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POTENTIAL METHANE PRODUCTION OF SPENT SAWDUST USED IN THE CULTIVATION OF *GYMNOPIIUS PAMPEANUS*

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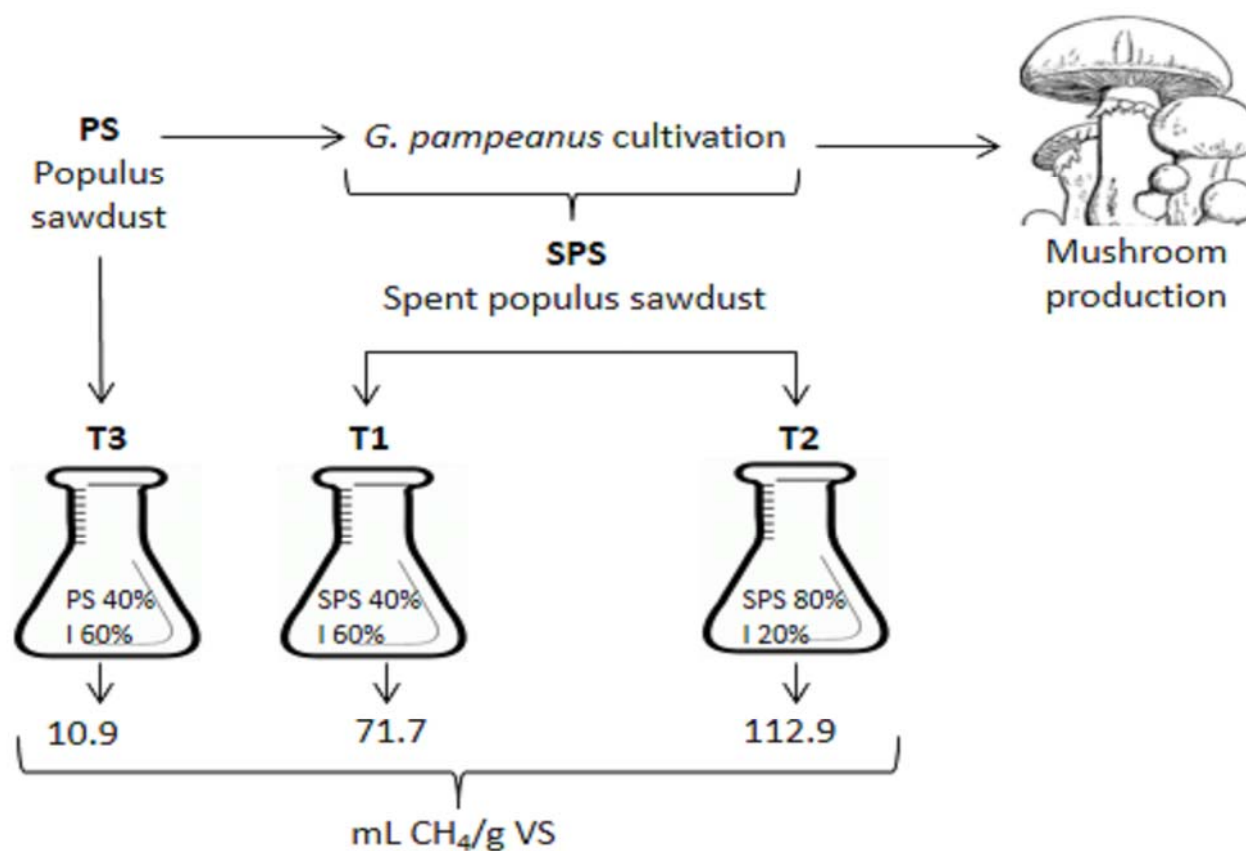
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Graphical abstract



HIGHLIGHTS

- *Gymnopilus pampeanus* (GP) demonstrated ability to improve the biodegradation of *Populus* sawdust (PS) by increasing methane production in 970%
- Spent PS produced higher methane and biogas productions than PS
- Lower substrate/inoculum ratios increased volatile solid removal and methane production.
- Gompertz equation adjusted adequately experimental data of methane production.
- The final value of methane potential resulted still low for energy recovery.

ABSTRACT

Gymnopilus pampeanus (GP) is a mushroom consumed in Argentina. Spent *Populus* sawdust (SPS) obtained from the cultivation of GP was used to test methane production. The effect of two substrate/inoculums (S/I) ratios was analysed. Three treatments were carried out, T1: 80% SPS + 20% I, T2: 40% SPS + 60% I and T3: 40% PS (*Populus* sawdust) + 60% I. After 105 days the cumulative biogas production resulted in 201.2 and 147.8 mL/g VS for T2 and T1 respectively. Methane production increased 62.2% when S/I decreased 83.3% (112.9 and 71.7 mL/g VS for T1 and T2 respectively) while for treatments which used the same percentage of inoculums (60%) the fungal action on the sawdust improved methane production in 970%. Regarding the kinetics of methane production, Gompertz equation demonstrated a good performance of the adjustment of experimental data ($R^2 > 0.98$) and the values of the kinetic parameters indicated that SPS structure showed better accessibility than PS to the methanogenic system. The long time of adaptation (32.2 days) and the low methane production rate (1.7 mL/g VS d) observed in SPS, revealed that the methane production is still not enough for energy purposes.

