Optimizing effects of various cultural conditions on efficiency of phosphate solubilization by *Aspergillus awamori*

Parmar Himanshu[1]*, Solanki Vikram[2] and Bhatt Shreyas[2]
1. Vrundavan Science College, Ganeshpura, Mehsana, Gujarat, INDIA
2. Department of Life Sciences, Hemchandracharya North Gujarat University, Patan, Gujarat, INDIA
*himanshuparmarr@gmail.com

Abstract

Phosphate solubilizing microbes possess the characteristic to convert the fixed form of phosphorus to available form for plants to absorb phosphorus from soil. Phosphorus is essential for storage and transfer of energy, photosynthesis as well as biochemical and genetic activities of plant. Various culture conditions like incubation time, inoculum size, temperature, pH, phosphate source, carbon source, nitrogen source, tricalcium phosphate (TCP) concentration and glucose concentration were optimized to obtain maximum phosphate solubilization by Aspergillus awamori fungi isolated from Bt-cotton rhizospheric soil.

Phosphate solubilizing efficiency of Aspergillus awamori was tested in PVK media with 0.5% tricalcium phosphate as phosphate substrate. Aspergillus awamori showed maximum phosphate solubilization at 6.5 pH of the medium incubated with 5% of inoculum size for 8 day incubation at 30 ^{o} C temperature. Aspergillus awamori showed maximum phosphate solubilization medium containing 2% of glucose as a sole source of carbon, $(NH_4)_2 SO_4$ as a nitrogen source and 0.7 % of tricalcium phosphate (TCP) as phosphate source.

Keywords: *Aspergillus* Sp., Optimization, BT, Biofertilizer and Fungi.

Introduction

Phosphate solubilizing microbes possess the characteristic to convert the fixed form of phosphorus to available form for plants to absorb phosphorus from soil[13].

Several studies have been made to find out the ability of fungi; mainly of genus *Aspergillus* and *Penicillium* that solubilize various forms of phosphate[15,16]. *Aspergillus spp.* is consisting dominant species capable of phosphate solubilization, siderophore production as well as indole acetic acid production[7,9,16].

In the present study, incubation time, inoculum size, pH, temperature, phosphate sources, carbon sources, nitrogen sources, TCP concentration and glucose concentration were optimized to enhance phosphate solubilization by potent strains *Aspergillus awamori.*

Material and Methods

Quantitative estimation of phosphate: Fungal strains were isolated from Bt-cotton rhizospheric soil and selected for optimization of phosphate solubilization. Phosphate solubilizing efficiency of *Aspergillus awamori* was tested in PVK media with 0.5% tri calcium phosphate as phosphate substrate. Flasks were inoculated with 8% v/v (1.5×10^7) of fungal cultures and incubated on shaker at 28^{o}C for 6 days at 100 rpm. After incubation, the fermented broth was centrifuged at 10,000 rpm for 15 min, the pH of the supernatant was measured and dissolved phosphate concentration in supernatant was determined by Vanado-Molybdate method [1], phosphate concentration was expressed in terms of µg ml^{-1} of phosphate released in culture.

Effect of various cultural conditions on efficiency of phosphate solubilization by *Aspergillus awamori*: Different cultural conditions were optimized for maximum phosphate solubilization by *Aspergillus awamori.*

Effect of incubation time: The study was carried out to optimize the incubation time for maximum phosphate solubilization. 100 mL of PVK broth was prepared, it was autoclaved at 121^{o}C for 15 lb and maintained for 15 min. Then it was inoculated with 8% (v/v) inoculum of *Aspergillus awamori*. Both flasks were incubated in shaker for 28^{o}C at 100 rpm after regular time interval (24 h) from 1 day upto 10 day for fungi and 7 days for bacteria. Then fermented broth was withdrawn, centrifuged and pH was checked. Dissolved phosphate concentration in supernatant was determined by Vanado-Molybdate method[1].

Effect of inoculum size: To determine the volume of inoculum required for maximum phosphate solubilization, PVK broth was inoculated with growing active pure culture of *Aspergillus awamori*, inoculum size ranging from 4 to 10% (v/v). Efficiency of phosphate solubilization was measured as described above.

Effect of temperature: To study the effect of different temperature on phosphate solubilization, the optimized parameter uptill were kept constant and the flasks were inoculated with fungal cultures separately and incubated at different temperatures i.e. 24^{o}C, 28^{o}C, 30^{o}C, 32^{o}C, 36^{o}C and 40^{o}C for fungi at 100 rpm. The amount of phosphate released was checked from Pikovskaya's broth after incubation period.

