International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(6): 2087-2096

Received: 04-09-2019
Accepted: 06-10-2019

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Isolation and kinetic growth study of rock phosphate solubilizing microbes

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Abstract
The present experiment is conducted on 'isolation & Kinetic Growth study of Rock phosphate solubilizing Microbes'. Rock Phosphate is the cheapest and abundant Phosphatic fertilizer available but due to its sparse solubility it is not always agronomically effective. Most agricultural soils contain large reserves of phosphorus (P), a considerable part of which accumulates as a consequence of regular applications of P fertilizers. However, a greater part of soil phosphorus, approximately 95-99% is present in the form of insoluble phosphates and hence cannot be utilized by the plants combined application of rock phosphate with phosphate solubilizing microorganisms has emerged as a logical solution to this issue. The selection of microorganisms capable of solubilizing phosphorus (P) from rock phosphates (RP) may contribute to reduce the dependence of imported fertilizers in grain crops, reducing the costs of agricultural production, and also the environmental impacts. In the present study bacterial and fungal strains were isolated from waste water, soil and sediment sample of ETP of Paradeep phosphate limited (PPL) and from Mahanadi river estuary (Paradeep), having potential to solubilize insoluble inorganic phosphates were characterized. Each isolates were tested for their tricalcium phosphate (TCP) and low grade rock phosphate (RP) solubilization efficiency in both solid and liquid medium. From the experiment it is found that out of 22 fungal isolates more than 50% were classified as efficient. There were significant differences in the availability of P among strains. From the Experiment it is also revealed that the solubilizing activity of both phosphates was associated with a reduction of pH which suggests that the acidification of the culture medium can be one of the mechanisms involved in the solubilization of P. Total number of 29 bacterial strains and 22 fungal strains are taken for experiment and studied for maximum solubilization of insoluble inorganic phosphorous. As an important bioremedial tool, the growth of microbe and substrate consumption is studied in the present study. From Monod kinetics, an equation is formulated to calculate the maximum cell growth in batch culture using certain parameters and optimal conditions. The contribution of these strains to increase the phosphorus nutrition of grain crops should be investigated further by *in vivo* experiments. From the above study, it is concluded that More than 40% isolates of bacterial and fungal strains show the effect of phosphate solubilizing activity. The bacterial strain with 11B and fungal strain with 6F has highest phosphorous solubilization. From the maximum specific growth rate it can be concluded that the microbial isolates 6F and 11B are copiotrophs.

Keywords: Isolation, tricalcium phosphate, paradeep phosphate limited, rock phosphate biosolubilization, phosphorus, kinetics, bioremedial

1. Introduction
In the soil, Phosphorus is one of the significant plant nutrients that are abruptly available. Phosphorus is imperative for morphological, physiological and biochemical growth of plants. It plays a vital part in plant germination. It holds an indispensable nutrient for plants which is an imperative integration of nucleosides, nucleotides, phospholipids etc. The plant will not accurately grow without an adequate quantity of phosphorus. A sufficient quantity of phosphorus in the initial stages of plant maturity improves physiological functions including initial root development and is essential for putting down the primordia for generative parts of plants. Externally phosphorus, crops do not transfer full yield, and animals do not succeed. Low Phosphorus levels in soils decrease crop yields by well over 50%. There are numerous animal illnesses associated with inadequate Phosphorus intake among which milk fever in high yielding cows. Lack of access to phosphorus (and other fertilizers) is one of the significant problems of agriculture in some areas.

In geological age, rocks and sediments especially phosphorous forms the largest reserve prior to minerals are concerned. The aspect of fixation and precipitation of P is extremely reliant on soil nature and pH. Therefore, iron fixes phosphorous in acid soils and Ca fixes it in alkaline soils. A broad scope of microbial Phosphate solubilization mechanisms endures in nature and many of the global cycling of soil phosphates are connected to bacteria and fungi.

Phosphate solubilising microorganisms (PSM) implies a collection of propitious organisms proficient of hydrolyzing inorganic phosphorus from insoluble compounds. Pseudomonas, Bacillus, and Rhizobium are the usual dominant phosphate solubilizers. Mineral phosphate solubilization transpires essentially through the generation of organic acids, and acid phosphates play a very influential part in the mineralization of organic phosphorus in soil. The phosphate solubilizing microorganisms, in addition, to provide Phosphorus to plants further facilitate plant germination by other mechanisms.

According to the basic concepts of microbiology, the growth of bacteria and fungus is linearly formulated by Jacques Monod. Although the concept remains simple still few basic concepts remains unanswered. Monod's work in 1950's implicated the basis of growth for bacteria, fungus, and remains stringent for few decades. In Industries, the physiology of bacteria and fungus is greatly helpful is optimizing.

Growth curves reflects the maturity of an individual, particularly how the individual's growth takes periodically. The growth of bacteria and fungus is characterized by succession of phases like lag phase, exponential phase, stationary phase and decay phase. The growth of bacteria and fungus is strictly depended on few factors like temperature, pH, growth media, substrate concentration. Summing up, Monod model derived the interrelationship between growth rate and the substrate concentration.

