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REMOVAL OF LEAD POLLUTANTS IN CULTIVATION WATER MODIFIED USING MARINE SPONGE SYMBIONT BACTERIA TO IMPROVE GROWTH OF TIGER SHRIMP (*Penaeus monodon*)

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Abstract
Brackish water is often contaminated with dangerous and toxic pollutants, such as heavy metals, microplastics, and polyaromatic hydrocarbons. Bioremediation methods using non-pathogenic bacteria can be applied to remove these pollutants. This research aims to eliminate lead pollutants in the cultivation media for tiger shirmp of the type Penaeus monodon (TSPM), while increasing daily growth. Engineering cultivation media with the addition of Bacillus pumilus (BP) and Pseudomonas stutzeri (PS) bioremediators to remove Pb^{2+} pollutants in TSPM media. The maintenance process lasts for 30 days. The effectiveness of the method is determined by measuring the physical and chemical properties, growth analysis, and survival rate of TSPM. The bioremediation performance of the bioremediator bacteria was determined using the Atomic Absorption Spectrophotometer instrument. The research results showed that all physical and chemical parameters of the cultivation media analyzed met quality standards. The presence of BP and PS remediation bacteria did not have a negative effect on TSPM growth. The survival rate of TSPM was relatively higher than in conventional shrimp cultivation. The bioremediation performance of BP bacteria is superior to that of PS bacteria and a mixture of BP+PS bacteria in removing lead pollutants. This bioremediation method is suitable for application to other crustacean cultivation.

Keywords: *Lead removal; Cultivation water engineering; Remediating bacteria; Specific growth; Tiger shrimp*

Introduction

The cultivation of Tiger Shrimp *Penaeus monodon* (TSPM) is growing rapidly and is popular with farmers because it contains essential amino acids, protein, vitamins, minerals, unsaturated fatty acids, and antioxidants [1]. The province of South Sulawesi was one of the centers of tiger shirmp production in Indonesia during the period 1980-2010 [2, 3]. Although there have been technological leaps in almost all aspects of the lives of people over time, the technology of tiger shrimp cultivation appears to have escaped government attention; as a results, research, knowledge, and innovation in this field have not progresses in line with the advancement of human lifestyles [4]. Currently, tiger shrimp farming is experiencing setbacks, both in terms of production volume, which is declining sharply, and the interest of the farming community, many of whom are turning to farming other commodities such as seaweed. This situation is caused by the failure of tiger shirmp farming, which occurs almost every year [5, 6].

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Several observations suggest that the failure of tiger shirmp production is caused by several factors, including very simple cultivation technology, saturation of cultivated land due to uncontrolled exploitation over the past three decades, use of chemical fertilizers, and possibly excessive use of pesticides [7]. Potential exposure to global trend pollutants (heavy metals, microplastics, polyaromatic hydrocarbons, pesticide residues from agricultural activities, medical waste, and household waste) in both cultivation, feed, and other components used in tiger shrimp cultivation is strongly suspected to be one of the causes of declining production and cultivation failure [8]. Preliminary observations from several community-owned culture sites indicate strong potential problems with the use of culture water, which is believed to be contaminated with heavy metal pollutants [9]. Sources of heavy metal pollutants in cultivated lands can come from the natural dynamics of shrimp ponds and also from cultivation water contaminated with heavy metals from industrial activities or human activities in upstream areas [10].

The contaminant lead is one of the most common heavy metals found in shrimp farming areas. Lead is classified as highly toxic and can accumulate in certain objects or tissues in both living and dead media [11]. The concentration of lead in shrimp culture media does not exceed 0.5mg/L (SNI 01-3553-2006) [2, 8], while the maximum concentration of lead in shrimp is < 10mg/Kg (BPOM No. 32/2019) [11]. The typical life behavior of shrimp is to swallow various substances and marine organisms, so shrimp are very vulnerable to lead exposure [12]. Heavy metals are non-biodegradable and exert toxic effects even at very low concentration. The toxic effects of heavy metals on aquatic ecosystems are due to assimilation, accumulation, precipitation, or combination with certain components flowing into abiotic components, eventually forming bioaccumulation and going through metabolic pathways to aquatic biota and animals [13].

Based on the above conditions, the treatment and prevention of exposure to heavy metal contaminants in shrimp is an important and urgent need. Innovation and technology, as well as the use of natural materials, both in the form of environmentally friendly organisms and microorganisms, are considered as a solution that can overcome this problem [4, 13]. Several research results have reported that different types of non-pathogenic bacteria can be used to remediate the toxic properties of lead pollutants by reducing toxic properties or even act as biomaterials for removing heavy metals [14]. Bioremediation methods using bacteria as remediation biomaterials are interesting to study because this method is environmentally friendly, simple, and economical, perhaps even efficient [5, 7].