Effect of pH: In the present study, the effects of different pH ranging from 5.0 to 9.0 were studied for maximum phosphate solubilization. To check effect of different pH on phosphate solubilization, the optimized incubation time, inoculum size and temperature were keep constant for *Aspergillus awamori*. The pH that gave maximum phosphate solubilization was utilized and exploited to optimize other parameters.

Effect of phosphate sources: After having optimized incubation time, inoculum size, temperature and pH, effect of various phosphate sources i.e. $Ca_3 (PO_4)_2$, $AlPO_4$ and $FePO_4$ were used to study their effects on maximum phosphate solubilization by fungal isolates. The optimized parameters uptill now were kept constant for both isolates. The broth was inoculated, incubated and then checked for amount of phosphate released.

Effect of carbon sources: In the present study, different carbon sources i.e. sugars like glucose, sucrose, fructose, lactose and galactose were used to check their effect on phosphate solubilization. The optimized parameters were kept as for *Aspergillus awamori*. The PVK broth was inoculated, incubated and then checked for amount of phosphate released.

Effect of nitrogen sources: For medium optimization study, various inorganic and organic nitrogen sources i.e. $(NH_4)_2SO_4$, $NaNO_3$, urea and casein were used to check their effect on phosphate solubilization. To check effect of various nitrogen sources on phosphate solubilization, the optimized parameters uptil now were keep constant. The amount of phosphate released was checked from each flask after completion of incubation period.

Effect of different TCP concentration: After having optimized incubation time, inoculum size, temperature and pH, phosphate sources, carbon sources, nitrogen sources and effect of tri calcium concentration ranging from 0.3 to 0.8% (w/v) were used to check their effects on maximum phosphate solubilization by fungi. The optimized parameters were kept as for *Aspergillus awamori*. Efficiency of phosphate solubilization was measured as described above.

Effect of different glucose concentration: For medium optimization study, glucose concentration ranging from 0.5 to 3% was used to check their effects on maximum phosphate solubilization by fungi. To check the effect of different glucose concentration on phosphate solubilization, the optimized parameters uptill now were kept constant for fungal strain. The amount of phosphate released was checked from each flask after completion of incubation period.

Results and Discussion

Effect of Incubation time: The incubation period plays a significant role in phosphate solubilizing activity of microorganisms. It varies from organism to organism. It may be in hours, days or sometime in weeks or more than that. *Aspergillus awamori* showed 561 ± 7.47µg mL^{-1} of phosphate solubilization after 6 days in unoptimized condition. To investigate the effect of incubation time on phosphate solubilization, the samples were checked for phosphate solubilization at regular time interval (24 h) starting from 1 day upto 10 days. *Aspergillus awamori* showed optimum phosphate solubilization when it was in logarithmic phase of growth curve.

Aspergillus awamori showed 589 ± 5.85 µg mL^{-1} of phosphate solubilization after 8 days, after then phosphate solubilization was slightly decreased (Figure 1). There are some evidences which showed that incubation time varies from genus to genus and species to species for optimum phosphate solubilization. Chadha et al[3] reported that phosphate solubilizing fungi showed maximum phosphate solubilization after 8 days. Darmwal et al[4] proved that *A. niger* was found to be the best phosphate solubilizer among several tested fungi and bacteria and maximum amount of phosphate solubilization reached after 7 to 10 days.

