

Bioethanol Production From Leaves of *Quercus Infectoria* in Kurdistan Region

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Research

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Abstract

Quercus infectoria is one of the most abundant native oak species in the Kurdistan region of Iraq. This study focused on utilizing leaves of *Quercus infectoria* for ethanol production in the region. A typical three-step conversion process of acid pretreatment, enzymatic hydrolysis, and yeast fermentation was investigated to produce ethanol from the leaves. Under the selected acid pretreatment and enzymatic hydrolysis conditions, the glucose and xylose concentrations in the hydrolysates reached 11.4 g/L and 16.8 g/L, respectively, with the corresponding sugar conversions of 42.8% and 99.8%. A yeast strain, *Kluyveromyces marxianus*, was used to ferment mono-sugars in the hydrolysates for ethanol production. The ethanol production rate and conversion of *K. marxianus* in the fermentation were 0.17 g/L/h and 27%. The techno-economic analysis further concluded that a regional ethanol biorefinery can be established in the Zawita sub-district, Iraq to utilize *Q. infectoria* leaves to produce 200,000,000 kg ethanol/year with a positive energy balance of 745,052,623 MJ/year. The net annual revenue of the biorefinery is \$123,692,804. The payback period of the biorefinery is 10 years.

1. Introduction

Kurdistan region, Iraq is one of the most quickly growing and actively explored regions in Middle East due to its rich oil and mineral resources. The region has more than 45 billion barrels of reserved oil and 200 trillion cubic feet of reserved natural gas (IEA 2013). However, activities related to the oil industry, such as oil extraction, transportation, and refining, lead to serious environmental concerns of toxic chemical and metal releases, greenhouse gas emission from fossil fuel burning, and deforestation. On the other hand, Kurdistan mountains are well covered by stocked and healthy forests. Approximately 30,000 square kilometers of the mountainous areas are forestlands, which is roughly 4% of the total Kurdistan area (438,466 square kilometers) (Mosa 2016). Kurdistan forests are mainly composed of *Quercus* species (oak) and *Pinus* species (pine). *Quercus* are the dominant species in Kurdistan forest, which represents more than 90% of the total forestry area (Mosa 2016). Leaves and tree branches from *Quercus* in the forest represent a large quantity of biomass. Partially removing them from the forest not only contributes to forestry health but also generates a large quantity of lignocellulosic biomass for potential biofuel production (Schnepf et al. 2009; Page-Dumroese et al. 2010).

Bioethanol production from lignocellulosic materials has been intensively researched in the past decades. Bioethanol has low greenhouse gas emissions, high octane number, good flammability (Chen and Fu 2016), and good blending capability with gasoline (Sun et al. 2016), which makes it one of the most promising biofuels to replace a large quantity of fossil fuels in the near future. However, due to the recalcitrant structure of lignocellulosic materials, the feedstock must be pretreated and hydrolyzed to release mono-sugars for ethanol bioconversion. Many pretreatment methods have been developed, including physical, chemical, physicochemical and biological approaches (Sun and Cheng 2002). Among them, acid pretreatment combining with enzymatic hydrolysis has been widely accepted as an effective approach to release mono-sugars from lignocellulosic biomass for biofuel production (Humbird et al. 2011; Quintero and Cardona 2011; Zanotti et al. 2016; Ruan et al. 2013), which was adopted by this study to treat leaves of *Quercus infectoria*.

Saccharomyces cerevisiae is the most widely used yeast species to carry out ethanol fermentation. However, *Saccharomyces* yeasts prefer to use C6 sugars, and has limited capability to consume C5 sugars. Considering high xylan content in the leaves, non-*Saccharomyces* strains that have diverse metabolic capacities to utilize a variety of mono-sugars are interest of the study. It has been reported that *Kluyveromyces marxianus* and *Pichia stipitis* are able to utilize C5 and C6 sugars for ethanol production (Lainez et al. 2019; Agbogbo et al. 2006; Fonseca et al. 2008). Agbogbo et al. reported that *P. stipites* generated 23 g/L ethanol at an ethanol production rate of 0.19 g/L/h on a medium with 50% glucose and 50% xylose (Agbogbo et al. 2006). Lainez et al. concluded that *K. marxianus* had an ethanol production rate of 1.77 g/L/h on leaves juice of agave plant (Lainez et al. 2019). Compared *K. marxianus* to *P. stipitis*, *K.*

marxianus has better ethanol production rate and C5 sugar utilization capability. Therefore, it was selected by this study to carry out ethanol fermentation.

The objective of this study is to investigate bioethanol production on leaves of abundant *Quercus infectoria* in Kurdistan region, Iraq. Acid pretreatment and enzymatic hydrolysis with detoxification were first applied to convert carbohydrates in *Q. infectoria* leaves into mono-sugars. *K. marxianus* fermentation then consumed the mono-sugars to produce ethanol. Consequently, the hydrolysis and fermentation data were used by techno-economic analysis to demonstrate a regional *Q. infectoria* leaves biorefinery and evaluate its performance of ethanol production.

2. Materials And Methods

2.1 Feedstocks

Q. infectoria leaves were collected during the peak growing season at a semi-arid area near to Zawita sub-district that locates 10 km north east of Duhok governorate, Iraq (37°03'N, 43°21'E). The sample was collected in May 2017. The leaves were washed thoroughly with tap water to remove dirt, and dried under the sun for a week. The dried sample was grinded using a Blendtec Total Classic Original Blender (Blendtec, Orem, Utah), and screened using a 40 mesh American standard sieve. The biomass powders passing the sieve were collected and stored in sealed plastic bags at room temperature prior to their use for ethanol production.

2.2 Pretreatment and enzymatic hydrolysis

Acid pretreatment and enzymatic hydrolysis were modified based on the method described by Ruan et al (Ruan et al. 2013). The dry biomass was pretreated using an autoclave (Brinkmann 2540M, Tuttnauer USA Co. Ltd., Hauppauge, NY). Sulfuric acid (H₂SO₄, 95% w/w) was used as the acid. Three reaction times (0.5, 1, and 2 hours), three acid concentrations (0.5, 1, and 2% w/w), and two temperature (105 and 120°C) were tested using a completely randomized design (CRD). The total solids content of biomass was fixed at 12.5% (w/w). The pretreatment was carried out in 125 mL glass bottles (Wheaton Industries, Millville, NJ). The amount of the solution in the bottle was 50 g. After the acid pretreatment, the solution was adjusted to pH of 5.0 using 30% w/w sodium hydroxide (NaOH). An enzyme mixture consisting of 69 µL cellulase (CTEC 3, protein content: 218 mg mL⁻¹; Novozymes North America, Franklinton, NC) with an enzymatic activity of 70 filter paper unit (FPU) and 13 µL xylanase (HTEC 3, protein content: 171 mg mL⁻¹; Novozymes North America, Franklinton, NC) per gram of initial dry biomass was directly applied on the pretreated solution to carry out the enzymatic hydrolysis at 50°C and 150 rpm (2.5 Hz) in a shaking incubator (Thermo Scientific, Odessa, TX) for 48 h. After the enzymatic hydrolysis, the hydrolysate was centrifuged at 7,025 ×g for 10 minutes to separate the liquid hydrolysate from the residual solids. Approximately 2 mL of the liquid hydrolysate was filtered through a 0.22 µm polyethersulfone membrane filter (SLGS033SS, EMD Millipore, Billerica, MA) for sugar analysis.

Glucose and xylose conversion were calculated as follows:

Glucose conversion = glucose amount in the hydrolysate / (glucan amount in the biomass for the hydrolysis x 1.1) x 100% (Eq. 1)

Xylose conversion = xylose amount in the hydrolysate / (xylan amount in the biomass for the hydrolysis x 1.14) x 100% (Eq. 2)

2.3 Preparation of the solution for ethanol fermentation

To further increase mono-sugar concentrations in the hydrolysate for ethanol fermentation, total solids (TS) content of the dry biomass was further increased to 15% (w/w) to run acid pretreatment and enzymatic hydrolysis using the

selected conditions that were concluded in the previous section. The hydrolysate after enzymatic hydrolysis was detoxified using the method reported by Zhong et al (Zhong et al. 2016). The detoxification was carried out in 500 mL Wheaton bottle. $\text{Ca}(\text{OH})_2$ powder was added in 300 ml liquid solution until pH reached 10. The bottles were placed in a shaking incubator (MaxQ 5000, Thermo Scientific, Odessa, TX) at 100 rpm (2.5 Hz) and 30°C for 5 hours. After detoxification, the pH of the detoxified solution was adjusted to 5.9 using 30% (w/w) H_2SO_4 . The neutralized, detoxified solution was then centrifuged at $7,025 \times g$ for 10 minutes to separate liquid and solids. The liquid was used for ethanol fermentation.

2.4 Ethanol fermentation

K. marxianus (ATCC 12424 and NRRL Y610) purchased from the American Type Culture Collection (ATCC, Manassas, VA) was used to carry out ethanol fermentation. The strain was stored in glycerol at -80°C prior to use. It was activated and inoculated into YM broth medium and cultured at 30°C and 200 rpm in a shaking incubator (MaxQ 5000, Thermo Scientific, Odessa, TX) for 24 hours to prepare the inoculum for the ethanol fermentation. The inoculum with a cell number of $1 \times 10^8/\text{mL}$ was mixed with sterilized liquid hydrolysate with additional nutrients (10 g/L of peptone and 5 g/L of yeast extract). The inoculum-to-solution ratio was 1:10 to carry out the fermentation in a sealed 125 mL glass bottle (Wheaton Industries, Millville, NJ) with 50 g of the fermentation broth under the conditions of 30°C and 150 rpm. Samples were taken from the fermenters periodically for mono-sugar and ethanol analysis.

