British Microbiology Research Journal
3(3): 378-392, 2013

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Enhancing Bio-Available Phosphorous in Soil Through Sulfur Oxidation by *Thiobacilli*

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Authors' contributions

This work was carried out in collaboration between all authors. Author IU performed all experiments, managed the literature searches and wrote the draft of the manuscript. Authors GJ and MIH designed and supervised the whole study while author AK assisted in analytical work. All authors read and approved the final manuscript.

Research Article

Received 25th March 2013
Accepted 30th May 2013
Published 16th June 2013

ABSTRACT

Aims: The objectives were to evaluate the phosphate solubilization efficiency of different *Thiobacilli* strains and to find out the best combination of sulfur and *Thiobacilli* for enhancing bio-available P in soil.
Study Design: An experimental study.
Place and Duration of Study: Microbiology and Soil Fertility Labs, Department of Soil Science and Soil and Water Conservation, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan and Microbiology and Soil Chemistry Labs, Auriga Research Center, Lahore, Pakistan, between May 2011 and November 2012.
Methodology: Fifty *Thiobacilli* strains were isolated from ten different ecologies. Then an incubation study of soil was performed wherein the most efficient four *Thiobacilli* strains were inoculated in combination with three different levels of elemental sulfur to determine pH, water soluble sulfur, sequential P fractions and bio-available phosphorous contents in the incubated soil.
Results: All the four *Thiobacillus* strains (IW16, SW2, IW1 and IW14) dropped pH of the incubated soil along with three doses of S° (50, 75 and 100 kg ha^{-1}). However, *Thiobacillus* strains IW16 and SW2 reduced soil pH quite sharply from 7.90 to 7.12 (net reduction of 0.78 points) and 7.28 (net reduction of 0.62 points) respectively where inoculated with S° @ 100 kg ha^{-1}. The best P solubilizer was *Thiobacillus* strain IW16 and the best dose of S°

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was @ 100 kg ha^{-1} and their combination enhanced maximum quantity of P (22.26 mg kg^{-1}) in the soil by solubilizing already present insoluble calcium bounded P fractions like octa-calcium phosphate (Ca8-P) and apatite (Ca10-P).
Conclusion: The present study suggests the use of *Thiobacilli* along with elemental sulfur for the dissolution and enhancement of bio-available P in alkaline and calcareous soils.

Keywords: Thiobacillus; sulfur oxidizing bacteria; sulfur; sulfur oxidation; phosphate solubilization.

1. INTRODUCTION

World's increasing population is demanding amplified agricultural production that is directly linked with soil phosphorous (P). Therefore, day by day soil P availability is getting more and more attention [1,2]. Phosphorus is an essential plant nutrient, which plays a vital role in crop production because plant's need for sufficient amount of P immediately starts as the plant growth begins [3]. Cultivated soils contain high quantity of total P ranging from 400-3,000 mg kg^{-1} [4] but the concentration of plant available P is as low as 1.0 mg kg^{-1}soil [5] which is unable to cope with the plant requirements due to the occurrence of P in calcium bounded plant unavailable forms in alkaline and calcareous soils. Furthermore, the efficiency of P fertilizers is very low (10 to 25 %) throughout the world [6]. Phosphorous after application immediately gets fixed in the soil and becomes unavailable to the plants. Major reasons for P fixation are oxides and hydroxides of iron in acidic soils and high amount of $CaCO_3$ in alkaline and calcareous soils [7]. Pakistani soils are mostly alkaline and calcareous in nature (pH > 7.5 and $CaCO_3$ > 3.0 %) having a serious problem of P fixation [8].

Sulfur oxidizing bacteria (SOB) oxidize elemental S° into sulfates to fulfill their energy requirements [9]. The genus *Thiobacillus* among SOB is very effective in biological S oxidation in soil [10]. Bacterial S oxidation mechanism generates huge quantity of sulfuric acid which attacks on insoluble calcium bounded P minerals like fluorapatite ($Ca_5(PO4)_3F$), calcite ($CaCO_3$) and lime (CaO) and converts them into soluble and plant available P forms [11-13]. Phosphate solubilization through bacterial S oxidation phenomenon is confirmed very effective [14]. Biological sulfur oxidation by *Thiobacilli* releases phosphate from rock phosphate [12,15] which has high positive correlation with the quantity of bacterially generated sulfuric acid [16]. However, there is insufficient information about phosphate solubilization in alkaline and calcareous soils through bacterial S oxidation.

Different inorganic fractions of P and especially the calcium bounded P fractions are very important for plant growth because these are directly or indirectly the major source of plant available P in soil. Phosphorous fractionation helps in getting knowledge and better understanding about different P sources and pools in the soil system [17,18]. Therefore, it is very crucial to have information about the relationship between different inorganic fractions of P and their solubilzation behavior by the interactive effect of *Thiobacillus* spp. and S° application in soil. Moreover, mechanism and rate of phosphate solubilization by *Thiobacillus* spp. through sulfur oxidation in soil are also very important. Therefore, the current study was planned to examine the influence of *Thiobacilli* and different doses of elemental S° to get their best combination for enhancing P bioavailability in soil.

