Efficiency of Nutrient Uptake by Microalgae From Livestock Wastewater

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***Abstract* — Increased industrialization results in the increased exploitation of water resources and generation of wastewater. Globally, wastewater generation increases resulting in water demand. Hence, we are in a great need to conserve it and treat it. There are several methods of wastewater treatment but bioremediation of wastewater by microalgae has receiving an ever increasing attention. Because they are less energy intensive and produces harmless end products which have higher economic value. The present study aims at explaining the efficiency of microalgae to trap nutrients from wastewater. Cattle farm wastewater were used because of their high nutrient content. A laboratory scale photo-bioreactor is designed to support the microalgae growth. The percentage reduction of COD, $PO^{-}4$ and $NO^{-}3$ at varying flow rate and hydraulic retention time were examined. Results reveal that percentage of nutrient reduction increases with increase in retention time and decreases in flow rate. The maximum percentage reduction of COD, $PO^{-}4$ and $NO^{-}3$ were obtained at a flow rate of 26.4ml/h, retention time of 24h namely 71%, 68% and 57.3% respectively.**

Keywords— Microalgae; Livestock wastewater; Eutrophication; Photo-bioreactor.

I. INTRODUCTION

The growth of livestock sector increases significantly and gradually every year. A majority of farmers depend on Animal Husbandry for their livelihood. Livestock plays a vital role in India and it has a very large livestock population of about 485 million animals, out of which 102.70 million where cattle. And Tamil Nadu contributes to 5.8% of total cattle Population. This sector contributes a huge to a nation's economy. In India it is found everywhere but in small scale. On a whole the wastewater generated are high and disposed of with no treatment.

In general, chemical oxygen demand (COD), Bio-chemical oxygen demand (BOD), nitrogen, phosphorus and suspended solids are usually found higher in livestock wastewater (Lee *et al.*, 2008).Nutrient pollution from animal manure, particularly excess nitrogen and phosphorus, has a major impact on the environment (Rui Chen, 2006). This nutrient rich wastewater when disposed of will contribute to eutrophication of receiving wastewater stream especially lakes and slow moving rivers. Livestock wastewater is a potential source of nutrients and can be applied potentially as nutrients for agricultural lands or crops. However, if the wastewater is applied at rates in excess of crop uptake rate, the excess wastewater could enter surface and groundwater sources. In such cases excessive nitrogen amount will robs oxygen of water and destroys the aquatic life. And nitrogen in the form of ammonia is volatized to the atmosphere to cause air pollution (Rui Chen, 2006).

In past the quantity of livestock wastewater generated are very negligible. Hence, they are disposed of without any treatment.

But now a day's the quantity and frequency of untreated livestock wastewater are discharged in higher proportion. So we are in a great need to treat it before its disposal.

High cost of reagent and poor performance of physico-chemical process in removal of soluble organics, biological treatment are most probably preferred. Interestingly, unicellular algae are proved to be best bioremediation agent who has a capacity to grow faster in wastewater, helping in wastewater treatment. The treatment of livestock effluents from dairy farms with microalgae has got an increased attention. This is due to the fact that the uncontrolled nutrient discharges coming with dairy waste may cause severe problems in aquatic ecosystem and pollute the groundwater resources. Conventional methods has some disadvantages namely, aerobic treatments methods, such as activated sludge process involve oxygen supply which are energy demanding. These process also entail the impractical recycling of valuable nutrients contained in dairy effluents (Gonzalez *et al.*, 2008 & Chynoweth. DP *et al.*, 1999). Anaerobic digestion is generally carried out to stabilize the organic matter in the wastewater. They are very effective in pathogen and organic matter reduction and they can be utilized as a bio-fuel (Chynoweth. DP *et al.*, 1999). But the major disadvantage of anaerobic digestion of livestock wastewater is characterized by high ammonia content that must be pretreated before its disposal (Dosta J *et al*., 2008). Digester, effluents increases the escaping rate of nitrogen in to the atmosphere by ammonia volatilization (Thompson RB *et al*., 2002). Hence, an environmentally friendly and economically sound wastewater management method is needed. A microalga offers low cost processes. They can be utilized as best bioremediation agents to remove inorganic nutrients from the wastewater and to and to improve water quality (Lincoln EP *et al.*, 1990). The major advantage of algae is its capacity to sequester CO_2. And the biomass produced can be utilized for various purposes namely, bio-gas and bio-fuel production. Since, it is used to treat wastewater it cannot be used in pharmaceuticals and other food sectors.

