

Biomass granulation in an upflow anaerobic sludge blanket reactor treating 500 m³/day low strength sewage and post treatment in high rate algal pond

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ABSTRACT

A pilot-scale upflow anaerobic sludge blanket-moving bed biofilm (UASB-MBB) reactor followed by a high rate algal pond (HRAP) was designed and operated to remove organic matter, nutrients and pathogens from sewage and to facilitate reuse. For an influent chemical oxygen demand (COD) concentration of 233 ± 20 mg/L, final effluent COD was 50 ± 6 mg/L. Successful biomass granulation was observed in the sludge bed of the upflow anaerobic sludge blanket (UASB) reactor after 5 months of operation. Ammonia removal in HRAP was $85.1 \pm 2.4\%$ with average influent and effluent ammonia nitrogen concentrations of 20 ± 3 mg/L and 3 ± 1 mg/L, respectively. Phosphate removal after treatment in the HRAP was $91 \pm 1\%$. There was a 2–3 log scale pathogen removal after treatment in HRAP with MPN of the final effluent being 600–800 per 100 mL, which is within acceptable standards for surface irrigation. The blackwater after treatment in UASB-MBBR-HRAP is being reused for gardening and landscaping. This proper hydro-dynamically designed UASB reactor demonstrated successful granulation and moving bed media improved sludge retention in UASB reactor. This combination of UASB-MBB reactor followed by HRAP demonstrated successful sewage treatment for a year covering all seasons.

Key words | anaerobic treatment, disinfection, high-rate algal pond, reuse, sewage treatment, UASB reactor

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INTRODUCTION

Population growth, industrialization, agricultural practices and urbanization all increase the water demand and thus the quantity of wastewater generated. Fresh-water scarcity is becoming an acute problem in several countries. Traditionally, wastewater treatment has focused on pollution abatement, public health protection and environmental protection by removing biodegradable material, nutrients and pathogens (Meneses *et al.* 2010). Wastewater recycling, reuse and resource recovery can be a very good approach to conserve water particularly in areas of water shortage (Moawad *et al.* 2009). Various options for treatment and reuse of treated water along with energy recovery (methane from anaerobic digestion) and materials recovery (biosolids and nutrients) are currently being researched, developed and implemented worldwide. Developing countries like India and China are gaining momentum in using treated water for potable and non-potable supplies, and decentralized treatment units are very helpful in these cases. The

reclaimed water application governs the type of treatment needed to protect public health and environment and the degree of reliability required for treatment processes and operation (Moawad *et al.* 2009).

Conventional sewage treatment processes involve high capital, maintenance and operational cost, huge energy requirements, which makes them unsuitable for use in developing countries (Sato *et al.* 2006). Energy efficient low-cost sewage treatment systems are the best choice for such countries. Anaerobic treatment systems excel in such respect. Upflow anaerobic sludge blanket (UASB) reactors are the most widely used high-rate anaerobic sewage treatment and several full scale reactors have been operated world-wide (Lim & Kim 2014). Most of the successful applications of UASB reactors are for treatment of high strength industrial wastewaters (Lim & Kim 2014). Municipal sewage treatment using UASB reactors is restricted to tropical regions, where temperature of the raw sewage allows fast

hydrolysis of organic matters and suspended solids (Zhang *et al.* 2013). Sato *et al.* (2006) evaluated the treatment efficiency of the 16 existing UASB reactor-based sewage treatment plants on the Yamuna river basin in India and observed that none of the plants met the discharge standards for biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS) or nutrients as total Kjeldahl nitrogen (TKN). In India, the discharge standards for BOD, COD, TSS and TKN are 30 mg/L, 250 mg/L, 100 mg/L and 100 mg/L, respectively (CPCB 1993). In order to improve the effluent quality up to disposal standards, polishing ponds, with short retention time, were used to treat the UASB reactor effluent in these sewage treatment plants. Unfortunately, effluent quality did not follow the desired standard limits even after the polishing ponds (Sato *et al.* 2006).

Granular sludge plays an important role in treating wastewater due to its advantages of denser and stronger aggregate structure, better settleability, better cell retention improving solid-effluent separation and establishing higher substrate conversion rate, supporting higher biomass concentration and greater resistance to shock loadings (Xing *et al.* 2015). This property is particularly important while dealing with low-strength wastewater, where adequate biomass retention can be possible even at higher upflow velocity (1.75 m/h) under peak flow conditions (Bhunia & Ghangrekar 2009). Biomass granulation depends on a number of factors starting from the source of inoculum, the type of organic matter, water hardness, presence of nutrients, hydraulic loading, pH, temperature, presence of metal ions, hydraulic retention time (HRT), liquid upflow velocity and even on the design of the reactor and applied organic loading rates (OLRs) (Ghangrekar *et al.* 1996). Formation of granules from non-granular inoculum occurs due to microbial self-immobilization and subsequently, aggregate formation and growth. Packing medium in a UASB reactor is expected to increase solids retention by dampening short circuiting, improving gas/liquid/solid separation, and providing surface for biomass attachment thus offering polishing treatment in the sludge blanket zone (Chatterjee *et al.* 2016).

