Utilization of enzymatic pretreatment for improving efficiency in the bio-methanation of mixed substrates

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Introduction: Sustainable waste management has become one of the key challenges facing humanity in the 21st century with the scientific community in a quest for the development of new methods and technologies able to protect the environment, the natural resources and the quality of human life. Human activities are always generating waste; for 1 ton of cereal product 1.5-3 tons of cellulose rich waste are disposed in the field, while 50% of human food products end up as a waste stream in landfill sites, while each dairy cow is generating approximately 10 tons of manure per year.

Over the years, a number of different technologies have emerged to offer high treatability of waste with minimal expenses. Most of these technologies are based on the advantages offered by aerobic microorganisms to generate high quality effluents through sedimentation of the generated sludge. Nonetheless, even though good quality effluent can be obtained these technologies require a lot of energy, while the generated sludge is not suitable for further utilization. A waste (i.e. substrate) valorization method offering the advantages of self-sustenance, income generation, and waste valorization with limited material requirements is the anaerobic digestion.

The anaerobic digestion process is being employed to a large number of different substrates including animal manures (Kalamaras and Kotsopoulos, 2014; Nie et al., 2015; Wang et al., 2014; Witarsa and Lansing, 2015; Zarkadas et al.) energy crops (Nges and Björnsson, 2012), the organic fraction of municipal solid wastes including food wastes (Koch et al., 2015; Zarkadas et al., 2015), industrial byproducts and biowaste(Pitk et al., 2013) and cellulose rich substrates including straw (Pohl et al., 2013).

Although anaerobic digestion is a well-established process, it is still suffering by a number of inhibitors (volatile fatty acid accumulation, ammonia, lack of trace elements, C/N imbalances, phenols) and the difficulties of the process to efficiently utilize a number of natural macromolecules (cellulose, hemicellulose, lignin and fat). While the problems generated by the inhibitors can be avoided through proper management, the inefficient digestion of some macromolecules can only be surpassed by technically updating the process.

Based on the above, the aim of this work was to assess the effect of enzyme addition on the anaerobic digestion of olive mill wastewater (OMW), olive mill solid waste (SMW), winery waste (WW), distillery waste (DW), dairy cattle manure (CM), slaughterhouse waste (SHW), white animal fat (WF) and sterilized mass–rendered byproducts of animal origin (SM). All experimentation took place under thermophilic conditions. The latter are superior when compared to the mesophilic conditions for bio-converting substrates into biomethane (Li et al., 2014). Furthermore, during the last years,industry has shifted towards thermophilic temperatures, especially when the generated heat cannot be utilized for any other purposes or when superior effluent quality is required or enforced (Angelidaki et al., 2006).

Materials and methods:

Enzymes

For these experiments four types of enzymes, lipase, cellulase, xylanase and protease, were used.

Substrates and inoculum

The inoculum was collected from a grid of 50 liter anaerobic reactor operating under steady state and sustained by different mixtures of cattle manure and food waste in OLRs ranging between 2 and 4kgVS/m^3-d.

Cattle manure (CM) were collected from a dairy farm. The dairy cattle in this farm were fed at a total mixed ratio composed of alfalfa pellets, straw, concentrate, mixed cereals and sweet sorghum. Fresh olive mill waste (OMW) and solid mill waste (SMW) were collected from a three-phase olive mill employing traditional olive pressing. The substrates were collected at the separation screw press. Slaughterhouse waste (SHW), and waste fat (WF) were collected from a large animal. The slaughterhouse wastes composed from parts of internal organs, waste soft tissue and fat, with all bones, hooves and skin excluded. Fat was collected from the same facility and from the dressing section where the meat is prepared to be send to the butchers. These different types of waste were frozen and subsequently grinded with the application of a bench top disk cutter through a 3mm

sieve, followed by manual mixing. Sterilisedmass (SM) was collected from a rendering facility where slaughterhouse waste is converted into sterilized mass that can be further utilized either as substrate to anaerobic digestion systems, pet feeds and soil amendments. This facility operates according to the EC Animal Byproduct Regulation and the recovered fat is further processed for biodiesel production. Winery waste (WW) and distillery waste (DW) were collected from a large alcoholic beverage production facility.

Results and discussion: The effect that four commercially available enzymes have on the anaerobic digestion of seven substrateswasexplored. While during the anaerobic digestion macromolecule hydrolysis takes place through enzymatic lysis, the addition of commercial products had limited and, in some instances, negative effects. Fat was one of the substrates that benefited by the addition of lipase both in the overall efficiency as well as the daily productivity. In contrast, the addition of protease in protein rich substrates resulted in the inhibition of the biomethane process. Two potential roots can explain the inhibition related to protease.The first mechanism can be directly related to the lysis of anaerobic bacteria. According to this mechanism the presence of protease does not allow the establishment of a microbial consortium able to efficiently bio-convert the substrate. Salazar and Asenjo(2007) describe a number of different forms of bacteria lysis by enzymes. A general lysis mechanism begins with the binding of the protease to the cell wall, followed by its rupture and release of the bacterium content. The second possible inhibitor is ammonia that is formed during the hydrolysis of protein and amino acids. Even though ammonia is an essential nutrient for microorganisms, at the same time it becomes one of the most powerful inhibitors of the anaerobic digestion process; the non-ion form of ammonia, known as free ammonia, presents the highest potential for inhibition over the anaerobic microorganisms. The maximum free ammonia concentration measured in this work was 2.02 g/L, which is a value significantly higher to these considered as inhibitory.

Conclusions: The effect of the different enzymes is shown to be a substrate specific process. Although protease is an important enzyme for tissue digestion,it inhibits the digestion process. On the other hand, the addition of lipase can improve the digestion though the release of nutrients that are bound within the fat polymers.

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