Malaysian Journal of Microbiology, Vol 18(2) 2022, pp. 215-221
DOI: http://dx.doi.org/10.21161/mjm.211272

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Comparison of nutrient-rich and limited media in the production of biosurfactant by *Achromobacter xylosoxidans* BP(1)5

Silvia Kurnia Sari[1,2], Ni'matuzahroh[1,2,3]*, Fatimah[1,2], Tri Nurhariyati[1,2], Nastiti Trikurniadewi[1,2], Ana Mariatul Khiftiyah[1,2], Achmad Zainal Abidin[1,2] and Khudrotun Nisa Indriyasari[1]

[1]Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia.
[2]University Center of Excellence - Research Center for Bio-Molecule Engineering, Universitas Airlangga, Surabaya, Indonesia.
[3]Faculty of Advanced Technology and Multidiscipline, Universitas Airlangga, Surabaya, Indonesia.
Email: nimatuzahroh@fst.unair.ac.id

Received 10 August 2021; Received in revised form 13 January 2022; Accepted 21 February 2022

ABSTRACT

Aims: This study aims to produce *Achromobacter* biosurfactant in nutrient-rich and nutrient-limited media.
Methodology and results: This study conducted fermentation on nutrient-rich and nutrient-limited media using a minimal salt medium (MSM). Dextrose and sodium citrate were used as sole carbon supplemented with 0.5% yeast extract for nutrient-rich media, while nutrient-limited media used molasses and rice straw hydrolysate (RSH) at variations of concentrations of 100 ppm and 200 ppm. The research was performed over 120 h and evaluated from growth response, surface tension and emulsification activity. The study revealed that the best surface tension value was when 2% (w/v) sodium citrate was used as C-source and 0.5% (w/v) yeast extract as N-source, after 72 h upon incubation at 30 °C/120 rpm having 45.45 ± 2.19 mN/m with emulsification activity 24.54 ± 3.42%. Whereas the best result of the nutrient-limited medium was obtained by RSH at a concentration of 200 ppm having 48.86 ± 5.36 mN/m.
Conclusion, significance and impact of study: The experiment showed that nutrient-limited medium from rice straw hydrolysate could compete with the nutrient-rich medium. The use of rice straw will contribute to the reduction of biosurfactant production costs and valorisation of agricultural waste.

Keywords: Achromobacter xylosoxidans, biosurfactant, agricultural and industrial wastes, green product

INTRODUCTION

Biosurfactant, which is a natural alternative to non-biodegradable and toxic synthetic surfactants, is produced mainly by microorganisms, including bacteria and has different types of surface-active properties such as reducing the surface tension between immiscible liquids or hydrocarbon mixtures (Marchant and Banat, 2012; Haloi and Medhi, 2019; Jahan *et al*., 2020). Research into biosurfactants has become more popular because of their low toxicity, high foaming ability, biodegradability, resistance to pH and salinity changes, and stability in a wide range of temperatures (Fakruddin, 2012; Ayed *et al*., 2014; Varjani and Upasani, 2017; Yaraguppi *et al*., 2020). There is, nevertheless, still a need for large-scale manufacturing of biosurfactant, despite its high price and limited production yield, due to its favorable feature (Nawawi, 2010). This challenge prompts different strategies such as improving potential strains, utilizing less valuable raw materials or waste substrates and optimizing environmental factors to reduce production costs (Makkar *et al*., 2011).

Concerning the problem stated, choosing an inexpensive raw material is a significant move to cut the overall expense as it accounts for half of the final production cost (Makkar and Cameotra, 1999). Generally, biosurfactants are produced by using petrochemical substrates (Henkel *et al*., 2015). In addition, some of the biosurfactant production today uses renewable substrates. To our knowledge, some examples of renewable substrates from agricultural and industrial wastes which have been studied for biosurfactant production are glycerol (Cruz *et al*., 2018), de-oiled seed cakes (Hazra *et al*., 2011), rice straw (Zhang *et al*., 2009; Panjiar *et al*., 2020), corn cob (Fatimah *et al*., 2020) and molasses (Rodrigues *et al*., 2017; Mouafo *et al*., 2018; Verma *et al*., 2020). Indonesia, a tropical country with a large agricultural area generates a lot of agricultural waste, including rice straw, a by-product of rice production (Ni'matuzahroh *et al*., 2020). Rice straw

*Corresponding author

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(2) 2022, pp. 215-221
DOI: http://dx.doi.org/10.21161/mjm.211272

consists of lignocelluloses that can be utilized for the fermentation of the biosurfactant-producing bacteria thus, it can be converted into the substrate. Additionally, agricultural waste was demonstrated in the same study to be a low-cost material for biosurfactants production. On the other hand, molasses, as a low value of a raw sugar by-product, has nutritional compositions that are valuable for fermentation (Makkar and Cameotra, 1997). Moreover, the amount of molasses and rice straw is abundant, and the use of these wastes can be beneficial for the environment.

