Evaluation of Pilot Scale Trickling Filter System for the Treatment of Wastewater

DISS
BIO
5298

By

Abdul Rehman

Department of Microbiology
Faculty of Biological Sciences
Quaid-i-Azam University
Islamabad
2018

Evaluation of Pilot Scale Trickling Filter System for the Treatment of Wastewater

A thesis submitted in partial fulfillment of the requirements for the Degree of

DOCTOR of PHILOSOPHY

In

Microbiology

By

Abdul Rehman

Department of Microbiology
Faculty of Biological Sciences
Quaid-i-Azam University
Islamabad
2018

In The Name Of Allah, The Most Beneficent, The Most Merciful

Importance of Water as Mentioned in the Holy Qur'an (63 times)

Water was the first thing to have ever existed

Allah said in His Book: *«And His throne was over water —that He might try you, which of you is best in conduct»* (Hud 7).

All organisms depend on Water

«We made from water every living thing» (Anbiyya' 30)

Water is the source of all vegetation

«It is he who sends down rain from the sky; from it ye drink, and out of it (grows) the vegetation on which you feed your cattle. With it He produces for you corn, olives, date palms, grapes and every kind of fruit: verily in this is a sign of those who give thought» (Nahl 11).

Water: promises and threats

«Say (O Muhammad): Tell me? if (all) your water were to sink away, who then can supply you with flowing water» (Mulk 30).

Water is a Source of Food

«Lawful to you is the pursuit of water-game and its use for food—for the benefit of yourselves» (Ma'ida 96).

«Nor are the two bodies of flowing water alike,- the one palatable, sweet, and pleasant to drink. And the other, salt and bitter. Yet from each (kind of water) do ye eat flesh fresh and tender, and ye extract ornaments to wear» (Fatir 12).

Quaid-i-Azam University
Reference Section

Dedicated to my Parents & Teachers

Specially to my Father Mr. Abdul Latif (Late), my
younger Brother Mr. Abdullah (Late) & my
Supervisor Prof. Dr. Safia Ahmed

List of External PhD Thesis Evaluator

1) Professor Dr. Lorna Dawson,
Head of Forensic Soil Sciences,
James Hutton Institute, Aberdeen, AB15 8QH.

2) Dr. Chunzhao Liu,
Professor of Biochemical Engineering,
State Key Laboratory of Biological Engineering,
Institute of Process Engineering, Chinese Academy of Sciences,
Beijing, China.

Author's Declaration

I **Mr. Abdul Rehman** hereby state that my Ph.D. thesis titled **"Evaluation of Pilot Scale Trickling Filter System for the Treatment of Wastewater"** is my own work and has not been submitted previously by me for taking any degree from Quaid-i-Azam University, Islamabad, Pakistan.

At any time if my statement is found to be incorrect even after I Graduate, the University has the right to withdraw my Ph.D. degree.

Mr. Abdul Rehman
Date: 13-12-2018

Plagiarism Undertaking

I solemnly declare that research work presented in the thesis titled **"Evaluation of Pilot Scale Trickling Filter System for the Treatment of Wastewater"** is solely my research work with no significant contribution from any other person. Small contribution / help wherever taken has been duly acknowledged and that complete thesis has been written by me.

I understand the zero tolerance policy of the HEC and Quaid-i-Azam University towards plagiarism. Therefore I as an Author of the above titled thesis declare that no portion of my thesis has been plagiarized and any material used as reference is properly referred/cited.

I undertake that if I am found guilty of any formal plagiarism in the above titled thesis even after award of Ph.D degree and that HEC and the University has the right to publish my name on the HEC/University Website on which names of students are placed who submitted plagiarized thesis.

Student / Author Signature:

Name: **Mr. Abdul Rehman**

Certificate of Approval

This is to certify that the research work presented in this thesis, entitled titled **"Evaluation of Pilot Scale Trickling Filter System for the Treatment of Wastewater"** was conducted by Mr./ Ms. **Mr. Abdul Rehman** under the supervision of **Prof. Dr. Safia Ahmed.** No part of this thesis has been submitted anywhere else for any other degree. This thesis is submitted to the Department of Microbiology, Quaid-i-Azam University, Islamabad in partial fulfillment of the requirements for the degree of Doctor of Philosophy in field of **Microbiology**.

Student Name: **Mr. Abdul Rehman** Signature:

Examination Committee:

a) External Examiner 1: Signature:

Prof. Dr. Muhammad Anwar Baig
Institute of Environmental Science and Engineering (IESE)
Department of Environmental Sciences
National University of Science and Technology (NUST)
H-12, Islamabad.

b) External Examiner 2: Signature:

Prof. Dr. Azra Yasmin
Dean, Environmental Sciences Programme
Fatima Jinnah University, Rawalpindi.

Supervisor Name: **Prof. Dr. Safia Ahmed** Signature: Safia Ahmed

Name of HOD: **Dr. Rani Faryal** Signature:

LIST OF CONTENTS

Title Page

LIST OF ABBREVIATIONS.. i
LIST OF TABLES.. ii
LIST OF FIGURES.. iii
ACKNOWLEDGEMENT.. vi
ABSTRACT.. viii

CHAPTER 1

INTRODUCTION... 1

CHAPTER 2

LITERATURE REVIEW.. 7

2.1 Overview of Water Crises and Searching for Alternate Resources... 7
2.1.1 Water and Wastewater Scenario in Pakistan 8
2.2 General Description of Wastewater and its Treatment; Advancement and Challenges.. 10
2.2.1 Chronological Outlook of Wastewater Treatment........................ 10
2.2.2 Why Treat Wastewater? 11
2.2.3 Essential Unit Operations in Wastewater Treatment.................... 11
2.2.4 Biological Wastewater Treatment Technologies 13
2.3 A Comprehensive Review of Attached Growth Systems................. 14
2.4 General Outlines of Trickling Filter System: Process Design and Operation 15
2.4.1 Distribution System.. 17
2.4.2 Biofilm Support Materials/Carriers................................... 19
2.4.3 Underdrain System.. 22
2.4.4 Recirculation Pattern.. 23
2.4.5 Classification of Trickling Filter System............................. 24
2.5 Biofilms or Microbial Aggregates 25
2.5.1 Biofilm Development and Arrangement 26
2.5.2 Application of Biofilms in Water and Wastewater Treatment........... 29
2.6 Mathematical Simulation or Modelling of Attached Growth Bioreactor for Wastewater Treatment 32

CHAPTER 3

MATERIALS AND METHODS.. 36

3.1 Experimental Setup-I Account.. 36
3.1.2 Experimental Setup-I Operational Scheme.................................. 36

3.2 Experimental Setup-II Account... 37
3.2.1 Experimental Setup-II Operational Scheme................................. 38
3.3 Physicochemical Investigation of Influent and Effluent Samples..... 40

Quaid-i-Azam University

3.3.1 Odor and pH.. 40
3.3.2 Electrical conductivity (EC).. 40
3.3.3 Dissolved Oxygen (DO).. 40
3.3.4 Biological Oxygen Demand (BOD_5).. 40
3.3.5 Chemical Oxygen Demand (COD)... 41
3.3.6 Total Dissolved Solids (TDS).. 41
3.3.7 Total Suspended Solids (TSS).. 42
3.3.8 Orthophosphates.. 42
3.3.9 Sulphates... 43
3.3.10 Nitrate-nitrogen (NO_3-N)... 43
3.3.11 Nitrite-nitrogen (NO_2-N)... 43
3.3.12 Total Nitrogen (TN).. 44
3.4 Microbiological Assessment of Influent and Effluent Samples........ 44
3.4.1 Most Probable Number Test (MPN/100 mL) for Fecal Coliforms....... 44
3.4.2 Quantitative Analysis in terms of Colony Forming Unit per milliliter (CFU/mL)........ 44
3.5 Bacteriological Assessment of Biofilm developed over Filter Media.. 45
3.5.1 Colony morphology.. 45
3.5.2 Microscopy.. 45
3.5.3 Biochemical Characterization.. 45
3.6 Statistical Analysis... 46

CHAPTER 4

4. APPRAISAL OF A LOW-COST PILOT SCALE TRICKLING FILTER SYSTEM FOR SEWAGE TREATMENT DURING VARIOUS SEASONS 47

4.1 Introduction... 47
4.2 Material and Methods... 48
4.2.1 Experimental Setup-I Description and Operation........................ 48
4.2.2 Microbial Profiling of Wastewater 48
4.2.2.1 Most Probable Number Test (MPN Index) for Fecal Coliforms........ 48
4.2.3 Chemistry of the Wastewater 48
4.2.4 Microbial Profiling of Slime Layer Developed on Stone Filter Media. 49
4.2.5 Model Expansion and Standardization 49
4.3 Results and Discussion 51
4.3.1 Efficacy of Pilot Scale Trickling Filter in the Removal of Fecal coliforms.. 52
4.3.2 Evaluation of Pilot Scale Trickling Filter System in the Removal of Carbonaceous Compounds.. 56
4.3.2.1 Pre-Treatment Characterization of Wastewater 56
4.3.2.2 Post-Treatment Characterization of Wastewater 56
4.3.3 Bacteriological Assessment of Slime Layer developed on Stone Filter Media.. 63
4.3.4 Model Applications 65

CHAPTER 5

5. EFFICIENCY OF LOCALLY DESIGNED PILOT SCALE TRICKLING BIOFILTER (TBF) SYSTEM IN NATURAL ENVIRONMENT FOR THE TREATMENT OF DOMESTIC WASTEWATER.......... 74

5.1 Introduction.......... 74
5.2 Material and methods.......... 74
5.2.1 Experimental Setup-II Scheme (Design and Operation).......... 74
5.2.2 Physico-Chemical Analysis of Wastewater.......... 75
5.2.3 Microbiological Characterization of the Influent and Effluent samples. 76
5.3 Results and Discussion.......... 76
5.3.1 Wastewater Treatment Efficiency of Pilot Scale TBF System.......... 76
5.3.2 Microbiological Characterization of Influent and Effluent Samples 86

CHAPTER 6

6. EFFECTS OF HYDRAULIC RETENTION TIME (HRT) ON THE PERFORMANCE OF PILOT SCALE TRICKLING FILTER SYSTEM TREATING DOMESTIC WASTEWATER.......... 88

6.1 Introduction.......... 88
6.2 Materials and Methods.......... 88
6.2.1 Experimental Setup-II TBF Scheme.......... 88
6.2.2 Sampling of wastewater 89
6.3 Results and Discussions.......... 89
6.3.1 Effects of Hydraulic Retention Time (HRT) on the Performance of TF System 89
6.3.2 Efficiency of Pilot Scale Trickling filter (TBF) System in terms of Pathogens Removal under Different HRTs 99

CHAPTER 7

7. ASSESSMENT OF AN INTEGRATED MEDIA PILOT SCALE TRICKLING BIOFILTER SYSTEM WITH VARIABLE FLOW RATES AND HYDRAULIC RETENTION TIME (HRT) TOWARDS TREATMENT OF DOMESTIC WASTEWATER.......... 101

7.1 Introduction.......... 101
7.2 Materials and Methods.......... 101
7.2.1 Experimental Setup-II TBF Scheme.......... 101
7.2.2 Samples Processing During Study.......... 102
7.3 Results and Discussions.......... 103
7.3.1 Efficiency of Integrated Media Pilot Scale TBF System Under Different Flow Rates and HRTs to Treat Domestic Wastewater.......... 103
7.3.2 Efficiency of Integrated Media Pilot Scale Trickling filter (TBF) System in the Removal Of Microbial Contaminants (CFU/mL) from Wastewater under Different Flow Rates and HRT.......... 111

7.3.3 Bacteriological Assessment of Slime Layer Developed on Integrated Filter Media………………………………………………………… 112

CHAPTER 8

GENERAL DISCUSSION……………………………………………....... 115

CONCLUSIONS…………………………………………………………... 123

FUTURE PROSPECTS…………………………………………………... 124

REFERENCES……………………………………………………………. 125

LIST OF PUBLICATIONS, WORKSHOPS ATTENDED AND PAPER/POSTER PRESENTATION……………………………......... 158

LIST OF ABBREVIATIONS

WHO	World Health Organization	**HLR**	Hydraulic Load Rate
EPA	Environmental Protection Agency	**EPS**	Extra cellular Polymeric Matrix
IWMI	International Water Management Institute	**SEM**	Scanning Electron Microscopy
USEPA	United State Environmental Protection Agency	**TEM**	Transmission Electron Microscopy
TF	Trickling Filter	**Q**	Flow Rates
COD	Chemical Oxygen Demand	**Q/A**	Hydraulic Loading
BOD	Biochemical oxygen demand	**PVC**	Polyvinyl Chloride
BOD$_5$	Biochemical oxygen demand of 5 days	**QAU**	Quaid-i-Azam University
TN	Total Nitrogen	**APHA**	American Public Health Association
S_o	Initial BOD$_5$ of treatment unit	**PST**	Primary Sedimentation Tank
X	Biomass	**EC**	Electric Conductivity
TBF	Trickling Biofilter	**DO**	Dissolved Oxygen
ABR	Anaerobic Baffled Reactor	**BaCl$_2$**	Barium Chloride
TSS	Total Suspended Solids	**MR**	Methyl Red
TDS	Total Dissolved Solids	**HRT**	Hydraulic Retention Time
MPN	Most Probable Number	S_t	Final BOD$_5$ of treatment unit
CFU	Colony Forming Unit	k_t	Specific bioreactor coefficient at specific temperature
PASA	Pakistan Agriculture Scientists Association	**O$_{LR}$**	Organic Loading Rate
rpm	Revolution per minute	**TSI**	Triple Sugar Iron
MBBR	Moving Bed Biofilm Reactor	**µS/cm**	Micro Siemen per centimeter

LIST OF TABLES

Table No.	Title	Page No.
2.1	Application of different types of filter media in wastewater treatment system.	20
2.2	Assortment of trickling filter systems on the basis of operational parameters.	25
4.1	Efficacy of pilot scale TF system in the removal of fecal coliforms under different seasonal conditions.	55
4.2	Mean values of different physico-chemical parameters of influent and effluent samples and average % reduction at day 1 and day 6 under different seasonal conditions.	59
4.3	Bacteriological assessment of slime layer developed on stone filter media.	64
4.4	Parameters of pilot scale TF system under different seasonal conditions.	67
5.1	Efficiency of pilot-scale TBF system in the removal of microbial contaminants (MPN index and CFU/mL) from wastewater for 15 weeks at temperature range of 14.5–36°C.	87
6.1	Effects of different hydraulic retention time (HRT) on the performance of pilot scale TBF System with respect to average reduction of different physico-chemical parameters.	97
6.2	Overall efficiency of pilot scale TBF system in the average percentage reduction of different physico-chemical parameters under different HRTs.	98
7.1	Efficiency of integrated media TBF system in the reduction of different physico-chemical parameters at different HRTs and flow rates.	110
7.2	Efficiency of integrated media pilot scale Trickling filter (TBF) System in the removal of microbial contaminants (CFU/mL) from wastewater under different flow rates and HRT.	112
7.3	Detailed description of microscopy, cultural and biochemical tests of bacterial spp., isolated from the slime layer (Biofilm) developed on integrated filter media.	114
8.1	Effect of different flow rates over the performance of integrated media TBF system in terms of average percent reduction of different physicochemical parameters.	121

LIST OF FIGURES

Figure No.	Title/Caption	Page No.
2.1	Water crises and alarming facts of water reservoirs in Pakistan.	9
2.2	Process design and operation of Trickling Filter System.	16
2.3	Hydraulically driven rotary distributor (**L**); Electrically driven rotary distributor (**R**).	19
2.4	Recirculation pattern of wastewater in fixed film bioreactor i.e. trickling filter.	24
2.5	Schematic illustration of biofilm development process.	28
3.1	Pilot scale Trickling filter system in a work station located at the vicinity of the Department of Microbiology, QAU Islamabad for the treatment of wastewater.	37
3.2	Schematic illustration of overall treatment units of TBF system installed at residential area of QAU Islamabad for the treatment of wastewater.	39
4.1	Schematic illustration of overall treatment units of pilot scale TF system installed in work station located at the vicinity of Department of Microbiology, QAU Islamabad.	49
4.2	Determination of "k_{20} value" from the model calibration of BOD_{exp} vs Time data at 20±1°C.	51
4.3	Monthly variation in the percentage removal of fecal coliform by pilot scale TF system during entire treatment operation.	54
4.4	Efficiency of pilot scale TF system in the average reduction of BOD_5 during entire treatment operation.	60
4.5	Efficiency of pilot scale trickling filter system in the average reduction of COD during entire treatment operation.	60
4.6	Efficiency of pilot scale trickling filter system in the average reduction of TSS during entire treatment operation.	61
4.7	Efficiency of pilot scale trickling filter system in the average reduction of TDS during entire treatment operation.	61
4.8	Efficiency of pilot scale trickling filter system to improve DO concentration during entire treatment operation.	62

4.9 Variation in the concentration of pH of influent and effluent during various seasons/months. 62

4.10 Removal of BOD_5 by pilot scale trickling filter system in the month of November, 2014 (18±3°C). 68

4.11 Removal of BOD_5 by pilot scale trickling filter system in the month of December, 2014 (12±2°C). 69

4.12 Removal of BOD_5 by pilot scale trickling filter system in the month of January, 2015 (10±2°C). 70

4.13 Removal of BOD_5 by pilot scale trickling filter system in the month of March, 2015 (23±3°C). 71

4.14 Removal of BOD_5 by pilot scale trickling filter system in the month of May, 2015 (32±2°C). 72

4.15 Removal of BOD_5 by pilot scale trickling filter system in the month of June, 2015 (38±3°C). 73

5.1 Schematic illustration of flow of wastewater from different units during treatment operation. 75

5.2 Changes in the concentrations of carbonaceous pollutants (COD and BOD_5) and dissolved oxygen (DO) in the effluent during 15 weeks of operation through stone media pilot-scale trickling biofilter at 14.5-36°C. 78

5.3 Changes in the pH and salinity levels during 15 weeks of operation through stone media pilot-scale trickling biofilter at 14.5-36°C. 80

5.4 Changes in the concentrations of total dissolved solid (TDS), total suspended solids (TSS) and electrical conductivity (EC) in the effluent during 15 weeks of operation through stone media pilot-scale trickling biofilter at 14.5-36°C. 82

5.5 Changes in the concentrations of phosphates (PO_4), sulphates (SO_4) and total nitrogen (TN) in the effluent during 15 weeks of operation through stone media pilot-scale trickling biofilter at 14.5-36°C. 85

6.1 Efficiency of pilot scale TBF system in the average reduction of **(a)** COD, **(b)** TDS and **(c)** EC contents under different HRTs. 91-92

6.2 Efficiency of pilot scale TBF system in the average reduction of **(a)** PO_4 and **(b)** SO_4 contents under different HRTs. 93-94

6.3 Efficiency of pilot scale TBF system in the average reduction of **(a)** pH levels and **(b)** TN contents under different HRTs. 96

6.4 Efficiency of pilot scale TBF system in terms of pathogens removal under different HRTs. 100

7.1 Schematic illustration of containment structure of TBF system installed at residential area of Quaid-i-Azam University, Islamabad 102

7.2 Efficiency of integrated media TBF system in the average reduction of COD at different HRTs and flow rates ($Q = 0.004\ m^3/day$, $0.0072\ m^3/day$ and $0.01\ m^3/day$). 104

7.3 Efficiency of integrated media TBF system in the average reduction of TDS at different HRTs and flow rates ($Q = 0.004\ m^3/day$, $0.0072\ m^3/day$ and $0.01\ m^3/day$). 105

7.4 Efficiency of integrated media TBF system in the average reduction of EC at different HRTs and flow rates ($Q = 0.004\ m^3/day$, $0.0072\ m^3/day$ and $0.01\ m^3/day$). 106

7.5 Efficiency of integrated media TBF system in the average reduction of PO_4 levels at different HRTs and flow rates ($Q = 0.004\ m^3/day$, $0.0072\ m^3/day$ and $0.01\ m^3/day$). 107

7.6 Efficiency of integrated media TBF system in the average reduction of TN levels at different HRTs and flow rates ($Q = 0.004\ m^3/day$, $0.0072\ m^3/day$ and $0.01\ m^3/day$). 108

7.7 Variation in the pH value of influent and effluent samples at different HRTs and flow rates ($Q = 0.004\ m^3/day$, $0.0072\ m^3/day$ and $0.01\ m^3/day$). 109

8.1 Comparative assessment of stone media (SM-TBF) and integrated media (IM-TBF) pilot scale TBF system. 122

ACKNOWLEDGEMENTS

Praise be to Allah, Lord of the Worlds. The Beneficent, the Merciful, Who is the entire source of knowledge and wisdom endowed to mankind; Who gave me courage and potential to pursue this goal Whom I believe that He never spoils any effort of good deeds. Blessings of Allah be upon His Prophet Mohammad (P.B.U.H.), The City of knowledge and blessing for entire creature, Who has guided his Ummah to seek knowledge from Cradle to Grave, and enabled me to win honor of life.

I am really thankful to Dr. Rani Faryal, Chairperson of the Department Microbiology, for her appreciable cooperation, kind help and practical guidance.

It is a matter of great pleasure to express my sincere regards to honorable Prof. Dr. Safia Ahmed, for her affectionate supervision, inspiring attitude, masterly advice and encouragement, and for providing me all the privileges during my research work. Without her useful intellectual suggestions, it would have been impossible for me to complete this tedious work.

I would like to extend my thanks to all respected Faculty Members of Department of Microbiology, Quaid-i-Azam University, Islamabad, whose guidance and support cannot be repaid.

I'm highly thankful to Pak-US Collaborative Research Project entitled "***Small Scale Sewage Treatment and Wastewater Reuse System for Pakistan***" and also thankful to Higher Education Commission (HEC), Government of Pakistan, for awarding me a scholarship under the head of International Research Support Initiative Program (IRSIP), to carry out some part of my research work under the kind supervision of Dr. Devendra P. Saroj at University of Surrey, Guilford, UK.

Heartfelt mention goes to my fellow friends Dr. Iffat Naz, Imran Rabbani, Dr. Muhammad Rafiq, Dr. Muhammad Irfan, Abdul Haleem, Ghadir Ali for their intense practical help and valuable suggestions throughout my research work. I cannot forget their family like support, care and scholarly suggestions throughout my research period. I also offer words of thanks to lab attendants, technical and clerical staff for their friendly attitude, support and presence all the time.

Words fail to express the intensity of gratitude to my loving father (Mr. Abdul Latif) and younger brother (Mr. Abdullah), although they are no more with us and the missing gap which created in my life due to their departure will never be filled but their prayers and inspiration gave me the courage to dream high and opened up avenues for me to explore my abilities. My sweet and loving mother whose self-sacrificing love will always be appreciated but can never be repaid. I wish to express my heartfelt and warmest thanks to my beloved brother (Kashif Latif) and sister for their extreme support and caring attitude. I have no words to thank my wife, whose love and caring support in every aspect enabled me to achieve what my parents had inspired me to dream. I am extremely thankful to my both Kids, who has suffered a lot during my PhD thesis write-up stage but I try my best to do more for their bright future.

Abdul Rehman

ABSTRACT

ABSTRACT

Attached growth wastewater treatment systems are considered to be effective in developing countries due to their low energy, operational and maintenance cost. A trickling filter system is a type of sewage treatment facility which is used to minimize the levels of carbonaceous compounds in terms of BOD_5 and COD from wastewater in addition to pathogens and NH_4-N level.

The present research study was mainly focused on to treat domestic wastewater by pilot scale trickling filter systems. For this tenacity, two different types of pilot scale trickling filter (TF) systems i.e. one was installed in work station (under shade) located at the vicinity of the Department of Microbiology, QAU, Islamabad, Pakistan (setup-I) and the other one was installed in open environment at residential area of QAU, Islamabad, Pakistan (setup-II), were evaluated with varied temperature conditions, hydraulic load rates (HLR), organic load rates (OLR) and continuous recirculation of effluent over the top of filter bed.

In the 1st phase, efficiency of setup-I system was assessed for potential water reuse, and development of a zero order kinetic model defining the efficiency of carbonaceous and microbial pollutant removal under various prevailing temperature conditions. Operational data (both influent and effluent) of 330 days were collected from pilot scale TF system for various parameters. In rainy monsoon, autumn, spring and summer seasons, average percentage reduction were observed in the range of 52-72, 51-73, 18-69 and 74-89 % for BOD_5, COD, TDS and TSS respectively. However in winter season, a high percentage reduction was observed only for TSS (74-81%) while for other parameters like BOD_5, COD and TDS, it was found in the range of 13-50, 12-49 and 23-61% respectively. It was also observed that pilot scale treatment facility showed high efficiency in the removal of fecal coliforms i.e. 43-55 and 57-86% average percentage reduction were observed after 1st and 6th day of treatment respectively in all seasons of the year, indicating potential for reuse in irrigation. Moreover, 13 different bacterial species (*E. coli, Salmonella, Pseudomonas, Enterobacter, Klebsiella, Shigella, Proteus, Alcaligenes, Staphylococcus, Streptococcus, Micrococcus, Corynebacterium* and *Bacillus* spp.) were isolated from the microbial slime layer developed on the surface of stone media. The mathematical model developed based on data can be used to design and optimize low-cost TF systems aimed at water quality improvement for potential wastewater reuse in developing countries.

In the 2nd phase, a cost-effective and simple stone media pilot-scale trickling biofilter (TBF) was designed, constructed and operated in a continuous recirculation mode for wastewater treatment with a hydraulic flow rate of 1.2 L/min ($Q = 0.072\ m^3/hr$) and hydraulic loading (Q/A) of $0.147\ m^3/day$ for 15 weeks at a temperature range of 14.5-36°C. A substantial reduction in the average concentrations of different pollution indicators, such as COD (85.6%), BOD_5 (85.6%), TDS (62.8%), TSS (99.9%), EC (15.1%), PO_4 (63.22%), SO_4 (28.5%) and TN (34.4%), was observed during 15 weeks of operational period. Whereas a considerable average increase in the levels of DO (63.2%) was found after treatment of wastewater by the TBF system. No significant reduction in most probable number (MPN) index of fecal coliforms was observed in the effluent in first 9 weeks of operation. However, a significant reduction in the MPN of fecal coliforms was observed, i.e. 80-90% in the last few weeks of treatment.

In the 3rd phase, stone media setup-II TBF system was operated under different hydraulic retention time (HRT) i.e. 24, 48 and 72 hrs at a constant flow rate of 0.04 m^3/day and it was perceived that stone media pilot scale TBF system showed high efficacy regarding removal of physicochemical and microbiological parameters under different HRTs. It was observed that by increasing HRT up to 48 and 72 hrs, the proficiency of setup-II TBF system increased significantly to reduce different parameters i.e. COD (70.9%), TDS (34%), EC (23.5%), SO_4 (37%), PO_4 (81.8%) and TN (66.6%). Furthermore, it was suggested that greater retention time and sand filtration play a key role in the pathogens removal and improvement of water quality.

In the 4th phase, a new strategy was evaluated by using integrated media containing pebbles and gravels instead of stone to provide support for the growth of microbial slime layer. Furthermore, the integrated media TBF system was operated at three different flow rates (Q) i.e. 0.004 m^3/day, 0.0072 m^3/day and 0.01 m^3/day and on each particular flow rate, effluent was operated under three different HRTs i.e. 48, 72 and 96 hrs in order to determine the efficiency of integrated media pilot scale TBF system treating domestic wastewater. It was observed that integrated bed material with air space had optimistic effect over TBF operation and the lowest and intermediate flow rates (0.004 m^3/day and 0.0072 m^3/day) showed promising results with respect to percent reduction of different physicochemical parameters i.e. COD (74.2-80.5%), TDS (60.3-69.5%), EC (62.8-68.6%) and PO_4 (45.3-60.3%). A significant reduction in TN (59-63.3%) was observed at flow rates of 0.004 m^3/day and 0.0072 m^3/day. Moreover, it was observed that the efficiency of integrated media pilot scale TBF system in terms of pathogen removal (CFU/mL) increased significantly with continuous recirculation of wastewater for an extended period of time under different flow rates. From comparative assessment of stone media and integrated media pilot scale setup-II TBF system, it was evaluated that integrated media TBF system showed significant activity in the percent reduction of different physicochemical parameters as compared to stone media TBF system.

Thus, overall results suggest that pilot-scale TBF has a great potential to be transferred to decentralized treatment system for handling sewage of small communities in developing countries, in order to produce effluent of good quality, which can be safely used for irrigation as well as ornamental purposes.

Chapter 1

INTRODUCTION

1. INTRODUCTION

Wastewater is a broad term and has been classified into domestic wastewater and industrial wastewater. Domestic wastewater also sometime termed as municipal wastewater, is a type of wastewater produced by anthropogenic activities occurring in residential areas and mostly includes effluent from toilets, bathrooms, kitchens and recreational areas. While industrial wastewater refers to the wastewater generated during manufacturing activities within different industries and each industry produced its own distinct composite hazardous wastes. Wastewater produced from either source have prodigious adverse effects on aquatic as well as terrestrial life, because of suspended and dissolved contents that are in the form of biodegradable organics and nutrients such as nitrogen, phosphorus etc. Therefore, when discharge as such to the open environment, suspended solids may leads to the deposition of sediments. Whereas biodegradable carbonaceous compounds may lead to the reduction of dissolved oxygen contents and produce septic conditions. While the presence of nutrients in excess may facilitate eutrophication process in streams and lakes (Soller *et al.*, 2003; Tee *et al.*, 2016). Properly designed wastewater treatment systems will be required to improve the quality of wastewater and to eradicate the possible hazard of wastewater such as transmission of water-borne diseases, mutagenesis and high levels of acute toxicity (Soller *et al.*, 2003).

Pakistan, once a water-surplus country is now a water deficit country. In developing countries like Pakistan, water is in crises due to unavailability of fresh water reservoirs as a result irrigation is highly affected (Archer *et al.*, 2010). In order to meet the food requirements of increasing population of Pakistan more food is required that means additional fresh water resources. Now-a-days, most of the farmers used untreated wastewater to cultivate agricultural lands because they considered wastewater as a nutrients rich source (Hameed *et al.*, 2010). But unfortunately they have no idea that sewage water also contain toxic organic and inorganic compounds, heavy metals and fecal coliforms which have extremely adverse effect on environment, soil fertility and public health (Scott *et al.*, 2000). Therefore, wastewater treatment is required to minimize the level of these toxic and pathogenic elements in wastewater prior to its reuse for agricultural activities.

Beside agricultural usage, treated wastewater may also use for aquaculture, industrial practices and revitalizing ground water reservoirs (Skinner *et al.*, 2015). However, in Pakistan, lack of funds, government and public awareness about environmental and health risks, no proper sewage treatment facility is available. Different agencies like Environmental Protection Agency (EPA), International Water Management Institute (IWMI) in collaboration with government institutes takes steps to aware people about the threats of wastewater towards public health (Feenstra *et al.*, 2000).

Wastewater treatment methods vary from country to country throughout the world. It has been reported by Doorn *et al.*, (2006) that in most developed countries, sewage treatment and discharge schemes are clearly different among provincial and central area users, being more advanced treatment systems in the central area with respect to countryside. In developing countries, facility for sewage treatment variate according to the needs. Proper and well operated wastewater treatment facilities are available in few developing states like Latin America and Brazil, while in major developing countries like Pakistan, Nepal, Bangladesh, Africa and India, a bulk of untreated wastewater (both from municipal and industrial sectors) is released as such to the open environment (USEPA, 2012) which may adversely affect the environmental system. Furthermore, it has been reported by many researchers that a number of wastewater treatment systems are available now a days and each system has capability to remove toxic chemical and physical contaminants from wastewater up to the desired standard limits (Danish *et al.*, 2011; Ali *et al.*, 2012). Comparatively, biological wastewater treatment systems are considered to be more promising in terms of low operational cost and less hazardous impact on aquatic and terrestrial ecology (Daigger and Boltz, 2011).

Oxidation ponds are simple, cost-effective and environmental friendly suspended growth technology for the treatment of wastewater. This technology mainly removes carbonaceous compounds from wastewater in terms of BOD_5 before its discharge to downstream ecosystem. These ponds are aerobic in nature because it consists of circular shaped channels assembled with automated aeration machines, continuously providing aeration to the ponds and make alive aerobic bacteria to oxidize toxic organic compounds present in wastewater. One of the major drawback of oxidation ponds is that they required large land area, prolonged detention and sludge retention time due to continuous aeration mode of operation (von Sperling and de Lemos, 2005).

Beside this the activated sludge process is the most widely used technology throughout the world for the biological treatment of wastewater. This technology differs from oxidation ponds in that it also removes pathogenic microorganisms and suspended solids from wastewater in addition to soluble carbonaceous compounds (Mara, 2004; Dewil *et al.*, 2006). There are certain drawbacks of activated sludge treatment technologies i.e. their efficiency of removing pathogenic and indicator microorganisms are not constant and mostly dependent on different factors such as retention time, temperature, pH and concentration of dissolved oxygen (Doorn *et al.*, 2006). Moreover, inappropriate settling of suspended solids may leads to the expanded solid treatment cost otherwise, effluent of high solid concentration will be produced which offer life threatened risks to the downstream aquatic system and public health (Martins *et al.*, 2004; Kim *et al.*, 2010).

In attached growth wastewater treatment system, microbial population and their particulate materials attached to the suitable packing material by secreting extra polymeric substrates and covers the packing material in the form of biofilm (Metcalf and Eddy, 2003). A variety of support materials have been used in attached growth system as filter media and it includes, stones, plastic cubes, ping pong balls, polystyrene sheets and glass etc. (Kargi and Karapinar, 1997; Clifford *et al.*, 2010). These filter media have been considered as heart of the attached growth system as they provides a larger surface area per unit volume for the development of microbial slime layer. Therefore, selection criteria for packing materials must be accurate and precise so that a high active biomass and microbial population is sustained (Hu and Gagnon, 2006). Examples of attached growth system are trickling filter system, rotating biological contactors, fluidized bed biofilm reactors and sub-surface flow constructed wetlands (Henze, 2008; Loupasaki and Diamadopoulos, 2013). As compared with suspended growth system, fixed-film bioreactor provides high retention time due to high active biomass concentration and thus bear high organic loading rates (Amorim *et al.*, 2005). Moreover, it requires low space, less energy, less operational and maintenance cost which makes them an attractive option for the treatment of all types of wastewater (Vartak *et al.*, 1997; Perez and Griebenow, 2001; Cheng and Liu, 2002). However, the only drawback of attached growth system is that void spaces among filter media are easy to clog due to climate changes and production of excess biomass, therefore time to time washing of filter media is required (Wang *et al.*, 2005).

A trickling filter system is a type of attached growth treatment facility which is used to minimize the level of carbonaceous compounds in terms of BOD_5 and COD from wastewater in addition to pathogens and ammonia-nitrogen level (Chaudhary *et al.*, 2003; Kornaros and Lyberatosa, 2006). According to Metcalf and Eddy, (2003), trickling filter was first introduced in 1893 in England for the treatment of small community wastewater and since then, it had been modified with the passage of time for the treatment of both domestic and industrial wastewater. Initially rocks or slags were used as filter media in trickling filter system, but now-a-days several types and shapes of packing materials are available in the market to be used as filter bed in trickling filter system for the development of biofilm.

Trickling filter (TF) system has three basic structural units i.e. "distribution system", as the name indicates used for distributing wastewater to the TF system, "filter media" (usually stones) used to support microbial growth and "underdrain system" to collect treated effluent. It has been reported that TF systems produce high quality effluents (e.g. < 20 mg/L BOD_5) without taking up a large space or using high amounts of electrical energy. It comprises a bed of stone covered with microbes, which played vital role in the oxidation of organic compounds present in wastewater as it percolates through it (Donlan and Costerton, 2002). Aeration is provided through continuous recirculation of wastewater to the bed of TF system (Pal *et al.*, 2010). The effluent after treatment has been allowed to settle down by gravity for a specific period of time and then released to the downstream aquatic ecosystem. It has also been reported that with the passage of time, biofilm over stone media in TF thickens which may lead to the decrease in metabolic activities associated with substrate mass-transfer limitation of microorganisms within biofilm (Metcalf and Eddy, 2003). Trickling filter system produce a small amount of sludge during treatment, which is removed in the secondary clarifier to produce a quality effluent suitable for agricultural and horticultural practices (Tawfik *et al.,* 2006).

In the present research study, it was planned that two different types of pilot scale trickling filter (TF) systems would be evaluated with varied environmental conditions i.e. one was installed in work station located at the vicinity of the Department of Microbiology, QAU, Islamabad, Pakistan and the other one was installed in open environment at residential area of Quaid-i-Azam University, Islamabad, Pakistan. As Pakistan's temperature varies a lot during the year and especially in Islamabad, a humid subtropical climate exists with five different seasons comprising winter (November, December, January and February), spring (March and April), summer (May and June), rainy monsoon (July and August) and autumn (September and October). Therefore, it was aimed to comprehend the philosophy of these pilot scale TF systems in the treatment and potential reuse of wastewater collected from residential area of Quaid-i-Azam University, Islamabad, Pakistan. Data was collected for carbonaceous and microbial loads for safe reuse of effluent in ornamental, recreational, horticultural as well as agricultural activities.

Aim and Objectives

The aim of the current research study is to determine the efficiency of pilot scale attached growth systems (trickling filters) with respect to wastewater treatment. While, the objectives of the current research study are;

1) To design and construct efficient pilot scale trickling filter systems consisting stone as filter media for the treatment of domestic wastewater.
2) To get optimum function of pilot scale trickling filter systems, different temperature conditions, hydraulic load rates (HLR), organic load rates (OLR) and continuous recirculation of treated water will be considered.
3) Characterization of microbial slime layer developed on stone filter media by conventional methods, wet-weight and dry-weight methods.
4) To evaluate the treatment efficiency of pilot scale trickling filter systems in terms of physicochemical (COD, BOD_5, TN, NO_3, NO_2, SO_4, PO_4, TSS, TDS) and microbiological (MPN/100 mL index and CFU/mL) parameters under different seasonal conditions.
5) To constitute a mathematical model for the optimum design and operation of pilot scale trickling filter systems by assuming the reduction of carbonaceous compounds (BOD_5) during wastewater treatment.

Chapter 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 Overview of Water Crises and Searching for Alternate Resources

Fresh and clean water is important for the existence of human beings, plants and animals on earth's planet, which offers numerous outfits such as water for household consumption, water for recreational activities, water for irrigation purposes and water for neutralizing streams (Aylwards *et al.*, 2005). About 97% of the earth's surface is covered by salt water of oceans while the rest 3% is found in iceberg, glacial mass or underground and difficult to be available for routine usage. Only minor fraction of fresh water (about 0.4%) is accessible for human consumption, which is usually sustained by natural water cycle (Abderrahman, 2000). It has also been reported that in the era of 21st century, water will be one of the rarest resources on the earth's planet due to substantial escalation in human population, which in turn greatly affect aquatic and public health, narrowing socio-economic and agricultural growth/production especially in developing countries (Bartram and Balance, 1996; Montgomery and Elimelech, 2007).

Water is an indispensable and vital element for endurance of all living beings, as plenty of fresh and clean water is required for their growth. According to survey report conducted by WHO in 2015, about 3.4 million people (mostly children less than five years) die per year in developing countries due to unhygienic conditions, poor sanitation and water-borne diseases (Andersson and Bohan, 2001; Craun *et al.*, 2006; Jury and Vaux, 2007; WHO, 2015). Water crises is a global issue now-a-days and it has been reported that one in every five persons had no way to reach clean and renewed water resources (Jury and Vaux, 2007). In developing countries, about 50% of community have no access to desired standard sanitation (Jayakumar *et al.*, 2009). Food and Agriculture Organization of the United Nations reported that in many parts of Asia, more than 800 million of population face severe water shortage i.e. possibility of fresh and potable water revenue would be less than 1500 m^3 per person per year, which might be expected to reach more than 2.5 billion of population in 2025 (Connor *et al.*, 2017). In order to overcome these challenges of water shortage, there is a need to establish functionable wastewater treatment schemes and projects especially in developing countries (Yang and Abbaspour, 2007). Though, the use of treated domestic wastewater effluent comes into three different sections i.e. **a)** Plain effluent reuse, where the effluent of one system to be used will be the influent of other system with or without supplementary treatment; **b)** Programmed usage of treated water, where the untreated wastewater first goes to the treatment facility such as trickling filter system, constructed wetlands, lagoons and then after treatment, discharge for further reuse; **c)** Haphazard water reuse, where the wastewater mixes into naturally occurring surface water reservoirs, acting as a source of water for other usages (Asano *et al.*, 2007; USEPA, 2012).

2.1.1 Water and Wastewater Scenario in Pakistan

Pakistan's total geographical and territorial area is 7,96,100 km^2 out of which 27% land has been used for irrigation purpose however, the complete data of Pakistan's irrigation is still not clear i.e. some reports suggested that irrigation has been done on more than 70% out of total land area (Ahmad *et al.*, 2009). Water erosion due to salinity and water shortage are the two key influences that expose the feasibility of irrigated agricultural land and effect yields production in Pakistan. Numerous exertions such as drilling tube-wells, discharge of salts by excess irrigation, use of fertilizers and expensive chemicals and physical methods have been performed to resolve water erosion and salinity issues in Pakistan, but still no progress has been observed in agricultural sectors (Salma *et al.*, 2012). Chairman of PASA (Pakistan Agriculture Scientists Association) in 2013 while conducting 30 days trial stated that less than 10% of clean and fresh water reservoirs are available for drinking and other routine activities in Pakistan while the remaining water reservoirs are either exploited or used for irrigation as shown in Fig. 2.1 (https://nation.com.pk/30-Dec-2013/water-storage-capacity-just-for-30-days). According to Hussain *et al.*, (2013) more than 33% of population in Pakistan have no access to safe and clean water reservoirs as a result more than 700 children lost their life per annum due to water-borne diseases such as cholera, typhoid, diarrhea and polio, which is a serious issue to be resolved.

In many urban areas of Pakistan, domestic wastewater is used to nourish agricultural land due to unavailability of ground and surface water reservoirs. The use of wastewater for agricultural activity is an approach to utilize municipal wastewater with considerable benefits i.e. it is rich in nutrients, which plants required for their growth and moreover, it has capability to increase soil fertility instead of using fertilizers, which makes irrigation cost effective (Viet Anh *et al.*, 2005; Morel, 2006; Asano *et al.*, 2007; Hameed *et al.*, 2010; Kalavrouziotis and Arslan-Alaton, 2008; Becerra-Castro *et al.*, 2015). Aside from these benefits, sewage water also contain toxic compounds, heavy metals and highly pathogenic microorganisms which have extremely adverse effects on the terrestrial and aquatic ecology, environment and public health (Scott *et al.*, 2000; Soller *et al.*, 2003). A set of standard guidelines had been proposed by WHO in 1989 for the harmless use of wastewater in agricultural sectors in order to secure agrarians and consumers of the said crops irrigated with wastewater. Therefore, it is essential to develop a suitable wastewater treatment technology in order to reduce the concentration of life-threatened components of sewage before its usage in agricultural lands. However in many developing countries including Pakistan, sewage water is still used as such or after initial treatment for horticulture and agricultural activities, so there is a growing demand to aware people about risks of raw sewage to the environment and public health (Feenstra *et al.*, 2000). Different international organizations in collaboration with national institutes now start step to aware people and also government of Pakistan about these serious issues of reclaimed wastewater and their possible treatment in the country region (Feenstra *et al.*, 2000).

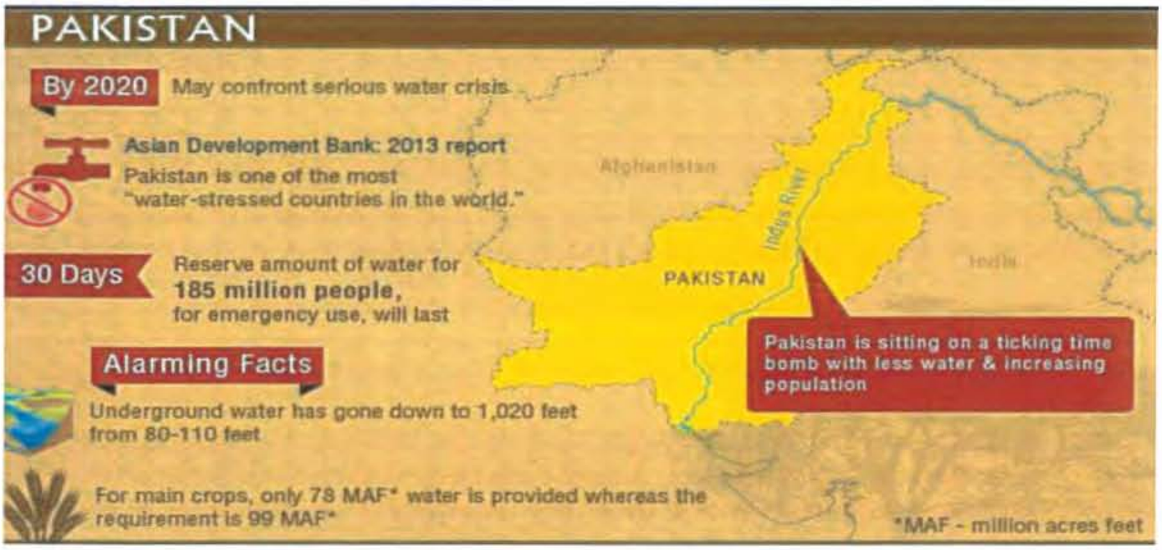


Fig. 2.1 Water crises and alarming facts of water reservoirs in Pakistan (Adopted from https://www.mapsofworld.com/around-the-world/water-crisis.html).

2.2 General Description of Wastewater and its Treatment; Advancement and Challenges

Wastewater is generally defined as a mixture of liquids, polluted with attenuated contents released from household, recreational, trading and industrial sectors (Zhou and Smith, 2002). In addition to this, it may also sometimes contain flooded as well as unclean surface water (Cheremisinff, 2002). Depending on the sources of production, wastewater has been classified as industrial or municipal wastewater. As the name indicates, industrial wastewater is a type of water generated during manufacturing processes going on in industrial division while the later one is a type of sewage spawned from domestic sites including houses, commercial areas and institutes. Chemistry of both of these are quite different from each other i.e. domestic wastewater mostly contain carbonaceous compounds such as carbohydrates, proteins, oil and greases etc. while each industry produced its own individual complex harmful wastewater mostly depending on the activities performed within industries (Cheremisinff, 2002; Zhou and Smith, 2002). Generally wastewater contain 99.6% water and 0.4% solids (USEPA, 2007). The solid part of wastewater is further divided into biodegradable organics (70%) and inorganics (30%) portion. The biodegradable organics in wastewater are usually composed of proteins, carbohydrates (starch, cellulose), fats, oils and grease etc. while the inorganic portion of wastewater is composed of sediments, salts and metals (Tebbutt, 1998).

