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Using visible light to stimulate or inhibit of *Pseudomonas stutzeri* and *Leuconostoc mesenteroides* ssp. cremoris growth

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ABSTRACT

Visible light represents a promising innovation for developing an activated sludge (AS) system. About 95% of AS microorganisms are bacteria, and their responses vary based on bacteria type, wavelength, intensity, and exposure time. An experimental apparatus is designed and constructed to identify the most effective wavelengths of visible light on *Pseudomonas stutzeri* and *Leuconostoc mesenteroides ssp. cremoris*. Three wavelengths (white, red, and blue light) were provided at fixed intensity. Optical density was used as an indicator to evaluate bacteria performance. The experimental results show stimulation for *P. stutzeri* reached (67.45, 62.47, and 38.1%), respectively under white, red, and blue relative to control at (1440 minutes). On the other hand, *L. cremoris* stimulated under a red light only reached (22.11%), while its inhibition under white and blue light reached (34.32, and 27.19%) relative to control. Maximum growth rate (μ) for both species of bacteria achieved under red light reached (0.004122, and 0.004214 min^{-1}), respectively compared to control (0.001579, and 0.002282 min^{-1}) at (960, and 720 minutes), while, maximum inhibition achieved under blue light was (0.002231, and 0.001362 min^{-1}) relative to control (0.001579, and 0.002282 min^{-1}) at (≤ 480 minutes), respectively.

Keywords: activated sludge, growth rate, inhibition, leuconostoc, light, pseudomonas, stimulation, wavelength.

INTRODUCTION

Photobioreactors are a novel strategy, environmentally and energy-friendly with broad prospects and stainability for the production of renewable fuels, wastewater treatment, and CO_2 fixation (Wang et al., 2025a; Wang et al., 2025b; Yas and Ibrahem, 2025). These systems' microorganisms depend on the light intensity, system design, hydrodynamics, CO_2, temperature, pH, and nutrients (Razzak et al., 2024). The productivity of activated sludge systems is famous for high-quality water (El Moussaoui, 2022) requiring limited setting up and operation space and low pests and odor (Noyola et al., 2019). However, 50 – 90% of all energy costs were consumed via the aeration operation (Drewnowski et al., 2019). To reduce the cost and improve the treatment process, the biological processes in the activated sludge system are integrated with visible light (Yas and Ibrahem, 2025). AS consists of a complex community of microorganisms containing many species of viruses, bacteria, algae, fungi, protozoa, and protists (Al-Hussieny et al., 2015). Bacteria play the most important role in decomposing and biological oxidation of organic substrates, nitrification of ammonia, accumulation of phosphorus, and denitrification of nitrate and form the main constituent of activated sludge (Bitton, 2011) about (95%) (Al-Hussieny et al., 2015). They respond differently to light depending on the wavelength, exposure period, light/dark cycle, and light intensity (Razzak et al., 2024). Among these bacteria, are *P. stutzeri*, a Gram-negative bacterium, and *L.cremoris*, a Gram-positive bacterium, which has ecological significance and a wide range of biotechnological applications according to (Hussein et al., 2022; Segundo et al., 2024).

