

**ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF
SCIENCE ENGINEERING AND TECHNOLOGY**

**MICROBIAL COMMUNITY MONITORING OF LANDFILL LEACHATE
TREATING SYSTEM AND IMPROVEMENT EFFORTS**

M.Sc. THESIS

Süleyman SOYEL

Department of Environmental Engineering

Environmental Biotechnology Programme

June 2019

ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF
SCIENCE ENGINEERING AND TECHNOLOGY

**MICROBIAL COMMUNITY MONITORING OF LANDFILL LEACHATE
TREATING SYSTEM AND IMPROVEMENT EFFORTS**

M.Sc. THESIS

**Süleyman SOYEL
(501141810)**

Department of Environmental Engineering

Environmental Biotechnology Programme

Thesis Advisor: Prof. Dr. Süleyman ÖVEZ

June 2019

Süleyman Soyel, a M.Sc. student of ITU Institute of Science and Technology student ID 501141810, successfully defended the thesis entitled “MICROBIAL COMMUNITY MONITORING OF LANDFILL LEACHATE TREATING SYSTEM AND IMPROVEMENT EFFORTS”, which he prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

Thesis Advisor: **Prof. Dr. Süleyman ÖVEZ**
Istanbul Technical University

Jury Members: **Prof. Dr. İsmail KOYUNCU**
Istanbul Technical University

Prof. Dr. Bestami ÖZKAYA
Yıldız Technical University

Date of Submission : 03 May 2019
Date of Defense : 11 June 2019



FOREWORD

I would like to thank my advisor Prof. Dr. Süleyman Övez for giving me valuable advice and support always when needed. I also would like to thank my friends Ayşe Güventürk, Yıldız Erdener, Sama Ali, Mustafa N. Al-mezory, Ayça Başar and Hande Ermiş who have always been there for support and sharing their knowledge with me.

I hope that experience and practice I have gained during the thesis, help me develop and improve conduct of future research in environment biotechnology.

June 2019

Süleyman Soyel
(Environmental Engineer)



TABLE OF CONTENTS

	<u>Page</u>
FOREWORD	v
ABBREVIATIONS	xi
LIST OF TABLES	xiii
LIST OF FIGURES	xv
SUMMARY	xix
ÖZET	xxi
1. INTRODUCTION	1
1.1 Purpose of Thesis	2
1.2 Layout of Thesis	2
1.3 Literature Review	3
1.3.1 Landfills	3
1.3.2 Landfill leachate.....	4
1.3.3 Landfill leachate discharge standards	7
1.3.4 Treatment options of leachate	8
1.3.5 Physical/Chemical treatment methods	10
1.3.5.1 Coagulation – flocculation.....	11
1.3.5.2 Chemical precipitation.....	11
1.3.5.3 Flotation.....	11
1.3.5.4 Activated carbon adsorption	11
1.3.5.5 Ammonia stripping	12
1.3.5.6 Membrane filtration	12
1.3.5.7 Treatment performance of physical/chemical methods	14
1.3.6 Biological treatment methods	15
1.3.7 Activated sludge, AS	16
1.3.8 Sequencing batch reactors, SBR.....	17
1.3.9 Nitrification-denitrification.....	18
1.3.10 Aerated lagoon	19
1.3.11 Up-flow anaerobic sludge blanket, UASB.....	19
1.3.12 Treatment performance of biological methods	20
1.4 Leachate and Activated Sludge Microbial Community	22
1.4.1 Bacteria & Archaea.....	22
1.4.2 Protozoa	24
1.4.2.1 Ciliates	24
1.4.2.2 Flagellates	25
1.4.2.3 Amoeba.....	25
1.4.3 Metazoa.....	26
1.4.3.1 Rotifers	26
1.4.3.2 Tardigrades	26
1.4.3.3 Worms.....	26
1.4.4 Fungi and algae	26
1.5 Description of Landfill Leachate Treatment Facility	27
1.6 Common Microbiological Problems in Activated Sludge Systems	30

1.6.1	Poor floc formation, pin floc and dispersed growth problems.	32
1.6.2	Toxicity of landfill leachate	33
1.6.3	Nitrification and denitrification problems	34
1.6.4	Nutrient deficiency and bulking & foaming	35
1.6.5	Filamentous bulking.....	35
1.7	State of Landfill Leachate Treatment Facility.....	37
1.7.1	Low COD, phosphate concentration and F/M ratio.....	37
1.7.2	High Ammonia Nitrogen, $\text{NH}_4^+\text{-N}$, concentration.....	38
1.7.3	Filamentous bulking and foaming	38
1.7.4	Poor sludge settling quality.....	39
1.8	Mathematical modelling study	39
2.	MATERIALS AND METHODS.....	41
2.1	Materials.....	41
2.1.1	Leachate sample.....	41
2.1.2	Laboratory equipment.....	41
2.1.3	Reagents and preparation.....	42
2.1.3.1	Gram staining	43
2.1.3.2	Neisser staining.....	43
2.2	Methods.....	43
2.2.1	Smear slide preparation.....	44
2.2.2	Gram staining procedure.....	44
2.2.3	Neisser staining procedure.....	45
2.3	Analytical Methods	46
2.3.1	Sludge volume index (SVI)	46
2.3.2	Microscopic examination methods	46
2.3.3	Filament counting	47
2.3.4	Floc characteristics and overall filament abundance	47
2.3.4.1	Floc size	47
2.3.4.2	Floc characteristics	48
2.3.4.3	Protozoa and other microorganisms	48
2.3.4.4	Bacterial colonies	49
2.3.4.5	Effects of filamentous organisms on floc structure	50
2.3.4.6	Filament abundance.....	51
2.3.5	Filamentous organism characteristics	53
2.3.5.1	Branching.....	53
2.3.5.2	Motility	54
2.3.5.3	Filament shape	54
2.3.5.4	Location	54
2.3.5.5	Sheath	54
2.3.5.6	Filament width.....	54
2.3.5.7	Filament length	54
2.3.5.8	Cell shape	55
2.3.5.9	Staining reactions	55
2.3.6	Filamentous organism identification.....	56
3.	RESULTS AND DISCUSSION.....	59
3.1	Main Parameters.....	59
3.1.1	Chemical oxygen demand 2013-2018	59
3.1.2	Orthophosphate 2013-2018.....	61
3.1.3	Total nitrogen 2013-2018	63
3.1.4	Ammonia nitrogen 2013-2018.....	65

3.1.5	pH and temperature data	68
3.1.6	SVI and MLSS	72
3.2	Filament Count.....	77
3.3	Filamentous Organism Identification.....	84
3.3.1	Filament 1- <i>Haliscomenobacter hydrossis</i>	86
3.3.2	Filament 2- <i>Microthrix parvicella</i>	87
3.3.3	Filament 3- Type 021N.....	88
3.4	Floc Size Count and Characteristics.....	90
3.5	Micrographs of Aerobic and Anoxic Tanks.....	97
3.5.1	2 nd month, aerobic tank	97
3.5.2	2 nd month, anoxic tank	99
3.5.3	6 th month, aerobic tank.....	101
3.5.4	6 th month, anoxic tank.....	104
3.5.5	10 th month, aerobic tank.....	106
3.5.6	10 th month, Anoxic Tank	108
4.	DISCUSSION.....	111
4.1	Landfill Leachate Parameter Comparison of 2013-2018 and Fluctuations in The Influent.....	111
4.2	Filament Count, Identification and Correlation	116
4.3	Filament Abundance, Floc Morphology and Microbial Diversity	120
5.	CONCLUSION AND RECOMMENDATIONS.....	125
5.1	System Improvement Suggestions	125
	REFERENCES.....	127



ABBREVIATIONS

AS	: Activated Sludge: AS
BOD	: Biochemical Oxygen Demand
COD	: Chemical Oxygen Demand
EPS	: Extracellular Polymeric Substance
MF	: Micro Filtration
MLSS	: Mixed Liqueur Suspended Solids
NF	: Nano Filtration
NH₄⁺-N	: Ammonia nitrogen
P	: Phosphate
PO₄-P	: Orthophosphate
RO	: Reverse Osmosis
SBR	: Sequencing Batch Reactor
SS	: Suspended Solids
SVI	: Suspended Volume Index
T	: Temperature
TN	: Total Nitrogen
TOC	: Total Organic Carbon
UASB	: Up-flow Anaerobic Sludge Bed
UF	: Ultra Filtration
FC	: Filament Count
VFA	: Volatile Fatty Acid



LIST OF TABLES

	<u>Page</u>
Table 1.1: Typical composition of landfill leachate parameters.	6
Table 1.2: Main groups of landfills.	7
Table 1.3: Discharge limit of leachate parameter concentrations in Germany and Turkey.	8
Table 1.4: Main objectives to be treated in leachate.	9
Table 1.5: Comparison of physical/chemical treatments on COD and NH_4^+ - N removal efficiency	15
Table 1.6: Performance of outstanding biological methods.	21
Table 1.7: Most commonly observed activated sludge microorganism groups.	24
Table 1.8: Treatment facility landfill leachate.	28
Table 1.9: Causes and effects of microbially-related activated sludge solids separation problems.	31
Table 2.1: Laboratory equipment.	41
Table 2.2: Reagents.	42
Table 2.3: Subjective scoring of filament abundance.	52
Table 3.1: Compared min., max., mean and std values of leachate parameters of 2013-2018.	67
Table 3.2: Min., max., mean and std values of pH and temperature in Inflow, aerobic and anoxic tanks.	72
Table 3.3: Min., max., mean and std data of 2018 MLSS data for aerobic and anoxic tanks.	74
Table 3.4: Correlation guideline.	83
Table 3.5: Pearson correlation analysis of Filament 1,2 and 3 in aerobic and anoxic tanks with parameters.	84
Table 3.6: Filamentous organism characteristics and Identifications.	85
Table 3.7: Pearson correlation analysis of floc size in aerobic and anoxic tanks with parameters.	94
Table 3.8: Filament abundance, floc characteristics and microbial diversity of aerobic and anoxic tanks.	96
Table 4.1: Input, aerobic and anoxic tank parameter data of 10th month...	118



LIST OF FIGURES

	<u>Page</u>
Figure 1.1: General Schematic of a waste deposited landfill site.	4
Figure 1.2: The membrane filtration spectrum for water and wastewater treatment	13
Figure 1.3: Schematic graph of activated sludge.	16
Figure 1.4: Process cycle of an aerobic SBR system.	17
Figure 1.5: UASB reactor.	20
Figure 1.6: Landfill leachate treatment facility.	27
Figure 1.7: Studied landfill leachate treatment facility flow diagram.	29
Figure 1.8: Phase contrast micrographs of floc characteristics: (a) compact, (b) diffuse, (c) firm, (d) weak. Original magnifications 100x, (a) and (b); 1000x, (c) and (d).	33
Figure 1.9: Phase contrast micrographs of effects of filamentous organisms on floc morphology and settleability: (a) and (b) interfloc bridging; (c) and (d) open floc structure, original magnification 100x.	36
Figure 1.10: Different F/M ratio and effects on activated sludge process.	38
Figure 2.1: Phase contrast micrographs of floc characteristics: (a) compact, (b) diffuse, (c) firm, (d) weak. (Original magnifications (Original magnifications 100x, (a) and (b); 1000x, (c) and (d)	48
Figure 2.2: Phase contrast micrographs of common flagellates and amoebae found in activated sludge: (a) Monas sp., (b) Bodo sp., (c) Polychaos sp., and (d) Arcella sp. Original magnifications 1000x, (a) and (b); 400x, (c), and (d).	49
Figure 2.3: Phase contrast micrographs of zoogloaeas: (a) and (b) fingered, (c) and (d) amorphous (globular). (Original magnifications 200, (a); 1000x, (b); 100x, (c); and 1000x (d).	50
Figure 2.4: Phase contrast micrographs of effects of filamentous organisms on floc structure: (a) and (b) interfloc bridging; (c) and (d) diffuse floc structure. Original magnification 100x.	51
Figure 2.5: Phase contrast micrographs of filament abundance categories using the subjective scoring system: (a) few, (b) some, (c) common, (d) very common, (e) abundant, and (f) excessive. Original magnification 100x.	53
Figure 2.6: Phase contrast micrographs of filamentous organism cell shapes: (a) square, (b) rectangular, (c) oval, (d) barrel, (e) discoid, (f) sausage, and (g) irregular. Original magnification 1000x.	55
Figure 2.7: Dichotomous key for identification of filamentous organism identification.	57
Figure 3.1: COD data comparison of 2013-2018.	60
Figure 3.2: Orthophosphate data comparison of 2013-2018.	62
Figure 3.3: Total nitrogen data comparison of 2013-2018.	64

Figure 3.4: Ammonia nitrogen data comparison of 2013-2018.....	66
Figure 3.5: Observed pH values of inflow and aerobic tank during 2018....	69
Figure 3.6: Temperature measurement of inflow, aerobic and anoxic tanks in 2018.	71
Figure 3.7: Aerobic and anoxic tank MLSS data of 2018.	73
Figure 3.8: Calculated SVI values of aerobic and anoxic tanks in 2018 from obtained samples.	75
Figure 3.9: SVI test results of aerobic and anoxic tanks; A and B, anoxic and aerobic tank samples of 06.09.2018, respectively; C and D, anoxic and aerobic tank samples of 07.11.2018, respectively. ..	76
Figure 3.10: Filament count of aerobic tank 2018.....	78
Figure 3.11: Filament count of anoxic tank 2018.....	80
Figure 3.12: Total filament count of aerobic and anoxic tanks 2018.	82
Figure 3.13: Micrographs of <i>Haliscomenobacter hydrossis</i> , Filament 1, found in activated sludge samples; gram staining-Negative, red/orange colour (original magnification 1000x).	86
Figure 3.14: Micrograph of <i>Haliscomenobacter hydrossis</i> , Filament 1, found in activated sludge samples; Neisser staining-Negative, light brown colour (original magnification 1000x).	87
Figure 3.15: Micrograph of <i>Microthrix parvicella</i> , Filament 2, found in activated sludge samples; gram staining-positive, navy blue (original magnification 1000x).....	88
Figure 3.16: Micrograph of <i>Microthrix parvicella</i> , Filament 2, found in activated sludge samples; Neisser staining-positive, poly-p granules green, outside light brown colour, (original magnification 1000x).	88
Figure 3.17: Micrograph of Type 021N, Filament 3, found in activated sludge samples; gram staining-negative, red/orange colour (original magnification 1000x).....	89
Figure 3.18: Micrographs of Type 021N, Filament 3, found in activated sludge samples; Neisser staining-negative, light brown colour (original magnification 1000x).....	90
Figure 3.19: Floc size data of aerobic tank.....	91
Figure 3.20: Floc size data of anoxic tank.....	93
Figure 3.21: Micrographs of anoxic tank samples; 1, 2, 3 are wet mount taken at original magnification 100x, 200x, 400x respectively and 4 is gram staining taken at original magnification 1000x with oil immersion technique	97
Figure 3.22: Micrographs of anoxic tank samples; 1, 2, 3 are wet mount taken at original magnification 100x, 200x, 400x respectively and 4 is gram staining taken at original magnification 1000x with oil immersion technique.	99
Figure 3.23: Micrographs of anoxic tank samples; 1, 2, 3 are wet mount taken at original magnification 100x, 200x, 400x respectively; 4 and 5 are gram staining taken at original magnification 1000x with oil immersion technique. <i>Actinomycetes spp.</i> is observed in microphotograph number 4.	101
Figure 3.24: Micrographs of anoxic tank samples; 1, 2, 3 are wet mount taken at original magnification 100x, 200x, 400x respectively	

and 4 is gram staining taken at original magnification 1000x with oil immersion technique.	104
Figure 3.25: Micrographs of anoxic tank samples; 1, 2, 3 are wet mount taken at original magnification 100x, 200x, 400x respectively and 4 is gram staining taken at original magnification 1000x with oil immersion technique.	106
Figure 3.26: Micrographs of anoxic tank samples; 1, 2, 3 are wet mount taken at original magnification 100x, 200x, 400x respectively and 4 is gram staining taken at original magnification 1000x with oil immersion technique.	108
Figure 4.1: 2018 rainfall and temperature data of Istanbul.....	114
Figure 4.2: SVI versus MLSS concentration graph of different treatment plant.	115
Figure 4.3: Effect of the sludge load on the occurrence of protozoa and metazoa.	122
Figure 4.4: Activated sludge microbial diversity predominance according to SVI, F:M and sludge age	123



MICROBIAL COMMUNITY MONITORING OF LANDFILL LEACHATE TREATING SYSTEM AND IMPROVEMENT EFFORTS

SUMMARY

The growing human population and their daily life needs have resulted in increased solid waste generation. Landfilling is the most conventional in solid waste handling however different kind of hazards are released into the environment, such as gas and leachate. Activated Sludge (AS) systems together with aerobic and anoxic (nitrification) units are commonly practiced in landfill leachate treatment. The treatment plant aims to treat 1.700 m³/day leachate with 80% efficiency. Landfill leachate entering the facility has low COD and phosphate and high ammonia nitrogen and MLSS levels. High ammonia nitrogen and MLSS input, bulking & foaming, low settling of flocs which causes solid-liquid separation problems and unstable inflow of nutrients are some of the problems faced at the facility. As a result of increased particle size and round compact flocs, it is expected to solve solid-liquid separation and bulking & foaming problems. Filament score of 6 regarding to Jenkins et al 2004 Filament abundance scoring system has been observed throughout the year, except 10th month where filament abundance reduced to aimed levels in both aerobic and anoxic tanks. Common morphology of flocs remained diffuse and weak with slight improvements where diversity of microbial community was below expected levels, poor to medium. The most abundant filamentous organisms' species were identified as *Haliscomenobacter hydrossis*, *Microthrix parvicella* and Type 021N. In monitoring study, eukaryotic microorganisms, protozoa such as *Paramecium* spp., *Euplotes* spp., *Aspidisca* spp. were also observed in low numbers and slightly more common after the operational changes made. In parallel to microscopic monitoring and examination of the system, the molecular analyses of the aerobic and anoxic treatment units have been sampled and analysed for further evaluation and improvement of the facility performance. It is suggested to perform a 16s/18s rRNA analysis on the problem causing filaments for better understanding of the system and in order to take actions in the name of improvement of the facility.



KATI ATIK DEPOLAMA SAHASININ SIZINTI SUYU ARITMA SİSTEMİNDEKİ MİKROBİYAL TOPLULUK İZLEMESİ VE İYİLEŞTİRME ÇALIŞMALARI

ÖZET

Dünya da hızlıca artan insan popülasyonu ve günlük hayatlarındaki ihtiyaçların giderek artması sonucunda katı atık üretimi artmıştır. Katı atık depolama sahaları, katı atıklarla başa çıkma konusunda en geleneksel yöntem olmakla beraber doğaya karşı farklı zararları vardır. En bilinen zararları arasında atmosfere gaz salımı ve yeraltı sularına sızıntı suyu karışmasıdır. Katı atık depolama sahası sızıntı suları genellikle yüksek kimyasal ve biyolojik oksijen ihtiyacı (KOİ ve BOİ) amonyak ve total nitrojen, (NH_4^+ -N ve TN), pH ve çeşitli ağır metalleri barındırır. Bu parametreler katı atık depolama sahasının yaşına göre değişimler göstermektedir. Sızıntı suyu arıtmasında fiziksel/kimyasal ve/veya biyolojik arıtma sistemleri kullanılmaktadır. Fiziksel/kimyasal arıtma metotlarından bazıları; koagülasyon/flokülasyon, kimyasal çökertme, amonyak uçurma, aktif karbon Emilimi ve membran filtrasyonu metodlarıdır. Biyolojik sistemler ise ardışık kesikli reaktörler, havalı lagünler, nitrifikasyon ve denitrifikasyon ve aktif çamur gibi sistemleri içerir. Sızıntı suyu arıtma sistemleri genellikle hibrid sistemlerden oluşup hem fiziksel/kimyasal metotları hem de biyolojik sistemleri içerir. Örnek olarak aerobik ve anoksik (nitrifikasyon-denitrifikasyon) üniteleri bulunan aktif çamur sistemleri sızıntı suyu arıtmasında en yaygın kullanılan uygulamadır. Sızıntı suyu içerisinde ki mikrobiyal yaşam bakteri ve arkeya, protozoa, metazoa, virus, mantar ve alg, rotifer ve kurtlardan oluşan geniş bir ekosisteme sahiptir.

İncelemelerin yapıldığı arıtma tesisi günde 1700 m³/gün sızıntı suyunu %80 verimlilikle arıtmayı planlamaktadır. Arıtma tesisinin depolama sahası orta yaşlı, ~10 sene, olup sızıntı suyunun parametreleri referans değerleriyle örtüşmektedir. Arıtma tesisinde, hibrid aktif çamur, nitrifikasyon/denitrifikasyon ve membran filtrasyonu sistemi kullanılmaktadır. Sistem içinde karşılaşılan problemlerin bazıları; düşük KOİ ve fosfat, yüksek amonyak nitrojeni ve partikül girişi, hacim artışı ve köpüklenme ve çamurun çökmemesidir. Bu problemlere bağlı olarak sistem verimi istediği seviyede seyir etmeyip, mekanik arızalara da sebep olarak maliyeti yükseltmektedir. Bu tezin amacı sızıntı suyu arıtma tesisindeki problemleri hem mikrobiyal topluluğun gözlemlenmesi hem de modelleme çalışmasıyla beraber çeşitli parametrelerde değişimler sağlayarak sistemi iyileştirmektir. Yapılan çalışmalarla iyileştirilen sistemde artırılan partikül boyutları ile kompakt ve yuvarlak flok oluşumları sayesinde köpüklenme ve katı-sıvı ayırma problemlerinin giderileceği düşünülmektedir.

Katı atık depolama sahası sızıntı suyu numuneleri düzenli olarak her 14 günde bir alınmış ve İstanbul Teknik Üniversitesi çevre mühendisliği mikrobiyoloji laboratuvarına incelenmek üzere getirilmiştir. Numuneler, aerobik ve anoxic tanklardan 2 metre derinliğinden alınıp, 1 litrelik hava geçirmez kaplarda incelenene kadar saklanmıştır. KOİ, amonyak nitrojeni, TN, pH, sıcaklık, fosfat ve askıda katı madde gibi parametreler artıma tesisinde ölçülmekle beraber, çamur hacim indeksi ile mikroskopik incelemeler İTÜ çevre mühendisliği mikrobiyoloji laboratuvarında yapılmıştır. Sızıntı suyunda yapılan mikroskopik incelemelerde

Olympus BX50 Model marka mikroskopa bağlı Kameram21 CMOS kamerası numunelerin mikro fotoğraflarını çekmek üzere kullanılmıştır. Filament tanımlaması için sızıntı suyu numuneleri 0.5 µl hacminde önce lam üzerine damlatılarak kurumaya bırakılmış daha sonra Gram ve Neisser boyamaya tabi tutulmuştur. Boya kuruduktan sonra 40x, 100x, 200x, 400x ve 1000x (yağlı immersiyon) büyültmelerinde incelenmiştir. Flok morfolojisi ve diğer mikrobiyal yaşam formlarını incelemek adına, sızıntı suyundan alınan 0.5 µl hacim yin lam üzerine damlatılmış ve kurumadan lamel ile kapatılarak, 40x, 100x 200x ve 400x büyültmeleri altında incelenmiştir.

Yapılan analizlerde 2013-2018 yılları arasında katı atık depolama sahası sızıntı suyu parametrelerinde ki değişimler gözlemlenmiştir. KOİ ve fosfat seviyesi yıllar içinde azalma gösterirken, amonyak nitrojeni ve TN giderek artmıştır. 2018 yılı içerisinde gözlemlenen KOİ, fosfat, amonyak nitrojeni, TN, pH, sıcaklık, çamur hacim indeksi ve askıda katı madde parametrelerinin düzensiz bir biçimde alçalarak veya artarak sisteme girdikleri tespit edilmiştir. Mikroskopik incelemeler sonucunda sistemdeki filament taşkınlığı, 10'uncu ay dışında, referans olarak alınan Jenkins filament skor skalasında en yüksek skor olan 6'ncı seviyede seyrettiği görülmüştür. Buna ek olarak flok morfolojisi istenilenin aksine genel olarak zayıf ve dağınık, mikrobiyal topluluk zayıf ve orta olarak tespit edilmiştir. Aerobik ve anoksik tanklardaki sızıntı suyu numunelerinde yoğun olarak üç adet filament gözlemlenmiş olup, filament 1; *Haliscomenobacter hydrossis*, filament 2; *Microthrix parvicella* ve filament 3; Type 021N olarak tanımlanmıştır. Mikroskopik incelemeler sırasında yaygın olarak görülen diğer organizmalar arasında protozoa'ya ait *Paramecium* spp., *Euplotes* spp. ve *Aspidisca* spp görülmüştür.

Analiz ve gözlem sonuçlarının ışığı altında sisteme giren dağınık ve değişken parametrelerin mikrobiyal çeşitliliği, flok yapısını ve dolayısıyla arıtma verimini düşürdüğü, düşünülmektedir. Modelleme çalışmasının da yardımıyla sisteme giren KOİ ve fosfat seviyesinin istenilen mikro organizmaların büyümesinde yetersiz olduğu ve ek olarak yüksek amonyak nitrojeninin sistem içerisinde toksisite yarattığı muhtemeldir. Askıda katı madde miktarının normalden yüksek ve sisteme sabit bir seviyede giriş yapmaması, çamur hacim indeksini yüksek seviyede tutmaktadır. Bunun sonucu olarak membran filtrasyon sisteminde tıkanıklık arızaları, çıkış suyunda bulanıklık ve diğer mekanik problemler yıl boyunca gözlemlenmiştir. Filament sayısında ki artış sebebiyle meydana gelen köpük sorunu da sistemi olumsuz olarak etkilemektedir. Gerek KOİ ve fosfata filamentlerin, flok yapıcı organizmalardan daha kolay ulaşabilmesi, gerekse çabuk değişen mikrobiyal çevreye daha toleranslı olmaları, özellikle pH ve sıcaklık değişimleri, üremelerine elveriş sağlayıp köpüklenme problemini ortaya çıkarmıştır. Modelleme çalışmasıyla beraber yapılan çamur yaşı, çamur atımı, geri besleme

süreleri ve oranlarında yapılan değişiklikler ile düzeltilmesi amaçlanan sistem sadece 10'uncu ayda arzu edilen filament skoru 5, aerobik tankı ve 4'e, anoksik tankı, indirilebilmiştir. Fakat filament skorunda elde edilen bu sonucun tamamıyla yapılan bu değişimler sonucunda kaynaklandı sonucuna varılmamalıdır. Bunun sebebi ise hale hazırda çalışmaya devam eden arıtma sisteminde gündelik veya aylık olarak meydana gelen mekanik arıza, inşaat başlangıcı, farklı kaynaktan sızıntı suyu aktarımı ve sabitlenemeyen parametre girişleridir.

Katı atık depolama sahası sızıntı suyu arıtma tesisindeki problemlerin giderilmesi için numune sıklığının artırılması, 16s/18s rRNA analizi ve daha sistematik bir şekilde sisteme kimyasal eklenmesi önerilmiştir. Mikroskobik analizlerde numune sıklığının 14 günde bir olması bazı değişimlerin gözden kaçabileceği ve ani parametre yüklemelerinde mikrobiyal topluluğun nasıl etkileneceği olumsuz etkilemektedir. 14 gün yerine, her 7 günde bir alınan numuneler düzenli olarak laboratuvarında incelenmeli ve imkanlar dahilinde petri kaplarına ekilip gözlemlenmelidir. Diğer bir öneri olan 16s/18s rRNA analizi ise filamentlerin daha etkin bir şekilde tanımlana bilmesi adına büyük önlem taşımaktadır. Gözlemler sonucu bahsedilen filament tanımlamasından emin olmak ve bu türlerin ırk özelliklerini bilmek, uygulanacak karşı stratejilerde mutlak rol oynamaktadır. Son olarak, işletme düzeninde ki pH, KOİ, vs gibi parametre değişimlerinde uygulanan düzensiz ve ani sabitleme çalışmalarının, daha sistematik, yaygın ve sisteme kimyasal çok oluşturmayacak şekilde yapılması önerilmektedir. Ani eklenen yüksek miktarlardaki kimyasalların, hâlihazırda var olan sızıntı suyu toksisitesine ek olarak mikrobiyal topluluğu olumsuz yönde etkileyebileceği bilinmektedir.

Netice olarak katı atık depolama sahası sızıntı suyu arıtma tesisinde istenilen filament skoruna ulaşamamış, flok morfolojisinde ve mikrobiyal topluluk çeşitliliğinde kısmen de olsa bir iyileşme gözlemlenmiştir. Yapılan öneriler doğrultusunda çalışmanın devam etmesi arıtma sisteminde ki problemlerin giderileceği ve iyileştirileceği kanaatindedir.



1. INTRODUCTION

Every year human population that inhabits the earth is increasing and so their needs to maintain their daily lives. The amount of consumption whether by a single person or an industry is generating way more waste than our habitat can sustain. Solid waste generation is one of the most important problem that most countries are facing and although there are several options to properly maintain it, none provides a hundred percent solution. The solid waste generation is directly correlated with the income level and urbanisation, meaning increased incomes allows higher living standards with more consumption of goods and services, resulting in far more waste generation. According to worldpopulationdata.org 2017 “worldwide population was approximately 7.5 billion people which is expected to reach 9.8 billion in 2050 and 1.2 kg of Municipal Solid Waste (MSW) is generated per person”. MSW generation levels are expected to increase to 2.2 billion tonnes per year by 2025, showing a significant increase in the next fifteen years [1]. Therefore, it is crucial to reduce the amount of waste produced, recycle more and reuse as much as possible.

As well as huge amounts of waste generation, improper management of generated solid waste has also resulted in grave ecological, environmental and health obstacle for us to yet overcome. Landfilling is adopted by most countries as it is the most conventional and economic method to deal with the solid waste. Although landfill proves to be the best option in solid waste handling, the landfill sites release different kind of hazards into the environment, such as gas and leachate, if not managed properly.

1.1 Purpose of Thesis

Purpose of this thesis is to monitor microbial community of landfill leachate treating system working with Activated Sludge (AS) and while providing problem solution and improvement. Leachate is a strong wastewater and has highly toxic character. Therefore, treatment of leachate is a must before discharging into either farmlands or water bodies, if not is a great threat to all living organisms. Thus, the treatment systems are modified to reduce the carbon amount, reduce toxicity and save available nutrients. In this study, it is also proposed to observe the effect of operational parameters on microorganisms, filaments and floc structure, aid favourable microorganism growth and finally to improve and increase effluent quality of the landfill leachate treating system.

For these purposes, samples from the landfill leachate treating system have been observed under a photographic microscope together with a computer software. These observations were used to understand the floc type, size and structure, as well as the number and type of microorganism inhabiting the system. Furthermore, filament size, structure and amount present in the system were also examined. Kameram digital mikroskopi software was used to do manual counting, measurements and photographing the microorganisms. Operational parameter changes together with the insight gained from modelling study were applied to achieve the desired floc size, structure and microorganism numbers as well as to solve the bulking and foaming problems at the landfill leachate treatment system.

1.2 Layout of Thesis

The scope of the thesis is given below:

- In the second chapter, the current literature survey related to leachate and its microorganism diversity, character, composition and heavy metal amounts, leachate treatment options, pollutants of concern, EPA & Turkey discharge standards, sanitary landfills, major problems – bulking & foaming and parameters affecting it, general information and current condition of the treatment plant.
- In the third chapter, materials and methods used in the experimental set-up and in the theoretical approaches were described in detail.

- In the fourth Chapter, results and discussions were divided into two parts. In the first part, the results of experimental studies were given, in which the experiments were carried out to find out the Sludge Volume Index (SVI), microbial diversity, number and type of present filaments, floc density, size and type. In the second part, the results and possible reasons behind problems were discussed together with suggestions to improve both the system and experiments.
- In the fifth chapter, the conclusion of experiment was explained.

1.3 Literature Review

1.3.1 Landfills

Landfilling is an engineered method of solid waste management system currently practiced by most countries due to being beneficial for both the environment and the economy. A well-engineered landfill is a specially constructed and protected cells of waste disposal on land without generating nuisances or hazards to public health or safety [2]. Biological, chemical and physical processes occur within the landfill and promote waste degradation which leads to leachate and gas production. The gas and leachate formed in landfill sites are used to produce energy, contributing to one of the vital needs of populations. Another advantage of landfills is deposited unrecyclable waste becomes “capped”; a lot of wildlife grow on top thus making these sites perfect for wildlife reserves. Even though landfill sites are most beneficial, there is only finite space to allow them and if recyclable materials are put into landfill sites, more energy and resources are used trying to create new materials instead of re-using or. The ecosystem diversity in landfill promotes stability; however environmental conditions such as temperate, pH, toxins, moisture content and the oxidation reduction potential strongly influence the system. The stabilization of wastes proceeds in five sequential and distinct phases:

- 1) **Initial adjustment phase:** Initial deposition of solid waste and accumulation of moisture within landfills. An acclimatization period is observed until enough moisture develops to support an active microbial community.
- 2) **Transition phase:** Transformation from aerobic to anaerobic environment occurs.

- 3) **Acid formation phase:** Production of intermediate volatile organic acids at high concentrations as a result of continuous hydrolysis of solid waste followed by the microbial conversion of biodegradable organic.
- 4) **Methane fermentation phase:** Intermediate acids are consumed by methanogenic bacteria and converted into methane and carbon dioxide.
- 5) **Maturation phase:** Nutrients and available substrate become limiting, gas production dramatically drops, and leachate strength stays steady at much lower concentrations.

A common schematic of a landfill is shown in Figure 1.1 [3].

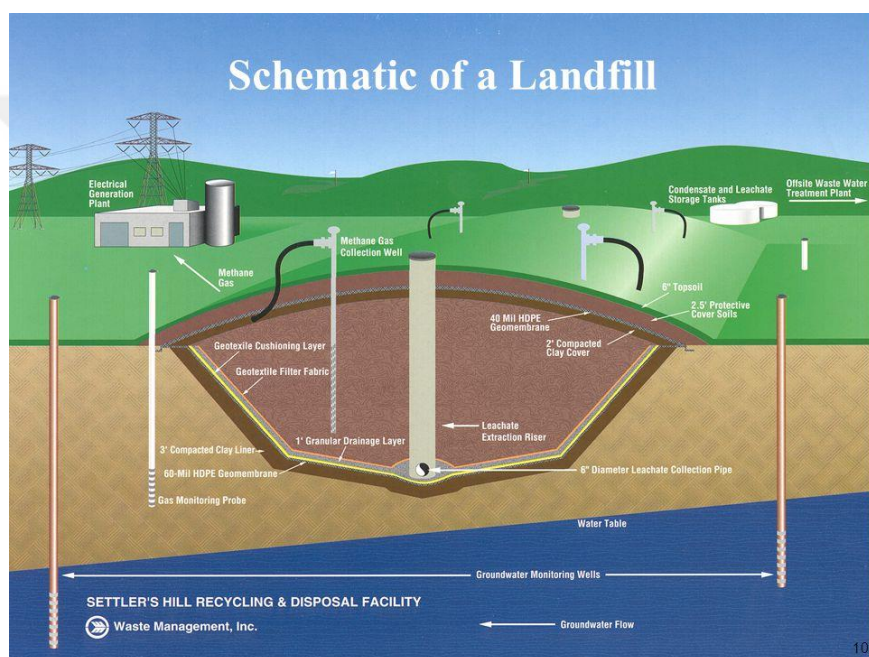


Figure 1.1: General Schematic of a waste deposited landfill site.

1.3.2 Landfill leachate

By definition “Leachate is the liquid that leaches or drains from a landfill.” [4]. Leachate is generated when excess rainwater percolates through the waste layers in a landfill or as the moisture of decomposition condenses through the layers and settles at the bottom of the landfill [5]. Direct discharge of leachate into receiving water bodies can cause eutrophication and toxicity to fauna due to a variety of contaminants. Extreme toxicity of leachate is a major threat surface and ground water bodies. The chemical composition of landfill leachate is determined by the waste composition of the landfill, climatic conditions, age of landfill and degradation rate of the solid waste buried. Generation and extraction of potential pollutants from the deposited waste in

landfills depends on factors such as; solid waste composition, degree of compaction, absorptive capacity of the waste and waste age, seasonal weather variations, levels of precipitation, landfill temperature, size, hydrogeological conditions in the vicinity of the landfill site, engineering and operational factors of the landfill, pH, landfill chemical and biological activities [6].

Generally, chemical characteristics of leachate includes high values of Chemical Oxygen Demand (COD) due to large amounts of organic matter, pH, ammonia and organic nitrogen, chlorinated organic and inorganic salts and heavy metals whereas physical characteristics are darkish-brown colour, very small granules and an unpleasant odour. However, these characteristics vary regarding to its composition and volume, as well as biodegradable matter present in the leachate versus time. Although high ammonia and organic nitrogen concentrations are one of the major problems in leachate treatment, presence of heavy metals are also equally important. Sources such as electronic waste, painting waste and used batteries increase heavy metals content in landfills [7]. Heavy metals are essential trace elements for living organisms in very small doses, however most are toxic to all forms of life at high concentrations. They cannot be biodegraded and persist indefinitely causing pollution of air, water and soils. Table 1.1 [8] shows common value range of leachate parameters:

Table 1.1: Typical composition of landfill leachate parameters.

Parameter	Value Range	Parameter	Value Range
COD (mg/L)	150 – 100000	SO ₄ (mg/L)	10 – 1200
BOD ₅ (mg/L)	100 – 90000	Cl (mg/L)	30 – 4000
pH	5,3 – 8,5	Fe (mg/L)	0,4 – 2200
Alkalinity	300 – 11500	Zn (mg/L)	0,05 – 170
HCO ₃ (mgCaCO ₃ /L)	500 -8900	Mn (mg/L)	0,4 – 50
NH ₄ (mg/L)	1 – 1500	CN (mg/L)	0,04 – 90
Norg (mg/L)	1 – 2000	AOX (µg CL/L)	320 – 3500
TN (mg/L)	50 – 5000	Phenol (mg/L)	0,04 – 44
NO ₃ (mg/L)	0,1 – 50	As (µg/L)	5 – 1600
NO ₂ (mg/L)	0 – 25	Cd (µg/L)	0,5 – 140
TP (mg/L)	0,1 – 30	Co (µg/L)	4 – 950
PO ₄ (mg/L)	0,3 – 25	Ni (µg/L)	20 – 2050
Ca (mg/L)	10 – 2500	Pb (µg/L)	8 – 1020
Mg (mg/L)	50 – 1150	Cr (µg/L)	30 – 1600
Na (mg/L)	50 – 4000	Cu (µg/L)	4 – 1400
K (mg/L)	10 – 2500	Hg (µg/L)	0,2 – 50

In general, the strength of leachate decreases with time due to biological breakdown of organic compounds and precipitation of heavy metals. Due to its biodegradable nature, the organic compounds decrease more rapidly than the inorganic compounds with increasing age of leachate production. Therefore, the ratio of total volatile solids to total fixed solids decreases with the age of the landfill [9]. Three main groups of landfills are classified as young (less than five years), intermediate (5-10 years), and old or stabilized (more than 10 years). Table 1.2 [10] summarizes the groups of landfills according to their age;

Table 1.2: Main groups of landfills.

