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Effect of environmental factors on *Bacillus* sp. and *Thioclava* sp. for phosphorus removal from saline wastewater

Rafitah Hasanah[1,2] and Tsuyoshi Imai[1,*]

[1]Graduate School of Sciences and Technology for Innovation, Yamaguchi University, Yamaguchi, Japan
[2]Faculty of Fisheries and Marine Sciences, Mulawarman University, Samarinda, Kalimantan Timur, Indonesia
[*]Corresponding author: imai@yamaguchi-u.ac.jp

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Abstract

In this study, *Bacillus* sp. (TR1) and *Thioclava* sp. (MA3) were assessed for their abiotic adaptability and phosphorus removal efficiency in saline wastewater. The effects of abiotic factors such as carbon source, pH, temperature, and salinity on bacterial growth were examined through a series of batch experiments. Both bacteria used carbon sources such as glucose, sucrose, and CH_3COONa for their growth. The pH study indicated that *Bacillus* sp. (TR1) preferred the pH range of 6–8 and *Thioclava* sp. (MA3) preferred the pH range of 6–9. *Bacillus* sp. favorably multiplied in the temperature range of 25–40 °C, while 25–35 °C was preferred by *Thioclava* sp. Salinity range of 0%–10% was favorable for TR1, with optimum growth observed at 3.5%–5%, and *Thioclava* sp. (MA3) preferred the salinity range of 1%–10% with optimal growth at 4% but was absent in non-saline water. *Bacillus* sp. and bacterial combination (TR1 and MA3) showed similar values for phosphorus removal efficiency (100%) at 1.0 mg-P/L total P compared to *Thioclava* sp. (38.2%). The initial phosphorus concentration of 2.5 mg-P/L in combination showed a slightly higher 72.35% P removal efficiency compared to the individual strains. However, phosphorus removal did not increase, but showed a downward trend with increasing at initial phosphorus. The results demonstrated that when used individually, *Bacillus* sp. showed a reasonably high phosphorus removal ability than *Thioclava* sp., and exhibited good synergy when used in combination to remove phosphorus from saline wastewater.

Keywords: Environmental factors, *Bacillus* sp., *Thioclava* sp., Phosphorus, Saline wastewater

1. Introduction

Phosphorus (P), an essential element for all life forms, is a structural constituent of several cell components and a functional component of all organisms [1]. However, under specific environmental conditions and high concentrations, it can be considered as a pollutant. Wastewater containing 1–10 g/L salt is usually defined as saline wastewater; otherwise, it is regarded as hypersaline or brine and necessitates treatment. Phosphorus-rich saline wastewater is often found in aquaculture, food processing, and saline farmlands. Excessive discharge of P into water bodies triggers eutrophication, which causes excessive growth of algae and other planktons, accelerating the depletion of dissolved oxygen (DO) that is fatal to the fish and other organisms [2]. Therefore, P removal from wastewater is of immediate concern, given its environmental impact; it can be detrimental and poses a threat to aquatic systems, requiring efficient treatment methods that is cost-effective in the long term [3].

The specification set by the US Environmental Protection Agency (1986) recommends that total phosphorus concentrations should be <0.10 mg/L in streams that do not discharge directly into reservoirs and should not exceed 0.05 mg/L in streams that do not discharge directly into reservoirs. The limits of phosphorus in the effluent of wastewater treatment plant (WWTP) discharging to aquatic systems in other countries vary from 0.5 to 2.0 mg/L. Presently, the concentration of inorganic and organic forms of phosphorus in municipal wastewater is usually from 5 to 20 mg/L [4]. Meanwhile, the concentration of phosphate levels are 0.015 mg/L for water supplies, 0.025 mg/L for aquatic life, 0.05 mg/L for lakes, and 0.02 mg/L for mountain lakes [5]. In addition, the

phosphate content in the seawater surface was 0.049 mg/L and deep seawater contained phosphate levels from 0.082 to 0.188 mg/L [6]. Phosphates are not toxic to people or animal unless they are present in very high levels. Phosphate level greater than 1.0 mg/L can cause digestive problems, and can interfere with freezing in water treatment plants [7].

