

Bioethanol Production from Mixed Temple Floral Waste and Pomegranate Peel using Alkaline Pretreatment and Oxalic Acid Hydrolysis

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Abstract

The Recent demand for sustainable energy and the associated environmental issues of unprofitable disposal of organic waste have led to growing interest in the production of from lignocellulosic biomass. In this study, the waste materials of temple flowers and bioethanol pomegranate peels were explored as the low-cost, renewable materials for bioethanol production by an integrated biochemical conversion system. The floral waste from the temple, pomegranate peel, and a mixture of both in the ratio 1:1 were characterized initially by pH analysis, proximate analysis, Fourier Transform Infrared Spectroscopy (FTIR), and Scanning Electron Microscopy (SEM). The mixed biomass had favorable physicochemical properties, such as a pH of 4.76, moisture content of 10.8%, volatile matter of 68.5%, ash content of 1.4%, and fixed carbon of 19.3%, which would increase their suitability for biochemical conversion.

In order to make cellulose more accessible for hydrolysis, the 1:1 mixed biomass was pretreated with NaOH and characterized. The 1:1 biomass mixture was chosen for alkaline pretreatment with NaOH to improve the accessibility of cellulose due to the reduction in lignin and hemicellulose content. The peaks around 1730 cm^{-1} and 1590 cm^{-1} were markedly decreased after the pretreatment, indicating the removal of hemicelluloses and partial delignification. SEM measurements also showed the structural disruption and increased surface irregularity following pretreatment. Fermentable components were then extracted from the pretreated biomass by hydrolysis with oxalic acid under autoclave-assisted conditions. Neutralized hydrolysate was fermented with activated *Saccharomyces cerevisiae* under anaerobic conditions for 3 days. Rotary vacuum evaporator was used to remove the ethanol and confirmed by FTIR analysis and potassium dichromate assay. The potassium dichromate test showed a color change from orange to green, giving confirmation of the formation of ethanol. The amount of ethanol recovered from 60 g of mixed biomass was 30 mL, which is equivalent to an ethanol yield of 0.5 mL/g of biomass.

Highlights

- Sodium hydroxide treatment enhanced the accessibility of cellulose via partial lignin removal.
- Hydrolysis with oxalic acid facilitated the conversion of lignocellulosic biomass to fermentation substrates.
- The resulting mixed biomass yielded 30 mL of ethanol from 60 g of feedstock at an efficiency of 0.5 mL/g biomass.

Key Words:

Bioethanol; Temple flowers; Pomegranate peel; Lignocellulosic biomass; Sodium hydroxide pretreatment; Oxalic acid hydrolysis;

1 Introduction

With the global demand for energy surging and concerns over environmental impacts of fossil fuels, the demand for sustainable and renewable energy sources has grown in recent years [1, 2]. Global energy consumption is still largely dependent on fossil fuels, which have led to the excessive use of non-renewable energy sources and, as a consequence, to high greenhouse gas emissions, climate change, environmental pollution, and depletion of non-renewable energy resources [3-5]. The energy consumption has been further increased by the growth of industrialization, urbanization, and population throughout the world, which makes the need for cleaner and environmentally friendly energy systems [6, 7] more of the need of the hour. To meet these challenges, alternative fuels derived from renewable sources have become promising fuels that can help to lower carbon dioxide emissions and to advance the long-term supply security [8, 9].

In the list of available renewable fuels, bioethanol has attracted a great deal of attention due to its biodegradability, renewability, and compatibility with the existing fuel supply systems [10, 11]. Carbohydrate-rich biomass materials can be fermented to produce bioethanol, which can be used as an alternative or additive source of gasoline and will be considered as a sustainable option [12]. The use of bioethanol leads to reduced GHG emissions compared to the use of fossil fuels and increased environmental sustainability [13]. As a consequence, there has been growing interest in recent years in the production of second-generation bioethanol from lignocellulosic biomass, which does not compete for land and food and can provide for the better utilization of biomass waste [14–16].

Lignocellulosic biomass mainly consists of cellulose, hemicellulose, and lignin, which together form a rigid and complex structural matrix [17]. Polysaccharides like cellulose and hemicellulose are soluble and can be broken down to fermentable sugars, which can then be fermented to ethanol by the use of microorganisms like *Saccharomyces cerevisiae* [18, 19]. The presence of lignin, however, is an obstacle that impedes the access of enzymes and acids to cellulose fibers and, thereby, the direct biomass conversion efficiency [20]. Therefore, pretreatment is an important process that breaks the lignocellulosic structure, decreases the lignin amount, makes cellulose more accessible, and increases hydrolysis efficiency [21–23]. Several pretreatment methods (alkaline, acidic, physical, and biological methods) have been explored for efficient biomass conversion [24]. In these techniques, alkaline pretreatment with sodium hydroxide has been extensively reported to be effective in partial delignification and structural modification of lignocellulosic [25–27].

Organic Waste Biomass (OWB) is a vast underutilized renewable resource from

households, religious activities, food processing industries, and agricultural activities that can be utilized for producing bioethanol [28, 29]. One such organic waste generated in large quantities is the floral waste produced at temples during religious rituals and ceremonies [30]. Flower waste from improper disposal in open areas and water bodies is responsible for the formation of odor, microbial contamination, and pollution of the environment [31]. Likewise, the pomegranate peel is a significant fruit processing waste with high lignocellulosic and organic content, which is suitable for biofuel production [32, 33]. The ethanol production from these biodegraded wastes can be used effectively to produce bioethanol, which will help in the generation of renewable energy along with sustainable waste management [34].

There are several studies that have examined the conversion of agricultural residues, fruit peels, and lignocellulosic wastes to bioethanol using various pre-treatment and hydrolysis methods [35-37]. Cellulose and hemicelluloses have been extensively used for acid hydrolysis to obtain fermentable sugars [38]. Owing to its relatively low environmental impact and efficient biomass degradation ability, among various hydrolyzing agents, oxalic acid has attracted much attention [39, 40]. In addition, *S. cerevisiae* is one of the most widely used yeast species for ethanol production because of its ethanol tolerance, high fermentation efficiency, and industrial applicability [41, 42].

