Influence of cow dung storage on methane production by anaerobic digestion and enhancement using the ligninolytic fungus *P.ostreatus*

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Introduction

The management of agricultural wastes is considered an important problem because of the enormous quantities produced, but at the same time it is an inexhaustible source of nutrients and energy which should not be wasted. For a long time, anaerobic digestion has been an optimal strategy to handle those organic wastes (Deublein, D., Steinhauser, 2011) becoming an alternative to waste disposal as well as a renewable energy source (Noor et al., 2021). However, biogas production is highly variable, since the amount of gas produced and its composition depend on the type of waste and the inoculum used (Rasi et al., 2007).

Although cattle manures sometimes show the suitable C/N ratio for the biomethanization process, they are usually mixed with the straw used as animal bedding, increasing the amount of lignin. This fact is considered a problem since lignin hinders the hydrolysis stage of the process (Schroyen et al., 2018).

In this work the presence of lignin in the manure was faced using two different strategies with the aim of facilitating the anaerobic digestion of the manure and increase the biomethane yield. On one hand the storage of the cow manure improved the biomethane production and on the other hand, a pre-treatment of cow manure and bedding material with ligninolytic fungi (*Pleurotus ostreatus*) made it easier for bacteria to reach compounds easily degradable to produce methane.

Materials and Methods

The manure used was obtained from a livestock farm located in Burgos (Spain). It was either pre-treated inoculating *P.ostreatus* mycelium embedded in wheat grains for two weeks or stored in a closed container of 1 m^3 with non-favoured leaching conditions for two months inside an umbraculum at temperature ranged between 2-17 ºC minimum to 10-31 ºC maximum . Once the period of storage was finished, the pile showed three visually different levels: the upper level (0-15 cm), the intermediate (15-30 cm) and the lower one (30-45 cm). An aliquot (1 kg) of each level was taken and underwent an anaerobic digestion process.

For the anaerobic digestion, sludge from a wastewater treatment plant located also inside the same farm in Burgos (Spain) was used as inoculum. The ratio inoculum: substrate was 2:1 (w:w) based on volatile solids (VS) of each one. The control parameters were VS (APHA, 2005), Chemical Oxygen Demand (COD) quantified by colorimetry using a spectrophotometer of Hanna instruments (Smithfield, RI 02917 USA) (James W. O'Dell, 1993), Total Kjeldhal nitrogen, ammonia nitrogen and volatile acids by titration using KjelFlex K-360 coupled with TitrinoPlus (Büchi Labortechnik, Flawil, Suiza), and total, partial and intermediate alkalinity (Ripley L. E., 1986).

The anaerobic digestion took place in micro digestors (500 mL) in batch on continuous basis in triplicate at 37 ºC. The volume of methane was daily measured using an equipment AMPTS II (Bioprocess Control, Lund, Sweden). The digestions were finished when the daily methane production was lower than 1% of the production of the day before.

A double control was performed, one with the inoculum and the other one with the same manure stored at 5 ºC. The pre-treated manure was also compared with non-treated manure in the same conditions.

Results and Discussion

In the stored cow manure the biomethane yield varied with the depth level. Thus, the lower level (30-45 cm) produced 50% more biomethane than the upper (0-15 cm) and the intermediate level (15- 30 cm) (Figure 1), concluding that the storage at those temperature conditions (between 2-17 ºC minimum to 10-31 ºC maximum) generated different settings throughout the pile of manure that resulted in significantly different biomethane production from the same original manure stored at 5ºC and used as a control (Figure 1).

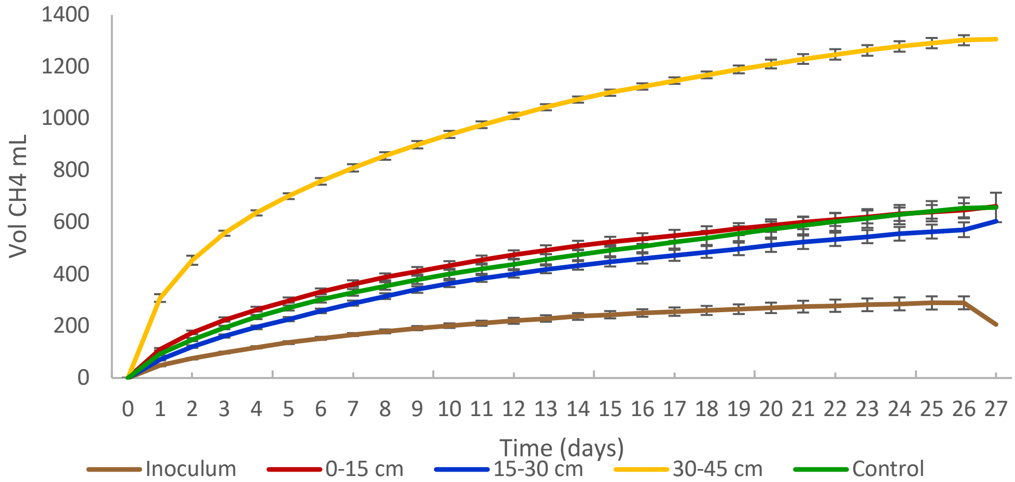

Figure 1 Cumulated methane production in the stored manure assay (n=3). Inoculum (control sludge), 0-15(upper level), 15-30 (intermediate level), 30-45 (lower level) and control (manure stored at 5 ºC)

Pre-treated cow manure with *P. ostreatus* before anaerobic digestion also yielded higher cumulated productions of biomethane than the non-pre-treated manure. The improvement in the hydrolysis stage due to ligninolisis by *P. ostreatus* resulted in 9% increase in methane production.

Conclusion

Biomethane production by anaerobic digestion from cattle manure can be influenced by several factors such as the conditions of storage as demonstrated in this work. Parameters such as moisture and oxygen availability determine the type of active microorganisms and thus, their ability to modify the structure of the raw material. In this work, the lower depth level (30-45 cm) of the manure pile, with more anaerobic condition due to the non-favored leaching conditions, enhanced 50 % the biomethane yield. The pre-treatment of the raw manure with ligninolytic fungi also improved the production of methane, although with lower increases than those obtained by storage. In any case, both, pre-treatment with ligninolytic fungi and storage, represent an increase in biomethane production that on a large scale could represent a significant boost in the yield of real biogas plants. So that, although anaerobic digestion of organic wastes is a well-known technique there is still scope for improvement.

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