Parameter	Young	Intermediate	Old
Age (years)	<5	5-10	>10
pH	6.5	6.5-7.5	>7.5
COD (mg/L)	>10,000	4,000-10,000	<4,000
BOD ₅ /COD	>0.3	0.1-0.3	<0.1
Organic compounds	80% volatile fat acids (VFA)	5-30% VFA + humic and fulvic acids	humic and fulvic acids
Heavy metals	Low-medium	Low	Low
Biodegradability	Important	Medium	Low

1.3.3 Landfill leachate discharge standards

Landfill leachate discharge standards varies in different countries and are defined according to several factors; continuous impact to the environment, quality of leachate, economic capabilities and the practical situation of the landfill. Following the discharge standards is crucial to protect the environment as it is the executive criteria to be obeyed. Table 1.3 [11] shows a comparison example of discharge limit concentrations of landfill leachate in Germany and Turkey.

Table 1.3: Discharge limit of leachate parameter concentrations in Germany and Turkey.

Parameter	Unit	Limiting concentration – Germany	Limiting Concentration – Turkey
COD	mg/L	200	700
BOD ₅	mg/L	20	-
Total Nitrogen	mg/L	70	20
Total Phosphorus	mg/L	3	2
Mercury	mg/L	0.05	-
Cadmium	mg/L	0.1	0.1
Chromium	mg/L	0.5	2
Nickel	mg/L	1	-
Lead	mg/L	0.5	2
Copper	mg/L	0.5	3
Zinc	mg/L	2	5
Cyanide	mg/L	0.2	1
Sulphide	mg/L	1	-
pH		-	6-9

1.3.4 Treatment options of leachate

The composition of leachate varies from landfill to landfill depending on mostly the type of waste deposited, therefore the leachate treatment approach should consider which methods are most suitable for that specific landfill leachate. The different composition of leachate, removal of diverse chemical compounds, e.g. COD, Biochemical Oxygen Demand (BOD), Ammonia nitrogen ($\text{NH}_4^+\text{-N}$), heavy metals, the advantages and disadvantages of treatment options should be taken into consideration carefully. In addition to varying leachate composition, leachate treatment is subject to national and regional level regulations of the country, therefore

treatment options should follow the regulations of that specific country, environmental aspects and evaluation alternatives.

Table 1.4 [12] summarises treatment methods depending on different removal purposes;

Table 1.4: Main objectives to be treated in leachate.

Treatment objectives	Main Treatment Options
Degradable organic (BOD) removal	Aerobic biological: Aerated lagoon/extended aeration Activated sludge Sequencing batch reactor (SBR)
Ammonium removal	Anaerobic biological: Up-flow sludge blanket Aerobic nitrification: Activated Sludge Aerated lagoon/extended aeration Rotating biological contractor Sequencing batch reactor Vegetated ditch Ammonia stripping
Denitrification	Anoxic biological: Sequencing batch reactor Vegetated ditch
Non-degradable organic and colour removal	Lime/coagulation addition Activated lagoon Reverse osmosis Chemical oxidation
Hazardous trace organic removal	Activated carbon Reverse osmosis Chemical oxidation

Table 1.4 (continued): Main objectives to be treated in leachate.

Treatment objectives	Main Treatment Options
Odour removal	Hydrogen peroxide
Dissolved iron and heavy metals, and suspended solids removal	Lime/coagulant addition, aeration and settling
Final polishing	Artificial wetlands (e.g. reed beds, ponds)
Disinfection	Hypochlorite
Volume reduction	Reverse osmosis Evaporation

Leachate treatment technologies are categorised into two basic groups; biological and physical/chemical treatment. However, depending on the treatment goals and composition of leachate, hybrid-treatment systems which combine both technologies are used in large scale systems.

1.3.5 Physical/Chemical treatment methods

Physical/Chemical treatment is used for stabilized landfill leachate with high concentration of toxic waste to biological life and has low degradable organic matter concentration. Suspended solids, colloidal particles, floating material, colour, and toxic compounds are reduced by several Physical/Chemical treatment methods. These methods are non-biological and used in addition as a pre-treatment and a post-treatment method or to treat a specific pollutant (ammonia stripping) [13].

Some of the applied physical/chemical treatment methods in landfill leachate treatment are coagulation – flocculation, chemical precipitation, flotation, activated carbon adsorption, ammonium stripping and membrane filtration. In the treatment facility coagulation – flocculation, chemical precipitation applied as pre-treatment in the pre-settling tank and membrane filtration is applied as a post-treatment right after nitrification/denitrification tanks.

1.3.5.1 Coagulation – flocculation

Coagulation – flocculation process involves removal of non-biodegradable organic compounds and heavy metals from leachate by addition of coagulants [14]. Addition of coagulants reduces the electric repulsion effects between the negatively charged colloidal particles and unite them for precipitation.

Result of Kurniawan et al 2006 [26] experiments show that successful COD removal of this method ranges between 30% and 86%, and heavy metal removal range between 74% and 98%.

1.3.5.2 Chemical precipitation

Chemical precipitation involves addition of chemical precipitation reagents to settle the non-biodegradable organic compounds and heavy metals, which then allows the use of filtration to remove the particles from the mixture. Main use of this method involves removal of heavy metals, non-biodegradable organic compounds and NH_4^+ -N as well as phosphorus, fluoride, ferro-cyanide and other inorganics

Kurniawan et al 2006 [26] shows 90% to 98% removal efficiency in Ammonium removal with an initial concentration range of 1380 mg/L to 5168 mg/L in leachate.

1.3.5.3 Flotation

Flotation is a process involving utilization of gas bubbles, attaching to solid particles in suspension to force them float onto the surface of the liquid, thus separating them from the mixture. The main purpose of this application is wastewater treatment by removal of solids, ions, macromolecules and fibres [15].

Flotation method has shown up to 99% humic acid removal efficiency in leachate as a post-treatment after biological treatment.

1.3.5.4 Activated carbon adsorption

Adsorption method involves adsorption of dissolved compounds to the surface of an adsorbing medium with a large internal surface. This process happens when the attractive forces of the carbon surface overcome the attractive forces of the liquid [16].

Powdered activated carbon (PAC) or granular activated carbon (GAC) could be used as adsorbing mediums which generally used to achieve a low adsorption affinity for

low molecular weight and polar organic species. GAC has a high surface area to volume ratio and thus is the most preferred adsorbent in wastewater treatments. Although, this method proved to be an efficient way of removing non-biodegradable compounds and heavy metals, it is not as efficient in NH_4^+ -N removal as other methods in wastewater treatment [17].

Kurniawan et al 2006 [26] results show that an initial heavy metal concentration of 184 mg/L and a COD concentration range of 940 mg/L to 7000 mg/L, achieves 80% to 96% and more than 90% removal efficiency, respectively.

1.3.5.5 Ammonia stripping

Ammonia stripping process reduces the concentration of ammonium in wastewater and final product, ammonia gas – NH_3 gas, is disposed as either air disposal, concentrated ammonium sulphate or thermal destruction. The equilibrium (1.1) reaction for ammonia stripping is:



The pH of the mixture plays an important role in ammonium/ammonia gas formation, as pH level 7 or lower promotes the formation of soluble ammonium from ammonia where at pH level 11 or above only dissolved ammonia gas is present. The dissolved ammonia gas then can be released from the wastewater; therefore, the pH of stripper should be adjusted to 11-12 by addition of NaOH.

Kurniawan et al 2006 [26] results of ammonia stripping show that 85 to 95% removal efficiency with an initial ammonia concentration of 220 mg/L to 2215 mg/L is achieved. This method is unaffected by the toxic materials present in the wastewater, and backwash or regeneration is non-existent. However, as the initial concentration in the wastewater/leachate increases, so as the economic burden of the method. Also, maintenance of the equipment due to high pH, temperature limitation on removal efficiency, disability to remove nitrite and nitrogen, and release of ammonia gas into environment are some of the disadvantages¹².

1.3.5.6 Membrane filtration

Membrane filtration methods involves one or multiple layers of thin, semi-permeable material linings forming a physical barrier that separates substances when a driving force is applied. These microporous plastic films are called membrane filters, and each

has specific pore size ratings. Membrane filtration is used for removal of bacteria, viruses, particulates, and natural organic matter. Innovations and investments in membrane production and module designs has helped the reduction of capital and operating costs furthermore. Four types of membrane filtration types will be explained below which are; microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO) [18].

RO is normally used in combination with coagulation to control fouling, ensure operational stability and improve removals of dissolved organics. UF can remove viruses, pyrogens and colloidal silica, thereby providing a physical disinfection barrier. MF that can remove common particles in water like bacteria and other microbial organisms [19].

The membrane filtration spectrum for water and wastewater treatment relative to the micropore size of the common materials is shown in Figure 1.2 [20]..

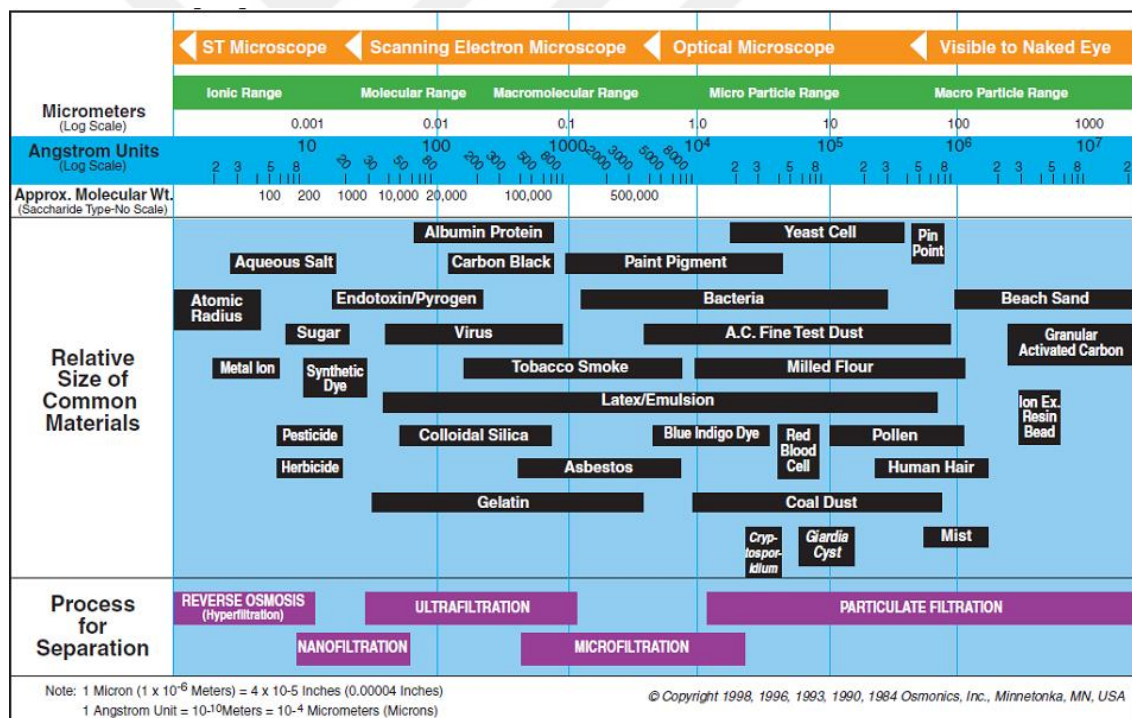


Figure 1.2: The membrane filtration spectrum for water and wastewater treatment

RO system can remove heavy metals, suspended/colloidal materials and dissolved solids from landfill leachate and has the smallest micropore size amongst other membranes. Water tends to diffuse through the membrane of the lower concentration side to the higher side until the pressure difference balancing the chemical potential differences [21]. RO is more efficient when compared to other filtration systems due

to high fluxes, operating at wide temperature range and pH. The utilization of RO system is affected by deposition of low retention small molecules, suspended and dissolved substances on the external surface, which reduces the efficiency of the filtration system [22].

The RO system has a relative high efficiency for the removal of COD and $\text{NH}_4^+\text{-N}$ in leachate treatment. In Kurniawan et al 2006 [26], COD and $\text{NH}_4^+\text{-N}$ removal performance of RO system showed over 95% and up to 96% removal efficiency when the initial concentrations were between 335-3840 mg/L and 33-1400 mg/L, respectively.

NF system involves larger micropore size than RO and the aim is the filtration of divalent and larger ions such as herbicides, synthetic dyes and heavy metals. Particles with a molecular weight more than 300 Daltons are removed in the NF process. The surface charge present in the NF allows charged molecules that are smaller than the membrane micropores to be rejected. The NF is not compatible with leachate treatment due to its loose membrane structure, unable to accept higher flux rate, and lower operating pressure.

The NF system has more than 65% of COD removal efficiency when the initial concentration is between 920-3000 mg/L.

UF system removes emulsified oils, metal hydroxides, colloids, emulsions, dispersed material, suspended solids, and other large molecular weight materials using pressure from the target liquid mixture [23]. Low molecular weight substances such as salts, surfactants and soaps can pass through the micropores of the UF system.

Oils and solids can be concentrated to 50% and produce permeates less than 50 mg/L of oil with the use of UF systems [24].

MF is also a pressure-driven system for water filtration; however, it operates at a relatively low pressure and the pore size of membrane is the largest compared to others, 0.04 μm to 0.10 μm .

1.3.5.7 Treatment performance of physical/chemical methods

The performance of different physical/chemical treatments on COD and $\text{NH}_4^+\text{-N}$ removal from stabilized leachate are shown below in Table 1.5 [26].;

Table 1.5: Comparison of physical/chemical treatments on COD and NH_4^+ -N removal efficiency

Treatment type	BOD5/COD	pH	Initial Concentration in leachate (mg/L)		Removal Efficiency (%)	
			COD	NH_4^+ -N	COD	NH_4^+ -N
AC adsorption	N/A	N/A	5690	2215	95	N/A
Ammonia Stripping	0.60	11.0	5850	1380	-	94
Precipitation	0.22	9.0	7511	5618	-	98
RO	0.31	6.0	3840	N/A	98	N/A
NF	0.03	6.4	17000	3350	96	N/A

Table 1.5 shows that ammonia stripping, and precipitation physical/chemical treatments have 94% to 98% NH_4^+ -N removal efficiency while NF, RO and AC adsorption have 95% to 98% COD removal efficiency.

1.3.6 Biological treatment methods

Biological treatment methods are the most common systems used in leachate treatment. Biological treatment methods involve aerobic, anaerobic and natural treatment systems. The basic principle of biological nitrogen removal is nitrification and denitrification. Nitrification is a process where ammonium turns into nitrate and nitrate in an aerobic environment, where then nitrite and nitrate turn into nitrogen gas in an anaerobic environment with denitrification.

Aerobic treatment methods in leachate treatment involves oxygen input into the system where microorganisms consume organic materials as energy in aerobic environment. Two types of aerobic processes are; suspended-growth system and fixed-growth biomass. Suspended-growth system is mainly applied in activated sludge and aerated lagoon systems, where fixed-growth biomass is applied in trickling filters and rotating biological contactors [25].

There are various types of aerobic treatment and some of the methods, including activated sludge, sequencing batch reactors (SBR), nitrification-denitrification and aerated lagoons will be introduced below.

Anaerobic process is the metabolization of organic materials to CO_2 , CH_4 and other metabolites without oxygen. Unlike aerobic process, anaerobic process conserves energy and produces very few solids, however reaction rates are much slower than the aerobic processes. The product of anaerobic process, Methane (CH_4), can be used to heat the digester or simply collected and turned into energy [8].

Anaerobic treatment methods are more suitable for the treatment of concentrated leachate streams. This method offers lower operating costs and usable biogas production. Below two anaerobic treatments will be introduced, up-flow anaerobic sludge blanket (UASB) and anaerobic filters.

1.3.7 Activated sludge, AS

Activated sludge (AS) is the most commonly used treatment method used in landfill leachate treatment. The process includes an oxygen supplied aeration tank where bacteria from an active microbial floc is added and grown with a steady inflow of wastewater/leachate, nutrient source. The optimum temperature for AC system should be mesophilic, around 35°C , and pH range should be between 6.0-7.5. Inflowing leachate into the aeration tank is mixed into the activated sludge by help of mechanical stirring or aerators. The organic matters in the mixture is metabolised into new microbial biomass, CO_2 and water, and passed into a settling tank/clarifier. In the settling tank/clarifier, sludge is separated from leachate and a portion of the settled sludge is recycled back to the aeration tank where excess is deposited generally to sludge beds.

A schematic graph of activated sludge is shown in Figure 1.3 [26];

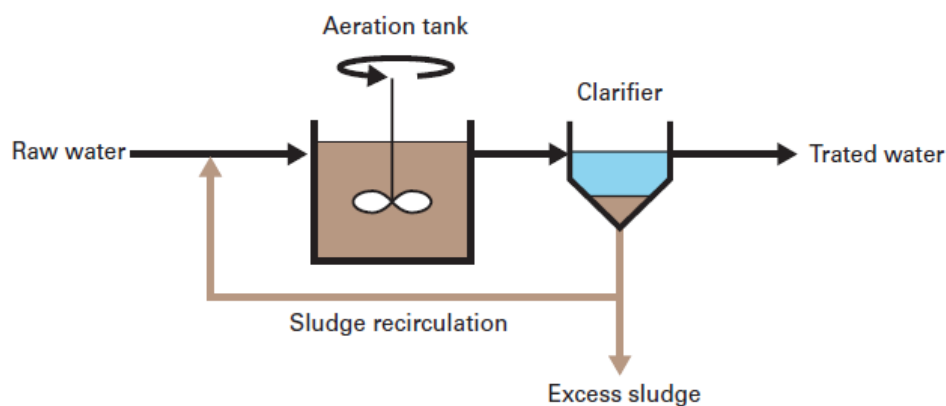


Figure 1.3: Schematic graph of activated sludge.

The COD removal by using activated sludge with young leachate with an initial COD concentration 1000 mg/L to 24000 mg/L and an organic loading rate range of 0.4 to 1.2 kg/m³/day, achieves more than 95% efficiency. This method also works well on NH₄⁺-N removal efficiency of 95% with the initial concentration ranging from 115 to 800 mg/L [27].

1.3.8 Sequencing batch reactors, SBR

Sequencing batch reactors, SBR is an activated sludge process designed to treat wastewater in one operation tank by a sequence of stages. SBR systems are easy to operate with minimum interaction, has much lower cost when compared to most systems and can treat a wide range of influent volume with high removal efficiency. SBR can be used both as an aerobic or anaerobic system. These properties have made SBR one of the most preferred leachate and wastewater treatment option worldwide.

The process cycle of an SBR system is shown in Figure 1.4 [21].;

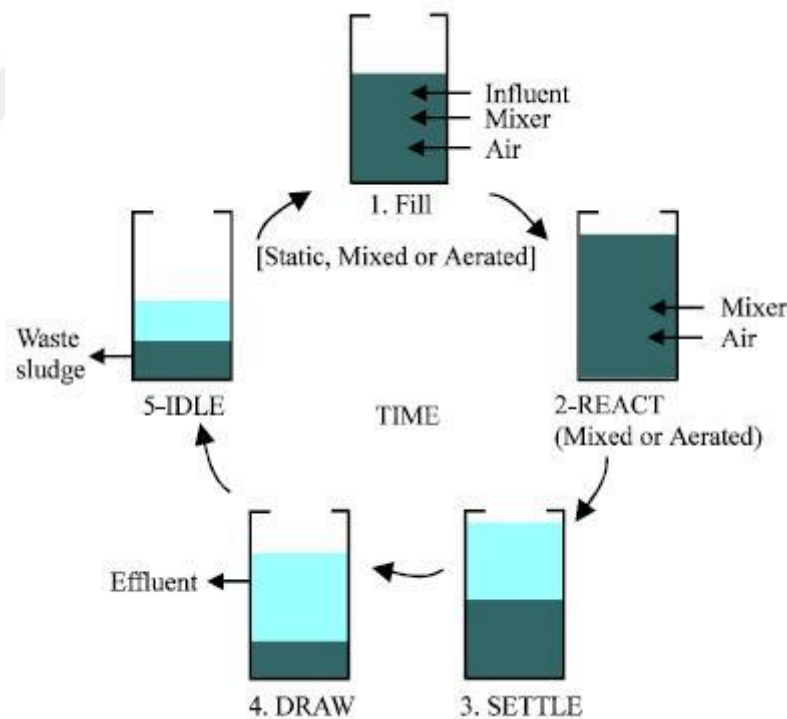


Figure 1.4: Process cycle of an aerobic SBR system.

SBR system works in five operating steps; fill, react (mixing and/or aeration), settle, draw and effluent, and idle. The first process, filling, involves feeding of the influent into the designated tank to a certain volume. Next step is the reaction process where

the targeted mixture is supplied with air, if it is an aerobic system and mixed to remove BOD and transfer ammonium nitrogen into nitrates. When the aimed retention time is reached, the aeration device is shut down and the content can precipitate at the bottom. During the third stage, settling, no liquid should enter or leave the tank to avoid turbulence in the tank. After the settling, the effluent is discharged from the tank and waste sludge is collected. Final stage before the cycle starts is the idle stage which is the wait time of the reactor to operate the next refilling with a new influent.

The SBR system can also be used as a nitrification-denitrification process by controlling the stages between filling and drawing with oxygen input into the tank. In this way the process can be operated by the periodical change of the concentration of O₂, substrates and inorganic nutrients [21].

1.3.9 Nitrification-denitrification

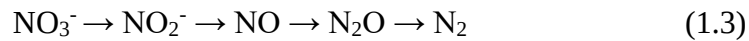
Nitrification/Denitrification processes are widely used in leachate treatment. Nitrification is a biological oxidation process that transfers ammonium into nitrate when oxygen is present by an irreversible two-step process. The first bacteria involved in this two-step process is named as ammonia oxidizers which convert ammonium to nitrite, followed by nitrite oxidizers which convert nitrite to nitrate [28]. This conversion process occurs rapidly, keeping the nitrite level low at any given time. The chemical equation (1.2) of this process is shown below;



The optimum pH for nitrification process is between 7.5 and 8.5, however in large scale treatment plants, successful nitrification is also achieved with a pH range of 6.5 to 7.0. The nitrification process produces acid which reduces the activity of nitrifying bacteria, therefore pH levels should be measured and adjusted to ensure the sustainability of the system. Suggested temperature range for nitrification is given as 30 °C – 35 °C in Kurniawan et al 2006 [26].

Denitrification is the biological reduction of NO₃ to nitrogen gas (N₂) by facultative heterotrophic bacteria under anoxic conditions. The facultative heterotrophic requires addition of a carbon source as food into the system in order to carry out the denitrification process. The N₂ gas has low water solubility, therefore it can escape

into atmosphere and will not cause any environmental problems. The chemical equation (1.3) of the denitrification process is:



The optimal pH range of for denitrification process is between 6.0 and 8.0, and the temperature range is from 5°C to 60°C. The process is strictly anaerobic/anoxic, therefore dissolved oxygen concentration should be less than 0.5 mg/L as suggested in Kurniawan et al 2006 [26].

Although the nitrification/denitrification process has a 90% NH_4^+ -N removal efficiency, with 270-535 mg/L initial concentrations, NH_4^+ -N and heavy metal concentrations affect the nitrification rate of the bacteria, hence reducing the removal efficiency of the system.

1.3.10 Aerated lagoon

Aerated lagoons are ponds with microorganisms that can degrade organic matters under aerobic or anaerobic conditions. Aerated lagoons can be artificially made to suit the needs or naturally formed lagoons. In this method, lagoons can act as a storage pond and as a reaction tank, however, occupies a much larger area and requires longer retention time, approximately 3 to 20 days, then other biological methods in order to break down the organic matters. Long retention time and large area requirements make it unsuitable for leachate treatment and is not used as widely as activated sludge systems.

In the study of Kurniawan et al 2006 [26], the performance of NH_4^+ -N removal by using lagoons is almost 100%. COD removal is over 80% with an initial COD concentration ranging from 104 mg/L to 175 mg/L.

1.3.11 Up-flow anaerobic sludge blanket, UASB

UASB process is an anaerobic wastewater and landfill leachate treatment method where can treat COD concentrations higher than 10,000 mg/L with high efficiency and short hydraulic retention time. In an UASB reactor the influent of targeted liquid goes from the bottom and flow upward through a blanket of biologically formed granules. The effluent is discharged, and produced biogas is collected at top of the system. The optimum pH level for the system is suggested as 7.0, where recommended COD: N:P

ratio and temperature are 300:5:1 and 20°C - 35°C, respectively. The chemical equation (1.4) of the reaction in the system is:



Figure 1.5 [29] shows a common UASB reactor. Abas et al 2009 shows that the COD removal of UASB is 70% at temperature 20 – 23 °C and 80% at temperature 35 °C. Some experiments show that high rate treatment at low temperature may minimize the need for heating the leachate prior to treatment, and the treatment may produce toxic substances.

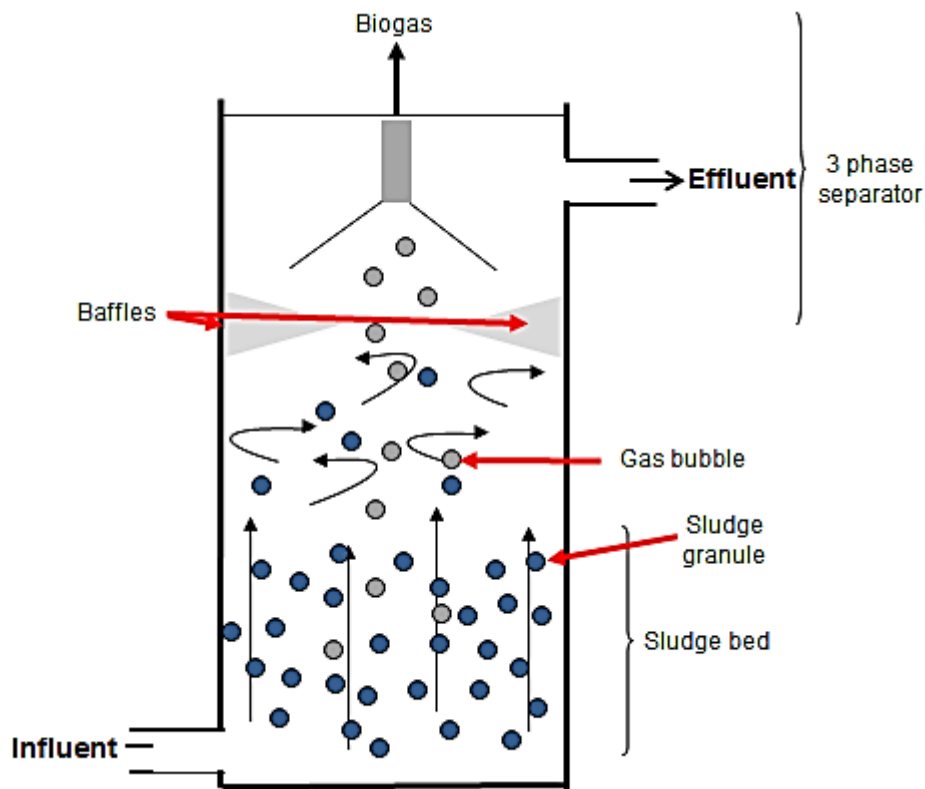


Figure 1.5: UASB reactor.

1.3.12 Treatment performance of biological methods

In order to compare the performance of each biological treatment method, some of the chosen parameters are pH, hydraulic retention time (HRT) (days), Oxygen uptake rate ($kg/m^3 \cdot day$) and initial concentration of COD and NH_4^+-N (mg/L) in the landfill leachate. Table 1.6 [26] below shows the best performance result of biological methods for removing COD and NH_4^+-N ;

Table 1.6: Performance of outstanding biological methods.

Type of treatment	HRT (day)	Leachate Volume in Reactor (L)	BOD ₅ / COD	Opt. pH	Initial leachate Concentration (mg/L)		Loading rate (kg/m ³ .day)		Removal Efficiency (%)	
					COD	NH ₄ ⁺ - N	COD	NH ₄ ⁺ - N	COD	NH ₄ ⁺ - N
Activated Sludge	20	N/a	0.5	6.0- 7.5	24000	790	1.2	N/a	98	99
SBR	1	5	N/a	7.5	26000	,000	120	0.58	97	99
Nitrification/ Denitrification	3-4	20	N/a	6- 8.8	26940	1810	15.2	0.84	90	N/a
Aerated lagoons	N/a	-	0.72	6.3	9750	175	N/a	N/a	98	99.5
UASB	4.5	2.5	N/a	7.0- 7.3	20000	679	16	16	98	99.6

From the results of Table 1.6 it can be concluded that aerated lagoon, activated sludge, SBR and UASB have the highest COD and $\text{NH}_4^+\text{-N}$ removal performance. However, selection of the most efficient method solely based on the treatment efficiency is not always gives the best outcomes. The leachate treatment method selection should be based upon the characteristics of the leachate, technical applicability, potential constraints, effluents limit required, cost effectiveness, regulatory requirements and long-term environmental impacts [20]. Therefore, there is no absolute the best treatment method for leachate.

1.4 Leachate and Activated Sludge Microbial Community

Landfill leachate is an excellent source for microbial community growth in some of the cases as it is composed of substrates that support the development of microorganism. However, it also has disadvantages for the microbial communities as heavy metals and toxic materials are also present. The microbial community of landfill leachate depends on the age and stage of the landfill, waste degradation and leachate composition, therefore different microorganism can be observed to dominate the micro-population at different times.

1.4.1 Bacteria & Archaea

Aerobic environment is dominated by aerobic heterotrophic bacteria that decompose cellulose and consume oxygen and nitrate present in the waste and produce carbon dioxide [30]. Anaerobic environment is dominated by cellulolytic bacteria such as *Clostridium* spp. and *Eubacterium* spp. which hydrolyses cellulose and further ferment it to produce alcohols and carboxylic acids [31]. Acetogens convert organic acids and alcohols to acetate, carbon dioxide and hydrogen [32].

Research on leachate treatment shows that it contains diverse microbial populations including both *Bacteria* and *Archaea*. The most common microbial populations observed belonged to the phyla *Proteobacteria*, *Firmicutes*, and *Bacteroidetes*.

A young landfill leachate research was observed to host *Bacteroidetes* in anaerobic niches. Strictly Anaerobes such as *Proteiniphilum* and *Petrimonas* was observed to be the predominant species which degraded the organic matter [33]. Dominant microorganisms involved in the denitrification of the young landfill leachate were

observed to be from phyla *Proteobacteria*, including *Pseudomonadaceae*, *Thiobacillus*, *Steroidobacter* [34].

In biological treatment of leachate, *Actinomycetes*, *Bacillus*, *Pseudomonas*, and *Burckholderia* predominantly observed in the system which can reduce Total Organic Carbon (TOC) up to 77% [35]. *Pseudomonas*, *Lysobacter*, and *Bacillus* are other abundant microorganisms in biological treatment of landfill leachate which can reduce organic carbon, volatile fatty acids (VFAs) and involved in denitrification process.

Within the domain *Archaeal*, *Methanosaeta* is the most popular organisms found predominantly in leachate related researches [36]. Methanogens were observed, including *Methanosaeta*, *Methanosarcina*, *Methanobacterium*, and *Methanoculleus* in young landfill leachate at pre-methanogenic stage [37].

Other microorganisms such as protozoa and metazoan are most often observed in activated sludge systems. Depending on species they can be observed as attached to the flocs or free in the space between wastewater and the flocs. Their “length” varies from 10 to 1000 μm , thus are considerably bigger than bacteria. Additionally, characteristic shape of each species greatly helps identification of these microorganisms, to some extent. Table 1.7 [41] shows most commonly observed microorganism groups in activated sludge systems.

Table 1.7: Most commonly observed activated sludge microorganism groups.

Group	Cell Length (µm)	Remarks
Bacteria	1-5	Occasional colonies, on filaments, or free in suspension
Protozoa		Occasional colonies or single
-flagellates	10-30	
-amoeba	30-400	
-heliozoa	40-200	
-ciliates	25-400	
Metazoan		Occasional colonies or single
-rotifers	100-500	
-nematodes	500-3,000	
-tardigrades	200-1,200	
-worms	3000-10000	

1.4.2 Protozoa

Protozoa are single celled organisms and are present expected to be present in every activated sludge system. The population percentage of protozoa is only a few of the total biomass, which means they are much smaller in number than that of the bacteria. Protozoa feed on the free or floc formed bacteria, sometimes even on other protozoa. Feeding on sludge flocs reduces the floc production if protozoa is present in high numbers. However, certain species of protozoa are relevant for the process conditions of wastewater treatment plants. Therefore, composition of microbial population holds great importance in sludge investigation.

As over 200 different strains could be observed in activated sludge samples only the identification of the important main groups is enough. Regularly observed subgroups of several species in activated sludge systems is further detailed below:

1.4.2.1 Ciliates

Most common characteristic of ciliates include the presence of cilia on their surfaces. Where for some of them the surface is completely covered by cilia, others are partly covered. The food diversity of ciliates ranges from organic fragments to bacterial cells

and even other protozoa. Some of the ciliates according to their food diet is given below;

- Mainly eating bacteria: *Aspidisca*, *Carchesium*, *Chilodonella*, *Verticella*;
- Omnivorous ciliates that prey on bacteria, flagellates and small ciliates; *Euplotes*, *Stentor*;
- Carnivorous ciliates that mainly prey on other ciliates; *Litonotus*, *Hemiohyrs*, *Suctorea*.

Sessile, crawling and free-living ciliates are most often observed in the activated sludge systems. Sessile ciliates are positioned on a stem that is usually attached on a floc. Some of the occurring sessile ciliates in activated sludge includes *Carchesium*, *Epistylis*, *Vorticella*. Crawling ciliates, as the name mentions, “crawl” their way around and over the flocs and graze the surface, removing any bacteria that is not attached to the flocs. *Aspidisca*, *Chilodonella* and *Trachelophyllum* are some of the most common “crawlers” observed in activated sludge. Free-living ciliates can move freely in the space of wastewater between the flocs. Most commonly seen examples include *Paramecium*, *Colpidium* and *Blepharisma*.

1.4.2.2 Flagellates

Defining characteristic of flagellates, as the name suggests, include one or more flagella, not more than eight, which are longer than the cilia of ciliates. Due to the rapid movement and relatively smaller sizes of their nature, the flagella are not easy to locate under microscopic investigation of activated sludge samples. Some species of flagellates includes bacterial cell diet where other species, mostly, absorb dissolved nutrient through their cell walls. Some examples include *Hexamitus*, *Peranema*, and *Pleuromonas*.

1.4.2.3 Amoeba

Amoeba has finger like appendages that change shape due to its flexible cell wall in order to move through its environment. During a microscopic investigation, careful examination is required due to their structure, size (30-400 µm) and extremely slow movement which could be mistaken for sludge flocs to the naked eye.

1.4.3 Metazoa

Metazoa are multicellular microorganism that are much bigger and “higher” organisms than bacteria and protozoa. Depending on their species their size can range from 100 μm to as big as 1-2cm. their diet include mostly free-living bacteria or small floc particles, however some species do consume whole sludge flocs. Some of the metazoan groups observed in activated sludge are given below;

1.4.3.1 Rotifers

Characteristics of Rotifers can be described as; elongated multi-celled organisms with large body length from 100 to 500 μm , distinct mobility and cilia existence on the head. Rotifers consume free living bacteria cells and small floc particles. Their tail is branched and plays an important role in the movement of the organism. The movement of rotifers often resembled to that of slugs as the tail attaches itself first and the body is dragged/stretched in that direction. Specific movement type is often helpful in identifying the rotifers.

1.4.3.2 Tardigrades

Tardigrades usually known as a water bear, are a multicellular organism that has pseudopodia with small claws. Like a bear, these claws help to crawl over flocs and graze them to feed. Their sizes range from 200 to 1200 μm .

1.4.3.3 Worms

Worms are the most easily observed and identified organisms on a wet slide due to their large sizes, approximately 0.1 to 10 mm. Worms are able to consume sludge flocs as food, thus are able to reduce the floc numbers when present in high numbers in activated sludge.

1.4.4 Fungi and algae

Although Fungi are seen in activated sludge, they do not become established in the sludge. This is presumably because of unfavorable habitat factors and competitive levels of other organisms. Exceptional situations in which fungi do become established include favorable oxygen, pH, and nitrogen levels which aid their competitive abilities in maintaining populations in competition with bacteria and protozoa. *Geotrichum candidum* is known to be an important sludge bulking fungi under favourable conditions.

Algae are not considered of importance in the constantly churning liquor, although they may form growths on the walls of tanks in which activated sludge is developed and settled, hence causing problems in the system.

1.5 Description of Landfill Leachate Treatment Facility

Landfill leachate treatment facility is built in 2008 in Istanbul, Turkey and daily solid waste deposit amount was 4500 tons. Leachate treatment capacity is 1700m³ per day and aimed to work with 80% efficiency in Chemical Oxygen Demand (COD), Suspended Solids (SS) and Ammonia-Nitrogen removal. Treated leachate is discharged into the receiving river according to the regulations in “Water Pollution Control Regulations” of Turkey [38]. Total area of the treatment facility is 233 hectares, which includes 140 hectares of solid waste deposit, total disposed solid waste was 26.69 tons in 2014 and expected life span of the landfill is until 2030. Furthermore, the treatment facility generates electricity from landfill gas (LFG) (10MW).

Figure 1.6 [39] below, shows the outline of landfill leachate treatment facility from above;



Figure 1.6: Landfill leachate treatment facility.

Table 1.8 [40] shows leachate analysis results from 2008 and the capacity of leachate treatment facility.

Table 1.8: Treatment facility landfill leachate.