Though many studies on bioethanol production from agricultural and lignocellulosic wastes were carried out, few studies were reported on the utilization of mixed biomass feedstock of temple floral waste and pomegranate peel [43]. Furthermore, there are relatively few studies on the recovery of ethanol from these combined wastes involving sodium hydroxide pretreatment, oxalic acid hydrolysis, FTIR characterization, SEM analysis, fermentation, and ethanol recovery from the combined wastes [44–46]. Hence, the present study aimed to explore the sustainable bioethanol production of mixed floral waste collected from the temple along with pomegranate peel (PP) using alkaline pretreatment and oxalic acid hydrolysis.

2 Methodology

Recent In this study, sustainable bioethanol production using mixed temple floral waste and pomegranate peels via alkaline pretreatment, oxalic acid hydrolysis, and microbial fermentation was studied through an experiment. The entire process of the study includes biomass collection, biomass characterization, pretreatment, hydrolysis, fermentation, ethanol production, and product verification. The biomass used in the experiment includes temple floral waste collected from temples located nearby and pomegranate peels from local fruit wastes. Prior to further processing, these biomass waste materials were washed thoroughly with distilled water to eliminate the dust particles and then dried, ground, and sieved into small particle sizes for subsequent analysis [14, 15].



Fig. 1. Experimental workflow for bioethanol production from mixed temple floral waste and pomegranate peel

The overall experimental workflow followed for bioethanol production from mixed temple floral waste and pomegranate peel is shown in Fig. 1. First of all, three kinds of biomass samples, including temple floral waste, pomegranate peels, and their mixture at a 1:1 ratio, were characterized using methods such as pH value measurement, proximate analysis, Fourier Transform Infrared Spectroscopy (FTIR), and Scanning Electron Microscopy (SEM). Based on the initial test results, the most suitable lignocellulosic biomass was selected for further processing. Afterward, the sodium hydroxide pretreatment step was used to disintegrate the lignocellulosic matrix, lower the lignin and hemicellulose concentration, and facilitate the exposure of cellulose for hydrolysis [54, 58]. Changes in structure before and after the pretreatment process were studied by means of FTIR and SEM [64, 85]. The pretreated biomass was hydrolyzed via oxalic acid under the action of an autoclave to transform carbohydrates into fermentable compounds [62, 82]. Subsequently, the hydrolyzed mixture was neutralized, followed by fermentation with activated *Saccharomyces cerevisiae* under anaerobic conditions for three days [77, 89]. Rotary vacuum distillation was used for ethanol purification, and FTIR analysis along with the potassium dichromate assay was applied for confirming ethanol production [71, 93].

3 Sustainable utilization of temple floral waste and pomegranate peel for bioethanol production

This constant buildup of biodegradable organic waste generated by religious activities and fruit-processing industries has posed severe environmental management problems in the world today [28, 29]. The floral waste generated from the temples, ceremonies, and religious events is generally dumped in open dump sites or water bodies, which causes the water bodies to become polluted, the floral waste to cause bad odors, and the water bodies to become contaminated with microorganisms [30, 31]. Likewise, the pomegranate peel is a large amount of food waste from the fruit processing industry, where it is discarded as solid waste, but it contains substantial amounts of lignocellulosic and organic compounds [32]. These wastes can be converted into renewable bioethanol, providing an environmentally friendly solution in waste management and production of renewable energy in one step [33, 34].

Due to its renewable, cheap, and readily available characteristics, lignocellulosic biomass has emerged as an important feedstock for the second-generation bioethanol production [14, 16]. The cellulose, hemicellulose, lignin, phenolic compounds, and biodegradable carbohydrates present in the floral waste of temples and pomegranate peel can be converted to fermentable sugar using the proper pretreatment and hydrolysis techniques [17, 18]. The compact lignocellulosic structure and the presence of lignin, however, make it difficult to break down the cellulose fibers by direct enzymatic or acid hydrolysis [20]. Thus, proper pretreatment is necessary for better digestibility of the biomass and to increase the ethanol yield [21-23].

Sodium hydroxide (NaOH) alkaline pretreatment has been widely explored as an effective method for delignifying and hemicellulose and maintaining the cellulose structure among the available pretreatment techniques [24, 25]. The alkaline pretreatment involved in the process gives improvement in the surface porosity and accessibility of cellulose and makes it easy for the subsequent hydrolysis processes [54, 58]. The mixed temple floral waste and pomegranate peel were selected as feedstocks for the present study due to their balanced physicochemical properties with higher volatile matter, lower ash content, and good suitability for biochemical conversion [47]. For this reason, oxalic acid hydrolysis was considered as a more environmentally friendly method in comparison to strong mineral acids and was found to be capable of

hydrolyzing the lignocellulosic biomass to fermentable components [39, 62].

Table 1 Physicochemical significance of selected biomass feedstocks for bioethanol production

Biomass feedstock	Major components	Significance in bioethanol production	Environmental relevance
Temple floral waste	Cellulose, hemicellulose, lignin, organic matter	Provides fermentable lignocellulosic biomass suitable for hydrolysis and fermentation	Reduces environmental pollution caused by improper floral waste disposal
Pomegranate peel	Cellulose, pectin, phenolics, carbohydrates	Enhances organic content and supports fermentable sugar generation	Utilizes fruit-processing waste for renewable energy production
Mixed biomass (1:1)	Combined lignocellulosic and organic composition	Improves volatile matter and overall biochemical conversion efficiency	Supports integrated waste valorization and sustainable biofuel generation

Table 2 Advantages and limitations of selected biomass feedstocks

Feedstock	Advantages	Limitations
Temple floral waste	Abundantly available from religious activities; renewable and biodegradable; rich in lignocellulosic content	Seasonal variation in composition; high moisture content before drying
Pomegranate peel	Rich in organic matter and carbohydrates; low-cost biomass source; easily available from fruit waste	Presence of phenolic compounds may inhibit fermentation if not treated properly
Mixed temple floral waste and pomegranate peel	Balanced physicochemical properties; higher volatile matter; lower ash content; improved suitability for ethanol production	Requires pretreatment and hydrolysis for efficient sugar release

