PRODUCTION OF MEXICAN BROWN MACROALGAE FUCOIDAN AND FUCOSIDASES UNDER AN INTEGRAL GREEN TECHNOLOGY BIOPROCESES BY THE BIOREFINERY CONCEPT

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RESUMO

Marine ecosystem can be considered a rather exploited source of natural substances with enormous bioactive potential. In Mexico macro-algae study remain forgotten for research and economic purposes besides the high amount of this resource along the west and east coast. For that reason the Bioferinery Group of the Autonomous University of Coahuila, have been studying the biorefinery concept in order to recover high value byproducts of Mexican brown macro-algae including polysaccharides and enzymes to be applied in food, pharmaceutical and energy industry. Brown macroalgae are an important source of fucoidan, alginate and laminarin which comprise a complex group of macromolecules with a wide range of important biological properties such as anticoagulant, antioxidant, antitumoral and antiviral and also as rich source of fermentable sugars for enzymes production. Additionally, specific enzymes able to degrade algae matrix (fucosidases, sulfatases, aliginases, etc) are important tools to establish structural characteristics and biological functions of these polysaccharides. The aims of the present work were the integral study of bioprocess for macroalgae biomass exploitation by the use of green technologies as hydrothermal extraction and solid state fermentation in order to produce polysaccharides and enzymes (fucoidan and fucoidan hydrolytic enzymes). This work comprises the use of the different bioprocess phases in order to produce high value products with lower time and wastes.

1. INTRODUCTION

Macroalgae have accounted for almost 3,000 natural products representing 20% of the chemistry reported from the marine realm. In general, brown algae are known to have a high polysaccharides and consists of three major types of components: (1) mineral or inorganic part, (2) alginates, (3) fucans and others carbohydrates. They have also proven to be rich sources of structurally diverse bioactive compounds with valuable potential in food, pharmaceutical, biomedical and energy industry (Shyamali et al., 1988; Morris et al., 1999; Merrifield et al., 2004; Veena et al., 2007; Ruiz et al., 2013). Fucoidans are marine hetero-polysaccharides, which consisting mainly of α-1-3-L-fucose, with a wide spectrum of activity in biological systems. Besides their well-attested anticoagulant and antithrombotic activity, they act on the inflammation and immune systems, have antiproliferative and antiadhesive effect on cells, protect cells from viral infection, and can interfere with mechanisms involved in fertilization (Berteau and Mulloy, 2003; Giordano et al., 2006). Usually, most of the processes to recover fucoidans from natural sources consist in acid extractions during long reaction times. Moreover, green chemistry technologies based in hydrothermal extraction with high temperature and low times using microwave and conventional heating are alternative processes to produce bioactive compounds at short times and without the use of chemical agents. Therefore, the establishment of the correct extraction parameters, such as temperature, solid/liquid relation, heating and cooling time, greatly influences the yield and structural characteristics of the target macromolecules.

On other hand, enzyme production is a growing field of marine biotechnology, principally in the last 10 years (Holtkamp et al., 2009). The global market for industrial enzymes is estimated at $3.3 billion in 2010. This market is expected to reach $ 4.4 billion by 2015 (BBC research, 2011). Additionally, enzymes with known specificities that catalyze the degradation of fucoidan to produce low molecular weight fucoidans are an important tool for studying the structural peculiarities and biological role of this class of polysaccharides, this special group of hydrolases are fucosidases. Fucosidases are reported found only from marine organisms, and their activities are usually extremely low and could be extracted from hepatopancreas of invertebrates, marine bacteria marine fungi and actually terrestrial fungi (Yaphe and Morgan, 1959; Kitamura et al., 1992; Bakunina et al., 2000; Sakai et al., 2003; Urvantseva et al., 2003; Tissot et al., 2006; El-Shahawi et al., 2009; Rodríguez-Jasso et al, 2010; Moyra et al. 2012).

Solid-state fermentation (SSF) has been widely used in recent years due to promote high productivity of several bioactive compounds (secondary metabolites) principally with agro-industrial residues, increasing the interest on their applications. For example, the reutilization of agro-industrial wastes for enzymes production using SSF minimizes the pollution and allows obtaining high added-value products using an economical technology (Aguilar et al., 2008). Compared to submerged fermentation, the solid media used in SSF contain less water but an important gas phase exists between the particles (Durand, 2003). This condition favors the development of filamentous fungi, given their unique capacity to colonize the interparticular spaces of solid matrices. For this reason, the utilization of macroalgae as substrates in solid-state fermentation process provides an alternative avenue and value-addition on the production of different products as enzymes and bioactive compounds (Rodríguez-Jasso et al, 2011; Rodríguez-Jasso et al, 2013).

The aim of this work was to apply the biorefinery concept in the global study of fucoidan and fucosidases production by green technology bioproceses based in Mexican brown macroalgae as raw material, including 1) two hydrothermal extraction (microwave and conventional), process optimization, physicochemical characterization and partial purification and 2) Macroalgae biomass pretreatment study for enzymes production by solid state fermentation.

