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Research article

Bioethanol production from *Ficus religiosa* leaves using microwave irradiation

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ABSTRACT

A microwave assisted feasible process for the production of bioethanol from *Ficus religiosa* leaves was developed. Under the process conditions (8 min. microwave irradiation, 1 M HCl), 10.1 wt% glucose yield was obtained from the leaves. Microwave based hydrolysis process yielded higher glucose content (10.1 wt%) compared to the conventional hydrothermal process (4.1 wt%). Upon fermentation of the hydrolysate using Baker's yeast, 3 wt% (dry wt. basis) of bioethanol was produced.

1. Introduction

The demand for alternate transportation fuels is continuously increasing due to the limited and diminishing supply of fossil based fuels. Biomass is an attractive feedstock for the production of biofuels (Kumar et al., 2016). Today, energy crisis and environmental problems are the greatest challenges for the society. Huge demand for energy, limited resources, and severe environmental problems such as global warming and air pollution have motivated the researchers to develop green, non-polluting and sustainable energy sources (Klein et al., 2015a,b).

Bioethanol, produced from the fermentation of sugars, is a potential biofuel (Kang et al., 2014c). Bioethanol is a unique transportation fuel, with economic, environmental and strategic benefits. The octane boosting as well as the non-toxic properties of bioethanol are particularly appealing (Ali and Zulkali, 2013). Production of bioethanol using lignocellulosic agricultural residues (second generation biofuel) has increased over the past 25 years. Biomass is an abundant, cheap, renewable and sustainable feedstock for bioethanol (Machado and Atsumi, 2012).

Traditionally, bioethanol (first generation) is produced from agricultural crops containing high amount of carbohydrates (sugar cane, corn, sweet potato starch, wheat, cassava) (Kang et al., 2014a). In the US, the grain corn is the major source for ethanol production. Cane sugar is extensively used for bioethanol production in Brazil (Chen et al., 2013; Jung et al., 2013). Maize is another alternative energy crop which is currently considered as a feedstock for bioethanol production in the North Central and Midwest United States. As these processes compete with human consumable food sources and also occupies agricultural land, interest in the second (cellulose) and third (algal biomass) generation processes is growing (Baeyens et al., 2015; Kang et al., 2014b).

Lignocellulose is the primary component in plant cell walls with a complex and rigid structure. Typical constituents of biomass involve cellulose, hemicellulose and lignin. Cellulose is tightly bound to lignin and hemicelluloses through hydrogen and covalent bonding in the matrix structure of the cell wall (Klein et al., 2015a,b). This linkage between the components is sensitive to alkali solutions (Li and Kim, 2011). Chemical delignification is a process of partial or total removal of lignin from biomass making the cellulose more accessible to acid hydrolysis (Victor et al., 2016; Gierer, 2013; Sathitsuksanoh et al., 2011).

Saccharification (hydrolysis) is a process to break down cellulose into glucose. In the subsequent fermentation reaction, the

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fermentable sugars from cellulose (glucose) and hemicellulose (xylose and other C5 sugars) are converted to bioethanol (Victor et al., 2015). For the production of bioethanol various kinds of feedstock were used previously, like sugarcane bagasse, corn stover, wheat straw, barley straw, rice straw, cotton-based textile, maize and date syrup (Moradi et al., 2013; Hong and Sun, 2013; Marei, 2010). Bermuda grass (*Cynodondactylon*) was also considered as a promising feedstock for bioethanol (Machado and Atsumi, 2012).

Ficus religiosa is widely distributed around the globe. *Ficus religiosa* is an important traditional medicinal plant distributed throughout India (Gregory et al., 2013; Singh et al., 2013). *Ficus religiosa* emerged as a good source of traditional medicine for the treatment of several ailments (Singh et al., 2011, 2013; Haneef et al., 2012). The leaves that fall from the tree are generally a waste whose disposal is an issue from both environment and economic viewpoints. Moreover, the leaves are a rich source of holocellulose. Thus, the objective of the current study is to evaluate the feasibility of bioethanol production from the leaves of *Ficus religiosa* and this is the first ever study on this biomass.

2. Experimental

Dry leaves of *Ficus religiosa* fallen from the trees were collected on the grounds of Bar Ilan University, Israel. The dry leaves of *Ficus religiosa* were subjected to grinding in a mechanical blunder and sieved with a USA standard testing sieve to a mesh size of 250 μm. So the particle size of the biomass used for further studies is ≤250 μm. The obtained powder material was hydrolyzed under either hydrothermal or microwave irradiation conditions using HCl as catalyst.

