

# Performance assessment of combination of an upflow anaerobic sludge blanket reactor and high rate algal pond for complete sewage treatment

Pritha Chatterjee<sup>1</sup>, Sumat C. Jain<sup>1</sup>, Chattulaal Maity<sup>1</sup>, M.M. Ghangrekar<sup>1,\*</sup>, A. Real<sup>2</sup>, C. A. Aragon<sup>2</sup>, I. Martin<sup>2</sup> and J.J. Salas<sup>2</sup>

<sup>1</sup>- Department of Civil Engineering, Indian Institute of Technology, Kharagpur – 721302, India.

<sup>2</sup>- Centre for New Water Technologies (CENTA). Autovia Sevilla-Huelva, km.28. 41820. Carrión de los Céspedes (Sevilla). Spain.

\*-Corresponding author: Tel.: +91-3222-283440; Fax: 03222-282254, E-mail: [ghangrekar@civil.iitkgp.ernet.in](mailto:ghangrekar@civil.iitkgp.ernet.in)

## Abstract

This study aimed at assessing the performance of a pilot-scale upflow anaerobic sludge blanket (UASB) reactor followed by a high rate algal pond (HRAP) to remove organic matter, nutrients and pathogen from sewage and facilitate reuse. Sampling was carried out at different heights of the UASB reactor (inlet, 0.2 m, 1.2 m, 2.2 m, 3.2 m, 4.2 m and outlet), and profile concentrations were determined for organic matter and solids. First-order kinetics showed the best fit to the decay of concentrations of chemical oxygen demand (COD) in the UASB reactor, after it reached steady state. For an influent COD concentration of  $233 \pm 20$  mg L<sup>-1</sup>, the effluent COD concentration was  $75 \pm 15$ . Successful biomass granulation was observed in the sludge bed of the UASB reactor. After reaching steady performance in HRAP, ammonia removal increased to  $85.1 \pm 2.4\%$  with influent ammonia nitrogen concentrations of  $20 \pm 3$  mg/L and effluent ammonia nitrogen concentration of  $3 \pm 1$  mg/L. Phosphate removal after treatment in the HRAP was  $91 \pm 1\%$ . There was a 4 log scale pathogen removal after treatment with HRAP with MPN of the final effluent being less than 1000/100 ml, which is within acceptable standards for surface irrigation. The blackwater after treatment using UASB/HRAP is being reused for gardening and landscaping.

**Keywords:** Anaerobic treatment; disinfection; kinetics; reuse; UASB/pond system; organic matter removal

## INTRODUCTION

Population growth, industrialization, agricultural practices and urbanization all increase the water demand and thus the quantity of wastewater generated. 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 (Azizi et al., 2013; Moawad et al., 2009). Various options for treatment and reuse of water like water resources (reclaimed water); energy (methane from anaerobic digestion) and materials (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 decentralised treatment units are very helpful in these cases (Mankad & Tapsuwan, 2011). The reclaimed water application governs the type of treatment needed to protect public health and environment and the degree of reliability required to treatment processes and operation (Moawad et al., 2009).

55 Conventional sewage treatment processes involve high capital, maintenance and operational  
56 cost, huge energy requirements, which makes them unsuitable for use in developing countries  
57 (Sato et al., 2006). Energy efficient low-cost waste treatment systems are the best choice for  
58 such countries. Anaerobic treatment systems excel in such respect. UASB reactors are the  
59 most widely used high rate anaerobic sewage treatment process and several full scale reactors  
60 have been operated world-wide (Buntner et al., 2013; Lim & Kim, 2014). Most of the  
61 successful applications of UASB reactors are to treat high strength industrial wastewaters  
62 (Lim & Kim, 2014). Municipal sewage treatment using UASB reactors is restricted to  
63 tropical regions where temperature of the raw sewage allows fast hydrolysis of organic  
64 matters and suspended solids (Zhang et al., 2013). Sato et al. (2006) evaluated the treatment  
65 efficiency of the sixteen UASB reactor based sewage treatment plants on the Yamuna river  
66 basin in India and observed that none of the plants met the discharge standards for  
67 biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solids (SS)  
68 or nutrients as total Kjeldahl nitrogen (TKN). The discharge standards for BOD, COD, SS  
69 and TKN are 30 mg/L, 250 mg/L, 100 mg/L and 100 mg/L, respectively (CPCB, 1993). In  
70 order to improve the effluent quality up to disposal standards, polishing ponds with short  
71 retention time were used to treat the UASB effluent. Unfortunately, effluent quality did not  
72 follow the desired standard limits even after the polishing ponds (Sato et al., 2006).