In the present study bacterial and fungal strains were isolated from waste water, soil and sediment sample of ETP of Paradeep phosphate limited (PPL) and from Mahanadi river estuary (Paradeep), having potential to solubilize insoluble inorganic phosphates were characterized. Each isolates were tested for their tricalcium phosphate (TCP) and low grade rock phosphate (RP) solubilization efficiency in both solid and liquid medium. Among 22 fungal isolates more than 50% were classified as efficient. There were significant differences in the availability of P among strains. The solubilizing activity of both phosphates was associated with a reduction of pH which suggests that the acidification of the culture medium can be one of the mechanisms involved in the solubilization of P. Total number of 27 bacterial strain and 27 fungal strain are investigated from which the highest insoluble inorganic phosphorous concentration is considered. From Monod kinetics, an equation is designed to calculate out the maximum cell growth in batch culture using certain parameters and optimal conditions. The contribution of these strains for increasing the phosphorus nutrition of grain crops should be investigated *in vivo* experiments

1.1 Growth kinetics of bacteria

Kinetic growth refers the rate at which the unicellular cells turns into a defined mass. The growth kinetics was carried out in batch growth, that refers to culturing cells in a vessel with an initial charge of medium that is not altered by further nutrient addition/removal. In microbiology, concentration of substrate and specific growth rate, is one of the basis to measure the growth of bacteria. Growth of bacteria follows the concept of binary fission; where one cell divides into 2 cell, 2 cell divides into 4, 4 cell divides into 8. If the cell growth takes place in a similar fashion, then we can easily plot the graph between cell count vs time.

2. Materials and Methods

2.1 Soil and water sample collection

Low grade rock phosphate was collected from Hindustan Zinc limited, Udaipur. Marine soil. Water and sediment sample has been collected from the vicinity of Paradeep Phosphates Limited (PPL) Paradeep, Odisha for isolation of phosphate solubilizing microbes which is shown in Table 2. Prior to sampling the collection bottles has been rinsed well and then filled up to neck and stoppered immediately to prevent any accidental entry/escape as well as interaction with outside atmosphere. It is composed of 7.5% total P,30% total Ca,0.6% total Mg,3.82% total Fe,0.9% total Na,2.93% total Al,15.45% total Si,1.23% total D,0.16%total total K,0.23% total T;10.3 mg kg^{-1} total Mn,45 mg kg^{-1} total La respectively. The following chemical composition is shown in table 1 for soil and rock phosphate (RP)

Table 1: Chemical composition of soil and rock phosphate

Parameters	Soil	Phosphate rock
pH	5.5± 0.2	7.5± 0.63
EC (ds/m)	0.12 ±0.02	0.92± 0.003
Bulk density (g/cm^3)	1.04± 0.02	2.8± 0.008
Organic carbon (%)	0.08± 0.04	0.002± 0.03
WHC (%)	35.23± 0.02	62± 5.3
Total N (ppm)	0.94± 0.003	540± 13.2
Total P (%)	1.5± 0.006	8.3± 2.8
Total K (%)	0.05± 0.0004	0.35± 0.002
Available N (ppm)	0.32± 0.02	0.003 ±0.0006
Available P (ppm)	4.4± 0.02	13.2 ±0.03
Available K (ppm)	6.2± 0.08	2.8± 0.8
Na (ppm)	4.06± 0.005	8300± 12.4
Mg (ppm)	1.32± 0.004	5400± 25.6
Cr (ppm)	0.319 0.0002	207 ±10.3
Co (ppm)	0.17± 0.0003	13.5± 3.8
Ni (ppm)	0.008± 0.0005	76.2 ±21.8
Pb (ppm)	0.056± 0.003	6.8± 0.8

Table 2: Different Sampling Stations at Paradeep Municipality

S. No.	Name of sampling station	Distance from PPL
1.	Drainage Canal	0.5km
2.	Shiva Temple	2km
3.	Ramachandipada	3km

2.2 Pure culture of microbes

2.2.1 Pure culture of fungus

A pure culture is developed from a mixed culture, by transferring the culture into a new sterile medium in order to isolate unicellular cells. In the present study, the pure culture of the fungus was spread on Potato Dextrose Agar (PDA) plates for fungus growth. PDA plates were made by dissolving 39gm of PDA in 1000ml of distilled water. This was then sterilized by autoclaving at a pressure of 15psi and temperature 121 ^{O}C for the duration of 45 minutes and then poured into empty Petri plates. With the help of autoclave and UV sterilization, the glassware was sterilized. Once it reaches room temperature the plates were streaked and incubated for 2-3 days and was kept in inverted position in order to avoid water vapor

2.2.2 Pure culture of bacteria

A pure culture is developed from a mixed culture, by transferring the culture into a new sterile medium in order to isolate unicellular cells. In the present study, the pure culture of the bacteria was spread on Nutrient Agar (NA) plates for bacteria growth. NA plates were made by dissolving 28 gm. of NA in 1000 ml of distilled water. This was then sterilized by autoclaving at a pressure of 15psi and temperature 121 ºC for the duration of 45 minutes and then poured into empty Petri plates. With the help of autoclave and UV sterilization, the glassware was sterilized. Once it reaches room temperature the plates were streaked and incubated for 2-3 days and was kept in inverted position in order to avoid water vapor.

