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# BIOGAS PRODUCTION FROM WHEAT STRAW PRE-TREATED WITH LIGNINOLYTIC FUNGI AND CO-DIGESTION WITH PIG SLURRY

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**Abstract**

This study carried out for the first time a comparison among ligninolytic (white-rot) and cellulosolytic or xylanolytic (*Trichoderma)* pre-treated wheat straw, for biogas production, potential, without or with pig slurry in co-digestion. Methane ($CH_4$) production from wheat straw pre-treated for 4 and 10 weeks with seven different fungal isolates was preliminarily measured. Then, the effects on biogas yield of the co-digestion with pig slurry were checked on straw pre-treated with 3 selected fungal strains. The maximum production of $CH_4$ from pre-treated straw with *Ceriporiopsis subvermispora* (SUB) for 4 and 10 weeks was higher than the control (16% and 37%, respectively). The accumulation daily rate was higher than control (42% and 81%, respectively). A positive correlation between $CH_4$ accumulation daily rate and straw enzymatic digestibility was found. In co-digestion with pig slurry, SUB pre-treated straw for 10 weeks showed an accumulation daily rate of 17.4 mL $d^{-1}$ $g^{-1}$ VS, significantly higher (17%) than that of the control. The time to reach the maximum $CH_4$ production was shortened on average from 34 to 21 days in co-digestion with pig slurry, in comparison with pre-treated mono-digested wheat straw. The biological pre-treatment with selected white-rot fungi appears a promising technology to increase methane production from wheat straw.

*Key words:* biogas, *Ceriporiopsis subvermispora*, co-digestion, enzymatic hydrolysis, manure

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## 1. Introduction

The interest in sustainable biogas production is currently oriented towards the use of agricultural byproducts instead of high-input dedicated crops. These byproducts contain large lignocellulosic fractions that could be exploited to increase methane production. A biomass pre-treatment step could facilitate anaerobic digestion (AD) by partial lignin removal. Biological pre-treatments seem suitable to achieve sustainable biogas production due to low energy requirement, low pollution generation, and simple procedures and equipment (Sun and Cheng, 2002).

Furthermore, this process is expected to avoid the production of inhibitors for the subsequent conversion steps compared to conventional thermo-chemical pre-treatments (Alvira et al., 2010; Gupta et al., 2011; Isroi et al., 2011).

Biological pre-treatment can be carried out by *Basidiomycetes* white-rot fungi which are considered among the most effective biological pre-treatment agents. They produce lignin degrading enzymes, like laccases and peroxidases, which increase the accessibility of holocellulose (cellulose and hemicellulose), for further biochemical transformation (Isroi et al., 2011). *Ceriporiopsis subvermispora*, *Cyathus stercoreus*, *Phanerochaete*

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*chrysosporium*, *Pleurotus ostreatus*, *Trametes versicolor* and other white-rot fungi have been examined alone or in combination with mild chemicals for the pre-treatment of different lignocellulosic biomasses (Akin et al., 1995; Keller et al., 2003; Salvachúa et al., 2011; Taniguchi et al., 2005). These studies indicate that considerable differences exist among fungal species in terms of the rate of pre-treatment and of mineralization of holocellulose, due to metabolic consumption.

Some authors studied the possibility of producing biogas from lignocellulosic material pre-treated with ligninolytic white-rot fungi. Amirta et al. (2006) tested *C. subvermispora* on cedar wood. Ghosh and Bhattacharyya **(**1999) studied biogas production from rice straw pre-treated with *P. chrysosporium* and *P. ostreiformis*. Cellulosolytic *Ascomycetes* like *Trichoderma* spp. have also been proposed as pre-treatment agents for biogas production from lignocellulosic materials with positive results (Phutela et al., 2011). These studies considered only a few fungal species and substrate types. There is a need to further investigate fungal isolates with different hydrolytic properties as pretreatments to increase biogas yields from lignocellulosic biomasses.

Wheat straw is the most abundant agricultural residue in Europe, following rice straw in the rest of the world (Kim and Dale, 2004). The use of straw for the production of biogas represents a sustainable option, since it does not compete with human food resources. In particular, wheat straw pre-treated by white-rot fungal isolates has been proposed as feedstock for sustainable biogas production in co-digestion with cow manure (Müller and Trösch, 1986). Co-digestion has received a great deal of attention in academic literature (Hoppe and Sanders, 2014).

Limited amounts of lignocellulosic biomass are commonly used in co-digestion with manure for biogas production in order to enrich manure with volatile solids without excessively enlarging the digester size. However, the frequency of AD using vegetal biomass without manure has recently increased, due to the incentive policies for renewable energies. In fact, biogas producers have reacted by increasing the electrical nominal power of the AD plants and by utilizing mainly high energy content biomass, like amylaceous dedicated crops (Bacenetti et al., 2014). Government incentives have also raised the interest of the agroindustry (such as olive oil mills, cheese factories, breweries) toward the exploitation of agro-industrial waste for biogas production with no connection with livestock.

A critical point of biological pre-treatment of lignocellulosic biomass is represented by the need to minimize the cellulose loss due to fungal metabolism, while increasing the biomass digestibility. In a previous study, the effect of fungal pre-treatment by white-rot and *Trichoderma* spp. isolates on wheat straw enzymatic digestibility was evaluated (Cianchetta et al., 2014).

