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Green synthesis of titanium dioxide photocatalyst using *Lactobacillus bulgaricus* for processing palm oil mill effluentL. Agustina¹, M. Romli², P. Suryadarma², S. Suprihatin^{2*}¹ Agroindustrial Engineering Study Program of Graduate School, IPB University, PO Box 220, Bogor, West Java, Indonesia² Department of Agroindustrial Technology, Faculty of Agricultural Engineering and Technology, IPB University, PO Box 220, Bogor, West Java, Indonesia

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ABSTRACT

BACKGROUND AND OBJECTIVES: To improve photocatalytic degradation performance, photocatalyst particles with a larger surface area preferred. The effectiveness of titanium dioxide as a photocatalyst depends on the synthesis method used. The method affect the particle size, crystallinity and phase composition of the produced catalyst. This study aims to develop a green synthesis process of nano- titanium dioxide photocatalysts for the advanced treatment of palm oil mill effluent.

METHODS: The green synthesis of titanium dioxide nanoparticles used de Man-Rogosa-Sharpe broth media containing *Lactobacillus bulgaricus* culture and titanium oxyhydroxide metal oxide. The factors investigated were the molarity of titanium oxyhydroxide (0.025 molar; 0.035 molar and 0.045 molar) and temperature (40; 50 and 60 degrees Celsius). The synthesized photocatalyst was characterized using a particle size analyzer to determine the particle size. The produced photocatalyst with a nanoparticle size range of 1-100 nanometer was further characterized using scanning electron microscopy-energy dispersive X-ray and X-ray diffraction. The photocatalyst was tested for advanced treatment of palm oil mill secondary effluent. The factors investigated in this test included the irradiation time and titanium dioxide photocatalyst dosage. The treatment performance was evaluated in terms of effluent quality and pollutant elimination efficiency.

FINDINGS: Nano titanium dioxide photocatalysts have been synthesized through titanium oxyhydroxide metal oxide biologically using *Lactobacillus bulgaricus*. The synthesis process at a temperature of 60 degrees Celsius and a 0.025 molar metal oxide solution produced a titanium dioxide photocatalyst with a size of 33.28 nanometer. The content of titanium and oxygen constituents in the photocatalyst was confirmed to be 39.06 percent and 47.95 percent respectively, with 67.6 percent titanium dioxide crystallinity in a theta degree of 25.4. This indicates that the green synthesis has produced an anatase diffraction nano titanium dioxide photocatalyst. Testing the titanium dioxide photocatalyst to treat palm oil mill secondary effluent yielded in elimination efficiency of 16.16-27.27 percent for chemical oxygen demand and 11.05-21.95 percent for biological oxygen demand. Phenol, which is toxic and difficult to degrade biologically, could eliminated significantly (up to 81.12 percent) using a photocatalyst dose of 1 gram per liter at a time irradiation of 2.5 hour.

CONCLUSION: The biological synthesis of nano titanium dioxide photocatalysts is affected by temperatures and metal oxide concentrations. The photocatalytic process for advanced treatment of palm oil mill secondary effluent shows that this synthesis process effectively eliminates phenols. Some compounds such as lignin, amino acids, and pectin are not significantly mineralized using this process.

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INTRODUCTION

The increase in crude palm oil (CPO) production increases in the amount of liquid waste (Palm Oil Mill Effluent/POME) produced (Agustina et al., 2021). Biological treatment (anaerobic and aerobic) has been widely applied to treat POME because the processing costs are low. However, the resulting effluent still contains contaminants in the form of suspended and dissolved materials difficult to degrade biologically (recalcitrant), such as pectin, lignin, tannins, and other phenolic compounds (Zainuri et al., 2018; Samimi and Shahriari-Moghadam, 2020). These materials can cause the effluent to be colored, causing water pollution problems and ecosystem disturbances. The remaining pollutants in the effluent need to be removed using appropriate methods to produce clean water to support palm oil mill activities, where the need for clean water at factory sites is now becoming a problem. Therefore, the development of a wastewater recycling process in palm oil mills can be a win-win solution for environmental and economic interests. Considering that pollutants in the effluent of biological processes are generally difficult to degrade biologically (recalcitrant), non-biological process is suitable for eliminating these pollutants. To achieve this goal, the research plan is intended to develop a photocatalytic degradation process for eliminating dissolved organic pollutants difficult to degrade biologically. A review of the results of previous research shows that this method has various advantages for advanced wastewater treatment (Wang et al., 2022), such as being effective for recalcitrant pollutants, and the process is fast, does not depend on microbial activity, and can utilize local resources (Li Puma, 2003; Fathinia and Khataee, 2013; Ng and Cheng, 2015; Ng et al., 2019). The performance of the photocatalytic process is mainly determined by the photocatalyst used (Nguyen et al., 2021; Hidayah et al., 2022). One of the prospective metal oxides is titanium dioxide (TiO₂) because it is inert, harmless, cheap, chemical corrosion resistant, and has good optical characteristics (Khan et al., 2015; Fares et al., 2022). The requirement for a good catalyst is to have a large surface area (Supriyanto et al., 2014) to increase the adsorption capacity so that a larger photocurrent is produced (Sucahya et al., 2016). Therefore, the particle size of a good catalyst needs to be reduced/reduced to nano size. Several studies in this field have used TiO₂ photocatalysts doped with other metal oxides in a photocatalytic system (Fadzil et al., 2013), where the