The biosurfactant-producing *Achromobacter xylosoxidans* BP(1)5 that was isolated from petroleum sludge in this research is the same as that of the previous study due to its potential to produce biosurfactant compared to other isolates (Ni'matuzahroh *et al*., 2019). Several studies have revealed the ability of *Achromobacter* to produce biosurfactants (Deng *et al*., 2016; Haloi and Medhi, 2019; Joy *et al*., 2019; Deng *et al*., 2020; Li *et al*., 2020). However, almost all of them were grown in nutrient-rich media, a high-priced media. Additional research on the fermentation of the biosurfactant-producing *Achromobacter* as a promising strain in the low-cost nutrient-limited medium is necessary to address the cost-cutting barrier. The main objective of this study is to find out effective media used for biosurfactant production by *A. xylosoxidans* BP(1)5 utilizing agricultural hydrolysate sugar and industrial waste. Additionally, this study is intended to not only identify cost-effective biosurfactant production options but also maximize the use of agricultural and industrial wastes, hence limiting the amount of waste in the environment.

MATERIALS AND METHODS

Microorganism

The microorganism used in this present work was isolated from petroleum sludge obtained from Balongan refinery, West Java, Indonesia and was identified as *Achromobacter xylosoxidans* BP(1)5 (NCBI accession number MN. 401689), showing similarity with query cover 98% to *Achromobacter xylosoxidans* IPA-CC9.

Raw materials

Rice straw, agricultural residue was procured from a local farmer in East Java, Indonesia. Agricultural hydrolysate production was performed according to the method as described by Ni'matuzahroh *et al*. (2020). The agricultural residue was milled and sieved using 40-mesh to obtain average particle sizes ranging from 1-5 mm. The raw agricultural residue was subjected to pretreatment by chemical delignification using 1% (w/v) NaOH at 100 °C for 1 h. Following delignification, the biomass was rinsed with distilled water to a pH of 7.0 and oven-dried for 24 h at 60 °C before being utilized for enzymatic hydrolysis. The enzymatic hydrolysis was carried out using *Penicillium citrinum* H9 in Mandel and Stenberg's medium for 144 h. The concentration of agricultural hydrolysate was determined by Somogyi-Nelson's method (Nelson, 1944). Sugarcane molasses was obtained as a by-product of the sugar processing industry in East Java, Indonesia. All chemicals and reagents used in this work were analytical-grade.

Optimization of biosurfactant production

Biosurfactant production was aerobically performed in two type of media, nutrient-rich and nutrient-limited media. Minimal salt medium (MSM) pH 7.0 comprised of (g/L) $(NH_4)_2SO_4$ 3.0; NaCl 10; $MgSO_4 \cdot 7H_2O$ 0.2; $CaCl_2$ 0.01; $MnSO_4 \cdot H_2O$ 0.001; H_3BO_3 0.001; $ZnSO_4 \cdot 7H_2O$ 0.001; $CuSO_4 \cdot 5H_2O$ 0.001; $CoCl_2 \cdot 6H_2O$ 0.005 and $NaMoO_4 \cdot 2H_2O$ 0.001; KH_2PO_4 5.000; K_2HPO_4 2.6207 and Fe_3SO_4 0.0006 was used as basal medium for biosurfactant production.

Nutrient-rich media utilized 2.0% w/v sodium citrate and 3.0% w/v dextrose containing sugar content of 7395 ppm as the main carbon source and added 0.5% (w/v) yeast extract as a nitrogen source. Meanwhile, nutrient-limited media used rice straw hydrolysate (RSH) and molasses (7.5% v/v) at concentrations of 100 and 200 ppm as carbon source, without the addition of yeast extract. Nutrient broth (NB) and MSM media were employed as positive and negative controls, respectively. Overnight-grown inoculum of *A. xylosoxidans* BP(1)5 (2% v/v) in nutrient broth was utilized as inoculum for biosurfactant production. The culture was incubated at 30 °C in a shaker incubator at 120 rpm for 0, 24, 72 and 120 h. All of the experiments were performed in triplicates. The growth and biosurfactant production of *A. xylosoxidans* BP(1)5 in the different media were evaluated by measuring the optical density (OD) at 600 nm, surface tension by tensiometer and emulsification activity.