The enhanced biological phosphorus removal system (EBPR) is a sewage treatment method for removing P from wastewater. In this process, microorganisms such as bacteria, yeast, protozoa, microalgae, and fungi are used to accumulate phosphate in wastewater [8]. In general, all bacteria contain a small portion (1%–2%) of phosphorus, as a part of their cellular components, such as phospholipid membranes and DNA. Therefore, in the wastewater treatment plant, the bacterial cells grow by consuming the nutrients in the wastewater, thereby, accumulating phosphorus in their biomass. Phosphorus is used by microorganisms for cell maintenance, nucleic acid synthesis, cell membrane construction (as phospholipids), and chemical energy transfer reactions with cells (such as ATP). Additionally, a quantity of phosphorus is also stored by cells for future use. The EBPR process uses specific bacterial metabolism, which in certain circumstances, accumulates large quantities of intracellular polyphosphate. In the anaerobic phase, carbon source is taken up by the bacteria and stored as polyhydroxyalkanoate (PHA) and is accompanied by the degradation of poly-P, which consequently releases orthophosphate. In the aerobic phase, bacteria grow aerobically and requires orthophosphate to restore poly-P levels using the stored PHA as a source of carbon and energy. Since PHA is a reduced polymer, its synthesis requires reducing power [9].

Abiotic conditions such as water quality, carbon sources, pH, temperature, and salinity can affect the EBPR system. Consequently, the operation and management of these parameters are essential for removing P from wastewater treatment [10]. The bacterial involvement in removing nutrients from wastewater and the removal efficiency has been studied. Bacterial communities are important in the activated sludge systems and are responsible in stabilizing the effluent entering the treatment plant [11]. Microbes play a fundamental role in the remediation of polluted water. Microbial activity is an essential parameter in understanding the ecological role of bacteria for pollutant removal processes in aquatic ecosystems [12]. Since microbial activity is a crucial parameter for the functioning of aquatic ecosystems, tracing the significance of various habitat changes for the entire environment is a serious research challenge. The marine bacteria are able to adapt and live in extreme abiotic conditions (such as high salinity and pressure, low temperature and nutrients) and play an essential role in P transformation in the oceans [13]. However, the literature on the effect of seawater on EBPR is lacking. The effect of saline wastewater has been studied in NaCl-supplemented wastewater processes or NaCl-based enrichment culture of *Ca*. Accumulibacter phosphatis [14]. Various studies have shown that *Bacillus* sp. is used in combination with *Pseudomonas* sp., *Aeromonas* sp., and *Enterobacter* sp. [3,15]. Meanwhile, current research on *Thioclava* sp. is related to sulfur oxidation [16,17]. Therefore, it is essential to understand the specific role of *Bacillus* sp. and *Thioclava* sp. in increasing phosphorus removal in saline wastewater that is adapted to seawater. In this study, the adaptability of *Bacillus* sp. and *Thioclava* sp. to abiotic factors such as carbon sources, pH, temperature, and salinity variations was investigated, and their individual and combined effects for P removal performance were examined at different P concentrations in saline wastewater, which was similar to that of seawater.

2. Materials and methods

2.1 Chemical and media

Chemicals used in this study were of analytic grade. The 216 L medium as described by (Liu et al. 2015 [18]) contained (g/L): CH_3COONa (1), tryptone (10), yeast extract (2), sodium citrate (0.5), NH_4NO_3 (0.2), synthetic sea salt (35) (Gex, Japan), and agar (15). The salinity wastewater contained: (g/L): CH_3COONa (1.0), sodium citrate (0.5), NH_4NO_3 (0.2), and synthetic sea salt (35) (Gex. Japan). A specified amount of KH_2PO_4 was added to the medium to achieve the desired P concentration (1, 2.5, 5, and 10 mg-P/L). The medium pH was adjusted to 7 using 0.1 M NaOH and 0.1 M HCl.

