International Journal of Modern Science and Technology
www.ijmst.co

2025;10(1):1-11.
ISSN: 2456-0235.

Research Article

Hydrolysis of waste rice water using immobilized α-Amylase for production of nutritional energy drink

Rajamurugan Shivani, Soundararajan Aniesha, Gurunathan Baskar*

Department of Biotechnology, St. Josephs College of Engineering, Chennai 600119, India.

*Corresponding author: basg2004@gmail.com

Abstract

Rice water, a nutrient-rich byproduct of cooking, acts as a low-cost substrate for value-added applications. In the present work, rice water starch was hydrolyzed using α-amylase immobilized on zinc oxide nanoparticles, enabling efficient glucose conversion. A maximum glucose yield of 6.2 mg/ml was obtained under optimized conditions (0.08% enzyme, 45 °C, 20 min). The resulting glucose-rich hydrolysate was evaluated for its potential as an energy drink base due to its high glucose content and nutritional value. Further fermentation of the hydrolysate was carried out by *Saccharomyces cerevisiae*. The characterization of the fermented hydrolysate identified the presence of valuable bioactive compounds such as 2,2-dimethyl-4-hexen-3-one, 2,6,10-dodecatrienoic acid (7,11-dimethyl-3-(trifluoromethyl)), 2,2,4-trimethylpentane (isooctane), and oxalic acid derivatives. These compounds are known for their potential uses in food, pharmaceutical, and industrial applications. Thus, this study demonstrated an innovative approach to converting food waste into functional beverages and bioactive ingredients, supporting circular bioeconomy and sustainable development goals.

Keywords: Rice water hydrolysate, Enzyme immobilization, Energy drink, Bioactive compounds.

Introduction

Worldwide, large amounts of nutrient rich food are discarded every year. It is an important issue as it results in economic losses and environmental degradation, and it is a challenge to global food security [1]. This issue is not just a matter of poor resource management it is a critical concern that intersects with economics, environmental health, and global food security. The underutilization of these resources not only represents a economic loss but it also poses critical environmental and ethical problems [2]. Economically, food waste results in loss of billions of dollars annually. In developing countries, food waste is more prevalent during post-harvest due to inadequate infrastructure, while in developed countries, food waste occurs due to over-purchasing and consumer unawareness about expiration dates and proper storage methods [3]. Thus, addressing food waste is not merely about reduction, it is about reimagining waste as a resource, transforming what was once discarded into something of nutritional, economic, or industrial value. Reuse of discarded resources can lead to innovative solutions and more sustainable practices [4,5]. Fruit peel, cereal residue, and rice water are some of the agro-industrial wastes that can be made into value-added products such as colorants, natural pigments, carbohydrates, prebiotics, and bioactive molecules according to circular economy philosophy [6].

One of the new applications is in the development energy drinks which are formulated to enhance endurance, alertness, and physical performance [7,8]. This study explores a novel approach to valorizing waste rice water into a functional glucose-rich hydrolysate through enzymatic treatment. Rice, a staple food in South India, generates significant by-products during processing [1]. The hydrolysis of the starch in rice water using α-amylase, immobilized on zinc oxide nanoparticles, enables efficient conversion of starch into glucose. The resulting hydrolysate, with high glucose content, is evaluated for its potential as a natural energy drink base, offering a clean-label, cost-effective alternative to commercial sugary beverages [9]. Furthermore, beyond its primary application as an energy drink base, the rice water hydrolysate serves as a versatile substrate for fermentation processes aimed at producing valuable bioactive compounds [10].

Received: 18.04.2025; Received after Revision: 12.09.2025; Accepted: 14.09.2025; Published: 20.09.2025

Four major compounds produced during fermentation of rice water hydrolysate are 2,2-dimethyl-4-hexen-3-one, 2,6,10-dodecatrienoic acid 7,11-dimethyl-3-(trifluoromethyl), 2,2,4-trimethylpentane, and oxalic acid. These have diverse industrial applications [11, 12]. 2,2-Dimethyl-4-hexen-3-one is a floral, fruity-scented volatile ketone that is commonly used in the perfume and flavor industries. It is used in perfumes, food flavor, and cosmetics to impart sensory appeal. Its presence in essential oils has also made it useful in plant metabolic research. Identification of the compound in rice hydrolysate confirms the ability of food waste to yield natural aroma compounds, which is an eco-friendly alternative to synthetic additives. 2,6,10-Dodecatrienoic acid, 7,11-dimethyl-3-(trifluoromethyl) is a trifluoromethylated polyunsaturated fatty acid derivative possessing considerable chemical stability due to its trifluoromethyl substitution. It is endowed with anti-inflammatory, antioxidant, and antimicrobial properties and is a valuable compound in the field of pharmacy and nutraceuticals. The fluorines increase its resistivity to metabolic degradation, enhancing its utility as a drug molecule and in research on membrane dynamics and lipid signaling [13].

The 2,2,4-Trimethylpentane (Isooctane) is widely used in the petrochemical industry as a reference for octane rating of gasoline. It is used as a solvent in biochemical and food science applications, particularly in chromatography and extraction but also in controlled chemical reactions and microbial cultures due to its low reactivity and inertness. It has also been utilized in characterizing mixtures employed in measuring physical properties like density and refractive index [14]. Oxalic acid is a naturally occurring organic acid found in plants. With excellent metal-chelating capacity, it is reported to be used on a large scale in the stripping of rust, leather production, and cleaning agents. Although toxic at high concentrations, its antimicrobial property is gaining more attention for application in food preservation. A fungal and plant natural metabolite, it is also a significant molecule in plant pathology and microbial metabolism [15,16]. By identifying these compounds, this research not only highlights the prospective industrial applications of waste rice water but also reinforces the notion that waste streams can be pivotal in discovering novel chemical entities with broad utility [10]. The recovery of such value-added compounds from food waste not only supports the principles of circular bioeconomy but also shows how nutrient-rich byproducts like rice water can be innovatively utilized to generate functional, marketable, and sustainable products across multiple domains.

Materials and methods

Chemicals and biochemicals

Fungal α-amylase of *Aspergillus oryzae*, Zinc acetate dihydrate, Glutaraldehyde, Potassium dihydrogen phosphate, Yeast extract, Peptone, Dextrose, Glucose Oxidase-Peroxidase kit (GOD/POD), Sodium hydroxide were purchased from Ravi Scientific Company, Chennai, Tamilnadu, India. *Saccharomyces cerevisiae* was purchased from local stores. Rice water was collected from household sources and stored at room temperature for subsequent use.

