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Research Report

THE PHOTODYNAMIC EFFECT OF LED-MAGNETIC EXPOSURE TO PHOTOINACTIVATION OF AEROBIC PHOTOSYNTETIC BACTERIA

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ABSTRACT

All photosynthetic bacteria have a major pigment of bacteriochlorophyl and accessor pigment e.g. the carotenoids, which both have an important role in photosynthesis process. This study aim to explore the exogenous organic photosensitizer from photosyntetic bacteria for photodynamic therapy application. This study is an experimental research aiming to test the potential illumination of LED with wavelength 409, 430, 528 and 629 nm, and power optimization and time exposure LED-magnetic for optimum photo activation Rhodococcus growth. The reseach design use a factorial completely randomized design with factor of power and exposure time. The number of bacterial colonies grown measure using of Total Plate Count (TPC) methods. The result of anova test shows that irradiation treatment with LED 409 nm, 430 nm, 528 nm and 629 nm significantly affects on bacterial colony growth. LED 409 nm exposure has the greatest potential to boost the growth of bacterial colonies by 77%. LED exposure and the addition of 1.8 mT magnetic field increases bacterial colony growth by 98%. Results of optimization of LED and magnetic fields show power 46 mW and a 40 minute (energy dose 110 J/cm^2) optimum growth of bacterial colonies increase by 184%. So LED and magnetic illumination has potentially increased the viability of an aerob photosyntetic bacteria colonies.

Key words: *photosynthetic bacteria, optimum energy dose, LED-magnetic, Rhodococcus*

ABSTRAK

Semua bakteri fotosintetik memiliki pigmen mayor yaitu bakterioklorofil dan pigemen asesoris seperti karotenoid, yang memiliki peran penting dalam proses fotosintesis. Penelitian ini bertujuan untuk mengeksplorasi eksogen fotosensitiser organic dari bakteri fotosintetik untuk aplikasi terapi fotodinamik. Penelitian ini merupakan penelitian eksperimental bertujuan untuk uji potensi iluminasi LED dengan panjang gelombang 409, 430, 528 dan 629 nm, dan optimasi daya dan lama waktu pemaparan LED-magnet fotoaktivasi pertumbuhan Rhodococcus. Desain penelitian ini menggunakan desain acak lengkap pola faktorial dengan faktor daya dan waktu pemaparan. Jumlah koloni bakteri yang tumbuh dihitung dengan menggunakan metode TPC. Hasil uji anova menunjukkan bahwa perlakuan penyinaran dengan LED 409, 430, 528 dan 629 nm berpengaruh signifikan terhadap pertumbuhan bakteri. Pemaparan LED 409 nm berpotensi terbesar untuk meningkatkan koloni bakteri 77% . Pemaparan LED-magnet meningkatkan pertumbuhan koloni bakteri 98%. Hasil optimasi LED-magnet menunjukkan daya 46 mW dan waktu 40 menit (dosis energi 110 J/cm^2) optimum meningkatkan pertumbuhan bakteri sebesar 184%. Jadi iluminasi LED dan magnet meningkatkan viabilitas koloni bakteri fotosintetik aerob.

Kata kunci: *bakteri fotosintetik, dosis energy optimum, LED-magnet, Rhodococcus*

INTRODUCTION

All photosynthetic bacteria have photosynthetic pigments that are sensitive to light (photosensitizer). The main pigment in photosynthetic bacteria is bacteriochlorophyl and accessory pigments which one carotenoid, which both have an important role in photosynthesis process. Bacteriochlorophyl have major role as a light harvesting which packaged in the form of first light harvesting (LH1) and second light harvesting (LH2) and as a charge separation in the form of the reaction center (RC)[1]. Carotenoid also have major role as same as bacteriochlorophyl that is as an accessory light-harvesting pigment and as a triplet quencher to provide protection again photooxidative damage[2]. That is the difference between absorbance spectrum when photophysical process.

Exposure light will be absorbed by the photosensitizer molecules in photosynthesis bacterial, light photon energy absorbance will excite the photosensitizer molecules in the singlet excitation and triplet. Excitation of photosensitizer molecules occurs only if of light photons spectrum correspond to the photosensitizer absorption spectrum. Subsequent excitation energy is transferred and converted into electrochemical potential energy in the form of transmembrane charge separation as well as the synthesis of adenosine triphosphate (ATP)[3] for the activation of photosynthetic bacteria. Amount of energy converted to ATP (photoactivation) depends on the number of photosensitizer molecules and the number of photons of light absorbed.

