

DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

**TREATMENT OF AN AGRICULTURAL,
INDUSTRIAL POLLUTED WASTEWATER WITH
PURPLE PHOTOTROPHIC BACTERIA**

by
Recep Tuğcan PAMUK

February, 2020
İZMİR

TREATMENT OF AN AGRICULTURAL, INDUSTRIAL POLLUTED WASTEWATER WITH PURPLE PHOTOTROPHIC BACTERIA

**A Thesis Submitted to the
Graduate School of Natural and Applied Sciences of Dokuz Eylül University
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Environmental Engineering, in Environmental Engineering Program**

**by
Recep Tuğcan PAMUK**

February, 2020

İZMİR

M.Sc THESIS EXAMINATION RESULT FORM

We have read the thesis entitled “**TREATMENT OF AN AGRICULTURAL, INDUSTRIAL POLLUTED WASTEWATER WITH PURPLE PHOTOTROPHIC BACTERIA**” completed by **RECEP TUĞCAN PAMUK** under supervision of **ASSOC. PROF. DR. SERPİL ÖZMIHÇI** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

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To me in the future: “Never lose the track of time! Expand it, if you are able to...”

Recep Tuğcan PAMUK

TREATMENT OF AN AGRICULTURAL, INDUSTRIAL POLLUTED WASTEWATER WITH PURPLE PHOTOTROPHIC BACTERIA

ABSTRACT

The aim of this thesis is to apply an alternative treatment method to conventional nutrient removal with *Rhodobacter sphaeroides* (RS) or with a co-culture of activated sludge (AS) and *R. sphaeroides* (RS) to an agricultural and industrial polluted wastewater in excessive conditions.

In the first experiment environmental conditions were tested to optimize the nutrient removal capacity of RS. Aerobic dark conditions were favored for COD removal. COD removal efficiency of RS with low and high COD/TN ratios with high COD concentrations were tested and the nitrogen fixing capacity of the culture gave COD removal efficiencies over 93% with low COD/TN ratios. In high COD/TN ratios the highest COD removal efficiency was obtained with a COD/TN ratio of 100/23 as 42%. A synthetic wastewater modeled from Porsuk River containing 1000 mg/L COD was used in evaluation of COD/TN (100/5-100/25) and COD/TP (100/0.5-100/2) ratio experiments. RS removed all the COD in a COD/TN ratio of 100/23 and the total phosphorus removal efficiency was around $54\pm3\%$. All the COD was removed in COD/TP ratio experiments. The TN removal efficiencies decreased with increasing COD/TP ratios and were over 82%. TP removal was low and it is understood that RS could not remove phosphate from the wastewater sufficiently.

A co-culture consisted of AS and RS with a high C/N/P ratio was used for nutrient removal. The co-culture (AS/RS) ratio of 1/5 was able to convert all the COD concentrations (100%) and TP and TN removal efficiencies were 72% and 89.5%, respectively.

The effects of hydraulic retention times (6-15.9 h) on a continuous activated sludge system with the co-culture consisted of AS and RS with a ratio of 1/5 was used for nutrient removal. COD, TN, NO_3 , NH_4 , PO_4 removal efficiencies were highest at 10.1 h with 80, 76, 86, 67 and 79%, respectively. It has to be considered

that the simulated surface water characteristic is very close to domestic wastewater which can be classified as open sewage. Besides, Porsuk River has much lower nutrient concentrations than the chosen sampling point of wastewater which can be treated well to meet the receiving water discharge standards. It can be concluded that using a co-culture (AS/RS) may be used as a natural treatment method.

Keywords: Nutrient removal, Porsuk River, COD/N/P, continuous system, co-culture, *Rhodobacter sphaeroides*, activated sludge



TARIMSAL, ENDÜSTRİYEL KAYNAKLI KİRLETİLMİŞ BİR ATIKSUYUN MOR FOTOTROFİK BAKTERİLER İLE ARITILMASI

ÖZ

Bu tezin amacı klasik nütriyent arıtma yöntemlerine bir alternatif yöntem uygulayarak, tarımsal ve endüstriyel kaynaklarca kirlenmiş bir atıksudan *Rhodobacter sphaeroides* ya da aktif çamur ve *Rhodobacter sphaeroides*'ten oluşan bir ko-kültür kullanarak nütriyent giderimi sağlamaktır. Bu yöntem, özellikle bazı havzalarda tarımsal uygulamalar nedeniyle aşırı besin içeren yüzeysel sulara uygulanabilecektir. Porsuk Çayı'ndan alınan yüksek miktarda nütriyente sahip model bir atık su simule edilmiş ve nütriyent giderimi yapılmıştır.

İlk deneyde, *R. sphaeroides*'in nütriyent giderme kapasitesini optimize etmek için çevresel koşullar test edilmiştir. KOİ giderimi için aerobik karanlık koşullar tercih edilmiştir. *R. sphaeroides*'in düşük ve yüksek KOİ/TN oranlarındaki KOİ giderme verimi test edilmiş ve bu kültürün azot bağlama kapasitesi, düşük KOİ/TN oranlarında %93'ün üzerinde KOİ giderme verimi göstermiştir. Yüksek KOİ/TN oranlarında en yüksek KOİ giderme verimi %42 ile 100/23'lük KOİ/TN oranında elde edilmiştir. Bir sonraki deneyler için Porsuk Nehri'nden alınan bir model atık su simülasyonu yapılmıştır. KOİ/TN (100/5-100/25) ve KOİ/TP (100/0,5-100/2) oranlarının değerlendirildiği deneylerde 1000 mg / L KOİ içeren sentetik bir atık su kullanılmıştır. *R. sphaeroides* ile KOİ/TN oranının 100/23 olduğu koşullarda KOİ tamamen giderilmiştir. KOİ/TN oranı deneylerinde toplam fosfor giderme verimi yaklaşık %54±3 olmuştur. 100/20'lik KOİ/TN oranında, toplam azot giderme verimi %82 ile en yüksektir. KOİ/TP oranı deneylerindeki tüm KOİ'nin tüm koşullarda giderilebildiği tespit edilmiştir. TN giderme verimleri KOİ/TP oranı arttıkça azalma göstermiş ve bu verimler %82'nin üzerinde olmuştur. TP gideriminin düşük olması nedeniyle *Rhodobacter sphaeroides*'in atık sudan yeterince fosfat gideremediği anlaşılmıştır. Deneyler sonucunda nihai TN, TP konsantrasyonlarının yüzeysel su deşarj standartlarını karşılayamadığı tespit edilmiştir. Nütriyent giderimi için yüksek C/N/P oranlı aktif çamur ve *Rhodobacter sphaeroides* içeren bir ko-kültür

kullanılmıştır. AS/RS ko-kültür oranının 1/5 olduğu koşullarda KOİ %100'lük bir giderme verimi ile tamamen giderilmiştir. Bu koşullar altında toplam fosfor ve toplam azot giderme verimleri sırasıyla %72 ve %89,5 olmuştur. Test edilen nihai nütriyent konsantrasyonları, yüzeysel su deşarj standartlarını karşılayamamıştır.

Sürekli aktif çamur sisteminde; hidrolik alıkonma sürelerinin (6-15,9 saat) etkileri, 1/5 (AS/RS) oranında aktif çamur ve *R. sphaeroides*'ten oluşturulan ko-kültür ile birlikte, nütriyentlerin giderimi araştırılmıştır. COD, TN, NO₃, NH₄, PO₄ giderme verimlerinin, yüzde 80, 76, 86, 67 ve 79 olan değerleriyle 10.1 saatte en yüksek seviyede olduğu gözlemlenmiştir. Atık su konsantrasyonları yönetmeliklere göre su kalite standartlarını karşılayamamıştır. Bununla birlikte, simüle edilmiş yüzeysel suyun özelliklerinin açık kanal suyu olarak sınıflandırılabilir evsel atık suya çok yakın olduğu düşünülmektedir. Ayrıca, Porsuk Nehri, seçilen noktadaki su numunesinin alındığı noktaya göre deşarj standartlarını karşılamak için çok daha düşük nütriyent konsantrasyonlarına sahiptir. Sonuç olarak, aktif çamur ve *R. sphaeroides*'ten oluşan bir ko-kültür nütriyent giderimi için kullanılabilir ve doğal bir arıtma yöntemi olarak tercih edilebilir.

Anahtar kelimeler: Besin giderimi, Porsuk Çayı, KOİ/N/P, sürekli sistem, ortak kültür, *Rhodobacter sphaeroides*, aktif çamur

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CHAPTER ONE

INTRODUCTION

1.1 Preface / The Problem Statement

Various rivers are nutrient rich, cause of agricultural applications. The characteristic of these surface waters need extra attention. Nutrients in surface water cause eutrophication, lack of dissolved oxygen, N and P limiting waters. Nutrient removal with biological treatment methods in modified activated sludge systems having anaerobic, anoxic and oxic zones, are widely used in municipalities. However the transfer of rivers to the plants needs additional effort and budget to remove these nutrients from surface waters. Also infiltration as a land application, different natural treatment applications with organisms, plants and trees may be used for nutrient removal of surface waters.

The excess nutrients in the rivers and lakes affect the quality, usability and the ecological diversity. Especially in sensitive environments, it may cause death of various species. Many conventional alternatives were developed for nutrient removal. In this study; the main aim was to find an effective biological treatment method for these areal nutrient polluted sources. For this purpose, as a novel approach, an organism which is able to grow in anaerobic phototrophic (photoheterotrophic and photoautotrophic) and aerobic chemoheterotrophic conditions along with activated sludge was used with a modeled surface water. The modeled surface water was taken from Sakarya Basin; Porsuk River polluted by Alpu plain, livestock breeding and industrial applications. The sampling point was chosen to have the highest excessive amounts of the nutrients along the river which the characteristics of the surface water showed a very close one to municipal wastewater with addition of excessive amounts of nutrients. In this approach, the quality of the surface water was tried to be improved with an economical treatment solution which may be applied close to the surface water with a conventional activated sludge system.

1.1.1 Treatment of Surface Waters

Natural waters contain inorganic compounds and microorganisms in suspended and dissolved form. These compounds are miscible in water due to natural resources or wastes. Inorganic components are calcium, magnesium, sodium and potassium salts of bicarbonate, chloride, sulfate, nitrate and phosphate. Also trace amounts of lead, copper, arsenic, iron and manganese can be found. Organic components are mixed with water sources due to degradation of plant and animal residues and flow from agricultural areas. Organic pollutants include; synthetic organic materials such as natural humic substances, detergents, pesticides, herbicides and solvents (Saatçi et al., 2015).

The primary purpose of surface water treatment is to remove the pollutants from the water and to prevent the use of the water in accordance with its natural qualities or the threats it may pose in the ecosystem where it is released. For this purpose, it is very important to treat the wastewater to certain standards. Treatment of water in accordance with the intended use or the area to be discharged is a priority aim (Fisher, 2018).

Resolution of the global water pollution crisis requires multiple approaches to improve the quality of our fresh water and move towards sustainability. The most deadly form of water pollution, pathogenic microorganisms that cause waterborne diseases, kills almost 2 million people in underdeveloped countries every year. The best strategy for addressing this problem is proper sewage (wastewater) treatment. Untreated sewage is not only a major cause of pathogenic diseases, but also a major source of other pollutants, including oxygen-demanding waste, nutrients (N and P, particularly), and toxic heavy metals. Wastewater treatment is performed at sewage treatment plants in urban areas and through septic tank systems in rural areas (Fisher, 2018).

Surface and stationary water sources (lakes, etc.) may contain impurities as silt (sand and mud-like impurities), microorganisms, and compounds of different organic

and inorganic structure. Surface and flowing water sources (rivers, etc.) have higher suspended matter concentrations than stationary water sources. The infiltration of surface water sources into the ground provides the removal of a very important part of the suspended solids content and bacteria. However, during infiltration into the ground, different minerals in the ground dissolve in contact water and mix into groundwater. Types and concentrations of dissolved minerals depend on groundwater layer (aquifer) thickness and geological properties (Saatçi et al., 2015).

The surface waters in the basins are collecting pollutants along their way, while the ingredients are treated via for example precipitation, infiltration, foto oxidation or with biological methods (soil filters, constructed wetland, stabilization pond, aquatic plant systems, irrigation by wastewater). Naturally the ecological system, cycles the nutrients very easily by organisms, animals and plants. However the excessive amounts of pollutants that come from municipal wastewater, polluted storm water runoff, industrial, agricultural, ballast water or discharge of untreated water from treatment plants are endangering the habitat (Rozkošný et al., 2014).

Natural treatment systems have various advantages over conventional treatment technologies when applied in small communities and natural surface waters. The integration of these systems offers an aesthetic view in the environment, increase in biodiversity of the landscape, affect the microclimate, energy efficient systems with low investment costs, low operation cost, simple construction, high treatment efficiencies (Rozkošný et al., 2014). Especially the excessive amounts of nutrients are removed with biomass uptake in surface waters affecting the quality improvement.

However the disadvantages of these systems need to be observed before construction. These are high surface requirement, low removal efficiencies when operation fails due to for example clogging of filters, unharvested plants, increase of sediment, decrease of temperature etc (Rozkošný et al., 2014).

CHAPTER TWO

LITERATURE SURVEY

2.1 Surface Waters in Basin

Ecosystem Services are events and processes providing benefits and services that are supplier (food, water, etc.), regulator (regulation of climate, air quality, water flow, cleaning of water, protection of soil quality, prevention of diseases, etc.), supportive (food and water chains, pollination of plants, etc.) cultural (recreation, aesthetics, educational values, etc.) that performed by ecosystems and the environment in the earth remains balanced and stable (T.C. Orman ve Su İşleri Bakanlığı, 2014). Basin Protection Action Plan is developed to protect the water resources potential for all uses, ensuring its use, preventing pollution and improving the water quality of the contaminated water resources.

Basin management is very important because there are significant differences between the expectations and related demands of different stakeholders from the economic, ecological, social and cultural versatile products and services and their priorities such as energy production, drinking, using, agricultural irrigation, meeting industrial water needs, utilization of forests and rangelands, increasing productivity in agricultural areas, income and livelihood supply from basin sources, protection of biological diversity, prevention of air pollution, recreation, natural landscape, ecotourism, hunting, basin to protect the culture of the region, national income and corporate budgets to provide income, the private sector to gain, etc. (T.C. Orman ve Su İşleri Bakanlığı, 2014). Basin needs to be managed and the strength of basin management can be summarized as:

- The experiences gained by institutions in basin projects and applications, and their recent inclination towards investments and plans based on basin integrity,
- Preparing of basin protection action plans and river basin management plans,
- The increase in state-provided financing for watershed investments,

- Increased soil conservation and basin rehabilitation practices,
- Knowledge and experience of stakeholders (NGOs, scientific organizations, etc.) outside the public institutions on integrated basin management projects.
- Significant increase in the number of protected areas,
- Biodiversity Monitoring Unit and Database (Noah's Ark) (T.C. Orman ve Su İşleri Bakanlığı, 2014).

Our study is focusing on natural treatment of surface waters which should be classified under basin rehabilitation practices as a strength of basin management that will help to improve surface water and watershed quality. The improvement of the water quality can also help to reorganize the water and land usage plans.

2.2 Current Situation and Overview of Basins in Turkey

Turkey is divided into 25 hydrological basins and total average annual flow of the basins is 186 billion m³. According to State Hydraulic Works (SHW) data, about one third of these belong to the Fırat (Euphrates)-Dicle (Tigris) basin located to the east of the country. The average annual flow rate comes from the Eastern Black Sea, East Mediterranean and Antalya basins after the Fırat-Dicle basin, while the Kızılırmak and Sakarya basins follow this as the area size (T.C. Orman ve Su İşleri Bakanlığı, 2014). With the 58,160 km² drainage area, Sakarya River Basin is the third biggest basin of Turkey (Erdeviren, 2010).

Population density in the Upper Basin and the East regions is generally lower, while it is increasing in the lower basins and basin areas in the western regions. Dependence on natural resources for rural poverty and livelihood is more prevalent in upstream regions and eastern and southeastern regions than in downstream areas and other regions. Upper basins are mostly used for livestock, small-scale agriculture and forestry, while agricultural activities are applied in large areas in lower basins (T.C. Orman ve Su İşleri Bakanlığı, 2014).

