Chapter X

Column Studies and Employment of Spent Materials as Nutrients for Phosphorus Solubilizing Bacteria (PSB) and Vegetation

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10.1 Column Experiments

Batch mode studies are the basic pilot studies performed to screen the sorption efficiencies of biosorbent in the removal of specific adsorbates through the determination of optimum conditions. However, the data obtained from batch studies are limited to the laboratory scale. Therefore, in order to scale up the methodology, continuous column flow screening has to be facilitated, before adopting the sorption process to the field levels[1]. Results of Batch equilibration method insist the feasibility and compatibility of the prepared TETS material and their magnetite beads (M@TETSB) as potential promising adsorbents. Fixed bed columns are effective in quantifying the sequestrating nature of sorbent materials. for the treatment of industrial leachates, through varied adsorption/ desorption cycles[2]. In view of this, the suitability of aforesaid materials was operated in column schemes to trap PO_4^{3-}, NO_3^-, SO_4^{2-} ions from aqueous solutions and further, extended to the collected effluent from the point source. The particle size of the sorbent was fixed as 0.71 mm for column operations, to minimize agglomeration at higher doses.

10.2 Fixed Bed Column – Short Term / Long Term Analyses

Fixed-bed column experiments were designed to conduct short term and long term analyses, in order to assess the sorbing ability of the studied materials. Cylindrical glass tubes of different required dimensions are tabulated below:

Analysis	Dimensions	
	Diameter (cm)	Height (cm)
Short term	3.5	30
Long term	5	50

The schematic diagrams of fixed-bed adsorption setup are shown in figures 10.1 & 10.2 referring to short term and long term column runs. Appropriate doses of TETS and M@TETSB, were packed between two supporting layers of glass wool/glass beads, to prevent channelling, support even distribution of sorbate species in the process of preventing sorbent loss from the packed bed and also avoidance of outlet clogging[3]. Operating parameters viz., doses, flow rate, bed depth were fixed through pilot reactions by pouring aqueous anionic solutions of appropriate concentrations from column top. The flow rates were adjusted through trial and error setup. Residual concentration of the anions were analysed using UV-Vis spectrophotometer, after collecting the samples through control value fixed at the column bottom.

The recorded values exhibiting maximum removal are noted in tables 10.1 and 10.2, wherein, TETS recorded marked removal. Better anion trapping ability of TETS than M@TETSB, shall be due to close packing of the coarse modified material against the filling gaps found between the sample beads[4]. This facilitates extensive reticulation in case of the former, promoting greater contact time between sorbent- sorbate species.

Figure 10.1 Short term Analysis

Figure 10.2 Long term Analysis

Table 10.1 Short term Analysis

Adsorbent	Sorbent Dose (g)	Flow Rate (mL/min)	Bed Depth (cm)	Aqueous Solution	Initial Conc. (mg/L)	Percentage Removal (%)
TETS	15	20/5	5	PO_4^{3-}	100	85.3
				NO_3^-	100	81.6
				SO_4^{2-}	250	83.2
M@TETSB	5	10/5		PO_4^{3-}	100	63.8
				NO_3^-	100	60.2
				SO_4^{2-}	250	62.7

Table 10.2 Long term Analysis

Adsorbent	Sorbent Dose (g)	Flow Rate (mL/min)	Bed Depth (cm)	Aqueous Solution	Initial Conc. (mg/L)	Percentage Removal (%)
TETS	40	50/5	10	PO_4^{3-}	100	99.9
				NO_3^-	100	98.5
				SO_4^{2-}	250	99.7
M@TETSB	25	50/5		PO_4^{3-}	100	75.5
				NO_3^-	100	70.9
				SO_4^{2-}	250	72.3

Column run for the first cycle resulted in collection of 22 and 18 litres for TETS and M@TETSB packing. At the end of the process, 70% removal was registered, beyond which the column materials appeared to be completely exhausted. In order to explore the reusability of the spent sorbent materials, desorption / regeneration studies were planned.

10.2.1 Desorption/ Regeneration Studies

The exhausted materials (TETS/ M@TETSB) were rinsed thoroughly with double distilled water, following by the addition of 1000 mL 0.1N HCl as eluent[5]. Thirty minutes standby period between acid and the packed samples ensured the desorbing process to progress. Finally, the materials were run through distilled water, so as to minimize the acidic nature of the columns. The eluted samples from water / acid media were tested for anionic concentrations, which registered least quantity, implying immobilization of the chosen anions. The regenerated materials were subjected to sorption- desorption cycles, six times, which subsequently, registered a similar trend as before. Beyond this stage, the process did not proceed, indicating complete saturation of the packed sorbents. Data pertaining to the aforesaid studies are given in Table 10.3.

