Novel methods for the conversion of biomass to bioethanol

שיטות מתקדמות להיפוך ביומסה לביואתנול

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Renewable energy

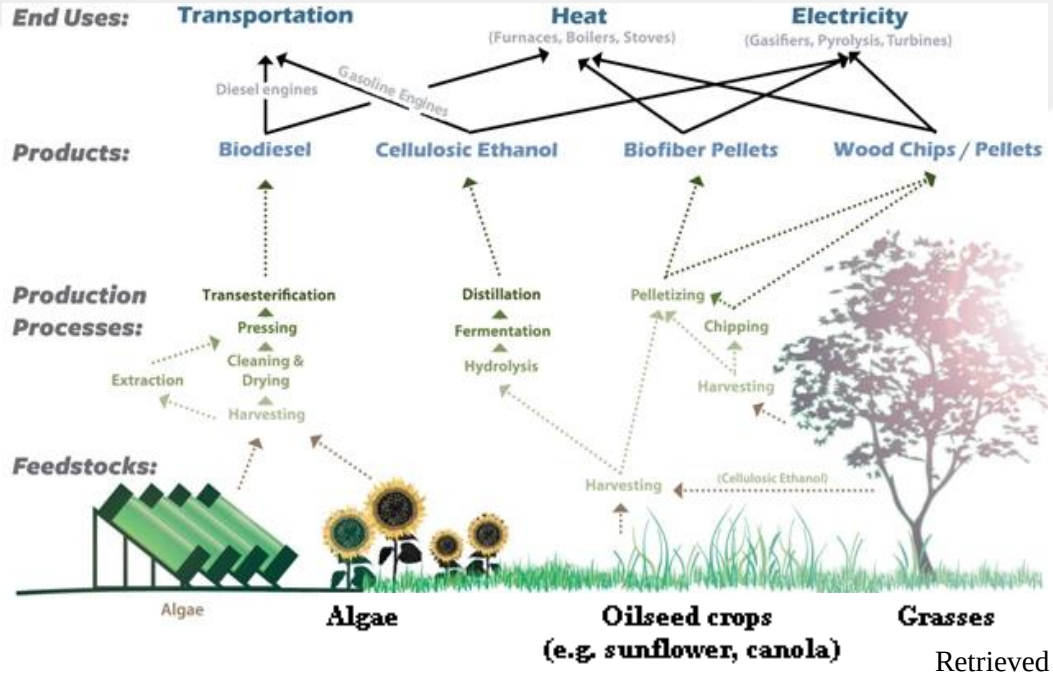
- In 1973, over 86 % of the world's total primary energy supply came from fossil fuels and in 2007, it was still over 81 % (gas: 20.9 %; oil: 34 %; coal: 26.5 %)
- Numerous experts predict that oil production will reach a ceiling by 2020, while the demand will continue to grow
- Concerns are increasing about climate change and the potential economic and political impact of limited oil and gas resources
- Due to the depletion of fossil fuels, renewable energy sources are vigorously being investigated
- Biofuels are potential substitutes for current transportation fuels

Retrieved from: http://www.staffordshireplumbingandheating.co.uk/renewable-energy-systems.html

https://usgbccrc.site-ym.com/store/ViewProduct.aspx?id=836649

Biofuels

- Biofuels from biomass significantly reduce dependence on imported oil and decrease the environmental impact of energy use
- Major commercial biofuels include bioethanol and biodiesel
- Brazil and the US started producing 1st-generation bioethanol and biodiesel from food products (grains and edible oil) and they are currently available at petrol stations
- 2nd-generation bioethanol from ligno-cellulosic materials is still under research
- The potential global production of bioethanol from waste crops and crop residues is estimated at 491 GL $year^{-1}$ which could replace 32 % of total gasoline consumption

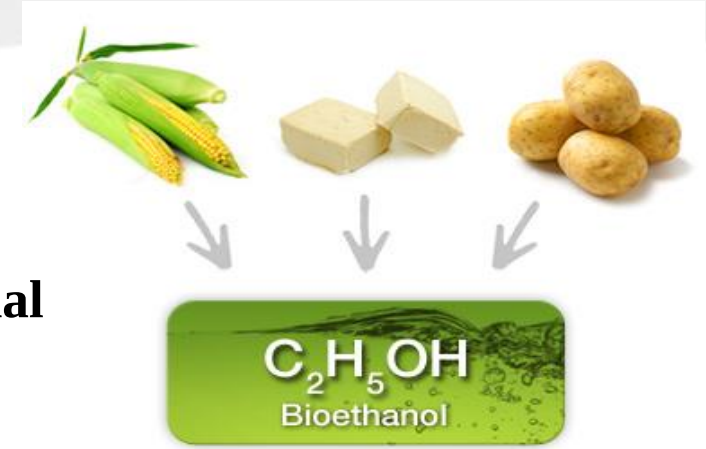


Retrieved from: http://www.vsjf.org/projects/what-are-biofuels

Bioethanol

- Bioethanol is one of the most promising alternatives to conventional transportation fuels because of its high octane value and high combustion efficiency
- It is carbon neutral on a lifecycle basis, meaning the CO_2 emitted during its use is counterbalanced by the absorption from the atmosphere during its growth
- It can be blended with petrol or used as neat alcohol in dedicated engines
- In comparison to gasoline, it contains only a trace amount of sulfur; therefore, ethanol blended with gasoline helps to decrease the overall emission of SOx
- Currently, it is the most dominant biofuel and its global production has shown an upward trend over the last 25 years