2.2.1 Chronological Outlook of Wastewater Treatment

In the start of 20th century, there was an outbreak of life-threatened water-borne diseases and most of the scientist at that time put forward to determine the actual cause and they concluded that it was due to microorganisms present in wastewater that seepage into naturally occurring drinking water reservoirs (Henze and Harremoes, 1983). In order to overcome these issues, they started chemical treatment of wastewater in an open fields, but soon they realized that this type of sewage treatment would affect the fertility of soil. Subsequent endeavors were made to withdraw raw wastewater directly into running streams but it was observed that it deteriorate quality of existing water. Furthermore, as time goes on, there was a demand to develop suitable methods for wastewater treatment, as population and urbanization increased day by day due to which production of sewage water also increased (Rosen *et al.,* 1998; Spellman, 2000). Therefore, advanced methods were established for the treatment of wastewater in the late 1990s with almost the same objectives as in the start i.e. remove suspended and dissolved contents from wastewater which make them toxic and also eradicate disease causing pathogenic microorganisms from wastewater. Later on, further initiatives were made to remove nutrients such as NO_3, NO_2 and PO_4 etc. from wastewater in order to enhance the quality of treated effluent (Barbose and Sant'Anna, 1989; McGhee, 1991; Rowe and Abdel-Magid, 1995; Metcalf and Eddy, 2003).

2.2.2 Why to Treat Wastewater?

Treatment of wastewater is one of the ultimate processes to eradicate possible toxicity of compounds present in wastewater. As in this process, multifarious carbonaceous compounds either by physico-chemical or biological means converted into simpler composites that are non-toxic and safe to the aquatic ecosystem (Cheremisinff, 2002). On the other hand, if untreated waste effluent is discharged as such to the environment, it may leads to the emission of putrid gases, reduction of dissolved oxygen contents from desired level required by aquatic organisms, eutrophication of the lakes and streams due to the presence of excessive nutrients which facilitate the growth of unnecessary aquatic plants and algae. Moreover, it contains pathogenic microorganisms belonging to enterobacterace family and are responsible for variety of water-borne diseases in living beings. Because of these reasons, wastewater treatment must be performed to produce an effluent suitable for agricultural or aquaculture reuse, or to produce an effluent that can be safely discharged into ground or surface water reservoirs (Spellman, 2000).

2.2.3 Essential Unit Operations in Wastewater Treatment

As mentioned earlier, treatment of wastewater can be done either by physico-chemical methods or by means of microorganisms (biological method) to achieve suitable treated effluent that fulfill the desired standard limits proposed by WHO and EPA for reuse of treated wastewater (Pol and Lettinga, 1986; Prabu *et al.,* 2011; USEPA, 2012; WHO, 2015). Examples of physical processes involved in wastewater treatment system are screening by screen bars to remove large suspended solids and particulate matters, mixing, flocculation, sedimentation and physical filtration (Metcalf and Eddy, 2003), while precipitation and adsorption are the examples of chemical unit processes used in sewage treatment (Lemji and Eckstadt, 2013). In case of biological methods, the primary goal is to eradicate biodegradable carbonaceous compounds in wastewater along with nutrients like phosphate, sulphate, nitrate and nitrite by means of microorganisms (Rosen *et al.,* 1998). Although, all these methods are an important unit of wastewater treatment systems but among them biological processes are the best option to treat wastewater especially in developing countries due to their low operational cost, low maintenance requirements and environmental friendly nature (Shalaby *et al.,* 2008).

These different treatment units provide diverse levels of treatment i.e. preliminary, primary, secondary and tertiary wastewater treatment. In preliminary wastewater treatment, substances such as dead animals, tree branches, wood, plastics and rags that causes damage to the treatment design and operational process, are removed by physical mean. It also removes organic and inorganic substances such as oils and greases from sewage thereby contribute to reduce BOD_5 up to 10-25% (McGhee, 1991; Cheremisinff, 2002) while, in primary treatment operation, suspended solids and other organic matters are removed by physical sedimentation process (Cripps and Bergheim, 2000; Spellman, 2000; Lu *et al.,* 2003; Koh *et al.,* 2004). Both of these treatments are categorized together under the term primary treatment, which acts as a forerunner to secondary treatment (Barbose and Sant'Anna, 1989; McGhee, 1991). Secondary wastewater treatment involves oxidation of carbonaceous compounds present in wastewater (coming from primary treatment unit) through biological means under both aerobic and anaerobic conditions. In these treatment units, microorganisms play a vital role to oxidize organic compounds present in wastewater and hence produce an effluent that fulfill standard limits (Henze and Harremoes, 1983). Tertiary treatment is an advanced wastewater treatment system used to further purify effluent of secondary treatment units (Mann *et al.,* 1998). In tertiary treatment process, excessive nutrients and organic compounds has been removed up to some extent but this method is most commonly used to kill and destroy pathogenic microbes from wastewater by chemical disinfection, flocculation, sedimentation and chlorination methods (Pal *et al.*, 2010; Prabu *et al.*, 2011).

2.2.4 Biological Wastewater Treatment Technologies

The existence of well-designed and operated wastewater treatment systems are very rare especially in developing countries due to lack of funds, lack of well-established pollution control laws and lack of expertise towards these environmental issues. However, a wide-range consolidated wastewater treatment facilities are available in developing countries which includes stabilization ponds, activated sludge, constructed wetlands and trickling filter system (von Sperling, 1996). Stabilization ponds are aerobic in nature and primarily remove organic compounds present in wastewater by their aerobic oxidation process. Moreover, it has been reported that most of these ponds removed 80-85% BOD_5, 40-45% ammonia and 70-95% indicator organisms from wastewater (Shuval *et al.*, 1986). However, the main drawback of stabilization ponds is that they required larger space and extended retention time due to continuous mode of aeration (von Sperling and de Lemos, 2005). On other side, activated sludge process is also a suspended growth process in which microbial suspension is used for the removal of carbonaceous compounds, nitrogen and phosphorus (Mara, 2004; Dewil *et al.*, 2006), but there are certain shortcomings of this process i.e. removal efficiency of pathogenic indicators is not constant and also settling of suspended solids are inappropriate which in turn enhanced treatment cost (Doorn *et al.*, 2006).

Constructed wetlands are ecological contrived systems that consume the natural processes comprising wetland foliage, territories, and the accompanying microbial biofilm to treat wastewaters (Vymazal, 2005). It usually provide two types of treatment systems i.e. free water surface system and sub-surface flow system. The main downsides of constructed wetlands are that they required large land, continuous monitoring of operating units and sludge removal. As compared to other conventional treatment methods, fixed-film bioreactors i.e. trickling filter provides extended retention time due to high active biomass concentration and thus bear high organic loading rates (Amorim *et al.*, 2005). Moreover, it requires low space, less energy, less operational and maintenance cost which makes them an attractive option for the treatment of all types of wastewater especially in developing countries (Vartak *et al.*, 1997; Perez *et al.*, 2001; Cheng and Liu, 2002).

2.3 A Comprehensive Review of Attached Growth Systems

Attached growth process is a well-developed technology as it permits high retention time and population dynamics. In this technique solid media provides a surface for the attachment of biofilms, as a result the efficiency of attached growth bioreactor increased in the removal of carbonaceous compounds from wastewater (Loupasaki and Diamadopoulos, 2013). In addition to this, biofilm systems have advantages of being eco-friendly, offers operational flexibility, adjustable hydraulic retention time (HRT), flexible to environmental changes and nature of the contaminant. Furthermore, the rate of degradation of organic pollutants increased by the increase in concentration of active biomass while, the slow growth of microbes results in less sludge production (Chen and Chen, 2000; Mahmoud *et al.*, 2010). Biofilm systems also take the advantage of removing the pollutant by employing a number of mechanisms such as bio-sorption, biodegradation, bioaccumulation and biomenralization (Pal *et al.*, 2010).

Attached growth reactors include rotating biological contactors, moving bed biofilm reactors and trickling filter systems (Henze, 2008; Loupasaki and Diamadopoulous, 2013). Rotating biological contactors (RBC) consists of a shaft having plastic discs stacked over the shaft. These plastic discs provide a surface for the growth of microbial slime layers which in turn play a vital role in the clarification of wastewater by utilizing and eliminating toxic hazardous waste (Kadu *et al.*, 2013). About 40% of discs are submerged in the water collection tank and shaft rotates at speed of 1-2 rpm. Kadu *et al.*, (2013) also reported that RBC consume more than 20% electric energy as compared to other conventional wastewater treatment technologies, moreover a single contactor is not enough to meet the strict discharge limits so a series of contactors are installed at a time that makes process expensive with respect to cost. Furthermore, proper maintenance of rotating shaft and discs will also be required to get maximum efficiency of RBC in terms of wastewater treatment. Due to these limitations and high energy demands, rotating biological contactors are not effective for the treatment of the wastewater in developing countries (Ramsay *et al.*, 2006).

Norwegian company in 1981, introduced the first use of moving bed biofilm reactors (MBBR) for the treatment of wastewater. MBBR works in similar mode as that of activated sludge reactors, but the only difference is that MBBR required presence of small particles that flow with water. Moreover, it provides better oxygen transfer rate even at low hydraulic retention time as compared to other conventional sewage treatment technologies (Chan *et al.*, 2009). Now-a-days, both aerobic and anaerobic MBBRs are in use worldwide for the treatment of wastewater and hence play a significant role in the removal of carbonaceous as well as nitrogenous and other hazardous compounds by employing oxidation, reduction, nitrification and denitrification processes (Hosseini and Borghei, 2005). The only drawback of MBBR is that they are highly expensive as compared to other traditional wastewater treatment technologies. Moreover, Aygun *et al.*, (2008) also reported that MBBRs only work at low organic loading rate, requires a high retention time and recirculation of the effluent.

Among all these reactors, trickling filter systems are supposed to be the most effective in terms of wastewater treatment in developing countries, as it requires low energy and maintenance requirements, easy to operate and have low operational cost as compared to other treatment technologies. Because of these advantages, trickling filter systems can be employed in a number of industries to remove toxic carbonaceous compounds from wastewater (Chowdhury *et al.*, 2010). This system also provide additional advantages over suspended growth systems of being simple in design, easy to construct, low space and maintenance requirements (DeFilippi *et al.*, 1998). Furthermore, it also plays an important role to achieve the goals of high biodegradation, high contact time between the wastewater and microbes, stable process and less washout of the cells (Loupasaki and Diamadopoulous, 2013).

2.4 General Outlines of Trickling Filter System: Process Design and Operation

Trickling filter system is a type of attached growth process, in which wastewater influent trickle down over the surface of packing material also termed as filter media, as a result organic compounds and other nutrients are ingested and utilize by the biomass grown on filter media. This is the main principle or mode of action of trickling filter system towards treatment of wastewater (Wik, 1999). It usually comprises of three main components that is distribution system, filter media and underdrain system as shown in Fig. 2.2. Distribution system maintains the hydraulic load by spraying and distributing the wastewater over the filter media. Wastewater trickles over the filter media and is collected in the underdrain system (Lekang and Kleppe, 2000; Soller *et al.*, 2003). Underdrain system receives treated waste and a fraction of biosolids i.e. sludge. Among these, filter media is an important part of trickling filter system because it provides a surface for the attachment of microorganisms in the form of extracellular matrix or layers, which then play a vital role in the removal of carbonaceous compounds from wastewater (Donlan and Costerton, 2002).

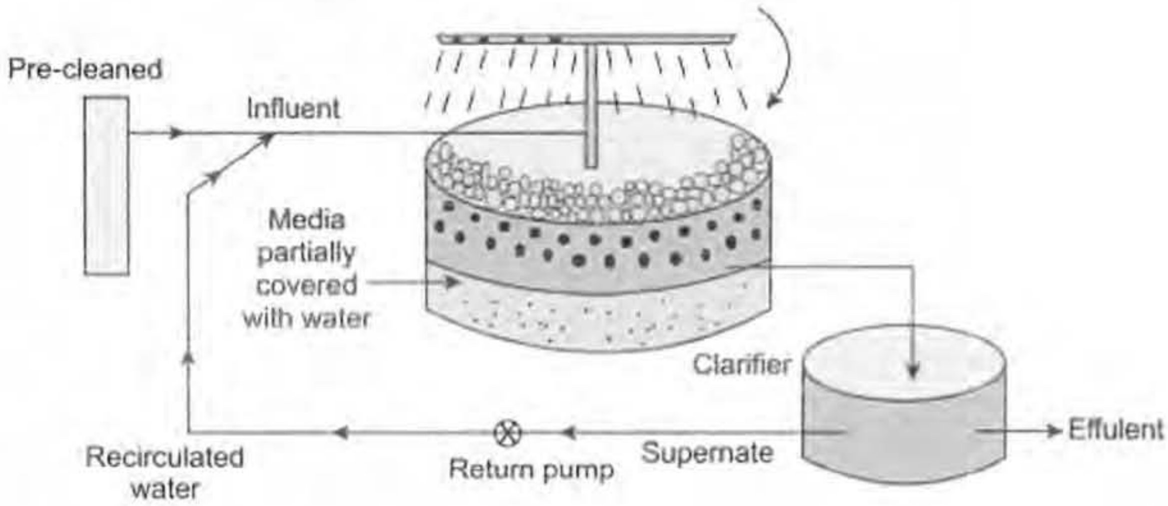


Fig. 2.2 Process design and operation of Trickling Filter System (Adopted from Krishna *et al.*, 2017).

Trickling filter system have a number of advantages over other biological systems however, it has certain limitations especially when require further treatment of the effluent for the removal of remaining organic compounds and pathogenic organisms (USEPA, 2012). Moreover, trickling filter system shows variation in their performance under different temperature and seasonal conditions. Thus such problems can be overcome by designing a system that can compensate with such limiting factors. For the designing of an absolute system, a thorough understanding of the microbial profile is necessary (Wagner and Loy, 2002). The most important thing is to correlate the composition of the wastewater and structure of the microbial communities for the process of nitrification, denitrification, oxidation of organic compounds, sulphur and phosphorus removal. Both structure and composition of the wastewater are important parameters in determining the efficiency, stability and robustness of the system (Wanger *et al.*, 2002).

Trickling filter was first introduced in 1893 in England for the treatment of small community wastewater but it had various design and operational complications like clogging of filter media due to frost in winter season which greatly affect its efficiency towards wastewater treatment (Metcalf and Eddy, 2003; Tchobanoglous and Burton, 1991). Its design was still unpredictable until the mid of 1950s, since then, it has been modified with the passage of time for the treatment of both domestic and industrial wastewater (Eckenfelder, 1961; Germain, 1966). In the start of 1970, USEPA stated that trickling filter had a problem to produce effluent that did not fulfill the desired standard limits for reuse due to immature and poor design of secondary clarifier (Parker *et al.*, 1999). After this statement, numerous researches were conducted to resolve this issue. In 1982, a group of scientists introduced a full scale trickling filter-solid contact process in which they used aeration basin and well-designed secondary clarifier in order to improve the quality of trickling filter treated effluent (Norris *et al.*, 1982).

A considerable amount of suspended solids would be produced during treatment of wastewater by trickling filter system. Therefore, there is a growing demand to separate solids from liquid which can be easily achieved by installing globular or quadrilateral secondary clarifiers/sanitizer. Moreover, trickling filter system stereotypically comprises of wastewater distribution system, support material/carrier for biofilm development, underdrain system, recirculation pump unit and secondary clarifiers (Daigger and Boltz, 2011).

2.4.1 Distribution System

Effluent from primary treatment unit is pumped to the distribution system of trickling filter system either by gravitational force (hydraulically driven) or by means of electric pumps. From distribution system, wastewater is sporadically distribute over the bed of trickling filter system. In order to get maximum efficiency of trickling filter system, influent distribution system will be efficient so that filter media must be properly wet otherwise, inappropriate wetting of filter media may lead to the formation of inadequate treatment regions. In case of trickling filter system, majorly two types of distribution systems are used i.e. fixed-nozzle and rotating arm distributor. Rotating arm distributors are most commonly used in pilot scale trickling filter system because they provide sufficient distribution of wastewater to the filter media as compared to fixed-nozzle distributors. Fixed nozzle distributors usually consist of circular shape holes along with upside down hook in the center to facilitate flow of wastewater. A major drawback of fixed nozzle distribution system is that it requires an extensive piping system to assure uniform distribution of wastewater.

On other hand, a rotating arm distributor used less piping system (usually 2 or more) as compared to fixed nozzle distribution system. The wastewater is fed from the column through the horizontal pipes and is distributed over the media through orifices located along the side of each of these pipe. Rotation of the arms is due either by hydraulically driven or by means of electric pumps. Hydraulically driven rotary distributors use back-spray vents, or contrary propelling jets, to minimize the speed of arm rotation in order to uphold the desired flow rate of wastewater. Fig. 2.3 shows both hydraulically and electrically driven rotary distributor that uses flexible-speed drive and electronic controller to provide optimum and controllable speed.

Fig. 2.3 Hydraulically driven rotary distributor (**L**); Electrically driven rotary distributor (**R**) (Adopted from https://www.environmental-expert.com/products/westech-biodoc-trickling-filter-rotary-distributor-2992).

2.4.2 Biofilm Support Materials/Carriers

Filter media is the heart of trickling filter system as it provides a specific surface area for the growth of biomass (Verma *et al.,* 2006). Selection of filter media or carrier for the development of biofilm in trickling filter system is critical, because different parameters or characteristics of support materials would be considered such as durability to resist toxins and shock loads, greater specific surface area for biomass, must be economically feasible and have high void ratio to evade clogging and ponding issues and also to facilitate aeration (Soller *et al.,* 2003; Christensson and Welander, 2004). Examples of carrier to be used as filter media in trickling filter system are; stones, rocks, variety of plastics sheets (random, vertical flow and cross-flow) of synthetic nature, polystyrenes, rubbers etc. Beside these, there are also some other commercially available synthetic media such as hanging plastic strips, wooden slats and plastic rings, but these media are highly expensive that's why rarely used in trickling filter system. Moreover, variety of filter media application in attached growth wastewater treatment system had been reported by different researchers in the past (Table 2.1).

Table 2.1 Application of different types of filter media in wastewater treatment system.

Source	Carrier	Experimental parameters	Major Observations
Wang *et al.*, (1995)	Anthracite, Sand, GAC	TDS, total organic carbon, Biodegradable organics.	GAC media facilitate growth of microbes as compared to anthracite and sand media.
Boon *et al.*, (1997)	Granite	BOD_5, TSS, NH_4	Efficiency of bioreactor depends on OLR, shape of filter media and environmental conditions.
Carlson and Amy, (1998)	Anthracite	Biodegradable and Dissolved organic carbon	Anthracite had capability to resist high hydraulic load rates.
Hozalski and Bouwer, (1998)	Glass beads, sand	Total organic carbon	Back wash with water did not affect the growth of slime layer.
Yang *et al.*, (2001)	Plastic sheets	BOD_5, TSS, Nitrate, Nitrite, Phosphate	Plastic sheets provide greater surface area and capable to resist toxins and shock loads.
Rehman *et al.*, (2012)	Plastic balls	BOD_5, COD, TDS, TSS, Nitrate, Nitrite, PO_4, SO_4	Plastic balls provide greater surface area for microbial growth which enhance lab scale reactor efficiency.
Khan *et al.*, (2015)	Stones	BOD_5, COD, TDS, TSS, Nitrate, Nitrite, PO_4, SO_4	Stone being a natural media improve lab scale reactor efficiency.
Naz *et al.*, (2016)	Stones, Plastic balls, polystyrene packing materials	Biofilm development process	Evaluate chemical nature of support materials and their effects on biofilm development process.

Naturally occurring media such as stones and rocks are commonly used in pilot scale trickling filter system for the treatment of different types of wastewater. These naturally occurring media are available in different sizes, shapes and have numerous rough surfaces which facilitate the growth of microorganisms. Generally rock filter media provides a low specific surface area for the development of biofilm. Moreover, it has been reported that due to low void spaces and larger unit mass of rock media, trickling filters are often facing reduced oxygen diffusion rate, clogging and ponding problems at high organic and hydraulic loading rates under different seasonal conditions (Grady *et al.,* 1999). Therefore, to enhance the proficiency and productivity of trickling filter using rock as a filter media, proper aeration and adjustable speed of rotary arm distributors would be required along with well operated secondary sedimentation tank (Boltz *et al.,* 2006). In situation where maximum efficiency is estimated from trickling filter system to treat rich organics wastewater, rock media will be replaced by synthetic media because they offer high void ratio and larger surface area for the growth of biomass. However, Grady *et al.,* (1999) concluded that for wastewater having low concentration of organics (< 1 kg.d^{-1}.m^{-3} BOD_5), well-designed and operated rock media trickling filter showed almost similar treatment efficiency as synthetic media trickling filter.

On other hand, several synthetic media such as plastic sheets, polystyrene packing materials etc. are exclusively used in advanced trickling filter systems because they provide a larger surface area as compared to stone/rock media for the development of biofilm. Moreover, they also possess wide emptied spaces to facilitate efficient organic and hydraulic loading rates, substrate-mass transfer rate and enhanced oxygen diffusion co-efficient (Bryan and Moeller, 1960; Harrison and Daigger, 1987; Parker and Bratby, 2001). Generally plastic media are accessible in diverse arrangements i.e. vertical flow, cross-flow, pierce form, quadrilateral and flat segments (Harrison and Daigger, 1987; Parker and Bratby, 2001). These different types of plastic media are reported to effectively removed BOD_5, COD, TDS and TSS from wastewater after systematic treatment.

Synthetic media as lighter in weight provide larger surface area and void space, therefore trickling filter using synthetic media could be constructed in a variety of dimensions and depth as compared to rock media trickling filter, to facilitate high organic shock loads. Selection of synthetic media, their size, shape and cost would be critical parameters as it may greatly affect the efficiency of trickling filter system. Synthetic media are available in both vertical and cross flow patterns which effectively removed carbonaceous compounds and suspended solids from wastewater (Aryan and Johnson, 1987; Harrison and Daigger, 1987). Furthermore, Harrison and Daigger, (1987) reported that the performance of cross flow synthetic media trickling filter is high as compared to vertical flow units at moderate organic loading rate (1-2 $kg.d^{-1}.m^{-3}$ BOD_5) whereas, the treatment efficiency of vertical flow trickling filter is high when the concentration of carbonaceous compounds in wastewater is high (> 5 $kg.d^{-1}.m^{-3}$ BOD_5). Mann and Stephenson, (1997) used granular type media (floating and sunken media) in trickling with aiming to remove colloidal as well as suspended solids from treated effluent in considerable amount. They observed that both of these media were highly effective during treatment operation and removed 65-80% COD from wastewater at hydraulic rate of 3.3 ml/s. Furthermore, Szogi *et al.,* (1997) used gravel as a support material in trickling filter system to treat wastewater of pigs form house and observed considerable reduction in COD, TSS and phosphate concentration after treatment. As mentioned earlier that selection of biofilm carrier is an important parameter in attached growth process therefore a group of scientists used clay and plastic media of different size and shape in nitrifying trickling filter system and observed profound results (Lekang and Kleppe, 2000). Moreover, Park *et al.,* (2006) used rubber as a filter media in three different types of wastewater treatment plants and checked the treatment efficiency of these plant and found efficient results. They also carry out toxicity analysis of treated effluent and found that rubber is non-toxic and suitable to be used as filter media in future sewage treatment plants. A variety of support materials have been reported to be used in attached growth system as filter media and it includes, stones, plastic balls, rubber, polystyrene packing materials, gravels and pebbles, which provides a larger surface area per unit volume for the development of microbial slime layer (Kargi and Karapinar, 1997; Clifford *et al.,* 2010; Rehman *et al.,* 2012; Khan *et al.,* 2015; Naz *et al.,* 2015; Rasool *et al.,* 2018).

2.4.3 Underdrain System

Underdrain system is a pierced support located at the bottom of trickling filter system that not only collect treated effluent, excess biomass and sludge but also provides a gangway for air at high filtration rate. A variety of materials are used to construct underdrain system and they usually include stainless steel tank, concrete and wooden blocks (Daigger and Boltz, 2011). As most of the trickling filters are planned to recirculate wastewater for specific period of time in order to keep filter media wet, in this case underdrain system also act as a recirculation tank. With the passage of time, the organisms in slime layer continuously grow due which biofilm becomes thick and hence anaerobic condition will form in the bottom of filter bed (USEPA, 2012). Due to successive microbial growth, production of secondary waste products and high hydraulic load often leads to the detachment of loosely bound surface organisms. The underdrain system collect these biosolids along with effluent and then transport it towards the final clarifier where it has been separated from effluent by sedimentation process (Grady *et al.,* 1999; Heeb *et al.,* 2012).

2.4.4 Recirculation Pattern

Recirculation is an important phenomena in trickling filter system as it improve the quality of treated effluent by minimizing the concentration of organic and pathogenic wastes in wastewater and also keep desired pumping rate. In trickling filter system, two different types of recirculation patterns are used i.e. optional recirculation of effluent and indirect recirculation of wastewater containing sludge through primary or secondary clarifier as shown in Fig. 2.4. Normally effluent from primary and secondary clarifier promote the efficiency of trickling filter system towards treatment of wastewater, but it must be kept in mind that recirculation rate required to wet filter media must be equal to that of hydraulic flow rate otherwise the performance of reactor will be greatly affected. Albertson and Eckenfelder, (1984) reported that maximum efficiency of trickling filter system would be achieved by keeping recirculation and distribution rates in desirable limit as it may greatly influence the growth of microbial slime layer on filter media. The desired recirculation rate for natural and synthetic media tricking filter to remove maximum organic compounds from wastewater is in the range of 0.5-4 m^3/m^2.h. However, Bryan and Moeller, (1960) used recirculation rate of about 0.4-1.1 m^3/m^2.h in small size trickling filter using cross-flow media and greater than 1.5 m^3/m^2.h in vertical flow synthetic media trickling filter system. Moreover, Grady *et al.,* (1999) stated that sufficient media wetting may be achieved at a total hydraulic load rate (HLR) of 1.8-2 m^3/m^2.h with rotary arm distributors.

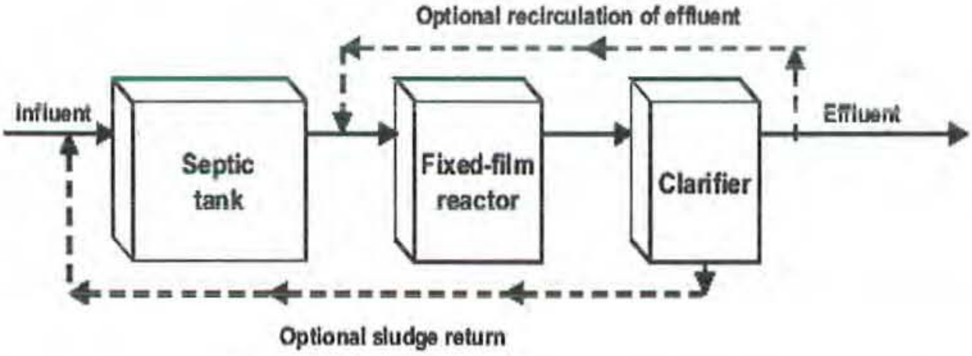


Fig. 2.4 Recirculation pattern of wastewater in fixed film bioreactor i.e. trickling filter.

2.4.5 Classification of Trickling Filter System

Trickling filters are categorized into different classes on the basis of hydraulic and organic loading rates i.e. low or standard rate trickling filter, intermediate, high and super-high rate trickling filters. Standard rate trickling filters typically designed to bear 1.1-4.3 $m^3/m^2/d$ hydraulic loads and 5-25 lb BOD/d/1000 ft^3 organic loading rates. Intermediate rate filters are generally used to manifest hydraulic loading rate of 4.3-10.8 $m^3/m^2/d$ and organic loading rate of 15-30 lb BOD/d/1000 ft^3 along with continuous recirculation of wastewater. High rate filters are generally assembled for significantly higher hydraulic and organic loading rates than that of standard or intermediate rate trickling filters. While, super high rate filters are projected to tolerate extremely high hydraulic loads of more than 162.3 $m^3/m^2/d$. These filters are in the form of towers with intensity of filter bed more than 40 feet and mostly synthetic media being lighter in weight and offer greater surface area to bear high hydraulic and organic loading rates. Moreover, detail of these different types of trickling filter system are presented in Table (2.2).

Table 2.2 Assortment of trickling filter systems on the basis of operational parameters.

Operating Parameters	**Low Rate**	**Intermediate Rate**	**High Rate**	**Super High Rate**
Hydraulic loading ($m^3/m^2/d$)	1.1-4.3	4.3-10.8	10.2-40.6	15.4-93
Organic loading (lb BOD/d/1000 ft^3)	5-25	15-30	25-300	≤ 300
Recirculation	Slightest	Frequently	Always	Frequently
Filter flies	More	Varies	Varies	Insufficient
Sloughing	Moderate	Varies	Continuous	Continuous
BOD_5 removal efficiency	80-85%	50-70%	65-80%	65-85%
Nitrification	Good	Slightly	Low	Low

2.5 Biofilms or Microbial Aggregates

Microorganisms degrade almost all types of toxic organic waste present in natural ecosystem. Generally, microorganisms participate in degradation processes occurring in natural water system are classified into two different group i.e. surface-attached organisms that take possession of all the surfaces occurring in the water, e.g. stones, plants, leaves and other barriers. On other hand, organisms that colonize water column itself instead of surfaces, usually in the form of bacterial flocs, plankton and microscopic shellfish are termed as suspended organisms (Gebara, 1999). Antoni van Leeuwenhoek was the first scientist who discovered and published microscopic interpretations of microorganisms (Madigan *et al.*, 2000). Though most of the organisms grow on earth in surface associated communities (Sutherland, 2001; Stoodley *et al.*, 2002), but still no reports had been published on microbial growth in slime layers until the era of 1940. The first report on microbial aggregates or biofilm was published in the mid of 1968 and then further research has been started on biofilm (Bryers, 2000).

A biofilm is a community of microorganisms that develops on suitable support media especially in humid condition. In other words, a biofilm is an organized community of different type of microorganisms encapsulated within polymeric matrix (EPS) and adherent to a living or inert support material. Common examples of biofilm in daily life are dental plaque, slippery surfaces in water streams, slime and smooth layer on infected wounds and blockage of water distribution pipes by biomass etc. It has been reported that microbes within slime layer or biofilm secrets polymeric matrix i.e. extracellular polymeric substances (EPS) that keep the cell mass together and hence form an organized biofilm community (Branda *et al.*, 2005; Stewart and Franklin, 2008). Beside this, extracellular polymeric substances (EPS) also serve as a source of nutrition during unfavourable and growth-limiting conditions and may also provide protection to the organisms against antibiotics and other environmental factors (Davey and O'Toole, 2000; Castonguay *et al.*, 2006). In addition to this biofilm lifestyle also helps in gene transfer among different bacterial species which is a beneficial act for microorganisms itself but from medical point of view, this is a major issue because in this way microorganisms modify themselves to show resistance to variety of antibiotics. It has been reported by Davey and O'Toole, (2000) that maximum genetic exchange occurred among bacterial species living in biofilm community, assurances an advanced evolution and genetic diversity and hence enhancing the competitiveness behaviour of the bacterial cells.

2.5.1 Biofilm Development and Arrangement

Development of biofilm on a suitable support matrix is an interesting and complex process. Microorganisms form a stable slime layer or biofilm on almost all type of surfaces in unfavorable humid conditions by utilizing different techniques, which includes expression of extracellular polymeric substances (EPS) secreting genes and exchange of essential messages through quorum sensing. Different steps involved in the formation of mature biofilm are depicted in Fig. 2.5. Initially an acclimatizing layer of adsorbed organic and inorganic molecules develop on the surface of support matrix in moist environment and then bacteria moves towards the moist surface by chemotaxis and hence results in the formation of transitory bacteria-surface interaction (Gottenbos *et al.*, 1999). After that bacteria secrets EPS, which irreversibly attached cells to the concerned surface. According to Dunne, (2002), irreversible attachment of bacterial cells to the surface was due to secretion of EPS by bacterial cells in humid condition, which is a mixture of exopolysaccharides and polypeptides complexes. Different forces participate in this initial attachment of bacterial cells and these includes weak hydrogen bonds, strong covalent and ionic bonds along with hydrophobic connections (Kumar and Anand, 1998). Palmer *et al.*, (2007) reported that initial attachment of bacterial cells on moist surfaces took less than few minutes and after that bacterial cells start cell division to form a mature community over the surfaces.

As mentioned earlier, organic and inorganic molecules deposits on moist surfaces in the initial step of biofilm synthesis, which act as a source of nutrients for bacterial cells to grow and form micro-colonies which then slowly expand to build a slime layer covering the whole surface (Kumar and Anand, 1998). Garrett *et al.*, (2008) studied genetics of bacterial cells during biofilm development process. They observed that genes responsible for motility would be down regulated while the genes for EPS production and other transport proteins would be up regulated during development of biofilm. Moreover, Dunne, (2002) reported that regulation and expression of different genes in bacterial cells mainly dependent on the bulk of their population mass as well as signal molecule induced communication.

Structure of mature biofilms are very complex mainly dependent on different environmental and nutritional factors such as temperature, pH, composition of nutrients and their availability as well as the composition of microbial associations or diversity (Pulcini, 2001). Transport of nutrients and oxygen to the inner side of mushroom like complex biofilm structure was observed by various researchers in their studies and found that water surrounding the biofilm facilitate these diffusion processes (Pulcini, 2001; Stoodley *et al.*, 2002; Kolter and Greenberg, 2006). Biofilm development process is relatively slow and takes round about two weeks (Stoodley *et al.*, 2002). In biofilm, microorganisms survived for a long period of time and hence through genetic exchange, they can modify themselves to survive in adverse conditions and search for favorable environment to be settled. This phenomena is commonly termed as detachment which is usually mediated by different factors such as nutrient deficiency, filth of extracellular polymeric substances, blockage of signal induced communications and operational instability i.e. variation in hydraulic loading rates (Chambless and Stewart, 2007).

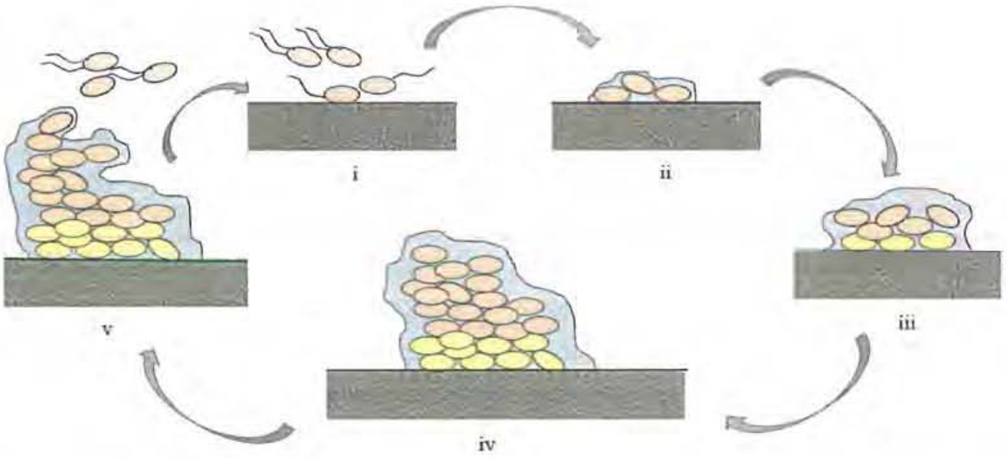


Fig. 2.5 Schematic illustration of biofilm development process; **i)** Bacterial cells adhere to the moist surface, **ii)** attached bacterial cells start cell division and excrete polymeric matrix, **iii)** expansion of polymeric matrix and formation of structural biofilm, **iv)** further expansion and microbial growth and **v)** detachment and dispersion of planktonic bacteria and hence searching for new habitat.

A very little evidence is available about the arrangement of bacterial cells in biofilm communities due to the absence of precise and accurate analytical techniques. Information about structure of biofilm was totally based on hypothesis of a regular, constant and mono-layer structure ascribed by empirical models (Watanabe *et al.,* 2016). With the passage of time, different techniques have been developed to examine the structural arrangement of biofilm, among which the light microscopy and confocal scanning laser microscopy are most commonly used techniques (Lazarova *et al.,* 1994). These methods are sensitive and give accurate image of microbial slime layer after staining of binocular magnifying glass with INT [2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenylt etrazolium chloride]. The only drawback of light microscopy is that resolution power is almost equal or slightly below to the limits of bacterial cell extents. In order to study the innermost parts of biofilm community, a highly resolution confocal scanning laser microscope was developed, which form basis for electron microscopy (Caldwell *et al.,* 1992). Many researchers used these techniques to demonstrate complex structure of biofilm and visualized active and inactive parts of biofilm community (Watanabe *et al.,* 2016).

Scanning electron microscopy (SEM) is another technique, commonly used now-a-days to visualize biofilm structure with high resolution. In addition to this, SEM is also equipped with X-ray dispersive microscopy to determine the composition of support matrix as well as biofilm (Robinson *et al.*, 1984). This technology gives a clear image of biofilm, which is not a modest form of microbial aggregates but of bacterial cells encapsulated within a polymeric environment (Lazarova *et al.*, 1992). Transmission electron microscopy (TEM) is a new version of SEM which gives a complete picture of internal structures of biofilm (Kinner *et al.*, 1983). Furthermore, Eighmy *et al.*, (1983) studied the structure of aerobic biofilm with TEM and observed three different kinds of matrix polymers with diverse physical possessions and three-dimensional distribution.

2.5.2 Application of Biofilms in Water and Wastewater Treatment

A lot of applications of microbial aggregates or biofilms in water and wastewater management processes have been reported for over a decade (Miranda *et al.*, 2017). In the early stage of 1980s, biofilm was an interesting topic for many researchers not only among environmental scientists but also among biotechnologists. But now-a-days, biofilm is a hot topic and a number of research activities has been performed on attached growth bioreactors for the production of bioactive compounds, enhancing the quality of ground water and also for sewage treatment. Importance of biofilm towards wastewater treatment was initially reported by Klein and Ziehr, (1990) which was further confirmed by other scientists (Blenkinsopp and Costerton, 1991). The phenomena of genetic exchange among microorganisms within biofilm would be utilize by some researcher in biofilm reactor for wastewater treatment (Fletcher, 1986) while others reported the activity of biofilm reactors with respect to nutrients concentration, production of variety of enzymes in different environmental conditions, presence of toxins and other inhibitory substances (Rittmann *et al.*, 1986; Manem, 1988). Furthermore, it has been reported that the activity of attached growth bioreactors would be affected less strongly as compared to suspended growth bioreactors under different environmental condition i.e. temperature, pH, concentration of carbonaceous compounds in wastewater, production of secondary metabolites and presence of growth limiting substances (Pedersen, 1990; Das *et al.*, 2017).

Activity of biofilm is not dependent on fixed biomass i.e. increase with increasing thickness of biofilm up to certain limits termed as active biomass thickness, above this limit, flow of nutrient to the biofilm is a limiting factor that differentiate active biomass from inactive biomass (Kornegay and Andrews, 1968; LaMotta, 1976). Thus in wastewater treatment plants, a steady, squeaky and active biofilm would be required to perform optimum activity of neutralizing wastes. In addition to this, a simple, rapid and well-designed methods will be required to determine the limits of active biomass. Attached growth system has numerous advantages in wastewater treatment as compared to suspended growth system such as low space and operational requirements, cost effective, environmental friendly, resist toxins and shock loads, operational compliance, increased retention time, enhanced biodegradation rate and poorer sludge production due to slower microbial growth rate (Chen and Chen, 2000; Lazarova and Manem, 2000; Wilderer and McSwain, 2004). Moreover, attached growth system also have capability to regulate reaction rates according to the demand (Lazarova and Manem, 2000).

Examples of wastewater treatment technologies that utilize biofilm or in other words used microbial aggregates on suitable support media are rock-media trickling filter systems, rotating biological contactors, fluidized bed bioreactor, high rate plastic media trickling filters, granular filters and immobilized cell membrane bioreactor (Seeger, 1999; Lazarova and Manem, 2000). Fixed bed and moving bed systems will be differentiated from each other on the basis of type, shape and nature of filter media. In fixed bed system, inert media such as stone, ping pong balls, plastic sheets and polystyrene sponges are used for the development of biofilm (Lazarova and Manem, 2000). Flow or passage of wastewater through filter media facilitate nutrients and dissolved oxygen supplies to the microorganisms living in biofilm community. In case of moving bed or fluidized bed system, moving or floating media has been used for biofilm development (Rodgers and Zhan, 2003). Selection of filter media in both fixed bed and moving bed treatment systems will be based on specific surface area of carrier materials, their size, shape and density, and hence made up of supreme quality materials in order to resist toxin and shock loads (Christensson and Welander, 2004).

There are wide ranges of application of attached growth bioreactors in wastewater treatment i.e. not only used to treat domestic or municipal wastewater but also industrial or high duty wastewater due to variety of removal mechanisms such as biodegradation or biomineralization of recalcitrant and carbonaceous compounds, bio-sorption of heavy metals and bio-accumulation of organic and inorganic compounds (Späth *et al.*, 1998; Guibaud *et al.*, 2006; Singh *et al.*, 2006). Mack *et al.*, (1975) analyzed growth pattern of microbial slime layer in trickling biofilter system and found that structure of biofilm largely depends on the nature of support matrix, microbial diversity, presence of nutrients, enzymes and environmental factors like temperature, pH etc. Furthermore, they used stones as support media for the development of biofilm in trickling filter system to treat domestic wastewater and found more than 85% reduction in BOD_5 after treatment. In addition to this, they also studied biofilm development process by using scanning electron microscopy and found that bacterial cells were the first to form thick slime layer on stone media, while protozoa and parasites invade it later to feed on metabolites secreted by bacterial cells.

Lens *et al.*, (1994) comparatively analyzed the behavior of sulphate reducing bacteria and methanogens towards treatment of wastewater in two different sewage handling plants i.e. trickling filter and anaerobic activated sludge. It was observed that both bacterial strains grow well in these treatment plants but the growth rate of methanogens in anaerobic activated sludge unit was high as compared to trickling filter system due to variation in reactor designs and organic loading rates. On other hand the growth pattern of sulphate reducing bacterial strains was almost same in both i.e. independent to reactor design, sulphate concentrations, organic and hydraulic loading rates. Furthermore, they analyzed that trickling filter using synthetic plastic media as a filter was more efficient in sulphate reduction as compared to anaerobic treatment plant and suggest that aerobic sewage treatment unit would serve as an easy target for redox-reaction processes.

Novotny *et al.*, (2010) evaluated the efficiency of bacterial and fungal attached growth bioreactors for the treatment of textile industry wastewater, which was a mixture of numerous recalcitrant dyes. First of all they studied the efficiency of both these reactor separately and found that bacterial attached growth system was effective only to remove simple biodegradable dye i.e. mano-azo dye from wastewater while no change was observed in the concentration complex dyes, while fungal fixed film bioreactor showed more than 85% overall efficiency in the degradation of mano-azo and complex dyes present in textile wastewater. After that they analyzed the combined effect of both these reactors towards treatment of textile wastewater and observed more than 95% treatment efficiency within 14 days. Almstrand *et al.*, (2011) found that biological wastewater treatment processes were more competent in the removal of ammonia and other nitrogenous compounds from wastewater as compared to physical and chemical treatment methods. They developed a nitrifying trickling filter utilizing biofilm of slow growing ammonia and nitrite oxidizing bacteria, and operate it under different organic and hydraulic loading rates. From their experiments, they concluded that the capability of these organisms to remove ammonia and nitrogenous compounds from wastewater was directly proportional to the organic and hydraulic loading rates.

2.6 Mathematical Simulation or Modelling of Attached Growth Bioreactor for Wastewater Treatment

In biological filtration scheme, biodegradation is a common episode for the removal of organic contaminants from wastewater. In attached growth system, microorganisms form a slime layer, also termed as biofilm over the surface of filter media, which usually takes control of all oxidation reduction reactions occurring during treatment of wastewater. Therefore, the efficiency of attached growth bioreactors to treat wastewater largely depends on the biofilm density, microbial diversity within biofilm and continuous source of nutrients. As mentioned earlier in this review, three major steps are involved in biofilm formation process which includes, a) adhesion and attachment of microbial cells to the support matrix, b) microbial cells division, expansion of polymeric matrix and formation of structural biofilm, and c) detachment and dispersion of planktonic cells (Chaudhary *et al.,* 2003). As in fixed film system, microorganisms are wedged to the surface, therefore the supply of nutrients to the microbes in slime layer will be restrained by bulk liquid and surface transport mechanism. Normally in fixed film growth system, nutrients or organic substances present in wastewater will be transported to the outermost surface of biofilm, from where it will diffuse into biofim for their utilization. According to Fick's law and Monod expression, there are different factors that affect the rate of substrate utilization within a biofilm i.e. (a) transport of nutrients or organic substances to the outer surface of biofilm from bulk liquid, (b) diffusion of nutrients or substances from outer surface into biofilm, (c) utilization kinetics within the biofilm. Beside this, there are some other factors which affect the efficiency of attached growth bioreactors that usually includes growth rate of biomass within reactor and physical factors like hydraulic loading rates, temperature, recirculation flow rates that greatly affect biofilm formation and slough-off process during wastewater treatment.

Mathematical or empirical modelling is an indispensable work to design, construct and understand the mechanism of wastewater treatment plant. Lu *et al.*, (2004) stated that for the optimum design and operation of a wastewater treatment system, empirical modelling is considered to be an integral part. Modelling of wastewater treatment technologies increases day by day and a number of theoretical, physical design and systematic models are available in practice (Morgenroth *et al.*, 2000). In the field of wastewater treatment, first empirical model was developed by Ottengraf and Van Den Oever in 1983 to understand the basic mechanism of biological filters towards treatment of wastewater. After that many new models have been reported for trickling filters that add sufficient information in the literature regarding to development of biofilm, substrate utilization and inhibition kinetics of microbial growth (Rene *et al.*, 2011). Shareefdeen *et al.*, (1993) developed a model that describes the restraining effects of dissolved substrates on microbial growth, which was further improved by developing another model using Freundlich isotherm, explaining the biofilm formation process on suitable support matrix.

Up till now, most empirical models of trickling filter system are designed to understand the behavior of trickling filter systems towards treatment of wastewater under prevailing temperature conditions, and normally three phase models are used for trickling filters. Zhu *et al.*, (1998) developed a three phase model for the removal of diethyl ether from industrial wastewater and they also determine the limitations of mass transfer within biofilm. Kim and Deshusses, (2003) proposed an efficient model to evaluate the efficiency of trickling filter system towards removal of hydrogen sulphide. In their model, they supplied hydrogen sulphide to the biofim directly from gaseous and liquid phase and suggested that proper flow of wastewater is required to completely wet the filter media. Iliuta *et al.*, (2005) established an active model that demonstrate the effects of microbial aggregates in trickling filter for toluene removal. These authors through their models provide sufficient information regarding the attitude and behavior of trickling filters in the removal of different wastes but still a lot of work is required to develop models for enhancing the efficiency of wastewater treatment technologies.

