HYBRID BIOLOGICAL SYSTEMS FOR WASTEWATER TREATMENT

Thesis

Submitted in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

by

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D E C L A R A T I O N

I hereby *declare* that the Research Thesis entitled **"*Hybrid biological systems for wastewater treatment*"** which is being submitted to the **National Institute of Technology Karnataka, Surathkal** in partial fulfilment of the requirements for the award of the Degree of **Doctor of Philosophy** in **Civil Engineering**, is a *bonafide* report of the research work carried out by me. The material contained in this Research Synopsis has not been submitted to any University or Institution for the award of any degree.

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C E R T I F I C A T E

This is to *certify* that the Research Thesis entitled **"*Hybrid biological systems for wastewater treatment*"** submitted by **Mr. Manu. D S** (Register Number: **138004CV13F05**) as the record of the research work carried out by him is accepted as the Research Thesis submission in partial fulfilment of the requirements for the award of degree of Doctor of Philosophy.

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A C K N O W L E D G E M E N T

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Abstract

The current trend in sustainable development deals majorly with the environmental management. There is a need for economically affordable, advanced treatment methods for the proper treatment and management of domestic wastewater containing excess nutrients (such as nitrogen and phosphorous) which otherwise may lead to eutrophication. In the present study, the effect of carbon to nitrogen (C/N) ratio, suspended biomass concentration (X), hydraulic retention time (HRT), and dissolved oxygen (DO) on nutrients removal in a lab-scale activated sludge biofilm (AS-biofilm) reactor was monitored. Based on various trials, it was seen that AS-biofilm reactor achieved good removal efficiencies with respect to COD-92%, NH_4^+-N- 93%, TN- 86% and TP-52%. Further, in order to improve the quality of the treated wastewater, photocatalysis by TiO_2 was investigated as a post-treatment technology, using solar and UV irradiations. The UV photocatalysis was found to be better than solar photocatalysis during the comparative analysis. The maximum removal efficiencies of COD, MPN and phosphorous at optimum conditions in the case of UV and solar irradiations were 72%, 95%, 52% and 71%, 99%, 50% respectively. Similarly, to enhance the performance of the system in terms of nitrogen and phosphorous in addition to carbon removal integrated anaerobic/anoxic/oxic activated sludge biofilm (A^2O-AS-biofilm) reactor was designed and operated by varying operating conditions such as C/N ratio, suspended biomass (X), HRT and DO. Based on various trials, it was seen that the A^2O-AS-biofilm reactor achieved good removal efficiencies of COD-95.5%, TP-93.1%, NH_4^+-N-98% and TN-80% when the reactor maintained C/N ratio - 4, suspended biomass (X) - 3 to 3.5 g/L, HRT-10hr, and DO - 1.5 to 2.5mg/L. Applicability of soft computing techniques viz, Adaptive Neuro Fuzzy Inference System (ANFIS), Genetic Algorithm Adaptive Neuro Fuzzy Inference System (GA-ANFIS) and Particle Swarm Optimization Adaptive Neuro Fuzzy Inference System (PSO-ANFIS) to performance prediction of hybrid system was studied. ANFIS was applied on real time WWTP of 43.5 MLD capacity. ANFIS models showed better efficiency while modeling wastewater using multivariate analysis. So in the current study, in order to improve the prediction ability of ANFIS, hybrid models such as GA-ANFIS and PSO-ANFIS have been applied for the prediction of effluent TN, COD and TP concentration yielded from a hybrid AS-biofilm reactor. From the results, both GA-ANFIS and PSO-ANFIS proved capable to predict the effluent parameters of the reactor with varying operation conditions and can be adopted for modeling the nonlinear data.

***Keywords**:* AS-biofilm, Biomass, Carbon/nitrogen, GA-ANFIS, PSO-ANFIS

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LIST OF ABBREVIATIONS

AOPs	Advanced Oxidation Processes
ANN	Artificial Neural Network
ANFIS	Adaptive Neuro-Fuzzy Inference System
AS	Activated Sludge
A^2O	Anaerobic Anoxic Oxic
AOA	Anaerobic Aerobic Anoxic
A^2 N–IC	Anaerobic–Anoxic/Nitrifying-Induced Crystallization
A^2N	Anaerobic-Anoxic/Nitrifying
AOB	Autotrophic Bacteria
ASFF	Aerated Submerged Fixed Film
AUR	Ammonia Uptake Rate
BOD	Biochemical Oxygen Demand
BPNN	Back Propagation Neural Network
BNR	Biological Nutrient Removal
BAF	Biological aerated filter
CAS	Conventional Activated Sludge
C/N	Carbon/Nitrogen
COD	Chemical Oxygen Demand
C/P	Carbon/Phosphorous
CPC	Compound Parabolic Collectors
CC	Correlation coefficient
CSTR	Continuous Stirred Tank Reactor
DO	Dissolved Oxygen
DOE	Design Of Experiments
DOC	Dissolved Organic Carbon
EPS	Extracellular Polymeric Substance
EGSB	Expended Granular Sludge Bed
EBPR	Enhanced Biological Phosphorus Removal

FBBR	Fluidized Bed Bioreactor
FTIR	Fourier transform infrared spectroscopy
GAC	Granular Activated Carbon
GA-ANFIS	Genetic Algorithm Adaptive Neuro-Fuzzy Inference System
HBR	Hybrid Biological Reactor
HRT	Hydraulic Retention Time
HASBR	Hybrid Activated Sludge Baffled Reactor
MBBR	Moving Bed Biofilm Reactor
MBR	Membrane Bioreactor
MPUF	Modified Polyurethane Foam
MPN	Most Probable Number
MUCT	Modified University of Cape Town
MF	Membrane Filtration
MLSS	Mixed Liquor Suspended Solid
MLVSS	Mixed Liquor Volatile Suspended Solid
MSBR	Multi-Stage Biological Reactors
NSE	Nash-Sutcliffe coefficient
OH*	Hydroxyl radical
OLR	Organic Loading Rate
PAC	Powdered Activated Carbon
PAO	Phosphorus Accumulating Organisms
POSBR	Pure Oxygen Sequencing Batch reactor
PU	Poly Urethane
PCL	Polycaprolactones
PHA	Polyhydroxyalkanoate
PSO-ANFIS	Particle Swarm optimization Adaptive Neuro-Fuzzy Inference System
RPM	Rotations per Minute
RBC	Rotating Biological Contactors

RMSE	Root Mean Squared Errors
RBNN	Radial Basis Neural Network
SBR	Sequencing Batch reactor
SC	Soft Computing
SS	Suspended Solids
SRT	Sludge Retention Time
SAC	Synthetic Activated Ceramic
SVI	Sludge Volume Index
SEM	Scanning Electron Microscopy
S/N	Signal/Noise
SND	Simultaneous nitrification and denitrification
SMBR	Submerged Membrane Bioreactor
TN	Total Nitrogen
TP	Total Phosphorous
TSS	Total Suspended Solids
TFB	Two-stage Fluidized-Bed
TOC	Total Organic Carbon
TKN	Total Kjeldahl Nitrogen
UV	Ultraviolet
UCT	University of Cape Town
UASB	Upflow Anaerobic Sludge Blanket
VFCW	Vertical Flow Constructed Wetlands
VSS	Volatile Suspended Solid
WWTP	Waste Water Treatment Plant
WWQI	Waste Water Quality Indices

CHAPTER 1

INTRODUCTION

1.1 General

Till the recent past, when the cities were small and sparsely populated, people used to dispose of the wastewater generated directly into natural water bodies wherein it was treated naturally either by dilution or bacterial breakdown (Helness et al., 2001). But due to the rapid increase in population, urbanization and industries it resulted in the generation of huge quantities of wastewater with a wide range of characteristics which when directly disposed into the natural water systems would directly or indirectly affect the surrounding environment, aquatic lives and human health thus demanding a reliable treatment technology which is sustainable and techno-economically viable.

Various wastewater treatment methods till date to remove organics & nutrients include physical, chemical and biological processes (Yuan et al., 2010). Among them, biological treatment systems are the most feasible option in handling wastewater. General classification of the biological treatment processes based on the condition of biomass is shown in Figure 1.1. For the past few decades, biological treatment by conventional activated sludge (CAS) process and attached growth systems are the most convenient and extensively used treatment techniques for domestic wastewater. The efficiency of the conventional activated sludge process is severely affected due to general operating conditions i.e. hydraulic and organic shock loadings and sludge bulking thus needing more attention and frequent monitoring.

Further, treating nutrients such as nitrogen and phosphorus by CAS proved to be much inferior, since the removal of these nutrients requires more hydraulic retention time compared to that of other organic matter thus increasing the land requirements. On the other hand though attached growth systems where in a layer of biofilm grows on inert media thus enhancing the microbial population in the system are slightly better than CAS, they still lack the popularity due to their inability to effectively transfer oxygen into thick biofilm.

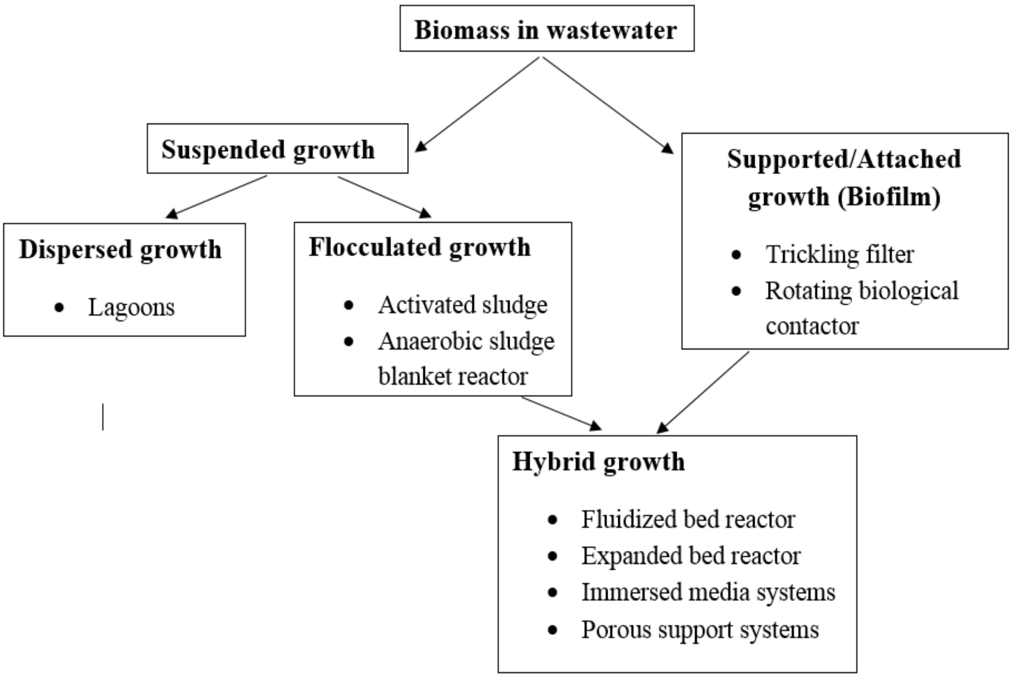

Figure 1.1 Classification of biological treatment processes (Jianlong et al., 2000)

However, due to the most stringent policies and regulations by government bodies across the world, there is a need to explore and develop new treatment techniques and process modifications to the existing conventional systems to meet the treatment & reuse requirements of these regulatory bodies. Since then the activated sludge process & attached growth systems have been modified and improved numerous times in order to produce better quality effluent. One such modification is membrane bioreactor (MBR) process that proved to provide high effluent water quality and high productivity (Maiti et al., 2013). However, the main drawbacks of MBR application include the membrane lifespan, sludge production, membrane fouling and chemical waste from the membrane cleaning (Duan et al.,2013; Meng et al.,2007). Researchers are experimenting to combine the two different processes viz. suspended and attached growths to enhance their performances and this combined system is commonly named as hybrid/aerobic hybrid system.

In early 1990's the first moving bed biofilm reactor (MBBR) which is a type of aerobic hybrid system wherein biomass carriers are introduced into the reactor to increase active biomass concentration for effective treatment without increasing the reactor size (Hauduc et al., 2013; Xu et al., 2013) became operational in Norway and later in other parts of Europe and the United State of America. The MBBR technology has been emerging as one of the standard methods in the field of wastewater treatment in the recent past because of its advantages such as high efficiency, high capacity, relatively small footprint in the treatment plant when compared with the conventional treatment technologies (Di Trapani., 2010). It also possesses the potential to withstand the challenges of wastewater treatment such as producing less sludge as a result of high biomass retention time, retrofitting the old treatment plants, eliminating the need of backwashing, minimizing process complexities and operators and so on.

Due to the ever increasing gap between water supply and demand, human society is forced to look at options to reduce this gap. One of the feasible option is recycling and reusing treated wastewater. But most often, wastewater effluent quality from secondary treatment units does not meet the requirements or regulations prescribed by government bodies for reusing the wastewater and so it needs further treatment known as tertiary or post-treatment. However, the degree of tertiary or post-treatment depends upon the intended use. Advanced oxidation processes (AOP's) incorporating a set of chemical treatment technologies are nowadays employed for the post-treatment of various wastewaters (Bandala et al., 2008). Photocatalysis is one such AOP's that has been extensively studied since last decade, to treat the effluent, especially in large-scale treatments (Hashimoto et al., 2005). Titanium dioxide is the most commonly used photocatalyst because of its efficient photocatalytic activity, higher stability and low cost (Hashimoto et al., 2005; Di Paola et al., 2012). TiO_2 Photocatalysis for remediation of wastewater has been widely studied on textile and pesticides industry wastewater (Neppolian et al., 2002; Al-Momani et al., 2002). Also, the use of solar radiation as a source of light for photocatalysis has given good results for the removal of pollutant load and disinfection (Spasiano et al., 2015). It is also effective in the removal of chemical compounds and pathogens from industrial wastewater.

Models are necessary for the reason that, the effects of tuning the operating variables can be studied more transiently on a computer than by doing experiments. Hence, many alternative schemes and operational strategies can be evaluated without the need for physical trials of each scenario. By simulating the performance assessment models using suitable influential variables, one can rapidly respond to any changes in the processes and devise operational strategies to shift the plant to new operating conditions which improve its stability, quality of the effluent and at the same time achieve a reduction in the running costs.

With this as background, a thorough literature survey was done in line with the above discussion, objectives were derived from the gaps identified in the area and addressed appropriately.

1.2 Thesis Outline

The thesis has been organized into six chapters as under

Chapter 1 is a general introduction and discusses the objectives and outline of the thesis.

Chapter 2 literature reviews on the aerobic hybrid system, an advanced oxidation process and data modelling.

Chapter 3 presents the materials and methods used for the experimental studies on laboratory scale aerobic hybrid system and describes the experiments.

Chapter 4 presents results and discussion for (a) performance evaluation of AS-biofilm reactor by varying operating condition such as variable Carbon to Nitrogen ratio, suspended biomass concentration, DO and HRT, followed by post-treatment using TiO_2 based UV and solar photocatalysis. (b) Performance evaluation of anaerobic-anoxic-oxic AS-biofilm reactor in series.

Chapter 5 presents data statistics and model evaluation to predict the performance of 43.5 MLD sewage treatment plant and lab scale hybrid AS-biofilm reactor.

Chapter 6 outlines conclusions and future scope of work.

1.3 Contribution of Thesis

Important contribution made from this study are:

Laboratory experiments were conducted on the activated sludge biofilm (AS-biofilm) reactor to study its performance under varying C/N ratio, biomass concentration, HRT and DO, followed by post-treatment using UV and solar photocatalysis using TiO_2 as a catalyst to check if the water meets the quality criteria specified by regulatory bodies for reuse of treated wastewater. Similarly anaerobic-anoxic-oxic (A^2O)-AS-biofilm reactor in series were studied for the performance of reactor in terms of nutrient removal using molasses based synthetic wastewater.

Data model or soft computing models are used to predict the performance of 43.5 MLD real time sewage treatment plant using ANFIS and lab scale hybrid AS-biofilm reactor for removal of nutrients using genetic algorithm optimized adaptive neuro-fuzzy inference system (GA-ANFIS) and particle swarm optimization optimized adaptive neuro-fuzzy inference system (PSO-ANFIS). The model predictions have been compared with experimental results.

CHAPTER 2

LITERATURE REVIEW

2.1 General

Due to the ever increasing stringent regulation across the world on wastewater management, there is always a need for the researchers to work on different technologies and their up gradations to meet the requirements. Among the various treatments available to handle wastewater, biological treatment is most reliable and cost effective alternative. Hybrid biological systems are the systems wherein two or more technical alterations are made to make the system more resilient and economical. In the present chapter efforts were made to present a summary of work being done in the current area of research to snap out the drawbacks or gaps.

In this chapter a short historical perspective on the development of the process and a review of the large number of existing process variants is given. A brief literature review of various modelling approaches for the activated sludge and biofilm process evolved during the last thirty years is presented. Special emphasis is placed on the description, design and modelling of the AS-Biofilm reactors.

2.2 Wastewater treatment technologies

The gap between supply and the water demand has grown and as a result there is a marginal growth in the cost of additionally supplied water (Mekala et al., 2008). The development of new treatment technologies has paved way for the economical fulfilment of the gap between demand and supply. However, the type and treatment scheme or technology to be chosen largely depends on the wastewater characteristics.

Any wastewater treatment scheme, until recent years, encompasses the conventional physical (screening, mixing, sedimentation, floatation, filtration etc.), chemical (precipitation, adsorption, oxidation using chemicals etc.) (Pradhan & Gogate, 2010) and biological methods. Some of the advantages of physical/chemical treatment in comparison to biological are easy control, adaptation with a wide range of flows, loading rates and variety of application usages. The above said, conventional approaches are either slow or ineffective for some or more persistent chemical and organic contaminants. While physical treatment often results in the partial reclamation of the effluent, chemical treatment in spite of its effectiveness, often increases the chemical load (solid waste) of the treated effluent. Biological treatment of effluent frequently meets the ultimate objectives of wastewater treatment with respect to the changes in physico-chemical properties and provides an opportunity for safe disposal of the effluent. Hence, biological treatment is the most widely accepted method all over the world for treating wastewater.

A brief description of merits and demerits of the treatment technologies used in wastewater treatment is given in Table 2.1. The construction and operation of some of these technologies are comparatively easy, dependable and economical. However, some of these elementary and inexpensive treatment methods may be uncertain for the systems that require constant maintenance and regular inspections for their smooth operations.

2.3 Biological treatment processes

The main objective of biological treatment is to transform or oxidize dissolved and particulate biodegradable constituents, suspended and non-settleable colloidal solids, nutrients such as nitrogen, phosphorous and specific trace organic constituents into simple products. Biological treatment refers to the stabilization of wastewater solids by decomposing them into harmless inorganic compounds either by aerobic or anaerobic processes with the help of microorganisms. Under aerobic conditions, the end products of decomposition are carbon dioxide and biomass, while during anaerobic conditions carbon dioxide and methane are produced (Metcalf & Eddy 2004).

Nitrogen is removed through continuous interconversion between various nitrogen species and the principal reactions involved are nitrogen fixation, ammonification by heterotrophic bacteria, biological oxidation of ammonia to nitrate (nitrification), subsequent reduction of nitrate to nitrogen gas (denitrification) and

Table 2.1 Different types of treatment processes used in wastewater treatment

Treatment methods	Brief description	Merits	Demerits	References
		Physicochemical treatment		
Adsorption	Adsorption process can be used for the removal of toxic, persistent organic and inorganic contaminates from wastewater. Adsorption is the process of adhering of atoms and ions from a gas, liquid or dissolved solid on to a surface of adsorbent. Most commonly used adsorbents are powder activated carbon and Granular activated carbon. Some of the waste materials can be used to for preparation of Adsorbents such as, sawdust, sugarcane bagasse, rice husk, coconut shell and many more.	Flexible and simple to design ,Low initial cost, easy to operate, insensitive to toxic pollutants	High maintenance cost, this process just transfers pollutants from one phase to another instead of eliminating from the environment.	(Rangabhashiyam et al., 2013; Yousif et al., 2012)
Chemical oxidation	Oxidation technologies which can be used for destruction of organic pollutants. Chemical oxidation brakes down the structures of the pollutants in wastewater into less harmful substances such as CO_2 and H_2O.	High oxidizing power. Increased biodegradability Decreased toxicity Not selective oxidant No residues (except homogeneous Fenton and Ozone catalytic)	Cost of UV Visible radiation and chemicals. Mass transfer control for ozone. UV light transmission to water rate decreases due to OH^* scavenging by the sensitizer.	(Gogate et al., 2004; Tony et al., 2012)
Biological treatment				
Aerobic treatment	The process of reduction of organic matter present in wastewater with the help of microorganisms such as bacteria and protozoa in the presence of oxygen is known as aerobic treatment. The reduction of organics is effectively achieved by a series of metabolic reactions carried out by the microbes.	Aerobic treatment can efficiently reduce BOD resulting in better quality effluent. It requires less space when compared to anaerobic	Requires skilled labor and continuous monitoring for maintaining the plant. Need of air blowers to provide aeration , which	(Yadav et al., 2016)

	Aerobic treatment processes based on suspended-growth biomass, such as sequential batch reactor, aerated lagoons, activated sludge process. Examples for attached growth bioreactor are rotating biological contactor, trickling filter, that are the mostly used aerobic treatment processes.	system. The treated effluents may contain dissolved oxygen which reduces the immediate oxygen demand on receiving water. Minimal odor is generated	results in high energy consumption Huge quantity of sludge is produced from the aeration tank which will increase the maintenance cost	
Anaerobic treatment	The process of biological breakdown of nutrients present in the wastewater by the help of bacteria in the absence of oxygen is known as anaerobic treatment. The microbial action in the absence of oxygen results in anaerobic conversions of organic pollutants into methane and carbon dioxide the latter of which can be collected and used as an energy source. Upflow anaerobic sludge blanket (UASB), septic tank, anaerobic lagoon, anaerobic fluidized bed reactor are the common anaerobic treatment methods.Anaerobic treatment is widely used to treat wastewater with high organic load.	Lower operating cost, Odour /flies typically removed from system, sludge occupies less volume, easier to dry. Methane recovery by most of the microbial biomass can be used as alternate fuel source.	Requires long startup, alkalinity should be sufficient, under mesophilic conditions optimum temperature is 35^0C. Nitrification not possible, low kinetic rates at low temperature. If COD <1000mg/L anaerobic treatment is not practically economical. Effluent from anaerobic treatment is generally not acceptable for direct discharge and polishing treatment is needed.	(Ganesh et al., 2010; Akil & Jayanthi, 2012; Murugesan et al., 2016; Yue et al., 2013)
Advanced treatment processes				
Membrane bioreactor (MBR)	MBR process is the process of combining both conventional aeration processes along with the membrane system. In this system membrane will be directly introduced into aeration tank to and the	Better quality of effluent can be achived compared to conventional treatment process.	High initial and maintenance cost, Skilled labor is needed to carefully monitor the	(Fenu et al., 2010)

	treated water will be taken out through the membrane, thus reducing the need for filters. The MBR system can be designed to treat wide range of wastewater from both domestic and industrial sources. The treated water produced from this system will have very low foot print, and can be reused. This system can also be used for the recovery of useful minerals present in the industrial wastewaters.	Less space is required and sludge production is less when compared to traditional treatment methods. Can be completely automated for easy operation and modulation.	process as the membranes can get damaged due to the presence of large solids and harmful chemicals, and Wastewater from chemical cleaning of membranes should be handled separately.	
Moving bed Biofilm reactor (MBBR)	Moving bed biofilm reactor is a type of aerobic hybrid systems, were suspended attached biomass is responsible for removal of nutrients such as nitrogen and phosphorous. Small cylindrical or circular polyethylene carrier media is incorporated into the reactor to support biofilm. Fine bubble diffusers are used to circulate Biofilm carriers inside the reactor. This treatment process is effective in removal of COD, BOD and nutrients such as nitrogen and phosphorous.	Reliability easy operation, Easy to retrofit for existing treatment plant, No return activated sludge, Utilize whole tank volume for biomass, No media clogging, Less sludge production and better settling, Smaller foot print.	Disadvantages would include that it is a biological process, which means that it would require a staff with higher qualifications & bacterial activity have to be monitored periodically. Some complain that the fixed film media tends to wash out of the systems over time, even after installing the various strainer systems	(Barwal et al.,2014)

and finally the assimilative nitrogen uptake. Biological phosphorus removal system is reliant on improving the ability of microorganisms to uptake more phosphorus into their cell. By subjecting the activated sludge to a cycle of anaerobic and aerobic conditions, the growth of phosphorus-accumulating organisms (PAOs) is favoured which accumulate the influent phosphorus intracellular as polyphosphate that is later removed from the system by wasting of excess sludge. During the N and P removal processes, the influent wastewater must contain sufficient biodegradable carbon source to satisfy the combined demand of heterotrophic denitrification and bacterial metabolism for phosphorus removal, a key factor affecting the treatment efficiency (Semerci, &Hasılcı, 2016; Yadav et al., 2017). The municipal sewage sometimes will suffer from low carbon to nitrogen (C/N) ratios, indicating inadequate carbon sources for efficient biological removal of nitrogen and phosphorus. In the successive sections, different types of basic WWT systems are discussed in brief.

2.3.1 Suspended growth Process

In most aspects, suspended growth process is operated under aerobic conditions while in the cases of high organic concentration industrial and organic sludge, treatment processes may operate under anaerobic conditions (Nemerow, 2007). In aerobic suspended growth process, microorganisms in presence of air or oxygen consume the dissolved and colloidal organic matter present in the wastewater and give up CO_2, new cells and energy. Some of the famous suspended growth processes are presented in brief below.

2.3.1.1 Activated sludge process

The activated sludge process was developed by Clark and Gage at the Lawrence experiment station in Massachusetts (Metcalf and Eddy, 2004) but the conception of activated sludge process was discovered by Ardern and Lockett (1914). In this process, organic matter removal is achieved by microorganisms in the presence of oxygen and in this process huge quantity of biomass or sludge is formed which is removed in secondary sedimentation tank (Haandel and Lubbe, 2012). However, to maintain a minimum Food to Microorganism ratio, appropriate amounts of biomass is recycled into the aeration tank as this is very important in maintaining the efficiency and stability of the system. This process has been applied in field to treatment wide range of wastewater effluents viz. domestic sewage, industrial effluents, leachates etc. (Renou et al., 2008). Although variations of this process can achieve high removal efficiencies in case of organic carbon, nutrients and ammonia content, disadvantages of activated sludge process led to the usage of other technologies (Renou et al., 2008). The first drawback of activated sludge is the dependence of this process on gravity settling technology which controls the activated sludge system design. Some other disadvantages of activated sludge processes being high operation and maintenance, problems in sludge settleability, need for longer retention times, sensitivity towards toxicants, organic and volumetric loads (Yeon et al., 2011).

Though the practical applications of ASP started much earlier i.e. 1900s, yet it took a long time to formulate the models for its design and operation. In fact the first efforts to formulate the mathematical form of the process started in 1960s and were made by Eckenfelder, (1989), McKinney and Ooten, (1969). Now with the most advanced computational facilities available, there are more advanced versions of these models available and some of the famous ones being ASM1, ASM2, ASM2d and ASM3 (Henze et al., 2000). But the practical application or usability of these models depends on the availability of large number of kinetic parameters, their accuracy which needs huge experimental practices and depends on accuracy at which these parameters are analysed.

2.3.2 Attached growth process

In attached growth process the microorganisms which convert organic materials and nutrients to gases and cell tissue are attached to an inert packing material. Packing materials or the media ranges from natural materials such as rock, gravel, slag, sand, redwood to synthetic media made out of high density polyethylene. In this process, biofilm consisting of a group of microorganisms are responsible for biological reactions which lead to removal of organic matter and nutrients from wastewater (Metcalf and Eddy, 2014). Evolution of biofilm is a four-stage process viz. the attachment of cells to the surface; cell growth and division, together with production of extracellular polymeric substances (EPS); a plateau phase in which biofilm’s physical properties approach a "steady-state"; and finally detachment ("sloughing off") of biofilm fragments ("erosion"). Biofilm accumulation is the net result of a number of physical, chemical and biological processes, each leading to either gain, or loss of biomass. Figure 2.1 shows various processes involved in the formation of biofilm and Figure 2.2, the various metabolic reactions that occur in it.

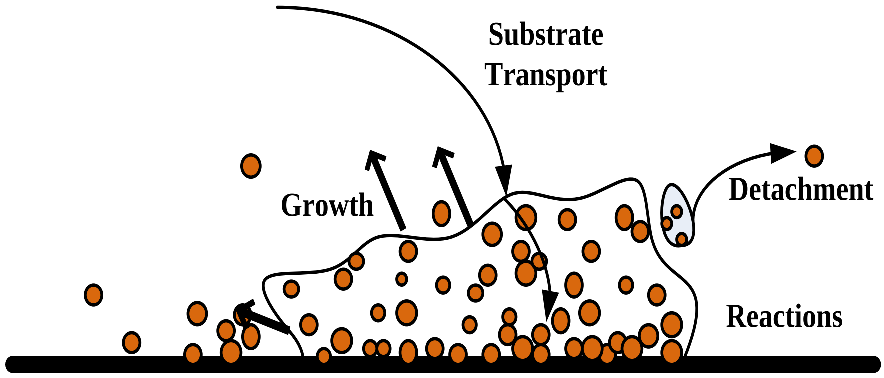

Figure 2.1 Processes occurring in a biofilm structure (Thalla, 2009)

As these systems have the ability to keep active biomass and have less of an effect on nitrification in low temperature, they were counted as high efficiency systems and high demanded ones for many years (Metcalf and Eddy, 2004). Although the most significant feature of the attached growth process is the performance of biofilm, they could mostly be diffusion limited. Therefore, overall removal rates can be affected by diffusion rates as well as the electron donor and electron acceptor concentrations at different locations in the biofilm (Tchobanoglous et al., 1998). Diffusion limitation should be considered more whilst measuring bulk liquid dissolved oxygen (DO) concentration in the attached growth process due to its effects on the biological reaction rate. In this context, while the suspended growth aerobic process requires DO concentration of 2-3 mg/L, this low DO concentration in the case of attached growth process could be a limitation. Thus, in order to achieve a high level of nitrification in attached growth bioreactors, higher DO concentrations are required when considering the ammonia removal (Odegaard, 2006). Some of the popular variants of attached growth process are trickling filters, rotating biological contactors, biofilters etc. and a detailed explanation on these processes can be found in research literature (Daigger & Boltz 2011; Loukidou & Zouboulis 2001).

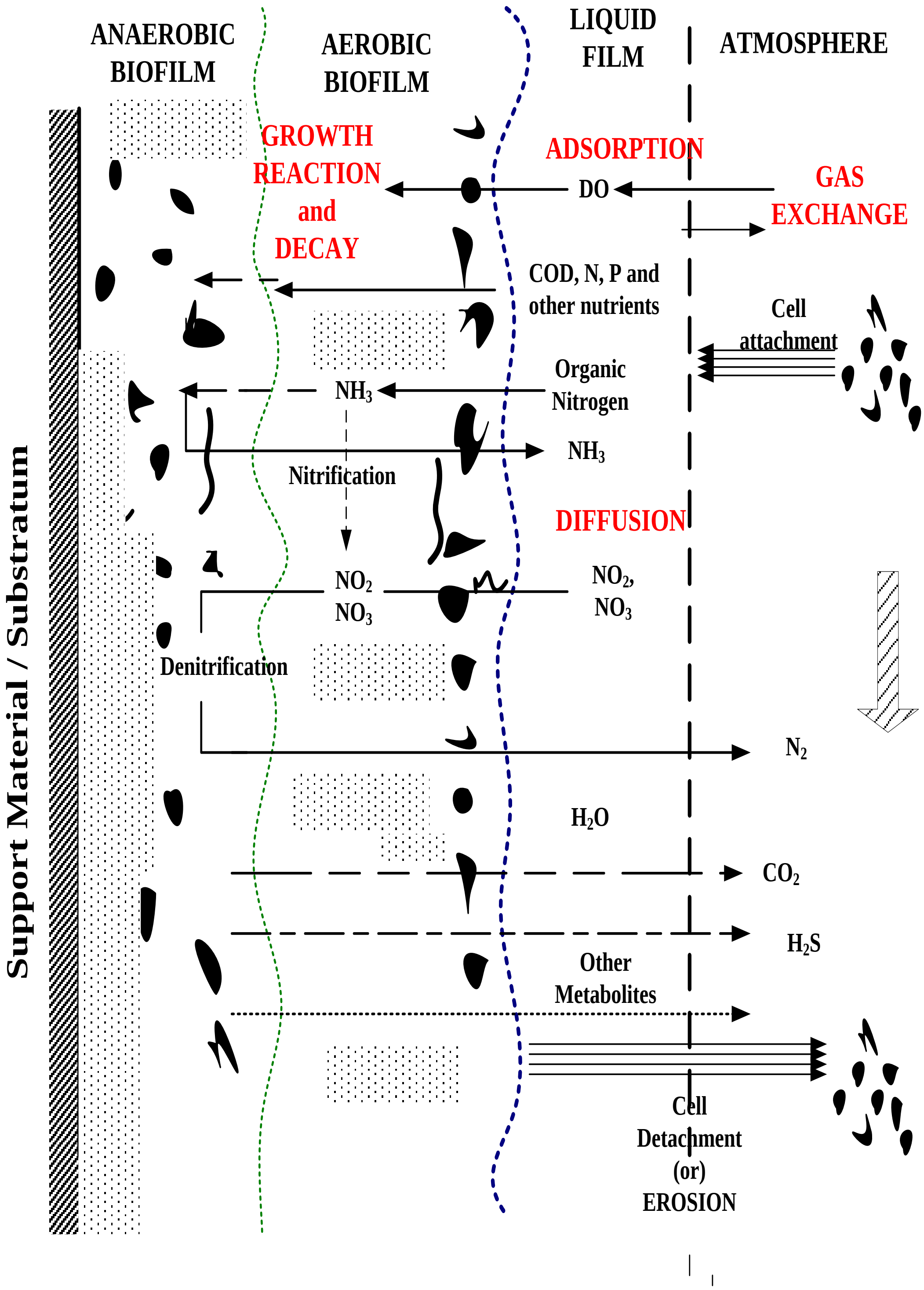

Figure 2.2 Schematic representation of metabolism in biofilm (Thalla, 2009)

2.3.2.1 Trickling filters

In trickling filters, wastewater is trickled over the surface of the filter from a rotating arm (Figure 2.3). Organisms that are in biofilm that is present on the surface of the media oxidize the organic matter in the wastewater. Some of the advantages of this process in comparison with ASP is that it is good at handling hydraulic and organic shock loads, no external air supply, simpler operation with no problems in mixed liquor inventory control and sludge wasting; No issues of sludge bulking in secondary clarifiers and low operation and maintenance costs (Lekang et al., 2000; Metcalf and Eddy, 2004). However, some of the disadvantages of this system are due to its greater sensitivity to lower temperature, poorer effluent quality in terms of BOD and TSS concentrations and production of odors.

