, as such WRF hydrolyse part of the available cellulose and hemicellulose to support their growth, metabolism and lignin degradation activity. Colonization of MD by *I. lacteus* led not only to a reduction in lignin concentration (18.8-28.4%), but also to a complete removal of hemicellulose (>97.3%) and a significant loss of cellulose (24.3-31.7%). Lignin mineralization was sufficient, however, the specific fungal strain belongs to the non-selective category and the quantities of cellulose and hemicellulose, consumed by the basidiomycete for retrieving nutrients for its growth and metabolism, were also significant (Sun et al., 2011). WRF colonization in CSD for 20 days influenced the concentration of polysaccharides in a similar manner with that in the case of MD. In particular, *I. lacteus* caused all polymers degradation with cellulose loss reaching a 17%, hemicellulose approaching complete removal (>97.3%) and lignin being depleted by 36%. A significant part of holocellulose, especially hemicellulose, was lost from both digestates, however, the delignification was also sufficient and could potentially lead to elevated methane yields from digestates provided that the absence of lignin could provide passages for AD microorganisms to reach the available holocelluloses in substrate that constitute suppliers of carbon to methanogenic archaea for their growth and activity (Abraham et al., 2020; Vasco-Correa et al., 2016).

3.2 BMP

3.2.1 Biogas and methane production from untreated digestate

Figure 2 shows cumulative biogas and methane generation curves for the inoculum and digestates. Biogas and methane yield attained a sufficient value during the first three days. Produced gas early in process suggests that AD microorganisms were promptly adapted to the substrates and exploited part of organics in digestates to generate gas (Hosseini Koupaie et al., 2019), which was considered reasonable, bearing in mind that both inoculum and sludges were generated by AD of biomass-based substrates. Besides, the production temperature for inoculum was within the mesophilic range which also favoured a prompt acclimatization of AD microorganisms to the conditions of BMPs.

Total methane generation from MD (0.119 $m^3kg_{VS}^{-1}$) was essentially equal, with insignificant statistical difference ($p>0.05$) to that from the inoculum (0.111 $m^3kg_{VS}^{-1}$) and CSD (0.132 $m^3kg_{VS}^{-1}$). Methane production from digestates and inoculum resembled with those found in the literature for AD effluents (0.117-0.134 $m^3kg_{VS}^{-1}$) (Menardo et al., 2011a; Ruile et al., 2015). Nevertheless, lower methane generation in the order of 0.023 to 0.087 $m^3kg_{VS}^{-1}$ was also reported for digestates deriving from typical AD feedstocks like animal manure, food waste and agricultural crops (Lindner et al., 2015; Uludag-Demirer and Demirer, 2022). In these cases, the low values were relevant to the AD system from which the digestate was derived and particularly to the operating conditions like the HRT (Menardo et al., 2011b). Ammonia concentrations larger than 2.5 gL^{-1} in an AD medium could be also capable to hamper the activity of methanogens and limit methane yields from digestates (0.003-0.070 $m^3kg_{VS}^{-1}$) (Menardo et al., 2011b; Sambusiti et al., 2015).

3.2.2 Effect of I. lacteus on methane production from digestates

Figure 3 provides total biogas and methane production results for the untreated and modified by *I. lacteus* (28°C, 20 days) MD. For biomass materials, pretreatment with non-selective WRF such as *I. lacteus* and *C. subvermispora* was reported to moderately or negatively influence the AD performance of the lignocellulose (Basinas et al., 2022). However, few reports (García-Torreiro et al., 2016; Wan and Li, 2011) existed where the impact of non-selective WRF on biomass degradability was reported to be positive. This is agreement with the observations in the current assays, where incubation of the non-selective *I. lacteus* in MD enhanced the total methane yields from the sludge by 67.2% (Figure 3). The same trend was observed for the CSD with the rise being at 59.1% (Basinas et al., 2025). *I. lacteus* activity for 20 days induced an almost complete removal of hemicellulose from the MD (>96.4%), provoked a mild cellulose reduction (from 16.22 to 12.28%wt_{TS}) and an extensive metabolization of lignin (28%) (Table 1). As such, the remaining quantity of cellulose was presumably the principal source of organics for AD microorganisms to produce biogas. Evidently, lignin polymerization was efficient to provide additional proportions of the available cellulose for digestion and promote methane production at a level that covered any depletion in methane yield provoked by the consumption of cellulose and hemicellulose by *I. lacteus* (Fang et al., 2018; Zanellati et al., 2021). In addition, despite the pH of the treated MD with *I. lacteus* was at a high level (8.94 for a treatment of 20 days) (Table 1) that would be expected to promote a generation of free ammonia inhibiting AD, the pH of reactor sludge after blending with inoculum was at 7.81 leading to insignificant ammonia inhibition effects.

