



Faculty of Research Science and Technology

**Characterisation and Utilisation of Bacterial Pigments for Dye-sensitised
Solar Cell Development Applications**

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**Master of Science
2024**

Characterisation and Utilisation of Bacterial Pigments for Dye-Sensitised
Solar Cell Development Applications

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A thesis submitted

In fulfilment of the requirements for the degree of Master in Science

(Bioprocess)

Faculty of Resource Science and Technology

UNIVERSITI MALAYSIA SARAWAK

2024

DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.



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ACKNOWLEDGEMENT

I would like to take this opportunity to thank my supervisor Dr Nurashikin Suhaili for her caring support and motivation throughout my study who never gave up on me. I am very blessed to be under her supervision. Thank you for all the guides and words of encouragement when I need it the most. I am sincerely appreciating all the nice gestures. Not to forget Dr Kashif and Dr Kudnie from Faculty of Engineering and Dr Tay Meng Guan on the endless help in overcoming the problems faced in the experimental stages. Their kind gesture had eased my master journey. I will always be in debt for their helps. My heartfelt gratitude also goes to my dear family and friends that support me in any conditions. Last but not least, I wanna thank all the people who had been involved in my study directly or indirectly. I will not be here if not for the support of everyone that I had encountered in my life.

ABSTRACT

Dye-sensitised Solar Cell (DSSC) is a new type of solar cells that uses dye to absorb sunlight and convert it into electrical energy. Comprehensive works on the use of plant and synthetic pigments as dye sensitisers in DSSC were evidently reported. However, harmful effects of ruthenium on environment and large number of plants needed for pigment extraction have increased the interest in the use of bacterial pigments as dye sensitisers. Therefore, this study aims to investigate the potential of bacterial pigments as dye sensitisers for DSSC. The work focused on three pigmented bacteria namely *Serratia marcescens*, *Chromobacterium violaceum* and *Ralstonia* sp. The bacterial pigments were extracted and were subjected to spectra analysis for pigment characterisation. Based on UV-vis spectrophotometry and FTIR analysis the pigments from *S. marcescens*, *C. violaceum* and *Ralstonia* sp. were identified as prodigiosin, violacein and carotenoid, respectively. Scanning Electron Microscopy (SEM) was performed to characterise the behaviour of bacterial pigments adsorbed in titanium dioxide. The efficiency of the DSSC converting sunlight energy to electrical energy established using prodigiosin, carotenoid, and violacein were 0.0242%, 0.0382%, and 0.0806%, respectively which indicate promising applications of all dyes the aforementioned for DSSC. This work gives useful insights into the potential of alternative bacterial dyes for the development of DSSC.

Keywords: Dye-sensitised solar cell, pigmented bacteria, bacterial pigments, solar cell efficiency

Penggunaan Pigmen Bakteria dalam Aplikasi Sel Suria Peka Pewarna

ABSTRAK

*Sel suria peka pewarna (DSSC) ialah sejenis sel suria baharu yang menggunakan pewarna untuk menyerap cahaya matahari dan menukarkannya kepada tenaga elektrik. Kerja-kerja komprehensif telah dilaporkan mengenai penggunaan pigmen tumbuhan dan sintetik sebagai pemeka pewarna dalam DSSC. Walau bagaimanapun, disebabkan oleh kesan berbahaya Ruthenium terhadap alam sekitar dan hasil besar tumbuhan yang diperlukan untuk pengekstrakan pigmen telah mengalihkan kerja ini ke arah pigmen bakteria. Oleh itu, kajian ini bertujuan untuk menyiasat potensi pigmen bakteria sebagai pemeka pewarna untuk DSSC. Hasil kerja tertumpu kepada tiga bakteria berpigmen iaitu Serratia marcescens, Chromobacterium violaceum dan Ralstonia sp. Pigmen bakteria telah diekstrak dan tertakluk kepada analisis spektrum untuk pencirian pigmen. Berdasarkan spektrofotometri UV-vis dan analisis FTIR *S. marcescens*, *C. violaceum* dan *Ralstonia* sp telah dikenal pasti sebagai prodigiosin, violacein dan karotenoid. Scanning Electron Microscopy (SEM) telah dilakukan untuk mencirikan kelakuan pigmen bakteria yang terserap dalam Titanium Dioxide (TiO₂). Kecekapan DSSC yang ditubuhkan dengan menukar tenaga cahaya matahari ke tenaga elektrik menggunakan prodigiosin, karotenoid, dan violacein ialah 0.0242%, 0.0382%, dan 0.0806% masing-masing menunjukkan aplikasi yang menjanjikan. daripada semua pewarna untuk DSSC. Kerja ini memberikan pandangan berguna tentang potensi pewarna bakteria alternatif untuk pembangunan DSSC.*

Kata kunci: *Sel suria peka pewarna, bakteria berpigmen, pigmen bakteria, kecekapan solar sel*

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LIST OF ABBREVIATIONS

ACP	Acyl carrier protein
API	Analytical Profile Index
A-carotene	Alpha Carotene
B-carotene	Beta Carotene
<i>Burkholderia sp.</i>	<i>Burkholderia pickettii</i>
-COOH	Carboxylic
<i>C. violaceum</i>	<i>Chromobacterium violaceum</i>
<i>Chryseabacterium sp.</i>	<i>Chryseabacterium</i> species
DCM	Dichloromethane
DNase	Deoxyribonuclease
DSSC	Dye-sensitised solar cells
<i>E. coli</i>	<i>Escherichia coli</i>
FF	Fill factor
FPP	Farnesyl diphosphate
FTIR	Fourier-transform Infrared Analysis
FTO	Fluorine Tin Oxide
GGPP	Geranylgeranyl diphosphate
HOMO	Highest Occupied Molecular Orbital

<i>Hymenobacter sp.</i>	Hymenobacter species
I ₂	Iodine
IR	Current-resistance
ISC	Short Circuit Current
ITO	Indium Tin Oxide
<i>Janthinobacterium sp.</i>	<i>Janthinobacterium</i> species
Jsc	Short Circuit Current
KI	Potassium Iodide
LHC	Light Harvesting Complex
LUMO	Lowest Unoccupied Molecular Orbital
MAP	Methy-amy-l-pyrrole
MBC	Methoxy-bipyrrole-carbaldehyde
MW	Molecular Weight
NaCl	Sodium chloride
NaCO ₃	Sodium carbonate anhydrous
NRPS	Non-ribosomal peptide synthase
-OH	Hydrolic
PCP	Peptidyl Carrier Protein
PV	Photovoltaic device

PVC	Photovoltaic cells
<i>Ralstonia</i> sp.	<i>Ralstonia</i> species
Rna	Recombinant ribonucleic acid
<i>S. marcescens</i>	<i>Serratia marcescens</i>
<i>Serrantia</i> sp.	<i>Serrantia</i> species
TCO	Transparent conductive oxide
TiO	Titanium Oxide
TSA	Tryptic Soy Agar
UNIMAS	University Malaysia Sarawak
UV-VIS	Ultraviolet-visible spectoscopy
V	Voltage
VOC	Open Circuit Current
ZnO	Zinc Oxide

CHAPTER 1

INTRODUCTION

1.1 Study Background

Increased global power consumption as a result of rising human population has heightened interest in sustainable renewable energy. In addition, modern silicon-based photovoltaic cells have received much interest (Leow et al., 2016). The establishment of Photovoltaic Cells (PVCs) since 20 years ago has led to the progress of devices designed to expand PVC applications and reduce their production costs in order to compete with energy generation systems. Due to easier fabrication along with lower cost for it and technology that adopted sustainable sources that environmentally friendly, the latest revolutionary process Dye-Sensitised Solar Cell (DSSC) has received wide attention recently (Ludin et al., 2014). DSSC is the third generation of solar cells comprised of wide bandgap semiconductors as substrate and the dyes that works as photon harvesting (Al-Alwani, 2018).

Performance of DSSC can be measured through photo-to-electric conversion efficiency. It measure ability of the DSSC to convert light energy to electrical energy. The efficiency of the DSSC depends on the photo sensitiser which is the dye layer that have the characteristics corresponding to the spectrum of available light (Grätzel et al., 2003). At the centre of the system is a nanoporous oxide layer, which is typically comprised of titanium dioxide in the form of anatase. The dye forms a monolayer film on the surface of the titanium dioxide. There are two types of dyes used in DSSC namely synthetic and natural dyes. The most common synthetic dyes used is ruthenium due to its high efficiency and excellent stability reaching 1-12% (Pratiwi et al., 2016). However, currently, there is an growing interest on the usage of natural dyes as environmental friendly alternatives. Natural dyes are

originated from plants or microorganisms such as bacteria and algae. The dye can be extracted from various part of plants such as leaves, fruits, and flowers. The successful DSSC fabrication using plants pigments such as betacyanin, chlorophyll, anthocyanin, tannin, β -carotene and flavonoid has been reported in the literature (Garcia et al., 2003). A number of works targeting natural pigments were reported. In a work by Chang and co-workers (2013) the use of chlorophyll extracted from wormwood and anthocyanin originated from red cabbage as dye-sensitiser in DSSC has been reported where the efficiency of the two dyes were reported to be 0.9% and 1.47%, respectively.

1.2 Problem Statement

Whilst various works have reported the use of natural dyes as sensitisers for DSSC, there remains a need to uncover the potential of dyes produced by various organisms especially bacteria. Presently, the natural pigments utilised in DSSC fabrication are primarily of plant derivation, such as betalainins, carotenoids, chlorophyll, and anthocyanins, which are derived from fruits, flowers, leaves, seeds, roots, and wood (Narayan, 2012). However, given the huge biomass production by bacterial cultures in a short period, acquiring bacterial pigments can be more efficient and less expensive than that from plants. In additions, the dye extraction methods for pigmented bacteria are relatively less complex. Although, there are some reports of bacterial pigments, the studies are limited to certain types of bacteria. Furthermore, the hunt for novel dyes with appropriate conversion efficiencies is a challenge.

Marizcurrena and co-workers (2021) assessed the usage of xanthophylls as DSSC dye sensitizers isolated from the *Hymenobacter* sp. UV11, Antarctic bacterium with a cell's efficiency of 0.03% in earlier research. In the same report, violacein was also reported to be extracted from *Janthinobacterium* sp. UV13 UV-resistant Antarctic bacterium where the efficiency was found to be 0.23%. In other report by Hernandez-Velasco et al., (2020),

prodigiosin extracted from *Serratia marcescens* 11E was found to have an efficiency of 0.032%. Carotenoids extracted from *Hymenobacteria* sp. and *Chryseobacterium* sp. were reported to result in the efficiency of 0.0332% and 0.0323%, respectively (Ordenes et al., 2016). In the latest report by Gadave et al., (2021), carotenoid usage using lycopene sensitized ZnO photoanode-based DSSC was reported with the efficiency of 0.39%. The hunt for novel pigments or dyes with appropriate efficiencies conversion is a challenge in this context.

Studies on *C. violacein*, *S. marcescens* and *Ralstonia* sp. are in general still scarce. These bacteria have great potential as sources of dyes because they contain dye molecules from their secondary metabolites known as pigment that able to absorbs sunlight energy. Hence, more research is needed to provide further insights into the potential of the aforementioned bacterial pigments as dye sensitiser candidates for DSSC.

1.3 Objectives

Therefore, this research aims to establish knowledge on the potential of selected bacterial dyes as dye sensitisers for DSSC. The objectives of this research are as follows:

- i. To characterise selected bacterial pigments.
- ii. To evaluate the potential of selected bacterial pigments as dyes for DSSC.
- iii. To fabricate DSSC using selected bacterial pigments and to determine the efficiency of DSSC established.

CHAPTER 2

LITERATURE REVIEW

2.1 Background of Selected Pigmented Bacteria

Novel bioactive compounds are abundant in microorganisms because, unlike higher organisms, they are a source of easy renewable resources that can lead to increased yields. (Wang et al., 2018). Microorganisms produce a different type of pigments with distinct properties as a result of environmental influences, most of which are related to the microorganism's relationship with the ecosystem. Bacteria have a lot of potential for making a variety of bioproducts, including pigments. Numerous researchers were researching the production and use of bacterial pigments as natural colourants (Ahmad et al., 2012). Production of bacterial pigment has become among the most promising research fields, since the pigments have potentials to be used in a variety of industries (Venil et al., 2009). Carotenoids, indigoidines, flavin, melanins, monascines, violaceins, quinones, and phenazines are examples of the microbial pigments that have been discovered in various environments (Pombeiro-Sponchiado et al., 2017). Amongst the genera that produce pigments include *Streptomyces*, *Nocardia*, *Thermomonospora*, *Microbispora*, *Streptosporangium*, *Rhodococcus*, and *Kitasatospora* (Lana et al., 2014).

2.1.1 *Serratia marcescens*

Serratia marcescens is associated with a long fanatical history that dates back to antiquity when it was identified as having 'masqueraded' as *S. marcescens* blood due to its ability to create a red pigment. The unique red colour of *S. marcescens* along with a distinct low-level pathogenicity, At the turn of the century, it was used as a biological indicator of infection. As a result, *S. marcescens* has been used in a variety of typical bacterial

transmission studies, contributing to a greater understanding of infection epidemiology. *S. marcescens* was utilised as infectious weapon test by US military performed public under more questionable circumstances (Mahlen, 2011).

In the clinical setting, non-pigmented *S. marcescens* isolates outnumbered pigmented strains from 1960 onwards, and were highly engaged in health-related infection, especially patients with a compromised immune system (Şimşek, 2019). *Serratia* sp. belongs to the *Enterobacteriaceae* family and are classified as gram negative bacillus which are motile which do not form endospore. *Serratia* common extraction were either from the bloodstream and wound sites in the laboratory culturing on blood agar or selective culture methods from the respiratory and urinary tract. Popular selective agar cultures include MacConkey agar that classifies *Serratia* isolates into a wide grouping of *Klebsiella*, *Enterobacter*, *Serratia* and *Citrobacter* (KESC) with other non-lactose fermenting *Enterobacteriaceae* or chromogenic agars (Abbott, 2011).

For the detection of *Serratia*, various form of PCR and serological typing may be used. It is also possible to use biotyping, bacteriocin typing, phage typing, plasmid analysis, and ribotyping (Menozzi et al., 2006). *Serratia* is one of the easiest taxa to recognise within the *Enterobacteriaceae* family. Unlike other *Enterobacteria*, *Serratia* strains generally produce extracellular deoxyribonuclease (DNase), gelatinase, and lipase, and are resistant to the antibiotics colistin and cephalothinin. *Serratia* sp. has historically been detected by the phenotypic microbial recognition method using the Analytical Profile Index (API) 20E (bio-Merieux) (Schaumann et al., 2013). *Serratia marcescens* can survive in the range of pH 5-9 and temperature of 5-40 °C which is also motile bacteria. They will swarm together on agar in lower concentrations as a group (0.5-0.8%) (Harshey, 2003).

It is characterised by its ability to carry out casein hydrolysis from other Gram-negative bacteria, which helps it to generate extracellular metalloproteinases that are thought to operate in cell-to-extracellular matrix interactions (Hemraj et al., 2013). This bacterium is a facultative anaerobe, meaning it can flourish in the presence of oxygen (aerobic) or in the absence of oxygen (anaerobic), and it can reduce nitrates in anaerobic conditions. Nitrate tests are also positive, as nitrate is commonly used instead of oxygen as the final electron acceptor. It uses fermentation mainly as a way of collecting energy and has enzymes (superoxide dismutase, catalase or peroxidase) that shield it from reactive oxygen species, enabling it to survive in oxygenated environments. *S. marcescens* also displays tyrosine hydrolysis and degradation of citrate. *S. marcescens* test positive on citrate test since it utilises citrate to make pyruvic acid. Therefore, citrate can be used as carbon source. A methyl red test can also be conducted when determining the organism, which decides whether a microorganism performs mixed-acid fermentation. *S. marcescens* leads in a test that is negative (Rebecca et al., 2013).

There are other biochemical studies in the laboratory that help to classify *Serratia marcescens*. Due to their synthesis of 2, 3, butanediol and ethanol, it is negative for the methyl red test, but positive for the Voges-Proskauer test, which indicates the capacity of an organism to transform pyruvate to acetoin. It is positive for decarboxylase, which is the elimination of an amino acid producing amine and carbon dioxide from the carboxyl group. A distinctive element is the red pigmentation (prodigiosin) for which *S. marcescens* is recognised, but it is only present in certain strains (Luthra et al., 2014).

2.1.2 *Chromobacterium violaceum*

Chromobacterium violaceum is a gram-negative bacterium that shaped-rod with facultative anaerobic ability. It grows easily at 35 to 37 °C on simple nutrient media such as

MacConkey agar. When tested with the catalase test and oxidation reaction, it produces positive results for both tests (Ray et al., 2014). The organism may lose pigmentation from the parental strain in future cultures or be exposed to harsh circumstances, such as freeze-thaw cycles in laboratories. *Aeromonas* or *Vibrio* species may be mistaken for nonpigmented isolates, despite the fact that pigmented colonies are easily distinguishable. Since *C. violaceum*, like most *Aeromonas* species, can be hemolytic, biochemical features like positive acid generation from maltose and mannitol by *Aeromonas* species can be used to distinguish these organisms. *C. violaceum* can currently be classified by automatic biochemical methods (Vitek, MicroScan, and Phoenix), MALDI-TOF MS, or 16S rRNA sequencing technologies in diagnostic laboratories (Abbot et al., 2003).

C. violaceum is resistant to most penicillins and cephalosporins, but commonly susceptible, to carbapenems, aminoglycosides, cotrimoxazole, chloramphenicol, tetracyclines and fluoroquinolones (Brown et al., 2006). *Chromobacterium* sp. strain C-61 environmental isolates have the ability to be used in other biotechnological uses, including the biocontrol of insect pest-induced plant diseases (Kim et al., 2014).

This non-pathogenic bacterium is a prevalent saprophyte found in water and soil in tropical and subtropical areas around the world (Savini et al., 2017). It is a low-grade pathogen that, despite its extensive geographic distribution, only causes a few cases of infection in immunocompetent individuals and is typically disregarded as a contaminant when cultures are studied. In 1881, it was first discovered, and its pathogenic potential was first described by P.G. Woolley, who isolated it from a deadly buffalo virus in the Philippines in 1905. It was first identified in Malaysia in 1927 by J E Lessler as a human pathogen (Ray et al., 2014).

There are both pigmented and non-pigmented strains, but there are uncommon non-pigmented strains. Pigmented strains produce violacein, a violet non-diffusible pigment that is soluble in ethanol but insoluble in water and chloroform. The violet colony is caused by violacein [3-(1, 2-dihydro-5-(5-hydroxy-1H-indol-3-yl)-2-oxo-3H-pyrrol-3-ilydene)-1,3-dihydro-2H-indol-2-one], a pigment with antibacterial, trypanocidal, tumoricidal, mycobacterial, and antioxidant properties (Ahmad et al., 2012).