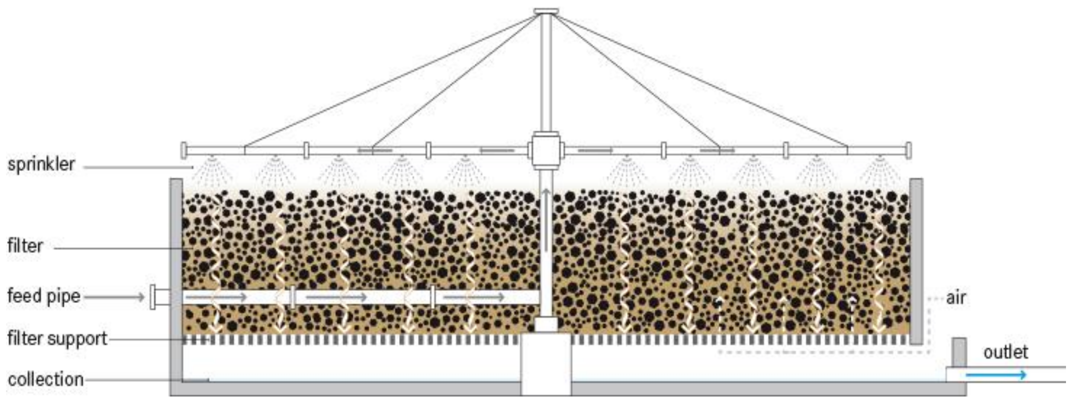

Figure 2.3 Trickling filter (Metcalf and Eddy, 2004)

Another popular version of attached growth process is Rotating Biological Contactors, where an assembly of closely packed circular disks are used as media for the growth of biofilm and these disks keep rotating at a very low RPM on the surface of the wastewater to be treated (Metcalf and Eddy, 2004). RBCs have many advantages like trickling filters i.e short HRT, high biomass concentration, high specific surface area, low operation and maintenance (Yamaguchi et al., 1999). RBCs have been successfully implemented for treatment of municipal and industrial wastewaters under medium and high organic loading rates and no limitation in the reactor was reported even at high OLR values. In addition, high COD removal efficiency of 88% from industrial wastewater with a COD surface loading rate of 38 g COD/m^2d via RBC was reported (Najafpour et al., 2005). Few disadvantage of RBCs are low operational flexibility, a covering requirement in cold climates, a primary sedimentation requirement and the dryness possibility of un-submerged biofilm portion in warm climates.

2.4 Aerobic hybrid system

Conventional activated sludge units are incorporated with biofilm carriers, thus containing both suspended and attached growth biomass in the same unit. The biofilm media can be rigidly arranged or kept floating in the reactor and based on this arrangement, the reactor can be classified as fixed media or movable media type. Biomass carriers provide significant surface area for the formation of biofilm and thus aid in retaining an increased concentration of biomass within activated sludge unit. Major advantages of these systems are (i) high biomass densities with shorter hydraulic retention times which enhance the techno-economic efficacy of the system with better controllability of system by altering surface area and amount of supporting media (Tsuno et al., 1992; Hamoda and AL-Sharekh 2000; Jianlong et al., 2000). (ii) enhanced nitrification as slow growing nitrifiers in sufficient amount exist throughout the depth of the biofilm (Wartchow, 1990; Lessel, 1994; Sen et al., 1994; Watanabe et al., 1994; Randall and Sen, 1996; Jones et al., 1998; Andreottola et al., 2000; Jianlong et al., 2000; Rodgers and Burke , 2003; Kumar and Chaudhari, 2003; Rodgers et al., 2006); (iii) Minimum to no operating problems such as bed clogging, high pressure drop, poor mixing, poor sludge settelability & wasting and oxygen transfer (Kargi and Karapinar, 1997; Rusten et al., 1997; Fouad and Bhargava, 2005c); (iv) good resistance against toxicity and shock loading (Hamoda and AL-Sharekh 2000). (v) Ease to upgrade the existing systems to takeup extra loads (Hegemann W, 1984; Lessel, 1994; Sagberg et al., 1992; Su and Ouyang, 1996).

Some of the popular names by which these systems are known are Powder Activated Carbon-ASP (PAC-AS) (Aktas and Cecen, 2001); Kaldnes Suspended Carrier Process (Dalentoft and Thulin, 1997 ; Ødegaard et al., 2000) ; Aerated Submerged Fixed Film (ASFF) (Hamoda and AL-Sharekh 2000; Belgiorno et al., 2003); Floating Biological Bed, Floobed®, (Hansen et al., 1999); Bio-2-Sludge Process, (Muller 1998); Hybrid Biological Reactor (HBR) (Jianlong et al., 2000); ASP-Biofilm or AS-Biofilm Process (Chuang et al., 1997 ;Gebara 1999); Suspended-Carrier Biofilm (Welander et al., 1997); Fluidized Bed Bioreactor (FBBR) (Kargi and Karapinar, 1997); Captor ®Process (Golla et al., 1994); Linpor® Process (Reimann 1990; Morper and Wildmoser, 1990; Morper 1994); moving bed biofilm reactor (Odegaard et al., 2000).

2.4.1 Factors affecting the performance of AS- biofilm system

The factors affecting the performance of AS-Biofilm are the type of media, specific surface area of media, media filling ratio, operating conditions such as DO, OLR and HRT.

2.4.1.1 Type of media

The media used in the process may either be fixed or floating in the reactor. The most commonly used fixed media for attached growth processes are stones, clinker, sand, activated charcoal, metals, plastic sheets, and foams. Media used in movable media hybrid system should have density less than water. The physical appearance and characteristics of these media are shown in Table 2.2. Polyurethane (PU) carriers have become well-demanded materials in the case of carbon and ammonium removal (Chu et al., 2014). This is due to the entrapment of microorganisms in the polyurethane pores which results in augmentation of the number of nitrifiers.

Table 2.2 Characteristics of media used in the attached growth processes (Asmita 2013)

Media	Shape	Size	Specific Surface area (m2/g)	Figure
KaldnesTM K1	Cylindrical	Dia-10 mm Length- 7mm	5.0×10^{-3}	
KaldnesTM K3	Cylindrical	Dia-10 mm Length- 7mm	0.5×10^{-3}	

Sponges	Cubic	15×15×15 mm	0.91	
Polyethylene Ball	Circular	Dia-10 mm Length 7 mm	0.32×10^{-3}	
Polypropylene grains	Granules	3.5 mm	1.001×10^{-3}	
Sand	Circular	Dia-3.0 mm	820 m^{-1}	
BioPortzTM	Cylindrical	Dia- 20 mm Length 20 mm	0.58×10^{-3}	
WD-F10-4bioMTM	Cylindrical	Dia- 25 mm	0.9×10^{-3}	
Ceramic spheres	Spherical	Outer Dia 20 cm inner Dia 18 cm Length 18 cm	1.032×10^{-3}	
Flocor RMP-HSP®	Cylindrical	Dia-10 mm Length 10 mm	2.77×10^{-3}	

Feng et al., (2008) worked with different structured porous carriers in a two-stage bioreactor for the removal of organic carbon along with the nitrogen and an average COD removal of about 95% was achieved. Similarly, high removal efficiencies of 93%, 89% and 81% were reported for NH_4^+-N, phenol and COD respectively using aerobic AS-biofilm with polyethylene as carrier material (Li et al., 2011).

Chu et al., (2014) investigated modified polyurethane foam carrier (MPUF) and polyurethane foam carrier (PUF) for biofilm attachment in AS-biofilm. It was noticed that at steady state the biofilm growth ratio on MPUF carriers to PUF carriers was 1.3µm. Zhuang et al., (2014) investigated advanced biological treatment of wastewater using suspended polyethylene circular carrier in a novel anoxic AS-biofilm, the maximum reduction of total organic carbon, COD, NH_4^+-N, total nitrogen (TN) and total phenols were 70.0%, 74.6%, 85.0%, 72.3% and 92.7%, respectively was observed.

2.4.1.2 Surface area of media

An increase in the effective area of the AS-biofilm carrier medium by 70% of the total surface area is reported to reduce the attachment of biofilm on the exterior portion of the media. In addition, surface shape as well as the size of the media is proven to be effective in the system's removal efficiency. This results in changes in carrier biofilm thickness inside and outside of the carrier. Ngo et al., (2007) demonstrated that polyurethane sponge (70–90 cells/in^2) with a designated slope of sponge tray at 10° led to a good performance in terms of both organic and nutrient removal efficiency. In addition, in terms of biomass growth and pollutant removal, medium sponge size of 2×2×2 cm was reported to show the best performance among all cube sizes (Nguyen et al., 2011). Similarly, Mostafa et al., (2008) examined hybrid activated sludge baffled reactor (HASBR), which had both suspended and attached-growth biomass. In the HASBR, results indicated that maximum reduction of total COD (98±2%) and ammonia (98±2%) from the influent could be achieved, when the influent wastewater had COD 593±11 mg /L and nitrogen of about 43±5 mg/L, at an HRT of 10 hours. Guo et al., (2010) investigated the effect of packing material's thickness on the removal efficiency of nutrient and organics. It was reported that as the thickness of packing material (i,e sponge) increased, the efficiency in terms of removal of organic matter and nutrients reduced. Sponge of size 1 cm exhibited the best T-P and T-N removals.

2.4.1.3 Media filling ratio

In addition to physical properties of carriers, it is reported that the ratio of packing media also affects AS-biofilm performance. This is due to the linear relationship between the packing media ratio in the reactor and the total biomass attachment on carrier's surface. It was proven that packing ratio significantly affected the nitrogen removal efficiency, while organic removal was not considerably affected. Gu et al., (2014) investigated the effect of carrier filling ratio ranging from 20% to 60% on the proficiency of AS-biofilm in the removal of COD, phenol, thiocyanate and ammonia from cooking wastewater at 20 h HRT. Removal efficiencies of 89%, 99% and 99% respectively for COD, phenol and thiocyanate, were achieved at 50% carrier filling ratio. Lopez et al., (2012) examined different media filling ratio 20%, 35% and 50% of Anox Kaldnes in AS-biofilm for removal of organic matter, 35% filling ratio the maximum removal of COD -75.3% occured at HRT- 7h. Thalla et al., (2012) investigated the performance of activated sludge biofilm reactor at various filling ratio. At 30% Bioflow 9 filling, an increase in the influent COD concentration reduced the efficiency of COD removal, and at 60% filling [Biofilm 9 (30%), Flocor (15%), and BioBall (15%)] it was observed that an increase in the feed composition while maintaining the same HRT reduced the percentage removal of COD and NH^{+}_{4} -N. As media packing ratio increased from 20% to 30% and then to 60%, organic removal increased from 96.6% to 97.3% and 97.8% and nitrogen removal increased from 9.6% to 26.0% and then to 41.3%, respectively.

Ngo et al., (2007) investigated an attached growth bioreactor with the usage of the sponge as a media. In this experiment, the high removal efficiency of 90% for ammonia and COD removal of 20-100% were achieved, which were related to the media material and properties. Consequently, it could be concluded that the types of packing material and its packing ratio played undoubtedly a major role in AS- biofilm efficiency and performance

2.4.1.4 Operating conditions

A moving bed biofilm reactor (AS-biofilm) facilitates aerobic and anoxic or anaerobic processes in which the performance of the system could be affected by various conditions like C/N ratio, organic loading rate (OLR), hydraulic retention time (HRT) and DO. Sheli et al., (2007) reported that as OLR increases, attached biomass in AS-biofilm was augmented as well. The rate of denitrification in an AS-biofilm is influenced by the biofilm area, type of external carbon source, bulk liquid carbon to nitrogen ratio (C: N), wastewater temperature, bulk liquid dissolved oxygen concentration, and bulk liquid macronutrient concentrations (McQuarrie et al., 2011). The C/N ratios of 5 and 6 were found to be suitable for denitrification, and any change if the composition of acidogenic liquid had no negative effect on denitrification (Zhang et al., 2016). In the denitrification process, denitrifying organisms such as Pseudomonas, Achromobacter, Acinetobacter, Agrobacterium, Alcaligenes, Arthrobacter and Bacillus are responsible for the conversion of oxidized nitrogen compounds such as nitrite or nitrate to nitrogen gas (Wang et al., 2006).

Yang et al., (2014) investigated the modified sequencing batch biofilm reactor (SBBR) which was bio-augmented with a consortium of 5 strains of indigenous bacteria (genus Pseudomonas and Bacillus). The upper part of the reactor consisted of fibrous filler and the lower part was filled by ceramsite filter media. An HRT of 10h was essential to attain good quality effluent when operated at low C/N ratio. During the N and P removal processes, the influent wastewater must contain sufficient biodegradable carbon source to satisfy the combined demand of heterotrophic denitrification and bacterial metabolism for phosphorus removal, a key factor affecting the treatment efficiency (Semerci, and Hasılcı, 2016). Similarly Mohan, et al., (2016) have shown that the influent C/N and C/P ratios were the key factors that affected the performance of biological nutrient removal systems.

Zou et al., (2014) investigated total nitrogen removal at low temperature (10^0 C) by excellent coupling of enriched autotrophic nitrifying and heterotrophic denitrifying consortiums at the sole aerobic condition. The aerobic denitrifying consortium could achieve a specific denitrifying rate of 32.93 mg N g SS under dissolved oxygen of 1.0–1.5 mg/L at 10^0 C. Shore et al., (2012) investigated AS-biofilm for tertiary ammonia treatment at a high temperature (35–45 ^{0}C) by successfully removing more than 90% of the influent ammonia (up to 19 mgL^{-1} NH_3–N) in both the industrial and synthetic wastewaters.

In addition, uniform movement of carriers inside the reactor enhances system performance by controlling the flow velocity (Odegaard, 2006). The water circulation pattern is controlled by flow control valves, which uniformly distributes plastic biofilm carriers (McQuarrier et al., 2011). Coarse bubble diffusers have the inherent benefits of being more resistant to scaling and fouling and less likely subjected to maintenance than fine bubble diffusers. This process causes the augmentation of the gas, liquid interfacial area and favourable oxygen transfer (Jing et al., 2009). The aerobic AS-biofilm is mostly involved in the removal of ammonium as the biofilm grown in the AS- biofilm were reported to mainly contain the autotrophic bacteria (AOB) and Anammox (>97% at an HRT of more than 1.25 d) (Cortez et al., 2011).

In addition, the aerobic AS-biofilm achieved more than 85% COD removal efficiency at an optimum carrier filling rate, HRT, OLR and dissolved oxygen (Chen et al., 2007). Javid et al., (2013) examined AS-biofilm at different HRTs and the Organic Loading Rate (OLR) was varied from 0.73-3.48 kgBOD_5/m^3.day in the system. It was observed that at lower HRT, the system produced effluents with good quality and obtained an average BOD_5 removal efficiency of about 88% during the operational period. Di Trapani et al., (2010) studied a hybrid AS-biofilm process, mostly for the reduction of organic matter and nitrification operated at different MLSS concentration and sludge retention time (SRT), and also for the effect of temperature in the process. Ammonia uptake rate (AUR) batch test illustrated that the nitrification activity of the biofilm increased when SRT decreased. Kumar et al., (2014) investigated the effect of mixed liquor volatile suspended solids (MLVSS) and hydraulic retention time (HRT) on the performance of activated sludge process during the biotreatment of real textile wastewater. The results depicted that the decolourization and chemical oxygen demand (COD) removal rate raised with increase in MLVSS and HRT.

2.5 Reactor Configurations/Multistage Biological systems

2.5.1 Sequential Batch Reactor (SBR)

This system is an adjusted activated sludge process which uses fill and draw mode of operation. The most significant advantage of the (SBR) system is the knowledge of biomass activity. This knowledge leads the operator to adjust the operation of SBR for optimal biomass activity, troubleshoot the SBR in order to identify problems and establish proper conditions. The SBR system can be operated in aerobic, anoxic and anaerobic conditions. The aerobic SBR system is appropriate for nitrification and denitrification processes due to the supplement operation regime as well as organic carbon oxidation and nitrification (Diamadopoulos et al., 1997). In this regard, high COD removal up to 75% and NH_4^+-N removal of 99% was reported during aerobic treatment of domestic wastewater in a SBR with a 20-40 days residence time. By comparison, anaerobic sequencing batch reactors have demonstrated good performance in the case of solid capture achievement, reduction of organics in one vessel and omission of the need for a clarifier (Renou et al., 2008). Thus, it was recommended to use aerobic-anaerobic SBRs in order to simultaneously bring down organic and nitrogen matter concentration in the effluent and increase the performance of the treatment system (Ahmed et al., 2011). Torrijos et al., (2001) investigated SBR process for the treatment of wastewater at small cheese making dairies in the Jura Mountains. The results obtained by this study showed that the SBR is capable of treating cheese production wastewater, with purification levels at 97.7% for total COD and 99.8% for BOD_5.

Chen et al., (2013) investigated two SBRs operated 3 cycles /day. The aerobic /extended idle (A/EI)-SBR cycle consisted of a 240 min aerobic period, followed by 30 min settling, 1 min decanting and 209 min idle periods, and the anaerobic-oxic (A/O)-SBR cycle consisted of an anaerobic period (2 h), and an aerobic period (4 h), with the remainder of the cycle time for settling (30 min), decanting (1 min), and idle (89 min). The results showed that the A/EI-SBR removed 1.32 ± 0.03–3.55 ± 0.04 mg of phosphorus per g of VSS during the steady-state operation, i.e. BPR from domestic wastewater removed effectively in the A/EI regime. Likewise, Wei et al., (2014) investigated the effect of different carbon sources on the efficiency of enhanced biological phosphorus removal (EBPR) from synthetic wastewater with acetate and two ratios of acetate/starch as a carbon source, indicated that the phosphorus removal efficiency decreased with the increase in starch concentration. It was also found that pressurized pure oxygen sequencing batch reactor (POSBR1) produced more polyhydroxyalkanoates (PHAs) than the other reactors. It was reported that hydraulic residence time (HRT) and temperature can affect the performance of SBR significantly. Thus, higher removal efficiency was achieved at higher HRTs and temperatures.

2.5.2 Completely Stirred Tank Reactor (CSTR)

Reactors are often advantageously connected in series or parallel. Generally, the purpose of wastewater treatment unit is to handle organic matter and nutrients, which is brought about by different types of microorganisms. Though these multiple reactions can be carried out in single completely stirred tank reactor (CSTR), the process is easy to control if two or more reactors are connected in series, the first reactor decomposes organic matter and second does nitrification, and so on. Another advantage of this multistage reactor configuration is that it can take care of organic and hydraulic shock loads by connecting CSTR in series. Biological treatment techniques such as four stage Bardenpho process, the anaerobic/anoxic/oxic (A^2O) process, the University of Cape Town (UCT) process, Modified University of Cape Town (MUCT) process and biological – chemical phosphorous and nitrogen removal, etc. (Rajagopal et al., 2011; Katam et al., 2017) are in wide use, among which A^2O process, a single sludge suspended growth system incorporating anaerobic, anoxic and aerobic zones in sequence are found to be more efficient in nutrient removal.

Xu et al., (2011) investigated novel anaerobic/aerobic/anoxic (AOA) process proposed for denitrifying phosphorous removal, and the characteristic of the AOA process is transferring part of the anaerobic mixed liquor to the post-anoxic zone for providing the carbon source needed for denitrification. The average removal efficiencies of NH_4^+ - N, TN and PO_4^{3-}- P were 93.0 ± 3.1%, 70.3 ± 2.9% and 87.3 ± 11.8%, respectively. Liu et al., (2013) investigated biological nutrient removal in a continuous anaerobic–aerobic–anoxic process treating synthetic domestic wastewater without the addition of external carbon source. On an enhanced biological phosphorus removal (EBPR) system with influent COD, NH_4^+ - N and PO_4^{3-}- P concentrations of 300, 50 and 3.8 mg/L, respectively. The removal efficiencies of total nitrogen (TN) and PO_4^{3-}- P reached above 90% and 99% when the DO concentration of the aerobic unit was controlled at 1.2 ± 0.2 mg/L and the HRT of the aerobic and anoxic units were at 2 h and 4 h, respectively.

Shi et al., (2014) investigated the new anaerobic–anoxic/nitrifying/induced crystallization (A^2N–IC) system and compared with anaerobic-anoxic/nitrifying (A^2N) process for nutrient removal under different influent COD and ammonia concentrations. Ammonia and COD removal rates were very stable in both processes, which were maintained at 84.9% and 86.6% when the influent ammonia varied from 30 mg/L to 45 mg/L and COD ranged from 250 mg/L to 300 mg/L. Nam et al., (1998) studied comparison of COD, nitrogen and phosphorus removal between anaerobic/anoxic/aerobic and anoxic/aerobic fixed biofilm reactor using synthetic activated ceramic (SAC) media. Results showed that anoxic and aerobic processes using SAC media could be possible for removing organics and nutrients from municipal wastewater, in case phosphorus removal was not considered for municipal wastewater with a low concentration of phosphorus. Xing et al., (2014) investigated modified A^2O (anoxic/anaerobic/aerobic/pre-anoxic)-membrane bioreactor (MBR) plant combined with the step feed strategy to improve the biological nutrient removal (BNR) from low C/N ratio municipal wastewater. Results showed that total phosphate (TP) removal efficiency increased by 18.0%. It was suggested that the external carbon source was needed to improve the BNR performance in treating low C/N ratio municipal wastewater in the modified A^2O-MBR process.

Zeng et al., (2011) investigated denitrifying phosphorus removal and impact of nitrite accumulation on phosphorus removal in a continuous anaerobic–anoxic–aerobic (A^2O) process treating domestic wastewater. The results showed that mean total nitrogen (TN) removal was only about 47% and phosphorus removal was almost zero without the pre-anoxic zone and additional carbon source. In contrast with the configuration of pre-anoxic zone, TN and phosphorus removal were increased to 75% and 98%, respectively, as well as denitrifying phosphorus removal of 66–91% occurred in the anoxic zone. Chen et al., (2011) investigated the effect of nitrate recycling ratio on simultaneous biological nutrient removal in a novel anaerobic/anoxic/oxic (A^2O) biological aerated filter (BAF) system. Results showed that nitrate recycling ratios had a negligible effect on the removal efficiencies of COD and NH_4^+ - N. Kermani et al., (2008) investigated the effect anaerobic, anoxic, and aerobic moving bed biofilm reactor for removal of organics and nutrients from synthetic wastewater by varying loading rates of nitrogen and phosphorous. In optimum condition, the average SCOD, TN and TP removal efficiencies were 96.9%, 84.6% and 95.8%, respectively.

2.6 Post treatment

Post treatment is necessary to remove residual organic matter and total suspended solids beyond that have been accomplished by conventional secondary treatment to meet stringent standards for reuse of the treated wastewater for various recreational purposes. It includes advanced oxidation processes, membrane filtration processes, Ion exchange processes. Advanced oxidation processes is a promising technique for treatment of wastewater containing refractory organics. This processes having some advantages such as high oxidizing power, increased biodegradability, decreased toxicity and no residue.

2.6.1 Advanced oxidation process

The term "Advanced oxidation" was first coined by Glaze et al., (1987). Advanced oxidation processes (AOPs) involve supplementation of traditional oxidants with additional stimuli such as high temperature or UV light to create highly reactive hydroxyl radicals to oxidize difficult to treat substance such as saturated organics molecules and pesticides. One example of AOP is the photocatalytic oxidation using titanium dioxide. TiO_2 is an active photo catalyst and is extremely stable, i.e. it does not dissolve or corrode under photoexcitation.

A. Mechanism of photocatalytic oxidation

As a result of solid-state quantum effects, semiconductor materials possess two allowable electron bands. The lower energy region is the valence band the electrons in this energy band are binding electrons and are somewhat restricted in movement. The high energy region is the conduction band. These electrons to a first approximation, are free to move throughout the solid and show reduce conductivity similar to that of metals. Between these two regions is a forbidden zone or band-gap. The occurrence of photoexcitation in a semiconductor is due to the absorption of radiation energy equal to or greater than the band gap energy which excites an electron (e^-) to the conduction band of the solid (Equation 2.1). There is correspondingly an electron vacancy or hole (h^+) that remains in valence band as shown in figure 2.4.

These holes, having an affinity for electrons, are very strong oxidizing agents. The number of electron-hole pairs depends on the intensity of the incident light and the material's electronic characteristics that prevent them from recombining and releasing the absorbed energy. The electron is free to move throughout the solid in the nearly unoccupied conduction band. Similarly, the hole can migrate by a valence band electron filling the vacancy, leaving another hole in the previous position. The corresponding wavelength required for excitation for some common semiconductors are given in table 2.3. The semiconductor potentials for the valence band and the conduction band are significantly different. This difference avoids recombination of the electrons and hole pairs. The band potentials are a function of pH and decrease by 0.059 V per pH unit increase as predicted by the Nernst equation (Turchi and Ollis, 1990)

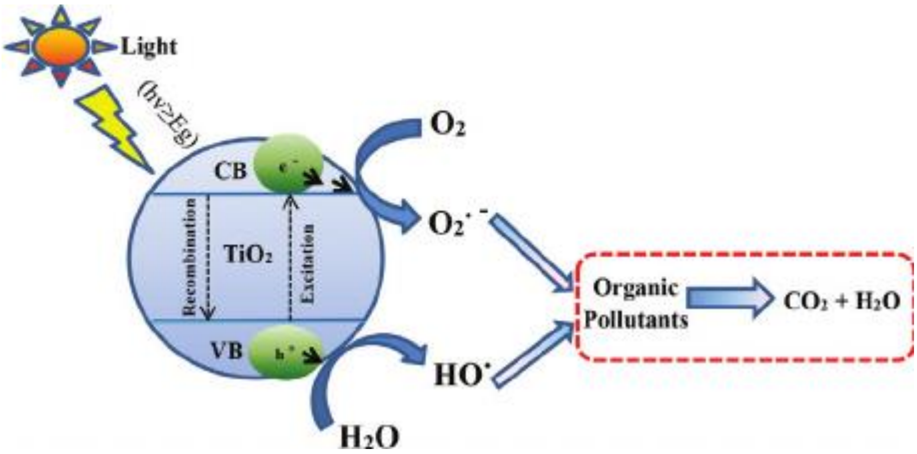

Figure 2.4 Basic mechanism of a heterogeneous photocatalytic process (Samsudin et al., 2015)

Table 2.3 Bandgap energy and corresponding excitation wavelength of some common semiconductors

Semiconductor	Bandgap (eV)	Wavelength (nm)
TiO_2	3.0-3.2	413-388
ZnO	3.2	388
ZnS	3.7	335
CoS	2.4	516
Fe_2O_3	2.3	539
WO_3	2.8	443

The holes in the semiconductor solid are attracted to the oxides/sulfide surface, where they oxidize an adsorbed water molecule or hydrogen ion as shown in (equations 2.2 & 2.3). Hydroxyl radicals are very reactive neutral species which hold an unpaired electron. Their rapid and non-selective reaction during the oxidation of organic compounds makes them a common oxidizers in photo catalytic oxidation and a high pH ozone systems.

$$\text{Semiconductor } e^- + h^+ \quad (2.1)$$

$$H_2O + h^+ \longrightarrow OH^\bullet + H^+ \quad (2.2)$$

$$h^+ + OH^- \longrightarrow OH^\bullet \quad (2.3)$$

Equation 2.2 & 2.3 are likely to occur for two reasons. One is the large quantities of OH^* groups are available as adsorbates, and the chances of holes reacting with these groups on the semiconductor surface are high. The second reason in that for several semiconductors the oxidation potential of these reactions is above the potential for valence band over the entire pH range. Equation 2.2 favoured at low pH while equation 2.3 is favoured at high pH.

The destruction of pollutants using strong photo-produced oxidation power of TiO_2 was first reported by Frank and Bard in 1977, in which the decomposition of cyanide occurred in the presence of aqueous TiO_2 suspensions. Titania (TiO_2), a most promising candidate for manufacturing of commercial solar applications, mainly due to its high photochemical stability and oxidation power, greater resistance to photo-corrosion in aqueous environments, assurance of safety and low priced when compared to other photocatalytic materials (Danilo et al., 2015).

2.6.2 Effect of Catalyst Loading

Many studies are focused on the effects of catalyst loading on photocatalytic degradation process of dyes in wastewaters. The TiO_2 assisted photocatalytic degradation rate in any reactor, was found to be increasing initially with respect to increasing the concentration of catalyst until optimum dosage is reached. Any further increase in the concentration of catalyst ended in the particle agglomeration of the catalyst, hence a part of the catalyst surface became elusive for the photon absorption and degradation rate declined (Akpan. et al., 2009).

2.6.3 Effects of Irradiation time

Illumination time has a direct relationship with the degradation and disinfection characteristics of Titanium dioxide photocatalysis. As the illumination time increases there is increase in degradation of pollutants, hence removal efficiency increases as the irradiation time is increased.

2.6.4 Use of TiO_2 photocatalysis in treatment of various wastewater

Photocatalytic property of TiO_2 to degrade textile dye reactive blue 4 was measured under sunlight irradiation in the study done by Neppolian et al. (2002). The influence of solar light intensity, the concentration of the dye, dosage of catalyst and pH on the rate of degradation of dye was performed in this study. The photodegradation was observed to be optimum in the pH range of 5-10 and the degradation rate of dye was found to be directly proportional to the amount of TiO_2 dosage. About 82% of degradation of dye was noted for 8 hours of solar irradiation. A blank study was conducted in the absence of solar irradiation to illustrate that the degradation of dye was due to the photocatalytic action of TiO_2 using solar irradiation.

Sakkas et al., (2004) conducted an experiment at University of Ioannina, Greece to show the photocatalytic degradation of herbicide (metolachor) using TiO_2 thin film and silver modified nanocrystalline TiO_2 under UV irradiation. The results of this experiment showed that TiO_2 was more efficient in reducing metlachor. TiO_2 photocatalysis was able to reduce about 82% metlachor concentration under UV light irradiation. In Carpio et al., (2005) study, titanium dioxide (TiO_2) photocatalysis was employed for the phenol degradation in aqueous medium using UV rays from an external light source along with the airflow which was provided to increase the rate of oxidative photodegradation. The effect of calcination temperature of TiO_2 crystals on photocatalytic activity of TiO_2 was also studied. It was found that a TiO_2 nanocrystal was able to reduce about 50% of the phenol content from the wastewater by 4 hours of solar irradiation and it was also mentioned that complete degradation of phenol could be achieved by increasing the irradiation time. Similarly, Alalm et al., (2014) investigated the photocatalytic decomposition of phenols with solar and UV compound parabolic collectors (CPCs) and results showed that after an irradiation time of 150 minutes, a degradation efficiency of 94.5% was achieved when initial concentration was taken as 100 mg/L.

Photocatalytic degradation of insecticides (imidacloprid, thiamethoxam, clothianidin) using immobilized TiO2 was observed in the study of Žabar et al., (2012). TiO_2 was found to be a good catalyst to reduce Imidacloprid by 98%, Thiamethoxam by 90.1% and Clothianidin by 92% under 2 hours irradiation of artificial UV lights generated by the six low-pressure mercury fluorescent lamps. The experiment was also duplicated in the absence of light and it was observed that there was no change in the concentration of all three insecticides indicating that the degradation was only due to the photocatalytic action of TiO_2.

Yeber et al., (2000) conducted an experiment to measure the degradation rate of cellulose bleaching effluent using TiO_2 and ZnO as the photocatalyst using artificial UV source. Reduction in COD, TOC and total phenol content was used as the indicator to measure the degradation rate. It was found that there was the presence of a large number of chlorinated compounds in the effluent which might inhibit the microbial activity as a result of the reduced efficiency of biological treatment. Increase in the colour of the effluent was observed when UV irradiation was applied; this may be due to the formation of complex molecules by radical condensation or quinone-like lignin derivatives. Around 50% reduction in colour was observed after 30 min of illumination time and after 90 min of treatment by illumination, the colour was found to be nil. The total phenolic content was found to be reduced in the similar profile as that of colour. TiO_2 was capable of reducing about 51% of TOC content while only 38% of reduction was observed when ZnO was used, thus indicating that TiO_2 photocatalysis was more efficient than to ZnO.

In the study of Kansal et al., (2008) it was found that ZnO had the better photocatalytic property than that of TiO_2 for the degradation of lignin. The results showed that the initial degradation rate due to photocatalysis increased with increase in catalyst dose up to an optimum dosage. Further addition of catalyst showed no significant effect. Also, initial photodegradation rate was observed to be high at lower concentrations of lignin.

Photocatalysis on TiO_2 for remediation of industrial wastewater has been widely been studied on textile industry (Neppolian et al., 2002; Bandala et al., 2008; Al-Momani et al., 2002) and pesticide removal (Echavia et al., 2009). Also, use of solar radiation as a source of light for photocatalysis has given good results for removal of pollutant load and disinfection (Spasiano et al., 2015).

2.7 Modeling of Aerobic hybrid system

Improper maintenance of Wastewater treatment plant (WWTP) can pose serious environmental and health related problems and also possibilities for the spreading of various water-borne diseases affecting human beings and aquatic life are also immense. Implementation of various control actions have to be done for the efficient controlling of the processes during the operation of the treatment plant (Hong et al., 2003). Models are significant as the studies on the operating variables can be done more transiently on a computer rather by conducting experiments. Hence, evaluation of many operational strategies and alternative schemes can be performed without any physical trials of each scenario (Pai et al., 2011). By the model simulation using the possible correction actions, there comes a possibility for rapid response to any process change and devise an operational strategy, which allows the plant to run under new operating conditions that improves its stability, quality of the effluent and reduce in the running costs (Muller et al., 1998). The development of several stochastic, deterministic and time-series based models have been done for prediction of efficiency of WWTPs. In modelling either aerobic or anaerobic hybrid systems, the competition that exists for the substrate between two types of biomass the suspended and attached has to be given due importance. This competition depends on the kinetics of both the processes, which makes it a complex biological system.

