

# Novel methods for the production of Biological Fuels

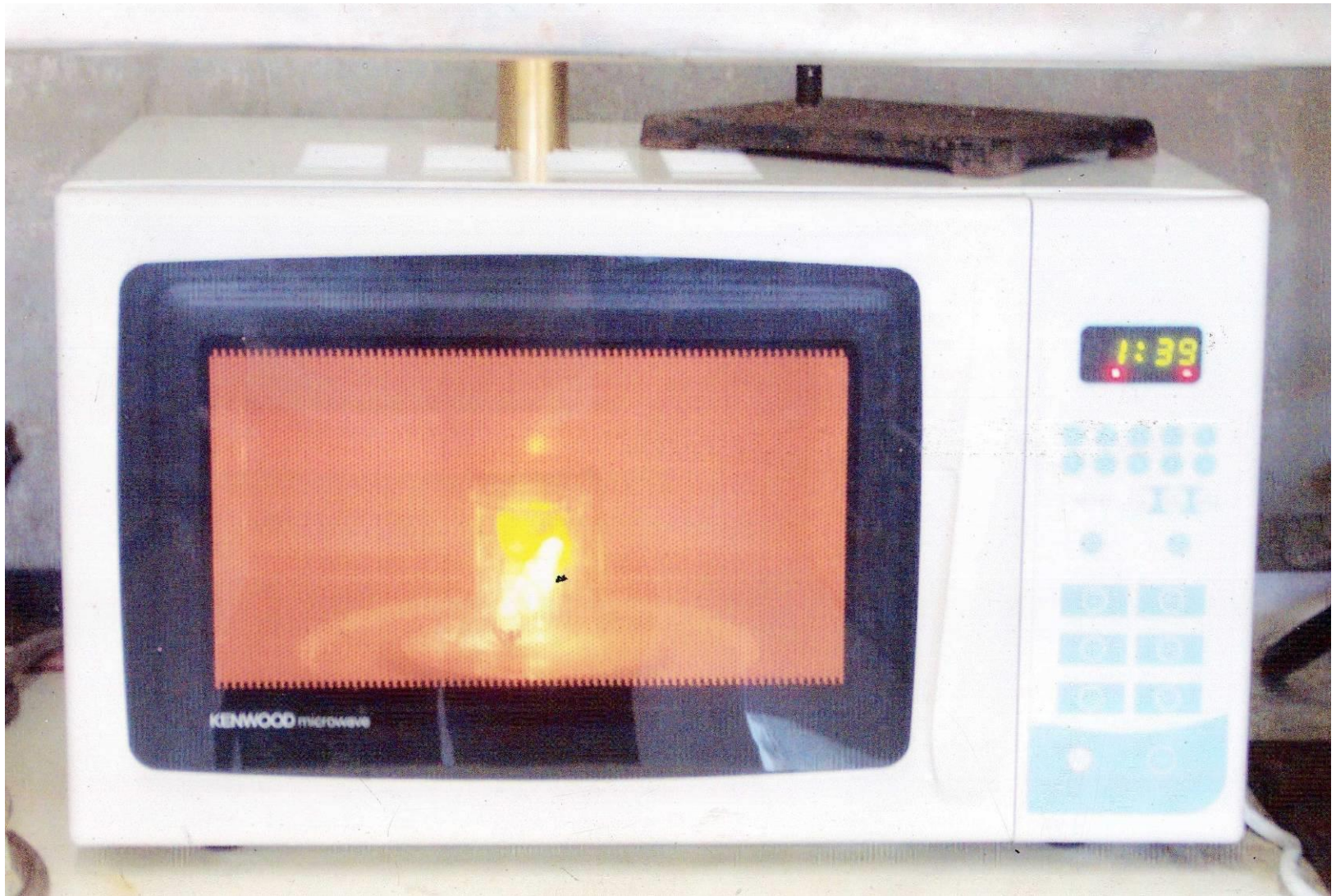


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**Israel**

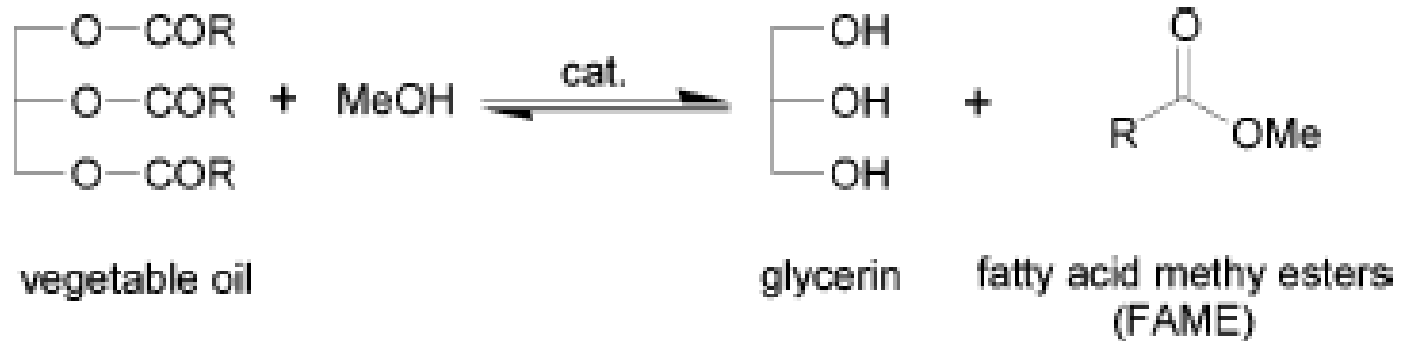
**February, 2012**

## Microwave oven with provision for stirring and refluxing employed for hydrolysis of biomass





# Preparation of Biodiesel



- *Biodiesel is made from vegetable oils or animal fats by transesterification with methanol using an acid or base catalyst.*
- *The most commonly used catalysts in transesterification reaction are: NaOH , KOH , H<sub>2</sub>SO<sub>4</sub>.*

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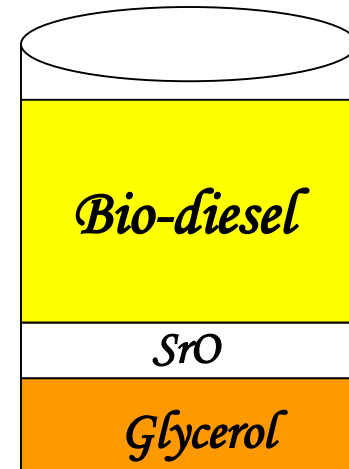
## *Important Parameters:*

- *The reaction time*
- *Catalyst amount and alcohol-oil ratio*
- *Recycling of the catalyst activity*
- *Separation between FAME phase, catalyst phase and glycerol*
- *Conversion*

*Optimization of Bio-diesel Production!*

## Optimization of Bio-Diesel Production:

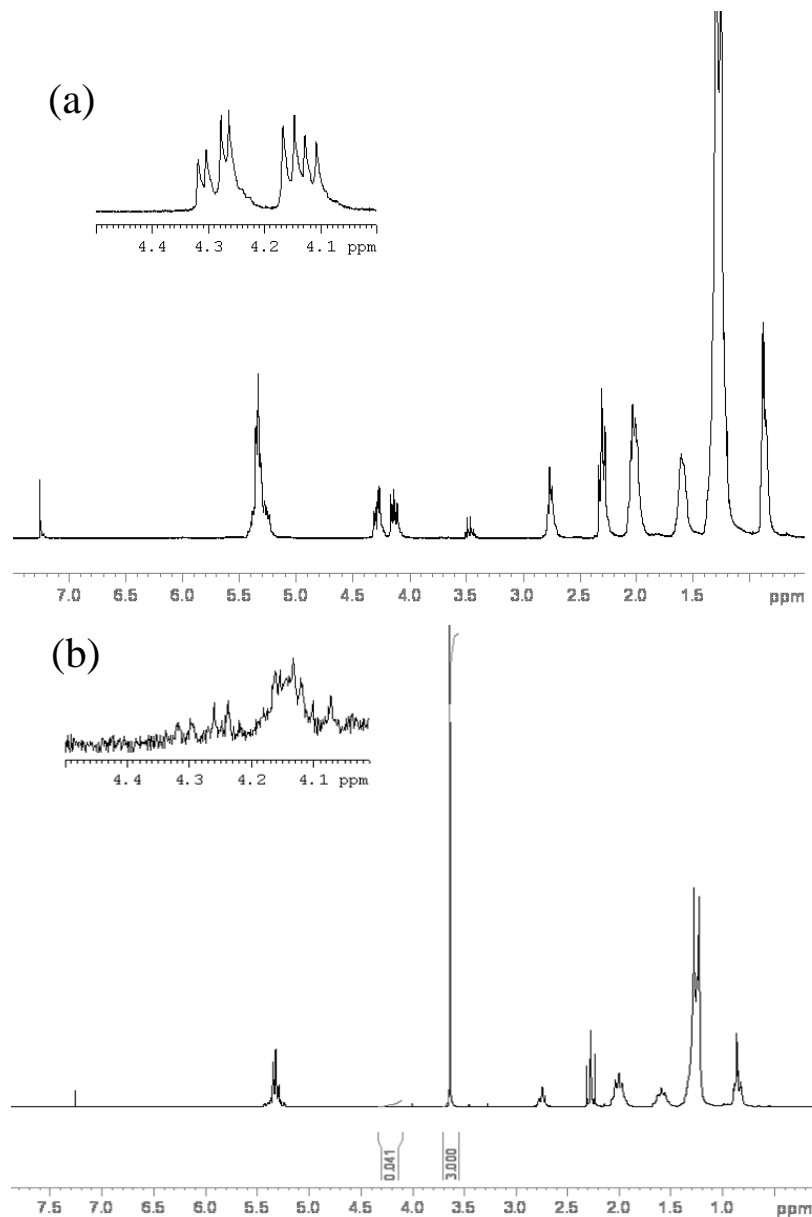
**Probing the New Combination of the Microwave Irradiation of Cooked Oil and SrO as a Solid Catalyst.**



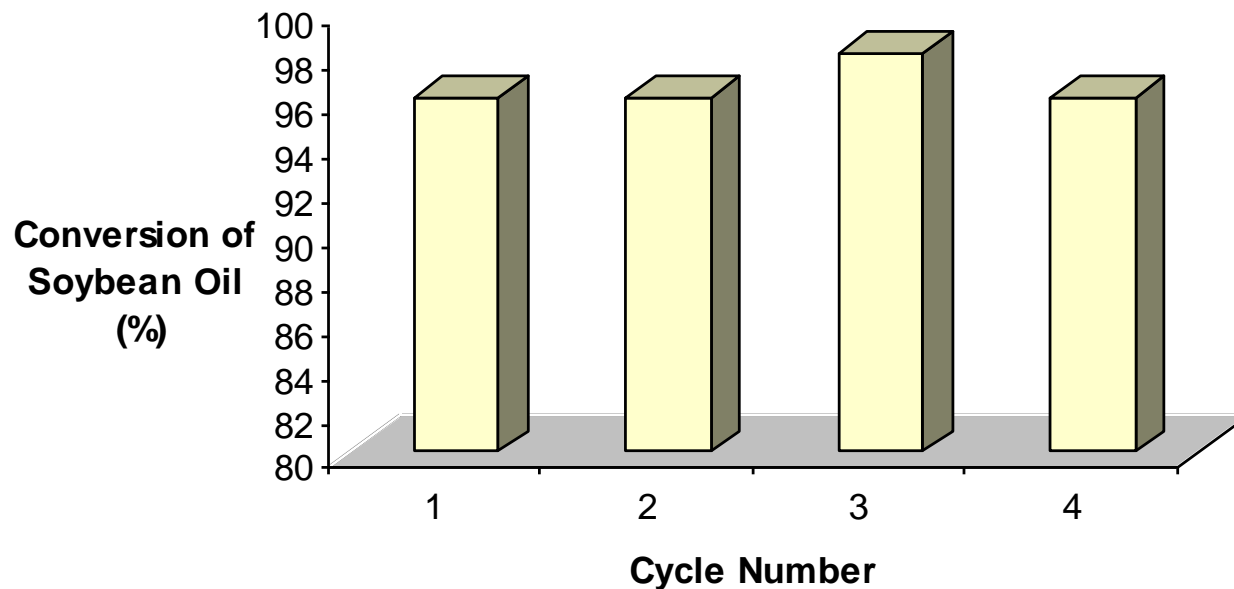
*Microwave Irradiation of a Reaction Mixture of SrO, Methanol, and Oil leads to Creation of 3 Phases*

## Optimum Result:

- 99% conversion in 40 seconds of microwave irradiation with SrO.  
Amounts: Oil / Methanol / SrO  
15 gr / 4.05 ml / 0.276 gr
- Using KOH under the same conditions leads to 83% conversion!
- (a)  $^1\text{H}$ NMR spectrum of wastes of cooked oil. (b)  $^1\text{H}$ NMR spectrum of bio-diesel production from wastes of cooked oil obtained after 40 sec using SrO as a catalyst.



## Repeated Activity of the Catalyst



*The result confirm the stability and the sustained activity of SrO, which is of great importance for industrial application*



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- In the next stage we have investigated the bio-diesel production directly from the microalgae biomass of *Nannochloropsis* by microwave and ultrasound radiation

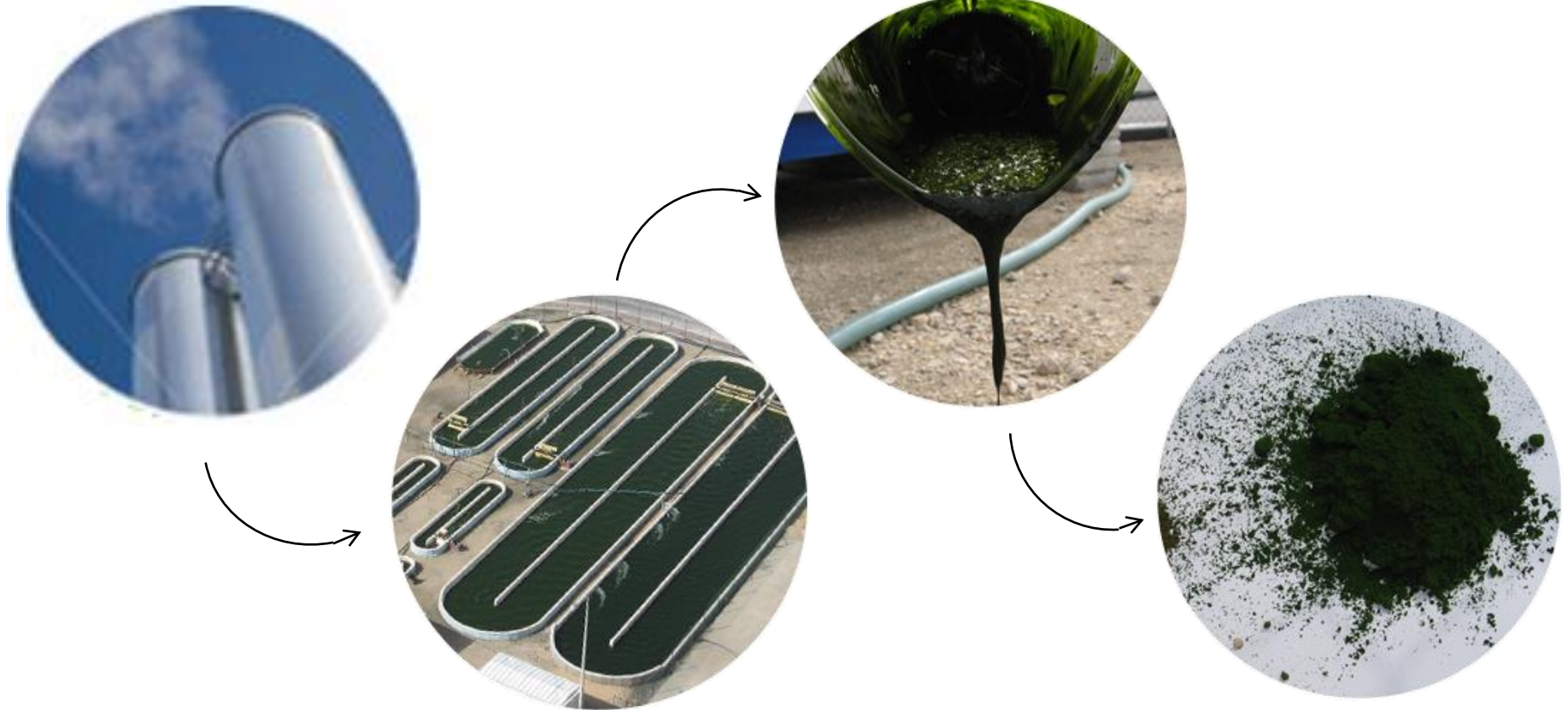
# How were the microalgae prepared?

- **The microalgae was provided to us by Seambiotic.** This company grows the algae on seawater. The cultures were supplied with N & P and C inorganic nutrients, where the C was supplied by coal burning scrubbed flue gas containing 13% CO<sub>2</sub>. A culture of the marine Eustigmatophyte *Nannochloropsis* sp. was inoculated in outdoor raceway ponds at a concentration of less than 0.25 g/L, grown for 7 days to a concentration of 0.5 g/L. The culture was harvested using a continuous centrifuge (GEA Westfalia) and the paste was dried with a spray dryer (Anhydro) to obtain a dry powder.

# Seamiotic microalgae ponds



# Seamiotic microalgae cultivation process



Culture Inoculation at concentration of 0.25 g/L (Raceway Pond)

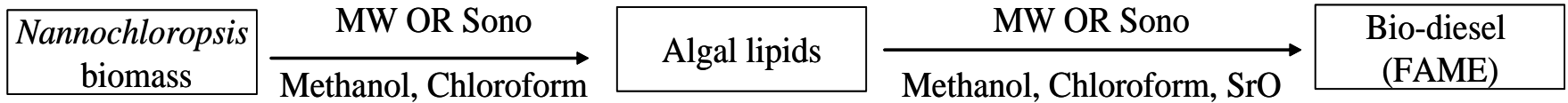
Culture concentration increase to 0.5 g/L

Harvest  
Paste at concentration of 150-200 g/L (Continuous centrifuge)

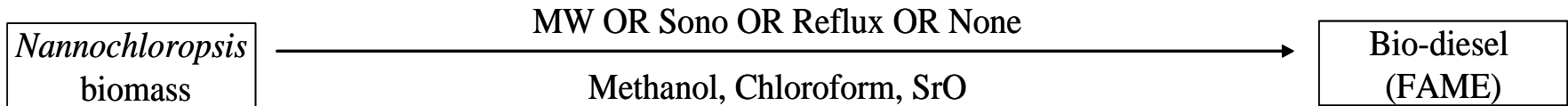
Drying  
Dry algae powder (Spray drier)

# Bio-diesel production from the microalgae biomass of *Nannochloropsis*

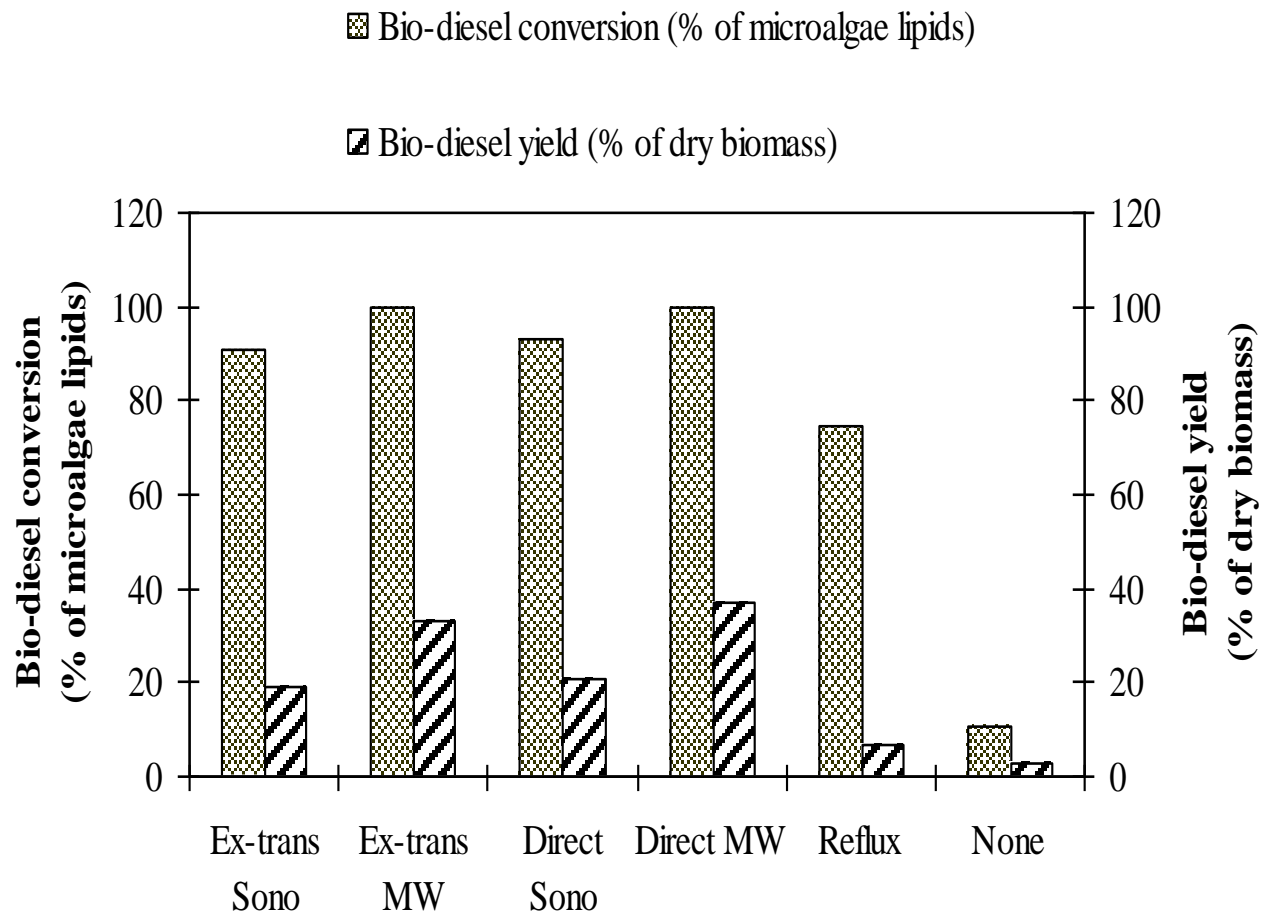
(a) A two-step reaction, namely, extraction and transesterification:



(b) A one-step direct transesterification



Direct transesterification reaction using microwave resulted the highest bio-diesel yield of **37.1%** with a conversion (**99.9%** conversion) of the triglyceride to bio-diesel.