Soil and water pollution caused by excessive use of chemical fertilizers and medicines in agriculture is increasing in the lower basins and in the western and southern basin areas, whereas in the upstream regions, agriculture is mostly carried out under conditions close to organic farming. Majority of rangeland areas are damaged because of over-irregular and irregular pastures, and close to half of forest areas due to irregular utilization in past years and most of ecosystems are degraded. Road construction also causes discoloration and erosion, which is not suitable for trenching. In the western regions of our country and in the lower basins, the urban population and industrial establishments have been intensified and the demand for residential areas, water and energy has increased accordingly. Environmental pollution, unplanned urbanization and industrialization threaten more and more natural resources and assets such as fertile soil, water, forest and pasture (T.C. Orman ve Su İşleri Bakanlığı, 2014).

The current utilization rate of 112 billion m³ of usable water resources is still around 36%. 32 billion m³ is used for irrigation, 7 billion m³ is used for drinking and use, 5 billion m³ is used in industry. Approximately 74% of water sources in Turkey are used for irrigation, 11% are used for industry, and 15% are used for urban consumption. These rates are respectively 70%, 22%, 8% in the world, 33%, 51% and 16% in Europe (T.C. Orman ve Su İşleri Bakanlığı, 2014).

Efficient management is essential to achieving sustainable development, as water resources are shrinking day by day and face the risk of pollution. Since water resources cover many factors with different characteristics, only the efforts made at the local level will not be sufficient to protect these resources. It has been scientifically proven that water resources can be utilized and protected only if managed as a basin. With this information, River Basin Management Plans have been started in 2014 in accordance with the EU Water Framework Directive and the plans have been put into practice. Institutional arrangements were made such as establishment of Water Management Coordination Committee and Turkish Water Institute (SUEN) in order to determine the necessary measures for the protection of water resources within the framework of integrated basin management, to ensure

inter-sectorial coordination for effective water management, to ensure cooperation and accelerate water investments. The main plans directly related to the management of basin and basin resources including “watershed conservation action plans”, “watershed management plans”, “watershed master plans” while “environmental plans”, “land use plans”, “protected area plans” are important plans for basin management and especially for preventing faulty land use in basins (T.C. Orman ve Su İşleri Bakanlığı, 2014).

2.3 Regulations Related with Basins Referring Surface Water Quality

In the Environmental Law, it is stated that in accordance with the principle of sustainable development in the physical space of the country, environmental plans on the basis of region and basin will be made and approved by the Ministry in order to prevent environmental pollution from urban and rural resources considering the balance of protection and use. It is stated that the procedures and principles regarding the construction of environmental plans are determined by the regulation issued by the Ministry (Law of Environment, 1983, 18132).

In the Soil Conservation and Land Use Law, in order to prevent the loss of soil in dams, ponds and similar reservoir basins which are affected by the physical, chemical and biological properties of the soil or which are likely to deteriorate, it is stated that erosion sensitive areas are determined by the decision of the President. It is stated that the usage plans and infrastructure projects of these lands were carried out by the relevant public institutions on the basis of the basin in order to identify and protect the erosion sensitive lands (Law of Land Conservation and Land Use, 2005, 25880).

Metropolitan Municipality authorities are stated in the Law of Metropolitan Municipality such as to ensure the protection of the environment, agricultural areas and watersheds in accordance with the principle of sustainable development; afforestation and taking measures to prevent environmental pollution within the municipal boundaries; to make metropolitan solid waste management plan; to perform services related to re-evaluation, storage and disposal of solid wastes and

excavation, to establish and operate facilities for this purpose; to carry out services related to industrial and medical wastes, to establish and operate necessary facilities for this purpose; to collect, treat and make the necessary arrangements for the waste of marine vessels (Law of Metropolitan Municipality, 2004, 25531).

In the Municipal Law, it is stated that municipalities cannot be established in drinking and potable water basins, protected areas and other protected areas and settlements that are closer than 5,000 meters to the boundaries of an established municipality (Law of the Municipality, 2005, 25874).

In the Law of Agriculture, agricultural watersheds have been defined for agricultural activity as one or several provincial or regional boundaries, which are the same ecological conditions and which are the continuation of each other. It was stated that the agricultural basins were determined by the President to concentrate, support, organize, specialize and integrate agricultural production in the fields appropriate to its ecology. The regulation on the principles and procedures related to the functioning of these basins is subject to the Ministry (Law of Agriculture, 2006, 26184).

In the Forest Law, it is stated that afforestation, erosion and flood control, prevention of avalanches and landslides, protection and development of ecosystems and improvement of living conditions of people living in the basin in forested areas are carried out as integrated projects prepared under the coordination of the Ministry (Law of Forest, 1956, 9402).

The Water Pollution Control Regulation has introduced extensive adjustments to water quality management. In this regulation, it is aimed to protect the quality of water resources within the framework of ecosystem principle and to improve water quality in line with country requirements. Within this framework, the regulation has introduced the necessary protection areas to be established around drinking and potable water reservoirs, and the regulations for the protection of domestic and industrial wastewater discharges and agricultural lands. It is essential that the existing

quality of the inland water resources is determined by the General Directorate of State Hydraulic Works by taking the opinion of the relevant institutions and determining the conformity of the existing quality with the necessary quality criteria for the usage areas. It is essential to make a watershed protection plan considering the characteristics of the watershed in order to protect the water resources of the continent for all uses, to prevent pollution and to improve the water quality of the contaminated water resources. The watershed protection plan sets out a long-term conservation program and conservation measures. In addition to this, quality classification is made for surface water and groundwater in the regulation. For surface water, four quality classes are defined: high quality, lightly polluted, contaminated and highly contaminated waters. The purposes of use for each class are specified separately. There are three quality classes for groundwater: Class I - water that can be used for drinking water, Class II - waters that can be used for drinking water after treatment or for industrial and agricultural purposes, Class III - water that can be used after treatment for intended use (Regulation on Water Pollution Control, 2004, 25687). Table 2.1 depicts the quality criteria according to inland water resources. Table 2.2 gives the wastewater standards for the discharge of wastewater to wastewater infrastructure facilities. In this study the treatment method is developed to give an overview of achieving high quality water for discharge to receiving waters.

Table 2.1 Quality criteria according to classes of internal water resources (Regulation on Water Pollution Control, 2004, 25687)

WATER QUALITY PARAMETERS	WATER QUALITY CLASSES			
	I	II	III	IV
Physical and inorganic-chemical parameters				
Heat (°C)	25	25	30	>30
pH	6.5 - 8.5	6.5 - 8.5	6.0 - 9.0	out of 6.0 - 9.0
Dissolved Oxygen (mg O ₂ / L)	8	6	3	<3
Oxygen saturation (%)	90	70	40	<40
Chloride ion (mg Cl ⁻ / L)	25	200	400	>400
Sulphide ion (mg SO ₄ ⁼ / L)	200	200	400	>400
Ammonium Nitrogen (mg NH ₄ ⁺ -N/ L)	0.2	1	2	>2
Nitrite Nitrogen (mg NO ₂ ⁻ -N/ L)	0.002	0.01	0.05	>0.05
Nitrate Nitrogen (mg NO ₃ ⁻ -N/ L)	5	10	20	>20
Total Phosphorus (mg P/ L)	0.02	0.16	0.65	>0.65
Total Dissolved Matter (mg/ L)	500	1500	5000	>5000
Color (Pt-Co unit)	5	50	300	>300
Sodium (mg Na ⁺ / L)	125	125	250	>250
Organic parameters				
Chemical Oxygen demand (COD) (mg/ L)	25	50	70	>70
Biological Oxygen demand (BOD) (mg/ L)	4	8	20	>20
Total organic Carbon (mg/ L)	5	8	12	>12
Total Kjeldahl-Nitrogen (mg/ L)	0.5	1.5	5	>5
Oil and grease (mg/ L)	0.02	0.3	0.5	>0.5
Surfactants reacting with methylene blue (mg/ L)	0.05	0.2	1	>1.5
Phenolic substances (volatile) (mg/ L)	0.002	0.01	0.1	>0.1
Mineral oils and derivatives (mg/ L)	0.02	0.1	0.5	>0.5
Total pesticides (mg/ L)	0.001	0.01	0.1	>0.1
Inorganic polluting parameters				
Mercury (µg Hg/ L)	0.1	0.5	2	>2
Cadmium (µg Cd/ L)	3	5	10	>10
Lead (µg Pb/ L)	10	20	50	>50
Arsenic (µg As/ L)	20	50	100	>100
Copper (µg Cu/ L)	20	50	200	>200
Chrome(total) (µg Cr/ L)	20	50	200	>200
Chrome (µg Cr ⁼⁶ / L)	Immeasurable	20	50	>50
Cobalt (µg Co/ L)	10	20	200	>200
Nickel (µg Ni/ L)	20	50	200	>200
Zinc (µg Zn/ L)	200	500	2000	>2000
Cyanide (total) (µg CN/ L)	10	50	100	>100
Fluoride (µg F ⁻ / L)	1000	1500	2000	>2000
Free Chlorine (µg Cl ₂ / L)	10	10	50	>50
Sulfur (µg S ⁼ / L)	2	2	10	>10
Iron (µg Fe/ L)	300	1000	5000	>5000
Manganese (µg Mn/ L)	100	500	3000	>3000
Boron (µg B/ L)	1000	1000	1000	>1000
Selenium (µg Se/ L)	10	10	20	>20
Barium (µg Ba/ L)	1000	2000	2000	>2000
Alluminum (µg Al/ L)	0.3	0.3	1	>1
Radioactivity (Bq/ L)				
-Alpha-activity	0.5	5	5	>5
-Beta-activity	1	10	10	>10
Bacteriological parameters				
Fecal coliform (EMS/ 100mL)	10	200	2000	>2000
Total coliform (EMS/ 100mL)	100	20000	100000	>100000

Table 2.2 Wastewater standards for the discharge of wastewater to wastewater infrastructure facilities
(Regulation on Water Pollution Control, 2004, 25687)

PARAMETER	WASTEWATER SUBSTRUCTURAL PLANTS WHICH FINISHES THE SEWER SYSTEMS WITH COMPLETE TREATMENT	WASTEWATER SUBSTRUCTURAL PLANTS WHICH FINISHES THE SEWER SYSTEMS WITH DEEP SEA DISCHARGE
Heat (°C)	40	40
pH	6.5-10.0	6.0-10.0
Suspended Solid Matter (mg/ L)	500	350
Oil and grease	250	50
Oils from tar and petroleum (mg/ L)	50	10
Chemical Oxygen Demand (COD) (mg/ L)	4000	600
Biological Oxygen Demand (BOD ₅) (mg/ L)	-	400
Sulfate (SO ₄ ²⁻) (mg/ L)	1700	1700
Total Sulfur (S) (mg/ L)	2	2
Phenol (mg/ L)	20	10
Free Chloride (mg/ L)	5	5
Total Nitrogen (N) (mg/ L)	-	40
Total Phosphorus (P) (mg/ L)	-	10
Arsenic (As) (mg/ L)	3	10
Total Cyanide (Total CN ⁻) (mg/ L)	10	10
Total Lead (Pb) (mg/ L)	3	3
Total Cadmium (Cd) (mg/ L)	2	2
Total Chrome (Cr) (mg/ L)	5	5
Total Mercury (Hg) (mg/ L)	0.2	0.2
Total Copper (Cu) (mg/ L)	2	2
Total Nickel (Ni) (mg/ L)	5	5
Total Zinc (Zn) (mg/ L)	10	10
Total Tin (Sn) (mg/ L)	5	5
Total Silver (Ag) (mg/ L)	5	5
Cl ⁻ (Chloride) (mg/ L)	10000	-
Surfactants reacting with methylene blue (mg/ L)	Discharge of biodegradable substances that do not comply with TSI standards is in principle prohibited.	

The main regulations covering surface waters like rivers in the basins and watershed is the Regulation on the Preparation, Implementation and Monitoring of Watershed Management Plans. The purpose of the Regulation on the Preparation, Implementation and Monitoring of Watershed Management Plans; is to preserve or improve water bodies in their current form with physicochemical, chemical and ecological quality components as basins, and to ensure the sustainable use of them in accordance with the needs priorities, to prepare the procedures and principles regarding the preparation and implementation of management plans. (Regulation on the Preparation, Implementation and Follow-Up of Basin Management Plans, 2012, 28444).

Surface Waters are classified in another regulation called Regulation on the Monitoring of Surface Waters and Groundwater. The purpose of the Regulation on the Monitoring of Surface Waters and Groundwater is to determine the current status of all surface waters and groundwater in terms of quantity, quality and hydromorphological elements, to monitor the water with an approach based on ecosystem integrity and to ensure coordination between monitoring institutions and organizations to determine the principles and procedures (Regulation on Monitoring of Surface Waters and Underground Waters, 2014, 28910). Especially the modelled surface water in this study was taken from an agricultural area contaminated with agricultural activities. The regulation covering the river passing through agricultural lands is defined in Regulation of Agricultural Basins and Regulation of Surface Water Quality. The aim of the Regulation of Agricultural Basins is to regulate the procedures and principles regarding the conduct, support, organization, specialization and preparation of agricultural inventory in an integrated manner in agricultural basins determined for the development of agricultural production in appropriate ecologies (Regulation on Agricultural Basins, 2010, 27695).

The purpose of the Regulation of Surface Water Quality, determination of the biological, chemical, physico-chemical and hydromorphological qualities of surface waters and coastal and transitional waters, monitoring the water quality and quantity, monitoring the balance of water use and purpose of these waters in accordance with sustainable development objectives and to determine the procedures and principles for the measures to be taken in order to establish, protect and achieve good water condition (Regulation on Surface Water Quality, 2012, 28483).

The other regulations that should be checked for surface waters in the Basins are, Regulation on Protection, Use and Planning of Agricultural Land (2017, 30265), Regulation on Protection of Drinking-Using Water Basins (2017, 30224), Regulation on Environmental Impact Assessment (2014, 29186), Communiqué on the Organization, Duties, Working Procedures and Principles of The Central Board of the Basin Management, Basin Management Delegations and Coordination Committee of Province Water Management (2019, 30659).

2.4 Sakarya Basin and Sub-Basins

Sakarya River Basin stays in the northwest area of Turkey. Susurluk Basin in the west, Akarçay and Konya Closed Basins in the south, Kızılırmak and West Blacksea Basins in the east covers the Sakarya Basin. Bolu Mountains to the north of the basin, Idris Mountain, Elmadağ and Haymana Plateau to the east, Emir Mountain, Murat Mountain, Bayat and Cihanbeyli Plateaus to the east, Domanic Mountain and Uludağ to the west are located in the north of the basin. Area of the basin covers about 7% of the surface area of Turkey (T.C. Orman ve Su İşleri Bakanlığı, Su Yönetimi Genel Müdürlüğü, 2016).

The drainage area of Sakarya River is 58,160 km². Considering that some of the resources at the beginning of the dried up total length of 720 km with arms. With a value of 479 mm, it is located in four basins with an average annual precipitation of less than 500 mm. Considering the total annual rainfall, it is one of the basins receiving the most precipitation with 32 billion m³. It is one of the basins with the highest flow value with an annual flow of 12 billion m³ due to the high population per capita rainfall amount (4,437 m³/ person) is one of the lowest basin. In the basin where the average annual yield is 3.6 L/ h/ km³, the contribution of flow to rainfall is 3.4% (T.C. Orman ve Su İşleri Bakanlığı, Su Yönetimi Genel Müdürlüğü, 2016).



Figure 2.1 Location of Sakarya Basin in Turkey (Hansen, 1996)

The important branches of the Sakarya River poured from Karasu district of Sakarya province to Karadeniz can be listed as;

- Seydi Water,
- Porsuk Stream,
- Ankara Stream,
- Kirmir Stream,
- Karasu Stream,
- Göksu Stream,
- Göynük Stream,
- Mudurnu Stream,
- Çarksuyu Stream.

Sakarya River at the northeast end of the Marmara Region is born from the sources in the Sakaryabaşı region, also called Sakarbaşı located in the south of Eskişehir Çifteler District center (Figure 2.2). After flowing in the northeast direction, the river turns east near Çifteler and draws a natural border between Eskişehir and Ankara. Further, after passing through the Polatlı district of Ankara, Porsuk River joins to the river which is one of the most important branches. Furthermore, it merges with the Ankara River coming from the east and then returns to the northwest and west. In this section, the river deepens its bed and joins the Kirmir River from the northeast and the Aladağ River from the north. It continues at the provincial border separating Eskişehir from Ankara, passes through the central part of the artificial lakes formed by the collection of waters on Sarıyar (Hasan Polatkan), Gökçekaya and Yenice Dams and then turns to northwest and north. Karasu and Göksu streams coming from the southwest and Göynük Creek coming from the east join the river in this section. After the Göksu intersection, the Sakarya River, which turns northeast and enters the Canbaz Strait, reaches Pamukova, an important agricultural area. It leaves Pamukova via Geyve Strait and then enters Adapazarı Plain, also called Akova. After the Adapazarı Plain, Mudurnu Stream from the south and Çarksuyu Creek, which comes from southwest and carries the excess waters of Sapanca Lake join to the river usually flows in north-south direction (T.C.

