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Reuse of secondary treated effluent of UASB reactor for bioremediation using algae as feed in small scale aquaculture system

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Abstract
The present experiment deals with the use of secondary treated sewage by Up flow Anaerobic Sludge Blanket (UASB) followed by algal pond for tertiary treatment of secondary treated effluent along with aquaculture practices. Water quality is an important parameter which governs fish growth. Water quality standards vary significantly due to different environmental conditions, ecosystem and intended human uses. This experiment was carried out during February to April months with algal pond water as culture medium. The water coming from UASB reactor is directly discharged into algal pond water for tertiary treatment along with aquaculture. The growth of tilapia (*Oreochromis niloticus*) fishes was observed and treatment efficiency was measured in terms of organic waste as well as pathogens removal. The results obtained from the experiment shows 80.10±2.4% of total nitrogen removal with influent total nitrogen concentrations of 20±3 mg/L. Phosphate removal after treatment in algal tank was 90±1%. There was 2-3 log scale pathogen removal was also observed after treatment in algal tank. The maintenance of good water quality is essential for both survival and optimum growth of culture organisms. In conclusion, apart from wastewater treatment UASB-Algal-tilapia ponds also offer marketable by-products as fish protein including algal biomass, which correspond to cost recovery for sewage treatment.

Keywords: bioremediation; aquaculture; algal pond; UASB treated secondary effluent; tertiary treatment and water quality

Introduction
Water scarcity affects all continent and around 2.8 billion people around the world as a minimum one month out of every year. More than 1.2 billion people lack, right to use to clean drinking water (WHO, 2005). As per CPCB reports, in India generate around 38,254 MLD (Million Litres per Day) of sewage of which only 11,787 (31%) is treated and balance is discharged untreated. The key regarding sewage collection, treatment and disposal at the national as well as state level is inadequate provision of sewage treatment facilities which is one of the main causes of water-bodies pollution in country. The rate of municipal waste generation in India in 2011 was 127458.1 T/day. This was divided by the then urban population to get the per capita waste generation rate of 0.356 kg/day. The amount of waste generated per capita is estimated to increase at the rate of 1-1.33% annually (Pappu *et al*. 2007) [25]. The global biochemical cycles had largely affected by human activities. In the course of agricultural practices, urbanization, industrialization and other alterations, humans had increased the use of nutrients into biochemical cycles, mainly nitrogen and phosphorus. An increase in algae and aquatic plants, loss of component species, and loss of ecosystem function are caused due to eutrophication (nutrient enrichment) of aquatic ecosystems. So many studies have focused on nitrogen and phosphorus removal regarding these reasons. Most of these studies are based on biological processes and different combinations anaerobic, aerobic and anoxic zones. The treatment of sewage by using a series combination of a UASB (Up flow Anaerobic Sludge Blanket) reactor and waste stabilization tank is a low cost solution and could satisfy the discharge standards with regards to physiochemical and biological parameters. The success of the UASB concept relies on the establishment of a dense sludge bed in the bottom of a reactor, in which biochemical conversion of organic matter into methane and CO_2 takes place. The sludge bed is basically consists of active anaerobic bacteria and incoming suspended solids, getting digested by action of bacteria. The effluent from UASB reactor usually needs further treatments, in order to remove organic matter, nutrient and pathogens. By taking ideas from the usual practices in different disciplines of wastewater