3.2.3 Effect of incubation time on the AD performance of digestate

For the investigation of the influence of incubation time on the methane generation potential of a digestate, MD was inoculated with *I. lacteus* for 20 (baseline) and 30 days and the modified materials were subjected to AD for 40 days. Cumulative methane generation results for modified digestates appear in Figure 4. Methane production results for the untreated MD are also included for comparison.

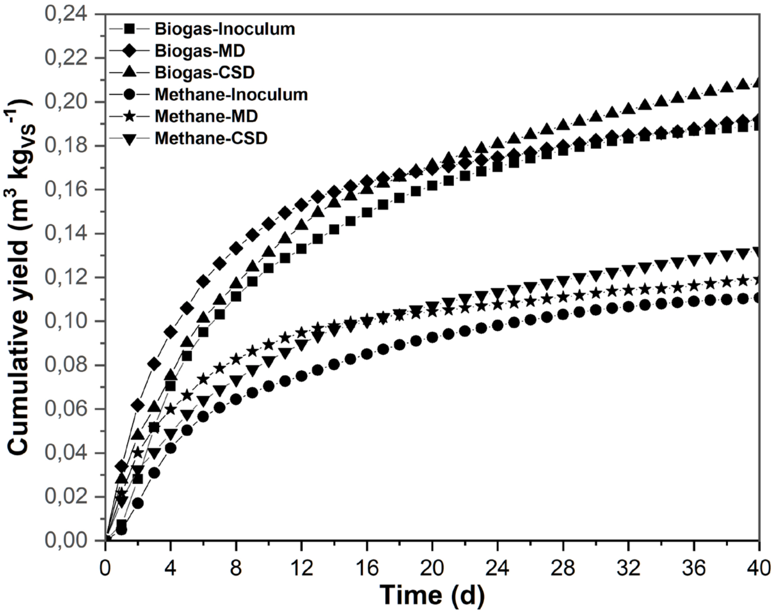

FIGURE 2: Biogas and methane production from non-treated digestates.

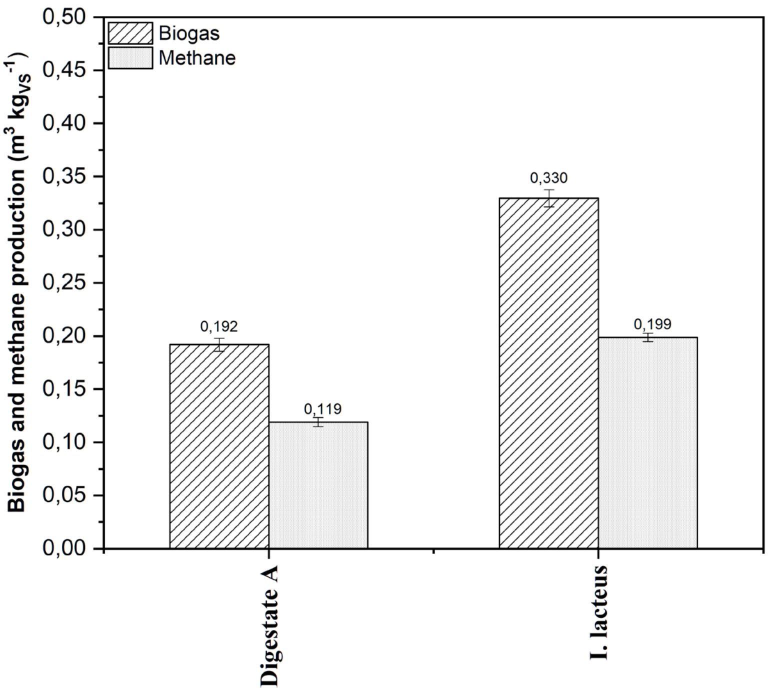

FIGURE 3: Effect of pretreatment with *I. lacteus* on biogas and methane generation potential of MD under mesophilic AD conditions (mean values±standard deviations from three replicates). Conditions of pretreatment: 28°C and 20 days.

Despite that the methane production from MD increased 1.24-fold with incubation for 30 days, the increment was lower ($p<0.05$) than the rise achieved with pretreatment for 20 days (1.67-fold). Given the non-selective characteristics of *I. lacteus*, the elongated time of 30 days resulted in a greater loss of cellulose by 31.7% and a lower reduction in lignin concentration (18.8%) with regard to the modification for 20 days where the corresponding decrease in concentration was at 24.3% and 28.4%, respectively (Table 1). As such, an extensive abatement in cellulose and a modest depolymerization of lignin by *I. lacteus* during the 30 days treatment were presumably reasons for the lower methane generation ability of the sludge sustained a prolong modification (Mustafa et al., 2017).

