Treatment of Nitrogenous Wastewater by using Microalgae and its Utilization for the Production of Value-Added Products

THESIS

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By

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Chapter - I : Introduction and Literature review

Algae have recently received a lot of attention as a new biomass source for the production of renewable energy. Some of the main characteristics which set algae apart from other biomass sources are that algae (can) have a high biomass yield per unit of light and area, can have a high oil or starch content, do not require agricultural land, fresh water is not essential, and nutrients can be supplied by wastewater and CO_2 by combustion gas. The first distinction that needs to be made is between macroalgae (or seaweed) versus microalgae. Microalgae have many different species with widely varying compositions and live as single cells or colonies without any specialization. This makes their cultivation easier and more controllable.

It is estimated, that one ton of algal biomass would produce net renewable fuel sufficient to abate a similar amount of fossil CO_2, based on a reasonable mix of natural gas, oil and coal. Therefore, the potential of microalgae for Greenhouse gas (GHG) abatement is the product of productivity times the total aggregate scale of processes that is hectares of ponds. Most importantly, microalgae production systems could use land and water resources not suitable for agriculture or aquaculture (e.g. saline, brackish, waste waters), and, in any event, their water use efficiency (tons of water per ton output) would be much higher than any terrestrial crop.

The major limitations of this technology are not land, water or CO_2 resources, but the technical feasibility and economic competitiveness of microalgae processes compared to other alternatives, including crop production and forestry, for examples. Integrating algal GHG abatement with other large-volume co-processes and co-products, assures that microalgae will make a significant contribution to different sustainable development goals.

In India 34% of household sewage flow virtually untreated into waterways. The fates of the key nutrients (chiefly nitrogen and phosphorus) that are present in sewage are wasted, either by discharging them untreated into the environment. The total wastewater generation from Class I cities (498) and Class II (410) towns in India is around 35,000 and 2,700 MLD respectively, while the installed centralized sewage treatment capacity is just 11,500 and 230 MLD. The rest of the population is dependent on *septic tanks* for decentralized sewage treatment. Septic tanks do not sanitize wastewater. Moreover, the effluent from septic tanks still contains most of the sewage nutrients. Centralized sewage treatment relies instead on conventional activated sludge (CAS), which achieves sufficiently low carbon, nitrogen and phosphorus effluent levels, but is not cost-effective, hardly achieves recovery, requires electricity equivalent to a fossil fuel consumption of 85 kWh per inhabitant equivalent (IE) per year and has an operational CO_2 footprint of 80 kg CO_2 IE^{-1} $year^{-1}$. Projected water and nutrient shortages and the need to lower greenhouse gas emissions force us to rethink wastewater treatment for a sustainable future by production of value- added products.

Microalgae have proven to be significant in recovery of pollutants from wastewater while generating high value biomass for extraction of various value-added products (Biodiesel, Ethanol, pharmaceutical products and Biomethane). Wastewater treatment using microalgae can be achieved by either Open Raceway Ponds (ORPs) or closed photobioreactors (Tubular Photo-Bioreactor-TPBR). There are two major limitations of microalgal wastewater treatment systems. Firstly, high solid content in wastewater increasing turbidity leading to loss of light penetration affecting their growth and decreasing their phytoremediation efficiency. Secondly, microalgae are sensitive to high ammonia concentration in wastewater. The first limitation can be overcome by integrating microalgal Treatment System (MTS) to SBR or Vertical Flow Constructed Wetlands (VFCWs). Whereas, the second limitation can be solved by using specific ammoniacal nitrogen (NH_4-N) tolerant algal strains depending on initial NH_4-N concentration present in wastewater. *Chlorella* sp. and *Spirulina* sp. have tolerance limit of 200 and 100 mg/L of NH_4-N and can be used for treatment of varieties of wastewater. Recently, use of microalgal consortium is gaining importance for treatment of wastewater as maintaining unialgal culture in wastewater is difficult. Use of algal consortium is preferred over unialgal strains in wastewater treatment as it is robust in nature and not very much sensitive to changes in wastewater composition. Fertilizer manufacturing industries generates huge amount of effluents rich in NH_4-N (5000 mg/L) and PO_4-P (4000 mg/L) and less carbon content. In this context, Conventional Sewage Treatment Plants (CSTPs) cannot be directly adopted for effluent treatment. The study of ammoniacal nitrogen tolerance of microalgae is of particular interests because, a) almost all types of wastewater contains ammoniacal nitrogen, b) little information is available about the problems resulting from microalgal cultivation at high ammoniacal nitrogen, c) little is known about the concentration at which ammonia toxicity becomes effective and d) the use of ammonium fertilizers in cultivation medium instead of nitrate fertilizers to reduce microalgal biomass production costs. Different microalgal species exhibit different ammoniacal nitrogen tolerance limits depending on other conditions. Microalgal strains also cannot grow in this effluent due to NH_4-N toxicity. So, attempts should be made to recover NH_4-N and PO_4-P as struvite and the residual effluent can be used for the growth of NH_4-N tolerant microalgal consortium. In this context, the focus the thesis was not only to increase the long-term pollutant removal efficiency by integrating MTS to currently available treatment systems but also to generate value-added products (lipids, biogas, ethanol, struvite etc.)