\Abbreviations

AD: Anaerobic digestion

e: mathematical constant (2.718).P

GHGs: Greenhouse gases

GP: *Gymnopilus pampeanus*

M: methane cumulative production (mL/g VS) at time t (d);

P: maximum methane production (mL/g VS);

PS: *Populus* sawdust

R: maximum methane production rate (mL/g VS/d)

S/I: substrate/inoculums

SPS: Spent *Populus* sawdust

TS: total solids

VS: volatile solids

λ : lag-phase time (d)

Keywords: methane production, biogas, spent substrate, *Gymnopilus pampeanus*, kinetics modelling

1. Introduction

The accumulation of agro-industrial and farming waste represents an important source of pollution, mainly due to the releasing of large amounts of greenhouse gases (GHGs) as well as the harmful consequences for the environment. Mushroom production is an activity that usually uses agro-industrial wastes as substrate. When mushroom production finishes, for every kilogramme of mushroom produced, about 5 kg of spent substrate is generated, which traditionally has been discharged as waste [1]. China, considered as the first mushroom producer country, produced 25,712,000 ton of edible mushroom in the year 2011 and this generated more than 100 hundred millions of tons of spent substrate [2]. This organic material could have added value if it can be transformed to produce clean energy that could be use in the same mushroom farm considering that most of them are located in rural areas where energy supply is not always provided [2].

Anaerobic digestion (AD) is a technological option that simultaneously contributes to mitigate the pollution caused by the inadequate disposal of waste and industrial effluents [3,4] and to provide a source of renewable energy. Many different organic materials can be processed by anaerobic digestion, such as paper [5], sewage sludge [6–8], organic solid [9–11] and animal waste as manure [3,12,13]. Lignocellulosic materials such as woody wastes are exceptions to this behaviour. They are hardly converted to biogas due to their chemical composition and complex structure. While cellulose and hemicellulose can be used by the anaerobic system, lignin however, cannot be degraded under anaerobic conditions [14,15]. In order to increase the biogas potential, treatments that facilitate the access of holocellulose (cellulose and hemicellulose) of bio-fibers are needed [14,16]. Positive effects on the biodegradability of lignocellulosic waste by bacteria have been obtained from physical, chemical or biological pre-treatments improving the production of biogas [14,17–19]. However, physical and chemical pre-treatment have been considered unattractive due to the high costs involved [20]. In contrast, the biological pre-treatments have the advantage of low energy requirements and mild environmental conditions [21]. Microorganisms, such as brown, white and soft rot fungi and bacteria can be used as biological treatments to

attack the raw material by using their enzymes [15,22]. Between these types of fungi, the genus *Gymnopilus* (Basidiomycetes) has a large number of xylophagous species [23,24]. *G. pampeanus*, also known in Argentina and Uruguay as *G. spectabilis* var *pampeanus* [25], is an edible species that usually grows under *Eucalyptus* trees and that has been recently cultivated to produce fruit bodies for human consumption [26]. For the cultivation of such fungi *Populus* and *Eucalyptus* sawdust are usually used [27–29]. Both are abundant raw material in Argentina since these two types of wood are used to produce furniture and fruit wooden boxes [30]. As a result of the production of this fungus, a large quantity of a spent substrate of low density that requires a large area for final disposal is generated. As this residue is rich in organic matter that has been biologically degraded during mushroom production, it is interesting to evaluate its potential to produce methane by AD.

A wide range of factors affect the production of methane during AD, therefore, the net energy that can be produced by an unknown waste is a complex task. One of the key parameters in a batch high solid AD process is the substrate (S) to inoculum (I) ratio (S/I), expressed as the amount of feedstock volatile solids (VS) added per amount of VS in the inoculum. The amount of inoculum added, as a source of a large number of microorganisms that promote methane production, influences not only the start-up of a batch digester but also determines the ultimate methane yield, as well as the rate of methane production in relation to a potential inhibition of the substrate [31–34]. The effect of inoculum on methane production of different kinds of substrates was studied by several researchers. Chynoweth et al [35,36] found out that the decrease in S/I may be necessary for recalcitrant substrates and suggested a rate of 1:1 to 1:2. Lesteur et al. [37] reported that S/I between 1:1 and 1:3 are the rate generally used by researchers, although the relationship that optimises the process strongly depend on the substrate used. Cheng and Zhong [33] showed the importance to determine the optimal S/I ratio for unknown substrates in order to minimise the requirement of active inoculum for the start-up of a digester to assure good methane production.

The objective of this work was to evaluate the capability of methane production of the spent substrate of *G. pampeanus* under AD. The effect of two S/I ratios on both, the methane production and the organic matter removal was analysed. The potentially degradable action of *G. pampeanus* on *Populus* sawdust was also analysed regarding the

methane production. Experimental data of methane production was modelled through a non-linear model in order to obtain the kinetics parameters of the process.