P. stutzeri has gained significant attention for its successful participation in the treatment of industrial pollutants such as uranium contamination remediation (Yu et al., 2022), the possibility of injecting it into wells to reduce the viscosity and density of oil, and the remediation of contaminated soil (Wei et al., 2023; Segundo et al., 2024). *L. cremoris* protects against radiation damage and forms biofilms with antimicrobial effects against ten pathogens such as *Escherichia coli* and *Pseudomonas aeruginosa*. The specific growth rate of *Pseudomonas syringae* was faster when exposed to blue LED light at (470 nm) (Kuo et al., 2012). Also, exposure to blue light at (410 nm) inhibited the formation of a membrane of Pseudomonas aeruginosa at increasing intensity levels at 75.225, and 450 J. cm^{-2} (Martegani et al., 2020). Wavelength affected pigment and biomass production of *P. aeruginosa NR1*. Biomass of *P. aeruginosa NR1* and pigment production was increased, especially with pigment the productivity was doubled under appropriate light wavelength. Maximum extracellular pigment production was achieved under red, blue light, and darkness. Yellow and green lights are appropriated for maximum intracellular pigment production (Rehman et al., 2020). Gram-positive and Gram-negative species were successfully inactivated by LED light's wavelength (405 nm) (Maclean et al., 2009). Govarthanan et al., (2019) studied the growth of *Erythrobacter spp*. at different wavelengths of light. The growth of *Erythrobacter spp*. was optimized under blue light at (470 nm), and the order of bacterial growth was blue light > white light > green light > red light > yellow light > control (without treatment). According to Yang and Zhao (2023), visible light enhanced the microbial metabolic/enzymatic degradation of aromatic hydrocarbon pollutants by microorganisms reaching 85% at 8 days. In addition, bacteria showed good hydrolytic ability of several insoluble active pharmaceutical substances, such as fluoxetine and diclofenac, reaching (88, and 20%), respectively. Al-Shammary et al. (2020) found that exposed *S. aureus*, *Pseudomonas aeruginosa*, and *Mycobacterium fortuitum* to visible light (405 nm) caused the most obvious killing effects at 10 min (power 230 mW) with loss of viability of ≥ 96%, while, decreased at 532, and 650 nm by ≥ 51%, and 37%, respectively. Lipovsky et al. (2022) found that high-intensity visible light (400–800 nm) kills bacteria, while low-energy white light promotes bacterial proliferation. The toxic effect of light was due to the induction of reactive oxygen species (ROS) production. The production of ROS after blue light (400–500 nm) illumination was higher than red light (500–800 nm). Blue light (415 nm) induced more ROS production than 455 nm, which caused a reduction in the colony counts of *S. aureus* and *E. coli* after illumination with equal intensities of these two wavelengths. With low intensities of wavelengths 415 and 455 nm, the proliferation of *S. aureus* increased but the viability of E. coli decreased. Galo et al., (2021) found that Gram-positive bacteria, *Staphylococcus aureus*, and Gram-negative, *Pseudomonas aeruginosa* exposed to blue light (470 nm) due to inhibition of the growth about 75% after 24 hours at 284.90 J/cm^2, 13.19 mW/cm^2 and 6 hours. However, these bacteria could resume growth within 48 hours after removing the light source. Besides that, red light (660 nm) did not have any inhibition with both bacteria at 603.44 J/cm^2, 27.93 mW/cm^2.

This paper presents a novel technological method to investigate the possibility of stimulation or inhabitation of *P.* stutzeri and *L. cremoris* isolates from activated sludge under modern conditions in a tube test exposed to visible light beams (white, red, and blue) at an exposed time of (240, 480, 720, 1200, and 1440 minutes) (Bertrand, 2019; Wang et al., 2023). According to the study by (Mira and Hall, 2022), optical density was used to evaluate the growth after determining the maximum wavelength absorption by bacteria. Three experimental groups were used under similar conditions in a self-controlled and manual system tightly isolated from the external environment. The effect of the wavelengths and exposed time on specific growth rates was studied. The model was used to describe bacteria growth. Meanwhile, the results of the experimental data and the specific growth rate of bacteria were discussed.

MATERIALS

All the glassware and Petri dishes used in the experiment were made by Zhejiang Bioland Biotechnology Co., Ltd., and Scott Duran manufacturer (DURAN, Germany). Tube test consists of colorless properties and low absorption spectra between 310 to 2200 nm. The light was provided via a light-emitting diode (LED) (Indian origin) of 50 W, 50 Hz, and 220–230 V. The chemical materials used in the study are displayed in Table 1.

Table 1. Chemicals used in this study

Chemical	Concentration (g/l)	Supplier
Blood Agar	50	Bhiwadi-301019, Rajasthan, India
Nutrient Agar	28	Bhiwadi-301019, Rajasthan, India
Nutrient Broth	15	Bhiwadi-301019, Rajasthan, India
Brain heart infusion (BHI) broth	34	Sisco Laboratories Pvt. Ltd., India.
Eosin-methylene blue agar	30	Model NO.CM0136 Nanjing, China
MacConkey Agar	40	Bhiwadi-301019, Rajasthan, India
Catalase (bovine liver)	-----	E0026-2000-5000u, Njduly, China
Mannitol Salt Agar	30	Verna, Goa-403722, India
Oxidase	-----	Verna, Goa-403722, India
Gram stain	-----	Model: E0218, Nanjing, China
Normal saline solution	0.85	HG-0.9-500-B China
Gram stains	-----	HiMedia Laboratories, India
Glycerin	-----	Infinator Pvt Ltd, India

