Title of the Thesis: Performance Modelling of a Combined System of Phycoremediation with a Fixed Film Bioreactor for the Treatment of Dairy Industry Waste Water

Submitted by: Abhishek Das

Doctor of Philosophy (Ph.D) (Engineering)

Department of Food Technology and Biochemical Engineering

Faculty Council of Engineering & Technology

Jadavpur University

Kolkata, India

Year: 2022

THESIS

On

"Performance Modeling of a Combined System of Phycoremediation with a Fixed Film Bioreactor for the Treatment of Dairy Industry Waste Water"

Under the Supervision of

Dr. Sunita Adhikari (Nee Pramanik)
(Associate Professor, Department of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata, West Bengal)

&

Dr. Pradyut Kundu
(Lecturer, Dept. of Food Processing Technology, Mirmadan Mohanlal Govt. Polytechnic, Plassey, Nadia, West Bengal)

List of Publications:

List of Journal Publications:

- ***Abhishek Das, Pradyut Kundu, and Sunita Adhikari, 2020 "Characteristics and treatments of dairy industry wastewater in a suspended growth batch reactor", Indian Chemical Society (Elsevier), 97(9a), pp. 1323-1327.***
- ***Abhishek Das, Pradyut Kundu, and Sunita Adhikari, 2021 "Biological treatment of dairy industry wastewater in a suspended growth batch reactor: performance evaluation and biodegradation kinetics", Bioremediation Journal (Taylor & Francis), pp. 1-19. DOI: 10.1080/10889868.2021.1988893 .***
- ***Abhishek Das, Pradyut Kundu, and Sunita Adhikari, 2022 "Two stage treatability and biokinetic study of dairy wastewater using bacterial consortium and microalgae", Biocatalysis and Agricultural Biotechnology (Elsevier), pp. 1-11. DOI: https://doi.org/10.1016/j.bcab.2022.102387***
- ***Abhishek Das, Pradyut Kundu, and Sunita Adhikari, 2022 "Treatability and Kinetic Study of Dairy Effluent using Microalgae Spirulina platensis in a Laboratory Scale Batch Reactor", Journal of Water Chemistry and Technology (Springer publication),44(3), pp. 208-215.***

List of Publications in National/International Book Chapter:

Abhishek Das, Pradyut Kundu, and Sunita Adhikari, 2019 "Bioremediation of wastewater using microalgae", **Environmental Biotechnology for Soil and Wastewater Implications on Ecosystems**

(Springer Publicatons), pp. 55-60. **ISBN 978-981-13-6846-2 (eBook)**

List of Patents:

Nil

List of Presentation in

National/International/Conferences/Workshops

- ***Oral presentation on the paper titled "Bioremediation of wastewater using microalgae" presented in International Conference BIOSPECTRUM-2017 organized by University of Engineering and Management, Kolkata, West Bengal***
- ***Oral presentation on the paper titled "Characteristics and treatments of dairy industry wastewater in a suspended growth batch reactor" presented in International Conference ATIPC-2018 organized by IIEST, Howrah, West Bengal***
- ***Oral presentation on the paper titled "A two stage bio- treatment of dairy industry wastewater with bacterial consortium and micro algae in a continuous suspended and fixed film bioreactor" presented in the National conference ICHEM-2022 organized by Hindustan Institute of Technology and Science (HITS), Chennai, Tamil Nadu***
- ***Oral presentation on the paper titled "Application of response surface methodology in optimization of process parameters for combined biological treatment of Dairy wastewater using consortium of bacteria and microalgae" presented in International conference BIOSPECTRUM-2022 organized by University of Engineering and Management, Kolkata, West Bengal***

PROFORMA-1

"Statement of Originality"

I, Abhishek Das, registered on 11.04.02018 do hereby declare that this thesis entitled **"Performance Modeling of a Combined System of Phycoremediation with a Fixed Film Bioreactor for the Treatment of Dairy Industry Waste Water"** contains literature survey and original research work done by the undersigned candidate as part of Doctoral studies.

All information in this thesis have been obtained and presented in accordance with existing academic rules and ethical conduct. I declare that, as required by these rules and conduct, I have fully cited and referred all materials and results that are not original to this work.

I also declare that I have checked this thesis as per the "Policy and Anti Plagiarism, Jadavpur University, 2019", and the level of similarity as checked by iThenticate software is 7%.

Signature of the Candidate: Abhishek Das

Date: 22.09.23.

Certified by Supervisor(s):

(Signature with date seal)

1. Sunita Adhikari (Nee Pramanik)

Dr. Sunita Adhikari (Nee Pramanik)
Associate Professor
Department of Food Technology
& Biochemical Engineering
Jadavpur University, Kolkata-700032

2. Pradyut Kundu

DR. PRADYUT KUNDU
LECTURER, DEPARTMENT OF
FOOD PROCESSING TECHNOLOGY
MIRMADAN MOHANLAL GOVT. POLYTECHNIC
GOVT. OF WEST BENGAL
KALIGANJ, PLASSEY, NADIA
PIN-741156

PROFORMA-2

CERTIFICATE FROM THE SUPERVISOR/S

This is to certify that the thesis entitled "**Performance Modeling of a Combined System of Phycoremediation with a Fixed Film Bioreactor for the Treatment of Dairy Industry Waste Water**" submitted by Shri. Abhishek Das, who got his name registered on 11.04.2018 for theaward of Ph. D. (Engineering) degree of Jadavpur University is absolutely based upon hisown work under the supervision of Dr. Sunita Adhikari (Nee Pramanik) and Dr. Pradyut Kundu and that neither his thesis nor any part of the thesis has been submittedfor any degree / diploma or any other academic award anywhere before.

1. Sunita Adhikari (Nee Pramanik) 22.09.23.

Dr. Sunita Adhikari (Nee Pramanik)
Associate Professor
Department of Food Technology
& Biochemical Engineering
Jadavpur University, Kolkata-700032

Signature of the Supervisor
Date with Office Seal

2. Pradyut Kundu

DR. PRADYUT KUNDU
LECTURER, DEPARTMENT OF
FOOD PROCESSING TECHNOLOGY
MIRMADAN MOHANLAL GOVT. POLYTECHNIC
GOVT. OF WEST BENGAL
KALIGANJ, PLASSEY, NADIA
PIN-741156

Signature of the Supervisor
Date with Office Seal

6 | P a g e

Acknowledgement

Firstly, I would like to thank both of my supervisors Dr. Sunita Adhikari (Nee Pramanik), Associate Professor, Department of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata and Dr. Pradyut Kundu, Lecturer, Dept. of Food Processing Technology, Mirmadan Mohanlal Govt. Polytechnic, Plassey, Nadia, West Bengal for their constant support, inspiration and supervision. Their immense knowledge, patient hearing, helping nature and professional approach were largely responsible for the completion of this thesis. Their extensive discussions and interesting advice have been very helpful in executing this research work.

I gratefully acknowledge my parents, all of my relatives for their unconditional support and love and I gratefully acknowledge DST SERB (GoI) for their funding.

I am very much grateful to all the faculty members and non-teaching staffs and also to the authorities of Department of FTBE, JU, for their cooperation and facilities accorded during my research work.

Finally I wish to thank Dr. Prasanta Kumar Biswas (HOD, FTBE, JU,), Ms. Mausumi Ray, Ms. Najmun Nahar, Ms. Asmita Bhattacharjee, Ms. Deblina Santra, Mr. Debapriya Maity, Mrs. Bijia Ghatak and Ms. Nagma Kasmi (Research Scholars) for their help and cooperation during my work.

Last but not the least, thanks to God for his support and blessings.

.....

(Abhishek Das)

7 | P a g e

Dedicated to my Parents and my Research supervisor(s)

Table of Contents

	reactor for pretreated simulated dairy wastewater (SDW) sample [Set 1: Initial NO_3^--N = 54 mg/L as N, initial PO_4^{3-}- P = 16 mg/L as P, initial inoculums of *Chlorella pyrenoidosa*= 0.8% v/v, batch contact time = 8 days]		

Executive Summary

This research was done to obtain the bio-kinetic coefficients for the bio-treatment of dairy-industry wastewater in a suspended growth batch reactor or SGBR. This research work was conducted in a laboratory scale batch reactor which is considered to be a prototype of the aeration tank segment of an activated sludge treatment system. Dairy industry wastewater samples were collected from a dairy industry located at Dankuni, near Kolkata on regular time interval and also in different seasons to neglect seasonal variation and establish mean values of the main wastewater parameters. A simulated synthetic sample was made according to the evaluated characteristics of dairy-wastewater. Study of Kinetic parameters in batch reactor was carried out to obtain kinetic coefficients (Y, Ks, k_d and k). Isolation and identification of the most potent strains for both the carbon oxidation and nitrification study was done and by using those strains biokinetic coefficients with pure culture was determined.

Purpose of the second phase of the study was to carry out nutrient removal and find out biokinetic coefficients of a bacterial pretreated dairy wastewater in a batch reactor using micro algal strain of *Chlorella pyrenoidosa*. The research work was done in a laboratory-scale batch reactor. Dairy-wastewater after a primary treatment with a consortium of bacterial population containing carbon oxidation and nitrification was taken as the feed material for algal treatment process using *Chlorella pyrenoidosa* strain. Nitrate nitrogen which was formed during bacterial nitrification still remaining in primary treated dairy wastewater along with Phosphorus have been removed by algal treatment using microalgae *Chlorella pyrenoidosa*. The present investigation was done for algal treatment of simulated dairy plant effluent containing nitrate nitrogen and phosphate in a lab-scale batch reactor with acclimatized seeds of microalgae *Chlorella pyrenoidosa* and also to evaluate various biokinetic coefficient of microalgae *Chlorella pyrenoidosa.* The maximum 99.67 % and 90.25 % of Nitrate nitrogen or NO_3^--N and

Phosphate as Phosphorus or PO_4^{3-}- P removal were achieved corresponding to initial Nitrate nitrogen and Phosphorus concentration of 54 and 16 mg/L respectively, with an initial inoculums concentration of microalgae *Chlorella pyrenoidosa*of0.8 % v/v after 8 days of detention period in batch reactor. Kinetics study was also carried out to obtain bio-kinetic coefficient for NO_3^--N and PO_4^{3-}- P removal using microalgae of *Chlorella pyrenoidosa*.

Third part ofthe research work was to evaluate the biokinetic coefficients of bacterial pre treated Dairy wastewater in a suspended growth batch reactor with the treatment of microalgae *Spirulina platensis.* This study was quite similar as done in the previous phase treatment with microalgae *Chlorella pyrenoidosa.*The study was performed in a laboratory scale batch setup. Samples of dairy plant; pre treated with a consortium of carbon oxidation and nitrification bacterial culture was the feed of this purification process. Bacterial pre treatment was done to reduce its organic carbon and Ammonium nitrogen content. Nitrate nitrogen which was formed during bacterial treatment and most of the Phosphate still remaining in the water was treated in this process with micro algal species to remove these nutrients in wastewater and consequently to meet with discharge standards of regulatory authorities. A simulated synthetic wastewater sample was prepared according to the concentration measured in the original pre treated wastewater where average Nitrate Nitrogen concentration was found 54 mg/L and phosphate concentration 16 mg/L. Kinetics study was carried out to obtain kinetic constants (Y, Ks and k) for both the nutrients.

A combined treatment process with bacterial consortium and microalgae was done after finding out an optimum inoculums combination for both the cases of bacteria and microalgae. A combination of 10% and 5% initial inoculums of carbon oxidation and nitrification respectively was seemed to be an optimum initial inoculums combination for bacterial consortium. In case

of combination of microalgae, an equal amount of *chlorella* and *spirulina* (in the ratio of 1:1) was giving the optimum result. Beyond around 90% of most of the SCOD and nutrients were removed from the wastewater, Time concentration study as well as kinetics study was carried out similarly after this optimization study, where an optimum condition was found out. In this phase of work real life dairy wastewater was used for the treatment process. Firstly the process was carried out in a small scale batch reactor and then finally in a pilot scale bioreactor.

A Response Surface Methodology or RSM study was carried out using Design Expert software ver. 13 and by performing sets of experiments accordingly with varying initial parameters or independent input variables. This study sets were prepared by synthetic chemicals present in the laboratory. Response Surface Methodology or RSM studies endorse and validate the results of the present research experiments.

Objectives

- On the basis of the critical reviews, available in the literature sources the objective of this present research work has been primarily set as to test the performance of a laboratory-scale reactor by bacteria and microalgae for the combined removal of nutrients (nitrogen and phosphorous) and soluble organics (SCOD) from synthetic and real-life Dairy wastewater.
- Evaluation of kinetic coefficients for carbon oxidation, nitrogen and phosphorous removalfor rational design of treatment plant system.
- In the subsequent phase of the study, the experimental results obtained have been used to validate a statistical model like RSM with factorial design models for prediction of the performance of a laboratory scale reactor in terms of SCOD, NH_4^+-N, NO_3^-N and phosphorousremoval from real life and synthetic simulated dairy wastewater as output parameters in correspondence to various input functions such as initial SCOD, NH_4^+-N, phosphorous concentrations, initial biomass concentration, contact time, pH, dissolved oxygen (DO) etc.
- This study also includes isolating and identifying the predominant bacteria species by morphological, physiological and biochemical examinations and 16S rDNA study.

Scope of the Present Work

The scopes of the present work for achieving the afore-said objective are given below

- Collection of Dairy industry wastewater from Effluent Treatment Plant (ETP) of Dairy Industry.
- Characterization of wastewater discharging from dairy wastewater
- Acclimatization of bacteria and microalgae in laboratory condition for removal of both SCOD and nutrients (N and P) under combined feeding condition with respect to specific target input substrate concentration.
- Determination of optimum time period for removal of maximum soluble organics carbon (SCOD) and nutrients (nitrogen and phosphorous)
- Assessment of the removal kinetics of SCOD, ammonium nitrogen (NH_4^+-N), Nitrate Nitrogen (NO_3^--N) and phosphorous with time and under different substrate concentration.
- Assessment of effect of influencing parameters such as initial SCOD, ammonium nitrogen (NH_4^+-N), phosphorous, biomass concentration, DO or dissolved oxygen level, pH level, phosphorous release and uptake rates on the performance of reactor.
- Evaluation of kinetic studies for nitrification and phosphorous removal under multi-component substrate feeding condition.
- Determination of kinetic constants based on microbial and micro algal growth and substrate removal for dairy wastewater
- Validation of Response Surface Methodology (RSM) with factorial design models using experimental data for combined Carbon oxidation and nutrient removal of dairy wastewater.
- Isolation and identification of the predominant microbial strain(s) present using morphological, physiological, biochemical examinations and 16S rDNA study in support of biological treatment of present investigation.

Introduction

Industries are the backbone of our civilization. It helps improving our country's Gross Domestic Product (GDP) and helps to develop our economy. Booming of industries is now becoming the hallmark of many developing nations. A phenomenon of rapid industrialization is also associated with our nation, in the last few decades. Expanding industrial development has its negative aspects as well. Pollution in the form of toxic gases, contaminants, solid wastes as well as liquid discharges is the major by-products of industrial production. Noise and other forms of pollutions coming from industries can't be neglected too. The impacts of these pollutants are moderate to severe depending on their physical and chemical nature. Impacts are not only limited in the sphere of human society but also affects the whole ecosystem. Food manufacturing and processing industries pollute the environment and ecosystem as well.

Fast development of enterprises has improved the productivity as well as brought about the creation and injection of harmful substances into the environment, which are responsible for creating health hazards to human, influencing various normal operations as well as producing harm to flora and fauna in the environment. These squander are potent toxins as they hit nature badly and delivered huge quantity of solids or liquid wastes including broad spectrum of synthetic compounds. Sewage treatment in plants to fulfill the discharge standard guidelines prescribed by different pollution controlling agencies has consistently been an issue for the enterprises. Prior to releasing the treated waste on land or in water body the enterprises should properly meet the standards. To maintain the proper processing in a wastewater treatment unit, wastewater characterization, treatability studies and proper planning for treatment procedure is quite essential.

In general food and agriculture industry wastewater is composed of mainly organics or organic carbon which contribute to its high Biochemical Oxygen Demand or BOD and Chemical Oxygen Demand or SCOD and high nutrients content mainly rich in Nitrogen and Phosphorus. High BOD and SCOD in the wastewater is the major cause for the depletion of dissolved oxygen or DO level in the surface water where this untreated or partially treated wastewater is discharged. This event gives rise to the death of aquatic organisms due to suffocation; a condition known as hypoxia.

High levels of nutrients mainly in the form of Nitrogen and Phosphorus which come from the processing of the foods and dairy products and also from the cleaning process or CIP system with various cleaning agents is the leading cause of the phenomenon known as eutrophication in the streams and enclosed bodies of water like ponds, lake etc.

In this situation availability of high levels of limiting nutrients like nitrate and phosphate gives rise to abundant growth of microalgae; a condition known as algal bloom. When these algae die, the decomposition of these algal mass by microorganisms deplete dissolved oxygen in the water body which again leads to death due to hypoxia of aquatic organisms.

Discharge of these wastewater in the land and agriculture field also cause nuisance. Potential toxicity of this wastewater may lead to make crops hazardous. Other problems of aesthetics are also there.

Different treatment processes are available for the treatment of wastewater. One of the best methods for this treatment process is considered as biological treatment method. Microbial and/or micro algal treatment method which takes up the organics and nutrients as feed or substrate and degrade them into innocuous or non-toxic products like carbon di oxide, water and they multiply into new cell mass by absorbing and assimilating nutrients in their cellular system.

A treatment system with the consortium of microbial population and microalgae has been employed for the treatment of dairy industry wastewater in both small scale and pilot scale

bioreactor in the system of bioremediation in suspended growth batch reactor and a fixed film phycoremediation. Biokinetic coefficients were evaluated from these studies. A statistical tool for the optimization of the process parameters was also introduced. This optimization study also has validated this research study. Literatures from other sources regarding this type of biological treatment of food and agro industry wastewater also endorse the outcomes of this study.

Literature Review

Production of milk in India has boomed significantly in the last few decades from a volume of a million ton to 110 million tons within 1951 and 2009(Chawla et. al. 2009, Sharma and Gulati 2003)

Untreated or partially treated sewage with elevated level of toxins can cause major environmental hazards when released into the environment. Dairy wastewater can contribute high organic load to the wastewater. In addition to these cleaning of plant, such as cleaning in place system (i.e. CIP) results in caustic wastewater. Dairy wastewater contains highly putrescible organic constituents (Raghunath et al. 2016).VFAs or Volatile fatty acids are one of the most common organic volatile compounds in dairy waste and are the prime cause of odor pollution (Page et al. 2014). The contributor of different types of waste material in the dairy wastewater stream from various steps of milk processing is showing in the following diagram.

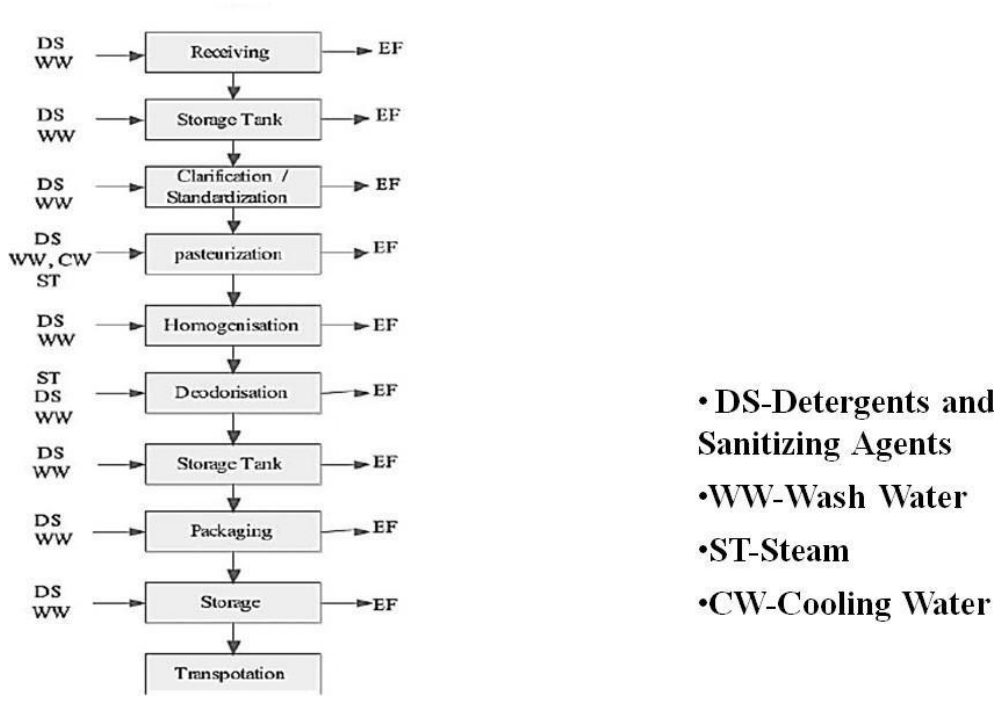


Fig. LR1: Contribution of different waste elements in different stages of dairy-industrial processing.

Most of the constituents in the dairy wastewater are biologically degradable and hence the wastewater should be treated biologically, either aerobic or anaerobic.

Throughout the process chain of the dairy industry affect the environment (Strydom et al.1993). Majority of dairy industries consider treatment of wastewater as a burden on their economy and so think of it as a futile investment. (Robinson 1997); In general, they produce whitish to off white color of wastewater containing different types of carbohydrates, proteins, fats and other biological materials. Dairy industry wastewater characteristics vary between industries according to their products and processing technique (Vidal et al. 2000). Composition of dairy wastewater from different sources has been summarized in the following table.

Table LR1: General Characterization of dairy wastewater from different sources.

Waste Type	SCOD	BOD	pH	TSS	TS	References
Milk & Dairy Products factory	10251.2	4840.6	8.34	5802.6		Cristian, O. 2010
Dairy effluent	1900-2700	1200-1800	7.2-8.8	500-740	900-1350	Deshannavar, etal.,2012
Arab Dairy Factory	3383±1345	1941±864	7.9 ±1.2	831±392		Tawfiket.al.,2007
Dairy wastewater	2,500-3,000	1,300-1,600	7.2-7.5	72,000-80,000	8,000-10,000	JavedIqbalQaziet.al., 2011
Dairy effluent(CPCB 1993)	1120-3360	320-1750	5.6-8	28-1900		Lata,et.al., 1999
Whey	71526	20000	4.1	22050	56782	Deshpande.et.al.,2012
Bhandara Co-operative Dairy industry wastewater	1400to2500	800to1000	7.1-8.2	1045 to 1800	1100 to 1600	Gotmareetal.,2011
Cheese Whey pressed	80,000-90,000	120,000-135,000	6	8000-11000		Kabbout,etal.,2011
Aavin dairy industry washwater	2500-3300		6.4 - 7.1	630-730	1300-1400	Sathyamoorthyetal.,2012
Dairy industry wastewater	2100	1040	7-8	1200	2500	Arumugam, A. 2008

Correct information about the true composition of chemicals present in dairy industry wastewater is scarce (Donkin 1997). Raw Milk has a Biological Oxygen Demand (BOD) almost around two hundred and fifty times larger than that of wastewater (Burton 1997). Dairy

wastewaters having comparatively high organic contents, of which the main contributors are milk sugar or lactose, fats, and milk protein (mainly casein), and is rich in elevated levels of nitrogen (Vidal et al. 2000,Danalewich et al. 1998). A basic characterization of dairy wastewater has been depicted in the following table.

Table LR2: Basic characterization of dairy wastewater.

Characteristics	Effects
High BOD and COD content	Indicates low do-aquatic organism find hard to survive
Nitrogen, phosphorous and other nutrients	Algal growth-eutrophication-depletion of oxygen, native species endangered
Fats	Decreases oxygen transmission, problems in sewage treatment facilities, biological treatment system
Fluctuating pH	Threats to pH sensitive species or metabolic activities, of inhabiting organism, affecting crop production when it used for irrigation purpose
Cleansing agent waste	Causes turbidity, toxicity. They are recalcited . Dissolved oxygen problem

Discharge standards prescribed by various authorities for the efficient and safe discharge of wastewater are also summarized in the table below.

Table LR3: Discharge standards of wastewater into aquatic/terrestrial ecosystem according to authorized agencies.

Parameters	As per World Bank Group(1996)	EPA	As per CPCB, INDIA
pH	6-9	5.5-9	6.5-8.5
BOD	50 mg/L	100 mg/L	100 mg/L
COD	250 mg/L	-	-
Oil & grease	10 mg/L	10 mg/L	10 mg/L
Total Nitrogen	10 mg/L	-	10 mg/L
Total Phosphorus	2 mg/L	-	-

*Source courtesy: Web portals of respective authority

Though a number of physical and chemical methods are available for the remediation of dairy wastewater, biological method using microorganisms have gained more importance and it was found to be most promising technique for the dairy wastewater treatment (Chaubey 2002). It was found that by activated sludge process with a batch contact time of 24hrs, 70% BOD_5 (5days Biological Oxygen Demand), 71% SCOD and 61% VOM (Volatile organic matter) could be removed from dairy wastewater (Naser et al. 2014). Similar effluent when treated in trickling filter, it could remove VOM by 90%.When the dairy wastewater was treated in activated sludge system removal efficiencies of 99%, 81% and 93% for BOD, SCOD and TKN (Total Kjeldahl

Nitrogen) respectively was obtained (Carta-Escobar et al. 2005). Investigation was carried out on the treatment of a typical dairy Wastewater by a bench-scale aerobic sequential batch reactor (SBR) and it was found that the SCOD removal efficiency was more than 90%at 8hr cycle time which demonstrated the ability of SBR to treat the dairy wastewater in terms of SCOD reduction (Mohseni-Bandpi et al. 2004). In the year 2004 another investigation was done in which settleddairy effluent was treated in a bench scale Continuous Flow Stirred Tank Reactor (CFSTR) without any solid recycle keeping cell residence time (θ_c) varying from 1 to 6 days (Venkatesanet al. 2004).The percentage of BOD removal they obtained in the range from 67-90 %.

A group of researchers treat dairy wastewater using an algae *Botryococcus sp.* (Gani et al. 2014).From concentrated wastewater, *Botryo coccus p*roduced wastewater-parameters such as BOD, TOC, TC, IC and SCOD as 73.3%, 65.1%, 61.4%, 58.3% and 48.8% respectively after 15 days of treatment. Another study found that treatment of wastewater using microalgae is a green technique and algal biomass generated can be utilized further and helps in efficient recycling of nutrients. This investigation was done for the purification of wastewater by *Chlorella vulgaris*. Treatment with *Chlorella vulgaris* was effective in removal of major

inorganic components form the wastewater. (Fathi et al. 2013). A group of researchers made a study in Konabari near Gazipur city-corporation (Bangladesh) in which textile-industry wastewater were treated by two indigenous species of cyanobacteria and different important parameters of the treated wastewater were analyzed for the evaluation of the treatment efficiency and the removal efficiency was found to be satisfactory. Two species of cyanobacteria , *Anabaena*and *Nostoc muscorum* were used for this purpose (Talukder et al. 2015).One investigation was done for simultaneous removal of nutrient (mainly N and P) and some of the heavy metals (such as iron, manganese, zinc). Two microalgae species *Scenedesmus and Chlorella* were isolated from municipal wastewater. *Chlorella vulgaris* showed better potency for the removal of total nitrogen (TN) in waste water than *Scenedesmus armatus* (Kozłowska et al.2014).

Chakraborty et al. in 2021 had performed similar study as of this present work with bakery and confectionery waste water. Bioremediation with mixed bacterial consortium was done in this case. Around 95% COD and over 75% Ammonium Nitrogen was removed within 24 hrs. Kinetic coefficients derived from this study was 0.765, 0.056, 9.09 and 350.69 for Y, Kd, k and Ks with Carbon oxidation study and 0.425, 0.063, 23.25 and 29.99 respectively with Nitrification study.

Bhattacharya et al. in 2019 had published their work on bioremediation of dairy waste water. For Carbon oxidation study kinetic constants were calculated and found to be 0.568 mg MLSS/mg COD, 72.134 mg/L, 0.031 per day and 8.84 per day for Y, Ks, Kd and k respectively.

Lateef et al. in 2013 had documented their work on biological treatment of dairy wastewater using activated sludge and their four kinetic constants were found to be around 0.933, 0.015, 867.76 and 2.5 for Y, Kd, Ks and k respectively.

A simultaneous reduction of Carbon oxidation and Nutrients study in a batch fed reactor by Pseudomonas sp. P1 was carried out with organic loaded wastewater and there is around 95%

removal of SCOD and 81.38% removal of Ammonium Nitrogen was found with an initial SCOD and Ammonium Nitrogen content of 600 mg/L and 50 mg/L respectively after twenty four hours. Moreover the book of Metcalf and Eddy has prescribed a range of those four kinetic parameters, Y, Ks, k and Kd for the biological treatment of municipal wastewater and these are as follows: 0.4-0.8, 25-100, 2-10 and 0.025-0.075 for Carbon oxidation study and 0.1-0.3, 0.2-5, 1-30 and 0.03-0.06 for Nitrification study respectively.

Valentine et al. in 2018, Aslan and Kapdan in 2006, Rowley in 2010 had performed their work on removal of nutrients especially Nitrogen and Phosphorus from wastewater with different potent microalgal strains and had developed their growth and substrate removal kinetics simultaneously by following the manner very similar to that of used by this study. A modified Monod kinetics was employed to find out kinetic coefficients for those nutrients assimilating processes. Coefficients found were in the range of 3-30, 2-40 and 0.01-0.06 for k, Ks and Y respectively for both Nitrogen and Phosphorus utilization studies while initial nutrient content was somewhat in the similar ranges of this present study and the contact time was for almost all cases around 5-10 days.

Critical Review

Based on the previous literatures regarding bioremediation of food and agro industrial wastewater it has been assessed that mixed sludge of carbonaceous and nitrifying bacteria is an important tool for the biodegradation of organic waste. Carbonaceous bacteria decompose organic materials and in turn reduce Chemical and Biological Oxygen Demand (COD and BOD). Nitrifying bacteria on the other hand oxidizes Ammonium Nitrogen into first Nitrite and then Nitrate Nitrogen with the help of Oxygen. This process reduces the organic loadings and Ammonium Nitrogen Species and simultaneously gives rise to the amount of Nitrate Nitrogen. Some researchers have used Denitrifying bacteria in the next step to remove the Nitrate Nitrogen species which is more or less a costly process as there is a requirement of dedicated anaerobic chamber for the process of denitrification. There is also some amount of Phosphorus in the wastewater in terms of Phosphate which should have been removed. For the removal of Phosphate-P, use of Phosphate Accumulating Organisms or Phosphate Solubilising Bacteria (PAOs or PSBs) has been used by some researchers for which an anoxic chamber is a basic requirement. For the fabrication of both anoxic and anaerobic chamber is an expensive process. Here in this study use of microalgae in the second phase of treatment provides a facility for the uptake of both Nitrate Nitrogen and Phosphate-P through the assimilatory process of microalgae and it also provides a good quantity of algal mass which can be further used for various purposes. Finally RSM was employed for the optimization of process parameters was done in this study. This combined treatment method helps in the treatment process both in saving large expenses, alongside giving a eco friendly process and to produce some kind of utilizable products as “Waste to Wealth”

Novelty of the present research work

Dairy Industries are one of the most pollution generating food and agro-processing industries. Milk sugar – lactose, different types of milk proteins, fats and different other compounds used in the production of various dairy products attribute to its high organic and nutrient loads which must be treated efficiently to prevent environmental and health hazards. In this study potency of combined treatment with consortium of bacteria and microalgae in two separate stages was evaluated by means of Response Surface Methodology (RSM). Obtained results suggest and upheld the significance of bio-treatment process for the treatment of wastewater.

Unlike most other researchers who have used Denitrifiers and Phosphorus removal bacteria (mainly Phosphate Accumulating Organisms or PAOs) for removal of Nitrate and Phosphorus from wastewater; this present study uses two stage sequential treatment of carbon oxidation and nitrificaion bacteria and micro algal combination to treat dairy industry wastewater which is quite cheap and eco-friendly treatment system which can turn the waste into wealth.

Chapter 1

Theoretical Considerations

Analytical Methods Followed for Monitoring of different Parameters

Determination of MLSS and MLVSS

Mixed Liquor Suspended Solids (MLSS)

A sample which was quite well mixed was filtered by using a whatmann (No 42) filter paper. Weight of the filter paper was taken previously. The residual mass retained by the filter paper was dried at a temperature of 105°C in a hot air oven. The increase in weight of the filter paper was measured as the mass of total suspended solids or TSS. The estimation of MLSS was done in a similar manner.

$$\text{Total suspended solids (TSS) in mg/L} = \frac{(A-B)\times 1000}{\text{mL of sample}}$$

Where, A = weight of filter with dried residue (mg) and B = weight of filter paper (mg)

Mixed Liquor Volatile Suspended Solids (MLVSS)

The residual mass from the Mixed Liquor Suspended Solids was burnt for a time period until constant weight has been found. Temperature was around 550±50°C, a laboratory scale Muffle furnace was used for this purpose. The residual mass left in porcelain dish was weighed again after this burning process. The residual solid mass is an estimate of the fixed solid while the weight lost on ignition represents the amount of volatile solids. Calculation of MLVSS was done by the equation given below.

$$\text{MLVSS in mg /L} = \frac{(A-B)\times 1000}{\text{mL of sample}}$$

Where, A = weight of porcelain dish + filter weight residue after ignition (mg) and B = weight of porcelain dish (mg)

Determination of NH_4^+- N by Electrode Method

The Ammonium ion (NH_4^+) electrode (Fig.-1.1) is an ISE or Ion selective Electrode,

made of a hydro-phobic and gas-permeable membrane that separates the sample-solution and the internal solution present in the electrode made of NH_4Cl. This Probe/ electrode

respond to Ammonium ion activity of the sample it has been dipped into. Mercury and silver ions present in the solution may interfere in the measurement process by complexing with ammonia. To avoid such interferences, Interference suppressant solution was added in a ratio of 1:50. Calibration process was done by taking two points as references. Calibration for the NH_4^+- N was accomplished with two different strengths of standard NH_4^+- N solutions; 100 mg/L and 10 mg/L. After this calibration process was over, strengths of different samples were obtained by direct measurement.

Fig.-1.1: Ammonium Nitrogen Electrode

Determination of NO_3^- N by Electrode Method

The nitrate ion (NO_3^-) electrode (Fig.-1.2, 1.3) is an ISE that develops a small potential difference across the thin and porous membrane. The electrode responds to theNO_3^- ion activity of the sample it has been dipped into. Chloride and bicarbonate ions may interfere in this measuring process. To eliminate such interferences Nitrate Interference Suppressor Solution or NISS has been added to the sample in a ratio of 1:1. Calibration process was done by taking two points as references. Calibration of nitrate solution was accomplished with two different strengths of standard Nitrate solutions; 100 mg/L and 10 mg/L. After this calibration process was over, strengths of different samples were obtained by direct measurement.

Fig.-1.2: Nitrate Nitrogen Electrode Probe

Determination of Phosphate

Chemical reagents required for this test**:**

Ammonium molybdate solution, Stannous chloride solution, Stock (20.0 mg/L) phosphate solution.

In a 25 mL capacity Erlenmeyer flask, water sample was to be analyzed. 1 mL of ammonium molybdate solution was put into the flask and mixed thoroughly. Two drops of stannous chloride reagent solution was added to it and mixed by swirling. If phosphate is present in the sample, a bluish color will appear in 5-15 minutes. It is measured in the spectrophotometer at 650 nm. The blank solution was set at zero absorbance. The intense the blue color, more phosphate is present in the sample. Amount of phosphate was interpolated from the standard curve of phosphate, which was made previously by using standard phosphate solution following the same procedure stated before.

Fig.-1.3:UV-VisibleSpectrophotometer (Lassany make)

Methodologies for Characterization of the isolates

Physiological characterization of the isolates

Different biochemical tests were done according to the standard protocols mentioned in Bergey's Manual of Systematic Bacteriology (Brenner et al., 2005) to find out the physiology of the isolated strains.

Gram Staining Process

Christian Gram discovered this method of staining in 1884. Bacterial smear was fixed in glass slides and the following staining reagents were given one after another in the order as follows: Crystal violet, Iodine solution, Ethyl alcohol and finally counter stain Safranin. Gram (+) bacteria, which retain crystal violet and appeared as violet, whereas gram (-) bacteria, which lose crystal violet and retain safranin and present themselves in red color.

Endospore Staining (Schaeffer-Fulton method)Process

Several bacteria have the potential to produce resistant spores. The spore staining technique was done with the following reagents.

- **Malachite Green:** Due to the presence of an impervious layer spores do not take the primary stain easily. For the penetration of color, heat is applied. After the application of primary stain with heat, both vegetative cells and spores become green in color.
- **Decolorizing Agent:** After the vegetative cells and spores have taken the primary stain malachite green, a wash with tap water removes the excess primary stain. Thus, spores retain their green color whereas vegetative cells do not retain their primary green color due to their small affinity with the stains and hence become colorless.
- **Safranin or Counter Stain:** This red colored stain is utilized as the next reagent in process to color the colorless vegetative cells, which take this stain and become red in color. Spores retain their primary greenish color from the Malachite Green.

Growth under Aerobic Condition

To find out optimum growth in aerobic condition, the isolates were grown in Luria broth medium. Incubation was done at 37ºC for 24 to 48 hrs. Optical Density (OD) was then measured at 600 nm in UV-Vis Spectrophotometer.

The compositions of Luria Broth (g/L of distilled water):

Tryptone; 10, Sodium Chloride; 10, yeast extract; 5, pH was around 7±0.2.

Growth under Anaerobic Condition

To find out optimum growth in anaerobic condition, the isolates were grown in Luria broth medium. The flasks were then flushed with CO_2 and capped tightly with sterilized corks to resist the entry of air as well as O_2. Flasks were kept for incubation in standing condition at 37ºC for around 7 to 9 days. OD was then measured at 600nm using UV-Vis Spectrophotometer.

Growth at Different Temperatures

Optimum temperature for growth has been obtained by growing the isolated culture in the Luria-Bertani (LB) medium and kept at incubator in shaking condition for around 24 to 48 hr at various temperatures; 4ºC, 25 ºC, 30ºC, 37ºC and 42ºC. The OD (optical density) of the culture was measured at 600nm using Spectrophotometer.

Growth in Different pH

To find the optimum pH for growth purpose, the isolated strain was grown in Luria- Bertani (LB) medium and kept at 37ºC temperature for around 24 to 48 for incubation with shaking condition. The pH of this LB medium was kept in both acidic and basic pH range spectrum. OD value was measured against blank at 600nm in the UV-Vis Spectrophotometer.

Growth in Different Sodium Chloride Concentrations

To determine the optimum NaCl concentration for growth purpose, the isolate was cultured in Luria-Bertani medium, then was kept for incubation with shaking for a period of 24 to 48 hrs at around 37ºC. The concentration of NaCl the above mentioned broth was varied; 0%, 1%, 2%, 4%, 5%, 6%, 7%, 8%, 9% and 10% was the different combination for different sets. After this, OD values were measured against blank at 600nm of wavelength using UV-Vis Spectrophotometer.

Acid Production from Carbohydrates

Bacterial species generally utilize carbohydrates as the main source of energy especially those bacteria which are heterotrophic in nature. Bacteria take up glucose, a form of simple sugar, because generally they have the ability to break it down through its enzymatic systems. Some

sorts of bacteria also use complex carbohydrates like lactose or sucrose or starch which are the dimer or polymer forms of the simple sugar units or monomers. Bacteria have to secrete certain enzymes those are able to cleave glycosidic linkages present in those complex sugars. If these bacteria are unable to make such enzymes then they can't use those complex carbohydrates. Each bacterium has its own specialized sets of enzymes that help it to use different sugars or carbohydrates and this feature is utilized in the identification of different bacterial species. Determination of any bacterial species through its metabolic byproducts while growing in different types of carbohydrate medium is being the fundamental principle of this test. pH indicators may also be added to the medium to detect acids that have been made by those bacteria after the utilization of different sugars.

The compositions of this test medium (g/L distilled water):

Tryptone; 15, yeast extract; 10, phenolred indicator; 0.05, carbohydrate; 10 (g/L distilled water) pH was maintained around 7. Tryptone is used as an alternative energy source and sugars are used as a carbohydratesource. Sugar is added to detect gas and metabolic acid production from bacterial metabolism of carbohydrate present in different medium. Phenol red is a pH indicator for the detection of organic acids. If the bacteria take up the sugar present in it and gives off different types of organic acids, the pH indicator will become yellow. Durham tubes are placed in submerged condition in the media to find out if there is any gas produced inside it or not. Durham tubes area special kind of small inverted tubes placed inside the test tubes in such a way that its enclosedend at the top and open end reside at the bottom of the test tubes. Small bubble inside this Durham tubes representing trapped gas that has been produced by the bacterial cultures in the process of Substrate utilization and metabolism. There are 4 possible outcomes in this test shown below.

- **No Reaction (-) with no Growth:** The broth remains red in color pointing out no utilization of carbohydrate present in the media and no growth of bacteria as well.
- **No Reaction (-) with Growth:** The broth retains it red color and the media becomes foggy or cloudy pointing out the growth of the microorganism, but is not utilizing carbohydrates; Microorganisms are using the alternative energy source.
- **Acid Production (A):** The broth turns yellow pointing out that the organism is using carbohydrate and gives off acids that bring down the pH of the media.
- **Acid and Gas production (A/G):** The broth becomes yellow and also small bubble is present there in the inverted Durham tube denoting that the organism is utilizing the carbohydrate

present in the media; producing both acids and gas in the process.

Different carbohydrates were used, viz, Mannitol, Fructose, Arabinose, Galactose, Glucose, Lactose, Sucrose etc. For each carbohydrate-test, a medium of 20 ml was made and distributed into two test tubes (10 ml each). Then in each of this test tube, Durham tube was placed in an inverted manner and then finally tubes were plugged with cotton and sterilized at 121°C temperature, 15 psi pressure, for around 15 minutes. Then for each carbohydrate solution, out of these two test tubes, two drops of 24-hr cell-suspension of the bacterial strain was added in one of the test tube and another was kept without any inoculum. This is the "Control/ Standard Reference" Test tube which will act as an equivalent to "Blank" for some other testing purpose. Then all test tubes were incubated in the incubator at 37° C and they have been observed in regular interval up to 15 days for any change in color and gas formation.

Catalase Test

This test is used to identify specific species of bacteria. It eases the decomposition of H_2O_2 into water and oxygen as a catalyst. Catalase enzyme, if present in the test isolate (i.e., are catalase (+)), when that isolate is added to H_2O_2 (3%), oxygen in bubble form are produced. Presence of catalase enzyme in bacterial cells depends on the growing condition and also the medium used for growing. This test was performed by adding a drop of H_2O_2 on a glass slide. Inoculation loop was used for the addition of H_2O_2 into the culture smear.

- Catalase (+)if bubbles form
- Otherwise the organism may be called as Catalase (-).

Nitrate Reduction Test

This biochemical investigation is for the determination of whether the bacteria possess the enzymes nitrate-reductase & nitrite-reductase or not. These two enzymes are able to catalyze two reactions which help in converting nitrate into N_2 gas. Few bacteria can produce both of these enzymes, some bacteria produce only nitrate-reductase, and some can produce neither of these two enzymes. If some bacteria are capable of producing the enzyme nitrate-reductase has been grown in a nitrate rich media, the enzyme can convert its nitrate content into nitrite form. If the microorganism is able to produce nitrite-reductase, N_2 gas will be given off.

The isolated cultured strain was aseptically transferred into a sterile tube containing nitrate broth and also a Durham tube in an inverted position. Then the tube is incubated in an incubator for

a period of around 24 hrs at 37ºC and the outcomes were observed.

Compositions of the nitrate broth (g/L of distilled water) are as follows:

Peptone; 5, beef extract; 3, potassium nitrate; 1, pH was around in the range of 7.0 ± 0.1.

If the bacteria possess both of these enzymes then nitrate will reduce to nitrite form which may be then be reduced/converted to nitric oxide, nitrous oxide, or N_2 gas. This test is based on the detection of nitrite and its ability to produce a red-colored compound when it reacts with sulfanilic acid to form a complex (i.e. nitrite-sulfanilic acid) which then reacts with α-naphthylamine to form a reddish colored precipitate which is known to be as prontosil. Reduction of nitrate is a kind of anaerobic respiration where an organism obtains oxygen from nitrate. Red color forms when nitrite is present in the media. No significant appearance of reddish color in the media after the addition of sulfanilic acid and α-naphthylamine points out that there is no nitrite present in the media. There are two possible interpretation of this observation.

- The nitrate present in the media may not have been reduced; i.e. the strain is nitrate (-).
- The nitrate in the media may have been reduced to nitrite which has been then totally reduced to NO, NO_2 or N_2 gas which is non-reactive with the chemicals that react with nitrite; the strain is then be called as nitrate(+) strain.

Urease Test

This test is for the identification of those microorganisms that have the potential of hydrolyzing urea and gives ofNH_3 and CO_2 in the process. Urease is an enzyme that hydrolyzes urea to form NH_3 and CO_2. Urease-test medium contain 2% urea and phenol red as an indicator. Increase in alkalinity through the production of NH_3leads to the change in color from yellow to pink.

Composition of test medium (gm /L of distilled water) is as follows:

potassium phosphate, monobasic; 9.1, potassium phosphate, dibasic; 9.5, urea; 20, phenol red; 0.01 and yeast extract; 0.1, pH was kept around, 7.0

A day-old pure culture of the bacterial inoculum was inoculated in the broth medium. The tube was agitated slowly to keep the bacteria in suspension. Incubation was done with cotton plugging at around 37^{o}C and was observed for any change in color at 8th, 12th, 24th, and 48th hrs. A uniform mixture of bright pink color is the indication of positive result or the production of urease.

Indole Production

This test checks the ability of bacteria to decompose the amino acid tryptophan and production of indole. A chain of several enzymes which are intracellular in origin are responsible for this phenomenon, referred as tryptophanase. Tryptophan can undergo de-amination and hydrolysis process by bacteria that possess the enzyme "tryptophanase" Tryptophan decomposed to form indole, pyruvic acid and NH_3. Organisms before this the indole test is cultured in a medium contains an ample amount of tryptophan.

Detection of indole depends on the reaction between indole and p-dimethylamino benzaldehyde, also called as DMAB under low pH conditions to produce the red chemical agent as indole.

Compositions for this tryptone broth medium (g/L of distilled water) are as follows:

Tryptone; 10, sodium chloride; 5, pH ~ 7.0

Inoculation was done with the isolate in the medium and incubated for around 24 to 48 hrs at 37℃, after that 4 to 5 drops of Kovac"s Reagent was given directly into the test tubes and the change of color was measured. A positive result is when the formation of red color in the upper layer within a few seconds of addition of the reagents and the layer will remain yellow if it is indole negative.

Starch Hydrolysis Test

This is the test that has been utilized to distinguish bacteria on the basis of their capability of starch hydrolysis with the aid of the enzyme α-amylase or oligo-l,6-glucosidase.Starchesare large molecules to permeate into the bacterial cell-membrane. So they have to cleave it into smaller fragments. Organisms that have the ability to make the extracellular enzymes α-amylase and oligo-l,6-glucosidase are potent enough to hydrolyze starch by breaking down the glycosidic bonds between the sugar monomers. The medium is nutrient agar with added which starch.

Compositions (g/L of distilled water) are peptone; 5, beef extract; 3, starch; 2, agar; 15, pH was maintained in the range of 7±0.3.

Iodine is used to detect the presence or absence of starch around the bacterial growth region. Iodine interacts with starch and creates a blue or brown color; and so microbial starch hydrolysis will be revealed as a clear zone around their growth. Isolated cultures were inoculated in the agar and the petridishes were incubated at 37 ℃ for 24 to 48 hrs, colonies were flooded with iodine after that time period is over and the results were observed.

Motility Test

The ability to move spontaneously is motility. Several ways are there to detect motility in microbes like bacteria. Motility agar test is one of these processes. A soft agar media is used for this test.

The bacteria those are non-motile will only grow in the very soft agar tube in the area only they are inoculated by stabbing. But motile bacteria, they will grow and spread around the stabbing zone by swimming.

Compositions for motility test agar medium (g/L of distilled water) are enzymatic digestion of gelatin; 10, beef extract; 3, sodium chloride; 5, and agar; 4, pH; 7±0.2. The isolated culture was inoculated through stabbing method in the mid portion of the medium with an inoculation needle to approximately half of the depth of the medium. Incubation was done at 37°C for 5 days, after that it was observed for results.

Voges Proskauer (VP) Test

Voges Proskauer or VP test is utilized for the identification of such bacteria those are capable of fermenting of 2, 3- butane diol in mixed-acid fermentation. It is a buffered broth of glucose and peptone. Microorganisms that have the ability of glucose fermentation release acid in the medium. Methyl red, an added coloring agent present in the system becomes red due to decrease in pH.

Medium composition for this test: Voges Proskauer (VP) media (g/L of distilled water) are casitone; 3.5, peptone; 3.5, dextrose or glucose; 5, potassium phosphate; 5, pH~7.0. Isolated culture was inoculated in the media and then the test tubes were kept in incubation at 37°C for a period of 24 to 48 hrs. 5 drops of methyl red was added after this period is over.

Test tubes were also shaken gently and let themto stand for 10 to 15 minutes and it was observed for color change. Positive test is indicated by a pink-red color developing in a few minutes and no color formation is an indicative of a negative result.

Response Surface Methodology

Response Surface Methodology (abbreviated as RSM) is a Statistical tool used in order to optimize responses, coming out from the experimental methods done in the laboratory. It is also used as a tool for the validation of the experimental results. Different software has been used for this purpose. Design Expert and Minitab are some of the examples of these softwares. All of these softwares analyze the input variables or the independent variables and give us the optimized response or the dependent variables in a statistical manner. In some cases these software also take help of the modern state of the art technologies like artificial intelligence. These softwares produce various two dimensional and three dimensional plots by analyzing independent input variables/data. Some parameters like R squared and adjusted R squared values ensure the accuracy of data fit, some of the parametric values like 'p' or probability values are the indicators of the viability or the significance of the obtained data individually or the model a a whole. Different 2D plots also give us ideas of accuracy and viability of the models. 3D diagrams are mainly the indicators of relationship between the explained and unexplained variables and also give the idea of zone of stability as well as sensitivity and zone of robustness regarding the correlation of different dependent and independent variables. Standard deviations or "S" values provide the deviations or the dispersed characteristics of the obtained data in several ways like scattered plots.

Residual values were also calculated by subtracting the experimentally obtained data from the model predicted data and these residual values were compared with standard values or ranges. These values must be very small in case of a model of "Good fit".

All of these parameters in a combined manner ultimately indicate the suitability and appropriation of the experimental research work by endorsement or rejection.