- **The total output in 2015 is expected to be more than 115 billion liters annually. Therefore, any breakthrough in bioethanol production is beneficial to the socio-economic well-being of humankind**



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Feedstocks for bioethanol production

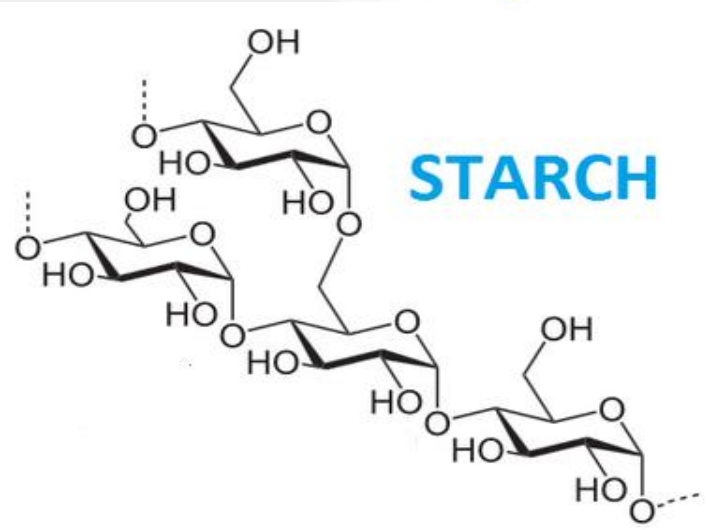
- Two processes are involved in the conversion of biomass to ethanol: the degradation of starting plant material into fermentable sugars and the conversion of sugar into alcohol (fermentation).8 Fermentation is a vital stage in bioethanol production and selection of suitable feedstock for fermentable sugars is a challenge. Homogenous crop materials are easily metabolized to sugars (e.g. molasses from sugar cane, starch from corn kernels).6, 8 The varied raw materials used in the manufacture of ethanol via fermentation are conveniently classified into three main types of raw materials: sugars, starches, and cellulose materials.9

Sugars

- Glucose
- Sucrose
- Molasses

Starch

- Most agricultural biomass containing starch can be used as a potential substrate for the ethanol fermentation by microbial processes. These substrates include corn, wheat, oats, rice, potato, and cassava. On a dry basis, corn, wheat, sorghum, and other grains contain around 60-75 % (wt./wt.) of starch and they offer a good resource in many fermentation processes.13 Fermentation of starch is more complex than fermentation of sugars because it must first be converted into sugar and then into ethanol. Starch is first hydrolyzed by adding α-amylase to avoid gelatinization, and then cooked at high temperature (140-180°C). Next, the liquefied starch is hydrolyzed to glucose with glucoamylase. The resulting dextrose is fermented to ethanol with the aid of microorganisms producing CO2 as a coproduct.9

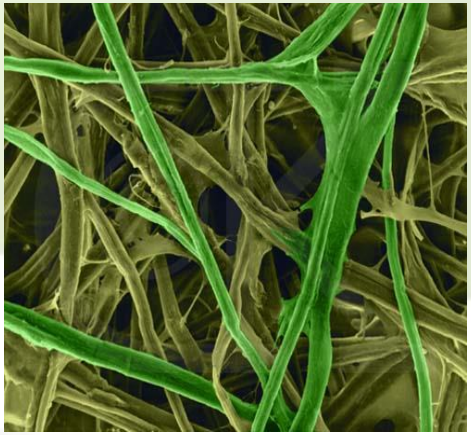


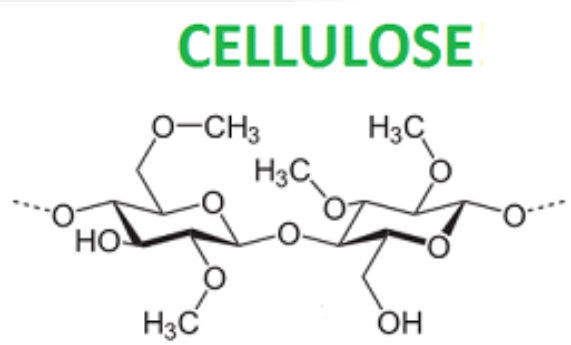
Retrieved from: http://sites.psu.edu/cellulosicbiofuels/2013/12/02/cellulose-to-ethanol-molecules-enzymes-chemical-reactions/

http://creepypasta.wikia.com/wiki/File:Corn.jpg

Cellulose

- Among the three main types of raw materials, cellulose materials represent the most abundant global source of biomass and have been largely unutilized. Over 90 % of the global production of plant biomass is lignocellulose and it is a more complex substrate than starch. It is composed of a mixture of carbohydrate polymers (cellulose and hemicellulose) and lignin. The biological process for converting the lignocellulose to fuel ethanol requires delignification to liberate cellulose and hemicellulose from their complex with lignin, depolymerization of the carbohydrate polymers to produce free sugars, and fermentation of mixed hexose and pentose sugars to produce ethanol. Extensive research has been carried out in this field and the first demonstration plant using lignocellulosic feedstocks has been in operation in Canada since April 2004.14 It is expected that the cost of lignocellulosic ethanol can undercut that of starch-based ethanol because low-value agricultural residues can be used.9