The results showed that *C. subvermispora* appeared the best performing agent, since it showed the highest digestibility after 4 and 10 weeks of pre-treatment, minimizing weight loss. On the contrary *P. chrysosporium* showed only a moderate increase of digestibility at 4 weeks and a very high mineralization rate, especially after 10 weeks. The other white-rot isolates showed intermediate behaviors, while *Trichoderma* isolates were almost ineffective. Following these findings, the same pre-treated materials were subjected to biomethanation tests. The aim of the study was to compare wheat straw pre-treated with ligninolytic (white-rot) and cellulosolytic or xylanolytic (*Trichoderma)* fungal isolates, for biogas production potential, without or with pig slurry in co-digestion. Wheat straw was chosen since it is considered a low cost residue easily available. Co-digestion of pre-treated wheat straw with pig slurry was also carried out in order to assess any possible synergistic effect of biological pre-treatment and animal manure on biogas production.

A preliminary experiment was carried out in order to evaluate biogas yields and identify the best fungal isolates and pre-treatment time. In a second experiment, possible ameliorative effects of co-digestion with pig slurry were evaluated on selected pre-treated materials.

## 2. Materials and methods

### *2.1. Wheat straw, pig slurry and fungal isolates*

Naturally dried wheat straw, provided by the CRA-CIN experimental farm at Budrio (BO, Italy), was used as lignocellulosic substrate. Its composition is reported in Table 1. Fresh pig slurry utilized for co-digestion with straw was obtained at CRA-SUI, after biomass mixing with a pumping system, from the farm storage tank collecting the liquid fraction of manure after separation of solids. The values of selected composition parameters are reported in Table 1. Pre-treated wheat straw with 7 fungal strains plus the non-inoculated control were previously obtained (Cianchetta et al., 2014) and samples were used in this study. Briefly, wheat straw had been pre-treated for 4 and 10 weeks with each isolate and aliquots had been stored at -20 °C before being employed in this study for further analysis and biogas experiments.

With regard to the fungal isolates, 5 were ligninolytic white-rot: *C. subvermispora* D-98698 (SUB), *T. versicolor* D-83211 (TRA) and *P. chrysosporium* D-85242T (PHA) from the VTT Technical Research Centre of Finland; *C. stercoreus* CBS 378.80 (CYA) from the CBS-KNAW Fungal Biodiversity Centre, Netherlands; *P. ostreatus* (PLE) from a commercial distributor (Funghi Mara, San Giorgio di Piano (BO), Italy. The remaining 2 isolates, used for comparison, were the hyper-cellulolytic mutant *Trichoderma reesei* Rut-C30 D-86271 (RUT) from VTT, and the xylanolitic wild-type *Trichoderma* sp. IK4 from CRA-CIN.

**Table 1.** Selected composition parameters for the organic materials used as substrates in the experiment. Standard deviation in parentheses (N=3). FM: fresh matter. TS: total solids

| *Composition parameter* | *Wheat straw* | *Pig slurry* | *Wheat straw + Pig slurry* |
|---|---|---|---|
| Total solids, TS (g $kg^{-1}$ FM) | 923 (13) | 18.6 (0.3) | 24.6 |
| Volatile solids (g $kg^{-1}$ FM) | 808 (18) | 14.3 (0.2) | 20 |
| Volatile solids (g $kg^{-1}$ TS) | 875 | 769 | 813 |
| Organic carbon (g $kg^{-1}$ TS) | 450 (5) | 399 (4) | 415 |
| Total N (g $kg^{-1}$ TS) | 9.1 (1.4) | 61.9 (3.4) | 46.6 |
| $NH_4$-N (g $kg^{-1}$ TS) | Nd | 35.1 (3.5) | 26.54 |
| C/N | 49.5 | 6.45 | 8.91 |
| pH in water (1.5:50) | 7.92 (0.2) | 7.04 (0.2) | 7.0 |
| Total P (g $kg^{-1}$ TS) | 0.10 (0.03) | 4.5 (0.01) | 3.22 |
| Lignin (g $kg^{-1}$ TS) | 125 (4) | 57 (1) | 77 |
| Hemicellulose (g $kg^{-1}$ TS) | 345 (4) | 106 (1) | 175 |
| Cellulose (g $kg^{-1}$ TS) | 371 (11) | 61(1) | 151 |

*Nd: not determined*

### *2.2. AD inoculum preparation*

Digestate prepared in the laboratory from pig slurry was used as the inoculum source. The inoculum was prepared as follows: pig slurry was withdrawn from the farm storage tank collecting the liquid fraction of pig manure after solid separation, at two-thirds depth. Two hundred mL of slurry were mixed with 200 mL definite synthetic medium for methanogens (phosphate buffered basal medium, PBBM, sterilized) without energy sources in 500-mL serum bottles, in an $N_2$-$CO_2$ (80:20) atmosphere. This mixture was left to incubate at 35 °C, in strictly anaerobic conditions, and the head space composition was analyzed for $CH_4$ accumulation.

The aim was to obtain a starving wild methanogenic population. The inoculum was considered as ready for use when $CH_4$ production had stopped, indicating the complete exhaustion of endogenous energy sources.

### *2.3. Analytical methods*

Total solids (TS), volatile solids (VS), organic C, total N, total P concentrations and pH were determined on straw and pig slurry (APHA, 1992). Total solids were determined gravimetrically by thermal treatment at 105 °C at constant weight. Analyses of the straw were conducted on samples dried at 65 °C at constant weight and milled at 1 mm. Organic C was determined by dichromate oxidation with external heating and reflux condenser. Total N was determined with the Kjeldahl apparatus. Total P was determined on ashes by colorimetry with ammonium molibdate, after solubilization by means of HCl 1N.