Parameters	Unit	Value
Inflow	m ³ /day	1700
BOD ₅	Mg/L	3000-30000
COD	Mg/L	5000-45000
SS	Mg/L	300-2500
Total Nitrogen	Mg/L	2000-4000

Highly polluted landfill leachate is treated with activated sludge, AS and filtration systems. Landfill leachate is collected with drainage pipes is first stored at collection tanks then pumped into Equalisation tank. In equalisation tank coagulants are added to allow suspended solids within the landfill leachate to settle as it flows slowly through and prior to transfer into nitrification/denitrification pools, parameters such as temperature, pH, COD, ammonia nitrogen, dissolved oxygen, etc (e.g pH between 6.5-7, temperature at 35°C) are conditioned to the desired values by addition of chemicals or with help of mechanical instruments (heating/cooling). After Equalisation tank, Leachate is pumped into the Bioreactor of 20171 m³ volume. Bioreactor is split into two sections; the outer channel is the Anoxic tank where denitrification takes place, volume: 9,467 m³, and the inner channel is the Aerobic tank where nitrification occurs, volume: 10,704 m³. Nitrate is recycled back into Anoxic tank from the end of Aerobic tank channel. Excess sludge in activated sludge system is sent to sludge dewatering, Decanter, and recycled back into the system and landfill. Treated Leachate in bioreactor then sent to ultra-filtration, UF, and nano-filtration, NF, systems for further treatment. A portion of the treated leachate is recycled back into the bioreactor before entering the UF units. UF effluent is forwarded into NF and UF concentrate is recycled back into the anoxic channel of the bioreactor. Effluent of NF is discharged into the receiving body, İSKİ sewage system and NF concentrate is sent to landfill. Sludge retention time (SRT) of the bioreactor is approximately 62 days and Food: Microorganism, F:M, ratio is 0.12 kg COD/COD.day. Figure 1.7 below shows flow diagram of landfill leachate;

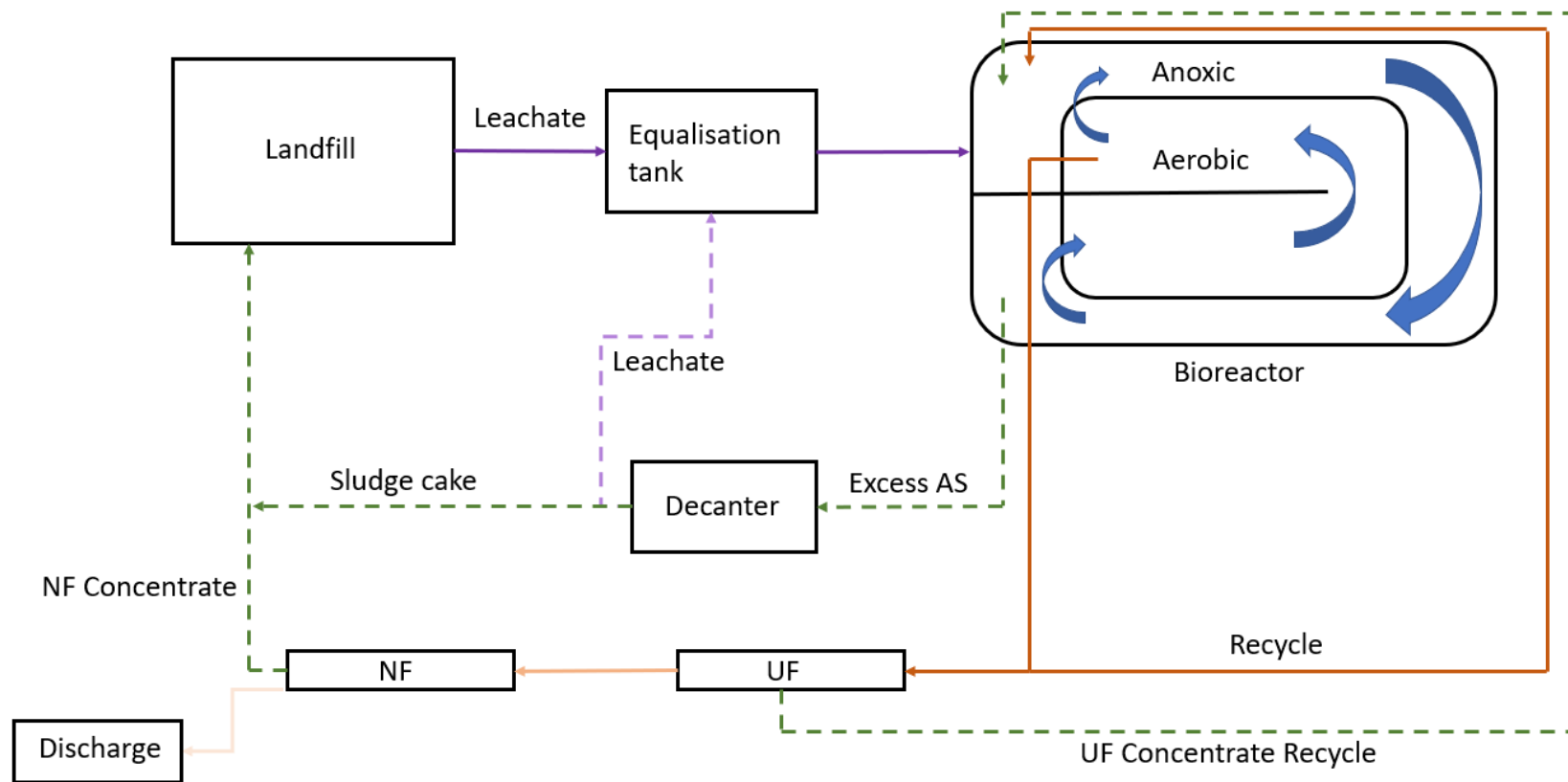


Figure 1.7: Studied landfill leachate treatment facility flow diagram.

1.6 Common Microbiological Problems in Activated Sludge Systems

The landfill leachate treatment facility worked with is using a hybrid system of activated sludge and filtration systems in order to achieve the discharge standards. The most important and crucial part of an activated sludge system is the development and maintenance of a mixed microbial culture that treats the wastewater. However, like every other treatment system, activated sludge system can develop many problems related with engineering, hydraulic and microbiological component operations. Any problem within the system can adversely affect effluent quality.

Some of the common microbiological problems that can occur in activated sludge operation include; dispersed (non-settleable) growth, pin floc problems, zoogloeal bulking and foaming polysaccharide ("slime") bulking and foaming, nitrification and denitrification problems, toxicity, and filamentous bulking and foaming [41]. Table 1.9 [36] shows some of the activated sludge system problems, cause of problems and Effect of problems;

Table 1.9: Causes and effects of microbially-related activated sludge solids separation problems.

Problem	Cause of Problem	Effect of Problem
Dispersed growth	Microorganisms are dispersed, forming only small clumps or single cells	Turbid effluent; no zone settling of activated sludge
Slime, viscous or zoogloeal bulking; non-filamentous bulking	Microorganisms are present in large amounts of extracellular material; in severe cases, extracellular material imparts a slimy or jelly-like consistency to activated sludge	Reduced settling and compaction rates; virtually no solids separation in severe cases, resulting in overflow of sludge blanket from secondary clarifier; viscous foam may appear in poor sludge dewatering
Pin floc	Small compact, weak roughly spherical flocs are formed; larger flocs settle rapidly; smaller flocs settle slowly	Low sludge volume index (SVI) and turbid, often high SS effluent
Filamentous bulking	Large amount of filamentous microorganism present bridge between the flocs or create diffuse flocs, interfering with compaction, settling and thickening	High SVI with very clear supernatant; low RAS and WAS solids concentrations; in severe cases, the sludge blanket overflows the secondary clarifier; solids handling processes become hydraulically overloaded
Blanket rising	Denitrification in the secondary clarifier releases poorly soluble N ₂ gas which attaches to activated sludge flocs and floats them to the secondary clarifier surface	A scum of activated sludge forms on surface of the secondary clarifier and on aeration basin anoxic zones
Foam/scum formation	Caused by (i) undegraded surfactants and by (ii) nocardioforms, <i>M. Parvicella</i> , or type 1863	Foams (scums) can float large amounts of SS to surfaces of treatment units; nocardioforms and <i>M. Parvicella</i> foams are persistent and difficult to break mechanically; foams accumulate and can putrefy; secondary effluent SS can be elevated; foams can overflow tank freeboards

1.6.1 Poor floc formation, pin floc and dispersed growth problems

Basic floc formation is due to growth form of many species of natural bacteria. Floc-forming species produces an extracellular polysaccharide called glycocalyx which forms a “slime” layer. Glycocalyx is consist of polysaccharide, protein and sometimes cellulose fibrils and glues the bacteria together as a whole to form a floc. It has been observed that “good” floc formation, non-dispersed and settleable growth, occurs at low growth rates and nutrient levels which is termed as “starvation” or “stationary” growth conditions. Dispersed and non-settleable growth, “bad” floc formation, is observed if the growth rate is too high. Dispersed growth is mostly observed in industrial waste treatment where the organic loading, food to microorganism ratio (F/M), is high, hence the growth rate. Free floating filaments can, at times, cause a dispersed growth problem. Structurally, dispersed growth shows no floc development and biomass settling does not occur, resulting in a very turbid effluent. The correct remedial action for a dispersed growth problem is a reduction in the F/M of the system, usually done by raising the MLSS concentration. Dispersed growth problem is most commonly observed when activated sludge biomass is low and F/M ratio is high in cases such as toxicity or hydraulic washout event. On the contrary, at very low F/M ratio and long sludge age conditions, small and weak flocs consist of only floc-forming bacteria without a filament backbone can be formed in activated sludge, which are called Pin flocs. Pin flocs can easily be sheared and affected by the hydraulic flotation leading to a turbid effluent. Chronic toxicity of the activated sludge system also promotes the formation of pin flocs. Compact and firm flocs are required for good settling properties, where when flocs are weak and diffused settling problems in clarifier and turbidity in effluent is observed. Figure 1.8 [42] shows characteristics of different flocs.

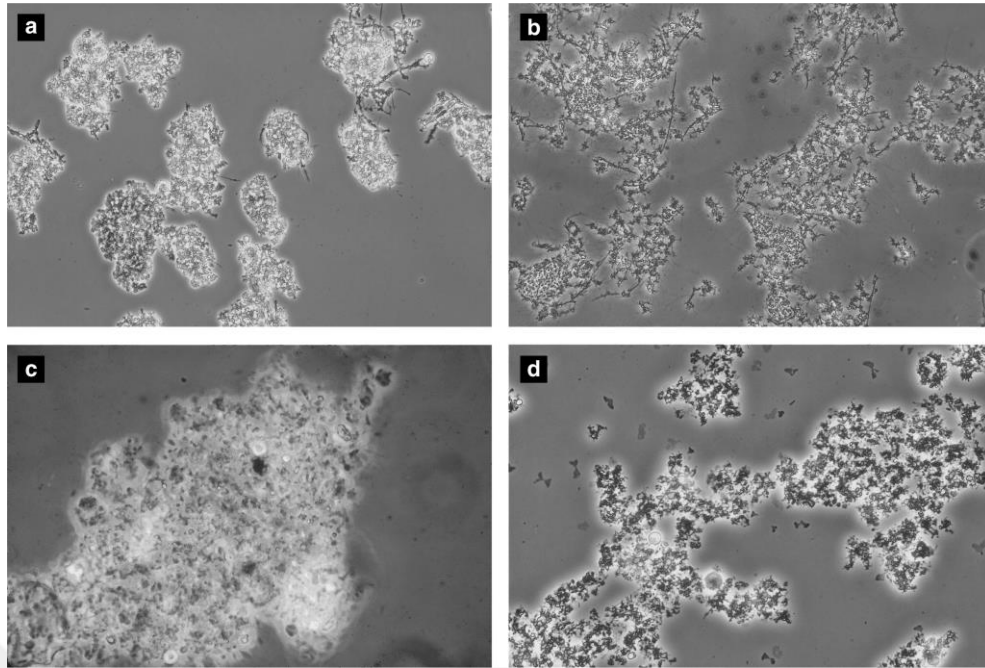


Figure 1.8: Phase contrast micrographs of floc characteristics: (a) compact, (b) diffuse, (c) firm, (d) weak. Original magnifications 100x, (a) and (b); 1000x, (c) and (d).

1.6.2 Toxicity of landfill leachate

Toxicity is the degree to which a chemical substance or a particular mixture of substances can damage an organism. In activated sludge systems, even too much of oxygen can create a toxic shock for the aerobically respiring organisms. Toxicity problems in small communities are more common due to lack of controlled toxic material dilution into the small systems. Some examples of toxicity events are; washing of cement or lime trucks to a manhole, dumping of congealed diesel fuel to the sewer system, dumping of metal, electronical goods into river bodies and overload of small systems with septage, which contains a high amount of organic acids and sulphides. As the landfill leachate composition could have different toxicities depending on the type of wastes buried in the landfill, hence the composition of leachate. Most common toxic shock caused by landfill leachate is heavy metal toxicity. Small concentrations of heavy metals are required for microorganisms to grow, however at higher does their toxicity is so powerful that the whole system can collapse. Toxicity can be diagnosed microscopically, often in the following sequence:

1. An initial flagellate "bloom";
2. Subsequent complete die-off of protozoa and other higher life forms;

3. Biomass de-flocculation, often accompanied by foaming;
4. Loss of BOD removal; and
5. Filamentous bulking upon process recovery.

Toxic wastes generally do not favour filaments directly, except H₂S toxicity; rather proliferates the growth of filaments in the system. For example, bulking by *Sphaerotilus natans* frequently follows a toxic upset due to a high F/M condition³³. Here the "true" F/M value may be many-fold that calculated based on total biomass present, due to low viability of the biomass.

1.6.3 Nitrification and denitrification problems

Nitrification can cause several problems depending on different sources in activated sludge systems. Dispersed growth and filamentous bulking problems are mostly observed during springtime when warmer temperature induces nitrification. Another problem is loss of chlorine disinfection during nitrification which is due to nitrite build-up in the system. Ammonia and nitrate do not have any chlorine demand however one chlorine is consumed per nitrite. Therefore, significant amount of chlorine dependency is present with nitrite. One of the most common problems is low pH, as low as pH 6, due to extensive nitrification and low wastewater alkalinity. As a result of low pH pin floc and high turbidity in the system is observed. Finally, denitrification in nitrification tank. When free oxygen amount is low in the system, nitrifying bacteria uses nitrate to respire and release nitrogen gas a by-product. This gas is slightly soluble in water and causes bubbles in the activated sludge. Rising bubbles of nitrogen gas result in sludge blanket flotation in the final clarifier.

Problems in denitrification are also more prevalent during warm weathers. As the biological rate increases, so does the release of nitrogen gas which causes sludge blanket flotation. The problem worsens if a filamentous sludge is already present, more extensive entrapment of the nitrogen gas.

Floating sludge in the aeration basin can lead to a significant amount of the sludge inventory in the foam, which can inhibit the process control. This problem occurs in systems with a high front-end organic loading and a long hydraulic detention time (2 days or more). Nitrification and denitrification occur at the back end of the system due to endogenous conditions there and the release of ammonia from the biomass.

Nitrification and denitrification often occur together within the floc, with no finding of free nitrate when examined [33].

1.6.4 Nutrient deficiency and bulking & foaming

Nutrient deficiency is a general problem in industrial wastes. Nitrogen and phosphorous are growth limiting nutrients and should be present in enough amounts in the influent wastewater. For complete removal of BOD, BOD₅:N:P weight ratio of 100:5:1 is required in the inflowing wastewater. Other nutrients such as iron or Sulphur are also growth limiting to activated sludge bacteria, however, is not observed as often as nitrogen and phosphorus deficiency.

Extracellular polysaccharide, responsible for floc formation, is overproduced at nutrient deficiency by the bacteria which is poorly degraded in the system. As the extracellular polysaccharide builds up in the system “slime bulking”, poor sludge settling, is observed which also leads to problems in sludge dewatering. Normal activated sludge contains from 10 to 20% polysaccharide on a dry weight basis with the higher polysaccharide content occurring at younger sludge ages. Signs of nutrient deficiency include filamentous bulking and foam on the aeration basin that contains polysaccharide [33].

1.6.5 Filamentous bulking

Filamentous bulking and foaming are common and serious problems in activated sludge operation, affecting most activated sludge plants. Many research and studies has been conducted over 20 years on filamentous bulking and foaming, the causative filaments and their causes and control, which contributed to the understanding and the database of Eikelboom and Buijsen identification system published in 1981 [43]. Later, between 1993 and 2004, Jenkins et al [41] has updated and modified the filament identification which is used worldwide. It is extremely crucial to identify the causative filaments and their causes in order to apply appropriate control measures to the systems.

A bulking sludge is defined as one that settles and compacts slowly, which generally accepted to have a sludge volume index (SVI) of >150 ml/g. However, each treatment plant is unique to suit the needs of targeted wastewater. Therefore, specific SVI values ranging from <100 ml/g to 300> ml/g is observed depending on the size and

performance of the final clarifier(s) and hydraulic considerations. Thus, sludge bulking may or may not be a “problem” for the treatment plant according to the containment of sludge within the clarifier(s).

Presence of filamentous bacteria in activated sludge system is not always a hindrance or a problem, on the contrary a certain amount is beneficial to the system. Some filaments serve as a “backbone” to floc structure, allowing formation of larger, stronger flocs helping sludge settling and some filaments act as a “net” for holding small particles which yields lower turbidity effluent. As the number of filaments in the system exceeds a certain amount, 10^7 μm filaments per gram of activated sludge, hindrance in sludge settling and compaction occurs.

Depending on the type of filament involved, two forms of interference in sludge settling occur: (1) interfloc-bridging - the filaments extend from the floc surface and physically hold the floc particles apart; and (2) open-floc structure - the filaments grow mostly within the floc and the floc grows around and attached to the filaments. Here, the floc becomes large, irregularly shaped, and contains substantial internal voids. Figure 1.9 [41] visually represents the differences between interfloc bridging and open-floc structure.

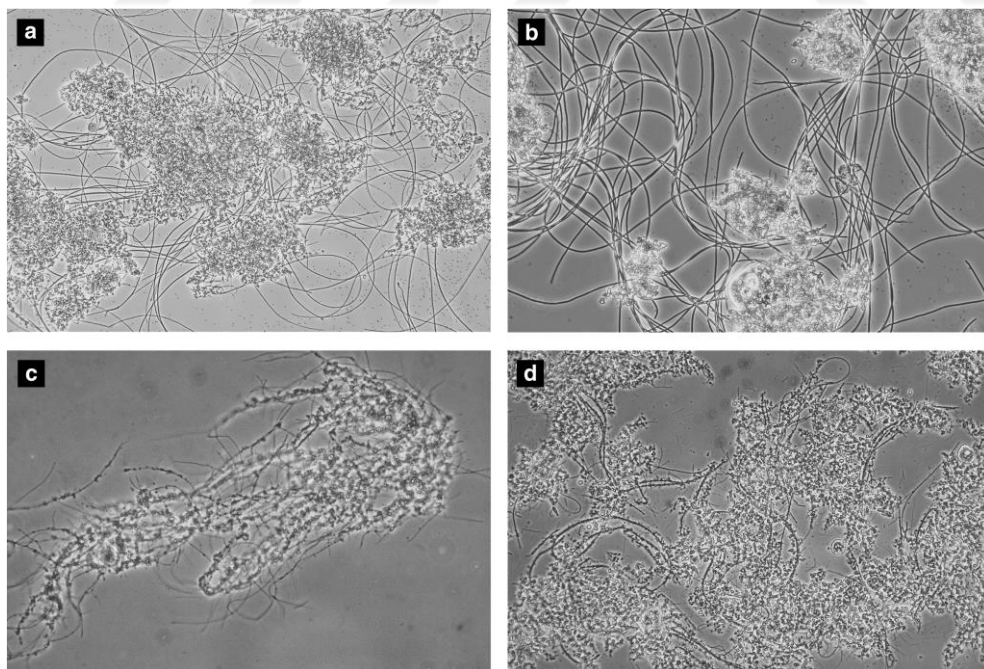


Figure 1.9: Phase contrast micrographs of effects of filamentous organisms on floc morphology and settleability: (a) and (b) interfloc bridging; (c) and (d) open floc structure, original magnification 100x.

A bulking sludge can lead to the loss of sludge inventory in the effluent, causing environmental damage and effluent that does not meet the discharge standards. If the problem is not solved, aggravated sludge inventory loss can seriously affect the treatment capacity of the plant and fail the process. Increased number of solids present during bulking also affects the disinfection of the treated wastewater negatively. Furthermore, sludge recycle rates are increased due to excessive sludge return which causes disproportionate activated sludge disposal. Many problems in waste sludge thickening are filamentous bulking problems [33].

1.7 State of Landfill Leachate Treatment Facility

Landfill treatment plant was studied for leachate treatment capability and whether any problems or drawbacks occurring in the system. The state of treatment plant at the start of 2018 was concluded and given below;

1.7.1 Low COD, phosphate concentration and F/M ratio

Chemical oxygen demand, COD, is the amount of dissolved oxygen required for oxidation of organic and inorganic matter. The treatment facility is receiving leachate with less COD and nutrients, e.g phosphate, as the landfill ages. As a result, substrate available for microorganism is getting scarce in the system which disrupts the Food to Microorganism ratio. Food to microorganism ratio, F/M, is a very important aspect in designing and operating activated sludge basins. If the F/M ratio is unbalanced several problems could occur in the system. At very high or very low F/M ratio dispersed floc formation and poor settling ability is observed in the settling tank. Figure 1.10 [44] below shows the results of High, Ideal and low F/M ratio on floc formation and microbial diversity of the system [44]. On the other hand, low nutrient inflow into the system also hinders the growth capability of targeted microorganisms, e.g *Zoogloea spp.*, as other microorganisms tend to absorb these nutrients faster.

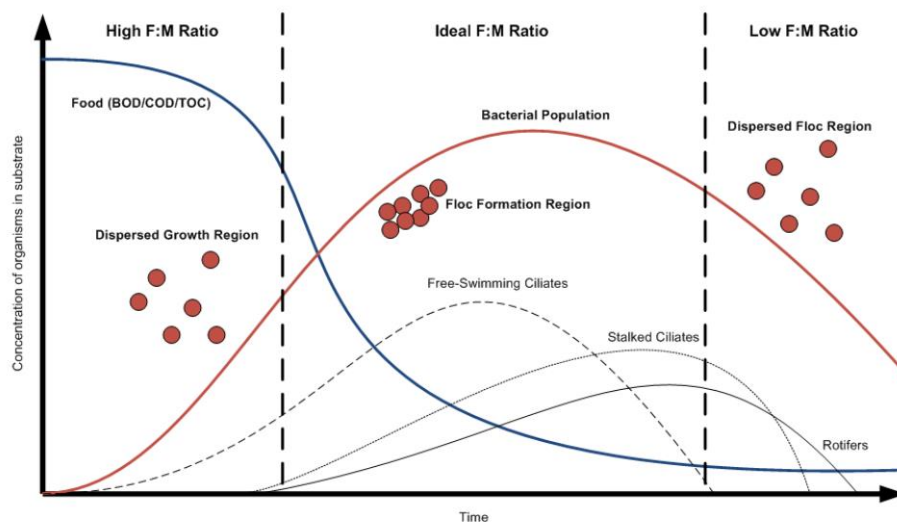


Figure 1.10: Different F/M ratio and effects on activated sludge process.

1.7.2 High Ammonia Nitrogen, $\text{NH}_4^+\text{-N}$, concentration

Both the Influent and Effluent Ammonia Nitrogen levels for the Landfill leachate treatment plant is high at the beginning of 2018. High concentration of $\text{NH}_4^+\text{-N}$ in the influent could become toxic for the microorganisms both in aerobic and anoxic tanks, resulting in incomplete treatment even shut down of the system. If the effluent $\text{NH}_4^+\text{-N}$ concentration is higher than the expected values, it means that nitrification/denitrification cycle is incomplete. Some of the $\text{NH}_4^+\text{-N}$ conversion effecting factors are F/M ratio, Temperature, pH etc., however loading of high $\text{NH}_4^+\text{-N}$ concentrations above the calculated capacity of treatment plant could also result in increased $\text{NH}_4^+\text{-N}$ effluent from the system [45].

1.7.3 Filamentous bulking and foaming

One of the most common and serious problems in the Activated sludge systems is filamentous bulking and foaming. Although filamentous organisms are required to form the backbone and help the formation of good quality settling sludge, at very low and high levels hinders the activated sludge operations with rising of sludge bed and turbid effluent. Landfill leachate treatment facility has been suffering from filamentous bulking at the start of 2018 and the causative filaments were unidentified in the system. Identification of causative filaments in the system helps to set a series of appropriate control measures rather than random application of precautions.

1.7.4 Poor sludge settling quality

In activated sludge systems with disturbed F/M ratio due to nutrient deficiency, low COD input or with high toxicity levels such as high ammonia nitrogen, the quality of settling sludge decreases. Under unfavourable environmental conditions, the growth and floc formation abilities of zoogloal cells are hindered. As a result of this, small flocs are formed, treatment capability reduced, settling of sludge disrupted and turbid effluent is formed. In 2018, The settling sludge of the Treatment facility showed below the expected agglomeration properties, resulting in blockage of UF and NF filters.

1.8 Mathematical modelling study

A modelling study has been conducted for the improvement of landfill leachate treatment plant rather than blindly applying a series of changes in the AS system. In this study, the most commonly used mathematical modelling program for activated sludge systems GPS-X version 6.5, developed by Hydromantis Environmental Software Solution inc. that include Mantis2 and Mantis3, modified versions of ASM1, ASM2 and ASM3 activated sludge models, by default was used [46]. GPS-X program was used to simulate process flow schematics of landfill leachate treatment plant in order to improve and stabilise the dynamic conditions [47]. GPS-X software and ASM1 activated sludge model was used in the simulation of landfill leachate treatment plant. ASM1 model includes carbon removal, nitrification and denitrification processes and is the most common model used for wastewater treatment plants [48].



2. MATERIALS AND METHODS

2.1 Materials

Detailed explanation and description of materials that have been used throughout the experiments have been given in the below sections.

2.1.1 Leachate sample

Leachate samples used in this study were supplied from the Sanitary solid waste landfill in Istanbul, Turkey. Samples were collected from 2 meter below the aerobic and anoxic tanks and are stored in 1L, airtight containers and delivered immediately to the Environmental Biotechnology Microbiology Lab, ITU once every two weeks for examination.

2.1.2 Laboratory equipment

Laboratory equipment used in this study is given in Table 2.1.

Table 2.1: Laboratory equipment.

Laboratory Equipment	Firm/Model
Microscopy slides	
• Isotherm microscope slides	• Straight cut, 50 pc
• Isotherm microscope cover glass	• 20x20 mm, 100pc
Microscope	Olympus BX50 Model
Microscope Attached Camera	Kameram21 CMOS
Pasteur pipettes	İsolab Pasteur Pipette plastic-3 ml
Drying Rack	-
Graduated Cylinder 1L	-
Timer	-

2.1.3 Reagents and preparation

Reagents used to prepare required solutions for experiments in this study are given in Table 2.2.

Table 2.2: Reagents

Reagents	Application Area
Crystal Violet	Gram and Neisser staining - the primary Stain
a. Ammonium oxalate	Gram staining - preparation of Crystal violet
Iodine Solution	Gram staining - the mordant
a. Iodine	Gram staining - preparation of Iodine solution
b. Potassium iodide	
Ethanol (95%)	Gram staining - decolorizer
Safranin O	Gram staining – the counterstain
Neisser Solution	Neisser staining – the primary Stain
a. Methylene Blue	Neisser staining – preparation of Neisser solution
b. Glacial acetic acid	
Bismark Brown	Neisser staining – the Counterstain
Water	Gram and Neisser staining – wash liquid

2.1.3.1 Gram staining

Reagents Prepare fresh every 3 to 6 months.

Solution 1: Prepare Solutions A and B separately, then combine them.

- Solution A: 2 g Crystal Violet + 20 mL ethanol 95%.
- Solution B: 0.8 g ammonium oxalate + 80 mL distilled water.

Solution 2: 1 g iodine + 2 g potassium iodide + 300 mL distilled water.

Decolorizing solution Ethanol 95%.

Solution 3: 10 mL Safranin O (2.5% w/v in 95% ethanol) + 100 mL distilled water.

2.1.3.2 Neisser staining

Reagents Prepare fresh every 3 to 6 months.

Solution 1: Prepare Solutions A and B separately.

- Solution A: 0.1 g Methylene Blue + 5 mL ethanol 95% + 5 mL glacial acetic acid + 100 mL distilled water.
- Solution B: 3.3 mL Crystal Violet (10% w/v in 95% ethanol) + 6.7 mL ethanol 95% + 100 mL distilled water.
- Mix two parts by volume of A with one part by volume of B.

Solution 2: 33.3 mL Bismarck Brown (1% w/v aqueous) + 66.7 mL distilled water.

2.2 Methods

Samples for microscopic examination were prepared from the delivered leachate samples taken from aerobic and anoxic tanks, contained in airtight containers. The original samples were immediately observed after sampling under bright field and phase-contrast microscopy by preparing wet-mounts slides. These viable original samples were prepared using 0.5 mL (about one drop) of leachate sample on a glass slide. Four air-dried smear slides, two for anoxic and two for aerobic tank samples, were also prepared for Gram and Neisser staining. The stained slides were prepared spreading by 0.5 mL activated sludge sample on glass smear slides and allowing them air dried in a room temperature. The Gram and Neisser staining methods were applied according to modifications of Jenkins

et al 2004 [41]. The wet mounts slides were examined by using 40X, 100X, and 200X magnifications of phase contrast and bright field microscope where Gram and Neisser stained slides were examined under bright field microscopy by using 40X, 100X, 400X, and 1000X magnifications. The dimensions of the filamentous bacteria were measured on taken micro photos by using software, called “Kameram digital mikroskopi”. The calibrations of measurements were corrected by using an ocular and an objective micrometre. The microscopic analyses were performed using an Olympus BX50 Model research microscope with attachments of phase contrast microscopy, Kameram21 CMOS camera and “Kameram digital mikroskopi” analysis computer program. The filamentous bacteria were identified by using schemas, pictures and microbiological characteristics that were defined by Jenkins et al 2004 3rd edition. The abundance of each filamentous bacterium was counted using the subjective scoring method developed by also Jenkins et al 2004 [41].

2.2.1 Smear slide preparation

Leachate samples for wet mount slide observation, Gram staining and Neisser staining were prepared by smear preparation procedure. The purpose is to fix the bacteria onto the previously prepared slide to prevent the loss of sample during a staining procedure. A solid or broth medium can be used to prepare a smear, however in our study only broth medium is used. Preparation steps are shown below;⁴⁹

1. Place one, drop of liquid leachate sample, approx. 0.5 mL, in the centre of a clean slide.
2. Mix the specimen with inoculating loop completely and spread the mixture out to cover about half of the total slide area.
3. Place the slide on a slide heater and wait for it to dry. The smear is ready for the staining procedure after its completely dried. If wet sample observation is the case, the prepared smear on the slide should be directly observed under microscope before it dries out.

2.2.2 Gram staining procedure

This procedure highlights bacterial cells against its background with the use of dyes. All reagents must be easily accessible on the bench before the staining procedure, to save

time, safety goggles and lab coat must be worn. Gram staining procedure steps are shown below;

1. A smear is prepared onto a clean slide.
2. The slide is placed on a slide holder or rack. Gently cover the entire slide completely with crystal violet reagent solution. Let the crystal violet stand for about 60 seconds. Rinse the slide gently after for 5 seconds with water. After the wash, the specimen should appear blue violet to the naked eye.
3. Gently flood the slide with iodine solution reagent and let it stand about 60 seconds. After the time expires, rinse the slide with water for 5 seconds and immediately proceed to step three. The specimen should still be blue-violet colour.
4. In this step, Ethanol is used as decolourizer. Add ethanol dropwise onto the slide until the blue-violet colour is no longer emitted and follow with a gentle water rinse for 5 seconds. This step is important as too much or too less decolourizer could result in false Gram (-) or Gram (+) results, respectively.
5. In the 4th, final step, the counter stain, safranin reagent solution is applied to the slide. Gently cover the slide with the dye and let it stand for 60 seconds in order to allow the bacteria to incorporate the safranin. Again, rinse with water for 5 seconds to remove any excess dye. DO NOT RUB THE SMEAR.
6. Blot the slide gently with bibulous paper or allow it to air dry before viewing it under the microscope.

After all the steps are completed the Gram-positive cells, that incorporated little or no counterstain and will remain blue violet in appearance and Gram-negative bacteria, however, take on a pink colour and are easily distinguishable from the Gram-positive⁵⁰.

2.2.3 Neisser staining procedure

This procedure is used to test for the presence of polyphosphates stored in the cells. Neisser staining aids identification of certain strains of filamentous bacteria and renders bio phosphorus bacteria visible. Neisser staining procedure steps are shown below;

1. A smear is prepared onto a clean slide.

2. The slide is placed on a slide holder or rack. Gently cover the entire slide completely with Neisser solution reagent, mixture of solution A and B. Let the solution stand for about 60 seconds. DO NOT RINSE WITH WATER! Gently tilt the slide to one side and allow the excess Neisser solution to runoff.
3. Gently flood the slide with Bismarck solution and cover surface of the specimen. Let the solution stand for about 60 seconds. Afterwards gently rinse the slide under water for 5 seconds.
4. Blot the slide gently with bibulous paper or allow it to air dry before viewing it under the microscope [51].

2.3 Analytical Methods

In this part the analytical methods that are used in this study are shown in detail.

2.3.1 Sludge volume index (SVI)

Sludge volume index (SVI) is used to measure the settling characteristics of the landfill leachate treatment facility sample. The method is defined as “the volume, in ml, occupied by 1 gram of activated sludge after settling the liquid for 30 minutes”. A graduate cylinder, 1L, and a stopwatch reading in minutes is used as apparatus for this method. Procedure follows as [52];

- 1) Pour 1 L freshly sampled mixed liquor into the graduate cylinder.
- 2) Allow to settle quiescently out of direct sunlight for 30 min.
- 3) After 30 min., record volume occupied by the settled sludge.
- 4) MLSS is measured at the treatment facility.

Equation (2.1), below shows the calculation formula of SVI values;

$$SVI, \frac{ml}{mg} = \frac{\text{Settled sludge Volume} \div \text{Sample Volume, ml/L}}{\text{Suspended Solids Concentration, mg/L}} \times \frac{1,000 \text{ mg}}{g} \quad (2.1)$$

2.3.2 Microscopic examination methods

Microscopic examination methods are separated into two sections. The first method is used for filament counting; the second methods include examination of activated sludge for floc and filamentous organism characterisation.

2.3.3 Filament counting

Filament counting (FC) method utilized the use of “Kameram dijital mikroskopi” software to draw a hairline to the side of the observed area. A known volume of sample is placed on a slide and covered with a cover slip. The entire width of the cover slip is observed in a series of consecutive fields and the number of times that filament intersects the hairline in each field is observed and totalled for the entire width of the slide. Procedure follows as [53];

- 1) Transfer 50 µl mixed liquor sample to a glass microscope slide.
- 2) Cover completely with a 20 x 20 mm glass cover slip
- 3) Using 100x total magnification and starting at the edge of the cover slip, observe consecutive fields across the entire 20 mm lengths of the cover slip. At 100x magnification width of each field is about 110 µm, hence number of fields are 20 mm divided by 110 µm is approximately 181 units.
- 4) Count the number of times that any filamentous organism is present in each field.
- 5) Sum the number of filaments for the entire length of the cover slip. To express the FC as a unit filament count, following calculation is used:

Equation (2.2), below, shows the calculation formula of unit filament count;

$$\text{Unit filament count, } \frac{\text{number}}{\text{uL}} = \frac{\text{FC} \times \text{number of fields in width of slide (181)}}{50 \text{ uL}} \quad (2.2)$$

2.3.4 Floc characteristics and overall filament abundance

2.3.4.1 Floc size

A set number of flocs, 20, from each mixed liqueur sample is measured at 100x and 200x total magnification and placed in the following size categories based on their minimum dimensions or diameters if they were approximately spherical:

- Small $\leq 150 \text{ µm}$
- Medium 150-500 µm
- Large $\geq 500 \text{ µm}$

2.3.4.2 Floc characteristics

Flocs were determined whether to be round or irregular and compact or diffuse; and texture was firm or weak all according to Jenkins et al. 2004 [41]. Figure 2.1 [41]. shows floc characteristics that were used as a base for the determination of sample characteristics.

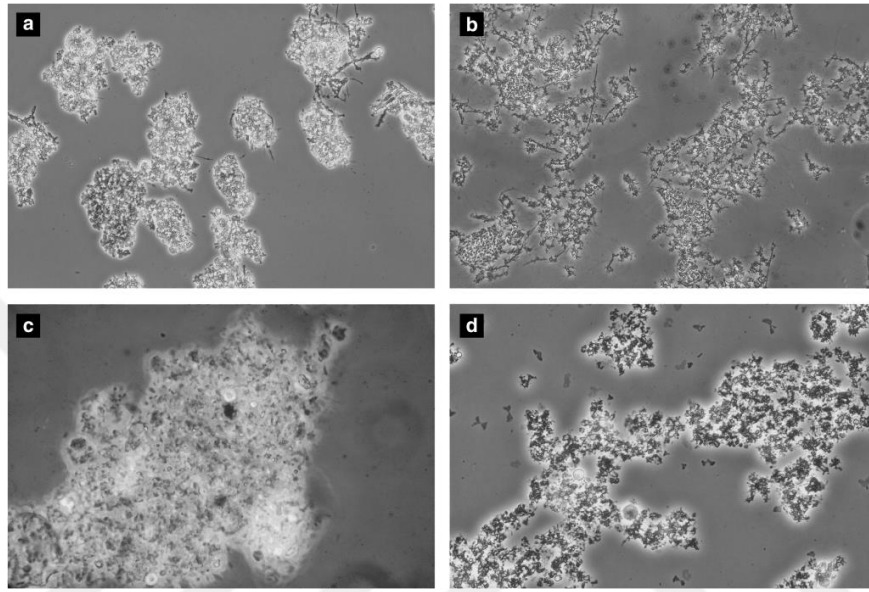


Figure 2.1: Phase contrast micrographs of floc characteristics: (a) compact, (b) diffuse, (c) firm, (d) weak. (Original magnifications (Original magnifications 100x, (a) and (b); 1000x, (c) and (d)

2.3.4.3 Protozoa and other microorganisms

The presence and types of protozoa and other microorganisms such as rotifers, nematodes, actinomyces were determined using several sources from the internet and books. Figure 2-2 [41] shows some of the pictures used as the base of determination of organisms from Jenkins et al 2004 [41];

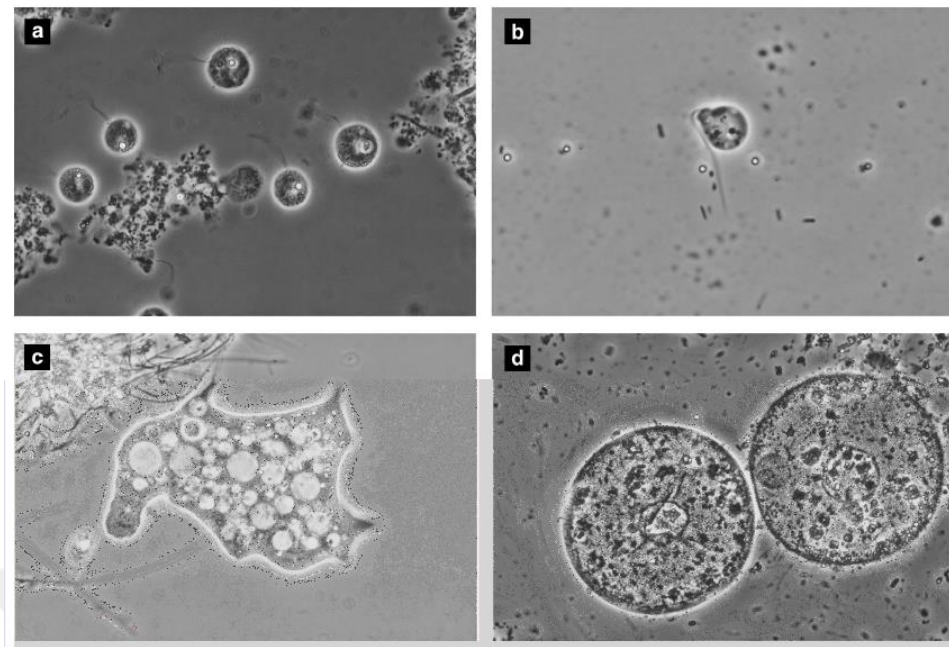


Figure 2.2: Phase contrast micrographs of common flagellates and amoebae found in activated sludge: (a) *Monas* sp., (b) *Bodo* sp., (c) *Polychaos* sp., and (d) *Arcella* sp. Original magnifications 1000x, (a) and (b); 400x, (c), and (d).

