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ORIGINAL ARTICLE

Stimulation of natural enzymes for germination of mimosa weed seeds to enhanced bioethanol production

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Abstract

Depleting fossil fuels target plant weeds which have the potential to be converted into efficient biofuels. In this study, mimosa seeds were utilized as a substrate for bioethanol production. This investigation was divided into three parts: breaking dormancy of seeds, mimosa seeds germination, and bioethanol production from mimosa seeds. Seed dormancy breaking was initiated by seeds soaked in hot distilled water to analyze the sugar quantity. Sugar content was measured relevance with root length results. According to results, root length obtained revealed that at 0.5–1.0 cm for root size has the most sugar availability. It was revealed that the total sugar 548.21 g/L and reducing sugar has a concentration of 248.67 g/L. Therefore, the broken dormancy of seeds using hot water at 95 °C for 10 min with a root length of 0.5–1 cm was used for ethanol fermentation. Ethanol fermentation was done by free yeast cell and immobilized yeast by injecting yeast directly. The ethanol yield was measured on the 3rd day of every fermentation. Results showed that the free cell yeast during the 1st day of fermentation afforded an ethanol production of 57.574 g/L, while the yield for immobilized yeast was 60.714 g/L. Consequently, the ethanol yield on the 2nd day of fermentation from the directly injected immobilized yeast was 60.088 g/L. Results revealed that the immobilization of yeast cells in fermentation provided a higher probability for bioethanol yield and could be utilized as a baseline for future bioethanol production. Stimulation of natural enzymes by germination of seeds for enhanced bioethanol production will be a novel approach towards next-generation biofuels.

Keywords Mimosa seeds · Bioethanol · Free cell yeast · Immobilized yeast · Seed germination

Introduction

The extensive use of fossil fuels is one of the causes of several other concerns, such as high levels of pollution, destruction of natural landscapes and habitats, and environmental catastrophes (Saengsawang et al. 2020). Global depletion of fossil fuels, rising fuel prices, environmental concerns, and oil independence pressures create a substantial biofuels market (Darzins et al. 2010). The biofuels global demand for food and feedstocks-derived biomass is expected to increase in the coming decades (Ramaraj et al. 2014). The increase in petroleum cost and global warming, and climate change result in an investigation to discover new renewable energy resources. Bioenergy is one of the most important sources concerning the academic and industrial sectors (Hwang et al. 2016; Sasujit et al. 2017). The current oil price situation is increasing tremendously, causing its effect on the economic impact for the world, due to which the globe is facing a choice of energy futures. Fossil energy for mechanized agriculture has been an essential driver of the "Green Revolution" of increasing farm productivity (Suganya et al. 2016).

Energy is a significant parameter for establishing a country's growth and progress, rather than the standard of living, which directly depends on per capita energy consumption (Tsai et al. 2015). The energy demand required to meet Thailand's economic growth is high and increasing tremendously per year (Aggarangsi et al. 2013).

Currently, energy is one of the Thailand's most sensitive issues, where almost 50% of the total commercial energy

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supply was imported (Kaewdiew et al. 2019). Renewable energy can address many concerns related to fossil energy use. It produces little or no environmental emissions and does not rely on imported fuels. Renewable resources are infinitive, and many are available throughout the country (Sasujit et al. 2017). Thailand has the campaign to use gasohol biodiesel and natural gas. As renewable energy and the production process found that ethanol is an essential alternative biofuel for the production of biodiesel and gasohol. Presently, one of the most promising alternatives for petro-fuels is bioethanol (Bhuyar et al. 2020a, b; Khammee et al. 2020). Ethanol is simple alkyl alcohol that can be used as a transport fuel in spark-ignition engines. It has high octane levels and can be either blended into petrol or used in unmodified vehicles or run as 100% ethanol in a converted machine (Banos et al. 2011). The Thai Government encourages and continues to promote domestic biofuel utilization. Production and consumption of biofuel in Thailand have increased rapidly due to the Thai Government's aggressive policies in reducing foreign oil imports and increasing domestic renewable energy utilization (Chaiyapa et al. 2018).

Ethanol or ethyl alcohol is alcohol fermentation produced from all plant's materials such as cassava, sweet potato, beet, and various grains such as rice, corn, sorghum (Vu et al. 2017; Bautista et al. 2019). Industrial products such as fruit and sweet cane, Lin Ji, longan, pineapple and sugar can be applicable for bioethanol production (Nguyen et al. 2020). Additionally, agriculture waste residues have rich cellulose and hemicellulose, which can be tapped as raw materials to produce ethanol which helps to reduce the amount of trash and agricultural leftovers (Manmai et al. 2017). Furthermore, all waste plant materials are appropriate for bioethanol production. Unfortunately, many weeds are growing throughout the country, and mimosa is one of the weeds that grow and spread quickly. Often, farmers who want to cultivate the area could not stop its growth because the mimosa plant is difficult to eliminate, and trees have a large number of thorns. Usually, the mimosa plant produces a lot of seeds (Ashraf and Foolad 2005).