The degree of crystallinity of the cellulose component in the powdered leaves was determined by X-ray diffraction (XRD) studies. The XRD patterns were collected using a Bruker AXS Advance powder X-ray diffractometer (Cu Kα radiation, λ = 1.5418 Å). The thermal stability of the leaves is deduced from thermogravimetric analysis (TGA). TGA analysis was carried out in the temperature range of 25–600 °C at a heating rate of 10 °C/min under Ar atmosphere using a Mettler TGA/STDA 851 device. The elemental analysis of the leaves (C, H, N and O) was carried out on C, H, N, S, O analyzer, Thermo Electron Corporation, Flash EA 1112 series (Italy).

Microwave irradiation was carried out in a modified domestic (Sharp model R390L(S)) microwave oven (Klein et al., 2012). The microwave oven was operated at 2.45 GHz in a batch mode under air at atmospheric pressure. The output of the microwave reactor was 1200 W. Typical hydrolysis in domestic microwave oven involved irradiation of leaves in the powder form (1.0 g) with 20 mL of 1 M HCl for different time periods (2–15 min) without stirring. The temperature at the end of the microwave reaction was measured immediately after the reaction by employing a pyrometer (Fluke, 65 Infrared thermometer) and varied in the range of 85–109 °C as the reaction time is increased from 2 to 15 min.

In addition to microwave irradiation, conventional hydrothermal method was also employed for the hydrolysis reaction. Hydrothermal hydrolysis was done in a stainless steel autoclave lined inside with polytetra fluro ethylene to resist corrosion by the acid catalyst. The volume of the hydrothermal reactor is 32 mL. Typical batch process of a hydrothermal reaction comprise of treating 1.0 g of leaves with 20 mL of 1 M HCl in the reactor without stirring. The effect of hydrothermal reaction conditions, namely, the time of heating (1–5 h) and the reaction temperature (80–120 °C) on the yield of glucose from *Ficus religiosa* leaves is studied.

Residual solid mass was separated from the hydrolysate by filtration through a filter paper (Whatman® 150 mm Φ), washed with excess distilled water and dried in an air oven at 120 °C overnight. The residual solid mass was calculated from difference in the weights of the initial and final amounts of the dry leaves (Ji et al., 2008). The filtrate was analyzed by ^{13}C NMR for fermentable sugars. The amount of glucose in the hydrolysate is determined by using a non enzymatic method based on the in situ generation of carbon nanoparticles from the glucose in the analyte (Pulidindi and Gedanken, 2014). Briefly, the pH of the hydrolysate was adjusted to 7. Then, 10 wt% urea was added. The analytes were treated at 120 °C for 20 min. in an autoclave (Tuttnauer cat 2007). The urea in the analyte under these conditions is converted to ammonia and the pH of the solution becomes basic. As a result, carbon nanoparticles from glucose are obtained and the solution turns to pale yellow color owning to the unique absorbing properties of the hydrophilic carbon nanoparticles. The UV absorbance measurements were done using a spectrophotometer (Varian Cary 100 scan UV/vis spectrophotometer) at 275 nm. For deriving a calibration plot, standard solutions containing known amount of glucose were prepared under identical conditions and subjected to the hydrothermal treatment along with the analytes.

Fermentation of the hydrolysate was carried out using Baker's yeast (*Saccharomyces cerevisiae*). Typical fermentation process comprise of taking 55 mL of neutral hydrolysate (from the hydrolysis of 3 g leaves) and 1.0 g of yeast in a 250 mL Erlenmeyer flask. The contents were incubated at 30 °C under shaking at 150 rpm. Aliquots of samples from the fermentation broth were collected at regular intervals of time. The ethanol amount in the broth was determined by ^{1}H NMR using D_2O as solvent and HCOONa as an internal standard. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance DPX 300 NMR spectrometer.

3. Results and discussion

A schematic representation of the methodology employed for the production for bioethanol from the leaves of *Ficus religiosa* is depicted in Scheme 1. The methodology adopted comprises of a direct acid hydrolysis of the leaves under either microwave irradiation or hydrothermal conditions followed by the fermentation of the sugars in the hydrolysate to bioethanol.

