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Utilization of Agricultural Waste Substrates with Inorganic Salt Medium as Nutrient Enhancer for Biosurfactant Production by MEOR-Oriented Bacterial Consortia

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Abstract

The decline in oil recovery during secondary processes has led to the exploration of tertiary methods such as Microbial Enhanced Oil Recovery (MEOR). This study investigates biosurfactant production by *Pseudomonas aeruginosa* and *Bacillus subtilis* in a co-culture system, optimizing fermentation media and evaluating effectiveness in oil recovery. Seven media were tested based on optical density (OD_{600}), emulsification index (E24), oil displacement area (ODA), and yield of wet crude biosurfactant, observed over five days. The highest E24 value, 89.66% was recorded on day two using used lubricant as the water-immiscible phase. The best ODA values were 44.49 cm² for supernatant (ODA SNT) and 55.86 cm² for wet crude biosurfactant (ODA CBS). The optimized production medium contained 2% (v/v) waste cooking oil, 1% (w/v) molasses, and 1% (v/v) tofu wastewater. Partial purification of the culture broth through acid precipitation and solvent extraction yielded the highest wet crude biosurfactant on day three, reaching 155 g/L. The co-culture system at a 1:1 P/B ratio effectively reduced oil-water interfacial tension, as demonstrated by the high ODA values, particularly in the purified crude biosurfactant. This highlights the eco-friendly potential of biosurfactants for MEOR applications.

Keywords: Bacillus subtilis**,** biosurfactant, fermentation, MEOR, pseudomonas aeruginosa

Abstrak

Penurunan perolehan minyak selama proses sekunder mendorong eksplorasi metode tersier seperti Microbial Enhanced Oil Recovery (MEOR). Studi ini mengevaluasi produksi biosurfaktan oleh Pseudomonas aeruginosa *dan* Bacillus subtilis *dalam sistem ko-kultur, dengan mengoptimalkan media fermentasi serta mengkaji efektivitasnya dalam perolehan minyak. Tujuh jenis media diuji berdasarkan nilai Kerapatan Optik (Optical Density, OD_{600}), Indeks Emulsifikasi (E24), Luas Penyebaran Minyak (oil displacement area, ODA), dan hasil biosurfaktan kasar basah, yang diamati selama lima hari. Nilai E24 tertinggi sebesar 89,66% diperoleh pada hari kedua dengan menggunakan pelumas bekas sebagai fase yang tidak tercampur dengan air. Nilai ODA terbaik diperoleh sebesar 44,49 cm² untuk supernatan (ODA SNT) dan 55,86 cm² untuk biosurfaktan kasar basah (ODA CBS). Media optimasi produksi terdiri dari 2% (v/v) minyak jelantah, 1% (w/v) molase, dan 1% (v/v) air limbah tahu. Proses purifikasi parsial terhadap kaldu fermentasi melalui presipitasi asam dan ekstraksi pelarut menghasilkan biosurfaktan kasar basah tertinggi pada hari ketiga, yaitu sebesar 155 g/L. Sistem ko-kultur pada rasio P/B 1:1 terbukti efektif dalam menurunkan tegangan antarmuka minyak-air, yang ditunjukkan oleh tingginya nilai ODA, terutama pada biosurfaktan kasar yang telah dimurnikan. Temuan ini menyoroti potensi ramah lingkungan biosurfaktan untuk aplikasi MEOR.*

Kata kunci: *Bacillus subtilis**,** biosurfactant, fermentasi, MEOR,* pseudomonas aeruginosa

I. INTRODUCTION

Indonesia continues to face environmental and waste management challenges due to the increasing generation of agro-industrial waste, particularly in urban and semi-urban areas such as Bandung. Three types of waste-waste cooking oil (WCO), tofu wastewater (TW), and molasses are abundant by products from households, small food enterprises, and the sugar industry, respectively. Despite their high organic content and potential economic value, these wastes are

often improperly disposed of, leading to environmental degradation such as clogged drainage, water pollution, and foul odors. In particular, Bandung, as a growing metropolitan hub in West Java, generates significant volumes of WCO and TW due to the city's dense population and high consumption of fried foods and tofu products. These waste streams, however, can be considered untapped natural resources with high potential for value-added applications such as biosurfactant production.