management, aquaculture is being purposed and standardized as a tool for post treatment of domestic sewage (CIFA, 1998) [8]. Mechanization of system gives better output as well as serves as a cost recovery option to minimize or recover operational investments, and is mostly eco-friendly (Kumar and Kumar, 2017) [15, 16, 17, 18]. Mechanization need be enhanced substantially in order to meet the recommended level and to enhance the productivity (Kumar *et al.* 2017; Kumar *et al.* 2018) [15, 16, 17, 18, 19]. Mechanization has been defined as the use of improved hand and animal operated tools (Kumar *et al.* 2016). Aeration affects the growth as well as metabolic activities of fish towards treatment efficiency of fish (Kumar *et al*. 2017) [15, 16, 17, 18]. There are so many studies which demonstrated a great potential for the nitrogen and phosphorus removal with the help of microalgae. The uptake into cell and stripping ammonia through elevated pH are main mechanisms in algal nutrient removal from wastewater (Hoffman, 1998; Bich *et al*. 1999) [14, 4]. Bioremediation with algae has advantages such as low cost of the operation, the recycling possibility of assimilated phosphorus and nitrogen into algae biomass as a fertilizer avoiding sludge handling difficulty, and the oxygenated effluent release into the water body. In secondary treatment effluent of domestic wastewater, the average value of NH_4^+ and PO_4-P were 27.4 mg/L and 11.8 mg/L respectively as reported by Martinez *et al*. (2000) [20] whereas 48 mg/L of NH_4-N and 16 mg/L of PO_4-P in the treated effluent of Upflow Anaerobic Sludge Blanket reactor, fed with domestic sewage (Van der Steen *et al*. 1999) [30].

Microalgae were used in many aspects. Due to their high photosynthetic efficiency and their faster growth rate comparatively any other energy crops, microalgae are one of the most promising sources for biodiesel production (Minowa *et al*. 1995) [22]. They can be harvested every day due to their ability of quick reproduce (Haag, 2007) [11]. There are so many factors in laboratory or nature on which microalgae depend, such as light, temperature, salinity and nutritional factors that growth, physiological activities and biochemical composition (Alsull and Omar, 2012) [2]. Microalgae can assimilate nitrogen from a range of sources (Paasche and Kristiansen, 1982; Dortch, 1990; Page *et al*. 1999) [23, 10, 24]. Ammonia, nitrate, nitrite and many dissolved form of organic nitrogen like free amino acids, urea and peptides are considered as the major nitrogen sources for microalgae (Abe *et al*. 2002; Converti *et al*. 2006) [1, 9]. In wastewater treatment, microalgae have been broadly used for nutrient removal (Hoffmann, 1998; Borges *et al*. 2005) [14, 5]. Compared to conventional wastewater treatment methods, microalgae are considered as one of the most efficient, relatively low-cost, environment friendly and simple alternative for wastewater treatment (Hii *et al*. 2011) [13]. A number of the algae species (*Tetraselmis sp.* and *Nannochloropsis sp.*) have high nutritional value, and for this reason they are widely used as a food supply in the aquaculture industry for hatchery-growth herbivores (Alsull and Omar, 2012) [2]. Integrated wastewater treatment processes are considered as an alternative technique to single stage algal pond systems to improve nutrient removal as well as algal separation. It was also reported that macrophyte treatment had no additional effect on nutrient removal after microalgae treatment. The aim of this study is to investigate the performance of algae fed aquaculture system in terms of nutrient removal.

Materials and Methods

Wastewater was collected from the algal pond of UASB reactor situated in the IIT, Kharagpur campus. Characterization of the wastewater was carried out by following the standard technique APHA, 1998 [3]. Sewage after treatment through the UASB reactor was treated further in algal pond. The treated secondary effluent water from UASB reactor passed to algal pond where tilapia fishes are grown. The experimental set-up (shown in Fig. 1) is set in Aqua cultural Engineering Workshop in following manner to full fill the study criteria and for proper observation:

Fig 1: Experimental set-up for study

After collection of wastewater samples, they were analyzed immediately for the parameters such as influent and effluent COD, pH, ammonia-nitrogen, nitrite-nitrogen and nitrate-nitrogen as per the procedures given in standard methods of APHA (1998) [3]. Water quality is usually defined as the suitability of water for survival and growth of fishes and it is normally governed by few variables as reported by Boyd (1978). Most of these properties can be measured with appreciable degree of accuracy and the data can be used for productivity management of the pond. Evaluation of water quality was carried out on each 5 days cycle by collecting the samples.

Dissolved oxygen and temperature: Dissolved oxygen (DO) was measured using YSI (MODEL 55) which is based on polarographic method. This DO meter is consisting of a probe comprising of two electrodes both bathed in potassium chloride and separated from the water by means of a membrane. One electrode measure DO and another one temperature. Both the parameters were measured on daily basis.

