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Study of *Chlorella vulgaris* from Different Growth Phases as Biosensor for Detection of Titanium and Silver Nanoparticles in Water

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ABSTRACT

The increased use of metallic nanoparticles has led to concern for environmental contamination and disruption in water quality. Therefore, effective screening of metallic nanoparticles is important for detecting metallic nanoparticles in aquatic environments. Biosensors offer several advantages, including high sensitivity to pollutants, short response time, energy efficiency, and low waste generation. In this study, a whole-cell biosensor was developed using microalga *Chlorella vulgaris* as a recognition element, and its fluorescence response was used as a measuring parameter for detecting the presence of titanium dioxide (TiO_2) and silver (Ag) nanoparticles in water. The responses of *C. vulgaris* at the lag, exponential, and stationary phases to different concentrations of TiO_2 and Ag nanoparticles were studied. The results showed that in TiO_2 and Ag nanoparticles exposures, the highest fluorescence change (50-150%) was observed at the lag phase, whereas the lowest fluorescence change (40-75%) was observed at the stationary phase. A significant fluorescence change was observed in 15 min. The immobilized *C. vulgaris* under TiO_2 and Ag nanoparticles exposures showed 30-180% higher fluorescence change than the negative control, indicating the potential of *C. vulgaris* as a biosensor for rapid detection of TiO_2 and Ag nanoparticles in water. The mathematical modeling of the responses of *C. vulgaris* to TiO_2 and Ag nanoparticles at 15 min of exposure with high R^2 indicated that this biosensor is sensitive to the concentration tested (0.010–10.000 $mg.L^{-1}$). Taken together, these results reveal that, for the first time, it is possible to detect TiO_2 and Ag nanoparticles in water within a very short time using a microalgae-based biosensor. Moreover, no genetic engineering requirement makes this biosensor simple, economical, and free from the restriction on genetically modified microorganisms for environmental applications.

INTRODUCTION

Metallic nanoparticles became popular owing to their unique physicochemical characteristics, such as substantial surface-to-volume ratio, high reactivity, and catalytic activity and use in electronics, energy, biomedicine, and the environment (Khan et al. 2019). The increased use of metallic nanoparticles raised concern over their potential negative environmental effect. Due to the diverse applications of nanoparticles in the daily production of textiles, cleaning agents, and personal care products, effluent streams receive significant quantities of nanoparticles of diverse compositions and concentrations (Bundschuh et al. 2018). These nanoparticles might bring negative consequences, such as toxicity to underwater organisms and ecosystem disruption (Lead et al. 2018).

Metallic nanoparticles are being observed to increasingly interact with the environment and produce toxic effects. Due

to their small size, metallic nanoparticles can pass through biological barriers, such as cell membranes, and interact with biological macromolecules, causing adverse effects, such as oxidative stress, inflammation, and genotoxicity (Nel et al. 2006). Furthermore, metallic nanoparticles can accumulate in various human organs and tissues, causing harm to essential organs, such as the liver, kidneys, and lungs (Zhang et al. 2012).

Some silver, iron oxide, gold, zinc oxide, and titanium oxide nanoparticles are commonly employed in biomedical, pharmaceutical, and cosmetic industries (Yaqoob et al. 2020). Among these nanoparticles, silver and titanium oxide nanoparticles have gained strong attention among researchers during the last few decades because of their potential hazards to humans and the environment. While small quantities of these nanoparticles do not pose a harmful risk upon exposure, studies have shown that they could gradually accumulate in the brain and intestinal mucosa (Lee et al. 2013). The presence of silver and titanium oxide nanoparticles exerts toxicity once a certain threshold is reached. Moreover, the study proves that titanium dioxide nanoparticles could cause cancer (Baranowska-wójcik et al. 2020). The International Agency for Research on Cancer and the National Institute for Occupational Safety and Health have classified them as possible human carcinogens (Skocaj et al. 2011). Although silver nanoparticles are not classified as a possible human carcinogen, Liao et al. (2019) indicated that even low concentrations of silver nanoparticles (<6.7 $\mu g.mL^{-1}$) could induce toxic effects, such as DNA damage and apoptosis of human red blood cells, liver cells, and bronchial epithelial cells. Furthermore, once disposed into the aquatic environment, titanium and silver nanoparticles can bioaccumulate in the human body through the food chain (Asztemborska et al. 2018, Yaqoob et al. 2020). Hence, detecting and monitoring titanium dioxide and silver nanoparticles in water is important to ensure that the water is safe to be discharged and utilized.