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http://bioserv.fiu.edu/~walterm/human_online/chemistry/water_and_molecules/water_and_organic_molecules.htm

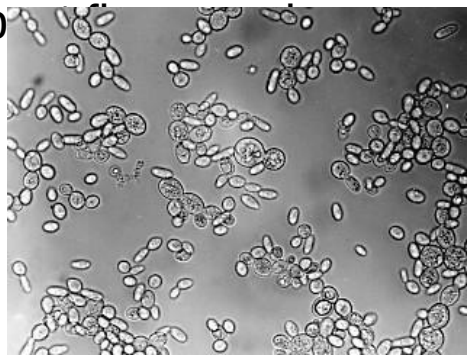
Marine macroalgae (seaweed)

- Seaweeds can serve as a promising sustainable source for biofuels due to their extremely high growth rates, low energetic demands, and high carbohydrate contents. Algal-based biofuel, as opposed to terrestrial biomass sources, does not compete with the cultivation of food crops, or freshwater supply, nor adversely affect vulnerable ecosystems. Thus developing novel approaches to produce biofuel from macroalgal biomass may have tremendous impact as a future renewable energy source.

Retrieved from: http://www.dreamstime.com/royalty-free-stock-photo-aquarium-fish-seaweed-image8094885

Yeast

- Bioethanol production using various substrates by free or immobilized cells of bacteria (*Clostridium sp.*) or yeasts (*Saccharomyces sp., Zymomonas sp.*) has been studied intensively over the past two decades. *Saccharomyces cerevisiae* is a facultative anaerobe able to live on various fermentable and non-fermentable carbon sources. When yeast is grown on fermentable substrates such as glucose, the metabolic energy essentially originates from glycolysis.16 *Saccharomyces cerevisiae* is one of the most effective ethanol-producing microorganisms for hexose sugars including glucose, mannose, and galactose. It is yeast with high ethanol productivity, high tolerance to ethanol, and also to the inhibitory compounds present in the hydrolysate of lignocellulosic biomass.17
- *Saccharomyces cerevisiae* is one of the most commonly used yeast in microbiology, and it was actually the first eukaryote to have its entire genome sequenced.18 There are many different strains of *Saccharomyces cerevisiae* and those that are used for baking are different from those used in brewing, and thus are cultured on different substrates. The enzymes produced by the yeast are involved in complex chemical reactions during fermentation and include not only the formation of different alcohols, but also organic acids [illegible] from strain to strain.



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http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/Y/Yeast.html

Research objectives

- 1. Designing a solar radiation based fermentation reactor for the continuous production of ethanol from biomass
- 2. Evaluating the kinetics of fermentation process using Ultraturrax
- 3. Developing a flow system for the continuous production of bioethanol from monosaccharides in the presence of yeast and soft sonication
- 4. Using fermentable sugars produced from renewable feedstocks, such as marine algea, sugar cane bagasse, and cotton for bioethanol production under sonication, microwave irradiation, and solar radiation
- 5. Experimenting on different yeast strains to enhance the ethanol yield (we propose to evaluate wine yeast as a substitute to currently used Baker's yeast)
- 6. Up-scaling the production of sugars from biomass and also the conversion of fermentable sugars to bioethanol