The main objective of this study was to test the efficiency of microalgae in nutrient removal and wastewater treatment.

II. MATERIAL AND METHODS

A. Livestock wastewater

Raw undiluted livestock wastewater is collected from Cattle farm weekly twice. The livestock wastewater contains urine, washing water, blood and feces which where sieved through a mesh of 1.00mm to eliminate large debris and solid materials. The initial parameters such as pH, COD, phosphate, nitrate and total solids were analyzed using a standard procedure. The characteristics of raw livestock wastewater was given in table 1

Table 1 Cattle farm wastewater characteristics

Parameters	**Characteristics***
pH	8.3
Electrical conductivity (Ec)	0.201
Total solids	2800
Total suspended solids	340
COD	3600
BOD5	1700
Nitrate (NO3)	2.0
Phosphate (PO4)	20.8

*All units are in mg/l except pH and Ec

B. Microalgae collection

The algae species are collected from a nearby agricultural land in a sterilized bottle to avoid contamination. The collected microalgae species are viewed through a microscope to find the native species. *Chlorella* and *Anabena* species were found predominant.

C. Experimental setup

An innovative tubular photo-bioreactor is designed for treating wastewater by microalgae. The proposed design consists of a continuous irradiance loop made up of an acrylic pipe of internal radius 15cm.

Three pipes of 90cm long were used, which are arranged at a distance of 10 cm from each. The total capacity of the reactor is 19 L. A peristaltic pump is used to feed the wastewater into the reactor. A sample port is provided at both the end of the reactor, at one end wastewater is feed inn and at another end wastewater effluent was collected. The reactor was operated at varying hydraulic retention time (HRT) and flow rate (FR) to check the efficiency of nutrient uptake by microalgae.

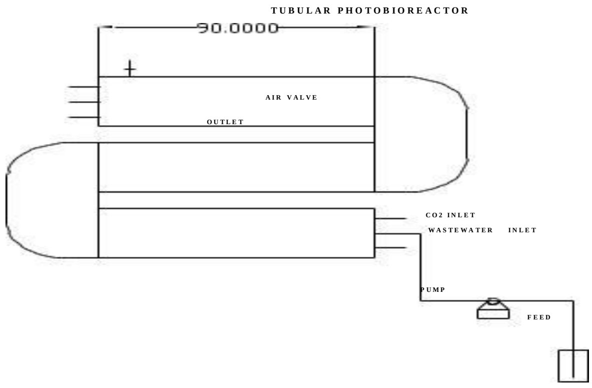

Fig 1 Schematic representation of photo-bioreactor

D. Characterization

The cattle farm wastewater once collected is characterized using standard methods (APHA 2005).

III. RESULTS AND DISCUSSIONS

In this study the capacity of microalgae to uptake nutrients from the cattle farm wastewater were studied. Cattle farm wastewater are slightly alkaline in nature with a COD concentration of around 3600mg/l. They are rich in their phosphate and nitrate content. Under different operating and processing condition the reduction efficiency were analyzed. Two different flow rate and hydraulic retention time were used.

Table 2 and 3 describes the percentage reduction of nutrients and COD at two different FR namely 26.4ml/h and 53ml/h and a HRT of 24h and 12h respectively.

A. Influence of microalgae in COD reduction

Carbon is very essential for microalgae growth. The prime source of carbon is CO_2 and it is the most preferable form of carbon too. This condition where carbon is utilized from CO_2 is known as phototrophic condition. In photoheterotrophic condition microalgae uses light as an energy source and dissolved organic carbon as a carbon source resulting in the depletion of COD concentration.

