

Repair and servicing facility

The government pays a job fee of Rs 800 per biogas plant constructed on turnkey basis with three years' warranty for trouble-free functioning of the plant in

north-eastern states, Sikkim, Jammu and Kashmir, Himachal Pradesh, Uttaranchal, hilly districts, and islands. In other regions, it is Rs 700 per plant. Financial support limited to 50% of the rate of

central subsidy is also provided for repair and revival of family-type biogas plants that are at least five years old and have developed structural defects thereafter.

Courtesy: MNRE, Government of India

Manufacturers of biogas burners

M/s Sunflame Industries (P) Ltd
Shed No. 2, Plot No. 58
P O Amar Nagar, Faridabad – 121 003

M/s Batra Investments Pvt. Ltd
14/1, Mathura Road
P O Amarnagar
Faridabad – 121 003

M/s Gas and Chemical Industries (P) Ltd
14/1 Mathura Road
Faridabad - 121 003

M/s Baroda Appliances
886/A, GIDC Makarpura
Baroda - 10

M/s Sweet Home Appliances Pvt. Ltd
3-E/16, BPNIT
Faridabad – 121 001

M/s Tulsi Domestic Appliances
30-A, Old Industrial Area
Alwar - 301 001

M/s Associated Engineering Works
Tanuku - 534 211
Andhra Pradesh

M/s Mitaso Appliances Ltd
Plot No. 63, Sec. 6
Faridabad – 121 006

M/s Agriculture Associates
Station Road
Alwar (Rajasthan)

M/s Malhotra Engineering Company (P) Ltd
572-B, Nangloi
New Delhi – 110 041

M/s Rupak Enterprises
1/46 Vishwas Nagar, Shahdra
New Delhi – 110 032

Bhawna Industries
8-A, Industrial Development Colony
Kunjapura Road, Karnal – 132 001, Haryana

M/s Mech-Ci-Co.
1-7, GIDC Industrial Township
Vatwa, Ahmedabad – 382 445

Inter Gas Appliances Pvt. Ltd
C-113, Sector-2
Noida – 201 301, Uttar Pradesh

Akshay Urja bids adieu to Mr Vilas Muttemwar, former Minister for New and Renewable Energy

Shri Vilas Muttemwar represents Nagpur Lokshabha constituency of Maharashtra and has been re-elected 7th time as a Member of Parliament of the 15th Lok Sabha in May 2009. Shri Vilas Muttemwar completed his full tenure of five years as Minister for New and Renewable Energy. During his tenure the renewable energy sector took a leap with installation of over 9500 MW of renewable powers in

the country, and the massive installation of solar water heating systems, solar lighting systems, family-size biogas plants, and a number of other renewable energy systems. The major initiatives taken by him for popularizing renewable energy among the common people include the Akshay Urja magazine, Rajiv Gandhi Akshay Urja Diwas, Renewable Energy Clubs, District Advisory Committees, and so on.

Akshay Urja Team wishes him success in all his future endeavours.

Inviting articles for Akshay Urja

Akshay Urja publishes news, articles, research papers, case studies, success stories, and write-ups on RE. Readers are invited to send material with original photographs and statistical data. The photographs should be provided on hard copy or as high resolution (minimum 300 DPI) files on a CD. *Akshay Urja* will pay suitable honorarium for each published article of about 1500 words and above to the authors. The publication material in two copies, along with a soft copy on CD/floppy/e-mail may be sent to

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Bioethanol production from biomass



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DILIP K ADHIKARI¹**

Lignocellulosic biomass is the most abundant renewable resource on earth. Lignocellulosic materials, including wood, grass, forest residues, agricultural residues, pulp and paper mill wastes, and municipal solid wastes can be used for bioethanol production. Among these resources, agricultural residues, such as sugarcane bagasse, dominate in terms of tonnage and can serve as feedstock. Sugarcane bagasse is plentiful in tropical and sub-

tropical regions such as Brazil, India, Thailand, Hawaii, and the southern USA. Other lignocellulosic feedstocks include agricultural residues such as corncob, corn stover, wheat, and rice straw; industrial residue such as pulp and paper processing waste; and energy crops such as switch grass.