While UASB reactor contributes to organic matter removal, high-rate algal pond (HRAP) abates nutrients and pathogens. Micro-algal cultivation generally requires inorganic nutrients and carbon dioxide in the presence of sunlight through photosynthesis. These organisms can also be used to accumulate nutrients, as they require less than one-tenth of the area to recover phosphorous compared to terrestrial crops (Mehta *et al.* 2015). As microalgae require

both organic and inorganic nutritional inputs for their survival, wastewater can be a potential source of these nutritional requirements and hence micro-algae can help in further polishing sewage treated by UASB reactor (Kiran *et al.* 2014). Microalgae also have broad bioenergy potential as the harvested biomass can be used to produce liquid transportation and heating fuels, such as biodiesel and ethanol, or can be anaerobically digested to produce biogas. The treated wastewater after UASB-HRAP can be used for landscape irrigation. In the present study, the possibility of biomass granulation in pilot-scale UASB reactor treating low strength wastewater like sewage is explored. A combination of UASB-HRAP was piloted to produce reusable quality of treated wastewater.

MATERIALS AND METHODS

Pilot plant description and operation

A novel combination of UASB reactor and HRAP was designed and constructed for treatment of sewage (Figure 1). Performance of UASB reactor was evaluated to examine the removal of organic matter and enhance biomass granulation, while HRAP was used to exploit nutrient (N and P) transformation processes and removal of pathogens. The UASB reactor was having a height of 5.3 m and diameter of 5.6 m, which is followed by a 15.8 m long and 7.9 m wide HRAP with a water depth of 0.9 m. Valves are provided over the UASB reactor height (5 nos) to facilitate sampling of reactor contents. A secondary sedimentation tank, having a diameter of 3.0 m and a side water depth of 2.75 m was provided after the HRAP. The settler was operated at three different surface overflow rates, 8 m³/m² day, 32 m³/m² day and 65 m³/m² day.

The UASB/HRAP system was fed with domestic sewage generated in IIT Kharagpur campus, India. The sewage was



Figure 1 | (a) UASB reactor, (b) HRAP.

passed through a screen located upstream of the sump where the wastewater was collected before being pumped into the UASB reactor. The sewage fed to the UASB reactor had COD, 224 ± 81 mg/L; pH, 7.24 ± 0.01 ; alkalinity, 224 ± 38 mg/L of CaCO_3 ; volatile fatty acids (VFAs), 140 ± 30 mg/L; ammonia, 20 ± 3 mg/L; phosphate, 4.0 ± 0.8 mg/L; TSS, 121 ± 5 mg/L; volatile suspended solids (VSS), 84 ± 1 mg/L and MPN, 3.6×10^4 to 1.01×10^5 MPN/100 mL. The pilot UASB reactor was inoculated with septic tank sludge with VSS concentration of 8 g/L and no separate inoculum was used for the HRAP. Spontaneous growth of mixed culture algae was observed as a response to effluent of the UASB reactor. The UASB reactor was operated at gradually increasing OLRs in the start-up phase (Table 1). The HRAP was operated at five different HRTs of 8, 6, 4, 2 and 1 days with 35 days of operation under each HRT. After operating the UASB reactor for a period of 400 days, polypropylene media was added to the reactor and it was operated as a hybrid UASB-MBBR for another 160 days.

Monitoring

During monitoring, analyses of raw sewage, samples from five sampling ports inside the UASB reactor, effluent from the UASB reactor and final effluent from the secondary settling tank after the algal pond were performed. The analyzed parameters were: COD (total and soluble), TSS, VSS, alkalinity, VFA, pH, total nitrogen (TN), nitrate nitrogen and temperature. The analyses were conducted according to the Standard Methods for the Examination of Water and Wastewater (APHA 1998). Total dissolved solids, pH and NO_3^- -N were measured using electrodes

(Thermo, USA). Turbidity was measured by Systronics Turbidity Meter. Biogas production rate was measured by water displacement method and methane content was measured by passing biogas through 5% NaOH solution.

