International Journal of
Advance in Molecular Engineering

Volume 2, Issue 1, 2024
January-June
DOI (Journal): 10.37591/IJAME

https://journals.stmjournals.com/ijame/

Research IJAME

Nanoyeast Supported on Silica Gel for The Continuous Flow Bioethanol Production

Dedicated to Our Honorable Prime Minister of India, Shri Narendra Damodardas Modi Ji on the Occasion of the 78th Independence Day of India.

Swara Pancholi[1], Parth Naghera[1], Manan Shah[1], Indra Neel Pulidindi[2], Suresh Selvaraj[3], Prakash Vaithyanathan[4,*]

Abstract

Nanomaterials outperform their bulk counterparts. Yet, production of nanomaterials, especially, nanoyeast is a challenge and is an upcoming field. Yeast is a fungal strain that consumes carbohydrates (glucose, sucrose, molasses and the like) and produces bioethanol, a potential transportation fuel. Impregnation methods are well known in catalysis to yield nanomaterials via better dispersion. An attempt has been made to modulate the size of yeast to increase the efficiency of carbohydrate conversion into bioethanol. Effective adsorbent, namely, silica gel, is developed to disperse small particles of yeast on the support. The supported biocatalyst (nanoyeast/silica gel) thus produced was packed in a fixed bed flow reactor. From a reservoir held vertically above the packed bed reactor, the carbohydrate feedstock (10 wt.%) was passed through the reactor. The reactor was packed with 50 g of the silica gel loaded with nanoparticles of yeast. In a very first attempt of its kind, successful conversion of sucrose to bioethanol is obtained at a modest flow rate of the feed. The product collected at the outlet after 24 hours of fermentation process in the fixed bed continuous flow reactor was characterized by ^{1}H NMR to see the potential of the nanoyeast under modest loadings for the fermentation of carbohydrates. Indeed, the formation of bioethanol was observed in the ^{1}H NMR spectrum (a 2 H, q at 1.029 ppm, and 3 H, t at 3.65 ppm) of the aliquot collected after 24 h of the fermentation of sucrose by the nanoyeast. This evidence is good enough to prove the formation of bioethanol from the metabolism of sucrose by the nanoyeast. However, in addition to peaks typical of bioethanol, the signals characteristic of unreacted sucrose were also seen. This shows that further optimization of the process is required. Thus a new avenue is opened to modulate the size of yeast particles using mild ultrasound irradiation (40 kHz) as a dispersing field and silica gel as dispersing medium. Much remains to be done in this direction pertaining to the characterization of the nanoyeast/silica gel biocatalyst. With the kind of synthetic strategy developed, even with no yeast particles visible to the naked eye, the supported catalyst could convert sucrose to bioethanol. It is, at the moment, surmised that the activity of the catalyst is attributable to the nanoyeast. However, detailed characterization of the size of the yeast particles with scanning electron microscopy is warranted, which will be a subject of our next communication.

***Author for Correspondence**
Prakash Vaithyanathan
E-mail: pvaithyanathan@gmail.com

[1]M.Sc. Graduate, Department of Chemical Sciences, GSFC University, Vadodara, Gujarat, India.
[2]Scientific Consultant, Jesus' Scientific Consultancy for Industrial and Academic Research (JSCIAR), Tharamani, India
[3]Associate Professor, Department of Science and Humanities, ACE Engineering College, Ghatkesar, Secunderabad, Telangana, India.
[4]Science Teacher and Innovator 50, L.B road, Adyar, Chennai, India.

Received Date: August 16, 2024
Accepted Date: August 21, 2024
Published Date: September 09, 2024

Citation: Swara Pancholi, Parth Naghera, Manan Shah, Indra Neel Pulidindi, Suresh Selvaraj, Prakash Vaithyanathan. Nanoyeast Supported on Silica Gel for the Continuous Flow Bioethanol Production. International Journal of Advance in Molecular Engineering. 2024; 2(1): 1–7p.