This research aims to reduce the toxic properties of Pb^{2+} contaminants in TSPM culture media by using marine sponge symbiont bacteria *Bacillus* and *Pseudomonas* as bioremediators [15]. The technology used is a new method of modifying the TSPM culture media. The evaluation parameters to determine the success include determining the level and performance of *Bacillus* and *Pseudomonas* bioremediation against Pb^{2+} contaminants, general and specific growth, TSPM survival rate, and also adsorption isotherm analysis based on the Langmuir and Freundlich theory approach [16].

Materials and methods

Materials

Materials used: bioremediator bacteria; stock bacteria have been characterized based on genotype and phenotype, namely *Bacillus pumilus* strain GLB197 (BP), isolated from the sponge *Niphates* sp., and *Pseudomonas stutzeri* RCH2 (PS) bacteria, isolated from the sponge *Clatheria (Thalysias) reinwardtii* [17]. Tiger shirmp *Peneus monodon* (TSPM) PL 20 (days), Pb^{2+} contaminant in preparation $(CH_3COO)_2Pb{\cdot}5H_2O$, p.a., 125mg/L. Chlorine, thiosulphate citrate bile salt sucrose agar (TCBPA) media, p.a. 70% alcohol, PF 100 MS Prima feed, formalin, p.a., aquabidest, and culture media (water) [18]. Equipment includes Atomic Absorption Spectroscopy (AAS) type AA240FS, a platinum triple scale RTD thermometer, a digital pH meter, Portable water DO (AZ 8361 quality, AZ Instrument Corp., Taiwan), a muffle furnace (Thermolyne F6010, China), stainless steel filters (5 and 0.3mm), a density separator, a test tube,

an Eppendorf tube, a digital balance, a vacuum pump, a sample bottle, a digital camera, a culture box 120×75×50cm^3, and caliper gauges [2, 19].

Procedure

Engineering aquaculture of tiger shrimp (*Penaeus monodo*n) (TSPM) was carried out using three treatment tanks with styrofoam material measuring 120×75×50cm^3, each tank was lined with black plastic, and the cultivation tank was filled with 120L of water that had previously been given a concentration of 20mg/L of chlorine, then aerated for one night [2, 20]. Add 5mL of bacterial suspension, density ± 2mg as a bioremediator to each treatment tank. Tank 1 (treatment I) added BP bacteria (3.10×10^{2}cells/mL), tank 2 (treatment II) added PS bacteria (3.67×10^{2}cells/mL) and tank 3 (treatment III) added both types of bacteria with relatively balanced volume and density [21]. Leave each treatment tank for 1×24 hours, then add ± 10mL of Pb^{2+} pollutant (125mg/L) to the treatment tank, homogenize for ± 5 minutes [22]. Cultivation media contaminated with Pb^{2+} pollutants were expected to reach a concentration range of 10.00 - 12.50mg/L (± 20), times higher than the maximum permitted Pb^{2+} concentration in cultivation media, i.e., 0.5mg/L. Each treatment tank contains 120 TSPM PL 20 individuals [1, 13]. Observations and measurements of analytical parameters were carried out 7 times (0, 5, 10, 15, 20, 25, and 30 days). Analytical parameters, water quality, or physical properties (temperature, salinity, pH, OTR, DO) [11, 23] are measured in situ, and chemical properties in the form of total organic matter (TOM), nitrite, nitrate, and ammonia are measured. Carried out 4 times (0, 10, 20, and 30 days). Measurement of TSPM weight and length, Pb^{2+} concentration in cultivation media, and TSPM [24, 25].

Data and analysis

Data from monitoring, analysis, and interpretation in terms of physicochemical characteristics of the cultivated water are presented in tabular form. The bioremediation rate or performance of BP and PS bacteria can be referred to as the bioremediation/biosorption capacity, which is determined using equations (1) and (2) [26]. The average growth of TSPM based on the physical condition of body weight and length is calculated using equations (3) and (4), while the daily specific growth rate and development of shrimp are processed using equations (5) and (6), regression analysis, and percentages. The Langmuir and Freundlich adsorption isotherms are calculated using equations (7) and (8), while the survival rate (SR) of TSPM is determined based on the mortality during the cultivation period using equations (9) [3, 13, 24]. Bacterial bioremediation capacity against TSPM:

$$BR\ (\%) = \frac{\ln C0 - \ln Ci}{\ln Co} \times 100\% \quad (1)$$

$$Ci = CCW + CPM \quad (2)$$

where BR in % is bioremediation performance of isolates BP and PS against Pb^{2+} contaminants; Co is the rate of change in concentration of Pb^{2+} contaminants in culture water (mg/L); CCW is the final concentration of Pb^{2+} in culture water (mg/L); CPM is the concentration of Pb^{2+} absorbed by TSPM at the end of culture (mg/kg); and Ci is the total concentration of Pb^{2+} in culture water and in TSPM (CCW + CPM) [27].