2. MATERIALS AND METHODS

2.1 Isolation, Characterization and Identification of Sulfur Oxidizing Bacteria

Sulfur oxidizing bacteria (SOB) were isolated from the samples collected from ten different ecologies viz., paddy fields (PF), wheat rhizosphere (WR), sugarcane rhizosphere (SR), maize rhizosphere (MR), industrial wastewater (IW), canal water (CW), sulfur mud (SM), sewage water (SW), industrial waste sludge (IS) and sewage sludge (SS). The composition of the thiosulfate medium used for isolation of SOB was:

$Na_2S_2O_3$, 5.0 g; K_2HPO_4, 0.1 g; $NaHCO_3$, 0.2 g; NH_4Cl, 0.1 g dissolved in 1.0 L distilled water. The pH of the medium was adjusted at 8.0 and the indicator used was bromo cresol purple. Change in colour from purple to yellow indicated the growth of SOB in the medium [19].

Fifty obtained isolates were purified and labeled according to their sampling ecologies. Four the best SOB isolates (IW14, IW1, IW16 and SW2) were selected on the basis of their efficiency to reduce pH and sulfates production in the growth medium [20,21]. Then different physiological, morphological and biochemical characteristics of the selected four SOB strains were studied [22-26].

2.2 Inoculation of *Thiobacilli* with Different Sulfur Levels for Enhancing Bio-Available Phosphorous in Soil

An incubation experiment with soil (1.0 kg soil / plastic bag) was conducted in completely randomized design (CRD) with three replications. Two factor treatments viz., (i) three levels of sulfur, and (ii) four strains of the most efficient *Thiobacillus* spp. (1 mL of 10^6 cells fresh culture g^{-1} S°) for P solubilization were employed. Facotor 1 consisted of (i) S_0 no sulfur (ii) S_1 50 kg S° ha^{-1} (iii) S_2 75 kg S° ha^{-1} and (iv) S_3 100 kg S° ha^{-1}. Factor 2 contained (i) control (No *Thiobacilli* spp. inoculation) (ii) *Thiobacillus* strain IW14 (iii) *Thiobacillus* strain IW1 (iv)*Thiobacillus* strain SW2 and (v) *Thiobacillus* strain IW16.

Basic soil analyses before incubation was performed. Then the soils with two factor factorial treatment combinations were incubated at 30 °C for 90 days. Soil samples were drawn after 30, 60 and 90 days for the determination of pH, water soluble sulfur, sequential P fractions and bio-available phosphorous contents in the incubated soil.

Metrohm High-precision 780 pH meter was used to determine pH and the amount of bio-available P in the soil was determined through Mo-blue method [27]. Sequential extraction of P was carried out according to the procedure given by Jiang and Gu [28] for the determination of various inorganic P fractions in calcareous soil. Total P was determined by digesting the soil with $HClO_4$ method [29]. The concentration of water soluble sulfates in the soil was determined by ion chromatography (conductivity detector L-2470, pump L-2130, column oven L-2350) as described by Oh *et al.* [30].

2.3 Procedure for Phosphate Dissolution from Soil by Bacterial Sulfur Oxidation

The added S° was oxidized by *Thiobacillus* strains to produce sulfuric acid. This sulfuric acid attacked on the insoluble calcium bounded minerals to form gypsum and phosphoric acid

[31]. Phosphoric acid reacted again with insoluble calcium bounded minerals to covert them into plant available calcium dihydrogen phosphate. These reactions are shown by the following chemical equations [16].

$$S^{\circ} + 1.5\ O_2 + H_2O \xrightarrow{\textit{Thiobacilli}} H_2SO_4$$

$$Ca_5(PO_4)_3F\ + 5H_2SO_4 + 10H_2O \longrightarrow 3H_3PO_4 + 5CaSO_4{\cdot}2H_2O\ +\ HF$$

(Fluorapatite -Insoluble calcium bounded P)

$$Ca_5(PO_4)_3F\ + 7H_3PO_4 \longrightarrow 5Ca(H_2PO_4)_2\ +\ HF$$

(Soluble calcium dihydrogen phosphate)

2.4 Statistical Analysis

Variance in pH, water soluble sulfur contents, sequential P fractions and bio-available phosphorous in the incubated soil were statistically analyzed using MSTAT-C software [32] taking *Thiobacilli* and different levels of sulfur amendment as source of variance. Simple linear correlation and regression were determined through MS Excel to evaluate the extent of interrelationship and interdependence among various variables.