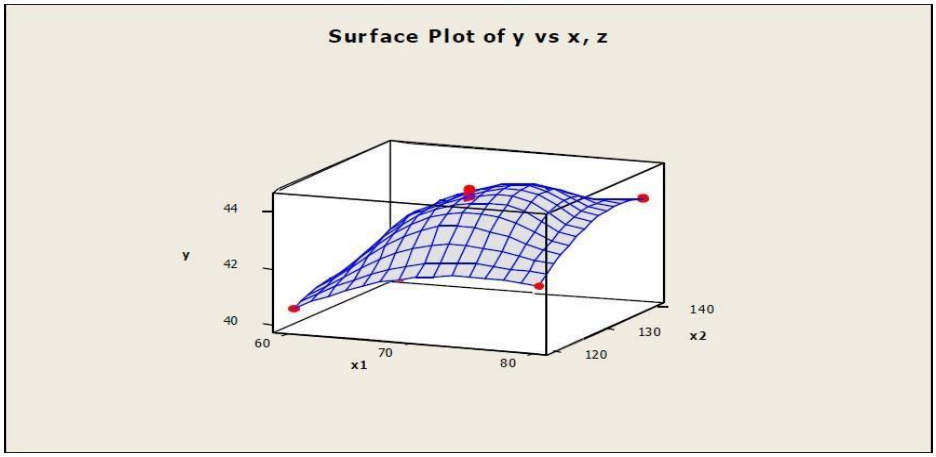


Figure 1. 4 Response surface plot

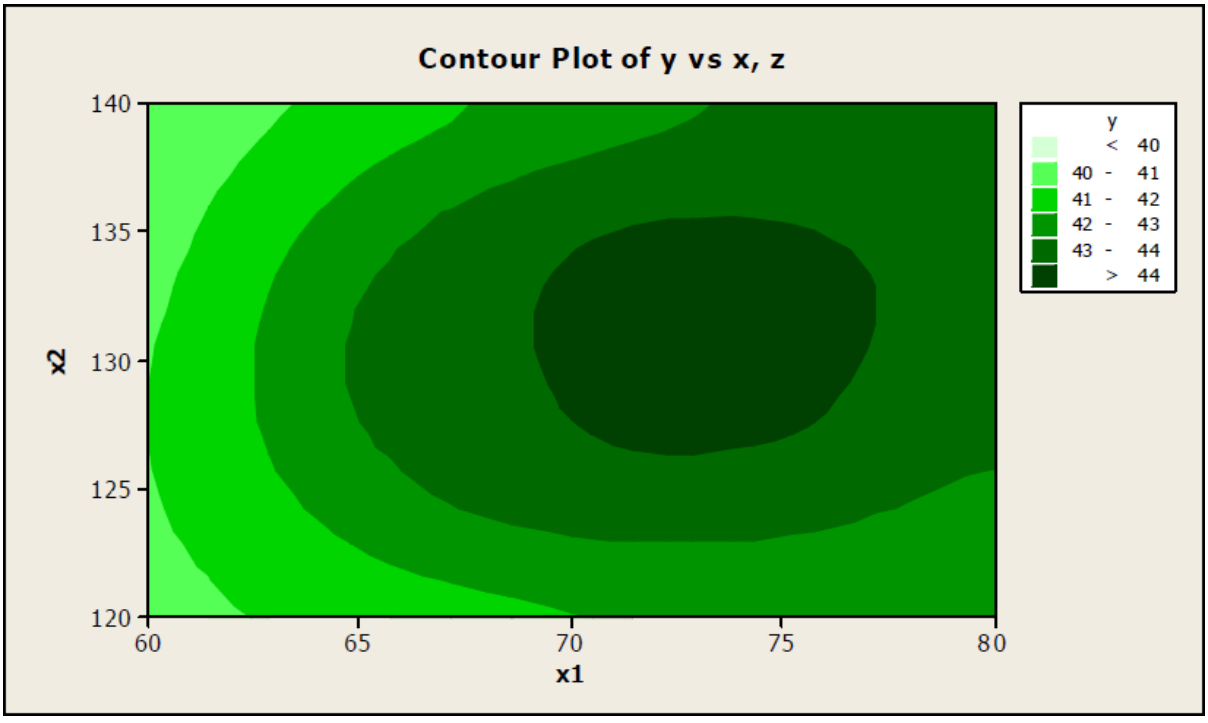


Figure 1.5. Contour plot

In the Fig. 1.4. and 1.5. X and Z and X1 and X2 axis represent independent input variables and Y axis represent the Response variable.

Chapter 2

Biological treatment of dairy industry wastewater in a suspended growth batch reactor: performance evaluation and biodegradation kinetics

Introduction and Literature Review

In many countries, dairy-industry is recognized as one of the biggest wellsprings of food processing wastewater which produces around 0.2-10 L (Raghunath et al. 2016) of waste water per liter of milk processed.Dairy industries are the major pollution generating industries among the food sector. As thedemand of milk is increasing rapidly, dairy industries in India are also growing at a fast rate andthe production of waste and related environmental issues are also increasing steadily. Productionof milk in India has boomed significantly in the last few decades from a volume of a million tonto 110 million tons within 1951 and 2009 (Chawla et. al. 2009, Sharma and Gulati 2003) Untreated or partially treated sewage with elevated level of toxins can cause major environmentalhazards when released into the environment. Dairy wastewater can contribute high organic load tothe wastewater. In addition to these cleaning of plant, such as Cleaning in Place system (i.e. CIP)results in caustic wastewater. Dairy wastewater contains highly putrescible organic constituents(Raghunath et al. 2016). Volatile Fatty Acids are one of major causes of odor pollution. (Page et al. 2014) This is the main reason why industries have to treat dairy wastewater adequately before discharging it to the environment.Majority of the constituents in the dairy wastewater is biologically degradable and hence thewastewater should be treated biologically, either aerobic or anaerobic.

Throughout the process chain of the dairy industry affect the environment (Strydom et al.1993). Majority of dairy industries consider treatment of wastewater as a burden on their economy and so think of it as a futile investment. (Robinson 1997); In general, they produce whitish to off white color of wastewater containing different types of carbohydrates, proteins, fats and other biological materials. Dairy industry wastewater characteristics vary between industries according to their

products and processing technique (Vidal et al. 2000). Correct information about the true composition of chemicals present in dairy industry wastewater is scarce (Donkin 1997). Raw Milk has a Biological Oxygen Demand (BOD) almost around two hundred and fifty times larger than that of wastewater (Burton 1997). Dairy wastewaters having comparatively high organic contents, of which the main contributors are milk sugar or lactose, fats, and milk protein (mainly casein), and is rich in elevated levels of nitrogen (Vidal et al. 2000,Danalewich et al. 1998). As dairy wastewater is full of organic pollutants, they have the potential to increase the BOD and SCOD of the water body where discharged which can create oxygen depleted environmental conditions and force the aquatic creatures to die off due to anoxia. Nutrient like nitrate and phosphate are also a major concern. Cleaning-In-Place system (CIP) in those dairy plants uses mostly phosphate containing detergents which ultimately end up in the aquatic body. The cleansing chemical agents used frequently for this purpose are lye or caustic soda, HNO_3, H_3PO_4, and NaClO or sodium hypochloride; all of those have impacts on environment. If the discharged water is not treated adequately, it goes into the aquatic system and can lead to eutrophication in lakes and ponds. Eutrophication is a condition when the nutrient level mainly Nitrogen and Phosphorus in the system becomes very high. This can causes algal bloom and that ultimately leads to an anoxic condition due to the death and decomposition of algae.So, the dairy wastewater is required to be treated before discharge. The properties of the sewage sludge that comes out from the dairy industries has its typical temperature and color, a pH of around 6.5 to 8.0, high BOD and SCOD, dissolved solids; measured by TDS, suspended solids measured by TSS, presence of high amounts of nutrients like nitrogen and phosphorus along with residual oil and grease. Characteristic of dairy wastewater is also a factor of the amount of milk processed and type of dairy product produced. The wastewater having ample amounts of milk constituents like casein, inorganic salts, alongside cleaning and washing agents like sanitizers and

detergents. It also has large quantities of sodium (Na) as its component which comes from the usage of caustic soda (NaOH) or lye used for cleaning purpose (Ferguson 1996)

The organic part of the wastewater from dairy farms can be classified as proteins, carbohydrates; majority of which is the sugar lactose and fat. These compounds have a harmful and negative impact on the water stream where it has been discharged; depending on their biodegradability and solubility. The organic material in the waste will lower the dissolved oxygen in the aquatic system which will lead to the higher biological and chemical oxygen demand in the system and that will ultimately lead to the death of aquatic plants and animals. Nutrients like nitrate and phosphate in the wastewater can cause eutrophication which will trigger algal bloom and when these algae die microbes in the water decompose them and doing that consumes a lot of Dissolved Oxygen (DO) in the water. This depletion of oxygen ultimately perishes the aquatic lives.

The effects of these toxic wastes on lands including those used for irrigation purposes were neglected. The toxic elements of these wastes can make the productive agricultural land unfertile along with creating other problems like vision pollution, aesthetics etc. So, the dairy industry wastewater should be treated in such a way so that it can meet the standard for discharge mentioned by regulating authorities.

Though various methods are available there for the remediation of dairy wastewater, biological method using microorganisms have gained more importance and have been proven to be the most efficient treatment technique for the dairy wastewater (Chaubey 2002). It was found that by activated sludge process with a batch contact time of 24hrs, 70% BOD_5 (5days Biological Oxygen Demand), 71% SCOD and 61% VOM (Volatile organic matter) could be removed from dairy wastewater (Naser et al. 2014). Similar effluent when treated in trickling filter, it could remove VOM by 90%.When the dairy wastewater was treated in activated sludge system removal

efficiencies of 99%, 81% and 93% for BOD, SCOD and TKN (Total Kjeldahl Nitrogen) respectively was obtained (Carta-Escobar et al. 2005). Investigation was carried out on the treatment of a typical dairy Wastewater by a bench-scale aerobic sequential batch reactor (SBR) and it was found that the SCOD removal efficiency was more than 90%at 8hr cycle time which demonstrated the ability of SBR to treat the dairy wastewater in terms of SCOD reduction (Mohseni- Bandpi et al. 2004). In the year 2004 another investigation was done in which settled dairy effluent was treated in a bench scale Continuous Flow Stirred Tank Reactor (CFSTR) without any solid recycle keeping cell residence time (θ_c) varying from 1 to 6 days (Venkatesan et al. 2004).The percentage of BOD removal they obtained in the range from 67-90 %. Another research team (Lateef et al. 2013) performed a kinetic study on dairy effluent treatment by activated sludge process operated for 3 months by varying batch contact time for 2-12 days. In another investigation it was observed the treatment of simulated dairy wastewater or SDW in terms of SCOD and TKN by using SBR in 24hr (Kushwaha and Srivastava 2013).Bacterial communities isolated from Mother Dairy ETP activated sludge was analyzed and *Proteobacteria sp.* of bacteria was isolated in predominance. This isolated bacteria along with other three *Bacillus sp*. can reduce nitrate contentof 93% within 20hr and 82% in immobilized condition in 96hr (Biswas et al. 2019). A synthetic wastewater was treated in fluidized bed bioreactor and SCOD removal of 84% and BOD removal of 75% was found at 30 °C, pH 7 and incubation period of 120hr (Purushothaman and Jena 2020) With the view point of above, a research investigation has been undertaken in the present study to evaluate the performance of a laboratory-scale SGBR for the treatment of soluble organic carbonand ammonium nitrogen with Carbon oxidation and nitrification kinetics using simulated dairy wastewater by development of acclimatized seed of mixed culture in laboratory condition. Kinetics study in batch reactor was done to find biokinetic coefficients (Y, Ks, k_d and k). The study also

includes isolating the predominant bacterial species from acclimatized sludge by morphological, physiological, biochemical examinations along with 16S rDNA study.

Materials and Methods

Collection and characterization of sample wastewater

Wastewater samples were taken from a nearby dairy plant processing unit located at Mother Dairy Kolkata located at Dankuni, Hooghly, West Bengal (W.B), India. Samples were collected from inlet of the aeration tank in 2liter polythene bottles and brought to the laboratory of Department of FTBE, Jadavpur University. It was subsequently kept at 4°C in the refrigerator for further analysis of quality parameters like pH, TSS, TDS, DO, SCOD, BOD_5 at 20º C, N_4H^+- N, phosphorous. Determination of analytical parameters was done by using standard methods (Rice et. al, 2012). For every parameter athree-set replicate was made.

Study with Bacterial consortium

Acclimatization of seed for carbon oxidation and nitrification

Acclimatization of microbial population seed under laboratory condition of both Carbon oxidation study and nitrification was done in a measuring cylinder unit of one liter capacity. Two separate arrangements of microbial seed acclimatization program were made to develop a consortium culture of carbon oxidizing and nitrifying bacteria. Culture of the microbial seed population under ambient condition in the lab settings was started by inoculating a sludge of 75mL; collected from a stabilization pond of Mother Dairy Calcutta unit located at Dankuni, Hooghly District in West Bengal, India, to a growth propagating diluted Carbon oxidation medium (1:4 dilution) of 800 mL composed of (g/L) Milk powder: 1.00, Dextrose: 0.50, Peptone: 0.25, Beef extract: 0.25, Lactose: 0.25, Di hydrogen potassium phosphate: 0.20, Di potassium hydrogen phosphate: 0.20 and 25 mL of trace elements solution in the above 1L cylinder. The trace element solution was made up of following compounds in g/L, EDTA: 0.01, $ZnSO_4.7H_2O$: 0.001, $CaCl_2.2H_2O$: 0.1, $MnCl_2.2H_2O$: 0.008, $FeCl_3.6H_2O$: 0.71, $(NH_4)_6Mo_7O_{24}$: 0.0011, $CuSO_4.5H_2O$: 0.001 and $CoCl_2.6H_2O$: 0.2. Two aquarium pumps were used to provide aeration by diffusing air into the culture medium system. Dextrose (D-Glucose) was supplied intermittently as a carbon source. The process of acclimatization was carried out for a period of around two months. Growth of biomass was observed by the amount of sludge volume through sludge volume index or SVI and Mixed Liquor Suspended Solids (MLSS) concentration. pH was kept

fixed within the range of 6.8 to 7.0 by adding phosphate buffer and sodium bi carbonate as per requirement.

Similarly in the case of nitrification study, 75 mL of concentrated biomass including nitrifier seed collected from dairy treatment plant was separately cultured under ambient condition in the laboratory to a growth propagating diluted nitrification medium (1:4 dilution) of 800 mL composed of (g/L)Milk powder: 0.05, Dextrose: 0.02, Peptone: 0.05, Lactose: 0.05, Ammonium sulphate: 0.25, Ammonium chloride: 0.25, Potassium nitrate: 0.05, Di hydrogen potassium phosphate: 0.20, Di potassium hydrogen phosphate: 0.2 and 25 mL of nutrient solution of trace elements in the 1L measuring cylinder. Aeration was made continuously by diffusing air within the system with the help of aquarium pumps.During acclimatization process pH in the medium was kept fixed in between 7 and 7.5 by adding required amount of sodium bi-carbonate ($NaHCO_3$) solution. This acclimatization process was carried out for a tenure of around two months. Growth of biomass in the system was measured through SVI and MLSS concentration having nitrifiers as predominant species.

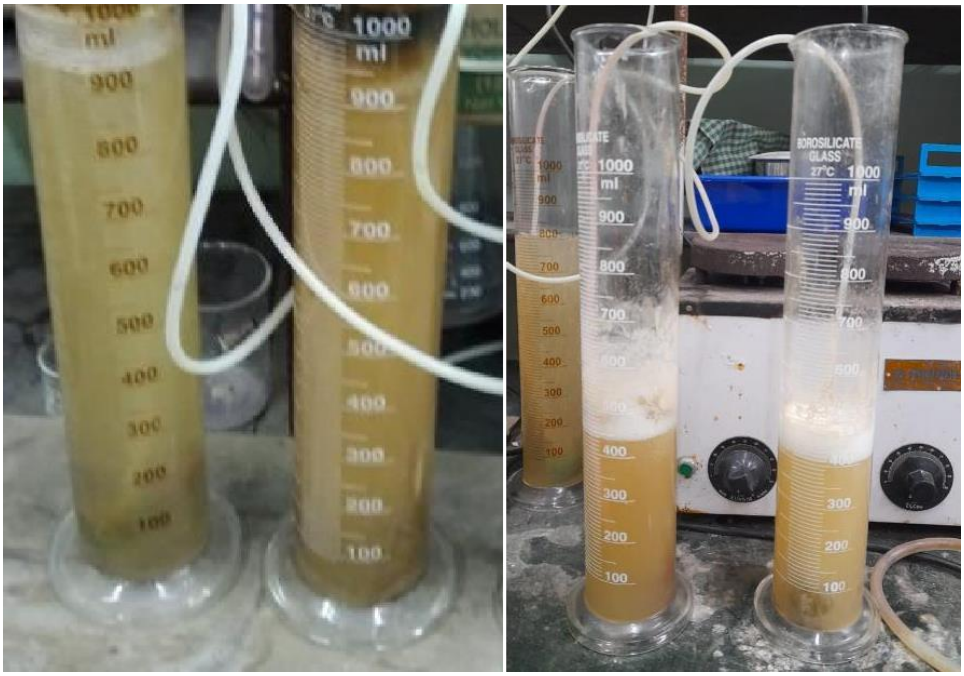


Fig. 2.1. During the process of Acclimatization

Experimental setup and procedure for treatment of dairy wastewater in batch reactor

Studies of batch kinetic with simulated dairy wastewater for carbon oxidation and nitrification have been done separately in measuring cylinder of 1 L capacity. Sample volume taken in each cylinder was around 800 mL with necessary feed solution. The feed solution was composed of

(g/L) Lactose: 0.50, Milk Powder: 1.00, Dextrose: 0.50, Peptone: 0.50, Beef extract: 0.25, Yeast extract: 0.25, Potassium nitrate: 0.60, Ammonium sulphate: 0.35, Ammonium chloride: 0.70, Di potassium phosphate: 0.20, Di hydrogen phosphate: 0.20. In each cylinder aeration was provided by injecting air through stone sparger fitted with lab scale compressor. The sludge volume was determined by the graduation mark in the cylinder for more convenient manner.

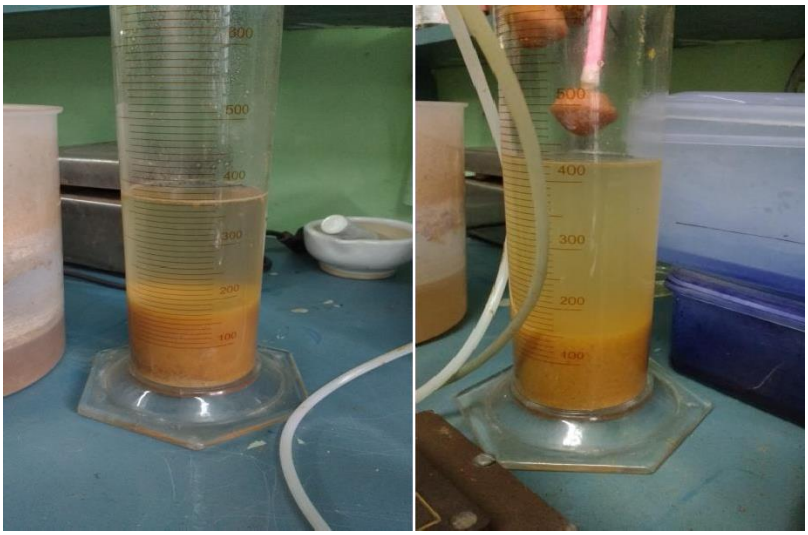


Fig. 2.2. Settled biomass after a 20 minutes of settling period

Carbon oxidation study in batch reactor

Inoculum concentration containing mixed carbonaceous sludge is considered to be one of the important parameters for optimization of performance of batch reactor for carbon oxidation. In the present study effect of initial inoculums concentration i.e. 4%, 6%, 8%, 10%, 12% and 14% was examined for simulated dairy wastewater having a constant initial SCOD concentration of 700 ± 50 mg/L. Samples were withdrawn after 24 hr of reaction time and the organic carbon removal rates were measured. The organic carbon (SCOD) removal was found to be reduced beyond the respective 10 % (v/v) inoculum levels for afore-said initial SCOD concentrations. After that time concentration study of simulated dairy wastewater was conducted using acclimatized seed for organic Carbon oxidation containing SCOD of 680 mg/L and 720 mg/L in a suspended growth batch reactor. Time concentration study is the study of reduction of substrates like organic carbon, Nitrogen and Phosphorus with respect to time. Throughout the time period of the study of Carbon oxidation, a sample of 25 mL were withdrawn from the batch reactor at different time interval (2, 4, 6, 8, 16, 18, 22, 24 hrs) and examined the SCOD removal efficiency and MLSS

concentration.

Nitrification study in batch reactor

Inoculum concentration containing nitrifier sludge is considered to be an effective parameter for optimization of performance of batch reactor for nitrification. In the present nitrification study effect of initial inoculum concentration was examined for simulated dairy wastewater with a constant initial ammonium nitrogen concentration of 47.25 ± 5mg/L. Samples were withdrawn after 24hr of reaction time and the ammonium nitrogen removal rates were measured. The ammonium nitrogen (NH_4^+- N) removal was found to be reduced beyond the respective 4 % (v/v) inoculum levels for afore-said initial NH_4^+- N concentrations. After optimization of initial inoculum concentration for nitrification, time concentration study of simulated dairy wastewater was conducted using acclimatized seed for nitrification with initial Ammonium nitrogen concentration of 54 mg/L & 48 mg/L in a suspended growth batch reactor. Throughout the time period of the study of nitrification, a sample of 25 mL were withdrawn from the batch reactor at different time interval (2, 4, 6, 8, 16, 18, 22, 24 hrs) and the NH_4^+- N removal efficiency and MLSS concentration was examined.

Carbon oxidation and nitrification kinetics in batch reactor

A reactor's performance can be assessed properly by evaluating its reaction kinetics. By conducting experiment, different values for kinetic coefficients for heterotrophs and nitrifiers are estimated as steady state kinetics for stabilization of organic carbon, ammonium nitrogen (NH_4^+-N). Kinetic coefficients viz. yield co-efficient (Y), endogenous decay co-efficient (K_d), half velocity constant (Ks) and maximum Substrate utilization rate (k) were obtained on the basis of aforesaid experiments by varying Mixed Liquor Suspended Solid (MLSS) concentration.

k is the maximum Substrate utilization rate per unit mass of microorganisms. This value of k has a particular significance. It's value deals with the volume of reactor. The value of k is inversely proportional with the volume of reactor. That is greater the value of k; little the volume of reactor would be needed (Benefield and Randall 1980). K_s is known as Half velocity constant. It is dimensionally similar to the concentration of substrate. Generally, its value bears no practical significance. It is more of theoretical nature. Y is the Biomass Yield coefficient. Its value indicates

the quantity of sludge produced by utilizing the substrate and lastly K_d is the decay coefficient which denotes the death or decay rate of the microorganisms in the system and the greater the value of K_d; lower will be the presence of net activated sludge (Lateef et. al. 2013).

Kinetic study was done on the basis of Monod kinetics model, both for carbon oxidation study and study of nitrification. In the case of carbon oxidation study the inverse of specific Substrate utilization rate i.e.$1/U_C$ were plotted against the inverse values of SCOD concentration (1/C) and the kinetics for removal of substrate was found using a simple linear equation which is in the form of y = mx + c where m being the straight line's slope and c being it's Y - intercept.

$1/U_C = (Ks/k)(1/S) + 1/k(1)$

A straight line of best fit was then drawn statistically by using a least square method using data obtained experimentally. On the other hand, Microbial-growth kinetics was prepared from the equation

$1/\theta = YU_C - k_d$ (2)

In case of nitrification study the inverse values of specific Substrate utilization rate i.e. $1/U_N$ were plotted against the inverse values of ammonium nitrogen concentration (1/N) and ammonium nitrogen (NH_4^+- N) reduction kinetics were prepared with the help of equation

$1/U_N = (Ks/k)(1/N) + 1/k$ (3)

$1/\theta = YU_N - k_d$ (4)

A straight line of best fit was then drawn statistically using experimental data.

Isolation, identification and characterization of predominant bacterial species from mixed sludge containing carbonaceous bacteria and nitrifier

Materials and Methods

Serial dilution or step-wise dilution of a solution causes dilution of concentration of the solute present in that specific solution in a manner of Geometric Progression or GP. In general, dilution factor or DF at each step is constant. Sample taken from experimental batch reactor containing carbonaceous sludge was spread on nutrient ager medium and kept in incubation at 37º C for 24hr. The composition of NA medium was (g/L)- peptone:5.0, beef extract: 3.0, Agar: 30.0, pH 6.8 - 7.2.To identify the most potent carbonaceous bacteria, cultures of selected six (BC 1 – BC6) well developed isolates were transferred on the individual slant. To select the most potent strain the culture was transferred into nutrient broth medium and incubated at temperature of 37º C for 24 hr. Inoculums of the isolated bacteria (10 % v/v) was added to the simulated dairy wastewater sample and cultivated by shaking at 120 rpm at 37º C. Samples were taken out from each flask after a period of 24 hr, then centrifuged at 5000 rpm (REMI, R-8C Model, RCF: 3600g) for 15 min at 4º C and finally the supernatant solution was tested for SCOD removal. Among the six isolates, the one (isolate BC 5) shows the maximum SCOD removal efficiency.

In case of nitrification study 1mL acclimatized sludge containing nitrifier was taken and mixed with 9mL of distilled water in a test tube, thereby diluting the sample 10^{-1} times. Then a series of dilution of the sample was carried out up to 10^{-6} dilution by taking 1 mL sample from each diluted sample and diluting it 10 times with distilled water. Then, 1mL solutions was taken from the 10^{-6} diluted sample and spread on nutrient ager medium (NA) and was incubated at 37ºC for 24hr. After 24hr, growth was observed on the NA plates. The isolated colonies were transferred into a separate Nutrient Agar (NA) plate medium and incubated at 37ºC for 24hr. Nitrifier cultures of selected as five (BN 1-BN 5) well developed isolates were grown on NA medium, taken into nutrient broth medium and incubated for 24 hr at temperature of 37º C. Inoculums of the isolated nitrifier (4% v/v) was added to the stimulated dairy wastewater sample and cultivated by shaking at 120 rpm at 37º C. Samples were withdrawn from each flask after a time period of 24 hr, then centrifugation was performed in a laboratory grade centrifuge of the brand “Remi” at 5000 rpm (REMI, R-8C Model, RCF: 3600g) for 15 min at 4º C and finally the supernatant solution was

tested for NH_4^+- N removal. Among the five isolates, the one (isolate BN 3) shows the maximum NH_4^+- N removal efficiency.

Study of identification of the isolated culture was conducted in lab by performing different biochemical tests following standard protocols (Holt et al. 1993) and 16S rDNA study (Weisburg et al. 1991) of the isolated culture.

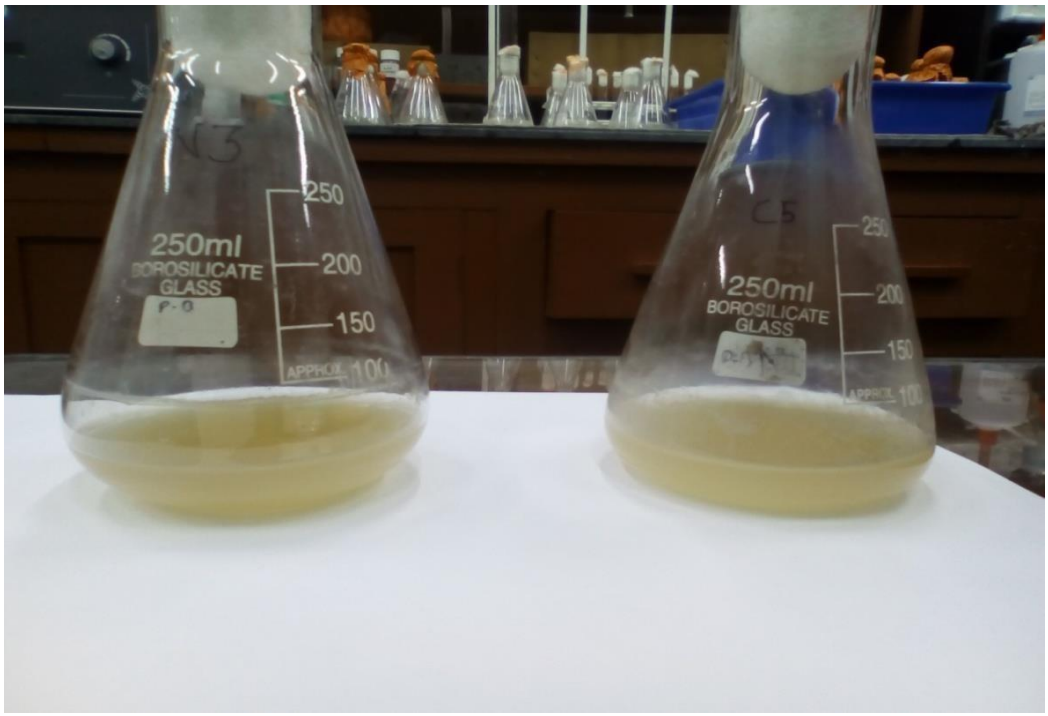


Fig. 2.3 Isolated most potent bacterial strains

Evaluation of removal kinetics by isolate of BC 5strain

Carbon oxidation study of the isolate BC 5was assessed batch wise in the aforementioned stimulated dairy wastewater sample. The sterilized media were inoculated with the isolate BC 5 (10 % v/v) and incubated with shaking at 37º C for 24 hr. Aliquots were removed from each flask at the interval of 2, 4, 6, 8, 16, 20, 24hrs and centrifugation was done in a laboratory grade centrifuge at 5000 rpm (REMI, R-8C Model, RCF: 3600g) for 10 min after which the supernatant solution was tested for SCOD and MLSS.

Evaluation of removal kinetics by isolate of BN 3strain

Batch wise removal of ammonium nitrogen by the isolate BN 3wasassessed in the aforementioned stimulated dairy wastewater sample. The sterilized media were inoculated with the isolate (4% v/v) and incubated with shaking at 37º C for 24 hr. Aliquots were removed from each flask at the interval of 2, 4, 6, 8, 16, 20, 24 hrs and centrifugation was performed in a laboratory grade centrifuge at 5000 rpm(REMI, R-8C Model, RCF: 3600g) for 10 min. The supernatant solution was tested for ammonium nitrogen and MLSS.

Results and discussion

Characterization of real life dairy wastewater

Wastewater samples were characterized in the FTBE laboratory, JU and the findings are displayed in Table 2.1. SCOD concentration of raw dairy effluent lies within the range of 700 ± 50 mg/L. SCOD of dairy wastewater generally remains high because of elevated levels of biodegradable organic compounds present in it. About 80 % of SCOD and BOD loadings are caused due to lactose. Dairy wastewater has a high soluble BOD and SCOD portion, indicates high biodegradability. $BOD_5$of the wastewater was around 440 ± 30 mg/L which is also high due to the presence of large amount of biodegradable organic components in wastewater. The ammonium nitrogen and phosphorus concentration were observed in a range of 47.25 ± 5 and 18 ± 2 mg/L, respectively. Ammonium nitrogen and phosphorus concentration was quite high in the dairy wastewater as milk protein contains a lot of nitrogen in its molecular structure and

phosphorus that mainly comes from the different cleansing agents used during cleaning process or CIP operation. The average pH and Total dissolve solid (TDS) of real-life wastewater sample were found to be 8.28 and 565 mg/L respectively. Temperature of wastewater during the time of sampling was in between 25 and 32°C.The major reason behind high TDS value might be due to the presence of various ions; in general, which are part of different disinfectants and sanitizing chemical agents used while cleaning operation namely Cleaning in Place system or CIP has been done. Sanitizers and fertilizers contain different compounds which can contribute to Nitrogen, phosphorus, Potassium, calcium etc. which can elevate the level of ionic concentration and Total Dissolved solids (TDS) upon dissolving in the final wastewater. As the raw effluent passes through the chemical treatment, a large portion of colloidal matter has been reduced due to the chemical coagulation and sedimentation. The pH values indicated the effluent was alkaline in nature when it entered to the inlet chamber of ETP due to the discharge of alkaline cleaning solutions, pH was found to be decreasing in nature after pre-treatment because of alum addition in primary sedimentation tank.

Table 2.1: Characterization of Dairy wastewater

Parameters	Set I	Set II	Set III	Set IV	Mean
pH	8.0	8.2	8.5	8.4	8.28±1
Dissolved Oxygen (mg/L)	3.2	3.5	3.9	3.0	3.4±0.5
SCOD (mg/L)	720	700	680	700	700±50
BOD_5 (mg/L)	450	440	440	430	440±30
NO_3^--N (mg/L as N)	22	24.5	21.2	25	23.18±5
NH_4^+-N (mg/L as N)	45	48.2	46.3	49.5	47.25±5
Total Phosphorus (mg/L as P)	20	16	18	18	18±2
Total Dissolved Solids (mg/L)	550	560	550	600	565±32
Total Suspended Solids (mg/L)	300	290	280	300	292.5±25

Study with Bacterial consortium

Study of Carbon oxidation of dairy wastewater in batch reactor

An inoculum concentration of 10 % (v/v) for carbon removal was found to be optimum in time-concentration study in batch reactor. Maximum 97 % SCOD removal was achieved after 24 hrs corresponding to inoculums concentration of 10% v/v. Removal potential of different percentage of inoculums was shown in Fig. 2.4. From the figure it had been also found that the SCOD removal percentage was increased from 4 to 10 % v/v inoculums, after that SCOD removal percentage decreased due to substrate limitation. The MLSS concentration for SCOD removal of 1050 mg/L at 10 % v/v inoculums was found to be optimum for maximum SCOD removal corresponding to an initial SCOD of 700 - 720 mg/L after 24 hr contact time. This is probably due to the fact that the Food to Microorganisms ratio or F/M ratio was being optimized at or near this specific MLSS of 1050 mg/L (corresponds to 10% v/v innoculum). F/M ratio is the ratio of the substrate (in terms of BOD or SCOD) and microorganisms (in terms of MLSS or MLVSS) which has an optimum range of around 0.2 to 0.6 depending on various other factors and process parameters. It is the ratio which determines the rate of degradation of substrate in the presence of microorganism. If the substrate concentration is high enough it is quite natural that the requirement of decomposing microorganisms will be more and vice versa. It is the matter of ratio

and proportion and not the matter of absolute values or quantum of MLSS.

Time concentration study of simulated dairy wastewater for Carbon oxidation carried out in a batch reactor with two different initial SCOD of 680 and 720 mg/L using 10 % (v/v) inoculums concentration. It had been found that the maximum SCOD removal of 98 and 90 % occurred after 24 hr with an initial SCOD concentration of 680 and 720 mg/L, respectively as shown in Fig. 2.5 and 2.6. It was also observed that in both cases, percentage of SCOD removal increased sharply within the first 8 hr contact period there after the rate of removal decreased and the curve becameasymptotic in nature as shown in Fig. 2.5 and 2.6. The treatment could be completed in two major

phases. During the first or assimilation phase, bacteria rapidly consumed organic carbon compounds from dairy wastewater and required oxygen at a high rate (Porges et al. 1957). During the second or endogenous phase, these bacteria did not receive any new organic carbon compounds from wastewater due to limitation of substrate and underwent endogenous metabolism (Hoover et al. 1952). MLSS concentration increased with time from initial MLSS concentration of 1050 mg/L in both cases as shown in Fig. 2.5 and 2.6.

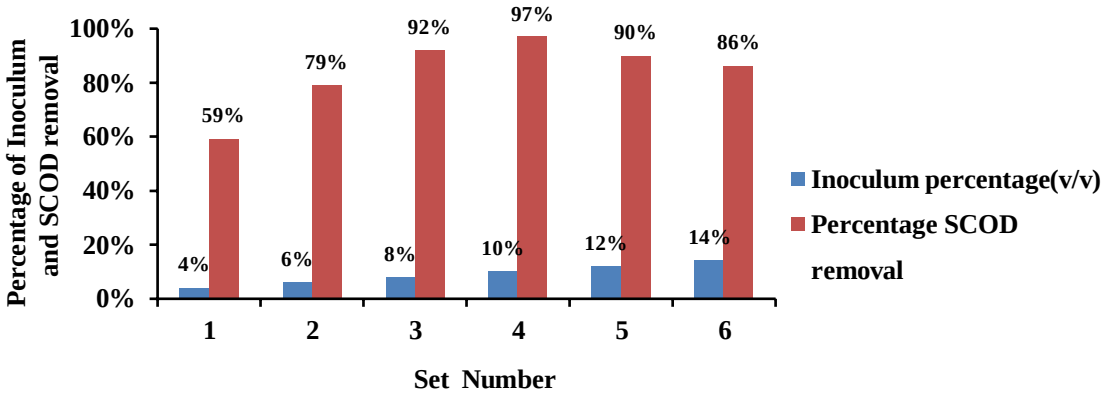


Fig. 2.4. SCOD Removal Efficiency under Different percentage Inoculums

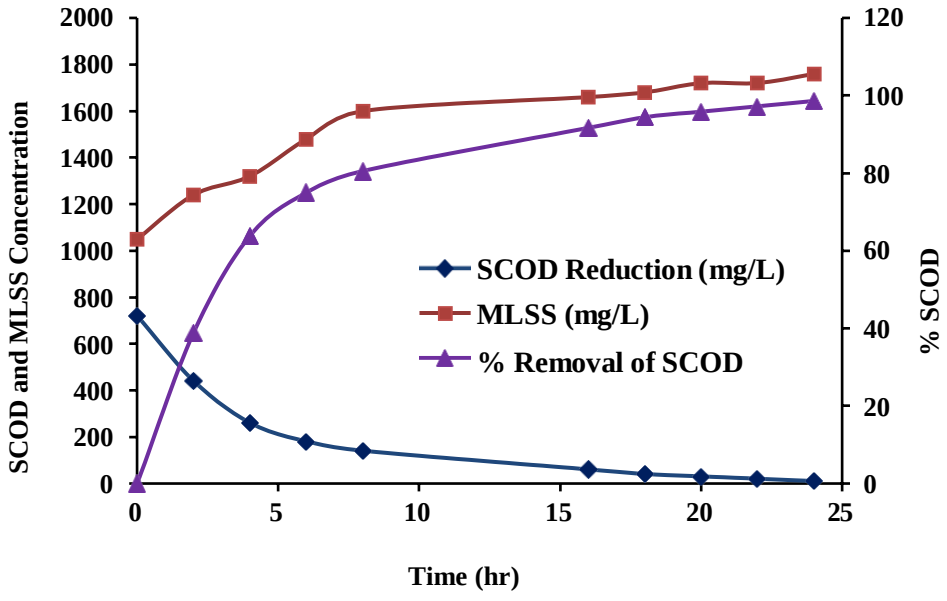


Fig 2.5.SCOD and MLSS Profile during Carbon oxidation Study in Batch Reactor[Initial SCOD Concentration = 720 mg/L]

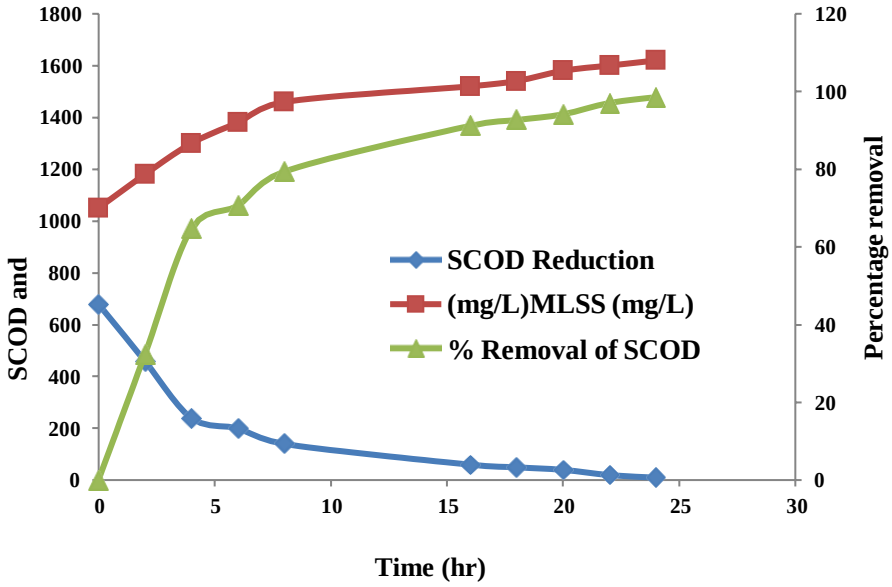


Fig 2.6. SCOD and MLSS Profile during Carbon oxidation Study in Batch Reactor [Initial SCOD Concentration = 680 mg/L]

Nitrification study of dairy wastewater in batch reactor

Removal potential of NH_4^+- N under different percentage of inoculums were shown in Fig. 2.7. From Fig. 2.7 it has been observed that NH_4^+- N removal percentage was increased from 2 to 4 % v/v inoculum, after that it decreased due to limiting concentration of NH_4^+- N in reactor. It had been also observed that 89 % NH_4^+- N was removed after 24 hr contact time corresponding to inoculum concentration of 4 % v/v. The MLSS concentration of 670 mg/L for nitrification at 4 %v/v inoculums was found to be optimum for maximum NH_4^+- N removal with an initial NH_4^+- N concentration of 48-54 mg/L after 24 hr contact time.

The change of ammonium nitrogen concentration with time might be due to assimilatory or dissimilatory uptake of NH_4^+- N. Assimilatory means that the ammonia was taken as nutrient by the heterotrophs for their biomass growth, however dissimilatory stands for uptake of ammonium nitrogen as electron acceptor. During dissimilation NH_4^+- N was oxidized to nitrite and subsequently to nitrate; the two step of ammonia oxidation was termed as nitrification. In the present study the concentration of nitrate was checked along with ammonium nitrogen, it was observed that nitrate concentration was elevated with elapsed of time whereas the ammonium nitrogen was found decreasing trend, signifies that dissimilatory uptake occurred during present

study. The results of the substrate (NH_4^+- N) reduction study with respect to time with varying amount of preliminary concentration of NH_4^+- N; 48 and 54 mg/L as N shown graphically in Fig. 2.8 and Fig. 2.9, respectively. From Fig. 5it was seen that the ammonium - Nitrogen concentration followed a declining trend for an initial value 48 mg/L as N, after 24 hr the concentration was measured in the range of 5.9 mg/L as N. Most of the ammonium nitrogen removed within 8 to 10hr of detention period, corresponding to around 61.85 % removal. From Fig. 2.8 it was also observed that the ammonium Nitrogen concentration decreased from an initial value of 54 mg/L as N, to 7.2 mg/L as N after around 24 hrs. Majority of the ammonium nitrogen was removed within 16 hr of batch contact time, corresponding to 79.17% removal. MLSS concentration for nitrification increased with time from an initial MLSS concentration of 670 mg/Lin both cases as shown in Fig. 2.8 and Fig. 2.9.Around 87.7% of substrate (NH_4^+- N) removal hasbeen observed in case where initial NH_4^+- N concentration was 48 mg/L whereas around 86.6% NH_4^+- N removal has been observed with an initial NH_4^+- N concentration of 54 mg/L after a detention period of 24 hrs. Slightly more removal in the former case is probably due to the lower initial substrate concentration.

In the present study nitrate concentration was increased with time due to nitrification. Both nitrite and nitrate were generated during nitrification but nitrite was unstable and it was subsequently oxidized to nitrate. The increase in nitrate concentration during nitrification was shown in Fig. 2.10 along with reduction NH_4^+- N concentration. Fig. 2.10 showed that initially some nitrate was present as the real wastewater and sludge contained some amount of nitrate; however, accumulation of nitrate could be occurred when the ammonia concentration decreased with time in the reactor. Fig. 2.10 showed the increase of nitrate concentration of 60 mg/L as N after twenty-four hours contact time from an initial nitrate concentration was 24 mg/L as N corresponding to initial NH_4^+- N concentration 54mg/L.

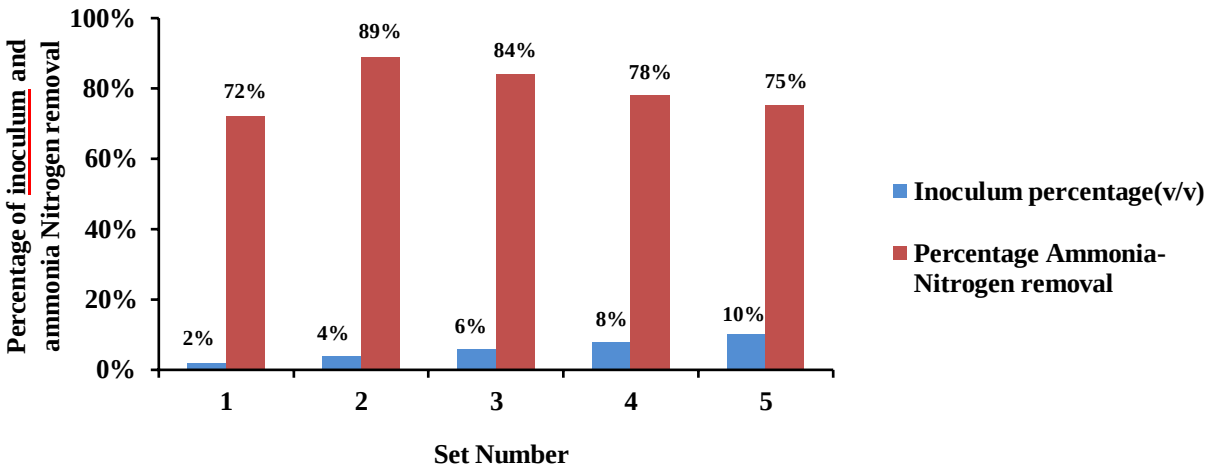


Fig 2.7. NH_4^+-N Removal Efficiency under Different Percentage Inoculums

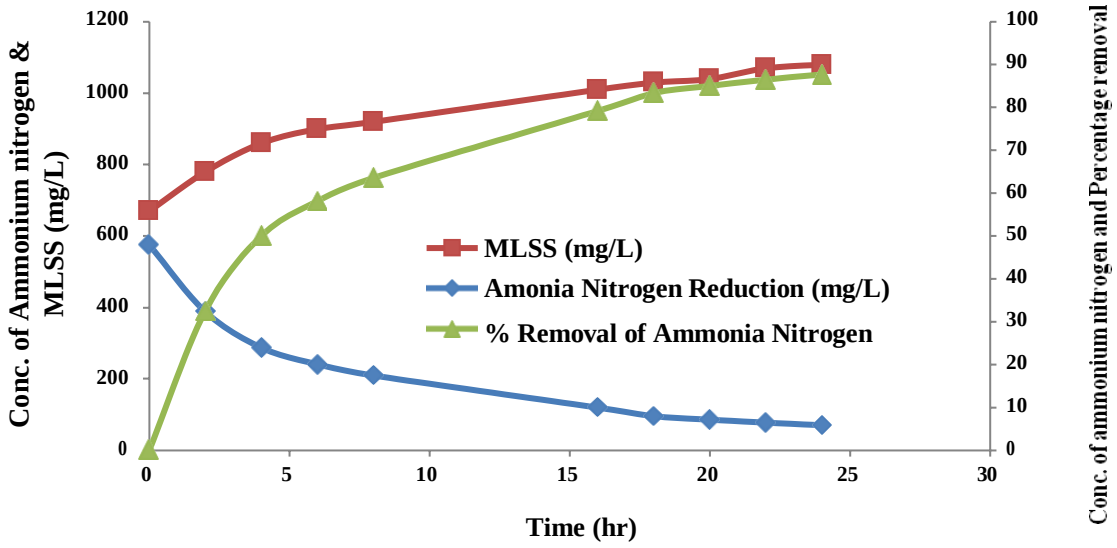


Fig 2.8. NH_4^+-N and MLSS Profile during Nitrification Study in Batch Reactor [Initial $NH4^+$-N= 48 mg/L]

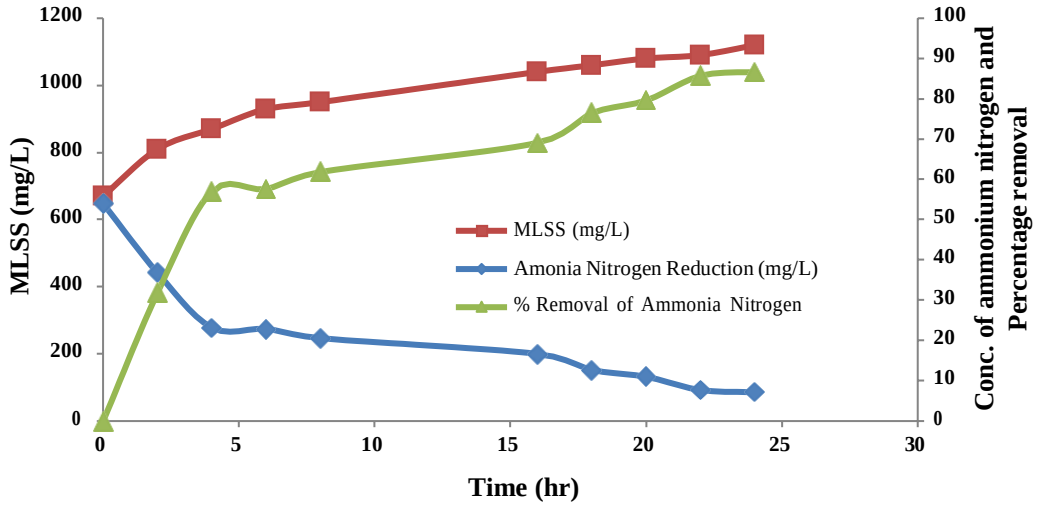


Fig 2.9.NH_4^+-N and MLSS Profile during Nitrification Study in BatchReactor [Initial NH_4^+-N= 54mg/L]

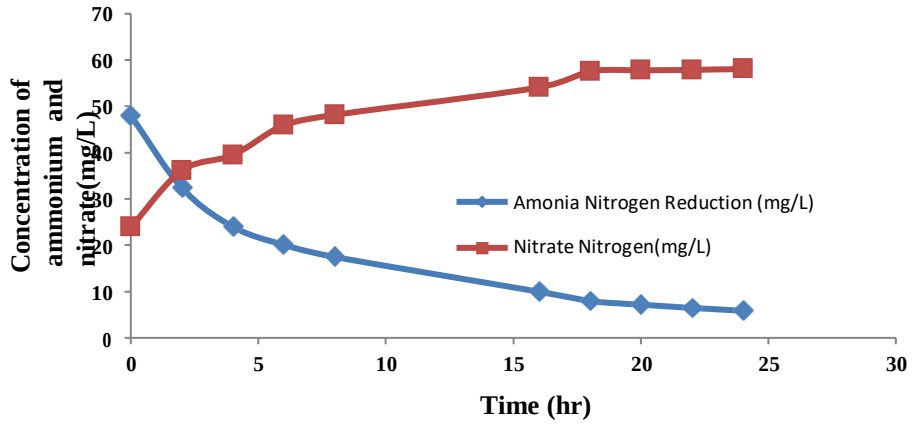


Fig 2.10. Ammonium and Nitrate nitrogen Profile during Nitrification Study in Batch Reactor

Batch kinetic study for Carbon oxidation

The inverse of U_C i.e. $1/U_C$, measured from kinetic study experiments were plotted against the inverse values of effluent Chemical Oxygen Demand or SCOD (1/C) by using simple linear equation(1). A straight line of best fit was drawn by applying statistical method; shown in Fig. 2.11a. The value of k was found to be 4.42 day^{-1}; Ks; 206.81 mg/L for an initial concentration of SCOD 700 - 720 mg/L. The value of K_s; the half velocity constant obtained in this study was to some extent higher than the values stated in the book of Wastewater Engineering (Burton et. al. 2004)probably due to the elevated initial levels of SCOD. In the next stage, values of $1/\theta$ i.e. reciprocal of the reaction time were plotted against U_C data by applying equation (2) which was also in the form of straight line as shown in Fig. 2.11b. Y was obtained from the slope of the straight line and k_d; from the intercept. The value of Y is 0.628 mg of MLSS/mg of SCOD and thevalue of k_d 0.051 per day for SCOD concentration of 700 – 720mg/L

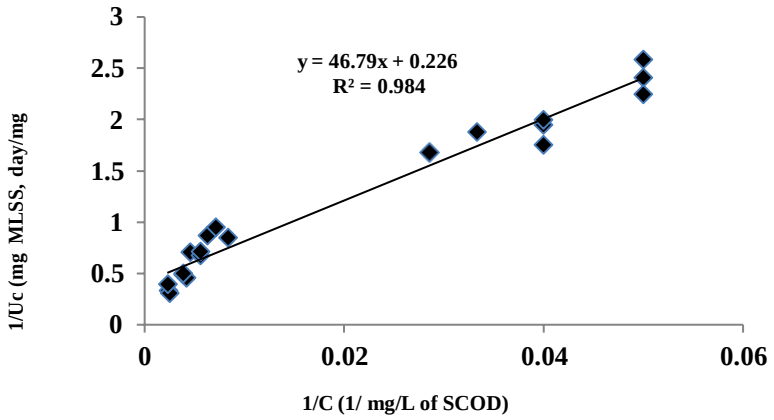


Fig 2.11a. Substrate-utilization kinetics for Carbon oxidation Study in Batch Reactor [Initial SCOD Concentration of 700-720 mg/L]

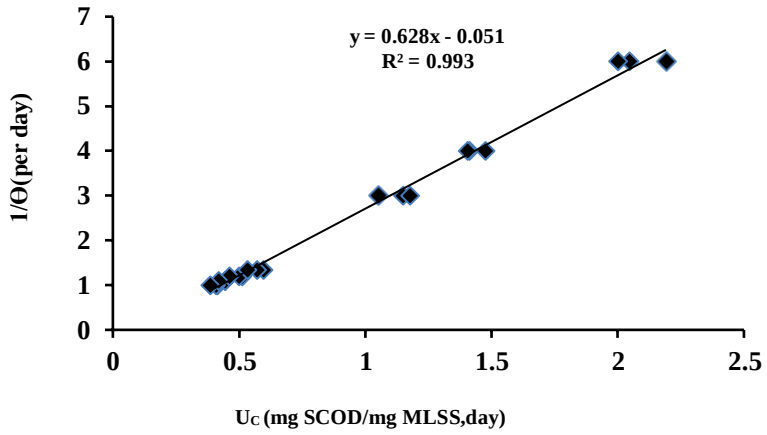


Fig 2.11b. Microbial-Growth kinetics for Carbon oxidation Study in Batch Reactor [Initial SCOD Concentration of 700-720 mg/L]

Batch kinetic study for nitrification

A line of best fit was drawn statistically in a similar manner as done in the case of Carbon oxidation study. Experimental data were plotted in equation (3) as inverse of the specific substrate (NH_4^+-N) utilization rate i.e.$1/U_N$ with the inverse of effluent ammonium nitrogen (1/N) as shown in Fig. 2.11c. The value of k; maximum substrate removal rate was found 2.5 per day and the value of Ks was measured to be 24.5 mg/L from the equation mentioned above for an initial NH_4^+- N level of 48-54 mg/L as N. The value of K_s; obtained in this study was found to be some extent higher than the values mentioned in the book of Wastewater Engineering (Burton et. al. 2004) a reasonable explanation of which might be the higher initial concentrations of the substrate (i.e.NH_4^+- N) inside the batch reactor. Another straight line was drawn statistically by minimizing error terms and applying equation (4) by plotting experimental value of inverse of the reaction time ($1/\theta$) with U_N as shown in Fig. 2.11d. From Fig. 2.11d the values of Y and k_d for nitrification was obtained as the slope and intercept of the straight line; and these values were0.552 mg of MLSS/mg of NH_4^+-N and 0.056 day^{-1}, respectively.