Lignocellulosic materials are comprised of lignin, hemicellulose, and cellulose in varying proportions. The general composition of lignocellulosic biomass is cellulose

(35%–50%), hemicellulose (20%–35%), polyphenolic lignin (10%–25%) and other extractable components. The utilization of both cellulose and hemicellulosic monosachharides like hexose and pentose present in a typical lignocellulosic biomass hydrolysate (composition of various lignocellulosic biomass is shown in Table 1) is essential for the economical production of ethanol. Therefore, microorganisms that are able to ferment both glucose and xylose are most desirable for an efficient bioconversion of biomass to

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Table 1 Composition of various lignocellulosic raw materials

Raw materials	Glucose	Mannose	Galactose	Xylose	Arabinose
Corn stover	39	0.3	0.8	14.8	3.2
Wheat straw	36.6	0.8	2.4	19.2	2.4
Rice straw	41	1.8	0.4	14.8	4.5
Sugarcane bagasse	38.1	--	1.1	23.3	2.5
Rice hulls	38.1	3.0	0.1	14.0	2.6

ethanol. The bioethanol production from lignocellulosic biomass requires two essential steps: saccharification of lignocellulosic biomass to fermentable sugars and fermentation of sugars to ethanol.

Worldwide, bioethanol is currently produced by fermentation of monomeric sugars by mesophiles, which ferment the sugars at 25–37 °C. These mesophiles have certain limitations in fermenting pentose sugars produced from lignocellulosic biomass. However, the thermophiles have certain advantages over mesophiles, which could be exploited for ethanol production. Solvent tolerance, energy savings through reduced cooling costs, higher saccharification and fermentation rates, continuous ethanol removal, and the reduced risk of contamination have stimulated a search for thermophilic or thermotolerant yeasts. Less energy is required for mixing and product recovery in thermophilic fermentations because of lower viscosity, surface tension, higher vapour pressure, and increased solubility of organic compounds.

This article describes the saccharification of sugarcane bagasse by acid treatment followed by sugar recovery from the hydrolysate by ion exchange chromatography and fermentation of sugars in batch and continuous mode with recycle of thermophilic yeast, *Kluyveromyces sp. IPE453* in the temperature range of 50 °C.

Materials and methods

Microorganisms and culture conditions

The strain used for ethanol production, *Kluyveromyces sp. IPE453*, was grown in salt medium containing 0.15 g/l (gram per litre) di-sodium hydrogen ortho phosphate, 0.15 g/l potassium di-hydrogen ortho phosphate, 2.0 g/l ammonium sulphate, 1.0 g/l yeast extract, and 10 g/l glucose with pH 5.5 at 45 °C. Fermentation was carried out in a medium prepared in hydrolysate containing 0.15 g/l di-sodium hydrogen ortho phosphate, 0.15 g/l potassium

di-hydrogen ortho phosphate, 1.0 g/l ammonium sulphate, and 1.0 g/l yeast extract with pH 5.0 at 45 to 60 °C in batch process and continuous process with recycling the cells.

Fermentation conditions

Batch fermentation process

Batch fermentation of sugarcane bagasse hydrolysate and cassava starch hydrolysate was performed in 2 litre Bioflow-110 bioreactor by free cells of *Kluyveromyces sp. IPE453* in batch mode. The temperature and agitation were controlled at 50 °C and 200 rpm respectively.