Bio-diesel yield of the *Nannochloropsis* microalgae and bio-diesel conversion of the microalgae lipids using various techniques. Five techniques used in bio-diesel production: (Ex-trans Sono) extraction and transesterification reaction steps occurred separately using sonication, (Ex-trans MW) extraction and transesterification reaction steps occurred separately using microwave, (Direct Sono) direct transesterification without the initial extraction step using sonication, (Direct MW) direct transesterification without the initial extraction step using microwave, (Reflux) direct transesterification without the initial extraction step using regular reflux technique, and (None) extraction and transesterification reaction occurred without heating and stirring.

# Direct transesterification of castor seeds for bio-diesel production by microwave and SrO catalyst



***Direct transesterification reaction using microwave resulted the highest bio-diesel yield of 57.23% with a conversion (99.95% conversion) of the triglyceride to bio-diesel.***

# Direct transesterification of jatropha seeds for bio-diesel production by microwave and SrO catalyst



*Direct transesterification reaction using microwave resulted bio-diesel yield of 41.08% with a conversion (99.71% conversion) of the triglyceride to bio-diesel.*



## Drive for research in Bioethanol – Cost reduction

- World wide focus - bioethanol as a substitute to gasoline
- Feedstocks for ethanol - Brazil (sugar cane molasses), United States (corn), India (sugar cane), Thailand (cassava), France (sugar beet), China (corn), Canada (wheat)
- Brazil in 2010 - 27 billion liters of bioethanol produced from sugar cane juice or molasse
- US in 2010 - produced 50 billion liters of bioethanol from corn

### Bioethanol Industry

- In Brazil - 243 bioethanol processing plants and nearly 33, 620 ethanol service stations
- In US - 113 ethanol biorefineries in operation with 1073 ethanol stations

### Possible ways of reducing bioethanol production costs

- ✓ Identifying appropriate feedstock
- ✓ Improving the feed stock pretreatment
- ✓ Lowering of enzyme dosage or developing a robust chemical hydrolysis process
- ✓ Improving over all starch and cellulose hydrolysis
- ✓ Shortening the fermentation time

## Advantages of Bioethanol

- Renewable and environmentally friendly and carbon neutral fuel
- Blended with petrol or used as neat alcohol
- Higher octane no. and higher heat of vapourisation

## Why Cellulose for ethanol?

- No food to fuel conflict
- Most common carbohydrate
- Annual world biosynthesis is  $10^{11}$  t
- Most widespread and readily available solid organic waste
- 1/3<sup>rd</sup> of the wt. of all trees, vines, grasses and straws comprise of cellulose
- Renewable and replenished by photosynthesis and growth
- Vast potential feed stock to chemical industry



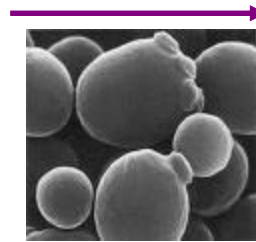
**Scheme 1.** Schematic representation of the formation of ethanol from biomass

***Hydrolysis of cellulose and fermentation of sugars are the two important reactions in the conversion of biomass to fuel ethanol***

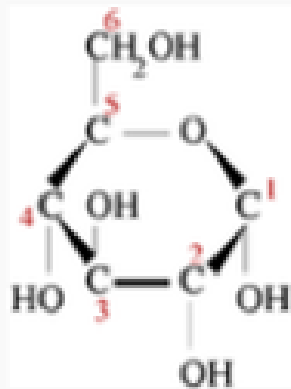
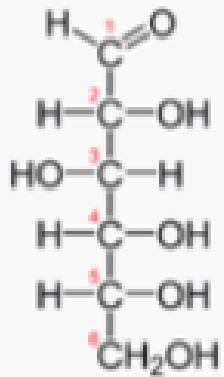
# Glucose fermentation to ethanol

Yeast (*Saccharomyces cerevisiae*)

Sugars  
(D-glucose, D-sucrose)



Ethanol + CO<sub>2</sub>



Theoretical yield of ethanol:

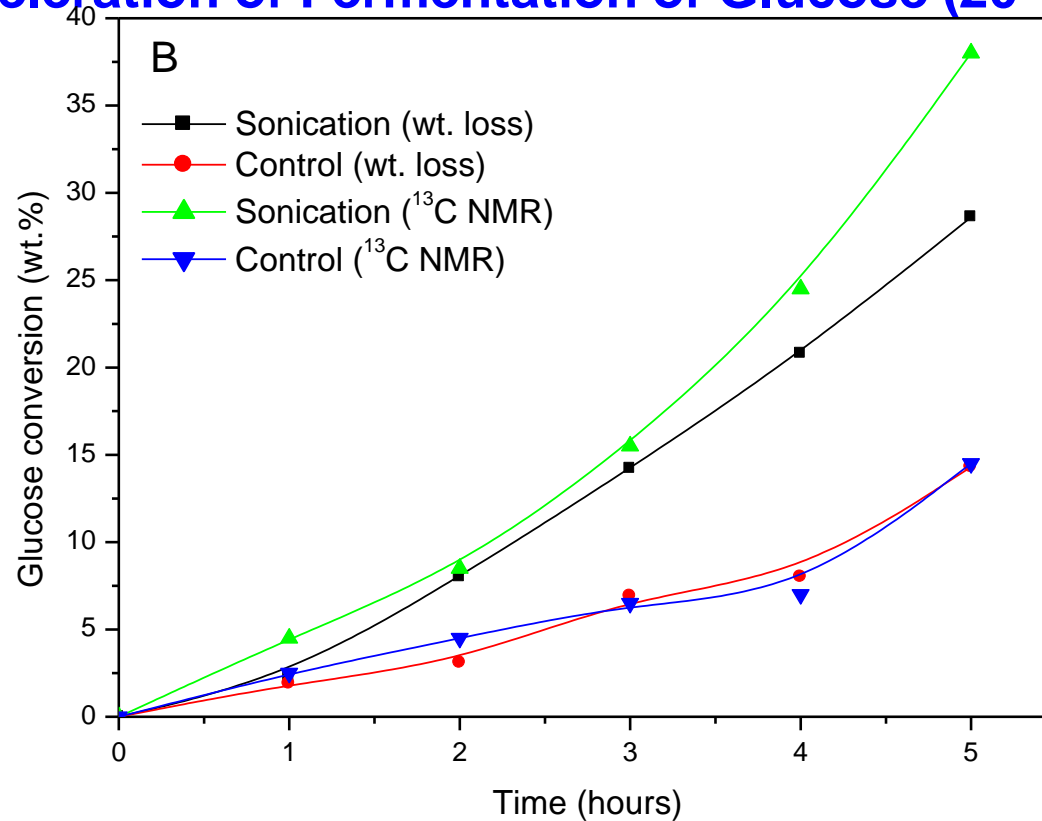
1 g glucose yields 0.51 g ethanol  
1 g sucrose yields 0.54 g ethanol

Glucose

Yeast strain employed: Bakers yeast (Bravo brand dry yeast)

*Fermentation reaction was carried out under unstirred, stirred and mild sonication conditions*

# Acceleration of Fermentation of Glucose (20 %) at 30 °C



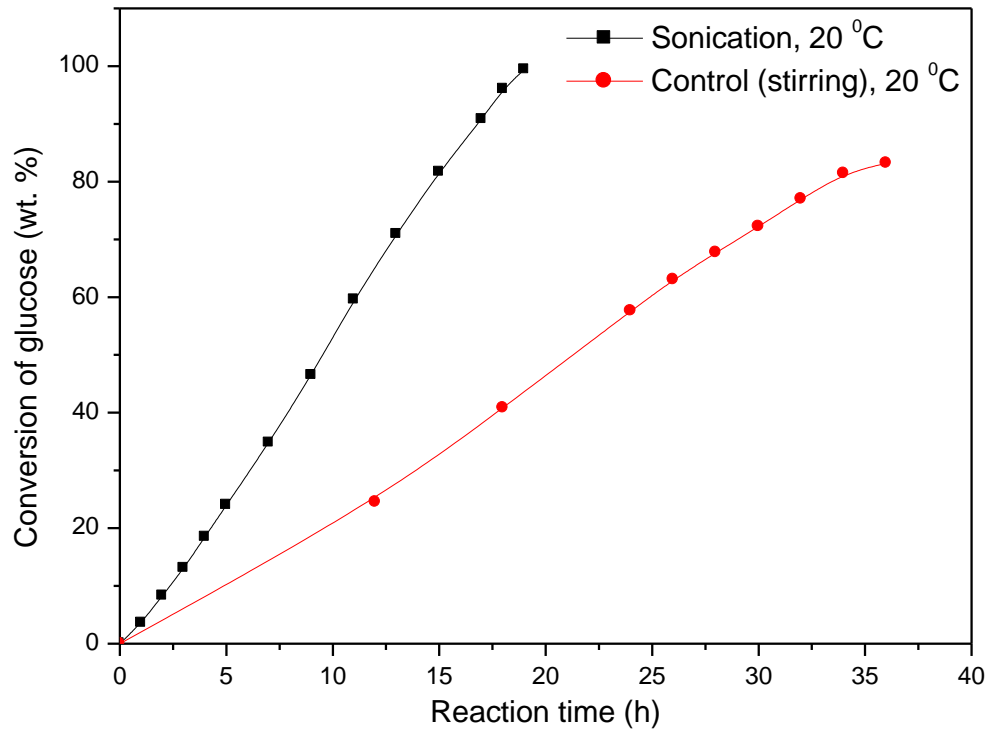
## Effect of ultrasonic sound on the conversion (wt. %) of glucose at 30 °C

Sonication – 38 wt. % conversion of glucose in 5 h  
Stirring - only 14.5 wt. % conversion of glucose in 5 h

***Reaction rate enhanced by a factor of 2.3 upon sonication of the fermentation broth compared to regular stirring***

A. Gedanken, R. Schwarz, P. Indra Neel, Accelerating the fermentation of glucose using a sonochemical process with the aid of yeast, US Patent, Application No. 124726, March 2011.

## Acceleration of Fermentation of Glucose at 20 °C

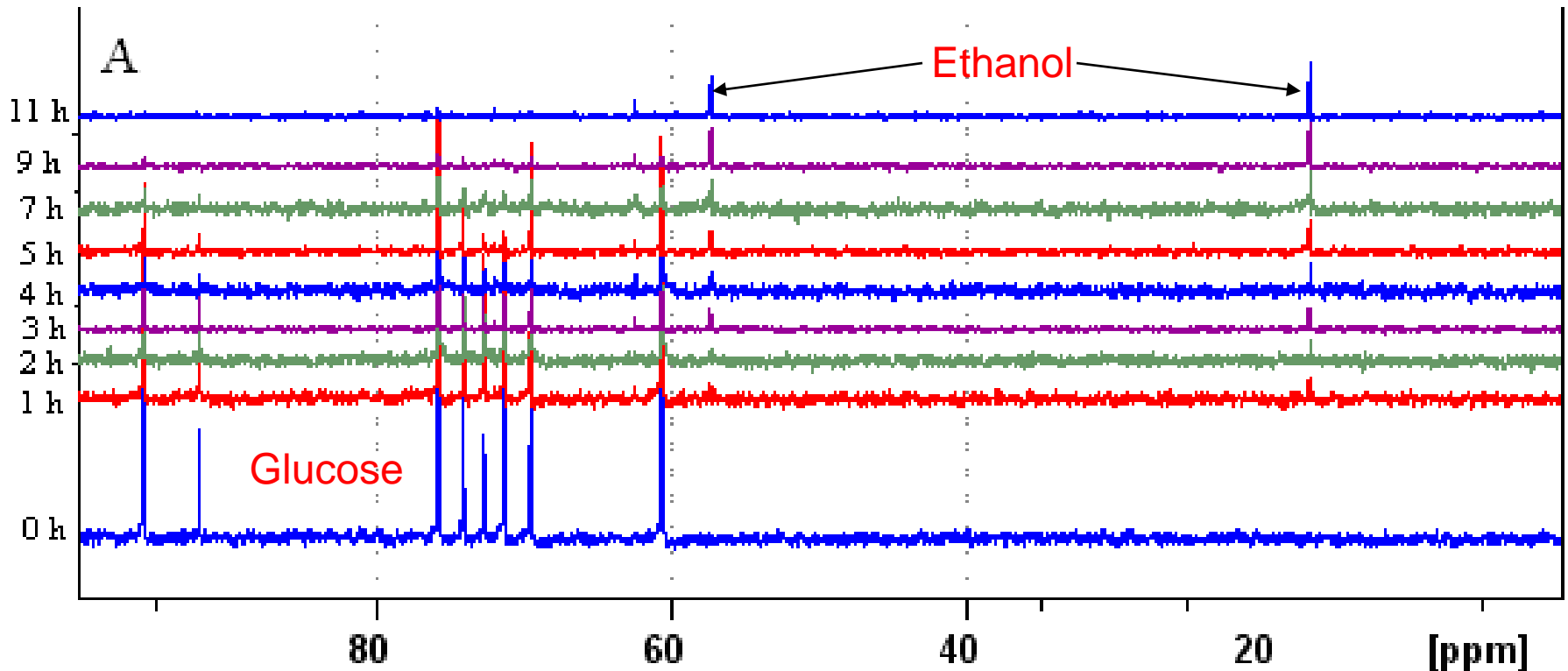


**Sonication – 100 wt. % conversion of in 19 h**  
**Stirring - 100 wt. % conversion of in 36 h**

### Effect of ultrasonic sound on the conversion (wt. %) of glucose at 20 °C

- ❖ *Reaction rate enhanced by a factor of 2.6 upon sonication of the fermentation broth compared to regular stirring*
- ❖ *Fermentation under stirred and bath sonication conditions were 6.5 and 10 times faster than the unstirred reaction*

## Glucose (20 %) fermentation reaction under sonication (30 °C)

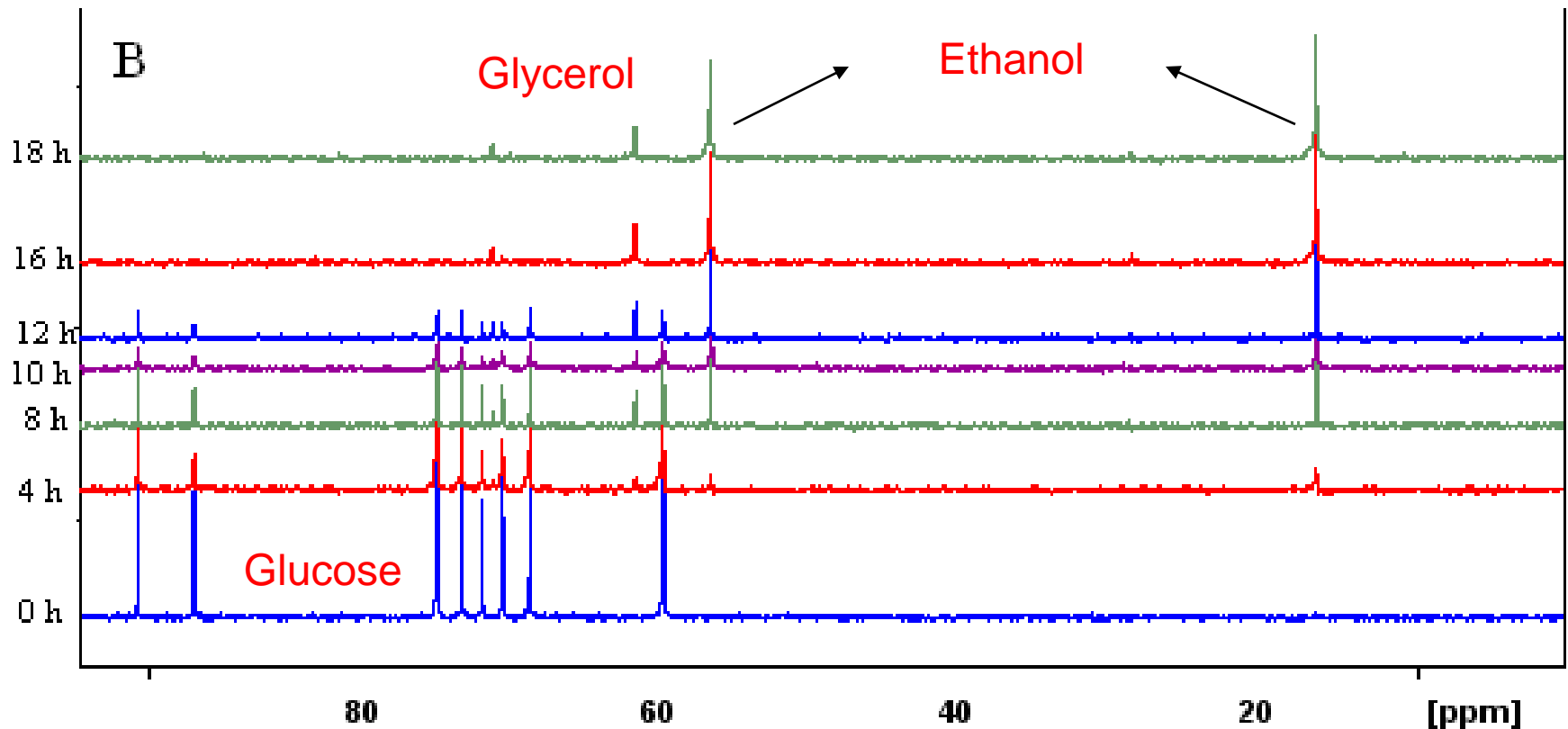


$^{13}\text{C}$  NMR spectra of aliquots from the fermentation broth under sonication

- With time the intensity of the peaks corresponding to ethanol (16.8 and 57.4 ppm) increased
- intensity of peaks corresponding to glucose (60.6 (C6), 69.5 (C4), 72.7 (C2), 75.7 (C3), 75.8 (C5) and 95.8 (C1)) decreased
- In the 11 h sample no trace of glucose is observed indicating the completion of the fermentation reaction

