Nitrogen Fixation in Activated Sludge Process of Pulp & Paper Mill


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ABSTRACT

There is a high potential idea of replacing the artificial nitrogen dosing of activated sludge process (ASP) by natural occurring processes such as bacterial fixation of atmospheric nitrogen. Nitrogen requirement of ASP could be met either by augmenting the system with the efficient isolates or by promoting the growth of nitrogen fixing bacteria already present in the activated sludge. Different isolates were collected from different sources and evaluated for their nitrogen fixation and pollutant removal efficiency. The results of the study revealed that some of isolates were found to be good in terms of nitrogen fixation and pollutant removal efficiency in pulp & paper mill effluent. The study revealed that conventional ASP can be successfully converted to nitro fix ASP by optimizing the system. The nitro fix ASP can be run with zero nitrogen addition with dissolved oxygen (DO) 1.0 mg/L and phosphorus dose (C:P;100:1), at the same time meeting the required treatment goal of pulp & paper mill wastewater.

Keywords: *Activated sludge process, COD, Color, Nitrogen fixation, Nutrient, Pulp & paper mill wastewater*

Introduction

Activated sludge process (ASP) has been widely used to treat pulp & paper mill effluent where removal and stabilization of organic matter is accomplished biologically with a variety of microorganisms, principally bacteria. Nutrients are necessary components for the growth of bacteria as well as to stimulate the production of surface biopolymers (extracellular polymeric substances) which play a part in settling sludge. Dosing of nitrogen and phosphorus is one of the key factors in most pulp & paper effluent treatment plants. Correct dosing is crucial as high dosage might lead to increased discharge of nutrients, increase in operation cost, whereas both low and high dosage may lead to operational problems like poor sludge quality, deprived plant performance etc.

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Performance of activated sludge process depends on the concentration of several key elements including nitrogen where a ratio of 100:5:1 for bioavailable carbon (C) to nitrogen (N) to phosphorus (P) in the feed (raw effluent) is usually recommended (1). Microorganisms involved in the removal of carbonaceous substances utilize nitrogen for the growth, synthesis of proteins, cell wall components and nucleic acids. The biomass has been universally accepted to have the chemical formula $C_5H_7O_2N$ or $C_{60}H_{87}O_{23}N_{12}P$ (1).

Effluent of pulp & paper mills is deficient in nutrients and has low concentration of the key nutrient; nitrogen which is well below the level conventionally recommended for aerobic treatment (2). Most of the nitrogen in the effluent is bound in suspended solids or in chelating agents, the total nitrogen amount can be high, but as those elements are not easily available for the bacteria, nitrogen has to be supplemented (3).

The mills routinely spend large amount on ammonia or urea which are added in the aeration tanks (bioreactors) to permit normal biomass growth (4). It has been reported that the nitrogen can also be provided through nitrogen fixation process (4-5). Nitrogen-fixing activity has also been reported in a number of pulp and paper aerated lagoons (4).

Theoretically the biological fixation of nitrogen requires the following conditions: (i) readily available carbohydrate as an energy source (ii) low fixed-nitrogen concentration, and (iii) absence or very low concentration of dissolved oxygen (4). The presence of ammonium has been reported to reversibly inhibit the nitrogenase enzyme. However, the microbial population was able to fix nitrogen at the BOD:N ratios of 100:0, 100:1.3 and 100:1.8, at feed ammonia concentration of up to 4 mg/L while at higher nitrogen level (BOD:N ratios of 100:2.7 and 100:4.9) nitrogen was lost from the system and the acetylene reduction assay (ARA) was negative (6).

The wastewater from pulp & paper mills is known to contain large number of heterotrophic nitrogen fixing bacteria where members of family enterobacteriaceae mainly Kleibseilla sp. have predominance over others. The presence of nifh or nitrogenase coding gene in the bacterial genome indicates the ability of bacteria to fix atmospheric nitrogen (4). The bacterial nitrogen fixation efficiency can be analyzed either by direct estimation of fixed nitrogen content through Kjeldhal method or by acetylene reduction assay.

In the present research paper, the effect of dosage of nutrients along with DO level has been discussed. The results revealed that some factors are responsible for nitrogen fixation and it is feasible to fix the atmospheric nitrogen using nitrogen fixing bacteria.