Characterization of biomass present inside the UASB reactor was done, with the determination of extracellular polymers, sludge volume index (SVI), sludge settling velocity and specific methanogenic activity (SMA) of the sludge. Settling velocity and strength of the sludge was measured following the procedure described by Ghangrekar *et al.* (1996). The results were expressed in terms of an integrity coefficient defined as, the ratio of solids in the supernatant to the total weight of the granular sludge, expressed in percentage. Settling velocity of sludge settled at bottom of settling column at fixed time intervals (0.5, 1, 2, 3, 5, 7, 15, 30, and 60 min) was considered to determine corresponding size (d_p) of biomass fractions, using Newton's law of particle settling theory as mentioned by Bhunia & Ghangrekar (2007). From percentage mass fractions of settled sludge at different time intervals, mean diameter of particles present in the sludge was calculated. Sludge produced, sludge yield and solid retention time (SRT) in the UASB reactor was calculated as described by Chatterjee *et al.* (2016). For algal biomass harvested protein, lipid, carbohydrate, chlorophyll a, chlorophyll b and algal biomass were determined. Protein (PN) content in algal biomass was measured by Bradford assay (Bradford 1976) and polysaccharide (PS) by Anthrone method (Morris 1948). SVI was calculated according to the procedure described in Standard Methods (APHA 1998). SMA was measured as described by Ghangrekar *et al.* (1996) and biomass granulation index (BGI) and granulation index (GI) were calculated based on the description by Bhunia & Ghangrekar (2007).

Table 1 | Monthly average of COD removal in the UASB reactor

Month	COD inlet (mg/L)	COD outlet (mg/L)	Total COD removal (%)	Soluble COD inlet (mg/L)	Soluble COD outlet (mg/L)	Soluble COD removal (%)	OLR (kg COD/m ³ ·day)	HRT (h)
Nov	167 ± 30	117 ± 30	30 ± 12	110 ± 12	80 ± 12	27 ± 8	0.14	24
Dec	162 ± 30	60 ± 41	60 ± 26	94 ± 21	48 ± 7	49 ± 7	0.25	21.4
Jan	193 ± 26	79 ± 19	59 ± 9	95 ± 3	48 ± 1	49 ± 1	0.33	15.6
Feb	187 ± 61	80 ± 17	57 ± 16	78 ± 23	34 ± 13	56 ± 9	0.43	12.5
Mar	250 ± 61	92 ± 18	63 ± 12	101 ± 21	42 ± 7	58 ± 10	0.48	9.1
April	195 ± 49	60 ± 11	70 ± 11	83 ± 19	32 ± 2	61 ± 8	0.52	8.6
May	215 ± 44	60 ± 31	72 ± 14	91 ± 29	34 ± 3	63 ± 7	0.60	8
June	233 ± 20	63 ± 15	73 ± 10	95 ± 23	32 ± 3	66 ± 6	0.61	7.5
July	210 ± 30	48 ± 23	77 ± 19	90 ± 22	31 ± 4	66 ± 7	0.77	7.2

RESULTS AND DISCUSSION

General performance of the UASB reactor

The UASB reactor was operated for a total of 400 days. After an initial start-up period, gradual improvement in performance occurred and the reactor stabilized and remained in pseudo steady state until the end of the experimental period. COD concentration profile was evaluated over reactor height. Majority of COD reduction took place only within 2 m from the bottom of the reactor (Figure 2(a)), which is also corroborated by the pH profile (Figure 2(b)). COD removal efficiency kept on increasing with operation time, with a maximum monthly soluble COD removal of $66 \pm 6\%$ and maximum monthly total COD removal of $73 \pm 10\%$, in the eighth month from start-up. Average effluent total COD concentration throughout the entire range of operation was 75 ± 15 mg/L.

It was observed that the pH at the bottom of the reactor was lower than the pH at the top portion of the reactor (Figure 2(b)). Low pH at the sludge bed zone is due to slight acidification of organic matter at this zone. As the wastewater moved upward, the VFAs produced were utilized by the acetogens and methanogens and resulted in increase of pH due the generated alkalinity from these reactions. The variation in slope of pH profile indicates presence of biochemical reactions within the reactor. Hence, it is evident from Figure 2 that major biochemical reactions occurred within the bottom 2 m of the reactor that is the sludge bed and sludge blanket zone.

The average biogas production rate was 0.39 ± 0.10 m³/kg COD removed and methane production was 0.26 ± 0.15 m³/kg COD removed. The produced biogas coupled with liquid upflow velocity and downward movement of sludge from settler is mainly responsible for providing mixing inside the reactor, which is essential for

proper distribution of substrate and to avoid any short circuit of the sewage through sludge bed. Theoretical biogas production should have been 0.52 m³/kg COD removed. Due to low biogas production rate and intermittent measurement of biogas some error might have caused lower biogas measurement. Methane content in the biogas was 68%.

