Energy Conversion and Management 119 (2016) 257–265

Contents lists available at ScienceDirect

Energy Conversion and Management

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

A biorefinery concept using the green macroalgae *Chaetomorpha linum* for the coproduction of bioethanol and biogas

Nesrine Ben Yahmed [a], Mohamed Amine Jmel [a], Monia Ben Alaya [a], Hassib Bouallagui [b], M. Nejib Marzouki [a], Issam Smaali [a,*]

[a] Laboratoire d'ingénierie des protéines et des molécules bioactives, Université de Carthage, INSAT-BP 676, Centre urbain nord, 1080 Carthage Cedex, Tunisia
[b] Laboratoire d'écologie et technologie microbienne, Université de Carthage, INSAT-BP 676, Centre urbain nord, 1080 Carthage Cedex, Tunisia

ARTICLE INFO

Article history:
Received 28 January 2016
Received in revised form 24 March 2016
Accepted 13 April 2016
Available online 22 April 2016

Keywords:
Macroalgae
Biorefinery
Pretreatment
Saccharification
Biomethane
Bioethanol

ABSTRACT

An innovative integrated biorefinery approach using the green macroalgae *Chaetomorpha linum* was investigated in the present study for the co-production of bioethanol and biogas. Among three pretreatments of *C. linum* biomass, consisting of acidic, neutral and alkali ones, 3% NaOH pretreatment gave the best result in terms of thallus disintegration, biomass recovery and enzymatic digestibility as demonstrated by scanning electron microscopy and saccharification tests. The hydrolysis of *C. linum* feedstock with a crude specific enzyme preparation, locally produced from fermentation of *Aspergillus awamori*, at 45 °C, pH 5 for 30 h gave the maximum yield of fermentable sugar of 0.22 ± 0.02 g/g dry substrate. An ethanol yield of 0.41 g/g reducing sugar corresponding to about 0.093 g/g pretreated algae was obtained after alcoholic fermentation by *Saccharomyces cerevisiae*. In the integrated proposed process, mycelium issued from the fungal fermentation, liquid issued from alkali pretreatment, residual from the non-hydrolysable biomass and all effluents and co-products represent a heterogeneous substrate that feed an anaerobic digester for biogas production. GC-analysis of this later showed that the biomethane yield reached 0.26 ± 0.045 L/gVS. This study presents therefore an eco-friendly biorefining process, which efficiently coproduce bioethanol and biomethane and generate only a single waste (0.3 ± 0.01 g/g) allowing an almost complete conversion of the algal biomass.

1. Introduction

The decrease of oil resources combined to the increase of the world population and therefore the energy consumption are the main requirements for using renewable energies. Among these, biomass constitutes a renewable source of biofuel, namely bioethanol, biogas and biodiesel [1]. It represents a promising alternative for the substitution, at least in part, of fossil fuels. Indeed, the development of reliable, cost-effective and ecological processes from biomass becomes a global priority despite the two known main limits for this energetic bioconversion. In fact, the culture of lignocellulosic plants is done in detriment of cultivable land used for human consumption which is not a long term solution to the increase of population [2]. Besides, lignocellulosic biomass which is consisted of cellulose, hemicelluloses and lignin, requires mechanical, thermal and/or chemical pretreatment steps to make the cellulose accessible to enzymes during the enzymatic hydrolysis [3,4]. These pretreatment steps usually affected the cost of energetic conversion. Thus, all the research on the biological transformation of lignocellulose were interested in several issues namely finding suitable pretreatments which do not generate harmful products to the environment and fermentation inhibitors [5] and producing specific and stable enzymes with reasonable cost [6,7]. Some research were also interested in developing strains of yeasts or bacteria able to ferment simultaneously hexoses and pentoses resulting from the enzymatic saccharification as well as resistant to the various inhibitors which may be generated [8,9].