3. RESULTS

3.1 Physiochemical Characteristics of the Experimental Soil

Different physiochemical properties of the soil used in the incubation study are presented in Table 1 which reveals that the soil was sandy clay loam, non saline, alkaline and calcareous in nature with EC_e 1.02 dS m^{-1}, pH 7.90 and $CaCO_3$ 7.14 % [33]. The concentration of total and plant available P in the soil was 664.74 mg kg^{-1} and 4.52 mg kg^{-1} respectively. The quantity of total P in soil was very high, however, the amount of plant available P was very low and came under deficient category [34,35]. Amongst the six P fractions determined in the fractionation process, di-calcium phosphate (Ca2-P) was the smallest fraction amounting 3.53 mg kg^{-1} (0.53 % of the total soil P) while Apatite (Ca10-P) fraction was the biggest fraction with values of 307.30 mg kg^{-1} constituting 40.23 % of the total P and 65.03 % of the total inorganic P in the soil. Total inorganic P (sum of all six fractions) was 472.48 mg kg^{-1} which constituted 71.08 % of the total P, out of which 401.28 mg kg^{-1} (84.93 % of the total inorganic P and 60.37 % of the total soil P) was calcium bounded P. Total soil organic P was calculated as 192.26 mg kg^{-1} (28.92 % of the total soil P). The amount of total S in the experimental soil was recorded as 214.47 mg kg^{-1} which was quite adequate but the concentration of water soluble sulfate was noted as 9.60 mg kg^{-1} and it was deficient (< 10 mg kg^{-1}) for plant growth [36].

Table 1. Physiochemical Characteristics of the Experimental Soil

Particulars	Value	Method
Clay (%)	18.87	Boyoucos Hydrometer method [37]
Sand (%)	71.59	
Silt (%)	9.54	
ECe (dS m^{-1})	1.02	Electrical condicutivty meter [37]
pH (1:1:: Soil : Water)	7.90	Metrohm High-precision 780 pH meter [37]
O.M (%)	0.42	Potassium dichromate method [37]
$CaCO_3$ (%)	7.14	CH_3COOH consumption mthod [38]
Total phosphorous (mg kg^{-1})	664.74	Olsen and Sommers method [29]
Available phosphorous (mg kg^{-1})	4.52	Olsen method [27]
Di-calcium phosphate (Ca2-P mg kg^{-1})	3.53	Phosphorous fractionation scheme given by Jiang and Gu [28]
Octa-calcium phosphate (Ca8-P mg kg^{-1})	90.45	
P-adsorbed by Al oxides (Al-P mg kg^{-1})	28.45	
P-adsorbed by Fe oxides (Fe-P mg kg^{-1})	24.25	
Occluded-P (O-P mg kg^{-1})	18.50	
Apatite (Ca10-P mg kg^{-1})	307.30	
Total sulfur (mg kg^{-1})	214.47	Gravitation method [39]
Water soluble sulfur (mg kg^{-1})	9.60	Ion chromatography method [40]

3.2 Isolation, Characterization and Identification of Sulfur Oxidizing Bacteria

Isolation data showed that out of 160 samples only 50 strains of SOB were isolated and purified by streaking on thiosulfate agar plates. Sulfur rich ecologies viz., industrial wastewater, sulfur mud and sewage water had the highest 80, 60 and 60 percent of SOB occurrence respectively. Amongst 50 strains 4 strains IW16, IW14, SW2 and IW1 were selected due to their high efficiency of pH reduction from 8.00 to 2.65, 3.44, 2.84 and 3.13 and sulfates production 2233.85, 340.25, 1398.81 and 721.51 mg L^{-1} respectively after 200 hours in the growth medium (Table 2). The strains IW16 and SW2 were more acidophilic than IW14 and IW1.

Different physiological, morphological and biochemical characteristics of the selected 4 SOB strains showed that all were short rods, Gram negative and motile having smooth round and yellow colonies with colony diameter between 0.7 to 1.7 mm. All can utilize elemental S° and Thiosulphate as the only source of energy and carbon dioxide as a sole source of carbon. Two strains SW2 and IW16 utilized only ammonium ion as nitrogen source while other two strains IW14 and IW1 can also use glutamate as an alternate source of nitrogen (Table 2). The characteristics of the two strains IW16, SW2 matched with the taxonomy of *Thiobacillus thiooxidans*, while the rest two strains IW1 and IW14 taxonomically resembled with *Thiobacillus thioparus* [41].

Table 2. Morphological, physiological and biochemical characterization of sulfur oxidizing bacterial strains

Characteristics	IW14	IW1	SW2	IW16
Morphology	Short-rods	Short-rods	Short-rods	Short-rods
Gram reaction	-	-	-	-
Colony character	Smooth-round-yellow	Smooth-round-yellow	Smooth-round-yellow	Smooth-round-yellow
Colony diameter (mm)	1.7	1.2	0.9	0.7
Elemental S° utilization	+	+	+	+
Thiosulphate utilization	+	+	+	+
Nutritional type	Autotrophic	Autotrophic	Autotrophic	Autotrophic
Source of nitrogen utilization	NH_4^+ and glutamate	NH_4^+ and glutamate	NH_4^+	NH_4^+
pH of the growth media (after 200 hours)	3.44	3.13	2.84	2.65
Sulfates production (mg L^{-1}) (after 200 hours)	340.25	721.51	1398.81	2233.85
Motility	Motile	Motile	Motile	Motile

