Journal of Cleaner Production 416 (2023) 137952

Contents lists available at ScienceDirect

Journal of Cleaner Production

journal homepage: www.elsevier.com/locate/jclepro

Microbial bioconversion of chemical waste effluents from marine gelatin isolation: Production of probiotics under circular economy philosophy

José Antonio Vázquez [a,*], Araceli Menduíña [a], Ana I. Durán [a], Margarita Nogueira [a], Javier Fraguas [a], Adrián Pedreira [a,b], Jesus Valcarcel [a]

[a] Grupo de Reciclado y Valorización de Materiales Residuales (REVAL), Instituto de Investigaciones Marinas (IIM-CSIC), C/ Eduardo Cabello, 6, CP 36208, Vigo, Galicia, Spain

[b] Grupo de Biosistemas e Ingeniería de Bioprocesos, Instituto de Investigaciones Marinas (IIM-CSIC), C/ Eduardo Cabello, 6, CP 36208, Vigo, Galicia, Spain

ARTICLE INFO

Handling Editor: Maria Teresa Moreira

Keywords:
Microbial bioconversion
Gelatin waste streams
Probiotics production
Lactic acid production
Sustainability
Circular economy

ABSTRACT

The marine gelatin is one of the most interesting biocompounds to recover from fish skin by-products. The initial processes commonly used for gelatin isolation are based on the alkaline and acid washing of skins. These streams, with remarkable levels of proteins, must be efficiently managed and depurated to avoid environmental pollution and to make the gelatin recovery viable. In the current study, we have evaluated the bioconversion of those contaminant gelatin effluents (GE) from tuna, shark, turbot and salmon by means of two probiotic lactic acid bacteria (LAB). These LAB, *Lactobacillus plantarum* and *L. brevis* were fermented in batch culture, under controlled conditions, in each of the effluents which simulated the common medium for LAB (Man, Rogosa, Sharp, MRS) but without commercial peptones. In the 50% of the media based on GE, the growth of both bacteria (achieving, for example, 5.2 g/L of *L. brevis* in alkaline-tuna stream) and lactic acid productions (20 g/L using citric acid-shark stream in *L. plantarum*) were similar or higher than those observed in MRS. Minimal GE media formulated only with effluents, glucose and salts demonstrated the essential presence of yeast extract as an ingredient to achieve optimal growths. Unstructured mathematical equations modelled accuracy the experimental kinetics of all LAB productions (R^2 = 0.92–0.99) and nutrient consumptions (R^2 = 0.75–0.99). From an economical viewpoint, productions on effluents reduced around 3 times the costs of production reported in MRS. GE showed to be a good substrate to support LAB productions and the approach exposed here is a sustainable solution to valorize and depurate such wastewaters will help to increase the profitability of fish gelatin industry.

1. Introduction

Gelatin is an important commercial biopolymer with wide application in food, pharmaceutical and cosmetic products, resulting in an annual global production of around 620,000 metric tonnes (Koutsoumanis et al., 2020). Production of gelatin involves partial denaturation of collagen, the main structural protein in animal connective tissue, typically from porcine skin and bovine hide and bones, which is also a high demanded bioproduct in nano and tissue regenerative devices (Abdullah et al., 2022, 2023; Carvalho et al., 2023; Silva et al., 2014). However, cultural constraints and safety concerns have spurred interest in alternative sources such as poultry and fish (Dille et al., 2021). Although extraction processes vary depending on the source tissue, these commonly involve initial washing followed by acid or alkaline treatments to eliminate impurities such as fat, minerals, and non-collagenous protein, and render the tissue more amenable to later thermal hydrolysis (Boran and Regenstein, 2010).

The gelatin extraction process produces a large volume of effluents, in the order of 300–1500 m^3 per tonne of raw material (Maree et al., 1990; Wang et al., 2018), of a heterogenic nature. The alkaline treatment releases fat, hydrolysed protein and ammonia, whereas soaking in acid results in wastewaters rich in inorganic ions and protein (Maree et al., 1990; Ghatnekar et al., 2010; Wang et al., 2018; Tawfik et al., 2021). Besides these liquid effluents, gelatin production also generates an important amount of sludge and solid waste (Awasthi et al., 2016). Treatment of such processing waste streams has traditionally focused on effluent cleaning by coagulation (Arturi et al., 2019), phosphate absorption to avoid eutrophication (Rai and Maheshwari, 2002), collagen hydrolysates production by enzimatic hydrolysis (Vázquez et al., 2021; Valcarcel et al., 2021a) and aerobic or anaerobic digestions (Maree

* Corresponding author.
E-mail address: jvazquez@iim.csic.es (J.A. Vázquez).

https://doi.org/10.1016/j.jclepro.2023.137952
Received 3 January 2023; Received in revised form 9 June 2023; Accepted 29 June 2023
Available online 30 June 2023

et al., 1990) before discharge.

As an additional step towards resource efficiency, some authors have proposed from basic co-composting of sludge with other residues (Awasthi et al., 2016), or even direct application in the soil (Silvano et al., 2018), to more sophisticated purification strategies integrating vermiculture, enzymes, and microorganisms to produce compost and clean water (Ghatnekar et al., 2010). In another approach aimed at increasing the efficiency of the process, hydrochloric acid from acidification wastewater can be recycled for reuse in the gelatin process, producing at the same time calcium sulfate (Wang et al., 2018). Associated to anaerobic digestion, further value addition might be possible through concomitant biofuel generation (Tawfik et al., 2021) or cultivation of microalgae with anaerobically digested wastewater as culture medium (Blanco et al., 2020).

This last biotechnological approach is particularly interesting for residual streams rich in nitrogen, not only for microalgae, but also for the culture of several microorganisms of industrial relevance. Peptones are the most expensive ingredient in the bacterial culture media, they are produced after some form of hydrolysis of animal by-products, and they supplied the essential organic nitrogen (in form of protein, peptides and free amino acids) suitable for the growth of microorganisms (Ummadi and Curic-Bawden, 2010; Vázquez et al., 2020a; Johnson et al., 2022; Vázquez et al., 2022; Barragán et al., 2022). In the case of gelatin waste streams, further hydrolysis may not be necessary, and these wastewaters could be directly a source of protein feedstock substitute of commercial peptones. This kind of application has not been reported yet for the case of gelatin effluents.

For the evaluation of these streams validity, as main protein component in culture media, lactic acid bacteria (LAB) are perhaps the most appropriate type of microorganisms due to (Vos et al., 2011; Kanmani et al., 2013; Kowalczyk et al., 2015; Nácher-Vázquez et al., 2015; Zhang et al., 2022): a) they are fastidious microbes in terms of nutritional demand of nitrogen source; b) they are bacteria of industrial relevance for the production of food ingredients –starters, dairy products, fermented meat and vegetables–; c) in many strains, aquaculture and human probiotic properties have been demonstrated; d) they are producers of bioactives, organic acids, biopolymers and other compounds very appreciate by chemical industry.

In recent years, replacement of traditional bovine and porcine sources of gelatin has concentrated on fish, not only because of advantages related to cultural or sanitary reasons, but also aiming at increased sustainability of the fishing industry (Tawfik et al., 2021). In previous works we have extracted and characterized gelatin from a number of fish species (Sousa et al., 2017; Vázquez et al., 2021; Valcarcel et al., 2021b) generating an important volume of effluents from alkaline and acid treatments.

In the present manuscript, we evaluate the performance of those resulting chemical waste streams from selected species –tuna, turbot, salmon and blue shark– as nitrogen source in the culture of two relevant LAB, *Lactobacillus brevis* and *Lactobacillus plantarum*, of technological and probiotic relevance (Martins et al., 2013; Pereira et al., 2022). Moreover, a brief and initial economical assessment of this bioconversion process is proposed to highlight its validity and thereby contribute to the sustainability of gelatin fish production.

2. Materials and methods

2.1. Chemical effluents from fish gelatin production

Gelatins were produced from skin wastes of four species, yellowfin tuna (YT, *Thunnus albacares*), turbot (Tu, *Scophthalmus maximus*), blue shark (BS, *Prionace glauca*) and salmon (Sa, *Salmon salar*) obtained from processing of canning (YT), aquaculture (Tu and Sa) and fishing (BS) industries. After peeling, fresh skins were immediately stored at −18 °C and cut frozen into 5 cm squares maximum for gelatin extraction. In all substrates, the production of gelatin was performed by a combination of initial chemical treatments, aqueous thermal extraction, purification of gelatin solution and drying (Fig. 1). To complete the integral valorization of skins, the remains of skins after gelatin recovery were also enzymatically hydrolysed to obtain collagen hydrolysates (Vázquez et al., 2021; Valcarcel et al., 2021b).