Keywords: Bioethanol, continuous flow process, fermentation, glucose, nanoyeast, silica gel, support.

INTRODUCTION

Bioethanol is a promising alternative to petroleum for transportation application.

Bioethanol can be used as such or it can be blended with fossil based fuels for mobile applications in day to day life leading to clean environment and sustainability as the dependence of fossil based fuels is reduced. There are major challenges involved in the field, especially, absence of a chemical catalyst for the conversion of carbohydrates to bioethanol. Only, the fungal strains, namely, yeast such as *Saccharomyces cerevisiae* and the like were used for the conversion of carbohydrates (glucose, sucrose, molasses) to bioethanol at an atom efficiency of 51 %. Per gram of glucose theoretically, 0.51 g of bioethanol is expected, with the rest being CO_2. However, in reality the biochemical reaction of fermentation of carbohydrates to bioethanol is complex, ranging from the rate of reaction to the kind of biochemical pathways operating as well as the complex cascade of metabolites and secondary metabolites that are formed. So huge surprises can be expected for the age old biochemical process, namely, fermentation of sugars with yeasts with the advances in characterization techniques, like the use of NMR and HPLC [1-12]. What is new in the field, the nanoyeast. Provided smart synthetic strategies were developed, nano size yeast particles can be obtained. As with chemical systems, in the biochemical world too performance enhancement with size reduction of microorganisms can be anticipated. In the words of the Nobel laureate Richard Feynman, "There is plenty of room at the bottom". This is true for biochemical world as well. Mat West et al., have already demonstrated the presence of nano sized yeast particles from their electron tomographic studies on wild type (wt) yeast. Nanoyeast buds of sizes, namely, 371, 383, 665, 908, 1095, 1255 nm were reported [13, 14], while conventionally, it was taught that any size reduction of yeast particles below 1 μm could not be achieved (Figure 1) [12]. The scanning electron micrographs of yeast (Baker's yeast procured from supermarket) after stirring (Figure 1 A) and sonication (Figure 1B) were shown in Figure 1. As expected mild sonication facilitates deagglomeration of yeast and single particles of yeast could be generated in aqueous medium that facilitates accelerated fermentation [12]. This shows that sonication appears to be a promising route for size reduction of yeast particles.

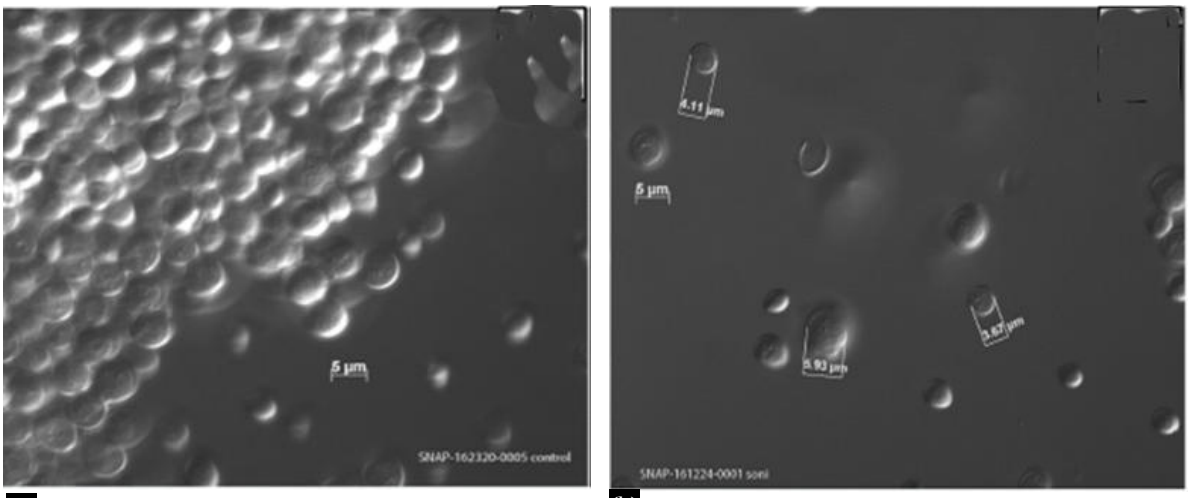

Figure 1. Scanning electron microscopic images of (a) commercial Baker's yeast (*Saccharomyces cerevisia*) after stirring in water and (b) after mild sonication in a bath sonicator (40 kHz) [reproduced from 12 with permission from the American Chemical Society].