2.7.1 Existing mathematical models

2.7.1.1 Lee model

Lee (1992) was first to propose the kinetic model for the hybrid system. The model was developed assuming that there exists a competition for rate limiting substrate between the suspended and fixed cultures within the hybrid reactor.

2.7.1.2 Gebara's model

Gebara (1999) proposed a mathematical model for evaluation of hybrid reactor and assessed it for its accuracy with the experimental studies he conducted by using plastic nets as a carrier for fixed biomass in the hybrid reactor. On the basis of mass balances done across the aeration tank and accounting for X_r, recycled flow rate Q_r, and the recycle ratio, a model was proposed for the hybrid system. This biofilm model is based on three assumptions: a) substrate utilized inside the biofilm follows Michaelis-Mention kinetics; b) the substrate utilization rate inside the biofilm is equal to rate at which substrate diffuses into the biofilm from bulk liquid and c) diffusion of substrate into the biofilm from the bulk liquid ensure Fick's law

2.7.1.3 Fouad and Bhargava model

Fouad and Bhargava et al., (2005) proposed a model based on Lee (1992) model with an exception that sludge is wasted from the settler. The model proposed to evaluate the mean cell residence time of the complete process (AS-biofilm). Thalla et al., (2010) developed a steady state mathematical model to describe nitrification process in an activated sludge biofilm reactor. The model developed uses the Monod's expression for both the growths (suspended and attached) simultaneously and the Ficks diffusion law for the biofilm in the basic equation for the aerobic hybrid reactor. In order to solve the developed model, modified expressions for the substrate flux proposed by Fouad and Bhargava (2005) have been used.

When it comes to modeling anaerobic hybrid system, AD1 proposed by IWA is the most popular among all and based on the purpose and operating condition it has been modified by many researchers.

Some of the draw backs of models are

- A model that oversimplifies may inaccurately reflect the real world situation.
- If the person who builds a model does not know what he is doing, output from the model will be incorrect.
- Models can sometimes prove too expensive to originate when their cost is compared to the expected return from their use.

As discussed earlier, the application and efficiency/accuracy of the model depend upon the assumptions made, input variables, solution technique used, accuracy of the input value etc. Soft computing technics are the alternative to address these issues

2.7.2 Soft computing models

In the recent past, soft computing tools like Artificial neural network (ANN), ANFIS have also been widely used for wastewater treatment prediction studies (Lee et al., 2011; Erdirencelebi et al., 2011; Ay et al., 2014; Bhattacharya et al., 2014; Nandagopal et al., 2016).

2.7.2.1 Artificial Neural Network (ANN)

Artificial neural network is a biologically inspired computational model formed from hundreds of single units, artificial neurons, connected with coefficients (weights) which constitute the neural structure. Raduly et al., (2007) developed a model for predicting WWTP using Artificial neural network (ANN), the data used for training the ANN were generated using ASM3 the input variables for the ANN were those not routinely measured at WWTP, for example, ammonium nitrogen, soluble inert material, particulate inert material and readily biodegradable substrate and heterotroph and autotroph bacteria concentrations. The model outputs were obtained as BOD, soluble ammonium, total COD, TKN, total nitrogen, and TSS. This confirms that the simulation of the model can readily be done using ANNs for WWTP.

Gulay et al., (2010) introduced an ANN model and applied it to a case study of a domestic WWTP. This model used treatment plant input and output parameters as well as independent treatment unit outputs. The treatment variables used in the first part were flow rate, suspended solids, pH, COD and BOD. In the second part, nitrogen and phosphorus compounds were modelled. Monika et al., (2011) analyzed the applicability of ANN operations to evaluate influent and effluent BOD for the effluent treatment process. A three-layered feedforward ANN using a back propagation learning algorithm was applied for predicting effluent BOD. Rangasamy and Latha (2012) investigated the use of Back Propagation Neural Network (BPNN) for modelling anaerobic tapered fluidized bed reactor. The input parameters measured for modelling were influent COD, Hydraulic Retention Time, influent pH, the flow of influent and two outputs namely methane (CH_4) gas and effluent COD. BPNN has the great flexibility to the variations of operation conditions, system configuration and a close resemblance of prediction results to experimental results were also found.

Similarly Guo et al., (2014) developed ANN model and used to simulate and predict the performance of sequential batch bioreactor (SBBR) and vertical flow constructed wetlands (VFCW). According to the ANN simulation results, the correlation coefficients (R^2) were all greater than 0.99, and the root mean squared errors (RMSE) were less than 0.0782. The influent concentrations of NH_4^+-N, DO and TP exhibited significant impacts on effluent. This study confirmed that the ANN was able to efficiently reflect the nonlinear function of each factor, and was best suited for the dynamic monitoring of SBBR-VFCW under various conditions. Haydera et al., (2014) developed ANN model for Multi-stage biological treatment of petroleum refinery wastewater using different biological conditions. Raw data obtained from two multi-stage biological reactors (MSBR) used for the treatment of different loads of petroleum refinery wastewater was used for developing a mathematical model that could predict the process trend. The model was then used for prediction; highest removal efficiency observed was 98% which was repeatedly recorded for various loads (Delnavaz et al., 2010). Rene et al., (2008) developed ANN model to predict BOD and COD from of a refinery wastewater. Results showed that ANN model could accurately predict the effluent concentration of water quality parameters.

2.7.2.2 Adaptive Neuro-Fuzzy Inference System

ANFIS, a hybrid fuzzy-logic based technique integrated with the learning power of Artificial Neural Network improves the performance of any kind of intelligent system by utilizing knowledge acquired after learning. For a real-time input-output dataset, a hybrid learning algorithm such as ANFIS constructs a backpropagation gradient descent and least squares methods associatively to frame a fuzzy inference system whose membership function parameters are iteratively tuned or adjusted. Adaptive Neuro Fuzzy inference systems comprise of mainly five layers viz. rule base, database, fuzzification interface, defuzzification interface and decision making unit. The generalized ANFIS architecture proposed by Jovanovic et al., (2004) is summarized below.

The structure of ANFIS is shown in Figure 2.5. The ANFIS is a fuzzy Sugeno model that allocates the structure of adaptive systems to assist learning and adaptation. ANFIS architecture comprises of five layers. Every single node in layer 1 is an adaptive node with a node function which may be anyone among the membership functions. Every node of layer 2 is a fixed node labeled ‘π’ which signposts the firing strength of each rule. All nodes of layer 3 are fixed nodes labeled as ‘N’ which demonstrates the normalized firing strength of each rule. The Layer 4 is as similar to layer 1 wherein every node is an adaptive node governed by a node function. The layer 5 being a single fixed node labeled 'Σ', representing the final output (f), defined as the summation of all arriving signals.

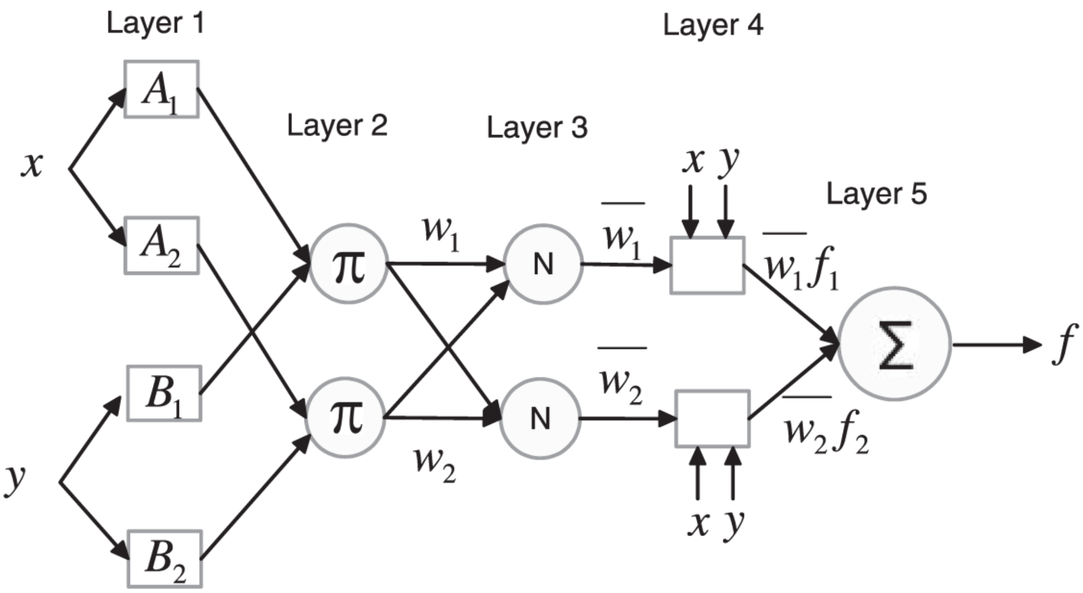

Figure 2.5 Schematic diagram of ANFIS Structure (Raghavendra & Deka, 2015)

Pai et al., (2009) conceptualized neural-fuzzy model-based adaptive neuro-fuzzy inference system (ANFIS) and artificial neural network (ANN) to predict effluent suspended solids (SS) and COD from hospital wastewater. The simulated results of ANFIS showed better correlation coefficient for SS_{eff} and COD_{eff} at 0.75 and 0.92. Pai et al., (2011) developed ANFIS model to predict effluent suspended solids (SSeff), chemical oxygen demand (CODeff), and pHeff from a wastewater treatment plant and the results showed that ANFIS statistically out performed ANN in terms of effluent prediction. The minimal mean absolute percentage errors of 2.67%, 2.80%, and 0.42% for SSeff, CODeff, and pHeff could be achieved using ANFIS. The maximum values of the correlation coefficients for SSeff, CODeff, and pHeff were 0.96, 0.93, and 0.95, respectively. Kisi and Ay (2012) developed the Radial Basis Neural Network (RBNN) and Adaptive Neuro-Fuzzy Inference System (ANFIS) methods to estimate Dissolved Oxygen (DO) concentration by using various input variables. From the study, it was found that the RBNN model resulted better in comparison with the ANFIS model in the DO estimation.

Kisi and Ay (2013) developed three ANFIS techniques Viz. ANFIS with fuzzy clustering, ANFIS with grid partition and ANFIS with subtractive clustering to determine COD concentration with varying input variables. These three ANFIS models were compared based on their performance measures such as root mean square, coefficient correlations and mean absolute error. The experimental results indicated that in the testing phase ANFIS with subtractive clustering model was found relatively better than the other alternative models and in the validation phase, ANFIS with fuzzy clustering model showed better performance than the other two models.

2.7.2.3 Genetic algorithm (GA) and Practical Swarm optimization (PSO)

Genetic Algorithm

Genetic algorithm (GA) is a stochastic search iterative method based on the evolutionary theory of natural selection and genetics that can provide optimal or near optimal solutions for the combinatorial optimization problems, such as traveling salesman problems, scheduling problems, heuristic search or process planning problems (Goldberg, 1989). The basic concept of Genetic Algorithm is to implement genetic operators like crossover, mutation and selection for up gradation and search of the best population by imitating the natural evolution process artificially. The genetic algorithm is initiated with an initial population of possible solutions, called individuals, with a specified objective where each individual is symbolized using distinct form of encoding as a chromosome. These chromosomes are evaluated for their fitness. Based on their fitness, chromosomes in the population are nominated for reproduction and selected individuals are manipulated using crossover and mutation. This idea is based on the expectation that better parents can probabilistically generate better offspring. The offspring in the next generation are generated by applying the GA operations, crossover and mutation, to the selected parent solution. This process is iterated until the GA search converges to the required searching level and the termination criterion is satisfied (Vijayabhanu et al., 2013). To implement the GA, the following basic components are to be considered:

- GA parameters (population size, maximum number of generation, crossover rate and mutation rate)
- Chromosome representation

- Initialization of population
- Evaluation of fitness function
- Selection process
- Genetic operators (crossover, mutation and elitism)

The advantages of GA includes: (1) fast convergence to near global optimum, (2) superior global searching capability in the space which has complex searching surface, and (3) applicability to the searching space where we cannot use gradient information of the space.

Practical swarm optimization

Particle Swarm optimization (PSO) is an evolutionary computational method developed to deal with the optimization of continuous and discontinuous function for decision making. The PSO algorithm is based on the biological and sociological behaviour of animals such as schools of fish and flocks of birds searching for their food. PSO is a population-based search method where each potential solution is represented as a particle in a population (called swarm). Particles change their position in a multidimensional search space until equilibrium or optimal state has been reached or until computation limitations are exceeded (Shi et al., 2003).

Suppose that the search space is *D*- dimensional and there are m particles from the colony. The i^{th} particle represents a *D*-dimensional vector $X_i = (i = 1, 2, 3.....m)$. It means that the i^{th} particle locates at $Xi = (X_{i1}, X_{i2}, X_{i3} \ldots\ldots X_{iD})$ $(i = 1,2,3.....m)$ in the search space. The position of each particle is the potential result. We could calculate the particles by putting their positions into the designed objective function. If the fitness value is higher, the corresponding particle X_i is "better". The i^{th} particle's "flying" velocity is also a *D*-dimensional vector and is denoted as $V_i = (V_{i1}, V_{i2}, V_{i3} \ldots\ldots V_{iD})$ $(i=1, 2, 3 .... m)$. Denote the best position of the i^{th} particle as $P_i = (P_{i1}, P_{i2}, P_{i3} \ldots\ldots P_{iD})$ and the best position of the colony as $Pg\ (P_{g1}, P_{g2}, P_{g3} \ldots. P_{gD})$ respectively and the PSO algorithm could be performed by the following eqs: (2.4 and 2.5)

$$V_{id} = V_{id} + C_1 \gamma_1 (P_{id} - X_{id}) + C2\gamma 2 (P_{gd} - X_{id}) \qquad (2.4)$$

$$X_{id} = X_{id} + V_{id} \qquad (2.5)$$

where $i=1, 2, 3 ,..., m$, $d=I, 2, 3... D$, $c1$ and c_2 are learning rates, r_1 and r_2 are generated randomly in intervals $[0. 1]$, $V_{id} = [-V_{max}, V_{max}]$, and V_{max} is a designated value. The termination criterion for the iterations is determined according to whether the maximum generation or a designated value of the fitness of P_g is reached.

2.7.2.4 Evolutionary ANFIS

The Fuzzy Inference System (FIS) based on fuzzy c-means (FCM) clustering is implemented in the Adaptive Neuro Fuzzy Inference System (ANFIS). The metaheuristics or evolutionary algorithms such as Genetic Algorithm (GA) or Particle Swarm Optimization (PSO) are applied to tune the parameters of ANFIS. The flow chart is shown in Appendix – A and Algorithm in Appendix B. Evolutionary-trained ANFIS is especially useful for non-linear regression problems. In the FIS based on FCM clustering, the input membership function type is ‘gaussmf’ by default and the output membership function type is ’linear’. The Evolutionary algorithm (GA or PSO) assists the ANFIS to adjust the parameters of the membership function. For additional information regarding GA, PSO, and ANFIS, their architecture, parameters, model building procedure, one may refer to Yu & Gen, (2010), Agrawal et al., (2008) and Jang, (1993).

Shabazien et al., (2017) developed a model for corrosion prediction of 3C steel considering different environmental factors using hybrid Adaptive Neuro-Fuzzy Inference System-Particle Swarm Optimization (ANFIS-PSO). Performance of the model was then compared with other two models, namely Adaptive Neuro-Fuzzy Inference System- Genetic Algorithm (ANFIS-GA) and Support Vector Regression (SVR) model. Results showed that PSO-ANFIS model had lower prediction error than the other two models. Sharma et al., (2015) investigated the application of Modified Particle Swarm Optimization (MPSO) to train ANFIS (Adaptive Neuro Fuzzy Inference System) for the efficient prediction of two major air pollutants namely, Sulphur dioxide (SO_2) and Ozone (O_3). The results obtained from MPSO-ANFIS model were compared with those obtained through training ANFIS. Results clearly indicate that ANFIS training using MPSO is far better at generalizing and achieving higher accuracy for the prediction of SO_2 and O_3 air pollutants. Jannaty et al., (2015) investigated Particle Swarm Optimization Adaptive Neuro Fuzzy Inference System

(PSO-ANFIS) model to predict scour depths. The empirical results showed that, PSO ANFIS model provided good results. Ravi et al., (2011) investigated GA ANFIS model controller design method for temperature control in plastic extrusion system. The results obtained from GA ANFIS controller showed improved performance in terms of time domain specification, set point tracking, and disturbance rejection with optimum stability. Similarly, Vijayabhanu et al., (2013) developed a model to improving the efficiency of the prediction system for anaerobic wastewater treatment process using Genetic Algorithm.

2.8 Summary of literature

Authors and researches worldwide have studied to remove harmful constituents such as organic matters and nutrients from wastewater. This has resulted in the development of numerous novel technologies to improve wastewater treatment plants. For instance, removal of organic carbon, nutrients and ammonia contents has been achieved through physical and chemical treatment such as adsorption, precipitation, chemical oxidation etc.; each method having its own advantages and disadvantages. Biological treatments such as conventional activated sludge (CAS) and attached growth systems though provide better quality treatment yet are not very reliable under stressful situations such as shock loads etc and further their costs are high. Therefore, combined Aerobic hybrid treatment systems which are combined processes where both suspended and attached microbes proliferate in the same reactor thus enhancing the efficacy. Furthermore, the type of media, surface area of the media, filling ratio, different hydraulic retention times (HRT) and organic loading rates (OLR) affect the performance of the system and have been investigated in few studies.

The conventional secondary treatment units must be supported by proper post treatment technology. Photocatalysis is one of the AOP's that has been extensively studied in last two decades, which not only reduces many chemical compounds but is also a major disinfection method. Photocatalysis on TiO_2 for remediation of industrial wastewater has been widely studied on textile industry, removal of insecticide and pesticides. Also use of solar radiation as a source of light for photocatalysis has given good results for removal of pollutant load and disinfection. Titania photocatalysis has therefore widely proven its effectiveness in removal of chemical compounds and pathogens from industrial wastewater.

Based on literature survey, it has been observed that the mathematical models are available for activated sludge biofilm process individually but there are only few models to understand the combined process. Kinetic parameters deduced for suspended and attached growth may be applied to describe the reactions in biofilm. Only few models have been developed, and they all have their own advantages and disadvantages.

Soft computing models are used to predict the efficiency of WWTP. In the recent days these models are necessary, mainly because the effects of tuning the operating variables can be studied more transiently on a computer than by doing experiments. Several deterministic and stochastic models have been developed for prediction of performance of WWTPs using ANN, ANFIS, GA-ANFIS and PSO-ANFIS etc.

2.9 Gaps Identified

Most literature on treatment of wastewater in a completely mixed hybrid systems deals with the specific type of media, filling ratio, and operating conditions such as organic loading rate (OLR) and DO. However, the performance of hybrid reactor is also influenced by the type of external carbon source, bulk liquid carbon to nitrogen ratio (C/N) and suspended biomass concentration (X) and there are only limited studies performed considering these parameters. Further, for a complex system like this, mathematical models could be effective in studying the effects and also evaluating the effects of operating variables on the overall performance of the system. Which otherwise would be possible only by doing a large number of experimental trials. Similarly, soft computing (SC) is an innovative approach to construct a computational intelligence system which deals with reliable models and gives solutions to complex real-life scenario. There are only limited soft computing models available to predict the efficiency of wastewater treatment plant. In the recent past, soft computing tools like, Artificial neural network (ANN), Adaptive neuro-fuzzy inference system (ANFIS) have also been widely used for wastewater treatment prediction studies. Based on the gaps identified the objectives mentioned in the next section have been decided for this research.

2.10 Objectives

The objectives of the proposed research work

1. To study the performance of hybrid biological system in treating wastewater under influence of variable C/N ratio, biomass concentration, hydraulic retention time and dissolved oxygen concentration for
    a. Ideal Aerobic AS-biofilm system
    b. In-series hybrid biological system i.e. anaerobic, anoxic, oxic (A^2O)-AS-biofilm system
2. Post-treatment of AS-biofilm system effluents using UV and Solar photocatalysis with TiO_2 as a catalyst.
3. To develop soft computing model to predict the performance of AS-biofilm system

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 General

All the laboratory experiments have been designed to study the performance of the biological hybrid system under various reactor configurations and operating conditions and also to train and validate the soft computing models for the aerobic hybrid system. The entire experimental work was done in two phases explained in successive sections.

Phase I: Performance evaluation of AS-biofilm reactor and post-treatment by photocatalysis.

The objective of phase-1 experiments was to study the effects of carbon to nitrogen ratio (C/N), suspended biomass concentration(X), hydraulic retention time (HRT) and dissolved oxygen concentration (DO) on the performance of AS-biofilm reactor and to evaluate the technical feasibility of TiO_2 based solar/UV photocatalysis in meeting the quality criteria specified by regulatory bodies for reuse of treated wastewater. Wastewater quality index (WWQI) was developed to judge the quality of treated water.

Phase II: Performance of anaerobic, anoxic, oxic (A^2O)-AS-biofilm reactor connected in series.

The objective of the Phase-2 study was to evaluate the performance of anaerobic, anoxic and oxic reactors connected in series to effectively removing carbon, nitrogen and phosphorous simultaneously under variable C/N, HRT, DO and biomass (X) concentrations.

3.2. Materials & Methods

3.2.1 Phase 1: Aerobic Hybrid System (AS-biofilm reactor) and post-treatment

3.2.1.1 AS-biofilm experimental model

A laboratory scale aerobic hybrid system of 15 L working volume was used in the present study as shown in Figure 3.1. The setup consisted of the overhead tank to store feed (100L capacity), a constant head tank of 30L capacity to maintain the head required so as to maintain the flow to the reactor by gravity and a settling tank of 10Lvolume. Constant head tank and the bio-reactor were fabricated from acrylic sheets.

Figure 3.1 Lab scale AS-biofilm reactor for Phase 1 studies

The reactor was aerated by fine bubble diffusers provided at the bottom of the reactor and the contents were stirred periodically using stirrer to avoid clogging of biomass between the biofilm carriers and dead zones in the reactor. Sludge was recycled by a peristaltic pump to maintain the desired biomass concentration in the reactor. Settling tank effluent was further fed to two post-treatment batch reactors each of 1000 ml capacity.

***3.2.1.2 Bio-Media used*:** Biofill® - Type C2 circular polyethylene media with characteristics as shown in Table 3.1 was used as biofilm carrier. They were procured from QM Environmental Services, Netherland.

Table 3.1 Characteristics of carrier media

Characteristics	Values
Specific surface area (m^2/m^3)	592
Weight per unit(gram)	2
No of pieces per m^3	87000
Density (kg/m^3)	172
Diameter (mm)	25
Material	Polyethylene
Max working temperature ^0C	65
Resistance to hydrocarbons	Good

3.2.1.3 Photocatalysis experimental setup

The settling tank effluent was further subjected to post-treatment in 1000 mL batch reactors (cylindrical glass beaker) using TiO_2 as catalyst (Figure 3.2). Aeroxide® P25, Titania, Titanium dioxide, Assay ≥ 99.5% trace metals bases, Form: Nanopowder (21 nm primary particle size) an Aldrich product was used as the photocatalyst. A magnetic stirrer was used for the continuous agitation of the catalyst in suspension and was subjected to UV and Solar irradiations. The UV-C Ultraviolet lamp of 8W at 254ɳm was used for UV irradiation

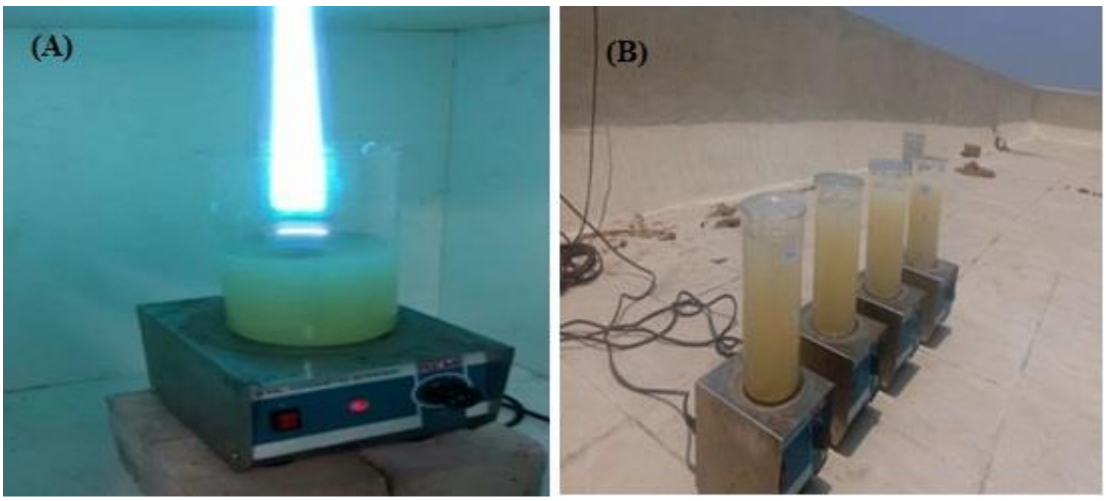

Figure 3.2 Lab scale setup (A) UV photocatalysis (B) Solar photocatalysis

3.2.1.4 Synthetic wastewater sample

In the present study, synthetic wastewater feeds of various combinations of C: N: P ratios (shown in table 3.4) were prepared out of diluted molasses (carbon source); ammonium bicarbonate (NH_4HCO_3) and potassium dihydrogen phosphate (KH_2PO_4), the phosphorus source. NH_4HCO_3 being used as nitrogen source, all the nitrogen initially was in the form of ammonia nitrogen (Kermani et al., 2009). In addition to C, N and P, trace metal solution to supplement micronutrient requirements was prepared composing 1500mg/L of $FeCl_3.6H_2O$; 30mg/L of $CuSO_4.5H_2O$; 150mg/L of H_3BO_3; 60mg/L of $NaMoO_4.2H_2O$; 15mg/L of $CoCl_2.6H_2O$; 12mg/L of $ZnSO_4.7H_2O$; 30 mg/L of KI; 120mg/L of $MnCl_2.4H_2O$; 10 mg/L of EDTA and 2mg/L yeast extract similar to the one prepared by Liu et al., (2013). Molasses was procured from a regional sugar factory located near Pandavapura Taluk, India. Characteristics of molasses and synthetic wastewater are shown in Table 3.2.

Table 3.2 Composition of diluted molasses and synthetic wastewater

Characteristics	Molasses (1:1000)	Synthetic Wastewater
COD	1075	400-1200
TOC	596	200- 600
BOD	380	NC (Not Checked)
NH_4^+-N	0.9	30- 50
KH_2PO_4	0.4	10
$MgSO_4$	32	50
$MgCl_2$	50.2	50
NO_3-N	0	0
NO_2-N	0	0
pH	6.3- 6.5	7.5- 7.8

** Except pH all other units in mg/L*

3.2.1.5 Operation

Initially, the reactors were seeded (30% of reactor volume) with activated sludge collected from municipal wastewater treatment plant. The system was aerated for 15h after transferring the sludge and before supplying the feed. Later the system was fed with molasses-based synthetic wastewater having a COD of 200mg/L, and the reactor was operated until it stabilized i.e. constant effluent concentration for a given influent. Once the stabilization was achieved, biofilm carriers (Biofill® Type C2 circular polyethylene media) were filled up to 30% of the reactor working volume which was decided based on the literature (Biswas et al., 2014) thus converting the system to an aerobic hybrid system and then the system was operated for development of biofilm around the carrier media. The reactor took 25 days to get stabilized (including the development of biofilm over carriers). The reactor was considered to be ready for operation soon after the development of biofilm around the carrier media and the real-time functioning of the reactor was started by feeding it with the desired amount of substrate (So). The biofilm developed on the surface of the carrier is shown in figure 3.3.

Figure 3.3 Biofilm growth upon the surface of the carrier media.

The AS-biofilm reactor was maintained at room temperature (28-32°C) and pH was maintained in the range of 7–8 using 0.01 N NaOH solution. The overview of phase-1 experiments is shown in Table 3.3.

3.2.1.6 Operating conditions

In the present study, reactor was operated under varied C/N ratio (4 to 13.33); suspended biomass concentration (X) (1 to 3.5 g/L); DO (1.5 to 4.5 mg/L) and HRT (6 to 10hrs). Taguchi method is a statistical approach to optimize process parameters in various engineering fields. In the present study Taguchi L_9- orthogonal array (Yu et al., 2010; Kaushik et al., 2009) design of experiment (DOE) was applied to reduce the number of experiments. Four independent variables viz. C/N, HRT, DO and X were selected as variables called as factors. The list of various operation scenarios incorporating different conditions is presented in Table 3.4. The samples were collected for analysis only after the system achieved steady state under each operating condition.

During post-treatment, the samples were irradiated for different radiation (contact) times at various dosages of titanium dioxide. The dosage of TiO_2 photocatalyst ranged from 50 mg/L to 700 mg/L, and the time of irradiation ranged from 60 min to 180min. Based on the removal efficiencies of the effluents (of known concentration), the optimal TiO_2 dosage and contact time were determined and then the effluent of AS-biofilm reactor was post-treated adopting the optimal TiO_2 dosage and contact time in the UV and Solar batch reactors.

Table 3.3 Overview of the phase-1 experiments

Parameters	**Descriptions** **Phase - 1 Experiments**	**Analysis performed**
Reactor configuration	Circular reactor 15L working volume	Daily analysis COD NH_4^+-N NO_2^- -N NO_3^- -N TN TP MLSS MLVSS
Seeding	Activated sludge collected from WWTP, 30% of reactor volume	
Movable media	Polyethylene media Type C2 (30% volume)	
Temperature	28 - 30^0c	
Rate of recirculation	Recirculation ratio (Qr/Q) Variable based on ‘X’ in the reactor	
Wastewater feed to reactor	Molasses-based synthetic wastewater	
Operating conditions	C: N ratio (in the range of 4 to 13.33); suspended biomass concentration (X) (in the range of 1 to 3.5 g/L); DO (in the range of 1.5 to 4.5 mg/L) and HRT (in the range of 6 to 10h)	
Post-treatment conditions	Photocatalysis using TiO_2 as catalyst under solar and UV irradiation(Dosage 50 to 700 mg/L; Irradiation time 60 to 120 min)	COD TP MPN

Table 3.4: Taguchi (L_9- orthogonal array) design of experiment (DOE) for present study

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Run no.	**Trial**	**Time (Days)**	**Factors**				**Influent**			**OLR**		**COD/N ratio**	**N/P ratio**
			C/N ratio	(X) Biomass g/L	HRT (h)	DO mg/L	COD mg/L	'N' mg/L	'P' mg/L	(Kg COD/m^3/d)	(Kg NH_4-N /m^3/d)		
1	1	0-5	4	3 to 3.5	10	1.5 to 2.5	400	50	10	0.96	0.12	8	5
	2	6-10	4	2 to 2.5	8	2.5 to 3.5	400	50	10	1.2	0.15	8	5
	3	11-15	4	1 to 1.5	6	3.5 to 4.5	400	50	10	1.6	0.2	8	5
	4	16-20	5	3 to 3.5	8	3.5 to 4.5	400	40	10	1.2	0.12	10	4
	5	21-25	5	2 to 2.5	6	1.5 to 2.5	400	40	10	1.6	0.16	10	4
	6	26-30	5	1 to 1.5	10	2.5 to 3.5	400	40	10	0.96	0.096	10	4
	7	31-35	6.67	3 to 3.5	6	2.5 to 3.5	400	30	10	1.6	0.12	13.33	3
	8	36-40	6.67	2 to 2.5	10	3.5 to 4.5	400	30	10	0.96	0.072	13.33	3
	9	41-45	6.67	1 to 1.5	8	1.5 to 2.5	400	30	10	1.2	0.09	13.33	3
2	10	46-50	8	3 to 3.5	10	1.5 to 2.5	800	50	10	1.44	0.12	12	5
	11	51-55	8	2 to 2.5	8	2.5 to 3.5	800	50	10	1.8	0.15	12	5
	12	56-60	8	1 to 1.5	6	3.5 to 4.5	800	50	10	2.4	0.2	12	5
	13	61-65	10	3 to 3.5	8	3.5 to 4.5	800	40	10	1.8	0.12	15	4
	14	66-70	10	2 to 2.5	6	1.5 to 2.5	800	40	10	2.4	0.16	15	4
	15	71-75	10	1 to 1.5	10	2.5 to 3.5	800	40	10	1.44	0.096	15	4
	16	76-80	13.3	3 to 3.5	6	2.5 to 3.5	800	30	10	2.4	0.12	20	3
	17	81-85	13.3	2 to 2.5	10	3.5 to 4.5	800	30	10	1.44	0.072	20	3
	18	86-90	13.3	1 to 1.5	8	1.5 to 2.5	800	30	10	1.8	0.09	20	3
3	19	90-135	12	2 to 2.5	10 8	1.5 to 2.5 2.5 to 3.5	1200	50	10	2.88 to 3.6	0.12 to 0.15	24	5

3.2.1.7 Analytical procedure

The effluent sample from AS-biofilm reactor was analyzed on daily basis. The sample was centrifuged at 10000 rpm for 10min in order to get supernatants which were then filtered through 0.45 micrometer whatman filter paper and analyzed for determining Chemical oxygen demand (COD), Total nitrogen (TN), Nitrate nitrogen (NO_3^-–N), Ammonia nitrogen (NH_4^+–N), Nitrite nitrogen (NO_2^-–N), Total phosphorous (TP), mixed liquor suspended solids (MLSS), and mixed liquor volatile suspended solids (MLVSS) according to the standard methods (APHA 2005). During the post-treatment, TiO_2 was separated by centrifuging it at 4800 rpm for 60 min from the batch reactors. An aliquot of centrifuged effluent samples was analyzed to determine chemical oxygen demand (COD), biochemical oxygen demand (BOD), most probable number (MPN), and phosphorous. The scanning electron microscopy (SEM) combined with energy dispersive spectrometry (EDS) analysis was conducted to clarify the microscopic behavior of sludge particles (JSM-6380LA, JEOL, Japan) operating at 20 kV (Zhang et al., 2016). X-ray diffraction (XRD) analyses were performed with a (DX-GE-2P, JEOL, Japan) diffractometer, with a cobalt tube scattering from 10 to 90^0 in 2θ (Manas et al., 2011). Fourier transformation infrared spectroscopy (FTIR) was performed to identify functional groups presented on the surface of the biomass. FTIR absorption spectra were performed with a (BRUCKER ALPHA) in the wave number range 4000-400cm^{-1}. Approximately 2 mg of biological material sample was prepared and compressed with 200 mg of dry KBr.