Gaps in research

Numerous studies focusing on wastewater treatment by using microalgae are available. However, there are certain loopholes that are described below.

- The composition of wastewater (COD, NH_4-N, TKN, and PO_4-P) varies depending on many factors like source of wastewater, drainage system and human habits. Many studies have focused on bioremediation of municipal or industrial wastewater with initial NH_4-N concentrations from 40-100 mg/L. Fertilizer industry wastewater is generally rich in NH_4-N, PO_4-P and has less organic matter. As dissolved ammonia is toxic to algal cells, it is essential to study the ammoniacal nitrogen tolerance studies of individual algal species or algal consortium at NH_4-N concentrations higher than 500 mg/L.
- The literature regarding the anaerobic digestion of biomass for the production of biogas is rich. However, the studies which are dealing with the determination of optimal loading rate of algal biomass for biogas production and effect of different biomass pre-treatment methods on biomass are scarce.
- Various studies have carried out the wastewater treatment by using microalgae and the production of single value-added product at a lab scale and not in the actual field. The studies focused on extraction of multiple products in sequential manner from algal biomass in biorefinery concept are scarce.
- The studies regarding integration of SBR, constructed wetland or struvite crystallization to microalgal treatment system for wastewater treatment are not available.

Aims and objectives of research work

The main focus of this study was to study the feasibility and potential of MTS in wastewater treatment to be integrated with currently available technologies (SBR, constructed wetland and struvite production) for parallel nutrient removal and production of value-added products. As domestic and fertilizer industrial wastewater vary in NH_4-N composition and variation in microalgal biomass composition, there should be an emphasis on microalga/consortium selection for wastewater treatment.

The proposed objectives of the study are given below.

- Isolation and enrichment of different microalgae in different wastewaters.
- Biomethane potential of different microalgae isolated in combination with ethanol production or other beneficial products.
- Photobioreactor development for wastewater treatment by using isolated microalgae.

- Pilot scale industrial waste water treatment by selected microalgae.

Chapter II and III :Tertiary treatment of domestic wastewater by using *Spirulina platensis* and *Chlorella vulgaris* integrated with microalgal biorefinery

Second and third chapter deals with the tertiary treatment of DW by using MTS integrated to SBR. The main aim of these studies was to explore parallel nutrient removal from secondary treated wastewater by microalgal production and their biomethanation potential. DW was initially treated with SBR and SBR effluent was further phycoremediated by using two individual microalgal species; *Spirulina platensis* and *Chlorella vulgaris*. *Spirulina platensis* and *C. vulgaris* were found to tolerate 100 mg/L and 200 mg/L of ammoniacal nitrogen. The observed removal efficiency of COD, PO_4-P, NH_4-N and TKN by *S. platensis* and *C. vulgaris* were 18%, 14%, 17%, 16% and 31%, 40%, 36% and 38 %, respectively. *S. platensis* and *C. vulgaris* biomass was observed to contain 26.65 % and 16.45 % lipids, respectively. The maximum biogas production (mL/g VS) was observed at 2 g VS/L. *S. platensis* and *C. vulgaris* was observed to produce 320 mL/g VS and 450 mL/g VS biogas, respectively. Effect of different pre-treatment methods (thermal, chemical, sonication and thermo-chemical) has also been studied. The biomass and biomass extract (before and after pre-treatments) were also analyzed for solubilization of complex compounds. Thermal pre-treatment of S. *platensis* and *C. vulgaris* biomass increased biogas production by 8.5% and 6.6 %, respectively. These studies have successfully demonstrated that microalgal cultivation in wastewater can be easily adopted in currently available wastewater treatment plants without any major modifications of existing available infrastructure.