Ethanol conversion from consumed mono-sugars and ethanol yield from leaf biomass were calculated as follows:

Ethanol conversion from consumed mono-sugars = Ethanol amount in the fermentation broth / the amount of glucose and xylose consumed in the fermentation $\times 100\%$ (Eq. 3)

Ethanol yield from leaf biomass = Ethanol amount in the fermentation broth / the amount of biomass used to produce the hydrolysate for ethanol fermentation $\times 100\%$ (Eq. 4)

2.5 Techno-economic analysis

Based on the hydrolysis and fermentation data, a techno-economic analysis (TEA) of the ethanol production from *Q. infectoria* leaves was conducted to investigate the feasibility of such a biorefinery in the region. Zawita sub-district, Duhok, Iraq is selected as the area for the TEA. Zawita sub-district has an area of approximately 415,910,000 m^2 with 54% of the area covered by *Q. infectoria* (Mosa 2016; Mustafa and Habeeb 2014). The average *Q. infectoria* density in the sub-district is 2,085 trees/10,000 m^2 . With the values of leave number (200,000/tree/year) and dry weight of leaf (0.0193 g/leaf), Zawita sub-district produces 18,075,340,463 kg/year of dry *Q. infectoria* leaves. This study assumes that 22% (4,000,000,000 kg) of the total dry leaves are collected and used by a regional biorefinery to produce 200,000,000 kg bioethanol/year.

The regional ethanol biorefinery of *Q. infectoria* leaves includes ten unit-operations: 1) leaves collection and transportation, 2) pretreatment and enzymatic hydrolysis and conditioning, 3) hydrolysate concentration, 4) ethanol fermentation, 5) ethanol distillation, 6) hydrolysis residue drying, 7) yeast drying, 8) wastewater treatment, 9) boiler and turbo-generator, and 10) utilities. Mass and energy balance analysis was first carried out to delineate mass flow and energy demand of the biorefinery, which was used to determine the size of individual unit operations. Capital expenditure (CapEx) and operational expenditure (OpEx) of the unit operations were calculated using linear scaling of reference numbers (Humbird et al. 2011; Quintero and Cardona 2011). Revenues include fuel ethanol, yeast biomass as animal feed, and energy saving. The Modified Accelerated Cost Recovery System (MACRS) was used to calculate the annual depreciation of CapEx. In addition, an annual inflation of 0.32% was set for OpEx and revenues based on the

five-year average inflation rate in Iraqi (from 2016 to 2020). The net cash flow based on depreciated CapEx and inflated OpEx and revenues was calculated to determine the payback period of the regional biorefinery.

2.6 Chemical analysis

Total solids (TS) and volatile solids (VS) were analyzed according to APHA (APHA 1998). Fiber composition (cellulose, xylan, and lignin) of the biomass were measured according to the National Renewable Energy Laboratory's (NREL) analytical procedure (Sluiter 2008). Glucose, xylose, and acetic acid in the hydrolysate were determined by High Performance Liquid Chromatography (HPLC) (Shimadzu prominence), equipped with a Bio-rad Aminex HPX-87H analytical column and a refractive index detector. The mobile phase was 0.005 mol L⁻¹ sulfuric acid at a flow rate of 0.6 mL min⁻¹. The oven temperature was set at 65°C. HPLC grade standards including glucose, xylose, and sodium acetate were purchased from Sigma-Aldrich, St. Louis, MO.

2.7 Statistical analysis

Statistical software R (Version 3.4.1) was applied to carry out the statistical analysis. Multiple-way analysis of variance (ANOVA) and Tukey's multiple comparison of means were conducted to evaluate effects of pretreatment time, temperature and acid concentrations on acid pretreatment and enzymatic hydrolysis of glucose and xylose production.

3. Results And Discussion

3.1. Effects of pretreatment conditions on enzymatic hydrolysis of mono-saccharides release

Characteristics of *Q. infectoria* was listed in Table 1. Glucan and xylan contents were 16.2% TS and 12.8% TS, respectively, which are similar with the reports on compositions of other oak leaves (Baber et al. 2014). Lignin content of 29.6 % was significantly higher than glucan and xylan in the leaf biomass.

Dilute acid pretreatment was first investigated to conclude the preferred reaction conditions. 12.5% of dry biomass were used for the pretreatment. Enzymatic hydrolysis was then applied on the pretreated slurry to release mono-sugars, which was used to evaluate performance of the pretreatment. Multiple-way ANOVA analysis shows that acid concentration and temperature had significant ($P < 0.05$) influences on glucose production. Xylose production was significantly ($P < 0.05$) impacted by all three factors of acid concentration, temperature, and reaction time. Two-way and three-way interactions did not have significant ($P > 0.05$) influences on glucose production. While, xylose production was significantly ($P < 0.05$) influenced by the interaction of acid concentration and pretreatment time.

Glucose concentrations of *Q. infectoria* in the hydrolysates under different pretreatment conditions were shown in Figures 1a-c. Compared to lower acid concentrations (0.5 and 1%) and lower reaction temperature (105°C), higher glucose concentrations of 8.7, 8.4, and 8.6 g/L under 120°C and 2% acid were achieved for 0.5, 1 and 2 hours, respectively, with no significant ($P > 0.05$) difference between each other. For the pretreatment at 105°C, there were also no significant ($P > 0.05$) differences between different reaction times and acid concentrations. The glucose concentrations were around 7.5 g/L at 105°C, which were significantly ($P < 0.05$) lower than them from the pretreatment at 120°C. The results clearly demonstrate that glucose release of *Q. infectoria* was mainly dependent on reaction temperature and acid concentration. 2% acid at 120 °C for 2 hours was the preferred pretreatment conditions for glucose release from *Q. infectoria*. In comparison with other major herbaceous energy crops of switchgrass and miscanthus (Ruan et al. 2013), the glucose conversion of the leaves was 43%, which is lower than 60 and 55% from switchgrass and miscanthus, respectively, under similar pretreatment and enzymatic hydrolysis conditions. The possible reason might be that high lignin content in the leaves (30%) absorbed cellulase and prevented some of them from hydrolyzing glucan in the pretreated leaves (Yang and Wyman 2006; Tengborg et al. 2001; Chen et al. 2012).

Xylose concentrations of *Quercus infectoria* under different pretreatment conditions are shown in Figures 1d-1f. Unlike glucose production, xylose concentration was significantly influenced by acid concentration, temperature and reaction time. Increasing acid concentration from 0.5 to 1% exhibits little impact on xylan conversion at 105 °C for all three reaction times, comparing to 30-60% increase of xylose concentration at 120°C at the corresponding reaction times. Xylose concentrations were further increased by 30-40% at 105°C and 20-30% at 120°C with the increase of acid concentration from 1% to 2%. Under 2% acid and 105°C, xylose concentrations reached 10.3, 13.3, and 14.1 g/L for 0.5, 1, and 2 hours of reaction time, respectively. Meanwhile, under 2% acid and 120°C, xylose concentrations reached 15.9, 15.4, and 17.0 g/L for 0.5, 1, and 2 hours of reaction time, respectively. The data show that xylose concentration was significantly ($P<0.05$) increased with extending the pretreatment time from 0.5 to 2 hours and increasing the reaction temperature from 105 to 120°C. The highest xylose concentration of 17.0 g/L was achieved under the conditions of 2 hours of reaction time, 2% of acid concentration, and 120°C of reaction temperature. Considering total mono-sugar conversion (both xylose and glucose), 2% acid at 120 °C for 2 hours was adopted to carry out pretreatment for enzymatic hydrolysis. Switchgrass and miscanthus were used again to compare xylose conversion. The xylose conversion of the leaves was 93%, which is higher than switchgrass (79%) and miscanthus (70%) (Ruan et al. 2013). Due to the fact that majority of xylose was released during the pretreatment step, lignin absorption of enzymes had less effect on xylose release than glucose release.

Figure 1. Effects of pretreatment conditions on mono-sugar release from *Quercus infectoria*

In order to increase the amount of mono-sugars for following fermentation, the biomass amount was further increased to 15% of TS for the pretreatment, which was the highest TS content with appropriate rheological property for the pretreatment and hydrolysis. Starting from 2.9 g/L in the pretreated slurry of *Q. infectoria*, glucose concentration quickly increased to 9 g/L in the first 6 hours and then leveled off after reaching 10.5 g/L at 12 hours (Figure 2). Compared to glucose production, xylose started from much higher concentrations of 16.8 g/L that was the result of acid pretreatment (Figure 2). The xylose concentration was increased to 22.0 g/L in 12 hours and then leveled off following the same trend of glucose production. Since acetate was mainly generated during the acid pretreatment [15], the acetate concentration remained relatively constant during the course of the enzymatic hydrolysis, which the acetate concentration was 1.3 g/L at the beginning and slightly increased to 1.9 g/L at the end of the hydrolysis.

Figure 2. Enzymatic hydrolysis of the acid treated *Q. infectoria*

3.2. Ethanol fermentation on the hydrolysate using *marxianus*

Ethanol production from the hydrolysate was subsequently carried out using *K. marxianus* fermentation. The fermentation data were shown in Figure 3. It has been reported that *K. marxianus* is able to utilize both glucose and xylose in the hydrolysates with a preference of glucose (Signori et al. 2014). The changes of glucose, xylose, acetate, and ethanol during *K. marxianus* fermentation were presented in Figure 3. *K. marxianus* exhibits relatively fast rates of sugar consumption and ethanol production. There was no lag phase for *K. marxianus* growth on the hydrolysate. Glucose concentration dropped to 1.8 g/L at 12 hours from 10.9 g/L at the beginning of the fermentation. The corresponding glucose consumption rate was 0.76 g/L/h. There was no further glucose consumption after 12 hours fermentation. Xylose consumption was accelerated after 6 hours fermentation when glucose concentration dropped below 4.0 g/L. A total of 6.3 g/L xylose was consumed during the 36 hours fermentation with a xylose consumption rate of 0.18 g/L/h. The data show that *K. marxianus* fermentation accumulated ethanol once glucose maintained at higher concentrations in the fermentation broth. Ethanol concentration reached 7.5 g/L within the first 12 hours, giving an ethanol production rate of 0.63 g/L/h. After glucose consumption leveled off at 12 hours of the fermentation, both ethanol production and xylose consumption were stopped. This result suggests that *K. marxianus* might require the existence of glucose to metabolize xylose to produce ethanol, which is consistent with the observations from the

literature reports (Signori et al. 2014; Du et al. 2019). Meanwhile, the data also demonstrate that a good amount of 9 g/L of xylose was consumed from 6 to 12 hours once glucose reached a low level of 3.3 g/L at 6 hours, which provides a potential strategy to apply *K. marxianus* to utilize xylose rich biomass for high-efficiency ethanol production. A small amount of glucose could be added into the fermentation broth to maintain the minimum amount of glucose for *K. marxianus* metabolism of xylose consumption and ethanol production. The fermentation results concluded that *K. marxianus* cultivation on the hydrolysate had a short fermentation time of 12 hours to produce 7.5 g/L with an ethanol production rate of 0.63 g/L/h.