2.3 Kinetics study methods

For the physical and chemical environment, growth of microbes is essential. Growth is an outcome of duplication of cell mass. Extracting nutrients from the medium and converting them into biological compounds is the process of microbial activity. By nutrient utilization, the cell growth and microbial mass increases w.r.t. to time and is simply described as

$$\text{Substrates + cells} \longrightarrow \text{Extracellular products + increase cells.}$$

Microbial growth is directly proportional to cell concentration. It is a good synonym of autocatalytic reaction. The rate growth step is characterized by

$$\mu_{net} = \frac{1}{X}\left(\frac{dx}{dt}\right)$$

Where, μ_{net} is specific growth rate (h^{-1}) and X is cell mass concentration (mg/ml) [15, 18, 19].

2.3.1 Microbes and media preparation

The organism isolated with the highest phosphorous solubilization efficiency are Fungus 6F and Bacteria 11B. The strain 6F fungus has a black color velvet morphology whereas the bacteria 11 B has a rod shaped white color morphology. The medium used was rock phosphate (RP) broth. The rock phosphate used was in the particle size of -75+45. The RP broth was prepared in the following manner.

Table 3: Ingredients of RP broth medium

Composition	gram/ml
Glucose	10
Yeast Extract	0.5
$(NH_4)_2SO_4$	0.5
Magnesium Sulphate ($MgSO_4.7H_2O$)	0.1
Rock Phosphate	10
NaCl	0.2
KCl	0.2
$MnSO_4.2H_2O$	0.002
$FeSO_4.7H_2O$	0.002
Agar	1.5

The pH of the broth was adjusted to 7.5. It was sterilized by autoclaving at 121 ºC and 15 psi pressure for 45 minutes. Then the broth was cooled and the initial phosphorous concentration was measured that accounted to 12 ppm.

2.3.2 Inoculation: The glassware's and the laminar air flow chamber was sterilized by autoclaving and UV sterilization respectively.50ml of the minimal media was added to each conical flask. Homogenous liquid aerobic cultures of the Fungus 6F and Bacteria 11B were obtained by growth in 50ml conical flask on a rotary shaker.1 ml of the respective suspension was added to the 50 ml minimal media flask and swirled to mix, sealed with cotton plug. The conical flask were incubated for 24,48,72,96 hours respectively on a rotary shaker at about 150 rpm and a temperature of 35 ± 3 ºC.

2.3.3 Harvesting: Once the growth formation starts, the flask were harvested at respective time periods. The broth culture was found to be turbid. The cell mass concentration (mg/ml), pH, phosphorous concentration (ppm) were measured.

2.3.4 Cell mass concentration: The determination of cellular dry weight is the most commonly used direct method. Samples of culture broth were centrifuged at 10000 rpm, -4 ^{O}C for 10minutes and washed with distilled water. The washed wet cell mass was then dried at 60 ^{O}C for 24 hours; then dry cell weight is measured.

2.3.5 pH: The pH of the culture broth samples were measured by water analyzer. The fungal pH varied in the range of 5-7 whereas the bacterial pH was in the range of 7-7.5.

2.3.6 Phosphorous concentration: The phosphorous standard was prepared by Bray's extraction method i.e. by using ammonium molybdate and stannous chloride solution. Samples of the culture broth were taken and phosphorous availability was calculated at 660nm with standard Monopotassium phosphate by using Spectrophotometer.

2.4 Calculation of the growth kinetic parameters specific growth rate (μ) and saturation or monod constant (K_S)

The effect of substrate concentration on specific growth rate (μ) in a batch culture is described with the relation known as the Monod rate equation given below

$$\mu = \mu_m\left(\frac{S}{K_s + S}\right)$$

Where μ is the specific growth rate, μm is the maximum specific growth rate in h-1, K_S is saturation or Monod constant and S is substrate in g^{-1}. The linearized form of the Monod equation is:

$$\frac{1}{\mu} = \left(\frac{Ks}{\mu m}\right)\frac{1}{s} + \frac{1}{\mu m}$$

Specific growth rates (μ) of the culture at different substrate concentrations were determined from the slope of semi-natural logarithmic plot of biomass vs. time and finally from the intercept and the slope of the 1/μ vs. 1/S plot a μm and Ks value is calculated.

3. Results and Discussion

Fig 1: Pure culture of fungus [6F]

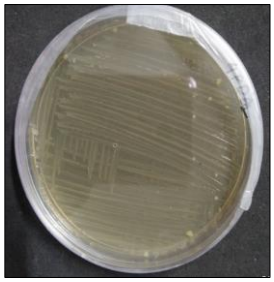

Fig 2: Pure culture of bacteria [11B]

3.1 Effect of particle size

Table 4: Particle size of rock phosphate and solubilization

Particle size (microns)	Phosphorous concentration (ppm)
500	0
250	6.8
180	10.1
150	10.5
106	8.7
75	11.9
45	9.4

