

# HYDROLYSIS AND THERMOCHEMICAL TECHNOLOGIES FOR THE RECOVERY OF BIO-BASED FERTILISER FROM FISHERY WASTE

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**ABSTRACT:** The growing demand for seafood consumption had led to the increase of the seafood production from 134.3 million tonnes in 2004 to 170.9 million tonnes in 2016 leading to an increase of the by-products volume, which can account for 30–70% of whole seafood after industrial processing. Biorefinery concept, based on circular economy model, is developed to recover bio-based fertilizer transforming such by-products into nutrients. Mollusc waste (non-edible products) and fish waste (viscera, heads, bones, etc) are valorized in liming agent (calcium carbonate) for correction soil pH, in protein hydrolysate with biostimulant properties and in biochar for the enhancement of soil fertility and productivity.

*Keywords:* fish waste, biorefinery, BBF, biochar, biostimulant

## 1. INTRODUCTION

Fishery plays a significant role for every country in the Adriatic basin. The largest catch, in 2007, was in Italy – 465,637 tons (Zonn et al., 2021). Marine activities are highly relevant in the economy of Ancona (Italy) where mollusc industry manages around 9830 t·y<sup>-1</sup> considering mussels, clams, murex, etc. At the same time, several small and medium companies operate in the marketing and processing of fish products from the Adriatic Sea (tub gurnard, monkfish, hake, salmon, gilthead, cuttlefish, etc).

Seafood processing is accompanied by the generation of tremendous amounts of by-products and discards: for instance, mollusc industry generates 500 t·y<sup>-1</sup> of biowastes. Such by-products (formerly considered as wastes) produced in fishery industry are usually rich in different elements as N, P, K, Ca, as well as, in micronutrients as Cu, Zn, Mo, and other relevant biomolecules such as proteins, polysaccharides, lipids, carotenoids, vitamins, minerals, etc. Recovering these valuable biomolecules and nutrients can mitigate environmental problems associated with seafood processing (Bruno et al., 2019) and close the loop in the fishery industry. One solution is to combine the excess of nutrients of these side-streams and wastes with the need for nutrients in agriculture. Currently, about 80% of fertiliser consumption comes from fossil sources and industrial processes that require large amounts of energy (especially in the case of N-fertilisers) or, from non-renewable sources such as phosphate rocks (as in the case of Phosphorous), which is a key element for agriculture and has been classified as critical material by the European Union (Cordell & White, 2013).

Depending on the kind of treatment, biowastes can be not only used to produce value-added

chemicals (Nisticò, 2017) but also other downstream processes (e.g., composting, incineration, etc.) can be applied to the by-products to obtain maximum productivity in a biorefinery scheme.

## 2. MATERIAL AND METHODS

### 2.1 By-products characterisation

Mollusc wastes were obtained from a company set in Ancona and was composed (on average) by clam (79%), mussel (13%) and murex (8%). It resulted in two different fractions that were separated to optimise the recovery of added-value compounds: 67-85% of the total waste, depending on the mollusc species, consisted of shells and only the 15-33% was residual meat. Shells have a low moisture content (<8%) and involve mainly inorganic compounds (>94% of ashes), more than 80% being calcium carbonate (CaCO<sub>3</sub>), while crude protein content is lower than 1% on a dry basis. On the other hand, the residual meat has only 19-28 % of dry matter and its organic fraction accounts for 74-89% (Table 1); crude protein consists of 49.5-64.4%, while fats are in the range 17.3-23.9%.

In both shell and residual meat, the concentration of heavy metals is comparable to that of the organic fraction of municipal solid waste (Fisgativa et al., 2016) commonly used as a by-product in aerobic and/or anaerobic treatments to produce fertilisers.

Concerning fish waste, it must be noted that their characteristics are similar to those of the organic fraction of mollusc, showing comparable amounts of dry matter and crude proteins (Table 1).

Table 1. Chemical characterisation of fishery by-products.