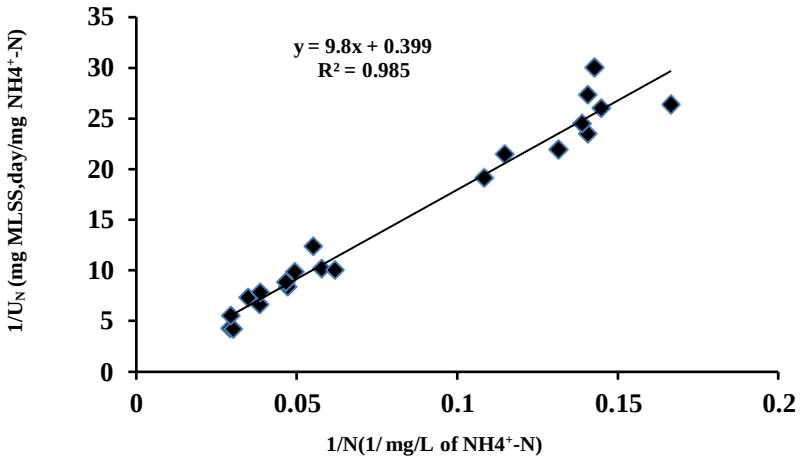


Fig 2.11c.Substrate-utilization Kinetics for batch nitrification study [Initial NH_4^+-N= 48 -54 mg/L as N]

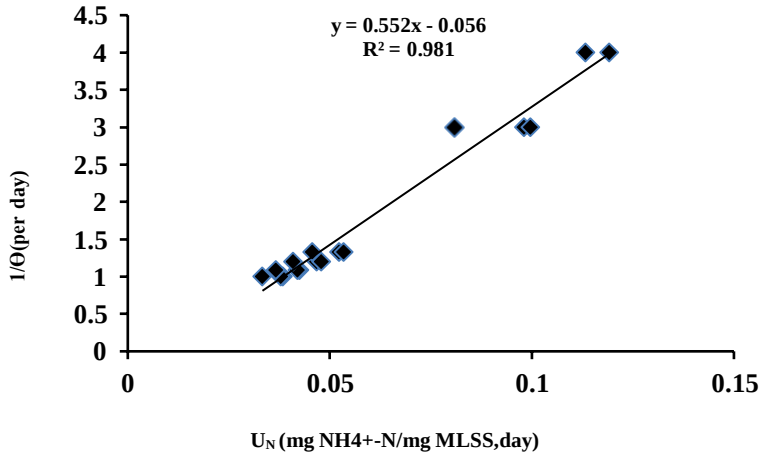


Fig 2.11d. Microbial-growth kinetics for batch nitrification study [Initial NH_4^+-N = 48 -54 mg/L as N]

Isolation, Identification and Characterization of Predominant Bacterial Species from Mixed Sludge Containing Carbonaceous Bacteria and Nitrifier

Results and Discussions

3.6. Isolation and identification of predominant bacterial species from mixed sludge containing carbonaceous bacteria

About six isolates were cultivated. Among six isolates, one with the maximum Carbon oxidation potential has been reported here. Result of the removal potential of six isolates, marked as BC1, BC 2, BC3, BC4, BC 5 and BC6 are shown in Fig 2.12. The isolated BC 5 showed the maximum SCOD removal potential of 93%for an initial SCOD of 700-720 mg/L.

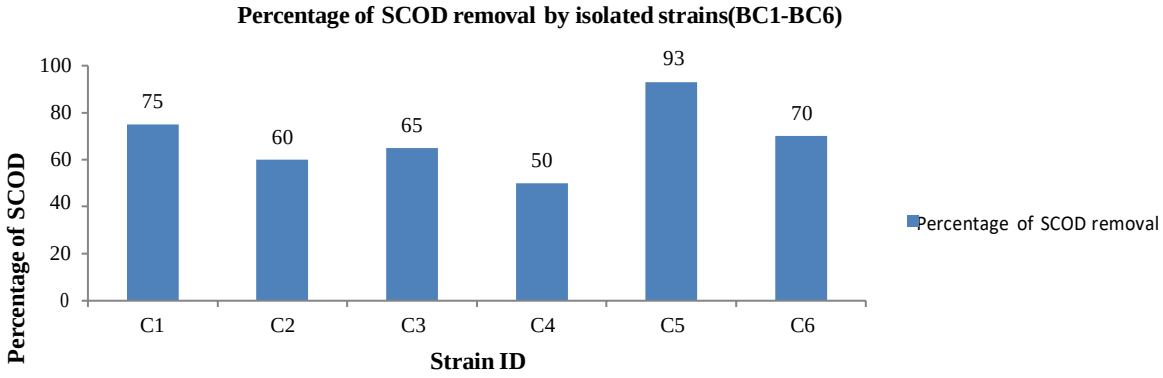


Fig 2.12. SCOD Removal Efficiency of Different Isolate of Carbonaceous Bacteria

Identification of the isolate BC 5predominated in mixed sludge containing carbonaceous bacteria

Morphological and physiological characterization of isolate BC 5

Morphological and physiological characteristics of the strain selected as the most efficient carbon oxidizer (BC 5) were done by standard biochemical test following the standard protocol(Holt et al. 1993).Colonies of pure bacterial culture were found on nutrient agar plates after incubation at 30º C for 48 hr aerobically. Spreading of colonies, diffuse growth emanating from the line of inoculation in motility medium after 24 hr of incubation at 30º C were examined microscopically in wet-mount preparations. Growth in 50 mL nutrient broth medium taken in 250 mL conical flask was observed after incubation in an aerobic environment for three consecutive days. Experimental results of Morphological and Cellular Characteristics of Strain BC 5has been shown in Table 2.2.It

was observed that the culture seed of strain BC 5 secrets a white coloured pigment in the plate. The isolated pure culture of strain BC 5 was identified through Gram staining. It was observed that the cell retained the crystal violet stain only, thereby appeared in blue violet colour indicated that the bacteria was Gram Positive. Different biochemical tests such as Ammonia from arginine, Arginine used as a sole source of energy, Nitrate reduction test, Catalase reaction test, Indole formation test, Litmus milk test, Voges-Proskauer test, Carbohydrate fermentation test, urease test, starch hydrolyses test and few growth tests in different medium with varying initial conditions were carried out and results were given in Table 2.3.

Table 2.2. Morphological and Cellular Characteristics of Strain BC5

Experiment	Observation
Morphology of vegetative cells	(a) Cell shape – Rod (b) Cell size – Moderate
Arrangement	Single, double, multiple
Gram's staining	(+) ve
Spore staining	(+) ve
Motility	(+) ve
Colony characteristics(Size)	Moderate
Opacity	Opaque
Surface growth	Smooth
Edge	Entire
Consistency	Good
Color	Off white
Pigmentation	Nil
Growth in 50 ml medium taken in 250 ml Erlenmeyer flask	**a. Stationary condition** i. After 24 hr. Moderate growth, No ring formation, no pellicle formation, sedimentation at the bottom. ii. After 48 hr. Same as after 24 hr but the growth was more.

	b. Shaking condition: i. After 24 hr Fair growth, turbid, no sedimentation, no pellicle, no pigmentation, ring formation ii. After 48 hr Same as after 24 hr.

Table 2.3.BiochemicalCharacteristics of isolated Strain BC5

Parameters	**Characteristics**	
1. Ammonia from arginine 2. Arginine used as a sole source of energy 3. Nitrate reduction 4. Catalase reaction 5. Indole formation 6. Litmus milk test 7. Voges - Proskauer test pH<6.0 pH>7.0	Positive Positive Positive Positive Negative Positive **Result:** Negative Positive	
8. Growth at anaerobic condition 9. Growth at different temperature 10^oC 30^oC 40^oC 50^oC 10. Growth at different NaCl Concentration 2% 5% 7% 10% 11. Growth at 6.5% NaCl & pH-9.6 12. Growth at pH 6.8 pH 5.4 13. Urease test 14. Starch Hydrolyses test 15. Carbohydrate fermentation	Positive **Result:** Poor Growth Vigorous Growth Vigorous Growth No Growth **Result:** Vigorous Growth Vigorous Growth Poor Growth No Growth Very Poor Growth **Result:** Positive Positive Negative Positive	
	Acidity	Gas Formation

i) Fructose	Positive	Negative
ii) Arabinose	Positive	Negative
iii) Galactose	Positive	Negative
iv) Glucose	Positive	Negative
v) Lactose	Positive	Negative
vi) Raffinose	Negative	Negative
vii) Sucrose	Positive	Negative
viii) Dextrin	Positive	Negative
ix) Salicin	Negative	Negative
x) Sorbitol	Negative	Negative
xi) Mannitol	Negative	Negative
xii) Glycerol	Negative	Negative
xiii) Inositol	Negative	Negative
xiv) Maltose	Positive	Negative

16S rDNA sequence and phylogenetic analysis of isolatedstrain BC 5

On the basis of nucleotide homology and phylogenetic analysis as carried out with the 1484 bp 16S rDNA gene sequence of the isolate strain BC 5and it was found that the isolate BC 5 strain (GenBank accession number MH900215.1)have a significant similarity with many species of *Bacillus*. Identity analysis on the EZ taxon server (Kim et al. 2012) revealed that the 16S rDNA gene sequence had closest similarity (99.73 %) with the gene sequence of the type strain of *Bacillus species* referred in Table 4. The phylogenetic position based on the NJ algorithm of the isolate BC 5having NCBI GenBank accession number MH900215.1 had been shown in the dendrogram Fig The isolated strain BC 5emerged as a distinctive phylogenetic line from the cluster containing the type strains of *Bacillus* species exhibited in Table 2.4.

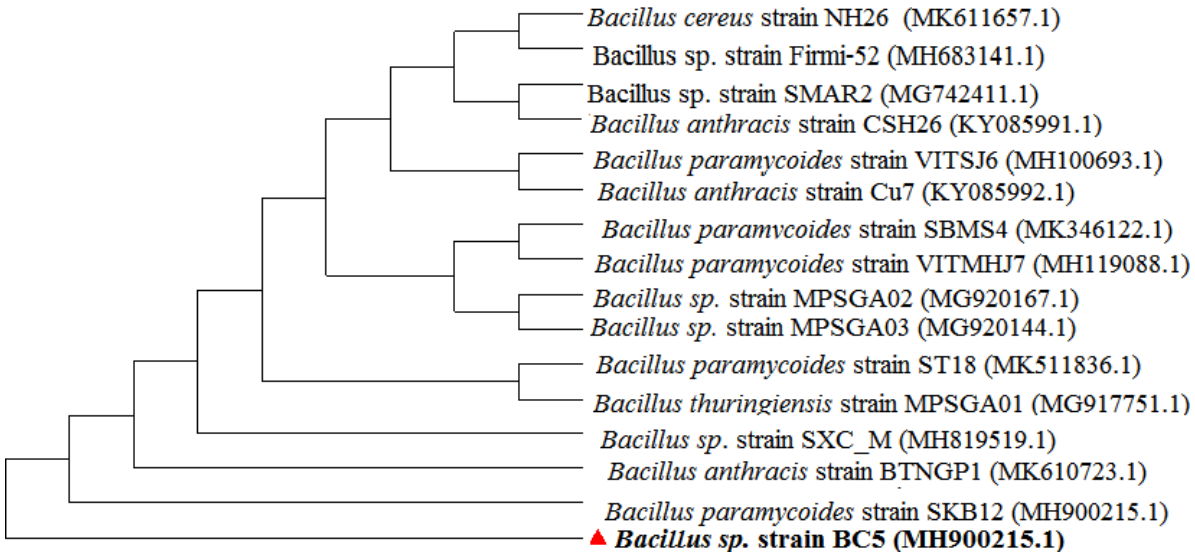


Fig. 2.13. Unrooted Phylogenetic Tree Based on 16S rDNA Sequences Obtained by the Neighbor-Joining (NJ) Method Showing the Position of Strain *Bacillus sp.* BC5 strain among its Phylogenetic Neighbors. NCBI Accession Numbers are provided in Parentheses.

Table-2.4: Results of the Identity Analysis of Strain *Bacillus sp.* BC5 based on the EzTaxon Server in Relation to the Pairwise similarity with other strains.

SL. No.	Similar Species According to Rank	NCBI Accession No.	Pairwise Similarity (%)
1.	*Bacillus paramycoides* strain ST18	MK511836.1	99.73%
2.	*Bacillus paramycoides* strain SBMS4	MK346122.1	99.73%
3.	*Bacillus paramycoides* strain VITMHJ7	MH119088.1	99.73%
4.	*Bacillus anthracis* strain BTNGP1	MK610723.1	99.73%
5.	*Bacillus cereus* strain NH26	MK611657.1	99.73%
6.	***Bacillus paramycoides* strain SKB12**	**MH900215.1**	99.73%
7.	*Bacillus sp.* (in: Bacteria) strain SXC_M	MH819519.1	99.73%
8.	*Bacillus sp.* (in: Bacteria) strain Firmi-52	MH683141.1	99.73%
9.	*Bacillus sp.* (in: Bacteria) strain SMAR2	MG742411.1	99.73%

10.	*Bacillus paramycoides* strain VITSJ6	MH100693.1	99.73%
11.	*Bacillus sp. (in: Bacteria)* strain MPSGA02	MG920167.1	99.73%
12.	*Bacillus sp. (in: Bacteria)* strain MPSGA03	MG920144.1	99.73%
13.	*Bacillus thuringiensis* strain MPSGA01	MG917751.1	99.73%
14.	*Bacillus anthracis* strain Cu7	KY085992.1	99.73%
15.	*Bacillus anthracis* strain CSH26	KY085991.1	99.73%

Isolation and identification of predominant bacterial species from mixed sludge containing nitrification bacteria

To identify the most potent nitrifying bacterial strain, cultures of five well-grown isolated strains from mixed acclimatized seed were grown in NA medium. About five isolates were cultivated. Among these five isolates, one with the maximum potential had been reported here. Result of the removal potential of five isolates, marked as BN1, BN2, BN 3, BN4 and BN5were shown in Figure The isolated BN 3 showed the maximum ammonium nitrogen removal potential of 77 %for an initial NH_4^+-N concentration of 48 – 54mg/L as N mg/L.

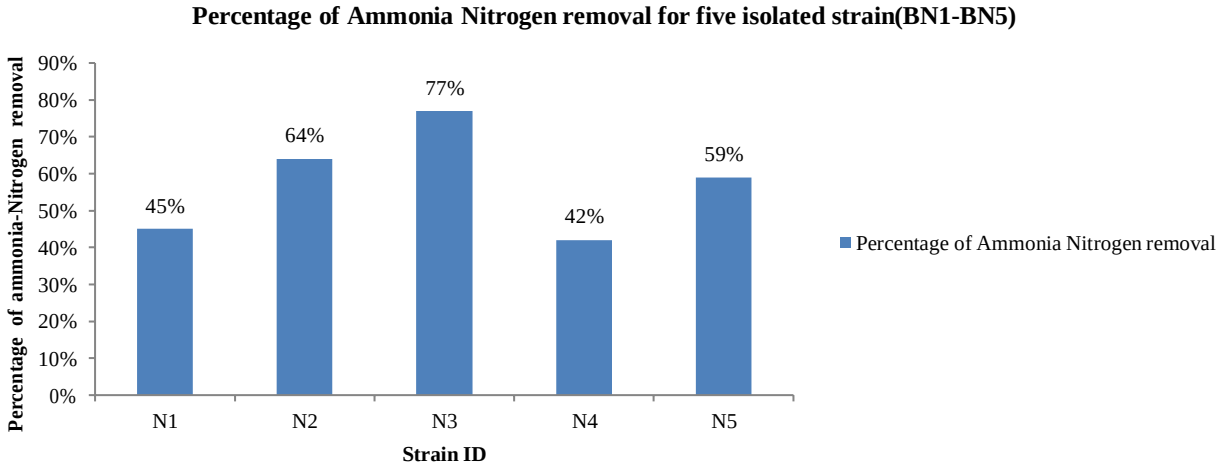


Fig 2.14.NH_4^+-N Removal Efficiency of Different Isolate

Identification of the isolate BN 3predominated in mixed sludge containing nitrifier

Morphological and physiological characterization of isolate BN 3

Morphological and physiological characteristics of the isolate BN3 have been determined following the standard methods (Holt et al. 1993).Experimental results of Morphological and Cellular Characteristics of Strain BN 3 had been shown in Table 2.5. It was observed that the culture seed of strain BN 3 secreted a yellowishcoloured pigment in the plate. The isolated pure culture of strain BN 3 was identified through Gram staining. It was observed that the cell retained the crystal violet stain only, thereby appeared in bluish violet color indicated that the bacteria was Gram (+ve).Numerous other biochemical tests were performed in the same manner as done with the isolated carbon oxidation strain with the isolated pure culture strain BN 3 and results were given in Table 2.6.

Table 2.5.Morphological and Cellular Characteristics of Strain BN3

Experiment	Observation
Morphology of vegetative cells	(a)Cell shape – Rod (b)Cell size - Moderate
Arrangement	Single, double, multiple
Gram's staining	(+) ve
Spore staining	(+) ve
Motility	(+) ve
Colony characteristics(Size)	Moderate
Opacity	Opaque
Surface growth	Smooth
Edge	Entire
Consistency	Good
Color	Yellowish
Pigmentation	yes
Growth in 50 ml medium in 250 ml Erlenmeyer flask	A. Stationary condition i. After 24 hr Moderate growth, No ring formation, no pellicle formation, sedimentation at the bottom. ii. After 48 hr Same as after 24 hr but the growth was more.

	B. Shaking condition: i. After 24 hr Fair growth, turbid, no sedimentation, no pellicle, light yellowish pigmentation, ring formation ii. After 48 hr Same as after 24 hr

Table 2.6.BiochemicalCharacteristics of isolated Strain BN3

Parameters	Characteristics
1. Ammonia from arginine	Positive
2. Arginine used as a sole source of energy	Positive
3. Nitrate reduction	Positive
4. Catalase reaction	Positive
5. Indole formation	Negative
6. Litmus milk test	Negative
7. Voges-Proskauer test	Result:
pH<6.0	Negative
pH>7.0	Positive
8. Growth at anaerobic condition	Positive
9. Growth at different temperature	**Result:**
10^oC	Poor Growth
30^oC	Vigorous Growth
40^oC	Vigorous Growth
50^oC	No Growth
10. Growth at different NaCl Concentration	**Result:**
2%	Vigorous Growth
5%	Vigorous Growth
7%	Poor Growth
10%	No Growth
11. Growth at 6.5% NaCl & pH-9.6	Very Poor Growth
12. Growth at	**Result:**
pH 6.8	Positive
pH 5.4	Positive
13. Urease test	Negative
14. Starch Hydrolyses test	Positive
15. Carbohydrate fermentation	

	Acidity	Gas formation
i) Fructose	Positive	Negative
ii) Arabinose	Positive	Negative
iii) Galactose	Positive	Negative
iv) Glucose	Positive	Negative
v) Lactose	Negative	Negative
vi) Raffinose	Negative	Negative
vii) Sucrose	Negative	Negative
viii) Dextrin	Positive	Negative
ix) Salicin	Negative	Negative
x) Sorbitol	Negative	Negative
xi) Mannitol	Negative	Negative
xii) Glycerol	Negative	Negative
xiii) Inositol	Negative	Negative
xiv) Maltose	Positive	Negative

16S rDNA sequence and phylogenetic analysis of isolatedstrain BN 3

On the basis of nucleotide homology and phylogenetic analysis as carried out with the 1473 bp 16S rDNA gene sequence of the isolate strain BN 3and it was found that the isolate BN 3 strain (GenBank accession number NR_156837.1) have a significant similarity with *Solibacillus isronensis* strain B3W22. Identity analysis on the EZ taxon server (Kim et al. 2012) revealed that the 16S rDNA gene sequence had closest similarity (96.16 %) with the gene sequence of the type strain of *Solibacillus isronensis* strain B3W22refer in Table 2.7.The phylogenetic position based on the NJ algorithm of the isolate BN3 having NCBI GenBank accession number NR_115952.1had been shown in the dendrogram Fig 2.15.

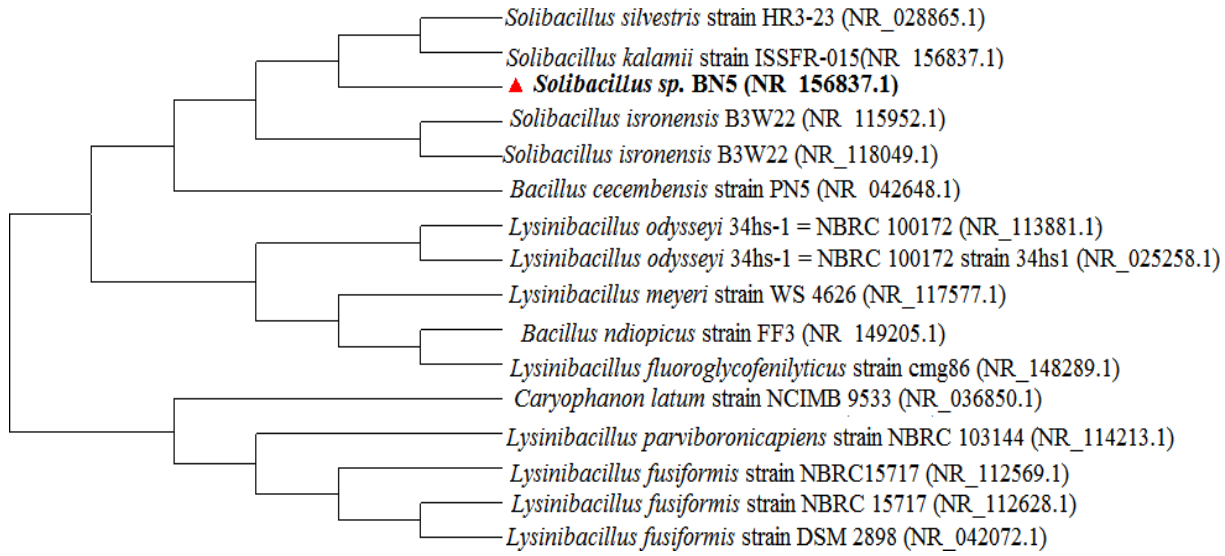


Fig 2.15. Unrooted Phylogenetic Tree Based on 16S rDNA Sequences Obtained by the Neighbor-Joining (NJ) Method Showing the Position of Strain *Solibacillus sp.* BN3 strain among its Phylogenetic Neighbors. NCBI Accession Numbers are provided in Parentheses.

Table 2.7: Results of the Identity Analysis of Strain *Solibacillus sp.* BN3 strain based on the EzTaxon Server in Relation to the Pairwise similarity with other strains.

SL. No.	Similar Species According to Rank	NCBI Accession No.	Pairwise Similarity (%)
1.	***Solibacillus isronensis* B3W22**	**NR_115952.1**	**97.84%**
2.	*Solibacillus isronensis* B3W22	NR_118049.1	97.37%
3.	*Solibacillus silvestris* strain HR3-23	NR_028865.1	97.56%
4.	*Solibacillus kalamii* strain ISSFR-015	NR_156837.1	96.16%
5.	*Bacillus cecembensis* strain PN5	NR_042648.1	95.89%
6.	*Lysinibacillus meyeri* strain WS 4626	NR_117577.1	95.54%
7.	*Bacillus ndiopicus* strain FF3	NR_149205.1	94.48%

8.	*Lysinibacillus fluoroglycofenilyticus* strain cmg86	NR_148289.1	94.47%
9.	*Lysinibacillus odysseyi* 34hs-1 = NBRC 100172	NR_113881.1	94.40%
10.	*Lysinibacillus fusiformis* strain NBRC15717	NR_112569.1	94.33%
11.	*Lysinibacillus fusiformis* strain NBRC 15717	NR_112628.1	94.33%
12.	*Lysinibacillus odysseyi* 34hs-1 = NBRC 100172 strain 34hs1	NR_025258.1	94.27%
13.	*Lysinibacillus fusiformis* strain DSM 2898	NR_042072.1	94.26%
14.	*Caryophanon latum* strain NCIMB 9533	NR_036850.1	94.19%
15.	*Lysinibacillus parviboronicapiens* strain NBRC 103144	NR_114213.1	94.07%

Carbon oxidation study of the identified isolate of strain BC 5

Carbon oxidation study of the selected most active bacterial stain was conducted through cultivation of isolate BC 5in a shake flask in nutrient broth medium. Duplicate sets of twelve numbers of conical flasks made of Borosilicate glass (total of 24) of capacity 250 mL were used for single batch experiment using sterilized synthetic dairy waste water and the sterilized media were inoculated with 10 % (v/v) of the isolate and incubated at 37º C with shaking at 100 rpm for 24 hr. The whole culture of 100 mL was withdrawn at every 2 hr intervals from one flask of each duplicate set for a period of twenty-four hours to examine. Afterwards centrifugation was done in a “Remi” made centrifuge at 5000 rpm for 15 min at 4°C. Obtained supernatant solution was then tested for parameters like pH, MLSS, MLVSS and SCOD, as per Standard Methods (APHA, 2012).

Stabilization of organic carbon by strain BC 5

The findings of the time - concentration study on the removal of SCOD was shown in Fig. 2.16. Results showed that within the contact period of 24hr, around 88 % SCOD had been reduced and after this time period the percentage removal of SCOD became gradual. The nature of plot indicated that the degradation of biodegradable portion of the synthetic dairy waste water took place withinthe reaction period.

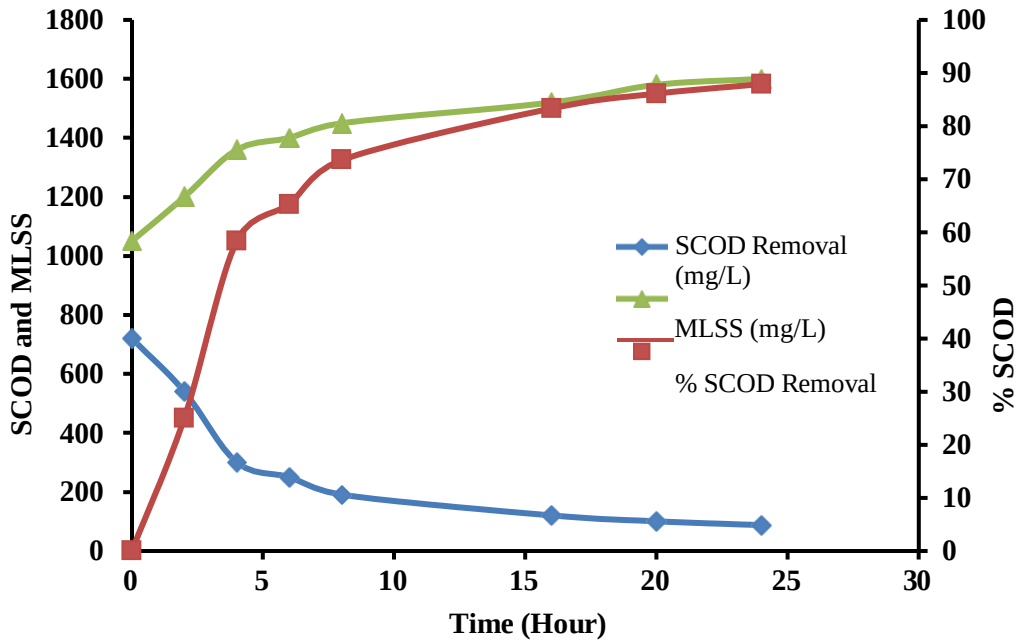


Fig 2.16. SCOD and MLSS Profile during Carbon oxidation Study in Batch Reactor of strain *Bacillus sp.* BC 5 [Initial SCOD Concentration = 720 mg/L]

Kinetic study for Carbon oxidation with isolate of BC 5

The experimentally obtained data were plotted for $1/U_C$ against 1/C(inverse of effluent SCOD) to make a straight line with best fit statistically as shown in Fig. 2.17a. Values of K_s; half velocity constant and k; maximum Substrate utilization rate, depends on environmental factors, such as temperature, pH, presence of nutrients and inhibitory factors. The K and K_s values were obtained to be 4.32 per day and 203.90mg/L for SCOD reduction. These values were closed to the values obtained for mixed sludge Carbon oxidation kinetic values. The experimentally obtained data for Microbial-growth kinetics of isolate of BC 5based on SCOD reduction was plotted against $1/\Theta$; inverse of reaction time and U_C; specific Substrate utilization rate to get a straight line of best fit with Yield coefficient (Y) as its slope and endogenous decay coefficient (K_d) as its intercept as shown in Fig. 2.17b. From this plot the values of Y and K_d were found to be 0.615 and 0.015 respectively.

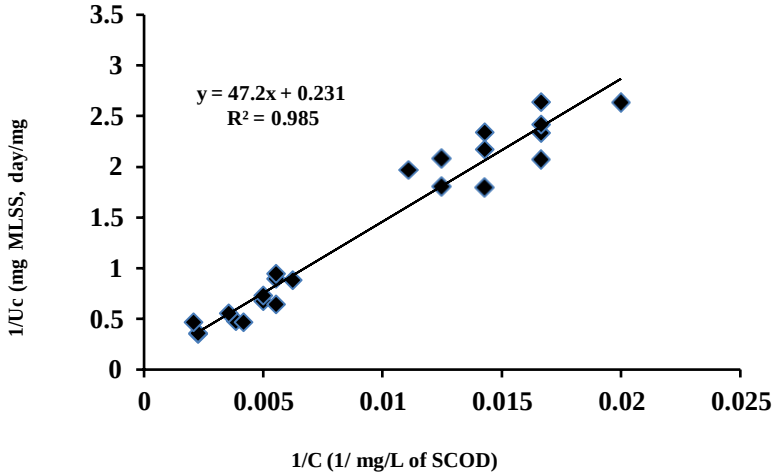


Fig 2.17a.Substrate-utilization kinetics for Carbon oxidation Study in Batch Reactor for strain *Bacillus sp.* BC 5 [Initial SCOD Concentration of 700-720 mg/L]

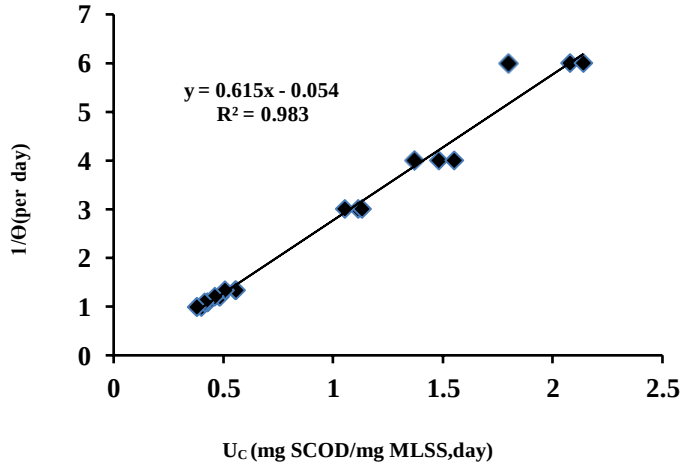


Fig 2.17b. Microbial-Growth kinetics for Carbon oxidation Study in Batch Reactor for strain *Bacillus sp.* BC 5 [Initial SCOD Concentration of 700-720 mg/L]

Nitrification study of the identified isolate of BN 3 strain

Nitrification study of the selected most active nitrifier stain was performed through cultivation of isolate BN 3 strain in a shake flask in nutrient broth medium. Duplicate sets of twelve numbers of conical flasks made of Borosilicate glass (total of 24) of capacity 250 mL were used for single batch experiment using sterilized synthetic dairy wastewater and the sterilized media were inoculated with 4 % (v/v) of the isolate and incubated at 37º C with shaking at 100 rpm for 24 hr. The whole culture of 100 mL was withdrawn at every 2 hr intervals from one flask of each duplicate set for a period of 24 hr. Afterwards centrifugation was done in a "Remi" made centrifuge at 5000 rpm for 15 min at 4°C. Obtained supernatant solution was then tested for parameters pH, MLSS, MLVSS, NH_4^+- N, NO_3^--N according to Standard techniques.(APHA, 2012)

Stabilization of ammonium nitrogen by isolate BN 3 strain

The most potent nitrifier species (i.e.BN 3) was identified as the strain which lowered the ammonium nitrogen concentration most efficiently for a detention period of 24 hr. Fig 2.18 showed the nitrification process by isolate BN 3 strain. Around 77± 0.2 % of ammonium nitrogen (NH_4^+-N) was removed within a batch contact time of 24 hr in the batch reactor. It was found that during initial phase, the rate of decomposition was faster due to the availability of higher amount of substrate, but due to reduced availability of degradable substrate to the microorganism in the laterstages, the rate of decomposition started falling. The results indicated that the microbial strain BN3 was capable of decomposition and stabilization of ammonium nitrogen (NH_4^+- N).

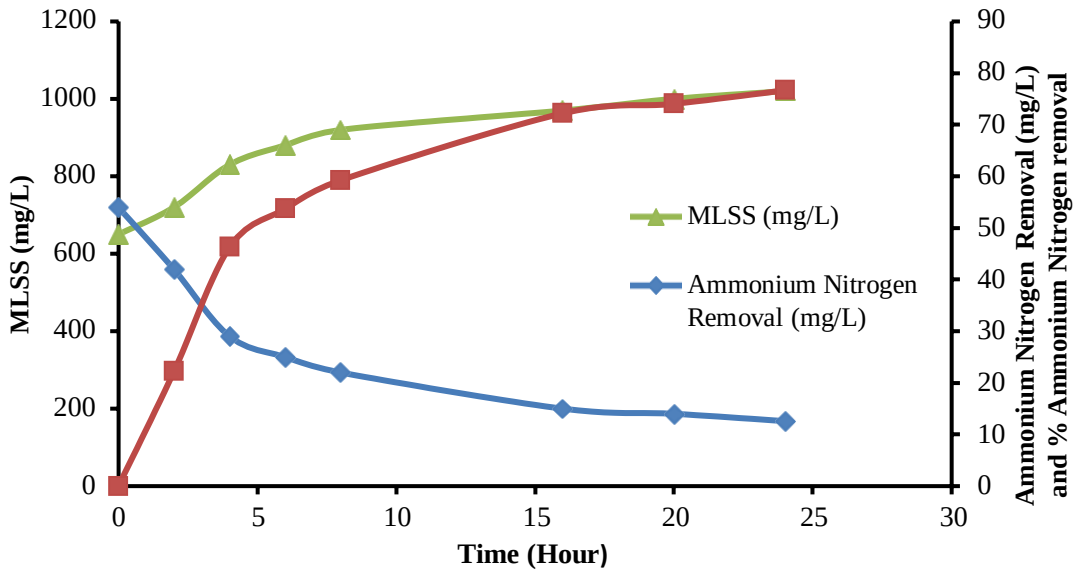


Fig 2.18.NH_4^+-N and MLSS Removal Profile during Nitrification Study in Batch Reactor for strain *Soli bacillus sp.* BN 3[Initial NH_4^+-N= 54mg/L]

Kinetic study for nitrification with isolate BN 3 strain

The experimental data obtained from kinetic study of BN 3 strain were plotted for $1/U_N$; inverse of the specific substrate (NH_4^+- N) utilization rate against 1/N; inverse values of effluent ammonium nitrogen to draw a line of best fit. The plots of kinetic study for NH_4^+- N removal was shown in Fig. 2.19a. Value of k and K_s was found to be 2.52 per day and 23.94 mg/L for NH_4^+- N removal. These obtained values were very much similar to those values obtained for mixed sludge nitrification kinetic study. The experimentally obtained data for Microbial-growth kinetics of strain BN 3 strain based on NH_4^+-N removal was plotted for$1/\Theta$; inverse of the reaction time againstU_N; shown in Fig. 2.19b. A line of best fit was drawn. From this plot values of Y and K_d. were found to be 0.564 and 0.052 respectively.

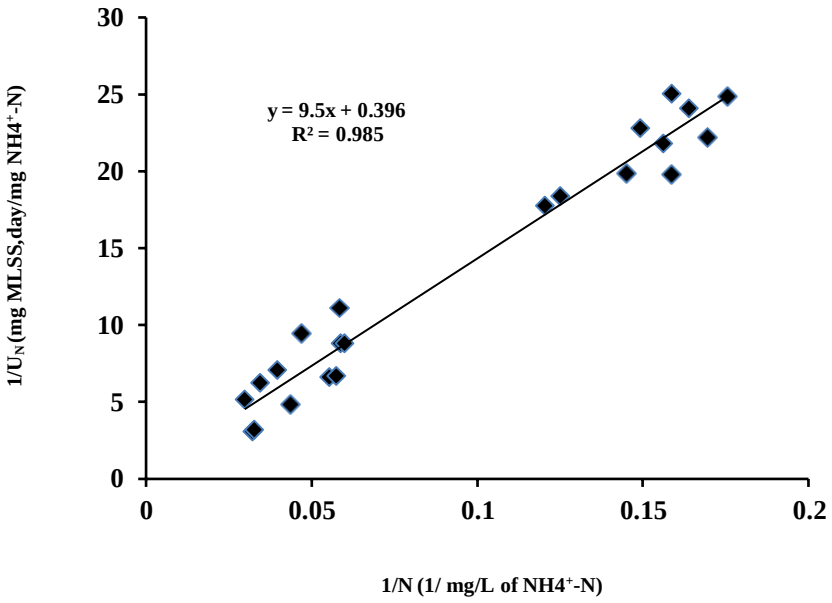


Fig 2.19a. Substrate-utilization Kinetics for batch nitrification study for strain *Solibacillus sp.* BN 3 [Initial NH_4^+-N= 48 -54 mg/L as N]

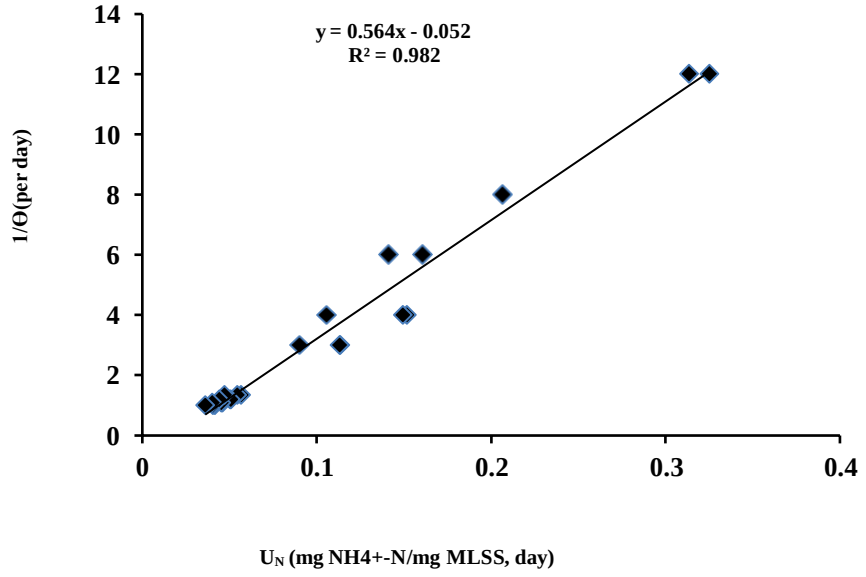


Fig 2.19b. Microbial-Growth kinetics for batch nitrification study for strain *Solibacillus sp.* BN 3 [Initial NH_4^+-N= 48 -54 mg/L as N]

Chapter 3-4

Treatibility study of Bacterial treated wastewater containing Nitrate and Phosphorus in Suspended Growth Batch Reactor using Micro algae Chlorella pyrenoidosa and Spirulina platensis

Chapter 3

Nutrient Removal and Bio kinetic Study of MicroalgaeChlorella pyrenoidosa in Suspended Growth Batch Reactor

Introduction and Literature Review

In numerous nations, dairy industry is viewed as one of the biggest wellspring of organic wastewater generating industry. Dairy wastewater incorporates lingering milk inside pipelines, tanks and compartments, cleaning synthetic compounds like cleansers, sanitizers, disinfectants (like sodium hypochlorite) and acids (phosphoric and nitric acids), residue during Cleaning in Place (CIP) system. The dairy wastewater contains huge amounts of milk components, for example, casein, inorganic salts, other than cleansers and sanitizers utilized for cleaning and washing. Each of these contributes towards the high natural BOD and SCOD. These Wastewater parameters are generally much higher than the discharge standards prescribed by the regulating agencies like WHO or CPCB. As these squanders are for the most part discharged to the nearby terrestrial or aquatic ecosystem with no earlier treatment, is accounted for genuine contamination problem. The major reason behind carrying out little or no wastewater treatment in case of medium or small companies or plants is associated with high cost of chemical treatment. An alternate treatment with biological agents like algae is trending now a days. Algae can grow in wastewater and have the capability of assimilating CO_2 from air and Nitrogen and Phosphorus from sewage (Kumar et al. 2010, Lu 2015, Lu 2016). Nitrogen and phosphorus are very important for the proper growth of algae. (Lu et al. 2015, Rowley2010)

Phosphorus in aquatic system is generally found bounded with sediments. (Engblom1998). Sequestering CO_2 from air by algae also help maintaining the level of Green house gases which accomplish the goals of Kyoto Protocol. (Mata et al. 2010)

A group of researchers treat dairy wastewater using an algae *Botryococcus sp.*(Gani et al. 2014).From concentrated wastewater, *Botryococcus sp* reduced wastewater-parameters such as BOD, TOC, TC, IC and SCOD as 73.3%, 65.1%, 61.4%, 58.3% and 48.8% respectively after

15

days of treatment. Another study found that treatment of wastewater using microalgae is a green technique and algal biomass generated can be utilized further and helps in efficient recycling of nutrients. This investigation was done for the purification of wastewater by *Chlorella vulgaris*. Treatment with *Chlorella vulgaris* was effective in removal of major inorganic components form the wastewater (Fathi et al. 2013). A group of researchers made a study in Konabari near Gazipur (Bangladesh) in which textile-industry wastewater were treated by two indigenous species of cyanobacteria and different important parameters of the treated wastewater were analyzed for the evaluation of the treatment efficiency and the removal efficiency was found to be satisfactory. Two species of cyanobacteria, *Anabaena* and *Nostoc muscorum* were used for this purpose (Talukder et al. 2015).One investigation was done for simultaneous removal of nutrient (mainly N and P) and some of the heavy metals (such as iron, manganese, zinc). Two microalgae species *Scenedesmus and Chlorella* were isolated from municipal wastewater. *Chlorella vulgaris* showed better potency for the removal of total nitrogen (TN) in waste water than *Scenedesmus armatus* (Kozłowska et al. 2014).

Some researchers in Indian state of Tamil Nadu collected leather-processing wastewater from a plant in Ranipet, and used *Chlorella vulgaris* as their remediating agent. This micro algae was isolated from the collected raw effluent. The study was conducted to treat the effluent wastewater and the solid sludge of Effluent Treatment Plant and find the difference in concentration of wastewater parameters before and after treatment. *Chlorella vulgaris* was proved to be efficient in removing much of the nutrients in both laboratory and pilot scale according to their findings (Rao et al. 2011). A study was carried out by using soft drink manufacturing plant's discharge in the western Indian city of Ahmedabad. Treating agent here was also micro algae. Parameters tested for reduction was BOD, SCOD, total hardness, etc. and removal efficiency was found highly

appreciable (Sivasubramanian et al. 2012). Another group of researchers found that the combination of two algal species namely *Chlorella vulgaris* of strain ID LS120 and *Scenedesmus obliquus* of strain ID LS121 at different concentrations effectively removed TDS, BOD and SCOD levels of effluents collected from leather industry after completion of 21 days of phycoremediation (Elumalai et al 2014).Dairy wastewater after a primary treatment with a consortium of bacterial population containing Carbon oxidation and nitrification was taken as the feed material for treatment process in this present investigation and *Chlorella pyrenoidosa* strain was used for this purpose. Pretreated wastewater mentioned was high in nitrate nitrogen and phosphate. After a certain time period during treatment process with this algal strain the concentration of nitrate and phosphate becomes reasonably low and met with the discharge standards prescribed by the environmental regulatory authorities like WHO and CPCB.

The present investigation was done for algal treatment of simulated dairy plant effluent containing nitrate nitrogen and phosphate in a lab-scale batch reactor with acclimatized seeds of microalgae *Chlorella pyrenoidosa* and also to evaluate various biokinetic coefficient of microalgae *Chlorella pyrenoidosa* require for design of an appropriate suspended growth biological reactor applicable for algal treatment of nutrient rich dairy plant effluent.

Materials and Methods

Preparation of simulated dairy wastewater and Primary treatment

Initially waste water was collected from the inlet of the aeration tank from the effluent treatment plant (ETP) of Mother Dairy Calcutta; located in Dankuni, West Bengal. Bacterial seed was also collected from the aeration tank. This seed culture was further used for the treatment of the simulated wastewater; that was prepared as identical to the collected wastewater according to the concentration measured through characterization process. During pretreatment process with acclimatized seed of carbonaceous bacteria and nitrifier in laboratory scale suspended growth batch reactor the organic carbon in terms of SCOD and ammonium nitrogen (NH_4^+-N) content of dairy wastewater become reduced. Initial high concentration of NH_4^+-N was transformed into nitrate nitrogen during pretreatment process. Phosphate concentration remained more or less similar as it was initially present in the wastewater. pH of this pretreated dairy wastewater was slightly in the acidic range as nitrifier bacteria produced some acid in the medium. Major objective of this pretreatment process of dairy wastewater was that to reduce SCOD and NH_4^+-N concentration as well as convert all NH_4^+-N into nitrate nitrogen (NO_3^--N). Now pretreated dairy wastewater sample containing high value of nutrients (NO_3^--N and phosphorous) was prepared synthetically in a laboratory and used for further treatment using microalgae *Chlorella pyrenoidosa*. This pretreated simulated dairy wastewater (SDW) was diluted in a ratio of 1:4 with distilled water during batch study using microalgae *Chlorella pyrenoidosa*. In every batch study prepared pretreated SDW was sterilized at 121^o C temperature for destroying all microorganism. After that the sterile pretreated SDW was innoculated with varying innoculum concentration of acclimatized pure culture of microalgae *Chlorella pyrenoidosa* in batch study.

Composition of SDW (g/L) was as Milk powder 0.01, Dextrose 0.01, Peptone 0.25, Beef extract 0.10, Lactose 0.01, Ammonium sulfate 0.1, Potassium nitrate 1.5, Di hydrogen potassium phosphate 0.20, Di potassium hydrogen phosphate 0.20. pH of SDW was kept around 7.0

Collection of Algal strain

A pure culture of *Chlorella pyrenoidosa* strain was obtained in solid growth medium as slant culture from the NICM laboratory, Pune. It was kept at refrigerated condition at 4°C temperature

before it was transferred aseptically in the liquid broth medium prepared in accordance with the composition mentioned below.

Preparation of Growth Culture

50 mL of growth culture medium was made in Erlenmeyer flask of 250 mL. Prepared medium was sterilized at 121°C temperature for 15 min in an autoclave. The composition of growth medium are Magnesium sulfate hepta-hydrate 0.2 g/L, Di-Potassium hydrogen phosphate 0.2 g/L, Calcium chloride monohydrate 0.1 g/L, Fe-EDTA solution 5.0 mL, Micronutrient solution 1.0 mL. Micronutrient solution containing Boric acid, Manganese chloride tetra hydrate, Zinc sulfate and some other inorganic salts in trace amounts. All the constituents were dissolved in water and pH of the medium was kept between 7.2 – 7.5 using dilute hydrochloric acid and dilute sodium hydroxide.

Transfer into Growth Culture

From the stock culture inoculums was taken with an inoculation loop and was transferred into previously prepared sterile growth medium. The whole process was performed aseptically inside a laminar air flow (LAF) chamber to avoid any cross contamination. This inoculated growth culture was kept in wooden racks in the laboratory after plugged with cotton at the open mouth of the Erlenmeyer flask. Light Emitting Diode (LED) tubes were lit inside the wooden racks for bright illumination purpose. Flask was kept under ambient temperature for about two weeks.