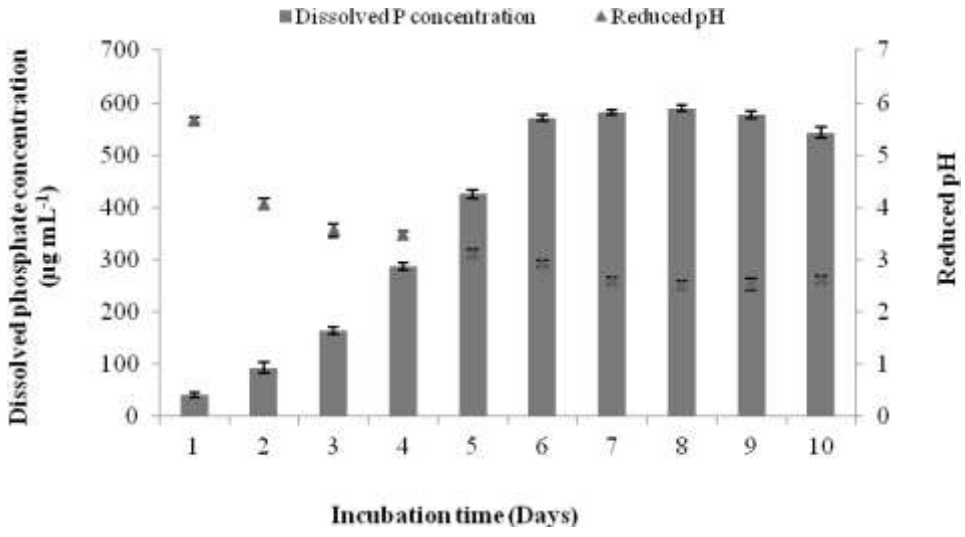

Figure 1: Effect of incubation time on phosphate solubilization by *A. awamori*

Effect of inoculum size: To investigate the optimum inoculum size, the Pikovskaya's broth was inoculated with 4.0 to 10.0% v/v inoculum of *Aspergillus awamori*. *Aspergillus awamori* showed 611 ± 8.18 µg mL^{-1} of phosphate solubilization and pH drop upto 2.45 by inoculum size of 5% (Figure 2). Pradhan and Sukla[10] studied that maximum phosphate was solubilized with 5% (v/v) spore suspension of *A. niger* and *Penicillum spp*.

Effect of temperature: For *Aspergillus awamori*, temperatures like 24ºC, 28ºC, 30ºC, 32ºC, 36ºC and 40ºC were selected for phosphate solubilization. The optimum temperature for *Aspergillus awamori* was 30ºC and phosphate solubilization was 643 ± 5.29 µg mL^{-1} and pH drop was 2.36 (Figure 3).

Wani et al[18] reported that 30ºC was optimum temperature for tri calcium phosphate solubilization by *Aspergillus awamori.* For *A. niger* and *Penicillium spp.*, optimum temperature for phosphate solubilization was 30ºC[10].

Effect of pH: The range of pH 5.0, 6.0, 6.5, 7.0, 8.0 and 9.0 was selected to obtain maximum phosphate solubilization. For *Aspergillus awamori*, pH 6.5 was found optimum which showed 658.7 ± 7.57 µg mL^{-1} of phosphate solubilization and pH of the medium decreased from 6.5 to 2.34 after completion of incubation period (Figure 4). However, acidification does not seem to be the only mechanism of phosphate solubilization, as the ability to reduce the pH in some cases does not correlate well with the ability to solubilize mineral phosphates[8]. Hefnawy et al[5] investigated that maximum rock phosphate and tri calcium phosphate solubilization were observed at pH 6.5 and 7.0 by *A. niger* and *A. fumigates*.