Determination of the physical growth of shrimp, especially the weight and length of the TSPM body, uses the following equation:

$$AGW\ (\%) = \frac{Wt - Wo}{Wo} \times 100\% \quad (3)$$

$$AGL\ (\%) = \frac{Lt - Lo}{Lo} \times 100\ \% \quad (4)$$

where AGW is the growth of TSPM body weight (%); Wt is the final weight of shrimp after 30 days of cultivation (g); Wo is the initial weight of shrimp before treatment (g); AGL is the growth of TSPM body length (%); Lt is the body length of TSPM at the end of cultivation (cm); Lo is the initial length of TSPM seed stocked at the beginning of treatment (cm) [28]. Determination of average specific growth in weight and body length of TSPM:

$$\text{SGRW (\%/day)} = \frac{\ln Wt - \ln Wo}{t} \times 100\% \quad (5)$$

$$\text{SGRL (\%/day)} = \frac{\ln Lt - \ln Lo}{t} \times 100\% \quad (6)$$

where SGRw is the specific growth rate of TSPM body weight (%/day); SGRL is the specific rate of change of TSPM body length (%/day); and t is the cultivation time (days) [29].

The isotreme bioadsorption performance of remediator bacteria against Pb^{2+} pollutants is determined by applying the Langmuir and Freundlich equations:

$$\frac{1}{Qe} = \frac{1}{(KL \times Qm)} X \left(\frac{1}{Ce} + \frac{1}{Qm}\right) \quad (7)$$

$$\ln Qe = \ln Kf + \left[\left(\frac{1}{n}\right) \times \ln Ce\right] \quad (8)$$

where Qm is maximum adsorption capacity (mg/g); Ce is adsorbate (remediator bacteria) concentration at equilibrium (mg/L); Kf is an undefined variable that is the Freundlich coefficient as a parameter that measures adsorption absorption (mg/g); KL is the Langmuir adsorption equilibrium constant (l/mg); Qe is equilibrium adsorption absorption (mg/g); and n is the cultivation period or adsorption intensity [2, 29].

The survival rate of TSPM cultivated in modified media by administration of Pb^{2+} contaminants and BP and PS bacterial bioremediators was determined using the equation:

$$\text{\% SR} = \frac{\Sigma PMo - \Sigma PM1}{\Sigma PMo} \times 100\ \% \quad (9)$$

where SR is the mean survival of TSPM (%); PMO is the amount of TSPM distributed at the beginning of treatment; and PM1 is the number of TSPM that died. TSPM that survived to the end of the 30-day cultivation period [30].

Results and discussion

The utilization of non-pathogenic bacteria to improve the quality of aquatic environments, particularly the management of wastewater through the application of bioremediation methods, has been widely used. The development of bioremediation techniques using marine sponge symbiont bacteria (*Bacillus pumilus* and *Pseudomonas stutzeri*) in TSPM cultivation to enhance growth may be novel [5, 26]. The selection of BP and PS bacteria types in this study was based on the history of these two types of bacteria as biomaterials against several types of heavy metal contaminants [31]. The results of the microscopic test for BP bacteria include gram-positive bacteria and PS, bacteria of gram-negative.

Physical and chemical dynamics of cultivation media

The success of the bioremediation method for Pb^{2+} pollutants by BP and PS bacteria can be evaluated by looking at the physico-chemical dynamics of the cultivation media during the cultivation period, as shown in Tables 1 and 2.

Table 1. The characteristics of the physical water quality medium for cultivating tiger shrimp (*Penaeus monodon*)

Treatment	Measurable parameters	Measurement and maintenance period (days)							Average	Reference
		0	5	10	15	20	25	30		
(I) Pb^{2+}; BP	Temp. (^{0}C)	28.7	28.9	28.4	28.1	28.9	28.7	29.4	28.37	[7]
	pH	7.96	8.32	7.72	7.83	7.31	7.69	8.25	7.85	[32]
	Salinity (‰)	37.29	36.32	36.34	39.26	38.27	38.47	38.31	37.75	[16]
	DO (mg/L)	3.57	3.93	4.32	3.79	4.43	4.70	4.59	4.19	[31]
	ORP (mV)	64.12	67.20	72.45	72.32	68.45	60.15	68.67	67.62	[33]
(II) Pb^{2+}; PS	Temp. (^{0}C)	28.9	28.7	28.12	28.03	29.5	29.7	28.8	28.82	[34]
	pH	7.58	8.35	8.02	7.87	7.65	7.18	7.99	7.81	[35]
	Salinity (‰)	37.18	36.24	36.21	39.16	39.24	38.91	38.95	37.98	[36]
	DO (mg/L)	4.63	3.95	5.01	3.72	3.75	4.36	4.57	4.28	[37]
	ORP (mV)	58.25	76.38	66.45	74.76	66.52	64.15	65.65	67.45	[27]
(III) Pb^{2+}; (BP+PS)	Temp. (^{0}C)	28.7	28.5	27.5	28.5	28.7	27.6	28.1	28.23	[38]
	pH	8.45	7.78	7.67	7.78	7.85	7.65	7.65	7.83	[32]
	Salinity (‰)	36.36	37.42	36.76	38.67	38.46	38.25	38.25	37.74	[39]
	DO (mg/L)	3.26	3.45	4.52	3.84	4.25	4.25	4.42	4.00	[37]
	ORP (mV)	47.25	70.54	63.65	68.35	56.65	53.45	51.45	58.76	[33]