***Under sonication, fermentation is completed in 11 h***

## Glucose (20 %) fermentation under stirring (30 °C)

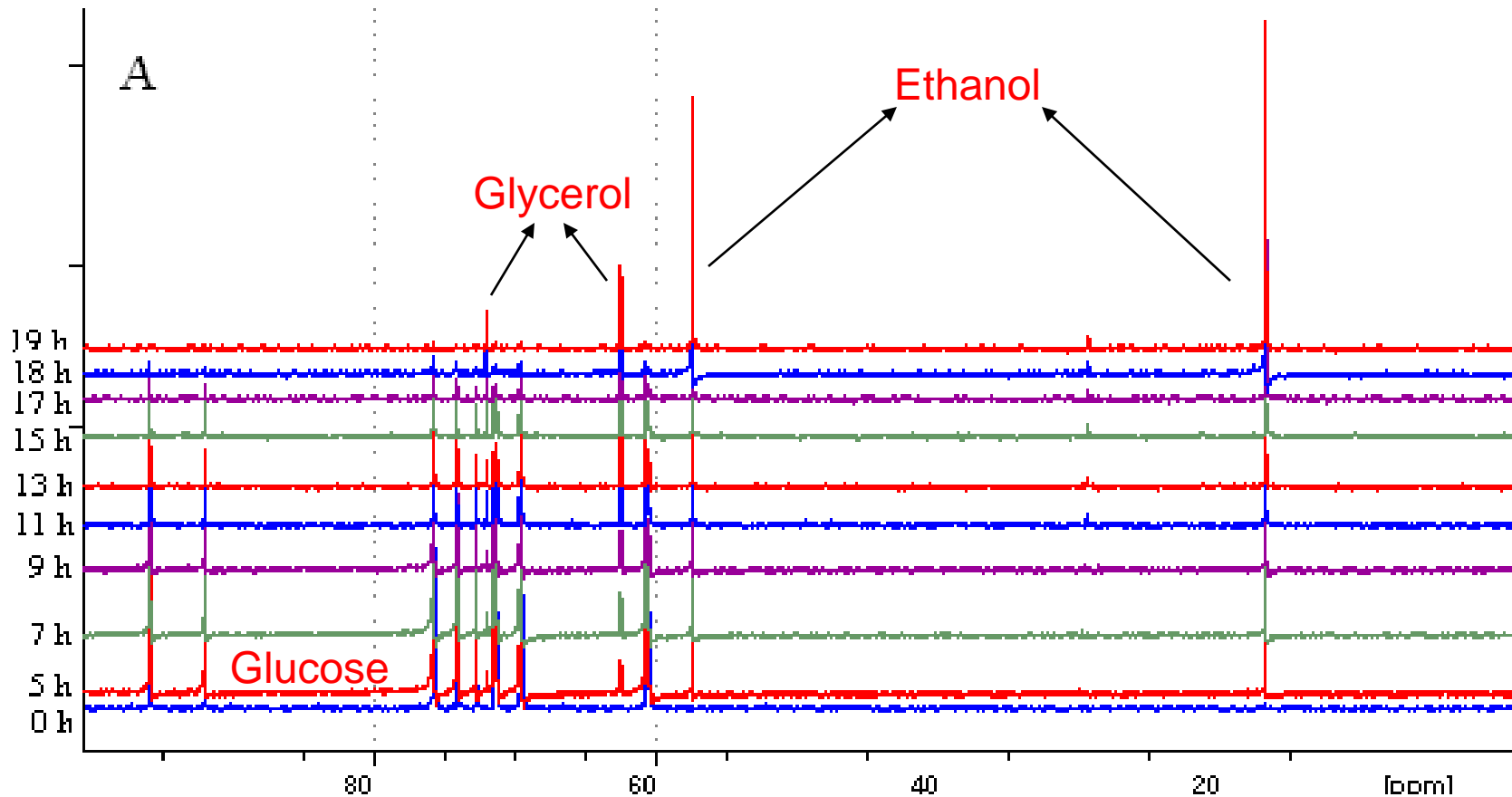


$^{13}\text{C}$  NMR spectra of aliquots from the fermentation broth under control (stirring)

➤ 18 hours needed for complete conversion of D-glucose (10.0 g) to ethanol at 30 °C under stirring

***Under stirring, fermentation requires 18 h relative to 11 h under sonication***

# Glucose (20 %) fermentation under sonication (20 °C)



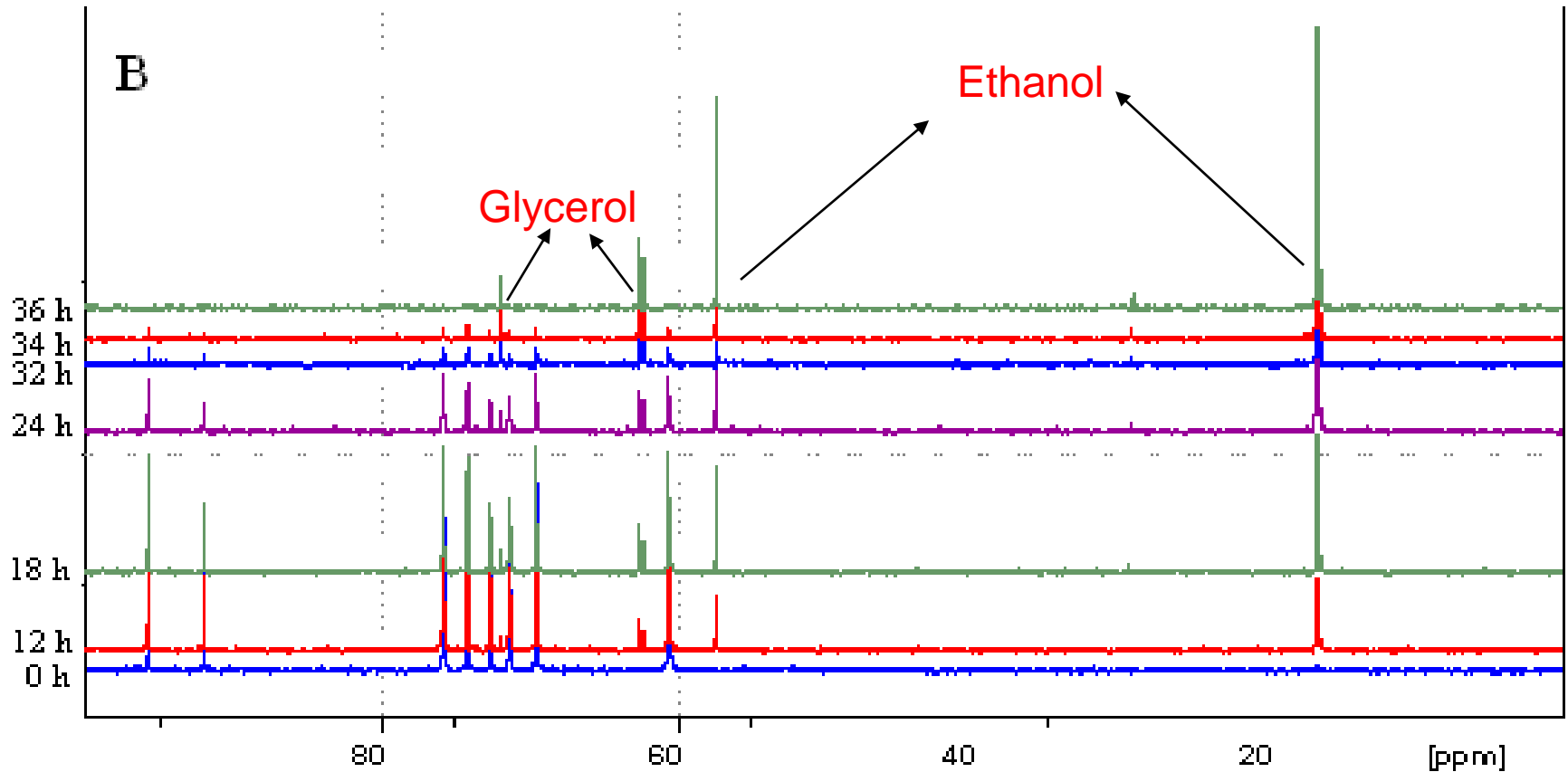
$^{13}\text{C}$  NMR spectra of aliquots from the fermentation broth under sonication at 20 °C

➤ In the 19 h sample no trace of glucose is observed indicating the completion of the fermentation reaction

***Under sonication, fermentation is completed in 19 h***



## Glucose (20 %) fermentation under stirring (20 °C)

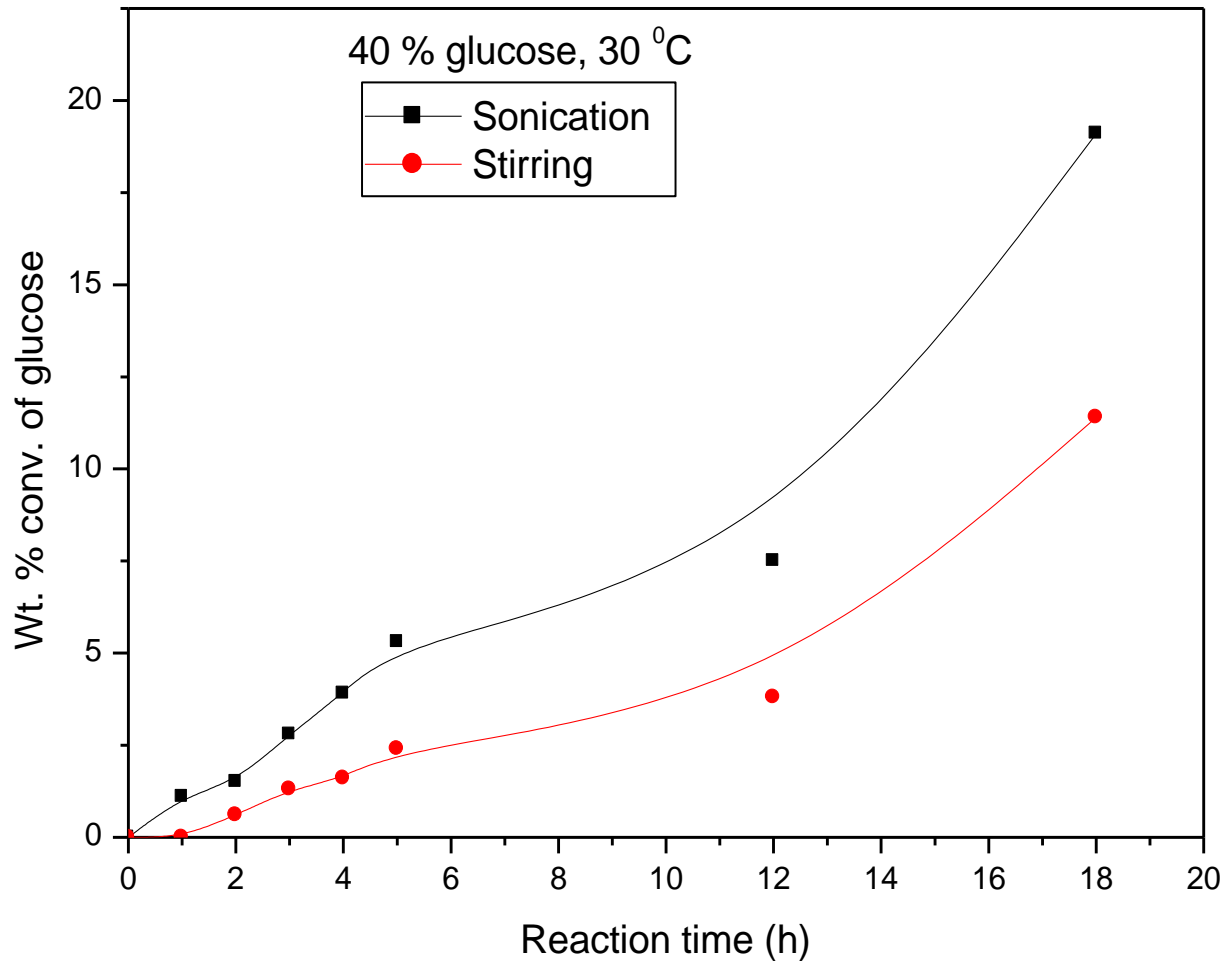


$^{13}\text{C}$  NMR spectra of aliquots from the fermentation broth under stirring at 20 °C

- 36 h required for the complete conversion of D-glucose (10.0 g) to ethanol at 20 °C

***Under stirring, fermentation requires 36 h relative to 19 h under sonication***

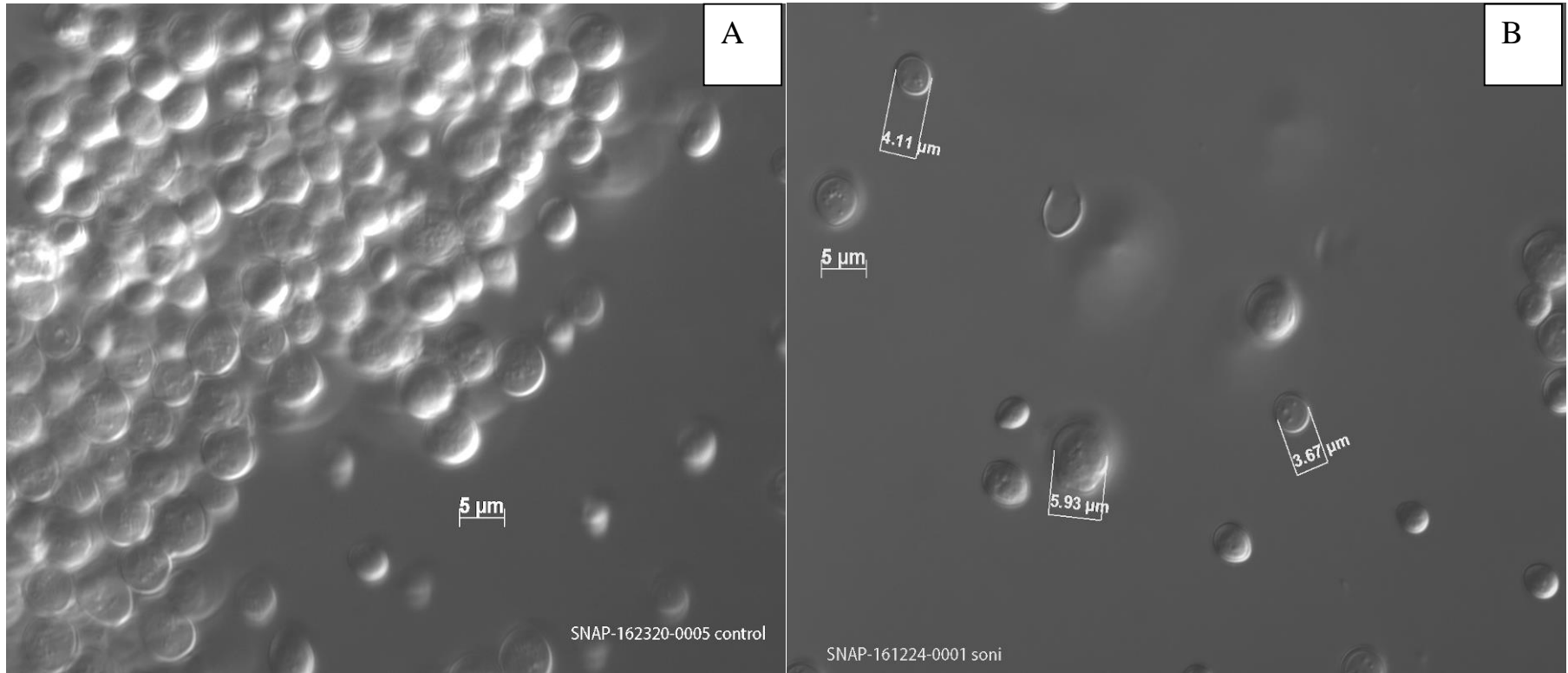
# Glucose Fermentation (40 %) : Sonication Vs stirring at 30 °C



**Effect of ultrasonic sound on the conversion of glucose**

***Sonication resulted in 19% glucose conversion relatively to only 11 % under stirring (in 18 h)***

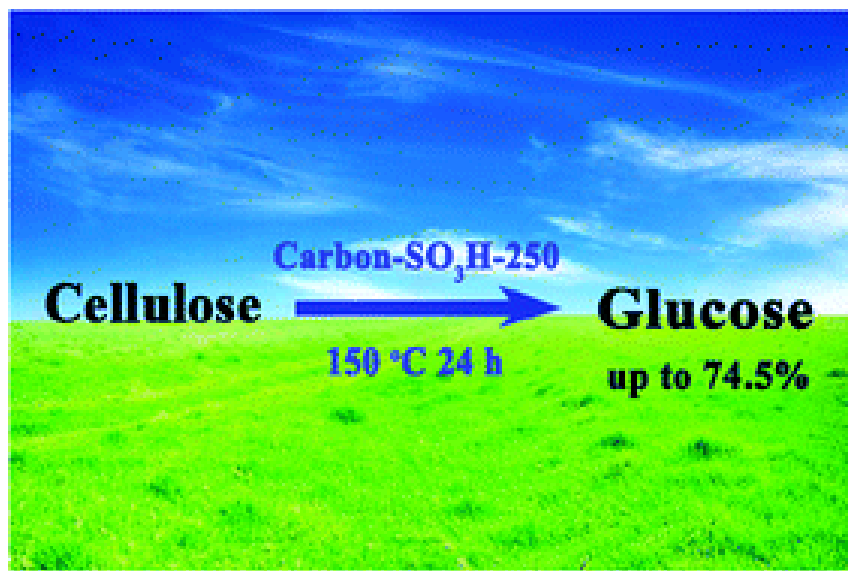
## Effect of sonication - Deagglomeration of yeast



Microscope images of yeast in the fermentation broth (20 % glucose) after 30 minutes (A) control (stirring) and (B) bath sonication at 30 °C

- ❖ *Yeast cells were well dispersed upon sonication of the broth*
- ❖ *The observed acceleration of the kinetics of the fermentation reaction is not due to the disaggregation of the yeast cells*
- ❖ *Soft sonication introduces in the system a strong stirring and this causes the acceleration of the fermentation. The stirring may help to remove the ethanol from the yeast surface, and also helps in the removal of CO<sub>2</sub> from the fermentation broth.*

# Hydrolysis of cellulose into glucose over carbons sulfonated at elevated temperatures



**Jifeng Pang, Aiqin Wang, Mingyuan Zheng and Tao Zhang**  
**Chem. Commun., 46 (2010) 6935-6937**

## Highlights:

- ✓ Increase in sulfonation temperature from 150 to 250 °C increased the glucose yield from 7 to 61%
- ✓ On sulfonated CMK-3, 94.4% conversion and 74.5% glucose yield were obtained

# Effect of method of sulfonation on the textural, acidic and catalytic properties of activated carbon

- ✓ Sulfonation of carbon materials - Under N<sub>2</sub> at 150-300 °C for 24 h using 15 mL conc. H<sub>2</sub>SO<sub>4</sub> per g of carbon

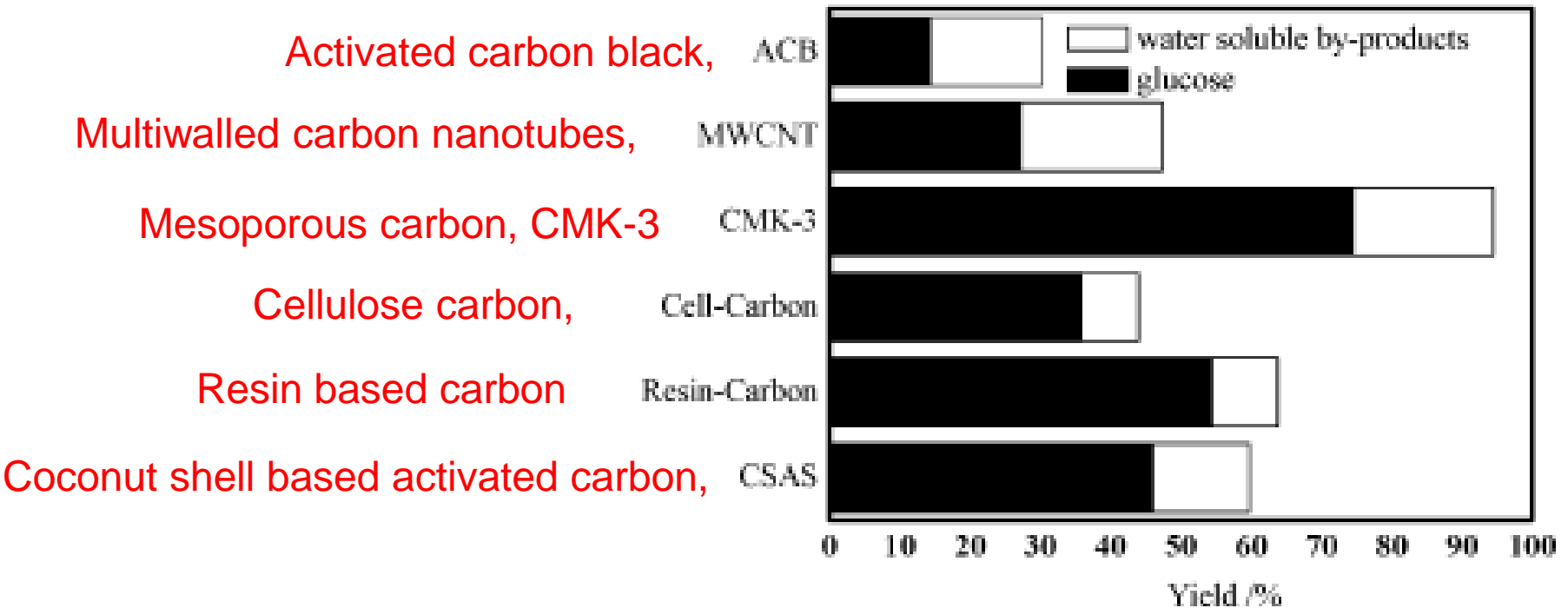
**Table 1** The surface properties of AC sulfonated at different temperatures and their catalytic activities for hydrolysis of cellulose<sup>a</sup>