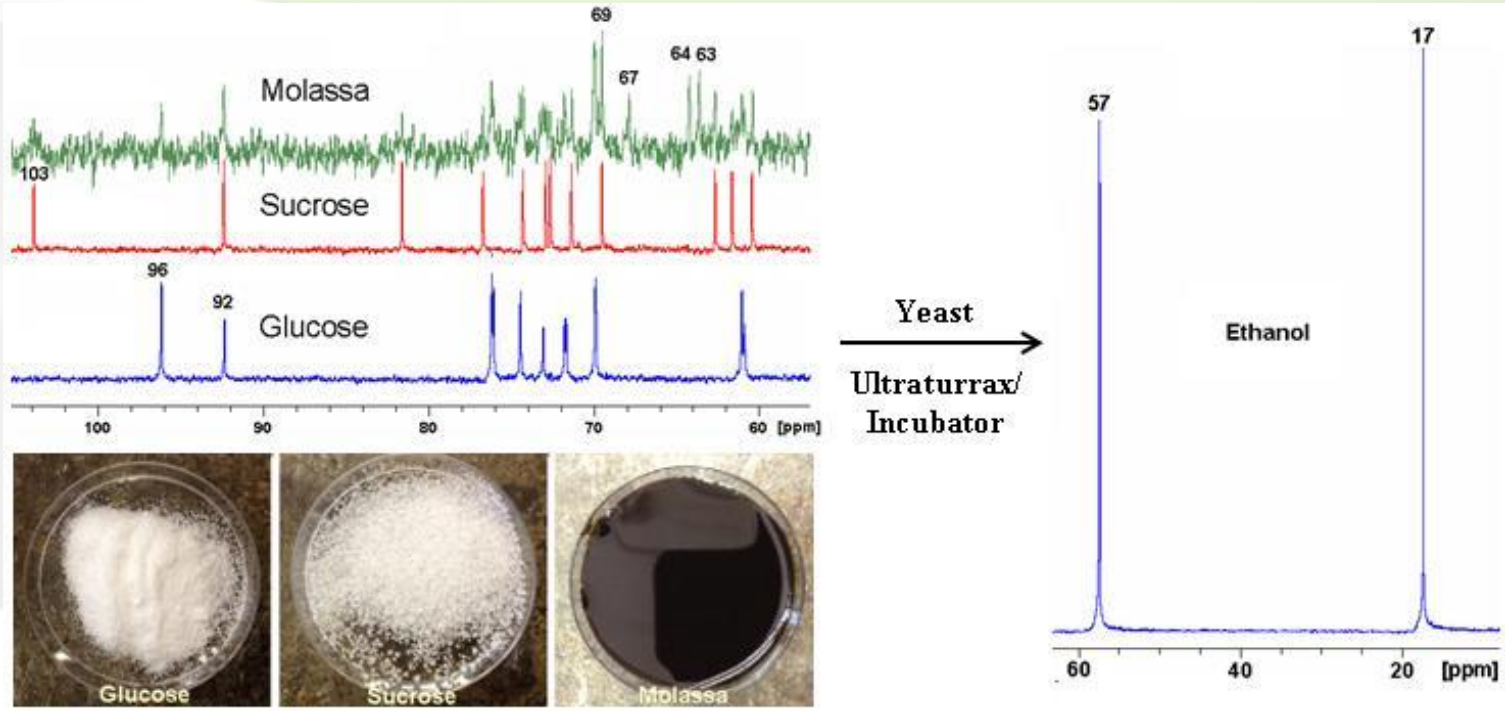
Expected significance

- The significance of the research will be developing novel processes for bioethanol production from renewable sources. Another important outcome of this research will be the development of a commercially viable process for the production of bioethanol. It should be noted that bioethanol could not only be blended with gasoline but also be converted to C2 hydrocarbons which indicates the possibility of complete replacement of oil by the biofuels (bioethanol and biobutanol).

Fermentation in batch cultures

- For all experiments, fermentation was performed with *Saccharomyces cerevisiae*, commercial Baker's yeast.
- Fermentation reactions were performed in 250 mL Erlenmeyer flasks and the flasks were closed with cotton plugs.
- The fermentation broth for both glucose and sucrose fermentation typically comprised 20 g (or 40 g for the 40 wt. % solution) of glucose or sucrose dissolved in 100 mL of water to which 2 g (or 4 g for the 40 wt. % solution) of yeast was added. For molasses fermentation, the broth typically comprised 10 g molasses dissolved in 100 mL of water with 1 g of yeast.
- To evaluate the effect of agitation speed on fermentation, the flasks were placed in a high speed stirrer ultraturrax device (Leroy Somer, Digidrive SK, make ESCO-LABOR).
- As control experiments, the fermentation reactions were performed in an incubator (MRC, LM-570, Orbital shaker incubator) without shaking.
- The fermentation reactions were performed at 30 °C and the effect of the stirring speed on the kinetics of fermentation was evaluated by NMR spectroscopy .

Bioethanol production from various carbon precursors



- Ethanol production was evaluated using glucose as a model carbohydrate
- In addition to glucose, the feasibility of sucrose and molasses as carbon sources for the production of bioethanol was also evaluated by using an ultraturrax
- Here, we report the effect of varying the following parameters: agitation speed, mechanical shaking, the effect of shelf-life of yeast, pH of the broth, and effect of additives such as activated carbon on the fermentation kinetics

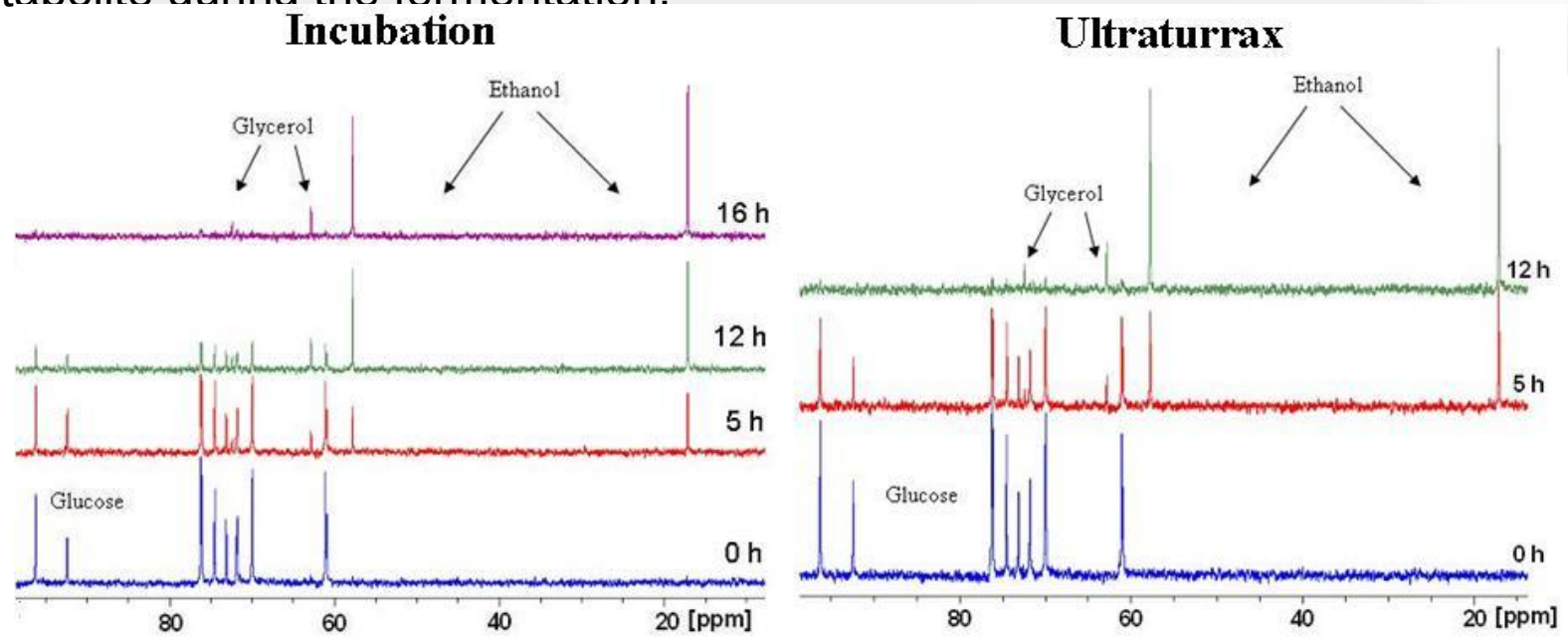
Effect of agitation speed on the rate of glucose fermentation