In general, the physical characteristics of the culture media, especially the 5 (five) parameters analyzed, meet the quality standards for shrimp culture. Data from Table 1 shows that the average temperature parameter for the three treatments is 85.42°C, which is within the standard temperature range for shrimp culture, 26-33°C. The standard pH parameter is in the range of 7.80-8.30 [35], while the results of the research conducted showed that the average pH of the three treatments was 7.83. Other parameters such as salinity, dissolved oxygen (DO), and oxidation-reduction potential (ORP) also meet quality standards for shrimp farming [40]. In general, however, there is no effect on TSPM growth when the dynamics of the physical parameters are considered in each observation and measurement (Table 1) [41]. From the data in Table 1, it can be interpreted that the Pb^{2+} bioremediation method in TSPM cultivation with modification by adding BP and PS bacteria, both treatments (I), (II), and (III), there are no physical changes in the cultivation media [42]. This condition shows that the presence of BP and PS bacteria has no effect on the growth of TSPM; bacteria actually improve the quality of the cultivation water, thus providing a positive effect on the growth and vitality of TSPM [43].

Table 2. Chemical characteristics of water quality medium for cultivating tiger shrimp (*Penaeus monodon*)

Treatment	Chemical measurement parameters	maintenance period (days)				Average (mg/L)	Reference
		0	10	20	30		
(I) Pb^{2+}; BP	TOM (mg/L)	58.15	52.37	40.15	22.49	43.29	[25]
	N-NO_2 (mg/L)	0.0028	0.0004	1.1578	0.6548	0.45	[44]
	N-NO_3 (mg/L)	0.2762	0.1880	2.1702	0.1610	0.70	[45]
	N-NH_3 (mg/L)	0.2123	0.2244	0.2279	1.3193	0.50	[46]
(II) Pb^{2+}; PS	TOM (mg/L)	59.07	50.49	43.71	23.09	44.09	[6]
	N-NO_2 (mg/L)	0.0028	0.0012	1.1142	0.5828	0.43	[26]
	N-NO_3 (mg/L)	0.1332	0.5315	3.2475	0.2131	1.03	[10]
	N-NH_3 (mg/L)	0.2340	0.2530	0.3217	1.9982	0.70	[46]
(III) Pb^{2+}; (BP+PS)	TOM (mg/L)	59.36	55.81	43.84	24.05	45.77	[30]
	N-NO_2 (mg/L)	0.0022	0.0011	1.2408	0.4648	0.43	[47]
	N-NO_3 (mg/L)	0.1906	0.1754	3.0244	0.1537	0.89	[48]
	N-NH_3 (mg/L)	0.2494	0.2803	0.2190	1.4409	0.55	[2]

The total organic material (TOM) standard for shrimp farming is < 90mg/L, while in this study, the average of the three treatments was 44.38mg/L (Table 2). The decomposition of organic matter produces inorganic compounds such as ammonia, nitrate, and nitrite [48]. The concentration of organic matter in the culture media increases with increasing shrimp biomass, accompanied by an increase in feeding frequency. The results showed an average concentration of N-NO_2 (0.44mg/L) from the three treatments, while the standard nitrite concentration was (< 0.5mg/L) [49]. The average ammonia concentration of the three treatments is N-NO_3 (0.87mg/L), while the nitrate quality standard is (0.4-0.8mg/L) [46]. The nitrate concentration of treatments (II) and (III) slightly exceeded the standard but was not considered to have a significant effect on the physical quality of the culture media and, therefore, was not considered to have a negative effect on TSPM growth [50]. Calculation results of the average N-NH_3 showed the concentration of the three treatments (0.58mg/L) and the standard value (< 0.80mg/L) (Table 2). These results show that all chemical parameters of the TSPM shrimp culture media using the bioremediation method, a cultivation period of 30 days, meet usual shrimp growth standards [51]. These results show that the presence of BP and PS remediation bacteria in the culture media does not cause relatively significant changes in the chemical content of the culture media, and it is hoped that TSPM growth can continue normally [13].

The bioremediation performance of bacteria against pollutants

The performance of BP and PS bacteria is defined as their ability to decrease the concentration of Pb^{2+} contaminants in the culture medium to more than 50% of the initial concentration [4]. The decreasing trend of the Pb^{2+} concentration in the cultivation media and the TSPM is shown in figure 1.