According to theory (Chen et al., 2008), the manner that H_2S inhibits the biochemical conversion of a substrate is related to the production of H_2S and a competitive consumption of intermediate fermenters by sulphate reducing bacteria in order to conduct a reduction of sulphates (SO_4^{-2}) to sulphides (S^{-2}) and a final conversion of sulphides to H_2S. A second way is relevant to the molecular form of H_2S and a toxic effect that can exert on methanogenic bacteria leading to a restriction of their methane generation capacity which becomes more intense when concentration of dissolved H_2S in the AD sludge exceeds 400 mgL^{-1} (Cai et al., 2021; Zhou et al., 2016). The proportion of H_2S in biogas liberated from the 30-day pretreated digestate was permanently greater than those from the untreated and 20-day pretreated sludge while concurrently surpassed the 400 ppmv throughout the AD process (Figure 4). This level likely corresponds to significantly low dissolved concentrations, well below the cited inhibitory threshold. While an inhibition by H_2S was unlikely, a mild inhibitory effect cannot be entirely ruled out and might even barelly contributed to the lower methane yield from the 30-day modified sludge than that from the digestate pretreated for a short period. The total methane production that was significantly greater than that from the untreated digestate and the fact that the difference in yields from the 20-day treated sludge could be justified by the non-selective delignification of the material by *I. lacteus* further support the conclusion for a minimal inhibition effect.

3.2.4 Fungal efficiency dependence on digestate origin

The type of material fed in a digester defines at a great extent the properties of an AD effluent (Ruile et al., 2015; Tišma et al., 2018; Uludag-Demirer and Demirer, 2022). The way, therefore, that a fungus modifies the methane production capacity of a digestate is presumably influenced by the origin of sludge. A second sludge (CSD) was employed to be incubated with *I. lacteus* at baseline conditions (28°C – 20 days) and the AD performance of the pretreated material was examined in BMP assays (Figure 5).

Modification of CSD induced a rise in the total methane production by 1.6-fold. As discussed above, *I. lacteus* impacted the composition of CSD in a similar way with MD, provoking an appreciable removal of lignin (36%) from the CSD and a relatively modest loss of cellulose (17%) (Table 1). Hence, the amount of cellulose remaining in the material could presumably be considered as major supplier of organics to AD microorganisms and together with the delignification effect principal reasons for the advancement in methane production from the sludge (Abraham et al., 2020). Nevertheless, total concentration of carbohydrates despite increased from <0.05%wt_{TS} in untreated CSD to 0.43%wt_{TS} in treated sludge was significantly small (Ta-

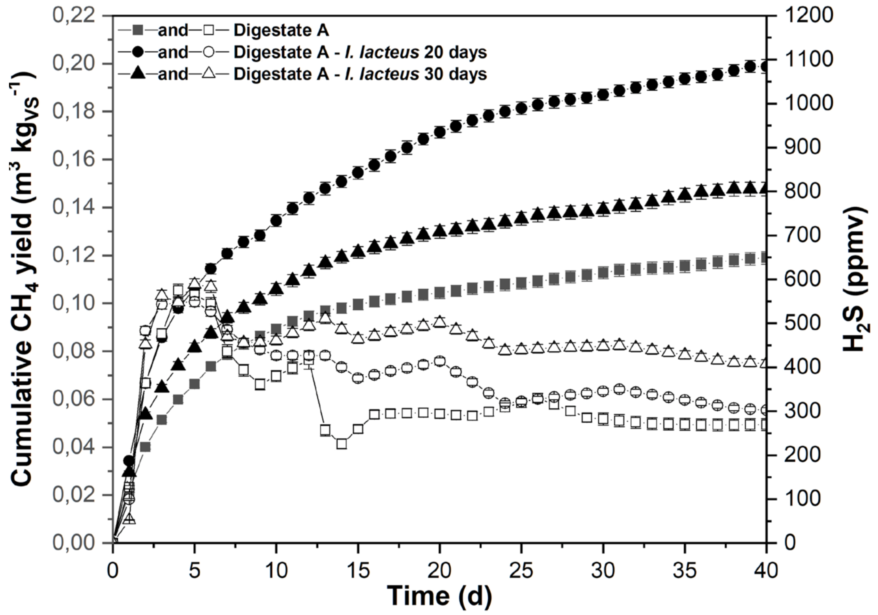

FIGURE 4: Effect of the length of pretreatment on cumulative methane (close points) and H2S (open points) production during AD of digestates pretreated with the non-selective *I. lacteus* at 28°C.