- The glucose (20 wt. %) fermentation was performed using an ultraturrax device at different shaking speeds. Mechanical agitation was found to have an accelerating effect on the rate of fermentation. Relative to a stand-still reaction in an incubator, mechanical agitation at 10 000 rpm showed twofold enhancement (kultraturrax,10 000/kincubator = 2) in the reaction rate constant value (at 30 ºC). The values of the reaction rate constants as a function of stirring speed are summarized in Table 1 and 10 000 rpm was found to be the optimum shaking speed where the k value was maximum.

Shaking speed (rpm)	Reaction rate constant, k (x 10^{-5} s^{-1})
0	1.9
5 000	1.8
10 000	3.8
20 000	4.2

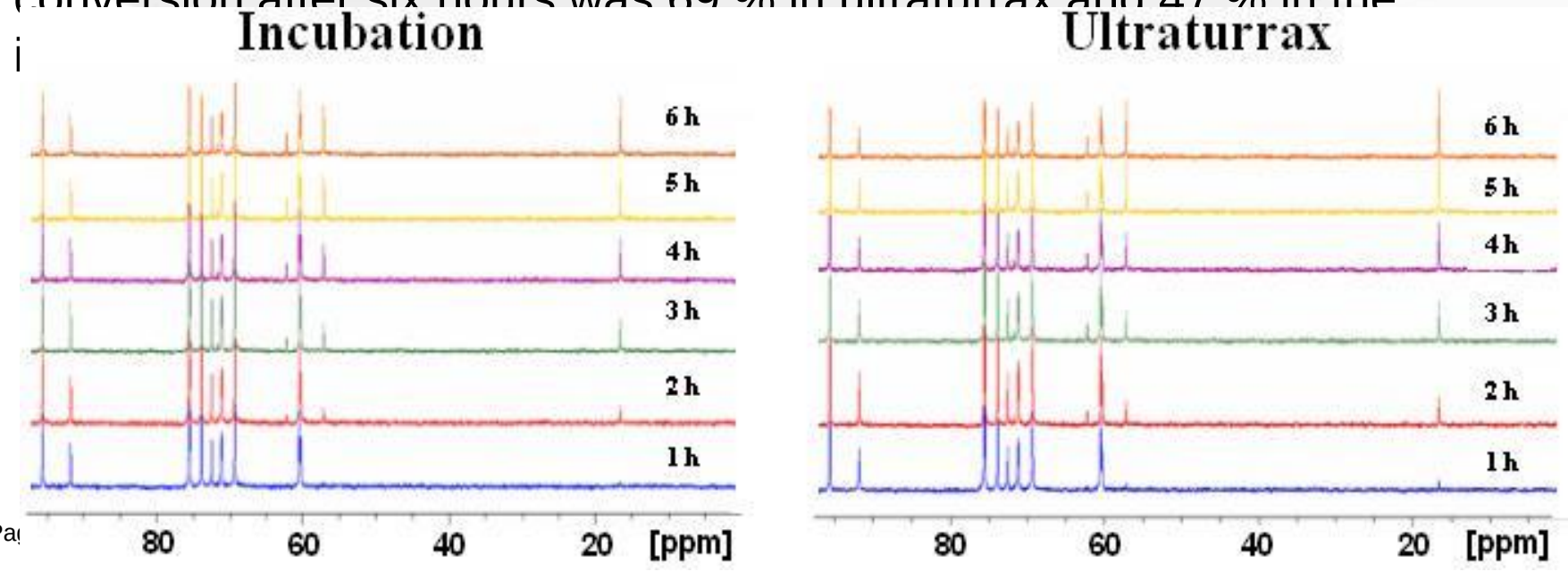
Monitoring complete glucose fermentation

- The entire glucose (20 wt. %) fermentation was monitored. As a function of time, the intensity of the peaks corresponding to ethanol (17 and 57 ppm) increased and the intensity of peaks corresponding to glucose (60.9 (C6), 69.9 (C4), 71.7 (C2), 74.4 (C3), 76.0 (C5), 92 (C1, β) 96 (C1, α) ppm) decreased. No trace of glucose was observed in 12 h and 16 h samples collected from the broth in ultraturrax and incubation, respectively, which indicated the completion of the fermentation reaction. With the use of an ultraturrax (at 30 °C) at 10 000 rpm, the time required for the completion of the fermentation reaction was reduced from 16 h to 12 h. In addition to ethanol, a negligible amount of glycerol (62.8 and 72.3 ppm) was also formed as a secondary metabolite during the fermentation.