3.1. Characterization of the dry leaves of Ficus religiosa

3.1.1. X-ray diffraction studies

The wide angle X-ray diffraction pattern of native *Ficus religiosa* leaves is shown in Fig. 1.

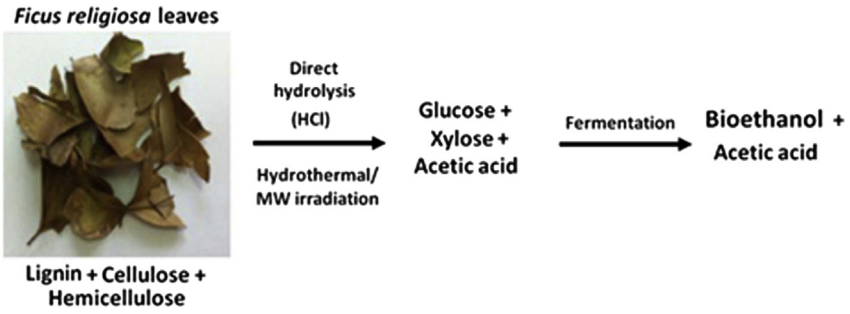
Peaks typical of holocellulose are observed at the 2θ values of 15, 22, 25 and 38°. The low angle reflections (15° and 22°) are broad whereas the reflections at 25 and 38° are sharp and intense. These reflections are attributed to amorphous ($I_{(am)}$) and crystalline components ($I_{(002)}$ and $I_{(040)}$) arising from hemicellulose and cellulose, respectively (Sathitsuksanoh et al., 2011; Obi Reddy et al., 2010).

The evaluation of degree of crystallinity, 34%, is based on the area under the peak indexed as (002). The cellulose fraction in the leaves is not highly crystalline compared to commercial cellulose samples. This result is in agreement with the crystallinity index values reported by Reddy et al. for *Ficus leaf fiber* (37.1%) (Obi Reddy et al., 2010). Lower the crystallinity, more feasible is the hydrolysis of cellulose to glucose (Zhang et al., 2013; Subhedar and Gogate, 2013).

3.1.2. Thermogravimetric analysis (TGA)

Thermal stability of the lignocellulosic material was evaluated from the thermal analysis of the leaves. Typical thermogravimetric curve (TGA) of *Ficus religiosa* leaves is shown in Fig. 2.

Primary weight loss (13%) observed at a temperature of 100 °C



Scheme 1. Schematic representation of bioethanol production from the leaves of *Ficus religiosa*.

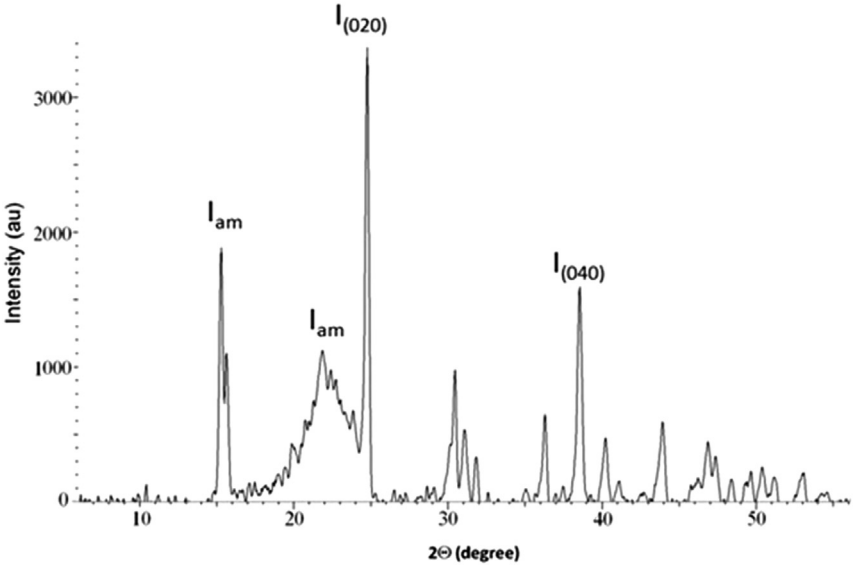


Fig. 1. X-ray diffraction pattern of *Ficus religiosa* leaves.

