Agro Bali : Agricultural Journal
Vol. 6 No. 1: 1-11, March 2023

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v6i1.1044

Substrate Optimization for Bioemulsification Using *Saccharomyces cerevisiae* 2031 by Response Surface Methodology

Justine Allen S. Malabuyoc[1], Virgie A. Alcantara[2], Rhebner E. Arocena[1] and Francisco B. Elegado[2♥]

[1]Department of Chemical Engineering, College of Engineering and Agro-Industrial Technology, University of the Philippines Los Baños, College, Laguna, Philippines
[2]National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños, College, Laguna, Philippines
♥Corresponding author email: fbelegado@up.edu.ph

Article history: submitted: September 23, 2022; accepted: March 2, 2023; available online: March 31, 2023

Abstract. Biosurfactants are microbially derived amphiphilic molecules that can be used as biodegradable emulsifiers in various applications. For biosurfactant production to be economically viable, inexpensive raw materials should be used. In this study, substrate optimization of biosurfactant production from *Saccharomyces cerevisiae* 2031 was done using molasses as an additional carbon source to glucose, and coco paring meal extract as a nitrogen source. Optimum conditions were determined as pH 5.69, 10.60% ($w.v^{-1}$) molasses and 7.27% ($v.v^{-1}$) coco paring meal extract using Box-Behnken design. At these conditions, the obtained responses: namely biomass concentration and % emulsification index determined with kerosene, were 6.43 $g.L^{-1}$ and 82.81%, respectively. The highest emulsification activity (84.60%) was attained after 4 days of shake-flask fermentation. On the other hand, a bioreactor system observed the maximum yield for emulsification activity (93.33) after 4 days. The biosurfactant extracted was characterized by its total sugar, protein content and surface tension reduction.

Keywords: biosurfactant; coconut paring meal; molasses; *Saccharomyces cerevisiae*

INTRODUCTION

Surfactants are amphiphilic molecules causing them to aggregate at the interfaces between fluids with different polarities such as water and hydrocarbons (Sharma et al., 2022). Biosurfactants are produced by microorganisms such as bacteria, yeasts and fungi, that feed on a water-immiscible substrate such as spent cooking oil in canals or crude oil spillage in the sea (Rocha e Silva et al., 2017; Almaral et al., 2010). By evolution, the bacteria have adapted themselves to feed on water-immiscible materials through the manufacture and use of a surface active product that aids them in absorbing, emulsifying, wetting and dispersing or solubilizing the water-immiscible material. Biosurfactants can be either cell bounds or secreted extracellularly (Chaprao et al., 2015).

Since the major characteristic of surfactants is their ability to reduce surface tension, they are considered to be the key ingredients used in consumer and industrial products such as detergents, shampoos, toothpaste and oil additives. In various industries, surface-active compounds commonly used are chemically synthesized. However, the interest in biosurfactants has increased considerably in recent years due to the present concern with the protection of the environment (Kumar et al., 2021; Jimoh & Lin, 2019). Therefore, the most significant advantage of a microbial surfactant over chemical surfactants is its ecological acceptance because it is biodegradable and nontoxic to the natural environment (Eras-Muñoz et al., 2022).

Apart from the biosurfactants' capability to reduce surface and interfacial tension, they are also able to form microemulsions where hydrophobic compounds can solubilize in water or vice versa, thereby making the hydrophobic parts more soluble and microbial degradation more favorable. Biosurfactants have also been found to stimulate the biodegradation of organic compounds, commonly alkanes (Freitas et al., 2016; Sajna et al., 2015). Other advantages of biosurfactants over synthetic ones are the possibility of their production

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v6i1.1044

through fermentation (Rahman & Gakpe, 2008), their potential applications in environmental protection and management, crude oil recovery, as antimicrobial agents in health care and food processing industries, selectivity and specific activity at extreme temperatures, pH and salinity (Akubude et al., 2021).

Nevertheless, biosurfactants are not yet competitive with synthetics since it is non-economical in terms of large-scale production. Replacing synthetic surfactants with biosurfactants can be possible if (1) cheaper and waste substrates are used to lower the initial cost of raw materials involved in the process; (2) efficient bioprocesses such as optimization of the culture conditions and cost-effective separation methods for maximum biosurfactant production and recovery be implemented; and (3) better strains for enhanced biosurfactant yields are used (Edding, 2009).