3.2.1.8. Determination of Wastewater Quality Index (WWQI)

WWQI is regarded as one of the most efficient way to convey wastewater quality in a collective way by interpreting wastewater quality parameters (Khambete et al., 2014). The WWQI was developed for effluents from UV and solar photocatalysis. The parameters used for obtaining WWQI were COD, BOD, MPN, and Phosphorous. The WWQI was only calculated when all parameters were obtained under or around the prescribed standard values. The weighted arithmetic mean method was used for calculating WWQI for the treated samples (Yogendra and Puttaiah, 2008). The superiority of UV and Solar photocatalysis were comparatively evaluated based on WWQI. (eq 3.1 & 3.2)

$$WWQI = \frac{\sum Q_n \times W_n}{\sum W_n} \quad (3.1)$$

Further, sub-index Q_n was calculated using equation

$$Q_n = \frac{(V_n - V_{io}) \times 100}{(S_n - V_{io})} \quad (3.2)$$

Where V_n = Calculated value of n^{th} parameter after treatment

V_{io} = Ideal value of n^{th} parameter

S_n = Standard value for n^{th} parameter

W_n = Unit weight for n^{th} parameter = 0.25

WWQI and the status of wastewater quality were established using standard values and ideal values as mentioned in Table 3.5. The range for wastewater quality status was set by using values of parameters under and above the ideal and standard values. Wastewater quality status and ranges are as given in Table 3.6.

Table 3.5 Standard values for wastewater disposal

Parameter	**Standard value (mg/l)**	**Ideal value (mg/l)**
	GoI, MoE, Forest and Climate change (2015)	United States Environmental Protection Agency Guide lines for water reuse (2004)
COD	60	30
BOD	30	10
MPN	100	0
TP	5	2

Table 3.6 Wastewater quality status and ranges

Wastewater quality index	**Wastewater quality status**
0 – 20	Excellent
20 – 40	Very Good
40- 60	Good
60 – 80	Average
80 – 120	Poor
> 120	Very Poor

3.2.2. Phase 2: Anaerobic, anoxic and oxic activated sludge biofilm (A^2O-AS-biofilm) reactor connected in series.

3.2.2.1 Experimental model

The laboratory scale A^2O-AS-biofilm process consisted of three ccylindrical biological reactors and a settler, which were made up of the acrylic sheet as shown in Figure 3.4. The volume of each biological reactor was 3L with total volume of 9L and the volume of the settler was 3L. Synthetic wastewater passed through the reactor by gravity. The aerobic reactor was aerated by fine bubble diffusers provided at the bottom of the reactor and the contents were stirred periodically using a magnetic stirrer to avoid clogging of biomass between the biofilm carriers and dead spaces of the reactor.

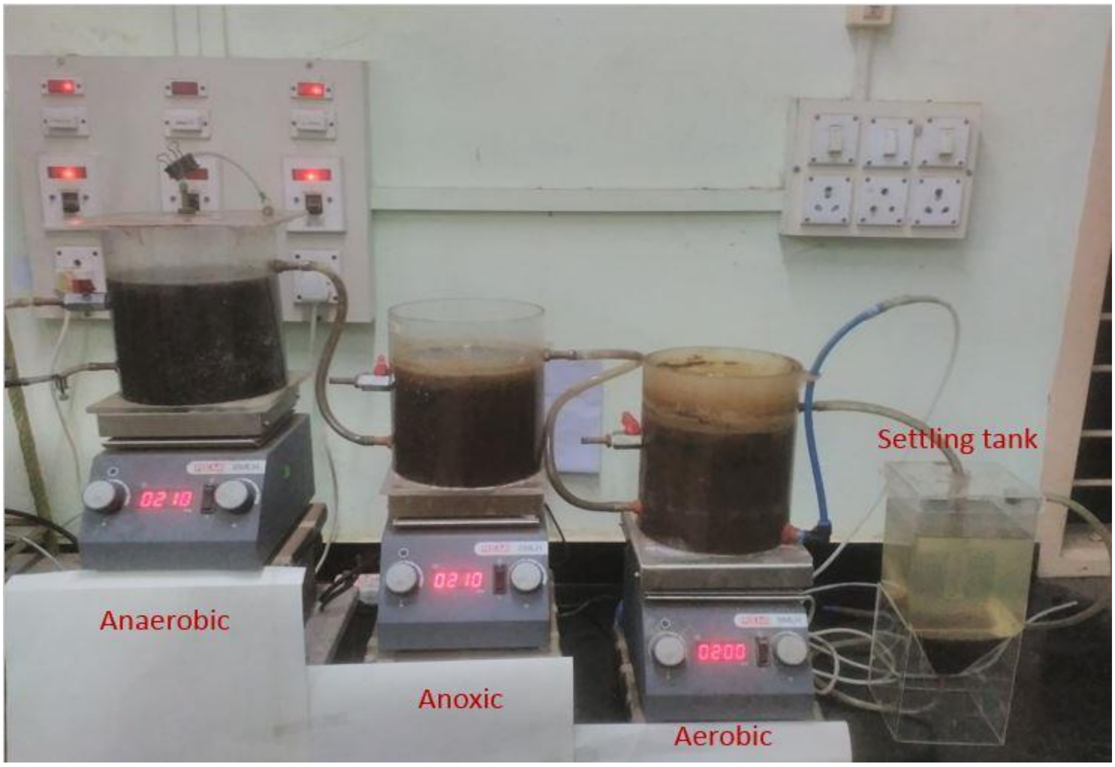

Figure 3.4 A^2O-AS-biofilm reactor connected in series and its accessories

3.2.2.2 Bio-Media used

Circular Polyethylene C2 type media with properties as shown in table 3.1 was used as biofilm carrier, and it filled 30% of the volume of the reactor in all the three reactors is described in section 3.2.1.2.

3.2.2.3 Preparation of synthetic wastewater

Synthetic wastewater used for this study has been described in section 3.2.1.4.

3.2.2.4 Operation

The overview of phase-2 experiments is shown in Table 3.7.

Table 3.7 Overview of the Phase 2 experiments

Parameters	Descriptions	Analysis performed
Reactor configuration	3Cylindrical reactor 9Lworking volume	Daily analysis Total COD pH NH_4^+-N NO_2^- -N NO_3^- -N Total phosphorous Total nitrogen
Wastewater feed to reactor	Molasses-based synthetic wastewater	
Movable media	Biofill® - Type C2	
% of media filling	30% of the volume of the reactor	
Temperature	28 - 30^{0}c	
Rate of recirculation	Recirculation ratio (Qr/Q) Variable based on ‘X’ in the reactor	
Operating Conditions	C: N ratio (in the range of 4 to 13.33); HRT (in the range of 6 to 10h); Except suspended biomass concentration(X) (in the range of 1 to 3.5 g/L); DO (in the range of 1.5 to 4.5 mg/L) is varied in aerobic reactor and the strategy of experimental design is as shown in Table 3.4.	MLSS MLVSS
Seeding	Anaerobic sludge is obtained from Kavoor wastewater treatment plant. Aerobic activated sludge is collected from NITK, wastewater treatment plant and filled to about 30% of reactor volume	SEM Analysis

CHAPTER 4

RESULTS AND DISCUSSION

4.1 General

Laboratory experiments had been designed to study the performance of AS-biofilm reactor in phase-1 and integrated A^2O-AS-biofilm reactor configuration in phase-2 under various operating conditions viz. C/N ratio, HRT, biomass (X) and DO for COD and nutrient removal as per the details given in chapter 3.

4.2 Performance of AS-biofilm reactor for COD and nutrient removal (Phase-1).

Taguchi (L_9- orthogonal array) design of experiment (DOE) has been applied to reduce the number of experiments and the operating conditions of the reactor was varied according to the experimental design by varying the factors (*Col. 4-7, Table 3.4*) i.e. independent variables, the complete experimental study is broadly reported as Run1, Run 2 and Run 3. The variation in COD, TN and TP in AS-biofilm reactor corresponding to each run is shown in Table 3.4.

4.2.1 COD removal

In Run 1, Run 2 and Run 3 trials, the influent COD concentration was maintained at around 400mg/L, 800mg/L and 1200mg/L respectively, in AS-biofilm reactor (Table 3.4). It can be seen that maximum COD removal in Run 1, Run 2 and Run 3 obtained were 82.96%, 92.5% and 94% which were corresponding to C/N ratio- 5, HRT- 8h, X - 3 to 3.5g/L and DO -3.5 to 4.5mg/L; C/N ratio- 10, HRT- 8h, X - 3 to 3.5g/L and DO -3.5 to 4.5mg/L; C/N- 12, HRT- 10h, X - 2 to 2.5g/L and DO - 2.5 to 3.5mg/L respectively. The COD removal efficiency of the optimal trail of Run 2 was 10% more than that of optimal trial of Run 1. From the Figure 4.1 it can be seen that the removal efficiency increased with increase in the influent COD concentration.

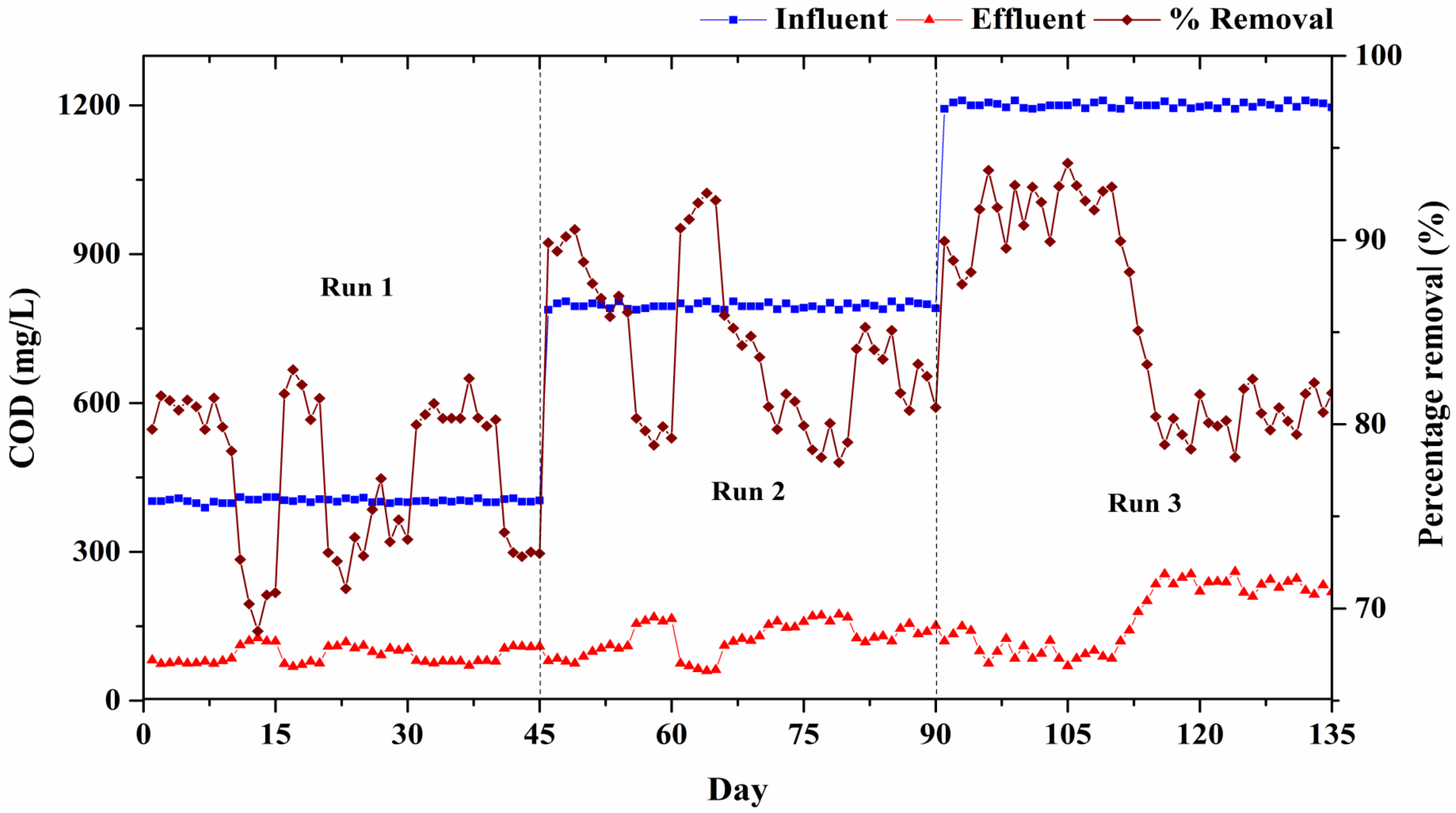

Figure 4.1 Performance of AS-biofilm reactor in COD removal during 135 days operation

A huge amount of COD (in the form of organic matter) is consumed by the micro-organisms (denitrifiers and polyphosphate accumulating organisms) during the simultaneous removal of nitrogen & phosphorous in the aerobic reactors (Yang et al., 2009). The average COD removal efficiency of Run 1, Run 2 and Run 3 trials were 78.58%, 85.24% and 90.5% respectively. Organic loading rate (OLR) and nutrient removal performance of AS-biofilm reactor with other hybrid systems is shown in Table 4.1. Similar results were observed by Rusten et al.,(1992) in their study treating dairy wastewater, the pilot-plant showed 85% and 60% COD removal at volumetric organic loading rates of 500 g COD/m^3h and 900 g COD/m^3h respectively. Similar results were obtained by Feng et al., (2008) on a two-stage fluidized-bed (TFB) bioreactor for COD influents between 500 – 600 mg/L with 95% removal.

4.2.2 Nitrogen removal

Total ammonium concentrations of influent, effluent and total removal efficiency with respect to time are presented in Figure 4.2. In Run 1, Run 2 and Run 3 trials the influent ammonia nitrogen concentration was varied between 30mg/L to 50mg/L. Maximum removal NH_4^+-N in Run 1, Run 2 and Run 3 obtained were 88.69%, 94.08% and 88% which were corresponding to C/N ratio- 5, HRT- 8h, X - 3 to 3.5g/L and DO - 3.5 to 4.5mg/L; C/N ratio- 13.3, HRT- 10h, X - 2 to 2.5g/L and DO -3.5 to 4.5mg/L; C/N- 12, HRT- 10h, X - 2 to 2.5g/L and DO - 2.5 to 3.5mg/L respectively. Similarly, maximum removal TN in Run 1, Run 2 and Run 3 obtained were 81.88%, 86.65% and 81% which were corresponding to C/N ratio- 6.67, HRT- 10h, X - 2 to 2.5g/L and DO-3.5 to 4.5mg/L; C/N ratio- 12, HRT- 10h, X - 2 to 2.5g/L and DO - 3.5 to 4.5mg/L; C/N- 12, HRT- 10h, X - 2 to 2.5g/L and DO - 2.5 to 3.5mg/L respectively. From the above results, the DO concentration of 3.5-4.5 mg/L is found to yield better removal at all the trials. The higher removal efficiency of NH_4^+-N could be attributed to sufficient nitrification in the system. It was because of the manifestation of a water thin stratum of heterotrophic biofilm over the interior and exterior surfaces of the 'Biofill type C2' carriers and a relatively thicker layer of nitrifiers grown over the carriers. The results obtained were in line with that obtained by Kermani et al., (2008).

Table 4.1 Comparison of organic loading rate and nutrient removal efficiency of AS-biofilm reactor and other hybrid systems

Reference	Reactor type	Organic loading rate		HRT (h)	Removal efficiency			
		kg COD/m^3*d	kg NH_4^+-N/m^3*d		COD%	NH_4^+-N%	TN%	TP%
Present study	AS-biofilm	0.96 - 2.4	0.09 - 0.2	6 - 10	82 -92	88 - 94	81 - 86	53 - 68
Amin et al., (2017)	Moving bed bioreactor	0.75 - 3.3	-	4 - 16	88 - 97	-	-	-
Rahimi et al., (2011)	Fixed bed sequencing batch reactor	0.56 - 2.23	55.71 – 222.86	-	90 - 96	-	70 - 88	79 - 90
Nguyen et al., (2011)	Sponge tray bioreactor	1.2 – 2.4	-		92	40.2	56	41.9
Eldyasti et al., (2010)	Liquid–solid circulating fluidized bed bioreactor	2.15	0.68	-	85	-	80	70
Jianlong et al., (2008)	Sequence hybrid biological reactor	4	-		92	93.5		
Jahren et al., (2002)	Moving bed biofilm reactor	2.5 - 3.5	-	13 - 22	60 -65	-	-	-
Rusten et al., (1992)	Moving bed biofilm reactor	0.5 -0.9	-	-	80 - 60	-	-	-

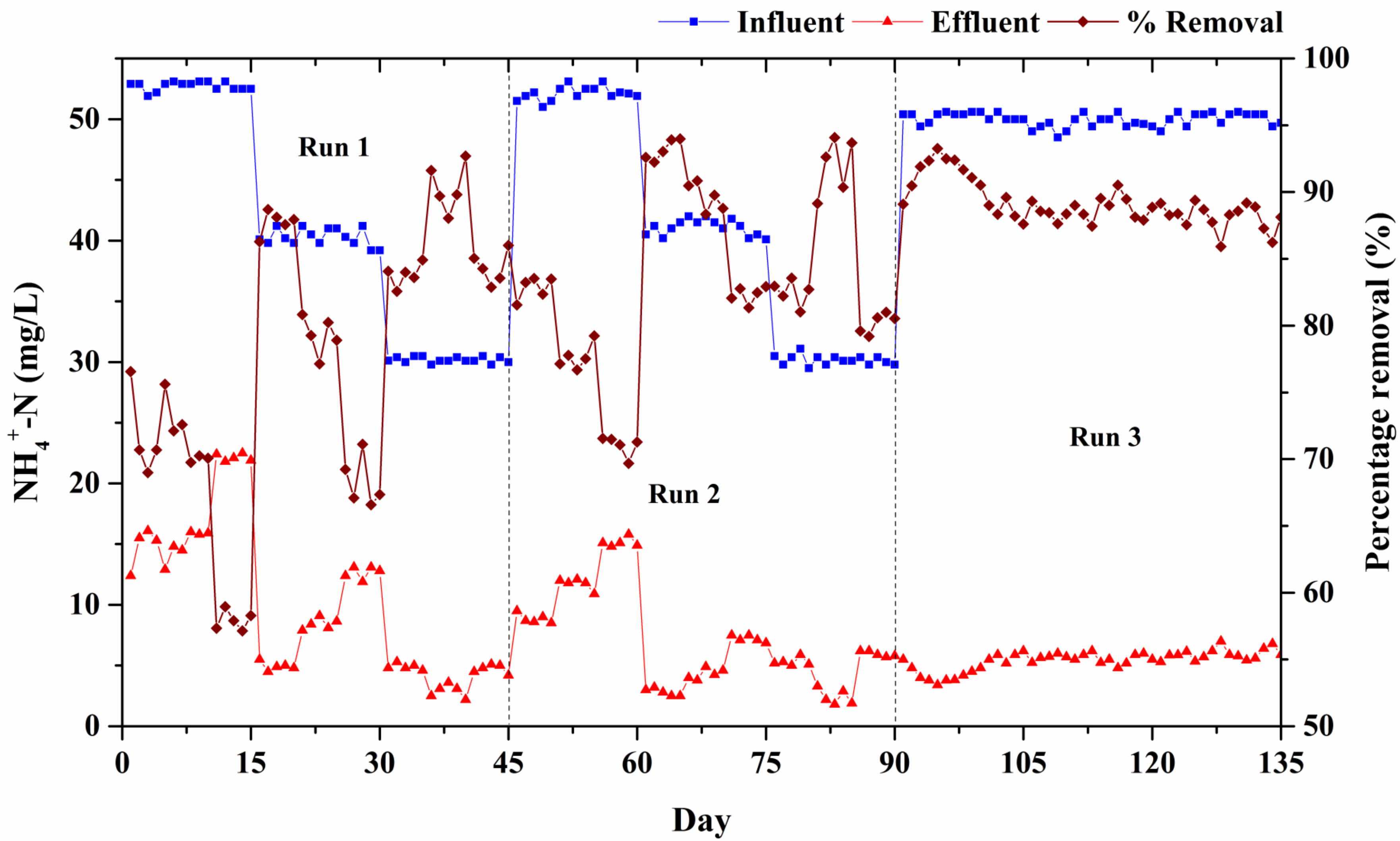

Figure 4.2 Performance of AS-biofilm reactor in NH_4^+-N removal during 135 days operation

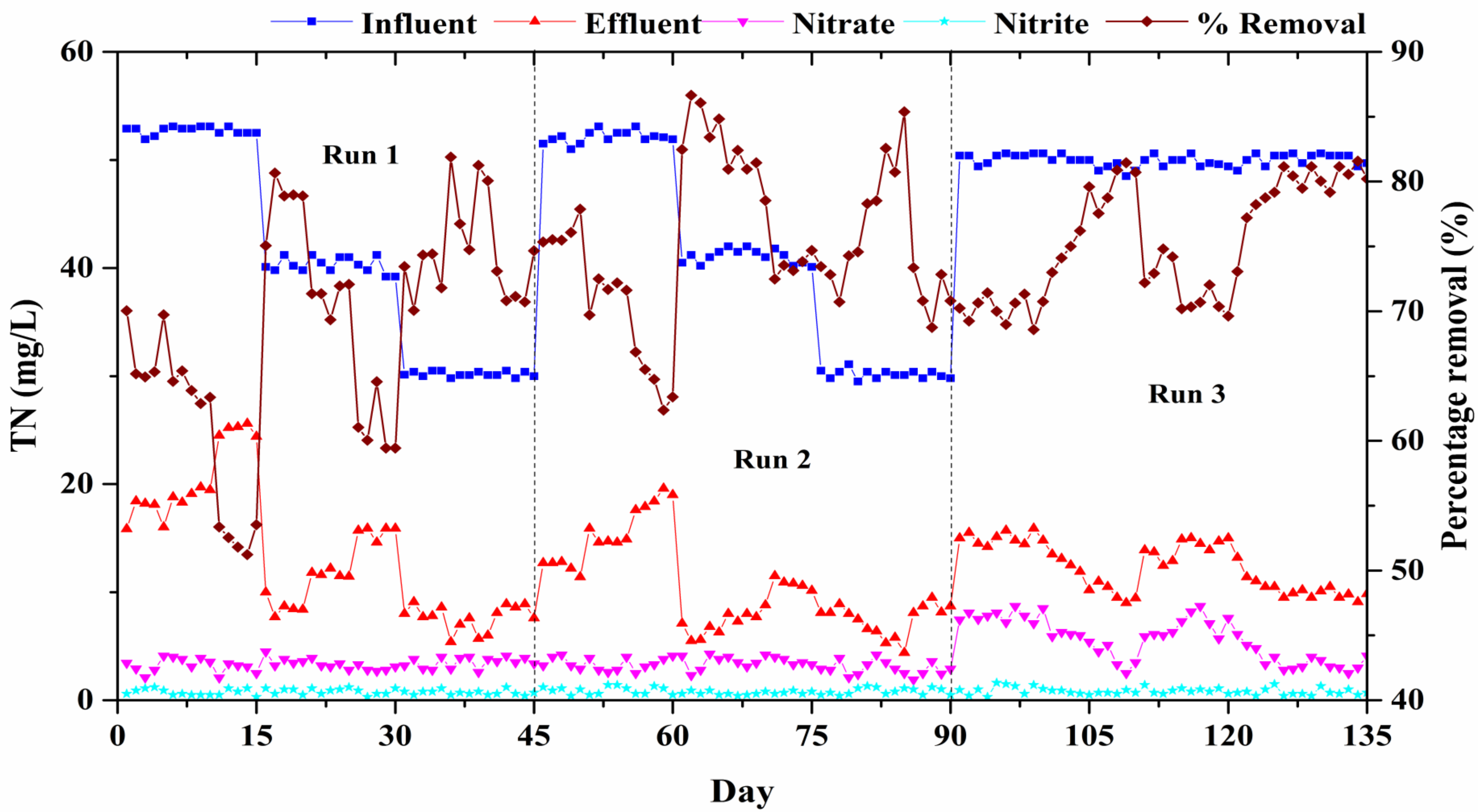

Figure 4.3 Performance of AS-biofilm reactor in TN removal during 135 days operation

However some of the nitrates produced were removed by denitrification, as the wastewater does contain a low level of COD. The low level of nitrate accumulation also strongly suggests the low rate of nitrate production, along with the high nitrogen removal. Figure 4.3 shows the variation in the total nitrogen concentration during the period of 135 days.

4.2.3 Phosphorous removal

TP removal from the system was monitored for about 135 days. The efficiencies of TP removal during this period is shown in Figure 4.4. The influent TP concentration of 10mg/L was kept constant for all the trails throughout the study. Maximum removal of TP in Run 1, Run 2 and Run 3 obtained were 53%, 68.63% and 74% which were corresponding to C/N ratio- 5, HRT- 8h, X - 3 to 3.5g/L and DO -3.5 to 4.5mg/L; C/N ratio- 10, HRT- 6h, X - 2 to 2.5g/L and DO - 1.5 to 2.5mg/L; C/N-12, HRT- 10h, X - 2 to 2.5g/L and DO - 2.5 to 3.5mg/L respectively. A remarkable increase of phosphorous removal efficiency could be noticed by decreasing the HRT and DO of the system. Carvalheira et al., (2014) in their study observed that at higher HRT and DO, glycogen accumulating organisms (GAOs) proliferate than phosphorus accumulating organisms (PAOs) thus decreasing the TP removal efficiency. PAOs possess a higher affinity for oxygen and showed a clear kinetic advantage over GAOs at low DO concentrations, thus EBPR systems should be operated at low DO levels to minimize the proliferation of GAOs. It might be attributed to the presence of considerable amount of PAO in AS-biofilm reactor than the GAO as generally found in conventional activated sludge process (Kong et al., 2005). The PAO's which utilize oxygen were the main contributors for the phosphorous removal in the aerobic phase while GAO's contribution is less. The extremely significant species like *acinetobacter* species may be some common bacterium in the biomass and may have been partly responsible for the TP removal in our experiments (Fuhs et al., 1975).

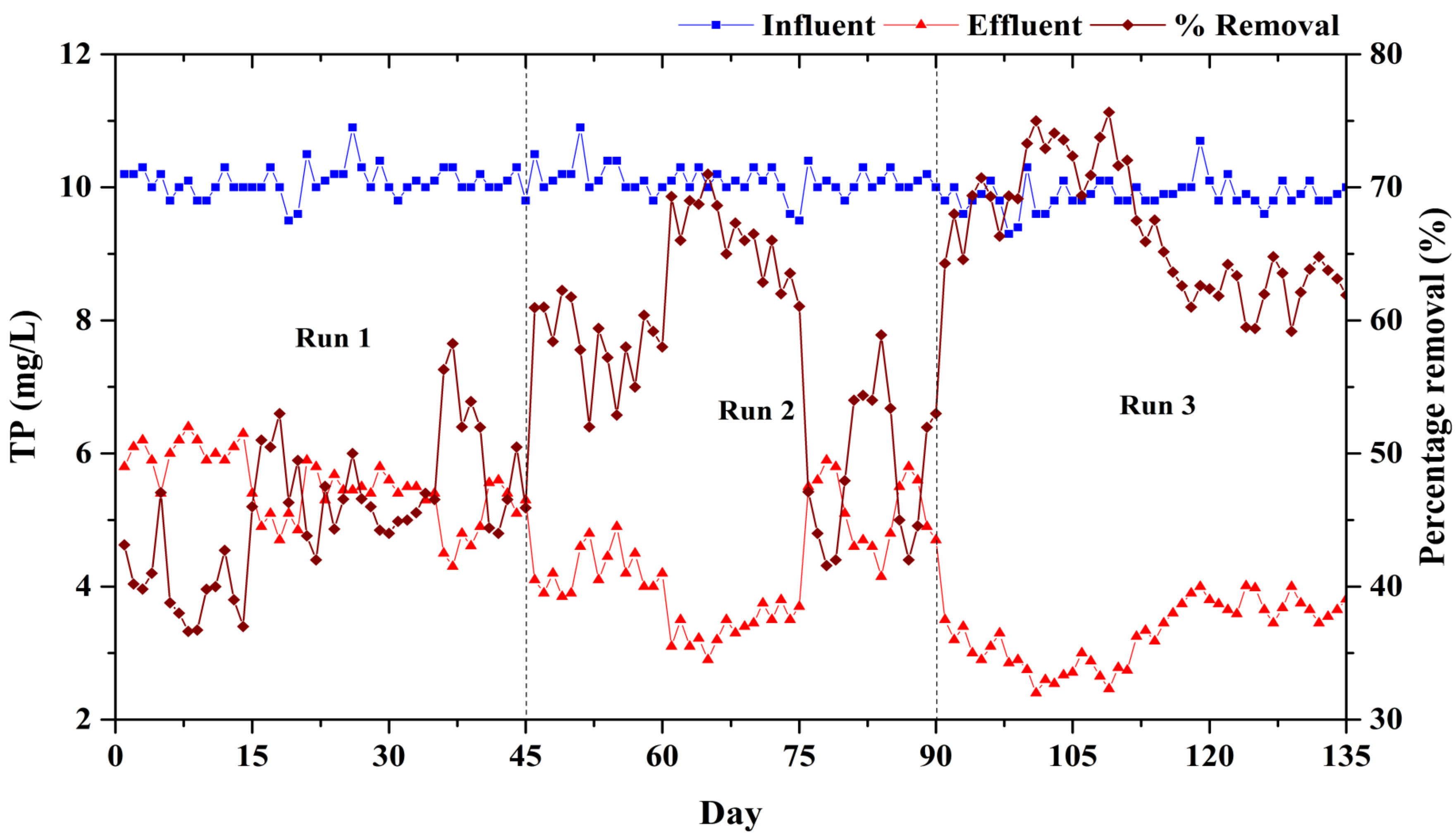

Figure 4.4 Performance of AS-biofilm reactor in TP removal during 135 days operation

4.2.4 The Effect of C/N ratio on the performance of system

In the biological nutrient removal process, the C/N ratio is one of the major factor that influences the performance of AS-biofilm reactor. The C/N ratio was altered in the range of 4 to 13.3. At C/N ratio=6.67 (Run1), the COD, NH_4^+-N and TN removal efficiencies were found to be much higher than the other C/N ratios. At C/N ratio=6.67, due to higher removal efficiencies of NH_4^+-N and TN, it could be inferred that more carbon source was utilized for the enrichment of denitrifying bacteria than the phosphorous accumulating organism (PAO) thus yielding a relatively lesser TP removal efficiency. Similarly, the COD, NH_4^+-N, TN, and TP removal efficiencies were higher at C/N ratio=10 (Run 2). The elevated C/N ratios might perhaps have speeded up the development of heterotrophic denitrifying bacteria present over the biofilm thus improving the TN removal efficiency (Zhang et al., 2016). A majority of the phosphorus accumulating bacteria were capable of utilizing nitrate as electron acceptor (an alternate to carbon source requirement) during TP removal. Figure 4.5(A) presents the plot of mean removal efficiencies of COD, NH_4^+-N, TN and TP v/s different C/N ratios from the data of all the trials.

4.2.5 The Effect of suspended biomass on the performance of system

The suspended biomass (X) is required in the AS-biofilm reactor to achieve reasonable rates of substrate utilization. In the present study, the optimal range of suspended biomass required to get higher nutrient removal efficiencies was determined by maintaining the AS-biofilm reactor at three different suspended biomass concentration ranges (i.e., 1-1.5, 2-2.5 and 3-3.5 g/L). The NH_4^+-N and TN removal efficiencies were found to be higher at X=2-2.5 g/L; and the COD and TP removal efficiencies were greater at X=3-3.5g/L. This showed that a higher concentration of suspended biomass was required for the effective removal of COD and TP. At X=3-3.5g/L, the NH_4^+-N and TN removal efficiencies were also high. Figure 4.5(B) shows the plot of mean removal efficiencies of COD, NH_4^+-N, TN and TP at different biomass concentration (X) from the data of all the trials. The reason for better removal of COD, NH_4^+-N, TN and TP at higher suspended biomass concentration could be that at higher biomass concentrations, dispersed microbial communities prevailed which were advantageous in the overall process. The competition among the microbes would be high at low F/M ratio, high sludge age and low substrate concentration. One disadvantage of maintaining a higher biomass concentration is that the sludge production rate within the reactor increases that reduces the contact time of sludge with the wastewater and also increases operating cost of the plant. More sludge production results in increased cost in managing it in terms of dewatering and digestion etc.

