

Isolation of sulphur oxidising bacteria and its characterisation for acid production

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ABSTRACT

Salt-affected and sodic soils pose a major constraint to agricultural productivity due to high pH, poor soil structure, and reduced nutrient availability. Biological oxidation of elemental sulphur by sulphur-oxidizing bacteria (SOB) offers an eco-friendly strategy for soil acidification and reclamation. The present study aimed to isolate and characterize efficient sulphur-oxidizing bacteria from sulphur-rich industrial and organic waste environments and evaluate their potential for acid production and sulphate generation. Samples including molasses, refinery sulphur-mixed soil, press mud, spent wash, fly ash, and compost were collected and cultured on thiosulphate and NCIMB media under varying pH conditions. Seven isolates were obtained, among which one strain demonstrated significant sulphur-oxidizing activity, evidenced by rapid pH reduction, colour change of bromocresol green medium, and high sulphate production. The isolate was identified as a short, rod-shaped, Gram-negative bacterium forming yellowish-orange colonies. Maximum acidification occurred at 32-37 °C with 2-4% elemental sulphur, where pH decreased to ~2.0 and sulphate concentration increased substantially. The strain effectively reduced pH and enhanced electrical conductivity and organic acid production in media with initial pH 5.0-6.5, whereas activity declined under strongly alkaline conditions. These findings demonstrate that indigenous SOB possess strong acidogenic and sulphur-oxidizing capabilities and can be exploited as bio-inoculants to accelerate elemental sulphur oxidation and improve sodic soil reclamation. The study highlights the potential of integrating microbial approaches with conventional amendments for sustainable soil management.

Keywords: Sulphur-oxidizing bacteria, Elemental sulphur oxidation, Acid production, Sulphate formation, Soil reclamation

INTRODUCTION

Salt affected soils are the major environmental concerns leading to land degradation in irrigated areas of arid and semi-arid regions across the world (Mahmoodabadi *et al.*, 2013; Kumar *et al.*, 2022). A total of 10.1 million hectare of land in the country is salt affected, of which about 2.5 million hectares occurs in the Indo-Gangetic Plains (Sehgal and Abrol, 1994; Mishra *et al.*, 2015). Sustainable management of salt-affected soils requires integrated chemical and biological approaches to restore soil productivity and resilience (Bhardwaj *et al.*, 2016; Kumar *et al.*, 2020). Sodic soil occupy more than

50% (3.77 m ha) of the salt affected area of India. Around 1.95M ha sodic land have been successfully reclaimed using the gypsum-based package in Haryana, Punjab, Uttar Pradesh and other parts of the country (Abrol *et al.*, 1988; Bhardwaj, *et al.*, 2016). Gypsum reclamation, though effective, often shows slow field response, highlighting the need for complementary biological interventions (Bhardwaj *et al.*, 2016; Singh *et al.*, 2024). Soil sodicity occurs due to the more accumulation of sodium salt relative to other types of salt cations, especially of calcium. An increase in soil pH and decrease in calcium and magnesium usually accompany this process. Sodic soils, characterized by a poor soil structure and low

infiltration rate, are poorly aerated and are difficult to cultivate (Bhardwaj *et al.*, 2021a). Excess exchangeable sodium deteriorates soil structure and restricts nutrient availability, severely limiting crop growth (Bhardwaj *et al.*, 2021b). Reclamation of sodic soils requires reduction of the amount of sodium present in the soil. Presently in India, it is done by the application of gypsum. Gypsum mainly consists of calcium sulfate, which are rich in calcium (Singh *et al.*, 2019). This calcium can replace the sodium present in sodic soils, which further can be leached from the root zone by irrigation water (Qadir *et al.*, 2001a; Ahmed *et al.*, 2023).

Sulphur (S) is one of the essential nutrient for plant growth with crop requirement similar to phosphorus. Sulphur is a non-metallic element. Sulphur is found in soil in the form of plant residues, animal wastes, chemical fertilizers, sulphur containing pesticides and in rainwater. Sulphur is an acidifier which reduces the pH of calcareous and alkaline soils and facilitates the absorption of some nutrients (Zhao *et al.*, 1999). Elemental sulphur acts as an acidifying amendment capable of lowering soil pH and improving nutrient solubility in alkaline soils (Malik *et al.*, 2021). An insufficient S supply can affect yield and quality of the crop; caused by the S requirement for protein and enzyme synthesis as well it is a constituent of the amino acids methionine and cysteine. Recently, interest in using elemental sulphur for reclaiming the calcareous sodic soils has increased. However, the characteristics of the fine S make it unacceptable for reclamation due to dustiness and fire hazards, and oxidation rate of prilled sulphur has been reported to be slow limiting its use as a reclaimant for sodic soils. Apart from the particle size, S oxidation being biological in nature depends upon the soil physico-chemical properties and soil biological activity specially sulphur oxidising microorganisms (Lawrence and Germida, 1988; Kirkby *et al.*, 2011).

The “Sulphur bacteria” are usually defined as those bacteria which are able to oxidize elemental sulphur and some sulphur compounds and utilize the energy derived for their maintenance and growth (Encyclopaedia Britannica, 1998). The genus *Thiobacillus* is a group of obligatory or facultative chemolithotrophic aerobic proteobacteria that are capable of growing with reduced inorganic sulphur compounds as sole energy source, but, at this time, is quite heterogeneous with members exhibiting a wide range of physiological, chemotaxonomic and

genetic characteristics (Kelly and Harrison, 1989; Moreira and Amils, 1997; Nicomrat *et al.*, 2008; Nuñez *et al.*, 2017). *Thiobacilli* play an important role in sulphur oxidation in soil. Sulphur oxidation is the most important step of sulphur cycle, which improves soil fertility. It results in the formation of sulphate, which can be used by the plants, while the acidity produced by oxidation helps to solubilize plant nutrients and improves alkali soils (Chaudhary *et al.* 2023; Zhou *et al.*, 2023). The oxidation of elemental sulphur brings about solubilization of soil minerals. The sulphuric acid formed during oxidation reacts with minerals and other insoluble material and increases the quantity of soluble phosphate, potassium, calcium, magnesium and aluminium in the soil. The transformation of sulphur in soil is mainly caused by microorganism. In pure culture, bacteria may attack on sulphur compound in various ways, resulting in yielding different end products. Sulphur-oxidizing microorganisms accelerate sulphate formation and enhance soil acidification processes (Singh *et al.*, 2024; Bhardwaj *et al.*, 2022). Biological sulphur oxidation also promotes micronutrient mobilization and soil fertility restoration (Bhardwaj *et al.*, 2022).