VSS concentration in the sludge bed of the UASB reactor, after reaching steady state was 30 ± 7 g/L. A higher SRT is required in anaerobic treatment processes like UASB reactor when compared to the aerobic ones. The potential of UASB reactor is mainly governed by the amount of sludge that can be retained within the reactor. In turn SRT is strongly dependent on the settling characteristics of the sludge. Average TSS and VSS in the effluent of the UASB reactor were 55 ± 17 mg/L and 30 ± 11 mg/L, respectively. During start-up phase low upflow velocity ranging from 0.25 to 0.45 m/h was maintained in the UASB reactor, which resulted in surface overflow rate of 15 m³/m² day in the settling compartment of the gas-liquid-solid separator. This led to the very low VSS concentration of 30 mg/L in the effluent. The average SRT during the entire range of operation was around 150 days and observed sludge yield including the VSS coming in with the influent sewage was less than 0.03 kg VSS/kg COD removed. The low effluent VSS supported high sludge hold up in the sludge bed of the UASB reactor resulting in the considerably high SRT of 150 days in the reactor. This high SRT had a contribution to make granulation possible in the UASB reactor.

The average values for influent COD were 167 mg/L, 177 mg/L, 218 mg/L and 214 mg/L, respectively, in the late autumn months, winter months, spring months and summer months, where the respective mean temperatures of sewage were 25, 13, 25 and 33 °C. Higher temperatures contributed to improve performance of the reactor and to obtain lower effluent COD concentration. The maximum rate of bacterial growth decreases by 11% per 1 °C decrease

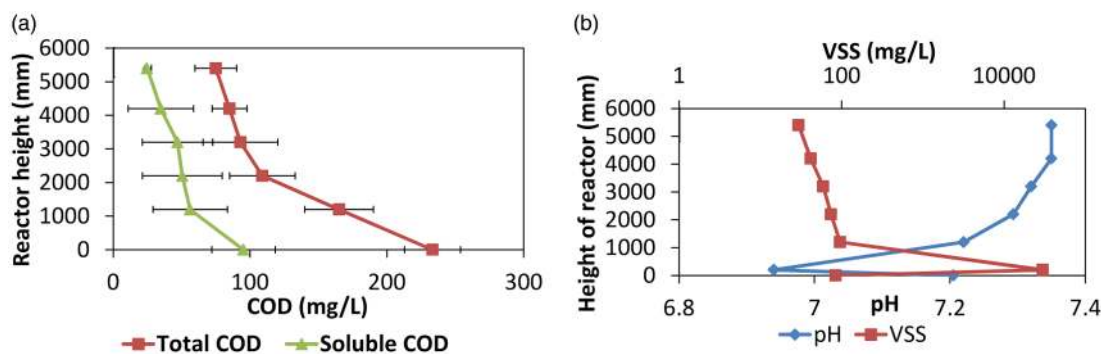


Figure 2 | Typical profile of (a) COD and (b) pH and VSS along the height of the UASB Reactor.

in temperature for anaerobic digesters operated below 30 °C (Pontes *et al.* 2014). The OLR applied to the UASB reactor was increased gradually from 0.14 kg COD/m³ day, with a scrupulous check on solid loading rate, during the operational phase to increase it to 0.81 kg COD/m³ day. Due to low inlet COD concentration, this was the maximum possible volumetric loading that could be applied to the UASB reactor. Organic matter removal efficiency was observed to improve with increasing OLR.

Decrease of stability constant (ρ) that is the ratio of VFA and alkalinity in anaerobic digestion process indicates process stability (Owamah & Izinyon 2015). When the VFA/alkalinity ratio exceeds 0.4, due to organic overloading (i.e. the rate of methane generation could not catch up with the rate of acid production) and could cause failure in digestion. This stability constant was higher than 0.4 in the start-up phase of November, December and January, indicating reduced activity of methanogens, leading to a higher COD in the effluent, even at higher HRTs. However, the reactor started to stabilize after the winter months and stability constant value decreased to 0.32 ± 0.09 .

