Journal of Biotechnology 317 (2020) 16–26

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Effects of oil substrate supplementation on production of prodigiosin by *Serratia nematodiphila* for dye-sensitized solar cell

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ARTICLE INFO

Keywords:
Dye-sensitized solar cell
Serratia nematodiphila
Prodigiosin
Oil substrate
Bioreactor

ABSTRACT

Bacterial pigments are potential substitute of chemical photosensitizer for dye-sensitized solar cell (DSSC) due to its non-toxic property and cost-effective production from microbial fermentation. *Serratia nematodiphila* YO1 was isolated from waterfall in Malaysia and identified using 16S ribosomal RNA. Characterization of the red pigment produced by the bacteria has confirmed the pigment as prodigiosin. Prodigiosin was produced from the fermentation of the bacteria in the presence of different oil substrates. Palm oil exhibited the best performance of cell growth and equivalent prodigiosin yield compared to olive oil and peanut oil. Prodigiosin produced with palm oil supplementation was 93 mg/l compared to 7.8 mg/l produced without supplementation, which recorded 11.9 times improvement. Specific growth rate of the cells improved 1.4 times when palm oil was supplemented in the medium. The prodigiosin pigment produced showed comparable performance as a DSSC sensitizer by displaying an open circuit voltage of 336.1 mV and a maximum short circuit current of 0.098 mV/cm^2. This study stands a novelty in proving that the production of prodigiosin is favorable in the presence of palm oil substrate with high saturated fat content, which has not been studied before. This is also among the first bacterial prodigiosin tested as photosensitizer for DSSC application.

1. Introduction

Renewable energy is becoming important in reducing greenhouse gas emission that leads to climate change and also in providing an alternative energy supply. Among the major resources of renewable energy such as wind (Guangul and Chala, 2019), water (Yah et al., 2017), biomass (Bhatia et al., 2018, 2019, 2020) and geothermal (Olabi et al., 2020), solar cell is considered one of the robust renewable energy resources and research is continuously being carried out to improve its operation. There are mainly three types of conventional solar cell which are monocrystalline silicon solar cell, polycrystalline silicon solar cell and thin film solar cell. Dye-sensitized solar cell (DSSC) is one type of the thin film solar cell which uses photosensitized dye as light absorber rather than semiconductor such as silicon. DSSC is the only solar cell that can offer both flexibility and transparency with its efficiency being comparable to the amorphous silicon solar cells but with a much lower cost (Gong et al., 2017). DSSCs convert inexpensive photon from solar energy to electrical energy based on sensitization of wide band gaps semiconductor dyes and electrolytes (Kimpa et al., 2012). Metal complex especially ruthenium complex has been widely applied as photosensitizer due to their photovoltaic properties and wide absorption spectra. However, the ruthenium complex is costly as compared to the

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https://doi.org/10.1016/j.jbiotec.2020.04.011
Received 7 August 2019; Received in revised form 10 April 2020; Accepted 15 April 2020
Available online 27 April 2020

organic dye and is harmful to the environment (Hagfeldt et al., 2010). A new alternative of organic sensitizer is therefore crucial to replace this toxic material.

A few studies have demonstrated the application of natural organic dye as a photosensitizer in DSSC. These pigments have been reported to be capable of serving as electron mediators because they can absorb light in the wavelength range of visible region and near-infrared region (Lee et al., 2017). Organic natural dyes for DSSC extracted from plants (Agarwal et al., 2018; Mansour and Yusuf, 2018) and microalgae (Orona-Navar et al., 2020) showed significant power conversions and efficiencies. Microbial synthesis of pigment, on the other hand, offers relatively low-cost process and easy to scale-up. Recent studies have also considered the pigments extracted from bacteria to be the photosensitizer for the DSSC. Various bacterial pigments such as pigment–-protein complexes from *Rhodopseudomonas palustris* CQV97 and *Rhodobacter azotoformans* R7 (Fu et al., 2014), bacteriorhodopsin and bacterioruberin from *Halobacterium salinarum* (Molaeirad et al., 2015), carotenoids from *Hymenobacter* sp. (red) and *Chryseobacterium* sp. (Órdenes-Aenishanslins et al., 2016), xanthophylls pigments from *Hymenobacter* sp. UV11 (Montagni et al., 2018), and melanine-like pigment from *Streptomyces fildesensis* (Silva et al., 2019) exhibited significant photovoltaic performance when tested on DSSC. Bacterial pigment as DSSC photosensitizer has recently received significant attention as it offers efficient production process with high biomass and easy to scale-up. Furthermore, the pigment extraction involves simple downstream process, making the whole production process cost-effective and environmentally friendly (Órdenes-Aenishanslins et al., 2016).

Prodigiosin is a prodigine family bacterial pigment produced by *Serratia nematodiphila, Serratia marcescens* and *Vibrio* spp. (Lin et al., 2019). It is mostly used in pharmacology as it shows anti-microbial activity (Haddix and Shanks, 2018), anti-cancer activity and low cytotoxicity in noncancerous cells (Lin et al., 2019). Because of its bright color, it has been used in textiles and as food colorant (Lin et al., 2019). The use of bacterial prodigiosin as photosensitizer for DSSC application has recently been reported (Hernández-Velasco et al., 2020). Production of prodigiosin from fermentation of *S. marcescens* and *S. nematodiphila* has been reported in various studies with nutritional supply significantly influenced the pigmentation (Haddix and Shanks, 2018). In the present work, *S. nematodiphila* YO1 was isolated from waterfall in Malaysia and prodigiosin pigment was characterized. The pigment was produced through fermentation of *S. nematodiphila* YO1 and the effects of medium pH and oil substrate supplementation was studied. The extracted prodigiosin was then tested for DSSC application.

2. Materials and methods

2.1. Bacteria isolation and cell characterization

Water sample was taken from waterfall in Malaysia, 10–15 cm from subsurface of the water body. Water sample was filtered using a 0.22 µm vacuum-driven bottle top filter (GP Millipore Express) and the filter paper was aseptically cut out and re-suspended in 50 ml distilled water. 100 µl of the re-suspended microorganisms were spread onto three different agar media plates; Nutrient agar CM003 (NA: Oxoid Hampshire, UK), Luria-Bertani agar (LB: Difco Laboratories, USA) and modified Bennett's agar (yeast extract, dextrose +, peptone). The plates were left at room temperature for 1–2 days. Red colored colonies developed on the plates were isolated and sub-cultured to give a pure culture of the bacteria species. The pigmented bacteria were grown on agar plate in a dark place and the non-pigmented colonies formed were used for Gram staining. The Gram staining was carried out by following methods from Benson (2006). For higher resolution and magnification of the bacteria, scanning electron microscope (SEM) was used. The SEM specimen preparation protocol stated in David and Robert (2006) was followed with slight modifications. First, overnight culture broth was centrifuged and supernatant was discarded. About 1 ml of 2.0% glutaraldehyde (prepared in 0.1 M phosphate buffer saline) was poured into the tube for fixation and left overnight at room temperature. The glutaraldehyde was then removed and a series of dehydration with ethanol (10%–100%). Cell pellets were coated with gold and examined by SEM.