Tofu, a popular source of plant-based protein in Indonesia, is widely produced across Java Island by small-scale industries. According to recent reports, there are more than 84,000 small tofu producers across the country, consuming over 2.5 million tons of soybeans annually. These industries generate around 20 million m^3 of liquid waste and 1 million tons of solid waste per year, with the majority discharged directly into local waterways (Nanda & Yurike, 2019). Inadequate waste treatment has caused environmental issues, urging regulatory bodies to implement stricter controls. The latest regulation, Minister of Environment and Forestry Regulation No. 2 of 2023**,** outlines updated wastewater quality standards for industrial sanitation and mandates improved management practices to prevent aquatic and atmospheric pollution in surrounding communities.

Rather than viewing these wastes solely as pollutants, they can be valorized as substrates for microbial biosurfactant production. Biosurfactants are amphiphilic compounds made by certain bacteria that can lower the tension between oil and water. They have gained significant attention due to their biodegradability, low toxicity, and effectiveness under extreme environmental conditions. Utilizing WCO (Awaludin & Sari, 2017) (Haba et al., 2000), TW, and molasses (Patel & Desai, 1997) as carbon sources offers a sustainable and cost-effective approach for biosurfactant synthesis, especially in countries like Indonesia where these resources are readily available and inexpensive. Furthermore, the integration of waste valorization into microbial processes contributes to circular economy principles by converting low-value residues into high-value bioproducts.

This study explores the use of a bacterial consortium consisting of *Pseudomonas aeruginosa* and *Bacillus subtilis* for biosurfactant production using a combination of WCO, TW, and molasses as mixed substrates. These bacteria are known for producing potent biosurfactants such as rhamnolipids and surfactin, which play essential roles in emulsification and hydrocarbon degradation (Wu B, Xiu J, Yu L, Huang L, Yi L, and Ma Y., 2023). Initial screening of various fermentation media will be conducted based on optical density (OD_{600}), emulsification index (E24), and oil displacement area (ODA) to identify optimal conditions for biosurfactant production. The process involves stepwise inoculation and co-culture in a pre-production

medium, followed by biosurfactant extraction using acid precipitation and solvent extraction, purification via rotary evaporation, and drying (Mardiah et al., 2022).

Although the application is currently limited to the laboratory scale, the long-term vision is to commercialize the process for industrial implementation, particularly in Microbial Enhanced Oil Recovery (MEOR) in oil and gas operations. As global petroleum recovery becomes more technically and economically challenging, the use of environmentally friendly biosurfactants offers a promising alternative to synthetic surfactants, with the added benefit of supporting waste reduction and sustainability in local communities.

II. METHODOLOGY

Microorganisms

The microorganisms used to produce biosurfactants, namely *Pseudomonas aeruginosa* bacterial isolates with strain code FNCC-0063, are gram-negative bacteria and will be co-cultured with *Bacillus subtilis* bacterial isolates with strain code FNCC-0059, which are gram-positive bacteria. Both bacteria have been grown on nutrient agar (NA) media before being used as starters.

Media Formulation and Growth Condition

The composition of the starter culture media used is as follows: Luria-Bertani (LB) (g/L) contains yeast (5.0), tryptone (10.0), and NaCl (5.0), pH adjusted to 7.0, then sterilized by autoclave at a temperature of 121ºC for 15 minutes. Next, take 1-2 ose cultures of pure bacterial isolates to inoculate into LB media and incubate at a temperature of 37ºC, 140 rpm until an optical density value (OD_{600}) of 0.8-1 is obtained. Then, the starter culture is stored as a stock in 15% glycerol (v/v) by transferring the glycerol solution and the starter culture into a microtube with a ratio (µl) of 150:850 and stored in a chiller at a temperature of -20ºC to maintain the viability of the cells.

There are seven modified media used in this study, including. (M1)(g/L): Molasses (10.0), NH_4NO_3 (4.0), KH_2PO_4 (2.0), Na_2HPO_4 (1.5), $MgSO_4.7H_2O$ (0.5), and $CaCl_2$ (0.25), as well as the addition of trace element solution sterile (L^{-1}) (CA syringe filters 0.22 µm) consisting of (g/L): $FeCl_3.6H_2O$ (0.5), $ZnSO_4.7H_2O$ (0.3), $CuSO_4.5H_2O$ (0.2), $MnSO_4.H_2O$ (0.5), K_2HPO_4 (0.5), $Na_2MoO_4.2H_2O$ (0.4), H_3BO_3 (1.0) as much as 1 ml. (M2)(g/L): M1 with the addition of waste cooking oil (WCO) substrate (2%, v/v). (M3)(g/L): Molasses (0.66%, v/v), NH_4NO_3 (1.0), KH_2PO_4 (2.0), Na_2HPO_4 (1.5), $MgSO_4.7H_2O$ (0.5), $CaCl_2$ (0.25), yeast (2.0), trace element solution (1ml/L). (M4)(g/L): Molasses (0.66%, v/v), NH_4NO_3 (1.0), KH_2PO_4 (2.0), Na_2HPO_4 (1.5), $MgSO_4.7H_2O$ (0.5), $CaCl_2$ (0.25), yeast (2.0), trace element solution (1ml/L), NaCl (0.3%, v/v). (M5)(g/L): Molasses (0.66%, v/v), NH_4NO_3 (1.0), KH_2PO_4 (2.0), Na_2HPO_4 (1.5), $MgSO_4.7H_2O$ (0.5), $CaCl_2$ (0.25); yeast (2.0), trace element solution (1ml/L), NaCl (0.3%, v/v),