3.3 Interactive Effect of *Thiobacillus* spp. and Sulfur Levels on pH and Water Soluble Sulfur contents in Soil

Data regarding soil pH under the interactive effect of *Thiobacillus* spp. and different levels of sulfur application after 30, 60 and 90 days of incubation are illustrated in Fig. 1 which predicted a significant decrease in soil pH in S oxidizing treatments having both *Thiobacilli* and S°. No change in soil pH was observed in control and in treatments containing *Thiobacillus* strain IW1 and *Thiobacillus* strain IW14. Maximum decline in soil pH from 7.90 to 7.52, 7.35 and 7.12 (net decrease of 0.38, 0.55 and 0.78 points) was recorded after 30, 60 and 90 days of incubation respectively in treatment S°100 IW16 (*Thiobacillus* strain IW16 plus maximum dose of S° @ 100 kg ha^{-1}). Minimum decrease in soil pH from 7.90 to 7.88, 7.88 and 7.12 (net decrease of 0.02, 0.02 and 0.03 points) was noted after 30, 60 and 90 days of incubation respectively in treatment where only *Thiobacillus* strain SW2 was inoculated. Both *Thiobacilli* strains and S° application alone could not reduce soil pH remarkably but when they were used collectively a clear significant decrease in pH was recorded.

The extent of biological S oxidation due to various treatments is portrayed in Fig. 2. Treatment S°100 plus *Thiobacillus* strain IW16 enclosed the highest significant increase in the quantities of water soluble S from 9.60 to 26.24, 33.57 and 40.24 mg kg^{-1} in the incubated soil while the treatment *Thiobacillus* IW14 strain contained the lowest values of water soluble S with quantities of 9.67, 9.93 and 10.20 mg kg^{-1} after 30, 60 and 90 days respectively. The treatments wherein bacterial S oxidation occurred under the influence of efficient *Thiobacillius* strains in combination with S° had the highest amount of water soluble S. The incubated soil in control treatment predicted no significant change.

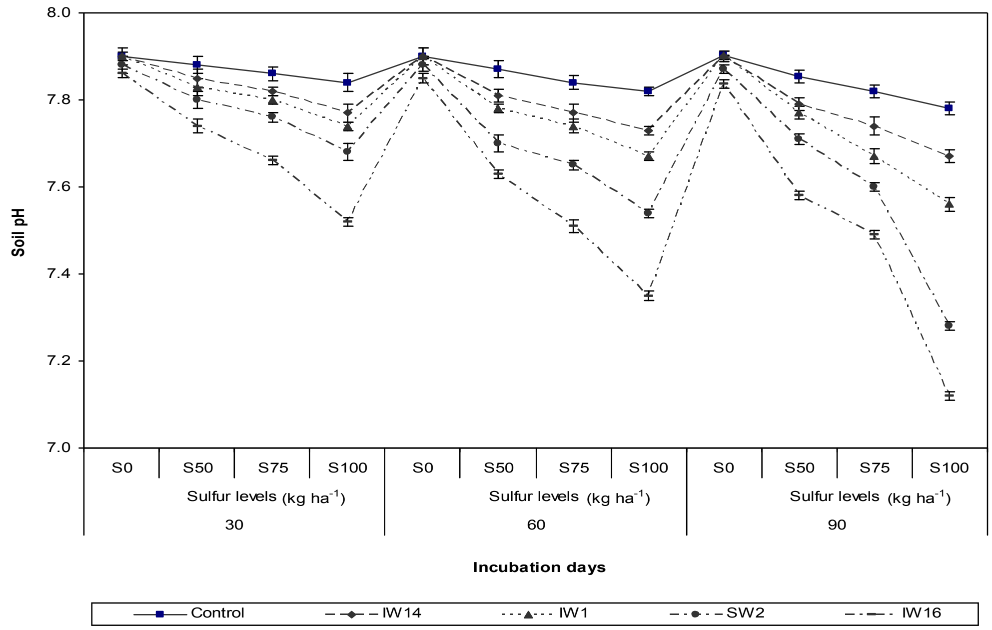

Fig. 1. Soil pH under the interactive effect of *Thiobacillus* spp. and different Levels of sulfur application

