# A pilot-scale study on PVA gel beads based integrated fixed film activated sludge (IFAS) plant for municipal wastewater treatment

Nitin Kumar Singh, Jasdeep Singh, Aakansha Bhatia and A. A. Kazmi

## ABSTRACT

In the present study, a pilot-scale reactor incorporating polyvinyl alcohol gel beads as biomass carrier and operating in biological activated sludge mode (a combination of moving bed biofilm reactor (MBBR) and activated sludge) was investigated for the treatment of actual municipal wastewater. The results, during a monitoring period of 4 months, showed effective removal of chemical oxygen demand (COD), biological oxygen demand (BOD) and $NH_3$-N at optimum conditions with 91%, ~92% and ~90% removal efficiencies, respectively. Sludge volume index (SVI) values of activated sludge varied in the range of 25–72 mL/g, indicating appreciable settling characteristics. Furthermore, soluble COD and BOD in the effluent of the pilot plant were reduced to levels well below discharge limits of the Punjab Pollution Control Board, India. A culture dependent method was used to enrich and isolate abundant heterotrophic bacteria in activated sludge. In addition to this, 16S rRNA genes analysis was performed to identify diverse dominant bacterial species in suspended and attached biomass. Results revealed that *Escherichia coli*, *Pseudomonas* sp. and *Nitrosomonas communis* played a significant role in biomass carrier, while *Acinetobactor* sp. were dominant in activated sludge of the pilot plant. Identification of ciliated protozoa populations rendered six species of ciliates in the plant, among which *Vorticella* was the most dominant.

**Key words** | bio-film activated sludge, hybrid process, integrated fixed film activated sludge (IFAS) system, municipal wastewater, MBBR, PVA gel

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## INTRODUCTION

Secondary treatment of municipal wastewater is usually accomplished by biological processes, which can be classified as suspended and attached growth processes (Singh *et al.* 2014, 2015a, b). Among the suspended growth processes, the activated sludge (AS) process is one of the most commonly used methods for biological treatment of municipal wastewater (Malmqvist *et al.* 2004; Singh *et al.* 2015a, b). Nowadays, most of the AS plants are suffering from frequent problems, most often due to unwanted biosolids-liquid separation (Jenkins *et al.* 1993). The low solids separation efficiency can lead to increased concentration of (1) organisms (measured in terms of biological oxygen demand (BOD)/chemical oxygen demand (COD)), (2) solids (in terms of total suspended solids (TSS), volatile suspended solids (VSS), etc.) and (3) nutrients (in terms of N and P bearing compounds) in the treated effluent; something which can then impact negatively on

wastewater recyclability as well as the receiving environment (Akker *et al.* 2010). This problem may be minimized by larger bioreactor volumes, which can be very expensive and sometimes impossible if space is limited (Almomani *et al.* 2014). Therefore, there is a need for compact hybrid systems that can increase capacity within existing volumes.

In last two decades, there has been an increasing interest in development of integrated fixed film activated sludge (IFAS, also known as hybrid biological reactor) systems for municipal as well as industrial wastewater treatment. These systems combine features of suspended and attached growth processes by incorporating specially designed biomass carriers, on which the biomass attach and populate, in bioreactors. This addition of biomass carrier increases the biomass inventory and subsequently the treatment capacity of the reactor (Ødegaard *et al.* 1994; Randall &

Sen 1996; Rusten et al. 2006; Wang et al. 2006; Rouse et al. 2007; Seetha et al. 2010; Li et al. 2012; AnoxKaldnes 2014; Chan et al. 2014). Based on arrangement of carrier, IFAS systems are categorized into two types: moving bed biofilm reactors (MBBRs), in which carrier moves freely inside the reactor, and fixed media activated sludge systems, in which carrier is fixed inside the reactor (Ødegaard et al. 1994; Randall & Sen 1996; AnoxKaldnes 2014).

Among these two modules of IFAS systems, MBBRs have gained much attention in developed countries, but very limited studies are available in developing countries such as India (Anterrieu et al. 2014; Jabari et al. 2014; Piculell et al. 2014; Zhang et al. 2014). These systems require low construction cost, minimal space requirement, simple operation combined with effective removal of organic and inorganic pollutants (Almomani et al. 2014; Yang et al. 2014), low sludge production, high biomass concentration, long sludge residence time (SRT), lower head loss and no clogging problems (Andreottola et al. 2000a, b; Ødegaard 2006; Rutt et al. 2006; Kim et al. 2010; Rosso et al. 2011; Chen et al. 2014). Due to all these specific features, various biomass carrier based MBBRs have been applied for the treatment of wastewaters in laboratory-scale, pilot-scale, and full-scale systems across the world (Sen et al. 1994; Ochoa et al. 2002; Trapani et al. 2010; Bassin et al. 2012). The carriers employed in MBBRs are designed to provide a large protected surface area and the key materials determining the efficiency and performance of treating wastewater in MBBRs (Larrea et al. 2007; Levstek & Plazl 2009). These biomass carriers are mainly grouped into two categories: porous and non-porous carriers. The non-porous carriers are usually made from polyethylene or polypropylene and have high durability and stability, and are widely used in the wastewater treatment plants and in the laboratory. However, they have some drawbacks of low specific surface area ($\sim$500 m$^2$/m$^3$), leading to slow start-up and easy detachment of biofilm due to the smooth surface. On the contrary, the porous carriers can escape from those drawbacks due to the excellent structure, which can not only trap and intercept the biomass efficiently as well as shorten the start-up period, but also promote biofilm accumulation by providing a large surface area (Falletti & Conte 2007; Sabzali et al. 2013; Chen et al. 2014). The applications of these types of carriers are still in their infancy due to lack of knowledge about their potential efficiency and application in conventional wastewater treatment systems.

