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Acid Hydrolysis of the wastes of *Opuntia Ficus-Indica (L.)* Miller in order to produce Bioethanol

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Abstract: *Opuntia ficus-indica* (L.), also referred to as prickly pear or nopal cactus, is commonly utilized in food, medicine, and cosmetics. Typically, cactus pear waste is discarded in nature or fed to cattle. It is composed mainly of water, cellulose, hemicellulose, pectin, extractives, ash, and lignin. Previous studies have indicated that the wastes of cactus pears have a high sugar yield which converts by fermentation into bioethanol. However, this process does not occur naturally; it needs physical pretreatment, acid hydrolysis, enzymatic hydrolysis, and fermentation. Acid hydrolysis pretreatment is necessary because it helps break the interchain linkages in hemicellulose and cellulose. This research aims to determine the optimal conditions for acid hydrolysis of cactus pear wastes using sulfuric acid and hot water to release total reducing sugars. This optimization resulted in 0.121 mol/l of total reducing sugars (TRS) after 20 minutes of reaction time with a 3% sulfuric acid solution at 121°C and a solid-liquid ratio of 1:10. The following were the best conditions for saccharose release: 1,561 mol/l saccharose, 15 minutes of reaction time, 3% sulfuric acid solution, 121°C temperature, and a solid ratio of 1:10.

Keywords: Bioethanol; Cactus Pears; Hydrolysis; Acid-Hydrolysis; Opuntia Ficus-Indica (L.); Biomass

1. Introduction

The considerable depletion of fossil energy supplies, the related costs, and the legislative constraints of sustainable development are the key motivations for the search for new natural and renewable resources. Bio-refineries generate bioethanol, bio-methanol, vegetable oils, biodiesel, biogas, biosynthetic gas (bio-syngas), bio-oil, bio-char, Fischer–Tropsch liquids, and biohydrogen (Balat. 2010). Alternatively, lignocellulosic biomass is a suitable, affordable, and safe substrate with tremendous promise for biorefineries (Kumar *et al.*, 2009). Green plants generate around 170 Gtons of dry biomass per year, of which 10 Gtons per year will be utilized for energy purposes by 2050 (representing 15% of the world's total energy requirement) (Lens *et al.*, 2005).

Despite their vast use, cactus crops are solely utilized to cultivate and sell prickly pears. In ideal conditions, the *Opuntia ficus-indica* species can produce more than 30 tons of dry matter per hectare per year (Ennouri *et al.*, 2006). Moroccan prickly pears (*Opuntia ficus-indica (L.) Mill.*), which have an average sugar content of around 140 g/kg of fruit, are mature and ready for harvest from July through August (Es-sbata *et al.*, 2022). This herbaceous feedstock has a low lignin content compared to more woody feedstocks, reducing its resistance to hydrolysis and saccharification and reducing processing costs (Yang *et al.*, 2010). Physical pretreatment, thermo-degradation, acid pretreatment, and enzymatic pretreatment are the steps involved in converting Opuntia ficus-indica biomass to bioethanol (Retamal *et al.* 1987, Kuloyo *et al.*, 2014, Pérez-Cadena *et al.*, 2018), by increasing the release of fermentable sugars, which are then converted into ethanol (Kuloyo *et al.*, 2014).

The physicochemical properties of cactus pear (*Opuntia ficus-Indica*), which seem to vary by geography, have been the subject of several research studies (Kadda *et al.*, 2022). Kadda *et al.* pointed out that the temperature increase increased the yields without any change in physicochemical properties as well as fatty acids, and tocopherols. O. dillenii fruits are a promising source for the isolation of novel compounds with antibacterial or antidiabetic activities as abundant phytochemicals which molecular docking simulations indicated their activity against human pancreatic α-amylase enzyme (Loukili *et al.*, 2022). Nanoparticles based on a new biocomposite of Hydroxyapatite and pectin, obtained from O. ficus-indica exhibited a high affinity for a variety of antimicrobial activity against both Gram-negative and Gram-positive bacteria, and fungi (Saidi *et al.*, 2022).