2.2. Molecular identification

Genomic DNA of the bacteria was extracted following methods obtained from Moore et al. (2004). Polymerase chain reaction (PCR) was performed to amplify and sequence the 16S ribosomal RNA (rRNA) from the bacteria genome. The protocol stated in Barghouthi (2011) was adopted with slight modifications. For this process, universal primers and PCR master mix (Vivantis, USA) were used. Fifty µl reaction was prepared by adding 36.5 µl distilled water, 5 µl PCR buffer (10 ×), 2 µl of $MgCl_2$ (50 mM), 1 µl dNTPs (10 mM), 1 µl 27 F primer (20 pmol), 1 µl 1525R primer (20 pmol), 3 µl DNA and 0.5 µl Taq polymerase (5µ/µl). The amplification reaction was hot started at 95 °C for 5 min. The PCR cycle was set at 94 °C for 90 s, 48 °C for 35 s, 72 °C for 105 s, and a final extension step at 72 °C for 3 min with a total of 30 cycles. PCR product of approximately 1500 bp was separated using 1.5% (w/v) agarose gel electrophoresis in 1 × TBE buffer at a constant voltage of 110 V for 35 min and was purified using GeneJET PCR purification kit (Thermo Scientific, USA) prior to sequencing. The 16S ribosomal RNA sequence was deposited to GenBank with accession number MH997835.1.

2.3. Extraction and purification of pigment

The overnight bacteria culture was harvested by centrifuging 1 ml of culture broth at 8000 rpm for 10 min. Supernatant was discarded and the pellet was re-suspended in pure methanol. The mixture was vortexed and then was centrifuged at 8000 rpm for 20 min to extract the prodigiosin pigment. The cell debris (pellet) was removed, and the colored supernatant liquid was collected. Equal amount of dichloromethane was added into the methanolic pigment. The mixture was then left in a separating funnel overnight. Three layers of fluid were formed. The bottom layer, which was pigment-containing dichloromethane was separated from the other layers. Next, equal amount of 5 M sodium chloride (NaCl) solution was poured into the pigment-containing dichloromethane. The mixture was then poured into a separating funnel. When two layers formed, the colored layer was retained. The step was repeated for three times. Then, one spatula of potassium bicarbonate anhydrous powder was added into the retained liquid to remove any remaining water. The mixture was swirled for few seconds and filtered to remove the potassium bicarbonate anhydrous powder. After filtration, the liquid was poured into a glass petri dish and allowed to evaporate. The solid leftover was the pure prodigiosin pigment.

2.4. Characterization of pigment

Ultraviolet-Visible Spectroscopy (UV/vis) analysis was conducted using a 200–800 nm scan of the pigment solution. The maximum absorption peak of the pigment was identified. The solvent used for the pigment extraction was used as blank. Fourier Transform Infrared Spectroscopy (FTIR) was performed according to Ahmad et al. (2012) with minor modifications using Perkin Elmer 1605 FTIR spectrophotometer. The pigment was dissolved in small amount of solvent and then smeared on a NaCl crystal and left to dry. The crystal was then inserted in the machine and FTIR spectra were obtained from the FTIR spectrometer. NaCl crystal without pigment smear was used as blank.

Nuclear Magnetic Resonance (NMR) Spectroscopy was conducted to accurately determine the structure of the pigment sample. Pigment dissolved in chloroform was analysed for ^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) using a JEOL ECA 500 spectrometer.

2.5. Production of prodigiosin from S. nematodiphila

The medium used in cell growth and prodigiosin production was lysogeny broth and was prepared by mixing the Difco™ Lysogeny broth powder with distilled water by 1:40 ratio. The medium was sterilized by autoclaving at 121 °C for 15 min before inoculated with the bacteria. For cultivation, the bacteria were revived from the glycerol stock and cultured in lysogeny broth at constant temperature of 26 °C with shaking at 200 rpm for 48 h.

2.6. Effects of pH and oil substrate supplementation

The effects of medium initial pH and oil substrate supplementation towards *S. nematodiphila* YO1 growth and prodigiosin productions were investigated. The effect of initial pH was studied by growing the bacteria in a shake flask containing medium with initial pH of 6, 7 and 8 at 26 °C for 48 h with shaking (200 rpm). The pH of medium was adjusted by titration using 1 M HCl or 1 M NaOH. The effect of oil substrate supplementation was studied by supplementing 3% (v/v) of palm oil, olive oil or peanut oil into the medium. The bacteria was grown in the medium containing oil substrate at pH 7 and temperature of 26 °C for 48 h with shaking (200 rpm). Samples were taken at several time intervals and were tested for cell density and prodigiosin concentration.

2.7. Batch fermentation in bioreactor

Labfors 4 benchtop 3.6 l bioreactor with X-DDC operating panel (Infors) was used for the bioreactor scale batch fermentation. Seed culture was prepared by growing a single colony of the bacteria in 900 ml medium for 12 h. Next, 200 ml of the seed culture was mixed with 2 l of fresh medium in the bioreactor. The culture medium was supplemented with the 3% (v/v) of the best oil substrate identified from the previous shake flask scale experiment. The reactor was maintained at temperature of 26 °C and at the optimum pH level as identified from the previous shake flask scale experiment. The pH level was regulated by built-in control system. Air flow was maintained at 1.5 m^3/m^3 broth per minute. The agitator speed was set at 120 rpm.

2.8. Analytical methods

The cell growth was quantified by measuring cell concentration in the medium. The absorbance at wavelength 600 nm was read using Shimadzu UV-1900 UV spectrometer. The absorbance was then being correlated with the concentration of cell which was determined by measuring the dry weight (Suryawanshi et al., 2014). The prodigiosin produced was quantified by using relativity of absorbance and known concentrations of prodigiosin (Sundaramoorthy et al., 2009) at wavelength of 499 nm.