Recently, polyvinyl alcohol (PVA™) gel beads (a porous biomass carrier), which have been shown to be effective for cultivation and retention of slowly growing bacteria, are attracting attention from many researchers. Successful implementation of MBBRs incorporating PVA gel beads as biomass carrier has also been reported for the treatment of municipal and industrial wastewater (Rouse et al. 2005; Rouse et al. 2007; Zhang et al. 2007; Levstek & Plazl 2009). These beads are typically used at volumetric packing ratios of only 5 to 15% versus much higher ratios of 50 to 70% common to other non-porous carriers, thus allowing more hydraulic loading to these systems (Levstek et al. 2010).

Among the various treatment configurations of MBBRs, recently two processes named as Hybas™ and BAS™ (AnoxKaldnes 2014), a combination of MBBR and AS processes, have shown significant potential in wastewater treatment (Chan et al. 2014). A similar configuration named the bio-film activated sludge (BAS) process, in which MBBR is followed by the AS process, has been tested successfully for food industry, textile, paper and pulp, petrochemical and dairy industry wastewaters, although its potential for municipal wastewater treatment is still to be investigated (Dalentoft & Thulin 1997; Malmqvist et al. 2004, 2007; Werker et al. 2004; Sivard et al. 2007; Haandel & Lubbe 2012). These systems are able to guarantee high COD removal performance at sludge age equal to the AS system, or similar performance at lower sludge age. The performance of a BAS process operated at 6–10 days of apparent sludge age and a F/M ratio of 0.2–0.4 kg COD. kg$^{-1}$. VSS.d$^{-1}$ is comparable to that of a conventional activated sludge system with a sludge age of 20 days and a F/M ratio of 0.2–0.25 kg COD.kg$^{-1}$. VSS.d$^{-1}$ or less. The treatment volume required by the BAS process is also 50–70% less than that of the AS process (Haandel & Lubbe 2012).

In this context, the feasibility of a PVA gel beads based IFAS (in BAS mode) plant for municipal wastewater treatment was addressed in this study to achieve effluent quality below the discharge standards of Punjab Pollution Control Board (PPCB), India (Table 1). In addition to this, the microbial community of this system was also investigated by culture dependent and independent techniques (Bhatia et al. 2013).

Table 1 | Discharge standards of Punjab Pollution Control Board, India for treated domestic effluents (as on 2 May 2015)

| S. No. | Parameter | Discharge limits* |
|---|---|---|
| 1 | BOD | <10 |
| 2 | COD | <50 |
| 3 | TSS | <20 |
| 4 | TKN | <50 |
| 5 | Ammonia nitrogen as N | <20 |
| 6 | Total phosphorus | <2 |

*All parameters are in mg/L.

## EXPERIMENTAL PROGRAMS

### Description of the pilot-scale IFAS plant (operated in BAS mode) and operating conditions

A pilot-scale IFAS plant was installed and operated in BAS mode at the sewage treatment plant (STP) of Jalandhar, Punjab, India. The pilot-scale plant consisted of two reactors (R1 & R2), each having 10 L capacity, in series followed by a secondary clarifier of 5 L capacity. The sludge was separated in a clarifier (5 L) and returned to the R2 reactor. Both reactors had the provision for excess sludge purging. Description of each reactor is given below:

*R1 reactor*: This reactor was filled with biomass carriers with 10% packing ratio and operated as MBBR. Influent wastewater was pumped to this reactor and its outlet directed to R2 reactor. Sieve arrangements were adopted to retain the carriers inside the R1 reactor. This reactor was operated with high dissolved oxygen to keep the carriers in suspension.

*R2 reactor*: This reactor was operated simply in activated sludge process mode, and settled activated sludge from clarifier was returned to R2 to maintain biomass in appropriate proportion. The excess sludge was disposed of from the clarifier daily.