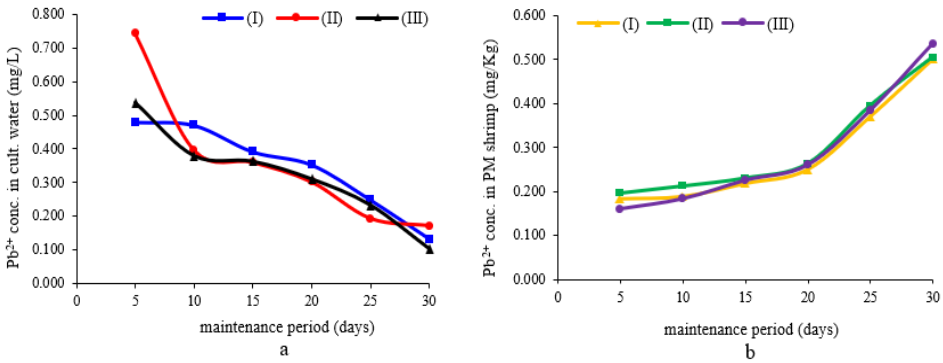

Fig. 1. Trend of Pb^{2+} pollutant concentration based on cultivation time: (a) Pb^{2+} concentration in water cultivation, (b) Pb^{2+} concentration in TSPM

Figure 1 shows that the Pb^{2+} concentration in the cultivation media of the three treatments decreased drastically during the first 0-10 days of cultivation. Although continuous, the decrease in Pb^{2+} concentration appeared to be sloping with increasing cultivation time of up to 30 days (Fig. 1a). The decrease in Pb^{2+} concentration showed a relatively similar pattern from the third treatment [13]. The decreasing trend in Pb^{2+} concentration in cultivation media is inversely proportional to the Pb^{2+} concentration in TSPM, which shows an increasing trend as cultivation time increases [52]. The increase in Pb^{2+} concentration in TSPM for the third treatment also showed the same pattern (Fig. 1b). Theoretically, the longer the contact between Pb^{2+} and TSPM, the higher the concentration of these pollutants in the TSPM body, because the nature of heavy metals (Pb^{2+}) is that they do not decompose and accumulate in an object, both in dead material and especially in living material [2, 53]. The Pb^{2+} pollutants introduced at the beginning of the treatment are distributed in three objects: (i) Most of the Pb^{2+} pollutants are remediated as a form of performance or remediation capacity of BP and PS

remediating bacteria; (ii) a small fraction remains in the culture medium; and (iii) a small fraction is also absorbed in the TSPM body [54]. The comparison of Pb^{2+} concentrations between cultivation media and TSPM for the same treatment is shown in figure 2.

The Pb^{2+} concentration in the cultivation media decreases with increasing cultivation time, which is inversely proportional to the Pb^{2+} concentration in TSPM. The decrease in Pb^{2+} concentration in the cultivation media was caused by at least two factors: first, some of the positive ions of the lead contaminant migrated or were absorbed by TSPM (Fig. 2a-c) [21, 55]. Second, the toxicity of Pb^{2+} pollutants is largely remediated or eliminated by BP and PS biomaterial bacteria. Compared to bioremediation performance, the two types of isolates against Pb^{2+} contaminants were relatively not significantly different [45]. This can be seen from the achievement of the equivalence point (equilibrium concentration of Pb^{2+}) in cultivation media vs. TSPM, determined by adding the equation of the linear trend of decreasing Pb^{2+} in air cultivation and the increasing trend of Pb^{2+} in TSPM (Fig. 2a-c). The results of this calculation obtained the equilibrium coordinate points for treatment I, namely (19.78 days; 0.313mg/L), treatment II (18.83 days; 0.318mg/L), and treatment III (18.46 days; 0.306mg/L) [56].