2.2 Source of strains

The marine bacteria were isolated from sediments and seawater in Yamaguchi Bay, Japan. They were enriched and cultivated in a fed-batch process during100 days. The marine bacteria with high P removal ability were isolated and screened by sequencing 1500-bp 16S rRNA. The screening results identified the bacteria as *Bacillus* sp. (TR1) and *Thioclava* sp. (MA3), which are gram-positive and gram-negative respectively, as previously reported [19]. The experiments were performed daily, and the cultures were maintained on plates at pH 7 using 216 L medium. In the present study, the bacteria were re-isolated and re-purified.

2.3 Carbon sources utilization on bacterial growth

The medium to assess the effect of carbon source contained different carbon sources (glucose, sucrose, and CH_3COONa), KH_2PO_4 and NH_4Cl with a C:P:N ratio of 100:5:1, used for acclimation purpose, were added to the medium in separate flasks. The pH of the medium was adjusted to 7 using 0.1 M NaOH and 0.1 M HCl before sterilization. The medium (200 mL) was dispensed in a 300 mL Erlenmeyer flask and sterilized in an autoclave (Hirayama; HA-300 MIV. Japan) at 121 °C and 15 psi for 15 min. After sterilization, a known concentration of the respective bacterial species was inoculated into individual flasks with different carbon sources. Each flask was mixed on a gyratory incubator shaker (145 rpm) (Eyela Multishaker MMS; Tokyo Rikakikai Co. Ltd. Japan) at 35 °C for 24 h. The optical density of the cell suspension was measured at 600 nm (OD_{600}) to determine cell growth using a UV-visible spectrophotometer (UV-VIS U2900; Hitachi Co., Ltd. Japan). All experiments were performed in triplicates.

2.4 Effect of pH, temperature, and salinity on bacterial growth

To determine the effect of optimum abiotic factors on the growth of selected strains, various pH values (4–10), temperature (25–50 °C), and salinity (0 %–10% w/v) were evaluated in this study. The flasks with 216 L medium were placed on a shaker for 24 h at 145 rpm. After 24 h, the cells in the suspension were estimated.

2.5 Estimation of phosphorus removal and bacterial growth

Phosphorus removal was performed in a salinity wastewater medium. Initially, 200 mL media was dispensed in a 300 mL Erlenmeyer flask and sterilized in an autoclave at 121 °C and 15 psi for 15 min. Bacteria were inoculated using an inoculating wire loop that was sterilized by flaming to redness and cooled by oscillating briefly in air. Three loopfuls of TR1 and MA3 were inoculated into each marked flask at 25 °C, following the method by Yusuf et al. (2013) [20]. Each flask was mixed on a gyratory incubator shaker (145 rpm) (Eyela Multishaker MMS; Tokyo Rikakikai Co. Ltd. Japan) at 35 °C for 24 h. The P removal ability was estimated by measuring the soluble phosphate content in the culture medium within 24 h of incubation. Within 24 h, 5 mL of agitated sample was drawn from the individual flasks and transferred to a centrifuge tube (15 mL) under aseptic conditions, which were centrifuged (Kokusan centrifugal machine H-103N. Japan) at 3500 rpm for 10 min, and the clear supernatant was used. The P concentration (as PO_4-P) was estimated using the molybdenum blue method (Japanese Industrial Standards-JIS K102.46.3-2000) and a UV-visible spectrophotometer analyzer (Spectrophotometer-800; Shimadzu Co., Ltd. Japan) at a wavelength of 880 nm. To determine cell growth within 24 h, the optical density of the cell suspension was measured using UV-visible spectrophotometer at 600 nm and expressed as OD_{600}.