Figure 3. Ethanol production from the hydrolysate using *K. marxianus*

3.3. Techno-economic analysis (TEA)

According to above experimental results, mono-sugar and ethanol production from *Q. infectoria* leaves are summarized in Table 2, and will be used for TEA. The selected pretreatment conditions are 120°C, 2% (w/w) H₂SO₄, and 2 hours followed by a 24-hour enzymatic hydrolysis. At 15% dry matter of *Q. infectoria* in the processing solution, the pretreatment and enzymatic hydrolysis had a glucose conversion of 42.8% and a xylose conversion of 99.8% with production of 0.076 kg glucose and 0.145 kg xylose per kg dry leaf biomass. The ethanol fermentation of the concentrated hydrolysate using *K. marxianus* for the TEA were set at 48 hours and 30°C of anaerobic cultivation. The extended fermentation time is due to the fact that a concentration step of the hydrolysate was introduced in the TEA analysis. The fermentation produced 0.050 kg ethanol per kg dry leaf biomass, respectively. The corresponding ethanol yield from mono-sugars consumed was 27.0%, and the corresponding ethanol yield from the dry leaf biomass was 5.0%.

Table 2. Sugar and ethanol production from *Q. infectoria* using *K. marxianus*

According to the leaves production in Zawita sub-district, this study assumed that 22% (4,000,000,000 kg) of the annual leaves production was used as the feedstock for an ethanol biorefinery. TEA was conducted based on the ethanol production 200,000,000 kg per year. The mass and energy balance calculation is detailed in the supplemental material.

3.3.1. Mass and energy balance

Mass balance data show that the amounts of fuel (diesel) for collecting and transporting the leaves to the biorefinery are 0.09 and 0.02 kg diesel/kg ethanol produced, respectively (Figure 3). Since the leaves available in Zawita sub-district is within 50 km radius, reference numbers of 5.43 and 1.45 L diesel/metric ton dry biomass for biomass collection and transportation, respectively, in the similar radius were used for the calculation (Morey et al. 2010). The corresponding energy consumption for the leaves collection and transportation is 6.22 MJ/kg ethanol produced (Table 3).

Once the leaves biomass arrives at the biorefinery, 20 kg of dry biomass is needed to be pretreated and hydrolyzed to release mono-sugars (glucose and xylose) for production of 1 kg of ethanol. During the pretreatment and hydrolysis and condition operation, 2.67 kg of sulfuric acid, 127 kg of water (64 kg of fresh water and 63 kg of condensation water from the hydrolysate concentration), 2 kg of calcium hydroxide, and 1.64 kg of enzymes are used to convert 20 kg of dry biomass into 102 kg of hydrolysate with glucose and xylose concentrations of 17 and 22 g/kg hydrolysate, 27 kg of wet hydrolysis residue with a moisture content of 36% (w/w), and 3.7 kg of CaSO₄ (Figure 3). Energy consumptions for acid pretreatment, enzymatic hydrolysis, detoxification (using Ca(OH)₂), and press filtration (liquid/solid separation) are 43.1, 0.9, 0, and 3.1 MJ/kg ethanol produced, which were calculated based on the approach described in a previous study (Zanotti et al. 2016). Total energy consumption for the pretreatment and hydrolysis step is 47.1 MJ/kg ethanol produced (Table 3), which is one of the largest energy demanding operations in the biorefinery.

Sugar concentrations of hydrolysate from the pretreatment and hydrolysis step are relatively low for ethanol fermentation, which leads to a large amount of water demand and a very low ethanol content from the fermentation step. A hydrolysate concentration step is needed to increase sugar concentration and recirculate water. A single-effect mechanical vapor recompression (MVR) evaporator is selected to carry out the concentration due to the better steam economy of MVR (Minton 1986). The evaporator produces 40 kg of concentrated hydrolysate with glucose and xylose concentrations of 44 and 56 g/kg hydrolysate, respectively, from 103 kg of dilute hydrolysate (Figure 3). Meanwhile, sixty-three kg of condensation water is recirculated back to the pretreatment and hydrolysis operation. The energy balance shows that the evaporator demands 13.8 MJ/kg ethanol produced to concentrate the hydrolysate and recovers 13.2 MJ/kg ethanol produced during the condensation. The recovered energy is used to raise and maintain the solution temperature of the pretreatment and hydrolysis and condition operation (Table 3).

During a 48-hour ethanol fermentation, thirty-seven kg of fermentation broth with an ethanol content of 2.7% (w/w) and 3 kg of wet yeast with a dry matter of 33% (w/w) are generated from 40 kg of concentrated hydrolysate for production of 1 kg ethanol (Figure 3). One tenths kg/kg ethanol produced of corn steep liquor (nitrogen source), 0.013 kg/kg ethanol produced of diammonium phosphate, and 0.01 kg/kg ethanol produced of air are used for seed culture and ethanol fermentation. Energy consumptions for yeast seed culture and ethanol fermentation are 2.94 and 0.91 MJ/kg ethanol produced, which were calculated based on a reference (Zanotti et al. 2016). Total energy consumption for the ethanol fermentation is 3.85 MJ/kg ethanol produced (Table 3).

A conventional distillation tower is then used to extract ethanol from the fermentation broth. One kg bioethanol stream with an ethanol content of 95% (v/v) is obtained from the distillation (Figure 3). The distillation also generates 36 kg of wastewater, which is treated by a wastewater treatment operation before discharging. Based on ethanol content in the fermentation broth (2.7%w/w or 3.42%v/v), an energy demand of 18.5 MJ/kg ethanol produced for the distillation was calculated based on the numbers from a reference (Katzen et al. 1999) (Table 3).

Since the leaf has a relatively high lignin content (Table 1), recovering the lignin-rich hydrolysis residue and using it as the fuel to power the ethanol biorefinery are critical to sustain ethanol production from the leaves. Twenty-seven kg of wet residue with a dry matter of 36% (w/w) obtained from a press filter is dried using a triple-pass rotary dryer to produce 10 kg of dry residue with a lignin content of 60% (w/w) (Figure 3). The energy demand of the drying is 45.9 MJ/kg ethanol produced, which is the second largest energy demand in the biorefinery (Table 3). However, the energy content of dry lignin-rich residue is 131 MJ/kg ethanol produced, which is much higher than the energy demand of the drying. Therefore, the lignin-rich residue is used by a boiler and a turbo-generator to power the biorefinery.

Meanwhile, yeast biomass from the fermentation process rich in protein can be another value-added product of animal feed. Another drying process using the triple-pass rotary dryer consumes 5.3 MJ of thermal energy to produce 1 kg of dry yeast biomass (Figure 3 and Table 3).

Due to a large amount of wastewater (7,190,104,864 kg/year) generated from the biorefinery, it must be treated before discharging. An activated sludge wastewater treatment operation is implemented in the biorefinery to handle the wastewater. Ninety percent (6,471,094,377 kg/year) of the wastewater is reclaimed. Based on an average energy demand of 1,14 kJ/kg wastewater for conventional activated sludge wastewater treatment process reported by the Water Environment Federation (WEF), energy consumption of wastewater treatment at the biorefinery is 0.041 MJ/kg ethanol produced or 8,203,910 MJ/year for 200,000,000 kg/year of ethanol production.

Figure 4. Mass balance of 1 kg bioethanol production from the leaves

Table 3. Energy balance of ethanol production from the leaves ^a

The mass and energy balance data demonstrate that due to the fact that *O. infectoria* leaf has a high lignin content, the lignin-rich hydrolysis residue contains the energy that is able to power the entire biorefinery with a surplus net energy of 3.8 MJ/kg ethanol produced or 753,256,533 MJ/year for the biorefinery. However, the water demand (64 kg/kg ethanol produced) of the biorefinery is much higher than other reports ranging from 5 to 12 kg/kg ethanol produced (Humbird et al. 2011; Mu et al. 2010). In order to address the high water demand issue, efficiencies of both mono-sugar production and ethanol fermentation from the leaves need to be further improved.

3.3.2. Economic analysis

Economic feasibility is another important factor that determines commercial applicability of the ethanol biorefinery in the region. CapEx, OpEx, and revenues are the parameters to assess economic performance of the biorefinery. As presented in Table 4, the CapEx to establish the studied biorefinery in Zawita sub-district is \$1,227,906,682. Due to the large amount of leaves required by the biorefinery and high lignin content in the leaves, the pretreatment/hydrolysis/condition of feedstock handling and the boiler and turbo-generator of hydrolysate residue utilization are the most expensive units (\$104,731,510 and \$413,881,582, respectively) in the biorefinery. The OpEx is \$170,129,613/year, including feedstock collection and transportation, water, chemicals, enzymes, maintenance, and labor costs. The revenue streams of the biorefinery are ethanol, dry yeast, and energy saving from hydrolysis residue utilization. Ethanol as a biofuel (\$1.11/kg), dry yeast as animal feed additive (\$0.5/kg), and energy saving (\$0.1/kwh) lead to a total revenue of \$293,822,418/year, which is 1.73 times higher than the OpEx. Correspondingly, a net positive revenue of \$123,692,804/year is realized from the biorefinery operation.