Green synthesis of ZnO nanoparticle using waste orange peel

A 10 g of orange peel was washed and cut as thinly as possible. The peels were then boiled in 250 ml of de-ionized water for 1 hr. Then it was stirred magnetically for 1 hr and the extract was filtered. 8 g of zinc acetate dihydrate was added to 170 ml of the extract. This mixture was magnetically stirred for 1 hr. Then it was kept in the water bath with the temperature being maintained at 60°C for 1 hr. The white precipitate was collected and oven dried overnight leading to the formation of Zinc oxide nanoparticles [17].

Surface modification of ZnO-NP by glutaraldehyde and immobilization of α-amylase on surface modified ZnO-NPs

The ZnO-NPs obtained was suspended in 0.5 M glutaraldehyde for 4 hr in a shaker at 200 rpm. Table top centrifuge was used to obtain the modified nanocatalyst. This step was followed by repeated washing with distilled water to remove traces of glutaraldehyde followed by a wash with assay buffer to remove any impurities. 1 g of glutaraldehyde modified ZnO-NPs were mixed with 1 g of α-amylase overnight (12 hr) with slow stirring in 0.1 M phosphate buffer with a pH 4.5 at 4-5°C in a refrigerator shaker. Enzyme conjugated to the modified nanocatalyst was obtained by centrifuging at 2000 rpm for 20 min. Enzyme-nanoparticle conjugate obtained was centrifuged thrice with the phosphate buffer to

remove minor impurities. Finally, the immobilized enzyme was stored at 4°C for analyzing the enzyme stability [18].

Characterization of immobilized α-amylase using NMR, XRD, FTIR and SEM with EDX

The immobilized α-amylase was characterized using SEM with EDAX, XRD, FTIR, and NMR. SEM (GRI-SEM, VEGA3, TESCAN, Czech Republic) analyzed surface morphology, while EDX (BRUKER Nano, GmbH, D-12489, Germany) confirmed elemental composition. XRD (GRI-XRDPHY FACILITY funded by DST-FIST) determined the crystalline phase of ZnO nanoparticles after Immobilization. FTIR (Make- Jasco; Model- FT/ IR- 4700) verified enzyme attachment by identifying functional groups and NMR (Make- Bruker Model- Avance III HD Nanobay 400 MHz FT-NMR SPECTROMETER) assessed structural modifications and stability.

Estimation of glucose

Experiments were conducted to study the effect of rice water starch concentration, temperature, enzyme concentration and time on enzymatic hydrolysis of starch by immobilized α -amylase. The glucose yield (Y) from hydrolysis of rice water starch was estimated using glucose oxidase-peroxidase kit. (GOD/POD) [19,20].

Optimization of rice water starch hydrolysis using immobilized α-amylase

The effect of rice water starch concentration on hydrolysis rate by immobilized α-amylase was studied by varying the starch concentration from 20% to 100% , while keeping other parameters constant. Similarly, the effect of enzyme concentration was varied from 0.02 to 0.10%, keeping starch concentration, temperature and time constant. The temperature of enzyme-starch mixture was varied from 10 to 50°C. while other parameters held constant. The reaction time was varied from 5 min up to 1 hr while maintaining while maintaining constant enzyme concentration, starch concentration, and temperature. All these works are conducted using 5 ml rice water.

Yeast activation and inoculum preparation

Dry *Saccharomyces cerevisiae* yeast cells were rehydrated in distilled water. Activation and inoculum preparation were carried out based on the method reported by [21] with some modifications. Activation was done by incubating at 30°C for 30 min. Active yeast was incubated in YPD broth (Yeast extract 10 g/l, peptone 20 g/l, glucose 20 g/l, pH 5.50 ± 0.05) in a shaker at 30°C at 200 rpm for 24 hr to give an initial yeast concentration of 10^7 colony forming units (CFU) per ml [22]. Then, 10 ml of this culture was added to 100 ml of freshly prepared YPD broth and incubated in a shaker at 30°C at 200 rpm for 24 hr for subculturing.

Fermentation of rice water hydrolysate

Fermentation was carried out in a batch system. To 90 ml of rice water which served as the fermentation medium; 10 ml of yeast inoculum (from YPD culture) was added. The pH was adjusted to 5.5 to create optimal conditions for *Saccharomyces cerevisiae* fermentation. This was then followed by incubation for 48-120 hrs at 35°C and 150 rpm, allowing for efficient glucose conversion. Samples were collected for every 24, 48, 72, 96 and 120 hrs to monitor glucose utilization throughout the fermentation process [23].

Characterization of fermented rice water using GC-MS

Gas Chromatography Mass Spectroscopy analysis was performed using a SHIMADZU, QP2010 PLUS system equipped with an RTX-5MS column. Helium was used as the carrier gas, and compounds were identified based on mass spectral matching with the NIST library.

Results and discussions

The **Fig. 1** illustrates the formulation of an energy drink using rice water through enzymatic hydrolysis and green nanotechnology. ZnO nanoparticles were synthesized using orange peel extract and used to immobilize α-amylase, enhancing enzyme stability and reusability. The immobilized α-amylase hydrolyzed rice water, breaking down starch into glucose. *Saccharomyces cerevisiae,* grown on YPD broth, is then introduced to the rice water hydrolysate to carry out fermentation, which was

characterized to confirm the presence of value added bioactive compounds. his eco-friendly approach supports food waste valorization and the development of a functional beverage rich in natural glucose and fermentation-derived compounds.