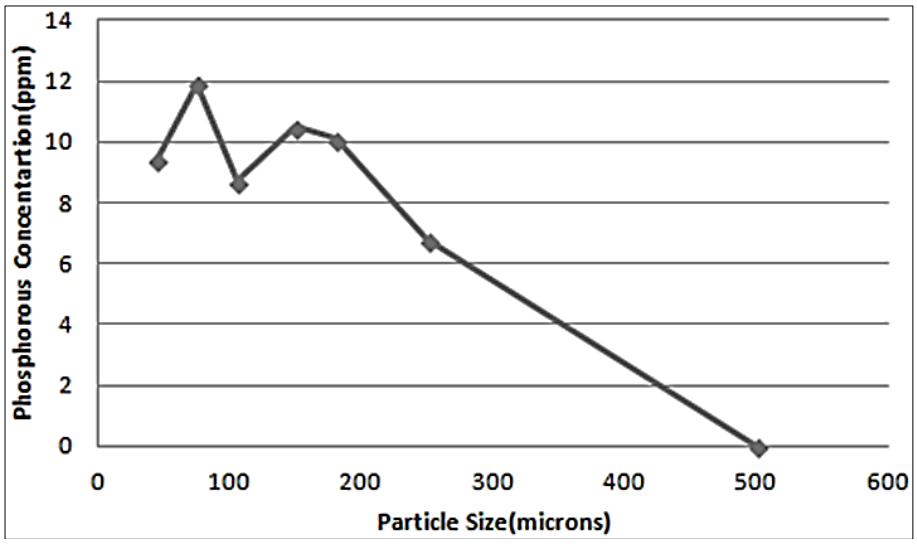

Fig 3: Particle size w.r.t. phosphorous concentration

The results of rock phosphate solubilized, are presented in Table 4. It is worth noted from figure 7 that with the increase in particle size the solubilization rate decreases and tends to increase the solubilization with the mono size fractions. Maximum solubilization of organisms was obtained when rock phosphate of -75+45 micron was used and the maximum phosphate solubilized amounted to 11.9 ppm.

3.2 Phosphorous solubilization study of fungus in PVK and RP broth

Table 5: Sample w.r.t. pH and phosphorous concentration in PVK broth

Sample	pH	Phosphorous concentration (ppm)
Controlled	7.11	33.3
1F	5.83	25.1
2F	5.6	21.7
3F	5.35	31.3
4F	5.73	25
5F	5.52	26.8
6F	2.69	38.6
7F	3.41	24.5
8F	3	35
9F	5.89	35.1
10F	5.84	23.9
11F	5	22
12F	5.95	39
13F	4.71	32.5
14F	4.52	32.6
15F	2.98	25.7
16F	5.98	18.8
17F	3.18	32.3
18F	5.15	20.3
19F	5.03	28.9
20F	2.71	47
21F	4.56	51.8
22F	4.95	33.3

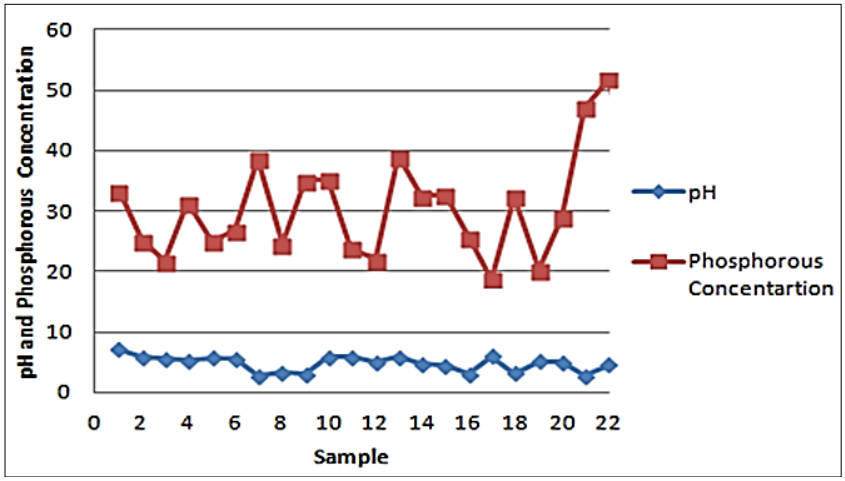

Fig 4: Sample w.r.t. pH and phosphorous concentration in fungus

In figure 4, it is reported that Phosphate solubilization was seen as a major drop in pH (2.5-5.0) of pH 7.21 after 72 hours. Maximum soluble phosphorous concentration varies between 20-52. Which is shown in the figure 8 in the new method, only 33.3 ppm soluble-P was identified as well no drop in pH was noted. The highest P solubilization was recorded by 21F (51.8 ppm) followed by 20F (47.0 ppm) with a highest decline in the pH to 3.6. The minimum concentration was observed to be 18.8ppm in the sample of 16F and pH of 6. Moreover maximum drop in pH was associated with higher levels of P solubilization, in some cases, for example, (12F) where pH was decreased only to 6.0, comparatively higher amounts of soluble-P (39 ppm) was detected in the medium

Table 6: Sample w.r.t. pH and Phosphorous concentration in RP broth

Sample	pH	$P_{conc.}$
1F	8.51	15.3
2F	9.57	28.9
3F	7.31	8.6
4F	7.67	12.5
5F	7.91	8.6
6F	8.21	27.3
7F	7.83	22.2
8F	9.39	8
9F	8.67	8.5
10F	8.47	13.5

11F	7.89	23.4
12F	7.9	14.4
13F	7.21	3.3
14F	8.51	20.2
15F	9.01	12.7
16F	9.43	11.7
17F	7.73	3.1
18F	8.14	9
19F	7.57	5.7
20F	8.89	1.2
21F	9.32	12.9

