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Utilization of water hyacinth (*Eichhornia crassipes*) for bioethanol production: A comprehensive approach from hydrolysis to purification

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Water hyacinth (WH) is a widely available invasive aquatic plant, making it a promising resource for sustainable bioethanol production. In the present study, WH has been utilized for bioethanol production through a sequential process involving preteatment, ultrasonic-assisted hydrolysis, fermentation, and final purification. Hydrolysis is carried out employing 1N NaOH, succeeded by fermentation through the yeast species *Saccharomyces cerevisiae*, culminating in the synthesis of bioethanol. The produced bioethanol is subsequently recovered following purification step. The presence of ethanol in the sample is confirmed by gas chromatography analysis, which is validated by comparison with a standard ethanol reference. The ceric ammonium nitrate test has further verified the presence of alcoholic groups in the prepared bioethanol. Additionally, the dinitrosalicylic acid (DNS) test has revealed a free sugar content of 18% post-hydrolysis. The bioethanol yield is determined to be 9.36%, which aligns with values reported in existing literature for WH-based bioethanol production.

Keywords: Bioethanol, Fermentation, Lignocellulosic biomass, Water Hyacinth, Yeasts

Introduction

Energy has served as a pivotal factor in promoting societal advancement, with its ramifications observable from the dawn of the industrial revolution to the contemporary technology-centric age. Each phase of development is contingent upon various forms of energy, engendering an incessant quest for more efficient and sustainable sources than those that have been previously accessible. For over decades, global society depended heavily on conventional, non-renewable energy resources such as coal and petroleum. This dependency arises from their widespread availability, economical pricing, technological inertia, reluctance to embrace superior alternatives, and the substantial costs associated with viable substitutes. Nevertheless, the extensive utilization of these traditional energy resources has resulted in considerable challenges, encompassing substantial greenhouse gas emissions, regional dependencies, unpredictable market-driven price fluctuations, and environmental calamities such as oil spills, monopolistic pricing behaviours, and other associated complications. Considering these challenges, biomass waste constitutes an underexploited resource for energy production. With its progressively increasing generation over the years, biomass embodies a promising avenue for the establishment of sustainable energy sources. The utilization of biomass not only mitigates waste management issues but also provides a renewable and ecologically responsible alternative to conventional energy, thereby facilitating the transition towards a more sustainable energy paradigm. As per prediction of International Energy Association, biomass contribution to renewable energy-based sources will be largest in near future; moreover, heating purposes and transportation sectors alone will consume about 30% of renewable energy, indicating its further contribution scope[1]. Among the diverse categories of biomass waste, water hyacinth (WH) (*Eichhornia crassipes*), a perennial floating aquatic macrophyte, is distinguished by its extensive global distribution. Initially indigenous to South America, it represents the singular species within the subgenus Oshunae[2]. Characterized by its rapid proliferation and invasive characteristics, WH has emerged as a notable ecological challenge in numerous locales. Nevertheless, its profusion and widespread prevalence concurrently position it as a viable candidate for biomass conversion, thereby presenting avenues for sustainable energy generation and effective waste management. WH, quick overgrowth tends to become nuisance in several possible ways: water-based crop yield reduction, oxygen level depletion, Biological Oxygen Demand (BOD) level reduction, aquatic life growth inhibition, biodiversity loss, and water quality degradation.

Lignocellulosic biomass (LCB) is well suited for bioethanol production, compared to conventional biomass source, as it offers option to obtain renewable fuels. Furthermore, due to non-renewable energy sources rapid depletion, attention has shifted toward renewable biomass as a viable alternative. Beyond its abundant availability and valorization capability, factors like technological advancements, the need to meet escalating energy demands, compliance with climate regulations, and its economic viability have significantly bolstered the intensified consideration of biomass as an energy source. Moreover, biomass originating from all sources such as industry[3], agriculture[4], natural products[5], households[6], etc. has capability to replace the conventional fossil fuel derived energy source. The principal components of lignocellulosic material are cellulose (~45%), hemicellulose (~30%), and lignin (~25%), on dry basis[7]. WH is regarded as suitable substrate for bioethanol production, owing to considerable LCB presence and its successive conversion to bioethanol by fermentation[8]. The bioethanol based on WH remains as emerging field owing to its relevance, presence, and benign environmental consequence[9]. Apart from transportation ease through flash pyrolysis or hydrothermal liquefaction for environment friendly oil production, the resulted oil has higher energy density and lower CO_2, SO_x, and NO_x emissions compared to contemporary fossil fuels[10]. Therefore, over the years, various research has been dealt the WH-based bioethanol production. The LCB, are biopolymers of cellulose, hemicellulose and lignin rich, contained in both dried as well as wet plant matter; these biopolymers can be converted into bioethanol, and other value-added products by biochemical routes. As a suitable LCB with high cellulose content, WH offers a sustainable substrate for the bioethanol production. Additionally, prior research demonstrated comparable yield of bioethanol derived from WH compared to other agricultural residues[11].