Biomass granulation and settling in UASB reactor

The septic tank sludge used as inoculum to start up the reactor was flocculent in nature rather than granular. Specific density of the sludge collected from the UASB reactor on 200th day was 40.80 g VSS/L and that for the inoculum sludge it was 8 g VSS/L. Specific gravity of the sludge from UASB reactor was 1.04 when compared to 1.023 for inoculum sludge. Particle size distribution of the inoculum at the start of the experiments and at day 100, 200, 300 and 400 expressed in the percentage of the biomass volume represented by the granules is shown in Figure 3. After 200 days of operation around 5.63% of the sludge granules had a diameter of 1.0 mm, while 3.53% of the sludge granules had a diameter more than 1.0 mm. In comparison the inoculum sludge had 2.82% of particles of diameter more than 1 mm. Particles of diameter greater than 3 mm had a settling velocity above 150 m/h, which concludes that they might contain inert precursor material and not completely consisting of biomass sludge granules. While size of granular sludge has been widely reported to range from 0.5 mm to 5 mm, some researchers have reported sludge of size 0.16 mm or less as granules (Tiwari *et al.* 2005). Based on Reynold's number, Bhunia & Ghangrekar (2007) calculated the minimum size of granules as 0.34 mm, which makes around 63.47% of the sludge inside the UASB reactor after 200 days of operation as granular sludge. For

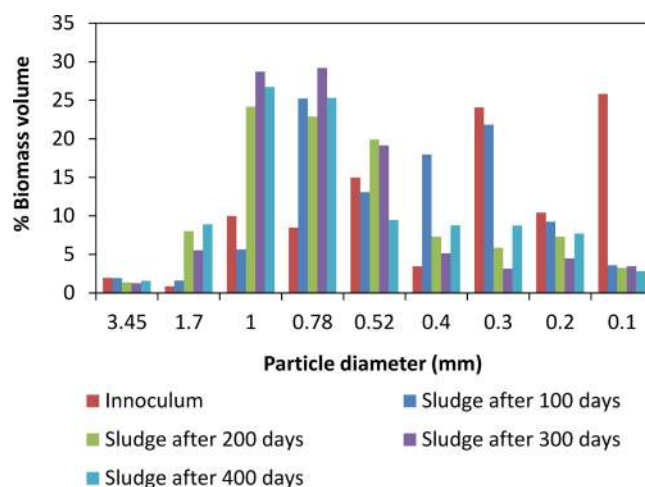


Figure 3 | Particle size distribution of inoculum sludge and sludge collected from UASB reactor at different days of operation.

the rest of the particles with size less than 0.34 mm, they can be regarded as pellets, which are aggregates with a more dense structure than flocs, which constituted around 60% fraction in the basic inoculum sludge (Bhunia & Ghangrekar 2007); whereas this fraction was only 34% after 200 days of operation. With duration of operation increase in percentage of higher granule size was observed. To the best of our knowledge such percentage of 63.47% of biomass granulation has not been reported while treating such low strength sewage in full scale UASB reactor.

SVI less than 20 mL/g TSS is reported for granular sludge; whereas for flocculent sludge the SVI ranges between 20 and 40 mL/g (Bhunia & Ghangrekar 2007). Equal values of SVI₅ and SVI₅₀ indicate good sedimentation properties. Similar phenomena were observed in the present study with SVI₅ and SVI₅₀ being 21.15 mL/g and 18.2 mL/g, respectively, after 200 days of operation of the pilot reactor. The SVI₅₀ of the inoculum sludge was 34.3 mL/g. It is worthy to note that the sludge samples used for determination of size distribution and settling velocity were collected from the reactor at a height of 20 cm from the bottom. Thus, the sludge granules at the bottom of sludge bed are expected to have even larger sizes and higher settling velocities (Li & Sung 2015).

Biomass granulation inside UASB reactors under favourable environmental conditions is mostly governed by the inoculum concentration and mixing in the sludge bed. The mixing is mainly induced by the upward movement of produced biogas and upflow velocity of wastewater in the reactor. A dimensionless number BGI and GI was developed by Bhunia & Ghangrekar (2009), to define favourable mixing conditions in the sludge bed of UASB reactor and

that was correlated with percentage granulation. Bhunia & Ghangrekar (2009) concluded that good granular sludge (percentage of granules more than 50%, w/w) can be developed in UASB reactor if BGI is maintained in the range of 240 to 560. To obtain proper granulation in UASB reactors (percentage granules greater than 50%, w/w), resulting in higher COD removal efficiency, GI values should be in the range of 15,000–57,000. For the present study, when the BGI was calculated initially with inoculum sludge concentration of 8 g/L, BGI was 185; however, with continued operation and increase in sludge concentration within the reactor the BGI value increased to an average of 280 and a maximum of 380, indicating a 50–60% of sludge granulation (Bhunia & Ghangrekar 2009), which is also evident from the settling velocity test. However, the GI value (12372) was slightly lower than the lower limit recommended for favourable granulation in the present study.