3. Results and discussion

3.1 Carbon sources utilization on bacterial growth

Carbon is a vital nutrient for microbial growth. Cell growth in the synthesized medium was investigated for different carbon sources. The effect of carbon sources on the cell growth of TR1 and MA3 after cultivating in shake flasks for 24 h is shown in Figure 1. From the figure, the standard deviation bars shows that the OD 600 results of MA3 using sucrose was more significant in the other results. The results showed that both TR1 and MA3 favored a medium with glucose, sucrose, or CH_3COONa as the carbon source. However, the rate of cell growth varied with different carbon sources. Glucose and sucrose exhibited the most prominent effect on *Bacillus* sp. (TR1), as shown in Figure 1. The growth of TR1 was approximately similar in glucose and sucrose, indicating that the bacteria preferred sugar. In contrast, MA3 shows that growth increased in CH_3COONa than in glucose and sucrose. Carbon sources play a significant role in cell growth, and the synthesis of various metabolites during cultivation. Certain species prefer specific carbon sources, while others can utilize several carbon sources [21].

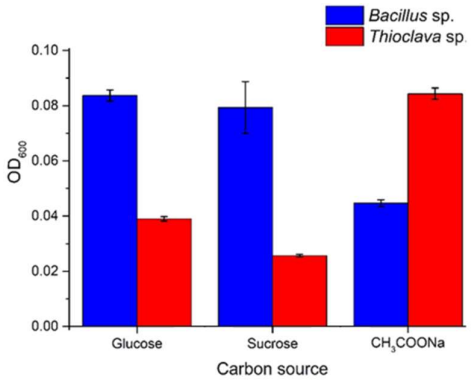

Figure 1 Effect of carbon sources on the growth of TR1 (*Bacillus* sp.) and MA3 (*Thioclava* sp.) at 35 ℃, salinity of 3.5 % w/v, and pH 7 for 24 h.

3.2 Effect of pH, temperature, and salinity variations on bacterial growth

Evidently, pH affects microbial growth and enzymatic reactions [22]. Bacteria are pH-sensitive, with each species comprising an optimum growth pH. Bacteria also have a tolerable pH range and are unable to survive beyond this range. pH plays a significant role in biological P removal systems. The influence of pH (4.0–10.0) on cell growth was studied and the results are shown in Figure 2. Based on statistical analysis the OD 600 in the pH range of 6-8 for TR1 and 6-9 for MA3 were more significant than below and above those ranges. Strain TR1 exhibited good survival in the pH range of 6–8. Meanwhile, the biomass concentration of strain MA3 was high (>1.0) at pH 6–9, which was more than the optimum pH range of TR1. These results are similar to those of Jiang et al. (2018), who reported that an ideal P removal performance could only be obtained at a pH between 7.0– 8.0. This study showed that the optimal pH range suitable for P removal differed in strains TR1 and MA3 [11].

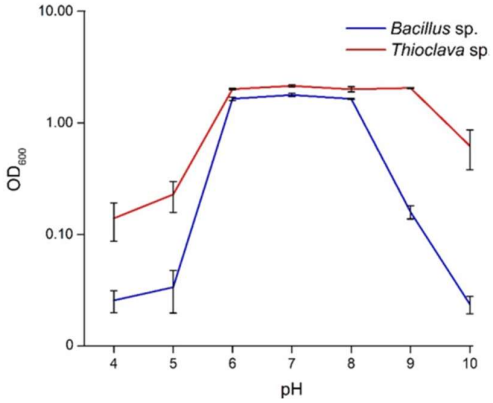

Figure 2 The effect of pH on the growth of TR1 (*Bacillus* sp.) and MA3 (*Thioclava* sp.) at 35 ℃ and salinity of 3.5% w/v for 24 h.