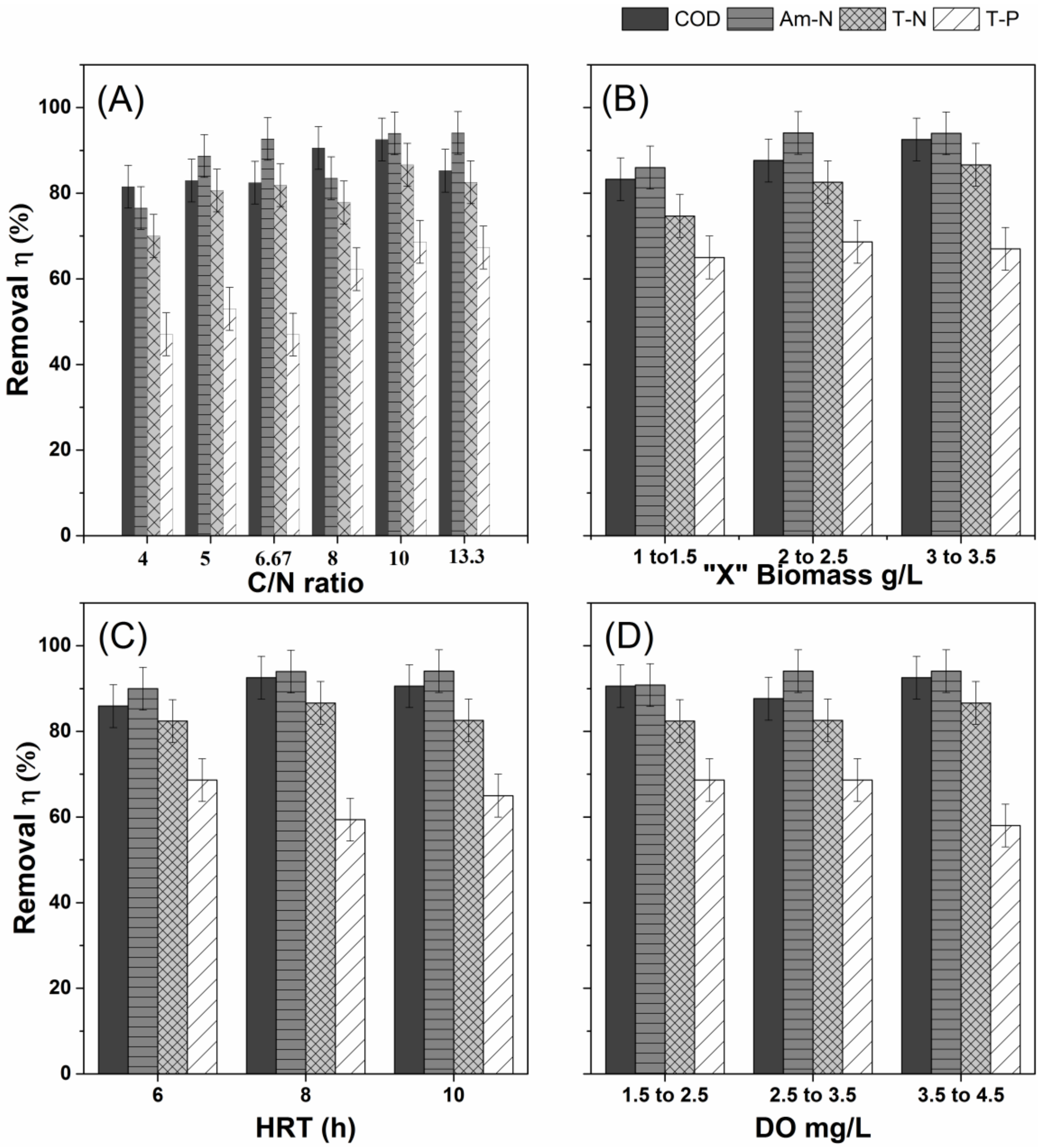

Figure 4.5 Effect of (A) C/N ratio; (B) 'X' Biomass; (C) HRT and (D) DO on the performance of system.

4.2.6 The Effect of HRT on the performance of system

It is average time a liquid remains in the reactor and can be defined as the ratio of volume of the reactor to the average influent flow rate. The HRT was varied from 6 to 10h, with the aim of understanding the effect of HRT on the performance of AS-biofilm reactor. The higher removal efficiencies of COD and TP were obtained for HRT=8h, and the NH_4^+-N and TN removal efficiencies were found to be higher at HRT=10h. For the efficient removal of NH_4^+-N and TN, the AS-biofilm reactor required a prolonged HRT of 10h. Figure 4.5(C) shows the plot of mean removal efficiencies of COD, NH_4^+-N, TN and TP at different HRT from the data of all the trials respectively. It can be observed that, at HRT=8h, the mean removal efficiencies of COD, NH_4^+-N and TN are substantially more than those the other HRTs. The mean phosphorous removal efficiency was found to be higher at lower HRT i.e 6h. The lower HRT is known to favor the multiplication of bacteria population inside the reactor thus increasing the nutrient removal efficiency. However, at higher HRT, the degradation rate increases decreasing the doubling rate of bacterium (Kumar et al., 2014).

4.2.7 Effect of DO on the performance of system

An improved efficiency of wastewater treatment plant can be obtained by proper aeration which is very significant for microbial oxidation and for maintaining turbulance in the wastewater (Rodriguez et al., 2014). Figure 4.5(D) shows the COD, NH_4^+-N, TN and TP removal efficiencies at different DO concentrations ranging from 1.5- 4.5 mg/L during the experimental period of 135 days. The average COD removal efficiency was 90, 87 and 92% at DO rates of 1.5-2.5, 2.5-3.5 and 3.5-4.5mg/L respectively. The average COD removal existed beyond 90% at all 3 different DO rates. The average NH_4^+-N removal at different DO concentrations were 90, 94 and 94.4% respectively. Likewise, the trend of TN removal efficiency in the AS-biofilm reactor at different DO were 82, 82 and 86% respectively which indicated that simultaneous nitrification and denitrification (SND) is happening in the reactor. The oxygen concentration, floc size and the C/N ratio are the key factors affecting simultaneous nitrification and denitrification (SND) processes in aerobic reactor. A minimum DO level is essential for the nitrifiers, but must not exceed a certain level at the same time for the denitrifiers for simultaneous nitrification & denitrification (Feng et al., 2011). The NH_4^+-N and TN removal rate at 2.5-3.5 mg/L DO rate was higher compared to 1.5-2.5 and 3.5-4.5 mg/L DO. In view of the fact that the reactor remained continuously under aerobic condition, the TN removal might perhaps be achieved due to DO of bulk solution inside the reactor. The average TP removal efficiency achieved were 68, 65 and 58% at DO of 1.5-2.5, 2.5-3.5 and 3.5 to 4.5mg/L, respectively. The effective TP removal was observed in the AS-biofilm reactor when DO rate was 2.5-3.5 mg/L. It could be concluded that a DO range between 2.5 -3.5 would be favorable condition for removal of nutrients.

4.2.8 Ranking the importance of operating parameters on the system performance: Taguchi analysis

The results obtained from the experiments conducted as per Taguchi orthogonal array design of experiments and their corresponding signal to noise (S/N) ratios pertaining to the removal efficiencies revealed that, the values of each factor and their levels are almost close to each other and these values were as shown in Table 4.2. It was seen that the delta value of C/N ratio was higher than that of other three parameters. The values were 1.23, 1.02, 0.75 and 0.37 for C/N ratio, Biomass (X), HRT and DO respectively and were correspondingly ranked as 1, 2, 3 and 4. It could be concluded that the parameter C/N ratio had more influence on the COD, NH_4^+-N, TN and TP removal in Run 1. Similarly in Run 2 delta value of X Biomass was higher than other three parameters and the values were 0.55, 0.70, 0.45 and 0.30 for C/N ratio, X Biomass, HRT, DO respectively and were ranked as 2, 1, 3 and 4 respectively. It could be concluded that X Biomass had more influence on the COD, NH_4^+-N, TN and TP removal in Run 2.

Table 4.2 Taguchi response table for percentage removal COD, NH_4^+-N , TN and TP removal verses A,B,C and D as signal to noise ratios(S/N) for Run 1 and Run 2 trials

		Run 1		
Level	C/N ratio (A)	X g/L (B)	HRT hrs (C)	DO mg/L (D)
1	35.14	36.48	36.24	36.12
2	36.36	35.92	36.13	35.76
3	36.36	35.46	35.49	35.98
Delta	1.23	1.02	0.75	0.37
Rank	1	2	3	4
		Run 2		
Levels	C/N ratio (A)	X g/L (B)	HRT hrs (C)	DO mg/L (D)
1	37.14	37.55	37.60	37.53
2	37.69	37.66	37.15	37.42
3	37.34	36.96	37.42	37.23
Delta	0.55	0.70	0.45	0.30
Rank	2	1	3	4

4.2.9 Microbial Study

SEM analysis was carried out to analyze the morphological characteristics of suspended and attached biofilm. EDS can be used to determine the elemental composition of surface films in the SEM. The morphology of microorganisms present on the sludge and biofilm is as shown in Figure 4.6. The microstructure presented in Figure 4.6(A) of initially seeded activated sludge had microporous gel layers with scattered deposition of microbial cells such as *Streptococcus sp*. Various forms of facultative aerobic coccus appearing in pairs, bead-like chains, or bunches were the dominant microbes over the surface of attached biofilm of Run 1 trials (Figure 4.6B). Several coccobacilli typically rod-shaped along with few *staphylococci* could be observed on the surface of attached biofilm of Run 2 trails (Figure 4.6C).

A bacillus similar to *Pseudomonas aeruginosa* along with cocci colonies was observed in the microstructure of suspended biomass of Run 1 trails (Figure 4.6D). The colony-forming ciliated protozoa communities along with several bacilli were observed in the microstructure of suspended biomass of Run 2 trails (Figure 4.6E). The size of different kinds of bacillus and cocci bacteria ranged from 0.5 to 1.0 µm diameter. Some compact layers of extra-cellular polymeric substances (EPS) composed of proteins were also observed in the attached and suspended biomass. From EDS analysis of initially seeded activated sludge showed that it composed of carbon 20.9%, oxygen 29%, magnesium 2.02%, aluminum 16.44%, silica 13.28%, Phosphorous 7.43%, calcium 2.8% and iron 8.1% as shown in Figure 4.7(F). XRD analysis of suspended granular sludge conformed that consists of inorganic minerals of quartz and calcite as shown in Figure 4.7(G). This phenomenon was similar to that observed by Li et al., (2011).The presence of quartz and calcite increased the bacterial metabolic activity both on the attached and suspended biomass (More et al., 2014; Rodríguez et al., 2012).

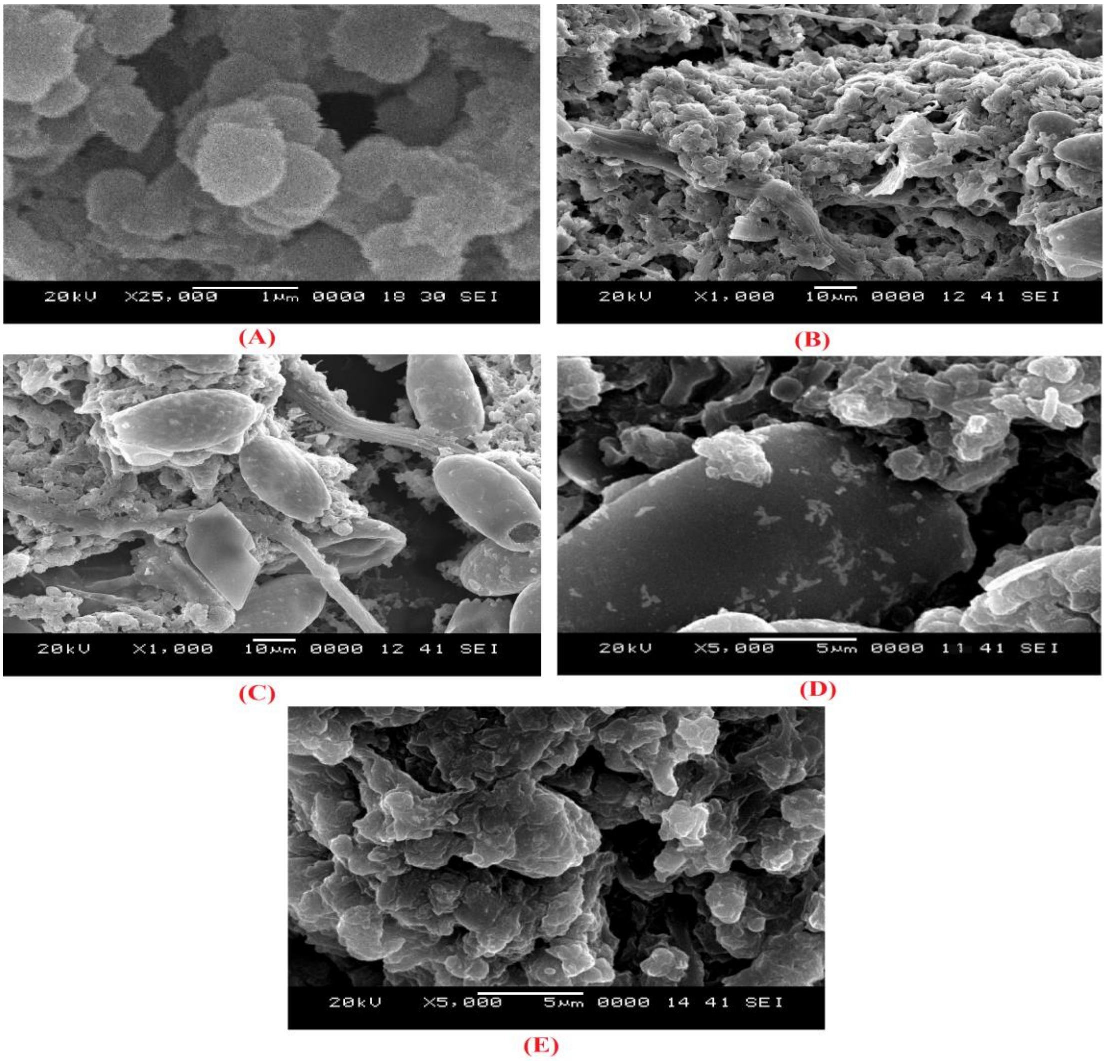

Figure 4.6 SEM images of AS biofilm reactor (A). Initial Seeded activated sludge; (B and C) attached biomass at end of Run1 and Run2; (D and E) suspended biomass at end of Run1 and Run2

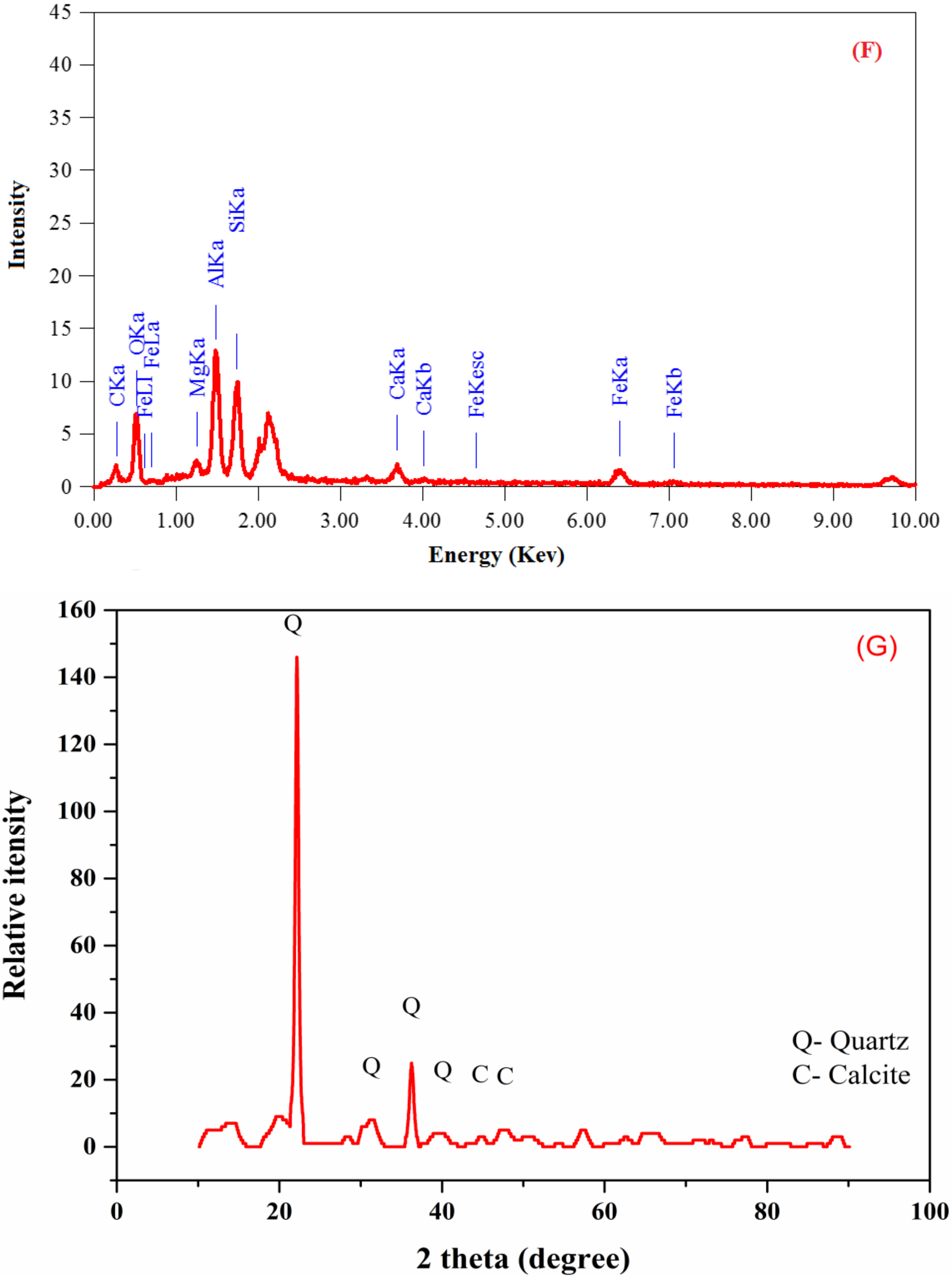

Figure. 4.7 (F) EDS energy spectrum analysis of sludge (G) XRD for granular sludge sample.

4.2.10 Fourier transform infrared spectroscopy (FTIR) Analysis

FTIR analysis was performed to identify the organic and inorganic functional groups present in the suspended and attached biofilm and also used to identify bacterial cell components and characterization of microorganisms based on the measurement of the molecular bond and vibration compounds, excited by radiation of a suitable frequency (Naumann et al., 1991; Duygu et al., 2009). FTIR spectra of initially seeded activated sludge, attached biofilm and suspended biomass (Figure 4.8, Table 4.3) in the range of 400-4000 cm^{-1} were taken in order to obtain information on biochemical composition of cells.

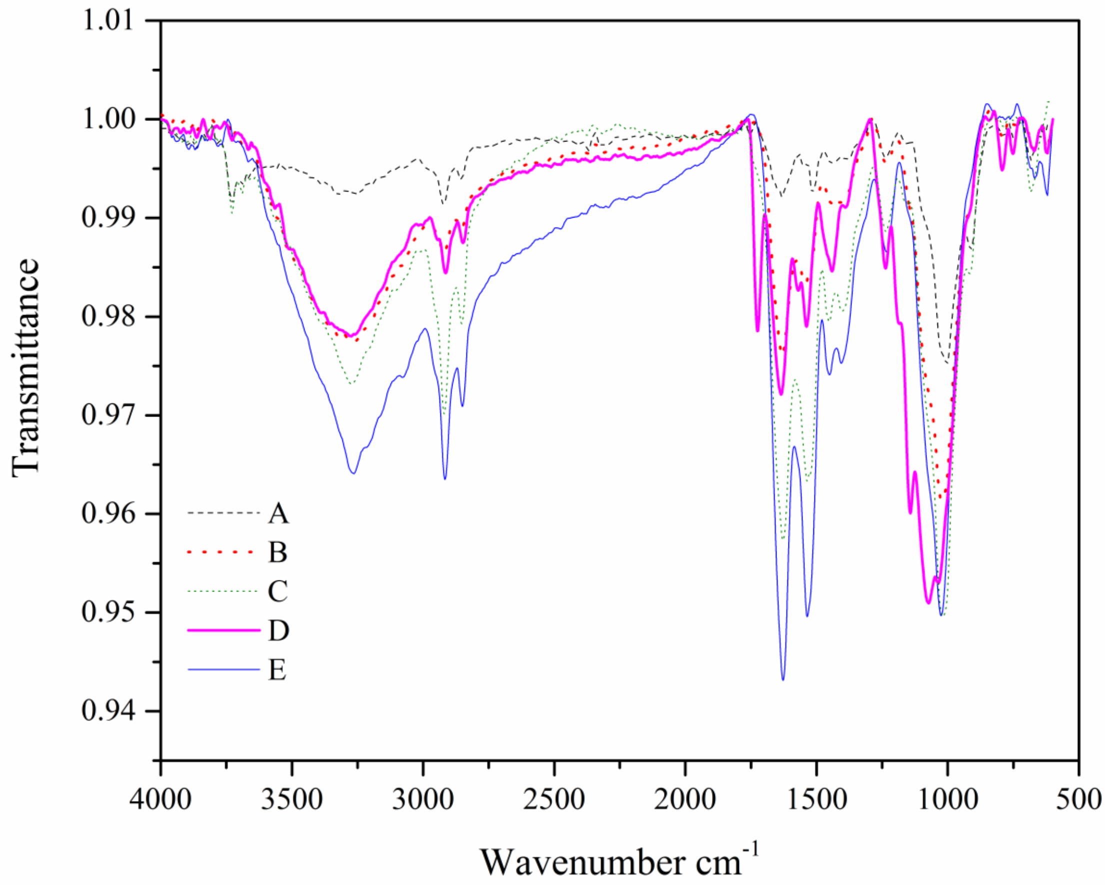

Figure 4.8 Fourier transformation infrared spectroscopy (FTIR) analysis ((A). Initial Seeded activated sludge; (B and D) suspended biomass at end of Run1 and Run2; (C and E) Attached biomass at end of Run1 and Run2.

The FTIR spectrum of initially seeded activated sludge exhibited broad absorption bands around 3253 to 3321 cm^{-1} indicating the presence of OH-stretch. The region 1800 – 700 cm^{-1} holds the absorption of two key distinctive cellular constituents: namely proteins (in the range 1700 – 1480 cm^{-1}) and carbohydrates (in

Table 4.3 Absorption bands obtained through FTIR spectrum for the suspended and attached biomass

Sl. no	Type of Biomass	Wavenumber cm-1	Functional group
1	Initial seeded activated sludge	3727.9 , 3665.9	Alcohol (O-H)
		3321.9 , 3253.1	Normal “polymeric” OH stretch
		2922.1 , 2853.9	Methylene C-H asym./ sym. stretch
		1632.2	Alkenyl C=C stretch
		1516.5	Aromatic ring stretch
		1003.0	Aliphatic fluoro compounds C-F stretch
2	Suspended Biomass Run-1	3272.8	Normal “polymeric” OH stretch
		2923.3 , 2853.7	Methylene C-H asym./ sym. stretch
		1631.5	Alkenyl C=C stretch
		1540.6	Carboxylate
		1235.4	Aromatic phosphates (P-O-C stretch)
		1021.0	Aliphatic fluoro compounds, C-F stretch
3	Attached Biomass Run-1	3264.0	Normal “polymeric” OH stretch
		2916.7 , 2850.5	Methylene C-H asym./ sym. stretch
		1627.4	Alkenyl C=C stretch
		1535.5	Aromatic nitro compounds
		1450.9	Aromatic ring stretch
		1025.3	Aliphatic fluoro compounds, C-F stretch
4	Suspended Biomass Run-2	3272.7	Normal “polymeric” OH stretch
		2913.9 , 2848.3	Methylene C-H asym./ sym. stretch
		1724.6	Aldehyde
		1635.0	Alkenyl C=C stretch
		1538.3	Carboxylate
		1441.0	Carbonate ion
		1142.7 , 1073.2	Aliphatic fluoro compounds, C-F stretch
5	Attached Biomass Run-2	3728.1 , 3689.0	Alcohol (O-H)
		3273.0	Normal “polymeric” OH stretch
		2919.7 , 2852.9	Methylene C-H asym./ sym. stretch
		1627.4	Alkenyl C=C stretch
		1235.3	Aromatic phosphates (P-O-C stretch)
		1018.0	Aliphatic fluoro compounds, C-F stretch

the range 1200 – 900 cm^{-1}). The typical bands assigned to organic compounds were observed in the range between 2900 to 2800 cm^{-1}; an aromatic nitro compound band at 1535.5 cm^{-1} was associated with Alkenyl C=C stretch at 1627.4 cm^{-1}. It was observed that protonation/carbonation hadn’t altered the reactive zones of the biomass. The normal "polymeric" OH stretch vibrations was the prominently observed band in the range 3253.1 cm^{-1} to 3321.9 cm^{-1} which mainly governed the thickness of the biofilm and its long-term stability. The phosphate solubilizing bacteria are known to increases due to the presence of aromatic phosphates (P-O-C stretch) at 1235.3 cm^{-1}. However, a continuous monitoring by FTIR analysis at regular intervals could have given a more detailed idea on the transition of groups. But due to the technical problems samples collected at the beginning and end of the experimental cycle were only subjected to FTIR analysis.

4.2.11 Post treatment using Solar and UV photocatalysis

As evident after secondary treatment the effluent was loaded with inorganic nitrogen and phosphorus which might cause eutrophication and more long-term problems because of organics (BOD & COD). After biological treatment, the values of average COD 60mg/L, TP 5mg/L and TN 10mg/L is recommended as per Ministry of Environment Forest and Climate change GOI (2015) effluent discharge standards for sewage treatment plant. Thus in order to effectively dispose or reuse the treated wastewater, secondary biological treatment should be supported by an appropriate post treatment option. In the present study as explained earlier, solar and UV photocatalysis has been tried to see its potential in meeting the standards COD: <30mg/L, TP: < 2 mg/L, TN: < 5 mg/L and MPN/100ml: < 2 as per US-EPA (2004) for the reuse of treated wastewater for industrial cooling and flushing toilets.

UV photocatalysis

Experiments were conducted on the effluents from biological reactor at various contact times and dosages as explained in section 3.2.1.6. The photocatalyzed wastewater was analyzed for various parameters like COD, BOD, MPN, and Phosphorous. The removal efficiencies obtained for COD, MPN, Phosphorous and variation in BOD/COD ratio to see the changes from non-biodegradable to biodegradable component for various irradiation times have been shown in Figure 4.9. It could be seen that, COD removal increased with increase in illumination time as well as with the dosage. Further, after a certain dosage the removal efficiency tends to reduced due to agglomeration of Titanium dioxide particles thus reducing the surface area available for effective photocatalysis. Similar behavior was observed even during MPN and Phosphorous removal.

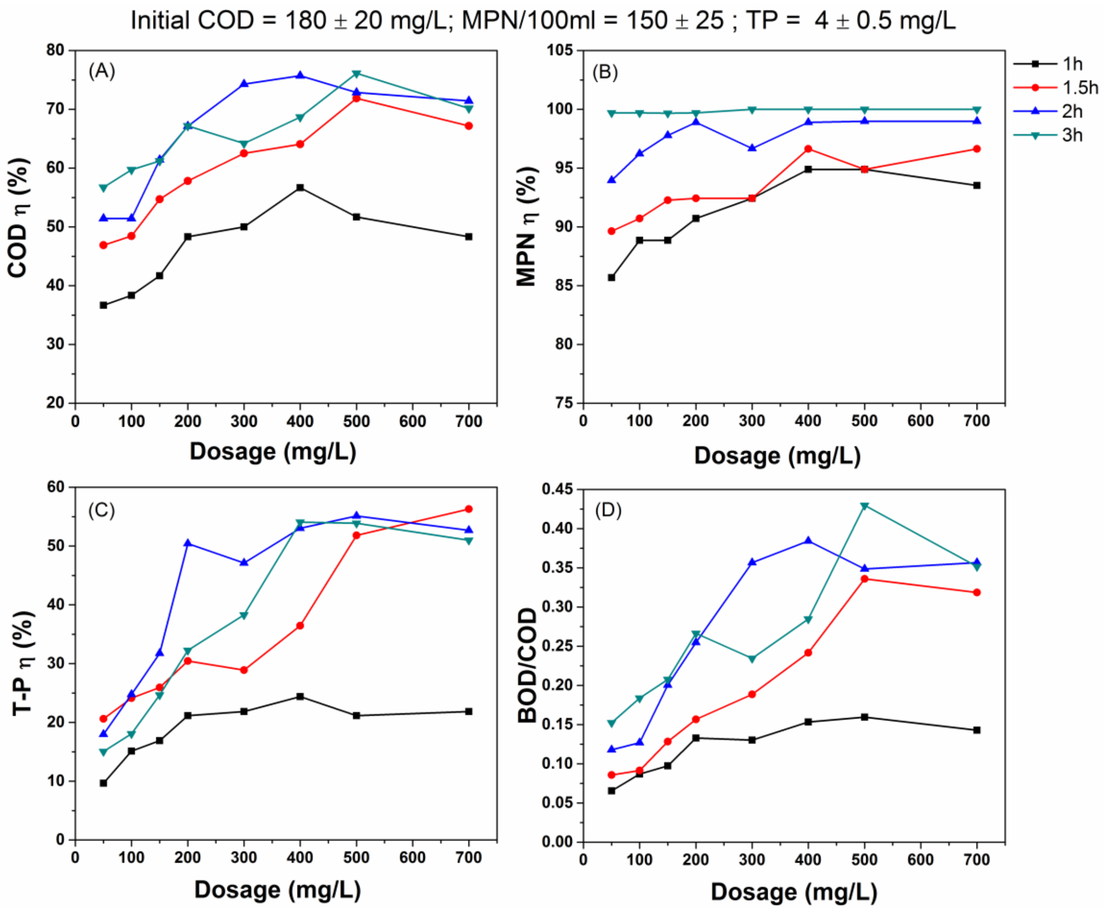

Figure 4.9 Percentage removal of (A) COD (B) MPN (C) TP (D) BOD/COD ratio Vs Dosage @ radiation time using UV photocatalysis

However, the biodegradability index i.e. BOD/COD ratio increasesd with the increase in dosage and irradiation time, but became steady thereafter. This may be attributed to the formation of biodegradable by-products. The maximum COD, MPN, and Phosphorous removal were found to be 80.32 %, 100 %, and 64.08 % after 1.5h of illumination time and the maximum increase in biodegradability was 0.43 as shown in Table 4.4 and Figure 4.9 and 4.10 respectively. The main mechanism of COD, MPN, and phosphorous removal by TiO_2 was by adsorption. Hence, the quantity of adsorbed phosphorus was higher due to titanium oxide surface that was charged positively (e.g. $TiOH_2^+$). Because, electrostatic attraction takes place between $TiOH_2^+$ and phosphate ions and this has to be taken into account with regard to adsorption (Moharami et al., 2014).