In the last two decades, biosensors have emerged as promising tools for detecting metal and other pollutants in environmental matrices (Odobašić et al. 2019). Generally, a biosensor contains three basic elements: biological recognition elements, transducer, and signal processing unit (Nigam & Shukla 2015). Using biosensors in detecting the pollutant of interest is based on the responses of biological recognition elements (Teo & Wong 2014). Upon exposure to metal pollutants, the responses produced by biological recognition elements are converted into detectable signals, such as optical and electrical signals through the transducer (Rocha et al. 2021). Subsequently, the signal is converted into readable information through a signal processing unit (Nigam & Shukla 2015). Biosensor offers several advantages over conventional analytical methods, including high sensitivity, real-time monitoring, portability, cost efficiency, and high speed (Bereza-Malcolm et al. 2015). Biosensors can also be designed to ideally fulfill the requirement of Sustainable Development Goals (SDG) and operated under very low energy or self-powered using biofuel cells (Grattieri & Minteer 2018, Wang et al. 2019), which is more energy-efficient than conventional analytical methods (SDG 7).

Generally, conventional analytical methods entail sample extraction with a high volume of organic solvent and sample before detection (Voon et al. 2022), whereas a biosensor requires trace amounts of reagents and produces less waste, minimizing its environmental impact (SDG 12, 13 and 14). A biosensor is easy to use, making single-person handling possible even without comprehensively understanding the separation and extraction chemistry (Kowalczyk et al. 2023).

In recent years, there has been increased interest in using microorganisms as biological recognition elements in biosensors for detecting metallic particles (Wan Jusoh & Wong 2014). Metallic nanoparticles have been shown to cause changes in growth rate, photosynthetic activity, and enzyme activity in microorganisms (Xiao et al. 2023), which can be used to signal metallic nanoparticles' presence. To date, various algae-based biosensors have been developed to detect different kinds of metallic bulk particles (Berezhetskyy et al. 2007, Scognamiglio et al. 2019, Singh & Mittal, 2012). Studies suggested that the metabolic response of microalgae to metal bulk particles and nanoparticles might be similar, probably due to the different mechanisms triggered by bulk particles and nanoparticles (AL-Ammari et al. 2021). Currently, studies concerning algae-based biosensors for detecting metallic nanoparticles, especially silver and titanium metallic nanoparticles, are yet to be developed.

Despite the potential of biosensors in detecting metallic nanoparticles, several issues are still to be addressed. One of the issues is the effect of exposure time on detecting metallic nanoparticles. The exposure time can affect the response of biosensors to metallic nanoparticles (Wan Jusoh et al. 2020). Many studies have focused on the response of the microorganisms to nanoparticles over relatively long exposure times, ranging from hours to days (Amaroa et al. 2014, Hou et al. 2014, Hui et al. 2022).

However, research on the response of microorganisms to nanoparticles over shorter exposure times (below two hours) is lacking. Studying the response of microorganisms to nanoparticles over shorter exposure times could help identify the immediate response of microorganisms to metallic nanoparticles.

Only a few studies have focused on the effect of microorganisms' growth phases on detecting metallic

nanoparticles. In many studies, cells at the exponential phase are often used as test organisms or biological recognition elements in biosensors (Amaro et al. 2011, Cheng et al. 2021, Hazeem et al. 2019). Cells from the lag or stationary phases are rarely used. Utilizing microorganisms from different growth phases might ease the construction of biosensors and the detection of metals. As microorganisms at different growth phases produce different metabolite levels (Canelli et al. 2020, Tiwari & Dhakal 2023), the responses of microorganisms at different growth phases to metallic nanoparticles might also differ.

In this study, immobilized microalga *Chlorella vulgaris* was used as the biological recognition element in the biosensor. The microalgae conduct photosynthesis in the aquatic environment and can produce fluorescence. The presence of metallic nanoparticles induces stress on the microalgae, leading change in fluorescence intensity. This study aims to investigate the response of *C. vulgaris* to the exposure of various concentrations of silver and titanium metallic nanoparticles by measuring the fluorescence intensity change for two hours. The potential for detecting silver and titanium metallic nanoparticles in a short exposure time (15 min to 2 h) was also examined. To the best of our knowledge, this is the first significant effort to detect TiO_2 and Ag nanoparticles in water within 15 min using a microalgae-based biosensor.

MATERIALS AND METHODS

Chemicals and Reagents

Bold-modified Basal freshwater nutrient solution, nanoparticles of titanium oxide (TiO_2), and silver (Ag) were purchased from Sigma-Aldrich (Darmstadt, Germany). The purity of TiO_2 and Ag nanoparticles was ≥ 99.5%. The size and surface area of TiO_2 nanoparticles were 21 nm and 35-65 $m^2.g^{-1}$, respectively, while the size and surface area of Ag nanoparticles were <100 nm and 5.0 $m^2.g^{-1}$, respectively. Both metallic nanoparticles were spherical. The freshwater nutrient solutions were diluted using distilled water before microalgae cultivation. All the glassware used was autoclaved at 121°C for 15 min before use for the experiment.