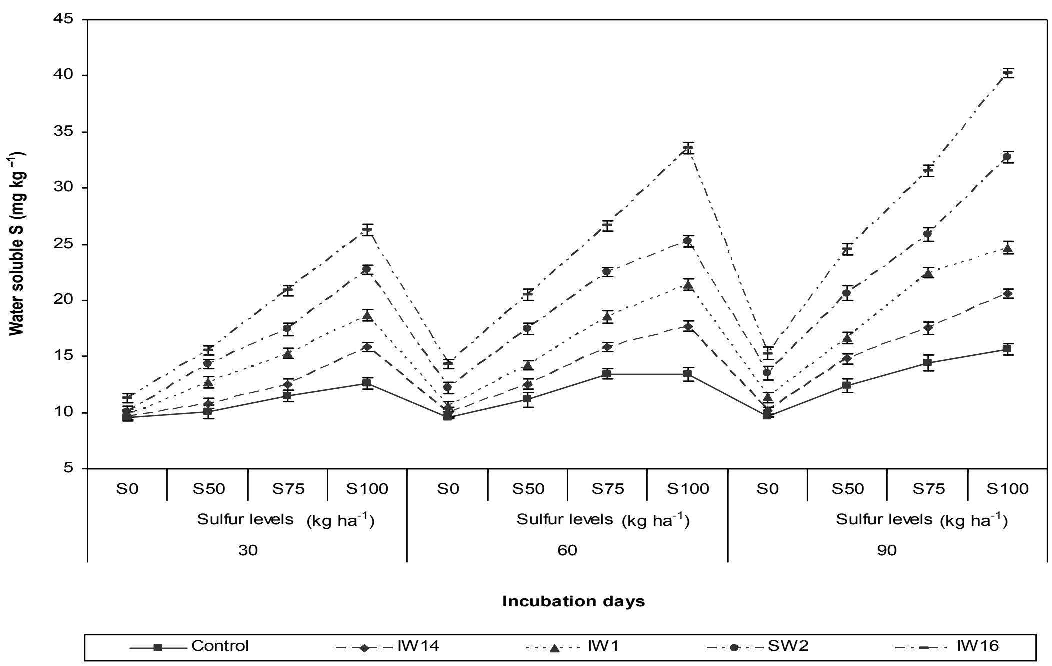

Fig. 2. Water soluble S contents in soil under the interactive effect of *Thiobacillus* spp. and different Levels of sulfur application

3.4 Interactive Effect of *Thiobacillus* spp. and Sulfur Levels on Various Phosphorous Fractions in Soil

Various P fractions in soil showed considerable changes in their quantities during 90 days of incubation under the influence of different treatments. Amongst the six P fractions the amount of di-calcium phosphate (Ca2-P) increased while the concentrations of octa-calcium phosphate (Ca8-P) and Apatite (Ca10-P) fractions decreased in all treatments except in control. However, no significant change was noted in case of P-adsorbed by Al oxides (Al-P), P-adsorbed by Fe oxides (Fe-P) and occluded phosphate (O-P) fractions. The highest increase in the concentration of Ca2-P from 3.53 to 12.56, 16.63 and 23.86 mg kg^{-1} (Fig. 3) and the maximum decrease in the concentrations of two P fractions Ca8-P from 90.45 to 84.52, 82.32 and 80.27 mg kg^{-1} (Fig. 4) and Ca10-P from 307.30 to 305.36, 304.12 and 302.58 mg kg^{-1} (Fig. 5) after 30, 60 and 90 days respectively was obtained with the combination of *Thiobacillus* strain IW16 and S° @ 100 kg ha^{-1}. Minimum increase in Ca2-P contents and minimum decrease in the quantities of Ca8-P and Ca10-P was noted in treatment where *Thiobacillus* strain IW14 was inoculated without S°. The amount of P solubilized as Ca2-P varied according to strains and dose of S°. With the increase in water soluble S contents and decrease in soil pH the quantity of P solubilized increased. Soil pH had a strong negative relationship with Ca2-P (-0.98, -0.96 and -0.98) and a massive positive association with Ca8-P (0.95, 0.95 and 0.97) and Ca10-P (0.94, 0.97 and 0.96) after 30, 60 and 90 days respectively. Coefficient of determination (R^2) values of soil pH with Ca2-P were 0.97, 0.93 and 0.96, with Ca8-P were 0.91, 0.91 and 0.94 and with Ca10-P were 0.88, 0.95 and 0.92 after 30, 60 and 90 days respectively.