EXPERIMENTAL PROGRAM

System setup

Figure 2a shows a system designed to acclimatize wastewater samples to obtain activated sludge. A plastic bucket (20 liters) was used, tightly isolated from the external environment by glass wool. A submersible pump (8L/h) was placed at the bottom of the bucket to provide the required ventilation. The temperature was set using a heater 600 W, that was automatically controlled by a thermostat. Figure 1 shows a laboratory-scale system designed for the treatment of activated sludge and bacteria diagnosis via light wavelengths. The batch reactor was designed using a plastic container (150L × 25W × 15H mm), with light transmittance of more than (95%). It was isolated from the surrounding environment using an aluminum cabinet (1900L × 950W × 400H mm) divided into several shelves. The window was covered with glass wool to prevent light from reaching an external source and maintain the temperature. A variable resistor was used to regulate the light intensity. An automatically controlled heating and cooling system was created using an Arduino device. The required wavelengths were provided by light–emitting diode lamps installed (200 mm) away from the samples on both sides of the cabinet, with a 200 mm gap between the reactor and the LED. An electric mixing motor (note, used only when taking samples for measurement) was installed above each reactor and connected

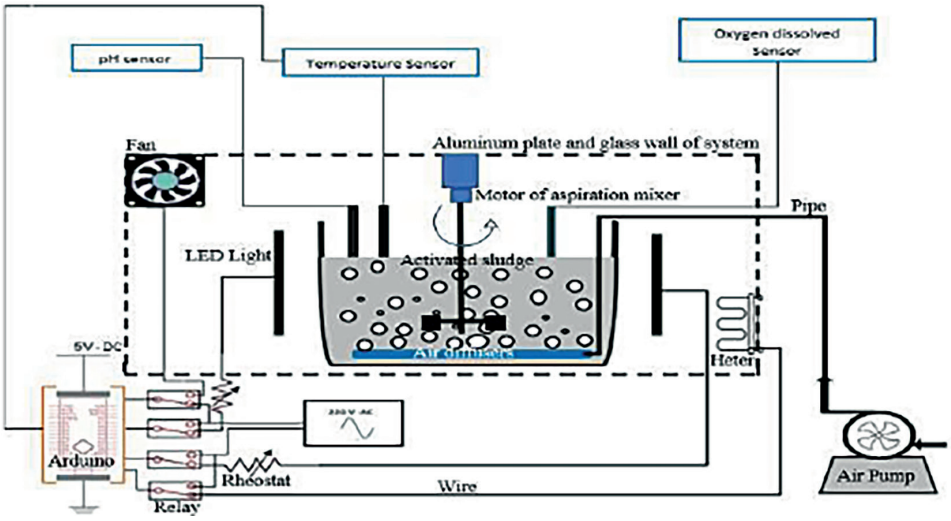

Figure 1. Schematic diagram of the experimental setup of activated sludge

Figure 2. The photograph illustrates: a – adaption of activated sludge for 10 days (unlit), b – activated sludge exposed to wavelengths of light for 5 days

to a finned fan raised above the bottom of the reactor base (50 mm). The reactor was equipped with two air diffusers connected by plastic pipes to two pumps. Electricity energy was provided by a solar power system of 50 Hz and 220 volts. Temperature, pH, and intensity were measured by a thermometer (DP–1K55–796C), a digital pH meter (pH–280: pen type), and a digital lux meter (MESTEK, LM610; 0~100000 Lux, made in China), respectively.

Experimental sets

The study was divided into two sets. Set 1, raw wastewater was collected from two positions to prepared of activated sludge. A system was designed to acclimatize the activated sludge for (10 days) as shown in Figure 2a. Then another system was designed to expose the activated sludge to wavelengths of light (white, red, blue) and control (unlit) for (5 days). This system detail was shown in Figures 1, (see Figures 2b, and 3a). At the end of (5 days) of treatment, samples from activated sludge were taken to isolate and diagnose bacteria. In set 2, diagnosed bacteria were inoculated into the nutrient broth in tubes. Test tubes were incubated in a cabinet (Figure 3a) and exposed to LED light (white, red, blue) and control (unlit) for 1440 minutes (Table 2) (Asgari et al., 2023).