Continuous fermentation process with recycling the cells

Continuous fermentation was performed in 2 litre Bioflow-110 bioreactor by using sugarcane bagasse hydrolysate solution containing glucose and xylose sugars. The hydrolysate solution, supplemented with inorganic salts, was fermented using previously



Table 2 Sugars and furfural (percentage of bagasse) recovered in first stage hydrolysis at different acid concentrations

Acid concentration (%) (w/w)	Solid to liquid ratio	Xylose (%)	Glucose (%)	Furfural (%)
2	1:10	9.2	0.6	0.12
4	1:8	13.6	0.9	0.16
6	1:8.8	19.6	1.4	0.23
8	1:8.3	23.1	4.4	0.31
	1:5.2	25	4.6	0.41
10	1:4.2	26.14	4.6	0.63
	1:8.4	25	5.8	0.95

grown cells of *Kluyveromyces sp. IPE453*. The air/N₂ flow in proper ratio was controlled in the bioreactor for in situ recovery of ethanol. The process was performed at different dilution rates, cell mass concentration, and temperatures. The pH and agitation were controlled at 5 and 250 rpm, respectively.

Hydrolysis of sugarcane bagasse

The sugarcane bagasse powder was collected from sugar mill. It was hydrolysed by sulphuric acid treatment in two stages. In the first stage hydrolysis, the sugarcane bagasse was soaked into 2%–10% w/w sulphuric acid with a solid-to-liquid ratio of 1:10 to 1:4.2. The temperature in the digester was maintained at 100 °C for an hour and agitation in the reactor was maintained 1000 rpm. The aqueous phase was separated from the residual bagasse followed by its washing to collect xylose-rich hydrolysate-rich stream.

In second stage hydrolysis 18%–65% w/w sulphuric acid was added to residual bagasse taken from first stage hydrolysis. The temperature in the digester was maintained at 80 °C for 1 hour and agitation was maintained 1000 rpm. The aqueous phase was separated from the residual bagasse followed by its washing to collect glucose rich hydrolysate rich stream.

Hydrolysis of starch biomass

Hydrolysis of different starch biomass like soluble starch, cassava, tapioca, sweet sorghum, and maize was performed in 1 litre flasks containing 5% starch biomass in 200 ml 0.05 M acetate buffer (pH 5.0) and 100 ml crude enzyme (5.29 mg/ml). All the flasks were incubated at 80 °C and monitored at an interval of 2 hours until the total starch was hydrolysed.

Recovery of sugars from hydrolysate

The sugars from the bagasse hydrolysate were recovered by ion exchange chromatography using strong anion and weak anion resins in the ratio of 5:1 to 1:1. A glass column with 100 cm length and 3 cm diameter was packed with 700 g resins. The bagasse hydrolysates obtained in the first stage of hydrolysis contained sugar concentration of 35 g/l and sulphuric acid concentration of 60 g/l. In the second stage of hydrolysis, it contained sugar concentration of 80 g/l and sulphuric acid concentration of 200 g/l. The hydrolysate was passed through the column with flow rate ranging from 4 to 17 ml/min. The acid was retained in the column and sugars were eluted through the column. The column was regenerated with water to recover the acid and the acid solution was recycled back for hydrolysis of fresh sugarcane bagasse.

Analytical methods

Reducing sugars in media and fermented broth were determined by DNS (di-nitrosalicylic acid) method. Ethanol was determined by using gas chromatography using a Chemito 8600 Refinery Gas Analyser with a 4-m-long and 1/8 diameter Porapak column with Chemosorb 80/60. Sample was injected at 120 °C, and the oven temperature and flame ionization detector temperature was 150 °C and 200 °C, respectively, using helium as a carrier gas. Ethanol was also determined by colorimetry method. Furfural was measured by Double Beam UV-VIS Spectrophotometer 2600 at 277 nm.