The pH was determined after suspension, 2-h stirring and sedimentation of 1.5 g dry matter in 50 mL distilled water. Ammonium N was also determined on pig slurry samples and digestates by distillation. Fiber fractions (neutral detergent fiber, NDF; acid detergent fiber, ADF; and lignin, ADL) were determined on wheat straw, pre-treated wheat straw, pig slurry and AD digestates according to Van Soest et al. (1991).

The hemicellulose content was estimated as the difference between NDF and ADF; cellulose as the difference between ADF and ADL. For each treatment a composite digestate sample, obtained by mixing the digestate of the 3 replicates, was analyzed.

The expected CV for the lignin, hemicelluloses and cellulose measurements was 8.8%, 3.8%, and 1.7%, respectively, as estimated for the same parameters on the basis of measurements done on a 6-sample population of wheat straw, with the same instrument.

## 3. Experimental

### *3.1. AD preliminary experiment*

AD was carried out using as substrate wheat straw pre-treated for 4 and 10 weeks with the 7 fungal isolates. Non-inoculated controls were also included. The reaction mixture included 1.5 g (fresh weight) of pre-treated wheat straw in 50 mL sterilized PBBM, without energy sources ("hydration medium"), and 5 mL inoculum, in 100-mL reactors (118.5 mL effective volume), in triplicate (48 reactors, in total). Since the comparison was based on the same sample fresh weight, the TS amount in each reactor was different, depending on the different water content of the fresh material utilized.

The biogas production parameters in this experiment were therefore related to the TS content. The initial pH of the mixture was on average equal to 6.3 ± 0.2. The head space of the reactors was gassed with $N_2$-$CO_2$ (80:20) throughout the preparation steps before inoculation.

Reactors were plugged with butyl rubber stoppers and aluminum seals and they were incubated at 35 °C for 90 days. During the incubation period they were randomly distributed on the incubator shelves.

*3.2. Co-digestion experiment*

Selected wheat straw pre-treated for 4 and 10 weeks with 3 different isolates (SUB, PHA and RUT) was utilized for an AD experiment with (PS+) or without (PS-) pig slurry in comparison with an untreated control.

The comparison was based on the same VS content of the reaction mixture: in each PS- reactor, 1g VS of wheat straw was added to 50 mL PBBM (i.e., 2% VS); in each PS+ reactor, instead, 0.29 g VS of wheat straw were added to 50 mL (0.71 g VS) pig slurry, for a total of 1 g VS. Pig slurry alone was inoculated as control. All reactors were in triplicate (51 reactors, in total). Five-mL inoculum was added to all 100-mL reactors (118.5 mL effective volume). The average pH of straw after mixing with PBBM was 6.3 ± 0.4, while in the presence of pig slurry it was 7.0 ± 0.04.

*3.3. Biomethanation tests*

The biogas production (volume and composition) was measured according to Owen et al. (1979) 2 days after the start of the incubation and then weekly for 3 months. Biogas was collected by means of 100-mL glass syringes. The incubation period was completed when there was no more biogas production in any of the reactors. No methane production was detected in the control reactors, where the inoculum had been suspended in PBBM without energy source.

Methane concentration in the biogas was determined by means of a MicroGC Agilent 3000 gaschromatograph, equipped with 2 columns: Molsieve and Plot U; detector: TCD. Carrier gas: argon.

*3.4. Parameters of the cumulative methane production curves (Gompertz parameters)*

In this paper *cumulative $CH_4$ production* means the $CH_4$ volume accumulated over time, including the amounts of $CH_4$ released in the syringe at each measurement date as well as the $CH_4$ volume remaining within the reactor. The comparison of the cumulative $CH_4$ production curves was based on the parameters: maximum cumulative $CH_4$ production, *Hmax* (mL $CH_4$); daily rate of $CH_4$ accumulation in the linear phase of $CH_4$ accumulation, *R* (mL $CH_4$ $d^{-1}$); and lag time duration (λ, d) that is the time of microbial adaptation before the starting of $CH_4$ production.

The *R* and *Hmax* values were expressed per weight unit of TS or VS, depending on the experiment. These parameters were estimated by fitting a modified Gompertz equation to measured data (Lay et al., 1997). This function is often utilized for interpolating growth curves, in general, and microbial growth curves, in particular (Zwietering et al., 1990).

Measurements from 3 replicates were merged for the parameter value estimation. Fitting was performed using the PROC NLIN of the SAS package (SAS, 1989); the parameter values were estimated according to the Gauss-Newton method. The time (d) necessary to reach *Hmax* was estimated by calculating the ratio *Hmax / R*.

*3.5. Correlation between enzymatic digestibility and AD parameters*

The values of enzymatic digestibility of the pre-treated wheat straw were used for a correlation study with the AD parameters described above (*R* and *Hmax*). These values were obtained from data reported in a previous work carried out on aliquots of the same pretreated materials (Cianchetta et al., 2014). The *enzymatic digestibility* is defined here as the amount of sugar released after 48 h of enzymatic hydrolysis by commercial cellulase, using final enzymatic loads of cellulase and xylanase of 10 FPU/g, 110 U/g, respectively and β-glucosidase in excess, on 3.75% straw slurries (w/w).