Biochemical oxygen demand (BOD): It is an important regulatory parameter used to indicate organic strength of the wastewater. For BOD measurement diluted sample was kept in the incubator for five days. The change in DO concentration in the water sample was measured over the three days incubation period at 20 degree Celsius. Then BOD value was calculated by following formula:

$$BOD = \frac{(D_1 - D_2) - (B_1 - B_2)f}{P}$$

Where

D_1=DO on first day B_1=DO of blank on first day

D_2=DO on third day B_2=DO of blank on third day

P=Dilution factor

The BOD was measured on every four days cycle.

Most Probable Number (MPN): The MPN is a method of estimating the total coliforms in water. It is more practical to examine water for indication of bacteriological contamination. For each sample tested following amounts were added:
5*10 ml to 10 ml double strength Lactose Broth (LB)
5*1 ml to 5 bottles single strength LB
5*0.1 ml to 5 bottles single strength LB
Durham tubes are completely filled
Incubation at 37 °C
Acid production-a yellow coloration
Gas-trapped in Durham tube
Those tubes that produce acid and gas are scored positive. By varying sample size and number it is possible to build up a statistical picture as to the numbers present. These are represented in the McCray tables. The procedure followed for this test is as described by APHA (1998) [3].

SGR (Specific Growth Rate): The growth of fish was represented in terms of SGR to evaluate the fish growth and health.

$$\mathrm{SGR} = 100 \times \frac{\ln(\frac{\text{Final weight}}{\text{Initial weight}})}{\text{Culture periods (days)}}$$

Determination of algal biomass: Algal Dry Weight Estimation: Dry cell weight (DCW) was estimated as per the protocol of Rai *et al*. (1991). A noted quantity of microalgae culture was centrifuged for 10 min at 5000 rpm, after which the harvested biomass was dried at 60 °C till a constant weight, was reached. The biomass concentration was expressed as g L^{-1}.

Results and Discussion

The results obtained during characterization of raw sewage, laboratory experiments on treatment of wastewater using UASB reactor and algal pond are discussed in this section. Wastewater recycling, reuse and resource recovery can be a very good approach to conserve water particularly in areas of water shortage. The treatment of sewage by using a series combination of a UASB (Up flow Anaerobic Sludge Blanket) reactor and waste stabilization tank is a low cost solution and could satisfy the discharge standards with regards to physiochemical and biological parameters. The success of the UASB concept relies on the establishment of a dense sludge bed in the bottom of a reactor, in which biochemical conversion of organic matter into methane and CO_2 takes place. The production of high biomass concentration is the first step of the cultivation method for microalgae. Microalgae biomass growth depend on an enough supply of carbon source, organic nutrients present in the water and light to carry out photosynthesis. The different water quality parameters of algal pond water were discussed in table 1.

Table 1: Characteristics of algal pond water quality

Sr. No.	Parameter	Concentration
1	Temperature	22 °C±7.89
2	pH	8.9 to 9.6
3	COD	45±5.66 mg/L
4	BOD	15±5.89 mg/L
5	TSS	85±7.65 mg/L
6	VSS	70±6.89 mg/L
7	NH_3	4±0.86 mg/L
8	PO_4	0.2-0.3 mg/L
9	Alkalinity	150±10.33 mg/L
10	DO	4±2.2 mg/L

The pH of aquaria pond water was measured on every 4 days interval. The pH values of all aquaria were ranges from 8.1 to 9.6. If the pH of water is too high or too low, the aquatic organisms living within it will die. pH can also affect the solubility and toxicity of chemicals and heavy metals in the water. The pH of the wastewater affects a lot of the bio-chemical processes related with algal growth and metabolism and availability and uptake of nutrients ions. The pH could not solitary influence algal growth algal growth but also nitrogen removal efficiency in waste water treatment (Park and Craggs, 2010; Park *et al*. 2011) [26, 27]. The pH usually increases in algal cultures due to assimilation of photosynthetic CO_2 (Borowitzka, 1997; Chevalier *et al*. 2000) [6, 7]. The fish growth was measured on every 15 days and ideal pH for fish culture in pond water is 8.2. For good growth, availability of choice food is enough in water body is desirable. If growth of fish is good, this shows that fish health is good. The good growth of fish also suggests that the genotype hormone and physiological conditions of individual species are also evenly important. Good fish growth also shows that the biological conditions of water body are also good. The growth of fish was represented in fig. 2.