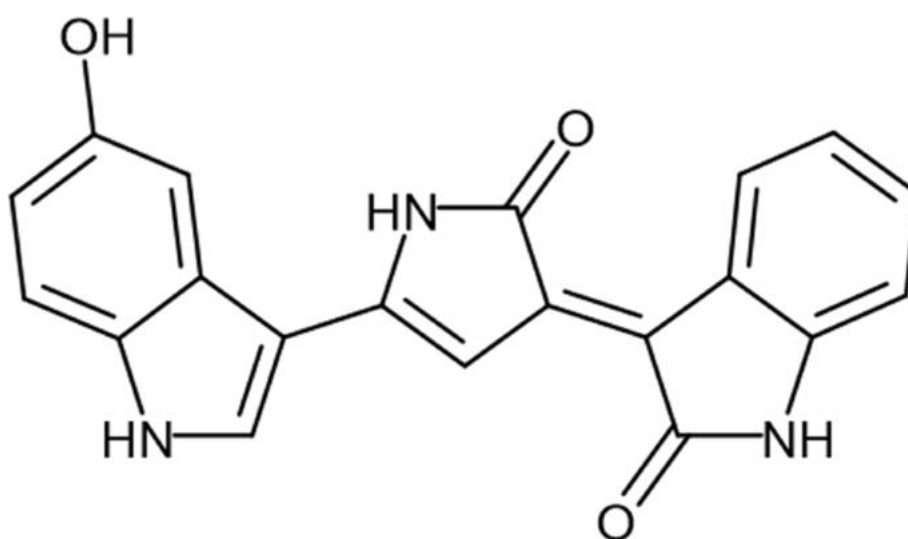


Figure 2.1: Chemical Structure of Violacein (Venegas et al., 2019)

2.1.3 *Ralstonia* sp.

Ralstonia is a Proteobacteria genus previously included in the *Pseudomonas* genus. It is named after Ericka Ralston, an American bacteriologist. *Ralstonia pickettii*, *Ralstonia insidiosa*, and *Ralstonia mannitolilytica* have been found in a variety of habitats, including clinical settings where they can act as pathogens (Lee et al., 2017). *Ralstonia* is a genus that contains former *Burkholderia* sp. members (*Burkholderia pickettii* and *solanacearum* *Burkholderia*) (Coenye & Vandamme, 2003). Aerobic Gram-negative, non-fermentative rods present in water and soil are *Ralstonia* sp. *Ralstonia pickettii* falls under *Ralstonia* genus. The bacteria has been found in blood, urine, and cerebrospinal fluid, among other

clinical specimens. The bacteria were isolated from various sources of water, including public supplies of drinking water, bottled water, dental water supplies, supplies of hospital water, space shuttle water systems, normal filtered water, high-purity laboratory-based water systems and ultra-pure/high-purity industrial water (Lee et al., 2010).

Ralstonia pickettii thrives in saline and other fluids, and has been linked to various outbreaks involving contaminated infusates and pseudo-outbreaks using tainted laboratory diagnostic solutions (Ryan et al., 2006). During the processing period and by extrinsic manipulation, the oxidation of solutions took place. In addition to bacteremia from contaminated intravenous medicines, contaminated respiratory treatment solutions have caused airway colonisation. In one epidemic caused by contaminated saline solution, only one of 19 patients with *R. pickettii* airway colonisation received antibiotic therapy, possibly due to the low virulence of the bacterium. There have since been many hospital-associated infections due to *R. mannitolilytica*. There are less common clinical isolates of other *Ralstonia* species. *Ralstonia* species grow on regular media, but development can be slow and colonial visualisation takes more than 72 hours of incubation. In motile species, *Ralstonia* species have one or two polar flagella, contain glucose acid and many other carbohydrates, and are resistant to colistin (Ryan et al., 2007).

Ralstonia solanacearum, a commonly available and economically important plant pathogen, invades the roots of different plant hosts from the soil and actively colonizes xylem tubes, producing a deadly wilting known as bacterial wilt disease. It is a soil-borne gram-negative bacterium that causes bacterial wilt disease in more than 200 plant families, including primary crops such as potatoes, tomatoes, peanuts, tobacco, bananas and plantains, as well as many native plant species. The *R. solanacearum* possesses swimming motility regulated by three to four polar flagella (Tans-Kersten et al., 2001). It is also tested negative

for potassium hydroxide solubility test and showed negative results in carbohydrate test for four simple sugars: dextrose, sucrose, mannitol and lactose (Rahman, 2010). Many reports on *Ralstonia* sp are on the pathogenesis of the bacterium towards plants and there are still limited published reports on yellow pigment produced by this bacterium.

2.2 Background of Selected Pigments

2.2.1 Prodigiosin

Prodigiosin, a secondary metabolite, has been reported to be formed by *S.marcescens*, *Vibrio psychroerythrous*, *Vibrio gazogenes*, *Pseudomonas magneslorubra*, *Serratia rubidaea*, *Rugamonas rubra*, *Alteromonas rubra* and Gram positive *actinomycets*, such as *Streptoverticillium rubrireticuli* and *Streptomyces longisporus* *ruber* forms prodigiosin and/or derivatives of this molecule (Giri et al., 2004; Khanafari et al., 2006).

The natural product prodigiosin group is a family of tripyrrole red pigment containing a common 4-methoxy 2,2 bipyrole ring system. The structure of prodigiosin consist of $C_{20}H_{25}N_3O$ as shown in Figure 2.1. Thus, the IUPAC Name for prodigiosin is (2Z)-3-methoxy-2-[(5-methyl-4-pentyl-1H-pyrrol-2-yl)methylidene]-5-(1H-pyrrol-2-yl)pyrrole with molecular weight of 323.4 g/mol (Juang et al., 2012).

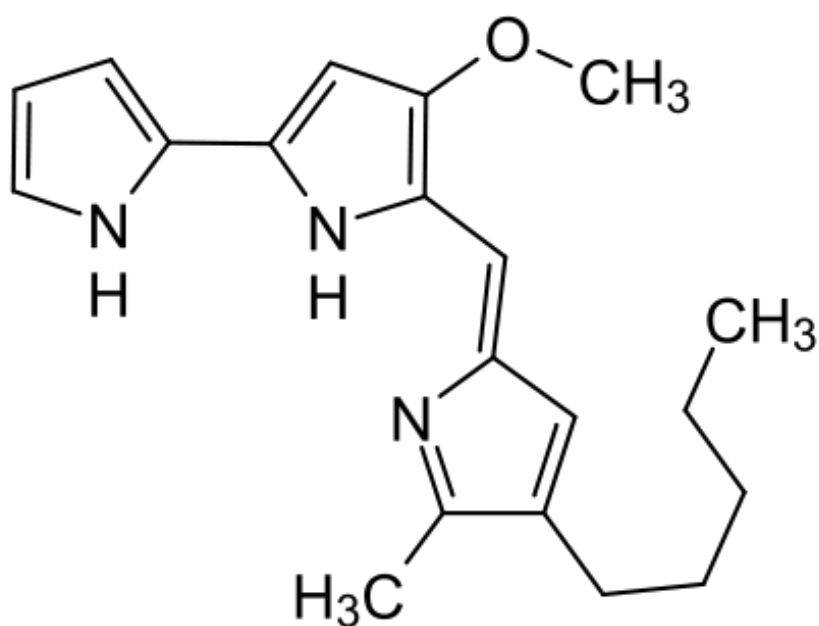


Figure 2.2: Prodigiosin Structure (Wals et al., 2006)

In the early 1960s, the structure of prodigiosin, an archetypal prodiginin, was elucidated by partial and complete chemical synthesis that exposed the pyrrolyl dipyrromethene core skeleton. The three prodigiosin pyrolic circles are conventionally known as pyrolic ring A, ring B, and ring C shown in Figure 2.2. Prodigiosin occurs in two interconverting rotamers, cis (or β) and trans (or α). The equilibrium of both types relies on the pH of the solution, since the trans form protonates more quickly (Darshan, 2015).

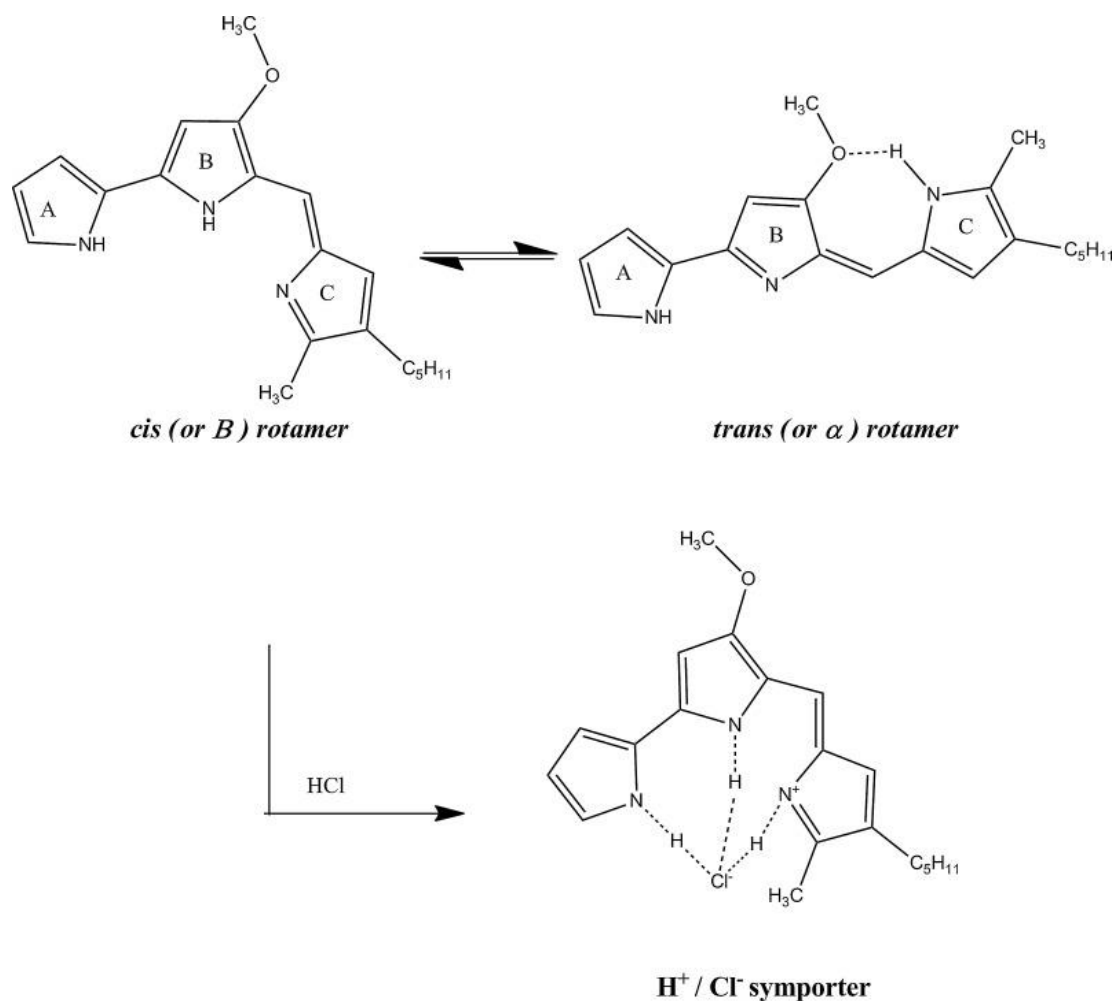


Figure 2.3: Prodigiosin Pyrolic Cycles (Darshan, 2015)

Recent works have shown that prodigiosin is formed via a bifurcated pathway, which results in the enzyme condensation of the two pathway terminal components, 2-methyl-3-n-amylyl-pyrrole (MAP) and 4-methoxy-2,2'-bipyrrole-5-carbaldehyde (MBC) (Williamson et al., 2006).

Prodiginines have been actively studied for medicinally important properties including antimalarial function, a distinct mechanism of immunosuppression from cyclosporine A, FK506, and rapamycin,¹ and their capacity to selectively trigger apoptosis in many human cancer cell lines. A synthetic prodiginine-based analog, obatoclax, was used in several step I and combination II cancer chemotherapy trials (Hu et al., 2016). Prodigiosin

biosynthesis requires the converging of three rings of the L-proline, L-Serine, L-methionine, pyruvate and 2-octenal pyruvate. Through non-ribosomal peptide synthase (NRPS) pathway. In Figure 2.3 adenylation of L-proline by the addition of peptidyl carrier protein (PCP) in L-proline structure is shown.

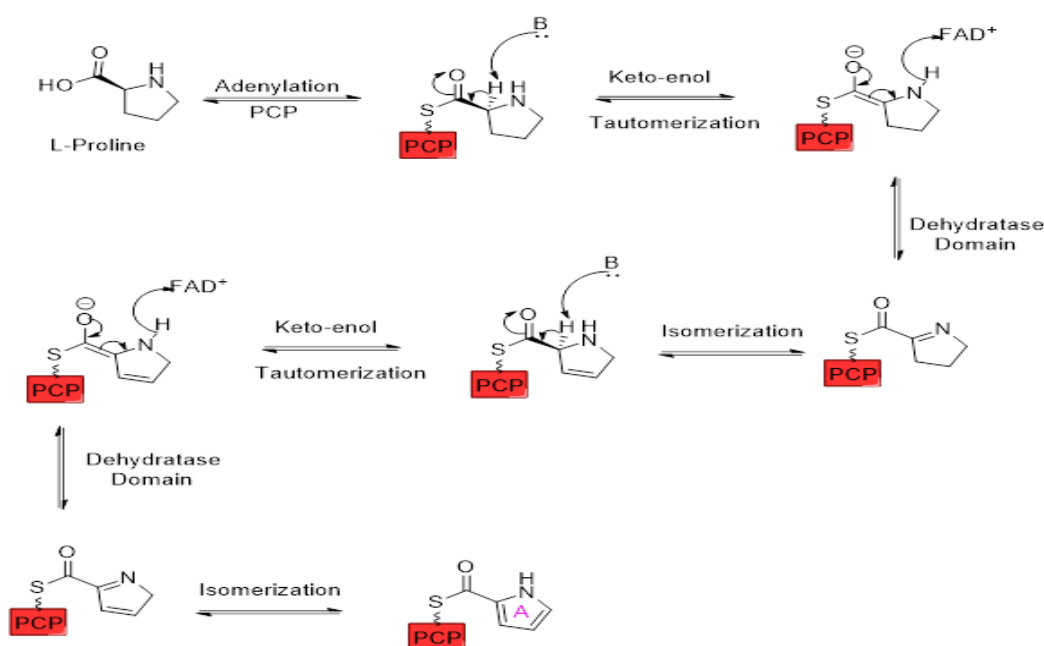


Figure 2.4: Formation of Ring A Fragment (Hu et al., 2016)

The pyrrolidine ring of proline undergoes oxidation twice through FAD^+ resulting in pyrrole ring A. Stretching of ring A is through integration of L-serine into ring B by polyketide synthase pathway. KS domain transfer ring A from PCP to acyl carrier protein (ACP). Then, decarboxylation Claisen condensation transfers it to malonyl-ACP. This fragment is then capable of interacting with the masked carbanion produced by the L-Serine induced PLP decarboxylation, which is cyclic in a dehydration reaction to create the second ring of pyrrole. Modification occurs to the intermediate by methylation where methyl group integrates onto the alcohol at the 6 position from L-methionine as illustrated in Figure 2.4.

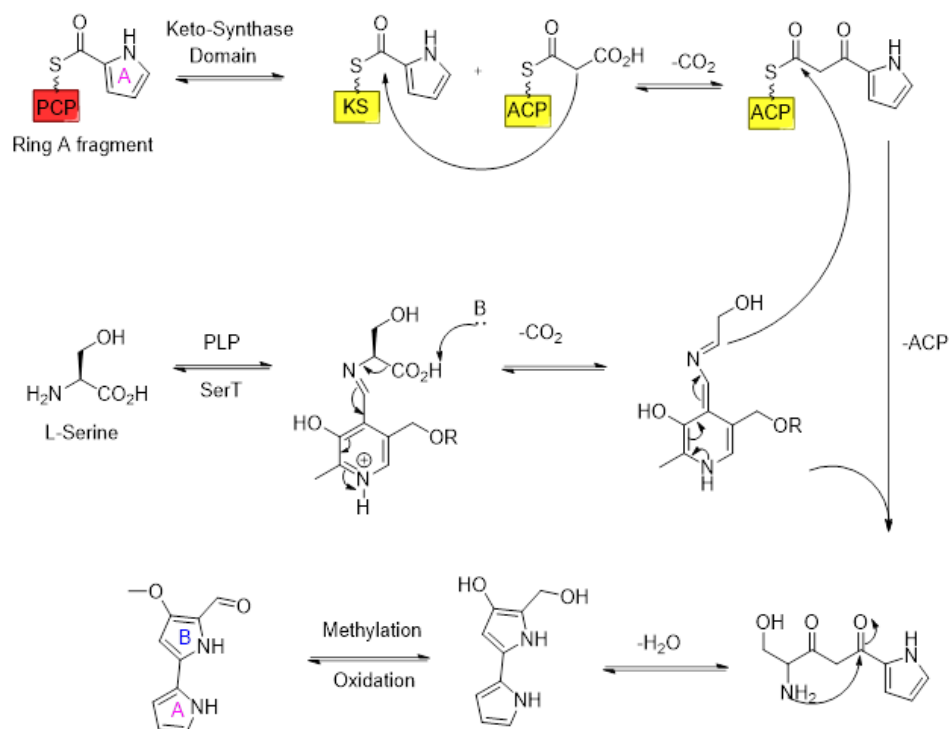


Figure 2.5: Formation of A-B Ring Fragment (Hu et al., 2016)

Resultant A-B ring structure is developed by the oxidation of the primary alcohol to the aldehyde. Ring C is generated from the TPP induced decarboxylative addition of pyruvate to 2-octenal, accompanied by a PLP reaction to produce an amine for intramolecular condensation. The oxidation of the resulting ring occurs in the final ring of the cylinder C is illustrated in Figure 2.5.