Table 10.3 Desorption / Regeneration Studies

Aqueous Solutions (Litres)														
Cycle	**I**	**II**	**III**	**IV**	**V**	**VI**	**I**	**II**	**III**	**IV**	**V**	**VI**	**Desorption capacity (%)**	
Anions	**Complete Exhaustion**													
	TETS						**M@TETSB**						**TETS**	**M@TETSB**
PO_4^{3-}	18	17	16	15	14	12	15	14	12	11	10	10	99.2	62.1
NO_3^-	17	15	14	13	12	11	12	10	10	9	8	7	98.1	57.7
SO_4^{2-}	18	16	15	14	13	12	13	12	11	10	9	9	98.9	59.5

TETS is preferred to be a propitious material in upscaling the laboratory conditions towards field sample analysis due to its low column saturation property with greater desorbing ability.

10.3 Fixed Bed Column Experiments - Laundry Effluent

The requirement of column dimensions for lab scale treatment is larger compared to those, designed for lab scale experiments. This is because the effluent sample contains many interfering ions, apart from those ions to be analysed, which in turn demands increase in the sorbent load, to match the sorption need under optimized conditions. Therefore, glass

column with diameter, threefold times than employed for long-term analysis was designed for effluent column run as shown in figure 10.3. Invariably, greater dose (150 g TETS) was packed and the column experiments were carried out for the diluted laundry effluent, as per the procedure mentioned in chapter IX under section 9.5. It is evident from table 10.4, that the sorbing ability of TETS declined in subsequent sorption/desorption cycles, implying least possible regeneration at the end of sixth cycle. The tested samples at the end of each cycle exhibited similar results as those accounted in lab scale studies.

Figure 10.3 Effluent Analysis – Column setup

Table 10.4 Effluent Analysis - TETS

TETS	Initial Conc. (mg/L)	Percentage Removal (%)					
		Cycle					
		I	II	III	IV	V	VI
PO_4^{3-}	317	98.5	95.7	92.9	90.7	87.8	85.6
NO_3^-	106	95.4	93.8	90.5	89.6	83.9	80.7
SO_4^{2-}	262	96.3	94.6	91.4	90.2	85.1	82.5

10.4 Breakthrough Curve Analysis and Parameters Design - Fixed Bed Column

The performance of fixed-bed adsorption column is described by the concept of breakthrough curves, indicative of breakthrough time / shapes of the curves. They are the key characteristics for sorption dynamic response and process designing of fixed-bed column as they directly reflect upon the feasibility and economics of adsorption process.[6] Column operating conditions such as influent concentration, flow rate and bed depth, decide the profile of these curves. A plot of breakthrough curves expressed in terms of normalized concentration, defined as the ratio of effluent adsorbate concentration (C_t) to the influent adsorbate concentration (C_0) as a function of time for a given bed height[5] is represented in figures 10.4 & 10.5. The corresponding column parameters along with breakthrough/exhaustion time frames are tabulated in table 10.5.

Percentage removal of anions increased with decreased flow rate and extended bed depth. This may be due to sufficient contact time between sorbent and sorbate species[7]. Also, the rise in residence time of anion solution inside the column permitted the ions to diffuse deeper into the adsorbent. Similar observations were reported by few researchers[8,9].

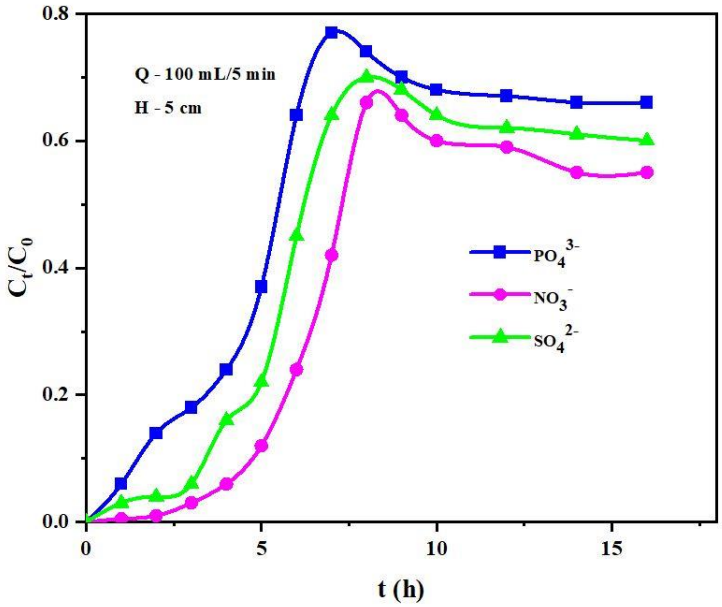

Figure 10.4 Impact of flow rate / bed depth (100 mL / 5min, 5 cm)