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74 In the present study, the possibility of producing reusable quality treated wastewater by a  
75 combined UASB-HRAP system is explored. While UASB will help in organic matter  
76 removal, HRAP will abate nutrients and pathogens. Microalgal cultivation generally requires  
77 inorganic nutrients and carbon dioxide in the presence of sunlight through photosynthesis.  
78 These organisms can also be used to accumulate nutrients, as they require less than one-tenth  
79 of the area to recover P compared to terrestrial crops (Mehta et al., 2015). As microalgae  
80 require both organic and inorganic nutritional inputs for their survival, wastewater can be a  
81 potential source of these nutritional requirements and hence forth micro-algae can help in  
82 bioremediation of UASB treated wastewater (Kiran et al., 2014). Microalgae also have broad  
83 bioenergy potential as they can be used to produce liquid transportation and heating fuels,  
84 such as biodiesel and ethanol, or anaerobically digested to produce biogas. The treated  
85 wastewater after UASB-HRAP can be used for landscape irrigation (parks, playgrounds, and  
86 school yards), fire protection, construction, ornamental fountains, recreational  
87 impoundments; in-building uses (toilets, air conditioning).

## 88 89 90 **MATERIALS AND METHODS**

### 91 92 **Pilot plant description and operation**

93 A novel combination of UASB reactor and HRAP was designed and constructed for  
94 treatment of sewage and performance was evaluated to examine the removal of organic  
95 matter and nitrogen transformation processes (Figure 1). The UASB reactor was having a  
96 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  
97 HRAP. The UASB/HRAP system was fed with domestic sewage generated in IIT Kharagpur  
98 campus, India. The sewage was passed through a screen located upstream the UASB reactor.  
99 The pilot UASB reactor was inoculated with septic tank sludge. It was operated at gradually  
100 increasing organic loading rates in the start-up phase. The HRAP was operated at a constant  
101 HRT of 8 days.



Figure 1: (a) UASB reactor, (b) HRAP

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### 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 analysed parameters were: chemical oxygen demand (COD, total and soluble), total suspended solids (TSS), volatile suspended solids (VSS), alkalinity, volatile fatty acids (VFA), pH, total kjeldahl nitrogen (TKN), 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 (TDS), pH and  $\text{NO}_3^-$ -N were measured using electrodes (Thermo, USA). Turbidity was measured by Systronics Turbidity Meter. Biogas production was measured continuously by water displacement method.

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The characterization of biomass was done for the UASB reactor, 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) and Bhunia and Ghangrekar (2008a). 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 and 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 polysachharide (PS) by Anthrone method (Morris, 1948).

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## RESULTS AND DISCUSSION

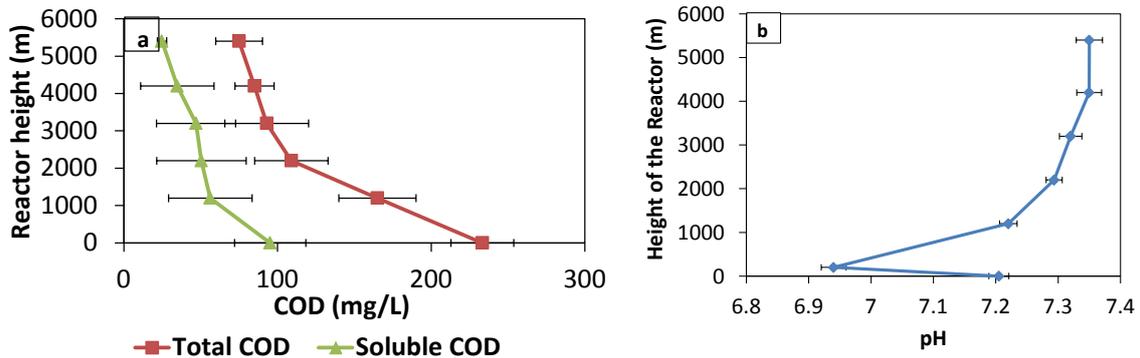
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### General performance

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The UASB reactor was operated for a total of 250 days. After an initial start-up period with gradually improving performance the system stabilised and remained in pseudo steady state until the end of the experimental period. COD profile was evaluated over reactor height. Majority of COD reduction took place only within 2 m from the bottom of the reactor (Figure 2a), which is also corroborated by the pH profile (Figure 2b). COD removal efficiency kept

143 on increasing with operation time, with a maximum soluble COD removal of  $73 \pm 6\%$  and  
 144 maximum total COD removal of  $66 \pm 10\%$ , in the month of June. Average effluent COD  
 145 throughout the entire range of operation was  $75 \pm 15$  mg/L.