Catalyst	-SO <sub>3</sub> H <sup>b</sup> / mmol g <sup>-1</sup>	Acid density <sup>c</sup> / mmol g <sup>-1</sup>	Acid density <sup>d</sup> / mmol g <sup>-1</sup>	S <sub>BET</sub> <sup>e</sup> / m <sup>2</sup> g <sup>-1</sup>	Adsorbed water <sup>f</sup> / %	Y <sub>Glu</sub> <sup>g</sup> C (%)	Conv <sup>h</sup> C (%)
AC	—	0.15	0.05	703	8.8	0	10.0
AC-NO <sub>3</sub>	0.02	1.49	0.36	762	10.7	9.7	31.5
AC-SO <sub>3</sub> H-150	0.19	0.80	0.22	709	13.8	7.1	25.1
AC-SO <sub>3</sub> H-200	0.24	1.03	0.42	728	15.4	17.9	43.4
AC-SO <sub>3</sub> H-250	0.24	1.58	0.47	945	17.9	61.0	70.4
AC-SO <sub>3</sub> H-280	0.23	2.03	0.59	462	18.3	56.0	66.6
AC-SO <sub>3</sub> H-300	0.20	2.19	0.54	278	19.6	36.7	63.1
AC-N-SO <sub>3</sub> H-150	0.12	2.15	0.37	723	14.1	19.0	42.1
AC-N-SO <sub>3</sub> H-200	0.34	2.18	0.43	741	15.4	56.1	64.3
AC-N-SO <sub>3</sub> H-250	0.44	2.23	0.50	762	19.6	62.6	74.3
AC-N-SO <sub>3</sub> H-280	0.27	2.38	0.70	362	20.8	58.4	66.3
AC-N-SO <sub>3</sub> H-300	0.27	2.39	0.78	30	21.3	54.2	68.6

- Acid density increased with increase in the sulfonation temperature
- 250 °C is the optimum temperature of sulfonation
- Above 250 °C, the surface area of the catalyst decreased
- Nitric acid treatment followed by sulphuric acid treatment is preferred
- A maximum of 74 % conversion and 62 % yield of glucose obtained with nitric acid followed by sulphuric acid treatment

# Cellulose hydrolysis

## Catalytic activity based on the type of carbon



### Cellulose hydrolysis over sulphonated carbon catalysts

- *Sulphonated mesoporous carbon, CMK-3 out performed other carbon materials*
- *Cellulose conversion of 94.4 % and glucose yield of 74.5 % were achieved which are the maximum values reported so far*
- *High acid density, large surface area and also the mesoporous structure contributed to the high value of cellulose conversion and glucose yield*

# Catalytic activity – Accessibility of active sites (-SO<sub>3</sub>H)

Cat.	-SO <sub>3</sub> H <sup>a</sup> / mmol g <sup>-1</sup>	Acid density <sup>b</sup> / mmol g <sup>-1</sup>	Acid density <sup>c</sup> / mmol g <sup>-1</sup>	S <sub>BET</sub> / m <sup>2</sup> g <sup>-1</sup>	
ACB	0.02	0.24	0.07	89	
MWCNT	0.02	0.26	0.05	96	
CMK-3	0.63	2.39	0.88	412	Best catalytic activity
Cell-Carbon <sup>d</sup>	2.01	3.96	1.22	6	High acid density
Resin-Carbon	0.28	2.08	0.45	834	
CSAC	0.33	1.10	0.28	981	High surface area

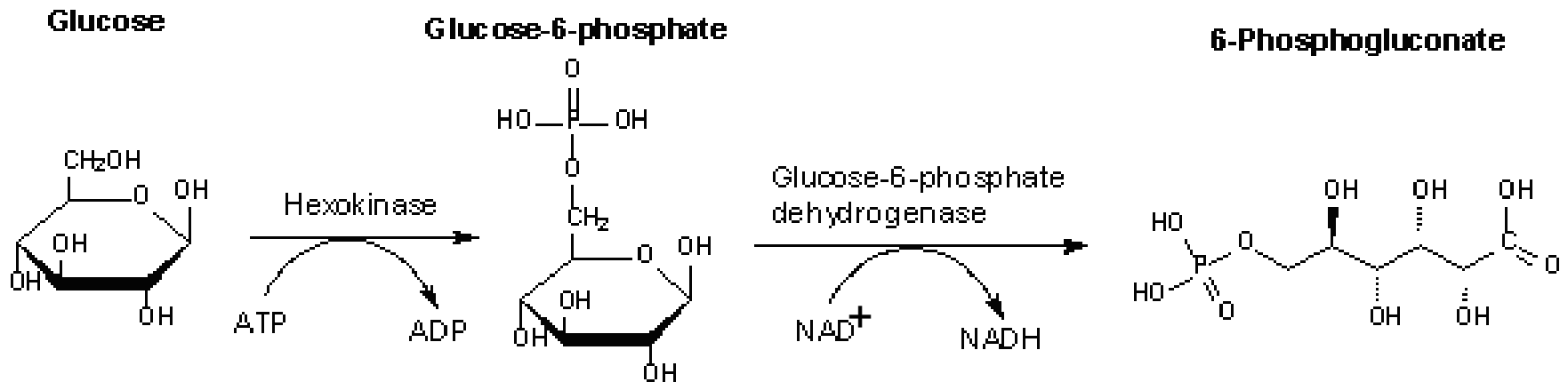
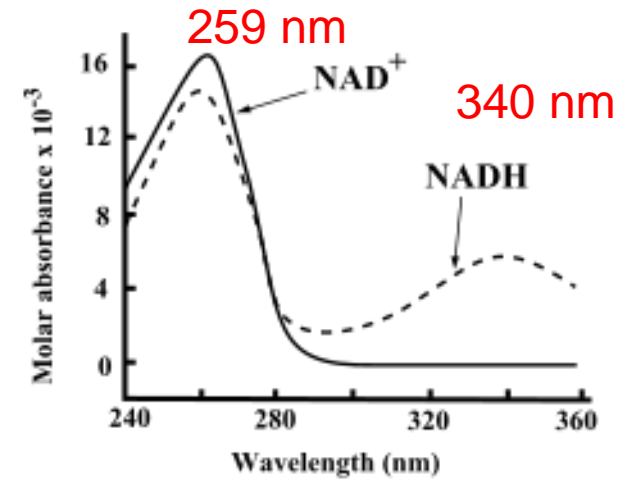
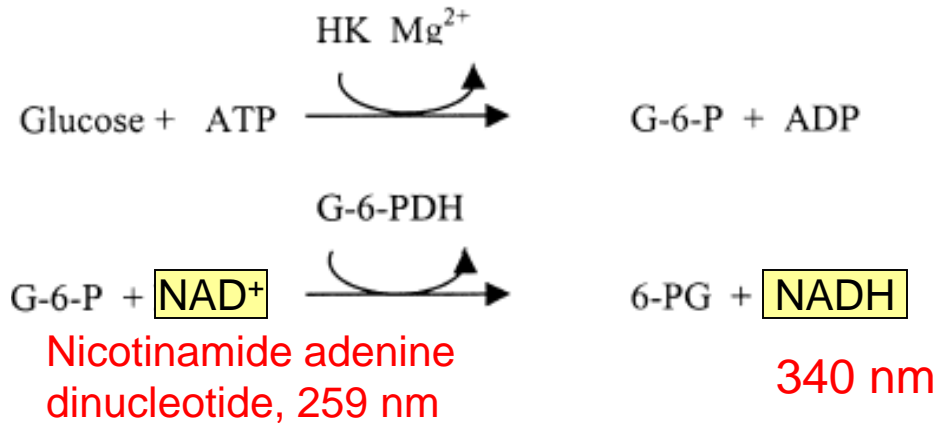
- Even though CSAC possessed high specific surface area it yielded only 45 % glucose
- Even though Cellulose-carbon showed high acid density it yielded only 35 % glucose
- In spite of lower surface area relative to CSAC and lower acid density CMK-3 yielded 75 % glucose
- Thus mesoporosity is the important criteria leading to the accessibility of active sites to the incoming bulky reactant molecules of cellulose

## **Summary:**

- ❖ **A highly active and selective solid acid catalyst was developed for the hydrolysis of cellulose into glucose**
- ❖ **Best result of cellulose conversion (94.4 %) and glucose yield (74.5 %) were obtained when CMK-3 is sulfonated at 250 °C**
- ❖ **Sulfonation temperature (250 °C) is crucial for obtaining optimum acid density required for cellulose hydrolysis**
- ❖ **Carbon source also has a great impact on the acid density derivable through the process of sulfonation**

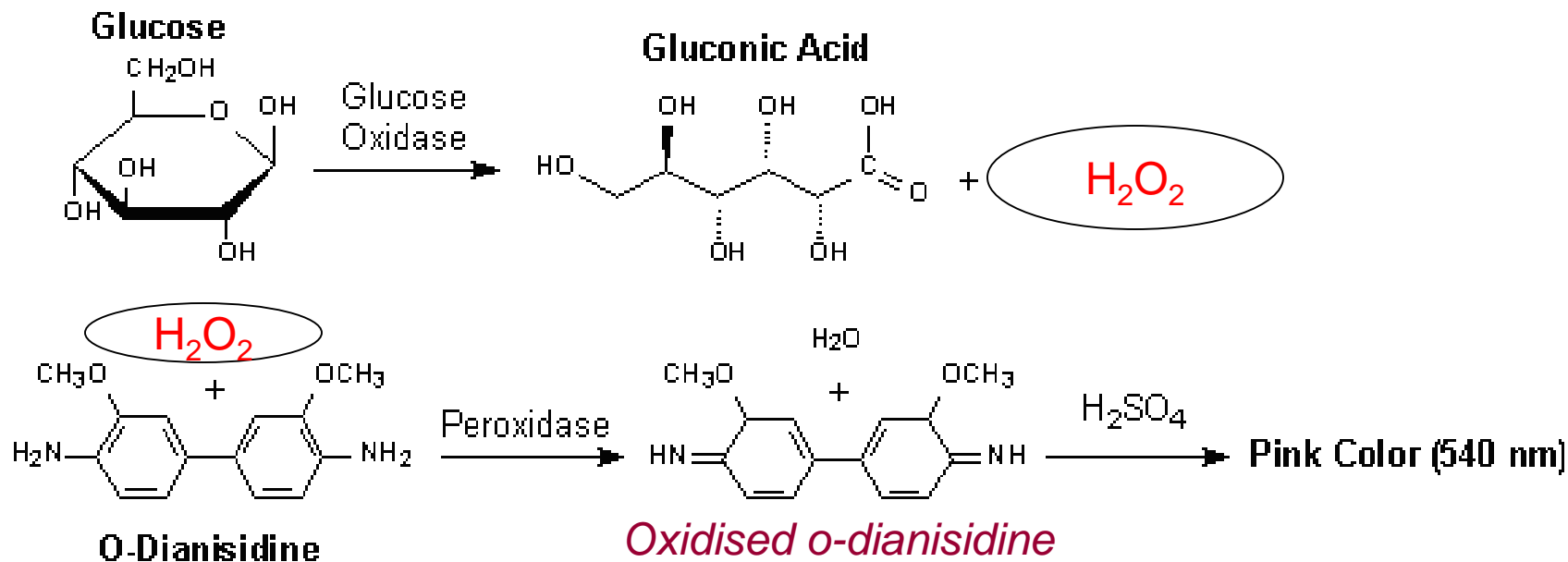


# Quantitative enzymatic (hexokinase) determination of glucose at 340 nm



- **Glucose is oxidized to 6-phosphogluconate.**
- **In turn nicotinamide adenine dinucleotide (NAD<sup>+</sup>) is reduced to NADH which absorbs at 340 nm**

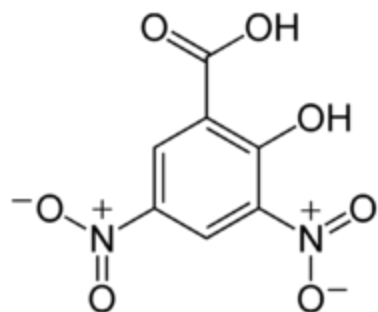
# Quantitative enzymatic (**glucose oxidase**) determination of glucose at 540 nm



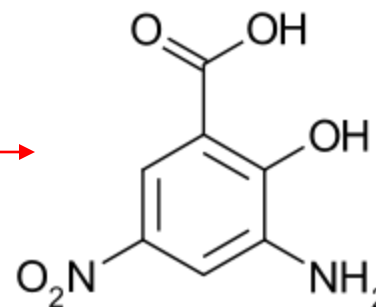
- **Glucose is oxidized to gluconic acid and hydrogen peroxide**
- **In turn the  $\text{H}_2\text{O}_2$  oxidises O-dianisidine to a pink coloured compound which absorbs at 540 nm**

# Quantitative (chemical) determination of glucose at 570 nm (DNS method)

oxidation  
aldehyde group -----> carboxyl group  
reduction  
3,5-dinitrosalicylic acid -----> 3-amino,5-nitrosalicylic acid



3,5-dinitrosalicylic acid (DNS)



3-amino,5-nitrosalicylic acid  
570 nm

## Method of estimation:

100  $\mu$ L of sample



1 mL DNS reagent



Absorbance measured  
at 570 nm

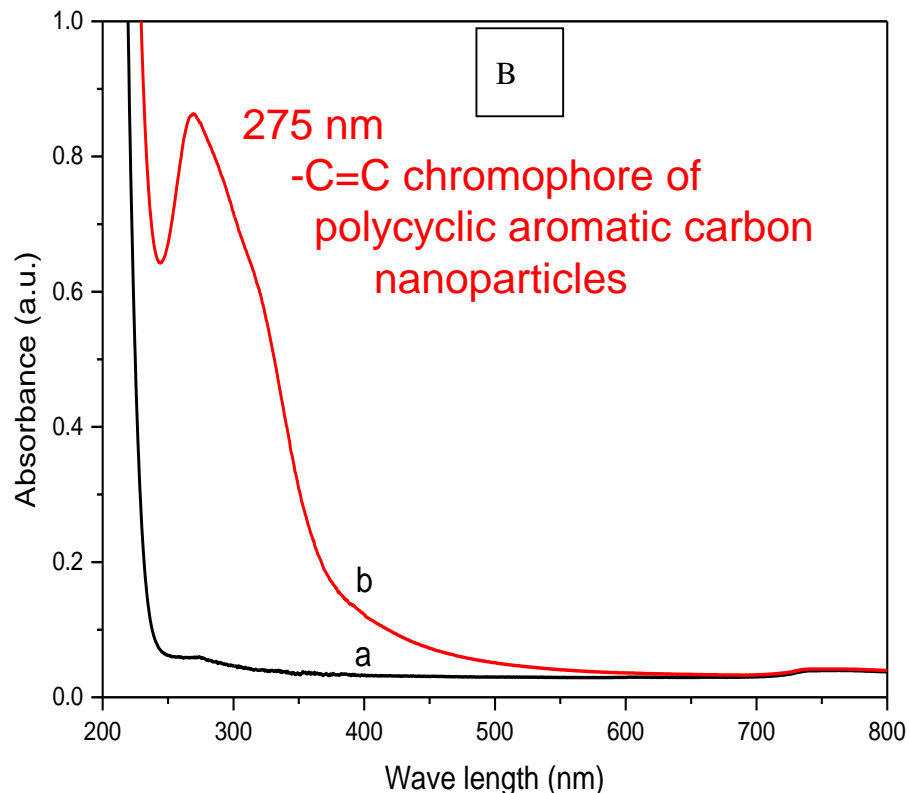
- **Glucose is oxidized to gluconic acid**
- **In turn the DNS is reduced to amino nitro salicylic acid which absorbs at 570 nm**

# Carbon nanoparticles as sensors for glucose



**Before  
autoclave**

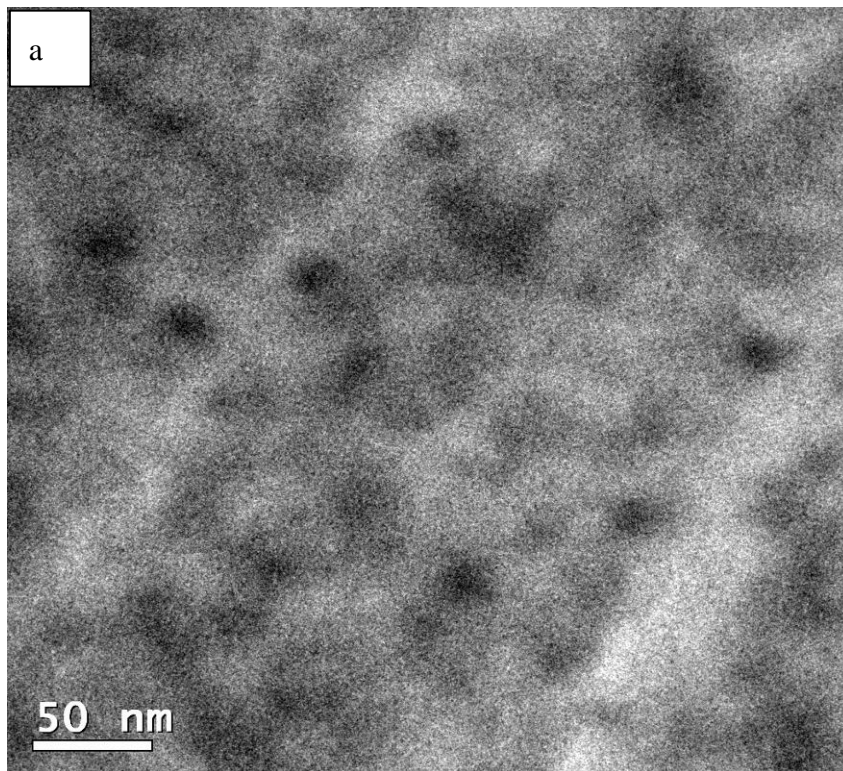
**After  
autoclave**



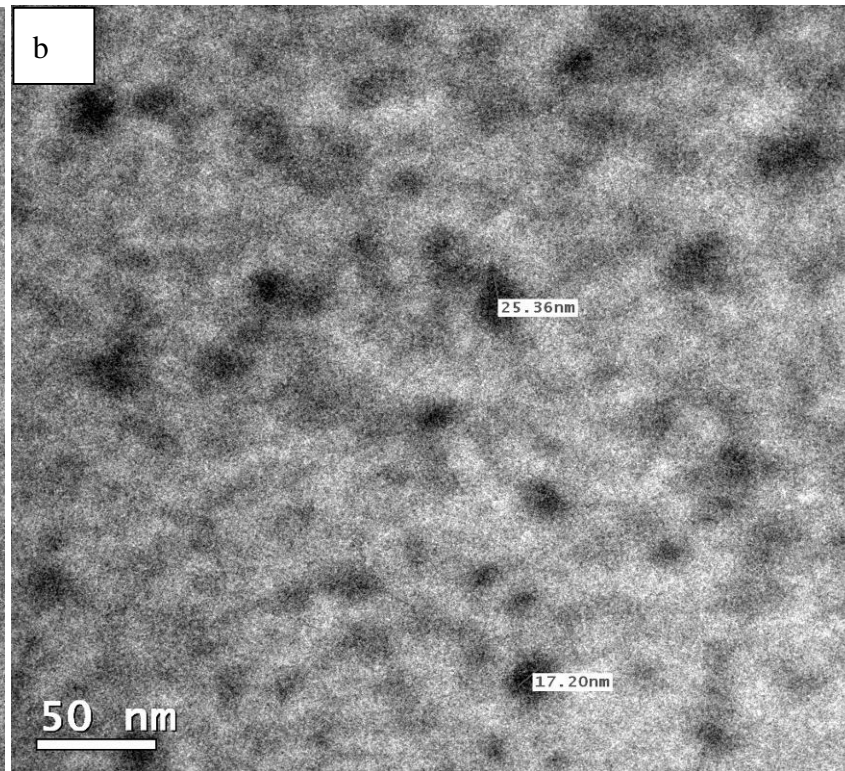
- *Autoclave treatment was done at 120 °C for 20 minutes*
- *Yellow colour indicate formation of carbon nanoparticles*
- *The carbon nanoparticles are hydrophilic*
- *-C=C- is the chromophore responsible for absorption at 275 nm*