Surface tension measurement

The fermentation culture was centrifuged at 6000 rpm for 15 min to remove cell debris and insoluble substances. The surface tension of cell-free supernatant was determined using a tensiometer calibrated with distilled water. The measurement followed the Du-Nouy ring method at room temperature. A decrease in the surface tension of the supernatant against distilled water indicates the presence of biosurfactants in culture. All measurements were carried out three times then, the values were averaged to increase the accuracy of the measurements. The surface tension value was expressed in mN/m.

Emulsification activity

Emulsification activity was detected by adding 1 mL cell-free supernatant of the fermentation culture with the equal volume of the kerosene then it was vortexed for 2 min. The emulsification was kept to settle for 24 h and was calculated as the percentage of height (cm) of the

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(2) 2022, pp. 215-221
DOI: http://dx.doi.org/10.21161/mjm.211272

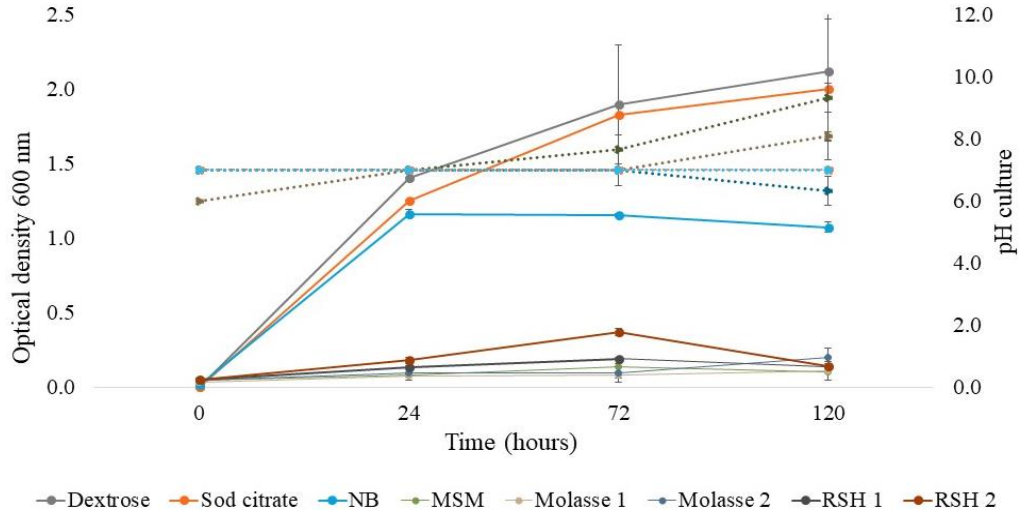

Figure 1: Effect of varying carbon sources in MSM on respond growth (straight lines) and pH culture of *Achromobacter xylosoxidans* BP(1)5 (dotted lines). Dextrose: Dextrose 3% (w/v), Sod citrate: Sodium citrate 2% (w/v), Molasse 1: Molasse 100 ppm, Molasse 2: Molasse 200 ppm, RSH 1: RSH 100 ppm, RSH 2: RSH 200 ppm, MSM: Control negative, NB: Control positive.

emulsion layer divided by the total height (cm) (Cooper and Goldenberg, 1987).

Statistical analysis

All of the data are expressed as mean ± standard deviation. Statistical analysis was carried out in each incubation time. Differences among the treatment groups were analysed using analysis of variance (ANOVA) SPSS version 25 (SPSS Inc., Chicago, IL, USA). *P*-value<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Growth response of *Achromobacter xylosoxidans* BP(1)5

Different hydrophilic carbon sources such as dextrose, sodium citrate, molasses and RSH were supplemented in MSM media to determine the best medium for biosurfactant production of *A. xylosoxidans* BP(1)5. As shown in Figure 1, *A. xylosoxidans* BP(1)5 is capable of growth in both nutrient-rich and nutrient-limited media. However, nutrient-rich media had a better growth response than nutrient-limited media. In that media, BP(1)5 showed they were the same growth trend. Moreover, a lag phase was observed at 24 h of incubation, followed by a stationary phase at 72 h.

The highest optical densities (2.12 ± 0.04 and 2.00 ± 0.09) were observed in a nutrient-rich medium culture when dextrose and sodium citrate were utilized as the sole carbon sources after 120 h, respectively. Meanwhile, the optical density of the limited-nutrient medium was very low (0.37 ± 0.02) in comparison to the optical density of the nutrient-rich medium due to the high dextrose content (3% w/v). This resulted from the culture's low carbon content, 200 ppm. Dextrose is a water-soluble simple carbon source that microorganisms may readily utilize for growth and has been found to enter the glycolytic pathway directly (Suh *et al.*, 2019).