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 147 Figure 2: Typical (a) COD and (b) pH profile along the height of UASB reactor  
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149 It was observed that the pH at the bottom of the reactor was lower than the pH at the top  
 150 portion of the reactor. Low pH at the sludge bed zone is due to slight acidification of  
 151 substrate at this zone. As the wastewater moved upward, the VFA produced was utilized by  
 152 the methanogens and resulted in increase of pH due the generated alkalinity from the  
 153 reaction. The variation in slope of pH profile indicates presence of biochemical reactions  
 154 within the reactor. Hence, it is evident from Figure 2 that major biochemical reactions occur  
 155 within the bottom 2 m of the reactor that is the sludge bed and sludge blanket zone.  
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157 Biogas production rate was measured by water displacement method and methane content  
 158 was measured by passing biogas through 5% NaOH solution. The average gas production rate  
 159 was  $0.39 \pm 0.10$  m<sup>3</sup>/kg COD removed. The produced biogas is mainly responsible for mixing  
 160 inside the reactor, which is essential for proper distribution of substrate and to avoid any short  
 161 circuit. Theoretical biogas production should have been 0.62 m<sup>3</sup>/kg COD removed. Due to  
 162 low gas production rate and intermittent measurement of biogas some error might have  
 163 caused lower biogas measurement. Methane content in the biogas was 68%.  
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165 VSS concentration in the sludge bed of the UASB reactor, after reaching steady state was  $30$   
 166  $\pm 7$  g/L. A higher solid retention time (SRT) is required in anaerobic treatment processes like  
 167 UASB reactor as compared to the aerobic ones. The potential of UASB reactor is mainly  
 168 governed by the amount of sludge that can be retained within the reactor. In turn SRT is  
 169 strongly dependent on the settling characteristics of the sludge. The average SRT during the  
 170 entire range of operation was around 150 days and average sludge yield was less than 0.3 kg  
 171 VSS/kg COD removed. Average TSS and VSS in the effluent of the UASB reactor was  $31 \pm$   
 172  $17$  mg/L and  $20 \pm 10$  mg/L, respectively.  
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174 Table 1 shows the monthly mean concentrations of total and soluble COD, in the influent and  
 175 effluent of the UASB reactor. The average values for influent COD were 167 mg/L, 177  
 176 mg/L, 218 mg/L and 214 mg/L, respectively in the late autumn months, winter months,  
 177 spring months and summer months, where the respective mean temperatures were 25, 13, 25  
 178 and 33 °C. Higher temperatures contributed to improve the performance of the reactor and to  
 179 obtain lower effluent COD concentration. The maximum rate of bacterial growth decreases  
 180 11% per 1 °C for anaerobic digesters operated below 30 °C (Pontes et al., 2014).  
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184 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 (%)	Remarks
Nov	167 ± 30	123 ± 30	27 ± 12	110 ± 12	77 ± 12	30 ± 8	Reactor getting commissioned
Dec	162 ± 30	79 ± 41	49 ± 26	94 ± 21	35 ± 7	63 ± 7	Performance improved
Jan	193 ± 26	98 ± 19	49 ± 9	95 ± 3	39 ± 1	59 ± 1	Sludge washout due to reduced temperature and performance instability
Feb	187 ± 61	76 ± 17	56 ± 16	78 ± 23	33 ± 13	57 ± 9	Performance getting stabilized
Mar	250 ± 61	100 ± 18	58 ± 12	101 ± 21	38 ± 7	63 ± 10	Improving Performance
April	195 ± 49	71 ± 11	61 ± 11	83 ± 19	23 ± 2	70 ± 8	Improving Performance
May	215 ± 44	78 ± 31	63 ± 14	91 ± 29	23 ± 3	72 ± 7	Improving Performance
June	233 ± 20	75 ± 15	66 ± 10	95 ± 23	25 ± 3	73 ± 6	Improving Performance

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186 Decrease of stability constant ( $\rho$ ) that is the ratio of VFA and alkalinity in anaerobic digestion  
187 processes indicates process stability (Nikolaeva et al., 2009; Owamah & Izinyon, 2015).  
188 When the VFA/alkalinity ratio exceeds 0.4, due to organic overloading (i.e. the rate of  
189 methane generation could not catch up with the rate of acid production) and could cause  
190 failure in digestion. This stability constant was higher than 0.4 in the start-up phase of  
191 November, December and January, indicating reduced activity of methanogens, leading to a  
192 higher COD in the effluent, even at higher HRTs. Hence, during regression analysis, such  
193 low values of  $R^2$  were obtained in these months.