Chemists have various synthetic strategies to generate nanoparticles from their bulk counterparts. One such a simple but effective technique is the wet impregnation of the active component (chemical or biochemical catalyst) on a support material (usually inert medium with high specific surface area and binding ability with the active species). Such a methodology yielded promising results via enhancing the catalyst performance and reducing the utilization of the active component and also enhancing the reusability leading to sustainability and minimum waste generation [15-19]. In this current work, silica gel is used as a support for commercial yeast (*Saccharomyces cerevisia*) to generate nanoyeast which was subsequently tested for its ability to convert sucrose to bioethanol in a continuous flow process.

EXPERIMENTAL

Sucrose used for fermentation process was procured from the super market in the GSFCU campus, Vadodara. Yeast, namely, *Saccharomyces cerevisiae*, used for the fermentation of sucrose was procured from Blue bird. The silica gel that was used as support for dispersing yeast was procured from Rankem. Typical method for the synthesis of nanoyeast comprised of taking 50.0 g of silica gel in 100 mL water and adding 1.0 g *Saccharomyces cerevisiae* (yeast produced from blue bird) to the aqueous suspension of silica gel in water under bath sonication conditions. The contents were subjected to ultrasound (40 kHz) irradiation for 10 minutes. The supernatant liquid was discarded. The residual solid (nanoyeast coated silica gel) was washed with excess water to remove any unadsorbed or weekly bound yeast particles to the silica gel surface. After decantation, the silica gel appeared as it was before the addition of yeast, meaning, the yeast particles on silica gel surface were so small that they cannot be seen by naked eye. Subsequently, the nanoyeast deposited silica gel was used a biocatalyst for the conversion of carbohydrates (sucrose) to bioethanol in a continuous flow fermentation process. The experimental set-up used for the continuous-flow production of bioethanol from sucrose using nanoyeast dispersed on silica gel packed bed catalyst was shown in Figure 2.