2.9. Statistical analysis

All experiments were conducted in triplicate. The data represented in graphs are means ± standard deviations. The statistical significance of the means data between two groups was determined by 2-tailed student's t-test. The statistical significance of the mean values of more than two groups was analyzed using one-way analysis of variance (ANOVA) and post hoc Tukey HSD. IBM SPSS software was used to run the analysis. A p-value of less than 0.05 is considered statistically significant and a p-value of less than 0.01 is considered highly significant.

2.10. Growth kinetic and prodigiosin production analysis

The specific growth rate of cell was calculated from Eq. (1),

$$\mu = t \cdot \ln\left(\frac{X}{X_0}\right) \tag{1}$$

where X and X_o are biomass concentration at time t and t = 0, respectively. To determine carrying capacity coefficient of the cell growth, the logistic Eq. (3) was derived from Eq. (2),

$$\frac{dX}{dt} = kX\left(1 - \frac{X}{X_{\max}}\right) \tag{2}$$

$$k = \frac{1}{\overline{X}} \times \frac{\Delta X}{\Delta t} \div \left(1 - \frac{\overline{X}}{X_{\max}}\right) \tag{3}$$

where $\overline{X}$ is the average biomass concentration during Δt and $X_{\max}$ is the maximum biomass concentration in the experiment. The cell growth yield, $Y_{X/S}$ was identified from Eq. (4). On the other hand, the production of prodigiosin was compared in terms of product yield, $Y_{P/S}$ as shown in Eq. (5),

$$Y_{X/S} = \frac{\Delta X}{\Delta S} \tag{4}$$

$$Y_{P/S} = \frac{\Delta P}{\Delta S} \tag{5}$$

whereas ΔS is the substrate consumed and ΔX and ΔP are biomass growth and prodigiosin production, respectively.

2.11. Fabrication of dye-sensitized solar cell

Titanium oxide (TiO_2) solution was prepared by adding 0.157 g of TiO_2 powder into 5 ml of 0.0035 M of acetic acid. Two drops of non-ionic surfactant triton X-100 were added into the final solution. A pair of fluorine-doped tin oxide (FTO) conductive glass slide of dimension 2.5 cm × 2.5 cm was prepared. One slide of the glass was placed above a burning candle with conductive side facing towards the flame to cover the glass with a layer of carbon. As for the other slide of glass, the predetermined conductive side was covered with a thin strip at the edge and exposing 1 cm^2 of glass (Mohammed et al., 2015). The TiO_2 was deposited on the 1 cm^2 exposed area by spin-coating technique. The depositor was set at 2000 rpm for 30 s at room temperature. The 30 µl of TiO_2 was dispensed on the glass with a pipette. After each layer of deposition, the glass was dried on a hot plate at 120 °C for 30 s. The TiO_2 deposition was repeated 7 times to obtain 7 layers. After that, the TiO_2-deposited glass was annealed at 450 °C for 30 min (Harry et al., 2016). The TiO_2-deposited glass was then immersed in the methanolic prodigiosin for 20 min. When the glass was taken out, the glass was rinsed with distilled water and air dried at room temperature for five minutes. Then, the pair of glass was sandwiched with conductive side facing each other. The position was fixed by a binder clip. The iodine triiodide electrolyte was prepared by adding 0.1269 g of pure iodine and 0.83 g of potassium iodide in 10 ml of ethylene glycol (Mohammed et al., 2015). Thirty µl of iodine triiodide electrolyte was added at the edge of the sandwiched glass at the non-overlapping side. The electrolyte was drawn into the cell by capillary action. The excess electrolyte was removed.

2.12. Photovoltaic test

The photovoltaic test for the fabricated DSSC was carried out by exposing the cell under irradiation with white light from a lamp in ambient atmosphere. The non-overlapping end of fabricated DSSC was attached to a pair of alligator clips. The alligator clips were connected to a multi-meter. The open circuit voltage and short circuit current were determined.