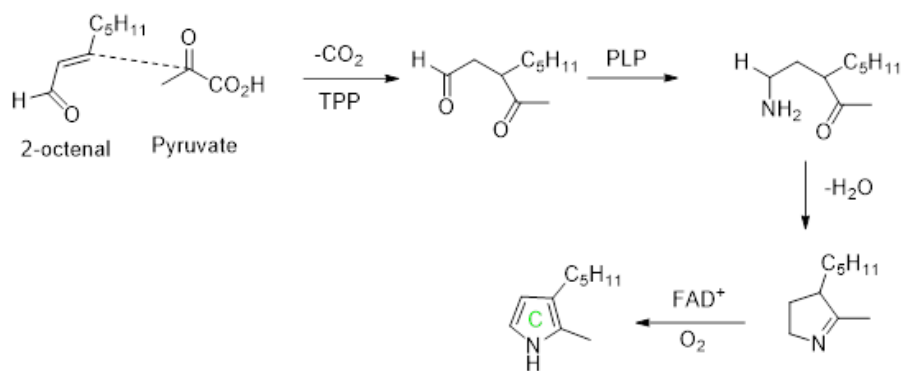


Figure 2.6: Formation of C Ring (Hu et al., 2016)

Figure 2.6 shows two parts of the structure (Ring A and Ring B) are eventually stitched together in a dehydration reaction pushed along by the creation of a conjugate network in all three rings. This ends the converging transformation of the natural component (Hu et al., 2016).

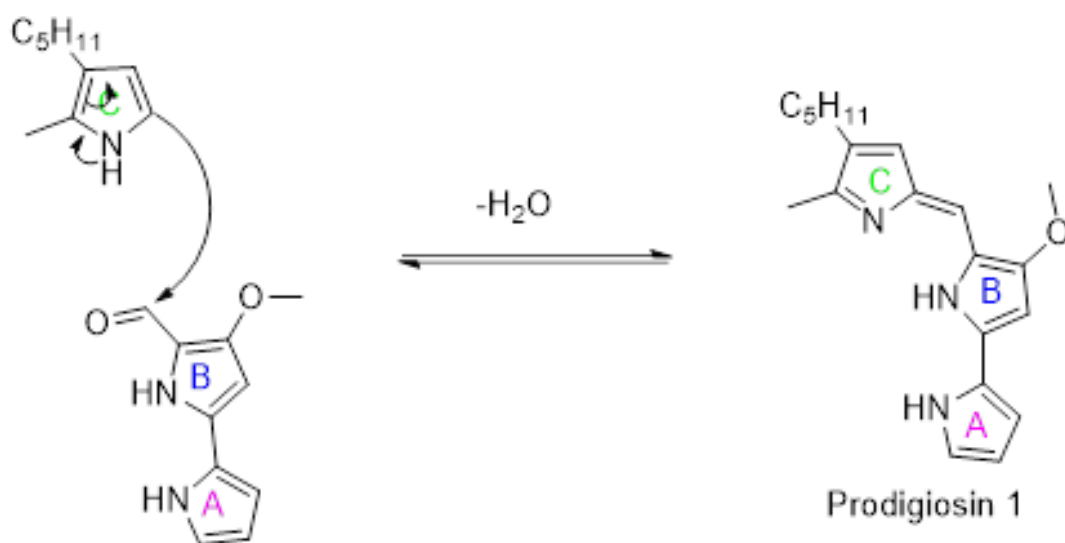


Figure 2.7: Prodigiosin Biosynthesis (Hu et al., 2016)

2.2.2 Violacein

Violacein is a violet, wide ranging-spectrum antibacterial pigment with a dimeric structure consisting of 5-hydroxyindole, oxindole and 2-pyrrolidone subunits produced by the condensation of two different tryptophan molecules as shown in Figure 2.7. Radiolabel incorporation research performed with L-tryptophan exhibits the biosynthesis of violacein. Nevertheless, the biosynthetic pathway and form of enzymes involved have remained unclear. Genes encoding the violacein biosynthetic pathway have previously been extracted from *C. violaceum* to a 14 kb fragment that was functionally expressed in *E. coli* (August et al., 2000).

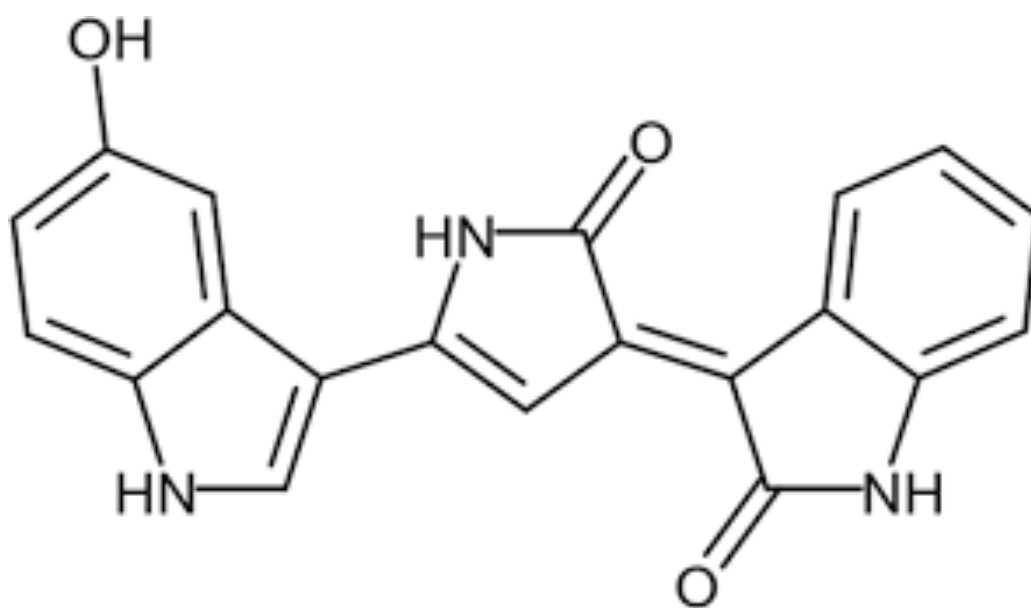


Figure 2.8: Structure of Violacein (Durán et al., 2016)

In 1934, Tobie observed that the time required to produce maximum violacein pigment profoundly shortened by oxygenation of *C. violaceum* culture. In order to synthesise violacein, the bacteria require molecular oxygen and that L-tryptophan had been introduced into violacein, with the exception of carboxylic acid, which had been added during biosynthesis by decarboxylation (Durán et al., 2016). From 1987 to 1990, Hoshino and co-

workers discovered that the condensation of the side chains of two L-tryptophan molecules, followed by a 1,2-shift indole ring on one tryptophan molecule formed the carbon structure of the pyrrolidone moiety. All carbon, nitrogen and hydrogen atoms in the pyrrolidone moiety were supplied exclusively by L-tryptophan and the oxygen atoms derived from molecular oxygen and the 5-hydroxy-L-tryptophan intermediate in violacein biosynthesis, because tryptophan hydroxylation was the first stage in violacein biosynthesis (Hoshino, 2011).

In 2007, Shinoda and co-workers announced the discovery of a true intermediate of violacein biosynthesis, called protoviolaceinic acid, generated by incubation of L-tryptophan with VioABDE in the presence of NADPH as shown in Figure 2.8. The development of this compound indicated that VioC acts to oxygenate the 2-position indole ring on the right side.

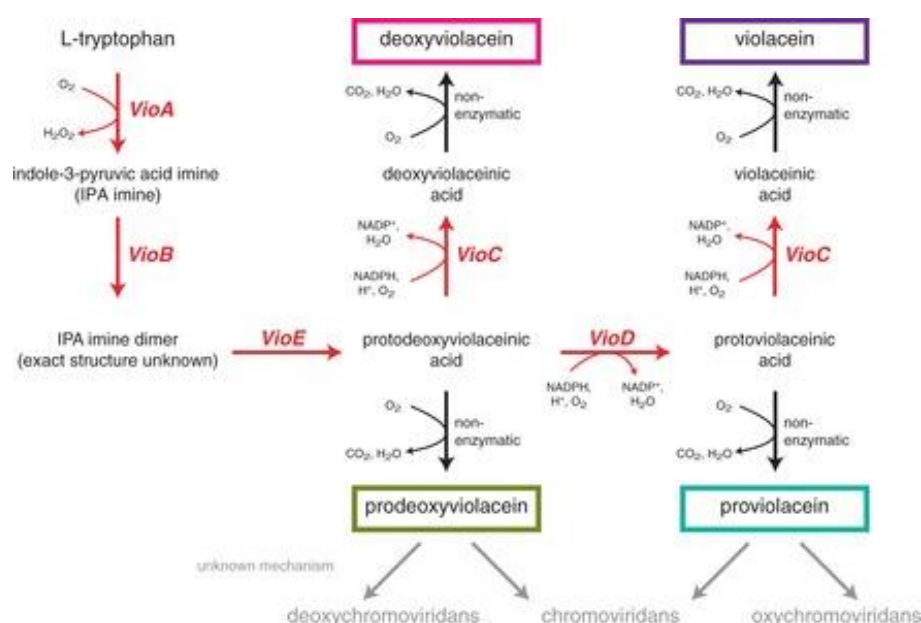


Figure 2.9: Violacein Biosynthesis (Dantas et al., 2012)

The oxidation of L-tryptophan with VioA to the imine indole-3-pyruvic acid (IPA) begins the process. IPA imine dimer is an uncharacterised intermediate resulted from VioB's oxidative coupling of two IPA imine molecules. Intramolecular rearrangement catalytically

done by olVioE to the structure of the indole ring forming protodeoxyviolaceinic acid. The transformation of prodeoxyviolacein can be through autoxidation or hydroxylation catalysed by VioD at the 5-position of the left indole ring resulting in formation of protoviolaceinic acid. Violaceinic acid created through hydroxylation of protodeoxyviolaceinic acid at the right-side indole ring 2 position by VioC. The oxidative decarboxylation process produces violacein (Dantas et al., 2012).

Bacteria that produces violacein are normally sessile, rendering them more vulnerable to predation. This shows that violacein serves as defence mechanism or competitive advantage for the bacteria. Consistent with this, violacein has been shown to have antibacterial effects, especially for Gram-positive bacterial strains. However, the biological function of violacein is not limited to prokaryotes, since it is also reported to have adverse effects on bacterivorous protozoans and metazoans. Research on purified violacein and violacein-producing biofilms with various protozoan species that include flagellates, ciliates, and amoeba showed that the bacterivorous amoeba *Acanthamoeba castellanii* had a decrease in feeding and morphological changes like cell rounding when exposed to violacein. In details, there is an increase in TUNNEL assay studies and caspase-3-like activity showing that they undergo apoptotic-like cell death (Lin et al., 2022).

2.2.3 Carotenoid

The central and most common category of pigments are thought to be the carotenoids (Misawa et al, 1998). Carotenoids are produced by a broad range of species, from non-phototrophic to higher plant prokaryotes, with more than 700 distinct structures described to date. They are as yellow, orange, or red and the carbon skeleton biosynthesis is based on isoprenyl unit condensation. A large number of structures are gained by various carbon backbone alterations such as oxidation, hydroxylation, and so on. Owing to their colour and

biochemical properties like antioxidant, carotenoids are used extensively for cosmetic and medicinal uses as food colourings, animal feed additives and, more recently, as pharmaceuticals. Commercial processing of carotenoids is currently largely focused on plant tissue extraction or chemical synthesis. Nonetheless, due to increasing global demand for such compounds microbial growth has tremendous potential both in terms of production quality and in terms of the variety of carotenoid structures (Stafsnes et al., 2010).

Carotenoids are divided into two groups namely carotenes (α -carotene and β -carotene) and xanthophylls (canthaxanthin, astaxanthin and zeaxanthin) (Asker et al., 2012). Astaxanthin, β -carotene and β -carotene were beneficial in food colouring industry, aquacultures and as animal feed additives in poultry farming. Recently, the other types of carotenoids were attraction to researchers due to its health beneficial benefits. The pigment was characterised from the chemical compositions. Hydrocarbon carotenoids are known as carotenes and oxygen-containing carotenoids are called xanthrophylls. Figure 2.9 depicts the structure of the carotenoid based on their properties.

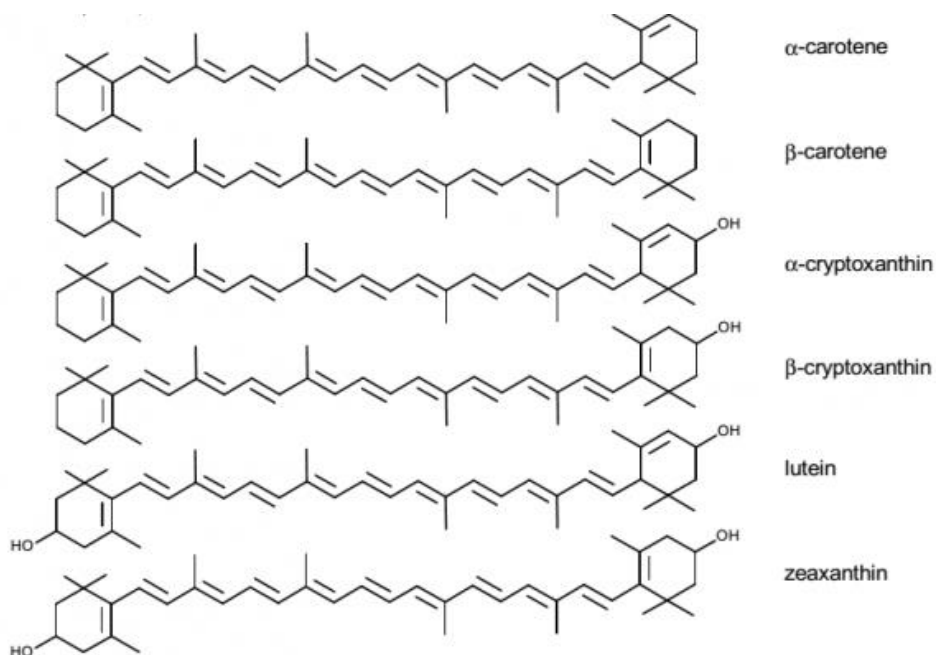


Figure 2.10: Carotenoid Structure (Fiedor et al., 2014)

The hydrocarbon phytoene (or 4,4'-diapophytoene in a minor case) is used to biosynthesise all carotenoids. Two molecules of geranylgeranyl diphosphate (pyrophosphate) (GGPP) and farnesyl diphosphate (FPP) are used to make phytoene and 4,4'-diapophytoene, respectively. Dehydrogenation of the hydrocarbon precursors forming conjugated double bonds, often cyclised and went through sequence of modifications forming vast assortment of xanthophylls (Schaller et al., 2010). All-trans lycopene undergoes a first reaction with α -LCY producing α -carotene, followed by a second reaction with β -LCY, whereas β -carotene undergoes two reactions with β -LCY (Nisar et al., 2015).

Carotenoids were previously studied for their provitamin A properties, and it is only recently discovered that they have a high antioxidant potential, allowing them to protect humans from life-threatening diseases such as cancer, age-related macular degeneration, delayed tooth or bone development, and impaired rough scaly skin (Rojas-Garbanzo et al., 2017). Thus far, carotenoid extracted from fruits and flowers were researched extensively as

dye sensitiser in DSSC (Hemalatha et al., 2012; Yu et al., 2013). A number of bacterial carotenoids were reported to be used as DSSC sensitiser (Ordenes et al., 2016).

2.3 Introduction to Photovoltaic devices

2.3.1 First Generation

Photovoltaic cells specifically transform the solar radiation into electrical energy. There are three generations of solar cells. The first generation comprises of silicone mono-crystalline solar cells referred to the p-n junction photovoltaic (Raut et al., 2018). Fabrication of crystalline solar cells based on silicon as it is branded to be the best material. Typical materials are mono and poly-crystalline silicon doped with other elements (Sharma et al., 2015). Silicon is an ample material and safe to environment. Fabrication of this type of solar cell using Czochralski method made up of silicon wafers. Despite high efficiency potentials, the fabrication of this solar cells cost a lot causing the high overall cost of commercially available crystalline solar cell. This is due to the large amount of silicon used and long fabrication process (Richhariya et al., 2017). Temperature influences crystalline solar cell performance thereby impacting the cell efficiency. Mono crystalline cell holds the highest efficiency. Hoffman in 1960 successfully developed a method increasing the PV cell efficiency from 2% to 14% (Perlin, 2004).

2.3.2 Second Generation

The thin film solar cells fall under second generation photovoltaic cells. It is an amorphous silicon solar cells that uses powdered materials thus decreasing the weight and improve the flexibility. Thin film solar cells divided into different categories such as cadmium telluride (CdTe), amorphous silicon, and copper indium gallium selenide (CIGS) (Tang, 2017). There are three types of thin film structure: mono junction, double junction,

and multiple junctions. The number of p – I – n junction layers affecting the structures (Bagher et al., 2015). The efficiency increases by depositing multiple layers of thin films with different band gaps. However, the deposition of multiple layers during fabrication requires multiple processes or methods leading to increase in cost. The efficiency of thin film solar cell is less than silicon solar cell that currently dominate PV market is the main obstacle (Le Roux, 2009). Improvement on the efficiency and decreasing the cost production are the main focuses of researchers. The first and second generation of solar cell are fundamentally constructed from semiconductors (Ludin et al., 2014).

2.3.3 Third Generation

Third generation of solar cells is Dye-Sensitised Solar Cell (DSSC). Its unique characteristics differentiate from other solar cells is the conversion of visible light into electricity based on wide band gap semiconductor sensitisation. DSSC comprises of semiconductor such as titanium dioxide coated photo anode electrode, a counter electrode as cathode, an electrolyte, and a sensitizer (Suhaimi et al., 2015). Fabrication of DSSC by O'Regan and Gratzel (1991) was the first leading to other experiments. The efficiency of DSSC is less than crystalline solar cells while more compared to thin films.

During the 1970s, researchers at the University of California investigated the phenomenon of photoexcitation in order to imitate the mechanism of photosynthesis by extracting the chlorophyll pigment from spinach and using ZnO as a semiconductor electrode material in electrochemical cells. When dye molecules were photo-excited, electrons were injected into the conduction band of the n-type semiconductor scaffold material, and it was discovered that dye molecules adsorbed on the semiconductor monolayer material were responsible for the highest yield. The bionic or biomimetic method to sensitising semiconductor materials for electron excitation is based on this research

(Tributsch & Calvin, 1971). Matsumura et al. (1980) concluded that fine-tuning the porosity of semiconductor oxide materials could improve the efficiency of dye molecules, but dye stability is just a challenge for dye-sensitized photocells. Advances in this area were initiated in 1991 by Professor Michael Graetzel, who performed sensitized electrochemical photovoltaic devices from dye sensitisation of semiconductor TiO_2 materials and called them "desensitized solar cells" (O'regan & Grätzel, 1991).

