European Journal of Applied Sciences 1 (1): 06-15, 2009
ISSN 2079-2077

Studies on the Efficiency of the Removal of Phosphate Using Bacterial Consortium for the Biotreatment of Phosphate Wastewater

Usharani Krishnaswamy, Muthukumar Muthusamy and Lakshmanaperumalsamy Perumalsamy

Department of Environmental Sciences, Bharathiar University, Coimbatore - 641 046. T.N., India

Abstract: The efficient phosphate reducing isolates were used to remove phosphate from synthetic medium was tested using batch scale process. The three most efficient phosphate reducers were isolated and screened from eutrophic lake water and forest soil samples. The total heterotrophic bacterial analysis of the samples showed the presence of about 38 phosphate reducers. Among them, *Bacillus* sp RS-1, *Pseudomonas* sp YLW-7 and *Enterobacter* sp KLW-2 were found to be efficient in phosphate reduction. The consortium combination of *Bacillus* sp RS-1*, Pseudomonas* sp YLW-7 and *Enterobacter* sp KLW-2 have efficiently removed the phosphate in the synthetic medium. The phosphate removal was observed to be maximum of 92.5% in MSM and 63.4% in synthetic phosphate solution with lactose by consortium after 72 h. Thus the microorganisms may use the contaminants as nutrients and as energy sources or it may be utilized by co-metabolism.

Key words: Synthetic waste water · *Bacillus* sp RS-1 · *Pseudomonas* sp YLW-7 · *Enterobacter* sp KLW-2 · Phosphate removal

INTRODUCTION

Phosphorus is recognized as one of the major nutrients required by living organisms involved in major physiological processes. However, it can also be considered a pollutant if the concentrations are high under specific environmental conditions. The addition of phosphorus as phosphate ion is one of the most serious environmental problems because of its contribution to the increased eutrophication process of lakes and other natural waters. It occurs in natural water, wastewater, sediments and sludges. The possible entry of this ion into aquatic environment is through household sewage water and industrial effluents- particularly fertilizer and soap industries. The main sources of phosphorus released into the environment include fertilizers, detergents, cleaning preparations and boiler waters to which phosphates are added for treatment [1]. It exists in three forms: organic phosphorus (associated with organic molecules), orthophosphate- (exists as an anion) and polyphosphates (from detergents). Only Orthophosphate can be chemically precipitated, however, most of the organic phosphorus and polyphosphates are converted to the orthophosphate form during biological treatment.

Biotreatment is a cost-effective method for wastewater before being discharged into the streams and rivers. Microbial strategies for the removal of environmental pollutants from waste streams or contaminated sites can provide an attractive alternative to traditional methods such as incineration or disposal in landfills. Currently, phosphates are biologically removed by wastewater treatment facilities by absorption of dissolved orthophosphate, polyphosphate and organic phosphate by living microorganisms, such as bacteria, microalgae, yeast, protozoa, fungi and macrophytes. The quantity of eliminated phosphate depends on the net production of living biomass. Enhanced Biological Phosphate Removal (EBPR) is an alternate biotechnological as well as eco-friendly approach to both the environment and its living beings. The process of EBPR uses the metabolism of specific bacteria, which under certain conditions accumulate large amounts of phosphate as intracellular polyphosphate. These bacteria use the degradation of polyphosphate to produce energy under anaerobic conditions with the release of phosphate into the wastewater (resulting in a transient increase in the phosphate content of the waste water). The waste water is then treated in an aerobic basin. Here the polyphosphate bacteria use stored carbon reserves to produce energy for growth and to replenish their stores of polyphosphate. The result is a net removal of phosphate from the waste water (due to replenishment

Corresponding Author: Usharani Krishnaswamy, Department of Environmental Sciences, Bharathiar University, Coimbatore- 641 046., T.N., India.

of polyphosphate in extant cells and de novo polyphosphate reserves in divided cells). Biological phosphate removal from wastewater is a generally accepted; less costly alternative to chemical phosphate removal [2]. Phosphorus is used by the microorganisms for their cellular maintenance, synthesis of nucleic acids, construction of cell membranes (as phospholipids) and chemical energy transfer reactions within cells (as ATP molecules). Some phosphorus is also stored for future use by the cells.

An important group of contaminants for which efficient treatment methods are needed are phosphates, since they may adversely affect and pose a threat to aquatic ecosystems. According to federal government standards, phosphate levels in water should not exceed 0.01 - 0.1 mgl^{-1} (EPA, 1991). Therefore it is essential to control the emission of phosphates from discharge of wastewater and reducing phosphorus concentrations to the lowest possible level is vital to the maintenance of unpolluted water supplies. Hence the objective of the present study was to examine the efficiency of bacterial species individually and in consortium for the removal of phosphate from synthetic phosphate solution and mineral salts medium (MSM).

MATERIALS AND METHODS

Sample Collection: The eutrophic lake waters were collected in sterile glass bottles from four different sampling stations of Yercaud and Kodaikanal Lakes (South India). The soil samples from the rhizosphere and non- rhizosphere region of teak trees from the Siruvani Forest (located in Westen Ghats of Coimbatore District, South India) were collected in sterile polyethylene bags.

Isolation and Identification of Phosphate Reducers: Pour plate technique was employed to enumerate total heterotrophic bacteria using Nutrient Agar (Hi-Media, Mumbai, India). Minimum inhibitory concentration (MIC) test with plate screening method was carried out to screen phosphate reducers using phosphate agar. The bacterial cultures isolated from nutrient agar and phosphate agar plates were classified to various genera based on their morphological and biochemical characters as given in Bergey's Manual of Determinative Bacteriology [3].