A great variety of alternative raw materials is currently available as nutrients for industrial fermentations, namely various agricultural and industrial by-products and waste materials. Such materials, aside from being considered as a potential substrate for biosurfactant production, can also alleviate many processing industrial waste management problems and pollution (Maneerat, 2005). Molasses, a by-product of the sugar cane industry, is an interesting raw material alternative for biosurfactant production due to its low price compared to other sources of sugar and due to the presence of nutritive compounds other than sucrose. The components of molasses such as minerals, organic compounds and vitamins were also found out to be valuable for fermentation processes (Rodrigues et al., 2006). Others use molasses as the sole carbon source or additional carbon source for the production of biosurfactants (Patel & Desai, 1997). Aside from molasses, coconut paring meal is considered a potential source of nutrients for biosurfactant production. Coconut paring meal, a by-product of desiccated coconut manufacture, is a stock feed source from the coconut fruit and has highvitamin content (Stein et al., 2015).

The use of high biosurfactant-producing microorganisms such as *Saccharomyces cerevisiae*, also known as baker's yeast or brewer's yeast, would also be very important in its economic feasibility (Alcantara et al., 2012). Compared to other microorganisms such as fungi and filamentous bacteria, yeasts do not produce endotoxins making them safe for food, beverage and pharmaceutical use (Ribeiro et al., 2022)

This study was designed to optimize the production of biosurfactants by *Saccharomyces cerevisiae* 2031 using low-cost and locally-available base substrates, namely: molasses and coco paring meal extract as additional carbon and nitrogen sources, respectively. Response Surface Methodology was used for the substrate and pH optimization of extracellular biosurfactant production.

METHODS

Yeast Strain and Growth Conditions

Saccharomyces cerevisiae 2031 was obtained from the collection of the laboratory of the Industrial, Energy and Environmental Biotechnology Program of the National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños. From the stock culture, the yeast strain was transferred and was incubated in yeast extract malt agar (YMAT) (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 0.01% $CaCl_2$, 1.8% agar, and 1% Tween 80) at 30°C. The slants were maintained in YMAT at 4°C. Yeast inoculum was prepared by transferring cells from YMAT slants to 125 mL Erlenmeyer flasks containing 50 mL of yeast malt extract broth (YMB) (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, and 1% glucose). Cultures were incubated at ambient temperature with shaking at 200 rpm for 24 h.

Media for Biosurfactant Production

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v6i1.1044

A modified Cooper and Paddock's medium (Cooper & Paddock, 1984), containing 5% glucose, 0.10 % KH_2PO4, 0.5% $MgSO_4 \cdot 7H_2O$, 0.01% $CaCl_2$ and 0.01 % NaCl, was used as the basal components. Different concentrations of coco paring meal extract (0-20% $v \cdot v^{-1}$) and sugar cane molasses (0-20% $w \cdot v^{-1}$) at varying pH (3-8) were prepared to make a 50 mL medium containing the modified Cooper and Paddock's supplements in 125 mL Erlenmeyer flasks which were used in the optimization trials. The coco paring meal extract was prepared by boiling 20% ($w.v^{-1}$) coco paring meal in distilled water for 30 min and removal of the solid residue after centrifugation at 3000 rpm for 10 min.

Analytical Methods

Determination of the biomass concentration by dry weight

Every day, samples (5 mL) from the fermentation culture were obtained after thorough mixing and then centrifuged at 3000 rpm for 10 min. The supernatant was removed and the resulting pellets were washed with distilled water. The washed cells were transferred to pre-weighed aluminum dishes. Pans were placed in an oven at 105 °C to constant weight.

Measurement of % emulsification index

Samples from the fermentation culture (25 mL) were obtained and centrifuged at 3000 rpm for 10 min. The supernatant was removed and distilled water was added to the yeast (30 mL dH2O per gram dry yeast). The mixture was boiled for 20 min and centrifuged for 10 min at 3000 rpm. Two mL supernatant (presumed to contain the biosurfactant) was obtained and mixed with kerosene (3 mL). The mixture was vortexed at high speed for 2 min. Measurement of emulsification activity was done after 24 h. The percent emulsification index (E24) was obtained by dividing the height of the emulsion layer by the total height and multiplying by 100 (Cooper & Goldenberg, 1987).

Optimization by Three Factor Interaction (3FI) and Response Surface Methodology (RSM)

Using the initial estimates (pH 3-8, 0-20% $w.v^{-1}$ molasses, 0-20% $v.v^{-1}$ coco paring meal extract) combination and their corresponding responses (biomass concentration and % emulsification index), the interaction of the parameters were determined by the Design Expert Version 8 software using 3FI design (pre-optimization). Upon detecting a curvature in the design space, RSM was used to determine the optimum conditions, using the software's Box-Behnken design as a quadratic model.