Briefly, the chemical procedures involved a sequential set of skin washes with 0.05 M NaOH, 0.02 M H_2SO_4 and 0.052 M citric acid, all conducted under the same conditions of a 1:4 solid:liquid ratio, at room temperature, and agitation at 50 rpm for 30 min (Vázquez et al., 2021). A water washing step was applied for 30 min between each treatment. Gelatin effluents (GE) –NaOH stream (Na), sulphuric acid stream (Su) and citric acid stream (Ci)– for each fish skin species were characterized in terms of pH, total sugars, soluble protein, total lipids and amino acids content (see section below) and stored at −18 °C until use. In Table 1 and Table S1 (supplementary material), the results of those analysis are summarized.

2.2. Bacterial procedures and culture media

Two lactic acid bacteria from CECT (Spanish Type Culture Collection) were used as target microorganisms: *Lactobacillus plantarum* CECT 220 and *Lactobacillus brevis* CECT 4043. Stock cultures were stored at −80 °C in Man, Rogosa and Sharpe medium (MRS, from Pronadisa, Hispanlab S.A., Spain) with 25% glycerol (w/w). Three types of low-cost media, based on GE as basic component, were evaluated (Table S2, supplementary material): a) broths similar to MRS (Vázquez et al., 2016) in which commercial peptones included in MRS (meat extract and bactopeptone) were not added (A); b) minima media similar to previous one but without yeast extract in the formulation (B); c) minima-minima media only including glucose and GE as ingredients (C). Media from (A) were prepared using individually each effluent (Na, Su, Ci) or a proportional mixture of the three streams (NSC). Media from (B) and (C) only were prepared with NSC. Mineral salts and Tween 80 were purchased to Sigma-Aldrich (Burlington, MA, USA), glucose was supplied by Vorquímica S.L. (Vigo, Spain), and yeast extract was obtained from Panreac Applichem (Barcelona, Spain). In all cases, initial concentration of glucose was fixed at 24 g/L, adjusting the initial pH to 6.0 with 5 N NaOH and sterilized separately at 121 °C for 15 min. These fermentations were carried out in triplicate at 30 °C and 200 rpm on an orbital shaker (New Brunswick Innova® 43/43R, Edison, NJ, USA) using 300 mL Erlenmeyer flasks with 180 mL working volume. Inocula (0.5%, w/v) were prepared with cellular suspensions of each bacterium produced after 12–16 h of cultivation in medium MRS.

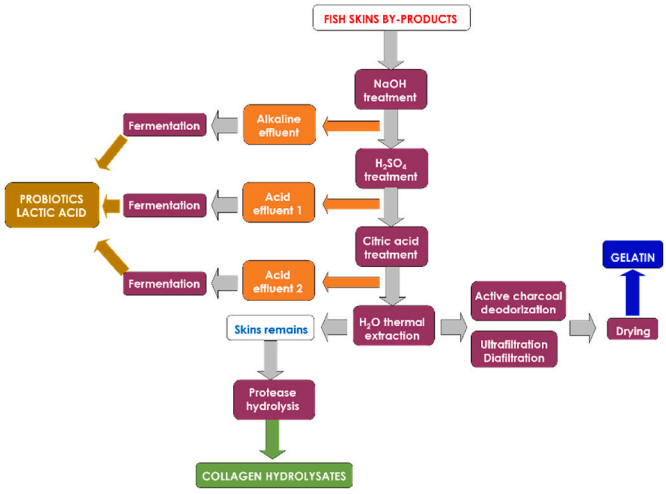

Fig. 1. Flowchart of steps involved in the production of marine gelatins and collagen hydrolysates showing the three contaminant effluents (one alkaline and two acids) used for the production of probiotics and lactic acid.

Table 1
Basic biochemical composition of GE (mean values ± confidence intervals for n = 3). Pr: Total soluble protein; TS: Total sugars; TL: Total lipids. Different letters in each row (as superscript) mean significant differences between effluents ($p < 0.05$).

SPECIES	EFFLUENTS	pH	Pr (g/L)	TS (g/L)	TL (g/L)
SHARK (BS)	**NaOH (Na)**	13.29 ± 0.15^{a}	2.71 ± 0.24^{a}	0.08 ± 0.01^{a}	0.32 ± 0.12^{a}
	H_2SO_4 (Su)	2.60 ± 0.07^{b}	1.85 ± 0.39^{b}	0.02 ± 0.00^{b}	0.13 ± 0.08^{b}
	Citric acid (Ci)	2.61 ± 0.09^{b}	2.46 ± 0.36^{a,b}	0.01 ± 0.00^{c}	0.07 ± 0.02^{b}
TUNA (YT)	**NaOH (Na)**	12.86 ± 0.21^{c}	3.52 ± 0.54^{c}	0.66 ± 0.08^{d}	1.64 ± 0.29^{c,d}
	H_2SO_4 (Su)	2.52 ± 0.09^{b}	1.95 ± 0.38^{b}	0.15 ± 0.03^{e}	1.47 ± 0.04^{c}
	Citric acid (Ci)	2.74 ± 0.13^{b,d}	2.30 ± 0.61^{a,b}	0.13 ± 0.05^{a,e}	1.43 ± 0.49^{c,d}
TURBOT (Tu)	**NaOH (Na)**	12.63 ± 0.19^{c}	3.95 ± 0.52^{c}	0.21 ± 0.04^{e}	2.01 ± 0.21^{d}
	H_2SO_4 (Su)	2.80 ± 0.11^{d}	3.01 ± 0.48^{a,c}	0.10 ± 0.02^{a}	1.65 ± 0.29^{c,d}
	Citric acid (Ci)	2.50 ± 0.06^{b}	5.05 ± 0.42^{d}	0.04 ± 0.00^{f}	1.17 ± 0.40^{c}
SALMON (Sa)	**NaOH (Na)**	12.85 ± 0.36^{c}	4.38 ± 0.52^{c,d}	0.31 ± 0.04^{g}	2.41 ± 0.50^{d}
	H_2SO_4 (Su)	3.69 ± 0.29^{e}	3.45 ± 0.60^{c}	0.14 ± 0.03^{e}	2.01 ± 0.36^{d}
	Citric acid (Ci)	2.96 ± 0.22^{d}	4.04 ± 0.42^{c}	0.07 ± 0.01^{a}	1.75 ± 0.40^{c,d}

2.3. Chemical and bacterial determinations

At pre-defined times, aliquots from each flask were taken to determine biomass, the production of metabolites (lactic and acetic acids), and the consumption of nutrients (glucose and soluble protein). They were centrifuged at 3,273×*g* for 15 min (Allegra X-12R centrifuge, Beckman-Coulter, Brea, CA, USA), from which the supernatant was employed in the determination of aforementioned nutrients and organic acids. The sediment was washed and suspended in distilled water at an appropriate dilution to measure the optical density (OD) at 700 nm (UV/Vis Lambda 365 spectrophotometer, Perkin Elmer, Waltham, MA, USA) and then dry weight was estimated from a calibration curve (OD *vs.* dry weight). Additionally, at 24 and 48 h (N_{48}) samples were also processed for the quantification of the viable cells using plate count (cfu/mL, colony-forming units per mL) in MRS agar medium (Vázquez et al., 2020a). The following chemical analysis for GE and/or samples from supernatants of fermentations were done in triplicate: 1) Total sugars using Dubois et al. method (Dubois et al., 1956); 2) total soluble protein by Lowry et al. (1951); 3) total lipids by Soxhlet extraction (Bligh and Dyer, 1959); 4) profile of amino acids by the ninhydrin reaction (Moore et al., 1958) employing an amino acid analyzer (Biochrom 30 series, Biochrom Ltd., Cambridge, UK); 5) metabolites (lactic and acetic acids) and glucose using a Beckman system gold HPLC (Brea, CA, USA) configured with refractive index detector (Thermo, Waltham, MA, USA) and ION-300 column (Transgenomic, San José, CA, USA) (Vázquez et al., 2020a).