Chapter IV: Integration of microalgal biorefinery to vertical flow constructed wetland and microalgal treatment system for treatment of raw domestic wastewater

Fourth chapter of the thesis deals with integration of microalgal biorefinery to a passive treatment system i.e. Vertical Flow Constructed Wetland (VFCW) and microalgal treatment system for treatment of raw DW. DW often contains solid matter limiting its direct use as a medium for microalgal growth. These limitations can be overcome by adopting hybrid treatment system i.e. VFCW and MTS. The main aim of this study is to treat DW in a hybrid VFCW-4.2 m^2 and MTS-1m^2. The objective is not only to treat DW but also to produce value added products from microalgal biomass. The DW was initially treated by VFCW (first stage alone) and the VFCW effluent was further phycoremediated by MTS. *Canna indica* was used for wetland vegetation and resident microalgal consortium from VFCW effluent was used in MTS. The integrated system was observed to remove 68.9% COD, 77.4% NH_4-N, 75.8%

TKN and 63.6% PO_4-P. The harvested Naive Biomass (NB) was observed to contain 16.7 % of lipids (W/W). The Residual Biomass after Lipid Extraction (RBLE) was used as a substrate for ethanol production. The observed yield of ethanol using RBLE as a substrate was 33.4 %. NB, RBLE, and Residual Biomass after Lipid and Sugar Extraction (RBLSE) indicated net biomethane yield (mL/g VS) of 211.8, 134.6 and 107.7, respectively. This study demonstrated an initial attempt of demonstrating hybrid wastewater treatment system for the production of value-added products in terms of biofuel.

Chapter V: Demonstration of pilot scale integrative treatment of nitrogenous industrial effluent for struvite and algal biomass production

Sixth chapter of the thesis deals with the demonstration of pilot scale integrative treatment of nitrogenous industrial effluent for struvite and algal biomass production. This study demonstrates the integration of pilot scale struvite production from fertilizer industrial wastewater in air-agitated reactor to phycoremediation of residual wastewater. The parameters required for the production of high yield and better quality of struvite was optimized. The microalgal consortium was isolated from anaerobic plant digestate and adapted to tolerate 1000 mg/L of NH_4-N using synthetic wastewater rich in NH_4-N. Pilot-scale struvite production was carried out in the air-agitated reactor (1 m^3 capacity) in batch mode and phycoremediation of residual effluent was carried out in tubular photobioreactor (200 L capacity) in fed batch mode. Pilot-scale struvite crystallization produced 60 kg of struvite from 1 m^3 of effluent. Due to integration, 64.58 % COD, 87.31 % NH_4-N, 89.0 % TKN (Total Kjeldahl Nitrogen) and 98.79 % of PO_4-P was removed. The observed yield (g/m^3 effluent) of biomass, lipids, ω-3 fatty acid and biogas (L/ m^3 effluent were 290, 56, 11.2 and 80 L, respectively. The individual microalgal species present in BPGC consortium were also identified by 18 S rDNA sequencing. BPGC consortium was found to contain five major microalgae; *Chlorella pyrenoidosa*, *Micractinium pusillum*, *Actinastrum hantzschii*, *Micractinium* sp. and *Chlorella coloniales*. In brief, the integration of struvite production and microalgae cultivation can be used as an effective treatment system for fertilizer industry wastewater.

Summary of results and conclusion

The major achievements of the present work may be summarized as;

1. Treatment of domestic wastewater

The domestic wastewater was phycoremediated by integrating active treatment system (SBR) and passive treatment system (VFCW) to MTS (by using *S. platensis* and *C. vulgaris* and algal consortium) for the production of value-added products.