*3.6. Statistical analysis*

Analysis of variance (ANOVA) was performed using the PROC MIXED procedure of the SAS statistical package (Littell et al., 1996). Multiple comparisons of the means were carried out using the SAS LSMEANS statement. Factor and factor interaction effects were considered significant at $P < 0.05$. Fisher's LSD test ($P < 0.01$) was used to compare treatment mean values.

## 4. Results and discussion

*4.1. AD preliminary experiment*

Biogas production (Fig. 1) started immediately after inoculation, thus the lag phase duration (λ) was equal to 0 for all the treatments, including the controls and with the exception of straw treated with IK4 for 10 weeks (λ = 0.11 d) and with PHA for 4 or 10 weeks (λ = 0.50 and 0.85 d, respectively). These differences between treatments should not be attributed to differences in pH values, since the pH of pre-treated wheat straw, being initially slightly acidic, increased to neutral values after mixing the straw with the hydration medium for all the treatments. This effect could be attributed to an inhibitor released by IK4 and PHA during the straw pre-treatment or to the lack of readily fermentable compounds removed by these fungi.

Larger differences among the materials were found for the 10-weeks pre-treatment than for the 4-week pre-treatment (Fig. 1 a, b). In particular, wheat straw pre-treated with SUB showed the fastest and highest $CH_4$ accumulation, achieving the maximum $CH_4$ production in about 30 days. Other pre-treated materials displayed values even lower than that of the untreated control, such as PHA 10-weeks (Fig. 1b).

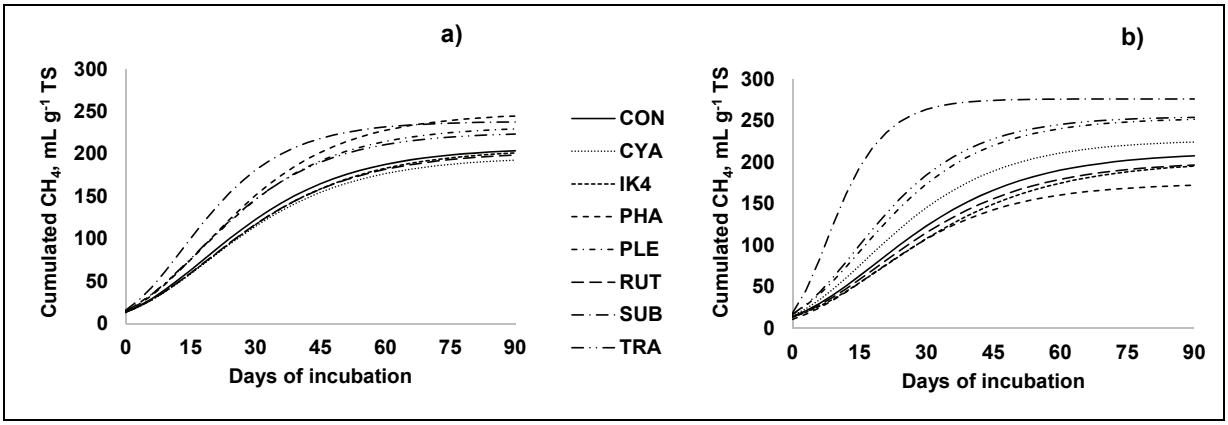


**Fig. 1.** Gompertz-estimated curves of methane accumulation from wheat straw pre-treated with fungal isolates for a) 4 weeks; b) 10 weeks. CON, untreated wheat straw; CYA, *C. stercoreus*; IK4, *Trichoderma* sp.; PHA, *P. chrysosporium*; PLE, *P. ostreatus*; RUT, *T. reesei*; SUB, *C. subvermispora*; TRA, *T. versicolor*

The daily rate of $CH_4$ accumulation (*R*) (Table 2) was on average higher for 10-weeks pre-treated straw (5.9 mL $CH_4$ $d^{-1}$ $g^{-1}$ TS) than for 4-weeks (4.8 mL $CH_4$ $d^{-1}$ $g^{-1}$ TS).

Four-weeks pre-treated straw with SUB, PHA, TRA and PLE gave *R* values higher than the control; thus, 4 weeks is a sufficient pre-treatment period to appreciate an increased biogas production rate, utilizing these isolates. In particular SUB displayed the highest *R* value (6.7 mL $CH_4$ $d^{-1}$ $g^{-1}$ TS). With regard to straw pre-treated for 10 weeks, all the white-rot isolates gave *R* values higher than control, except PHA.

This result is not surprising, considering that the straw pre-treated with PHA for 10 weeks showed very low digestibility values (Table 2), due to a high mineralization rate and poor selectivity towards lignin (Cianchetta et al., 2014). The straw pre-treated with SUB for 10 weeks gave *R* values (13.8 mL $CH_4$ $d^{-1}$ $g^{-1}$ TS) three-fold higher than the control, thus confirming its high performance in increasing biogas production from lignocellulosics, as already reported (Amirta et al., 2006).