In several parts of the world tropics, *Mimosa pigra* is a small prickly shrub that infests wetlands and is also an agricultural weed in rice fields. The shrub transforms open grasslands into dense thorny thickets in natural wetlands, reducing native biodiversity. It is considered one of the worst alien invasive weeds in tropical Africa, Asia, and Australia, and control is always expensive. Mimosa, commonly known as the giant sensitive tree (pigra = lazy, slow), is a genus *Mimosa* species in the family Fabaceae. It is native to neotropics but has been listed as one of the world's 100 worst invasive species and forms dense, thorny, impenetrable thickets, particularly in wet areas. The genus *Mimosa* contains 400–450 species, which are primarily native to South America. *M. pigra* is a woody invasive shrub that originates from tropical America and has become widespread throughout the tropics (Grice 2006). These seeds contained high nutritious substances and can be utilized for bioethanol production. In the renewable energy sector, mimosa seeds are efficient in producing ethanol to fuel. The seeds contain a high amount of protein and carbohydrates; these carbohydrates can be digested into sugars and ferment them into ethanol. This study aimed to produce bioethanol from germinated mimosa seeds using *Saccharomyces cerevisiae*. Moreover, the process of activation or stimulation of natural enzymes for germination of mimosa seeds is studied to enhance bioethanol production from the free and immobilized yeast cells.

Materials and methods

Sample collection and preparation

Mimosa seeds were collected from the area around Maejo University, Chiang Mai, Thailand. Seeds were collected from the branches, as shown in Fig. 1a: mimosa tree and Fig. 1b is the sheath of mimosa, and Fig. 1c is only seeded out from the sheath. After the collection, the seeds were brought to the laboratory for further processing. The seeds were stored at room temperature.

Sample preparation and dormancy breaking of mimosa seeds

Collected mimosa seed samples were studied for the seed germination percentage (Silveira and Fernandes 2006). The sample was prepared by soaking the seeds in hot water for control, 5 min, 10 min and 15 min of time duration. Next, all the seeds were incubated at 30 °C to find the best germination percentage. The washing seeds initiated the procedure with 70% ethanol for 1 min, followed by washing with distilled water and then soaked in hot water (95 °C), and allowed them for 5-, 10-, and 15-min time duration. Finally, the last part of the seed was washed and used for control on wet tissue paper wait for growth monitoring.

Study about mimosa seed germination per quantity of sugar.

Mimosa seed germination was studied by the root length analysis (Chen et al. 2001). Totally 180 g of mimosa seeds was added into a 600 ml beaker and then washed with 70% of ethanol for 1 min and washed with distilled water. Then, the seeds were washed with the hot water of 95 °C for 10 min and allowed for seeding in the nursery trays lined with a white cloth. Next, the seeds were spread on

Fig. 1 The pictures of sample collection of mimosa **a** plant, **b** seeds, and **c** mimosa seed sheath

white muslin cloth soaked in water, followed by a regular sprinkling of water to maintain a moist environment. Once the seed starts germinating with the roots, they were blended for fermentation of mimosa seed paste. The ferment solution was a mixture of mimosa 12.5 g with 75 ml of water and then heated by water bath at 50 °C for 30 min to digest the protein by an activated/stimulated enzyme protease. After the digestion, the protease enzymes were filtered from the extractor using a white cloth and a continuous water supply. Simultaneously, the stored mixture was stirred to get the fermented juice. Then, wort water samples were analyzed for total sugar to ferment (total sugar) by phenol–sulfuric method and to reduce sugars by DNS method. Next step, the roots are separated according to their root length from 0 to 0.5 cm, 0.5 to 1 cm and 1 to 1.5 cm, and kept for 0–5 days for drying in a hot air oven at 50 °C for 24 h. After drying, the powder was utilized for fermentation.

Preparation and immobilization of yeast

The YPD media are composed of yeast extract, peptone, and dextrose, also known as yeast extract peptone dextrose media. The media were subjected to steam sterilization by autoclave at 121 °C and 15 lb inch pressure for 15 min, and then cooled. After the fermentation, cooling was initiated by the addition of *Saccharomyces cerevisiae* TISTR 5020 in the medium by loop ashtray infections, media was shaken to RT for 48 h. Two different kinds of fermentation procedures were carried out. For the first fermentation: the best-infected balls were added to the scott duran bottle and were shaken at RT for 48 h. The second fermentation includes the preparation of yeast cells followed by centrifugation at 1500 RCF for 15 min. Yeast cells were injected into tightly wrapped cotton stockings and closed with the thread. The injection was maintained to the proportion of 3 ml: 1 ball.

Scanning electron microscopy

S. cerevisiae sample was collected with a loop needle from Yeast Peptone Dextrose (YPDA) Agar medium and swabbing on carbon tape, then air-drying for a few minutes until dry. Ion sputter MC1000 was used to coat the sample (Hitachi Corp.). Even though the sample has been coated with ion sputter, the fresh sample of *S. cerevisiae* B-18 for SEM evaluation cannot be processed. Therefore, this process, particularly for isolate screening prior to adequate SEM preparation methodology, was used. The maximum time of a sample that has been air-dried for around three hours: if the sample has been air-dried for longer, the resolution will be inadequate. The ideal time for observation in the chamber under a high vacuum is around two hours. The accelerating voltage (Vacc) 3 kV and 5 kV, spot strength 30%, working distance 6 mm, and magnification 5 K and 10 K were all set on the Hitachi SU3500 SEM.

Bioethanol production

The germinated seed was blended to paste using distilled water and filtered for fermentation. Produced wort was sterilized by autoclave at 121 °C and 15 lb per square inch pressure for 15 min. The samples were stored at frozen stage 0 °C, and 3 ml of the sample was kept for total sugar and reducing sugar analysis. The Wort sample was prepared in the Erlenmeyer flask, and about 1% glucose was added to start up the reaction for a carbon source into the fermentation broth; finally, yeast culture was inoculated. Samples were centrifuged to remove the sediment of yeast cells. The flask mouth was covered with cotton and aluminum foil to prevent outside contamination. Then, fermentation was initiated at 30 °C temperature for 3–5 days. After the scheduled time duration, the broth sample was used to determine the ethanol concentration using Ebulliometer (Whangchai et al.