In literature, very few empirical models were reported that can forecast the efficiency of trickling filter system and among these, most models were based on steady state condition (DiGiano and Speitel, 1993). In steady state model, the transport of substrates to the biofilm and their utilization kinetics were expressed by Fick's laws and Monod expression (Rittmann and McCarty, 1980) and it was predicted that minimum substrate concentration in wastewater was required for optimum performance of biofilm activities within reactor. A major drawback of steady state model is that it does not give sufficient informations regarding to biomass growth with time. Chang and Rittmann, (1987) established a model that incorporate with all essential biological process such as biofilm kinetics on activated carbon, rate of substrate transfer to the biofilm, utilization rate within biofilm and also described biomass growth rate. However, there are certain limitation of this model i.e. it does not give information about the voids ratio of filter media and recommended depth for filter bed.

Huck *et al.,* (1994) proposed first order kinetic model for trickling filter system by considering the efficiency of reactor to remove carbonaceous compounds from wastewater. The model of first order kinetics which they proposed was most accurate to predict the efficiency of trickling filter system. Furthermore, Billen *et al.,* (1992) developed a model for the removal of biodegradable organics which showed that removal rate for biodegradable organic carbon was dependent to their concentration in wastewater i.e. higher the concentration of organic compounds in wastewater, higher would be the rate of removal and vice versa. Moreover, in their model they considered different variable such as concentration of biodegradable organics in wastewater, activity of microbial community present in wastewater also those attached to the support matrix in the form of biofilm. The model was also calibrated and validated with pilot and full size filters. Hvala *et al.,* (2017) used the same model to evaluate the efficiency of pilot scale facility in terms of BOD_5 and ammonia removal and it was found that bioreactor efficiency was greatly affected by bringing changes in the organic and hydraulic loading rates.

Chapter 3

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present study comprised of setting up two pilot scale trickling filter systems and evaluation of these systems at different conditions for the treatment of domestic waste water.

3.1 Experimental Setup-I Account

The pilot scale TF system (setup-I) was installed in work station located at the vicinity of the Department of Microbiology, Quaid-i-Azam University, Islamabad as shown in Fig. 3.1. The pilot scale facility was used to treat about 0.195 m^3 (51.5 US gallons) of wastewater per day, with a hydraulic flow rate (Q) of 0.193 m^3/day and hydraulic loading (Q/A) of 0.151 m/day. The main body of TF system was made up of stainless steel (Diameter, 1.28 m and height, 2.29 m) to support the stones used as filter media and each stone has an average diameter of 4 inches (0.1 m provided a surface area of about 0.093 m^2) for bacterial growth. A rotating arm distributor (Diameter, 0.05 m and length, 0.73 m) was installed at the top of filter bed to distribute wastewater uniformly. Electric pumps of 1.3 horsepower were connected to the wastewater distribution system through poly vinyl chloride (PVC) pipe system. To collect treated effluent and sludge, an under drain system (Diameter, 0.92 m and height, 0.37 m) was installed at the bottom of TF system and having total capacity of 0.24 m^3. Moreover, a recirculation tank also assisted as final clarifier having water-holding capacity of 0.267 m^3.

3.1.2 Experimental Setup-I Operational Scheme

For the development of biofilm, stones were collected from a nearby stream and were kept in a container containing untreated wastewater for about one week. An activated sludge was used for seeding purpose in order to facilitate the biofilm development process. Treatment of wastewater through pilot scale trickling filter system was carried in a batch mode using standard protocols i.e. initially about 200 L (52 US gallon) of wastewater was collected from the out flow of septic tank located at residential area of QAU Islamabad and then immediately transferred to the work station located near Department of Microbiology, QAU, Islamabad and then 1-2 hrs settling time was given to it in influent feed tank in order to settle down colloidal particles (APHA, 2005). After this, it was circulated and recirculated through the pilot scale TF at a flow rate (Q) of 0.193 m^3/day for about 6 days and the effluent was collected after every 1st and 6th day of treatment. Furthermore, after completion of one cycle, another cycle started with a new sample of wastewater following a similar scheme of treatment. This batch mode of treatment operation was followed for 11 months (August 2014-June 2015). Temperature was regularly monitored during whole experimental trial.

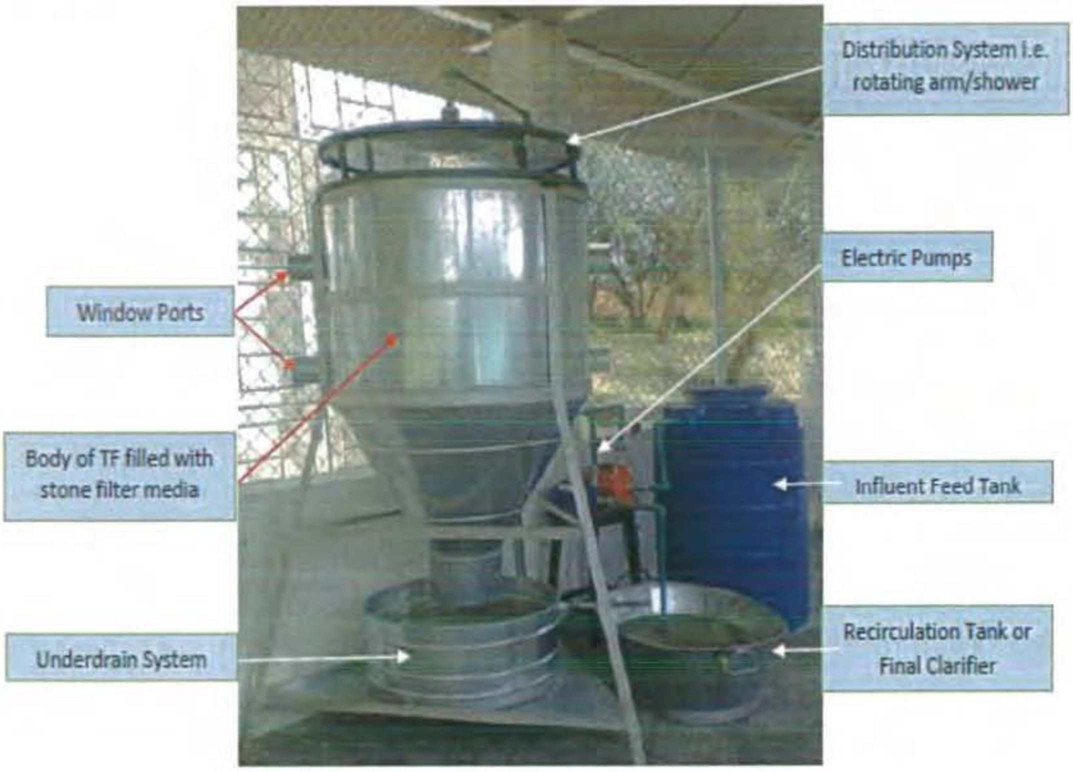


Fig. 3.1 Pilot scale TF system installed in a work station located at the vicinity of the Department of Microbiology, QAU Islamabad for the treatment of wastewater.

3.2 Experimental Setup-II Account

A pilot scale trickling biofilter (TBF) was designed and installed in an open environment at residential area of Quaid-i-Azam University, Islamabad, Pakistan. The main components of TBF system were made up of concrete and usually include primary sedimentation tank

(Diameter, 1.53 m and height, 1.4 m) to carry about 2300 US gallons (88 m^3) of influent and was followed by main body of TBF (Diameter, 1.53 m and height, 1.67 m) to support the stones used as filter media for bacterial growth and each stone has an average diameter of 0.12 m. Furthermore, it was followed by a recirculation tank having same dimensions as that of primary sedimentation tank (PST). A sand bed (Length, 0.4 m; depth, 0.91 m and width, 0.91 m) was also incorporated at the outlet of recirculation tank. A rotating arm distributor (length, 1.28 m), having numerous small pores was installed at the top of filter bed to distribute wastewater uniformly. Electric pumps were connected to the wastewater distribution system through PVC pipe system. To collect treated effluent and sludge, an underdrain system (Diameter, 1.53 m and height, 0.5 m) was installed at the bottom of TBF component and the total gap between the TBF component and underdrain system was about 0.5 m in order to facilitate aeration. The stone bed has a void space of 35% and the start-up time was 12 days.

3.2.1 Experimental Setup-II Operational Scheme

The pilot scale facility was used to treat about 0.4 m^3 (400 L = 105.7 US gallons) of wastewater per day for about 15 weeks. The frequency of the influent from the distribution system over the stone media bed was maintained at a hydraulic flow rate of 1.2 L/min (Q = 0.072 m^3/ hr) and hydraulic loading (Q/A) of 0.147 m^3/day. For the development of active biofilter media, mixture of wastewater and activated sludge (1:9) was pumped into the TBF for 7 days (1 week) before the formal start-up of the system for wastewater treatment and the start-up time was 12 days. The activated sludge used as a seed was collected from wastewater treatment plant (I-9) Islamabad, Pakistan.

Wastewater from the residential area entered into the septic tank, the outlet of the septic tank opens into the anaerobic baffled reactor (ABR), where bulky suspended particles were restrained and hence primary screening and anaerobic digestion would take place. The wastewater from the outlet of ABR entered into the PST. The flow of wastewater into the PST was controlled by a manual valve and once PST was filled completely, the valve was closed to stop flow of wastewater. Water pump P1 was installed on the PST that pumped wastewater to the hydraulically compelled rotary distributor through the PVC pipe. The exclusion of water from the holes of arms generates dynamism and revolves the water distribution system at uniform velocity to distribute wastewater over the filter bed. The ON/OFF mechanism of pumps was under the control of digital timers, i.e. after 4.5 hrs, P1 switched off automatically and it turns on the pump P2 installed on the recirculation tank, then wastewater was continuously pumped and recirculated for about 19.5 hrs. After the completion of 24 hr cycles, the effluent was discharged into the adjacent fields or stream. Next day at fixed timing (10:00 AM), P2 switched off automatically and it turns on P1, which pumped new sample of wastewater from PST to the reactor and the next cycle begins. Digitally controlled system has the capacity to evaluate its running time, so in case of any load shedding, each pump finalized its running time. The samples of the influent and effluent were collected at a fixed time (10:00-11:00 AM) every day, for the estimation of the wastewater treatment efficiency of the TBF system for 15 weeks and sent to laboratory for physicochemical analysis. Other physical parameters such as color and odor were observed at the spot. The schematic illustration of overall treatment units of pilot scale TBF system is shown in Fig. 3.2.

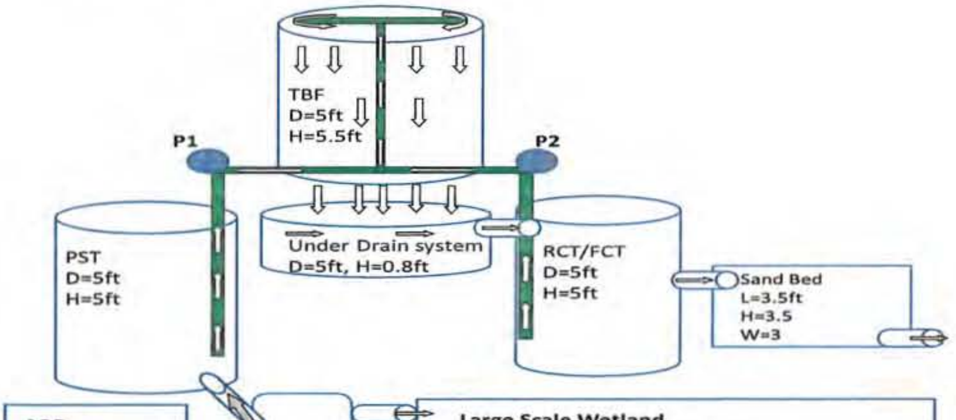


Fig. 3.2 Schematic illustration of overall treatment units of pilot scale TBF system installed at residential area of QAU Islamabad for the treatment of wastewater.

Legends: *ABR = Anaerobic Baffled Reactor, PST = Primary Sedimentation Tank, TBF = Trickling Bio Filter, P1 = Pump-1, P2 = Pump-2, RCT = Recirculation Tank, FCT = Final Clarification Tank, SB = sand bed, H = Height, D = Diameter, L = Length and W = Width.*

3.3 Physicochemical Investigation of Influent and Effluent Samples

Samples collected during the working of the tricking filter systems were analyzed for different physiochemical and microbiological parameters as follows:

3.3.1 Odor and pH

Odor and pH are important parameters to examine the quality of water samples. In the current study odor and pH of influent samples were determined by using standard method 2150 and potable pH meter device (APHA, 2005).

3.3.2 Electrical Conductivity (EC)

During wastewater treatment, electrical conductivity (EC) is also an important parameter to determine treatment efficiency of bioreactor. The EC of both influent and effluent samples were measured in μS/cm by using conductivity meter device.

3.3.3 Dissolved Oxygen (DO)

In the current study, the level of dissolved oxygen (DO) in the samples of both influent and effluent were determined by using potable DO meter device in mg/L. The bulb of DO meter was rinsed each time with distilled water during operation.

3.3.4 Biological Oxygen Demand (BOD_5)

The concentration of BOD_5 in both influent and effluents samples were determined by standard dilution technique (APHA, 2005). In this technique, four different types of reagents i.e. phosphate buffer solution, magnesium sulphate solution, calcium chloride solution and ferrous chloride solution were used in a ratio of 1:1000 (1 mL of each reagent was placed in 1000 mL of distilled water) to prepare dilution water. After this, about 5 mL of both influent and effluent was mixed with almost 295 mL of dilution water in aspirator bottle which was then further transfer into two BOD bottles. Among these two bottles, one was kept in incubator for 5 days at 25°C while the other one was processed at spot to determine initial DO concentration while final DO concentration was determined after 5th day of incubation. From these two different DO values, the BOD_5 of both influent and effluent was determined by using following equation;

$$BOD5\left(\frac{mg}{L}\right) = \frac{(DOi - DOf)}{volume\ of\ sample\ used\ (mL)}\ x\ 1000$$

Where,

DO_i = Initial DO concentration

DO_f = Final DO concentration after 5th day of incubation

3.3.5 Chemical Oxygen Demand (COD)

In the current study, COD kit method was used to determine the level of COD in influent and effluent samples (APHA, 2005). In this method different ranges of COD kits i.e. 0-40 mg/L, 40-1500 mg/L and 1500-5000 mg/L were used. About 3 mL of both influent and effluent samples were added into COD kits and then agitate for about 5-10 min and after agitation, they were placed on Thermoreactor at 148°C for about 2 hrs. After digestion of 2 hrs, kits were allowed to cool down and then reading were taken on spectroquant (Merck, Pharo 300).

3.3.6 Total Dissolved Solids (TDS)

TDS is another important parameter to determine the quality of water samples. In the current study, water quality standard guidelines were followed to determine TDS level in both influent and effluent samples (APHA, 2005). About 50 mL of influent and effluent samples were filtered out through whatman filter paper and after filtration, filtrate was shifted to pre-weight china dish (mg) and then evaporated to dryness on a hot plate. After dryness on hot plate, china dishes were shifted to hot oven for further dryness at 150°C. After complete dryness of influent and effluent samples in china dish, allowed them to cool down in a desiccator and then weighed (mg). The TDS was then determined by using following equation;

$$TDS\left(\frac{mg}{L}\right)=\frac{(wt.of\ dried\ sample\ along\ with\ china\ dish-wt.of\ empty\ dish)}{Volume\ of\ Sample\ used\ (mL)}x1000$$

3.3.7 Total Suspended Solids (TSS)

Standard water quality guidelines were also followed to determine TSS level in both influent and effluent samples (APHA, 2005). In case of TSS assessment, whatman filter papers were inserted in to filtration assembly and later washed them three time with distilled water in order to remove any particulate material and after washing, dried them for about 1 hr in oven at 100-105°C. Afterward, initial weight (mg) of filter papers were recorded using digital weighing balance. These filter papers were again inserted into filtration assembly and about 50 mL of influent and effluent samples were filter out by applying vacuum. After filtration, similar process of drying was followed again for about 1 hr in oven at 100-105°C and consequently final weight (mg) of dried filter papers along with residue were noted down after the accomplishment of drying process. The TSS was then determined by using following equation;

$$TSS\left(\frac{mg}{L}\right)=\frac{(Final\ weight\ of\ filter\ paper-initial\ weight\ of\ filter\ paper)}{Volume\ of\ Sample\ used\ (mL)}x1000$$

3.3.8 Orthophosphates

Spectrophotometric analysis was used for the determination of orthophosphate level in both influent and effluent samples according to standard guidelines. In this method, four different types of reagents were used and these include strong acid solution, ammonium molydate solution, standard phosphate solution and chloride solution. Moreover, phenolphthalein was used as an indicator. For assessment of orthophosphate level, about 50 mL of influent and effluent samples were taken in a flask and few drops of indicator were added to it. After addition of an indicator solution, if pink color appeared, small amount of strong acid solution was added to neutralize the color of the solution. After this, about 4 mL of ammonium molybdate reagent was added slowly, followed by the addition of few drops of stannous chloride with thorough mixing was done after each addition. Then, it was allowed to stand for about 8-10 min and absorbance was measured at wavelength of 610 nm and then further computed it by comparing with orthophosphate standard curve.

3.3.9 Sulphates

Spectrophotometric analysis was also used for the determination of sulphate levels in both influent and effluent samples according to standard guidelines. In this method, first of all about 25 mL of influent and effluent samples were filtered out by using whatman filter paper and then 5 mL of NaCl-HCl and glycerol solutions were added at 1:1 ratio. After this, 150 mg of $BaCl_2$ was added to it and then allowed for 30 min to withstand and absorbance were measured at wavelength of 420 nm which were then converted to concentration units comparing with sulphate standard curve.

3.3.10 Nitrate-Nitrogen (NO_3-N)

Assessment of NO_3-N test was carried by standard method as described by APHA, (2005). About 50 mL of pre-filtered samples of both influent and effluent were taken in labeled china dish and then placed on hot plate until it become completely dry. After formation of dry residues, 500 μL of phenol disulphonic acid was added to each china dish and then allowed to cool down at room temperature. Then 600-800 μL of ammonia solution was added and mix well and after mixing diluted them up to 100 mL with distilled water and then absorbance readings were measured at wavelength of 410 nm. The absorbance readings of each sample were then computed by comparing with standard NO_3-N curve.

3.3.11 Nitrite-Nitrogen (NO_2-N)

Standard method 4500 NO_2-N was used to assess nitrite's concentration in influent and effluent samples. In this method, about 50 mL of influent and effluent samples were taken in 100 mL Nessler's tubes and 2 mL of buffer reagent was supplement into each tube and then mixed carefully for 10 min until color developed. Then absorbance readings were measured at wavelength of 540 nm, which were then computed by comparing with standard NO_2-N curve.

3.3.12 Total Nitrogen (TN)

In the current study, the concentration of TN in both influent and effluent samples were determined by kit method according to standard protocol (APHA, 2005). In case of TN assessment, about 9 mL of distilled water and 1 mL of influent and effluent samples were added into blank kits and after this one tea spoon of N_1K and 6 drops of N_2K reagents were added to each kit. After addition of reagents, allowed them to be digested in Thermoreactor at 120°C for 1 hr. After digestion, the kits were allowed to cool down and the 1 mL of N_3K reagent was added to each kit and then after few minutes reading was noted down through spectroquant.

3.4 Microbiological Assessment of Influent and Effluent Samples

3.4.1 Most Probable Number Test (MPN/100 mL) for Fecal Coliforms

Most probable number test was performed for the examination and validation of fecal coliforms within influent and effluent samples. In this test, three sets of test tubes (each set contain 3 test tubes filled with lactose broth along with upside-down Durham tubes) were inoculated with sample and then incubated at 35°C for 48 hrs. After incubation, test tubes with gaseous bubbles in Durham tube were considered positive that generate a code and from this code, MPN/100 mL were measured against standard dilution table for MPN index. Furthermore, about 100 µL of sample from each positive tubes were streaked on nutrient agar plates (NA) to determine fecal coliforms. These plates were incubated at 37°C for 24 hrs and after incubation bacterial isolates were confirmed by pure culturing technique.

3.4.2 Quantitative Analysis in terms of Colony Forming Unit per milliliter (CFU/mL)

Quantitative analysis of influent and effluent samples was performed for enumeration of pathogenic bacteria (*E.coli* and other *Enterobacteraceae*, such as *Salmonella*, *Shigella*, *Klebsiella*, *Enterobacter* and *Citrobacter*) and serial dilution along with plate count method was used. The colonies appeared on plates were counted by using colony counter and then CFU/mL was determined by using the formula:

$$\frac{\text{CFU}}{\text{mL}} = \frac{\text{No. of colonies x Dilution factor}}{\text{Inoculum size}}$$

3.5 Bacteriological Assessment of Biofilm Developed over Filter Media

3.5.1 Colony Morphology

Colony morphology is an aid in identifying bacterial cultures on growth media and different parameters would be considered to determine morphology of bacterial cultures and these include, size, pigmentation, form, opacity, margin and elevation of colonies appeared over the surface of different growth media.

3.5.2 Microscopy

Standard Gram staining procedure was used for microscopy of bacterial isolates. In microscopy, smear of bacterial isolates were prepared, dried and heat fixed on clean slides. Few drops of crystal violet were put down on smear and allowed to stand for 1-2 min and then it was washed with distilled water. In the second step, flooded the smear with Gram's iodine, allowed to stand for 45 sec and then washed with distilled water. In the third step, it was decolorized with 95% ethyl alcohol and again washed with distilled water. After that, it was counter stained with safranin for 1 min and washed with distilled water. This Gram's staining procedure was followed for all bacterial isolates. All the slides were air dried and examined under the oil emulsion objective (100X). According to Gram's reaction; the Gram negative bacteria appeared pink whereas the Gram positive bacteria appeared purple in color under microscope.

3.5.3 Biochemical Characterization

Different biochemical tests were performed in the current study to characterize bacterial isolates. These tests include carbohydrate fermentation tests, triple sugar iron test, indole and H_2S production test, citrate utilization test, nitrate reduction test, catalase test, urease test and methyl red vogas proskauer test. All these tests were performed according to their standard procedure as described in Bergey's Manual of Determinative Bacteriology (9th Edition).

3.6 Statistical Analysis

For different physicochemical parameters such as BOD_5, COD, DO, pH, TSS, TDS orthophosphates, sulphates, nitrates, nitrites and TN of both influent and effluent samples, the mean values were calculated and graphically epitomized as bar charts, using Microsoft Excel program. In order to determine treatment efficiency of pilot scale TF system, different parameters values of both influent and effluent samples were compared by t-test and $p < 0.05$ was considered the minimum value for statistical significance.

Chapter 4

4. APPRAISAL OF A LOW-COST PILOT SCALE TRICKLING FILTER SYSTEM FOR SEWAGE TREATMENT DURING VARIOUS SEASONS

4.1 Introduction

The pilot scale TF system was installed in work station located at the vicinity of the Department of Microbiology, QAU, Islamabad, Pakistan as reported previously by the same research group Naz *et al.,* (2016). In the present research study, it was planned that a pilot scale TF system would be evaluated with varied environmental conditions. As Pakistan's temperature varies a lot during the year and especially in Islamabad, a humid subtropical climate exists with five different seasons comprising winter (November, December, January and February), spring (March and April), summer (May and June), rainy monsoon (July and August) and autumn (September and October), therefore the main objective of this research study was to comprehend the efficiency of TFs in the treatment and potential reuse of wastewater collected from residential area in Pakistan. Data was collected for carbonaceous and microbial loads for reuse of effluent for ornamental, recreational and agricultural purposes. Furthermore, to learn about the mechanisms of different processes and to reduce experimental cost and time, numerical modelling and development of mathematical equations is considered to be indispensable (Lu *et al.,* 2004). Likewise, mathematical modelling of wastewater treatment plant provides information on how the reactor works under different treatment conditions, which was considered to be a fundamental tool for design analysis and optimization of wastewater treatment processes. Hence, modelling gives a detailed description of biochemical reactions taking place within reactor during wastewater treatment and also provides sufficient information on how organic compounds within the reactor are converted into CO_2, N_2 and biomass (Chirila *et al.,* 2009). The key role of modelling and simulation of wastewater treatment plants is to achieve two major goals i.e. reduction of carbonaceous compounds up to standard level and to remove all hydraulic conflicts in order to achieve effluent of good quality (Harremoes *et al.,* 1993). In the current study, a simplified zero order kinetic model was developed to judge the productivity of pilot scale TF system in the oxidation of carbonaceous compounds.

4.2 Materials and Methods

4.2.1 Experimental Setup-I Description and Operation

The detail description of experimental setup-I (pilot scale TF system) was given in section 3.1 of chapter 3. However, the schematic illustration of overall treatment units is shown in Fig. 4.1. The pilot scale facility was operated in a batch mode to treat about 0.195 m^3 (51.5 US gallons) of wastewater per day, with a hydraulic flow rate (Q) of 0.193 m^3/day and hydraulic loading (Q/A) of 0.151 m/day. For the development of biofilm, stones were collected from a nearby stream and were kept in a container containing untreated wastewater for about one week. An activated sludge was used for seeding purpose in order to facilitate the biofilm development process. Treatment of wastewater through pilot scale trickling filter system was carried in a batch mode using standard protocols i.e. initially about 1-2 hours' settling time was given to the wastewater in the influent feed tank (septic tank) in order to remove large suspended particles (APHA, 2005). Then it was circulated and recirculated through the pilot scale TF at a flow rate (Q) of 0.193 m^3/day for about 6 days and the effluent was collected after every 1st and 6th day of treatment. Furthermore, after completion of one cycle, another cycle started with a new sample of wastewater following a similar scheme of treatment. This batch mode of treatment operation was followed for 11 months (August 2014-June 2015). Temperature was regularly monitored during whole experimental trial.

4.2.2 Microbial Profiling of Wastewater

4.2.2.1 Most Probable Number Test (MPN Index) for Fecal Coliforms

Most probable number test was performed according to their standard protocol.

4.2.3 Chemistry of the Wastewater

To assess the efficiency of pilot scale TF system with respect to wastewater treatment, chemistry of the influent and effluent samples including BOD_5, COD, TDS, TSS, pH and DO was carried out in triplicate after 6 days (every 1st and 6th day) of operations by using standard protocols for water and wastewater analysis (APHA, 2005; EPA, 2007).

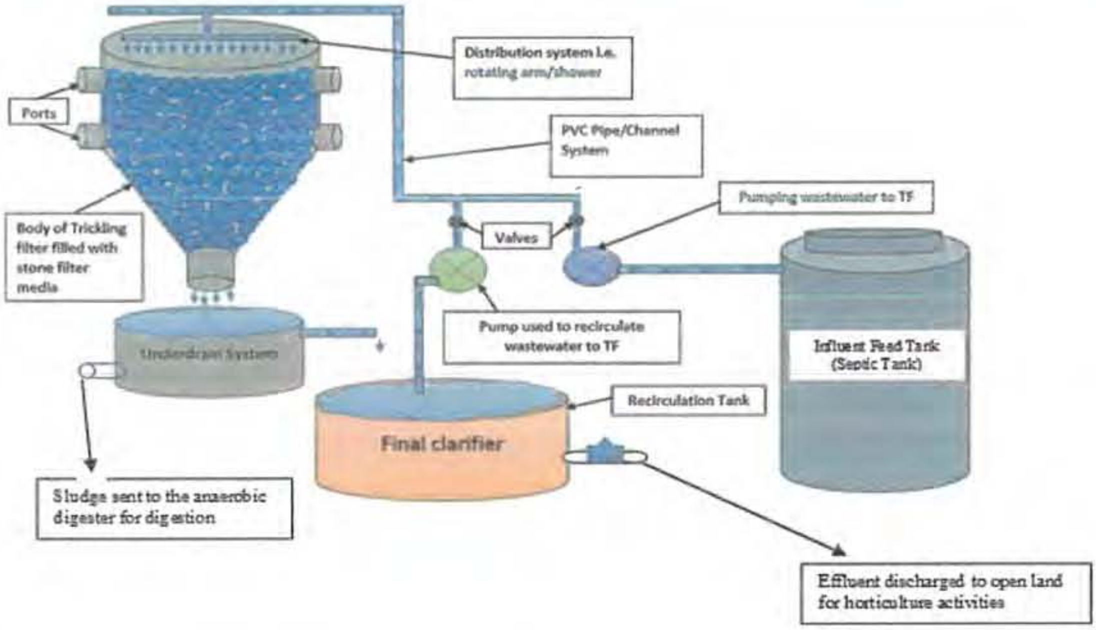


Fig. 4.1 Schematic illustration of overall treatment units of pilot scale TF system installed in work station located at the vicinity of Department of Microbiology, QAU Islamabad.

4.2.4 Microbial Profiling of Slime Layer Developed on Stone Filter Media

After the development of slime layer on stone filter media, the analysis and identification of the microbial community was carried out by pure culturing technique i.e. cultural characteristics, microscopy and biochemical tests according to Bergey's Manual of Determinative Bacteriology (9th Edition).

4.2.5 Model Expansion and Standardization

For the optimum design and operation of a wastewater treatment system, empirical modelling is considered to be an integral part. Modelling of wastewater treatment technologies increases day by day and a number of theoretical, physical design and systematic models are available now-a-days in common practice. In the current research study, a basic systematic model was developed by assuming that reduction of carbonaceous compounds (BOD_5) in wastewater by pilot scale TF system is a single phase process. Heterotrophic bacteria within biofilm of TF system are most commonly involved in the degradation of organic compounds to obtain energy for their growth, as a result, the concentration of substrates in wastewater decreases with time during treatment. A zero order kinetic model was used to mathematically describe the nature of pilot scale TF system in this study i.e.

$$\frac{dS}{dt}\,\alpha - X \qquad (1)$$

$$\frac{dS}{dt} = -\,k_t X \qquad (2)$$

$$dS = -\,k_t X dt \qquad (3)$$

$$\int_{So}^{St} dS = -\,k_t X \int_{0}^{t} dt \qquad (4)$$

$$S_t - S_o = -\,k_t X(t-0) \qquad (5)$$

$$S_t - S_o = -\,k_t X t \qquad (6)$$

$$S_t = S_o - k_t X t \qquad (7)$$

Where, "S_t" denotes final BOD_5 of one treatment unit at any time, "S_o" represents initial BOD_5 of untreated wastewater, 'k_t' is a specific bioreactor coefficient at specific temperature and 't' is the time required (in days) for the treatment of wastewater. Moreover, 'X' represents biomass that should be remained unchanged during the steady state. For practical purposes, 'X' can be assumed to be constant. As reported by Naz *et al.*, (2016), the initial BOD_5 can be represented as volumetric loading rate (O_{LR}) i.e.

$$O_{LR} = \frac{QS_o}{HA} \qquad (8)$$

$$S_o = O_{LR}\frac{HA}{Q} \qquad (9)$$

Putting "S_o" in eq. (7) from eq. (9) we get,

$$S_t = O_{LR}\frac{HA}{Q} - k_t X t \qquad (10)$$

Quaid-i-Azam University

This equation can be used to mathematically describe the nature of pilot scale TF system to reduce carbonaceous compounds within wastewater. As in this research study, 11 months data was collected by operating pilot scale TF system under a range of temperature conditions, therefore the model can be applied to all experimental results. The model calibration was done on experimental data obtained at 20°C in order to determine "k_{20}" value by using an equation i.e. $k_{20} = (S_o-S_t)/Xt$ as shown in Fig. 4.2, which has been further validated for each experimental setup to get "k_t" value for a particular temperature range.

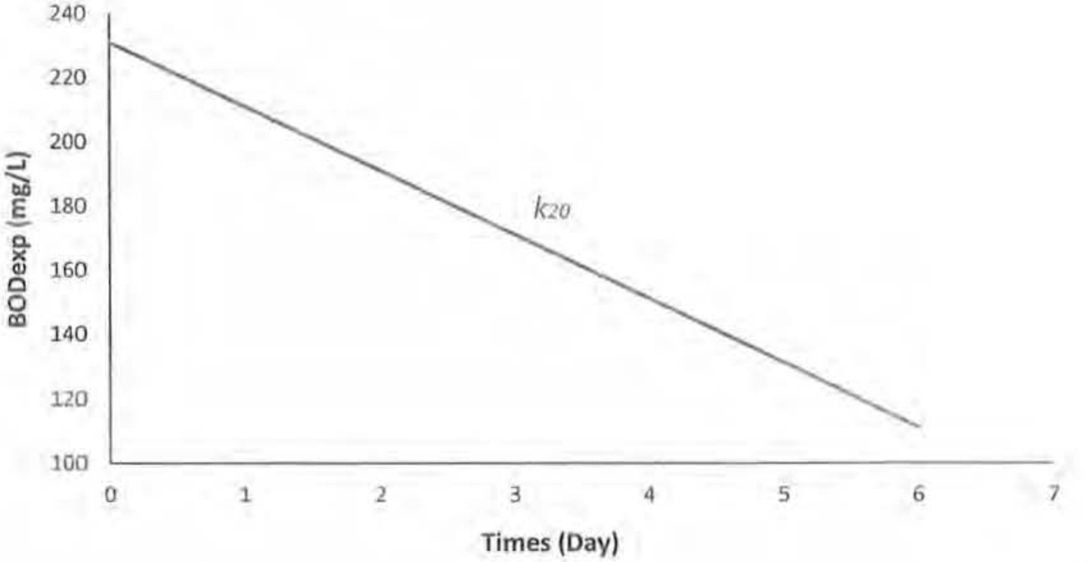


Fig. 4.2 Determination of "k_{20} value" from the model calibration of BOD_{exp} vs Time data at 20±1°C.

4.3 Results and Discussion

The wastewater treatment efficiency of the locally designed and constructed pilot scale TF system was evaluated for 11 months under varying temperature conditions. It was observed that the TF treatment system was effective at all temperatures but showed higher performance in the removal of carbonaceous compounds as well as pathogenic organisms at temperature range of 20-38°C. As it was reported previously that in many developing countries, it is a common practice that wastewater from residential and recreational areas discharged directly into natural environment and also used for irrigation purposes, thus resulting in many water borne diseases (Ensink *et al.*, 2004). Moreover, the presence of high nutrient contents in wastewater may lead to eutrophication in the receiving water bodies and hence decreasing water quality that results in the accumulation of toxic compounds within vegetables and crops, which ultimately affects human life (Bouwer and Idelovitch, 1987; Murtaza and Zia, 2011). Therefore, it is essential to develop a low cost biological wastewater treatment facility suitable for reuse in Pakistan to eradicate potential hazards from wastewater before its disposal to the natural environment or safe use for irrigation. Furthermore, the experimental data on BOD_5 was used to formulate a mathematical equation which would help to design an efficient field scale domestic wastewater treatment facility in future to improve the public health as well as agricultural productivity.

4.3.1 Efficacy of Pilot Scale TF System in the Removal of Fecal Coliforms

Fecal coliforms, are commonly used as an indicator of disease-causing pathogens in the aquatic environment. Influent from a municipality would contain large quantities of fecal coliforms including pathogenic microorganisms in the range of 1,100-5,000 per 100 mL (Servais *et al.*, 2007; Bailey *et al.*, 2018). Various researchers reported that traditional wastewater treatment processes were effective in removing a third of fecal coliforms (Lucena *et al.*, 2004; Souissi *et al.*, 2018) and as the influent contained a large quantity of these organisms thus a huge amount of fecal coliforms were being discharged directly to the water bodies.

In the present research study, it was observed that the MPN index of influent samples were more than 1,100 per 100 mL during the entire treatment process but the removal efficiency of fecal coliforms by the pilot scale TF system varied from 43-55% and 57-86% after the 1st and 6th day of treatment respectively (Fig. 4.3). Similar observations in the reduction of coliforms by bench-scale stone media TF systems were found by Khan *et al.* (2015). Higher removal of pathogens was in rainy monsoon (August), autumn (September, October), spring (March and April) and summer (May and June) seasons as compared to winter season (Table 4.1). A basic reason for this might be that during these seasons the average temperature was in the range of 20-38°C therefore, metabolic activities of microorganisms would be at their peak, as a result natural die-off processes and predation by protozoa would take place (Rehman *et al.*, 2012; Latrach *et al.*, 2018). Another reason for pathogens removal during treatment of wastewater by pilot scale TF system at 20-38°C might be extended retention time, reactor configuration, microbial competition for survival and chemical interaction (Ruiz-Espinoza *et al.*, 2012). The removal efficiency of fecal coliforms by the reactor decreased to 43-47% in winter seasons (10-15°C) which clearly indicated that fecal coliforms resist inactivation when temperatures were lower than 20°C. This resistance to inactivation was valid because this group of bacteria were mesophilic in nature and the method to enumerate the fecal coliforms bacteria includes incubation at 35°C. However, many of the pathogenic bacteria found in wastewater were either bound to the solids matrix or trapped to form floc by absorption, coagulation, or precipitation processes that in turn provide better conditions for the survival of pathogenic microorganisms (Bitton, 2010).

In developing countries, it's a common practice to use untreated wastewater directly for irrigation purposes due to the lack of investment in infrastructure as well as the requirement for valuable nutrients (Musazura *et al.*, 2018). However, treatment of wastewater is considered to be an essential component with respect to public health protection, local social and religious beliefs (Starkl *et al.*, 2018). Safe use of treated water simply was the use of retrieved water following use in the home, industry or agriculture. The major factors that ensure treated water suitable for safe use in public and irrigation sectors are low levels of microbial load and toxic compounds. Furthermore, treated water allows a community to become less reliant on natural water reservoirs (USEPA, 2003; Woldetsadik *et al.*, 2017). Tang *et al.* (2018) also reported that treated water enhanced agricultural productivity by 10-30% as compared to using untreated wastewater. WHO, (2001) provides guidelines that treated water used for irrigation of fodder crops must contain less than 200 fecal coliforms/100 mL while USEPA, (2003) suggested that the acceptable MPN index for irrigation with river water containing wastewater discharge was up to 1,000 fecal coliforms/100 mL. In the current study, the MPN index of the treated water lies within WHO and USEPA standard limits, therefore, it is highly recommended that wastewater treatment plants should be designed in such manner that not only reduce organic pollution of river and streams but also reduce the count of pathogenic microorganisms.

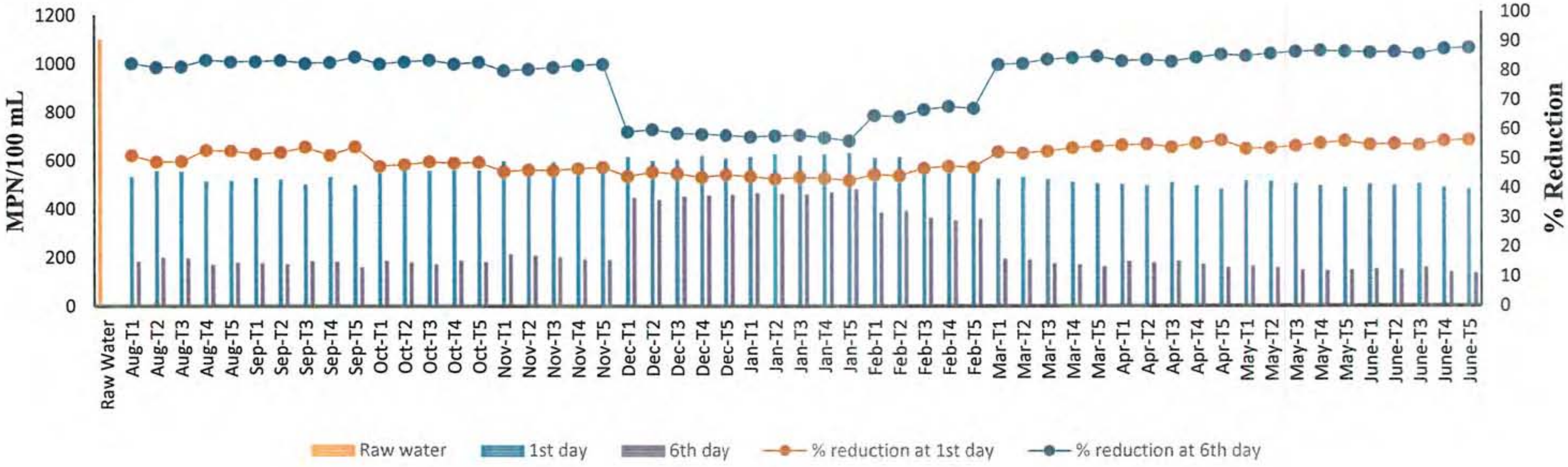


Fig. 4.3 Monthly variation in the percentage removal of fecal coliform by pilot scale TF system during entire treatment operation.

Table 4.1 Efficacy of pilot scale TF system in the removal of fecal coliforms under different seasonal conditions (± *indicates Standard error*).

Seasons	**Months-Year**	**Temperature** (°C)	**Average Fecal coliforms MPN/100 mL**			**Average % Reduction**	
			Influent	**Effluent at 1st day**	**Effluent at 6th day**	**At 1st day**	**At 6th day**
Rainy Monsoon	Aug-14	38±1.2	>1100	534.2	185.4	51.44	83.15
Autumn	Sep-14	24±3	>1100	516	175.2	53.10	84.07
	Oct-14	20±1	>1100	563.4	181.6	48.78	83.51
Winter	Nov-14	18±3	>1100	586.8	200.6	46.65	81.8
	Dec-14	12±2	>1100	607.2	447.6	44.8	59.31
	Jan-15	10±2	>1100	620.8	464.8	43.56	57.75
	Feb-15	15±1	>1100	591	368.2	46.273	66.53
Spring	Mar-15	23±3	>1100	515.2	177.6	53.2	83.85
	Apr-15	27±2	>1100	492.8	173.2	55.2	84.32
Summer	May-15	32±2	>1100	499	151	54.64	86.31
	June-15	38±3	>1100	490	145.4	55.45	86.78

4.3.2 Evaluation of the Pilot Scale TF System in the Removal of Carbonaceous Compounds

4.3.2.1 Pre-Treatment Characterization of Wastewater

Domestic wastewater is generally defined as a mixture of liquids, polluted with attenuated contents released from household, recreational and trading centers. Generally, it contains 99.6% water and 0.4% solids. The solid part of wastewater is further divided into biodegradable organics (70%) and inorganics (30%). The biodegradable organics in wastewater are usually composed of proteins, carbohydrates (starch, cellulose), fats, oils and grease etc. while the inorganic portion of wastewater is composed of sediments, salts and metals (Fan *et al.,* 2013). In the current study, the quality of domestic wastewater was examined in triplicates. It was dark grey in appearance and average values of-parameters such as BOD_5 (227.3±23.4 mg/L), COD (334.3±34.5 mg/L), TDS (355.26±26.6 mg/L) and TSS (466.3±45 mg/L) during the whole treatment operation showed considerable deviation from the prescribed limits of WHO, indicating a high level of contamination. Several research studies in the field of wastewater have focused on the physicochemical characteristics of wastewater samples (Waziri *et al.,* 2009; Gwaski *et al.,* 2013). In the present study, the physico-chemical parameters showed that the influent obtained from the residential area, of the Quaid-i-Azam University (QAU), Islamabad, Pakistan contained high loads of organic pollutants represented by high COD, BOD_5 values, total suspended solids (TSS) and total dissolved solids (TDS) as shown in Table 4.2. Moreover, the BOD_5/COD ratio for whole period ranged from 0.64 to 0.72 with an average of 0.68. High BOD_5/COD ratio indicated a decline in DO concentration because the free oxygen in water was utilized by microbes and organic compounds leading to the failure of fish and other aquatic organisms to thrive. Based on these values, wastewater obtained from the residential area of QAU had been considered as heavy-duty wastewater due to high loads of organic pollutants. Therefore, a pilot scale TF system was designed to receive a high BOD concentration in the range of 195.3 to 264.8 mg/L.

4.3.2.2 Post-Treatment Characterization of Wastewater

Average variation in the physico-chemical parameters of effluent at day 1 and day 6 during treatment operation are presented in Table 4.2. As mentioned earlier the pilot scale TF system operates in five different seasons for about 11 months, therefore its efficiency was evaluated under different environmental conditions. In the present study, it was observed that pilot scale facility worked in all seasons but the highest average BOD_5 percentage reduction i.e. 70-74, 67-72, 68-71 and 53-65% was observed in summer, spring, rainy monsoon and autumn respectively, while in winter, it achieved removal of carbonaceous compounds in the range of 13-57% after day 6 treatment (Fig. 4.4). Similar pattern of percentage reduction was also noted for COD during entire treatment operation under different environmental conditions (Fig. 4.5). On the other hand, a considerable average BOD_5 and COD percentage reduction were observed in summer (29-37%), spring (30-44%), rainy monsoon (32-34%) and autumn (8-29.5%) seasons while a very low percentage reduction were observed in winter season (5-32%) after Day 1 treatment operation (see Table 4.2). Different researchers found similar removal proficiencies for BOD_5 and COD, and reported that a primary mechanism for BOD_5 and COD removal was sedimentation, adsorption and microbial digestion of organic compounds present in wastewater (Lekeufack *et al.*, 2017; Iribarnegaray *et al.*, 2018). Furthermore, it was observed that trickling filters operate more effectively in a warm rather than a cold climate i.e. the performance of the pilot scale TF system began to decline from October to February because in these months the temperature was in the range of 10-20°C. A basic reason might be that the biochemical reactions are temperature dependent and the activity of the microorganism increases with the increase in temperature up to certain value, and drop with decrease in temperature. Similar investigations were reported by a group of researchers and observed that seasonal variation had a tremendous effect on BOD_5 and COD removal efficiencies by the bioreactor, with the best performance occurring during the summer while in winter seasons, it started decline due to reduction in the metabolic activities of organisms (Kishimoto *et al.*, 2014; Kim *et al.*, 2016).

Furthermore, a high degree of reduction in TSS concentration (74-88%) during the entire treatment operation clearly indicated that TSS is a temperature independent parameter as shown in Fig. 4.6. In addition, TSS is a significant factor in observing water clarity. Heavier particles, such as gravel and sand, often settle out in the primary sedimentation tank, ~~while,~~ the remaining particles that do not settle out are termed colloidal or non-settleable solids. These non-settleable solids enter into the reactor where they are retained on the surface of the filter bed and microbes present in slime layer have sufficient contact time to utilize them as a source of nutrients (EPA, 2014; Ali *et al.*, 2016). In addition to this, Pungrasmi *et al.* (2015) reported that continuous recirculation of wastewater over the surface of filter bed at preeminent flow rates played a significant role in the removal of TSS from wastewater. While in the current study, a 0.193 m^3/day recirculation flow rate was maintained throughout the study, which produced the highest percentage reductions in all seasons for TSS.