Effect of Fermentation Time

Shake-flask fermentation

Biosurfactant production was carried out in 125 mL Erlenmeyer flasks using the modified Cooper and Paddock's medium (50 mL) containing 5% glucose and optimized amounts of molasses and coco pairing meal extract at the optimum pH. A corresponding amount of YMB was added for each flask. The flasks were incubated at ambient temperature with shaking (200 rpm) for 0 to 7 days. Biomass concentration and % emulsification index were determined for each sample.

Bioreactor fermentation

Biosurfactant production was carried out in a 5 L bioreactor (Biostat A-plus, Sartorius, Germany) using modified Cooper and Paddock's medium (3 L), containing 5% glucose, and optimized amounts of molasses and coco pairing meal extract at the optimum pH. The yeast inoculum, grown in YMB, was added to an initial O.D. (optical density) of 1.0. The system was carried at 300C with agitation at 100 rpm for 1 to 4 days. Sampling was done every 24 hours. Biomass concentration and % emulsification index weredetermined for each sample.

Characterization of Biosurfactant

Carbohydrate content was determined by phenol-sulfuric acid assay using glucose

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v6i1.1044

as standard. On the other hand, protein content was determined by the method of Lowry using bovine serum albumin (BSA) as standard. Lastly, the surface tension reduction was qualitatively determined using a modified drop-collapse technique of Bodour & Miller-Maier (1998).

Statistical Analysis

The 3FI and Box-Behnken designs by the Design Expert software were used for the data analysis of the pre- optimization and optimization steps. A significant difference was evaluated at 5% confidence level. ANOVA using Duncan's Multiple Range Test (DMRT) for the effect of fermentation time was obtained by employing Statistical Packages for Social Sciences (SPSS). The whole experiment was carried out in duplicate trials.

RESULTS AND DISCUSSION

Initial Optimization Using 3FI Design

The Three Factor Interaction (3FI) design was first used to have an estimate of the location of the optimum conditions. The three parameters were pH, molasses concentration (% $w.v^{-1}$), and coconut paring meal extract concentration (% $v.v^{-1}$) while the response variables were the biomass concentration ($g.L^{-1}$) and the % emulsification index. Using this design, the presence of a curvature in the design space was determined which indicated that the optimum condition was a point somewhere in the design space. Based on the experimental responses, ANOVA was done using Design Expert. The obtained F-values for the model and curvature were significant for both the responses, biomass concentration and % emulsification index (data not shown).

Evaluation of Parameters by Box-Behnken Design

To further evaluate the optimum parameter levels, their interaction, and the response levels, the Box- Behnken Design (BBD) was chosen. Analysis of variance was made to ensure the reliability of the models that were used in the analyses. Based on the significant model terms for both biomass concentration (molasses concentration, coco paring meal concentration, square of pH and square of molasses concentration) and % emulsification index (squares of the three parameters: pH, molasses concentration and coco paring meal concentration), the effects of the three parameters were better analyzed using a quadratic model which made the design model more reliable (ANOVA data not shown). The BBD employed has five center points, each with replicates. These five center points are close to one another such that they overlap and the other points are hidden below the response plane. The predicted response surface fits the model points well. However, the differences between the actual data points and the response plane are greater than that between the center points. In simpler terms, when the center points are fitting better than the model points, the lack of fit (LOF) becomes significant (Stat-Ease Inc., 2004). Although there is significant LOF, it doesn't mean that the model is not effective. The replicates were from independent set-ups but the results have little variation from each other.

Numerical optimization

The optimum condition should give high yields of the responses and should require the least amounts of input variables as well. Numerical analyses were employed to determine the optimum conditions. The optimum conditions are: pH, 5.69; molasses concentration, 10.60 (%$w.v^{-1}$); and coco paring meal extract concentration of 7.27 (% $v.v^{-1}$), which gave 6.43 ($g.L^{-1}$) biomass and % emulsification index of 82.81. The desirability of the model was 0.943.