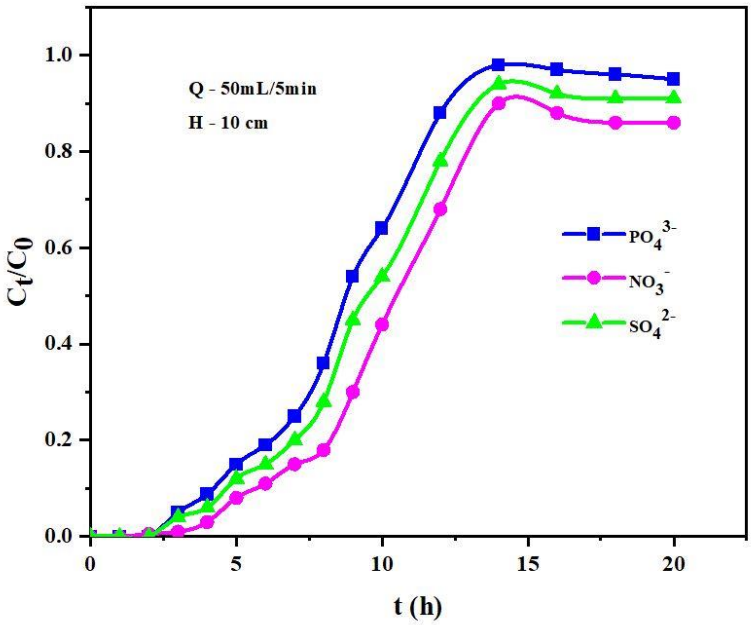

Figure 10.5 Impact of flow rate / bed depth (50 mL / 5min, 10 cm)

Table 10.5 Parameters in Fixed Bed Column for Anion Adsorption by TETS

Anions	C_0 (mg/L)	Q (mL/min)	H (cm)	t_b (h)	t_e (h)
PO_4^{3-}	100	100/5	5	8	16
NO_3^-	100				
SO_4^{2-}	250				
PO_4^{3-}	100	50/5	10	15	20
NO_3^-	100				
SO_4^{2-}	250				

10.4.1 Kinetic Modelling

Kinetic models were applied to analyse the breakthrough behaviour in the process of evaluating the adsorption rate constant and the maximum uptake by the sorbent.[10] Column experimental results were fit into Thomas (fig 10.6), Adams-Bohart (fig 10.7) and Yoon–Nelson (fig 10.8) models. Kinetic constant values calculated from the slopes and

intercepts of the corresponding linear plots along with their respective correlation coefficients (R^2) are summarized in table 10.6.