Experimental

Isolation of nitrogen fixing bacteria

The nitrogen fixing bacteria were collected from four different sources i.e., lab scale ASP reactor (continuous & batch), soil and commercial sample. Selective isolation was done using nitrogen deficient media. All the isolates were characterized for their morphological features. Acetylene Reduction Assay (ARA) was used for estimation of nitrogenase activity through Gas Chromatography with FID detector. The ethylene production was determined at varying time interval by gas chromatography. All other parameters were tested as per international standard test methods viz. IS and APHA.

MLSS & MLVSS: For MLSS and MLVSS, 100 ml of mixed sludge sample was centrifuged and washed with distilled water before transferring to pre-weighed silica crucible. The sample was oven dried at 103±2 °C for overnight. Dried material was taken as MLSS and the same crucible was ignited at 550 °C and loss in weight was taken as MLVSS.

HRT: For determination of HRT, effluent was collected in bucket for known time for estimation of influent volume and calculated as follow:

HRT (h) = Volume of aeration tank (m^3)/ Influent flow rate (m^3/h)

Results and discussion

The Activated sludge process in pulp & paper mills is provided with artificial source of nutrients like nitrogen to achieve the required treatment efficiency while this can be replaced completely or partially by some natural occurring process i.e., bacterial nitrogen fixation. The present study was aimed to convert conventional ASP into nitro fix ASP by optimizing certain parameters like DO and phosphorus level in order to activate the population of nitrogen fixing bacteria already present in activated sludge.

Optimization of DO level

The impact of varying DO level on the nitrogen fixation & treatment efficiency of nitrogen deficient activated sludge process was studied. The reactors operated at varying DO were not provided with any artificial source of nitrogen as the presence of ammonical nitrogen suppresses the activity of nitrogen fixing bacteria. Four reactors named R1, R2, R3 and R4 were operated where R1, R2 and R4 were nitrogen deficient with varying DO i.e., 1, 2 & 0.5 mg/L whereas R3 was kept as control with nitrogen supplementation at the dose of 100:2.5 and DO≈1. The study was divided into two phases (I & II) and each phase consisted of 25 days. The operating conditions maintained in all the reactors during both the phases were as given in table 1. The MLSS and MLVSS were aimed to be 4.0 g/L and 3.2 g/L, respectively and were able to maintain the same in R1, R2 & R3 while in the case of R4 (DO 0.5) the values were slightly on lower side (i.e., 3.5 g/L & 2.9 g/L, respectively) probably due to unavailability of the required DO level (Table 1). The sludge from R1, R2 & R3 was settling in nature while the sludge from R4 (DO≈0.5) was comparatively bulking in nature (as the SVI was around 200 ml/g due to low DO level) (Table 1). In terms of COD reduction, R1, R2 & R3 were almost similar with around 70% of reduction while the least reduction, 53%, was observed in the case of R4 (DO≈0.5) during phase II (Table 1). In phase-I, R1 & R2 were slightly lower than R3 (nitrogen supplemented) while they were similar to R3 in phase-II which reflects the initial lag phase of nitro fix ASP. Similar trend was observed in case of color and lignin reduction where maximum color and lignin reduction of around 65 and 60%, respectively was observed in R1, R2 & R3 while least was observed in the case of R4 (Table 1).