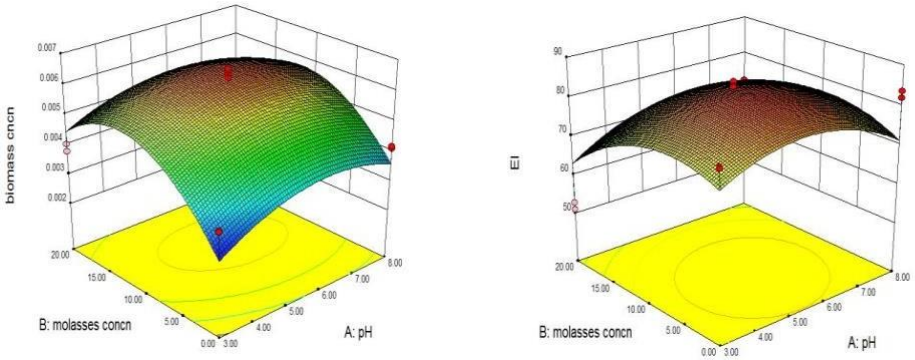
It can be seen that the biomass concentration and % emulsification index yields in these optimum conditions are close to the actual amount of the maximum yields from the experiment wherein biomass was 6.54 $g.L^{-1}$ while % emulsification index was

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v6i1.1044

measured as 85.04, confirming the validity of the model and optimizationresults.

Interactions among parameters

To complete the analysis of optimization, interactions of the parameters around the optimum condition were established. Contour plot and 3D graph best illustrate the interaction which exists between two variables at a time while the other factors are fixed at a certain level. The contour plots of the biomass concentration and emulsification index obtained using the software package also confirmed the optimum condition that exists in the design space as shown in Figures 1 to 3. In these plots, the red-colored areas signify high response levels while the blue-colored areas signify low response levels. These graphs were generated by the software by fitting the quadratic model of the Box-Behnken design to the actual response levels.



A B

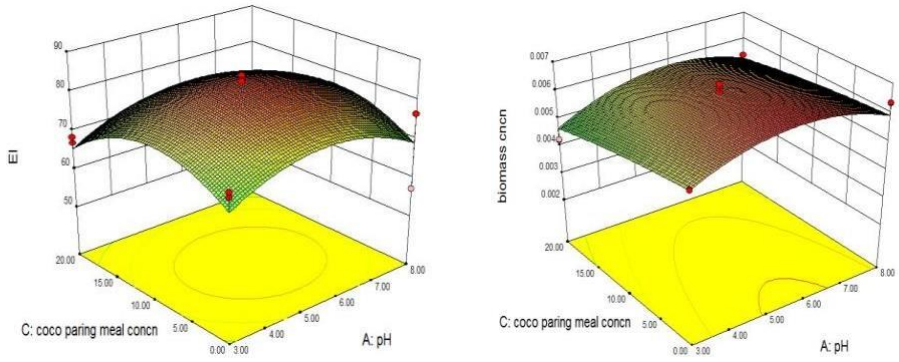
Figure 1. 3D graphs and contour plots showing the effect of molasses concentration (% w/v) and pH on the biomass concentration (g/L) (A) and biosurfactant's emulsification index (B). [Hold value: Coconutparing meal concentration = 10 % (v/v)].

pH - molasses concentration interaction

For the biomass concentration (**Figure 1A**), the optimum yield has been observed at pH 5.5 and a molasses concentration of 10% ($w.v^{-1}$). According to Alcantara et al. (2012), the pH of the media has an important role in biosurfactant production. Their study cited that low values for biomass concentration were obtainedat very acidic and highly alkaline pH values and that the maximum biomass production was observed at pH 5-8. The biomass concentration increased when the molasses concentration increased but only up to 10% ($w.v^{-1}$). At higher concentrations of the carbon source (20 % $w.v^{-1}$), the biomass concentration decreased. These results may be due to substrate inhibition or diversion of the substrate to the other by-products (Yabes, 2002) supported this observation and stated that high substrate concentrations also express growth-inhibitingproperties.

For the % emulsification index (**Figure 1B**), the optimum yield was also observed at pH 5.5 and a molasses concentration of 10% ($w.v^{-1}$). Above pH 5.5 and when molasses concentration was increased further, the % emulsification index values obtained decreased. The lower yield for the % emulsification index may be due to growth inhibition which in turn produced less biosurfactant, therefore, a corresponding decrease in emulsion activity.

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v6i1.1044



A B

Figure 2. 3D graphs and contour plots showing the effect of coco paring meal concentration (% v/v) andpH on the biomass concentration (g/L) (A) and biosurfactant's emulsification index (B). [Hold value: Molasses concentration = 10 % (w/v)].

pH - coconut paring meal concentration interaction

As illustrated in **Figure 2A**, the effect of pH and coco paring meal concentration on the biomass concentration did not show a distinct optimum response. The biomass has relatively high yields at pH 5.5 and a coconut paring meal concentration of 10% ($v.v^{-1}$). However, the biomass concentration has almost the same yield at pH 8 when no coconut paring meal was added. The increase in biomass can probably be attributed to the use of yeast extract as a nitrogen source. (Perez-Guevarra et al., 1994). Cooper and Paddock's medium originally has yeast extract as a component which makes the medium more efficient for cell growth. Yet, this component is expensive.