A feed tank was used during this study to maintain continuous supply of influent wastewater to the pilot plant by using a peristaltic pump. The feed tank was periodically (twice a day) filled with wastewater collected from the primary (mechanical) stage of a 25 million litre per day (MLD) sequential bioreactor (SBR) plant of Jalandhar, Punjab, India. Necessary aeration (to keep the carriers in suspension in R1 and to run R2 in conventional AS mode) was achieved by providing

pressurized air via a blower by which two lines were derived for each reactor. To ensure the homogeneity of the mixed liquor in R2, the reactor was equipped with a mechanical stirring system. A schematic diagram of the IFAS plant at optimum operating conditions (phase 3) is shown in Figure 1.

### Characteristics of biomass carrier

PVA™ gel beads, a trademark biomass carrier from Kuraray Co., Tokyo, Japan, were used in the present study. They are hydrophilic in nature and have a very porous structure with only 10% solids and a continuum of passages of 10 to 20 μm in diameter tunneling throughout each bead. This carrier allows the growth of aerobic bacteria on the outer surface of the carrier and anaerobic growth at the core, with occupying capacity of 1 billion microbes per bead (Ramteke *et al.* 2015; Kuraray Aqua Ltd 2015). Typical characteristics of these carriers are shown in Table 2.

### Analysis and measurements

#### Physicochemical analysis

Reactor performance was monitored by analysing the water quality parameters of influent and effluent samples of the pilot plant. Spot samples (daily basis in phases 1 and 2; alternate days in phase 3) were collected and analysed for BOD, COD, TSS, VSS, ammonia nitrogen ($NH_3$-N), nitrate nitrogen ($NO_3$-N), and ortho and total phosphorus (OP and TP). Samples for determination of soluble components were passed through Whatman 42 (0.45 μm) filter paper prior to analyses. Activated sludge from R2 tank was also characterized by
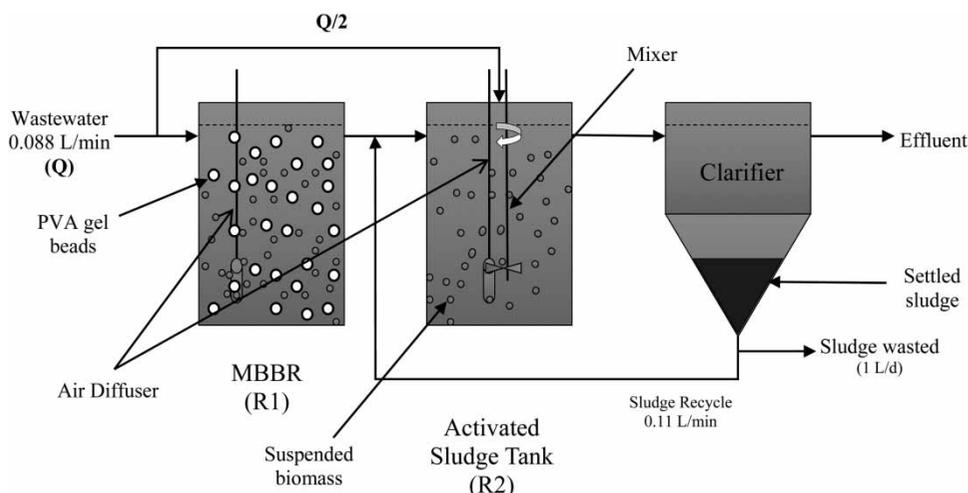


**Figure 1** | Schematic flow diagram of pilot-scale IFAS plant at optimum conditions (third phase).

**Table 2** | Characteristics data for biomass carrier used in present study (Rouse *et al.* 2005, 2007; Levstek & Plazl 2009)

| Characteristics of media | Unit | Value |
|---|---|---|
| Effective surface area | $m^2/m^3$ | 2,500 |
| Specific surface area | $m^2/m^3$ | 1,000 |
| Shape | – | spherical |
| Diameter | mm | 4 |
| Specific gravity | – | 1.025 |

mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS) and sludge volume index (SVI). All analyses of spot samples were conducted in accordance with *Standard Methods* (APHA 2005). Other operational parameters, like dissolved oxygen (D.O.), pH, temperature, flow rate and hydraulic retention time (HRT), were also monitored at the pilot plant site. The pH and D.O. were measured by a table pH meter (Cyberscan 510 digital, Thermo Scientific, Mumbai, India) and a portable D.O. meter (HACH- Model OX-2P, Hach, Bangalore, India), respectively.

## Microbial measurements

Total and faecal coliforms (TC and FC) were measured according to *Standard Methods* (APHA 2005). The pour plate technique and serial dilution method was used to enumerate the bacterial communities in the pilot plant. Total heterotrophic bacterial (HPC) count, fecal *Staphylococcus* and *Escherichia coli* were determined as described in Bhatia *et al.* (2013).