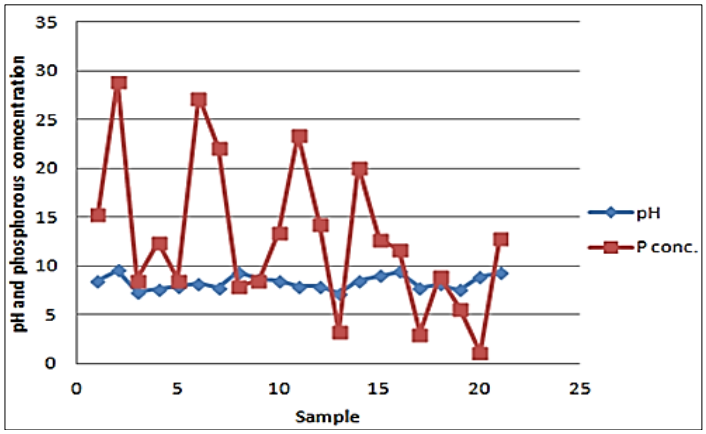

Fig 5: Sample w.r.t. pH and phosphorous concentration in RP broth for fungus

In figure 5, it is reported that Phosphate solubilization was seen as a major variation in pH range 7.2-7.7. Maximum soluble phosphorous concentration varies between 11-29 which is shown in the figure 6 the highest P solubilization was recorded for sample 2 (28.9 ppm) followed by sample 6 (23.6 ppm) with a highest decline of 3.1 ppm in sample 17. The minimum pH was observed to be 7.21 in sample 13 and highest pH observed at sample 2, 9.57. Moreover maximum drop in pH was associated wi.th higher levels of P solubilization.

3.3 Phosphate solubilization study of Bacteria in PVK and RP

Table 7: Effect of pH and phosphorous concentration of bacteria in PVK broth

Sample	pH	Pconc.
1B	5.5	19.1
2 B	6.1	14.3
3 B	6.2	19.9
4B	5.8	9.7
5B	5.3	10.5
6B	5.5	11.5
7B	5.4	6.4
8B	5.8	12.6
9B	6.2	8
10B	6.1	13.7
11B	7.05	67.5
12B	5.6	24.2
13B	6.05	65.9
14B	5.6	10.4
15B	5.7	40.2
16B	5.8	24.7
17B	5.9	33.5
18B	6.1	40.4
19B	6.02	41.4
20B	6.05	40.5
21B	5.54	41
22B	5.39	38.6
23B	6.07	10.2
24B	5.76	39.1
25B	6.02	39.8
26B	6.03	40.2
27B	5.67	9.8
28B	6.03	42.8
29B	5.99	38.9

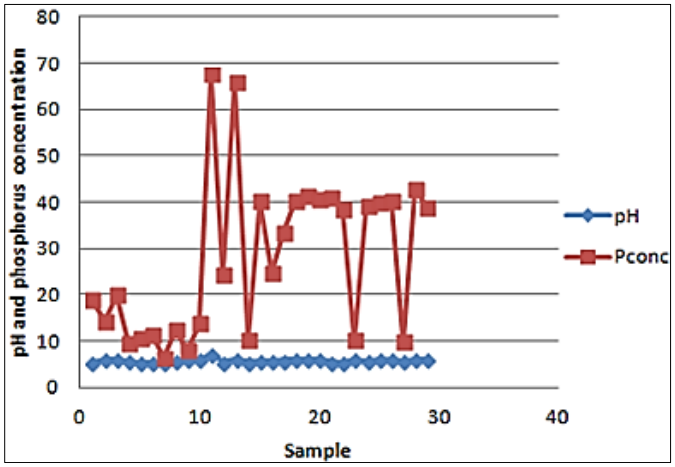

Fig 6: Sample of bacteria w.r.t. pH and phosphorous concentration in PVK

In figure 6, it is reported that, Phosphate solubilization has seen a major variation in the pH of sample 10-14, and phosphorous concentration in the sample of 15-20. Maximum pH is observed at sample Maximum pH of the sample is observed in sample 11 and with a minimum pH at sample 9. Similarly, maximum phosphorous concentration is observed in sample 11 and minimum at sample 8. It is observed that with the increase of pH, high value of phosphorous solubilization takes place.

Table 8: Effect of pH and phosphorous concentration of bacteria in RP broth

Sample	pH	Phosphorous conc., ppm
1B	7.02	25.4
2B	7.76	22.3
3B	5.75	19.5
4B	7.07	14.6
5B	7.38	24.7
6B	7.64	26.8
7B	7.64	29.7
8B	7.56	31.2
9B	7.18	31.2
10B	7	30.1
11B	7.34	42.8
12B	7.59	23.5
13B	7.43	21.8
14B	7.55	32.3
15B	7.36	26.4
16B	7.71	21.2
17B	7.47	29.8
18B	7.49	28.1
19B	7.65	39.2
20B	7.26	12.3
21B	7.46	15.2
22B	7.15	29.6
23B	7.86	27.2
24B	7.01	28.5
25B	6.89	22.3
26B	7.36	24.1
27B	7.44	23
28B	7.25	38.6
29B	6.9	35.4