Production of bioethanol from mixed organic biomass is not only a way to enable the production of renewable fuels; it is also a contribution to the development of the circular bioeconomy and sustainable valorization of waste [36, 37]. The integrated utilization of the waste from the temple flowers and the pomegranate peels can be used for bioethanol production with a low production cost and can eliminate the environmental pollution caused by the inappropriate disposal of the flower and peel waste [60, 72]. In addition, *Saccharomyces cerevisiae* is easy to ferment and highly

tolerant of ethanol, which makes it highly productive and applicable for ethanol production in an industrial setting [41, 77]. Table 1 shows the physicochemical significance of temple floral waste and pomegranate peel for bioethanol production, and Table 2 summarizes the advantages and limitations of the selected biomass feedstocks.

The utilization of the mixed temple floral waste and pomegranate peel as lignocellulosic feedstocks is found to have significant potential in sustainable bioethanol production. They are highly effective in the conversion to renewable fuels and can be important in reducing waste, producing renewable energy, and developing environmentally sustainable bioprocessing technologies [74, 80].

4 Bioethanol production from mixed temple floral waste and pomegranate peel

Bioethanol is a liquid biofuel, which is mainly synthesized by using microbial fermentation of fermentable sugars obtained from biomass feedstocks [10, 16]. Lignocellulosic biomass has been given a lot of attention in recent years due to its widespread availability, biodegradability, and lack of competition with food resources for the production of the second generation of bioethanol [14, 45]. Alternative sustainable feedstocks derived from agricultural residues and fruit-processing wastes and floral wastes have come to the fore as replacements for the traditional sugar and starch feedstocks [20, 36]. The cellulose and hemicellulose present in the floral wastes of the temple and pomegranate peel, along with organic acids and biodegradable carbohydrates, can be pretreated, hydrolyzed, and fermented to convert them into ethanol [32, 42]. Using such organic wastes, in addition to producing renewable energy, can help in environmental management and waste valorization [29,39].

In the present study, the mixed lignocellulosic substrates of temple floral waste and pomegranate peel were investigated for production of bioethanol. Initial characterization suggested that the properties of the combined biomass were favorable regarding the physicochemical characteristics, with a more balanced acidic pH, a higher volatile matter, lower ash content, and improved biochemical conversion potential [47]. To increase the accessibility of cellulose to hydrolysis, the cellulosic cell wall structure was broken up through sodium hydroxide pretreatment [54, 58]. Thereafter, the biomass was hydrolyzed into fermentable components for microbial fermentation under the autoclave-assisted conditions using oxalic acid [62, 82]. The yeast, activated *Saccharomyces cerevisiae*, was used for the ethanol fermentation due to its high ethanol tolerance, fermentation efficiency, and industrial applicability [77, 99].

4.1 Characteristics of bioethanol

The major component of bioethanol is ethyl alcohol (C_2H_5OH), which has some fuel properties that make it a suitable renewable fuel alternative to conventional fossil fuel [40]. Bioethanol possesses a higher octane number than gasoline, which in turn gives better knock-free combustion in engines [41]. It also has oxygen in its molecular structure, which promotes cleaner combustion and reduces the emission of harmful gases like CO and PM [44]. Bioethanol has, however, a lower energy density than gasoline, which results in lower mileage of vehicles fueled with high ethanol blends [40]. Table 3 summarizes and specifies the important physicochemical properties of bioethanol and their implications for fuel applications.

Table 3 Key properties of bioethanol and their implications in fuel performance

Property	Value/Characteristic	Implication for fuel performance
Chemical formula	C ₂ H ₅ OH	Renewable alcohol fuel produced from biomass
Octane rating	108-112	Enhances combustion efficiency and reduces engine knocking
Energy density	~21-23 MJ/L	Lower than gasoline, resulting in reduced mileage
Oxygen content	~35%	Improves combustion and lowers harmful emissions
Boiling point	78.5 °C	Supports efficient vaporization during combustion
Water solubility	Fully miscible	May absorb moisture during storage
Renewability	Produced from biomass feedstocks	Reduces dependence on fossil fuels
Carbon emissions	Lower than gasoline	Supports reduction of greenhouse gas emissions

4.2 Production of bioethanol from lignocellulosic biomass

The second generation of bioethanol production uses the hydrolysis of lignocellulosic biomass into fermentable sugars and then microbial fermentation [14, 45]. Cellulose and hemicellulose of the temple floral waste and pomegranate peel can be hydrolyzed to simple sugars that can be used for ethanol production [17, 18]. Lignocellulosic biomass, however, has a rather rigid structure of lignin, which makes it difficult to access the cellulose for hydrolysing agents [20]. Thus, the pretreatment process is deemed to be an essential process for enhancing the efficiency of hydrolysis and ethanol production [21–23].

In the present study, partial delignification and hemicellulolysis were achieved by sodium hydroxide treatment [54]. The results of FTIR analysis following the pretreatment showed that the peaks corresponding to hemicellulose and lignin-associated peaks at 1730 cm⁻¹ and 1590 cm⁻¹, respectively, were found to be reduced, which resulted in structural modification of the biomass [64]. The pretreated biomass carbohydrates were then subjected to oxalic acid hydrolysis to obtain fermentable components in an autoclave-assisted process [62]. The resulting hydrolysate was neutralized and fermented with the help of activated *Saccharomyces cerevisiae*, under anaerobic conditions, for three days [77]. The ethanol recovery was performed with rotary vacuum evaporation, while the FTIR analysis and potassium dichromate assay confirmed the ethanol formation [71].