Table 4.4 Initial Characteristics of wastewater from AS- biofilm taken for post treatment and there removal efficiency

Run no.	Trial	Time (Days)	Initial concentration			UV Treatment Dosage-500mg/L, Irradiation time - 1.5h						Solar Treatment Dosage-500mg/L, Irradiation time - 3h					
			COD mg/L	MPN/ 100 ml	TP mg/L	COD mg/L		MPN/100 ml		TP mg/L		COD mg/L		MPN/100 ml		TP mg/L	
						Eff	η (%)	Eff	η (%)	Eff	η (%)	Eff	η (%)	Eff	η (%)	Eff	η (%)
1	1	0-5	74.2	168	5.4	18	75.74	0	100	2.5	53.70	23	69.00	5	97.02	2.7	50.00
	2	6-10	74.5	138	6	17	77.18	0	100	3	50.00	26	65.10	3	97.83	3.2	46.67
	3	11-15	112	112	6.2	24	78.57	0	100	2.9	53.23	38	66.07	2	98.21	2.9	53.23
	4	16-20	68.5	151	4.7	15	78.10	0	100	2.4	48.94	22	67.88	5	96.69	2.6	44.68
	5	21-25	105	135	5.1	29	72.38	0	100	2.3	54.90	30.5	70.95	3	97.78	2.6	49.02
	6	26-30	92	130	4.85	25.5	72.28	0	100	2.6	46.39	27.2	70.43	3	97.69	2.6	46.39
	7	31-35	75.3	170	5.5	21	72.11	0	100	2.5	54.55	25.2	66.53	6	96.47	2.8	49.09
	8	36-40	70.4	161	5.3	20.6	70.74	0	100	2.5	52.83	20.1	71.45	6	96.27	2.8	47.17
	9	41-45	105	136	5.4	28.2	73.14	0	100	1.9	64.81	28	73.33	4	97.06	2.2	59.26
2	10	46-50	75	178	3.85	18	76.00	0	100	1.5	61.04	22.1	70.53	7	96.07	2	48.05
	11	51-55	99	152	4.1	27	72.73	0	100	2	51.22	25.2	74.55	4	97.37	2.3	43.90
	12	56-60	155	139	4.45	30.5	80.32	0	100	2.1	52.81	42	72.90	3	97.84	2.5	43.82
	13	61-65	60	167	4.9	18.5	69.17	0	100	2.5	48.98	23.4	61.00	5	97.01	2.9	40.82
	14	66-70	111	150	3.2	26	76.58	0	100	1.4	56.25	31.1	71.98	5	96.67	1.9	40.63
	15	71-75	147	131	3.5	36.2	75.37	0	100	1.6	54.29	40.2	72.65	3	97.71	2	42.86
	16	76-80	160	182	3.3	50.2	68.63	0	100	1.4	57.58	52.1	67.44	6	96.70	1.8	45.45
	17	81-85	118	141	4.15	33.1	71.95	0	100	1.8	56.63	39.2	66.78	4	97.16	2.3	44.58
	18	86-90	134	155	4.8	29.2	78.21	0	100	2.2	54.17	36.4	72.84	5	96.77	2.5	47.92

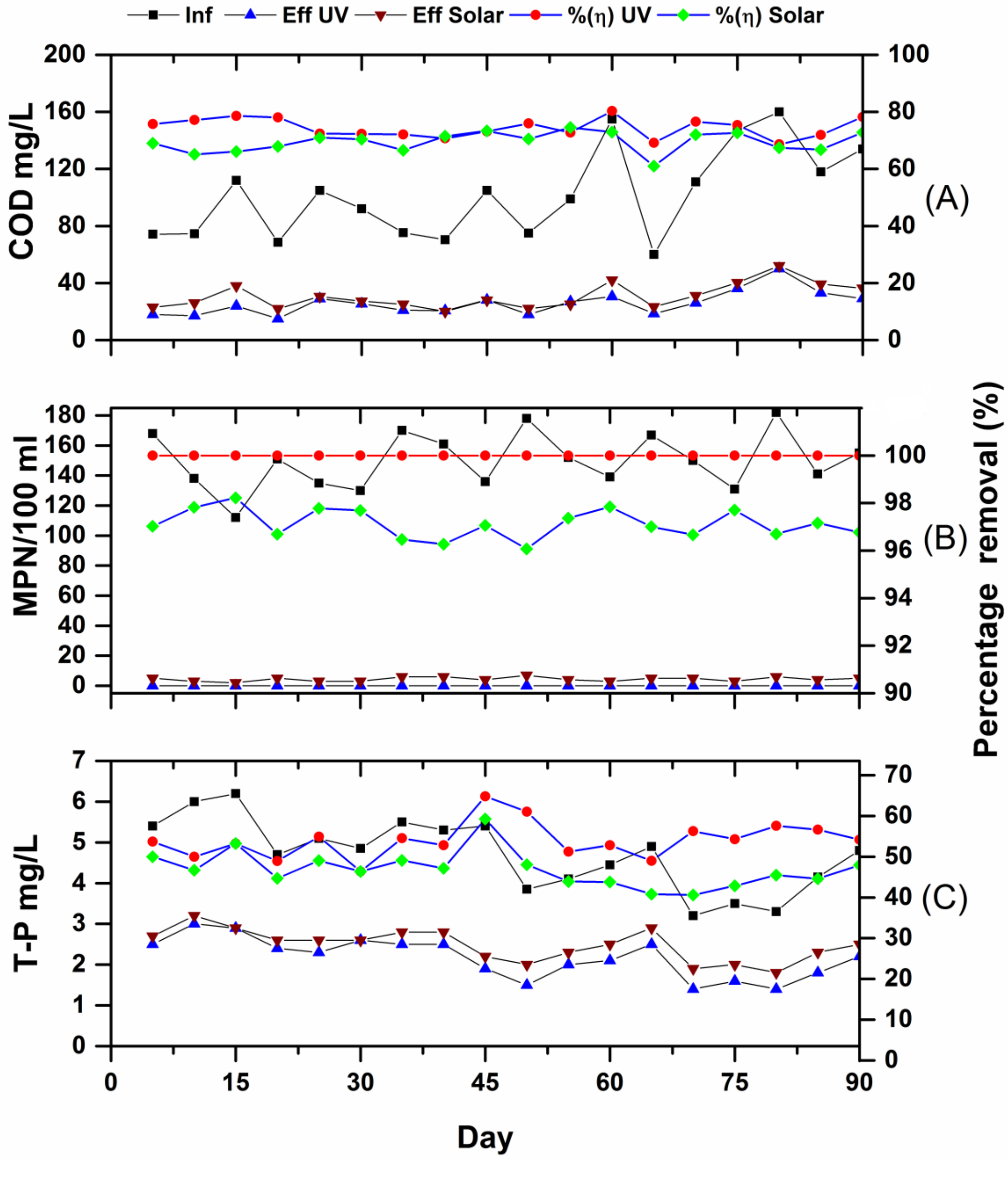

Figure 4.10 Post treatment using UV and Solar for removal of COD, MPN and TP

Solar photocatalysis

Solar photocatalysis yielded similar efficiency patterns when compared to UV photocatalysis. However, the percentage removal was slightly lesser than the former. The removal efficiencies obtained have been shown in Figure 4.10. It was observed that BOD in some cases reduced from initial concentration. This might be due to degradation of biodegradable compounds that were formed during photocatalysis after a certain limit of BOD/COD ratio. The maximum COD, MPN, and Phosphorous removal were found to be 74.55 %, 98.21 %, and 59.84 % respectively (refer to Table 4.4), after illumination for 3 hrs. Whereas, the maximum increase in biodegradability was 0.3875 as shown in Figure 4.11

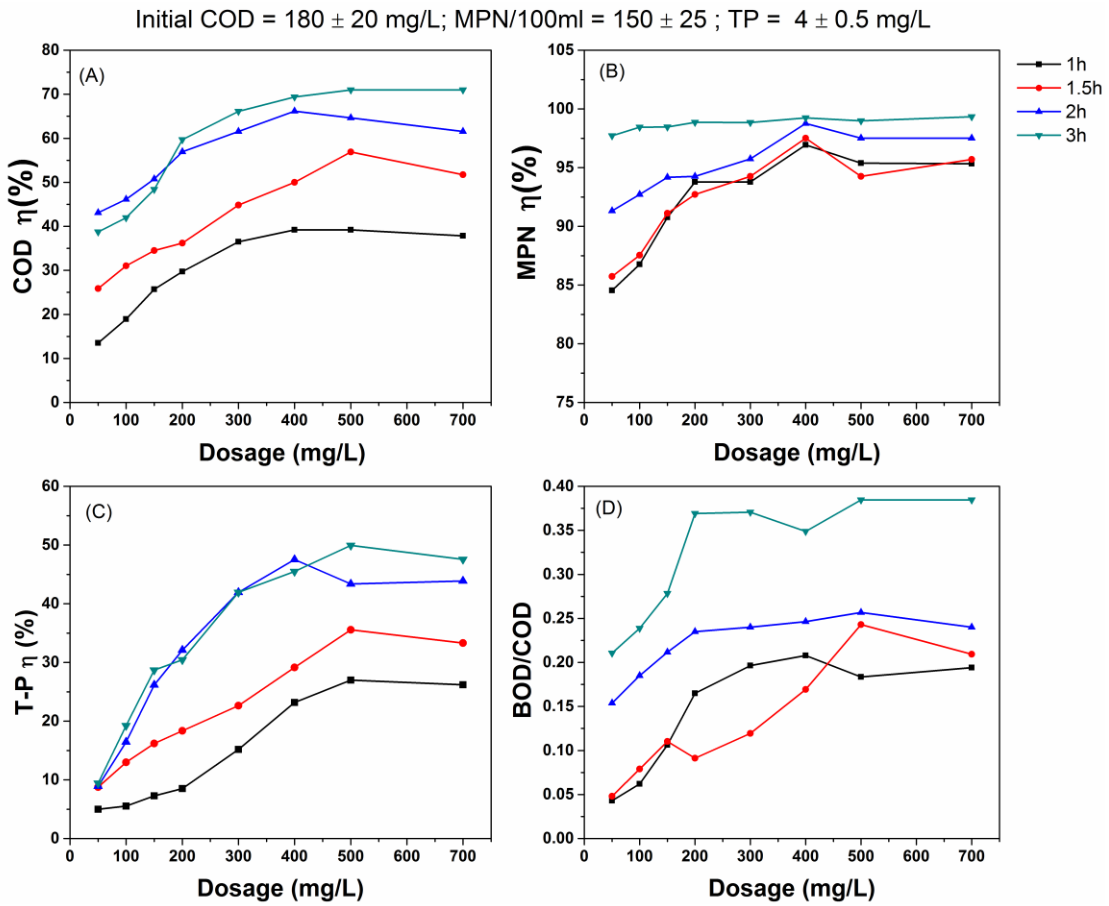

Figure 4.11 Percentage removal of (A) COD (B) MPN (C) TP (D) BOD/COD ratio Vs Dosage (radiation) time using solar photocatalysis

4.2.12 Water Quality Indices (WWQI) for UV and solar photoctalysis

The WWQI obtained for UV and solar photocatalysis have been shown in Table 4.5. Wastewater quality status corresponding to optimized dosage of UV photocatalyst and illumination time, was 500 mg/L and 90 min respectively and is regarded as 'Good'. The COD, MPN and Phosphorous removal for the optimal combination were 71.88 %, 94.89 %, and 51.8 % respectively. Similarly, the calculated results showd that, dosage of 500 mg/L and irradiation time of 180 min were the optimized values with respect to solar photocatalysis. The wastewater quality status corresponding to WWQI obtained was 'Average'. The COD, MPN and Phosphorous removal for the optimal combination were 74.55 %, 98.21 %, and 59 % respectively.

Table 4.5 WWQI values for UV and Solar photocatalysis

UV photocatalysis				**Solar photocatalysis**			
Time (min)	Dosage (mg/l)	WWQI	Status	Time (min)	Dosage (mg/l)	WWQI	Status
90	500	48.24	Good	180	400	82.53	Poor
120	300	66.75	Average	180	500	77.32	Average
120	400	58.89	Average	180	700	78.37	Average
120	500	63.95	Average	-	-	-	
180	500	52.54	Average	-	-	-	

4.2.13 Recovery studies

The removal efficiencies of COD, MPN and Phosphorous were monitored after using recycled photocatalyst and the WWQI was calculated to understand the performance of recycled titanium dioxide. The results for WWQI after recycling in case of UV and solar photocatalysis have been tabulated in Table 4.6. From the results, it could be clearly observed that, in case of solar as well as UV photocatalysis, the wastewater quality status louvered as the number of cycles of recycling increased. This might be because of adsorption of contaminants over the surface of titanium dioxide. The efficiency after recycling can be increased by proper washing of catalyst powder after centrifuging. Also, mixing of new catalyst powder in some proportion with recycled photocatalyst can help to improve the removal efficiency.

Table 4.6 WWQI values obtained after recycling for UV and Solar photocatalysis

Cycle	UV Photocatalysis		Solar Photocatalysis	
	WWQI	Wastewater quality status	WWQI	Wastewater quality status
1	53.62	Good	72.57	Average
2	71.64	Average	99.1	Poor
3	88.3	Poor	130.95	Very poor

4.3 Performance of A^2O-AS-biofilm reactor connected in series for organic matter and nutrient removal (Phase 2).

Operating conditions of the reactor were varied according to the experimental design as explained in 3.1 and accordingly the variation of various factors have been shown in *Col. 4-7, Table 3.4*. The complete experimental details have been broadly reported as Run1 and Run 2.

4.3.1 COD removal

The COD removal rate of A^2O-AS-biofilm reactor system during Run 1 and Run 2 are shown in Figure 4.12. Experimental results indicate a slight decrease in the COD removal efficiency with a decrease in HRT. The COD removal efficiency ranged between 85.71-96.1 % in Run 1 and 88.3-95.5% in the Run 2 and infers that Run1 shows slightly better COD removal than Run 2. The maximum COD removal efficiency of 96.1% in the Run 1 was observed in the trial having a C/N ratio of 4, HRT of 10hr, DO within 1.5-2.5 mg/L and biomass concentration(X) in the range 3-3.5g/L. The COD removal efficiency in the individual reactors was also analysed. For the above mentioned, optimal trial of Run 1, the COD removal efficiencies in the individual anaerobic, anoxic and aerobic reactors were 51.34%, 12.34%, and 31.89% respectively. Similarly, the maximum COD removal efficiency of 95.53% in the Run 2 was observed in the trial having a C/N ratio of 10, HRT of 8hr, DO within 3.5-4.5 mg/L and biomass concentration (X) in the range 3-3.5g/L. For this optimal trial of Run 2, the COD removal efficiencies in the individual anaerobic, anoxic and aerobic reactors were 55.2%, 9.39%, and 30.94% respectively.

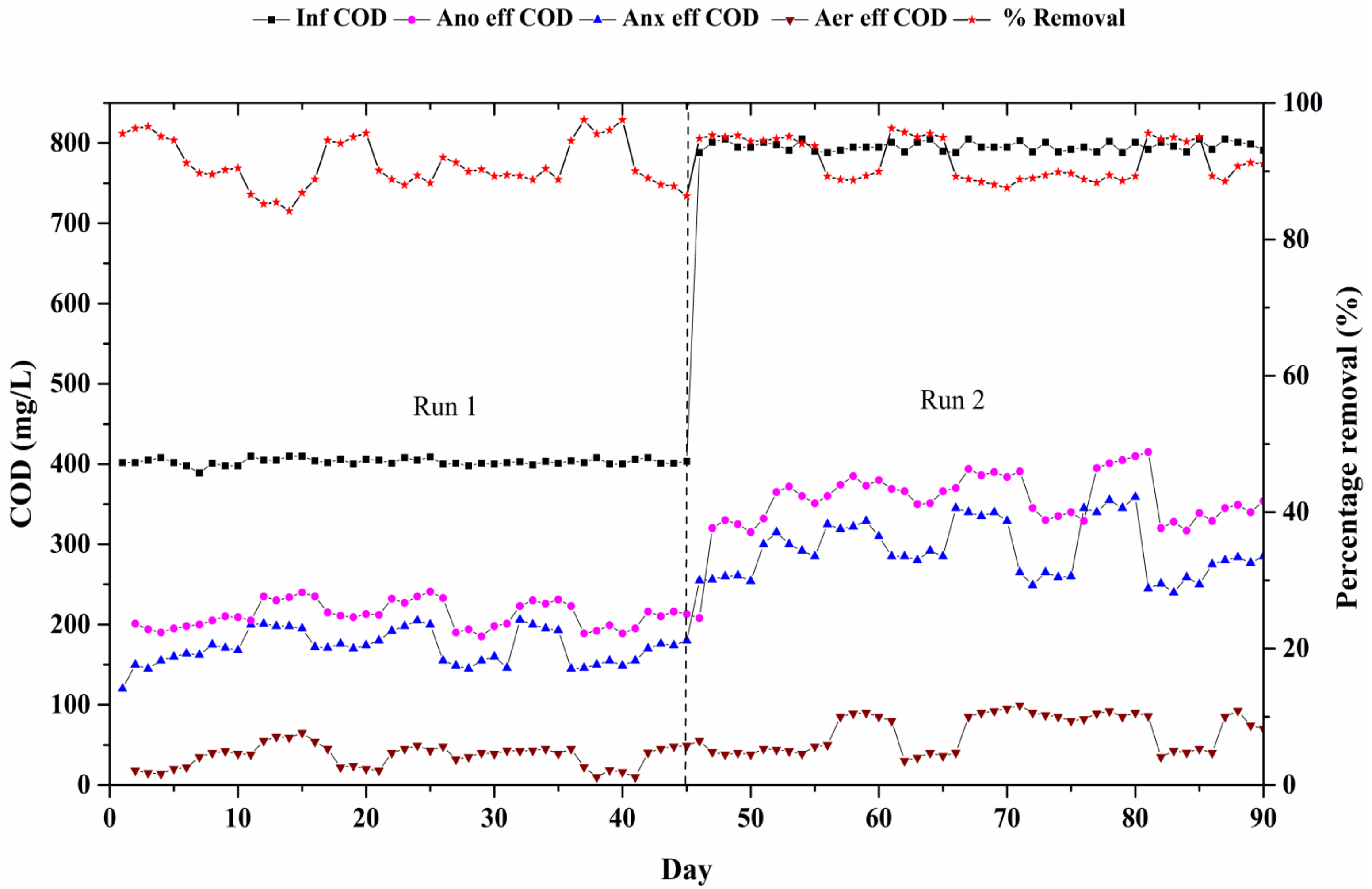

Figure 4.12 COD removal during 90 days operation of A^2O-AS-biofilm reactor

Similar results were observed in the study carried out by Nam et al., (1998) where the COD removal efficiencies ranged between 90.5-97.5%. Su & Ouyang (1996) also observed about 89.7-91.3% COD removal efficiency in a combined process having activated sludge and fixed biofilm at HRT 8-12 hr. The COD removal occured due to a huge population of anaerobic bacteria in the anaerobic reactor; whilst in the anoxic reactor, the denitrification process instigates the COD reduction and finally the residual COD reduction has been observed in the aerobic reactor due to organic matter oxidation by heterotrophic bacteria and phosphorus-accumulating bacteria.

4.3.2 Total Phosphorous removal

Figure 4.13 depicts the changes in the TP concentration in Run 1 and Run 2. The TP removal efficiency ranged between 72.18-93.14% in the Run 1 and 75.96-90.38% in the Run 2 and it infered the high rate of TP removal in the Run 1 than that in Run 2. The maximum TP removal efficiency of 93.14% in the Run 1 was observed in the trial having a C/N ratio of 4, HRT of 10hr, DO within 1.5-2.5 mg/L and biomass concentration (X) in the range 3-3.5g/L. Su & Ouyang (1996) have also observed about 68.0-98.0 % of TP removal efficiency at HRT 8-12 hr in a combined process with activated sludge and fixed biofilm reactor. The TP removal efficiency in the individual reactors was also analyzed. For the above mentioned, optimal trial of Run 1, the TP removal efficiencies in the individual anaerobic, anoxic and aerobic reactors were 17.64%, 44.11%, and 31.39% respectively. Similarly, the maximum TP removal efficiency of 90.38% in the Run 2 was observed in the trial having a C/N ratio of 10, HRT of 8hr, DO within 3.5-4.5 mg/L and biomass concentration (X) in the range 3-3.5g/L. For this optimal trial of Run 2, the TP concentration in anaerobic reactor increased by about 1.54% on an average in all the runs which could be from the recycled sludge which contained adsorbed phosphorous in it, leading to an increase in concentration in anaerobic reactor with less or no TP removal.

However, TP removal efficiency in the anoxic and aerobic reactors were 50.96%, and 39.42% respectively. During the anaerobic phase due to the sludge recirculation phosphorus may get released into the liquid by phosphorus-accumulating bacteria like the Acinetobactor species etc. there by increasing phosphorous content in the sample of the anaerobic reactor (Kerrn-jespersen 1994). In the anaerobic reactor the microorganisms uptake carbon from the influent feed and store it as polyhydroxybutyrate (PHB). In the subsequent anoxic and aerobic reactor, the degradation of these stored PHBs occurs for glycogen restoration and thus phosphorus is removed.

4.3.3 Total Nitrogen removal

Figure 4.14 and 4.15 shows the concentrations of NH_4^+-N and TN, for Run 1 and Run 2. The influent NH_4^+-N concentration varied between 30 to 50 mg/L throughout the study. The effluent NH_4^+-N concentration and its removal efficiencies in Run 1 and Run 2 of the anaerobic reactor were 15.5 mg/L, 53% and 12 mg/L, 55% respectively. Dilution by the external recirculation and adsorbed cells from cell layers by anaerobic bacteria and attached biofilm on the surface of the media cause the reduction of concentrates in the anaerobic reactor (Lee et al. 1998; Nam et al.1998). Similarly, the effluent NH_4^+-N concentrations and its removal efficiencies in Run 1 and Run 2 was the effect of internal and external recycle dilution and cell synthesis, partly in the anoxic reactor. The major part of the NH_4^+-N removal takes place in the aerobic reactor and the fraction of NH_4^+-N removed is caused by nitrification of nitrifiers and assimilation by both heterotrophic and autotrophic bacteria in aerobic reactor. The effluent NH_4^+-N concentration in the aerobic reactor was 0.9 mg/L and 0.5mg/L and its removal efficiencies were 18% and 20% during Run 1 and Run 2 respectively. The maximum overall NH_4^+-N and TN removal efficiencies were 98%, and 80% during Run 1 and were observed form the trail having a C/N ratio of 4, HRT of 10h, DO in the range of 1.5 to 2.5 mg/L and biomass concentration (X) in the range of 3-3.5 g/L. The maximum overall NH_4^+-N and TN removal efficiencies were 99%, 82.17% during Run 2 corresponding to the trail having C/N ratio of 10, HRT of 8hrs, DO in the range of 3.5 to 4.5 mg/L and biomass concentration (X) in the range of 3-3.5 g/L. Similar results were observed by Xu et al. (2011) in a novel anaerobic/aerobic/anoxic (AOA) process without media, the average removal efficiencies of NH_4^+ -N, and TN and were 93.0 ± 3.1% and 70.3 ± 2.9% respectively.

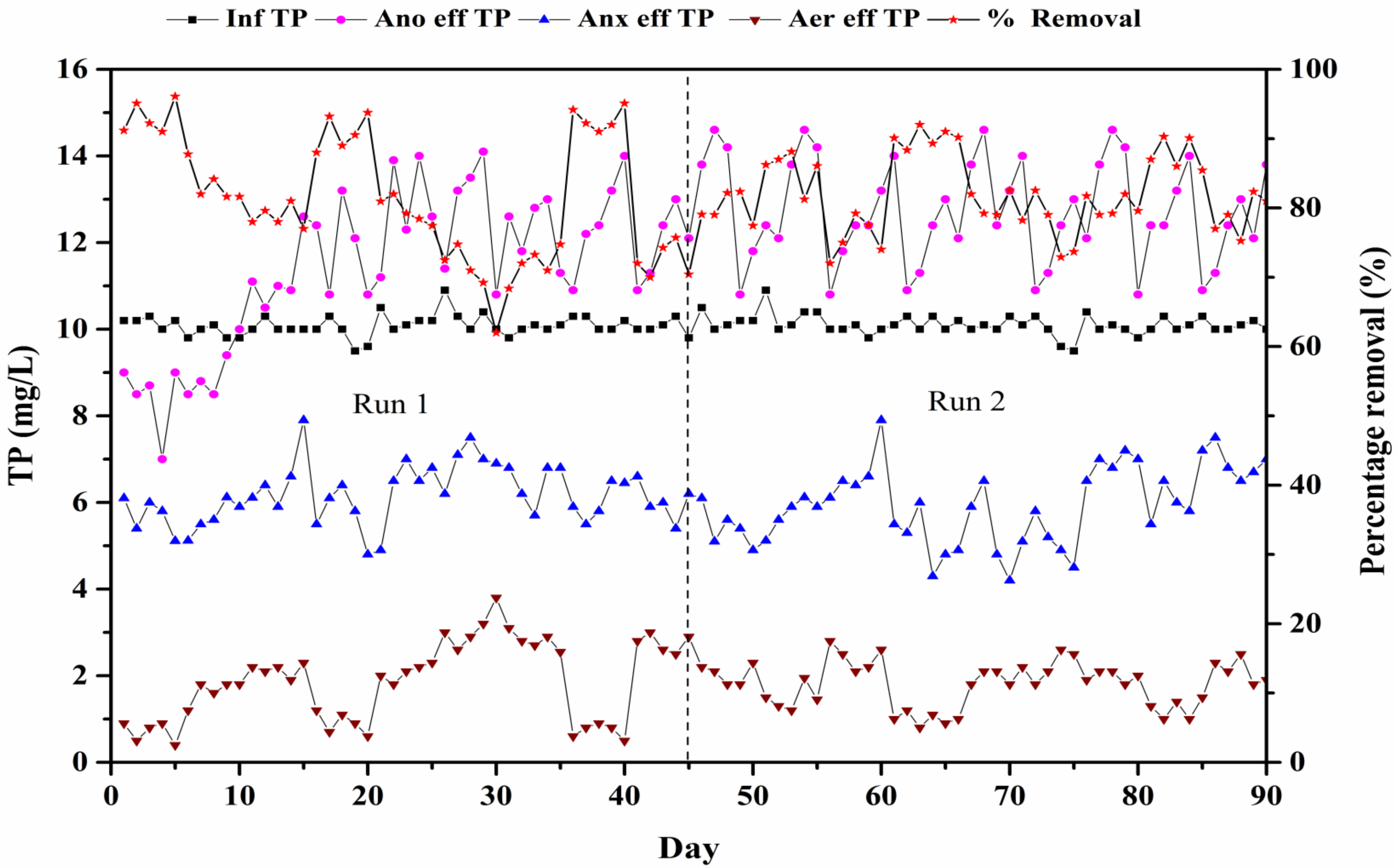

Figure 4.13 TP removal during 90 days operation of A^2O-AS-biofilm reactor

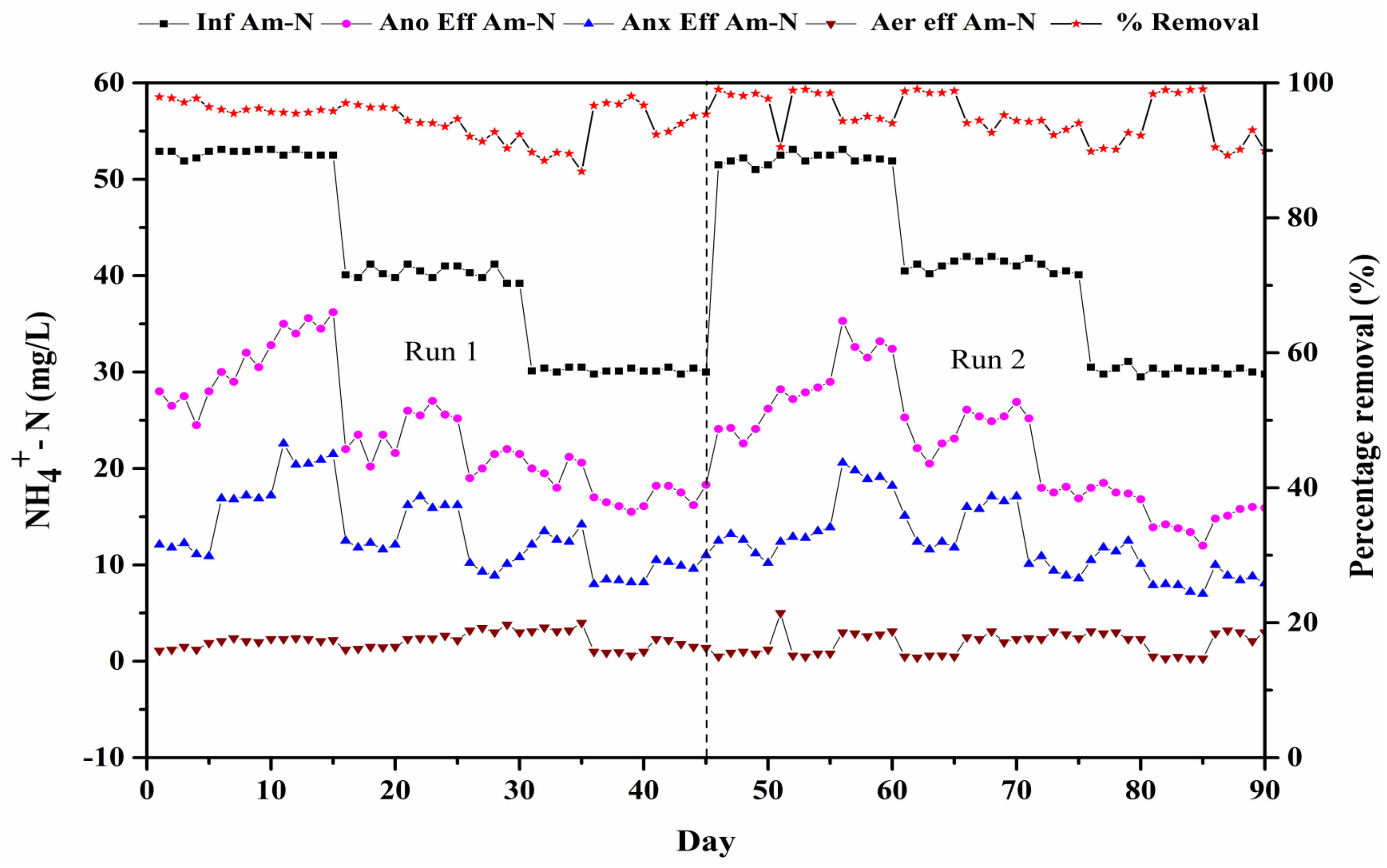

Figure 4.14 NH_4^+-N removal during 90 days operation of A^2O-AS-biofilm reactor

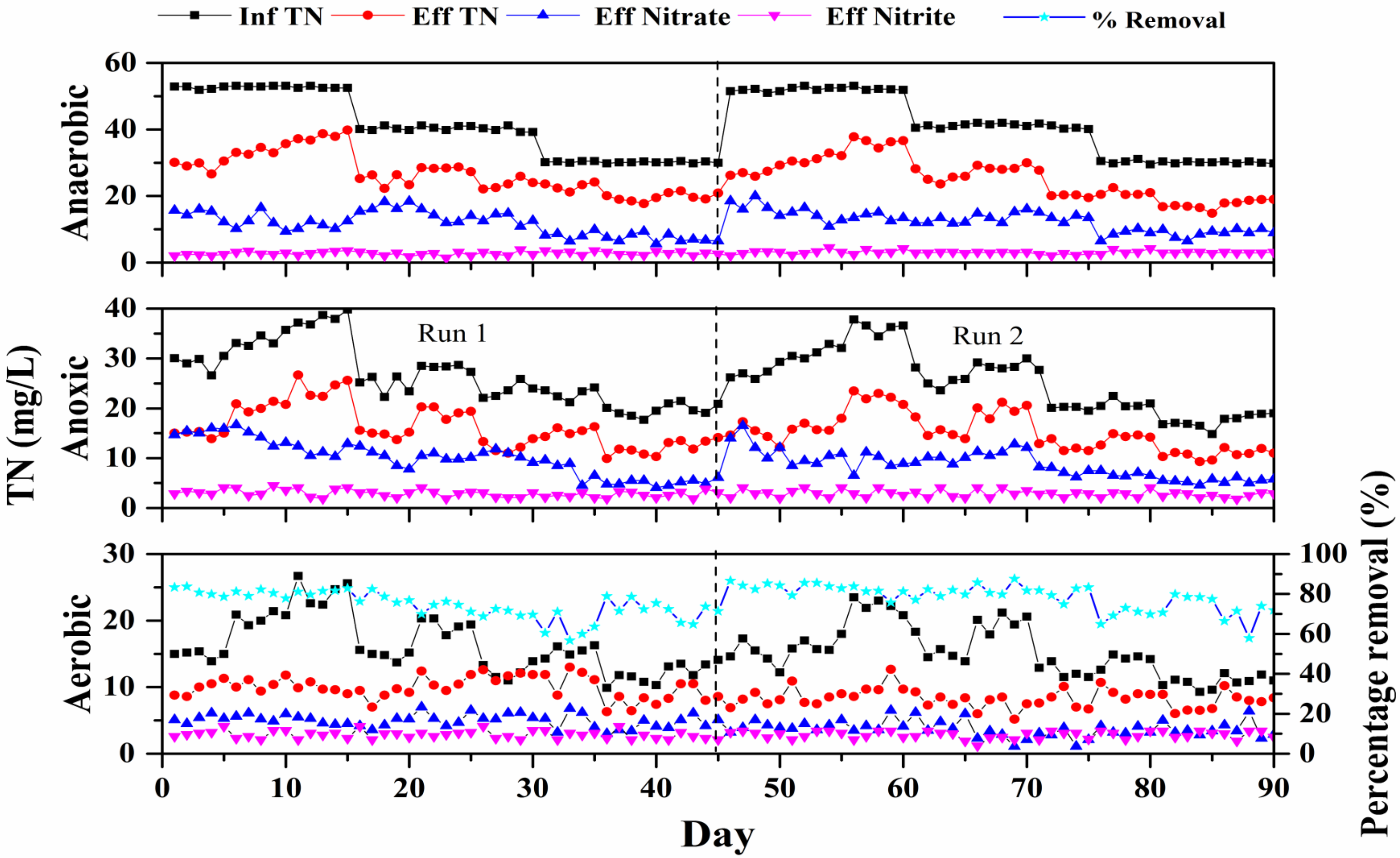

Figure 4.15 TN removal during 90 days operation of A²O-AS-biofilm reactor

4.3.4 Microbial study for attached biofilm and suspended biomass

SEM analysis was carried out to analyze the morphological characteristics of suspended and attached biofilm of the series of reactors at the end of the experimental cycle. In the suspended and attached biofilm, different kinds of Bacillus and Cocci bacteria of diameter ranging from 0.3 to 1.2 μm and some dense layers of extracellular polymeric substance (EPS) comprising proteins and carbohydrates were observed on the surface of biofilm as shown in Figure 4.16. Some micro-pores and passages which aid for sufficient DO circulation within the biofilm were also noticed. The surface suspended sludge had sloppier bacterial layers on their surface than that on the biofilm. In figure 4.16(A) of suspended anaerobic sludge, microporous gel layers with scattered deposition of microbial cells, similar to *Streptococcus pneumoniae* or *Neisseria gonorrhoeae* were noticed. A bacillus similar to *Pseudomonas aeruginosa* along with cocci colonies was observed in the microstructure of anoxic suspended sludge (figure 4.16C). The floc-like colony-forming ciliated protozoa communities along with several bacilli were observed in the microstructure of aerobic suspended sludge (figure 4.16E).

Various forms of facultative anaerobic coccus appearing in pairs, bead-like chains, or bunched (such as *Streptococci, Citrobacter, Enterococci* etc.) were the chief microbes over the surface of the anaerobic attached biofilm (figure 4.16B). Several coccobacilli typically rod-shaped along with few staphylococci were observed on the surface of the anoxic and aerobic attached biofilm (figure 4.16D and figure 4.16F). These rod-shaped bacteria consume nutrients and also store them in their intracellular spaces. Dominant heterotrophic bacteria such as *Pseudomonas, Coccus, Bacillus*, etc. are highly correlated to COD, TN and TP removal (Lin et al. 2008). The bacteria such as *Bacillus, Neisseria gonorrhea, Citrobacter* etc. are highly effective in mineralization and solubilizing organic phosphate.