Attached and suspended sludge samples were collected from the R1 reactor of the pilot plant. Culture independent methods using polymerase chain reaction (PCR) amplification of specific 16S rRNA sequences (16S ribosomal RNA) were used for identification of dominating bacteria in suspended and attached biomass (Bhatia *et al.* 2013). The experimental analysis was performed in the following steps. The total genomic DNA was extracted from samples with the bead-beating method, using a DNA extraction kit (Hi PurA™ Soil DNA

kit, Hi Media, Mumbai, India) as per manufacturer's instructions. The universal primer pair 1492 r (5′-TAC CTT GTT ACG ACT T-3′) and 27f (5′-AGA GTT TGA TCC TGG CTC AG-3′) was used to amplify the 16S rRNA gene. The PCR reaction was done using 2 μM of primers, 50 ng of genomic DNA, 200 μM of each dNTP, $1 \times$ PCR buffer, 2 mM $MgCl_2$, and 2.5 units of *Taq* DNA polymerase (Biochem Biotech products, Sigma Aldrich, Bangalore, India). The PCR reaction was carried out at 94 °C for 2 min, followed by 35 cycles at 94 °C for 1 min, then at 48 °C for 1 min and 72 °C for 1.5 min. The final elongation step was carried out at 72 °C for 10 min (Bhatia *et al.* 2013). Thereafter DNA sequencing was done (BioAxis DNA Research Center (P) Ltd, Hyderabad, India) and sequence data from 16S rRNA gene fragments were analysed by a Genetic analyser (ABI model 3100, Applied Biosystems, New Delhi, India) and compared using the biocomputing tools provided online by the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov). The basic local alignment search tool (BLAST) program (Altschul *et al.* 1990) was used for sequence similarity analysis.

Characterization of ciliate species was done by taking 25 μL sub-samples of mixed liquor with an automatic micropipette, and a minimum of four replicates of this volume were counted each time. For the image acquisition, a drop of sludge was deposited on a slide and carefully covered with a cover slip. Image acquisition of the flocs on the slide was carried by phase-contrast illumination technique (magnification ×100) using a photonic microscope (Olympus Medical Systems India Private Limited, Gurgaon, India) and keeping the illumination constant for all samples (Martín-Cereceda *et al.* 2001).

## Experimental methodology

At start-up, the pilot plant was inoculated with 5 L of activated sludge from the SBR plant (5 day SRT) located in Jalandhar. The pilot plant was operated for 4 months, in three consecutive phases with varying D.O., influent flow (Q) and external recycle ratio (ERR). The operational parameters in each phase are shown in Table 3.

**Table 3** | Different phases of IFAS plant process optimization

| | | | | D.O. (mg/L) | | Loading applied (kg COD/$m^3$.d) | | Loading applied (kg NH$_3$-N/$m^3$.d) |
|---|---|---|---|---|---|---|---|---|
| Phase | Q (L/d) | ERR (L/d) | HRT (h) | R1 | R2 | MBBR | Total (MBBR + AS) | Total (MBBR + AS) |
| 1 | 80 | 100–240 | 6 | 5–6 | 3–4 | 4.22 ± 2.23 | 2.11 ± 1.11 | 0.092 ± 0.044 (28.55 ± 13.77) |
| 2 | 64 | 159 | 7.5 | 5–6 | ≤1 | 2.10 ± 1.02 | 1.05 ± 0.51 | 0.071 ± 0.036 (22.15 ± 11.20) |
| 3* | 127 | 159 | 3.75 | 5–6 | 2-3 | 2. 56 ± 1.67 | 2.50 ± 1.59 | 0.213 ± 0.079 (67.07 ± 24.54) |

*Optimum conditions.
Values presented in brackets are in g/$m^2$.d.

At optimum conditions (phase 3), half of the inflow and effluent from MBBR were fed to the AS tank as shown in Figure 1. Typical composition of influent fed to plant is shown in Table 4. It was necessary to maintain appropriate food (BOD) to microorganism (MLSS) ratio, i.e. F/M (0.2–0.5) in the R2 reactor, so that it could behave like a conventional activated sludge process (Metcalf et al. 1991).

## RESULTS AND DISCUSSION

In the initial phase of process optimization, sewage temperature was high, nearly around 30–35 °C. This temperature range was found to be detrimental to microbial species and the sludge settleability, and resulted in floating sludge in the clarifier (Malmqvist et al. 2004). Another reason behind this unwanted phenomenon may be denitrification at the bottom of the settling tank, which is very common in AS based systems (Wanner 1994). Beside this, the recycling of an inappropriate amount of active biomass could be a possible reason during the process (Metcalf et al. 1991; Qasim 1998). However, the process was optimized by maintaining suitable operational conditions (ERR and D.O. were maintained as shown in the third phase of optimization), and this improved the settleability of the activated sludge. After the optimization, the process has shown stable results for long periods. Operating parameters of each reactor of the pilot in third phase (optimum conditions) are shown in Table 5.