# Urea-glucose solution after autoclave treatment – TEM analysis

Fresh nanoparticle solution



After three months of long standing



TEM images of carbon nanoparticles

- *Carbon nanoparticles < 30 nm were formed in situ in urea (0.2 %)-glucose (2 %) solution after autoclave treatment*
- *The carbon nanoparticles solution is stable even after 3 months*

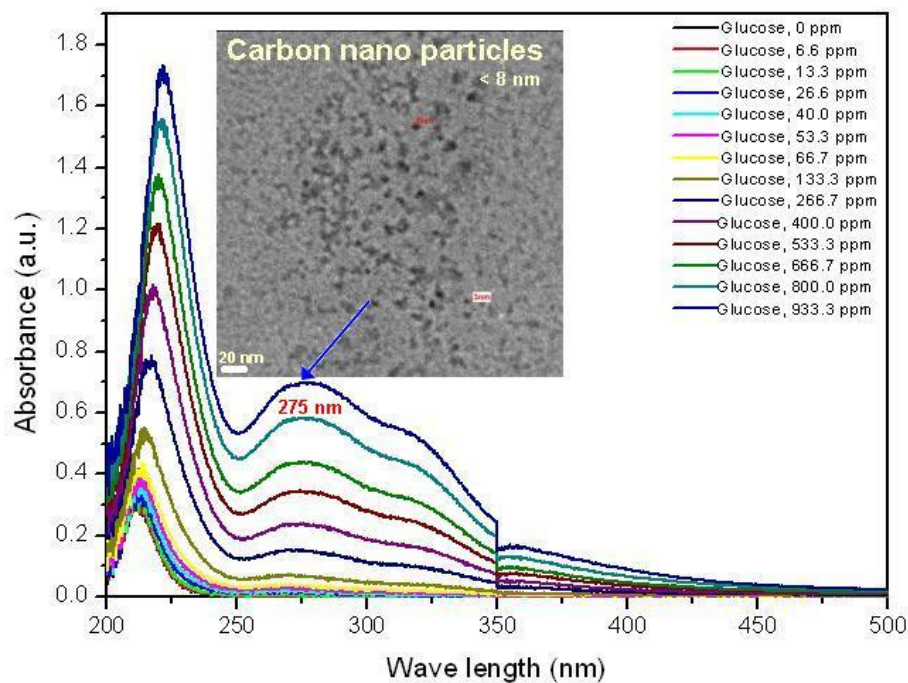
# Assay for glucose estimation

Analytes with varying amounts (7 to 933 ppm) of glucose

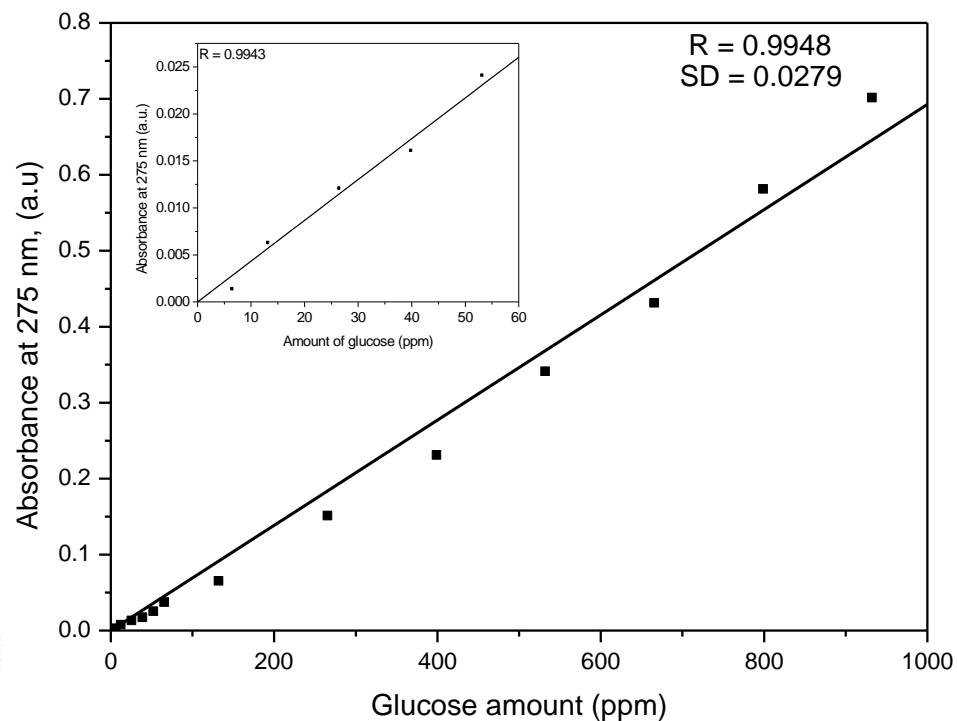
Sample code	S0	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13
Glucose (0.1 %), mL	0	0.1	0.2	0.4	0.6	0.8	1.0	2.0	4.0	6.0	8.0	10.0	12.0	14.0
Glucose, ppm	0	6.6	13.3	26.6	40.0	53.3	66.7	133.3	266.7	400	533.3	666.7	800	933.3
Urea (10 %), mL	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Water, mL	14.0	13.9	13.8	13.6	13.4	13.2	13.0	12.0	10.0	8.0	6.0	4.0	2.0	0
Total volume, mL	15	15	15	15	15	15	15	15	15	15	15	15	15	15

- **Glucose concentration varied between 0 – 1000 ppm**
- **Urea concentration fixed in the analytes S1 to S13**
- **Urea (10 %) -glucose (0.1 %) solutions autoclaved for 20 minutes at 120 °C**
- **The absorbance of solutions S1-S13 measured at 275 nm**

# Glucose sensor based on Carbon nanoparticles



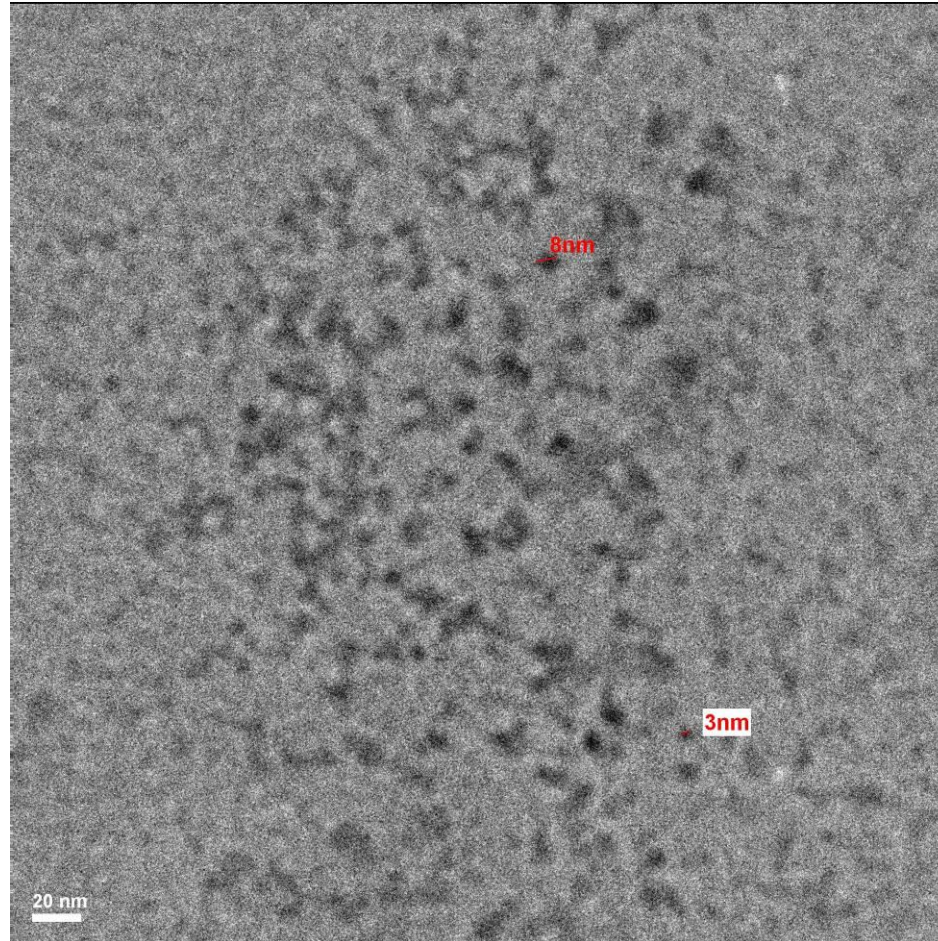
**UV Vis spectra of glucose-urea solutions (0-1000 ppm glucose)**



**Calibration plot for glucose estimation absorbance at 275 nm Vs glucose amount**

- **Absorbance at 275 nm increased as a function of the amount of glucose**
- **Good linearity over a wide range of glucose concentration (7-933 ppm or 0.04-5 mM)**
- **Limit of detection – 40  $\mu$ M**
- **Thus a simple and reliable non-enzymatic method for sensing glucose is developed**

## *In situ* generation of small (< 8 nm) carbon nanoparticles

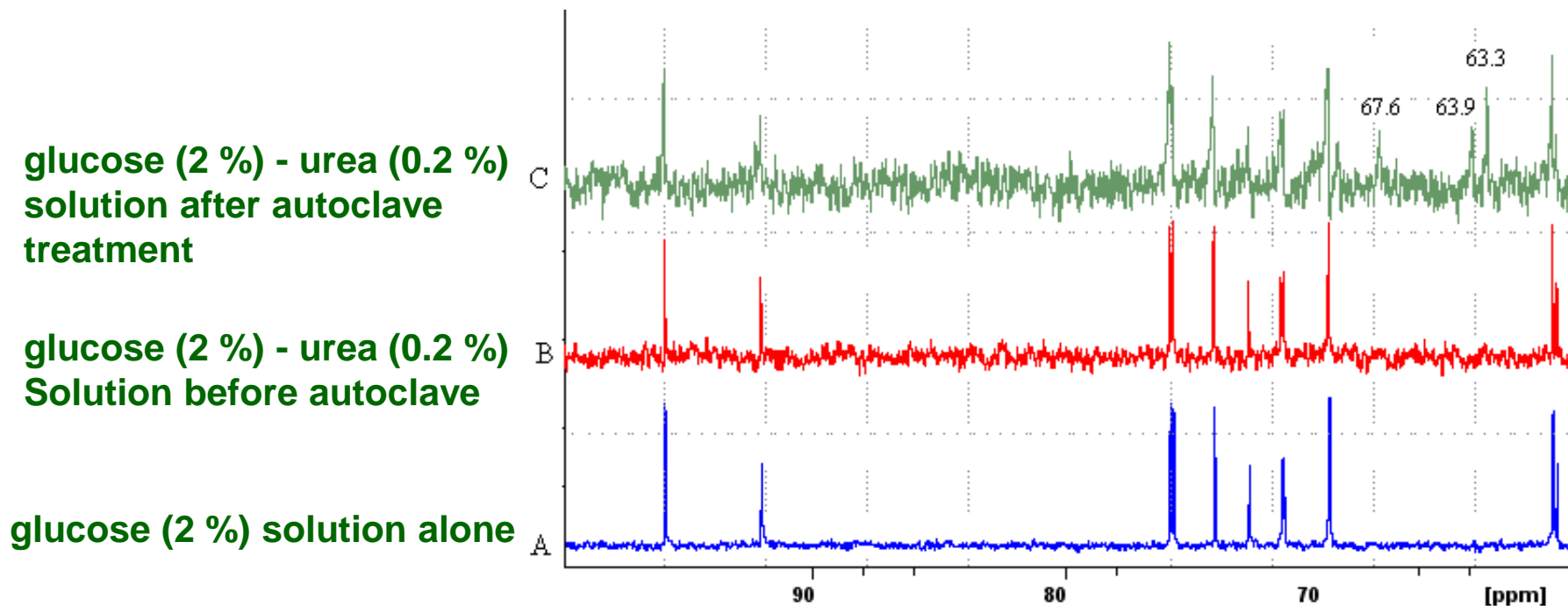


**HRTEM image of carbon nanoparticles (< 8 nm)**

- Even at a high concentration of 933 ppm of glucose, very small (< 8 nm) carbon nanoparticles are generated *in situ* in S13 sample



## chemical nature of carbon nanoparticles - NMR analysis



glucose (2 %) - urea (0.2 %) solution after autoclave treatment

glucose (2 %) - urea (0.2 %) Solution before autoclave

glucose (2 %) solution alone

$^{13}\text{C}$  NMR spectra

- The three new peaks at 63.3, 63.9 and 67.6 ppm in addition to the regular glucose peaks indicate the presence of carbon nanoparticles in the glucose urea solution after autoclave treatment
- The three peaks at 63.3, 63.9 and 67.6 ppm indicate that the carbon nanoparticles comprise of carbon nuclei in three different chemical environments

# Marine alga (*Ulva lactuca*) to glucose

0.5 g algae

1 M HCl, 10 mL

Microwave irradiation  
for 5 minutes

77 wt.% conversion of alga



*Ulva lactuca*

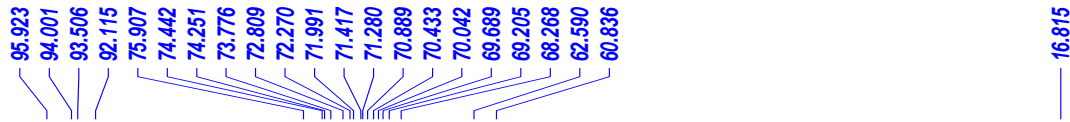
## Chemical composition

Fibers – 54%  
Minerals – 19.6 %  
Proteins – 8.5 %  
Lipids – 7.9 %  
Hemicellulose – 20.6 %  
Cellulose – 9.0 %  
Lignin – 1.7 %

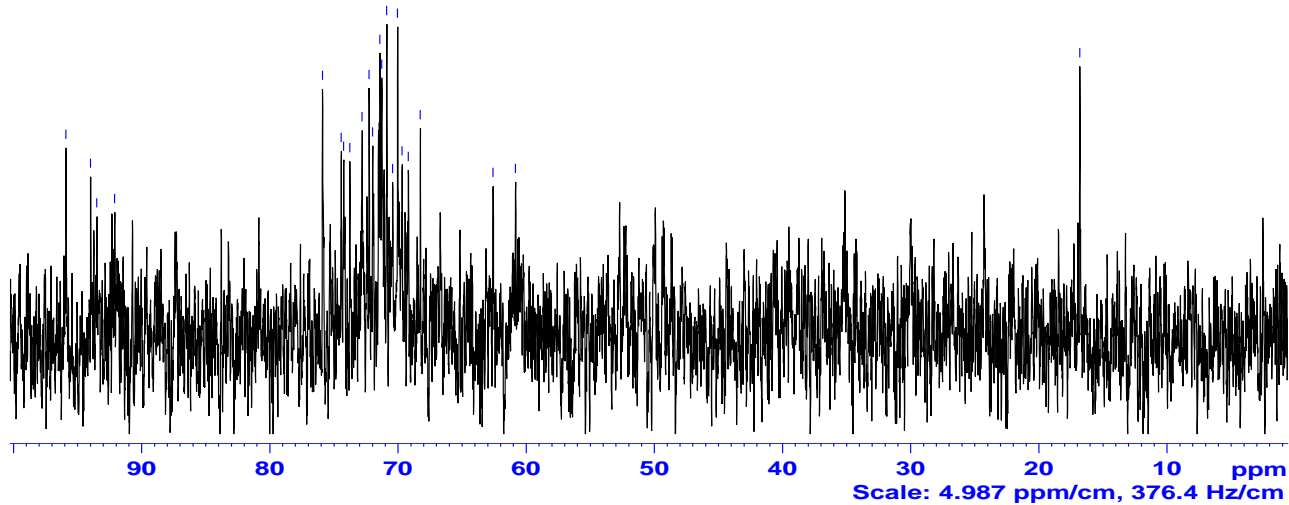
Hela Yaich et al.,  
Food Chemistry, 128, 2011, 895.

- *In addition to 0.5 g batch, higher amounts like 2 and 4 g alga could be converted to sugars with 70 - 77 % conversion and 10 wt. % yield of sugars*
- *Fast chemical hydrolysis method based on the use of microwave irradiation is developed for the production of sugars from marine alga*

# Production of sugars from *Ulva lactuca* (marine biomass)



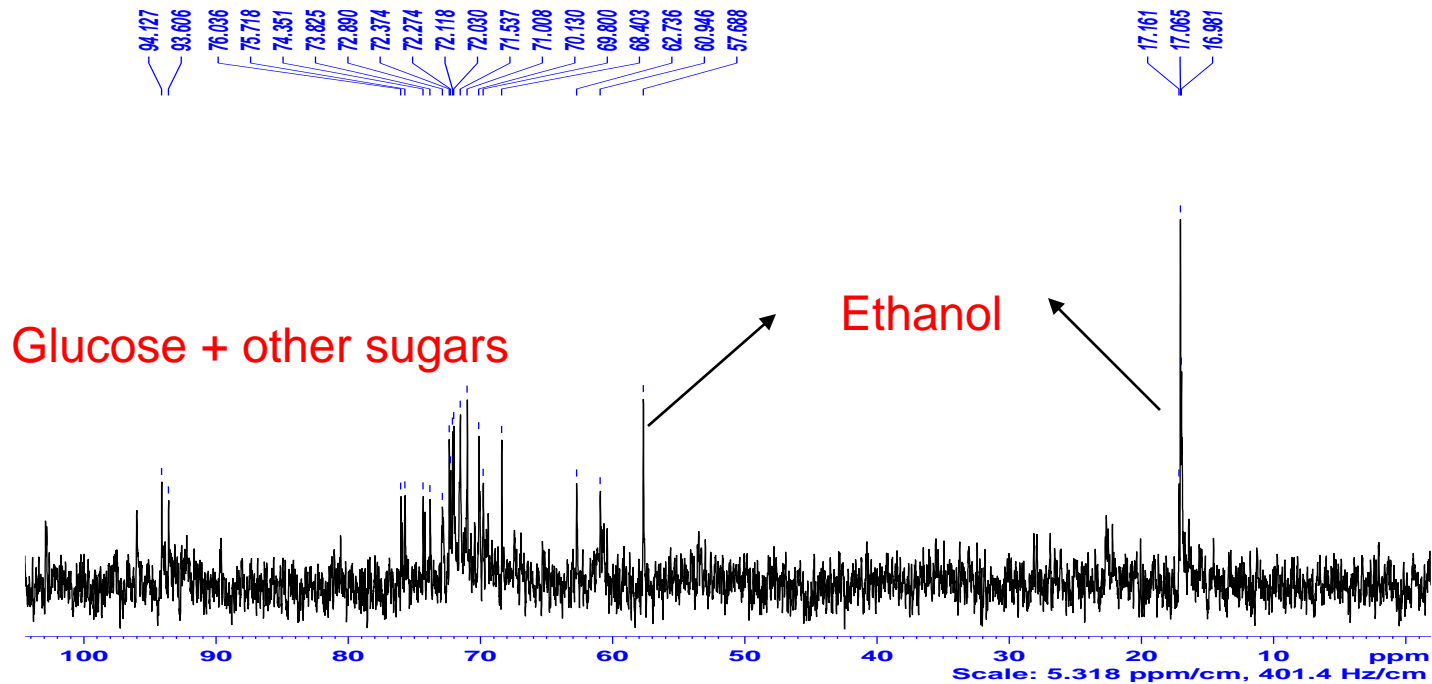
Glucose + other C<sub>5</sub> and C<sub>6</sub> sugars



**<sup>13</sup>C NMR spectrum of hydrolyzate of the hydrolysis of *Ulva lactuca***

- Presence of peaks typical of glucose (between 60 – 95 ppm)
- 77 wt.% conversion of *Ulva lactuca* is observed upon microwave irradiation for 5 min
- Yield of sugars – 10 wt. % (quantified by DNS and Urea-glucose assays)

# Production of ethanol from the sugars obtained from *Ulva lactuca* (marine biomass)



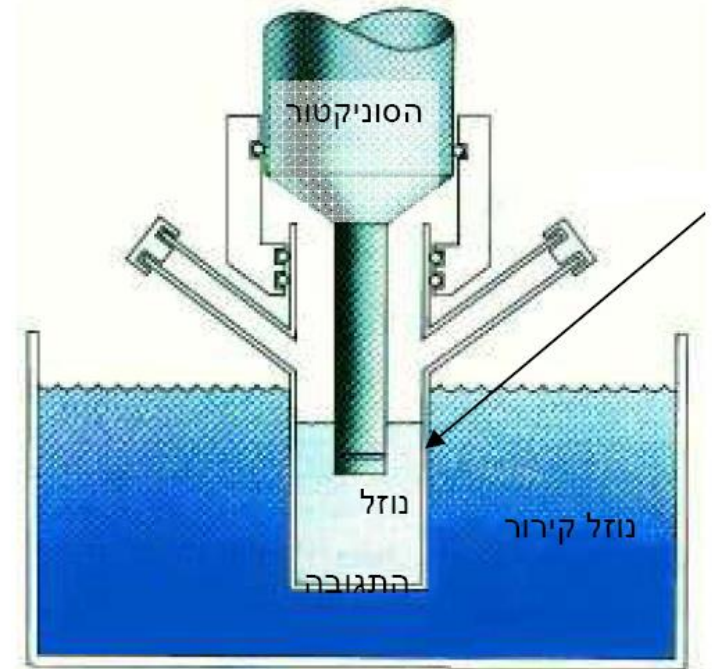
$^{13}\text{C}$  NMR spectrum of aliquot of sample from the fermentation broth