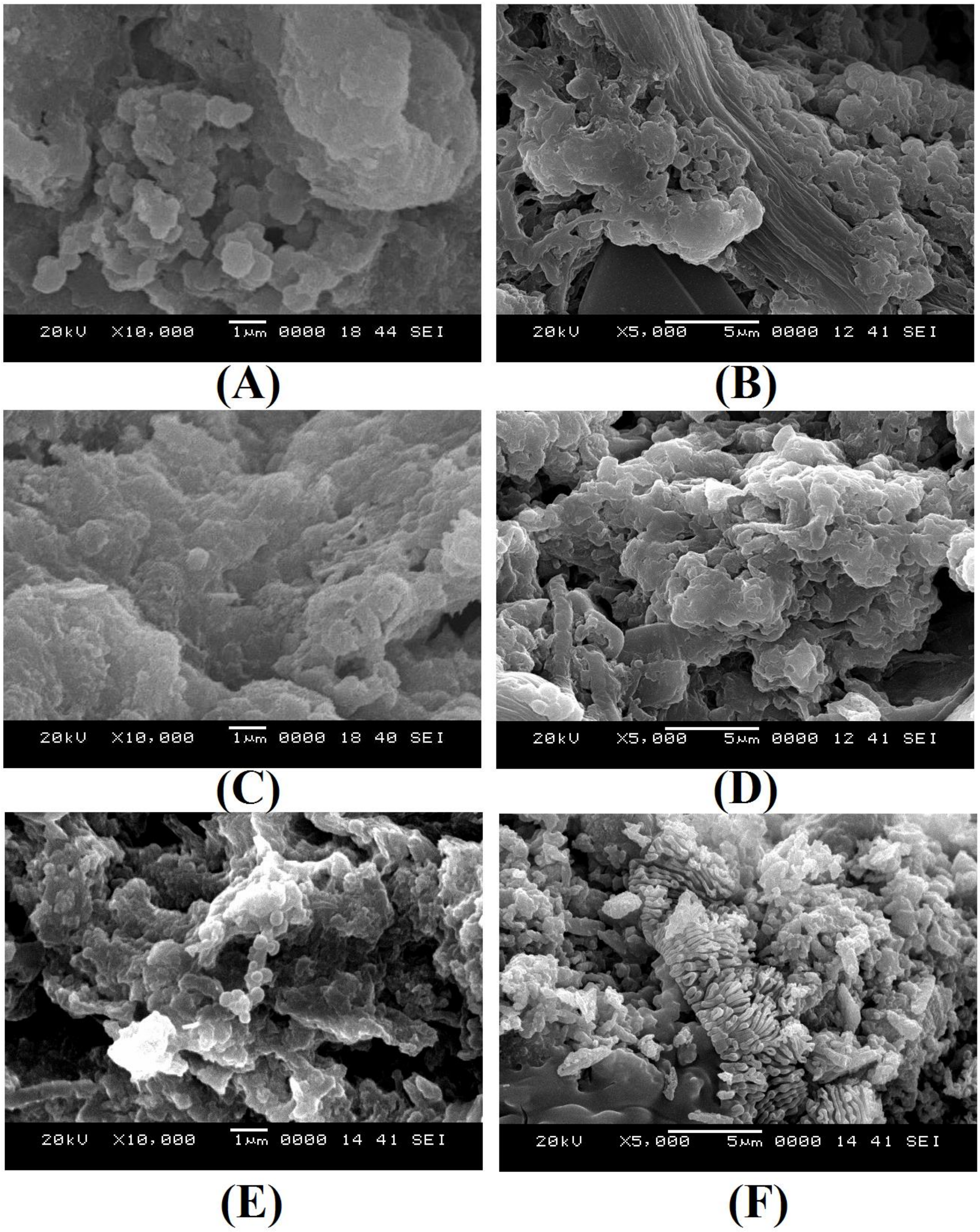

Figure 4.16 SEM images of A^2O-AS-biofilm reactor (anaerobic suspended sludge (A); anaerobic attached biofilm (B); anoxic suspended sludge (C); anoxic attached biofilm (D); oxic suspended sludge (E); oxic attached biofilm (F)

4.3.5 FTIR analysis of suspended biomass and attached biofilm

The FTIR spectroscopy was performed to identify the organic and inorganic functional groups present in the suspended and attached biofilm of the reactor (refer figure 4.17 and Table 4.7). Each microbial genus has a compound cell wall which gives a unique IR fingerprint due to the stretching and bending vibrations of molecular bonds present in its proteins, lipids, nucleic acids, lipopolysaccharides (LPS) and sugars. The band 3750- 3650 cm^{-1}corresponds to the O-H and N-H groups of alcohol. Kamnev (2008) discussed that similar O-H and N-H vibrations in the region between 3700-3300 cm^{-1} for these amino and hydroxyl groups showed the presence of polysaccharides which aided in metal adsorption to a significant level owing to negatively charged sol-gel coated particles present in polysaccharides (Davis maMauer 2010).

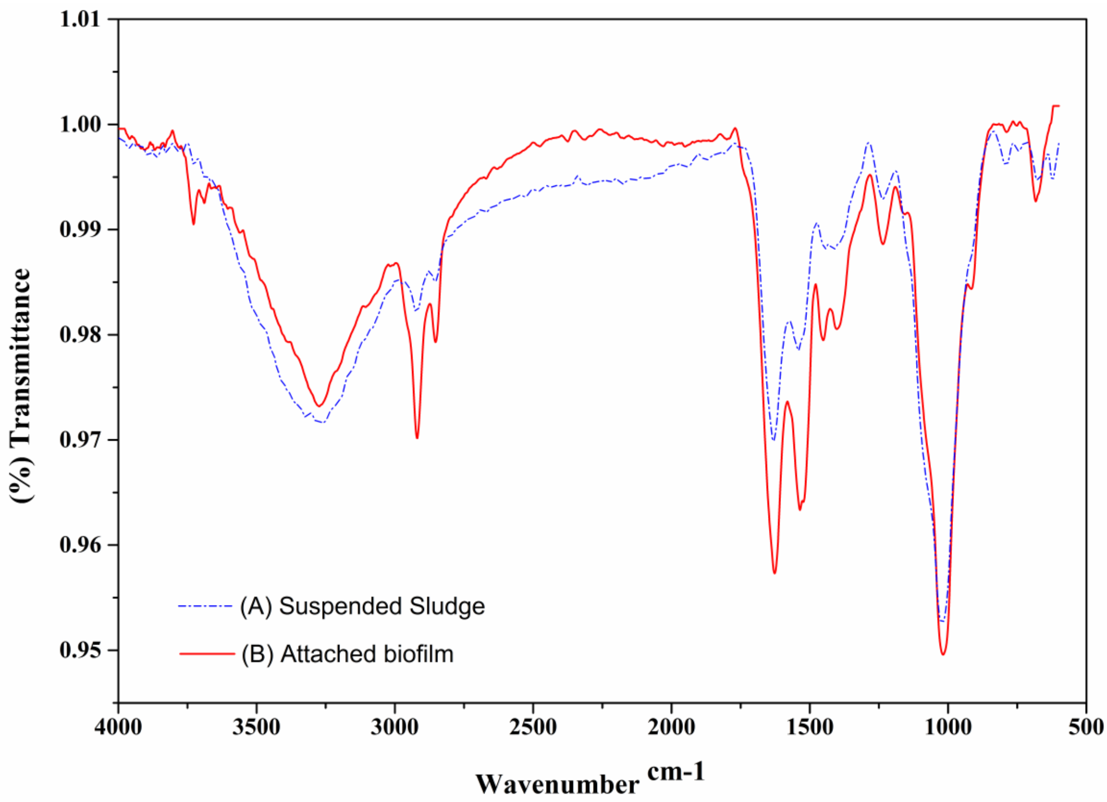

Figure 4.17 Fourier transformation infrared spectroscopy (FTIR) analysis of suspended sludge and attached biomass.

Table 4.7 Absorption bands obtained through FTIR spectrum for the suspended and attached biomass

	Type of Biomass	Wavenumber cm-1	Functional group interpretation and comments
1	Suspended sludge	3272	Normal "polymeric" OH stretch H-bonding (due to water) and possible metal binding of amide groups in proteins
		1631	Alkenyl C=C stretch
		1540	Carboxylate
		1021	C-0, C-C, C-O-C and C-0-P. These groups mainly occur in carbohydrates and cellular polysaccharides in the fungal biomass.
2	Attached Biomass	3765	Alcohol (O-H)(N-H) These hydroxyl groups especially present in all polysaccharides can become negatively charged thereby contributing the metal adsorption to a significant level
		3270	Normal "polymeric" OH stretch H-bonding (due to water) and possible metal binding of amide groups in proteins
		2925	Methylene C-H asym./ sym. Stretch
		1628	Alkenyl C=C stretch
		1536	Aromatic nitro compounds
		1018	C-0, C-C, C-O-C and C-0-P stretching vibrations of polysaccharides. These groups mainly occur in carbohydrates and cellular polysaccharides in the fungal biomass.

The band 3200 cm^{-1} relates to the normal polymeric O-H stretch vibration of polysaccharides and H-bonding (due to water) which usually supports binding of phospho-amides and proteins. The peaks at 2950-2850 cm^{-1} endorse the methylene C-H stretch and aromatic ring stretch vibrations which are typically the long-chain carbon-carbon stretching vibrations of -CH3, -CH2, CH and CHO functional groups (Simonescu 2012). The strong peak at 1630 cm^{-1} was assigned to be the alkenyl C-C stretch vibration of the carboxylate group of proteins (Byler & Susi 1986). The peak 1500 cm^{-1} indicated a few carbohydrate binding proteins of exopolysaccharides mainly belonging to the C-H bending vibrations (Wolkers et al. 2004). The band 1020 cm^{-1} is associated with C-O-H, C-O-C and C-C-O vibrations associated with accumulation of polyesters. The region from 1200 to 900 cm^{-1} show a sequence of bands caused by microbial cells particularly the C-O, C-C, C-O-C and C-O-P stretch (Davis & Mauer 2010). These groups majorly transpire in biomass which helps in digesting carbohydrates and intra-cellular proteins

4.4 Summary

Laboratory studies were conducted on aerobic hybrid reactors in two phases with objectives i) effect of C/N ratio, HRT, Biomass (X) and DO on AS-biofilm reactor for removal of nutrients followed by post treatment using UV and solar photocatalysis ii) effect of C/N ratio, HRT, Biomass (X) and DO on A^2O-AS-biofilm reactor.

Phase -1 Performance of AS-biofilm reactor

It is observed that the overall percentage removal efficiency of COD, NH_4^+-N, TN and TP increases with increase in C/N ratio, HRT and Biomass (X) concentration. When there was increase in the percentage removal of TN was higher ethanol of other parameters. Fourier transformation and infrared spectroscopy results indicated the presence of extra cellular aromatic and aliphatic protein like substance. In order to effectively dispose or reuse the wastewater treated by secondary biological process, UV and solar photocatalysis have been tried as post treatment option to see their potential in meeting standards for reuse. Experimental results shows that COD, MPN, and TP removal efficiencies increased with increase in irradiation time and catalyst dosage. However after certain dosages removal efficiency reduce due to the agglomeration of titanium dioxide particles thus reducing the surface area for the effective photocatalysis. UV photocatalysis was found to be better than solar photocatalysis during comparative analysis.

Phase -2 Performance of A^2O-AS-biofilm reactor connected in series

Experiments were conducted on a three reactor assembly connected in series. It has been observed that the increase in C/N ratio (4 - 13.3), there was no significant change in percentage removal of COD, NH_4^+-N, TN and TP. The variation in HRT significantly influenced the performance of A^2O-AS-biofilm reactor; removal efficiency increases with increase in HRT. Scanning electron microscopy of suspended biomass and attached biofilm showed dense bacterial structure of cocci and bacillus microorganisms ranging from 0.3 to 1.2 µm. The FTIR results indicated phosphorylated macromolecules and carbohydrates mixed or bound with extracellular proteins in exopolysaccharides.

4.5 Comparison study

Comparison between AS-biofilm, AS-biofilm reactor with UV-Photocatalysis, AS-biofilm reactor with solar photocatalysis and A^2O-AS-biofilm reactor, in terms of nutrient removal is shown in Table 4.8. The maximum removal efficiency of COD, NH_4^+-N, TN and TP were 82%, 93%, 83% and 47% respectively at C/N ratio 6.67 and HRT 10hr. Further to improve the treatment performance of AS - biofilm reactor followed by UV and solar photocatalysis as a post treatment. The results shows that the maximum removal efficiency COD, MPN and TP in AS - biofilm + UV photocatalysis and AS- biofilm + solar photocatalysis were 98%, 100% and 75%; 97%, 97% and 73% respectively. Similarly the performance of A^2O-AS-biofilm reactor, in terms of nutrient removal was studied. The results shows that maximum removal efficiency of COD, NH_4^+-N, TN and TP were 96%, 98%, 82% and 93% respectively C/N ratio 4 and HRT 10hr. From the results A^2O-AS-biofilm reactor shows better performance than AS-biofilm reactor with UV and solar photocatalysis.

4.6 Cost analysis

Cost analysis for lab - scale AS-Biofilm ractor, A^2O - AS - bioflm ractor, AS - biofilm reactor + UV photocatlysis and AS- bioflm reactor + Solar photocatalysis for nutrient removal is shown in Table 4.9. However, the degree of secondary and tertiary treatment (post treatment) depends upon the intended use.

1) Cost requred for treating 1L wastewater using AS-biofilm reactor = 0.064 rupees
2) Cost requred for treating 1L wastewater using A^2O - AS - biofilm reactor = 0.161 rupees
3) Cost requred for treating 1L wastewater using AS - biofilm reactor + UV photocatalysis = (0.064 + 0.171 + 0.528) = 0.76 rupees
4) Cost requred for treating 1L wastewater using AS - biofilm reactor + Solar photocatalysis = (0.064 + 0.198 + 0.528) = 0.79 rupees

Table 4.8: Comparission between AS-biofilm ractor, A^2O - AS - biofilm ractor, AS - biofilm reactor + UV photocatlysis and AS-bioflm reactor + Solar photocatalysis for nutrient removal.

Reactor	Run no	Hybrid reactor Operating conditions	Post treatment Operating Condition	% Removal				
				COD	NH_4^+-N	TN	TP	MPN
AS - Biofilm	1	C/N -6.67, HRT-10hr, (X)- 2 to2.5 g/L DO- 2 to 4 mg/L	-	82	93	82	47	-
	2	C/N -10, HRT-8hr, (X)- 2 to2.5 g/L DO- 2 to 4 mg/L	-	93	94	87	53	-
A^2O - (AS - Biofilm)	1	C/N - 4, HRT- 10hr, (X) - 2 to2.5 g/L, DO - 2 to 4 mg/L	-	95.6	97.9	80.2	93.1	-
	2	C/N -10, HRT-8hr, (X)- 2 to2.5 g/LDO- 2 to 4 mg/L	-	95.5	98.8	82.2	90.4	-
AS-Biofilm + UV Photocataysis	1	C/N -6.67, HRT-10hr, (X)- 2 to2.5 g/L DO- 2 to 4 mg/L	Dosage-500mg/L, Irradiation time- 1.5 hours	95	-	-	75.7	100
	2	C/N -10, HRT-8hr, (X)- 2 to2.5 g/L DO- 2 to 4 mg/L		97.7	-	-	75.0	100
AS-Biofilm + Solar photocataysis	1	C/N -6.67, HRT-10hr, (X)- 2 to2.5 g/L DO- 2 to 4 mg/L	Dosage-500mg/L, Irradiation time-3 hours	95.0	-	-	72.8	96.3
	2	C/N -10, HRT-8hr, (X)- 2 to2.5 g/L DO- 2 to 4 mg/L		97.1	-	-	71.0	97.1

Table 4.9: Cost analysis of lab - scale AS-biofilm reactor, A^2O - AS - bioflm reactor, AS - biofilm reactor + UV photocatlysis and AS- bioflm reactor + Solar photocatalysis for nutrient removal.

Sl. No	Parameters	AS - biofilm reactor 45 L/day		UV photocataysis		Solar photocataysis		A^2O - AS- biofilm reactor 27 L/day	
1	**Capital cost**								
		Nos.	Price (Rs)	Nos.	Price (Rs)	Nos.	Price (Rs)	Nos.	Price (Rs)
	Reactor Fabrication	1	15000.00	1	1000.00	1	1000.00	1	12000.00
	Stirer	1	19000.00	1	5500.00	1	5500.00	3	16500.00
	Peristaltic pump	1	55000.00	-	-	-	-		55000.00
	Diffusers	2	500.00	-	-	-	-	1	250.00
	Carrier media	300	1500.00	-	-	-	-	200	1000.00
	Total		**91000.00**		**6500.00**		**6500.00**		**84750.00**
2	**Maintenance cost**								
	Power cosumption (W)/L*	10.6	0.064	28.5	0.171	33	0.198	26.96	0.161
	Chemicals TiO_2 gram/L	-	-	0.5 g	0.528	0.5g	0.528		-

Note*:1000W = 1Unit = 10rupees

CHAPTER 5

SOFT COMPUTING TOOLS FOR MODELING HYBRID SYSTEMS

5.1 General

Improper maintenance of WWTP can trigger serious ecological and public health problems and also it may be a reason for various water borne diseases affecting human health and aquatic life. Nitrogen and phosphorous are the key nutrients supporting the growth of algae and organic matter which instigate eutrophication in water bodies. Various control actions have to be implemented for efficient monitoring of process performance during the operation of Wastewater Treatment Plant (WWTP) (Boelee et al., 2011). Many modeling studies have attempted to capture this tightly regulated system, and three common approaches are compartmental or mathematical, simulation and stochastic models. Some of the disadvantages of mathematical models are requirement of huge input variables and computational efforts due to the complex nature of the wastewater treatment systems. It may further aggravate the complexity if combined with faulty or hidden assumptions and may over or under predict the performance of systems. A model that oversimplifies may inaccurately reflect the real world situation. So soft computing techniques are gaining importance in real time operation of wastewater treatment plants these days.

Though the objective of the present thesis work is to address modeling of AS-biofilm system, to understand the applicability of soft computing model on real time wastewater treatment plants, the applicability of adaptive neuro-fuzzy inference system (ANFIS) modeling approach for predicting the Kjeldahl nitrogen removal from a 43.5 MLD domestic WWTP was done and from the understanding gained from the study a modified ANFIS or hybrid GA-ANFIS and PSO-ANFIS modeling approaches for nonlinear time series data modelling for predicting TN, COD and TP removal from AS-biofilm reactor was proposed and results have been discussed in the present chapter.

5.2 Data Statistics

5.2.1 Sewage treatment plant of 43.5 MLD

The data sets were obtained from the Kavoor Wastewater treatment plant (WWTP) treating wastewater from a population of 4,40,000 and situated at Mangalore, INDIA. The designed capacity of the WWTP is 43.5 MLD consisting of. screening, grit chamber, anaerobic, aerobic reactors and a secondary clarifier units. The flow diagram of the plant is shown in Figure 5.1. The data sets contained daily time series data recorded at the WWTP plant during the period June 2014–Sept 2014 with a total 120 data points (period of 4 months) of seven variables i.e. pH, COD, TS, Temperature, Ammonia nitrogen, Free ammonia and Kjeldahl Nitrogen. The dataset was split into training dataset which included 74% (74 data points) of data in the period from 2 June 2014 to 31 August 2014 and test dataset composed of the remaining 26% (26 data points) in the period from 2 June 2014 to 30 September 2014.

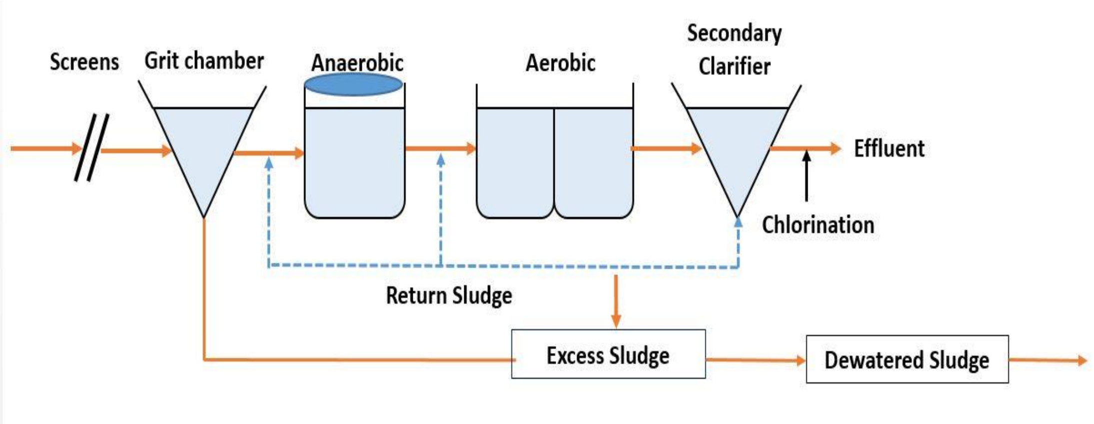

Figure 5.1 Schematic flow diagram of Kavoor WWTP

The descriptive statistics of the observed parameters of WWTP have been presented in Table 5.1. The X_{max}, X_{min}, X_{mean}, S_d, & C_v denotes the maximum, minimum, mean, standard deviation and variance respectively. The training dataset was used to build the model and testing dataset was employed to evaluate the performance of the built model. In order to investigate the dependency between variables that influence Total Kjeldahl Nitrogen (TKN), cross-correlation coefficients between effluent Total Kjeldahl Nitrogen (TKN) and each input parameter were analyzed and have been presented in Table 5.2

Table 5.1. Statistical Indices of Various Parameters of WWTP

		Parameters	Statistical Indices				
			X_{max}	X_{min}	X_{mean}	S_d	C_v
Training	Input	Influent pH	6.70	6.30	6.45	0.09	0.0078
		Influent TS	670.00	367.00	487.05	56.84	3230.64
		Influent COD	592.00	264.00	389.72	73.14	5349.11
		Influent T (˚C)	34.00	27.00	29.29	1.60	2.55
		Influent FA	0.16	0.05	0.09	0.025	0.0006
		Influent AN	29.00	10.00	17.12	4.40	19.33
		Influent TKN	37.00	16.00	23.71	5.35	28.59
	Output	Effluent TKN	32.00	11.00	19.72	5.00	24.98
Testing	Input	Influent pH	6.70	6.30	6.4478	0.1039	0.0108
		Influent TS	626.00	382.00	460.78	51.68	2671.27
		Influent COD	504.00	200.00	329.39	84.87	7202.34
		Influent T (˚C)	30.00	27.00	28.4783	0.6653	0.4427
		Influent FA	0.08	0.02	0.0436	0.0126	0.0002
		Influent AN	11.00	5.00	8.45	1.53	2.3550
		Influent TKN	16.00	9.00	13.13	1.96	3.8458
	Output	Effluent TKN	14.00	7.00	10.83	1.80	3.2411

** Except pH and Temperature all are mg/L*

Table 5.2 Cross-Correlation between effluent Total Kjeldahl Nitrogen (TKN) and other parameters

Parameter	Effluent Total Kjeldahl Nitrogen (TKN)	
	Train Data	***Test Data***
Influent pH	-0.597	-0.532
Influent TS	0.654	0.628
Influent COD	0.723	0.698
Influent T (˚C)	0.646	0.622
Influent FA	0.872	0.765
Influent AN	0.916	0.853
Influent TKN	0.952	0.920

* Except pH and Temperature all are mg/L

This data was analysed to assist in selecting input variables for ANFIS models. From Table 5.2, it could be noticed that the effluent Total Kjeldahl Nitrogen (TKN) at the time (t) was strongly correlated with the influent Total Kjeldahl Nitrogen (TKN) concentration (with a correlation value of 0.952(in train dataset) and 0.92(test)); Influent Ammonia Nitrogen concentration (with a correlation value of 0.916(train) and 0.85(test)); and the influent Free Ammonia concentration (with a correlation value of 0.87(train) and 0.76(test)). The cross-correlation coefficients between the effluent Total Kjeldahl Nitrogen (TKN) and other variables (influent Total Solids, COD concentrations, Temperature) were also found to be fairly influential. The cross-correlation coefficients between the effluent Total Kjeldahl Nitrogen (TKN) and the influent pH ranged from −0.597 (train) and −0.532 (test). The negative correlation indicated that a high occurrence or amount of Total Kjeldahl Nitrogen (TKN) rendered in the effluent during decreased pH of the influent.

5.2.2 Laboratory scale AS-biofilm reactor

Experimental setup and operating conditions have been explained in section 3.2.1.1 and 3.2.1.6 the experimental data were obtained for a period of 135 days by varying operating conditions such as C/N ratio; suspended biomass concentration (X) ; DO and HRT. The data set contained five variables, namely COD, C/N ratio, TN, MLSS, and TP. Hence, in the present context, the factors such as influent COD, MLSS, C/N ratio, TN and TP are used as predictors for the effluent TN, COD and TP concentrations using Artificial Intelligence (AI) models. The descriptive statistics of the observed variables have been presented in Table 5.3. The X_{max}, X_{min}, X_{mean}, S_d, & C_v denotes the maximum, minimum, mean, standard deviation and variance of the data respectively. In order to investigate the dependency between variables that influence TN, COD and TP, cross-correlation coefficients between effluent TN, COD and TP of each input parameter were analysed and have been presented in Table 5.4. From Table 5.4, it could be noticed that the effluent total nitrogen was fairly correlated with the influent Total nitrogen concentration with a correlation value of 0.69; similarly effluent COD concentration was fairly correlated with influent COD value 0.58 and C/N ratio value 0.69. The cross-correlation coefficients between the effluent Total phosphorous were strongly correlated with influent COD value 0.82 and the influent total phosphorous.

Table 5.3 Statistical Indices of Various Parameters of AS-biofilm reactor

		Parameters (mg/L)	Statistical Indices				
			X_{max}	X_{min}	X_{mean}	S_d	C_v
Training	Input	Influent COD	1210	398	792	326	106005
		Influent TN	53.1	29.5	43.3	8.7	75.2
		Influent MLSS	3256	1488	2325	434	187953
		Influent TP	10.9	9.3	10.0	0.25	0.06
		C/N ratio*	13.6	3.7	9.2	3.3	10.7
	Output	Effluent TN	25.6	4.4	11.7	4.4	19.6
		Effluent COD	260	60	133	56	3127
		Effluent TP	6.4	2.5	4.4	1.1	1.2
Testing	Input	Influent COD	1210	389	829	331	109653
		Influent TN	53.1	30	45.6	8.2	67.4
		Influent MLSS	3330	1484	2333	452	203987
		Influent TP	10.9	9.4	10.0	0.26	0.07
		C/N ratio*	13.3	3.7	9.1	3.2	10.4
	Output	Effluent TN	25.2	5.3	12.7	4.8	23
		Effluent COD	244	62	120.7	51.4	2638.5
		Effluent TP	6.2	2.4	4.2	1.1	1.2

* C/N ratio - No units

Table 5.4 Cross-Correlation between effluent TN, COD and TP with other parameters

Parameter mg/L	Inf COD	Inf TN	Inf C/N ratio	Inf TP	Inf MLSS	Eff TN	Eff COD	Eff TP
Inf COD	1.00							
Inf TN	0.44	1.00						
Inf C/N ratio	0.86	-0.05	1.00					
Inf TP	-0.32	-0.16	-0.05	1.00				
Inf MLSS	0.13	0.09	-0.16	0.03	1.00			
Eff TN	-0.08	0.69	0.09	-0.01	-0.28	1.00		
Eff COD	0.58	0.20	0.69	-0.16	-0.17	0.01	1.00	
Eff TP	-0.82	-0.35	0.20	0.29	-0.19	0.23	-0.22	1.00

* C/N ratio - No units

5.3 Model development

5.3.1 ANFIS model for sewage treatment plant

The analysis was carried out to predict the concentration of effluent Kjeldahl Nitrogen using influent pH, Temperature, TS, COD, Free Ammonia, Ammonia nitrogen and Kjeldahl Nitrogen as input parameters. The modeling of ANFIS was carried out in MATLAB platform. The results obtained from ANFIS model with Gbell and trapezoidal MFs have been depicted in the form of various statistical indices like RMSE, CC and NSE through tables and various plots. The optimal ANFIS architecture as presented in Table.5.5 was obtained after tuning fuzzy MF and rules of certain number and type.

Table 5.5. Details of ANFIS Architecture

ANFIS Architecture	
No of membership function (MF)	2
Alogoritham selected	Hybrid
No.Epoch given	30
FIS generated	Grid partition
No of membership (MF) type	Constant
Member ship function (MF) used	Gbell & Trapezoidal

5.3.2 Hybrid GA-ANFIS and PSO-ANFIS model for AS-biofilm reactor

The optimal parameters obtained after tuning the GA and PSO-ANFIS model have been tabulated in Table 5.6. The modeling of GA-ANFIS and PSO-ANFIS was carried out in MATLAB platform. The results obtained from GA-ANFIS and PSO-ANFIS models have been depicted in the form of various statistical indices like RMSE, CC and NSE through tables and various plots.

Table 5.6 PSO and GA algorithm parameter

PSO-ANFIS Parameter		GA-ANFIS Parameter	
Population Size	25	Population Size	25
Max No of Iterations	1000	Max No of Iterations	1000
PSO parameters C1	1.5	Mutation rate	0.15
PSO parameters C2	1.5	Crossover percentage	0.4
PSO momentum or inertia	0.73	Mutation percentage	0.7
		Selection pressure	8

5.4 Performance evaluation

The level of confidence over the predictions of any developed model is assessed by using suitable statistical indices. Correlation coefficient (CC), root mean square error (RMSE) and Nash-Sutcliffe Error (NSE) were used to evaluate the model accuracies. Although RMSE values were used to distinguish model performance in training and testing period, they can also be used to compare the performance of individual model to other predictive models. To assess the performance of ANFIS models the following statistical indices (equation. 5.1-5.3) were adopted.

Correlation coefficient (CC)

$$CC = \frac{\sum_{i=1}^{n}\left(X_i - \overline{X}\right)\cdot\left(Y_i - \overline{Y}\right)}{\sqrt{\sum_{i=1}^{n}\left(X_i - \overline{X}\right)^2 \cdot \sum_{i=1}^{n}\left(Y_i - \overline{Y}\right)^2}} \quad (5.1)$$

Root mean square error (RMSE)

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}\left(X - Y\right)^2}{n}} \quad (5.2)$$

Nash-Sutcliffe coefficient (NSE)

$$NSE = 1 - \frac{\sum_{i=1}^{n}\left(X_i - Y_i\right)^2}{\sum_{i=1}^{n}\left(X_i - \overline{X}\right)^2} \quad (5.3)$$

Where X = Observed/Actual Values

Y = Predicted Values

$\overline{X}$ = Mean of Actual Data values

n = Total Number of Values

5.5 Results and Discussion

5.5.1: ANFIS model for sewage treatment plant

Table 5.7 presents the statistical results of the ANFIS model developed for Gbell and trapezoidal MF. The magnitude of RMSE and NSE infer that the ANFIS model with Gbell membership function closely predicts the effluent Kjeldahl Nitrogen concentration than that of trapezoidal membership function. Here, the RMSE=0.852 mg/L, NSE=0.741 and CC=0.814 of ANFIS model with Gbell membership function during testing verifies the close agreement of concentration of effluent Kjeldahl Nitrogen with the observed concentration. The comparative evaluation of results obtained from Gbell and trapezoidal ANFIS models for prediction of effluent Kjeldahl Nitrogen were as presented in the form of graph Figure5.2.

Table 5.7 Statistical results of ANFIS models

STATISTICAL INDICES	ANFIS MODELS			
	GBELL MF		TRAPEZOIDAL MF	
	TRAIN	TEST	TRAIN	TEST
CC	0.999	0.814	0.999	0.746
RMSE (mg/L)	0.075	0.852	0.479	1.171
NSE	0.999	0.741	0.984	0.510

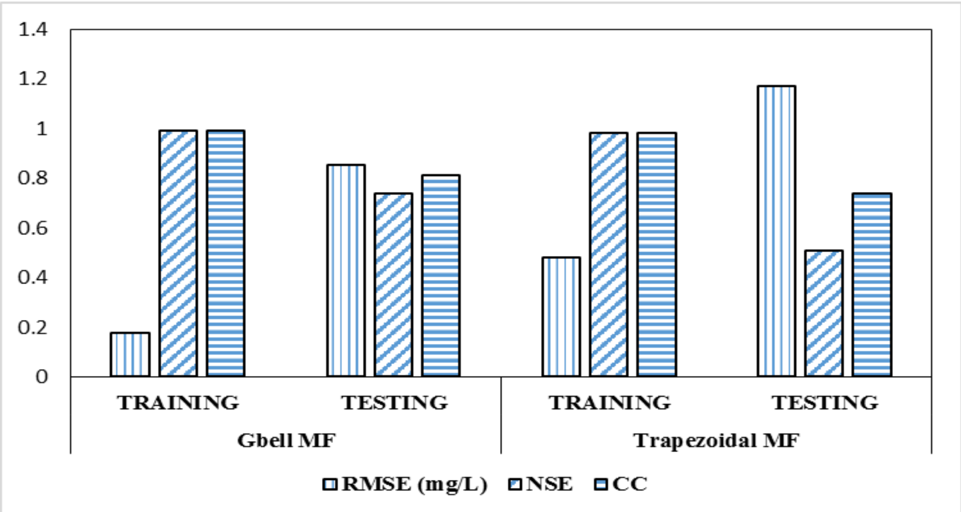

Figure 5.2 Comparative performance evaluation of ANFIS models with Gbell and trapezoidal MFs.

From the time series graphs as presented in Figure 5.3 for effluent Kjeldahl Nitrogen prediction, it is observed that ANFIS model with Gbell MF closely follows the observed time series. The ANFIS model with Gbell MF appears to have the accepted accuracy during testing.

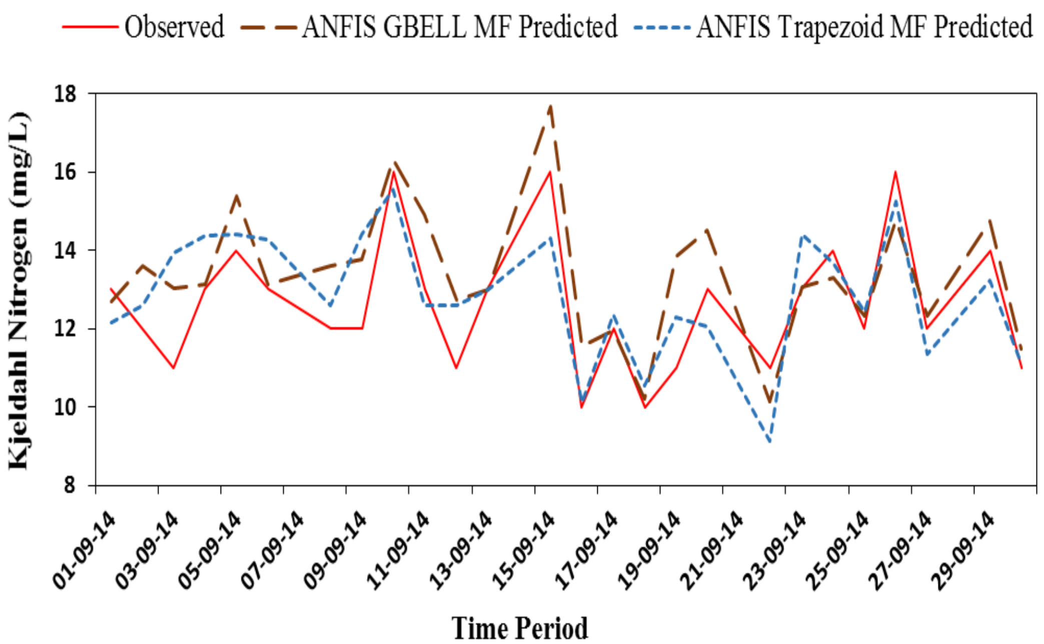

Figure 5.3 Predicted effluent Kjeldahl Nitrogen concentration of ANFIS models during testing

Figure 5.4 shows closely spaced scatters of the computed and observed effluent Kjeldahl Nitrogen concentrations of ANFIS models with Gbell MF and Trepizodal MF function during testing. It can be observed that ANFIS model with trapezoidal MF has more number of outliers than that of the Gbell ANFIS model during the testing. From this, it can be ascertained that ANFIS model with Gbell MF has higher consistency and robust performance.