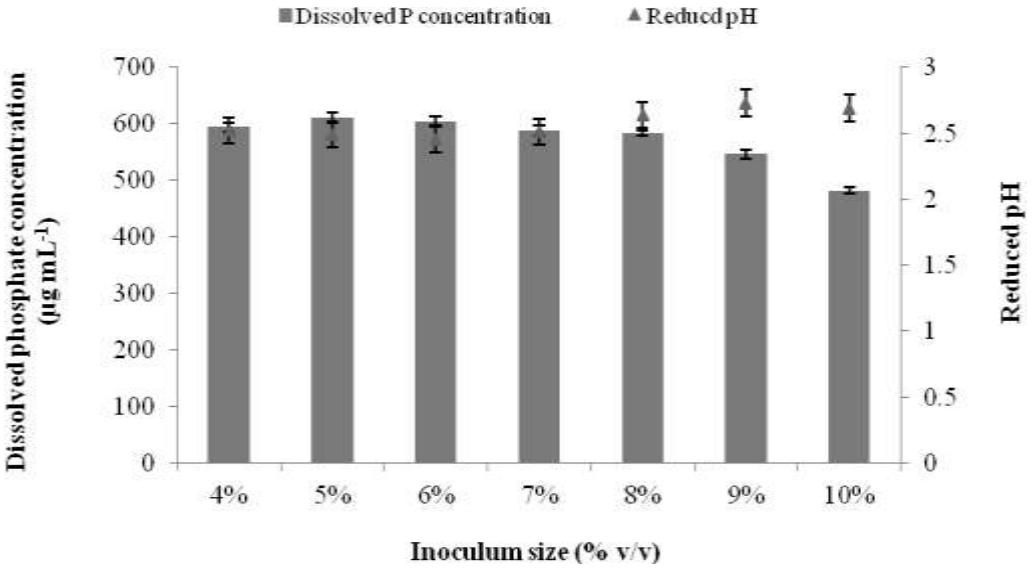

Figure 2: Effect of inoculum size on phosphate solubilization by *A. awamori*

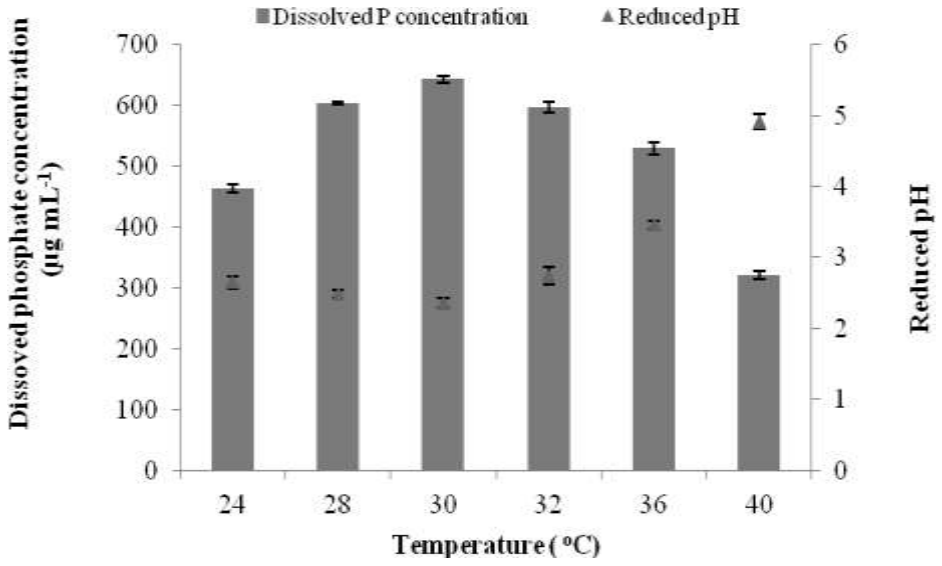

Figure 3: Effect of temperature on phosphate solubilization by *A. awamori*

Effect of phosphate sources: The phosphate solubilization is greatly dependent on phosphate source as substrate. *Aspergillus awamori* was solubilizied maximum phosphate with TCP compared to $FePO_4$ and $AlPO_4$. This may be probably due to the adaptive nature of the enzyme that is responsible for solubilizing Ca_3 $(PO_4)_2$ [2]. *Aspergillus awamori* solubilized 668 ± 7.23 µg mL^{-1} TCP to 2.35 ± 0.08 and pH dropped from 6.5 (Figure 5). There was a very vast difference observed in phosphate solubilization between TCP to other phosphate sources. The majority of the phosphate solubilizing microorganisms mobilizes calcium phosphate complexes and only a few can solubilize iron phosphate and aluminium phosphate complexes.

As a result of acidification of the surrounding medium, soluble orthophosphate ions can be readily released. More precisely, the organic acids secreted can either directly dissolve the mineral phosphate as a result of anion exchange of PO_4^{-3} by acid anion or can chelate both iron and aluminium ions associated with phosphate[8].

Walpola et al[17] investigated that *A. awamori* highly solubilized phosphate using tri calcium phosphate as phosphate source compared to $AlPO_4$, $FePO_4$ and rock phosphate.

Effect of carbon sources: The various carbon sources were used e.g. glucose, fructose, sucrose, lactose and galactose to check their effect on phosphate solubilization by *Aspergillus awamori.* For *Aspergillus awamori*, 637 ± 10.69 µg mL^{-1} of phosphate solubilization was observed with glucose and pH drop was 2.37. The remaining sugars sucrose, fructose, lactose and galactose showed 588.7 ± 9.07, 582 ± 10.92, 291 ± 6.24 and 186 ± 5.03 µg mL^{-1} of phosphate solubilization.