2.3.4.4 Bacterial colonies

Bacterial colonies composed of one type of microorganisms such as zoogloea and nitrifying bacteria were noted during the observations. In general, some of the bacterial colonies that may be observed to include fingered and amorphous zoogloea and nitrifying bacteria. A common problem in activated sludge treatment of industrial wastewaters is the poor diversity of flocs. In this case, an entire floc seems to consist of one morphological type of microorganism. Figure 2.3 [41] shows fingered formation of zoogloea cells below;

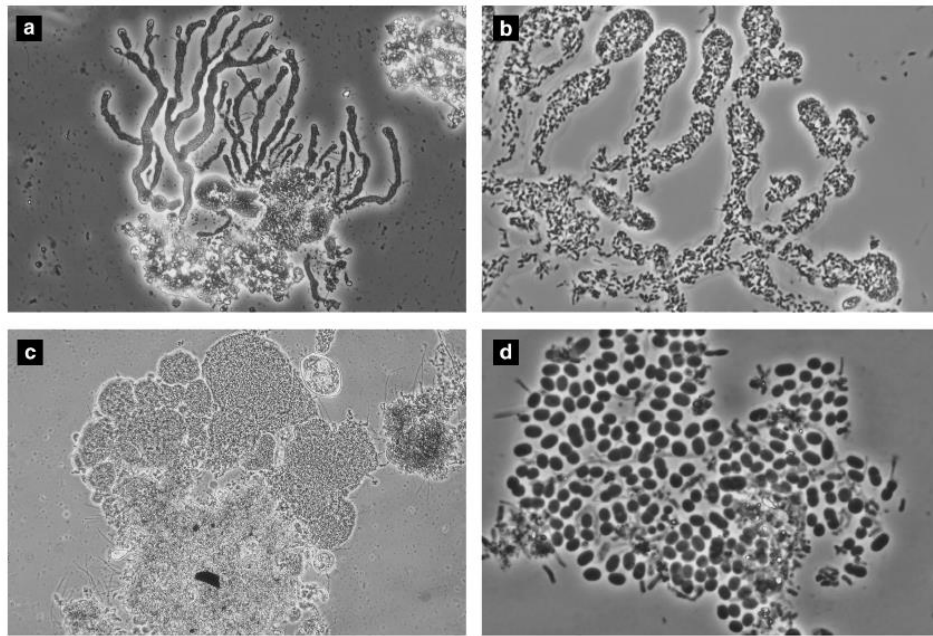


Figure 2.3: Phase contrast micrographs of zoogloea: (a) and (b) fingered, (c) and (d) amorphous (globular). (Original magnifications 200, (a); 1000x, (b); 100x, (c); and 1000x (d).

2.3.4.5 Effects of filamentous organisms on floc structure

The effect of filamentous organisms on floc structures were identified as follows:

- 1) None
- 2) Bridging: Extension of filaments from floc surface into the bulk solution and acting as bridges between the flocs
- 3) Diffuse floc structure: Irregularly shaped flocs with internal voids as a result of bacterial colonies growing around the filamentous organisms.

Figure 2.4 [41] shows filamentous organism effects on floc structure;

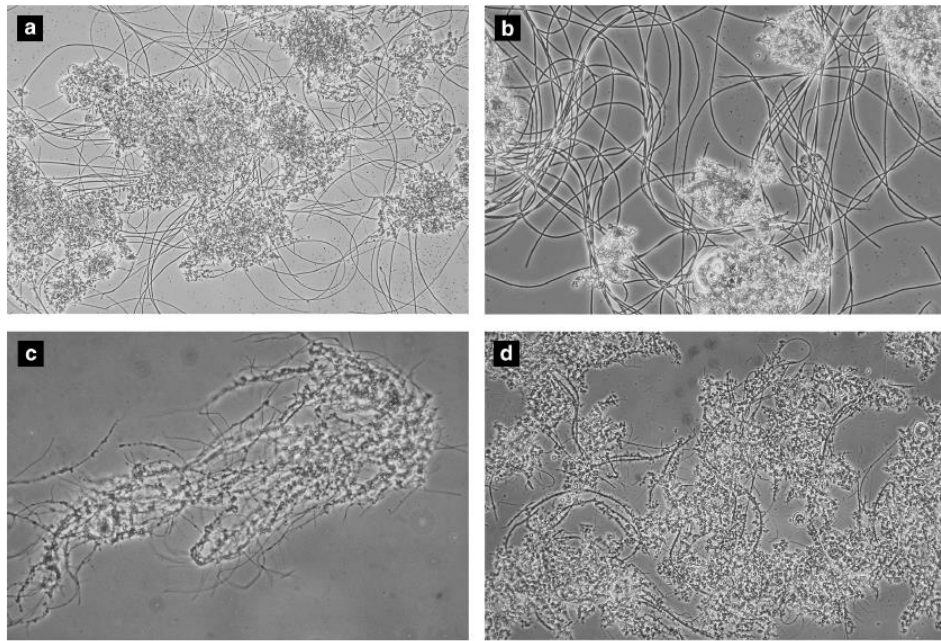


Figure 2.4: Phase contrast micrographs of effects of filamentous organisms on floc structure: (a) and (b) interfloc bridging; (c) and (d) diffuse floc structure. Original magnification 100x.

2.3.4.6 Filament abundance

Subjective scoring system of Jenkins et al 2004 [41], The filaments were observed at 40x, 100x and 1000x, and subjectively rated the overall filament abundance on a scale from 0 (none) to 6 (excessive) (in table 10). Three of the most abundant and dominant filaments were chosen to be studied and considered responsible if they are scored very common or greater. Table 2.3 [41] was used to determine filament abundance.

Table 2.3: Subjective scoring of filament abundance.

Numerical Value	Abundance	Explanation
0	None	No filaments observed
1	Few	Filaments present, but only observed in an occasional floc
2	Some	Filaments commonly observed, but not present in all flocs
3	Common	Filaments observed in all flocs, but at low density (1 to 5 filaments per floc)
4	Very Common	Filaments observed in all flocs at medium density (5 to 20 per floc)
5	Abundant	Filaments observed in all flocs at high density (>20 per floc)
6	Excessive	Filaments observed in all flocs (more filaments than floc and/or filaments growing in high abundance in bulk solution)

Figure 2.5 [41] below, shows the filament abundance categories taken from Jenkins et al 2004 [41].

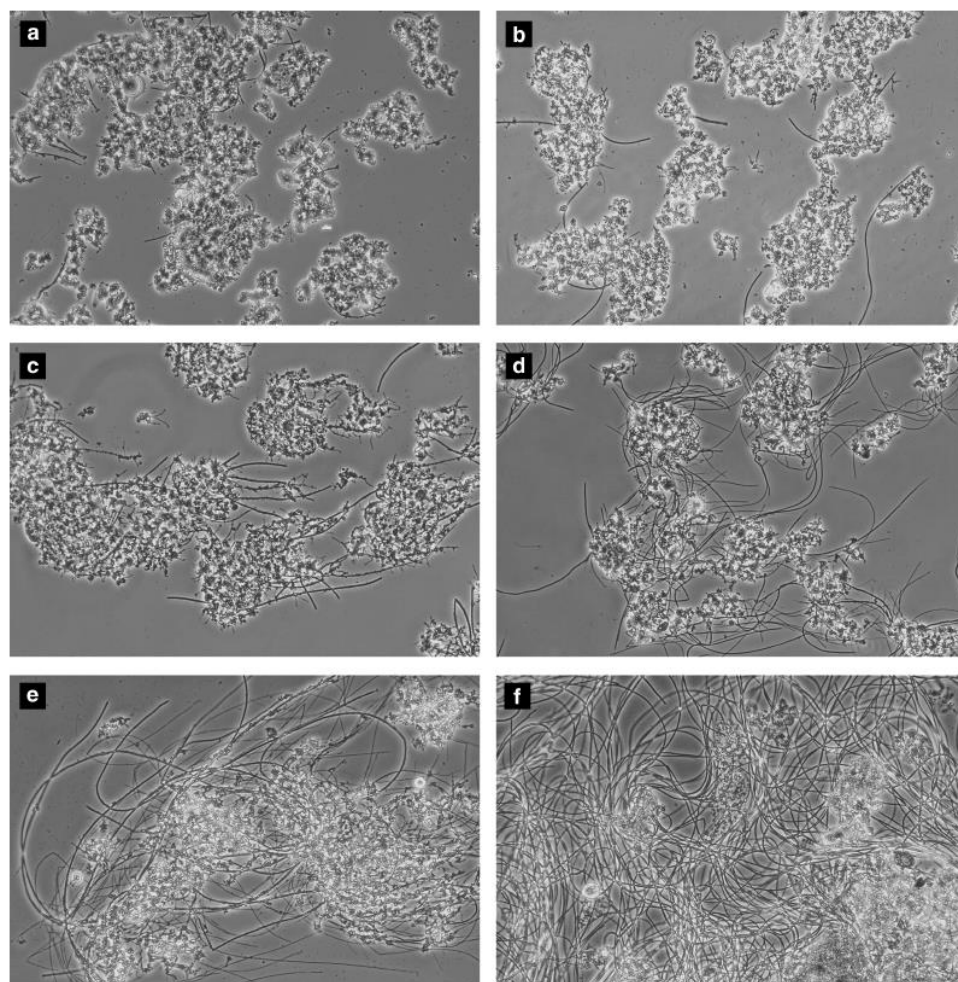


Figure 2.5: Phase contrast micrographs of filament abundance categories using the subjective scoring system: (a) few, (b) some, (c) common, (d) very common, (e) abundant, and (f) excessive. Original magnification 100x..

2.3.5 Filamentous organism characteristics

Filamentous organism types and abundances were determined by observing each type of filament under 1000x phase contrast. Worksheets provided in Jenkins et al. 2004 [41] were used to characterise the filamentous organisms. Characteristics that helped to distinguish different types of filamentous organisms are given below:

2.3.5.1 Branching

Absence or presence of branching is reported and, if present, true or false branching is distinguished. True branching shows contiguous cytoplasm between branched

filaments where false branching merely has two filaments stuck together and grown outward.

2.3.5.2 Motility

The organism reported to be motile or nonmotile and, if motile, the type of motility is described. Only a few filamentous organisms in activated sludge are motile. *Beggiatoa* spp., *Flexibacter* spp., and some blue green *Cyanophyceae* bacteria exhibit gliding motility; *Thiothrix* spp. and Type 021N may display limited twitching or swaying motions.

2.3.5.3 Filament shape

Filamentous organisms determined whether to be straight, smoothly curved, bent, irregularly shaped chains of cells, coiled or mycelial.

2.3.5.4 Location

Location of filamentous organisms reported whether to extend from the floc surface, exist within the floc, or were free in the liquid between flocs.

2.3.5.5 Sheath

Presence or absence of a sheath is reported. Due to having a clear structure outside the cell wall, it is difficult to distinguish a true sheath. Sheaths are most easily seen when filaments are isolated from flocs and several feature of filamentous organisms can be confused with sheaths, therefore a critical eye on identification is required.

2.3.5.6 Filament width

Kameram djital mikroskopi software was used to measure both average width and its range in μm and categorized as thick or thin filament, $>1\ \mu\text{m}$ or $<1\ \mu\text{m}$ respectively.

2.3.5.7 Filament length

Kameram djital mikroskopi software was used to report the average length and range of lengths in μm .

2.3.5.8 Cell shape

Observed cell shaped determined to be square, rectangular, oval, barrel, discoid, sausage-shaped, or irregular.

Figure 2.6 [41], below, shows some of the most common filamentous organism cell shapes;

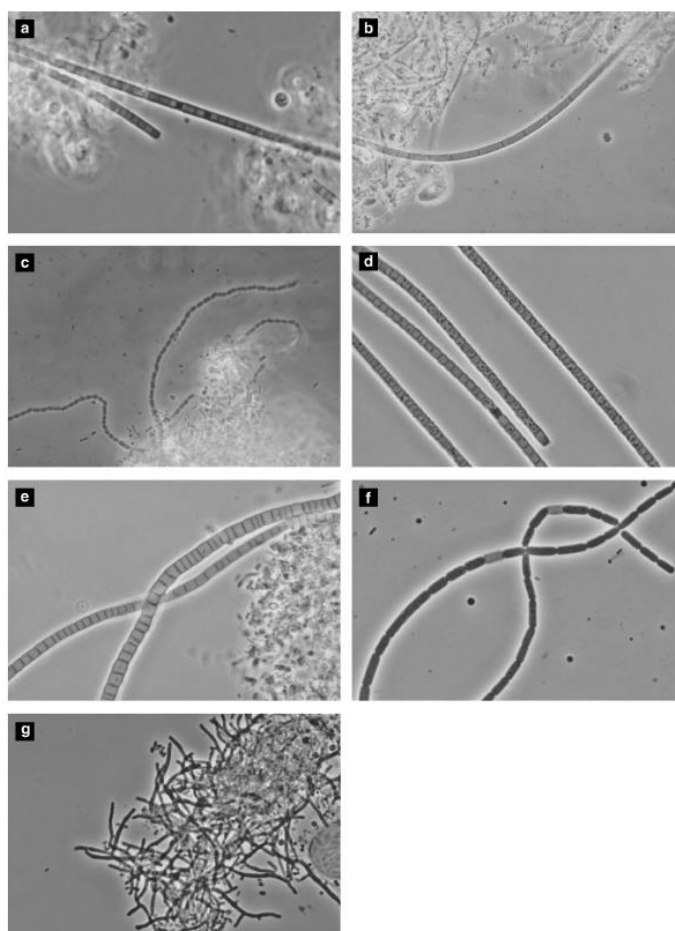


Figure 2.6: Phase contrast micrographs of filamentous organism cell shapes: (a) square, (b) rectangular, (c) oval, (d) barrel, (e) discoid, (f) sausage, and (g) irregular. Original magnification 1000x.

2.3.5.9 Staining reactions

Staining reactions of each filamentous organisms with Gram and Neisser stains were evaluated at 1000x using the direct illumination. Results reported based on whether the organisms were positive or negative with the stains.

2.3.6 Filamentous organism identification

The results of filamentous organism characteristics were used in the dichotomous key provided by Jenkins et al 2004 [41] in order to identify the organisms. The dichotomous key is shown below in Figure 2.7 [41]. The identification process was simplified by using a limited number of characteristics to describe the organism, which then the most common filamentous bacteria in activated sludge was listed to make a match. It is important to take into consideration that using the dichotomous key may or may not address all variable and identification is not absolute. Other DNA identification techniques, such as 16s rRNA, provides an absolute identification of the filamentous organism. However, identification with the dichotomous key provides a fast and easy first impression for the researchers to understand what type/kind of filamentous organisms they are dealing with.

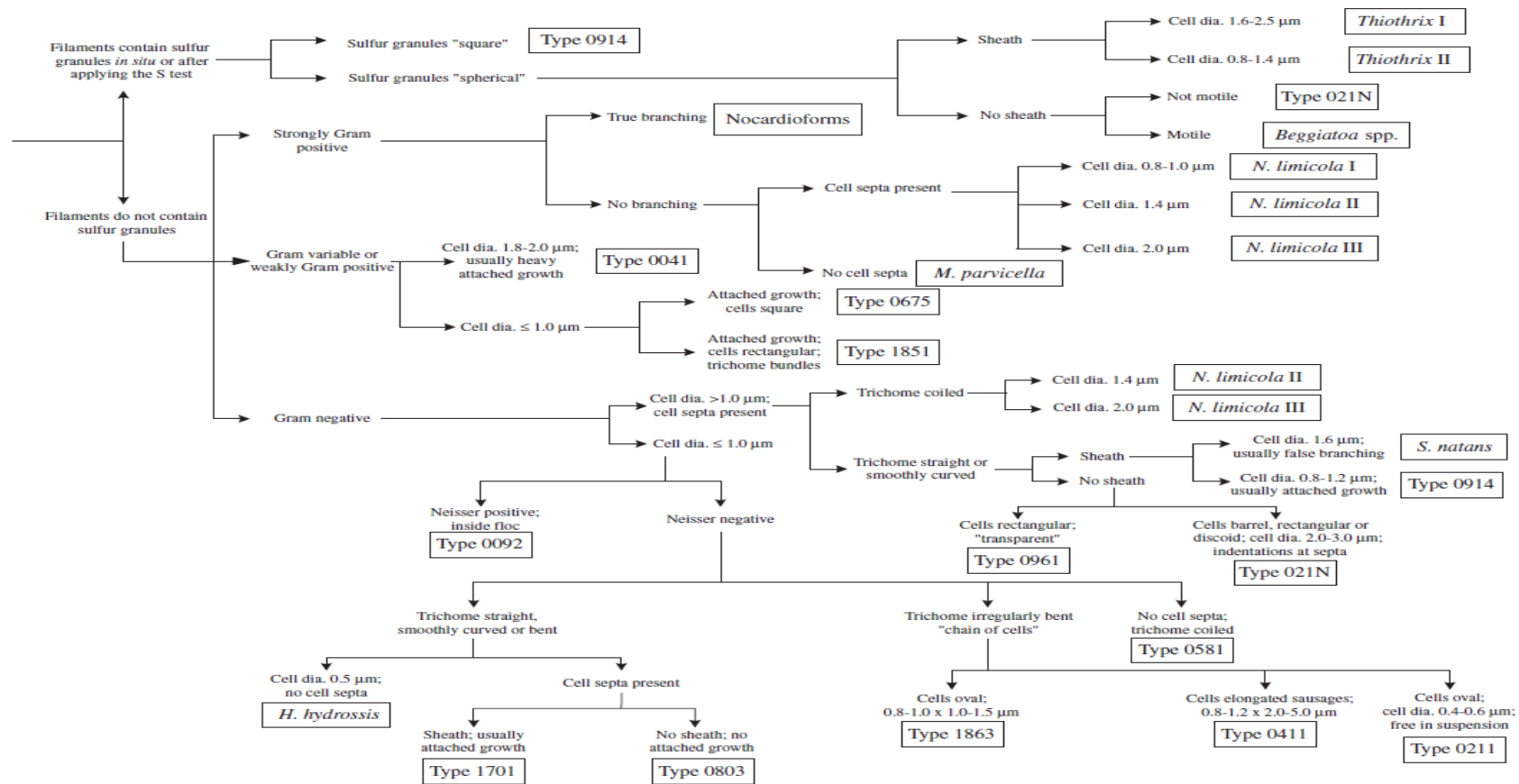


Figure 2.7: Dichotomous key for identification of filamentous organism identification.



3. RESULTS AND DISCUSSION

3.1 Main Parameters

Characteristics of landfill leachate changes as the time passes. In this section, main parameters; Chemical Oxygen Demand (COD), Total Nitrogen (TN), Orthophosphate ($\text{PO}_4\text{-P}$) and Ammonia Nitrogen ($\text{NH}_4^+\text{-N}$), were compared against data of 5 years ago. The results are shown below:

3.1.1 Chemical oxygen demand 2013-2018

Figure 3.1 shows that COD fluctuation in 2013 was much higher than in 2018. The COD value reaches as high as ~54000 mg/L during 2013 where in 2018, it hardly reaches ~20000 mg/L. The COD-2013 data fluctuates within a wide range of 15000-54000 mg/L, where COD-2018 data shows fluctuation within a smaller band of 6500-18500 mg/L.

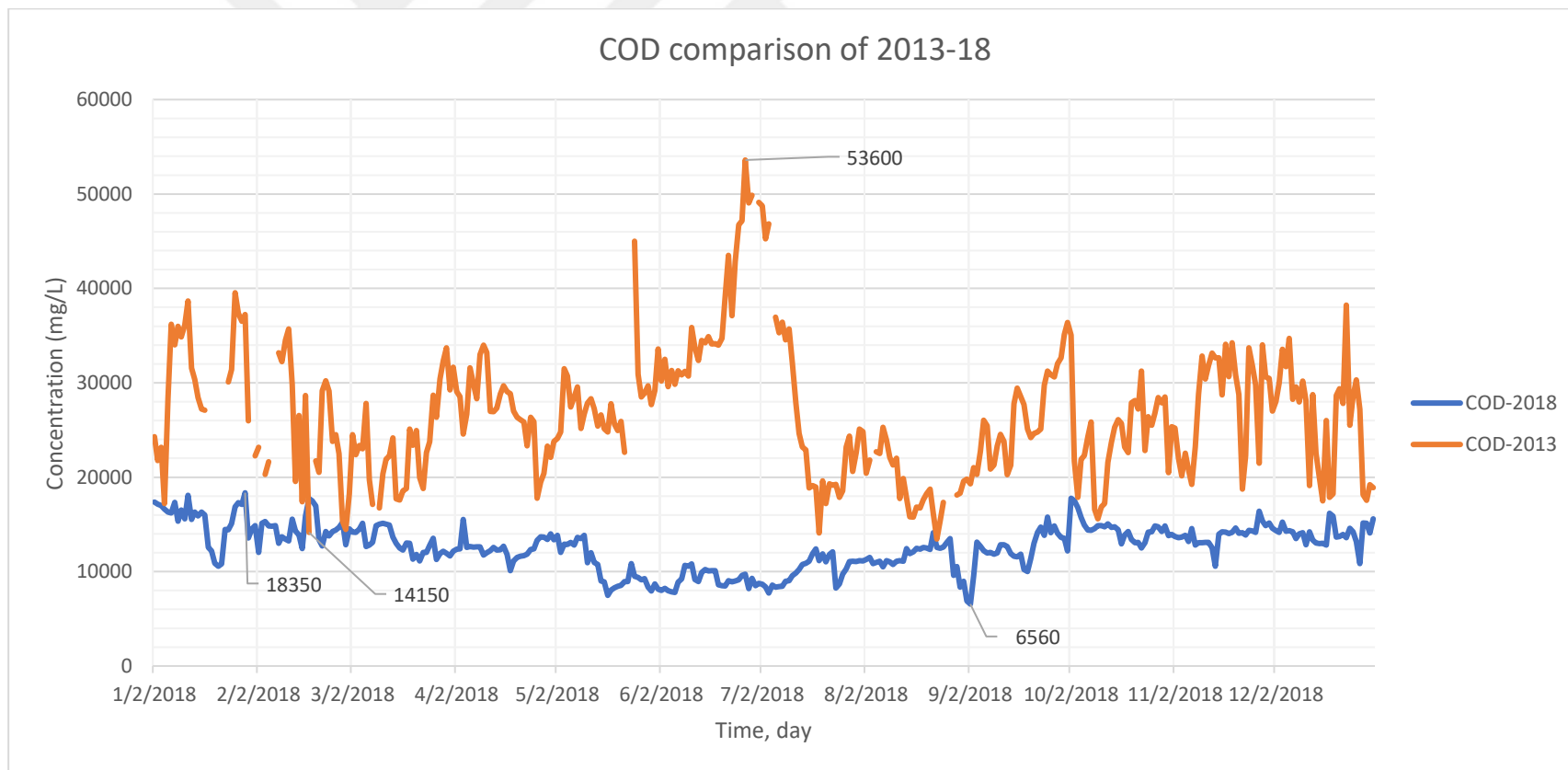


Figure 3.1: COD data comparison of 2013-2018.

3.1.2 Orthophosphate 2013-2018

Figure 3.2 shows that $\text{PO}_4\text{-P}$ fluctuation in 2013 was much higher than in 2018, especially at 7th moth. $\text{PO}_4\text{-P}$ concentration dropped as low as 5 mg/L and reaches as high as 16 mg/L within the same month, 2nd, of 2018. In 2013, lowest $\text{PO}_4\text{-P}$ value was 8 mg/L and peaks as high as 46mg/L. 2013 data shows vast fluctuations within a band of 8-46 mg/L where 2018 data shows less fluctuation within a range of 5-16 mg/L.



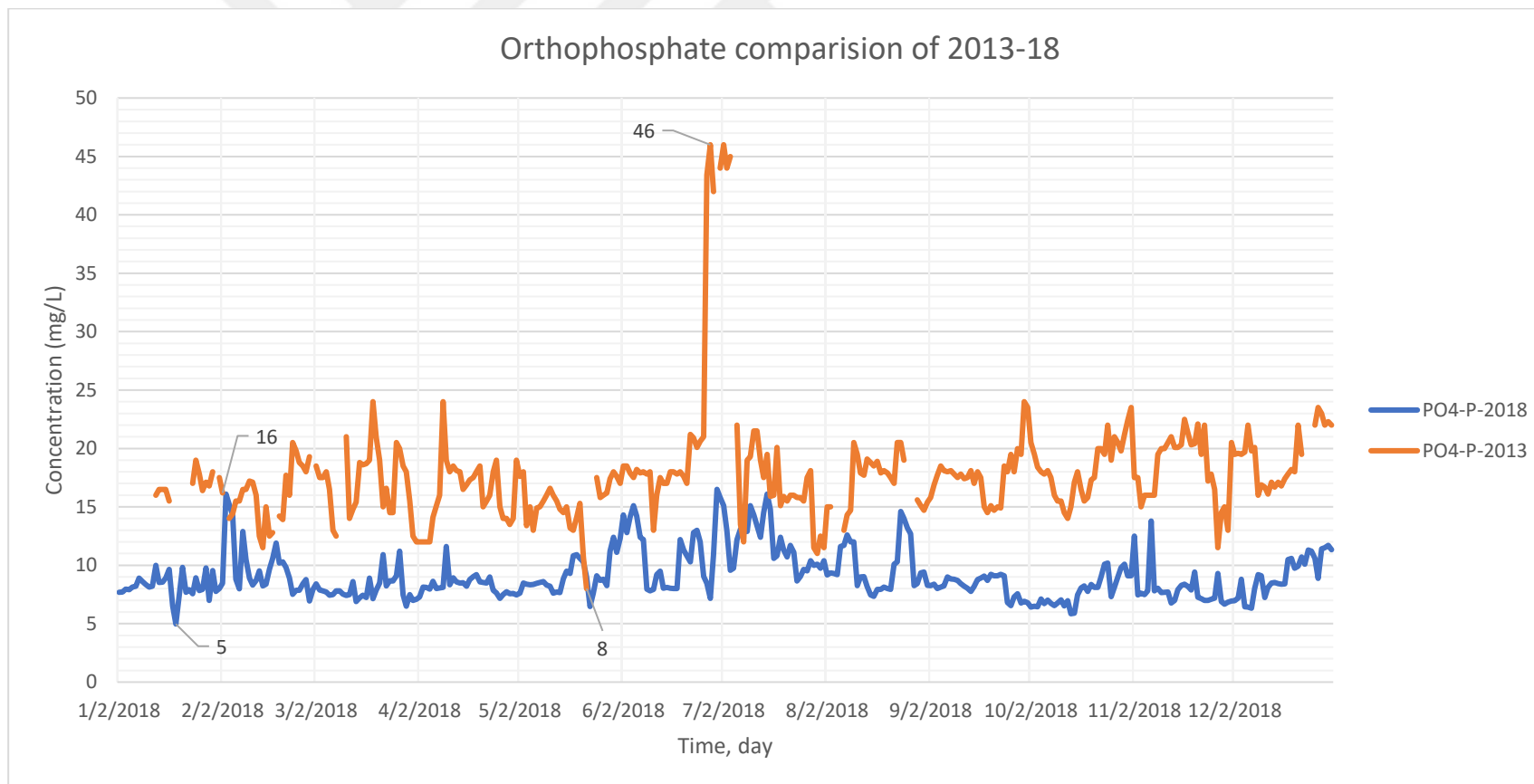


Figure 3.2: Orthophosphate data comparison of 2013-2018.

3.1.3 Total nitrogen 2013-2018

Figure 3.3 shows that TN fluctuation in 2018 was much higher than in 2013, especially in later months of the year. The TN value reaches as high as ~7200 mg/L during 2018 where in 2013, it hardly reaches ~3900 mg/L. The TN-2013 data fluctuates within a short band of 2000-3900 mg/L, where TN-2018 data shows high fluctuation within a wide range of 1900-7200 mg/L.



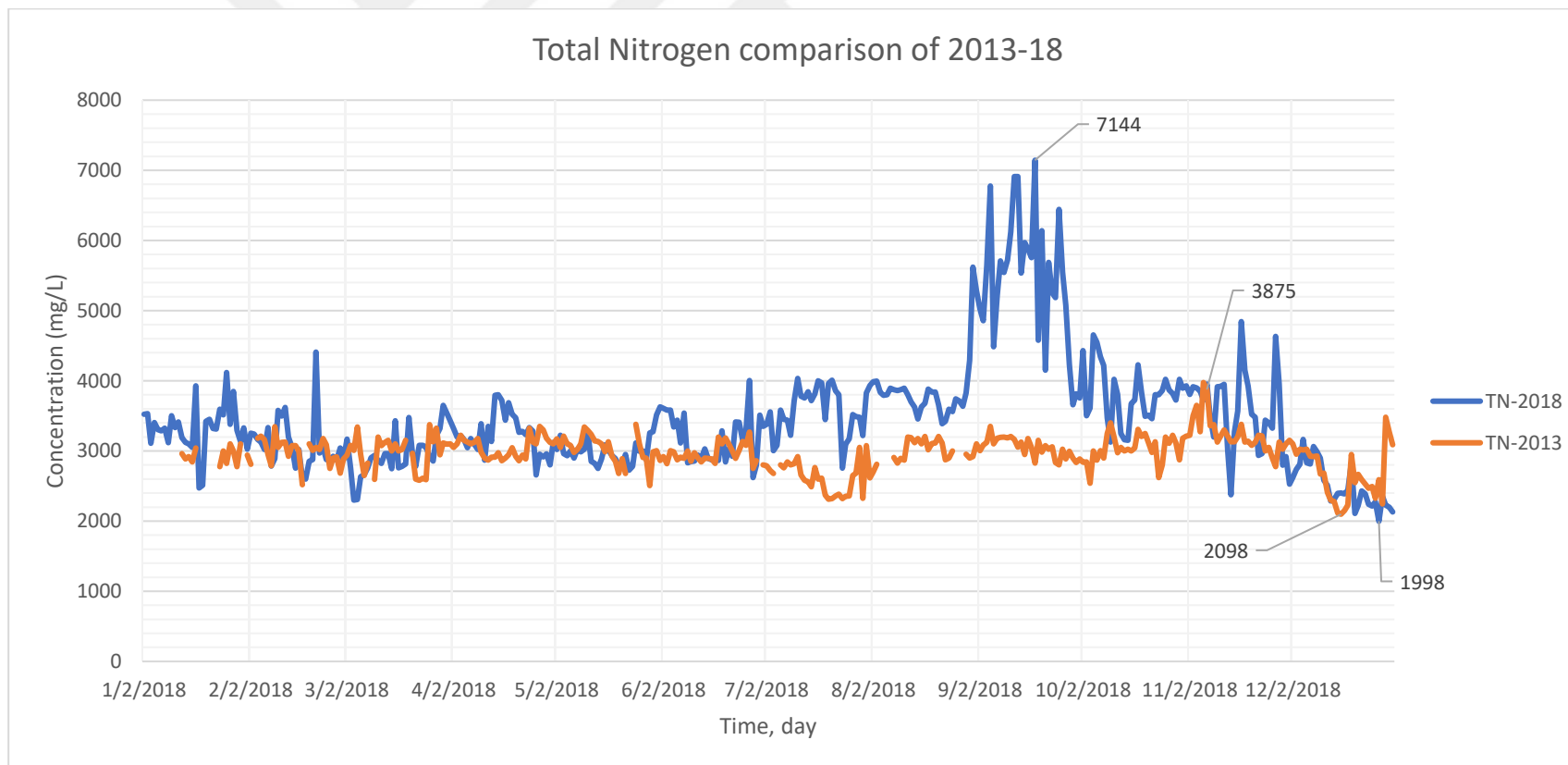


Figure 3.3: Total nitrogen data comparison of 2013-2018.

3.1.4 Ammonia nitrogen 2013-2018

Figure 3-4 shows that $\text{NH}_4^+\text{-N}$ fluctuation in 2018 was much higher than in 2013, especially in later months of the year. $\text{NH}_4^+\text{-N}$ concentration was lowest at 10th month in 2018 and reaches as high as ~4000 mg/L during 2018 where in 2013, it hardly reaches ~2750 mg/L. $\text{NH}_4^+\text{-N}$ -2013 data fluctuates within a band of 500-2750 mg/L, where $\text{NH}_4^+\text{-N}$ -2018 data shows high fluctuation within a wide range of 500-4000 mg/L.



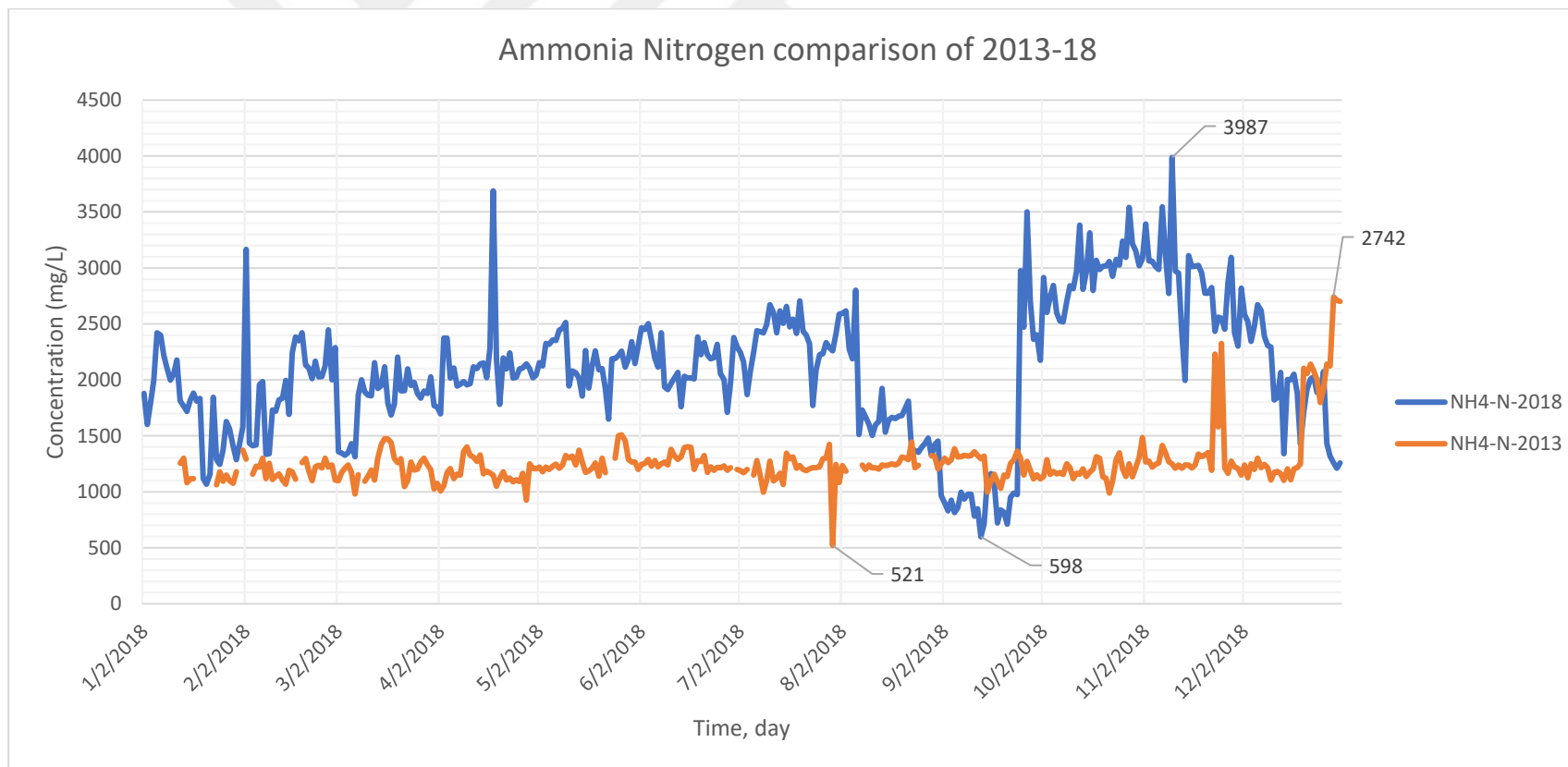


Figure 3.4: Ammonia nitrogen data comparison of 2013-2018.

A comparison table of minimum, maximum and mean values are given below in Table 3.1. COD and PO₄-P mean dropped almost in half from 2013 to 2018, where TN and NH₄⁺-N mean increased 1.45 times in 2018, compared to 2013.

Table 3.1: Compared min., max., mean and std values of leachate parameters of 2013-2018.

Parameter	COD		TN		NH ₄ -N		PO ₄ -P	
Unit	Mg/L		Mg/L		Mg/L		Mg/L	
Year	2013	2018	2013	2018	2013	2018	2013	2018
Min	13450	6560	2098	1998	521	598	8	5
Max	53600	18350	3975	7144	2742	3987	46	17
Mean	26726	12596	2964	3460	1262	2095	18	9
Std	6909	2383	265	821	232	593	5	2

3.1.5 pH and temperature data

Figure 3.5 and 3.6 shows pH and Temperature data of 2018 in the treatment plant. In Figure 3.5, pH of inflow, which is the pH in equalisation tank, was observed to have a maximum value of 8.37, a minimum value of 7.56 and a mean value of 8.03 in 2018. Overall, inflow and aerobic tank showed fluctuations within a range of 7.5-8.6 and 7.8-8.4, respectively.



