RE-FISH TO FOOD biorefinery:
Valorization of seafood and cannery industry resources into alternative proteins

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Introduction:

The global consumption of animal products is ever increasing, but their production through current agricultural practices results in detrimental environmental effects. The seafood industry processes around 80% of the total catch into transform products. Centralised processing operations, including beheading, de-shelling, skinning, gutting, removing fins and scales, filleting and washing, among others, generate significant amounts of solid waste and wastewater as effluents (Choudhury et al., 2022). Anaerobic digestion and dark fermentation are established technologies that can help reduce non-edible effluent volumes while producing biogases (mainly CH_4, H_2 and CO_2) that can be used to produce energy and high value-added products.

Microbial protein (MP) or single-cell protein (SCP) could alleviate the environmental and socioeconomic pressure caused by the limitations of conventional agriculture, because it can provide nutritional protein based on recovered resources. MP is the biomass of microbes, such as fungi, yeasts, microalgae and bacteria, which contain all the essential amino acids (EAA) and can replace conventional protein food/feed sources. A recent life cycle assessment (LCA) revealed the lower environmental impact of MP compared to the use of soybean meal as a feed ingredient (Spiller et al., 2020). Methane oxidizing bacteria (MOB), also known as methanotrophs, use CH_4 as carbon and energy source. Hydrogen oxidizing bacteria (HOB), use H_2 as an energy source and can use CO_2 as a carbon source. Both MOB and HOB are biotechnologically interesting organisms, capable of producing a variety of products.

Within the framework of the circular economy, this work explores the feasibility of producing MP through the biorefinery proposed in the Re-Fish to Food project (**Figure 1**). Treatment of 25 different effluent and by-product streams from the seafood industry has been studied in AD and DF processes. Furthermore, biomass growth and accumulated protein amount of different MOB and HOB strains fed CH_4 and H_2 have been analysed.

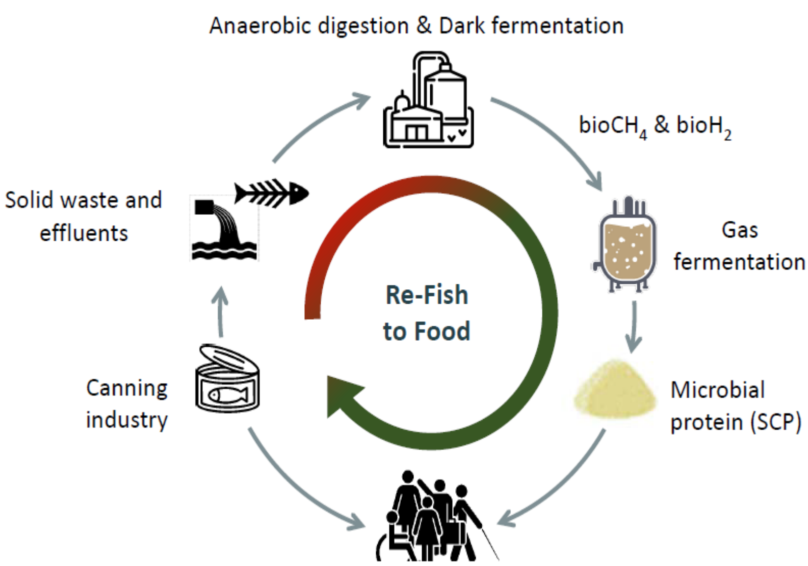

Figure 1. Biorefinery proposed in Re-Fish to food project.

Materials and Methods:

Twenty-five samples (named S1 to S25) were obtained from 11 relevant seafood and cannery industries in Galicia, Spain. These samples were characterized by standard methods. The tests were performed in triplicate, at mesophilic temperature and following existing protocols both for Biomethane potential (BMP) for AD (Angelidaki et al., 2009) and Biohydrogen potential (BHP) for DF (Carrillo-Reyes et al., 2020). To produce protein by gas fermentation, 3 MOB strains (*Methylococcus capsulatus*, *Methylomonas methanica* y *Methylomonas koyamae*) and 2 HOB strains (*Cupriavidus necátor* y *Xanthobacter autotrophicus*) were studied separately on sterilized media and on the mixture of the 3 MOBs (mixMOB). All tests were carried out in triplicate in 100 ml bottles in an incubator at constant temperature (28°C) with synthetic macro and micronutrients following Kerckhof et al. (2021). The MOB were fed a gas mixture of 50% CH_4 and 50% air and the HOB with 50% H_2, 30% air and 20% CO_2 by volume at a pressure of 1 bar. At the end of the experiment, the gas remaining, the volatile suspended solids (VSS) present to determine the maximum biomass growth and the proteins present in the solid were measured using the KjeldahlNitrogen method (Met.4500-Norg B Standard Method).

Results:

According to the physico-chemical characterisation of the samples studied, the 4 samples with a content of more than 10% in carbohydrates were chosen to undergo DF, while the rest went to DA. All results are shown in **Figure 2**. The BMP results show yields between 200-520 $LCH_4\ kgSV^{-1}$, yields within the high range of the literature (Choudhury et al., 2022). The BHP results show yields between 50-100 $LH_2\ kgSV^{-1}$. These results are promising for the development of the proposed biorefinery, and the most promising samples are currently under continuous-mode evaluation in CSTR reactors.