They designed the device towards the new concept of photovoltaic energy production. The new conceptual approach is based on the three-billion-year-old idea of photosynthetic activity in nature. They deduced that the PV device was modeled on the mechanism of light absorption and electron transmission in plants. When sunlight hits a chlorophyll molecule, the electrons produced from the chlorophyll are transferred from one molecule to another until it reaches the reaction center of the chlorophyll. From the reaction center, electrons are transferred to the energy-storage molecule. The lack of chlorophyll with one electron will be captured by the surrounding water molecule. This cyclic process is simulated to capture energy using solar radiation through synthetic dyes. On the first attempt, they got < 2% proficient from DSSC. It has its own disadvantages with chlorophyll dyes such as the range of adsorption and stability of the dye molecules. To increase the device's performance, they used a ruthenium-based dye and a polymer gel electrolyte that had good thermal stability in 2003 and achieved a maximum efficiency of about 6% (Wang, 2003).

In 2005, analysts concentrated on progressing the quantum effectiveness of DSSCs by altering the morphology of the photoanode scaffold material and moving forward the electron exchange within the semiconductor, designing the particle with a small size having high surface area for more dye adsorption. At that point, the supplanted the ruthenium-based

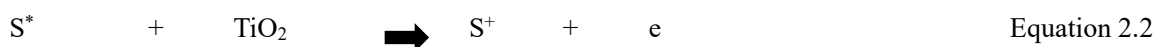
colour with less costly dyes and accomplished around 5% effectiveness (Wang et al, 2005). The use of an iodine-based redox liquid electrolyte resulted in an efficiency of 8% in 2008. The researchers next sought to resolve the electrolyte's corrosive character, which improved photovoltage, efficiency, and device stability. During this time, the efficiency of the device grew by 11.55%. G24 Power Limited, South Lake Drive, Imperial Park, Newport, United Kingdom, was the first to market DSSC in 2009, (Karthick, 2013).

2.3.4 Principle of Dye Sensitised Solar Cell (DSSC)

The method used in the DSSC is photo-electrochemical processes. Dye molecular layer acts as medium for the absorption of photon energy. Molecules of dye bound to the surface of nanocrystalline material. Found at the anode of semiconductor material where it is at the peak energy level, the dye molecules are at ground level. Photoexcitation of the dye molecules occurs when sunlight passes through the transparent conductive glass such as Fluorine Tin Oxide (FTO) or Indium Tin Oxide (ITO) striking the dye adsorbed with the TiO₂ molecules, resulting in a higher energy content of the excited molecules, thereby closing the semiconductor band gap. S in the equations below represent dye molecule. As can be seen from the equation, the dye molecule getting excited (S*) after receiving light energy.



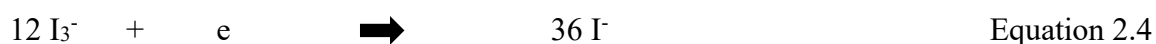
Afterwards, the excited dye molecule oxidized, and the electron released into the conduction band. The electron will travel easily inside the semiconductor that is conductive at this stage.



The electron emitted is transferred through diffusion to the present collector at the anode. Electrical load may be powered if it is connected. The oxidized molecule can be produced by donation of the electron from iodide to the electrolyte.



Iodide regenerated by reduction in triiodide on cathode.



The performance of the DSSC depends on four energy levels of the component: the excited state (approximately LUMO) and the ground state (HOMO) of the photosensitiser, the Fermi level of the TiO_2 electrode, and the mediator redox potential (I^{-}/I_3^{-}) of the electrolyte (Ludin et al., 2014).

2.3.5 Compositions and Functions of DSSC

The standard solar device uses the semiconductor as both light absorbance and charge carrier while the DSSC distinguishes all processes. The dye sensitiser in DSSC receives light and the semiconductor serves as a charge collector. Upon illumination of the solar cell by light source, the photoexcitation of the dye molecules absorbed on the nanoparticle surface of the semiconductor in contact with the electrolyte would shift the excited electrons to the titanium dioxide conduction band (Mehmood et al., 2014).

DSSC comprised of two transparent conductive oxide (TCO) material to facilitate the transport of photo-generated charge carriers to the electrode (Nazeeruddin et al., 2011). The typical TCO used is either indium tin oxide (ITO) or fluorine doped tin oxide, FTO (Hug et al., 2013). In the initial version of Grätzel and O'Regan, the top of the DSSC serves as an anode. It's in the back of the plate. The substrate for the plate is normally translucent glass

for the absorption of light. The light absorbing dye used is sandwiched between the TCO glasses (Henning et al., 2013). Nano-porous broad band gap semiconductor is stored at the TCO anode. Titanium dioxide (TiO_2) is used as a semiconductor owing to its nano-crystalline structure sizes varying from 20 to 50 nm. It would increase the surface area by about a factor of 1000 and reduce optical absorption. TiO_2 is a negative charged semiconductor due to the excess of free electrons (O'regan & Grätzel, 1991).

2.3.5.1 Photoanodes

In DSSC, a common photoanode material is dye molecules-adsorbed semiconductor oxide material coated on a TCO substrate, which serves as the working electrode. An anatase phase of titanium dioxide (TiO_2) with a band gap of 3.2 eV is the most extensively used semiconductor material. It is a low-cost, non-toxic, and plentiful substance found in the earth's crust (Yan et al., 2013). Karthick et al. (2019) reported that for a strong adhesion to the FTO glass plate, TiO_2 paste might be created using binders such as terpineol, ethyl cellulose, polyethylene glycol, and ethylene glycol. The viscous TiO_2 paste is coated onto the substrate using screen printing or doctor blade processes. Binder is normally removed by annealing the metal oxide paste-coated Fluorine Tin Oxide (FTO) glass plate for 30 minutes at 450–500 °C. The metal oxide has high adherence to the Transparent Conductive Glass (TCO) glass substrate, inter-particle contact, and finally forms the nanostructured porous electrode as a result of the annealing process. The generated porous electrode film is 10 m to 15 m thick, with interlinked nanoparticles ranging in size from 15 nm to 30 nm. The common TCO used is titanium dioxide. However, there are other options for this semiconductor oxide such as Zn_2SnO_4 (Tan et al., 2007), SiO_2 (Choi et al., 2014), CeO_2 (Sayyed et al., 2020), ZnO (Lin et al., 2011), Nb_2O_5 , and SrTiO_3 (Kim et al., 2013).

2.3.5.2 Counter Electrodes

There are three crucial requirements for a counter electrode to serve as a potential catalyst. Firstly, it is in charge of completing the process, in which the oxidised redox pair is reduced at the counter electrode's surface by receiving electrons. The oxidised redox pair is reduced in solid-state DSSC by collecting electrons from the counter electrode via an ionic transport material (Hamann et al., 2011). Secondly, from the outer circuit, it takes part in the electron collection and then transports them to the electrolyte solution in the inner cell. Therefore, the counter electrode's primary function is to collect electrons from the load and transport them to the cell for circulation (Liu et al., 2015). Lastly, the counter electrode serves as a mirror, reflecting unabsorbed light to the photo-anode for better solar utilisation (Wu et al., 2017). The counter electrode should have high catalytic activity, high conductivity and reflectivity, high surface area with porous nature, chemical corrosion resistance, high chemical and mechanical stability, energy level of counter electrode should match the potential of the redox electrolyte, and good adhesiveness toward TCO substrate to fulfill these basic functions.

In standard cells, platinum paste is utilised as a counter electrode material to complete the redox process. The counter electrode was responsible for the reduction of triiodide. The rate of reduction of triiodide to iodide at the counter electrode is crucial because formation of iodide is required to replenish dye molecules at the anode side of the cell following the redox reaction. The anode side of a working cell undergoes a slow reaction, whereas the cathode side undergoes a quick reaction. Carbon, graphite, and conductive polymers, in addition to platinum, are utilised as counter electrode materials. Various preparation procedures, including sputtering, chemical vapour deposition, hydrothermal, and electrodeposition, are used to make counter electrodes (Zhong et al., 2011).

2.3.5.3 Dyes in DSSC

In DSSC, the dye plays a vital role by ejecting electrons and activating the process when irradiated. The following are some of the advantages of dyes. It should be adsorbed on the photoanode and capture all wavelengths (λ) below 920 nm. For photoanode adsorption, it should contain strong anchoring groups such as $-\text{COOH}$, $-\text{SO}_3\text{H}$, and $-\text{H}_2\text{PO}_3$. For efficient electron transport from the dye to the photoanode, the dye's excited state should have a greater energy than the photoanode's conduction band. It should have a higher positive oxidised state level than the electrolyte's redox potential for dye regeneration. Dye aggregation on the semiconductor electrode surface is prevented by optimising the dye's molecular structure. The dye should be very resistant to light and heat. Metal complexes, porphyrins, phthalocyanines, and metal-free organic dyes have all been used as photosensitisers in the past (Karthick et al., 2019)

Metal complexes usually have a core metal ion and at least one anchoring group in the auxiliary ligand. The visible light absorption in the solar spectrum is caused by the metal ligand charge transfer mechanism. The N719 dye, a metal ligand complex (cis-bis (4,4' - dicarboxy-2,2' -bipyridine) diisothiocyanato-ruthenium (II)). Another sensitizer used as a reference for the standard cell is cis-(SCN) 2bis (2,2'-bipyridyl-4,4'-dicarboxylate) ruthenium (II) coded as N3 dye, which has a 10% efficiency and a longer excited state duration of 20 seconds. N749 dye, often known as black dye, is made up of Ru linked to three thiocyanato and one terpyridine ligands that are replaced with three carboxyl groups. Using black dye, the power conversion efficiency was 10.4 % (Shalini et al., 2016)

Wang et al. (2011) utilised coumarin dyes to demonstrate a power conversion efficiency of 7.6% using the donor- π -conjugated bridge-acceptor idea with $-\text{CN}$ groups as electron acceptor. The natural sensitisers carotenoid and anthocyanin dye were isolated from

Rosa chinensis and *Kerria japonica* flowers, respectively, and sugar molecules were added to the dye for dye stability. The power conversion was relatively poor, with a maximum of 0.29% (Hemalatha et al., 2012).

2.3.5.4 Electrolytes

Another key compound found in DSSC is the electrolyte. Between the counter electrode and the photoanode, the presence of redox mediator in the electrolyte works as a charge or ion transporter. The light in the visible range should not be observed by the redox couple. It should be reversible and chemically stable. It should have a low viscosity to allow charge carriers to diffuse quickly. It should not desorb the dye or spread the semiconductor material of FTO plate. The iodide/tri-iodide redox pair is the most prevalent, stable, and highly effective redox mediator. Because of its strong penetration power into semiconductor nanoporous films, minimal recombination loss, and quick dye molecule regeneration, this redox mediator seems promising.

The redox pair is to promote charge transfer between both the counter electrodes and photoanode. The triiodide in the electrolyte aids in lowering the oxidised dye to the ground state as rapidly as possible when dye molecules inject electrons into the semiconductor metal oxide. The iodide, which is an electron acceptor, travels to the counter electrode and recovers the electrons it has lost, regenerating as triiodide. The electron migration across the external load completes the circuit. The redox potential of the iodide/triiodide should be used for dye and electrolyte regeneration (Wu et al., 2015).

2.3.6 Photoelectrical Parameters

Solar energy-to-electricity conversion efficiency can be attained using the following formula (Alhamed et al., 2012):

$$\eta = \frac{J_{sc} \times V_{oc} \times FF}{P_{in}} \quad \text{Equation 2.4}$$

Where J_{sc} is the short circuit current, V_{oc} is the open circuit voltage, FF is the measure of junction quality and series resistance of DSSC and P_{in} is the power of incident light where it can be measured in mW cm^{-2} respectively. The solar cell's open circuit voltage (V_{oc}) is specified in the cell's open terminals. The V_{oc} decreases as the temperature of the cell increases. The cell V_{oc} is described in the following terms:

$$V_{oc} = V_t \ln\{(I_{sc}/I_o) + 1\} \quad \text{Equation 2.5}$$

The short circuit current (J_{sc}) can be located at the cell's short circuit terminals. The current in the short circuit decreases with temperature change. The I_{sc} demonstrates following expression:

$$I_{sc} = I + I_o \{\exp(V/V_t) - 1\} \quad \text{Equation 2.6}$$

The solar cell's fill factor (FF) can be specified as the actual power ratio (maximum voltage product; V_{max} and maximum current; J_{max}) to the dummy power (V_{oc} and J_{sc} product). It is expressed in the following formula (Alhamed et al., 2012):

$$FF = \frac{J_{max} \times V_{max}}{J_{sc} \times V_{oc}} \quad \text{Equation 2.7}$$

2.3.7 Past and Current Works on DSSC

Several works targeting bacterial pigments as dye sensitizer for DSSC have been reported in the literature. Anand et al. (2018) reported the extraction of prodigiosin from *S. marcescens* NCIM 5246 and used it as a dye-sensitizer in their experiment. The study aimed to improve the efficiency of DSSC by using prodigiosin extracted from *S. marcescens*. The

results showed that using dye at a concentration of 4162.574 ug/ml resulted in the highest efficiency of 0.5071%. It was also reported that with sunlight illumination at 1002.38 W/m², the efficiency was 0.4597%.

The strain *S. marcescens* 11E, which was isolated from a natural spring in the state of Nuevo Leon in northeastern Mexico, was grown on peanut oil broth 0.01 v/v, a culture medium known to boost prodigiosin production. Based on spectroscopic and structural properties acquired by UV–Vis spectrophotometry, FTIR, and ¹H NMR spectroscopies, the resulting pigment was identified as prodigiosin. After five days, the initial absorbance decomposition test on the bacterial pigment revealed that prodigiosin had a high photostability, while the photovoltaic performance test of the sensitised DSSC yielded an open voltage circuit of 560 mV, a current density of 0.096 mA/cm², and an efficiency of 0.032%. The DSSC comprised a titanium dioxide photoanode sensitised with the pigment by direct adsorption, an electrolyte comprising a redox pair, and a carbon paste cathode or counter electrode (Hernández-Velasco et al., 2020).

The use of UV-resistant Antarctic bacterium pigments as photosensitisers in (DSSC) is described by Órdenes-Aenishanslins et al. (2016). Red and yellow coloured psychrotolerant bacteria were discovered from soils on King George Island, Antarctica, and used to make pigments. Pigmented bacteria were identified as *Hymenobacter* sp. (red) and *Chryseobacterium* sp. based on metabolic features and 16s DNA sequence (yellow). Based on their spectroscopic and structural features, measured by UV-Vis spectrophotometry and FTIR, the pigments were identified as carotenoids. For the red pigment, solar cells based on these photosensitisers have an open circuit voltage (V_{oc}) of 435.0 (mV), a short circuit current density (I_{sc}) of 0.2 (mA/cm²) an efficiency of 0.0332%., while the yellow pigment has a VOC of 548.8 (mV), an ISC of 0.13 (mA/cm²) and an efficiency of 0.0323%.

Antarctic bacterial pigments were used as sensitisers in conjunction with exogenous co-sensitisers as reported by Marizcurrena et al. (2021). Different amounts of the purple pigment violacein, as well as xanthophylls and silver nanoparticles, were used to make DSSC. The violacein produced by the Antarctic bacterium *Janthinobacterium* sp. UV13 is a dye with physical/chemical properties that make it suitable for use as a sensitiser in the creation of high-efficiency DSSC. The conversion efficiency of the cells improved after they were sensitised with pigment and silver nanoparticle preparations in order, resulting in a conversion efficiency of 0.230%.

Currently, there is no published reports on violacein produced by *C. violaceum* as well as carotenoid produced by *Ralstonia* sp. and their applications for DSSC. The summary of the use of several bacterial pigments for DSSC as reported in the literature is outlined in Table 2.1.

Table 2.1: Comparison of the efficiency of different bacterial pigments used as sensitisers in DSSC.

Bacteria	Pigment	Efficiency (%)	References
<i>Janthinobacterium</i> sp. UV13	Violacein	0.230	Marizcurrena et al. (2021)
<i>Serratia marcescens</i> 11E	Prodigiosin	0.032	Hernández-Velasco et al. (2020)
<i>Hymenobacter</i> sp.	Carotenoid	0.03	Órdenes-Aenishanslins et al. (2016)
<i>Chryseobacterium</i> sp.	Carotenoid	0.032	Órdenes-Aenishanslins et al. (2016)
<i>Streptomyces fildesensis</i>	Melanin	0.014	Silva et al. (2019)

CHAPTER 3

CHARACTERISATION OF BACTERIAL PIGMENT FOR DYE-SENSITISED SOLAR CELLS APPLICATIONS

3.1 Introduction

Solar energy is seen as an alternative and efficient renewable energy that could replace the role fossil fuels. Dye Sensitised Solar Cell (DSSC) was developed to be an alternative in the development of solar energy. DSSC has sensitising element as its main component whereby it acts as a photon harvesting system to generate electricity (Al- Awani, 2018). Throughout the years, conventional photosensitisers such as organic dyes, Ruthenium complexes and platinum complexes are used in DSSC. Nonetheless, these dyes display high toxicity, expensive and not ecologically friendly (Pandey et al., 2019). Recently, there is a trend towards using natural dyes as photosensitisers, which can be extracted from plants and pigmented microorganisms. Extraction of dyes from plants and fruits however is less convenient than microorganisms as the latter process is more simple, rapid, requires low-cost culture medium and easier to be manipulated. Therefore, research into microbial pigments is now one of the emerging areas in the development of cost effective and environmentally friendly DSSC.

Pigmented bacteria contain dye molecules that work as light harvesting complex (LHC), which transfer excited energy to photosynthetic reactions. This is the primary process for photosynthesis in bacteria and plants (Hu et al., 1997). Bacterial dyes can be easily obtained and can be mass-produced in short amount of time. Different pigmented bacteria exhibit different colours such as *S. marcescens* that produce prodigiosin, *Streptomyces coelicolor* that produce prodigiosin and actinorhodin, *C. violaceum* that

produce violacein and *Thialkalivibrio versutus* that produce natronochrome and chloronatronochrome (Ahmad, 2012).

This work focused on three bacteria, which were isolated from Kota Samarahan, Sarawak, Malaysia namely *S. marcescens*, *C. violaceum*, and *Ralstonia* sp. *S. marcescens* is a gram-negative bacillus falls under Enterobacteriaceae. It is originally considered as a harmless, nonpathogenic saprophytic water organism. *S. marcescens* is commonly used as a biological marker due to its easily recognized red colonies. Prodigiosin is a pigment resulted from secondary metabolites of *S. marcescens* (Giri et al., 2004). The natural product of prodigiosin group is a family of tripyrrole red pigment containing a common 4-methoxy 2,2 bipyrole ring system. The pigment biosynthesis is a bifurcated process in which mono-and bipyrole precursors are synthesized independently and then gathered to form prodigiosin (Shahitha and Poornima, 2012).