2021). The water samples were analyzed for total sugar by phenol–sulfuric and reducing the sugar by DNS protocol.

Total sugar and reducing sugar analysis

The total sugar and reducing sugar analysis were done by phenol–sulfuric and DNS analysis, respectively (Dubois et al. 1956; Miller 1959). The standard curve of total sugar was plotted using 250 µg/ml standard glucose with a range of five serial dilutions. Then the sample was determined by adding added 0.5 ml of the sample in a test tube, filled 0.5 ml of 5% Phenol (w/v), 2.5 ml of 98% sulfuric acid, mix well with a vortex, leave it for 10 min, and the absorbance was measured at 490 nm and compared with the standard curve. The standard curve for reducing sugar was studied with 625 µg/ml of standard with five serial dilutions. The reducing sugar was calculated from the samples by placing a 0.5 ml volume sample in a test tube, filling it with 0.5 ml of DNS reagent, vortexing it thoroughly, and then sealing it with plexiglass. The test tubes were boiled in boiling water for 15 min and allowed to cool at RT, then the absorption of the sample was measured at 540 nm wavelength and the obtained results were compared to a standard curve.

Results and discussion

Breaking dormancy of mimosa seeds

The seed dormancy was studied by germination and root length measurement. The control seed was washed with distilled water, and the sample seeds were soaked in hot water at 95 °C for 5 min, 10 min and 15 min, respectively. The obtained results revealed that the control with distilled water has shown 4% of germination. The samples soaked in hot water at 95 °C for 5 min has shown 95.6% of germination; for 10 min, 97.2% of germination; and for 15 min, 92.4% of germination. Figure 2 demonstrates the dormancy-breaking and seed germination process: (a) mimosa seed soaked on the white cloth for germination, (b) seed germination of 1 cm root length, and (c) developed root size of 1.5 cm length.

The observed results confirm that hot water treatment enhanced the germination rate; these findings confirm that the investigated species' seed coats are resistant to thermal damage. This dormancy is caused by tissues surrounding the seeds acting as an impediment, causing impermeability of the seed coat or pericarp to water and oxygen, the presence of chemical inhibitors in the pericarp or seed coat, such as coumarin or sorbic acid, or merely by the embryo's physical growth (Fowler and Bianchetti. 2000). The mimosa seed coat is harder in nature and difficult to get self-broken which leads to delay in self-germination. Sperandio et al. (2013) found that sulfuric acid and hot water (70 and 80 °C) treatments were effective for *Mimosa setosa*. However, for *Mimosa ophthalmocentra*, sulfuric acid therapy for 5 and 10 min was the most effective dormancy-breaking approach (Brito et al. 2014).

Mimosa seed germination

The obtained results of root length measurement and the correlated best sugar content are shown in Fig. 3a. The hot water treatment of 95 °C for 10 min is the best temperature of breaking mimosa seed's dormancy, as observed in Fig. 3a. The mimosa paste sample of different root lengths was subjected to total sugar analysis by phenol–sulfuric method and reduced sugar analysis by DNS method. The obtained results showed that mimosa seeds having a root length of ≤ 0.5 cm provided the highest total sugar concentration of 423.02 g/L

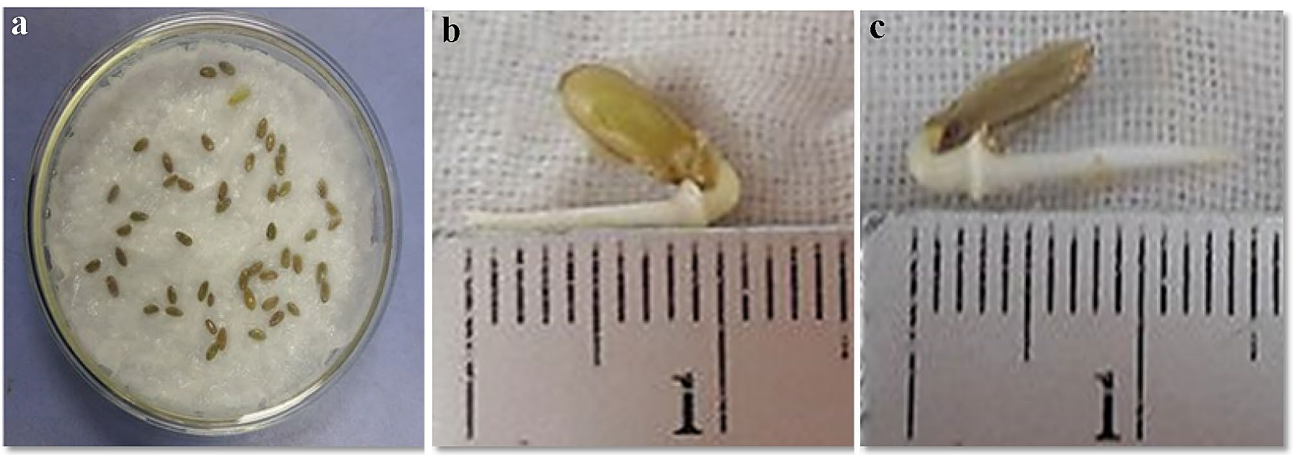

Fig. 2 Mimosa sees germination **a** water-soaked seed on the white cloth, **b** germination of root length of 1 cm, and **c** germination of root length of 1.5 cm