- Peaks at 16.9 and 59.7 ppm are typical of ethanol
- The sugars obtained from *Ulva lactuca* could be converted to ethanol through fermentation using yeast (*Saccharomyces cerevisiae*)
- The fermentation reaction was carried out at 25 °C for 19 h under stirring
- Quantification of the ethanol produced is being studied by  $^1\text{H}$  NMR

# Decomposition of cellulose (Avicel<sup>®</sup>) under microwave irradiation



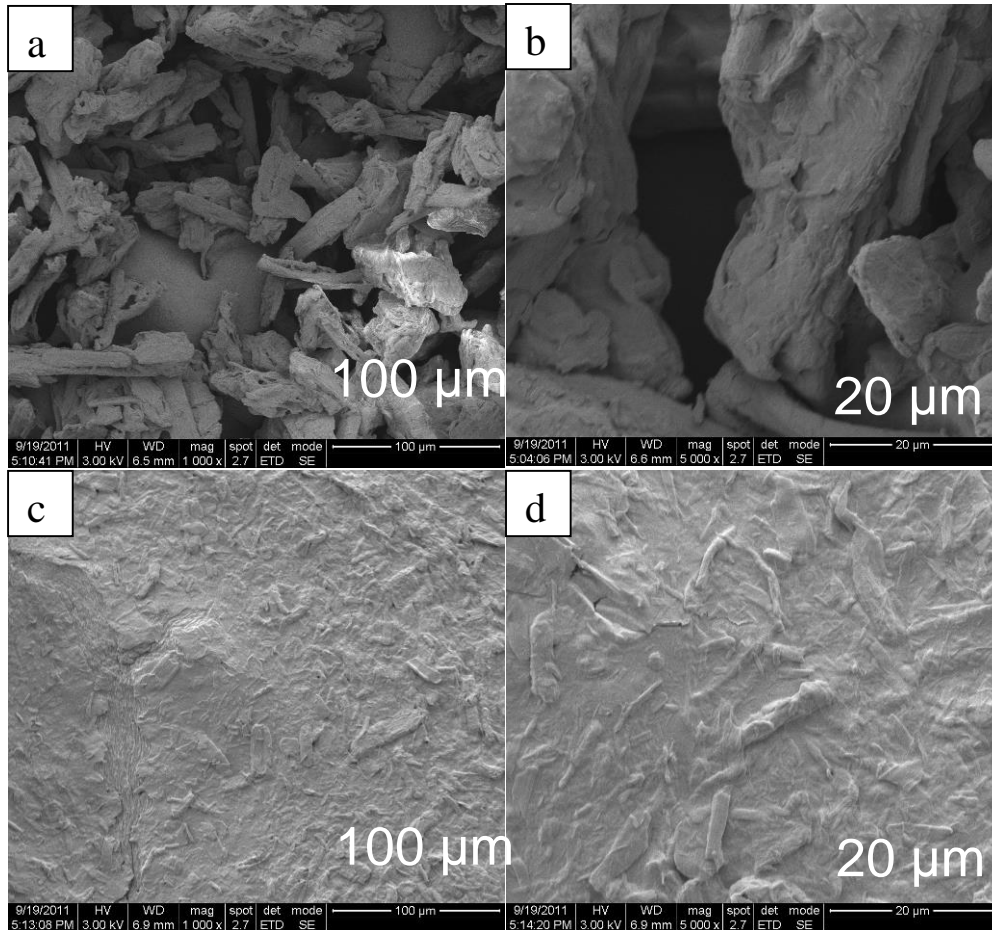
Pictorial representation of the 10 wt.% cellulose powder in water  
(a) before and (b) after sonication



תרשים כללי של מערכת הסונוכימית

**Aharon Gedanken, P. Indra Neel, Mariana Rodrigue, Patricia Mayer,  
Provisional Patent Application No. 131772, US patent dated 11.10. 2011**

# SEM analysis of cellulose before and after sonication



**Before sonication**

**After sonication**

## SEM images of the 10 wt.% cellulose (Åvicel) powder in water

- *Sonication had a profound influence on the morphology of the cellulose material*
- *Cellulose particles which appeared like blocks and logs of wood changed to thin layers upon sonication for 1h*
- *Change in the physical form of the  $\alpha$ -cellulose was attributed to the disruption of the hydrogen bonding network*

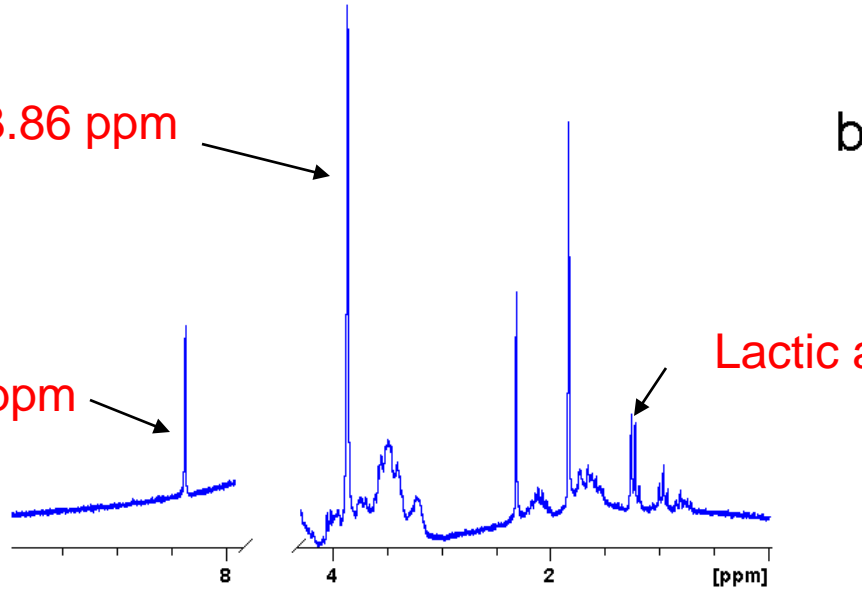
# Microwave irradiation of 1 wt. % cellulose in the presence of NaOH

Ethylene glycol, 3.86 ppm  
4H, s

**<sup>1</sup>H NMR**

Formic acid, 8.3 ppm  
1H, s

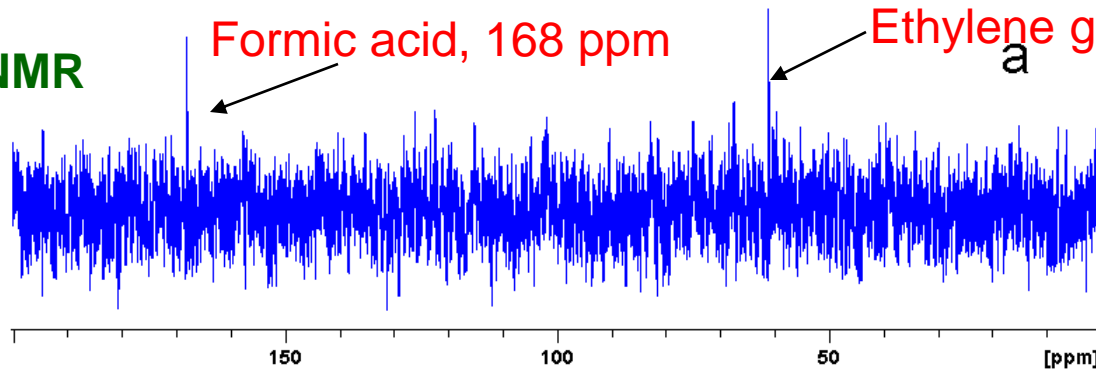
Lactic acid, 1H, d (1.18, 1.2 ppm)



**<sup>13</sup>C NMR**

Formic acid, 168 ppm

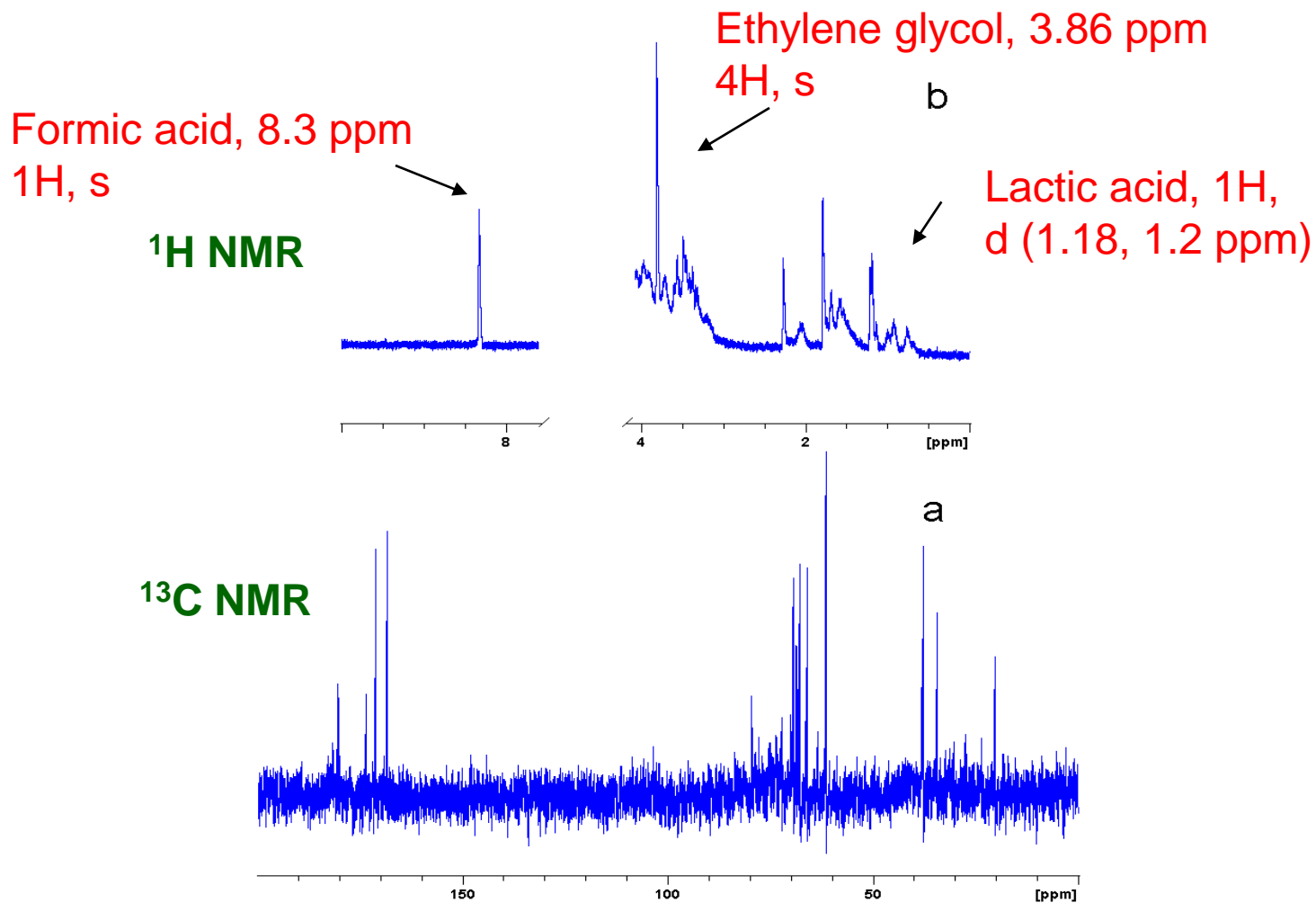
Ethylene glycol, 61.1



**NMR spectra of reaction product from cellulose (1 wt.%) degradation**

- 10 mL, 1 (wt.%) cellulose dispersion with 0.1 g NaOH exposed to microwave irradiation for 5 minutes
- 44 wt.% conversion of cellulose observed
- Ethylene glycol, formate, lactate and acetate were observed as cellulose decomposition products

# Microwave irradiation of 4 wt. % cellulose

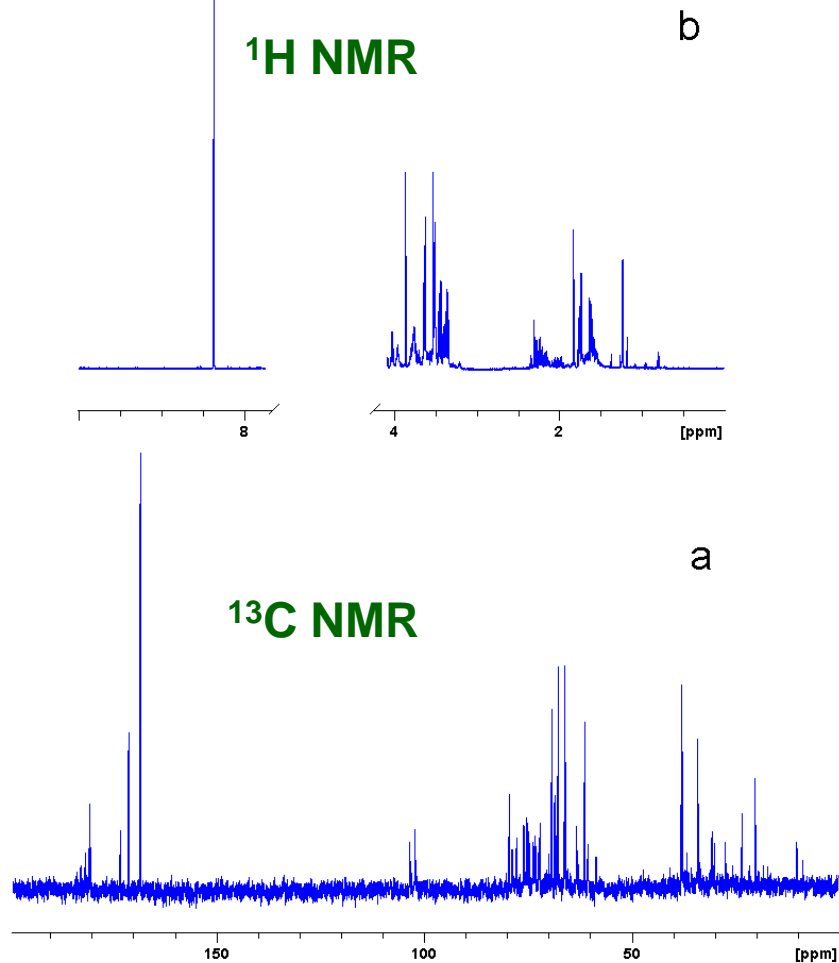


## NMR spectra of the reaction product from 4 wt.% cellulose

- *Product distribution obtained is similar to that of 1 wt.% cellulose dispersion*
- *58 wt.% conversion of cellulose is observed upon irradiation for 5 minutes*
- *In addition to ethylene glycol, formic acid and lactic acid, isosaccharinic acid is also observed*



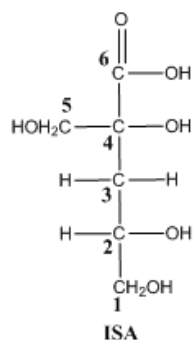
# Microwave irradiation of 10 wt. % cellulose



## NMR spectra of the reaction product from 10 wt.% cellulose

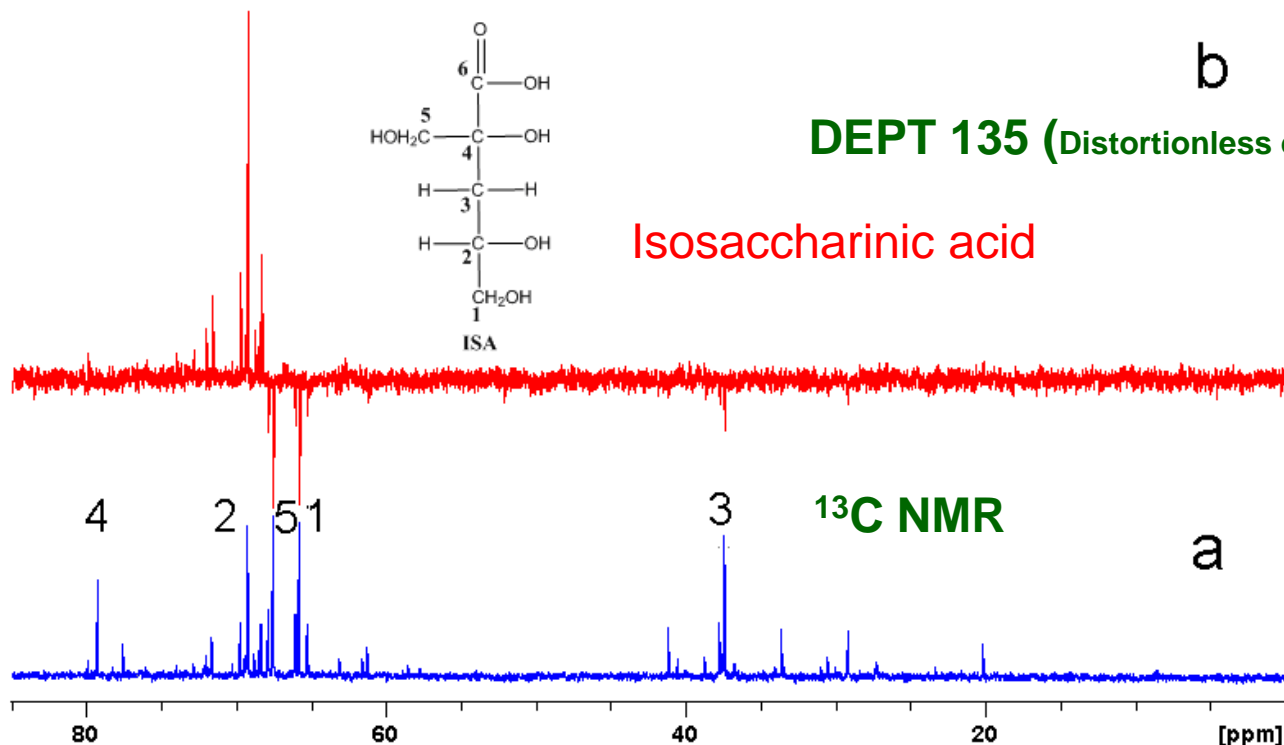
- *Product distribution obtained is similar to that of 1 wt.% cellulose dispersion*
- *54 wt.% conversion of cellulose is observed upon irradiation for 5 minutes*
- *In addition to ethylene glycol, formic acid and lactic acid, isosaccharinic acid is also observed*

# Isosaccharinic acid – the reaction intermediate of Cellulose degradation



**DEPT 135** (Distortionless enhancement by polarization transfer)

Isosaccharinic acid



**NMR spectra of the reaction product from  
10 wt.% cellulose upon microwave irradiation for 30 seconds**

- *DEPT studies reveal the substitution of carbon atoms in a compound*
- *In DEPT 135, CH and CH<sub>3</sub> appear as positive peaks and CH<sub>2</sub> will appear as negative*
- *10 wt.% cellulose dispersion exposed to microwave for 30 sec to know the reaction intermediate*
- *The reaction product exhibited peaks typical of Isosaccharinic acid (37.5, 65.7, 67.5, 69.2 and 79.2 ppm)*
- *Thus isosaccharinic acid is the reaction intermediate in the degradation of cellulose*

## Summary:

1. The study comprises of employing highly crystalline commercial cellulose (Avicel®) for the production of chemicals like formic acid, ethylene glycol and lactic acid.
2. The strategy for decomposing cellulose comprises of two stages: (i) to reduce the crystallinity of cellulose by sonication and (ii) expose the aqueous dispersions of cellulose in alkaline medium to microwave irradiation for a maximum of 5 minutes.
3. The developed method is applicable for 1, 4 and 10 wt.% cellulose (initial concentration) aqueous dispersions. The wt. % conversion values of 44, 58 and 54 % were observed with 1, 4 and 10 wt.% cellulose dispersions.
4. Thus an easy and fast process has been developed for the degradation of cellulose to small organic molecules with the aid of sonication and microwave irradiation under alkaline (NaOH) conditions.