Orman ve Su İşleri Bakanlığı, Su Yönetimi Genel Müdürlüğü, 2016). The morphometric parameter values of Sakarya Basin are given in Table 2.3.

Table 2.3 The morphometric parameter values of Sakarya Basin (T.C. Orman ve Su İşleri Bakanlığı, Su Yönetimi Genel Müdürlüğü, 2016)

Morphometric Parameter	Value
Main Arm Length, L(km)	847
Basin Area, A (km ²)	63,273
Basin Total River Length, L _{total} (km)	19,725
Basin Form Factor, m	0.234
Basin View Ratio, a	0.377
Basin Form Factor, L ² / A	11,348
Drainage Density, Dd (km/ km ²)	0.31
Main Arm Slope, s	0.0021
Basin Mean Slope, Sb (%)	18.09
Basin Average Height, Havg (m)	969
Basin Storage Index	0.007

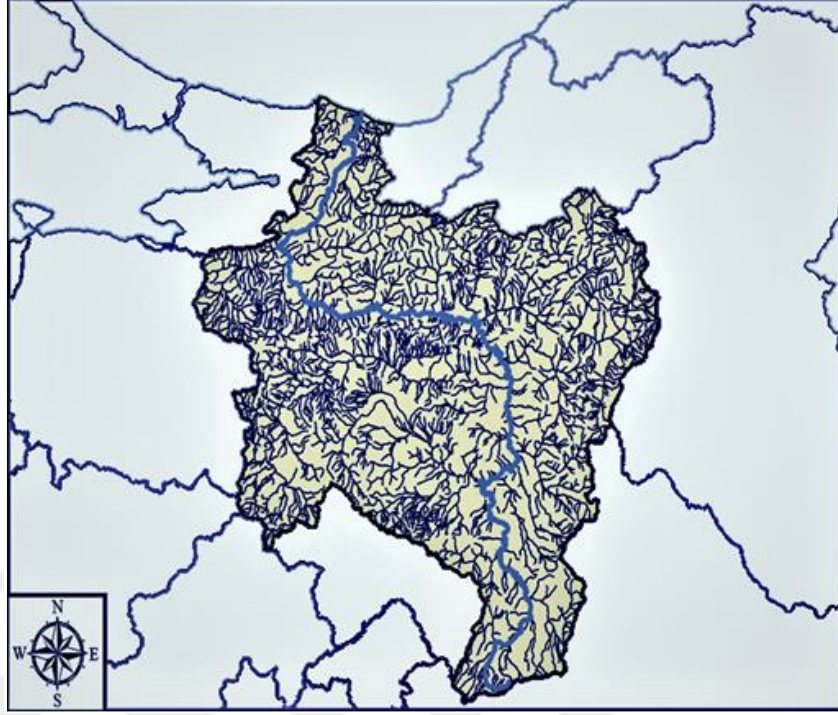


Figure 2.2 Sakarya River, Branches and the Basin (T.C. Orman ve Su İşleri Bakanlığı, Su Yönetimi Genel Müdürlüğü, 2016)

All of Eskişehir and Sakarya provinces, almost all of Bilecik province (97.2%), approximately 70% of the central districts of Ankara province, 42% of Bolu, and 35% of Kütahya with its center, 23% of Afyonkarahisar, 20% of Konya, 17% of Bursa, 10% of Kocaeli and less than 2% of Düzce, Çankırı and Uşak provinces located within the boundaries of Sakarya Basin (Table 2.4).

Table 2.4 Cities and covered areas in the Sakarya Basin (T.C. Orman ve Su İşleri Bakanlığı, Su Yönetimi Genel Müdürlüğü, 2016)

Cities	Total Area(ha)	Area in Basin (ha)	Section into the Basin (%)	Distribution of Basin by Cities (%)	Number of municipalities in the basin	Average Altitude (m)
Ankara	2,561,500	1,784.520	69.7	28.2	31	890
Eskişehir	1,373,843	1,373.843	100	21.7	28	788
Konya	4,082,400	814.102	19.9	12.9	36	1,106
Sakarya	450,472	450.472	100	7.1	28	31
Bolu	1,071,600	449.854	42	7.1	5	726
Kütahya	1,211,900	425.450	35.1	6.7	9	970
Bilecik	418,100	406.348	97.2	6.4	15	850
Afyonkarahisar	1,453,200	380.975	26.2	6	13	1,034
Bursa	1,108,700	188.216	17	3	6	155
Kocaeli	363,500	34.546	9.5	0.5	0	28
Düzce	259,200	5.485	2.1	0.1	0	160
Çankırı	841,100	8.237	1	0.1	0	730
Uşak	517,400	3.762	0.7	0.1	0	921

The Sakarya River Basin is divided into 6 sub-basins to the point where it flows into the Black Sea. These are sub-basins;

- Upper Sakarya Sub-Basin
- Porsuk Stream Sub-Basin
- Ankara Stream Sub-Basin
- Mid-Sakarya Sub-Basin
- Göksu-Karasu Stream Sub-Basin
- Lower Sakarya Sub-Basin

The Pressures and impacts on Sub-Basins and the threats on the basin are listed in Table 2.5 and Table 2.6, respectively. Mines, domestic and industrial wastewater discharges, livestock wastewater, agricultural wastewater discharges are the dominant pressures affecting the water quality.

Table 2.5 Pressures and impacts on Sub-Basins (T.C. Orman ve Su İşleri Bakanlığı, Su Yönetimi Genel Müdürlüğü, 2016)

Sub-Basin	Pressure Fact
Upper Sakarya	Domestic Wastewater Discharges, Eti Kırka Bor Plant
Porsuk Stream	Domestic and Industrial Wastewater Discharges, Kütahya Eti Gümüş Plant
Ankara Stream	Wastewater Treatment Plant Discharges, Industrial Wastewater Discharges
Mid-Sakarya	Domestic Wastewater Discharges, Chicken Farm Wastes
Göksu-Karasu Streams	Domestic and Industrial Wastewater Discharges, Solid Waste Leachate Waters
Lower Sakarya	Domestic and Industrial Wastewater Discharges, Material Quarries

Table 2.6 Hot points on Sakarya Basin and possible threats (T.C. Orman ve Su İşleri Bakanlığı, Su Yönetimi Genel Müdürlüğü, 2016)

Sub-Basin	Hot Point Name	Possible Threats
Upper Sakarya	Ilgın Lake (Çavuşçu Deposition)	Domestic WW Discharges, Agricultural Pollutants, Agricultural Water Use
Upper Sakarya	Yunak Akgöl Wetlands	Domestic WW Discharges, Agricultural Pollutants, Agricultural Water Use
Upper Sakarya	Balıkdami Wetlands	Agricultural Pollutants, Agricultural Water Use
Upper Sakarya	Çatören and Kunduzkar Dams	Boron Pollution
Porsuk Stream	Porsuk Dam	Domestic and Industrial WW Discharges, Agricultural Pollutants
Ankara Stream	Çubuk II, Kurtboğazi, Kavşakkaya Dams	
Ankara Stream	Ankara Stream	Domestic and Industrial WW Discharges
Ankara Stream	Mogan Lake	Fertilizer Pollution, Agricultural Pollutants
Mid-Sakarya	Çamlidere, Eğrekaya, Akyar Dams	
Mid-Sakarya	Sarıyar Dam Lake/ Nallıhan Bird Heaven (Davutoğlu Wildlife Development Course)	Domestic and Industrial WW Discharges, Agricultural Pollutants
Göksu-Karasu Streams	Karasu Stream	Domestic and Industrial WW Discharges, Solidwaste Leachate
Göksu-Karasu Streams	Boğazköy Dam	Domestic and Industrial WW Discharges, Solidwaste Leachate
Göksu-Karasu Streams	Kızıldamlar Dam	Domestic and Industrial WW Discharges, Solidwaste Leachate
Lower Sakarya	Sapanca Lake	Pressure on Water Budget, Domestic and Industrial WW Discharges, TEM and D100 Highways, Spread Pollution Sources
Lower Sakarya	Çarksuyu Stream	Domestic and Industrial WW Discharges, Livestock Waste
Lower Sakarya	Longoz of Acarlar Lake	Domestic and Industrial WW Discharges, Agricultural Pollutants
Lower Sakarya	Ballıkaya Dam	

2.5 Porsuk Stream Sub-Basin

Porsuk River Basin constitutes a sub-basin of Sakarya River and reaches to Sakarya by crossing Kütahya and Eskişehir provinces. The Porsuk River, which was born cleanly at the peaks of Murat Mountain, is consumed both for agricultural, domestic and industrial uses. Although the water potential of Porsuk started to be used more efficiently with the realization of some water structures in the past, water pollution issues have also come to the fore. This situation reveals that, as in other river basins, water should be managed with a basin management approach in the Porsuk basin (Büyükerşen & Efelerli, 2008).

Porsuk Stream is divided in studies by GWW into three sections; Kütahya Section, Porsuk Dam Lake, Eskişehir Section. From the Murat Mountain to the entrance to Kütahya, Porsuk is polluted by wastewater from irrigated waters and agricultural pollution, Kütahya city wastewater, Kütahya slaughterhouse wastes, Sugar Factory, Tügsaş, Kümaş, Thermal Power Plants and Ceramic factories. The water which is the 1st class while entering the city is the Class III in terms of dissolved oxygen, BOD₅, COD and Class IV in terms of ammonia nitrogen when it leaves (Büyükerşen & Efelerli, 2008).

Porsuk Dam is absorbing this pollution from the lake with large water masses and large surfaces it accumulates, but in the meantime it is going in the negative direction. According to observations made by SHW different from time to time, each passing day the lake gains a eutrophic character (Büyükerşen & Efelerli, 2008).

After the exit of Porsuk dam, it is entering to Eskişehir as relatively renewed Porsuk. It is polluted here with Sarar textile, Tülomsaş, Sugar Factory, Air Supply, Organized Industry and urban wastes. Later, Alpu and Beylikova pollution and leather industry pollution are loaded. From Porsuk Dam to Eskişehir, it is Class I in terms of dissolved oxygen, BOD₅; COD and Ammonia Nitrogen Class II water. After loading, it becomes Class IV water (Büyükerşen & Efelerli, 2008).

Paleozoic, Mesozoic and Cenozoic units can be seen in the Porsuk River Basin to the west of Central Anatolia. With magmatic activity developed in different phases in the region, it is possible to come across units formed by granitic intrusions, volcanic lava and pyroclastics. There are plains of Aslanapa, Altıntaş, Kütahya and Köprüören in the south, Eskişehir and İnönü in the north, Alpu and Aşağı Porsuk plains in the northeast. These plains are wide plains that are cultivated. Most of the basin soils are poor in organic matter. The geological structure and climatic conditions of our country and the basin have provided sufficient potassium levels in the soils. Soils are rich in lime and very rich in lime. Soil is no problem in terms of salinity. Alluvial soils, colluvial soils, brown soils, red-brown soils, brown forest soils, lime-free brown forest soils, hydromorphic alluvial soils and non-calcareous brown soils can be seen as big soil bodies in the area (Öztürk & Altan, 2008).

The rivers in the Porsuk Basin form the Porsuk River and its tributaries. The annual average water potential of the basin is 481 hm³. Porsuk River is born from the foothills of Murat Mountain, after taking Porsuk resources and passing Kütahya, draws an arc towards the northeast and enters the Porsuk Dam Lake and exits. It flows east through Eskişehir, Alpu, Beylikova, Sarıköy and mixes with Sakarya River around Beylikköprü. The major tributaries of the river are Kokar Stream, Murat Stream, Porsuk Creek, Çat Creek, Değirmen Creek, Felent Stream, Kınık Creek, Kuduzlu Creek, Kargın Creek, Uludere and Musaözü Creek. In the north and east, Sarısu Creek, Sarıungur Creek, Muttalip Creek, Mihalıççık Creek and Pürtek Creek are located. Other water surfaces in the basin are Porsuk, Enne, Darıdere, Musaözü, Akçaköy, Beşkarış, Kureyşler Dams and Erenköy and Dereyalak Ponds (Öztürk & Altan, 2008).

Since the Mediterranean, Euro-Siberian and Iranian-Turanian phytogeographic regions are in transition, plants of all three regions are seen. In general, oak (*Quercus* ssp.) and juniper (*Juniperus* ssp.) species between 800-900 m elevations, larch (*Pinus nigra* subsp. *pallasiana*) between 900-1200 m elevations, and scotch pine (*Pinus sylvestris* subsp. *hamata*) constitutes the basic forest cover (Öztürk & Altan, 2008). Among the bird species seen in the basin, day and night predators such as eagles

(*Aquila chrysaetos*), vultures (*Aegypius* ssp.), hawks (*Buteo Buteo*), owls (*Athena* sp.) are among the endangered species. Species at risk in Turkey such as red-legged partridge (*Alectoris chukar*), goatsucker (*Caprimulgus europaeus*), green woodpecker (*Picus viridis*), turtledove (*Streptopelia turtur*), hoopoe (*Upupa epops*), wild goose (*Anser* sp.), raven (*Corvus corax*), and turaç (*Frankolinus frankolinus*) are also located in the basin. Altıntaş Plain is one of the breeding grounds of the last bustard birds (*Otis tarda*) in Anatolia and they live only in this region. While the population in the provincial centers increased in the Porsuk Basin, the population of rural areas decreased. Road transport is highly developed. The Ankara-Eskişehir-Bursa-Balıkesir highway in the east-west direction, the İstanbul-Bilecik-Kütahya-Afyon highway in the north-south direction and the railway between Ankara-İstanbul-İzmir pass in the basin area (Öztürk & Altan, 2008).

Wheat, barley, rye, oats, corn, rice and sugar beet are cultivated in the basin. However, due to the fact that the cultivated land is dry land, the whole land cannot be cultivated every year. Although the agricultural lands in the Porsuk River Basin have changed over the years, 81.34 % are planted, 17.28 % are left fallow, 0.42 % are produced fruit and vineyard and 0.96 % are produced vegetables (Öztürk & Altan, 2008).

As a result of development and industrialization in recent years, it is the receiving environment for domestic and industrial wastewater of the two provinces in the basin. According to SHW IIIrd Regional Directorate measurements, Porsuk River is Class II between the location of source region and Kütahya City Center, and Class IV between the location of Kütahya City Center and Porsuk Dam. At the output of Porsuk Dam the quality gets lower to the Class II and III. After the Eskişehir City Center, it becomes Class IV and gets bound to the Sakarya River in this quality. With the current situation, Porsuk River is one of Turkey's most polluted rivers. Urbanization has accelerated due to the industrialization process that started in the 1950's and across the country. The rapid industrialization, migration, population growth and housing needs of the basin caused productive agricultural lands to become threatened and residential in time. During the 35 year period between 1960

and 1995, approximately 2/ 3 of the lands in Eskişehir city center were considered as settlements. The mentioned areas are Class I and II arable land (Öztürk & Altan, 2008).

An important problem in the Porsuk Stream is floods and inundations. In 1964, a major flood disaster occurred in Eskişehir. After the construction of the Porsuk Dam, which was built at that time, the greatest danger was reported to be the sediments deposited in the Porsuk deposit. According to SHW reports, there is a risk of flooding if these sludges are neglected. Agricultural activities are also the factors that have a significant impact on pollution in Porsuk Stream. There are important irrigation areas in the Porsuk Basin and new ones are planned to be built. The rich nitrogen and phosphorus compounds in the waters returning from irrigation cause pollution in Porsuk Stream and Porsuk Dam. Excessive irrigation for the purpose of agricultural production leads to salinization or infertilization of soils. In addition, in the arid and semi-arid regions, the water-soluble salts given to meet the water needs of the plants accumulate in the soil and cause important agricultural problems. Salts accumulated in the soil adversely affect seed germination and plant growth (Öztürk & Altan 2008).

Porsuk Stream is clean in its sources. It carries a small amount of pollution from agricultural areas and domestic wastes until Kütahya Wastewater Treatment Plants. With the discharge from these facilities, the pollution load increases intensely. This is followed by Kütahya Sugar Factory, Güral Cam, Kütahya Nitrogen Factory, Kütahya Porselen and Seyitömer Thermal Power Plant. The waters of the Porsuk River, which has been rested in the Porsuk Dam Lake and the pollution load has been reduced to a certain extent, continues from here to be heavily polluted by Sarar Giyim Textile wastes, TÜLOMSAŞ Eskişehir Locomotive Factory wastes, Eskişehir Sugar Factory wastes, Eskişehir Municipal Slaughterhouse wastes, Eskişehir 1st Air Supply Maintenance Center Command wastes, Eskişehir Organized Industrial wastes, Ford Automotive wastes, Demircanlar Oil Industry, Eskar Meat Combination, Paşlıgil Dairy Industry, Dairy wastes, Eskişehir Municipality Sewerage wastes, Irrigation Canal wastes and Beylikova Dersan Leather Processing Industry waste (Öztürk &

Altan, 2008).