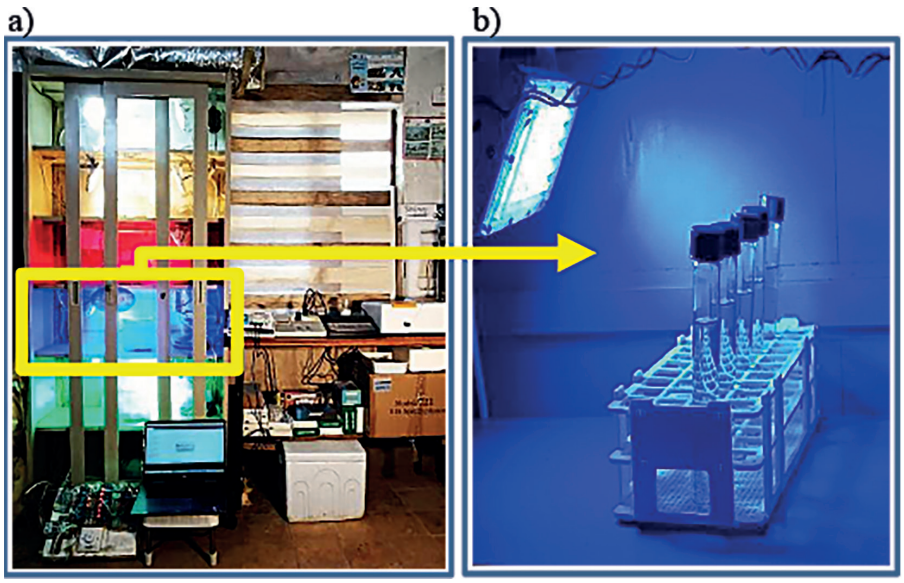

Figure 3. (a) Photograph of the experimental setup, (b) enlargement of the highlighted section of the photograph (a)

Activated sludge preparation

All experimental steps were done according to standard methods (Rice et al., 2012). Raw wastewater (RWW) was collected from sewage systems at a depth of 0.1–0.3 m) taken from Al-Zab, and Al-Hawija cities, affiliated to Kirkuk Governorate. RWW was acclimated for 10 days under continuous feeding and aeration until the mixed liquor-suspended solids (MLSS) reached 2000–3500 mg/l. Samples from MLSS were taken into batches reactors with an operating volume of 3 liters. The pH and temperature were adjusted to 6.6–7.8, and 30±0.5 – 33±0.5 °C, for photobioreactors. These reactors were exposed to wavelengths (white, red, and blue light) and the control (unlit) for a specified time according to Table 1. The AS samples were obtained from a laboratory-scale activated sludge system (aerobic butch reactor) as described in (Hassuon, 2008).

Isolation and diagnosis of bacteria

Bacteria were isolated after exposing activated sludge to wavelengths of visible light for 5 days. During the experiment required to isolate bacteria, (1 ml) of the activated sludge sample was taken from the photobioreactor and diluted 10^{-5},10^{-10}, and 10^{-15} by a normal saline solution (Figures 4a). A single colony of strains was inoculated onto nutrient agar. These plates were kept in an incubator at 37 °C for 24 hours, and then left at 28 °C for 5–7 days (Sam, 2021). The isolated bacteria were subjected to complete cultural, microscopic (Model; Leica DM2000 microscope), morphological, and biochemical examinations and even the species was identified using the Vitek2 (Bergey, 1994; Da Silva, 2018). Treatment, analysis, and testing were conducted in laboratories of the Technical Institute Hawija, General Hospital Hawija, and Al-Raya Central Specialized, Bab Al-Muadham, Baghdad.

Pre-culture preparation

P. stutzeri, and *L. remoris* (Figure 5a, and 5b) were reactivated. Strains were grown in a sterile nutrient-rich (BHI) broth. Before each experiment, bacteria were inoculated into normal saline solution (ten-fold dilutions up to a factor of 10^{-6}), by spread 100 µL)by Drygalski spatula onto nutrient agar to obtain a colony count of approximately 3×10^5 CFU/ml. Plates were incubated at 37 °C for 24 hours (Figure 4b) (Lalucat et al., 2006; Alegría et al., 2013; Da Silva et al., 2018). pH was maintained at 7.00±0.02 (Koupaie, 2017). Hand count and ImageJ software determined the number of colonies grown on plates. The number of

Table 2. Operating parameters in the experimental

LED light	Intensity, (W m^{-2})	Wavelength, (nm)	Exposure time, (min)
White, Red, Blue, and Control (unlit)	96±4.02, 40±4.89, 120±2.07, and 0.00±0.05	(620-650), (620-700), (430-480), and (~0.0)	240, 480, 720, 960, 1200, and 1440