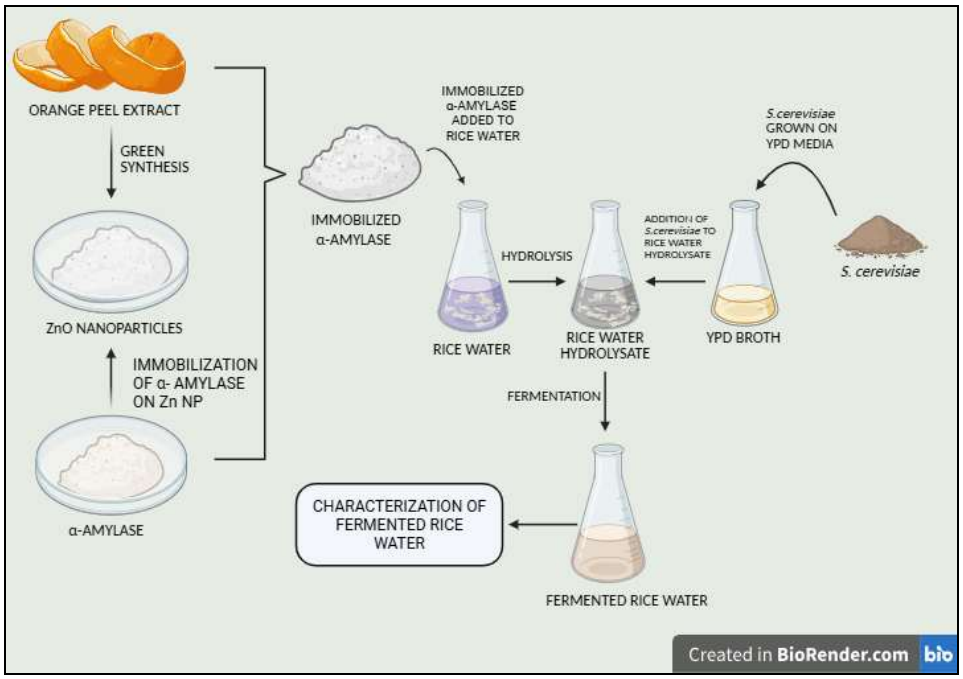

Fig. 1. A schematic overview of hydrolysis and fermentation of rice water

Effect of temperature on hydrolysis of rice water by immobilized α-amylase

The **Fig. 2** illustrates the impact of temperature on enzymatic hydrolysis of rice water using immobilized α-amylase. The temperature was varied from 30 up to 60°C. The glucose concentration increased with an increase in temperature, reaching an optimum temperature at 40-45°, beyond which there was a gradual decrease. This shows that α-amylase exhibited maximum activity within this temperature range.

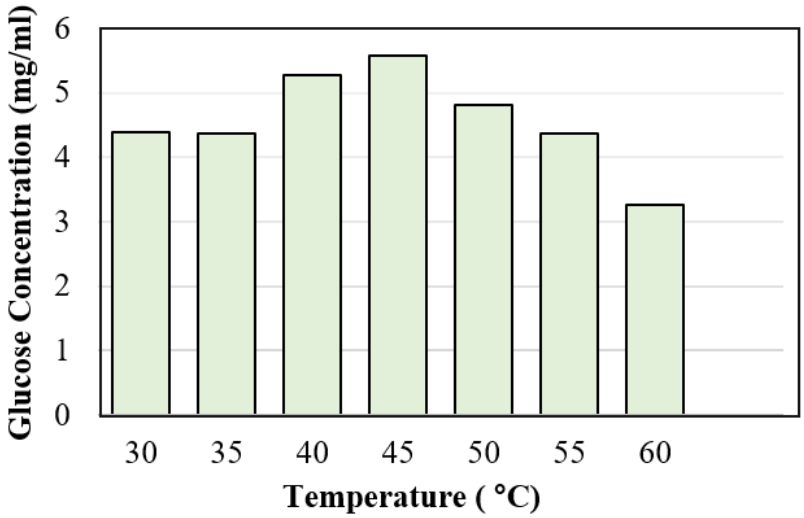

Fig. 2. Effect of temperature on hydrolysis of rice water by immobilized α-amylase

Effect of time on hydrolysis of rice water by immobilized α-amylase

As shown in **Fig. 3,** the hydrolysis of rice water was monitored at different time intervals to determine the optimal time taken for the reaction. Glucose concentration was measured from 5 min to 30 min. The glucose concentration increased steadily and was found to be maximum at 20 min, after which a slight decrease was observed. These results indicate that 20 min is the ideal time for hydrolysis.

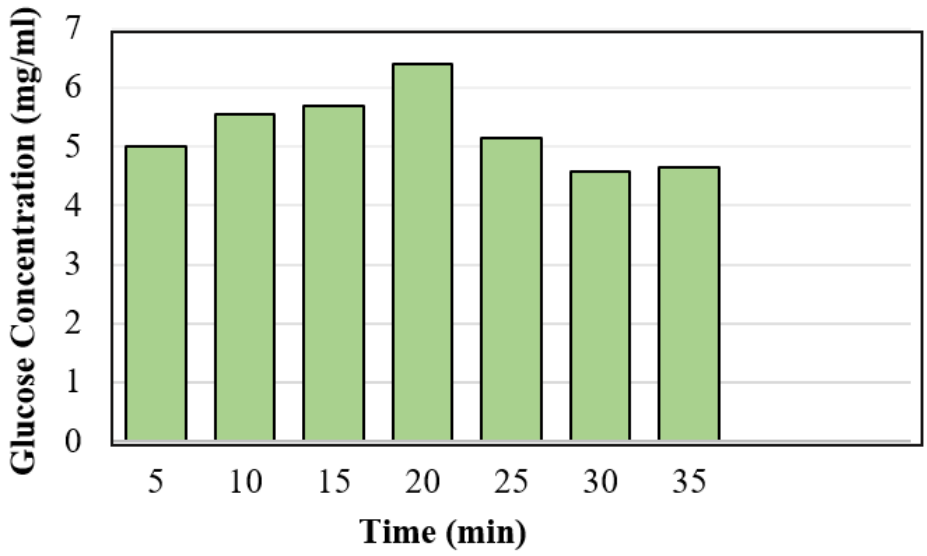

Fig. 1. Effect of time on hydrolysis of rice water by immobilized α -amylase

Effect of enzyme concentration on hydrolysis of rice water by immobilized α-amylase

The **Fig. 4** depicts the effect of varying enzyme concentration from 0.1 mg/ml to 0.5 mg/ml. The maximum concentration of glucose was observed when the enzyme concentration as 0.4 mg/ml. Hence, 0.4 mg/ml is the optimal enzyme concentration for efficient hydrolysis under the given conditions.