2.4. Mathematical modelling of LAB fermentations

In order to model the experimental kinetic data of growth (X, biomass as dry weight), lactic acid production (L_a) and uptakes of glucose (G) and protein (Pr), we have used well-known unstructured mathematical models (Vázquez and Murado, 2008a):

$$\frac{dX}{dt} = \mu_X X\left(\frac{X_m - X}{X_m}\right) \quad [1]$$

$$\frac{dL_a}{dt} = \mu_L L_a\left(\frac{L_m - L_a}{L_m}\right) \quad [2]$$

$$-\frac{dG}{dt} = \frac{1}{Y_{x/g}}\frac{dX}{dt} + m_g X \quad [3]$$

$$-\frac{dPr}{dt} = \frac{1}{Y_{x/p}}\frac{dX}{dt} + m_p X \quad [4]$$

In addition, we have included two differential equations dealing with the relationship between L_a *vs.* X and L_a *vs.* G in order to obtain the corresponding yields of L_a production/nutrients consumption:

$$\frac{dL_a}{dt} = Y_{L/x}\frac{dX}{dt} \quad [5]$$

$$\frac{dL_a}{dt} = -Y_{L/g}\frac{dG}{dt} \quad [6]$$

All these differential equations can be integrated and reparametrized to obtain the analytical forms (Vázquez and Murado, 2008a, 2008b). Thus, kinetic profiles of X, L_a, G and Pr were fitted to the corre-sponding integrated equations:

$$X = \frac{X_m}{1+\exp\left[2+\frac{4v_X}{X_m}(\lambda_X - t)\right]} \quad \text{with} \quad X_0 = \frac{X_m}{1+\exp\left(2+\frac{4v_X\lambda_X}{X_m}\right)} \quad [7]$$

$$L_a = \frac{L_m}{1+\exp\left[2+\frac{4v_L}{L_m}(\lambda_L - t)\right]} \quad \text{with} \quad L_0 = \frac{L_m}{1+\exp\left(2+\frac{4v_L\lambda_L}{L_m}\right)} \quad [8]$$

$$G = G_0 - \frac{1}{Y_{x/g}}\left[\frac{X_m}{1+\left(\frac{X_m}{X_0}-1\right)\exp\left(-\frac{4v_X}{X_m}t\right)} - X_0\right] - \left(\frac{m_g X_m^2}{4v_X}\right)\ln\left[\frac{X_0\left(e^{\frac{4v_X}{X_m}t}-1\right)+X_m}{X_m}\right] \quad [9]$$

$$Pr = Pr_0 - \frac{1}{Y_{x/p}}\left[\frac{X_m}{1+\left(\frac{X_m}{X_0}-1\right)\exp\left(-\frac{4v_X}{X_m}t\right)} - X_0\right] - \left(\frac{m_p X_m^2}{4v_X}\right)\ln\left[\frac{X_0\left(e^{\frac{4v_X}{X_m}t}-1\right)+X_m}{X_m}\right] \quad [10]$$

$$L_a = L_0 + Y_{L/x}\left[\frac{X_m}{1+\left(\frac{X_m}{X_0}-1\right)\exp\left(-\frac{4v_X}{X_m}t\right)}\right] - Y_{L/x}X_0 \quad [11]$$

$$L_a = \frac{-X_0 Y_{L/g}}{Y_{x/g}} + \frac{Y_{L/g}}{Y_{x/g}}\left[\frac{X_m}{1+\left(\frac{X_m}{X_0}-1\right)\exp\left(-\frac{4v_X}{X_m}t\right)}\right] + \left(\frac{m_g X_m^2 Y_{L/g}}{4v_X}\right)\ln\left[\frac{X_0\left(e^{\frac{4v_X}{X_m}t}-1\right)+X_m}{X_m}\right] \quad [12]$$

All the parameter definitions and corresponding units are summarized in Table 2. These unstructured models are based on the macroscopical description of growth (biomass as dry weight), metabolites and nutrients kinetics including the following assumptions (Bouguettoucha et al., 2007; Vázquez and Murado, 2008a; Panikov, 2019): (a) the rate of biomass production and biomass concentration are proportional; (b) the rate of lactic acid production and lactic acid concentration are also directly proportional; (c) the bacteria need substrates (glucose and

Table 2
Parameter definitions (symbolic notations) and corresponding units.

X	Biomass. Unit: g/L
t	Time. Unit: h
X_m	Maximum biomass. Unit: g/L
μ_m	Specific maximum growth rate (biomass production per unit of biomass and time). Unit: h^{-1}
X_0	Initial biomass. Unit: g/L
v_X	Maximum growth (biomass production) rate. Unit: g $L^{-1}h^{-1}$
λ_X	Growth lag phase. Unit: h
L_a	Lactic acid. Unit: g/L
L_{a0}	Initial lactic acid. Unit: g/L
L_m	Maximum lactic acid production. Unit: g/L
μ_L	Specific maximum rate of lactic acid production. Unit: h^{-1}
v_L	Maximum rate of lactic acid production. Unit: g $L^{-1}h^{-1}$
λ_L	Lactic acid lag phase. Unit: h
G	Glucose. Unit: g/L
G_0	Initial glucose. Unit: g/L
$Y_{x/g}$	Yield factor for biomass formation on glucose. Unit: g biomass/g glucose
m_g	Maintenance coefficient for glucose. Unit: g (glucose) g^{-1} (biomass) h^{-1}
$Y_{L/g}$	Yield factor for lactic acid production per glucose consumed. Unit: g lactic acid/g glucose
$Y_{L/x}$	Yield factor for lactic acid formation per biomass produced. Unit: g lactic acid/g biomass
Pr	Protein. Unit: g/L
Pr_0	Initial protein. Unit: g/L
$Y_{x/p}$	Yield factor for biomass formation on protein. Unit: g biomass/g protein
m_p	Maintenance coefficient for protein. Unit: g (protein) g^{-1} (biomass) h^{-1}
A_m	Maximum acetic acid production. Unit: g/L
v_A	Maximum rate of acetic acid production. Unit: g $L^{-1}h^{-1}$
λ_A	Acetic acid lag phase. Unit: h

proteins) and can synthesize metabolic compounds (*e.g.*, lactic acid) even when growth is depleted; (d) the time-course of growth (biomass rate) presents an asymptote as upper limit (saturation level or plateau phase) different for each nutrient or level of nutrient used; (e) the kinetics of glucose and protein consumption are jointly proportional to the rate of growth and to the concentration of biomass; (f) the rate of main metabolite production is individually proportional to the rate of glucose consumption and to the rate of growth.

2.5. Bioeconomy evaluation

A simple and preliminary study of economical sustainability for the LAB bioproduction costs was also carried out. Taking as reference the market prices of the MRS ingredients and the values of X_m and L_m compiled in Tables 3–4 and Tables S3–S6 (supplementary material), we have quantified the cost of production of biomass (in €/g) and lactic (in €/g) acid in each cost-effective media formulated with GE as well as in MRS.

2.6. Numerical fittings and statistical analyses

Fitting procedures of experimental data and parametric estimations were calculated by minimizing the sum of quadratic differences between the observed and model predicted values, using the non-linear least-squares (GRG non-linear) method provided by the macro-'Solver' of the Microsoft Excel spreadsheet. Confidence intervals from the parametric estimates (Student's *t*-test) and consistence of mathematical models (Fisher's F test) were evaluated by "SolverAid" macro (Levie's Excellaneous web-site:http://www.bowdoin.edu/~rdelevie/excellaneous). One-way ANOVA test followed by means of Tukey test was applied to know the existence of significant differences between fermentation parameters in the different growth media tested. This procedure was also used to determine the difference in composition between gelatin effluents. Statistical significance was also defined as $p < 0.05$.

3. Results and discussion

3.1. Chemical characterization of gelatin effluents

In most processes designed for the valorization of food by-products, it is almost impossible not to generate solid and/or water streams. Generally, these wastewaters contain important amounts of organic matter, remarkable levels of biological oxygen demand (BOD) and chemical oxygen demand (COD), are produced at huge volumes and even present extreme values of pH (Maree et al., 1990; Mistry and Patel, 2016; Alves et al., 2022). GE meet several of these properties, requiring their effective management in order to: a) avoid environmental pollution problems; and b) to ensure economic viability and sustainability to the process of gelatin extraction from fish skins. The approaches, but to treat GE from terrestrial animals (pigs and cattle), were based on the anaerobic digestion and biofuels/biogas production using conventional methanogenic microorganisms (Tawfik et al., 2021). The present work is the first one aimed to valorize GE from fish wastes by LAB fermentation.

The chemical characterization of GE is shown in Table 1. As expected, the values of pH were higher than 12.6, in all species, for alkaline effluent and lower than 3.7 for both acid streams (Su and Ci). The total sugars content was negligible and the protein extracted (ranging 1.85–5.05 g/L) was highest in aquaculture species and in Na and Ci treatments. The presence of lipids in GE was greatest in skins from fatty species (YT, Tu and Sa) and always largest in alkaline procedure.

All amino acids –including essential ones for lactobacilli (Ile, Leu, Cys, Glu and Val) (Jensen and Hammer, 1993; Biswas et al., 1991; Vázquez et al., 2004)– are present in all effluents (Table S1, supplementary material), but important differences were observed in the amino acidic composition among streams. The treatment of skins with citric acid released the highest amounts of glycine (Gly), proline (Pro) and hidroxyproline (OHPro) to the wastewater, indicating the presence of collagen derivative material in these effluents (Gauza-Włodarczyk et al., 2017; Nitsuwat et al., 2021).

In the alkaline washes, glutamic (Glu) and aspartic (Asp) acids together with lysine (Lys) were the most abundant amino acids. The application of sulphuric acid led to heterogeneous results without a common profile between species –higher Lys and His in YT, Glu and Gly in aquaculture fish, and Asp and Glu in BS–. In general, Na showed the best levels of essential amino acid ratios (TEAA/TAA>30%), followed by Su (20–30%) and Ci (16–22%).