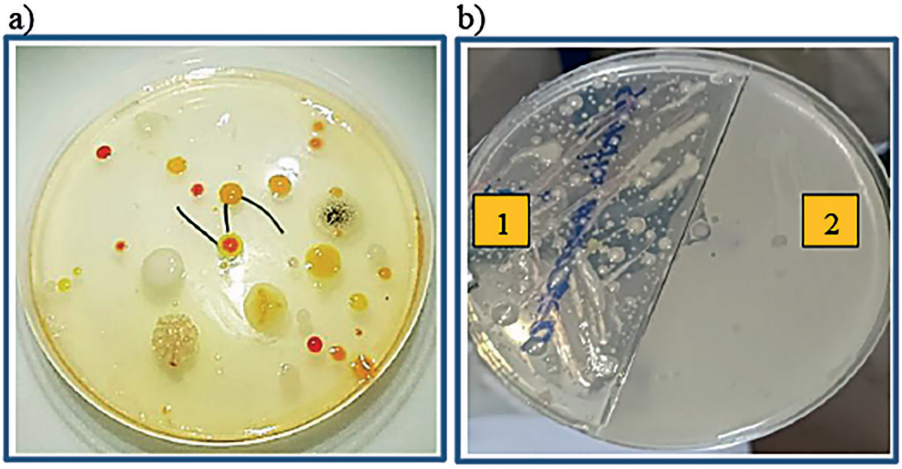

Figure 4. (a) – colony-forming of AS (exposed to wavelength light at 5 days) sample inoculated on nutrient agar at 21 days, 21–25 °C, (×10^{15}/ml); (b) – a pure colony of *L.cremoris* inoculated on nutrient agar and incubated for 48 hours at 28 °C, at diluted: (1) ×10^4/ml, and (2) ×10^2/ml

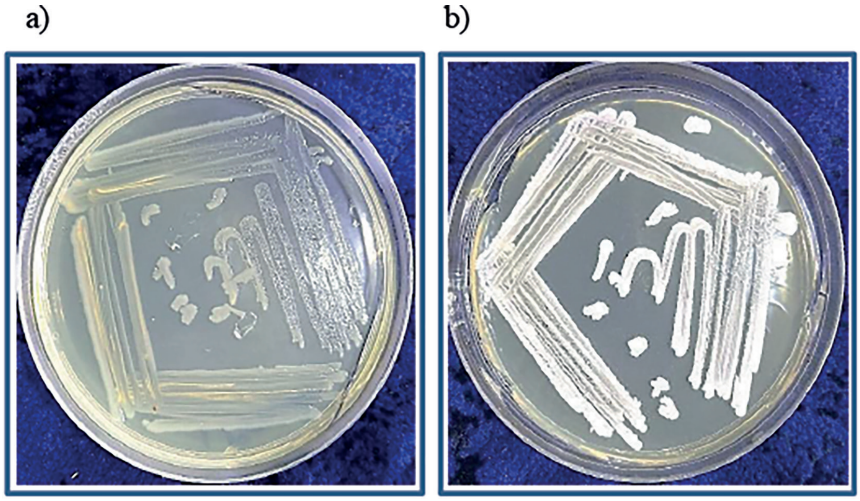

Figure 5. (a) *P. stutzeri*; (b) *L. cremoris* on nutrient agar after 48 hours at 28 °C

colony-forming units (CFU/ml) was calculated using Equation 1 (Da Silva et al., 2018).

$$CFU/ml = \frac{c}{d \cdot v} \quad (1)$$

where: c – the number of colonies on the count plate; d – the dilution rate of the counted plate; v – the inoculated volume of this dilution (ml).

Rinsing procedure

The isolated *P. stutzeri*; and *L. cremoris* were cultured in tubes containing nutrient broth and incubated at 37 °C for 24 h in aerobic conditions. The nutrient broth was incubated at 37 °C for 24 h in aerobic conditions and was inoculated via *P. stutzeri* and *L. cremoris* in test tubes (25 ml) using a pipette (100 µml) of (~3 $\times 10^5$ CFU ml^{-1}). It was exposed to white, red, and blue LED lights with control (without treatment) for 1440 minutes as specific parameters and values in Table 2 (Figure 2a, and b). The procedures were performed under sterile conditions and bacterial cultures were incubated at a temperature from 30±0.5 to 33±0.5 °C. Spectrophotometric measurement of developing turbidity at regular intervals can be used as an indication of increasing cellular mass of *P. stutzeri,* and *L. cremoris* and was recorded in the wavelengths (500, and 340 nm) in a cuvette with a path length of 1 cm. The concentration of bacteria was evaluated at 240, 480, 720, 960, 1200, and 1440 minutes (as an average of three experiments). The bacteria strains were exposed to LED light white, red, blue, and control (unlit) for 1440 minutes (Pham and Nguyen, 2020; Asgari et al., 2023).

Analytical methods

The collection of raw wastewater samples and accumulation of activated sludge was done according to the Standard Methods for Examination of Water and Wastewater (Rice et al., 2012). The effects of exposure to bacterial strains were evaluated by optical density every (240–1440 minutes) (Da Silva et al. 2012). The growth of bacteria was estimated by measuring OD_{500} and OD_{340} for *P. stutzeri*; and *L. cremoris*, respectively, using a VIS spectrophotometer model 721. The specific growth rate (µ) was described through a first-order kinetic model (Equation 2) (Sorokin and Krauss,1958).

$$u = log_2 \cdot \frac{OD_1}{OD_0} \cdot \frac{1}{t} \quad (2)$$

where: OD_0 and OD_1 – optical densities (nm) at the beginning and end of the time (t).