Seed Acclimatization for *Chlorella*:

The study was conducted in an Erlenmeyer flask of 500 mL capacity as an acclimatization unit. About 10 mL of the algae from the cultured broth medium was added to 100 mL of Synthetic Feed medium diluted with distilled water in a ratio of 1:4. The composition of original undiluted Synthetic Feed medium (g/L) Milk powder 0.01, Dextrose 0.01, Peptone 0.20, Beef extract 0.20, Lactose 0.02, Ammonium sulphate 0.1, Potassium nitrate 1.5, Di-Hydrogen potassium phosphate 0.20, Di-Potassium hydrogen phosphate 0.20. pH of Synthetic Feed medium was adjusted in between the range of 7.0 – 7.2using dilute hydrochloric acid and dilutes sodium hydroxide.

Efficacy study

After the acclimatization process was over; different percentages of inoculums were transferred from the acclimatized culture of *Chlorella pyrenoidosa* to the Bacterial treated dairy wastewater (BTDW). 100 mL of BTDW was used for this purpose in five Erlenmeyer flasks of 500 mL and kept for 10 days in shaking condition at ambient temperature. Inoculums concentrations of 0.2, 0.5, 0.8, 1.0 and 1.2% (v/v) were taken in five different Erlenmeyer flasks. Samples were withdrawn from all five flasks and concentration of phosphate and nitrate nitrogen was measured using standard protocol (Rice et al. 2012)

Time concentration study with microalgae *Chlorella pyrenoidosa*

100 mL of BTDW was taken in five conical flasks of 500 mL capacity and kept for 8 days in shaking condition at ambient temperature with added inoculums of different percentages as used in the efficacy study. Inoculum concentrations in five flasks were 0.2, 0.5, 0.8, 1.0 and 1.2% (v/v). Samples were withdrawn periodically in an interval of two days from all five flasks and concentration of algal biomass, phosphate and nitrate nitrogen concentration was measured using standard protocols. (Rice et al. 2012**)** Phosphate concentration and algal biomass was measured through colorimetric method by using a UV Spectrophotometer made by Lassany and Nitrate nitrogen concentration was measured using Nitrate Ion Selective Electrode (ISE) probe made by Thermo Fischer Limited. Biomass was also measured using gravimetric method to establish a correlation between Mixed Liquor Suspended Solids (MLSS) in g/L value with their corresponding Absorbance (ABS) value.

Substrate utilization and algal growth kinetics study

A reactor's performance can be properly evaluated and optimized by evaluating its' reaction kinetics. These kinetic parameters bear technological importance in designing Bioreactor. (Lee, 1992) By conducting experiment, different values of kinetic coefficients are estimated as steady state kinetics for stabilization of Nitrate Nitrogen and Phosphate. Kinetic coefficients were obtained on the basis of experimental results by varying MLSS concentration. Kinetic study was done by using Monod kinetics model equation.

For Nitrate nitrogen removal study reciprocal values of specific substrate (NO_3^--N) utilization rate ($1/ R_{xiN}$) were plotted against the inverse values of NO_3^--N concentration ($1/N_0$) and substrate-

removal kinetics was obtained from the linear equation, $1/R_{xiN} = (Ks/k)(1/N_0) + 1/k$. A line with best-fit was drawn by using statistical method from obtained data. The values of the change in algal biomass in Y axis with consumption of Nitrate nitrogen as substrate (N_0-N_f) in X axis gives the growth kinetics equation in the form of y = mx where m, the slope of the straight line is Yield Coefficient (Y_N)

For Phosphate removal study the inverse values of specific substrate (PO_4^{3-} - P) utilization rate ($1/R_{xiP}$) were plotted against the inverse values of Phosphate concentration ($1/P_0$) and Phosphate removal-kinetics were also evaluated from the straight-line equation, $1/R_{xiP} = (Ks/k)(1/P_0) + 1/k$. A graph with best fit was then drawn by using similar technique followed in NO_3^-N study.

The values of the change in algal biomass in Y axis with consumption of Phosphate as substrate (P_0-P_f) in X axis gives the growth kinetics equation in the form of y = mx where the slope of the straight line, m is Yield Coefficient (Y_P)

Results and Discussions

Efficacy study of algae

Removal potential of Nitrate nitrogen and Phosphorus under different percentage of inoculums has been shown in fig. 3.1. It has been observed that both NO_3^--N and PO_4^{3-}- P removal percentage was increased from 0.2 to 0.8% v/v inoculums, after that it decreased due to limiting substrate concentration of NO_3^--N and PO_4^{3-}- P in reactor. It has been also observed that 99.67% and 90.25% of NO_3^--N and PO_4^{3-}- P were removed after 8 days batch contact time respectively, corresponding to initial inoculums concentration of 0.8% v/v. The MLSS concentration of 0.08 g/L equivalent to

0.8 % v/v inoculums was found to be optimum for maximum NO_3^--N and PO_4^{3-}- P removal corresponding to an initial NO_3^--N and PO_4^{3-}- P concentration of 54 mg/L as N and 16 mg/L as P after 8 days batch contact time.

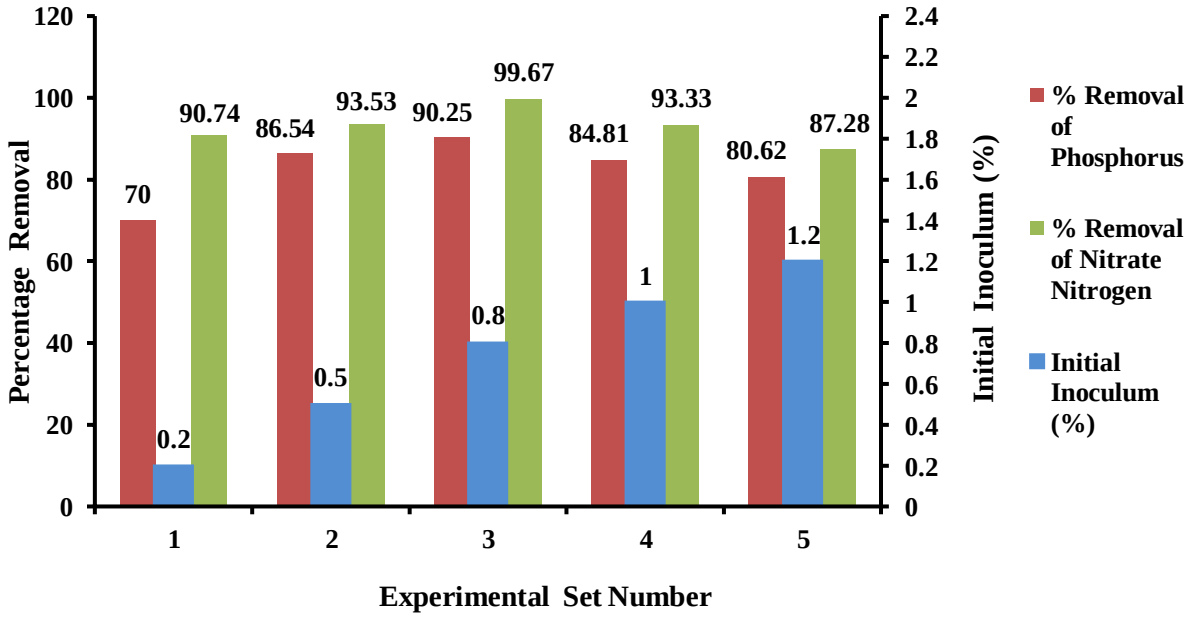


Fig. 3.1Time concentration study of Bacterial treated dairy wastewater (BTDW) for removal Nitrate Nitrogen and Phosphorus in Batch reactor

Five set of time concentration study for removal of nitrate nitrogen and phosphorus in batch reactor with an initial NO_3^--N and PO_4^{3-}- P concentration of 54 mg/L as N and 16 mg/L as P using acclimatized culture of *Chlorella pyrenoidosa* has been shown in Fig. 3.2a-3.2e. corresponding to five different initial inoculums percentages of 0.2, 0.5, 0.8, 1, 1.2 v/v. From Fig.3.2a it was observed that 90.74 % and 70 % of NO_3^--N and PO_4^{3-}- P were removed after 8 days batch contact time respectively, corresponding to initial inoculums concentration of 0.2% v/v. Fig.3.2b demonstrates that there is a slightly increase of NO_3^--N and PO_4^{3-}P removal of 93.53 % and 83.54 % after 8 days respectively, corresponding to initial inoculums concentration of 0.5 % v/v. The maximum

99.67 % and 90.25 % of NO_3^--N and PO_4^{3-}- P removal were achieved respectively with an initial inoculums concentration of 0.8 % v/v after 8 days has been shown in Fig. 3.2e. When an initial inoculums concentration increases further, removal of NO_3^--N and PO_4^{3-}- P decrease to 93.33 % and 84.80 % respectively for 1 % v/v initial inoculums concentration as shown in Fig. 3.2c and also decrease to 87.28 % and 80.62 % respectively for 1.2 % v/v initial inoculums concentration as shown in Fig. 3.2d after 8 days batch contact time.

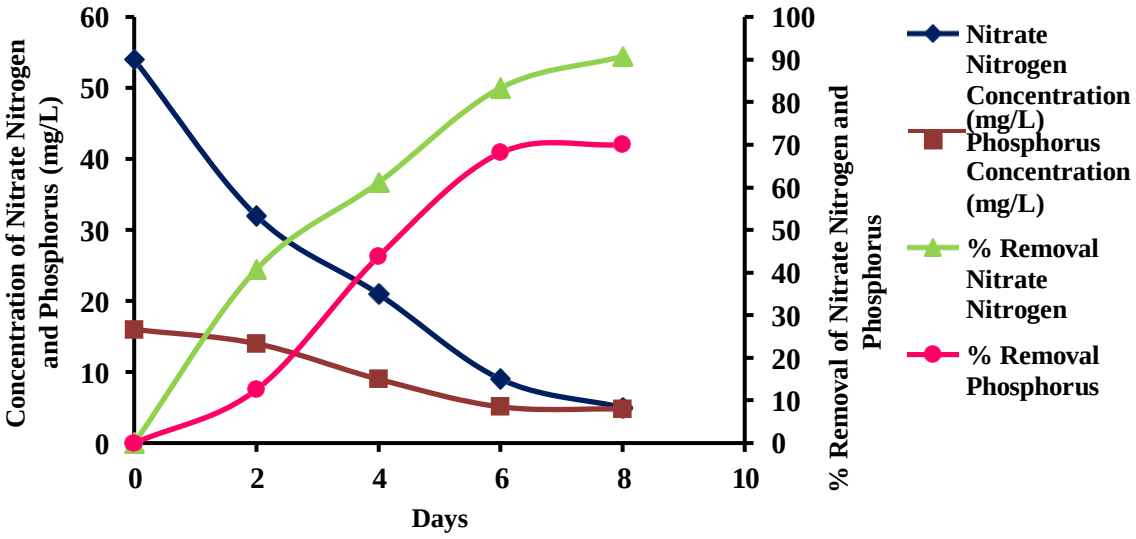


Fig. 3.2aNitrate nitrogen and phosphorus removal profile in batch reactor for pretreated simulated dairy wastewater (SDW) sample [Set 1: Initial NO_3^--N = 54 mg/L as N, initialPO_4^{3-} - P = 16 mg/L as P, initial inoculums of *Chlorella pyrenoidosa* = 0.2% v/v, batch contact time = 8days]

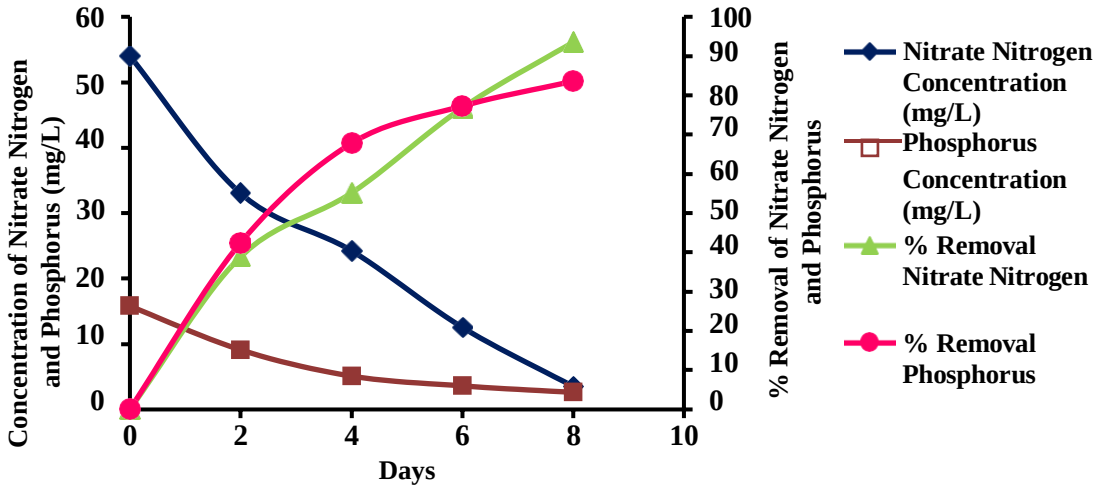


Fig. 3.2bNitrate nitrogen and phosphorus removal profile in batch reactor for pretreated simulated dairy wastewater (SDW) sample [Set 1: Initial NO_3^--N = 54 mg/L as N, initialPO_4^{3-} - P = 16 mg/L as P, initial inoculums of *Chlorella pyrenoidosa*= 0.5% v/v, batch contact time = 8days]

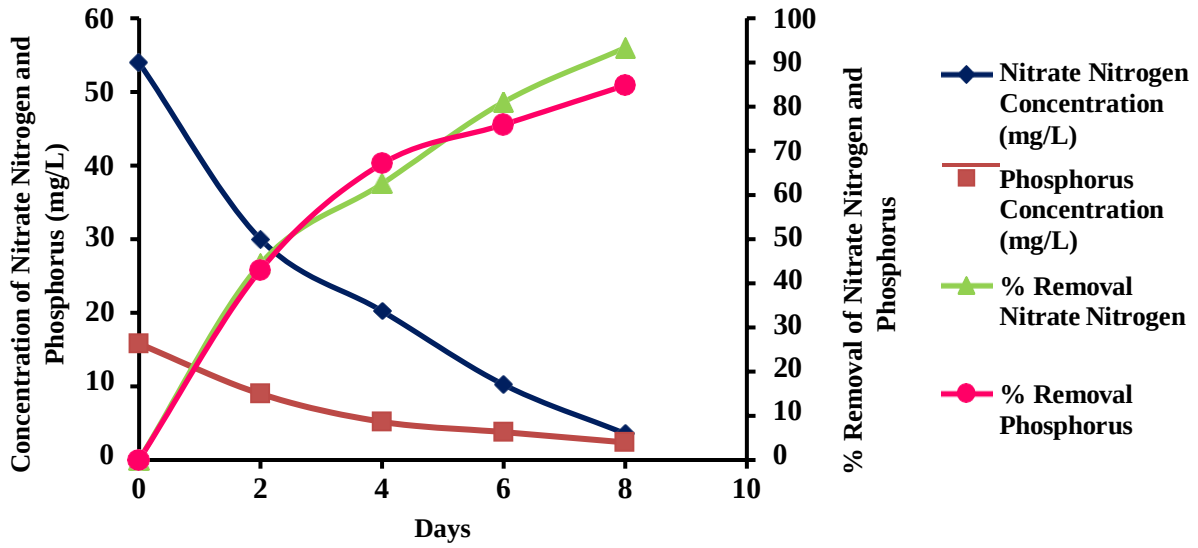


Fig. 3.2cNitrate nitrogen and phosphorus removal profile in batch reactor for pretreated simulated dairy wastewater (SDW) sample [Set 1: Initial NO_3^--N = 54 mg/L as N, initialPO_4^{3-} - P = 16 mg/L as P, initial inoculums of *Chlorella pyrenoidosa*= 1.0% v/v, batch contact time = 8days]

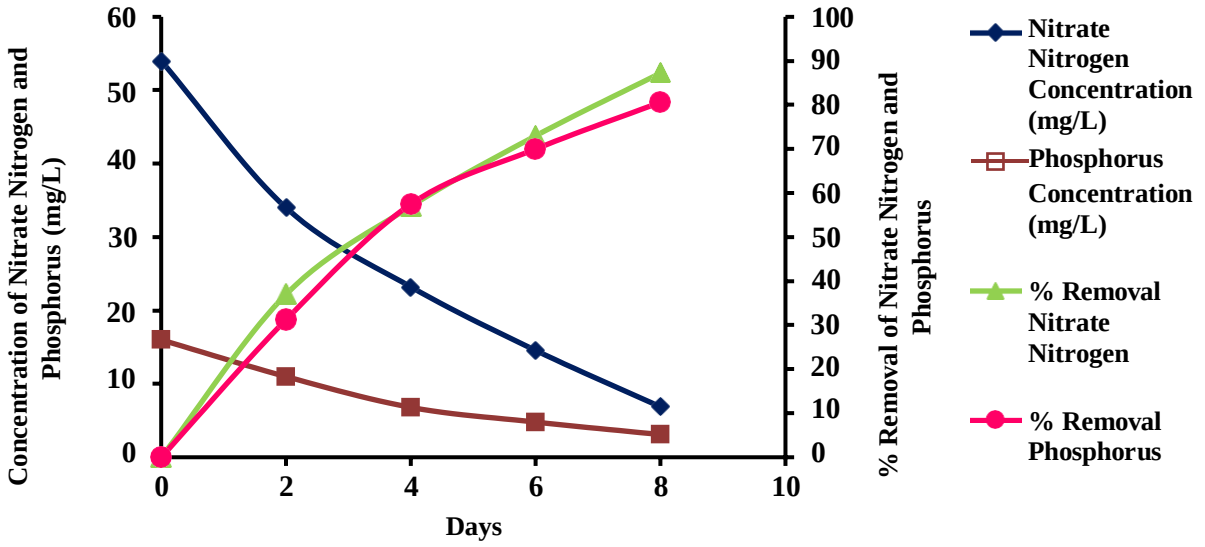


Fig. 3.2dNitrate nitrogen and phosphorus removal profile in batch reactor for pretreated simulated dairy wastewater (SDW) sample [Set 1: Initial NO_3^--N = 54 mg/L as N, initialPO_4^{3-} - P = 16 mg/L as P, initial inoculums of *Chlorella pyrenoidosa*= 1.2% v/v, batch contact time = 8days]

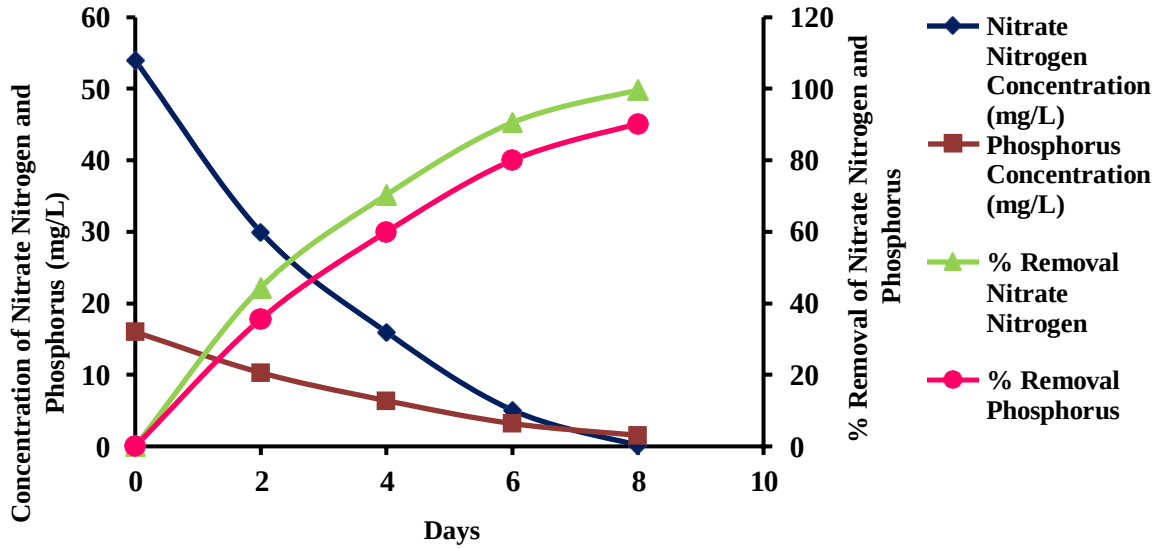


Fig. 3.2eNitrate nitrogen and phosphorus removal profile in batch reactor for pretreated simulated dairy wastewater (SDW) sample [Set 1: Initial NO_3^--N = 54 mg/L as N, initialPO_4^{3-} - P = 16 mg/L as P, initial inoculums of *Chlorella pyrenoidosa*= 0.8% v/v, batch contact time = 8 days]

MLSS Profile in Batch reactor

From the different plots in Fig. 3.3 corresponding to five different initial inoculums percentages of 0.2, 0.5, 0.8, 1, 1.2 v/v, it exhibited that a steady increase in MLSS level was occurred starting from the commencement of the batch study. Fig. 3.3 demonstrates that initial MLSS concentration have different values of 0.02, 0.05, 0.08, 0.09, 0.13 g/L corresponding to five different initial inoculums percentages of 0.2, 0.5, 0.8, 1, 1.2 v/v respectively. After 8 days of batch contact time in batch reactor the MLSS concentration increased to 0.76, 0.80, 0.84, 0.78, 0.80 g/L corresponding to five different initial inoculums percentages of 0.2, 0.5, 0.8, 1, 1.2 v/v respectively.

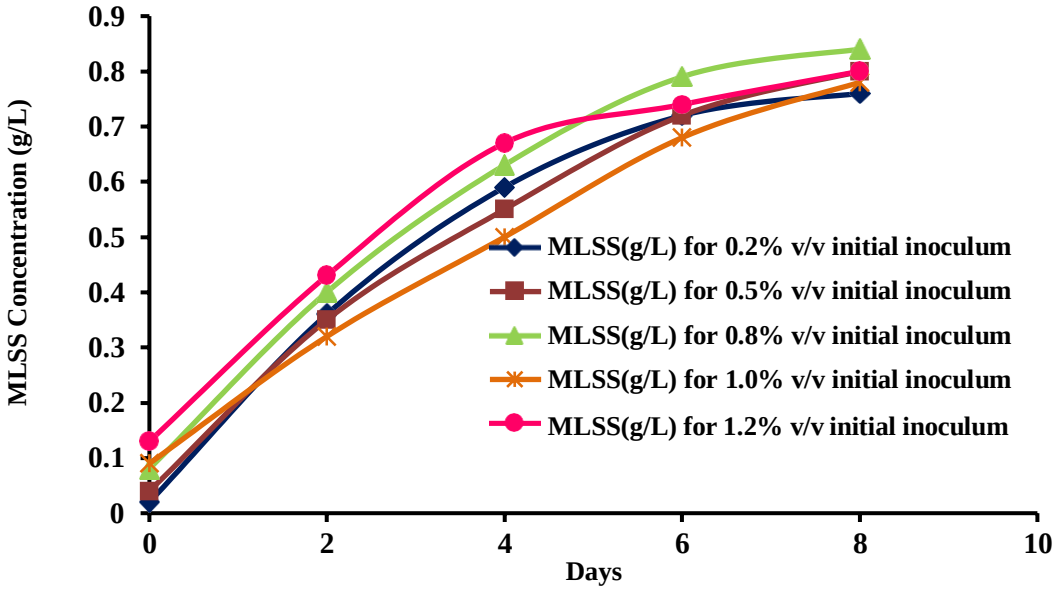


Fig. 3.3MLSS profilein batch reactor for pretreated simulated dairy wastewater (SDW) sample under different initial inoculums percentage of *Chlorella pyrenoidosa*

Kinetic study for Nitrate-Nitrogen removal

The experimental data were plotted between $1/R_{xiN}$ and $1/N_0$, $1/R_{xiN} = (Ks/k)(1/N) + 1/k$ to make a straight line with K_s/k and $1/k$ as its slope and intercept respectively as shown in Fig. 3.4a. The values of k and K_s were 43.48 per day and 16.21 mg/L for nitrate nitrogen reduction. The growth kinetics of *Chlorella pyrenoidosa* based on nitrate nitrogen reduction was plotted against change in algal biomass with consumption of nitrate nitrogen as substrate (N_0-N_f) shown in Fig. 3.4b. From this plot the value of Yield coefficient Y_N was found to be 0.013 g of biomass/mg of NO_3^--N as N.

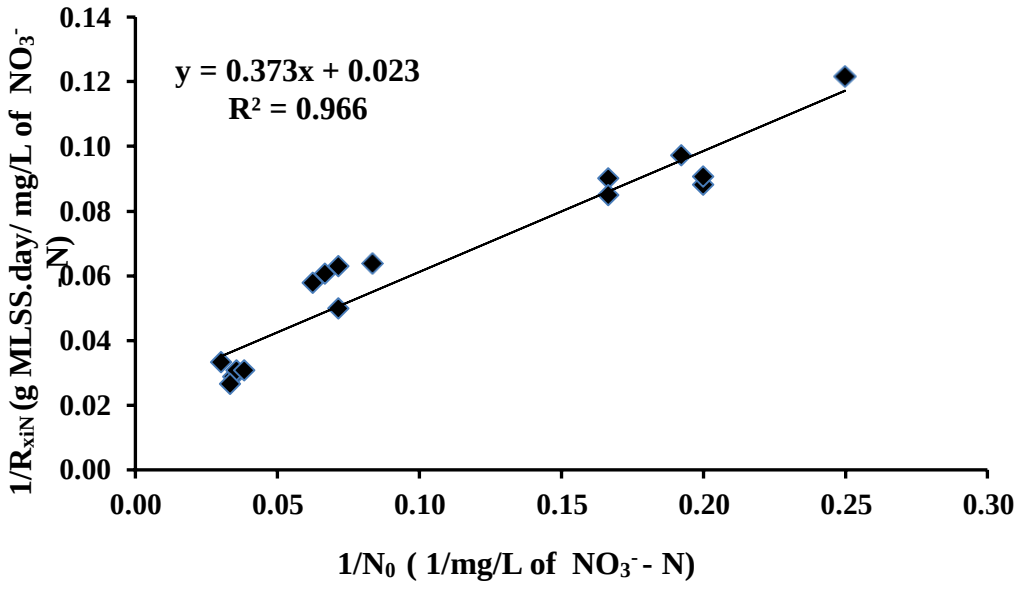


Fig. 3.4aSubstrate-utilization Kinetics of *Chlorella pyrenoidosa* for nitrate nitrogen removal in batch reactor from pretreated simulated dairy wastewater (SDW) [Initial NO_3^--N = 54 mg/L as N]

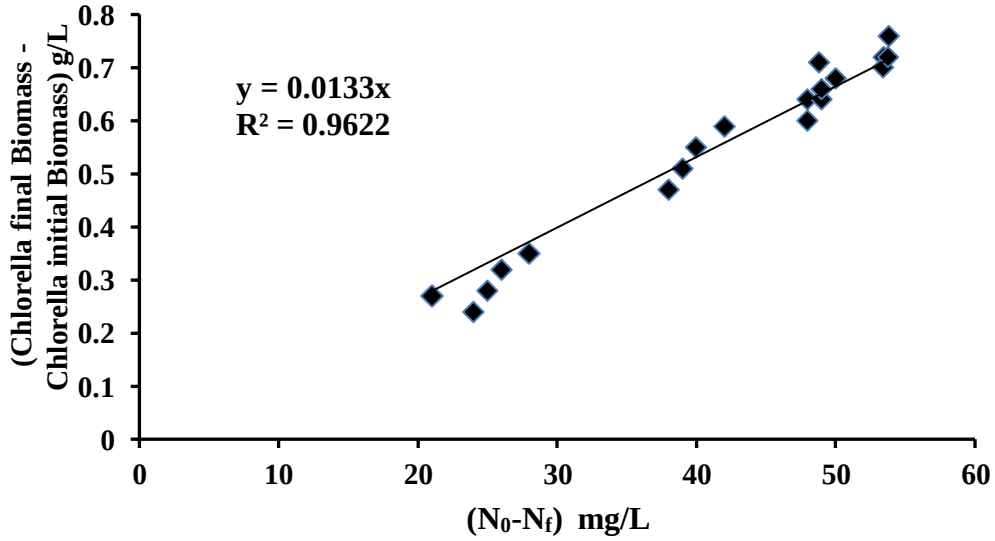


Fig. 3.4bMicroalgal growth kinetics *Chlorella pyrenoidosa* for nitrate nitrogen removal in batch reactor from pretreated simulated dairy wastewater (SDW) [Initial NO_3^--N = 54 mg/L as N]

Kinetic study for Phosphorus removal

The experimental data were plotted between $1/R_{xiP}$ and $1/P_0$ similarly as was done in previous kinetic study of Nitrate Nitrogen removal, $1/R_{xiP} = (Ks/k)(1/P_0) + 1/k$ to make a straight line with best fit with K_s/k and $1/k$ as its slope and intercept respectively as shown in Fig. 3.4c. The k and K_s values were found to be 7.14 per day and 5.41 mg/L for phosphorus removal. The growth kinetics based on phosphorus removal was plotted against change in algal biomass with consumption of phosphorus as substrate (P_0-P_f) shown in Fig. 3.4d. From this plot the value of Yield coefficient Y_P was found to be 0.053 g of biomass/mg of PO_4^{3-}- P as P.

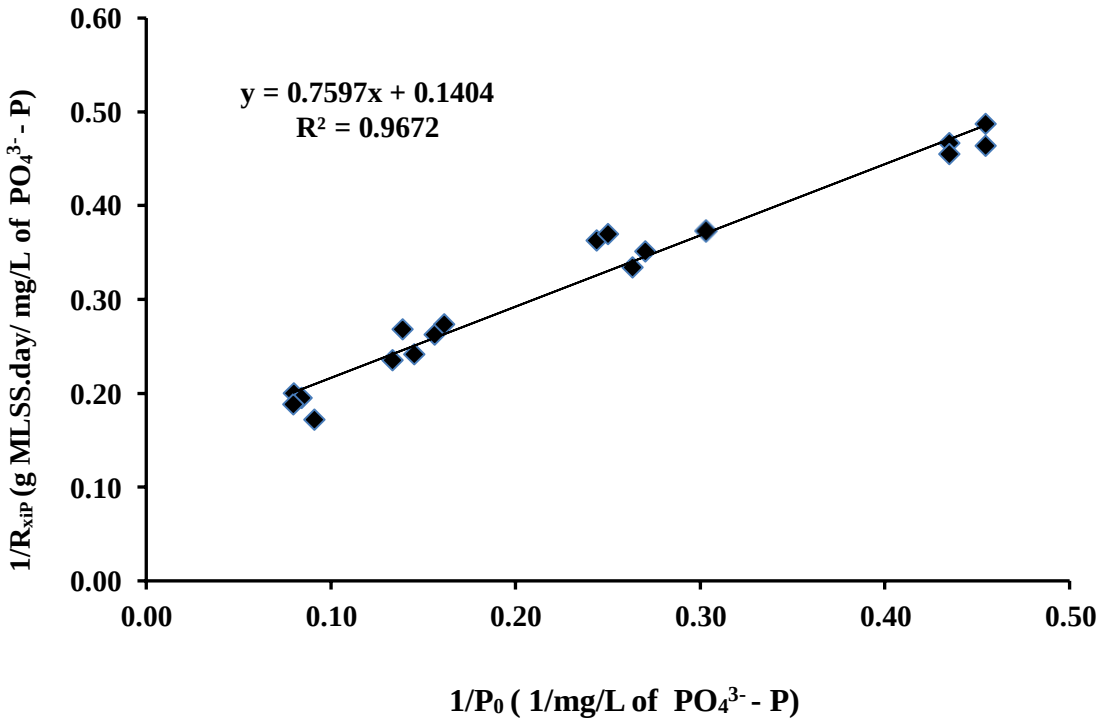


Fig. 3.4cSubstrate-utilization Kinetics of *Chlorella pyrenoidosa* for phosphorus removal in batch reactor from pretreated simulated dairy wastewater (SDW) [Initial PO_4^{3-} - P = 16 mg/L as P]

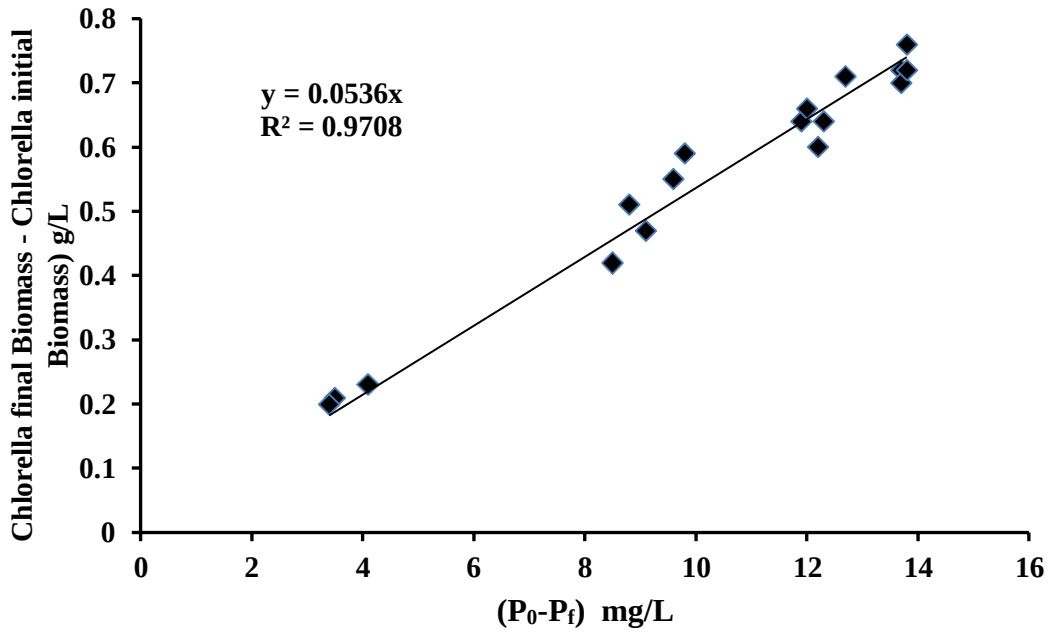


Fig. 3.4dMicroalgal growth kinetics of *Chlorella pyrenoidosa* for phosphorus removal in batch reactor from pretreated simulated dairy wastewater (SDW) [Initial PO_4^{3-} - P = 16 mg/L as P]

Chapter 4

Treatability and Kinetic Study of Dairy Effluent using Microalgae Spirulinaplatensis in a Laboratory Scale Batch Reactor

Introduction and Literature Review

Dairy industry is seen as one of the greatest source of wastewater producing industry. The dairy wastewater contains gigantic measures of milk constituents, like casein, inorganic salts along with other chemicals and sanitizers used for washing and cleaning. These organics and nutrients have potential to cause nuisance if left untreated. To reduce these waste loads different types of treatment methods are available. These removal techniques may be physical, chemical or biological. Physical treatment methods alone are not so efficient and so it may be done in line with biological and chemical treatment. Chemical treatments are generally much expensive. Treatment with biological agents like plants, microbes or algae is a relatively cost effective and green technique of waste removal as very small amount of secondary pollutants and contaminants are generated which cause less impact on the environment unlike chemical treatment methods where a number of hazardous chemicals are produced during wastewater processing. The biggest problem especially the developing nations are facing is the discharge of wastewater into the streams, agricultural field or in other sites with having very little treatment. The major reason behind carrying out little or no wastewater treatment in case of medium or small companies or plants is associated with high cost of chemical treatment. An alternate treatment with biological agents like algae is a novel approach. Algae can grow in wastewater and have the capability of assimilating CO_2 from air and Nitrogen and Phosphorus from sewage.(Kumar et al. 2010, Lu 2015, Lu 2016). Nitrogen and phosphorus are very important for the proper growth of algae (Lu et al. 2015, Rowley2010). Phosphorus in aquatic system is generally found bounded with sediments (Engblom 1998). Sequestering CO_2 from air by algae also help maintaining the level of Green house gases which accomplish the goals of Kyoto Protocol (Mata et al. 2010). Research has been carried out

to evaluate the treatment efficiency of algae in treating wastewater where lots of organic nutrients are present. The findings of some of these researches are highlighted in next passage.

A group of researchers found that the combination of two algal species namely *Chlorella vulgaris* of strain ID LS120 and *Scenedesmus obliquus* of strain ID LS121 at different concentrations effectively removed TDS, BOD and SCOD levels of effluents collected from leather industry after completion of 21 days of phycoremediation (Elumalai et al 2014).Some studies suggested that *Spirulina platensis is* capable of growing in low cost seawater and consumption of ample amount of nutrients(Sandeep et al. 2015). Another study conducted to indicate the influence of nitrogen and phosphorus concentration on the growth of *Spirulina platensis* and observed that there was nosignificant difference in growth and substrate removal with the marginal variation in nitrogen andphosphorus; two of the major nutrients for their growth (Fernandes et al. 2014).

The aim of this study is to evaluate the efficiency of microalgae *Spirulina platensis* in the pretreated wastewater rich in Nitrogen and Phosphorus. Dairy wastewater after a primary treatment with a consortium of bacterial population containing Carbon oxidation and nitrification was taken as the feed material for treatment process in this present investigation and *Spirulina platensis* strain was used for this purpose. Pretreated wastewater rich in high nitrate nitrogen and phosphorous concentration. After treatment the concentration of nitrate as nitrogen and phosphate as phosphorus become reasonably low and met with the discharge standards prescribed by the environmental regulatory authorities like WHO and CPCB. The present investigation was done for algal treatment of simulated dairy effluent containing nitrate nitrogen and phosphate in a lab-scale batch reactor with acclimatized seeds of microalgae *Spirulina platensis* and also to evaluate various bio kinetic coefficients of microalgae *Spirulina platensis* require for design of an appropriate suspended growth biological reactor applicable for algal treatment of nutrient rich dairy effluent.

Materials and Methods

Preparation of simulated dairy wastewater and Primary treatment

During pretreatment process with acclimatized seed of carbonaceous bacteria and nitrifier in laboratory scale. Suspended growth batch reactor the organic carbon in terms of SCOD and ammonium nitrogen (NH_4^+-N) content of dairy effluent become reduced. Initial high concentration of NH_4^+-N was transformed into NO_3^--N during pretreatment process. Phosphorous concentration remained more or less similar as it was initially present in the wastewater. pH of this pretreated-dairy wastewater was slightly in the acidic range as nitrifier bacteria produced some acid in the medium. Major objective of this pretreatment process of dairy wastewater was that to reduce SCODand NH_4^+-N concentration as well as convert all NH_4^+-N into nitrate nitrogen (NO_3^--N). Now pretreated dairy wastewater sample containing high value of nutrients (NO_3^--N and phosphorous) was prepared synthetically in a laboratory and used for further treatment using microalgae *Spirulina platensis*. This pretreated simulated dairy wastewater (SDW) was diluted in a ratio of 1:4 with distilled water during batch study using microalgae *Spirulina platensis.* In every batch study prepared pretreated SDW was sterilized at 121^o C temperature for destroying all microorganisms. After that the sterile pretreated SDW was inoculated with varying innoculum concentration of acclimatized pure culture of microalgae *Spirulina platensis* in batch study.

Composition of SDW (g/L) was as Milk powder 0.01, Dextrose 0.01, Peptone 0.25, Beef extract 0.10, Lactose 0.01, Ammonium sulfate 0.1, Potassium nitrate 1.5, Di hydrogen potassium phosphate 0.20, Di potassium hydrogen phosphate 0.20. pH of the SDW medium was adjusted around 7.0

Collection of Algal strain

A pure culture of *Spirulina platensis* strain was obtained in solid growth medium as slant culture from the NICM laboratory, Pune. It was kept at refrigerated condition at 4°C temperature before it was transferred aseptically in the liquid broth medium prepared in accordance with the composition mentioned below.

Preparation of Growth Medium

50 ml of growth culture medium was prepared in 250 ml conical flask. Prepared medium was sterilized at 121℃ temperature for 15 min in an autoclave. Composition of medium for *Spirulina platensis* (in g/L): Sodium bi carbonate -10.0, Sodium nitrate - 2.5, Sodium chloride – 1.0, Di potassium hydrogen phosphate – 0.5, Potassium sulphate – 1.0, Magnesium sulphate hepta-hydrate – 0.2, Calcium chloride – 0.04 and Ferrous sulphate – 0.01. All the constituents were dissolved in water and pH was adjusted in between the range of 7.2 – 7.5 using dilute HCl and dilute NaOH.

Transfer of stock culture into Growth Medium

From the stock culture inoculum was taken with an inoculation loop and was transferred into previously prepared sterile growth medium. The whole process was performed aseptically inside a laminar airflow (LAF) chamber to avoid cross contamination. This inoculated growth culture was kept in wooden racks in the laboratory after plugged with cotton at the open mouth of the Erlenmeyer flask. Light Emitting Diode (LED) tubes were lit inside the wooden racks for bright illumination purpose. Flask was kept under ambient temperature for about two weeks.

Seed Acclimatization for *Spirulina platensis*:

The study was conducted in an Erlenmeyer flask of 500 ml capacity as an acclimatization unit. About 10 mL of the algae from the cultured broth medium was added to 100 ml of Synthetic Feed medium diluted with distilled water in a ratio of 1:4. The composition of original undiluted Synthetic Feed medium (g/L) Milk powder 0.01, Dextrose 0.01, Peptone 0.20, Beef extract 0.20, Lactose 0.02, Ammonium sulphate 0.1, Potassium nitrate 1.5, Di-hydrogen potassium phosphate 0.20, Di-potassium hydrogen phosphate 0.20. pH of Synthetic Feed medium was adjusted in between the range of 7.0 – 7.2 byusing dilute HCl and dilute NaOH.

Efficacy study

After the acclimatization process was over; different percentages of inoculums were transferred from the acclimatized culture of *Spirulina platensis* to the prepared stimulated dairy wastewater (BTDW). 100 ml of sterilized BTDW was used for this purpose in five Erlenmeyer flasks of 500 mL and kept for 10 days in shaking condition at ambient temperature. Inoculum concentrations of 0.2, 0.5, 0.8, 1.0 and 1.2% (v/v) were taken in five different Erlenmeyer flasks. Samples were withdrawn from all five flasks and concentration of phosphate and nitrate nitrogen was measured using standard protocol (Rice et al. 2012).

Time concentration study

100mL of sterilized SDW was taken in five conical flasks of 500 mL capacity and kept for 8 days in shaking condition at ambient temperature with added inoculums of different percentages as used in the efficacy study. Inoculums concentrations in five flasks were 0.2, 0.5, 0.8, 1.0 and 1.2% (v/v). Samples were withdrawn periodically in an interval of two days from all five flasks and concentration of algal biomass, phosphate and nitrate nitrogen concentration was measured using standard protocols (Rice et al. 2012). Phosphate concentration and algal biomass was measured through colorimetric method by using a UV Spectrophotometer made by Lassany and Nitrate nitrogen concentration was measured using Nitrate Ion Selective Electrode (ISE) probe made by Thermo Fischer Limited. Biomass was also measured using gravimetric method to establish a correlation between Mixed Liquor Suspended Solids (MLSS) in g/L value with their corresponding Absorbance (ABS) value.

Substrate utilization and algal growth kinetics study

Kinetic parameters bear technological importance in designing Bioreactor (Lee 1992). By conducting experiment, different values of kinetic coefficients were estimated as steady state kinetics for stabilization of Nitrate Nitrogen and Phosphate. Kinetic coefficients viz. Y, Ks and k were obtained on the basis of experimental results by varying MLSS concentration. Kinetic study was done by using Monod kinetics model equation.

For Nitrate nitrogen removal study inverse values of specific substrate (NO_3^--N) utilization rate ($1/R_{xiN}$) were plotted against the inverse values of Nitrate nitrogen (NO_3^--N) concentration ($1/N_0$) and substrate-removal kinetics was obtained by using simple linear equation, $1/R_{xiN} = (Ks/k)(1/N)$

+ 1/k. The values of the change in algal biomass in Y axis with consumption of Nitrate nitrogen as substrate (N_0-N_f) in X axis gives the growth kinetics equation in the form of y = mx where the slope (m) of the straight line is Yield Coefficient (Y_N)

Phosphorus removal-kinetics were also evaluated using linear equation, $1/R_{xiP} = (Ks /k)(1/P) + 1/k$.

The values of the change in algal biomass in Y axis with consumption of Phosphate as substrate (P_0-P_f) in X axis gives the growth kinetics equation (y = mx) where the slope (m) of the straight line is Yield Coefficient (Y_P)

Results and Discussions

Efficacy study

Removal potential of Nitrate nitrogen (NO_3^--N) and Phosphorous (PO_4^{3-}- P) under different percentage of inoculums have been shown in Fig. 4.1. From Fig 4.1 it has been observed that both NO_3^--N and PO_4^{3-}- Premoval percentage was increased from 0.2 to 0.8% v/v inoculums, after that it was decreased due to limiting concentration of NO_3^--N and PO_4^{3-}- P concentration in reactor. It has been also observed that almost 99.00% and 90.38% of NO_3^--N and PO_4^{3-}- P were removed after 8 days batch contact time respectively, corresponding to initial inoculums concentration of 0.8% v/v. The MLSS concentration of 0.10 g/L equivalent to 0.8 % v/v inoculums was found to be optimum for maximum NO_3^--N and PO_4^{3-}- P removal corresponding to an initial NO_3^--N and PO_4^{3-}P concentration of 54 and 16 mg/L after 8 days batch contact time.

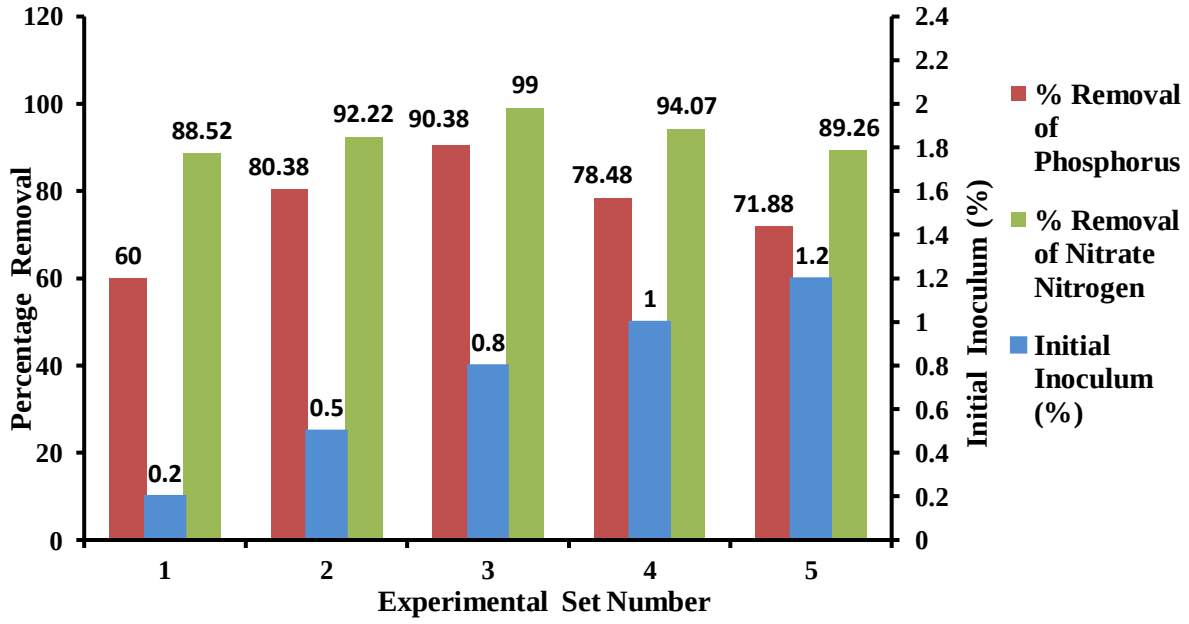


Fig. 4.1: Nitrate nitrogen and phosphorus removal profile in batch reactor for pretreated simulated dairy wastewater (SDW) sample under different initial inoculums percentage of *Spirulina platensis*

Time concentration study of pretreated simulated dairy wastewater (SDW) for removal of Nitrate Nitrogen and Phosphorus in Batch reactor

Five set of time concentration study for removal of nitrate nitrogen and phosphorus in batch reactor with an initial NO_3^-N and PO_4^{3-}- P concentration of 54 mg/L as N and 16 mg/L as P using acclimatized culture of *Spirulina platensis* has been shown in Fig 4.2a-4.2e corresponding to five different initial inoculums percentages of 0.2, 0.5, 0.8, 1, 1.2 v/v. From 4.2a it was observed that 88.52 % and 60 % of NO_3^--N and PO_4^{3-}- P were removed after 8 days batch contact time respectively,corresponding to initial inoculums concentration of 0.2% v/v. It also demonstrated in fig 4.2b thatthere was a slight increase of NO_3^--N and PO_4^{3-}P removal of 92.22 % and 80.38 % after 8 days respectively, corresponding to initial inoculum concentration of 0.5 % v/v. The maximum 99.00

% and 90.38 % of NO_3^--N and PO_4^{3-}- P removal were achieved respectively with an initial inoculum concentration of 0.8 % v/v after 8 days has been shown in the Fig 4.2c. When an initial inoculums concentration was increased further, removal of NO_3^--N and PO_4^{3-}- P decreased to 94.07 % and 78.48% respectively for 1 % v/v initial inoculums concentration and for 1.2 % v/v initial inoculums concentration it wasdecreased to 89.26 % and 71.88 % respectivelyas shown in Fig 4.2d and 4.2e respectively after 8 days batch contact time.