Results and discussion

Hydrolysis of sugarcane bagasse

In first stage hydrolysis, sulphuric acid was used at different concentrations (2% w/w to 10% w/w) and different solid-to-liquid ratios. The temperature was maintained at 100 °C for 1 hour. The hydrolysate was separated from the residual bagasse and sugars and furfural in hydrolysate were estimated. The maximum 26.14% xylose and 4.6% glucose (47% sugars of total cellulose and hemicellulose present in the sugarcane bagasse) was obtained at 8% w/w acid concentration and at 1:4.2 solid-to-liquid ratio, as shown in Table 2.

In second stage hydrolysis 6%–18% glucose and 2.5%–4.64% xylose were obtained, as shown in Table 3. The maximum 33% sugars of total cellulose and hemicellulose present in sugarcane bagasse was achieved at 65% w/w sulphuric acid concentration when the digester temperature was maintained at 80 °C for 1 hour followed by diluting the acid to 20% w/w and again maintained the temperature 100 °C for 30 minutes and agitation was maintained 1000 rpm. The furfural concentration was negligible.

Table 3 Sugars and furfural (percentage of bagasse) recovered by second hydrolysis at different acid concentrations

Acid concentration (%) (w/w)	Acid (%) (w/w) (acid/sugar)	Glucose (%)	Xylose (%)	Furfural (%)
18	50	6	3.5	0.21
26.4	75	9.7	2.5	0.16
33.8	100	14.5	4.64	0.14
65	125	18	3.34	0.11

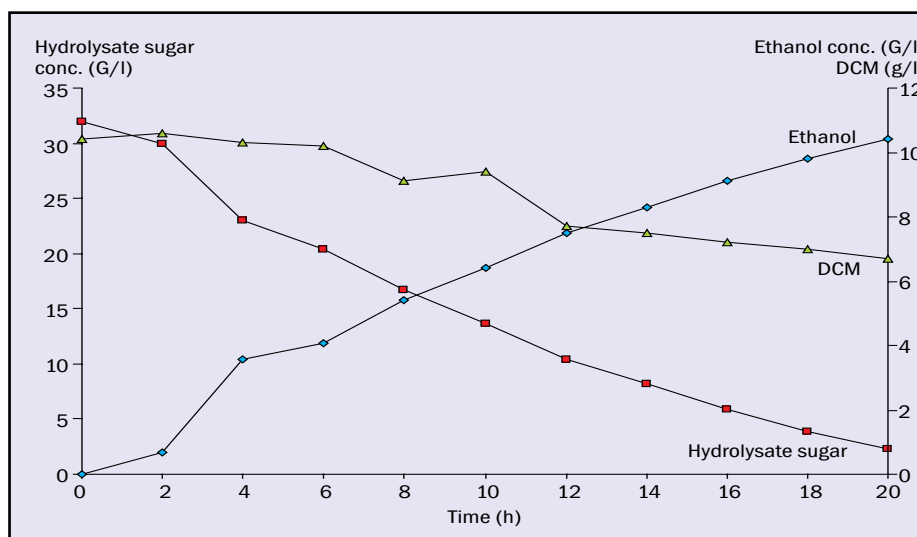


Figure 1 Ethanol production in batch mode by *Kluveromyces sp. IIPE453* in sugarcane bagasse hydrolysate at 50 °C

Recovery of sugars from hydrolysate

The sugarcane hydrolysate, containing fermentable sugars and sulphuric acid, was passed through the column containing ion exchange resins. About 95%–100% acid-free sugars were recovered, with strong anion and weak anion mixture in the ratio of 5:2 and flow rate 17 ml/min. Ninety five per cent acid was recovered in regeneration of the column. The recovered acid was recycled for further hydrolysis of fresh sugarcane bagasse.

Ethanol fermentation from sugarcane bagasse hydrolysate

Batch fermentation process

The concentration of thermophilic yeast, *Kluveromyces sp. IIPE453*, was kept at about 10 g/l. Total sugar was

consumed within 20 hours as shown in Figure 1. The final ethanol concentration in broth was 10.2 g/l with ethanol yield of 35% and productivity of 0.52 g/l/h.