The phosphate reducers screened from the different sample source were given symbols (code name) based on the source of isolation; Kodaikanal Lake Water- KLW, Yercaud Lake Water - YLW, Rhizosphere Soil - RS, Non- Rhizosphere Soil - NRS.

Preparation of Inoculum: Nutrient broth (Hi-Media, Mumbai, India) was prepared and selected bacterial isolates were inoculated separately and incubated for 24 h at room temperature. The cells were recovered by centrifugation (10,000 rpm for 15 min) and were transferred to sterile saline. The cell concentration of each strain was adjusted to an optical density at 600 nm (OD_{600}) of 0.1 and used as inoculum. Three efficient phosphate reducers (*Bacillus* sp RS1, *Pseudomonas* sp *YLW*7 and *Enterobacter* sp KLW2) were used for removal of phosphate.

The mixed bacterial consortium [A+B+C] were prepared in combination from the three isolates *Bacillus* sp RS1-(A), *Pseudomonas* sp *YLW* 7-(B) and *Enterobacter* sp KLW 2-(C), by adjusting the cell concentration of A, B and C to 0.1 of OD_{600}. About 97 (RS-1), 105 (YLW-7) and 92 (KLW- 2) X 10^4 CFU/100ml (1ml of 0.1OD) of the cells was used as an inoculum.

Experimental Study: Shake flask batch culture experiments were performed. Phosphate removal was carried out by adding phosphate to synthetic wastewater (mineral salts medium and plain distilled water). The synthetic phosphate solution (distilled water with phosphate concentration of 100 mgl^{-1} using Potassium dihydrogen phosphate; pH-7.2) and in mineral salts medium containing 100 mgl^{-1} phosphate concentration and 0.5% of different carbon substrates such as sucrose, starch, glucose and lactose were prepared. Then the medium containing flasks were sterilized at 121°C and at 15 lbs for 15 min in an autoclave. One ml of inoculum (0.1 OD) from the selected phosphate reducers A, B, C and consortium of combination A+B+C were inoculated in the individual flasks. They were incubated at room temperature in a shaker maintained at 150 rpm for a period of three days. Samples were collected at 0, 24, 48, 72 h and analyzed for growth of bacteria, pH change and change in total phosphate concentration of the medium. All experiments were performed in triplicates.

Analytical Methods

Growth of Bacteria and pH: The increase in growth of bacteria for every 24 h was monitored by measuring OD at 600nm on a UV-Visible Spectrophotometer (UV-VIS Hitachi - U3210). The pH change in the culture medium after treatment was measured using a pH meter.

Estimation of Phosphate: The phosphate uptake activities of different strains were quantified by stannous chloride

reduced molybdophosphoric acid blue method. The soluble phosphate content in the culture medium was estimated after 24, 42 and 72 h of incubation by using the stannous chloride calorimetric method [4] and (APHA, 1998). After every 24 h, 10 ml of the agitated sample was drawn from the series of individual flask and transferred into the centrifuge tubes of 15ml capacity under aseptic conditions. Then the tubes containing samples were centrifuged at 10,000 rpm at 15 min and the clear supernatant was used for soluble phosphate estimation at 690 nm by using spectrophotometer (UV-VIS Hitachi - U3210).

Phosphate uptake efficiency (E) was calculated using the formula,

$$E = [(I-F)/I] \times 100$$

Where:

I and F are the initial and final concentrations of phosphorous respectively.

An efficiency value of 100% was obtained when no phosphate appeared in the water sample (i.e., F = 0).

RESULTS

Isolation and Screening of Efficient Phosphate Reducers: The isolation and screening of phosphate reducers from the samples mentioned above were carried out to determine the efficient phosphate removal which showed that wide range of these bacteria was occurred in rhizosphere soil and in eutrophic lake water. Table 1 shows the maximum population of total heterotrophic bacteria (THB-64.7 CFU x 10^3 $g^{-1}$of soil) and phosphobacteria (4.7 CFU x 10^3 g^{-1} of soil) were observed in soils collected from rhizosphere soil region of teak trees from Siruvani forest, whereas in water samples, the maximum THB population of 28.5 CFU x 10^3 ml^{-1} and phosphate reducing bacterial population of 3.3 CFU x 10^3 ml^{-1} was noticed in Yercaud lake water sample.

It was found that among the 38 isolates, A - *Bacillus* sp (RS-1), B - *Pseudomonas* sp (YLW-7) and C - *Enterobacter* sp (KLW-2) were screened and identified as predominant phosphate utilizers based on the minimum inhibitory concentration (MIC) test after 72 h of incubation, each from different sources Rhizosphere Soil-RS, Yercaud Lake Water-YLW and Kodaikanal Lake Water-KLW respectively as in Figure 1. These isolates were used in this study to remove phosphate in synthetic phosphate solution and mineral salts medium with two different phosphate concentrations.

Effect of Carbon Source in Medium: Carbon sources - enriched synthetic medium at the experimental concentration greatly influenced the growth and phosphate removal efficiency of the bacteria. The results obtained from different bacterial species and its combinations were plotted in Figures 2-5.