inducer WCO (2%, v/v). (M6)(g/L): Molasses (0.3%, v/v), tofu wastewater (1%, v/v), KH_2PO_4 (2.0), Na_2HPO_4 (1.5), $MgSO_4.7H_2O$ (0.5), $CaCl_2$ (0.25), yeast (2.0), trace element solution (1ml/L), NaCl (0.3%, v/v), inducer WCO (2%, v/v). (M7)(g/L): Molasses (0.3%, v/v), tofu wastewater (1%, v/v), yeast (2.0), inducer WCO (2%, v/v).

The modified media above was adjusted to pH 7.0 using HCl 1N and NaOH 1N before being sterilized with an autoclave at 121°C for 15 minutes.

Cells grown in LB media are used as inoculum (2%, v/v). Growth was carried out in a test tube containing 5 ml of modified media, then incubated at 37ºC, 140 rpm for 48 hours to standardize the treatment on the seven media, because in the M5-M7 medium after 24 hours, the addition of WCO inducers was added. Every 24 hours, a sample of 1 ml is taken for OD_{600} testing. At the 48th hour, the cell suspension is centrifuged (3500 rpm, 30 minutes, 4°C) to separate the biomass from its supernate, and the cell-free supernatant is subsequently used in the emulsification index (E24) and oil displacement area tests.

Determination of Emulsification Index (E24)

Biosurfactants produced in the co-culture system of *P. aeruginosa* and *B. subtilis* were used, emulsification testing was carried out by adding 2 ml of used lubricant as an emulsifier to the water phase consisting of 2 ml of cell-free supernatant, then mixing in the vortex at high speed for 2 minutes and leaving at room temperature (Câmara et al., 2019). After 24 hours, the value of E24 can be determined by the following equation.

$$E24(\%) = \frac{Emulsion\ Height\ (cm)}{Total\ Height\ Liquid\ (cm)} x\ 100 \quad \text{.....(1)}$$

Acclimatization of Microorganisms

The inoculum of *P. aeruginosa* and *B. subtilis* used in this study was prepared through the process of acclimatization of microorganisms, followed by the simultaneous inoculation stage of pre-production media. This stage aims to improve the viability of the cell and maximize the adaptability of microbes to the conditions of the medium different from the initial growth medium (starter). 2%, v/v starter cultures of *P. aeruginosa* and *B. subtilis* were grown on different inoculum media, respectively. *P. aeruginosa* inoculum media (g/L): NH_4NO_3 (1.0), KH_2PO_4 (2.0), Na_2HPO_4 (1.5), $MgSO_4.7H_2O$ (0.5), $CaCl_2$ (0.25), yeast (0.5), trace element solution (1ml/L). The starter culture of *P. aeruginosa* was inoculated into the inoculum medium, then incubated (37°C, 160 rpm) until it enters the exponential phase. *B. subtilis* inoculum media (g/L): NH_4NO_3 (1.0), KH_2PO_4 (2.0), Na_2HPO_4 (1.5), $MgSO_4.7H_2O$ (0.5), $CaCl_2$ (0.25), yeast (0.5), sucrose (10.0), tofu wastewater (1%, v/v), trace element solution (1ml/L). The starter culture of *B. subtilis* is inoculated into the inoculum medium, then incubated (37°C, 160 rpm) until it enters the exponential phase.

This process allows each microorganism to adapt to a medium that corresponds to its characteristics so that at the consortium stage, one of the microorganisms does not dominate but can synergize to produce biosurfactants.

Pre-Production of Biosurfactants

The pre-production stage uses M2 media, where this medium produces the highest E24 value and oil displacement area (cm^2), among other modified media. A total of 2%, v/v inoculum with a ratio of 1:1 *P. aeruginosa* and *B. subtilis* was inoculated into M2 medium (50 ml), followed by fermentation (37°C, 160 rpm, 5 days). Every day, fermentation cell suspension samples are taken to test the values of OD_{600}, E24, oil displacement area supernatant, and oil displacement wet crude biosurfactant.