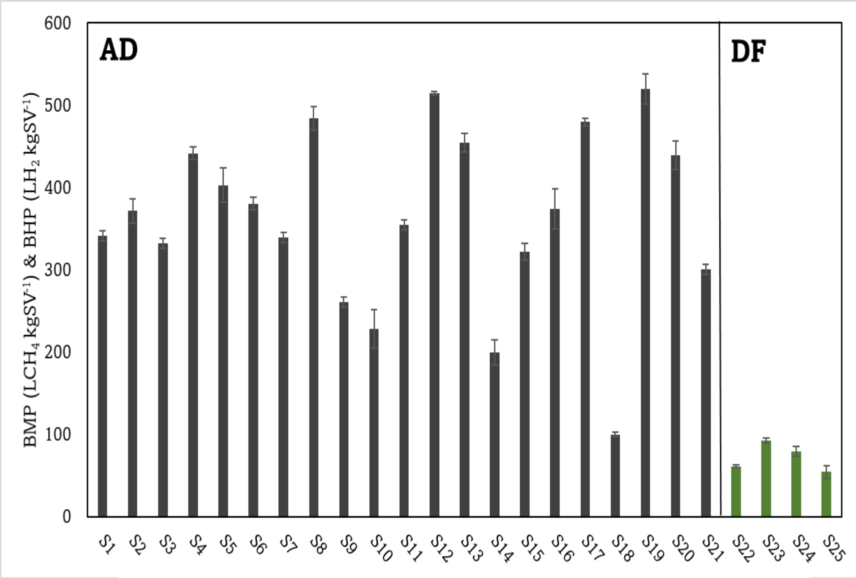

Figure 2. BMP (AD) and BHP (DF) results for all samples.

In studies of protein production by gas fermentation, more than 70% of the gas present in the bottle was consumed in less than 5 days. However, it is relevant to add that in the case of HOB bacteria, about 95% of the hydrogen present was consumed, suggesting a possible limitation of growth due to the lack of H_2. Regarding the maximum biomass growth, we can see in **Figure 3** that *Methylomonas koyamae* was the MOB with the highest growth, with a mean of 1.42 ± 0.18 g L^{-1} of biomass. As for HOB, *Cupriavidus necator* had the highest growth of up to 1.28 ± 0.04 gSSV L^{-1}. Surprisingly, the highest growth occurred with the co-culture of MOB (MixMOB) with a biomass of up to 1.66 ± 0.9 g L^{-1}.

We can observe that there are no significant differences between the amount of protein present in the different MOB (between 58% and 63%). However, we observed some difference in the HOB, where *Xanthobacter autotrophicus* accumulated 58% protein while *Cupriavidus necator* reached 65%. The MixMOB test accumulated 63% protein and was therefore considered the best option for scaling. The next step currently underway is the cultivation in continuous mode in an airlift reactor.

These results are above of those observed in the literature, where protein percentages of 50% are normally achieved (Sahoo et al., 2021). However, integration of the process is essential for its scaling as well as a study of the quality of the essential amino acids of the proteins obtained. Both aspects are currently being studied.

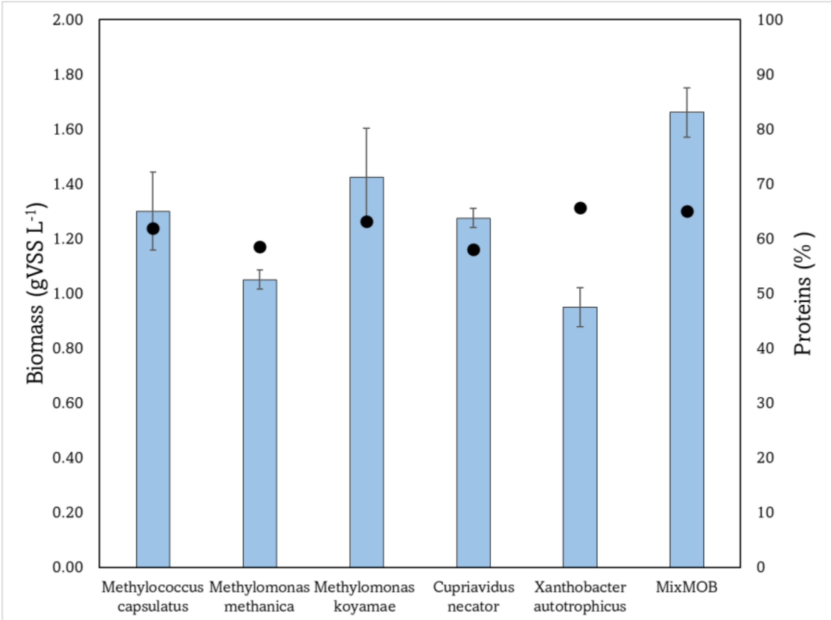

Figure 3. Biomass growth (bars) and protein accumulation (dots).

Conclusion:

Our research suggests that seafood non-edible by-products could be a valuable source to produce alternative proteins. The treatment of these by-products by AD and DF produces high amounts of biogas (up to 520 LCH_4 $kgSV^{-1}$ and 105 LH_2 $kgSV^{-1}$). This biogas is fed to MOB and HOB bacteria capable of accumulating proteins up to 65% of their mass. This work has the potential to contribute to the sustainable development of alternative proteins using the precepts of the circular economy.

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