TiO₂ photocatalyst is combined with feroxalate and exposed to a stream of ozone, yielding a 54 percent (%) decrease in organic compound content (as measured by the decrease in chemical oxygen demand (COD) value). A decolorization effect of 90% on congo red with the use of TiO₂ combined with gadolinium has also been achieved in previous research (Khademalrasool et al., 2016). This research used a TiO₂ nanoparticle as the photocatalyst to provide an improved effect on degradation of palm oil mill wastewater. Efforts were made in this research to improve the characteristics of TiO₂ through green synthesis. Method for creating nanoparticles, include physical, chemical, and biological processes. However the biological method is the simplest and uses no chemicals (Agustina et al., 2020). This method can improve the properties of TiO₂ photocatalysts thus, the biological approach is thought to be more ecologically friendly (Mukherjee and Nethi, 2019) and efficient. TiO₂ nanoparticles with a size of 30 nanometer (nm) with a tetragonal structure and anatase phase (which has the best ability to degrade organic compounds because it has the highest bandgap) have been proven in research (Jha et al., 2009) using Lactobacillus on nutrient-broth media. To increase the adsorption ability of a TiO₂ photocatalyst (Azhar et al., 2011) performed synthesis with the help of Lactobacillus on de Man-Rogosa-Sharp (MRS) broth media, however the size of the TiO₂ photocatalyst produced was still large (150 nm). Based on the results obtained from their research, green synthesis of TiO₂ was performed in this research to improve its physical and chemical characteristics so that it can be used as a photocatalyst in the processing of palm oil liquid waste using the photocatalytic method. The green synthesis method is expected to provide advantages that are not obtained in physical and chemical synthesis (Bandeira et al., 2020). Polysaccharides from MRS broth media, which promote the stability of synthesis, are the medium to be used in green synthesis (Makarov et al., 2014). According to Jha et al. (2009), Lactobacillus is a non-pathogenic, partially oxygen-tolerant, gram-positive, prokaryotic, anaerobic-mesophilic bacterium with adaptability and high metabolism. It can be used to enhance the synthesis condition by assisting in the formation of nanoparticles from TiO₂. The green synthesized TiO₂ photocatalyst also has other potential applications besides POME treatment. For example, it can be used for air purification (removal of air pollutants, including volatile organic compounds,

nitrogen oxides, and sulfur oxides (Prasetya *et al.* 2021); coating self-cleaning surfaces such as glass, ceramics, and metals; solar energy conversion; environmental remediation, which is related to this study (degradation of organic recalcitrant in case of a water purification process). Table 1 shows some important research conducted related to the synthesis of nanoparticles for photocatalysts.

To date, no specific information about the successful implementation of green synthesis using *Lactobacillus bulgaricus* (*L. bulgaricus*) for palm oil mill secondary effluent (POMSE) treatment on an industrial scale has been found. This study was conducted to solve the following problems; 1) How to determine the best conditions for performing green synthesis of TiO₂ photocatalysts with superior characteristics to degrade recalcitrant pollutants in advanced processing of palm oil mill wastewater; 2) How to determine the performance of the photocatalytic process using green synthesized TiO₂ photocatalysts for advanced treatment of POMSE. The main objective of this study is to develop a photocatalytic process with a focus on green synthesis of TiO₂ photocatalysts so that the produced photocatalyst can be used for advanced processing of liquid waste, as an effort to apply the concept of recycled wastewater in palm oil mills.

Furthermore, the specific aims of this research are as follows: 1) To produce TiO₂ photocatalysts through a green synthesis process using *L. bulgaricus* as a bio-reductor; 2) To obtain the performance value of the photocatalytic process (using the main parameter: COD, biological oxygen demand (BOD₅) and phenol degradation). This study was conducted from 2021 to 2022, using samples of palm oil liquid waste from a factory located in South Kalimantan, Indonesia.

MATERIALS AND METHODS

Material

The materials used were metal oxide titanium oxyhydroxide: TiO(OH)₂, MRS broth, MRS agar, *L. bulgaricus* culture, POME, POMSE, TiO₂ photocatalyst from green synthesis, polyaluminium chloride (PAC), filter paper, distilled water and other materials for analysis. The tools used are photoreactors, hot plates (with stirrers), water bath, incubators and glassware for synthesis and analysis.

Green synthesis of TiO₂ photocatalyst

Synthesis using specific media (MRS broth containing *L. bulgaricus* and metal oxide TiO(OH)₂) was performed in the following steps (work procedures adjusted from the study of (Jha *et al.*, 2009):

Table 1: Research on photocatalyst synthesis

No.	Results	Sources
1.	Nano TiO ₂ films have significantly better climatic resistance than blank polyethylene films. TiO ₂ nanoparticles were added, which increased in water vapor transmission from 18.1 to 24.6 g/m ² in 24 h. Results showed that TiO ₂ nanoparticles incorporated into polyethylen-based films have a good potential application as an active food packaging solution.	Xing <i>et al.</i> (2012)
2.	TiO ₂ nanoparticles made from <i>Planomicrobium sp.</i> , a microbial species isolated from melting ice, were resistant to the growth of <i>Bacillus subtilis</i> , <i>Klebsiella planticola</i> , and <i>Aspergillus niger</i> bacteria. This nanoparticle synthesis used an eco-friendly, cost-effective process.	Chelladurai <i>et al.</i> (2013)
3.	Nanobiotechnology procedures involving biological synthesis have a tremendous potential to increase the production of nanoparticles without the use of harsh, expensive, and toxic chemicals typically used in conventional physical and chemical processes.	Shah <i>et al.</i> (2015)
4.	Catalytic activity during the photodegradation of dyes significantly diminished when TiO ₂ was substituted with zinc oxide (ZnO) because ZnO is unstable and results in inconsistent dissolution processes to form zinc hydroxide (Zn(OH) ₂) on the ZnO particle surfaces, leading to catalyst deactivation.	Amini and Ashrafi (2016)
5.	Analyses of the experimental conditions, nanoparticle properties, and possible uses of nanoparticles in pharmaceuticals and biomedical applications were presented for the production of high-value nanoparticles from food waste (in aquaculture and horticulture).	Ghosh <i>et al.</i> (2017)
6.	The reduction of aromatic aldehydes was facilitated using produced metal oxide nanoparticles. Ammonium formate was used as a green hydrogen donor during the reduction, and the corresponding alcohols were produced in excellent yields in 2–24 h.	Muthuvinothini and Stella (2019)
7.	Current analysis of various biological substrate sources, green synthesis processes, and effects on the characteristics of zinc oxide nanoparticles.	Bandeira <i>et al.</i> (2020)