Partial Purification of Biosurfactants

The pH of the supernatant liquid obtained after centrifugated cell transfer (3500 rpm, 30 min, 4°C) was adjusted to 2.0 using HCl 6N and left for 24 hours at 4°C (Patel & Desai, 1997); the precipitation formed after centrifugal acidification (10000 rpm, 20 min, 4°C) to separate the biosurfactant from the residual liquid. Biosurfactant deposits were further extracted in a 100 mL Erlenmeyer using a mixture of chloroform:methanol (2:1, v/v) and incubated (100 rpm, 37°C, 30 min) (Sari et al., 2020) (Goyal & Singh, 2022). Next, the solution is separated using a separatory funnel until two layers are formed. This process is carried out in two stages. The organic phase at the bottom is carried out by evaporating solvents using a water bath at a temperature of 60°C (Mardiah et al., 2022) until crude biosurfactant is obtained. The crude biosurfactant is further dried by oven at 60°C until a foamy clear layer is obtained, indicating that the biosurfactant has been successfully extracted in Figure 1.

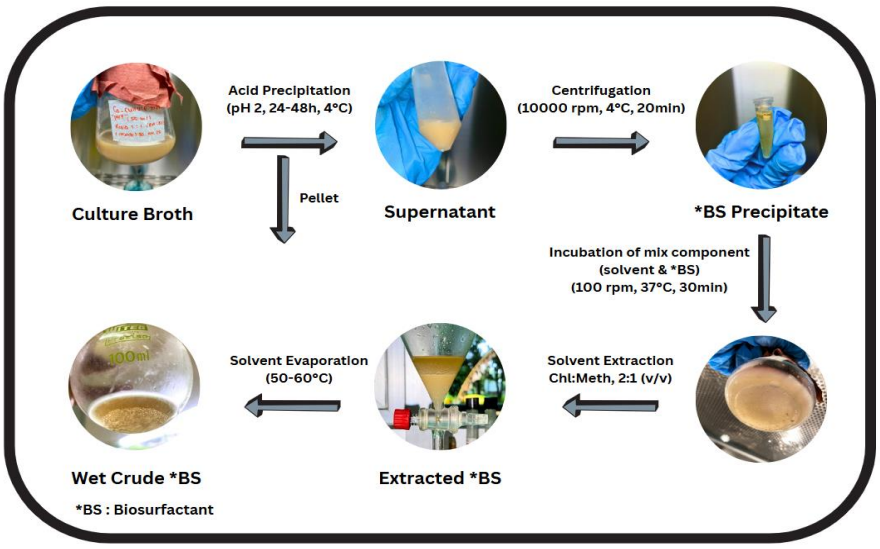

Figure 1. Stages of the Pre-Production Process of Wet Crude Biosurfactant

Oil Displacement Area (ODA)

Ten µl of crude oil is dripped onto the surface of 40 ml of distilled water in a 15 cm diameter petri dish to form a thin layer of oil. Next, a supernatant sample solution (10 µl) is dripped into the center of the oil layer. The area of the circle formed due to oil displacement is measured as an indicator of biosurfactant activity. The area of this shifted oil indicates a linear relationship with the amount of biosurfactants tested (Morikawa et al., 2000). The determination of the area was determined using the ImageJ software (Kang et al., 2024).

III. RESULT AND DISCUSSION

Growth, emulsification index, and oil displacement area in co-culture systems at various inoculum ratios

The growth performance of microorganisms and their ability to produce biosurfactants are important factors in the implementation of microbial enhanced oil recovery *(*MEOR). Growth, biosurfactant production, and emulsification testing by microorganisms can be quantified by performing biosurfactant characterization. The growth of microorganisms can be evaluated by determining the optical density (OD) value of the cell suspension at a wavelength of 600 nm. The effectiveness of biosurfactants was determined by conducting preliminary testing, namely studying the co-culture system in emulsifying used lubricants and lowering the surface tension in oil displacement area testing using liquid paraffin (H. Li et al., 2023).

There are seven media to be screened before reaching the pre-production stage. The variety of media used in the screening stage, using inorganic salt medium (ISM) media which consists of NH_4NO_3 as a source of inorganic nitrogen, is commonly used in *P. aeruginosa* strains which can produce higher biosurfactant yields than other inorganic nitrogen sources such as $NaNO_3$, $(NH_4)_2SO_4$ and KNO_3. Macronutrients and trace elements are used in production media, as they can play a role in the balancing process of cell wall communication and aid in the mechanism of protein synthesis (Nurfarahin et al., 2018).