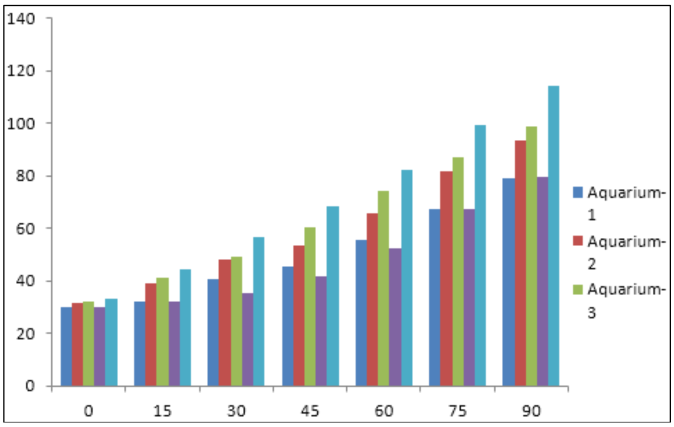

Fig 2: Comparison of fish growth under different aeration and controlled conditions along with proper aquaculture

The maintenance of good water quality is essential for both survival and optimum growth of culture organisms. Under controlled condition over 90 days period the SGR values of aquaria 1, 2, 3, 4 and 5 was measured as 1.075, 1.199, 1.242, 1.082 and 1.370% body weight/day respectively.

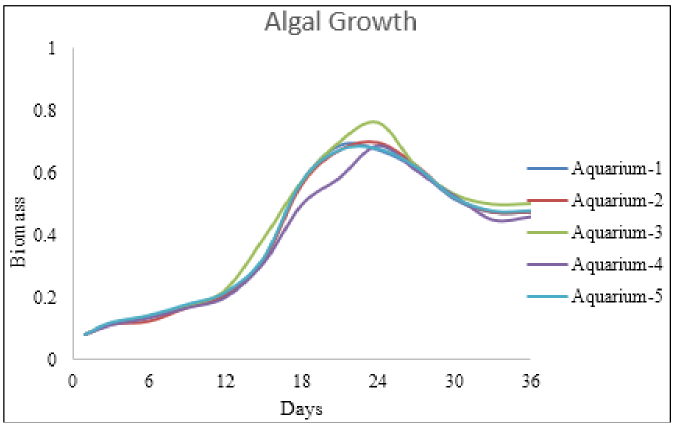

Fig 3: Growth behaviour of algae under different aeration condition

The good growth of algae was achieved while experiment and the good algal growth showed bioremediation of significant amount of organic waste. Han *et al*. (2008) [12] reported integrated cultivation for wastewater purification of aquaculture and algal biomass production and established that the average removal rate of total nitrogen, nitrate and nitrite was more than 80% for each; the total phosphorus removal rate was 94.17%. Menke *et al*. (2011) [21] also reported that after culture of *Nannochloropsis sp.* for 10 days grown in 75% wastewater, nitrogen and phosphorus was reduced to 80.4% and 72.8%. Patel *et al*. (2012) [28] experimented how microalgae differ in uptake of phosphorus from wastewater. They established that the phosphorus removal rate by *Nannochloropsis sp. was* poor however *Tetraselmis sp.* removed 79.4% of total phosphorus.