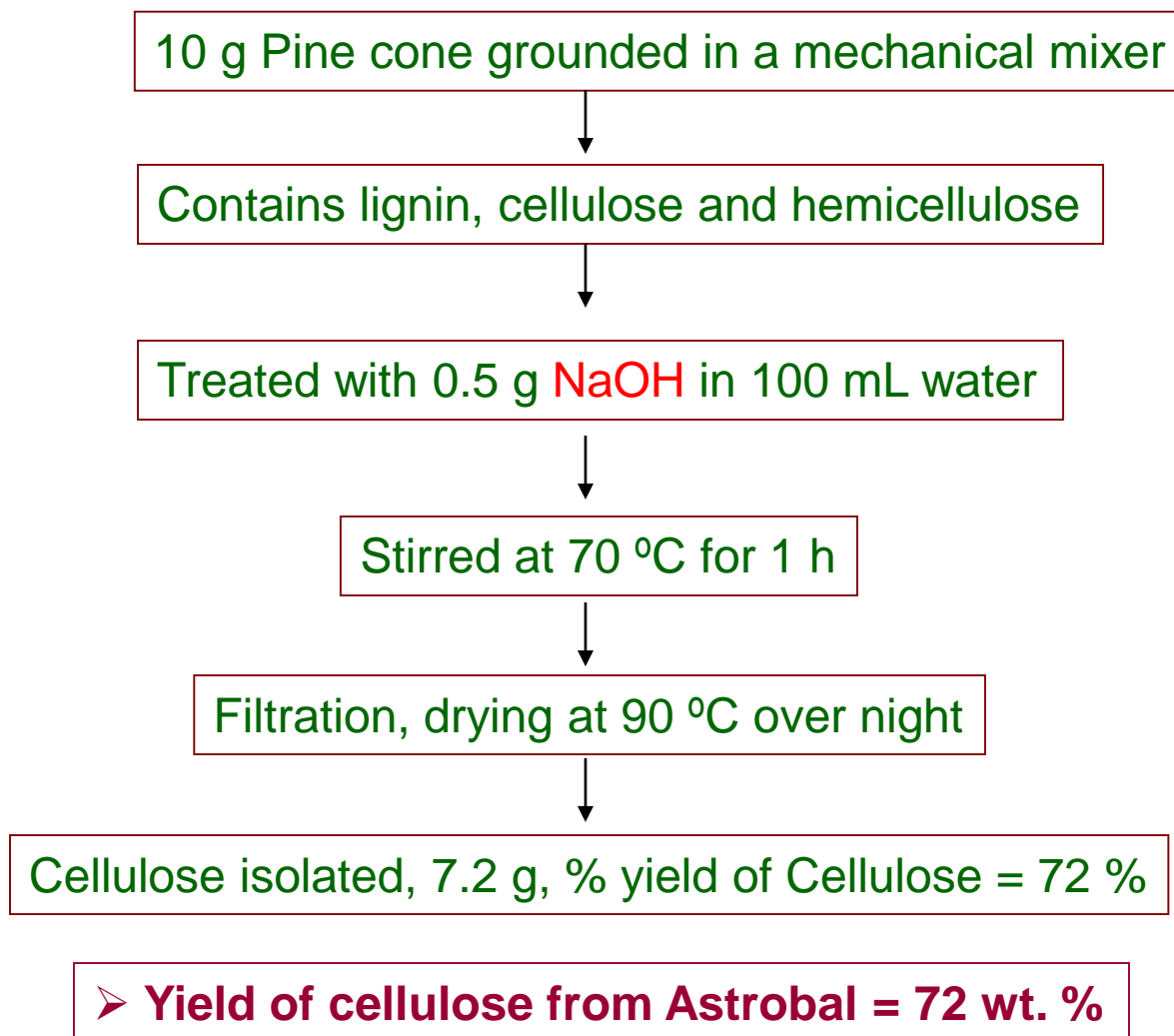
## Glucose from pine cone , Astrobol, (*pinus radiata*)



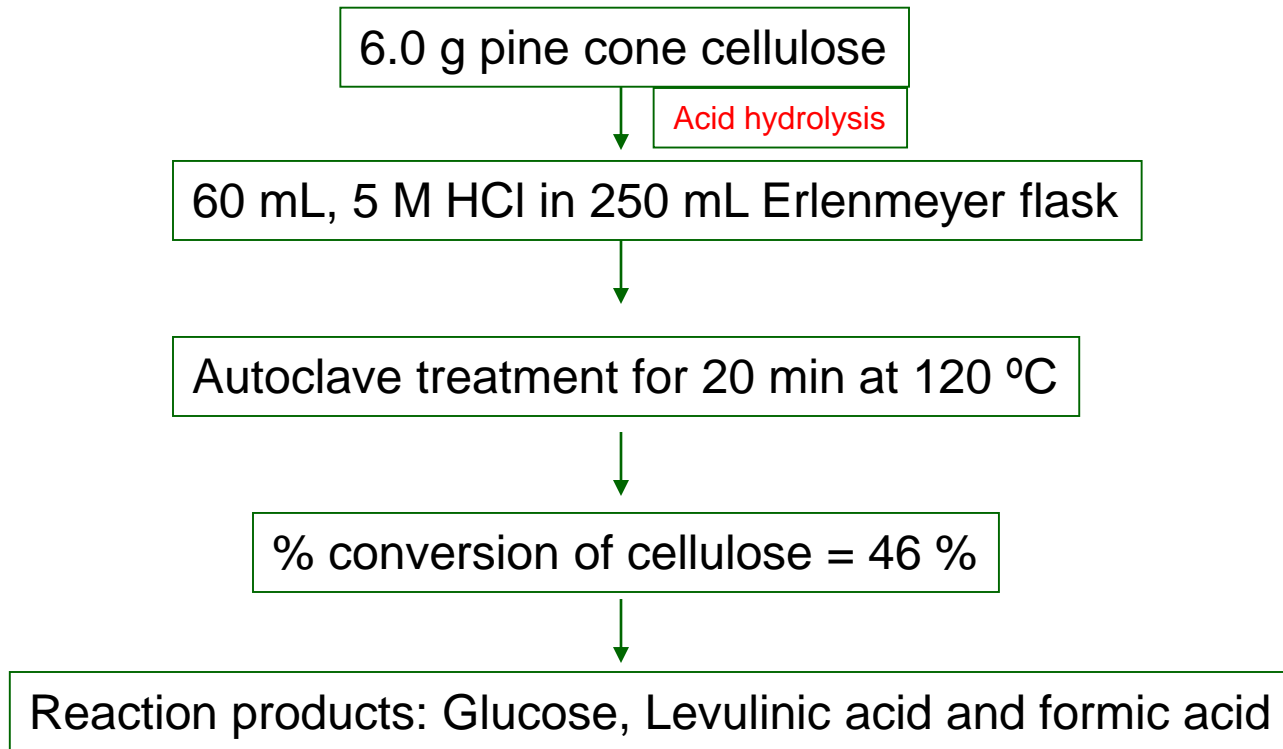
Pictures of the pine cone, needles and spikes and the bark of the tree *Pinus radiata*

- ***Pine cone comprises of lignin, cellulose and hemicellulose***
- ***Lignin do not yield sugars***
- ***Hemicellulose yields C5 sugars like Xylose***
- ***Unlike lignin and hemicellulose, cellulose upon hydrolysis yields glucose, an easily fermentable sugar***
- ***Thus it is necessary that the cellulose from pine cone is isolated to obtain glucose***

## Cellulose from pine cone, Astrobal, (*pinus radiata*)

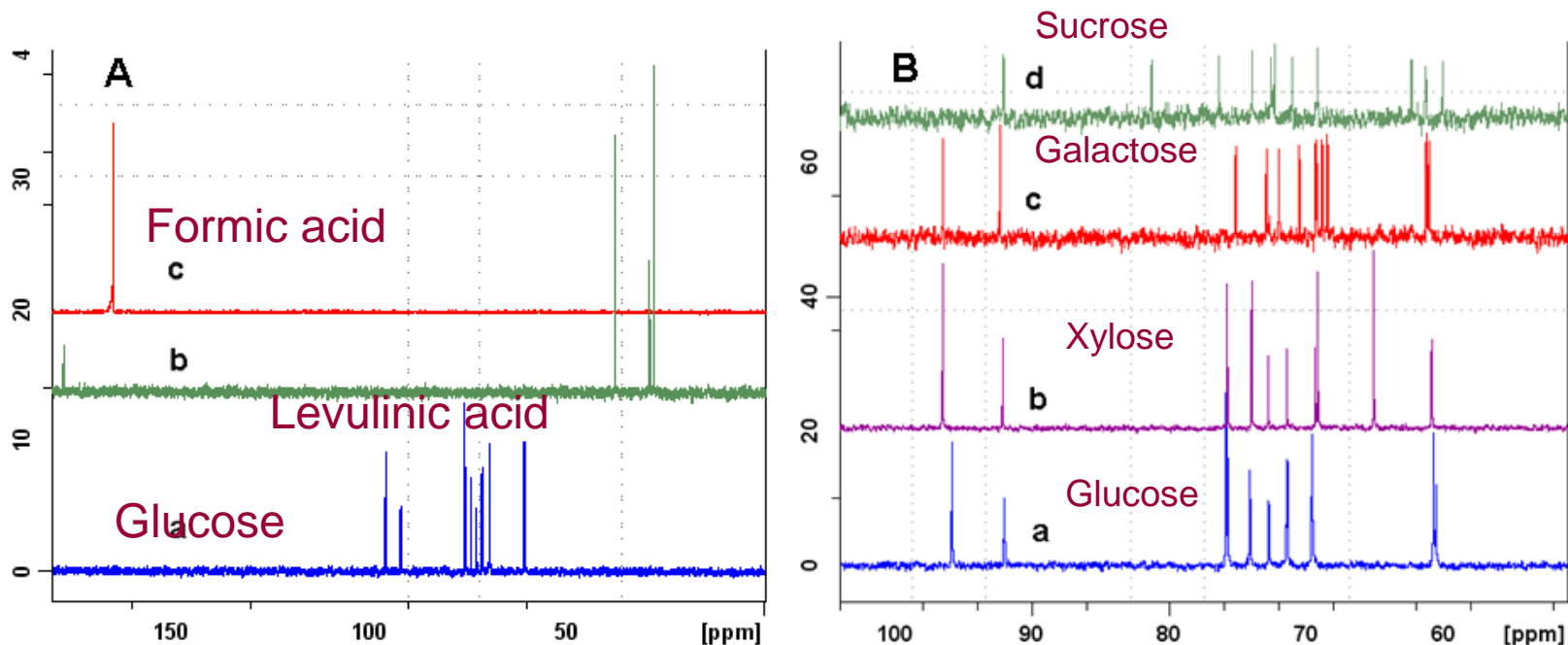


# Glucose from the pine cone cellulose



- Except glucose no other C5 or C6 sugars are observed
- In addition to glucose, levulinic acid and formic acid were formed
- Levulinic acid ( $C_5H_8O_3$ ,  $CH_3COCH_2CH_2COOH$ ) is a valuable bulk chemical and a fuel additive
- Formic acid is a fuel by itself. It can be a substitute to methanol in Direct methanol fuel cells

## $^{13}\text{C}$ NMR – A potential tool in distinguishing various sugars



$^{13}\text{C}$  NMR spectra of the authentic samples of (A). (a) glucose, (b) levulinic acid and (c) formic acid and (B). (a) glucose, (b) xylose, (c) galactose and (d) sucrose

- The spectra of various sugars have striking differences and each of them is distinguishable.
- For instance, xylose can be distinguished from glucose by the peak at 65.1 ppm which is present only in xylose but not in glucose

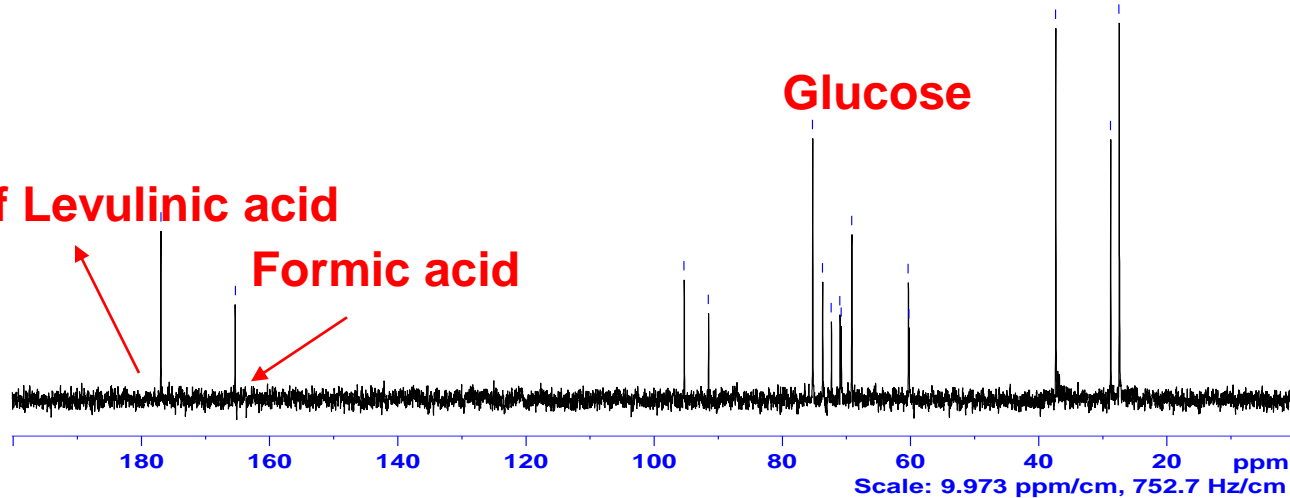
# Glucose from the pine cone cellulose



## Levulinic acid

## Glucose

**-COOH of Levulinic acid**  
**Formic acid**



## Reaction product from the acid (HCl) hydrolysis of cellulose from pine cone

- 20 minutes autoclave treatment of pine cone cellulose in the presence of 5 M HCl at 120 °C, yielded 46 %
- Glucose, Levulinic acid and formic acid were produced upon acid hydrolysis
- Glucose is the only sugar produced from pine cone; no other C5 or C6 sugars observed
- No fermentation inhibitors like, acetic acid, hydroxy methyl furfural, furfural, glycol aldehyde and soluble phenolic compounds are formed



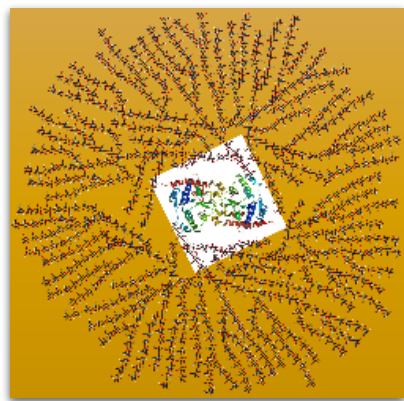
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## Summary:

- ❖ Lignocellulosic biomass derived from the Pine cones of *pinus radiata* was effectively delignified to yield holocellulose.
  - ❖ Holocellulose upon hydrolysis yielded glucose as the only fermentable sugar and no other C5, C6 sugars are produced.
  - ❖ Formic acid and levulinic acid were also found in the hydrolyzate along with glucose upon the acid (5 M HCl) hydrolysis of the holocellulose from pine cone.
  - ❖ No trace of fermentation inhibitors like, acetic acid, hydroxy methyl furfural, furfural, glycol aldehyde and soluble phenolic compounds, were found in the hydrolyzate.
  - ❖ The glucose in the hydrolyzate is transformed to bioethanol upon fermentation at 30 °C in the presence of *Saccharomyces cerevisiae*.
  - ❖ Thus pine cones from *pinus radiata* were demonstrated to possess the ability of potential feedstock for fermentable sugars which serve as a precursor to the production of bioethanol.
-

# Glucose from glycogen

❖ Glycogen is a biopolymer which is a rich and abundant source of glucose



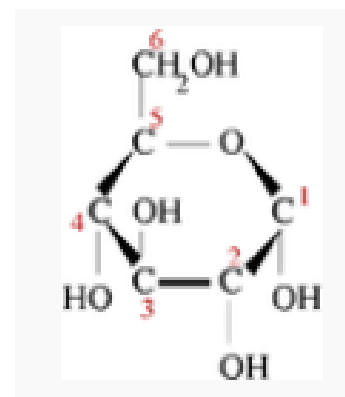
**Glycogen**



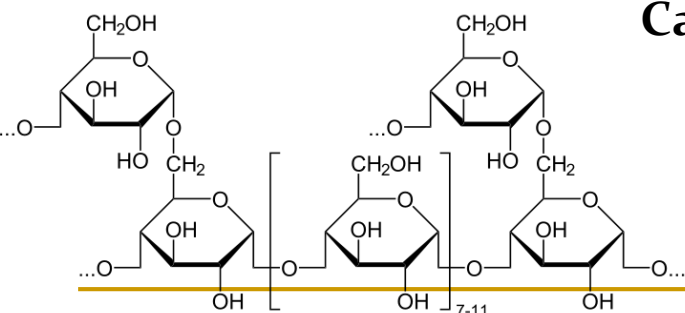
**Acid  
Catalyst**



**Microwave  
irradiation,  
10 min**

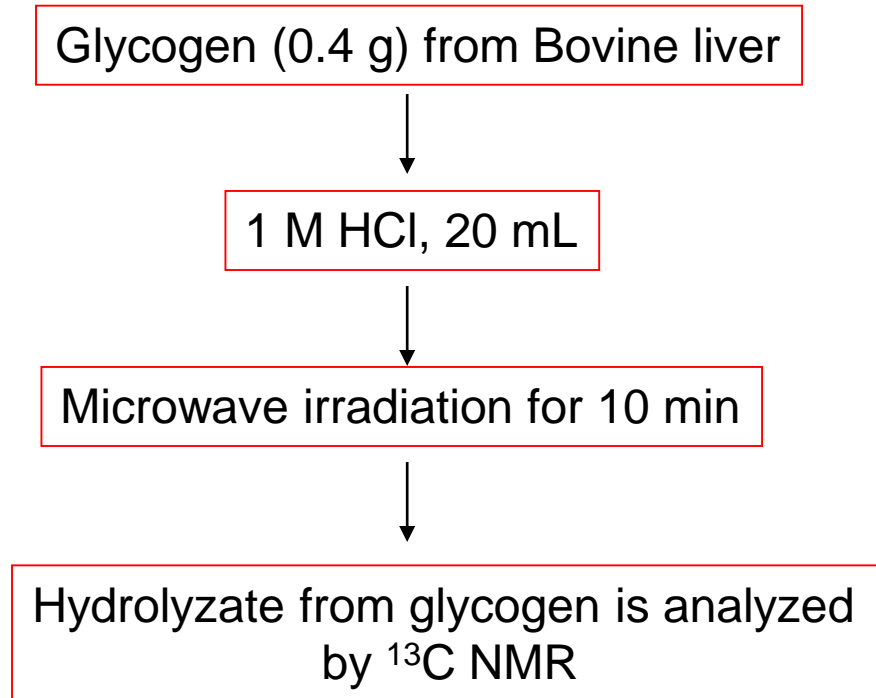


**Glucose**

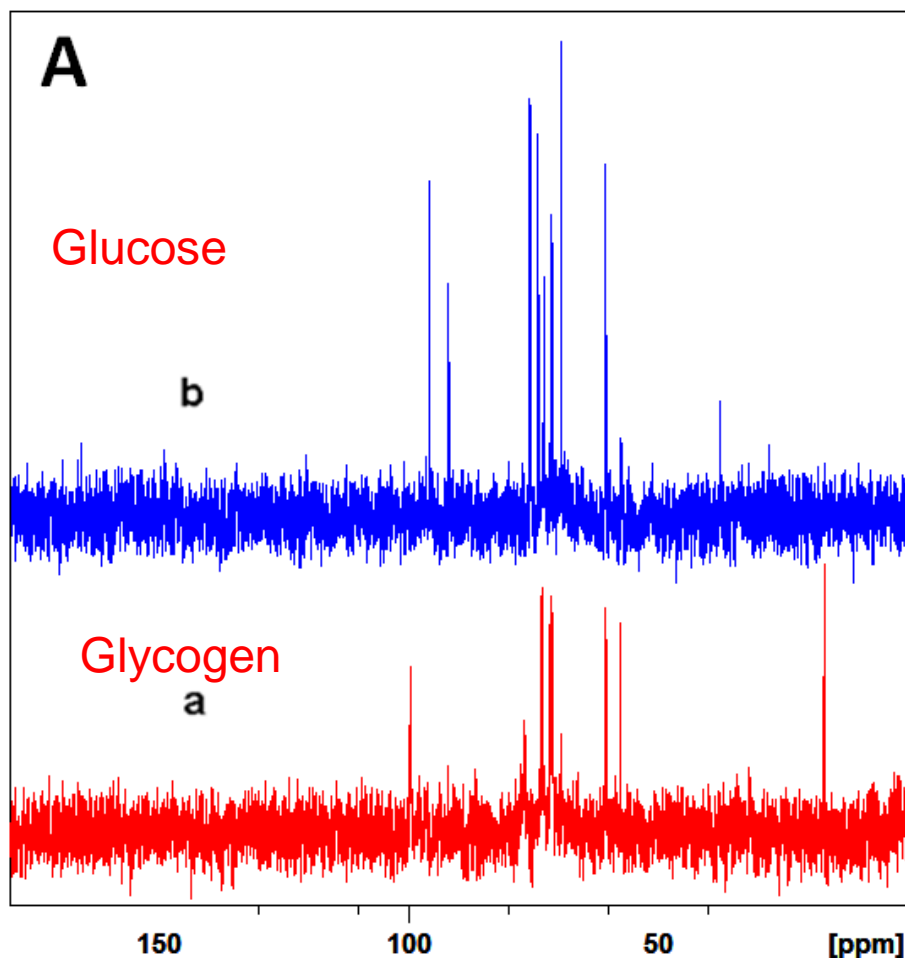


**Chemical structure of glycogen**

# Glycogen hydrolysis under microwave irradiation



## Glucose from glycogen



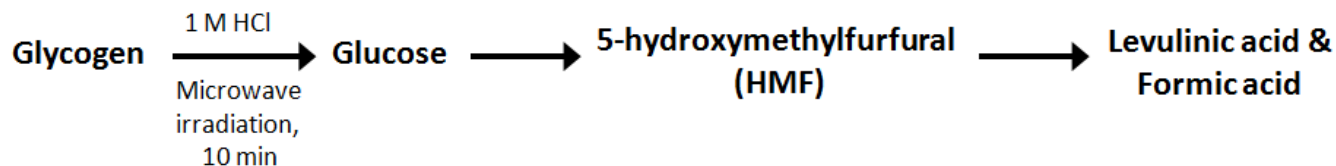
- ❖ Peak typical of glycogen at 99 ppm disappeared completely upon microwave irradiation for 10 min
- ❖ Complete conversion of glycogen in the presence of 1 M HCl
- ❖ Peaks typical of glucose (95 and 92 ppm) are observed in the hydrolyzate
- ❖ Only glucose but not other C5 and C6 sugars are produced from glycogen
- ❖ Glucose is the most easily fermentable sugar

$^{13}\text{C}$  NMR spectra of (a) authentic sample of glycogen and (b) hydrolyzate from glycogen in 1 M HCl upon microwave irradiation;

## Effect of HCl concentration on the hydrolysis of glycogen

Conc. of HCl (M)	Reactant	Reaction products		
	Glycogen	Glucose	L.A	F.A
5	–	+	+	+
3	–	+	+	+
1	–	+	+	+
0.5	+	+	–	–
No acid	+	–	–	–

L.A– levulinic acid; F.A– formic acid. "+" present; "-" absent.