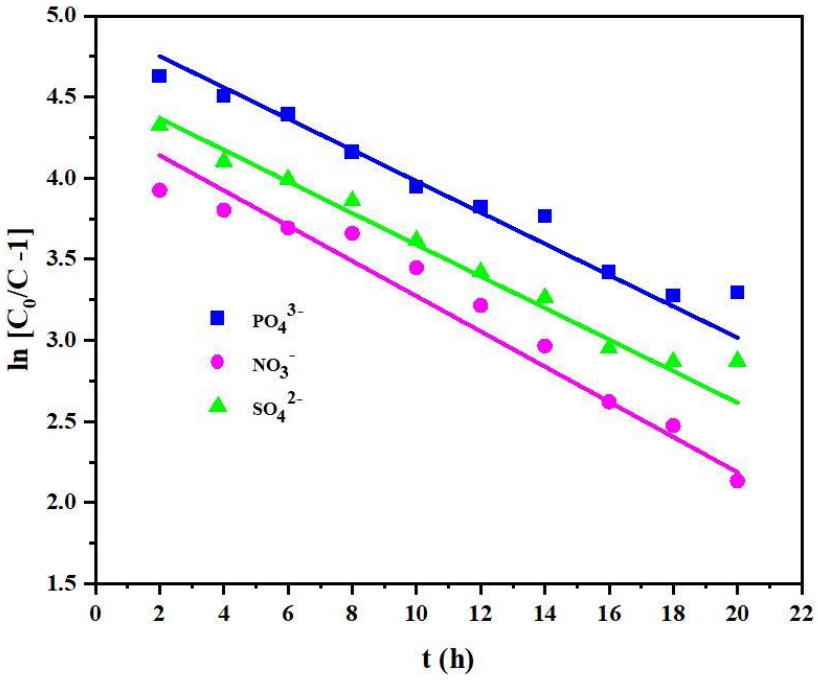

Figure 10.6 Thomas Plot

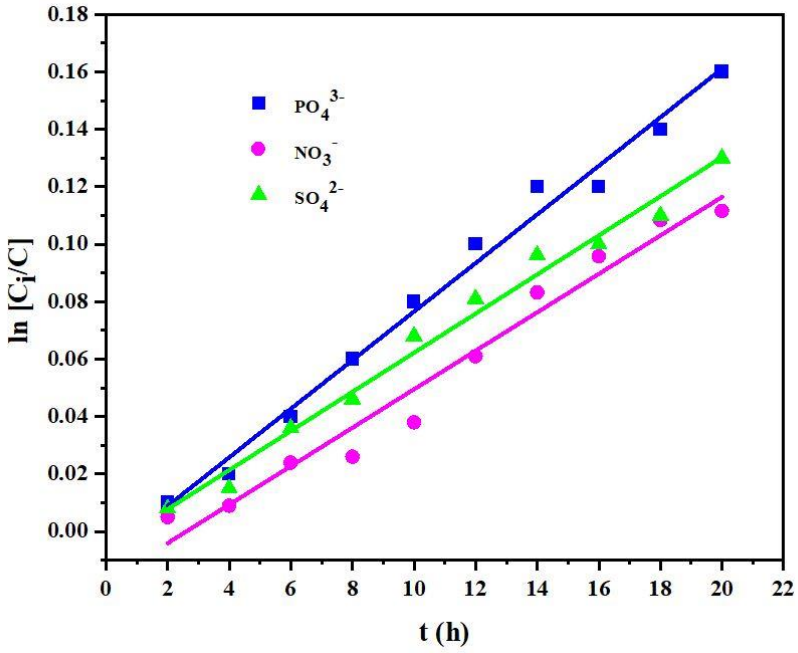

Figure 10.7 Adams–Bohart Plot

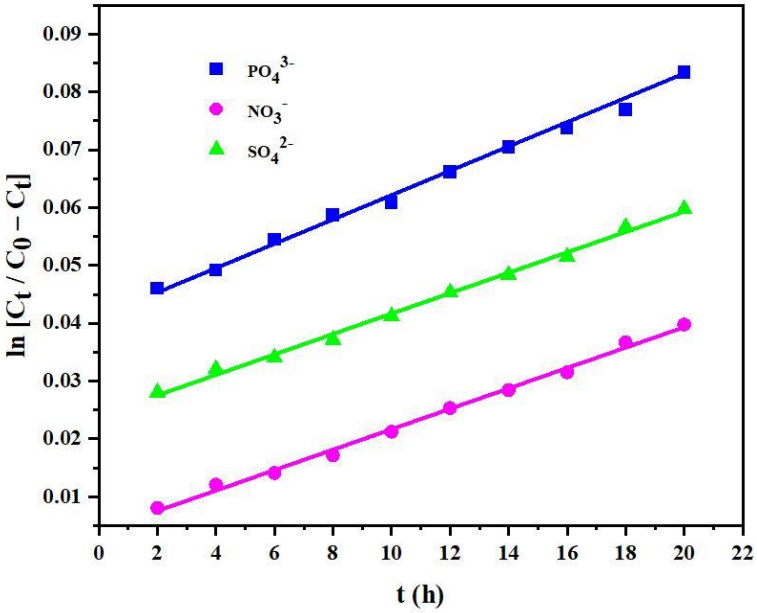

Figure 10.8 Yoon-Nelson Plot

Table 10.6 Kinetic Parameters

Anions	C_0 (mg/L)	F (mL/ min)	H (cm)	Thomas Model			Adams Bohart Model			Yoon-Nelson Model		
				K_{TH}	q_0	R^2	K_{AB}	N_0	R^2	K_{YN}	τ_0	R^2
PO_4^{3-}	100	50/5	10	0.09	8.94	0.9525	0.008	4.86	0.9589	0.21	**45.39**	0.9985
NO_3^-	100			0.05	8.35	0.9303	0.006	4.12	0.9386	0.17	**40.26**	0.9958
SO_4^{2-}	250			0.08	8.56	0.9489	0.007	4.47	0.9513	0.19	**41.98**	0.9932

Adsorption capacity values τ_0 as expressed by Yoon-Nelson model is excellent against q_0 / N_0 values of the other two models. The transient stage of the breakthrough curves are in line with high driving force between the anions present on the sorbent and the anions in the solution, supports better column performance[11], which substantiates the fitness of this model.