**Table 4** | Typical characteristics of sewage during different phases of reactor optimization

| Parameters* | Phase 1 | 2 | 3 | Average |
|---|---|---|---|---|
| pH | 7.4 | 7.5 | 7.5 | 7.4 |
| Temp. (°C) | 30.6 | 32.9 | 31.7 | 31.7 |
| COD | 528 ± 280 | 329 ± 159 | 374 ± 250 | 410 |
| BOD | 233 ± 117 | 135 ± 49 | 173 ± 113 | 180 |
| TSS | 410 ± 424 | 258 ± 149 | 262 ± 206 | 310 |
| VSS | 197 ± 205 | 121 ± 69 | 161 ± 120 | 159 |
| $NH_3$-N | 23 ± 11 | 22 ± 11 | 33 ± 13 | 26 |
| $NO_3$-N | 6.5 ± 3.1 | 9.2 ± 6 | 5.6 ± 3.1 | 7.1 |
| TP | ND | ND | 8.6 | 8.6 |

*All parameters are in mg/L except pH and temperature; ND: not done.

**Table 5** | Typical operational parameters of R1 and R2 at optimum state

| Parameter | Unit | R1 | R2 |
|---|---|---|---|
| pH | – | 7.4–7.8 | 7.2–7.7 |
| Temperature | °C | 27–37 | 28–38 |
| HRT | h | 1.87 | 1.87 |
| MLSS | mg/L | <1,000 | 3,000–5,000 |

### Organics removal

The long-term variation in effluent COD and BOD, along with applied and removed COD loadings for the BAS process, is presented in Figure 2. It was observed that applied loadings were almost comparable with a previous study in which moving carriers were used (Aygun et al. 2008). However, these values were slightly higher for the BAS process, as reported by Van Haandel & Van der Lubbe 2012. During the first and second phases of process optimization, there was much variation in effluent quality (COD and BOD values). This may be attributed to the discharge of suspended solids (floating sludge particles) in effluent. At the startup of the third phase (i.e. HRT = 3.75 h and ERR = 125%), the pilot started to give stable results, as shown in Figure 2. These parameters represent the effectiveness of the process and are mainly used to determine the treatment efficiencies. During the whole study, a significant variation in influent COD was observed, and at the end, under optimized conditions, the pilot achieved the effluent BOD and COD of <10 mg/L and <50 mg/L respectively, which can easily meet the discharge standards of PPCB, India (Punjab Pollution Control Board, India). The removal of total COD and BOD over the BAS process was highly variable during the operation (Punjab Pollution Control Board 2015). After 90 days of operation, when the process was stabilized, the mean removal of COD and BOD was found to be ~91% and 92%, respectively. The mean removal of soluble COD in the process was ~81% at stable conditions.