Figure 3 shows the effect of temperature, ranging between 25–50 °C, on the cell growth of TR1 and MA3. The statistical analysis of standard deviation of both TR1 and MA3 exhibited more significant results in the range of 25- 35 °C than 40-50 °C. Both strains TR1 and MA3 could grow at temperatures in the range of 25 to 40 °C. When the temperature increased to 50 °C, the growth decreased significantly, suggesting that the bacteria were unable to grow at high temperatures (>40 °C). The results indicated that the growth of TR1 and MA3 was affected by environmental temperature. Additionally, the optimum temperatures for phosphate removal using bacteria studied by Akpor et al. (2014) [23] were 30–40 °C [26].

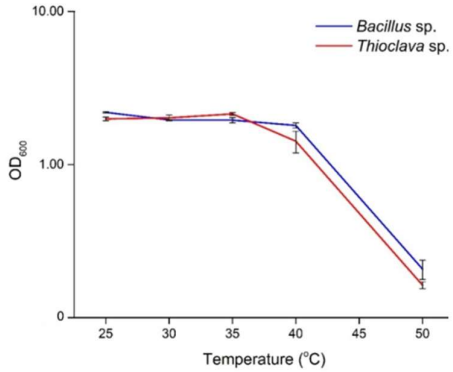

Figure 3 The effect of temperature on the growth of TR1 (*Bacillus* sp.) and MA3 (*Thioclava* sp.) at pH 7 and salinity of 3.5% w/v for 24 h.

Salinity has selective effects on the microbial community structure and influences the degradation rate by inhibiting microbial or enzyme activity [24]. According to Munn (2011) [25], the major ionic components of seawater are sodium, chloride, sulfate, magnesium, calcium, and potassium, including key nutrients such as nitrate, phosphate, silica, and iron. The concentration of each component is crucial in determining the growth of marine microbes [28]. The effect of salinity on the growth of TR1 and MA3 cells is shown in Figure 4. The results showed that both TR1 and MA3 exhibited a certain adaptability to survive in different salinities from 0 to 10%. In the optimum salinity condition range (3-5 % w/v), standard deviation showed that the calculation of OD 600 of *Bacillus* sp was more significant than *Thioclava* sp. Figure 4. shows that TR1 prefers the salinity range of 0%–10% and peaked at 3.5% to 5% salinity. However, MA3 indicates can grow from 1 to 10% salinity, and was absent in non-saline water, as opposed to TR1. The maximum growth of MA3 was recorded at a salinity of 4%. This result indicated that salinity had a significant influence on the growth of these two phosphate reducers.

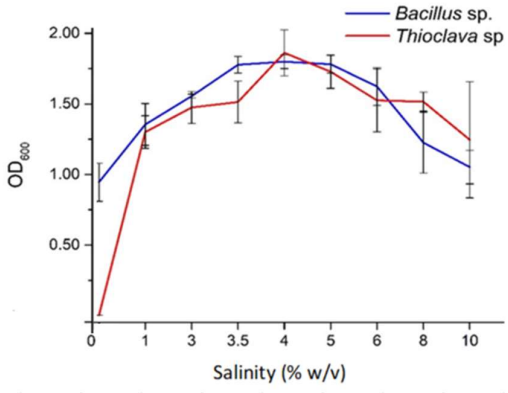

Figure 4 The effect of salinity variations on the growth TR1 (*Bacillus* sp.) and MA3 (*Thioclava* sp.) at 35 °C and pH 7 for 24 h.

3.3 Phosphorus removal capability and bacterial growth of the isolated strains of the isolated strains

Figure 5 shows the results of phosphorus removal by TR1 and MA3 at different P concentrations. The performance of the strains differed in batch experiments designed to estimate phosphorus removal. Each strain, individually and combined, removed phosphorus at various concentrations after 24 h. Phosphate is consumed by the cells to grow and modify polyphosphates under aerobic conditions [3]. Figure 5 shows that TR1 had a higher P removal efficiency than MA3 at every experimental P concentration. Based on the standard deviation, the calculation of the removal of phosphorus concentration by TR1 and the combination showed more significant results compared to other calculations. The results showed that 1 mg-P/L of P in synthetic saline wastewater could be completely removed by TR1. However, MA3 could only reduce P to 38.2%. The percentage of phosphorus removal decreased as the P concentration increased. The phosphorus removal percentages of TR1 decreased from 67.1% to 15.0% as the P concentration increased from 2.5 to 10 mg-P/L. Meanwhile, MA3 could only remove 11.5% and 2.4% when the P concentration were 2.5 and 5 mg-P/L, respectively.