Recently, several studies are interested in finding an alternative to the use of lignocellulosic biomass. In fact, beside the use of microalgae as a source of sustainable biodiesel production [10], marine macroalgae have received considerable attention as source of third-generation biofuels [11] such as bioethanol [12–16] and biogas [17–19]. Compared to microalgae, macroalgae are multicellular plants that possess plant-like characteristics with thallus-type morphology, composed mainly of carbohydrates. They can be therefore considered as a good candidates for biofuel production like biogas, bioethanol and bio-oils [11]. Additionally, their harvesting were also easier, they represent a renewable abundant

* Corresponding author at: INSAT, Centre Urbain Nord, University of Carthage, BP 676, 1080 Tunis Cedex, Tunisia.
E-mail address: issam.smaali@insat.rnu.tn (I. Smaali).

http://dx.doi.org/10.1016/j.enconman.2016.04.046
0196-8904/

biomass that could be easily cultivated with low cost of collection and null environmental damage [20]. Obviously, they do not compete with land use (avoiding arable land) and water consumption, necessary for food crops [2]. Furthermore, macroalgae are characterized by a higher biomass production due to its fast growing rate in the open aquatic media [20] and does not require agricultural additives such as fertilizer and pesticides [21,22]. Moreover, they have higher photosynthetic activity than terrestrial plants [20] and they contain little or no lignin-like molecules [23].

Using macroalgae in biorefinery concepts would reduce petroleum dependence while assuring a positive environmental impact [20]. The bioethanol has been the most biofuel type produced from the macroalgae [24]. Nevertheless, the cost-effectiveness of biorefinery concept which is based on the production of bioethanol is debatable for the low cellulose content (15–25%) and the seasonal and environmental variation of macroalgae which influences its biochemical composition including the content of cellulose principal source of fermentable sugars [25].

Thus, the objective of our study is to develop a novel integrated biorefinery concept based on the co-production of both bioethanol and biogas from the green macroalgae *Chaetomorpha linum* with one coproduct. *C. linum* is very abundant in the coasts of Tunisia but it is not very valued. The feasibility of different stages of the process such as pretreatment of macroalgae, alcohol fermentation and anaerobic digestion was demonstrated. In this work environmental friendly cell-wall degrading enzymes, locally produced, were used for the saccharification of *C. linum*.

2. Materials and methods

2.1. Biological materials

The green macroalgae *C. linum* was collected in September 2013 from the shores of Tunis lagoon (GPS: 36.813095, 10.192673, salinity: 33.8 psu) suffering from eutrophication problem. A bioremediation of this ecosystem could be attempted using these stranded algae as feedstock of a biorefinery process. Samples were washed, dried, finely ground and stored until they were used.

Aspergillus awamori (NBRC 4033, Osaka, Japan) was maintained at 4 °C in potato dextrose agar plates. The spores were collected in 4 mL of sterile water containing 0.1% Tween 80 and transferred into a 250 mL Erlenmeyer flask containing 75 mL of medium.

Baker's yeast (*Saccharomyces cerevisiae*) was purchased from the local market (la Patissiere Company). The strain stored at −20 °C in 25% glycerol was firstly purified by subculture on YPG (yeast peptone glucose) agar medium. One purified colony was transferred in YPG broth to start liquid precultures for 12 h with 180 rpm agitation on rotary shaker. Microscopic observations were performed to ensure the presence of *S. cerevisiae* and its purity. The culture was used at 10% v/v as inoculum for alcohol fermentation.