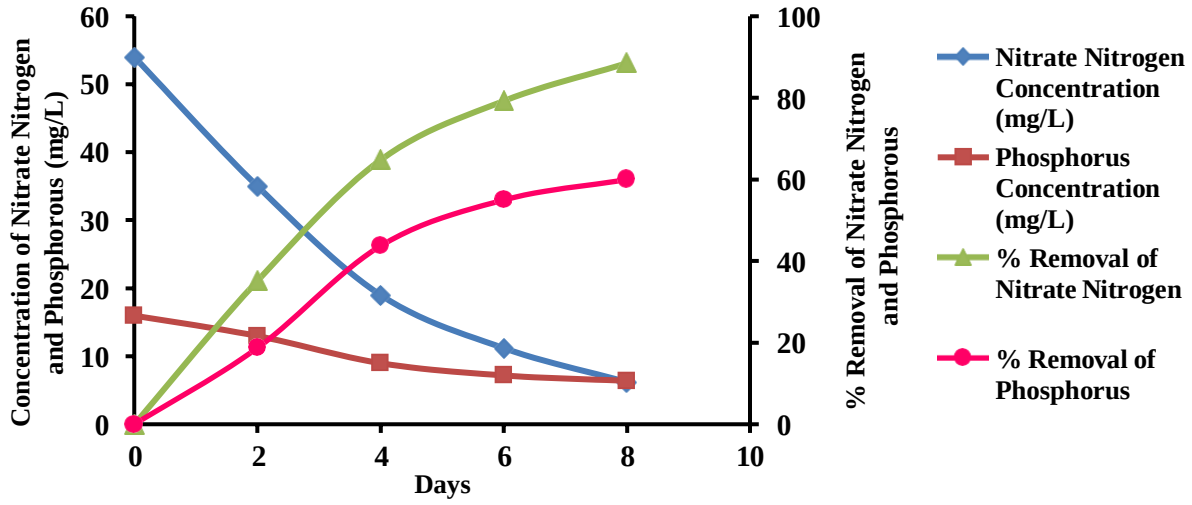


Fig. 4.2a: Nitrate nitrogen and phosphorus removal profile in batch reactor for pretreated simulated dairy wastewater (SDW) sample [Set 1: Initial NO_3^--N = 54 mg/L as N, initialPO_4^{3-} - P = 16 mg/L as P, initial inoculums of *Spirulina platensis*= 0.2% v/v, batch contact time = 8 days]

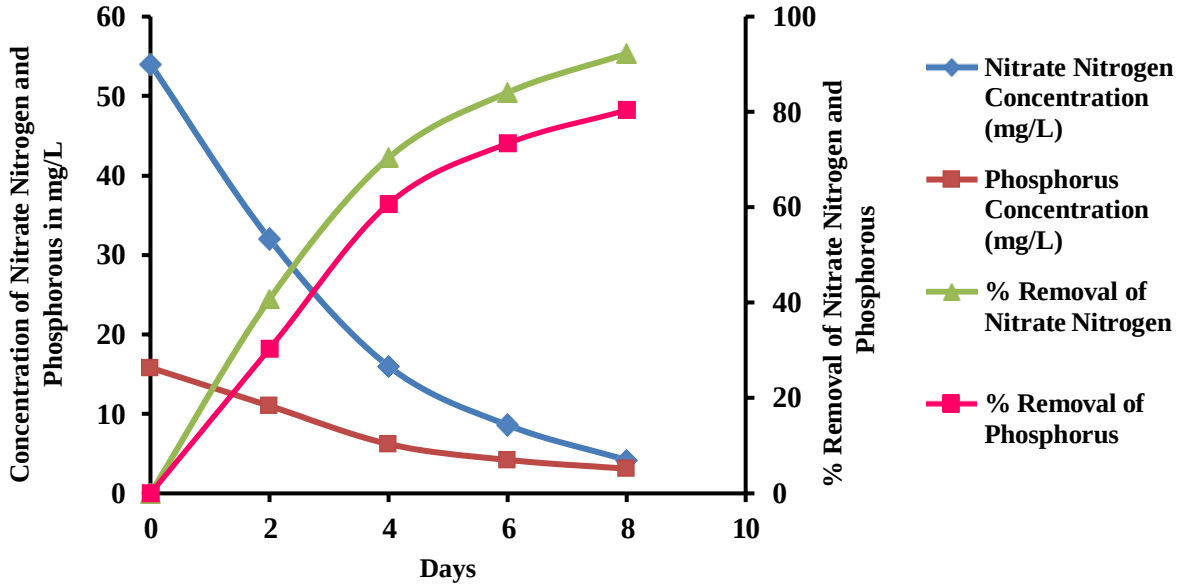


Fig. 4.2b: Nitrate nitrogen and phosphorus removal profile in batch reactor for pretreated simulated dairy wastewater (SDW) sample [Set 2: Initial NO_3^--N = 54 mg/L as N, initialPO_4^{3-} - P = 16 mg/L as P, initial inoculums of *Spirulinaplatensis*= 0.5 % v/v, batch contact time = 8 days]

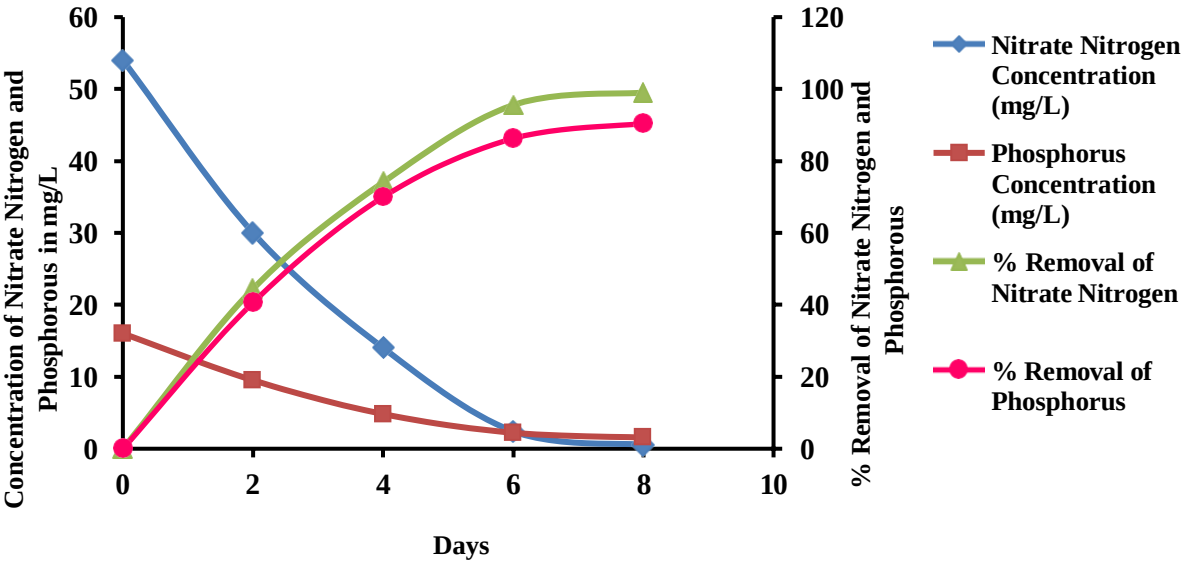


Fig. 4.2c: Nitrate nitrogen and phosphorus removal profile in batch reactor for pretreated simulated dairy wastewater (SDW) sample [Set 3: Initial NO_3^--N = 54 mg/L as N, initialPO_4^{3-} - P = 16 mg/L as P, initial inoculums of *Spirulina platensis*= 0.8 % v/v, batch contact time = 8days]

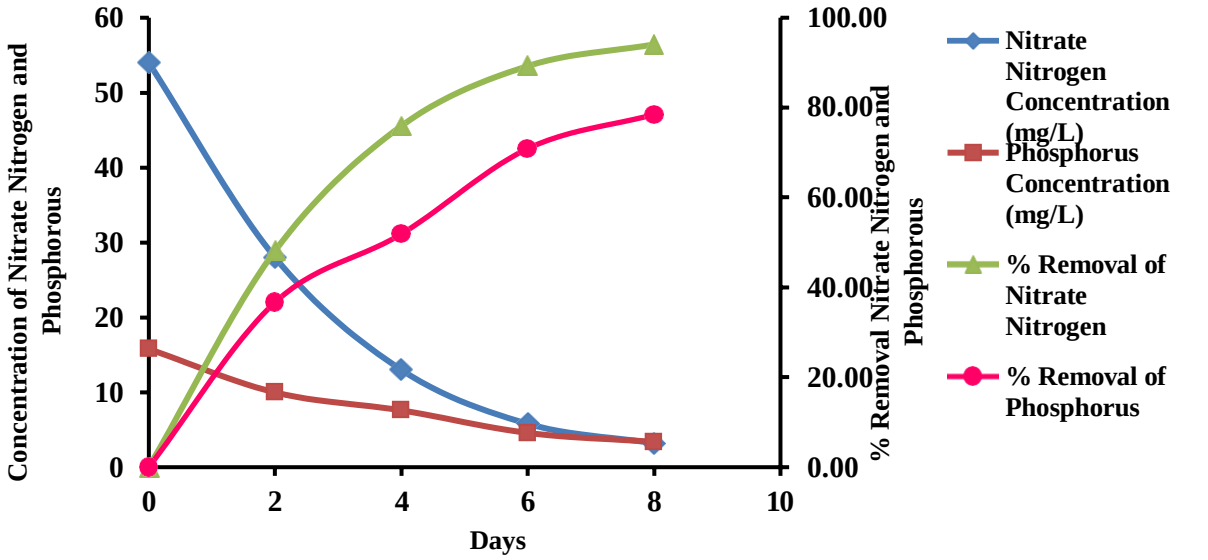


Fig. 4.2d: Nitrate nitrogen and phosphorus removal profile in batch reactor for pretreated simulated dairy wastewater (SDW) sample [Set 4: Initial NO_3^--N = 54 mg/L as N, initialPO_4^{3-} - P = 16 mg/L as P, initial inoculums of *Spirulina platensis*= 1 % v/v, batch contact time = 8 days]

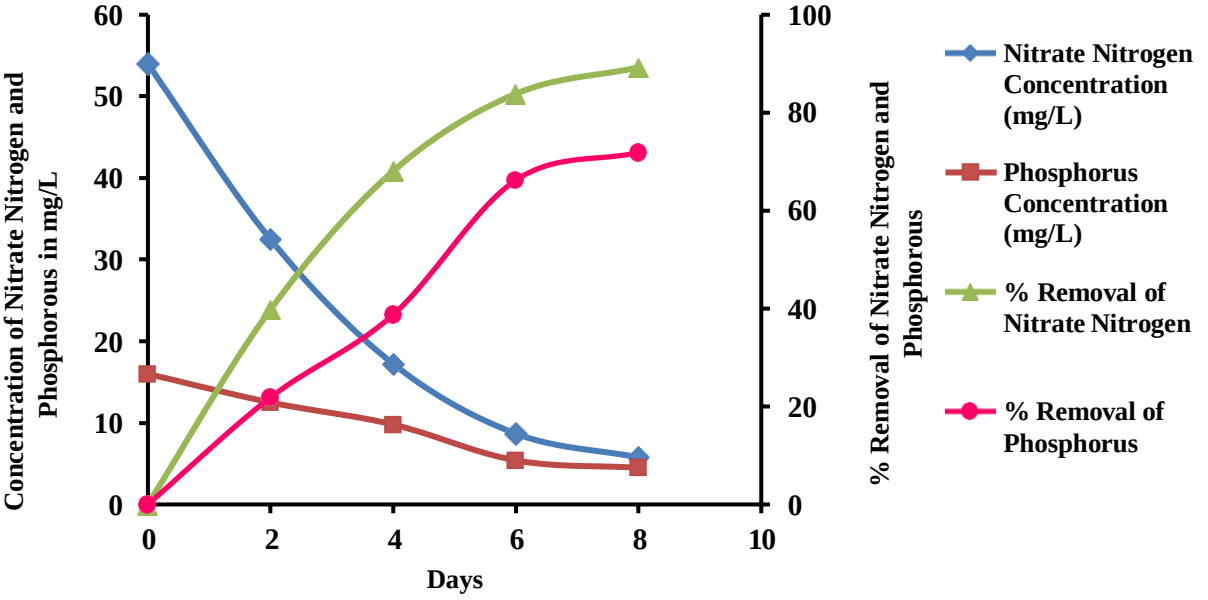


Fig. 4.2e: Nitrate nitrogen and phosphorus removal profile in batch reactor for pretreated simulated dairy wastewater (SDW) sample [Set 5: Initial NO_3^--N = 54 mg/L as N, initialPO_4^{3-} - P = 16 mg/L as P, initial inoculums of *Spirulina platensis*= 1.2 % v/v, batch contact time = 8days]

MLSS Profile in Batch reactor

Fig. 4.3 corresponding to five different initial inoculums percentages of 0.2, 0.5, 0.8, 1, 1.2 v/v, it exhibited that a steady increase in MLSS level was occurred starting from the commencement of the batch study. It demonstrates that initial MLSS concentration have different values of 0.03, 0.07, 0.10, 0.14, 0.16 g/L corresponding to five different initial inoculums percentages of 0.2, 0.5, 0.8, 1, 1.2 v/v respectively. After 8 days of batch contact time in batch reactor the MLSS concentration increased to 0.66, 0.75, 0.78, 0.82, 0.79 g/L corresponding to five different initial inoculums

percentages of 0.2, 0.5, 0.8, 1.0, 1.2 v/v respectively.

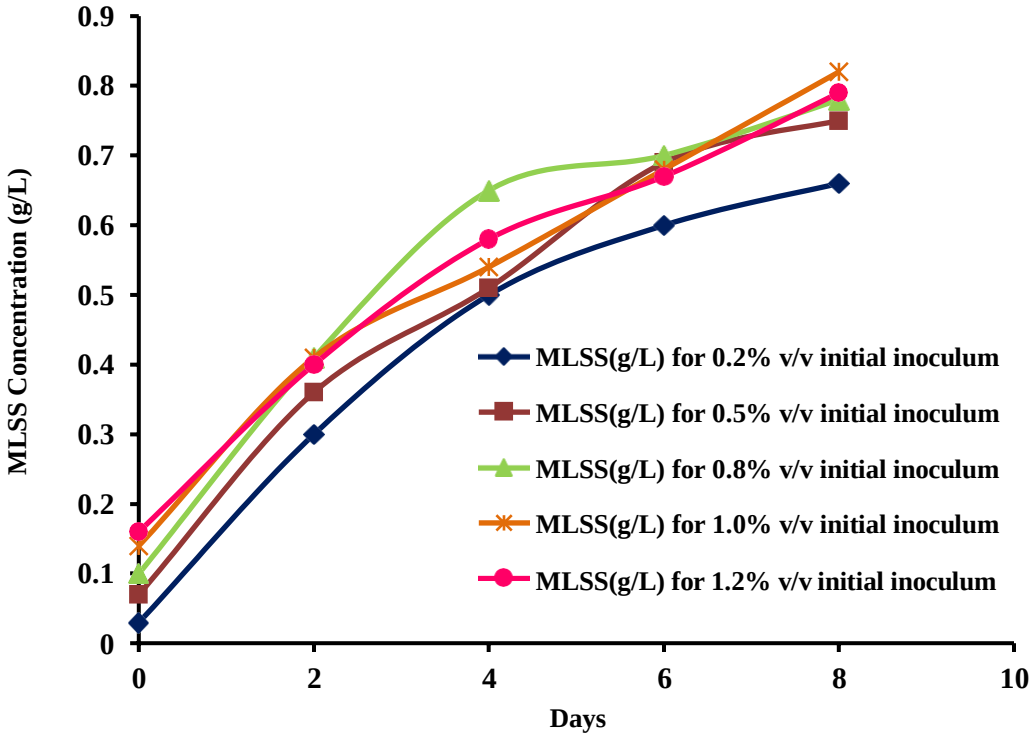


Fig.4.3: MLSS profilein batch reactor for pretreated simulated dairy wastewater (SDW) sample under different initial inoculums percentage of *Spirulina platensis*

Kinetic study for Nitrate-Nitrogen removal

The experimental data were plotted between $1/R_{xiN}$ and $1/N_0$, by $1/R_{xiN} = (Ks/k)(1/N) + 1/k$ to make a straight line of best-fit with K_s/k and $1/k$ as its slope and intercept respectively as shown in Fig. 4.4a. The k and K_s values were obtained as21.74 per day and 1.61 mg/L for nitrate nitrogen reduction The growth kinetics of *Spirulina platensis* based on nitrate nitrogen reduction was plotted against change in algal biomass with consumption of nitrate nitrogen as substrate (N_0-N_f) shown in Fig. 4.4b. From this plot the value of Yield coefficient Y_N was found to be 0.011 g of biomass/mg of NO_3^--N as N.

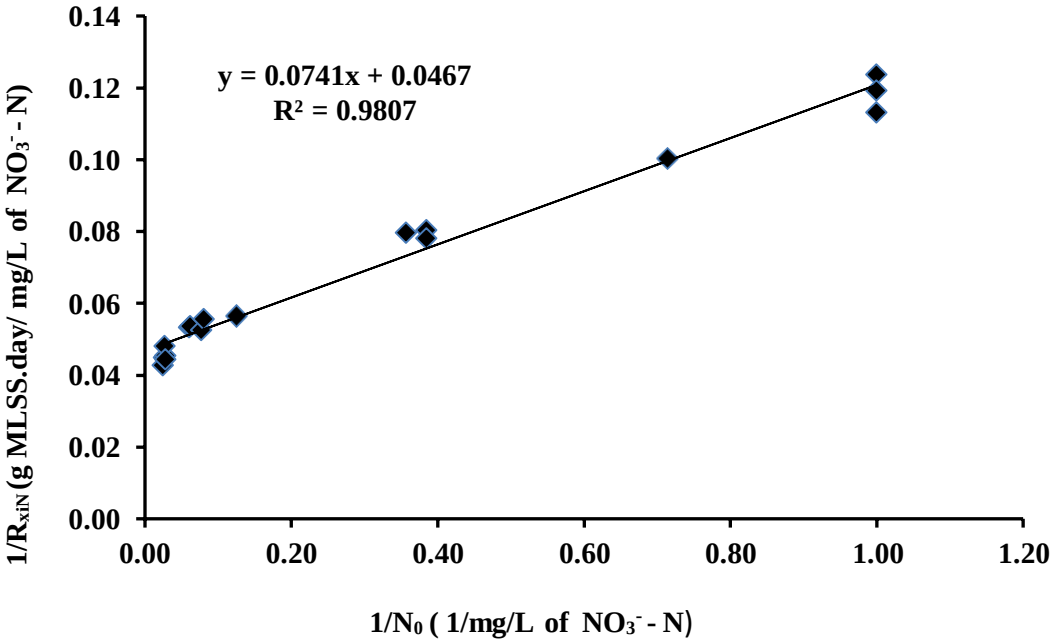


Fig 4.4a. Substrate-utilization Kinetics of ***Spirulina platensis*for nitrate nitrogen removal in batch reactor from pretreated simulated dairy wastewater (SDW) [Initial NO_3^--N = 54 mg/L as N]**

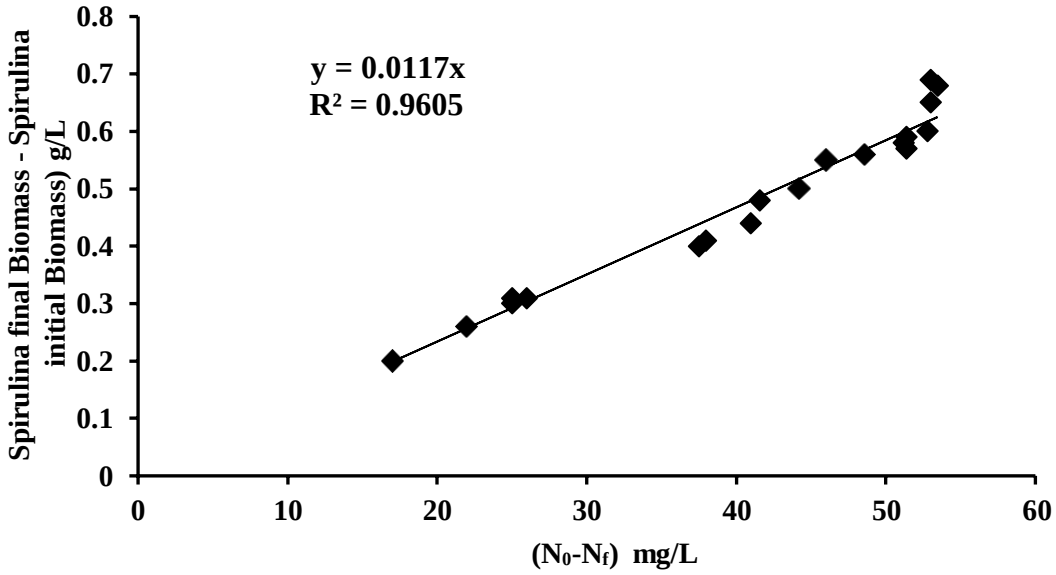


Fig 4.4b.Microbial-growth kinetics *Spirulina platensis*for nitrate nitrogen removal in batch reactor from pretreated simulated dairy wastewater (SDW) [Initial NO_3^--N = 54 mg/L as

Kinetic study for Phosphorus removal

The experimental data were plotted between $1/R_{xiP}$ and $1/P_0$ similarly as was done in previous kinetic study of Nitrate Nitrogen removal, by using equation $1/R_{xiP} = (Ks/k)(1/P_0) + 1/k$ to make a straight line with best fit with K_s/k and $1/k$ as its slope and intercept respectively as shown in Fig. 4.4c. The k and K_s values were found to be 14.49 per day and 16.63 mg/L for phosphorus removal. The growth kinetics based on phosphorus removal was plotted against change in algal biomass with consumption of phosphorus as substrate (P_0-P_f) shown in Fig. 4.4d. From this plot the value of Yield coefficient Y_P was found to be 0.052 g of biomass/mg of PO_4^{3-}- P as P.

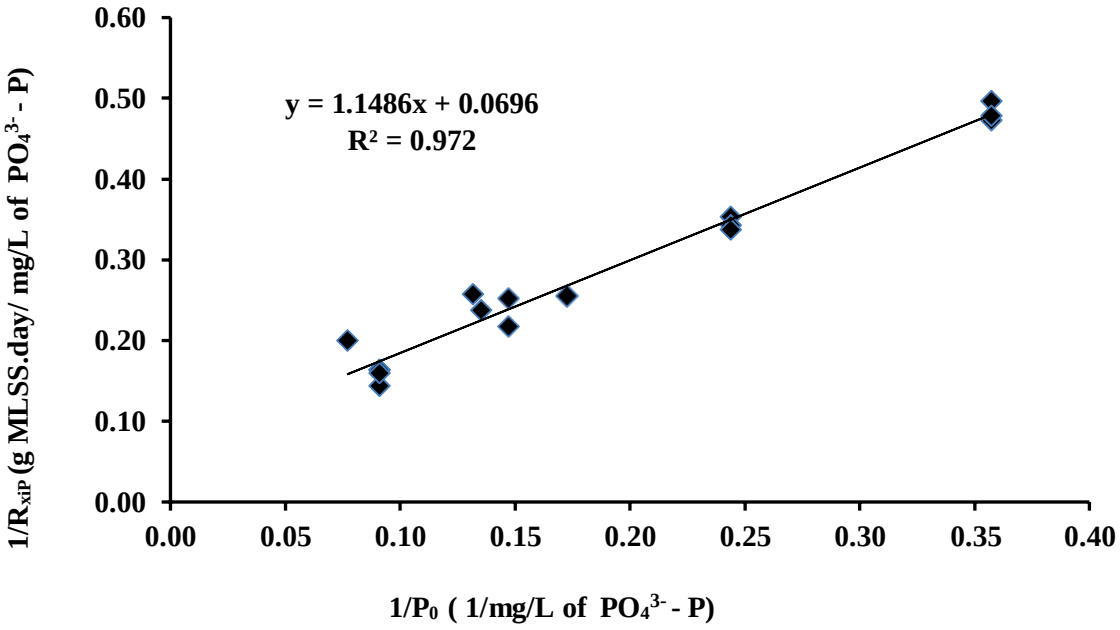


Fig 4.4c. Substrate-utilization Kinetics of ***Spirulina platensis*****for phosphorus removal in batch reactor from pretreated simulated dairy wastewater (SDW) [Initial PO_4^{3-} - P = 16 mg/L as P]**

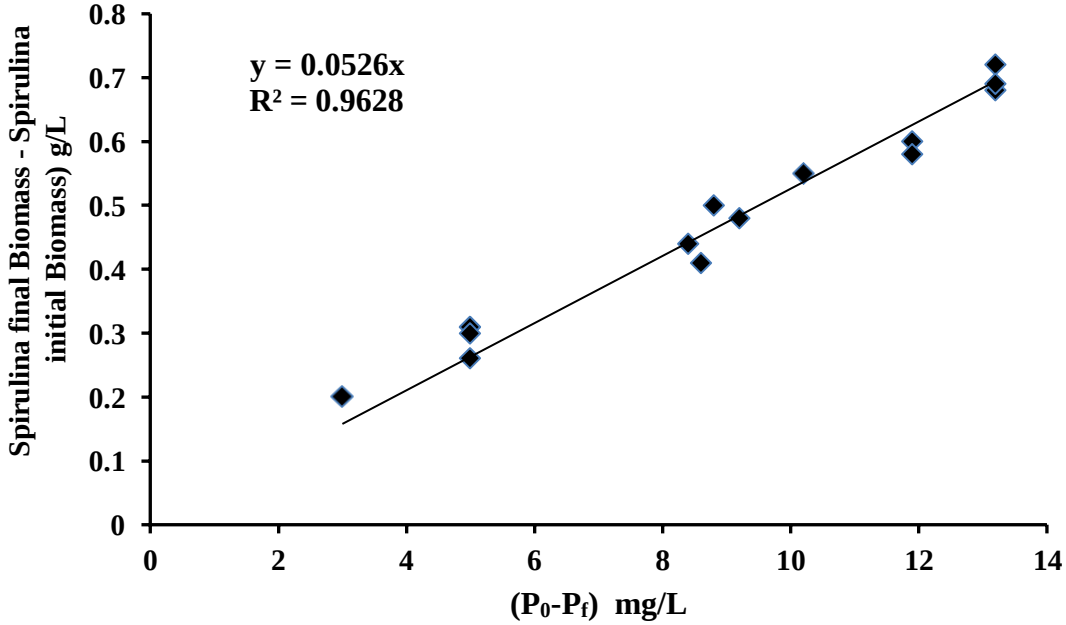


Fig 4.4d.Microbial-growth kinetics of ***Spirulina platensis*** **for phosphorus removal in batch reactor from pretreated simulated dairy wastewater (SDW) [Initial PO_4^{3-} - P = 16 mg/L as P]**

Chapter 5

Combined treatment of Real life dairy wastewater in a laboratory scale Suspended Growth Batch Reactor using mixed bacterial consortium and micro algae

Introduction

Dairy Industries are one of the most polluting food and agro-processing industries. Milk sugar – lactose, different types of milk proteins, fats and different other compounds used in the production of various dairy products attribute to its high organic and nutrient loads which must be treated efficiently to prevent environmental and health hazard. In this study potency of combined treatment with consortium of bacteria and microalgae in two separate stages was evaluated by means of Response Surface Methodology (RSM). Obtained results suggest and upheld the significance of biological treatment process for wastewater.

This study was conducted to investigate the combined two-stage biological-treatment-process using consortium of bacteria and microalgae for removal of SCOD, NH_4^+-N, NO_3^--N, PO_4^{3-}-P from dairy wastewater in SGBR. The study also investigated the removal of SCOD, NH_4^+-N, NO_3^--N, PO_4^{3-}-P under different process parameters, like initial SCOD, NH_4^+-N,NO_3^--N, PO_4^{3-}-P, MLSS, pH and react timeusing RSM with a five-factor-three-level Central Composite Design(CCD). The experimental results were analysed by ANOVA and second-order polynomial mathematical-models were developed with high correlation efficiency for SCOD, NH_4^+-N removal and NO_3^--N, PO_4^{3-}-P removal using bacterial and micro algal-consortia respectively in SGBR.Individual and combined effects of process variables on responses were studied using three-dimensional response surface plots. Under the optimum operating conditions(i.e. initial SCOD,NH_4^+-N, pH, MLSS of 720 mg L^{-1},55 mg L^{-1} as N, 7 and 1500 mg L^{-1} respectively and react time of 24 hrs) highest removal efficiencies of SCOD (98.61%) and NH_4^+-N(97.42%) were obtained using bacterial consortium and in second-phase using micro algal consortium under optimum conditions(initialNO_3^--N; 55 mg L^{-1} as N, PO_4^{3-}-P; 15 mg L^{-1} as P, pH; 7.5, MLSS; 0.1 g L^{-1} and react time; 8 days)highest removal efficiencies of nitrate nitrogen and phosphorus 98.64% and 90.53% respectively. All results were confirmed by validation experiment. Different combination of input parameters with certain ranges were used in performing RSM studies to optimize output parameters. Which input parameters are significant were also determined through RSM study. In case of both the

treatments initial substrate concentration and initial MLSS were the most significant parameters and significance of pH and React time were little less. Cross factor interaction was also studied with different input parameters to optimize response. Values with significance were selected by their respective p-values of less than 0.05 obtained from ANOVA test.

Optimization of process parameters by using RSM study for the two stage treatment process with bacteria and micro algae is supposed to be helpful for the treatment of real life dairy wastewater in pilot/large scale by designing bioreactor system in effluent treatment plants.

Materials and Methods

(A) Experimental setup with bacterial consortium

Optimization study

Different percentage of inoculums was combined in such a manner by keeping the optimum initial concentration in mind found from the previous individual studies discussed before. Different combination of Carbon oxidation and nitrification bacteria consortium were utilized to make the Optimization study viz. (10 + 4)%, (10 + 5) % and (10 + 6) % ; % Carbon oxidation and nitrification corresponding to 1420, 1500 and 1610 mg/L biomass in terms of MLSS respectively. Initial pH was around 7 and initial SCOD and ammonium nitrogen concentration was 720 and 55 mg/L respectively. This experimental work was done in 1 L measuring cylinders with continuous aeration was done using aquarium pump. Samples were withdrawn from the measuring cylinder after 24 hours to measure the sample SCOD and Ammonium nitrogen using standard protocol. (Rice et al. 2012)

Time concentration study with bacterial consortium

A combination of (10 + 5) %; % Carbon oxidation and nitrification corresponding to around 1500mg/L biomass in terms of MLSS was utilized for performing the time concentration study as it was found to be optimum initial inoculums concentration in the previous optimization study.

Initial pH was around 7 and SCOD and ammonium nitrogen was measured as 720 and 55 mg/L respectively. This experimental work was done in 1 L measuring cylinder with continuous aeration was done using aquarium pumps. Samples were withdrawn from the measuring cylinders periodically after every two hours and SCOD and Ammonium nitrogen was measured for each sample using standard protocol. (Rice et al. 2012)

Substrate utilization and bacterial growth kinetics study

A reactor's performance can be properly evaluated and optimized by evaluating its' reaction kinetics. These kinetic parameters bear technological importance in designing Bioreactor. (Lee, 1992) By conducting experiment, different values of kinetic coefficients are estimated as steady state kinetics for stabilization of SCOD and Ammonium Nitrogen. Kinetic coefficients viz. yield co-efficient (Y), half velocity constant (Ks) and maximum Substrate utilization rate (k) were

obtained on the basis of experimental results by varying MLSS concentration. Kinetic study was done by using Monod kinetics model equation.

A combination of three different Carbon oxidation and nitrification bacteria consortium were utilized to prepare the kinetic study; viz. (10 + 4)%, (10 + 5) % and (10 + 6) % ; % Carbon oxidation and nitrification corresponding to 1420, 1500 and 1610 mg/L biomass in terms of MLSS respectively. Initial pH was around 7 and initial SCOD and ammonium nitrogen concentration was 720 and 55 mg/L respectively. This experimental work was done in 1 L measuring cylinders with continuous aeration by using aquarium pump. Samples were withdrawn from the measuring cylinder periodically at every two hours to measure the sample SCOD and Ammonium nitrogen using standard protocol. (Rice et al. 2012)

(B) Experimental setup with Combination of microalgae

Optimization study

Two species of pure algae *C. pyredenosa* and *S. platensis* were combined in different initial percentage form of (40+60)%, (50+50)% and (60+40)% v/v inoculums respectively by keeping the MLSS value fixed as 100 mg/L which was found to be quite ideal in the previous individual studies of both *C. pyredenosa* and *S. platensis.* These two algal species were utilized for removing the residual phosphate and nitrate nitrogen content present in the wastewater. Conical flask of 1 L capacity was taken for performing the task. Two CFL lights of 10 watts each were used as an illuminating device. It was kept for 8 days under ambient temperature of around 27 degree Celsius. Results were obtained from those three different sets after the optimum batch contact time of 8 days.

Time concentration and Kinetics study

After obtaining an optimum combination of these two species (the mixture of initial inoculums) conical flask of 1 L capacity was taken for performing the time concentration study. Two CFL lights of 10 watts each were used as an illuminating device. Optimum batch contact time was 8 days in the conical flask under ambient temperature of around 27 degree Celsius. Samples from the conical flasks were withdrawn in a periodic manner of two days. Samples were analyzed thoroughly using prescribed standard techniques for necessary parameters (Rice et. al, 2012). Obtained data was utilized for the time concentration plot.

The kinetic study was carried out by varying the initial inoculums concentration in the same way done in the optimization study. Samples were withdrawn periodically from each set at the interval of 2 days for testing the necessary parameters. Obtained data was utilized for the making of kinetic plots.

Results and Discussions

A. Experimental setup with bacterial consortium

Optimization study

Three different sets of initial percentage inoculum of Carbon oxidation and nitrification bacteria consortium; viz. (10 + 4)%, (10 + 5) % and (10 + 6) % ; % Carbon oxidation and nitrification corresponding to 1420, 1500 and 1610 mg/L of MLSS has produced different removal percentages after 24 hours, shown in the following fig 5.1. As the overall removal percentage of SCOD and Ammonium Nitrogen was found to be maximum in case of 1500 mg/L MLSS i.e. (10+5)%; this MLSS was chosen as the optimum MLSS concentration and it has been further utilized for the time concentration study for the combined treatment process.

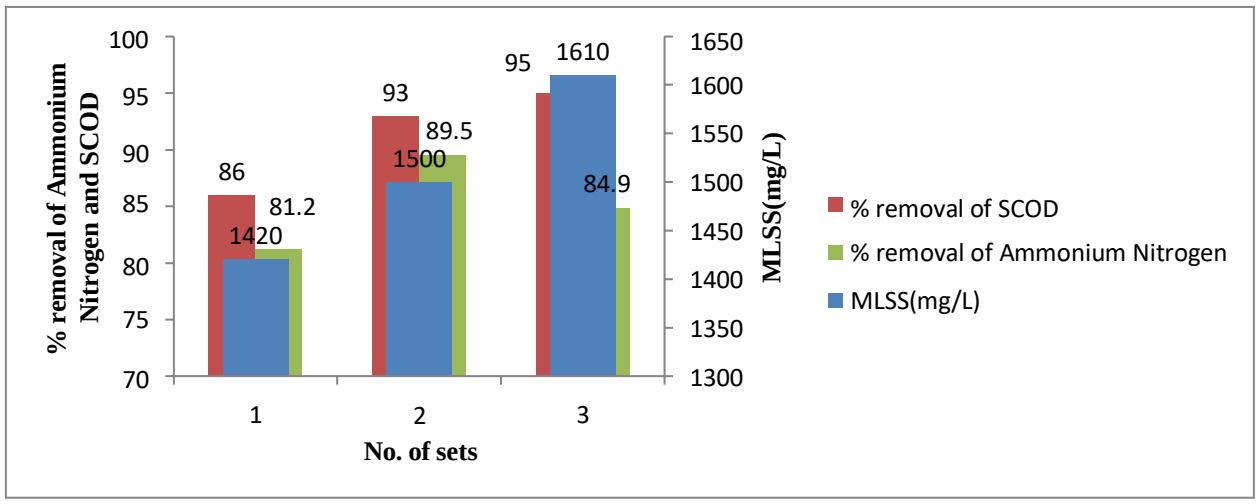


Fig. 5.1.Results of Optimization study

Time concentration study with bacterial consortium

Around 95% of both SCOD and Ammonium nitrogen was removed after 24 hours of batch contact time. Over 60% of SCOD and Ammonium nitrogen was removed from the initial solution after around 6 to 8 hours of detention shown in the fig. 5.3-5.4. A steep slope and then gradually flattening of the curve shows slow removal of both SCOD and Ammonium nitrogen. Both removal of SCOD and Ammonium Nitrogen, percentage removal and growth of bacterial biomass in termsof MLSS is depicted in fig no. 5.2-5.4.

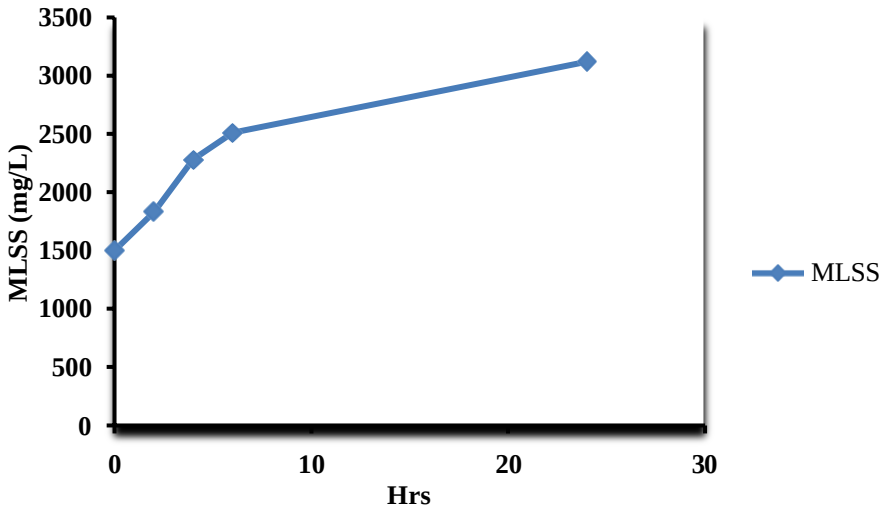


Fig. 5.2. MLSS profile of microbial consortium

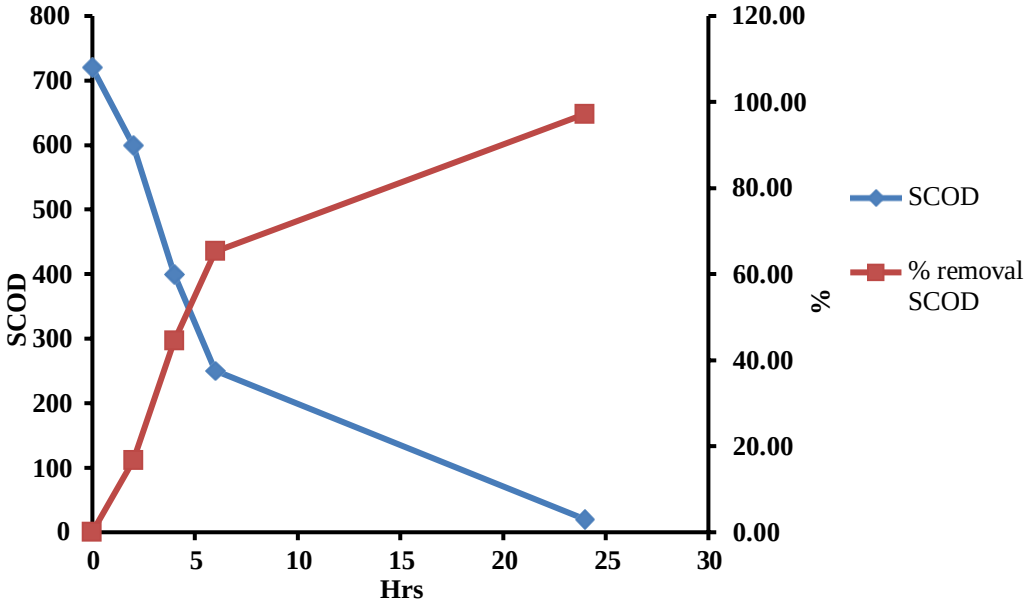


Fig. 5.3. Time concentration study of Carbon oxidation (Initial SCOD=720 mg/L)

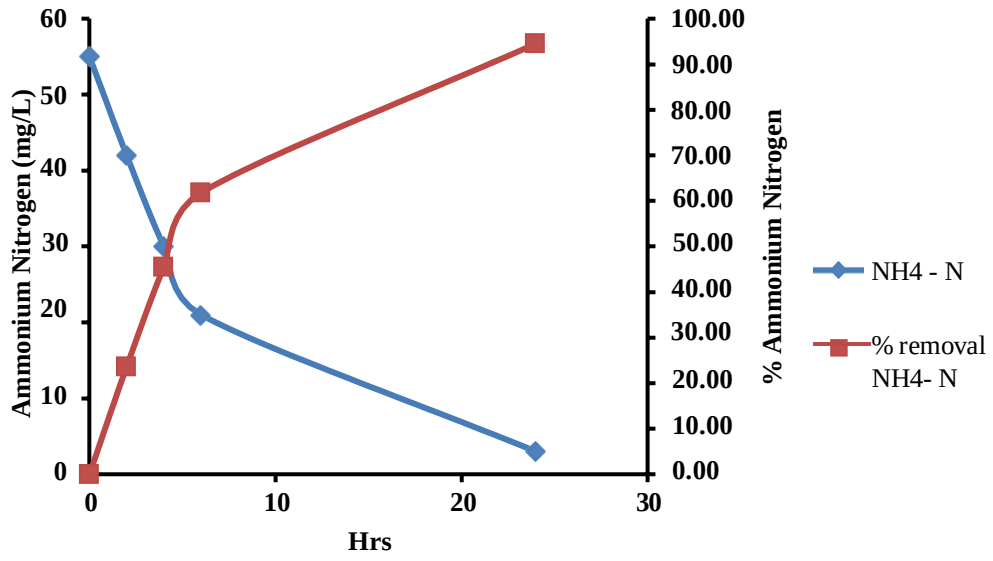


Fig. 5.4. Time concentration study of nitrification (Initial Ammonium Nitrogen = 55 mg/L)

Substrate utilization and bacterial growth kinetics study

Four kinetics coefficients each for Carbon oxidation and nitrification were obtained using modified version of Monod kinetics called Lawrence and McCarty equation. These kinetic coefficients were useful for further designing and fabrication of bioreactor. Similar techniques were applied to obtain the biokinetic coefficients from the kinetic plots as stated in the previous segments of kinetic studies. The values of k, Ks, Y and K_d were obtained from those two straight-line equation. Values of kinetic coefficients obtained by plotting three different data sets in graphical plot of slightly different initial MLSS concentration are as follows: For SCOD removal values of K, K_s, Y and K_d are 1.06 day^{-1}, 69.4 mg/L, 6.097 mg of MLSS/mg of SCOD, and 0.538 day^{-1} respectively and for Ammonium nitrogen removal 0.08 day^{-1}, 19.12 mg/L, 6.28 mg of MLSS / mg of NH_4^+- N, 0.609 day^{-1} respectively obtained from fig. 5.5a-5.5d

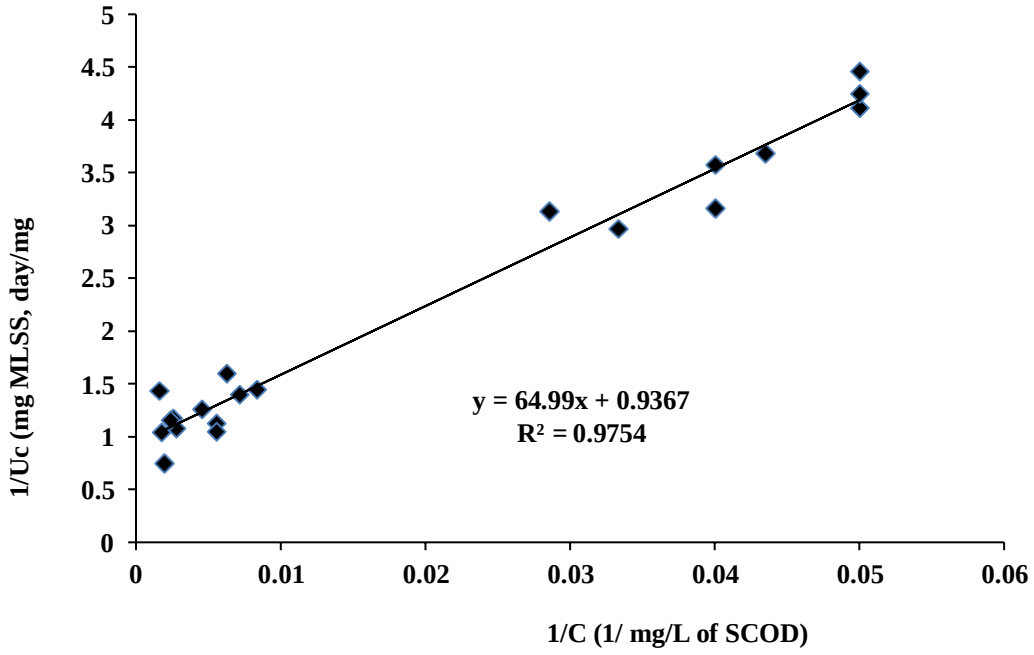


Fig. 5.5aSubstrate-utilization kinetics for Carbon oxidation Study in Batch Reactor [Initial SCOD Concentration of 700-720 mg/L]

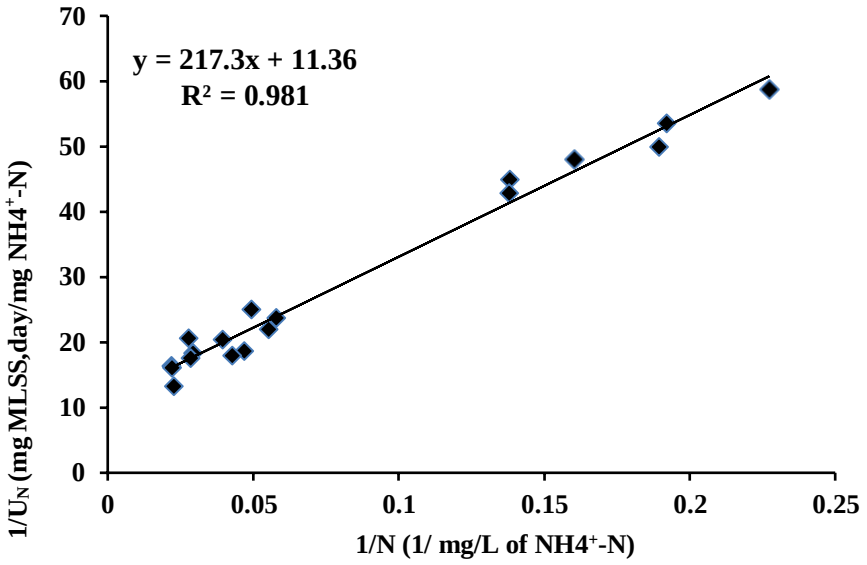


Fig. 5.5b.Substrate-utilization Kinetics for batch nitrification study [Initial NH_4^+-N= 48 -54 mg/L as N]

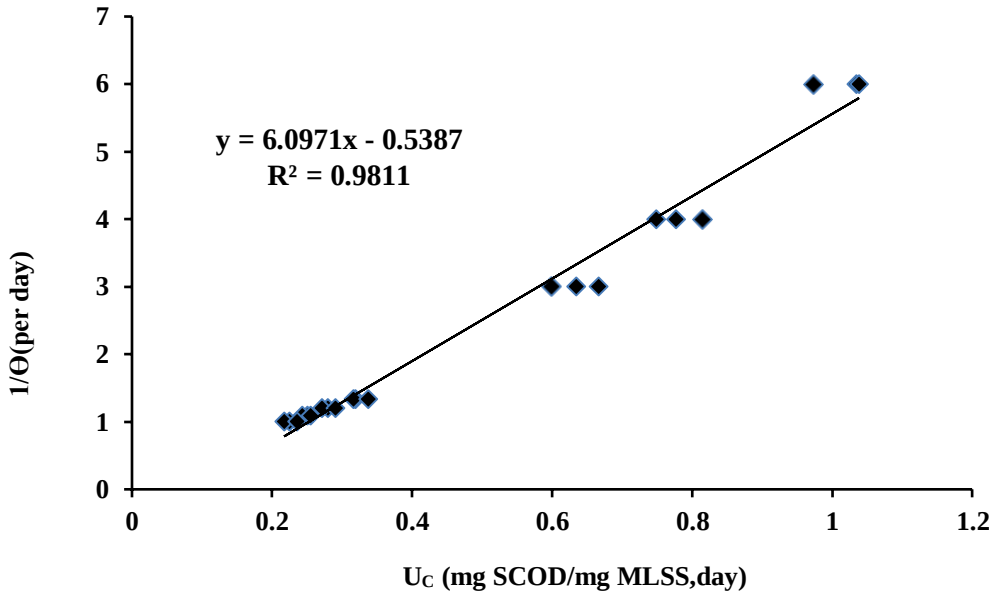


Fig. 5.5cMicrobial-Growth kinetics for Carbon oxidation Study in Batch Reactor [Initial SCOD Concentration of 700-720 mg/L]

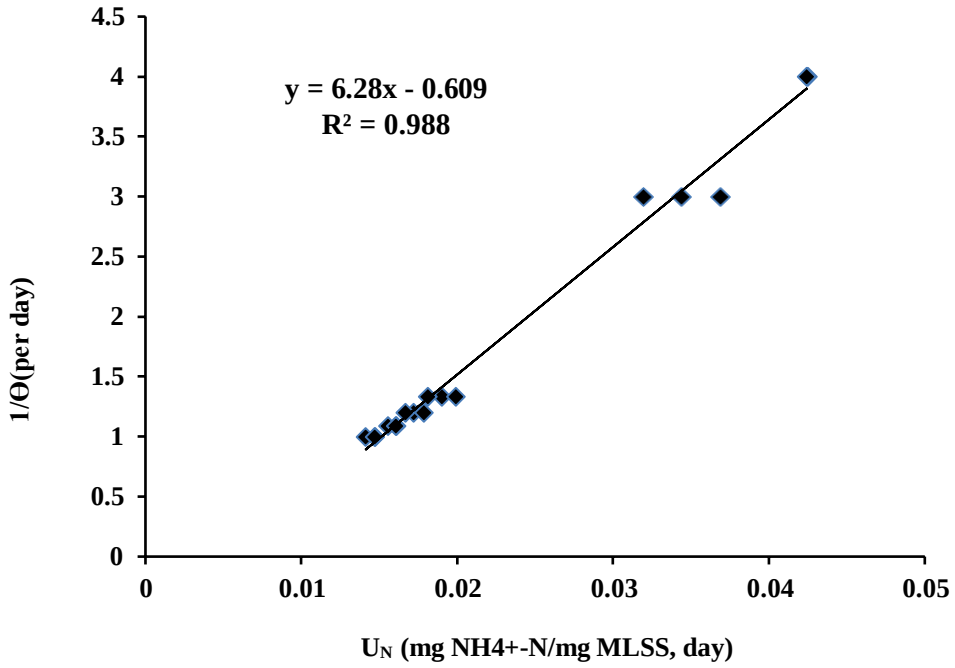


Fig. 5.5dMicrobial-growth kinetics for batch nitrification study [Initial NH_4^+-N = 48 -54 mg/L as N]

B. Experimental setup with micro algae

Optimization study

Three different sets of initial percentage inoculums of microalgae *C. pyredenosa* and *S. platensis*; viz. (40+60)%, (50+50) % and (60+40) % ; % *C. pyredenosa* and *S. platensis*; while keeping the initial MLSS constant at 100 mg/L which has been found to be quite optimum initial MLSS concentration in the pure culture study for both *C. pyredenosa* and *S. platensis*; gives different removal percentages of Nitrate Nitrogen and Phosphorus after 8 days, shown in the following fig 5.6. As the overall removal percentage of Nitrate Nitrogen and Phosphorus was found to be maximum in case of (50+50) % this percentage combination was chosen as the optimum percentage combination and it has been further utilized for the time concentration study for the combined treatment process.