The cash flow analysis predicts that payback period of the biorefinery is 10 years (Figure 5). A sensitivity analysis was then conducted on five key items (from both CapEx and OpEx) of pretreatment/hydrolysis/condition unit, boiler and turbo-generator unit, sulfuric acid for pretreatment, enzyme protein for hydrolysis, and ethanol revenue to elucidate the impacts of them on the payback period (Table 5). An increase of 25% on ethanol production could reduce the payback period by 30% to 7 years, which is the largest reduction among these five key items. A capacity reduction of 25% on the boiler and turbo-generator can also decrease the payback period by 15% to 8.5 years. 25% reduction of sulfuric acid usage, enzyme protein usage, and size of the pretreatment/hydrolysis/condition unit could shorten the payback period by 8, 10, and 5%, respectively. According to the sensitivity analysis, the conclusion is similar with that from the mass and energy balance analysis. Increasing ethanol conversion from mono-sugars in the hydrolysate and improving the hydrolysis efficiency of mono-sugar release are two key steps to greatly reduce CapEx and OpEx, increase ethanol revenue, and significantly enhance the performance of the biorefinery.

Table 4. Economic assessment of a bioethanol plant with a capacity of 200,000 kg ethanol per year from *Q. infectoria* leaves in Kurdistan region of Iraq

Table 5. Sensitivity analysis of key CapEx, OpEx, and revenue items on the payback period of the biorefinery

Figure 5. Cash flow of the bioethanol plant with a capacity of 200,000 kg ethanol per year from *Q. infectoria* leaves

4. Conclusions

Ethanol production using a local, abundant biomass – *Q. infectoria* leaves in Zawita sub-district, Duhok, Iraqi has been developed by this study. With selected conditions of pretreatment, enzymatic hydrolysis, and fermentation, one kg of leaves can produce 0.076 kg glucose and 0.145 kg xylose, and consequently generate 0.05 kg ethanol. According to the mono-sugar and ethanol data, a regional ethanol biorefinery can be established to utilize 22% of the total available *Q. infectoria* leaves to produce 200,000,000 kg ethanol/year. The biorefinery has a positive net energy balance of

745,052,623 MJ/year. With final products of fuel ethanol, dry yeast, and energy saving, a net annual revenue of \$123,692,804 can be obtained, which leads to a payback period of 10 years for the biorefinery.

5. Abbreviations

CRD	Completely randomized design
FPU	Filter paper unit
TS	Total solids
TEA	Techno-economic analysis
CapEx	Capital expenditure
OpEx	Operational expenditure
MACRS	Modified accelerated cost recovery system
VS	Volatile solids
NREL	National Renewable Energy Laboratory
ANOVA	Analysis of variance

6. Declarations

Availability of Data and Materials

All data generated and analyzed from this study are included in the published article.

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Author's contributions

Bawar Sh. Tahir: Conceptualization, Methodology, Investigation, Data curation, Writing the original draft. Xiaoqing Wang: Conceptualization, Methodology, Investigation, Data curation, Validation, Writing the original draft. Yuan Zhong: Methodology, Investigation. Hassan A. Mezeri: Reviewing and editing. Yan Liu: Methodology, Investigation, Data curation, Reviewing and editing. Wei Liao: Conceptualization, Methodology, Data curation, Reviewing and editing, Supervision.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Tables

Table 1. Characteristics of the biomass*

Characteristics	<i>Quercus infectoria</i>
Total solids (%)	88.86 ± 0.23
Volatile solids (% TS)	93.36 ± 0.03
Glucan (% TS)	16.19 ± 0.23
Xylan (% TS)	12.77 ± 0.16
Lignin (% TS)	29.57 ± 1.95

*: Data are average of three replicates with standard deviation.

Table 2. Mon-sugar release and ethanol production from the leaves under the selected reaction conditions

	<i>Quercus infectoria</i>
Total dry leaf amount (kg)	1
Enzymatic hydrolysis	
Glucose (kg)	0.076
Glucose conversion (%)	42.8
Xylose (kg)	0.145
Xylose conversion (%)	99.8
Ethanol fermentation – <i>Kluyveromyces marxianus</i>	
Ethanol (kg)	0.050
Xylose consumption (%)	99.8
Glucose consumption (%)	42.8
Ethanol conversion from consumed mono-saccharides (%)	27.0
Ethanol yield from leaf biomass (%)	5.0

Table 3. Energy balance of bioethanol production from the leaves ^{a, b}

	Energy (MJ/kg ethanol produced)	Energy (MJ/biorefinery/year)
Energy demand		
1. Leaves collection and transportation ^c	-6.22	-1,245,206,398
2. Pretreatment and hydrolysis and condition ^d	-47.05	-9,409,638,344
3. Hydrolysate concentration	-13.80	-2,760,831,628
4. Ethanol fermentation ^e	-3.85	-769,890,545
5. Ethanol distillation	-18.54	-3,708,842,803
6. Hydrolysis residue drying ^f	-45.93	-9,186,268,754
7. Yeast drying ^f	-5.30	-1,059,564,232
8. Wastewater treatment	-0.04	-8,203,910
Energy generation (recovery)		
3. Hydrolysate concentration	13.17	2,635,339,282
6. Hydrolysis residue drying ^g	131.29	26,258,159,958
Overall energy balance		
Net energy ^h	3.73	745,052,623

a. Energy balance calculation is based on the ethanol production of 200,000,000 kg per year.

b. Negative numbers are energy demand, and positive numbers are energy generation.

c. The transportation distance in Zawita sub-district is within 50 km radius. The reference number of diesel consumption for corn stover collection and transportation was used here (Morey et al. 2010).

d. The operation includes: acid pretreatment, enzymatic hydrolysis, Ca(OH)₂ detoxification, and press filtration.

e. Ethanol fermentation includes seed culture and ethanol fermentation.

f. Triple-pass rotary dryers are used for both drying operations. The energy consumption was calculated based on a reference (Zanotti et al. 2016).

g. The dry hydrolysis residue contains 60% w/w of lignin. The low heating value of lignin is 22.2 MJ/kg (Demirbas 2017).

h. Net energy = Energy generation – Energy demand.

Table 4. Economic assessment of a bioethanol plant with a capacity of 200,000 kg ethanol per year from *Q. infectoria* leaves in Kurdistan region of Iraq

	Unit cost	Unit	Cost	Reference
Capital expenditure (CapEx)				
Pretreatment/hydrolysis/condition ^a	\$104,731,510	1	\$104,731,510	(Quintero and Cardona 2011)
Hydrolysate concentration ^a	\$36,443,241	1	\$36,443,241	(Quintero and Cardona 2011)
Ethanol fermentation ^b	\$31,293,154	1	\$31,293,154	(Quintero and Cardona 2011)
Ethanol distillation ^b	\$17,445,078	1	\$17,445,078	(Quintero and Cardona 2011)
Hydrolysis residue drying ^c	\$8,801,496	1	\$8,801,496	(Amos 1999)
Yeast drying ^c	\$1,027,180	1	\$1,027,180	(Amos 1999)
Boiler and turbo-generator ^d	\$413,881,582	1	\$413,881,582	(Humbird et al. 2011)
Utilities ^{b, e}	\$7,564,929	1	\$7,564,929	(Humbird et al. 2011)
Wastewater treatment plant ^b	\$54,160,506	1	\$54,160,506	(Humbird et al. 2011)
Added direct and indirect cost (45% of total CapEx) ^f	\$552,558,007	1	\$552,558,007	(Humbird et al. 2011)
Total CapEx			\$1,227,906,682	
Operational expenditure (OpEx)				
Diesel fuel for leaves collection and transportation	\$0.7/kg	22,870,016 kg/year	\$16,009,011/year	Local price
Sulfuric acid for pretreatment	\$0.08139/kg	533,333,332 kg/year	\$43,408,000/year	(Humbird et al. 2011)
Water for pretreatment	\$0.0002/kg	12,856,098,619 kg/year	\$2,571,220/year	(Humbird et al. 2011)
Enzyme protein for hydrolysis	\$6.27/kg	11,050,800 kg/year	\$69,288,516/year	(Liu et al. 2016)
Ca(OH) ₂ for conditioning	\$0.05/kg	399,999,999 kg/year	\$20,000,000/year	Current price

Corn steep liquor for ethanol fermentation	\$0.05155/kg	19,753,425 kg/year	\$1,018,289/year	(Humbird et al. 2011)
Diammonium phosphate for ethanol fermentation	\$0.89532/kg	2,607,452 kg/year	\$2,334,504/year	(Humbird et al. 2011)
Maintenance ^g			\$168,136,513/year	
Labor cost				
Plant manager	\$50,000/employee/year	1 employee	\$50,000/year	Local price
Plant engineer	\$30,000/employee/year	2 employees	\$60,000/year	Local price
Maintenance supervisor	\$22,000/employee/year	1 employee	\$22,000/year	Local price
Maintenance technician	\$15,000/employee/year	12 employees	\$180,000/year	Local price
Lab manager	\$22,000/employee/year	1 employees	\$22,000/year	Local price
Lab technician	\$17,000/employee/year	4 employees	\$68,000/year	Local price
Shift supervisor	\$20,000/employee/year	4 employees	\$80,000/year	Local price
Shift operator	\$17,000/employee/year	28 employees	\$476,000/year	Local price
Yard employee	\$10,000/employee/year	4 employees	\$40,000/year	Local price
Clerk and secretary	\$17,000/employee/year	3 employees	\$51,000/year	Local price
Labor burden (90% of total salary)			\$944,100/year	
Total labor cost			\$1,993,100/year	
Total OpEX			\$170,129,613/year	
Revenue				
Ethanol	\$1.11/kg	200,000,000 kg/year	\$222,000,000/year	Current price
Dry yeast	\$0.5/kg	102,253,023 kg/year	\$51,126,512/year	Current price
Energy saving	\$0.1/kwh	206,959,062 kwh/year	\$20,695,906/year	Current price
Total revenue			\$293,822,418/year	
Net revenue^h			\$123,692,804/year	

a. The number was linearly scaled using the biomass feeding rate from the reference.

b. The number was linearly scaled using the ethanol production rate from the reference.