Similarly to what was observed for *R* values, 4 weeks pre-treated straw with SUB, PHA, TRA and PLE gave *Hmax* values higher than the control (Table 2); in particular SUB and PHA gave the highest values (16% and 21% higher than control, respectively). Equally, all the straw pre-treated with white-rot isolates for 10 weeks gave *Hmax* values equal to or higher than the control, except the straw types with PHA (13% lower than control). This result confirms the observations about the poor PHA performance at longer pre-treatment times reported by Cianchetta et al. (2014). The straw pre-treated with SUB for 10 weeks gave a 37% higher *Hmax* value than the control. These data show that SUB 10-weeks was the most effective pre-treatment, since it reduced AD duration from 48 to 20 days (58% reduction) in comparison to the control and gave the highest methane yields (276 mL $CH_4$ $g^{-1}$ TS) (Table 2). The SUB, PHA, PLE and TRA isolates confirmed their suitability to be used for the pre-treatment of lignocellulosic materials for biogas production, in agreement with the findings of other authors. In a screening of white-rot fungi for the pre-treatment of straw, Müller and Trösch (1986) were able to obtain an amount of biogas twice that of the untreated control by using *P. ostreatus*, probably due to the more intense ligninolytic activity of this fungus (Taniguchi et al., 2005).

Ghosh and Bhattacharyya (1999) pre-treated rice straw with the white-rot fungus *P. chrysosporium* (PC) and the brown-rot fungus *P. ostreiformis* (PO). Biogas and $CH_4$ production were increased by about 35% and 46 % in PC-treated straw and 21% and 31% in PO-treated straw, respectively.

In our study, *Trichoderma* isolates did not show any effectiveness, unlike what was observed by other authors for a similar substrate, like paddy straw (Puthela et al., 2011). This result probably depends on the relatively low N content of wheat straw (Table 1), which can affect ligno-cellulosolytic enzyme production (Mutschlechner et al., 2015).

The improvement in AD performances of straw pre-treated with *C. subvermispora* can be related to its higher cellulose accessibility. A positive correlation was in fact observed between *R* or *Hmax* and the enzymatic digestibility of pre-treated wheat straw (r = 0.91 and 0.79, respectively), measured on the same samples used for the biomethanation tests. This correlation can be interpreted considering that hydrolysis represents the rate-limiting step for the AD of the lignocellulosic materials. In fact the hydrolysis of lignocellulosic materials may be constrained by high lignin content and cellulose crystallization, resulting in low biogas output. Biomass pre-treatments increase digestibility by lignin removal, hemicellulose solubilization, reduction of cellulose crystallization and increased surface for enzymatic attack (Di Girolamo et al., 2013). Analogous effects can be envisaged from biological pre-treatments.

The good correlation observed between *R* and digestibility could indicate that this latter parameter

may represent a useful index to evaluate the effectiveness of a fungal pre-treatment in terms of biogas production rate.

*4.2. Co-digestion experiment*

The co-digestion experiment was carried out on a selection of pre-treated straw including representative isolates in the screening test: SUB and PHA (white-rot fungi), which had shown the best performances, and RUT *(Trichoderma)*, not differing from the untreated control.

The use of pig slurry in co-digestion with straw remarkably increased the rate and amount of biogas production, in comparison with the straw in mono-digestion, for the same amount of added VS (Fig. 2). In particular, no differences were observed for the AD lag-phase duration, except for the substrate effect: λ for straw in co-digestion with pig slurry (0.70 d, on average) was slightly longer than without pig slurry (0.13 d; LSD, at $P<0.01$: 0.48 d). The *R* value for the PS+ reactors was on average 155% and 178% higher than that in the PS- reactors, for 4- and 10-weeks pre-treatment duration, respectively (Fig. 3 a, b).

The *Hmax* value was 61% and 70% higher than that in the PS- reactors, for 4- and 10-weeks pre-treatment duration, respectively (Fig. 3 c, d). When the straw was digested alone (PS-), $CH_4$ production was significantly faster and higher than the control only for the straw treated with SUB, and more pronounced after a 10-weeks pre-treatment. In particular, *R* increased by 42% and 81% and *Hmax* increased by 14% and 31%, for the 4- and 10-weeks pre-treatments, respectively (Fig. 3). RUT and PHA pre-treated straw gave *R* and *Hmax* values not different from the control, or even lower (PHA 10-weeks) (Fig. 3). These results are consistent with those obtained in the preliminary experiment.

When the straw was in co-digestion with pig slurry, differences in AD performances determined by the fungal isolates were reduced, due to the relatively low contribution to VS from the pre-treated straw (29%). Only the straw pre-treated with SUB for 10 weeks had an *R* value (17.4 mL $CH_4$ $d^{-1}$ $g^{-1}$ VS) significantly higher (17%) than that of the control and of the other treatments (included between 14.9 and 15.6 mL $CH_4$ $d^{-1}$ $g^{-1}$ VS) (Fig. 3b). The *Hmax* value obtained by SUB 10-weeks was the highest (350 mL $g^{-1}$ VS) even if not statistically different from the control (Fig. 3d). Besides the straw/pig slurry volume ratio, the straw lignin content could have affected the overall performance. In fact, Pourcher et al. (2013) did not find any significant increase in biogas production from low-lignin straw (6.3%), pre-treated with selected white-rot isolates, in co-digestion with pig slurry. On the contrary, other authors, working with high-lignin straw (16%), found an increase up to 27% of biogas production with *Pleurotus* sp. "florida" pre-treatment in co-digestion with cow manure (Müller and Trösch, 1986). Thus, in co-digestion, biological pre-treatment by selected white-rot isolates would give the best results with relatively high lignin content biomass.