Table 1: Optimization of DO for Nitro fix ASP in two phases

Parameter		R1 C:N:P (100:0:1) DO 1	R2 C:N:P (100:0:1) DO 2	R3 C:N:P (100:2.5:1) DO 1	R4 C:N:P (100:0:1) DO 0.5
pH (outlet)	P-I	8.3 ± 0.1	8.4 ± 0.06	8.5 ± 0.03	8.4± 0.1
	P-II	8.3 ±0.0	8.4 ±0.0	8.6 ±0.0	8.4 ±0.0
Temperature (^{0}C)	P-I	35.2 ±0.3	35.6 ±0.6	35.7 ±0.5	36.2 ±0.3
	P-II	35.0 ±0.0	35.2 ±0.3	35.6 ±0.4	35.9 ±0.4
DO (mg/L)	P-I	1.4 ± 0.6	2.4 ± 0.6	1.2 ± 0.3	0.6 ± 0.5
	P-II	1.4 ±0.3	2.2 ± 0.3	1.3 ± 0.3	0.4 ± 0.1
Sludge yield (g/g COD s removal)	P-I	0.08 ±0.26	0.08 ±0.22	0.25 ±0.22	0.01 ±0.15
	P-II	0.16 ±0.5	0.14 ±0.5	0.23 ±0.5	0.14 ±0.26
F/M ratio	P-I	0.32 ± 0.08	0.32 ± 0.07	0.32 ± 0.04	0.30 ± 0.08
	P-II	0.33 ± 0.06	0.37 ± 0.07	0.37 ± 0.04	0.34 ± 0.06
Organic load (kg/m^3/d)	P-I	1.03 ± 0.24	1.13 ± 0.28	1.14 ± 0.13	0.85 ± 0.25
	P-II	1.08± 0.08	1.14 ± 0.09	1.16 ± 0.07	0.86 ± 0.1
HRT (h)	P-I	7.5 ± 0.8	7.2 ± 0.6	7.6 ± 0.7	8.3 ± 0.3
	P-II	7.8 ± 0.3	7.7 ± 0.3	7.6 ± 0.3	8.0 ± 0.3
MLSS (g/L)	P-I	3.9 ±0.4	4.2 ±0.4	4.2 ±0.4	3.5 ±0.6
	P-II	4.1 ±0.5	4.2 ±0.7	4.3 ±0.4	3.7 ±0.6
MLVSS (g/L)	P-I	3.3 ±0.3	3.5 ±0.3	3.5 ±0.3	2.9 ±0.5
	P-II	83.5 ± 1.3	83.5 ± 1.2	84.2± 1.1	83.4 ± 1.2
Organics (%)	P-I	80.5 ± 4.1	80.6 ± 3.2	80.4± 4.4	79.3 ± 6.2
	P-II	83.5 ± 1.3	83.5 ± 1.2	84.2± 1.1	83.4 ± 1.2
SVI (ml/g)	P-I	27.0 ± 4.3	26.0 ± 2.5	23.0 ± 3.4	186.0 ± 36.0
	P-II	21.0 ± 2.5	23.0 ± 2.3	23.0 ± 2.0	244.0 ± 38.6
COD_{out} (mg/L)	P-I	216 ± 51.0	212 ± 31.7	180 ± 13.8	257 ± 54.3
	P-II	160 ± 14.4	156 ± 18.1	154 ± 9.4	241 ± 19.8
CODs reduction (%)	P-I	60.2 ± 8.5	60.6 ± 7.1	66.6 ± 3.3	52.4 ± 10.7
	P-II	69.3 ± 2.3	69.8 ± 3.3	70.2 ± 1.4	53.5 ± 4.6
Color removal (%)	P-I	53.7 ± 5.7	54.0± 7.4	59.0 ± 5.7	37.1 ± 16.3
	P-II	62.3 ± 5.6	65.4 ± 6.0	65.3 ± 2.5	56.8 ± 5.3
Lignin removal (%)	P-I	55.0±7.6	51.7±6.6	60.1±4.3	40.0±14.7
	P-II	59.3±3.2	60.2±0.8	63.5±0.5	53.2±6.7

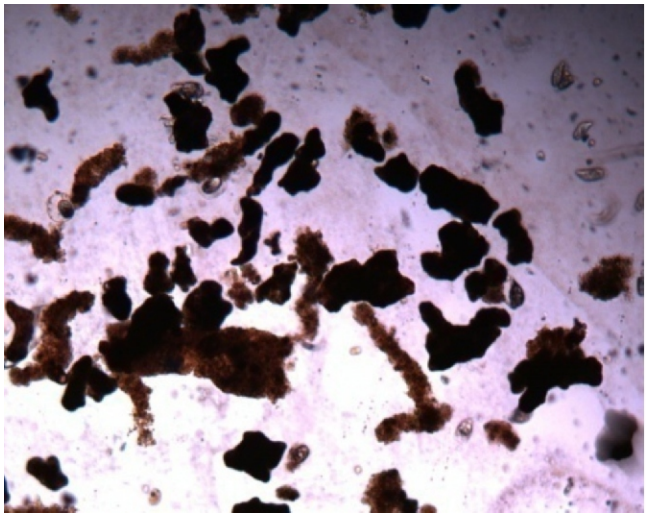
Phase I: Inlet COD (mg/L): 500 ± 20; color (Pt-Co unit): 935 ± 24; lignin (mg/L): 97 ±12
Phase II: Inlet COD (mg/L): 430 ± 47; Color (Pt-Co unit):791±21; Lignin (mg/L): 100 ±14

