Upgrading municipal biowaste to high value feedstuff

P. Tsapekos[1], E. Papadopoulou[1], J.A. Madsen[2], N. Ahrensberg[3], I. Angelidaki[1]

[1]Department of Environmental Engineering, Technical University of Denmark, Kgs Lyngby, 2800, Denmark
[2]EnviDan A/S, Fuglebækvej 1, Kastrup, 2770, Denmark
[3]Biofos A/S, Refshalevej 250, Copenhagen, 1432, Denmark
Keywords: circular economy, biowaste, digestate valorization, singe cell protein.
Presenting author email: ptsa@env.dtu.dk

Nowadays, seas and arable lands are over-utilized due to activities related to aqua- and agriculture, respectively. Nevertheless, the traditional feeding strategies of the livestock sector should be reconsidered in order to meet the globally increasing demand of food and feed production. On this topic, the huge amounts of municipal biowaste that are underutilized could have a vital role. For example, the Municipality of Copenhagen in Denmark has lately decided to sort organic household waste. As a consequence, more than 14.000 tonnes of municipal biowaste were collected which amount was 30% more than the municipality had anticipated. A traditional approach would have been to pretreat the waste in a pulping facility in order to discard the non-degraded material and simultaneously, break down the organic matter (Khoshnevisan et al., 2018). Then, the pretreated municipal biowaste can be efficiently converted into biogas to produce electricity and heat, while digestate free of micro-pollutants can be spread in agricultural land for organic farming.

Within the frame of United Nations sustainable development goals (SDGs), anaerobic digestion (AD) can contribute on ensuring affordable and sustainable energy (Goal 7). However, with respect to SDGs, it is also an urgent need to ensure the existence of adequate amount of proteins to avoid starving population (Goal 2). On this topic, methanotrophs could accumulate high amounts of protein and thus, appear as a solution to help on the forthcoming global protein scarcity. Specifically, the methanotrophic upcycle of both gas and liquid streams of AD to high value feedstuff is lately considered as a promising alternative (Tsapekos et al., 2019). Biogas rich in methane can provide the needed carbon for biomass assimilation (Henard et al., 2018). On the other hand, nitrogen rich AD digestates can provide the macro-nutrients for microbial protein production (Matassa et al., 2015). Through this valorization route, municipal biowaste can significantly contribute on the replacement of fishmeal and soybean meal proteins. Hence, land, water, and natural resources could be alleviated from intense exploitation to produce feed for aqua- and agri-culture. In the present work, a novel integrated concept is suggested to upcycle the underutilized municipal biowaste into single cell protein (SCP) in a quality that can substitute traditional sources of proteins for animal feeding based on the frame of circular economy. More specifically, the well-established AD process and the trending concepts of biological biogas upgrading and nutrients recovery are combined with the cutting-edge technology of methanotrophic SCP production.

A naturally methanotrophic culture mainly consisted of two strains (i.e. *Methylomonas* and *Methylophilus* genera) was explored as mixed consortium. For pure culture cultivation, selected strains within *Methylococcus*, *Methylosinus* and *Methylomicrobium* genera were elucidated. Initially, the consortia were fed with purified synthetic CH_4 and grown at diluted mineral salt medium (dAMS). Subsequently, biologically upgraded biogas was explored as substitute to the standard gas stream. In parallel, digestate collected after the AD of municipal biowaste was treated in a two chamber electrochemical cell for nitrogen extraction and explored as alternative nitrogen source. Optical density at 600 nm (OD_{600}), biomass growth (gVSS/L), biomass yield on methane (g-VSS/g-CH_4), phospholipids and amino acids content were monitored to elucidate process performance. Amino acids are presented as percent of lysine because it is one of the most essential for human and animal nutrition and also, is considered to be limiting in main cereal species and legumes (Leinonen et al., 2019).

Initially, biowaste was anaerobically digested at mesophilic conditions and at hydraulic retention time of 20 days, producing more than 1.0 $L_{-CH4}/L_{r.day}$ when the feedstock contained 8% total solids. Raw biogas (~60% CH_4) after AD was biologically upgraded at a trickle bed reactor coupling the produced CO_2 with exogenous H_2. The produced biomethane was consisted of 95% CH_4, 1% CO_2 and 4% H_2. Moreover, 70% of ammonia was removed from digested biowaste applying 3.5 V at an electrodialysis cell which was equipped with a Ti alloy electrode coated with IrO_2 at the anode and a Ti woven wire mesh at the cathode. As a next step, the methane oxidizing bacteria were grown at the biologically upgraded biogas and extracted nitrogen. Biomass growth was compared with the usage of pure chemicals (Fig. 1a). Remarkably, no extension of lag phase, decreased growth rate, lower biomass yield or other signs of inhibition were detected. In addition, higher biomass yield was measured (1.08 ± 0.01 g-VSS/g-CH_4) compared to the control (0.71 ± 0.01 g-VSS/g-CH_4). The higher biomass production could be also attributed to the presence of *Methylomonas* genera of *Gammaproteobacteria* class, which contains strains able to fixate CO_2 (Tsapekos et al., 2019).