One of the photosynthetic pigment producing bacteria is *Rhodococcus*. The *Rhodococcus* include Eubacteria subkingdom members, a group of bacteria autotrophs (able to make their own food from inorganic substances), have chlorophyl and is able to photosynthesize like plants. *Rhodococcus* have chlorophyl pigment (green), carotenoids (orange) and pigment phicobilin consisting of phycocyanin (blue) and phicoeritin (red). Combine of these pigments produce the color to be turquoise. Cell wall contain peptides, hemicellulose and cellulose, and have a slimy membrane. These bacteria use two photosystems to split water and produce oxygen as a byproduct. These bacteria like to live in fresh water, but there are some that live in the sea.[4]

This study is an experimental research laboratory, aimed to determine the potential irradiation of LED Purple 409, LED Blue 430 nm, LED Green 528 nm and LED red 629 nm and 1.8 mT magnetic field for Rhodococcus growth activation as well as effectivel dose energy optimization of LED-magnetic for photoactivation. Giving of 1.8 mT magnetic field from a bar magnet aims to increase the biosynthesis of photosensitizer molecules, thereby increasing the amount of light-absorbing molecules.

MATERIAL

The Sample *Rhodococcus* bacteria were isolated from water river Mas, Surabaya. The Bacteria are grown on the photosynthetic media (PMS).

Irradiation Equipment

Irradiation equipment is a source LED light instrument, microcontroller, cervo motors, temperature sensor and LCD display. The Source LED light is used i.e. 409nm Purple, 430 nm Blue, 528 nm Green and 629 nm Red for exposure the in vitro bacterial. The type AVR 8535 microcontroller is used for setting an exposure time and power LED. The Paralax Continous servo motors is used for rotating bacterial petri dish on holder for flatten irradiation. The temperature sensor type LM35 is used for controlling the room temperature remains constant. The LCD display is used for showing given the pulse width modulation of irradiation and equipped by the timer running according to the length of time a given input, and the room temperature is detected by the temperature sensor. Before the LED instrument used for irradiation, temperature and time exposure calibration were done before.

METHODS

This study are prepared using a completely randomized design (RAL = Fully Randomized Design) factorial[5], which consists of two factors; A factor (irradiation power) with 4 levels (i.e. 17 mW, 28 mW, 34 mW and 46 mW) and B factor (exposure time) with 4 levels (i.e. 20 min, 30 min, 40 min and 50 min). Each treatment is accompanied by the control group using 3 times replication.

The Bacterial Growth

The Bacteria are grown on sterile PMS medium (photosynthetic medium) for 48 hours at 37°C temperature on the shaker incubator until the absorbance values obtained (OD) solution in 0.15 at 600 nm wavelength. The two ml of each bacterial sample is put in a sterile plastic dish diameter 3.5 cm and ready for irradiated.

Irradiation Bacteria by LED

Petri dishes containing bacteria are placed on the holder in the acrylic box above platform servo motors. The distance between the LED and the cup is permanently made in 2 cm. The source LED light are performed at various power and exposure time. Subsequently, the bacteria in the treatment group and the control are grown in PMS *agar* medium.

Counting the number of bacterial colonies

Samples are taken out from the incubator and counted the number of bacterial colonies growing by Total Plate count method using a Quebec Colony Counter. Next step, calculating the percentage would decrease the number of bacterial colonies that grow on each treatment using the equation:

| (ý Treatment colonies – ý control colonies)/ ý control colonies | × 100%

Statistical Analysis

Analysis of research data use SPSS statistical analysis (Statistical Package For Social Science) 13.0 for windows, i.e. factorial ANOVA for determining the effect of each factor and the interaction between factors. To show couple of different treatment groups, the analysis use multiple comparison test, on SPSS using Multiple Comparison Post Hoc5.

RESULTS AND DISCUSSION

The LED instrument has a temperature gauge and a time duration of irradiation designed with precision calibrator. Data of temperatur and time exposure calibration were showed in Table 1 and 2.

In LED instrument performance measurement, the time duration and temperature of LED exposure was set up by calibrator ENKO Sport Digital Stopwatch Timer and calibrators *Atech Thermo* L87AD (Figure 1). The regression graph of time duration and temperature LED instrument yield $R^2 = 1$ and $R^2 = 0.9995$ that means the LED instrument has a good performance.

Table 3 and 4 showed the percentage of bacterial colonies growth after LED exposure and with 1.8 mT magnetic field exposure.