The pH of the molasses and RSH substrate cultures remained constant during the incubation period (pH 7.0). Meanwhile, the pH of the dextrose substrate culture gradually decreased to 6.5 at 120 h. This may be due to the accumulation of organic acid secondary metabolites in the culture (Abouseoud *et al.*, 2008). Compared to other results, the pH culture that used sodium citrate as a carbon source rose gradually, around 7.6 at 72 h and 9.3 at 120 h.

Biosurfactant production

During the biosurfactant production of *A. xylosoxidans* BP(1)5, the surface tensions (ST) of the fermentation broth and emulsification activity (EA) against kerosene were evaluated and its result was shown Figure 2 and Figure 3. The highest reduction in surface tension was seen when isolate BP(1)5 was cultivated in a nutrient-rich medium culture with sodium citrate as the substrate (45.45 2.19 mN/m) for 72 h.

Many researchers revealed that genera *Achromobacter* synthesized biosurfactants during the stationary phase until the end of the stationary phase (Haloi and Medhi, 2019; Joy *et al*., 2019; Fatimah *et al*., 2020). In this study, *Achromobacter* showed a stationary phase at 72 h incubation. A similar result was reported by Subudhi *et al*. (2016) in which *Achromobacter xylosoxidans* strain TERI L1 also showed a stationary phase at 72 h incubation. Deng *et al.* (2016) also reported that *Achromobacter* sp. HZ01 was able to reduce the surface tension to a minimum value of 24.20 mN/m at 72 h of incubation.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(2) 2022, pp. 215-221
DOI: http://dx.doi.org/10.21161/mjm.211272

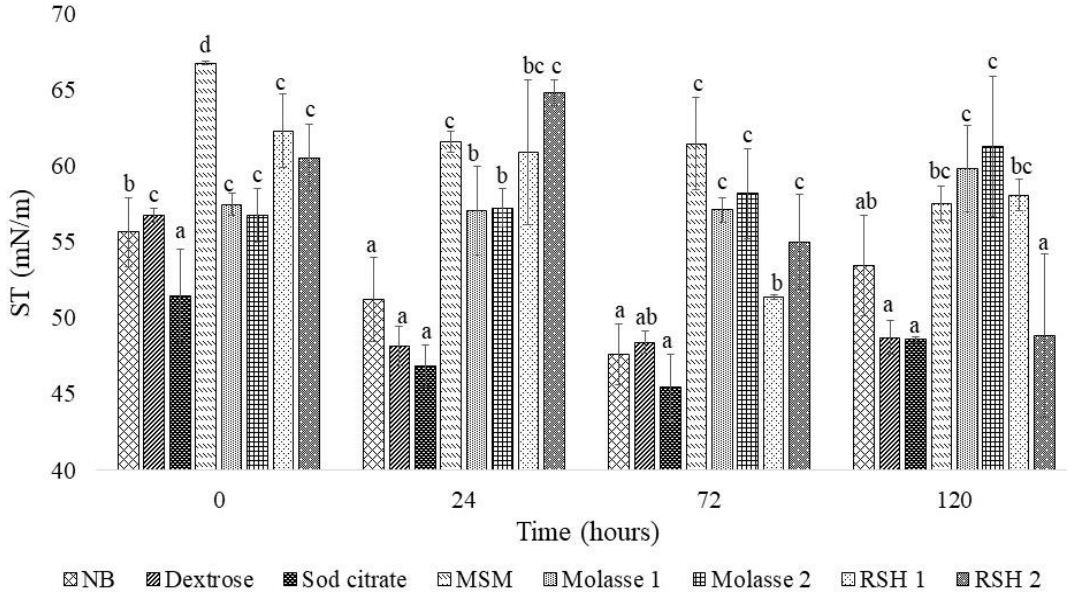

Figure 2: Surface tension of biosurfactant production in the MSM supplemented with different carbon sources. Dextrose: Dextrose 3% (w/v), Sod citrate: Sodium citrate 2% (w/v), Molasse 1: Molasse 100 ppm, Molasse 2: Molasse 200 ppm, RSH 1: RSH 100 ppm, RSH 2: RSH 200 ppm, MSM: Control negative, NB: Control positive.