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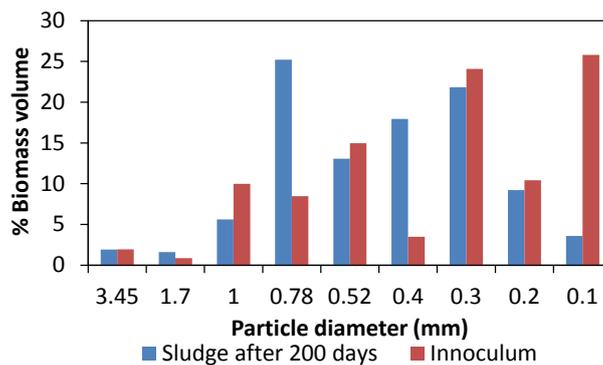
### 195 Biomass granulation and settling

196 The inoculum used to start up the reactor was flocculent in nature rather than granular.  
197 Specific density of the sludge collected from the UASB reactor on 200<sup>th</sup> day was 40.80 g  
198 VSS/L and that for the inoculum sludge it was 8 g VSS/L. Specific gravity of the sludge from  
199 UASB reactor was 1.04 as compared to 1.023 for inoculum sludge. Particle size distribution  
200 of the inoculum at the start of the experiments and at day 200, expressed in the percentage of  
201 the biomass volume represented by the granules is shown in Figure 3. After 200 days of  
202 operation around 5.63% of the sludge had a diameter of 1.0 mm, while 3.53% of the sludge  
203 had a diameter more than 1.0 mm. In comparison the inoculum sludge had 2.82% of particles  
204 of diameter more than 1 mm. Particles of diameter greater than 3 mm had a settling velocity  
205 above 150 m/h which concludes that they might contain inert precursor material and not  
206 completely consisting of biomass sludge granules. While size of granular sludge has been  
207 widely reported to range from 0.5 mm to 5 mm, some researchers have reported sludge of

208 size 0.16 mm or less as granules (Bhunia & Ghangrekar, 2007). Based on Reynold's number,  
 209 Bhunia and Ghangrekar (2007) calculated the minimum size of granules as 0.34 mm which  
 210 makes around 63.47% of the sludge inside the UASB reactor after 200 days of operation as  
 211 granular sludge. For the rest of the particles with size less than 0.34 mm, they can be regarded  
 212 as pellets, which are aggregates with a more dense structure than flocs, which constituted  
 213 around 60% fraction in the basic inoculum sludge (Bhunia & Ghangrekar, 2007); whereas  
 214 this fraction was only 34% after 200 days of operation. To the best of the knowledge of the  
 215 authors such percentage of granulation has not been reported while treating sewage in full  
 216 scale UASB reactor.

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 218 SVI less than 20 mL/g is reported for granular sludge, whereas for flocculent sludge the SVI  
 219 ranges between 20 and 40 mL/g (Bhunia & Ghangrekar, 2008a). Equal values of SVI<sub>5</sub> and  
 220 SVI<sub>30</sub> indicate good sedimentation properties (Jin et al., 2013; Tang et al., 2011). Similar  
 221 phenomena were observed in the present study with SVI<sub>5</sub> and SVI<sub>30</sub> being 31.15 mL/g and  
 222 28.2 mL/g, respectively, after 200 days of operation of the pilot reactor. The SVI<sub>30</sub> of the  
 223 inoculum sludge was 34.3 mL/g. It is worthy to note that the sludge samples used for  
 224 determination of size distribution and settling velocity were collected from the reactor at a  
 225 height of 200 cm from the bottom. Thus, the sludge at the bottom of sludge bed are expected  
 226 to have even larger sizes and higher settling velocities (Li & Sung, 2015).