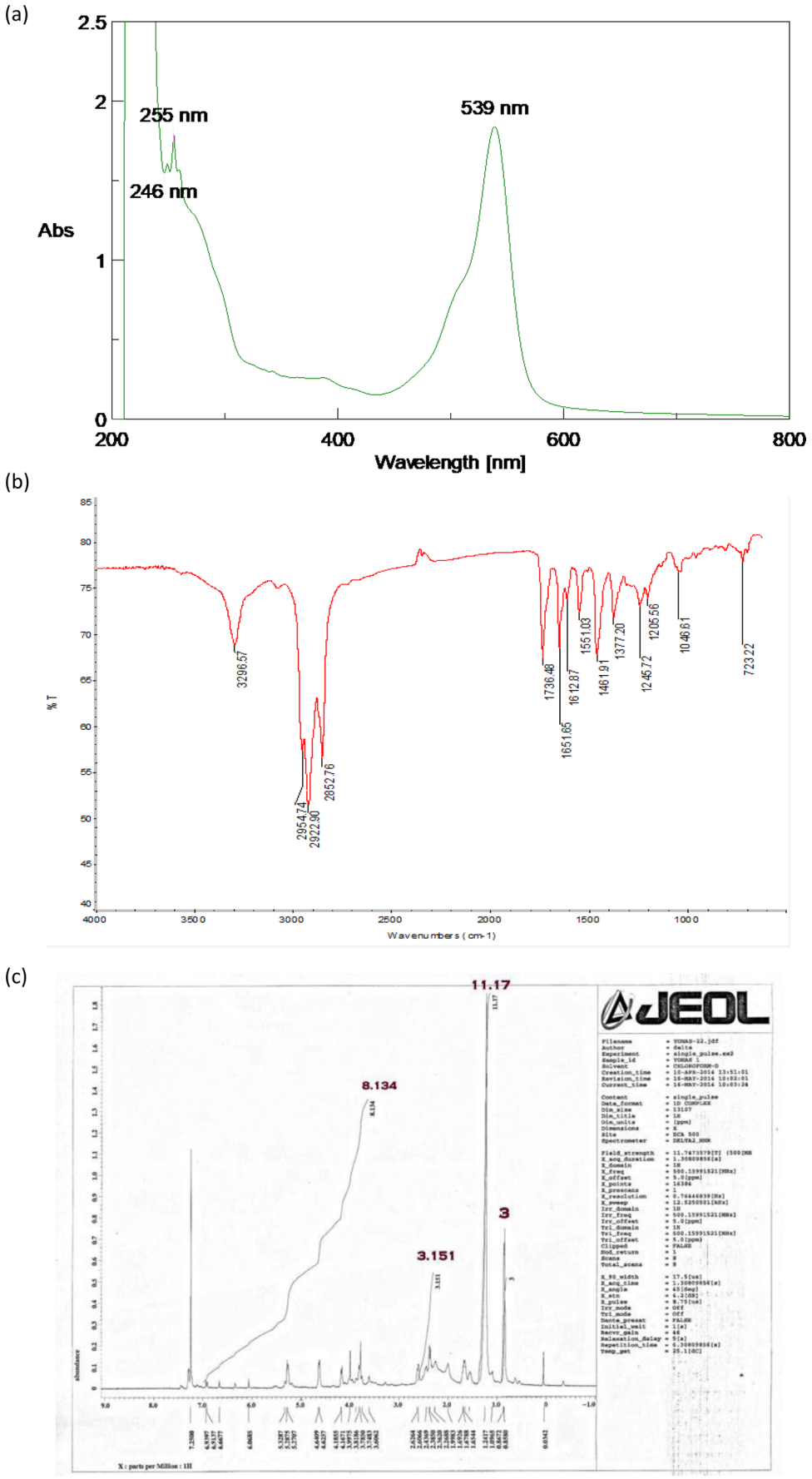

Fig. 1. Identification of pigment using (a) UV–vis spectroscopy, (b) FTIR spectroscopy and (c) H-NMR spectroscopy.

3. Results

3.1. Isolation of pigmented bacteria and pigment identification

From the water sample, red pigmented bacterium was isolated from Bennet's agar and the genome was extracted for 16S rRNA amplification. The sequence of the purified PCR product was subjected to nucleotide BLAST against NCBI database to find similarity of the sequence. From the alignment result, the 16S rRNA sequence was most similar to *Serratia nematodiphila* DZ0503SBS1 16S ribosomal RNA (accession number NR_044385.1) with 99.59% identity. The isolated bacterium was named *Serratia nematodiphila* YO1 and the 16S rRNA sequence was deposited to GenBank with the accession number of MH997835.1. Gram staining identified the bacteria as a Gram negative and SEM image showed the coccus shape of the bacteria, which is similar as described by Zhang et al. (2009). Methanolic extraction produced a bright red pigment from *S. nematodiphila* YO1 cells. The pigment was characterized using UV–vis spectrometry, FTIR spectrometry and NMR spectrometry for identification. As shown in Fig. 1a, the UV–vis spectroscopy analysis yielded a spectrum containing two main peaks of absorption at 246 nm and 539 nm. The maximum absorption peak at 539 nm corresponds to prodigiosin which agrees with the result obtained by de Araújo et al. (2010). FTIR spectroscopy analysis produced major peaks at 3292.57, 2922.90, 1736.46, 1651.65, 1551.03, 1461.91 and 1065.0 cm^{-1}, as depicted in Fig. 1b. FTIR analysis also produced a spectrum that has a good degree of similarity to the spectrum of prodigiosin obtained from previous studies. These peaks correspond to the functional groups of pyrrole, methylene, alkene and alkane, respectively, which are present in prodigiosin as described by Ahmad et al. (2012). The ^{1}H NMR analysis (500 MHz) depicted in Fig. 1c shows the integral number of hydrogen atoms present in the compound which is 25.455, similar to the number of hydrogen atoms in prodigiosin that is 25.

3.2. S. nematodiphila growth and prodigiosin production at different initial pH level

The effects of medium initial pH to the bacteria growth and prodigiosin production were observed in this study. The growth curves of *S. nematodiphila* are comparable at pH 6, pH 7 and pH 8 as shown in Fig. 2a. No significant difference in growth was observed at this range of pH. The growth rate, μ of *S. nematodiphila* growing under pH 6, 7 and 8 also shows comparable values of 0.181 h^{-1}, 0.186 h^{-1}, and 0.184 h^{-1}, respectively. However, growth at pH 7 demonstrated slightly higher carrying capacity coefficient, k, which was 0.241 h^{-1} compared to 0.163 h^{-1} at pH 8 and 0.159 h^{-1} at pH 6. The cell growth decelerated at approximately 13th hour. The deceleration in growth was commonly due to depletion of nutrient (Shuler and Kargı, 1992). The prodigiosin productions began after 12th hour for all pH level (Fig. 2b), which was at the end of exponential growth. The highest prodigiosin production was observed at pH 7 as indicated by its significant difference compared to that at pH 6 and pH 8 with p values of < 0.05 and < 0.01, respectively at 49th hour. For pH 6 and 7, the production of prodigiosin ceased since 37th hour as concentration of prodigiosin was constant beyond 37th hour at these pHs. Production of prodigiosin was observed to proceed after 37th hour at pH 8.

3.3. Effect of oil substrate towards S. nematodiphila growth and prodigiosin production

Fig. 3a portrays *S. nematodiphila* growth under effect of different oil substrates supplementation. The growth rate of *S. nematodiphila* at beginning stage was much slower with oil supplementations compared to that without oil supplementation. The exponential growth begun at approximately 13th hour which was relatively much later than the *S. nematodiphila* that grew without the oil substrate. The *S. nematodiphila* grew under effect of oil substrates has longer lag phase. The specific growth rate and carrying capacity coefficient of *S. nematodiphila* for different oil substrates in this experiment are shown in Table 1. Among the studied oil substrates, the specific growth rate of *S. nematodiphila* under the effect of palm oil substrate supplementation was the highest with 0.255 h^{-1} which is almost 1.4 times of the specific growth rate of *S. nematodiphila* without any oil substrate. Palm oil supplementation significantly promoted higher biomass concentration compared to without oil supplementation with p value of < 0.005 followed by peanut oil and olive oil with P values of < 0.05 at 49th hour.

Fig. 3b shows the production profile of prodigiosin with different oils supplementation. Prodigiosin production improved significantly with oil susbtrate supplementation compared to that without oil supplementation with p values of < 0.005. Delayed production of prodigiosin was observed in all oil substrates. The production of prodigiosin began at the end of exponential growth. The production of prodigiosin under effect of palm oil began much later than the others and was the lowest before 40th hour. However, the rate of prodigiosin production for palm oil increased greatly and exceeded the prodigiosin produced under effect of olive oil and peanut oil beyond 40th hour. The concentration of prodigiosin with palm oil as oil substrate reached its maximum at 49th hour. On the other hand, the maximum concentration of prodigiosin with peanut oil and olive oil as substrate was at 37th hour. The concentration of prodigiosin for peanut oil and olive oil dropped after 37th hour. As observed from the graph, addition of oil substrate has increased the prodigiosin production by at least 11 times. Hence, it was deduced that the effect of supplemented oil substrates towards prodigiosin production is significant.