4.3 Environmental impact and sustainability

The production of bioethanol from the floral waste of temples and pomegranate peel has a great environmental impact since it uses biodegradable organic waste, which is converted into renewable energy [28, 39]. Inadequate disposal of temple floral waste into any river, lake, or open dumping site results in environmental pollution, microbial contamination, and generation of foul odors [30, 31]. In the same manner, fruit-processing wastes like pomegranate peels generate huge quantities and are frequently not effectively utilized [32].

Bioethanol from their conversion helps to achieve sustainable waste management and minimizes environmental impact caused by waste disposal of organic origin [60]. The combustion of bioethanol emits fewer greenhouse gases and particulate pollutants than the combustion of fossil fuels [48, 49]. Bioethanol production has a relatively balanced carbon cycle because it sequesters CO₂ in the atmosphere during plant growth. Moreover, waste-derived lignocellulosic biomass is not in competition with food resources, and it helps the development of a circular bioeconomy [36]. Therefore, renewable biomass conversion technologies can have a significant contribution toward providing sustainable energy generation and environmental protection [80].

4.4 Applications of bioethanol

In addition to its use for fuel, bioethanol has various industrial and energy applications because of its renewability and environmental friendliness [54]. It is well established as an additive for gasoline that enhances combustion and lowers carbon emissions in E10 and E85 gasoline blends [44]. Bioethanol can also be used in the pharmaceutical, cosmetic, disinfectant, industrial solvents, and chemical industries (other than fuel applications) [55-57]. Bioethanol is used for cooking and heating in rural and developing areas as a clean fuel [58].

The bioethanol from the temple floral waste and pomegranate peel shows that biodegradable waste material can be used to produce renewable energy. Lignocellulosic biomass residue produced during the hydrolysis and fermentation process can also be used for biofertilizer preparation, composting, or biofuel generation (such as biogas), which will enhance both the fuel and fertilizer value of biomass [87]. A summary of the different uses of bioethanol and bioconversion processes is shown in Fig. 2.

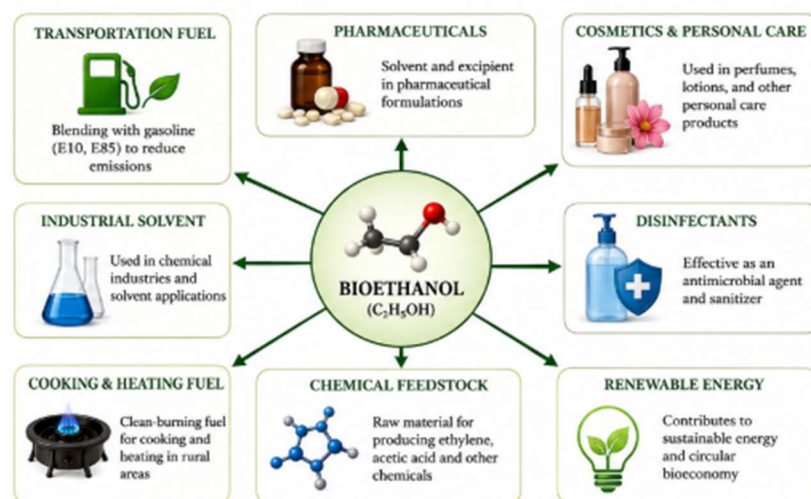


Fig. 2. Applications of bioethanol produced from lignocellulosic biomass

4.5 Pretreatment of lignocellulosic biomass

It is known that one of the most critical steps in the production of bioethanol from lignocellulose is the pretreatment, which increases the accessibility of the cellulose and hemicellulose for hydrolysis [60]. Lignin has a compact structure that limits the high efficiency of enzymatic and acid hydrolysis, which results in low efficiency of sugar conversion [20]. Various pretreatment techniques have been developed to convert biomass, including physical, chemical, thermal, biological, and enzymatic [61]. Sodium hydroxide alkaline pretreatment was chosen in this study due to its ability to partially delignify and remove hemicelluloses [54] from the biomass. Alkaline pretreatment changes the structure of the biomass surface, enhances porosity, and

makes cellulose easily accessible [58]. From SEM characterization of the untreated mixed biomass, it was seen that the biomass structure consisted of irregular lignocellulosic particles with low porosity [35]. In the case of sodium hydroxide pretreatment, there was observed disruption of biomass structure with higher accessibility of cellulose fibers, hence making it suitable for hydrolysis [64]. To further facilitate the conversion of pretreated biomass into fermentable components, oxalic acid hydrolysis was performed [62]. The entire process of pretreatment and hydrolysis is illustrated in Fig. 3.



Fig. 3. Process of pretreatment and hydrolysis for bioethanol generation from mixed biomass

4.6 Fermentation process

It is a process by which microorganisms produce ethanol and carbon dioxide under anaerobic conditions using fermentable sugar [65]. Several fermentative microorganisms have been used for ethanol production, but *Saccharomyces cerevisiae* is commonly employed due to its ability to tolerate high ethanol levels and its fast fermentation process [41, 77]. In the current work, activated *Saccharomyces cerevisiae* was introduced to the oxalic acid hydrolysate after neutralization and fermentation was carried out for 3 days.

Observation of bubbles, alcohol smell, and precipitation indicated that fermentation was taking place [47]. Factors affecting the efficiency of fermentation include substrate composition, pH, nutrients, temperature, and microbial action [45, 70]. Although lignocellulosic hydrolysate might have some inhibitors because of pretreatment and hydrolysis, proper neutralization and fermentation process control could lead to increased ethanol yield [71–75]. Production of ethanol from mixed floral temple waste and pomegranate peels shows the viability of using biodegradable biomass waste as an alternative energy source [96, 113].

5 Optimization process

Optimization is also important to enhance the efficiency, sustainability, and ethanol yield of lignocellulosic bioethanol production systems [74]. In the optimization process, the selection of biomass feedstocks and the improvement of pretreatment efficiency, hydrolysis and fermentation conditions, and the effective utilization of process byproducts are all key considerations [55, 66]. In the last few years, it has been shown that with optimized pretreatment and fermentation conditions, ethanol yield from lignocellulosic biomass can be significantly enhanced [58, 75]. Effective pretreatment technologies, environmentally friendly hydrolytic processes, and microbial fermentation processes are also part of this integration, which helps to minimize waste production and to support the sustainable production of renewable fuels [57, 68].