In synthetic phosphate solution with 0.5% carbon source, lactose was observed to yield maximum phosphate removal (63.4%) and bacterial growth by the consortium. In the individual strains, *Pseudomonas* sp showed maximum (52.3%) phosphate removal and growth in glucose as carbon source followed by starch, lactose and sucrose as shown in Figures 2 and 4.

The MSM contains lactose as carbon source was found to be maximum for phosphate removal as well as growth of phosphate reducers when compared to synthetic phosphate solution. From the Figures 3 and 5, it was observed that, in MSM at 100 mgl^{-1} of phosphate concentration with 0.5% of different carbon sources, the lactose source showed a maximum phosphate removal of 92.5% and maximum bacterial growth by the consortium after 72 h. But in the individual strains (*Pseudomonas* sp YLW-7), the glucose showed a maximum (68.2%) phosphate removal trend as well as the growth. The results of this study showed that the synthetic medium without carbon sources (control) showed less removal when compared to synthetic phosphate solution and MSM amended with carbon sources.

Table 1: Enumeration of Total heterotrophic bacteria (THB) and Phosphate reducers present in different samples

Source of Isolation	THB Population	Phosphate reducers
Rhizosphere Soil [RS] (Siruvani forest soil)	64.7 cfu x10^3 g^{-1}	4.7 cfu x10^3 g^{-1}
Non- Rhizosphre Soil [NRS] (Siruvani forest soil)	53.8 cfu x10^3 g^{-1}	3.8 cfu x10^3 g^{-1}
Kodikanal Lake Water [KLW]	17.8 cfu x10^3 ml^{-1}	2.2 cfu x10^3 ml^{-1}
Yercaud Lake Water [YLW]	28.5 cfu x10^3 ml^{-1}	3.3 cfu x10^3 ml^{-1}

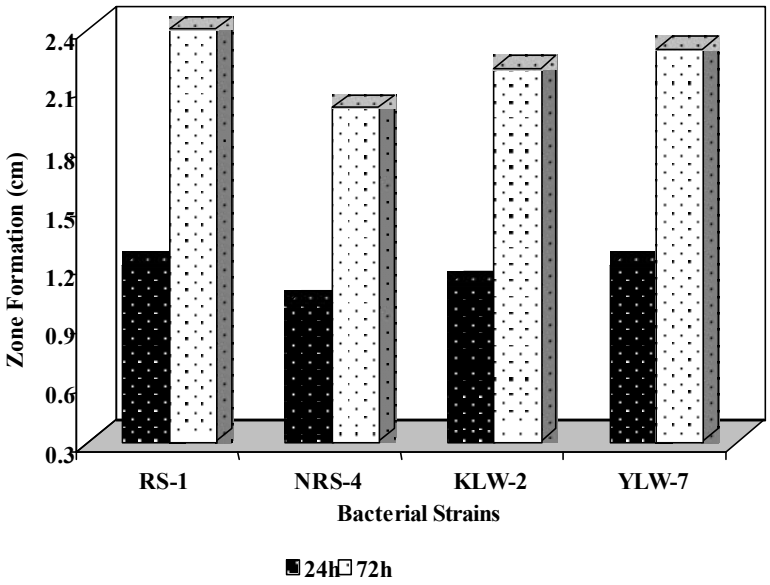

Fig. 1: Screening of efficient phosphate reducers based on the Minimum Inhibitory Concentration test (A - *Bacillus* sp RS-1, B - *Pseudomonas* sp YLW-7, C - *Enterobacter* sp KLW-2 and A+B+C -Consortium)

Growth of Bacterial Cultures: The growth of bacterial species was analyzed using different carbon sources in phosphate medium after 72 h of incubation period. The results were shown in Figures 4 and 5.

In synthetic phosphate solution with 0.5% carbon sources, the individual strain of *Pseudomonas* sp was observed a maximum growth of 0.6886 OD in glucose and minimum of 0.2786 OD in sucrose by *Bacillus* sp as shown in Figure 4. But the consortium showed maximum growth of 0.6997 OD in the presence of lactose.

Where as in MSM contains 100 mgl^{-1} of phosphate with 0.5% carbon source, the individual strains of *Pseudomonas* sp was observed a maximum growth of 0.9886 OD in the presence of glucose and minimum of 0.3280 OD in sucrose by *Bacillus* sp as shown in Figure 5. Whereas in case of consortia, the maximum growth was found to be 1.1428 OD in lactose source.

The metabolism of phosphate by *Bacillus* sp (RS-1), *Pseudomonas* sp (YLW-7) and *Enterobacter* sp (KLW-2) were indicated by a visible increase in growth (OD) with time. Initially, the growth was suppressed in presence of phosphate, but after adaptation to phosphate it was grown rapidly exhibiting high growth rate. In later stage the amount of growth produced in the medium containing phosphate was much higher as compared to the growth in medium without carbon sources. This could be due to the availability of additional carbon source upon reduction of phosphate in the medium.

pH Change of the Medium: In order to find the relationship between metabolic activities and reduction of phosphate, pH of the culture medium was monitored. The pH changes in culture medium with time were shown in Figure 6 and 7.

The pH value of the culture medium with 0.5% of carbon source was reduced during the process. In synthetic phosphate solution, the maximum reduction of pH from 7.2 to 6.0 was recorded in consortium with various carbon sources (Figure 6). Whereas in MSM, the reduction was from 7.2 to 5.7 in sucrose and glucose carbon sources by *Enterobacter* sp after 72 h (Figure 7). In mixed cultures, maximum reduction of pH (7.2 to 4.5) was recorded in the consortium (A+B+C) where the glucose was used as carbon source (Figure 7). In contrast, there is no significant change of pH was monitored in the medium without carbon source.