Table 2: Oxygen uptake rate (OUR) Phase-I & Phase-II at varying DO

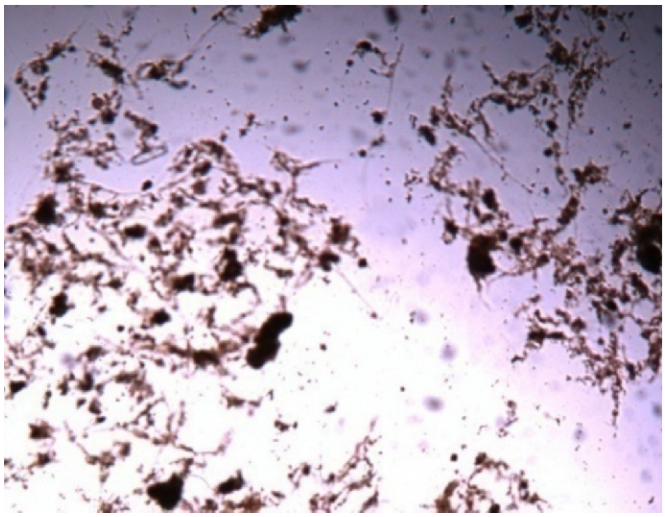
Parameter		R1	R2	R3	R4
OUR (mg/s)	P-I	0.004	0.004	0.004	0.006
SOUR (mg/g/h)		4.14	3.47	3.47	5.93
OUR (mg/s)	P-II	0.003	0.003	0.003	0.014
SOUR (mg/g/h)		3.21	2.95	2.74	17.4

Table 3: Nitrogenase activity at varying DO

Parameter	R1 C:N:P (100:0:1) DO 1	R2 C:N:P (100:0:1) DO 2	R3 C:N:P (100:2.5:1) DO 1	R4 C:N:P (100:0:1) DO 0.5
Nitrogenase activity (ng)	4.7	3.3	0.02	7.2



1a- R1 (DO 1)



1b- R4 (DO 0.5)

Fig 1: Sludge morphology at varying DO

The oxygen uptake rate (OUR) was also determined for R1 to R4 where highest uptake rate was observed in R4 while it was followed by R1, R2 & R3 which were almost similar to each other. The significantly high oxygen uptake rate in R4 clearly indicated the suppressed metabolic activity of the sludge under low DO condition (0.5 mg/L) (Table 2).

The nitrogenase activity of the sludge from R1-R4 reactors was also determined where maximum potential was observed in R4 (DO 0.5) followed by R1 (DO 1) and R2 (DO 2) which was significant too and the least was observed in nitrogen supplemented R3 (Control - C:N ratio100:2.5) (Table 3). The nitrogenase activity in R4 was high as low DO enhances the activity of nirogenase enzyme. But on the other hand, sufficient DO level is required for the degradation of organic material and therefore its treatment efficiency was low.

Sludge morphology was also observed for the entire reactors. Sludge in R1-R3 was having compact flocs while in