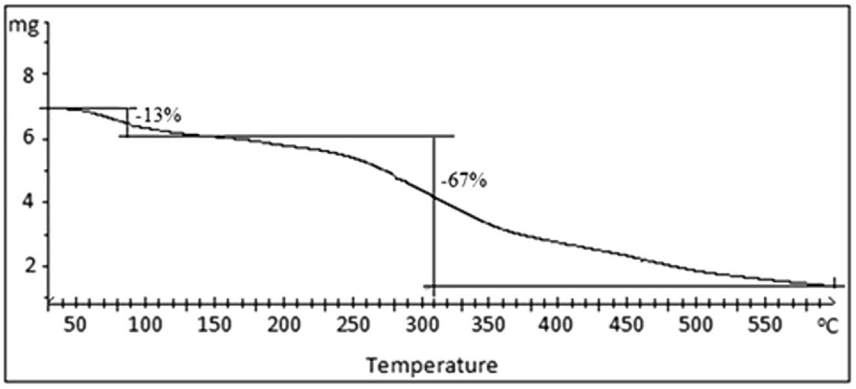


Fig. 2. Thermogravimetric curve of *Ficus religiosa* leaves under Ar at a heating rate of 10 °C/min.

was attributed to dehydration (loss of water molecules). A second and major weight loss (67%) occurred in the temperature range of 150–550 °C. This weight loss is attributed to the thermal decomposition of lignocellulosic material. As the hydrolysis temperature (80–120 °C) of the biomass is below the decomposition temperature (150 °C), *Ficus religiosa* leaves are an appropriate feedstock for glucose production under microwave irradiation/hydrothermal conditions. The 20% weight remaining after 550 °C is attributed to the char that formed as a result of thermal decomposition of the biomass. Similar thermal degradation features were observed earlier for cellulose and native *Ficus* leaf fiber (Obi Reddy et al., 2010; Kim et al., 2000).

3.2. Hydrolysis of Ficus religiosa leaves

3.2.1. Acid hydrolysis using microwave irradiation

The lignocellulosic biomass was subjected to acid hydrolysis (1 M HCl) using microwave irradiation for 10 min. The hydrolysate

was separated from the residual solid biomass by filtration. The filtrate containing the hydrolysis products was analyzed with ^{13}C NMR. The ^{13}C NMR spectrum showed peaks characteristic of glucose (60.3 (C6), 69.2 (C4), 72.4 (C2), 73.7 (C3), 75.3 (C5), 92.9 (C1 α) and 96.7 (C1 β)) (Fig. 3). In addition to glucose, xylose peaks (92.2 (C1α), 72.8 (C2α), 74.0 (C3α), 71.4 (C4α), 60.9 (C5α) and 96.6 (C1β), 75.8 (C2β), 75.8 (C3β), 69.2 (C4β), 65.1 (C5β)) were also found in the hydrolysis product (Victor et al., 2015).

The formation of glucose (C6 sugar) is due to the hydrolysis of cellulose. The formation of xylose (C5 sugar) is due to the hydrolysis of hemicellulose component. In addition to the release of the fermentable sugars, a peak at 21 ppm is also observed which is typical of acetic acid. Hemicellulose was identified as the source of acetic acid in the hydrolysate (Rackemann et al., 2014). As the hydrolysis reaction is carried out at a low pH (≪1, 1 M HCl), no detectable change in pH could be observed as a result of acetic acid formation.

Microwave irradiation accelerated the acid hydrolysis of cellulose (Avicel® PH-101) (Pulidindi et al., 2014). To examine the effect of the acid treatment, the leaves were treated only with 1 M HCl for 1 h under stirring at room temperature. The acid extract of the leaves was analyzed using ^{13}C NMR (Fig. S1). Major and intense peaks were observed at 43, 161 and 173 ppm. The peak at 161 is typical of formic acid. This is further substantiated by the presence of a peak (1H, s) at 8.2 ppm in the ^{1}H NMR typical of formic acid. In addition, some minor and less intense peaks are observed at 67, 70 and 73 ppm possibly attributable to soluble starch from the leaves. Unlike the microwave reaction which yielded glucose and xylose, no fermentable sugars are identified in the products of the reaction without microwave irradiation. Thus, microwave irradiation facilitates selective as well as high yields of glucose in cellulose hydrolysis. Fan et al. investigated the mechanism of interaction between cellulose and microwave irradiation leading to glucose production. It was reported that microwave irradiation weakens the hydrogen bond network by activating the CH_2OH pendant groups at high temperatures (>180 °C). At lower temperatures the amorphous regions of cellulose are activated leading to high glucose yields than possible under conventional hydrothermal heating (Fan et al., 2013).