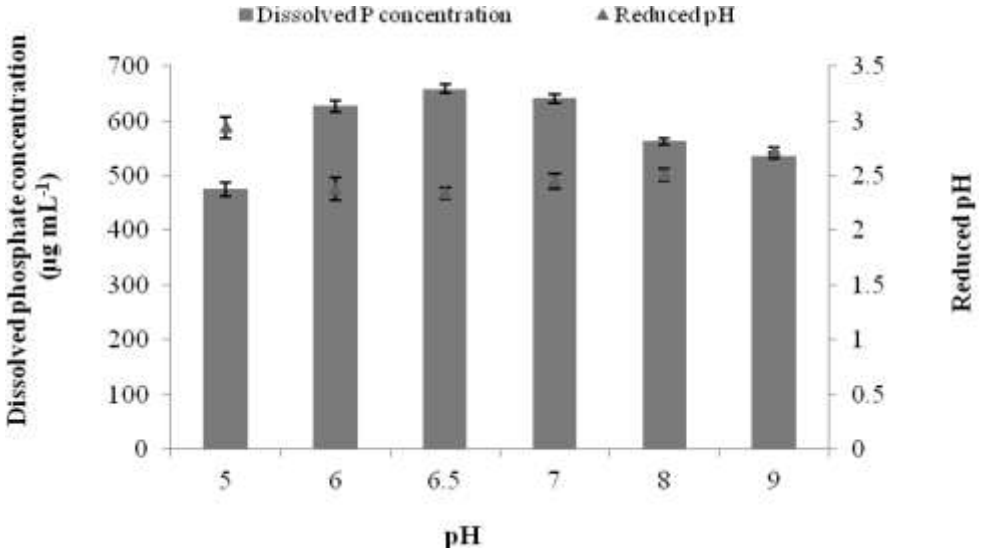

Figure 4: Effect of pH on phosphate solubilization by *A. awamori*

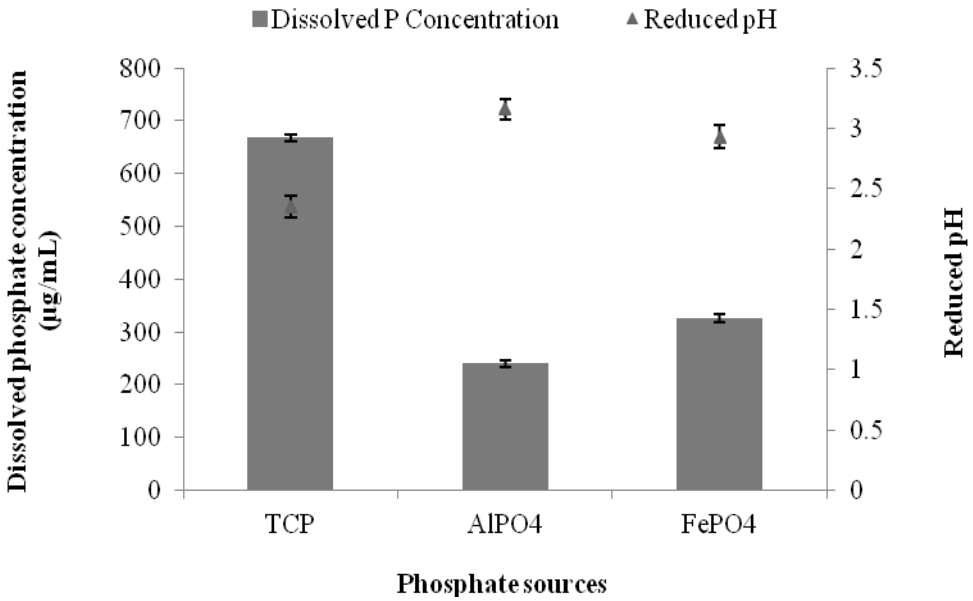

Figure 5: Effect of phosphate sources on phosphate solubilization by *A. awamori*

There was some little difference in phosphate solubilization observed by glucose, sucrose and fructose but there was a vast difference in phosphate solubilization and pH observed by lactose and galactose (Figure 6). Rathore[11] reported that glucose was the best carbon source for phosphate solubilization by *Aspergillus* species. Relwani et al[12] showed that glucose and sucrose were the best sources for phosphate solubilization by *Aspergillus tubingensis*.

Effect of nitrogen sources: The phosphate solubilization is also dependent on nitrogen sources. Different inorganic and organic nitrogen sources e.g. $(NH_4)_2SO_4$, $NaNO_3$, urea and casein are checked for phosphate solubilization of *Aspergillus awamori*. *Aspergillus awamori* showed highest phosphate solubilization (649 ± 6.55 µg mL^{-1}) and pH drop was 2.41 with $(NH_4)_2SO_4$ followed by urea> $NaNO_3$> casein (Figure 7).

Pradhan and Sukla[10] reported that $(NH_4)_2SO_4$ was the best nitrogen source for maximum phosphate solubilization (411 µg mL^{-1}). Illmer and schinner[6] reported the number of fungi solubilized phosphate only in the presence of ammonium as the nitrogen sources.

Effect of TCP concentration: To study the effect of different concentrations of TCP from 0.3 to 0.8% for phosphate solubilization by *Aspergillus awamori*, the highest phosphate solubilization was observed with 0.7% of TCP (714 ± 8.54 µg mL^{-1}) and pH drop was 2.27 for *Aspergillus awamori* (Figure 8). In range of 0.3 to 0.8% of TCP concentration, phosphate solubilization was gradually increased upto optimum level, then there was decrease in phosphate solubilization with further increase in TCP concentration. There was no drastic difference observed in phosphate solubilization by various TCP concentrations.