In the basin, saplings are planted for reforestation and erosion control by Kütahya and Eskişehir AGM directorates. Various models of erosion mapping were carried out in Sarısu Basin, which is a branch of Porsuk Stream, in 2005. Erosion maps provide preliminary data for better results of studies on erosion (Öztürk & Altan, 2008).

2.5.1 Wastewater Characteristics

In the study conducted by Kaçaroğlu and Günay (1997) in 1996, the sources of pollution were investigated and regular measurements were made at various stations between 1986 and 1988. Of these stations, the A-8 station was established just after the organized industrial zone around Alpu. As a result of the analysis; temperature 15 °C, pH 7.75, electrical conductivity 686 µS/ cm, dissolved Oxygen 3.8 mg/ L and NO₃ 29.6 mg/ L average values were obtained. The minimum value for NO₃ was 4.2 mg/ L and the maximum value was 57.1 mg/ L.

Büyükerşen and Efelerli (2008) have classified water quality parameters according to the average data values in 2004 in Alpu Region are given in Table 2.7.

Table 2.7 Average quality parameter data of Alpu Region (Büyükerşen & Efelerli, 2008)

Parameter	Unit	Quality Class
pH		I
Total dissolved solids	mg/ L	II
Dissolved Oxygen	mg/ L	IV
BOD ₅	mg/ L	IV
COD	mg/ L	IV
Ammonium Nitrogen	mg NH ₄ ⁺ -N/ L	IV
Nitrate Nitrogen	mg NO ₃ ⁻ -N/ L	I
Orto-Phosphate	mg o-PO ₄ / L	IV

In 2008, Altın et al. has finished a study of observing data in Eşenkara in Odunpazarı region, where water quality parameters determined between 1995 and 2005. They have made the research for 29 parameters and observed the data for

seasons in Table 2.8.

Table 2.8 Average data in Odunpazarı region between 1995-2005 (Altın et al., 2008)

Parameter	Abbreviation	Unit	Winter Data Average	Spring Data Average	Summer Data Average	Fall Data Average
Flow	Q	m ³ / sn	5.5	7.91	12.17	7.36
Temperature	T	°C	5.34	8.83	13.48	11.53
pH	pH		8.1	8.08	7.97	7.93
Electrical Conductivity(25°C)	EC	umho/ cm	541.1	518.61	578.15	581.33
Total Dissolved Solids	TDS	mg/ L	337.75	319.94	361.39	370.38
Suspended Solids	SS	mg/ L	34.72	23.42	17.76	20.76
Total Alkalinity	M-Al	mg/ L CaCO ₃	242.09	231.11	248.68	264.73
Chloride	Cl ⁻	mg/ L	12.54	11.59	13.89	13.23
Ammonical Nitrogen	NH ₄ ⁺ -N	mg/ L	0.26	0.3	0.61	0.49
Nitrite Nitrogen	NO ₂ ⁻ -N	mg/ L	0.02	0.02	0.06	0.05
Nitrate Nitrogen	NO ₃ ⁻ -N	mg/ L	1.15	0.8	0.87	1.26
Dissolved Oxygen	DO	mg/ L	11.04	10.43	8.9	8.74
Organic Matter	pV	mg/ L	2.81	2.88	2.89	2.93
Biochemical Oxygen Demand	BOD	mg/ L	3.04	3.01	3.39	3.36
Chemical Oxygen Demand	COD	mg/ L	20.46	27.24	25.74	24.92
Total Hardness	TH	mg/ L CaCO ₃	269.44	256.18	284.95	291.51
Ortho-Phosphorus	o-PO ₄ ³⁻	mg/ L	0.38	0.37	0.84	0.86
Sulphate	SO ₄ ³⁻	mg/ L	34.73	34.89	43.26	37.31
Iron	Fe	mg/ L	0.35	0.35	0.27	0.33
Manganese	Mn	mg/ L	0.11	0.08	0.09	0.11
Sodium	Na ⁺	mg/ L	13.68	12.88	13.92	14.85
Potassium	K ⁺	mg/ L	3.95	4.01	3.98	4.48
Calcium	Ca ²⁺	mg/ L	46.93	46.5	48.31	52.63
Magnesium	Mg ²⁺	mg/ L	36.96	34.00	39.89	38.93
Total Coliforms	T.coli	MPN/ 100 mL	998.98	1,184.73	1,496.91	2,248.58
Fecal Streptococ	F.strp.	MPN/ 100 mL	147.97	48.18	106.70	364.08
<i>Escherichia coli</i>	<i>E. coli</i>	MPN/ 100 mL	214.69	164.45	292.60	925.07
Boron	BOD	mg/ L	0.16	0.14	0.16	0.17
Fluoride	F ⁻	mg/ L	0.25	0.23	0.27	0.28

In the study of Bakış et al. (2011) which was finished in 2010, surface and groundwater pollution level of Porsuk River and its basin has been researched. In 2005 and 2006, samples are taken from 20 stations in summer and winter seasons for

2 years and 18 parameters were analyzed. The data of Station 16 in Alpu Region are listed in Table 2.9.

Table 2.9 Data detected in Alpu Region of the basin in 2005-2006 (Bakış et al., 2011)

Parameter	Unit	2005 Winter	2005 Summer	2006 Winter	2006 Summer
pH		7.7	7.6	6.75	6.5
Electrical Conductivity (EC)	µS/ cm	880	750	910	900
Temperature	°C	12.1	22	11.2	21
Nitrate Nitrogen (NO ₃ ⁻ -N)	mg/ L	0.4	0.5	0.3	0.4
Nitrite Nitrogen (NO ₂ ⁻ -N)	mg/ L	0.7	0.8	0.6	0.7
Sulphate (SO ₄ ³⁻)	mg/ L	42	75	42	59
Ortho-Phosphate (o-PO ₄)	mg/ L	0.65	1.7	0.65	1.3
Sodium (Na ⁺)	mg/ L	18	10	24	7.5
Potassium	mg/ L	7.5	8	13.5	8
COD	mg/ L	33	81	34	63
Lead (Pb)	mg/ L	125	120	180	10
Zinc (Zn)	µg/ L	65	30	1500	400
Copper (Cu)	µg/ L	10	10	920	520
Nickel (Ni)	µg/ L	65	60	820	820
Cadmium (Cd)	µg/ L	10	10	30	10
Chrome (Cr)	µg/ L	10	10	700	410
Manganese (Mn)	µg/ L	10	10	30	10
Boron (B)	µg/ L	75	30	120	290

Köse (2016) et al. has finished their study in in 2016. The purpose of this study is to determine the water quality parameters in 13 stations established along Porsuk River between May 2010 and February 2011. The average data obtained from samples taken from Station 6 around Alpu Region in the coordinates of “N: 39°46'17.0", E: 030°58'13.3" which is in 782 m elevation are given in Table 2.10 (Köse 2016).

Table 2.10 Water quality data detected in Alpu Region of the basin in 2010-2011(Köse 2016)

Parameter	Unit	Average Data
Temperature	°C	15.0
pH		7.43
Dissolved Oxygen	mg/ L	2.96
Electrical Conductivity	µS/ cm	833
Sulphate(SO ₄ ³⁻)	mg/ L	64.7
NH ₄ ⁺ -N	mg/ L	4.41
NO ₂ ⁻ -N	mg/ L	0.093
NO ₃ ⁻ -N	mg/ L	1.48
Phosphate (PO ₄ ³⁻)	mg/ L	2.46
BOD	mg/ L	15.25
COD	mg/ L	54.02
Sodium (Na ⁺)	mg/ L	40.98
Total Phosphorus	mg/ L	1.49

In the study conducted by Köse et al. in the summer of 2015, water quality parameters determined in the stations established along the Porsuk Stream and water quality classes were determined in terms of the parameters examined. The data obtained at Station 15 in Alpu Region are given in Table 2.11 (Köse et al., 2016).

Table 2.11 Water quality data detected in Alpu Region of the basin in 2015 (Köse et al., 2016)

Parameter	Unit	Obtained Data	Quality Class
Heat	°C	21.3	I
Dissolved Oxygen	mg/ L	4.33	III
Oxygen Saturation	%	53.7	-
Turbidity	NTU	20.9	-
pH		7.22	I
Electrical Conductivity	µS/ cm	911	-
Salinity	‰	0.45	-
Sulphate	mg SO ₄ / L	82.5	-
Ammonium Nitrogen	mg NH ₄ ⁺ -N/ L	1.53	II
Nitrite Nitrogen	mg NO ₂ ⁻ -N/ L	0.251	IV
Nitrate Nitrogen	mg NO ₃ ⁻ -N/ L	2.76	I
Total Phosphorus	mg/ L	0.503	III
COD	mg/ L	21.1	I
BOD	mg/ L	6.92	II

In the researches, very different sampling points were determined in Porsuk River and the characteristics of Porsuk River were studied to be determined. The studies carried out within this scope are the wastewater characteristics determined in recent years and the mentioned study groups tried to determine the characteristics of Porsuk River. In this thesis; since the water sample was taken from a point after the Eskişehir exit, only the Porsuk Stream characterization after the Eskişehir exit was given in the tables.

2.6 Nutrient Removal

The substances that plants and animals need to live and grow are called nutrients. These nutrients are Nitrogen (N), Phosphorus (P), Carbon (C), Magnesium (Mg), Potassium (K), Sulfur (S), Calcium (Ca), Sodium (Na), Chlorine (Cl). Nitrogen and

Phosphorus are nutrients which are natural parts of aquatic ecosystems (USEPA, 2018). Nitrogenous compounds cause dissolved oxygen consumption during disintegration. In the receiving environment where it is discharged, the dissolved oxygen concentration decreases. Uncontrolled discharge of wastewaters containing high concentrations of phosphorus into the receiving environment may result in significant deterioration in water quality. Phosphorus discharge into the receiving environment causes excessive growth of algae and other aquatic plants known as eutrophication (Doğan, 2010).

Wastewater contains nitrogen and phosphorus from human wastes, food and various soap and detergents (USEPA, 2018). Nitrogen and phosphorus sources can be coming from domestic and industrial applications. In addition to urea, protein and $\text{NH}_3\text{-N}$, which may be of domestic origin, organic and inorganic nitrogenous and phosphorus substances from the industrial, fertilizer, meat, milk and nitrogenous industries produce excessive nitrogen and phosphorus pollution in the receiving environment (Metcalf & Eddy, 2003). Excess nutrients from agricultural activities can lead to a significant deterioration of surface and groundwater quality (Sooknah & Wilkie, 2004). Agricultural activities such as fertilizer application, aquaculture and animal husbandry are among the main sources of high levels of nitrogenous compounds in wastewater. The most abundant of these pollutants is nitrate. In the natural environment, nitrate can easily be converted to less toxic compounds with the aid of various microorganisms involved in the nitrogen cycle reactions. However, human activities have drastically altered the nitrogen cycle, causing a significant impact on both aquatic organisms and surrounding water bodies (Idi et al., 2015). Anthropogenic activities may include ammonium nitrogen source, nitrogen fertilizer production and release of unused ammonium nitrogen in agricultural flow. Discharging of products of human and animal origin is another important source of nitrogenous waste (Nancharaiah et al., 2016).

Nitrogen is present in various forms in natural systems and its compounds are involved in numerous biological and abiotic processes. The presence of approximately 80 % of Nitrogen in the atmosphere forms the main storage pool in

the complex nitrogen cycle through the ecosystem. The conversion of some of these gases into ammonia (NH_3), ammonium (NH_4^+) and a number of other nitrogen compounds in the soil and in water is known as nitrogen fixation, and in the absence of industrial fertilizers, it is one of the primary sources of nitrogen for living. On land, special nitrogen-fixing bacteria and algae, often living in nodules in legume roots, mediate biological nitrogen fixation to provide the energy they need. In freshwater and marine systems, cyanobacteria stabilize nitrogen (Kapdan & Çelebi, 2014). Nitrogen is primarily fixed in soil or aquatic systems, followed by two different paths. It can be oxidized for energy by nitrification or assimilated by an organism by assimilation of ammonia into biomass. After the nitrification process, reduction reactions produce N_2 gas via denitrification. (Kapdan & Çelebi, 2014, Baeten et al., 2019, Wang et al., 2018, Chen et al., 2017).

Plants incorporate fixed forms of fixed nitrogen into their tissues through their root systems and then use them to produce amino acids and convert them into proteins. Nitrogen, which is fixed as protein in the body of living organisms, eventually returns to the original form of nitrogen, the nitrogen gas form. The denitrification process begins when plants containing fixed nitrogen are eaten or die. In dead plants, animal bodies and animal feces, the fixed nitrogen products are confronted with denitrifying bacteria that take back the work done by the nitrogen-fixing bacteria. Generally, the final product of denitrification is N_2 , but very little nitrogen oxide is also produced (Kapdan & Çelebi, 2014, Baeten et al., 2019, Wang et al., 2018, Chen et al., 2017).

The deterioration of the nitrogen cycle of human activities leads to a wide range of environmental problems, such as fog formation in the troposphere, degradation of stratospheric ozone, and contamination of surface water. For example, nitrogen oxide is a greenhouse gas, such as carbon dioxide and water vapor, which causes heat generation on the earth's surface. It also destroys stratospheric ozone. Ozone in the stratosphere is decomposed by ultraviolet rays and converts to nitrogen dioxide and nitric oxide, causing ozone to decrease. Nitrogen oxides are chemically recycled to N_2 , nitrate or nitrite compounds (Kapdan & Çelebi, 2014).

Phosphorus has only one form in nature. This form is phosphate containing phosphorus atom and four oxygen atoms. This powerful molecule never follows a path in the atmosphere, but is always part of the organism, dissolved in water or in the form of rock. Phosphate rock dissolves when exposed to water, especially water containing a small amount of acid. Autotrophic organisms incorporate this phosphorus and use it in various ways. Heterotrophic organisms receive phosphorus from the plants they eat. Only the fungi species obtain phosphorus as a result of mutualistic symbiotic relationships with plant roots. When animals and plants die, phosphate is returned to soil or water by separators. Here they are taken up by other plants and re-used. This cycle occurs repeatedly until the phosphorus is lost in the deepest part of the ocean until it becomes part of the sedimentary rocks formed here. If sedimentary rocks reach the surface of the water, phosphorus is finally released and separated. Excess phosphorus causes excessive algae growth in water, consuming oxygen in water and destroying aquatic life (Kapdan & Çelebi, 2014, Nielsen et al., 2019).

When too much Nitrogen and Phosphorus are discharged, the natural environment may be polluted. Nutrient pollution has affected many streams, rivers, lakes, bays and coastal waters in the last few decades, causing serious environmental and human health problems and affecting the economy (USEPA, 2018).

The release of Nitrogen and Phosphorus-containing waters into surface waters affects lakes, rivers, estuaries and oceans. Excess Nitrogen and Phosphorus cause nutrient pollution in surface and semi-surface waters. In addition, Nitrogen and Phosphorus cause algae and aquatic plant growth in surface waters, which deteriorates water quality and reduces dissolved oxygen concentration. Algal explosion reduces dissolved oxygen and causes death of fish and other organisms. Eutrophication kills fish by causing loss of color, odor, turbidity and dissolved oxygen (Nancharaiah et al., 2016). Nutrient pollution in animal source wastewater becomes a more serious problem if improperly disposed of. This not only has a negative impact on agricultural development, but can also affect the improvement of the environment and the quality of life of people (Zhu et al., 2011).

2.6.1 Nutrient Removal Methods

After the wastewater is collected in canals, it is discharged to a receiving waterbed such as a lake or a sea, estuary, river or land for disposal. The wastewater to be discharged into the receiving environment must first be treated in order not to cause any environmental problems (Balçık, 2013). Biological nutrients in wastewater to be discharged play an important role in water quality. Based on nitrogen and phosphorus as nutrients, they can both cause eutrophication. The reasons for using biological nutrient removal methods can be listed as environmental, economic and functional gains. Since eutrophication may be prevented by nutrient removal methods, environmental gains are the most important ones (Akyol, 2011).