the main culture with various dilutions was added to MRS broth (culture: MRS broth; 25 millilitre (mL): 75 mL) and incubated for 24 h at 27 degrees Celsius (°C). Then a 20 mL TiO(OH)₂ solution was added with the molarity according to the factor applied to the treatment combination. Heat the solution in a water bath (temperature adjusted according to the treatment combination) for 20-25 min until a white precipitate forms at the bottom of the Erlenmeyer flask. The solution was then incubated at room temperature (25°C) for 48 h until a stable white precipitate formed on the Erlenmeyer flask bottom. The goal of the synthesis is to obtain the appropriate size (1-100 nm) in the size range of nanoparticles (Carvalho *et al.*, 2018); thus the initial design applied was a-completely randomized (two replications) at different temperatures of 40°C, 50°C, and 60°C and molarity levels of 0.025, 0.035, and 0.045 M. The Determination of the levels was based on the research results of Jha *et al.* (2009). The molarity level and temperature chosen to synthesize *Lactobacillus sp.* in this study were 0.025 M TiO(OH)₂ and 50°C, respectively. The mesophilic *Lactobacillus* bacterium can thrive between 35°C and 45°C. The ideal growth temperature for *L. bulgaricus* is between 45°C and 47°C. According to previous research findings, green synthesis of TiO₂ nanoparticles was conducted over a temperature range of 37°C–60°C; thus, the temperature was set at 40°C, 50°C, and 60°C. Determination of the molarity levels of 0.025, 0.035, and 0.045 M considered the research results of

Jha *et al.* (2009) that used a minimum molarity level of 0.025 M. It was then expected that the higher concentrations would help the synthesis process to produce the desired nanocatalyst characteristics. The synthesized photocatalyst was tested for size using a particle size analyzer (PSA) in Laboratory of Materials Physics, Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia, if the size of the produced photocatalyst is within the nanoparticle size range, proceed with another characterization using scanning electron microscopy-energy dispersive X-ray (SEM EDX) spectroscopy at Integrated Laboratory and Technology Innovation Center, University of Lampung and X-ray diffraction (XRD) Institut Teknologi Bandung Nanoscience and Nanotechnology Research Center.

Photocatalytic process

The performance of the photocatalyst obtained through the green synthesis process was tested by applying the photocatalytic process to POMSE in a suspended photoreactor, as shown in Fig. 1. In this study stage, the POMSE sample was first coagulated and filtered using filter paper to homogenize the solution, and then, it was tested for the COD, BOD₅ and phenol. The analysis was performed in South Kalimantan Province Health Laboratory. The factors studied were catalyst dose and irradiation time for the photocatalytic process using a completely randomized trial design with two replications. The photocatalytic process was performed under the following conditions: (a) the photocatalyst used

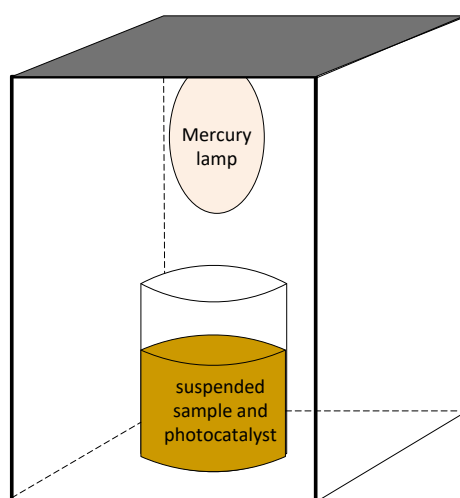


Fig. 1: Scheme of photoreactor

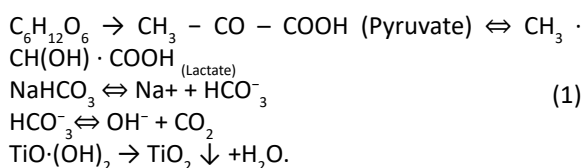
was TiO₂ resulting from green synthesis; (b) the photocatalyst dosages used were 1 gram (g), 1.5 g, and 2 g (Thota *et al.*, 2014; Lestari, 2017);

(c) irradiation times (contact time) in the photocatalytic process were 1.5, 2, and 2.5 h; (d) the light source used was a 350-W Philips mercury lamp.

RESULTS AND DISCUSSION

Green synthesis of TiO₂ photocatalyst

The research phase begins with the preparation of the microorganism culture (*L. bulgaricus*) used for experiments. Furthermore, the main culture of *L. bulgaricus* was made on MRS broth media with four times dilution. In this synthesis, atoms and molecules mix to create precursor blocks, which are later self-assembled into nanoparticles (Ealia and Saravanakumar, 2017). This method is known as a bottom-up technique. The primary biological factor affecting the synthesis of a substance is the cell membrane, which is composed of lipids and membranes. In the cell membrane, synthetic biochemical conversion occurs following the oxidation and reduction mechanisms. Because the composition of the membrane is dynamic and flexible, the effect of the composition is not always constant and facilitates the synthesis (Capeness *et al.*, 2019). It is anticipated that green synthesis of TiO₂ nanoparticles will be able to use extracellular and intracellular microorganisms under suitable environmental conditions. Electrostatic interaction between the membranes of microorganisms containing negatively charged phospholipids and the combination of metal oxides used can also result in the extracellular creation of nanoparticles (Anandgaonker *et al.*, 2019). Eq. 1 is the chemical reaction that probably occurs (Makarov *et al.*, 2014).