Phosphate Removal by Bacteria in MSM and Synthetic Phosphate Solution: Among the medium, the MSM with lactose as carbon source showed maximum phosphate removal when compared to synthetic phosphate solution with and without carbon sources.

In synthetic phosphate solution at 100 mgl^{-1} of phosphate concentration in 0.5% carbon source, it was found to be maximum removal of 63.4% by the consortium (A+B+C) where lactose was used as a carbon source and minimum of 48.3% in glucose source as shown in Figure 2d. But in the individual strains, 52.3% removal by

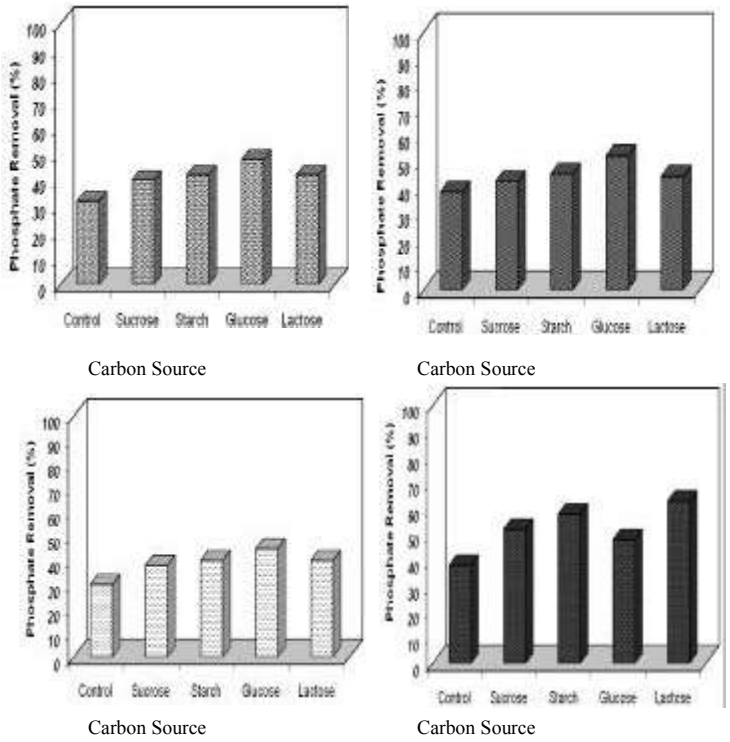

Fig. 2: Effect of carbon source on the removal of phosphate by the bacterial species in synthetic phosphate solution (a-'A' - *Bacillus* sp RS-1, b-'B' - *Pseudomonas* sp YLW-7, c-'C' - *Enterobacter* sp KLW-2 and d-'A+B+C' -Consortium)

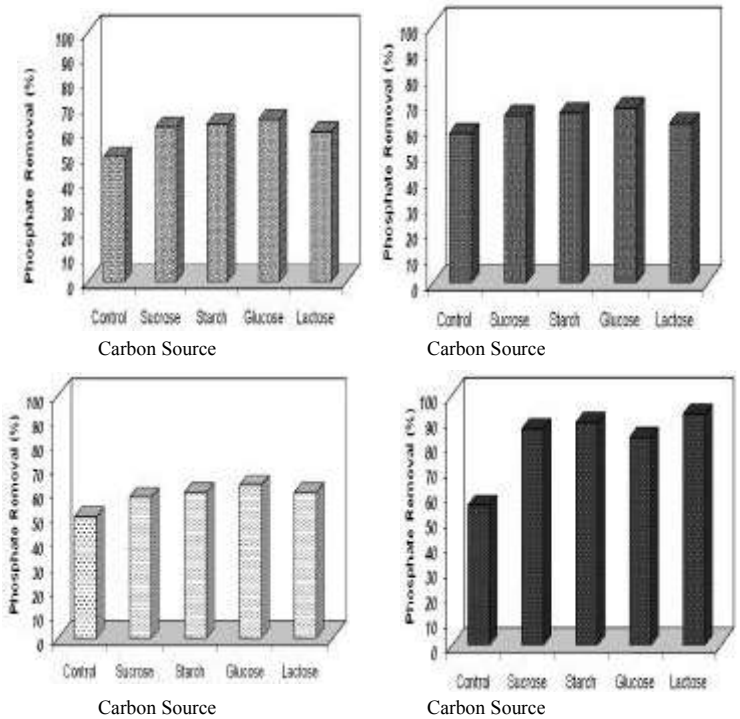

Fig. 3: Effect of carbon source on the removal of phosphate by the bacterial species in Mineral salts medium (MSM) (a-'A' - *Bacillus* sp RS-1, b-'B' - *Pseudomonas* sp YLW-7, c-'C' - *Enterobacter* sp KLW-2 and d-'A+B+C' -Consortium)