Test results of the potential LED irradiation and 1.8 mT magnetic field exposure can be summarized in Table 4. The test results indicate that in LED exposure has potentially increase the number of *Rhodococcus* growth colonies at 77% and increase to 98% by the addition of 1.8 mT magnetic field exposure.

In Figure 3 and Figure 4 show that potentialy percentage reduction in the number of *Rhodococcus* colonies in 469 nm, 541 nm, 626 nm and 409 nm of LED exposure and with 1.8 mT magnetic field exposure.

Table 4 showed the percentage of bacterial colonies growth after LED exposure and with 1.8 mT magnetic field exposure in varying intensity and time exposure.

Figure 5 shows a graph of the percentage growth of *Rhodococcus* colony on a variety of power and duration time in LED irradiation and 1.8 mT magnetic field exposure. Exposure to 409 nm violet LED optimal *Rhodococcus* increase growth by 119% in power 28 mW and 60 minutes duration time exposure (dose 101 J/cm^2). Results of exposure optimization in 409 nm purple LED and 1.8 mT magnetic field shows the percentage of *Rhodococcus* growth by 184% in the power of 46 mW and 40minute duration time exposure (dose 110 J/cm^2). Exposure to

Table 1. Time exposure calibration data of LED instrument

time (s)	Time of LED instrumen (s)										tmean	Time of calibrator(s)
	1	2	3	4	5	6	7	8	9	10		
300	300	300	300	300	300	300	300	300	300	300	300	300
600	600	600	600	600	600	600	600	600	600	600	600	600
900	900	900	900	900	900	900	900	900	900	900	900	900
1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200

Table 2. Temperatur calibration data of LED instrument

Temperature (ºC)	Temperature of LED instrumen (ºC)										T mean	ðT	Calibrator temperature
	1	2	3	4	5	6	7	8	9	10			
21	20	20	20	21	21	21	21	21	21	21	20,7	0,48	21
22	22	22	21	21	22	22	22	22	22	22	21,8	0,42	22
23	23	23	22	24	22	23	23	23	23	23	22,9	0,57	23
24	23	23	24	24	24	24	24	24	24	24	23,8	0,67	24
25	25	25	25	25	24	25	25	25	25	25	24,9	0,32	25
26	26	26	26	25	25	26	26	26	26	26	25,8	0,42	26
27	27	27	27	28	27	27	26	27	27	27	27	0,47	27
28	28	28	28	28	27	28	28	28	28	28	27,9	0,32	28
29	29	29	28	28	29	29	29	29	29	30	28,9	0,57	29
30	30	30	30	30	30	30	30	31	30	30	30,1	0,32	30

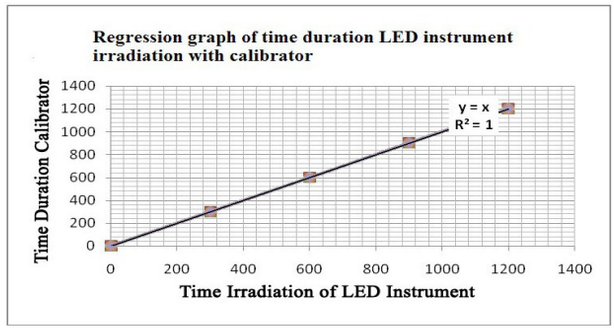

Figure 1. Regression graph of time duration LED instrument irradiation with calibrator.

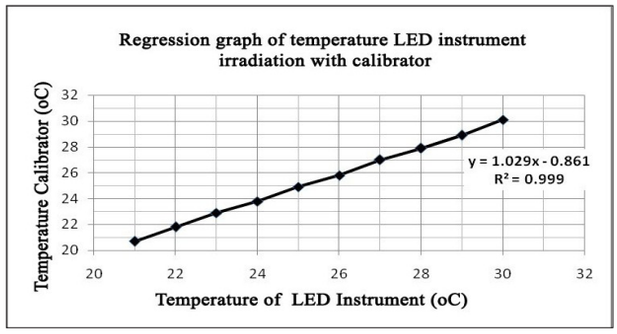

Figure 2. Regression graph of temperature LED instrument irradiation with calibrator.