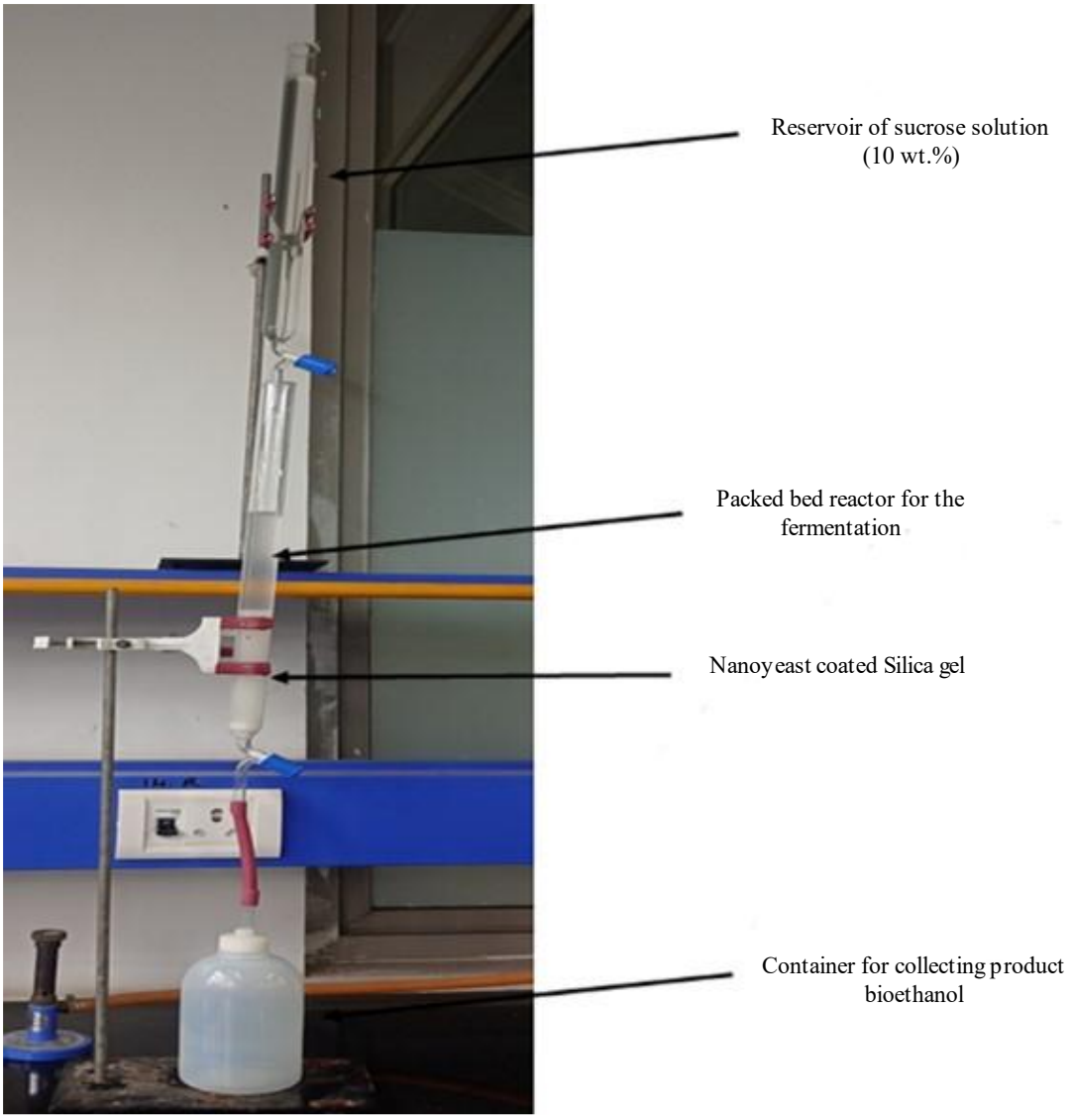

Figure 2. Experimental set-up for the continuous flow production of bioethanol from the fermentation of sucrose on a fixed bed catalyst (nanoyeast dispersed on silica gel)

^{1}H NMR analysis of the product from the fermentation of sucrose by nanoyeast in a continuous flow process was carried out on Ascend 400 (BRUKER) NMR machine using D_2O as a solvent. Typical analyte comprised of 200 μL solvent (D_2O), and 400 μL of the analyte with the number of scans being 16.

RESULTS AND DISCUSSION

Typical fermentation reaction of sucrose comprise of two steps, namely, the preliminary hydrolysis of sucrose (disaccharide) to glucose and fructose (monomeric sugars) by the action of the enzyme invertase that is present in the cell wall of the yeast (*Saccharomyces cerevisiae*). The monomeric sugars (glucose and fructose) thus produced in the hydrolysis process were subsequently metabolized by yeast to yield bioethanol and CO_2 as the metabolites as shown in Figure 3. For each mole of glucose/fructose that is metabolized by yeast, two moles of bioethanol and two moles of carbon dioxide are formed. The gaseous byproduct CO_2 goes out from the fermentation broth leading to weight loss which is a measure of the progress of the fermentation reaction [20].