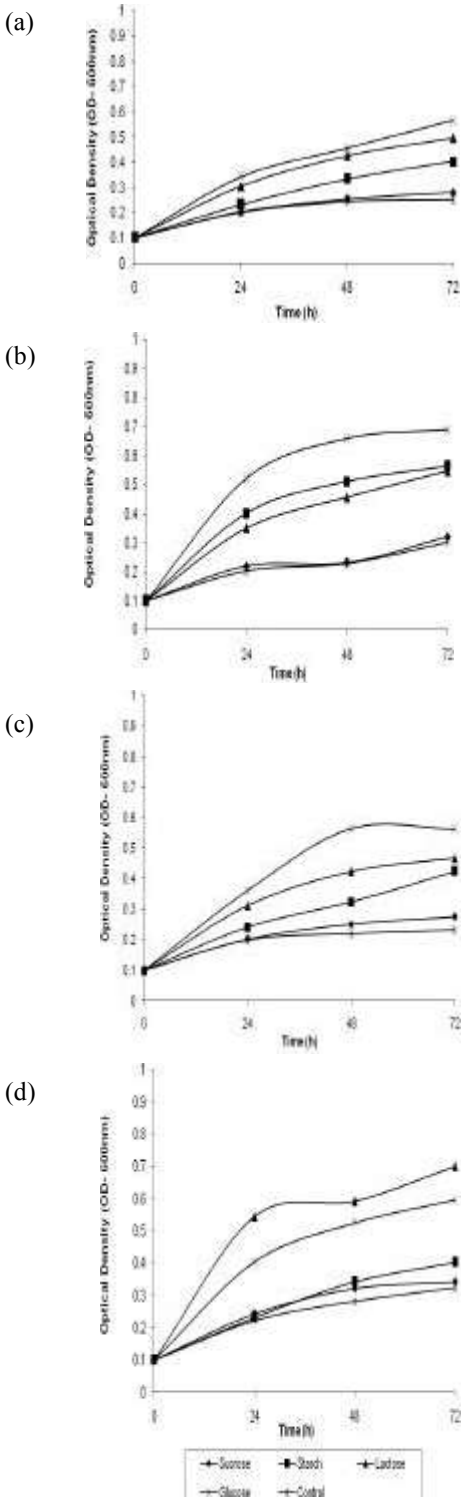

Fig. 4: Effect of carbon source on the growth of bacteria in synthetic phosphate solution (a-'A' - *Bacillus* sp RS-1, b-'B' - *Pseudomonas* sp YLW-7, c-'C' - *Enterobacter* sp KLW-2 and d-'A+B+C' - Consortium)

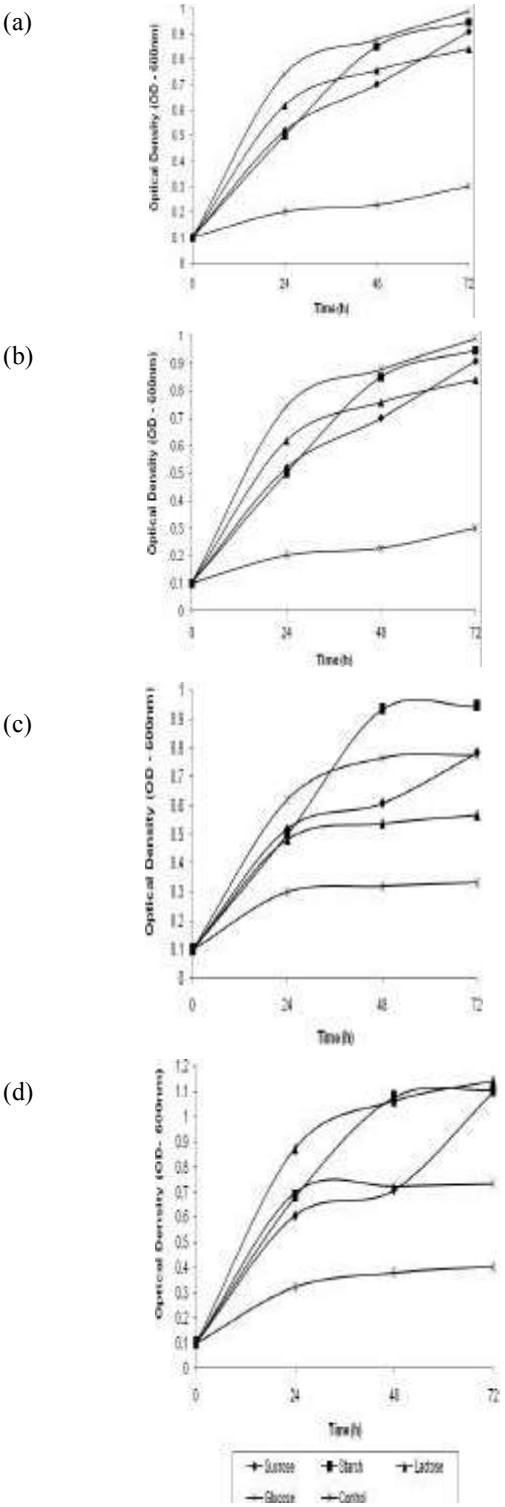

Fig. 5: Effect of carbon source on the growth of bacteria in Mineral salts medium (MSM) (a-'A' - *Bacillus* sp RS-1, b-'B' - *Pseudomonas* sp YLW-7, c-'C' - *Enterobacter* sp KLW-2 and d-'A+B+C' - Consortium)