Physicochemical characteristics

Table 3 presents the physicochemical characteristics of the sampled raw wastewater. It was analyzed according to Standard Methods for Examination of Water and Wastewater, and standard for the Ministry of Environmental, Iraq.

RESULTS AND DISCUSSION

Results of colonies formation

Table 4 shows colonies forming on sterile nutrient agar after exposing activated sludge to wavelengths (white, red, and blue light) with control

Table 3. Mean values (±standard deviation) physicochemical properties of samples

Sample position	pH	EC (mohs/cm)	Temp. (˚C) *	COD (mg/l)	BOD (mg/l)	TDS (mg/l)	TSS (mg/l)	PO_4^{3-} (mg/l)	NO_3^- (mg/l)	NH_3^- (mg/l)
Al-Hawija	6.156	3207.33	23.9	1720	478.5	2190	1061.33	44.467	2	20.8
	±0.1405	±130.693	±0.509	±180.55	±21.5	±277.96	±138.123	±20.724	±1.471	±11.492
Al–Zab	6.552	2133.8	22.9	1122.6	412.5	1832.2	634.4	7.962	1.887	19
	±0.114	±285.793	±0.829	±91.38	±55.39	±452.9	±197.137	±2.636	±1.073	±10.318

Note: * relevant to these tests (Table 3) only, not to treatment conditions.

Table 4. Mean values (±standard deviation) of colony-forming count after exposure activated sludge to wavelengths of light at 5 days

Bacteria ssp.	White light		Red light		Blue light		Control	
	$\times10^{10}$/ml	$\times10^{15}$/ml	$\times10^{10}$/ml	$\times10^{15}$/ml	$\times10^{10}$/ml	$\times10^{15}$/ml	$\times10^{10}$/ml	$\times10^{15}$/ml
P. stutzeria[1]	16±3	0	28±6	3±2	13±2	11±1	0	0
*L. cremoris*2	23±3	0	18±5	0	2±1	0	43±13	1±1

Note: 1, 2 Isolated from samples from sewage systems for Al – Zab, and Al – Hawija cities, respectively.

(unlit) for 5 days. Two serial dilutions (10^{10}, and 10^{15}) were counted, while, the colonies at (10^5) were neglected because it too numerous to count. A high percentage of *P. stutzeri* and *L. cremoris* were observed in the photobioreactor of red light (49.397, and 13.33%), otherwise, high inhibition occurred in the photobioreactor of white, and blue light (30, and 63.334%) relative to the control, respectively.

Result of optical density

OD_{500}, and OD_{340} of Gram-negative *P. stutzeri* and Gram-positive *L. Cremoris*, respectively, at different wavelengths of LED light, exposed time (240, 480, 720, 960, 1200, and 1440 minutes), and fixed intensity, are shown in Figures 6 and 7. One-way analysis of variance (ANOVA) revealed that the variation of wavelengths of light was significant ($p < 0.01$) with exposed time.

Figure 6 shows all wavelengths of stimulation growth of *P. stutzeri* at (240–1440 minutes). Maximum OD_{500} concentration was achieved under white light and reached (0.4913) compared to control (0.2934). Following that red and blue light was (0.4052, and 0.2934) at 1440 minutes. It was noted that the optimum growth path was achieved with the red light. The exposure period (720–960 minutes) represented the exponential