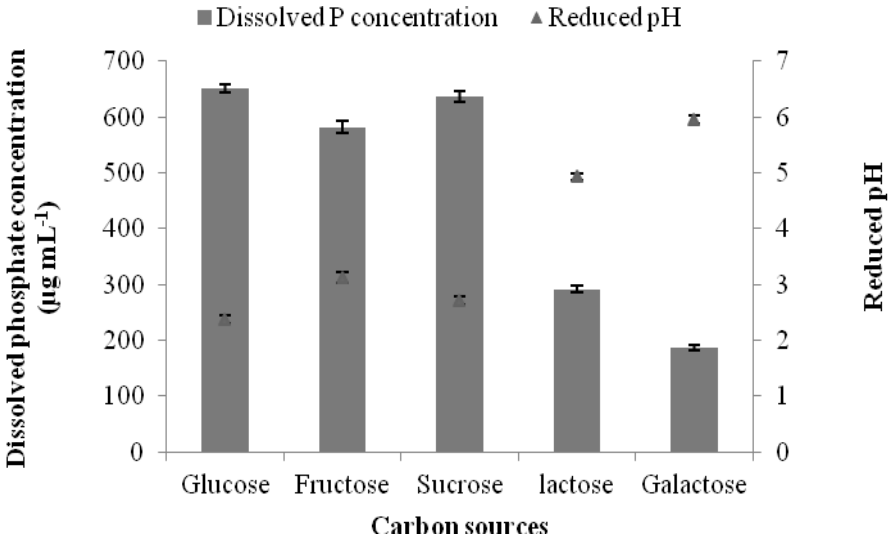

Figure 6: Effect of carbon sources on phosphate solubilization by *A. awamori*

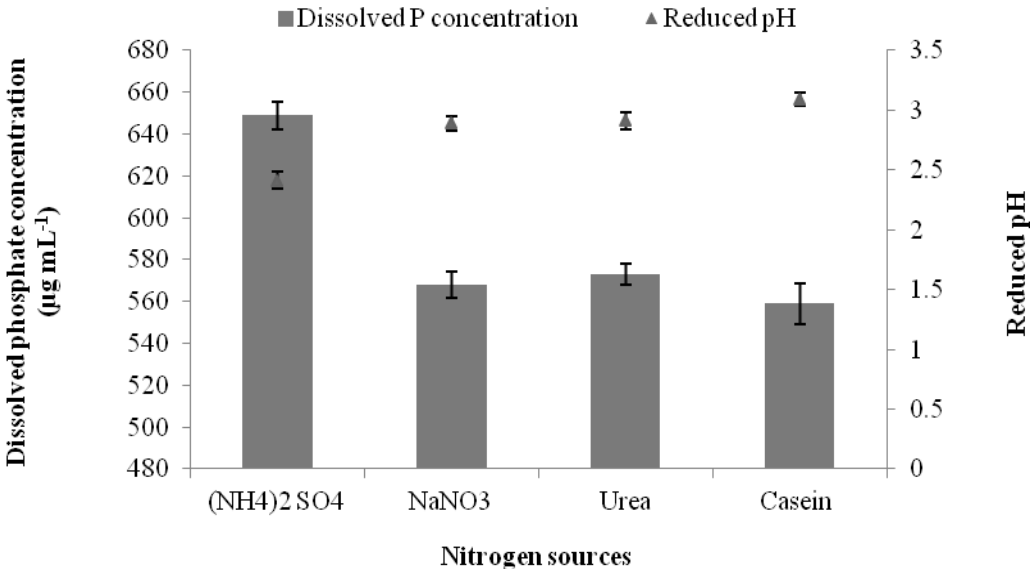

Figure 7: Effect of nitrogen sources on phosphate solubilization by *A. awamori*

Hefnawy et al[5] studied efficiency of rock phosphate and tri calcium phosphate solubilization at different concentrations ranging from 0.5 to 8% and maximum phosphate solubilization activity was observed at 1% of ore concentration by *A. niger* and *A. fumigates*.

Effect of glucose concentration: Scervino et al[14] reported that the concentration and the nature of the carbon source affected phosphate solubilization. In present study glucose concentration like 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 % were selected for phosphate solubilization. Phosphate solubilization was high at 2.0 % glucose concentration and low at 0.5% glucose (Figure 9). For *Aspergillus awamori*, 768 ± 6.08 µg mL^{-1} of phosphate solubilization and pH drop of 2.19 was observed at 2.0% glucose concentration.

Walpola et al[17] reported that *Aspergillus awamori* bxq33110 gave the maximum phosphate solubilization with 2% of glucose concentration.