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Table 2 Percentage reduction in COD and nutrient content for a FR of 26.4ml/h and a HRT of 24 h

SI NO	DATE	INF pH	EFF pH	INF COD	EFF COD	% R	INF $PO^{-}4$	INF $PO^{-}4$	% R	INF $NO^{-}3$	INF $NO^{-}3$	% R
1	10.01.2017	8.20	8.32	3060	2860	6.53	16.00	15.28	4.50	4.00	3.80	5.00
2	11.01.2017	8.30	8.36	3800	3400	10.52	14.50	13.18	9.10	3.80	3.42	10.00
3	12.01.2017	7.80	8.10	3600	3000	16.66	12.00	10.00	16.66	3.60	2.98	17.22
4	13.01.2017	7.30	7.42	3700	2850	24.86	15.00	11.28	24.80	4.00	4.28	-
5	14.01.2017	8.36	8.40	3400	2300	32.35	12.00	8.26	31.16	3.70	2.86	22.70
6	15.01.2017	8.20	8.40	3200	1900	40.63	11.00	6.82	38.00	3.80	2.68	29.47
7	16.01.2017	8.08	8.20	3050	2000	47.37	10.50	5.64	46.29	3.40	2.20	35.29
8	18.01.2017	7.92	7.80	3200	1400	56.25	10.00	4.62	53.8	2.80	1.65	41.07
9	19.01.2017	8.10	8.22	2860	1050	63.28	11.00	4.20	61.88	3.00	1.56	48.00
10	**20.01.2017**	**8.24**	**8.30**	**2000**	**580**	**71**	**10.00**	**3.20**	**68.00**	**3.00**	**1.28**	**57.33**

NOTE: INF: Influent; EFF: Effluent; % R: Percentage of reduction; All units are in mg/l except pH

Table 3 Percentage reduction in COD and nutrient content for a FR of 53ml/h and a HRT of 12h

SI NO	DATE	INF pH	EFF pH	INF COD	EFF COD	% R	INF $PO^{-}4$	INF $PO^{-}4$	% R	INF $NO^{-}3$	INF $NO^{-}3$	% R
1	22.01.2017	8.00	8.12	3800	3600	5.26	15.2	14.60	3.90	3.80	3.68	3.16
2	23.01.2017	8.30	8.40	3700	3300	10.81	15.0	13.80	8.00	4.00	3.62	9.50
3	24.01.2017	8.12	8.36	4000	3300	17.5	14.5	12.60	13.10	3.60	3.10	13.88
4	25.01.2017	7.80	7.92	3800	2950	22.36	14.8	12.00	18.91	3.70	2.98	19.46
5	26.01.2017	8.10	8.18	3600	2600	27.77	15.0	11.42	23.86	3.30	2.50	24.24
6	27.01.2017	8.24	8.32	3500	2300	34.28	13.0	9.20	29.23	3.00	2.10	30.00
7	28.01.2017	7.70	7.90	3400	2000	41.18	12.0	7.60	36.66	2.80	1.80	35.71
8	29.01.2017	8.20	8.42	3100	1600	48.38	9.0	5.22	42.00	3.00	1.72	42.66
9	30.01.2017	8.36	8.40	2900	1350	53.44	10.0	5.18	48.20	2.80	1.48	47.14
10	31.01.2017	8.00	8.20	2800	1100	60.71	8.0	3.62	54.75	2.60	1.24	52.30

NOTE: INF: Influent; EFF: Effluent; % R: Percentage of reduction; All units are in mg/l except pH