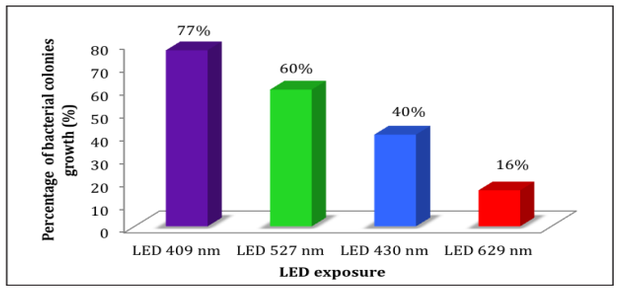

Figure 3. The Graph Percentage of *Rhodococcus* bacteria colonies growth in LED exposure.

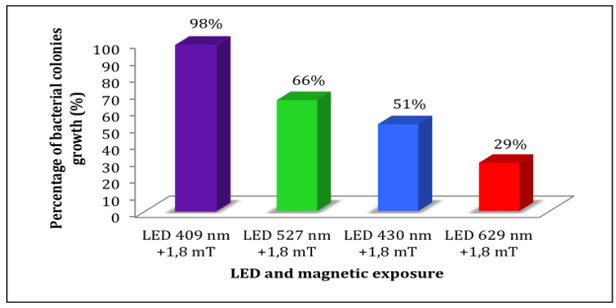

Figure 4. The Graph Percentage of *Rhodococcus* bacteria colonies growth in LED exposure and 1,8 mT magnetic field exposure.

Table 3. Potential of LED irradiation and 1.8 mT magnetic field exposure on the percentage growth of photosynthetic bacteria

		Percentage of Bacterial Colonies Growth (%)
LED exposure	LED 409 nm	77
	LED 527 nm	60
	LED 430 nm	40
	LED 629 nm	16
LED and 1.8 mT magnetic exposure	LED 409 nm	98
	LED 527 nm	66
	LED 430 nm	51
	LED 629 nm	29

430 nm blue LED and 1.8 mT magnetic field *Rhodobacteria* optimal increase by 111% growth in power of 34 mW and 50 minute duration time exposure (dose 102 J/cm^2).

One way ANOVA test results showed that there have influence of LED irradiation treatment 409 nm, 430 nm, 528 nm and 629 nm against *Rhodococcus* colony growth (Table 5).

Statistical test results of exposure energy dose optimization purple LED 409 nm and 1.8 mT magnetic field on *Rhodococcus* data show significance (p) = 0.624 > α (0.05), which means the data i.e. the percentage growth of bacterial colonies on 409 nm purlple LED irradiation normally distributed. The test results showed homogeneity (p) = 0.109 > α (0.05), which means homogeneous data. Factorial ANOVA test results showed that the power factor of 409 nm purple LED irradiation has a significance level (p) = 0.043 < α 0.05, which means that the radiation has an effect on the percentage growth in the number of *Rhodococcus* bacteria colonies. Summary of statistical test results are shown in Table 6 below.

Figure 5 shows a graph of the percentage growth of *Rhodococcus* colony on a variety of power and duration time in LED irradiation and 1.8 mT magnetic field exposure. Exposure to 409 nm violet LED optimal *Rhodococcus* increase growth by 119% in power 28 mW and 60 minutes duration time exposure (dose 101 J/cm^2). Results of exposure optimization in 409 nm purple LED and 1.8 mT magnetic field shows the percentage of *Rhodococcus* growth by 184% in the power of 46 mW and 40 minute duration time exposure (dose 110 J/cm^2). Exposure to 430 nm blue LED and 1.8 mT magnetic field *Rhodobacteria* optimal increase by 111% growth in power of 34 mW and 50 minute duration time exposure (dose 102 J/cm^2).

Table 4. The percentage of bacterial colonies growth after LED 406 nm and magnetic exposure in varying LED time and power exposure

Power time	Percentage of *Rhodococcus* bacteria colonies growth (%)			
	25 mW	46 mW	68 mW	93 mW
20 minutes	102	90	109	111
30 minutes	111	123	98	85
40 minutes	116	148	112	114
50 minutes	126	117	95	81

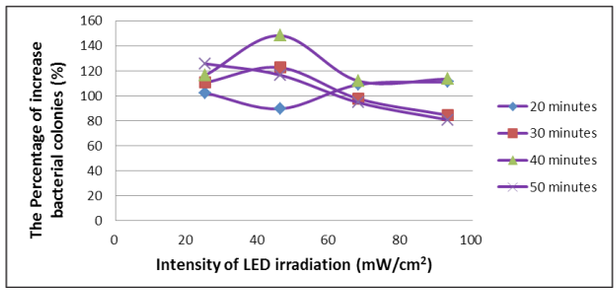

Figure 5. The Graph Percentage of Increase *Rhodococcus* bacteria colonies in 409 nm purple LED irradiation and 1,8 mT magnetic field exposure.