- c. The number was calculated based on the cost per kg water removal for a triple-pass rotary dryer.
- d. The number was linearly scaled using the steam demand from the reference.
- e. Utilities include equipment for water cooling/heating, electricity converter and transportation, and steam delivery etc.
- f. Additional direct costs include warehouse, site development, and additional piping. Indirect costs include field expenses, home office and construction, proratable costs, and other costs.
- g. The maintenance cost was set at 2% of total equipment cost without considering added direct and indirect cost.
- h. The net revenue = Total revenue – Total OpEx

Table 5. Sensitivity analysis of key CapEx, OpEx, and revenue items on the payback period of the biorefinery ^{a, b}

Item	Base value	Sensitivity range	Change on payback period (%)
CapEx of pretreatment/hydrolysis/condition	\$104,731,510	\$78,548,633 - \$130,914,388	±5
CapEx of boiler and turbo-generator	\$413,881,582	\$310,411,186 - \$517,351,977	±15
OpEx of sulfuric acid for pretreatment	\$43,408,000/year	\$32,556,000 - \$54,260,000	±8
Enzyme protein for hydrolysis	\$69,288,516/year	\$51,966,387 - \$86,610,645	±10
Revenue of ethanol ^c	\$222,000,000/year	\$166,500,000 - \$277,500,000	±30

- a. All values are adjusted by ±25% of their base values.
- b. The base payback period is 10 years.
- c. The revenue change of ethanol is from changes of ethanol production.

Figures

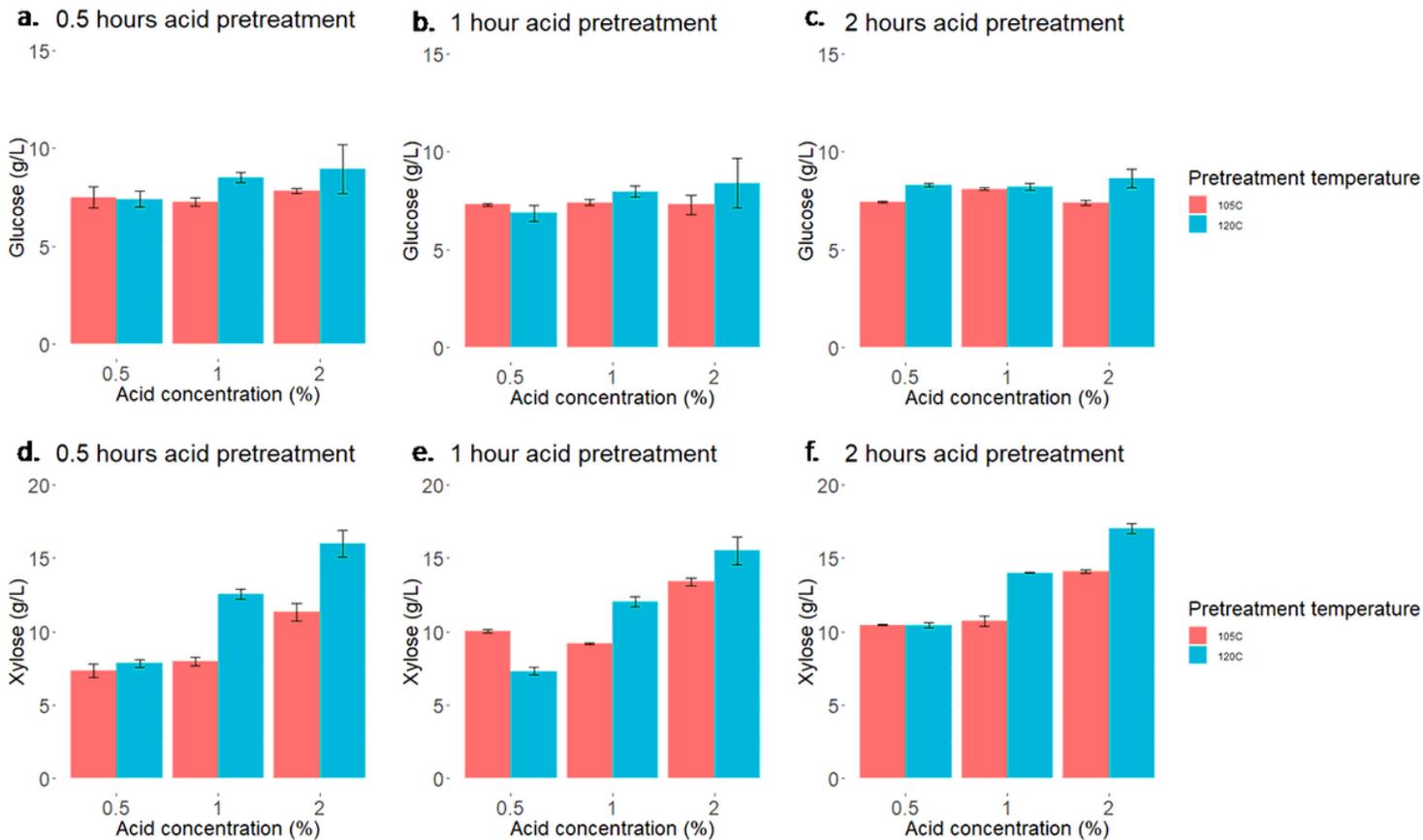


Figure 1

Effects of pretreatment conditions on mono-sugar release from *Quercus infectoria** *: The experimental runs were on the raw biomass at TS of 12.5%.