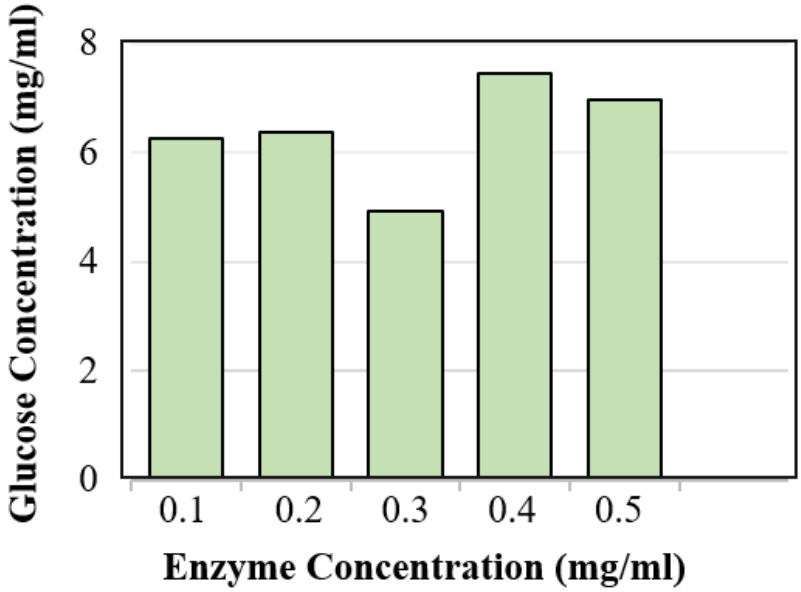

Fig 4. Effect of enzyme concentration on hydrolysis of rice water by immobilized α -amylase.

The X-Ray Diffraction (XRD) analysis of α-amylase immobilized on ZnO nanoparticles

The XRD pattern of α- amylase immobilized on ZnO nanoparticles confirms the presence of characteristic diffraction peaks corresponding to hexagonal wurtzite structure of ZnO. **Fig. 5** shows that the most intense peak appeared at 2θ = 36.46°, corresponding to the (101) plane, which confirms that ZnO maintained its crystalline structure after enzyme immobilization. The calculated crystalline size (D) is approximately 27.42 nm. It was calculated using Scherrer's equation in Eq. (1).,

$$D = \frac{K\lambda}{\beta cos\theta} \qquad (1)$$

where, K = 0.9 (Scherrer's constant) , λ= 1.5406 Å (X-ray wavelength for Cu-Kα), β= 0.3011° (FWHM) = 0.00525 radians. Thus θ = 31.56° / 2 = 15. 78° = 0.274 radians.

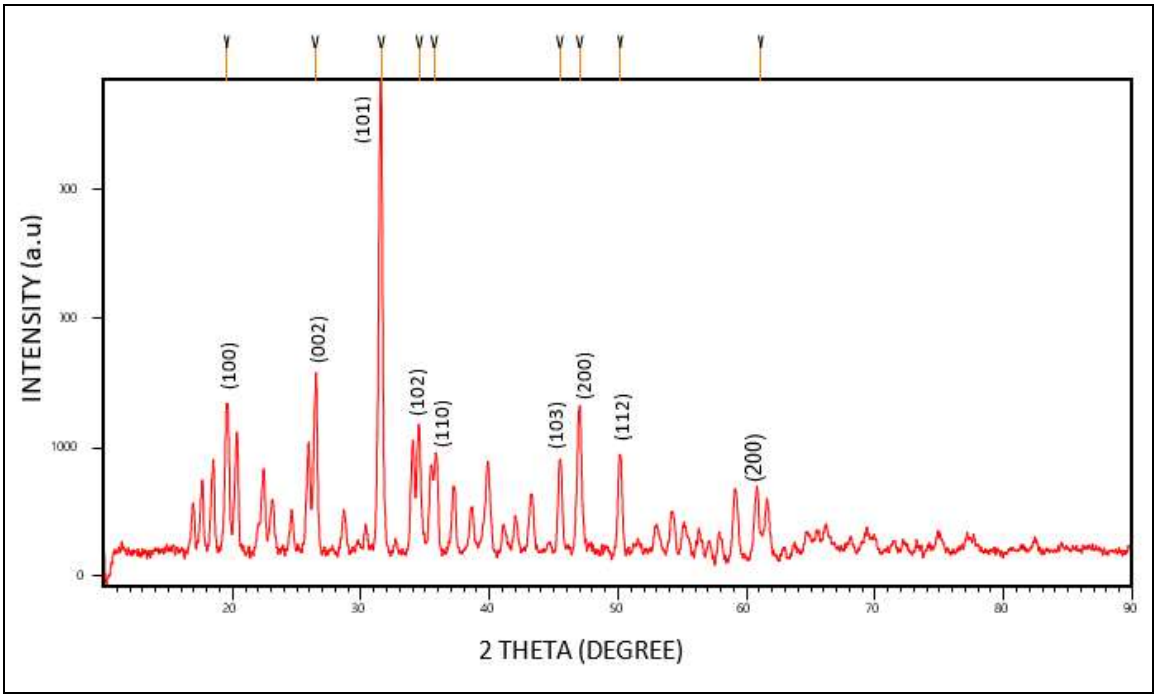

Fig. 5. XRD Pattern analysis of α- amylase immobilized on ZnO nanoparticles

Nuclear Magnetic Resonance (NMR) spectral analysis of α- amylase immobilized on ZnO nanoparticles

The ^{1}H NMR of α-amylase immobilized on ZnO nanoparticles exhibit notable chemical shifts, indicating successful enzyme attachment (**Fig. 6**). A broad peak at 4.70 ppm in ^{1}H NMR corresponds to hydroxyl (-OH) and amine (-NH) groups, suggesting strong hydrogen bonding between α-amylase and ZnO. Additionally, the appearance of a deshielded peak at 14.43 ppm suggests coordination of enzyme functional groups, likely carboxyl (-COOH) or amide (-$CONH_2$), with Zn^{2+} Overall, the observed peak shifts, broadening, and intensity variations confirm the successful immobilization of α-amylase onto ZnO nanoparticles, preserving its structural integrity and enzymatic functionality.