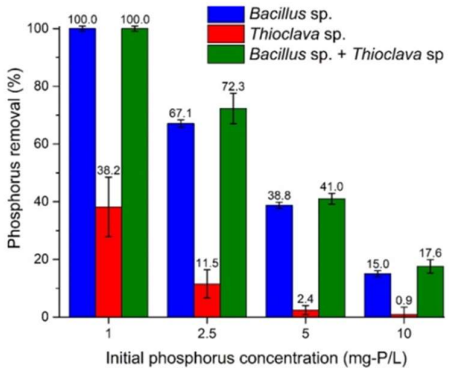

Figure 5 The effect of initial phosphorus concentration on phosphorus removal of TR1 (*Bacillus* sp.), MA3 (*Thioclava* sp.), and the combination of strain TR1 and MA3, under aerobic condition at 35 °C, pH 7, and salinity 3.5% w/v.

Furthermore, a very small portion of P was reduced by MA3 at a P concentration of 10 mg-P/L. This is supported by Zhang et al. (2018) [26], who found that an initial low phosphorus concentration creates conditions conducive for the growth of *Bacillus* sp. PK1 and stimulates enzyme activity induced by phosphoric acid that increases the total phosphorus uptake, but does not increase the rate of phosphorus removal, but a decreasing trend with an increase in the initial phosphorus concentration is observed. According to Choi et al. (2013) [27], conventional biological methods are effective in reducing wastewater phosphate levels to ~1 mg/L, and necessitates long durations of microbial adaptation for effective phosphate removal [30]. In addition, perhaps the loopful of each strain inoculated in this experiment was insufficient for removing phosphorus at concentrations of 5 and 10 mg-P/L. Figure 6 shows The effect of initial phosphorus concentration on bacterial growth of TR1, MA3, and the combination under aerobic conditions. The figure shows that the calculation of OD 600 of the combination was more significant than the others. The lower P accumulation may be associated with the slower growth rate of TR1 and MA3 at cell density (OD_{600} of TR1 0.304 and 0.204; MA3 0.041 and 0.038, respectively) concurring with the result that the removal efficiency of this method is relatively poor at higher phosphorus concentrations, as shown in Figure 6. Besides, the lower P accumulation of MA3 compared to TR1 was related to the slower growth rate of MA3 at the salinity used (OD_{600} of TR1 and MA3 at salinity 3.5% are 1.8 and 1.5, respectively). Moreover, the fact that *Bacillus* sp. is gram-positive and *Thioclava* sp. is gram-negative can also be a reason for the low P uptake in MA3. Several studies reported that gram-positive organisms exhibit a reasonably higher phosphate accumulation than gram-negative organisms [15,28]. Another possibility for the low P uptake is that MA3 may be classified as a low phosphate accumulator similar to the strain YG-24 (*Pseudomonas stutzeri*), which accumulates low P concentration to maintain its growth [29].

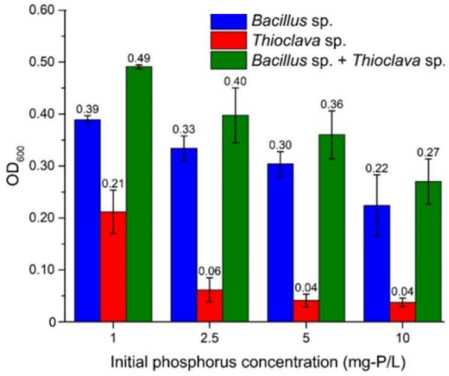

Figure 6 The effect of initial phosphorus concentration on bacterial growth of TR1 (*Bacillus* sp.), MA3 (*Thioclava* sp.), and the combination of strain TR1 and MA3 under aerobic condition at 35 °C, pH 7, and salinity 3.5% w/v for 24 h.