3.2. Fermentation of LAB on GE

The two LAB selected, *L. brevis* and *L. plantarum*, are bacteria with well-known technological (Martins et al., 2013; Laranjo et al., 2019) and probiotic properties (Rao and Samak, 2013; Linares et al., 2017; Pereira et al., 2022). They are also ideal target microorganisms to evaluate the capacity of food substrates as ingredient of culture media, due to its nutrient requirements to support growth and metabolite productions (Horn et al., 2007; Shi et al., 2018). LAB are classified as fastidious since they need complex media, as MRS, containing glucose, several inorganic salts, tensioactive and various sources of organic nitrogen (proteins).

Initially, the fermentations were carried out in complete media (as MRS) formulated with GE, as protein source, instead of commercial peptones. Alkaline and acid streams, and a proportional mixture of the three effluents (NSC), were studied in individual fermentations for both probiotic. Fig. 2 depicts the kinetics of *L. plantarum* on Na effluents and MRS, including the production of biomass (growth) and lactic acid, and the consumption of glucose and soluble protein. The rest of *L. plantarum* cultures are presented in supplementary material (Figs. S1–S3). The time-course of pH (data not shown) were parallels to glucose uptake and similar, but decreasing, to lactic acid generation.

Data of L. *plantarum* productions and nutrient uptakes were accurately predicted by the unstructured mathematical models [7–10] exposed in M&M. These predictions included all the kinetic phases of

Table 3
Numerical values and confidence intervals for parameters obtained from experimental data of *L. plantarum* growth on Na and Su effluents modelled by equations [7–12]. R^2 are the determination coefficients among experimental and predicted data. Different letters in each column (as superscript) mean significant differences between media ($p < 0.05$). MRS 1: numerical values for the fermentations on control medium for this set of experiments. Na: media formulated with NaOH effluent. Su: media formulated with sulphuric acid effluent. BS: blue shark. YT: yellowfin tuna. Tu: turbot. Sa: salmon. All fermentations were done in triplicate.

	Na_BS	Na_YT	Na_Tu	Na_Sa	Su_BS	Su_YT	Su_Tu	Su_Sa	MRS 1
X_m	2.03 ± 0.12[a]	2.88 ± 0.26[b]	2.05 ± 0.12[a]	2.65 ± 0.22[b]	1.33 ± 0.15[c]	1.71 ± 0.10[d]	2.06 ± 0.17[a]	0.959 ± 0.104[e]	2.57 ± 0.15[b]
ν_x	0.172 ± 0.050[a]	0.191 ± 0.072[a]	0.231 ± 0.098[a]	0.169 ± 0.059[a]	0.072 ± 0.024[b]	0.175 ± 0.063[a]	0.139 ± 0.047[a]	0.076 ± 0.037[b]	0.175 ± 0.043[a]
λ_x	6.44 ± 1.94[a]	5.76 ± 3.15[a]	3.63 ± 2.11[a]	3.98 ± 3.06[a]	9.80 ± 3.31[a]	7.36 ± 1.98[a]	5.83 ± 2.77[a]	8.70 ± 3.44[a]	6.78 ± 2.02[a]
R^2_x	0.990	0.981	0.986	0.981	0.978	0.988	0.984	0.974	0.992
$Y_{x/g}$	0.137 ± 0.016[a]	0.142 ± 0.019[a]	0.189 ± 0.052[a]	0.171 ± 0.026[a]	0.100 ± 0.035[a,b]	0.174 ± 0.040[a]	0.187 ± 0.032[a]	0.077 ± 0.014[b]	0.131 ± 0.015[a]
m_g	0.118 ± 0.033[a,b]	0.018 (NS)	0.155 ± 0.046[a,c]	0.057 ± 0.031[b]	0.090 (NS)	0.159 ± 0.051[a,c]	0.098 ± 0.035[a,b]	0.247 ± 0.091[c]	0.012 (NS)
$R^2_{x/g}$	0.995	0.992	0.983	0.992	0.973	0.988	0.993	0.992	0.994
$Y_{x/p}$	1.50 ± 0.37[a]	1.37 ± 0.31[a]	1.43 ± 0.22[a]	1.91 ± 0.75[a]	1.69 ± 1.20[a]	2.13 ± 1.01[a]	2.71 ± 1.41[a]	1.75 ± 1.03[a]	2.01 ± 0.86[a]
m_p	0.003 (NS)	−0.003 (NS)	−0.001 (NS)	−0.003 (NS)	−0.004 (NS)	−0.001 (NS)	−0.001 (NS)	0.008 (NS)	0.004 (NS)
$R^2_{x/p}$	0.973	0.970	0.983	0.907	0.747	0.878	0.873	0.910	0.929
L_m	18.80 ± 1.71[a]	18.91 ± 1.62[a]	18.09 ± 4.34[a]	17.85 ± 1.80[a]	16.32 ± 1.93[a]	17.25 ± 2.00[a]	16.33 ± 2.26[a]	15.85 ± 1.80[a]	19.14 ± 1.07[a]
ν_L	0.988 ± 0.298[a,b]	1.14 ± 0.37[a,b]	0.575 ± 0.267[b]	0.816 ± 0.240[a,b]	0.731 ± 0.243[a,b]	0.810 ± 0.281[a,b]	0.709 ± 0.278[a,b]	0.736 ± 0.221[b]	1.25 ± 0.28[a]
λ_L	5.76 ± 3.14[a]	4.65 ± 2.99[a]	2.12 (NS)	4.56 ± 3.45[a]	4.94 ± 3.96[a]	4.76 ± 3.97[a]	3.48 (NS)	4.05 ± 3.67[a]	5.92 ± 1.92[a]
R^2_L	0.983	0.984	0.924	0.983	0.978	0.981	0.967	0.980	0.993
$Y_{L/x}$	8.75 ± 1.57[a,b]	6.53 ± 0.75[a]	7.10 (NS)	6.63 ± 1.13[a]	11.50 ± 2.26[b]	8.81 ± 2.03[a,b]	7.34 ± 1.36[a]	16.47 ± 2.27[c]	7.14 ± 0.57[a]
$R^2_{L/x}$	0.953	0.980	0.777	0.957	0.943	0.923	0.958	0.949	0.902
$Y_{L/g}$	0.897 ± 0.101[a]	0.916 ± 0.122[a]	0.782 ± 0.096[a]	0.908 ± 0.102[a]	1.33 ± 0.12[b]	1.03 ± 0.11[a]	1.05 ± 0.14[a]	1.02 ± 0.13[a]	0.951 ± 0.098[a]
$R^2_{L/g}$	0.985	0.977	0.984	0.993	0.934	0.989	0.975	0.967	0.989
A_m	0.631 (NS)	1.19 ± 0.024[a]	0.789 ± 0.089[b]	0.847 ± 0.178[b]	0.767 ± 0.533[a,b]	0.674 ± 0.117[b]	0.822 ± 0.306[a,b]	0.858 ± 0.064[b]	1.43 ± 0.30[a]
ν_A	0.030 (NS)	0.035 ± 0.006[a]	0.060 ± 0.019[b]	0.028 ± 0.009[a]	0.021 ± 0.012[a]	0.026 ± 0.008[a]	0.032 ± 0.022[a,b]	0.032 ± 0.005[a]	0.050 ± 0.016[a,b]
λ_A	27.65 (NS)	15.64 ± 2.72[a]	26.31 ± 2.30[b]	11.13 ± 4.41[a]	14.12 ± 9.52[a]	11.26 ± 4.10[a]	11.56 ± 8.73[a]	6.36 ± 2.13[a]	11.78 ± 4.42[a]
R^2_A	0.721	0.975	0.973	0.957	0.802	0.962	0.856	0.992	0.958

sigmoid profiles (Vázquez and Murado, 2008b). These agreements between experimental and predicted data can be graphically observed in Fig. 2 and Figs. S1–S3, and numerically demonstrated –by means of R^2 values– in Table 3 and Table S3 (supplementary material). R^2 varied in the following intervals: 0.974–0.996 for biomass, 0.924–0.989 for lactic acid, 0.866–0.995 for glucose, 0.777–0.993 for protein. The consistency of fits was always excellent ($p < 0.005$ from F-Fisher test, data not shown) and the parameters were almost already statistically significant (for $\alpha = 0.05$, t-Student test) less in various coefficient of maintenance.

Monod and logistic equations are the two unstructured models most widely applied to the macroscopic description of microbial growth (Panikov, 2019; Xu, 2020). The latter, is independent on the substrate concentration, is based on a mechanism of autocatalytic reaction, is formulated with parameters of clear biological meaning, is able to describe all phases of sigmoid profiles (including lag, exponential and plateau), and it is commonly used in the prediction of multiple microorganism growths and even bacterial metabolites produced under batch experimental conditions with limited substrate (Thilakavathi et al., 2007; Quintas et al., 2007; Saman et al., 2019).