The main emphasis of the present study was to optimize the use of a suitable mixture of biomass, pretreatment with NaOH, hydrolysis with oxalic acid, and fermentation with *Saccharomyces cerevisiae*. The pH analysis, proximate analysis, FTIR, and SEM preliminary characterization confirmed that the 1:1 ratio of temple floral waste and pomegranate peel had better physicochemical properties than the individual substrates [47]. The favorable volatile matter, balanced acidic pH, low ash content, and increased

biochemical conversion potential of the mixed biomass were the reasons for selecting it for further processing [79]. Sodium hydroxide pretreatment was tuned to partially remove lignin and hemicellulose barriers to enhance the hydrolysis of cellulose [54, 58].

5.1 Biomass selection and pretreatment optimization

One of the most important factors that affect the efficient production of bioethanol is specific selection of the appropriate lignocellulosic biomass [78]. In the present study, floral waste of temples and pomegranate peel were selected because of their abundant availability, renewability, and high lignocellulosic content [30, 32]. The 1:1 mixed biomass was found to have better physicochemical properties than individual substrates, which means that the mixed biomass is more suitable for hydrolysis and fermentation [47].

Sodium hydroxide was used as pretreatment for optimization, as it has been reported to be able to break the structure of lignocellulosic and increase the exposure of cellulose [54]. However, FTIR analysis after pretreatment in Fig.4. shows that the lignin and hemicelluloses-associated peaks at 1730 cm^{-1} and 1590 cm^{-1} decreased, which indicates the successful structural modification of the biomass [64]. SEM analysis has also shown that the surface becomes more irregular and porous following the pretreatment, thereby improving the hydrolysis accessibility [85]. This optimized pretreatment process thus enhanced the overall suitability of the biomass for oxalic acid hydrolysis and fermentation.

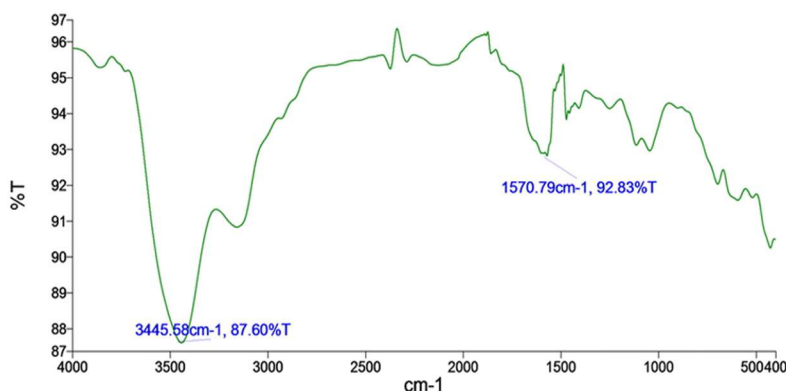


Fig.4. FTIR analysis after NaOH pretreatment of mixed biomass

From the results obtained from the FTIR analysis, it can be seen that there have been changes in the chemical composition of the mixture biomass, especially after the addition of NaOH. This can be evidenced by the peak at about 1730 cm^{-1} , which shows that there is no presence of the hemicellulose group, and the peak at about 1590 cm^{-1} , which denotes that there is partial removal of the lignin group. Besides, the cellulose peak ranging from 1050 to 1100 cm^{-1} was not affected.

Table.4. Major FTIR peaks observed after NaOH pretreatment

Peak region	Interpretation
Around 1730 cm^{-1}	Reduction indicates hemicellulose removal

Peak region	Interpretation
Around 1590 cm^{-1}	Reduction indicates partial lignin removal
1050-1100 cm^{-1}	Cellulose-related peak retained

5.2 Optimization of hydrolysis and fermentation

To achieve efficient ethanol production from the lignocellulosic biomass, hydrolysis and fermentation processes must be efficient [84]. In the present work, hydrolysis was carried out using oxalic acid, as it is a relatively environmentally friendly organic acid that can be used to hydrolyze cellulose and hemicellulose to fermentable components [62]. Elevated temperature and pressure conditions were used to break down the biomass by autoclave-assisted hydrolysis [82].

After hydrolysis, the hydrolysate was neutralized to ensure optimal growth and fermentation by the yeast [71]. Activated *Saccharomyces cerevisiae* was used because of its high tolerance to ethanol, high fermentation rate, and industrial applicability [77]. The fermentation had been practiced for 3 days under anaerobic conditions to get the maximum ethanol production. The gas generation was observed, an alcohol-like smell was detected, and the broth color changed, indicating active microbial fermentation [47]. The optimum fermentation conditions led to the successful production of ethanol from mixed biomass feedstock.

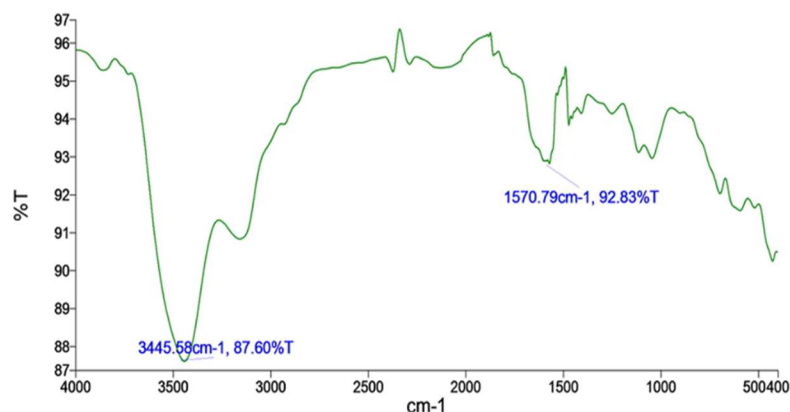


Fig.5.FTIR analysis of recovered ethanol

The FTIR analysis was conducted for the recovered distillate to verify the existence of functional groups related to alcohol and to prove that the distillate is composed of ethanol. In the spectrum of the recovered distillate, Table 5 shows that there exists a broad band with wavenumber 3445.58 cm^{-1} , which indicates the existence of O-H bond stretching vibration, implying the existence of a hydroxyl group in the compound. There is another sharp band with wavenumber 1570.79 cm^{-1} , which shows the existence of organic group vibrational motion.