In the M3 to M7 media, the carbon source of molasses was limited to see its effect on the growth and effectiveness of biosurfactants. Likewise, the inorganic nitrogen source of NH_4NO_3 reduced to 1 g/L because the production of biosurfactants is more optimal under nitrogen stress conditions (A. Li et al., 2011). In M3 to M7 media, yeast is added to enrich nutrients and maintain the stability of the growth of the co-culture system during the fermentation process. NaCl (0.3%, w/v) was used on M4, M5, and M6 media to see the effect of the addition of osmotic sources on the performance of biosurfactants (Nurfarahin et al., 2018).

Tofu wastewater is added to the M6 and M7 media, used as a source of nitrogen and carbon because it is easy to obtain and has a relatively low price. This waste has the potential to be an

organic substrate that can be used in the production of biosurfactants. Some previous studies have shown that the use of soybean oil, whose raw material is derived from soy, is capable of producing competitive biosurfactants (Ebadipour et al., 2016; Quraishi et al., 2021; K.S.M. Rahman, Thahira J.Rahman, Stephen McClean, 2002). However, the use of soybean oil has several obstacles, such as its expensive price, limited availability, and its status as a human consumption production, which is mainly used for food needs. Therefore, in this study, soybean raw materials were substituted with tofu wastewater, while the oil source was replaced with waste cooking oil as a more economical alternative.

The use of co-culture of *P. aeruginosa* and *B. subtilis* with tofu wastewater substrates and waste cooking oil in biosurfactant fermentation systems is still rarely reported. The results obtained from this study, using tofu wastewater substrate, showed the highest emulsification index of 80% at a P/B ratio of 1:1, compared to the study of a single culture of bacterial isolation, which showed an emulsification index value of 64% (Kamallia & Hasbi, n.d., 2021), and the study of a single culture of *Bacillus subtilis* ATCC 19659 obtained an emulsification index result of 40.0% (Fachria, 2021). The use of waste cooking oil as a carbon source without tofu wastewater in this study resulted in the highest emulsification index value of 65.38% at a P/B ratio of 1:1, while in the previous study using a carbon source from used oil with a single culture of *Pseudomonas sp*. 55T1 (olive oil) produced an emulsification index value of 61.3% (Haba et al., 2000).

The determination of the effectiveness of biosurfactants of seven modified media at the screening stage was tested using an oil displacement area test using liquid paraffin as a hydrophobic medium. In contrast to other hydrophobic media, liquid paraffin does not form a uniform thin layer but forms droplets; therefore, the method used to measure the displacement area is not based on the clear zone but based on the area of the distribution of the droplets produced. Area analysis was carried out digitally using the ImageJ software by utilizing the difference in color contrast between the droplets and the hydrophobic media background above the water to identify the boundaries of the scattered area. The best oil displacement area results were obtained from M2 media with a value of 12.32 cm^2. Compared to the positive control of the 1% SDS (sodium dodecyl sulfate) which showed the result of an oil displacement area of 2.28 cm^2. This value indicates the competitive activity of biosurfactants in lowering the interface tension between oil and water, as explained that the wider the dispersion zone, the higher the biosurfactant's ability to lower the surface tension (Uyar & Avcı, 2023).

