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An environmental management industrial solution for the treatment and reuse of mussel wastewaters

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HIGHLIGHTS

- Mussel processing wastewaters (MPW) have been continuously dumped into the sea.
- MPW is causing the progressive deterioration of marine ecosystems in the NW of Spain.
- A biotechnological process to transform MPW into profitable bioproducts
- A sustainable process to integrate the mussel industry into the ecosystem

GRAPHICAL ABSTRACT

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ABSTRACT

In the North-West of Spain, the annual production of mussel is 2×10^6 t (35% of the world). The industrial thermal treatment of mussels generates between 300 and 400 L/t wastewaters that are continuously disposed into the sea without previous treatment and or further reuse. These effluents, relatively rich in organic matter (7 g glycogen/L and 25 g COD/L), contribute to the progressive deterioration of the marine ecosystem. We wish to suggest a biotechnological process, based on a laboratory optimization and industrial pre-scale trials, to transform these industrial effluents into a growth culture medium to produce microbial biomass. Furthermore, this biomass is isolated and treated by different optimized separation and purification processes to produce several bioproducts: 1) single cell protein; 2) cell wall material with a high content in glucans and glycoproteins 3) fractions of 1,3-β-glucans and mannoproteins from yeast cell walls hydrolysis; and 4) a potential antioxidant extract. Finally, the authors propose a scaled process for its industrial application. In consequence, we believe that this work provides an environmentally friendly, eco-designed and profitable solution that allows integrating the mussel industry into the ecosystem in a sustainable way.

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1. Introduction

Since many years, the Galician scientific community has searched for a process to treat the effluents derived from the mussel processing industry that are being released to the estuary coastal waters at the NW of Spain (~100,000 m^3/year). Previous authors have suggested (González et al., 1987, 1992; Murado et al., 1993a,b) to transform the mussel processing wastewaters (MPW) into a suitable growth medium able to provide the carbon source for microbial fermentation showing clearly the viability of this approach. Also, this residual effluent has been successfully used for several bioproductions including citric acid, gibberellins, amylases, pediocin, glucose oxidase, hyaluronic acid, among others (Murado et al., 1997; Omil et al., 1996; Pastrana et al., 1995; Pintado et al., 1993; Ramasamy and Abbasi, 2000; Slater et al., 2009; Vázquez et al., 2013), encouraging its industrial application. Despite of the considerable environmental effects of MPW on the

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marine ecosystem, the seasonal uncertainty (Omil et al., 1996) and the low profitability of the by-products obtained, made a treatment, which is able to reduce the environmental impact of these continuous effluents, difficult at industrial level (Prieto et al., 2011).

Among the diverse number of microbial bioproductions, one alternative for the MPW, not yet examined, is to optimize the production of glucans from yeasts (Suphantharika, 1997). Glucans and glycoproteins are a diverse group of glycoconjugates, mainly made of carbohydrates with significant differences in their molecular mass, solubility, viscosity, and three-dimensional configuration. In vegetables, these compounds are associated with cellulosic cover structures, and in bacteria, yeast and fungi are components of the cell walls. In general, these compounds have several beneficial properties and many useful applications. The differences between soluble and insoluble β-glucans, as well as the differences between the types of links and their spatial structure are significant in regards to solubility, application, mode of action, and overall biological activity (Janusz et al., 1989; Kulicke et al., 1997). Mannoproteins, 1,3-β-D-glucans and insoluble fiber fractions showed beneficial effects on human health (Diez et al., 2010; Kim and Yun, 2006), such as immunostimulatory, antitumoral and anti-inflammatory properties (Bagni et al., 2005; Bohn and BeMiller, 1995; Marques et al., 2006). Authors have pointed out that glucans and glycoproteins have some ability to capture hydrophilic and lipophilic radicals (Altan et al., 2009; Jaehrig and Rohn, 2007; Jaehrig et al., 2008; Martínez-Tomé et al., 2004) and, therefore, they have been proposed as food additive with antioxidant features. Apart from of all these potential applications, the animal feed and veterinary industries are the sectors in which glucans applications are more extensively applied. Furthermore, a broad spectrum of living organisms (earthworms, shrimp, fish, pigs, etc.) have shown health benefits when they were fed with diets formulated with glucans and glyoproteins as ingredients (Beschin, 1998; Buddle et al., 1988; Skjermo et al., 2006; Suphantharika and Khunrae, 2003). In addition, glucans have demonstrated to be active compounds, increasing the natural resistance mechanisms to microbial pathogens (Figueras et al., 1998; Santarem et al., 1997), which has a direct application in the aquaculture field, substituting the typical misuse of antibiotics which is applied to reduce the high mortalities of fish larvae (Rodríguez et al., 2009).

