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# ASSESSMENT OF FUNGAL AND THERMO-ALKALINE POST-TREATMENTS OF SOLID DIGESTATE IN A RECIRCULATION SCHEME

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## 1. Keywords

Anaerobic digestion, Solid digestate recirculation, Fungal post-treatment, *Pleurotus ostreatus*, Thermo-alkaline post-treatment

## 2. Highlights

- Necessary sterilization and high spawn level to allow fungal SSF of solid digestate.
- Observation of losses in biodegradability and methane yield after fungal SSF.
- No specific ligninolytic activities noted during fungal SSF.
- Thermo-alkaline sterilization treatment increases methane potential/lignin removal.

## 3. Purpose

Recirculation of solid digestate (SD) within the biogas plant was identified as a relevant strategy that allow to recover additional methane from feedstocks and increase biogas plant efficiency [1]. The presentation will focus on the impact of an intermediate post-treatment, before recirculation, that aims to enhance the residual potential methane of SD. Effect of fungal solid state fermentation (SSF) of SD on subsequent methane production has never been explored before. It was hypothesized that: (i) ligninolytic fungi would be able to specifically use the complex fraction of SD for their growth and (ii) energy generation from the subsequent anaerobic digestion (AD) of the colonized SD will be enhanced. The presentation will provide the main outcomes.

## 4. Materials and methods

SD was collected from a French agricultural CSTR plant, directly on-site after digestate mechanical separation. Two Basidiomycetes fungus strains were ordered from Mycelia bvba (Deinze, Belgium). Strain M2191 of *Pleurotus Ostreatus* (PO), also called winter oyster mushroom, and strain M5012 of *Stropharia rugoso-annulata* (SRA), also called garden giant mushroom, were received under spawn form. They were selected as they are both robust, little demanding in cultivating techniques and known to efficiently degrade lignin when growing on wheat straw [2].

Fungal SSF was performed in a designed isolated wooden box, into which temperature, luminosity as well as relative humidity were controlled. A screening to determine potential growing conditions was performed. Then, applying best determined growing conditions, optimal fungal SSF duration was evaluated for both fungi. Five durations were tested: 5.5, 7.5, 10, 15 and 21 days. Determination of the impact of fungal SSF on SD: (i) methane yield and biodegradability were performed using BMP tests (VS loss due to respiration was taken into account thanks to additional TS/VS measurement); (ii) VS characterisation was performed via sequential chemical extractions according to existing protocol [3]. Similar analysis were performed on SD only subject to the sterilization process achieved by thermo-alkaline post-treatment (CaO: 2% w/w of SD and 20 min at 121 °C autoclaving). Based on these results, feasibility at full scale of fungal SSF post-treatment can be evaluated

## 5. Results and discussion

Screening shows that best growing conditions were obtained when: (i) Sterilization by thermo-alkaline treatment was performed before inoculation; (ii) a high spawn level of 20% w/w of SD was applied.

No colonization of SD was feasible when no sterilization was performed. This is likely to be due to the competition with endogenous SD microbial community ( $1.8 \times 10^{11}$  bacterial cells·g<sup>-1</sup> fresh matter). **Figure 1** provides examples of SD colonized by the two fungi following these conditions.

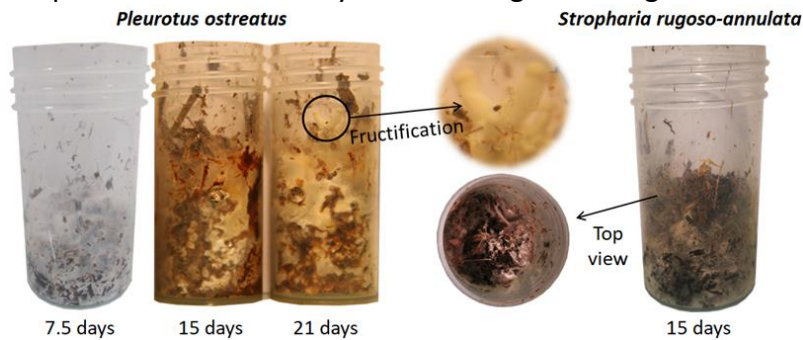


Figure 1: Photographs of sterilized solid digestate colonized by PO and SRA after SSF at different durations

For all SSF durations tested and for both fungal strains, BMP test results show that SD methane potential decreased following this post-treatment (between 15 to 50% lower as a function of the duration). This is due to the combination of two interrelated phenomena: (i) Under aerobic conditions and in presence of fungi, VS losses, mainly due to respiration, were important and reached up to 20% of the initial VS; (ii) This VS loss was not compensated by a gain in biodegradability as fungal activities were not specifically directed towards the lignin-like fraction (see **table 1**). Easily accessible fractions (e.g. proteins) that would have given methane under anaerobic conditions were also depleted. Thus, tested ligninolytic fungi were not able to specifically use the complex fraction of SD for their growth and subsequent AD of the colonized SD was not improved.

Table 1: Impact of treatments on the evolution of the different fractions of the VS and BMP in comparison to the untreated SD

	Soluble fraction	Protein-like fraction	Hollocellulose-like fraction	Lignin-like fraction	BMP (mL/gVS <sub>pret</sub> )	BMP (mL/gVS <sub>init</sub> )
Thermo-alkaline	+123%	+9%	+7%	-26%	+13%	+13%
PO colonization 15 d	-7%	-53%	-30%	-14%	-20.7%	-35.8%
SRA colonization 15d	-26%	-21%	-17%	-14%	-16.8%	-1%

Finally, only sterilization process, consisting of a thermo-alkaline post-treatment, increase energy recovery from SD of 13%. This is due to a specific degradation towards the lignin like-fraction (see **table 1**). Further optimization of this post-treatment may enhance efficiency of SD recirculation.

## 6. Conclusions and perspectives

Fungal SSF of SD, before its recirculation into biogas plants, appears not to be a viable strategy to enhance energy recovery from SD within the tested conditions. Overall, looking at the energetic loss (sterilization and lower methane yield) and the additional economic cost (mycelium and labour) this strategy will not be profitable at full scale. Tracks that explore production of higher value products than methane (enzymes, biomolecules...) are likely to be the only way to enhance economic profitability and render viable fungal SSF of SD.

## 7. References

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