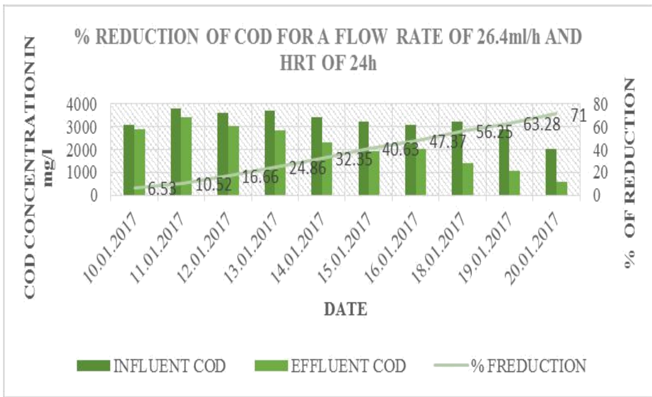

Fig (a) % reduction of COD for a HRT of 24h

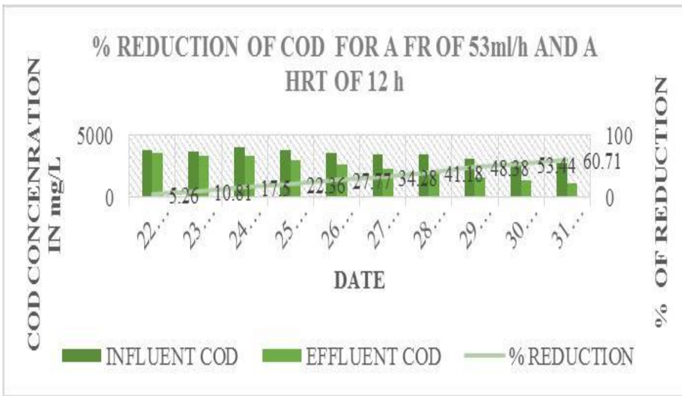

Fig (b) % reduction of COD for a HRT of 12h

Figure a and b represents the % reduction of COD at varying flow rate. To a maximum of 60.71% and 71% of COD reduction was observed at a FR of 53ml/h and 26.4ml/h and a HRT of 12h and 24h respectively. Retention time plays a major role in COD reduction. Maximum reduction efficiency of 71% was achieved at a retention time of 24h with a flow rate of 26.4ml/h. And it is evident from the result that COD reduction increases with increase in retention time and decrease in flow rate.

B. Nutrient uptake capacity

Besides carbon, nitrogen (N) and phosphorus (P) are the two major macronutrients essential for algae. There are some mechanism through which microalgae helps us to reduce the nutrients from wastewater.

- Diffusion of substances through cell wall (or) Uptake by microalgae (Mostert et al., 1987 and Borowitzka, 1998).
- Precipitation as a result of high pH.
- Stripping of ammonia as a result of elevated pH.

i. Nitrogen uptake efficiency

Nitrogen is widely available in wastewater in many forms. It is an essential nutrient required by microalgae whose cell comprises 16% of nitrogen by its dry weight. Microalgae can assimilate only the inorganic nitrogen. Ammonia is the most favorable nitrogen form for assimilation. After depletion of ammonia (NH_3-N) in wastewater, microalgae will start to assimilate nitrate (NO_3^-) as a source of nitrogen for their cells. Several microalgae can take up nitrogen in excess of metabolic needs, so called luxury consumption. This can be used later in the case of nitrogen starvation (Karin Larsdotter 2006).

Fig (c) % reduction of NO^-3 for a HRT of 24h Note: The % increase in NO^-3 is differentiated by blue color