10.5 Field Study - Implementation of FRP Column

Fibre Reinforced Polymer column of larger dimensions was fabricated based on the batch and column experiment conducted for both aqueous and effluent samples. This FRP column was installed as a protype model to trap phosphate ions from the enriched effluent discharged at Perfect Laundry Unit, Ootacamund, Nilgiris, Tamil Nadu, India as shown in figure 10.9. References regarding the designed column are detailed below:

Volume: 3 Litre capacity	Dosage: 2 Kg
Diameter: 6”	Particle Size: 0.71 mm
Height: 20”	Column Bed: 15 cm
Maximum Flow rate: 100 mL/ 5 min	pH: 7.2

Phosphate laden TETS was found to exhaust at the end of column operation, were 36 litres of laundry effluent was collected at a span of 12 hours. Desorption and regeneration of the spent material was ensured by the addition of 0.1 N HCI. This material was dried and reloaded, further, second cycle of column operation was monitored to assess the phosphate removal efficiency of the regenerated material. The successful installation and satisfactory functioning of the protype device have been appreciated by the industrial authorities, expressed through a consent letter referred at the end of this chapter.

Figure 10.9 FRP installation

10.6 Application of PO_4^{3-} Enriched TETS as Nutrient

Phosphorus is an essential macronutrient for plant growth and development. 95-99% of it, present in the soil is insoluble and seldom utilized by plants[12]. Solubilization of insoluble phosphate compounds in soil is brought about by microorganisms like Phosphate Solubilizing Bacteria[12] (PSB). In view of this, the modified biosorbent (TETS) laden with phosphate ions, instead of being disposed off as a solid waste, further creating secondary pollution, was tested for its performance as a bionutrient, to enrich the plant growth. This property of phosphate enriched TETS shall be due to the uptake of notable nutrients by the java plum woody shell seeds, this in turn,[13] when used for vegetation purpose, is expected to enhance the soil fertility. Myriad of microorganisms, Pseudomonas and Bacillus are reported to be one of the most powerful phosphate solubilizers[14]. Isolation of these phosphate solubilizing bacterias was carried out as follows:

According to the literature, Pikovskaya's agar medium (PVK) is found to be a selective media for the isolation of PSB[15]. A composition of the agar medium as per table 10.7, was prepared as a control to culture bacterias (Pseudomonas & Bacillus). Apart from this, 5 g of phosphate loaded TETS was added to replace calcium phosphate present in the control to prepare the medium with the exhausted TETS, to serve as enriched PO_4^{3-} nutrient for the bacterial growth. 5 g each of the selected bacteria were added to 20 mL compositions of control (C) / loaded phosphate nutrient (P) media taken in the conical flasks and the flasks were sealed. These sealed conical flasks are shown in figure 10.10. The contents of these flasks were subjected to biological reaction in an autoclave, maintained at a temperature of 70 ^{0}C.

Table 10.7 Composition of Pikovskaya's Agar medium

Composition (gm/mL)	
Yeast Extract	0.5
$(NH_4)_2SO_4$	0.5
$MgSO_4.7H_2O$	0.1
$Ca_3(PO_4)_2$ – Control	5.0
Biosorbent (TETS) - Nutrient	5.0
NaCl	0.2
KCl	0.2
$MnSO_4.2H_2O$	0.002
$FeSO_4.7H_2O$	0.002
Agar	1.5

Approximately, 1 gm of soil collected from an agricultural field in Coimbatore, Tamil Nadu, India was serially diluted five times using distilled water, to remove any impurities present in the sample. 1 mL each of this soil water suspension was poured into four petri plates and inoculated in hot air oven (60 ˚C) for a time period of 2 hours, to ensure the thorough sterilization of these suspensions. Later, these inoculated petri dishes were dispensed with the autoclaved agar media and further incubated (28 ˚C) for about 5 to 7 days to promote the ambience of bacterial growth[16]. The incubated plates with the culture of Pseudomonas and Bacillus bacterias are picturized in figures 10.11 & 10.12 respectively. Left hand side plates referring to controls show seldom growth of bacteria in the agar medium against a notable Pseudomonas culture growth in right hand side plate of figure 10.12. Also, non-growth of Bacillus bacteria in right hand side plate of figure 10.13 suggest that only Pseudomonas bacteria acts as a phosphate solubilizing bacteria. This is confirmed by the colony formation, as indicated by a clear zone against the halo zone as found in Bacillus culture[17]. The Pseudomonas colonies were subjected to morphological testing at Bioline Laboratory Coimbatore, Tamil Nadu, India (fig10.13), where a visible pearlescent

appearance imply that the chosen Pseudomonas is a genus of gram-negative bacteria, favouring plant growth and also inhibiting the influence of pathogenic microorganisms. Also, it is stated in literature that this type of bacteria plays a key role in the synthesis of hormones pertaining to plant growth, contributing to an increased resistance in plants to fight against diseases[18].