Table 5 FTIR peak interpretation of recovered ethanol

Observed peak	Functional group	Interpretation
3445.58 cm ⁻¹	O–H stretching	Indicates alcohol-related hydroxyl group
1570.79 cm ⁻¹	Organic functional group vibration	Supports presence of organic compound in recovered distillate

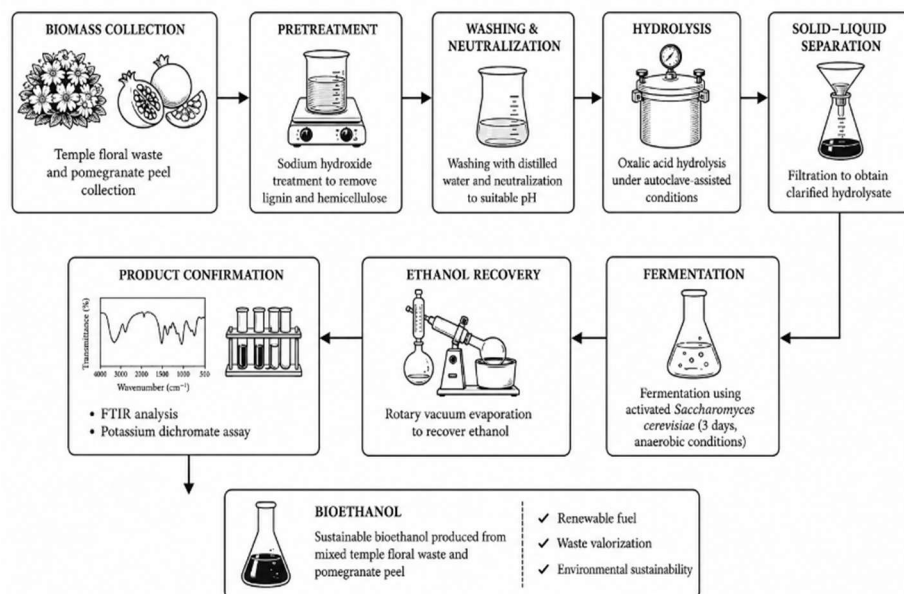


Fig. 6. Flowchart of bioethanol production process from mixed temple floral waste and pomegranate peel

The overall process followed for bioethanol production from mixed temple floral waste and pomegranate peel is summarized in fig.6.

5.3 By-product utilization and waste management

The effective utilization of biomass residues and process by-products is a key factor in the better sustainability of bioethanol production systems [87]. Converting temple floral waste and pomegranate peel into bioethanol not only minimizes the environmental impact of improperly disposed organic waste but also helps preserve the natural environment. The utilization of temple floral waste and pomegranate peel in bioethanol production is a significant factor in reducing the environmental impact of improper disposal of organic waste and also contributes to the conservation of the natural environment [29, 60]. Residual biomass after hydrolysis and fermentation can also be used for composting, anaerobic digestion, or biofertilizer production and so contributes to the development of a circular bioeconomy [88].

The utilization of biodegradable wastes for renewable energy generation also helps to alleviate open dumping and environmental pollution [31]. Moreover, by valorizing the agricultural and organic residues into bioethanol, new economic possibilities are provided, and sustainable waste management plans are promoted [89].

5.4 Sustainability and environmental assessment

The production of bioethanol must rely on suitable feedstock and efficient processes, while minimizing waste generation, for environmental sustainability [49]. When burning fossil fuels, bioethanol causes fewer greenhouse gas emissions and a lower amount of particulate pollutants [48]. It is essential to use renewable organic waste like temple floral waste and pomegranate peel for ethanol production to overcome the competition with food crops and to promote environmentally friendly conversion of biomass [36].

In the present study, it was found that biomass conversion efficiency was enhanced along with the effective degradation of lignocellulosic using sodium hydroxide pretreatment and oxalic acid hydrolysis [58, 62]. Furthermore, waste biomass conversion to renewable fuels helps reduce environmental pollution of temple and fruit processing waste [39]. Therefore, the use of integrated biomass conversion technologies can be used to promote renewable energy generation, waste reduction, and circular resource utilization [90].

5.5 Economic feasibility and future prospects

The economic feasibility of bioethanol production can be greatly enhanced by the use of low-cost waste biomass feedstocks [95]. The floral waste from the temple and pomegranate peel are ever-available biodegradable waste, which is of no commercial value before processing [30, 32]. They can thus be used as raw material for bioethanol production and lower raw material costs while at the same time providing waste management.

In the present research, the production of bioethanol from mixed biomass was achieved successfully, but the hydrolysis conditions, recovery of sugar, and efficiencies of the fermentation can be optimized for higher yields of ethanol at a larger scale [97]. Product characterization and process evaluation can be further improved using advanced analytical methods, like high-performance liquid chromatography (HPLC) and gas chromatography (GC) [103]. In the future, further research on process scale-up, continuous fermentation systems, and integrated biorefinery approaches could enhance the industrial potential of bioethanol production from temple floral waste and pomegranate peel [114, 115].

6 Emerging research trends and future opportunities in bioethanol production from temple floral waste and pomegranate peel

Producer gas is an alternative energy source, and with the growing demand for renewable energy and sustainable waste management, research on the production of lignocellulosic bioethanol from biodegradable waste biomass has gained momentum [1, 14]. The potential of the organic residues, like wastes from the temple floral offerings and fruit processing wastes, has received a great deal of attention due to their renewability, abundance in supply, and environmental importance [29, 30]. Conversion of these waste materials into bioethanol is a step towards advancing a circular bioeconomy and, at the same time, mitigating environmental pollution due to unauthorized waste disposal [36, 39]. The research areas of bioethanol production today are primarily directed towards novel biomass utilization, improved pretreatment methods, optimization of fermentation processes, utilization of by-products, assessment of the environmental impact, and scaling up processes to industrial scales [57, 74].