According to Malik *et al.* (2014), additional research into other metal oxides and culture variables is necessary to produce certain nanoparticles. However, the types of nanoparticles produced also depend on the temperature used during the synthesis. The color change of the media to a lighter

shade is one of the early signs of the production of high-quality nanoparticles (Ridhawati and Fajar, 2017). Based on the self-defense mechanisms of microbes, microorganisms are used in the fabrication of nanoparticles (Ghosh *et al.*, 2017). The biological mechanism converts reactive ions into stable atoms because high ion concentrations are typically lethal to bacterial cells. In this investigation, *L. bulgaricus* cells were cultivated for 36 h in sterile distilled water containing carbon and nitrogen sources. A white precipitate appeared at the bottom of the Erlenmeyer flask after 20 mL of TiO(OH)₂ was heated in a water bath at 50°C for 20–25 min to create the metal oxide. This precipitate appearance is proof that TiO₂ nanoparticles have formed. The solution was then incubated at room temperature (25°C) for 48 h until a stable white precipitate formed on the Erlenmeyer flask base. Fig. 2 depicts the green synthesis process of TiO₂.

Measurement using PSA

The results of particle size analysis using the PSA showed a different pattern. From Fig. 3a, at a constant treatment temperature of 40°C but increasing concentrations (0.025, 0.035, and 0.045 M), the size of the particles formed increases. From Fig. 3b, using the same concentration of 0.025 M but at different temperatures of 40°C, 50°C, and 60°C produced better synthesis results, with the particle size decreasing. At 60°C and 0.025 M the results were significant, with the obtained particle size within the size range of nanoparticles (33.28 nm). TiO₂ nanoparticles have high protein affinity for binding metals, which prevents particle aggregation. Proteins and polysaccharides are produced when *L. bulgaricus* is used as a bio-reductor during the synthesis of TiO₂ nanoparticles, which is not the case when the synthesis is performed using physical or chemical methods. This aids in the process of synthesizing metal oxide bonds so that they do not cause protein agglomeration. (Makarov *et al.*, 2014). Regulation of the synthesis media is necessary to create an optimal synthesis environment.

Factors affecting the synthesis include pH, metal oxide concentration, and temperature. Generally, glucose-containing media support the growth of microorganisms, particularly *Lactobacillus* grown on MRS media. As glucose is a reducing agent, it tends to reduce the value of the oxidation reduction

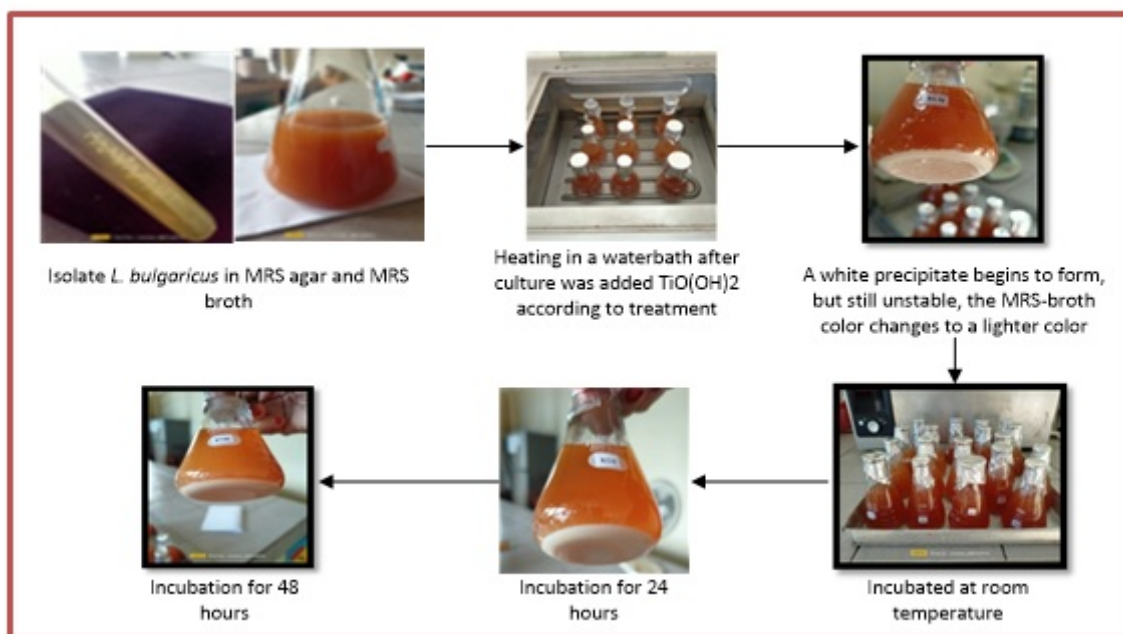
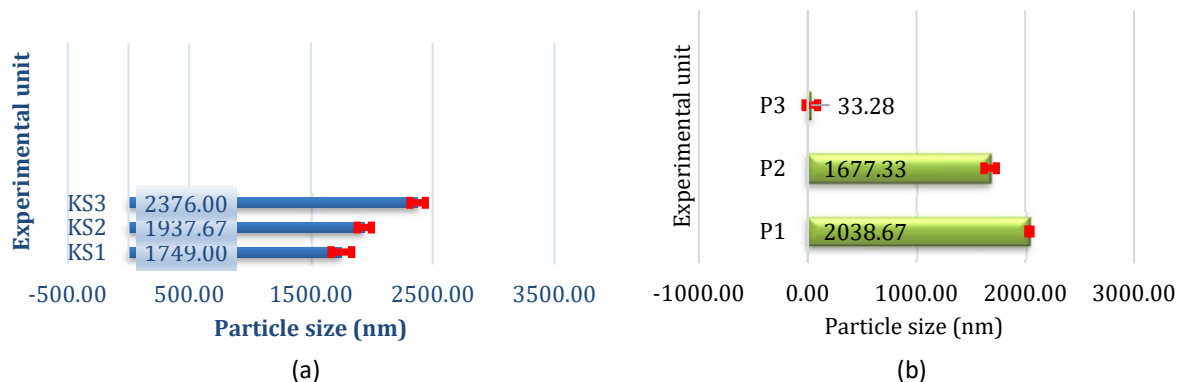


Fig. 2: Green synthesis of TiO_2 photocatalyst using *L. bulgaricus*



Experimental unit:

KS1 : 40° C; 0.025 M;

KS2 : 40° C; 0.035 M;

KS3 : 40° C; 0.045 M;

P1 = 40° C; 0.025 M;

P2 = 50° C; 0.025 M;

P3 = 60° C; 0.025 M;

Fig. 3: Graph of particle measurement results a) fixed temperature with increasing concentration, b) increasing temperature with fixed concentration

potential. The degree of aerobiosis is quantified by the oxidation-reduction potential, which is written as the oxidation reduction potential ($r\text{-H}_2$); the negative logarithm of the partial pressure of hydrogen gas. The

pH of the medium and the overall $r\text{-H}_2$, both of which are partially controlled by bicarbonate, have an impact on the synthesis conditions of TiO_2 nanoparticles in addition to the use of glucose, an energy-

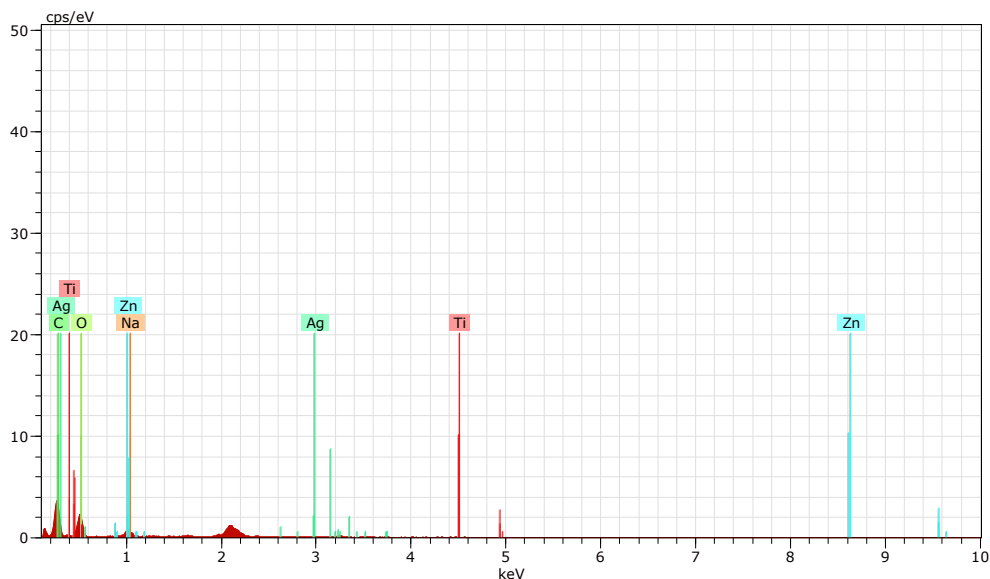


Fig. 4: Spectrum of TiO_2 based on SEM EDX analysis

generating substance (in the synthesis medium). The membrane-bound oxidoreductase is activated by the anticipated slightly acidic pH and decreased $r\text{-H}_2$. The temperature also plays a significant role in the synthesis process. The mesophilic *Lactobacillus* bacterium can thrive between 35°C and 45°C . The ideal growth temperature for *L. bulgaricus* is 45°C and 47°C . According to earlier research findings, green synthesis of TiO_2 nanoparticles was conducted over a temperature range of 37°C – 60°C (Jha *et al.*, 2009). However, the present study's findings indicate that the best green synthesis conditions occurred at 60°C . This suggests that for nanoparticle synthesis to occur, the temperature must be raised above the range for growth. The concentration of metal oxides used during catalyst synthesis may have effects on the process. Higher concentrations may lead to agglomeration of metal oxide particles, resulting in decreased surface areas and reduced accessibility of active sites. Conversely, lower concentrations can promote better dispersion and higher surface areas, which can enhance catalytic activity. In this study the main factors studied were the process temperature and concentration of the metal oxide used.

Nanoparticle characterization (using SEM EDX and XRD)

Based on the results of particle measurements resulting from the green synthesis, the treatment

product was determined at 60°C with a molarity level of 0.025 M which was further characterized using SEM EDX. The characterization results were also used to determine whether the product can be synthesized in larger quantities so that it can be used as a photocatalyst in processing palm oil mill wastewater using the photocatalytic technique. Confirmation of the content of the synthesized TiO_2 elements was performed using SEM EDX. As shown in the spectrum in Fig. 4, the amounts of the titanium (Ti) and oxygen (O) elements are 39.06% and 47.95%, respectively.

Table 2 shows the overall results of the constituent elements in the TiO_2 nanocatalyst synthesis process (in atomic and weight percentages). Through SEM EDX it is possible to identify the constituent elements in the synthesis in detail. In addition, to Ti and O, the other elements were carbon (C), zinc (Zn), silver (Ag) and sodium (Na).

The peak area formed (Fig. 5) shows that the crystallinity of TiO_2 is 67.6%, which also indicates the amount of TiO_2 in the mixture is 67.6%, with the remaining 32.4% being an amorphous component. The highest peak is in the range of 25.4 degrees theta, indicating anatase diffraction formed by TiO_2 .

Photocatalyst performance

Through photocatalysis, the TiO_2 photocatalyst is crucial in the treatment of POMSE, which is a waste

Table 2: Elements in TiO₂ nanocatalyst synthesis process

Formula	Mass (%)	Atom (%)
C	0.41	0.13
O	47.95	59.98
Zn	8.62	1.98
Ag	2.63	0.37
Na	1.33	0.87
Ti	39.06	36.68
Total	100	100