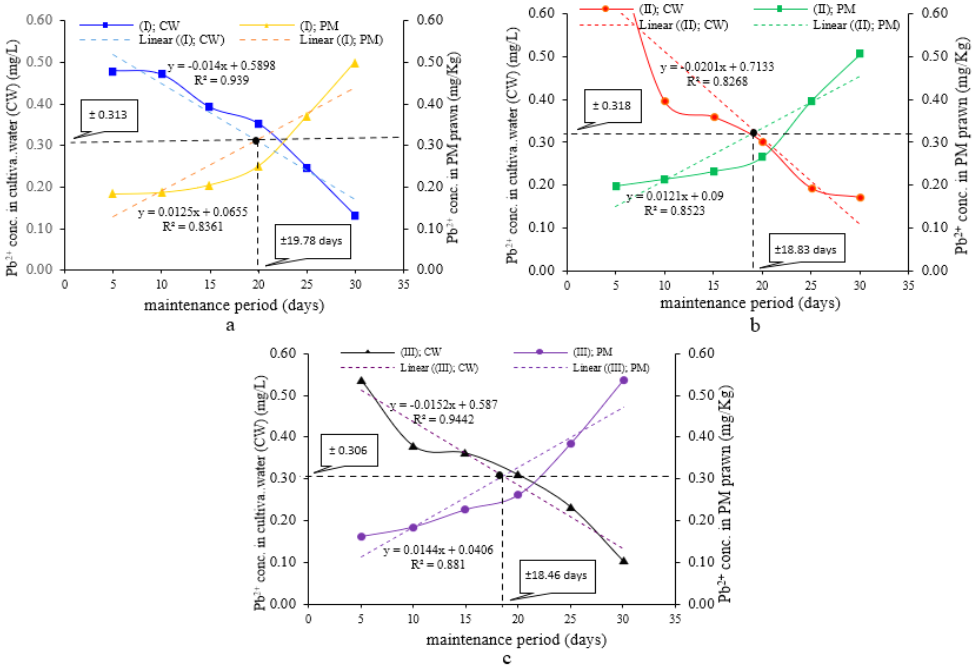

Fig 2. Comparison of Pb^{2+} pollutants in air cultivation vs. TSPM based on cultivation time: (a) treatment (I), (b) treatment (II), and (c) treatment (III)

These results illustrate that the maximum bioremediation performance of BP and PS bacteria occurs in the first ten days of cultivation (Fig. 1a). After the equivalence point, the decrease in Pb^{2+} concentration in the cultivation air still varies both when considering the third treatment and in other aspects. Increase in cultivation time for each treatment [57]. The decrease of Pb^{2+} in the air cultivation also shows that there is a mutual migration of ions from the air cultivation media into the TSPM body, so in shrimp cultivation treatments I-III, this shows an increasing trend with increasing cultivation time [58]. Based on the R^2 value (Fig. 2a-c), it appears that treatment (III) using a mixture of BP and PS bacteria is the most stable, followed by treatment (I) and finally treatment (II). The consistent increase in Pb^{2+} exposure in TSPM

occurred in treatment (III), followed by treatment (II) and lastly, treatment (I) [59]. Bioremediation of Pb^{2+} pollutants by PS and BP bacteria is presented in Table 3.

Table 3. The bioremediation performance of BP and PS bacteria against Pb^{2+} contaminants in TSPM cultivation media

Treatment	*Co* (mg/L)	C_{CW} (mg/L)	C_{PM} (mg/Kg)	*Ci* (mg/L)	*BR* (%)
(I); Pb^{2+}; BP	10.7211	0.1221	0.3711	0.4341	95.95
(II); Pb^{2+}; PS	10.4780	0.2450	0.4767	0.7217	93.11
(III); Pb^{2+}; BP + PS	11.4077	0.2529	0.9152	1.1681	89.76

Bioremediation capacity of BP and PS bacterial isolates against Pb^{2+} contaminants. Based on Table 3, it appears that the bioremediation capacity of BP bacteria after treatment (I) is more dominant than PS bacteria after treatment (II) [47, 59]. The bioremediation performance of the bacterial mixture (BP + PS), treatment (III), appears to be lower than treatments (I) and (II) [60]. This may be due to the competition between the two types of bacterial cells during remediation, which may decrease the ability of bacterial cells to divide [61]. Results from the analysis aspect of bacterial remediation capacity show that the bacterial bioremediation performance of BP and the combination of BP and PS is better than PS.

Langmuir and Freundlich Biosorption Isotherms

The Langmuir isotherm is assumed to be the first level of adsorption by the negative pole of the BP/PS bacterial cell so that at a certain point, saturation or ionic binding of Pb^{2+} contaminants to the positive pole occurs. The ability of the bacterial cells to defend themselves allows the second, third, and subsequent stages of ionic binding to occur, depending on how many times the bacterial cells divide. This process can be described as a stratified equilibrium [44]. The Langmuir and Freundlich adsorption isotherms of bioremediator bacteria in culture media are shown in Tables 4 and 5.

Table 4. Langmuir and Freundlich adsorption of Pb^{2+} on TSPM culture media

Treatment	C_0 (mg/L)	*Ce* (mg/L)	C_0-*Ce* (mg/L)	massa (g)	volume (*L*)	*Qe* (mg/g)	Langmuir		Freundlich		
							Ce/Qe	K_L (L/mg)	Log *Ce*	Log *Qe*	*Kf* (L/g)
(I)	10.7411	0.1320	10.6091	0.0020	0.0050	26.5228	0.0050	-0.4903	-0.8794	1.4236	0.0007
(II)	10.7380	0.0635	10.6745	0.0020	0.0050	26.6863	0.0024	-0.4904	-1.1972	1.4263	0.0002
(III)	11.4077	0.2529	11.1548	0.0020	0.0050	27.8870	0.0091	-0.4908	-0.5971	1.4454	0.0023

Based on Table 4, the Langmuir adsorption in the first stage of the culture media was relatively the same in the three treatments, which can be seen in the relatively similar KL values. If we look at the Ce/Qe value on the Langmuir isotherm, we can see that the BP bacterial cells in treatment (I) work harder to remove Pb^{2+} ions than the PS bacterial cells in treatment (II) [2, 47]. The number of bacterial cells in treatment (III) worked much more but was less successful in remediating Pb^{2+} pollutants. This is due to competition between the cells of the two types of bacteria (BP and PS) [62]. The same can be seen in the Freundlich isotherm of the culture media, where saturation is maximal in the first stage, while in the second stage and so on, it is present but very weak. This condition indicates that the bacterial cells are not dividing optimally to form the second- and third-generation divisions [63]. This is because, in the first stage of saturation (formation of Langmuir isotherm biosorption), it is believed that many bacterial cells are unable to divide due to poisoning in the ionic reaction event to reach the saturation point.