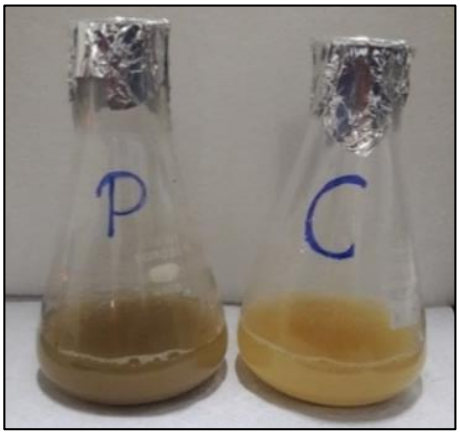

Figure 10.10 Pikovaskaya's Agar medium

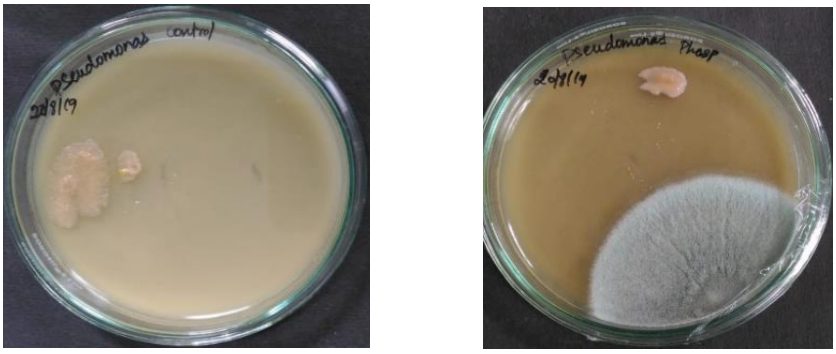

Figure 10.11 Pseudomonas culture

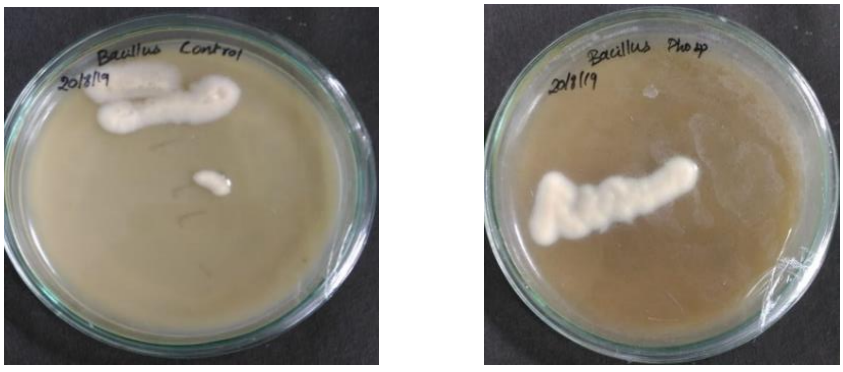

Figure 10.12 Bacillus culture

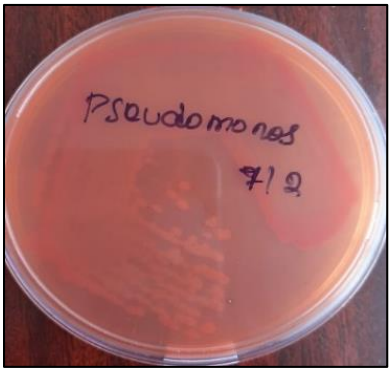

Figure 10.13 Pseudomonas Morphology

10.7 Plant Cultivation Assay

Mentha (mint leaves) and *Raphanus raphanistrum* (pink radish) were the two plants selected to test the nutrient nature of the derived Pseudomonas culture as discussed in 10.6. Seeds of mint and radish were purchased from Nilgiris Cooperative Marketing Society (NCMS), Ootacamund, Tamil Nadu India. 3 g of each of these seeds were added to 20 mL of Pseudomonas bacterial suspension taken in 250 mL beaker and left undisturbed for 2 hours in room temperature[19], to ensure the growing ability of the bacteria upon the surface of the seeds. A similar setup was made using distilled water to serve as the control in another beaker. The soaked seeds from both the beakers were air dried. Four clay pots (25 cm height) were filled with sterilized red soils upto 2/3rd level. Later, the pre treated seeds were sown in four pots: (1 & 2 containing mint seeds with control/nutrient media; pots 3 & 4 with radish seeds under similar environment.