As the maximum production of $CH_4$ from straw was 197 mL $g^{-1}$ VS (Fig. 3c, d), and the maximum production of $CH_4$ from pig slurry alone was 342 mL $g^{-1}$ VS (data not shown), the theoretical production of $CH_4$ from a mixture containing 29% VS from straw and 71% VS from pig slurry should have been 300 mL (197 x 0.29 + 342 x 0.71). Actually, the maximum production of $CH_4$ in the CON PS+ treatment was on average 334 mL (Fig. 3c, d), that is 10% higher than the theoretical one. These results seem to indicate that there was a synergistic effect on methane production when using pig slurry in co-digestion with straw. A synergic effect of co-digestion of animal manure with lignocellulosic materials was already reported (Duong, 2014).

The time to reach the maximum $CH_4$ production was shortened on average from 34 to 21 days, when the straw was in co-digestion with pig slurry, in comparison with mono-digestion, without differences due to the pre-treatment duration.

The improvement in the rate and extent of biogas production when using pig slurry in co-digestion with straw may be attributed to a better composition of the substrate in terms of readily available nutrients for microbial consortia in the anaerobic digester.

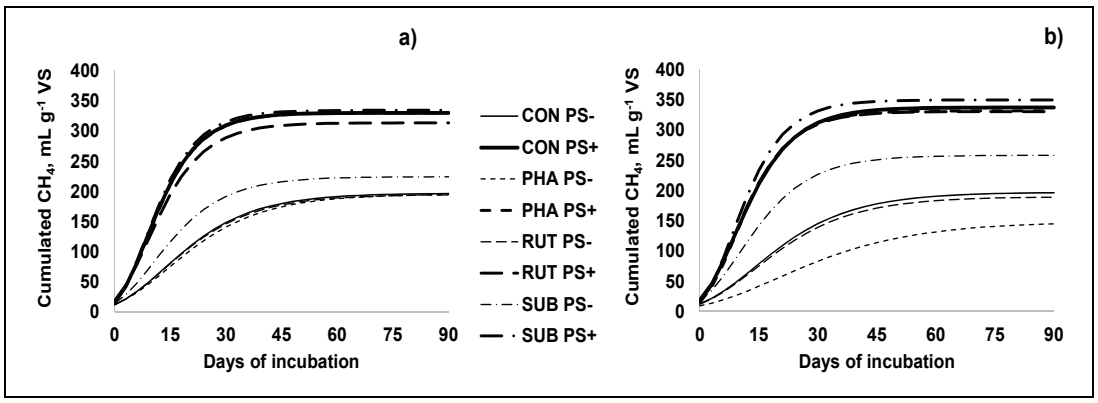


**Fig. 2.** Gompertz-estimated **c**urves of methane accumulation from pre-treated or untreated wheat straw, digested alone (PS-) or in co-digestion (PS+) with pig slurry. Duration of the pre-treatment a) 4 week; b) 10 week. CON, untreated wheat straw; PHA, *P. chrysosporium*; RUT, *T. reesei*; SUB, *C. subvermispora*

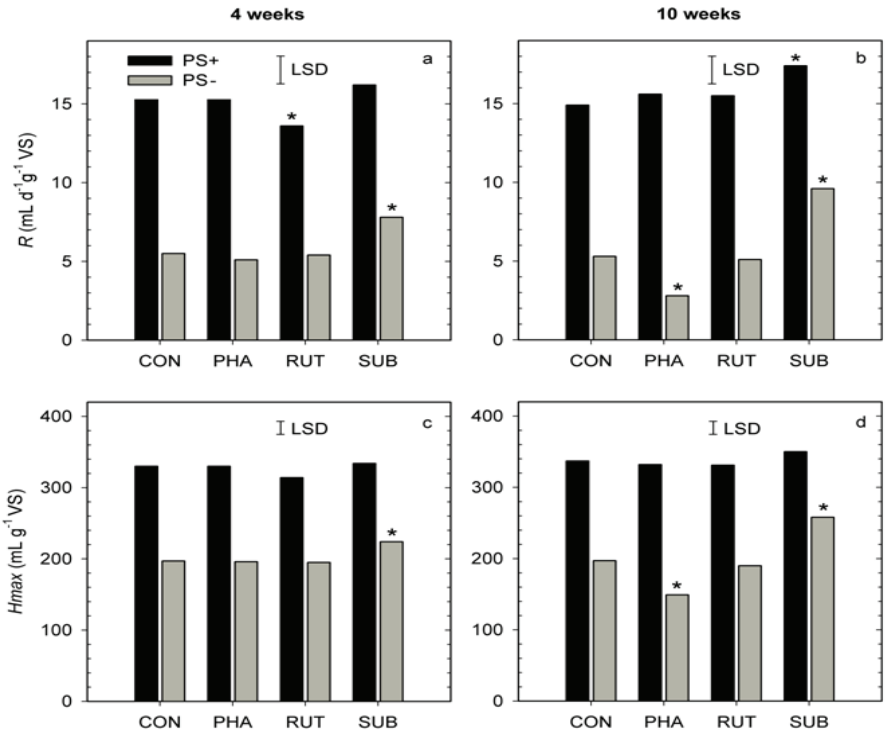


**Fig. 3.** Daily rate of $CH_4$ accumulation (*R*) (a, b) and maximum $CH_4$ cumulative production (*Hmax*) (c, d) for the differently pre-treated straw, as a function of the pre-treatment duration and substrate composition. Columns represent mean values for a 4- (left panels) or 10-weeks (right panels) pre-treatment with PHA, *P. chrysosporium*; RUT, *T. reesei* or SUB, *C. subvermispora* in comparison to CON, untreated wheat straw. Substrate was straw with (PS+) or without (PS-) pig slurry. In each panel, asterisks highlight statistically significant differences from the corresponding control, according to Fisher's LSD test ($P < 0.01$)

In our experiment, the C to N ratio of the untreated wheat straw in co-digestion with pig slurry (8.9) was considerably lower than in mono-digestion (49.5) (Table 1). The reduction of this ratio as well as differences in the prevailing forms of carbon (i.e., lignin) or nitrogen (ammonium or organic) could be the reasons for the improvement in the rate and extent of biogas production in the co-digestion experiments (Wang et al., 2012).