Microalgae Culture

Chlorella vulgaris was obtained from Universiti Tunku Abdul Rahman, Malaysia. The microalgae were grown and maintained in a conical flask containing 200 mL of Bold's Basal Medium. The culture was maintained at room temperature (25±2°C) and illuminated with 16 hours of cool-white fluorescence light followed by 8 hours of dark period.

Determination of Cell Growth

Growth phases of the cells were determined by cell count using a hemocytometer (Marianela-Superior, Neubauer), light microscope (Eclipse E-100 LED, Nikon), and spectrophotometer (GeneQuant 1300, GE Healthcare Life Sciences) at an optical density of 700 nm.

Immobilization of Microalgae

Before exposure to nanoparticles, immobilization was performed using a 1% (w/v) agarose medium. The volume of 0.5 mL of cells and 0.5 mL of agarose medium were added into a four-sided clear cuvette at 45°C. Then, the cuvette was sealed with plastic paraffin film (Parafilm M, Pechiney Plastic Packaging) and left at room temperature until the mixture solidified.

Exposure of Nanoparticles

The exposure test on nanoparticles is summarized in Fig. 1. The fluorescence was measured using a spectrofluorometer (Promega Glomax Multi Jr., Promega, United States of America). Five concentrations (0.001, 0.01, 0.1, 1.0, and 10 $mg.L^{-1}$) each of TiO_2 and Ag nanoparticle solutions were prepared using deionized water. A 3 mL of each concentration of nanoparticle solution was added into cuvettes containing the immobilized cells. Each sample was illuminated with light at 490 nm wavelength, and the emitted fluorescence intensity at 680-690 nm wavelength was measured using a spectrofluorometer before and every 15 min after the exposure. The immobilized cells exposed to 3 mL of deionized water were used as the negative control. All exposure tests were conducted in triplicate and repeated using cells in lag, exponential, and stationary phases. The percentage of change in fluorescence intensity after the exposure was determined using the following equation:

Change of fluorescence intensity (%) = change of fluorescence for analyte - change of fluorescence for negative control …(1)

Where the change of fluorescence for the analyte is the fluorescence change before and after exposure to metallic nanoparticles, the change of fluorescence for negative control is the fluorescence change before and after exposure to distilled water.

RESULTS AND DISCUSSION

Growth Phase of *Chlorella vulgaris*

The cell density was measured to determine the growth phase of *Chlorella vulgaris*. The growth phase of *C.*

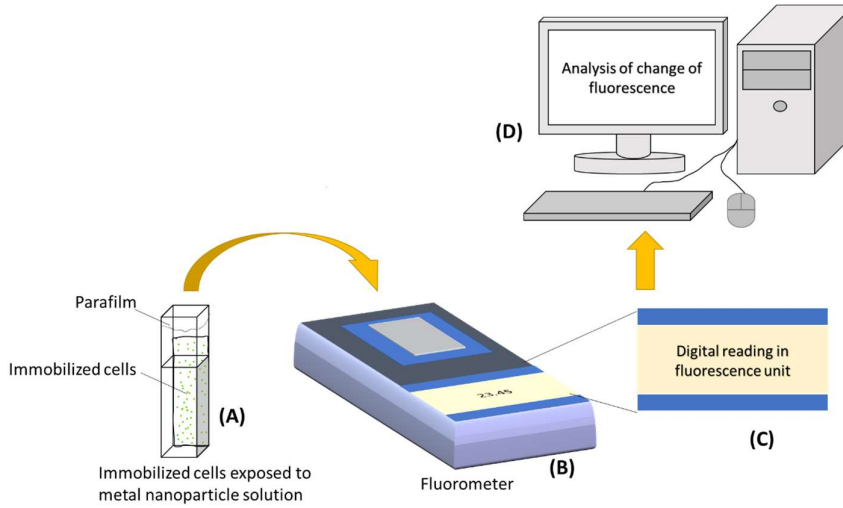

Fig. 1: Summary of exposure test on nanoparticles. Microalga *C. vulgaris* cells were immobilized in the cuvette. Then, 3 mL of the sample was added to the cuvette for the exposure test (A). The cuvette was inserted into the spectrofluorometer (B). The fluorescence intensity of 490 nm was illuminated and emitted fluorescence intensity of 680-690 nm was measured by a spectrofluorometer. The fluorescence intensity was displayed digitally (C) and analyzed using a computer (D).