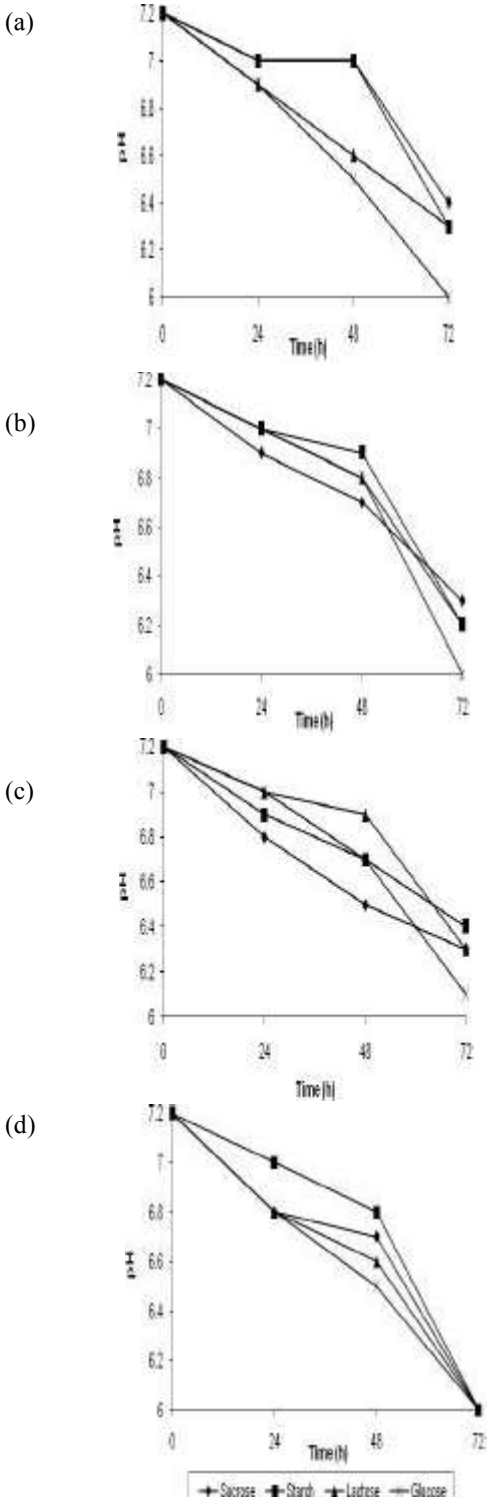

Fig. 6: Change in pH of culture medium (synthetic phosphate solution) during phosphate removal by bacteria (a-'A' - *Bacillus* sp RS-1, b-'B' - *Pseudomonas* sp YLW-7, c-'C' - *Enterobacter* sp KLW-2 and d-'A+B+C' -Consortium)

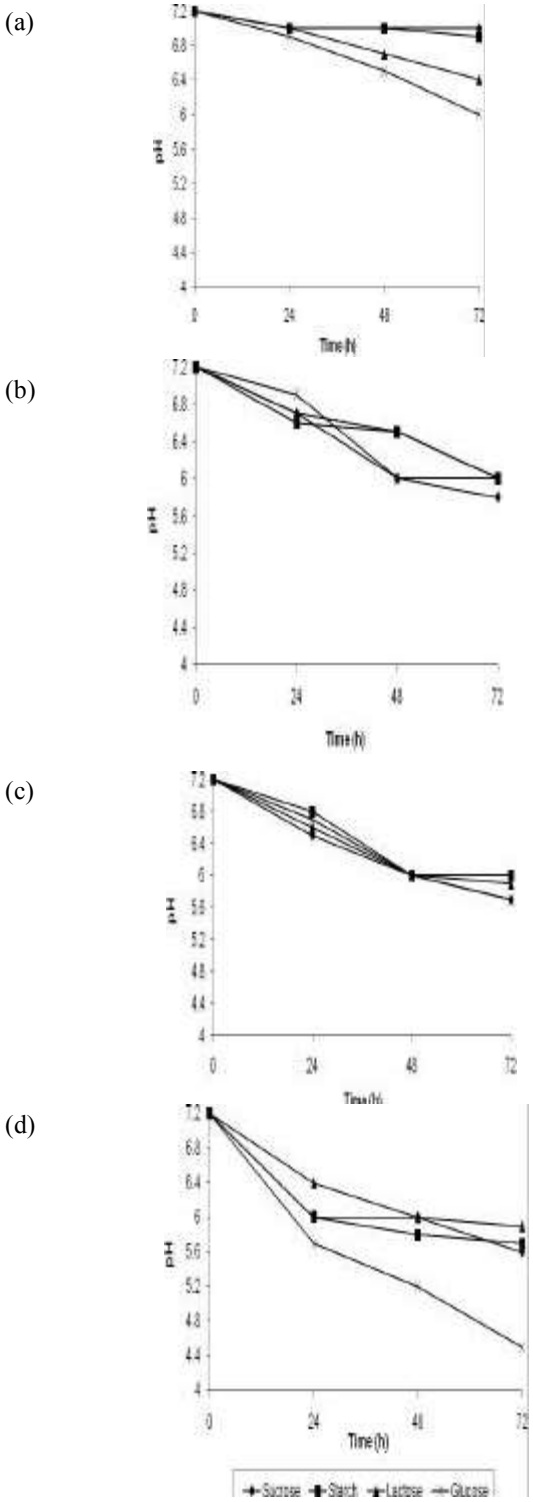

Fig. 7: Change in pH of culture medium (MSM) during phosphate removal by bacteria (a-'A' - *Bacillus* sp RS-1, b-'B' - *Pseudomonas* sp YLW-7, c-'C' - *Enterobacter* sp KLW-2 and d-'A+B+C' - Consortium)

Pseudomonas sp in glucose carbon source and minimum of 45.2% removal by *Enterobacter* sp in sucrose carbon source were observed (Figure 2).