2. Materials and methods

2.1. Spent *Populus* sawdust

The preparation of the substrate used in the experience included the following steps:

- 2.1.1. **Strains used:** *Gymnopilus pampeanus*: ICFC 748/12, Chascomús, Buenos Aires, Argentina; growing on *Eucalyptus*, 04-X-2011, leg M. B. Colavolpe. It is conserved in IIB-INTECH Collection of Fungal Cultures (ICFC), Laboratory of Mycology and Mushroom Cultivation, IIB-INTECH; Chascomús, Argentina (reference in the WDCM database: 826).
- 2.1.2. **Substrate preparation:** *Populus* sp. sawdust was used as the substrate. 25 x 45 cm polypropylene bags were filled with 1000 g of wet substrate mixture with 1% of CaCO₃ powder. Moisture was adjusted to 70% using distilled water. Bags were sterilised using an autoclave during 2 h at 120° C and 1.2 psi.
- 2.1.3. **Substrate inoculation:** Once bags reached room temperature they were inoculated at 5% w/w in laminar flow with the spawn of *G. pampeanus* which was prepared according to Lechner and Albertó [29].
- 2.1.4. **Substrate fermentation:** bags were transferred to an incubation room for 75 days under controlled environmental conditions: temperature 25°C; humidity 60% and darkness. After this period, bags were removed and the colonised substrates were moved to fruiting room under controlled conditions: temperature 18-20°C; humidity 80-90 % and photoperiod of 9 h light/15 h dark to induce basidiome production. Basidiomes were regularly harvested during 60 days. After this time, blocks were removed from production room, were frozen at -20 °C and considered as spend substrate.
- 2.1.5. **Substrate sampling:** Blocks were defrosted at room temperature; four blocks were put in a plastic box; they were disassembled and mixed by hand to obtain a homogeneous sample.

2.2. Inoculum used for anaerobic digestion assay

Sewage sludge obtained from the local wastewater treatment plant was used as inoculum. In order to ensure the degradation of the easily degradable organic matter that could be still present in the inoculum, it was maintained in a batch reactor at mesophilic conditions at $35^{\circ}\text{C} \pm 1$ until use [38,39].

2.3. *Experimental design*

Fractional factorials design with two factors each one at two levels was applied (Fig. 1). The factors selected were the percentage of I and the type of substrate. Inoculum was applied at 20 and 60%. The substrates selected were spent *Populus* sawdust (SPS) and *Populus* sawdust (PS). The response variable was the methane production. This design was selected in order to identify the potential degradation action of the fungus on PS and to evaluate the effect of the quantity of inoculum on the methane production. Three treatments (T) were performed in duplicate as follows (Table 1): T1: 80% SPS + 20% I, T2: 40% SPS + 60% I, T3: 40% PS + 60%. For all the treatments S/I ratio was expressed in g VS in S/g VS in I.

Bioreactors of 1000 mL capacity were filled with each one of the mixtures and were kept in a thermostatic water bath at 35°C according to Córdoba et al (2016) [40], as is shown in Fig. 1. In order to achieve a final content of total solids (TS) between 5-6%, a calculated volume of distilled water was added to each bioreactor to assure the degradation of the organic matter under wet anaerobic digestion [10]. The experiment was daily monitored during 105 days, and it was stopped when the daily cumulated methane production difference was lower than 0.2%.

2.4. *Physical and chemical analysis of substrates and inoculum*

The characterization of S and I was performed through the determination of the following parameters % TS, % VS and % ashes. For the inoculum, the chemical oxygen demand (COD, mg/L), total nitrogen (TN, mg/L), nitrogen as ammonium (AN, mg/L), pH and alkalinity (mg/L) were also measured. All these parameters were performed through APHA methods [41]. To determine organic matter removal, VS was measured at the beginning and at the end of the experiment in each treatment. pH values were also measured at the beginning and at the end of the process in order to verify a possible system upset.

2.5. *Biogas analysis*

The volume of biogas (mL) was determined in all the samples by volume displacement according to Córdoba et al (2016) [40]. The biogas quality was evaluated by its percentage of methane. Measurements were done periodically (at least daily) using a portable instrument Landgem GA2000 (Landtec, UK) with infrared cells to measure methane and carbon dioxide (maximum error $\pm 0.5\%$) and electrochemical cell for oxygen content (maximum error $\pm 1.0\%$). Calibration was done with certified standard type gas mixture 60-40 (CH₄-CO₂) from AGA (Certification Number 165342).

In order to identify the biogas and methane productions produced exclusively from S, it was conducted a blank with inoculum. The methane production of the blank was therefore subtracted from the methane production of the mixture samples [39].

2.6. *Kinetics of the methane generation*

Kinetic studies of AD models can provide useful information for the analysis, design and operation of a fermentation process [42,43]. First-order kinetic models are the simplest models applied to the AD of complex substrates as they provide a simple basis for comparing steady process performance under practical conditions. The cumulative methane production in a batch of high-solids digestion can be described by the Gompertz equation [44,45]. This is a non-linear regression used in the simulation of methane and hydrogen productions for several systems such as granular sludge [42,46,47], co-digestion of swine manure and food waste [48], and co-digestion of the organic fraction of municipal solid waste with ashes from the incineration of these waste [49]. The following equation describes the Gompertz equation:

$$M(t) = P \exp[-\exp(R/P)(\lambda - t)e + 1] \quad \text{Eq. 1}$$

where M is the methane cumulative production (mL/g VS) at time t (d); λ is the lag-phase time (d); P is the methane production potential or maximum methane production (mL/g VS); R is the maximum methane production rate (mL/g VS/d), and e is a mathematical constant that represents the base of the natural logarithm (2.718).

The experimental data of the cumulative methane production was non-linearly fitted by applying the Equation 1 by using Statgraphics Centurion XVI (v.16.2.04).