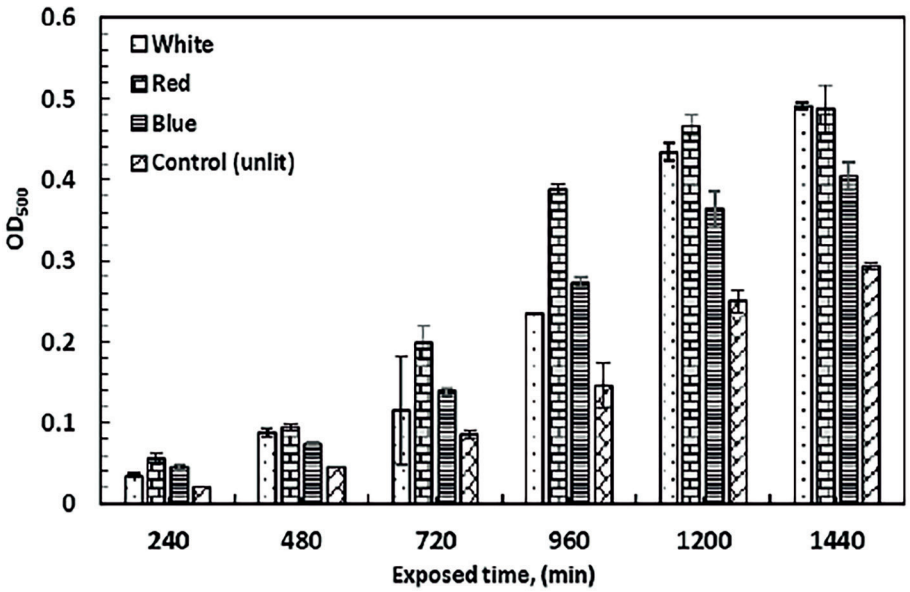

Figure 6. OD_{500} of *P. stutzeri* exposed to LED lights with time

growth phase of the bacteria, while control was between 960–1200 minutes. Finally, the results presented here are in agreement with several authors who demonstrated the effect of blue light on bacterial growth (Govarthanan et al., 2019; Galo et al., 2022) regarding the white and red light, while contradicting the blue light, the reason is attributed to the difference in the targeted microorganism. The blue light (430–480 nm) was used due to stimulated bacteria otherwise wavelengths ≤ 405, the latter causes cell destruction due to its high energy due to its proximity to ultraviolet rays were consistent with Kuo et al. (2012) and Martegani et al., (2020). In general, *P. stutzeri* growth was directly proportional with wavelength (that is, growth increases with decreasing wave energy) according to Equation 3 citation (Zwinkels, 2015):

$$\mathcal{E} = h \cdot v = \frac{h \cdot c}{\lambda} \quad (3)$$

where: $\mathcal{E}$ – the photon's energy; h – Planck's constant (6.626×10^{-34} J·s); v – the frequency of the wave (Hz); c – the speed of light (299,792,458 m/s); $h\times c$=1239.8 (eV. nm); eV = 1.6022×10^{-19} (Joule).

Figure 7 shows that exposed *L. cremoris* to white and blue light led to growth inhibition relative to control, otherwise, red light stimulated growth relative to control; maximum OD_{500} reached (0.2618, 0.2902, 0.48675, 0.3986) respectively, at 1440 minutes. The optimum growth was achieved with the red light and control (unlit). Also, the exponential growth phase was recorded between 720–960 minutes with red light and control, while it was achieved between 960–1200 minutes with white and blue light. The effects of red and blue light were similar to those reported by Rehman and Dixit (2020) and Galo et al. (2021). The inhibitory effect of blue light was consistent with both (Govarthanan, et al., 2019; Martegani et al., 2020). This agreement indicates a high probability of inhibitory or lethal effects of *L. cremoris* within these wavelengths with varying intensity. But it contrasts with Kuo et al. (2012) maybe because of the type of microorganism target. The effect of white was contrary to Govarthanan et al. (2019) because of the low light intensity used relative to the intensity of this study.

Result of specific growth rate

Figures 8 and 9 show the relationship between the specific growth rate for exposed *P. stutzeri* and *L. cremoris* to light wavelengths (white, red, blue light) with time. Red light achieved a higher growth rate for both species of bacteria relative to control at the same time, it was (0.004122, and 0.004214 min^{-1}) compared to control (0.001579, and 0.002282 min^{-1}) at (960, and 720 min), respectively. In contrast, blue light achieved maximum inhibition (0.002231, and 0.001362 min^{-1}) relative to control (0.001579, and 002282 min^{-1}) at (≤ 480 minutes). Finally, the results of the effect of blue light presented here are not in agreement with Kuo et al. (2012) because of the different microorganism targets, the most important reason was the big difference between the intensity used (1.3334 Wm^{-2}) and the intensity for this study (120±2.07 Wm^{-2}). Besides that, the red light stimulated the growth rate, perhaps because of its ability to raise energy in cells due to its closeness