vulgaris is illustrated in Fig. 2. The cell density of the *C. vulgaris* culture increased slowly in the first 2 days and then rose exponentially from day 2 until day 8. No significant increment in cell density was observed after day 8, where the cell density of 3.82×10^6 cells/mL was determined. Generally, the growth pattern of microalgae involves a lag phase, exponential phase, stationary phase, and death phase (Lee et al. 2015). At the beginning of the culture, which was known as the lag phase, microalgae species were accommodating to a new environment. Therefore, they were growing slowly. After fully accommodating to the new environment, the cells entered the exponential phase in which the cells quickly utilized the nutrients available therefore, the cell density was greatly increased. When the nutrients in the culture medium were depleted, the cells declined to grow with no significant change in cell density. When the nutrient in the medium cannot maintain the microalgal metabolism, the cells die and enter the death phase. The growth phase of *C. vulgaris* in this experiment was similar to other studies in which *C. vulgaris* has a short lag phase and quickly enters an exponential phase (Loh et al. 2021, Yatirajula et al. 2019). In the experiment, lag, exponential, and stationary phases were identified from day 0 to day 2, day 2 to day 8, and day 8 to 13, respectively. No death phase was observed in this experiment, probably due to the short cultivation period. The cells from day 1 (lag phase), day 3 (exponential phase), and day 9 (stationary phase) were chosen for the study of nanoparticle exposure.

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Exposure to Ti and Ag Nanoparticles

In this study, *C. vulgaris*, immobilized using agarose gel, was used as a biological recognition element, while the spectrofluorometer was used as a transducer and signal

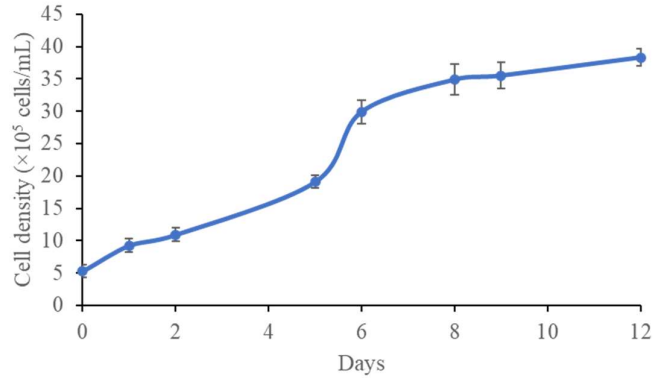

Fig. 2: Growth phase of *C. vulgaris* (n=3).

processing unit. During exposure to nanoparticles, TiO_2 and Ag nanoparticles enter the cells and disrupt the metabolism, including chlorophyll, protein, lipids, and β-carotene, of *C. vulgaris (Hazeem et al. 2019, Romero et al. 2020)*. The disruption of chlorophyll content in *C. vulgaris* changed the fluorescence, which the spectrofluorometer can detect and display.

The different growth phases of cells are critical in biosensing because the cells at various growth phases might respond differently to metal nanoparticles (Wong et al. 2018). In this study, the *C. vulgaris* cells at days 1, 3, and 9 were exposed to 0.001–10 $mg.L^{-1}$ of TiO_2 and Ag nanoparticles.

A significant increase in change of fluorescence intensity compared to negative control was observed in all concentrations of metal nanoparticles from 15 min to 120 min exposure (Fig. 3 and Fig. 4). The overall mean showed that the changes of fluorescence intensity were 84.24-174.08%, 43.59-81.44% and 43.93-64.34% respectively using immobilized *C. vulgaris* at lag, exponential and stationary phases when exposed to TiO_2 nanoparticles. For exposure to Ag nanoparticles, the changes in fluorescence intensity were 53.19-164.60%, 72.41-95.71%, and 43.07-75.09%, respectively, using immobilized *C. vulgaris* at lag, exponential, and stationary phases. Therefore, *C. vulgaris* in the lag phase showed the highest change in fluorescence intensity, whereas *C. vulgaris* in the stationary phase showed the lowest change in fluorescence intensity. The increase in fluorescence intensity detected by spectrofluorometer could be attributed to several reasons. Generally, during the measurement, the spectrofluorometer illuminates a specific wavelength of fluorescence, and it is captured by photosynthetic pigment, especially chlorophyll molecules. After absorbing the specific wavelength of fluorescence, photosynthetic pigments emit another longer wavelength of fluorescence as a byproduct that can be detected by a spectrofluorometer (Huot & Babin 2012). The high fluorescence intensity detected by the spectrofluorometer may indicate a high concentration of photosynthetic pigments. However, when photosynthetic molecules are harmed or destroyed, they can release more fluorescence than they normally would (Maxwell & Johnson 2000). Consequently, higher fluorescence intensity may be detected.