3.2.2. Effect of time of microwave irradiation on the glucose yield

To evaluate the optimum hydrolysis time of irradiation, the biomass was irradiated for 5, 8, 10 and 15 min in the presence of HCl. The hydrolysates obtained were analyzed qualitatively using ^{13}C NMR and quantitative estimation of the yield of glucose is carried out using a non-enzymatic method (Pulidindi and Gedanken, 2014). Irrespective of the time of irradiation, all the hydrolysates contained glucose, xylose, acetic acid and formic acid. In the case of 10 and 15 min. of irradiation, in addition to the aforementioned products hydroxy methyl furfural (HMF) is also observed. HMF is known as a dehydration product of glucose (Table S1). The residual solid masses as well as the glucose yield values as a function of microwave irradiation time were shown in Fig. 4.

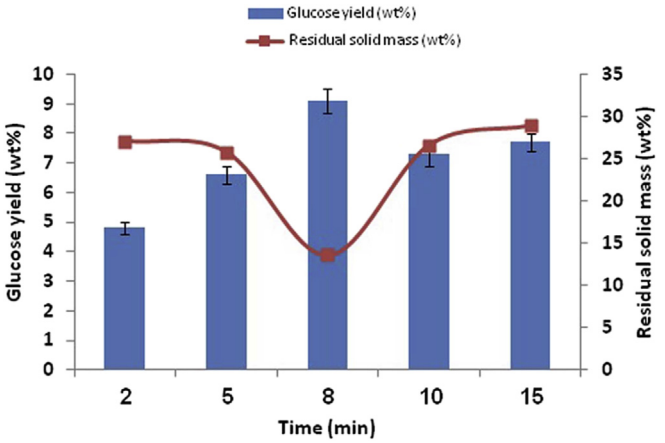


Fig. 4. Effect of microwave irradiation time on the glucose yield (wt%) and residual solid mass (wt%).

As the time of irradiation increased from 2 to 8 min. the yield of glucose value raised from 4.8 to 9.1 wt%. Correspondingly a decrease in the residual solid mass from 27 to 14 wt% is noticed. A maximum glucose yield value of 9.1 wt% was obtained for a microwave irradiation time of 8 min. Beyond 8 min. the residual solid mass gradually increased from 14 to 29 wt%. Under these reaction conditions the formation of water insoluble humins from the condensation of glucose is observed leading to an increase in the amount of the solid residue in the hydrolysate. Humin formation is reported to be the preferred pathway in the case of glucose relative to fructose in the presence of a Bronsted acid catalyst (Yang et al., 2012). In addition, beyond 8 min. of irradiation, dehydration product of glucose, HMF, is formed. For the selective formation of fermentable sugars the optimum time of irradiation is 8 min.

3.2.3. Effect of concentration of HCl on the microwave hydrolysis of *Ficus religiosa*

The microwave-assisted hydrolysis of the *Ficus religiosa* leaves

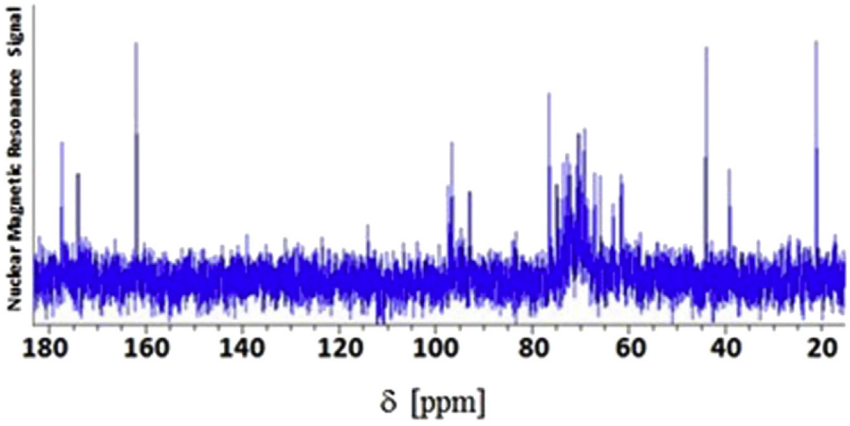


Fig. 3. ^{13}C NMR spectrum of the hydrolysate from *Ficus religiosa* leaves using microwave irradiation for 10 min. in 1 M HCl.

was carried out with different concentrations of HCl (0.5, 1, 2 and 3 M) for 8 min. The product selectivity as a function of HCl concentration was summarized in Table S2. The glucose yield and residual solid mass for different HCl concentrations is shown in Fig. 5.