3.4. Prodigiosin production in bioreactor-scale fermentation

The cultivation of *S. nematodiphila* in a stirred tank batch bioreactor has been carried out under controlled condition at 120 rpm agitation speed, pH 7 and 26 °C for prodigiosin production. Fig. 4 shows the cell growth and prodigiosin production level in bioreactor fermentation. The trend for cell growth and prodigiosin production in bioreactor under palm oil supplementation is similar to the flask cultivation. The depression of cell growth by increasing amount of prodigiosin was present, however in a less significant way. At around 32nd hour, the concentration of biomass plunged slightly as the prodigiosin increased. As seen from the growth curve, diauxic growth was observed at 4th hour. The exponential growth began at 10th hour and ended at 28th hour. The cultivation of *S. nematodiphila* in bioreactor has shown 5.2 times better growth yield which is 0.089 g cell/g oil substrate as compared to flask cultivation (Table 2). The cultivation of *S. nematodiphila* under controlled condition in bioreactor produced 0.875 g of biomass from 1 l of culture medium which is much higher than the flask cultivation. The specific growth rate is 0.24 h^{-1} and the carrying capacity coefficient of the cell growth in bioreactor is 0.17 h^{-1} which is higher than the shake flask experiment (Table 2). Similar to shake flask cultivation, the prodigiosin production increased rapidly at the end of exponential growth. Production of prodigiosin in this controlled condition reached 3.499 mg/g oil substrate compared to 1.880 mg/g oil substrate achieved in shake flask cultivation.

3.5. Performance of prodigiosin as sensitizer for dye-sensitized solar cell (DSSC)

The prodigiosin was extracted from the bacterial culture after fermentation under palm oil supplementation and was tested for its performance as photosensitizer in DSSC. The photovoltaic properties of DSSC using prodigiosin was measured by reading the open circuit voltage and short circuit current produced from the fabricated DSSC. The DSSC fabricated in this study had an open circuit voltage of 336.1 mV and a maximum short circuit current of 0.098 mV/cm^2. Since the open circuit was measured by connecting both terminal to the

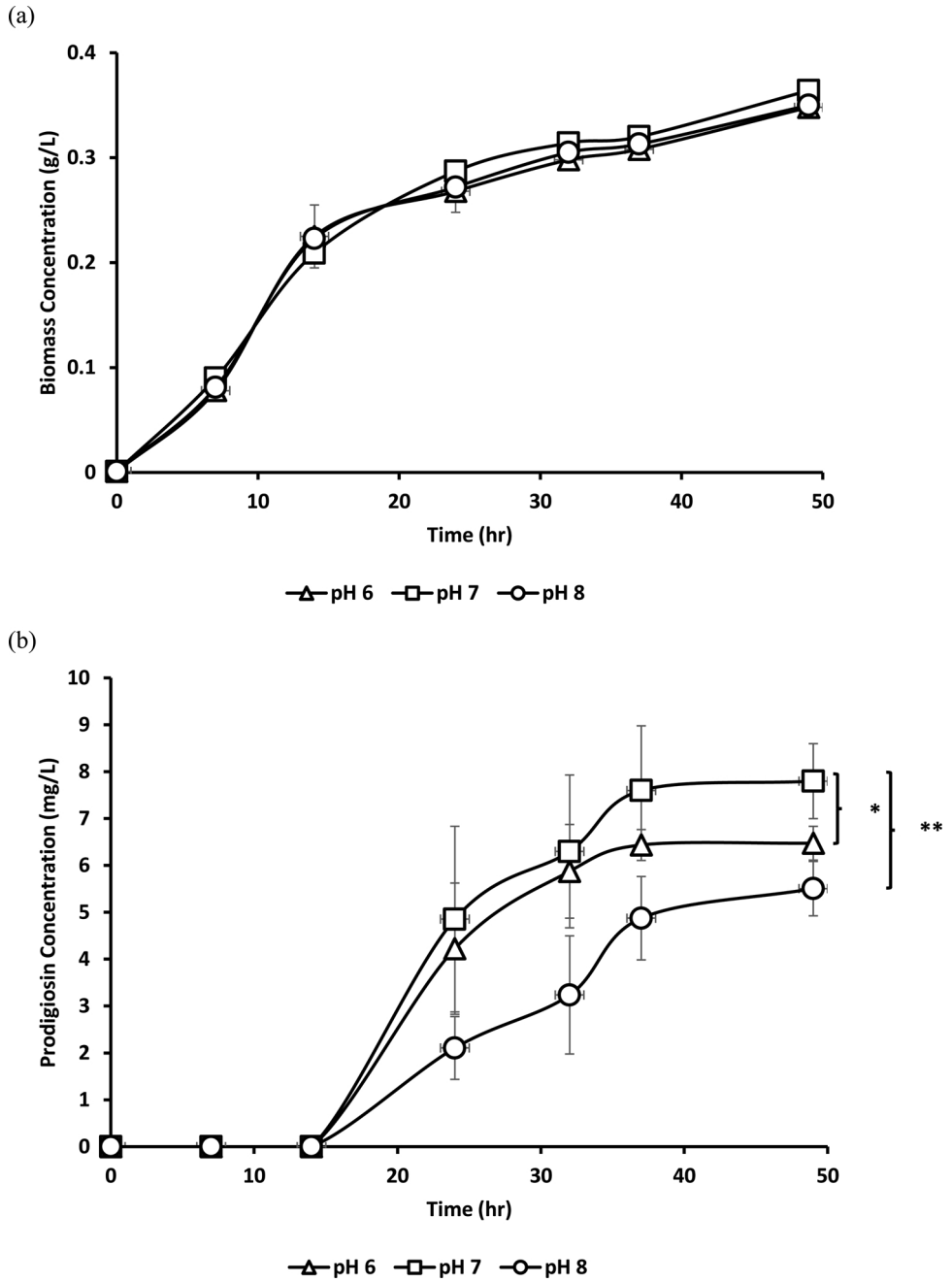

Fig. 2. Growth of *Serratia nematodiphila* (a) and production of prodigiosin (b) at different pH at 26 °C. The data presented as the mean ± S.D (n = 3). The statistical significance in mean values of more than two groups was determined using one- way analysis of variance (ANOVA) and post hoc Tukey HSD were applied using pH 7.0 as a control.
*, $P < 0.05$; **, $P < 0.01$.

multi-meter, no current flow in the circuit. Therefore, open circuit voltage was the maximum available voltage in a solar cell. On the other hand, the short circuit current was the maximum current flow in a circuit at zero voltage.