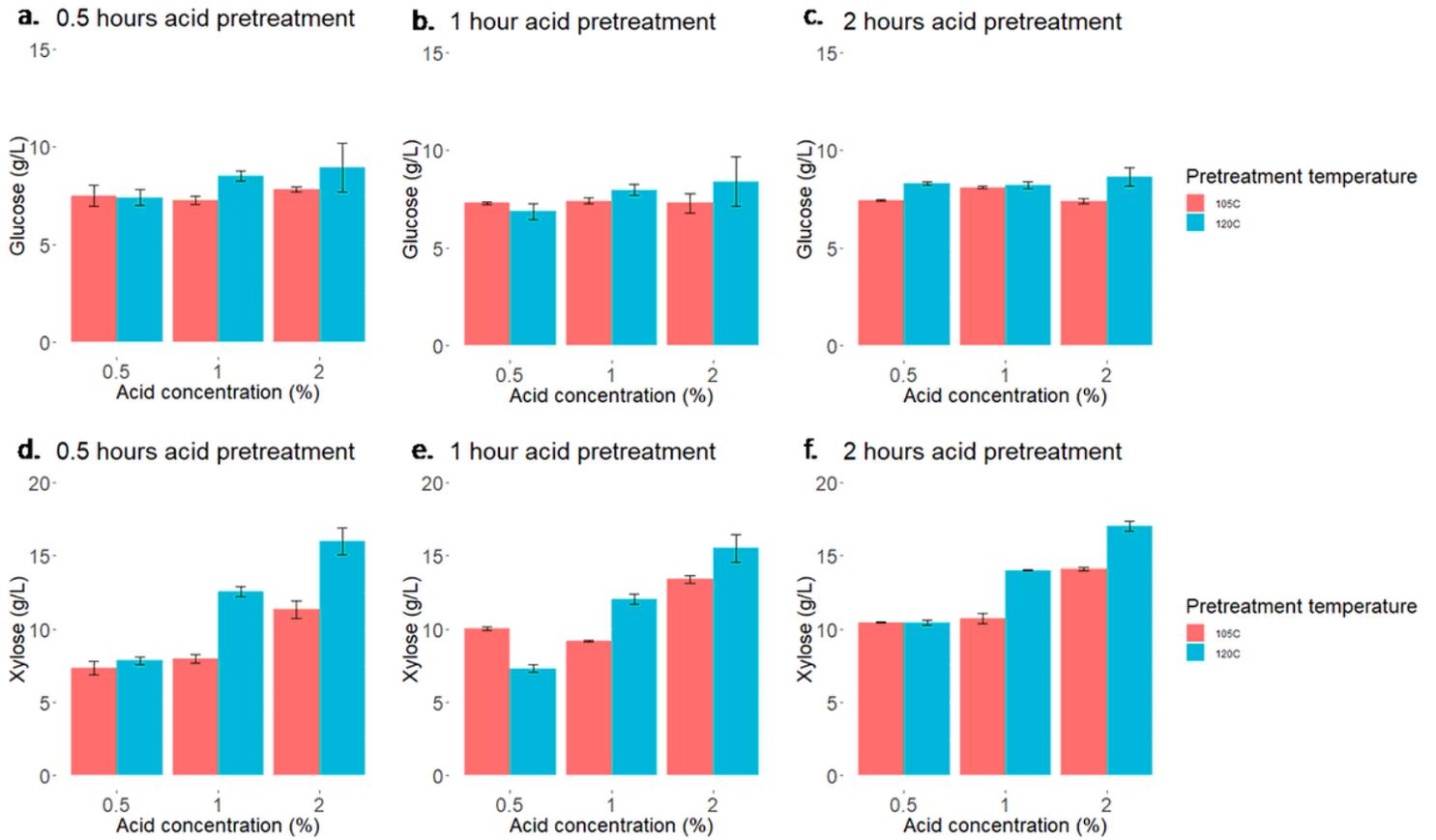


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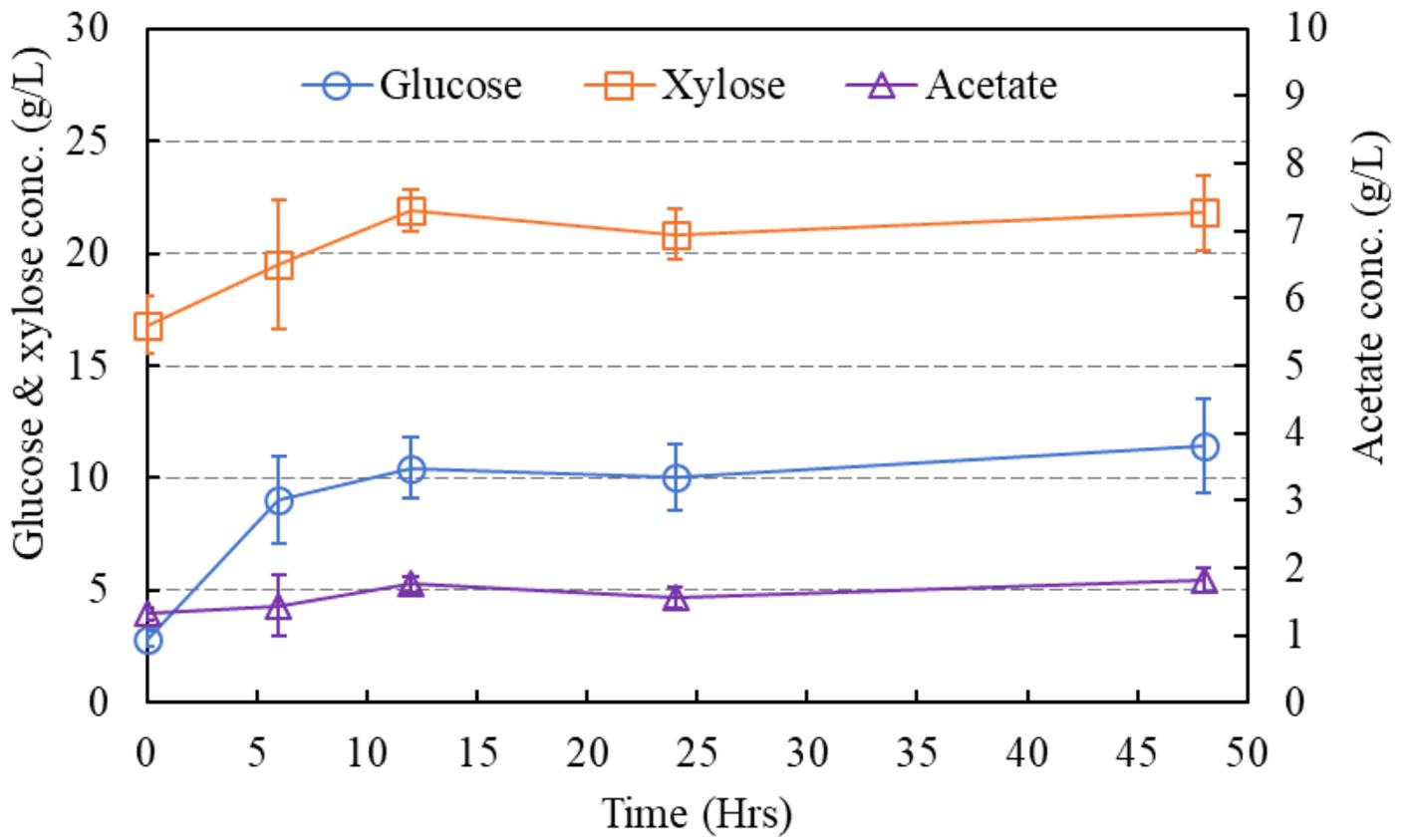


Figure 2

Enzymatic hydrolysis of the acid treated *Quercus infectoria** *: The experimental runs were on the raw biomass at TS of 15%.

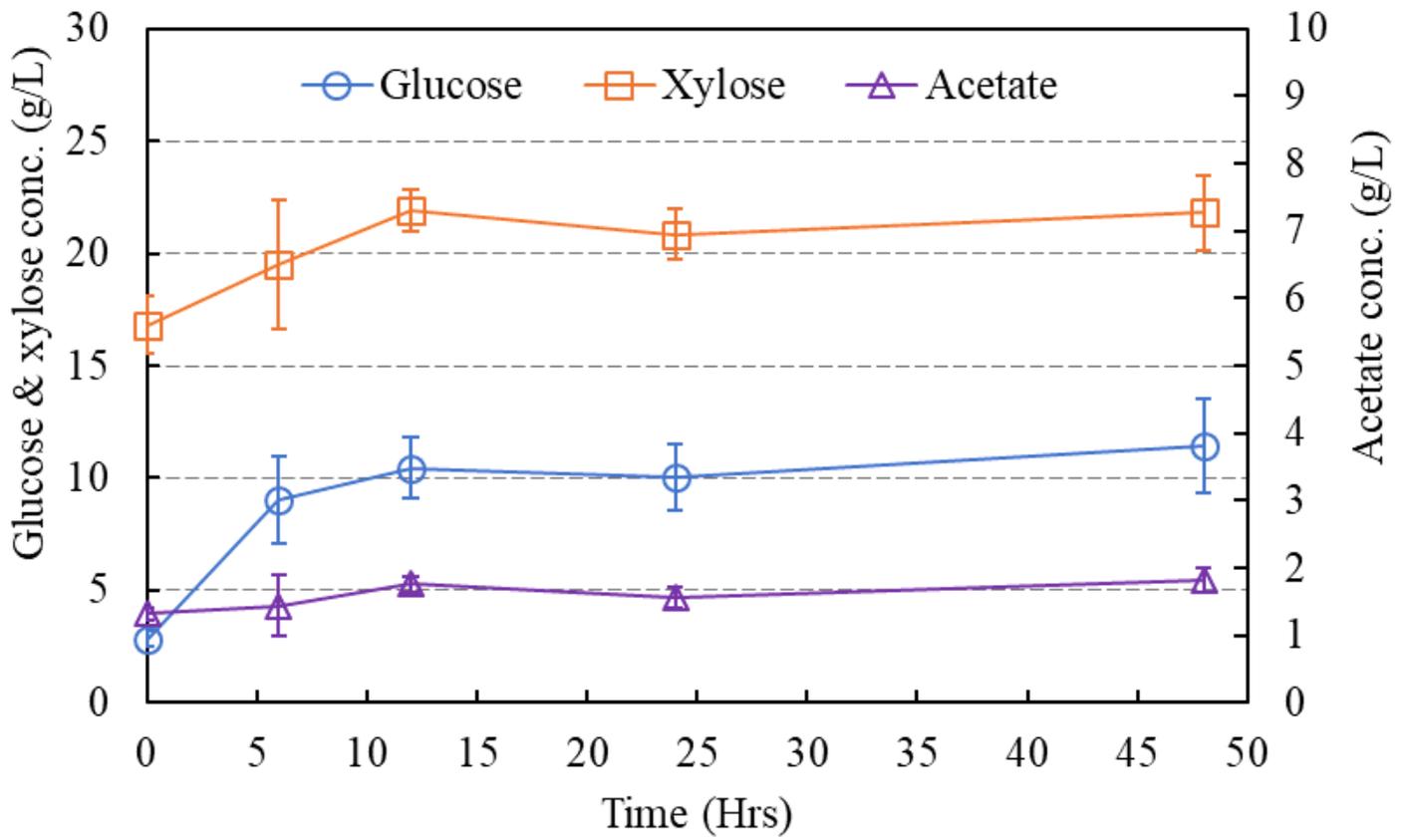


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Enzymatic hydrolysis of the acid treated *Quercus infectoria** *: The experimental runs were on the raw biomass at TS of 15%.

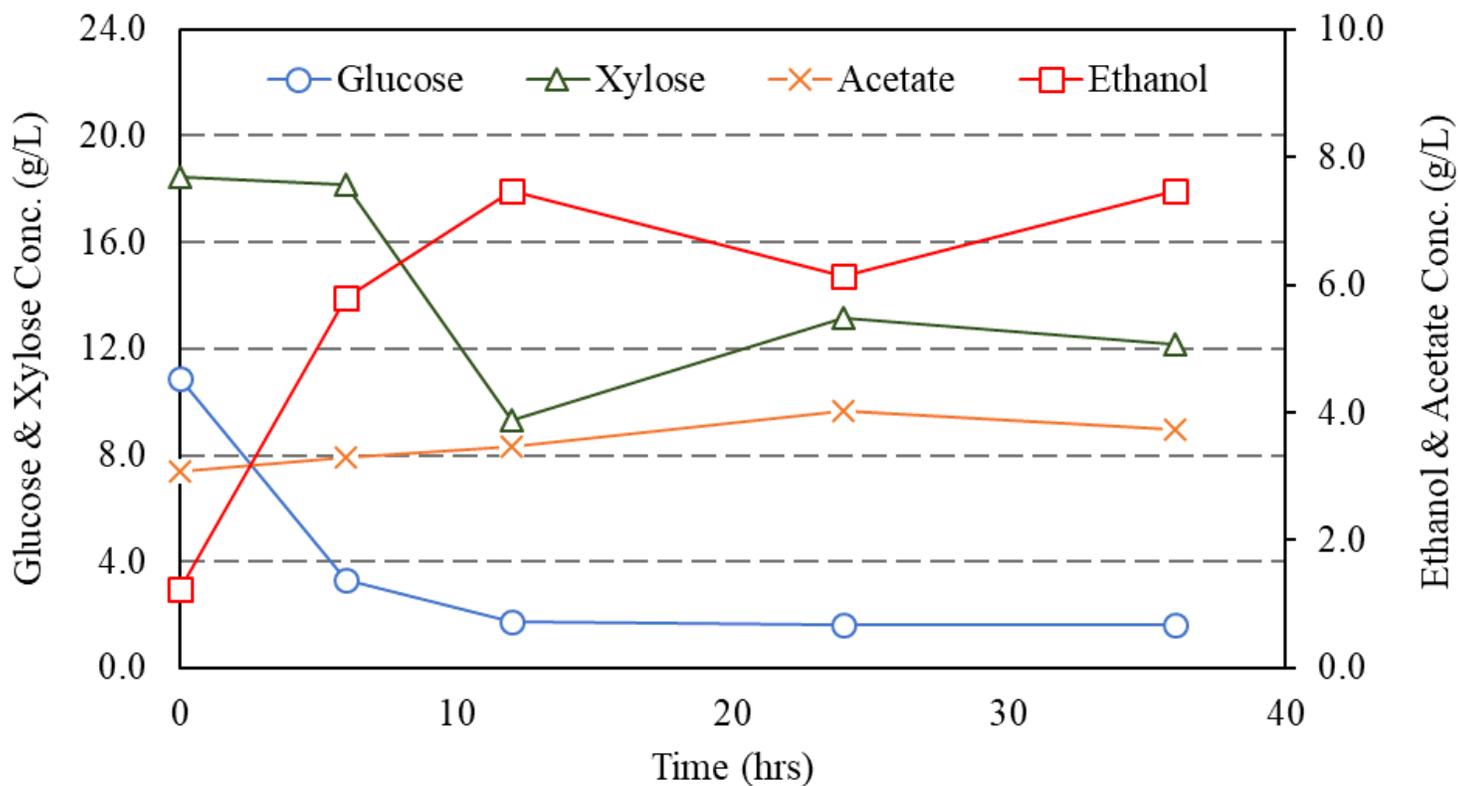


Figure 3

Ethanol production from the hydrolysate using *Kluyveromyces marxianus* (ATCC 12424)