2.7. *Statistical analysis*

Statistical analysis of the results was performed using t-Student at 95.0 % confidence level through Statgraphics Centurion XVI (v.16.2.04). Data was expressed as the mean value (\pm) the standard deviation of replicates ($n = 2$). ANOVA test was performed to determine the significance of mean values. Fisher's least significant difference (Fisher's LSD) was calculated at $\alpha = 0.05$. Statistical methods were carried out by using Statgraphics Centurion XVI (v.16.2.04).

3. **Results and discussion**

3.1. *Physical and chemical analysis of substrates and inoculums*

Table 2 shows the parameters evaluated on S and I. SPS has a lower percentage of total solids than PS indicating higher water content as a result of the process suffered by the sawdust during the fungus production. On the other hand, volatile solids, a useful parameter to evaluate the methane production since it represents the source of the organic matter [50], was also measured in each substrate. SPS contains 10.1% lower VS content than PS but resulted 85.2% lower when is considered by unit of mass of substrate (Table 2). The higher ash content in SPS regarding PS (Table 2) is coincident with previous results obtained by Colavolpe and Albertó [26]. Rajarathnam et al. [51] observed a relative increase in ash content during substrate degradation in shiitake (*Lentinula edodes*). Chantaraj [52] and Sánchez et al. [53], meanwhile, proposed that the mineral content increase is one of the changes suffered by the substrates during the enzymatic degradation while fungi are in the vegetative development stage. The presence of minerals is favourable for biogas production since they provide the required macro and micronutrients for the cell growth and the methanogenic bacteria concentration [34].

The characterization of I revealed significant higher ash content than both, SPS and PS while VS content was 34.4 and 49.6% lower than SPS and PS respectively. pH value did not strongly differ from the optimal range (6.7–7.5) for methane production [38]. The value of alkalinity (6109 ± 90 mg/L) indicated a proper buffer capacity of the system since it was higher than the value of 3,000 mg/L suggested by several authors [40,54,55] to assure the stability of the process. AN value resulted adequately lower than the range of 3000 – 5000 mg/L proposed by Drogg [50] as cause of inhibition while

the value of 1825 mg/l for TN revealed a low ratio COD/TN (122/5) regarding the recommended range between 350/5 to 1000/5 suggested by Ghasimi et al. [56] to assure adequate microorganisms growth.

3.2. Analysis of the substrate degradation by the removal of the organic matter

In an anaerobic digester, the VS removal is a measure of the efficiency of the process since indicates the degradation of the organic matter. Table 3 details the values of this parameter for each treatment. T1 and T2 achieved 5.5% and 9.3% of VS removal respectively showing that higher inoculum percentages increased VS removal; however, no statistical differences were found between these values. T3 showed the lowest percentage of VS removal (0.25%) indicating that anaerobic bacteria were not able to degrade lignin compounds of sawdust as has been reported by Bruni et al [14], despite using a high percentage of inoculum. In general, the organic matter removal observed for all the treatments in this experiments seems to be low compared with some conventional substrates such as pig slurry, which usually achieve a degradation of organic matter of about 50% [40,57]. The low capacity to remove organic matter could be the result of the poor biological degradation suffered by the sawdust that was not able to destroy the lignocellulosic structure. Colavolpe and Albertó [26] reported that *G. pampeanus* demonstrated a strong capacity to degrade *Populus* sawdust since the organic matter decreased 83.9% at the end of production period. The same authors revealed that lignin content of *Populus* sawdust decreased 34.18% by the biodegradation action of *G. pampeanus*. On the other hand, Montgomery and Boch [58] questioned the effect of the fungal action on biogas yield based on the biodegradation action of white-rot fungi that not only deslignified the substrate, but also removed part of its organic matter that could be used for AD.

3.3. Effect of S/I ratio on biogas and methane production

Fig. 2 shows the cumulative biogas production for each treatment along time. T2 showed the highest biogas production (201.2 ± 2.3 mL /g VS) followed by T1 (147.8 ± 16.1 mL/g VS) pointing out that higher I proportion, higher biogas production. The 83% decrease of the S/I ratio caused 36.2% increase in biogas production. This behaviour is in agreement with the results reported by Eskicioglu and Ghorbani [59] which

demonstrated that a decrease of 87% in S/I ratio produced 14% increase in biogas production when whole corn stillage was used as substrate.

As biogas is composed mainly of carbon dioxide and methane, in terms of energy purposes, the concentration of methane in biogas is a key parameter to evaluate the process. The evolution of the percentage of methane for each treatment is shown in Fig. 3. T1 behaved as the slowest treatment since during the first 28 days the methane production was nil and then slowly grew to reach its maximum concentration of 66,7 % on day 95th. As pointed out several authors, the high S/I ratio in T1 (16.2) could be one of the reasons for the delay in methane production. González-Fernández and García-Encina [60] demonstrated that when sewage sludge is applied as inoculum in the AD of swine slurry the S/I ratio determines the rate of methane production, showing that when that ratio is higher than 1, a process stress takes place resulting in a delay in methane production. In this sense, the lower S/I ratio in T2 (2.7) was the reason for the fast increase in methane percentage reaching 45% at day 15th with slower increase up to reach 72.6 % on day 95th (Fig. 3). Methane concentration was considerably lower in T1 than in T2 from the start of the process until day 82nd from which slight differences were observed (Fig. 3).