Conclusion

Aspergillus awamori showed 561 ± 7.47 µg mL^{-1} of phosphate solubilization under unoptimized conditions. *Aspergillus awamori*a fungal isolates showed maximum phosphate solubilization at different culture conditions like 8 days incubation time, 5% inoculum size, 30°C temperature, pH 6.5, TCP as a phosphate source, $(NH_4)_2SO_4$ as a nitrogen source, glucose as a carbon source, 0.7% TCP concentration and 2% glucose concentration. After completion of optimization study, *Aspergillus awamori* showed 768 ± 6.08µg mL^{-1} of phosphate solubilization.

Moreover, this study makes the *Aspergillus awamori* attractive phosphate solubilizer and might be used as biofertilizers to increase the available phosphorus in soil, to minimize chemical fertilizers and to reduce environment pollution in order to achieve a sustainable agriculture.

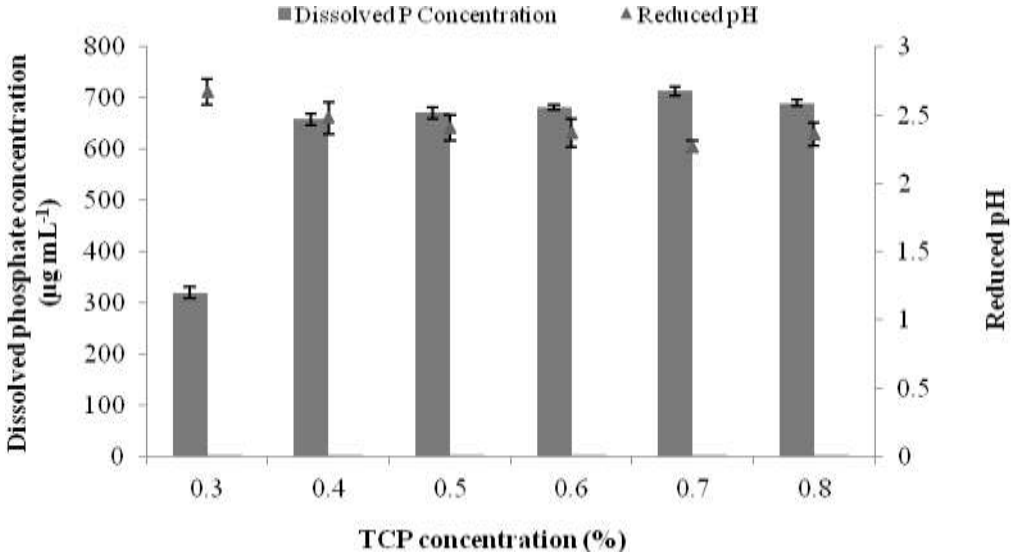

Figure 8: Effect of different concentrations of TCP on phosphate solubilization by *A. awamori*

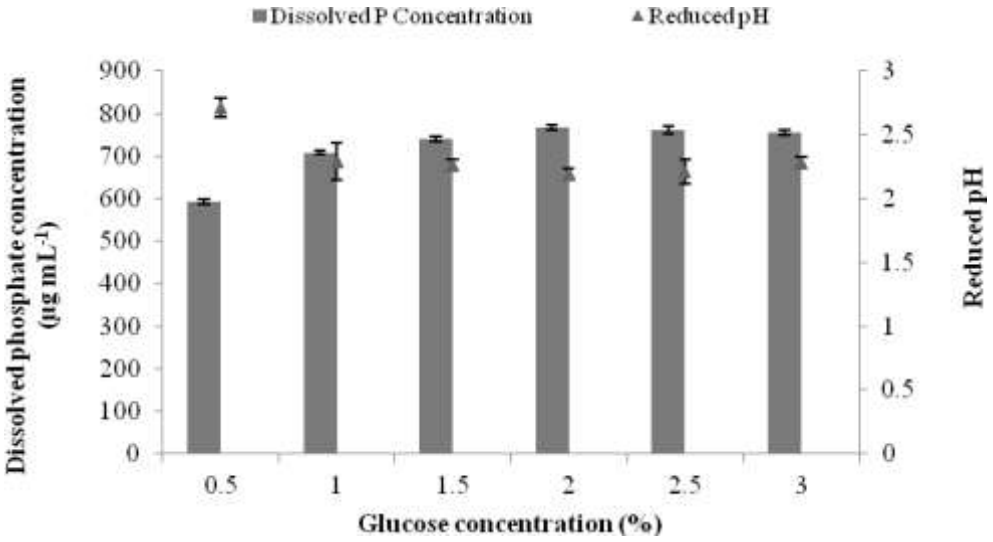

Figure 9: Effect of different concentrations of glucose on phosphate solubilization by *A. awamori*

Acknowledgement

We are thankful to the Principal of Biogas Research and Extension Centre, Gujarat Vidyapeeth, Sadra, Gandhinagar, Gujarat for providing research facility to carry out present work.