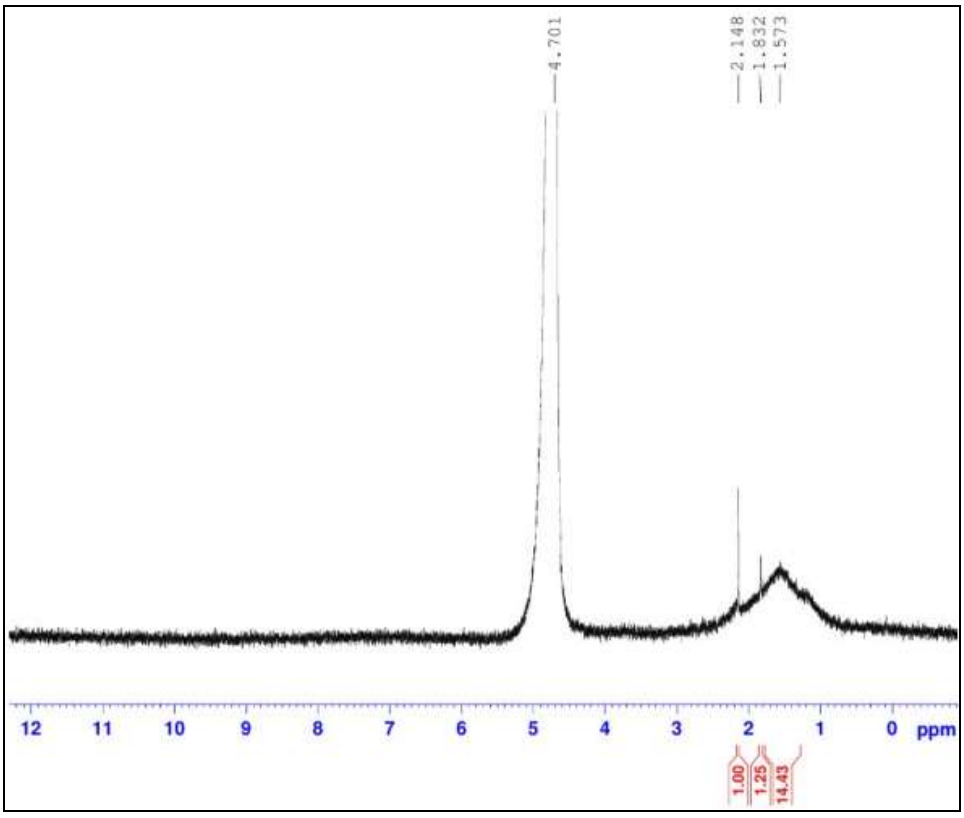

Fig. 6. ^{1}H NMR analysis of α- amylase immobilized on ZnO nanoparticles

Scanning Electron Microscope with Energy Dispersive X-ray analysis (SEM/EDX) analysis of α - amylase immobilized on ZnO nanoparticles

The SEM analysis of the sample reveals a rough and porous surface morphology of ZnO nanoparticles, which is advantageous for enzyme immobilization. The observed particle size falls within approximately 300- 600 nm range, indicating successful nanoparticle synthesis. The surface characteristics suggest an increased surface area, which can enhance the interaction between the immobilized enzyme and the substrate. (**Fig. 7 (a) & (b)). Table 1** shows the elemental composition of the sample confirmed by EDX spectrum., The presence of zinc (Zn) and oxygen (O), verifies the ZnO nanoparticle structure. Additionally, the presence of potassium (K), phosphorus (P), and carbon (C) indicates successful immobilization of amylase, as these elements are typically found in biomolecules. The presence of aluminum (Al) could be due to sample preparation or external sources. Importantly, no significant contamination was detected, confirming the purity and stability of the immobilized enzyme system (**Fig. 8**).

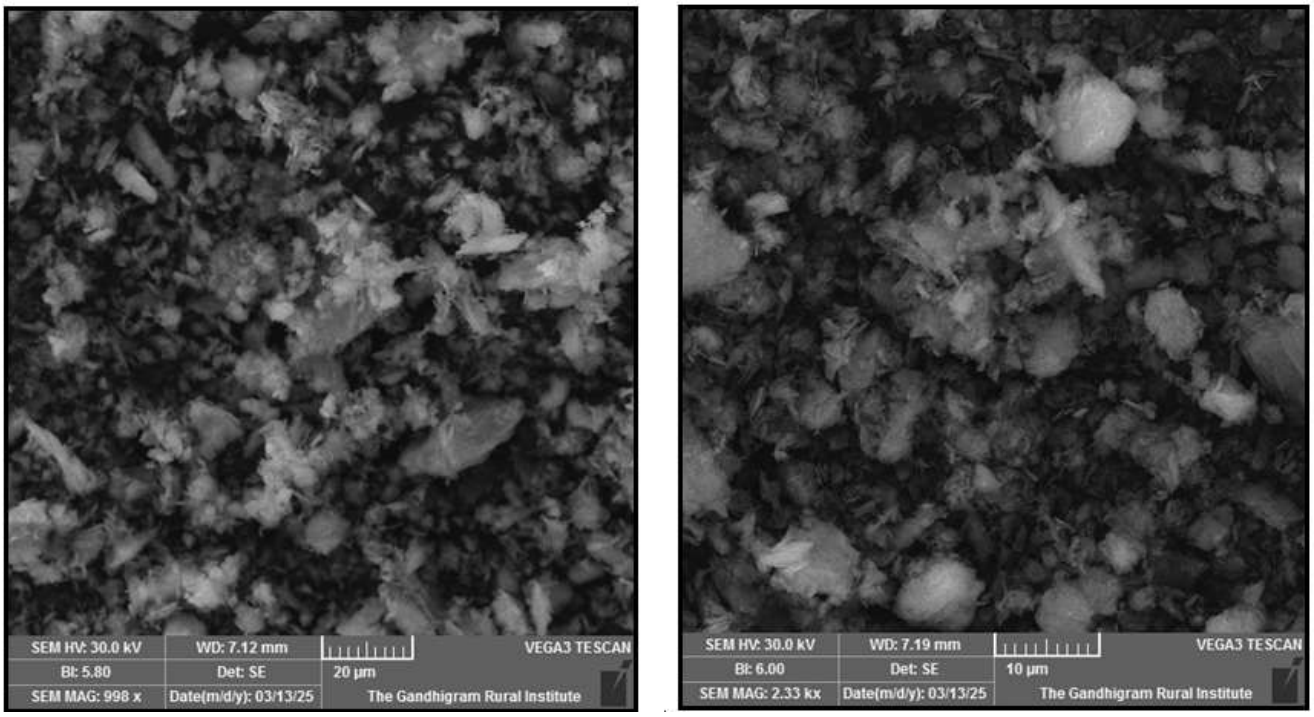

Fig. 7. SEM analysis of α-amylase immobilized on ZnO nanoparticles at different resolution **(**a) at 20 µm (b) 10 µm