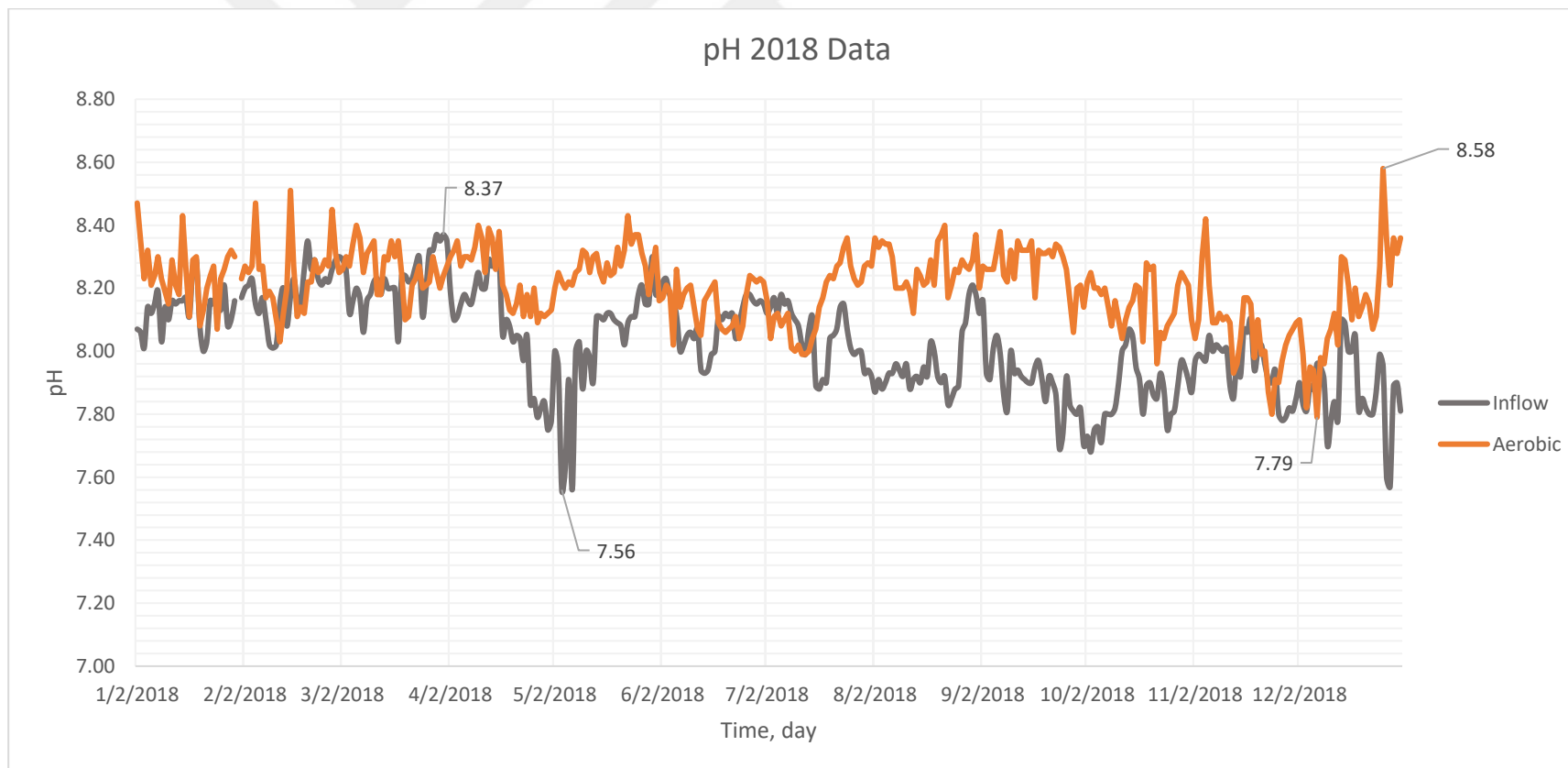


Figure 3.5: Observed pH values of inflow and aerobic tank during 2018.

In Figure 3.6, values obtained from temperature measurement of inflow, equalisation tank, aerobic and anoxic tank is represented. During 2018, minimum temperature value of 10 °C and maximum value of 40 °C was observed in the Inflow, where for both aerobic and anoxic tank minimum and maximum value was 19.4 °C and 39.5 °C, respectively. Overall the temperature range changed accordingly with the seasons. Even though aerobic and anoxic tanks stayed within mesophilic temperature band, 20°C – 45°C, temperature changes were not considered smooth and fluctuations were observed throughout the year.



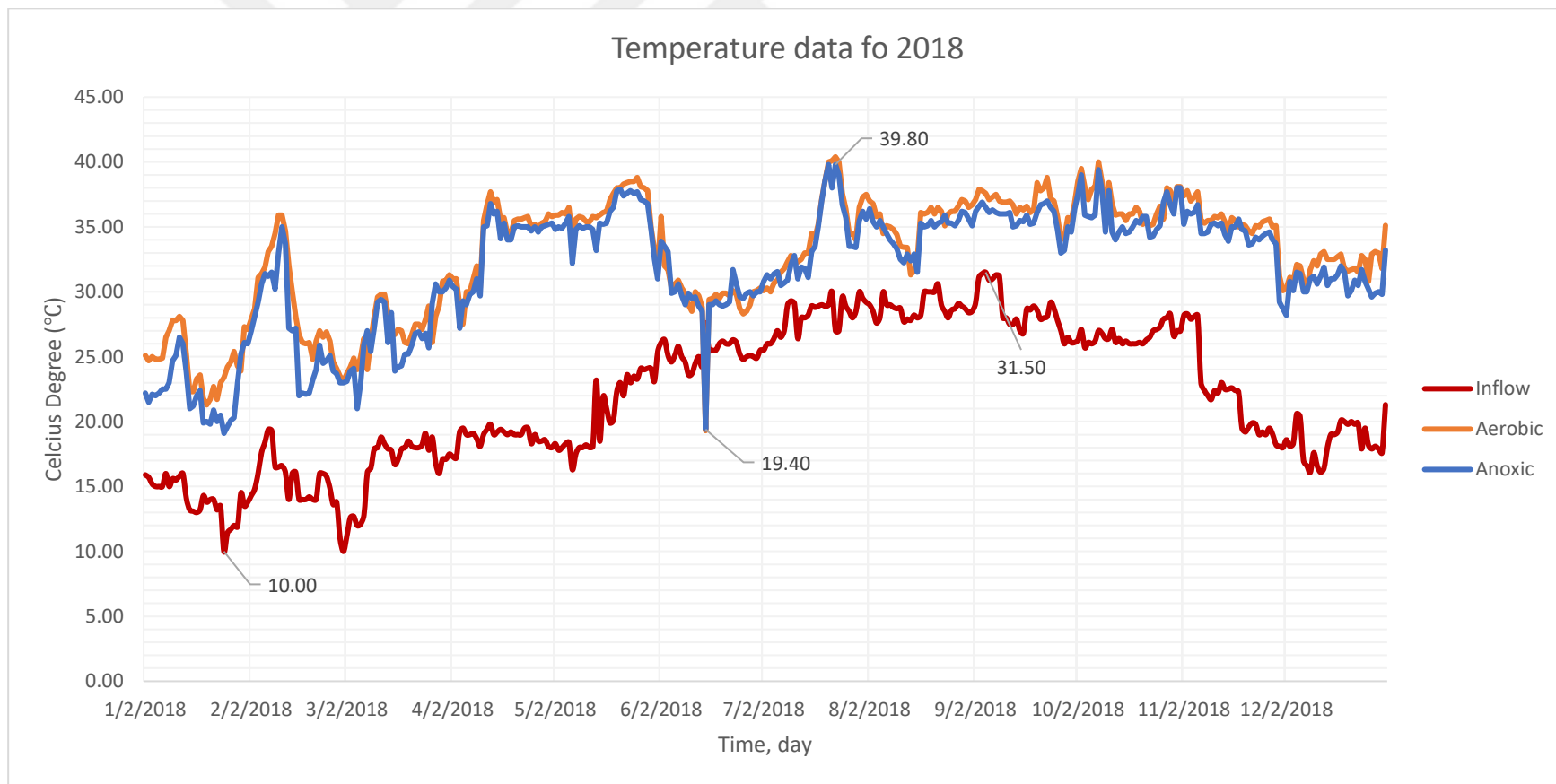


Figure 3.6: Temperature measurement of inflow, aerobic and anoxic tanks in 2018.

Table 3.2 below shows the minimum, maximum and mean values of pH and temperature observed in the inflow, aerobic and anoxic tanks for 2018.

Table 3.2: Min., max., mean and std values of pH and temperature in Inflow, aerobic and anoxic tanks.

Parameter	pH		Temperature, °C		
	Inflow	Aerobic	Inflow	Aerobic	Anoxic
Min	7.56	7.79	10.00	19.30	19.10
Max	8.37	8.58	31.50	40.40	39.80
Mean	8.03	8.20	21.99	32.68	31.68
Std	0.16	0.12	5.46	4.52	4.79

3.1.6 SVI and MLSS

Aerobic and anoxic tank MLSS data of 2018 are shown in Figure 3.7 and Table 3.3 below. For both tanks a similar trend in MLSS values was observed. In aerobic tank, MLSS value peaked at 19000 mg/L and observed to drop as low as 6350 mg/L, where in anoxic tank, MLSS reached its highest value as 17740 mg/L and 6930 mg/L as its lowest value. Overall, MLSS entry into the system was never stable and fluctuating concentrations were observed.

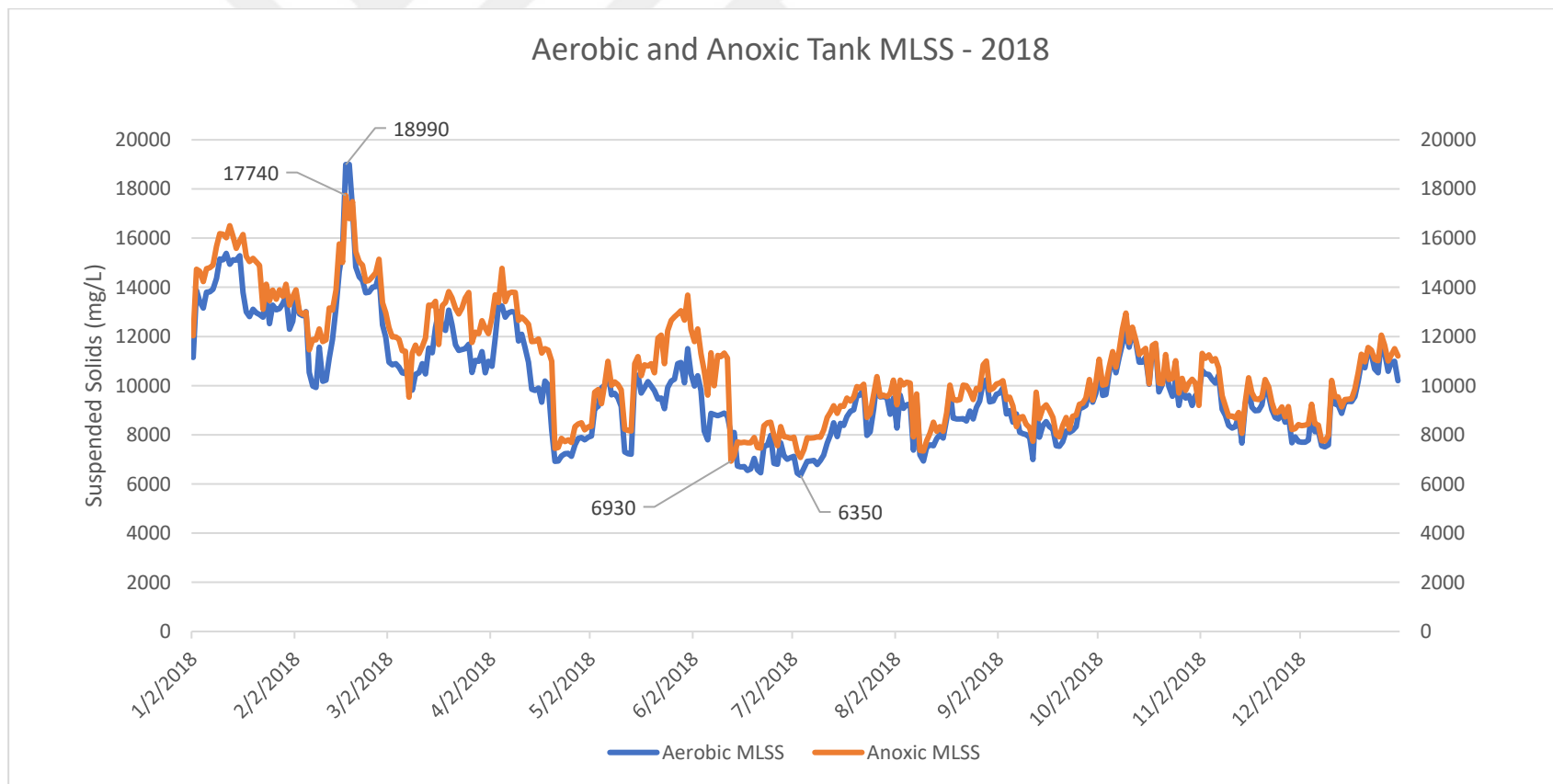


Figure 3.7: Aerobic and anoxic tank MLSS data of 2018.

Table 3.3: Min., max., mean and std data of 2018 MLSS data for aerobic and anoxic tanks.

Parameter	MLSS, mg/L	
	Aerobic	Anoxic
Tank		
Min	6350	6930
Max	19000	17740
Mean	10009	10770
Std	2213	2302

Figure 3.8 and Figure 3.9 represents the test results of SVI for the obtained samples from landfill leachate treatment facility. For aerobic tank minimum value of 86 ml/g and maximum value of 145 ml/g was observed in 12th and 6th months, respectively. Anoxic tanks maximum and minimum values were 37 ml/g and 123 ml/g in 9th and 6th months, respectively.

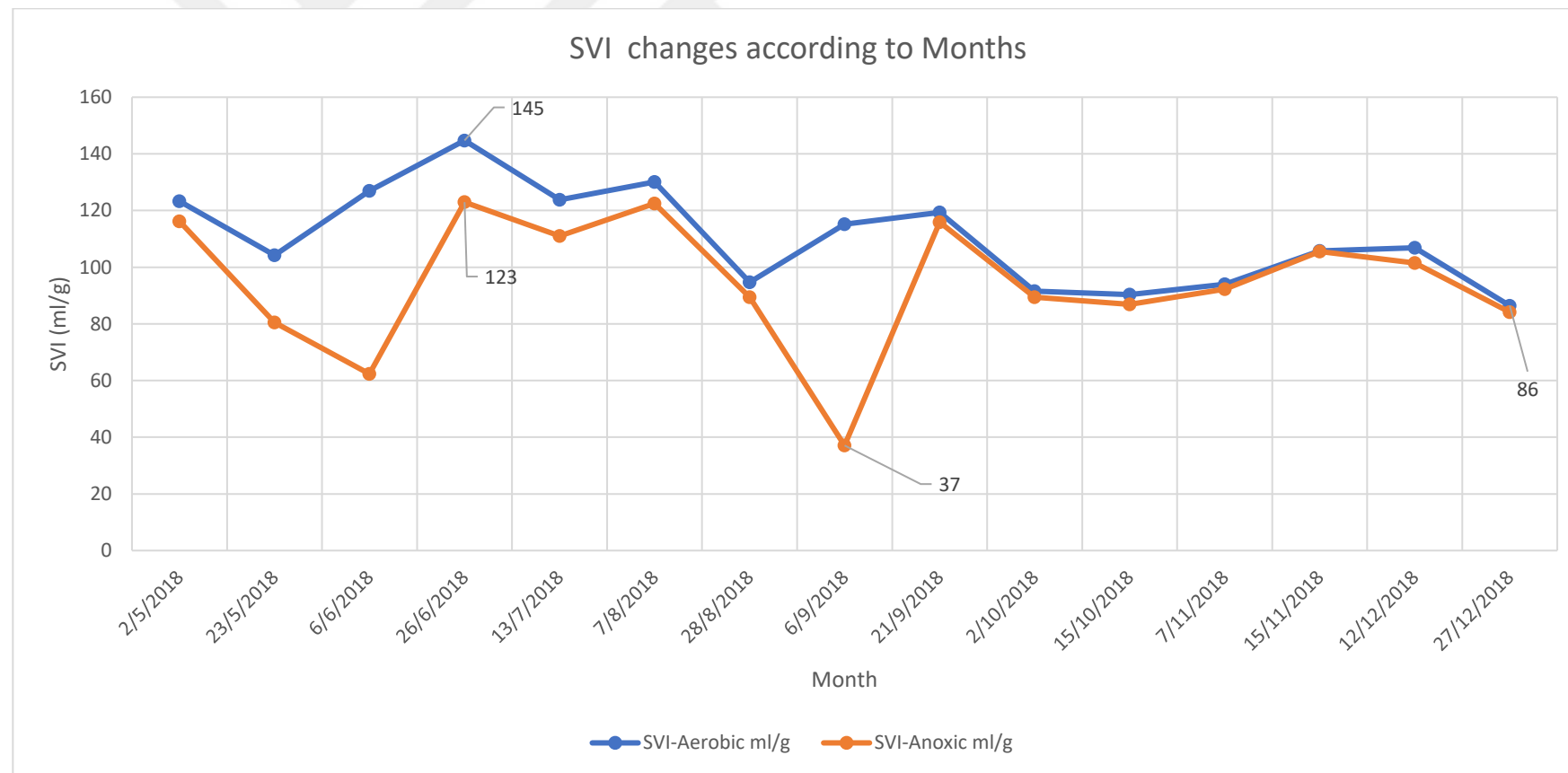


Figure 3.8: Calculated SVI values of aerobic and anoxic tanks in 2018 from obtained samples.

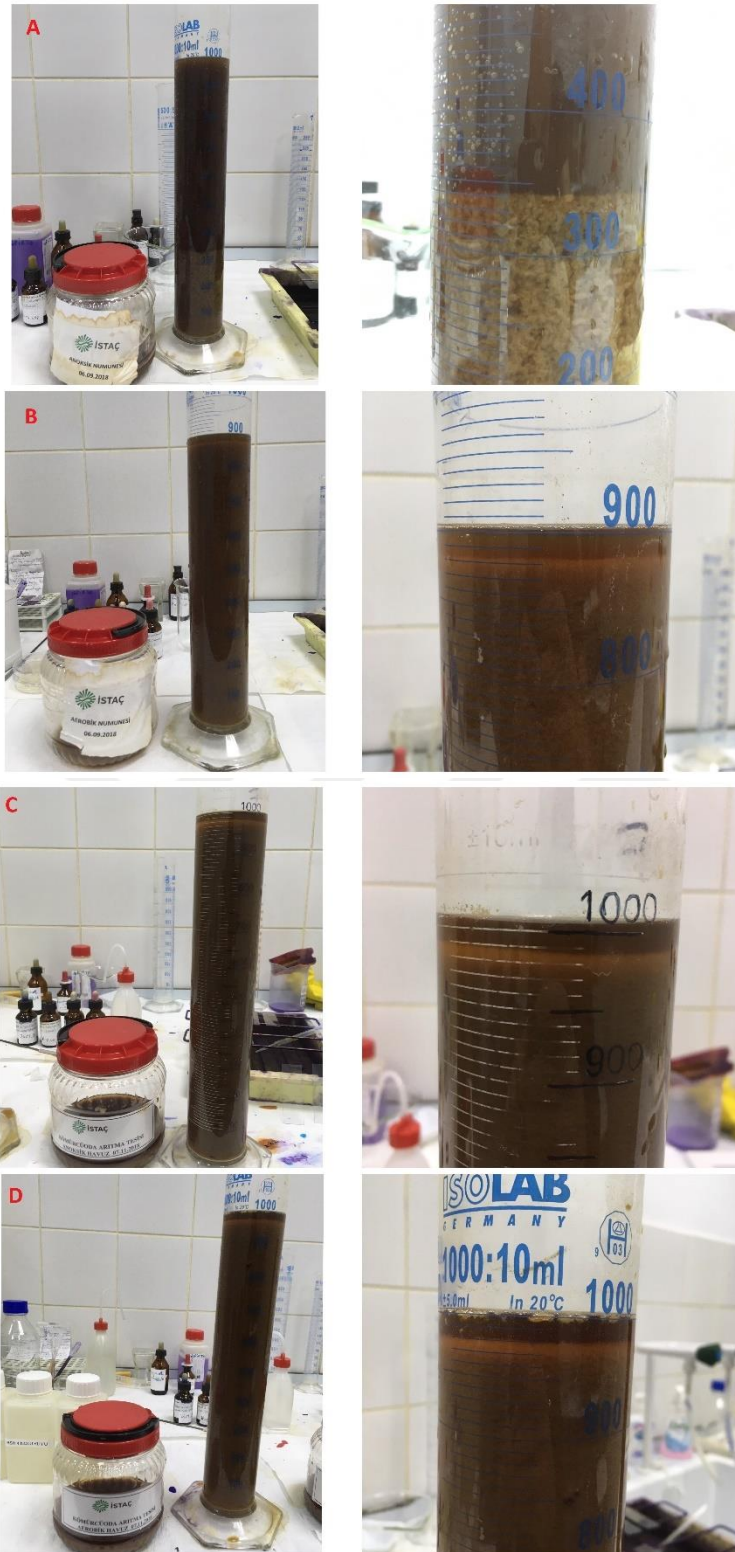


Figure 3.9: SVI test results of aerobic and anoxic tanks; A and B, anoxic and aerobic tank samples of 06.09.2018, respectively; C and D, anoxic and aerobic tank samples of 07.11.2018, respectively.

3.2 Filament Count

Under this section several figures of filament count results have been shown. Each figure is included with “Filamentous organism abundance scale” Jenkins et al 2004 [41] shown in yellow. The most abundant three filaments named as Filament 1, 2 and 3, respectively in the figures.

Figure 3.10 shows that Filament 1 had the highest, 19657 filament number/ μl , through half of the year. Filament 2 reached its peak value at 6th month in growth, 10156 no./ μl . Finally, Filament 3 was observed to reach 1966 no/ μl as its peak value in 4th month.



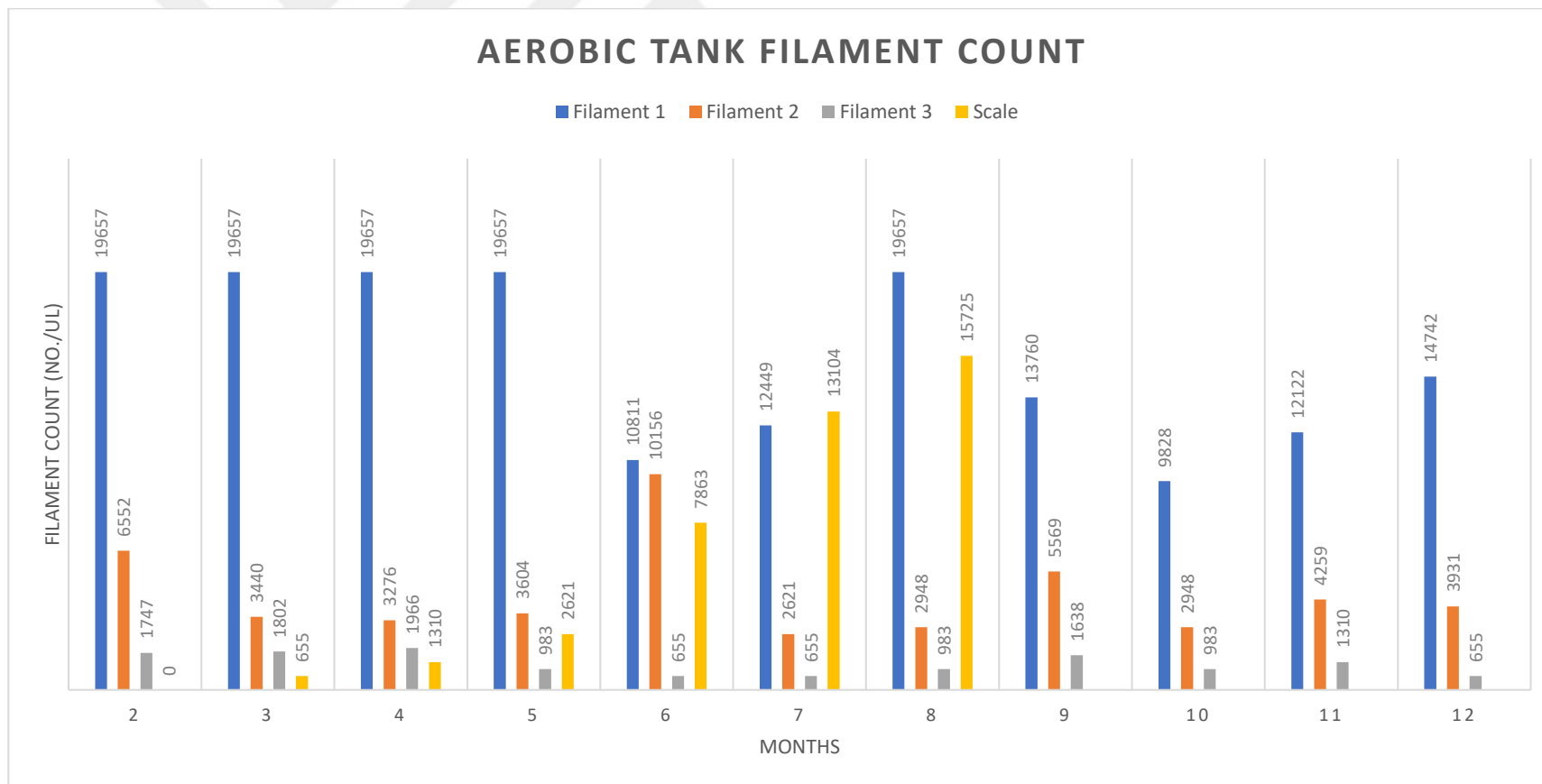


Figure 3.10: Filament count of aerobic tank 2018.

Figure 3.11 shows that Filament 1 was highest, 19657 no./ μl through one-third of the year. Filament 2 reached its peak value at 6th month in growth, 8518 no./ μl . Finally, Filament 3 was observed to reach 2948 no./ μl as its peak value in 5th month.



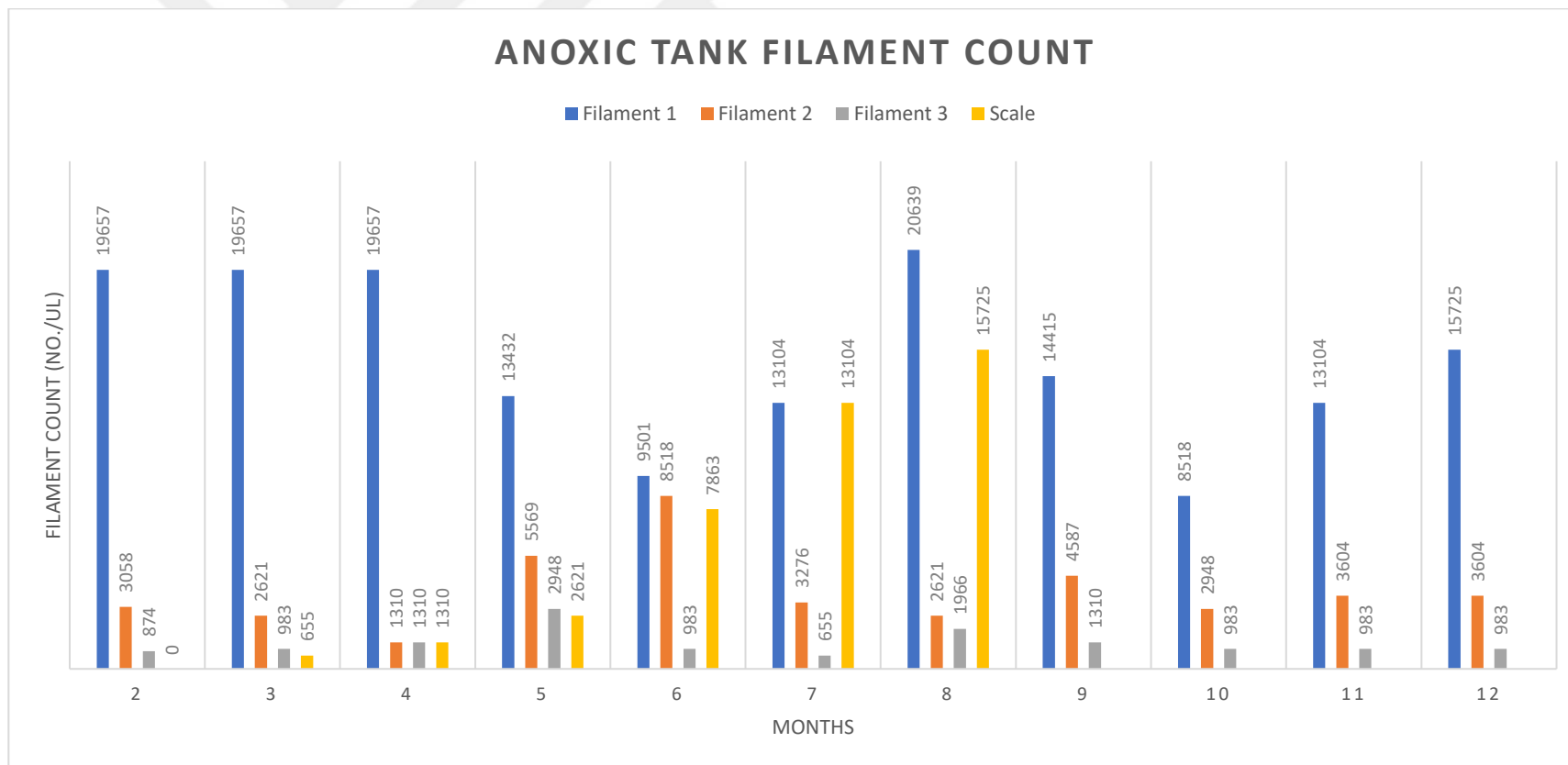


Figure 3.11: Filament count of anoxic tank 2018.

Figure 3.12 shows the total filamentous organism count in both aerobic and anoxic tanks. Total filament counts in Aerobic tank peaked at 2nd month with 27956 no./ μ l and showed least amount in 10th month with 13760 no./ μ l. Total filament count in Anoxic tank was highest at 8th and lowest in 10th month with 25226 no./ μ l and 12449 no./ μ l, respectively. Only in 10th month, total filament count of Aerobic tank and Anoxic tank was marked at the score of 5 and 4, respectively.



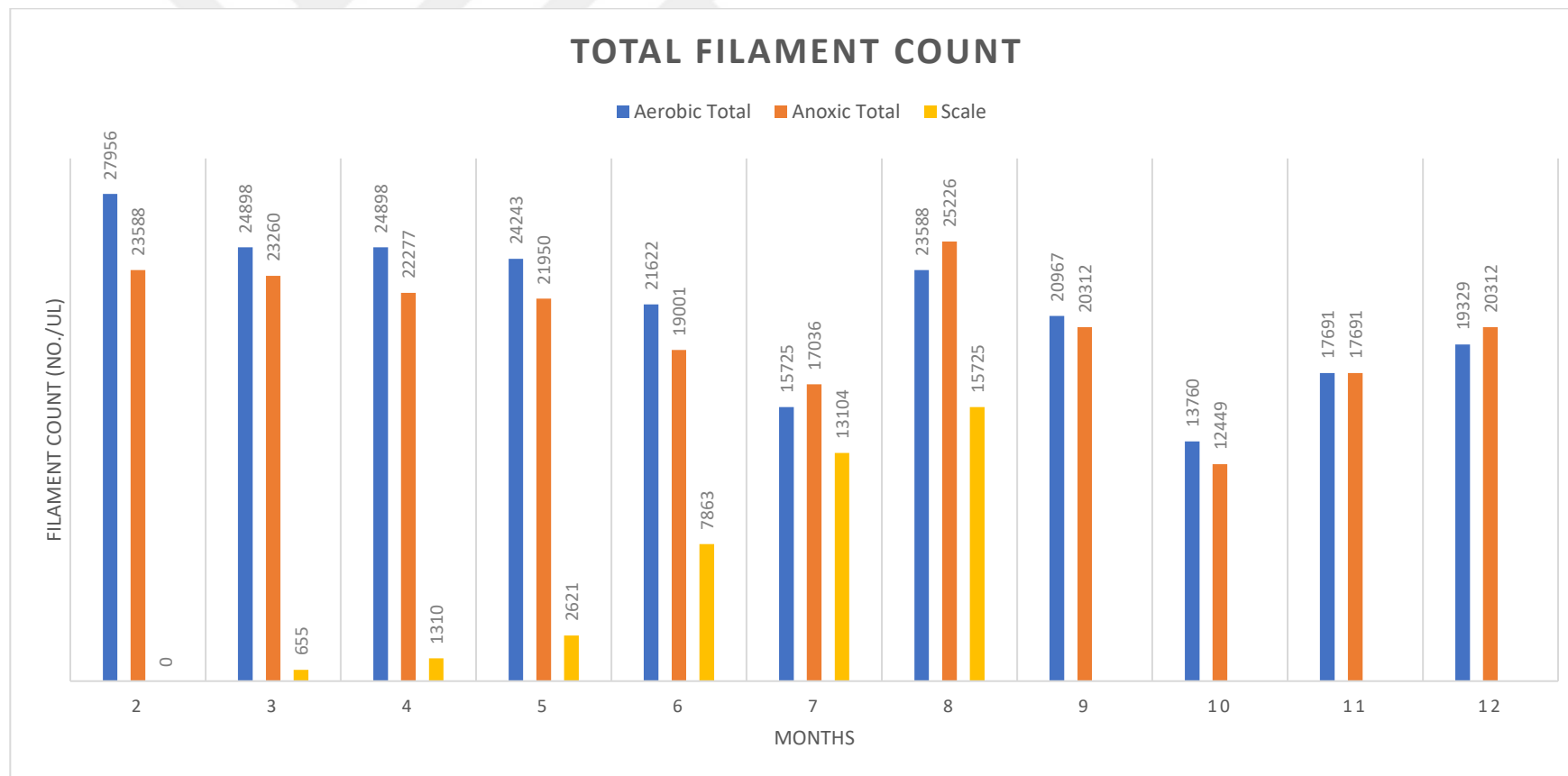


Figure 3.12: Total filament count of aerobic and anoxic tanks 2018.

Pearson correlation coefficient, r , was used to inspect the strength and direction of the parameters with Filament 1, 2 and 3. Strength is indicated by the larger absolute value of the coefficient and is between 1 and -1, where Direction is shown by the “+” or “-“ where a positive value indicates that both variables increase or decrease together and a negative value indicates that one variable increases as the other decreases, and vice versa [54]. Table 3.4 [62] below represents the commonly accepted guidelines for Pearson correlation coefficient values:

Table 3.4: Correlation guideline.

Strength of Association	Coefficient, r	
	Positive, +	Negative, -
None	0	0
Small	0.1 to 0.3	-0.1 to -0.3
Medium	0.3 to 0.5	-0.3 to -0.5
Large	0.5 to 1	-0.5 to -1

Pearson Correlation analysis values of Filament 1, 2 and 3 with measured parameters (MLSS, COD, $\text{NH}_4^+\text{-N}$, TN, $\text{PO}_4\text{-P}$, temperature and pH) are given in Table 3.5. The results show that Filament 1 showed a large positive correlation with MLSS (both tanks) and pH (aerobic tank), 0.50 and 0.51 respectively, and a large negative with temperature (anoxic tank), -0.54. Filament 2 did not show any large strength with any of the parameters, but negative and positive medium correlation values was observed with COD (anoxic tank) and $\text{PO}_4\text{-P}$ (Aerobic tank), -0.48 and 0.42 respectively. Filament 3 was only seen to have a large positive correlation with temperature (anoxic tank).

Table 3.5: Pearson correlation analysis of Filament 1,2 and 3 in aerobic and anoxic tanks with parameters.

Tank	Aerobic	Anoxic
	<i>MLSS</i>	<i>MLSS</i>
MLSS	1	1
Filament 1	0.43	0.50
Filament 2	-0.13	-0.30
Filament 3	0.43	-0.30
	<i>COD</i>	<i>COD</i>
COD	1	1
Filament 1	-0.12	-0.10
Filament 2	-0.29	-0.48
Filament 3	0.26	-0.34
	<i>NH₄-N</i>	<i>NH₄-N</i>
NH ₄ -N	1	1
Filament 1	-0.41	-0.40
Filament 2	-0.07	-0.07
Filament 3	-0.22	-0.25
	<i>TN</i>	<i>TN</i>
TN	1	1
Filament 1	-0.38	-0.27
Filament 2	0.01	0.00
Filament 3	-0.02	0.05
	<i>PO₄-P</i>	<i>PO₄-P</i>
PO ₄ -P	1	1
Filament 1	-0.24	0.00
Filament 2	0.42	0.22
Filament 3	-0.28	-0.04
	<i>Temperature</i>	<i>Temperature</i>
Temperature	1	1
Filament 1	-0.43	-0.54
Filament 2	-0.23	0.20
Filament 3	-0.32	0.50
	<i>pH</i>	<i>pH</i>
pH	1	
Filament 1	0.51	
Filament 2	0.09	
Filament 3	0.33	

3.3 Filamentous Organism Identification

Filamentous organisms were identified using the dichotomous key provided by Jenkins et al 2004 [41] and the results are given below in Table 3.6;

Table 3.6: Filamentous organism characteristics and Identifications.

Filament number		1	2	3
Characteristics				
Filament shape	Branching	not branched	not branched	not branched
	Motility	Immobile	immobile	immobile
	Straight/ slightly bent	Straight/slightly bent	Straight/slightly bent	Straight/slightly bent
	Coiled/ twisted	-	-	-
	Filament location	Protruding from the flocs	Free in water or in/around the flocs	usually free in the liquid
	Attached growth	Rarely	usually no attached growth	no attached growth
	Sheath	Present	no	no
Filament diameter, μm	Septa Clearly visible	Invisible septa	septa not clearly visible	septa clearly visible
	<1.0 μm	~0.3-0.4 μm	~0.5 μm	-
	1.0-3.0 μm	-	-	~1.5-2.0
	>3.0 μm	-	-	-
Filament length, μm	<200 μm	<200	<200	-
	>200 μm	-	-	>200
Cell shape	Square or Rectangular	-	-	Variable (disc shaped, almost square and rod shaped)
	Round, disc or rod	-	-	
Gram stain	Positive	-	Positive	-
	Negative	Negative	-	Negative
Neisser stain	Positive	-	Positive (poly-p granules are + inside, but get smaller if the growth stagnates)	-
	Negative	Negative	-	Negative
	Sulphur Storage	No	No	Little or no sulphur storage
	Commonness in AS	Common	Common	Frequently observed
Identification		<i>Haliscomenobacter hydrossis</i>	<i>Microthrix parvicella</i>	Type 021N

3.3.1 Filament 1- *Haliscomenobacter hydrossis*

Filament 1 was identified to be non-branching, immobile and straight/slightly bent, generally protruding from the flocs and showing rare attached growth. Sheath was present; however, septa was none/invisible. Filament length was variable but never more than 200 μm and diameters were around 0.3-0.4 μm . Both Gram and Neisser staining was negative for Filament 1 and no Sulphur storage observed. All the identified characteristics above matched with given properties of *Haliscomenobacter hydrossis* in the dichotomous key provided by Jenkins et al. 2004 [41].

Figure 3.13 and 3.14 shows the micrographs of *Haliscomenobacter hydrossis* taken during the sample examination:

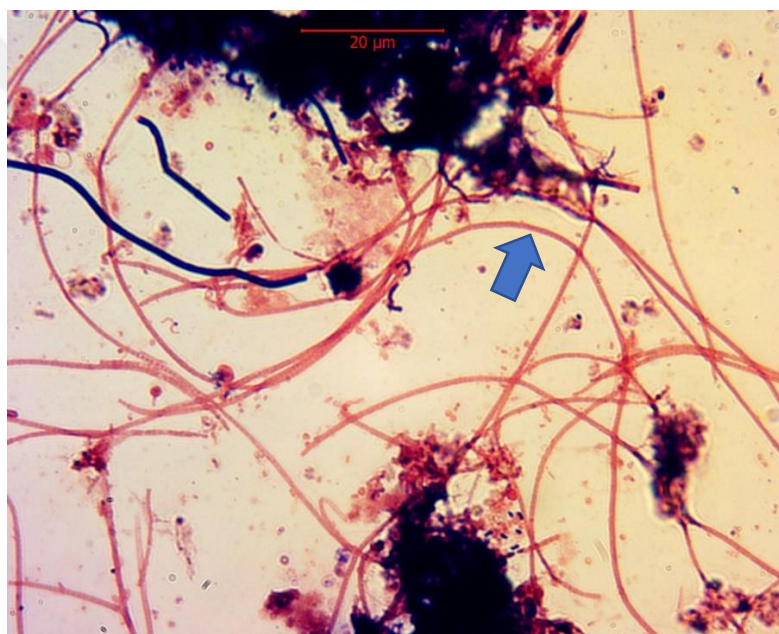


Figure 3.13: Micrographs of *Haliscomenobacter hydrossis*, Filament 1, found in activated sludge samples; gram staining-Negative, red/orange colour (original magnification 1000x).