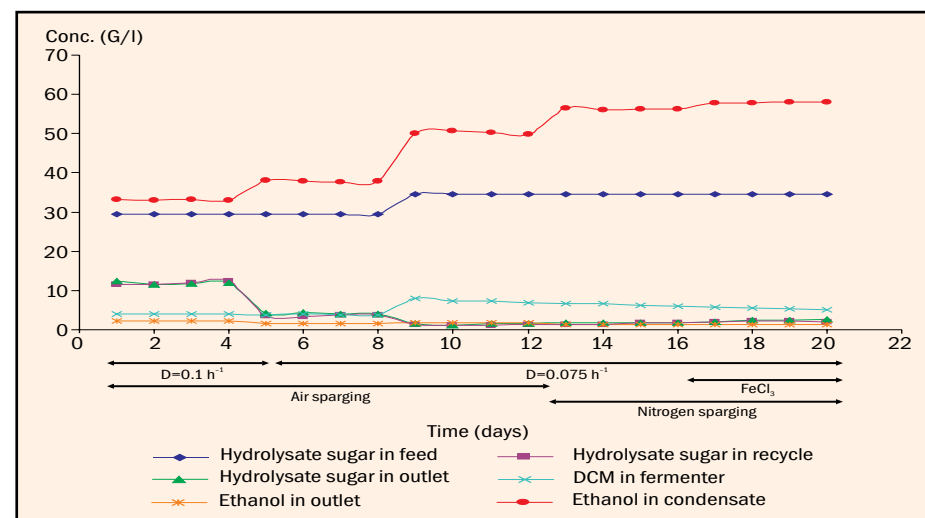


Figure 2 Continuous fermentation with cell recycle at 50 °C and air/N₂ stripping in sugarcane bagasse hydrolysate by *Kluveromyces sp. IIPE453*

Continuous fermentation process with recycling the cells

The sugarcane bagasse was fed into the bioreactor with a dilution rate of 0.075/h and 0.1/h at 45–60 °C. The highest ethanol yield and productivity of 42% and 2.3 g/l/h, respectively, was obtained at 45 °C and 0.1/h. At 50 °C, air/N₂ was passed through the bioreactor for in situ recovery of ethanol at different conditions as shown in Figure 2. The overall ethanol yield on the basis of total fermentable sugars present in hydrolysate in continuous fermentation with cell recycle at 50 °C was 35%–38% with ethanol productivity of 0.216–1.86 g/l/h. Almost 90% of ethanol was recovered during fermentation on stripping by air/N₂ and five times concentrated ethanol was obtained as compared to ethanol in fermented broth.

Simultaneous liquification and saccharification of starch biomass

Different starchy biomass like soluble starch, cassava starch, tapioca starch, sweet sorghum, and maize were hydrolysed by thermoamylase isolated from *Geobacillus sp. IIPTN* at 80 °C as shown in Figure 3. The total starch for each substrate was hydrolysed in