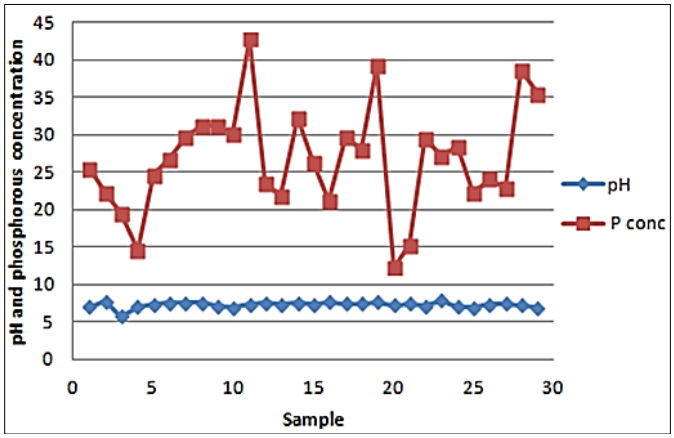

Fig 7: Sample of bacteria w.r.t. pH and phosphorous concentration in RP broth**.**

Maximum phosphorous solubilization is shown in the sample 11 with phosphorous concentration of 42.8 ppm and highest pH is observed at sample 2. Variation of phosphorous concentration is between sample 5-15, lowest phosphorous concentration is observed at sample 20 and lowest pH at sample 29 [17, 19].

3.4 Kinetic growth calculation

3.4.1 Effect of pH of bacteria and fungus w.r.t time

Table 9: Time variation w.r.t pH for bacteria and fungus

Time, hrs	pH, Bacteria 11B	pH, Fungus 6F
0	7.5	7.5
24	7.05	5.39
48	7.12	6.55
72	7.12	6.74
96	7.17	6.90

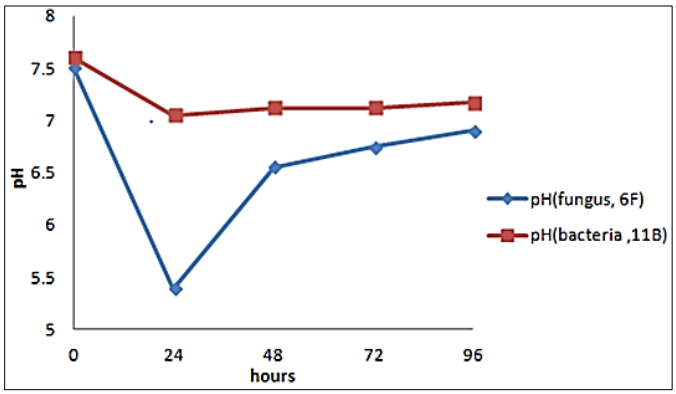

Fig 8: Sample of bacteria and fungus w.r.t. pH and hours

From the figure 8, it is noted that pH varies because of the production of organic acid. There are also several mechanisms such as production of bacterial metabolites and siderophores that leads to solubilization. In microbial-mediated P-solubilization, the addition of 0.1 or 0.2 g RP to media raises the pH of the media by 0.5-1.0 pH units. This was attributed to the carbonate component of the RP.

3.4.2 Effect of Phosphorous concentration of bacteria and fungus w.r.t. Time

Table 10: Time variation w.r.t. P_{conc} bacteria and fungus

Time, hrs	P_{conc}, bacteria 11B	P_{conc}, fungus 6F
0	12	12
24	7.7	13.2
48	15.1	18.6
72	5.9	10.6
96	5.3	11.1

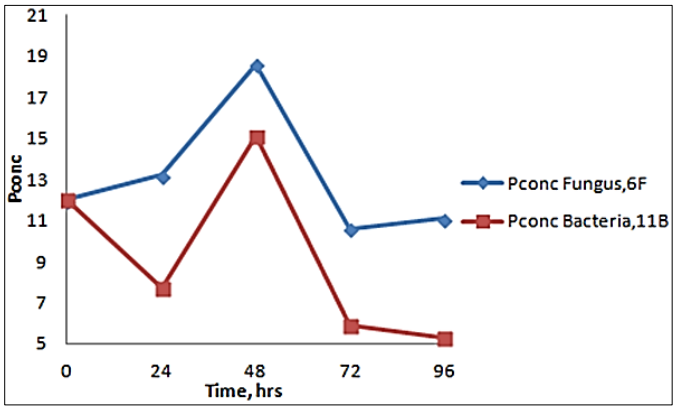

Fig 9: Sample of bacteria and fungus w.r.t. pH and P_{conc}

From the figure it id reported that highest phosphorous concentration was at 48 hours for bacteria 15.1 ppm and fungus 18.6 ppm.