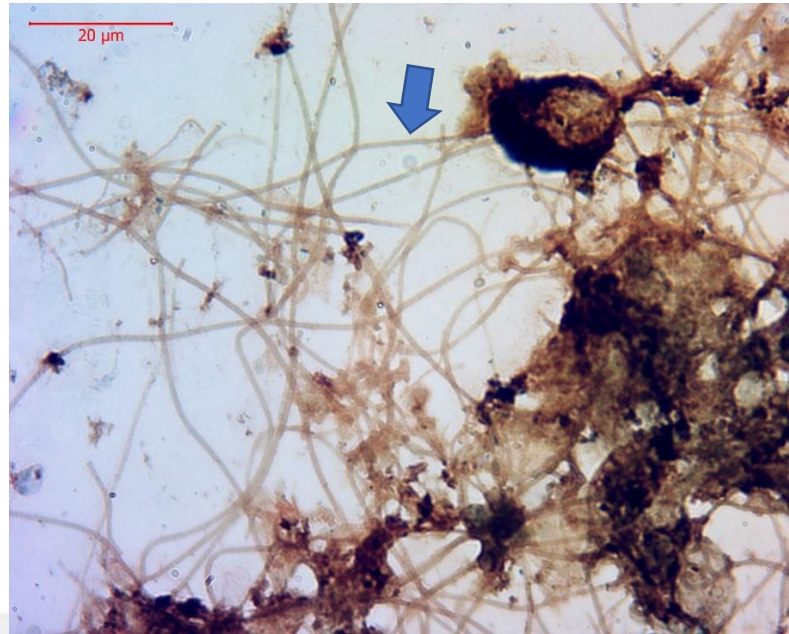


Figure 3.14: Micrograph of *Haliscomenobacter hydrossis*, Filament 1, found in activated sludge samples; Neisser staining-Negative, light brown colour (original magnification 1000x).

3.3.2 Filament 2- *Microthrix parvicella*

Filament 2 was identified to be non-branching, immobile and straight/slightly bent, generally free in water or in/around flocs and showing usually no attached growth. Sheath was not present, and septa was not present. Filament length was variable but never more than 200 μm and diameters were around 0.5 μm . Gram staining was strongly positive and poly-p granules are Neisser positive inside but get smaller if the growth stagnates for Filament 2 and no Sulphur storage observed. All the identified characteristics above matched with given properties of *Microthrix parvicella* in dichotomous key provided by Jenkins et al. 2004 [41].

Figure 3.15 and 3.16 shows the micrographs of *Microthrix parvicella* taken during the sample examination:

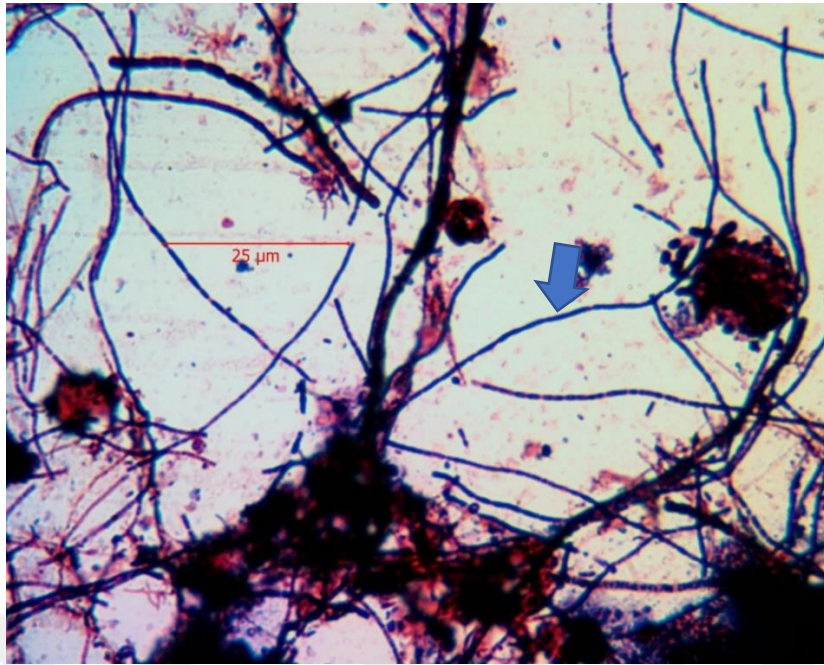


Figure 3.15: Micrograph of *Microthrix parvicella*, Filament 2, found in activated sludge samples; gram staining-positive, navy blue (original magnification 1000x).



Figure 3.16: Micrograph of *Microthrix parvicella*, Filament 2, found in activated sludge samples; Neisser staining-positive, poly-p granules green, outside light brown colour, (original magnification 1000x).

3.3.3 Filament 3- Type 021N

Filament 3 was identified to be non-branching, immobile and bent/twisted, generally free in the liquid and showing no attached growth. Sheath was not present, and septa

was clearly visible. Filament length was variable but usually more than 200 μm and diameters were between 1.5-2.0 μm . Gram and Neisser staining were negative for filament 3 and little/no sulphur storage observed. All the identified characteristics above matched with given properties of Type 021N in dichotomous key provided by Jenkins et al 2004 [41].

Figure 3.17 and 3.18 below shows the micrographs of Type 021N taken during the sample examination:

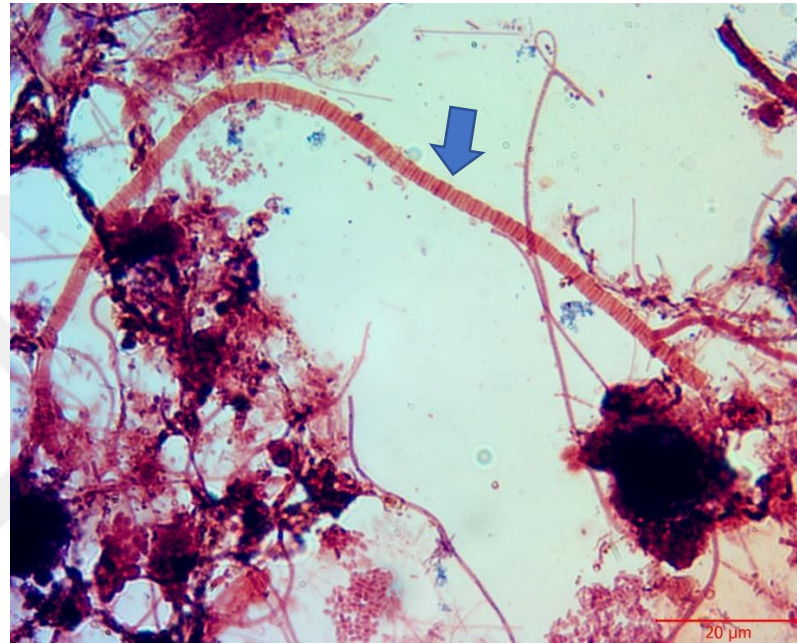


Figure 3.17: Micrograph of Type 021N, Filament 3, found in activated sludge samples; gram staining-negative, red/orange colour (original magnification 1000x).

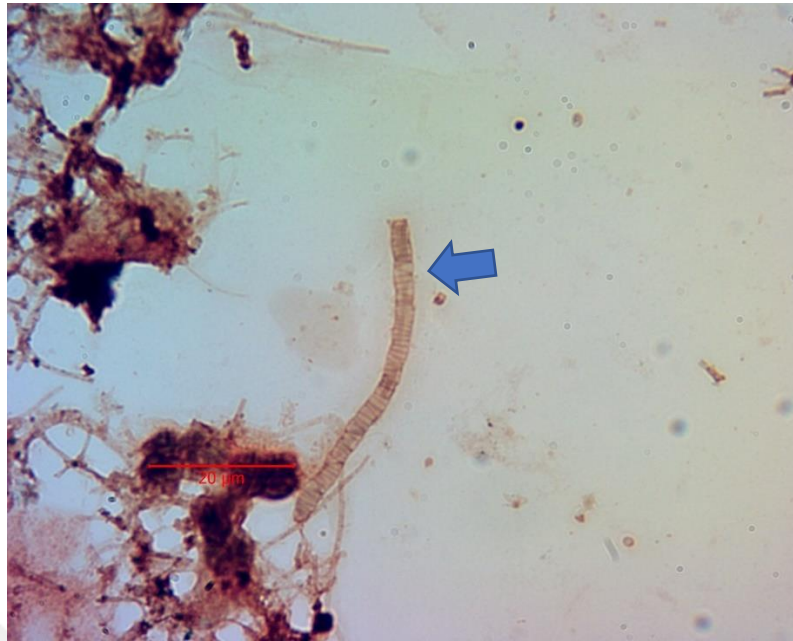


Figure 3.18: Micrographs of Type 021N, Filament 3, found in activated sludge samples; Neisser staining-negative, light brown colour (original magnification 1000x).

3.4 Floc Size Count and Characteristics

Micrographs of mixed liquor samples obtained were observed under 100x and 200x original magnification as wet mount and gram stained in order to collect the below data on filament abundance, floc size and characteristics and microorganism diversity.

Figure 3.19 shows the data of aerobic tank floc size count. Maximum amount of small, medium and large floc size was ~18, 9 and 1, respectively. Large flocs showed a decrease after 5th month and stayed at 0 after 7th month. Medium size flocs followed a steady decrease after 5th month and started to increase at 9th month, 1.5, peaking at 9 at 12th month. Small floc size was always highest in numbers, where it showed steady increase from 4th to 9th month, reaching its peak value of 18.5 then dropping to 11 at 12th month

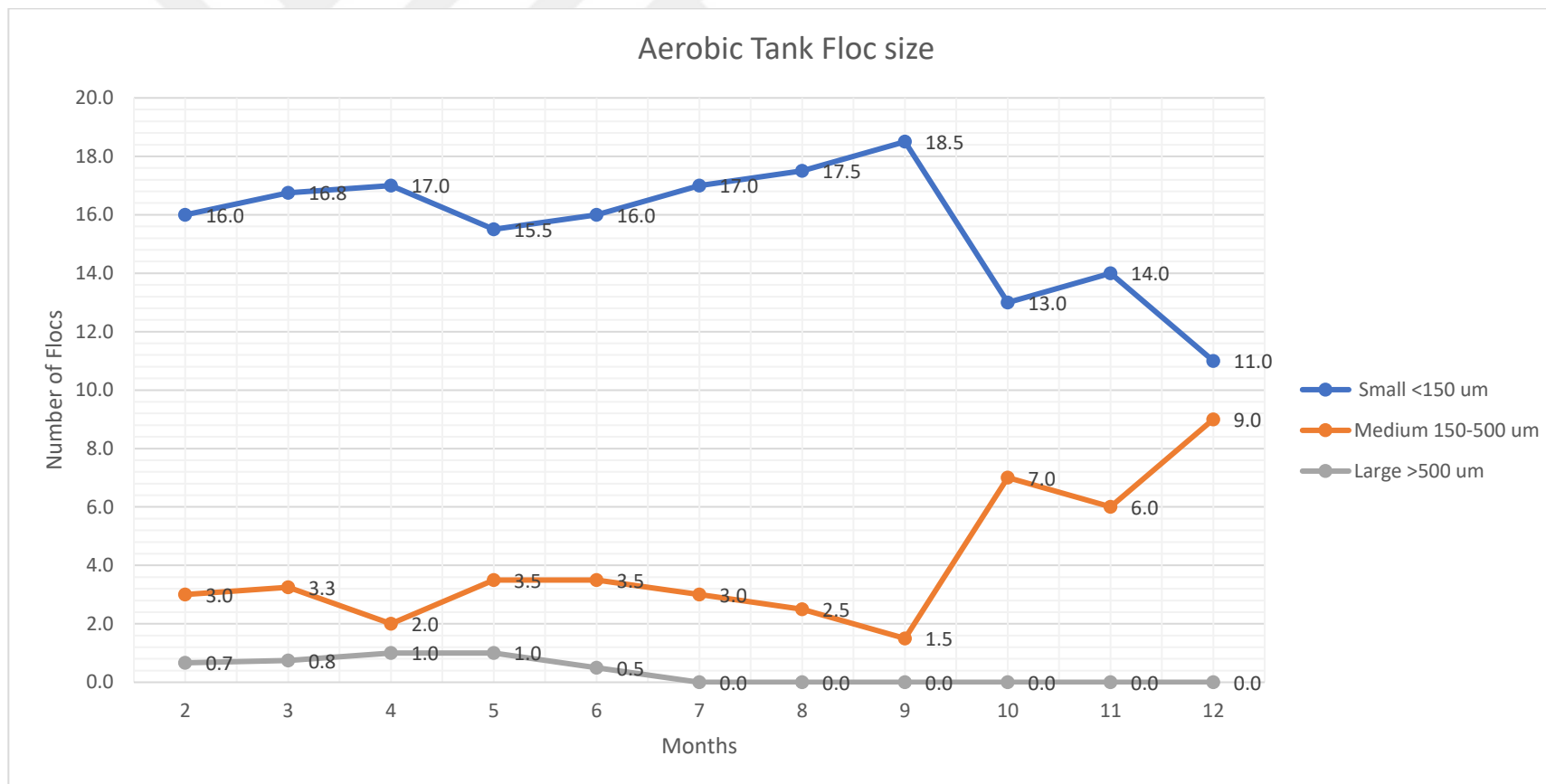


Figure 3.19: Floc size data of aerobic tank.

Figure 3.20 shows the data of anoxic tank floc size count. Maximum amount of small, medium and large floc size was ~18, ~9 and 3, respectively. Large flocs showed an increase after 7th month and peaked at 3 in 12th month. Medium size flocs followed a steady range between 4th and 9th which then increased up to 8.5 in 11th month. Small floc size was always highest in numbers, where it showed steady increase from 3rd to 9th month, except 6th, and dropped to 10 at 12th month.



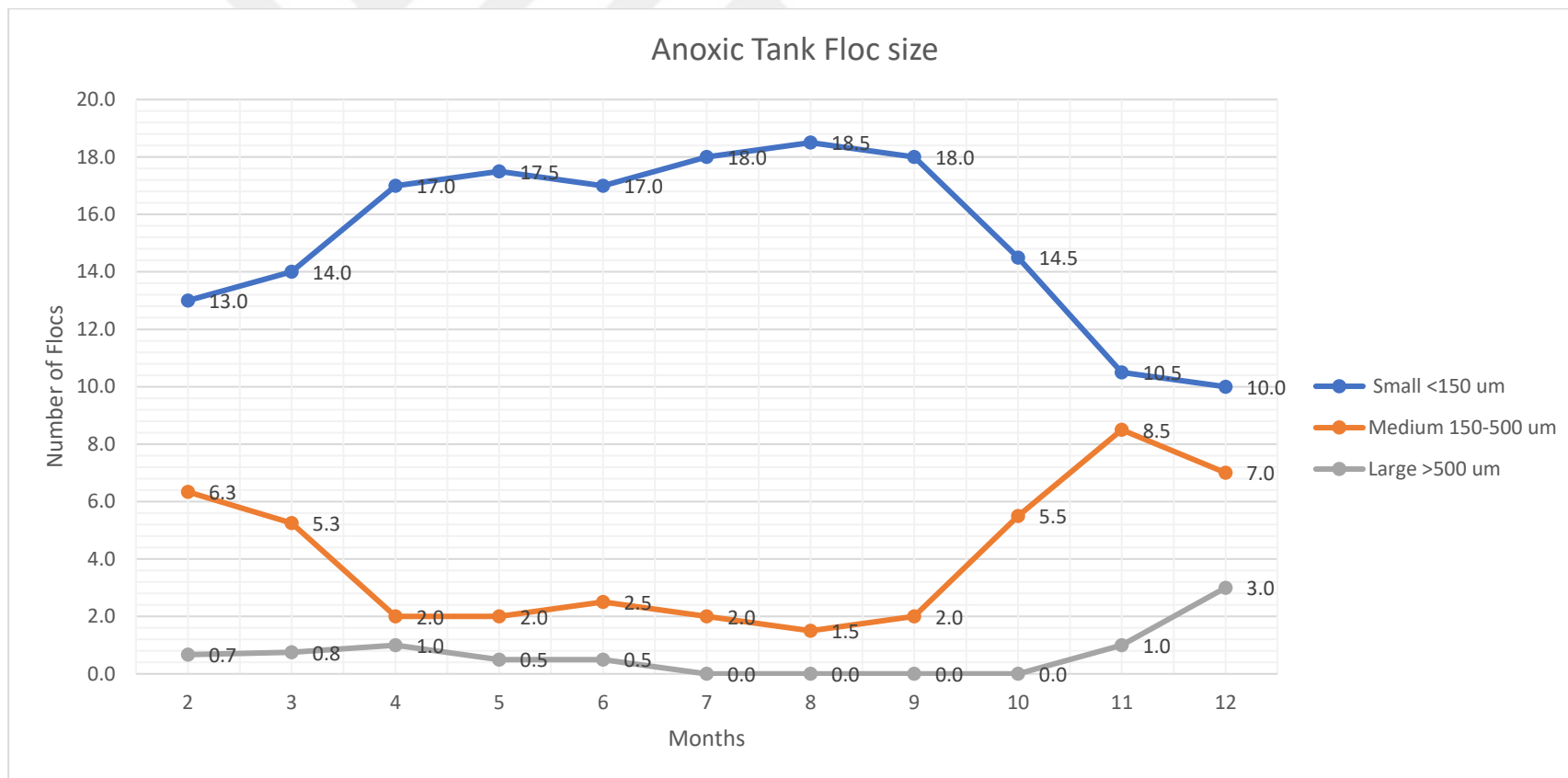


Figure 3.20: Floc size data of anoxic tank

Pearson correlation analysis values of floc size with measured parameters (MLSS, COD, NH₄-N, TN, PO₄-P, temperature and pH) are given in Table 3.7.

Table 3.7: Pearson correlation analysis of floc size in aerobic and anoxic tanks with parameters.

Tanks	Aerobic	Anoxic
	<i>MLSS</i>	<i>MLSS</i>
MLSS	1.00	1.00
Small <150 µm	-0.16	-0.33
Medium 150-500 µm	0.08	0.40
Large >500 µm	0.42	0.01
	<i>COD</i>	<i>COD</i>
COD	1	1
Small <150 µm	-0.39	-0.47
Medium 150-500 µm	0.39	0.52
Large >500 µm	-0.16	0.13
	<i>NH₄-N</i>	<i>NH₄-N</i>
NH ₄ -N	1	1
Small <150 µm	-0.49	-0.45
Medium 150-500 µm	0.46	0.53
Large >500 µm	0.04	0.05
	<i>TN</i>	<i>TN</i>
TN	1	1
Small <150 µm	0.27	0.44
Medium 150-500 µm	-0.20	-0.38
Large >500 µm	-0.28	-0.35
	<i>PO₄-P</i>	<i>PO₄-P</i>
PO ₄ -P	1	1
Small <150 µm	0.03	0.07
Medium 150-500 µm	-0.07	-0.08
Large >500 µm	-0.01	-0.01
	<i>Temperature</i>	<i>Temperature</i>
Temperature	1	1
Small <150 µm	0.20	0.39
Medium 150-500 µm	-0.12	-0.42
Large >500 µm	-0.43	-0.12
	<i>pH</i>	<i>pH</i>
pH	1	
Small <150 µm	0.55	n/d
Medium 150-500 µm	-0.63	n/d
Large >500 µm	0.60	n/d

Small and Medium size flocs showed large negative, -0.47, and large positive, 0.52, respectively, correlation with COD data in Anoxic tank. NH₄-N correlation analysis

with Small floc size showed a large negative correlation of -0.49 in aerobic tanks and -0.44 in anoxic tank, where medium floc size showed large positive correlation value of 0.46 in aerobic tank and 0.53 in anoxic tank. pH correlation analysis with small and large floc sizes showed large positive values of 0.55 and 0.60, respectively, where medium sized flocs had a large negative correlation value of -0.63.

Table 3.8 shows the data obtained from observation of sample on their filament abundance, floc characteristics and microbial diversity for each month. Throughout the months filament abundance score stayed at 6, except at 10th month where aerobic and anoxic tank score dropped to 5 and 4, respectively. Morphology of flocs for both tanks were always observed to be diffuse and weak, however 6th, 8th, 9th and 10th months showed slight improvements. However, morphology of slightly better flocs couldn't not be accepted as compact and firm, hence considered somewhere between diffuse & weak and compact & firm. Free cells in suspension for both tanks shared similar results, where it started in high amounts until 4th month then decreased steadily until 6th month. From 7th month onward free cell amounts stayed at a level of medium until the end, 12th month. Microbial diversity of both aerobic and anoxic tanks was considered poor until 8th month, which then elevated to medium level between 9th and 11th month. In the final 12th month, microbial diversity again dropped to poor level.

Table 3.8: Filament abundance, floc characteristics and microbial diversity of aerobic and anoxic tanks.

Months	Aerobic				Anoxic			
	Filament Abundance	Morphology of floc	Free cells in suspension	Diversity	Filament Abundance	Morphology of floc	Free cells in suspension	Diversity
2	6	Diffuse & Weak	High	Poor	6	Diffuse & Weak	High	Poor
3	6	Diffuse & Weak	High	Poor	6	Diffuse & Weak	High	Poor
4	6	Diffuse & Weak	High	Poor	6	Diffuse & Weak	High	Poor
5	6	Diffuse & Weak	Medium	Poor	6	Diffuse & Weak	Medium	Poor
6	6	Diffuse & Weak*	Low	Poor	6	Diffuse & Weak*	Low	Poor
7	6	Diffuse & Weak*	Medium	Poor	6	Diffuse & Weak*	Medium	Poor
8	6	Diffuse & Weak*	Low-Medium**	Poor	6	Diffuse & Weak*	Low-Medium**	Poor
9	6	Diffuse & Weak*	Medium	Medium	6	Diffuse & Weak*	Medium	Medium
10	5	Diffuse & Weak	Medium	Medium	4	Diffuse & Weak	Medium	Medium
11	6	Diffuse & Weak	Medium	Medium	6	Diffuse & Weak	Medium	Medium
12	6	Diffuse & Weak	Medium	Medium	6	Diffuse & Weak	Medium	Medium

*slightly less diffuse & weak than others but not enough to be called compact & firm.

** First observed sample was low, where second observed sample was medium in free cells in suspension

3.5 Micrographs of Aerobic and Anoxic Tanks

3.5.1 2nd month, aerobic tank

Figure 3.21 shows Free cell suspension: High, Floc morphology: Diffuse & weak, Diversity: poor and Filament Abundance Score: 6

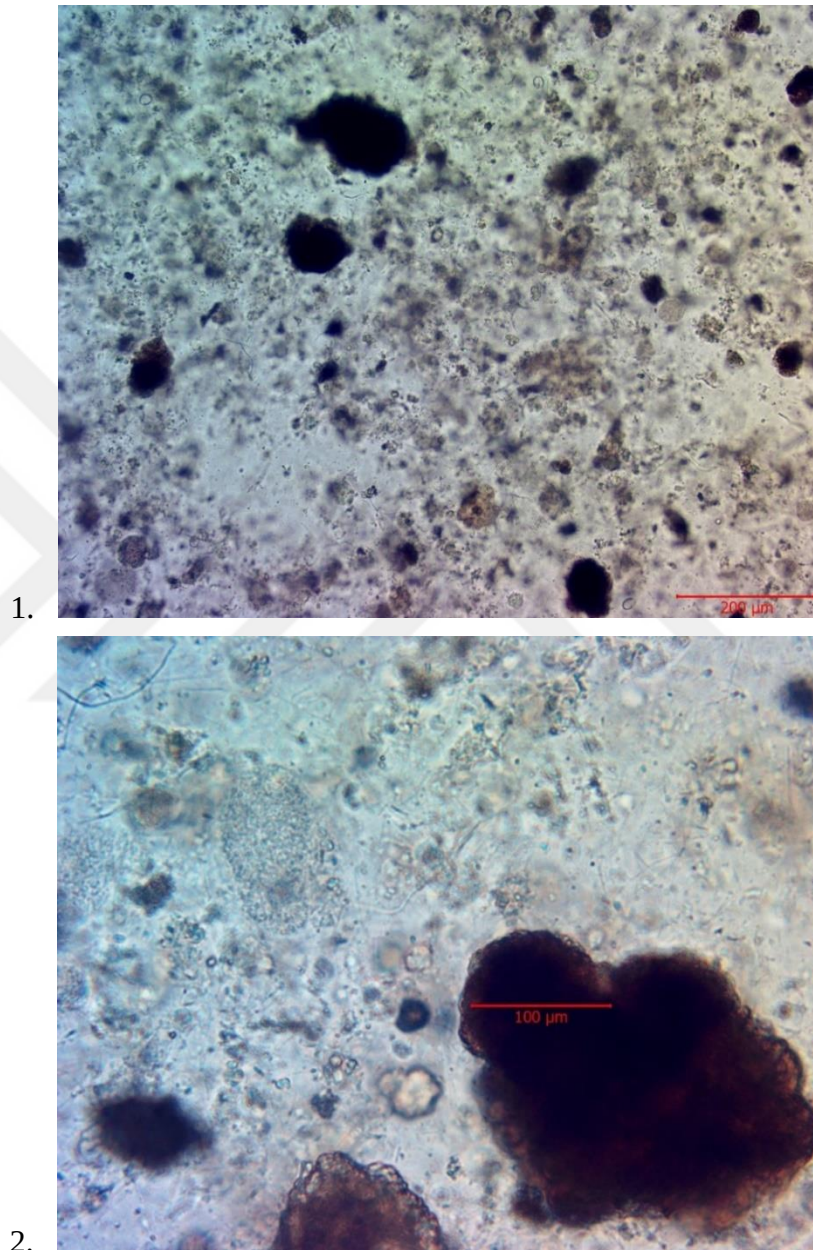
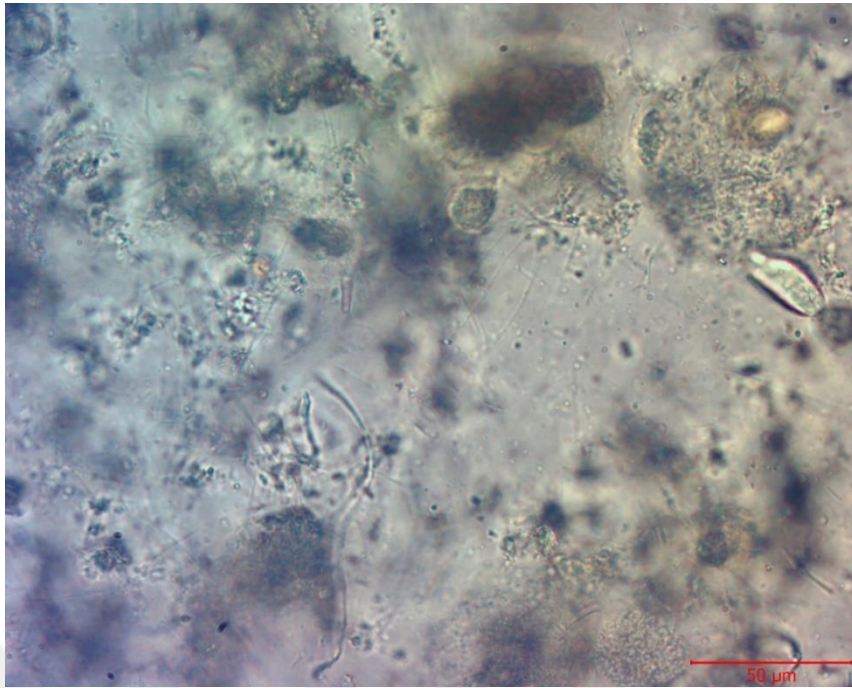


Figure 3.21: Micrographs of anoxic tank samples; 1, 2, 3 are wet mount taken at original magnification 100x, 200x, 400x respectively and 4 is gram staining taken at original magnification 1000x with oil immersion technique

3.



4.

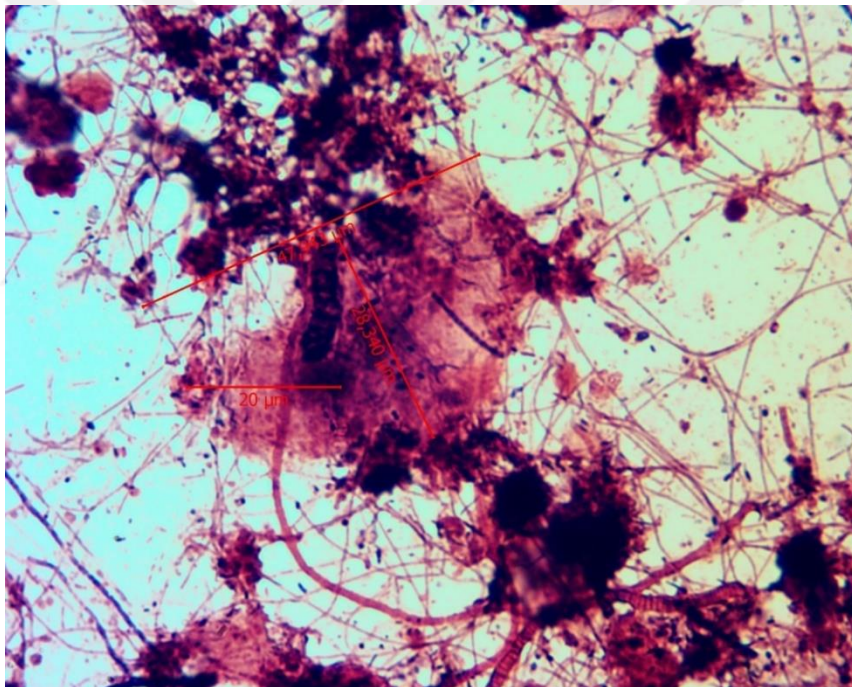


Figure 3.21 (continued): Micrographs of anoxic tank samples; 1, 2, 3 are wet mount taken at original magnification 100x, 200x, 400x respectively and 4 is gram staining taken at original magnification 1000x with oil immersion technique.

3.5.2 2nd month, anoxic tank

Figure 3.22 shows Free cell suspension: High, Floc morphology: Diffuse & weak, Diversity: poor and Filament Abundance Score: 6

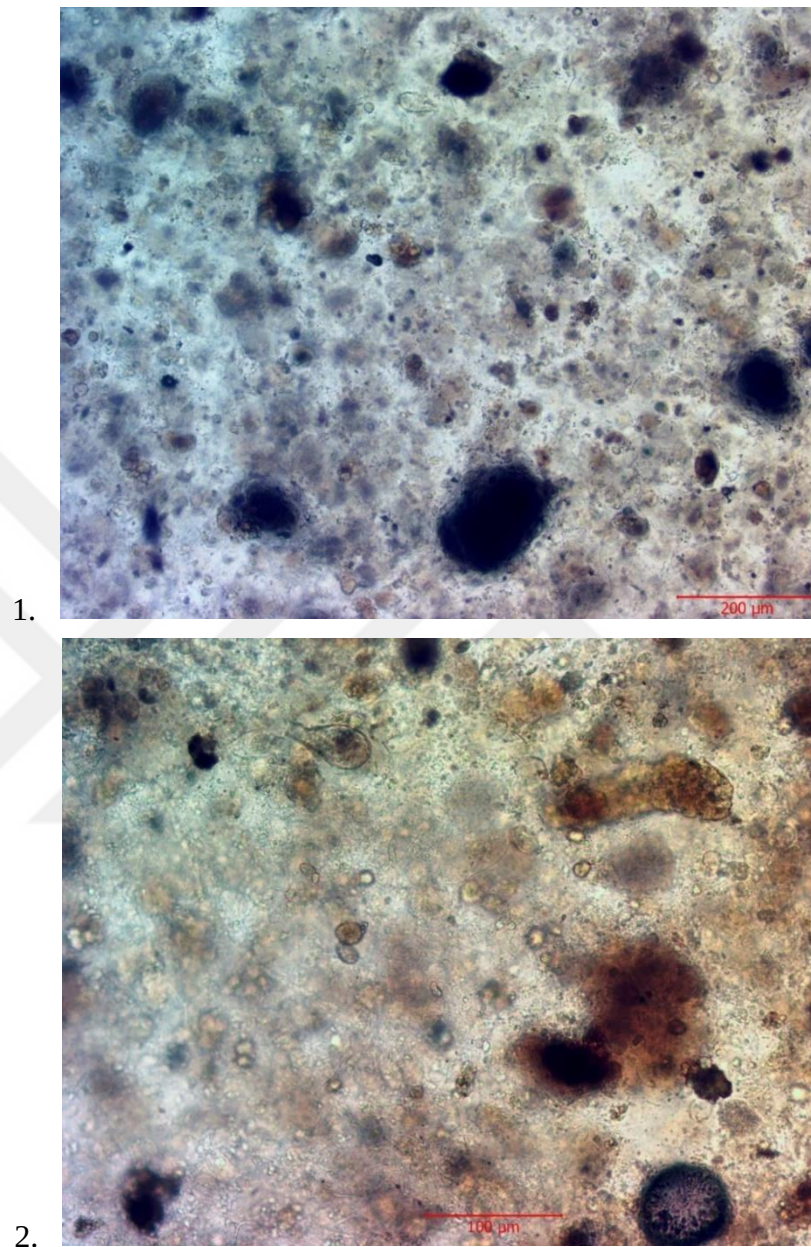
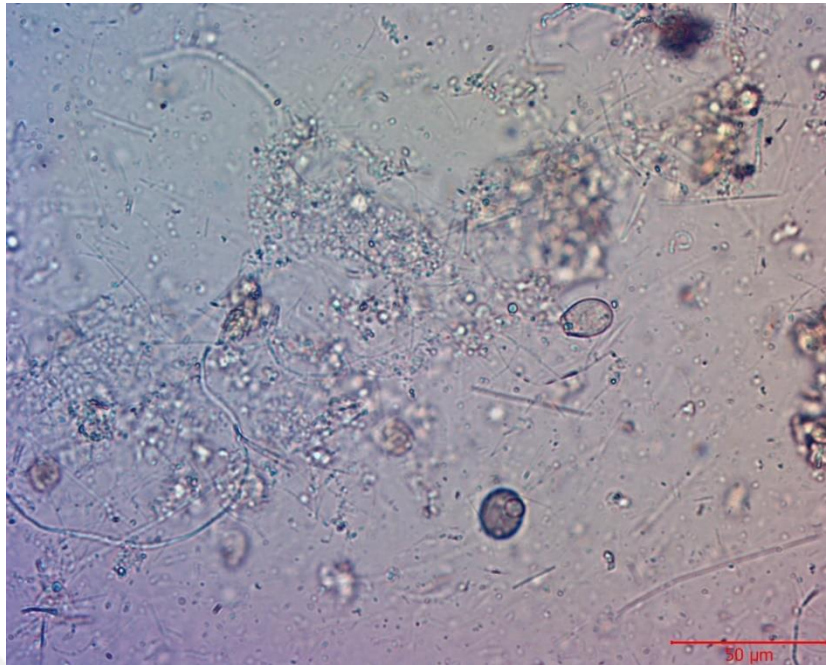


Figure 3.22: Micrographs of anoxic tank samples; 1, 2, 3 are wet mount taken at original magnification 100x, 200x, 400x respectively and 4 is gram staining taken at original magnification 1000x with oil immersion technique.

3.



4.

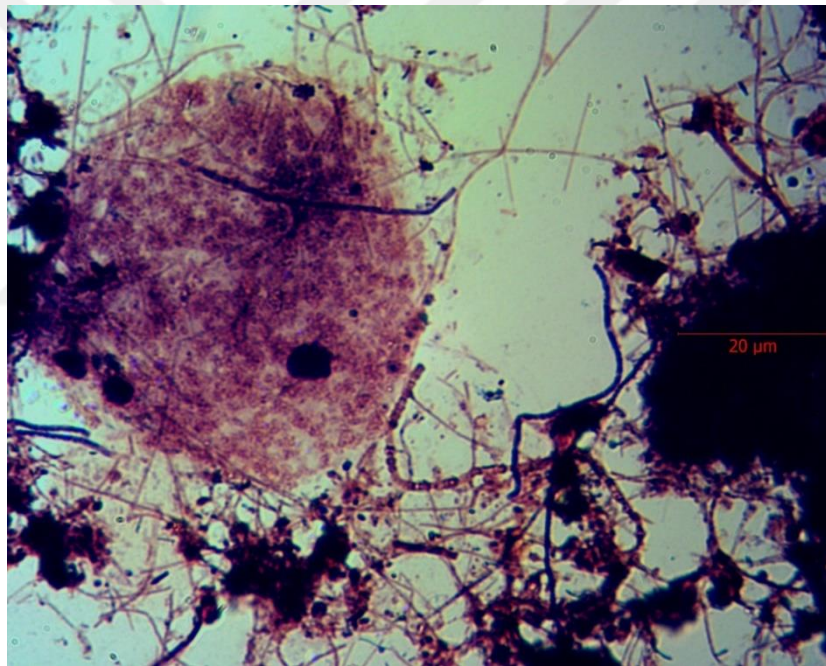


Figure 3.22 (continued): Micrographs of anoxic tank samples; 1, 2, 3 are wet mount taken at original magnification 100x, 200x, 400x respectively and 4 is gram staining taken at original magnification 1000x with oil immersion technique.