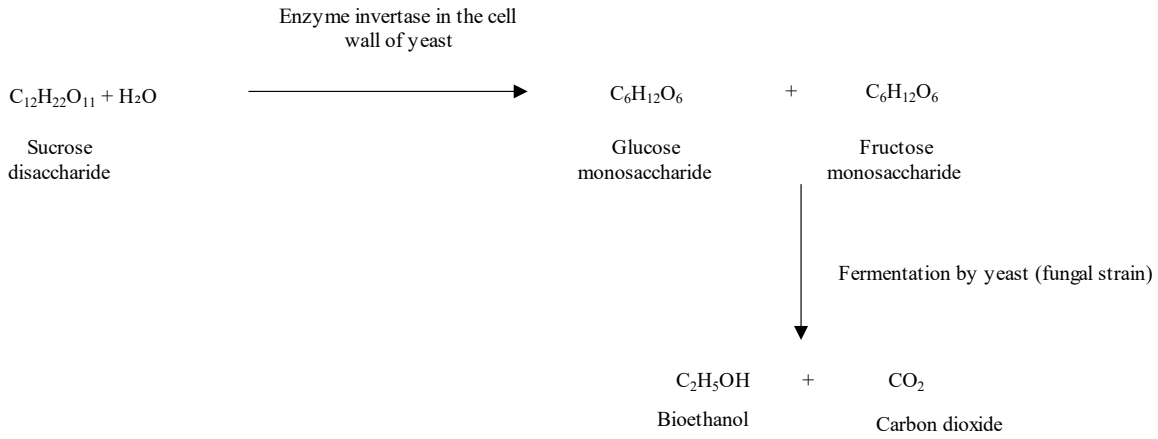

Figure 3. Schematic representation of the fermentation of sucrose to bioethanol using Baker's yeast.

Continuous Flow Bioethanol Production Using Fixed Bed Reactor Packed with Nanoyeast Deposited on Silica Gel

The flow rate of the feed (sucrose 10 wt.% solution) is 6 mL/h. The fixed bed flow reactor is packed with 50 g of silica gel coated with trace amount of yeast (from blue bird). After 24 h of the continuous flow process from the 144 mL of the product collected at the outlet, an aliquot of the sample is taken and tested by ^{1}H NMR for detecting the presence of the desired product ethanol resulting from the metabolism of sucrose by nanoyeast.

The aliquot of the product collected at the outlet was tested using ^{1}H NMR. The ^{1}H NMR of the product of fermentation of sucrose (10 wt. %) in a continuous flow packed bed reactor is shown in Figure 4.

A 3 H triplet at 1.029 ppm and a 2 H quartet at 3.572 ppm signify the formation of bioethanol with the new biocatalyst that is designed based on nanoyeast impregnated on silica gel indicating the success of the synthetic strategy opening a new avenue in this field. However, as can be noticed, the 2H quartet at 3.572 ppm is masked by many other signals typical of sucrose signifying the fact that not all sucrose (10 wt.%) solution that passed through the fixed bed reactor got converted to bioethanol. Typical ^{1}H NMR of sucrose [7] supports the statement that the unreacted sucrose masked the 2 H quartet of the bioethanol produced in the packed bed continuous flow reactor. Thus the flow rate of the feed needs to be optimized. Ideally the bioethanol produced from sucrose fermentation gives the characteristic and well resolved peaks, namely, a 2 H triplet at 1.029 ppm and a 3 H quartet at 3.65 ppm as shown in Figure 5.