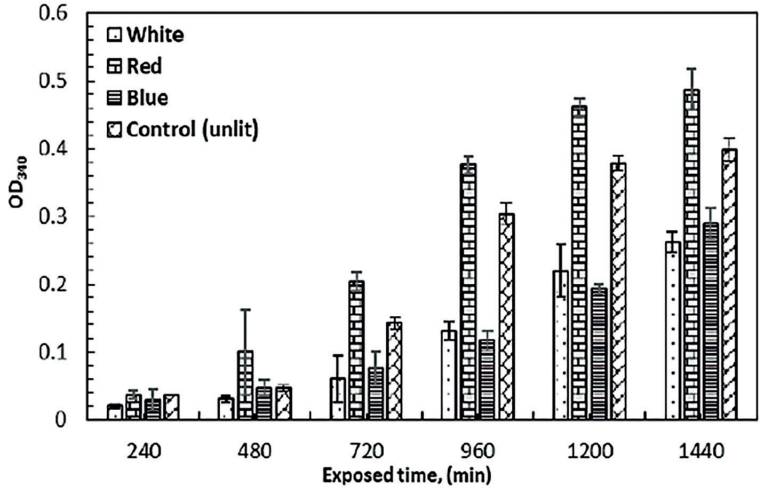

Figure 7. OD_{340} of *L. cremoris* exposed to LED lights with time

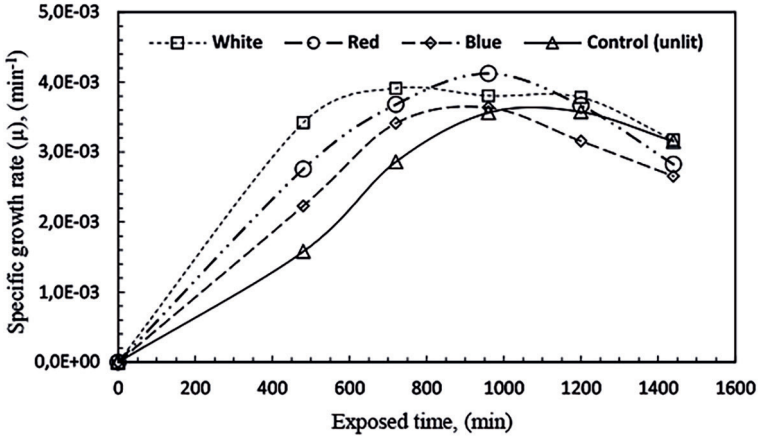

Figure 8. Specific growth rate (μ) of *P. stutzeri* with time

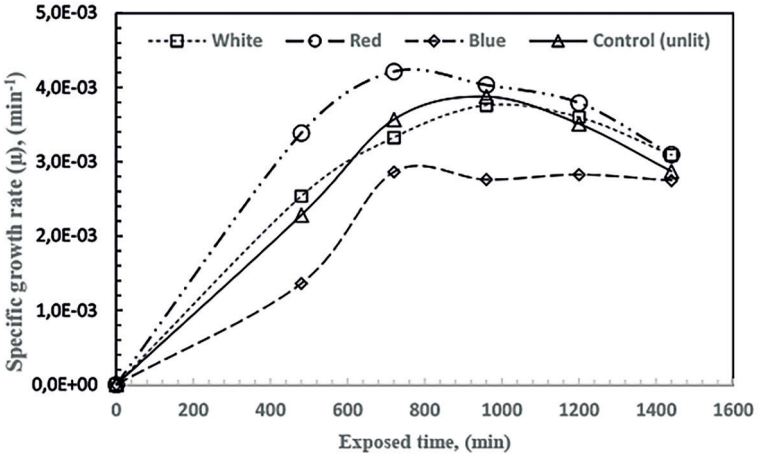

Figure 9. Specific growth rate (μ) of *L. cremoris* with time

to infrared radiation. Cultivation of bacteria for both species required 24 hours to describe the lag, exponential, stationary stage. The curves for all wavelengths had a lag phase of ≤ 240 min. After this time, exponential growth (log phase) was observed until 280 minutes, after slow-down bacteria growth happened. The stationary phase was observed after 960–1200 minutes.

STATISTICAL ANALYSIS

Statistical analysis used correlations and one-way analysis of variance (ANOVA) significant differences between the levels of study using EXCEL version 21 at P-value <0.05.

Acknowledgements

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CONCLUSIONS

This study tested the effectiveness of visible light on bacteria performance. *P. stutzeri and L. cremoris* were treated via (white, red, and blue lights). All wavelengths stimulated *p. stutzeri*, and a higher growth was achieved under white light reached (OD_{500}, 0.4913), and control (OD_{500}, 0.2934) at 1440 min. Also, the red light stimulated *L. cremoris* and reached (OD340, 0.48675), and control (0.3986) at (1440 min). While, white and blue light caused inhibition of *L. cremoris* reached 0.2618 and 0.2902, and control 0.3986. The specific growth rate (μ) was maximum under red light

reach 0.004122 and 0.004214 min^{-1}, and control 0.001579, and 0.002282 min^{-1} at 960, and 720 min, for both species of bacteria, while, blue light due to inhibition reached 0.002231, and 0.001362 min^{-1}, and control 0.001579, and 002282 min^{-1} at ≤ 480 min^{-1}, respectively. From the above, it is clear that each wavelength has a negative or positive effect on the growth, activity, and reproduction of bacteria that can be employed according to the situation to serve environmental applications.

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