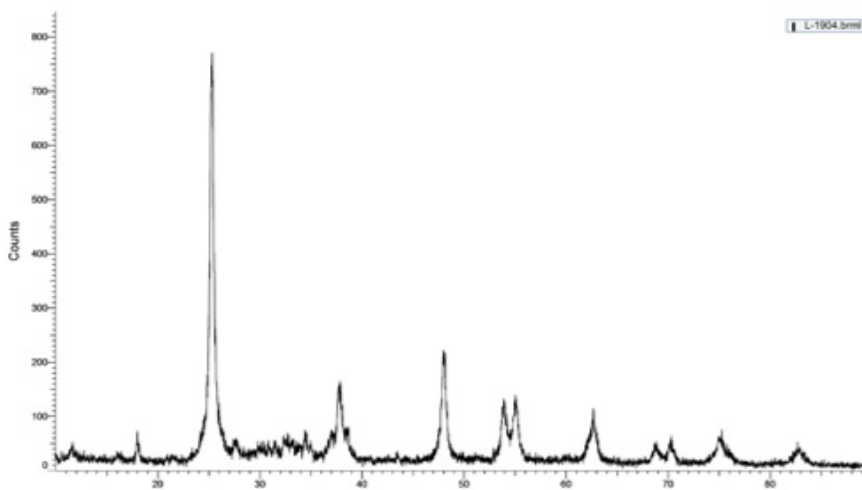
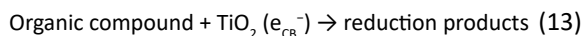
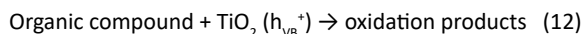
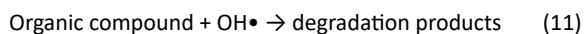
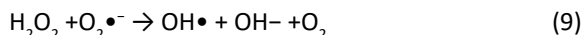
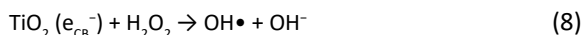
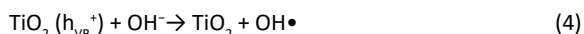
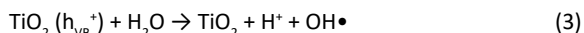
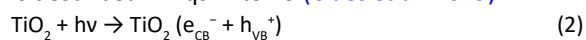


Fig. 5: Peak area of TiO₂ based on XRD analysis

product of the extraction of palm oil that contains significant concentrations of organic contaminants, suspended particles, and nutrients making it hazardous to the environment if released into the environment untreated. When exposed to light energy, especially in the ultraviolet spectrum, the TiO₂ photocatalyst can start and accelerate chemical reactions through the photocatalytic process. When TiO₂ is exposed to ultraviolet light, electron hole pairs are produced, where electrons and holes develop into highly reactive species. The TiO₂ photocatalyst can assist with the overall remediation process in the context of treating POMSE in organic pollutant degradation. The reactive species generated by TiO₂, such as hydroxyl radicals (•OH), can oxidize and break down complex organic compounds in POMSE. These radicals react with organic pollutants, converting them into simpler and less harmful substances, such as carbon dioxide and water. The general mechanism is described in Eqs. 2 to 13 (Claes *et al.* 2019).



Increasing the amount of O₂ in the liquid waste affects the BOD₅ and COD values, because the availability of sufficient O₂ in the liquid waste helps the degradation process of organic components, thereby reducing the BOD₅ and COD values in the liquid waste. The reduction and percentage of the elimination parameters COD, BOD₅ and phenol are presented in Table 3. According to Table 3, the measurement results of the waste quality parameters after the photocatalytic process was applied did not meet the specified quality standards.

The percentage reductions of COD (Table 3 and Fig. 6) ranged from 16.16% to 27.27%. This shows that the ability of the TiO₂ catalyst to degrade organic pollutants ranges from 16.16% to 27.27% for 2.3 h. The assumption that can be given from these results is that in 150 min, organic pollutants which account for approximately 16-27% of the total organic pollutants have been mineralized. Meanwhile, the rest, which are complex compounds (lignin, amino acids, pectin) and some phenols remain in the depolymerization stage to form molecules with smaller molecular weights (micro-molecules) as intermediate compounds, which require a longer total mineralization time. This is based on visual observations of the color of POMSE (presumably from lignin) before and after 150 min of photocatalysis, which did not exhibit a significant color change. The presence of sufficient dissolved oxygen in the reactor, which enables microorganisms to break down polluting substances, is one environmental aspect that contributes to the high COD value (Elystia *et al.*, 2022). Palm liquid waste contains high protein (in the form of amino acids); thus, it also contains high organic matter and causes pollutant concentrations to remain high (Alhaji *et al.*, 2016). This statement is reinforced

by research conducted by (Chang and Wu, 2010) who reported that under the same pH conditions (7-8), it takes 960 min of degradation time, to degrade lignin up to 88% with a TiO₂ photocatalyst concentration of 10 gram per liter (g/L) without the addition of air. Concerning the degradation of amino acids, some researchers have stated that the degradation of amino acids is affected by the molecular structure of its constituents (especially the side chains) and the interactions between the amino acid side chains and the catalyst surface which vary with pH (Tran *et al.*, 2006). Thus, not all amino acids can be degraded at similar pH values.

Research related to the application of the photocatalytic process to POMSE has been conducted, but the TiO₂ photocatalyst used is a result of chemical synthesis. The TiO₂ photocatalyst can degrade organic compounds (COD) and effectively eliminate phenolic pollutants in POMSE (31.36% COD degradation performance and up to 96.66% phenol elimination by TiO₂ loading of 1.5 g/L; air flow rate of 10 cc/min) at 120 min (Lestari *et al.* 2017). Another research by Nawaz *et al.* (2023) investigated the effectiveness of photocatalytic remediation of treated POME-containing phenolic compounds using TiO₂ nanomaterials. Chemical precipitation was used to prepare the TiO₂ nanomaterial. Under visible light illumination, 78.32% of 224.85 milligram per liter (mg/L) phenolic compounds were broken down in 180 min at the optimal TiO₂ dosage (0.9 g/L). The reaction of hydroxyl free radicals is non-selective, thus, some free radicals attack phenols, whereas others attack other organic pollutants; when almost all phenols are mineralized, the hydroxyl radicals formed oxidize other organic pollutants in POMSE. Based on this, the

Table 3: Measurement results and percentage of elimination parameters COD, BOD₅ and phenol