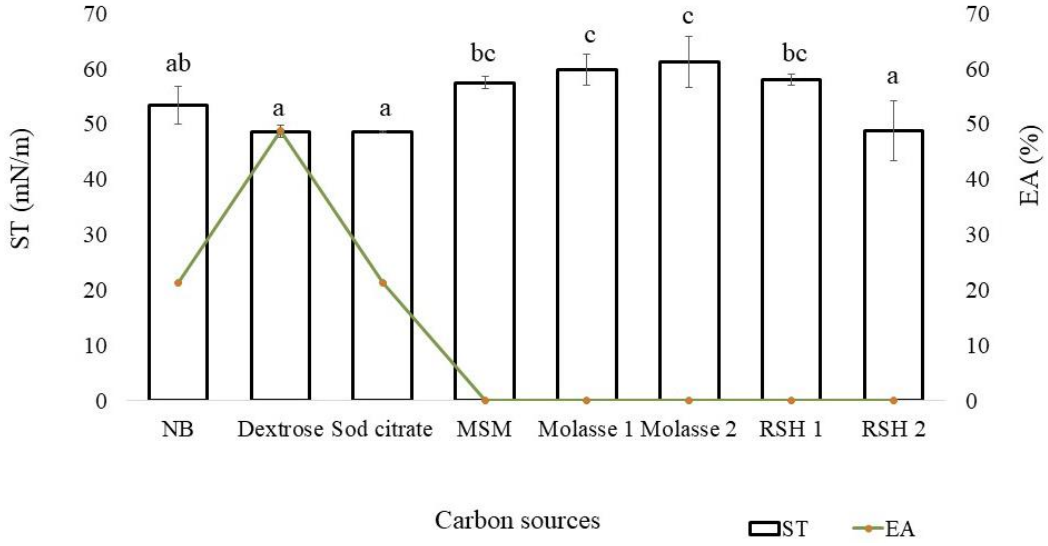

Figure 3: Surface tension (ST) and emulsification activity (EA) of biosurfactant production in the MSM supplemented with different carbon sources at 120 h incubation. Dextrose: Dextrose 3% (w/v), Sod citrate: Sodium citrate 2% (w/v), Molasse 1: Molasse 100 ppm, Molasse 2: Molasse 200 ppm, RSH 1: RSH 100 ppm, RSH 2: RSH 200 ppm, MSM: Control negative, NB: Control positive.

As seen in Figure 3, the surface tension of free cell supernatant at 120 h of incubation on a nutrient-rich and nutrient-limited medium (RSH 200 ppm) was found to be inconsequential. The surface tension values of the free cell supernatant which used dextrose and sodium citrate as substrates in nutrient-rich media were 48.73 ± 1.12 and 48.59 ± 0.20, respectively. Whereas, the surface tension value in the nutrient-limited medium was 48.86 ± 5.36, with 200 ppm RSH as substrate. Previous studies have reported the ability of *A. xylosoxidans* BP(1)5 to produce biosurfactants (ST 58.8 mN/m, AE 27.22%) in rice straw hydrolysis (RSH), but it requires a high carbon source, 600 ppm (Ni'matuzahroh *et al*., 2020). Meanwhile, even though no emulsification activity was observed in this study, 200 ppm RSH was able to generate a surface tension of 48.86 (Figure 3). The decrease of the result in

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

surface tension was better than positive control in nutrient broth media and negative control. Generally, the surface tension value of *Achromobacter* biosurfactant ranges around 56.20–25.00 mN/m (Deng *et al*., 2016; Haloi and Medhi, 2019; Li *et al*., 2020; Ni'matuzahroh *et al*., 2020).

At the same time, emulsification activity was only observed in nutrient-rich media and the positive control culture. This was due to the medium's composition, which affects the type and activity of biosurfactants, particularly the range of carbon sources employed in biosurfactant production (Miguez *et al*., 1986; Sen, 1997; Panjiar *et al*., 2020). Biosurfactants are not always capable of emulsification. Some biosurfactants are capable of reducing surface tension and forming an emulsion, whereas others can only reduce surface tension or produce an emulsion. Emulsan and liposan are biosurfactants that have a limited capacity to reduce surface tension but create stable emulsions when combined with vegetable oil (Cirigliano and Carman, 1985). In contrast, *Torulopsis bombicola* produces sophorolipids which have been shown to reduce surface tension but cannot form an emulsion properly (Fracchia *et al*., 2015).

However, both growth response and biosurfactant production indicated better results in nutrient-rich media. The high response of growth and biosurfactant production was due to the high carbon source in this medium. Joy *et al*. (2019) revealed that substrate concentration greatly influenced the growth and biosurfactant production of *Achromobacter insolitus* (PS1), even an increase in dextrose concentration of 1-4% increased the biosurfactant productions. A similar observation was also reported by Silva *et al*. (2010); they observed 3% glycerol concentration showed the best result in terms of surface tension and surfactant yield compared to the 2% glycerol concentration.