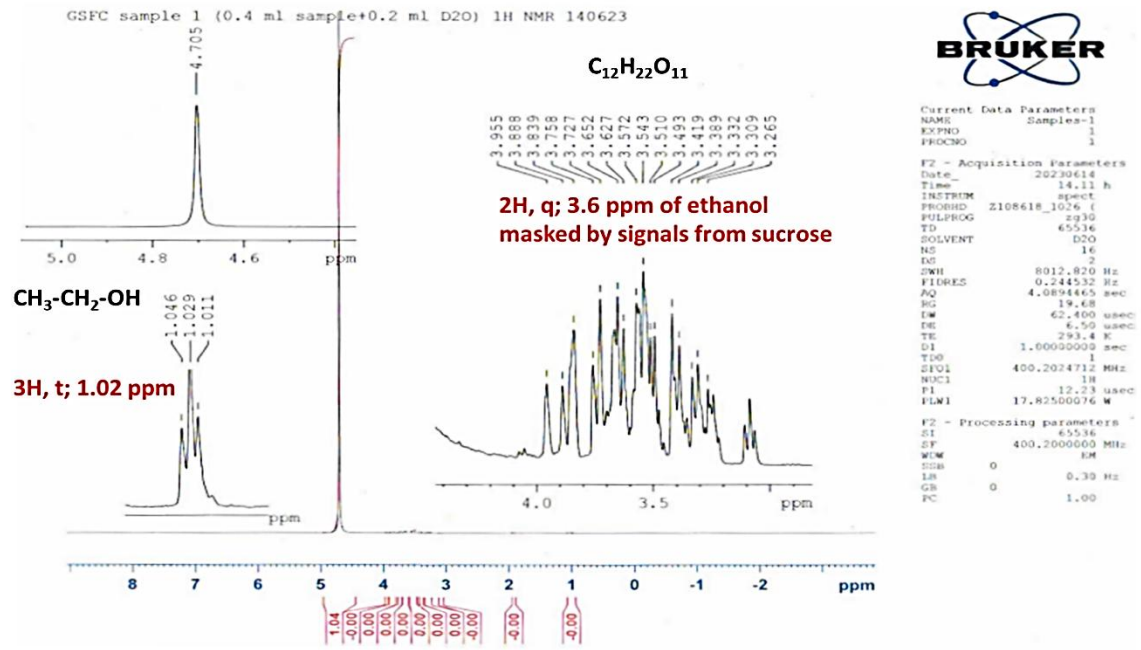

Figure 4. [1]H NMR spectrum of the aliquot of sample collected at the outlet of the continuous flow fixed bed reactor packed with nanoyeast deposited on silica [Amount of catalyst: trace amount of yeast deposited on 50 g of silica gel; sucrose feed: 10 wt.%; sucrose flow rate: 6 mL/h]

