Upgrading of biogas plants to produce microbial proteins from agro-waste

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The world population is set to reach 9 billion by 2050, with food demand expected to increase by 56% in the same timeframe (Van Dijk *et al*, 2021). The traditional sources of proteins - animals and plants – cannot be produced at the required pace, while their production greatly impacts the environment (Vermeulen *et al*, 2012). Therefore, new approaches must be sought to meet protein needs, such as the use of alternative protein sources like insects, algae, cultured meat and microbes. Microbial proteins (MPs) are organisms such as bacteria, algae, fungi and yeasts used for the manufacture of food, feed and various commodities (Ravindra, 2000). They can be produced at moderate costs by using low-value feedstocks like wastes and residues created by the agricultural sector (agro-waste), therefore contributing to the reduction of waste (Nasseri *et al*, 2011). Biogas plants already utilise agro-waste and transform it into biogas and fertilisers through the process of anaerobic digestion (AD). There is at present a great opportunity for taking advantage of existing AD plants and turning them into biorefineries not only of biogas and fertilisers, but also of other bio-based products like MPs destined for the aquaculture sector.

In this work, we evaluated the feasibility of a process where the production of biogas by AD was coupled with the production of MPs in the same plant (Fig. 1). Volatile fatty acids (VFA)-rich streams resulting from the acidogenic fermentation of agro-waste were used as feedstocks to evaluate the production of MPs by mixed microbial cultures (MMC) in a continuous stirred tank reactor (CSTR) at different hydraulic retention times (HRT). The obtained MPs were characterized through proximate analyses and amino acid profiling to assess their potential use as feed ingredients in aquaculture. The feasibility of the entire supply chain was evaluated through mass and energy balances, enabling a preliminary assessment of the economic feasibility of the integrated production process of biogas, biofertilisers and MPs in a medium-sized European biogas plant.

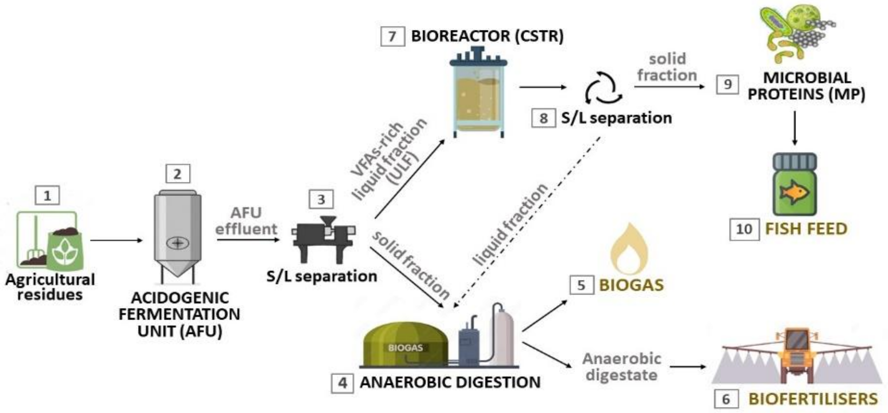

Figure 1. Schematic representation of the experimental set-up.

The process of acidogenic fermentation (the second step of AD) allowed the valorisation of the agro-waste into a VFA-rich fraction (ULF, Table 1), which was used as a culture medium for the MMC in the bioreactor. The microbial biomass obtained from the CSTR during the periods with the highest productivity in terms of volatile suspended solids (HRT = 2 and 3 days) was characterised to evaluate its potential application for aquafeed formulations. The protein content

Parameter	Unit	Average (min-max)
pH	-	5.2 (3.6-5.8)
Chemical Oxygen Demand (COD)	gCOD/L	42.5 (41.2-45.3)
Fraction of biodegradable COD	%	90.0
Total VFAs	gCOD/L	9.0 (7.5-18.4)
Acetic acid	g/L	6.4 (4.8-14.4)
Propionic acid	g/L	0.7 (0.5-4.8)
Butyric acid	g/L	1.9 (0.2-3.9)
Ammonia as nitrogen (NH_4-N)	gN/L	1.6 (0.4-1.9)

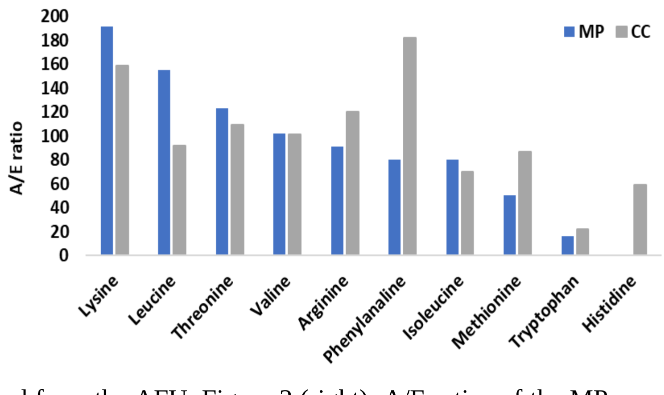

Table 1 (left): chemical-physical properties of the ULF collected from the AFU. Figure 2 (right): A/E ratios of the MPs compared to the dietary essential amino acids requirements for the common carp *Cyprinus carpio* (CC).