The pots were watered regularly and the growth of the plants was monitored upto a period of 25 days and 90 days for mint leaves and radish vegetable respectively. Shoots growth of these plants are represented as photographs shot at the end of specific days (figs 10.14 & 10.15). These pictures clearly explain the action of nutrient reusability in the enriched growth of mint leaves and fully grown radish with nourished roots (fig 10.16), against those observed in control pots (fig 10.17). From these studies, it is confirmed that phosphate solubilizing bacteria (Pseudomonas) serves as an effective bio-nutrient, for growing plants.

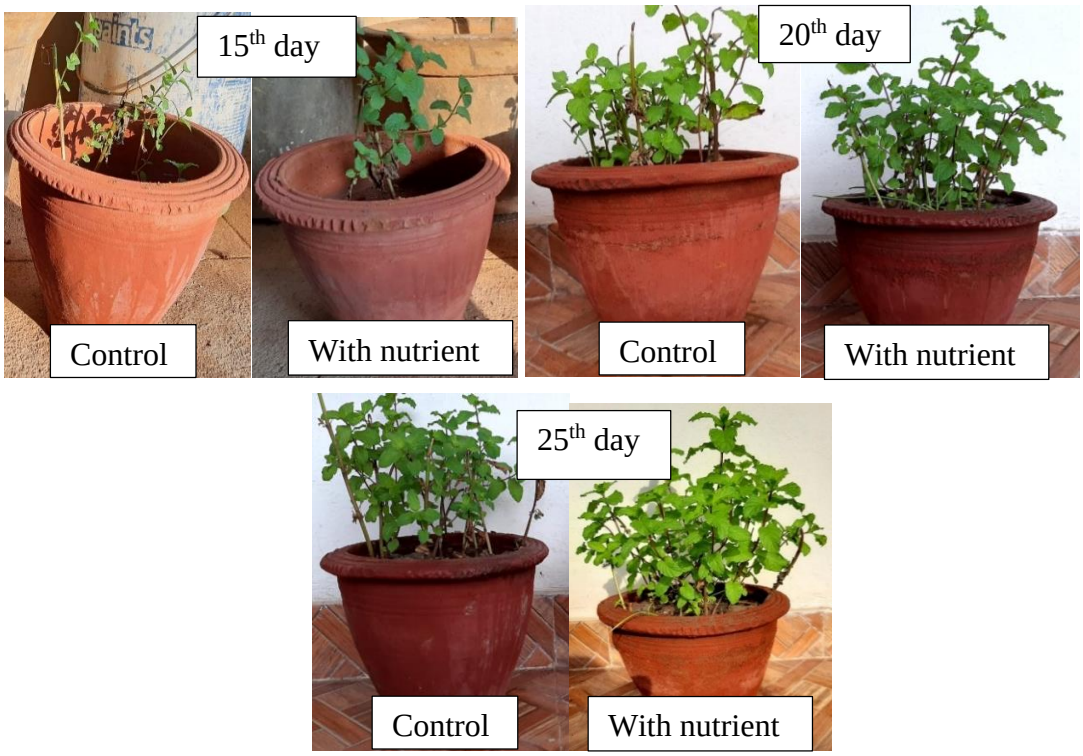

Figure 10.14 *Mentha* Growth

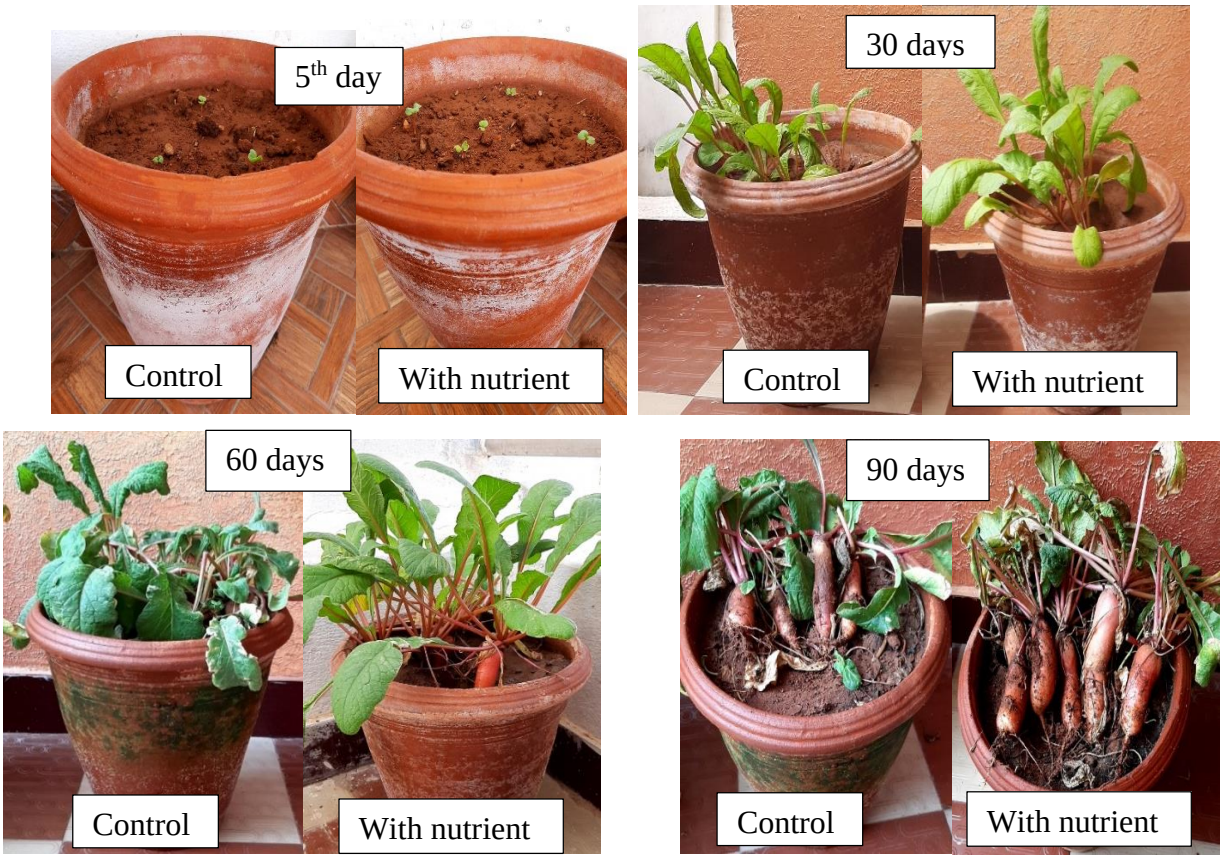

Figure 10.15 *Raphanus raphanistrum* Growth

Figure 10.16 Plant Yield (control) Figure 10. 17 Plant Yield (with nutrient)

10.8 Phytotoxicity Test

Matured sample parts (leaves, stems, roots) of mint leaves and radish were examined to explore the possibility of phosphate bioaccumulation. One gram of the dissected, and were pre-cleaned with double distilled water, shredded into small pieces and dried in an hot air oven (65 ^{0}C)[20]. The dried samples were pounded into a fine mixture. 