Parameter	Unit of measure	Meat residue	Shell fraction	Fish waste
Dry matter	%	23.7±4.6	93.02±0.59	26.2±6.9
pH	-	5.9±0.5	8.5±0.1	6.0±0.4
Ashes	% d.b.	23.5±8.6	95.9±11.5	13.7±4.4
Crude protein	% d.b.	52.1±7.9	1.6±0.5	43.0±7.6
Fats	% d.b.	22.6±3.5	-	-
P	g/kg d.b.	8.69±1.08	0.41±0.2	16.7±9.9
Na	g/kg d.b.	15.85±2.85	6.11±1.24	7.72±1.96
Mg	g/kg d.b.	3.20±1.31	0.47±0.16	2.05±0.42
K	g/kg d.b.	9.40±0.21	0.42±0.23	5.12±1.11
Ca	g/kg d.b.	41.7±14.5	347.5±13.6	39.1±18.4

### 2.2 Biorefinery scheme

According to the by-products characterisation, a biorefinery scheme was proposed to valorise the different fractions of the waste stream.

Since the shell fraction and meat residue of mollusc present so different characteristics, mollusc waste management requires a first wet separation step to split both fractions: crushed shells settled at the bottom of the tank (recovered shells); and a liquid fraction composed of the added water and the residual meat. The former, once dried and milled, could be used as an alternative to the mineral calcium carbonate obtained by a quarry to correct the pH of acidic soil, i.e., as a liming agent.

On the other hand, as the organic fraction of mollusc waste contains significant amounts of proteins (Table 1), these waste streams are suitable materials for protein hydrolysis, obtaining protein hydrolysates (FPH) through enzymatic hydrolysis of the liquid stream. Fish waste, similar in composition to the residual meat, could be added at this biorefinery step to increase hydrolysate production. Hydrolysis is followed by a filtration and/or centrifugation step to separate the raw FPH from the solid residue (bones, not hydrolysed substrate, etc). The high nitrogen concentration is often considered as a limiting factor for the valorisation of fishery waste through consolidated technologies such as anaerobic digestion and composting. Protein hydrolysis thus permits to obtain added-value products like biostimulants for the agriculture sector and, at the same time, a solid residue with a reduced nitrogen content and increased C:N ratio that could be further valorised.

In particular, the solid residue could be composted and/or pyrolysed to obtain compost and biochar, respectively. Biochar and compost present significant potential for soil C sequestration. The biochar-compost blending would enhance the composting performance by adding more stable C and creating a value-added product (biochar-compost blend) that can offset potential negative effects of the composting system and of the pyrolysis biochar system (Oldfield et al., 2018).