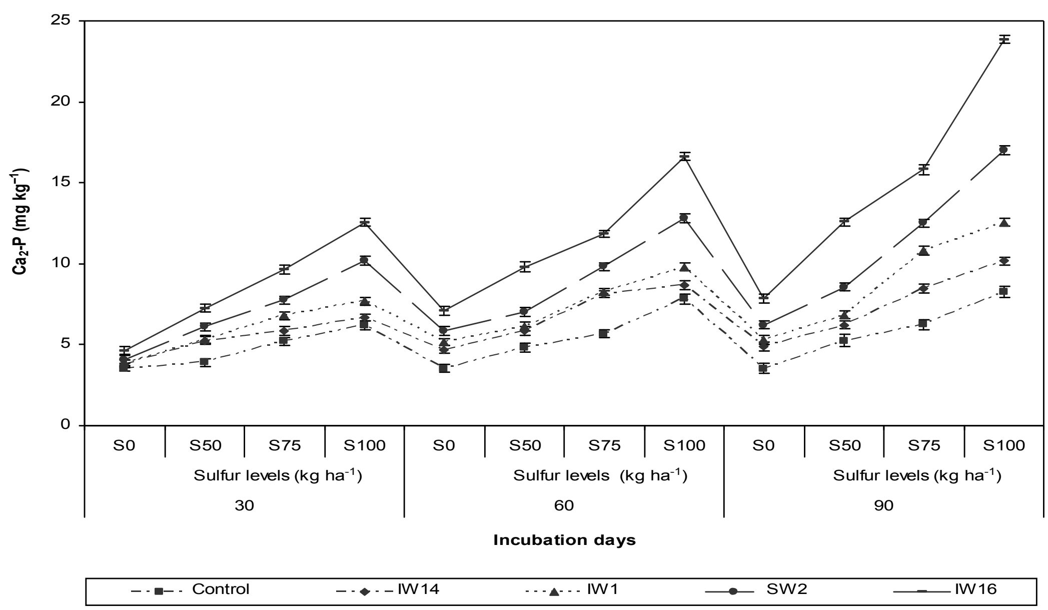

Fig. 3. Ca_2-P contents in soil under the interactive effect of *Thiobacillus* spp. and different Levels of sulfur application

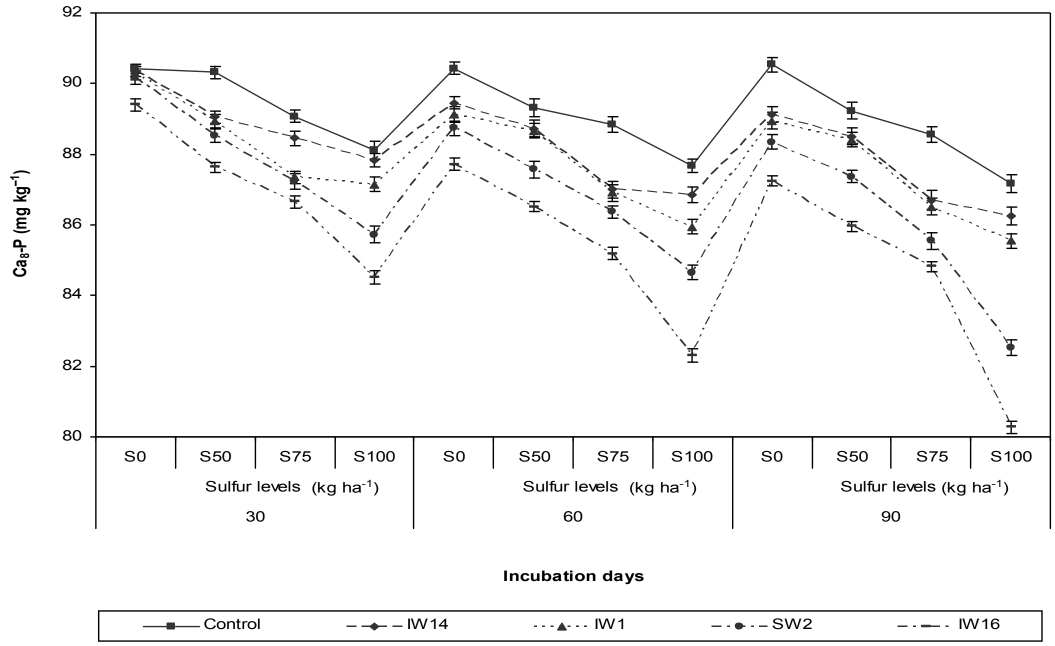

Fig. 4. Ca_8-P contents in soil under the interactive effect of *Thiobacillus* spp. and different Levels of sulfur application

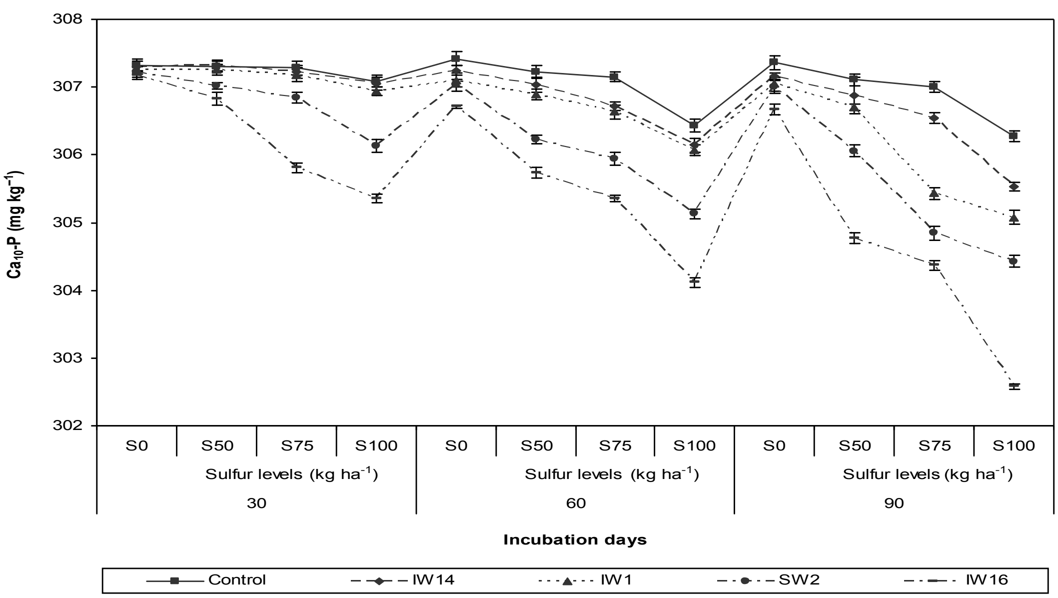

Fig. 5. Ca_{10}-P contents in soil under the interactive effect of *Thiobacillus* spp. and different Levels of sulfur application