Table 1. Elemental Composition of α- amylase immobilized on ZnO nanoparticle

Element	Weight % (wt %)	Atomic % (at %)
Carbon (C)	19.79%	36.84%
Oxygen (O)	15.21%	30.63%
Zinc (Zn)	59.17%	31.74%
Aluminum (Al)	0.64%	0.36%
Potassium (K)	5.19%	0.43%

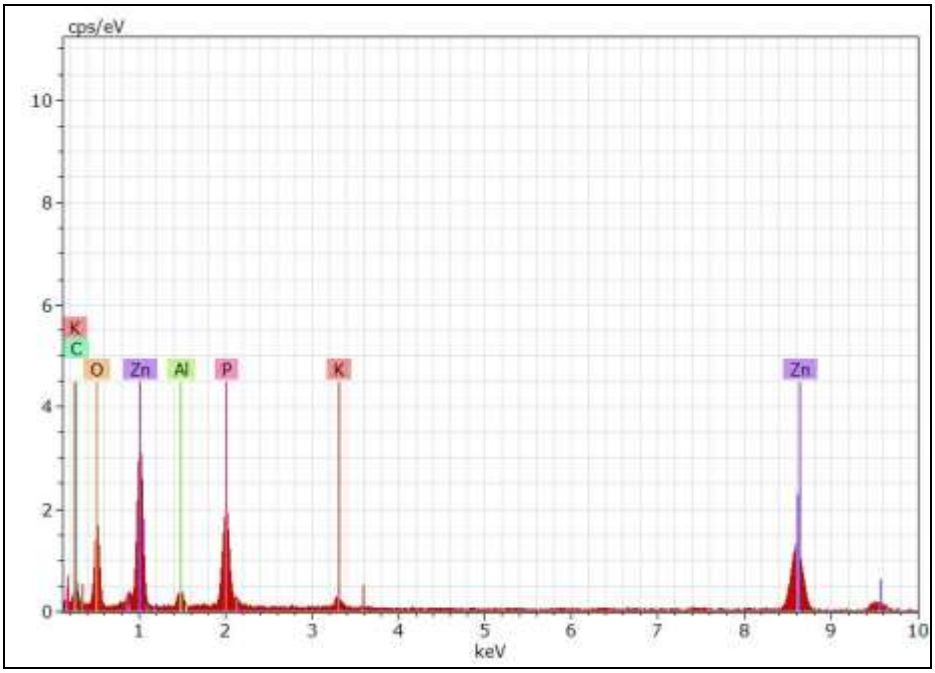

Fig. 8. EDX analysis of α- amylase immobilized on ZnO nanoparticles

Fourier Transform Infrared Spectroscopy (FT-IR) analysis of α-amylase immobilized on ZnO nanoparticles

Fig. 9 shows the FT-IR spectrum of α-amylase immobilized on ZnO nanoparticles that confirms the characteristic absorption peaks corresponding to functional groups involved in enzyme attachment. A broad peak at 3531.0229 cm^{-1} corresponds to O-H and N-H stretching vibrations, indicating the presence of hydroxyl and amine groups from α-amylase. The absorption band at 3256.2163 cm^{-1} further supports the presence of protein functional groups, confirming enzyme immobilization. A prominent peak at 1638.2327 cm^{-1} is assigned to C=O stretching (amide I), indicative of the protein's secondary structure. The peaks observed at 1110.797 cm^{-1}, 1001.8386 cm^{-1}, and 940.1276 cm^{-1} correspond to C-O and C-N stretching vibrations, which are characteristic of enzyme-linked carbohydrate and peptide bonds. Additionally, peaks at 630.6087 cm^{-1} and 566.9693 cm^{-1} are attributed to Zn-O stretching vibrations, confirming the presence of ZnO nanoparticles as the immobilization matrix. These spectral findings indicate successful immobilization of α-amylase onto ZnO nanoparticles while retaining the functional integrity of the enzyme, enhancing its stability and reusability.

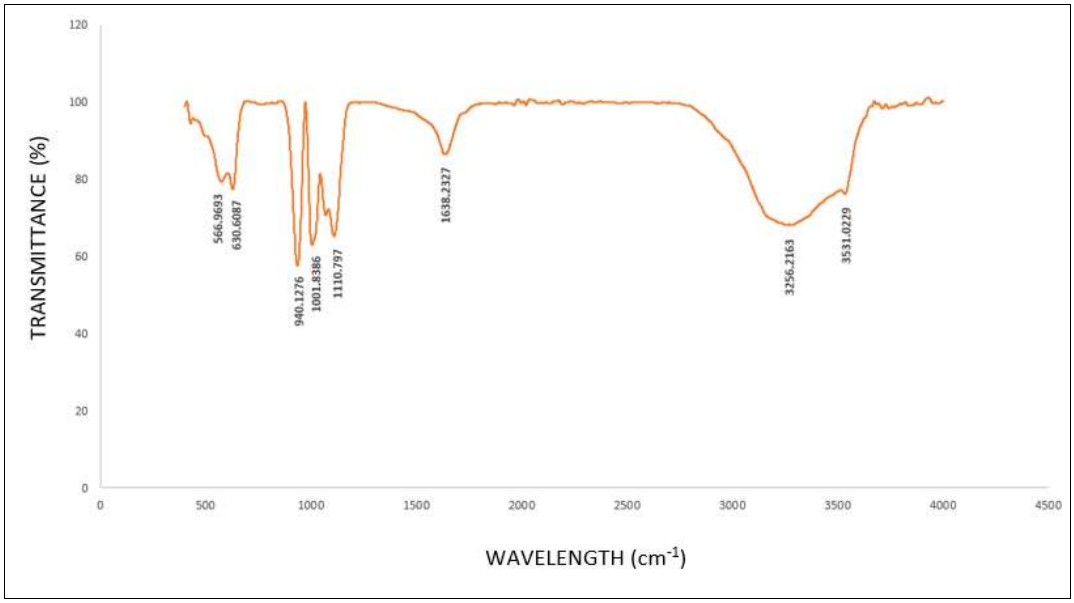

Fig. 9. FT-IR spectra of α-amylase immobilized on ZnO nanoparticles

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis of fermented rice water

Fig. 10 shows Gas Chromatography-Mass Spectrometry (GC-MS) analysis of fermented rice water. This revealed the presence of several bioactive compounds. Notably, 2,2,4-trimethylpentane, 2,2-dimethyl-4-hexen-3-one, oxalic acid dineopentyl ester, and 2,6,10-dodecatrienoic acid (7,11-dimethyl-3-(trifluoromethyl), methyl ester (Z,E)) were identified as major components which are shown in **table 2.** These compounds exhibit potential applications in the formulation of functional beverages and bio-based products, reflecting the valorization of rice water as a source of value-added metabolites.