In addition, nutrient-rich media was supplemented with an organic nitrogen source, yeast extract. It is known that yeast extract is an inducer for the production of biosurfactants (Deng *et al*., 2016; Singh and Tiwary, 2016). Yeast extract contains free amino acids, nucleic acids, protein, carbon source and some essential trace elements (Nakajo and Sano, 2002). The composition of yeast extracts, particularly those containing amine groups, will have an impact on the production of peptide-containing biosurfactants or the enzymes involved in the biosynthesis of biosurfactants, either directly or indirectly (Qazi *et al*., 2013). Yeast extract supplementation has been shown to increase biosurfactant production compared to a single inorganic nitrogen source (Deng *et al*., 2016; Haloi and Medhi, 2019).

The results in Figure 3 suggest that a nutrient-limited medium derived from rice straw hydrolysate was capable of competing with a nutrient-rich medium and so represent prospective media options for enhancing biosurfactant production for a variety of applications. Several studies have also reported that *Achromobacter* biosurfactant is widely used in microbial enhanced oil recovery (MEOR) and bioremediation of petroleum (Deng *et al*., 2020), biodegradation and detoxification of endosulfan insecticides (Li *et al*., 2009) and bioremediation of polycyclic aromatic hydrocarbons (PAHs) (Dave *et al*., 2014).

There is currently a dearth of research on *Achromobacter*'s production of biosurfactants in a nutrient-limited medium that depend largely on agricultural waste and industrial by-products as the primary carbon source. Consequently, further research is needed to optimise the biosurfactant production of *Achromobacter* in low-cost and eco-friendly substrates.

CONCLUSION

In conclusion, the result of this study indicates that conventional, costly nutrient-rich medium can be substituted with nutrient-limited medium from agricultural waste hydrolysate and could be potential candidates in biosurfactant production. The use of agricultural waste and the industrial by-product will contribute to the reduction of biosurfactant production costs and valorisation of organic waste.

ACKNOWLEDGEMENTS

This work was financially supported by "Direktorat Riset dan Pengabdian Masyarakat, Deputi Bidang Penguatan Riset dan Pengembangan Kementerian Riset dan Teknologi/ Badan Riset dan Inovasi Nasional" through "Penelitian Pendidikan Magister Menuju Doktor Sarjana Unggul" Universitas Airlangga scheme 1208/UN3.14/PT/2020.

REFERENCES

Abouseoud, M., Maachi, R., Amrane, A., Boudergua, S. and Nabi, A. (2008). Evaluation of different carbon and nitrogen sources in production of biosurfactant by *Pseudomonas fluorescens*. *Desalination* **223(1-3), 143-151.**

Ayed, H. B., Jridi, M., Maalej, H., Nasri, M. and Hmidet, N. (2014). Characterization and stability of biosurfactant produced by *Bacillus mojavensis* A21 and its application in enhancing solubility of hydrocarbon. *Journal of Chemical Technology and Biotechnology* **89(7), 1007-1014.**

Cirigliano, M. C. and Carman, G. M. (1985). Purification and characterization of liposan, a bioemulsifier from *Candida lipolytica*. *Applied and Environmental Microbiology* **50(4), 846-850.**

Cooper, D. G. and Goldenberg, B. G. (1987). Surface-active agents from two *Bacillus* species. *Applied and Environmental Microbiology* **53(2), 224-229.**

Cruz, J. M., Hughes, C., Quilty, B., Montagnolli, R. N. and Bidoia, E. D. (2018). Agricultural feedstock supplemented with manganese for biosurfactant production by *Bacillus subtilis*. *Waste and Biomass Valorization* **9(4), 613-618.**

Dave, B. P., Ghevariya, C. M., Bhatt, J. K., Dudhagara, D. R. and Rajpara, R. K. (2014). Enhanced biodegradation of total polycyclic aromatic

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

hydrocarbons (TPAHs) by marine halotolerant *Achromobacter xylosoxidans* using Triton X-100 and β-cyclodextrin – A microcosm approach. *Marine Pollution Bulletin* **79(1-2), 123-129.**

Deng, M. C., Li, J., Hong, Y. H., Xu, X. M., Chen, W. X., Yuan, J. P., Peng, J., Yi, M. and Wang, J. H. (2016). Characterization of a novel biosurfactant produced by marine hydrocarbon-degrading bacterium *Achromobacter* sp. HZ01. *Journal of Applied Microbiology* **120(4), 889-899.**

Deng, Z., Jiang, Y., Chen, K., Li, J., Zheng, C., Gao, F. and Liu, X. (2020). One biosurfactant-producing bacteria *Achromobacter* sp. A-8 and its potential use in microbial enhanced oil recovery and bioremediation. *Frontiers in Microbiology* **11, 247.**

Fakruddin, M. (2012). Biosurfactant: Production and application. *Journal of Petroleum and Environmental Biotechnology* **3(4), 124.**