Other characteristics of sludge from UASB reactor

VSS/SS ratio of sludge indicates the viable micro-organisms present and percentage of inert matters content in the sludge. Specific gravity of granules is predominantly governed by the combined effects of percentage of inert matters content and density of cells (number of cells). Increase in VSS/SS ratio, indicates decrease in inert material content of sludge. Bhunia & Ghangrekar (2007) reported an optimum VSS/SS ratio of 0.5 for granular sludge of 1.5 mm size. The average VSS/SS ratio in the sludge collected from the bottom of the pilot UASB reactor was 0.56 and it was above 0.5 throughout the entire range of operation, with the ratio being above 0.6 for the winter months. The SMA of the sludge collected from the bottom of the reactor was around 0.45 kg CH₄ COD/kg VSS day. Bhunia & Ghangrekar (2007) also observed a decrease in SMA of the sludge with increase in VSS/SS ratio, which is evidenced by the lower biogas production in the winter months from the reactor.

Extracellular polymeric substances (EPS) can mediate both cohesion and adhesion of cells, and play a crucial role in maintaining structural integrity of microbial matrix (Liu *et al.* 2003). Total EPS content of the sludge collected from the bottom of the UASB reactor was 12.94 mg/g VSS. Besides EPS content, their distribution in different fractions namely slime EPS (S-EPS), loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS), is important as well to understand bacterial cell stability (Figure 4). The presence of TB-EPS provides a shield to bacterial cells and it is often considered as the ‘skeleton’ of sludge, mediating

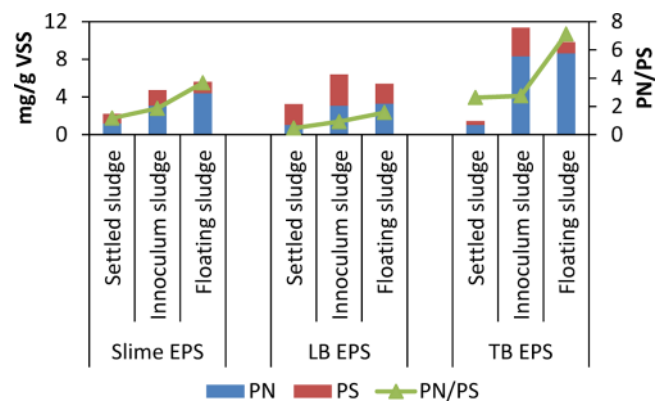


Figure 4 | PN and PS content in inoculum, settled and floating sludge.

both cohesion and adhesion of cells (Lu *et al.* 2015). On the other hand higher concentration of S-EPS and LB-EPS gives sludge a porous and fluffy structure with higher amount of bound water inside. The ratio of proteins and carbohydrates in sludge is used to determine its strength, stability and settling ability, with a higher ratio indicating low strength granules with bad settling properties and poor stability (Xing *et al.* 2015). Similar phenomenon was also observed in our study. The PN/PS ratio of the raw sludge was around 2.13; whereas that for the sludge collected on day 200 from the UASB reactor it was 0.94. The PN/PS ratio of floating sludge was significantly higher about 3.36 (Figure 4). The increase in PN content was observed to be much less than the increase in PS content in the sludge inside the UASB reactor thus leading to the formation of high strength granules.

Strength of granules was measured as integrity coefficient, which should be less than 20%, as reported by Ghangrekar *et al.* (1996), where strength of granules is inversely proportional to the value of integrity coefficient. The high strength of settled sludge (settling velocity more than 20 m/h), indicated by a low integrity coefficient of 3.74% was obtained for the sludge collected from the UASB reactor.

Effect of moving media in UASB reactor

The UASB reactor was operated for a total of 160 days after addition of polypropylene media into the sludge blanket zone of the reactor. As mentioned earlier maximum soluble COD removal of $66 \pm 6\%$ and maximum total COD removal of $73 \pm 10\%$ could be observed in the UASB reactor. To improve organic matter removal efficiency by providing a polishing treatment at the sludge blanket zone of the UASB, polypropylene media was added to it. Total COD removal efficiency increased to an average of $84 \pm 1\%$ and

soluble COD removal efficiency increased to an average of $76 \pm 3\%$. Average TSS and VSS in the effluent of the UASB reactor was 31 ± 17 mg/L and 20 ± 10 mg/L, respectively, which was 55 ± 17 mg/L and 30 ± 11 mg/L in UASB reactor without media addition. The presence of media helped in better retention of biomass and lower sludge wash-out resulting in low effluent VSS. Also bio-flocculation of particulate COD present in the raw sewage helped in increasing COD removal efficiency of the reactor.