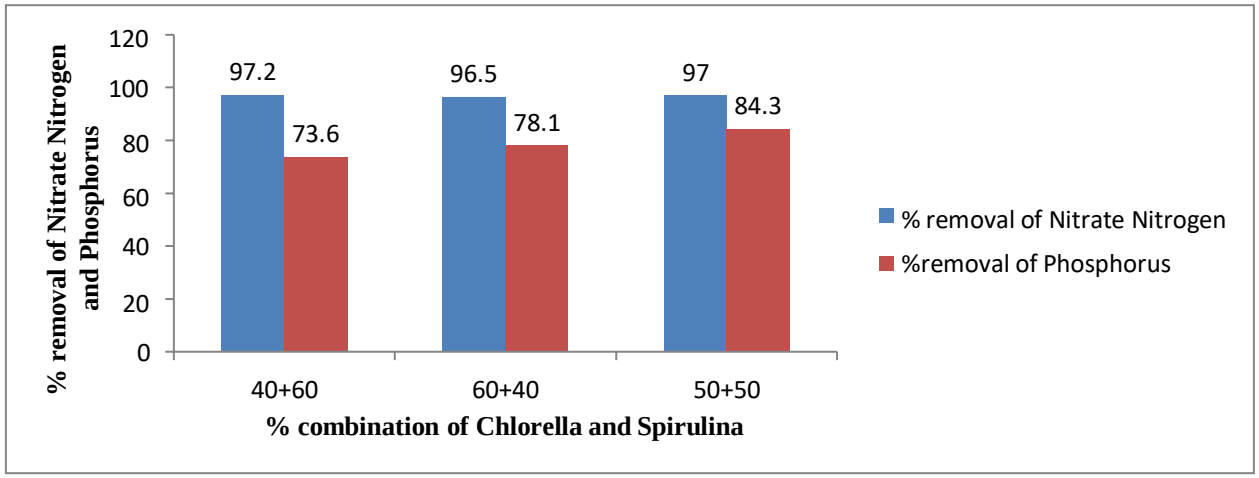


Fig. 5.6. Result of Optimization study

Time concentration and Kinetics study

Around 76% of Phosphorus and 94.70% of Nitrate Nitrogen was removed by the optimum combination of microalgae i.e. (50+50) %found in the time concentration study performed in the bacterial treated real life wastewater sample after 8 days of retention time. In the earlier period comparatively fast growth of microalgae as well as fast removal of substrate was observed; after that micro algal growth and rate of substrate removal became marginalized. This trend of substrate removal and growth of microalgae is shown in the following Time concentration plots (Fig 5.7 and 5.8)

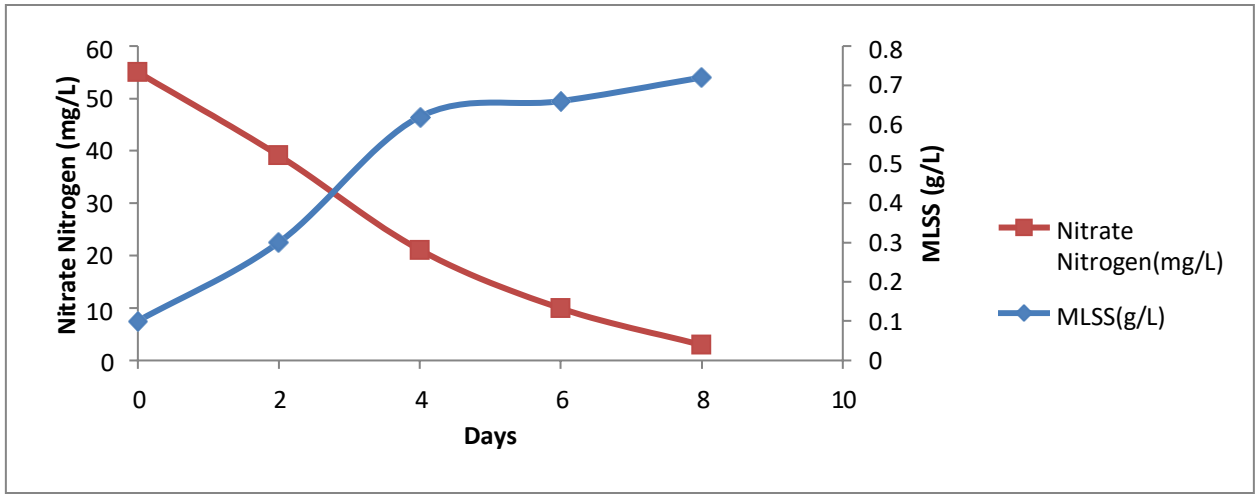


Fig. 5.7.Time concentration study with combination of microalgae (50+50) % for the removal of Nitrate Nitrogen

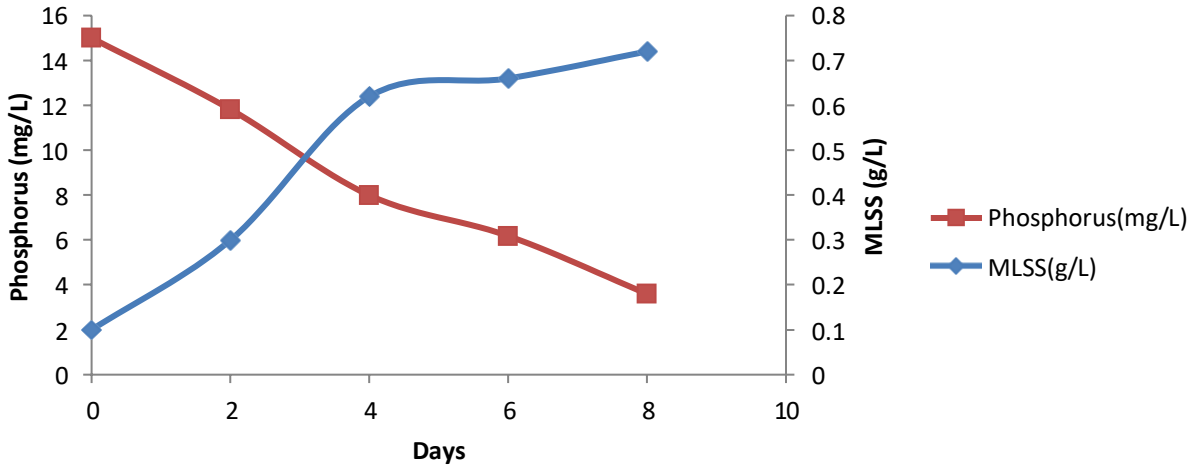


Fig. 5.8.Time concentration study with combination of microalgae (50+50) % for the removal of Phosphorus

The kinetic study was carried out by varying the initial inoculums concentration in the same way done in the optimization study i.e. (40+60) %, (50+50) % and (60+40) % respectively for % *C.*

pyredenosa and *S. platensis*,; samples were withdrawn periodically from each set in the interval of 2 days for testing the necessary parameters. Obtained data was has been plotted in the Lawrance-Maccurty model to produce different kinetic plots for this combined treatment process shown in the following graphs (Fig. 5.9a-5.9d)

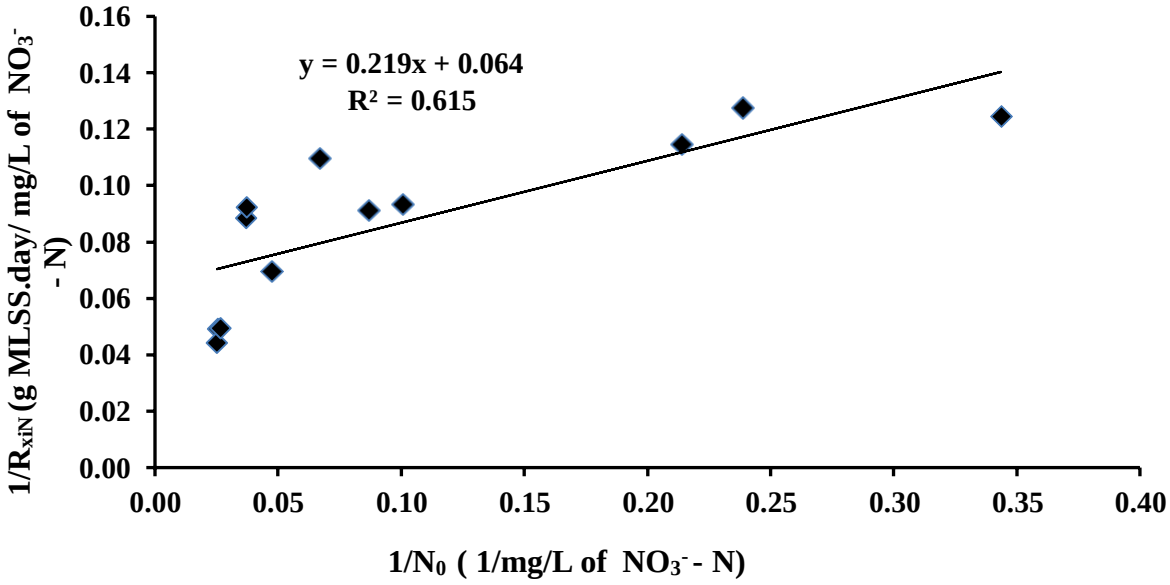


Fig. 5.9a.Substrate-utilization Kinetics of combination of microalgae for nitrate nitrogen removal in batch reactor from pretreated real life dairy wastewater [Initial NO_3^--N = 55 mg/L as N]

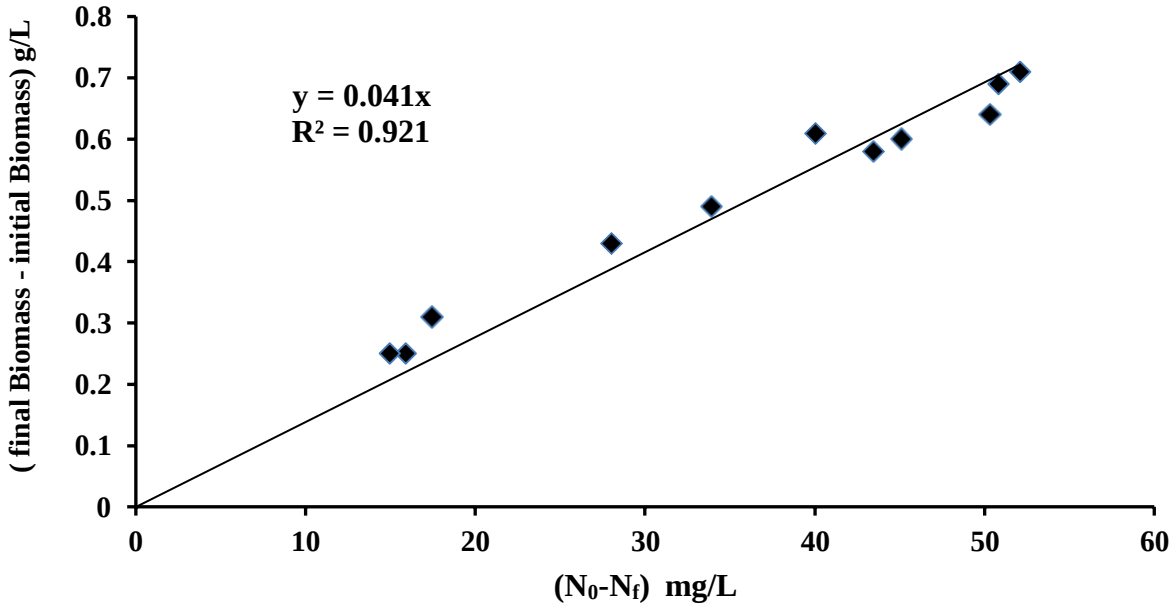


Fig. 5.9b.Micro algal growth kinetics of combination of microalgae for nitrate nitrogen removal in batch reactor from pretreated real life dairy wastewater [Initial NO_3^--N = 55 mg/L as N]

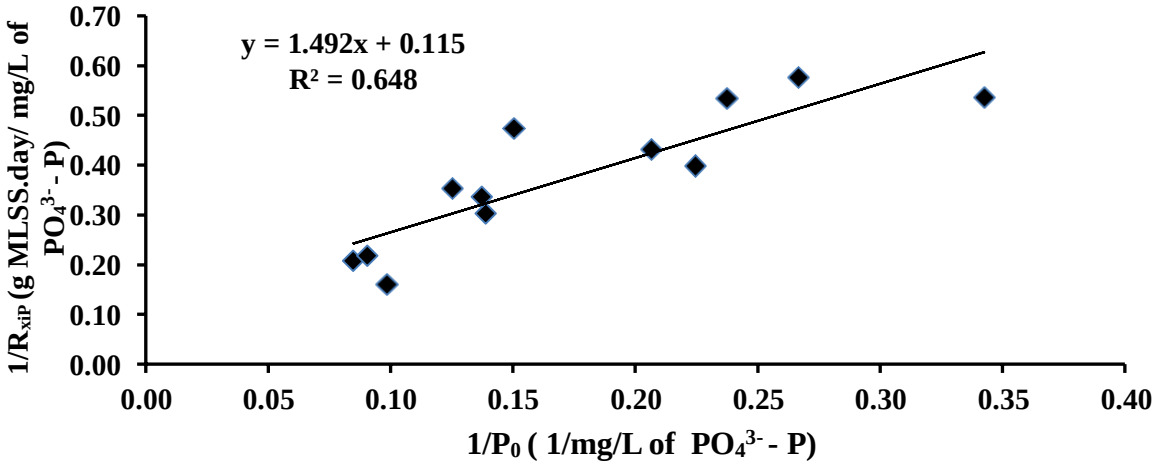


Fig. 5.9c.Substrate-utilization Kinetics of combination of microalgae for Phosphorus removal in batch reactor from pretreated real life dairy wastewater [Initial PO_4^{3-} - P = 16 mg/L as P]

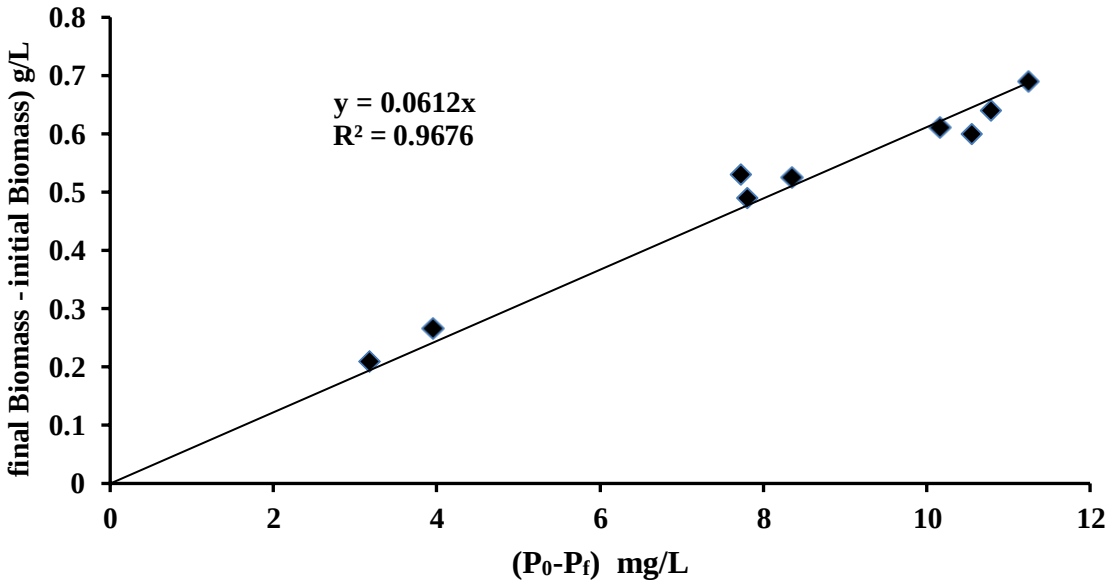


Fig. 5.9d Micro algal growth kinetics of combination of microalgae for phosphorus removal in batch reactor from pretreated real life dairy wastewater [Initial PO_4^{3-} - P = 16 mg/L as P]

Table 5.1a and b. List of kinetic coefficients for bacterial (Top) and micro algal (Bottom) treatment

Kinetic Coefficients	SCOD	Ammonium Nitrogen
K_s (mg/L)	69.4	19.122
k (per day)	1.068	0.088
Y (mg/mg)	6.097	6.28
K_d (per day)	0.538	0.609

Kinetic Coefficients	Nitrate Nitrogen	Phosphorus
K_s (mg/L)	3.421	12.96
k (per day)	15.625	8.69
Y (mg/mg)	0.041	0.061

Application of RSM in optimization of process parameters for combined treatment using mixed consortium of bacteria and microalgae

For Bacterial Study

Experimental design and optimization

A five-factor and three-level CCD clubbed with RSM was done in order to optimize percentage SCOD and percentage ammonium nitrogen removal. Independent factors in this case are influent concentration of SCOD (X_1), ammonium nitrogen (X_2), pH (X_3), MLSS (X_4) and react time (X_5). Response factors are percent SCOD removal (Y_1) and percent ammonium nitrogen removal (Y_2). Each input parameter was enSCODed to three levels from -1, 0 and +1 where "0" represent themid values or mean of each parameter and "-1" and "+1" represent equidistance less and more from that mid value for each parameters; depicted in the table 5.2 as three different values for each five factors in factorial run in multiple input factor combination.

Table 5.2. Independent variables with SCODed levels based on a five-factor, three level CCD for the bacterial treatment process

Sr. No.	Name	Units	Low	High	Variable SCODedas -1	Variable SCODedas +1	Variable Name
A	Initial SCOD	mg/L	700	740	700	740	X_1
B	Initial Ammonia Nitrogen	mg/L	50	60	50	60	X_2
C	pH		6	8	6	8	X_3
D	Initial MLSS	mg/L	1400	1600	1400	1600	X_4
E	React Time	hr	12	36	12	36	X_5

Model Equation

Following Equation was utilized to predict the output parameters as a quadratic model and also the estimation of the parametric coefficients was done by correlating independent and response variables by using the regression model.

$$Y_i = \alpha_1 + \sum \alpha_i x_i + \sum \alpha_{ii} x_i^2 + \sum\sum \alpha_{ij} x_i x_j + c$$

where α_1, α_i, α_{ii} and α_{ij} are the constant, linear, quadratic, and cross-factor interaction coefficients, respectively; X_i and X_j represent the independent variables; Y_i is the predicted response; and c; the residual term.

Parametrical Optimization

The software "Design-Expert (Version 13)" was used in order to analyze regression and graphical representation to predict the response functions' coefficients. The importance of the input parameters, interactions between them, and model equations were verified by the ANOVA technique at 95% CI. 2D-contour plots and 3D response surfaces were generated by keeping other terms fixed or constant in this model. Experiments were done for the validation of the statistical models for maximum percentage of SCOD and Ammonium nitrogen.

Operating conditions were optimized by using the optimization method. An additional experimental run was also performed by the RSM software for the validation of the predicted optimal conditions for all output parameters mentioned previously.

For Micro algal Study

Experimental design and Optimization

A five-factor and three-level CCD clubbed with RSM was done similarly in case of algal treatment study in order to optimize percentage Nitrate nitrogen and phosphorus removal. Independent factors in this case are Initial Nitrate Nitrogen (Z_1), Initial Phosphorus (Z_2), Initial pH (Z_3), Initial MLSS (Z_4) and react time (Z_5). Response factors are percent Nitrate Nitrogen removal (R_1) and percent phosphorus removal (R_2). Each parameter was enSCODed to three levels from -1 to +1; depicted in Table 5.3.

Table 5.3. Independent variables with SCODed levels based on a five-factor, three level CCD for the micro algal treatment process

Sr. No.	Name	Units	Low	High	Variable SCODedas -1	Variable SCODedas +1	Variable Name
1	Initial Nitrate Nitrogen	mg/L	50	60	50	60	Z_1
2	Initial Phosphorus	mg/L	12	18	12	18	Z_2
3	Initial pH		7	8	7	8	Z_3
4	Initial MLSS	g/L	0.5	1.5	0.5	1.5	Z_4
5	React Time	Days	6	10	6	10	Z_5

Model Equation

Following Equation was utilized in order to predict the output parameters as a quadratic model and also the estimation of the parametric coefficients was done by correlating independent and response variables by using the regression model.

$$Y_i = \alpha_1 + \sum \alpha_i x_i + \sum \alpha_{ii} x_i^2 + \sum\sum \alpha_{ij} x_i x_j + c$$

The notation for each symbols in the above stated mathematical equation are same as given in the model equation for bacterial model equation for the RSM study in the previous segment.

Parametrical Optimization

The software "Design-Expert version 13" was used in order to analyze regression and graphical representation in order to predict the response or output function's coefficients. The importance of the input parameters, interactions between them, and model equations were verified by the ANOVA method at 95% CI.

2D contour-plots and 3D response surfaces were generated by keeping other terms constant in this model. Experiments were done for the validation of the statistical models for maximum percentage of Nitrate nitrogen and Phosphorus removal.

Operating conditions were optimized by using the optimization technique. An extra run was also done by the RSM software for the validation of the predicted optimal conditions for all output parameters mentioned previously.

Experimental Design and Statistical Analysis

Bacterial treatment:

RSM was employed to estimate the relationship among the explained and unexplained parameters, depicted in the Equations below. In order to predict the response or output functions for percentage SCOD removal and percentage ammonium nitrogen removal, second-order polynomial Equations 1 and 2 were developed separately:

Equation 1.

Percentage SCOD removal (Y_1)=$94.01-3.16\ X_1-1.13\ X_2+0.4444\ X_3+12.32\ X_4+3.43\ X_5+0.6034\ X_1X_2+0.8109\ X_1X_3-0.0091\ X_1X_4-0.3291\ X_1X_5+1.80\ X_2X_3-0.6384\ X_2X_4+0.2903\ X_2X_5+0.4366\ X_3X_4+1.21\ X_3X_5-1.82\ X_4X_5+5.93\ X_1^2+1.03\ X_2^2-41.17\ X_3^2-11.48\ X_4^2+1.23\ X_5^2$

Equation 2.

Percentage ammonium nitrogen removal (Y_2)=$92.15-1.94\ X_1-3.76\ X_2+0.6565\ X_3+11.66\ X_4+2.93\ X_5-1.20\ X_1X_2+1.22\ X_1X_3-1.39\ X_1X_4-0.0319\ X_1X_5+0.2850\ X_2X_3-+0.6763\ X_2X_4+1.60\ X_2X_5-0.7506\ X_3X_4+2.72\ X_3X_5-2.38\ X_4X_5+9.27\ X_1^2-2.07\ X_2^2-41.52\ X_3^2-11.39X_4^2+1.97\ X_5^2$

Negative coefficients for components of X_1, X_2, X_1X_2,X_1X_4, X_1X_5, X_2X_4, X_4X_5, X_3^2 and X_4^2 in response Y_1 and X_1, X2, X_1X_2, X_1X_4, X_1X_5,X_3X_4,X_4X_5, X_2^2,X_3^2 and X_4^2 in response Y_2, is indicative of effects that is unfavorable on the percent SCOD removal and percent ammonium nitrogen removal respectively. Whereas, positive coefficients for X_3,X_4,X_5,X_1X_2,$X_1X_3X_2X_3$,X_2X_5,X_3X_4,X_3X_5, X_1^2,X_2^2 and X_5^2 in Y_1 and X_3, X_4,X_1X_3, X_2X_3,X_2X_4, X_2X_5, X_3X_5,X_1^2 and X_5^2 in $Y_2$is indicative of effects that is favorable on the percent SCOD removal and percent ammonium nitrogen removal respectively.

Here values of coefficients which are very close to zero represent very small intensity as compare to others, X_1X_4 does not intensely affect the percentage SCOD removal and X_1X_5 does not intensely affect percentage ammonium nitrogen removal.

This model estimation gives us a quick analysis of the parametric impact on the dependent or output response factors. ANOVA with a CI of around 95% was implemented to obtain statistical-significance of models for both percentage SCOD removal and percentage ammonium nitrogen removal. Thus, statistical significance of each factor coefficient; shown in Equations (1) and (2), was calculated from Fisher's (*F*) exact test, comparing probability values(*p*) greater than *F*. *F*-values of 59.14and 49.99for percentage SCOD removal and percentage ammonium nitrogen removal respectively indicate the significance of model.

Alongside, small p-values (i.e. $p < 0.05$) imply that the model-terms are significant, which ensures that the developed model is accurate enough to predict the response functions. Table 5.5 and 5.6 depicts the significant and insignificant terms in the ANOVA response table. In this case X_1, X_4, X_5, X_2X_3, X_4X_5, X_3^2, X_4^2 andX_1, X_2, X_4, X_5, X_3X_5, X_4X_5, X_1^2, X_3^2, X_4^2 are significant model terms for percentage SCOD removal and percentage ammonium nitrogen removal respectively. Values greater than 0.05point out the terms are not significant. For percentage SCOD removal; value of the predicted R^2 (0.9258) is quite similar to the value of adjusted R^2 (0.9596).

Adequate precision provides the ratio of signal to noise. Its' desirable value is greater than four. Obtained value of 24.286 implies much more signal than noise. So it may be said that, this model may be used to navigate the design space. For percentage ammonium nitrogen removal; value of the predicted R^2 (0.9153) is also very much comparable with the adjusted R^2 (0.9524).Obtained ratio of 23.671 implies much more signal than noise. So this model may also be used.

Table 5.4. Five-factor, three-level CCD with observed and predicted percent SCOD and Ammonium-Nitrogen Removal

Run	Space Type	Factor 1 Initial SCOD mg L^{-1}	Factor 2 Initial Ammonium Nitrogen mg L^{-1}	Factor 3 Initial pH	Factor 4 Initial MLSS mg L^{-1}	Factor 5 React Time hr	Actual Response 1 SCOD Removal %	Predicted Response 1 SCOD Removal %	Actual Response 2 Ammonium Nitrogen Removal %	Predicted Response 2 Ammonium Nitrogen Removal %
1	Axial	700	55	7	1500	24	96.34	103.10	96.68	103.36
2	Center	720	55	7	1500	24	98.61	94.01	97.42	92.15
3	Factorial	700	60	6	1600	12	55.36	59.80	64.26	63.08
4	Factorial	700	50	8	1600	36	66.32	68.55	64.25	65.39
5	Center	720	55	7	1500	24	98.61	94.01	97.42	92.15
6	Axial	740	55	7	1500	24	94.36	96.79	95.62	99.48
7	Center	720	55	7	1500	24	98.61	94.01	97.42	92.15
8	Factorial	740	60	8	1600	12	55.36	58.28	48.36	49.02
9	Factorial	700	50	6	1600	12	68.35	68.72	66.32	70.62
10	Factorial	700	60	6	1400	12	38.26	33.64	34.26	29.35
11	Axial	720	55	8	1500	24	48.25	53.29	45.36	51.29
12	Factorial	740	50	6	1400	12	32.15	31.55	38.62	38.52
13	Factorial	740	50	8	1600	12	60.35	57.58	62.35	60.23
14	Factorial	740	60	8	1400	12	28.35	30.41	24.35	23.86
15	Factorial	740	60	6	1600	36	52.36	54.42	50.32	50.44
16	Axial	720	55	7	1500	36	94.65	98.67	92.85	97.05
17	Factorial	740	60	6	1600	12	58.26	53.71	52.34	51.63
18	Factorial	740	60	6	1400	36	36.25	35.60	32.65	31.81
19	Factorial	740	60	8	1600	36	65.32	63.83	62.58	58.70
20	Axial	720	55	6	1500	24	48.25	52.40	45.36	49.97
21	Factorial	700	50	6	1400	12	36.25	40.02	38.62	39.60
22	Factorial	740	50	8	1600	36	60.25	61.98	57.36	63.50
23	Axial	720	55	7	1400	24	65.36	70.22	62.35	69.09
24	Factorial	700	50	8	1400	12	36.25	32.39	32.62	33.97
25	Factorial	700	60	8	1400	12	34.25	33.22	28.25	24.87
26	Factorial	740	60	8	1400	36	44.25	43.26	41.36	43.06
27	Factorial	700	50	8	1400	36	40.35	45.39	45.36	46.90
28	Factorial	700	60	6	1600	36	64.35	61.82	61.52	62.02
29	Factorial	700	60	6	1400	36	38.62	42.97	34.62	37.82
30	Factorial	740	50	6	1400	36	35.62	38.40	40.25	40.46
31	Factorial	700	50	8	1600	12	64.35	62.84	68.35	61.99
32	Center	720	55	7	1500	24	98.61	94.01	97.42	92.15
33	Factorial	740	50	6	1600	12	60.35	60.22	66.32	63.98
34	Center	720	55	7	1500	24	98.61	94.01	97.42	92.15
35	Factorial	700	50	6	1600	36	72.35	69.59	66.35	63.15
36	Axial	720	50	7	1500	24	92.54	96.18	89.36	93.84
37	Axial	720	60	7	1500	24	88.36	93.92	80.26	86.32
38	Center	720	55	7	1500	24	98.61	94.01	97.42	92.15
39	Center	720	55	7	1500	24	98.61	94.01	97.42	92.15
40	Factorial	740	50	8	1400	12	24.56	27.17	36.25	37.77
41	Center	720	55	7	1500	24	98.61	94.01	97.42	92.15
42	Axial	720	55	7	1500	12	86.65	91.82	84.85	91.19
43	Factorial	700	60	8	1600	36	70.25	67.99	68.52	65.39
44	Factorial	700	60	8	1400	36	48.36	47.38	44.36	44.20
45	Factorial	740	50	6	1600	36	58.63	59.77	55.45	56.39
46	Factorial	740	60	6	1400	12	28.65	27.59	22.35	23.47
47	Factorial	700	50	6	1400	36	54.36	48.18	44.36	41.66
48	Axial	720	55	7	1600	24	90.52	94.86	88.62	92.42
49	Factorial	700	60	8	1600	12	62.35	61.12	50.26	55.59
50	Factorial	740	50	8	1400	36	44.36	38.85	56.35	50.57

Table 5.5.Response 1: SCOD Removal

ANOVA for Quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value	Significance
Model	27530.54	20	1376.53	59.14	< 0.0001	significant
A-Initial SCOD	338.56	1	338.56	14.55	0.0007	significant
B-Initial Ammonia Nitrogen	43.44	1	43.44	1.87	0.1824	
C-Initial pH	6.72	1	6.72	0.2885	0.5953	
D-Initial MLSS	5159.37	1	5159.37	221.67	< 0.0001	significant
E-React Time	399.53	1	399.53	17.17	0.0003	significant
AB	11.65	1	11.65	0.5006	0.4849	
AC	21.04	1	21.04	0.9041	0.3495	
AD	0.0026	1	0.0026	0.0001	0.9916	
AE	3.47	1	3.47	0.1489	0.7024	
BC	103.86	1	103.86	4.46	0.0434	
BD	13.04	1	13.04	0.5604	0.4601	
BE	2.70	1	2.70	0.1159	0.7360	
CD	6.10	1	6.10	0.2620	0.6126	
CE	46.88	1	46.88	2.01	0.1665	
DE	106.40	1	106.40	4.57	0.0411	significant
A^2	87.08	1	87.08	3.74	0.0629	
B^2	2.64	1	2.64	0.1135	0.7386	
C^2	4191.54	1	4191.54	180.09	< 0.0001	significant
D^2	325.77	1	325.77	14.00	0.0008	significant
E^2	3.76	1	3.76	0.1617	0.6906	
Residual	674.98	29	23.28			
Lack of Fit	674.98	22	30.68			
Pure Error	0.0000	7	0.0000			
Cor Total	28205.52	49				

Table 5.6.Response 2: Ammonia Nitrogen Removal

ANOVA for Quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value	Significance
Model	27120.23	20	1356.01	49.99	< 0.0001	significant
X_1-Initial SCOD	128.43	1	128.43	4.73	0.0378	significant
X_2-Initial Ammonia Nitrogen	481.28	1	481.28	17.74	0.0002	significant
X_3-Initial pH	14.65	1	14.65	0.5402	0.4683	
X_4-Initial MLSS	4625.06	1	4625.06	170.52	< 0.0001	significant
X_5-React Time	292.82	1	292.82	10.80	0.0027	significant
X_1X_2	46.22	1	46.22	1.70	0.2020	
X_1X_3	47.53	1	47.53	1.75	0.1959	
X_1X_4	61.83	1	61.83	2.28	0.1419	
X_1X_5	0.0325	1	0.0325	0.0012	0.9726	
X_2X_3	2.60	1	2.60	0.0958	0.7591	
X_2X_4	14.63	1	14.63	0.5395	0.4685	
X_2X_5	81.98	1	81.98	3.02	0.0927	
X_3X_4	18.03	1	18.03	0.6647	0.4215	
X_3X_5	236.10	1	236.10	8.70	0.0062	significant
X_4X_5	181.45	1	181.45	6.69	0.0150	significant
X_1^2	212.55	1	212.55	7.84	0.0090	significant
X_2^2	10.60	1	10.60	0.3906	0.5369	
X_3^2	4263.77	1	4263.77	157.20	< 0.0001	significant
X_4^2	321.14	1	321.14	11.84	0.0018	significant
X_5^2	9.60	1	9.60	0.3540	0.5565	
Residual	786.59	29	27.12			
Lack of Fit	786.59	22	35.75			
Pure Error	0.0000	7	0.0000			
Cor Total	27906.83	49				

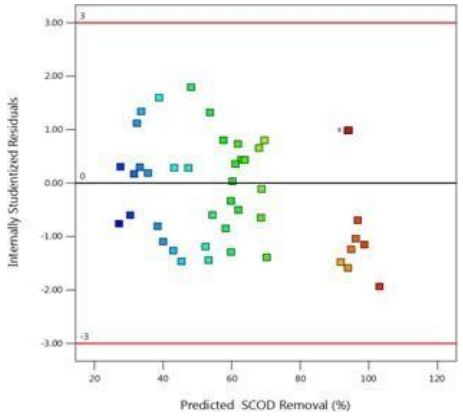
Assumption of the constant variance was verified by plotting internally studentized residual against predicted values (Fig. 5.10(a) &Fig. 5.10(b)). The studentized residuals were found

dividing the residuals by their standard deviations showing a randomly scattered pattern within the outlier detection limits –3 and +3. Therefore, model predictions, described in Equations (1) and (2), for both the percentage SCOD removal and percentage ammonium nitrogen removal, respectively, are satisfactory.

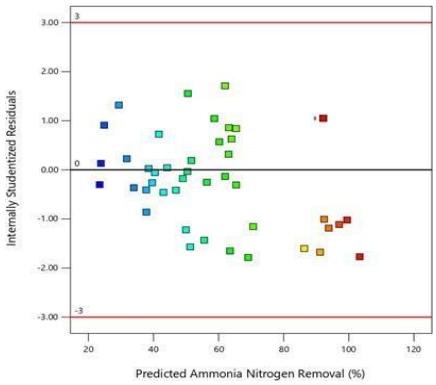
The normal probability plots of residuals for the percentage SCOD removal and percentage ammonium nitrogen removal; depicted in Figure 5.10(c) and Figure 5.10(d) respectively, showed a linear pattern on the plot, not a curve which is S-shaped. Transformation of the response is not needed because the residuals are distributed normally. (Bustillo - Lecompte et al. 2016).

The correlation between the actual and predicted values is depicted in Figure 5.10(e) and Figure 5.10(f) for the percentage SCOD removal and percentage ammonium nitrogen removal, respectively. Small discrepancies are shown by a linear trend, which implies similarity between actualand predicted values. So it may be concluded that the modelpredictions for both percentage SCOD removal and percentage ammonium nitrogen removal responses may be considered as satisfactory.

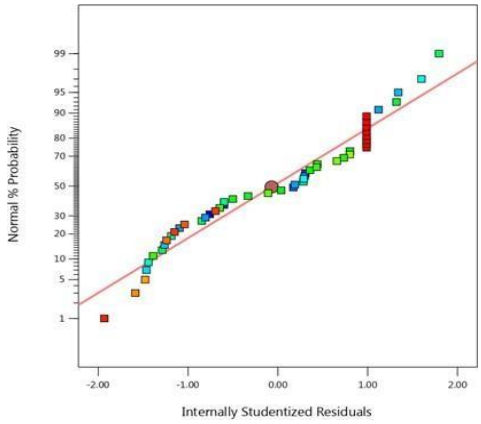
(Table 5.4 has summed up all the actual and predicted values for the response variables for all 50 runs)



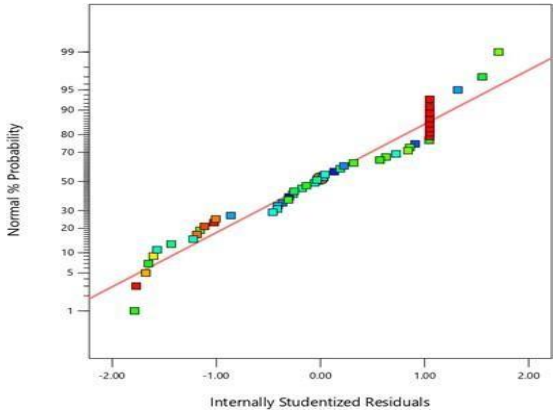
5.10a)



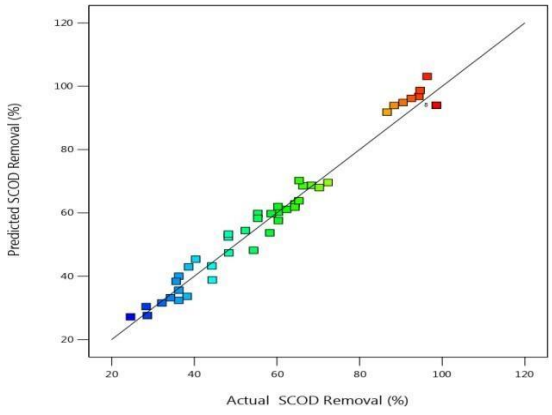
5.10b)



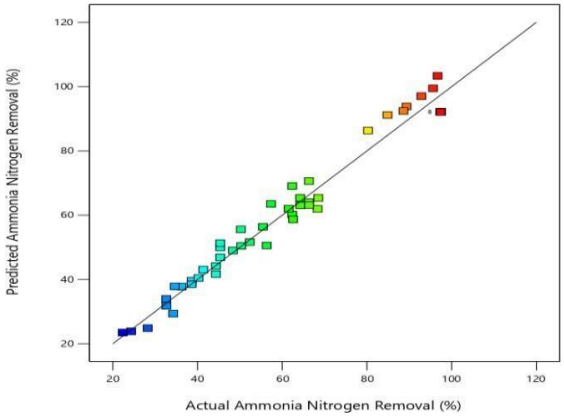
5.10c)



5.10d)



5.10e)



5.10f)

Fig. 5.10a and 5.10b. internally studentized residual against predicted values

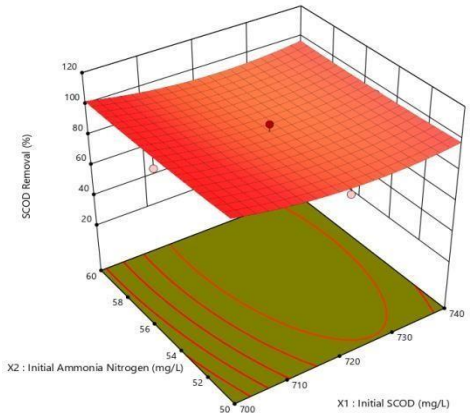
Fig. 5.10c and d. The normal probability plot of residuals for the percentage SCOD removal and percentage ammonium nitrogen removal

Fig. 5.10e and 5.10fThe correlation between the actual and predicted values for percentage SCOD removal and percentage ammonium nitrogen removal

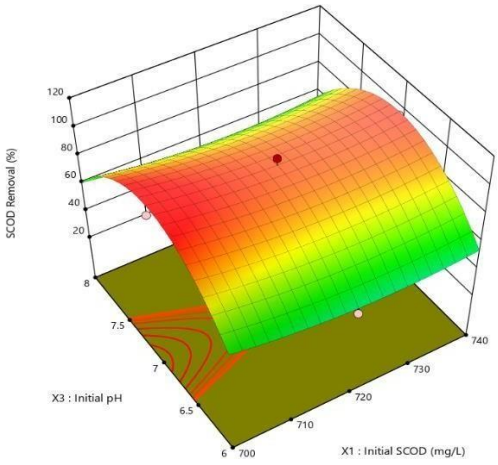
Individual and cross-factor interaction effects of model parameters

Four factors Initial SCOD (X_1), Initial Ammonium Nitrogen (X_2), Initial MLSS (X_4) and React time (X_5) have significant impacts on both responses as their *p*- values are found lower than 0.05. Initial pH (X_3) does not influence output parameters significantly as the p values in cases with both output parameters greater than 0.05. Beside that Initial Ammonium Nitrogen concentration (X_2) does not significantly affect Response 1; SCOD removal as depicted by the larger p value of 0.1824 (>0.05). Alongside these, the cross-factor interactions of different parameters, such as Initial MLSS and React time (X_4X_5) has shown significant impact on SCOD removal and both Initial MLSS and React time (X_4X_5) and Initial pH and React time (X_3X_5), has shown significant effect on Ammonium Nitrogen removal. (As their respective p values found is less than 0.05 as shown in Table 5.5 and 5.6). The effects of cross factor terms on the response or output parameters keeping other terms constant are depicted in Fig. 5.11a-h.

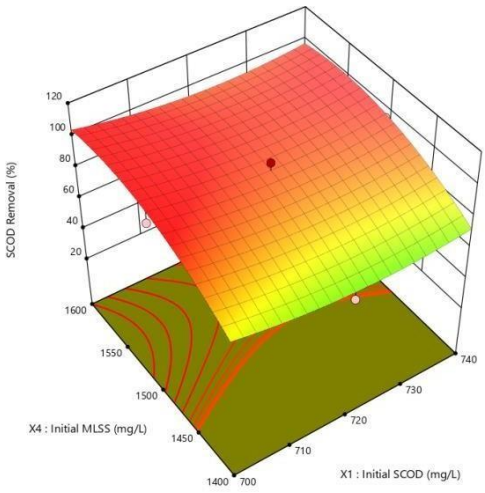
5.11a)



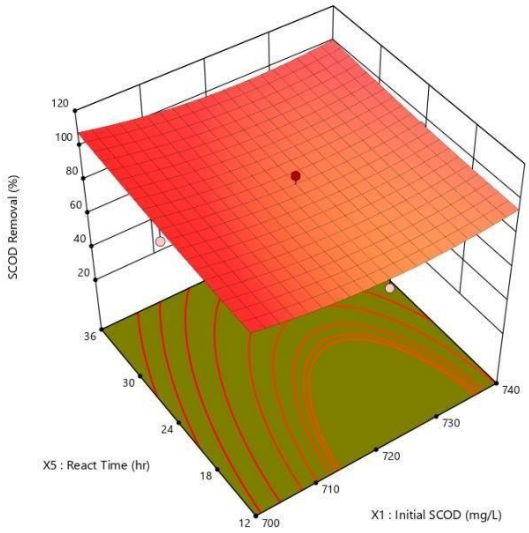
5.11b)



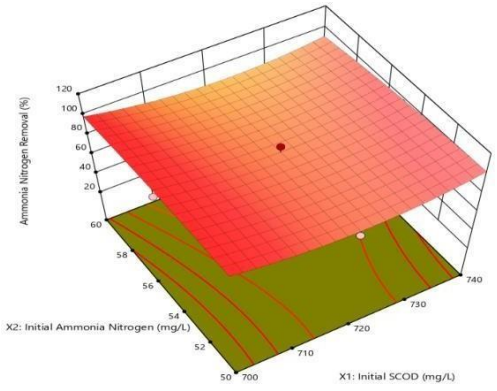
5.11c)



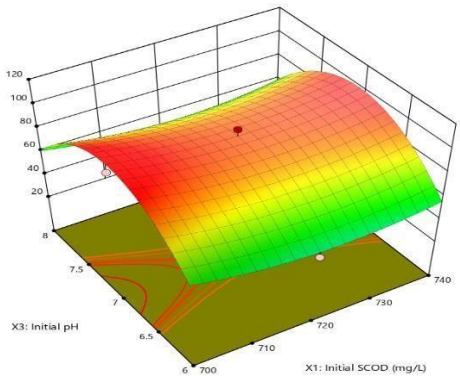
5.11d)



5.11e)



5.11f)



5.11g) **5.11h)**

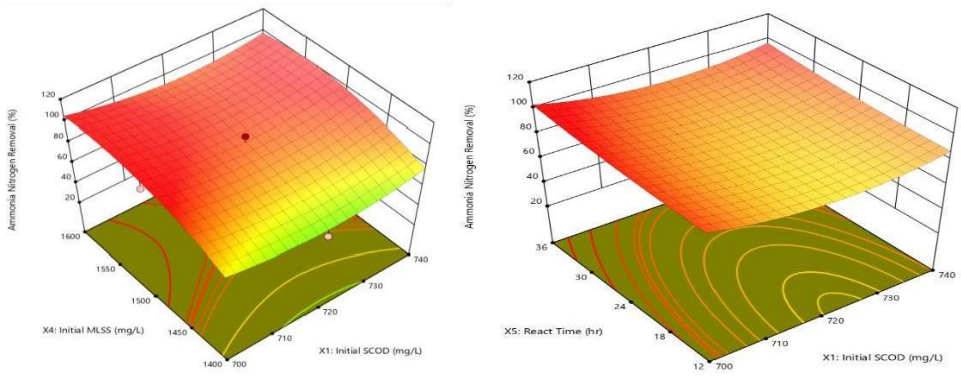


Fig 5.11. Three-dimensional (3D) surfaces and two-dimensional (2D) contour plots of Cross-Factor Interaction Effects on (A)SCOD removal of (a) X_1-Initial SCOD and X_2-Initial Ammonium Nitrogen (b) X_1-Initial SCOD and X_3-Initial pH (c) X_1-Initial SCOD and X_4-Initial MLSS (d) X_1-Initial SCOD and X_5-React Time &

(B)Ammonium nitrogen removal of (e) X_1-Initial SCOD and X_2-Initial Ammonium Nitrogen (f) X_1-Initial SCOD and X_3-Initial pH (g) X_1-Initial SCOD and X_4-Initial MLSS (h) X_1-Initial SCOD and X_5-React Time

Experimental Design and Statistical Analysis

For Micro algal treatment

RSM was employed to estimate the relationship among the explained and unexplained parameters, depicted in the Equations below. In order to predict the response or output functions for Percentage Nitrate Nitrogen Removal (R_1) and Percentage Phosphorus Removal (R_2) the second-order polynomial Equations (3) and (4) were developed, respectively:

Equation 3.

Percentage Nitrate Nitrogen Removal (R_1) = $95.54-2.66Z_1-0.2271Z_2-3.94Z_3+4.72Z_4+9.89Z_5+0.1297\ Z_1Z_2-0.4353\ Z_1Z_3-0.1822\ Z_1Z_4-0.3734\ Z_1Z_5-0.2359\ Z_2Z_3-0.1328\ Z_2Z_4-0.1866\ Z_2Z_5-0.6866\ Z_3Z_4-0.7453\ Z_3Z_5-2.62\ Z_4Z_5+0.3711\ Z_1^2+1.87\ Z_2^2-20.18\ Z_3^2-19.18\ Z_4^2-6.14\ Z_5^2$

Equation 4.

Percentage Phosphorus Removal (R_2) = $88.02 - 0.0174Z_1 - 5.38Z_2 - 3.20\ Z_3 + 5.62\ Z_4 + 10.48Z_5 -0.2822Z_1Z_2 - 0.0172Z_1Z_3 + 0.2309Z_1Z_4 + 0.1428Z_1Z_5 -0.4622\ Z_2Z_3 -0.4622\ Z_2Z_3+0.2747Z_2Z_4-2.53\ Z_2Z_5-0.8491\ Z_3Z_4+0.4766\ Z_3Z_5-1.78\ Z_4Z_5+2.78\ Z_1^2 -13.22\ Z_2^2 -14.27\ Z_3^2 -12.27\ Z_4^2 +0.2258\ Z_5^2$

Negative coefficients of the components Z_1, Z_2, Z_3, Z_1Z_3, Z_1Z_4, Z_1Z_5, Z_2Z_3, Z_2Z_4, Z_2Z_5, Z_3Z_4, Z_3Z_5, Z_4Z_5, Z_3^2, Z_4^2, Z_5^2 in R_1 and Z_1, Z_2, Z_3, Z_1Z_2, Z_1Z_3, Z_2Z_3, Z_2Z_3, Z_2Z_5, Z_3Z_4, Z_4Z_5, Z_2^2, Z_3^2, Z_5^2 in R_2, are indicative of effects that is unfavorable on the Percentage Nitrate Nitrogen Removal and Percentage Phosphorus Removal respectively. Whereas, positive coefficients for Z_4, Z_5, Z_1Z_2, Z_1^2, and Z_2 in R_1 and Z_4, Z_5, Z_1Z_4, Z_1Z_5, Z_2Z_4, Z_3Z_5, Z_1^2 and Z_5^2 in R_2 are indicative of effects that is favorable on the Percentage Nitrate Nitrogen Removal and Percentage Phosphorus Removal respectively.

Z_1, Z_1Z_3 does not affect the Percentage Phosphorus Removal significantly as the values of their coefficient are very close to zero.

Statistical significance of each factor coefficient; shown in Equations (3) and (4), was calculated from Fisher's (F) exact test, comparing probability values (p) greater than F. F-values of 165.22 and 183.66 for percentage Nitrate Nitrogen removal and percentage Phosphorus removal indicate the significance of model.

In this case Z_1, Z_3, Z_4, Z_5, Z_4Z_5, Z_3^2, Z_4^2, Z_5^2 are significant model terms and Z_2, Z_3, Z_4, Z_5, Z_2Z_5, Z_3Z_4, Z_4Z_5, Z_2^2, Z_3^2, Z_4^2 are significant model terms for Percentage Nitrate Nitrogen Removal and Percentage Phosphorus Removal respectively as their p-values are small ($p<0.05$). Table 5.8 and Table 5.10 depict both significant and insignificant terms. For Percentage Nitrate Nitrogen Removal the Predicted R^2 (0.9763) is quite similar to the value of Adjusted R^2 (0.9853).

Adequate Precision provides the ratio of signal to noise. Its' desirable value is greater than four. Obtained value of 40.214 implies much more signal than noise.

For Percentage Phosphorus Removal Predicted R^2 (0.9775) is also very much similar with the Adjusted R^2 (0.9868).

Adequate Precision was 48.446; which indicates much more signal than noise.