Sulphur oxidizing bacteria play important role in reclamation of sodic soils by converting elemental sulphur into sulphate. Sulphur-oxidizing microorganisms have a wide metabolic diversity which is isolated from hypersaline, as well as from acid, neutrophilic and alkalophilic environments. According to their response to pH, sulphur-oxidizing species include acidophiles (optimum pH 2–5), neutrophiles (optimum pH 6–8), and alkaliphilic (optimum pH 10–11) microorganisms (Sorokin and Kuenen, 2005; Aliyu *et al.*, 2024). They can also be found in a wide range of temperatures such as thermophilic (optimal temperature 50–75 °C), psychrophilic (optimal –5–5 °C) or mesophilic (optimal temperature 25–40 °C) microorganisms (Jørgensen and Bak, 1991; Hallberg and Lindström, 1994; Sievert *et al.*, 2000). Most of the known sulphur oxidising bacteria (SOB) belongs to the genera *Thiobacillus*, *Thiothrix*, *Thiomicrospira*, *Achromatium* and *Desulfuromonas* (Kelly and Wood, 2000; Friedrich *et al.*, 2001). Soil SOB increase the availability of sulphate (SO₄²⁻) for plant absorption and improve plant growth and agricultural production (Eriksen, 2009; Singh *et al.*, 2024). These types of bacteria are widely present in the environment and are involved in sulphur oxidizing reactions in the environment.

The most important factors governing the rate of sulphur oxidation are temperature and water potential (Germida and Janzen, 1993), variation in particles size (Laishely *et al.*, 1986; Watkinson and Bolan, 1998), organic amendments (Malik *et al.*, 2021), soil fertility (Schoenau and Malhi, 2008) and other environmental conditions (Lee *et al.*, 2021; Ranadev *et al.*, 2023). Sulphur oxidizing bacteria play important role in the reclamation of sodic soils by converting elemental sulphur into sulphate. Objective of this study was to isolate these sulphur oxidizing bacteria from different sources and their introduction into soil samples to improve the process of conversion of elemental sulphur to sulphate.

MATERIALS AND METHODS

Collection of samples

For the isolation of sulphur oxidizing bacteria (SOB) those sites were identified where sulphur is used directly or indirectly. Different samples were collected from sugar mill of Kaithal and Panipat (Table 1). Press mud from the sugar industries are rich source of nutrients. It is a by-product in the making of sugar from sugar cane. Press mud is mainly used in bio-composting where it is treated with spent wash which is also a by-product from the distillery. Molasses is a viscous liquid which is separated from sugar syrup during the process of sugar formation.

Table 1. Description of samples

Sr. no.	Sample no.	Sample type
1.	S-1	Molasses
2.	S-2	Refinery sulphur mixed with soil
3.	S-3	Soil + press mud
4.	S-4	Fly ash
5.	S-5	Press mud infected with fungus
6.	S-6	Spent wash
7.	S-7	Press mud compost

Isolation and characterisation of sulphur oxidising bacteria

For the isolation of SOB two types of media were used- Thiosulphate medium and NCIMB medium and the pH of medium was kept variable (pH 4.5, pH 6, pH 8) for giving wide range of growth conditions. Samples were appropriately diluted and 0.1 ml of diluted samples were inoculated in the petri-plates by two methods- Pour plate technique and spread plate method. Samples like pressmud

and spent wash along with elemental S were used to enrich the soils. The petriplates having samples were kept for incubation at $35\pm 2^\circ\text{C}$ for 7 days.

Screening of isolates was done by growing them on the thiosulphate selective medium with 0.4% of bromocresol green having 4.5 pH and by observing color change of the medium as mentioned by Konde and Jadhav (1979) and Starosvetsky *et al.*, (2013). Selected isolates were observed for gram staining and microscopic studies for their appearance and morphology.

Evaluation of screened isolate

For the evaluation of isolates, the pH reduction was observed and for this the thiosulphate broth was prepared with different concentration of elemental S as 1%, 2%, 3%, 4% and 5%. After autoclaving, 100 microlitre of bacterial culture was inoculated in tubes except controls. The tubes were incubated on different temperatures- 27°C , 30°C , 32°C , 37°C , 42°C and 47°C for 7-8 days.

Sulphate estimation

After incubation the sulphate was estimated in the filtrate by the turbid metric method (Chesnin and Yien, 1950).

Effect of sulfur-oxidizing bacteria on pH and EC of media

In this study, reduction of pH at regular interval of time was observed in submerged conditions to see the efficiency of isolate for the reduction of pH. Thiosulphate broth was prepared and pH of media was set at 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, and 8.5. The pH of the samples was determined on the 5th day, 10th day and 15th day of the incubation.

Organic acid production

Titration activity or organic acid of the samples was determined by titrating 10 ml sample against 1N NaOH using 3-5 drops of phenolphthalein solution (0.5%) indicator with continuous stirring till light purple color persist.