On the other hand, based on the concentric circles formed, the interaction of pH-coconut paring meal extract gave an optimum % emulsification index response (**Figure 2B**) observed at pH 5.5 and coconut paring meal extract concentration of 10% ($v.v^{-1}$). At acidic (pH 3) and basic (pH 8) pH, the emulsification activity ranges from 57.3 % to 76.52 %. In a study by Ribeiro et al. (2020), they obtain a wider range of emulsification index from 3.77% to 94.58%. The change in pH causes variability in the % emulsification index. According to Edding (2009), variation in emulsification activity is due to the alteration of the conformational structure of the protein component. It must also be noted that coco paring meal has saturated fatty acid content, which makes the emulsion less at a higher concentration of coconut paring meal extract (20% $v.v^{-1}$).

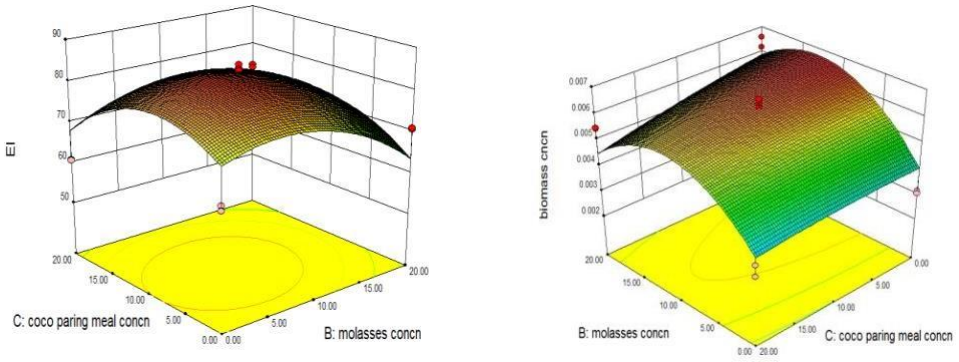
Interaction between molasses – coco paring meal extract concentrations

For the biomass concentration (**Figure 3A**), no optimum response was observed. However, for the % emulsification index, the optimum yield has been observed at 10% concentration of both molasses ($w.v^{-1}$) and coco paring meal extract ($v.v^{-1}$). A synergistic relationship exists between the carbon (carbohydrate) and nitrogen (protein) sources. When both carbohydrate and protein were considered, efficient emulsifying activity was observed (Edding, 2009). The emulsification properties were due to the protein component while the stabilization of the emulsion was caused by the carbohydrate component. The carbohydrate residue decreases the solubility of the protein, keeping it at the oil/water interface. At the right proportions of molasses (10% $w.v^{-1}$) and coco paring meal extract (10% $v.v^{-1}$), the % emulsification index is at its highest.

The optimum conditions based on the

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v6i1.1044

interaction of parameters used are pH 5.5, molasses concentration of 10% (w.v^{-1}), and coco paring meal extract concentration of 10% (v.v^{-1}) (**Figures 1A, 1B, 2B** and **3B**). However, these were not the ones suggested by the numerical optimization. The optimum conditions simulated by the numerical optimization also considered the interactions featured in **Figures 2A** and **3A**, even though no optimum response was obtained. Therefore, the numerical optimization incorporated all the interactions before suggesting the optimum conditions of pH 5.5, molasses concentration of 10.60 % (w.v^{-1}) and coco paring meal extract concentration of 7.27% (v.v^{-1}).



A B

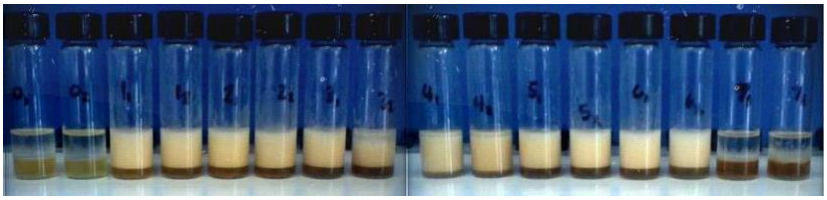
Figure 3. 3D graphs and contour plots showing the effect of coco paring meal concentration (% v/v) and molasses concentration (% w/v) on the biomass concentration (g/L) (A) and biosurfactant's emulsificationindex (B) [Hold value: pH = 5.5].

