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Research paper

Characterization, pretreatment and saccharification of spent seaweed biomass for bioethanol production using baker's yeast

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ABSTRACT

Seaweeds are marine macroalgae found abundantly and viewed as potential source of phycocolloids to produce biofuel. In this study, seaweed spent biomass obtained from alginate production industry and biomass obtained after pigment extraction were found to contain a considerable amount of phycocolloids. These two spent biomasses were investigated for the production of ethanol. In this study, the red seaweed spent biomass of *Gracilaria corticata* var *corticata* showed higher content of polysaccharide (190.71 ± 30.67 mg g^{-1} dry weight) than brown seaweed spent biomass (industrial) (136.28 ± 30.09 mg g^{-1} dry weight). Hydrolysis of spent biomasses with different concentrations of sulfuric acid (0.1%, 0.5% and 1%) was also investigated. Brown seaweed spent biomass and red seaweed spent biomass exhibited high amount of sugar in 0.5% and 1% sulfuric acid treatment, respectively. Proximate and ultimate composition of seaweed spent biomasses were analysed for energy value. The FT-Raman spectra exhibited similar stretches for both acid hydrolysed spent biomasses with their respective standards. Ethanol produced through a fermentation process using spent hydrolysates with baker's yeast at pH 5.3 was found to be significant. The ethanol yield from brown seaweed spent biomass and red seaweed spent biomass was observed to be 0.011 g g^{-1} and 0.02 ± 0.003 g g^{-1} respectively, when compared with YPD (0.42 ± 0.03 g g^{-1}) and D-galactose (0.37 ± 0.04 g g^{-1}) as standard on day 4. The present study revealed the possibility of effective utilization of spent biomass from seaweed industry for ethanol production.

1. Introduction

Fossil fuels accounted for about 88% of the global primary energy consumption [1]. The depletion of fossil fuels, increased cost of fuels, concern about global climatic changes and increased CO_2 emission have led to the discovery of bio-fuels [2]. Biofuels are liquid or gaseous fuels produced from plants, including microalgae, and seaweeds [3], municipal wastes [4] and agricultural or forest by-products [5,6]. Among biofuels, bioethanol, a renewable source of energy, has been accepted more widely as an alternative to fossil fuels.

Seaweeds are macroalgae found abundantly on east and west coast of India and broadly classified into red, brown and green forms based on colour and biochemical composition [7]. Seaweeds have many bioactive compounds like pigments, sulfated polysaccharides such as agar, carrageenan and alginates, that are used for various industrial applications [8,9]. *Gracilaria* sp., *Kappaphycus* sp. and *Sargassum* sp., are well known for agar, carrageenan and alginates production at an industrial scale level, respectively.

Nearly 7.5–8 million tonnes of wet seaweeds are harvested worldwide per year [10]. The production of macroalgae is 15.5 million tonne fresh weight per annum and contributes 93% commercial value of seaweeds in 2008 worldwide [11–14]. *Saccharina latissima* (previously known as *Laminaria saccharina*) is the fastest-growing seaweed called gigantic kelp species. This seaweed is similar to*Saccharina japonica* of which 4 million tonnes fresh weight is harvested annually in Northern China, and almost 0.3 million tonne fresh weight of *S. japonica* was also harvested in Korea whereas from Japan reported for about 50,000 tonnes [9,15–17]. Commercially important seaweeds such as *Gracilaria* sp.,

Abbreviations: BS, brown seaweed spent; RS, red seaweed spent; DW, dry weight.

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Sargassum sp., were cultivated long before since 1960 and 1970 in India and even today Central Salt and Marine Research Institute (CSMCRI), Mandapam and Bhavnagar, India successfully cultivated *Gracilaria edulis* commercially and developed a technology to grow onshore [10,18,19].

Agar (1179 tonne) and alginate (3180 tonne) production in India seems to be very less when compared to world scenario up to 2003–2004 [10]. The huge amount of spent biomass generated from seaweed industry worldwide and effective utilization of those spent is really a challenging task. In India, some seaweed industries are converting the spent seaweed biomass to agricultural manures through composting. Though composting is a simple process, the current status of biofuel production through waste has become an innovative method to convert waste into a more valuable product.

The seasonal variations in algal sugar quantity may vary depending upon climatic conditions. The phycocolloids such as agar, carrageenan and alginates after acid pretreatment can be effectively utilised by yeast for ethanol production [20–22]. However, yeast has a narrow substrate range such as six-carbon sugars [23] for its growth and ethanol production. Mostly seaweed yields five-carbon and six-carbon sugars on hydrolysis by acid or alkali. In order to convert polysaccharides to monosugars, an effective pretreatment process is necessary. Mild acid treatment was found to be effective on hydrolysis of polysaccharides at a particular temperature [24–26]. Saccharification and hydrolysis are essential for bioconversion of substrate. Enzymatic hydrolysis is a method for converting of polysaccharides into monosaccharides and can be widely used for ethanol production but cost increases invariably [27]. Acid cleaves β-1-4-glycosidic bond of cellulose and other complex polysaccharides [28]. Monosugars formed due to hydrolysis are found to be valuable substrates for bioethanol production [29–31].