RESULTS

Electrical conductivity and pH of the samples

The electrical conductivity (EC) and pH of the collected samples were determined and are presented

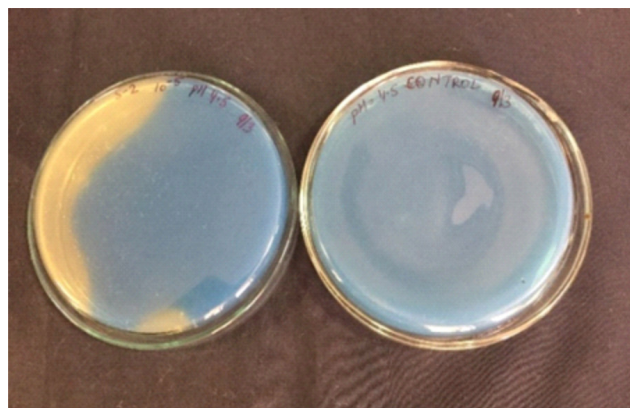
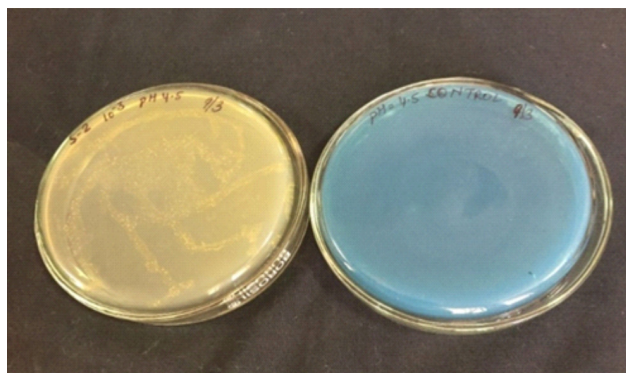
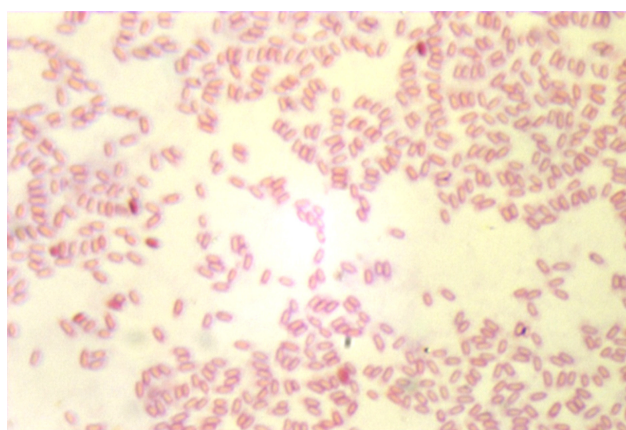
Table 2. Electrical conductivity and pH of samples

Sr. No.	Sample	EC (dSm ⁻¹)	pH
1.	S-1	21.00	4.95
2.	S-2	3.51	2.15
3.	S-3	1.31	7.30
4.	S-4	6.15	8.92
5.	S-5	4.58	7.02
6.	S-6	29.6	3.38
7.	S-7	8.36	6.61

in Table 2. EC values ranged from 1.33 to 21.00 dS m⁻¹, while pH ranged from 2.15 to 8.92. Sample S 6 exhibited the highest EC (29.6 dS m⁻¹) and a strongly acidic pH of 3.38, whereas sample S 4 had a pH of 8.92 with lower EC.

Isolation and characterisation of SOB

Isolation of sulfur-oxidizing bacteria (SOB) was carried out on thiosulfate and NCIMB media. The plates were incubated at 25 °C were selected and further tested for pH reduction. In thiosulphate agar medium reduction in pH causes change in colour of media from blue to yellow. Seven isolates were initially obtained, but only one isolate—from sulfur-enriched refinery soil—demonstrated significant pH reduction on thiosulfate agar. On this medium, pH reduction was indicated by a pronounced color change from blue green (bromocresol green indicator at pH >3) to yellow (pH <3), as seen at day 7 and day 9 (Figs. 1a and 1b). The active isolate was characterized as short, rod-shaped, gram-negative, with yellowish-orange, smooth, round colonies under microscopic examination (Fig. 2).

**Fig. 1a.** Change in colour of the thiosulphate selective medium due to bacterial growth (Day-7)**Fig. 1b.** Change in colour of the thiosulphate selective medium due to bacterial growth (Day -9)**Fig. 2.** Microscopic view of Gram staining

Bacterial population

Total bacterial count of sulphur oxidising bacteria was done on thiosulphate agar plates. The colonies on the agar plates were counted after 7 days incubation. The results revealed diverse colony morphologies—white, green, yellow, and off-white—at varying pH levels (4.5, 6, 8). In fresh sugar-mill soil samples, sample S-7 displayed the highest SOB counts, with 111 green and 1 white brown center colony at pH 4.5; 132 green at pH 6; and 216 green colonies at pH 8 (Table 3).

Following the addition of 1% elemental sulphur, SOB counts increased significantly across samples and pH conditions (Table 4), notably in S 7 with 88 green and 46 white colonies at pH 4.5.

Evaluation of isolate with different sulphur concentration

The active isolate was further evaluated for its pH-reducing ability in thiosulfate broth amended with varying sulphur concentrations (1–5%) and

Table 3. Microbial count of fresh sugar mill sample

Sample	Number of colonies *10 ³		
	pH – 4.5	pH – 6	pH – 8
S-1	-	-	3 green, 2white with brown centre
S-2	32 green and 2 yellow	14 green, 22 white	-
S-3	2 green	-	2 white with brown centre
S-4	-	2 white with brown centre	-
S-5	47 green and 16 white	42 green, 38 white	-
S-6	-	-	-
S-7	111 green, 1 white with brown centre	132 green, 1 white with brown centre	216 green

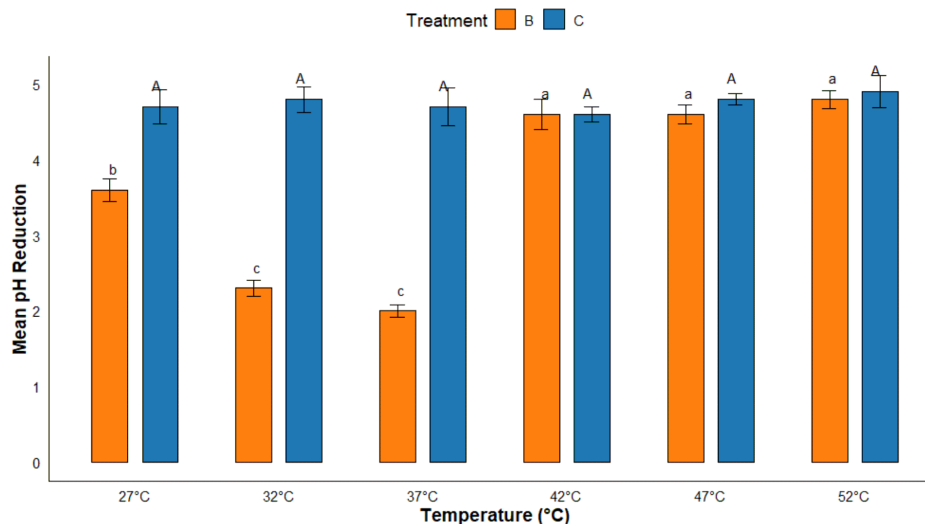
Table 4. Microbial count of fresh sugar mill sample with addition of sulphur