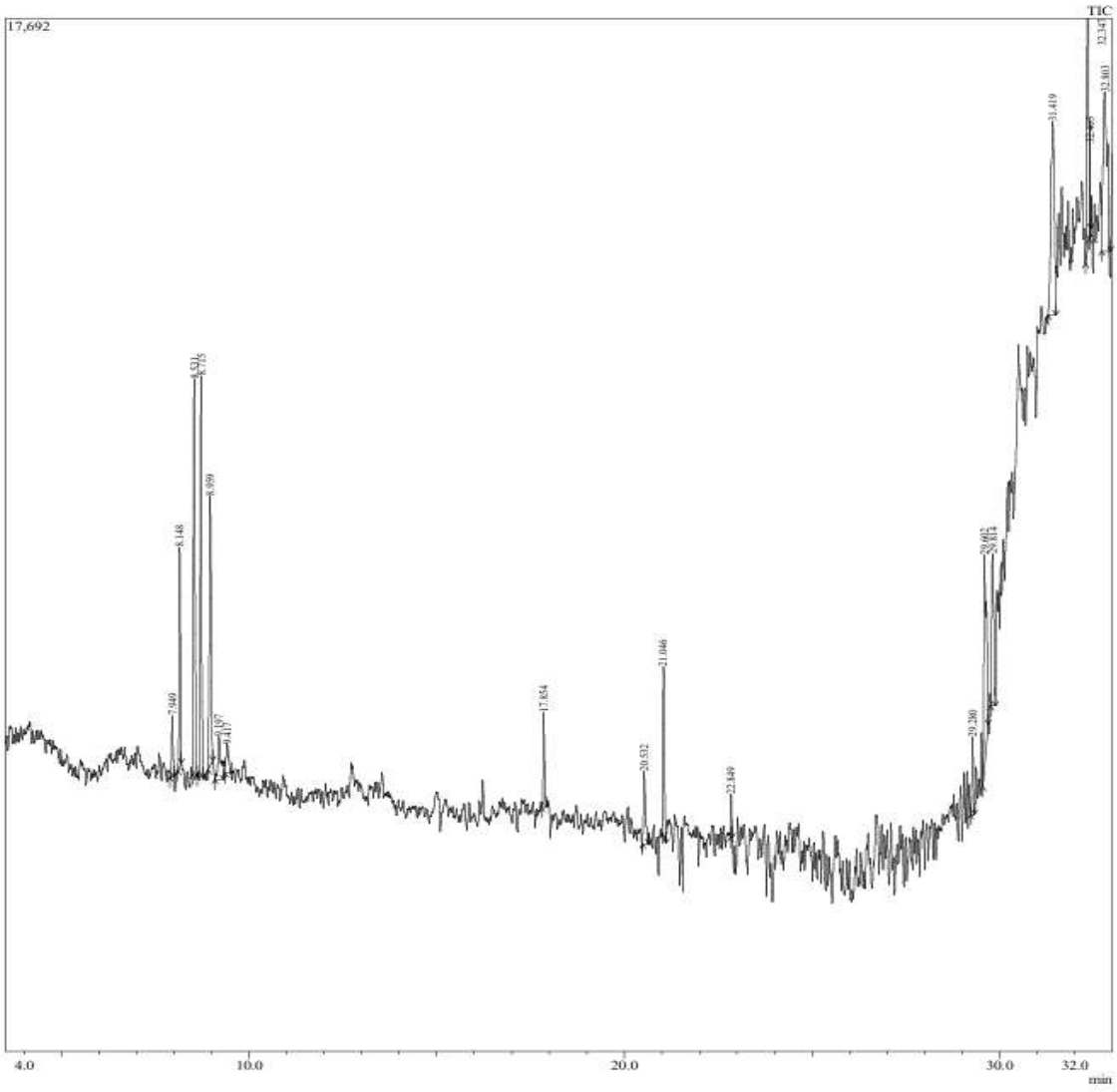

Fig. 10. GC-MS analysis of fermented rice water

Table 2. Major Bioactive Compounds Identified in Fermented Rice Water by GC-MS

Peak	Retention Time (min)	Area	Area %	Height	Compound Name
3	8.531	21059	11.77	6779	Pentane, 2,2,4-trimethyl
4	8.715	18835	10.53	6867	Pentane, 2,2,4-trimethyl
15	31.419	22099	12.35	3321	2,6,10-Dodecatrienoic acid, 7,11-dimethyl-3-(trifluoromethyl)-, methyl ester, (Z,E)
16	32.347	14285	7.98	3966	Oxalic acid, dineopentyl ester
18	32.803	19230	10.75	2719	2,2-Dimethyl-4-hexen-3-one

Conclusions

The fungal α-amylase enzyme was efficiently immobilized on zinc oxide nanoparticles and used for the hydrolysis of rice water starch into glucose. A maximum glucose concentration of 6.2 mg/ml was obtained using 100% rice water starch with 8% nanocatalyst of α-amylase in 20 minutes of reaction time at 45°C. The immobilized α-amylase enabled easy enzyme recovery and reuse, significantly reducing the production cost of starch hydrolysate. The resulting glucose-rich hydrolysate was formulated into an energy drink, targeting food and beverage applications. Further GC-MS analysis of the fermented rice water showed the presence of bioactive compounds such as 2,2-dimethyl-4-hexen-3-one, 2,6,10-dodecatrienoic acid (7,11-dimethyl-3-(trifluoromethyl)), 2,2,4-trimethylpentane, and oxalic acid, indicating additional value-added potential. These findings demonstrate the feasibility of rice water valorization through enzyme-assisted hydrolysis for glucose syrup production and recovery of industrially relevant bioactive compounds, contributing to sustainable and circular bioeconomy practices.

Conflict of interest

The authors declare that there is no conflict of interest. This research was conducted solely for academic purposes, with no financial, commercial, or personal interests influencing the outcomes.