Variables	Measurement results (mg/L)			Elimination percentage (%)		
	BOD ₅	COD	phenol	BOD ₅	COD	phenol
Quality standard	50	100	0.5	-	-	-
POMSE	2061.50	4852.50	4.28	-	-	-
F1 (1 g/L TiO ₂ ; 1.5 h of irradiation)	1790.5	3940.4	3.37	13.21	18.86	21.25
F2 (1,5 g/L TiO ₂ ; 1.5 h of irradiation)	1740	3700.6	3.45	15.66	23.79	19.48
F3 (2 g/L TiO ₂ ; 1.5 h of irradiation)	1609	3529.1	1.86	22.01	27.32	56.57
F4 (1 g/L TiO ₂ ; 2 h of irradiation)	1801	3949.3	1.93	12.70	18.67	54.85
F5 (1,5 g/L TiO ₂ ; 2 h of irradiation)	1786.5	3918.1	1.83	13.40	19.31	57.20
F6 (2 g/L TiO ₂ ; 2 h of irradiation)	1833.75	3856.9	1.09	11.11	20.57	74.62
F7 (1 g/L TiO ₂ ; 2.5 h of irradiation)	1746.1	3828.65	0.81	15.36	21.16	81.12
F8 (1,5 g/L TiO ₂ ; 2.5 h of irradiation)	1814.95	3930.3	1.55	12.02	19.06	63.73
F9 (2 g/L TiO ₂ ; 2.5 h of irradiation)	1813.5	4067.8	1.65	12.09	16.23	61.54

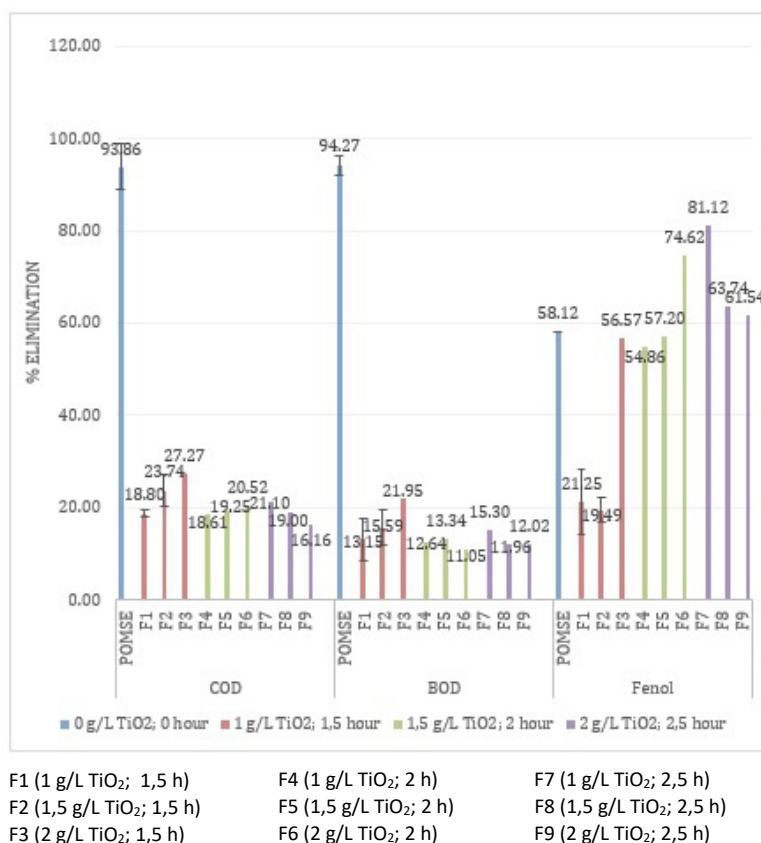


Fig. 6: Effect of irradiation time and TiO₂ photocatalyst dosage on elimination percentage of COD, BOD₅ and Phenol

assumption that can be given is that approximately 70% of the pollutants not been mineralized are intermediate compounds resulting from the degradation of lignin, amino acids, phenols, pectin, oils and greases, as well as macromolecules, which are constituent of organic pollutants in POMSE. However, COD could only be reduced by 16.16%-27.27%. Phenol, which is a hazardous substance, toxic, and difficult to degrade by decomposer organisms, can be reduced significantly to close to environmental quality standards (0.5 part per million). The experimental unit F7 (dose of 1 g/L; irradiation of 2.5 h) resulted in phenol elimination of up to 81.12%, producing an effluent phenol concentration of 0.81 mg/L.

Benefits of using a green synthesis approach for TiO₂ photocatalyst production

Utilizing a green synthesis approach for TiO₂ photocatalyst production can offer several

environmental benefits compared with conventional methods. Here are some key advantages,

- Reduced energy consumption: green synthesis methods typically employ milder reaction conditions, such as lower temperatures and pressures, resulting in reduced energy requirements. This decrease in energy consumption contributes to lower greenhouse gas emissions and overall environmental impact.
- Decreased chemical waste: green synthesis approaches typically emphasize the use of environmentally friendly solvents and reagents, minimizing the generation of hazardous byproducts and chemical waste. This aspect reduces the risk of water and soil contamination and helps preserve ecosystem health.
- Lower toxicity and pollution potential: conventional synthesis methods may involve the use of toxic chemicals and hazardous reagents, which can pose risks to human health and the environment. Green

synthesis techniques prioritize the use of nontoxic or low-toxicity substances, thereby minimizing the potential for pollution and associated adverse effects.

- Renewable feedstock: green synthesis approaches typically utilize renewable feedstock or bio-based materials as starting materials, reducing reliance on non-renewable resources. By utilizing sustainable sources, such as biomass or agricultural waste, the environmental impact of the synthesis process is reduced, and the overall sustainability is improved.

- Water and energy conservation: certain green synthesis methods emphasize water-based reactions or solvent-free approaches, reducing water consumption and minimizing the need for organic solvents. Furthermore, by optimizing the synthesis process, green approaches can reduce the overall reaction time, thereby saving energy.