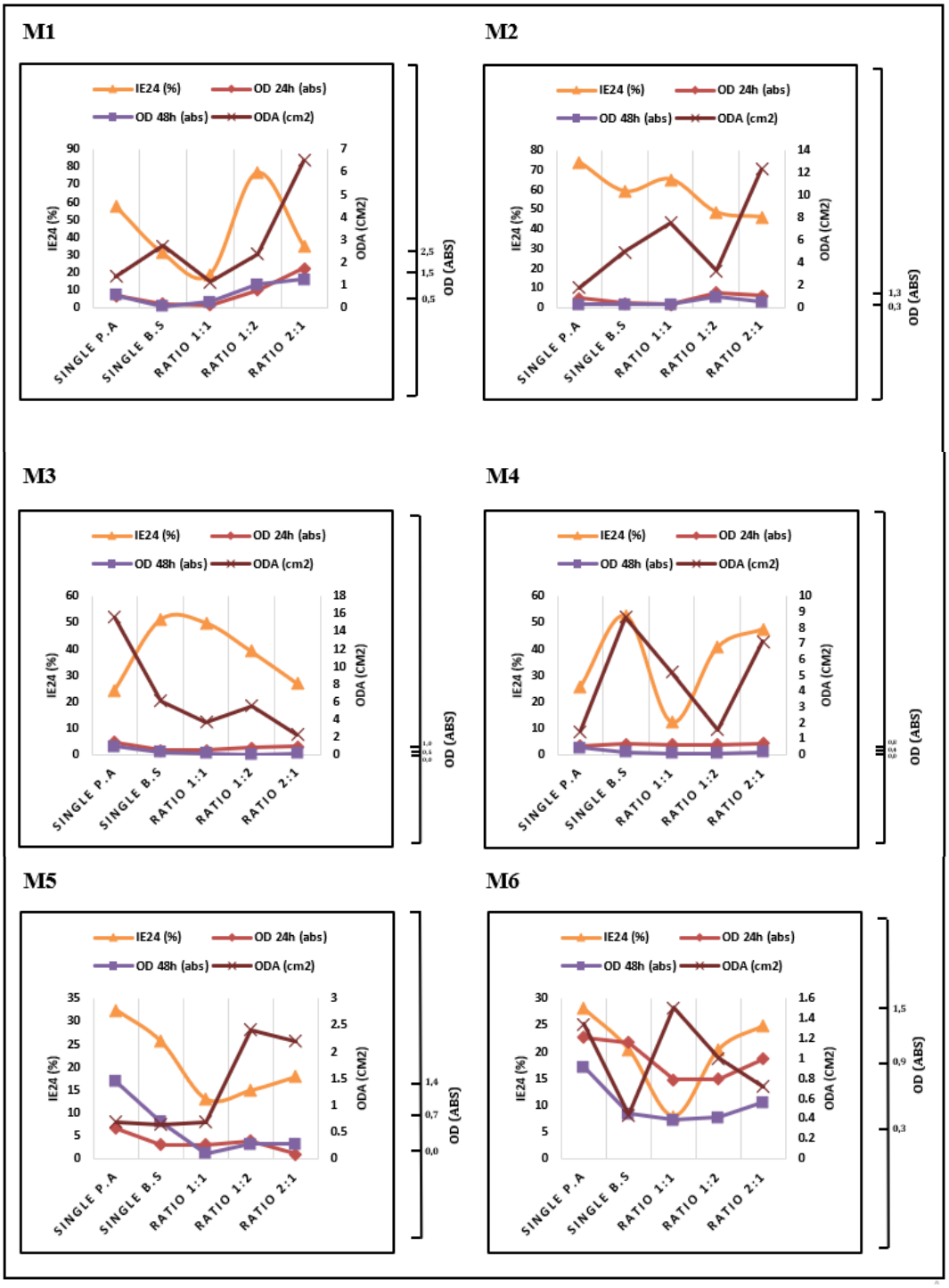

Figure 2. The Results of OD_{600}, E24, and ODA Value Test Parameters at Various Inoculum Ratios (P/B Ratio). **Emulsifier* = Used Lubricant, **Oil = Liquid Paraffin ($\rho = 0{,}87\ kg/m^3$)

In the pre-production stage, biosurfactant fermentation is carried out under operating conditions (37ºC, 160 rpm, 5 days); every 24 hours, cell suspension is taken to measure the OD_{600} value to evaluate the growth phase in the co-culture system, and biosurfactant performance test

parameters are carried out by testing the emulsification index using used lubricants from supernatant samples, oil displacement areas with kerosene, crude oil, and paraffin from supernatant samples, and CBS (crude biosurfactant). The highest OD_{600} value as an indicator of microbial cell growth was recorded on day 3 (2.19 abs), while the highest emulsification index (E24) was achieved on day 2 (89.66%) using crude oil. Compared to the previous study, the value of E24 using crude oil is 54.22% conducted by (Wu B, Xiu J, Yu L, Huang L, Yi L, and Ma Y., 2023) with the same co-culture system of *P. aeruginosa* and *B. subtilis*. Meanwhile, the value of the oil displacement area actually showed an increase to reach the maximum on day 5, which was 44.49 cm^2 and 55.86 cm^2 from the supernatant and CBS samples, respectively. This difference in pattern indicates that the biosurfactant activity of the co-culture system of *P. aeruginosa* and *B. subtilis* strains is not always aligned with cell growth or emulsification ability directly. This is because biosurfactants are secondary metabolites that are extracellular and remain active in the medium even though the microbial cells have entered the death phase. The highest oil displacement area on day 5 reflects the presence of stable and effective biosurfactants in lowering the surface tension of oil-water; biosurfactant compounds from this co-culture system can still maintain their function even though they have passed a stationary growth phase. These biosurfactants are not only emulsionically active at the beginning of fermentation but also exhibit strong interface activity at the end of fermentation.