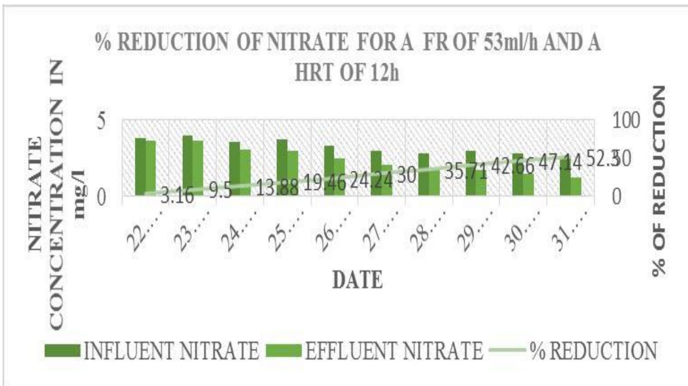

Fig (d) % reduction of NO^-3 for a HRT of 24h

Figure c and d shows the % reduction of NO^-_3 at varying flow rate. Microalgae have good ability to trap nitrate at the same time an increase in nitrate level was also observed.It may be due to nitrification of ammonia in the presence of oxygen. However an increase in nitrate was less than 0.3mg/l, but the decrease in ammonia was nearly above 8mg/l. To a maximum of 52.33% and 57.3% of NO^-_3 reduction was observed at a FR of 53ml/h and 26.4ml/h and a HRT of 12h and 24 h respectively. And it was evident that reduction in nitrate was related to microalgae uptake rather than nitrification. Grobbelaar 2004 revealed that about 80% TN removal was due to micro algal assimilation. And from the result it is sure that retention time plays a major role in reduction. Maximum reduction efficiency of 57.3% was achieved at a retention time of 24 h with a flow rate of 26.4ml/h. And it is evident from the result that NO^{-}_{3} reduction increases with increase in retention time and decrease in flow rate.

ii. Phosphate reduction

Phosphorus is the second essential nutrient required by microalgae for their growth. It is an important element that comprises 1% of the cells dry weight (Brown *et al.*, 2014) and it is found higher in cattle farm wastewater. Which can be utilized for algal growth and its treatment. Microalgae has ability to assimilate phosphorus in excess, which are stored in the cells in the form of polyphosphate (volutin) granules. These are sufficient for the prolonged growth of algae even in the absence of available phosphate (Karin Larsdotter 2006).