Fatimah., Sari, S. K., Trikurniadewi, N., Ibrahim, S. N. M. M., Khiftiyah, A. M., Indriyasari, K. N., Nurhariyati, T., Surtiningsih, T., Yuliani, H. and Ni'matuzahroh. (2020). Corn cob hydrolyzate from *Penicillium citrinum* H9 as an alternative substrate for biosurfactant production by hydrocarbonoclastic bacteria. *Ecology Environment, and Conservation* **26, 169-175.**

Fracchia, L., Ceresa, C., Franzetti, A., Cavallo, M., Gandolfi, I., Van Hamme, J., Gkorezis, P., Marchant, R. and Banat, I. M. (2015). Industrial applications of biosurfactants. *In*: Biosurfactants: Production and Utilization-Processes, Technologies, and Economics. Kosaric, N. and Sukan, F. V. (eds.). CRC Press, Boca Raton. **pp 245-260.**

Haloi, S. and Medhi, T. (2019). Optimization and characterization of a glycolipid produced by *Achromobacter* sp. to use in petroleum industries. *Journal of Basic Microbiology* **59(3), 238-248.**

Hazra, C., Kundu, D., Ghosh, P., Joshi, S., Dandi, N. and Chaudhari, A. (2011). Screening and identification of *Pseudomonas aeruginosa* AB4 for improved production, characterization and application of a glycolipid biosurfactant using low-cost agro-based raw materials. *Journal of Chemical Technology and Biotechnology* **86(2), 185-198.**

Henkel, M., Syldatk, C. and Hausmann, R. (2015). The prospects for the production of rhamnolipid on renewable resources evaluation of novel feedstocks and perspectives of strain engineering. *In*: Biosurfactants: Production and Utilisation-Processes, Technologies, and Economics. Kosaric, N. and Sukan, F. V. (eds.). CRC Press, Boca Raton. **pp 83-100.**

Jahan, R., Bodratti, A. M., Tsianou, M. and Alexandridis, P. (2020). Biosurfactants, natural alternatives to synthetic surfactants: Physicochemical properties and applications. *Advances in Colloid and Interface Science* **275, 102061.**

Joy, S., Rahman, P. K., Khare, S. K. and Sharma, S. (2019). Production and characterization of glycolipid biosurfactant from *Achromobacter* sp. (PS1) isolate using one-factor-at-a-time (OFAT) approach with feasible utilisation of ammonia-soaked lignocellulosic pretreated residues. *Bioprocess and Biosystems Engineering* **42(8), 1301-1315.**

Li, J., Wang, Y., Zhou, W., Chen, W., Deng, M. and Zhou, S. (2020). Characterization of a new biosurfactant produced by an effective pyrene-degrading *Achromobacter* species strain AC15. *International Biodeterioration and Biodegradation* **152, 104959.**

Li, W., Dai, Y., Xue, B., Li, Y., Peng, X., Zhang, J. and Yan, Y. (2009). Biodegradation and detoxification of endosulfan in aqueous medium and soil by *Achromobacter xylosoxidans* strain CS5. *Journal of Hazardous Materials* **167(1-3), 209-216.**

Makkar, R. S. and Cameotra, S. S. (1997). Utilization of molasses for biosurfactant production by two *Bacillus* strains at thermophilic conditions. *Journal of the American Oil Chemists' Society* **74(7), 887-889.**

Makkar, R. S. and Cameotra, S. S. (1999). Biosurfactant production by microorganisms on unconventional carbon sources. *Journal of Surfactants and Detergents* **2(2), 237-241.**

Makkar, R. S., Cameotra, S. S. and Banat, I. M. (2011). Advances in utilization of renewable substrates for biosurfactant production. *AMB Express* **1(1), 5.**

Marchant, R. and Banat, I. M. (2012). Biosurfactants: A sustainable replacement for chemical surfactants? *Biotechnology Letters* **34(9), 1597-1605.**

Miguez, C. B., Beveridge, T. J. and Ingram, J. M. (1986). Lipopolysaccharide changes and cytoplasmic polyphosphate granule accumulation in *Pseudomonas aeruginosa* during growth on hexadecane. *Canadian Journal of Microbiology* **32(3), 248-253.**

Mouafo, T. H., Mbawala, A. and Ndjouenkeu, R. (2018). Effect of different carbon sources on biosurfactants' production by three strains of *Lactobacillus* spp. *BioMed Research International* **2018, Article ID 5034783.**

Nakajo, Y. and Sano, H. (2002). U.S. Patent No. 6,344,231. U.S. Patent and Trademark Office, Washington, DC.