3.5 Interactive Effect of *Thiobacillus* spp. and Sulfur Levels on Bio-Available Phosphorous Concentration in Soil

Fig. 6 depicts the amount of bio-available P during 90 days of incubation period. Bio-available P contents in soil increased in all treatments except in control treatment but the highest and the most significant increase was found (4.52 to 14.34, 21.43 and 26.78 mg kg^{-1} after 30, 60 and 90 days respectively) in treatment S°100 plus *Thiobacillus* strain IW16 due to dissolution and solubilization of fixed P forms in the soil. The lowest increase in the quantity of soil available P was recorded (4.52 to 4.86, 5.14 and 5.25 mg kg^{-1} after 30, 60 and 90 days respectively) in treatment containing *Thiobacillus* IW14 strain without S° application. Like Ca2-P the concentration of bio-available P increased with time and depended on the type of strain and rate of S° application. The strains *Thiobacillus* IW16 and *Thiobacillus* SW2 were more P solubilizers and P enhancers than *Thiobacillus* strain IW1 and *Thiobacillus* strain IW14. The association between soil pH and bio-available P contents in soil was found huge negative significant with coefficient of correlation (r) values of -0.97, -0.97, and -0.99 after 30, 60 and 90 days respectively. Coefficients of determination (R^2) values between these two variables were recorded as 0.92, 0.94 and 0.98 after 30, 60 and 90 days respectively. Further, bio-available P had a high significant positive relationship (r = 0.98, 0.99 and 0.99 after 30, 60 and 90 days respectively) with Ca2-P fraction in soil.

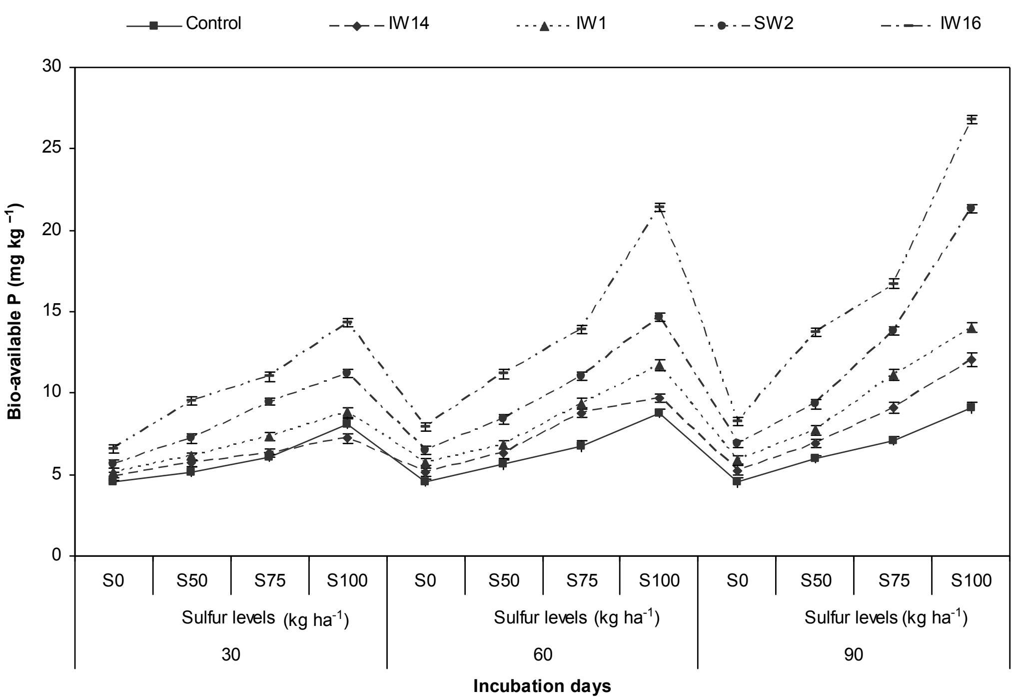

Fig. 6. Bio-Available-P Contents in Soil under the Interactive Effect of *Thiobacillus* spp. and Different Levels of Sulfur Application

4. DISCUSSION

This study describes phosphorous solubilizing potential of *Thiobacilli* in soil through bacterial sulfur oxidation mechanism. From the isolation data it was evident that maximum percentage of *Thiobacilli* were found in sulfur rich ecologies viz., industrial wastewater, sulfur mud and sewage water because sulfur or reduced sulfur compounds are essential for the existence of *Thiobacilli* like other sulfur oxidizing prokaryotes as they totally depend on S oxidation for their energy requirements. Biological sulfur oxidation is a unique characteristic of all sulfur oxidizing prokaryotes through which they oxidize S or S compounds and produce sulfuric acid [9,42]. Thus sulfur oxidation is a sulfuric acid generating process shown in the following chemical equation:

$$S^{\circ} + 1.5\ O_2 + H_2O \xrightarrow{\textit{Thiobacilli}} SO_4^{2-} + 2H$$
$$(\Delta\ G^{o} = -587.1 \text{kJ / reaction})$$