4. Discussion

The isolated *S. nematodiphila* YO1 in this study produced red pigment identified as prodiosin. Prodigiosin was successfully extracted using polar solvent methanol, which shows its polar nature. Optimization of *S. nematodiphila* YO1 growth was carried out to improve the prodigiosin production from fermentation process. During exponential growth, the cell growth was assumed to be the first order. High specific growth rate, μ at pH 7 indicates faster growth of *S. nematodiphila* YO1 as compared to the other pHs. The higher k value indicates that the pH 7 medium able to sustain larger cell population. For equal amount of nutrient provided at a certain period, the *S. nematodiphila* YO1 undergone more frequent cell division at pH 7. It could be because of macromolecular synthesis was inhibited at some degree outside the optimum pH. Optimum pH for prodigiosin production was reported at pH 7 (Sundaramoorthy et al., 2009). However, in this study, no significant difference of prodigiosin production was observed at pH 6, pH 7 and pH 8. Prodigiosin was continuously produced after 37th hour at pH 8, whereas the production was ceased at pH 6 and 7. This phenomenon could be due to decrease of the medium pH as the bacteria grew, hence the pH level might gradually decrease from pH 8 to a pH

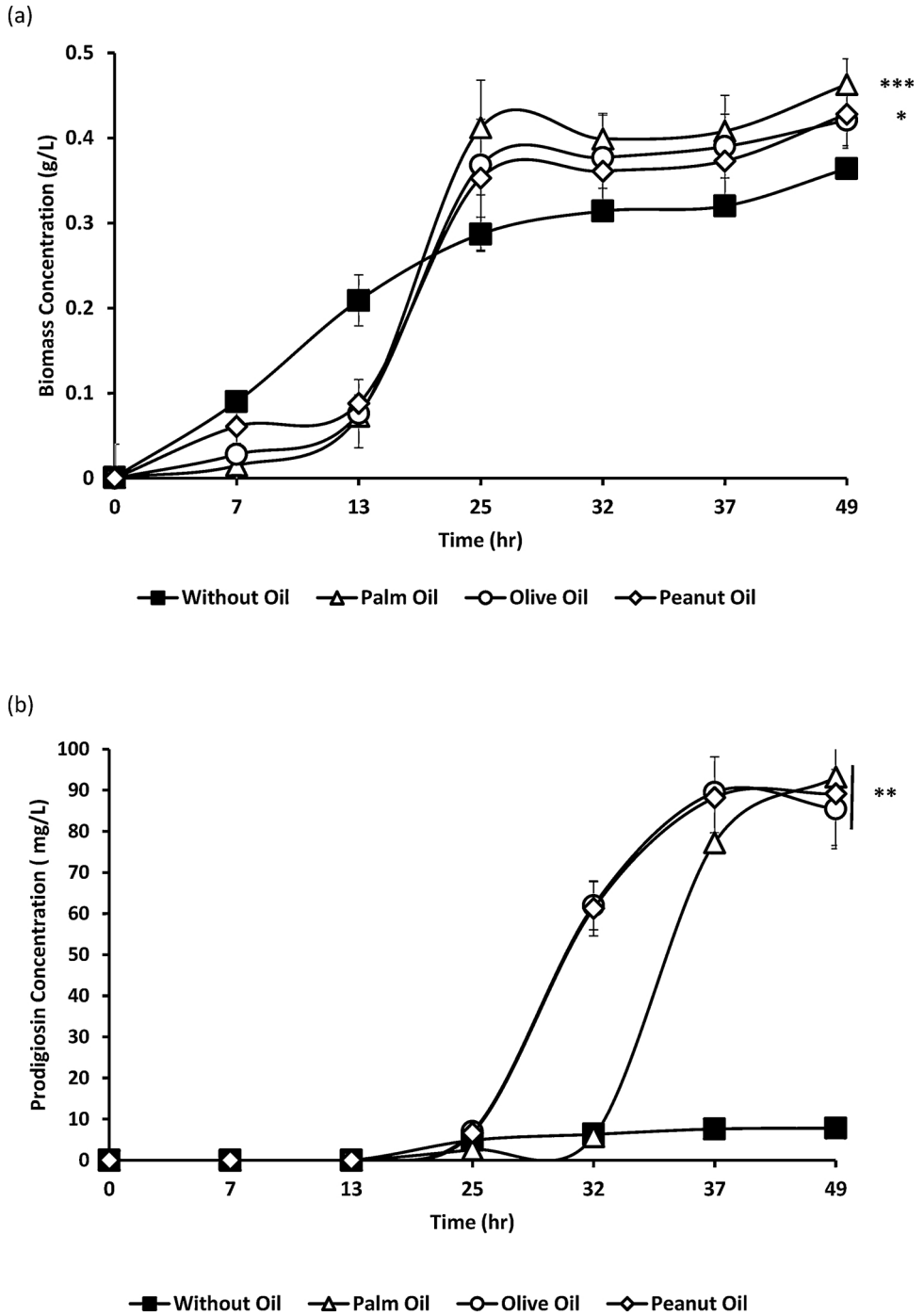

Fig. 3. Growth of *Serratia nematodiphila* (a) and production of prodigiosin (b) under supplementation of different oil substrate at pH 7 and 26 °C. The data presented as the mean ± S.D (n = 3). The statistical significance in mean values of more than two groups was determined using one- way analysis of variance (ANOVA) and post hoc Tukey HSD were applied using flask cultivation of *S. marcescens* without addition of oil substrate as a control.
*, $P < 0.05$; **, $P < 0.01$. ***, $P < 0.005$; ****, $P < 0.001$.

Table 1
Specific growth rate, μ, carrying capacity coefficient, k, growth yield of *S. nematodiphila* and prodigiosin yield under different oil substrates.