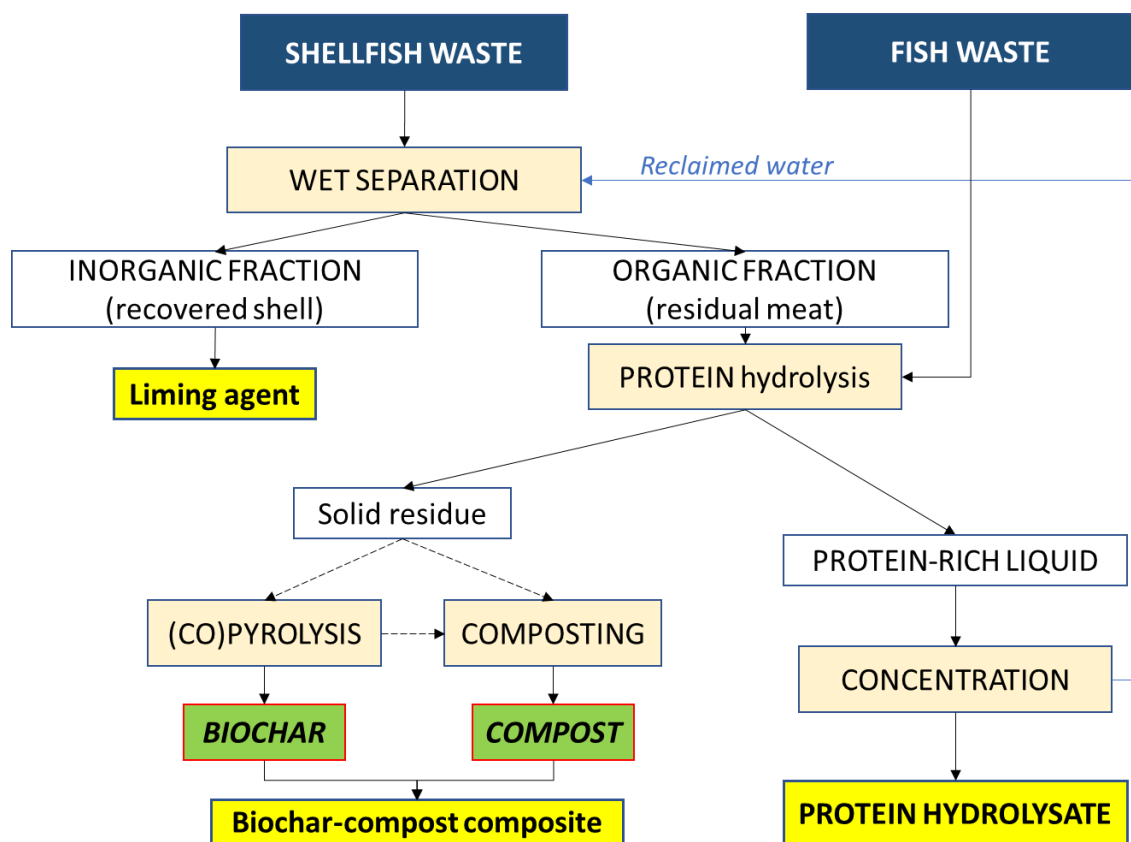


Figure 1. Scheme of the proposed biorefinery from fishery by-products.

### 2.2.2 Enzymatic hydrolysis of proteins

Different enzymatic hydrolysis tests were carried out at lab-scale to test the effect of temperature, pH and the type of enzyme in the protein hydrolysis of mollusc and fish. The set parameters are shown in Table 2. It has to be noted that the mix (50% each) of both types of wastes (fish waste and organic fraction from mollusc) were also tested.

Table 2. Lab-scale conditions of the hydrolysis tests.

Factor	Test conditions
Substrate	Mollusc, fish and mix (1:1)
Substrate:Water (w/v)	1:2
Temperature	28, 40, 60 °C
pH	Natural, 7.5, 8.5
Type of enzyme	Alcalase, Protana
Enzyme: substrate (v/w)	1:100
Hydrolysis duration	4 hours

It must be highlighted that the tests were done with fresh waste samples collected the same day of test. This implied that the characteristics of raw materials differed slightly from one test to another. For this, the comparison of results between tests was carried out by the nitrogen recovered (NR) which is referred to the initial nitrogen content of the substrate (Eq. 1):

$$NR(\%) = \frac{TKN_{liquid}}{TKN_{substrate}} * 100 \quad (\text{Eq. 1})$$

where  $TKN_{liquid}$  is the total Kjeldhal nitrogen ( $\text{mgN}\cdot\text{L}^{-1}$ ) measured in the liquid fraction of the hydrolysed sample;  $TKN_{substrate}$  is the total Kjeldhal nitrogen ( $\text{mgN}\cdot\text{kg}^{-1}$ ) measured in the raw sample before the hydrolysis test.

### 2.2.3 Pyrolysis of solid residue

The solid residues from the hydrolysis tests were dried at 105°C and then grounded by an impact mill (IKA A11 basic). Thermo-gravimetric properties of the solid residue were assessed through a thermogravimetric analyser (LINSEIS STA PT-1600). The sample (20 mg) was heated from 25 °C to 900 °C at Bi and a heating rate of 10 °C/min.

Once reached 900°C, the nitrogen flux was stopped and the temperature of 900°C was maintained for 15 min at an air flowing rate of 100 mL·min<sup>-1</sup>. This permitted to determine the amount of fixed carbon present in the sample. Tests were conducted in triplicate.

## 3. RESULTS AND DISCUSSION

### 3.1 Wet separation

Prior mixing of the solid waste with water is needed to carry out the separation step of the two fractions: i) crushed shells (recovered at the bottom of the tank); and ii) the liquid fraction composed of the added water, the residual meat, and also some lighter particles that came out from shredding (impurities).

Different waste to water (W:W) ratios were assessed (1:0.3, 1:1, 1:1.5, 1:2, weight:weight) with the goal to check if this ratio had a prominent effect on separation performance. W:W ratio did not influence the separation efficiency, i.e., the organic matter content in the recovered shell did not show significant variations between different tests, being in the range of 2.7-3.0% on dry basis.

The recovered shell (solid fraction), to be used as liming agent, is certainly of interest considering that the European median pH is 5.8 for Ap soil (0-20 cm topsoil) (Fabian et al., 2014).

### 3.2 Protein hydrolysis

In all conditions, hydrolysis carried out with Alcalase enzyme showed significantly higher nitrogen recovery than Protana enzyme (data not shown). This was not surprising since Protana is an exo-protease enzyme while Alcalase is a wide range endoprotease, cutting especially on hydrophobic amino-acids.