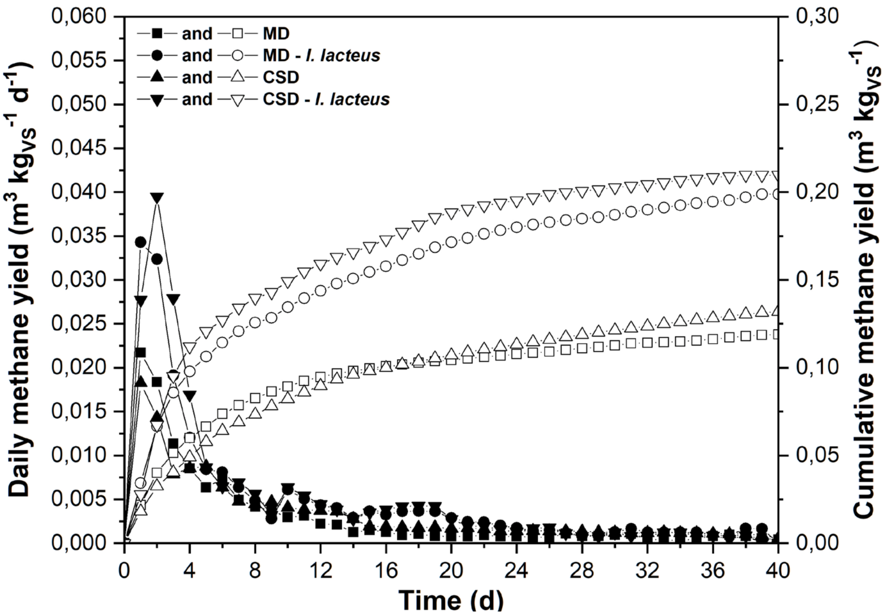

FIGURE 5: Daily (filled points) and cumulative (open points) methane production during AD of different digestates and their pretreated samples with *I. lacteus* at 28°C for 20 days.

ble 1) to comprehensively justify an increment of 59% in methane generation potential. Notwithstanding the carbohydrates proportion, concentration of lipids and proteins were increased from 2.76 and 11.90 in untreated digestate to 2.81 and 12.70%wt_{TS}, respectively in its pretreated counterpart. Fungal mass encloses amounts of lipids and proteins, and therefore mycelial growth on digestate fibres could be regarded responsible for the augmented concentration of the specific components and considered complementary to delignification for the increase in methane generation from the sludge (Zanellati et al., 2021). Comparison of cumulative methane generation from MD and CSD before and after treatment (Figure 5) indicates that the gas yields from the two untreated substrates were comparable (statistically insignificant difference, $p>0.05$) and subsequently the increment in methane release attained with inoculation of MD was merely 7.6% greater than that accomplished with modification of CSD. As discussed above, the advancement in AD performance of modified MD was likely related to a metabolization of lignin during the rotting process that improved the proportion of carbohydrates and the accessibility of AD microorganisms to the available cellulose. Therefore, it would be reasonable to be assumed that pretreatment with *I. lacteus* at certain conditions feasibly enhanced the biodegradability of both sludge types, however, the degree of improvement in methane production was depended on the increment in lipid and protein concentration in the case of a modified sludge with a small carbohydrate fraction and on the available for digestion cellulose in the case of a pretreated digestate rich in carbohydrates.

4. CONCLUSIONS

Digestates produced from different substrates were subjected to treatment with the basidiomycete *I. lacteus* and the methane generation potential of the modified materials was examined in AD assays to determine the influence of the fungal strain, modification time and digestate type on the AD performance of sludge. Pretreatment of sludge with the non-selective *I. lacteus* for 20 days at 28°C increased the methane generation potential of the sludge by 1.67-fold. For the non-selective fungus, prolonging incubation time to 30 days led to an increment in methane yields (1.24-fold), which, however, was lower than that accomplished at a shorter pretreatment period due to a substantial loss of fermentable organics, especially cellulose. Independently of the digestate type, pretreatment of digestate with *I. lacteus* enhanced the digestibility of sludge by at least 1.6-fold. The degree of improvement was depended on the amount of lipids and proteins supplied by the fungus when the modified sludge contained an insignificant carbohydrate proportion and on the available for digestion cellulose when the pretreated digestate was rich in carbohydrates. A combination of biological fermentation post-treatment and recirculation of the modified digestate into the biogas reactor could alter the overall efficiency of the anerobic digestion of organic materials.

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