1.1 Integration of Active Treatment System (SBR) to MTS

- *Spirulina platensis* and *C. vulgaris* were found to tolerate 100 mg/L and 200 mg/L of ammoniacal nitrogen.
- The observed removal efficiency of COD, PO_4-P, NH_4-N and TKN of *S. platensis* and *C. vulgaris* were 18%, 14%, 17%, 16% and 31%, 40%, 36%, 38 %, respectively.
- *S. platensis* and *C. vulgaris* biomass was observed to contain 26.65 % and 16.45 % lipids, respectively.
- The maximum biogas production (mL/g VS) was observed at 2 g VS/L.
- *S. platensis* and *C. vulgaris* was observed to produce 320 mL/g VS and 450 mL/g VS biogas, respectively.
- Thermally pre-treatment of *S. platensis* and *C. vulgaris* biomass increases biogas production by 8.5% and 6.6 %, respectively.
- These studies have successfully demonstrated that microalgal cultivation in wastewater can be easily adopted in currently available wastewater treatment plants without any major modifications of existing available infrastructure.

1.2 Integration of Passive Treatment System (VFCW) to MTS

- The integrated system was observed to remove 68.9% COD, 77.4% NH_4-N, 75.8% TKN and 63.6% PO_4-P.
- The harvested Naive Biomass (NB) was observed to contain 16.7 % of lipids (W/W).
- The Residual Biomass after Lipid Extraction (RBLE) was used as a substrate for ethanol production. The observed yield of ethanol using RBLE as a substrate was 33.4 %.
- NB, RBLE, and Residual Biomass after Lipid and Sugar Extraction (RBLSE) indicated net biomethane yield (mL/g VS) of 211.8, 134.6 and 107.7, respectively.

2. Treatment of industrial wastewater

2.1 Ammoniacal nitrogen tolerance studies of microalgae.

- The observed ammoniacal nitrogen tolerance limits of *Chlorella vulgaris*, *Spirulina platensis* and BPGC consortium were 200 mg/L, 100 mg/L and 1000 mg/L.
- The individual microalgal species present in BPGC consortium were also identified by 18 S rDNA sequencing. BPGC consortium was found to contain five major microalgae; *Chlorella pyrenoidosa*, *Micractinium pusillum*, *Actinastrum hantzschii*, *Micractinium* sp. and *Chlorella coloniales*.

2.2 Phycoremediation of industrial wastewater

- This study also demonstrates the integration of pilot scale struvite production from fertilizer industrial wastewater in air-agitated reactor to phycoremediation of residual wastewater.
- The optimized pH and $MgCl_2$ concentration for struvite production were 9.0 and 5 % W/V, respectively.
- Pilot-scale struvite crystallization produced 60 kg of struvite from 1 m^3 of effluent.
- Due to integration, 64.58 % COD, 87.31 % NH_4-N, 89.0 % TKN (Total Kjeldahl Nitrogen) and 98.79 % of PO_4-P was removed.
- The observed yield (g/m^3 effluent) of biomass, lipids, ω-3 fatty acid and biogas (L/ m^3) effluent were 290, 56, 11.2 and 80 L, respectively. In brief, the integration of struvite production and microalgae cultivation can be used as an effective treatment system for fertilizer industry wastewater.

The potential of microalgal biorefineries integrated to wastewater treatment is depicted in Table blow.

Table 1 -The potential of microalgal biorefineries integrated to wastewater treatment.

Wastewater Type	Microalgae	Biomass yield (g/m^3)	Lipid yield (g/m^3)	Biogas yield (L/m^3)	Ethanol yield (g/m^3)	Struvite yield (Kg/m^3)
SBR effluent	*S. platensis*	600	160	161.28	NA	NA
SBR effluent	*C. vulgaris*	260	42.77	77.35	NA	NA
VFCW effluent	*Consortium*	320	53.44	110.656	83.2	NA
Fertilizer Industry effluent	*BPGC Consortium*	290	56.46	78.88	NA	60

Publications from PhD thesis

1. Ram Chavan and **Srikanth Mutnuri.** (2020) Domestic wastewater treatment by constructed wetland and microalgal treatment system for the production of value-added products, **Environmental Technology**, 1-14. DOI: 10.1080/09593330.2020.1726471.

2. Ram Chavan and **Srikanth Mutnuri.** (2020) Demonstration of pilot scale integrative treatment of nitrogenous industrial effluent for struvite and algal biomass production, **Journal of Applied Phycology**, 32,1-15. DOI:10.1007/s10811-019-01978-4.

3. Ram Chavan & **Srikanth Mutnuri** (2019) Tertiary treatment of domestic wastewater by *Spirulina platensis* integrated with microalgal biorefinery, **Biofuels,** 10:1, 33-44. DOI: 10.1080/17597269.2018.1461509.