Performance of HRAP

Effluent of UASB reactor was treated further in HRAP. As evident from Figure 5, start-up of the algal pond comprised of two steps based on the nitrogen removal performance: lag phase (1–50 days) and propagation phase. Lag phase was characterized by sharp variations in effluent nitrogen concentrations and nitrogen removal efficiency (Figure 5). During this lag period a reduction of TN was observed, but only after 1 month of operation, before which an increase of TN was detected. This may be due to organic hindrance or self-degradation of nutrients in wastewater thus making it unavailable to species, hence leading to less chlorophyll concentration in wastewater as well (Kiran *et al.* 2014).

The HRT of the pond was gradually reduced from an HRT of 7.8 days to 1 day. After 50 days, when the algal pond reached into propagation phase the HRT of the pond was 5 days and it was gradually reduced further. In the early days at HRT of 7.8 days, there was a negligible removal of nitrogen and pathogen due to presence of less concentration of algal culture; however, nitrogen removal stabilized at HRTs lesser than 5 days and stable pond performance could be observed even up to an HRT of 1.3 days. Further reduction of HRT led to detrimental effects on nutrient and pathogen removal.

After reaching steady state, TN removal increased to $85.1 \pm 2.4\%$. With influent TN concentrations of 20 ± 3 mg/L, the average effluent TN concentration was 3 ± 1 mg/L. Similar nitrogen removal rate was reported by Ruiz-Marin *et al.* (2010) while using pure culture of *Chlorella vulgaris* and *Scenedesmus obliquus* for treating municipal wastewater. The ammonium ion tolerance of different algae species is around $1,000 \mu\text{mol}$ (Cai *et al.* 2013). The inlet ammonia concentration to the HRAP was slightly higher than this concentration. However, ammonium is not only removed by cell metabolism, but also by volatilization, where significant amounts of ammonia can be volatilized at increased pH and temperature (Cai *et al.* 2013). Hence, presence of ammonia stripping significantly reduced ammonia concentration below the maximum tolerance level for algal cells. Similar results were also reported by researchers like Li *et al.* (2011). Nitrification was not observed in the pond due to low DO availability with a nitrate concentration of only 1.3 ± 0.2 mg/L.

Though during lag phase, there was very little biomass built up or nitrogen removal; however there was a stable phosphate removal starting from the initial days, which reached to a maximum of $91 \pm 1\%$. Similar to the removal of nitrogen, phosphorus removal in wastewater is not only governed by the uptake by the cells, but also by external conditions such as pH and dissolved oxygen. Phosphorus cannot exist in a gaseous state, thus phosphate precipitates from the wastewater as a result of elevated pH (above 9) leading to such high phosphate removal rate. Higher phosphate removal rates when compared to nitrogen removal, indicates majority of phosphate removal occurred due to precipitation rather than cell synthesis and assimilation (Cai *et al.* 2013). A final effluent total COD of around 50 ± 6 mg/L could be obtained after treatment in HRAP. There was a 2–3 log scale pathogen removal after treatment in HRAP with MPN of the final effluent being 600–800 per

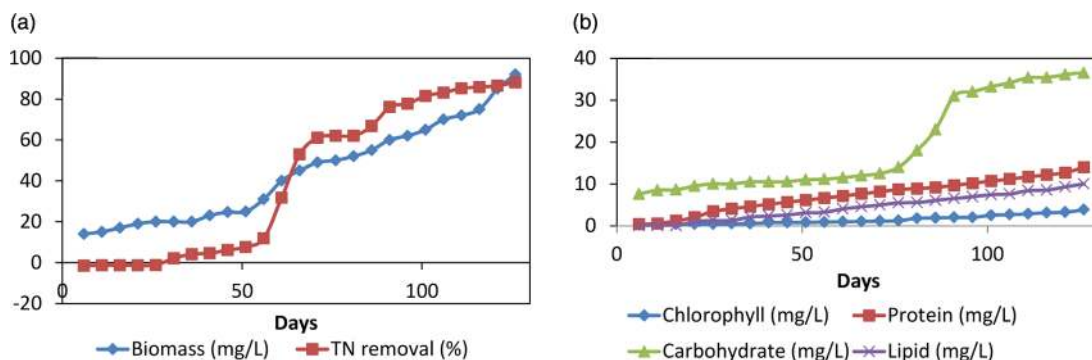


Figure 5 | Variation of (a) algal biomass and TN removal efficiency, (b) biochemical composition of algae with time.

100 mL, which is within acceptable standards for surface irrigation (CPCB 1993).