Over the years WH applications have evolved in several fields such as adsorbent[12], compost[13], construction[14], handmade paper[15], composites fibers[16], and biofuel[17]. Among several mentioned domains of applications, WH utilization as biofuel remains most attractive due to current shifting trend toward renewable energy-based sources, depleting current energy sources and future energy security. In bioethanol production from biomass containing LCB, four steps are involved: pre-treatment, hydrolysis, fermentation and purification. For LCB processing, alkali liquor has been widely employed; wherein, bases such as sodium, potassium, calcium, and ammonium hydroxide are employed. Owing to high biopolymers content in LCB, it can generate many valuable products including bioethanol; moreover, LCB of WH possess suitability for fermentative method because of its high cellulosic contents. Therefore, bioethanol production from WH biomass involving fermentative method is extensively reported in the literature; various microbes and other such as bacteria[18], recombinant bacteria[11], fungi[19], sewage sludge[20], acidogenic culture[21], anaerobic inoculum[22], methanogenic sludge[23], microbial culture[24], anaerobic sludge[25], cow dung[26], waste-activated sludge[27] have been employed in fermentation, other than yeast[28]. Among various employed microbes, yeast provides sustainable option for LCB conversion into bioethanol. Moreover, among several available strains of yeast, Saccharomyces cerevisiae is known for high ethanol productivity, high ethanol tolerance and wide ranges of sugar fermentation capability[29]. Biomass material, belonging to renewable energy resources, balances atmospheric CO_2 content; since combustion or thermally conversion, and emission of CO_2 is assimilated by the plant during photosynthesis for life propagation. In this way biomass available on earth becomes part of cyclic process, instead of relying on exhaustible fossil-based energy sources.

As per estimation related to biomass waste usage for energy production, biomass based energy production would cross above 50% by 2050; since, biomass have high cellulosic contents, which are crucial in biofuels production, a step in direction of waste reduction and meeting energy needs without hampering food security[30]. To address the above said, in the current work, we have prepared bioethanol from WH. After WH pretreatment, ultrasonic treatment, (maintained at frequency, 30 kHz) prior to alkali hydrolysis is introduced, the conditions for ultrasonic-assisted alkali hydrolysis were selected based on literature demonstrating that ultrasonic pretreatment effectively disrupts lignocellulosic structure leading to specific surface area enhancement and polymerization reduction, thereby enhancing subsequent alkaline hydrolysis efficiency[31–33]. In next fermentation step, yeast microbe is utilized. In last purification step, simple distillation is employed for bioethanol separation. The obtained bioethanol is analyzed using gas chromatography. On the obtained sample, confirmatory test and DNS test are performed. Moreover, yield of bioethanol production is estimated, and compared with literature reported values.

Experimental Section

Materials

Sodium Hydroxide, Dextrose, Dinitro salicylic acid, Phenol, Sodium Sulfite, Potassium sodium tartrate, and Hydrochloric acid are procured from Researchlab; and Agar, Peptone, Yeast Extract, Methyl Blue, and Gram's Iodine are procured from Himedia. All analytical grade reagents are used in current work.

Methods

The bioethanol formation from WH consisting of four steps pre-treatment, hydrolysis, fermentation, and purification stage is depicted with brief details in Fig. 1.