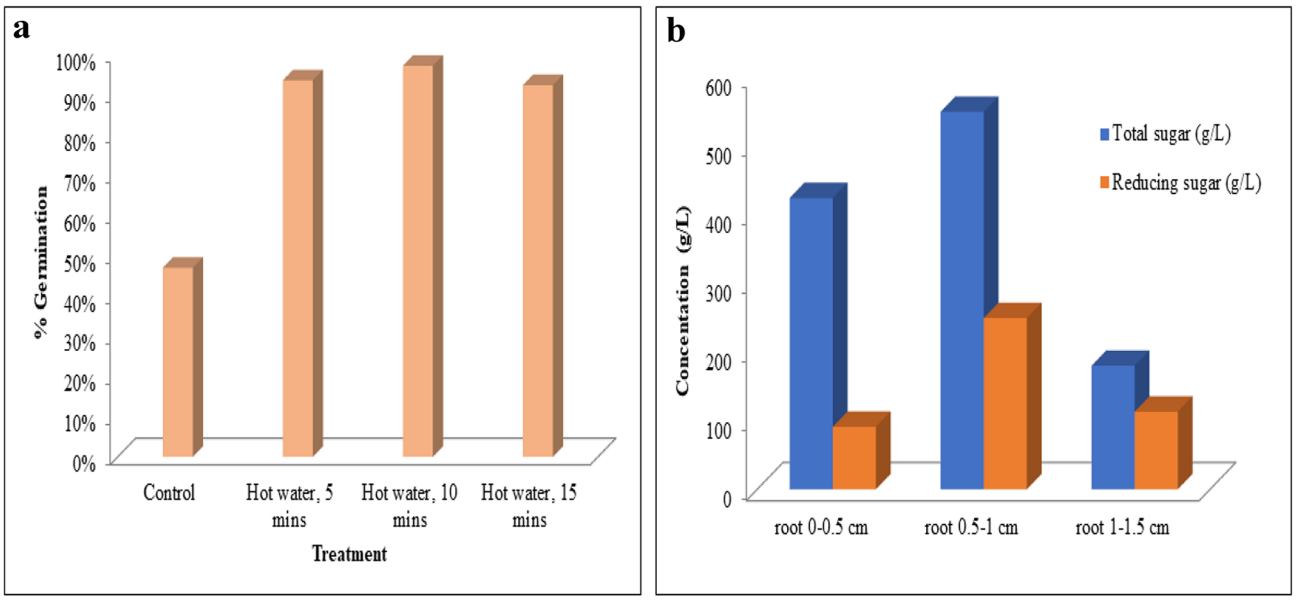

Fig. 3 **a** Percentage of Mimosa seed germination at different time treatment such as 5, 10 and 15 min, **b** the total and reducing sugar concentration of germinated root of mimosa seed

and the highest reducing sugar of 90.67 g/L (Fig. 3b). While the length of 0.5–1 cm has a concentration of total sugar 548.21 g/L and reducing sugar has a concentration of 248.67 g/L, and the length of 1–1.5 cm has a concentration of total sugar 179.37 g/L and has a concentration of reducing sugar 112.67 g/L Fig. 3b. Obtained results confirmed that the root length of 0.5–1 cm has the highest amount of total and reducing sugar. Mimosa seeds have been found to germinate in a wide range of temperatures. Seeds of *M. tenuiflora* germinate best between 10 and 30 °C, according to Camargo-Ricalde et al. (2002); while *M. similis, M. lacertata*, and *M. depauperata* seeds germinate best between 20 and 35 °C, and *M. texana var. texana* seeds germinate best between 30 and 35 °C, according to Silveira and Fernandes (2006). *M. foliolosa* seeds germinated in a wide range of temperatures once the seed coat became permeable. High temperatures influence several cerrado species' germinative behavior. Seed germination of *M. taxifolia* (a sympatric species of *M. foliolosa*; Silveira et al. 2004) was prevented by a constant temperature of 35 °C, but seed germination of *Vellozia* species was favored (Silveira and Fernandes. 2006). At 35 °C, an increase in the germination of *M. foliolosa* control seeds could be attributed to a tegument break that allows for water uptake and, as a result, imbibition.

Bioethanol production

Fermentation by free cell and immobilized yeasts

The mimosa seed paste obtained from a root length of 3.2 cm was subjected to fermentation with the addition of 1% glucose for two kinds of fermentation conditions. The first treatment was added with the 2% yeast (free cells) and the second fermentation using immobilized yeast. The fermentation was carried out for 3–5 days for ethanol production, which was later quantified and recorded. Simultaneously, the concentration of total and reducing sugars were also calculated before and after the fermentation. At the beginning of fermentation by free yeast cells, the initial total sugar was 546.02 g/L, while the reducing sugar was 133.73 g/L, and from the immobilized cell set-up, the total and reducing sugars were 448.84 g/L and 134.18 g/L, respectively (Fig. 4a, b). On the 3rd day, the free cell fermentation had a total sugar of 64.67 g/L, a reducing sugar of 35.62 g/L, and an alcohol content of 57.57 g/L (Table 1). The immobilized cells fermentation obtained a total sugar of 52.55 g/L, reducing sugar of 34.05 g/L, and alcohol content 60.71 g/L. By the 5th day, free cells fermentation had a total sugar of 63.83 g/L, reducing sugar of 41.55 g/L, and alcohol content of 45.01 g/L, while the immobilized cell set-up afforded a total sugar of 50.86 g/L, reducing sugar of 36.62 g/L, and the alcohol content is 52.34 g/L (Table 1).