Sample	Number of colonies *10 ²		
	pH 4.5	pH 6	pH 8
S-1	16 green	2 green and 34 white	23 white
S-2	25 orange and 6 green	37 orange and 12 green	12 white and 16 orange
S-3	17 green and 21 white	16 green and 35 white	87 green, 7 orange and 92 yellow
S-4	-	6 green and 98 white	2 green and 3 white
S-5	55 green	-	-
S-6	64 green	120 green	12 white
S-7	88 green and 46 white	52 white and 68 green	1 green and 47 white

temperatures (27–52 °C). At 1% S, pH reduction was most pronounced at 37°C (final pH 1.98), whereas at 52°C the effect was minimal (final pH 4.67) (Fig. 3). At 2% S, similar trends were observed, with final pH around 2.32 at 32–37°C (Fig. 4). Increasing sulphur to 3% resulted in minimum final pH values of 2.37 at 37°C (initial pH 4.81) (Fig. 5). With 4% S, the lowest pH (2.26) was again at 37°C (Fig. 6). At 5%, substantial pH drop to 2.35 occurred at 37°C (Fig. 7). Sulphate ion concentration peaked at 37°C across all treatments.

Slope-based sensitivity of pH reduction to temperature

The regression slope values quantify how much pH reduction changes per degree Celsius increase in temperature for each sulphur level and treatment (Fig. 8). Treatment B consistently exhibited higher slope values than Treatment C across all sulphur levels, indicating that pH reduction in Treatment B is more sensitive to rising temperature. The highest sensitivity was observed in Treatment B at 2% sulphur, with a slope of 0.098, suggesting significant

**Fig. 3.** pH reduction in 1 % S at different temperature. (C=control and B= bacterial strains)

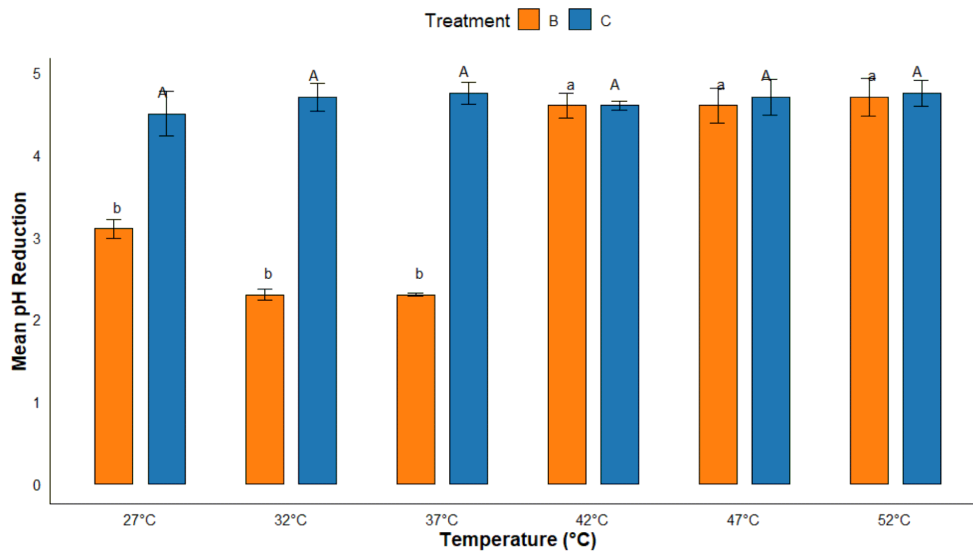


Fig. 4. pH reduction in 2 % S at different temperature. (C=control and B= bacterial strains)

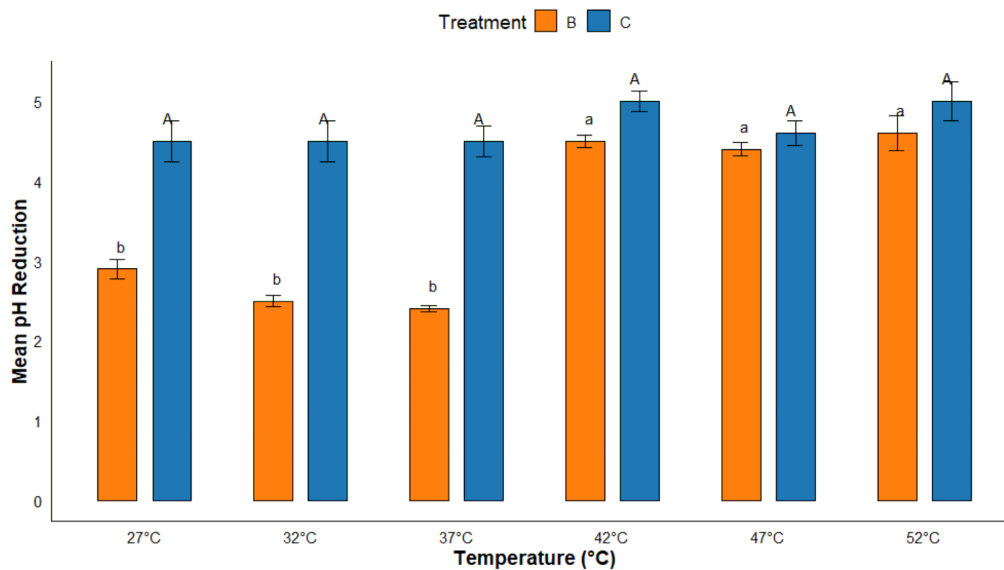


Fig. 5. pH reduction in 3 % S at different temperature. (C=control and B= bacterial strains)

acidification with temperature rise. Conversely, Treatment C showed minimal sensitivity, indicating that pH reduction in Treatment C remained relatively stable across the temperature range. Highest slope of 0.019 in group C was recorded at 3 percent Sulphur level. In the control (Treatment C) at different temperatures pH shows consistency and the SOB (treatment B) shows reduction of pH.