Table 5.7.Five-factor, three-level CCD with observed and predicted percent Nitrate-Nitrogen Removal

		Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Actual Response 1	Predicted Response 1	Residuals
Run	Space Type	Initial Nitrate Nitrogen	Initial Phosphorus	Initial pH	Initial MLSS	React Time	Nitrate Nitrogen Removal	Nitrate Nitrogen Removal	Nitrate Nitrogen Removal
		mg/L	mg/L		g/L	Days	%	%	%
1	Axial	55	15	7.5	0.08	8	68.25	71.63	-3.38
2	Axial	55	15	7.5	0.12	8	78.25	81.08	-2.83
3	Center	55	15	7.5	0.1	8	98.64	95.54	3.10
4	Factorial	60	18	8	0.12	6	42.35	42.66	-0.3142
5	Factorial	50	18	7	0.08	6	39.24	39.40	-0.1637
6	Factorial	60	12	7	0.12	6	52.36	51.38	0.9784
7	Factorial	50	12	7	0.12	6	56.24	55.71	0.5321
8	Factorial	60	12	8	0.12	10	55.36	55.90	-0.5378
9	Center	55	15	7.5	0.1	8	98.64	95.54	3.10
10	Center	55	15	7.5	0.1	8	98.64	95.54	3.10
11	Factorial	50	12	8	0.12	6	48.25	49.29	-1.04
12	Axial	55	12	7.5	0.1	8	94.25	97.64	-3.39
13	Factorial	60	12	7	0.08	10	61.25	61.56	-0.3135
14	Factorial	60	18	7	0.12	6	52.32	51.77	0.5537

15	Factorial	60	12	7	0.08	6	35.24	35.41	-0.1691
16	Factorial	60	12	7	0.12	10	66.35	67.04	-0.6874
17	Axial	55	15	7	0.1	8	76.25	79.29	-3.04
18	Axial	50	15	7.5	0.1	8	95.36	98.57	-3.21
19	Factorial	60	12	8	0.08	10	54.26	53.17	1.09
20	Factorial	50	18	7	0.12	6	56.24	55.57	0.6662
21	Factorial	50	18	8	0.08	6	36.25	34.79	1.46
22	Factorial	60	12	8	0.12	6	43.25	43.22	0.0267
23	Axial	55	15	8	0.1	8	68.25	71.41	-3.16
24	Factorial	60	18	7	0.12	10	68.25	66.68	1.57
25	Center	55	15	7.5	0.1	8	98.64	95.54	3.10
26	Axial	60	15	7.5	0.1	8	90.25	93.25	-3.00
27	Factorial	50	12	8	0.12	10	66.25	63.46	2.79
28	Center	55	15	7.5	0.1	8	98.64	95.54	3.10
29	Factorial	50	12	7	0.08	10	68.25	66.65	1.60
30	Factorial	50	18	8	0.12	10	62.35	61.63	0.7151
31	Axial	55	15	7.5	0.1	10	96.24	99.29	-3.05
32	Factorial	60	18	8	0.12	10	54.36	54.59	-0.2324
33	Center	55	15	7.5	0.1	8	98.64	95.54	3.10
34	Factorial	60	12	8	0.08	6	31.25	30.00	1.25
35	Center	55	15	7.5	0.1	8	98.64	95.54	3.10
36	Axial	55	15	7.5	0.1	6	76.35	79.50	-3.15
37	Factorial	50	18	7	0.08	10	67.25	66.31	0.9443
38	Factorial	60	18	7	0.08	10	62.35	61.73	0.6168
39	Factorial	60	18	8	0.08	6	30.25	29.97	0.2809
40	Factorial	60	18	8	0.08	10	52.36	52.40	-0.0361
41	Factorial	50	12	8	0.08	10	58.24	60.00	-1.76
42	Center	55	15	7.5	0.1	8	98.64	95.54	3.10
43	Factorial	50	12	8	0.08	6	35.24	35.34	-0.0957
44	Factorial	50	18	7	0.12	10	70.25	71.98	-1.73
45	Axial	55	18	7.5	0.1	8	94.36	97.18	-2.82
46	Factorial	50	12	7	0.12	10	72.35	72.86	-0.5074
47	Factorial	50	12	7	0.08	6	39.24	39.01	0.2334
48	Factorial	50	18	8	0.08	10	58.24	58.71	-0.4699
49	Factorial	60	18	7	0.08	6	35.24	36.32	-1.08
50	Factorial	50	18	8	0.12	6	48.25	48.21	0.0370

Table 5.8. ANOVA of the prediction results for the percent Nitrate Nitrogen Removal by Quadratic modeling.

Source	Sum of Squares	df	Mean Square	F-value	p-value	Significance
Model	23380.94	20	1169.05	165.22	< 0.0001	**Significant**
Z_1-Initial Nitrate Nitrogen	240.57	1	240.57	34.00	< 0.0001	**Significant**
Z_2-Initial Phosphorus	1.75	1	1.75	0.2477	0.6224	**Not Significant**
Z_3-Initial pH	527.41	1	527.41	74.54	< 0.0001	**Significant**
Z_4-Initial MLSS	758.88	1	758.88	107.25	< 0.0001	**Significant**
Z_5-React Time	3328.38	1	3328.38	470.41	< 0.0001	**Significant**
Z_1Z_2	0.5382	1	0.5382	0.0761	0.7847	**Not Significant**
Z_1Z_3	6.06	1	6.06	0.8570	0.3622	**Not Significant**
Z_1Z_4	1.06	1	1.06	0.1501	0.7013	**Not Significant**
Z_1Z_5	4.46	1	4.46	0.6307	0.4335	**Not Significant**
Z_2Z_3	1.78	1	1.78	0.2518	0.6196	**Not Significant**
Z_2Z_4	0.5645	1	0.5645	0.0798	0.7796	**Not Significant**
Z_2Z_5	1.11	1	1.11	0.1574	0.6945	**Not Significant**
Z_3Z_4	15.08	1	15.08	2.13	0.1550	**Not Significant**
Z_3Z_5	17.78	1	17.78	2.51	0.1238	**Not Significant**
Z_4Z_5	220.45	1	220.45	31.16	< 0.0001	**Significant**
Z_1^2	0.3407	1	0.3407	0.0482	0.8279	**Not Significant**
Z_2^2	8.66	1	8.66	1.22	0.2777	**Not Significant**
Z_3^2	1007.61	1	1007.61	142.41	< 0.0001	**Significant**
Z_4^2	910.24	1	910.24	128.65	< 0.0001	**Significant**
Z_5^2	93.21	1	93.21	13.17	0.0011	**Significant**
Residual	205.19	29	7.08			
Lack of Fit	205.19	22	9.33			
Pure Error	0.0000	7	0.0000			
Cor Total	23586.13	49				

Table 5.9.Five-factor, three-level CCD with observed and predicted percent Phosphorus Removal

Run	Space Type	Factor 1 Initial Nitrate Nitrogen mg/L	Factor 2 Initial Phosphorus mg/L	Factor 3 Initial pH	Factor 4 Initial MLSS g/L	Factor 5 React Time Days	Actual Response 2 Phosphorus Removal %	Predicted Response 2 Phosphorus Removal %	Residuals Phosphorus Removal %
1	Axial	55	15	7.5	0.08	8	68.25	70.14	-1.89
2	Axial	55	15	7.5	0.12	8	78.25	81.37	-3.12
3	Center	55	15	7.5	0.1	8	90.53	88.02	2.51
4	Factorial	60	18	8	0.12	6	40.34	40.40	-0.0634
5	Factorial	50	18	7	0.08	6	34.25	34.21	0.0418
6	Factorial	60	12	7	0.12	6	55.36	55.20	0.1573
7	Factorial	50	12	7	0.12	6	55.24	54.46	0.7776
8	Factorial	60	12	8	0.12	10	72.35	70.73	1.62
9	Center	55	15	7.5	0.1	8	90.53	88.02	2.51
10	Center	55	15	7.5	0.1	8	90.53	88.02	2.51
11	Factorial	50	12	8	0.12	6	46.25	46.37	-0.1241
12	Axial	55	12	7.5	0.1	8	78.35	80.18	-1.83
13	Factorial	60	12	7	0.08	10	68.25	67.70	0.5494
14	Factorial	60	18	7	0.12	6	50.24	50.41	-0.1693
15	Factorial	60	12	7	0.08	6	38.25	38.79	-0.5382
16	Factorial	60	12	7	0.12	10	76.25	76.98	-0.7338
17	Axial	55	15	7	0.1	8	74.25	76.95	-2.70
18	Axial	50	15	7.5	0.1	8	88.25	90.82	-2.57
19	Factorial	60	12	8	0.08	10	64.25	64.85	-0.5960
20	Factorial	50	18	7	0.12	6	51.24	50.80	0.4423
21	Factorial	50	18	8	0.08	6	27.25	27.67	-0.4174
22	Factorial	60	12	8	0.12	6	47.25	47.05	0.2044
23	Axial	55	15	8	0.1	8	68.25	70.56	-2.31
24	Factorial	60	18	7	0.12	10	64.25	62.09	2.16
25	Center	55	15	7.5	0.1	8	90.53	88.02	2.51
26	Axial	60	15	7.5	0.1	8	88.35	90.79	-2.44
27	Factorial	50	12	8	0.12	10	70.24	69.49	0.7498
28	Center	55	15	7.5	0.1	8	90.53	88.02	2.51
29	Factorial	50	12	7	0.08	10	70.24	67.31	2.93
30	Factorial	50	18	8	0.12	10	54.25	53.87	0.3770
31	Axial	55	15	7.5	0.1	10	96.25	98.73	-2.48
32	Factorial	60	18	8	0.12	10	52.34	53.99	-1.65
33	Center	55	15	7.5	0.1	8	90.53	88.02	2.51
34	Factorial	60	12	8	0.08	6	34.25	34.03	0.2226
35	Center	55	15	7.5	0.1	8	90.53	88.02	2.51

36	Axial	55	15	7.5	0.1	6	75.24	77.77	-2.53
37	Factorial	50	18	7	0.08	10	51.32	52.45	-1.13
38	Factorial	60	18	7	0.08	10	50.24	51.70	-1.46
39	Factorial	60	18	8	0.08	6	26.35	26.29	0.0636
40	Factorial	60	18	8	0.08	10	48.32	47.00	1.32
41	Factorial	50	12	8	0.08	10	62.34	64.53	-2.19
42	Center	55	15	7.5	0.1	8	90.53	88.02	2.51
43	Factorial	50	12	8	0.08	6	35.21	34.28	0.9304
44	Factorial	50	18	7	0.12	10	62.35	61.90	0.4462
45	Axial	55	18	7.5	0.1	8	66.25	69.43	-3.18
46	Factorial	50	12	7	0.12	10	74.25	75.67	-1.42
47	Factorial	50	12	7	0.08	6	38.27	38.97	-0.7017
48	Factorial	50	18	8	0.08	10	49.32	47.81	1.51
49	Factorial	60	18	7	0.08	6	34.25	32.90	1.35
50	Factorial	50	18	8	0.12	6	41.21	40.86	0.3494

Table 5.10. ANOVA of the prediction results for the percent Phosphorus Removal by Quadratic modeling.

Source	Sum of Squares	df	Mean Square	F-value	p-value	Significance
Model	19556.76	20	977.84	183.66	< 0.0001	**Significant**
Z_1-Initial Nitrate Nitrogen	0.0102	1	0.0102	0.0019	0.9653	**Not Significant**
Z_2-Initial Phosphorus	983.14	1	983.14	184.65	< 0.0001	**Significant**
Z_3-Initial pH	347.71	1	347.71	65.31	< 0.0001	**Significant**
Z_4-Initial MLSS	1073.53	1	1073.53	201.63	< 0.0001	**Significant**
Z_5-React Time	3735.07	1	3735.07	701.52	< 0.0001	**Significant**
Z_1Z_2	2.55	1	2.55	0.4786	0.4946	**Not Significant**

Z_1Z_3	0.0095	1	0.0095	0.0018	0.9667	**Not Significant**
Z_1Z_4	1.71	1	1.71	0.3205	0.5756	**Not Significant**
Z_1Z_5	0.6527	1	0.6527	0.1226	0.7288	**Not Significant**
Z_2Z_3	6.84	1	6.84	1.28	0.2665	**Not Significant**
Z_2Z_4	2.41	1	2.41	0.4535	0.5060	**Not Significant**
Z_2Z_5	204.17	1	204.17	38.35	< 0.0001	**Significant**
Z_3Z_4	23.07	1	23.07	4.33	0.0463	**Significant**
Z_3Z_5	7.27	1	7.27	1.36	0.2522	**Not Significant**
Z_4Z_5	101.71	1	101.71	19.10	0.0001	**Significant**
Z_1^2	19.13	1	19.13	3.59	0.0681	**Not Significant**
Z_2^2	432.21	1	432.21	81.18	< 0.0001	**Significant**
Z_3^2	503.60	1	503.60	94.58	< 0.0001	**Significant**
Z_4^2	372.32	1	372.32	69.93	< 0.0001	**Significant**
Z_5^2	0.1261	1	0.1261	0.0237	0.8787	**Not Significant**
Residual	154.40	29	5.32			
Lack of Fit	154.40	22	7.02			
Pure Error	0.0000	7	0.0000			
Cor Total	19711.16	49				

The assumption of the constant variance was verified by plotting internally studentized residual against predicted values (Fig 5.12(a) and Fig 5.12(b)). The studentized residuals were found similarly as it was found in the previous case. So model predictions, shown in Equations (3) and (4), for both the Percentage Nitrate Nitrogen and Percentage Phosphorus Removal, respectively, are satisfactory.

The normal probability plot of residuals, depicted in Fig.5.12(c) and Fig. 5.12(d) for the Percentage Nitrate Nitrogen and Percentage Phosphorus Removal, respectively, showed a linear pattern, not a curve with S-shape. Transformation of the response is not needed because the residuals are distributed normally. (Bustillo-Lecompte et al. 2016).

The correlation between the actual and predicted values is depicted in Fig. 5.12(e) and Fig 5.12(f) for the Percentage Nitrate Nitrogen and Percentage Phosphorus removal. Small discrepancies are shown by a linear trend, which implies similarity between actual and predicted values. So it may

be concluded that the model predictions for Percentage Nitrate Nitrogen and Percentage Phosphorus Removal responses may be considered as satisfactory.

(Table 5.7 and 5.9 has highlighted all the actual and predicted values for the response variables for all 50 runs.)

5.12a) **5.12b)**

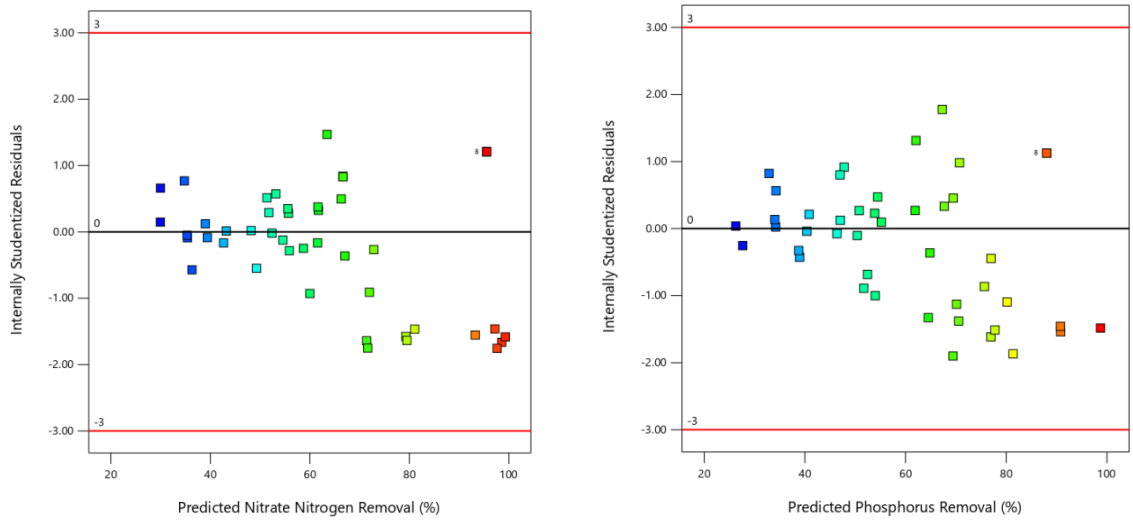


Fig. 5.12a and 5.12b. internally studentized residual versus predicted values for the Percentage Nitrate Nitrogen (left) and Percentage Phosphorus Removal (right)

5.12c) **5.12d)**

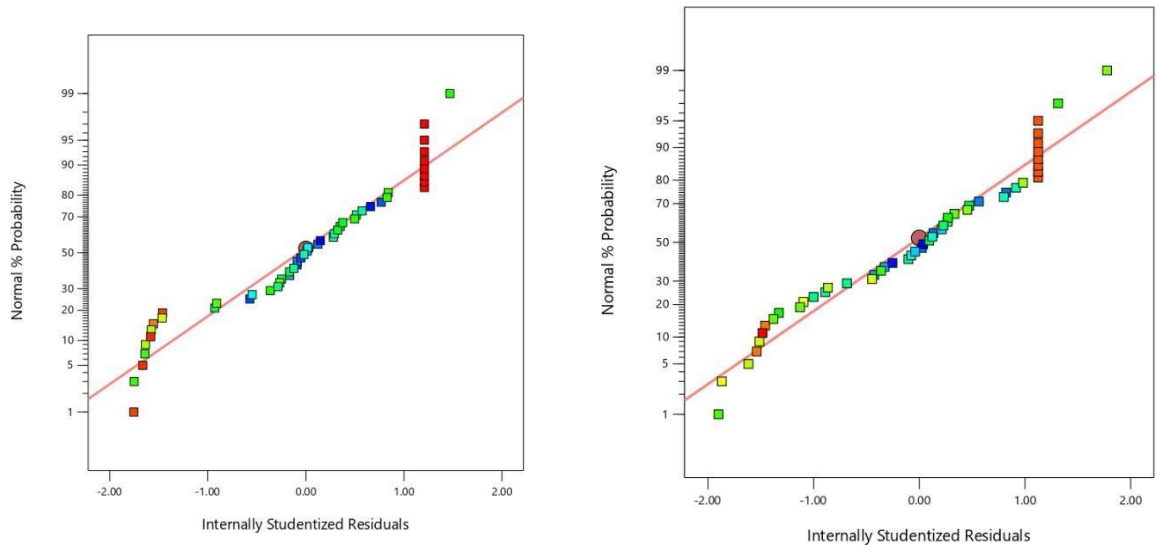


Fig.5.12c and 5.12d.Normal probability plot of residuals for the Percentage Nitrate Nitrogen (left) and Percentage Phosphorus Removal (right)

5.12e) **5.12f)**

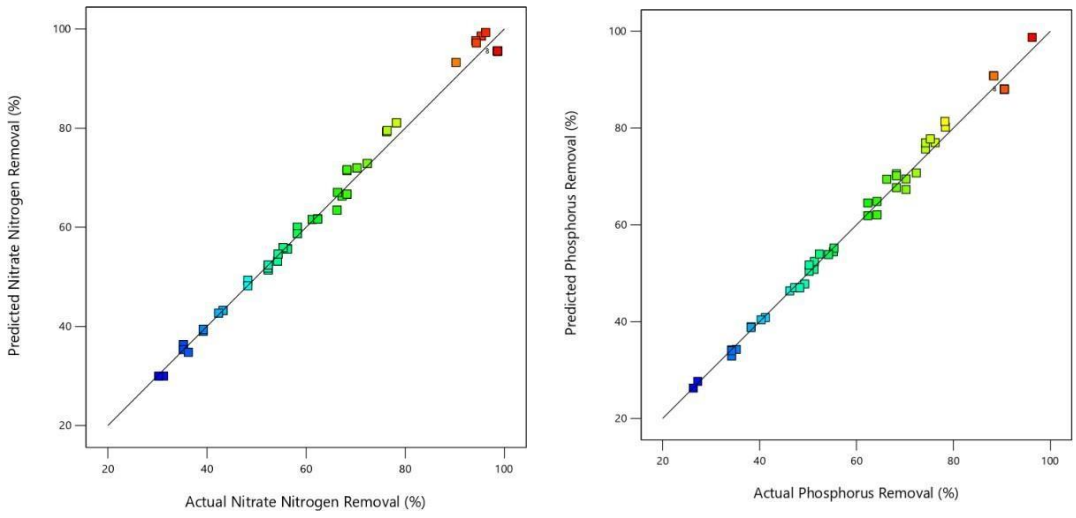


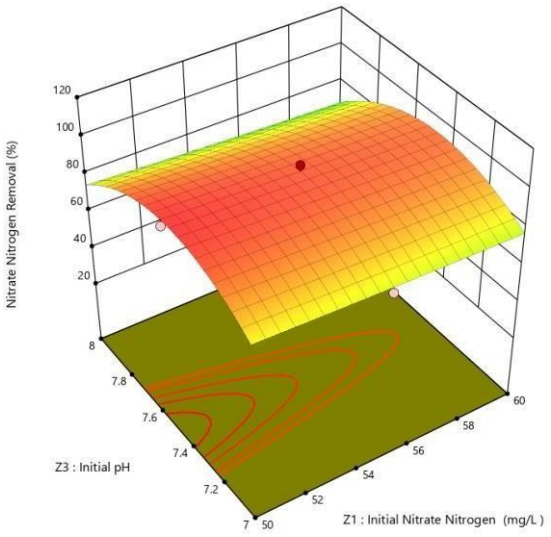
Fig. 5.12e and 5.12f. The correlation between the actual and predicted values for the Percentage Nitrate Nitrogen (left) and Percentage Phosphorus Removal (right)

Individual and Cross-Factor Interaction Effects of Model Parameters

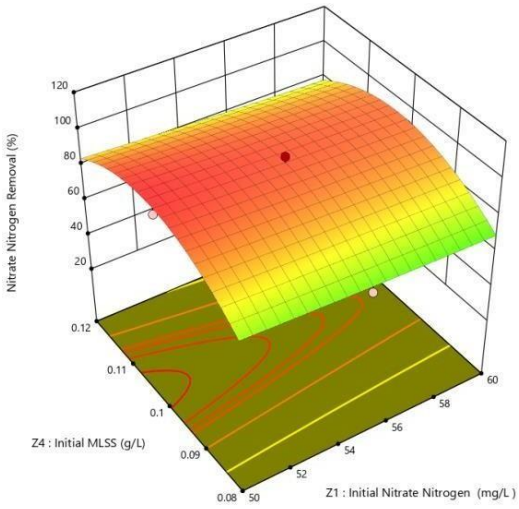
Five input factors; Initial Nitrate Nitrogen (Z_1), Initial Phosphorus (Z_2), Initial pH (Z_3), Initial MLSS (Z_4) and React time (Z_5) all have significant effects on two response parameters R_1 and R_2; Nitrate Nitrogen Removal and Phosphorus removal respectively except the fact that Initial Phosphorus concentration and Initial Nitrate Nitrogen concentration have no significant effect on response R_1 and R_2 respectively as shown by their greater p values.

Cross-factor interactions of various parameters, such as initial MLSS and React time (Z_4Z_5) has significant impact on Nitrate Nitrogen Removal and Initial MLSS and React time (Z_4Z_5), Initial pH and Initial MLSS (Z_3Z_4) and Initial Phosphorus and React time (Z_2Z_5) has showed significant impact on Phosphorus removal (Shown by their respective p values in table no. 5.8 and 5.10) The effects of cross factor terms on the response parameters keeping other terms constant are depicted in Fig. 5.13 and 5.14.

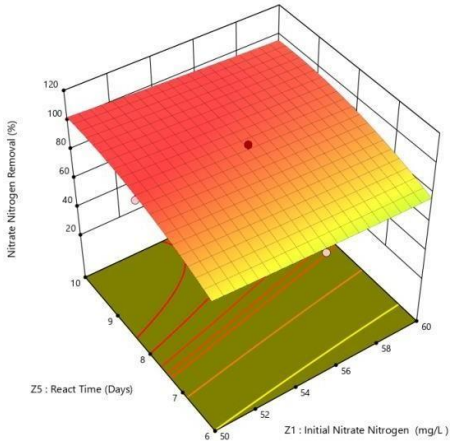
5.13a)



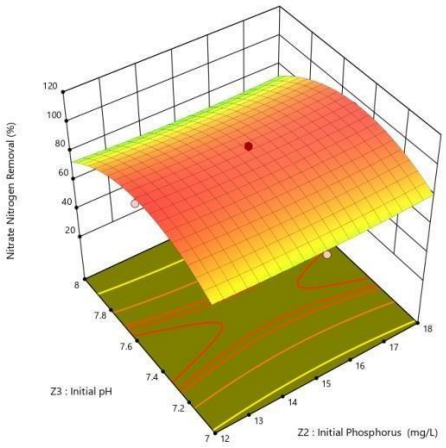
5.13b)



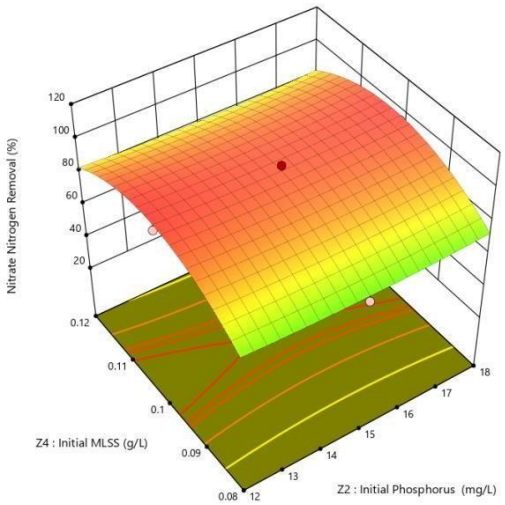
5.13c)



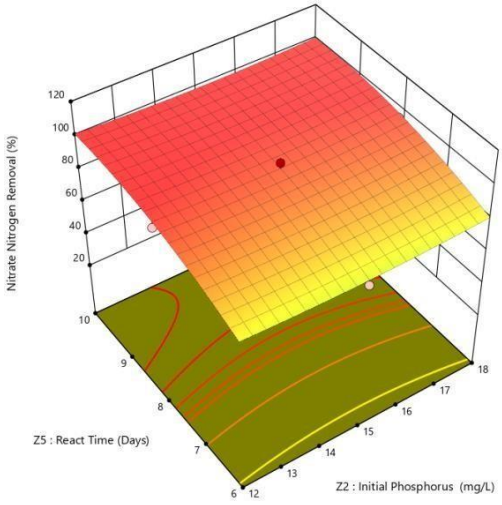
5.13d)



5.13e)



5.13f)



5.13g) **5.13h)**

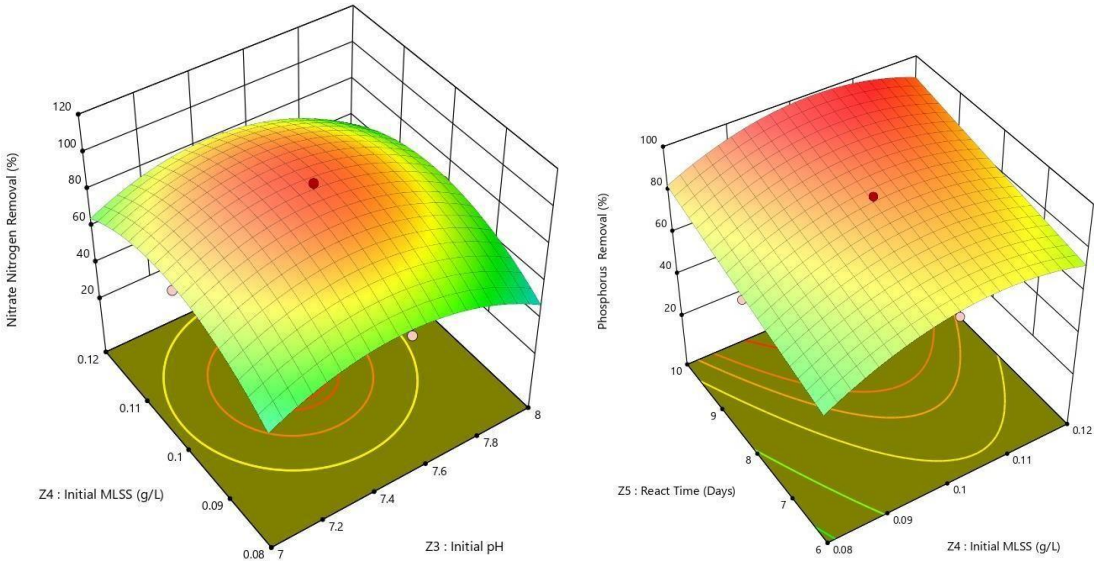
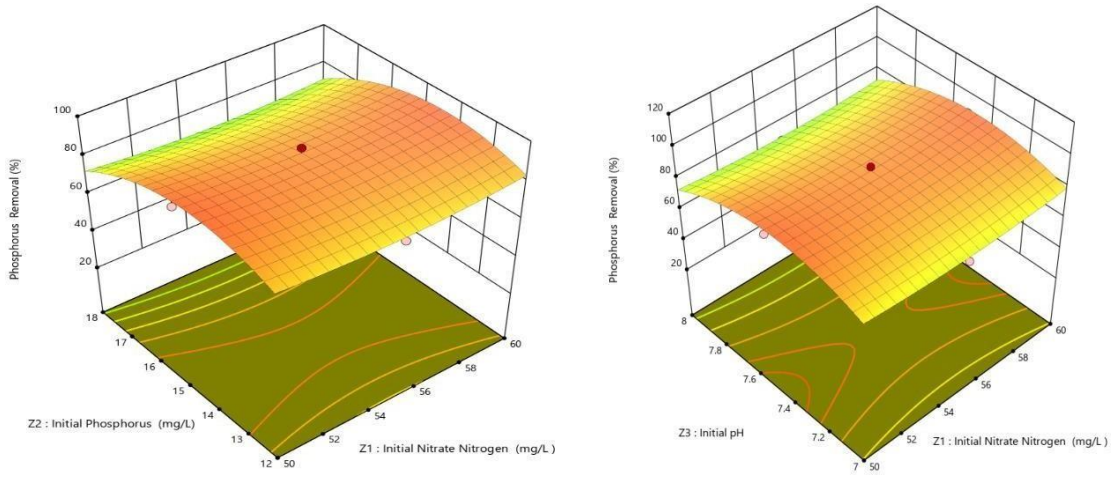
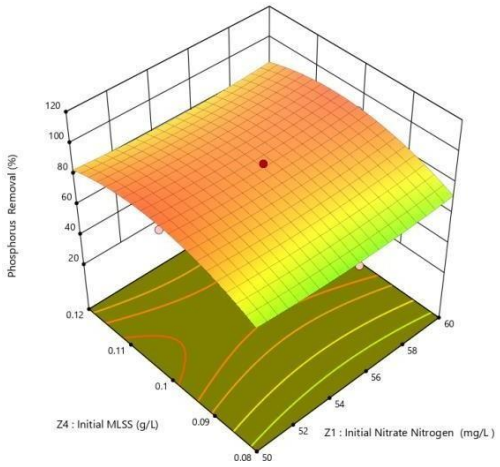


Fig 5.13. Three-dimensional (3D) surfaces and two-dimensional (2D) contour plots of Cross-Factor Interaction Effects on Nitrate nitrogen removal of (a) Z_1-Initial Nitrate Nitrogen and Z_3-Initial pH (b) Z_1-Initial Nitrate Nitrogen and Z_4-Initial MLSS (c) Z_1-Initial Nitrate Nitrogen and Z_5-React Time (d) Z_2-Initial Phosphorus and Z_3-Initial pH (e) Z_2-Initial Phosphorus and Z_4-Initial MLSS (f) Z_2-Initial Phosphorus and Z_5-React Time (g) Z_3-Initial pH and Z_4-Initial MLSS (h) Z_4-Initial MLSS and Z_5-React Time

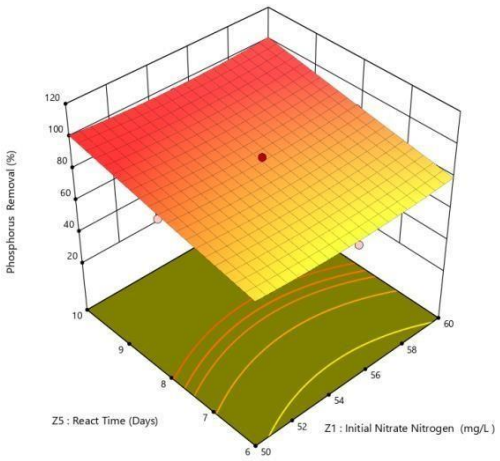
5.14a) **5.14b)**



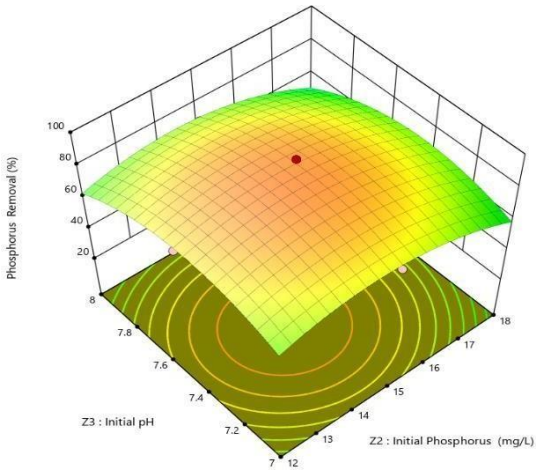
5.14c)



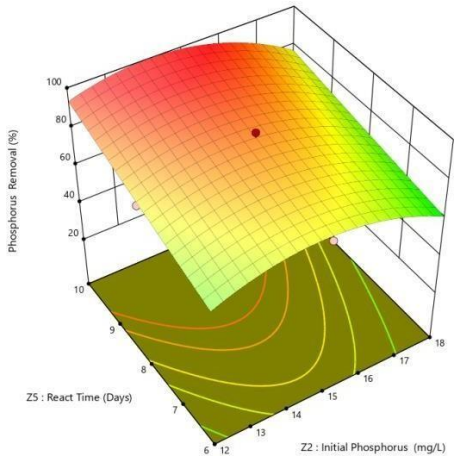
5.14d)



5.14e)



5.14f)



5.14g) **5.14h)**

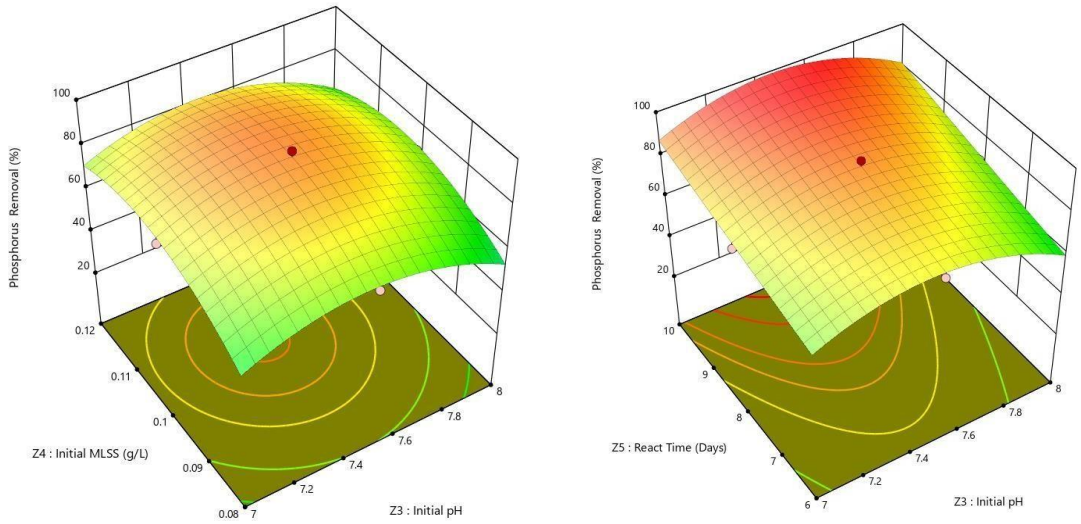


Fig 5.14.Three-dimensional (3D) surfaces and two-dimensional (2D) contour plots of Cross-Factor Interaction Effects on Phosphorus removal of (a) Z_1-Initial Nitrate Nitrogen and Z_2-Initial Phosphorus (b) Z_1-Initial Nitrate Nitrogen and Z_3-Initial pH (c) Z_1-Initial Nitrate Nitrogen and Z_4-Initial MLSS (d) Z_1-Initial Nitrate Nitrogen and Z_5-React Time (e) Z_2-Initial Phosphorus and Z_3-Initial pH (f) Z_2-Initial Phosphorus and Z_5-React Time (g) Z_3-Initial pH and Z_4-Initial MLSS (h) Z_3-Initial pH and Z_5-React Time

Chapter 6

Combined treatment of Real life dairy wastewater in a Pilot-scale Suspended Growth Batch Reactor and fixed film Phycoremediation using mixed bacterial consortium and micro algae

Introduction and Literature review

Population in the world is increasing in a disproportionate manner with the growth of resources. Growth and availability of resources is of no match with the increasing demand proportional to the fast pace of growth in population. Standard of living of people has been improved since Industrial revolution had taken place in different countries in the middle of eighteenth century which has also put pressure on the resources of the earth. One of the most important resources for human being to live on the earth is fresh water. Industries have developed to meet the ever-increasing demand of human being by supplying mechanized product. Proportionately wastewater discharge from the drain pipes of industrial plants has been increased which is making fresh water bodies polluted along with polluting marine ecosystem. Wastewater must be treated to make it free of contaminants before discharging into any water bodies. Discharge standards have been prescribed by different agencies like WHO internationally, CPCB in India and Different State Pollution Control Boards (SPCB) in different states and provinces in India to maintain the Recycling of waste or the waste to wealth policy.

Dairy wastewater is one of the major polluting industries among the Food and Agricultural Food processing sectors. Organic loads contribute to high BOD and SCOD which have the potential to deplete the Dissolved Oxygen (DO) in a water body and this can be fatal for fishes and other creatures dwelling in those water bodies. Nutrients like nitrogen and phosphorus loads are also high which may lead to cultural eutrophication; a dying condition of water bodies created artificially by humans through this kind of discharge unlike natural eutrophication which occurs naturally. Although throughout the process chain; dairy industries affect the environment (Strydom et al.1993) majority of dairy industries consider treatment of wastewater as a burden on their economy and so think of it as a futile investment (Robinson 1997). Dairy industry wastewater characteristics vary between industries according to their products and processing technique (Vidal

et al. 2000). Correct information about the true composition of chemicals present in dairy industry wastewater is scarce (Donkin et al. 1997).

Objective of this present research work is to treat dairy industry wastewater in a continuous bioreactor in two-stage treatment system where in the first phase consortium of Carbon oxidation and nitrifying bacteria consume organic carbon, reduce both BOD and SCOD and oxidize Ammonium Nitrogen present in wastewater to Nitrate Nitrogen. These Nitrate Nitrogen and residual phosphorus has been taken as nutrients by microalgae *Chlorella pyrenoidosa* and *Spirulina platensis* for their cellular growth and reproduction, in the second stage treatment process where fixed film reactor was used; ultimately reduce residual nitrate nitrogen and phosphorus load that met the discharge standards prescribed by CPCB and WHO. Finally kinetic coefficients were also obtained based on modified Monod-Kinetics for both bacterial and micro algal studies which are necessary for further designing of bioreactors in pilot scale.

Materials and Methods

Description of Pilot-scale Bioreactor:

For the treatment with Microbial consortium:

This suspended growth pilot scale bioreactor; shown in Fig. 6.1, was made of transparent plastic sheet. It is of around 10L capacity, It contains inlet and outlet valves connected with pipelines as injection and outlet port, there is a "Remi" made Stirrer to make the whole mixture homogeneous. An antenna shaped aeration device with perforated surface on the antenna like structure was used for injection of air in the mixed liquor. Numerous small bubbles were incorporated into the system through aeration unit which uses a moderate size-capacity pump sitting outside the bioreactor unit. Feed is given to it in the form of real life raw dairy wastewater. The air from the aeration unit in the form of bubbles and the stirrer in the reactor system makes the bacterial growth-culture in a suspended form inside the bioreactor.

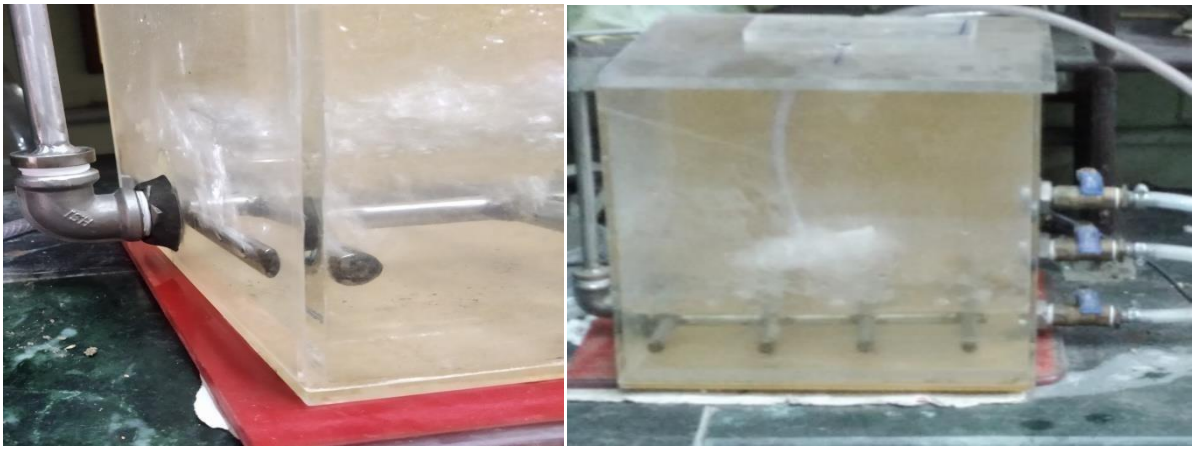


Fig. 6.1.Image of the Suspended Growth Pilot Scale Bioreactor

For the treatment with combined Micro algal system:

This a type of open-air tray-type reactor made of translucent plastic sheet which is of around 10 L of capacity shown in Fig. 6.2 and 6.3. On the base of the tray reactor there lie small white pebbles with rough surfaces which provide a platform for the growth of micro algae which become loosely attached to the surface of the pebbles and can carry out the performance of phycoremediation. Feed given here is the bacterial treated dairy wastewater. Phycoremediation with fixed film of microalgae takes place in this bioreactor.

Fig. 6.2. Image of individual open air tray type Fixed film Phycoremediation Chamber

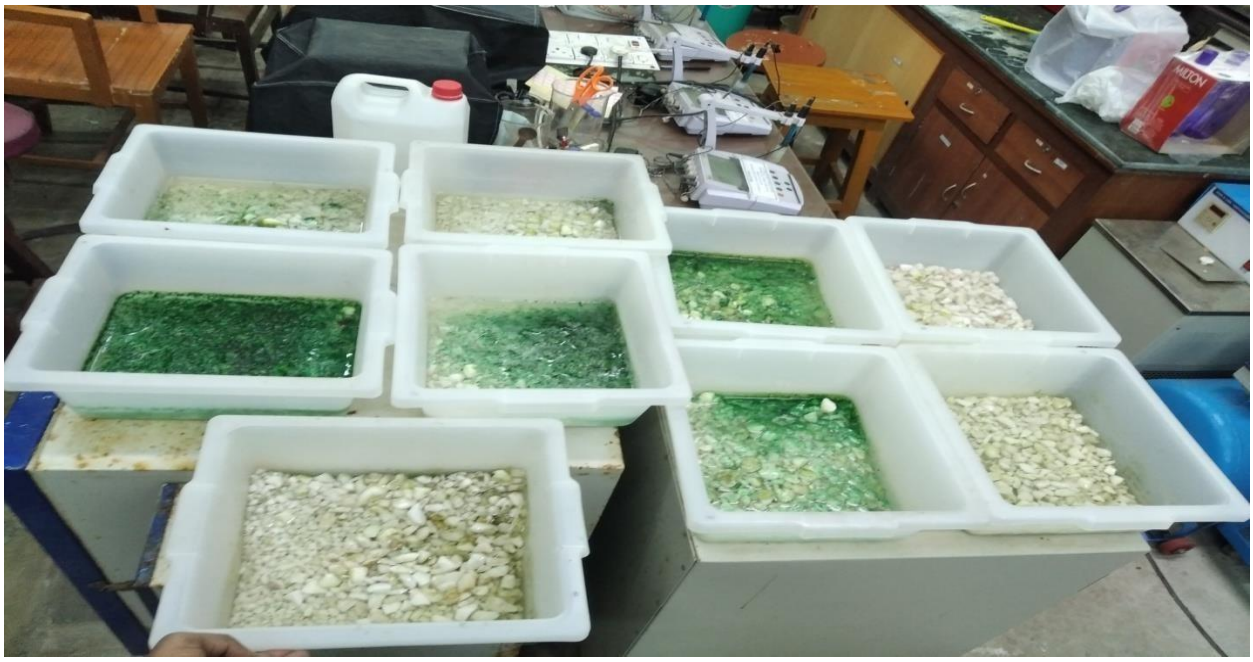


Fig. 6.3. Image of open air tray type Fixed film Phycoremediation Chambers

A. Study with bacterial consortium

Time concentration study with bacterial consortium

A combination of (10 + 5) %; % Carbon oxidation and nitrification corresponding to around 1500mg/L biomass in terms of MLSS was utilized for performing the time concentration study as it was found to be optimum initial inoculums concentration in the previous optimization study.

Initial pH was around 7 and SCOD and ammonium nitrogen was measured as 720 and 55 mg/L respectively. This experimental work was done in 10 L capacity pilot scale bioreactor. Samples were withdrawn from the bioreactor periodically after every two hours and SCOD and Ammonium nitrogen was measured for each sample using standard protocol. (Rice et al. 2012)

Substrate utilization and bacterial growth kinetics study

By conducting experiment, different values of kinetic coefficients are estimated as steady state kinetics for stabilization of SCOD and Ammonium Nitrogen. Kinetic coefficients; k, Y, Ks and K_d were obtained on the basis of experimental results by varying MLSS concentration. Kinetic study was done by using modified linearized Monod kinetics model equation.

A combination of three different Carbon oxidation and nitrification bacteria consortium were utilized to prepare the kinetic study; viz. (10 + 4)%, (10 + 5) % and (10 + 6) % ; % Carbon oxidation and nitrification corresponding to 1420, 1500 and 1610 mg/L biomass in terms of MLSS respectively. Initial pH was around 7 and initial SCOD and ammonium nitrogen concentration was 720 and 55 mg/L respectively. This experimental work was done in 10 L capacity pilot scale bioreactor. Samples were withdrawn from the bioreactor periodically after every two hours and SCOD and Ammonium nitrogen was measured for each sample using standard protocol. (Rice et al. 2012)

B. Study with Micro Algae

Time concentration and Kinetics study

Utilizing the optimum combination of these two species *C. pyredenosa and S. platensis*; (50+50) %, Time concentration study was performed in the open tray type bioreactor, Two CFL lights of 10 watts each were used as an illuminating device. Batch contact time was 8 days in the Tray reactor under ambient temperature. Samples from the reactor were withdrawn in a periodic manner of two days. Samples were analyzed thoroughly using prescribed standard techniques for necessary parameters (Rice et al. 2012). Obtained data was utilized for the time concentration plot.

The kinetic study was carried out by varying the initial inoculums concentration in the same way done in the previous small scale combined batch reactor study. Samples were withdrawn periodically from each set in the interval of 2 days for measuring the necessary parameters. Obtained data was utilized for the making of kinetic plots.

Results and Discussions

A. Study with bacterial consortium

Time concentration study with bacterial consortium

Around 97.22% of SCOD and 87.64% of Ammonium Nitrogen was removed by the optimum combination of microbial consortium composed of Carbon oxidation and nitrification sludge. Time concentration study performed in the real life dairy industry wastewater sample for 24 hrs of retention time. Trend of substrate removal and growth of microbial biomass is shown in the Time concentration table. 6.1.

Substrate utilization and bacterial growth kinetics study

Four kinetics coefficients each for Carbon oxidation and nitrification were obtained using modified version of Monod kinetics called Lawrence and McCarty equation. These kinetic coefficients were useful for further designing and fabrication of bioreactor. Similar techniques were applied to obtain the biokinetic coefficients from the kinetic plots as stated in the previous kinetic studies. The values of k, Ks, Y and K_d were obtained from those two straight-line equation. Values of kinetic coefficients obtained by plotting three different data sets in graphical plot of slightly different initial MLSS concentration are as follows: For SCOD removal values of K, K_s, Y and K_d are 1.05day^{-1}, 96.16 mg/L, 4.589mg of MLSS/mg of SCOD, and 0.049 day^{-1} respectively and for Ammonium nitrogen removal 1.08day^{-1}, 83.106 mg/L, 6.976mg of MLSS / mg of NH_4^+- N, 0.048 day^{-1} respectively obtained from fig. 6.4a-6.4d.

B. Study with Micro Algae

Time concentration and micro algal kinetics study

Around 80.55% of Phosphorus and 94.71% of Nitrate Nitrogen was removed by the optimum combination of microalgae in the time concentration study performed in the bacterial treated real life wastewater sample for 8 days of retention time. Percentage of substrate removal and growth of microalgae is shown in the Time concentration table. 6.2.

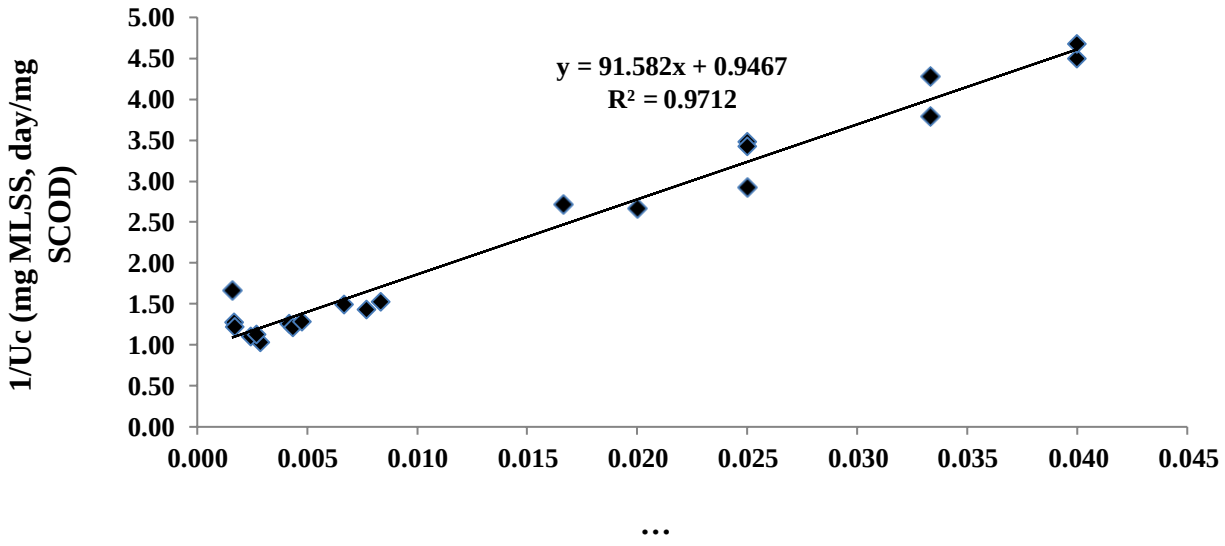
The kinetic study was carried out by varying the initial inoculums concentration in the same way done in the previous small scale batch study.

Samples were withdrawn periodically from each set in the interval of 2 days for testing the necessary parameters. Obtained data was has been plotted in the same manner in linearized Lawrance-Maccurty model to produce different kinetic plots for this combined treatment process shown in the following graphs; Fig. 6.5a-6.5d.

Table 6.1. and 6.2. Data obtained in the Time concentration study for both microbial consortium (Top) and combination of microalgae (Bottom) in suspended growth bioreactor and fixed film phycoremediation respectively.