References

1. APHA, Standard methods for the examination of water and waste water, American Public Health Association, Washington, D.C. **(1995)**

2. Banik S. and Dey B.K., Available phosphate content of an alluvial soil as influenced by inoculation of some isolated phosphate-solubilizing microorganisms, *Plant and Soil*, **69(3)**, 353-364 **(1982)**

3. Chadha N., Prasad R. and Varma A., Plant promoting activities of fungal endophytes associated with tomato roots from central Himalaya, India and their interaction with *Piriformospora indica*, *International Journal of Pharmacy and Bio Sciences*, **6(1)**, 333-343 **(2015)**

4. Darmwal N.S., Singh R.B. and Rai R., Isolation of phosphate solubilizers from different sources, *Current Science*, **58**, 570-571 **(1991)**

5. Hefnawy M.A., Gharieb M.M., Eissa O.A. and Ammar A.M., Evaluation and optimization of rock phosphate and tricalcium phosphate solubilization by some soil fungi, *The Egyptian Society of Experimental Biology*, **5**, 75-84 **(2009)**

6. Illmer P. and Schinner F., Solubilization of inorganic calcium phosphate solubilization mechanisms, *Soil Biology and Biochemistry*, **27(3)**, 257-263 **(1995)**

7. Milagres A.M.F., Machuca A. and Napoleao D., Detection of siderophore production from several fungi and bacteria by a modification of chrome azurol S (CAS) agar plate assay, *Journal of Microbiological Methods*, **37(1)**, 1-6 **(1999)**

8. Muleta D., Microbial inputs in coffee (*Coffea Arabica L.*) production system, Southwestern Ethiopia, Doctoral thesis, Uppsala, Sweden, 21-23 **(2007)**

9. Nath R., Sharma G.D. and Barooah M., Plant growth promoting endophytic fungi isolated from tea (*Camellia sinensis*) shrubs of Assam, India, *Applied Ecology and Environmental Research*, **13(3)**, 877-891 **(2015)**

10. Pradhan N. and Sukla L.B., Solubilization of inorganic phosphates by fungi isolated from agricultural soil, *African Journal of Biotechnology*, **5(10),** 850-854 **(2005)**

11. Rathore P., Role of different carbon sources in phosphate solubilization, *International Journal of Scientific Research*, **3(2)**, 457-458 **(2014)**

12. Relwani L., Krishna P. and Reddy M.S., Effect of carbon and nitrogen sources on phosphate solubilization by a wild-type strain and UV-induced mutants of *Aspergillus tubingensis*, *Current Microbiology*, **57(5),** 401-406 **(2008)**

13. Rodriguez H. and Fraga R., Phosphate solubilizing bacteria and their role in plant growth promotion, *Biotechnology Advances*, **17**, 319-339 **(1999)**

14. Scervino J.M., Papinutti V.L., Godoy M.S., Rodriguez M.A., Monica I.D., Recchi M., Pettinari M.J. and Godeas A.M., Medium pH, carbon and nitrogen concentrations modulate the phosphate solubilization efficiency of *Penicillium purpurogenum* through organic acid production, *Journal of Applied Microbiology*, **110(5),** 1215-1223 **(2011)**

15. Seshadri S., Ignacimuthu S. and Lakshminarasimhan C., Effect of nitrogen and carbon sources on the inorganic phosphate solubilization by different *Aspergillus niger* strains, *Chemical Engineering Communications*, **191(8)**, 1043-1052 **(2004)**

16. Wakelin S.A., Warren R.A., Harvey P.R. and Ryder M.H., Phosphate solubilization by *Penicillium spp.* closely associated with wheat roots, *Biology and Fertility of Soils*, **40(1),** 36-43 **(2004)**

17. Walpola B.C., Song J.S., Jang K.Y. and Yoon M.H., Stress induced phosphate solubilization by *Aspergillus awamori* bxq33110 isolated from waste mushroom bed of *Agaricus bisporus, Korean Journal of Soil Science Fertilizer*, **45(3)**, 428-434 **(2012)**

18. Wani P.V., More B.B. and Patil P.L., Physiological studies on the activity of phosphate solubilizing microorganisms, *Indian Journal of Microbiology*, **19(1)**, 23-25 **(1979)**.

(Received 04th March 2021, accepted 08th May 2021)