EFFECT OF SULFUR-OXIDIZING BACTERIA AT VARIED PH MEDIA ON PH, EC AND ORGANIC ACID PRODUCTION

The influence of sulphur-oxidizing bacteria (SOB) on medium acidification was assessed at

various initial pH levels over a 15-day incubation period (Table 5). Across all initial pH values (4.5–8.5), samples inoculated with SOB exhibited a significantly greater reduction in pH compared to uninoculated controls. At lower initial pH values (4.5–6.5), SOB-inoculated media showed pronounced acidification. For instance, at an initial pH of 5.5, the pH dropped sharply from 5.21 ± 0.18 in the control to 1.89 ± 0.02 after 15 days with SOB. Similarly, at pH 6.0, a marked reduction to 1.93 ± 0.01 was recorded with SOB, compared to a relatively stable pH of 6.20 ± 0.18 without inoculation. The strongest acidification was observed between pH 5.0 and 6.0, with pH reductions of more

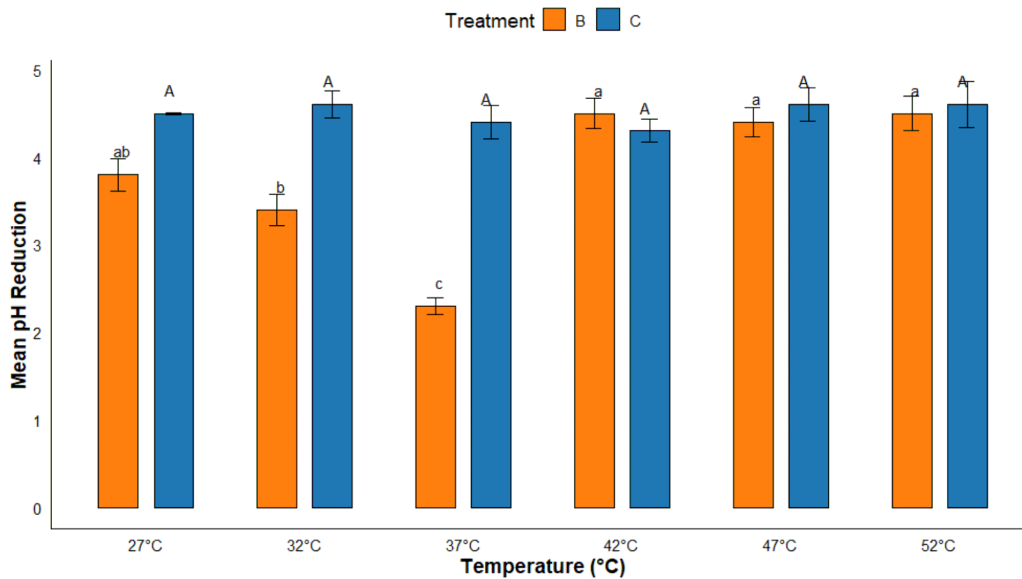


Fig. 6. pH reduction in 4 % S at different temperature (C=control and B= bacterial strains)

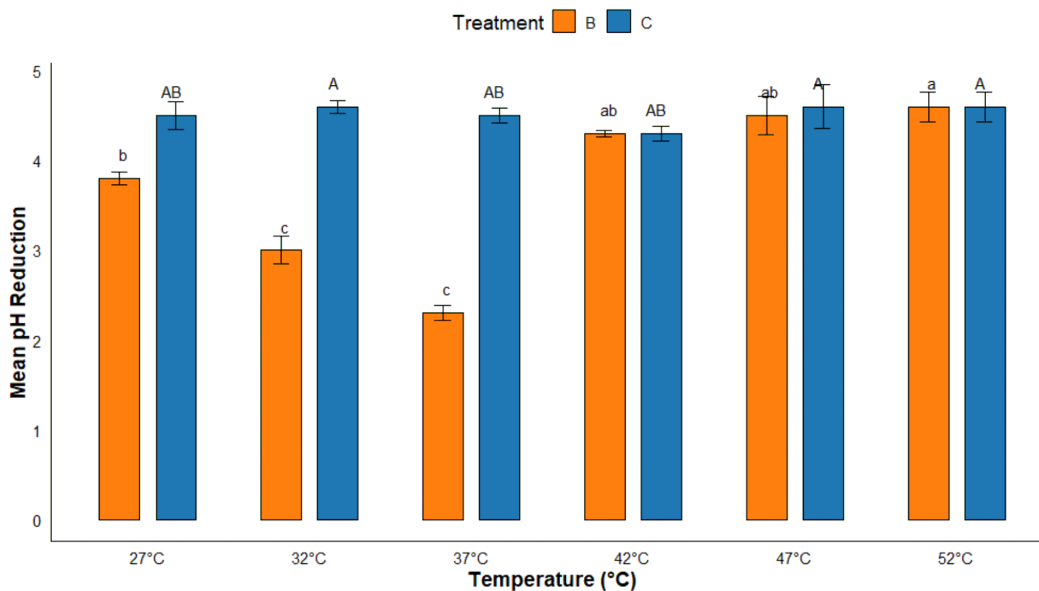


Fig. 7. pH reduction in 5 % S at different temperature (C=control and B= bacterial strains)

than 3 units over the incubation period. At pH 6.5, SOB activity still caused a significant pH drop (to 3.44 ± 1.44), whereas the control pH increased to 6.62 ± 0.27 . However, at initial pH values of 7.0 and above, SOB-mediated acidification was negligible. For instance, at pH 7.5 and 8.0, the final pH values remained above 6.9 in SOB treatments and showed minimal deviation from control samples.