3.4.3 Bacteria kinetics growth calculation

Table 11: Bacteria growth w.r.t time

Time, hrs	S, mg/ml	μ, hr^{-1}	1/s	1/μ	μ_{max}
0	0	0	0	0	
2	0.05	0.0005	20	2000	
4	0.075	0.0008	13.33333	1250	
6	0.1	0.0012	10	833.3333	
8	0.12	0.0015	8.33333	666.6667	
10	0.15	0.002	6.666667	500	
12	0.2	0.0025	5	400	
14	0.21	0.0028	4.761905	357.1429	
16	0.28	0.0031	3.571429	322.5806	
18	0.3	0.0033	3.333333	303.0303	
20	0.34	0.0035	2.941176	285.7143	
22	0.4	0.00394	2.5	253.8071	
24	0.5	0.00397	2	251.8892	
26	0.6	0.003971	1.666667	251.8257	0.00398
28	0.7	0.003972	1.428571	251.7623	
30	0.8	0.003973	1.25	251.699	
32	0.85	0.003974	1.76471	251.6356	
34	0.9	0.003975	1.111111	251.5723	
36	1.	0.003976	1	251.5091	
38	1.07	0.003977	0.9345579	251.4458	
40	1.09	0.003978	0.917431	251.3826	
42	1.13	0.003979	0.884956	251.3194	
44	1.14	0.00398	0.877193	251.2563	
46	1.172	0.00398	0.853242	251.2563	
48	1.175	0.00398	0.851064	251.2563	
72	1.178	0.00399	0.848896	251.6266	
96	1.801	0.00108	0.565247	929.9259	

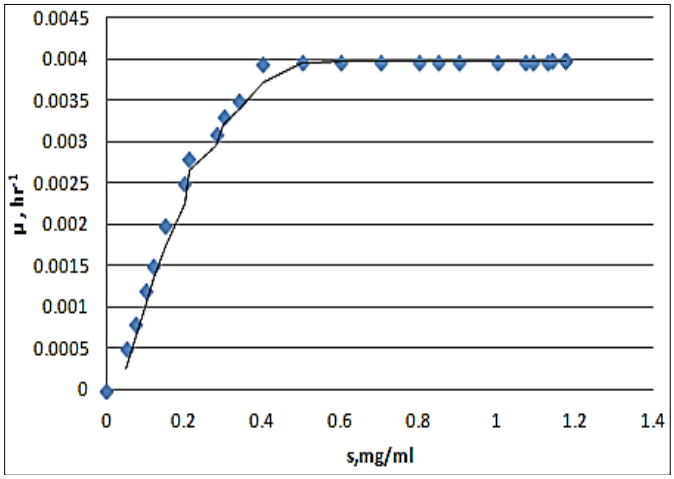

Fig 10: Graph between s w.r.t. μ for bacteria.

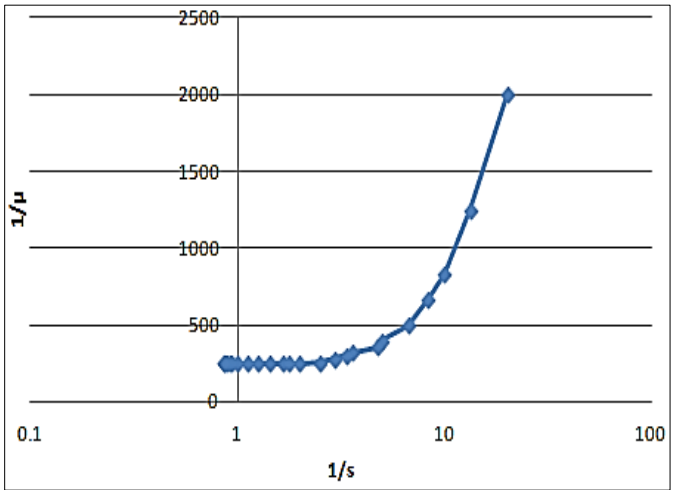

Fig 11: Graph between 1/s w.r.t. 1/μ for bacteria.

For 24 hr,
s =0.5
μ =0.00397

$\frac{K}{\mu max}$ = 0.0021

K = 0.000008358
We know that,

$\mu = \mu_m \frac{S}{Ks+S}$

Putting the values we get, μ_m = 0.00398.
Similarly for the substrate concentration of s = 1.175, 1.178, 1.801 we get, μ_m = 0.00398.
From the table, it is observed that the growth rate tends to increase with the subsequent time interval 0, 24, 48, 72, 96. A graph is plotted between S and μ, from which the slope $\frac{K}{\mu max}$ is noted, from which K value is calculated to be 0.000008358.Taking the Monod kinetics into consideration μ_m value is calculated to be 0.00398.

3.5 Fungal kinetics growth calculation

Table 12: Fungus growth w.r.t. time

Time, hrs	S, mg/ml	μ, hr^{-1}	1/s	1/μ	μ_{max}
0	0	0	0	0	
2	1	0.01	1	100	
4	2.4	0.0015	0.416667	66.666667	
6	2.98	0.0023	0.33557	43.47826	
8	3.4	0.0028	0.294118	35.71429	
10	3.75	0.003	0.266667	33.333	
12	4.1	0.0034	0.243902	29.41176	
14	4.76	0.004	0.210084	25	
16	5.4	0.0054	0.185185	18.51852	
18	5.75	0.061	0.173913	16.39344	
20	6.2	0.068	0.16129	14.70588	
22	6.98	0.0758	0.143266	13.19261	
24	7.24	0.0774	0.138122	12.9199	
26	8.5	0.07745	0.117647	12.91156	0.078
28	10.6	0.07748	0.09434	12.90656	
30	12.3	0.0775	0.081301	12.90323	
32	14.6	0.07752	0.068493	12.8999	
34	15.6	0.077	0.064103	12.98701	
36	16.8	0.077	0.059524	12.98701	
38	17.4	0.077	0.057471	12.98701	
40	18.4	0.077	0.054348	12.98701	
42	20.93	0.077	0.047778	12.98701	
44	21.84	0.077	0.045788	12.98701	
46	22.34	0.077	0.044763	12.98701	
48	22.57	0.077	0.43535	12.98701	
72	23	0.078	0.043478	12.82051	
96	29	0.08	0.034483	12.5	