Most treatment technologies involve chemical, physical and biological processes. Conventional wastewater treatment basically involves in three steps: primary, secondary and advanced (tertiary) treatment. Initial treatment cleans and removes sediment and floating materials. The second stage is the biological treatment methods in which organic substances are degraded and nutrients are removed by a series of aerobic and anaerobic treatments (Santos & Pires, 2018).

Ammonium is one of the most important parameters to be removed from domestic and industrial wastewater. Ammonium removal from wastewater is generally achieved in an aerobic environment in biological systems by nitrification of ammonium with bacteria such as *Nitrosomonas* or *Nitrososipira* followed by oxidation (nitrification) of nitrite to nitrate by bacteria such as *Nitrobacter* followed by reduction of nitrate to nitrogen gas (denitrification) by means of an organic carbon source in an anoxic medium (Güven & Sözen, 2003, Baeten et al, 2019, Wang et al. 2018, Winkler & Straka, 2019). Nitrogen is found in wastewater in the form of Ammonia (NH_3), Ammonium ion (NH_4^+), Nitrite (NO_2^-), and Nitrate (NO_3^-).

There are many biological processes for the combined treatment of nitrogen and phosphorus, which are in the form of an activated sludge process consisting of a combination of aerobic, anaerobic and anoxic zones. Some of these processes have

been developed primarily for phosphorus removal and then converted into systems where nitrogen and phosphorus can be treated together. There are widely used systems such as A²/O process, five-stage Bardenpho process, UCT process, VIP process (Toprak, 1994). Nowadays these methods are enlarged with different aspects.

In recent studies, it has been observed that nitrification and denitrification processes need to be separated according to time and place in order to operate effectively. Conventional nitrification and denitrification reactions are long-term processes. High levels of oxygen are needed during nitrification reactions. In denitrification reactions, organic carbon is required as electron donor. In order to completely remove nitrogen from wastewater containing a high concentration of nitrogen, a large amount of additional carbon resources is required, but the limited amount of naturally occurring organic carbon leads to increased costs. Existing wastewater plants are generally designed for nitrogen removal and it is difficult to meet the needs of nitrification and denitrification processes (Can Doğan & Kırılı, 2008). In biological nitrogen removal nitrification/ denitrification is the most energy demanding step. There are lower energy intensive ones like nitrification/ denitrification and partial nitrification/ Anammox processes (Winkler & Straka, 2019). Anaerobic/ aerobic/ partial nitrification-anammox granular sludge reactors are one of modified systems to achieve higher nutrient removal efficiencies with less energy needs (land footprint 50 %- 75 %; energy 30 %- 48 % lower compared with conventional systems). Organics and ammonium was widely treated till 1970's in aerobic treatment systems with spherical carriers where biofilms can form. In ten years nitrate and nitrite removal via denitrification was adopted to these systems under anoxic conditions. Nowadays also biological phosphorus removal with sequencing batch reactors is modified with self sustained granules under aerobic conditions. Also anammox process is used to treat nitrogen rich wastewaters with addition of granules allowing phosphorus to accumulate inside the particles under various conditions. The self- immobilized microbial granules have high biopolymeric structures allowing higher settling velocities. They have good biomass water separation allowance, high biomass concentrations where nitrification, denitrification and phosphate removal takes place simultaneously in a tank, lower

sludge production, ability to use the excess sludge as a resource due to alginate-like exopolymers serving sludge management. However there are disadvantages like long term start-up periods, disintegration of the system, and stability issues of the organisms depending on different operational conditions (Baeten et al., 2019, Nancharaiah & Sarvajith, 2019). Also there are methods where nitrogen is recovered by phototrophs via cell assimilation (Discussed later). However this method depends on space requirement limiting the applications and beside solid separation is a challenge in these methods. Microbial electrochemical cells as side streams are also tried to recover nitrogen. However, the effluent nitrogen concentrations do not meet discharge standards properly (Winkler & Straka, 2019, Nancharaiah et al., 2016, Kelly & He, 2014). Enhanced phosphorus removal applications are modified in activated sludge systems by adding membrane bioreactors (Zuthi et al., 2013). Liu et al. (2017) provided nutrient removal by microalgae and bacterial consortium. In the table given below, the input and output concentrations of total nitrogen and phosphorus are given.

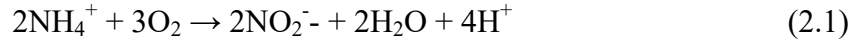
Table 2.12 Nutrient removal in bioreactors using attached microalgae and bacteria (Liu et al., 2017)

	Bioreactor Type	Input (mg/ L)		Output (mg/ L)	
		TN	TP	TN	TP
Bioreactor used in the bound microalgae and bacteria consortium	Algae remover	0.5	0.03	0.3	0.01
	Phototrophic plane biofilm reactor	5.57	0.97	<0.1	<0.05
	Rotating algae biofilm reactor	10	2.3	<1	<1.5
	Algae biofilm membrane photobioreactor	15	0.8	2.6	0.1

According to a study by Conthe et al. (2019), nitrification refers to the oxidation of ammonium ($\text{NH}_4\text{-N}$) to nitrite ($\text{NO}_2\text{-N}$) and then to nitrate ($\text{NO}_3\text{-N}$) by a two-stage biological process. Aerobic autotroph bacteria are responsible for nitrification. In the two-step reaction, ammonium is first oxidized to nitrite by some autotrophic bacteria. In the second stage, nitrite is oxidized to the nitrate by another group of autotrophic

bacteria. These two groups of bacteria are completely different from each other. The reactions of nitrite ammonium to nitrite and nitrite oxidation to nitrate by these two groups of bacteria are given below:

Oxidation of NH_4^+ to NO_2^- by *Nitrosomonas*;

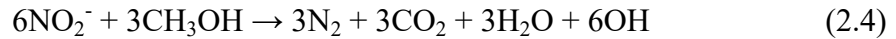
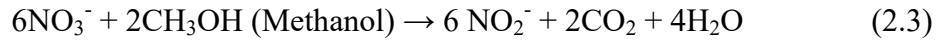


Oxidation of NO_2^- to NO_3^- by *Nitrobacter*;



Nitrification bacteria in activated sludge have a lower reproduction rate and population compared to heterotrophic bacteria. They are aerobic bacteria, so they need sufficient dissolved Oxygen in the system (Conthe et al., 2019).

The Nitrate formed as a result of nitrification is reduced to Nitrogen gas after successive steps by heterotrophic bacteria in anoxic medium in the biological denitrification process. Reduction reactions are as follows:



Methanol, ethanol and acetic acid are mostly used as carbon sources to meet the energy needs of the bacteria in the process. The order of nitrification and denitrification processes depends on the wastewater composition. If wastewater contains only carbon and ammonium, nitrification takes precedence in the process. In wastewater containing excess nitrate, priority is given to denitrification process (Conthe et al., 2019).

Since phosphorus is one of the most important nutrients found in many water sources, one of the most effective ways to control it is to prevent anthropogenic eutrophication. Phosphate removal from wastewater can be achieved by physical,

chemical and biological methods or by their combined use. Many biological processes have been developed to provide nitrogen and phosphorus removal together. Most of these are registered and most of them use activated sludge process. Nevertheless, anaerobic, anoxic and aerobic zones should be used to achieve nitrogen and phosphorus removal (Horan, 1989). There are many novel nutrient removing organisms up to date which can be summarized as ammonia oxidizing archaea (AOA), Complete ammonia oxidation (COMAMMOX) bacteria, Anaerobic ammonium oxidation (ANAMMOX), anaerobic ammonium oxidation coupled to iron reduction (FEAMMOX) bacteria, and denitrifying anaerobic methane oxidation (DAMO) microorganism. These explained organism types are also taking place in natural treatment helping to reduce nutrients (Ren et al., 2020).

ANAMMOX (Anaerobic Ammonium Oxidation) Process: According to the studies conducted by Can Doğan and Kırılı (2008), the ANAMMOX process was discovered in a fluidized bed reactor that purifies the methanogenic reactor effluent. As a result of long-term studies, the presence of anaerobic ammonium oxidation (ANaerobic AMMonium OXidation) bacteria (anammox bacteria) in a fluidized bed reactor in a laboratory-scale denitrification pilot plant was proved. The process can remove ammonium from concentrated wastewater at low cost. With the release of nitrogen gas as the main product, some nitrate is also formed. Nitrate is produced by the ANAMMOX process and begins to accumulate in the reactor over time. In the process, Ammonia is used as an electron donor and nitrite is used as an electron acceptor. The main disadvantage of the process, which is suitable for the treatment of industrial wastewater, is the slow growth rate of anammox microorganisms. In order to observe the increase in *Anammox* biomass, it is stated that the most suitable reactor is sequential batch reactor (Can Doğan & Kırılı, 2008).

CANON Process: According to studies by Khin and Annachhatre (2004), elemental nitrogen loss is high in wastewaters containing high ammonium and low concentration of organic carbon. In these cases, the CANON process is a very economical option. The process takes place in a single reactor or biofilm with limited oxygen. This process is based on partial nitrification and oxidation of anoxic

ammonium. Its oxygen consists of aerobic and anaerobic ammonium oxidizing bacteria under limited conditions, and this combined culture forms CANON activity. This process is based on the interaction between two autotrophic microorganisms (*Nitrosomonas*, *Anammox*) under limited conditions of oxygen. This group of autotrophic microorganisms converts ammonium directly into nitrogen gas through nitrite. Oxidation of nitrite to nitrate is prevented by high ammonium concentration and oxygen limiting conditions in the reactor. Approximately 85% of nitrogen removal efficiency is obtained (Can Dogan & Kırılı, 2008).

Compared to conventional nitrification and denitrification processes, although the rate of nitrogen removal is low, there is no need to add COD because the CANON process is carried out entirely by autotroph microorganisms. In addition, it can be said that the removal of all nitrogen in the process can be carried out in a single reactor and with very little ventilation. This results in a very energy and space saving result. This process consumes 63% less oxygen and 100% less organic matter than conventional nitrogen removal methods (Can Doğan & Kırılı, 2008).

SHARON Process: This process is the most effective process for reducing ammonium concentration at higher temperature (55 °C) and pH (>7) and without biomass retention in a single aeration reactor compared to other processes. The SHARON process involves partial nitrification where Ammonium is reduced to nitrite. This may reduce the ventilation cost to a minimum level in the treatment plant. Furthermore, it is a very suitable process for Nitrogen removal in wastewaters with high Ammonia concentration. (Can Dogan & Kırılı, 2008). As a result of this process, 90 % yield is obtained in Nitrogen removal. The use of the reactor tank will be sufficient for this process. Chemical sludge formation is not observed and smaller amounts of biological sludge are observed. Since there is oxidation in the Nitrite step, the Oxygen demand is not much, which saves energy. When compared to the conventional nitrification-denitrification process, the SHARON process requires 25% less in ventilation and 40% less in carbon requirements (Khin & Annachhatre, 2004).

A/O Process: It is the only sludge suspended growth system which connects anaerobic and aerobic parts to each other respectively, developed to provide phosphorus removal and oxidation of carbon in wastewater. The sludge formed in the sedimentation tank due to the entering wastewater comes to the anaerobic chamber and dissolves and leaves the system as phosphate. In the aerobic state, phosphorus is stored as polyphosphate (Toprak, 1994). In this process, nitrification does not occur and the residence time for biological phosphorus removal in the anaerobic phase ranges from half an hour to 1 hour. In the aerobic phase, the retention time varies from 2 to 4 days depending on the temperature (Doğan, 2010). According to the research conducted by Önel (2003), it does not provide a good removal for high amounts of nitrogen and phosphorus, cannot be provided working conditions in cold weather requires a high BOD₅/ P ratio, requires a high rate of Oxygen for aerobic phase are the disadvantages of this system.

PhoStrip Process: It consists of both biological and chemical methods. Some of the recycled activated sludge is transported to the anaerobic stripping tank. The retention time in this tank varies from 8 to 12 hours. To ensure phosphorus release, acetic acid or inlet water is added to the scraper tank (Önel, 2003). According to a study by Öztürk (2013), phosphorus mixed with the supernatant in anaerobic environment is chemically precipitated in another tank. In addition, phosphorus removal is provided by excess sludge drawn from the system. The PhoStrip process is theoretically less sensitive to the input phosphorus concentration. It is preferred in the treatment of weak wastewaters.

A²/O Process: The A²/O process is a modification of the A/O process and also includes an anoxic portion for denitrification. The residence time in the anoxic zone is approximately one hour. In the anoxic section, nitrite and nitrate in the form of chemically bound oxygen is fed back into the system from the air section. At the outlet there may be less than 2 mg/ L Phosphorus concentration without filtration. By filtration of the effluent, the Phosphorus concentration may be lower than 1.5 mg/ L (Toprak, 1994). This system has been developed to remove both Nitrogen and Phosphorus at the same time, provides alkalinity for the realization of nitrification,

releases good sediment sludge, the process is relatively simple and saves energy. In addition, they require a higher BOD/ P ratio than the A/O process, and Nitrogen removal is limited by the internal recycle rate (Önel, 2003). According to Coşkun et al. (2018), the advantages of A²/O process are; easier to operate, to provide energy conservation, to provide the necessary alkalinity for nitrification in nutrient removal and the sludge produced has good precipitation properties. Disadvantages of the system are that Nitrogen removal depends on the rate of return and the effect of Phosphorus removal rate due to the Nitrate content of the recycling.

Five-Stage Bardenpho Process: This system, which operates in five stages, provides the removal of Phosphorus, Nitrogen and Organic Carbon by using anaerobic, anoxic and aerobic phases. In addition to BOD removal, nitrification and polyphosphate accumulation is also provided in this process (Öztürk, 2013). According to the research of Coşkun et al. (2018), with the Five-Stage Bardenpho Process; it can reach 3-5 mg/ L total Nitrogen value at the outlet of the system without the need of filter process and the sludge obtained from the process system provides advantages such as good deposition property like VIP, UCT, A²/O processes. However, the lower Phosphorus removal efficiency and the need for large tank volumes are disadvantages of this process.

UCT (University of Cape Town) Process: The UCT process developed by the University of Cape Town is similar to the A²/O process, except in two cases. The activated sludge recycle is returned to the anoxic section instead of the aerobic section, and the inner cycle is from the anoxic section to the anaerobic section. By recycling the activated sludge into the anoxic section, the Nitrate does not enter the anaerobic section, thereby providing better release of Phosphorus in the anaerobic section. The internal cycle provides an increase in the use of organic in the anaerobic section. The mixture in the anoxic section contains a significant amount of dissolved BOD and a small amount of Nitrate. The recycling of the anoxic mixture provides optimum conditions for the fermentation rate in the anaerobic section (Toprak, 1994). In the work of Coşkun et al. (2018), the advantages of UCT Process; Nitrogen removal is good, the sludge produced has good precipitation properties as in A²/O

process, decrease of nitrate in the system and increase Phosphorus removal efficiency on the contrary. The disadvantages of the process are the complex operating structure and the need for additional recycling systems.

VIP (Virginia Initiative Plan) Process: This system is similar to A²/O and UCT processes except for recycling. The activated sludge recycle is introduced into the anoxic section together with the aerobic section recycle. The mixture of the anoxic part is fed back to the anaerobic part. Based on experimental data, some of the organic matter in the wastewater is stabilized by the anerobic mechanism, which reduces the Oxygen demand of the process (Toprak, 1994). In the studies of Coşkun et al. (2018), the advantages of VIP Process are; similar to the UCT Process, the reduction of Nitrate in the system does not reduce the Phosphorus removal efficiency, but rather the lower BOD/ P requirement compared to UCT, and the sludge produced has good precipitation properties as in UCT and A²/O processes. The disadvantage of this process is that it is as complex as the UCT Process and that it requires additional operating equipment and additional recycling systems.

Sequencing Batch Reactor (SBR): This process is been used, especially in recent years, to improve biological phosphorus removal. These systems do not require continuous maintenance and a certain time is waited after the wastewater is filled into the reactor and some of the water reaching the desired height is taken from the reactor. Reactor number, retention time, mixing speed and Oxygen amount may vary depending on the situation (Öztürk, 2013). According to the study conducted by Coşkun et al. (2018), the Sequencing Batch Reactor Process provides advantages such as low design data, sufficient Nitrogen and Phosphorus removal together and ease of use. However, the disadvantage of the process is that it is used for low flow treatment systems, the need for extra units and changes in leaving water quality are observed. When the studies carried out with the process examined Nitrogen and Phosphorus removal rates; it has been reported to range between 90- 95% and 77-100% (Abufayed & Schroeder, 1986).