Oil substrate	Specific growth rate, μ (h^{-1})	Growth yield (g cell/g oil substrate)	Prodigiosin yield (mg prodigiosin/g oil substrate)	Carrying capacity coefficient, k (h^{-1})
Without oil Substrate	0.186	–	–	0.241
Palm oil	0.255	0.017	3.499	0.155
Olive oil	0.241	0.015	3.280	0.131
Peanut oil	0.240	0.016	3.265	0.116

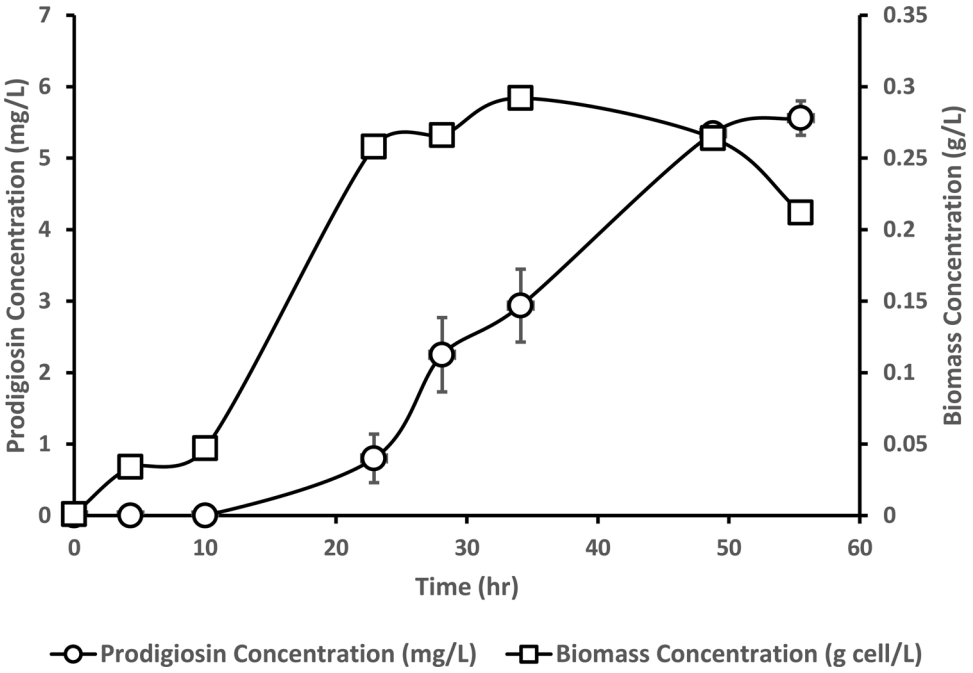

Fig. 4. Biomass concentration and production of prodigiosin in bioreactor at 120 rpm, pH 7 and 26 °C. The data presented as the mean ± S.D (n = 3).

level which able to support further production of prodigiosin (Shaikh, 2016). Prodigiosin was produced at the end of exponential growth which in accordance with several reported findings (Elkenawy et al., 2017; Fender et al., 2012; Wei and Chen, 2005). The delay in prodigiosin production is due to the characteristic of prodigiosin as secondary metabolite, which is not required for primary metabolism during cell growth and multiplication (Elkenawy et al., 2017). Pigment being secondary metabolite gives us access to easily manipulate their production without compromising the bacterial growth provided that we know the environmental factors and optimum conditions needed for their production.

The presence of glucose inhibited prodigiosin production due to catabolic repression. Hence, *S. nematodiphila* requires alternative carbon source for sustaining growth. Fatty acid has been suggested as one of the alternative carbon sources (Giri et al., 2004). The presence of palm oil, olive oil and peanut oil produced pink prodigiosin compared to red prodigiosin produced without oil supplementation. The cultivation medium affected the color of prodigiosin pigment. For example, in corn steep medium, the prodigiosin pigment produced was yellow in color (de Araújo et al., 2010). Under oil supplementation, the lag phase is lengthened. This phenomenon has also been observed in the experiment carried out by Fu and Mathews who added lactose as alternative carbon source for *Lactobacillus plantarum* (Fu and Mathews, 1999). The length of lag phase period is substrate concentration dependent (Fu and Mathews, 1999). The addition of oil substrate has lowered the carrying capacity coefficient. There is no related study found to explain this phenomenon. Further study must be done to understand the effect of oil as carbon source towards growth of *S. nematodiphila*. However, it was observed that the carrying capacity coefficient of *S. nematodiphila* growth under effect of palm oil was the highest among studied oils. The effect of palm oil for cell growth was more apparent than peanut oil and olive oil as shown by the significant improvement of biomass concentration compared with its effect toward prodigiosin production. The improvement of prodigiosin yield under these oil substrate supplementations however very significant compared to that of without oil supplementation. Palm oil has the highest content of saturated fatty acid among three types of oil as shown in Table 3. High content of saturated fatty acid has led to high yield of prodigiosin (Giri et al., 2004; Ramani et al., 2014). The delayed production of prodigiosin for palm oil could be due to high viscosity of palm oil. High viscosity will cause the carbon source in palm oil to be slowly consumed by the bacteria due to mass transfer resistance (Giri et al., 2004).

Table 3
Properties of oil substrate.

Oil substrate	Saturated fat g/100 g	Viscosity (mPas)		References
		25 °C	35 °C	
Olive oil	14	56.2	46.29	(Fasina and Colley 2008)
Peanut oil	17	57.4	45.59	
Palm oil	49	77.19	44.68	USDA food composition databases

The performance of palm oil, olive oil and peanut oil in promoting prodigiosin production was compared with other substrates as shown in Table 4. The low level of biomass and prodigiosin production could be due to the difference in bacteria strain. For example, the *S. marcescens* SMΔR is an isogenic spnR insertion-deletion mutant of *S. marcescens* which is designed to portray higher ability of sliding and prodigiosin production (Wei and Chen, 2005). From Table 4, it is shown that the *S. marcescens* SMΔR shows 4 times cell growth and 7.6 times prodigiosin production as compared to the *S. nematodiphila* YO1 that being used in

Table 2
Specific growth rate, carrying capacity coefficient and growth yield of *S. nematodiphila* for bioreactor and flask cultivation.

Cultivation	Specific growth rate, μ (h^{-1})	Growth yield (g cell/g oil substrate)	Prodigiosin yield (mg prodigiosin/g oil substrate)	Carrying capacity coefficient, k (h^{-1})
Flask cultivation	0.255	0.017	3.499	0.155
Bioreactor	0.240	0.089	1.880	0.170

Table 4
Maximum biomass and prodigiosin production by flask cultivation of *Serratia* sp. in different medium.