Cumulative methane production of T2 resulted 112.9 mL/g VS being 62.2% higher than T1 (Table 3). Consequently, the use of higher amount of inoculum not only promoted higher methane yield, but also a substantial decrease in the time process (Fig. 3). The influence of the percentage of inoculum on methane production was statistically analysed. The obtained p-value resulted lower than 0.05 revealing that the quantity of inoculums used significantly influenced the methane production (Table 4).

3.4. Action of the *Gymnopilus Pampeanus* on methane production

The lower biodegradability of sawdust was confirmed by the low values of biogas and methane production obtained (23.9 ± 0.6 mL/g VS y 10.9 ± 0.6 mL/g VS respectively) and the low organic matter removal (0.25 %VS) observed in T3. Comparing the daily methane production between T2 and T3 (Fig. 3), it can be observed that between days 10th and 30th, T2 produced higher percentage of methane than T3; from this day and until day 70th both treatments produced similar percentage of methane (50-60%) and after day 70th T2 continued increasing up to 72% meanwhile T3

drastically decreased. Methane production increased 970% because of the fungal action of *G. pampeanus* on *Populus* sawdust considering that both treatments, T2 and T3, used the same inoculum percentage. These results were confirmed through the statistical analysis shown in Table 4 ($p = 0.0007$), which indicated that the methane production was influenced not only by the S/I ratio but also by the substrate type. The absence of acidification process observed through the final pH value in T2 and T3 (6.95 ± 0.49 and 6.74 ± 0.05 respectively) revealed that the low methane production can be attributed to the nature of the substrate instead to a process stress.

Colavolpe and Albertó [26] analysed the biodegradation of *Populus* sawdust by *G. pampeanus* after 5 months of biological treatment and observed that cellulose, hemicellulose and lignin contents decreased 14.3, 41.04 and 34.18% respectively. The decaying of lignin in substrate could improve the absorption of nutrients by bacteria increasing the volume and the quality of the biogas produced. Mackul'ak et al [61] evaluated the wood rot mushroom *Auricularia auriculata – judae* as a pre-treatment in different lignocellulose materials (sweet chestnut *Castanea sativa* leaves and hay) and observed an increase of 15% in biogas production. Zhong et al [15] evaluated a microbial complex with yeasts (*Saccharomyces cerevisiae*, *Coccidioides desimmitis* and *Hasenulaanomalasp*), cellulolytic bacterias (*Bacillus licheniformis*, *Pseudomonas sp* and *Bacillus subtilis*), a white rot fungi (*Pleurotus florida*) and an acid lactic bacteria (*Lactobacillus deiliehii sp*) on rice straw and reported that biogas and methane productions increased 33 and 75 % respectively and the process time decreased 34% compared with the untreated sample. Moreover, Zhong et al [15] reported an increase of 33% in methane production in oranges waste treated with selected strains of fungi. Muthangya et al [62], which used the fungus *Trichoderma reesei* as a pre-treatment in sisal leaf decortication residues obtained 101% increase in methane production. The values here obtained are considerably higher than those reported by these authors indicating that *G. pampeanus* could be an interesting species to be used as a pre-treatment with different lignocellulose materials.

3.5. Kinetics study results

3.5.1. Technical Digestion Time (T_{80})

A technological parameter known as Technical Digestion Time (T_{80}) defined as the required time to produce 80% of the maximum digester gas production was

evaluated to analyse the performance of the process [33]. According to Kafle and Kim [63], this parameter could be assimilated to the hydraulic retention time in a continuous anaerobic digester. Fig. 4 shows T_{80} of T1 and T2. T_{80} only increased 5.8% when the S/I ratio increased 500%. The observed differences did not result statistically different ($p=0.3947$) revealing that the S/I ratio did not significantly influence this parameter for the substrates studied. For other biomass, it has been found different behaviours (Fig. 4). For vinegar residue Feng et al [64] reported values of T_{80} from 28 (S/I=1) to 60 days (S/I=6), which represented an increase of 114% while Cheng and Zhong [33] found an increase of 44% from 16 (S/I=2) to 23 days (S/I=6) when used cotton stalk (Fig 4). These results revealed that this parameter depends not only on the S/I ratio but also of the type and characteristics of the substrates and inoculums used.

3.5.2. Estimation of the methane production potential using Gompertz equation

The kinetics of the methane production was analysed through the Gompertz equation in order to identify the behaviour of both substrates SPS and PS through its kinetic parameters. The experimental data adjusted adequately to Gompertz equation for all the treatments since the correlation coefficients (R^2) resulted higher than 98% (Fig. 5). The experimental and calculated values of the parameters P, R, λ and their statistics are detailed in Table 5.

The highest methane production potential P was obtained for T2 followed by T1, pointing out that higher proportion of inoculums improved this parameter. The overestimated P values obtained from the modelling could be explained on the basis that the remainder cumulated methane generated after 105th day was not measured. Methane production potential of other lignocellulosic materials such as cotton stalk resulted in a similar range to those obtained for SPS (116 mL/g VS; Cheng and Zhong [33]) while other biomass such as vinegar residue showed higher methane production potential. Fig. 6 describes the values of the methane production potential reported in the literature as a function of the S/I ratio. The highest values (above 400 mL/g VS) were found for whole corn stillage, showing an increase of 12.5% when S/I ratio increased from 0.27 to 2.17 while vinegar residue increased from 183 to 250 mL/g VS (36.4%) when S/I increased from 1 to 6 (500%). As can be observed from Fig. 6 the ratio S/I is only one of the several factors that influence methane production.