Nearly 2 wt% increase in glucose yield is observed as the concentration of HCl is increased from 0.5 to 1 M. A raise of only 0.5 wt % in the glucose yield is observed as the HCl concentration is varied from 1 to 2 M, which is within the experimental error, which means that the conversion values remained unchanged with the increase in the HCl concentration beyond 1 M. At a high concentration of 3 M HCl, the sugars generated were converted to humins reducing the glucose yield. So, 1 M HCl was the optimum concentration for obtaining highest glucose yield (10.1 wt%) with lower amount of residual solid mass (13.7 wt%).

3.2.4. Acid hydrolysis of Ficus religiosa leaves using hydrothermal treatment

In addition to the microwave irradiation method, the effect of hydrothermal treatment on the hydrolysis of *Ficus religiosa* was also evaluated. The hydrothermal treatment was carried out at different reaction temperatures (80, 100 and 120 °C) for 1 h. The glucose yield and residual solid mass after the hydrolysis reaction were summarized in Table 1.

With an increase in the reaction temperature, an increase in the yield of glucose is observed. A maximum glucose yield value of 3.9 ± 0.2 wt% was obtained at 120 °C. The product distribution remained the same at different reaction temperatures except that at 120 °C, the dehydration product of glucose, HMF is formed. As the dehydration product of glucose appeared to form at 120 °C, higher reaction temperatures were not evaluated. Thus the optimum temperature of hydrolysis under hydrothermal conditions is 120 °C.

The hydrolysis reaction was further studied at 120 °C as a function of reaction time so as to see if the yield of glucose could be increased beyond 3.9 wt%. Even after a duration of 5 h of hydrothermal treatment at 120 °C, the glucose yield value remained at 4.1 wt% indicating that the optimum hydrolysis time is 1 h. Unlike the hydrolysis in hydrothermal treatment where the glucose yield is only 3.9 wt% (1 h), under microwave irradiation the glucose yield value as high as 10.1 wt% was achieved indicating the superiority of microwave irradiation compared to hydrothermal reaction which is attributed to the superheating (generation of hotspots) that could be achieved using microwave irradiation. Thus, microwave irradiation is used effectively for the production of glucose from *Ficus religiosa* leaves in a fast hydrolysis process (Klein et al., 2012).

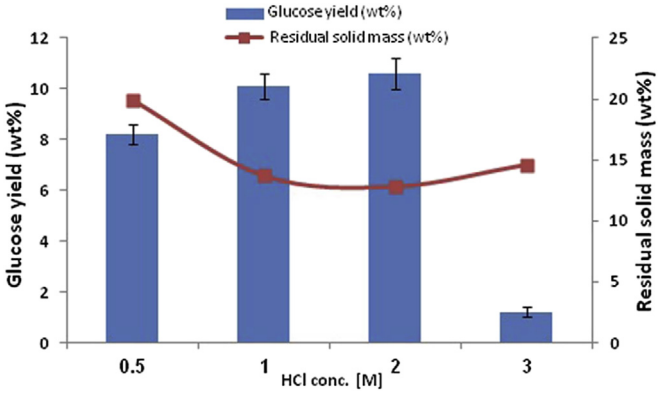


Fig. 5. Effect of HCl concentration on the glucose yield (wt%) and residual solid mass (wt%) (8 min. microwave irradiation).

3.3. Fermentation of the neutral hydrolysate of Ficus religiosa leaves

The fermentation of the hydrolysate obtained from the acid hydrolysis under optimal reaction conditions (3 g of biomass, 60 mL 1 M HCl, 8 min. of microwave irradiation) was carried out in an incubator at 30 °C. Aliquots of samples from the fermentation broth were collected at regular intervals of time and the amount of ethanol in the analyte was quantified using ^{1}H NMR. The amount of bioethanol produced as a function of time is depicted in Fig. 6.