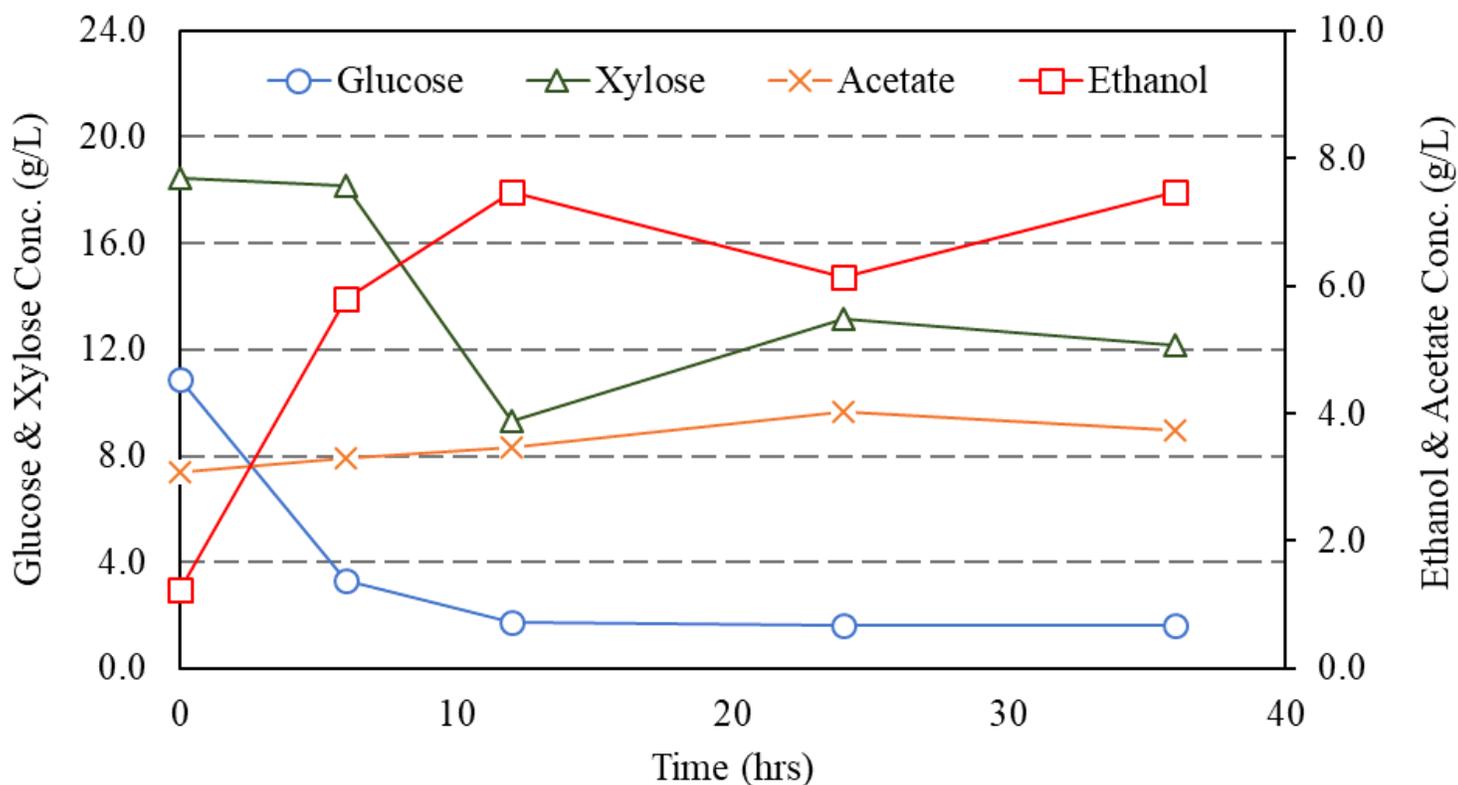


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Ethanol production from the hydrolysate using *Kluyveromyces marxianus* (ATCC 12424)