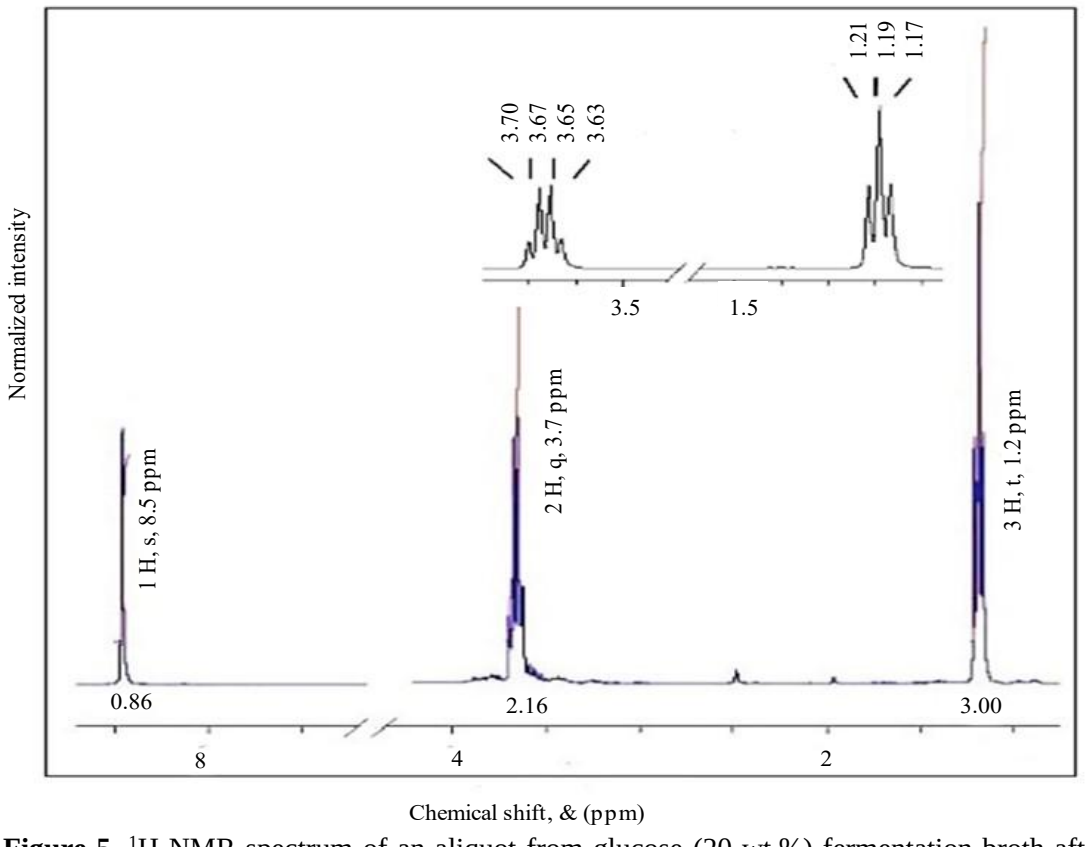

Figure 5. [1]H NMR spectrum of an aliquot from glucose (20 wt.%) fermentation broth after 12 h of ultraturrax exposure at 10,000 rpm, 30 °C. Inset shows the bioethanol peaks, a 3H (t) at 1.2 ppm and a 2 H (q) at 3.7 ppm. The singlet peak at 8.5 ppm is from the internal standard, HCOONa [Adapted from reference 9 with permission from Omics].

A comparison of Figures. 4 and 5, indicate the formation of bioethanol from the nanoyeast. However, the sucrose was not completely metabolized by yeast and so the product contained traces of sucrose. Further optimization studies are required on the reusability of the fixed bed packed column made of nanoyeast deposited on silica. We repeat, it is surmised that the nanoyeast buds orphaned by the mother yeast granules due to sonication in the presence of silica gel could be the source of formation of nanoyeast though no experimental evidence is provided in this communication.

CONCLUSION

A new avenue is opened in designing the biocatalyst with small particles of yeast deposited on a silica gel support. The supported yeast catalyst was successfully utilized for the fermentation of sucrose to bioethanol in a continuous flow process in a fixed bed reactor. ^{1}H NMR analysis of aliquot of the sample from the nanoyeast catalyst showed the formation of bioethanol. However, in addition to bioethanol unreacted sucrose was also present. This show that the process needs to be optimized further. The column height of the packed bed need to be increased so that the feedstock will have longer time of interaction with the nanoyeast fungal strain and there by whole of the sucrose will be converted to bioethanol. Such studies on optimization, especially, examination of size of the yeast particles deposited on silica gel using electron microscopy are underway and will be the subject of discussion in the next communication. Breakthrough in size reduction of yeast particles is expected that would lead to demand based supply of bioethanol for transportation application.

Acknowledgements

Grateful thanks are due to Mr. Rajesh B Vyas, Mr. Praimal Prabhat, and Ms Satyaswini, heavy water plant, Baroda, for the NMR analysis of the bioethanol produced. Thanks are due to the faculty at the GSFCU, namely, Dr. Santhosh Kumar Koppula, Dr. Saroj Shekhawat, Dr. Arti Bhadouria, Dr. Archana Deokar, Dr. Rajni Pania, Dr. Trupti Gajaria and the staff, namely, Mr. Kirankumar Parmar, Ms. Chaitali Karpe, Mr .Samir Soni, Miss. Vaibahvi Bhatia, Ms. Khyathi Joshi for the timely help. Thankfulness is due to the former provost Professor Nikhil Zaveri, GSFCU for the facilities and for the competitive research environment. I would like to express my sincere thanks to the staff of the Alumni Association, IIT Madras, for providing free access to their resources. I am also deeply grateful to Professor Mahendra N. Jadhav, the librarian, and the staff of the Central Library for maintaining a calm environment and offering valuable knowledge resources.