100 mg these homogenized samples were transferred to 25 mL beaker to which, 8 mL of 65% nitric acid was added slowly. The contents were digested on a hot plate at 700 ^{0}C for 2 hours. The digested residues were cooled, dissolved in 20 mL of 30 % hydrogen peroxide and further diluted to 50 mL[21,22]. The prepared samples complexed with specified reagents recorded seldom phosphate concentration when analysed in an UV-vis Spectrophotometer. Thence, it is obvious that the digested samples of leaves, stems and roots did not have traces of the anion, clearly indicating the solubilization of phosphate ions by the bacterial culture.

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Date: 29/2/2020

LETTER OF CONSENT

This is to acknowledge the work done by Mrs. K. Vivithabharathi, Ph.D Scholar, Department of Chemistry, PSGR Krishnammal College for Women, Coimbatore, towards the installation of Fibre Reinforced Polymer Column packed with a potential sorbent material for the treatment of the Phosphate effluent discharged with a concentration of 317 mg/L from our Laundry unit. Continuous FRP column operations registered 1.2 mg/L of Phosphate ion in the treated sample exhibiting 99.3 % removal.

For PERFECT LAUNDRY SERVICE

Authorised Signatory

Contact No:9865549598;8903915886.
Address: Murugan lodge, Ettines road,Ooty-643001.

10.9 Refernces

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