The lag phase λ decreased 44.6% between T1 and T2 revealing that higher inoculums concentration reduced the adaptation time. This behaviour confirmed that the lag phase is a phenomenon inherent to microbial kinetics that is affected by several factors one of which is the amount of inoculum used as pointed out Swinnen et al. [65]. However, the values obtained in the present study resulted longer than those obtained for other substrates such as vinegar residue (18 days for S/I=6; Feng et al. [64]) or for cotton stalk (4 days for S/I=6; Cheng and Zhong [33]).

It was observed non-significant differences ($p < 0.05$) between T1 and T2 on the maximum methane production rate R , revealing that this parameter is independent of the quantity of inoculum used. The experimental values obtained resulted lower than those reported by Feng et al. [64] and Cheng and Zhong [33] when digested vinegar residue (6.89 mL/g VS/d with S/I=4) and cotton stalk (5.3 mL/g VS/d with S/I=6). The analysis of the kinetics parameters demonstrated that while SPS structure is more accessible to the methanogenic system than PS, the achieved degradation of SPS is still insufficient to complete a good performance of methane production. The low values of P and R obtained in T3 are indicators of the inability of the anaerobic bacteria to degrade *Populus* sawdust.

4. Conclusions

This work described the behaviour of spent substrates of mushroom industry based on sawdust under anaerobic digestion for methane production. The inoculum and fungal actions were analysed along the process revealing that higher inoculums percentage (lower S/I ratio) increased VS removal and methane production. The treatment with SPS produced higher biogas production and methane concentration than those obtained with PS. This difference can be attributed to the *G. pampeanus* action on the *Populus* sawdust since the structure to SPS resulted more accessible to the methanogenic system than PS. The lower biodegradability of sawdust confirmed the lower biogas production obtained for T3 proving that anaerobic bacteria were not able to degrade lignin compounds in spite of use highly degradable inoculums such as sewage sludge. The non-linear Gompertz model adequately adjusted the experimental data highlighting differences in data kinetics according to the biologic pre-treatment undergo by the substrate. Results obtained demonstrated the ability of *G. pampeanus* to improve the biodegradation of *Populus* sawdust although the final value of methane potential

resulted 112.9 mL/g VS which is still low for energy purposes. Therefore, additional studies should be performed in order to improve biogas production using spent substrate of mushroom industry.

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Figure captions

Fig. 1. Experimental setup

Fig. 2. Cumulative biogas production for the experiment.

Fig. 3. Evolution of the methane production during the experiment

Fig. 4. Technical digestion time (T_{80})

Fig. 5. Gompertz model adjustments (symbols represent mean values of experimental data for each treatment).