Time(Hrs)	SCOD(mg/L)	% SCOD removal	Ammonium Nitrogen (mg/L)	% Ammonium Nitrogen removal	MLSS (mg/L)
0	720	0.00	55.00	0.00	1500
2	600	16.67	39.50	28.18	1832
4	260	63.89	20.21	63.25	2280
6	200	72.22	18.65	66.09	2410
8	150	79.17	16.20	70.55	2550
16	60	91.67	9.40	82.91	2680
20	40	94.44	7.80	85.82	2840
24	20	97.22	7.00	87.64	3120

Time(Days)	Nitrate Nitrogen(mg/L)	% Nitrate Nitrogen removal	Phosphorus (mg/L)	% Phosphorus removal	MLSS (mg/L)
0	55.00	0.00	15.00	0.00	100
2	39.10	28.91	10.15	32.33	360
4	21.08	61.67	7.20	52.00	590
6	9.92	81.69	4.45	70.33	700
8	2.91	94.71	2.92	80.55	780



…

Fig. 6.4aSubstrate-utilization kinetics for Carbon oxidation Study in Batch Reactor [Initial SCOD Concentration of 700-720 mg/L]

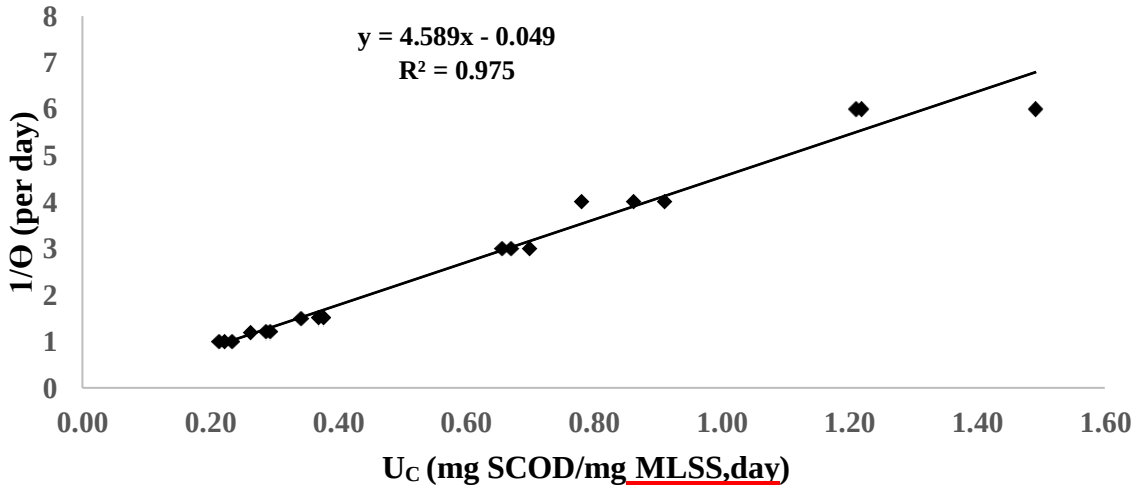


Fig. 6.4bMicrobial-Growth kinetics for Carbon oxidation Study in Batch Reactor [Initial SCOD Concentration of 700-720 mg/L]

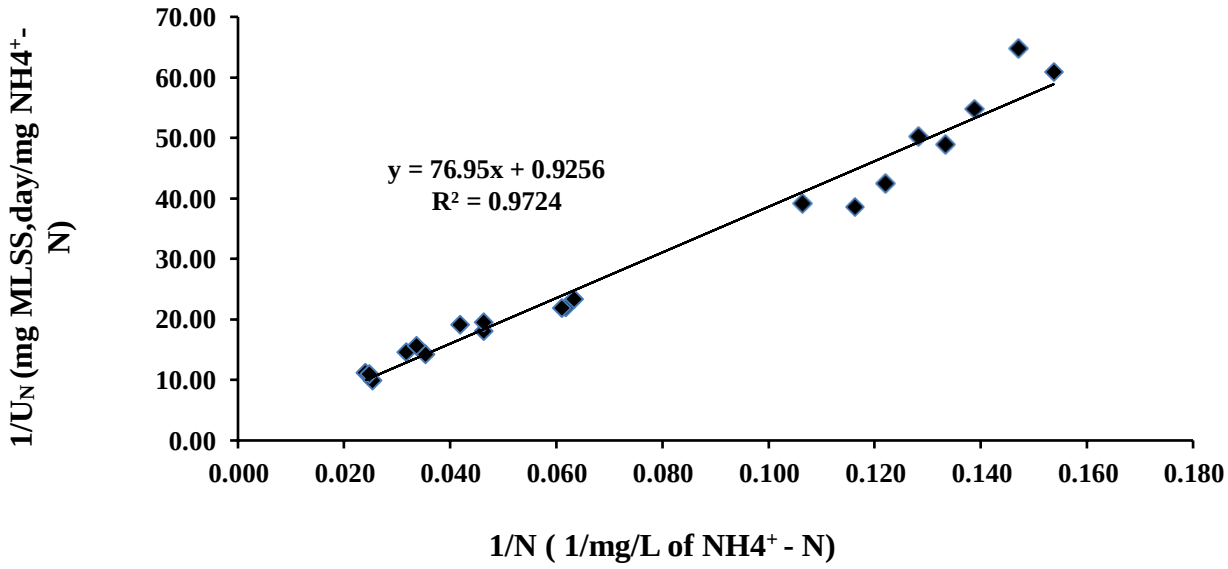


Fig. 6.4c.Substrate-utilization Kinetics for batch nitrification study [Initial NH_4^+-N= 48 -54 mg/L as N]

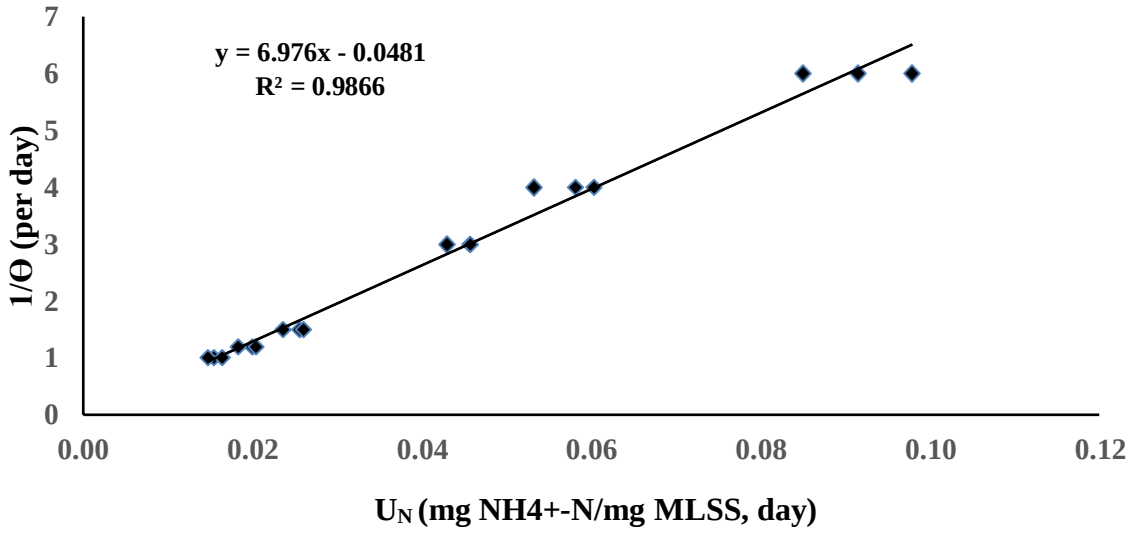


Fig. 6.4d Microbial-growth kinetics for batch nitrification study [Initial NH_4^+-N = 48 -54 mg/L as N]

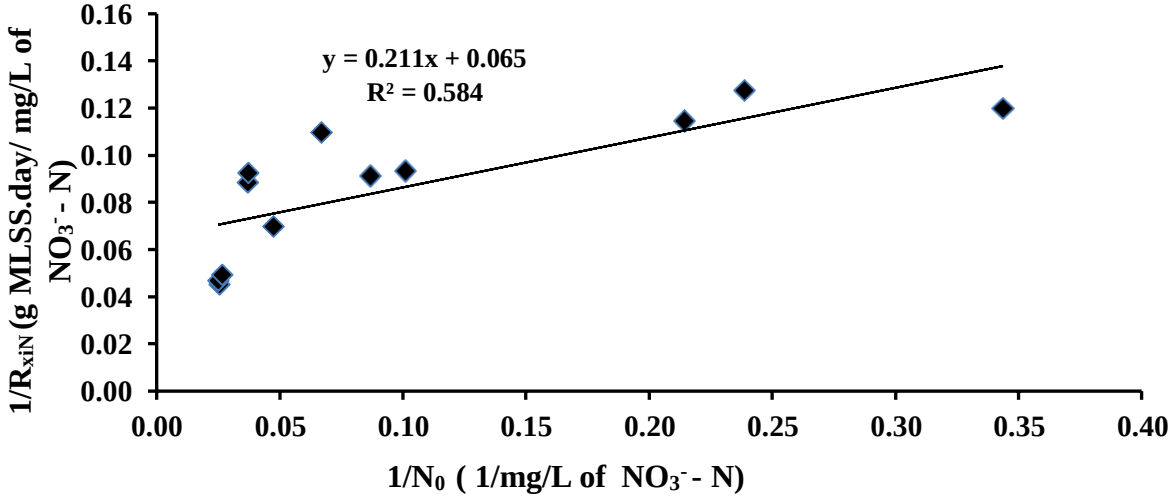


Fig. 6.5a.Substrate-utilization Kinetics of combination of microalgae for nitrate nitrogen removal in batch reactor from pretreated real life dairy wastewater [Initial NO_3^--N = 55 mg/L as N]

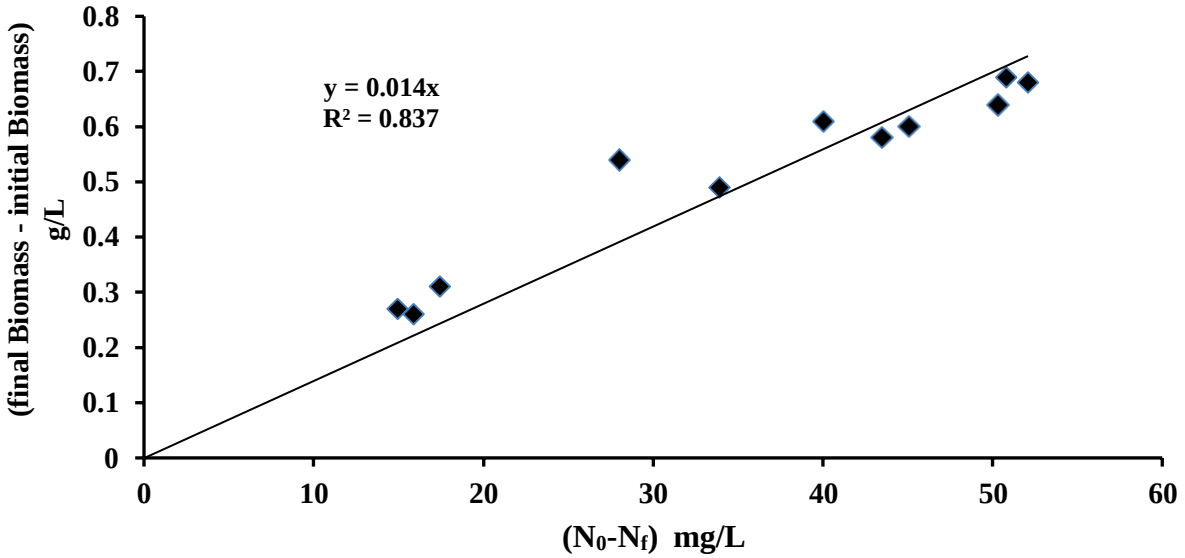


Fig. 6.5b. Micro algal growth kinetics of combination of microalgae for nitrate nitrogen removal in batch reactor from pretreated real life dairy wastewater [Initial NO_3^--N = 55 mg/L as N]

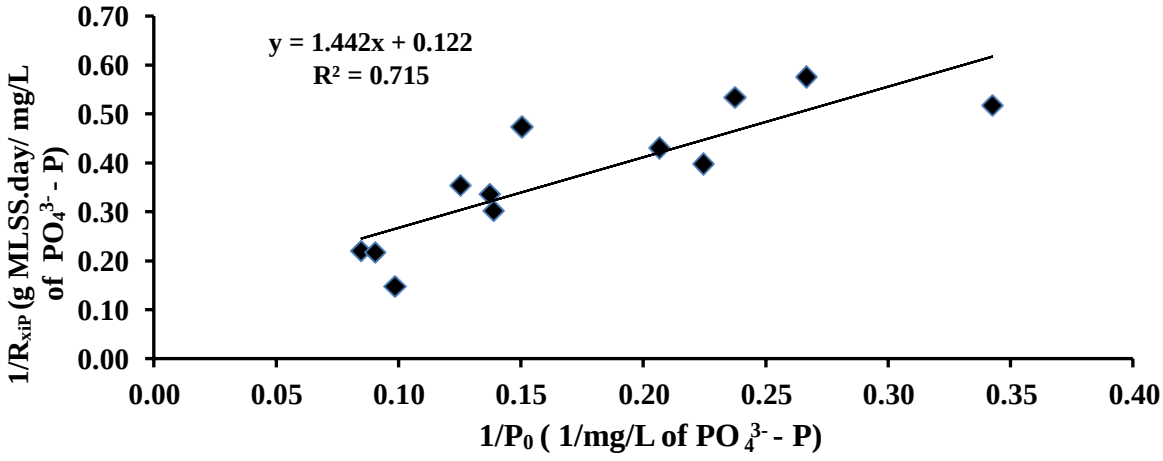


Fig. 6.5c.Substrate-utilization Kinetics of combination of microalgae for Phosphorus removal in batch reactor from pretreated real life dairy wastewater [Initial PO_4^{3-} - P = 16 mg/L as P]

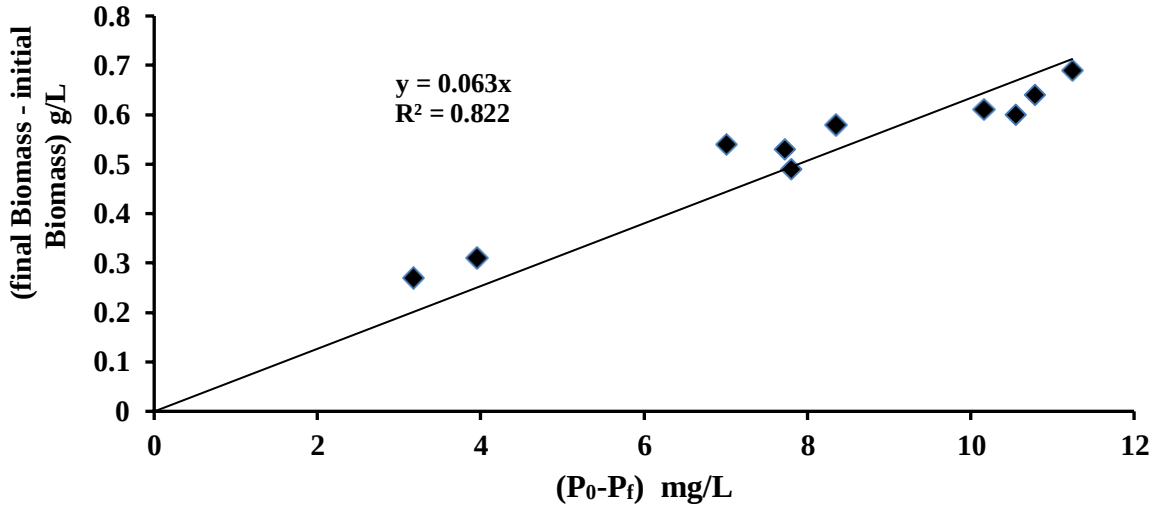


Fig. 6.5d Micro algal growth kinetics of combination of microalgae for phosphorus removal in batch reactor from pretreated real life dairy wastewater [Initial PO_4^{3-} - P = 16 mg/L as P]

Table 6.3a and b. List of kinetic coefficients for bacterial (Top) and micro algal (Bottom) treatment

Kinetic Coefficients	SCOD	Ammonium Nitrogen
K_s (mg/L)	96.16	83.106
k (per day)	1.05	1.08
Y (mg/mg)	4.589	6.976
K_d (per day)	0.049	0.048

Kinetic Coefficients	Nitrate Nitrogen	Phosphorus
K_s (mg/L)	3.245	11.818
k (per day)	15.38	8.196
Y (mg/mg)	0.014	0.063

General Discussions and Interpretation

Interpretation of some of the biological processes is necessary as these processes follow a certain trend. Time-Concentration study for both bacteria and microalgae is one of those processes; follow a certain trend. In the time concentration studies the removal of substrates in the form of organics and nutrients in the form of Nitrogen and Phosphorus follows a quite similar pattern. Removal is quite faster in the early phases and there is a reduction in the later halves of the removal process. It may be due to the optimum or most suitable Food to Microorganisms ratio or F/M ratio; which is an important parameter in case of microbial decomposition. As with respect to time substrates are consumed by the microorganisms along with their growth and multiplication F/M ratio deviates from its optimum range to a not so suitable one with time passing by. Therefore the rate of substrate utilization also declines. It is also comparable to the growth curve of bacteria, in which in the earlier times after a short lag or acclimatization phase exponential growth phase is observed with a rapid multiplication and increase in number of microorganisms and then there comes a stable plateau phase where growth and death rate are almost equal and finally the death and declining phase where more microorganisms are dying than those which reproduces new one because of severe competition and food or substrate shortage which may be easily explained by a very unsuitable F/M ratio. For the growth of microalgae the pattern is quite similar although the growth rate along with decay or death rate is quite slow with respect to bacteria. As Nutrients in terms of Nitrogen and Phosphorus are also taken as feed material by algae as in cases of substrate like organics for bacteria it follows a similar growth and decomposition pattern.

In the kinetic study section a modified version of Monod Kinetics model is employed which is similar to the modified Michaelis-Menten model Equation called Linewever-Burk plot. These give a simple linear plot which is very convenient for the derivation of the Kinetic constants. For the

growth and substrate utilization study of both bacteria and microalgae it has application in more or less similar manner. In most of the research studies where bacterial consortium is employed to derive kinetic coefficients applied this model and also some of the authors who has been worked with microalgal population used this linear model equation (Valentine et al., 2018, Aslan et al., 2006, Rowley 2010). For the study of substrate utilization Y=mx+c is the form of equation whereas for the micro algal growth kinetics study authors used the intercept less version that is y=mx. This is quite natural in case of microalgal growth kinetics plot as the intercept (c) or K_d in the original equation denotes the decay or death coefficient which is the implication of the death or decay rate of microalgae which is very small as compared to bacterial death rate.

The four kinetic coefficients K_s, Y, k and K_d have its own implications. K_s is not so specific parameter although it indicates the affinity of substrates to the microbial population. Y and k are the important indicators of their growth and multiplication. K_d which is the death coefficient which may in some cases also indicate the presence of any toxic compounds that leads to a faster than normal death of microbes population. A high yield or growth rate is a clear indication of a good F/M ratio, presence of simple biodegradable substrate and absence of inhibitory or toxic compounds in the growth media.

Chakraborty et al. in 2021 had performed similar study as of this present work with bakery and confectionery waste water. Bioremediation with mixed bacterial consortium was done in this case. Around 95% COD and over 75% Ammonium Nitrogen was removed within 24 hrs. Kinetic coefficients derived from this study was 0.765, 0.056, 9.09 and 350.69 for Y, K_d, k and K_s with Carbon oxidation study and 0.425, 0.063, 23.25 and 29.99 respectively with Nitrification study.

Bhattacharya et al. in 2019 had published their work on bioremediation of dairy waste water. For Carbon oxidation study kinetic constants were calculated and found to be 0.568 mg MLSS/mg COD, 72.134 mg/L, 0.031 per day and 8.84 per day for Y, Ks, K_d and k respectively.

Lateef et al. in 2013 had documented their work on biological treatment of dairy wastewater using activated sludge and their four kinetic constants were found to be around 0.933, 0.015, 867.76 and for Y, Kd, Ks and k respectively.

A simultaneous reduction of Carbon oxidation and Nutrients study in a batch fed reactor by Pseudomonas sp. P1 was carried out with organic loaded wastewater and there is around 95% removal of SCOD and 81.38% removal of Ammonium Nitrogen was found with an initial SCOD and Ammonium Nitrogen content of 600 mg/L and 50 mg/L respectively after twenty four hours (Kundu et al., 2016) These removal percentage values are very much similar to the findings made by the present study.

Moreover the book of Metcalf and Eddy has prescribed a range of those four kinetic parameters, Y, Ks, k and Kd for the biological treatment of municipal wastewater and these are as follows: 0.4-0.8, 25-100, 2-10 and 0.025-0.075 for Carbon oxidation study and 0.1-0.3, 0.2-5, 1-30 and 0.03-0.06 for Nitrification study respectively. These ranges of kinetic coefficients and the coefficient values found in those studies mentioned previously has corroborated the kinetics study of this present research work.

Valentine et al. in 2018, Aslan and Kapdan in 2006, Rowley in 2010 had performed their work on removal of nutrients especially Nitrogen and Phosphorus from wastewater with different potent microalgal strains and had developed their growth and substrate removal kinetics simultaneously by following the manner very similar to that of used by this study. A modified Monod kinetics was employed to find out kinetic coefficients for those nutrients assimilating processes. Coefficients found were in the range of 3-30, 2-40 and 0.01-0.06 for k, Ks and Y respectively for both Nitrogen and Phosphorus utilization studies. These values are more or less similar to the values of kinetic coefficients found in the present study while initial nutritent content was somewhat in the similar ranges of this study and the contact time was for almost all cases around 5-10 days.

Results of these studies endorse and corroborate the kinetics study made in this present study.

Conclusions

- Around 90% SCOD removal, around 80-90% ammonium nitrogen removal, beyond 95% of Nitrate nitrogen and around 75-88% of phosphorus reduction was found in almost all cases in the two stage biological treatment process with microbial consortium and microalgae in Suspended growth bioreactor and fixed film phycoremediation including small and pilot scale reactor respectively.

- Batch contact time for the case of bacterial consortium and microalgae was 24 hours and 8 days respectively. Initial concentration of the major input parameters were SCOD, Ammonium Nitrogen, Nitrate nitrogen and Phosphorus were in the ranges of 680-720 mg/L, 45-55 mg/L, 50- 55 mg/L and 15-18 mg/L respectively which was found in the characterization process of real life dairy waste water taken in four different times of a year to cancel out the seasonal variation in wastewater characteristics; from a nearby dairy industry plant located in Hooghly, West Bengal, India.

- Bio-kinetic coefficients were evaluated as the major measuring parameters of the bio reactorsystem. These kinetic coefficients were also compared with the results of similar bio-treatment processes carried out by other researchers studied in the similar field or domain of other allied researches.

- Identification of the most potent bacteria(s) for the degradation of organics and nutrients present in the wastewater was also carried out among the bacterial consortium.

- Kinetics study with these most potent bacterial strains was also performed and finally identification of these strains through16S r DNA process was done. Heterotrophic genus of bacteria namely *Bacillus* and *Solibacillus* strains were identified after genetic

identification process (16S rDNA) for Carbon oxidation and nitrification respectively.

- A statistical analysis was also done for the optimization as well as validation of the obtained experimental results. Response surface methodology which was employed for this purpose has made sure that the treatment process was operating in optimum conditions and has also endorsed this research work through the process of validation.

Scope for Future Studies

Principal Component Analysis (PCA) can be employed for the determination of strong positive and negative correlation between the physicochemical components. Validation and endorsement of the experimental work could be done through Artificial Neural Network or ANN study which is a better and modernized statistical tool that uses artificial intelligence in a better way.

References:

Arumugam, A., and P. Sabarethinam. 2008. Performance of a three-phase fluidized bed reactor with different support particles in treatment of dairy wastewater. ARPN Journal of Engineering and Applied Sciences 3(5).

Aslan, S. and IK. Kapdan. 2006. Batch Kinetics of Nitrogen and Phosphorus removal from synthetic wastewater from algae. Ecological Engineering, 28. 64-70.

Benefield L., and C. Randall. 1980. Biological Process Design for Wastewater Treatment, Prentice Hall, Inc., Englewood Cliffs.

Bergey, DH., RE. Buchanan, and NE. Gibbons. 1974. American society for Microbiology.8thEdition.Williams and Wilkins. Baltimore, USA.

Bhattacharya, R., P. Kundu Mukherjee, J. Mukherjee and S. Mukherjee. 2019. Kinetics Study of a Suspended Growth System for Sustainable Biological Treatment of Dairy Wastewater. Environmental Biotechnology For Soil and Wastewater Implications on Ecosystems, 15-20.

Brenner, DJ., NR. Kreig, and JT. Staley. 2005. The Proteobacteria. In G.M Garrity (ed.). 2nd Edition Vol: 2. Springer-Verlag, New York, USA.

Burton. C. 1997. FOG clearance. Dairy Ind. Int. 62 (12), 41–42.

Bustillo-Lecompte, C., M. Mehrvar, and E. Quiñones-Bolaños. 2016, Slaughterhouse Wastewater Characterization and Treatment: An Economic and Public Health Necessity of the Meat Processing Industry in Ontario, Canada. Journal of Geoscience and Environment Protection. 4, 175-186.

Carta-Escobar, F., J. Pereda-Marín, P. Álvarez-Mateos, F. Romero-Guzmán, and M. Durán Barrantes. 2005. Aerobic purification of dairy wastewater in continuous regime, Kinetic study of the organic matter removal in two reactor configurations. Biochem. Eng. J22, 117–124.

Chakraborty, B., P, Kundu &, J. Mukherjee and S. Mukherjee. 2021. Kinetics Study of a Suspended Growth System for Biological Treatment of Bakery and Confectionery Wastewater. Advances in Bioprocess Engineering and Technology, 339-348.

Chaubey, M. 2002. Treatment of dairy effluent with fixed film bioreactor technology. Environ. Pollut. Cont. J. 5(2), 36-38.

Chawla, A., N. Chawla, and Y. Pant. 2009(Retrieved 3rd August 2014). Milk and Dairy Products in India – Production, Consumption and Exports: Introduction, Hindustan Studies & Services Ltd., India.

Cristian, O. 2010. Characteristics of the untreated wastewater produced byfood industry Analele Universităţiidin Oradea, Vol. XV.

Danalewich, J., T .Papagiannis, R. Belyea, M.Tumbleson, and L. Raskin. 1998. Characterization of dairy waste streams, current treatment practices, and potential for biological nutrient removal. Water Res 32 (12), 3555–3568.

Deshannavar, U., R. Basavaraj, and M. Naik. 2012.High ratedigestion of dairy industry effluent by up flow anaerobic fixed-bed reactor.JournalofChemicalandPharmaceuticalResearch,4(6), 2895-2899.

Deshpande D., P. Patil, and S. Anekar. 2012. Biomethanation ofDairy Waste. Research Journal of Chemical Sciences. 2(4),35-39.

Donkin, J. 1997. Bulking inaerobic biological systems treating dairy processing waste waters. Int. J. Dairy Tech 50, 67–72.

Engblom, S., 1998. The phosphate sensor. Biosensors & Bioelectronics. 13, 981-994.

Elumalai, S., S. Ramganesh, S. Anuvarshini, T. Sangeetha, and D. Roopsingh. 2014.Phycoremediation for Leather Industrial Effluent - Treatment and Recycling Using Green Microalgae and its Consortia. Int.J.Curr.Biotechnol, 2(10), 1-9.

Fathi, A., M. Azooz, and A. Mohamed. 2013. Al-Fredan: Phycoremediation and the Potential of Sustainable Algal Biofuel Production using Wastewater. American Journal of Applied Sciences. 10(2), 189-194.

Ferguson,A. 1976. Acceptability of waste water effluents by soils. In: Sanks RL, Asano T

(eds) Land treatment and disposal of municipal and industrial waste water. Ann Arbor Science, Ann Arbor: 85–100.

Gani, P., N. Sunar, H. Matias-Peralta, et al. 2014. Er. Phycoremediation of Dairy Wastewater by Using Green Microalgae: Botryococcus sp. Applied Mechanics and Materials. 773, 1318-1323.

Gotmare, M., R. Dhoble, and A.Pittule. 2011. Biomethanation of Dairy Waste Water Through UASB at Mesophilic Temperature Range. IJAEST. 8(1),001-009

Holt.J., G. Kreig, J. Sneath, S. Williams, 1993. Bergey's Manual of Determinative Bacteriology, 9th Edition.

Hoover.S., and N. Porges. 1952. Assimilation of dairy wastes by activated sludge. II. The equation of synthesis and the rate of oxygen utilization, Sewage and Ind. Wastes 24, 306-312.

Kabbout, R., M. Baroudi, F. Dabboussi, J. Halwani, and S. Taha. 2011. Characterization, Physicochemical and Biological Treatmentof Sweet Whey (Major Pollutant in Dairy Effluent). InternationalConferenceonBiology.EnvironmentandChemistryIPCBEE.Vol.2.IACSITPress,Singapore

Kim. O., Y. Cho, K. Lee, S. Yoon, M. Kim, and other authors. 2012. Introducing EzTaxon-e: A prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62, 716–721.

Kozłowska, A., L. Dembiczak, and B. Bańka. 2014. Phycoremediation of Wastewater: Heavy Metal and Nutrient Removal Processes. Ochrona Srodowiskai Zasobów Naturalnych. 25(4), 51–54.

Kundu, P., V. Datta, and S. Mukherjee. 2016. Simultaneous Carbon-Oxidation and Nutrient (N and P) Reduction in a Batch-Fed Reactor by Pseudomonas sp. P1 Isolated from a Small-scale Slaughterhouse Wastewater. Journal of Advances in Biology & Biotechnology. 6. 1-13.

Kushwaha, J., and V. Srivastava. 2013. Sequential batch reactor for dairy wastewater treatment:

Parametric optimization; Kinetics and waste sludge disposal. Journal of Environmental Chemical Engineering 1(4), 1036-1043.

Kumar, A., 2010. Enhanced CO_2 fixation and biofuel production via microalgae: recent developments and future directions. Trends in Biotechnology. 28, 371–380.

Lata, K., A. Kansal, M. Balakrishnan, K. Rajeshwari, and V.Kishore. 1998. Tata Energy Research Institute, Biogas Users Survey inNepal,EvaluationofBiomethanationPotentialofSelectedIndustrialOrganicEffluentsinIndia.

Lateef, A., M. Nawaz Chaudhry, and S. Ilyas. 2013. Biological treatment of dairy wastewater using activated sludge, Science Asia Journal 39:179-185.

Lee , JM. 1992. Biochemical Engineering: Prentice Hall international. E Book Ver. 2.32. New Jersey, USA.

Lu, Q., 2016. Mitigating ammonia nitrogen deficiency in dairy wastewater for algae cultivation. Bioresource Technology. 201, 33-40.

Lu, Q., 2015. Growing *Chlorella sp.* on meat wastewater for nutrient removal and biomass production. Bioresource Technology. 198, 189–197.

Lu, W., Z. Wang, X. Wang, and Z. Yuan. 2015. Cultivation of *Chlorella sp.* using raw dairy wastewater for nutrient removal and biodiesel production: Characteristics comparison of indoor bench-scale and outdoor pilot-scale cultures. Bioresource Technology. 192, 382–388.

Mata, T., A. Martins, and N. Caetano. 2010. Microalgae for biodiesel production and other applications: A review. Renewable and Sustainable Energy Reviews. 14, 217-232.

Metcalf & Eddy Inc. 2004. Wastewater Engineering: Treatment, Disposal and Reuse, 4th edition, McGraw-Hill New York. 709-721.

Metcalf & Eddy Inc. 2009. Wastewater Engineering: Treatment, Disposal and Reuse, 4th edition, McGraw-Hill New York. 704-716.

Mohseni-Bandpi, A., and H. Bazari. 2004. Biological Treatment of Dairy Wastewater by Sequencing Batch Reactor. Iranian J Env Health Sci Eng. 1(2), 65-69.

Naser, M., A. Khalil, A. Abdel-Kader, W. Elbarki, and M. Moustafa. 2014. Comparative performance between conventional and hybrid anaerobic baffled reactors for treating dairy wastewater. International Journal of Chemical and Environmental Engineering 5(4), 123-129.

Page, L., N. Ji-Qin, A. Heber, N. Mosier, X. Liu, H. Joo, P. Ndegwa, and J. Harrison. 2014. Characteristics of volatile fatty acids in stored dairy manure before and after anaerobic digestion, Biosys. Eng. 118, 16–28.

Porges , R., H. Heukelekian, R. Manganelli, and H. Ambergand other authors.1957. Industrial Wastes, Sewage and Industrial Wastes29(6), 613-639.

Qazi, J . , M. Nadeem,S. Baig, and Q. Syed. 2011. Anaerobic Fixed Film Biotreatment of Dairy Wastewater.Middle-East Journal of Scientific Research. 8 (3), 590-593,ISSN1990-9233,©IDOSIPublications.

Raghunath, B., A. Punnagaiarasi, G. Rajarajan, A. Irshad, A. Elango and G. Mahesh kumar. 2016. Impact of Dairy Effluent on Environment—A Review. Springer International Publishing Switzerland,239-249.

Rao, P., R. Kumar, BG. Raghavan, VV. Subramanian, and V. Sivasubramanian. 2011. Application of phycoremediation technology in the treatment of wastewater from a leather-processing chemical manufacturing facility, Water SA, 37(1).

Rice, E., and A. Eaton. 2012. Apha Standard Methods for the Examination of Water and Waste water.23rd Edition. American Public Health Association, Washington DC.

Robinson, T. 1997. The real value of dairy waste. Dairy Ind. Int. 62(3), 21–23.

Rowley, W.M. 2010. Nitrogen and phosphorous biomass-kinetic model for *Chlorella pyrenoidosa* in a biofuel production scheme. Airforce Institue of Technology. Ohio. USA.

Rowley, WM. 2010. Nitrogen and Phosphorus Biomass Kinetics Model for Chlorella vulgaris in a Biofuel Production Scheme. Department of the Air Force University. Ohio, USA

Sathya moorthy, G., and M. Saseetharan. 2012. Dairy WastewaterTreatmentbyAnaerobicHybridReactor–astudyontheReactorPerformance and Optimum Percentage of Inert Media Fill inside Reactor. Res. J. Chem. Environ.16(1).

Sharma, V., and A. Gulati. 2003. Trade liberalization, market reforms and competitiveness of indian dairy sector, MTID Discussion Paper No.61, Markets, Trade and Institutions Division, International Food Policy Research Institute (IFPRI), Washington, D.C.

Shete, B., and N. Shinkar. 2013. Dairy Industry Wastewater Sources, Characteristics & its Effects on Environment. International Journal of Current Engineering and Technology. 3(5), 1611-1615.

Sivasubramanian. V., V. Subramanian, and M. Muthukumaran. 2012. Phycoremediation of effluent from a soft drink manufacturing industry with a special emphasis on nutrient removal – a laboratory study. J. Algal Biomass Utln, 3 (3), 21–29.

Strydom, J., J. Mostert, and T. Britz. 1993. Effluent production and disposal in the South African dairy industry–a postal survey. Water SA 19 (3), 253–258.

Talukder, A., S. Mahmud, S. Lira, and M. Abdul. 2015. Phycoremediation of Textile Industry Effluent by Cyanobacteria (Nostoc muscorum and Anabaena variabilis). Bioresearch Communications. 1(2), 124-127.

Tawfika, A., M. Sobheyb, and M. Badawya. 2008. Treatment of a combined dairyand domestic wastewater in an up-flow anaerobic sludge blanket (UASB)reactor followed by activated sludge (AS system). Desalination227, 167–177

Venkatesan, K., M. Saseetharan, and V. Arutchelvan. 2004. Determination of bio-kinetic coefficients for dairy waste water. Jr. of Industrial Pollution Control 20(1), 7- 16.

Vidal, G., A. Carvalho, R. Me´ndez, and J. Lema. 2000. Influence of the content in fats and proteins on the anaerobic biodegradability of dairy waste waters. Biores. Technol. 74, 231–239.

Weisburg, W., S. Barns, D. Pelletier, and D. Lane. 1991. 16S Ribosomal DNA Amplification for Phylogenetic Study. Journal of bacteriology. 173(2), 697-703.

Valentine, C., B. Sharon, AH. Garcia and others. 2018. Kinetic Modeling of microalgae cultivationfor wasteweater treatment and CO2 sequestration. Algal research, 32. 131-141.

Online Web References:

https://cpcb.nic.in/
https://www.who.int/
https://www.epa.ie/
https://www.worldbank.org/en/home

Annexure - I

List of frequently used Abbreviations/Symbols:

Abbreviations:

Standard Abbreviated Terms:

BOD: Biological/Biochemical Oxygen Demand
CCD: Central Composite Design
SCOD: Chemical Oxygen Demand
CPCB: Central Pollution Control Board
DNA: De-Oxyribo Nucleic Acid
DO: Dissolved Oxygen
EPA: Environmental Protection Agency
ETP: Effluent Treatment Plant
LED: Light Emitting Diode
MLSS: Mixed Liquor Suspended Solids
MLVSS: Mixed Liquor Volatile Suspended Solids
nm: Nanometer
rpm: Rotation per Minute
RSM: Response Surface Methodology
SCOD: Soluble Chemical Oxygen Demand (Used as an equivalent and interchangeable term of SCOD)SPCB: State Pollution Control Board
TDS: Total Dissolved Solids
TKN: Total Kjeldahl Nitrogen
TN: Total Nitrogen
TS: Total Solids
TSS: Total Suspended Solids
VFA: Volatile Fatty Acids
WB: World Bank
WHO: World Health Organization
ISE: Ion Selective Electrode

Special Abbreviated Terms used in Thesis:

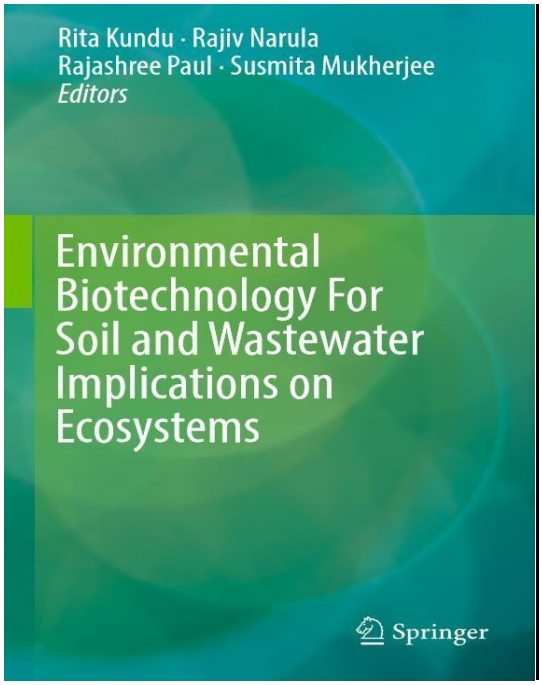
BTDW: Bacterial Treated Dairy Wastewater
FTBE: Food Technology and Biochemical Engineering
JU: Jadavpur University
SDW: Synthetic Dairy Wastewater

Symbols:

K_s: Half Saturation Constant
k: Maximum Substrate utilization rate
Y: Yield Coefficient
K_d: Death/Decay coefficient
U: Specific Substrate utilization rate
S: Concentration of Substrate
$\sum$: Summation
Θ (Theta): Residence/Retention Time
C1...-C6/BC1...-BC6: Carbon Oxidation Strain 1....-6/ Bacterial Carbon Oxidation Strain 1....-6 (Used synonymously as C1-C6 and BC1-BC6)
N1...-N5/BN1...-BN5: Nitrification Strain 1....-6/ Bacterial Nitrification Strain 1....-5 (Used synonymously as N1-N5 and BN1-BN5)

Annexure – II

Scanned Images of Published Papers



Contents

xi

Bioremediation of Wastewater Using Microalgae

8

Abhishek Das, Sunita Adhikari (Nee Pramanik), and Pradyut Kundu

Abstract

This study describes the feasibility for treatment of waste waters in batch cultures by using selected strains of green microalgae *Chlorella sp.* A5. To investigate the role of algae in wastewater treatment algal samples were collected from polluted water. These samples were used to isolate most dominant and pollution tolerant algae and used for the treatment. The wastewater samples used in this study was prepared in laboratory synthetically having composition of Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Kjeldahl Nitrogen (TKN) and Total Phosphorus (TP) of 398, 705, 38 and 25 mg/L respectively further reduced by ~98.5%, 97.8%, 92.6% and 99.2% by the algal treatment respectively at the end of the process. The experiments were carried out in the laboratory of Food Technology and Biochemical Engg, Jadavpur University using synthetic medium. *Chlorella sp.* A5 was found to be the most dominant one. Synthetic Dairy Waste Water (SDWW) was prepared in such proportion that could mimic the original Dairy wastewater. Those isolated species of *Chlorella* was then added to the prepared SDWW. A control sample (C-SDWW--sample without the algal species) was also prepared. Both the SDWW and C-SDWW was analyzed periodically for the waste water quality parameters in an interval of 3 days to evaluate the performance of the algae. At the end of our experiment the nutrients and BOD, COD removal rate was found to be sufficiently high especially the phosphate removal.

Keywords

Algae · SDWW · BOD · COD

A. Das (✉) · S. Adhikari (Nee Pramanik)
Department of Food Technology & Biochemical Engineering, Jadavpur University, Kolkata, India

P. Kundu
Department of Food Processing Technology, A. P. C Ray Polytechnic, Jadavpur Campus, Kolkata, India

Special Issue on "Innovative Technologies for Industrial Waste Management"
J. Indian Chem. Soc.,
Vol. 97, No. 9a, September 2020, pp. 1323-1327

Characteristics and treatments of dairy industry wastewater in a suspended growth batch reactor

Abhishek Das[a], Pradyut Kundu[b] and Sunita Adhikari (nee Pramanik)*[a]

[a]Department of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata-700 032, India

[b]Department of Food Processing Technology, Mirmadan Mohanlal Govt. Polytechnic, Plassey, Nadia-741 156, West Bengal, India

E-mail: sunitapramanik@gmail.com

Manuscript received online 02 January 2020, accepted 20 June 2020

This research work was embraced to assess the kinetic coefficients values of a suspended growth type batch reactor for the dairy industry wastewater treatment. This examination was done in a research facility batch type arrangement which was comparable to the aeration tank of Effluent Treatment Plant (ETP). Wastewater samples were withdrawn on regular basis to characterize wastewater. Simulated dairy wastewater was made synthetically according to the characterization done which was found the COD of 640 mg/L and ammonium nitrogen concentration of 43 mg/L. Kinetic study was conducted to find kinetic coefficients (Y, K_s, k_d and k).

Keywords: Dairy wastewater, COD, kinetic coefficients, suspended growth system, ammonium nitrogen.

Introduction

Food manufacturing and processing industries pollute the environment and ecosystem adversely. Dairy industry is one of the major polluting industries among the food sector. Throughout the process chain of the dairy industry affect the environment[1]. Majority of the dairy plants perceive treatment of wastewater as an evil thing[2]. In general, they produce whitish to off white colour of waste water containing different types of carbohydrates, proteins, fats and other biological materials. Dairy industry wastewaters characteristics vary to anoxia. Nutrient like nitrate and phosphate are also a major concern. Cleaning-In-Place system (CIP) in those dairy plants uses mostly phosphate containing detergents which ultimately end up in the aquatic body. Common CIP agents are nitric acid, caustic soda, phosphoric acid, and sodium hypochloride[6], all of those have impacts on environment. If the discharged water not treated adequately; goes into the aquatic system can lead to eutrophication in lakes and ponds. Eutrophication is a condition when the nutrient level in the system becomes very high which causes algal bloom and

BIOREMEDIATION JOURNAL
https://doi.org/10.1080/10889868.2021.1988893

Taylor & Francis
Taylor & Francis Group

Check for updates

Biological treatment of dairy industry wastewater in a suspended growth batch reactor: performance evaluation and biodegradation kinetics

Abhishek Das[a], Pradyut Kundu[b], and Sunita Adhikari (Nee Pramanik)[a]

[a]Department of Food Technology & Biochemical Engineering, Jadavpur University, Kolkata, India; [b]Department of Food Processing Technology, Mirmadan Mohanlal Government Polytechnic, Plassey, Nadia, India

ABSTRACT

This research work was carried out to evaluate the kinetic constants for the biological treatment of dairy industry wastewater in a suspended growth batch reactor. The study was performed in a lab-scale batch reactor which is a prototype of an aeration tank unit of the activated sludge system. Samples of dairy wastewater were collected from a dairy plant near Kolkata on regular time interval and in different seasons of a year to establish mean values of the major wastewater parameters. A synthetic simulated sample was made on the basis of evaluated characteristics of dairy wastewater. Kinetics study in batch reactor was done to find kinetic coefficients (Y, Ks, k_d and k). The value of the maximum substrate removal rate (k) at preliminary concentration of COD 700–720 mg/L was 4.42 day^{-1}; half velocity constant (Ks) was 206.81 mg/L and k value at initial NH_4^+–N level of 48–54 mg/L was found 2.5 per day, Ks was 24.5 mg/L. Isolation and identification of the most potent strains for the carbon oxidation and nitrification was also done and by using those isolated strains kinetic coefficients for pure culture was determined.

KEYWORDS

Carbon oxidation; dairy wastewater; isolation of predominant bacterial species; kinetic constants; nitrification; suspended growth batch reactor

Introduction

Industries are the backbone of our civilization. It helps improving our country's Gross Domestic Product (GDP) and helps to develop our economy. Booming of industries is now becoming the hallmark of many developing nations. A phenomenon of rapid industrialization is also associated with our nation, in the last few decades. Expanding industrial development has its negative aspects as well. Pollution in the form of toxic gases, contaminants, solid wastes as well as liquid discharges are the major by-products of industrial production. Noise and other forms of pollutions coming from industries can't be neglected too. The impacts of these pollutants are moderate to severe depending on their physical and chemical nature. Impacts are not only limited in the sphere of human society but also affects the whole ecosystem. Food manufacturing and processing industries pollute the environment and ecosystem as well.

Fast development of enterprises has improved the productivity as well as brought about the creation and injection of harmful substances into the environment, which are responsible for creating health hazards to human, influencing various normal operations as well as producing harm to flora and fauna in the environment. These squander are potent toxins as they hit nature badly and delivered huge quantity of solids or liquid wastes including broad spectrum of synthetic compounds. Sewage treatment in plants to fulfill the discharge standard guidelines prescribed by different pollution controlling agencies has consistently been an issue for the enterprises. Prior to releasing the treated waste on land or in water body the enterprises should properly meet the standards. To maintain the proper processing in a wastewater treatment unit, wastewater characterization, treatability studies and proper planning for treatment procedure is quite essential.

In many countries, dairy industry is recognized as one of the biggest well springs of food

ISSN 1063-455X, Journal of Water Chemistry and Technology, 2022, Vol. 44, No. 3, pp. 208–215. © Allerton Press, Inc., 2022.

BIOLOGICAL METHODS OF WATER PURIFICATION

Treatability and Kinetic Study of Dairy Effluent Using Microalgae *Spirulina platensis* in a Laboratory Scale Batch Reactor

Abhishek Das[a], Pradyut Kundu[b], and Sunita Adhikari (Nee Pramanik)[c, *]

[a] *Department of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata-32, India*

[b] *Department of Food Processing Technology, Mirmadan Mohanlal Govertment Polytechnic, Plassey, Nadia, India*

[c] *Department of Food Technology & Biochemical Engineering, Jadavpur University, Kolkata-32, India*

**e-mail: sunitapramanik@gmail.com*

Received May 30, 2021; revised June 5, 2021; accepted December 29, 2021

Abstract—Purpose of this research work is to evaluate the bio kinetic coefficients of a pretreated Dairy wastewater in a suspended growth batch reactor with the treatment of microalgae *Spirulina platensis*. The study was performed in a laboratory scale batch setup. Samples of dairy plant was collected and it was pretreated with a consortium of carbon oxidation and nitrification process with bacterial culture that was made as the feed of this purification process. Bacterial pretreatment was done in order to reduce its organic carbon and ammonium nitrogen content. Nitrate nitrogen which was formed during bacterial treatment process and most of the phosphate still remaining in the water was treated in this process with micro algal species to remove these nutrients in wastewater and consequently to meet with discharge standards of regulatory authorities like Central Pollution Control Board (CPCB) of India and World Health Organization (WHO). A simulated synthetic wastewater sample was prepared according to the concentration measured in the original pretreated-wastewater where average nitrate nitrogen concentration was found 54 mg/L and phosphate concentration was 16 mg/L. The maximum 99.00 and 90.38% of nitrate nitrogen (NO_3^--N) and phosphorus (PO_4^{3-}-P) removal were achieved corresponding to initial nitrate nitrogen and phosphorus concentration of 54 and 16 mg/L respectively, with an initial inoculum concentration of microalgae *Spirulina platensis* of 0.8% v/v after 8 days of detention period in batch reactor. Kinetics study was also carried out to obtain bio-kinetic coefficient for nitrate nitrogen and phosphorus removal using microalgae *Spirulina platensis* in order to obtain kinetic constants (Y, K_s and k). The values of k, K_s and Y_N were found to be 21.74 per day, 1.61 mg/L and 0.011 g of biomass/mg of NO_3^--N as N for nitrate nitrogen removal. Values of k, K_s and Y_P were found to be 14.49 per day, 16.63 mg/L and 0.052 g of biomass/mg of PO_4^{3-}-P as P for PO_4^{3-}-P removal. Corresponding kinetic coefficients were compared to studies done by other researchers which corroborate the findings of this present investigation.

Keywords: dairy wastewater, batch reactor, nitrate nitrogen, phosphate, microalgae, kinetic coefficients

DOI: 10.3103/S1063455X22030031

INTRODUCTION

Dairy industry is seen as one of the greatest sources of wastewater producing industry. The dairy wastewater contains gigantic measures of milk constituents, like casein, inorganic salts along with other chemicals and sanitizers used for washing and cleaning. These organics and nutrients have potential to cause nuisance if left untreated. To reduce these waste loads different types of treatment methods are available. These removal techniques may be physical, chemical or biological. Physical treatment methods alone are not so efficient and so it may be done in line with biological and chemical treatment. Chemical treatments are generally much expensive. Treatment with biological agents like plants, microbes or algae is a relatively

Biocatalysis and Agricultural Biotechnology 43 (2022) 102387

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Two stage treatability and biokinetic study of dairy wastewater using bacterial consortium and microalgae

Abhishek Das [a], Pradyut Kundu [b], Sunita Adhikari (Nee Pramanik) [a,*]

[a] Dept. of Food Technology & Biochemical Engineering, Jadavpur University, Kolkata, 32, India
[b] Dept. of Food Processing Technology, Mirmadan Mohanlal Govt. Polytechnic, Plassey, Nadia, India

ARTICLE INFO

Keywords:
Dairy wastewater
Batch reactor
Carbon oxidation
Nutrient removal
Microalgae *C. pyrenoidosa*
Bio-kinetic coefficients

ABSTRACT

Purpose of present research work is to perform nutrient removal and evaluate bio-kinetic co-efficients of a two-stage dairy wastewater treatment process in batch reactor using bacterial consortium and microalgae *Chlorella pyrenoidosa*. The research work was performed in laboratory-scale batch reactor. Primary treated real life dairy wastewater after a treatment with a consortium of bacterial population containing carbon oxidation and nitrification bacteria was taken as the feed material for algal treatment process using microalgae *C. pyrenoidosa* strain. Nitrate nitrogen which was formed during bacterial nitrification still remaining in bacterial pre-treated dairy wastewater along with phosphorus that have been removed by algal treatment using microalgae of *C. pyrenoidosa*. The present investigation was done for bacterial and algal treatment of dairy plant effluent in a lab-scale batch reactor with acclimatized seeds of both carbon oxidation, nitrification bacteria, microalgae *C. pyrenoidosa* and also to evaluate various biokinetic coefficients. Around 95% ammonium nitrogen and soluble chemical oxygen demand (SCOD), around 99.67% and 90.25% of Nitrate nitrogen (NO_3^--N) and Phosphorus (PO_4^{3-}- P) removal were achieved corresponding to initial ammonium nitrogen and SCOD of 55 and 720 mg/L, nitrate nitrogen and phosphorus concentration of 54 and 16 mg/L respectively, with an initial inoculum concentration of bacterial consortium of 15%; corresponding to 1500 mg/L and microalgae *C. pyrenoidosa* of 0.8% v/v; corresponding to 80 mg/L after 24 h and 8 days of detention period in batch reactor respectively. Kinetic study was also carried out to obtain bio-kinetic coefficient for SCOD, ammonium nitrogen, nitrate nitrogen and phosphorus removal using bacterium and microalgae *C. pyrenoidosa*.

1. Introduction

In numerous nations, dairy industry is viewed as one of the biggest wellsprings of organic wastewater generating industry. Dairy