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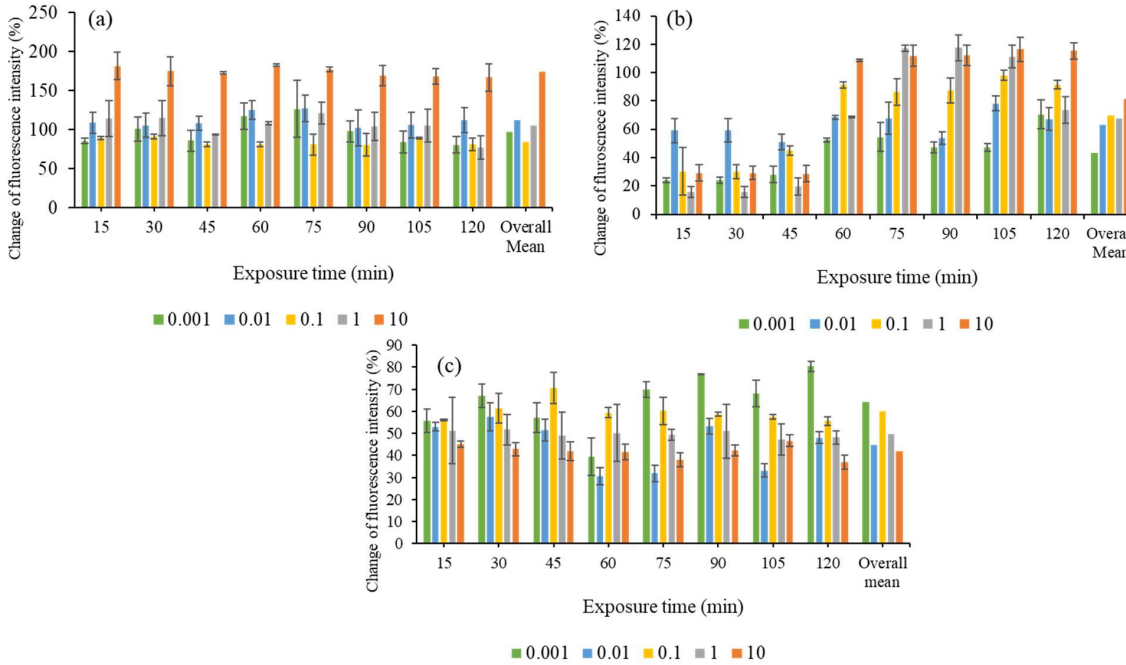

Fig. 3: Responses of *C. vulgaris* to different concentrations of TiO_2 (mg/L) during (a) lag phase (day 1), (b) exponential phase (day 3), and (c) stationary phase (day 9) (n = 3).