The water coming from UASB reactor is directly discharged into algal pond water for tertiary treatment along with aquaculture. The growth of tilapia (*Oreochromis niloticus*) fishes was observed and treatment efficiency was measured in terms of organic waste as well as pathogens removal. The results obtained from the experiment shows 80.10±2.4% of total nitrogen removal with influent total nitrogen concentrations of 200±3 mg/L. Phosphate removal after treatment in algal tank was 90±1%. There was 2-3 log scale pathogen removal was also observed after treatment in algal tank. The maintenance of good water quality is essential for both survival and optimum growth of culture organisms. The permeate generated in UASB-Algal pond combined process with 90 days of HRT of treatment can be utilized for agro-irrigation, re-circulatory aquaculture system or disposed safely in inland water channels. The black water after treatment using UASB/Algal pond is being reused for gardening and landscaping. Utilization of nutrients present in the treated sewage for the growth of microalgae species will not only control eutrophication but will also help in sustainable energy development. The findings of this study suggest that sewage can be directly used for mass cultivation of microalgae without requiring additional nutrient supplements. The effluent of the algal pond can be directly reused for surface irrigation of non-food crops. In conclusion, apart from wastewater treatment UASB-Algal-tilapia ponds also offer marketable by-products as fish protein including algal biomass, which correspond to a cost recovery for sewage treatment. The findings of this study suggest that sewage can be directly used for mass cultivations of microalgae without requiring additional nutrient supplements. The effluent of the algal pond can be directly reused for surface irrigation of non-food crops. Operating cost as an important point of view the algal biomass correspond to a cost recovery for sewage treatment by producing the biodiesel which can be further used as a fuel as 10-20% in addition with diesel.

Conclusions

Our results showed that wastewater from aquaculture could promote good algal growth and thus can be used for nitrogen and phosphorus removal in aquaculture and they could take a part in biological treatment of wastewater originate from such production. In conclusion, apart from wastewater treatment UASB-Algal-tilapia ponds also offer marketable by-products as fish protein including algal biomass, which correspond to a cost recovery for sewage treatment.

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References

1. Abe K, Imamaki A, Hirano M. Removal of nitrate, nitrite, ammonium and phosphate ions from water by the aerial microalgae Trentepohlia aurea. Journal of Applied phycology. 2002; 14(2):129-134.
2. Alsull M, Omar WMW. Responses of Tetraselmis sp. and Nannochloropsis sp. isolated from Penang National Park coastal waters, Malaysia, to the combined influences of salinity, light and nitrogen limitation. In International Conference on Chemical, Ecology and Environmental Sciences (ICEES), 2012.
3. APHA, Standard Method for Examination of Water and Wastewater, 20th ed. American Public Health Association, American Water Works Association, Water pollution Control Federation, Washington DC, USA, 1998.
4. Bich NN, Yaziz MI, Bakti NAK. Combination of Chlorella vulgaris and Eichhornia crassipes for wastewater nitrogen removal. Water Research. 1999; 33(10):2357-2362.
5. Borges MT, Silva P, Moreira L, Soares R. Integration of consumer-targeted microalgal production with marine fish effluent biofiltration-a strategy for mariculture sustainability. Journal of Applied Phycology. 2005; 17(3):187-197.
6. Borowitzka MA. Microalgae for aquaculture: opportunities and constraints. Journal of Applied Phycology. 1997; 9(5):393-401.
7. Chevalier P, Proulx D, Lessard P, Vincent WF, De la, Noüe J. Nitrogen and phosphorus removal by high latitude mat-forming cyanobacteria for potential use in tertiary wastewater treatment. Journal of Applied Phycology. 2000; 12(2):105-112.
8. CIFA. Sewage treatment through aquaculture. Central Institute for freshwater aquaculture, Bhubaneshwar, India, 1998, 8.
9. Converti A, Scapazzoni S, Lodi A, Carvalho JCM. Ammonium and urea removal by Spirulina platensis. Journal of Industrial Microbiology and Biotechnology. 2006; 33(1):8-16.
10. Dortch Q. The interaction between ammonium and nitrate uptake in phytoplankton. Marine ecology progress series. Oldendorf. 1990; 61(1):183-201.
11. Haag AL. Algae bloom again. Nature. 2007; 447:520-521.
12. Han S, Yan S, Fan C, Recycling of Aquaculture wastewater and reusing the resources of redundant algae Biomass. Journal of Natural Resources. 2008; 23(4):560-567.
13. Hii YS, SOO CL, Chuah TS, Mohd-Azmi A, Abol-Munafi AB. Uptake by Nannochloropsis sp. Journal of Sustainability Science and Management. 2011; 6(1):60-68.
14. Hoffmann JP. Wastewater treatment with suspended and nonsuspended algae. Journal of Phycology. 1998; 34(5):757-763.
15. Kumar D, Kumar M. Influence of different age seedlings mat type characteristics at different speed of self-propelled 8-row rice transplanter in district of