2.2. Cell wall degrading enzymes production

Cell wall degrading enzymes production was carried out in triplicate with a batch fermentation of *A. awamori* (NBRC 4033) in mineral medium according to Mandel and Weber [26]. Mandels' salts solution (0.3 g L^{-1} urea, 1.4 g L^{-1} $(NH_4)_2SO_4$, 2.0 g L^{-1} KH_2PO_4, 0.3 g L^{-1} $CaCl_2$, 0.3 g L^{-1} $MgSO_4$, 0.25 g L^{-1} yeast extract, 0.75 g L^{-1} peptone, 5 mg L^{-1} $FeSO_47H_2O$, 20 mg L^{-1} $CoCl_2$, 1.6 mg L^{-1} $MnSO_4$ and 1.4 mg L^{-1} $ZnSO_4$) supplemented with 0.5% (w/v) ground *C. linum* as carbon source was used. The pH of the medium was adjusted to 5 with 50 mM sodium acetate buffer. The flasks were autoclaved for 30 min at 120 °C and then inoculated with the *A. awamori* preculture. After 8 days incubation at 40 °C the cocktail of enzymes was obtained by simple filtration on gauze filter followed by ultrafiltration on 10 kD membrane cut-off.

2.3. Pretreatment conditions

C. linum were pretreated by thermohydrolysis carried out for 20 min at 120 °C in an autoclave (1.5 bars) without catalyst for neutral pretreatment and in the presence of 3% NaOH and 0.6% H_2SO_4 for alkali and acid pretreatment respectively.

Pretreated biomass was recovered by centrifugation (solid residue) and used at 4% (w/v) for the enzymatic saccharification after pH adjustment with sodium acetate buffer 100 mM. The supernatant corresponding to the liquid issued from pretreatment was also recovered and served to feed the anaerobic digester for the production of biogas.

For the determination of the residual mass, pretreated and filtered samples were dried in an oven at 104 °C overnight and then weighed by precision balance. Experiments were done in triplicate.

2.4. Biomass saccharification and alcohol fermentation

Enzymatic hydrolysis was carried out with 10% of alkali pretreated *C. linum* at pH 5. The saccharification reactions were conducted in triplicate in a laboratory incubator at 150 rpm and 45 °C for 30 h.

Ethanol production was studied in triplicates using the broth of enzymatic saccharification of *C. linum*. The saccharification reaction products were concentrated by evaporation at 60 °C for 12 h to reduce the water content and make the reducing sugars content of about 40 g/L. The saccharification broth was sterilized by filtration (0.2 μm filter membrane) and added to Yeast extract Peptone medium (YP) (10× concentrate) with the proportion (9/1). The prepared medium was inoculated with 10% v/v of fresh culture of *S. cerevisiae* and incubated at 28 ± 2 °C on an orbital shaker with a shaking speed of 150 rpm.

After 48 h of fermentation, the ethanol concentration was assayed by HPLC. The ethanol is recovered at high purity by conventional distillation at 60 °C. Vinasse (residue after fermentation / distillation) was recovered to feed the stage of anaerobic digestion.

2.5. Anaerobic digestion

The anaerobic digestion was achieved in duplicate in a batch stirred anaerobic reactor with a working volume of 0.5 L. The reactor was operated under mesophilic conditions (38 ± 1 °C) during 30 days. Over the anaerobic digestion heterogeneous substrate corresponding to all liquid, solid and gaseous effluent produced in the different stages of the manufacturing process of bioethanol was recovered. Inoculation with active methanogenic bacteria with an inoculum ratio of 50%/50% (v/v) for the digestion of *C. linum* was achieved. Initial anaerobic sludge used as inoculum was collected from an active digester located in a municipal wastewater treatment plant of Chotrana (Tunisia). The biogas produced was collected daily in plastic bags at room temperature. The total volume was later determined with wet gas-meter (Ritter, Germany). The methane content in the biogas was measured using a FID–PID Unichrom gas chromatograph.

2.6. Biochemical characterization of the cellulase crude extract

The optimal pH of the endoglucanase (CMCase) was determined using the following buffers at 100 mM: Glycine–HCl (for pH from 2.2 to 3), sodium acetate (for pH from 4.0 to 6.0), potassium phosphate (for pH from 6.0 to 8.0) and glycine–NaOH (for pH from 8.0 to 10.0). For temperature stability, the enzyme was pre-incubated in the standard buffer (sodium acetate pH 5) at 40, 50, 60 and 70 °C for 30 min and the activity was thereafter assayed by DNS method under the standard conditions.