Effect of substrate (glucose) concentration on the rate of fermentation

- In addition to 20 wt. % glucose concentration, the fermentation was also performed with 40 wt. % glucose concentration. The objective of the study was to verify whether in high glucose concentrations, which usually inhibit yeast performance, the fermentation rate was also accelerated using an ultraturrax. Ethanol concentration greater than 4 % will have a poisoning effect on yeasts and induce stress by retarding their productivity of further ethanol production.32 The relative reaction rate constant value (kultraturrax/kincubator) for the fermentation of glucose (40 wt. %) in an ultraturrax vs an incubator (at 30 ºC) was 1.7 (Fig. 7) and the glucose conversion after six hours was 69 % in ultraturrax and 47 % in the i



Evaluation of sucrose and molasses as feedstock for ethanol production

- Sucrose is a disaccharide composed of glucose and fructose. Relative to glucose, sucrose as an extract from sugar cane is more available. Molasses is a by-product of the cane sugar manufacturing process. Typical molasses is composed of sucrose, glucose, and fructose. Industrial-grade ethanol, a key product in the conversion of sugars and starches into energy and chemical feedstocks, is produced in India exclusively through the fermentation of sugarcane molasses using yeasts.33 The effect of mechanical agitation on sucrose and molasses fermentation was also evaluated. Aliquots of samples from the fermentation broths (maintained in an ultraturrax and in an incubator) were collected at regular time intervals and the 13 C NMR analyses were done. The effect of mechanical agitation on the fermentation rates is summarized in Table 2.

Carbohydrate precursor	Sucrose	Molasses
Ultraturrax	$9.1 \times 10^{-5}\ s^{-1}$	$5.5 \times 10^{-5}\ s^{-1}$
Incubation	$5.2 \times 10^{-5}\ s^{-1}$	$2.6 \times 10^{-5}\ s^{-1}$
$k_{ultraturrax}/k_{incubation}$	1.7	2.1
Comparison with glucose fermentation	2.4 times faster	1.4 times faster
Temperature	30 °C	30 °C

Evaluation of kinetics of fermentation using ^{13}C NMR

- Typical peaks of D-glucose include 60.9 (C6), 69.9 (C4), 71.7 (C2), 74.4 (C3), 76.0 (C5), 92 (C1, β) 96 (C1, α). Ethanol yielded two characteristic peaks at 17 ppm and 57 ppm. The ratio of intensity of peak of ethanol (at 17 ppm) to that of glucose (at 96 ppm) was found to increase with time during the fermentation process. This ratio was employed as a measure of the conversion of glucose (Eq. 2).
- Conversion of glucose (wt %) = (*I*E, 17 ppm / 2 *I*G, 96 ppm) x 100 (Eq. 2)
- where *I*E is the intensity of the ethanol peak at 17 ppm and *I*G is the intensity of the glucose peak at 96 ppm.
- Sucrose resulted in a spectrum with peaks at 103.9, 92.4, 81.6, 76.7, 74.3, 72.9, 71.3, 69.5, 62.6, 61.6, and 60.4 ppm. The ratio of the intensity of peaks of ethanol (at 17 ppm) to that of sucrose (at 92 ppm) was found to increase with time during the fermentation process and was employed as a measure of the conversion of sucrose (Eq. 3).
- Conversion of sucrose (wt %) = (*I*E, 17 ppm / 4 *I*S, 92 ppm) x 100 (Eq. 3)
- where *I*E is the intensity of the ethanol peak at 17 ppm and *I*S is the intensity of the sucrose peak at 92 ppm.
- Molasses resulted in a spectrum with peaks at 98.4, 96.2, 92.4, 81.6, 76.2, 76, 72.9, 71.8, 71.35, 69.9, 69.5, 67.9, 64.2, 63.6, 62.6, 61, and 60.4 ppm. The ratio of the intensity of peaks of ethanol (at 17 ppm) to that of molasses (at 70 ppm) was found to increase with time during the fermentation process and was employed as a measure of the conversion of molasses (Eq. 4).
- Conversion of molasses (wt %) = (*I*E, 17 ppm / 4 *I*M, 70 ppm) x 100 (Eq. 4)

- where *I*E is the intensity of the ethanol peak at 17 ppm and *I*M is the intensity of the

Calculation of the reaction rate constant

- The conversion (wt.%) values of glucose, sucrose, and molasses as a function of time obtained from 13C NMR analysis were inserted into the first-order reaction rate equation (Eq. 5) to obtain the reaction rate constant as a function of time.
- $k = (2.303 / t) \log [a / (a-x)]$ (Eq. 5)
- where t is the time, a is the initial concentration of glucose, and x is the conversion of carbohydrate (glucose, sucrose or molasses) at time t.