Another important parameter in water quality studies is TDS, although there is no health risk associated with TDS. However, the WHO suggested about 500 mg/L value of TDS in water provisions (WHO, 2006). In case of TDS, the highest percentage reduction was obtained in spring (37-55%), summer (56-68%), rainy monsoon (60%) and autumn (53-57%). In the winter season, the concentration of TDS reduced in the range of 18-37% (Fig. 4.7). Reduction in TDS content with increasing hydraulic retention time (HRT) was also due to continuous recirculation of wastewater for an extended period of time. As a result, microorganisms within the biofilm were allowed sufficient time to degrade dissolved organic components (Rehman *et al.*, 2012). Furthermore, Khan *et al.* (2015) reported 23 and 66% reduction in the TDS values after 24 and 48 hours of recirculation respectively during their study while using a stone media TF system integrated with a sand column filter. Furthermore, it was also noticed that the influent TDS seemed to decrease as the temperature decreased and also reactor efficiency in the removal of TDS decreased in comparison with high temperature months. These differences might be a result of the varying domestic waste loads on the QAU campus, impacted by resident numbers, commercial activities, sampling and analytical procedures (Wang *et al.*, 2018).

Sa and Boaventura, (2001) reported that COD/BOD_5 removal was positively correlated with increase in DO concentrations. In the present study, it was observed that the average initial value of DO in all seasons was very low but after treatment, a significant improvement ($p = 0.002$) in the quality of water was observed in terms of DO as shown in Fig. 4.8. Increase in the DO and reduction in the BOD_5 and COD concentration indicates that microorganisms within slime layer actively participate in the degradation of carbonaceous compounds present in wastewater. Moreover, the value of pH showed no substantial changes during treatment operation (Fig. 4.9).

Table 4.2 Mean values of different physico-chemical parameters of influent and effluent samples and average % reduction at day 1 and day 6 under different seasonal conditions.

Season	Months & Temp (°C)	Parameters	Mean Influent	Mean Effluent Day 1	Mean Effluent Day 6	Avg. % Red. Day 1	Avg. % Red. Day 6
Rainy Monsoon	Aug-14 (38±1.2°C)	BOD_5 (mg/L)	222.4±5.7	147.7±9.03	69.4±6.7	33.58	68.84
		COD (mg/L)	327±8.4	217.6±13.3	102±9.95	33.45	67.83
		TDS (mg/L)	388.53±7.4	248.7±11.8	155±16.9	35.9	60.1
		TSS (mg/L)	429.3±10.2	228.5±17	103.2±13.7	46.77	76.1
Autumn	Sep-14 (24±3°C)	BOD_5 (mg/L)	195.3±7.02	137.65±7.2	69.7±4.3	29.51	64.34
		COD (mg/L)	287.2±10.3	202.4±10.6	102.6±6.4	29.52	63.2
		TDS (mg/L)	378.1±8.8	264.4±10.6	161.4±3.7	30.08	57.32
		TSS (mg/L)	430.1±3.3	259.8±2.75	108.37±3.2	39.59	74.82
	Oct-14 (20±1°C)	BOD_5 (mg/L)	235.5±3.2	215.6±3.2	112.8±3.1	8.45	52.1
		COD (mg/L)	346.36±4.7	317±4.67	165.89±4.1	8.47	51.85
		TDS (mg/L)	365.0±14.6	267.7±17.1	168.1±4.5	26.6	53.7
		TSS (mg/L)	419.7±10.1	257.53±7.8	109.1±6.4	38.63	74.04
Winter	Nov-14 (18±3°C)	BOD_5 (mg/L)	244.2±4.5	194.63±3.3	128.55±7.6	20.29	47.33
		COD (mg/L)	359.1±6.7	286.23±4.9	189.1±11.2	20.29	46.77
		TDS (mg/L)	327.1±3.3	276.4±5.4	204.2±12.1	15.5	37.6
		TSS (mg/L)	419.8±7.3	178.41±4	90.06±5.6	57.50	78.55
	Dec-14 (12±2°C)	BOD_5 (mg/L)	264.8±4.2	241.05±2.1	193.22±6.0	8.96	27.03
		COD (mg/L)	389.5±6.1	354.5±3.1	284.2±8.6	8.98	26.84
		TDS (mg/L)	341.7±9.4	291.6±1.92	271.9±11.2	14.6	20.4
		TSS (mg/L)	478.1±8.2	256.84±3.3	122.7±3.1	46.27	74.33
	Jan-15 (10±2°C)	BOD_5 (mg/L)	245.8±3.5	231.67±3.3	212.78±3.9	5.74	13.42
		COD (mg/L)	361.5±5.14	340.7±4.85	312.9±5.8	5.75	12.94
		TDS (mg/L)	331.6±7.1	298.4±5.01	269±24.5	10.01	18.87
		TSS (mg/L)	463.5±7.3	217.8±8.3	107.4±7.3	53.0	76.82
	Feb-15 (15±1°C)	BOD_5 (mg/L)	196.6±12.5	132.5±4.6	96.97±2.75	32.6	50.48
		COD (mg/L)	289.1±18.2	194.85±6.8	142.6±4.05	32.6	49.37
		TDS (mg/L)	384.2±9.3	323.5±8.89	290.6±7.64	15.8	24.4
		TSS (mg/L)	518.8±4.1	220.23±2.1	99.8±3.1	57.5	80.76
Spring	Mar-15 (23±3°C)	BOD_5 (mg/L)	218.5±4	153.13±5.22	71.38±3.73	29.9	67.34
		COD (mg/L)	321.3±5.7	225.2±7.7	104.9±5.48	29.9	67.11
		TDS (mg/L)	338.4±10.4	270.7±17	211.1±7.96	19.9	37.63
		TSS (mg/L)	484.1±6.7	226.35±5.4	106.7±3.12	53.2	77.96
	Apr-15 (27±2°C)	BOD_5 (mg/L)	197.8±4.8	110.01±6.8	55.75±4.5	44.38	71.8
		COD (mg/L)	290.8±7.1	161.78±10	81.98±6.63	44.36	72.5
		TDS (mg/L)	326.13±4.5	241.0±20.5	147.0±18.2	26.1	54.9
		TSS (mg/L)	562.5±9.9	244.9±21.7	64.08±6.8	56.46	88.62
Summer	May-15 (32±2°C)	BOD_5 (mg/L)	247.8±2.87	174.86±9.5	74.67±2.72	29.43	69.88
		COD (mg/L)	364.5±4.2	257.15±13.9	109.8±4	29.45	68.76
		TDS (mg/L)	333.9±5.3	227.4±5.7	144.4±5.5	31.9	56.76
		TSS (mg/L)	481.7±3.8	239.4±10.8	110.84±8.6	50.3	77.2
	Jun-15 (38±3°C)	BOD_5 (mg/L)	231.5±2.3	145.9±2.46	61.26±1.45	36.9	73.54
		COD (mg/L)	340.5±3.4	214.57±3.63	90.08±2.14	36.98	72.63
		TDS (mg/L)	393±8.8	227.5±10.6	121.87±3.7	42.1	69
		TSS (mg/L)	441.3±6	212.1±6.3	93.1±3.25	51.9	79.22

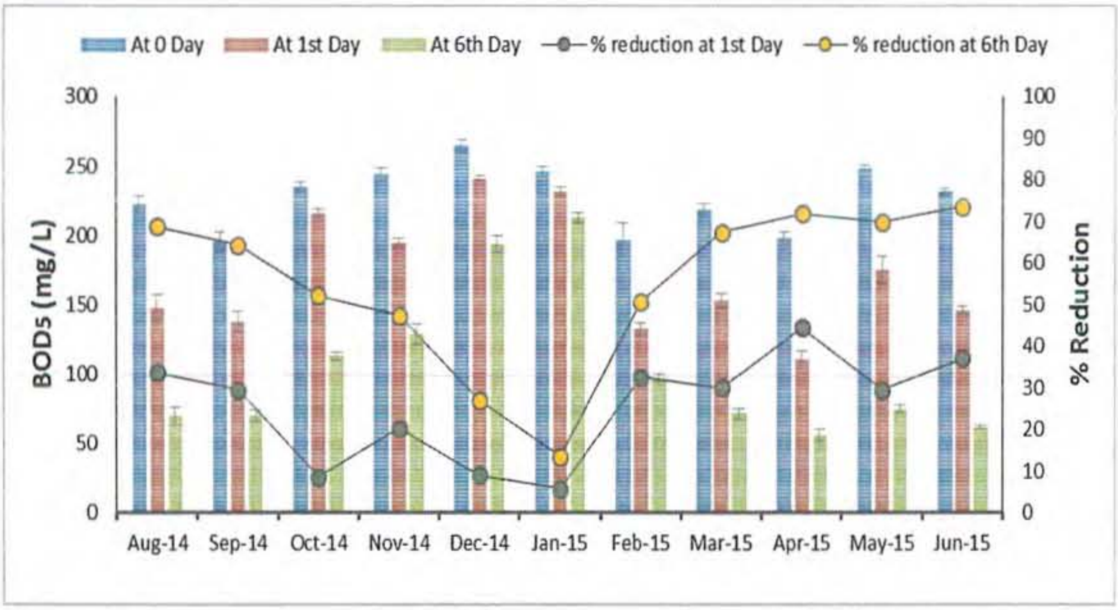


Fig. 4.4 Efficiency of pilot scale TF system in the average reduction of BOD_5 during entire treatment operation (*Bar indicates Standard error*).

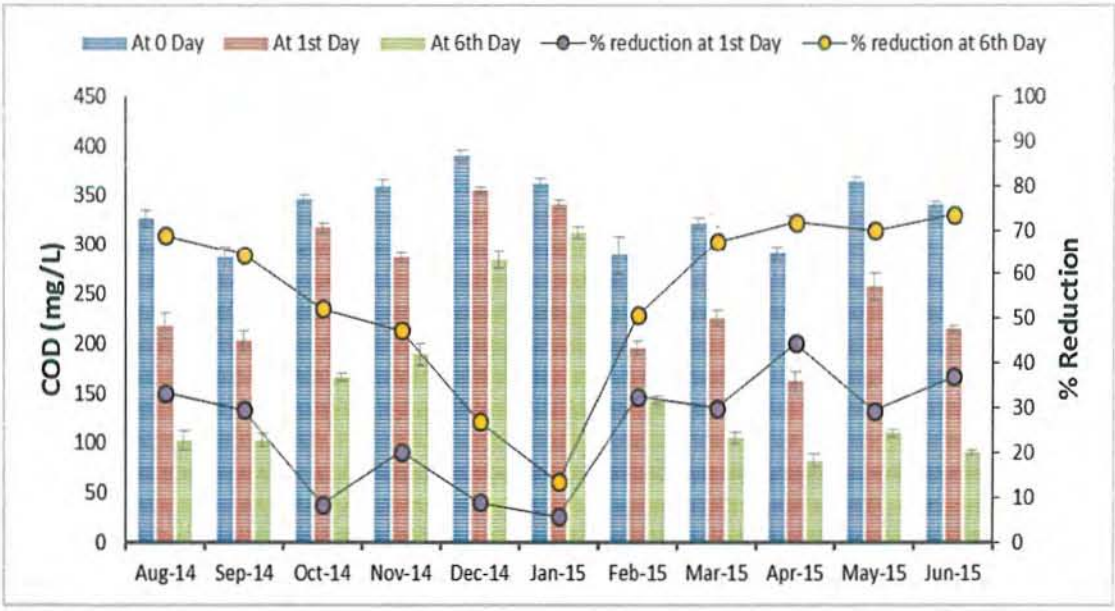


Fig. 4.5 Efficiency of pilot scale trickling filter system in the average reduction of COD during entire treatment operation (*Bar indicates Standard error*).

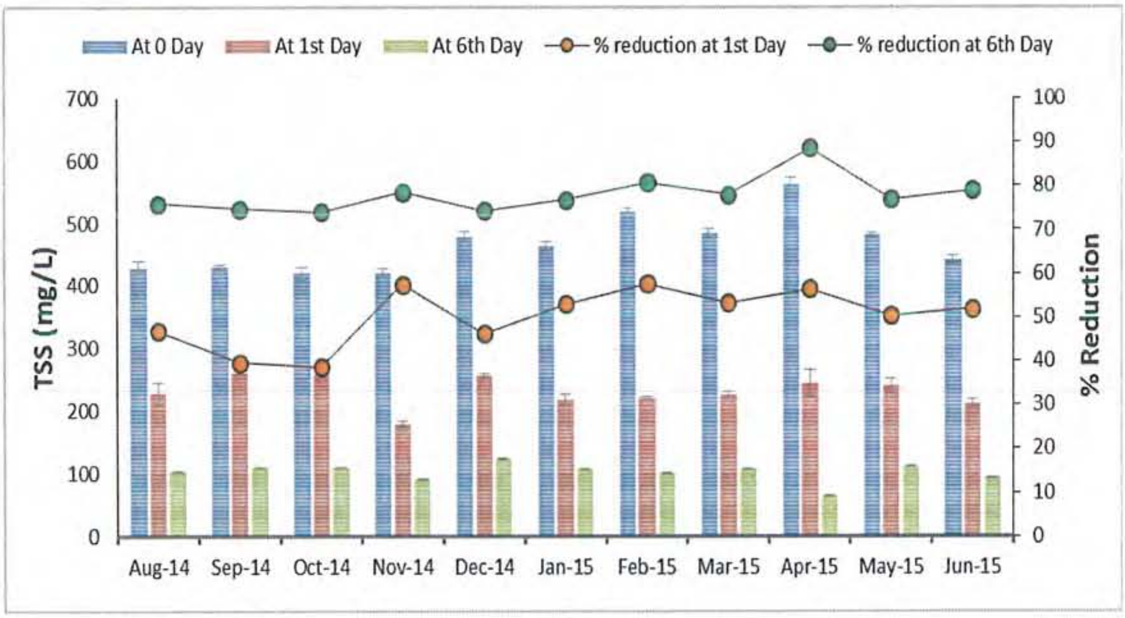


Fig. 4.6 Efficiency of pilot scale trickling filter system in the average reduction of TSS during entire treatment operation (*Bar indicates Standard error*).

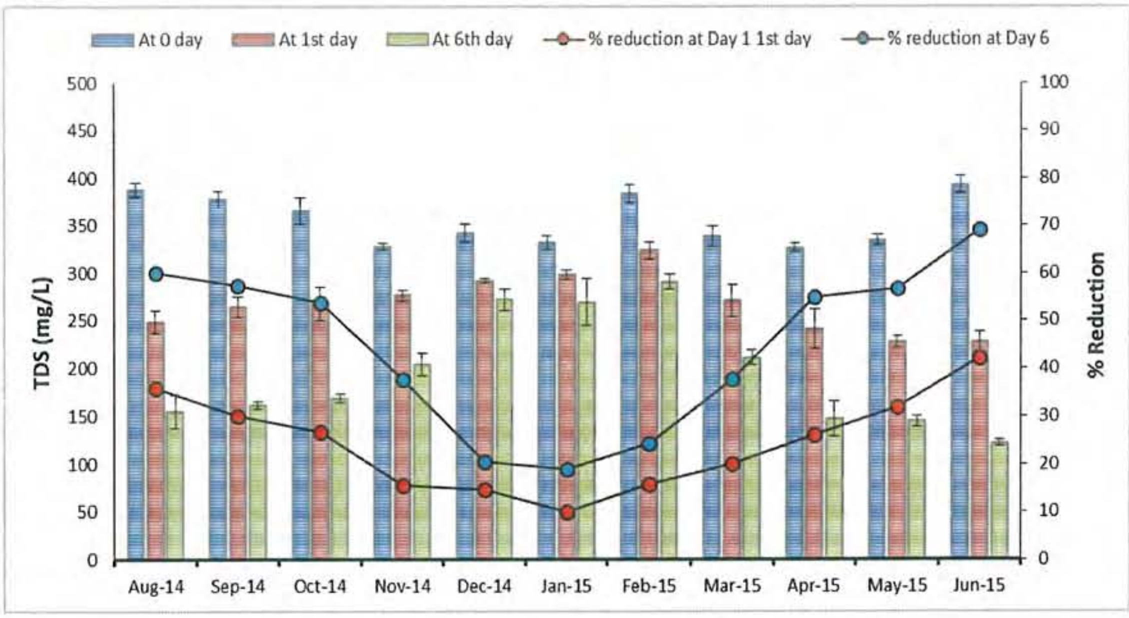


Fig. 4.7 Efficiency of pilot scale trickling filter system in the average reduction of TDS during entire treatment operation (*Bar indicates Standard error*).

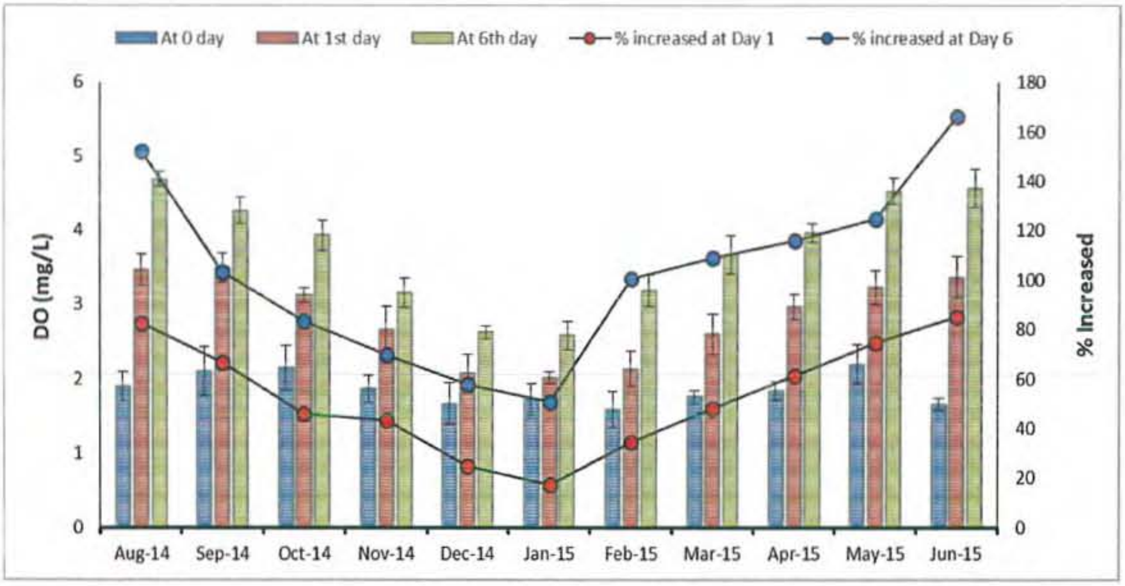


Fig. 4.8 Efficiency of pilot scale trickling filter system to improve DO concentration during entire treatment operation (*Bar indicates Standard error*).

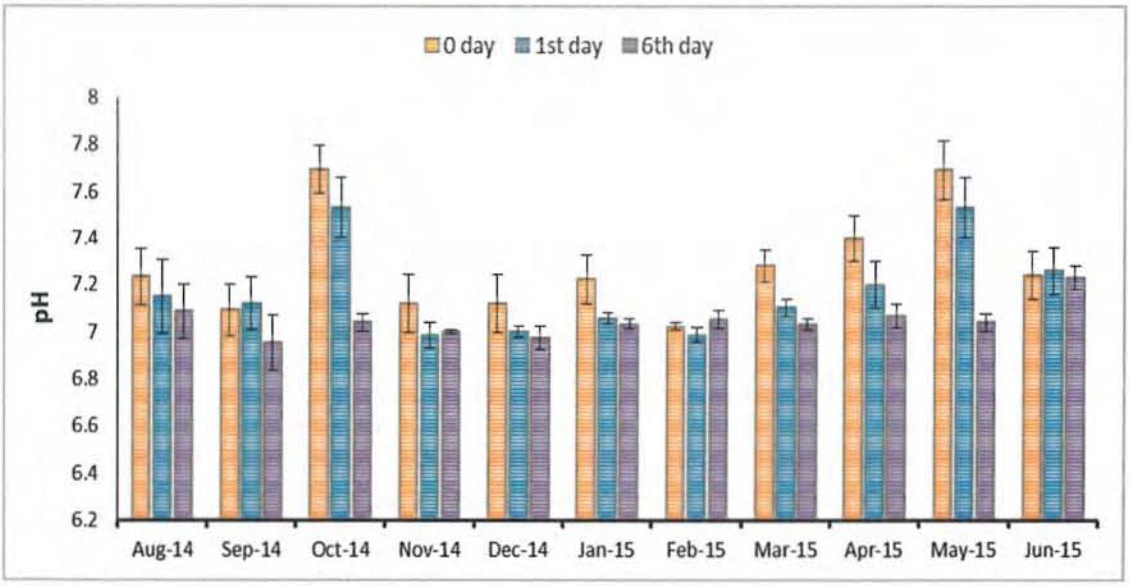


Fig. 4.9 Variation in the concentration of pH of influent and effluent during various seasons/months (*Bar indicates Standard error*).

4.3.3 Bacteriological Assessment of Slime Layer Developed on Stone Filter Media

Isolated bacteria were characterized according to Bergey's Manual of Determinative Bacteriology (9th Edition). On the basis of microscopy, cultural characteristics on different media and biochemical tests, 13 different bacterial isolates were identified in biofilm. Out of 13 different bacterial isolates, five were Gram positive and eight were Gram negative. After microscopy, these bacterial isolates were sub-cultured on nutrient agar media and different cultural characteristics were observed. After cultural characteristics, a complete identification of these bacterial isolates were carried out by performing biochemical tests. Isolate # 1, 2, 5, 9 and 12 produced acids as well as gas during sugar fermentation, isolate # 4, 6, 7, 8 and 11 were only ferment dextrose and sucrose while isolate # 3, 10 and 13 did not ferment sugar. Isolate # 7 and 11 showed positive results in term of H_2S production while all other isolates displayed negative results. Isolate # 12 and 13 showed negative results in case of NO_3 reduction while all other gave positive results. Isolate # 5, 6 and 11 showed positive indole test while all other isolates produced negative results. In case of MR test, isolate # 2, 5, 6, 7, 9, 11 and 12 showed positive response while other gave negative response while in case of VP test, isolate # 1, 2, 5, 6, 7, 9, 11 and 12 showed positive results while the remaining isolates showed no response. In case of urease test, isolates encoded as 2, 3 and 11 showed positive results while all other isolates displayed negative result. On other hand, all isolates except 12 showed positive results in case of catalase test. Isolate # 2, 4, 10, 11, 12 and 13 showed negative responses in case of TSI test while the remaining isolates showed positive response according to their biochemical nature. On the basis of microscopic, cultural and biochemical tests bacterial isolates were identified as *E.coli, Salmonella, Pseudomonas, Enterobacter, Klebsiella, Shigella, Proteus, Alcaligenes, Staphylococcus, Streptococcus, Micrococcus, Corynebacterium* and *Bacillus* spp. The detailed description of microscopy, cultural and biochemical analysis of all identified bacterial isolates is given in the Table (4.3).

Table 4.3 Bacteriological assessment of slime layer developed on stone filter media.

Isolate	Cultural characteristics	Gram's Reaction	Fermentation			H_2S test	NO_3 test	Indole test	MR test	VP test	Citrate test	Urease test	Catalase test	TSI test	Identified Organisms
			L	D	S										
1	Abundant, white, brilliant growth	–	AG	AG	AG	-	+	-	-	+	+	-	+	K/A	*Enterobacter* spp.,
2	Slimy white, translucent, convex	–	AG	AG	AG	-	+	-	±	±	+	+	+	-	*Klebsiella* spp.,
3	Soft, smooth, yellow growth	+	-	-	-	-	±	-	-	-	-	+	+	K/NC	*Micrococcus* spp.,
4	Grayish, granular, slow growth	+	-	A±	A±	-	+	-	-	-	-	-	+	-	*Corynebacterium* spp.,
5	White, moist, brilliant growth	–	AG	AG	A±	-	+	+	+	-	-	-	+	A/NC	*Escherichia coli*
6	Thin, even, grayish growth	–	-	A	A±	-	+	±	+	-	-	-	+	K/A, H_2S	*Shigella* spp.,
7	Thin, even grayish growth	–	-	AG±	A±	+	+	-	+	-	+	-	+	K/A, H_2S	*Salmonella* spp.,
8	Abundant, opaque, white growth	+	-	A	A	-	+	-	-	±	-	-	+	A/NC	*Bacillus* spp.,
9	Abundant, opaque, golden growth	+	A	A	A	-	+	-	+	±	-	-	+	A/A	*Staphylococcus* spp.,
10	Abundant, thin white growth	–	-	-	-	-	+	-	-	-	+	-	+	-	*Pseudomonas* spp.,
11	Thin, blue gray spreading growth	–	-	AG	AG	+	+	+	+	-	±	+	+	-	*Proteus* spp.,
12	Thin, even growth	+	A	A	A	-	-	-	+	-	-	-	-	-	*Streptococcus* spp.,
13	Light creamy, spreading growth	–	-	-	-	-	-	-	-	-	±	-	+	-	*Alcaligenes* spp.,

Key: L = Lactose; D = Dextrose; S = Sucrose; AG = Acid and gas; + = Positive; - = Negative; ± = Variable reaction; A = Acid production; K = alkaline reaction; NC = No change; H_2S = Sulfur reduction; K/A = Red/yellow; K/NC = Red/no color change; K/A, H_2S = Red/yellow with bubble and black precipitate; A/NC = Acid/no color change; A/A = Yellow/yellow.

4.3.4 Model Applications

Modelling was considered to be an essential component to describe the reality of all biochemical processes occurring during wastewater treatment (Boltz *et al.*, 2017). The empirical model was designed using simulation data of BOD_5 obtained from experimental work done under different prevailing environmental temperature conditions. Such a model should be simple and do not increase the level of intricacy and-computational demand when coupled with other sub-models.

In the current study, the pilot scale TF model was based on zero order kinetics, which was calibrated at 20°C using experimental BOD_5 data obtained in the month of October, 2014 to calculate the "k_{20} value" which was further validated to determine "k_t value" for each BOD_5 experimental setup performed under different temperature conditions (Table 4.4). The value of "k_t" obtained from experimental results showed better performance at six different temperature data sets i.e. November (18°C), December (12°C), January (10°C), March (23°C), May (32°C) and June 2015 (38°C). In these different temperature data sets, the value of "k_t" relates to higher removal of BOD_5 through pilot scale treatment system due to the excellent mass-transfer coefficient as shown in Figs. 4.10-4.15.

From zero order kinetic modelling, it had been observed that the value of "k_t" was specific for each experimental data set. However, minor differences between the experimental data and the modelling ones were observed on some days especially Day 1. A basic reason for this might be that in a fixed film system, microorganisms are attached to the surface, therefore the supply of nutrients to the microbes in the slime layer might be restrained by bulk liquid and surface transport mechanisms. Normally in fixed film growth systems, nutrients or organic substances present in wastewater would be transported to the outermost surface of biofilm, from where it would diffuse into the biofilm for their utilization. According to Fick's law and the Monod expression, there are different factors that affect the rate of substrate utilization within a biofilm i.e. transport of nutrients to the outer surface of biofilm from bulk liquid, diffusion of nutrients from outer surface into biofilm, and utilization kinetics within the biofilm. Beside this, there are some other factors which affect the efficiency of attached growth bioreactors that usually includes hydraulic loading rates, temperature, recirculation flow rates etc. In the literature, very few empirical models were reported that can forecast the efficiency of a trickling filter system and among them, most models were based on steady state conditions. A major drawback of the steady state model is that it does not give sufficient information regarding biomass growth with time, voids ratio of filter media and recommended depth for the filter bed (Rene *et al.*, 2011; Hvala *et al.*, 2017).

Moreover, simulated results described that about 21-45% removal of BOD_5 in low temperature range data (Nov, Dec and Jan, 2014) and 67-90% in high temperature range data sets (Mar, May and June, 2015) were observed as shown in Fig. 4.10-4.15, which exhibited that the model for the pilot scale TF system worked seamlessly in the removal of carbonaceous compounds from wastewater. Hvala *et al.* (2017) also used an empirical model to evaluate the efficiency of wastewater treatment in terms of BOD_5 and observed considerable reduction in the BOD_5 content. Furthermore, they concluded that bioreactor efficiency was greatly affected by bringing changes in the organic and hydraulic loading rates. In interpretation of this study, a significant correlation might exist in experimental as well as mathematical data for BOD_5 under different temperature conditions, therefore it gives more reliable and precise appraisals with regard to hydraulic load rates, biomass concentration and their specific activity in wastewater research as well as design of wastewater treatment plants. The model foretells that percentage reduction of BOD_5 surpasses the maximum possible for the assumed size distribution of soluble organics. The key drawback of the existing model was that wastewater must have related features to those used for model calibration. This drawback not only incorporates the diffusion coefficients of dissolved organics but also microbial activities for these substrates. The model also assumes that recirculation of wastewater enhances the overall level of wastewater treatment efficiency by the pilot scale TF system. Oliynyk and Kolpakova, (2014) also developed a mathematical model describing removal of BOD_5 from domestic wastewater by attached growth systems under many loading and design conditions. They reported that their simulated model was perfect to precisely predict percent removal of BOD_5 as a function of hydraulic loading rate, recirculation rate, depth of filter bed and microbial activity within biofilm under different temperature conditions.

.

Table 4.4 Parameters of pilot scale TF system under different seasonal conditions (± *indicates Standard error*).

Seasons	Months-Year	Temperature (°C)	Biomass "X" (mg/m^3)	Flow Rate "Q" (m^3/day)	Height * Area of Reactor (m^3)	K_t value	OLR (mg/m^3/day)
Rainy Monsoon	Aug-14	38±1.2	44	0.193	2.945	1.11	145.23
Autumn	Sep-14	24±3	42	0.193	2.945	0.809	127.55
	Oct-14	20±1	32	0.193	2.945	0.624	153.82
Winter	Nov-14	18±3	31	0.193	2.945	0.589	159.50
	Dec-14	12±2	21	0.193	2.945	0.496	172.94
	Jan-15	10±2	19	0.193	2.945	0.468	160.56
	Feb-15	15±1	34	0.193	2.945	0.54	128.4
Spring	Mar-15	23±3	37	0.193	2.945	0.68	142.67
	Apr-15	27±2	42	0.193	2.945	0.763	129.18
Summer	May-15	32±2	42	0.193	2.945	0.882	161.84
	Jun-15	38±3	47	0.193	2.945	1.048	151.20

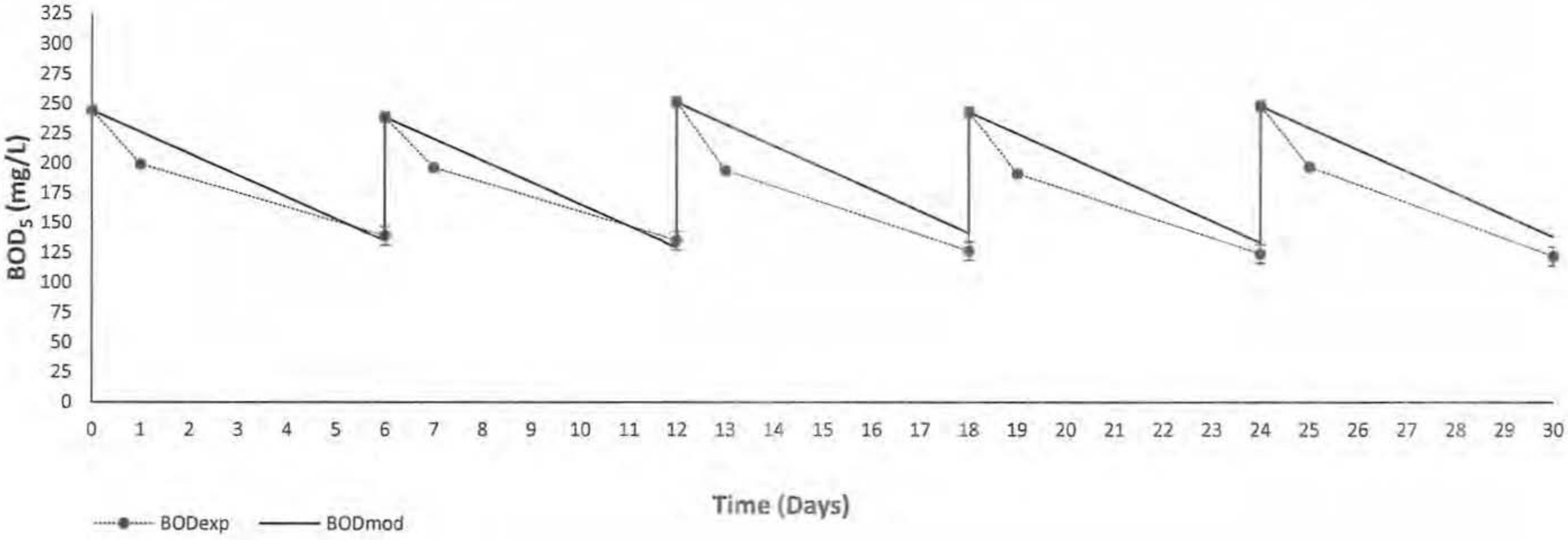


Fig. 4.10 Removal of BOD_5 by pilot scale trickling filter system in the month of November, 2014 (18±3°C).

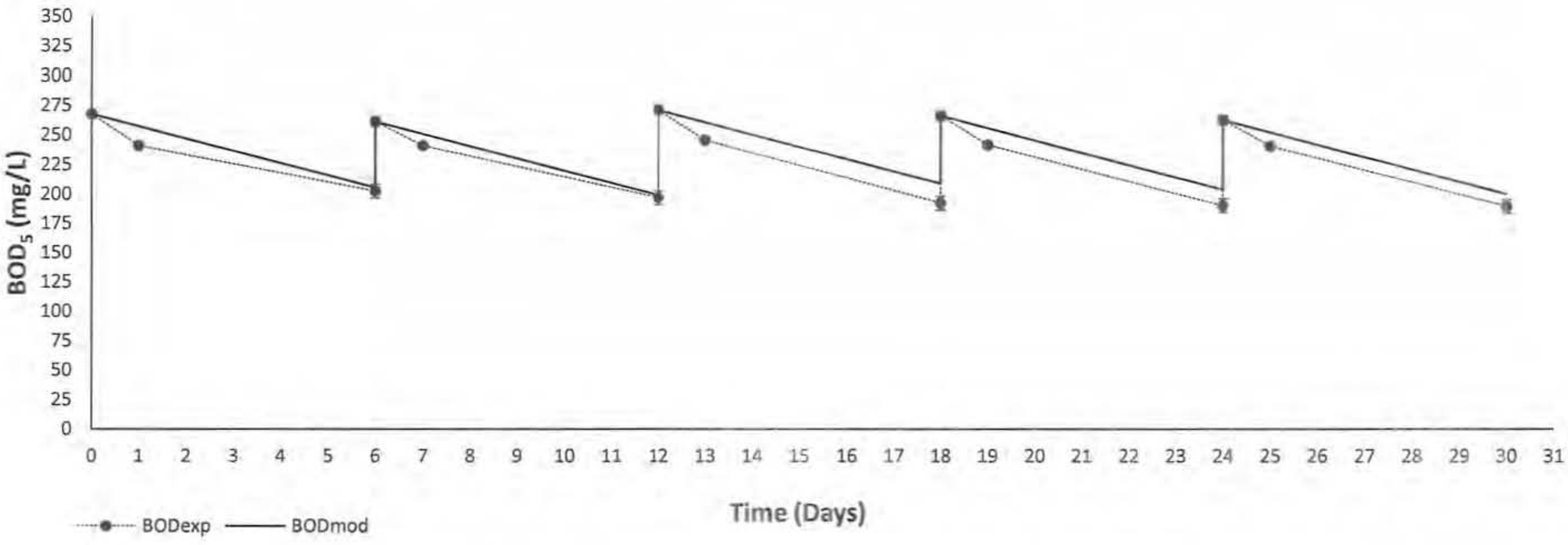


Fig. 4.11 Removal of BOD$_5$ by pilot scale trickling filter system in the month of December, 2014 (12±2°C).

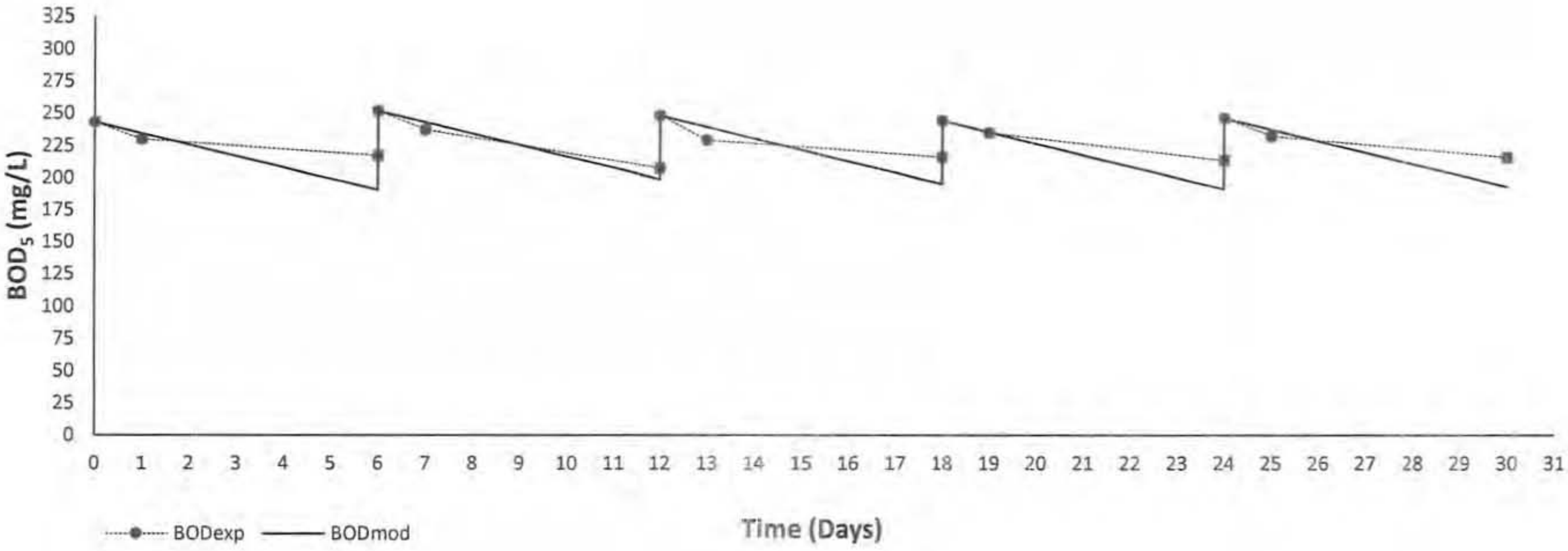


Fig. 4.12 Removal of BOD$_5$ by pilot scale trickling filter system in the month of January, 2015 (10±2°C).

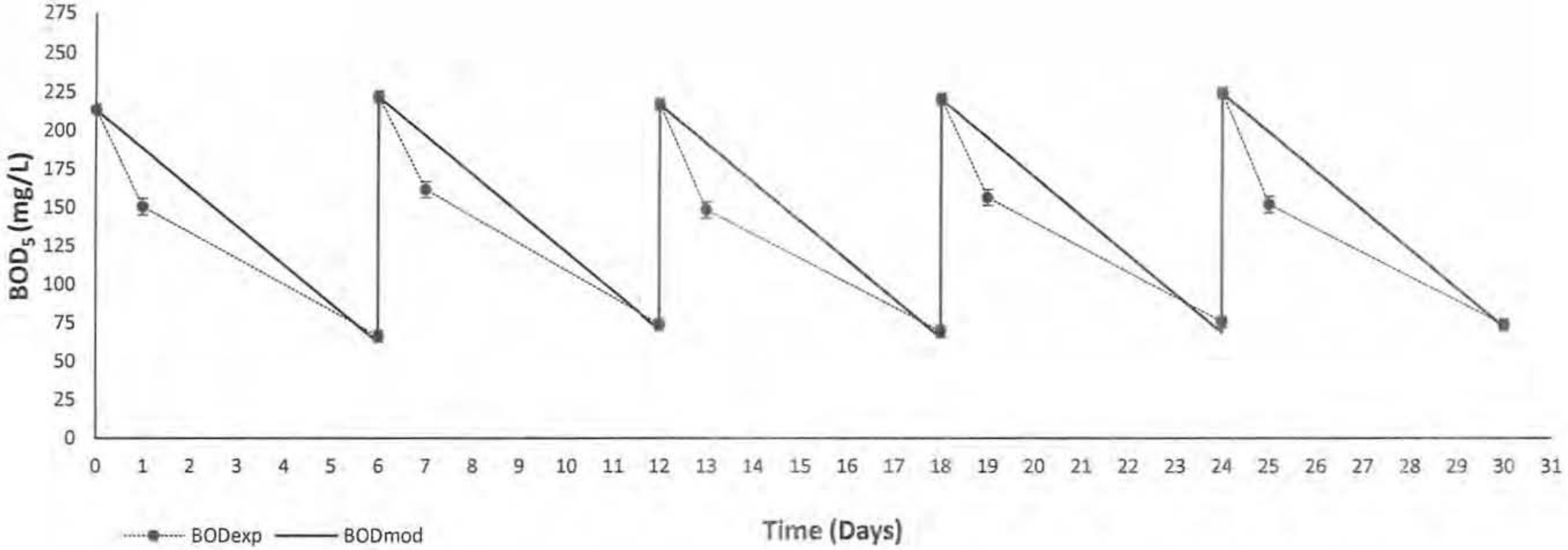


Fig. 4.13 Removal of BOD$_5$ by pilot scale trickling filter system in the month of March, 2015 (23±3°C).

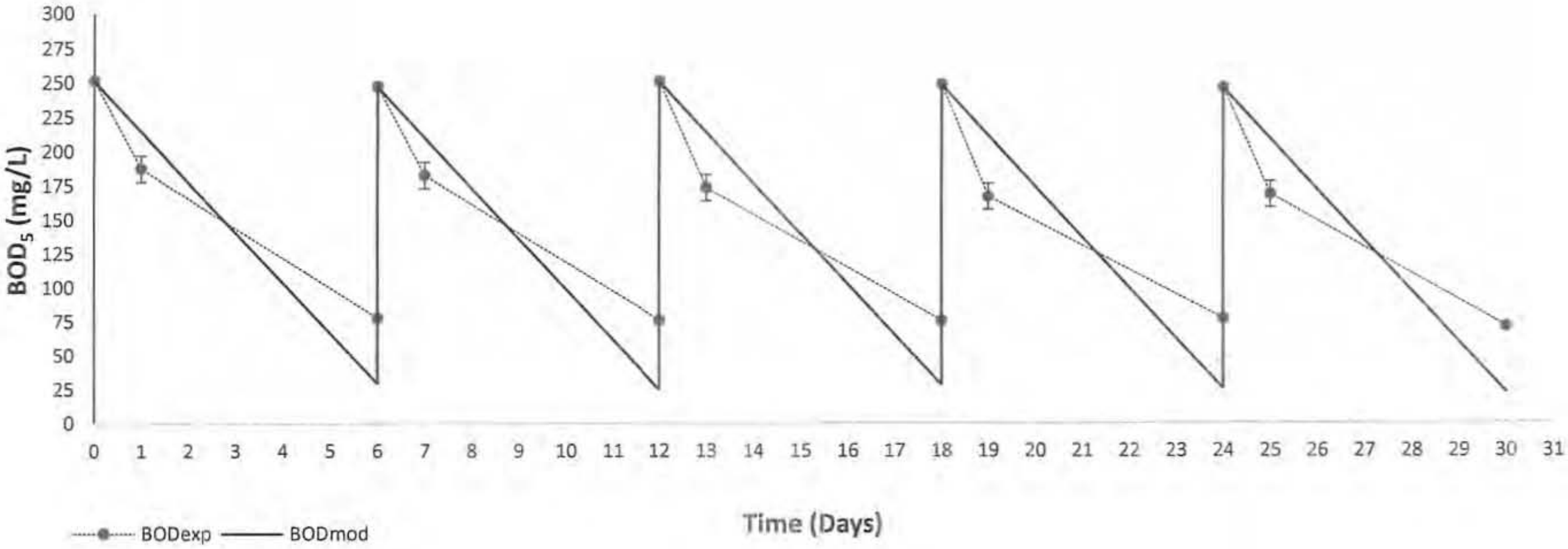


Fig. 4.14 Removal of BOD_5 by pilot scale trickling filter system in the month of May, 2015 (32±2ºC).

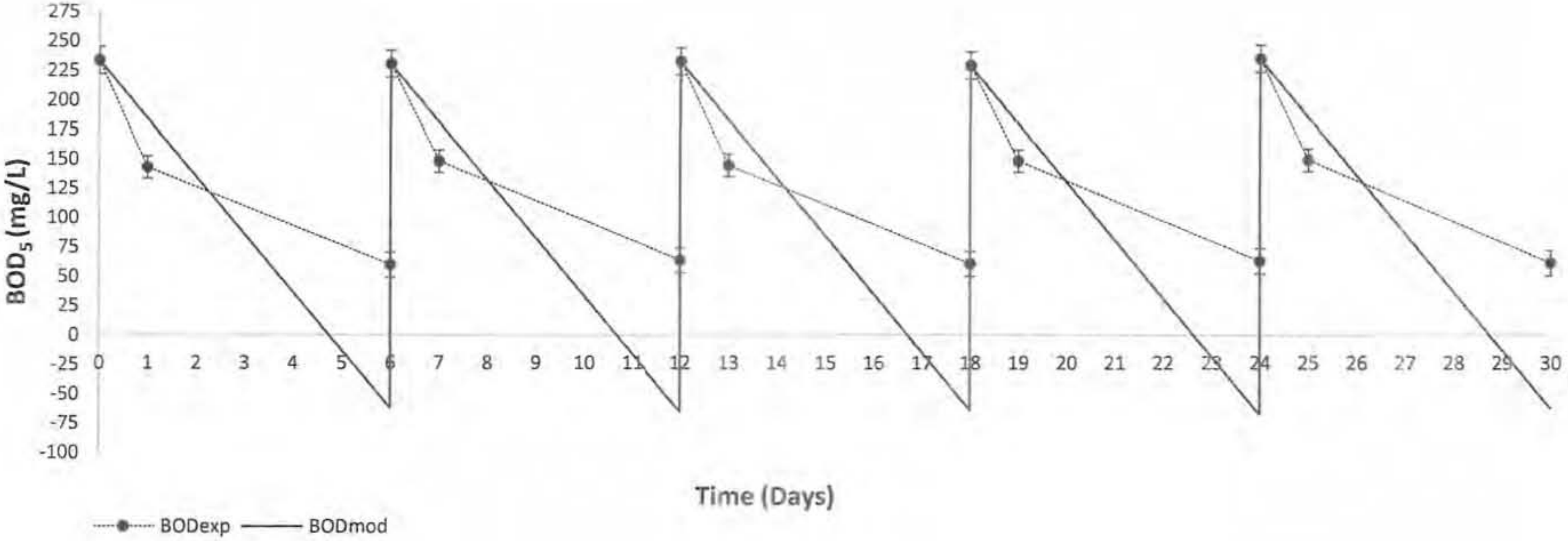


Fig. 4.15 Removal of BOD$_5$ by pilot scale trickling filter system in the month of June, 2015 (38±3°C).