During the study period, high variation was also observed in influent quality, i.e. COD ranging from 48 to 1,118 mg/L and BOD ranging from 28 to 456 mg/L. The average concentrations of BOD and COD in influent were also quite high, as shown in Table 3. But the performance of the reactor was observed to be stable at optimum state. This indicates that the system is able to bear the organic shock load and provided consistent values, indicating good quality of effluent in terms of BOD and COD. The results obtained in this study were in good agreement with previous studies (Andreottola et al. 2000a, b; Javid et al. 2013; Van Haandel & Van der Lubbe 2015).
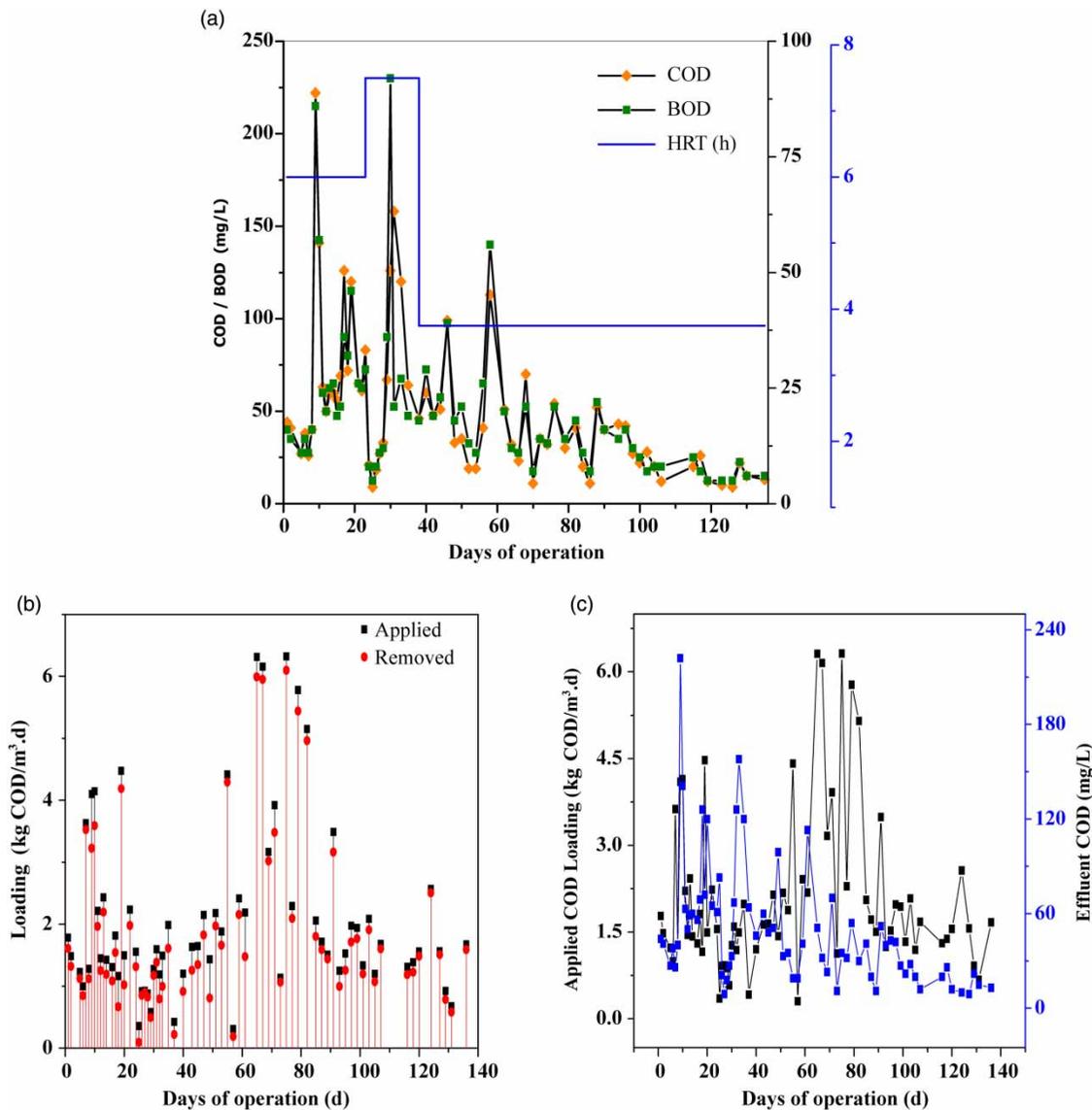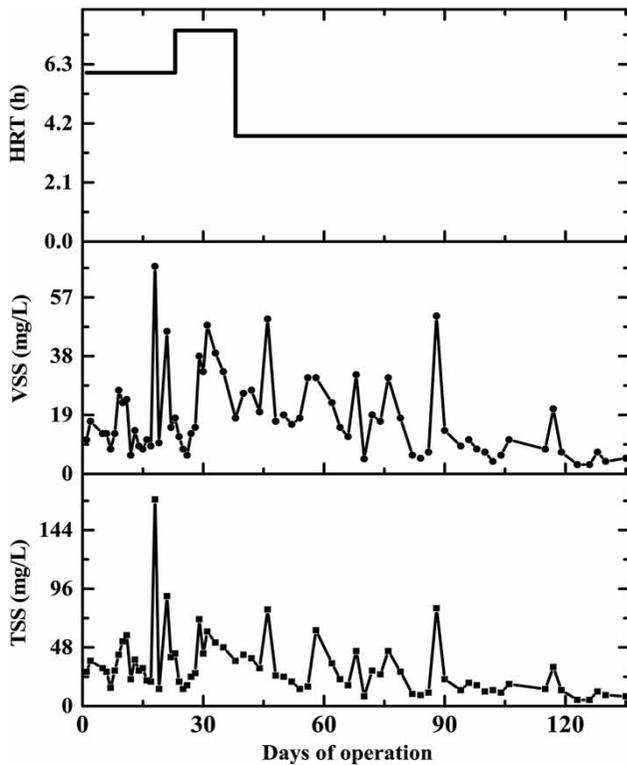
**Figure 2** │ (a) Time series plot of effluent BOD and COD from IFAS pilot plant at different HRT; (b) applied and removed COD loading from pilot; (c) time series plot of effluent COD concentrations vs. applied loading.

## Solids removal

The separation of bio-solids from treated effluent plays an important role in the overall performance of a treatment facility. Similarly, the volatile fraction of the suspended portion may cause deterioration of water bodies if high concentrations of organic solids are discharged into water bodies (Metcalf *et al.* 1991). Considering the average values (as shown in Table 4), the ratio of VSS to TSS in influent was observed as 0.48, 0.46, 0.61 in phase 1, 2 and 3, respectively, while for the treated effluent, these values were found to be 0.43, 0.61, and 0.66. Figure 3 shows the time series plots of effluent solids (TSS and VSS) concentrations. It