Nawawi, W. M. F. W., Jamal, P. and Alam, M. Z. (2010). Utilization of sludge palm oil as a novel substrate for biosurfactant production. *Bioresource Technology* **101(23), 9241-9247.**

Nelson, N. (1944). A photometric adaptation of the Somogyi method for the determination of glucose. *Journal of Biological Chemistry* **153(2), 375-380.**

Ni'matuzahroh, Sari, S. K., Ningrum, I. P., Pusfita, A. D., Marjayandari, L., Trikurniadewi, N., Ibrahim, S. N. M. M., Fatimah., Nurhariyati, T., Surtiningsih, T. and Yuliani, H. (2019). The potential of indigenous bacteria from oil sludge for biosurfactant production using hydrolysate of agricultural waste. *Biodiversitas Journal of Biological Diversity* **20(5), 1374-1379.**

Ni'matuzahroh, Sari, S. K., Trikurniadewi, N., Ibrahim, S. N. M. M., Khiftiyah, A. M., Abidin, A. Z., Nurhariyati, T. and Fatimah (2020). Bioconversion of agricultural waste hydrolysate from lignocellulolytic mold into biosurfactant by *Achromobacter* sp. BP(1)5.

Biocatalysis and Agricultural Biotechnology **24, 101534.**

Panjiar, N., Mattam, A. J., Jose, S., Gandham, S. and Velankar, H. R. (2020). Valorization of xylose-rich hydrolysate from rice straw, an agroresidue, through biosurfactant production by the soil bacterium *Serratia nematodiphila*. *Science of The Total Environment* **729, 138933.**

Qazi, M. A., Malik, Z. A., Qureshi, G. D., Hameed, A. and Ahmed, S. (2013). Yeast extract as the most preferable substrate for optimised biosurfactant production by *rhl*B gene positive *Pseudomonas putida* SOL-10 isolate. *Journal of Bioremediation and Biodegradation* **4, 204.**

Rodrigues, M. S., Moreira, F. S., Cardoso, V. L. and de Resende, M. M. (2017). Soy molasses as a fermentation substrate for the production of biosurfactant using *Pseudomonas aeruginosa* ATCC 10145. *Environmental Science and Pollution Research* **24(22), 18699-18709.**

Sen, R. (1997). Response surface optimization of the critical media components for the production of surfactin. *Journal of Chemical Technology and Biotechnology* **68(3), 263-270.**

Silva, S. N. R. L., Farias, C. B. B., Rufino, R. D., Luna, J. M. and Sarubbo, L. A. (2010). Glycerol as substrate for the production of biosurfactant by *Pseudomonas aeruginosa* UCP0992. *Colloids and Surfaces B: Biointerfaces* **79(1), 174-183.**

Singh, P. and Tiwary, B. N. (2016). Isolation and characterization of glycolipid biosurfactant produced by a *Pseudomonas otitidis* strain isolated from Chirimiri coal mines, India. *Bioresources and Bioprocessing* **3(1), 42.**

Subudhi, S., Bisht, V., Batta, N., Pathak, M., Devi, A. and Lal, B. (2016). Purification and characterization of exopolysaccharide bioflocculant produced by heavy metal resistant *Achromobacter xylosoxidans*. *Carbohydrate Polymers* **137, 441-451.**

Suh, S. J., Invally, K. and Ju, L. K. (2019). Rhamnolipids: Pathways, productivities, and potential. *In*: Biobased Surfactants: Synthesis, Properties, and Applications. Hayes, D. G., Solaiman, D. K. Y. and Ashby, R. D. (eds.). Academic Press and AOCS Press, United States. **pp. 169-203.**

Varjani, S. J. and Upasani, V. N. (2017). Critical review on biosurfactant analysis, purification and characterization using rhamnolipid as a model biosurfactant. *Bioresource Technology* **232, 389-397.**

Verma, R., Sharma, S., Kundu, L. M. and Pandey, L. M. (2020). Experimental investigation of molasses as a sole nutrient for the production of an alternative metabolite biosurfactant. *Journal of Water Process Engineering* **38, 101632.**

Yaraguppi, D. A., Bagewadi, Z. K., Muddapur, U. M. and Mulla, S. I. (2020). Response surface methodology-based optimization of biosurfactant production from isolated *Bacillus aryabhattai* strain ZDY2. *Journal of Petroleum Exploration and Production Technology* **10(6), 2483-2499.**

Zhang, Q., He, G., Wang, J., Cai, W. and Xu, Y. (2009). Mechanisms of the stimulatory effects of rhamnolipid biosurfactant on rice straw hydrolysis. *Applied Energy* **86, S233-S237.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538