Table 4: Optimization of phosphorous for nitro fix ASP

Parameter	R1 C:N:P (100:0:1)	R2 C:N:P (100:0:0.5)	R3 C:N:P (100:0:0)	R4 C:N:P (100:2.5:1)
pH (outlet)	7.8±0.1	7.8±0.1	7.8±0.2	7.8±0.2
Temperature (^{0}C)	35.1±0.3	35.1±0.3	35.2±0.4	34.8±0.8
DO (mg/L)	1.3 ± 0.3	1.2 ± 0.2	1.2 ± 0.3	1.3 ± 0.3
Sludge yield (g/g CODs removal)	0.24	0.30	0.20	0.29
F/M ratio	0.2±0.0	0.24±0.1	0.24±0.03	0.22±0.0
Organic load (kg/m^3/d)	1.06±0.09	1.13±0.11	1.12±0.08	1.14±0.09
HRT(h)	8.9± 0.4	8.2± 0.6	7.9± 0.4	8.3± 0.5
MLSS (g/L)	4.4±0.5	4.3±0.7	4.3±0.5	4.0±0.8
MLVSS (g/L)	3.5±0.4	3.5±0.5	3.5±0.4	3.2±0.7
Organics (%)	79.4±1.0	81.9±1.1	82.1±3.3	78.1±1.7
SVI (ml/g)	33.0±7.3	32.0±9.2	24.0±6.1	33.0±13.7
COD_{out} (mg/L)	157.0± 25.5	164.0±24.4	182.0±27.9	164.0±14.3
CODs reduction (%)	71.2 ± 3.8	70.0 ± 3.4	66.7 ± 3.7	70.0 ± 3.2
Color reduction (%)	57.6±3.8	50.3±2.6	46.2±8.6	59.7±4.5
Lignin removal (%)	55.2±6.8	53.6±3.6	47.0±4.6	54.3±9.0

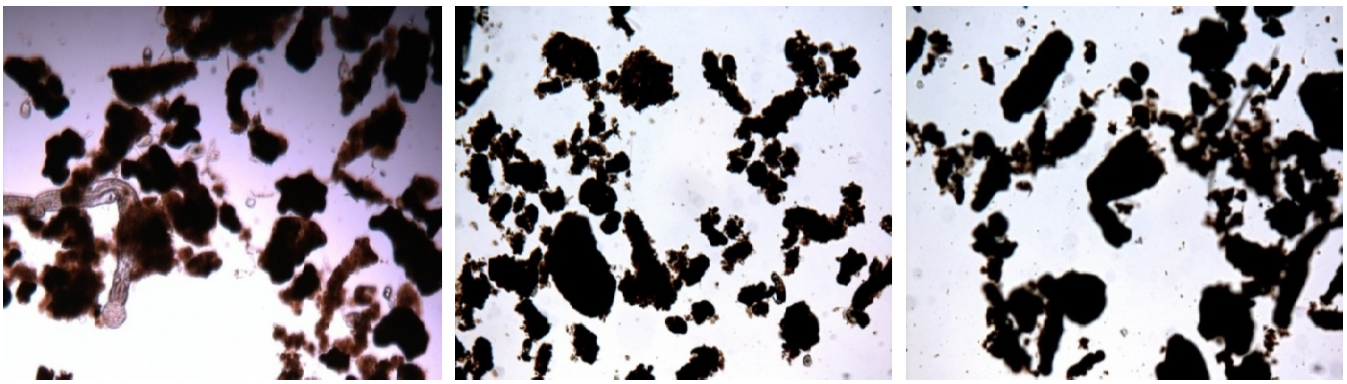
Phase I: Inlet COD (mg/L): 500 ± 20; color (Pt-Co unit): 935 ± 24; lignin (mg/L): 97 ±12
Phase II: Inlet COD (mg/L):430 ± 47; Color (Pt-Co unit):791±21; Lignin (mg/L): 100 ±14

Table 5: Oxygen uptake rate (OUR) Phase-I & Phase-II at varying phosphorus level

Parameter	R1	R2	R3	R4
OUR (mg/s)	0.002	0.003	0.002	0.003
SOUR (mg/g/h)	2.26	3.43	2.28	2.84

Table 6: Nitrogenase activity at varying phosphorus level

Parameter	R1 C:N:P (100:0:1)	R2 C:N:P (100:0:0.5)	R3 C:N:P (100:0:0)	R4 C:N:P (100:2.5:1)
Nitrogenase activity (ng)	9.8	4.6	1.8	0.14



2a- R1 (C:P≈100:1) 2b- R2 (C: P≈100:0.5) 2c- R3 (C: P≈100:0.0)

Fig 2: Sludge morphology at varying phosphorous dosage

R4 there was pin point and diffused flocs with filamentous organisms (Fig 1).

On the basis of the obtained findings, conventional ASP can be converted to nitro fix ASP by maintaining DO 1 mg/L without addition of nitrogen source, achieving all the treatment goals. In further experiments, DO was maintained to 1 mg/L.