According to a previous study (Kim *et al.*, 2006), *Opuntia ficus-indica (L.)* contains 12-15% sugars, 0.6% protein, and 0.1% lipids; 2200 ppm calcium, 490 ppm potassium, and 850 ppm magnesium. The second research (Nebbache *et al.*, 2010), found that *Opuntia Ficus-Indica (L.)* has an average of 84.14% water in the pulp and 90.33% water in the skin, along with 29% glucose and 24% fructose. However, saccharose levels in the pulp (0.19%) were much lower than in the skin (2.25%). The seed has absolutely no sugar. Potassium was rich compared to other minerals in all three fruit sections.

According to (Dehbi *et al.*, 2014), the moisture content of cactus fruit juice ranges from 89.13% to 91.18 %, with total soluble solids (TSS) ranging from 11.33°Brix to 15.47°Brix. The pH scale runs from 5.45 to 5.92. The pulp's acid value varied from 0.46 to 0.98%. The total sugar content of the pears was 154.16 g/kg, while the reducing sugar content was 149.16 g/kg. We may conclude from these studies that *Opuntia ficus-indica* possesses a large amount of sugars that can be converted into bioethanol using several pretreatment methods, including physical pretreatment, acid pretreatment, and enzymatic pretreatment. In the field of biomass pretreatment, acid hydrolysis is a pioneering and commonly used technique. Due to its low cost and effectiveness in hydrolyzing hemicellulose fractions into monomeric components while generating structural changes in solid fractions for better enzyme accessibility and cellulose conversion (Zheng *et al.*, 2014), researchers and industrials recommend this method over other pretreatments. In recent work, Opuntia Ficus Indica (OFI) cladodes slow pyrolysis conducted in a fixed-bed reactor at various temperature (500–600–700 °C) and residence time (30–60–120 min) with a constant heating rate (10 °C min^{-1}) provide the highest biochar yield (40.08 %). The produced biochar is an alkaline carbon rich with minerals and nutrient (N, P, K, Ca, Mg) that could be applied in agriculture to balance acidic soil. This integrated approach of local OFI biomass conversion into biofuels and bio-based materials is an opportunity for the implementation of a local circular economy (Maaoui *et al.*, 2023).

In this research, the best conditions for the full acid hydrolysis of *Opuntia Ficus-Indica* with the fewest potential side reactions and the maximum sugar-ethanol yield were determined. The main goal was to establish the processing parameters, such as residence time and sulfuric acid concentration.

2. Materials and methods

2.1 Sample collection

The cactus pear was collected in Morocco's Khouribga region (32°5′′N, 6°54′W) in October 2020. Under flowing tap water, the prickles and glochids on the surface of the fruit peel were removed by rubbing. Fruits were pressed at Flora Cooperative (**Figure 1)** to create by-products that would be utilized in medicinal, cosmetic, and culinary applications, including juice, jam, oil, and other cactus products. The used juice and peels were then brought to the lab of the National School of Applied Sciences "ENSA" Khouribga, where they were kept at -18°C until they were needed.

Figure 1. Collection and physical pretreatment of *Opuntia Ficus-Indica* (L.)

The physicochemical analyses performed on this fraction were: water content (by the drying oven method (CENT/TC 335, 2004) and the total reducing sugars TRS, i.e., glucose and fructose, which are the substrates converted into ethanol by the iodine method (Y.F.A.O, 1976). The results are described in Tables 1 and 2. For analysis, triplicate determinations were performed on each sample; to have an accurate result.

2.2 Water content determination

Plants with crassulacean acid metabolism (CAM) have a high moisture content. These plants create hydrocolloids in their tissues to conserve water. This characteristic is necessary for the survival of plants that grow in dry climates (Yang *et al.,* 2015). To determine the water content of the jus from

cactus pear wastes, we collected a 2 g sample of the jus in triplicate and dried it in an oven set to 105°C with a precision of ± 2 °C. It took 24 hours to completely dry the biomass materials to a constant weight. We weighed the final samples and used the following formula in **Eqn. 1** to measure their water content (CENT/TC 335, 2004) :

$$Moisture\% = \frac{wi-wf}{wi} \times 100 \qquad \textbf{Eqn. 1}$$

Where w_i and w_f are, respectively, the initial and final weight of the sample.