In fermentation procedures, the use of immobilized cells has several well-known benefits over free cells (Herrero et al. 2001). Environmental factors, as well as the cellular morphology of the organism, affect the ethanol fermentation process. Understanding the physiological regulation of yeast for ethanol production is vital when using standard fermentation methods, which has been studied extensively for many years. On the other hand, the regulation in immobilized yeast cells is still being studied. Yeasts trapped in an immobilizing

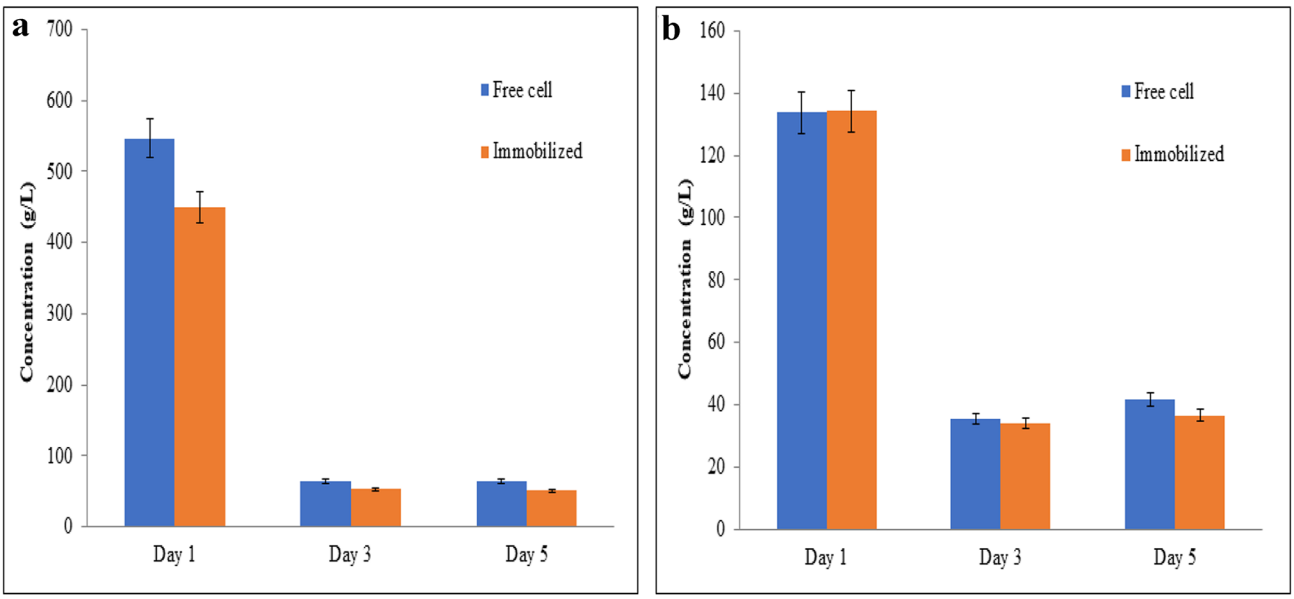

Fig. 4 The percentage of **a** total, and **b** reducing the sugars by free cells and immobilized cells, respectively, on the 1st, 3 rd, and 5th days of fermentation

Table 1 Ethanol production by free and immobilized yeast cells during the course of fermentation

Day	Percent of ethanol (% v/v)	Immobilized
1	0	0
3	57.574	60.7144
5	45.0124	52.34

matrix are exposed to a very different environment than cells in free suspension, and little information about yeast metabolism in these cells has been gathered (Williams and Munnecke 1981).

Fermentation by immobilized yeast inoculated by injection

The fermentation was improved by the immobilized yeast cells. The yeast cells were injected into the mini ball. The free yeast cells (Fig. 5a) were inoculated in the mini ball, as shown in Fig. 5b. The scanning electron microscopy of free and immobilized cells in the mini ball is shown in Fig. 6. (a) The free yeast cells are observed outside the cell wall and (b) Yeast cells encapsulated inside the mini ball were observed. Immobilized yeast was used to initiate the fermentation system. It was found that on the 1st day has the total sugar and reducing sugar was 545.683 g/L and 222.88 g/L. On the 3rd day, there was a total sugar, and reducing sugar was 32.842 g/L and 4.48 g/L (Table 2), and the alcohol content was 60.089 g/L (Table 3). On the 5th day, the total sugar was reduced to 17.684 g/L, and reducing sugar 8.4 g/L. The alcohol production was recorded as 1.963 g/L. The obtained results demonstrated that ethanol percentage on the 3rd day of immobilized yeast fermentation was higher than the other day's treatments.

The environment of immobilized cells differs from that of free cells, altering their physiological conditions. As a result, it contributes to our understanding of cell metabolism and the efficacy of consumption under internal bead conditions. The use of the immobilization method in bioprocess has been shown to have several benefits over traditional chemostat procedures, and it has been used to reduce plasmid instability and increase bioreactor efficiency (Chau et al. 2000).

Traditional cell immobilization procedures have been linked to cell physiology changes, reduced nutrient and product transport efficiency, and an increased risk of contamination during the immobilization process (Yang and Shu 1996). Fibrous matrices (some soft and highly porous materials like cotton, polyester, glass, nylon, rayon, polymer foam, and sponge) have recently been developed as alternative support for cell immobilization due to their high surface-to-volume ratios, constant surface-to-volume ratios, and lower mass-transfer resistance when compared to microcarrier (Hsu et al. 2004). Fibrous matrices are still more appealing as cell immobilization supports than other materials because of their availability, maximum loading, low diffusion problems, nontoxicity, biodegradability, and longevity (Melo and D'Souza 1999; Yang et al. 1995).