Optimization of phosphorus dosage

The impact of varying phosphorus level on the nitrogen fixation & treatment efficiency of nitrogen deficient activated sludge process was also studied. As discussed above, four reactors R1-R4 were operated. R1-R3 were provided with phosphorus doses of 100:1, 100:0.5 & 100:0 with no nitrogen supplement while R4 was taken as control with nitrogen and phosphorus at the dose of 100:2.5:1. The study was conducted for 27 days. The operating conditions maintained in all the reactors during both the phases were, as per given in table 4. The DO level was maintained around 1 mg/L (optimized for nitrogen fixation mode). The nitrogen and phosphorus deficiency in R2 (COD:P≈100:0.5) & R3 (COD:P≈100:0) did not lead to bulking in sludge (Table 4). In terms of COD reduction R1, R2 & R4 were almost similar with around 70% of reduction while the least reduction, 66%, was observed in the case of R3 (COD:P≈100:0) . Almost similar trend was observed in case of color and lignin reduction, with maximum color and lignin reduction of around 59% (R1 & R4) & 54% (R1, R2 & R4), respectively, whereas least (46%) was observed in the case of R3 (Table 4).

The specific oxygen uptake rate (SOUR) was almost similar in all the reactors varying from 2.3-3.4 mg/g/h (Table 5). The nitrogenase activity of the sludge at varying phosphorus level was also determined where maximum potential was observed in R1 followed by R2, R3 and least was observed in R4 (Table 5). The nitrogen fixation process is not directly dependent on the phosphorus level but the growth of the nitrogen fixing biomass is dependent on the phosphorus level. As it cannot be fixed naturally, therefore the nitrogen fixation potential has decreased with decrease in phosphorus level. Sludge morphology was also observed where sludge in R1-R3 was having compact flocs while in R4 there were few filamentous organisms (Fig 2).

It is concluded that the nitrogen deficient ASP can work efficiently at the phosphorus dose of 100:1 &100:0.5 but not at 100:0 phosphorus level with DO level around 1 mg/L.

Table 7: Augmentation of efficient nitrogen fixing bacteria (B, T, 124) into nitro fix ASP

Parameter		RI C:N:P (100:2.5:1)	R2 C:N:P (100:0:1)	R3 C:N:P (100:0:0) (B)	R4 C:N:P (100:0:0) (T)	R5 C:N:P (100:0:0) (124)
pH (outlet)	P-I	7.7±0.1	7.8±0.1	7.7±0.1	7.8±0.1	7.8±0.1
	P-II	7.7±0.1	7.6±0.2	7.7±0.1	7.7±0.2	7.7±0.1
Temperature (^{0}C)	P-I	35.5±0.4	36.0±0.5	35.7±0.3	35.3±0.4	35.1±0.1
	P-II	35.7±0.4	35.7±0.6	36.0±0.8	35.0±0.00	35.0±0.2
DO (mg/L)	P-I	1.4±0.1	1.1±0.2	1.2±0.3	1.4±0.1	1.2±0.2
	P-II	1.2±0.1	1.2±0.1	1.2±0.3	1.1±0.3	1.3±0.3
F/M ratio	P-I	0.28±0.04	0.27±0.03	0.33±0.04	0.36±0.07	0.3±0.04
	P-II	0.23±0.04	0.24±0.03	0.21±0.03	0.24±0.04	0.23±0.04
Organic load (kg/m^3/d)	P-I	1.07±0.16	0.91±0.14	1.02±0.13	1.0±0.12	0.99±0.21
	P-II	0.86±0.12	0.82±0.13	0.82±0.15	0.83±0.15	0.86±0.2
HRT (h)	P-I	7.7±0.3	7.8±0.2	7.5±0.1	7.5±0.2	7.6±0.2
	P-II	7.5±0.1	7.6±0.3	7.6±0.3	7.6±0.2	7.6±0.2
MLSS (g/L)	P-I	4.8±0.3	4.6±0.5	4.3±0.3	3.4±0.4	3.9±0.4
	P-II	4.4±0.8	4.6±0.6	4.6±0.5	4.2±0.4	4.7±0.5
MLVSS (g/L)	P-I	3.5±0.5	3.2±0.5	3.0±0.5	2.5±0.3	2.9±0.3
	P-II	3.4±0.5	3.5±0.4	3.5±0.4	3.1±0.3	3.5±0.4
Organics (%)	P-I	74.9±1.6	72.8±2.1	74.1±1.8	74.5±2.3	73.9±2.5
	P-II	74.9±6.8	74.3±1.5	76.2±1.5	73.9±1.9	73.5±1.1
SVI (ml/g)	P-I	32±6	38±5	30±3	30±5.4	30±5
	P-II	30 ±1	32±6	25±6	25±2	39±4
COD_{out} (mg/L)	P-I	160±16	200±25	175±20	180±18	182±32
	P-II	161±20	171±22	171±27	165±15	157±13
CODs reduction (%)	P-I	68±3	60±4	66±2	64±3	63±8
	P-II	63±3	60±3	60±4	61±6	63±4
Color removal (%)	P-I	59±3	52±9	54±11	55±13	47±1
	P-II	59±3	45±0.4	54±0.5	52±2	52±6
Lignin removal (%)	P-I	47±6	40±12	41±12	42±13	40±2
	P-II	37±1	28±2	32±0.5	30±1	31±7