Using numerical values of the parameters (Table 3 and Table S3, supplementary), we can establish multiple comparisons between low cost and control media. The values of G_0 and Pr_0 are not included in the Tables since they are not interesting parameters in comparative terms and neither from a kinetic viewpoint. Regarding growth, blue shark effluents induced lowest maximum production of biomass (X_m) and media including sulphuric acid stream (Su) were in general lower than Na and Ci. The largest concentration of biomass was reached in NSC from salmon, significantly higher than MRS ($p < 0.05$). NSC_YT, NSC_Tu, Na_YT and Na_Sa showed similar capacity than commercial peptones incorporated in the control. The faster cultures, in terms of maximum growth rate (ν_x), were observed in NSC_Tu and NSC_Sa, being the formulates with Su slower than those prepared with effluents treated with alkalis and citric acid ($p < 0.05$). Lag phases of growth (λ_x) were similar in all situations ($p > 0.05$). The yields of biomass production in relation to glucose consumption ($Y_{x/g}$), obtained from equation [9], varied from 0.105 to 0.190 gX/gG and were statistically similar in all case less for Su_Sa (lowest), Su_Tu and NSC_Sa (highest). The response for growth respect to protein intake, was much wider (1.2–2.71 gX/gPr) but without significant differences between most media ($p > 0.05$), except in Ci_Tu and Ci_Sa that showed a huge efficiency in protein consumption (6.05 and 7.48 gX/gPr, respectively). No comparisons were performed for maintenance coefficients due to, in most cases, this parameter was not significant (NS) according t-Student test.

Based on data of maximum production (L_m), maximum rate of production (ν_L) and lag phase (λ_L), the production of lactic acid was statistically similar between media evaluated. Two exceptions must be done, Ci_BS led to the maximum value of L_m (19.81 ± 1.49 g/L) significantly superior to Su_BS (16.32 ± 1.93 g/L), and MRS showed higher ν_L than observed in Su_Sa. The efficiency of *L. plantarum* to produce lactic acid in function of biomass production ($Y_{L/x}$) and glucose uptake ($Y_{L/g}$) was calculated from equations [11–12]. In the first case, the best options followed the next order: Su_Sa > Su_BS > Na_BS = Ci_BS = NSC_BS. For the second one, the highest yield was obtained in Su_BS (1.33 gL_a/gG) with the rest of media in the interval of 0.782 gL_a/gG (Na_Tu) to 1.06

Table 4
Numerical values and confidence intervals for parameters obtained from experimental data of *L. brevis* growth on Na and Su effluents modelled by equations [7–12]. R^2 are the determination coefficients among experimental and predicted data. Different letters in each column (as superscript) mean significant differences between media ($p < 0.05$). MRS 1: numerical values for the fermentations on control medium for this set of experiments. Na: media formulated with NaOH effluent. Su: media formulated with sulphuric acid effluent. BS: blue shark. YT: yellowfin tuna. Tu: turbot. Sa: salmon. All fermentations were done in triplicate.

	Na_BS	Na_YT	Na_Tu	Na_Sa	Su_BS	Su_YT	Su_Tu	Su_Sa	MRS 1
X_m	3.17 ± 0.33^{a}	4.11 ± 0.22^{b,c}	5.22 ± 0.35^{d}	4.61 ± 0.37^{c}	2.23 ± 0.10^{e}	3.78 ± 0.33^{a,b}	4.04 ± 0.15^{b}	3.98 ± 0.16^{b}	4.30 ± 0.15^{b,c}
v_x	0.280 ± 0.157^{a,b}	0.406 ± 0.125^{a}	0.506 ± 0.206^{a,b}	0.255 ± 0.070^{b}	0.143 ± 0.037^{b,c}	0.282 ± 0.115^{a,b}	0.369 ± 0.057^{a,b}	0.274 ± 0.065^{a,b}	0.493 ± 0.043^{a}
λ_x	5.53 ± 3.60^{a}	6.07 ± 1.77^{a}	4.06 ± 2.38^{a}	6.14 ± 2.73^{a}	6.09 ± 3.44^{a}	5.39 ± 3.06^{a}	6.10 ± 2.43^{a}	4.64 ± 2.64^{a}	6.87 ± 2.02^{a}
R^2_x	0.976	0.991	0.983	0.986	0.989	0.981	0.992	0.994	0.998
$Y_{x/g}$	0.224 ± 0.035^{a}	0.263 ± 0.042^{a,b}	0.294 ± 0.034^{b}	0.173 ± 0.057^{a}	0.152 ± 0.049^{a}	0.209 ± 0.043^{a}	0.273 ± 0.078^{a,b}	0.248 ± 0.038^{a,b}	0.212 ± 0.041^{a,b}
m_g	0.072 ± 0.026^{a}	0.049 ± 0.022^{a}	0.036 ± 0.013^{a}	−0.021 (NS)	0.070 (NS)	0.041 ± 0.034^{a}	0.048 ± 0.040^{a}	0.048 ± 0.023^{a}	0.011 (NS)
$R^2_{x/g}$	0.992	0.991	0.994	0.948	0.967	0.985	0.970	0.992	0.979
$Y_{x/g}$	3.96 ± 0.78^{a}	2.22 ± 0.71^{b}	2.71 ± 0.71^{a,b}	1.97 ± 0.54^{b}	2.83 ± 1.93^{a,b}	2.96 ± 1.12^{a,b}	3.53 ± 0.07^{a}	3.45 ± 0.65^{a,b}	2.38 ± 0.46^{b}
m_p	0.002 ± 0.002^{a}	0.001 (NS)	0.007 ± 0.003^{a}	0.001 (NS)	−0.002 (NS)	0.000 (NS)	0.000 (NS)	0.000 (NS)	0.003 ± 0.003^{a}
$R^2_{x/p}$	0.983	0.939	0.977	0.970	0.784	0.931	0.830	0.982	0.982
L_m	18.33 ± 1.80^{a}	18.60 ± 1.84^{a}	18.07 ± 1.97^{a}	18.96 ± 1.83^{a}	17.52 ± 2.05^{a}	18.44 ± 1.99^{a}	18.40 ± 2.35a	19.42 ± 1.80^{a}	18.41 ± 0.59^{a}
v_L	0.903 ± 0.274^{a}	0.956 ± 0.316^{a}	0.939 ± 0.370^{a}	1.03 ± 0.37^{a,b}	0.958 ± 0.364^{a}	0.888 ± 0.302^{a}	0.881 ± 0.359^{a}	0.977 ± 0.305^{a}	1.61 ± 0.28^{b}
λ_L	5.44 ± 3.33^{a}	4.35 ± 3.51^{a}	2.12 (NS)	2.38 ± 1.69^{a}	3.66 (NS)	4.03 ± 3.83^{a}	2.95 (NS)	3.32 (NS)	5.09 ± 1.13^{a}
R^2_L	0.984	0.981	0.970	0.975	0.977	0.979	0.967	0.981	0.997
$Y_{L/x}$	5.38 ± 1.25^{a,c}	4.15 ± 0.85^{a}	3.26 ± 0.68^{a,b}	3.89 ± 0.81^{a}	7.99 ± 1.26^{c}	4.62 ± 0.78^{a}	4.09 ± 0.86^{a}	4.72 ± 0.66^{a}	4.09 ± 0.34^{a}
$R^2_{L/x}$	0.923	0.939	0.936	0.937	0.963	0.957	0.936	0.971	0.902
$Y_{L/g}$	0.875 ± 0.301^{a}	0.873 ± 0.199^{a}	0.790 ± 0.251^{a}	0.857 ± 0.412^{a}	1.080 ± 0.207^{a}	0.829 ± 0.210^{a}	0.930 ± 0.309a	0.966 ± 0.412^{a}	0.871 ± 0.177^{a}
$R^2_{L/g}$	0.995	0.993	0.986	0.940	0.965	0.989	0.978	0.985	0.988
A_m	0.734 ± 0.136^{a}	0.695 ± 0.274^{a}	1.38 ± 0.09^{b}	1.20 ± 0.07^{b}	1.06 ± 0.12^{a,b}	1.18 ± 0.66^{a,b}	1.69 ± 0.17^{c}	1.45 ± 0.22^{b,c}	2.13 ± 0.35^{c,d}
v_A	0.048 ± 0.024^{a}	0.026 ± 0.017^{a}	0.090 ± 0.023^{b}	0.058 ± 0.015^{a,b}	0.229 (NS)	0.030 ± 0.014^{a}	0.084 ± 0.018^{b}	0.097 ± 0.057^{a,b}	0.082 ± 0.023^{a,b}
λ_A	19.49 ± 4.07^{a}	27.73 ± 2.80^{b}	7.84 ± 2.15^{c}	3.73 ± 2.08^{c}	17.18 ± 3.61^{a}	9.00 ± 8.92^{a,c}	17.76 ± 2.16^{a}	8.37 ± 4.86^{c}	12.55 ± 3.68^{a,c}
R^2_A	0.957	0.960	0.992	0.992	0.971	0.847	0.986	0.956	0.969

gL_a/gG (NSC_BS). The concentration of acetic acid was lower than 1.7 g/L (inferior to 10% of lactic acid production) and, therefore, it was not incorporated to the prediction global executed by unstructured equations. Nevertheless, we have employed a similar logistic equation [8] to fit the experimental data of acetic acid (Table 3 and Table S3, supplementary). Ci streams supported higher maximum production of acetic (A_m) than Su, but maximum rates of production (v_A) were indistinguishable. Lag phases (λ_A) varied in the range of 11–26 h depending on the media. This behavior is in line with the Luedeking and Piret definition of a mixed metabolite with a larger component of secondary metabolite (Luedeking and Piret, 1959). Lactic acid was mainly primary with, in general, a low level of secondary numerical coefficient according the same definition (modeling and parameters not shown).