Ethanol production of hexose sugars is easy and red–ox balanced, while production from pentoses or mannitol generates surplus hydrogen. Many bacteria overcome by transhydrogenase production, but yeast cannot produce transhydrogenase to solve this problem. Yeasts can overcome the problem by controlling supply of oxygen, which leads to complete oxidation of the sugar to CO_2 and water, and reduces ethanol yields [32–34].

The ethanol production has been reported from seaweeds such as green (*Ulva lactuca*, *Ulva pertusa*); red (*Kappaphycus alvarezii*, *Gelidium amansii*, *Gelidium elegans*, *Gracilaria salicornia*); and brown (*Laminaria japonica*, *Laminaria hyperborea*, *Saccharina latissima*, *Sargassum fulvellum*, *Undaria pinnatifida*, *Alaria crassifolia*) [35]. Apart from whole seaweeds, the industrial spent, such as floating residues, spent biomass can also be used for ethanol production [5].

The prospective of ethanol production from seaweeds is based on the carbohydrate content (60% of dry weight) and a conversion (90%) ratio to produce ethanol. Through fermentation, 1 g of sugar can yield 0.4–0.5 g of ethanol or 0.22 kg or 0.27 L ethanol from 1 kg dry weight seaweed biomass, equivalent to approximately 0.05 L ethanol per kg wet weight [34].

However, the global demand for bioethanol continues to be an interesting research for human benefit and industrial applications. World production of bioethanol reached over 51,000 million litres in 2007 [36]. The world ethanol market is projected to reach 100 billion litres per annum by the year 2012 [37]. Global demand of ethanol is currently 86 billion litres [38].

The present study is aimed at production of ethanol by utilizing spent biomass generated from seaweed processing industry using baker's yeast and its potential of converting galactose and alginate monomers to bioethanol through fermentation.

2. Materials and methods

2.1. Seaweed spent collection and processing

Brown seaweed spent (BS) biomass was collected from SNAP Alginates and Natural Products Pvt. Ltd., Ranipet, Tamilnadu, India. The collected spent was dried under sunlight to remove water content up to 80–90%. The dried spent was powdered using a mixer grinder and sieved in 100 mesh sizes for further use. Red seaweed spent (RS) biomass were prepared in the laboratory. Red seaweed *Gracilaria corticata var corticata* was collected from a Manapad coastal area, Tamilnadu, India. The phycobiliproteins were extracted using 0.1 M potassium phosphate buffer [39–41] and the remaining spent was processed similar to industrial spent (BS) and utilised for further experiment. The processed spent biomass was stored at room temperature.

2.2. Proximate, ultimate and biochemical analysis of seaweed spent biomass

The collected spent biomass was screened for pH, moisture content, ash content, volatile matter, fixed carbon, C, H, N, S analysis and total organic carbon [42]. Apart from these parameters, the biochemical content of spent biomass, such as total carbohydrates [43], reducing sugar [44], total phenol [45] and total protein [46,47], was quantified as explained below.

2.2.1. Analysis of pH

BS biomass collected from industry contains water along with biomass and appears to be semi solid in nature which was analysed using pH meter at room temperature; RS biomass prepared in the laboratory (after extraction of phycobiliproteins using buffer), obtained as solid biomass, was analysed using pH paper at room temperature.

2.2.2. Moisture analysis

One gram of spent biomass was taken in a known weight of crucible and dried in a hot air oven at 60 °C till the constant weight is attained. The moisture content was calculated by using the formula below.

$$\text{Moisture}\ (\%) = [(\text{Initial biomass weight} - \text{Final biomass weight}) \times /\text{Initial biomass weight}] \times 100$$

2.2.3. Analysis of total carbohydrates

Known quantity (100 mg) of spent biomass was taken into a boiling tube and hydrolysed by keeping in boiling water bath for 5 h with 5 mL of 2.5 N HCl and then cooled to room temperature. The hydrolysate was neutralized with solid sodium carbonate until the effervescence ceases and the volume was made up to 100 mL, then centrifuged. The supernatant 0.2 mL was taken in three test tubes, the volume of each tube was made up to 1 mL with distilled water. Distilled water was used as blank. About 1 mL of 5% phenol was added to each tubes including blank followed by the addition of 5 mL 96% sulfuric acid to each tube. The content in the tubes was shaken well and incubated at 25–30 °C for 20 min. The absorbance was recorded at 490 nm. The amount of total carbohydrate present in the sample solution was calculated using glucose as standard and was expressed in mg g^{-1} [43].