A recent work by Anand et al. (2021) reported the extraction of prodigiosin from *S. marcescens* NCIM 4526. Hernandez et al. (2020) isolated a strain of *S. marcescens* 11E from northeastern Mexican state of Nuevo Leon. With the distinct red colour, these bacteria were further investigated with peanut oil broth to enhance the prodigiosin production. The dye was extracted using chloroform and further purified to be used as dye sensitizer in DSSC. Thus far, cultivation of *S. marcescens* on agar combined with the extraction method of using methanol have not been studied comprehensively. With respect to that, the yield of prodigiosin grown on nutrient agar and extracted using methanol is one of the focuses of this research.

Ralstonia sp. are reported to be found in various habitat such as water and soils. This type of bacteria is known for infecting plants through soil (Lee et al., 2010). One of the species is *Ralstonia solanacearum*, which is known as an economically important plant

pathogen, normally invade roots of plant hosts from soil and colonise xylem tubes producing a deadly wilting known as bacterial wilt disease. The bacterium produces carotenoids, a significant class of compounds derived from isoprenoids. It consists of β -carotene (two β rings) and α -carotene (one β ring and one ϵ ring) (Park et al., 2002). Coloured carotenoids are synthesised by phytoene or dehydrosqualene desaturation reactions, which create double conjugated bonds.

Currently, research on *Ralstonia* sp. mainly focus on the pathogenic characteristics of the bacteria. Safni et al., (2018) the ecology and epidemiology *R. solanaraceum*. Seo and Choi (2020) reported the pigment produced by *Ralstonia pickettii* isolated from soil sources that is known as melanin-type pyomelanin. They researched on the genes that are responsible for producing the pigment in *R. pickettii*. To date, the yellow pigmented bacteria reported for DSSC application is *Chryseobacterium* sp. which isolated from soils of King George Island, Antarctica. The pigment extracted from the bacteria was identified as carotenoid based on the spectroscopic and structural characteristics (Órdenes et al., 2016). There are still limited reports on yellow pigment produced by *Ralstonia* sp. With that regards, this research focuses the characterisation of the pigment produced by *Ralstonia* sp. as candidate for dye sensitizer in DSSC.

Violaceum is a gram-negative bacterium that is rod in shape with facultative anaerobic ability. This non-pathogenic bacterium is a prevalent saprophyte found in water and soil in tropical and subtropical areas around the world (Rettori and Durán, 1998). The bacterium is capable of producing a pigment called violacein [3-(1, 2-dihydro-5-(5-hydroxy-1H-indol-3-yl)-2-oxo-3H-pyrrol-3-ilydene)-1,3-dihydro-2H-indol-2-one], which has bactericidal, trypanocidal, tumoricidal, mycobacterial and antioxidant characteristics (Ahmad et al., 2012). Violacein is violet in colour, and it has a dimeric structure consisting

of 5-hydroxyindole, oxindole and 2-pyrrolidone subunits produced by the condensation of two different tryptophan molecules. As mentioned, Section 2.3.7 in Chapter 2, to date, there is only one report on violacein as dye sensitiser where the pigment was extracted from *Janthinobacterium* sp. (Marizcurrena et al., 2021). The extracted pigment was characterised to determine its potential as a dye sensitiser in DSSC. More in-depth studies on violacein produced by other bacteria such as *C. violaceum* has yet to be reported. Hence, this is another focus of this research. In this chapter, the findings of the first and the second objectives of this research as outlined in Section 1.3 will be presented.

3.2 Materials and Methods

3.2.1 Bacterial Culture

The three targeted pigmented bacteria namely *S. marcescens*, *Ralstonia* sp. and *C. violaceum* were inoculated on nutrient agar (Oxoid, UK) for both *S. marcescens* and *Ralstonis* sp. and Tryptic Soy Agar (TSA) for *C. violaceum* respectively. *S. marcescens* and *C. violaceum* were incubated at room temperature for 24 hours meanwhile the *Ralstonis* sp. was incubated at room temperature for 48 hours.

3.2.2 Bacteria Pigment Extraction

Pigments from *S. marcessence*, *C. violaceum* and *Ralstonia* sp. extracted using methanol (Sigma-Aldrich, Canada) with a slight modification of the method by Anand et al. (2021). The coloured bacterial cultures were scrapped and mixed with 1:1 ratio of methanol (Sigma-Aldrich, Canada) (VELP Scientifica, Italy) using vortex. The solution was subjected to centrifugation at 0°C using 10000 rpm for 5 minutes. Supernatant were taken and further concentrated using rotary evaporator (IKA, China). Resultant extracted pigments undergone partial purification steps by mixing with dichloromethane (DCM) (Sigma-Aldrich, Canada)

in 1:1 ratio with the dye. Two layers were formed, and the colourless solution were discarded. Then, 1:1 ratio volume of sodium chloride (NaCl) (Sigma-Aldrich, Canada) with coloured solution was added in the dye resulting in double layers. Colourless solution was discarded. One tablespoon of sodium carbonate anhydrous (NaCO₃) were added to the solution to remove excess water and the filtered by using filter paper Whatman 110mm (Sigma-Aldrich, Canada). The resulted solution was put into glass petri dish and left until the solvent evaporated. Methanol (Sigma-Aldrich, Canada) were added to re-liquify the sample for further investigation.

3.2.3 Characterisation and Measurement

3.2.3.1 UV-VIS Spectroscopy Characterisation

Determination of absorbance rate in the visible light spectrum and the intensity composition of dye colour was performed by testing the wavelength of light absorbed using UV-vis spectrophotometer machine (Jasco V-630). Dye etching method based on Shahzad et al. (2013) was used to dissolve the sample into filling the UV-vis cuvette. This method was done by using methanol to rinse the sample from the glass. The solution were collected. A cuvette was placed into the sample holder. The pigment was analyzed for maximum UV-vis absorbance using methanol as blank between 650 nm and 400 nm (Indra et al., 2014). The peaks in the UV-vis results can be used for the determination of pigment's concentration and band gap. The pigment content in the sample was calculated using the following formula:

$$\frac{A \times MW \times DF \times 10^3}{\epsilon \times l} \quad \text{Equation 3.1}$$

Where $A = A_{\max} - A_{700}$, MW is the molecular weight for the pigments (323.432 g/mol for prodigiosin (Lapenda et al., 2015), 343.34 g/mol for violacein (Subramaniam et al., 2014) and 536.888 g/mol for carotenoid (Breeman et al., 1996), DF is the dilution factor, 10^3 is factor for conversion from g to mg, l is the assumed path length of incident light in cm and ϵ is molar extinction coefficient with the unit of $L \text{ mol}^{-1} \text{ cm}^{-1}$ can be calculated using the following formula:

$$\epsilon = \frac{A}{lc} \quad \text{Equation 3.2}$$

A represents absorbance value of the pigment, l is the length of cuvette and C is the molarity of sample. The samples were dried to obtain the dry weight for the calculation of molarity.

Band gap can be calculated using the following formula:

$$E = \frac{hc}{\lambda} \quad \text{Equation 3.3}$$

E represents photon energy, h is the plank constant 6.63×10^{-24} Js, c is the speed constant 3.0×10^8 m/s and λ is the corresponding wavelength.

Absorption coefficient determined how far into material; light of a wavelength can penetrate before it was absorbed. The absorption coefficient of respective wavelength was used using the formula stated below:

$$\frac{4\pi k}{\lambda} \quad \text{Equation 3.4}$$

Based on the formula above, K represented the Boltzman constant; 3.6173324×10^{-5} eV and λ stands for the cut-off's wavelength of the dyes.

3.2.3.2 Fourier-Transform Infrared (FTIR) Analysis

A small amount of dried pigments were dissolved in methanol which act as solvent and a drop of sample dropped onto the receiver and continued for analysis in Thermo scientific Nicolet iS10 between 4000 cm^{-1} to 400 cm^{-1} (Ahmad et al., 2013).

3.2.3.3 Scanning Electron Microscope (SEM)

The morphological of TiO_2 nanoparticles were investigated by using Scanning Electron Microscope (SEM) (JEOL JSM-639OLA) using 10kV accelerated voltage. The annealed TiO_2 onto ITO glass was mounted on metal stub by using conductive carbon tape. The mounted samples were then gold coated to further increase the conductivity. The samples then view under the scanning electron microscope.

3.3 Results and Discussion

3.3.1 Extraction and Purification of pigment

Coloured solution were obtained from this method as shown in diagram below. The coloured solutions were separated successfully.



Figure 3.1: Purified Purple Pigment Extracted from *C. violaceum*



Figure 3.2: Purified Red Pigment Extracted from *S. marcescens*



Figure 3.3: Purified Yellow Pigment Extracted from *Ralstonia sp.*

Nutrient agar provides the necessary nutrient for the growth of the targeted bacteria. The cultured bacteria were scrapped and dissolved in methanol due to its polarity making it suitable for the pigment's extraction as reported by Zang et al. (1997), Pantanella et al. (2007) and Casullo de Araújo et al. (2010). Fleming-Jones (2003) stated that the occurrence of polar bonds regulate the polarity of the compounds exist in polar functional groups such as amines and alcohol. The mixture were mixed thoroughly using a vortex to break down the bacterial cell wall to ease the pigment extraction. The resulted mixture of solvent and bacterial pigment were centrifuged to separate the pigment that already dissolved in the

solvent and other debris from the lysed bacteria. The presence of amines on the parol rings and pentile chain results in hydrophobic and polarity properties of prodigiosin (Park et al., 2012). Therefore, this research utilised methanol to separate the targeted pigment from other compound.

Purification was carried out to remove other impurities inside the dissolved pigment by using solvent-solvent purification. Chaugule et al. (2019) stated that the net transfer from one liquid phase to another liquid phase as from aqueous state to organic environment resulting in the separation of hydrophobic pigments towards the organic solvent. Purification of the pigment was performed on the basis of different densities of the solvent with water. Solvent with high boiling point were used in solvent-solvent purification such as Dichloromethane (DCM) due to its insolubility and higher density than that of water. The high boiling point is favourable in fast evaporation after the organic impurities such as fat, water and lipid are separated from the pigment resulting in double layer as shown in the Figure 3.1, Figure 3.2 and Figure 3.3, where distinct double layer can be seen. The separated residual water was drained out from the DCM and the pigments were obtained after the evaporation of DCM.

3.3.2 Ultraviolet-Visible Spectroscopy (UV-vis) of Red Pigment

Figure 3.4 depicts the absorbance spectra of red pigment extracted from *S. marcescens* showing the maximum absorbance peak at 540 nm with the absorbance value of 0.76. This result is in parallel with that described by Hernandez et al. (2020). The black line in the graph represents red pigment without TiO₂ meanwhile the blue line represents red pigment adsorbed in TiO₂. The pigment showed the absorption characteristics reported for prodigiosin, which is between 460 to 540 nm. Due to the presence of chromophore structure of the O-C group in prodigiosin, the peak absorbance resulted in wavelength of between 532

nm (Arivizhivendhan et al., 2015) and 535 nm (Zhao et al., 2021). The deprotonation of nitrogen atoms in pyrrole rings by the alkaline solution is responsible for the shift in absorption maxima towards higher wavelength (Setiyono et al., 2020). This tally with the presence of nitrogen functional group described by FTIR analysis in Section 3.3.4 later in this chapter. Prodigiosin displays strong absorption in the visible region with the molar extinction coefficient (ϵ) of up to $7.8486 \text{ M}^{-1} \text{ cm}^{-1}$ at 540 nm. The pigment adsorbed in TiO_2 exhibited a peak of 538 nm with the absorbance value of 2.004. The high absorbance peak value of pigment adsorbed in TiO_2 was due to the presence of TiO_2 .

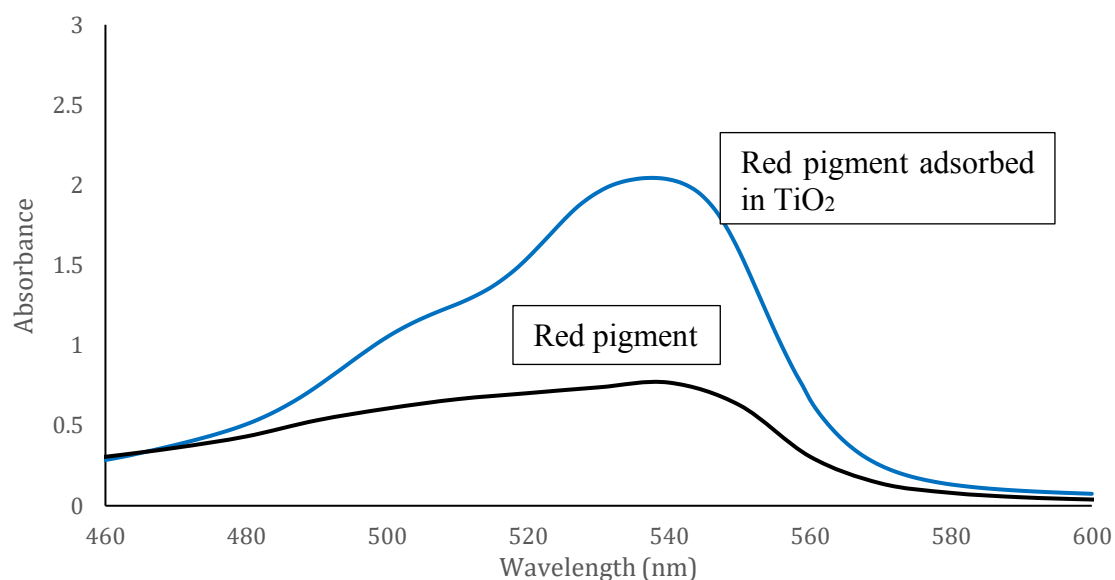


Figure 3.4: Absorption Spectra for Red Pigment where Blue Line Represent the Results for Red Pigment Adsorbed in TiO_2 meanwhile Black Line Represent the Results for Red Pigment Only

The profile of red pigment was found to extend broader than the profile of red pigment adsorbed in TiO_2 . The peaks of both profiles show the absorption in green region within the visible region spectrum. After being adsorbed with TiO_2 . The peak was not shifted farther from the visible region which is suitable for DSSC performance.

3.3.3 Ultraviolet-Visible Spectroscopy (UV-vis) of Purple Pigment

As illustrated in Figure 3.5, the maximum absorbance peak for purple pigment when absorbed in the presence and absence of TiO₂ and not absorbed in TiO₂ are 570 nm wavelength (blue line) with absorbance value of 1.339 and 570 nm with absorbance value of 0.669 (black line) respectively. Neshati (2010) stated that the conjugation of C=C in violacein resulted in the absorbance peak ranging from 550 nm to 580 nm. Therefore, the measured spectra correlated with the absorption range of violacein. This corresponded to the report by Ahmad et al. (2012) who describe the UV-vis absorbance peak at 567 nm with the absorbance value of 0.707. Similarly, the violacein extracted from *Janthinobacterium* sp. were found to exhibit the maximum absorption at 757 nm (Marizcurrena et al., 2021). The presence of chromophores such as alkene and carbonyl groups result in mentioned UV-vis absorption range. The carbonyl group absorbs strongly at short UV vis wavelengths, but as the wavelength increases, the strength diminishes due to the presence of n electrons. As a result, the alkene groups play an important part in the conjugation effect, where the more conjugated double bonds present, the longer the absorption wavelength (bathochromic shift) will be (Ahmad et al., 2012).

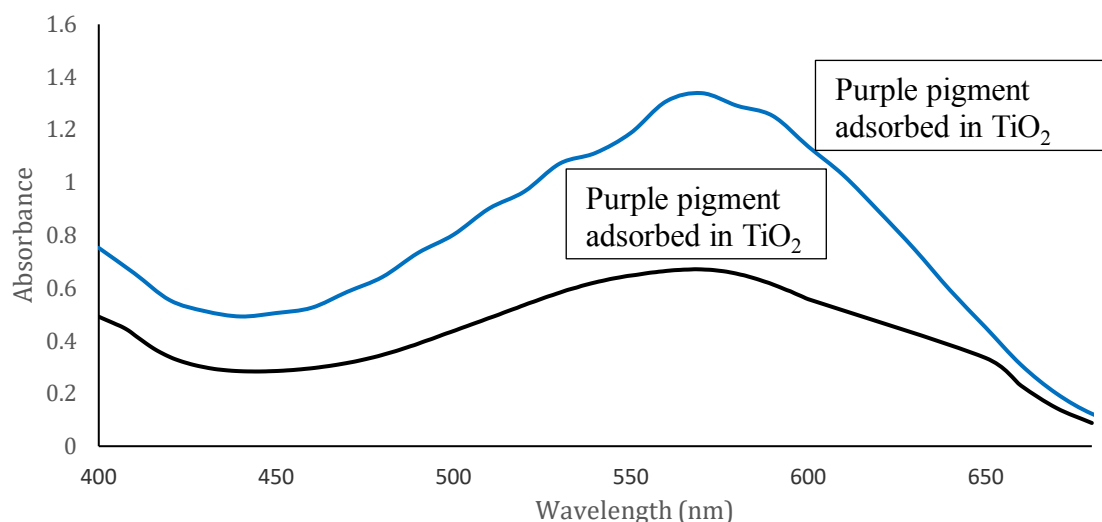


Figure 3.5: Absorption Spectra for Purple Pigment where Blue Line Represent the Results for Purple Pigment Adsorbed in TiO₂ meanwhile Black Line Represent the Results for Purple Pigment Only

At 570 nm wavelength, violacein exhibit molar coefficient (ϵ) of $34.243 \text{ M}^{-1} \text{ cm}^1$. Absorbance value of violacein adsorbed with TiO₂ shifted higher than that of violacein only. Both graphs broadly extended to form a single peak in the visible region spectrum falls within the yellow region.

3.3.4 Ultraviolet-visible spectroscopy (UV-vis) of Yellow Pigment

The characteristic absorption of carotenoid is between 400 nm to 550 nm (Dieser et al., 2010). As shown in the Figure 3.6, the extracted yellow pigment was found to exhibit broad maximum peak at 450 nm (black line) with the absorbance value of 0.5395. This is in parallel with previous works on the absorbance spectra of carotenoid. Tátraaljai et al. (2014) reported maximum absorption at 456 nm from β -carotene akin to zeaxanthin. The presence of chromophore group in carotene's responsible for the measured spectra (Ordenes et al., 2016).