3.5.3 6th month, aerobic tank

Figure 3.23 shows Free cell suspension: Low, Floc morphology: Diffuse & weak*, Diversity: poor and Filament Abundance Score: 6

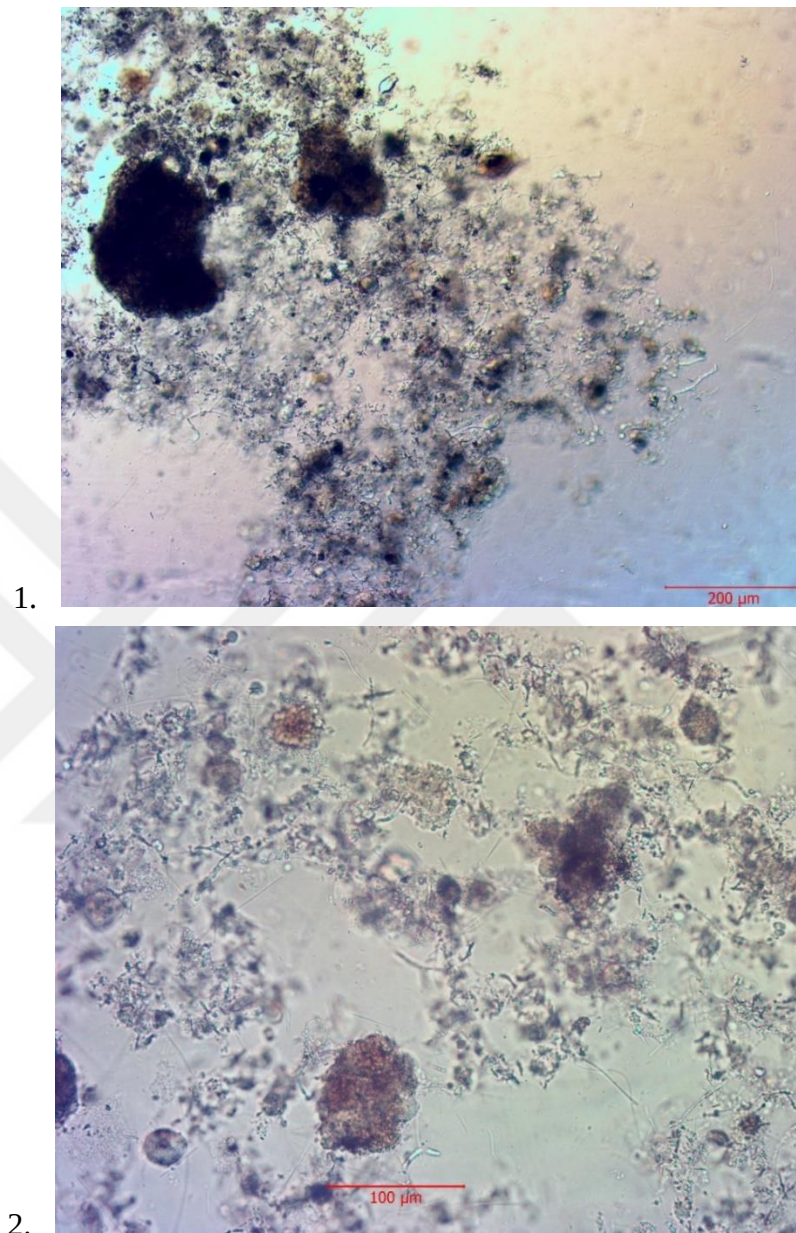
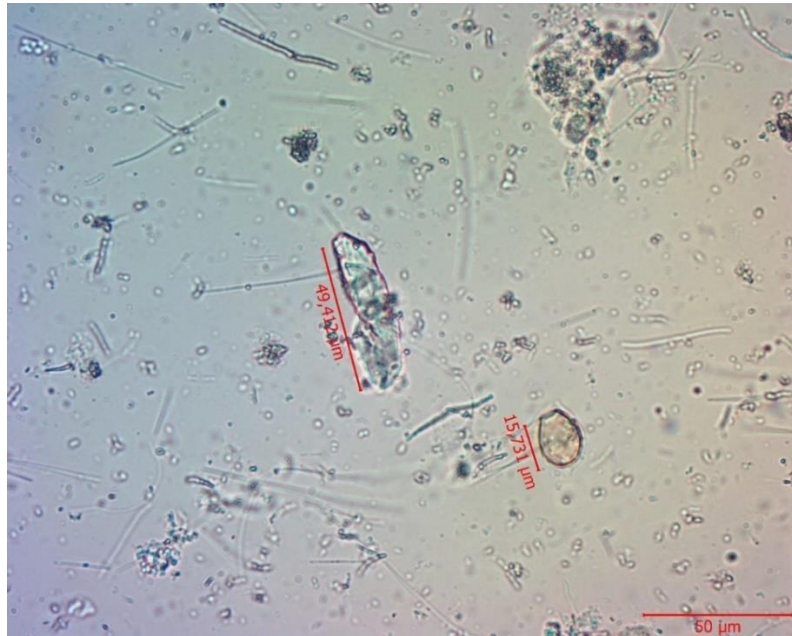
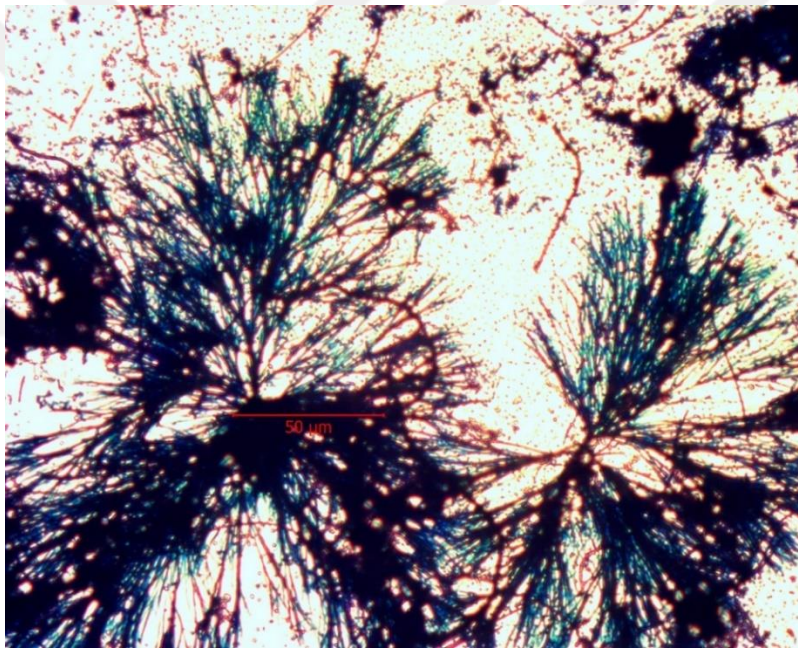


Figure 3.23: Micrographs of anoxic tank samples; 1, 2, 3 are wet mount taken at original magnification 100x, 200x, 400x respectively; 4 and 5 are gram staining taken at original magnification 1000x with oil immersion technique. *Actinomyces* spp. is observed in microphotograph number 4.

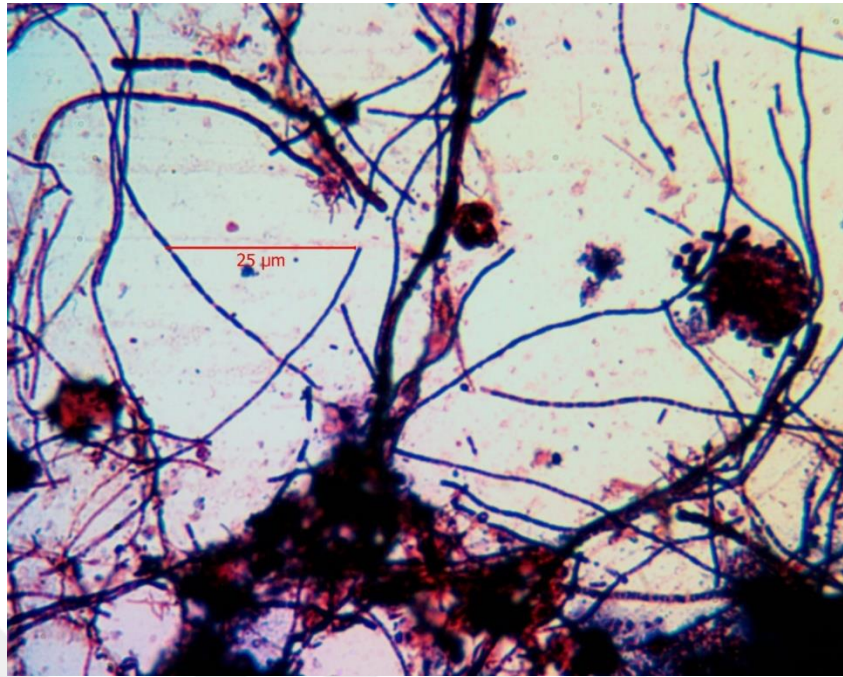


3.



4.

Figure 3.23 (continued): Micrographs of anoxic tank samples; 1, 2, 3 are wet mount taken at original magnification 100x, 200x, 400x respectively; 4 and 5 are gram staining taken at original magnification 1000x with oil immersion technique. *Actinomyces spp.* is observed in microphotograph number 4.



5.

Figure 3.23 (continued): Micrographs of anoxic tank samples; 1, 2, 3 are wet mount taken at original magnification 100x, 200x, 400x respectively; 4 and 5 are gram staining taken at original magnification 1000x with oil immersion technique. *Actinomycetes spp.* is observed in microphotograph number 4.

3.5.4 6th month, anoxic tank

Figure 3.24 shows Free cell suspension: Low, Floc morphology: Diffuse & weak*, Diversity: poor and Filament Abundance Score: 6

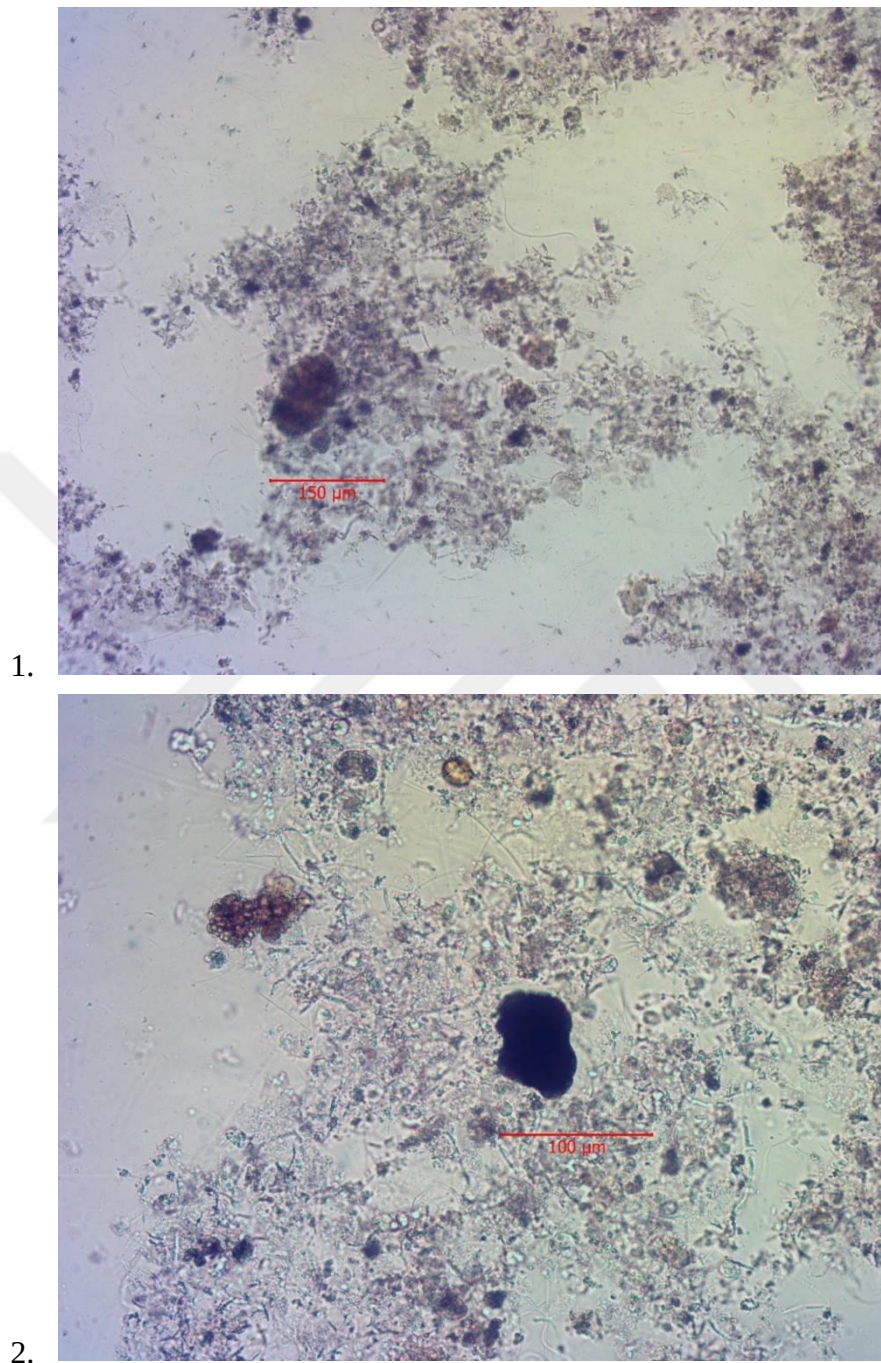
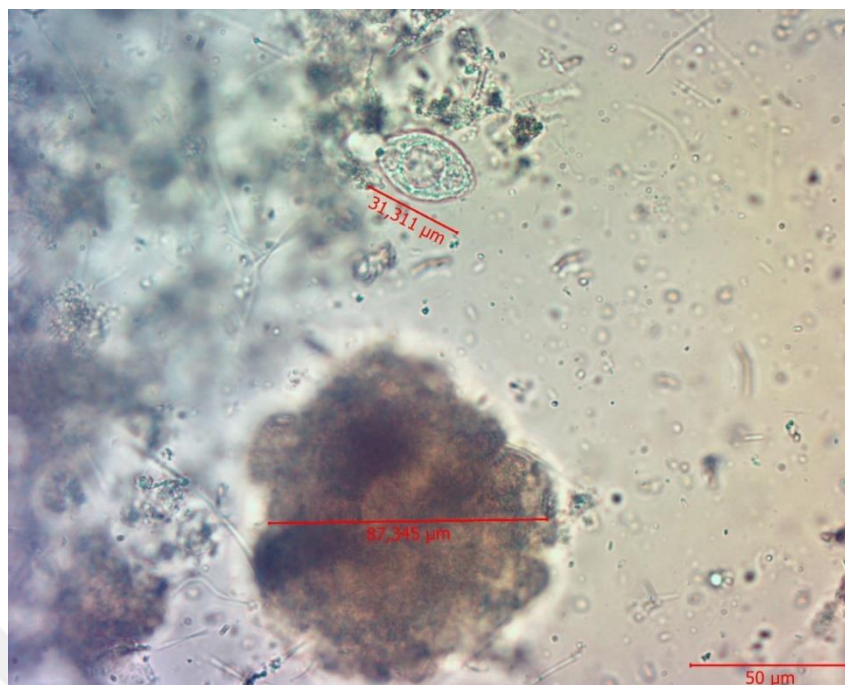


Figure 3.24: Micrographs of anoxic tank samples; 1, 2, 3 are wet mount taken at original magnification 100x, 200x, 400x respectively and 4 is gram staining taken at original magnification 1000x with oil immersion technique.

3.



4.

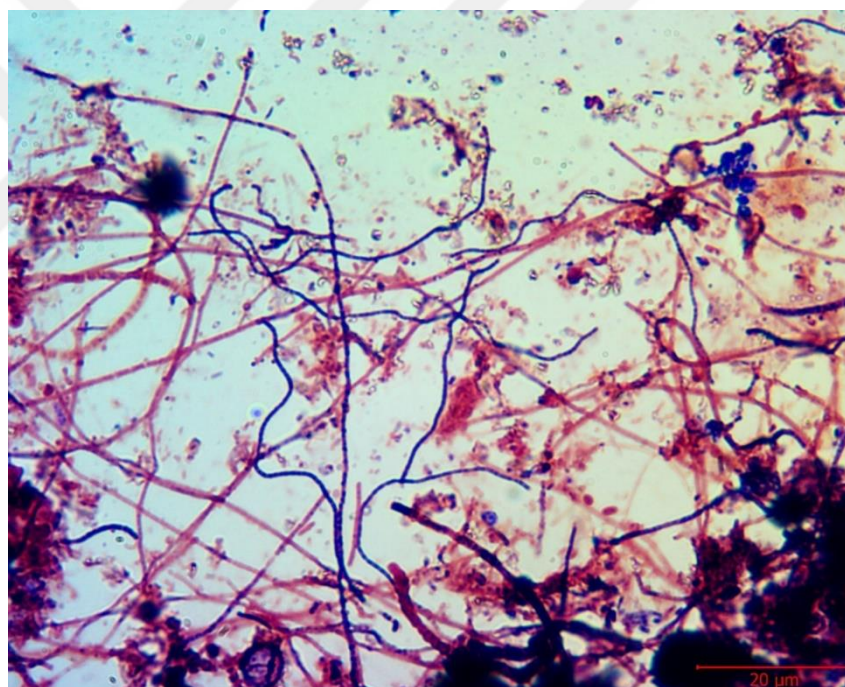


Figure 3.24 (continued): Micrographs of anoxic tank samples; 1, 2, 3 are wet mount taken at original magnification 100x, 200x, 400x respectively and 4 is gram staining taken at original magnification 1000x with oil immersion technique.

3.5.5 10th month, aerobic tank

Figure 3.25 shows Free cell suspension: Medium, Floc morphology: Diffuse & weak*, Diversity: medium and Filament Abundance Score: 5

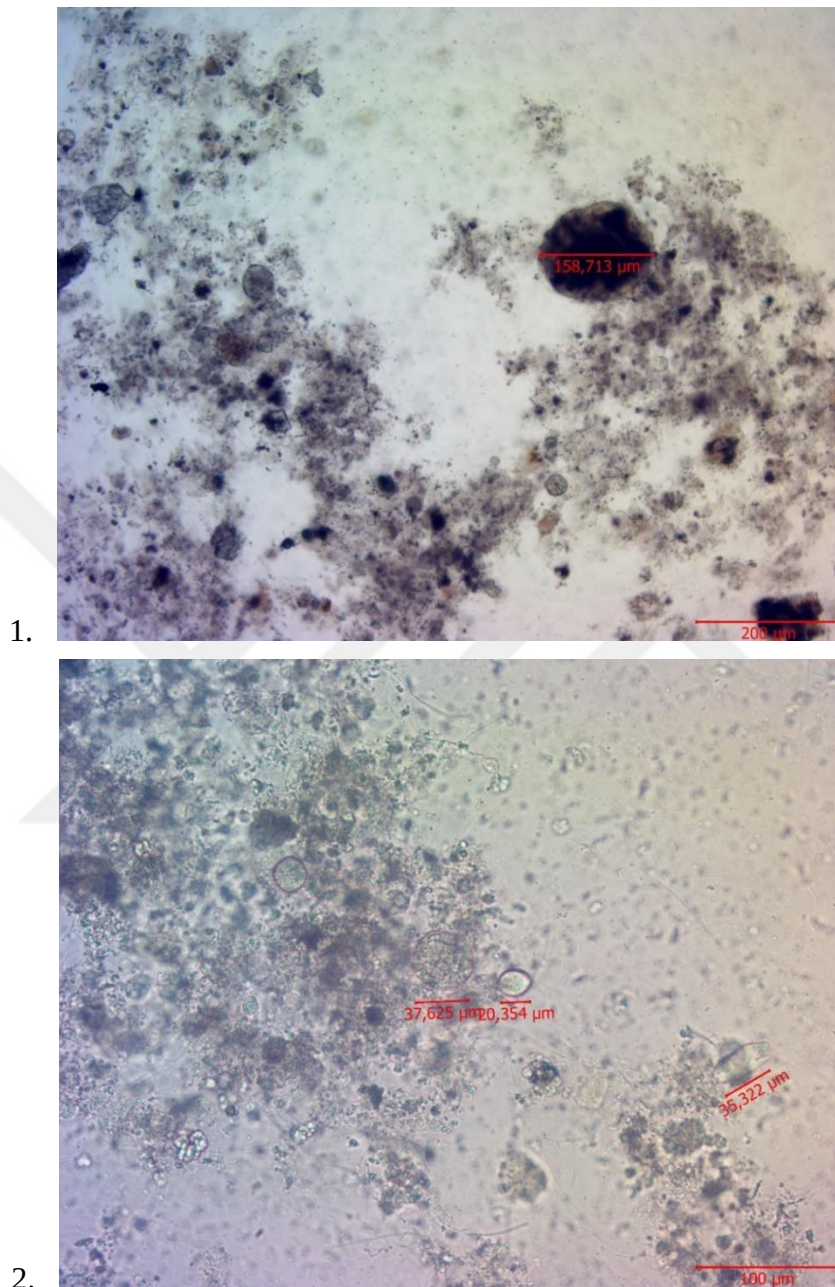
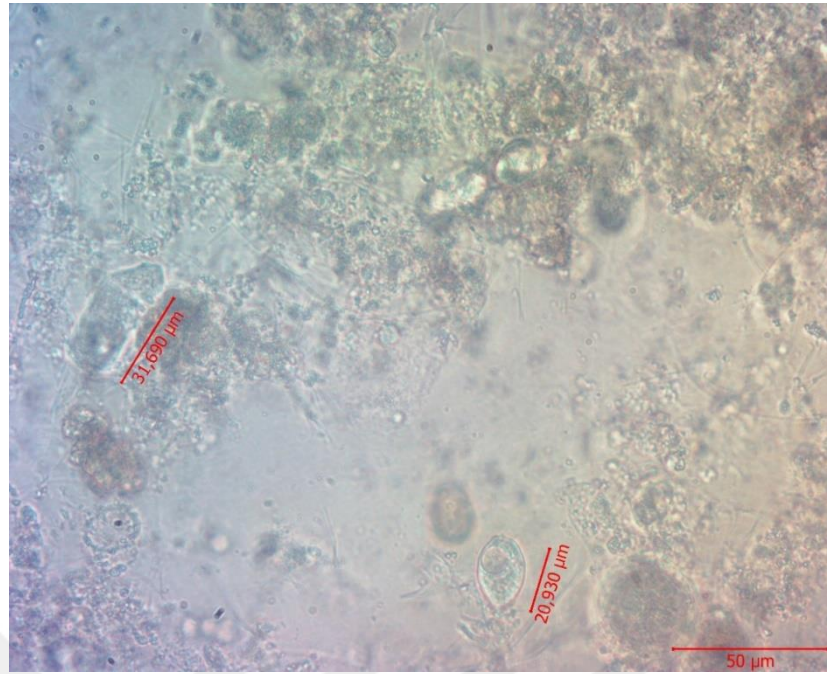


Figure 3.25: Micrographs of anoxic tank samples; 1, 2, 3 are wet mount taken at original magnification 100x, 200x, 400x respectively and 4 is gram staining taken at original magnification 1000x with oil immersion technique.

3.



4.

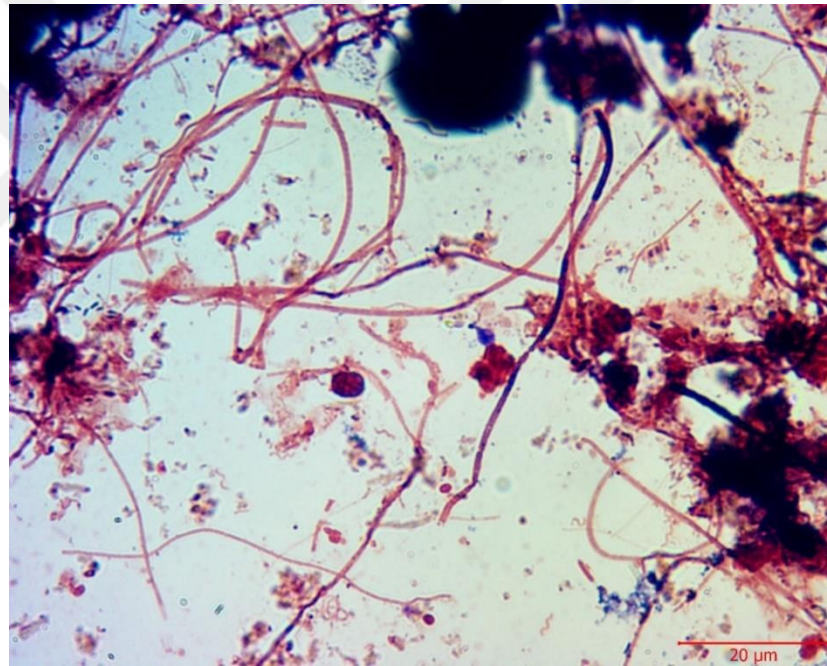


Figure 3.25 (continued): Micrographs of anoxic tank samples; 1, 2, 3 are wet mount taken at original magnification 100x, 200x, 400x respectively and 4 is gram staining taken at original magnification 1000x with oil immersion technique.

3.5.6 10th month, Anoxic Tank

Figure 3.26 shows Free cell suspension: Medium, Floc morphology: Diffuse & weak*, Diversity: medium and Filament Abundance Score: 4

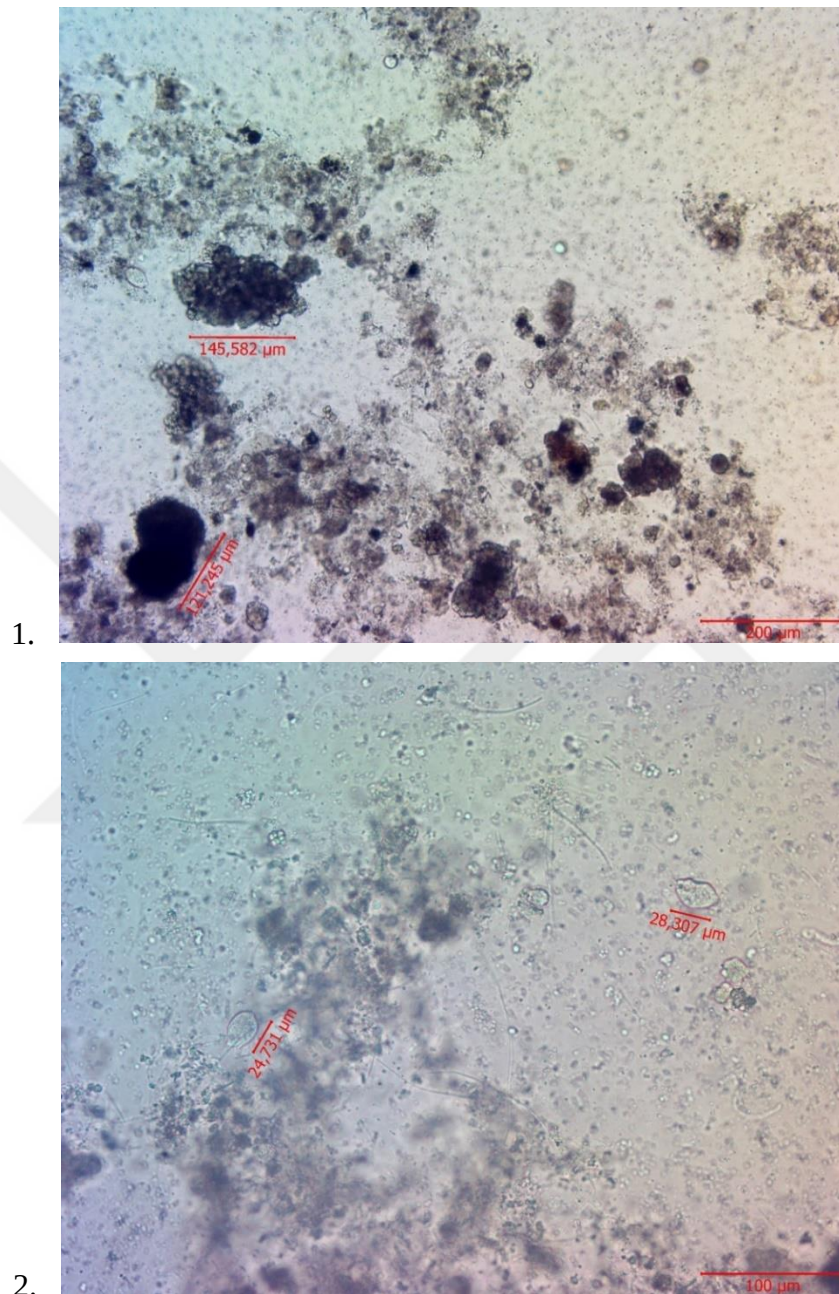


Figure 3.26: Micrographs of anoxic tank samples; 1, 2, 3 are wet mount taken at original magnification 100x, 200x, 400x respectively and 4 is gram staining taken at original magnification 1000x with oil immersion technique.

3.



4.

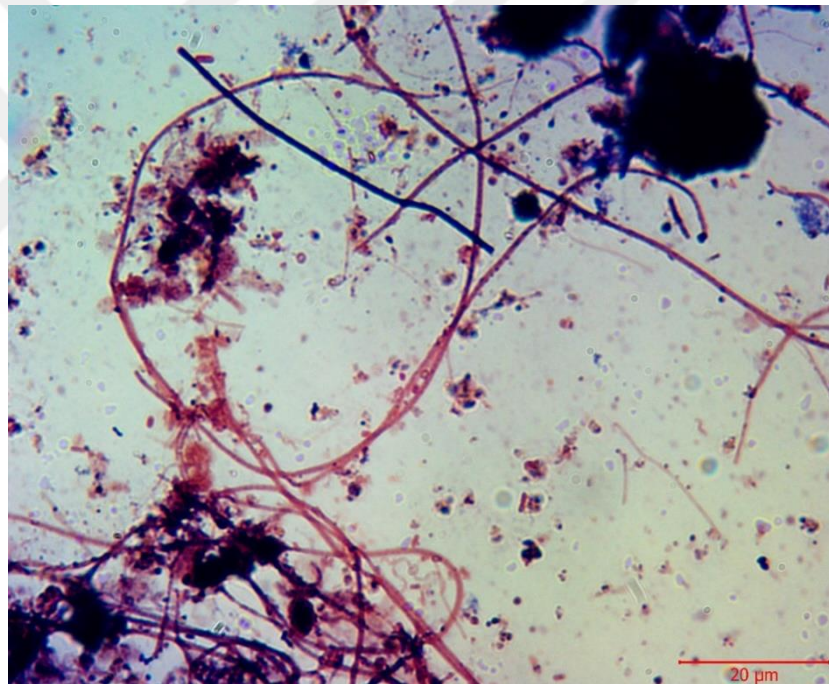


Figure 3.26 (continued): Micrographs of anoxic tank samples; 1, 2, 3 are wet mount taken at original magnification 100x, 200x, 400x respectively and 4 is gram staining taken at original magnification 1000x with oil immersion technique.



4. DISCUSSION

In this section the results are discussed and possible explanation on the causes are given.

4.1 Landfill Leachate Parameter Comparison of 2013-2018 and Fluctuations in The Influent

Landfill leachate parameters of COD, Orthophosphate, total nitrogen and ammonia nitrogen of 2018 were compared against the data obtained in 2013. The reason behind this comparison is to observe the changes occurring in the landfill leachate.

As the landfill ages, the COD of leachate decreases as a result of biological decomposition of organic materials [55]. Applying this knowledge to our landfill leachate, it is observed that COD and $\text{PO}_4\text{-P}$ has shown significant decrease, 2 folds, however TN and $\text{NH}_4^+\text{-N}$ has shown an approximately 1.45 folds increase in average inflow, mg/L. Gradual decrease of COD and $\text{PO}_4\text{-P}$ from 2013 to 2018 signals to the reduction of organic content in the landfill leachate. The COD reduction is typical of the transition from acetogenic to methanogenic leachates, where volatile fatty acids are reduced further to methane and carbon dioxide by methanogenic bacteria with a reduction in the COD of the leachate. The moisture content of the landfill is increased to accelerate the decomposition process by recirculation of the sludge from sludge dewatering system [56]. This approach reduces the COD by enhancement of methanogenesis.

Different scenarios performed by the modelling study showed that the denitrification capacity of the treatment plant was insufficient. The amount of $\text{NH}_4^+\text{-N}$ and TN entering the system calculated to require a COD input of at least 20000 mg/L for biological system to grow and sustain itself. At the beginning of 2018, it was suggested to mix in a high COD concentration containing leachate from another landfill to increase the COD value. However, even with the applied high COD leachate, required range was never met, COD-2018 maximum 18,350 mg/L, and mean COD flowing into the system was observed to be around ~12,000

mg/L. It was also concluded that $\text{PO}_4\text{-P}$ levels were below the required threshold too, thus Phosphoric acid, H_3PO_4 , addition into the system was suggested for 2018. Due to unforeseen consequences in the treatment plant, phosphoric addition to the system was neither at adequate volumes nor at given time intervals, thus fluctuations in $\text{PO}_4\text{-P}$ -2018 data was observed.

The TN and $\text{NH}_4^+\text{-N}$ data of 2013 could be considered stable as fluctuations were not as steep. 2018 data of TN and $\text{NH}_4^+\text{-N}$ shows sharp drops and sudden peaks within a large concentration range throughout the year. TN values of 2013 and 2018 share approximately the same concentration range, however at 7th month TN of 2018 starts to increase and reaches a peak value of 7144 mg/L at 10th month. At the end of 10th month TN-2018 concentration starts to gradually decrease until reaches approximately the same concentrations of TN-2013 at 12th month. Unlike TN, $\text{NH}_4^+\text{-N}$ concentrations of 2013 and 2018 shows vast differences, $\text{NH}_4^+\text{-N}$ - 2018 being extremely high and fluctuating throughout the year. Where $\text{NH}_4^+\text{-N}$ - 2013 observed to follow a steady concentration range, mostly, between 1,000-1,500 mg/L, $\text{NH}_4^+\text{-N}$ -2018 data shows abruptly scattered concentration inflow, between 600-4,000 mg/L throughout the year. Mostly noticeable and long-term changes for $\text{NH}_4^+\text{-N}$ -2018 data observed in 8th and 9th month where concentration levels dropped and stayed around 600 mg/L followed by a continuous increase at the start of the 10th month till 11th month showing a peak value of 3987 mg/L. One reason for these concentration fluctuations could be the addition of different source leachate to the system. Unfortunately, $\text{NH}_4^+\text{-N}$ concentration data of the added leachate to the system is not presented by the facility, therefore only assumptions can be made. However, it is also known that ammonia nitrogen of landfill leachate is high due to hydrolysis and fermentation of biodegradable materials and recirculation of sludge back into the landfill further increases the toxic waste solubilisation rate from the decomposition process hence also resulting more ammonia release into the landfill leachate inflow [57]. In addition, anaerobic decomposition processes do not assimilate nitrogen into biomass to the same extent as aerobic processes [58]. The modest amount of ammonia uptake from decomposition of landfill waste for anaerobic bacterial growth and further nitrogen release from protein decomposition increases the TN and $\text{NH}_4^+\text{-N}$ value of the landfill leachate [59]. Burton & Weston-Craik 1998 [58] has mentioned that

ammonia concentration of approximately 3000 mg $\text{NH}_4^+\text{-N}$ /L can significantly inhibit methanogenesis in their work. Therefore, any time spent with $\text{NH}_4^+\text{-N}$ at 3000 mg/L or above is assumed to be affecting the target microorganisms and microbial diversity of the system.

On average, the pH value of the system agrees with the age of the landfill that it should be more than 7.5 and based on pH data, landfill leachate can be classified as in methanogenic phase. In this phase, the volatile fatty acid in leachate is depleted and lead to the increasing of pH values [60]. The pH fluctuations in the system is assumed to root from two conditions; first is the addition of different source leachate, and Second is the HCl addition to the equalisation tank. The pH data of different source leachate is not present, however in order to have a high COD value this leachate must be from a young age landfill which has high COD as mentioned in the literature review section. Young leachate characteristics generally show pH range around 6.5, hence it is highly expected to decrease the pH of the system which could explain the pH drops in system. On the other hand, addition of HCl to the system is also observed to balance the pH value. However, the pH addition was neither at constant time intervals nor at constant volumes. Addition of up to 20000 litres of HCl a day was observed, and it is highly likely that sudden addition of such amount could disrupt the microbial diversity of the system.

As an open system, equalization, aerobic and anoxic tanks being exposed to open air, Temperature was influenced by the weather at landfill site. The rainfall and Sun exposure are known to change the temperature of the system [61]. It is expected to observe a temperature decrease during rainy and increase during sunny days in the system. The facility is in Istanbul hence is affected by the weather conditions of this area.

Figure 4.1 below shows weather conditions for Istanbul in 2018 [62]. In General, the temperature of Treatment facility equalisation, aerobic and anoxic tanks were above the weather conditions.

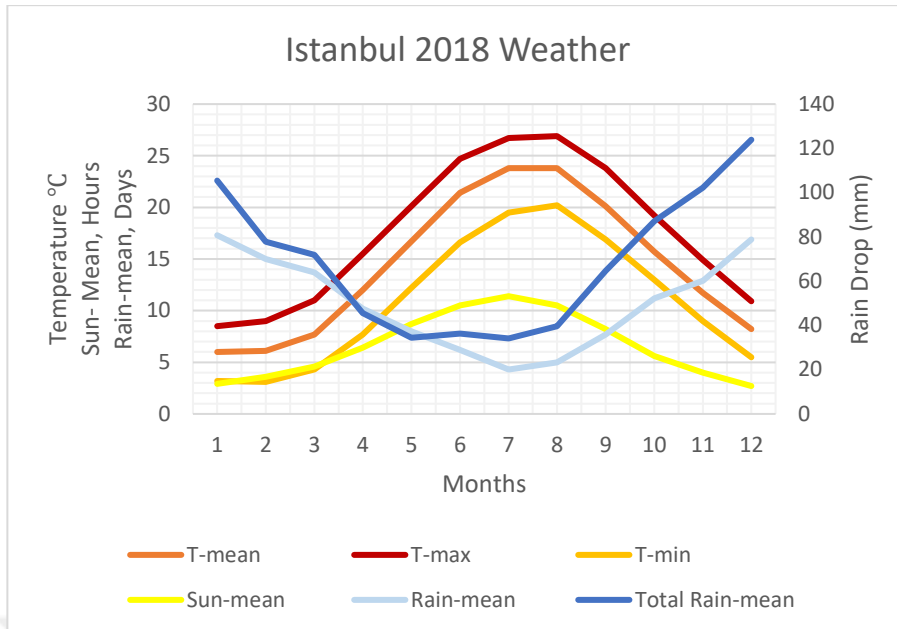


Figure 4.1: 2018 rainfall and temperature data of Istanbul.

Apart from above mentioned conditions effecting the system, technical problems encountered by the facility should also be taken into consideration. The daily reports submitted in the facility shows that pipe clogging and blockages, pump, aerator and automatic-mixer breakdowns, electricity cuts and generator failures, system pathway changing constructions were some of the technical disabilities occurred through the year. It is important to mention that all these factors can affect the microbial diversity of the system.

The Mixed Liqueur Suspended Solids, MLSS, inflowing into the system showed major fluctuations in both tanks throughout the year. The concentration of MLSS and the SVI is accepted to have an orderly relationship. The Increasing MLSS concentration, increases the SVI value, to a certain value, of the sludge as it fails to agglomerate into a coarse, open lattice to permit settling. Study of Richard I. Dick and P. Aarne Vesilind, July 1969, has shown that the SVI sludge highly dependent on MLSS concentration. Commonly accepted SVI values and their interpretations are as follows;

- Less than 80 ml/g; old sludge with possible pin floc and increased effluent turbidity.
- Between 80 and 150 ml/g; intended sludge with good settling and low effluent turbidity.

- More than 150 ml/g; bulking sludge with poor settling and high effluent turbidity.

Figure 4.2 [63] below is taken from their study to show that at very high MLSS concentrations even though the sludge may not agglomerate, the possible SVI of a sludge decreases at greater MLSS concentrations. In Figure 4-2, plant E and A2 has a MLSS concentration more than 6000 mg/L, even though their SVI is not within intended sludge interval, the sludge still agglomerated. In addition, it illustrates that a sludge of 10000 mg/L MLSS still would have a maximum SVI equal to 100 ml/g which between the intended well settling sludge gap.