Additionally, the P removal by combining the two strains (TR1 and MA3) was studied, and the results are shown in Figure 5. At a concentration of 1 mg-P/L, P was completely removed by this combination. Moreover, the combination of TR1 and MA3 at initial P concentrations of 2.5, 5, and 10 mg-P/L were 72.4%, 41.0%, and 17.6%, respectively, which was higher than their (TR1 and MA3) individual removal abilities. A study by Krishnaswamy and Muthuchamy (2011) [3], and Oljira et al. (2018) [15] showed that the combination of *Bacillus* sp. and *Pseudomonas* sp. efficiently removed phosphate from synthetic wastewater, suggesting that the combination of bacteria can encourage the increase in growth for a greater phosphate uptake capacity. This was attributed to an increase in the nutrient utilization rate of the polyphosphate organisms [3,15]. In addition, Cho et al. (2018), used the combination of *Bacillus* sp. KGN1 and *Vibrio* sp. KGP1 isolated from marine sediment, which showed high removal efficiency of 99.9% phosphorus with a total P concentration of 1.0 mg-P/L in marine wastewater [30].

This is consistent with our study of combined *Bacillus* sp. (TR1) and *Thioclava* sp. (MA3), which effectively removed phosphorus from saline wastewater. In addition, probably that the number of bacterial cell densities (Figure 6) that grow is greater for phosphorus removal of the combined bacteria in the ratio of 1:1 compared to individual bacteria. Moreover, Roller and Schmidt (2015) stated that changes in cellular P demand can affect how efficiently cells grow, or how much biomass is produced per unit nutrient [31]. Figure 7 shows that in the aerobic zone the different bacteria species utilized sodium acetate as a carbon source to uptake the different concentrations of phosphorus for metabolisms and cell growth, producing new bacteria replication and the other products such as, carbon dioxide, water, and energy. Furthermore, the efficient removal of P by the combination as compared to the single culture of bacteria may be due to the synergistic activity between the individual strains in a combination.

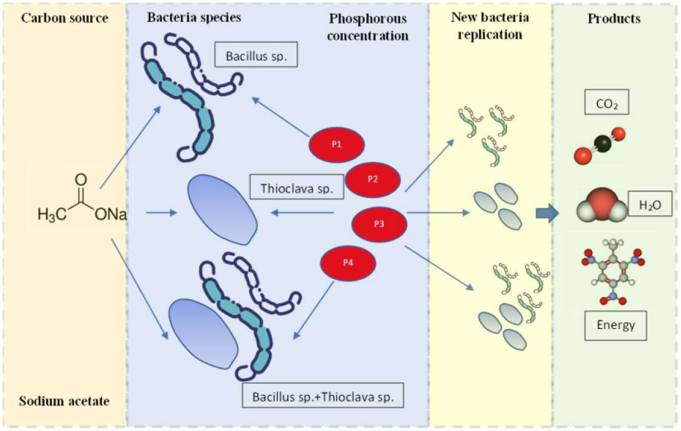

Figure 7 Phosphorus uptake mechanisms.

Table 1 compares the phosphorus removal efficiency of TR1 and MA3 in this study with prior studies strains YG-16 (*Sphingomonas* sp.), BL-21 (*E. coli*), and recombinant *E. coli* [27,29]. The P accumulating ability of YG-16 and BL-2 was evaluated at pH 7, 28 °C and in non-saline synthetic wastewater. It was demonstrated that TR1 has a significantly higher removal efficiency than *Sphingomonas* sp., *E. coli*, and recombinant *E. coli* at a P concentration of 1 mg-P/L; TR1 could completely remove P, whereas *Sphingomonas* sp., *E. coli*, and recombinant *E. coli* eliminated only 50%, 55%, and 58% of P, respectively. In addition, TR1 also displayed a higher P removal efficiency (38.8%) than *Sphingomonas* sp. (30%) and *E. coli* (26%) at initial P concentrations of 5 mg-P/L. However, when the initial concentration was increased to 10 mg-P/L, *Sphingomonas* sp. and *E. coli* removal efficiencies (20% and 19%, respectively) slightly outperformed the P removal efficiency of TR1(15%). On the other hand, *Thioclava* sp. (MA3) showed the least P removal ability, as shown in Table 1.