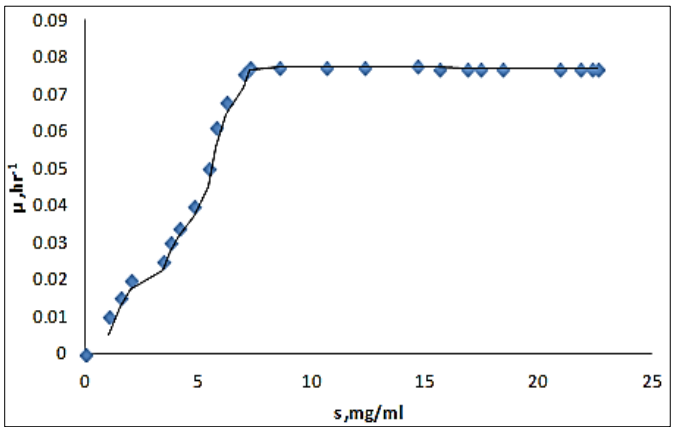

Fig 12: Graph between s w.r.t. μ for fungus.

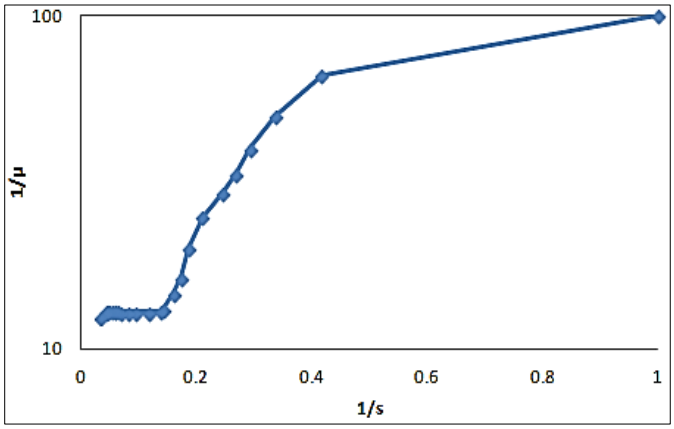

Fig 13: Graph between 1/ s w.r.t. 1/μ for fungus.

For 24 hrs,
S = 7.24

μ = 0.0774

$\frac{\mu}{\mu \max}$ = 0.003

Ks = 0.000234
We know that,

$\mu = \mu_m \frac{S}{K+S}$

Putting the values we get, μ_m = 0.078.
Similarly putting the value of 22.97, 23 and 29 we get, μ_m = 0.078.
From the figure, it is observed that the growth rate tends to increase with the subsequent time interval 0, 24, 48, 72, 96. A graph is plotted between S and μ, from which the slope $\frac{K}{\mu max}$ is noted, from which K value is calculated to be 0.000234. Taking the Monod kinetics into consideration μ_m value is calculated to be 0.078.

4. Conclusion

The present investigation of bacterial and fungal strains were isolated from waste water, soil and sediment sample of ETP of Paradeep phosphate limited (PPL) and from Mahanadi river estuary (Paradeep), having potential to solubilize insoluble inorganic phosphates were characterized. More than 40% isolates of bacterial and fungal strains shows the effect of phosphate solubilizing activity. The bacterial strain with 11B and fungal strain with 6F has highest phosphorous solubilization. The present study also highlighted the growth and utilization of microbes using inorganic chemicals. Nowadays the most important prospect of bio industry is the control of optimum factors. For this particular reason an relationship is established to know the cell growth at each time period and the expression formulated will be used for further microbial processes. But the unreal structure makes the kinetic study difficult because of non-uniformity, use of heterogeneous medium. In the present study, the main objective was to find out the optimizing conditions influencing batch growth. The parameters were evaluated using optimum conditions.

- Different graphs were plotted of fungus and bacteria for PVK broth and RP broth and it is worth noted that with the decrease of pH value the solubilization rate decreases.
- The graph plotted for fungi and bacteria between dry weight w.r.t. to time assumes to be a sigmoid shape. Maximum dry weight is being observed at particular 72 hours.
- For bacteria from the table, it is observed that the growth rate tends to increase with the subsequent time interval 0, 24, 48, 72, 96. A graph is plotted between S and μ, from which the slope K/μ_{max} is noted as 0.0021, from which K value is calculated to be 0.000008358. Taking the Monod kinetics into consideration μ_{max} value is calculated to be 0.00398.
- For Fungus from the table, it is observed that the growth rate tends to increase with the subsequent time interval 0, 24, 48, 72, 96. A graph is plotted between S and μ, from which the slope K/μ_{max} is noted as 0.003, from which K value is calculated to be 0.00023.Taking the Monod kinetics into consideration μ_{max} value is calculated to be 0.078.

- From the maximum specific growth rate it can be concluded that the microbial isolates 6F and 11B are copiotrophs.
- Overall the present study serves an initial point for the future studies in terms of biomass calculation which is an essential factor in the estimation of kinetic parameters.

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