Table 8: Nitrogenase activity at varying phosphorus level

Parameter	RI C:N:P (100:2.5:1)	R2 C:N:P (100:0:1)	R3 C:N:P (100:0:0)	R4 C:N:P (100:0:0)	R5 C:N:P (100:0:0)
Nitrogenase activity (ng)	-	6.5	8.3	4.7	7.9

Augmentation of efficient bacteria into nitro fix ASP

The study involved the augmentation of efficient bacteria i.e., B, T & 124 into nitro fix ASP to improve the overall nitrogen fixation and pollutant removal efficiency of the system. The experimental set up for the following study comprised of three starter reactors of 2L capacity for strains B, T & 124 where around 24 h of retention time was provided. Five reactors (R1-R5) of 6L capacity were set where R1 was nitrogen supplemented control reactor (100:2.5:1), R3, R4 and R5 were augmented with individual strains i.e., B, T & 124 from

respective starters and R2 was nitro fix ASP. The study was divided into two phases (I & II). The pH, temperature, DO & HRT varied from 7.6-7.8, 35-36°C, 1.1-1.4 mg/L and 7.5-7.8 h, respectively in phase I & II (Table 7). In phase I, MLSS varied from 4.0 - 4.8 g/L in case of R1, R2, R3 & R5 while in R4 it was 3.4 g/L. In phase II, it was 4.2-4.7 g/L in R1-R5. Similar trend was observed in case of MLVSS (Table 7). The organic content was 73-76% in both the phases. The sludge from all the reactors was settling in nature in both the phases. In phase I, significant COD reduction efficiency (64-68%) was observed in case of R1 (cnt), R3 (nitro fix B), R4 (nitro fix T) & R5 (nitro fix 124), whereas least efficiency (60%) was observed in R2 i.e., nitro fix ASP without any bacterial supplementation. The significant efficiency in supplemented nitro fix ASP in comparison with non supplemented nitro fix ASP in early phase clearly indicates that the supplementation with efficient nitrogen fixing strains may eliminate the initial lag phase of nitro fix ASP. In phase II almost similar COD reduction efficiency of around 60% was observed in R1-R5. Almost similar trend was observed in the case of color & lignin reduction with color removal was 50-60% in phase I & phase II while lignin reduction was 40-47% in phase I and 30-37% in phase II, respectively (Table 7).

The nitrogenase activity was also performed where considerable activity was observed in nitro fix ASP, isolate B and 124 augmented reactor (Table 8).

Conclusion

The artificial dosing of nitrogen into activated sludge process treating pulp & paper mill wastewater may be avoided by natural fixing of nitrogen using nitrogen fixing bacteria in order to resolve the issue of cost addition and nutrient discharge. The approach followed was supplementation of nitrogen to ASP by natural occurring process i.e., biological fixation of atmospheric nitrogen by nitrogen fixing bacteria. Process was developed for replacing chemical addition of nitrogen into ASP by natural supplementation of nitrogen by augmentation of efficient nitrogen fixing bacteria under optimized conditions, meeting treatment norms at the same time.

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