Samastipur, Bihar, India. Internat. J Agric. Engg. 2017; 10(2):268-274.
16. Kumar D, Kumar M. Identification of mechanization gaps for different farm operations for fodder and crop cultivation in Pusa (Bihar) region. International Journal of Forestry and Crop Improvement. 2017; 8(1):17-23.
17. Kumar D, Kumar M, Kant R. Mechanization planning to bridge the mechanization gap for different farm operations to meet fodder and crop production in region, Pusa, Bihar, India. Internat. J Agric. Engg. 2017; 10(2):1-9.
18. Kumar D, Mehta R, Dutta D. Effect of aeration on the suitability of secondary treated sewage by UASB reactor followed by algal pond for tertiary treatment with aquaculture. International Journal of Environment Technological Sciences. 2017; 4:141-146.
19. Kumar M, Kumar S, Kumar D, Rani P. Impact assessment of PAU maize dehuskar-cum-sheller with the local variety devaki. International Journal of Current Microbiology and Applied Sciences. Special. 2018; 7:3526-3532.
20. Martınez ME, Sánchez S, Jimenez JM, El Yousfi F, Munoz L. Nitrogen and phosphorus removal from urban wastewater by the microalga Scenedesmus obliquus. Bioresource technology. 2000; 73(3):263-272.
21. Menke S, Seeniwasan R, Huchzermeyer B, Rath T. Screening of microalgae for wastewater purifcation. 1st World Student conference on environment and Sustainability, United Nations environment Program, Shanghai, china June, 2011, 5-8.
22. Minowa T, Yokoyama SY, Kishimoto M, Okakura T. Oil production from algal cells of Dunaliella tertiolecta by direct thermochemical liquefaction. Fuel. 1995; 74(12):1735-1738.
23. Paasche E, Kristiansen S. Nitrogen nutrition of the phytoplankton in the Oslo fjord. Estuarine, coastal and shelf science. 1982; 14(3):237-249.
24. Page S, Hipkin CR, Flynn KJ. Interactions between nitrate and ammonium in Emiliania huxleyi. Journal of Experimental Marine Biology and Ecology. 1999; 236(2):307-319.
25. Pappu A, Saxena M, Asolekar SR. Solid wastes generation in India and their recycling potential in building materials. Building and Environment. 2007; 42(6):2311-2320.
26. Park JBK, Craggs RJ. Wastewater treatment and algal production in high rate algal ponds with carbon dioxide addition. Water Science and Technology. 2010; 61(3):633-639.
27. Park JBK, Craggs RJ, Shilton AN. Wastewater treatment high rate algal ponds for biofuel production. Bioresource technology. 2011; 102(1):35-42.
28. Patel A, Barrington S, Lefsrud M. Microalgae for phosphorus removal and biomass production: a six species screen for dual-purpose organisms. Gcb Bioenergy. 2012; 4(5):485-495.
29. Rai LC, Mallick N, Singh JB, Kumar HD. Physiological and biochemical characteristics of a copper tolerant and a wild type strain of Anabaena doliolum under copper stress, Journal of Plant Physiology. 1991; 138:68-74.
30. Van Der Steen P, Brenner A, Van Buuren J, Oron G. Post-treatment of UASB reactor effluent in an integrated duckweed and stabilization pond system. Water research. 1999; 33(3):615-620.
31. World Health Organization. International Decade for Action Water for Life, 2005-2015=Décennie internationale d'action sur le thème «L'eau, source de vie», 2005-2015. Wkly Epidemiol Rec. 2005; 80(22):195-200.