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229 Figure 3: Particle size distribution of inoculum sludge and sludge collected from UASB  
 230 reactor  
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233 Biomass granulation inside UASB reactors under favourable environmental conditions is  
 234 mostly governed by the inoculum concentration and mixing in the sludge bed. The mixing is  
 235 mainly induced by the upward movement of produced biogas and upflow velocity of  
 236 wastewater in the reactor. A dimensionless number biomass granulation index (BGI) and  
 237 granulation index (GI) was developed by Bhunia and Ghangrekar (2009) and Bhunia and  
 238 Ghangrekar (2008b), respectively to define favourable mixing conditions in the sludge bed of  
 239 UASB reactor and that was correlated with percentage granulation. Bhunia and Ghangrekar  
 240 (2009) concluded that good granular sludge (percentage of granules more than 50%, w/w)  
 241 can be developed in UASB reactor if BGI is maintained in the range of 240 to 560. To obtain  
 242 proper granulation in UASB reactors (percentage granules greater than 50%, w/w), resulting  
 243 in higher COD removal efficiency, Bhunia and Ghangrekar (2008b) recommended to  
 244 maintain GI values in the range of 15,000–57,000. For the present study, when the BGI was  
 245 calculated initially with inoculum sludge concentration of 8 g/L, BGI was 185; however, with  
 246 continued operation and increase in sludge concentration within the reactor the BGI value  
 247 increased to an average of 280 and a maximum of 380, indicating a 50 – 60% possibility of  
 granulation (Bhunia & Ghangrekar, 2009) which is also evident from the settling velocity

248 test. However, the GI values were not in the range for favourable granulation in the present  
 249 study.

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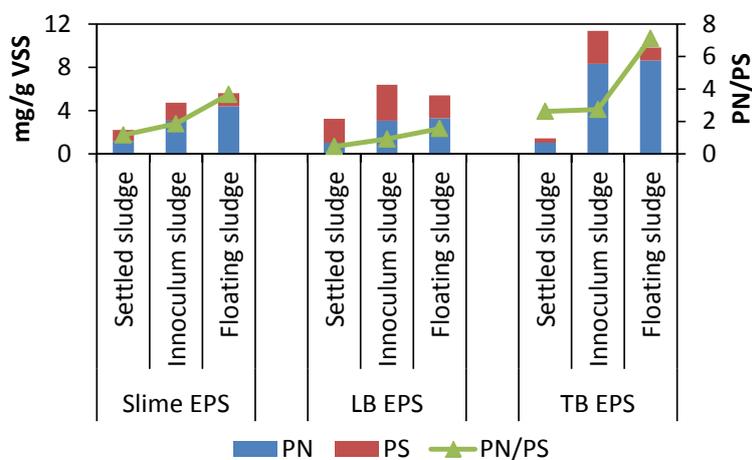
251 **Biomass characterization**

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

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263 Extracellular polymeric substances (EPS) can mediate both cohesion and adhesion of cells,  
 264 and play a crucial role in maintaining structural integrity of microbial matrix (Liu et al.,  
 265 2003). Total EPS content of the sludge collected from the bottom of the UASB reactor was  
 266 12.94 mg/g VSS. Besides EPS content, their distribution in different fractions namely slime  
 267 EPS (S-EPS), loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS), is important as  
 268 well to understand bacterial cell stability (Figure 4). The presence of TB-EPS provides a  
 269 shield to bacterial cells and it is often considered as the “skeleton” of sludge, mediating both  
 270 cohesion and adhesion of cells (Lu et al., 2015; Zhen et al., 2013). On the other hand higher  
 271 concentration of S-EPS and LB-EPS gives sludge a porous and fluffy structure with higher  
 272 amount of bound water inside (Bergmans et al., 2014). The ratio of proteins and  
 273 carbohydrates in sludge is used to determine its strength, stability and settling ability, with a  
 274 higher ratio indicating low strength granules with bad settling properties and poor stability  
 275 (Chen et al., 2015; Lu et al., 2015; Tang et al., 2011; Xing et al., 2015). Similar phenomenon  
 276 was also observed in our study. The PN/PS ratio of the raw sludge was around 2.13; whereas  
 277 that for the sludge collected on day 200 from the UASB reactor was 0.94. The PN/PS ratio of  
 278 floating sludge was significantly higher about 3.36 (Figure 4). The increase in PN content  
 279 was observed to be much less than the increase in PS content in the sludge inside the UASB  
 280 thus leading to the formation of high strength granules.