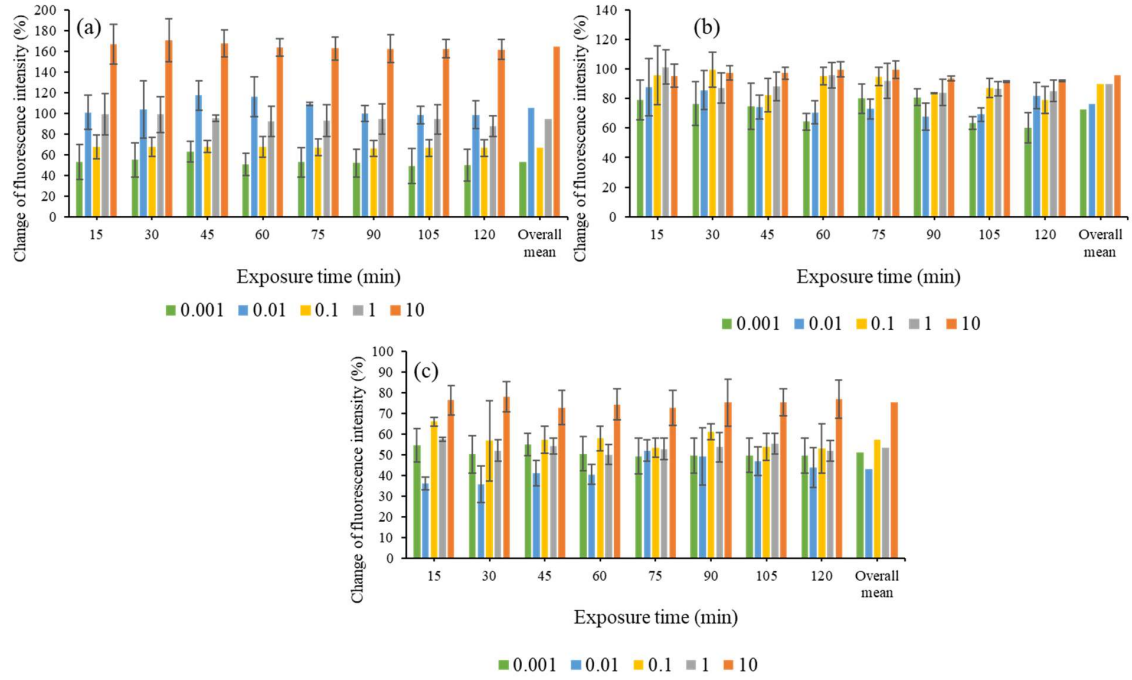

Fig. 4: Responses of *C. vulgaris* to different concentrations of Ag ($mg.L^{-1}$) during (a) lag phase (day 1), (b) exponential phase (day 3), and (c) stationary phase (day 9) (n = 3).

Previous studies showed that metal nanoparticles such as Ag and TiO_2 could directly decrease the chlorophyll content or induce the production of reactive oxygen species (ROS) in microalgae, which in turn impairs the photosynthetic pigments and other cellular molecules(Hazeem et al. 2019, Li et al. 2020). To reduce negative impacts caused by damage

to photosynthetic pigments and the production of ROS, microalgae cells can dissipate the excess energy through a non-photochemical quenching mechanism (Quaas et al. 2015). In this mechanism, excess energy is dissipated as heat within a timeframe ranging from seconds to several min (Goss & Lepetit 2015). Besides of non-photochemical quenching mechanism, microalgal photosynthetic pigments also have ROS scavenging systems to minimize the damage (Nowicka 2022). ROS scavenging systems and photochemical quenching mechanisms are affected by the microalgal growth phase (Nowicka et al. 2021). During the lag phase of growth, microalgae grow slowly and have a lower ability for non-photochemical quenching mechanism and synthesis of ROS scavenging systems because of the lower concentrations of photosynthetic pigments and other cellular components. As a result, the highest change in fluorescence intensity was detected during the lag phase. When the microalgae enter the exponential phase or stationary phase, the concentration of photosynthetic pigments and other components is increased, resulting in the greater ability for non-photochemical quenching mechanism and ROS scavenging systems (Sigaud-Kutner et al. 2002, Yusuf & Athirah 2021). Whereas the high cell density in exponential and stationary phases may also reduce the change of fluorescence intensity due to the reabsorption of fluorescence emission by neighboring cells (Wong et al. 2013).

The toxicity of metallic nanoparticles to microalgae depends on concentration, type, and exposure time. Several studies have reported that exposure to TiO_2 and Ag nanoparticles under low concentration can stimulate hormetic response. For instance, studies found that the photosynthetic activity of microalgae exposed to low concentrations of TiO_2 and Ag (<10 $mg.L^{-1}$) was increased from the beginning of the experiments (Chen et al. 2012, Zhao et al. 2021). This hormetic response in photosynthetic activity might be due to the photosensitizer effect of nanoparticles (AL-Ammari et al. 2021). During exposure, TiO_2 binds to the microalgal cell membrane and helps transfer photo-generated electrons to the thylakoid membrane of chlorophyll, enhancing photosynthesis. However, exposure to high concentrations of TiO_2 and Ag could decrease chlorophyll due to the damage to chlorophyll structure. TiO_2 and Ag nanoparticles bind with photosystem II and induce the production of ROS in chloroplast and mitochondria. These ROS render oxidative stress and damage to the chloroplast molecules, disrupting their ability to capture light energy and potentially inhibiting photosynthesis (Li et al. 2015, Sendra et al. 2017, Thiagarajan et al. 2019). To minimize the damage of nanoparticles, microalgae produce ROS-scavenging enzymes, such as superoxide dismutase and peroxidase, to convert the ROS into benign molecules (Adochite & Andronic 2021, Hazeem et al. 2019). Most studies determined the response of microalgae after 24 h of exposure to nanoparticles. In this study, the microalgae were exposed to TiO_2 and Ag from 15 to 240 min. No study has explored how the metabolism of microalgae was changed within a very short exposure time to TiO_2 and Ag. This scenario should be further studied.