For L. *brevis* fermentations (Fig. 3 and Figs. S4–S6 supplementary material), the equations proposed showed, once again, to be an excellent mathematical tool to model all experimental data of such cultures. The consistency and robustness of equations was always confirmed (p-values<0.005) and the accuracy between experimental and predicted data, in terms of goodness of fit (R^2), was ranged as 0.976–0.998, 0.967–0.997, 0.966–0.996 and 0.780–0.983 for biomass, lactic acid, glucose, and protein, respectively.

The values of maximum biomass X_m was numerically larger in Na_Tu (5.22 ± 0.35 g/L) than Na_Sa (4.61 ± 0.37 g/L) and (4.64 ± 0.19 g/L), varying the productions in the other media between 2.23 and 4.45 g/L, observing that the growths obtained from blue shark effluents were significantly lower (Table 4 and Table S4, supplementary). Because of amino acid profile is quite similar between species, these experimental results may be caused by the inferior protein content in BS streams. In the same line, MRS and Na_Tu supported the faster bacterial growths with significant improvements in relation to many other broths, especially with Su_BS. $Y_{x/g}$ was significantly higher in Na_Tu and lower in Na_Sa and BS cultures. However, Na_BS was the most efficient substrate for protein consumption yield. There were no differences either among media for biomass lag phase parameter ($p > 0.05$).

The concentration of lactic acid achieved levels around 20 g/L (prediction of 19.41 g/L for L_m in NSC_YT and Su_Sa) without significant variance among cultures. Ci_BS and MRS are the streams where highest v_x were found, observing similar rates for the remainder fermentations. In many situations, production latencies were not graphically clear and, therefore, λ_L not statistically significant (Table 4 and Table S4, supplementary). The kinetics of biomass and acid lactic production were concomitant each other, as expected for a metabolite defined mainly as primary (Luedeking and Piret, 1959). For both lactobacillus, a little coefficient of secondarily was also observed (data not shown). Nevertheless, lag phases for growth and lactic acid formation were almost equal ranging from 3 to 7 h. The conversion between L_a production versus growth or glucose uptake selected to Su_BS as the most effective regarding biomass ($Y_{L/X} = 7.99$ gL_a/gX), but without significant variations between effluents for the case of the carbon source ($p > 0.05$), although the values moved in the interval of $Y_{L/g} = 0.76$–1.08 gL_a/gG. The data of acetic acid maximum formation, calculated by means of logistic prediction, changed in the interval of 0.7–2.7 g/L depending on the media assessed and, as in the previous strain, it was not incorporated to the calculation of production yields. MRS was the most productive medium and alkaline effluents the worst. The delay in the acetic acid formation was also observed among 13 and 28 h.

Additionally, count viable cells (N, with N_0 as inoculum at initial time) were also determined at 24 and 48 h of fermentation for each culture from both LAB. Data are not represented, but we are able to summarize that: 1) most of *L. plantarum* growths on Na, Ci and NSC media were in the same magnitude order ($\ln(N_{48}/N_0) = 11.8$–12.2) than those refereed by MRS ($\ln(N_{48}/N_0) = 12.2$–12.4), and in some cases even

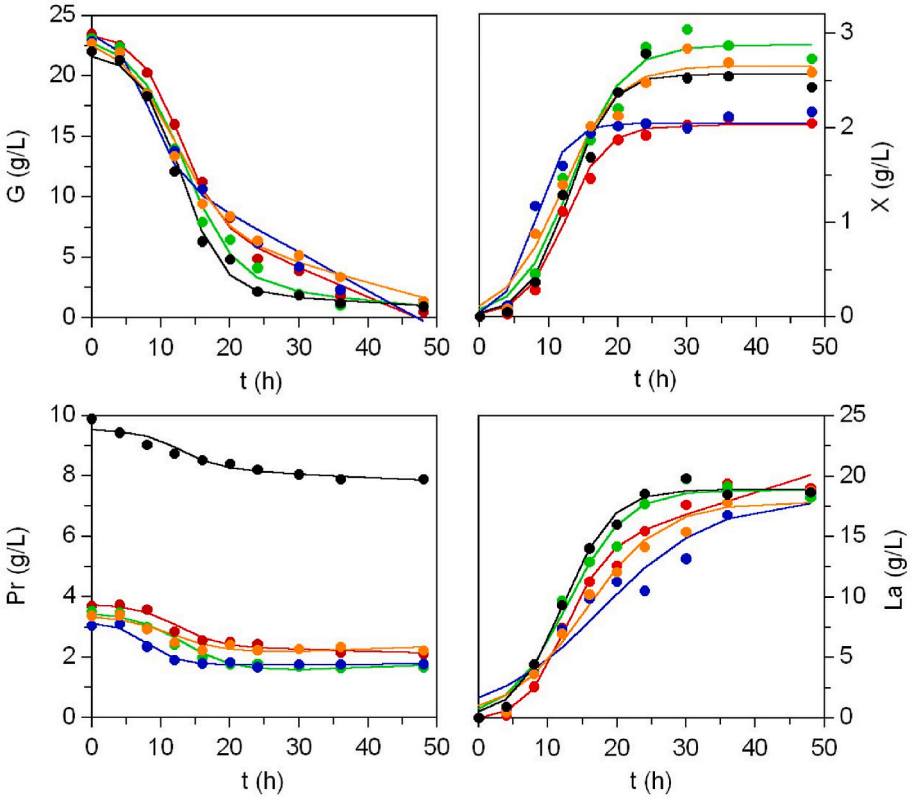

Fig. 2. Fermentations of *L. plantarum* on media formulated with NaOH effluents (Na) of shark (●), tuna (●), turbot (●) and salmon (●). Culture in MRS (●) was used as comparative control. X: biomass production, L_a: lactic acid production, G: glucose uptake, Pr: soluble protein consumption. Experimental data (points) were fitted to the [7–10] equations (continuous line). The confidence intervals of experimental data (for three replicates) were in all cases less than 15% of the experimental mean values and omitted for clarity.

slightly larger (Na_YT and NSC_Sa both around 12.5); 2) similar findings were found for *L. brevis*, the worst media in the production of biomass were less cell formed than the control. These highlights are in agreement with previously reported studies using several peptones from fish by-products (Vázquez et al., 2020b), in which fish peptones were able to promote viable LAB in similar or higher extent than MRS.

All present results demonstrated that lactobacillus are able to grow in formulated media with levels of soluble protein much lower than those included in MRS media, provided that the amino acid profile of GE residual sources is balanced (Jensen and Hammer, 1993; Kobayashi, 2019). In fact, protein consumption at the end of the culture was not exhaustive in almost any case, on average around 1.5–2.5 g/L was consumed, except for Na_Tu and Na_Sa in the case of *L. brevis* observing almost full metabolisation. This fact not only induces a reduction and cheaper use of nutrients, but also enables an improvement in the potential downstream processes that could be applied in the purification of metabolites of interest typical from LAB (*i.e.*, bacteriocins, lactic acid, etc.) (Zacharof et al., 2013; Komesu et al., 2017; Jamaluddin et al., 2018). GE revealed their capacity of promoting probiotic biomass, viable cell and lactic acid instead of commercial and expensive meat extract and bactopeptone. Different LAB, including lactobacilli, have been successfully growth –in addition to the concomitant production of antimicrobials and metabolites– using, as protein source, other types of fish by-products such as viscera, heads, hydrolysates, etc., from commercial and by-catch species by fishing industry (Aspmo et al., 2005; Juarez del Valle et al., 2017; Linares-Morales et al., 2022). Besides, the application of a bioconversion step mediated by LAB demonstrated to be a valuable alternative for the valorization of several wastewaters generated in the processing of seafood as follows: squid pen streams (Vázquez et al., 2016), shrimp cooking juice waste (Djellouli et al., 2021), effluents from cooking octopus (Vázquez and Murado, 2008c), tail water from fish meal (Huang et al., 2007), and tuna canning effluents (Vázquez et al., 2022).