2.3 Acid hydrolysis of the wastes of Opuntia ficus-indica

We carried out the hydrolysis in a stainless-steel autoclave set to a pressure of 1 atm and a temperature of 121 °C, with flasks inside and beaker lids on to prevent leaking throughout the different residences' times (**Figure 2)**. We selected a solid-liquid ratio of 1:10 (Juice of *Opuntia Ficus-Indica*: dilute sulphuric acid solution in mol/l) since it is equivalent to the water content of cactus pears (Dehbi *et al.* 2014, Méndez *et al.*, 2015, Oussaid *et al.*, 2020). Under these conditions, we conducted the hydrolysis (in triplicate) at various reaction times ranging from 5 to 30 minutes using sulphuric acid solutions at concentrations of 1, 2, 3, 4, and 5%.

Figure 2 Process of Acid Hydrolysis of *Opuntia Ficus Indica* (L.)

We analyzed the total reducing sugars (TRS) at the end of the hydrolysis and selected the procedure that liberated the greatest amount of TRS. Then, we determined two residence times of 15 and 20 minutes.

2.4 Autohydrolysis pretreatment (AP)

Using the same method as for the acid hydrolysis described above, we combined cactus pear jus and water to make a solid/liquid (v/w) ratio of 1:10 and 1:20, respectively, and carried out the autohydrolysis for 15 and 20 minutes at 121°C and 1 atm.

2.5 Analysis

To prepare solution J, we mixed 10 g of juice with 100 ml of a sulfuric acid solution with concentrations ranging from 1% to 5%. Cj represents the glucose concentration in solution J, and Cg represents the molar concentration of glucose in the raw juice. The factor of dilution is equivalent to 11:

$$Cg = 11 \times Cj \qquad \textbf{Eqn. 2}$$

Eqn. 2 represents the relationship between Cg and Cj.
We then autoclaved the mixes for durations ranging from 5 to 30 minutes at 121°C.

At the end of the acid hydrolysis, we removed the flasks and let them cool to ambient temperature before adjusting the pH to a range of 4 to 5 using 2.5 mol/L sodium hydroxide. Afterward, we transferred the solution to a 250 mL volumetric flask and added distilled water to the mixture until it reached 250 mL, resulting in the H solution. The symbol C_H represents the glucose concentration in solution H. **Eqn. 3** shows the relationship between C_H, C_g, and C_S.

$$C_s + C_g = 25 \times C_H \qquad \textbf{Eqn. 3}$$

3. Results and Discussion

3.1 Results

The physicochemical analysis of cactus pear wastes indicated a high-water content (93%). This level of moisture is comparable to what is suggested for cactus pear fruits (Abd El-Razek and Hassan, 2011). The pH of the cactus pear waste solution ranged between 4.58 and 5.0. The pH is found to be lower than the values given for Moroccan cultivars (5.95–6.34) (El-Gharras *et al.*, 2006) and the published data from Mexican and Argentina cultivars, which reported that the pH varied from 5.6–6.5 (Stinting *et al.* 2001, Felker *et al.*, 2005). Based on this pH, this fruit is classified within the low-acid group (pH >4.5) because of the extremely low content of organic acids. Using acid hydrolysis on samples with a solid: liquid ratio of 1:10 and a temperature of 121°C, concentrations of acid ranging from 1 to 5%, and residence times ranging from 5 to 30 minutes, we discovered that the more time passes and the more acid we add, the more the solution turns brown at the end of hydrolysis, indicating that the sugars burn in the solution. Table 1 indicates that we got less TRS at 5 and 10 minutes, the highest amounts of TRS at 15 and 20 minutes, and less TRS after those residence durations, indicating that the sugars burn in the solution as more time passes in Figure 3a. The auto-hydrolysis using just water instead of sulfuric acid is represented by 10% and 20%, respectively, since the water at high temperatures acts as an acid. We limited ourselves to 15 and 20 minutes since these were the most effective durations for acid hydrolysis.