In the present study, the preparation of bioethanol using mixed temple floral waste and pomegranate peel by sodium hydroxide pretreatment and oxalic acid hydrolysis was successfully demonstrated. The process was shown to be efficient for ethanol production, but other developments in biomass conversion technologies, fermentation optimization, and integrated biorefinery processes could enhance process efficiency and its industrial application [58, 75]. In the next sections, emerging research opportunities related to the waste from temple flowers and pomegranate peels are discussed.

6.1 Innovative biomass utilization for bioethanol production

The use of biodegradable wastes as a lignocellulosic raw material is emerging as an area of research with great interest in the production of renewable energy [45]. Temple floral waste produced in religious activities and pomegranate peel produced in fruit processing industries are sources of biomass with a high potential for biochemical conversion but have low costs. In this way they can be effectively used for bioethanol production, which means they can be used instead of edible feedstocks, thus decreasing the amount of waste in the

environment [36].

The applications of agricultural residues, fruit peels, and floral wastes for the production of the second generation of bioethanol have been discussed in several studies [35, 42]. However, only a few studies have concentrated on the combined use of the floral waste from temples along with pomegranate peel as combined biomass substrates [43]. Optimization of biomass ratios and co-substrate combinations, nutrient supplementation, and integrated pretreatment strategies for enhanced fermentable sugar release and ethanol yield could be examined in future studies. Furthermore, for industrial applications, variations in biomass composition over the seasons and the presence of large-scale biomass collection systems should be explored [103].

6.2 Improvements in pretreatment and bioprocessing technologies

The pre-treatment and hydrolysis are also key steps in the lignocellulosic bioethanol production process, as they have a direct impact on cellulose accessibility and generation of fermentable sugars [60]. The present study showed that sodium hydroxide pretreatment was able to break down the lignin and hemicellulose barriers, and oxalic acid hydrolysis was effective in biomass degradation under autoclave-assisted conditions [54, 62]. While successful, further optimization of the alkali concentration, hydrolysis temperature, pressure, and time could result in higher yields of ethanol [75].

The recent bioprocessing technology developments such as consolidated bioprocessing (CBP), simultaneous saccharification and fermentation (SSF), and co-fermentation systems have been demonstrated to be potential methods for enhancing the efficiency of lignocellulosic bioethanol production [68, 76]. In addition, microbial strains are being engineered to improve their inhibitor tolerance and ethanol productivity, and synthetic biology techniques could also be used to create microbial strains with enhanced inhibitor tolerance and improved ethanol productivity [74]. More sophisticated analytical methods, including High-Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), and Nuclear Magnetic Resonance (NMR), could be used to further reinforce biomass characterization and ethanol purity assessment [104].

6.3 Value-added products and by-product utilization

The use of the biomass residues and by-products in a sustainable manner is gaining in significance to make bioethanol production systems economically more feasible [87]. In the current study, a solution has been developed to transform mixed floral waste from temples as well as pomegranate peel into renewable bioethanol without causing environmental pollution due to the disposal of organic waste [60]. The residual biomass after hydrolysis and fermentation can, however, be further used for the production of compost, biofertilizers, biogas, activated carbon, and biochar [88].

Incorporation of bioethanol production and value-added byproduct production can have a significant improvement in the process sustainability and resource utilization efficiency [102]. Table 6 shows the possible value-added products and future prospects of utilization of mixed temple floral waste and pomegranate peel biomass.

Table 6 Potential value-added products from temple floral waste and pomegranate peel biomass

Biomass/Residue	Current application	Potential value-added products	Environmental significance
Temple floral waste	Bioethanol production	Compost, biochar, biogas, organic fertilizer	Reduces floral waste pollution and open dumping
Pomegranate peel	Bioethanol production	Antioxidant extraction,	Supports fruit waste

Biomass/Residue	Current application	Potential value-added products	Environmental significance
		biofertilizer, activated carbon	valorization and waste minimization
Fermentation residue	Residual biomass after ethanol recovery	Animal feed supplement, compost, anaerobic digestion substrate	Improves circular resource utilization
Lignocellulosic hydrolysate by-products	Process residue	Industrial enzymes and biochemical precursors	Supports integrated biorefinery development

6.4 Sustainability assessment and economic feasibility

Feedstock availability, process efficiency, waste management, and energy consumption are important factors in the environmental sustainability of bioethanol production [49]. Bioethanol produced from lignocellulosic biomass is a promising alternative to the fossil fuels that can drastically lower GHG emissions and environmental pollution [48]. The use of floret waste from the temples and pomegranate peel does not compete with the food crops and supports sustainable biomass conversion pathways [36].

However, the economic viability of a large-scale bioethanol production technology is also a key consideration for large-scale implementation [95]. The utilization of temple floral wastes and pomegranate peels as raw material can be reduced due to their ample availability as low-cost wastes [30, 32]. But for industrial-level bioethanol production, optimization of bioethanol production costs, hydrolysis efficiency, ethanol recovery systems, and the transportation of biomass logistics may be needed [105]. Further research can be conducted using techno-economic analysis and life cycle assessment (LCA) to gain further understanding into the commercial viability and environmental sustainability of mixed biomass bioethanol production systems [90].

6.5 Interdisciplinary and collaborative research approaches

The future development of bioethanol from lignocellulosic will need interdisciplinary teamwork between scientists, industry, environmental agencies, and policymakers [106]. Advanced characterization of biomass, microbial engineering, and optimization of the processes may be provided by research organizations, while pilot-scale implementation and commercialization strategies can be provided by industries [107]. Sustainable use of biodegradable biomass as energy fuel can be further encouraged by governmental policies and regulations on renewable energy, waste management, and financial support [96].