Cells may be preserved in suspension (free cells) or immobilized in different supports within bioreactors. Attachment to a surface, entrapment within a porous matrix, cell

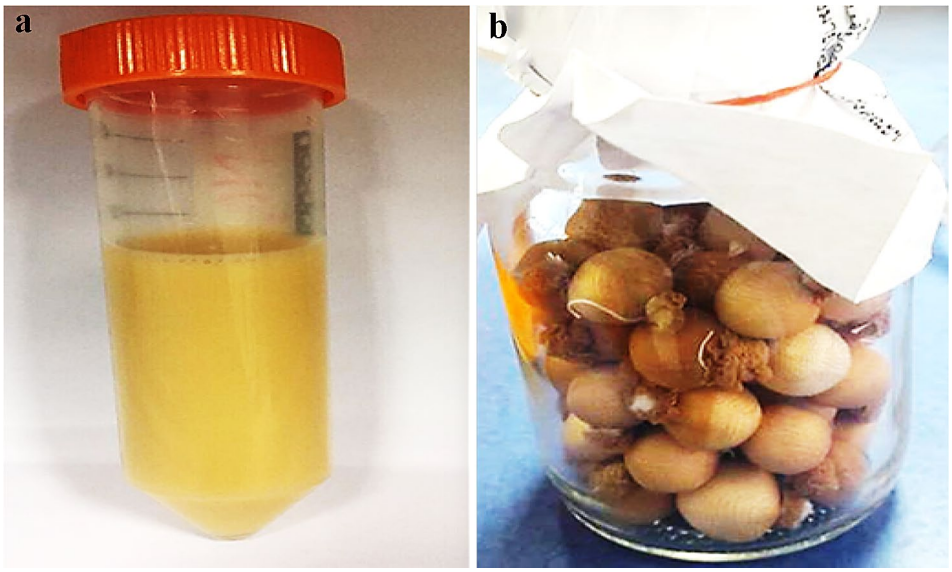

Fig. 5 Suspension of **a** free yeast cells, **b** cells embedded inside the mini ball

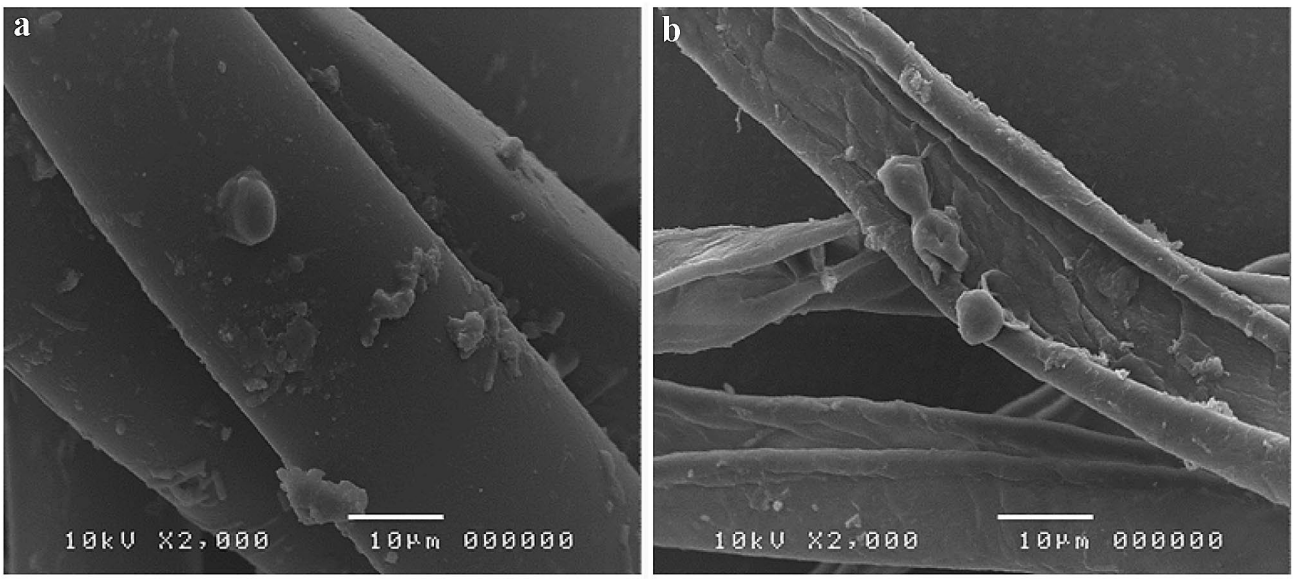

Fig. 6 Scanning electron microscopic image of **a** free yeast cells outside, and **b** inside the mini ball

Table 2 First fermentation total sugar and reducing sugar on fermentation by immobilizing yeast

Day	Treatment	Total sugar	Reducing sugar	SD
1	Free cell	546.02	133.73	±1.15
	Immobilized yeast	448.84	134.18	±2.42
3	Free cell	64.67	35.62	±2.19
	Immobilized yeast	52.55	34.05	±0.83
5	Free cell	63.83	41.55	±5.19
	Immobilized yeast	50.86	36.62	±0.9

Table 3 Total and reducing sugar concentration by fermentation of injected immobilized cells and ethanol percentage

Immobilized cells			
Day	Total sugar (g/L)	Reducing sugar (g/L)	Ethanol (g/L)
1	545.6842 g/L	222.88	0
3	32.8421	4.48	60.0888
5	17.68421	8.4	1.96275

aggregation (flocculation), and containment behind barriers are the four primary immobilization methods for yeast cells (Fig. 5) (Kourkoutas et al. 2004; Verboven et al. 2006). In principle, no binding between cell and carrier can occur with the entrapping procedure, and the preparations should have high activity retention. However, there are some restrictions to activity, and this approach is restricted to low molecular weight substrate and product molecules due to steric resistances to macromolecule diffusion. Non-chemical methods are preferred in cell viability, mainly when non-toxic materials such as carrageenan, calcium alginate, and adhesion materials are used. Immobilized cells provide a large surface area for fermentation which enables alcohol fermentation.