Table 1 Comparison table of the phosphorus removal efficiency of TR1 (*Bacillus* sp.) and MA3 (*Thioclava* sp.) in this study with YG-16 (*Sphingomonas* sp.), BL-21 (*E. coli*), and recombinant *E. coli*.

Initial P concentration (mg-P/l)	P removal efficiency (%)				
	TR1 *Bacillus* sp. (This study)	MA3 *Thioclava* sp. (This study)	YG-16 *Sphingomonas* sp. [29]	BL-21 *E. coli* [29]	Recombinant *E. coli* [27]
1	100	38.2	50	55	58
5	38.8	2.4	30	26	-
10	15	0.9	20	19	-

The marine bacteria in this study are likely to be useful in removing phosphorus from saline wastewater. The high P removal efficiency of TR1 in saline wastewater was also confirmed by a study conducted by Eom et al. (2018) using *Bacillus* sp. to remove phosphate from saline wastewater [32]. Meanwhile, current research on *Thioclava* sp. deals with the sulfur-oxidizing genus, which has demonstrated chemoautotrophic growth on intermediate sulfur compounds, including thiosulfate and heterotrophic growth on simple organic matter, including glucose [1,16]. In addition, Chen et al. (2018) suggested that adding *Thioclava* sp. could improve nitrogen and phosphorus removal for the treatment of saline wastewater. However, our research showed that these bacteria (TR1 and MA3) had the ability to reduce phosphorus, both in pure culture and in combination [33]. Both marine bacterial strains (TR1 and MA3) are suitable for phosphorus removal in saline wastewater, where TR1 had a higher phosphorus removal than MA3 based on the amount of phosphorus accumulated. This indicates that both TR1 and MA3 can be used for phosphorus removal in marine environments.

4. Conclusion

The results of this study indicated that strains TR1 (*Bacillus* sp.) and MA3 (*Thioclava* sp.) utilized different carbon sources, such as glucose, sucrose, and CH_3COONa, for their growth. A study on environmental pH adaptation showed that the strain TR1 prefers pH 6–8 and MA3 favors pH 6–9 for growth. Both bacteria can grow in the temperature range of 25–40 °C. Strain TR1 preferred a salinity range of 0%–10 % with the optimum growth observed at 3.5%-5%, while MA3 favored a salinity range of 1%–10% and grew optimally at a salinity of 4%. *Thioclava* sp. (MA3) growth was undetectable in non-saline water. Phosphorus removal studies in various phosphorus concentrations at 3.5% salinity revealed a higher phosphorus removal efficiency of TR1 than the MA3. Furthermore, a combination of TR1 and MA3 showed P removal efficiency of 100% at an initial phosphorus concentration of 1 mg-P/L that showed similar values to TR1. The initial phosphorus concentration of 2.5 mg-P / L showed a slightly higher 72.35% P removal efficiency compared to the individual strains. The combination possibly built a synergistic activity between the individual strains to remove phosphorus. However, phosphorus removal did not increase, but showed a downward trend with increasing at initial phosphorus concentration of 5 and 10 mg-P/L. Therefore, the loopful of culture inoculated into the batch process must be increased by varying the ratios, and long duration for microbial adaptation should be provided. These experimental results indicate that two marine bacteria can be utilized as an economically and environmentally viable biological treatment method for developing phosphorus removal processes, especially in removing low concentrations of P from saline wastewater.

5. Acknowledgements

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