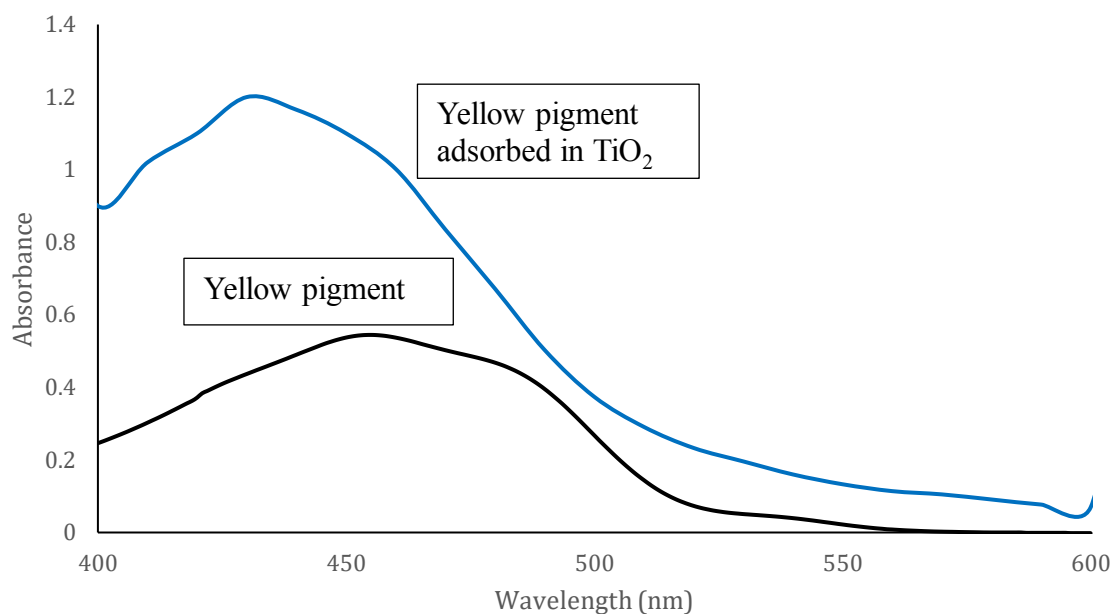


Figure 3.6: Absorption Spectra for Yellow Pigment where Blue Line Represent the Results for Yellow Pigment Adsorbed in TiO₂ meanwhile Black Line Represent the Results for Yellow Pigment Only

The blue line in Figure 3.6 represents the absorption spectra of pigment adsorbed in TiO₂ with the absorbance value of 1.2 at 430 nm. The absorption of the pigment was within the blue region and shifted to the green region after being adsorbed in TiO₂.

3.3.5 Optical Characteristic of Bacterial Dye

Photon energy, absorption coefficient and pigment content of a pigment was calculated using formula mentioned in above methodology extracted from the peak absorbance. Table 3.1 outlines the optical characteristics of the bacterial pigments studied in this work. All the three targeted bacterial pigments absorb wavelength within the visible lights, which is the characteristics of the pigment that can acts as dye sensitiser in DSSC (Narayan, 2012).

There are two distinct energy levels in electrons namely Lowest Unoccupied Molecular Orbital (LUMO) and Highest Occupied Molecular Orbital (HOMO) levels in bio

molecular organic dyes. Upon illumination, the excited electrons move from lower energy (LUMO) to higher energy (HOMO). The distance between LUMO with HOMO represented the band gap energy (Calogero et al., 2012). Dye reduction and oxidation potentials are found in the excited state (LUMO) and the ground state (HOMO), according to electrochemistry calculations. Correlations of dye band gaps in contributing to DSSC performance in past reports include anthocyanins with band gaps of 2.46 eV followed with 1.67% efficiency (Hao et al., 2006). The dye band gap can be credited for determining the redox potential (Kim et al., 2009). However, the DSSC efficiency is not solely linked to the band gap as there are other parameters that can also affect it. Table 3.1 shows that the photon energy and absorption coefficient for prodigiosin, carotenoid and violacein. The pigment containing the highest concentration was carotenoid with the pigment content of $1.39 \times 10^5 \text{ mgL}^{-1} \text{ cm}^{-1}$.

Table 3.1: Optical Characteristics of Bacterial Pigments Studied in This Work

Dyes	Peak absorbance (nm)	Photon energy (eV)	Absorption coefficient, α (km^{-1})	Pigment content ($\text{mgL}^{-1} \text{ cm}^{-1}$)	Sample concentration (molL^{-1})
Prodigiosin	540	2.302	1.902	2.03×10^6	0.2072
Carotenoid	430	2.967	2.848	1.39×10^5	0.1229
Violacein	570	2.181	1.802	1.07×10^5	0.0641

The results show that violacein containing the lowest band gap energy and second highest pigment content compared to the other targeted pigments. High pigment content resulting in more dye molecules available to bind with TiO_2 molecules. This ensures better interaction of both molecules to transport electron. This shows that the extracted violacein is able to transfer lower energy electrons to higher energy electron upon illumination of light faster than the other two pigments due to lower band gap. Figure 3.7 displays the graph

dependence of absorption coefficient on the wavelength of pigment based on data obtained from table 3.1. The results show that higher wavelength absorbed by the dye resulted in small band gap, which favour better DSSC efficiency.

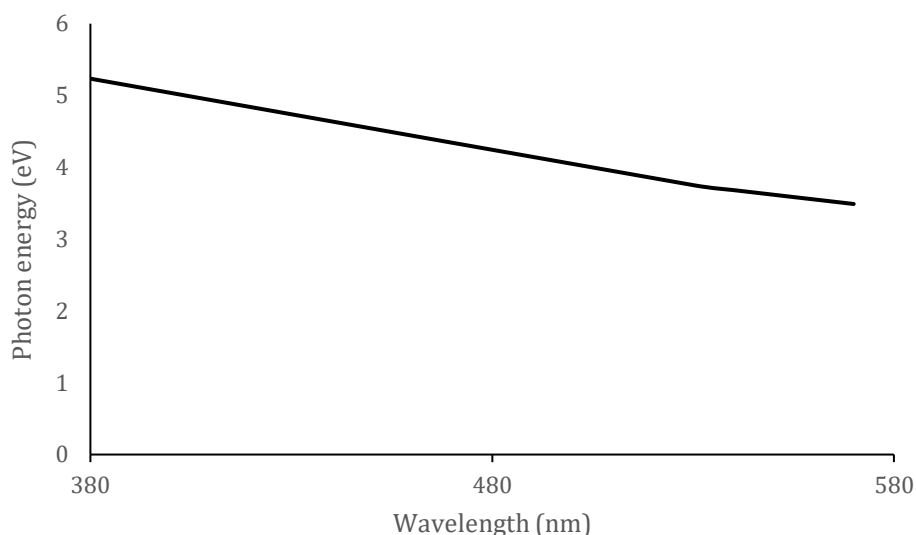


Figure 3.7: Dependence of Absorption Coefficient on the Wavelength of Pigment

The lowest photon energy occurred at 570 nm wavelength contributing to small band gap, which is favourable to faster electron transfer from lower energy to higher energy. The difference between valence band and conduction band is defined as energy band gap, which is used in analysing the performance of DSSC related to solar energy absorbed.

3.3.6 FTIR Spectra Analysis

Some of the parameters that should be considered in the selection of an ideal bio-pigment for DSSC include available functional groups, chemical (covalent) and physical bonds, number of conjugated double-bonds (n), and length of the organic hydrocarbon structure chain. For strong binding and attachment of dye molecules onto the semiconductor surface, hydrocarbon chains with a lot of hydroxylic ($-\text{OH}$), carboxylic ($-\text{COOH}$), and ($=\text{O}$)

radicals are preferred, as the compounds results in lower electron resistance and easier electron injection (Wang et al., 2005; Ahmad et al., 2013). FTIR analysis gives insights into the functional group possessed by the extracted pigments.

3.3.6.1 FTIR of Red Pigment

Figure 3.8 depicts the FTIR results from red pigment extracted from *S. marcescens*. The peaks in the FTIR result indicate the functional groups in the red pigment.

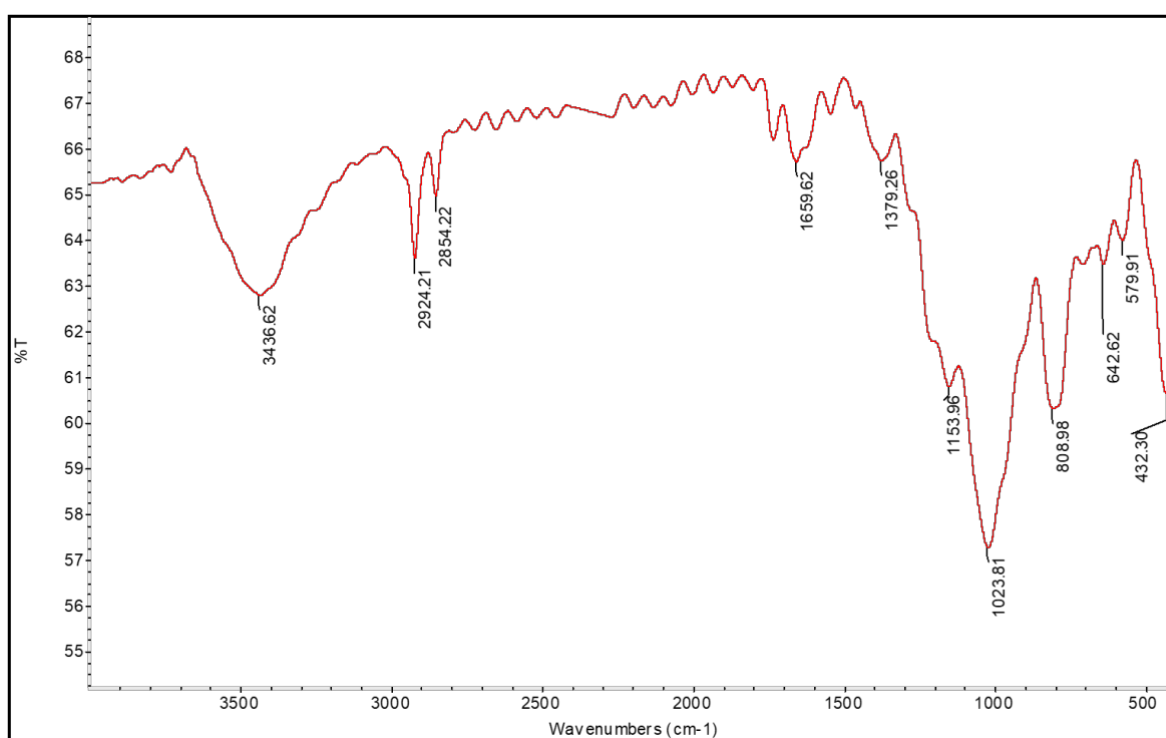


Figure 3.8: FTIR Analysis of Red Pigment From *S. marcescens*

FTIR characterisation stipulates the presence of peaks associated with prodigiosin characteristic groups, such as the strong peak seen at 3436.62 cm^{-1} indicating the presence of N-H stretch in the hetero-cyclic amines in ring A and ring B, augmented by the occurrence at 1379.26 cm^{-1} , which corresponds to ϕ -N stretch adjacent to a double bond as can be seen in Ring C as Figure 2.9 in literature review (Coates et al., 2000). The presence of this chromophore helps in the binding of pigment in TiO_2 molecules for better DSSC

performance. Further significant signals were obtained where the peaks at 2924.21 cm^{-1} resulted from aromatic C – H due to the asymmetrical stretching of methylene groups. The adjacent peak of 2854.22 cm^{-1} point was due to symmetrical stretching of methylene groups in the molecule structure. Both peaks were similar to report from Sumathi et al. (2014) who reported peaks between 2900 cm^{-1} to 2800 cm^{-1} . Likewise, the FTIR results of prodigiosin by Mathlom et al. (2014) stated the presence of N-H group at 3444.87 and 3429.43 peaks along with 2926.01 peak due to C-H bond. In addition, the peak at 1379.26 cm^{-1} was due to the presence of C – O group for carbonyl group. The feature of aromatic C=C was shown in peak at 16530.62 cm^{-1} and peak 1153.96 cm^{-1} contributing to C–O stretching (Faraag et al., 2017). Similarly, prodigiosin extracted by Hernandez et al. (2020) showed 2031 cm^{-1} , 1652 cm^{-1} , 1552 cm^{-1} and 1462 cm^{-1} peaks demonstrating the presence of conjugated C=C stretches as well as C=O detection through 1736 cm^{-1} peak indicating the presence of carbonyl group instead of the expected methoxy group.

In similar manner, this FTIR spectra of extracted red pigment from *S. marcescense* corroborate with the findings by Prabha et al. (2017) who reported the presence of OH stretching band due to the vibration of functional group molecular bands in protein and mucopolysaccharides at peak 3362.07 cm^{-1} . Furthermore, peak of 2937 cm^{-1} is responsible for C-H stretching of methylene groups and C=O, carbonyl strong band appearance from peak 1809.31 cm^{-1} . Concurrently, revelation of pyrole group was from the peak with wavelength of 1544.08 cm^{-1} together with C=C stretching at peak 1651.14 cm^{-1} .

3.3.6.2 FTIR of Purple Pigment

Spectral analysis of FTIR for violacein dissolved in methanol gave values of 3327.92 cm^{-1} , 2944.21 cm^{-1} , 2832.59 cm^{-1} , 1656.73 cm^{-1} , 1448.76 cm^{-1} , 1112.12 cm^{-1} , and 1019.44 cm^{-1} . Figure 3.9 shows the result of FTIR for purple pigment extracted from *C. violaceum*.

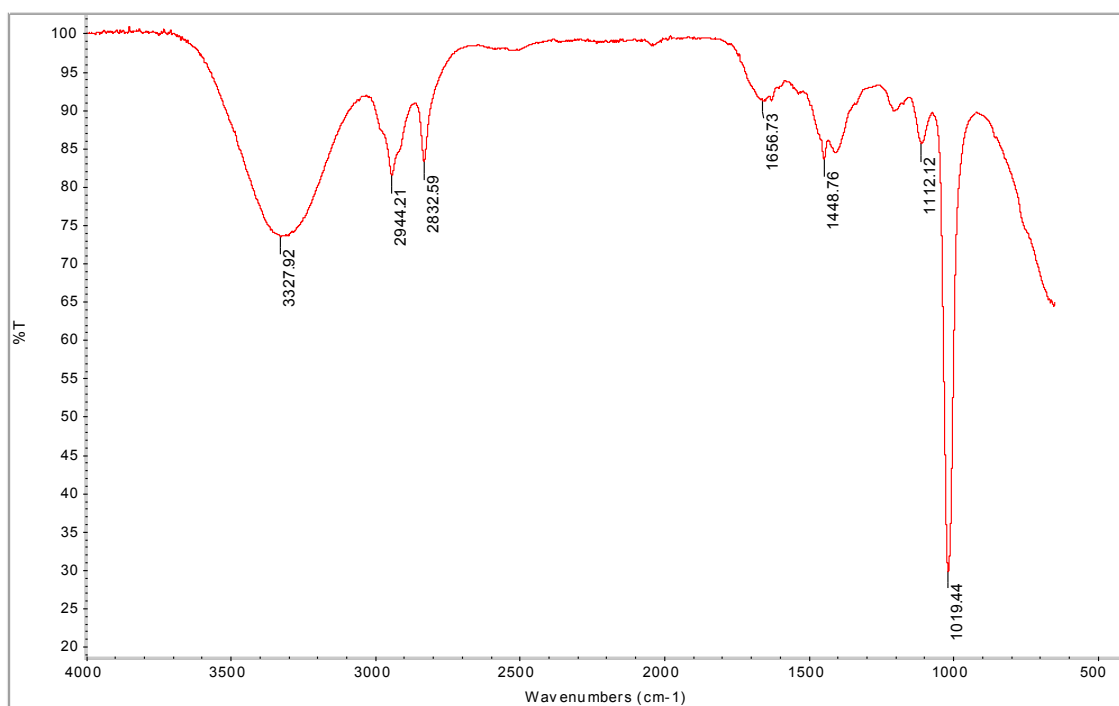


Figure 3.9: FTIR Analysis of Purple Pigment from *C. violaceum*

The broad spectrum in 3500 – 3300 range centred at 3327.92 was resulted from the presence of N–H group on the indole nucleus which is preferred in binding and attachment onto TiO_2 where it facilitates the electron injection in DSSC. This broad peak in the range was similar to that of violacein reported by Subramaniam et al. (2014). Secondary amides form dimers of cis and trans configuration through hydrogen bonding resulting in the replacement of the free N-H stretching band. Two peaks stretch at 2944.21 cm^{-1} and 2832.59 cm^{-1} which were contributed by the stretching of C-H of the alkane group.

The peaks at 1656.73 cm^{-1} and 1448.76 cm^{-1} were resulted from C=O amide groups. The lower frequency band is predominantly N–H in – plane bending (amide II band) and the higher frequency is predominantly C=O (amide I band). This doublet is quite prevalent in amides, where the higher frequency band is primarily C=O (amides I band) and the lower frequency band is primarily N-H in plane bending (amide II band). Since the carbonyl group is conjugated to an aromatic ring, the electrons of both unsaturated groups delocalised, reducing the double bond character of both bonds. Therefore, lowering the carbonyl frequency from 1718 cm^{-1} (normal absorption frequency of carbonyl) to 1656.73 cm^{-1} . The resonance is responsible for the lower absorption frequencies of C=O groups which will be an anchoring group to bind with TiO_2 (Mohan, 2007). Intermediate C – N band was observed from the two peaks at 1112.12 cm^{-1} and 1019.44 cm^{-1} (Kamarudin & Ahmad, 2017).

Similarly, FTIR results from a report by Ahmad et al. (2012) on violacein agrees with the purple pigment extracted in this work. They described the violacein functional group from FTIR spectra with the stretching of O-H group that might overlap with N-H stretching of a secondary amide that corresponded to 3256.53 cm^{-1} . Depiction of C=O is similar to this work with the value of 1657.07 cm^{-1} and stretching of C=C frequency with the value of 1613.18 cm^{-1} . C-O stretching was characterised with the peak at 1223.69 cm^{-1} .

3.3.6.3 FTIR of Yellow Pigment

Evaluation of chemical composition of yellow pigment from *Ralstonia* sp. resulted in the transmittance value of 3279.20 cm^{-1} , 1639.59 cm^{-1} , 1404.17 cm^{-1} , 1014.38 cm^{-1} , 590.33 cm^{-1} , and 555.39 cm^{-1} as shown in Figure 3.10.