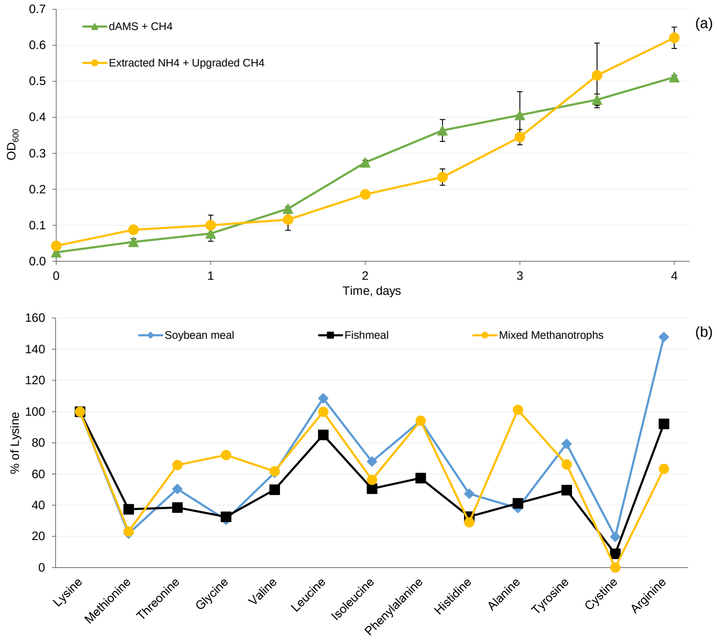

Figure 1. Mixed methanotrophic cultures: (a) grown at different sources of carbon and nutrients (a) and (b) amino acid profile in comparison with commercial soybean meal and fishmeal expressed as percent of lysine.

An efficient upcycling of used nitrogen and upgraded methane could stand as an innovative approach to decrease our dependency on traditional agriculture for food and feed supply. The obtained results are consistent with our proof-of-concept study showing that upgraded biogas and recovered nitrogen from digestate could be potential substitutes for natural gas and pure chemicals (Khoshnevisan et al., 2019). Furthermore, the amino acid profile of the methanotrophic biomass was compared with commercially available soybean and fishmeal (Fig. 1b). Among detected amino acids, the essential threonine was higher in the methanotrophs than the market products. The rest essential amino acids (i.e. methionine, valine, leucine, isoleucine, phenylalanine, histidine) were detected at insignificantly different fractions as percent of lysine. In contrast, the non-essential glycine and alanine were measured at significantly higher portions, while the conditionally essential cysteine and arginine were lower. Finally, methanotrophs accumulated more than 50% of their dry mass to proteins settling them a potential candidate for feed supplement.

The present research validated that upcycled AD gas (i.e. biologically upgraded biogas) and liquid (i.e. recovered nitrogen from digestate) streams can be used for fermentative SCP production. High biomass yield and relatively high protein content (>50% of dry mas) were detected. The produced biomass contained essential amino acids for animal feed and thus, could release over-exploited protein sources suitable for direct human nutrition.

Acknowledgement
The authors would like to acknowledge financial support by the Danish Agency of Energy program under MUDP “From Urban Bio-waste to Animal Feed – FUBAF” (J.nr. Mst-11700508).

References

Henard, C.A., Franklin, T.G., Youhenna, B., But, S., Alexander, D., Kalyuzhnaya, M.G., Guarnieri, M.T., 2018. Biogas Biocatalysis: Methanotrophic Bacterial Cultivation, Metabolite Profiling, and Bioconversion to Lactic Acid. Front. Microbiol. 9, 1–8.

Khoshnevisan, B., Tsapekos, P., Alvarado-Morales, M., Rafiee, S., Tabatabaei, M., Angelidaki, I., 2018. Life cycle assessment of different strategies for energy and nutrient recovery from source sorted organic fraction of household waste. J. Clean. Prod. 180, 360–374.

Khoshnevisan, B., Tsapekos, P., Zhang, Y., Valverde-Pérez, B., Angelidaki, I., 2019. Urban biowaste valorization by coupling anaerobic digestion and single cell protein production. Bioresour. Technol. 290, 121743.

Leinonen, I., Iannetta, P.P.M., Rees, R.M., Russell, W., Watson, C., Barnes, A.P., 2019. Lysine Supply Is a Critical Factor in Achieving Sustainable Global Protein Economy. Front. Sustain. Food Syst. 3, 1–11.

Matassa, S., Batstone, D.J., Hülsen, T., Schnoor, J., Verstraete, W., 2015. Can direct conversion of used nitrogen to new feed and protein help feed the world? Environ. Sci. Technol. 49, 5247–5254.

Tsapekos, P., Khoshnevisan, B., Zhu, X., Zha, X., Angelidaki, I., 2019. Methane oxidising bacteria to upcycle effluent streams from anaerobic digestion of municipal biowaste. J. Environ. Manage. 251.