Effect of yeast shelf-life on the kinetics of glucose fermentation

- To evaluate the effect of yeast shelf-life on fermentation kinetics, glucose (20 wt. %) fermentation was performed with fresh (up to one month) as well as old (six months) yeast. The fermentation was performed using an ultraturrax at 5 000 rpm and at 10 000 rpm. The k values calculated from each experiment are summarized in Table 3. Using yeast with long shelf-life reduced the fermentation rate. Long shelf-life, nearly six months, was found to decelerate the fermentation rate by almost 3-fold and 5.6-fold compared to fresh yeast when the stirring speeds were 5 000 rpm and 10 000 rpm, respectively. Thus, yeast shelf-life is an important parameter that affects the fermentation rate. Although dry yeast can be stored at room temperature and performs well for the duration of the package shelf-life, it will always lose some of its viability and activity over time. However, at colder temperatures these losses are less compared to warmer temperatures

Stirring speed (rpm)	Yeast	Reaction rate constant, k (x 10^{-5} s^{-1})	k_{fresh}/k_{old}
5 000	Old	0.61	2.95
	Fresh	1.8	
10 000	Old	0.68	5.6
	Fresh	3.8	

Effect of additives on the glucose fermentation rate

- To investigate whether the addition of activated carbon alters the kinetics of fermentation of glucose (20 wt. %), 2 g of activated carbon was added to the broth
- The reaction rate was found to decelerate 1.5-fold with the addition of activated carbon ($k_{without\ additive}/k_{with\ additive} = 3.8x10^{-5}\ s^{-1}/2.6x10^{-5}\ s^{-1}$)
- The reason for the deceleration is a drastic pH decrease from 6.8 to 2 caused by the addition of the activated carbon. To attribute this deceleration to the decrease in pH, the pH of the broth after the addition of activated carbon was raised to 6.8
- At a pH of 6.8, in the presence of the additive, the rate (4.4x10-5 s^{-1}) was similar to the rate without any additives (3.8x10-5 s^{-1}). Thus, the presence of activated carbon had no significant effect on the rate if the pH remained unaltered
- Due to the presence of surface oxygen-containing functional groups such as –COOH, which are formed as a result of the activation process of the carbon material, the activated carbon is acidic
- The pH of the neutral glucose solution (pH = 6.7) decreased to 2.2 solely due to the addition of activated carbon which verifies the inherent acidity of activated carbon. Therefore, the decrease in pH was not due to the presence of the yeast

Effect of pH on the kinetics of glucose fermentation

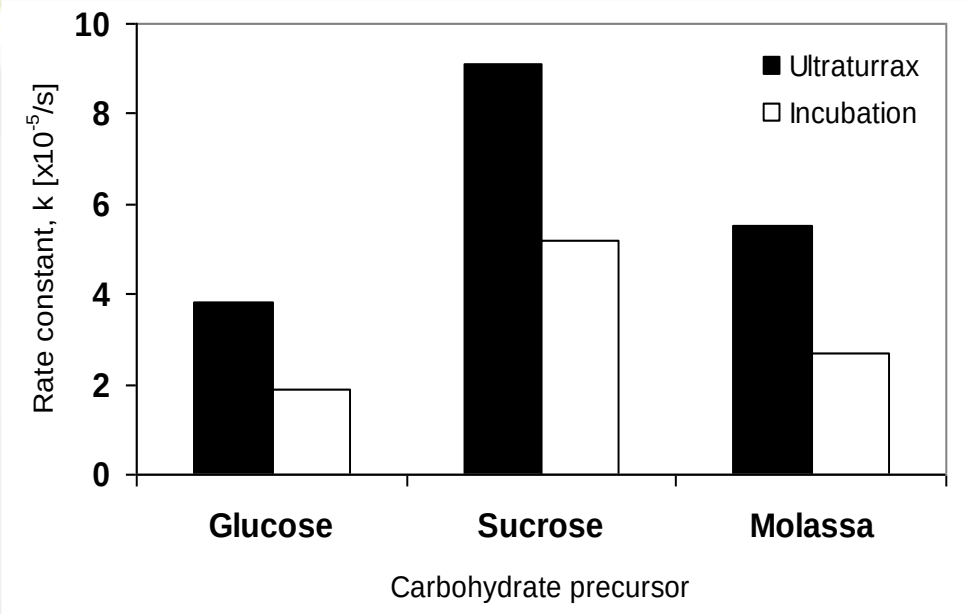
- One of the main factors that has a significant effect on the performance of yeast is the pH
- The pH of the fermentation broth has been varied (2, 4, and 6.8) using diluted HCl and NaOH
- The reaction rate constants at a pH of 2, 4, and 6.8 were calculated as $2.5x10^{-5}$, $3.6x10^{-5}$, and $3.8x10^{-5}$ s^{-1}, respectively
- Highly acidic pH (= 2) decelerated the rate of glucose fermentation by 1.5 times compared to the rate at a pH of either 4 or 6.8
- A pH between 4 and nearly neutral pH (= 6.8) had no significant effect on the fermentation rate, thus the optimum pH range for glucose fermentation was found to be 4-6.8
- Lee et al. (2013) reported an optimum pH value of 6.0 for the fermentation of glucose. Chiang et al. (1981) reported a pH range of 4-6 as an optimum range for the fermentation of D-xylose using commercial Baker's yeast