In MSM medium at 100 $mgl^{-1}$of phosphate concentration, 0.5% carbon sources with consortium shows the maximum phosphate removal of 92.5% was observed in lactose and minimum 83.2% was observed in glucose as carbon source by the consortium (A+B+C) (Figure 3d). The MSM with individual strains, the phosphate removal was found to be 68.2% by *Pseudomonas* sp in glucose carbon source and minimum of 58.3% by *Enterobacter* sp in sucrose as carbon source (Figure 3). The control was recorded very less removal when compared to other carbon sources as shown in Figures.

The results of this study showed that the synthetic phosphate solution without carbon sources showed less removal when compared to synthetic phosphate solution and MSM amended with carbon sources. Carbon source - enriched synthetic phosphate solution and MSM at the experimental concentrations enhance the phosphate removal and greatly influenced the growth of bacteria.

DISCUSSION

Phosphate Reducers: Phosphate utilizing bacteria were known to be present in various environments [5] and [6]. The strains of *Bacillus* sp RS-1, *Pseudomonas* sp YLW-7 and *Enterobacter* sp KLW-2 were isolated from Rhizosphere Soil, Kodaikanal Lake Water and Yercaud Lake Water respectively.

The removal efficiency of soluble phosphates varied with strains. Various microorganisms are capable of utilizing phosphate as a sole carnon source of phosphsphorus [7] and these microbial transformations have been proposed as key steps in the phosphorous cycle in nature. The *Pseudomonas* sp*., Aerobacter* sp*., Moraxella* sp*., Escherichia coli, Mycobacterium* sp*., Beggiatoa* sp and *Klebsiella* sp have the ability to accumulate phosphorus at approximately 1 to 3% of the cell dry mass reported [8].

Effect of Carbon Source: The carbon source was provided in the medium in order to enrich synthetic medium which in turn enhance the growth and phosphate uptake capacity of bacteria. Bacteria which can accumulate phosphate in the aerobic conditions and their internal phosphate had been depleted under anaerobic conditions.

Among the carbon sources, the glucose source showed maximum phosphate removal of 68.2% by the *Pseudomonas* sp in MSM, the glucose may be oxidized to gluconate which is further converted to other compounds. Glucose carbon source could induce good enhanced biological phosphate removal performance reported [9]. The carbon, i.e. glucose is oxidized to gluconate, which is converted into other compounds, such as 2-keto-3-deoxygluconate, pyruvate or glyceraldehydes was reported [10] and [11]. The results of [10] suggested that the presence of organic acids (formate) and the mechanism such as the release of protons associated with biological ammonium assimilation that enhances the utilization of phosphates.

Growth of Bacterial Cultures: From the experimental study, the maximum growth was observed in consortium (1.1428 OD) after 72 h in MSM with 0.5% lactose. The bacterial consortium of A+B+C combination showed maximum phosphate removal of 92.5%. The phosphate was taken up by cells for growth and to reform polyphosphate under aerobic condition. Increase in biomass concentrations showed a greater phosphate uptake capacity. This was attributed to an increase in the nutrient utilization rate of the polyphosphate organisms. The MSM medium enriched with carbon source at the experimental concentration greatly influenced the growth of bacteria and enhances the efficiency of phosphate removal.

pH Change of the Medium: The consortium showed maximum phosphate removal of 92.5% with pH change of the culture medium from 7.2 to 5.9. The reduction in pH may be due to the production of various organic acids by the phosphate reducers in the culture medium. Similar observations of the previous reports mentioned that the phosphate utilizing microorganisms produced various organic acids and consequently a fall in pH of the medium [12] and [13]. Reports suggested that the presence of organic acids (formate) release protons which involves in biological ammonium assimilation that enhances the utilization of phosphates [10].

The individual strain of *Pseudomonas* sp showed maximum phosphate removal of 68.2% with pH change from 7.2 to 6.0 after 72h in 0.5% glucose as the carbon source. The culture medium, pH 6.0 was favored for acid phosphatase secretion was reported [14]. *Burkholderia cepacia* maximum phosphate removal and accumulation of polyphosphate at pH 5.5 reported [15]. In contrast, high phosphate utilization was observed without detectable pH change [16].

Phosphate Removal in MSM and Synthetic Phosphate Solution by Phosphate Reducers: Microbes play a central role in the natural phosphorus cycle on a global scale. Biological phosphorus removal techniques are based on the principle that, given optimal conditions, some heterotrophic bacteria in the activated sludge biomass are able to remove solubilized phosphates by accumulating them intracellularly in the form of polyphosphates. These bacteria use the stored carbon reserves to produce energy for growth and to replenish their stores of polyphosphate. The result is a net removal of phosphate from the wastewater.

There is some specific phosphorus accumulating organisms in the active biomass that are responsible for EBPR, which under certain conditions accumulate large amounts of phosphate as intracellular polyphosphate. The results showed that the strain could grow rapidly and remove phosphate efficiently in MSM when compared to synthetic phosphate solution with 0.5% carbon source. This may be the influence of various mineral salts (magnesium sulphate, sodium acetate, potassium nitrate) present in MSM when compared to synthetic phosphate solution. The genes and proteins of microbial cells involved in the hydrolysis of organic phosphates were observed [17]. Some microorganisms can accumulate phosphate as polyphosphate [18] and [19].