3.3. Fermentation of LAB on minima GE media

After evaluating the good performance of GE as substitute source of organic nitrogen (protein substrate) of commercial peptones (bactopeptone and meat extract), the next step was to evaluate its capacity to replace also yeast extract and even mineral salts included in MRS. Initially, LAB were fermented in media B without yeast extract but within NSC (Table S2, supplementary material). The experimental kinetics for both bacteria were also easily modelled by equations [7–10] (Fig. 4 and S7, supplementary material), showing R^2 ranges of 0.986–0.997 for *X*, 0.862–0.987 for *G*, 0.705–0.951 for *Pr*, and 0.953–0.991 for L_a. Unfortunately, growths and lactic acid productions were much lower in these minima media (NSCm) than MRS: maxima biomasses only achieved 0.6 g/L (*L. plantarum*) and 0.93 g/L (*L. brevis*), and lactic acids lower than 7.2 g/L for the two LAB (Tables S5 and S6, supplementary material). Furthermore, the intakes of glucose and proteins in NSCm were far from exhaustive, remaining unmetabolized more than 15 g/L of glucose and almost the majority of soluble protein. All kinetic parameters and yields favored the fermentations carried out in MRS.

Although the results were not very satisfactory, the last option assessed was based on the fermentation of LAB in media formulated only with NSC and glucose (medium C, Table S2, supplementary material). As in previous cultures, MRS showed much larger bioproductions than NSCmm for all parametric estimations (Tables S5 and S6, supplementary material). The best values of X_m and L_m were 0.60 g/L and 4.21 g/L for *L. plantarum*, 0.83 g/L and 3.9 g/L for *L. brevis*. These results revealed that, although GE are composed by all amino acids in remarkable concentrations and small fermentative capacity was supported by GE + glucose, the presence of salts and, mainly, the complex B vitamins that

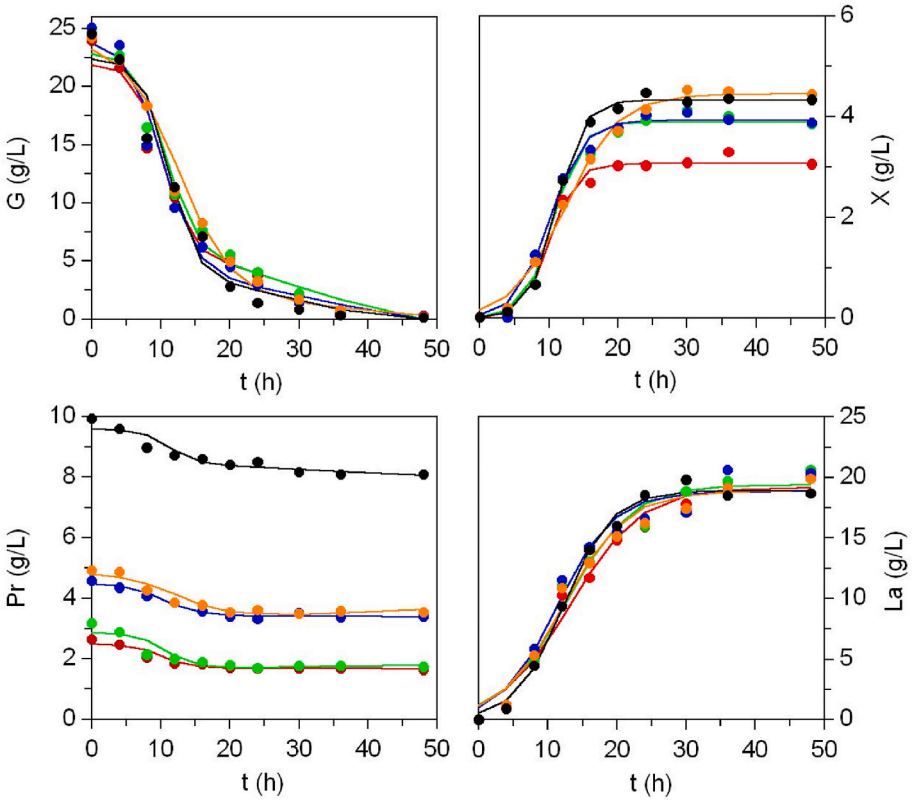

Fig. 3. Fermentations of L. *brevis* on media formulated with a proportional mixture of the three effluents of shark (●), tuna (●), turbot (●) and salmon (●). Culture in MRS (●) was used as comparative control. X: biomass production, L_a: lactic acid production, G: glucose uptake, Pr: soluble protein consumption. Experimental data (points) were fitted to the [7–10] equations (continuous line). The confidence intervals of experimental data (for three replicates) were in all cases less than 15% of the experimental mean values and omitted for clarity.

yeast extract supplies, is essential for the massive growth of lactobacilli (Ik-Keun et al., 1997). This relevance of yeast extract source for LAB was extensively confirmed in studies of purification and fractionation by ultrafiltration membranes at different molecular weights cut-offs (Gaudreau et al., 1999). Also, the replacing of yeast extract by different percentages of potato extracts was not efficient in various strains of Streptococcus and lactobacilli (Gaudreau et al., 2002). Recently, Johnson et al. (2022) reported that yeast extract is a fundamental LAB growth stimulator, irreplaceable by other protein sources, but it is not sufficient for the correct growth of LAB as *L. pentosus*, since an additional source of organic nitrogen is needed. It is hence evident that commercial peptones can be succesfully replaced by fish wastes and effluents, but yeast extract presence in LAB broths is also mandatory.

3.4. Circular economy pre-evaluation of GE for probiotic production

A simple test of cost-effectivity was performed to assess the validity of GE in bioeconomical terms with the aim to verify our proposal of GE valorization and to enhance the sustainability of the global process of fish gelatin production. Thus, taking into consideration the prices of MRS ingredients and the numerical data of X_m and L_m, obtained from Tables 3–4 and Tables S3–S6 (supplementary material), the costs of probiotic biomass and lactic acid productions were estimated for each culture media, and they were compared to MRS results in order to determine the reduction in costs of production when we replace commercial peptones by gelatin water streams.

The cost of biomass for *L. plantarum* can be reduced at least 3.4 folds using Na_YT or NSC_Sa (Fig. 5). The best effluents in economical comparisons were Na and NSC ranging from 2.4 to 3.4 reduction folds regarding MRS, and Su was the effluent in which minor reductions were reached (1.1–2.4 folds). The production costs of lactic acid were three fold larger in MRS than Na, Ci and NSC streams, and 2.6–2.8 folds in Su (Fig. 5). The decreasing costs of *L. brevis* growth were quite similar than previously commented with a global range of variation of 1.6–3.7. Na was the most valuable effluent (Na_Tu = 3.7 fold) and media formulated using blue shark species and/or Su treatment drove to the worst results. However, in the case of lactic acid, the reduction costs were equal in all kind of wastes using complete broths (intervals of 3.1–3.3 folds). These results demonstrated that a concentration of biomass higher than 1 g/L of L. plantarum and 2.1 g/L of L. brevis, as in the cases of Su_Sa and Su_BS, respectively, are metabolically capable of producing at least 16 g/L of lactic acid. This trend was also confirmed. The reduction of costs using minima media, NSCm without yeast extract and NSCmm only effluents and glucose, are not so exciting (Fig. S8, supplementary material). In those cases, GE media are more expensive alternative than MRS in order to produce biomass and somewhat cheaper to produce lactic acid.

An example of integral valorization (biorefinery) of fish skin –*e.g.*, for the case of turbot wastes– mass balance was also calculated and displayed in Fig. 6. Based on data of gelatin and collagen hydrolysates production previously published (Valcarcel et al., 2021b) jointly with current LAB productions, and using 1 kg of turbot fresh skin as basis of calculation, the obtained biocompounds are: 31 g of *L. plantarum* (or 47 g of *L. brevis*), 10^{17} cfu of *L. plantarum* (or 2.4×10^{17} cfu of *L. brevis*), 223–226 g of lactic acid, 52 g of gelatin, 10 mL of oil and 45 g of collagen hydrolysates with bioactive properties, spending around 15 L of water. We must indicate that the later water generated from washing steps between chemical treatments were not included in the valorization bioprocesses since its soluble protein concentration were negigible and lower than 0.1 g/L.