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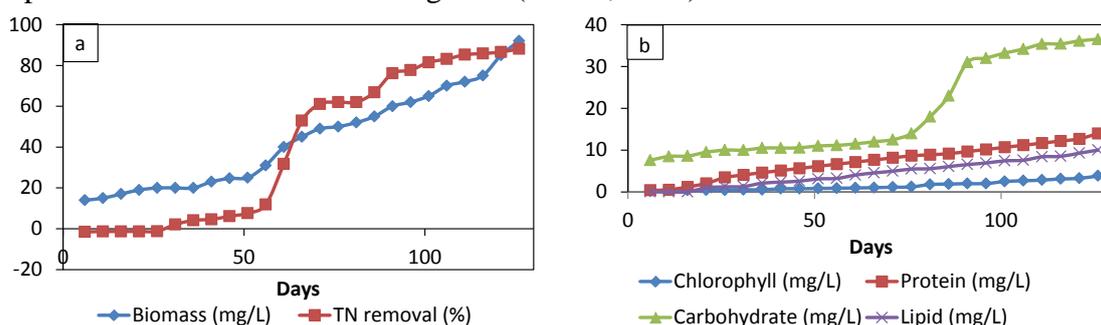
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Figure 4: PN and PS content in inoculum, settled and floating sludge

285 Strength of granules was measured as integrity coefficient, which should be less than 20%, as  
 286 reported by Ghangrekar et al. (1996), where strength of granules is inversely proportional to  
 287 the value of integrity coefficient. The highest strength of settled sludge, indicated by lowest  
 288 integrity coefficient as 3.74% was developed for the sludge collected from the UASB reactor.  
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### 290 Performance of HRAP

291 As evident from Figure 5, start-up of the algal pond comprised of two steps based on the  
 292 ammonium removal performance: lag phase (1 – 50 days) and propagation phase (still  
 293 continuing). Lag phase was characterized by sharp variations in effluent ammonium  
 294 concentrations and ammonium removal efficiency (Figure 5). During this lag period a  
 295 reduction of ammonium was observed, but only after one month of operation, before which  
 296 an increase of ammonium ion was detected. This may be due to organic hindrance or self-  
 297 degradation of nutrients in wastewater thus making it unavailable to species, hence leading to  
 298 less chlorophyll concentration in wastewater (Kiran et al., 2014). After reaching steady  
 299 ammonia removal increased to  $85.1 \pm 2.4\%$ . With influent ammonia nitrogen concentrations  
 300 of  $20 \pm 3$  mg/L the average effluent ammonia nitrogen concentration was  $3 \pm 1$  mg/L.  
 301 Though during lag phase, there was very little biomass built up or nitrogen removal; however  
 302 there was a stable phosphate removal starting from the initial days which reached to a  
 303 maximum of  $91 \pm 1\%$ . A final effluent total COD of around  $50 \pm 6$  mg/L could be obtained  
 304 after treatment with HRAP. There was a 4 log scale pathogen removal after treatment with  
 305 HRAP with MPN of the final effluent being less than 1000/100 ml, which is within  
 306 acceptable standards for surface irrigation (CPCB, 1993).



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 309 Figure 5: Variation of (a) algal biomass and TN removal efficiency, (b) biochemical  
 310 parameters of algae with time  
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312 Biochemical analysis of microalgae was also done to observe the changes in terms of  
 313 proteins, carbohydrates and lipids concentration in the presence of wastewater. Accumulation  
 314 of these has been found to be increasing with time. High levels of protein in the later stages  
 315 of growth support the decrease in levels of nitrogen or rapid uptake of nutrients for metabolic  
 316 activity. Decreasing levels of nitrogen content also plays important role in the production of  
 317 lipids (Kiran et al., 2014).  
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### 320 CONCLUSIONS

321 Wastewater contains many organic and inorganic nutrients, which are discharged into water  
 322 streams causing environmental pollution and health hazards. UASB reactor successfully gave  
 323 more than 70% COD removal with biomass granulation. Utilization of nutrients present in the  
 324 treated sewage for the growth of microalgal species will not only control eutrophication but  
 325 will also help in sustainable energy development. The findings of this study suggest that  
 326 sewage wastewater can be directly used for mass cultivation of microalgae without requiring

327 additional nutrient supplements. The effluent of the algal pond can be directly reused for  
328 surface irrigation of non-food crops.

329

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