Effect of Fermentation Time

The effect of fermentation time was carried out first by using the shake-flask method. Results showed that as the fermentation time increases, the biomass concentration also increases but up to the third day of shaking only and thereafter decreased (**Table 1**). According to Santelices (2005), biomass increases with increasing days of fermentation. Moreover, Held (2010) discussed that the metabolism of cells slows downwhen there is complete substrate consumption or high waste concentration. Using DMRT, the biomass obtained for days 5 and 7 were proven to be not significantly different from each other (**Table 1**). Five daysare enough for biomass fermentation since cell growth was already in its stationary phase.

The percent emulsification index obtained has a direct relationship with increasing fermentation time but up to four (4) days only. After 7 days of shaking, the emulsification activity dramatically decreased (Table 1). As seen in **Figure 4**, the emulsion layer started to become unstable after two days for those samples with 3 and 4 days of shaking. Apart from growth inhibition, the non-uniformity of mixtures due to uneven agitation may also be the cause of the instability of emulsion. Moreover, based on the DMRT, the % emulsification index obtained for the shake flask method for days 1, 2, 5 and 6 are not significantly different from each other (**Table 1**). Again, like in the case of biomass concentration, biosurfactant production in shake-flask can be done up to 4 days of shaking only.

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v6i1.1044



Day 0 Day 1 Day 2 Day 3 Day 4 Day 5 Day 6 Day 7

Figure 4. Emulsification activity of *Saccharomyces cerevisiae* 2031 biosurfactant at various fermentationtimes.

Table 1. Effect of fermentation time on the biomass concentration and emulsification activity of *Saccharomyces cerevisiae* 2031 biosurfactant.

Fermentation time (day)	Biomass concentration* ($g \cdot L^{-1}$)	Emulsification index* (%)
Shake-flask method		
0	4.30 ± 0.10^{b}	15.46 ± 0.00^{b}
1	4.70 ± 0.10^{c}	79.31 ± 0.10^{de}
2	5.15 ± 0.05^{d}	79.75 ± 0.66^{de}
3	5.55 ± 0.05^{e}	63.75 ± 5.75^{c}
4	5.20 ± 0.10^{d}	84.60 ± 0.25^{e}
5	4.15 ± 0.05^{b}	76.85 ± 0.70^{d}
6	3.65 ± 0.15^{a}	76.25 ± 0.25^{d}
7	4.35 ± 0.15^{b}	6.98 ± 0.24^{a}
Bioreactor		
0	5.25 ± 0.05^{a}	35.83 ± 11.79^{a}
1	5.40 ± 0.00^{ab}	89.57 ± 0.05^{b}
2	5.55 ± 0.05^{b}	90.48 ± 0.00^{b}
3	6.05 ± 0.05^{c}	91.47 ± 0.05^{b}
4	6.25 ± 0.05^{d}	93.33 ± 0.00^{b}

Note: *Values with no common letters are significantly different from each other at p=0.05 using Duncan's Multiple Test.

Bioreactor fermentation

The results obtained in shake-flask were applied in the bioreactor fermentation runs but this time agitation speed was kept constant at 100 rpm and the sample was evenly mixed. Results showed that both biomass concentration and % emulsification index increased over time (**Table 1**). Biomass has a direct relationship with fermentation time and analysis of variance using DMRT showed that measured biomass for samples after 0, 2, 3 and 4 days of agitation were significantly different from each other.

The emulsions formed by the biosurfactant produced in the shake-flask were less stable because agitation was at 200 rpm. In contrast, the emulsifying activity of the biosurfactant, when subjected to moderate bioreactor agitation (100 rpm), was enhanced and produced more stable emulsions for all samples. The maximum emulsification activity was achieved after 4 days although not significantly different from those samples after 1, 2 and 3 days.

Biosurfactant characterization

Total sugar

Carbohydrates are important because they aid in decreasing the solubility of protein components to attain a stable emulsion. Being the major group of compounds, carbohydrates are also one of the main sources of nutrients essential in biosurfactant production as these are the base

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v6i1.1044

substrate in the formation of glycolipids, particularly in *Saccharomyces cerevisiae* 2013. The total sugar content of the biosurfactant was calculated as 17.51 ± 0.04 $mg.mL^{-1}$.

Protein content

Protein moiety is also an important factor to consider for the emulsion activity of biosurfactants (Cameroon et al., 1998). It is said that a synergistic relationship exists between the protein and glycolipid components; emulsification properties were due to the protein component while the stability of the emulsion is affected by the carbohydrate and lipid components. The protein content of the biosurfactant sample was computed as 6.970 ± 0.003 $mg.mL^{-1}$. In a study by Rufino et al. (2014), the biosurfactant of *Candida lipolytica* was characterized as an anionic lipopeptide composed of 50% protein, 20% lipids, and 8% of carbohydrates.