4. Ram Chavan and **Srikanth Mutnuri.** (2019) Phycoremediation and biogas production potential of *Chlorella vulgaris* grown in secondarily treated wastewater, International Journal of Recent Technology and Engineering **(IJRTE)**, 8:3,1939-1945. DOI:10.35940/ijrte.C4477.098319

Future Prospects:

Future research will be on demonstrating at a full scale the integration of Microalgal treatment systems with other active or passive treatments system for producing multiple value-added products so as to make the wastewater treatment system sustainably viable. Focus will also be on studying ammoniacal nitrogen tolerance behavior of BPGC consortium at genetic level and protein expression profiles.

Brief biography of candidate

Name	Mr. Chavan Ram Indrajit
Education	M.Tech (Biotechnology,2014)-LPU ,Punjab
	M.Sc. (Biotechnology,2009)-University of Mumbai
	B.Sc. (Biotechnolgy,2006)-SRTMU, Nanded
State and national level tests qualified	GATE (2011,2013), CSIR NET (2013), ARS NET (2013) and MH SET (2013,2014)

Conferences attended

1. Ram Chavan and Srikanth Mutnuri (2017). Development and demonstration of pilot scale integrative treatment of nitrogenous industrial effluent for struvite production and microalgae cultivation. (Poster Presentation) at ABO summit-October, 2017, Salt Lake City-USA.
2. Ram Chavan and Srikanth Mutnuri (2019). Value added products from sea-Macrolagal biorefinery concept with special focus on *Ulva* sp. and *Porphyra* sp. (Poster presentation at India International Seaweed Expo and Summit-January, 2019.
3. Participated in Novel Sanitation Approaches and wastewater treatment systems (November 2017) at BITS Pilani, KK Birla Goa Campus.
4. Participated in Terra-preta sanitation and decentralized wastewater systems (November 2015) at BITS Pilani, KK Birla Goa Campus.
5. Participated in Decentralized biogas digesters and their slurry management (DBDSM-November 2014) at BITS Pilani, KK Birla Goa Campus.

Research Experience:

1. Worked as JRF in project entitled "Treatment of nitrogenous wastewater by using microalgae and its utilization for the production of value-added products" (May 2014-April 2017) funded by BITS Pilani-CORE-WWE under the under the supervision of Dr. M. Srikanth at BITS Pilani, KK Birla Goa Campus.

2. Awarded as Water Advanced Research and Innovation (WARI) Intern (May 2017-November 2017) at University of Nebraska-Lincoln-USA under the supervision of Prof. Concetta DiRusso. The internship was funded by DST-INDIA, DWFI-USA, IUSSTF-India and University of Nebraska-Lincoln.

3. Worked as Research Fellow on consultancy project by German Technical Cooperation on GIZ-BMU – Waste to Energy project (November-December 2017) under the supervision of Dr. M. Srikanth at BITS Pilani, KK Birla Goa Campus.

Brief biography of supervisor

Dr. M. Srikanth was a recipient of DAAD-UGC Scholarship to complete his Doctoral Research at UFZ – Centre for Environmental Research, Germany and obtained his degree from Anna University Chennai in the year 2004. He joined BITS Pilani K.K Birla Goa Campus as a full-time faculty by 2005. He worked as convener for three International Conferences in Environmental Biotechnology held in the year 2009, 2011 and 2014 at BITS Pilani K.K Birla Goa Campus. Dr. M. Srikanth conducted International Workshop on Bioremediation in association with Dr. Max Haggblom, Rutgers University USA for two weeks from January 4 – 16, 2010. He was principal investigator for four research projects funded by DST, DBT, UGC and GEDA and currently he has research projects funded by CSIR and DBT BIRAC & Bill and Mellinda Gates foundation. He has published 30 research papers in International Journals and written two Book Chapters. He received Helmholtz association's Junior Scientist Award and FEMS Young Scientist Award to participate in International conference on Environmental Biotechnology, Leipzig, Germany, 2006 and 14th International Biodeterioration and Biodegradation symposium Sicily, Italy, 2008 respectively. He had attended National and International Conferences to present his research work as Oral and Poster presentations. He has Research Collaborations with Scientists from IISc Bangalore, INRA France, UFZ Germany, GTZ-BMU Germany and Rutgers University USA. He is a Recipient of American Society for Microbiology & Indo US Science and Technology Forum (ASM IUSSTF) Indo US Research Professorship for October 2010.