Chapter 5

5. EFFICIENCY OF LOCALLY DESIGNED PILOT SCALE TRICKLING BIOFILTER SYSTEM IN NATURAL ENVIRONMENT FOR THE TREATMENT OF DOMESTIC WASTEWATER

5.1 Introduction

The present research study was aimed to design and construct a simple, cost-effective and efficient setup-II stone media pilot-scale TBF system in open environment at the residential area of QAU, Islamabad, Pakistan after optimizing the efficiency of setup-I pilot scale TF system towards wastewater treatment in closed environment (work station) for about one year. Objectives of the setup-II stone media pilot-scale TBF system were to overcome the challenges of the pollution indicators such as chemical oxygen demand (COD), biological oxygen demand (BOD_5), nutrients (sulphur, nitrogen, phosphorous, etc.) and also pathogens from domestic wastewater in order to produce effluent of good quality, which can be safely used for irrigation as well as ornamental purposes. The idea was to operate this setup-II system initially for 15 weeks to optimize its operational conditions for the treatment of domestic wastewater under natural environment and then transfer this model system for decentralized wastewater treatment of small residential colonies typically disconnected from main residential areas or lacking nearby wastewater treatment system. Furthermore, it was observed that the environmental temperature was in the range of 14.5-36°C during the study period.

5.2 Materials and Methods

5.2.1 Experimental Setup-II Scheme (Design and Operation)

The detail scheme of setup-II pilot scale TBF system was given in section 3.2 and 3.2.1 of chapter 3. However, the schematic illustration of flow of wastewater from different units during treatment operation is shown in Fig. 5.1. Environmental temperature was continuously monitored during the research study, and the maximum, minimum and average ambient temperatures were 36°C, 14.5°C and 28°C, respectively, whereas the maximum, minimum and average ambient temperatures of the influent were 25.5°C, 6.5°C and 11°C, respectively, during the study period.

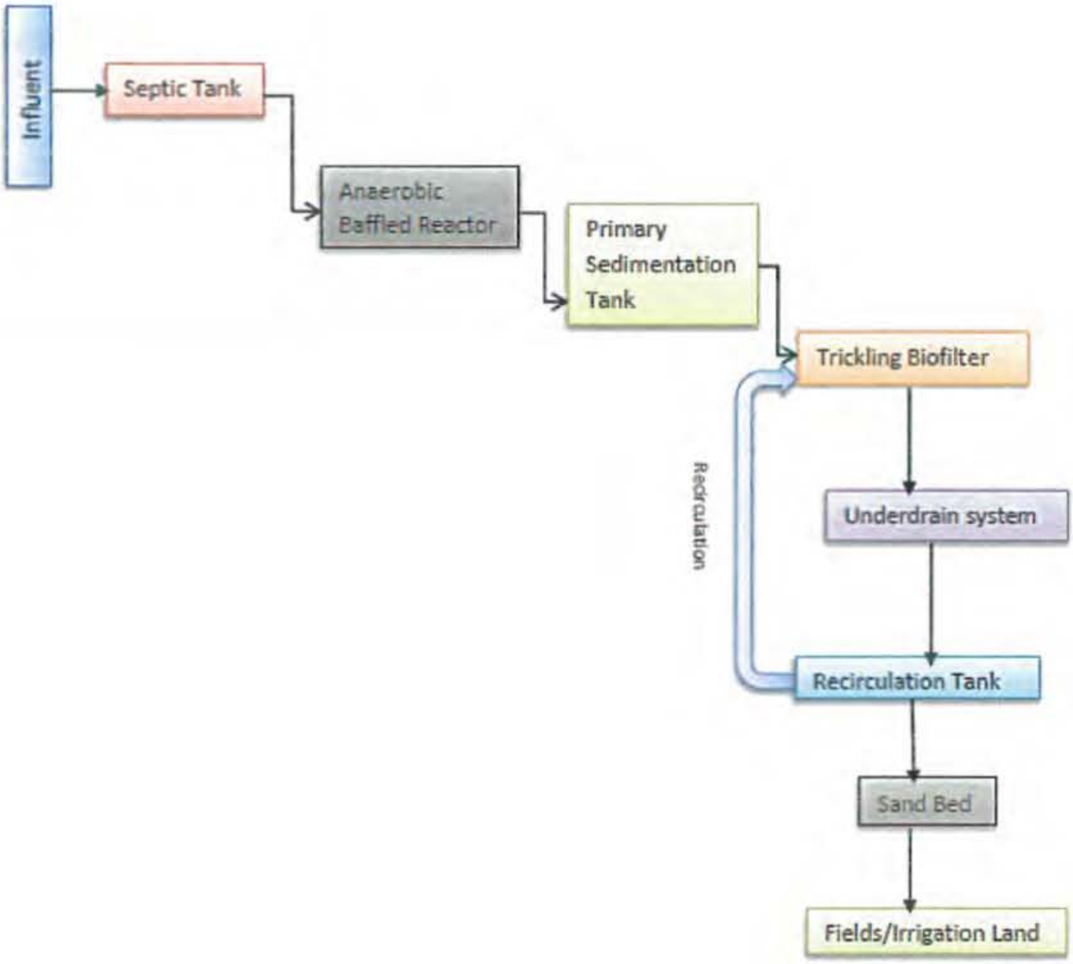


Fig. 5.1 Schematic illustration of flow of wastewater from different units during treatment operation.

5.2.2 Physico-Chemical Analysis of Wastewater

Physico-chemical analysis of various parameters, such as pH, DO, EC, BOD_5, COD, TDS, PO_4, SO_4, TN was determined by using their standard procedures given in section 3.4 of chapter 3 (APHA, 2005). However, salinity of influent and effluent samples were measured by a PCS Multi test meter. The values of the influent and effluent for whole week (7 days) were expressed as an average, and were indicated by 15 values for each parameter for 15 weeks.

5.2.3 Microbiological Characterization of Influent and Effluent Samples

Microbiological characterization of influent and effluent samples were determined in terms of most probable number (MPN index) and colony forming unit (CFU/mL). MPN and CFU tests were performed according to their standard protocols and detail procedure of MPN and CFU tests were given in section 3.5.1 and 3.5.2 of chapter 3.

5.3 Results and Discussion

The wastewater treatment efficiency of the locally designed and constructed pilot-scale TBF system was evaluated for 15 weeks under variable temperature conditions (14.5-36°C). It was observed that the TBF treatment system was effective in the removal of organic compounds, nutrients and pathogenic indicators from wastewater of the residential area of QAU, Islamabad, Pakistan at temperature range of 14-36°C.

5.3.1 Wastewater Treatment Efficiency of Setup-II Pilot-Scale TBF System

The presence of any color or odor in water is the sign of contamination and esthetically unpleasant. The odor in the water is due to several factors such as the presence of excessive nutrients, sulphur containing aromatic compounds and oxidation of aldehydes and ketones. The removal of the odor from wastewater was also the objective of this study and increased contact time of pollutant with the biofilm helps to eliminate the odorous compounds from the wastewater. In the present study, it was observed that several time recirculation of wastewater over the biofilm facilitates the degradation of the organic compounds and removal of the odor (Gupta, 2004).

Different physico-chemical parameters were used in the study to characterize the quality of water samples before and after treatment. Among physico-chemical parameters, BOD_5 is the most used parameter to determine organic pollution load and its biodegradation rates in wastewater (Eke and Scholz, 2008). Results of BOD_5 removal rates from domestic wastewater in the TBF system at 14.5-36°C are presented in Fig. 5.2. In the present study, the average value of BOD_5 was 104.95 mg/L in the wastewater used as an influent and declined to an average value of 13.68 mg/L in the effluent. It showed an average removal of 85.65% during the whole operational period of 15 weeks. The results indicate that BOD_5 elimination efficiency from influent significantly increases with operational time from the 1st to 15th week and range from 72.15% to 91.57% at a flow rate of 1.2 L/min. The highest removal rate was recorded (92.02%) on the 10th week of the reactor operation. However, > 80% wastewater treatment efficiency of the TBF system was observed after the 4th week of operation. Likewise, the average COD of influent and effluent observed over a study period of 15 weeks was 153.66 and 20.03 mg/L, respectively. COD removal efficiency of the TBF with an operational period from the 1st week (72.15%) to 15th week (91.57%) is shown in Fig. 5.2. However, the highest COD removal values (92.02%) were recorded for the 10th week of operation, showing an average COD decrease from 276 to 22 mg/L. This constant increase in the decline of BOD_5 and COD can be attributed to the increase in temperature and to the development of efficient biofilm community on stones capable of treating wastewater. The recirculation of the wastewater through the TBF bed provides both organic and inorganic nutrients to the microbial community of the biofilm, thus resulting in the establishment of metabolically competent biofilm (Naz *et al.*, 2016). Various researchers reported positive correlation between COD and BOD_5 removal efficiency with an increase in DO levels (Sa and Boaventura, 2001). In the present study, it was observed that the average initial value of DO was very low (2.6 mg/L), but after treatment a significant increase in DO concentration (27-110%) was observed (Fig. 5.2). Increase in the DO and reduction in the BOD_5 and COD contents indicate the active catabolism of organic pollutants by microbes present in the biofilm. Soontarapa and Srinapawong, (2001) reported that the active metabolism of pollutant by microbes is converted into biomass, carbon dioxide and water. Moreover, the removal of BOD_5 was also initially reported by Shipin *et al.*, (1999) during the treatment of wastewater by the TBF system. Khan *et al.*, (2015) reported 89.67% and 88.3% reduction in the BOD_5 and COD values after 48 hrs of treatments using laboratory-scale stone media fluidized bed reactor and concluded that the reduction in the carbonaceous content by microbes leads to increased level of dissolved oxygen. Presently, the ability of the TBF system to obtain high DO levels in the effluent was due to consistent arrangement of the filter stone bed providing high voidage for efficient passive aeration during recirculation of wastewater. Thus, sufficient aeration was provided by natural draft even without forced ventilation in the TBF system, which increased DO levels and decreased carbonaceous pollutants (COD and BOD_5) concentrations in the effluent.

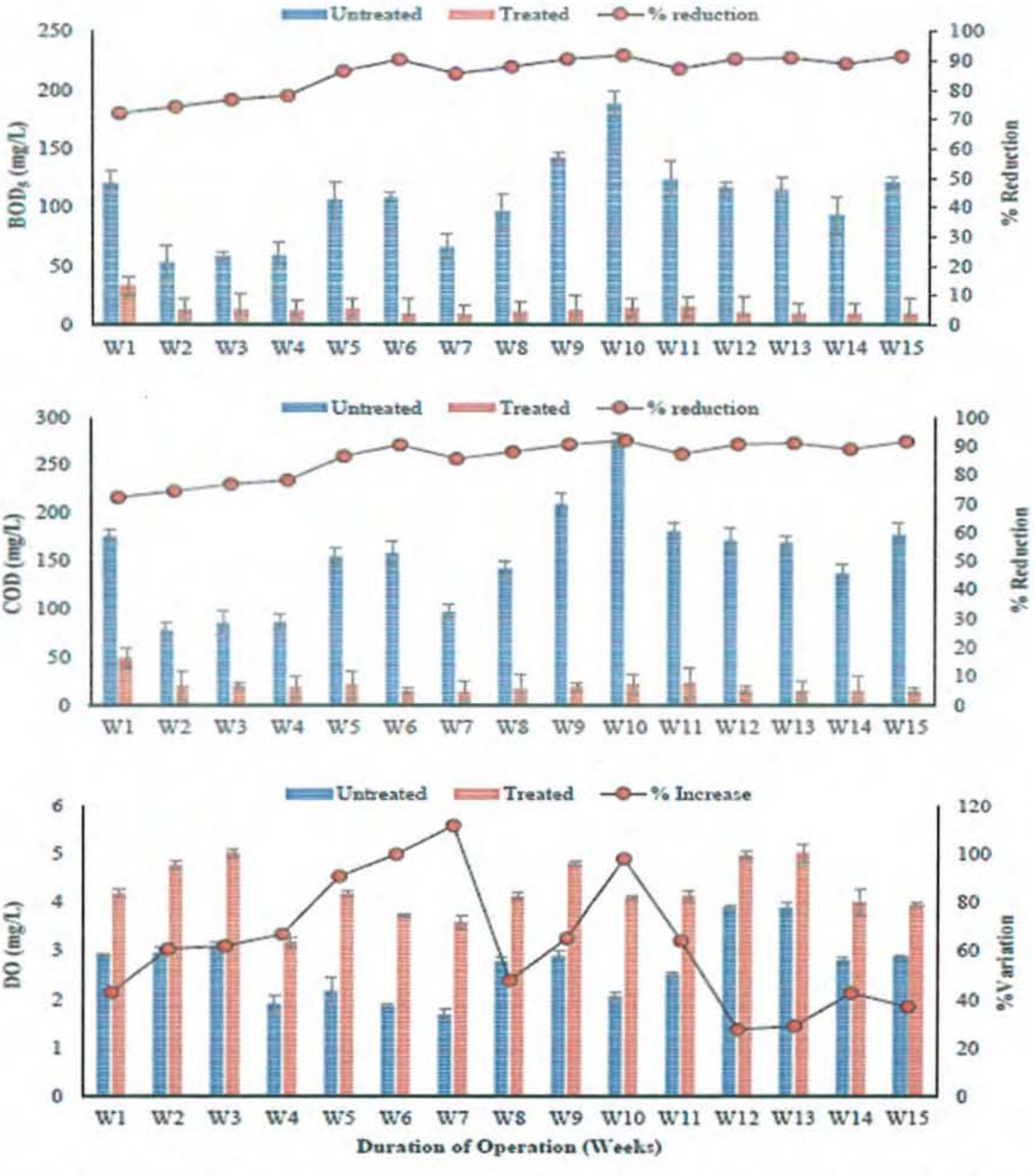


Fig. 5.2 Changes in the concentrations of COD, BOD_5 and dissolved oxygen (DO) in the effluent during 15 weeks of operation through stone media pilot-scale trickling biofilter at 14.5-36°C (*Bar indicates Standard error*).

The pH value variations in the influent and effluent were monitored over time and it was observed in the range of 6.8–8.4 during 15 weeks of operation at the temperature range of 14.5-36°C as shown in Fig. 5.3. Although pH has no direct effect on aquatic as well as terrestrial life, but it has been considered as one of the important parameters to define quality of wastewater before and after treatment. Moreover, it has been expected that pH has a negative impact on the microbial consortia involved in wastewater clarification. According to WHO, (2006) and US-EPA, (2007) reports, the rate of ammonium oxidation decreased significantly in acidic pH range. Therefore, for optimum performance of treatment systems, it is best to maintain a pH in the range of 6.5-8.5. The outcomes of study in this regard revealed the same range, indicating that the TBF system as one of the best options for domestic wastewater treatment. Likewise, Sakuma *et al.,* (2008) reported equivalent pH range and its alteration with redox as well as nitrification and denitrification reactions. The pH values diminution after treatment through the TBF system might be due to the denitrification phenomenon converting nitrates to molecular nitrogen (N_2) (Khan *et al.,* 2015). Moreover, in the present study, about 8.66–51.2% reduction in the salinity of residential colony wastewater was observed during 15 weeks of biological operation as shown in Fig. 5.3. Although, salinity is not considered as a big dispute with respect to other TBF concerns such as sewage upholding capacity and meeting discharge parameters. Mostly, TBF is designed to treat a variety of wastewater containing different organic and inorganic salts from different sources, which ultimately enhance the TDS level of wastewater. TDS, also referred to as salinity, can impact the operation of the TBF, limit reuse options or result in violations of effluent discharge standards. Rosenthal and Otte, (1979) reported that salinity affects microbial consortia within biofilm of TBF as a result the efficiency of TBF would be affected in terms of reduction in nitrogenous and carbonaceous compounds.

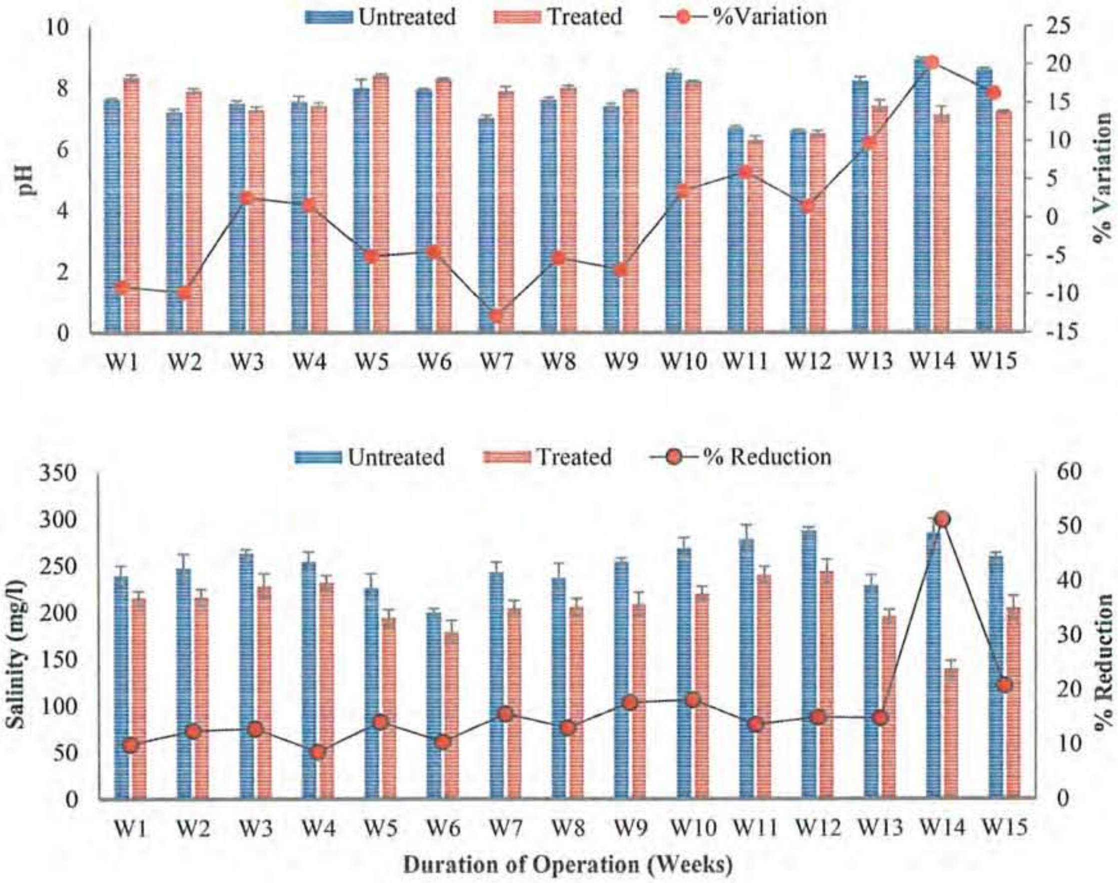


Fig. 5.3 Changes in the pH and salinity levels during 15 weeks of operation through stone media pilot-scale trickling biofilter at 14.5-36°C (*Bar indicates Standard error*).

Other important parameters of influent and effluent are total suspended solids (TSS) and TDS. According to WHO, (2006) and US-EPA, (2007) reports, the recommended value of TDS is less than 1000 mg/L, whereas for TSS lies in the range of 25-80 mg/L. In the present study, influent had TSS values in the range of 1000-1421 mg/L due to high concentration of colloidal or non-settleable solids varying from larger sand particles to fine slit and clay, depending on the flow rate of wastewater. While TDS values were in the range of 227-373 mg/L, much higher than the values of BOD_5 and COD due to the presence of various inorganic salts in addition to dissolved organic contaminants. It was also reported previously that household wastewater differs in composition from typical municipal sewage and is characterized by higher dissolved and suspended biodegradable contents (Makowska and Spychała, 2014). The high biodegradable compounds and relatively large reaction rate coefficients positively affect the efficiency of pollutant removal of the reactors (Makowska and Spychała, 2014). However, in the present research study, after treatment through the pilot-scale TBF system, the concentrations of TSS and TDS were reduced to 0.8 and 62 mg/L, respectively, as shown in Fig. 5.4. Szogi *et al.*, (1997) also discussed the reduction of TSS and TDS in the trickling filter systems. Reduction in TDS was due to continuous recirculation of wastewater over filter bed for 24 hrs, as a result contact time between dissolved contents and microbes present in biofilm increased and hence microbes performed their activities to utilize and degrade dissolved contaminants of wastewater (Rehman *et al.*, 2012). Khan *et al.*, (2015) reported 66% reduction in TDS and 100% reduction in TSS during their study by using the stone media TBF system integrated with sand column filter. Another parameter is EC, which is the measure of free ions present in wastewater. The normal limits of EC, as defined by WHO, are 400-1200 μS/cm (WHO, 2006). In the present study, about 33.1% reduction in EC values were observed during treatment (Fig. 5.4). There were many phenomena responsible for the reduction of EC concentration in water samples during biological operation and a basic reason for this might be that at 14.5-36°C temperature range ammonium, nitrates and nitrites converted into molecular nitrogen (N_2), as a result the concentrations of free ions were reduced to conduct electric current. Pritchard *et al.*, (2007) reported that decline in the TSS and fluoride ions also play an important role in the reduction of EC values. Moreover, Khan *et al.*, (2015) reported 29.4% reduction in the EC with a retention time of 48 hrs in the stone media TBF integrated with sand column filter.

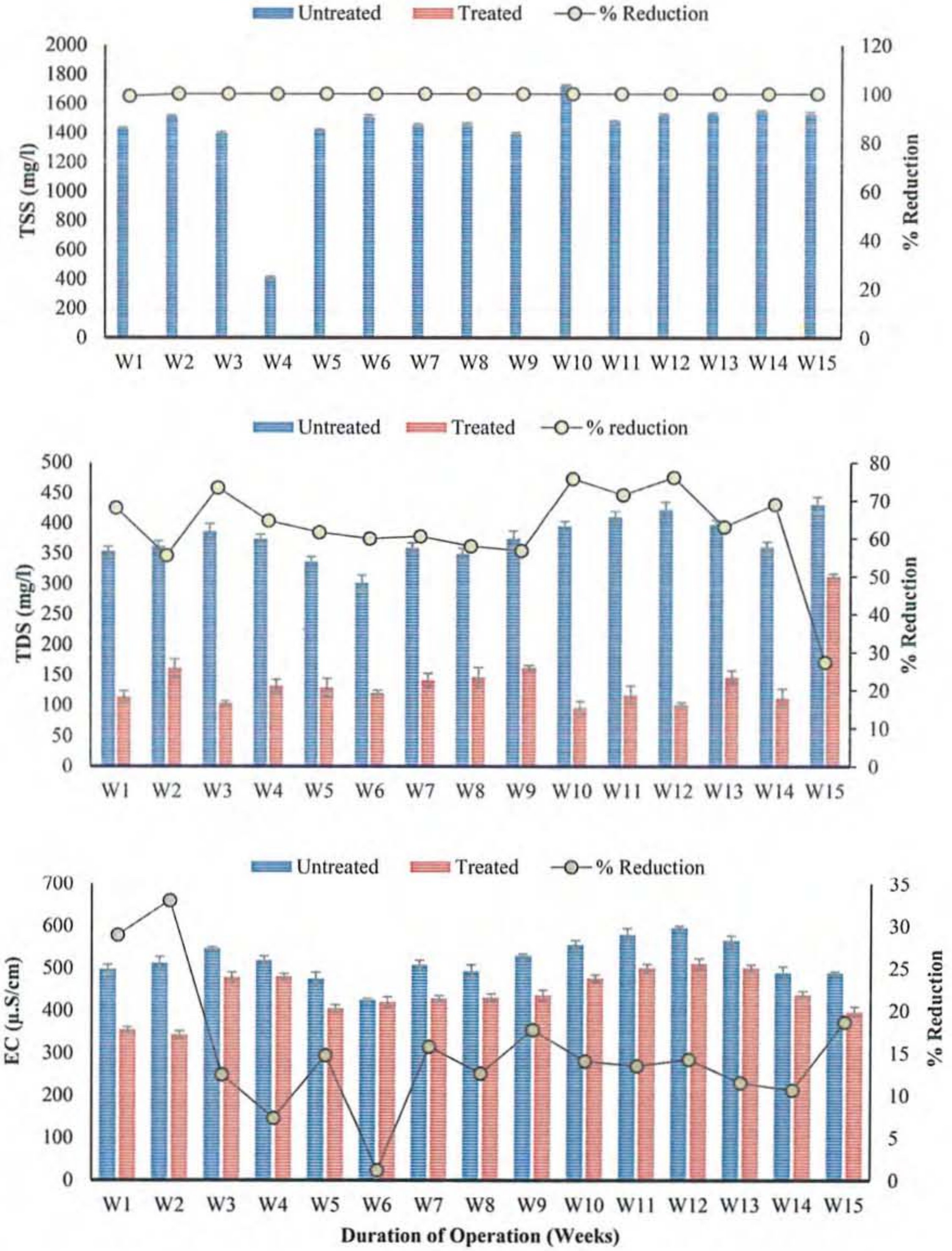


Fig. 5.4 Changes in the concentrations of total dissolved solid (TDS), total suspended solids (TSS) and electrical conductivity (EC) in the effluent during 15 weeks of operation through stone media pilot-scale trickling biofilter at 14.5-36°C (*Bar indicates Standard error*).

The sources of phosphates (PO_4) in wastewater are soaps, detergents, human and animal excreta as well as use of fertilizers. High level of PO_4 in the wastewater stream causes eutrophication because phosphates are removed from the wastewater by intracellular microbial accumulation. WHO has not described the limits of phosphate in the effluent water; however, US-EPA, (2007) defined that permissible limit of the PO_4 should be $\leq$ 0.05 mg/L. In the present study, an average reduction of 65% in the concentration of PO_4 was observed, which gives an indication of the presence of phosphate accumulating bacteria (PAOs) as shown in Fig. 5.5. Previously, PAOs, i.e. *Dechloromonas* (relative abundance of 38%), were reported in the biofilm retrieved from the aerobic stone media bioreactor by next-generation pyrosequencing (Naz *et al.*, 2015). Moreover, the same research group has reported the presence of nitrifiers such as *Nitrosonomas* and *Nitrobacter* spp., in the biofilm grown on stone media of the TBF system for treatment of domestic wastewater (Naz *et al.*, 2015). Jin *et al.*, (2006) reported about 80% PO_4 removal efficiency during their study. On the other hand, sulphates (SO_4) are of prime importance in the water and wastewater samples and are present normally in all types of effluents like industrial effluent, domestic discharge of water and natural runoff. In the present study, maximum up to 78% with an average reduction of 28.5% in SO_4 concentrations was observed during entire treatment operation (Fig. 5.5). This is due to increased concentration of DO in the effluent, which is necessary to oxidize the reduced form of sulphur containing compounds (Pritchard *et al.*, 2007). Lens *et al.*, (1995) reported that SO_4 containing salts are more soluble in water and impact hardness. Khan *et al.*, (2015) reported average 39.84–79.60% reduction in SO_4 from municipal wastewater by using the laboratory-scale stone media TBF system after 48 hrs of recirculation.

TN removal rate in the pilot-scale TBF system is directly related to the development of biofilm. In the present study, an average reduction of 34.4% in TN concentrations was observed during the entire treatment operation (Fig. 5.5). The key to achieve a better efficiency of TN removal was might be the concentration of organic substrates in the influent, favorable temperature, dissolved oxygen levels and rationale wastewater distribution frequencies. Presently, an average carbon (BOD_5) to nitrogen (TN) ratio during the 15 weeks of operation varies from 2:1 to 12:1. However, a high reduction in

TN of 60.6% was observed under the condition of C/N ratio at 3:1 during the 8th week of reactor operation. While minimum reduction (20%) of TN was obtained on the 1st week of reactor operation at a C/N ratio of 12:1. These results are contrary to Zhang *et al.*, (2015) who observed a maximum TN removal at high C/N ratio (7:1) in rural sewage by the vertical flow trickling filter and the horizontal flow multi-soil layering reactor. Thus, it indicates that nitrogenous pollutants' removal rates depend on an increase in temperature and development of biofilms on filter media (Khan *et al.*, 2015; Naz *et al.*, 2016). Temperature has a positive significant effect on the nitrogen removal because it increases the population of nitrifiers (Mann *et al.*, 1998; He *et al.*, 2007; Naz *et al.*, 2013). Moreover, the decrease in the organic/inorganic pollutants removal has a positive effect on oxygenation of the effluent and nitrifiers growth, resulting in efficient nitrification/denitrification (Naz *et al.*, 2016). Thus, presently the increase in the removal rate of the TN can be correlated with the removal of BOD/COD and increase in DO of the treated wastewater due to the recirculation of wastewater in the TBF bed (Fig. 5.2 and 5.5). Moreover, this pilot-scale stone media TBF has shown high nitrogenous pollutants' removal efficiency as reported for textile biofilter, i.e. 40% (Luo *et al.*, 2014). Further, Lacasse *et al.*, (2001) also present the results obtained in the development of a denitrification unit with innovative synthetic and organic filtering media installed between a conventional septic tank and an Ecoflo a Peat-based Biofilter and demonstrated approximately 65% TN removal. Thus, the present results clearly depict that attached growth bioreactors using stone as a filter media are efficient in nitrogen removal as it is less expensive and needs less maintenance requirement as compared to other reactors using a synthetic media like sponge (Mahmoud *et al.*, 2011).

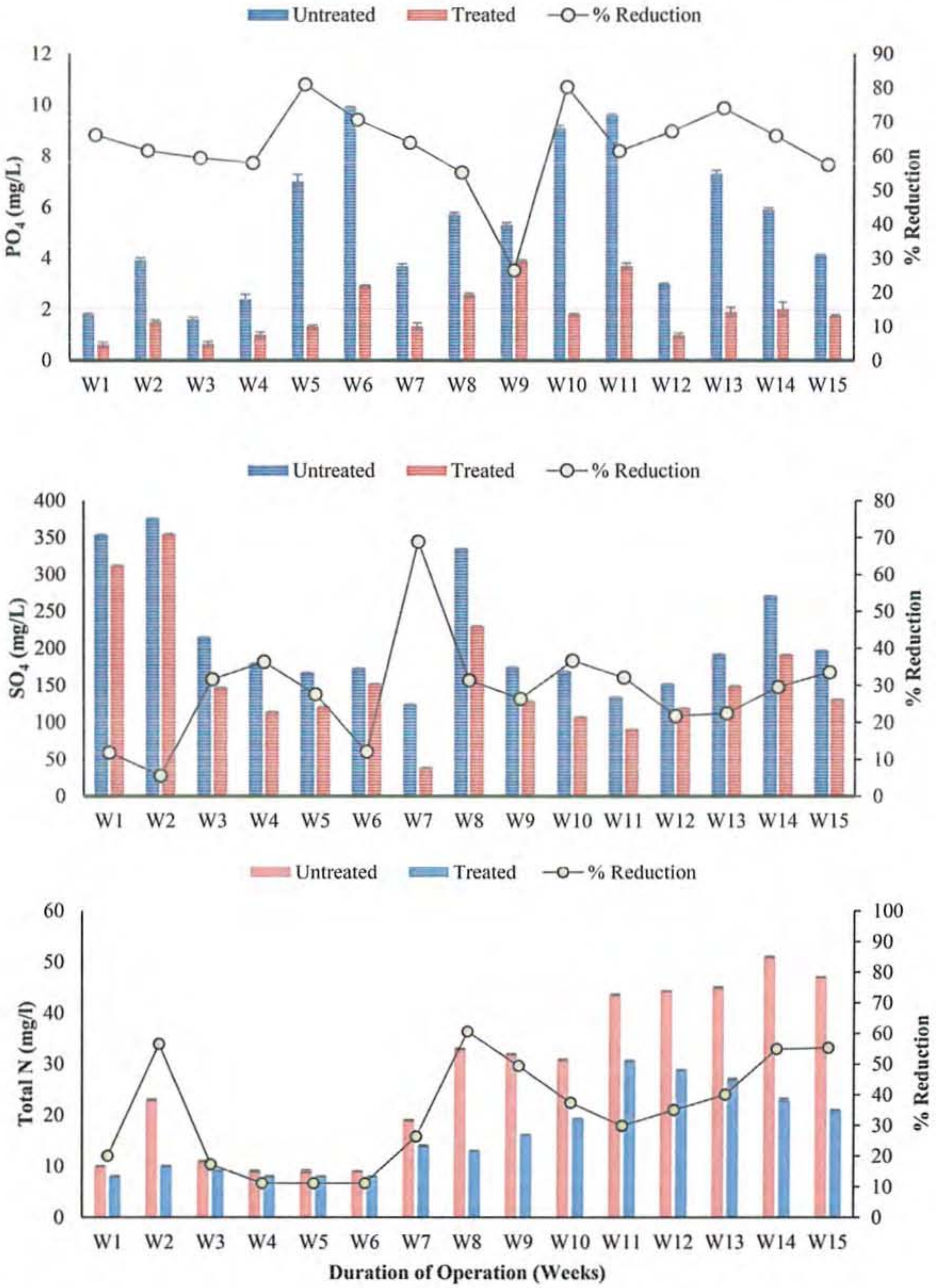


Fig. 5.5 Changes in the concentrations of phosphates (PO_4), sulphates (SO_4) and total nitrogen (TN) in the effluent during 15 weeks of operation through stone media pilot-scale trickling biofilter at 14.5-36°C (*Bar indicates Standard error*).

5.3.2 Microbiological Characterization of Influent and Effluent Samples

The quality of wastewater is allied with the presence or absence of fecal coliforms, also assisted as indicator bacteria and it indicates the presence of organic pollution (Grady *et al.*, 2011; Danish *et al.*, 2011). According to WHO, (2006) guidelines, the quantity of fecal coliforms should not be more than 1000 per 100 mL in water typically used for irrigation and ornamental purposes (Leonard, 2009). As MPN is both a qualitative and quantitative test for the detection of fecal coliforms in the water; therefore, in the present study it was observed that in the first 9 weeks, no significant reduction (0-54%) in the MPN index was observed. However, with an increase in the operational period, and development of biofilm, a significant reduction in the fecal coliforms was observed (80-90%) in the last few weeks of treatment. The CFU is another important biological parameter for the determination and enumeration of total bacteria present in the influent and effluent. About 54-92% reduction in CFU/mL was observed during treatment of wastewater by pilot-scale TBF (Table 5.1). This higher efficiency of the pilot-scale stone TBF system could be the greater hydraulic retention time in the reactor, which significantly affects the adsorption of pathogenic bacteria in the biofilm on stone surfaces. After becoming a part of the metabolically active biofilm, they are involved in the removal of organic and inorganic pollutants from wastewater, increasing DO levels, which leads to nitrification. The removal efficiency of pathogenic indicators has also been directly linked with the removal of organic and inorganic pollutants in the reactor and indirectly linked with parameters such as DO, pH and temperature (Naz *et al.*, 2016).

Table 5.1 Efficiency of pilot-scale TBF system in the removal of microbial contaminants (MPN index and CFU/mL) from wastewater for 15 weeks at temperature range of 14.5-36°C (± *indicates Standard deviation*).

Week	Temperature (°C)	Average CFU/mL		Treatment Efficiency (%)	Avg; Fecal coliforms MPN/100 mL		Treatment Efficiency (%)
		Influent	Effluent		Influent	Effluent	
1	15±2	12000	2000	83.33	2400	2400	0
2	15±2	19600	3100	84.18	2400	2400	0
3	20±1	19500	4100	78.97	2400	1100	54.16
4	21±2	16100	1800	88.81	2400	1100	54.16
5	23±1	12000	1800	85	2400	1100	54.16
6	22±1	16100	1700	89.44	2400	1100	54.16
7	23±3	14200	1000	92.95	2400	1100	54.16
8	20±2	19100	1600	91.62	2400	1100	54.16
9	22±2	15900	3100	80.50	2400	1100	54.16
10	28±2	18300	1900	89.61	2400	460	80.83
11	27±3	19000	8700	54.21	2400	460	80.83
12	32±3	17900	5000	72.06	2400	240	90
13	31±4	18900	7800	58.73	2400	240	90
14	34±1	19100	8100	57.59	2400	240	90
15	33±3	15600	4500	71.15	2400	240	90

Chapter 6

6. EFFECTS OF HYDRAULIC RETENTION TIME (HRT) ON THE PERFORMANCE OF PILOT SCALE TRICKLING BIOFILTER SYSTEM TREATING DOMESTIC WASTEWATER

6.1 Introduction

Different researchers in the past used both laboratory and pilot scales TBF systems under different HRT (few hrs to days) for the treatment of wastewater (Rehman *et al.*, 2012; Naz *et al.*, 2013; Khan *et al.*, 2015). Therefore, after initial 15 weeks operational study through setup-II TBF system in open environment, another study was designed to evaluate the effect of different hydraulic retention time (HRT) on the performance of setup-II stone media TBF system while treating domestic wastewater at a flow rate of 0.004 m^3/day. Generally, HRT has significant effects on the efficiency of wastewater treatment systems but several other factors such as seasonal variations, temperature, precipitation, humidity, organic loading and flow rates are obvious. In the present research study, three different HRT i.e. 24, 48 and 72 hrs were selected in order to determine the effects of HRT on the performance of stone media pilot scale trickling biofilter system (TBF) installed at residential area of Quaid-i-Azam University Islamabad, Pakistan with respect to wastewater treatment. The study period covered the months from January 2016 to July 2016. The temperature of the environment was routinely checked during biological operation and it was in the range of 16-38°C. This study will helps to figure out and fix the most appropriate HRT for the treatment of wastewater through pilot scale TBF system in future studies.

6.2 Material and Methods

6.2.1 Experimental Setup-II TBF Scheme

The design of stone media pilot scale TBF system installed in open environment at residential area of Quaid-i-Azam University Islamabad, Pakistan and its operational scheme was previously discussed in detail in section 3.2 of chapter 3. But in the current study, experiments were conducted in three different phases to determine the effect of HRTs (24, 48 and 72 hrs) on the efficiency of pilot scale stone media TBF with respect to wastewater treatment. As pilot scale TBF contained two pumps i.e. P1 (installed on

PST) and P2 (installed on RCT) therefore, initially influent was distributed for about 4.5 hrs from PST to the top of filter bed through P1 at a hydraulic flow rate of 0.64 m^3/day and after this, P1 automatically switch off and the pump P2 turned on, that maintained the frequency of water distribution from RCT over the top of stone filter bed at a hydraulic flow rate of 0.04 m^3/day. But here in the 1st phase of biological operation at HRT of 24 hrs, P2 continuously pumped and recirculated water from RCT for about 19.5 hrs while in the 2nd and 3rd phase of biological operation at HRT of 48 and 72 hrs, P2 recirculated water for about 43.5 and 67.5 hrs respectively. After the completion of 24, 48 and 72 hrs cycles, the effluent was allowed to flow into the sand bed compartment by gravitational force and then it was allowed to be discharged in to the adjacent fields or stream. After this, P2 was switched off automatically and it turned P1 on, which pumped new sample of influent from primary sedimentation tank to the reactor and next cycle begins. Digitally controlled system had the capacity to evaluate its running time, so in case of any load shedding, each pump finalized its running time.

6.2.2 Sampling of Wastewater

Standard methods were followed for sampling during study (APHA, 2005). Influent and effluent samples of 24, 48 and 72 hrs HRT were collected in triplicates, in 1000 mL separate clean plastic bottles and then shifted to the laboratory in ice box in order to be preserved at 4°C in a refrigerator before determining changes in the concentration of different physicochemical (pH, EC, COD, TDS, PO_4, SO_4 and TN) and microbiological (CFU/mL) parameters.

6.3 Results and Discussion

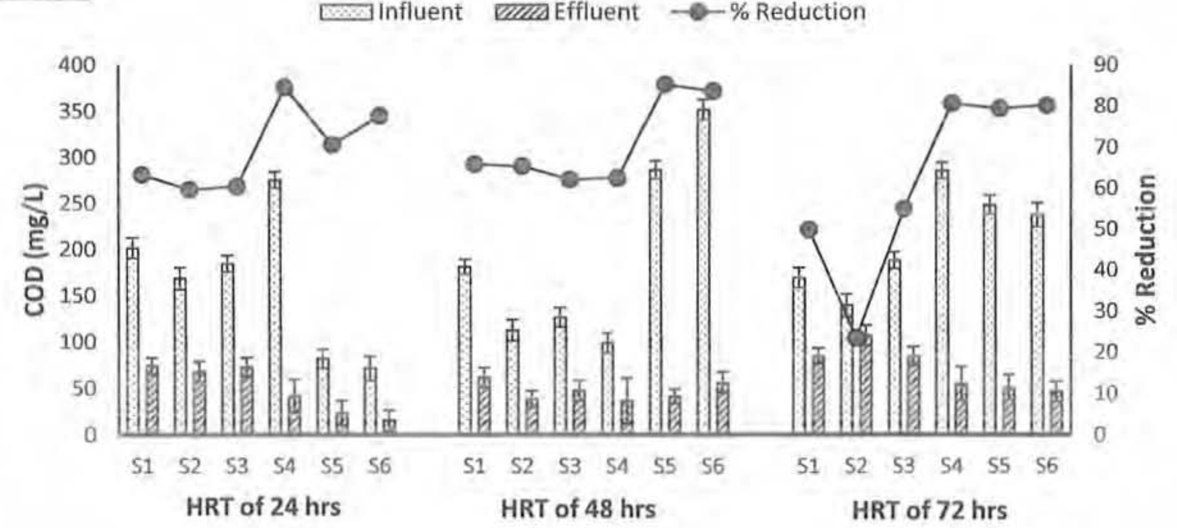
6.3.1 Effects of Hydraulic Retention Time (HRT) on the Performance of TBF System

The hydraulic retention time (HRT) is the time duration by soluble compounds to be remained in a bioreactor for a specified period of time. It is directly related to the flow of wastewater towards bioreactor (Rajakumar *et al.*, 2011). Furthermore, an adequate flow or recirculation rate and retention time offers sufficient contact between microbial biofilm and wastewater resulting in an enhanced organic as well as nitrogenous pollutions removal efficiency of the treatment facility (Rajakumar *et al.*, 2011; Luiz *et* *al.*, 2015). The effect of different HRTs on the performance of pilot scale TBF system are shown in Table (6.1). It was observed that increase of HRT (from 24 hrs to 72 hrs) positively affected the efficiency of the system with respect to wastewater treatment. In the case of COD content reduction, the efficiency of pilot scale TBF system under an HRT of 24 hrs were in the range of 59.7-84.6% while at HRT of 48 and 72 hrs, they were in the range of 62.2-85.3% and 23.5-80.8% respectively ($p = 0.003$) as shown in Fig. 6.1a. This agreed with the findings of Ladu *et al.*, (2014), who studied the effects of three different HRTs on the efficiency of anaerobic filter while treating domestic wastewater and found that the efficiency of reactor increased by increasing HRT (from one to three days). Furthermore, they observed 32% reduction in COD values at HRT of one day, 40% at HRT of two days and 44% at HRT of three days. The maximum removal percentage of COD at higher HRT might be due to continuous recirculation of wastewater, as a result microorganisms present in slime layer actively oxidize organic compounds present in wastewater (Naz *et al.*, 2016). Moreover, Laing, (2016) reported 84% reduction in COD values at HRT of 24 hrs while using sequential anaerobic batch reactors to treat synthetic winery wastewater Leyva-Díaz *et al.* (2016) reported 85% reduction in COD contents at HRT of 18 hrs during their studies while using a hybrid moving bed membrane bioreactor.

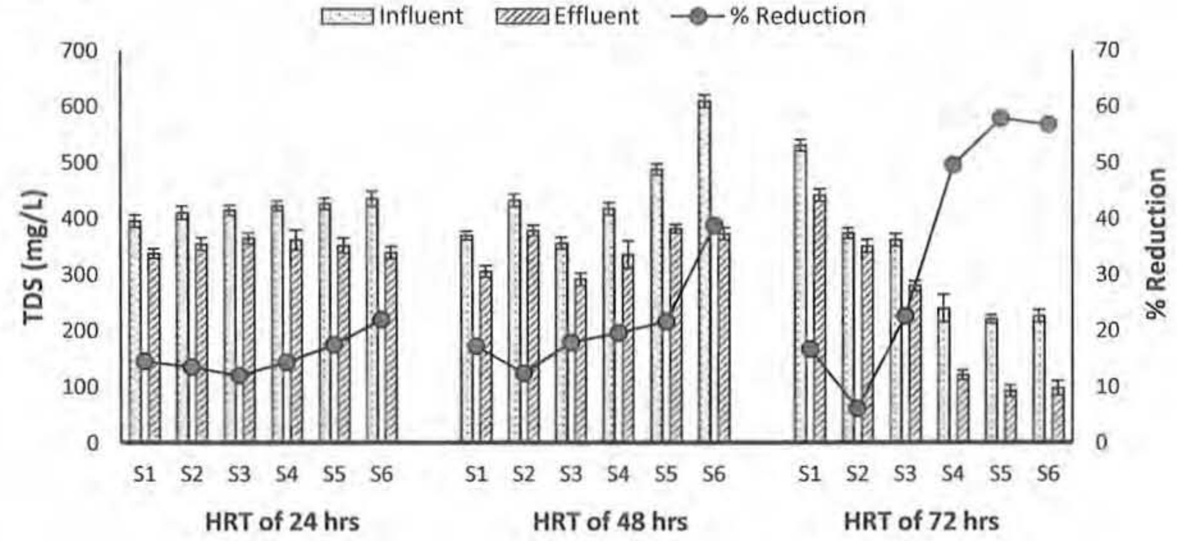
In the current study, it was observed that the TDS removal efficiency of TBF system increased with increase of HRT. About 22% maximum reduction in TDS level were observed at HRT of 24 hrs while 38.8 and 57.8% reduction were found in TDS level at HRT of 48 and 72 hrs respectively ($p = 0.008$) as shown in Fig. 6.1b. Reduction in TDS contents with increasing HRT was also due to continuous recirculation of wastewater for an extended period of time as a result, microorganisms within biofilm got sufficient time to degrade dissolved organic components (Rehman *et al.*, 2012; Fernandes *et al.*, 2017). Furthermore, Khan *et al.*, (2015) reported 23% and 66% reduction in the TDS at HRT of 24 hrs and 48 hrs respectively during their study using stone media TFs integrated with sand column filter. According to WHO, (2006) the approved range of EC in wastewater is 400-1500 µS/cm. It has a direct relationship with TDS, COD and fluorides present in water samples. Moreover, it was found that EC values of wastewater decreased up to 13.4-28.6% at HRT of 24 hrs while 14-39% and 5.87-

23.04% reduction were observed at HRT of 48 and 72 hrs respectively ($p = 0.03$) as shown in Fig. 6.1c. A basic reason for reduction in EC content might be that ammonium, nitrates and nitrites present in wastewater were converted to molecular nitrogen (Rasool *et al.*, 2018), as a result the concentration of free ions were reduced to conduct electric current. Khan *et al.*, (2015) also reported significant reduction in EC values by increasing HRT from 24-48 hrs while using laboratory scale fixed biofilm reactor.