Surface tension reduction

The Pennzoil drop-collapse technique was used to qualitatively determine the ability of the biosurfactant to reduce surface tension. Results indicated that the biosurfactant produced was not effective in decreasing the surface tension, but it was effective as an emulsifying agent. This is supported by the findings of Cameron et al. (1998) wherein the mannoprotein biosurfactant produced by *S. cerevisiae* was an excellent emulsifier. Mannoprotein belongs to the high-molecular-weight biosurfactants, therefore, it is more efficient in emulsion stabilization.

CONCLUSION

Molasses concentration, coco paring meal concentration, and pH affect the biosurfactant production needed for high biomass concentration and % emulsification index. Molasses and coconut paring meal can serve as cheap alternative carbon and nitrogen sources, respectively. The production of biosurfactants by *Saccharomyces cerevisiae* 2031 using low-cost and locally-available base substrates, namely: molasses and coco paring meal extract were optimized at the following conditions: (4 days fermentation, pH 5.5, molasses concentration of 10.60 % $w \cdot v^{-1}$ and coconut paring meal extract concentration of 7.27% $w \cdot v^{-1}$). Yeast biosurfactants are considered to be non-toxic, biodegradable and eco-friendly but still, the cost of production is high. Scale-up experiments on the production of yeast biosurfactants using inexpensive substrates should be done to make them more economically feasible and compete with their chemical counterparts.

ACKNOWLEDGMENTS

The authors would like to thank T.C. Ilagan, V.F. Gomez, N.S. Panting and B. R. Collado for the technical assistance. This research was funded in parts by BIOTECH-Core Research fund and by the Department of Agriculture Biotechnology Program Implementation Unit.

REFERENCES

Akubude, V.C., Sule, S., Chinweuba, D.C. & Okafor, V.C. (2021). Chapter 5 – Application of biosurfactant in food industry. In: Microbially-Derived Biosurfactants for Improving Sustainability in Industry. *Green Sustainable Process for Chemical and Environmental Engineering and Science*. pp. 109- 125. https://doi.org/10.1016/B978-0-12-823380-1.00004-6

Alcantara, V.A., Pajares, I.G., Simbahan, J.F. & Rubio, M.L.D. (2012). Substrate dependent production and isolation of an extracellular biosurfactant from *Saccharomyces cerevisiae* 2031. *Philippine Journal of Science*, 141: 13-24.

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v6i1.1044

Almaral P.F.F., Coelho, M.A.Z., Marrucho, I.M.J. & Coutinho, J.A.P. (2010). Biosurfactants from yeasts: characteristics, production and application. *Advances in Experimental Medicine and Biology*, 672:236-49. Doi: 10.1007/978-1-4419-5979-9_18.

Bodour, A.A. & Miller-Maier, R.M. (1998). Application of a modified drop-collapse technique for surfactant quantitation and screening of biosurfactant-producing microorganisms. *Journal of Microbiology*, 32: 273-280.

Cameron, D.R., Cooper, D.G. & Neufeld, R.J. (1998). The mannoprotein of *Saccharomyces cerevisiae* is an effective emulsifier. *Applied and Environmental Microbiology*, 54: 1420-1425.

Chaprao, M.J., Ferreira, I.N.S., Correa, P.F., Rufino, R.D., Luna, J.M., Silva, E.J. & Sarubbo, L.A. (2015). Application of bacterial and yeast biosurfactants for enhanced removal and biodegradation of motor oil from contaminated sand. *Electronic Journal of Biotechnology*. 18(6):471-479. http://dx.doi.org/10.1016/j.ejbt.2015.09.005

Cooper, D.G. & Goldenberg, B.G. (1987). Surface-Active Agents from Two Bacillus Species. Appl Environ Microbiol 53: 224-229.

Cooper, D.G. & Paddock, D.A. (1984). Production of a Biosurfactant from *Torulopsis bombicola*. *Applied and Environmental Microbiology*, 47(1):173–176.