*4.3. Input-material and digestate composition*

The AD of untreated or pre-treated straw led to a reduction of VS, in comparison with input materials, on average by 46% in mono-digestion and by 56% in co-digestion with pig slurry (Table 3). In particular, the highest and lowest reductions were observed in mono-digestion for SUB 10-weeks (62%) and RUT 10-weeks (36%); the untreated material showed a reduction of 39%.

The hemicellulose content was greatly reduced in mono-digestion (77% reduction on average) with appreciable differences between pre-treatments with the highest reduction displayed by PHA 4-weeks and SUB 10-weeks (89% and 91% respectively). In co-digestion with pig slurry a 76% reduction of hemicellulose was observed, on average, with 86% reduction for SUB 4- and 10-weeks. The untreated material showed a reduction of hemicellulose by 73% in mono-digestion and by 69% in co-digestion.

The cellulose content was almost halved (58% reduction on average for PS- and 48% for PS+). The lowest utilization was observed for RUT 4-weeks in co-digestion (35%) while SUB 10-weeks in mono-digestion showed the highest reduction (83%). In co-digestion PHA 4-weeks displayed the highest reduction of cellulose (68%). The untreated material showed a reduction of cellulose by 49% in mono-digestion and by 45% in co-digestion.

The lignin content, which had been partially reduced during pre-treatment of the straw, remained substantially unchanged after AD. Any fluctuations in the measured valued should be attributed to measurement variability (CV = 8.8%). Actually, no changes were expected for lignin, because the ligninolytic microorganisms, usually aerobic, are not active components of the anaerobic reactor communities.

At the start of the AD, the wheat straw types had different cellulose and hemicellulose contents depending on the fungal pre-treatment (Table 3). However, these available amounts were not equally exploited in AD, given the fact that both the PHA 4-weeks and the SUB 10-weeks treatments permitted a holocellulose consumption during AD higher in percentage than that allowed by the other treatments or by the untreated straw. Therefore the straw treated with these fungi showed a higher accessibility to holocellulose by the AD microorganisms, with higher methane yields.

**Table 2.** Biomethanation of wheat straw treated with various ligninolytic fungal isolates: daily rate of $CH_4$ accumulation (*R*) and maximum cumulative $CH_4$ production (*Hmax*). Enzymatic digestibility (amount of sugar released after 48 h of enzymatic hydrolysis) of pre-treated wheat straw is also reported

| | *4 weeks* | | | | | | *10 weeks* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***R*** ***mL $CH_4$ $d^{-1}$ $g^{-1}$ TS*** | | ***Hmax*** ***mL $CH_4$ $g^{-1}$ TS*** | | ***Digestibility*** ***mg sugar $mL^{-1}$*** | | ***R*** ***mL $CH_4$ $d^{-1}$ $g^{-1}$ TS*** | | ***Hmax*** ***mL $CH_4$ $g^{-1}$ TS*** | | ***Digestibility*** ***mg sugar $mL^{-1}$*** | |
| | ***mean*** | ***sd*** | ***mean*** | ***sd*** | ***mean*** | ***sd*** | ***mean*** | ***sd*** | ***mean*** | ***sd*** | ***mean*** | ***sd*** |
| CON | 4.2 | 0.2 | 205 | 4 | 1.2 | 0.1 | 4.2 | 0.5 | 202 | 15 | 1.5 | 0.1 |
| CYA | 3.9 | 0.2 | 197 | 30 | 3.6 | 0.1 | 5.0 | 0.5 | 227 | 14 | 6.2 | 0.2 |
| IK4 | 4 | 0.1 | 206 | 9 | 0.9 | 0.1 | 3.7 | 0.1 | 202 | 3 | 0.9 | 0.0 |
| PHA | 5.3 | 0.6 | 248 | 9 | 3.5 | 0.1 | 3.8 | 0.4 | 175 | 52 | 1.3 | 0.3 |
| PLE | 5.0 | 0.4 | 233 | 2 | 3.6 | 0.2 | 6.1 | 0.2 | 254 | 5 | 7.9 | 0.2 |
| RUT | 4 | 0.1 | 203 | 5 | 0.6 | 0.0 | 3.9 | 0.1 | 201 | 6 | 0.5 | 0.1 |
| SUB | 6.7 | 0.1 | 238 | 2 | 7.3 | 0.2 | 13.8 | 1.3 | 276 | 1 | 15.0 | 0.0 |
| TRA | 5.1 | 0.3 | 226 | 7 | 2.1 | 0.1 | 6.7 | 0.3 | 255 | 5 | 3.1 | 0.0 |
| Mean | 4.8 | | 220 | | | | 5.9 | | 225 | | | |

*CON, untreated wheat straw; CYA, C. stercoreus; IK4, Trichoderma sp.; PHA, P. chrysosporium; PLE, P. ostreatus; RUT, T. reesei; SUB, C. subvermispora; TRA, T. versicolor*