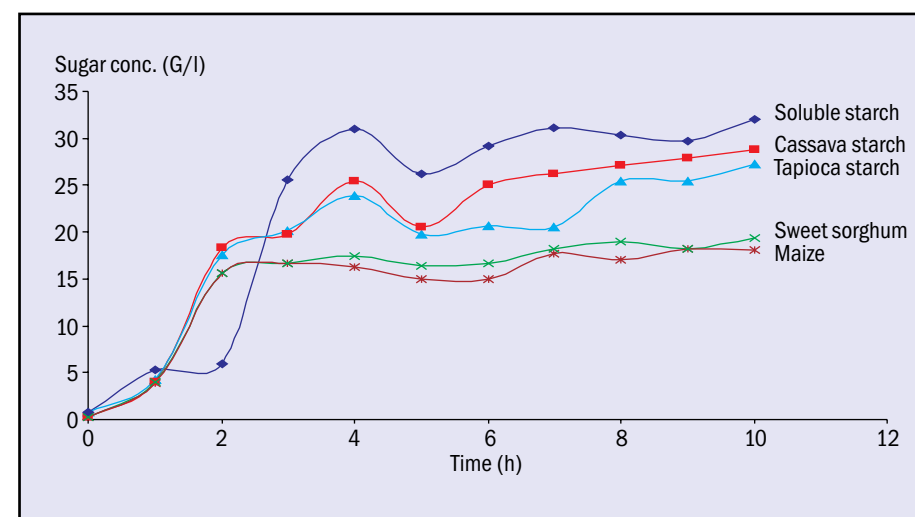


Figure 3 Enzyme hydrolysis of starch base biomass at 80 °C by thermoamylase produced by *Geobacillus sp. IIPTN*

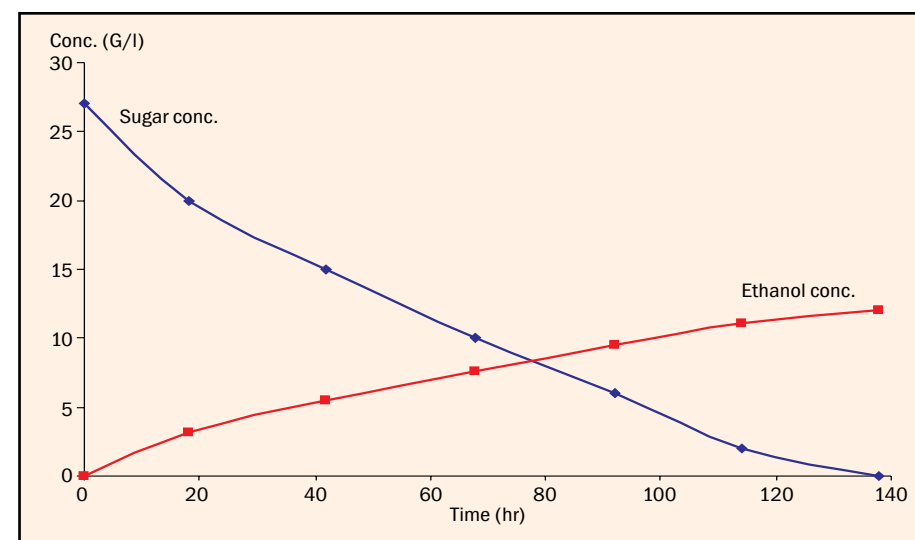


Figure 4 Ethanol production in batch mode by free cells of *IIPE453* from cassava hydrolysate.

10 hours. The sugar yield on tapioca and sweet sorghum were obtained at 81.7% and 58%, respectively.

Ethanol production from cassava hydrolysate

Fermentation was carried out with cassava hydrolysate in batch mode by free cells of *Kluveromyces sp. IIPE453* as shown in Figure 4. The total sugar in hydrolysate was consumed in 138 hours with productivity of 0.09g/l. The ethanol yield was obtained

at 45% on the basis of fermentable sugars and the overall ethanol yield on dry cassava basis was 33%. The dry cell mass was almost constant through out the fermentation.

Conclusion

The overall yield of fermentable sugars in acid treatment was 65%–80% (w/w) of total cellulose and hemicellulose present in sugarcane bagasse under different operating conditions. The overall ethanol yield on the basis of total fermentable sugars present in hydrolysate was obtained 35% in batch process with ethanol productivity of 0.52 g/l/h at 50 °C. The overall ethanol yield on the basis of total fermentable sugars present in hydrolysate in continuous fermentation with recycling the cells at 50 °C was 35%–38% with ethanol productivity of 0.216–1.86 g/l/h. Almost 90% of ethanol was recovered during fermentation on stripping by air/N₂ and five times concentrated ethanol was than ethanol in broth. The thermoamylase was found very effective for hydrolysing different types of starch biomass with a considerable yield at high temperature.