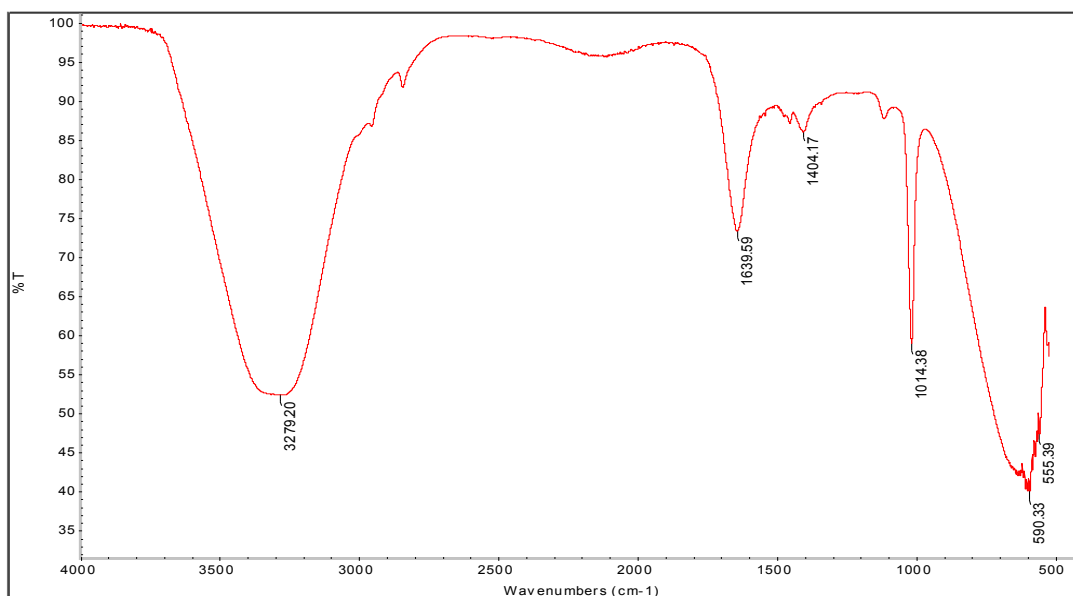


Figure 3.10: FTIR Spectra of Yellow Pigment from *Ralstonia* sp.

Based on Supriyanto et al. (2018), wide range of values from 3300 cm^{-1} – 2800 cm^{-1} resulted from the vibration of OH in the carboxylic acid in the carotenoid structure which crucial in TiO_2 binding with the bacterial pigment. The resilient vibration occurs due to the compression of the band. Ordenes et al. (2016) reported the carotenoid FTIR spectra of yellow pigment from antarctic bacteria with the spectra frequency of 3300 cm^{-1} which is corresponded to the absorption band for the vibration of the OH group. This is in agreement with the result of this work where the spectra frequency was found to be 3279.20 cm^{-1} . A feature of carbonyl group ($\text{C}=\text{O}$) was observed from the signal at 1639.59 cm^{-1} . This could be due to the oxidation of zeaxanthin, lycopene, or lutein. Oxidation happens when oxygen is incorporated into either of the $\text{C}=\text{C}$ double bonds or when the OH group in the chemical structure of the carotenoid is oxidised (Zheng, 2013). The peaks at 1404.17 cm^{-1} resulted in the bending $\text{C}-\text{H}$ of the alkane and 1014.38 cm^{-1} due to bending of $\text{C}=\text{C}$ with disubstitution. The pattern of this result shows similarities in carotenoid (Saha et al., 2015). The FTIR

results and UV spectra from the previous works suggest the yellow pigment from *Ralstonia* sp. falls under the carotenoid family (zeaxanthin, lutein or mixture of both).

3.3.7 Scanning Electron Microscopy of Bacterial Pigments with TiO₂

Scanning electron microscopy of bacterial pigments with titanium dioxide was performed in order to characterise the surface morphology of TiO₂ when adsorbed with pigments. TiO₂ is a nanoporous particles which is a suitable photoanode to carry pigment molecules. The presence of hydroxyl groups on TiO₂ nanostructure has made the chemical adsorption of bacterial pigments possible via condensation with methoxy protons and hydroxyl groups of targeted pigment (Khammee et al., 2020). Figure 3.11 shows the SEM images of TiO₂ nanomaterial which has been coated on top of ITO glass after the heat treatment.

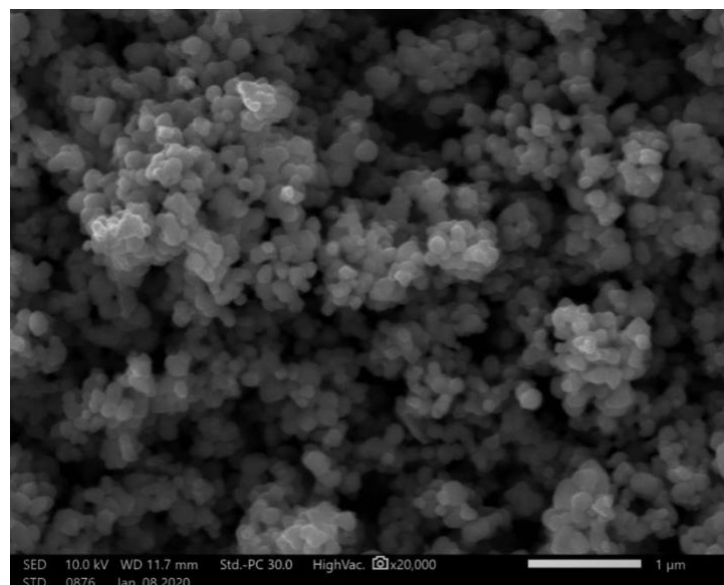


Figure 3.11: Micrograph Image of TiO₂

The analysis shows that TiO₂ investigated in this work is in spherical form. Clusters of TiO₂ spheres were observed together with the holes along the ITO surfaces. The porous matrix together with the absence of fracture in the surface facilitates higher dye adsorption

surface area with excellent inter-particle connectivity. This promotes the continuity of charge transport path (Hossain et al., 2017). Figure 3.12, 3.13, and 3.14 show the Scanning Electron Microscopy (SEM) of TiO₂ coating after adsorbed with prodigiosin, carotenoid and violacein respectively.

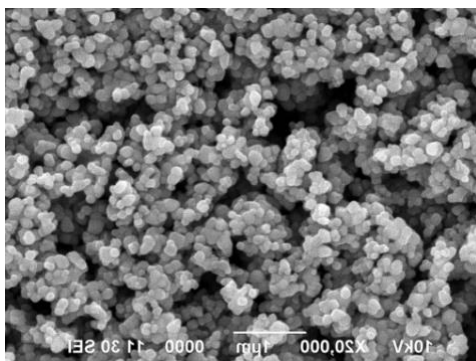


Figure 3.12: Micrograph Image of TiO₂ Adsorbed with Prodigiosin

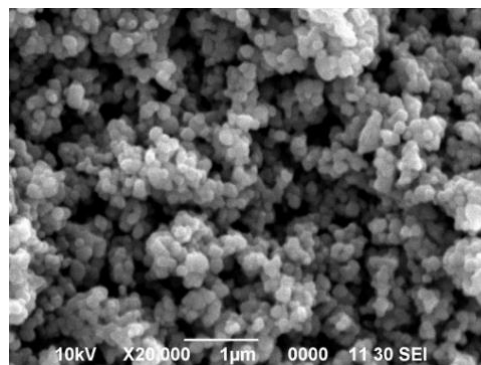


Figure 3.13: Micrograph Image of TiO₂ Adsorbed with Carotenoid

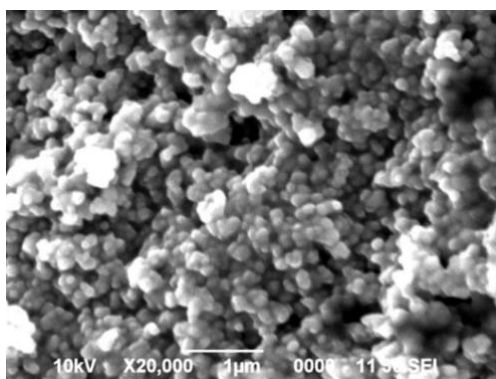


Figure 3.14: Micrograph Image of TiO₂ Adsorbed with Violacein

Photocatalytic processes are based on chemical reactions on the surface of the photocatalyst, TiO₂ spheres typically have a high specific surface area, resulting in an enhanced photocatalytic performance. These structural elements improve these materials'

light-harvesting capacities by enabling as much light as possible to enter the interior (Susanti et al., 2014).

Comparing Figure 3.11 with Figure 3.12, 3.13, and 3.14, a slight change is observed revealing most of the pores in the TiO₂ coating surface filled with dye molecule layer. The dye molecules uncovered the arrangement of the TiO₂ the particles to adhere onto it during adsorption of pigment resulting in the aggregation of molecules that can be seen (Shanmugam et al., 2013). The pigments aid the movement of excited electrons during the transfer of electrons from the excitation of sunlight by filling in the holes in the porous molecules of conductive TiO₂ (Hossain et al., 2017).

Triton X-100 was used in the preparation of TiO₂ solution to stabilise the particle interconnection during microemulsion production. According to Xiangcu et al. (2014), when the temperature of the microemulsion system rises during hydrothermal treatment, the microemulsion system becomes unstable or more flexible, enhancing micelle exchange and this encourage the particle size growth. The numerous tiny cavities observed in Figures 3.12, 3.13, and 3.14 may delay the movement of electrons and decrease the short-circuit photo current (Chou et al., 2008). This will be further discussed in the next chapter.

3.4 Conclusion

In summary, the main objectives of this chapter have been achieved. The three bacterial pigments derived from *S. marcescense*, *C. violaceum* and *Ralstonia* sp. were successfully characterised based on UV-Vis spectrophotometry analysis, FTIR analysis and Scanning Electron Microscopy analysis. The results showed that the red pigment from *S. marcescense* matched the characteristics of prodigiosin. Meanwhile, the purple pigment from *C. violaceum* was found to match the properties of violacein whilst the yellow pigment from *Ralstonia* sp. was found to resemble the characteristics of carotenoid. As these bacterial

dyes were revealed as capable to be used for dye-sensitised solar cell, their potential as photosensitisers will be further discussed in the following chapter.

CHAPTER 4

FABRICATION AND CURRENT-VOLTAGE CHARACTERISATION OF DSSC

4.1 Introduction

Photovoltaic device (PV) is a device that converts sunlight to electrical energy without pollution, moving parts or sound. The mechanism of PV devices is based on the principle of charges, which separates two materials with different conductive mechanisms at an interface. The first fabrication of PV was in the 19th century by Charles Fritts where the PV composed of selenium and a thin layer of gold. After 1873, A technique for increasing the sensitivity of photographic emulsions and associated silver halide emulsions with dyes to create photographic films in black and white was invented by Hermann Wilhelm Vogel's. Due to band gaps of 2.7-3.2 eV, silver halides are insensitive to much of the visible light. This achievement can be considered the first important study of sensitisation of semiconductor dye (Narayan, 2012).

Hernández-Velasco et al. (2020) reported the usage of prodigiosin from *S. marcescens* 11E in DSSC with the conversion efficiency of 0.032%. Moreover, carotenoid extracted from *Chryseobacterium* sp. and *Hymenobacter* sp. from Órdenes-Aenishanslins et al. (2016) was reported to be 0.0323% and 0.0332%, respectively. With a range of light absorption between 400 to 500 nm. To date, the natural carotenoid extraction in DSSC prospect were mainly from plants (Hemalatha, 2012) or those that are chemically synthesized (Yamazaki, 2007). Therefore, research into microbial pigments is now one of the emerging areas in the development of cost effective and environmentally friendly DSSC. In this chapter, the findings of the use of the characterised dyes from *S. marcescens*, *C. violaceum* and *Ralstonia* sp as sensitisers for DSSC will be discussed.

4.2 Materials and Method

4.2.1 TiO₂ Paste Preparations

Two ml of TiO₂ anatase solution (Across, USA) was mixed with 1 g of TiO₂ anatase powder (Across, USA) in a mortar. Then, 0.8 g of polyethylene glycol 10, 000 (Sigma-Aldrich, Canada) was mixed with the resulted solution. A few drops of concentrated ethanol (Sigma-Aldrich, Canada) and concentrated nitric acid (Sigma-Aldrich, Canada) were then added, and the suspension was mixed thoroughly using a pestle and mortar resulting in white sludge. Lastly, 3 drops of triton-X (Across, USA) were added into the white sludge and the mixture was left for 5 minutes to prevent bubbles.

4.2.2 Conductive Glass Preparation

Indium Tin Oxide (ITO) glass was cleaned in a series using 30ml of propanol (Sigma-Aldrich, Canada), 30 ml of acetone (Sigma-Aldrich, Canada), and 30ml of deionised water in a sonicator (Branson, Singapore) for 10 minutes for each medium. The glasses then air-dried. Conductive side was tested using multimeter (XEOLE, China). The edges of the glasses were covered with a tape with 1cm² active area. The active area is considered as area covered with TiO₂ that adsorb in pigments.

4.2.3 Tri-iodide Electrolyte Preparation

The electrolyte was prepared by mixing 0.5 M potassium Iodide (KI) (Sigma-Aldrich, Canada) and 0.05 M iodine (I₂) (Sigma-Aldrich, Canada) in polyethylene glycol (water free) (Sigma-Aldrich, Canada). In this experiment, 8.45 g of KI and 1.27 g of I₂ was mixed in 100 ml of ethylene glycol in a 250 ml conical flask. The solution was mixed using magnetic stirrer. The solution was stirred until both KI and I₂ dissolved. Dark brown solution observed. This electrolyte was kept in dark places before used.

4.2.4 DSSC Assembly

Two glasses were used as a sandwich for DSSC. One glass layered with a smooth titanium dioxide using a clean glass rod and air dried for one minute. The glass then sintered in a furnace for 30 minutes at 450 °C and cooled to room temperature. The TiO₂ glass coated slide were immersed in extracted dye for 24 hours without the exposure to light. It was washed gently with ethanol and dried. The other glass was prepared as carbon counter electrode using carbon sourcing from carbon paint using the same method as layering the TiO₂ as mentioned above. A drop of iodide electrolyte solution was sandwiched between positive and negative side of electrodes and clipped together (Rosana et al., 2014).

4.2.5 Fabrication and Current-Voltage (IV) Characterisation

The fabrication of DSSC were carried out following Alhamed et al. (2012) method with a slight modification using a resistor box (DoMore, China), alligator clip, lamp, and voltmeter (XEOLE, China). The sandwiched DSSC, resistor box and voltmeter were built in a parallel circuit as shown in diagram below.

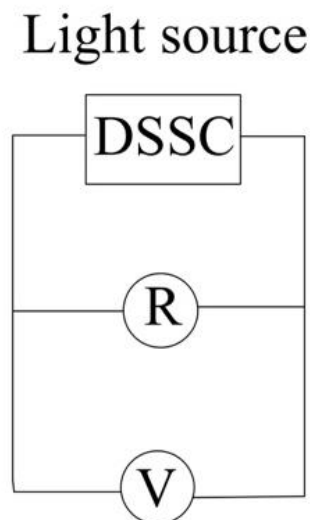


Figure 4.1: Circuit Set Up of DSSC Diagram



Figure 4.2: DSSC Set Up

Current values were calculated using the formula, $I=V/R$. The resistance of the circuit was manipulated in decreasing order to gain the current values. The corresponding voltage values were measured. Current density was calculated by dividing the current values to 1cm^2 of active site area. Graphs of current density (mA/cm^2) against voltage (mV) were plotted. The fabrication of the DSSC was started by immersing the treated TiO_2 in the targeted dye overnight resulting in adsorption of the dye. Changes of colour can be observed to the white colour of titanium dioxide originally to red, purple, and yellow after being immersed in prodigiosin, violacein and carotenoid pigments, respectively. Traces of pigments and other constituents were washed off by ethanol and blotted off dry to ensure that no other particles disrupting the circuit flow of electrons. The method used in depositing

the photoanode which is the titanium dioxide in this research is doctor blading technique. This method was used as it was easy to be carried out as it needs only a glass rod to distribute the photoanode onto the ITO glass uniformly.

The efficiency (η) of DSSC was calculated by measuring the area under the graph of current density vs voltage using the formula mentioned in Section 2.3.6.

$$\eta = \frac{J_{sc} \times V_{oc} \times FF}{P_{in}} \quad \text{Equation 4.1}$$

The Fill Factor (FF) of the cells was calculated with the formula stated in Section 2.3.6 as shown in Equation 4.2.

$$FF = \frac{J_{max} \times V_{max}}{J_{sc} \times V_{oc}} \quad \text{Equation 4.2}$$

The power input was the light illuminated from the white lamp which is 100 mW/cm².

4.3 Results and Discussion

4.3.1 Measurement of DSSC Performance

The performance of DSSC was carried by connecting the circuit to multimeter and resistor in series which were exposed to white light to obtain the efficiency curve graph which describes the relationship between voltage and density current. From the efficiency graph, power was determined by multiplying the current with the voltage. Figure 4.1, 4.2, and 4.3 shows the efficiency of DSSC developed using prodigiosin, carotenoid and violacein, respectively.