References

[1] Vargas-Escobar P. Flórez-Acosta O, Corrales-García LL. Renewing the potential of rice crop residues as value-added products in the cosmetics industry. Heliyon 2024;10(7):e28402. https://doi.org/10.1016/j.heliyon.2024.e28402

[2] Saadoun JH, Bertani G, Levante A, Vezzosi F, Ricci A, Bernini V, Lazzi, C. Fermentation of Agri-Food waste: a promising route for the production of aroma compounds. Foods, 2021;10(4): 707. https://doi.org/10.3390/foods10040707

[3] Petljak K. Food waste and food loss in the retail supply chain. Intereulaweast 2021;8(2):119-132. https://doi.org/10.22598/iele.2021.8.2.6

[4] Mahato N, Sharma K, Sinha M, Dhyani A, Pathak B, Jang H, Park S, Pashikanti S, Cho S. Biotransformation of citrus Waste-I: production of biofuel and valuable compounds by fermentation. Processes 2021;9(2):220. https://doi.org/10.3390/pr9020220

[5] Jin Q, Yang L, Poe N, Huang H. Integrated processing of plant-derived waste to produce value-added products based on the biorefinery concept. Trends in Food Science & Technology 2018;74:119–131. https://doi.org/10.1016/j.tifs.2018.02.014

[6] Leichtweis MG, Oliveira MBPP, Ferreira ICFR, Pereira C, Barros L. Sustainable Recovery of Preservative and Bioactive Compounds from Food Industry Bioresidues. Antioxidants 2021;10(11):1827. https://doi.org/10.3390/antiox10111827

[7] Karmee SK. A spent coffee grounds based biorefinery for the production of biofuels, biopolymers, antioxidants and biocomposites. Waste Management 2017;72:240–254. https://doi.org/10.1016/j.wasman.2017.10.042

[8] Tomke, P. D., & Rathod, V. K. Additionally added ingredients and enrichment of beverages: an overview. In *Elsevier eBooks*. 2019;pp. 1–35. https://doi.org/10.1016/b978-0-12-816687-1.00001-1

[9] Mishra A, Ghosh S. Saccharification of kans grass biomass by a novel fractional hydrolysis method followed by co-culture fermentation for bioethanol production. Renewable Energy 2019;146:750–759. https://doi.org/10.1016/j.renene.2019.07.016

[10] Socas-Rodríguez B, Torres-Cornejo MV, Álvarez-Rivera G, Mendiola JA. Deep Eutectic Solvents for the Extraction of Bioactive Compounds from Natural Sources and Agricultural By-Products. Applied Sciences 2021;11(11):4897. https://doi.org/10.3390/app11114897

[11] Liu G, Sun J, He X, Tang Y, Li J, Ling D, Li C, Li L, Zheng F, Sheng J, Wei P, & Xin M. (2018). Fermentation process optimization and chemical constituent analysis on longan (*Dimocarpus longan* Lour.) wine. Food Chemistry 2018;256:268–279. https://doi.org/10.1016/j.foodchem.2018.02.064

[12] Samyor D, Das AB, Deka SC. Pigmented rice a potential source of bioactive compounds: a review. International Journal of Food Science and Technology 2017;52(5):1073–1081.

https://doi.org/10.1111/ijfs.13378

[13] Shabir G, Saeed A, Zahid W. Naseer F, Riaz Z, Khalil N, Muneeba N, Albericio F. Chemistry and Pharmacology of fluorinated Drugs approved by the FDA (2016–2022). Pharmaceuticals 2023;16(8):1162. https://doi.org/10.3390/ph16081162

[14] Muñoz R, Burguet MC, Morlanés N, García-Usach F. Densities, refractive indices, and excess molar volumes of binary and ternary systems containing isobutyl alcohol, ethanol, 2-Methylpentane, and methyl tert-Butyl ether at 298.15K. Journal of Chemical & Engineering Data 2002;45(4):585-589. https://doi.org/10.1021/je000017x

[15] Riemenschneider, W., & Tanifuji, M. (2011). Oxalic acid. *Ullmann's Encyclopedia of Industrial Chemistry*. https://doi.org/10.1002/14356007.a18_247.pub2

[16] Teneva D, Denev P. Biologically active compounds from probiotic microorganisms and plant extracts used as biopreservatives. Microorganisms 2023;11(8):1896. https://doi.org/10.3390/microorganisms11081896

[17] Doan Thi TU, Nguyen TT, Thi YD, Ta Thi KH. Phan BT, Pham KN. Green synthesis of ZnO nanoparticles using orange fruit peel extract for antibacterial activities. RSC Advances 2020;10: 23899-23907. https://doi.org/10.1039/D0RA04926C

[18] Ahmed Ansari S, Abdelghany Damanhory A. Biotechnological application of Aspergillus oryzae β-galactosidase immobilized on glutaraldehyde modified zinc oxide nanoparticles. Heliyon 2023;9(2):e13089. https://doi.org/doi:10.1016/j.heliyon.2023.e13089

[19] Visvanathan R, Jayathilake C, Liyanage R. A simple microplate-based method for the determination of α-amylase activity using the glucose assay kit (GOD method). Food Chemistry 2016;211:853-859. https://doi.org/10.1016/j.foodchem.2016.05.090

[20] Jain BP, Pandey S, Goswami SK. Estimation of Blood Glucose by Glucose Oxidase Method. In Protocols in Biochemistry and Clinical Biochemistry 2025. pp.203-204. Elsevier BV. https://doi.org/10.1016/b978-0-443-13945-1.00059-6

[21] Joannis-Cassan C, Riess J, Jolibert F, Taillandier P. Optimization of very high gravity fermentation process for ethanol production from industrial sugar beet syrup. Biomass and Bioenergy 2014;70:165-173. https://doi.org/10.1016/j.biombioe.2014.07.027

[22] Sanchez N, Yolanda Ruiz R, Cifuentes B, Cobo M. Hydrogen from glucose: A combined study of glucose fermentation, bioethanol purification, and catalytic steam reforming. International Journal of Hydrogen Energy 2016;41:5640-5651. https://doi.org/https://doi.org/10.1016/j.ijhydene.2016.01.155

[23] Hadiyanto H, Ariyanti D, Aini AP, Pinundi DS. Optimization of ethanol production from whey through fed-batch fermentation using *Kluyveromyces Marxianus*. Energy Procedia 2014;47:108-112. https://doi.org/10.1016/j.egypro.2014.01.203

****