The industrial applicability of bioethanol production from temple floral waste and pomegranate peel could be significantly improved for a collaborative research project encompassing integrated biorefineries, renewable energy systems, waste valorization technologies, and green chemistry approaches. These interdisciplinary endeavors can be part of large-scale sustainable energy production, protection of the environment, and development of a circular bioeconomy [115].

7 Research in bioethanol production

In recent years, there has been considerable research on the production of lignocellulosic bioethanol from biodegradable waste biomass for the generation of renewable energy [14, 45]. The low cost, renewability, and reduced nature of competition with food resources have made agricultural residues, fruit-processing wastes, and floral wastes all attractive

second-generation bioethanol feedstocks [20, 36]. Specifically, the conversion of waste from temple flowers to bioethanol and pomegranate peels to bioethanol is gaining momentum due to the ecological issues resulting from their improper disposal and the need for sustainable bioethanol technologies [30, 32].

The development of better pretreatment and hydrolysis technologies that can improve the accessibility of the cellulose and that can generate fermentable sugars is one of the greatest advances in bioethanol research in recent years [58, 75]. Sodium hydroxide treatment has been proven to be very efficient in lowering the lignin and hemicellulose barriers and enhancing biomass digestibility [54]. Likewise, environmentally friendly biomass conversion methods like oxalic acid hydrolysis have been shown to be promising for the conversion of biomass as compared to strong mineral acid systems [62]. This study demonstrates that sodium hydroxide pretreatment and oxalic acid hydrolysis are an effective combination to enhance the conversion of lignocellulosic biomass and also as an effective means to produce ethanol from mixed temple floral waste and pomegranate peels.

8 Future research directions for bioethanol generation

The further studies on the production of bioethanol from temple floral waste and pomegranate peel should be directed towards the enhancement of biomass conversion efficiency, increasing ethanol yield, process sustainability, and scale-up of the processes for industrial production [74]. The current study has successfully proved bioethanol production from sodium hydroxide pretreatment, oxalic acid hydrolysis, and *Saccharomyces cerevisiae* fermentation, but there are still several opportunities for the advancement of the technology and optimization of the process [75].

The optimization of the pretreatment and hydrolysis conditions to maximize the amount of fermentable sugars that can be released from the lignocellulosic biomass is a major future research direction [60].

Another important direction for the future is to integrate advanced analysis tools to enable accurate process monitoring and product characterization [104]. The information that can be gathered from techniques like Gas Chromatography (GC), High-Performance Liquid Chromatography (HPLC), Nuclear Magnetic Resonance (NMR), and Mass Spectrometry (MS) can give detailed information on the composition of sugars, ethanol purity, inhibitor concentration, and fermentation efficiency [103]. These analytical developments will help to develop a better understanding of biomass conversion pathways and optimize the processes.

Additionally, cross-disciplinary research and development efforts involving academia, industries, policymakers, and environmental groups will greatly facilitate the further development of lignocellulosic bioethanol technologies [106]. Renewable energy policies and incentives for using biomass and sustainable waste management systems can promote the uptake of bioethanol production systems based on biodegradable organic wastes [96]. In the future, the use of sustainable biofuel technologies derived from temple floral waste and pomegranate peel can play a significant role in achieving sustainable energy generation, environmental protection, and the development of a circular bioeconomy [115].

9 Conclusion and recommendations

In the present study, the sustainable production of bioethanol from mixed temple floral waste and pomegranate peel was successfully achieved by using sodium hydroxide pretreatment, oxalic acid hydrolysis, and microbial fermentation using *Saccharomyces cerevisiae*. Biodegradable temple and fruit processing wastes are potential sources of lignocellulosic feedstocks that can be utilized for generating renewable energy in an environmentally sustainable way and also for tackling the challenges of organic waste disposal. The pH analysis, proximate analysis, FTIR, and SEM were used for preliminary

characterization and confirmed that the 1:1 mixed biomass had optimum physicochemical properties for biochemical conversion and ethanol production.

Sodium hydroxide pretreatment was found to be effective in loosening the lignocellulosic structure, thus facilitating the accessibility of cellulose for hydrolysis in the absence of lignin and hemicellulosic barriers. The subsequent hydrolysis in the presence of oxalic acid performed under autoclave-assisted conditions helped convert biomass polysaccharides to fermentable entities. By using activated *Saccharomyces cerevisiae*, ethanol was successfully produced using fermentation, and the final ethanol yield was 30 mL/60g mixed biomass, which is 0.5 mL/g biomass. This was further verified by FTIR analysis and $K_2Cr_2O_7$ assay.

The results obtained from the present study indicate that the temple floral waste and pomegranate peels have great potential as low-cost and renewable substrates for second-generation bioethanol production. Integrated waste biomass utilization as renewable fuel helps in sustainable waste management, decrease of environmental pollution, and development of a circular bioeconomy. Further investigations based on advanced analytical characterization and reducing sugar estimation, large-scale process optimization, and techno-economic assessment are needed for improving industrial feasibility and commercialization potential. In conclusion, the study illustrates that eco-friendly pretreatment and hydrolysis processes to valorize lignocellulosic biomass could be an effective way for sustainable production of renewable energy sources and future green biofuels.

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Author contributions

Developed the review framework, performed an in-depth literature review, and thoroughly analyzed advancements, challenges, and trends in bioethanol production from diverse feedstocks. Drafted the initial manuscript, created visual representations to highlight key findings, and refined the content to ensure precision, coherence, and academic quality. Additionally, verified the accuracy of the information, oversaw the manuscript's structure, and granted final approval for submission to Discover Biomass Conversion and Biorefinery.

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Data availability

The datasets generated and analyzed during the current study, including biomass characterization data, proximate analysis results, FTIR spectra, SEM observations, pretreatment and hydrolysis records, fermentation observations, and ethanol yield data, are available from the corresponding author upon reasonable request.

Declarations

Consent to publish

Not applicable.

Ethics and consent to participate declarations

Not applicable.

Competing interests

The authors declare no competing interests.

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