**Table 3**. Composition parameters of total and volatile solids**,** lignin, hemicellulose and cellulose of pre-treated or untreated wheat straw with or without pig slurry, before and after anaerobic digestion

| | | *4 weeks* | | | | | | | | | *10 weeks* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Without pig slurry*** | | ***Input material*** | | | | | ***Digestate*** | | | | | ***Input material*** | | | | | ***Digestate*** | | | | |
| ***Composition parameter*** | ***CON*** | ***PHA*** | ***RUT*** | ***SUB*** | ***mean*** | ***CON*** | ***PHA*** | ***RUT*** | ***SUB*** | ***mean*** | ***CON*** | ***PHA*** | ***RUT*** | ***SUB*** | ***mean*** | ***CON*** | ***PHA*** | ***RUT*** | ***SUB*** | ***mean*** |
| Total solids (g $kg^{-1}$ FM) | 21 | 21 | 21 | 21 | 21 | 18 | 15 | 19 | 17 | 17 | 21 | 22 | 21 | 19 | 21 | 19 | 15 | 20 | 14 | 17 |
| Volatile solids (g $kg^{-1}$ $TS_{Input}$) | 966 | 957 | 962 | 966 | 963 | 583 | 450 | 571 | 462 | 516 | 966 | 922 | 962 | 966 | 954 | 611 | 507 | 616 | 372 | 527 |
| Lignin (g $kg^{-1}$ $TS_{Input}$) | 121 | 100 | 122 | 108 | 113 | 123 | 93 | 124 | 115 | 114 | 128 | 75 | 137 | 89 | 107 | 136 | 75 | 138 | 90 | 110 |
| Hemicellulose (g $kg^{-1}$ $TS_{Input}$) | 341 | 262 | 320 | 263 | 297 | 92 | 28 | 77 | 80 | 69 | 341 | 250 | 333 | 195 | 280 | 95 | 61 | 102 | 17 | 69 |
| Cellulose (g $kg^{-1}$ $TS_{Input}$) | 370 | 383 | 359 | 368 | 370 | 192 | 132 | 189 | 106 | 155 | 371 | 308 | 375 | 414 | 367 | 185 | 156 | 204 | 72 | 154 |
| ***With pig slurry*** | | ***Input material*** | | | | | ***Digestate*** | | | | | ***Input material*** | | | | | ***Digestate*** | | | | |
| ***Composition parameter*** | ***CON*** | ***PHA*** | ***RUT*** | ***SUB*** | ***mean*** | ***CON*** | ***PHA*** | ***RUT*** | ***SUB*** | ***mean*** | ***CON*** | ***PHA*** | ***RUT*** | ***SUB*** | ***mean*** | ***CON*** | ***PHA*** | ***RUT*** | ***SUB*** | ***mean*** |
| Total solids (g $kg^{-1}$ FM) | 25 | 25 | 25 | 25 | 25 | 15 | 12 | 15 | 16 | 15 | 25 | 25 | 25 | 25 | 25 | 15 | 15 | 15 | 16 | 15 |
| Volatile solids (g $kg^{-1}$ $TS_{Input}$) | 816 | 813 | 813 | 816 | 815 | 367 | 390 | 350 | 394 | 375 | 816 | 806 | 813 | 816 | 813 | 358 | 341 | 362 | 351 | 353 |
| Lignin (g $kg^{-1}$ $TS_{Input}$) | 75 | 69 | 75 | 71 | 73 | 76 | 61 | 63 | 68 | 67 | 75 | 62 | 80 | 66 | 71 | 71 | 68 | 76 | 55 | 68 |
| Hemicellulose (g $kg^{-1}$ $TS_{Input}$) | 174 | 151 | 168 | 151 | 161 | 48 | 42 | 54 | 22 | 42 | 176 | 148 | 172 | 132 | 157 | 61 | 26 | 42 | 18 | 37 |
| Cellulose (g $kg^{-1}$ $TS_{Input}$) | 151 | 154 | 147 | 150 | 151 | 83 | 50 | 97 | 80 | 77 | 151 | 133 | 152 | 163 | 150 | 83 | 71 | 87 | 75 | 79 |

Hemicellulose was degraded to a higher extent in comparison with cellulose probably because it is more accessible to bacteria in mesophilic conditions, as reported by Ghosh et al. (1985).

A positive correlation was observed between *Hmax* and absolute holocellulose loss during AD in mono-digestion (r = 0.84). In the co-digestion experiment a lower correlation was found between holocellulose loss and *Hmax* (r = 0.74). These findings could be interpreted considering that pig slurry contains other digestible compounds which may have acted in competition with holocellulose released by the pre-treatment.

## 5. Conclusions

Pre-treated wheat straw with *C. subvermispora* for 10 weeks significantly increased the daily rate of $CH_4$ accumulation, both in mono- and in co-digestion with pig slurry, in comparison with the control (by 81% and 17%, respectively). In addition, the maximum cumulative production of $CH_4$ increased by 31% in mono-digestion. In co-digestion, a synergistic effect on the rate and extent of $CH_4$ production was observed, and the time to reach the maximum $CH_4$ production was shortened in comparison with the straw in mono-digestion.

The biological pre-treatment of wheat straw with selected white-rot fungal strains appears a promising technology to increase methane production from wheat straw because it increases $CH_4$ production and reduces the time required for AD without any need for energy input, and can thus conveniently be used as a suitable alternative to conventional thermo-chemical pre-treatments.

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