The optimum duration of fermentation was 8 h resulting in maximum ethanol yield (90 mg or 3 wt% on dry mass basis). Signals characteristic of bioethanol, 3H (t) at 1.19 ppm and 2H (q) at 3.66 ppm, were observed in the ^{1}H NMR spectrum (Fig. S2). Beyond 8 h of fermentation, the yield of ethanol leveled off and no subsequent increase in the amount of ethanol was observed. The lower yield of ethanol (3 wt%) could be due to the fact that the leaves are used as such without any pretreatment (delignification). Direct hydrolysis of the lignocellulosic biomass resulted in the formation of acetic acid (from hemicellulose), which is inevitably present in the hydrolysate along with the fermentable sugars. The peak at 21 ppm (^{13}C NMR) is typical of the presence of acetic acid in the hydrolysate (Fig. 3). Acetic acid is a well-known inhibitor of fermentation reaction (Huang et al., 2011). The inhibition of the fermentation reaction is further confirmed by the presence of fermentable sugars (signals in the range of 60–100 ppm) in the broth collected at the 8 h of fermentation. Intense signals at 17 ppm ($-CH_3$) and 58 ppm ($-OCH_2$) are characteristic of the formation of bioethanol (Fig. S3).

A comparison of the ethanol yield values from *Ficus religiosa* relative to other biomass namely de-oiled rice barn and sugar beet pulp is made. Beliya et al have reported a 10–11 wt% yield of bioethanol from the de-oiled rice bran (Beliya et al., 2013). Rezic et al. have obtained 10 wt% bioethanol yield from sugar beet pulp (Rezic et al., 2013). Victor et al produced 5.7 wt% bioethanol from pretreated pine cones (Victor et al., 2015). The relatively low yield of 3 wt% ethanol from *Ficus religiosa* could also be due to the difference in the nature of biomass type as well as methodology adopted by Beliya et al. and Rezic et al. relative to the current process. Beliya et al. and Rezic et al. have employed enzymatic process of hydrolysis. As the hydrolysis process was based on enzymes, the process is obviously slow and expensive compared to the hydrolysis process employed in the current work which is relatively inexpensive (as HCl is used in the place of enzymes) and much faster due to the use of microwave irradiation. The drawback of lower yield of ethanol could be compensated by the faster and inexpensive nature of the methodology suggested here.

4. Conclusion

The studies demonstrated that the waste leaves of *Ficus religiosa* could be a potential feedstock for the production of bioethanol because of the abundance and wide global distribution. Such a process will be both environmentally friendly, in terms of waste management, regulating CO_2 emissions, and also an economically viable alternative. It was elucidated that the waste leaves of *Ficus religiosa* could yield 3 wt% of ethanol without any pretreatment or delignification. Further studies in the direction of biomass pretreatment and use of solid acid catalyst could develop a potential process for the sustainable ethanol production for transportation applications.

Compliance with ethical standards

All the authors of the manuscript disclose that they do not have

Table 1
Effect of temperature (hydrothermal treatment) on the hydrolysis of *ficus religiosa leaves*.

Temperature (°C)	Yield of glucose (wt%)	Residual solid mass (wt%)	Products
80	1.5 ± 0.1	71.7 ± 2.2	Glucose, xylose, formic acid, acetic acid
100	1.6 ± 0.1	72.9 ± 1.8	Glucose, xylose, formic acid, acetic acid
120	3.9 ± 0.2	73.8 ± 1.7	Glucose, xylose, HMF, formic acid, acetic acid

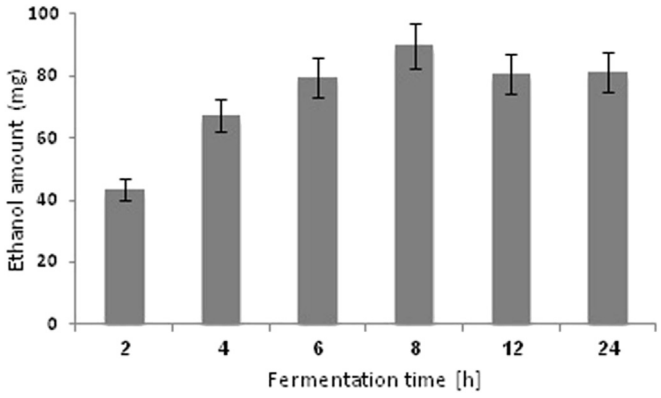


Fig. 6. Production of bioethanol from the hydrolysate of *Ficus religiosa* leaves (microwave treatment) as a function of fermentation time.

any potential conflict of interest.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jenvman.2016.03.050.

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