The previous study reports the immobilized system reached a greater initial glucose concentration of 176 g l^{-1} for all significant parameters. The ethanol concentrations attained in both the free and immobilized batch systems (Ca-alginate) were comparatively similar, according to Singh et al. (1998). According to the present research, immobilized cells showed greater tolerance to the higher substrate and product concentrations than free cells.

Substrate inhibition happened with immobilized cells at an initial glucose concentration of 200 g l^{-1}, but not with free cells, which were inhibited at a lower initial substrate concentration of 176 g l^{-1} (Nikolić et al. 2010). According to the findings, it can be inferred that the immobilized system was more productive under certain optimum process conditions. In a continuous fermentation system with immobilized cells, the rise in productivity may be far more critical. A more thorough economic analysis of the entire procedure, including the immobilization step (Nikolić et al. 2010), may confirm the immobilized system's advantage.

Conclusion

This study presented that mimosa seeds can be broken down into dormancy and utilized for fermentable sugar production for bioethanol generation. This study established that mimosa seeds can be broken down and used to generate fermentable sugars for bioethanol production. Therefore, it is confirmed that soaking in hot water at 95 °C for 10 min is the best to break down mimosa seeds' dormancy. The highest sugar content of mimosa seeds with a length of 0.5–1 cm has a concentration of total sugar 548.21 g/L, and reducing sugar 248.67 g/L. For this experiment, a root length of 0.5–1 cm has the most sugar content to produce bioethanol production. Compared with free cell's fermentation at 3rd day by treatment and immobilized cells, fermentation has the most ethanol production which was 52.34 g/L. The results recommend that immobilized yeast cells can produce an adequate amount of bioethanol. Consequently, this study results suggested that mimosa weed could be valid for bioethanol production and scale up for sustainable application in the future.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

Aggarangsi P, Tippayawong N, Moran JC, Rerkkriangkrai P (2013) Overview of livestock biogas technology development and implementation in Thailand Energy for Sustainable development. Energy Sustain Dev 17(4):371–377

Ashraf M, Foolad MR (2005) Pre-sowing seed treatment—A shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. Adv Agron 88:223–271

Banos R, Manzano-Agugliaro F, Montoya FG, Gil C, Alcayde A, Gómez J (2011) Optimization methods applied to renewable and sustainable energy: A review. Renew Sustain Energy Rev 15(4):1753–1766

Bautista K, Unpaprom Y, Ramaraj R (2019) Bioethanol production from corn stalk juice using *Saccharomyces cerevisiae* TISTR 5020. Energy Sour Part A Recover, Util Environ Eff 41(13):1615–1621

Bhuyar P, Sathyavathi S, Math RK (2020a) Production of bioethanol from starchy tuber (*Amorphophallus commutatus*) and antimicrobial activity study of its extracts. Afr J Biol Sci 2(2):70–76

Bhuyar P, Yusoff MM, Rahim MHA, Sundararaju S, Maniam GP, Govindan N (2020b) Effect of plant hormones on the production of biomass and lipid extraction for biodiesel production from microalgae *Chlorella* sp. J Microbiol Biotechnol Food Sci 10(1):671–674

Brito AS, Pinto M, Araújo AV, Souza VN (2014) Superação de dormência em *Mimosa ophthalmocentra* Mart Ex Benth. Enciclopédia Biosfera 10(18):2792

Camargo-Ricalde SL, Dhillion SS, Grether R (2002) Community structure of endemic *Mimosa species* and environmental heterogeneity in a semi-arid Mexican valley. J Veg Sci 13(5):697–704

Chaiyapa W, Esteban M, Kameyama Y (2018) Why go green? Discourse analysis of motivations for Thailand's oil and gas companies to invest in renewable energy. Energy Policy 120:448–459

Chau TL, Guillan A, Roca E, Nunez MJ, Lema JM (2000) Enhancement of plasmid stability and enzymatic expression by immobilizing recombinant *Saccharomyces cerevisiae*. Biotech Lett 22:1247–1250

Chen WM, Laevens S, Lee TM, Coenye T, De Vos P, Mergeay M, Vandamme P (2001) *Ralstonia taiwanensis* sp nov, isolated from root nodules of *Mimosa species* and sputum of a cystic fibrosis patient. Int J Syst Evol Microbiol 51(5):1729–1735

Darzins A, Pienkos P, Edye L (2010) Current status and potential for algal biofuels production Bioind Partnes NREL. In: Report prepared for the International Energy Agency, Bioenergy Task 39:131

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28(3):350–356

Fowler JAP, Bianchetti A (2000) Dormancy in forest seeds. Documentos-Embrapa Florestas 40:31

Grice AC (2006) The impacts of invasive plant species on the biodiversity of Australian rangelands. Rangel J 28(1):27–35