Besides the aforementioned factors, the size and shape of nanoparticles are also critical factors affecting the toxicity of metallic nanoparticles to microalgae. The studies showed that smaller nanoparticles could induce more censorious toxicity in reducing growth and increasing fluorescence intensity compared to larger nanoparticles during the same cultivation period (He et al. 2019, Ivask et al. 2014, Li et al. 2020). Wang et al. (2019) extrapolated that smaller nanoparticles easily pass through the microalgal cell wall, and cell wall pore sizes larger than the size of nanoparticles lead to toxicity. However, this is not yet proven. The biocomponents on microalgal cell walls can also promote the uptake of smaller nanoparticles into the cells; smaller nanoparticles release more metal ions that facilitate more metal uptake (Zhang & Wang 2019). In addition, as particle size decreases, the surface area per volume increases greatly, rendering them to be reactive with biocomponents in microalgal cells (Abbasi et al. 2023, Zhang & Wang 2019).

Some studies revealed that spherical metallic nanoparticles are more lethal than those of other shapes (Auclair & Gagné 2022). For instance, Wang et al. (2020) found that TiO_2 nanosphere has higher inhibition on microalgal growth and stimulated more ROS production compared to TiO_2 nanotubular. Spherical metallic nanoparticles are smaller and have a high surface-to-volume ratio, which dramatically enhances the dissolution rate of nanoparticles, leading to an abundant release of metal ions 70. The size of TiO_2 and Ag spherical nanoparticles in this study were <100 nm and 21 nm, respectively, resulting in fast uptake of TiO_2 and Ag nanoparticles and remarkable fluorescence intensity change within 15 min.

Analytical Performance of Biosensor

The exposure tests confirmed that *C. vulgaris* responded to all concentrations of TiO_2 and Ag nanoparticles with a more remarkable fluorescence intensity change than the negative control, even in 15 min (Fig. 5). The mathematical modeling in Table 1 indicates the relationship between the responses of *C. vulgaris* (y symbol) at 15 min of exposure and the concentration of TiO_2 and Ag nanoparticles (x symbol). The mathematical modeling with high R^2 (Table 1) showed that the response of *C. vulgaris* highly correlates to the concentration of TiO_2 and Ag (Wong et al. 2013),

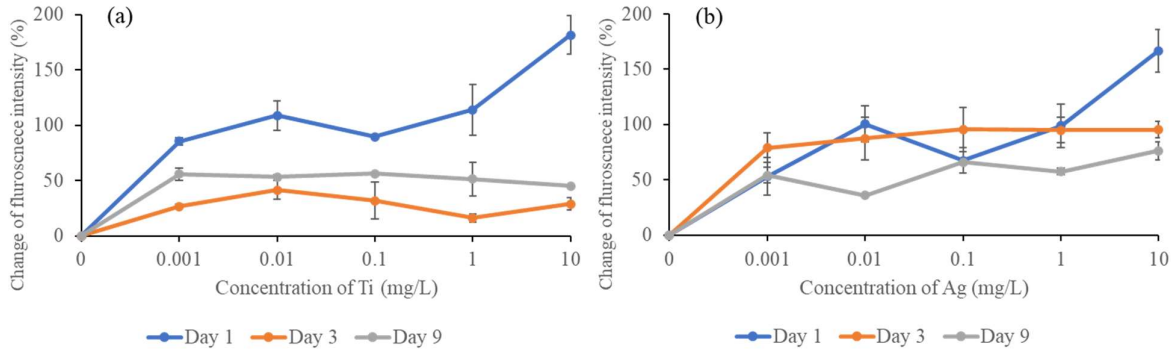

Fig. 5: Responses of *C. vulgaris* to different concentrations of TiO2 and Ag at 15 min (n = 3).

Table 1: The mathematical modeling and the correlation of the response of C. vulgaris to Ti and Ag at different growth phases.

Metals	Growth phase	Detection range [mg.L^{-1}]	Equation	R^2
TiO_2	Lag	0.001 – 10.000	$y = -1.173x^2 + 20.459x + 94.057$	0.9444
	Exponential	0.100 – 10.000	$y = 1.741x^2 - 17.674x + 31.927$	1.000
	Stationary	0.001 – 10.000	$y = 0.294x^2 - 3.940x + 55.050$	0.9207
Ag	Lag	0.010 – 10.000	$y = 85.313e^{0.0676x}$	0.8802
	Exponential	0.010 – 1.000	$y = 3.235\ln(x) + 102.030$	0.9931
	Stationary	0.010 – 10.000	$y = 34.478x^3 - 381.55x^2 + 372.09x + 32.427$	1.000

*x represented the concentration of metallic nanoparticle while y represented fluorescence intensity change

indicating the potential of using *C. vulgaris* to anticipate the concentration of metals in unknown samples (Wong et al. 2018). Moreover, the concentration of TiO_2 and Ag allowed in drinking water by water quality monitoring bodies was 0.1 mg.L^{-1} (Dong et al. 1993, World Health Organization 2021), which is in the detection range of the present developed biosensor.