The most efficient *Thiobacilli* isolates oxidize S compounds quickly and produce sulfates in huge quantity and drop pH sharply [43,10]. In the same way highly efficient *Thiobacilli* strains IW16 and SW2 produced sulfates (2233.85 and 1398.81 mg L^{-1}) rapidly and dropped pH of the growth media from 8.00 to 2.65 and 2.84 respectively. Therefore, *Thiobacilli* isolates could be scrutinized on the basis of sulfate concentration detected from their growth media [44,10]. Sulfur or *Thiobacillus* spp. alone could not produce significant amount of sulfates whereas S° along with *Thiobacillus* strains significantly increased the concentration of water soluble S in the incubated soil and decreased soil pH [14]. Several researchers also reported high amount of sulfates production and pH reduction during the process of S oxidation in combination with S° and *Thiobacillus* spp. [44,45].

Phosphorous bioavailability is highly dependent on soil pH. The optimum range of soil pH for P availability to plants is between 6 to 7 [46]. It was noted that efficient *Thiobacillus* strains and S° application rate were very important in the reduction of soil pH. Amongst the four *Thiobacillus* strains *Thiobacillus* strain IW16 along with S° @ 100 kg ha^{-1} gave the outstanding results. Bacterial S° oxidation depended on both quantity of S° and efficient *Thiobacillus* strains. Combination of these two factors resulted in maximum biological S° oxidation which generated enormous quantity of sulfuric acid to reduce pH of the incubated soil [10,11].

Soil P is present in different forms having different nature and behavior in soil [47,48]. These forms occur in soil from solution P (available to plants) to extremely stable P (unavailable to plants) present in equilibrium with each other. Inorganic soil P forms have different solubility rates (conversion rates from insoluble P forms to soluble P forms) depending on soil pH [49,50]. Amongst all P forms in soil, solution form of P is very important because the plants can only take and utilize it for their growth and development. A Significant change in different P fractions was recorded under treatments effect. High sulfuric acid producers (IW16 and SW2) in combination with S° @ 100 kg ha^{-1} dissolved insoluble calcium bounded P fractions octa-calcium phosphate (Ca8-P) and Apatite (Ca10-P) into sparingly soluble plant available di-calcium phosphate (Ca2-P) fraction. This dissolution trend continued upto 90 days of incubation which predicted that S oxidation process was going on till the end of the experiment. Maximum solubilization and conversion of insoluble calcium bounded P compounds Ca8-P and Ca10-P into sparingly soluble Ca2-P fraction and bio-available P was carried out by the combined effect of *Thiobacillus* strain IW16 and S° @ 100 kg ha^{-1}. During P solubilization mechanism one part of the bacterially produced sulfuric was consumed in solubilizing P while the other part reduced pH of the soil. Therefore, pH decline also predicts the efficiency and capability of *Thiobacilli* strains in P solubilization [44]. Soil pH had a strong negative correlation with Ca2-P fraction and bio-available P in soil. Results described by Lee et al. [51] were similar with these findings who concluded that microbial activities in the soil converted insoluble P fractions into bio-available P fractions in soil. Similarly Bhatti and Yawar [16] reported that high quantity of bacterially generated sulfuric acid through S oxidation resulted in high amount of P dissolution. Moreover, enhancement of soil available P was found associated with the quantity of S° applied and the efficiency of *Thiobacillus* strains inoculated. Best combination of these two critical factors (S° @ 100 kg ha^{-1} plus *Thiobacillus* strain IW16) helped in generating huge amount of sulfuric acid which solubilized complex insoluble calcium bounded P compounds (Ca8-P and Ca10-P) into simple soluble plant available P compounds (Ca2-P fraction and Bio-available P).

5. CONCLUSION

Best ecologies for *Thiobacilli* occurrence are sulfur based such as industrial wastewater, sulfur mud and sewage water. The genus *Thiobacillus* of SOB is tremendously and extraordinarily competent in sulfuric acid production and proved its worth in pH reduction both in broth and soil media. Bacterial sulfur oxidation is a sulfuric acid generating phenomenon and this bacterially produced sulfuric acid has a key role in P solubilization from different fixed forms of P present in the soil. Interaction between *Thiobacillus* spp. and S° is highly significant in enhancing bio-available P in soil. Soil treatment with *Thiobacilli* in combination with S° is a best approach to improve soil P fertility by solubilizing already present huge quantity of fixed P in soil.

ACKNOWLEDGEMENTS

The authors are indebted to Mr. Jamshed Iqbal cheema, Chief Executive and Mr. Muhammad Azam Cheema, Director (Admen & Finance), Auriga Group, Lahore, Pakistan for their financial help and cooperation for this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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