(a)



(b)



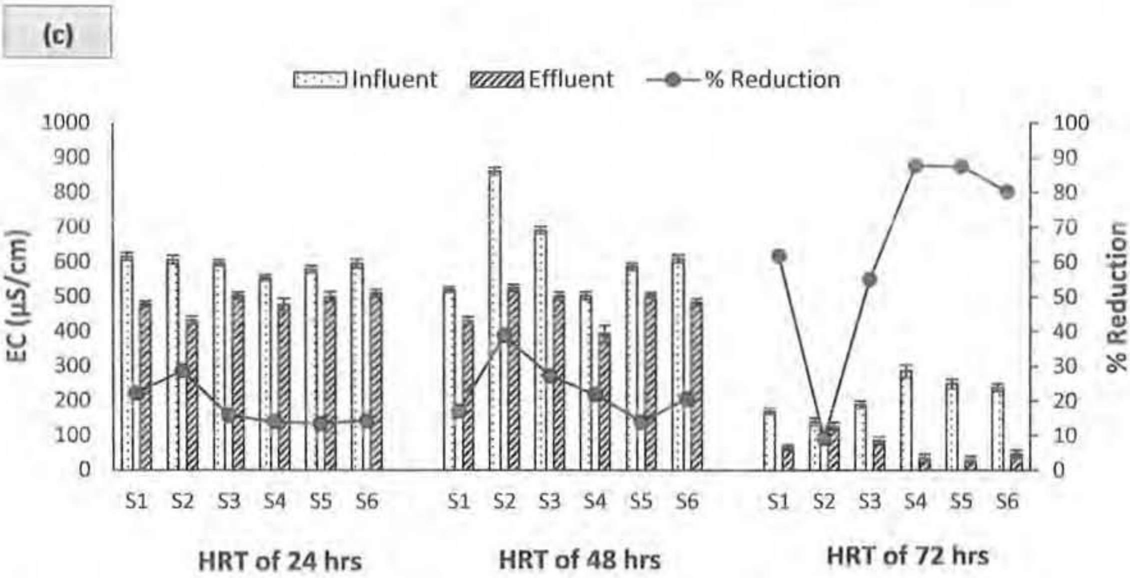
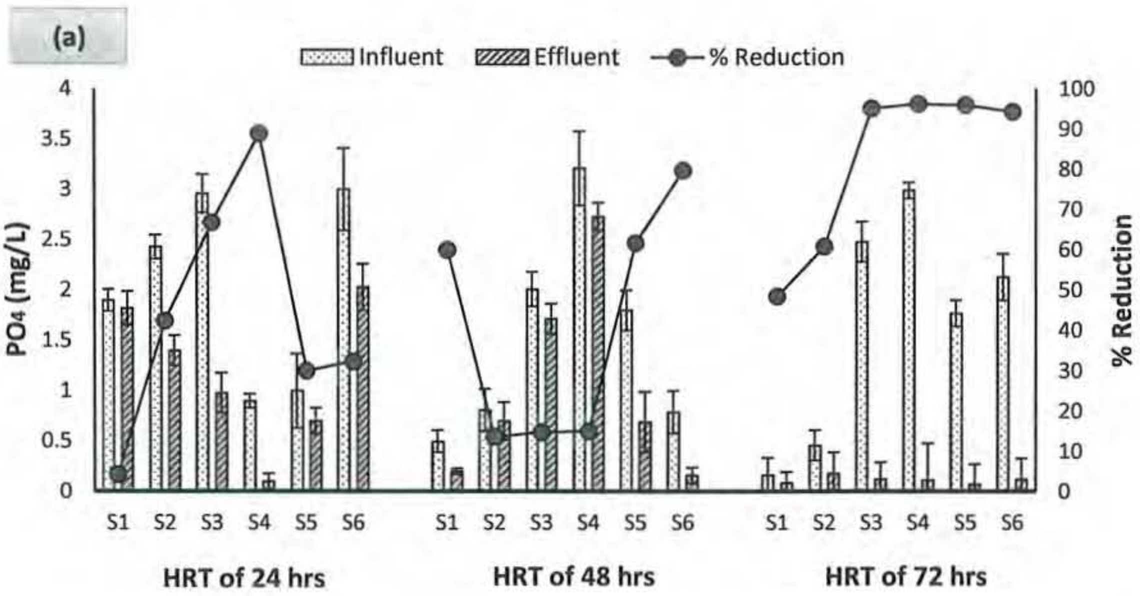


Fig. 6.1 Efficiency of pilot scale TBF system in the average reduction of **(a)** COD, **(b)** TDS and **(c)** EC contents under different HRTs (*Bar indicates Standard error*).

Phosphate (PO_4) in domestic wastewater came in the form of polyphosphates, causing eutrophication in water bodies leading to adverse effects on aquatic life by minimizing light penetration (hypoxia) and dissolved oxygen (DO) concentrations (Khan *et al.*, 2015; Naz *et al.*, 2016). Moreover, detergents, soaps, shampoos, oil and grease are being considered the most prominent source for PO_4 contamination in domestic wastewater (Shi *et al.*, 2007). In the present study, it was observed that an average reduction of 44.12%, 40.77% and 82% were achieved in PO_4 concentrations at HRT of 24, 48 and 72 hrs respectively (for an overview, see Table 6.2). However, the overall efficiency of pilot scale TBF in the reduction of PO_4 concentration is shown in Fig. 6.2a, where individually up to 88.8%, 79.7% and 96.3% reduction were observed at HRT of 24, 48 and 72 hrs respectively which was statistically highly significant (p = 0.007). These results showed consistency and compatibility with previous research work, where about 80-97% reduction in PO_4 concentrations were observed using "submerged membrane reactor" and "combined up flow anaerobic fixed bed in combination with suspended aerobic reactor having membrane unit (Shi *et al.*, 2007). Khan *et al.* (2015) showed an average reduction of 23% and 38% at HRT of 24 and 48 hrs respectively in PO_4 level using laboratory scale stone media TBF to treat domestic wastewater and concluded that the removal of PO_4 was associated with metabolic activities of microbial community flourishing on filter media. Furthermore, Zeng *et al.*

(2003) reported that nitrification and denitrification processes had promising effects on PO_4 elimination from wastewater during biological treatment.

In the present study, the efficiency of the pilot scale TBF to remove sulphates (SO_4) from wastewater increased with increase of HRT from 24-72 hrs. It was observed that during 1st phase of biological operation, an average reduction of 12.9% whereas 18.2% and 36.96% reduction in SO_4 concentration were observed during 2nd and 3rd phases of biological operations with respect to HRTs as shown in Table 6.2. However, the overall efficiency of pilot scale TBF in the reduction of SO_4 concentration is shown in Fig. 6.2b where significant ($p = 0.006$) reduction were observed in SO_4 content. Higher removal efficiency of SO_4 would be related to continuous recirculation and extended treatment time and furthermore, higher HRT provides favorable environment for sulphate oxidizing bacteria because SO_4 become oxidize in the presence of oxygen (Leyva-Diaz *et al.*, 2016). Ehlers and Turner, (2012) reported that sulphate oxidizing bacteria oxidize the sulphur to sulphates which was then reduce to sulphide by sulphate reducing bacteria. Sulphate oxidizing bacteria were widely detected in domestic sewage and played significant role in carbon, sulphur and nitrogen cycles. Laing, (2016) reported that anoxic condition developed within biofilm reactor with the passage of time, as a result sulphur reducing bacteria actively participate in the reduction of sulphur compounds.



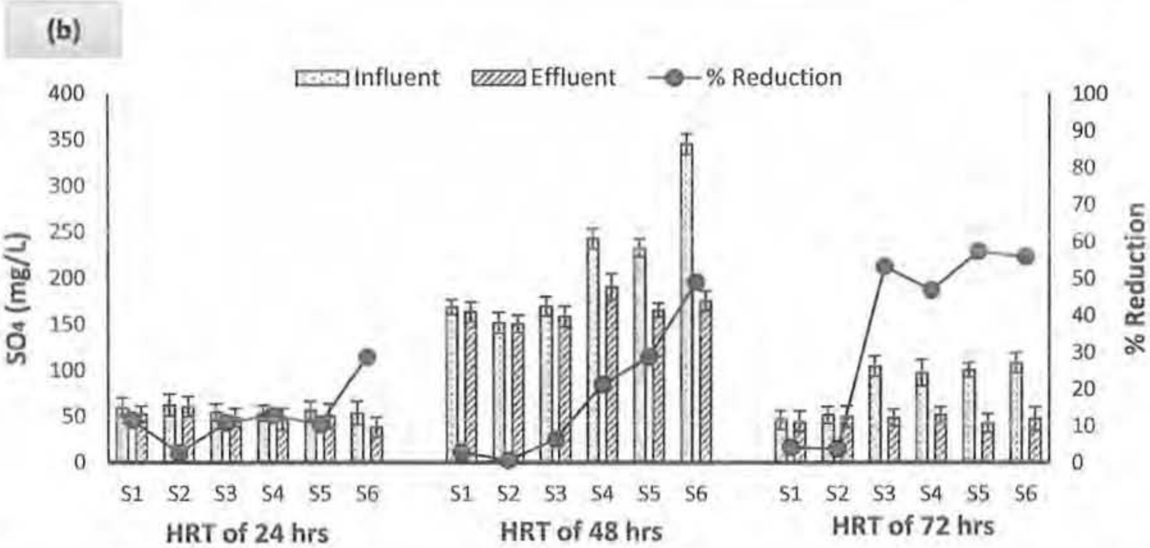


Fig. 6.2 Efficiency of pilot scale TBF system in the average reduction of **(a)** PO_4 and **(b)** SO_4 contents under different HRTs (*Bar indicates Standard error*).

The pH value variations in the influent and effluent samples were monitored over the time and it was observed in the range of 6.3-7.5, 6.8-8.5 and 7.1-8.2 during 1st, 2nd and 3rd phases of biological operation ($p = 0.01$) under different HRTs i.e. 24, 48 and 72 hrs respectively (Fig. 6.3a). According to WHO, (2006) and USEPA, (2007), the rate of ammonium oxidation decreased significantly in acidic pH range. Consequently, for optimum performance of treatment systems, it is best to maintain pH in the range of 6.8-8.0. The outcomes of study in this regard revealed the same range indicating pilot scale TBF as one of the best options for domestic wastewater treatment. Likewise, Khan *et al.* (2015) reported equivalent pH range and its alteration with redox as well as nitrification and denitrification reactions and furthermore, described that the pH values diminution after treatment through pilot scale TBF might be due to denitrification phenomenon converting nitrates to molecular nitrogen.

Untreated domestic wastewater contain large amount of nitrogen either in organic or in inorganic forms such as ammonia, nitrates and nitrites (Gangal *et al.*, 2015). Therefore, total nitrogen (TN) refers to the total amount of organic and inorganic fractions of nitrogen present in wastewater, while the term Kjeldahl nitrogen refers to the sum of organic and inorganic fractions of nitrogen from ammonium (NH_4^+) (Rasool *et al.*, 2018). The principle sources of TN in domestic wastewater are urea and proteins. It is an important component required for the growth of microorganisms and plants however, the excess amount of nitrogen in the effluent of wastewater treatment system facilitate hypertrophication and algal growth and hence deplete the oxygen supply (Sakuma *et al.,* 2008). In the present study, it was observed that during 1st phase of biological operation, an average reduction of 11.2% whereas 24.1% and 66.68% reduction in TN concentrations were observed during 2nd and 3rd phases of biological operations respectively (for an overview, see Table 6.2). Furthermore, in the present study, the highest percentage reduction i.e. 20.3, 32.8 and 87.2% in TN concentrations were observed at HRT of 24, 48 and 72 hrs respectively, which was statistically highly significant ($p = 0.003$) as shown in Fig. 6.3b. Different researchers reported that the efficiency of reactor to remove TN from wastewater largely depends on the ratio of COD to TN concentrations (COD/TN) along with reactor configuration (Ladu *et al.,* 2014; Wang *et al.,* 2017). Ladu *et al.* (2014) reported more than 75% of the TN removal efficiency in a sequencing batch reactor at a COD/TN ratio greater than 3.9 while Wang *et al.* (2017) reported 37-41% reduction in TN concentration at a COD/TN ratio of 7.2. In the present study, 5.1-20.3% reduction in TN concentrations at COD/TN ratio of 0.9 were observed at HRT of 24 hrs and during 2nd phase of operation (HRT of 48 hrs), 3.1-32.8% reduction in the concentrations of TN at COD/TN ratio of 1.75 while at HRT of 72 hrs, 28.4-87.2% reduction in the concentrations of TN at COD/TN ratio of 2.07 were observed. The subsequent increased in COD/TN ratio with HRT confirmed that the rate of nitrification increased therefore, to get optimum function of pilot scale TBF, maximum HRT of 72 hrs would be preferred.

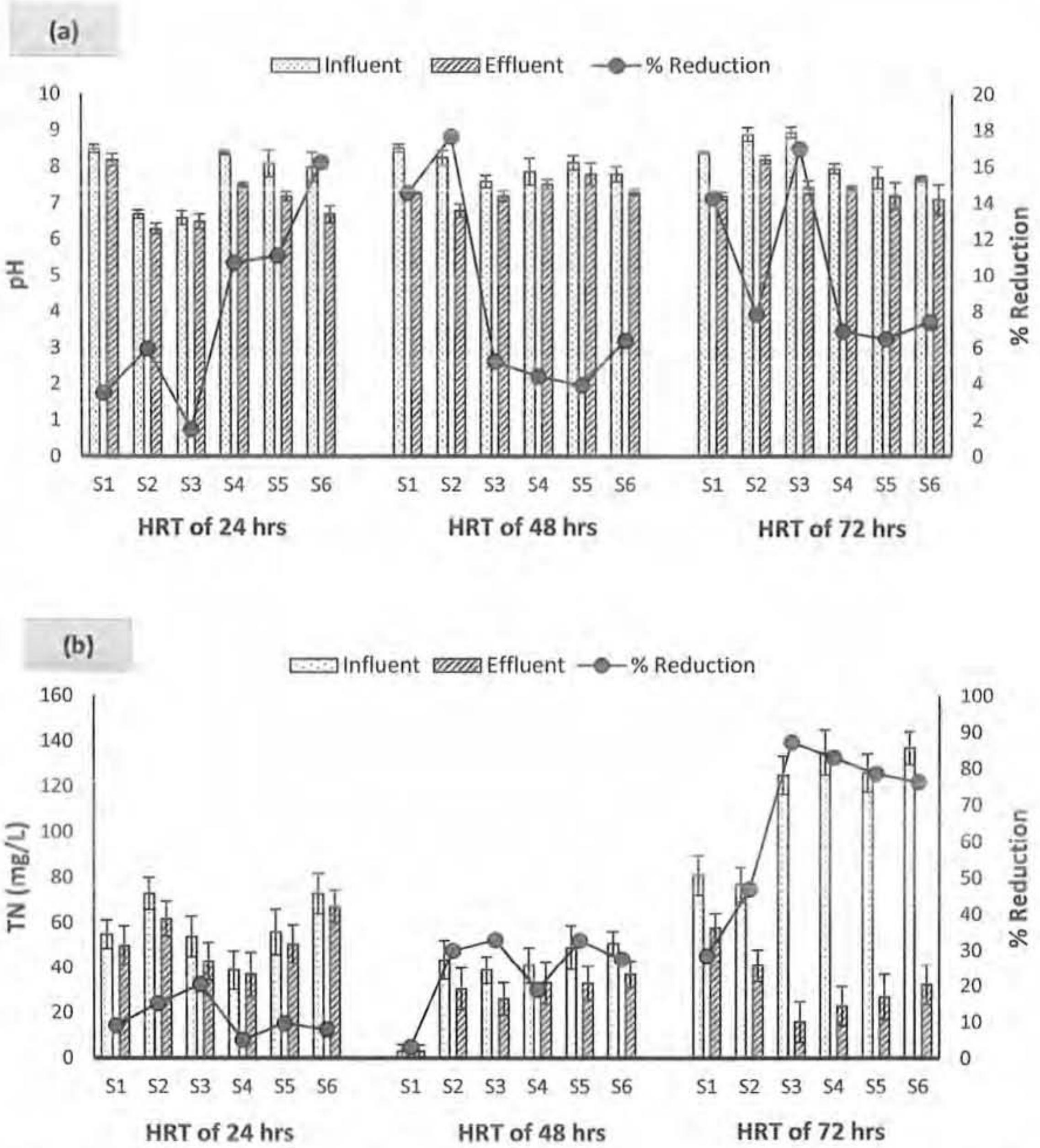


Fig. 6.3 Efficiency of pilot scale TBF system in the average reduction of **(a)** pH levels and **(b)** TN contents under different HRTs (*Bar indicates Standard error*).

Table 6.1 Effects of different hydraulic retention time (HRT) on the performance of pilot scale TBF System with respect to average reduction of different physico-chemical parameters.

	Parameters	S1			S2			S3			S4			S5			S6		
		UTs	**Ts**	% Efficiency	**UTs**	**Ts**	% Efficiency	**UTs**	**Ts**	% Efficiency	**UTs**	**Ts**	% Efficiency	**UTs**	**Ts**	% Efficiency	**UTs**	**Ts**	% Efficiency
HRT- 24 hrs	COD (mg/L)	202	74	63.4	169	68	59.8	185	73	60.5	276	42	84.6	82	24	70.7	72	16	77.7
	TDS (mg/L)	396	338	14.64	411	355	13.63	415	365	12.05	423	362	14.42	427	352	17.56	436	340	22.02
	EC (µS/cm)	616	479	22.24	607	433	28.66	598	503	15.88	555	477	14.05	579	501	13.5	596	511	14.26
	pH	8.5	8.2	3.5	6.7	6.3	5.97	6.6	6.5	1.52	8.4	7.5	10.7	8.1	7.2	11.11	8.0	6.7	16.25
	PO_4 (mg/L)	1.9	1.82	4.21	2.43	1.4	42.38	2.96	0.98	66,89	0.9	0.1	88.8	1.0	0.7	30.0	3.0	2.03	32.33
	SO_4 (mg/L)	60.0	53.0	11.67	62.7	61.0	2.71	55.0	49.0	11.0	54.0	47.0	12.96	57.0	51.0	10.53	54.0	38.48	28.74
	TN (mg/L)	55.0	50.0	9.09	73.0	62.0	15.06	54.0	43.0	20.37	39.0	37.0	5.13	56.0	50.6	9.64	73.0	67.2	7.95
HRT- 48 hrs	COD (mg/L)	182	62	66	113	39	65.5	127	48	62.2	99	37	62.6	287	42	85.3	252	57	83.8
	TDS (mg/L)	370	306	17.3	433	379	12.5	356	292	18.0	418	336	19.6	488	382	21.7	610	373	38.85
	EC (µS/cm)	521	432	17.08	861	526	40.0	691	502	27.35	503	393	21.86	587	505	14.0	610	484	20.66
	pH	8.52	7.28	14.6	8.26	6.8	17.67	7.6	7.2	5.26	7.87	7.52	4.44	8.12	7.8	3.94	7.8	7.3	6.41
	PO_4 (mg/L)	0.5	0.2	60.0	0.81	0.7	13.6	2.01	1.72	14.67	3.21	2.73	14.95	1.8	0.69	61.66	0.79	0.16	79.75
	SO_4 (mg/L)	169.0	164.0	2.96	152.0	151.0	0.66	170.0	159.0	6.47	243.0	191.0	21.4	233.0	166.0	28.75	346.0	176.0	49.13
	TN (mg/L)	3.2	3.1	3.13	43.6	30.6	29.82	39.0	26.2	32.82	41.0	33.3	18.78	49.0	33.0	32.65	51.0	37.0	27.45
HRT- 72 hrs	COD (mg/L)	170	85	50	140	107	23.57	189	85	55.02	286	55	80.76	249	51	80.25	238	47	80.25
	TDS (mg/L)	531	442	16.76	374	351	6.14	362	280	22.65	240	121	49.58	221	93	57.91	227	98	56.82
	EC (µS/cm)	744	620	16.66	528	497	5.87	512	394	23.04	338	310	8.28	353	303	14.16	339	300	11.50
	pH	8.4	7.2	14.28	8.9	8.2	7.86	8.95	7.43	16.98	7.95	7.4	6.91	7.7	7.2	6.49	7.67	7.1	7.43
	PO_4 (mg/L)	0.165	0.085	48.5	0.46	0.18	60.86	2.48	0.12	95.16	2.99	0.11	96.32	1.77	0.07	96.05	2.13	0.12	94.36
	SO_4 (mg/L)	47	45	4.25	52	50	3.84	105	49	53.33	98	52	46.93	101	43	57.42	109	48	55.96
	TN (mg/L)	81	58	28.39	77	41	46.75	125	16	87.2	135	23	82.96	126	27	78.57	137	32.6	76.20

Key: UTs = Untreated sample, Ts = Treated sample, S1, S2, S3, S4, S5, S6 = Codes of samples got at HRT of 24, 48 and 72 hrs

Table 6.2 Overall efficiency of pilot scale TBF system in the average percentage reduction of different physico-chemical parameters under different HRTs.

HRT	Parameters	Avg. Percentage (%) Reduction
HRT- 24 hrs	COD (mg/L)	69.45
	TDS (mg/L)	15.72
	EC (µS/cm)	18.09
	pH	8.17
	PO_4 (mg/L)	44.1
	SO_4 (mg/L)	12.93
	TN (mg/L)	11.20
HRT- 48 hrs	COD (mg/L)	70.9
	TDS (mg/L)	21.32
	EC (µS/cm)	23.49
	pH	8.72
	PO_4 (mg/L)	40.77
	SO_4 (mg/L)	18.22
	TN (mg/L)	24.10
HRT- 72 hrs	COD (mg/L)	61.64
	TDS (mg/L)	34.98
	EC (µS/cm)	13.25
	pH	9.99
	PO_4 (mg/L)	81.87
	SO_4 (mg/L)	36.96
	TN (mg/L)	66.68

Key: COD = Chemical oxygen demand; TDS = Total dissolved solids; EC = Electric conductivity; PO_4 = Phosphate; SO_4 = Sulphate; TN = Total Nitrogen; % = Percentage; HRT = Hydraulic retention time

6.3.2 Efficiency of Pilot Scale Trickling Filter (TBF) System in terms of Pathogens Removal under Different HRTs

The use of raw wastewater for irrigation purposes is an approach to utilize domestic wastewater with considerable benefits such as it is rich in nutrients which plants required for their growth and moreover, it has capability to increase soil fertility instead of using fertilizers, which makes irrigation cost effective (Hamid and Eskicioglu, 2012). Beside this, sewage water also contain toxic compounds, heavy metals and highly pathogenic microorganisms which have extremely adverse effects on the terrestrial and aquatic ecology, environment and public health (Purnell *et al.*, 2016; Perujo *et al.*, 2018). A set of standard guidelines had been proposed by WHO, (2006) for the harmless use of treated water in agricultural sectors in order to secure agrarians and consumers of the said crops. Therefore, it is essential to develop a suitable wastewater treatment technology in order to reduce the number of life-threatened and extremely pathogenic organisms before using treated effluent in agricultural lands. In the present study, it was observed that pilot scale TBF showed significant efficiency under different HRTs to remove pathogenic organisms from wastewater ($p = 0.0021$). About 37-86.4%, 41.6-82.5% and 42.4-83% reduction in total bacterial count were observed at HRT of 24, 48 and 72 hrs respectively as shown in Fig. 6.4. Pattern of reduction in microbial count varied in different samples taken during the study period. The highest reduction (86.4%) was observed in S1 at 24 hrs HRT while at 48 hrs and 72 hrs HRTs, the percentage reduction was lower in samples of low temperature months i.e. S1-S3 which was greater than 70% in samples of high temperature months i.e. S4, S5 and S6 (Fig. 6.4). This pattern showed that environmental temperature also played a role in the pathogen reduction in the trickling filter (Luostarinen *et al.*, 2007; Daija *et al.*, 2016). The decrease in the number of pathogenic organisms within effluent samples might be due to the reduction of carbonaceous components in wastewater with treatment or due to the confinement of microorganisms in the biofilm by adsorption (Leonard, 2011). Later on, it was associated with detachment and deactivation or natural die-off processes (Lucas *et al.*, 2014). Furthermore, similar percentage reduction (80-87%) in microbial count at HRT of 48 hrs were reported by Khan *et al.* (2015) during their studies using laboratory scale stone media trickling filter system integrated with sand column.

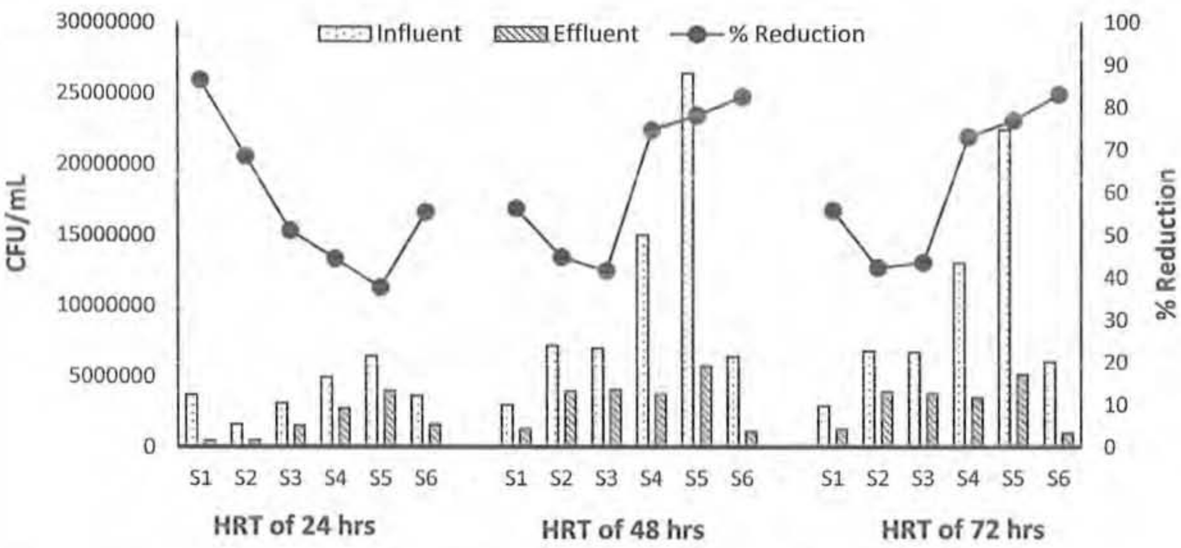


Fig. 6.4 Efficiency of pilot scale TBF system in terms of pathogens removal under different HRTs.

Chapter 7

7. ASSESSMENT OF AN INTEGRATED MEDIA PILOT SCALE TRICKLING BIOFILTER SYSTEM WITH VARIABLE FLOW RATES AND HYDRAULIC RETENTION TIME (HRT) TOWARDS TREATMENT OF DOMESTIC WASTEWATER

7.1 Introduction

After optimizing operational condition of stone media pilot scale TBF system installed in open environment at residential area of Quaid-i-Azam University Islamabad under different HRT (24, 48 and 72 hrs) at a constant flow rate of 0.004 m^3/day, another study was designed to evaluate the efficiency of pilot scale TBF system by making a little bit modification in the containment structure i.e. instead of using stone as a filter media, integrated media containing pebbles and gravels in two stage system were used as filter media to provide support for the growth of microbial slime layer. Furthermore, the integrated media TBF system was operated at three different flow rates (Q) i.e. 0.004 m^3/day, 0.0072 m^3/day and 0.01 m^3/day. On each particular flow rate, wastewater was operated under three different hydraulic retention time (HRT) i.e. 48, 72 and 96 hrs in order to determine the effect of different flow rates and HRTs on the performance of integrated media TBF system. The experimental data of the present research study will help us to do further modification in the structure of TBF system by using different filter media in future study to get maximum efficiency and to implement it on field scale to treat wastewater of larger communities.

7.2 Materials and Methods

7.2.1 Experimental Setup-II TBF Scheme

The design and operational scheme of pilot scale TBF system installed at residential area of Quaid-i-Azam University Islamabad, Pakistan was already discussed in section 3.2. But in the current study, modification had been made in the containment structure of pilot scale TBF system i.e. it contained three layers which were separated by using stainless steel perforated disc. Top portion of the containment was filled with pebbles having a size of 0.08 m (3 inches). Below this a voidage space of up to 0.3 m (1 ft) was left for air so that the air could be freely pass from upper to lower portion while the third and deepest portion contained small size gravels. Furthermore, experiments were conducted in three different phases to determine the effect of different flow rates (Q = 0.004 m^3/day, 0.0072 m^3/day and 0.01 m^3/day) under three different hydraulic retention time (HRT) i.e. 48, 72 and 96 hrs towards wastewater treatment. The schematic illustration of containment structure of integrated media TBF system is shown in Fig. 7.1.

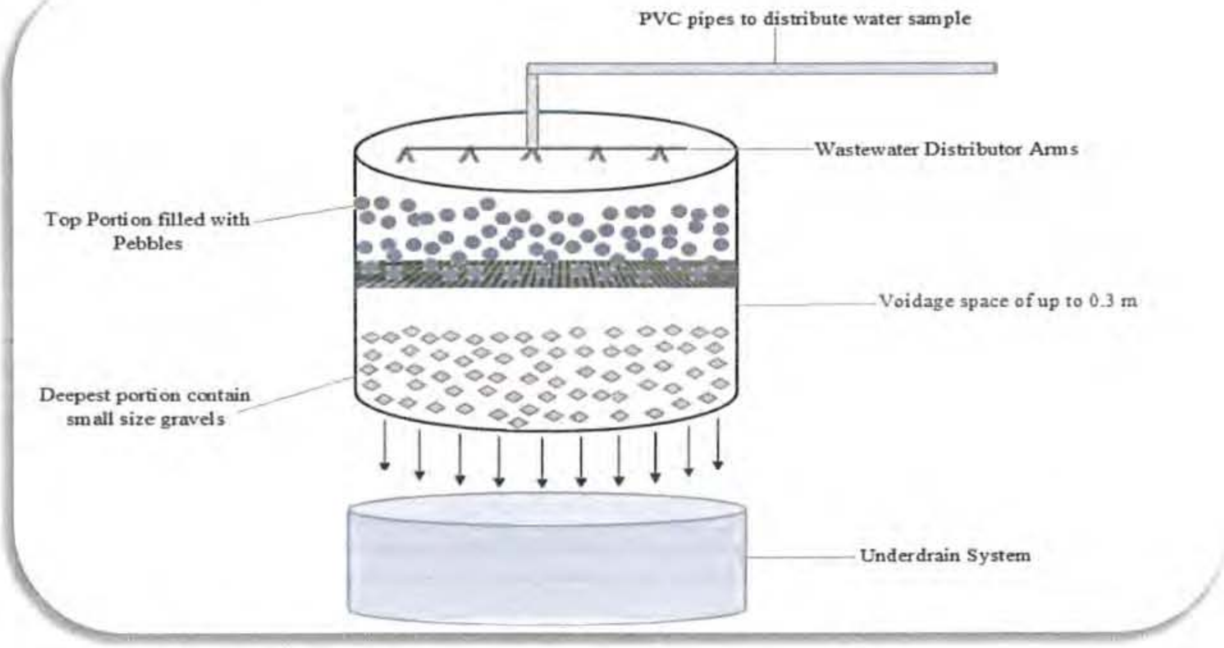


Fig. 7.1 Schematic illustration of containment structure of TBF system installed at residential area of Quaid-i-Azam University, Islamabad.

7.2.2 Samples Processing During Study

Standard methods were followed for sampling of influent and effluent samples throughout study (APHA, 2005). Influent and effluent samples of different flow rates and HRTs were collected in triplicates, in pre-sterilized plastic bottles and then shifted to the Microbiology Research Laboratory (MRL) in ice box in order to be preserved at 4°C in a refrigerator before determining changes in the concentration of different physicochemical (pH, EC, COD, TDS, PO_4, SO_4 and TN) and microbiological (CFU/mL) parameters. The study period covered the months from July 2016 to July 2017. The temperature of the environment was routinely checked during biological operation and it was in the range of 14-38.5°C. Furthermore, bacteriological assessment of biofilm developed on integrated filter media were performed according to their standard procedure as described in Bergey's Manual of Determinative Bacteriology (9th Edition).

7.3 Results and Discussion

7.3.1 Efficiency of Integrated Media Pilot Scale TBF System under Different Flow Rates and HRTs to Treat Domestic Wastewater

In the current research study, integrated media TBF system was operated in three different phases on the basis of flow rates (Q) i.e. 0.004 m^3/day, 0.0072 m^3/day and 0.01 m^3/day and furthermore on each particular flow rate, three different HRTs (48, 72 and 96 hrs) were selected to evaluate the efficiency of integrated media TBF system towards treatment of domestic wastewater. The effects of HRTs on the performance of pilot scale TBF system on each particular flow rate are shown in Table (7.1). In the first phase ($Q = 0.004$ m^3/day) of current study it was observed that, 48 hrs effluent's recirculation reduced the COD level by 65% from an average of 568 mg/L to 198 mg/L, whereas the COD concentrations reduced by 89% from an average of 466 mg/L to 51 mg/L and 87.5% from an average of 441 mg/L to 55 mg/L after 72 hrs and 96 hrs of recirculation respectively ($p = 0.001$). While, in the second phase ($Q = 0.0072$ m^3/day) and also in third phase ($Q = 0.01$ m^3/day) of experiment, maximum reduction up to 80.7 % ($p = 0.003$) and 52 % in COD contents ($p = 0.006$) were observed after 72 and 96 hrs of recirculation respectively as shown in Fig. 7.2. It was reported that as the effluent is being recirculated at low and moderate flow rate, surplus oxygen for aerobic microbial activities can be transmitted into the wastewater that is being frequently pumped and redistributed which will also bring benefit to the treatment facility by augmenting interactions between pollutants in the wastewater and microorganisms attached on the surface of integrated media (Sun *et al.*, 2003). That's why maximum reduction in COD values were observed at low and intermediate flow rate as compared to slightly higher flow rate.

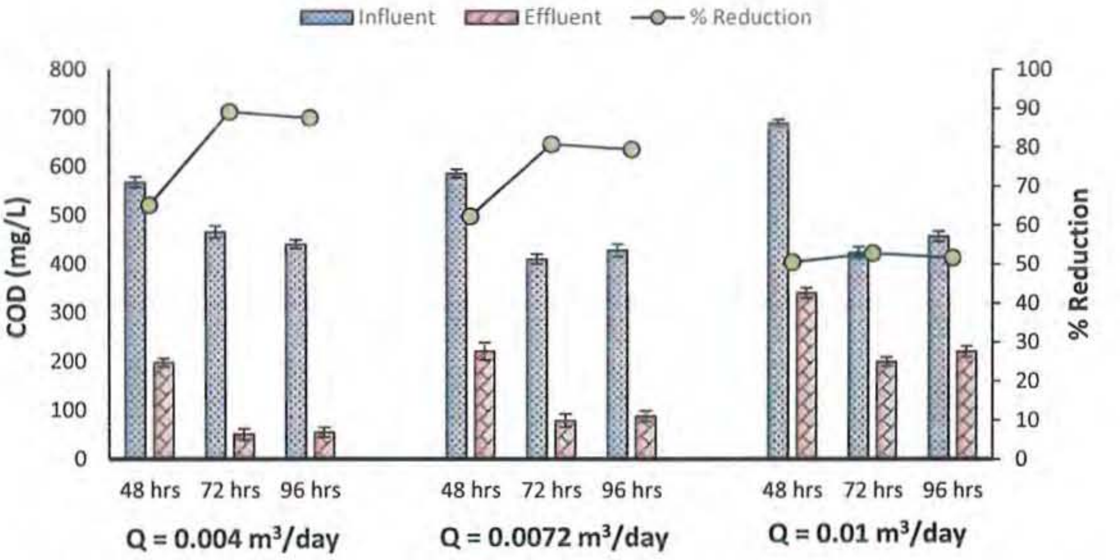


Fig. 7.2 Efficiency of integrated media TBF system in the average reduction of COD at different HRTs and flow rates (Q = 0.004 m^3/day, 0.0072 m^3/day and 0.01 m^3/day) (*Bar indicates Standard error*).

In the current study it was observed that the TDS removal efficiency of TBF system increased with increase in HRT under different flow rates. In the first phase (Q = 0.004 m^3/day) of experiment, it was observed that, 48 hrs recirculation reduced the TDS level by 61.6% whereas the TDS level reduced by 74.8% and 72% after 72 hrs and 96 hrs of recirculation of wastewater over the top of filter bed respectively. Similar pattern of TDS reduction were observed in 2nd and 3rd phases of experiment under different flow rates, where maximum reduction were observed in the range of 60.3-61% and 50.3-51% after 72 hrs and 96 hrs recirculation respectively as shown in Fig. 7.3. Reduction in TDS content with increasing HRT might be due to continuous recirculation of wastewater for an extended period of time as a result, microorganisms within biofilm got sufficient time to degrade dissolved organic components (Szogi *et al.,* 1997; Rehman *et al.,* 2012). Furthermore, it was perceived that at low and intermediate flow arte, maximum reduction in TDS were observed under different HRT as compared to higher flow rate. A basic reason for this might be that at slightly higher flow rate, microbes within biofilm did not get sufficient time to interact with dissolved organic contents and degrade them.

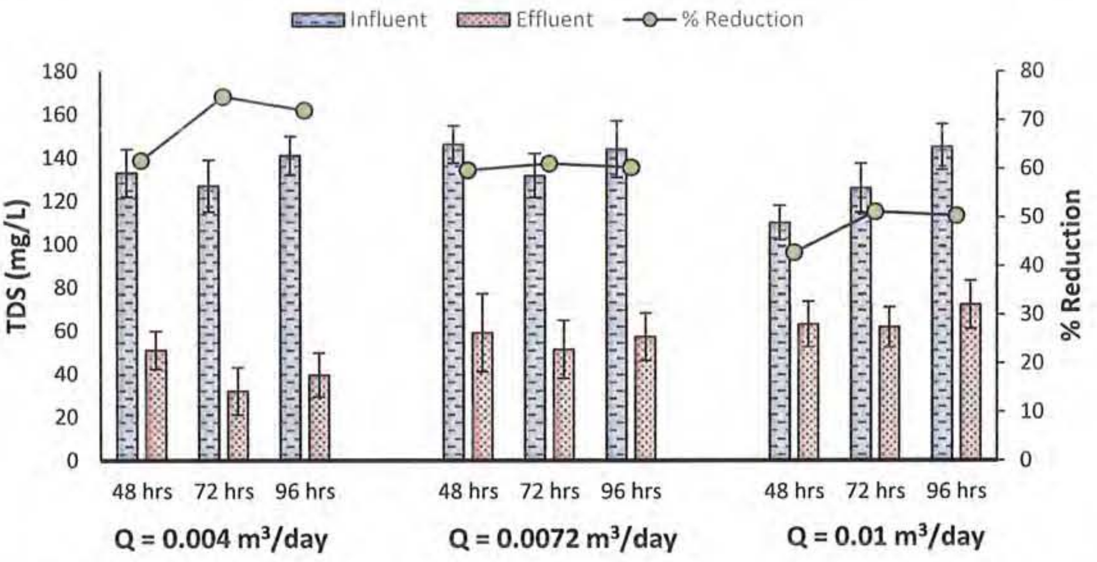


Fig. 7.3 Efficiency of integrated media TBF system in the average reduction of TDS at different HRTs and flow rates (Q = 0.004 m^3/day, 0.0072 m^3/day and 0.01 m^3/day) (*Bar indicates Standard error*).

According to WHO, (2006) electric conductivity (EC) has direct co-relation with total dissolved contents and COD i.e. higher the concentration of TDS and COD, higher will be the concentration of EC in wastewater. However, the permissible limit of EC in influent samples is 400-1500 μS/cm. In the current study, it was observed that reduction in EC contents increased with increase in HRT in all experimental phases operated at different flow rates. However, reduction in EC contents were observed in the range of 57.9-76% (1st phase), 46.4-71.4% (2nd phase) and 38.5-67.1% (3rd phase) under different HRTs and significant reduction ($p = 0.003$) were observed at 48 hrs and 72 hrs of recirculation as shown in Fig. 7.4. A basic reason for reduction in EC content might be that complex charged and reactive compounds present in wastewater were converted into inert simpler compounds, as a result the concentration of free ions were reduced to conduct electric current (Sawyer *et al.*, 1994).

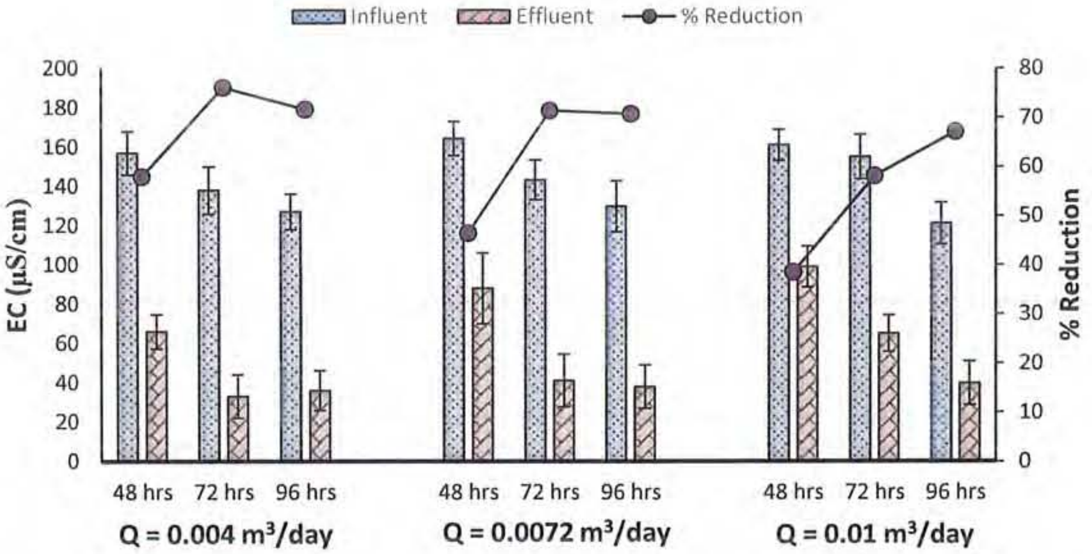


Fig. 7.4 Efficiency of integrated media TBF system in the average reduction of EC at different HRTs and flow rates (Q = 0.004 m^3/day, 0.0072 m^3/day and 0.01 m^3/day) (*Bar indicates Standard error*).

In the present study, it was observed that an average reduction of 42.4%, 66.8% and 71.6% ($p = 0.003$) were achieved in phosphate (PO_4) concentrations at HRT of 48, 72 and 96 hrs respectively at a flow rate of 0.004 m^3/day. While, at a flow rate of 0.0072 m^3/day, an average reduction of 31.1%, 52% and 52.2% were observed in PO_4 level at HRT of 48, 72 and 96 hrs respectively, whereas an average reduction of 43.9%, 54.3% and 53.5% were attained at HRT of 48, 72 and 96 hrs respectively at a flow rate of 0.01 m^3/day (Fig. 7.5) which is statistically significant ($p = 0.005$). The actual phenomena of PO_4 removal in integrated media TBF facility is still unknown. However, it is usually assumed that due to chemical interaction of inorganic phosphorus rich compounds with heavy metals present in wastewater contributes to the removal of PO_4 contents. Furthermore, other processes such as adsorption and nutrient uptake by microorganisms present in the slime layer may also play a key role in PO_4 accumulation, as a result the concentration of PO_4 reduced significantly with treatment (Sun *et al.*, 2003).

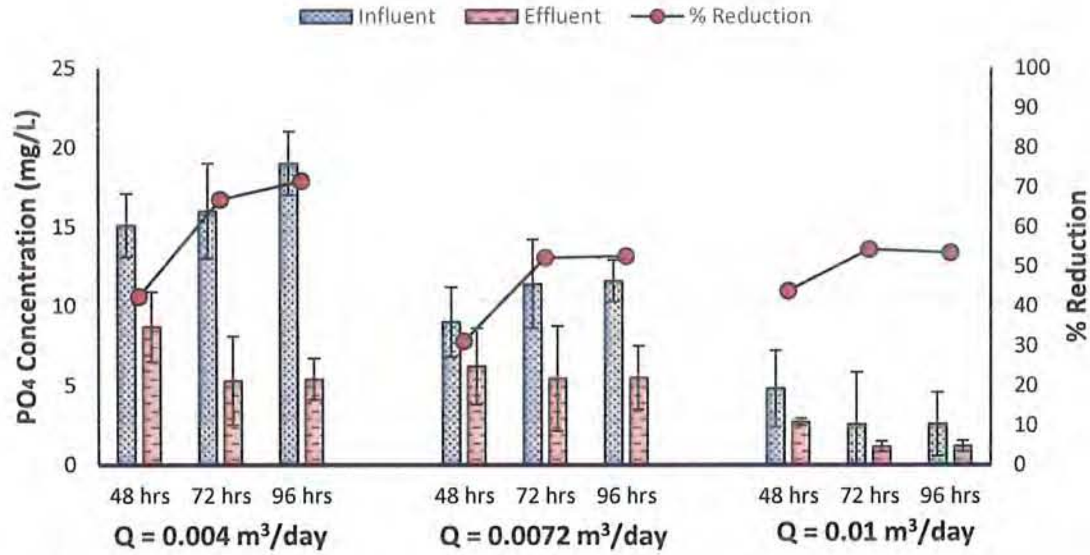


Fig. 7.5 Efficiency of integrated media TBF system in the average reduction of PO_4 levels at different HRTs and flow rates (Q = 0.004 m^3/day, 0.0072 m^3/day and 0.01 m^3/day) (*Bar indicates Standard error*).