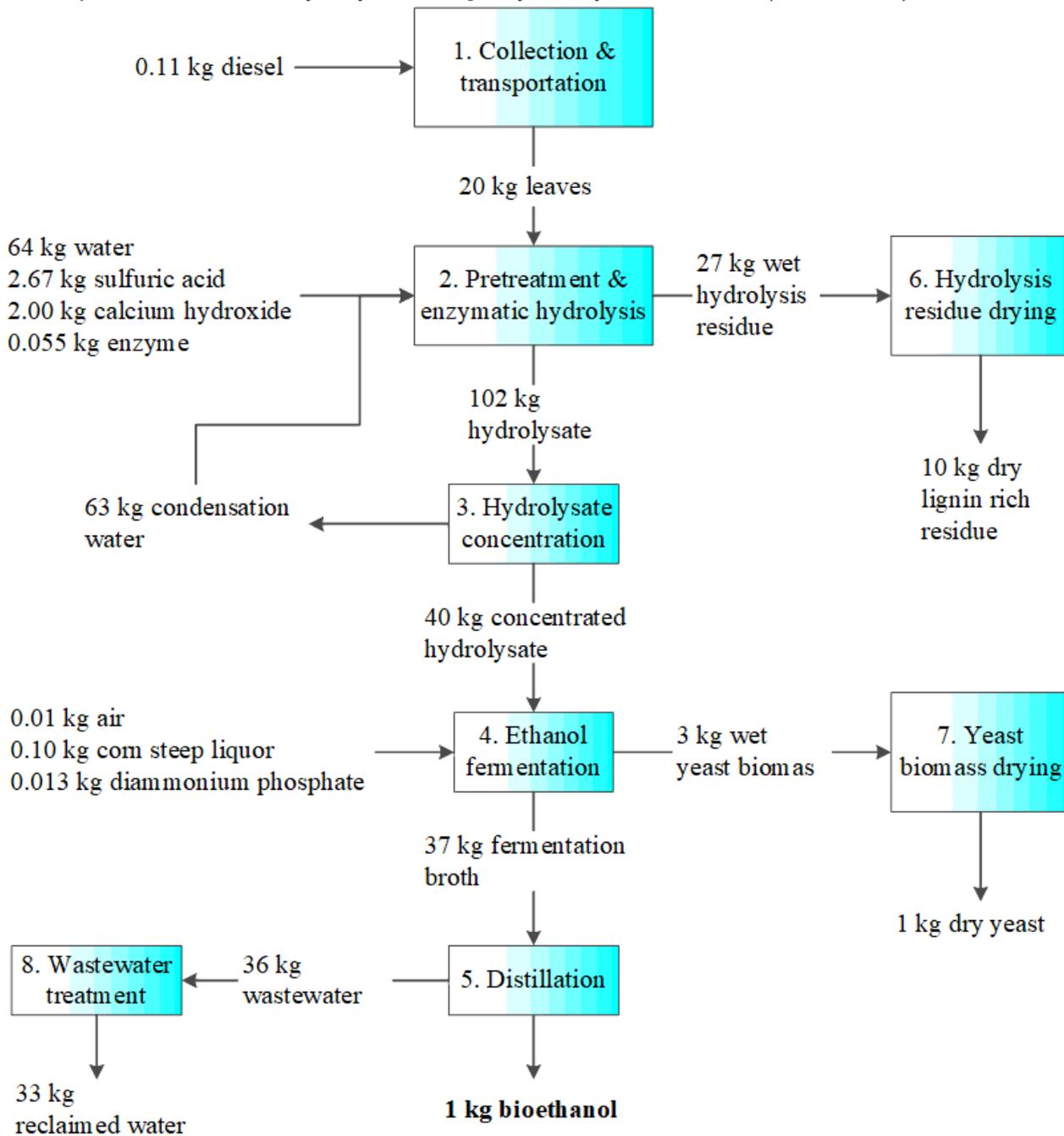


Figure 4

Mass flows of 1 kg bioethanol production from the leaves

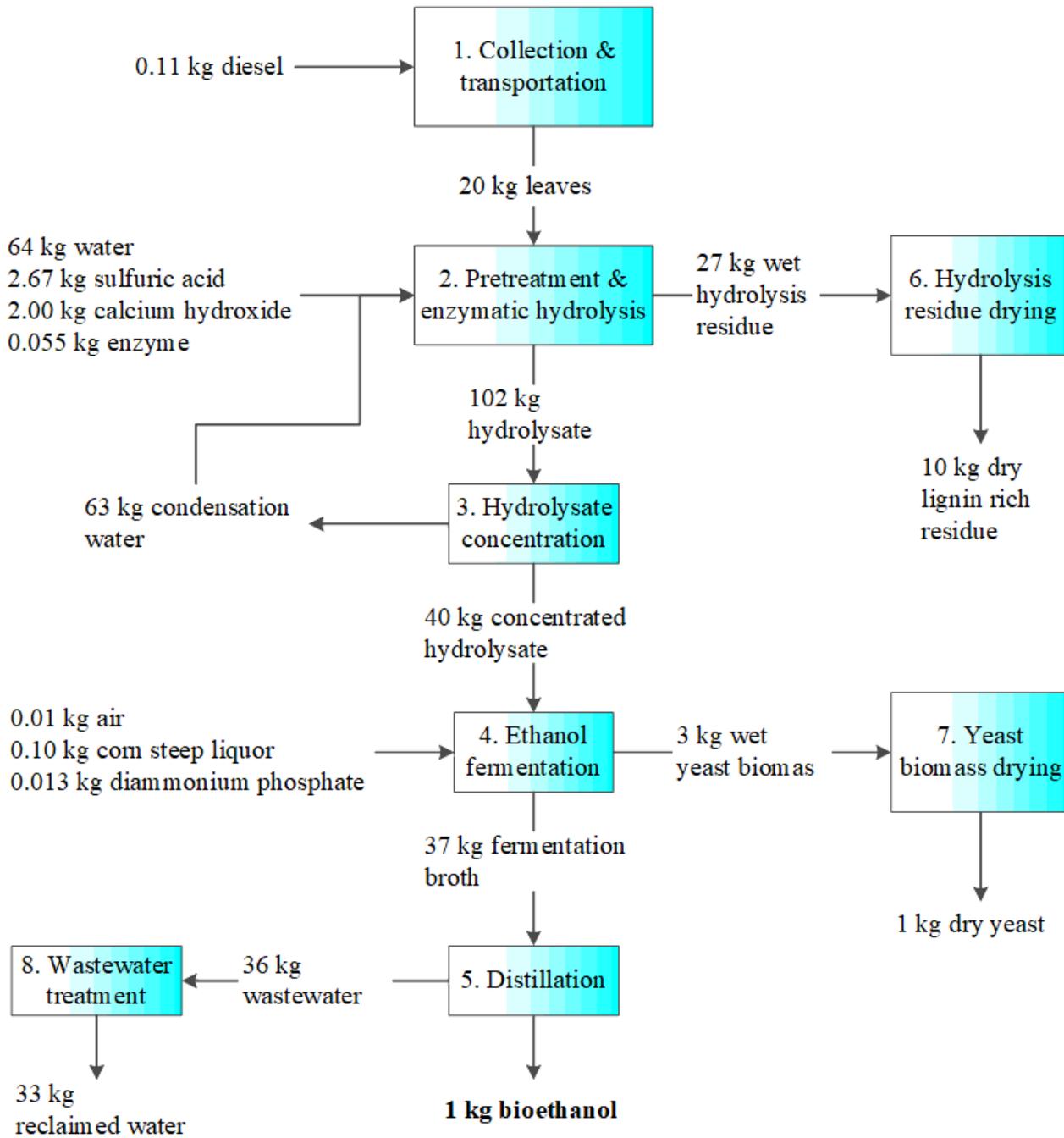


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Mass flows of 1 kg bioethanol production from the leaves

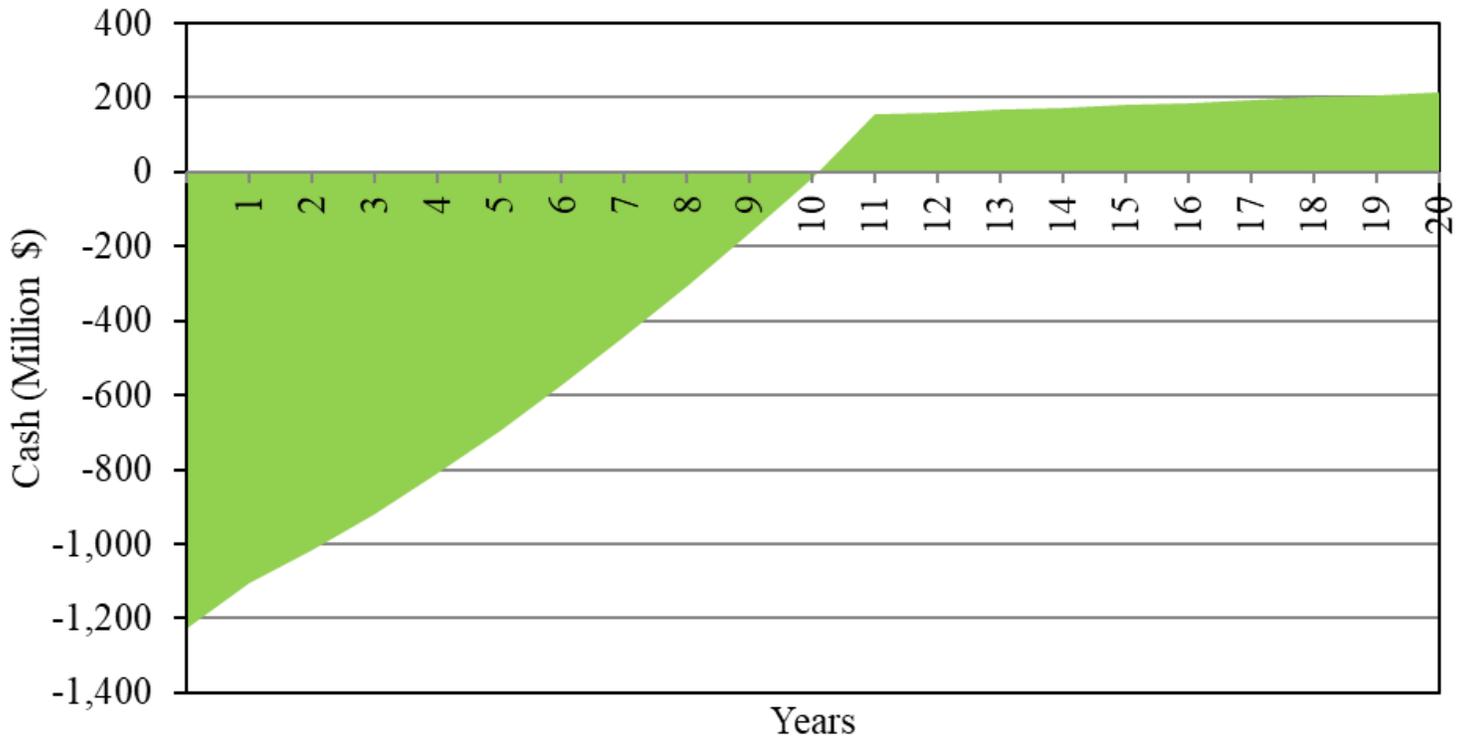


Figure 5

Cash flow of the bioethanol plant with a capacity of 200,000 kg ethanol per year from *Q. infectoria* leaves

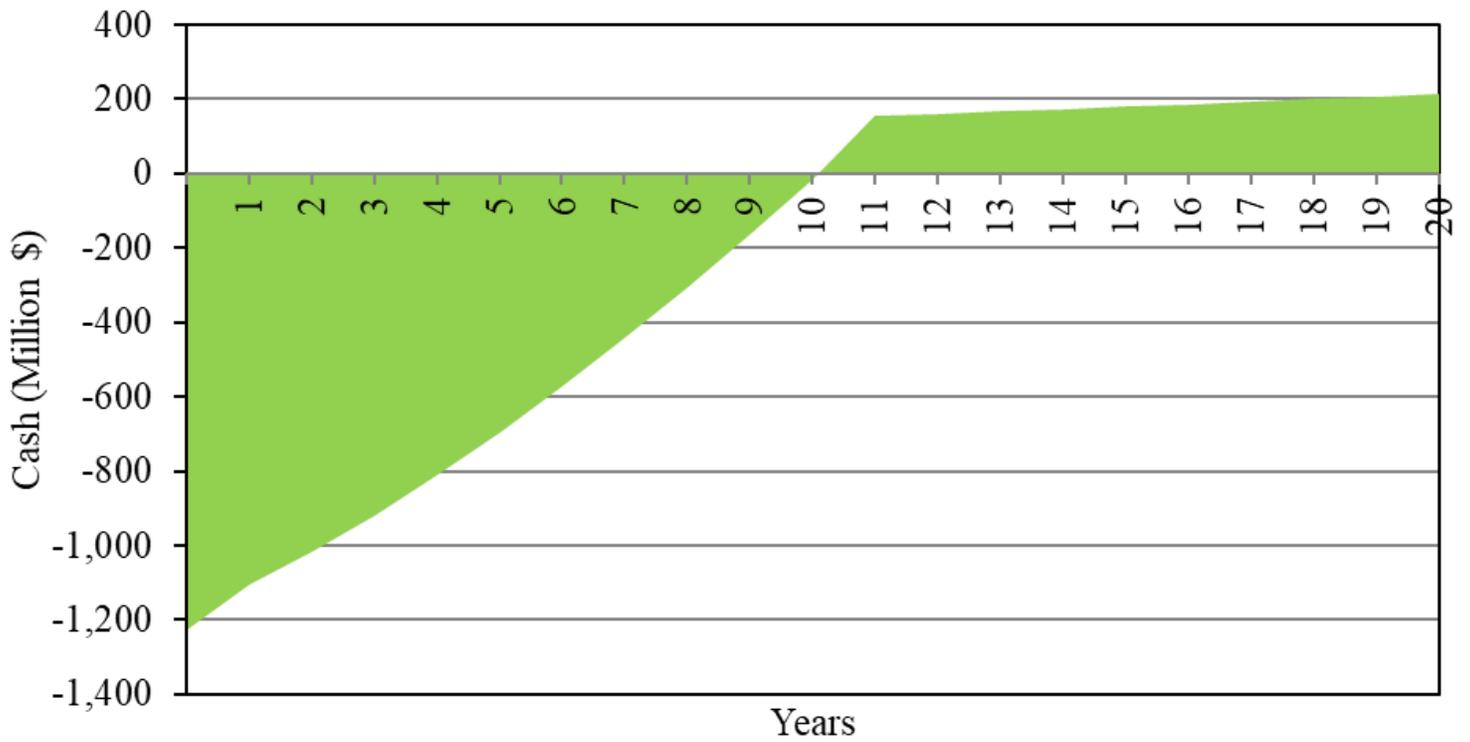


Figure 5

Cash flow of the bioethanol plant with a capacity of 200,000 kg ethanol per year from *Q. infectoria* leaves

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