The objectives of the present study were: 1) to select an appropriate microbial species for the biotechnological transformation of industrial MPW effluents into single cell protein (SCP); 2) to optimize the laboratory extraction of cell wall (CW), in order to obtain high value products for diverse applications; 3) to analyze the main composition of the glucans and glycoproteins products obtained and to quantify the corresponding yields; 4) to determine the potential antioxidant capacity in vitro of these products derived from biomass cell walls; and 5) to design a recycling process for MPW effluents in order to minimize the environmental impact by a profitable and cleaner technology and to integrate the mussel industry into the ecosystem in a sustainable way.

2. Material and methods

2.1. Microbial methods

2.1.1. Strains

The microbial species tested (all of them GRAS: Generally Recognized As Safe) were: a) the yeasts *Saccharomyces cerevisiae* (CBS 1907; abbreviation as Sc), *Rhodotorula rubra* (CECT 12891; Rr) and *Saccharomyces fibuligera* (other non-used synonyms: *Endomyces fibuligera*, *Saccharomycopsis fibuligera* CBS 2521; Sf); and b) the fungus *Aspergillus niger* (CBS 513.88; An) and *Aspergillus oryzae* (CBS 102.07; Ao). The inclusion of the first two yeasts obeyed only for comparative purposes in a conventional medium, since they are not amylolytic species and cannot metabolize the glycogen present in the MPW.

2.1.2. Culture media

2.1.2.1. Commercial malt culture (CMC) medium. Malt culture media (20 g/L) with 1 g/L of yeast extract.

2.1.2.2. Mussel process wastewaters medium (MPWm). The MPW were kindly supplied by Marcelino S.A. (Galicia, Spain), and their chemical composition was as follows: 7 g/L glycogen, 0.10 g/L reducing sugars, 3.5 g/L proteins and 1.6 g/L total nitrogen. Sediments were not observed in these effluents, and the initial pH was 7.2. The effluents were clarified by centrifugation after acidification (5 N HCl to pH 4.5) to precipitate the greater part of their protein content, and concentrated to 20 g/L of glycogen by ultrafiltration with a 100 kDa cut-off membrane (Gonzalez et al., 1992; Murado et al., 1993b).

Both media were supplemented with 400 mg/L of P (as KH_2PO_4) and 1.2 mg/L of N (as $NaNO_3$ and NH_4Cl, so that the relationship between the reduced and oxidized form of N was 0.8:0.2) then, they were sterilized in an autoclave with steam vapor during 60 min. Afterwards, the pH was brought to an initial value of 6.0.

2.1.3. Inoculum and growth culture conditions

The inocula (vegetative cells in yeast and spores in microfungi) were incubated in slant in malt agar at 30 °C for 48–96 h. Using a calibrated spectrophotometer analysis at 700 nm, suspensions in sterile water were prepared, whereby a volume of 1 mL provided an initial population of 5×10^4 cells/mL in 50 mL of CMC medium.

Microbial growths were conducted in 250 mL Erlenmeyer flasks with orbital shaking at 200 rpm. At appropriate times, post-incubated medium and biomass were separated by centrifugation (4000 *g*/30 min). The supernatant was used for the basic composition analysis (proteins, total and reducing sugars, nitrogen, chitin and amylolytic activity), and the biomass pellet was washed twice with distilled water, lyophilized and stored at −18 °C until processing.

2.1.4. Kinetic analysis

The growth analysis was carried out by adjusting the values of the produced biomass to the logistic-growth equation (Verhulst, 1845):

$$X(t) = \frac{K}{1 + \exp(c - \mu_m \cdot t)}; \quad c = \ln\left[\left(\frac{K}{X_0}\right) - 1\right] \tag{1}$$

where X is the biomass (g/L) with X_0 and K as initial and maximum values, respectively; t the time (h) and μ_m the specific maximum growth rate (h^{-1}). Other values of interest defined from Eq. (1) were the maximum growth rate (v_m, in $gL^{-1}\ h^{-1}$), the lag-phase (λ, in h) and the time (τ, in h) corresponding to the production of the half-maximum biomass, which is obtained by considering $X = K/2$ in model (1):

$$v_m = \frac{K \cdot \mu_m}{4} \tag{2}$$

$$\lambda = \frac{c - 2}{\mu_m} \tag{3}$$

$$\tau = \frac{c}{\mu_m} \tag{4}$$

Yields of biomass were referred to the consumptions of both T_S and T_P and quantified as:

$$Y_{X/Ts} = \frac{\Delta X}{\Delta Ts} = \frac{X_f - X_0}{Ts_0 - Ts_f} \quad \text{and} \quad Y_{X/Tp} = \frac{\Delta X}{\Delta Tp} = \frac{X_f - X_0}{Tp_0 - Tp_f}. \tag{5}$$

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