Total nitrogen (TN) is an important component required for the growth of microorganisms and plants however, the excess amount of nitrogen in the effluent of wastewater treatment system facilitate hypertrophication and algal growth and hence deplete the oxygen supply. The principle source of TN in domestic wastewater are urea and other protenaceous compounds used in daily life. In the present study, 42.4%, 74.5% and 72.8% reduction in TN concentration were achieved at HRT of 48, 72 and 96 hrs respectively at a flow rate of 0.004 m^3/day which is statistically showing highly significant efficiency (p = 0.002). While, at a flow rate of 0.0072 m^3/day, an average reduction of 37.5%, 70.6% and 69.5% were observed in TN level at HRT of 48, 72 and 96 hrs respectively (p = 0.003), whereas 30.1%, 66.6 % and 65.7 % reduction in TN were attained at HRT of 48, 72 and 96 hrs respectively (p = 0.005) at a flow rate of 0.01 m^3/day as shown in Fig. 7.6. Recirculation has a positive significant effect on the nitrogen removal because it increases the population of nitrifiers (Mann *et al.*, 1998; Sun *et al.*, 2003; He *et al.*, 2007). Moreover, the decrease in the organic/inorganic pollutants removal has a positive effect on oxygenation of the effluent and nitrifiers growth, resulting in efficient nitrification/denitrification processes (Naz *et al.*, 2016).

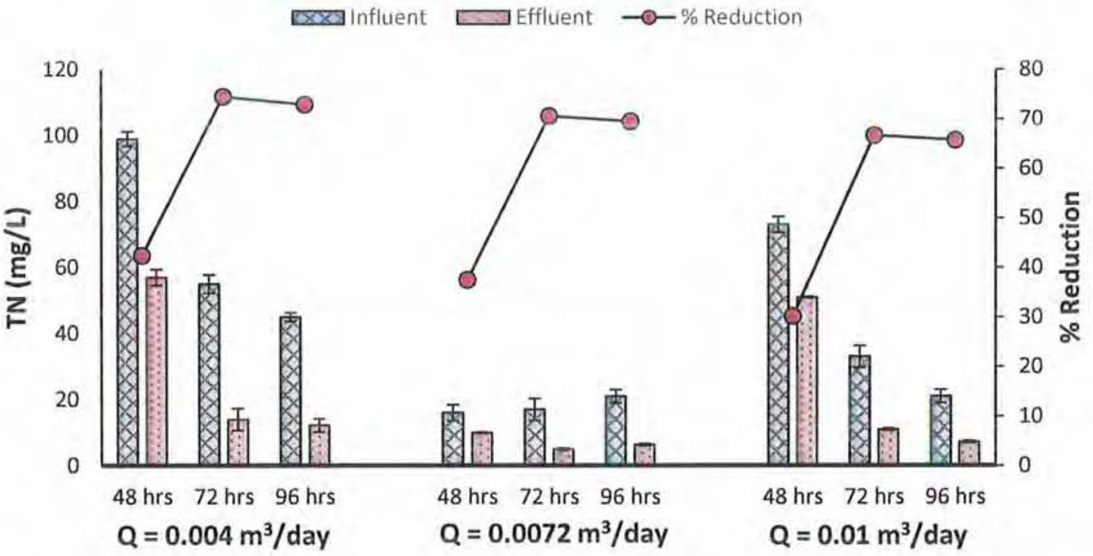


Fig. 7.6 Efficiency of integrated media TBF system in the average reduction of TN levels at different HRTs and flow rates (Q = 0.004 m^3/day, 0.0072 m^3/day and 0.01 m^3/day) (*Bar indicates Standard error*).

Variation in pH values of influent and effluent samples during treatment by integrated media TBF system under different flow rates and HRTs are shown in Fig. 7.7. According to Metcalf and Eddy, (1991) during nitrification process in integrated media TBF system, a large amount of alkalinity is consumed as an inorganic carbon source by the nitrifying bacteria due to which reduction in pH values were observed during treatment. Although pH has no direct effect on aquatic as well as terrestrial life, but it has been considered as one of the important parameters to define quality of wastewater. According to WHO, (2006) and US-EPA, (2007) reports, the rate of ammonium oxidation decreased significantly in acidic pH range. Therefore, for optimum performance of treatment systems, it is best to maintain a pH in the range of 6.5-8.5. The outcomes of study in this regard revealed the same range, indicating that the integrated media TBF system as one of the best options for domestic wastewater treatment.

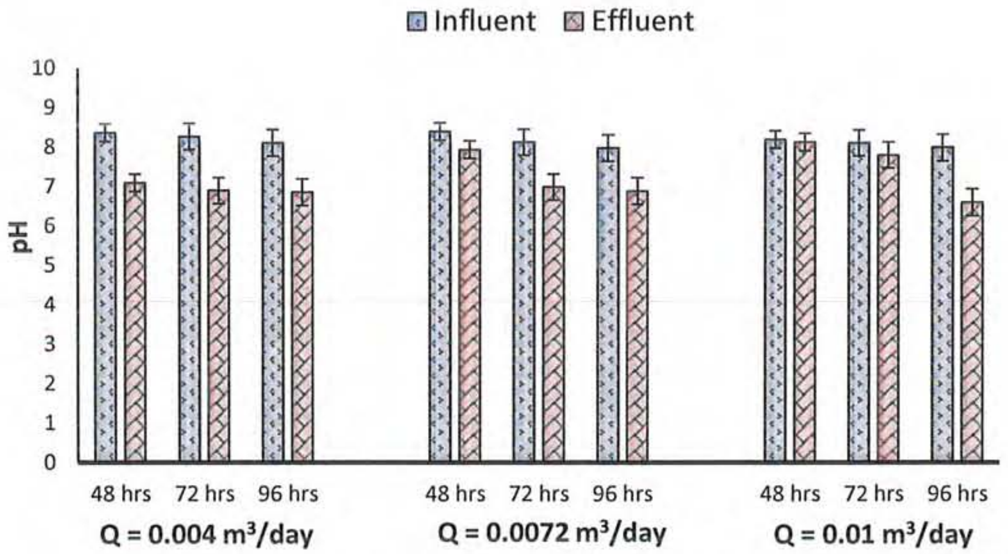


Fig. 7.7 Variation in the pH value of influent and effluent samples at different HRTs and flow rates (Q = 0.004 m^3/day, 0.0072 m^3/day and 0.01 m^3/day) (*Bar indicates Standard error*).

Table 7.1 Efficiency of integrated media TBF system in the reduction of different physico-chemical parameters at different HRTs and flow rates.

Flow Rates	HRTs	Different Physico-chemical Parameters of Influent and Effluent Samples																	
		COD (mg/L)			pH			EC (µS/cm)			TDS (mg/L)			PO_4 (mg/L)			TN (mg/L)		
		UT	T	% Red.	UT	T	% Red.	UT	T	% Red.	UT	T	% Red.	UT	T	% Red.	UT	T	% Red.
0.004 m^3/day	48 hrs	568	198	65.2	8.37	7.1	15.2	156.9	66	57.9	133	51	61.7	15.11	8.7	42.4	99	57	42.4
	72 hrs	466	51	89.0	8.27	6.9	16.6	138	33	76.1	127	32	74.8	16	5.3	66.8	55	14	74.5
	96 hrs	441	55	87.5	8.11	6.86	15.4	127	36	71.7	141	39.5	72.0	19	5.4	71.5	45	12.2	72.8
0.0072 m^3/day	48 hrs	586	221	62.3	8.4	7.94	5.5	164.3	88	46.4	146.2	59	59.6	9	6.2	31.1	16	10	37.5
	72 hrs	410	79	80.7	8.13	6.99	14.0	143.3	41	71.4	131.8	51.3	61.1	11.4	5.45	52.2	17	5	70.6
	96 hrs	428	88	79.4	7.98	6.89	13.7	129.8	38	70.7	144	57.2	60.3	11.6	5.5	52.6	21	6.4	69.5
0.01 m^3/day	48 hrs	689	341	50.5	8.2	8.13	0.85	161	99	38.5	110	63	42.7	4.81	2.7	43.8	73	51	30.2
	72 hrs	424	200	52.8	8.11	7.8	3.82	155	65	58.1	126	61.6	51.1	2.54	1.16	54.3	33	11	66.6
	96 hrs	457	221	51.6	7.99	6.6	17.4	121	39.8	67.1	145	72	50.3	2.56	1.19	53.5	21	7.2	65.7

7.3.2 Efficiency of Integrated Media Pilot Scale Trickling Filter (TBF) System in the Removal of Microbial Contaminants (CFU/mL) from Wastewater under Different Flow Rates and HRTs

The quality of wastewater is linked with the presence or absence of fecal coliforms, also assisted as indicator bacteria and it indicates the presence of organic pollution (Grady *et al.*, 2011; Danish *et al.*, 2011). In the present study, it was observed that the efficiency of integrated media pilot scale TBF system in terms of pathogen removal (CFU/mL) increased with continuous recirculation of wastewater for an extended period of time under different flow rates. Moreover, it was observed that at a flow rate of 0.004 m^3/day, an average reduction of 39.82%, 52.94% and 62.56% were achieved in CFU/mL at HRT of 48, 72 and 96 hrs respectively ($p = 0.007$). While, at a flow rate of 0.0072 m^3/day, an average reduction of 35.9 %, 36.77 % and 48.59 % ($p = 0.01$) were observed in microbial load at HRT of 48, 72 and 96 hrs respectively, whereas an average reduction of 25.77%, 40.1% and 57.3% were attained in CFU/mL at HRT of 48, 72 and 96 hrs respectively ($p = 0.009$) at a flow rate of 0.01 m^3/day (Table 7.2). In the current study, almost significant reduction in CFU/mL were observed in all phases of operation and this might be due to the greater hydraulic retention time in the reactor, which significantly affects the adsorption of pathogenic bacteria in the slime layer on the surface of integrated media. After becoming a part of the metabolically active biofilm, they are involved in the removal of organic and inorganic pollutants from wastewater, increasing dissolved oxygen levels, which leads to nitrification. Furthermore, the removal efficiency of pathogenic indicators has also been directly linked with the removal of organic and inorganic pollutants in the reactor, therefore due to nutrient depletion, natural die-off process would take place (Naz *et al.*, 2016).

Table 7.2 Efficiency of integrated media pilot scale Trickling filter (TBF) System in the removal of microbial contaminants (CFU/mL) from wastewater under different flow rates and HRT.

Flow Rates (Q)	HRTs	Average CFU/mL		Treatment Efficiency (%)
		Influent	Effluent	
$0.004\ m^3/day$	48 hrs	$2.21x10^5$	$1.33x10^5$	39.8
	72 hrs	$2.04x10^5$	$9.6x10^4$	52.9
	96 hrs	$2.19x10^5$	$8.2x10^4$	62.6
$0.0072\ m^3/day$	48 hrs	$1.89x10^5$	$1.21x10^5$	36
	72 hrs	$1.55x10^5$	$9.8x10^4$	36.8
	96 hrs	$1.77x10^5$	$9.1x10^4$	48.6
$0.01\ m^3/day$	48 hrs	$1.94x10^5$	$1.44x10^5$	25.8
	72 hrs	$2.02x10^5$	$1.21x10^5$	40.1
	96 hrs	$2.32x10^5$	$9.9x10^4$	57.3

7.3.3 Bacteriological Assessment of Slime Layer Developed on Integrated Filter Media

In the present study, integrated media (gravels and pebbles) were used as filter media, on which the slime layer was developed for wastewater treatment. Integrated media is highly efficient as compared to other media in wastewater clarification, allowing good microbial growth due to high specific surface area and low molecular weight (Mack *et al.*, 1975; Lens *et al.*, 1994; Filipkowska and Krzemieniewski, 1998). In the present study, bacteriological analysis of biofilm was performed by pure culture technique and 7 different bacterial isolates were isolated from the slime layer developed on the surface of integrated media. Out of 7 different bacterial isolates, five were Gram negative and two were Gram positive. After microscopy, these bacterial isolates were sub-cultured on nutrient agar media plates and different cultural characteristics were observed. After cultural characteristics, a complete identification of these bacterial isolates were carried out by performing different biochemical tests. The detailed description of microscopy, cultural and biochemical analysis of all identified bacterial isolates is given in the Table (7.3). Anderson *et al.*, (2008) studied the capability of 13 bacterial strains found in wastewater to form biofilm. About 9 pure cultures of bacteria were used for the formation of biofilm in TF by Butterfield and Wattie, (1941) and above 200 different microbial species including bacteria, protozoa, fungi, algae, worms and insects were used for the development of biofilm on a suitable media in TF by Cooke, (1959).

Table 7.3 Detailed description of microscopy, cultural and biochemical tests of bacterial spp., isolated from the slime layer (Biofilm) developed on integrated filter media.

Isolate	Cultural characteristics	Gram's Reaction	Fermentation			H_2S test	NO_3 test	Indole test	MR test	VP test	Citrate test	Urease test	Catalase test	TSI test	Identified Organisms
			L	D	S										
1	Slimy white, translucent, convex	-	AG	AG	AG	-	+	-	±	±	+	+	+	-	*Klebsiella* spp.,
2	Soft, smooth, yellow growth	+	-	-	-	-	±	-	-	-	-	+	+	K/NC	*Micrococcus* spp.,
3	White, moist, brilliant growth	-	AG	AG	A±	-	+	+	+	-	-	-	+	A/NC	*Escherichia coli*
4	Thin, even grayish growth	-	-	AG	A±	+	+	-	+	-	+	-	+	K/A, H_2S	*Salmonella* spp.,
5	Abundant, opaque, white growth	+	-	A	A	-	+	-	-	±	-	-	+	A/NC	*Bacillus* spp.,
6	Abundant, thin white growth	-	-	-	-	-	+	-	-	-	+	-	+	-	*Pseudomonas* spp.,
7	Thin, blue gray spreading growth	-	-	AG	AG	+	+	+	+	-	±	+	+	-	*Proteus* spp.,

Key: L = Lactose; D = Dextrose; S = Sucrose; AG = Acid and gas; + = Positive; - = Negative; ± = Variable reaction; A = Acid production; K = alkaline reaction; NC = No change; H_2S = Sulfur reduction; K/A = Red/yellow; K/NC = Red/no color change; K/A, H_2S = Red/yellow with bubble and black precipitate; A/NC = Acid/no color change; A/A = Yellow/yellow.

Chapter 8

GENERAL DISCUSSION

8. GENERAL DISCUSSION

With rapidly increasing population, urbanization/industrialization and decreasing water resources, wastewater in spite of having contrary impacts on public health, is becoming a significant resource for irrigation. Therefore, wastewater treatment is essential to prevent adverse impacts of wastewater disposal on the environment and public health (Llorens *et al.*, 2013). Furthermore, rapid growth in agriculture sectors and demand for clean water as a source of energy focused the attention of many environmental scientists towards the spoilage of renewable clean water reservoirs. It has been estimated that globally and especially in developing countries, more than 35% of children die due to unhygienic condition, lack of appropriate wastewater treatment facilities and scarcity of drinking water reservoirs (Ho, 2005; Massoud *et al.*, 2009). Moreover, it has also been reported that more than 38% of the population in developing countries live in life-threatened and unhygienic environment whereas, 30% of the developing countries lack proper infrastructure for wastewater treatment (Abegglen and Siegrist, 2006; Fach and Fuchs, 2010; Chong *et al.*, 2012).

Due to inadequate economic conditions in developing countries, environmental engineers/scientists would be requested to design cost-effective, environmental friendly and economically sustainable wastewater treatment facility for the treatment of municipal wastewater. From financial point of view, the differentiation in centralized and decentralized systems is of significance and details of both these systems with their respective merits and demerits are available in literature (Starkl *et al.*, 2012). Centralized systems are normally available in many developed countries for management of environmental problems associated with wastewater disposal but on other hand decentralized wastewater treatment facilities are often considered as more sustainable option especially for treating wastewater of small and rural communities in developing countries (Schories, 2008; Nanninga *et al.*, 2012). Different researchers described in detail the importance of decentralized wastewater treatment facilities in their literature i.e. they are low income projects and required less energy as compared to other conventional wastewater treatment systems (Fane & Fane, 2005; Galvao *et al.*, 2005; Beausejour and

Nguyen, 2007). Furthermore, it requires less space and maintenance requirement, environmental friendly and effluent of such treatment facilities have low impacts on terrestrial ecology (Meuler *et al.*, 2008; Wang, 2014).

Decentralized wastewater treatment systems can be further categorized in to different classes i.e. oxidation ponds, activated sludge (suspended growth systems) and attached growth systems. Oxidation ponds are simple, cost-effective and environmental friendly suspended growth technology used for the treatment of wastewater. This technology mainly removed carbonaceous compounds from wastewater in terms of BOD_5 before it's discharged to downstream ecosystem. There are certain limitations of oxidation ponds that they required large land area, prolonged detention and sludge retention time due to continuous aeration mode of operation (von Sperling and de Lemos, 2005). Activated sludge process (suspended growth system) is most widely used technology throughout the world for the biological treatment of wastewater. This technology differs from oxidation ponds in that it also removes pathogenic microorganisms and suspended solids from wastewater in addition to soluble carbonaceous compounds (Mara, 2004; Dewil *et al.*, 2006). But their efficiency depends on different factors such as retention time, environmental temperature, pH and level of dissolved oxygen (Doorn *et al.*, 2006). While, in attached growth systems, microorganisms present in wastewater attached to the suitable packing materials and form a slime layer over the surface of packing materials in the form of biofilm (Metcalf and Eddy, 2003).

Wastewater treatment with fixed film reactors like trickling filter is not a new technique. Trickling filters are filled with materials where biofilm grows. Structure of biofilms varies according to the composition of wastewater (Lewandowski and Filippi, 1998; Wagner *et al.*, 1998). The biofilm grows on filling materials with varied thickness as well and at ticker areas partially anaerobic zones develop. This leads to flushing off of population (Steinmann, 1989). Four different packing media (rubber, polystyrene, plastic and stone) for TBFs were investigated in previous research studies at laboratory scale experiments and also develop a simplified model for describing the capacity of BOD removal in TBF systems. It has been concluded that highly efficient TBFs can be designed using various filter media, which may be capable of treating organic loading rates of more than 3 kg BOD/m^3 day. These types of TBFs can be applied for the BOD and microbial contaminants removal of wastewater for potential reuse in developing countries (Naz *et al.*, 2015).

A variety of support materials have been reported by many researchers to be used in attached growth system as filter media and it includes, stones, plastic cubes, rubber, polystyrene packing materials, gravels and pebbles etc. (Kargi and Karapinar, 1997; Clifford *et al.*, 2010; Rehman *et al.*, 2012; Khan *et al.*, 2015; Naz *et al.*, 2015). These filter media have been considered as heart of the attached growth system as they provides a larger surface area per unit volume for the development of microbial slime layer. Therefore, selection criteria for packing materials must be accurate and precise so that a high active biomass and microbial population is sustained (Hu and Gagnon, 2006). Beside this, different characteristics of support materials would be considered such as durability to resist toxins and shock loads, greater specific surface area for biomass, must be economically feasible and have high void ratio to evade clogging and ponding issues and also to facilitate aeration (Soller *et al.*, 2003; Christensson and Welander, 2004). Stone media was selected for pilot scale filters in the present study as they are cost effective and could not easily be degraded.

The present research study was mainly focused on to evaluate the efficiency of pilot scale trickling filter systems towards treatment of domestic wastewater. Pilot scale TF systems were installed for the treatment of domestic wastewater and evaluated with varied temperature conditions, hydraulic load rates (HLR), organic load rates (OLR) and continuous recirculation.

Efficiency of a low cost stone media setup-I TF system was evaluated for sewage treatment during various seasons and almost one year experimental data was collected for carbonaceous and microbial loads. The wastewater treatment performance of the locally designed and constructed pilot scale TF system was good in all seasons apart from the winter in terms of the removal of organic as well as microbial pollutants from domestic wastewater. Furthermore, it was observed that in all seasons except winter the average percentage reduction were in the range of 52-72, 51-73, 61-81 and 74-89% for BOD_5, COD, TDS and TSS respectively. However in the winter season, excellent percentage reduction was only observed for TSS (74-81%) while for other parameters like BOD_5, COD and TDS, it was in the range of 13-50, 12-49 and 23-61% respectively. Steer *et al.*, (2002) also found similar removal proficiencies for BOD_5 and COD in their literature. A noteworthy reduction in microbial content of wastewater was also perceived in all seasons of treatment due to nutrient depletion in the treated wastewater, predation and natural die off-processes. The MPN index of effluent decreased considerably to lower values in all the seasons. During non-winter seasons, the TF system showed high potential of wastewater reuse in irrigation sector when MPN per 100 mL of lower than 200 is desired. This is particularly relevant to developing countries such as Pakistan where water sources are under significant threats due to increasing pollution load. The zero order kinetic model is a mathematical interpretation of a composite set of biological, chemical and physical interfaces that occur in TF systems. The model had resulted in more accurate measurements of pilot scale TF designs and would help to improve its ability to handle different types of wastewater in the future.

In the second experimental level, stone media setup-II TBF system was operated for 15 weeks to optimize its operational conditions for the treatment of domestic wastewater. It was observed that pilot scale TBF system proved efficient at a temperature range of 14-36°C by constant improvement in the quality of wastewater in terms of the reduction of BOD_5 (85.6%), COD (85%), TSS (99.8%), TDS (89%), EC (33.2%) and pathogenic microbes. A considerable reduction in the nutrients such as sulphates, phosphates and TN contents was also observed, which indicates that sulphate reducing, phosphate accumulating, nitrifying and denitrifying bacteria in the biofilm developed on stone media. The proposed study suggested that greater retention time in the bed of the TBF system plays a key role in the pathogen reduction. The TBF system employed in the present investigation was constructed on a slope and can be operated by force of gravity. Thus, power consumption by doing and recirculation pumps (0.3–0.6 kWh/m^3) is comparatively less than aerobic (2 kWh/m^3) and anaerobic membrane bioreactors (0.03–3.572 kWh/m^3), as reported by Martin *et al.*, (2011).

In the third experimental level, stone media setup-II TBF system was operated under different hydraulic retention time at a constant flow rate of 0.04 m^3/day and it was perceived that stone medial pilot scale TBF system (setup-II) showed high efficacy regarding removal of physico-chemical and microbiological parameters under different HRTs (24, 48 and 72 hrs). It was observed that by increasing HRT up to 48 and 72 hrs, the proficiency of setup-II TBF system increased significantly to reduce different parameters i.e. COD (70.9%), TDS (34%), EC (23.5%), SO_4 (37%), PO_4 (81.8%) and TN (66.6%) as shown in Table (6.2). Furthermore, setup-II TBF system suggested that greater retention time and sand filtration play a key role in the pathogen reduction and improvement of water quality. Albertson and Eckenfelder, (1984) also reported that maximum efficiency of trickling filter system would be achieved by keeping recirculation and distribution rates in desirable limit as it may greatly influence the growth of microbial slime layer on filter media. Moreover, stone media as natural filter media showed proficiency at pilot scale TBF operation, thus it can be a promising and favorable technology for wastewater treatment especially in the water stressed countries like Pakistan.

In the last experimental phase, a new strategy i.e. integrated media containing pebbles and gravels was evaluated to provide support for the growth of microbial slime layer (Fig. 7.1). Furthermore, the integrated media TBF system was operated at three different flow rates (Q) i.e. 0.004 m^3/day, 0.0072 m^3/day and 0.01 m^3/day and on each particular flow rate, effluent was operated under three different hydraulic retention time (HRT) i.e. 48, 72 and 96 hrs to determine the efficiency of integrated media pilot scale TBF system treating domestic wastewater. It was observed that integrated bed material with air space had optimistic effect over TBF operation and the lowest and intermediate flow rates (0.004 m^3/day and 0.0072 m^3/day) showed promising results with respect to percent reduction of different physico-chemical parameters i.e. COD (74.2-80.5%), TDS (60.3-69.5%), EC (62.8-68.6%) and PO4 (45.3-60.3%) as shown in Table (8.1). Furthermore, a substantial reduction in TN (59-63.3%) was observed at flow rates of 0.004 m^3/day and 0.0072 m^3/day.

Moreover, it was observed that the efficiency of integrated media pilot scale TBF system in terms of pathogen removal (CFU/mL) increased significantly with continuous recirculation of wastewater for an extended period of time under different flow rates (Table 7.2). On the other hand, continuous recirculation of effluent increases the operational cost of integrated media TBF system as huge amount of electricity is utilized by pumping which is not pointed in this study. However, it is obvious that recirculation increases the treatment efficiency of integrated media pilot scale TBF system and this assistance may well-compensate the slightly higher operational cost.

Lemji and Eckstadt, (2015) evaluated pilot scale trickling filter for removal of organics and nutrients from brewery wastewater at different hydraulic loading rates under aerobic and anaerobic biofilm systems. More than 80% COD removal was observed as the flow rates changed from 900 to 1100 L per day at an influent COD concentration of 600 mg/L. A linear regression model revealed that there was a very high correlation between mass loading rate and mass removal rate. The results suggested that a significant nutrient removal efficiencies can be achieved using the trickling filter at the design hydraulic load and organic load of 8.36 $m^{-3}m^{-2}day^{-1}$ and 0.75 $kg.m^{-3}day^{-1}$ respectively.

Trickling filters showed higher performance at higher hydraulic loading rate, which was due to the increased microbial growth and increased activity of microbial population associated with increase in organic loading with increase in hydraulic loadings. Further, higher hydraulic load controls the biofilm thickness and reduce the internal diffusion limitation thereby enhancing substrate transfer (Lazarova and Manem, 1994; Westerman *et al.*, 2000). Enhancement of aeration at higher hydraulic loads can be another reason. The attached growth systems maintain a high concentration of microorganisms while high removal rates occur at relatively small hydraulic retention times. This leads to the conclusion that the trickling filter is an ideal treatment technology under wide variations in wastewater flow, which is typically encountered in the domestic wastewater.

From comparative assessment of stone media and integrated media pilot scale setup-II TBF system operated under their specified flow rates, it was evaluated that integrated media

TBF system showed significant activity in the percent reduction of different physicochemical parameters as compared to stone media TBF system (Fig. 8.1). Generally stone filter media provides a low specific surface area for the development of biofilm. Moreover, it has been reported that due to low void spaces and larger unit mass of stone media, TBF system often facing reduced oxygen diffusion rate, clogging and ponding problems at high organic and hydraulic loading rates under different seasonal conditions (Grady *et al.*, 1999). Therefore, to enhance the proficiency and productivity of trickling filter using stone as a filter media, proper aeration and adjustable speed of rotary arm distributors would be required (Boltz *et al.*, 2006). In situation where maximum efficiency is estimated from trickling filter system to treat rich organics wastewater, stone media will be replaced by integrated media (pebbles and gravels) because they offer high void ratio and larger surface area for the growth of biomass.

Table 8.1 Effect of different flow rates over the performance of integrated media TBF system in terms of average percent reduction of different physicochemical parameters.

Flow Rates	**Avg. % reduction of different physicochemical parameters**				
	COD	TDS	EC	PO_4	TN
0.004 m^3/day	80.5	69.5	68.6	60.3	63.3
0.0072 m^3/day	74.2	60.3	62.8	45.3	59.2
0.01 m^3/day	51.6	48.1	54.6	50.6	54.2

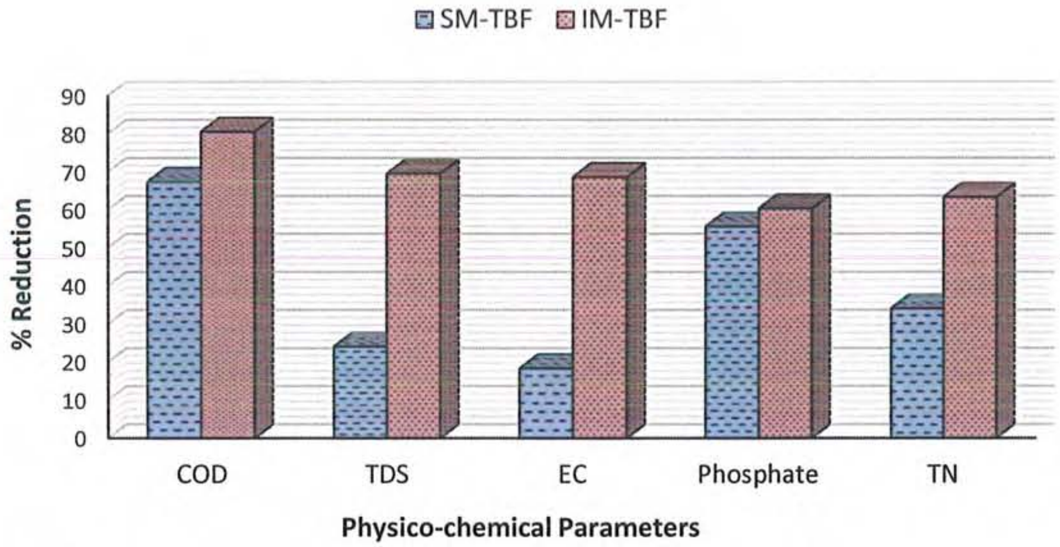


Fig. 8.1 Comparative assessment of stone media (SM-TBF) and integrated media (IM-TBF) pilot scale TBF system.

CONCLUSIONS

FUTURE PROSPECTS

CONCLUSIONS

1) A single stage trickling filter without the need to integrate it with other treatment technologies and without external aeration can be utilized to reduce the organic and nutrient load of domestic wastewater in a cost-effective and environmentally friendly manner.
2) The wastewater treatment performance of the locally designed and constructed pilot scale TF system was good in all seasons apart from the winter in terms of the removal of organic as well as microbial pollutants from domestic wastewater.
3) The zero order kinetic model has resulted in more accurate measurements of pilot scale TF designs and would help to improve its ability to handle different types of wastewater in the future.
4) Different bacterial spp., (*E. coli, Salmonella, Pseudomonas, Enterobacter, Klebsiella, Shigella, Proteus, Alcaligenes, Staphylococcus, Streptococcus, Micrococcus, Corynebacterium* and *Bacillus* spp.) were isolated from the microbial slime layer developed on the surface of stone media, so being a natural filter media showed proficiency at pilot scale TBF operation.
5) However, integrated bed material (pebbles and gravels) with air space had optimistic effect over TBF operation and the lowest and intermediate flow rates (0.004 m^3/day and 0.0072 m^3/day) showed promising results with respect to percent reduction of different physico-chemical parameters.
6) From comparative assessment it was concluded that integrated media TBF system displayed significant proficiency with respect to percent reduction of different physico-chemical parameters.

FUTURE PROSPECTS

1) Other types of media like plastic, ceramics, rubber, charcoal, wood chips etc. can be integrated in different combinations for the evaluation of pilot scale trickling filters proficiency.
2) Biofilm communities could be studied in detail with highly advanced technology such as next generation sequencing, genetic finger printing, clone library method, DNA micro assay and pyro-sequencing.
3) The design of the pilot scale TBF could be further modified in future studies to implement it on field scale for large communities providing socio-economic benefits.
4) This technology can also be tested for different industrial wastewater treatments.
5) Field scale tricking filters could be designed for local communities.

REFERENCES

REFERENCES

Abderrahman, W.A., 2000. Water demand management and Islamic water management principles: A case study. *International Journal of Water Resources Development*, *16*(4), pp.465-473.

Abegglen, C. and Siegrist, H., 2006. Domestic wastewater treatment with a small-scale membrane bioreactor. *Water Science and Technology*, *53*(3), pp.69-78.

Ahmad, M.U.D., Turral, H. and Nazeer, A., 2009. Diagnosing irrigation performance and water productivity through satellite remote sensing and secondary data in a large irrigation system of Pakistan. *Agricultural Water Management*, *96*(4), pp.551-564.

Albertson, O. E.; Eckenfelder, W. (1984) Analysis of Process Factors Affecting Plastic Media Trickling Filter Performance. Proceedings of the Second International Conference on Fixed Film Biological Processes; Washington, D.C

Ali, I., Asim, M. and Khan, T.A., 2012. Low cost adsorbents for the removal of organic pollutants from wastewater. *Journal of Environmental Management*, *113*, pp.170-183.

Ali, I., Khan, Z.M., Sultan, M., Mahmood, M.H., Farid, H.U., Ali, M. and Nasir, A., 2016. Experimental study on Maize Cob trickling filter-based wastewater treatment system: design, development, and performance evaluation. *Polish Journal of Environmental Studies*, *25*(6), p.2265.

Almstrand, R., Lydmark, P., Sörensson, F. and Hermansson, M., 2011. Nitrification potential and population dynamics of nitrifying bacterial biofilms in response to controlled shifts of ammonium concentrations in wastewater trickling filters. *Bioresource Technology*, *102*(17), pp.7685-7691.

Amorim, M.J., Römbke, J., Scheffczyk, A. and Soares, A.M., 2005. Effect of different soil types on the enchytraeids *Enchytraeus albidus* and *Enchytraeus luxuriosus* using the herbicide *Phenmedipham. Chemosphere*, *61*(8), pp.1102-1114.

Andersson, S., Kuttuva Rajarao, G., Land, C.J. and Dalhammar, G., 2008. Biofilm formation and interactions of bacterial strains found in wastewater treatment systems. *FEMS Microbiology Letters*, *283*(1), pp.83-90.

Andersson, Y. and Bohan, P., 2001. *Disease surveillance and waterborne outbreaks* (pp. 115-133). IWA Publishing, London.

APHA, A., 2005. WPCF, Standard methods for the examination of water and wastewater. *American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA.*

Archer, D.R., Forsythe, N., Fowler, H.J. and Shah, S.M., 2010. Sustainability of water resources management in the Indus Basin under changing climatic and socio economic conditions. *Hydrology and Earth System Sciences*, *14*(8), p.1669.

Aryan, A.F. and Johnson, S.H., 1987. Of: A Comparison of Trickling Filter Media. *Journal of Water Pollution Control Federation*, *59*(10), pp.915-918.

Asano, T., Burton, H., Leverenz, H., Tsuchihashi, R. and Tchobanoglous, G. 2007. Water Reuse: Issues, Technologies, and Application, McGraw-Hill Professional, New York; 1570.

Aygun, A., Nas, B. and Berktay, A., 2008. Influence of high organic loading rates on COD removal and sludge production in moving bed biofilm reactor. *Environmental Engineering Science*, *25*(9), pp.1311-1316.

Aylward, B., Bandyopadhyay, J., Belausteguigotia, J.C., Borkey, P., Cassar, A.Z., Meadors, L., Saade, L., Siebentritt, M., Stein, R., Tognetti, S. and Tortajada, C.,

2005. Freshwater ecosystem services. *Ecosystems and Human Well-Being: Policy Responses*, *3*, pp.213-256.

Bailey, E.S., Casanova, L.M., Simmons, O.D. and Sobsey, M.D., 2018. Tertiary treatment and dual disinfection to improve microbial quality of reclaimed water for potable and non-potable reuse: A case study of facilities in North Carolina. *Science of The Total Environment*, *630*, pp.379-388.

Barbosa, R.A. and Sant'Anna, G.L., 1989. Treatment of raw domestic sewage in an UASB reactor. *Water Research*, *23*(12), pp.1483-1490.

Bartram, J. and Balance, R., 1996. Water Quality Monitoring: A Practical Guide to the Design and Implementation of Fresh Water Quality Studies and Monitoring Programmes. Chapman & Hall, London, p.383.

Beausejour, J. and Nguyen, A.V., 2007. Decentralized sanitation implementation in Vietnam: a peri-urban case study. *Water Science and Technology*, *56*(5), pp.133-139.

Becerra-Castro, C., Lopes, A.R., Vaz-Moreira, I., Silva, E.F., Manaia, C.M. and Nunes, O.C., 2015. Wastewater reuse in irrigation: A microbiological perspective on implications in soil fertility and human and environmental health. *Environment International*, *75*, pp.117-135.

Billen, G., Servais, P., Bouillot, P. and Ventresque, C., 1992. Functioning of biological filters used in drinking water treatment-the Chabrol model. *Aqua*, *41*(4), pp.231-241.

Bitton, G., 2010. Wastewater Microbiology, 4th Ed.; Wiley & Sons: Hoboken, NJ.

Blenkinsopp, S.A. and Costerton, J.W., 1991. Understanding bacterial biofilms. *Trends in Biotechnology*, *9*(1), pp.138-143.

Boltz, J.P., La Motta, E.J. and Madrigal, J.A., 2006. The role of bioflocculation on suspended solids and particulate COD removal in the trickling filter process. *Journal of Environmental Engineering*, *132*(5), pp.506-513.

Boltz, J.P., Smets, B.F., Rittmann, B.E., van Loosdrecht, M., Morgenroth, E. and Daigger, G.T., 2017. From biofilm ecology to reactors: a focused review. *Water Science and Technology*, *75*(8), pp.1753-1760.

Boon, A.G., Hemfrey, J., Boon, K. and Brown, M., 1997. Recent Developments in the Biological Filtration of Sewage to Produce High-Quality Nitrified Effluents. *Water and Environment Journal*, *11*(6), pp.393-412.

Bouwer, H. and Idelovitch, E., 1987. Quality requirements for irrigation with sewage water. *Journal of Irrigation and Drainage Engineering*, *113*(4), pp.516-535.

Branda, S.S., Vik, Å., Friedman, L. and Kolter, R., 2005. Biofilms: the matrix revisited. *Trends in Microbiology*, *13*(1), pp.20-26.

Bryan, E. H.; Moeller, D. H. 1960. Aerobic Biological Oxidation Using Dowpac. Proceedings of the Conference on Biological Waste Treatment; Manhattan College: New York.

Bryers, G., 2000. *Protocol for monitoring trophic levels of New Zealand lakes and reservoirs* (No. 99/2). Lakes Consulting, Report.

Butterfield, C.T. and Wattie, E., 1941. Studies of Sewage Purification: XV. Effective Bacteria in Purification by Trickling Filters. *Public Health Reports (1896-1970)*, pp.2445-2464.

Carlson, K.H. and Amy, G.L., 1998. BOM removal during biofiltration. *Journal of American Water Works Association*, *90*(12), p.42.

Castonguay, M.H., Van der Schaaf, S., Koester, W., Krooneman, J., Van der Meer, W., Harmsen, H. and Landini, P., 2006. Biofilm formation by *Escherichia coli* is stimulated by synergistic interactions and co-adhesion mechanisms with adherence-proficient bacteria. *Research in Microbiology*, *157*(5), pp.471-478.

Chambless, J.D. and Stewart, P.S., 2007. A three-dimensional computer model analysis of three hypothetical biofilm detachment mechanisms. *Biotechnology and Bioengineering*, *97*(6), pp.1573-1584.

Chan, Y.J., Chong, M.F., Law, C.L. and Hassell, D.G., 2009. A review on anaerobic–aerobic treatment of industrial and municipal wastewater. *Chemical Engineering Journal*, *155*(1), pp.1-18.

Chang, H.T. and Rittmann, B.E., 1987. Mathematical modeling of biofilm on activated carbon. *Environmental Science and Technology*, *21*(3), pp.273-280.

Chaudhary, D.S., Vigneswaran, S., Ngo, H.H., Shim, W.G. and Moon, H., 2003. Biofilter in water and wastewater treatment. *Korean Journal of Chemical Engineering*, *20*(6), pp.1054-1065.

Chen, C.Y. and Chen, S.D., 2000. Biofilm characteristics in biological denitrification biofilm reactors. *Water Science and Technology*, *41*(4-5), pp.147-154.

Cheng, J. and Liu, B., 2002. Swine wastewater treatment in anaerobic digesters with floating medium. *Transactions of the ASAE*, *45*(3), pp.799.

Cheremisinoff, N.P., 2002. Membrane separation technologies. *Handbook of Water and Wastewater Treatment Technologies*, pp.335-371.

Chirila, E., Drăghici, C. and Puhacel, A., 2009. Performances of biochemical treatment stage from municipal wastewater treatment plant. *Environmental Engineering and Management Journal (EEMJ)*, *8*(4).

Chong, M.N., Sharma, A.K., Burn, S. and Saint, C.P., 2012. Feasibility study on the application of advanced oxidation technologies for decentralised wastewater treatment. *Journal of Cleaner Production*, *35*, pp.230-238.

Chowdhury, P., Viraraghavan, T. and Srinivasan, A., 2010. Biological treatment processes for fish processing wastewater–A review. *Bioresource Technology*, *101*(2), pp.439-449.

Christensson, M. and Welander, T., 2004. Treatment of municipal wastewater in a hybrid process using a new suspended carrier with large surface area. *Water Science and Technology*, *49*(11-12), pp.207-214.

Clifford, E., Nielsen, M., Sørensen, K. and Rodgers, M., 2010. Nitrogen dynamics and removal in a horizontal flow biofilm reactor for wastewater treatment. *Water Research*, *44*(13), pp.3819-3828.

Connor, R., Renata, A., Ortigara, C., Koncagül, E., Uhlenbrook, S., Lamizana-Diallo, B.M., Zadeh, S.M., Qadir, M., Kjellén, M., Sjödin, J. and Hendry, S., 2017. The United Nations World Water Development Report 2017. Wastewater: The Untapped Resource. *The United Nations World Water Development Report*.

Cooke, W., 1959. Trickling filter ecology. *Ecology*, *40*(2), pp.273-291.

Craun, M.F., Craun, G.F., Calderon, R.L. and Beach, M.J., 2006. Waterborne outbreaks reported in the United States. *Journal of Water and Health*, *4*(S2), pp.19-30.

Cripps, S.J. and Bergheim, A., 2000. Solids management and removal for intensive land-based aquaculture production systems. *Aquacultural Engineering*, *22*(1), pp.33-56.

Daigger, G.T. and Boltz, J.P., 2011. Trickling Filter and Trickling Filter-Suspended Growth Process Design and Operation: A State-of-the-Art Review. *Water Environment Research*, *83*(5), pp.388-404.

Daija, L., Selberg, A., Rikmann, E., Zekker, I., Tenno, T. and Tenno, T., 2016. The influence of lower temperature, influent fluctuations and long retention time on the performance of an upflow mode laboratory-scale septic tank. *Desalination and Water Treatment*, *57*(40), pp.18679-18687.

Danish, M., Hashim, R., Mohamad Ibrahim, M.N., Rafatullah, M., Sulaiman, O., Ahmad, T., Shamsuzzoha, M. and Ahmad, A., 2011. Sorption of copper (II) and nickel (II) ions from aqueous solutions using calcium oxide activated date (*Phoenix dactylifera*) stone carbon: equilibrium, kinetic, and thermodynamic studies. *Journal of Chemical and Engineering Data*, *56*(9), pp.3607-3619.

Das, N., Basak, L.V.G. and Salam, J.A., 2017. Application of biofilms on remediation of pollutants–an overview. *Journal of Microbiology and Biotechnology Research*, *2*(5), pp.783-790.

Davey, M.E. and O'Toole, G.A., 2000. Microbial biofilms: from ecology to molecular genetics. *Microbiology and Molecular Biology Reviews*, *64*(4), pp.847-867.

DeFilippi, L.J., Lupton, S. and Lewandowski, G.A., 1998. Introduction to microbiological degradation of aqueous waste and its application using a fixed-film reactor. *Biological Treatment of Hazardous Wastes*. Wiley, New York, pp.1-34.

Dewil, R., Appels, L. and Baeyens, J., 2006. Energy use of biogas hampered by the presence of siloxanes. *Energy Conversion and Management*, *47*(13), pp.1711-1722.

DiGiano, F.A. and Speitel Jr, G.E., 1993. Aqualink--Biofilm Computer Models: Time for Practical Applications?. *Journal of American Water Works Association*, *85*(5).

Donaldson, W.T., 1977. Trace organics in water. Refine methods are needed to 460 document their occurrence and concentration. *Environmental Science Technology*, *11*(4), pp.348-461.

Donlan, R.M. and Costerton, J.W., 2002. Biofilms: survival mechanisms of clinically relevant microorganisms. *Clinical Microbiology Reviews*, *15*(2), pp.167-193.

Doorn MRJ, Towprayoon S, Maria S, Vieira M, Irving W, Palmer C, Pipatti, R, Wang C (2006). Wastewater treatment and discharge. In 2006 IPCC Guidelines for National Greenhouse Gas Inventories volume 5. (Waste). WMO, UNEP. pp. 6: 1-6, 28.

Dunne, W.M., 2002. Bacterial adhesion: seen any good biofilms lately? *Clinical Microbiology Reviews*, *15*(2), pp.155-166.

Eckenfelder, W.W., 1961. Design and performance of aerated lagoons for pulp and paper waste treatment. In *Proc. 16th Industrial Waste Conference Purdue University. Engineering Extension, 109*, pp. 115-125.

Ehlers, G.C. and Turner, S., 2012. Biofilms in wastewater treatment systems. *Microbial Biofilms-Current Research and Applications*, pp.99-110.

Eighmy, T.T., Maratea, D. and Bishop, P.L., 1983. Electron microscopic examination of wastewater biofilm formation and structural components. *Applied and Environmental Microbiology*, *45*(6), pp.1921-1931.

Eke, P.E. and Scholz, M., 2008. Benzene removal with vertical-flow constructed treatment wetlands. *Journal of Chemical Technology and Biotechnology*, *83*(1), pp.55-63.

Ensink, J.H., Mahmood, T., Van der Hoek, W., Raschid-Sally, L. and Amerasinghe, F.P., 2004. A nationwide assessment of wastewater use in Pakistan: An obscure activity or a vitally important one? *Water Policy*, *6*(3), pp.197-206.

EPA (Environmental Protection Agency). 2007. Bureau of Water Supply and Wastewater Management: Department of 468 Environmental Protection Agency, Wastewater Treatment Plant Operator 469 Training (Module 20: Trickling filters). 470

EPA (Environmental Protection Agency). 2014. Sediments. In Water: Pollution Prevention & Control. Retrieved from http://water.epa.gov/polwaste/sediments/.

Fach, S. and Fuchs, S., 2010. Design and development of decentralized water and wastewater technologies: a combination of safe wastewater disposal and fertilizer production. *Water Science and Technology*, *62*(7), pp.1580-1586.

Fan, B. and Kirchhofer, D.K., Genentech Inc, 2013. *Methods and compositions for modulating prostasin*. U.S. Patent Application 13/615,122.

Fane, A.G. and Fane, S.A., 2005. The role of membrane technology in sustainable decentralized wastewater systems. *Water Science and Technology*, *51*(10), pp.317-325.

Feenstra, S., Hussain, R. and van der Hoek, W., 2000. Health risks of irrigation with untreated urban wastewater in the southern Punjab, Pakistan.

Fernandes, P.M., Pedersen, L.F. and Pedersen, P.B., 2017. Influence of fixed and moving bed biofilters on micro particle dynamics in a recirculating aquaculture system. *Aquacultural Engineering*, *78*, pp.32-41.

Filipkowska, Z. and Krzemieniewski, M., 1998. Effectiveness of indicatory microorganism removal on trickling filter with biofilm in magnetic field. *Polish Journal of Environmental Studies*, *7*, pp.201-206.

Fletcher, P.D., 1986. The partitioning of solutes between water-in-oil microemulsions and conjugate aqueous phases. *Journal of the Chemical Society, Faraday Transactions 1: Physical Chemistry in Condensed Phases*, *82*(9), pp.2651-2664.