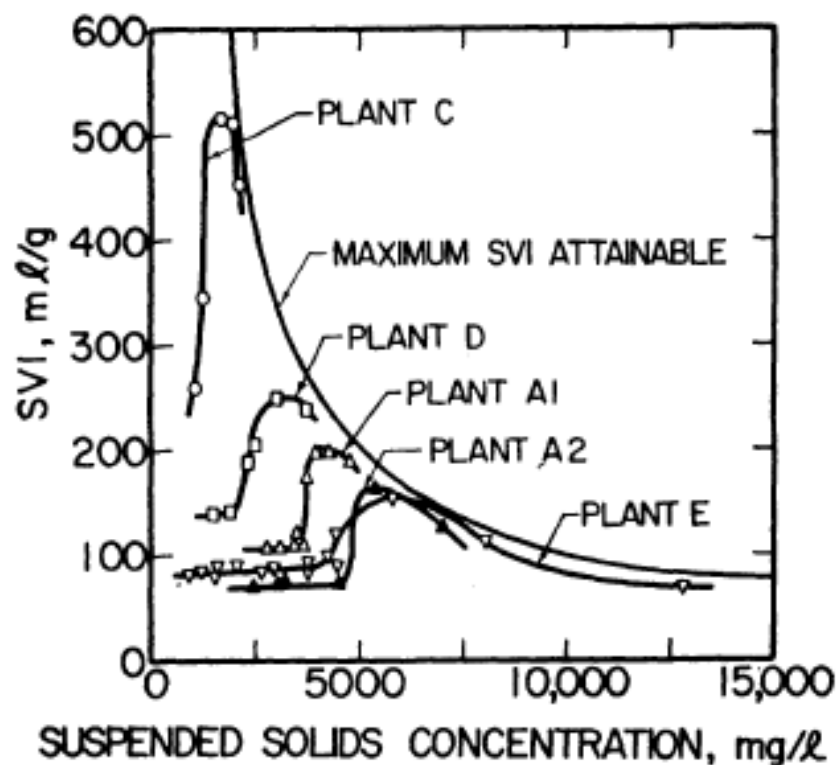


Figure 4.2: SVI versus MLSS concentration graph of different treatment plant.

The SVI values of samples taken from treatment plant never exceeded the 150 ml/g level at any given time throughout the year. However, even though the most of SVI values for samples were within the 80 and 150 mg/L SVI band, the sludge had poor settling and high turbidity. This is due to MLSS concentration being too high and excessive filamentous microorganisms, therefore even though the sludge is not settling, the calculated SVI is still within the accepted values. Each plant has a specific SVI value which can vary depending on the size and performance of the final clarifier(s) and hydraulic considerations. On the other hand, the sample taken from

anoxic tank on 06.09.2018 has shown the best settling quality and low turbidity. Hence, it can be assumed that acceptable SVI value for ~11000 mg/L MLSS concentration for our system is around 40 ml/g.

The fluctuation in MLSS concentration cannot be pinpointed to one reason however, once again, the addition of leachate from different could cause a rapid increase and the recirculated sludge into the landfill which enhances the decomposition of organic materials faster can cause a slower increase over the years. High sludge retention time also contributes to the increase of MLSS value in the system. The sudden decreases can be assumed to be the effects of sludge removal and scrapping of sludge from the bottom of the equalization tank. MLSS concentration decrease are not observed to be occurring at a set time interval which could result from several reasons; first, unscheduled, random sludge removal or scrapping of the bottom of tanks; Second, technical problems involving break down or service requirement of the equipment and finally the addition of different source leachate to increase required parameters, COD. Very high MLSS values is also observed to clog and break the filtration equipment and increase the number of backwashes required to re-set the whole treatment system, hence increasing the cost effectiveness of treatment plant.

4.2 Filament Count, Identification and Correlation

Most abundant and commonly observed filaments were named as Filament 1, 2 and 3, where later identified according to Jenkins et al. 2003. Filament count for aerobic and anoxic tanks were calculated and compared against the scoring system of Jenkins et al. 2003. It is important to mention that score 6, 20 or more filaments, is the highest score however does not reflect the exact number of filaments per μl . This is due to counting being stopped as maximum score is reached. In this study, filaments were counted to 30 instead of 20 as mentioned in the scoring system, to certain that score 6 is passed or not. The maximum number of filaments per μl in aerobic and anoxic tanks are shown as 19,657 no./ μl , however it is important to notice that there might be more filaments per μl as after counting 30 filaments the experiment was discontinued.

The aerobic tank filament count shows that Filament 1 was the most abundant, followed by Filament 2 as second most common and lastly Filament 3. Same results were observed in the anoxic tank filament count too. The data for both tanks shows that approximately the same amount of decrease and increase occurred for all the

filaments throughout the year. Least amount of Filament 1 was observed in 10th month for both tanks with 8,518 no/μl. The minimum amount of filament 2 in aerobic tank was on 7th month with 2,621 no/μl and in anoxic tank was on 8th month with 2,621 no/μl. As for filament 3 showed minimum value of 655 no/μl at several months (6th, 7th and 12th) in aerobic tank and in anoxic tank only in 7th month reached to its minimum value of 655 no/μl.

Total filament count showed that for most of the year both aerobic and anoxic tanks stayed at score 6. Only in 10th month total filament count score of aerobic and anoxic tank was reduced to score 5 and 4, respectively. In order to understand the decrease in both aerobic and anoxic tanks parameters of 10th month are given in Table 4.1.



Table 4.1: Input, aerobic and anoxic tank parameter data of 10th month.

Parameter	COD	TN	NH ₄ ⁺ -N	PO ₄ -P	MLSS			pH			Temperature			Oxygen	
Tank	Input				Input	Aerobic	Anoxic	Input	Aerobic	Anoxic	Input	Aerobic	Anoxic	Aerobic	Anoxic
Units	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	-	-	-	°C	°C	°C	mg/L	mg/L
Min	12185	3126	2175	6	1200	9200	9700	7.68	7.96	-	25.70	35.10	34.00	2.00	0.11
Max	17786	4654	3541	10	2300	12200	12950	8.07	8.28	-	28.30	40.00	39.40	3.76	0.26
Mean	14476	3799	2934	8	1618	10526	10893	7.86	8.16	-	26.58	36.90	35.94	3.12	0.16
Std	1245	387	275	1	269	898	846	0.10	0.08	-	0.64	1.29	1.43	0.48	0.03

Solely aerobic and anoxic tank parameter data however is not enough to make a correlation with the filament decrease. Each filament is unique and shows different reaction to each of these parameters. From the microscopic investigations and with the help of dichotomous key provided by Jenkins et al. 2003, Filament 1, 2 and 3 was identified as *Haliscomenobacter hydrossis*, *Microthrix parvicella* and Type 021N, respectively. Knowledge obtained from Jenkins et al 2004 shows that;

- *Haliscomenobacter hydrossis* can develop en masse in industrial plants mostly with high easily biodegradable matter, high concentration of nitrogen compounds, low oxygen concentrations in the aeration tank and under phosphate deficiency conditions. Peptides, proteins and polysaccharides stimulate the growth of *Haliscomenobacter hydrossis* in activated sludge. Growth at pH 7.5 is much faster than that at pH 6.4, Temperature range for growth: 8-30 °C optimum: approximately 26 °C [64].
- *Microthrix parvicella* is seen to develop commonly in wastewater treatment plants with low loading rates. *M. parvicella* shows a seasonal growth pattern reaching maximum population at the end of winter and minimum in summer. Significant amounts of higher fatty acids, especially oleic acid, in the influent also promotes the growth positively. The optimum growth temperature is around 22 °C however at 7 °C the maximum growth rate is still appreciable, and no growth observed above 30 °C. Growth rate of *M. parvicella* decreases gradually from pH 8.0 to 7.2 and at an initial pH value of ≤ 7.1 , no growth is observed [65]. Other factors effecting growth are; a long sludge retention time in the anaerobic zone with Bio-P processes, low oxygen level in the aeration tank and Supply of reduced sulphur and nitrogen compounds is also a possible cause, especially recycling of water from the sludge dewatering unit.
- Type 021N is frequently observed in activated sludge systems and is strictly aerobic. Nutrient-deficient wastes, completely mixed activated sludge systems at moderately low F:M and influents with high readily biodegradable organic matter such as fatty acids are associated with Type 021N occurrence. Optimum temperature range for Type 021N growth is between 25 °C – 30 °C, below 20 °C and above 35 °C significantly less growth observed. pH range for Type

021N growth is accepted to be between 6.0 and 9.3, where below pH 6.0 and above 9.6 no growth is observed [66].

Factors such as addition of high amounts of HCl, long sludge age, disorganised sludge removal, nonperiodically addition of phosphate, high amount NH_4^+ -N entering the system, rainfall, weather changes as well as mechanical problems occurring in the treatment facility could all affect the filamentous organism growth. Thus, altered conditions could favour their growth over the target organisms resulting in bulking and foaming problems. Even though Pearson correlation results show some degree of large, medium and small correlations between the parameters and filament amounts, the variety of factors affecting the filamentous microorganism growth is too many to be included. Therefore, it cannot be concluded that a certain parameter is interfering or promoting the growth of filamentous microorganisms.

4.3 Filament Abundance, Floc Morphology and Microbial Diversity

Filament abundance experiment results showed that both aerobic and anoxic tanks suffered greatly from filamentous overgrowth all year except at 10th month. Highest filament abundance score of 6 in both Aerobic and anoxic tanks could only be reduced to score 5 and score 4, respectively. As mentioned before, filaments act as backbone of the flocs when present in certain amounts, however when recommended filament number is exceeded floc formation and morphology is disrupted. Too many filamentous microorganisms protruding like tentacles from the flocs could prevent the formation of bigger flocs as they are in a more favourable position to compete for the available substrate than the unicellular microorganisms within the flocs [69] and could disturb the surface charge of the floc.

A microbial floc structure is a composition of heterogenous microorganisms, particles, organic polymers, colloids, dead cells and cations [67]. The main constituents of a floc matrix are the extracellular polymeric substances which form a matrix to encapsulate the cells and play a major role in binding the floc components together. Extracellular polymeric substances are made up out of polysaccharides, humic compounds, protein, lipids and nucleic acids [68]. These substances are produced depending on nutritional conditions, particularly nutrient limitation such as nitrogen, phosphorus or sulphur [69]. Therefore, parameter fluctuations in the inflow could be said to influence floc formation and morphology as much as the filament abundance. On the other hand,

Study of S. Bala Subramanian et al 2015 shows that EPS concentration and charge are not directly related to the sludge settling [70]. Pearson correlation analysis of this study between floc size and measured parameters also suggest that COD (anoxic tank), $\text{NH}_4^+\text{-N}$ (aerobic and anoxic tank) and pH (aerobic tank) has large correlations with floc size, meaning these parameters could have a more direct influence on both floc size and morphology. Although only a medium correlation is observed with MLSS, TN and temperature with floc sizes in tanks, it would be unwise to ignore them. MLSS and free cells in suspension was observed to show parallel changes through the months, however whether there is a correlation or not cannot be concluded.

Foster, 1968 has shown that surface charge of sludge particles was directly proportional to the SVI. Zeta potential and Van der Waals are two of the major physiochemical parameters effecting whether bacteria will form into flocs [71]. The greater the zeta potential, the larger the repelling surface charge of the particle than the attractive Van der Waals forces. Hence floc forming bacteria will not aggregate and stay dispersed in the landfill leachate. In order to promote aggregation and floc formation by bacteria, low surface charge or zeta potential is required which enables Van der Waals forces to dominate [60].

It could be assumed that free cells in suspension, floc size and morphology is due to low surface charge or zeta potential of the bacterial flocs, fluctuating parameters creating unbalanced environment, high amounts of filamentous microorganisms and poor diversity. Hence smaller floc sizes were more often observed in tests rather than expected large flocs.

It is reported that the composition and diversity of the microbial community had the greatest impact on stability and performance of the wastewater treatment systems. The biological community of activated sludge has a large biological diversity and contains a variety of viruses, bacteria, protozoa, fungi, algae, and metazoan [72].

In case of microbial diversity, simultaneous protozoa population increase is expected within the systems depending on the characteristic succession of species. In general, when high amounts of free cells present in suspension, protozoa and metazoa groups are expected to develop *en masse* due to increased food in the environment. Increase in sludge concentration and subsequent scarcity of nutrients generally results in decrease of free-living ciliates. Attached ciliates such as *Vorticella* or crawling ciliates

such as *Aspidisca* are not easily washed out at low sludge loading levels as free-living ciliates, thus expected to be observed in activated sludge samples often. On the other hand, Metazoa is also expected to eventually appear with long sludge ages [73]. The connection between the sludge load and the composition of the population is shown systematically in Figure 4.3 [42].

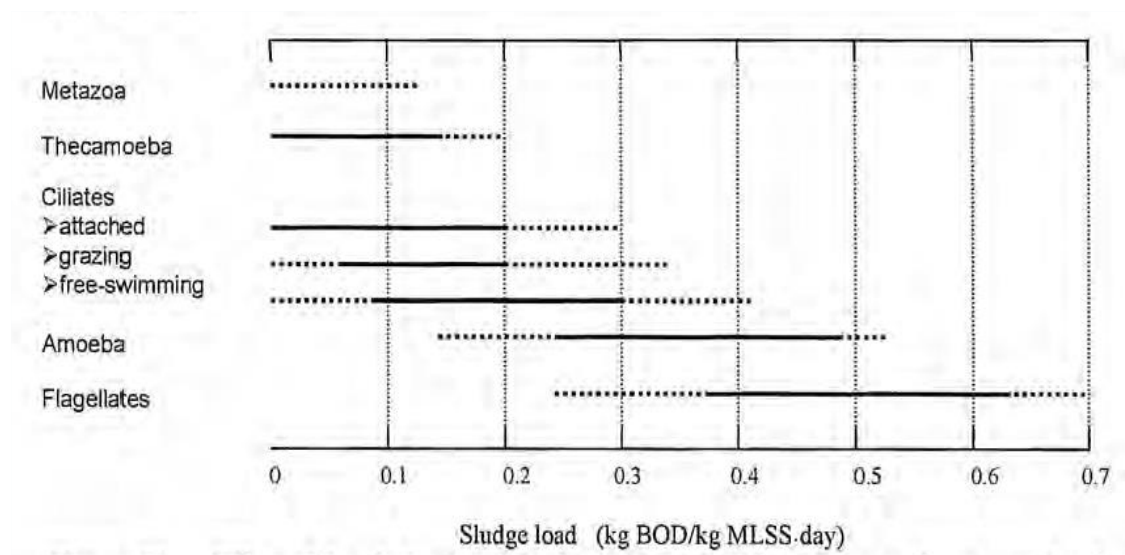


Figure 4.3: Effect of the sludge load on the occurrence of protozoa and metazoa.

Figure 4.3 is a good representation to understand what kind of microorganism is expected at different sludge loadings. Unfortunately, the treatment facility has not been measuring BOD levels, hence only an assumption over BOD:COD ratio from literature of young and old landfills could be used to relate our data. The study of Chian and DeWalle [74] reported that the BOD/COD ratio decreased rapidly in the aging landfills, from 0.7 to 0.04. A young landfill contains a BOD/COD ratio greater than 0.5 where a landfill with 0.1 ratio is considered mature and stable [75]. According to Samudro G. 2010 a value between $0.1 <$ and < 0.5 could be assumed for our landfill as it is an intermediate landfill. If the BOD:COD ratio is assumed 0.3 then BOD: MLSS (mean MLSS total year) would be 0.8 for our treatment plant. Based upon these assumptions mostly Flagellates are expected to be observed. On the other hand, Figure 4.4 [76] shows relative predominance of activated sludge microbial diversity according to low or high SVI, F:M and Sludge age. SVI of the system could be accepted to be high where sludge age and F:M of the system is low.

Courtesy of CSU Sacramento, Operation of Wastewater Treatment Plants (Vol II, 6th ed.)

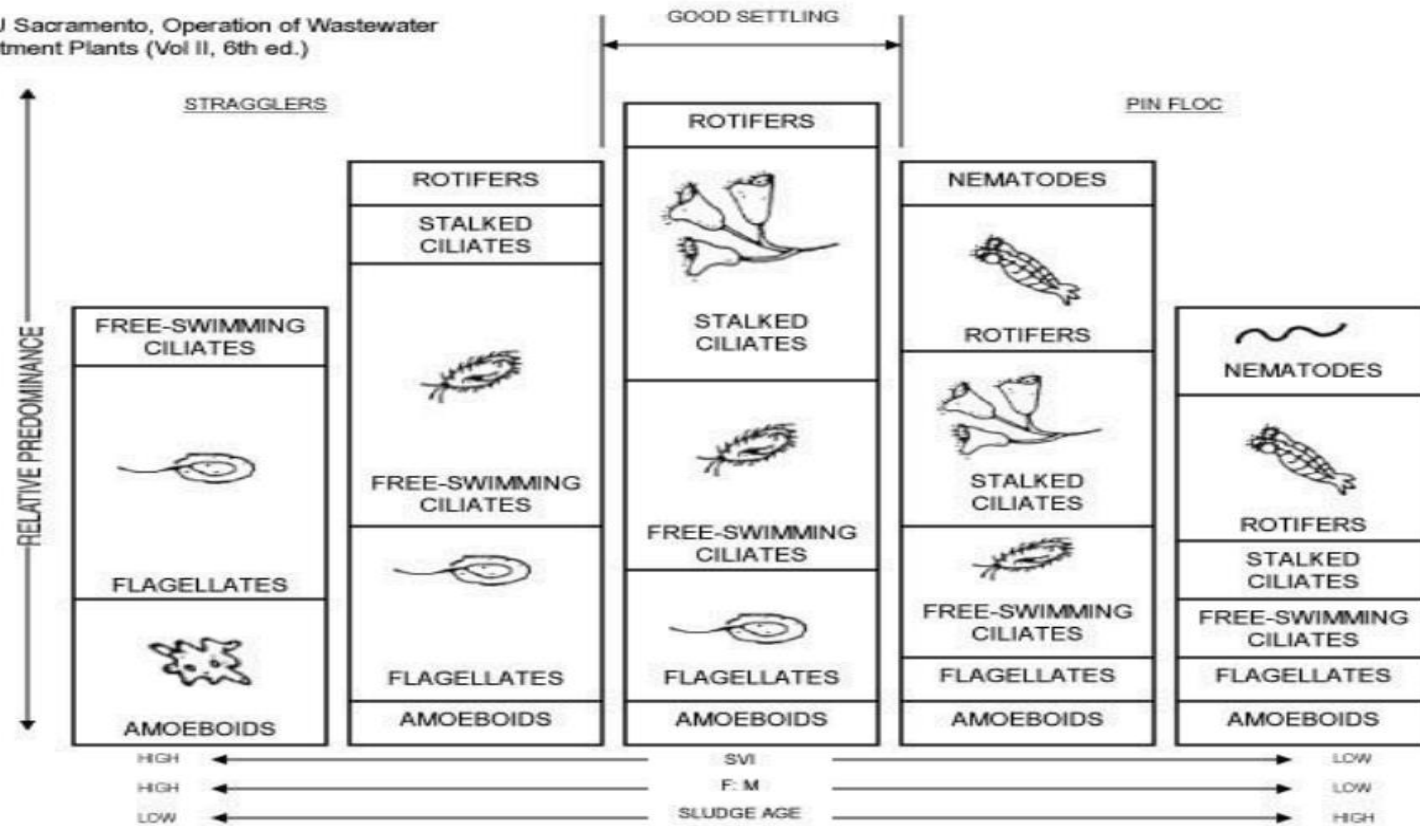


Figure 4.4: Activated sludge microbial diversity predominance according to SVI, F:M and sludge age

The microbial diversity observed in leachate treatment plant samples was poor until 9th month where increased to medium as mostly protozoa of free-swimming ciliates and flagellates were observed, very rarely actinomyces and no metazoa was seen during the examinations. Some of the identified protozoa included *Aspidisca*, *Paramecium*, *Euplotes*, *Blepharisma* and *Peranema* where no rotifers, nematodes, tardigrades or worms were observed.

The absence of protozoa and metazoa in the system could be related to toxic components present in the influent. Even though the free cells in suspension is medium/high for most of the year, elevated level of $\text{NH}_4^+\text{-N}$ entering the system, fluctuations in parameters especially pH, temperature, COD and $\text{PO}_4\text{-P}$ could have resulted in poor/medium diversity observation.

5. CONCLUSION AND RECOMMENDATIONS

In conclusion, low COD and $\text{PO}_4\text{-P}$, High $\text{NH}_4^+\text{-N}$ and MLSS, hence SVI, inflow to the system resulted in filamentous overgrowth, clogging of UF and NF filters, and poor microbial diversity. Attempts to stabilise the fluctuations in parameter values, reduction of filamentous microorganisms and improving floc morphology has not been at the level of our expectations. Even though filamentous microorganism abundance and floc size has shown some degree of correlation with the parameters, the variety of ever-changing factors disable our judgement on this issue. It came clear that increased amount of sampling and examination together with 16s/18s rRNA identification method is a must in order to truly improve the activated sludge system. Optimisation studies on identified and isolated filamentous microorganisms could be mimicked at lab scale to better understand how to deal with these organisms under our treatment plants conditions.

5.1 System Improvement Suggestions

Modelling study of the landfill leachate treatment facility suggested several precautions to improve the treatment and overcome the problems such as reducing current sludge age, elevated COD input to raise F:M ratio, increasing the anoxic area, stabilised inflow of nutrients and MLSS, and scheduled addition of chemicals in smaller doses to balance pH. Some of these conditions were applied such as increased COD input by mixing in younger landfill leachate with high COD, where others could not be applied due to unforeseen reasons. As a real time working treatment facility, mechanical problems such as breaking down of equipment, construction of new pipelines, pumps aerators, etc was also had some impact on microbiology and efficiency of the treatment plant too. Apart from the suggested improvements by modelling study, first and most important issue to be addressed is identification of filamentous microorganisms with 16s-18s rRNA sequencing method. Microscopic observations of filamentous microorganisms can only reveal the species that these organisms belong to, however different strains within species could show dissimilar

growth rate, resistance to stress and other factors. Therefore, it is very important to identify the strains of these species and apply improvement efforts accordingly to eliminate or proliferate them. Regarding to the methodology of this study, samples were received from treatment facility twice a month however increasing the number of samples received to every week is concluded to be better to keep a sharp eye on microorganism diversity. For example, if a toxic shock is applied due to chemical addition to balance pH, etc on the start of first week, it is only observed after 14 days later which during this time the changes in microbial community might go unnoticed. As for stabilised input of certain parameters mentioned, which has been the most challenging for the treatment facility, addition of chemicals, such as HCL for pH adjustment or phosphate to improve nutrient concentration, and different source leachate to the system was delayed, unevenly distributed and had no set time intervals. It is not known whether this was something to do with the treatment facility management or logistic issues resulting from suppliers.

REFERENCES

- [1] Hoornweg, D., & Bhada-Tata, P. (2012). What a waste: a global review of solid waste management (Vol. 15, p. 116). World Bank, Washington, DC.
- [2] Raghab, S. M., El Meguid, A. M. A., & Hegazi, H. A. (2013). Treatment of leachate from municipal solid waste landfill. HBRC journal, 9(2), 187-192.
- [3] URL1: <https://slideplayer.com/slide/4689087/15/images/10/Schematic+of+a+Land+fill.jpg>. date retrieved 21.02.2019
- [4] URL2: www.corrosionpedia.com. date retrieved 21.03.2019
- [5] Maiti, S. K., De, S., Hazra, T., Debsarkar, A., & Dutta, A. (2016). Characterization of leachate and its impact on surface and groundwater quality of a closed dumpsite—a case study at Dhapa, Kolkata, India. *Procedia Environmental Sciences*, 35, 391-399.
- [6] Di Iaconi, C., Ramadori, R., & Lopez, A. (2006). Combined biological and chemical degradation for treating a mature municipal landfill leachate. *Biochemical Engineering Journal*, 31(2), 118-124.
- [7] Al Raisi, S. A. H., Sulaiman, H., Suliman, F. E., & Abdallah, O. (2014). Assessment of heavy metals in leachate of an unlined landfill in the Sultanate of Oman. *International Journal of Environmental Science and Development*, 5(1), 60.
- [8] Polat, A., Öztürk, M., Topçu, U. S., & Aslan, Ş. Application of Partial Nitrification/Anammox Processes for Landfill Leachate Treatment.
- [9] Chian, E. S., & Dewalle, F. B. (1976). Sanitary landfill leachates and their leachate treatment. *Journal of the Environmental Engineering Division*, 102(2), 411-431.
- [10] Bhalla, B., Saini, M. S., & Jha, M. K. (2012). Characterization of leachate from municipal solid waste (MSW) landfilling sites of Ludhiana, India: a comparative study. *International Journal of Engineering Research and Applications*, 2(6), 732-745.)
- [11] Liu, S. (2013). Landfill leachate treatment methods and evaluation of Hedeskoga and Måsalycke landfills. Lund: Lund University.
- [12] (Johannessen, L. M. (1999). Guidance note on leachate management for municipal solid waste landfills. World Bank.)
- [13] Renou, S., Givaudan, J. G., Poulain, S., Dirassouyan, F., & Moulin, P. (2008). Landfill leachate treatment: review and opportunity. *Journal of hazardous materials*, 150(3), 468-493.
- [14] Diamadopoulos, E. (1994). Characterization and treatment of recirculation-stabilized leachate. *Water Research*, 28(12), 2439-2445.
- [15] Rubio, J., Souza, M. L., & Smith, R. W. (2002). Overview of flotation as a wastewater treatment technique. *Minerals engineering*, 15(3), 139-155.
- [16] Mojiri, A., & Branch, I. K. (2011). Review on membrane bioreactor, ion exchange and adsorption methods for landfill leachate treatment. *Aust. J. Basic Appl. Sci*, 5(12), 136.

-
- [17] Rocha, E. M., Vilar, V. J., Fonseca, A., Saraiva, I., & Boaventura, R. A. (2011). Landfill leachate treatment by solar-driven AOPs. *Solar Energy*, 85(1), 46-56.
- [18] PA website, November 2005; National Drinking Water Clearing House Web site, March 1999. From <https://www.mrwa.com/WaterWorksMnl/Chapter%2019%20Membrane%20Filtration.pdf>
- [19] Liu, S. (2013). Landfill leachate treatment methods and evaluation of Hedeskoga and Måsalýcke landfills. Lund: Lund University.
- [20] Radcliffe, J. C. (2004). Water recycling in Australia: a review undertaken by the Australian academy of technological sciences and engineering. Australian Academy of Technological Sciences and Engineering.
- [21] Bradley, B. R., Daigger, G. T., Rubin, R., & Tchobanoglous, G. (2002). Evaluation of onsite wastewater treatment technologies using sustainable development criteria. *Clean Technologies and Environmental Policy*, 4(2), 87-99.
- [22] Choo, K. H., & Lee, C. H. (1996). Membrane fouling mechanisms in the membrane-coupled anaerobic bioreactor. *Water research*, 30(8), 1771-1780.
- [23] Alzahrani, S., Mohammad, A. W., Hilal, N., Abdullah, P., & Jaafar, O. (2013). Comparative study of NF and RO membranes in the treatment of produced water—Part I: Assessing water quality. *Desalination*, 315, 18-26.
- [24] Ruiz-Martinez, A., Garcia, N. M., Romero, I., Seco, A., & Ferrer, J. (2012). Microalgae cultivation in wastewater: nutrient removal from anaerobic membrane bioreactor effluent. *Bioresource technology*, 126, 247-253.
- [25] Connolly, M. (2004). U.S. Patent No. 6,827,036. Washington, DC: U.S. Patent and Trademark Office.
- [26] Kurniawan, T. A., Lo, W. H., & Chan, G. Y. (2006). Degradation of recalcitrant compounds from stabilized landfill leachate using a combination of ozone-GAC adsorption treatment. *Journal of hazardous materials*, 137(1), 443-455.
- [27] Kurniawan, T. A., Lo, W. H., & Chan, G. Y. (2006). Degradation of recalcitrant compounds from stabilized landfill leachate using a combination of ozone-GAC adsorption treatment. *Journal of hazardous materials*, 137(1), 443-455.
- [28] Wang, B., He, S., Wang, L., & Shuo, L. (2005). Simultaneous nitrification and de-nitrification in MBR. *Water science and technology*, 52(10-11), 435-442.
- [29] Sandra, C. A., Jamil, N. A. M., Jabbar, S., Sakyat, S., & Gomes, C. (2017). Aerobic and anaerobic sewage biodegradable processes: the gap analysis. *Int J Res Environ Sci*, 3(3), 9-19.
- [30] Pourcher, A. M., Sutra, L., Hébé, I., Moguedet, G., Bollet, C., Simoneau, P., & Gardan, L. (2001). Enumeration and characterization of cellulolytic bacteria from refuse of a landfill. *FEMS Microbiology Ecology*, 34(3), 229-241.
- [31] Van Dyke, M. I., & McCarthy, A. J. (2002). Molecular biological detection and characterization of *Clostridium* populations in municipal landfill sites. *Appl. Environ. Microbiol.*, 68(4), 2049-2053.
- [32] Mackie, R. I., & Bryant, M. P. (1981). Metabolic activity of fatty acid-oxidizing bacteria and the contribution of acetate, propionate, butyrate, and CO₂ to methanogenesis in cattle waste at 40 and 60 °C. *Appl. Environ. Microbiol.*, 41(6), 1363-1373.
- [33] Köchling, T., Sanz, J. L., Gavazza, S., & Florencio, L. (2015). Analysis of microbial community structure and composition in leachates from a young landfill by 454 pyrosequencing. *Applied microbiology and biotechnology*, 99(13), 5657-5668.

-
- [34] Liu, J., Wu, W., Chen, C., Sun, F., & Chen, Y. (2011). Prokaryotic diversity, composition structure, and phylogenetic analysis of microbial communities in leachate sediment ecosystems. *Applied microbiology and biotechnology*, 91(6), 1659-1675.
- [35] Yahmed, A. B., Saidi, N., Trabelsi, I., Murano, F., Dhaifallah, T., Bousselmi, L., & Ghrabi, A. (2009). Microbial characterization during aerobic biological treatment of landfill leachate (Tunisia). *Desalination*, 246(1-3), 378-388.
- [36] Dong, H., Zhang, G., Jiang, H., Yu, B., Chapman, L. R., Lucas, C. R., & Fields, M. W. (2006). Microbial diversity in sediments of saline Qinghai Lake, China: linking geochemical controls to microbial ecology. *Microbial Ecology*, 51(1), 65-82.
- [37] Leachate characteristics, microbial community structure, and efficiency of a full-scale leachate treatment system (Doctoral dissertation, Nagaoka University of Technology Japan 2016).
- [38] “Atık Yönetimi”; Çevre Koruma ve Kontrol Daire Başkanlığı, 2014
- [39] İBB Halkla İlişkiler; @ibbPR; From <https://twitter.com/ibbpr/status/387494926969081856>
- [40] Duran, E. B., & Yakup, C. U. C. İ. (2016). Katı Atık Düzenli Depolama Sahası Sızıntı Suyunun Kimyasal ve Elektrokimyasal Arıtım Yöntemlerle Arıtılabilirliğinin Araştırılması. *Kahramanmaraş Sutcu Imam University Journal of Engineering Sciences*, 19(2), 104-110.
- [41] Richard, M., Brown, S., & Collins, F. (2003, June). Activated sludge microbiology problems and their control. In 20th annual USEPA national operator trainers conference (pp. 1-21).
- [42] Jenkins, D., Richard, M. G., & Daigger, G. T. (2003). Manual on the causes and control of activated sludge bulking, foaming, and other solids separation problems. Crc Press.
- [43] Eikelboom, D. H., & Van Buijsen, H. J. J. (1981). Microscopic sludge investigation manual. Report A94a. Delft, NL, IMG-TNO.
- [44] Kapley, A., Liu, R., Jadeja, N. B., Zhang, Y., Yang, M., & Purohit, H. J. (2015). Shifts in microbial community and its correlation with degradative efficiency in a wastewater treatment plant. *Applied biochemistry and biotechnology*, 176(8), 2131-2143.
- [45] URL3: <https://www.water-research.net/index.php/ammonia-in-groundwater-runoff-and-streams>. date retrieved 05.03.2019
- [46] Hydromantis Environmental Software Solution Inc.; Products: GPSX; 2018; Website: <https://www.hydromantis.com/GPSX.html>
- [47] Gülhan, H., Erşahin, M. E., Dereli, R. K., Özgün, H., Erkan, F. D., Özdemir, Ö., & Öztürk, İ. Tam Ölçekli Bir İleri Biyolojik Evsel Atıksu Arıtma Tesisinin Matematiksel Modellemesi.
- [48] Henze, M., Grady Jr, C. L., Gujer, W., Marais, G. V. R., & Matsuo, T. (1987). A general model for single-sludge wastewater treatment systems. *Water research*, 21(5), 505-515.
- [49] URL4: <http://coproweb.free.fr/pagbac/introbac/smeapre.htm>; date retrieved 13.03.2019
- [50] URL5: <http://coproweb.free.fr/pagbac/introbac/gramproc.htm>; date retrieved 13.03.2019
- [51] URL6: https://www.asissludge.com/NeisGram.htm#Neisser_Staining; date retrieved 13.03.2019

-
- [52] Federation, W. E., & American Public Health Association. (2005). Standard methods for the examination of water and wastewater. American Public Health Association (APHA): Washington, DC, USA.
- [53] Beebe, R. D., Jenkins, D., & Daigger, G. T. (1982, October). Activated Sludge Bulking Control at the San Jose/Santa Clara, California, Water Pollution Control Plant. In 55th Annual Water Pollution Control Conference, St. Louis, Mo.
- [54] Pearson Product-Moment Correlation Guide; Laerd Statistics 2018 Lund Research LTD; <https://statistics.laerd.com/statistical-guides/pearson-correlation-coefficient-statistical-guide.php>
- [55] Poznyak, T., Bautista, G. L., Chaírez, I., Córdova, R. I., & Ríos, L. E. (2008). Decomposition of toxic pollutants in landfill leachate by ozone after coagulation treatment. *Journal of hazardous materials*, 152(3), 1108-1114.
- [56] Barlaz, M. A., Milke, M. W., & Ham, R. K. (1987). Gas production parameters in sanitary landfill simulators. *Waste Management & Research*, 5(1), 27-39.
- [57] Bhalla, B., Saini, M. S., & Jha, M. K. (2012). Characterization of leachate from municipal solid waste (MSW) landfilling sites of Ludhiana, India: a comparative study. *International Journal of Engineering Research and Applications*, 2(6), 732-745.
- [58] Sturz, H., & Robinson, J. (1986). Anaerobic decomposition of chitin in freshwater sediments. In *Chitin in nature and technology* (pp. 531-538). Springer, Boston, MA.
- [59] Burton, S. A., & Watson-Craik, I. A. (1998). Ammonia and nitrogen fluxes in landfill sites: applicability to sustainable landfilling. *Waste management & research*, 16(1), 41-53.
- [60] Zakaria, S. N. F., Abdul Aziz, H., Amr, A., & Salem, S. (2015). Performance of Ozone/ZrCl₄ oxidation in stabilized landfill leachate treatment. In *Applied Mechanics and Materials* (Vol. 802, pp. 501-506). Trans Tech Publications.
- [61] Zakaria, S. N. F., & Aziz, H. A. (2018, April). Characteristic of leachate at Alor Pongsu Landfill Site, Perak, Malaysia: A comparative study. In *IOP Conference Series: Earth and Environmental Science* (Vol. 140, No. 1, p. 012013). IOP Publishing.
- [62] T.C Tarım ve Orman Bakanlığı, Meteoroloji Genel Müdürlüğü; from <https://www.mgm.gov.tr/veridegerlendirme/il-ve-ilceler-istatistik.aspx?k=undefined&m=ISTANBUL>
- [63] Dick, R. I., & Vesilind, P. A. (1969). The sludge volume index: what is it? *Journal Water Pollution Control Federation*, 1285-1291.
- [64] Van Veen, W. L., Krul, J. M., & Bulder, C. J. E. A. (1982). Some growth parameters of *Haliscomenobacter hydrossis* (syn. *Streptothrix hyalina*) a bacterium occurring in bulking activated sludge. *Water Research*, 16(5), 531-534.
- [65] Rossetti, S., Tomei, M. C., Nielsen, P. H., & Tandoi, V. (2005). “*Microthrix parvicella*”, a filamentous bacterium causing bulking and foaming in activated sludge systems: a review of current knowledge. *FEMS Microbiology Reviews*, 29(1), 49-64.
- [66] Aruga, S., Kamagata, Y., Kohno, T., Hanada, S., Nakamura, K., & Kanagawa, T. (2002). Characterization of filamentous Eikelboom type 021N bacteria and description of *Thiothrix disciformis* sp. nov. and *Thiothrix flexilis* sp. nov. *International journal of systematic and evolutionary microbiology*, 52(4), 1309-1316.

-
- [67] Jorand, F., Zartarian, F., Thomas, F., Block, J. C., Bottero, J. Y., Villemin, G., ... & Manem, J. (1995). Chemical and structural (2D) linkage between bacteria within activated sludge flocs. *Water research*, 29(7), 1639-1647.
- [68] Zita, A., & Hermansson, M. (1994). Effects of ionic strength on bacterial adhesion and stability of flocs in a wastewater activated sludge system. *Appl. Environ. Microbiol.*, 60(9), 3041-3048.
- [69] Steiner, A. E., McLaren, D. A., & Forster, C. F. (1976). The nature of activated sludge flocs. *Water Research*, 10(1), 25-30.
- [70] Subramaniam, S. B., Yan, S., Tyagi, R. D., & Surampalli, R. Y. (2007). Characterization of extracellular polymeric substances (EPS) extracted from both sludge and pure bacterial strains isolated from wastewater sludge for sludge dewatering. *Wat. Res*, 12, 1-7.
- [71] Sobek, D. C., & Higgins, M. J. (2002). Examination of three theories for mechanisms of cation-induced bioflocculation. *Water research*, 36(3), 527-538.
- [72] Xu, S., Yao, J., Ainiwaer, M., Hong, Y., & Zhang, Y. (2018). Analysis of bacterial community structure of activated sludge from wastewater treatment plants in winter. *BioMed research international*, 2018.
- [73] Eikelboom, D. H. (2000). Process control of activated sludge plants by microscopic investigation. IWA publishing.
- [74] Chian, E. S., & Dewalle, F. B. (1976). Sanitary landfill leachates and their leachate treatment. *Journal of the Environmental Engineering Division*, 102(2), 411-431.
- [75] Samudro, G., & Mangkoedihardjo, S. (2010). Review on Bod, Cod And Bod/Cod Ratio: A Triangle Zone For Toxic, Biodegradable And Stable Levels. *International Journal of Academic Research*, 2(4).
- [76] Rosso, D., Larson, L. E., & Stenstrom, M. K. (2008). Aeration of large-scale municipal wastewater treatment plants: state of the art. *Water Science and Technology*, 57(7), 973-978.