In conclusion, the plate screening method of minimum inhibitory concentration (MIC) test and shake flask culture study performed for analysing growth, pH change and change in total phosphate concentration after biotreatment were proved to be effective, easy and reliable method of screening the phosphate reducing cultures. The results from this study indicates that the mineral salts medium with carbon sources showed maximum phosphate removal when compared to synthetic phosphate solution (with and without carbon and other nutrient sources). The bacterial consortium (*Bacillus* sp, *Pseudomonas* sp and *Enterobacter* sp) used in this study efficiently removed the phosphate. The phosphate could be reduced below the permissible limit as prescribed by Environmental Protection Agency (EPA, 1991) within 72hrs using lactose carbon source and could be useful to remediate waste water containing phosphate. The efficient removal of phosphate by the consortium may due to the synergistic activity among the individual strains. The various mineral salts present in the MSM may influence the growth of the phosphate reducers and utilize the phosphate compound when compared to synthetic phosphate solution. Therefore, the mineral salts medium with carbon source support the removal of phosphate at higher level. Thus, the simple method of phosphate removal is possible microbial strains viz., *Bacillus* sp, *Pseudomonas* sp and *Enterobacter* sp may use the contaminants as nutrient and as energy source or it may be degraded by co-metabolism. Hence these microorganisms could be used in the remediation of phosphate contaminated environments.

REFERENCES

1. Pradyot, P., 1997. Hand book of Environmental Analysis, Chemical pollutants in Air, Water, Soil and Solid wastes. Lewis Publishers, CRC Press. Inc., pp: 233.
2. Van Loosdrecht, M.C.M., C.M. Hooijomans, D. Brdjanovic and J.J. Heijnen, 1997. Biological phosphate removal processes. 1997. Mini-Review. Appl. Microbiol. Biotechnol., 48: 289-296.
3. Buchanan, R.E. and N.E. Gibbons, 1994. Bergey's Manual of Determinative Bacteriology, 9th ed. William and Wilkins Publishers, Baltimore.
4. Saxena, M.M., 1994. Environmental Analysis - Water, Soil and Air. Agro Botanical Publishers (India).
5. Illmer, P. and F. Schinner, 1992. Solubilization of inorganic phosphates by microorganisms isolated from forest soils. Soil Biol. Biochem., 24: 389-395.
6. Illmer, P., A. Barbato and F. Schinner, 1995. Solubilization of hardly-soluble aluminium phosphate with phosphorus solubilizing microorganism. Soil Biol. Biochem., 27(3): 265-270.
7. Malacinski, G.M. and W.A. Konetzka, 1967. Orthophosphite-nicotinamide adenine dinucleotide oxidoreductase from *Pseudomonas fluorescens*. J. Bacteriol., 93: 1906-1910.
8. Bitton, G., 1994. Wastewater Microbiology. John Wiley and Sons, Inc., Publication; USA., 3: 63-65.
9. Jeon, C.O. and J.M. Park, 2000. Enhanced phosphorus removal in a sequencing batch reactor fed with glucose as a sole carbon source. Water Res., 34: 2160-2170.
10. Kim, K.Y., D. Jordan and G.A. Mcdonald, 1998. *Enterobacter agglomerans*, phosphate solubilizing bacteria and microbial activity in soil: effect of carbon sources. Soil Biol. Biochem., 30: 995-1003.
11. Reyes, I., L. Bernier, R. Simard and H. Antoun, 1999. Effect of nitrogen source on the solubilization of different inorganic phosphates by an isolate of *Penicillium rugulosum* and two UV- induced mutants. FEMS Microbiol. Ecol., 28: 281-290.
12. Kundu, B.S., 1984. A.C. Gaur. Rice response to inoculation with N- fixing and p-solubilizing microrganims. Plant and Soil, 79: 227-234.

13. Satar, M.A. and A.C. Gaur, 1984. Solubilization of insoluble and fixed phosphates in crop rhizobium by phosphomicroorganisms. Proc. Int. Symposium on soil test crop responses correlation studies. Internaional congress meeting of Soil Sci., Feb., 7-10, Dhaka, pp: 237-251.
14. Bouquet, P.L., C. Manoil and J. Beckwith, 1987. Use of Tn pho A to detect genes for exported protein in *Escherichia coli*. Identification of the plasmid encoded gene for a periplasmic acid phosphatase. J. Bacteriol., 169: 1663-1669.
15. Mullan, A., J.P. Quinn and J.W. McGrath, 2002. Enhanced phosphate uptake and polyphosphate accumulation in *Burkholderia cepacia* grown under low-pH conditions. Microbial Ecol., 44: 69-77.
16. Asea, P.E.A., R.M.N. Kucey and J.W.B. Stewart, 1988. Inorganic phosphate solubilization by two *Pencillium* sp. In solutions culture and soil. Soil Biology and Biochemistry, 20: 459-464.
17. Torriani-Gorini, A., 1987. The birth and growth of the pho regulation. In phosphate metabolism and regulation in microorganisms. American Society for Agronomy press, Madison, pp: 3-11.
18. Kornberg, A., 1995. Inorganic polyphosphate: toward making a forgotten polymer unforgettable. J. Bacteriol., 177: 491-496.
19. Keasling, J.D. and G.A. Hupf, 1996. Genetic manipulation of polyphosphate metabolism affects cadmium tolerance in *Escherichia coli*. Appl. Environ. Microbiol., 62: 743-746.