Electrical conductivity (EC) varied significantly with both initial pH levels and the presence of sulphur-oxidizing bacteria (SOB) over the 15-day incubation period (Table 6). In general, SOB-

inoculated samples exhibited higher EC values than the uninoculated controls, particularly in the mildly acidic to near-neutral pH range (5.5–6.5), indicating greater ionic release due to active sulphur oxidation. At pH 5.5, EC in the SOB treatment peaked at 9.48 ± 0.03 dS m^{-1} by day 15—the highest observed EC across all treatments—compared to only 5.32 ± 0.28 dS m^{-1} in the control. Similarly, pH 6.0 showed consistently high EC with SOB (8.24–9.44 dS m^{-1}) across all incubation periods. In contrast, at lower pH values (4.5–5.0), EC remained comparatively moderate in both SOB-treated and

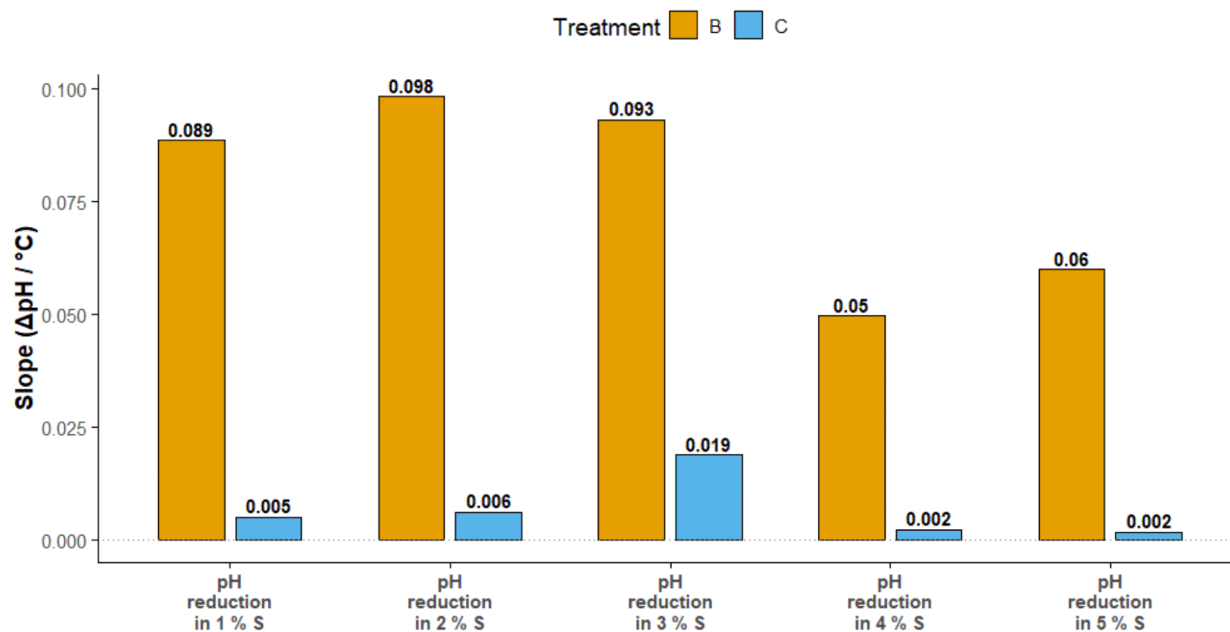


Fig. 8. Temperature sensitivity of pH reduction as influenced by sulphur level and treatment, represented by regression slopes ($\Delta\text{pH per } ^\circ\text{C}$)

Table 5. Effect of pH level and treatment on pH in growth media

Initial pH	Without culture			With SOB		
	5 days after incubation	10 days after incubation	15 days after incubation	5 days after incubation	10 days after incubation	15 days after incubation
4.5	4.67 ± 0.19e	4.88 ± 0.28d	4.65 ± 0.24e	3.60 ± 0.01d	3.66 ± 0.04c	3.95 ± 0.11b
5.0	5.15 ± 0.25de	5.26 ± 0.19d	4.99 ± 0.12e	3.60 ± 0.08d	3.63 ± 0.05c	2.49 ± 0.59bc
5.5	5.21 ± 0.18d	5.19 ± 0.17d	5.55 ± 0.23d	2.28 ± 0.27e	1.86 ± 0.01d	1.89 ± 0.02c
6.0	6.12 ± 0.16c	6.12 ± 0.08c	6.20 ± 0.18c	2.06 ± 0.06e	1.86 ± 0.02d	1.93 ± 0.01c
6.5	6.50 ± 0.16bc	7.01 ± 0.21b	6.62 ± 0.27cd	6.23 ± 0.06c	4.80 ± 1.04b	3.44 ± 1.44b
7.0	7.00 ± 0.33b	7.17 ± 0.16b	6.75 ± 0.16b	6.66 ± 0.05b	6.80 ± 0.01a	6.78 ± 0.07a
7.5	7.60 ± 0.13a	7.44 ± 0.10b	7.72 ± 0.08a	6.86 ± 0.07b	6.92 ± 0.13a	6.97 ± 0.08a
8.0	8.02 ± 0.10a	7.99 ± 0.06a	8.01 ± 0.09a	7.24 ± 0.04a	7.14 ± 0.06a	7.14 ± 0.12a
8.5	8.07 ± 0.15a	8.05 ± 0.09a	8.06 ± 0.14a	7.26 ± 0.03a	7.28 ± 0.06a	7.40 ± 0.03a