Fig. 6. Methane production potential of different lignocellulosic biomass

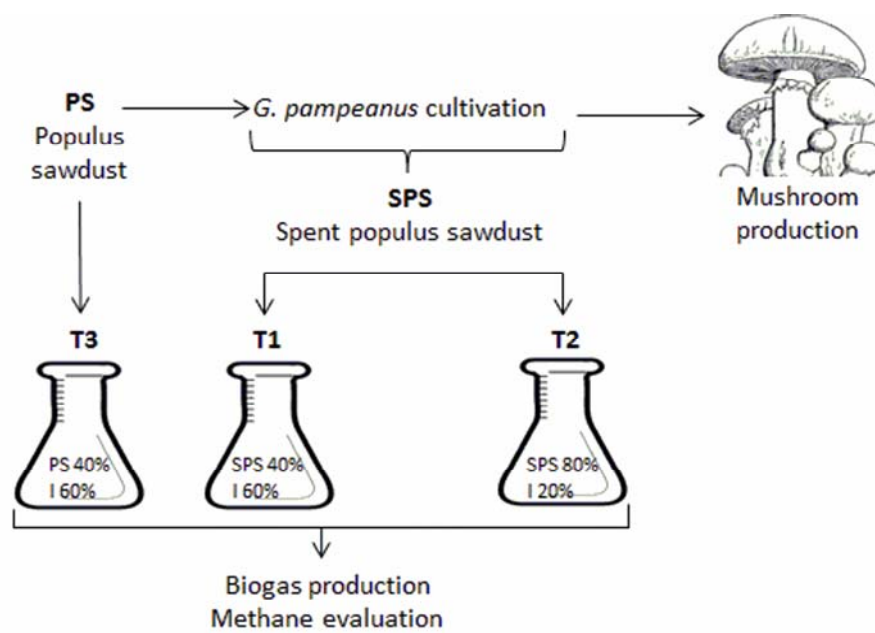


Fig 1

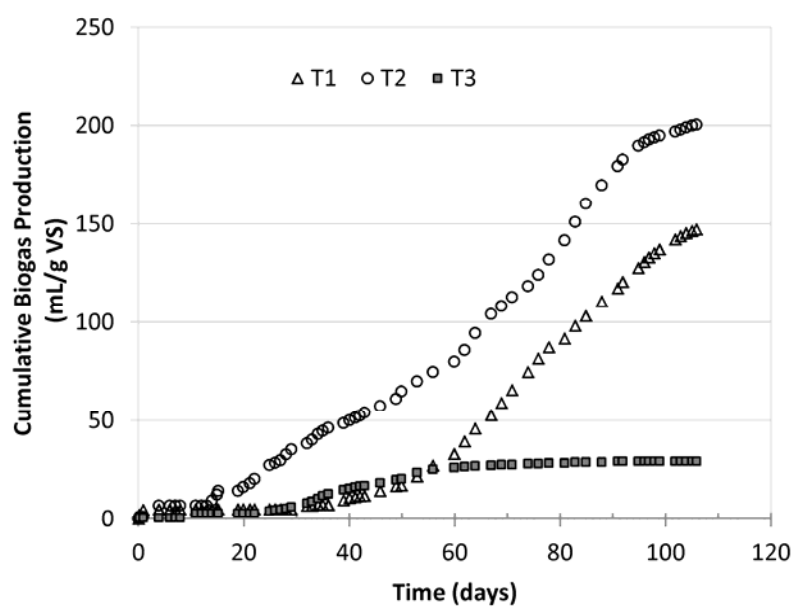


Fig 2

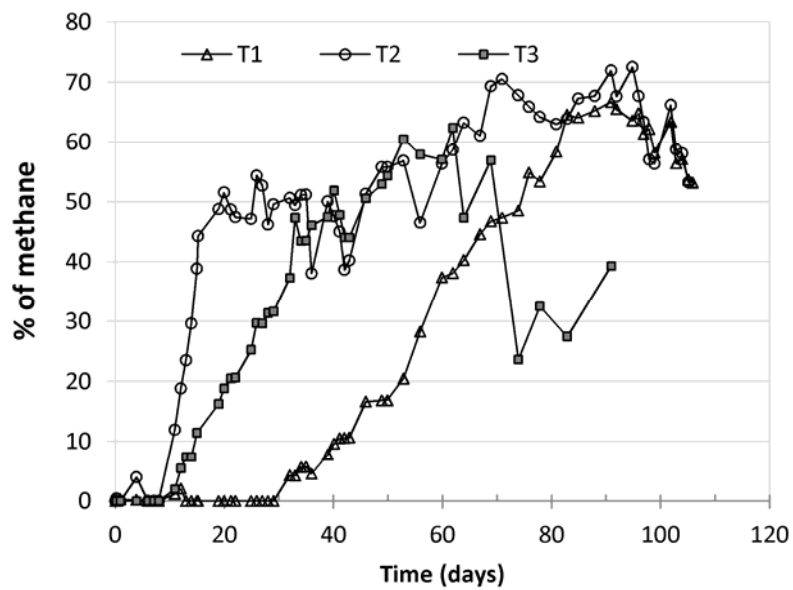


Fig 3

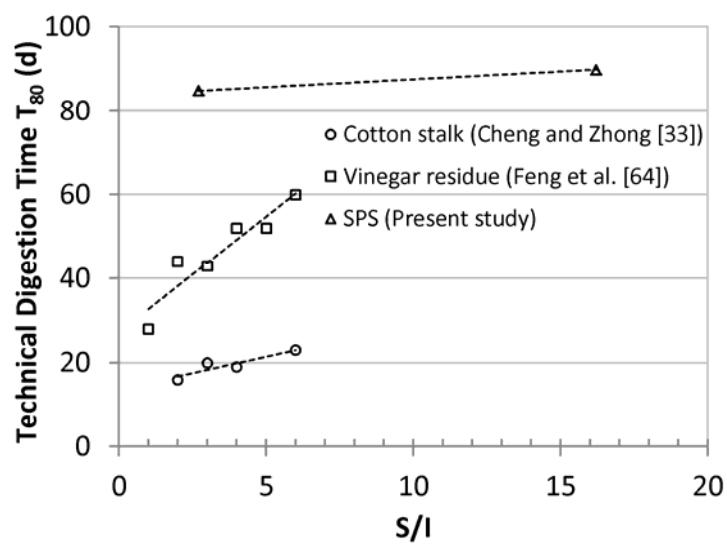


Fig 4

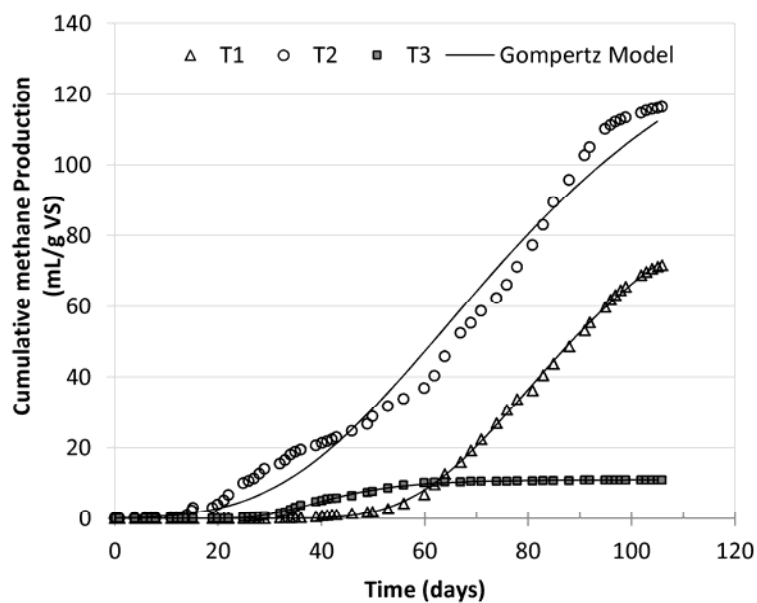


Fig 5

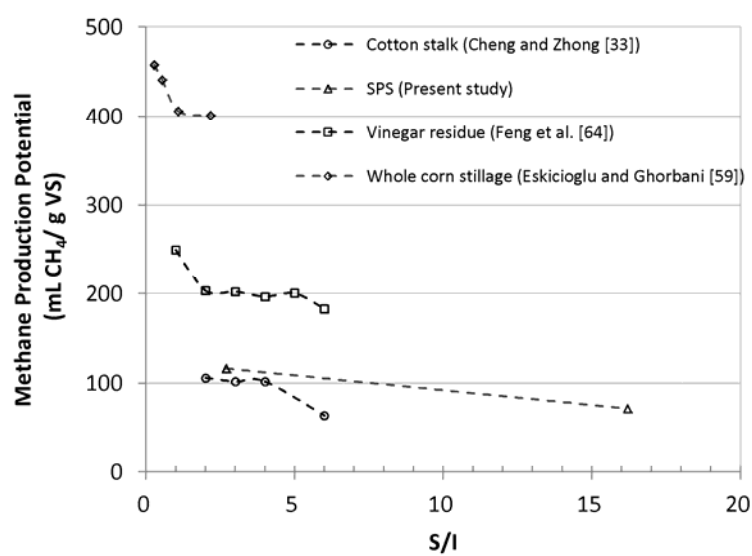


Fig 6

Legend of Tables

Table 1. Experimental design of anaerobic digestion experiment.

Table 2. Physical and chemical characterization of substrates and inoculum.

Table 3. Percentage of volatile solids (%VS) removal, cumulative biogas and methane production

Table 4. Analysis of variance for methane production

Table 5. Gompertz equations estimated parameters

Table 1. Experimental design of anaerobic digestion experiment.

Treatment ⁺	S		I	Water*	S/I
	Type	%	(%)	(mL)	g VS S/g VS I
T1	SPS	80	20	300	16.2
T2	SPS	40	60	300	2.7
T3	PS	40	60	560	18.2

*water added to the reactor to achieve a TS content between 5-6%

⁺ treatments were performed in duplicate

Table 2: Physical and chemical characterization of substrates and inoculum.

Parameter	I	SPS	PS
TS (%)	5.01 ± 0.07 ^a	15.06 ± 0.53 ^b	91.13 ± 0.19 ^c
VS (%)	66.22 ± 1.24 ^a	88.98 ± 0.53 ^b	99.08 ± 0.15 ^c
VS (g VS/g substrate)	0.033 ^a	0.134 ^b	0.903 ^c
Ashes (%)	33.78 ± 1.24 ^c	11.02 ± 0.85 ^b	0.92 ± 0.15 ^a
COD (mg/L)	44750 ± 2616		
pH	7.92 ± 0.01	-	-
Alkalinity (mg/L)	6109 ± 90	-	-
AN (mg/L)	1330.5 ± 36.9	-	-
TN(mg/L)	1825 ± 120	-	-
Cellulose (%)*		54.46 ± 0.51	63.54 ± 0.61
Hemicellulose (%)*		8.06 ± 0.31	13.67 ± 0.27