Bacterial cells can divide into second, third, and subsequent generations if the right conditions are met, such as nutrient availability, pH, and temperature. The defending cells then mature and can form ionic bonds. Theoretically, bacterial cells can defend themselves up to the fourth generation or more, but in this experiment, there was no clear evidence of bacterial regeneration or weak cell division (Table 5). This can be seen in the log Qe and Kf values [64].

The presence of the toxic pollutant Pb^{2+} in the TSPM culture media limits the ability of bacterial cells to divide in the second generation and beyond. The second and subsequent stage of ionic binding is the formation of a bilayer. The situation where all the negative poles provided by the bacterial cell are saturated with Pb^{2+} ions is considered to be the formation of the Freundlich isotherm. This is based on the empirical correlation of solutes represented by bacterial cells with solutes in the liquid, which describes the formation of an adsorption equilibrium [65]. Langmuir and Freundlich's isotherm adsorption analysis showed that Langmuir's isotherm adsorption occurred by both BP and PS bacteria and the BP + PS mixture.

Table 5. Langmuir and Freundlich adsorption of Pb^{2+} on TSPM

Treatment	*Co* (mg/L)	*Ce* (mg/L)	*Co-Ce* (mg/L)	massa (g)	volume (*L*)	*Qe* (mg/g)	Langmuir		Freundlich		
							Ce/Qe	*KL* (L/mg)	Log *Ce*	Log *Qe*	*Kf* (L/g)
(I)	0.0004	0.3711	0.3707	0.002	0.005	0.9268	0.4004	0.0857	-0.4305	-0.0330	0.1486
(II)	0.0006	0.3657	0.3651	0.002	0.005	0.9128	0.4007	0.1058	-0.4369	-0.0396	0.1465
(III)	0.0012	0.9152	0.9140	0.002	0.005	2.2850	0.4005	-0.3600	-0.0385	0.3589	0.3665

Growth and survival of Tiger shirmp ***(Penaeus monodon)***

The assessment of TSPM growth during cultivation can be considered from several aspects: physical condition, health status, and TSPM vitality. Physical condition includes growth trends based on average measurements over a while, growth based on initial and final conditions, and specific daily growth. The health status aspect can be seen in cultured shrimp, especially whether the shrimp has diseases or health symptoms that affect the quality and grade of the shrimp, while the survival aspect can be seen in the survival rate value of the shrimp [66].

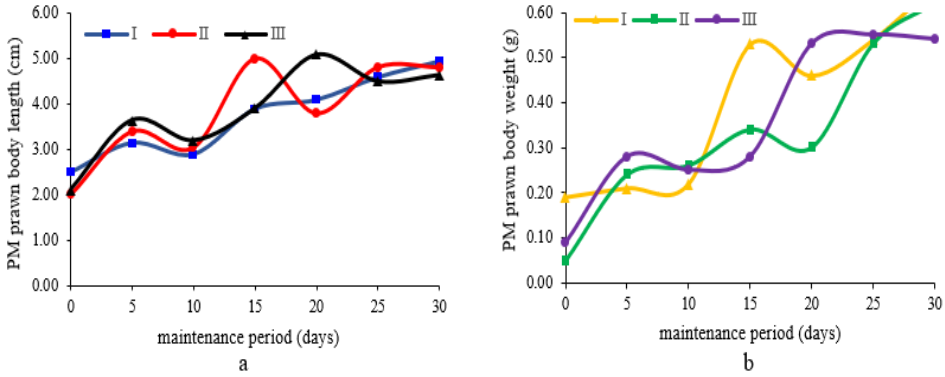

Fig. 3. The trend in TSPM growth rate based on cultivation time, (a) TSPM body weight Increase rate, (bb) TSPM body length increase rate

Although the increase in TSPM weight occurred in all three treatments as cultivation time increased (Fig. 3a), the increase in TSPM weight was variable. The same was true for TSPM length, which increased with increasing cultivation time (Fig. 3b). The increase in TSPM weight and length during cultivation appears inconsistent when compared to all measurements [67, 68]. This is due to the fact that TSPM measurements, both weight and cultivation length, were taken randomly, and TSPM samples in the first measurement were either discarded or not returned to the cultivation pond, as they had experienced severe stress and there was a concern that they would interfere with further growth [29].

Table 6 shows the average TSPM weight and body length increase for the three treatments. Based on the trend of increasing body length of TSPM during 30 days of cultivation,

it appears that treatment III > II > I, but the growth stability, by looking at the R^2 value, is best demonstrated by treatment I > III > II [2, 68].