Galvao, A., Matos, J., Rodrigues, J. and Heath, P., 2005. Sustainable sewage solutions for small agglomerations. *Water Science and Technology*, *52*(12), pp.25-32.

Gangal, M., Kaldate, A., Graveleau, L. and Desmottes, C., 2015. Evaluation of Low Energy Requirements in Deammonification Systems. *Proceedings of the Water Environment Federation*, *2*, pp.1-12.

Garrett, T.R., Bhakoo, M. and Zhang, Z., 2008. Bacterial adhesion and biofilms on surfaces. *Progress in Natural Science*, *18*(9), pp.1049-1056.

Gebara, F., 1999. Activated sludge biofilm wastewater treatment system. *Water Research*, *33*(1), pp.230-238.

Germain, J.E., 1966. Economical treatment of domestic waste by plastic-medium trickling filters. *Journal of Water Pollution Control Federation*, pp.192-203.

Gottenbos, B., Van der Mei, H.C. and Busscher, H.J., 1999. Models for studying initial adhesion and surface growth in biofilm formation on surfaces. *Methods in Enzymology*, *310*, pp.523-534.

Grady Jr, C.L., Daigger, G.T., Love, N.G. and Filipe, C.D., 2011. *Biological Wastewater Treatment*. CRC press.

Grady, J., Leslie, C.P., Daigger, G.T., Lim, H.C., 1999. Biological Wastewater Treatment, second ed. Marcel Dekker, Inc., New York.

Guibaud, G., van Hullebusch, E. and Bordas, F., 2006. Lead and cadmium biosorption by extracellular polymeric substances (EPS) extracted from activated sludges: pH-sorption edge tests and mathematical equilibrium modelling. *Chemosphere*, *64*(11), pp.1955-1962.

Gupta, PK., 2004. Methods in environmental analysis water, soil and air. 1st 370 (edn). *India Jodhpur Agro House: Updesh Purohit for Agrobios*, pp.47–48.

Gwaski, P.A., Hati, S.S., Ndahi, N.P. and Ogugbuaja, V.O., 2013. Emergent pollution bio-monitoring triad (target species-tissues-analyte) for the aquatic environment of Chad lake. *Advances in Applied Science Research*, *4*, pp.181-189.

Hameed, M., Ashraf, M., Naz, N. and Al-Qurainy, F., 2010. Anatomical adaptations of Cynodon dactylon (L.) Pers. from the Salt Range Pakistan to salinity stress. I. Root and stem anatomy. *Pakistan Journal of Botany*, *42*(1), pp.279-289.

Hamid, H. and Eskicioglu, C., 2012. Fate of estrogenic hormones in wastewater and sludge treatment: A review of properties and analytical detection techniques in sludge matrix. *Water Research*, *46*(18), pp.5813-5833.

Harremoes, P., Capodaglio, A.G., Hellström, B.G., Henze, M., Jensen, K.N., Lynggaard-Jensen, A., Otterpohl, R. and Søeberg, H., 1993. Wastewater treatment plants under transient loading-Performance, modelling and control. *Water Science and Technology*, *27*(12), p.71.

Harrison, J.R. and Daigger, G.T., 1987. A comparison of trickling filter media. *Journal of Water Pollution Control Federation*, pp.679-685.

He, S.B., Xue, G. and Kong, H.N., 2007. The performance of BAF using natural zeolite as filter media under conditions of low temperature and ammonium shock load. *Journal of Hazardous Materials*, *143*(1-2), pp.291-295.

Heeb, F., Singer, H., Pernet-Coudrier, B., Qi, W., Liu, H., Longrée, P., Müller, B. and Berg, M., 2012. Organic micropollutants in rivers downstream of the megacity Beijing: sources and mass fluxes in a large-scale wastewater irrigation system. *Environmental Science and Technology*, *46*(16), pp.8680-8688.

Henze, M. and Harremoes, P., 1983. Anaerobic treatment of wastewater in fixed film reactors–a literature review. *Water Science and Technology*, *15*(8-9), pp.1-101.

Henze, M., 2008. Biological wastewater treatment: principles, modelling and design. London, UK.

Ho, G., 2005. Technology for sustainability: the role of onsite, small and community scale technology. *Water Science and Technology*, *51*(10), pp.15-20.

Hosseini, S.H. and Borghei, S.M., 2005. The treatment of phenolic wastewater using a moving bed bio-reactor. *Process Biochemistry*, *40*(3), pp.1027-1031.

Hozalski, R.M. and Bouwer, E.J., 1998. Deposition and retention of bacteria in backwashed filters. *Journal of the American Water Works Association*, *90*(1), p.71.

https://nation.com.pk/30-Dec-2013/water-storage-capacity-just-for-30-days [Accessed at 02-02-2018].

https://www.environmental-expert.com/products/westech-biodoc-trickling-filter-rotary-distributor-2992 [Accessed at 02-02-2018].

https://www.mapsofworld.com/around-the-world/water-crisis.html [Accessed at 02-02-2018].

Hu, Z. and Gagnon, G.A., 2006. Impact of filter media on the performance of full-scale recirculating biofilters for treating multi-residential wastewater. *Water Research*, *40*(7), pp.1474-1480.

Huck, P.M., Zhang, S. and Price, M.L., 1994. BOM removal during biological treatment: a first-order model. *Journal of the American Water Works Association*, *86*(6).

Hussain, A., Alamzeb, S. and Begum, S., 2013. Accumulation of heavy metals in edible parts of vegetables irrigated with waste water and their daily intake to adults and children, District Mardan, Pakistan. *Food Chemistry*, *136*(3), pp.1515-1523.

Hvala, N., Vrečko, D., Levstek, M. and Bordon, C., 2017. The use of dynamic mathematical models for improving the designs of upgraded wastewater treatment plants. *Journal of Sustainable Development of Energy, Water and Environment Systems*, *5*(1), pp.15-31.

Iliuta, I., Iliuta, M.C. and Larachi, F., 2005. Hydrodynamics modeling of bioclogging in waste gas treating trickle-bed bioreactors. *Industrial & Engineering Chemistry Research*, *44*(14), pp.5044-5052.

Iribarnegaray, M.A., Rodriguez-Alvarez, M.S., Moraña, L.B., Tejerina, W.A. and Seghezzo, L., 2018. Management challenges for a more decentralized treatment and reuse of domestic wastewater in metropolitan areas. *Journal of Water, Sanitation and Hygiene for Development*, *8*(1), pp.113-122.

Jayakumar, O.D., Ganguly, R., Tyagi, A.K., Chandrasekharan, D.K. and Krishnan Nair, C.K., 2009. Water dispersible Fe3O4 nanoparticles carrying doxorubicin for cancer therapy. *Journal of Nanoscience and Nanotechnology*, *9*(11), pp.6344-6348.

Jin, Y.L., Lee, W.N., Lee, C.H., Chang, I.S., Huang, X. and Swaminathan, T., 2006. Effect of DO concentration on biofilm structure and membrane filterability in submerged membrane bioreactor. *Water Research*, *40*(15), pp.2829-2836.

Jury, W.A. and Vaux, H.J., 2007. The emerging global water crisis: managing scarcity and conflict between water users. *Advances in Agronomy*, *95*, pp.1-76.

Kadu, P.A., Landge, R.B. and Rao, Y., 2013. Treatment of dairy wastewater using rotating biological contactors. *European Journal of Experimental Biology*, *3*, pp.257-260.

Kalavrouziotis, I.K. and Arslan-Alaton, I., 2008. Reuse of urban wastewater and sewage sludge in the Mediterranean countries: case studies from Greece and Turkey. *Fresenius Environmental Bulletin*, *17*(6), p.625.

Kargi, F. and Karapinar, I., 1997. Performance of fluidized bed bioreactor containing wire-mesh sponge particles in wastewater treatment. *Waste Management*, *17*(1), pp.65-70.

Khan, Z.U., Naz, I., Rehman, A., Rafiq, M., Ali, N. and Ahmed, S., 2015. Performance efficiency of an integrated stone media fixed biofilm reactor and sand filter for sewage treatment. *Desalination and Water Treatment*, *54*(10), pp.2638-2647.

Kim, J.R., Premier, G.C., Hawkes, F.R., Rodríguez, J., Dinsdale, R.M. and Guwy, A.J., 2010. Modular tubular microbial fuel cells for energy recovery during sucrose wastewater treatment at low organic loading rate. *Bioresource Technology*, *101*(4), pp.1190-1198.

Kim, S. and Deshusses, M.A., 2003. Development and experimental validation of a conceptual model for biotrickling filtration of H2S. *Environmental Progress and Sustainable Energy*, *22*(2), pp.119-128.

Kim, S.H., Cho, J.S., Park, J.H., Heo, J.S., Ok, Y.S., Delaune, R.D. and Seo, D.C., 2016. Long-term performance of vertical-flow and horizontal-flow constructed wetlands as affected by season, N load, and operating stage for treating nitrogen from domestic sewage. *Environmental Science and Pollution Research*, *23*(2), pp.1108-1119.

Kinner, N.E., Balkwill, D.L. and Bishop, P.L., 1983. Light and electron microscopic studies of microorganisms growing in rotating biological contactor biofilms. *Applied and Environmental Microbiology*, *45*(5), pp.1659-1669.

Kishimoto, N., Ohara, T., Hinobayashi, J. and Hashimoto, T., 2014. Roughness and temperature effects on the filter media of a trickling filter for nitrification. *Environmental Technology*, *35*(12), pp.1549-1555.

Klein, J. and Ziehr, H., 1990. Immobilization of microbial cells by adsorption. *Journal of Biotechnology*, *16*(1), pp.1-15.

Koh, I.O., Chen-Hamacher, X., Hicke, K. and Thiemann, W., 2004. Leachate treatment by the combination of photochemical oxidation with biological process. *Journal of Photochemistry and Photobiology A: Chemistry*, *162*(2), pp.261-271.

Kolter, R. and Greenberg, E.P., 2006. Microbial sciences: the superficial life of microbes. *Nature*, *441*(7091), pp.300-302.

Kornaros, M. and Lyberatosa, G., 2006. Biological treatment of wastewaters from a dye manufacturing company using a trickling filter. *Journal of Hazardous Materials*, *136*(1), pp.95-102.

Kornegay, B.H. and Andrews, J.F., 1968. Kinetics of fixed-film biological reactors. *Journal of Water Pollution Control Federation*, pp.R460-R468.

Krishna, I.M., Manickam, V., Shah, A. and Davergave, N., 2017. Environmental Management: Science and Engineering for Industry. Butterworth-Heinemann.

Kumar, C.G. and Anand, S.K., 1998. Significance of microbial biofilms in food industry: a review. *International Journal of Food Microbiology*, *42*(1), pp.9-27.

Lacasse, R., Belanger, G., Henry, Y., Talbot, P., Mlynarek, J. and Vermeersch, O., 2001. A denitrification process based on a new filtering media for onsite wastewater treatment. In On-Site Wastewater Treatment. *American Society of Agricultural and Biological Engineers*, p-235.

Ladu, J.L.C., Lu, X.W. and Osman, A.M., 2014. Integrated Processes of Anoxic/Oxic Bioreactor and Artificial Wetland for Rural Domestic Wastewater Treatment. In *Advanced Materials Research*, (955), pp. 2526-2529.

Laing, M., 2016. Investigating the performance of a novel Anaerobic Sequencing Batch Reactor (AnSBR) and optimisation of operational parameters to treat synthetic winery wastewater (Doctoral dissertation, Stellenbosch: Stellenbosch University).

LaMotta, E.J., 1976. Internal diffusion and reaction in biological films. *Environmental Science and Technology*, *10*(8), pp.765-769.

Latrach, L., Ouazzani, N., Hejjaj, A., Mahi, M., Masunaga, T. and Mandi, L., 2018. Two-stage vertical flow multi-soil-layering (MSL) technology for efficient removal of coliforms and human pathogens from domestic wastewater in rural areas under arid climate. *International Journal of Hygiene and Environmental Health*, *221*(1), pp.64-80.

Lazarova, V. and Manem, J., 1994. Advances in biofilm aerobic reactors ensuring effective biofilm activity control. *Water Science and Technology*, *29*(10-11), pp.319-327.

Lazarova, V. and Manem, J., 2000. Innovative biofilm treatment technologies for water and wastewater treatment. *Cheminform*, *31*(32).

Lazarova, V.Z., Capdeville, B. and Nikolov, L., 1992. Biofilm performance of a fluidized bed biofilm reactor for drinking water denitrification. *Water Science and Technology*, *26*(3-4), pp.555-566.

Lekang, O.I. and Kleppe, H., 2000. Efficiency of nitrification in trickling filters using different filter media. *Aquacultural Engineering*, *21*(3), pp.181-199.

Lekeufack, M., Fonkou, T. and Tedonkeng, E.P., 2017. Growth Characteristics of Fuirena umbellata in a Surface Flow Constructed Wetland and Its Influence in Nutrients and Faecal Bacteria Removal from Domestic Wastewater in Cameroon. *Journal of Environmental Protection*, *8*(02), p.171.

Lemji, H.H. and Eckstädt, H., 2013. A pilot scale trickling filter with pebble gravel as media and its performance to remove chemical oxygen demand from synthetic brewery wastewater. *Journal of Zhejiang University Science B*, *14*(10), pp.924-933.

Lemji, H.H. and Eckstädt, H., 2015. Efficiency of a pilot scale trickling filter to treat industrial brewery wastewater: Influence of hydraulic loading. *Journal of Chemical Technology and Biotechnology*, *90*(1), pp.201-207.

Lens, P.N., De Poorter, M.P., Cronenberg, C.C. and Verstraete, W.H., 1995. Sulfate reducing and methane producing bacteria in aerobic wastewater treatment systems. *Water Research*, *29*(3), pp.871-880.

Lens, P.N., Vochten, P.M., Speleers, L. and Verstraete, W.H., 1994. Direct treatment of domestic wastewater by percolation over peat, bark and woodchips. *Water Research*, *28*(1), pp.17-26.

Leonard, A., 2011. Management of wastewater sludge's: a hot topic at the European level. *Journal of Residuals Science and Technology*, *8*(2).

Leonard, M., 2009. Biotransformation of sewage in a trickling filters. *Foundation for Research Science and Technology's Envirolink Scheme*, Client Report CSC 0906.

Lewandowski, GA., Filippi, LD., 1998. Biological treatment of hazardous waste. New York, (NY).

Leyva-Díaz, J.C., Muñío, M.M., González-López, J. and Poyatos, J.M., 2016. Anaerobic/anoxic/oxic configuration in hybrid moving bed biofilm reactor-membrane bioreactor for nutrient removal from municipal wastewater. *Ecological Engineering*, *91*, pp.449-458.

Llorens, E., Obradors, J., Alarcón-Herrera, M.T. and Poch, M., 2013. Modelling of arsenic retention in constructed wetlands. *Bioresource Technology*, *147*, pp.221-227.

Loupasaki, E. and Diamadopoulos, E., 2013. Attached growth systems for wastewater treatment in small and rural communities: a review. *Journal of Chemical Technology and Biotechnology*, *88*(2), pp.190-204.

Lu, C., Chang, K. and Hsu, S., 2004. A model for treating isopropyl alcohol and acetone mixtures in a trickle-bed air biofilter. *Process Biochemistry*, *39*(12), pp.1849-1858.

Lu, M.C., Lin, C.J., Liao, C.H., Huang, R.Y. and Ting, W.P., 2003. Dewatering of activated sludge by Fenton's reagent. *Advances in Environmental Research*, *7*(3), pp.667-670.

Lucas, F.S., Therial, C., Gonçalves, A., Servais, P., Rocher, V. and Mouchel, J.M., 2014. Variation of raw wastewater microbiological quality in dry and wet weather conditions. *Environmental Science and Pollution Research*, *21*(8), pp.5318-5328.

Lucena, F., Duran, A.E., Moron, A., Calderon, E., Campos, C., Gantzer, C., Skraber, S. and Jofre, J., 2004. Reduction of bacterial indicators and bacteriophages infecting faecal bacteria in primary and secondary wastewater treatments. *Journal of Applied Microbiology*, *97*(5), pp.1069-1076.

Luiz, A., Handelsman, T., Barton, G., Coster, H. and Kavanagh, J., 2015. Membrane treatment options for wastewater from cellulosic ethanol biorefineries. *Desalination and Water Treatment*, *53*(6), pp.1547-1558.

Luo, W., Yang, C., He, H., Zeng, G., Yan, S. and Cheng, Y., 2014. Novel two-stage vertical flow biofilter system for efficient treatment of decentralized domestic wastewater. *Ecological Engineering*, *64*, pp.415-423.

Luostarinen, S., Sanders, W., Kujawa-Roeleveld, K. and Zeeman, G., 2007. Effect of temperature on anaerobic treatment of black water in UASB-septic tank systems. *Bioresource Technology*, *98*(5), pp.980-986.

Mack, W.N., Mack, J.P. and Ackerson, A.O., 1975. Microbial film development in a trickling filter. *Microbial Ecology*, *2*(3), pp.215-226.

Madigan, M.T., Martinko, J.M. and Parker, J., 2000. Nutrition and metabolism. *Brock biology of microorganisms, 9th ed. Prentice-Hall, Upper Saddle River, NJ*, p.113.

Mahmoud, M., Tawfik, A. and El-Gohary, F., 2010. Simultaneous organic and nutrient removal in a naturally ventilated biotower treating presettled municipal wastewater. *Journal of Environmental Engineering*, *136*(3), pp.301-307.

Mahmoud, M., Tawfik, A. and El-Gohary, F., 2011. Use of down-flow hanging sponge (DHS) reactor as a promising post-treatment system for municipal wastewater. *Chemical Engineering Journal*, *168*(2), pp.535-543.

Makowska, M. and Spychała, M., 2014. Organic Compounds Fractionation for Domestic Wastewater Treatment Modeling. *Polish Journal of Environmental Studies*, *23*(1).

Manem, J., 1988. Interactions between heterotrophic and autotrophic bacteria in fixed-film biological processes used in drinking water treatment (Doctoral dissertation, University of Illinois at Urbana-Champaign).

Mann, A.T. and Stephenson, T., 1997. Modelling biological aerated filters for wastewater treatment. *Water Research*, *31*(10), pp.2443-2448.

Mann, A.T., Mendoza-Espinosa, L, Stephenson, T., 1998. A comparison of floating and sunken media biological aerated filters for nitriücation. *Journal of Chemical Technology and Biotechnology*, (72), pp.273-279.

Mara, D., 2004, September. To plant or not to plant? Questions on the role of plants in constructed wetlands. In *joint session of the Ninth IWA International Conference on Constructed Wetlands and the Sixth IWA International Conference on Waste Stabilization Ponds, Avignon, France*(Vol. 30).

Martin, I., Pidou, M., Soares, A., Judd, S. and Jefferson, B., 2011. Modelling the energy demands of aerobic and anaerobic membrane bioreactors for wastewater treatment. *Environmental Technology*, *32*(9), pp.921-932.

Martins, A.M., Pagilla, K., Heijnen, J.J. and van Loosdrecht, M.C., 2004. Filamentous bulking sludge-a critical review. *Water Research*, *38*(4), pp.793-817.

Massoud, M.A., Tarhini, A. and Nasr, J.A., 2009. Decentralized approaches to wastewater treatment and management: applicability in developing countries. *Journal of Environmental Management*, *90*(1), pp.652-659.

McGhee, T.J., 1991. Water Resources and Environmental Engineering. McGraw-Hill, New York.

Metcalf, E. and Eddy, H.P., 1991. Biological unit processes. *Wastewater Engineering: Treatment Disposal Reuse*, pp.411-467.

Metcalf, E.E. and Eddy, H.P., 2003. Wastewater engineer treatment disposal, reuse. New York: McGRaw.

Meuler, S., Paris, S. and Hackner, T., 2008. Membrane bio-reactors for decentralized wastewater treatment and reuse. *Water Science and Technology*, *58*(2), pp.285-294.

Miranda, A.F., Ramkumar, N., Andriotis, C., Höltkemeier, T., Yasmin, A., Rochfort, S., Wlodkowic, D., Morrison, P., Roddick, F., Spangenberg, G. and Lal, B., 2017. Applications of microalgal biofilms for wastewater treatment and bioenergy production. *Biotechnology for Biofuels*, *10*(1), p.120.

Montgomery, M.A. and Elimelech, M., 2007. Water and sanitation in developing countries: including health in the equation.

Morel, A., 2006. *Greywater management in low and middle-income countries* (No. 628.2 G842g). Dubenforf, CH: Swiss Federal Institute of Aquatic Science and Technology.

Morgenroth, E., Van Loosdrecht, M.C.M. and Wanner, O., 2000. Biofilm models for the practitioner. *Water Science and Technology*, *41*(4-5), pp.509-512.

Murtaza, G. and Zia, M.H., 2011. Individual's capacity development on the safe use of wastewater in agriculture in Pakistan. *Institute of Soil and Environmental Sciences*, University of Agriculture, Faisalabad.

Musazura, W., Odindo, A.O., Tesfamariam, E.H., Hughes, J.C. and Buckley, C.A., 2018. Decentralised wastewater treatment effluent fertigation: preliminary technical assessment. *Water SA*, *44*(2), pp.250-257.

Nanninga, T.A., Bisschops, I., López, E., Martínez-Ruiz, J.L., Murillo, D., Essl, L. and Starkl, M., 2012. Discussion on sustainable water technologies for peri-urban areas of Mexico City: Balancing urbanization and environmental conservation. *Water*, *4*(3), pp.739-758.

Naz, I., Hodgson, D., Smith, A., Marchesi, J., Ahmed, S., Avignone-Rossa, C. and Saroj, D.P., 2016. Effect of the chemical composition of filter media on the microbial community in wastewater biofilms at different temperatures. *RSC Advances*, *6*(106), pp.104345-104353.

Naz, I., Khan, Z., U., Ali, N. & Ahmed, S. 2013. Removal of pollution indicators from sewage by fixed film bioreactor and sand filter at low temperature. *Global Journal on Advances in Pure and Applied Sciences*, (1), pp. 259-270.

Naz, I., Saroj, D.P., Mumtaz, S., Ali, N. and Ahmed, S., 2015. Assessment of biological trickling filter systems with various packing materials for improved wastewater treatment. *Environmental Technology*, *36*(4), pp.424-434.

Naz, I., Ullah, W., Sehar, S., Rehman, A., Khan, Z.U., Ali, N. and Ahmed, S., 2016. Performance evaluation of stone-media pro-type pilot-scale trickling biofilter system for municipal wastewater treatment. *Desalination and Water Treatment, 57*(34), pp.15792-15805.

Norris, D.P., Parker, D.S., Daniels, M.L. and Owens, E.L., 1982. High-quality trickling filter effluent without tertiary treatment. *Journal of Water Pollution Control Federation*, pp.1087-1098.

Novotny, V., Ahern, J. and Brown, P., 2010. *Water centric sustainable communities: planning, retrofitting and building the next urban environment*. John Wiley & Sons.

Oliynyk, O.Y. and Kolpakova, O.A. 2014. Modelling and calculations of biological wastewater treatment at drip biofilters. *Environment Safety and Environmental Management,* (16), pp. 68-86.

Pal, S., Sarkar, U. and Dasgupta, D., 2010. Dynamic simulation of secondary treatment processes using trickling filters in a sewage treatment works in Howrah, west Bengal, India. *Desalination, 253*(1), pp.135-140.

Palmer, J., Flint, S. and Brooks, J., 2007. Bacterial cell attachment, the beginning of a biofilm. *Journal of Industrial Microbiology and Biotechnology, 34*(9), pp.577-588.

Park, H.D., Wells, G.F., Bae, H., Criddle, C.S. and Francis, C.A., 2006. Occurrence of ammonia-oxidizing archaea in wastewater treatment plant bioreactors. *Applied and Environmental Microbiology, 72*(8), pp.5643-5647.

Parker, D.S. and Bratby, J.R., 2001. Review of two decades of experience with TF/SC process. *Journal of Environmental Engineering, 127*(5), pp.380-387.

Parker, W.J., Shi, J., Fendinger, N.J., Monteith, H.D. and Chandra, G., 1999. Pilot plant study to assess the fate of two volatile methyl siloxane compounds during municipal wastewater treatment. *Environmental Toxicology and Chemistry*, *18*(2), pp.172-181.

Pedersen, K., 1990. Biofilm development on stainless steel and PVC surfaces in drinking water. *Water Research*, *24*(2), pp.239-243.

Perez, C. and Griebenow, K., 2001. Improved activity and stability of lysozyme at the water/CH2Cl2 interface: enzyme unfolding and aggregation and its prevention by polyols. *Journal of Pharmacy and Pharmacology*, *53*(9), pp.1217-1226.

Perujo, N., Romaní, A.M. and Sanchez-Vila, X., 2018. Bilayer infiltration system combines benefits from both coarse and fine sands promoting nutrient accumulation in sediments and increasing removal rates. *Environmental Science and Technology*, *52*(10), pp.5734-5743.

Pol, L.H. and Lettinga, G., 1986. New technologies for anaerobic wastewater treatment. *Water Science and Technology*, *18*(12), pp.41-53.

Prabu, G., Kawar, P.G., Pagariya, M.C. and Prasad, D.T., 2011. Identification of water deficit stress upregulated genes in sugarcane. *Plant Molecular Biology Reporter*, *29*(2), pp.291-304.

Pritchard, M., Mkandawire, T. and O'neill, J.G., 2007. Biological, chemical and physical drinking water quality from shallow wells in Malawi: Case study of Blantyre, Chiradzulu and Mulanje. *Physics and Chemistry of the Earth, Parts A/B/C*, *32*(15-18), pp.1167-1177.

Pulcini, E.D., 2001. Bacterial biofilms: a review of current research. *Nephrologie*, *22*(8), pp.439-441.

Pungrasmi, W., Chaisri, R., Malaphol, E. and Powtongsook, S., 2015. Efficiency of a hybrid solid digestion-denitrification column in suspended solid and nitrate removal from recirculating aquaculture system. *Environmental Engineering Research*, *20*(2), pp.175-180.

Purnell, S., Ebdon, J., Buck, A., Tupper, M. and Taylor, H., 2016. Removal of phages and viral pathogens in a full-scale MBR: implications for wastewater reuse and potable water. *Water Research*, *100*, pp.20-27.

Rajakumar, R., Meenambal, T., Banu, J.R. and Yeom, I.T., 2011. Treatment of poultry slaughterhouse wastewater in upflow anaerobic filter under low upflow velocity. *International Journal of Environmental Science and Technology*, *8*(1), pp.149-158.

Ramsay, J., Shin, M., Wong, S. and Goode, C., 2006. Amaranth decoloration by *Trametes* versicolor in a rotating biological contacting reactor. *Journal of Industrial Microbiology and Biotechnology*, *33*(9), p.791.

Rasool, T., Rehman, A., Naz, I., Ullah, R. and Ahmed, S., 2018. Efficiency of a locally designed pilot-scale trickling biofilter (TBF) system in natural environment for the treatment of domestic wastewater. *Environmental Technology*, *39*(10), pp.1295-1306.

Rehman, A., Naz, I., Khan, Z.U., Rafiq, M., Ali, N. and Ahmad, S., 2012. Sequential Application of Plastic Media-Trickling Filter and Sand Filter for Domestic Wastewater Treatment at Low Temperature Condition. *British Biotechnology Journal*, *2*(4), pp. 179-191.

Rene, E.R., Montes, M., Veiga, M.C. and Kennes, C., 2011. Styrene removal from polluted air in one and two-liquid phase biotrickling filter: steady and transient-state performance and pressure drop control. *Bioresource Technology*, *102*(13), pp.6791-6800.

Rittmann, B.E. and McCarty, P.L., 1980. Design of fixed-film processes with steady-state-biofilm model. *Progress in Water Technology*, *12*(6), pp.271-281.

Rittmann, B.E., Crawford, L., Tuck, C.K. and Namkung, E., 1986. In situ determination of kinetic parameters for biofilms: isolation and characterization of oligotrophic biofilms. *Biotechnology and Bioengineering*, *28*(11), pp.1753-1760.

Robinson, R.W., Akin, D.E., Nordstedt, R.A., Thomas, M.V. and Aldrich, H.C., 1984. Light and electron microscopic examinations of methane-producing biofilms from anaerobic fixed-bed reactors. *Applied and Environmental Microbiology*, *48*(1), pp.127-136.

Rodgers, M. and Zhan, X.M., 2003. Moving-medium biofilm reactors. *Reviews in Environmental Science and Biotechnology*, *2*(2), pp.213-224.

Rosen, M., Welander, T., Löfqvist, A. and Holmgren, J., 1998. Development of a new process for treatment of a pharmaceutical wastewater. *Water Science and Technology*, *37*(9), pp.251-258.

Rosenthal, H. and Otte, G., 1979. Adaptation of a recirculating fish culture system to salinity change. *Meeresforschung*, *27*(3).

Rowe, D.R. and Abdel-Magid, I.M., 1995. *Handbook of Wastewater Reclamation and Reuse*.

Ruiz-Espinoza, J.E., Méndez-Contreras, J.M., Alvarado-Lassman, A. and Martínez-Delgadillo, S.A., 2012. Effect of low temperature thermal pre-treatment on the solubilization of organic matter, pathogen inactivation and mesophilic anaerobic digestion of poultry sludge. *Journal of Environmental Science and Health, Part A*, *47*(12), pp.1795-1802.

Sa, C.S.A. and Boaventura, R.A.R., 2001. Biodegradation of phenol by Pseudomonas putida DSM 548 in a trickling bed reactor. *Biochemical Engineering Journal*, *9*(3), pp.211-219.

Sakuma, T., Jinsiriwanit, S., Hattori, T. and Deshusses, M.A., 2008. Removal of ammonia from contaminated air in a biotrickling filter–denitrifying bioreactor combination system. *Water Research*, *42*(17), pp.4507-4513.

Salma, S., Shah, M.A. and Rehman, S., 2012. Rainfall trends in different climate zones of Pakistan. *Pakistan Journal of Meteorology*, *9*(17).

Sawyer, C.N., Mc-Carty, P.L., Parkin, G.F., 1994. Chemistry for Environmental 542 Engineering, 4th ed. *McGraw- Hill Int. Editions*, 543

Schories, G., 2008. IWAPIL—innovative wastewater treatment applications for isolated locations. *Desalination*, *224*(1-3), pp.183-185.

Scott, R.L., Shuttleworth, W.J., Goodrich, D.C. and Maddock III, T., 2000. The water use of two dominant vegetation communities in a semiarid riparian ecosystem. *Agricultural and Forest Meteorology*, *105*(1), pp.241-256.

Seeger, H., 1999. The history of German waste water treatment. *European Water Management*, *2*, pp.51-56.

Servais, P., Garcia-Armisen, T., George, I. and Billen, G., 2007. Fecal bacteria in the rivers of the Seine drainage network (France): sources, fate and modelling. *Science of the Total Environment*, *375*(1-3), pp.152-167.

Shalaby, I.M., Altalhy, A.D. and Mosallam, H.A., 2008. Preliminary field study of a model plant for sewage water treatment using gravel bed hydroponics method. *World Applied Science Journal*, *4*(2), pp.238-243.

Shareefdeen, Z., Baltzis, B.C., Oh, Y.S. and Bartha, R., 1993. Biofiltration of methanol vapor. *Biotechnology and Bioengineering*, *41*(5), pp.512-524.

Shi, J., Podola, B. and Melkonian, M., 2007. Removal of nitrogen and phosphorus from wastewater using microalgae immobilized on twin layers: an experimental study. *Journal of Applied Phycology*, *19*(5), pp.417-423.

Shipin, O.V., Rose, P.D. and Meiring, P.G.J., 1999. Microbial processes underlying the PETRO concept (trickling filter variant). *Water Research*, *33*(7), pp.1645-1651.

Shuval, H.I., Yekutiel, P. and Fattal, B., 1986. An epidemiological model of the potential health risk associated with various pathogens in wastewater irrigation. *Water Science and Technology*, *18*(10), pp.191-198.

Singh, R., Paul, D. and Jain, R.K., 2006. Biofilms: implications in bioremediation. *Trends in Microbiology*, *14*(9), pp.389-397.

Skinner, S.J., Studer, L.J., Dixon, D.R., Hillis, P., Rees, C.A., Wall, R.C., Cavalida, R.G., Usher, S.P., Stickland, A.D. and Scales, P.J., 2015. Quantification of wastewater sludge dewatering. *Water Research*, *82*, pp.2-13.

Soller, J.A., Olivieri, A.W., Crook, J., Cooper, R.C., Tchobanoglous, G., Parkin, R.T., Spear, R.C. and Eisenberg, J.N., 2003. Risk-based approach to evaluate the public health benefit of additional wastewater treatment. *Environmental Science and Technology*, *37*(9), pp.1882-1891.

Soontarapa, K. and Srinapawong, N., 2001. Combined membrane-trickling filter wastewater treatment system. *Journal of Scientific Research Chulalongkorn University*, *26*(2), pp.59-70.

Souissi, M., Laabidi, R., Aissa, P., Pringault, O. and Said, O.B., 2018. Influence of Bizerte city wastewater treatment plant (WWTP) on abundance and antibioresistance of culturable heterotrophic and fecal indicator bacteria of Bizerte Lagoon (Tunisia). *Ecotoxicology and Environmental Safety*, *148*, pp.201-210.

Spath, R., Flemming, H.C. and Wuertz, S., 1998. Sorption properties of biofilms. *Water Science and Technology*, *37*(4-5), pp.207-210.

Spellman, F.R., 2000. *Safe work practices for wastewater treatment plants*. CRC Press.

Starkl, M., Anthony, J., Aymerich, E., Brunner, N., Chubilleau, C., Das, S., Ghangrekar, M.M., Kazmi, A.A., Philip, L. and Singh, A., 2018. Interpreting best available technologies more flexibly: A policy perspective for municipal wastewater management in India and other developing countries. *Environmental Impact Assessment Review*, *71*, pp.132-141.

Starkl, M., Parkinson, J., Narayanan, D. and Flamand, P., 2012. Small is beautiful but is large more economical? Fresh views on decentralized vs centralized wastewater management. *Water*, *21*, pp.45-47.

Steinmann, G., 1989. Sedimentation and coagulation processes in final settling tanks from trickling filters with suggestions for dimensioning. *Reports from Water Quality and Waste Management*, (88).

Stewart, P.S. and Franklin, M.J., 2008. Physiological heterogeneity in biofilms. *Nature Reviews Microbiology*, *6*(3), pp.199-210.

Stoodley, P., Sauer, K., Davies, D.G. and Costerton, J.W., 2002. Biofilms as complex differentiated communities. *Annual Reviews in Microbiology*, *56*(1), pp.187-209.

Sun, G., Gray, K.R., Biddlestone, A.J., Allen, S.J. and Cooper, D.J., 2003. Effect of effluent recirculation on the performance of a reed bed system treating agricultural wastewater. *Process Biochemistry*, *39*(3), pp.351-357.

Sutherland, I.W., 2001. The biofilm matrix–an immobilized but dynamic microbial environment. *Trends in Microbiology*, *9*(5), pp.222-227.

Szogi, A.A., Humenik, F.J., Rice, J.M. and Hunt, P.G., 1997. Swine wastewater treatment by media filtration. *Journal of Environmental Science and Health Part B*, *32*(5), pp.831-843.

Tang, C.Y., Yang, Z., Guo, H., Wen, J.J., Nghiem, L.D. and Cornelissen, E., 2018. Potable water reuse through advanced membrane technology. *Environmental Science and Technology*, 52(18), pp. 10215–10223.

Tawfik, A., Ohashi, A. and Harada, H., 2006. Sewage treatment in a combined up-flow anaerobic sludge blanket (UASB)–down-flow hanging sponge (DHS) system. *Biochemical Engineering Journal*, *29*(3), pp.210-219.

Tchobanoglous, G., Burton, F.L. and Stensel, H.D., 1991. Wastewater Engineering *Management*, *7*, pp.1-4.

Tebbutt, T.H.Y., 1998. Principles of Water Quality Control, 5th Edition.

Tee, P.F., Abdullah, M.O., Tan, I.A.W., Amin, M.A.M., Nolasco-Hipolito, C. and Bujang, K., 2016. Performance evaluation of a hybrid system for efficient palm oil mill effluent treatment via an air-cathode, tubular upflow microbial fuel cell coupled with a granular activated carbon adsorption. *Bioresource Technology*, *216*, pp.478-485.

US-EPA. 2003. National Primary Drinking Water Standards, United States Environmental Protection Agency, EPA 816-F-03-016, Washington, D.C.

USEPA. 2007. Bureau of water supply and wastewater management: department of environmental protection agency, wastewater treatment plant operator training. United State Environmental Protection Agency.

USEPA. 2012. Guidelines for Water Reuse. United State Environmental Protection Agency, Washington, DC.

Vartak, D.R., Engler, C.R., McFarland, M.J. and Ricke, S.C., 1997. Attached-film media performance in psychrophilic anaerobic treatment of dairy cattle wastewater. *Bioresource Technology*, *62*(3), pp.79-84.

Verma, M., Brar, S.K., Blais, J.F., Tyagi, R.D. and Surampalli, R.Y., 2006. Aerobic biofiltration processes—Advances in wastewater treatment. *Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management*, *10*(4), pp.264-276.

Viet Anh, N., Nga, P.T., Nhue, T.H. and Morel, A., 2005. Potential Decentralized Wastewater Management for Sustainable Development from Vietnamese Experience. *Proceedings of the Water Environment Federation*, *2005*(5), pp.917-946.

von Sperling, M. and de Lemos Chernicharo, C.A., 2005. Biological Wastewater Treatment in Warm Climate Regions Vol. 1 IWA publishing.

von Sperling, M., 1996. Comparison among the most frequently used systems for wastewater treatment in developing countries. *Water Science and Technology*, *33*(3), pp.59-72.

Vymazal, J., 2005. Horizontal sub-surface flow and hybrid constructed wetlands systems for wastewater treatment. *Ecological Engineering*, *25*(5), pp.478-490.

Wagner, M. and Loy, A., 2002. Bacterial community composition and function in sewage treatment systems. *Current Opinion in Biotechnology*, *13*(3), pp.218-227.

Wagner, M., Brumelis, D. and Gehr, R., 2002. Disinfection of wastewater by hydrogen peroxide or peracetic acid: development of procedures for measurement of residual disinfectant and application to a physicochemically treated municipal effluent. *Water Environment Research*, *74*(1), pp.33-50.

Wagner, M., Hutzler, P. and Amann, R., 1998. Three-dimensional analysis of complex microbial communities by combining confocal laser scanning microscopy and fluorescence in situ hybridization. *Digital image analysis of microbes. Wiley, Chichester*, pp.467-486.

Wang, H., Zhi, W., Deng, N. and Ji, G., 2017. Review on the Fate and Mechanism of Nitrogen Pollutant Removal from Wastewater Using a Biological Filter. *Polish Journal of Environmental Studies*, *26*(5).

Wang, J., Tai, Y., Man, Y., Wang, R., Feng, X., Yang, Y., Fan, J., Guo, J., Tao, R., Yang, Y. and Chen, Z., 2018. Capacity of various single-stage constructed wetlands to treat domestic sewage under optimal temperature in Guangzhou City, South China. *Ecological Engineering*, 115, pp. 35-44.

Wang, J.Z., Summers, R.S. and Miltner, R.J., 1995. Biofiltration performance: part 1, relationship to biomass. *Journal of the American Water Works Association*, *87*(12), pp.55-63.

Wang, S., 2014. Values of decentralized systems that avoid investments in idle capacity within the wastewater sector: A theoretical justification. *Journal of Environmental Management*, *136*, pp.68-75.

Wang, S., Boyjoo, Y., Choueib, A. and Zhu, Z.H., 2005. Removal of dyes from aqueous solution using fly ash and red mud. *Water Research*, *39*(1), pp.129-138.

Watanabe, K., Nakao, R., Fujishima, M., Tachibana, M., Shimizu, T. and Watarai, M., 2016. Ciliate Paramecium is a natural reservoir of Legionella pneumophila. *Scientific Reports*, *6*.

Waziri, M., Ogugbuaja, V.O. and Dimari, G.A. 2009. Heavy metal concentrations in Surface and groundwater samples from Gashua and Nguru areas of Yobe State-Nigeria. *International Journal of Science and Engineering*, *8*(1), pp. 58-63.

Westerman, P.W., Bicudo, J.R. and Kantardjieff, A., 2000. Upflow biological aerated filters for the treatment of flushed swine manure. *Bioresource Technology*, *74*(3), pp.181-190.

Wik, T., 1999. Rational transfer function models for nitrifying trickling filters. *Water Science and Technology*, *39*(4), pp.121-128.

Wilderer, P.A. and McSwain, B.S., 2004. The SBR and its biofilm application potentials. *Water Science and Technology*, *50*(10), pp.1-10.

Woldetsadik, D., Drechsel, P., Keraita, B., Itanna, F. and Gebrekidan, H., 2017. Heavy metal accumulation and health risk assessment in wastewater-irrigated urban vegetable farming sites of Addis Ababa, Ethiopia. *International Journal of Food Contamination*, *4*(1), p. 9.

World Health Organization (WHO). 1989. Health guidelines for the use of wastewater in agriculture and aquaculture: report of a WHO scientific group [meeting held in Geneva from 18 to 23 November 1987].

World Health Organization (WHO). 2001. Water Quality: Guidelines, Standards and Health Assessment of Risk and Risk Management for Water-related Infectious Diseases. Cited at http://www.who.int/water_sanitation_health/dwq/whoiwa/en/.

World Health Organization (WHO). 2006. *Guidelines for the safe use of wastewater, excreta and greywater* (Vol. 1). World Health Organization.

World Health Organization (WHO). 2015. *Progress on sanitation and drinking water: 2015 update and MDG assessment*. World Health Organization.

Yang, H. and Abbaspour, K.C., 2007. Analysis of wastewater reuse potential in Beijing. *Desalination*, *212*(1), pp.238-250.

Yang, L., Chou, L.S. and Shieh, W.K., 2001. Biofilter treatment of aquaculture water for reuse applications. *Water Research*, *35*(13), pp.3097-3108.

Zeng, R.J., Yuan, Z. and Keller, J., 2003. Enrichment of denitrifying glycogen-accumulating organisms in anaerobic/anoxic activated sludge system. *Biotechnology and Bioengineering*, *81*(4), pp.397-404.

Zhang, Y., Cheng, Y., Yang, C., Luo, W., Zeng, G. and Lu, L., 2015. Performance of system consisting of vertical flow trickling filter and horizontal flow multi-soil-layering reactor for treatment of rural wastewater. *Bioresource Technology*, *193*, pp.424-432.

Zhou, H. and Smith, D.W., 2002. Advanced technologies in water and wastewater treatment. *Journal of Environmental Engineering and Science*, *1*(4), pp.247-264.

Zhu, S., Deltour, J. and Wang, S., 1998. Modeling the thermal characteristics of greenhouse pond systems. *Aquacultural Engineering*, *18*(3), pp.201-217.

PUBLICATIONS

CONFERENCE PRESENTATIONS

LIST OF PUBLICATIONS

1) **Rehman, A.,** Ayub, N., Naz, I., Perveen, I., and Ahmed, S. (2018). Effects of Hydraulic Retention Time (HRT) on the Performance of Pilot Scale Trickling Filter System Treating Low-Strength Domestic Wastewater. *Accepted for publication in Polish Journal of Environmental Studies* (Impact Factor: 1.120).
2) **Rehman, A.,** Haris, M., Naz, I., and Ahmed, S. (2018). Assessment of an integrated media pilot scale trickling biofilter system with variable flow rates and hydraulic retention time (HRTs) towards treatment of domestic wastewater. *Accepted with minor revision in Environmental Technology* (Impact factor; 1.751).
3) **Rehman, A.,** Naz, I., Jones, J., Saroj, D.P., and Ahmed, S. (2018). Performance evaluation of a locally designed pilot scale trickling filter treating domestic wastewater under varying temperature conditions. *Accepted with revision and revision has been submitted in Water and Environment Journal* (Impact Factor: 1.224).
4) Rasool, T., **Rehman, A.,** Naz, I., Ullah, R. and Ahmed, S., (2018). Efficiency of a locally designed pilot-scale trickling biofilter (TBF) system in natural environment for the treatment of domestic wastewater. *Environmental technology*, 39(10), 1295-1306. (Impact factor; 1.751).
5) Naz, I., **Rehman, A.,** Sehar, S., Perveen, I. and Ahmed, S., (2017). Assessment of an integrated tire-derived rubber media-fixed biofilm reactor (TDR-FBR) and sand column filter (SCF) for wastewater treatment at low temperature. *Desalination and Water Treatment*, 99, 185-195. (Impact Factor: 1.631).
6) Naz, I., Ullah W., Sehar, S., **Rehman, A.,** Khan, Z. U., Ali, N., Ahmed, S., (2016). Performance evaluation of stone media pro-type pilot-scale trickling biofilter system for municipal wastewater treatment. Desalination and Water Treatment. 57: 15792–15805 (Impact Factor: 1.631).
7) Khan, U. Z., Naz, I., **Rehman, A.,** Rafiq, M., Ali, N., and Ahmed, S. (2015). Performance Efficiency of an Integrated Stone Media Fixed Biofilm Reactor and

Sand Filter for Sewage Treatment. Desalination and Water Treatment. 54 (10): 2638-2647 (Impact Factor: 1.631).

8) Irum, A., Mumtaz, S., **Rehman, A.,** Naz, I., and Ahmed, S. (2015). Treatment of Simulated Textile Wastewater Containing Reactive Azo Dyes Using Laboratory Scale Trickling Filter. World Academy of Science, Engineering and Technology. *International Journal of Chemical, Nuclear, Metallurgical and Materials Engineering*: 9 (1), 1-7.

9) **Rehman, A.,** Naz, I., Khan, Z. U., Rafiq, M., Ali, N., and Ahmad, S. (2012). Sequential Application of Plastic Media-Trickling Filter and Sand Filter for Domestic Wastewater Treatment at Low Temperature Condition. British Biotechnology Journal, 2(4): 179-191.

WORKSHOPS ATTENDED AND PAPER/POSTER PRESENTATION

1) One day International Seminar on Wastewater Treatment and its Reuse, May 16th 2012, Quaid-i-Azam University, Islamabad and achieved 1st prize in poster competition.
2) Appropriate and Advanced Wastewater Treatment Technologies: Workshop. January 30th 2014, Department of Environmental Engineering, George Washington University, Washington DC, USA.
3) One day Seminar on Constructed wetland: A cost Effective Solution for Wastewater Treatment (June 12, 2014), Quaid-i-Azam University, Islamabad.
4) Poster competition and Tree plantation Day, June 5, 2014 on world Environmental day, Department of Microbiology , Quaid-i-Azam University, Islamabad.
5) As Presenter in 3rd Abasyn International Conference on Technology and Business Management (AiCTBM), March 28-29, 2018.