2. MATERIALS AND METHODS

Mexican macroalgae *Macrocystis pyrifera* was used in the present study to recover fucoidan and fucosidases. The physicochemical characterization of *M. pyrifera* was performed to evaluate the lipid content, protein, crude fiber, moisture, ash, micro and macro elements (AOAC). The evaluation of total sugars, sulfates and phenols were carried out by spectrophotometric methods. Fucoidan extraction studies were conducted under a Central Composite Design varying time (1 to 10 min), temperature (160 and 200 ° C) power (800 and 1600 W power), with a ratio of 1:25 (w / v; algae / water). Recovering the sulfated oligosaccharides was conducted under sequential precipitation with $CaCl_2$ and ethanol. Response variables analyzed at various stages of the process were the total sugar, sulfates, phenols, extraction yield; higher treatments were analyzed by anion exchange chromatography using a cellulose column. Fucoidan, alginate and laminarin content were also quantified by HPLC.

Macroalgae biomass for solid state fermentation were based in *M. pyrifera* substrate after hydrothermal extraction of fucoidan using microwave and autoclave hydrothermal heating as pretreatment process, varying time, pressure and alga/water ratio. Fungal strains from Coahuila, Mexico region were screened for microbial radial growth using fucoidan and algae as sole carbon source. Microbial growth and enzymes induction were evaluated under different culture media and supports. Productivity responses and kinetic parameters were evaluated.

3. RESULTS AND DISCUSSION

The results of the physicochemical characterization were similar to those reported in the literature, with 23% and 34% of total sugars content (TS) and minerals, respectively. Minerals with the highest content of potassium and sodium were 915.44 and 363.72 mg/L, respectively, and the quantity of heavy metals was below the limit value approved by the Mexican Official Norm. NOM-247-SSA1-200.

The hydrothermal extraction MAE under the conditions of 10 min, 160 ° C and 1600 W showed the highest yield of fucoidan oligosaccharides 9.6%. The extraction temperature was the only factor significant ($p \leq 0.05$) in yield. Additionally was optimized extraction process being 3.32 min, 171.9 °C and 1313.3 W the optimal extraction conditions (Figure 1). Based on the elution peak obtained in the treatments studied was observed, under the conditions of 10 min, 160 ° C and 1600 W, the highest content of TS and S03 with 8.6% and 14.1% respectively.

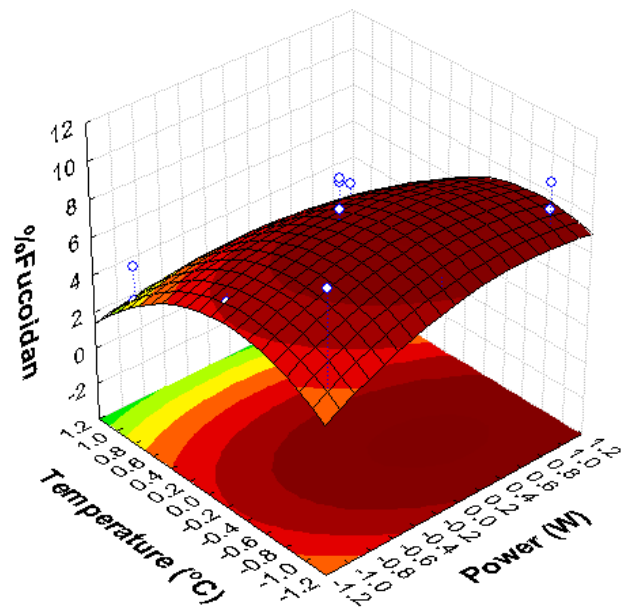

Figure 1. Surface plot for fucoidan optimization extraction

The algae biomass yield recovery and total sugar of *M. pyrifera* substrate after hydrothermal pretreatments for solid state fermentation were 52.8 and 22.6% for autoclave, repectively, and 50.32% and 22.68 for microwave, respectively.

Fungal strain *Aspergillus niger* PSH was able to growth on different fucoidan and algae substrate pretreated by microwave and autoclave process, being thus important tool for the synthesis of sulfated fucan-degrading enzymes. Besides, plate invasion capacity and radial growth rate were directly proportional to measured morphometric parameters. Such strain synthesize acting metabolites toward fucoidan matrix, being selected for subsequent use in fermentative processes.

4. CONCLUSIONS

Hydrothermal extraction, as a green technology, showed to be an effective method for fucoidan from *M. pyrifera* brown macroalgae with shorter times than those reported in the literature. *Aspergillus niger* PSH has the capacity for synthesize fucoidan hydrolytic enzymes, being potential microbiology tools for used in fermentation process and microbial growth induction. Algae substrate after fucoidan extraction is viable biomass to be used in solid state fermentation for enzymes production, causing a reduction in process residues.

6. BIBLIOGRAPHY

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