Table 6. Increase in weight and body length of TSPM

Treatment	Average measurements	Equality linear	Correlation (R^2)
Changes in body length of TSPM			
(I); Pb^{+2}; BP	3.73 cm	y = 0.0818x + 2.5018	0.9460
(II); Pb^{+2}; PS	3.84 cm	y = 0.0855x + 2.5575	0.6919
(III); Pb^{+2}; BP + PS	3.88 cm	y = 0.0803x + 2.6714	0.7364
Changes in body weight of TSPM			
(I); Pb^{+2}; BP	0.40 g	y = 0.0165x + 0.1539	0.8651
(II); Pb^{+2}; PS	0.33 g	y = 0.0166x + 0.0846	0.8961
(III); Pb^{+2}; BP + PS	0.36 g	y = 0.0155x + 0.1275	0.8616

The development of TSPM body weight, on average, was in treatment I > III > II, and based on the stability aspect, it can be seen that the increase in TSPM body weight was more consistently shown by treatment II > I > III (Table 6). This picture shows that average growth and growth stability are influenced by many factors, especially the physical and chemical parameters of the culture media, nutrient availability, oxygen adequacy, and the ratio of the number of seeds to the area of the culture pond [38]. The average growth based on the initial and final state of TSPM after cultivation and the specific daily growth are presented in Table 7.

Table 7. Growth and development of tiger shrimp *Penaeus monodon* (TSPM) on modified culture media

Treatment	W_0 (g)	Wt (g)	Lo (cm)	Lt (cm)	t (days)	AG_W (%)	AG_L (%)	SGR_W (%.day^{-1})	SGR_L (%.day^{-1})
(I); Pb^{+2}; BP	0.19	0.67	2.60	4.95	30	71.64	47.47	4.2033	2.1000
(II); Pb^{+2}; PS	0.10	0.62	2.00	4.80	30	83.87	58.33	6.0667	2.9333
(III); Pb^{+2}; BP + PS	0.08	0.54	2.05	4.65	30	85.19	55.91	6.3667	2.7333

The average growth is based on the initial and final TSPM body condition (weight and length); for TSPM body weight treatment, III > II > I is indicated according to the AGw value. Meanwhile, the average development of TSPM body length is shown by treatment II > III > I, according to AGL values (Table 7) [8, 69]. The daily specific growth rate for TSPM body weight is based on SGRw values, treatment III > II > I, and the daily specific growth in TSPM body length according to SGRL is II > III > I (Table 7).

Table 8. Survival rate and relationship between TSPM mortality rate and treatment

Treatment	ΣPM_O	Percent (%)	ΣPM_I	Percent (%)	ΣPM_t	Percent (%)	$\frac{(\Sigma PM_O - \Sigma PMI)}{\Sigma PM_O}$	SR (%)
(I); Pb^{2+}; BP	120	100	26	21.67	94	78.33	0.7833	78.33
(II); Pb^{2+}; PS	120	100	38	31.67	82	68.33	0.6833	68.33
(III); Pb^{2+}; BP + PS	129	100	31	25.83	89	74.17	0.7416	74.17

The viability of TSPM is influenced by several factors, particularly the physical and chemical quality of the culture media, as shown in Tables 1 and 2. In general, cultured shrimp such as TSPM have high viability when all the conditions necessary for their life stability are proportionally met. Table 8 shows treatment I > III > II had the highest TSPM viability [57]. This shows that good shrimp growth does not automatically result in higher viability; conversely, high viability is not directly proportional to better physical growth of shrimp [70]. Table 8 also

shows that the effect of BP bacteria on SR TSPM is better than that of PS bacteria and the BP + PS mixture.

Conclusions

Several important conclusions can be drawn from this research, including that the presence of *Bacillus pumilus* strain GLB 197 (BP) and *Pseudomonas stutzeri* RCH2 (PS) remediator bacteria does not affect the physical and chemical conditions of TSPM cultivation media. The bioremediation performance of BP bacteria against Pb^{2+} pollutants is more dominant than PS bacteria and the BP + PS mixture. Langmuir isotherm biosorption occurred in all treatments, while Freundlich isotherm could not be well determined. The order of stability of TSPM body length growth was treatment I > III > II, while the order of stability of body weight growth was treatment II > I > III. The daily specific growth rate of TSPM body weight follows treatment III > II > I, and the daily specific growth rate of body length is II > III > I. The highest TSPM viability is shown in the treatment sequence I > III > II. The results of this research, in general, are that BP and PS bacteria and the BP + PS mixture can carry out a high bioremediation function against Pb^{2+} pollutants and do not have a resistant effect on the physical and chemical parameters of the cultivation media. Likewise, TSPM still shows high growth in the AGw/AGL of the analysis aspect, SGRw/SGRL, and survival rate. Thus, this method is suitable for shrimp farming, especially for *Peneus monodon* tiger shrimp, to remove heavy metal contaminants while increasing the average growth and specific daily growth.

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