- Enhanced product performance: green synthesis methods can prepare TiO₂ photocatalysts with improved performance characteristics, such as higher surface areas, better crystallinity, or narrower particle size distributions. Enhanced product performance can lead to more efficient photocatalytic reactions, reducing the amount of catalyst required and thus minimizing the overall environmental impact.

CONCLUSION

Green synthesis of TiO₂ photocatalysts was performed using TiO(OH)₂ metal oxide, yielding a TiO₂ photocatalyst with a particle size of 33.28 nm at 60°C and a 0.025 M metal oxide solution. The content of Ti and O constituents in the TiO₂ photocatalyst was confirmed to be 39.06% and 47.95%, respectively, with TiO₂ crystallinity of 67.6% in the range of 25.4 degrees theta, indicating that the results of the green synthesis have formed anatase diffraction. The process temperature and metal oxide concentration are the factors influencing the formation of nanoparticles. By applying a photocatalytic to POMSE using synthesized TiO₂ photocatalyst, the following results were obtained; COD can be reduced by 16.16%-27.27%, BOD₅ by 11.05%-21.95%, and phenol by 19.49% up to 81.12%. The concentration of dissolved carbon dioxide (CO₂) in water influences the increase in the amount of oxygen (O₂). Generally, this is because CO₂ triggers an increase in the concentration of hydrogen (H) ions which decreases the pH of water to neutral, from 8.39 to a neutral pH value of 6.83-6.91. In the mechanism of the photocatalytic process, the photocatalyst used

(in this case TiO₂) functions to absorb photon energy and produce holes (h⁺). h⁺ oxidizes organic molecules and reacts with hydroxide (OH⁻) or hydrogen dioxide (H₂O). Increasing the amount of oxygen (O₂) in the liquid waste affects the BOD₅ and COD values, because the availability of sufficient O₂ in the liquid waste helps the degradation process of organic components, thereby reducing the BOD₅ and COD values in the liquid waste. The synthesized TiO₂ photocatalyst seems to specifically degrade phenol. Phenol experienced a significant reduction of up to 81.12% at a photocatalyst dose of 1 g/L and time irradiation of 2.5 h. Overall, adopting a green synthesis approach for TiO₂ photocatalyst production offers significant environmental benefits, including reduced energy consumption, decreased chemical waste, lower toxicity and pollution potential, renewable feedstock utilization, water and energy conservation, and improved product performance. These advantages contribute to a more sustainable and environmentally friendly production process for TiO₂ photocatalysts. Notably, while there are challenges associated with the green synthesis of TiO₂ photocatalysts using *L. bulgaricus*, these limitations can be addressed through further research in terms of process optimization and technological advancements. Green synthesis methods hold great potential for sustainable and eco-friendly production of TiO₂ nanoparticles, and ongoing efforts aim to overcome these challenges to make them viable alternatives to conventional synthesis methods.

AUTHOR CONTRIBUTIONS

L. Agustina contributed to the process of sampling and experimental research on laboratory (green synthesis and photocatalytic process), manuscript preparation and revision. M. Romli sharpened the background, supervised the experimental research on laboratory related to photocatalytic process, extended the discussion, and improvement recommendations. P. Suryadarma sharpened the implemented methodology, supervised the experimental research on laboratory related to green synthesis, extended the discussion, and improvement recommendations. S. Suprihatin, the corresponding author, supervised the experimental research on laboratory, organized the discussion, and proofread the manuscript.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been completely observed by the authors.

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ABBREVIATIONS

%	Per cent
Ag	Argentum
BOD ₅	Biochemical oxygen demand
C	Carbon
CO ₂	Carbon dioxide
COD	Chemical oxygen demand
CPO	Crude palm oil
Eq.	Equation
F1	1 g/L dosage of TiO ₂ ; 1.5 hour irradiation time

F2	1.5 g/L dosage of TiO ₂ ; 1.5 hour irradiation time
F3	2 g/L dosage of TiO ₂ ; 1.5 hour irradiation time
F4	1 g/L dosage of TiO ₂ ; 2 hour irradiation time
F5	1.5 g/L dosage of TiO ₂ ; 2 hour irradiation time
F6	2 g/L dosage of TiO ₂ ; 2 hour irradiation time
F7	1 g/L dosage of TiO ₂ ; 2.5 hour irradiation time
F8	1.5 g/L dosage of TiO ₂ ; 2.5 hour irradiation time
F9	2 g/L dosage of TiO ₂ ; 2.5 hour irradiation time
g	Gram
g/L	Gram per liter
H	Hydrogen
h+	Holes
H ₂ O	Hydrogen dioxide
ITB	Institut Teknologi Bandung
KS1	40° C; 0.025 M
KS2	40° C; 0.035 M
KS3	40° C; 0.045 M
L	Liter
L. <i>bulgaricus</i>	Lactobacillus bulgaricus
M	Molarity
mg	Milligram
mL	Milliliter
MRS	de Man Rogosa and Sharpe
Na	Natrium
nm	Nanometer
O	Oxygen
O ₂	Oxygen
OH ⁻	Hydroxide
°C	Celcius degrees
P1	40° C; 0.025 M
P2	50° C; 0.025 M
P3	60° C; 0.025 M
PAC	Poly aluminium chloride
pH	Potential hydrogen
POME	Palm oil mill effluent
POMSE	Palm oil mill secondary effluent

<i>ppm</i>	Part per million
<i>r-H₂</i>	oxidation-reduction potential
<i>SEM-EDX</i>	Scanning Electron Microscope-Energy Dispersive X-ray
<i>Ti</i>	Titanium
<i>TiO(OH)₂</i>	Titanium oxyhydroxide
<i>TiO₂</i>	Titanium dioxide
<i>TSS</i>	Total Suspended Solid
<i>W</i>	Watt
<i>XRD</i>	X-ray Diffraction
<i>Zn</i>	Zinc

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