All photosynthetic bacteria have main pigment such as bacteriochlorophyll (BChl) and accessory pigments namely carotenoids. Both of these pigments have an important role in the process of photosynthesis. Bacteriochlorophyll is magnesium porphyrin which has more saturated tetrapyrrole ring. This Porphyrin causes BChl absorbs at a wavelength near-infrared around 620–700 nm[2]. The main role of Bacteriochlorophyll is as a light-harvesting and charge separation. Light-harvesting Bacteriochlorophyll is packaged in first light harvesting (LH1) and second light harvesting (LH2) complex, but charge separation Bacteriochlorophyll is packaged in the form of the reaction center (RC). Reaction center is in the middle of the circle of LH1 and functionally very closely related, so that the unity between LH1 and RC often called RC-LH1 core complex.

Light harvesting is also carried by several major carotenoid pigments that give main pigmentation at visible spectrum area, between 450 to 550 nm.[6] Carotenoids are long chain isoprenoid, usually the amount of carbon is 40. Carotenoids are synthesized from 8 isoprene units (C5), the bonds between the carbon system alternately (double and single). This double bond is able to absorb light.[15] Carotenoids have important roles as light-harvesting especially in environments with limited light conditions and photo-protector bacteriochlorophyll against excessive light. In addition, carotenoids also act to prevent photo oxidation, due to the presence of oxygen in photosynthesis.[11]

The mechanism of energy transfer on photosynthetic bacteria by using inductive resonance and delocation excited. In inductive resonance energy transfer occurred energy transfer. When the bacteria are exposed by appropriate spectrum to the light spectrum, photon absorption then occurs, followed by electron excitation of photosensitizer. The excited electron is unstable and will transfer its energy to another molecule before it fell to the ground state. On excited transfer energy mechanism, the excited electrons move from one molecule to another molecule. This movement is very short and it just occurred on adjacent molecules less than 2 nm at distance.

According Vermeglio and Joliot[12] photosynthesis of bacteria originated from the absorption of light by an antenna system which contains the chromophore such as bacteriochlorophyll and carotenoid polyenes. Singlet excitation energy is quickly transferred between the antenna chromophores, and finally to the reaction center. The role of the reaction center is changing the excitation energy into

Table 5. The result of One Way ANOVA tests for determining the effect of LED irradiation 409 nm, 430 nm, 528 nm and 629 nm

Bacterial Isolates	Group	N	Percentage increase in bacteria colony (%)		T test	
			Rate	SD	Significance	Conclusion
Rhodococcus by LED irradiation	**409 nm LED**	**5**	**76,8**	5,5	p = 0,000	There is a significant difference
	528 nm LED	5	59,8	6,4		
	430 nm LED	5	40,0	5,0		
	629 nm LED	5	15,8	2,7		
	Total	20	45,10	23,8		
Rhodococcus by 1,8 mT magnetic exposure	**409 nm LED**	**5**	**98,2**	12,8	p = 0,000	There is a significant difference
	528 nm LED	5	66,8	12,8		
	430 nm LED	5	51,4	10,1		
	629 nm LED	5	28,4	7,7		
	Total	20	60,95	27,9		

Tabel 6. Statistical test results of exposure energy dose optimization purple 409 nm LED and 1.8 mT magnetic field on *Rhodococcus* colonies

Factor	Group	N	Percentage increase in bacteria colony (%)		Anova	
			Means	SD	significance	Conclusion
Power	46 mW (a)	3	97,4	19,6	p = 0,000	There is a significant difference
	34 mW (a,b)	3	103,3	20,8		
	17 mW (a,b)	3	113,7	22,1		
	28 mW (b)	3	119,2	25,6		
	Total	12	108,4	23,1		

electrochemical energy in the form of a trans membrane charge separation.

The absorption of light is followed by electron transfer from bacteriopheophytin to bacteriochlorophylls, QA quinone, QB quinone and binds with hydrogen to form hydroquinone. Hydroquinone which is produced diffuses to the cytochrome bc complex, which is a trans membrane proton pump.[11] The next step is transferring electrons to cytochrome-c by realizing H+ ions. The energy released is used to transfer protons across the membrane and the resulting energy drives the synthesis of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and inorganic phosphate (Pi) with catalysis by ATP synthase.