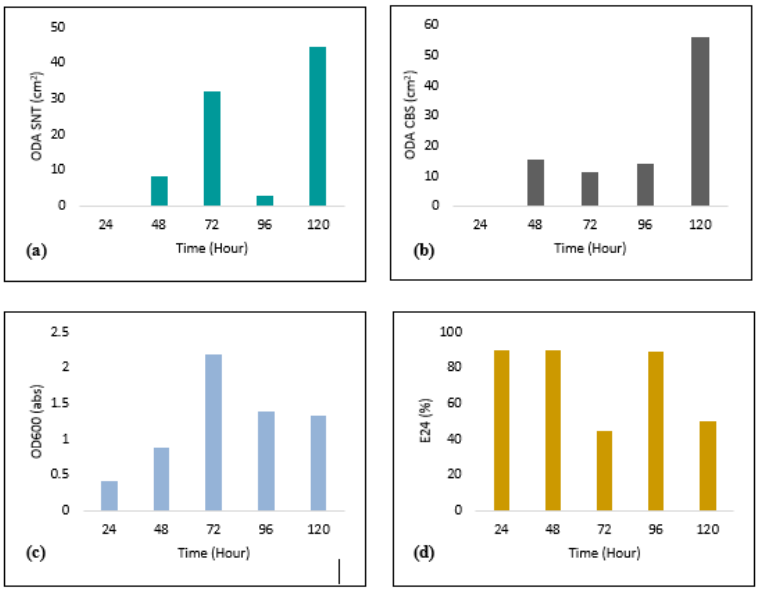

Figure 3. (a) The ODA SNT Values; (b) ODA CBS Values; (c) OD_{600} Values: (d) Emulsification Index Values in Co-Culture Systems with Different Inoculum Ratios

The partial purification process of biosurfactants to obtain the yield value of biosurfactants by the process of acid precipitation and solvent extraction using chloroform:methanol (2:1, v/v) has been carried out. The selection of this type of solvent is because biosurfactants have semipolar properties with a tendency towards polarity, so the solvent used for extraction and purification is generally a mixture of chloroform:methanol, which can be effective in dissolving polar compounds from biosurfactants (Fachria, 2021). In the fermentation of biosurfactants on the 1st to 4th day, the precipitate has not been significantly seen, so the solvent extraction process is continued using samples and their supernatants. On the 5th day, precipitate begins to form, so the solvent extraction stage only uses biosurfactant precipitate. This phenomenon illustrates that after passing through the stationary phase and even almost entering the death phase, the tendency of biosurfactant production increases compared to the time in the exponential phase (Figure 3).

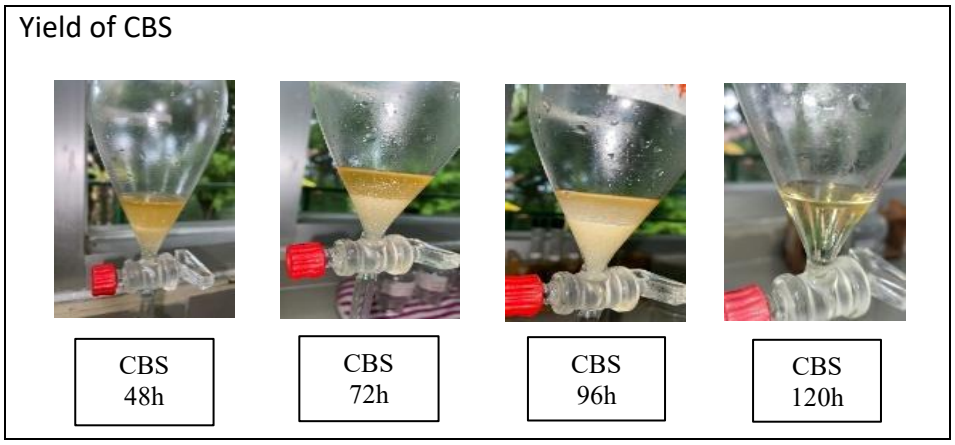

Figure 4. Process of Solvent Extraction in 120h Fermentation

To determine the biosurfactant yield, 8 ml sample of cell-free supernatant was obtained after centrifugation of the fermentation broth. This volume was consistently used as the basis for all yield calculations to ensure comparability across different media and time points. Following acid precipitation and solvent extraction (Figure 4), the recovered biosurfactant was not dried completely but weighed in its wet form due to the small amount of material recovered, which made accurate volumetric measurement difficult and prone to losses during handling. The wet weight approach was chosen to avoid data inaccuracy caused by evaporation or sample adhesion to tube surfaces. A yield of 1.24 g from 8 ml of culture corresponds to 155 g/L when scaled to the original culture volume. It should be noted that this value reflects the wet crude biosurfactant, which is typically higher than dry weight due to retained water, residual proteins, and other cell-derived materials.