Indeed, Protana is commonly more effective if it is used after a previous hydrolysis step to increase the quality of the final biostimulant by enhancing the free amino-acids contents.

After testing the already mentioned enzymes, the effect of pH on the hydrolysis of the organic fraction of mollusc, fish waste and a mixture of them (50% each) was assessed. As can be seen in Figure 2, no significant differences were observed at variable pH for any of the fractions tested. This means that natural pH (without reagent addition) was the most beneficial one since it avoids the use of reagents that are costly economically and environmentally.

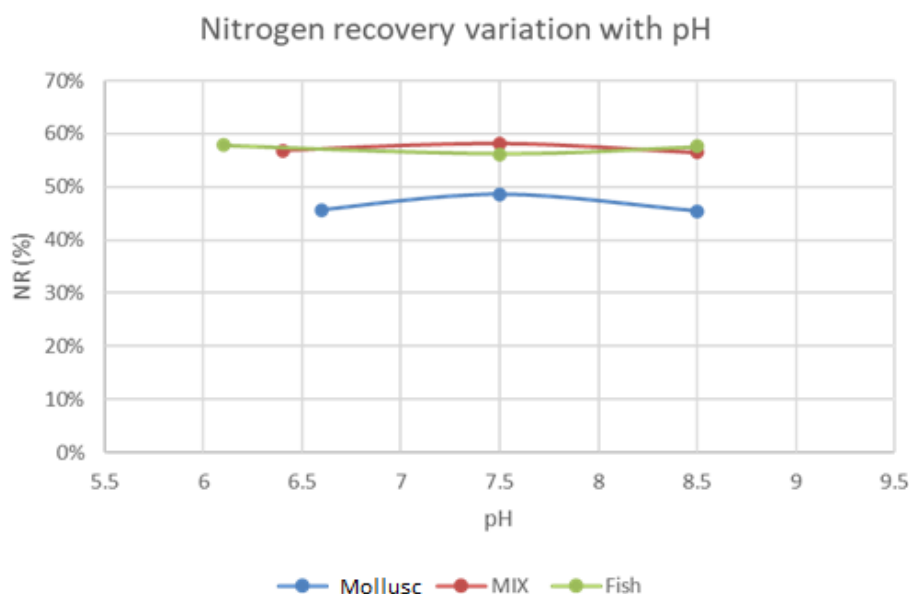


Figure 2. Nitrogen recovery (NR) obtained at different pH in hydrolysis tests.

Regarding temperature, three different values were analysed: 28 °C (room temperature), 40°C and 60°C. In this case, only mollusc and mix (50% mollusc and 50% fish) were tested. As observed in Figure 3, the mix fraction obtained significantly higher NR than the mollusc one. This trend was previously observed when the effect of pH was analysed, achieving similar nitrogen recovery for fish

and mix (Figure 2). This indicates that adding fish waste to the organic fraction of mollusc can help to increase the hydrolysate production from this raw material.

It must be also noticed that both fractions showed a logarithmic relationship between NR and temperature. This means that at mid temperatures up to 45-50°C the temperature has a more significant effect on NR but after surpassing these temperature values, the nitrogen recovered tend to be almost constant with temperature increases. This suggests that if this process were upscaled, temperatures should be in the aforementioned range to maintain the process as feasible as possible since further temperature increases (which implies higher operating costs) will not entail higher nitrogen recoveries. In any case, cost-benefit analysis should be carried out to corroborate this statement.