reached 61.8% of the total solids (TS, w/w) and 74.0% of volatile suspended solids (VSS, w/w), in line with the values of 50-83% reported for the typical concentration of crude protein in bacterial cells dry weight (Matassa *et al*, 2016), representing a good source of protein for a carnivorous fish, which normally requires 24-50% of protein in their diets (Cowey, 1994). The ten amino acids that are essential for fish growth were present in our sample, apart from histidine, which was below the level of quantification. The essential amino acid ratio (A/E) compared favourably to that of the dietary essential amino acid requirements for the common carp *Cyprinus carpio*, a fish species commonly used in aquaculture in Europe and Asia (Fig. 2).

Based on the results obtained from the CSTR, literature data and the input from the biogas plant operators, we estimated average production costs and break-even financial price balances for a typical existing Italian AD plant (current scenario, CS) and we compared them to an AD plant upgraded to produce MP (MP scenario, MPS) (Fig. 3).

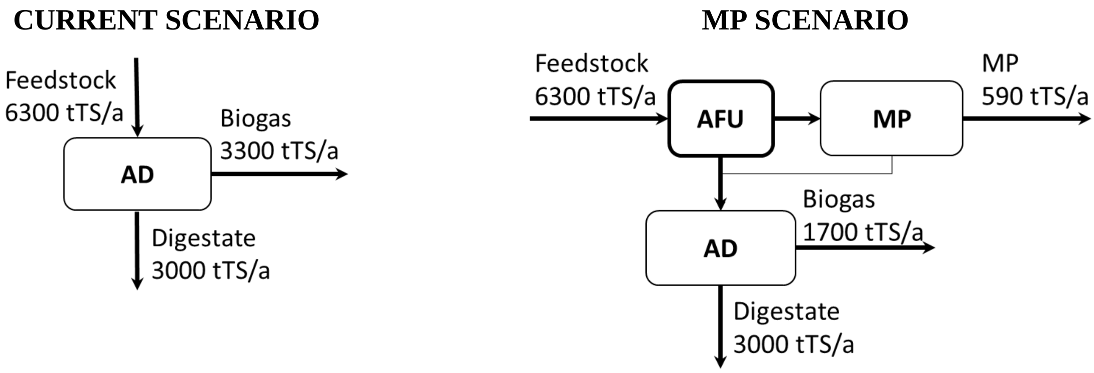

Figure 3. Simplified mass balance diagram featuring the major inlet and outlet flows in the current scenario (left, typical AD biogas plants in Italy) and in the MP scenario (right, upgraded AD plant for MPs production).

Under the CS, a biogas plant produces up to 6 GWh of electrical energy annually through the cogeneration of 2.5-2.8 NMm^3 of biogas with a methane content of 50-55%. On the other hand, the MPS plant has the potential to produce around 590 t MP/year with a crude protein content of 74% (based on VSS). However, the MP production comes with the trade-off of a 50% reduction in biogas production - an important factor in the economic assessment of the MPS. In terms of financial aspects, the total capital expenditures (CAPEX) for construction and equipment of the MPS are estimated at 4750 €/m^3 reactor (based on Peters *et al*, 2003) and the operational expenditures (OPEX) of the MPS are estimated at 360 €/t MP (based on Pikaar *et al*, 2018). Taking the OPEX into account and assuming a depreciation period of 25 years with an interest rate of 5% for CAPEX, the average estimated MP production costs in the MPS amount to 620 €/t MP. Finally, considering the costs of biogas and MP production, along with the revenue from selling the biogas-derived electricity at 70 €/MWh, without applying green incentives, an average break-even price for MP is estimated at 1,300 €/t of MP or 1,750 €/t of crude protein. This price does not reflect the socio-economic and environmental benefits of MP, while already being in the range or lower of the market prices of soymeal ($450 USD/t, i.e. 1,125 USD/t-protein with a crude protein content of 40%) and fishmeal (1,833 USD/t, i.e. 2,700 USD/t-protein with a protein content of 70%). This illustrates the competitiveness of MPS when evaluated against established market benchmarks. With the recent REPowerEU Action Plan of the European Commission (https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=SWD%3A2022%3A230%3AFIN&qid=1653033922121), which mobilises finances for supporting the sustainable maximisation of biogas production from agro-waste, and its subsequent upgrade to biomethane, it is anticipated that plants in Italy and in the EU will undergo expansion to enhance the economic feasibility of biomethane production. Consequently, there is a promising prospect for the expansion of MP production capacity, positively influencing both the economic aspects of production and the financial value of MPs derived from agro-waste proposed in this study.

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