Table 1. The concentration of total reducing sugars C_H

C_H	5 min	10 min	15 min	20 min	25 min	30 min
1%	0.035052	0.015423	0.115944	0.118694	0.024957	0.021031
2%	0.027761	0.012058	0.116069	0.117944	0.029443	0.035052
3%	0.021031	0.021031	0.116569	0.118069	0.035052	0.039538
4%	0.037856	0.032247	0.117444	0.119444	0.029443	0.06814
5%	0.015423	0.029443	0.117444	0.121194	0.03393	0.041781
10%			0.119991	0.120459		
20%			0.12375	0.12375		

Figure 3a. Acid hydrolysis of *Opuntia ficus-indica*

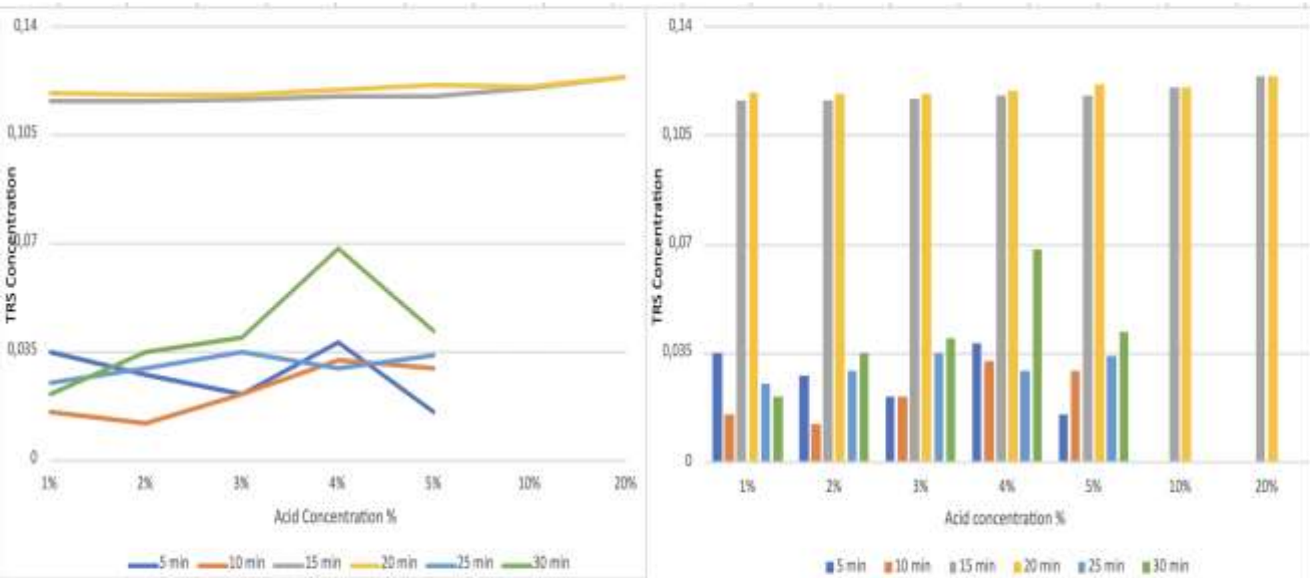

Figure 3b. The concentration of total reducing sugars

Table 2 and **Figure 3b** represent the results of the saccharose concentration. As shown by these data, the release of saccharose started after 10 minutes of residence time and decreased after 20 minutes due to the burning of sugars in the solution. Data demonstrates that the hydrolysis of *Opuntia ficus-indica* wastes occurs quickly and stabilizes between 10 and 20 minutes. This may be partly because most neutral polysaccharides are made up of simple sugars like arabinose, rhamnose, galactose, and xylose that are easy to break down (Wyman *et al.*, 2005).

Table 2. The concentration of *Saccharose* C_s *after acid hydrolysis*

Cs	5 min	10 min	15 min	20 min	25 min	30 min
1%	0.97993	0.257876	1.48625	1.17375	0.608588	0.464177
2%	0.711738	0.134096	1.56125	1.01125	0.773629	0.97993
3%	0.464177	0.464177	1.53625	1.16125	0.97993	1.14497
4%	1.08308	0.876779	1.43625	1.13625	0.546698	0.773629
5%	0.257876	0.773629	1.26125	0.86125	0.938669	1.227491
10%			1.26125	0.51125		
20%			0.751875	0.658125		