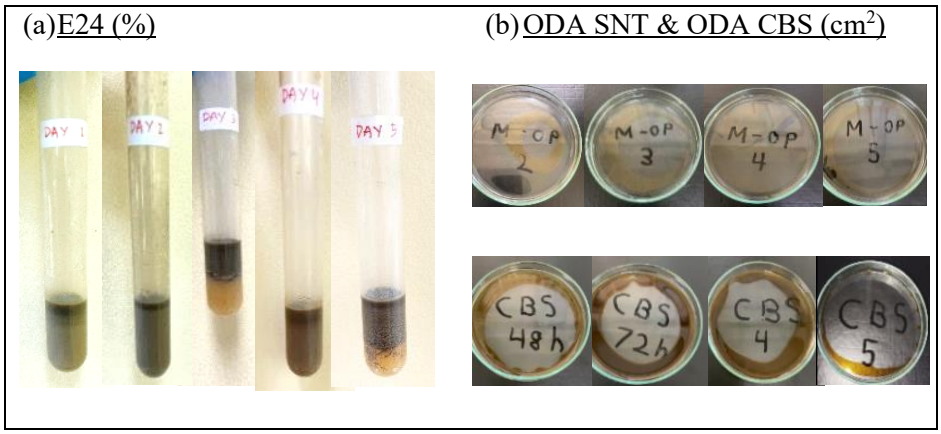

Figure 5. (a) Emulsification Index Using Used Lubricant (b) ODA SNT & ODA CBS Using Crude Oil

Used lubricant was selected for the E24 assay due to its high density and viscous nature, which simulate the characteristics of recalcitrant hydrocarbons found in aged oil reservoirs. Its properties enable clearer observation of stable emulsion layers, providing a realistic challenge for evaluating the emulsifying activity of biosurfactants toward heavy oil analogs. This approach aligns with recent findings emphasizing the relevance of lubricant-based models for assessing biosurfactant efficiency in heavy oil biotreatment (Rahman, P. K. S. M., Gakpe, E., & Marchant, R. 2020; Zhang, Y., Zhou, X., & Wang, L., 2021).

Whilst crude oil was selected for the ODA test due to its ability to form a uniform thin layer on the water surface, allowing clearer visualization and more accurate measurement of the biosurfactant's oil-displacing activity. This method has been widely adopted to assess surface activity, a key property in microbial enhanced oil recovery, especially under reservoir-mimicking conditions (Kumar, M., Singh, D., & Yadav, R., 2019; Wang, S., Li, Y., & Zhang, J., 2020). By incorporating both lubricant and crude oil across distinct assays, the evaluation provides a comprehensive understanding of biosurfactant effectiveness against varying hydrocarbon types.

IV. CONCLUSION

This study shows that biosurfactants produced by the co-culture systems of *Pseudomonas aeruginosa* and *Bacillus subtilis* can maintain their activity even though the producing microbes have passed the stationary phase. The highest oil displacement activity of the CBS area on the 5th day of fermentation (55.86 cm^2), indicates the stability of the biosurfactant, which is effective in lowering the oil-water surface tension. Meanwhile, the result of the emulsification index (E24) was highest on the 2nd day (89.66%) in the exponential phase. This result demonstrates excellent emulsification ability, which is indicative of a biosurfactant capable of forming stable oil-in-water emulsions. In the context of MEOR, such high emulsification potential is advantageous as it promotes oil mobilization by reducing interfacial tension and altering wettability, thereby

facilitating the displacement of residual oil trapped in porous media. The yield of wet crude biosurfactant reached its maximum value of (155 g/ L) on the 3rd day of fermentation, corresponding to the stationary growth phase. The high yield observed suggests that the producing strain exhibits strong biosynthetic potential under the given fermentation condition, making it a promising candidate for industrial-scale biosurfactant production.

In addition, the use of waste cooking oil (2%, v/v), tofu wastewater (1%, v/v), and molasses (2%, w/v) as modification media has proven to be an economical solution that has the potential for more sustainable industrial applications.

Further research is expected to focus on production scale, stability of biosurfactant application in reservoirs, and industrial economic analysis.

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