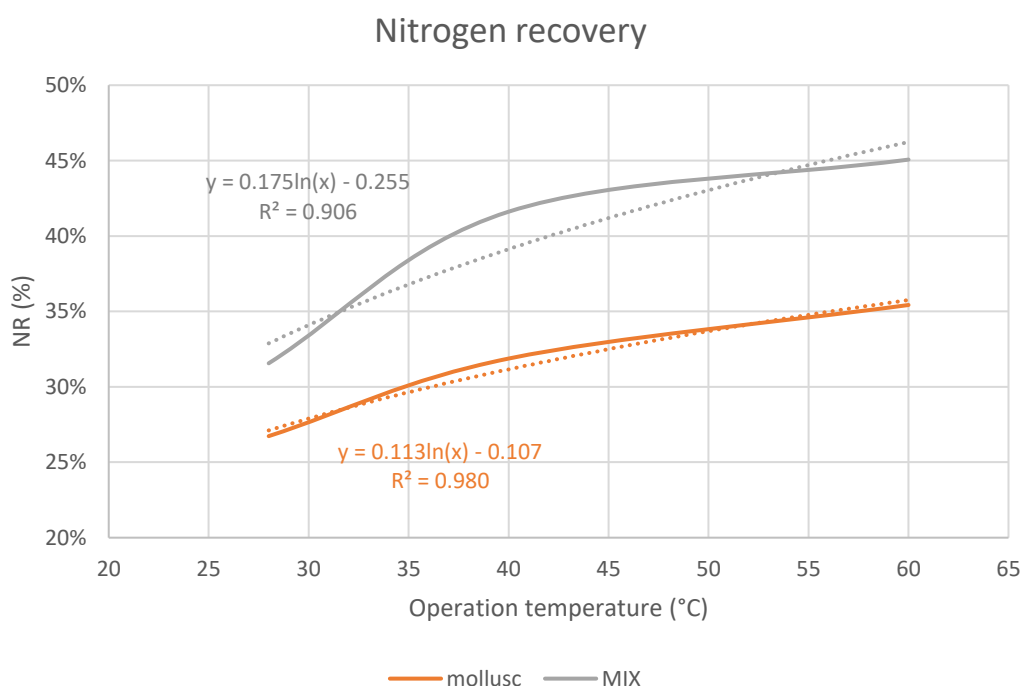


Figure 3. Nitrogen recovery (NR) obtained at different temperatures in hydrolysis tests.

### 3.3 Pyrolysis assessment

To assess if the residues from the protein hydrolysis process were suitable for pyrolysis, thermogravimetric analysis (TGA) of those residues were carried out. Thermogravimetric properties of the solid residue are summarized in Table 3.

The organic content, referred to the weight losses at temperatures lower than 550°C, of mollusc residue was around 22 %. It was slightly increased when fish waste was added (Table 3). On the other hand, fish residue showed a weight loss two times higher. This was probably since mollusc residue from hydrolysis contained some impurities (rests of shells, sand, etc.) that decreased the organic content of the residue.

During pyrolysis this lost fraction under 580 °C will be converted in bio-oil (condensable gases) and syngas (H<sub>2</sub>, CO, CO<sub>2</sub>, CH<sub>4</sub>), while the residual mass consists of biochar. Thermal valorisation of the formers helps pyrolysis to achieve self-sustained energy requirements. Biochar can be, instead, applied to the agriculture sector to improve crop yields as well as for carbon sequestration. Biochar used for soil amendment seems to improve nutrient density of soils, water holding capacity, reduce fertiliser requirements, enhance soil microbiota, and increase crop yields (Allohverdi et al., 2021).

Table 3. Thermogravimetric properties of solid residue.

Sample	Weight loss % 150-550°C	Weight loss % 150-900 °C	Fixed Carbon %
Mollusc	21.9±0.3	52.6±0.1	0.8±0.05
Fish	53.7±1.3	69.2±0.6	10.6±1.2
Mix	22.7±1.9	54.3±1.5	0.3±0.03

Preliminary results obtained from the thermogravimetric analysis will be used to set operation conditions of pyrolysis. The slow pyrolysis process (heating rate in the range of minutes to hours) will be promoted since produces the highest biochar yield (Roy & Dias, 2017).

On the other hand, one promising option is using the “terra preta concept” combining biochar and composting technologies. This concept could enhance the quality and material properties of compost products leading to a higher added value and to a much better C sequestration potential due to the long-term stability of biochar (Fischer & Glaser, 2012). According to recent studies, biochar has exhibited great potential for enhancing composting. Biochar addition in composting can: (1) improve compost mixture physicochemical properties, (2) enhance microbial activities and promote organic matter decomposition, (3) reduce ammonia (NH<sub>3</sub>) and greenhouse gas (GHG) emissions, and (4) upgrade compost quality by increasing the total/available nutrient content, enhancing maturity, and decreasing phytotoxicity (Xiao et al., 2017).

## CONCLUSIONS

The biorefinery scheme presented in this study is strongly based on circular economy principles and allows transforming wastes from the fishery industry into nutrients and other value-added products for crops. Specifically, 1 ton of waste (800 kg from mollusc industry and 200 kg from fish processing) could be valorized in 595 kg of a liming agent through separation, drying and milling of mollusc shell, 30 kg of biostimulant by enzymatic hydrolysis of organic fraction and 22 kg of biochar through pyrolysis of solid residue.

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