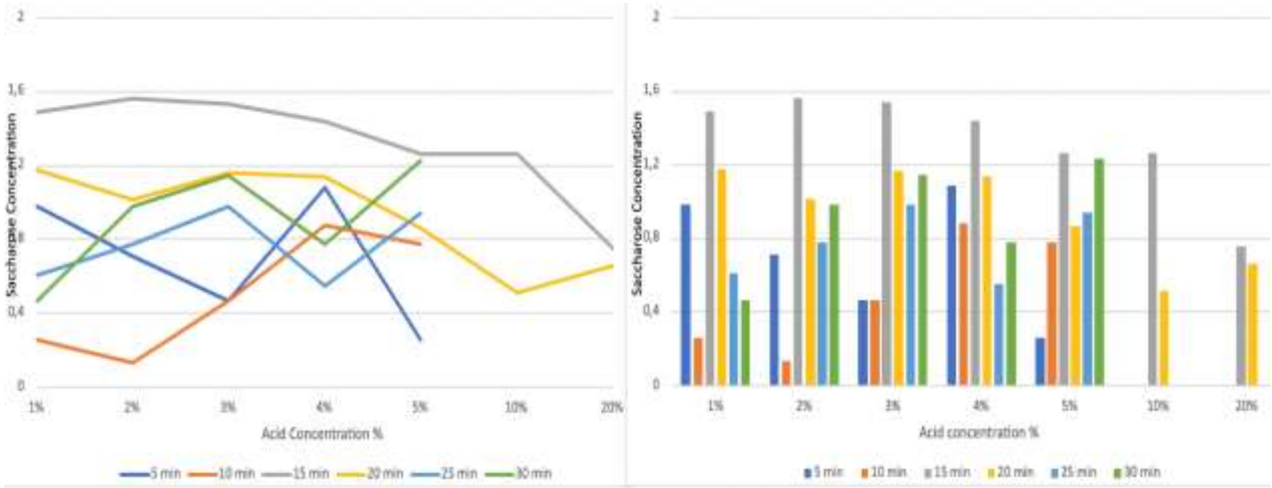

Figure 3c . The concentration of Saccharose in the solution

3.2 Analysis of the results of acid hydrolysis

3.2.1 Total reducing sugars

Following are the results of the two methods of analysis (acid hydrolysis and autohydrolysis):

The results of the treatments with various concentrations of acid solution and a Solid: Liquid ratio of 1:10 indicated that the best concentration of TRS was achieved at 4% sulfuric acid (C_H = 0.119 mol/l) and that TRS release decreases after that concentration (**Table 1.** and **Figure 3b.**). There is a relation between the amount of acid used and the release of TRS, up to the point where no additional TRS can be extracted from samples regardless of the addition of more acid. This may be because acid hydrolysis of hemicellulose, some proteins, lipids, and other biomolecules such as polyphenols forms crystalline cellulose, which is resistant to degradation. (Wyman *et al.*, 2005, Börjesson and Westman,

2015). Despite doubling the amount of water, the autohydrolysis results were the same at 15 and 20 minutes (C_H = 0.12 mol/l).

3.2.2 Saccharose concentration

For the acid hydrolysis, we achieved the best results for saccharose concentration using a 15-minute residence period and a 2% acid concentration (Cs= 1,56 mol/l), a solid: liquid ratio of 1:10, a temperature of 121°C, and a pressure of 1 atm. After 15 minutes, the saccharose content decreased, which can be explained by the burning of sugars in the juice, as shown in **Figure 3c**. We achieved the best results for autohydrolysis when the solid: liquid ratio was 1:10 and the residence time was 15 minutes: Cs=1.26 mol/l.

Conclusion

The goal of this study was to find the best way to acid hydrolyze the waste from cactus pears to get the most total reducing sugars, which will then be fermented into bioethanol.

We did a series of tests in a steel autoclave at a constant temperature of 121°C and a solid-to-liquid ratio of 1:10, as suggested in the literature. We tried different reaction times and acid concentrations to find the best conditions for acid hydrolysis of cactus pear wastes.

At the end of the acid hydrolysis, we stopped the reaction by adjusting the pH to 4–5, and we analyzed the concentrations of reduced total sugars and saccharose using the iodine method.

We achieved the best results for the total reducing sugars at a residence time of 20 min, a concentration of 3% of sulfuric acid solution at 121 °C, and a solid-liquid ratio of 1:10.

As for the saccharose, we obtained the best results at 15 minutes of reaction time, 2% sulfuric acid solution, 121 °C temperature, and a solid ratio of 1:10.

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