Reusability of yeast using an ultraturrax

- To study whether the yeast can be reused even after exposure to an ultraturrax, the yeast was separated from the fermentation broth soon after the complete conversion (12 h) of glucose (20 wt. %) into ethanol
- The fermentation broth was centrifuged and the supernatant was analyzed for ethanol estimation (using ^{1}H NMR). To remove the traces of ethanol, residual yeast was washed repeatedly with distilled water
- The regenerated yeast was used for another cycle of fermentation of glucose. The reaction rate using the regenerated yeast was found to be 8 times lower than that of the fresh yeast ($k_{\text{fresh yeast}}/k_{\text{regenerated yeast}} = 3.8\text{x}10^{-5}/0.47\text{x}10^{-5}\ s^{-1}$)
- The lower kinetics may also be due to the loss of some yeast during recycling. However, in industrial bioethanol production, yeast is not regenerated and reused. For every batch of ethanol production, fresh yeast is used
- In this study, the reusability was tested to verify that ultraturrax did not damage the yeast cells. The reusability was confirmed as the regenerated yeast could still yield ethanol.

Estimation of ethanol from glucose fermentation using 1H NMR

Effect of carbohydrate feedstock on the kinetics of fermentation



- The accelerating effect of the mechanical agitation by ultraturrax exposure was also dependent on the feedstock
- With either sucrose or glucose, a 1.7-fold acceleration in reaction rate was observed whereas the acceleration rate was 2.1-fold for molasses
- In addition, the fermentation rate in general was found to be a function of the feedstock used
- The rate of sucrose fermentation was nearly two times faster than either glucose or molasses regardless of the fermentation method used

Why is sucrose fermentation faster?

- D'Amore et al. (1989) reported that, in the initial stages of fermentation, sucrose is rapidly hydrolyzed into glucose and fructose by the action of the periplasmic enzyme invertase, prior to the sugars being transported across the cell membrane. Glucose was taken up preferentially over the other hydrolysis product fructose
- Growth of *Saccharomyces cerevisiae* on a medium consisting of a mixture of glucose and fructose also resulted in the preferential uptake of glucose. However, when glucose and fructose were added separately, the uptake profile for each sugar was very similar
- Since glucose is a monosaccharide, it is a common misconception that it should provide a higher rate of fermentation. However, glucose enters the yeast cells by facilitated diffusion which requires carrier proteins. When glucose is being absorbed, there will be a point where the rate reaches its maximum and all the carrier proteins are being used
- When sucrose is the substrate, it splits into glucose and fructose. When all the carrier proteins are transporting glucose, different proteins are used for fructose (due to the tertiary structure of the protein), so the composite monosaccharides of sucrose can enter the yeast at a higher rate. Hence, the rate is faster

Conclusions

- A critical analysis of mechanical agitation (ultraturrax) on the kinetics of fermentation was performed
- A 2-fold increment in the reaction rate was observed in the rate of fermentation of glucose, sucrose, and molasses
- According to our results, it is evident that sucrose is an ideal feedstock for the production of ethanol as it was found to be more feasible to be fermented
- Use of an ultraturrax is suggested as a new method for enhancing the catalytic function of *Saccharomyces cerevisiae* leading to ethanol production
- Thus, an effective, fast, and green method was developed for bioethanol production using renewable feedstocks

Solid state fermentation (SSF)

Solar energy based solid state fermentation for bioethanol production

Manuscript

Solar energy driven bioethanol production process

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Future Research

- **Developing a flow system for the continuous production of bioethanol from monosaccharides in the presence of yeast and sonication**

➢ The sugar solution obtained from the previous works will be neutralized with a diluted solution of NaOH and subsequently subjected to fermentation process under mild ultrasonic radiation conditions in the presence of yeast

➢ The batch process will later be replaced with a continuous flow system employing yeast immobilized on a solid matrix

➢ Parameters to be changed in the batch experiments are the concentration of glucose, the sonication time, and the temperature

➢ In the flow system, the flow rate of the sugar solution and the amount of the immobilized yeast will be optimized

➢ 13C NMR, GCMS, and HPLC will be used for determining the conversion of sugar and the yield of ethanol

Future Research

- **Using fermentable sugars, produced from renewable feedstocks, such as marine algae, sugar cane bagasse, and cotton, for bioethanol production under sonication, microwave irradiation, and solar radiation**

➢ Our source of marine algae is the research group of Prof. Alvaro Israel from the National Institute of Oceanography. The marine algae will be subjected to acid hydrolysis under MW radiation for obtaining fermentable sugars

➢ Parameters to be changed in this research for optimizing the yield of sugars are the concentration of the acid, the MW irradiation time, and the MW power. A similar strategy will be employed for terrestrial biomass (sugar cane bagasse and cotton)

➢ For the solar reactor, the marine algae will be placed in the fermentation chamber along with the yeast and the enzymes that could convert the starch in the algae to glucose (α amylase and glucoamylase) and the enzyme that could convert the cellulose in the algae to glucose (cellulose). The glucose thus generated in situ will be converted to ethanol by the yeast

Future Research

- **Experimenting on different yeast strains to enhance the ethanol yield**
  - The currently employed yeast, *Saccharomyces cerevisiae*, will be replaced by other strains of yeast based on literature scientific reports
- **Upscaling the production of sugars from biomass and the conversion of fermentable sugars to bioethanol**
  - Further efforts will be focused on taking the laboratory results to pilot plant level

Thank you for your attention

Special thanks to:

Prof. Aharon Gedanken

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