Schematic representation of hydrolysis of glycogen

❖ 1 M HCl is the optimum conc. with complete conversion of glycogen  
Upon 10 min microwave irradiation

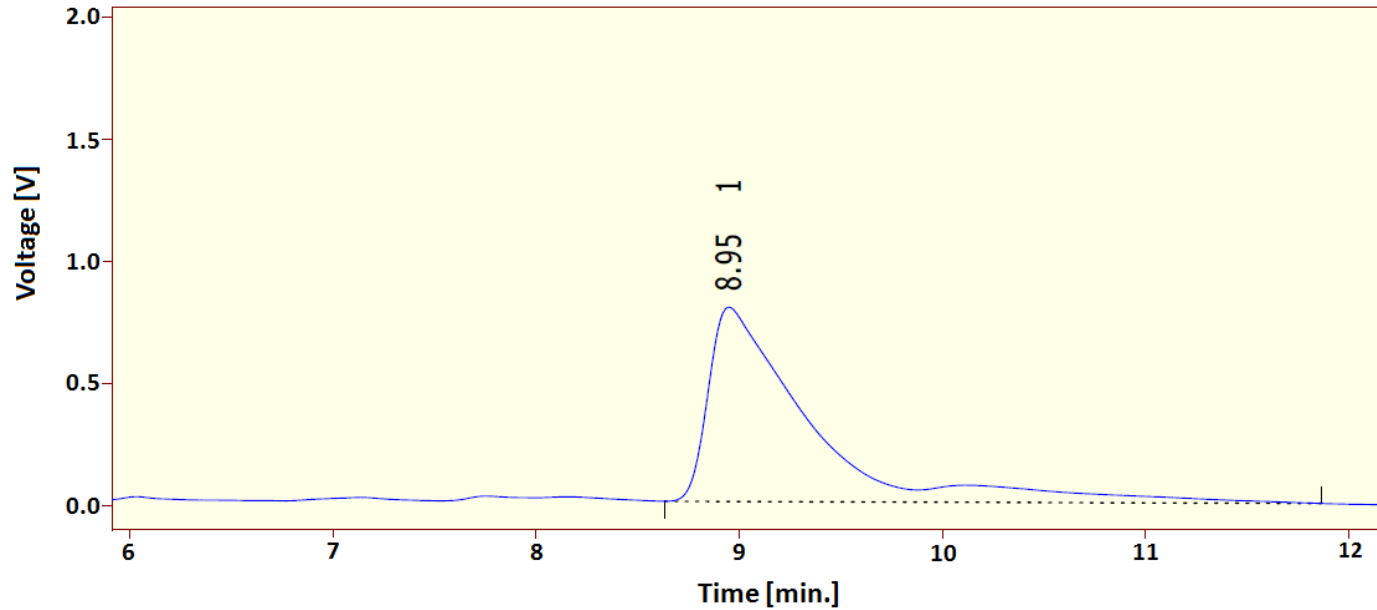
## Effect of reaction time on the hydrolysis of glycogen under microwave irradiation with 1 M HCl

Reaction time (minutes)	Reactant	Reaction products		
	Glycogen	glucose	L.A	F.A
10	—	+	+	+
6	+	+	—	—
4	+	+	—	—
2	+	+	—	—

L.A. - levulinic acid; F.A. - formic acid. "+" present; "-" absent.

❖ 10 minutes of irradiation is the optimum time required for the complete conversion of glycogen

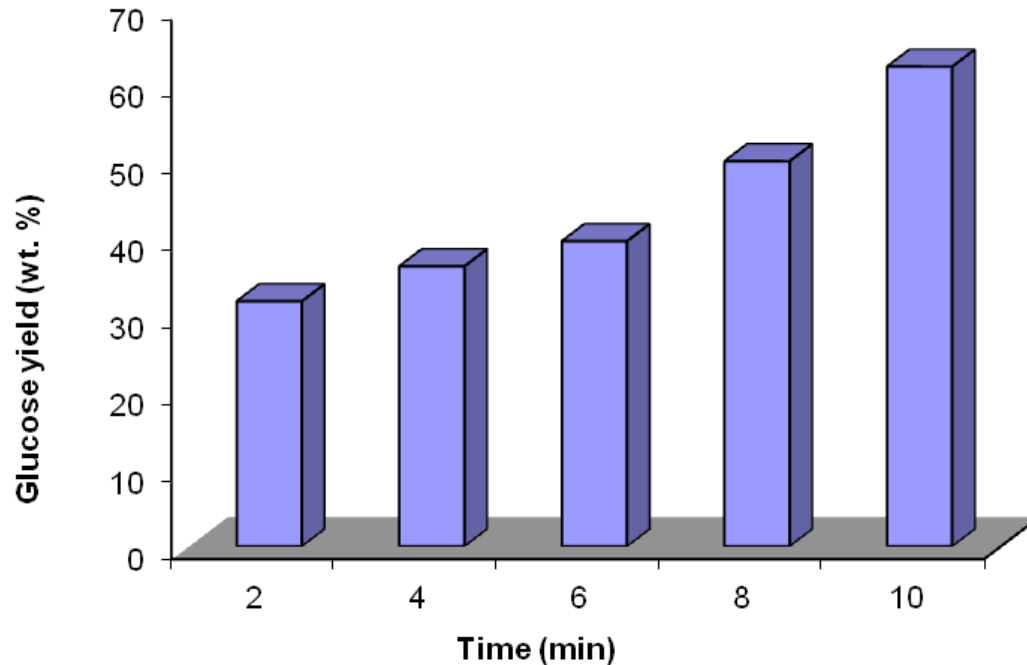
## Determination of the glucose content by HPLC



**Typical HPLC chromatogram of the hydrolyzate (from 0.4 g glycogen in 20 mL, 1 M HCl for 10 minutes)**

➤ A peak with a retention time of 8.95 minutes is typical of glucose

## Effect of time of microwave irradiation on the yield of glucose from glycogen

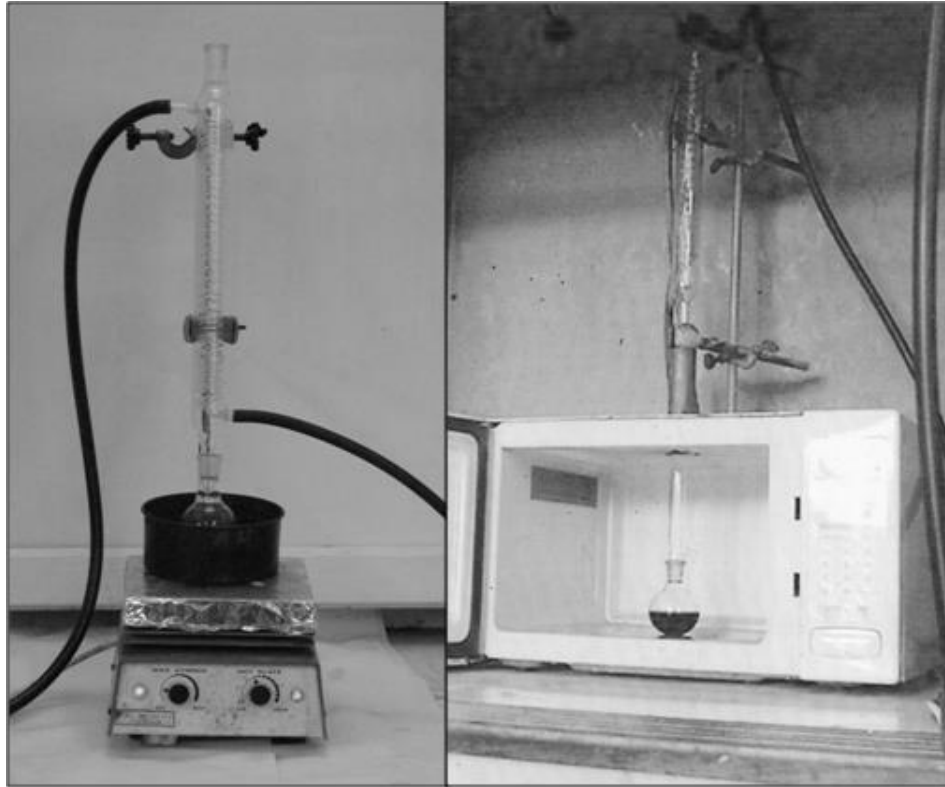


Glucose yield (wt. %) as a function of time of irradiation of the hydrolyzate (glycogen, 0.4 g, in 1 M HCl, 20 mL)

➤ Glucose yield increased steadily from 32 to 62 wt. % upon increasing the time of microwave irradiation from 2 – 10 minutes



## Effect of microwave heating Vs conventional heating for glycogen hydrolysis reaction



Pictorial representation of the hydrolysis of glycogen carried out under (A) oil bath at 80 °C and (B) microwave irradiation

- In the presence of microwave irradiation complete conversion of glycogen is observed in 10 minutes.
- Even after 6 h of hydrolysis reaction at 80 °C under conventional heating (oil bath with reflux condensation) no complete conversion of glycogen is observed

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## Summary:

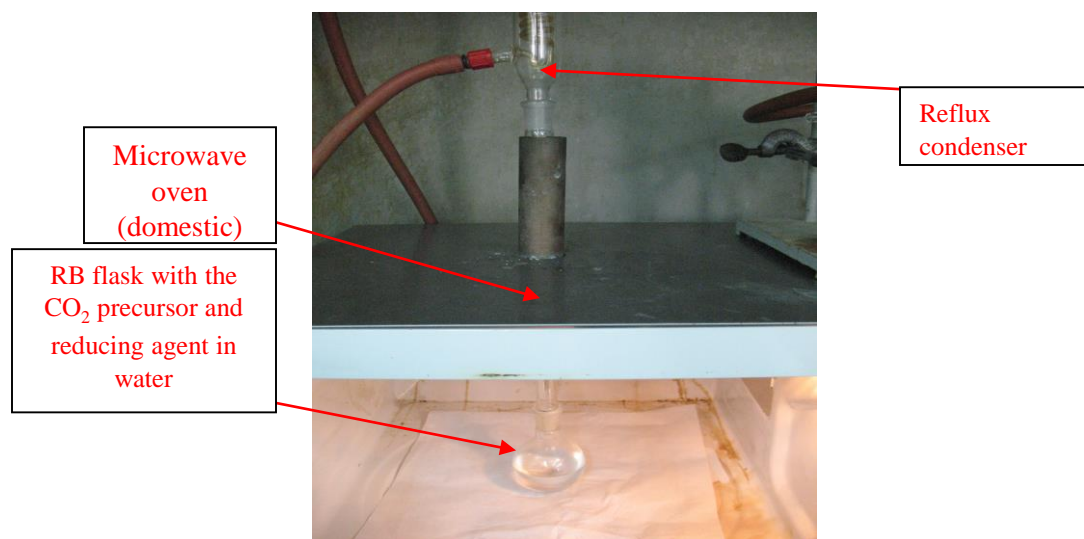
- Microwave irradiation of glycogen under acid conditions (1M HCl) for 10 minutes yielded complete conversion
- The highest yield of glucose is 62 wt.% in a time period as short as 10 minutes
- When the hydrolysis reaction was carried out under conventional heating (oil bath) more than 6 h was needed for the complete conversion of glycogen.
- Thus the accelerating effect of microwave energy for the production of fermentable sugar (glucose) from glycogen has been demonstrated.
- The glycogen upon acid hydrolysis yielded exclusively glucose as the fermentable sugar and no other C5 or C6 sugars are produced which are inevitable when lignocellulosic biomass is employed as feedstock for the generation of sugars.
- As glycogen can be produced from the abundant and renewable chemical feed stock like CO<sub>2</sub>, the use of glycogen as a precursor for glucose generation is an economically and environmentally benign alternative to cellulose.

## Chemical reduction of CO<sub>2</sub> to HCOONa

- Chemical reduction of carbon dioxide (CO<sub>2</sub>) to fuels and chemicals is one of the dream reactions of chemists.
- CO<sub>2</sub> is a very stable molecule (Standard free energy of formation,  $\Delta G^\circ = -394.3$  kJ/mol)
- Transformation of CO<sub>2</sub> to chemicals not only reduces the major green house gas but also convert the same to a useful material.
- There are currently no known chemical reduction methods operating at modest reaction conditions to convert carbon dioxide to formate.
- Radiochemical, photochemical, electrochemical and bio photo electrochemical methods are known to yield formic acid from carbon dioxide. But none of the aforementioned approaches could be employed industrially.

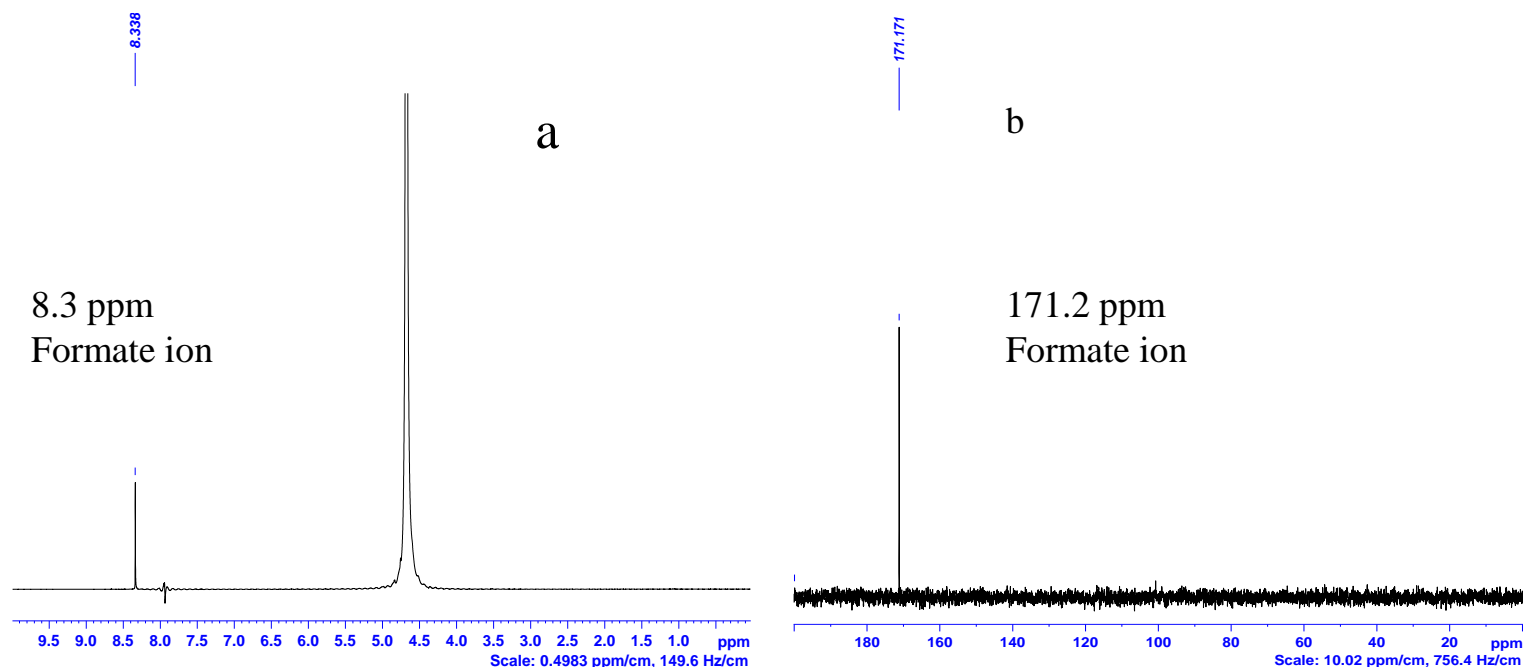
## Process for the chemical reduction of CO<sub>2</sub>

- Typical process comprise of placing in a round bottom flask, 1.0 g ammonium carbonate (CO<sub>2</sub> precursor) and 0.5 g sodium boro hydride in 10 mL water and subsequently subjecting the contents to microwave irradiation for 5 minutes



Pictorial representation of the CO<sub>2</sub> conversion taking place under microwave irradiation

## HCOONa from CO<sub>2</sub> generated in situ from (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub>



(a) <sup>1</sup>H NMR and (b) <sup>13</sup>C NMR spectra of reaction product from the chemical reduction of CO<sub>2</sub> (from ammonium carbonate)

- A peak at 8.3 ppm in the <sup>1</sup>H NMR (Fig. 1a), 171 ppm in <sup>13</sup>C NMR (Fig. 1b) establish the presence of formate anion and rule out the presence of formaldehyde or methanol or even carbonic acid.

## Summary:

- CO<sub>2</sub>, either generated in situ by the decomposition of ammonium carbonate or dry ice in aqueous medium is reduced selectively to formate using NaBH<sub>4</sub> as reducing agent under microwave irradiation for 5 minutes.
- The exclusive formation of sodium formate as the CO<sub>2</sub> chemical reduction product is established authentically through <sup>1</sup>H, <sup>13</sup>C, DEPT-135 NMR, FT-IR and UV-Vis spectroscopic studies.
- The chemical reduction process involving the conversion of CO<sub>2</sub> to formate in aqueous medium is fast owing to the use of micro wave irradiation.
- NaBH<sub>4</sub> is an efficient reducing agent for the reduction of CO<sub>2</sub> in aqueous medium under microwave irradiation conditions.
- Thus a new technology is invented for the chemical reduction of the thermodynamically stable CO<sub>2</sub> molecule to be converted to a valuable fuel, formate, which can be used as such in fuel cells as a substitute of methanol or can be employed as a reservoir for hydrogen fuel or can be used as an industrial chemical.

## Acknowledgement:

I thank Prof. Ami Ben-Amots from Seambiotic,

Dr. P. Indraneel,

Miri Koberg,

Miri Klein,

Barry Kimchi

The group of Prof. Dubinsky,

Prof. Alvaro Israel, NIO

**It is finished!**  
**St. John, 19:30**