Table 6. Effect of pH level and treatment on EC (dSm^{-1}) in growth media

Initial pH	Without culture			With SOB		
	5 days after incubation	10 days after incubation	15 days after incubation	5 days after incubation	10 days after incubation	15 days after incubation
4.5	6.64 ± 0.20bc	7.09 ± 0.15bcd	6.79 ± 0.34c	6.89 ± 0.08cd	6.91 ± 0.37e	6.94 ± 0.09c
5.0	6.47 ± 0.49bc	6.48 ± 0.31d	7.02 ± 0.13bc	6.82 ± 0.08d	6.70 ± 0.02e	8.18 ± 1.04b
5.5	5.51 ± 0.23c	6.79 ± 0.24cd	5.32 ± 0.28d	7.89 ± 0.78a	9.20 ± 0.08a	9.48 ± 0.03a
6.0	6.87 ± 0.81abc	6.83 ± 0.13cd	7.05 ± 0.14bc	8.24 ± 0.21a	9.44 ± 0.22a	7.27 ± 0.20bc
6.5	6.64 ± 0.42bc	7.22 ± 0.07bcd	7.25 ± 0.56abc	6.95 ± 0.18bcd	7.17 ± 0.08de	8.25 ± 0.48b
7.0	7.66 ± 0.53ab	7.52 ± 0.56bc	7.38 ± 0.61abc	7.50 ± 0.20abcd	7.59 ± 0.21cd	7.75 ± 0.09bc
7.5	8.11 ± 0.58a	8.66 ± 0.19a	8.35 ± 0.46a	8.08 ± 0.21a	8.19 ± 0.06b	8.15 ± 0.01b
8.0	7.68 ± 0.59ab	7.87 ± 0.40ab	8.09 ± 0.26ab	7.73 ± 0.10abc	7.67 ± 0.02c	7.83 ± 0.03bc
8.5	7.84 ± 0.17ab	7.94 ± 0.34ab	7.98 ± 0.60ab	7.78 ± 0.02ab	7.70 ± 0.10c	7.97 ± 0.27bc

Table 7. Effect of pH level and treatment on organic acid production (meq L⁻¹) in growth media

Initial pH	Without culture			With SOB		
	5 days after incubation	10 days after incubation	15 days after incubation	5 days after incubation	10 days after incubation	15 days after incubation
4.5	1.54 ± 0.05a	1.43 ± 0.13a	0.93 ± 0.02c	1.57 ± 0.55b	1.70 ± 0.10b	1.63 ± 0.21bc
5.0	0.79 ± 0.01c	1.49 ± 0.02a	1.31 ± 0.04a	1.50 ± 0.50b	1.57 ± 0.06b	2.57 ± 0.90ab
5.5	1.63 ± 0.12a	1.51 ± 0.02a	0.72 ± 0.01e	2.60 ± 0.50a	2.83 ± 0.49a	3.43 ± 1.52a
6.0	1.08 ± 0.06b	0.95 ± 0.04b	1.05 ± 0.04b	2.70 ± 0.10a	3.17 ± 0.06a	3.40 ± 0.17a
6.5	0.19 ± 0.01e	0.80 ± 0.03c	0.81 ± 0.05d	0.78 ± 0.21bc	1.23 ± 0.25bc	2.13 ± 0.86b
7.0	0.20 ± 0.01e	0.10 ± 0.01e	0.18 ± 0.01f	0.47 ± 0.06c	0.67 ± 0.06cd	0.47 ± 0.06c
7.5	0.38 ± 0.01d	0.30 ± 0.02d	0.19 ± 0.01f	0.50 ± 0.10c	0.46 ± 0.15d	0.50 ± 0.10c
8.0	0.20 ± 0.01e	0.31 ± 0.03d	0.10 ± 0.01g	0.30 ± 0.10c	0.45 ± 0.05d	0.40 ± 0.19c
8.5	0.10 ± 0.002e	0.20 ± 0.02de	0.11 ± 0.01g	0.27 ± 0.06c	0.23 ± 0.06d	0.50 ± 0.20c

untreated samples, with only a marginal increase from controls (e.g., 6.94±0.09 vs. 6.79±0.34 at pH 4.5 on day 15). At neutral to slightly alkaline pH (7.0–8.5), differences in EC between treatments were smaller and often statistically insignificant. For example, at pH 8.0 on day 15, EC values were 7.83±0.03 dS m⁻¹ (SOB) and 8.09±0.26 dS m⁻¹ (control).

Organic acid production in the growth medium varied significantly with initial pH and the presence of sulphur-oxidizing bacteria (SOB) over the 15-day incubation period (Table 7). Across all pH levels, inoculated treatments consistently produced higher concentrations of organic acids compared to the uninoculated controls, particularly in the mildly acidic to near-neutral pH range (5.0–6.5). At initial pH 5.5 and 6.0, SOB-inoculated media exhibited the highest organic acid concentrations, reaching 3.43±1.52 meq L⁻¹ and 3.40±0.17 meq L⁻¹ respectively by day 15. These values were significantly higher than the corresponding control treatments, which declined to 0.72±0.01 meq L⁻¹ and 1.05±0.04 meq L⁻¹, indicating enhanced metabolic activity and acidogenesis by SOB at these pH levels. At initial pH 5.0, acid production with SOB also increased progressively, peaking at 2.57±0.90 meq L⁻¹ by day 15, whereas the control remained lower at 1.31±0.04 meq L⁻¹. Similarly, at pH 6.5, SOB treatment reached 2.13±0.86 meq L⁻¹, a nearly threefold increase over the uninoculated control (0.81±0.05 meq L⁻¹). In contrast, at higher pH values (≥7.0), organic acid production was minimal and showed limited enhancement by SOB. For example, at pH 8.0, final acid concentrations with SOB were only 0.40±0.19 meq L⁻¹, compared to 0.10±0.01 meq L⁻¹ in the control. The lowest acid production was recorded at pH 8.5, with just 0.50±0.20 meq L⁻¹ in the SOB treatment.