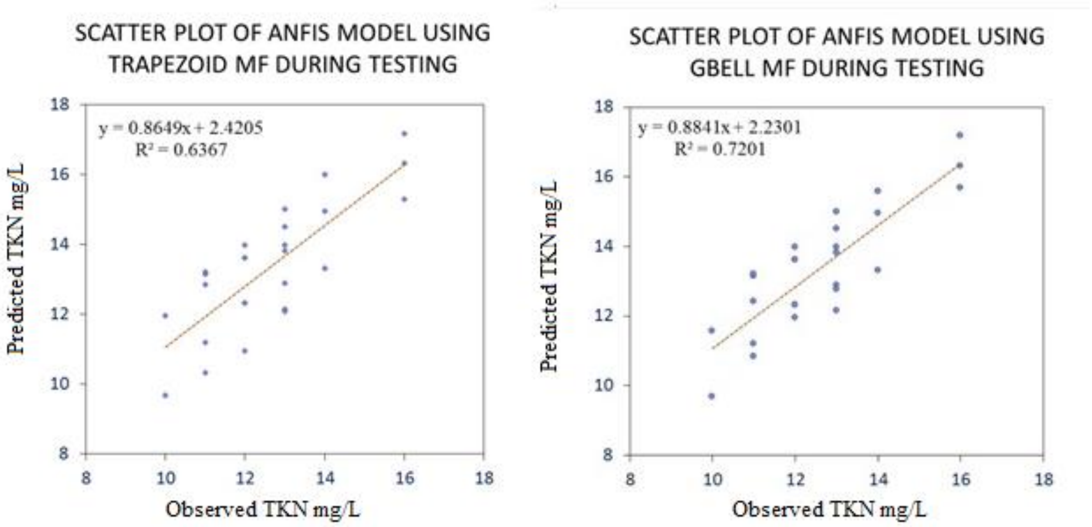

Figure 5.4 Scatter plot of observed v/s Predicted of ANFIS model with Gbell MF and trapezoidal MF during testing

5.5.2 Hybrid GA-ANFIS and PSO-ANFIS model for AS-biofilm reactor

The results obtained from GA-ANFIS and PSO-ANFIS models are depicted in the form of various statistical indices like RMSE, CC and NSE through tables and various plots. The prediction errors of the models in the training and testing are as presented in Table 5.8. In the PSO-ANFIS model, the RMSE and NSE are significantly less in both training and testing stages when compared to that of GA-ANFIS models in predicting the performance of TN and COD. The magnitude of RMSE and NSE computation infer that the PSO-ANFIS model closely predicts the effluent TN concentration than that of GA-ANFIS model. Here, the RMSE = 0.795 mg/L, NSE = 0.787 and CC = 0.88 of PSO-ANFIS model during testing of predicting TN verifiy the close agreement of concentration of effluent TN with the observed concentration as presented in figure 5.5. Similarly, the RMSE = 42.52 mg/L, NSE = 0.31 and CC = 0.62 of PSO-ANFIS model during testing of predicting COD verifiy the close agreement of concentration of effluent COD with the observed concentration as presented in figure 5.6.

Table 5.8 Statistical results of GA-ANFIS and PSO-ANFIS models

Parameters	Statistical Indices	GA-ANFIS		PSO-ANFIS	
		Training	Testing	Training	Testing
TN	CC	0.951	0.868	0.957	0.861
	RMSE	1.352	2.391	1.267	2.471
	NSE	0.906	0.751	0.917	0.734
COD	CC	0.721	0.520	0.816	0.624
	RMSE	38.61	47.79	32.11	42.52
	NSE	0.523	0.134	0.670	0.314
TP	CC	0.930	0.897	0.950	0.880
	RMSE	0.399	0.487	0.339	0.530
	NSE	0.866	0.801	0.903	0.764

Similarly, GA-ANFIS model closely predicts the effluent TP concentration than PSO-ANFIS model during the testing. Here, the RMSE = 0.5 mg/L, NSE=0.75 and CC = 0.88 of GA-ANFIS model during the testing of predicting TP verifies the close agreement of concentration of effluent TP with the observed concentration is as shown in figure 5.7. Figure 5.8 shows scattered plots of the predicted and observed effluent TN, COD and TP concentrations in GA-ANFIS and PSO-ANFIS models during the testing. The reasonable dependence of a variable can be verified through the coefficient of determination (R^2) which ranges between 0 and 1 signposting the predictable extent of the dependent variable. The data points in the upper and lower extremes of the scatter plot of GA-ANFIS and PSO-ANFIS model do not deviate to a great extent from the line of best fit indicating the goodness of the fit/model.

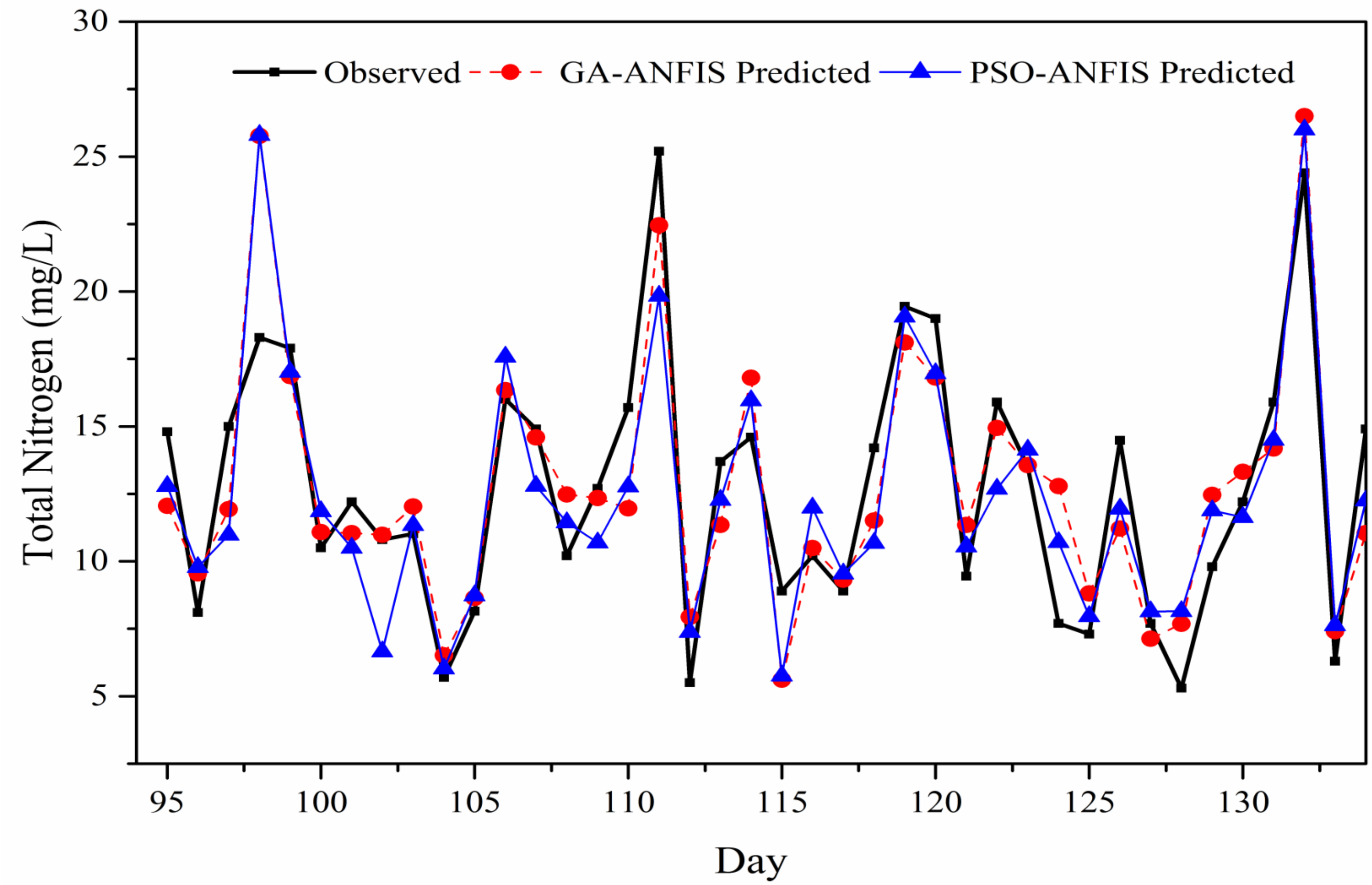

Figure 5.5 Predicted effluent Total Nitrogen concentration of GA-ANFIS and PSO-ANFIS models during testing

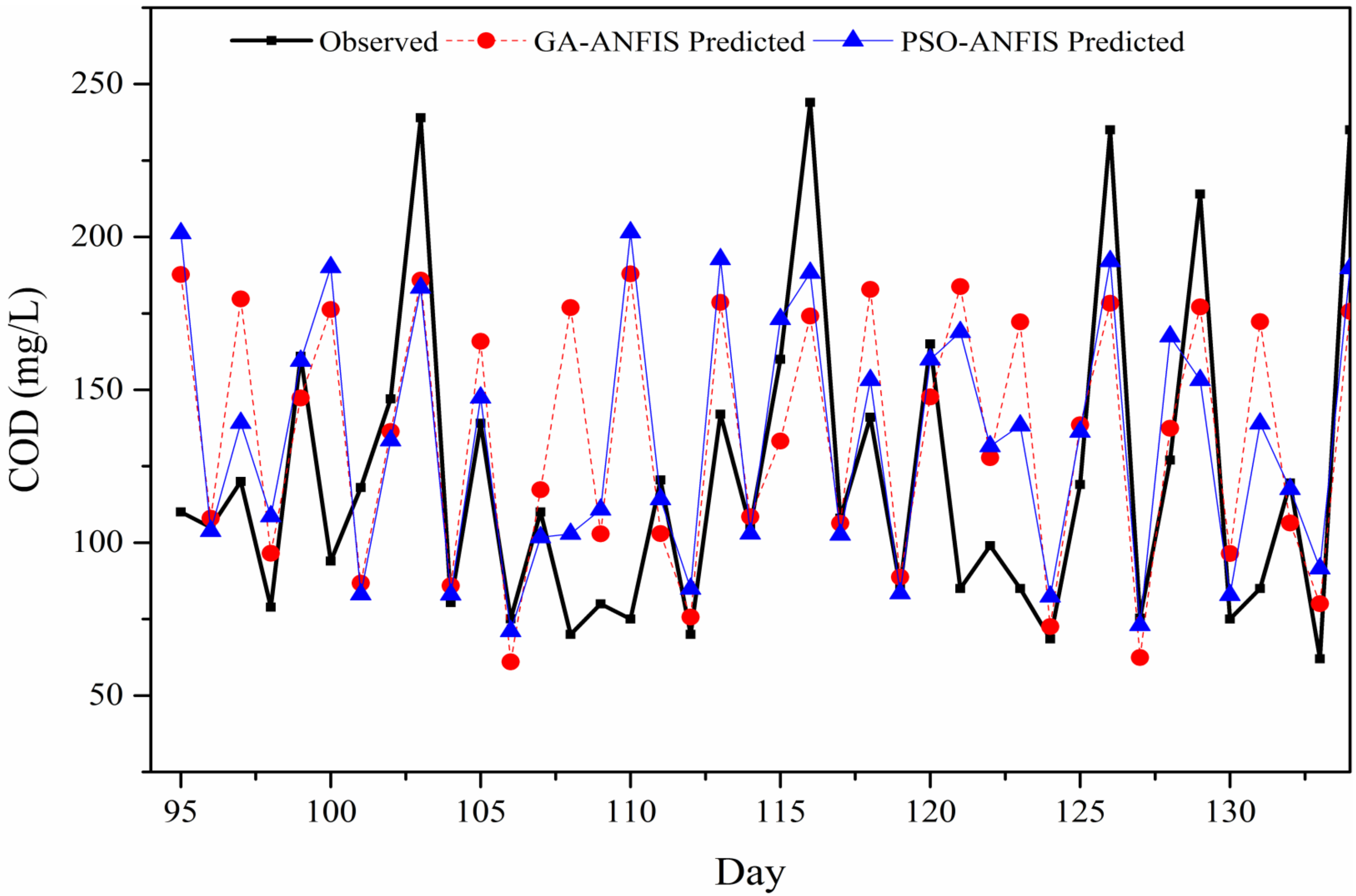

Figure 5.6 Predicted effluent COD concentration of GA-ANFIS and PSO-ANFIS models during testing

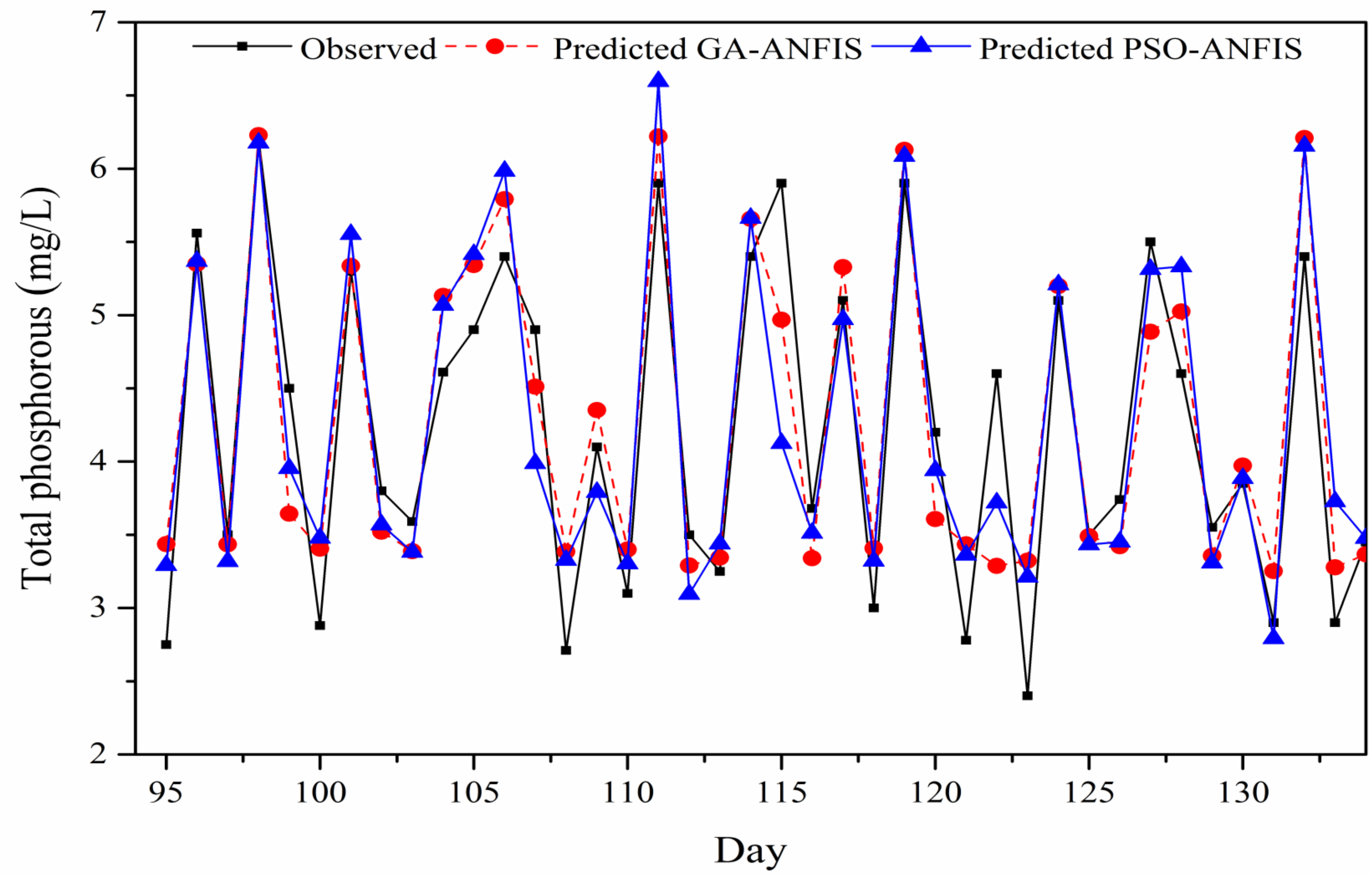

Figure 5.7 Predicted effluent Total phosphorous concentration of GA-ANFIS and PSO-ANFIS model during testing

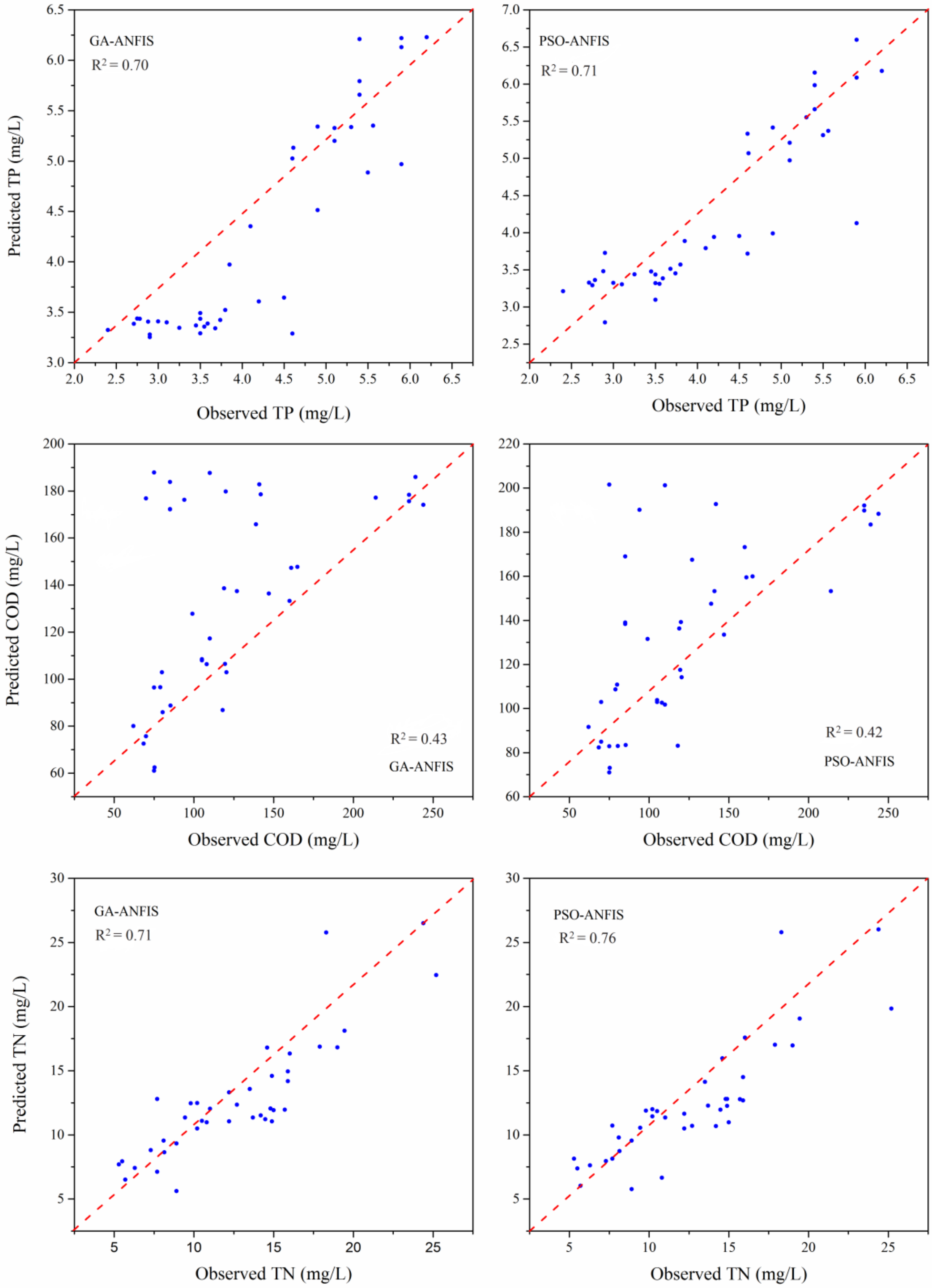

Figure 5.8 Scatter plot of observed v/s Predicted of GA-ANFIS and PSO-ANFIS models during testing

5.6 Summary

According to Indian regulations, the total phosphorus, nitrogen and COD in treated effluent should be in the range of 1 - 5 mg/L, 5 - 10 mg/L and 30 - 60 mg//L respectively. In many situations, where the risk of public exposure to the reclaimed water exists, effective monitoring of effluent quality is necessary. The data related to influent pollutants, including the total suspended solids (TSS) and COD, are utilized for immediate or short-term effluent quality prediction in order to provide information for efficient operation of the treatment process

In this study, (1) an artificial intelligence model – ANFIS, which combines the advantages of Artificial Neural Network and Fuzzy Logic is applied for prediction of effluent Kjeldahl Nitrogen concentration from a biological wastewater treatment plant (43.5 MLD capacity). ANFIS model with Gbell and trapezoidal membership functions has been tested in the study with input parameters as influent pH, TS, COD, and Temperature, Ammonia nitrogen, free ammonia and Kjeldahl Nitrogen. Gbell MF has been found to be very efficient in modeling the nonlinear time series and the results obtained from the ANFIS model by Gbell MF were found to be very promising in predicting the effluent concentrations and estimate the performance of the WWTP. However, due to the computational complexity, trapezoidal membership function was found to be incompatible to model the effluent Kjeldahl Nitrogen concentration in the present study.

So in the current study, in order to improve the predicting ability of ANFIS, GA-ANFIS and PSO-ANFIS were applied for predicting the effluent TN, COD and TP concentration from a hybrid AS-biofilm reactor. GA-ANFIS and PSO-ANFIS models were tested in the study with input variables such as influent COD, TN, TP, MLSS and C/N ratio. From the results presented above, Cross validation search PSO-ANFIS model was found to be slightly effective in modeling the nonlinear data.

CHAPTER 6

CONCLUSIONS

Based on the present study, the following conclusions are drawn

1. Effects of C/N ratio, biomass (X), HRT and DO variations on the performance of AS-biofilm reactor in terms of carbon and nutrient removal were investigated. In addition TiO_2 based photocataysis was tried to see its feasibility to improve the quality of the treated water. Some specific findings of this study can be drawn as follows.

- A thin layer of mature attached biofilm was formed over the carriers within few days after feeding the system with molasses based wastewater.
- When the C/N ratio was varied from 4 to 6.67, the maximum removal efficiencies of COD, NH_4^+-N, TN, and TP were 82%, 92%, 81% and 47% respectively when the reactor was maintained at C/N ratio of 6.67, HRT of 10hrs, DO in the range of 3.5- 4.5 mg/L, biomass (X) in the range of 2-2.5g/L and OLR of 0.96 kg COD/m^3.d during Run-1. In Run 2, when the C/N ratio was varied from 8 to 13.33, the maximum removal efficiencies of COD, NH_4^+-N, TN, and TP were 92%, 93%, 86% and 52% respectively when the reactor was maintained at C/N ratio of 10, HRT of 8hrs, DO in the range of 3.5- 4.5 mg/L, biomass (X) in the range of 3-3.5g/L and OLR of 1.8 kg COD/m^3.d. Similarly, at C/N ratio 12, the maximum COD, NH_4^+-N, TN, and TP removal efficiencies increasesd with increase in HRT of 10hrs, DO in the range of 2.5- 3.5 mg/L, biomass (X) in the range of 2-2.5g/L and OLR of 2.88 kg COD/m^3.d during Run-3.
- With an increase in C/N ratio from 4 to 13.37 the COD, NH_4^+-N, TN and TP removal efficiencies increased, from 81% to 92%, 76% to 94%, 71% to 82% and 47% to 69%, respectively, which supported the statement that AS-biofilm process was good at handling OLR. System when operated with 2.5 g/L of suspended biomass (X) concentration showed better removal efficiency.

- From the Taguchi analysis, it was found that the delta value of C/N ratio, biomass, HRT and DO were 1.23, 1.02, 0.75 and 0.37 respectively and are ranked as 1, 2, 3 and 4. It could be concluded that the parameter C/N ratio had more influence on the COD, NH_4^+-N, TN and TP removal in Run 1. Similarly, in Run 2, delta value of C/N ratio, biomass, HRT and DO were 0.55, 0.70, 0.45 and 0.30 and ranked as 2, 1, 3 and 4 respectively.
- Degradation and disinfection both were achieved by using UV and solar photocatalysis. It was clearly observed that the optimum dosage of photocatalyst was same for both UV and solar photocatalysis i.e. 500 mg/L. However, the irradiation time required for achieving similar efficiency was double in case of solar photocatalysis. TiO_2 photocatalysis in addition to disinfection also reduced other parameters like COD and Phosphorous. Based on the WWQI, if the treated effluent was indexed as 'good', it could be utilized for residential flushing and boiler cooling. Similarly the treated water of 'average' index would be suitable for usage in irrigation and gardening.

2. Integrated lab-scale A^2O-AS-biofilm system was established and smoothly operated with molasses based synthetic wastewater by varying C/N ratio, HRT, Biomass (X) and DO and these factors played a key role in enhancing biological nutrient removal. Some specific findings of this study can be drawn as follows.

   - When the C/N ratio was varied from 4 to 6.66 in Run-1 and 8 to 13.3, in Run-2 the maximum removal efficiencies of COD, TP, NH_4^+-N, and TN were 95.5%, 93%, 98% and 80% respectively when the reactor was maintained at C/N ratio of 4, HRT of 10hrs, DO in the range of 1.5- 2.5 mg/L and the biomass (X) in the range of 3-3.5g/L during Run- 1 and proved to be a good alternative for the treatment of wastewater with low C/N ratio.
   - The increase in C/N ratio from 4 to 13.3 showed no significant change in COD, NH_4^+-N, TN, and TP removal efficiencies.
   - The optimal simultaneous nitrogen and phosphorous removal efficiencies were achieved at HRT of 10 hrs, which might be due to efficient utilization of carbon sources (PHA) and higher denitrification capacity.

- The increase in suspended biomass concentration (X) from 1-1.5 mg/L to 3-3.5 mg/L in the A^2O-AS-biofilm reactor, increased the overall TN removal efficiency by 5% due to more volume of suspended biomass.
- The SEM analysis of suspended and attached biofilm revealed the presence of some dense layers of EPS of proteins and carbohydrates. Various forms of facultative aerobic cocci appearing in pairs, bead-like chains, or bunched (such as streptococci, citrobacter, enterococci etc.) were the chief microbes over the surface of the attached biofilm.

3. GA-ANFIS and PSO-ANFIS were used to predict the effluent concentration of TN, TP, and COD of AS-biofilm reactor operated under varying conditions. The GA-ANFIS model performed better with CC= 0.868, RMSE=2.391, NSE=0.751 while the results of PSO-ANFIS were CC=0.861, RMSE=2.471, NSE=0.734 for TN prediction. Similarly, for TP, and COD predictions, the GA-ANFIS models performed relatively better than PSO-ANFIS models with CC= 0.897 and 0.520 respectively. From the results presented, both GA-ANFIS and PSO-ANFIS models were capable of prediction the effluent parameters of the reactor with varying operation conditions and could be adopted for modeling the nonlinear time series data.

Scope for future work

Based on the findings of this research, the following recommendations can be made:

- For future research, it is suggested that studying the effect of microbial diversity with respect to various operating condition could be interesting.
- Though there are numerous laboratory studies available on photocatalysis by TiO_2, yet when it comes to scaling of this process, separation of these TiO_2 particles from treated water is a limiting factor. Studies on immobilized TiO_2 doped with other metal oxides under parabolic solar concentrators could be conducted to measure its efficiency which could pave a way for the pilot plant operations.
- To improve the performance of the model using other optimization technique such as ant colony optimization with clustering methods.

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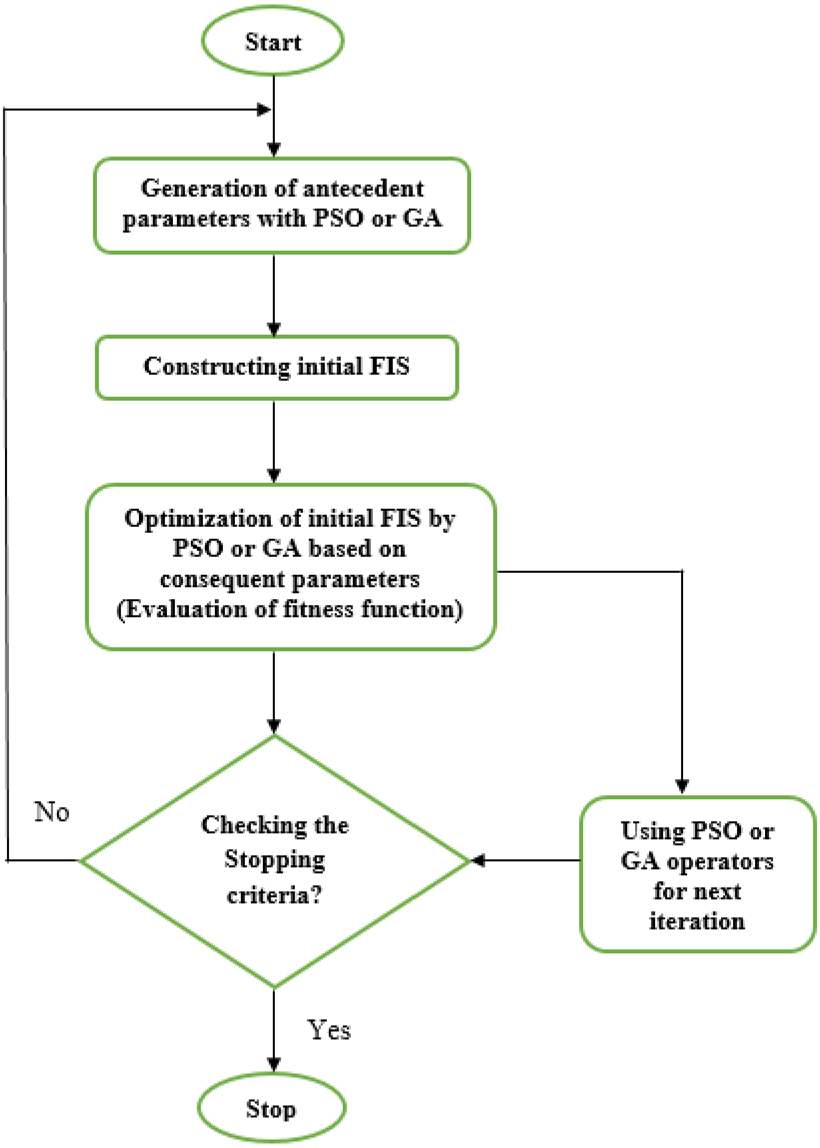
APPENDIX - A

Flow chart for GA and PSO-ANFIS



APPENDIX - B

Algorithm GA and PSO-ANFIS

```
%DATA LOADING
load Inputs.mat
load Targets.mat
nSample=size(Inputs,1);
     % Train Data
     pTrain=0.7;
     nTrain=round(pTrain*nSample);
    TrainInputs=Inputs(1:nTrain,:);
    TrainTargets=Targets(1:nTrain,:);
    % Test Data
    TestInputs=Inputs(nTrain+1:end,:);
    TestTargets=Targets(nTrain+1:end,:);
    % Export
    data.TrainInputs=TrainInputs;
    data.TrainTargets=TrainTargets;
    data.TestInputs=TestInputs;
    data.TestTargets=TestTargets;

%% Generate Basic FIS
fis=CreateInitialFIS(data,10);
%% Train Using GA or PSO
Options = {'Genetic Algorithm', 'Particle Swarm Optimization'};
[Selection, Ok] = listdlg('PromptString', 'Select training method for ANFIS:', ...
                   'SelectionMode', 'single', ...
                   'ListString', Options);

pause(0.01);
```

```
if Ok==0
    return;
end
switch Selection
    case 1, fis=TrainAnfisUsingGA(fis,data);
    case 2, fis=TrainAnfisUsingPSO(fis,data);
end

%% Results
% Train Data
TrainOutputs=evalfis(data.TrainInputs,fis);
PlotResults(data.TrainTargets,TrainOutputs,'Train Data');
% Test Data
TestOutputs=evalfis(data.TestInputs,fis);
PlotResults(data.TestTargets,TestOutputs,'Test Data');
```

APPENDIX - C

PUBLICATIONS

International Journals:

1. Manu D S and Arun Kumar Thalla, (2018), “Influence of various operating conditions on wastewater treatment in an AS-biofilm reactor and post-treatment using TiO_2 based Solar/UV Photocatalysis ". Environmental Technology, 1-18. (Taylor and Francis)

2. Manu D S and Arun Kumar Thalla, (2018), “The Combined effects of C/N ratio, suspended biomass, hydraulic retention time and dissolved oxygen on nutrient removal in a lab-scale anaerobic-anoxic-oxic activated sludge biofilm reactor ". Water Science and Technology, 77(1), 248- 25.(IWA)

3. Manu D S and Arun Kumar Thalla, (2017), “Artificial intelligence models for predicting the performance of biological wastewater treatment plant in the removal of Kjeldahl Nitrogen from wastewater”. Applied Water Science, 7(7), 3783-3791. (Springer)

International Conferences:

1. Manu D S and Arun Kumar Thalla, (2016), “Simultaneous nitrogen and phosphorus removal by an aerobic moving bed biofilm reactor for wastewater treatment”. Trends and Recent Advances in Civil Engineering (TRACE 2016), Amity University Noida, INDIA.

2. Manu D S and Arun Kumar Thalla, (2015), “Performance evaluation of Sequencing Batch Moving Bed Bioflm Reactor using synthetic wastewater ". Sustainable Energy and Built Environment VIT University, Vellore, INDIA.

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