For instance; the nutrients found in rotten pig manure are described by Obaja et al. (2005) by using the sequential batch reactor (SBR). 99.6% $\text{NH}_4^+\text{-N}$, 100% $\text{NO}_x^-\text{-N}$, 98.4% $\text{PO}_4^{3-}\text{-P}$ removal was performed in a 4-hour cycle with 0.5 day retention time (Obaja et al., 2005).

2.6.2 Natural Treatment Methods

New searches in wastewater treatment are of great importance due to high equipment and chemical costs (Sahu & Dubey, 2014). Due to the environmental impacts of the use of fossil fuels, the use of electricity, plastic, concrete and chemicals in conventional treatment plants results in some pollution elsewhere, despite cautious approaches. Since wastewater residues and sludge formation in traditional treatment processes pose a disposal problem, the use of natural treatment technologies where appropriate provides the potential for pollution control and the potential to reduce future environmental consequences. All natural treatment methods are relatively land-oriented, but they may need some complementary elements that can reveal strengths and weaknesses, such as fossil fuel use and special treatment methods (Kadlec et al., 1995).

All natural methods use a rooting medium for the growth of highland plants that filter an unsaturated soil layer or wastewater solids and absorb dissolved pollutants for final harvest and discharge to ensure direct filtration and removal of contaminants. In on-land flow methods, some of the wastewater infiltrates into groundwater by using lower permeability plateau soils. On-site systems form residential septic tanks and associated drainage areas, while larger systems include large community systems comprising a septic or Imhoff tank and a larger drainage area. The septic tank provides a built-in basin for sedimentation of solids and anaerobic digestion of solids. The components are here partially converted by anaerobic decomposition and converted into more stable particulate and dissolved forms before entering the leach lines. The leaching area consists of branched, perforated pipes embedded in a highly porous medium such as coarse gravel and buried in a permeable soil with an unsaturated zone of at least about 1.5 m above any

existing shallow groundwater. The unsaturated zone can be made in areas with low permeability using a mound system. The size required for this area is region-specific and depends on the current conditions. Slow-rate land application of wastewater uses the irrigation of plant systems for final disposal. The amount of irrigation is generally low and sparse, allowing for regular reconstruction of aerobic soil conditions. These conditions are essential for the growth of dry soil vegetation, necessary for nutrient removal, filtration of wastewater solids, and provision of permeable soil texture. High rate land application systems use permeable soils for discharge application. High speed land application systems are generally designed as small, narrow, shallow basins or ponds with a height of less than 1.5 m. Smaller basin areas and higher length-to-width ratios increase this infiltration rate. Drying and resting is provided by using multiple basin areas. By careful application, it can eliminate the problem of groundwater mounds beneath the basins. Alternatively, water-resistant plant species can be planted to protect soil texture and ventilation. Unlike other upland alternatives, on-land flow treatment systems rely on low permeability soils to limit infiltration and thus a surface discharge. The pre-treated wastewater is applied by terraces, through-pass pipes or spray injectors, and gravity is sloped into a series of collection channels. As water flows through the dense vegetation, pollutants precipitate and dissolved components are absorbed by plants and soils. In the absence of treatment during the rest periods, the organic fraction of the precipitated particles can be converted to microbially oxidized and absorbed nutrients (mainly inorganic nitrogen and phosphorus), microbially converted (nitrogen nitrification of ammonia nitrogen) or bound to soil (Kadlec et al., 1995).

Water and wetland treatment systems differ from upland systems mainly because they are constantly inundated and develop an anaerobic sediment and soil layer. This excludes the growth of plant species that require oxygen and causes simultaneous aerobic and anaerobic assimilation processes in a single-layer. Facultative ponds are designed to hold a naturally ventilated surface layer on a deeper anaerobic layer. Natural aeration is caused by the effect of atmospheric oxygen diffusion and the oxygen generated during photosynthesis of algae in water. Oxygen concentration may vary periodically. In these ponds, extreme anaerobic conditions are controlled

by the BOD loading rate limitation and retention time. Pond systems can be intentionally infused with floating water plant species to provide waste water treatment. Floating aquatic plant processing systems are functionally different from ponds because the photosynthetic component releases oxygen on the water surface and effectively reduces atmospheric oxygen diffusion. Therefore, floating aquatic plant systems are insufficient in oxygen and aerobic processes are largely limited to the plant root zone. In floating aquatic plant systems, the majority of the water column is generally anaerobic with the degree of exhaustion of oxygen, depending on the organic loading rate. Wetland treatment systems use soil conditions saturated with water-resistant plant species. The three basic types of wetland treatment systems include natural wetlands, constructed surface flow (SF) wetlands and constructed subsurface flow (SSF) wetlands. Although there are many naturally occurring wetland species, only those with plant species adapted to continuous floods are suitable for receiving continuous effluent flow. Furthermore, due to their protected legal status, discharges to natural wetlands should receive a high level of pretreatment. They can be used to treat primary and secondary wastewater, as well as storm water, wastewater, leachate, industrial and agricultural wastewater (Kadlec et al., 1995).

For the treatment of wastewater, intermittent sand filtration has been used for more than a hundred years. Sand filters can be operated in single pass or recirculation mode. In single pass mode, after primary sedimentation, the waste water is intermittently applied to a layered sand filter. In one pass through the system, organic carbon removal, ammonification and nitrification occur. Factors affecting the retention of bacteria in porous media include filtration, grain size of the filter media and hydraulic loading speed (Sahu & Dubey, 2014).

Phytoremediation is used for nutrient removal from wastewater and is a field of great potential and demand, considering that high organic wastewaters, surface and groundwater bodies in various parts of the world suffer from eutrophication (Olguin, 2003). Phytoremediation is the use of plants such as grasses and trees to remove, incorporate, or energize hazardous harmless substances from polluted environments

and groundwater. It is known that the plant can absorb a wide variety of nutrients through the roots. Recently, it has been discovered that plants can easily absorb organic and metallic wastes to be metabolized as a food source (Zodrow, 1999). There are several methods of phytoremediation.

Phyto-extraction or Phytoaccumulation; in this process, plant roots absorb contaminants together with nutrients and water. The pollutant mass is not removed, but it accumulates in plant shoots and leaves. This method is mainly used for metal-containing wastes. Phytotransformation or Phyto-degradation; plants actually metabolize and destroy pollutants in plant tissues. With this method, organic pollutants are removed from soil, sediments or water and then converted to a more stable, less toxic or less mobile form. Phytostabilization; pollutants are immobilized by chemical compounds produced by the plant. Phytostabilization does not show degradation, but plants prevent or reduce the movement of pollutants. Rhizodegradation is the degradation of pollutants with increased microbial activity around the root region (rhizosphere) and is a process much slower than phytodegradation. Microorganisms consume and digest organic materials for nutrition and energy. In Hydraulic Control system; trees create a dense root mass that absorbs large amounts of water by acting as natural pumps by lowering the roots towards the water table. In this way, they regulate and control groundwater movement. Phyto-evaporation; plants that incorporate water containing organic pollutants by releasing pollutants from the leaves into the air to ensure the uptake of pollutants from the soil or water and the removal of water by respiration. Rhizofiltration is a water treatment technique involving the uptake of pollutants by plant roots to reduce contamination in natural wetlands and estuaries (Zodrow, 1999).

Bioremediation is defined as the process in which organic wastes are reduced to harmless state by biological method or levels are reached below the concentration limits by various arrangements. The principle of bioremediation is based on the use of living organisms, particularly microorganisms, to reduce environmental pollutants to less toxic forms. Uses naturally occurring bacteria, fungi or plants to reduce or detoxify substances harmful to humans or the environment. Pollutant compounds are

converted by organisms through reactions that take place as part of their metabolic processes. Biodegradation of a compound is often a result of the interaction of multiple organism groups. In-situ bioremediation can be used for soil and groundwater by in situ purification. Ex-situ bioremediation techniques are carried out separately and solid-phase tillage processes include soil cultivation, soil biographies and composting. Slurry phase bioremediation is a relatively faster process than other treatment processes. Contaminated soils are combined with a large tank called a bioreactor and mixed with microorganisms in soil that are in contact with contaminants in the soil (Kumar et al., 2011).

2.7 Photosynthetic Bacteria and Nutrient Removal with *Rhodobacter sphaeroides*

In wastewater treatment technologies using Photosynthetic bacteria (PSB) for waste minimization is extensively researched in last decades. *Rhodospirillaceae* (purple non-sulfur bacteria, PNSB), *Chromatiaceae* (purple sulfur bacteria), *Chlorobiaceae* (green sulfur bacteria) and *Chloroflexaceae* (Gliding filamentous green sulfur bacteria) are the members of phototrophic bacteria family (Chen et al. 2019). The new approach of using PSB's which researchers focused on in wastewater treatment is the recovery technologies. PSB's are able to grow both in photoautotrophic and photoheterotrophic (under light conditions) and chemo-heterotrophic (under dark conditions) conditions (Larimer et al., 2004). The high content of protein, high economic valued product ingredients such as coenzyme Q10, polyhydroxyalkanoates, 5-aminolevulinic acid etc., ability to use as animal feed enriches the worth of PSB's. It is been reported that high COD removal efficiencies (90- 95%) can be obtained additionally with high carotenoids, coenzyme Q10, polyhydroxyalkanoates, 5-aminolevulinic acid production. PSB cells have the ability of including high protein content up to 80% giving an advantage to remove high nitrogen concentrations while normally they have a protein content of 40- 60%. It is been revealed that PSB's can remove $\text{NH}_4\text{-N}$ up to 2300 mg/ L without pretreatment saying that this is an important inference while organic carbon sources are not toxic to microorganisms however ammonia and nitrite at high concentrations (for instance

nitrogen concentrations over 1000 mg/ L inhibit methanogens) have toxic effects on microorganisms (Meng et al. 2018 a, Meng et al. 2018 b, Koder et al., 2017, Korosh et al. 2018, Zhong et al. 2018). A review written by Chen et al. (2019) compared traditional wastewater treatment methods with wastewater treatment with PSB's and exposed removal efficiencies for COD as 80- 95% and 13- 99%, TN as 5- 96% and 22- 98.2%, TP as 12- 93% and 64.7- 96.1%, respectively. They also collected data from the literature to point out the importance of adding trace elements such as Fe^{2+} , Mg^{2+} , Co^{2+} , nickel ions (Ni^{2+}), manganese ions (Mn^{2+}), molybdenum ions (Mo^{6+}), copper ions (Cu^{2+}), and zinc ions (Zn^{2+}) to the media to establish the active side of various enzymes or other key metabolites of the species. Furthermore, they mention that some trace elements are responsible for transferring electrons during energy metabolism enhancing the metabolism of PSB's.

Rhodobacter sphaeroides is purple, photosynthetic, gram-negative prokaryotic bacteria that convert light energy to chemical energy by anoxygenic photosynthesis process and can grow autotrophically by containing photosynthetic pigments. It belongs to the purple non-sulfur bacteria group in the α -3 subdivision of proteobacteria (Ceylan, 2012). However, photosynthesis occurs only under anoxic conditions. They participate in the anoxic cycle of carbon, both as primary producers and as light-induced consumers of reduced organic compounds. These bacteria provide oxidation of the sulphide, giving non-toxic sulfur forms such as elemental sulfur and sulphate, ensuring that the upper water layers remain oxygenated and suitable for plants, animals and aerobic bacteria. In the beginning, those which were able to store intracellular storage by tolerating and oxidizing low sulfur levels were identified as purple sulfur bacteria and those that did not contain any type of purple non-sulfur bacteria. However, subsequent studies have shown that many species of purple non-sulfur bacteria are actually highly sulphide tolerant (Madigan & Jung, 2009).

Some purple non-sulfur bacteria, such as *Rhodobacter sphaeroides*, are physiologically versatile, well-developed in both light and dark environments, as they contain growth properties in the dark under phototrophic conditions with CO_2 or

organic carbon or by respiration, fermentation or chemolithotrophy. With only a few exceptions, purple non-sulfur bacteria can make nitrogen fixation ($\text{N}_2 + 8\text{H} \rightarrow 2\text{NH}_3 + \text{H}_2$). *Rhodobacter* species such as *R. capsulatus* and *R. sphaeroides* grow rapidly with N_2 as a nitrogen source and are the best among enrichment cultures for purple nonsulfur bacteria using nitrogen fixation, showing the highest rates of nitrogenase activity in their metabolism. *R. sphaeroides* have true denitrification ability which reduces NO_3^- to N_2 using non-fermentable carbon sources as electron donors (Madigan & Jung, 2009). It is a very diverse organism in terms of its metabolism, known for its ability to grow in a wide variety of environmental and nutritional conditions, as well as aerobic or anaerobic respiration and extensive energy-gaining mechanisms such as photosynthesis or chemosynthesis (Özmeral, 2013). Carbon dioxide is used as the only carbon source under autotrophic growth conditions, and also organic compounds are used as both a carbon source and reductant for photoheterotrophic and chemoheterotrophic growth. The mechanism of nitrogen fixation is very important because it uses molecular nitrogen as a source of organic nitrogen (Özmeral, 2013). They can also synthesize important tetrapyrrols, chlorophyll, hem and vitamin B12 (Ceylan, 2012). The cells of these bacteria are rod-shaped and have intracytoplasmic membranes adjacent and parallel to the cytoplasmic membrane, and budding or polar growth, asymmetric division occurs as the mode of reproduction and allows movement using flagella (Imhoff et al., 1984).

Like other organisms, *R. sphaeroides* has developed different defense mechanisms for the different types of stress they are constantly facing in their environment. The defense mechanisms developed by *R. sphaeroides* for protection may vary depending on the type of stress encountered. Bioremediation of heavy metals is one of their important properties. Su et al. (2020) used *Rhodobacter sphaeroides* SC01 to uptake soluble and particulate forms of heavy metals such as mercury (Hg), lead (Pb) and cadmium (Cd). They were successful of removing Pb with a removal rate of 98% with cell death.

Among the photosynthetic organisms capable of producing hydrogen, it is one of the most active biohydrogen producers. Polyhydroxybutyrate (PHB) is the most

common polyhydroxyalkanoate (PHA) synthesized and deposited by *R. sphaeroides* under both aerobic and anaerobic growth conditions. In environments where nutrients are depleted, PHBs may be degraded by bacteria to obtain energy. It is biodegradable, biocompatible and non-cytotoxic to cells and therefore has great potential in many areas such as pharmacology, transplantology and tissue engineering (Özmeral, 2013).

It is recommended to use photosynthetic organisms in the bioremediation process to remove or recover contaminants from contaminated environments. Like most microorganisms, *R. sphaeroides* has the ability to reduce oxyanions and metals. It is capable of tolerating high concentrations of heavy metals, bioaccumulating cobalt and nickel, and reducing oxyanions which are toxic to other microorganisms. It is highly tolerant to certain heavy metals such as Hg^{+2} , Cu^{+2} , Fe^{+2} , Co^{+2} , Ni^{+2} , especially molybdate (Özmeral, 2013).

Synthesis of vitamin B12, which shares a common pathway with porphyrin, chlorophyll, both and 5-aminolevulinic acid (ALA), can be carried out with *R. sphaeroides* both aerobically and anaerobically. It is one of the most important vitamin B12 producer microorganisms. *R. sphaeroides* can produce coenzyme Q10 twice as much under anaerobic light conditions as in aerobic dark conditions. It is the microorganism in which porphyrin is mostly produced from photosynthetic bacteria (Özmeral, 2013). Carotenoids can be produced and compared with the extraction of carotenoids from other sources; the extraction of carotenoids from *R. sphaeroides* has a commercial value (Gu et al., 2008). There are also studies on their use as microbial fuel cells, but improvements in technology are needed as power density and electrogenic capacity are still too low for industrial uses (Çadırcı, 2018).

Nitrogenase and hydrogenase enzymes are two main types of enzymes used in nutrient consumption. Nitrogenase is the main enzyme that catalyzes the production of hydrogen in purple non-sulfur bacteria. With this enzyme, the free nitrogen in the atmosphere is bound and converted into ammonia and involved in the nitrogen cycle. Nitrogenase enzyme is of three types and each consists of two separate protein units,

dinitrogenase containing Mo-Fe protein, V-Fe protein or Fe-Fe protein and, dinitrogenase reductase containing Fe protein. The function of the reductase protein unit is to carry nitrogenase, which reduces nitrogen to ammonia. It also provides the hydrogen molecule. The conversion of nitrogen to ammonia is a reaction requiring high energy due to the triple bonds of the nitrogen. Nitrogenase activity is inhibited in the presence of oxygen and ammonium or at high N/ C ratios. In the presence of high concentrations of nitrogen, nitrogen-binding metabolism predominates over hydrogen production metabolism and also increases cell growth (Ceylan, 2012). The cell growth enhances nutrient uptake for maintenance.