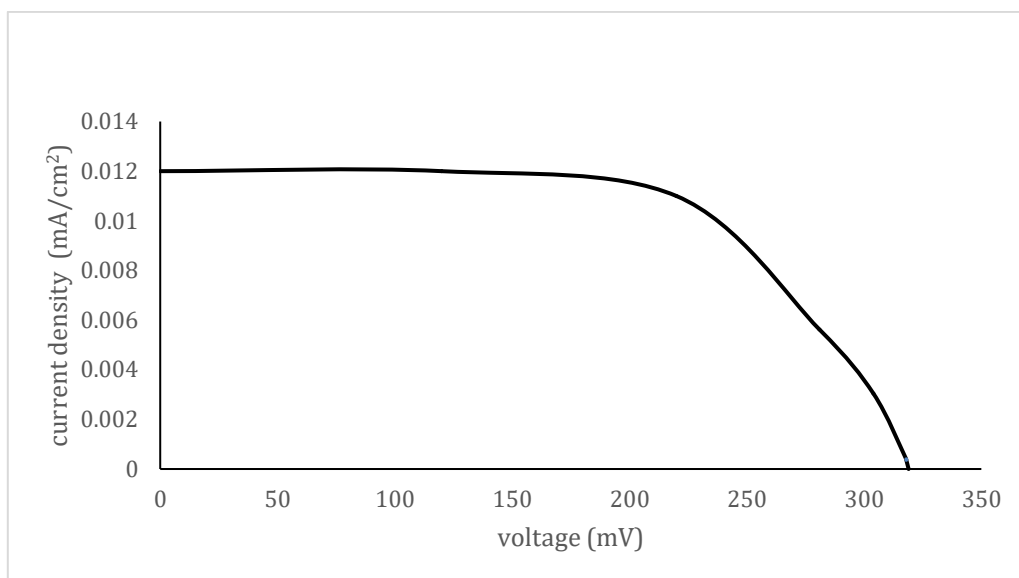


Figure 4.3: Efficiency of DSSC Developed using Prodigiosin

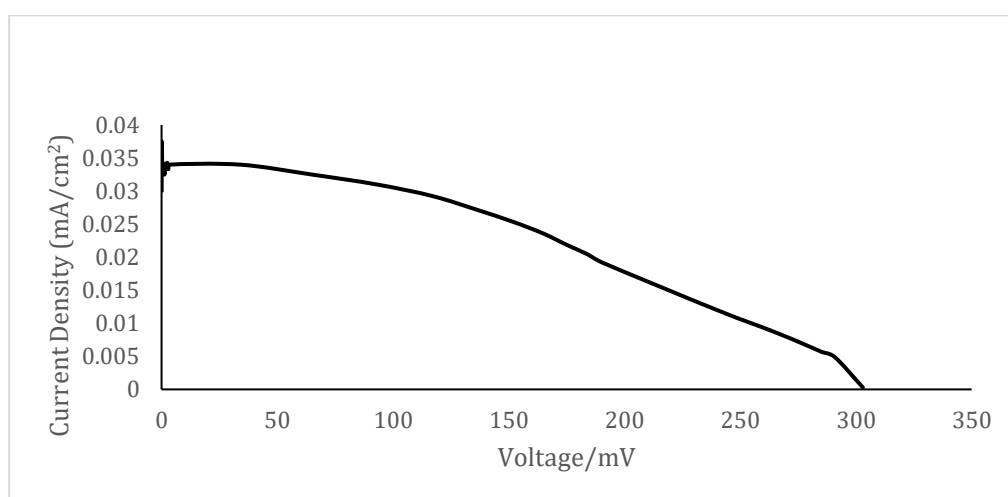


Figure 4.4: Efficiency of DSSC Developed using Carotenoid

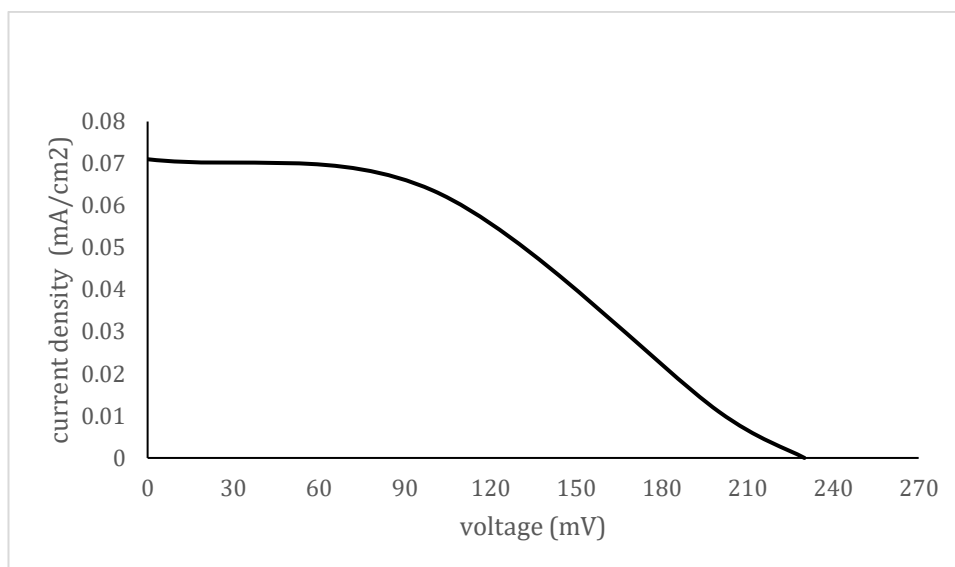


Figure 4.5: Efficiency of DSSC Developed using Violacein

From the efficiency graphs two parameters namely Open Voltage (V_{oc}) and short circuit (I_{sc}) can be determined. The x-intercept is V_{oc} whilst the y-intercept is I_{sc} . Therefore, V_{oc} from the prodigiosin efficiency graph was 318 mV whilst the I_{sc} is 0.012 mA/cm². V_{oc} obtained from the carotenoid and violacein efficiency graphs were 303 mV and 240 mV, respectively. Furthermore, the short circuit current (I_{sc}) retrieved from the carotenoid and violacein efficiency graphs above were 0.035 and 0.012 mA/cm², respectively. Bacterial carotenoid from Ordenes et al. (2016) works reported V_{oc} of 548.79 mV paired with I_{sc} of 0.13 mA/cm² which are higher than carotenoid from this research. There is a high possibility that the dye molecules did not adhere well to TiO₂ film halting the clear pathway for electrons transport.

Upon the illumination of light, pigment dyes molecule becomes excited by injecting electrons to conduction band of TiO₂ nanoparticle films. The redox electrolyte provides recirculation of electrons and at the same time creating a path for electron transport (O'Regan & Grätzel, 1991). Therefore, the DSSC device convert sunlight energy into electrical energy

when illuminated with light and current and voltage data can be obtained from the external circuit.

As shown in Figure 4.1, 4.2, and 4.3, the DSSC utilises bacterial pigments exhibit expected behaviour of solar cells attest by expected current-voltage curve. No photo-voltage responses were observed under darkness due to minimal density of charge carriers in the valence band and presence of the indication of diode character in sensitized TiO_2 and electrolyte interface named Schottky barrier. In the presence of light, the dynamic response in the intermittent status implicates the occurrence of processes such as photo-regeneration, recombination, and diffusion of electrons (Tello et al., 2012).

Figures 4.4, 4.5 and 4.6 show the relationship between power and voltage of DSSC developed using prodigiosin, carotenoid and violacein respectively. The power curves show the maximum power points, which are the optimal working points of the cells. The maximum power for prodigiosin, carotenoid and violacein were 2.42 mW, 3.89 mW and 6.36 mW respectively. It can be observed that DSSC that used violacein generated the highest power whilst the lowest power was generated by DSSC that used prodigiosin.

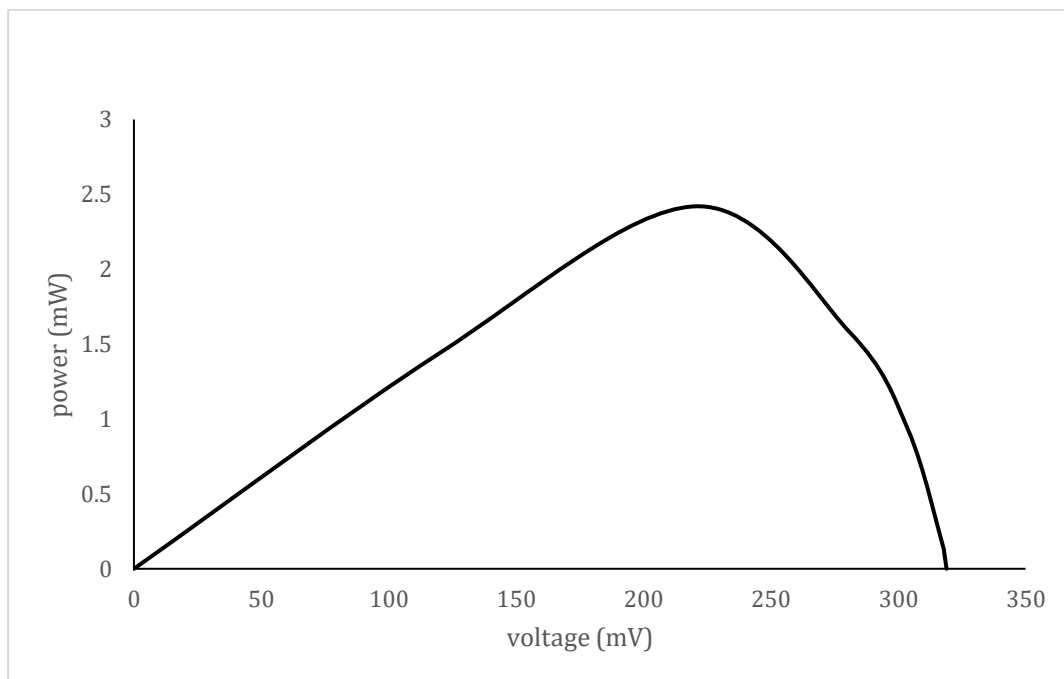


Figure 4.6: Relationships between Power and Voltage of DSSC Developed using Prodigiosin

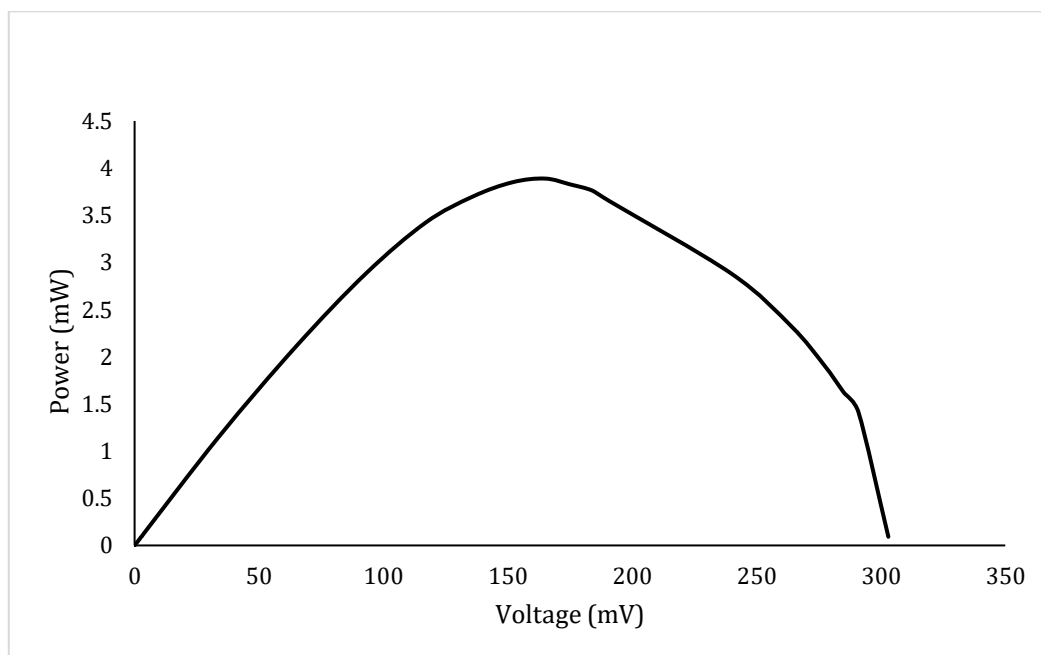


Figure 4.7: Relationships between Power and Voltage of DSSC Developed using Carotenoid

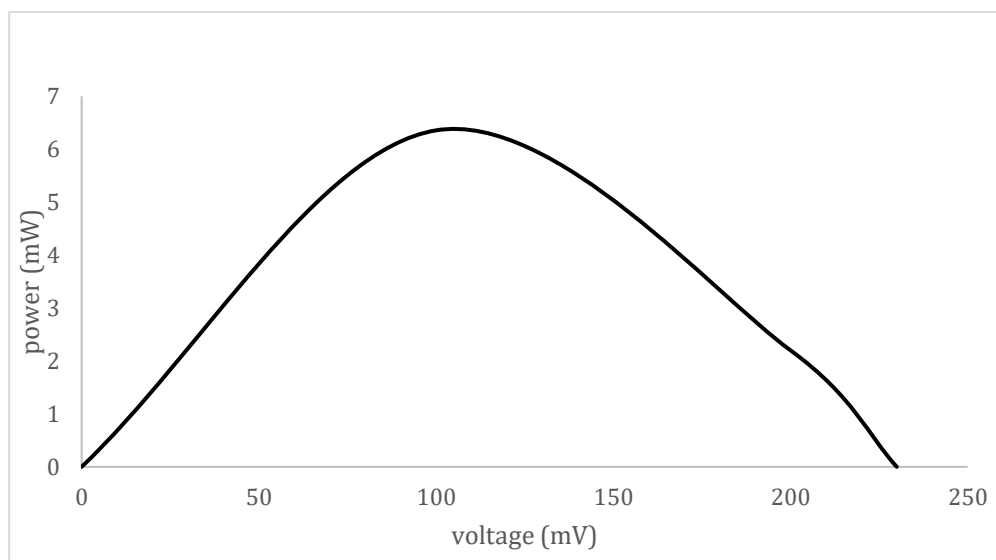


Figure 4.8: Relationships between Power and Voltage of DSSC Developed using Violacein

Red dragon fruit pigment was used as dye sensitiser in DSSC reported the maximum power of 35 mW which is much higher than all of the three targeted bacteria (Siregar, 2021). Silva and coworkers (2019) reported the maximum power exhibited by bacterial pigment (melanin) isolated from *S. fildesensis* of 3910 mW with pigment concentration of 6500 µg/ml. Compared to the concentration of bacterial pigment in this work, melanin with significant concentration resulting in notable maximum power production.

4.3.2 Photoelectrical Properties of DSSC

Table 4.1 shows the short-circuit current (J_{sc}), open circuit voltage (V_{oc}), fill factor (FF) and efficiency percentage of DSSC developed using prodigiosin, carotenoid and violacein. Prodigiosin has the highest FF amongst the three pigments which was 0.6342 %. The FF represents the measure of closeness in a solar cell and how it acts as an ideal source. Solar cell fill factor is a metric that measures the efficiency of a solar PV module. The solar cell fill factor has an impact on the solar panel because it influences the efficiency of the panel by affecting the cell series values. It also has an influence on solar panel energy

efficiency by altering the shunt resistances and diode losses. When the shunt resistance of a solar cell is increased, the series resistance lowers, resulting in a larger FF. Meanwhile, larger FF in a solar cell means that the cell is more efficient, resulting in a higher output power that matches the theoretical maximum (Jao et al., 2016). However, even though with the highest fill factor value, prodigiosin produces the lowest power input compared to the other two pigments due to lower short circuit current produced. This may cause by high resistance in the circuit limiting the current production.

Table 4.1: Photoelectrical Properties of Extracted Bacterial Pigments

Pigments	Source of pigment	Short circuit current, J_{sc} (mA/cm ²)	Open circuit voltage, V_{oc} (mV)	Fill Factor, FF (%)	Efficiency, η (%)
Prodigiosin	<i>S. marcescens</i>	0.012	318	0.6342	0.0242
Carotenoid	<i>Ralstonia</i> sp.	0.035	313	0.3488	0.0382
Violacein	<i>C. Violaceum</i>	0.071	318	0.5802	0.0806

The result from this work shows that the violacein pigment had the highest efficiency of 0.0806%, with the highest FF of 0.5802% while the prodigiosin pigment had the lowest efficiency of 0.0242%. This implies that the violacein pigment in the DSSC converted 0.0806% of the solar energy into electrical power. In comparison to Hernández-Velasco et al. (2020) the efficiency of prodigiosin was reported to be 0.032%. Meanwhile, in a work by Ordenes-Aenishanslins et al. (2016), only *Chryseobacterium* sp. and *Hymenobacter* sp. were reported as sources of carotenoid. This study is amongst the first to report the extraction of carotenoid from *Ralstonia* sp. and its use for DSSC application. The efficiency of carotenoid in this work (0.0382 %) is comparable to that extracted from *Chryseobacterium* sp. and *Hymenobacter* sp. which were 0.0323% and 0.0332% respectively (Ordenes et al., 2016).

The efficiency of DSSC developed using violacein in this research (0.0806%) is lower than the efficiency of DSSC using a pigment produced by *Janthinobacterium* sp. UV13 (0.230%) (Marizcurrena et al., 2021). The low efficiency in this work may be caused by improper interaction of bacterial pigments with TiO₂ which decreases the optimal electron transport in the circuit. The instability of electrolyte also affecting the efficiency of electron reload within the cell.

As discussed in Section 3.35, the concentration of bacterial pigment presence in the sample facilitates the electron transportation by increasing the chances of dye molecules adhering onto the TiO₂ nanoparticles. This can be supported by the results showed in Table 4.1 where violacein with the highest pigment concentration exhibiting highest efficiency among the three targeted bacteria. There are a few factors that can explain the behaviour of the DSSC efficiency in this work. Violacein has the lowest band gap energy as calculated in Section 3.35 which insinuates low energy is required for the pigment to excite the dye molecules which are essentials in improving electron transportation. Electron transportation is crucial in DSSC efficiency as the movement of the electron along the circuit generates current.

4.4 Conclusion

In summary, the third objectives of this work have been achieved. Fabrication of DSSC using prodigiosin from *S. marcescense*, carotenoid from *Ralstonia* sp., and violacein from *C. violaceum* was successfully performed. Furthermore, the efficiency of DSSC established using three dyes was successfully determined. The efficiencies of DSSC established using prodigiosin, carotenoid and violacein were 0.0242%, 0.0382%, and 0.0806% respectively. Violacein was identified as the most efficient pigment for DSSC in this work.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In general, the aim of this investigation was successfully achieved. This work has established novel knowledge on the potential of the targeted bacterial dyes for DSSC application. The selected bacterial pigments were successfully characterised based on certain analyses. The UV-vis analysis showed that the absorbance peak of red pigment extracted from *S. marcescens* was at 540 nm. Meanwhile the absorbance peaks of yellow pigment extracted from *Ralstonia* sp. and purple pigment extracted from *C. violaceum* were at 420 nm and 570 nm, respectively. This indicates that all the three pigments can absorb sunlight within visible region.

FTIR analysis showed the functional groups of each of the bacterial pigment. Purple pigment was found to consist of amino group, carboxyl group, hydroxyl group and cyano group. Red pigment was found to consist of carboxyl group, amino group, alkene group and alkane group. Yellow pigment was found to comprise hydroxyl group, alkene group and alkane group. These functional groups assist the pigments to adhere with TiO₂ for better electron transportation. The SEM analysis of the TiO₂ after being adsorbed with bacterial dyes shows the dye molecules filling up the porous TiO₂ molecules. Based on the collective analysis, the red pigment from *S. marcescens* was identified as prodigiosin. Meanwhile, the purple pigment from *C. violaceum* shares the characteristics of violacein. The yellow pigment derived from *Ralstonia* sp. was identified as carotenoid.

Fabrication of DSSC was carried out to determine the capability of all the three bacterial pigments as dye sensitisers. All the pigments; prodigiosin, carotenoid and violacein were found capable of generating electric by utilising sunlight yielding efficiency of 0.0242%, 0.0382% and 0.0806% respectively. Maximum power generated from 1 cm² active site was 2.42 mW for prodigiosin, 3.89 mW for carotenoid and 6.36 mW for violacein. This indicates the promising applications of aforementioned dyes for DSSC applications.

In conclusion, the results achieved in this work have confirmed the ability of all the targeted bacterial pigment as alternative dye sensitisers for DSSC development

5.2 Recommendations

In continuation to the current study, future works should consider several aspects. Firstly, the range of TiO₂ thickness during DSSC fabrication should be broadened in order to investigate its effect on dye adsorption. This will give insights into suitable thickness that can improve the cell's efficiency.

Next, future research should consider improving the capability of electrolyte to reload ions faster and with better stability in cells. This can be performed by testing the thermosetting gels that can increase the stability of electrolyte in order to avoid it from drying too fast.

Lastly, other parameters such as different types of dye derived from other pigmented bacteria such as *Pseudomonas* sp. should be investigated. This will give more options of potential dye sensitisers derived from bacteria

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