Table 2 compares the metal ions between a few biosensors and the currently developed biosensors in work. Although the biosensors in other studies can detect a lower concentration of metals than the present developed biosensor, all the listed biosensors in Table 2 can be used to detect the concentration lower than 1.0×10^{-1} mg.L^{-1}, the permission limit allowed in drinking water (Dong et al. 1993, WHO 2021). The microalga *C. vulgaris* in this study can detect TiO_2 and Ag within 15 min, which is faster than in other studies (Table 2). Moreover, the *C. vulgaris* used in this study was derived from nature without any genetic engineering. Genetic engineering on an organism usually involves identifying specific traits, extracting specific genes, inserting specific genes into vector/bacterial plasmid, cloning of plasmid, transforming the transgenic plasmid into a desired organism, and growing of transgenic organism (Liu et al. 2022). More chemicals and complicated processes are required to achieve successful genetic modification. Whereas the method to construct biosensors in this study is simple and more economical. Using non-genetically modified microalga *C. vulgaris* as a biological recognition element is free from the restrictions on genetically modified microorganisms for environmental applications. Nevertheless, the biosensor used in this work intends to screen the presence of Ag and TiO_2 without identifying specific metals in the water sample.

Several possible strategies are employed to improve the application of currently developed biosensors in work. Besides TiO_2 and Ag nanoparticles, other types of metals, such as copper, zinc, and cadmium, can also disrupt the chlorophyll content of *C. vulgaris*, changing fluorescence intensity (Kondzior & Butarewicz 2018). Therefore, the currently developed biosensor could also detect the presence of these metals. As different metal nanoparticles stimulate various responses, it is indispensable to study their effects on the change of fluorescence intensity of *C. vulgaris* and the growth phase before exposure to real samples. To improve the portability of the currently developed biosensor and the possibility of in situ detection, an electrical-operated spectrofluorometer can probably be replaced by a battery-operated spectrofluorometer (Alarie et al. 1993).

CONCLUSIONS

A microalgae-based biosensor using *Chlorella vulgaris* for detecting TiO_2 and Ag has been successfully developed, with fluorescence emission utilized as a measuring parameter.

Table 2: Comparison of the metal ions (non-nanoparticles) detection using cell-based biosensors and currently developed biosensors in work.

Cell type	Metals	Exposure time [min]	The lowest limit of detection [mg.L^{-1}]	Reference
Engineered *Bacillus megaterium* with transformed *gfp* gene	Cd, Cu (II), and Zn	240	$1.42 \times 10^{-4} - 3.16 \times 10^{-4}$	(Rathnayake et al. 2021)
Engineered *E. coli* with *gfp* gene	Cu and Ag	240	1.27×10^{-3} - 4.30×10^{-3}	(Martinez et al. 2019)
Engineered *E. coli*	Hg and Cd	480	1.34×10^{-3} - 2.59×10^{-3}	(Hui et al. 2022)
Engineered *Tetrahymena thermophila*	As Cd, Cu, Zn, Hg, Pb	120	$1.80 \times 10^{-3} - 5.60 \times 10^{-1}$	(Amaro et al. 2011)
Adenine deficient yeast	Ag	120 - 180	8.90×10^{-6}	(Sun & Wang 2022)
C. vulgaris	TiO_2 and Ag	15	1.00×10^{-3} - 1.00×10^{-1}	This work

Microalga *C. vulgaris* at different growth phases responded differently to various concentrations of TiO_2 and Ag nanoparticles. The overall mean showed that the highest responses of TiO_2 and Ag nanoparticles, 84.24-174.08% and 53.19-164.60%, respectively, exposure was obtained during the lag phase, whereas the two lowest responses of TiO_2 and Ag nanoparticles exposure, 43.93-64.34% and 43.07-75.09%, respectively, were observed at the stationary phase. A significant response was observed even within 15 min. The immobilized *C. vulgaris* under TiO_2 and Ag exposure showed 30-180% higher fluorescence change than the negative control, indicating the currently developed biosensor could rapidly detect the presence of TiO_2 and Ag. The biosensor with high R^2 showed high sensitivity to TiO_2 and Ag nanoparticles, and it could be used to screen TiO_2 and Ag nanoparticles in drinking water. Taken together, these results reveal that, for the first time, it is possible to detect TiO_2 and Ag nanoparticles in water within a very short time using a microalgae-based biosensor. Furthermore, the construction of currently developed biosensors is simple, economical, and free from genetically modified microorganism concerns.

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