Medium	Bacteria	Biomass production (g/l)	Prodigiosin production (mg/l)	Reference
LB broth	*Serratia nematodiphila* YO1 isolated from fresh water	0.364	7.8	This study
LB broth with 3% (v/v) peanut oil supplement		0.428	89.1	
LB broth with 3% (v/v) olive oil supplement		0.421	89.5	
LB broth with 3% (v/v) palm oil supplement		0.463	93.2	
Medium broth with 16.97 g/l of peanut powder and 11.29 ml/l of olive oil	*Serratia marcescens* FZSF02	–	13.622	Lin et al. (2019)
LB Glucose medium	*Serratia marcescens* UCP 1549	2.865	13,500	de Araújo et al. (2010)
Modified LB broth with 4% (v/v) olive oil supplement	*Serratia marcescens* SMΔR	–	579	Wei and Chen (2005)
Modified LB broth with 6% (v/v) sunflower oil supplement		–	790	
LB broth		1.5	60	
Modified LB broth		3.8	150	
Cassava waste mannitol medium	*Serratia marcescens* UCP 1549	7.562	49,500	de Araújo et al. (2010)
Peanut oil cake extract	*Serratia marcescens* CF-53	50	29	Naik et al. (2012)
Powdered peanut seed broth	*Serratia marcescens* isolated from soil	–	39,000	Giri et al. (2004)
Peanut oil broth			2890	

Table 5
Photovoltaic properties of fabricated DSSC using various bacterial pigments as photosensitizer.

Pigment (Color)	Pigment source	Open circuit voltage, V_{OC} (mV)	Short circuit current density, I_{SC} (mA/cm^2)	Reference
Prodigiosin (Pink)	*S. nematodiphila*	336.1	0.098	This study
Prodigiosin	*S. marcescens* 11E	560	0.096	Hernández-Velasco et al. (2020)
Canthaxanthin (Red)	*Hymenobacter* sp.	435.02	0.200	Órdenes-Aenishanslins et al. (2016)
Lutein (Yellow)	*Chryseobacterium* sp.	548.79	0.130	
Xanthophyll (PURE orange)	*Hymenobacter* sp. UV11	260	0.078	Montagni et al. (2018)
Xanthophyll (RAW orange)	*Hymenobacter* sp. UV11	460	0.127	
Mixture of acteriorhodopsin and bacterioruberin (purple)	*Halobacterium salinarum.*	570	0.450	Molaeirad et al. (2015)

this experiment. Furthermore, in this study, only pH and substrate supplementation were optimized. Parameters such as temperature which will affect oil solubility, concentration of oil, light intensity and other significant growth parameter need further optimization. The prodigiosin production in peanut seed broth was higher than the prodigiosin production in this study due to the peanut seed broth has large amount of unextracted oil to be served as nutrient to support *S. marcescens* growth and production of prodigiosin (Giri et al., 2004). From Table 4, it is noticed that the peanut oil cake extract did have positive effect on cell growth and prodigiosin production although the prodigiosin production was not as much as the prodigiosin produced in this study. The peanut oil cake may have limited amount of oil after extraction. The concentration of oil does affect the cell growth and prodigiosin production (Giri et al., 2004; Wei and Chen, 2005). Although the biomass and prodigiosin production in this study were relatively lower as compared to the result from most studies, the role of palm oil substrate in promoting the cell growth and production of prodigiosin was confirmed when compared with the lower yield obtained by some studies (Lin et al., 2019; Naik et al., 2012). This comparable performance of palm oil as a substrate to improve prodigiosin production provides an equally effective substrate alternative especially when the prodigiosin is to be produced in Malaysia. As Malaysia is among the major palm oil producer, an affordable carbon source is abundantly available as a raw material. Furthermore, this is the first study on the effect of palm oil to the production of bacterial prodigiosin so far.

Comparing the fermentation under controlled condition in bioreactor, the specific growth rate of *S. nematodiphila* YO1 was slightly lower than the flask fermentation. The metabolite toxic by prodigiosin which is commonly occurring in high-cell-density cultivation in bioreactor could be one of the reasons causing the lower specific growth rate in bioreactor (Bae et al., 2001; Tao et al., 2005). From Fig. 4, it is also observed that the production of prodigiosin begun at 24th hour. The second exponential growth which supposed to spike at 28th hour was inhibited at some degree by the presence of prodigiosin. The growth yield in bioreactor should be much higher than 0.089 g cell/g oil substrate to accommodate high prodigiosin yield. The diauxic growth shown by *S. nematodiphila* YO1 in bioreactor is due to shifting in metabolic pathway by the cell to utilize a second available carbon source which is palm oil in this case (de Araújo et al., 2010). The diauxic growth was also observed at 10th hour in a study by de Araújo et al. (2010) using cassava waste mannitol as cultivation medium. The cultivation in bioreactor able to sustain larger population of *S. nematodiphila* as shown by higher carrying capacity coefficient. The higher prodigiosin production under this controlled condition of bioreactor by 1.86 times proves the easy scale-up process of bacterial pigment production.

The photovoltaic properties of fabricated DSSC using prodigiosin pigment produced under palm oil supplementation in this study was compared with other bacterial pigments in Table 5. Recently, Hernández-Velasco et al. (2020) has reported the application of prodigiosin pigment from *S. marcescens* 11E as DSSC sensitizer. The performance of prodigiosin from *S. nematodiphila* YO1 is comparable with this study and other bacterial pigments tested in other studies. The reported open circuit voltage was in the range of 260–570 mV and short circuit current density was in between 0.078–0.45 mA/cm^2. Both values of prodigiosin lays between these range, thus proving that prodigiosin shows superior photovoltaic performance.

5. Conclusions

This study has produced prodigiosin from the fermentation of *S. nematodiphila* for the application of dye-sensitized solar cell. *S.*

nematodiphila growth and prodigiosin production was optimal at pH 7 and the performance of palm oil substrate as a carbon source is comparable with other oil substrates. The biomass yield was the highest with palm oil substrate supplementation and prodigiosin yield with palm oil, peanut oil and olive oil showed at least 11 times improvement compared to that without oil supplementation. To date, no study has been performed on the effect of palm oil to the production of bacterial prodigiosin. Therefore, palm oil is a potential alternative substrate that can be used to enhance the production of bacterial prodigiosin. The improved production in the controlled condition of bioreactor indicates the possible large-scale production of this pigment. The DSSC fabricated using the pink prodigiosin pigment as photosensitizer showed comparable photovoltaic properties with other bacterial pigments from other studies. Further studies on agitation speed, oil substrate concentration as well as temperature effects for oil substrate supplemented cultivation could be carried out to optimize the production of prodigiosin from *S. nematodiphila* for the application of dye-sensitized solar cell. The maximum voltage and current could be measured for determining efficiency of DSSC in the future.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Funding

This study was supported by Research University Grant (Tier 2), Universiti Teknologi Malaysia (Grant no. R.J130000.2651.17J89).

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Acknowledgment

The authors thank Faculty of Engineering and Faculty of Resource Science and Technology Universiti Malaysia Sarawak for providing facilities to carry out the research.

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