REFERENCES

1 Tabah B, Pulidindi IN, Chitturi VR, et al. Solar-energy-driven conversion of biomass to bioethanol: a sustainable approach, *J Mat Chem* A. 2017; 5: 15486-506p.
2 Tabah B, Varvak A, Pulidindi IN, et al. Production of 1, 3-propanediol from glycerol via fermentation by Saccharomyces cerevisiae. *Green Chem*. 2016; 18: 4657-59p.
3 Klein M, Griess O, Pulidindi IN, et al. Bioethanol production from Ficus religiosa leaves. *J Environ Manag*. 2016; 177: 20-5p.
4 Tabah B, Pulidindi IN, Chitturi VR, et al. Utilization of solar energy for continuous production of bioethanol for energy applications. *RSC Adv*. 2016; 6: 24203-09p.
5 Kumar VB, Pulidindi IN, Kinel Y, et al. Evaluation of the potential of *Chlorella vulgaris* for bioethanol production. *Energy Fuels*. 2016; 30: 3161–6p.
6 Tabah B, Pulidindi IN, Chitturi VR, et al. Solar energy driven simultaneous saccharification and fermentation (SSF) of starch to bioethanol for fuel cell applications. *ChemSusChem*. 2015: 8: 3497-503p.
7 Victor A, Pulidindi IN, Gedanken A. Assessment of Holocellulose for the production of Bioethanol by conserving *Pinus radiata* cones as renewable feedstock. J Environ manag. 2015; 162: 215-20p.
8 Korzen L, Pulidindi IN, Israel A, et al. Marine integrated culture of carbohydrate rich *Ulva rigida* for enhanced production of bioethanol. *RSC Adv*. 2015; 59251-6p.
9 Tabah B, Pulidindi IN, Gedanken A. A study on fermentation kinetics for accelerated production of bioethanol from glucose, sucrose, and molasses. *J Bioprocess Biotech*. 2015; 5: 232.

10 Korzen L, Pulidindi IN, Israel A, et al. Single Step Production of Bioethanol from the Seaweed *Ulva rigida* using Sonication. RSC Adv. 2015; 5: 16223-9p.
11 Pulidindi IN, Kimchi BB, Gedanken A. Can cellulose be a sustainable feedstock for Bioethanol production? *Renew energy*. 2014; 71: 77-80.
12 Pulidindi IN, Gedanken A, Schwarz R, et al. Mild sonication accelerates ethanol production by yeast fermentation. *Energy Fuels*. 2012; 26: 2352-6p.
13 West M, Zurek N, Hoenger A, et al. A 3D analysis of yeast ER structure reveals how ER domains are organized by membrane curvature. *J. Cell Biol*. 2011; 193 (2): 333-46p.
14 Willaert R, Kasas S, Devreese B, et al. Yeast Nanobiotechnology, Ferment. 2016; 2: 118p.
15 Rajesh D, Pulidindi IN, Pandurangan A, et al. Pd-NiO decorated multiwalled carbon nanotubes supported on reduced graphene oxide as an efficient electrocatalyst for ethanol oxidation in alkaline medium. *Appl Surf Sci*. 2018; 442:787-96p.
16 Tangy A, Pulidindi IN, Gedanken A. Continuous flow biodiesel production from waste cooked oil using microwave irradiation and supported SrO catalyst. *Bioresour. Technol*. 2017; 224:333-41p.
17 Victor A, Pulidindi IN, Kim TH, et a;.Design of active solid acid catalyst for the optimization of glucose production from *Oryza Sativa* straw. *RSC Adv*. 2016; 6:31-8p.
18 Kumar VB, Pulidindi IN, Gedanken A. Selective conversion of starch to glucose using carbon based solid acid catalyst. *Renew Energy*. 2015; 78: 141-5p.
19 Klein M, Varvak A, Segal E, et al. Sonochemical synthesis of HSiW/graphene catalysts for enhanced biomass hydrolysis. *Green Chem*. 2015. 17, 2418-25p.
20 Caylak B, Sukan FV. Comparison of different production processes for bioethanol. *Turk. J. Chem*. 1998; 22: 351-9p.