Carotenoids are lipids so that this pigment is liposoluble (fat soluble) and soluble in nonpolar solvents.[13] Carotenoid pigments are very efficient in absorbing light at wavelengths (450–550 nm). When the carotenoid molecule is exposed by light, it will be excited to a certain energy level. Opportunities excitation energy levels that occur can be divided into two kinds, namely the singlet and triplet state. The function of carotenoids as photo protector occurs on triplet-triplet (TT) energy transfer and singlet-singlet energy transfer mechanism.[11]

When the light absorption, carotenoid excited to singlet state and immediately transfers the excitation energy to bacteriochlorophyll by using singlet-singlet energy transfer.[11] The process of singlet- singlet (SS) energy transfer is more common than the triplet-triplet (TT) energy transfer.[14] This is due to the energy transfer from carotenoids to bacteriochlorophyll does not take a long time and does not require too much energy to work in the process of photosynthesis so that the cycle of photosynthesis can take place. While the triplet-triplet energy transfer occurs at bacteriochlorophyll have excess energy so that the energy transfer to the carotenoid is happened.

Carotenoids can be excited to a triplet state, i.e. when it receives a transfer of energy from triplet-bacteriochlorophyll via triplet-triplet energy transfer mechanism[7]. The energy transfer happens in order to the carotenoid makes photo protection to bacteriochlorophyll. Photo protector carotenoids function is as a photo protector through suppression mechanism (quenching), either directly or indirectly.

In the directly extinction process, carotenoids receive energy transfer from triplet bacteriochlorophyll directly and disposed the excess energy in the form of heat (energy dissipation). The process of extinction (quenching) is indirectly done by carotenoids in a manner involving singlet oxygen. Singlet oxygen formed naturally from receiving oxygen triplet energy transfer from triplet bacteriochlorophyll. Singlet oxygen is radical. In the process of extinguishing, carotenoid accepts energy transfer from singlet oxygen, so the carotenoid gets the transition to the triplet state. Finally, the triplet carotenoids release the excess energy as heat.

Photophysical process also can be occurred in bacteriochlorophyl. But if bacteriochlorophyl absorb more photon energy, excitation state bacteriochlorophyl can be exchange to triplet state. An excited electron spin singlet S_n can be reversed, leaving the molecule in the excited state triplet T_n, called intersystem crossing[9]. Intersystem crossing probability increases if the lowest singlet of vibrational level experience overlap with one of the higher vibrational levels of the triplet state. The triplet state bacteriochlorophyl will interact with oxygen molecule so that produce reactive oxygen species (ROS). ROS is a toxic molecule so that make cell damage. In order to avoid such that carotenoids neutralize it with excitation singlet oxygen transfer energy to carotenoid so that carotenoid is at high vibrational levels of the triplet excited state and back to the ground state through transfer heat in environment, the mechanism is called triplet-triplet energy transfer. This energy is used to prevent photooxidation and photoprotection.[1]

Exposure to magnetic fields significantly influence the growth of bacterial colonies. Giving magnetic field causes stress on bacterial cells and activates genes ALA dehydratase (ALAD).[4] ALAD is a key enzyme of porphyrin biosynthesis.[10] Thus giving the magnetic field increases the synthesis of porphyrin photosensitizer in pigment-producing bacteria. Exposure to light will be absorbed by the photosensitizer molecules in bacterial photosynthesis, light energy is absorbed photon will excite the photosensitizer molecules. Further excitation energy is converted into electrochemical potential energy in the form of transmembrane charge separation and synthesis of adenosine triphosphate (ATP).[3]

According to the result, wavelength light having increase of the number colonies is 409 nm purple LED which it is accordance with maximum Soret absorbance. Photophysical process initiate the photochemical process.[8] Almost photophysical mechanism occurs in carotenoid. Photon light absorption by carotenoids will excite the molecule from the singlet ground electronic vibrational levels S_0 to one of the vibrational levels of the electronic excitation. Excitation of the molecule to the higher energy state is likely to return to the ground state, either through

chemical reactions or changes to the heat released into the environment in the process of internal conversion or vibrational relaxation. In the singlet excited state, the carotenoids transfer energy to Bacteriochlorophyls via singlet-singlet energy transfer.[6]

CONCLUSIONS

As briefly described in this article, the research results indicate that in 409 nm purple LED irradiation has potentially increased the number of *Rhodococcus* growth colonies at 77% and increase to 98% by the addition of 1.8 mT magnetic field exposure. Results of exposure optimization in 409 nm purple LED and 1.8 mT magnetic field shows the percentage of *Rhodococcus* growth by 184% in the power of 46 mW and 40 minute duration time exposure (dose 110 J/cm^2).

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