Lignin (%)*		11.28 ± 0.05	17.14 ± 0.07

Values obtained are means of replicates ± standard deviation (SD). Values with the same letter in the same row have no significant differences (p>0.05).

*[26]

Table 3: Percentage of volatile solids (%VS) removal, cumulative biogas and methane production

Treatment	VS removal	Cumulative Biogas production	Cumulative CH ₄ production
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	(%)	mL	mL /g VS	mL	mL /g VS
T1	5.5 ± 1.25 ^b	3960 ± 431 ^b	147.8 ± 16.1 ^b	1922 ± 196 ^b	71.7 ± 7.3 ^c
T2	9.3 ± 2.26 ^b	5392 ± 62 ^c	201.2 ± 2.3 ^c	3025 ± 132 ^c	112.9 ± 4.9 ^b
T3	0.25 ± 0.09 ^a	863 ± 21 ^a	23.9 ± 0.6 ^a	393 ± 22 ^a	10.9 ± 0.6 ^a
p-value	0.0207	0.0008	0.0007	0.0017	0.0015

Values obtained are means of replicates ± standard deviation (SD). Values with the same letter in the same column have no significant differences ($p > 0.05$).

Table 4: Analysis of variance for methane production

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Main effects					
Inoculum percentage	1993.62	1	1993.62	39.49	0.0081
Substrate type	11140.8	1	11140.8	220.68	0.0007
Residual	151.45	3	50.4833		
Total (corrected)	11380.3	5			

Table 5: Gompertz equations estimated parameters.

Treatment	Methane Final Production P			R	λ	R ²
	Measured	Calculated	Diff			
	(mL/g VS)		%			
T1	71.7±7.3	98.3±3.1 ^b	36.9	1.67±0.03 ^b	58.0±0.4 ^b	99.96
T2	112.9±4.9	149.3±12.3 ^c	28.3	1.70±0.12 ^b	32.2±2.6 ^a	98.36
T3	10.9±0.6	10.9±0.1 ^a	0.35	0.4±0.02 ^a	27.9±0.5 ^a	99.86
p-value		0.0020		0.0014	0.0015	