DISCUSSION

The present study demonstrates the robust sulphur-oxidizing activity of an indigenous bacterial isolate under diverse sulphur concentrations, temperature ranges, and initial pH conditions, underscoring its potential for sodic soil reclamation. The isolate exhibited remarkable acidification capabilities at moderate temperatures (32–37°C), with pH dropping to as low as ~1.98 in 1% sulphur broth and ~2.26 in 4% sulphur broth. This pronounced acidogenicity reflects efficient oxidation of elemental sulphur to sulfuric acid, mediated by metabolic processes that are likely catalysed via enzymes such as sulphur oxygenase reductase (SOR) or components of the Sox system, commonly found in *Acidithiobacillus* and related chemolithoautotrophs (Friedrich *et al.*, 2005; Meyer *et al.*, 2007). The steepest temperature-dependent pH reductions (highest regression slopes ~0.098 at 2% sulphur) similarly support this inference: the isolate's enzymatic machinery responds dynamically to rising temperatures, enhancing acid production within the mesophilic temperature window.

Our findings align with the observations of Konde and Jadhav (1979), who recorded substantial pH drops (from 8.5 to ~3.0) via elemental sulphur oxidation by SOB strains. Likewise, Starosvetsky *et al.* (2013) employed thiosulfate-based isolation to identify potent sulphur-oxidizers, and Donati *et al.* (1996) noted that their activity waned at elevated temperature thresholds, mirroring the reduced pH reduction seen at 52°C in our experiments. These parallels reinforce the notion that while SOB are adaptable, their acid production and metabolic efficiency are maximized in a specific temperature band—typically around 30–40°C.

The sulphate concentrations achieved in this study (up to ~887/ ppm) exceed previously reported values—Behra *et al.* (2014) observed 125–245/ ppm sulphate, Ravichandra *et al.* (2007) reported 14–150/ mg mL⁻¹, and Babana *et al.* (2011) measured up to 243 mg L⁻¹ sulfuric acid—highlighting the superior efficacy of this isolate. This may be due to enhanced sulphur bioavailability, optimal inoculum density, or particularly high enzyme activity. The strong correlation between sulphate accumulation and pH decline further underlines acid production as a reliable proxy for microbial sulphur oxidation. Further, the isolate displayed a favourable operating pH range, maintaining growth and acid production up to initial pH 6.5, but failing to acidify media above pH 7 even after 15 days. This suggests limitations in survivability or metabolic stability under highly alkaline conditions—a critical consideration for field applications in sodic soils, which often exhibit initial pH values around 8.5–9 (Bao *et al.*, 2016; Twible *et al.*, 2024). The active range within neutral to moderately alkaline soils (pH 6–7) underscores the importance of pre-conditioning field sites or applying incremental sulphur/generic acidification prior to bio-augmentation.

The results of table 6 showed high EC with SOB at pH 6, confirming enhanced mineralization and solubilization under optimal microbial activity. Very little difference in EC over the time with lower pH conditions reflects reduced SOB metabolic efficiency under more acidic initial conditions. The results of EC at higher pH indicate limited sulphur oxidation activity in alkali conditions which is consistent with the known pH sensitivity of SOB. Notably, EC trends over time in SOB-inoculated treatments reflect dynamic sulphur oxidation and associated release of sulphate, hydrogen ions, and potentially other solubilized ions (e.g., Ca²⁺, Mg²⁺), contributing to improved chemical activity in the medium. The elevated EC observed in inoculated samples, compared to controls, corroborates ionic release from sulphur mineralization—particularly sulphate, H⁺ ions, and potential cation exchange with sodium on clay surfaces (Abrol *et al.*, 1988). These physicochemical changes are indicative of the reclamation process: as acid leaches soil minerals, soluble calcium and magnesium can displace exchangeable sodium, ultimately improving soil structure and permeability (Qadir *et al.*, 2001b; 2006). Visualization of color shifts from blue-green to

yellow in thiosulfate media (Figs 1a & 1b) further illustrates robust SOB metabolic activity and acidification (Malviya *et al.*, 2022).

The trends of organic acid production highlight that optimal production occurred at pH 5.5–6.0, aligning with the preferred activity range for SOB. Acid yields declined progressively at pH levels above 6.5, suggesting that higher alkalinity suppresses microbial metabolism and/or enzyme function responsible for organic acid biosynthesis. An additional insight is the role of organic acid production (1.7–3.4 meq L⁻¹ at pH 4.5–6). Such acids may include gluconic, citric, or oxalic acids, which can chelate metals and enhance nutrient solubilization — contributing further to the breakdown of carbonate-rich sodic soils (Jones, 1998; Mahmoodabadi *et al.*, 2013; Etemadian *et al.*, 2017). Their presence suggests a multifaceted mode of action: while sulphur oxidation drives primary pH change, organic acids may broaden the spectrum of beneficial soil reactions, including phosphorus mobilization and microbial synergism (Richardson and Simpson, 2011; Ranadev *et al.*, 2023).

These findings suggest that inoculation with an active SOB strain, especially in tandem with 2–4% sulphur, could significantly expedite sodic soil restoration, reducing reliance on gypsum alone (Stamford *et al.*, 2002; Rai *et al.*, 2024). The optimal temperature range and pH adaptability position this bacterium as a robust candidate for field trials in tropical and subtropical regions, such as the Indo-Gangetic Plains, where soil temperatures frequently fall within the active window and initial pH falls within a manageable range.

Despite its promise, the isolate's inability to function effectively above pH 7 necessitates a combined approach in field settings—preliminary pH reduction via gypsum or acidulant amendments may be necessary before bio-inoculation. Additionally, environmental factors—soil moisture, organic matter, native microbiome—can impact SOB efficacy (Germida and Janzen, 1993; Chaudhary *et al.*, 2023). Field trials under varied real-world settings are thus essential to validate lab results. Genomic or enzymatic characterization of this strain should also be pursued to elucidate pathways of sulphur oxidation and identify potential for genetic or metabolic optimization (Meyer *et al.*, 2007; Yin *et al.*, 2014; Umezawa *et al.*, 2016).

CONCLUSION

In conclusion, the isolated SOB demonstrates high potential for bioaugmentation in elemental sulphur-based sodic soil reclamation. Its physiological traits-broad temperature tolerance, strong acidogenicity, moderate pH adaptability, and organic acid secretion-suggest practical viability. Integrating this microbial approach with conventional ameliorants could accelerate soil recovery and enhance sustainable crop productivity on salt-affected lands.

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