Ruiz-Marin *et al.* (2010) operated a semi-continuous microalgal cultivation system on municipal wastewater, using immobilized beads of pure culture as inoculum. Use of high concentration of such inoculum prevented spontaneous growth of other species of microalgae. Though initially a high ammonia and phosphate removal efficiency was reported by Ruiz-Marin *et al.* (2010), however both ammonia and phosphate removal efficiency dropped to almost 30% just after four cycles of operation due to collapse of pure culture of bacteria. In this respect, allowing spontaneous growth of mixed culture algae as a response to secondary treated sewage in the current study proved to be a better way at management of post-treatment options for UASB effluent.

Biochemical analysis of microalgae was also done to observe the changes in terms of proteins, carbohydrates and lipids concentration in the presence of wastewater. Accumulation of these has been found to be increasing with time. Some microalgae display heterotrophic behaviour, using organic forms of carbon, while others may possess both autotrophic and heterotrophic traits, simultaneously (Cai *et al.* 2013). Decrease in organic matter content of the influent waste-stream indicates presence of heterotrophic algal or bacterial population in the HRAP. High levels of protein in the later stages of growth support the decrease in levels of nitrogen or rapid uptake of nutrients for metabolic activity. Decreasing levels of nitrogen content also plays important role in the production of lipids (Kiran *et al.* 2014). Average protein and lipid content of the algal cells of around 15–16% and 10–11%, was in similar range of high lipid accumulating species like *Chlorella vulgaris* when grown in municipal wastewater (Ruiz-Marin *et al.* 2010). The energy efficiency for biodiesel production from this harvested mixed culture algae needs to be worked out to avail energy recovery.

Performance of algal settler

Harvesting of microalgae typically employs methods such as filtration, centrifugation, or flocculation, which can be technically and economically challenging when considering larger production scales (Cai *et al.* 2013). Hence, simple secondary sedimentation was explored for harvesting of microalgae. A maximum settling of $85.71 \pm 4.29\%$ of algal biomass was observed in the algal settler at an HRT of 6 hour. A primary assumption for design of settler is that there is no biochemical activity in the settler. Initially when the settler was operated at an HRT of 1 day due to

controlled flow rate in the algal pond, percentage of algal biomass settled was just $59.17 \pm 6.67\%$. This HRT is not suitable for a settler since, it allows algal growth in the settler compartment leading to reduced settling efficiency. Settling efficiency improved upon reduction of HRT from 1 day to 6 hours. However, further reduction of HRT to 3 hour showed a decline in performance with settling efficiency being reduced to $79.54 \pm 5.45\%$.

Cost of treatment

To get a qualitative idea of cost of treatment, capital, operation and maintenance, and depreciation cost was estimated. Total capital cost of construction of the $500 \text{ m}^3/\text{day}$ UASB-HRAP was Rs 3,400,000 (\$ 53,000) as per Indian market rates. Three semi-skilled personnel were employed for routine monitoring of plant performance and operation of the plant. Salary of each personnel was Rs 8,000 (\$ 125)/month, as per DST, Govt. of India mandate for attendant. Annual maintenance required was assumed as Rs 100,000 (\$ 1,556) with an annual depreciation of 10% of the capital cost. Electricity cost @ Rs 8 (\$ 0.12) per unit, for operating two pumps of 5 HP and 2 HP was Rs 654,080 (\$ 10,177) annually. Hence, the treatment cost was estimated as Rs 7.57/kL (\$ 0.12/kL). Considering the cost of transport and treatment of same volume of potable water above Rs 25/kL (\$ 0.39/kL), presently incurred in IIT Kharagpur campus India, net benefit of above Rs 15/kL (\$ 0.23/kL), along with added benefits of saving freshwater reserves is being achieved.

CONCLUSIONS

This study aimed at assessing the performance of a pilot-scale UASB reactor to achieve granulation while treating sewage followed by a HRAP to remove organic matter, nutrients and pathogen from sewage to facilitate reuse. The sewage after treatment using UASB/HRAP is meeting the standards for cultivation of non-food crops and being reused for gardening and landscaping. Utilization of nutrients present in the treated sewage for the growth of microalgal species will not only control eutrophication but will also help in sustainable energy development from this harvested biomass. The findings of this study suggest that anaerobically treated sewage can be directly used for mass cultivation of microalgae without requiring additional nutrient supplements. A net gain above Rs 15 (\$ 0.23)/kL, considering the cost of treatment of sewage @ Rs 7.57

(\$ 0.12)/kL, by reusing the treated wastewater, emphasizes the cost-effectiveness of the treatment scheme proposed.

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