Herrero M, Laca A, García LA, Díaz M, (2001) Controlled malolactic fermentation in cider using *Oenococcus oeni* immobilized

in alginate beads and comparison with free cell fermentation. Enzyme Microb Technol 28(1):35–41

Hsu CH, Chu YE, Argin-Soyal S, Hahm TS, Lo YM (2004) Effects of surface characteristics and xanthan polymers on the immobilization of *Xanthomonas Campestris* to fibrous matrices. J Food Sci 69(9):E441–E448

Hwang JH, Church J, Lee SJ, Park J, Lee WH (2016) Use of microalgae for advanced wastewater treatment and sustainable bioenergy generation. Environ Eng Sci 33(11):882–897

Kaewdiew J, Ramaraj R, Koonaphapdeelert S, Dussadee N (2019) Assessment of the biogas potential from agricultural waste in northern Thailand. Maejo Int J Energy Environ Commun 1(1):40–47

Khammee P, Ramaraj R, Whangchai N, Bhuyar P, Unpaprom Y (2020) The immobilization of yeast for fermentation of macroalgae *Rhizoclonium* sp for efficient conversion into bioethanol. Biomass Convers Biorefinery 11:827–835

Kourkoutas A, Kourkoutas A, Bekatorou A, Banat IM, Marchant R, Koutinas AA (2004) Immobilization technologies and support materials suitable in alcohol beverages production: a review. Food Microbiol 21:377–397

Manmai N, Siriboon T, Tipnee S, Unpaprom Y, Ramaraj R (2017) Bioconversion of lignocellulosic biomass, *Sorghum bicolor* L into bioethanol. In: Proceedings of academics World 73 rd International Conference. Taipei, Taiwan, 41–45

Melo JS, D'Souza SF (1999) Simultaneous filtration and immobilization of cells from a flowing suspension using a bioreactor containing polyethylenimine coated cotton threads: application in the continuous inversion of concentrated sucrose syrups. World J Microbiol Biotechnol 15(1):23–27

Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31(3):426–428

Nguyen T, Unpaprom Y, Ramaraj R (2020) Enhanced fermentable sugar production from low grade and damaged longan fruits using cellulase with algal enzymes for bioethanol production. ELSR 6(2):26–31

Nikolić S, Mojović L, Pejin D, Rakin M, Vukašinović M (2010) Production of bioethanol from corn meal hydrolyzates by free and immobilized cells of *Saccharomyces cerevisiae* var ellipsoideus. Biomass Bioenerg 34(10):1449–1456

Ramaraj R, Tsai DDW, Chen PH (2014) An exploration of the relationships between microalgae biomass growth and related environmental variables. J Photochem Photobiol, B 135:44–47

Saengsawang B, Bhuyar P, Manmai N, Ponnusamy VK, Ramaraj R, Upaprom Y (2020) The optimization of oil extraction from macroalgae, *Rhizoclonium* sp by chemical methods for efficient conversion into biodiesel. Fuel 274:117841

Sasujit K, Dussadee N, Homdoung N, Ramaraj R, Kiatsiriroat T (2017) Waste-to-Energy: Producer gas production from fuel briquette of energy crop in Thailand. Int Energy J 17(1):37–45

Silveira FAO, Fernandes GW (2006) Effect of light, temperature and scarification on the germination of *Mimosa foliolosa* (Leguminosae) seeds. Seed Sci Technol 34(3):585–592

Silveira FA, Negreiros D, Fernandes GW (2004) Influência da luz e da temperatura na germinação de sementes de Marcetia taxifolia (A St-Hil) DC (Melastomataceae). Acta Bot Bras 18(4):847–851

Singh D, Nigam P, Banat IM, Marchant R, McHale AP (1998) Ethanol production at elevated temperatures and alcohol concentrations: Part II–Use of *Kluyveromyces marxianus* IMB3. World J Microbiol Biotechnol 14(6):823–834

Sperandio HV, Lopes JC, Matheus MT (2013) Superação de dormência em sementes de *Mimosa setosa* Benth. Comunicata Scientiae 4(4):385–390

Suganya T, Varman M, Masjuki HH, Renganathan S (2016) Macroalgae and microalgae as a potential source for commercial applications along with biofuels production: a biorefinery approach. Renew Sustain Energy Rev 55:909–941

Tsai DDW, Chen PH, Chou CM, Hsu CF, Ramaraj R (2015) Carbon sequestration by alga ecosystems. Ecol Eng 84:386–389

Verboven P, Flick D, Nicolai BM, Alvarez G (2006) Modeling transport phenomena in refrigerated food bulks, packages and stacks: basics and advances. Int J Refrig 29(6):985–997

Vu PT, Unpaprom Y, Ramaraj R (2017) Evaluation of bioethanol production from rice field weed biomass. Emergent Life Sci Res 3:42–49

Whangchai K, Inta W, Unpaprom Y, Bhuyar P, Adoonsook D, Ramaraj R (2021) Comparative analysis of fresh and dry free-floating aquatic plant *Pistia stratiotes* via chemical pretreatment for second-generation (2G) bioethanol production. Biores Technol Rep 14:100651

Williams D, Munnecke DM (1981) The production of ethanol by immobilized yeast cells. Biotechnol Bioeng 23(8):1813–1825

Yang ST, Shu CH (1996) Kinetics and stability of GM-CSF production by recombinant yeast cells immobilized in a fibrousbed bioreactor. Biotechnol Prog 12:449–456

Yang ST, Huang Y, Hong G (1995) A novel recycle batch immobilized cell bioreactor for propionate production from whey lactose. Biotechnol Bioeng 45:379–386