Ogbonna et al. (2000) studied on treating high strength wastewater by using *R. sphaeroides* along with different species. They found that this species grows better under aerobic dark conditions and the cell growth was not inhibited with 10 000 ppm acetate, 1000 ppm propionate, 700 ppm nitrate, 100 ppm phosphate, 400 ppm of ammonia. The species was able to remove all the nutrients except nitrate.

There are two types of hydrogenase enzymes involved in hydrogen metabolism: reversible hydrogenase and uptake hydrogenase. While reversible hydrogenase catalyzes the formation of hydrogen, membrane-bound hydrogen uptake hydrogenase primarily catalyzes hydrogen dissipation. These enzymes are suppressed or activated according to the redox state of the cells. Hydrogenase is less inhibited by carbon monoxide and oxygen than nitrogenase. The limited amount of Nickel may reduce the synthesis of the hydrogenase enzyme (Ceylan, 2012).



Denitrification has a very important role in wastewater treatment and nitrogen cycle. Accumulation of high amounts of nitrate and nitrite inhibits processes in the nitrogen cycle. For this purpose, Idi et al. (2015) have isolated a denotified phototrophic bacteria to investigate the removal of toxic nitrogen compounds by 16s rRNA characterization (*Rhodobacter sphaeroides* ADZ101). Nitrogen removal performance of the bacteria was investigated at different nitrogen input

concentrations and biokinetic constants were identified by Michaelis-Menten equation. *Rhodobacter sphaeroides* can remove 71% nitrate under any photoheterotrophic growth condition without any nitrite or ammonium build-up, while the input nitrate is 85 mg/ L. In addition, such an input ammonium concentration was 62 mg/ L, while under anoxic growth conditions; it removed 62% ammonium, without the accumulation of nitrite and nitrate. In addition, the results show that the increase in concentrations leads to a reduction of NO_3^- and $\text{NH}_4\text{-N}$ removal (Idi et al., 2015).

Meng et al. (2018 a) used *Rhodopseudomonas* to remove COD ranging between 100- 40,000 mg/ L sugar based wastewater. They tried aerobic dark and anaerobic light conditions at 26- 30 °C with a C/ N/ P ratio of 100/ 5/ 1. They found a similar trend of COD removal in both conditions at low initial COD concentrations; however at high initial COD contents light anaerobic fermentation enhanced COD removal. They found out that escalating COD contents also increase cell growth and cell growth rate. They mentioned that the best COD removal efficiency was obtained with 500 mg/ L initial COD concentration where it was close to aerobic dark conditions. The same group (Meng et al., 2018 b) worked with *Rhodobacter sphaeroides* KD131 at 26- 30 °C at aerobic dark conditions to remove $\text{NH}_4\text{-N}$ with an initial range of 3- 16.000 mg/ L (COD: 500- 40000 mg/ L; PO_4 : 0.6- 3200 mg/ L). They found out that nitrogen can not be degraded when it is over 8000 mg/ L, which was reported in the literature in conventional activated sludge systems. Kantachote et al. (2005) reported a study where latex rubber sheet wastewater was treated with PSB's. They isolated 92 PSB's and grew them in microaerobic light conditions (3000 lux) at a pH of 6.5- 7.5 and 30 °C where the media was dominated by *Rhodopseudomonas blastica*. They treated 54% of COD, 70% of BOD and found a protein content of 66.7% dry weight in the cells which may have a potential to be used as single cell protein. A study in Yemen at waste stabilization ponds having poor BOD removal and odour problems, and including high ammonia concentrations such as 150- 200 mg N- $[\text{NH}^{3+}, \text{NH}^{4+}]$ / L found out that combination of high temperature, high organic loading rates, presence of sulfide favors development of photoheterotrophic purple-non sulphur bacteria *Rhodopseudomonas sp.* in facultative

ponds. They found out that, algal growth is been disfavored, at high ammonia concentrations and where sulfate reduction occurs under photoheterotrophic conditions at low DO and poor C metabolisation causing reddish-pink colour. They also monitored that, to outcompete with purple bacteria at facultative ponds in Yemen, 2025 days are needed to provide ecological conditions allowing algae growth.

The removal of phosphorus compounds from the mud sediments of the oyster field was performed under anaerobic conditions by Takeno et al. (1999) by a series of bioprocesses. Anaerobic acidogenic fermentation of a slurry sediment suspension (200 g wet weight/ L artificial sea water) was initially performed. Acidogenic fermentation was achieved by adding vitamins such as thiamine, nicotinic acid and biotin to give about 2 g/ L acetic acid. In addition, about 20 mg/ L PO_4^{3-} (10% of total phosphorus in sludge weight) and 5300 mg/ L COD_{Cr} (82% organic matter in sludge weight) were left in the culture bath for 7 days after fermentation. The supernatant of this culture extract was used to grow *Rhodobacter sphaeroides* IL 106, denitrified photosynthetic bacteria. At the end of 4 days, 3.32 g/ L biomass containing carotenoid and ubiquinone was obtained, 58% and 72% COD_{Cr} and acetic acid were removed respectively. In addition, reduction of PO_4^{3-} by 97% suggests that removal of PO_4^{3-} from sludge may be possible with *Rhodobacter sphaeroides* (Anaerobic acidogenic fermentation) (Takeno et al., 1999).

According to the study of Takeno et al. (1999), COD, $\text{NH}_4^+\text{-N}$, $\text{NO}_3\text{-N}$, TP and PO_4^{3-} removal efficiencies with *Rhodobacter sphaeroides* IL 106 in a four days cultivation was determined as 58.2%, 69.2%, 97.7%, 97.2%, 97.2%, respectively.

R. sphaeroides exhibit an unusual spectrum of metabolic diversity by allowing the organism to grow under various environmental conditions (Zeilstra-Ryalls & Kaplan, 1995).

Bioremediation with microorganisms promises a technique for soil contaminated with heavy metals. In the study of Li et al. (2016), *R. sphaeroides* was first isolated

from the oilfield injection water and used for bioremediation of soil contaminated with lead. Optimum conditions for remediation of microorganism were investigated and optimum temperature for *R. sphaeroides* was 30- 35 °C, optimum pH 7, optimum inoculation amount was 2×10^8 mg/ L. *R. sphaeroides* did not remove Lead from the soil but changed the properties of the Lead. During the bioremediation process, more suitable fractions are converted to less accessible and inert fractions. In particular, while the residual phase is increased, the convertible phase decreases. Wheat seedling test shows that the lead content of the plant is reduced in the healed soil. The results show that the main mechanism by which *R. sphaeroides* treats soil contaminated with Lead is the formation of precipitation of inert compounds, including Lead Sulphate and Lead Sulphide. *R. sphaeroides* provide an alternative method of Lead remediation in contaminated soil, although the bioremediation efficiency in wheat is not very high (14.78% on the root, 24.01% on the leaf) (Li et al., 2016).

In the study of Yetiş et al. (2000), the pre-treated sugar refinery wastewater (SRWW) was used for Hydrogen production by *Rhodobacter sphaeroides* O.U.001 in a 0.4 L column photo-bioreactor. Hydrogen was produced at a 20% dilution of SRWW at a culture rate of 0.001 L Hydrogen/ h/ L. Sucrose or L-malic acid was added as the Carbon source to adjust the Carbon concentration to 70 mM and the Nitrogen concentration to 2 mM. Sodium Glutamate as Nitrogen source was added to a 20% dilution of SRWW. With these adjustments, the Hydrogen production rate was increased to 0.005 L Hydrogen/ h/ L culture. Continuous Hydrogen production was achieved for 100 days at three different dilution rates in the same medium. The maximum Hydrogen produced was 2.67 L (within 100 days) at a dilution rate of 0.0013 hL (Yetiş et al., 2000).

Under anaerobic light conditions, *R. sphaeroides* produces PHB as the main component (97%) and a small amount (3%) of PHV reported by Brand et al. (1991). The production of PHA from some waste materials, respectively, from sugar refineries and palm oil-based wastewater inspected in the studies of Yiğit et al. (1999) and Ali Hassan et al. (1996). Noparatnaraporn et al. (2001) reported the

production of PHA from acetic acid in *R. sphaeroides* S and *R. sphaeroides* IL 206.

Mukhopadhyay et al. (2005) examined the effect of cultural conditions on the synthesis and accumulation of PHB by *Rhodopseudomonas palustris* SP5212. Khatipov et al. (1999) had studied various carbon and nitrogen substrates combinations by *Rhodobacter sphaeroides* RV deposition of poly- β -hydroxybutyrate and on the evolution of H₂. Also, an attempt was made to obtain PHB from two phototrophic bacteria isolated from tannery wastes and to examine the effect of phosphate restriction on production (Merugu et al., 2010).

2.8 Mechanisms of *R. sphaeroides*

For energy production, *R. sphaeroides* can grow under aerobic respiratory and aerobic chemotrophic conditions, as well as under anaerobic anoxygenic phototrophic conditions using cyclic photophosphorylation. Phototrophic growth requires anaerobic conditions because light reactions can produce destructive oxygen derivatives and also require oxidation and reduction (redox) homeostasis because redox imbalance can stop cyclic photophosphorylation (Krieger-Liszkay, 2004, Ziegelhoffer & Donohue, 2009). Redox sensitivity occurs because reduced electron carriers need to transfer their electrons to oxidized carriers that establish the flow of electrons used for energy (Lavergne et al., 2009, McEwan, 1994). In case of imbalance, the number of carriers decreases, thereby slowing the electron flow of the loop. If this situation does not improve, the cell will no longer generate energy and eventually die (McEwan, 1994, Falcone & Tabita, 1991, Hallenbeck et al., 1990, Zannoni et al., 2009). The cell must maintain redox balance in order for phototrophic growth to occur.

The carbon metabolism of *R. sphaeroides* can occur in two growth modes. In heterotrophic conditions, the cell consumes reduced carbon, especially acidic fermentation end products (Madigan & Jung, 2009). However, for autotrophic growth, the cell must reduce its carbon from carbon dioxide (CO₂) using the Calvin-Benson-Bassham (CBB) reductive pentose phosphate cycle (Tabita, 1988). The CBB

cycle starts from the catalysis of three enzymes and converts CO₂ into cell material. Ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) is one of two unique enzymes of the CBB cycle. It performs the main carbon fixation step by catalyzing the carboxylation of ribulose-1,5-bisphosphate (RuBP) to form two molecules of 3-phosphoglycerate. Phosphoglycerate kinase hydrolyzes ATP after this step to produce 1,3-bisphosphoglycerate from 3-phosphoglycerate. Finally, 3-phosphoglycerate dehydrogenase reduces 1,3-bisphosphoglycerate to form glyceraldehyde-3-phosphate, which leaves the cellular metabolism cycle. To complete the cycle stoichiometrically, for each 1 leaving the cycle remain 5 molecules glyceraldehyde-3-phosphate in the cycle. It then converts the remaining glyceraldehyde-3-phosphates into 3 RuBP molecules, the substrate of RubisCO, by multiple enzymatic reactions. The final stage of formation of RuBP is catalyzed by phosphoribulokinase (PRK), another unique enzyme of this cycle. This pathway is used to supply carbon to the cell. However, it can also function as a fermentative way in energy metabolism to maintain cellular redox homeostasis through the reducing activity of 3-phosphoglycerate dehydrogenase (Tabita, 1995).

Whether the fermentative aspect of the CBB cycle is required for photoheterotrophic growth is determined by the oxidation states of the growth substrates. When the oxidation state of the carbon source is greater than or equal to that of the cell, the need for the CBB cycle depends on how the carbon source is metabolized. For organic acids malate and succinate, the first steps in their metabolism are reductive (NADH) and CO₂-producing oxidative, and in these steps more NADH is produced than consumed later. Thus, *R. sphaeroides* activates the reductive processes of the CBB cycle to oxidize NADH and recycle NAD⁺ and is used as a metabolically derived CO₂ electron acceptor as a result (Laguna et al., 2011). Although the CBB cycle is the only redox balance path naturally expressed as photoheterotrophic, other systems can be induced to alleviate the extra reductant. For example, nitrogen can be expressed under nitrogen depleted conditions.

Nitrogenase is the enzyme complex that catalyzes nitrogen fixation, which is the reduction of nitrogen gas to ammonia, and there are three types of nitrogenases

classified by the metal cofactor in its active part. However, *R. sphaeroides* encode only one copy of the most common type: molybdenum (Mo) nitrogenase (Masepohl & Kranz, 2009). The nitrogenase products, the three genes that make up the nitrogenase structure, are encoded by *nifHDK*. However, more protein is needed for its activity. There are two complex and unique sets of metals that require accessory proteins for assembly and maturation. P set is a set of [8Fe-7S] located in the relay of electrons to the active zone containing iron-molybdenum cofactor (Burgess & Lowe, 1996, Dixon & Kahn, 2004, Seefeldt et al., 2012). A unique feature of Mo-nitrogenases is that Mo can be replaced by tungsten (W). These W-nitrogenases lack the ability to reduce acetylene, a suitable alternative substrate used to measure nitrogenase activity. Such W-nitrogenases still retain limited proton reduction (hydrogenase) activity (Siemann et al., 2003). Even under optimal conditions, hydrogen production is a mandatory product of nitrogen fixation, but may be the only product if nitrogen is not available (Hillmer & Gest, 1977). Both nitrogenase and hydrogenase activities are energetically difficult and require hydrolysis of two ATP molecules per electron sent to the active site; therefore nitrogenase is synthesized only under conditions that do not have an easily assimilated nitrogen source, such as ammonia (Dixon & Kahn, 2004, Masepohl & Kranz, 2009).

To use nitrogenase catalysis to maintain redox balance under nitrogen repletion conditions, normal pressure from ammonia must be overcome. Thanks to its metabolic versatility and excess, *Rhodobacter sphaeroides* can preserve homeostasis like no other organism can (Farmer & Tabita, 2015).

2.9 Objectives and Scopes

A novel approach is developed as an alternative treatment method to conventional treatment technologies for excessive nutrients in wastewater. This novel approach given in this thesis includes uptake of excessive nutrients to a special organism. This special organism is capable of treating nitrogen and phosphorus and was used along with activated sludge for treatment of nutrients. This method can be applied especially to surface waters containing excessive nutrients due to agricultural

applications in certain basins. There are some major advantages of this approach over conventional methods:

- Lower investment cost of treatment plant
- Lower operational costs
- Applicability directly in to the surface water or land that needs to be treated in special conditions.

The major objectives and scopes are summarized as follows:

- To develop an effective and low cost treatment alternative for nutrient removal in surface waters
- To select the operation condition of the selected culture
- To investigate the nitrogen removal performance in low C/ N ratios of the selected culture
- To investigate the nitrogen removal performance in high C/ N ratios of the selected culture
- To investigate the phosphorus removal performance in different C/ P ratios of the selected culture
- To investigate the nitrogen and phosphorus removal performance of a culture conducted with the selected culture and activated sludge
- To investigate the effects of operating parameters (HRT) on nutrient removal in an activated sludge system with the co-culture
- To prove the advantages of using this special organism in biological treatment of polluted surface waters.

The experiments except investigation of operating parameters in an activated sludge system on nutrient removal was carried out with shake flask experiments to understand the nutrient removal performance of the culture and the co-culture.

Treatment of nutrients in the surface waters in basins need low cost solutions. This thesis gives a novel approach which can be applied with low investment and

operational costs in the basins to achieve high quality surface waters polluted with areal pollution sources which are not easy to control.



CHAPTER THREE

MATERIAL AND METHODS

3.1 Experimental Set-Up

The experiments were carried out in two steps. Batch experiments have been studied with pure *Rhodobacter sphaeroides* for selecting the operational conditions, C/ N and C/ P ratio. Then co-culture experiments were carried out in batch mode to determine the best *R. sphaeroides*/ activated sludge co-culture ratio. The chosen co-culture ratio then was conducted to continuous experiments with different hydraulic retention time to determine the best operational conditions giving the highest nutrient removal performance.

Batch experiments were performed in an incubator shaker at 150 rpm and 30 °C, plugged 500 mL erlenmayer flasks were filled with 200 mL synthetic wastewater, and were inoculated with *R. sphaeroides* and in co-culture experiments they were inoculated with *R. sphaeroides* and activated sludge culture. pH was adjusted to 7 with 1 M NaOH solution. In the first experiments four different ambient conditions (aerobic-light, aerobic-dark, anaerobic-light, and anaerobic-dark) were tried. The light source used in light conditions was applied under fluorescent lamp LTD36W/54-270 W/ m² light intensity with a reaction time of 168 hours. Then different COD/ TN (100/ 0- 100/ 25) and COD/ TP (100/ 0.5- 100/ 2) ratios were tried with the chosen environmental condition subjected in the first experiment. After verifying the ratios of COD/ TN and COD/ TP with the wastewater simulated from Porsuk River a co-culture of activated sludge and *R. sphaeroides* was subjected to the model wastewater with ratios of 1/ 1 to 1/ 20 (AS/ RS). In these experiments also pure culture of *R. sphaeroides* and activated sludge was tested as positive controls along the experiment.