can be clearly observed from Figure 3 that there was a great variation in effluent quality, which may be attributed to the floating sludge phenomenon. In the optimization process, variation in effluent solids (TSS and VSS) corresponded to effluent COD (which can be correlated to TSS) and BOD (which can be correlated to VSS) values (Metcalf *et al.* 1991; Qasim 1998). During this study, the first and second phases showed high variation in effluent solids due to an operational problem (high concentrations of suspended solids escaped from the plant due to floating sludge). Suspended solids levels in the effluent from the clarifier varied between 20 and 80 mg/L during this period. Results revealed that the pilot scheme is capable of
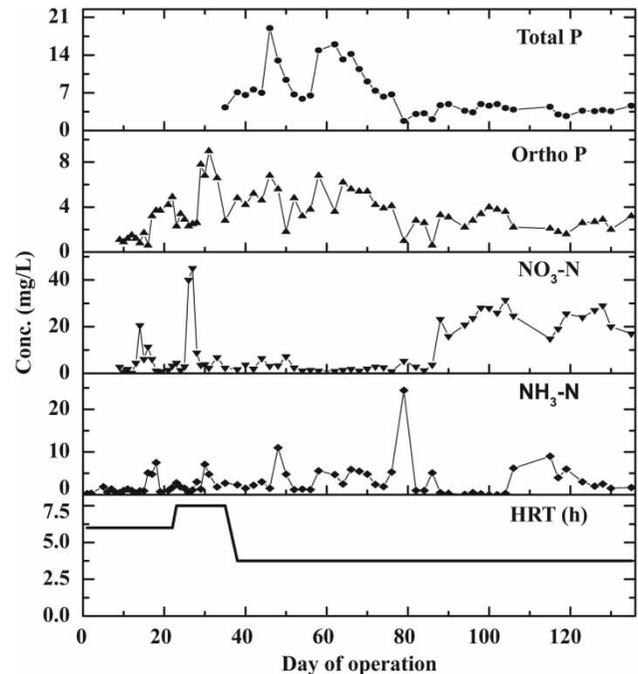
Figure 3 | Effluent concentration of TSS and VSS during the pilot run period.



Figure 4 | Time series plot of effluent nutrient (N and P forms) concentrations from the IFAS pilot plant at different HRT.

removing TSS and VSS significantly to a level of ~20 mg/L and ~10 mg/L, respectively (Figure 3). After 90 days, when the process was stabilized, the effluent suspended solids concentration decreased to a minimum level ~5 mg/L. Beside this, the average concentration of suspended solids was observed to be 24 mg/L at optimum state, while in the first and second phases these values were ~41 mg/L and ~38 mg/L, respectively.

## Nutrient removal

The time series plots of effluent ammonia, nitrate nitrogen, and ortho and total phosphorus are illustrated in Figure 4. It was observed that during the first and second phases of process optimization, the effluent nutrient parameters ($NH_3$-N, $NO_3$-N, OP and TP) fluctuated too much. This could be attributed to floating sludge and inappropriate sludge recycle ratio. Average ammonia concentrations throughout the study period in the influent and effluent of the pilot were quantified as 28.7 mg/L and 2.8 mg/L, respectively. This resulted in an average ammonia removal efficiency recorded as 90.15%, while $NO_3$-N removal was not significant at level of extent as compared with ammonia nitrogen. The highest ammonia nitrogen removal during the

overall experimental period was 99%. When the process was found to be stabilized, the concentration of effluent ammonia nitrogen was observed to vary between 1 and 5 mg/L. The effluent total phosphorus was not so much different from the influent value. Although under stable conditions, between 90 and 130 days, the effluent soluble phosphorus concentrations were recorded in the range of 1.6–4 mg/L, while total phosphorus varied between 2 and 4.9 mg/L, and these values do not meet the requirement of PPCB, India.

## Settling characteristics

Sludge from the AS tank of the pilot plant was characterized in terms of MLSS, MLVSS and SVI. After optimization, the sludge characteristics improved slowly, and SVI reached up to 60 mL/g on the 110th day. Typical values of SVI varied in the range of 25–72 mL/g at stable conditions. These values of SVI represent good settling in the current treatment scheme. For a pure suspended growth system, the sludge yield coefficient varies in the range of 0.4–0.6 g per g of COD removed (Metcalf et al. 1991), while in the present study sludge production was calculated as 0.18 kg VSS/kg COD removed at optimum condition. This indicates that the sludge production was much lower in comparison with other suspended growth systems.

## Microbial examination of water samples and PVA/AS biomass

Microbiological experiments were performed for pilot influent and effluent at optimum conditions (third phase in Table 3). The results demonstrated that the removal percentage for TC, FC, HPC, *E. coli*, and *Staphylococcus* was ~96%, 99%, 99%, 99% and 85%, respectively.

To explore the microbial consortium in PVA gel, 16S rRNA gene based identification (molecular method) was done. The 16S rRNA gene sequences of the three most dominant distinct bacteria were detected to be most similar to gene sequences of *E. coli*, *Pseudomonas* sp. and *Nitrosomonas communis* (98, 97, and 98% similarity), respectively. The presence of rod shaped Gram-negative *E. coli* bacteria confirms the anaerobic zones in biofilm of carrier biomass. All *Pseudomonas* sp. are rod shaped Gram-negative and are supposed to be responsible for biofilm formation, while *Nitrosomonas communis* are mainly responsible for high nitrification rates in the current treatment scheme (Siripong & Rittmann 2007; Qin *et al.* 2008; Wang *et al.* 2010).