Edding, S.N. (2009). A multi-step recovery strategy for the isolation of a biosurfactant from *Saccharomyces cerevisiae* 2031. [BS thesis]. Ermita District, Manila: University of the Philippines, Manila. (Available at the University of the Philippines Manila Library)

Freitas, B.G., Brito, J.G.M., Brasileiro, P.P.F., Rufino, R.D., Luna, J.M., Santos, V.A. & Sarubbo, L.A.(2016). Formulation of a commercial biosurfactant for application as a dispersant of petroleum and by-products spilled in Oceans. *Frontiers in Microbiology*, 7. https://doi.org/10.3389/fmicb.2016.01646

Held, P. (2010). Monitoring the growth of beer brewing strains of *Saccharomyces cerevisiae*- The utility of synergy H1 for providing high-quality kinetic data for yeast growth applications. http://www.biotek.com

Jimoh, A.A. & Lin, J. (2019). Biosurfactant: A new frontier for greener technology and environmental sustainability. *Ecotoxicology and Environmental Safety* 184: 109607. https://doi.org/10.1016/j.ecoenv.2019.109607

Kumar, A., Singh, S.K. & Kant, C. (2021) Microbial biosurfactant: a new frontier for sustainable agriculture and pharmaceutical industries. *Antioxidants*, 10(9):1472. https://doi.org/10.3390/antiox10091472

Maneerat, S. (2005). Production of biosurfactants using substrates from renewable resources. Songklanakarin J Sci Technol 27: 675-683. http://www.asksilicone.com/pdfs/Biosurfactants.pdf

Patel, R.M. & Desai, A.J. (1997). Biosurfactant production by *Pseudomonas aeruginosa* GS3 from

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v6i1.1044

molasses. *Letters in Applied Microbiology*, 25: 91-94.

Perez-Guevara, F., Riba, J.P. & Strehaiano, P. (1994). Effect of yeast extract/sugar ratio on growth of *Schizosaccharomyces pombe*. *Bioresource Technology*, 48(1):13-15. https://doi.org/10.1016/0960-8524(94)90128-7

Ribeiro, B.G., Campos Guerra, J.M. & Sarubbo, L.A. (2022). Production of biosurfactant from *S. cerevisiae* and its application in salad dressing. *Biocatalysis and Agricultural Biotechnology*, 42: https://doi.org/10.1016/j.bcab.2022.102358

Ribeiro, B.G., Dos Santos, M., Da Silva, A.I., Meira, M.H., Campos Guerra, J.M. & Sarubbo, A.L. (2020). Study of the biosurfactant production by *Saccharomyces cerevisiae* URM 6670 using agro- industrial waste. *Chemical Engineering Transactions*, 79:61-66. https://doi.org/10.3303/CET2079011

Rocha e Silva, F.C.P., Roque, B.A.C., Rocha e Silva, N.M.P., Rufino, R.D., Luna, J.M., Santos, V.A., Banat, I.M. & Sarubbo, L.A. (2017). Yeasts and bacterial biosurfactants as demulsifiers for petroleum derivatives in seawater emulsions. *AMB Express.* 202. DOI 10.1186/s13568-017-0499-6

Rodrigues, L.R., Teixeira, J.A. & Oliveira, R. (2006). Low-cost fermentative medium for biosurfactant production by probiotic bacteria. *Biochemical Engineering Journal*, 32: 135-142. https://doi.org/10.1016/j.bej.2006.09.012

Rufino, R.D., De Luna, J.M., De Campos Takaki, G.M. & Sarubbo, L.A. (2014). Characterization and properties of the biosurfactant produced by *Candida lipolytica* UCP 0988. *Electronic Journal of Biotechnology*, 17(1):34-38. https://doi.org/10.1016/j.ejbt.2013.12.006

Sajna, K.V., Sukumaran, R.K., & Gottumukkala, L.D. (2015) Crude oil biodegradation aided by biosurfactants from *Pseudozyma* sp. NII 08165 or its culture broth. *Bioresource Technology*, 191:133–139.

Sharma, N., Lavania, M. & Lal, B. (2022) Biosurfactant: A next-generation tool for sustainable remediation of organic pollutants. *Frontiers in Microbiology*.12. https://doi.org/10.3389/fmicb.2021.821531

STAT- EASE INC. (2004). FAQ – Interpreting lack of fit. http://www.statease.com

Stein, H.H., Casas, G.A., Abella, J.J., Liu Y. Liu & Sulabo, R.C. (2015). Nutritional value of high fiber co-products from the copra, palm kernel, and rice industries in diets fed to pigs. *Journal of Animal Science and Biotechnology*. (2015) 6:56. DOI 10.1186/s40104-015-0056-6

Yabes, M.P. (2002). Isolation, screening, and characterization of yeasts for biosurfactant production using waste cooking oil as substrate. [MS thesis]. Los Baños, Laguna: University of the Philippines, Los Baños. (Available at the University of the Philippines Los Baños Library)