Present outcomes are perfectly in agreement with other previously reported using peptones from fish discards (Safari et al., 2012), effluents generated from cephalopods processing (Vázquez et al., 2016) and wastes from canning industry (Vázquez et al., 2022). The excellent

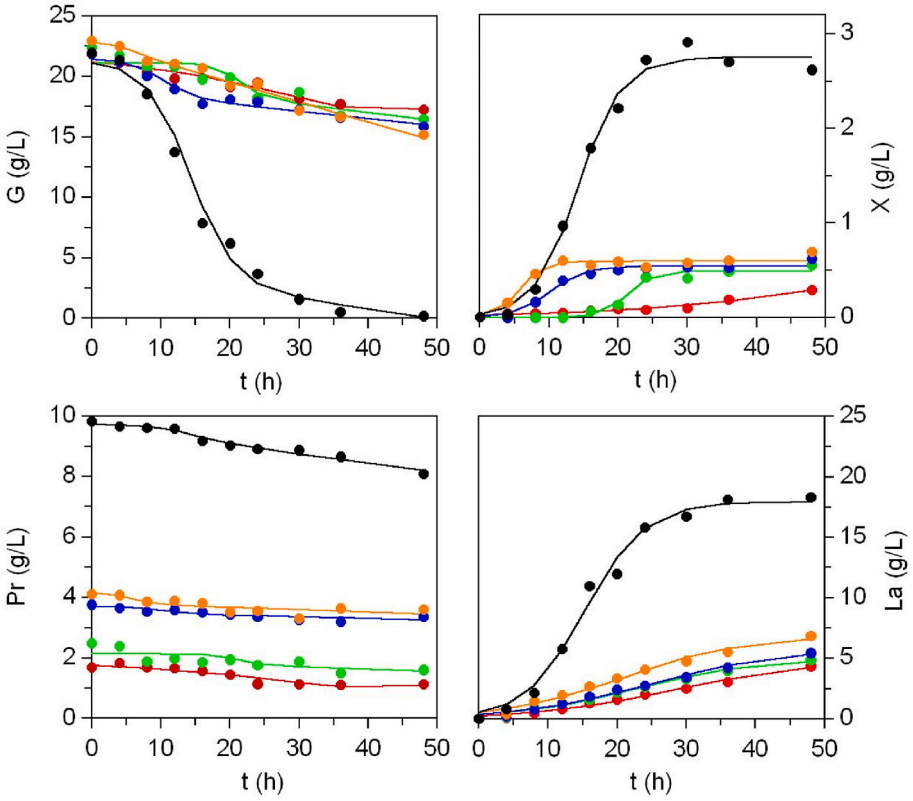

Fig. 4. Fermentations of L. *plantarum* on media formulated with a proportional mixture of the three effluents (but without yeast extract) of shark (●), tuna (●), turbot (●) and salmon (●). Culture in MRS (●) was used as comparative control. Xfi: biomass production, L_a: lactic acid production, G: glucose uptake, Pr: soluble protein consumption. Experimental data (points) were fitted to the [7–10] equations (continuous line). The confidence intervals of experimental data (for three replicates) were in all cases less than 15% of the experimental mean values and omitted for clarity.

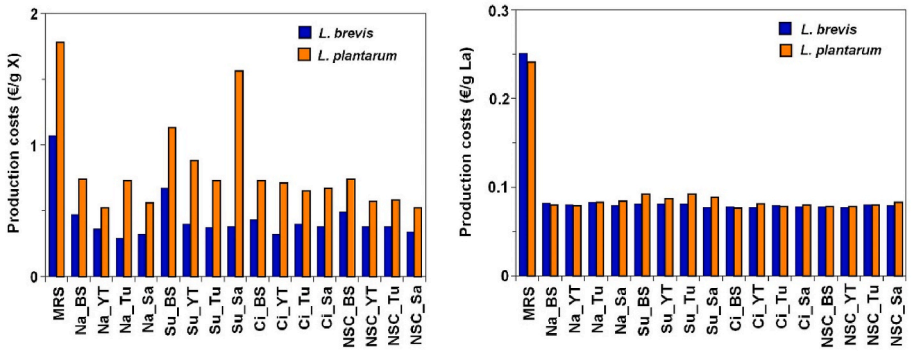

Fig. 5. Bioeconomical evaluation of LAB costs required for the production of biomass (left) and lactic acid (right) in the culture media studied.

productions of biomass (as dry weight and viable cells) and lactic acid achieved together with the corroborated cost-effectivity of present approach should serve to consolidate this sustainable bioprocess as main strategy to management, depurate and convert GE in high-added value bioproducts. Similar type of biobased economy perspective for the conversion of organic wastes was also reported by other authors (Pleissner et al., 2016; Pleissner et al., 2019; Lübeck and Lübeck, 2019; Ladakis et al., 2022).

4. Conclusions

This study shows that the chemical and contaminant streams generated in the pro-duction of gelatins from fish skin wastes (tuna, blue shark, turbot and salmon) can be successfully valorized and bio-converted by means of probiotic lactic acid bacteria. In majority of cases, alternative media formulated with gelatin effluents, as main source of proteins, had comparable and even higher performance than commercial medium MRS in both, biomass and lactic acid productions. For the two bacteria studied, *L. plantarum* and *L. brevis*, cost-effective media generated reductions in the probiotic production costs around 3 fold. This bioprocess supposes a sustainable strategy useful to aim the integral valorization of industrial fish skin wastes and the production of multiple valuable biocompounds in a biorefinery way. This strategy can be also expanded to other microorganisms for the enhanced production of desirable metabolites and microbial biomass using gelatin wastewaters as feedstock. Besides, life cycle assesments and carbon footprint analysis must be performed to complete circular economy evaluation of proposed bioprocesses.

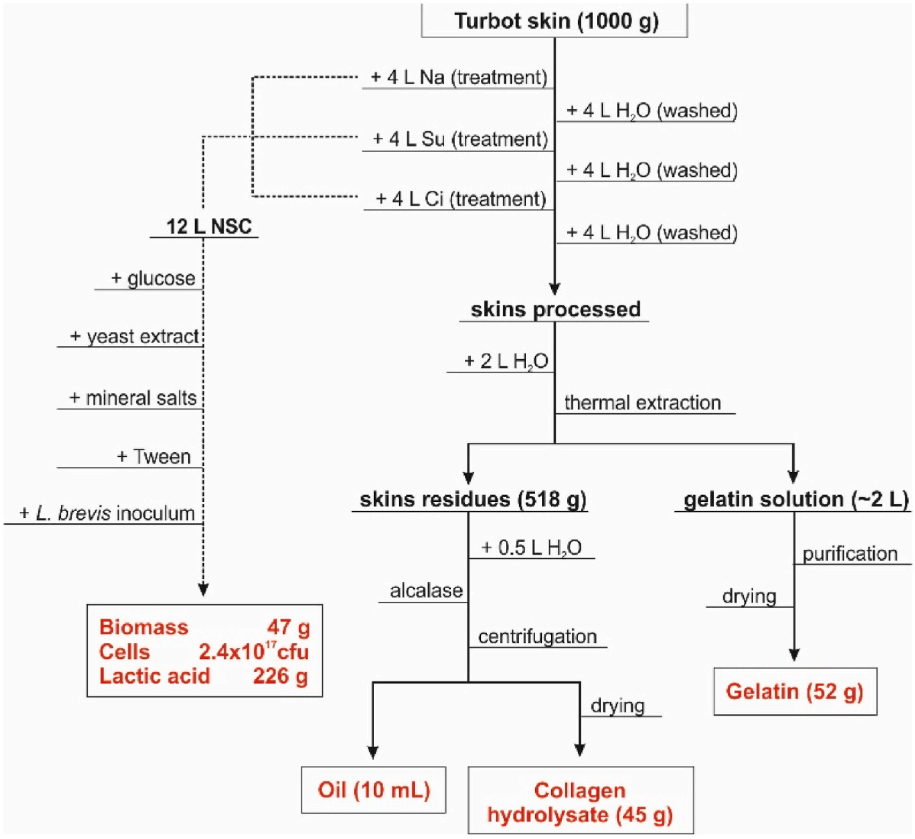

Fig. 6. Mass balance diagram of the biorefinery designed for the integral valorization of turbot skins wastes from aquaculture activities (combining chemical, enzymatic and fermentation processed) in order to produce gelatin, FPH, oil, probiotic biomass and lactic acid.

Funding

This research was funded by the projects LIFE-REFISH (European Union's LIFE PROGRAMME under Grant Agreement No. 101047323, LIFE21-ENV-ES-LIFE REFISH), Intramural PIE-CSIC (202130E070) and Xunta de Galicia (Grupos de Potential Crecimiento, IN607B 2021/11).

CRediT authorship contribution statement

José Antonio Vázquez: Conceptualization, Methodology, Formal analysis, Validation, Investigation, Resources, Visualization, Supervision, Project administration, Funding acquisition, Writing – original draft, Writing – review & editing. **Araceli Menduíña:** Methodology, Investigation. **Ana I. Durán:** Methodology, Investigation. **Margarita Nogueira:** Methodology, Investigation. **Javier Fraguas:** Methodology, Investigation. **Adrián Pedreira:** Methodology, Investigation, Validation, Writing – review & editing. **Jesus Valcarcel:** Methodology, Validation, Investigation, Supervision, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

The authors wish to thank to Conservas Rianxeira S.A.U. (Jealsa Corporation, Boiro, A Coruña, Spain), Prodemar (Stolt Sea Farm S.A., Carnota, A Coruña, Spain), Protea Productos del Mar S.A. (Marín, Spain) and Dr. Johan Johansen (Norwegian Institute of Bioeconomy Research, NIBIO, Bodø, Norway) for the kindly supplied of skin wastes of tuna, turbot, blue shark and salmon, respectively. We acknowledge support of the publication fee by the CSIC Open Access Publication Support Initiative through its Unit of Information Resources for Research (URICI).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2023.137952.

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