For species in activated sludge, the sludge sample was analysed by a culture independent molecular method. Two non-chimeric and non-redundant partial 16S rRNA genes (clone 1 and clone 2) of the bacterial species in sludge were sequenced and submitted to NCBI (www.ncbi.nlm.nih.gov/) to acquire accession numbers of the most similar genes. The metagenomic study using the BLAST tool demonstrated that both clone 1 and clone 2 were observed to be most identical (~98%) to the *Acinetobacter* sp. as shown in Figure 5.

Moreover, ciliate species (protozoa) are one of the most common components in anthropogenic systems (biological wastewater treatment plants) and play an important role in wastewater treatment processes. These species are responsible for improving the quality of the effluent, maintaining the density of dispersed bacterial populations by predation. Studies of the relationships between protozoa and physico-chemical and operational parameters have also revealed that the species structure of these communities is an indicator of plant efficiency (Madoni 2011). The indicator species of the representative group of ciliated protozoa growing in the activated sludge of the IFAS plant are shown in Table 6. The most dominant species of ciliates identified in the IFAS plant were of the attached type, as reported earlier (Madoni 2011).

## CONCLUSIONS

The results of the present study demonstrated the steady state efficiency of a PVA gel beads based IFAS plant with respect to removal of organics and nutrients (N and P forms) from a real municipal wastewater, in spite of significant fluctuations in influent organic pollution load. The following insights were drawn from the present study:

- PVA gel beads were found to be a promising carrier which allows up-gradation of existing wastewater treatment plants with small packing ratio in comparison with other available biomass carriers, thus allowing more hydraulic loadings on treatment systems.
- The pilot-scale PVA gel beads based IFAS plant was found to be effective in municipal wastewater treatment. The removal rates of COD, BOD, TSS, and $NH_3$-N were 91%, 92%, 92.3% and 90.3%, respectively, which meets the discharge standards of PPCB, India. However, effluent discharge levels of total P could not meet the requirements of PPCB.
- SVI values obtained from the present study revealed that the sludge has good settling properties, having SVI in the range of 25–72 mL/g. The production of sludge has been
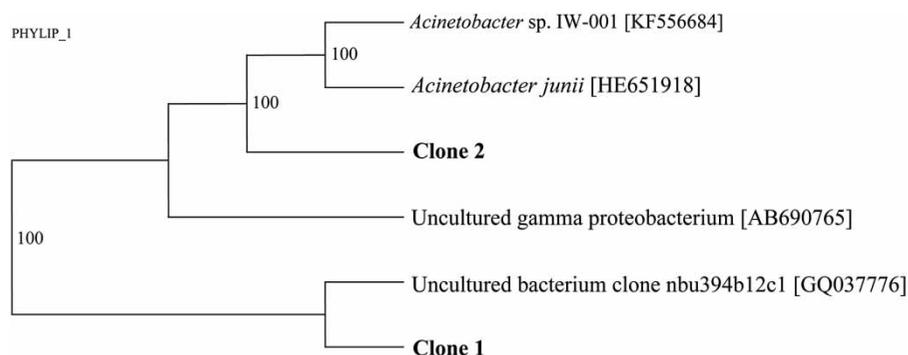


**Figure 5** | Phylogenetic tree derived from16S rRNA sequence data of suspended and attached biomass of IFAS plant.

**Table 6** │ Ciliate species identified in activated sludge of IFAS plant at optimum state

| S. no. | Mixed liquor fauna | Characteristics |
|---|---|---|
| 1 | *Vorticella* (+++) | Well working system; Satisfactory effluent quality; Good floc formation |
| 2 | *Arcella* (++) | Satisfactory effluent quality; Good nitrification; Longer sludge retention time (SRT) |
| 3 | *Entosiphon* (++) | Excellent effluent quality |
| 4 | *Litonotus* (++) | Well working system; Satisfactory effluent quality |
| 5 | Filamentous bacteria (+) | Low concentration of filamentous organisms helps in good floc formation |
| 6 | Worm (++) | Helps in minimizing sludge |

+= Scarce; ++ = Moderate; +++ =Abundant; ++++= More Abundant; ND = not detected.

determined as 0.18 kg VSS/kg COD removed, which was low as compared with stoichiometric values. Further research is required in this direction to reveal the facts behind low sludge production in these kinds of systems.

- Microbial examinations of PVA gel and activated sludge using 16S rRNA gene analysis showed richness of *E. coli*, *Pseudomonas* sp. and *Nitrosomonas communis* in PVA gel and *Acinetobacter* sp. in suspended biomass, respectively. Also, microscopic observation of activated sludge showed that *Vorticella* were the most dominant protozoa in the IFAS plant.

- The results obtained in the present study may aid in the design of full-scale processes. In addition, these innovative processes can be applied to existing plants to enhance the treatment efficiency without major new construction.

## ACKNOWLEDGEMENT

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