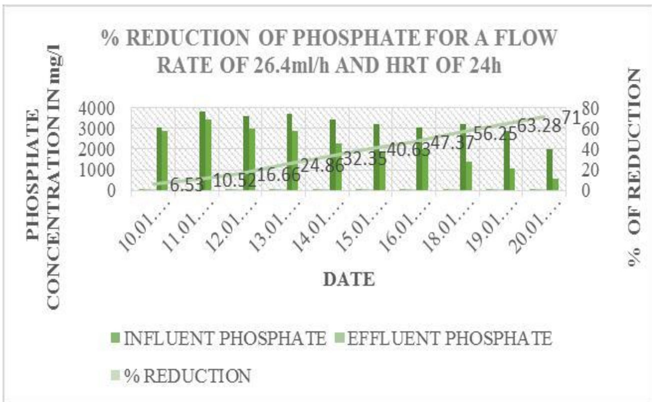

Fig (e) % reduction of $PO^{-}4$ for a HRT of 24h

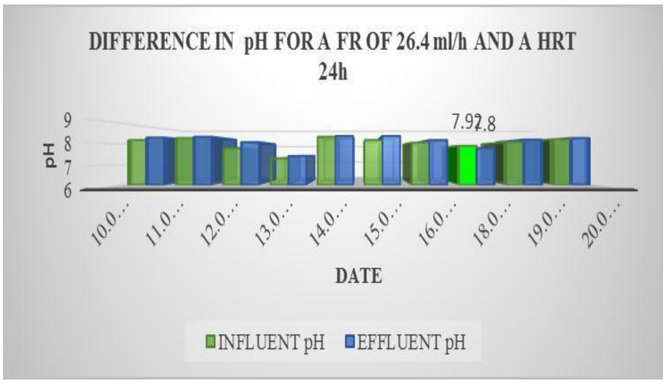

Fig (g) Difference in pH for a HRT of 24h

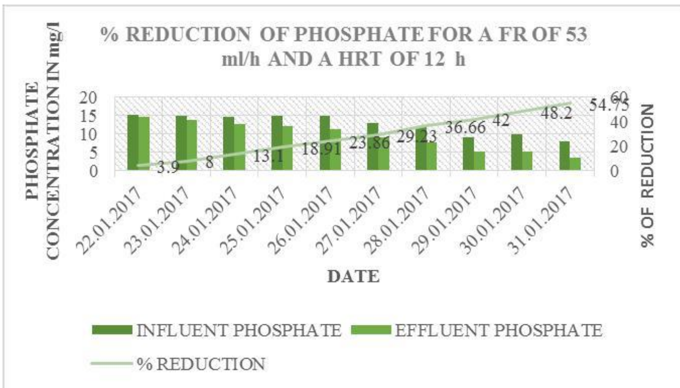

Fig (f) % reduction of $PO^{-}4$ for a HRT of 12h

Figure e and f represents the % reduction of phosphate at two different flow ate. To a maximum of 54.75% and 71% of NO^{-}_{3} reduction was observed at a FR of 53ml/h and 26.4ml/h and a HRT of 12h and 24 h respectively. There are two mechanism that support the removal of dissolved phosphate namely assimilation of phosphate by algae and precipitation of phosphorus as a result of high pH (Munoz *et al.*, 2004). But, it was evident from the result that the maximum removal of phosphate is due to the assimilation of nutrients by microalgae and it was proved true by the effluent pH. Because the resultant pH is neither acidic nor alkaline. And FR and HRT time also plays a predominant role in phosphate reduction. Increase in RT increases the % reduction of phosphate.

A. Difference in pH

pH is a important factor for algae growth and it also affects it. Usually in any wastewater treatment system by algae pH increases due to photosynthetic assimilation of CO_2. Absorption of nitrogen by microalgae also increases pH of the system, as every nitrate ion reduces to produce one OH^{-} ion (Xu *et al.*, 2012). If ammonia is used as a source of nitrogen it results in the reduction of pH. Even the pH is reduced to 3, which is too acidic to support algae growth (Karin, 2006).

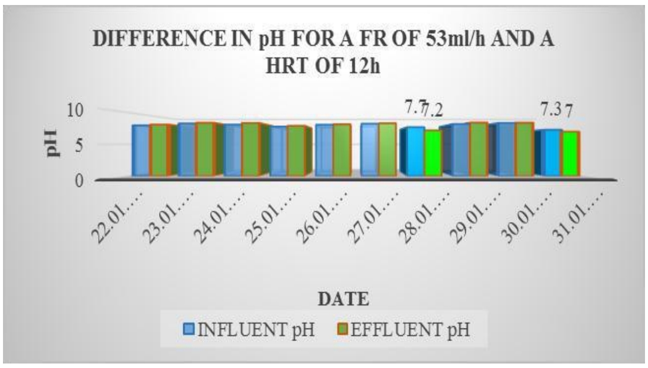

Fig (h) Difference in pH for a HRT of 12h

Figure g and h represents the difference in pH for the varying FR and HRT. Compared to the influent pH there is a gradual increase in effluent pH. But at the same time a gradual decrease in pH is also observed. Increased level of oxygen is a main reason for decrease in pH. And ammonia is used as a source of nitrogen which results in the depletion of pH and it is very slightly acidic in nature. Similar observations was recorded by Aarti *et al.*, 2008 and Mostafa *et al.*, 2016.

IV. CONCLUSION

The main goal of the study was to determine the efficiency of microalgae to uptake nutrients from the wastewater. From the result it is evident that microalgae can be incorporated to conventional wastewater treatment. The results shows that 60.71% and 71% of COD, 54.75% and 57.33% of NO^-_3, and 54.75% and 68% of PO_4^- were reduced under various operating condition. It also reveals that retention time plays a major role in nutrient removal. The maximum efficiency of COD and nutrient removal namely 71%, 57.33% and 68% were observed at a retention time of 24h. Microalgae treatment can provide dual benefits of removing nutrients and residual COD as well as producing microalgae biomass. Microalgae biomass has higher calorific value which can be used for biogas production. Hence, microalgae can be adopted for wastewater treatment and act as a source of renewable energy. And algae proves to be a best bioremediation agent.

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