In pretreatment method, at first, raw WH with long stem sample was collected from nearby Godavari River, Nashik. At first, WH was washed thoroughly multiple times with tap water for any possible dirt removal, followed by roughly chopping into small pieces (~2-2.5 cm). In next step, chopped WH air drying was followed by hot air oven (Make: Bio-technics, Model: BIT-29, Country: India) drying at 100-105°C for 5-6 h. Then, WH was ground fine with domestic mixer (Make: Bajaj electricals) and stored in closed container prior to further experimentation. In the second step of bioethanol production, NaOH-assisted hydrolysis is performed in autoclave (Make: Bio-Technics India) using the following settings: 20 min, 15 psi, 121°C. Prior to abovesaid step, WH was treated using ultrasound bath reactor (Make: Dakshin Ultrasonics, Model: NA, Operating Frequency: 22 to 40 kHz ±3 kHz, Irradiation power: 100W) for 15 min, so that the lignocellulosic material broke down to higher sugars by high frequency ultrasonic waves in small duration.

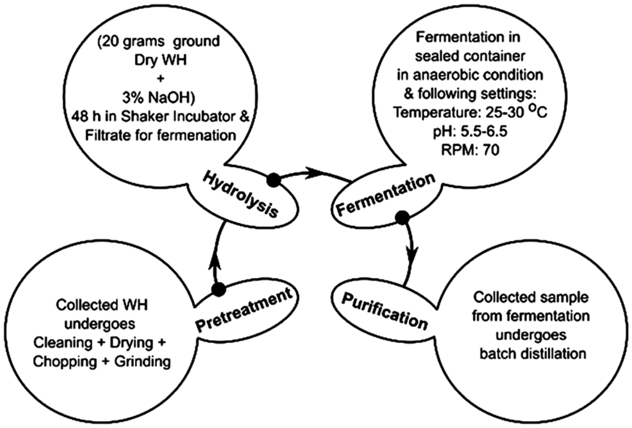

Fig. 1 — Schematic of steps involved in bioethanol production

In the current work, the alkaline chemical hydrolysis method was employed, owing to benefits like lenient substrate washing regime and method universality[34], effective fractionation[35], and improved hydrolysis[36]. With the formed hydrolysate by hydrolysis, polymeric compounds possessing long chained can degrade into furfural, hydroxymethylfurfural, acetate, and lignin molecules[37]. Consequently, during this process, WH releases sugars such as fructose, galactose, arabinose, mannose, which are acted upon by fermentation microorganism.

In order to perform fermentation, first of all, yeast batch was prepared in Ultraviolet laminar flow bench (Make: Sai Samarath Enterprises); wherein, yeast batch (10 mL yeast inoculation in 200 mL broth) is added to the hydrolysate for the fermentation to commence. The fermentation was carried out in fermenter (Make: Dhruv Arbortech, Model: Laboratory Fermenter Machine, Capacity: 10 L). In fermentation, complex molecules are broken down into simpler ones with help of microbes; particularly, yeast converts carbohydrates into ethanol and carbon dioxide. Then, prepared broth solution containing dextrose (20 g/L), added for yeast cultivation; yeast microbe (10 g/L); and peptone (20 g/L) is kept in batch fermenter of 10 L capacity, whose pH is adjusted (5.5-6.5) by equipment with provided 1 N HCl and 1 N NaOH solution. The fermentation is carried out for 21 days, with intermittent pH checking at 7 days interval and continuous stirring. In purification process, sample collected from fermentation is filled in round bottom flask at 90-95°C for 3 h. Then, bioethanol is collected from the process, utilized further in characterization process.

Results and Discussion

Gas chromatography analysis

To assess the presence the functional group in the sample obtained from distillation post bioethanol purification step, gas chromatography (Make: Analytical Technologies Ltd., Model:GC-2979N) analysis was performed, like other similar study[38]. The performed analysis is depicted in Fig. 2; wherein, peak position of bioethanol closely matches with standard ethanol, confirming ethanol presence. Likewise, for standard ethanol, bioethanol single sharp peak confirms separation of bioethanol by batch distillation in purest form.

Ceric ammonium nitrate test

To support ethanol presence in prepared sample, standard ceric ammonium nitrate test is performed. In this test, ceric ammonium nitrate solution was added to obtained sample post purification, leading to red colour appearance, which confirms presence of ethanol in the sample.

Dinitrosalicylic acid (DNS) test

A standard test was performed to assess the saccharification resulted for indirect measurement of starches or cellulose conversion into fermentable sugars during production of bioethanol[39]. With this test, presence of reducing sugars or free carbonyl group is detected. At first, oxidation of ketone and aldehyde functional groups present in different sugar types is facilitated. Thereafter, DNS is converted in 3-amino-5-nitrosalicylic acid in the alkaline condition. To begin DNS test, 3 mL of DNS reagent added with same proportion of glucose in a lightly capped test tube, followed by 5-15 min heating at 90°C, leading to red-brown colour formation; for colour stabilization, 1 mL of 40% potassium sodium tartrate solution is added, followed by room temperature cooling in cold-water bath, and recording with help of UV-visible spectrophotometer (Make: Shimadzu, Model: UV-1780, Country: Japan) at 575 nm. With 3% NaOH usage, spectrophotometer depicts 0.2955 absorbance alongside following specific parameter values: refractometer reading indicating a sugar concentration of 18%, a sugar concentration of 26.4 mg per 100 mL of filtrate, and a sugar concentration of 4.8 mg per gram of the sample.

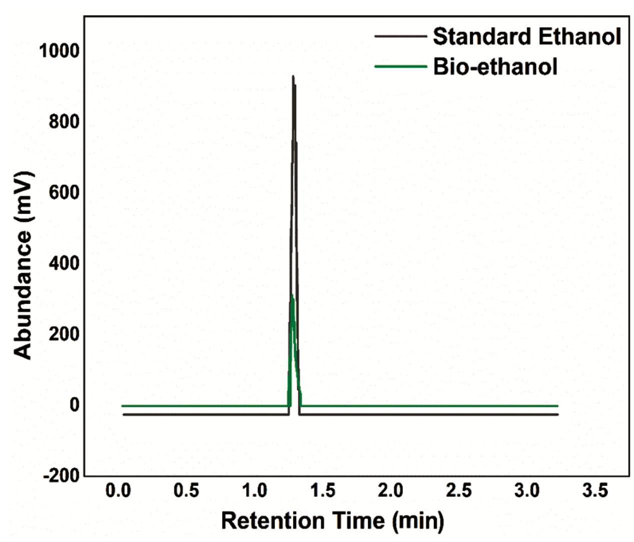

Fig. 2 — Gas chromatograph of bioethanol compared with standard ethanol

Yield analysis and comparison

The bioethanol yield is calculated as ratio of mass of bioethanol and mass of dried water hyacinth, represented using Eq. (1); in bioethanol mass calculation, its density (760 kg/m^3) is considered, which is close to ethanol. The bioethanol yield of current work is determined to be 9.36 (w/w). This yield is significantly higher than many values reported in recent literature, as shown in Table 1. The comparison considers studies from year 2010 to the present and demonstrates the effectiveness of the sequential process involving ultrasonic-assisted hydrolysis in this study.

$$Yield = (Mass\ of\ bio-ethanol\ produced)/(Mass\ of\ drid\ water\ Hyacinth) \quad \ldots (1)$$

Conclusion

In the current study, bioethanol is prepared from water-hyacinth, an invasive pollutant, employing ultrasonic treatment-cum-hydrolysis and fermentation. The collected and cleaned WH is converted into powder form. Before, basic hydrolysis, ultrasonic

Table 1 — Bioethanol yield compared with reported values from literature

S. No.	Process	Yield (w/w)	References
1	Alkali-treatment	0.09-0.21	40
2	Acid-treatment	0.23-0.42	41
3	Acid-treatment	0.30	42
4	Acid-treatment	0.23	28
5	Acid-treatment	0.42	17
6	Alkali-treatment	9.36	Current Study

treatment is performed. In next step, fermentation is performed, followed by purification to get bioethanol. In gas chromatography analysis, bioethanol peak coincides with ethanol, confirming ethanol production. Moreover, confirmatory analysis is performed to cross-check ethanol presence. Additionally, DNS test is also performed for degree of sugar formation from LCB, which depicts 18% free sugar content. A significant yield of bioethanol 9.36(w/w)is attained, which is high compared with literature reported values. In summary, ahigh bioethanol yield is possible with the employed bioethanol production method, which may have potential to meet future ethanol demand.

Conflict of Interest

The authors declare no conflict of interest.

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