Continuous experiments were conducted in laboratory scale activated sludge unit. The experimental set-up consisted of a wastewater tank including the wastewater taken from Porsuk River, feed wastewater pump, activated sludge reactor, blower and an effluent tank. The continuous experimental set-up is depicted in Figure 3.1. The

reactors were made of plastic, with 16x16x10 cm dimensions having 1 L total liquid volume. The activated sludge system was divided into two zones with a plastic plate including holes to let the upflow through the plate. The plate separates the aeration tank from the sedimentation tank. The aeration and sedimentation tanks were 0.875 L and 0.125 L, respectively. Small diffusers were used along with the blower for aeration.



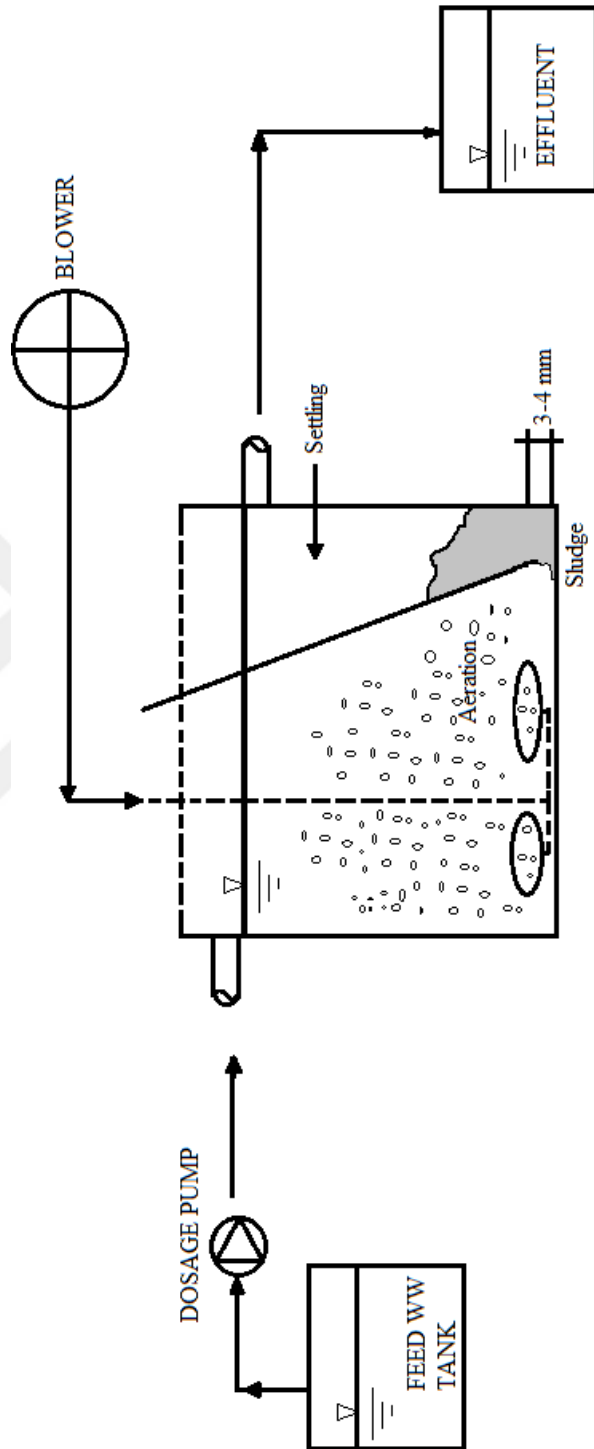


Figure 3.1 Schematic diagram of the activated sludge unit used in the experimental studies

3.2 Wastewater

In the batch experiments the inoculum cultures were either only *Rhodobacter sphaeroides* or *Rhodobacter sphaeroides* with activated culture. Depending on the experiments synthetic *R. sphaeroides* growth medium or wastewater taken from Porsuk River (Sevinç Village) was simulated as C, N and P source.

3.2.1 Synthetic Wastewater Composition for Batch Experiments

The nutrient media contained Glucose as Carbon source. In the first experiment the initial COD concentration was chosen 10000 mg/ L to select the best conditions. The conditions were chosen to be a strength wastewater to also remove industrial discharges that might affect surface waters. In the other batch experiments 1000 mg COD/ L was used. This concentration was chosen because the real wastewater taken from Porsuk River included 1000 mg/ L COD. In the experiments, 0.05 g/ L MgSO_4 was added to the media. Synthetic wastewater composition contained also $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.02 g/ L), $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (0.01 g/ L). In total volume of 200 mL for each Erlenmeyer flask, 20 mL inoculum was added in 180 mL media for 10% ratio.

1 g/ L KH_2PO_4 was used as P source and 0.195 g/ L NH_4Cl was for N source at the first experiment. After the best operational condition determination, N source was changed to urea.

In the experiment with low TN/ COD ratios, where COD removal efficiencies of *R. sphaeroides* were investigated, the TN/ COD ratios were 100/ 0, 100/ 1, 100/ 2, 100/ 4, 100/ 6, 100/ 8 and the NH_4Cl concentration varied between 0- 3.3 g/ L. 2 g/ L KH_2PO_4 was added as Phosphorus source in this experiment.

In the experiments, where nutrient removals with different TN/ COD ratios (100/ 5, 100/ 10, 100/ 15, 100/ 20, 100/ 23, 100/ 25) were investigated, the urea concentration varied between 0.11- 0.55 g/ L. The P ratio was kept constant at 1.4 and KH_2PO_4 was added (0.061 g/ L) into the media.

In the experiments where nutrient removals with different TP/ COD ratios (100/ 0.5, 100/ 0.8, 100/ 1, 100/ 1.4, 100/ 2) were investigated, the KH_2PO_4 concentration varied between 0.022- 0.088 g/ L. The N ratio was kept constant at 20 and urea was added (0.44 g/ L) into the media.

In the co-culture experiments the COD/ N/ P ratio was kept at 100/ 23/ 1.4. Glucose (0.93 g/ L) urea (0.51 g/ L), and KH_2PO_4 (0.061 g/ L) was used as macro nutrients, and the other micro nutrients mentioned above were added.

3.2.2 Wastewater used in Continuous Experiments

Real wastewater taken from Porsuk River was used as influent of the activated sludge system. The wastewater was taken from Sevinç village, Eskişehir. The location where the wastewater was taken from has latitude of $39^\circ46'52.95''$ N and longitude of $30^\circ41'36.64''$ E. This place was chosen because the village has many activities of livestock farming and the water is discharged to the channel passing through the village. The channel passing through the village is a branch connected to Porsuk River. This sampling point was chosen cause of the open sewage characteristic of this channel. The extreme conditions of this channel will serve as the maximum nutrient levels that can be found through the river. The characteristic of the surface water was analyzed and given in Table 3.1. To support the cultures used in continuous activated sludge system 0.05 g/ L MgSO_4 , 0.02 g/ L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g/ L $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ was added into the influent.



Figure 3.2 Sampled Wastewater Coordinates on GoogleEarth Taken From Sevinç Village

One set of experiments with different hydraulic retention times was carried out with a co-culture of *R. sphaeroides*/ activated sludge. In order to avoid any contamination while feeding the reactor the wastewater was autoclaved at 121 °C for 15 min. The feed wastewater was preserved at 4 °C in the refrigerator. The pH of the wastewater was set to pH 7- 7.5 by adding NaOH or H₂SO₄ when necessary.

Table 3.1 Characteristic of sampled wastewater taken from Porsuk River, Sevinç Village

Parameter	Value
pH	6.85
ORP (mV)	408.4
Dissolved Oxygen (mg/ L)	4.51
Electrical Conductivity (µS/ cm)	580
Turbidity (Ntu)	44.35
Total Solid Matter (g/ L)	1.4
Suspended Solid Matter (g/ L)	1.2
COD (mg/ L)	1080
TOC (mg/ L)	287.9
Total Phosphorus (mg/ L)	13.51
Total Nitrogen (mg/ L)	220
TKN (mg/ L)	145.6
NH ₄ ⁺ -N (mg/ L)	57.2
NO ₃ ⁻ -N (mg/ L)	0.195
NO ₂ ⁻ -N (mg/ L)	1.859

3.3 Organisms

A pure culture of *Rhodobacter sphaeroides* (DSM-158) was obtained from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, GERMANY. Pure culture of *R. sphaeroides* was cultivated in the laboratory in growth conditions by using an incubator shaker at 150 rpm and 30 °C. Cultivation pH was kept between 6.8 and 7.1. Chemicals added for growth media were glucose (0.93 g/ L), KH₂PO₄ (0.061 g/ L), NH₄Cl (0.3 g/ L) or urea (0.44 g/ L), MgSO₄ (0.05 g/ L), FeSO₄.7H₂O (0.02 g/ L), Na₂MoO₄.2H₂O (0.01 g/ L).

Culture of activated (aerobic) sludge was obtained from wastewater treatment plant of PAKMAYA Baker's Yeast Company, İzmir, Turkey. The sludge was

cultivated in the laboratory in growth conditions by using an incubator shaker at 150 rpm and 30 °C. Cultivation pH was kept between 7.0 and 7.5. Chemicals added for growth media were glucose (1 g/ L), peptone (0.15 g / L), KH₂PO₄ (0.065 g/ L), urea (0.174 g/ L), MgSO₄ (0.01 g/ L).

Both cultures were checked by using microscope for investigating their growth and activeness before inoculation into the experimental flasks. The remaining cultures were preserved and stocked by freezing.

3.4 Experimental Procedure

3.4.1 Experiments with Erlenmeyer Flasks

In the first set of experiments pure culture of *R. sphaeroides* was conducted to four different growth conditions. The growth conditions were; aerobic/ anaerobic and dark/ light; checked for their COD removal performances. The aerobic erlenmayer flasks were cotton plugged while the anaerobic erlenmayer flask were plugged with rubber stoppers. Fluorescent lamps (LTD 36W/54-270 W/ m²) were used when light was applied to erlenmayer flasks.

After the growth condition of the pure culture was chosen *R. sphaeroides* was conducted to different COD/ N (100/ 0, 100/ 1, 100/ 2, 100/ 4, 100/ 6, 100/ 8, 100/ 5, 100/ 10, 100/ 15, 100/ 20, 100/ 23, 100/ 25) and COD/ P (100/ 0.5, 100/ 0.8, 100/ 1, 100/ 1.4, 100/ 2) ratios.

After investigation of COD/ N and COD/ P ratios *Rhodobacter sphaeroides* was mixed with activated culture in appropriate ratios to compose a co-culture. For this purpose, activated sludge/ *R. sphaeroides* co-culture ratios were chosen as 1/ 1, 1/ 2, 1/ 5, 1/ 10, 1/ 20. The suspended solid matter of the activated sludge and *R. sphaeroides* were 7 g/ L and 3 g/ L, respectively. Also two flasks containing pure cultures of *R. sphaeroides* and activated sludge were added along with the experiments to investigate the nutrient removal performances of the cultures alone.

All of the experiments included control flasks with no inoculation. In total volume of 200 mL for each flask, 20 mL inoculum was added to the flasks.

3.4.2 Experiments with Activated Sludge System

Continuous experiments started with inoculation of the appropriate co-culture ratio of activated sludge/ *R.sphaeroides* in the reactor. They were grown on shake flasks. Each experiment lasted for five days. Different hydraulic retention times (2-14 h) were tried along with the co-culture. The system reached to steady state conditions within several days and samples were taken at last three days after the steady state conditions were conducted. Small diffusers were located at the bottom of the aeration tank to obtain agitation and aeration in the activated sludge tank.

3.5 Sampling

The samples taken from each flask once a day were 15 mL in sterilized conditions and centrifuged at 7000 rpm for 15 min. to remove the organisms and to clear supernatant and kept with small amount of diluted sulphuric acid to preserved in the refrigerator for analysis. pH, Oxygen Reduction Potential (ORP) and Dissolved Oxygen (DO) was measured by probes. COD, TN, NH₄-N, NO₃-N, PO₄-P analysis were carried out with the supernatant.

In continuous experiments, the system was operated until steady state conditions were reached and the samples were collected at the steady state. Samples were taken once a day and centrifuged at 7000 rpm for 15 min. The supernatant was preserved in the refrigerator for COD, TN, NH₄-N, NO₃-N, and PO₄-P. The pH, ORP, DO was analyzed everyday and the pH was adjusted to around 7 if needed with NaOH or H₂SO₄.

3.6 Analytical Methods

COD, TN, TP, NO₃-N and NH₄-N analysis were carried out on clear supernant after centrifugation. Suspended solids were taken while sampling and measured according to procedure.

3.6.1 Chemical Oxygen Demand (COD) Analysis

COD measurements were carried out daily according to Standard Methods by using closed reflux colorimetric method. In this method, borosilicate culture tubes with 10 mL capacity were used for reaction process. Absorbances of the samples were measured on a spectrophotometer wavelength at 600 nm. Digestion solution was prepared by adding dried 10.22 g K₂Cr₂O₇ and 167 mL concentrated H₂SO₄ into distilled water in the volume of 1 L and cooled until room temperature reached. Suphuric acid solution was prepared by addition of 5.5 g Ag₂SO₄/ kgH₂SO₄ to H₂SO₄ and stands for 2 days to be dissolved. Potassium Hydrogen Phtalate (KHP) standard was dried at 120 °C for half an hour and cooled and used for preparation of calibration curve. Different initial KHP concentrations were dissolved in distilled water for different COD concentrations and the theoretical COD of KHP is 900 mg/ L for 0.765 g KHP. For determination of COD concentrations of the samples, KHP standards prepared to obtain COD concentrations of 50 to 900 mg COD/ L. The calibration equition was obtained by regression of the samples (Greenberg, 1989).

3.6.2 Total Nitrogen Analysis

Nitrogen compounds are transformed into Nitrate by treatment with an oxidizing agent in a thermoreactor. This Nitrate reacts with a benzoic acid derivative which is Nitrospectral to form a red-purple nitro compound that can be determined by photometer. 517 nm of spectrum is used for absorbance measurement and the range was between 0.5 and 15 mg/ L N. After the pretreatment, the 10 mL diluted sample is taken and 1 microspoon of Reagent N-1K is added. 6 drops of the Reagent N-2K is added and the cell is shaken. For 1 hour the cell is heated at 120 °C and after heating

cooled until room temperature is reached. After reaching, the cell is waited for 10 minutes and shaken briefly. 1 microspoon of the Reagent N-3K is added to measurement cell and for 1 minute it is shaken. 1.5 mL sample is taken from the pretreated cell and added to measurement cell slowly. The measurement cell is closed tight and shaken. After 10 minutes reaction time and cooling the absorbance value was read from the photometer (Merck Spectroquant[®] Nitrogen (total) Cell Test Cat. No: 1.14537.0001).

3.6.3 Total Phosphorus Analysis

Photometer is used for absorbance measurement and the range was between 1.0 and 30 mg/ L P. After the pretreatment, the 5 mL diluted sample is taken and 1.2 mL Reagent PO₄-1 is added. The absorbance value was read from the photometer after shaking (Merck Spectroquant[®] – Phosphate Test Cat. No: 1.14842.0001).

3.6.4 Nitrate Nitrogen Analysis

The absorbances were measured and the range was between 0.5 and 18 mg/ L NO₃-N. 1 microspoon of the Reagent NO₃-1K is added in the measurement cell, and the cell is closed tight and shaken. After the pretreatment, the 1.5 mL diluted sample is taken and added into the measurement cell and the cell is closed tight. After shaking and waiting for 10 minutes, the absorbance value was read from the photometer (Merck Spectroquant[®] – Nitrate Cell Test Cat. No: 1.14542.0001).

3.6.5 Ammonium Nitrogen Analysis

The range from absorbance measurement was between 0.05 and 3 mg/ L NH₄-N. After the pretreatment, the 5 mL diluted sample is taken and added to the test cell. 0.60 mL of the Reagent NH₄-1 and 1 microspoon of the Reagent NH₄-2 is added in the test cell, and the cell is closed tight and shaken until the solution becomes homogeneous. After waiting for 5 minutes until the reaction finished, 4 drops of the Reagent NH₄-3K added into the test cell. After waiting for 5 minutes until the

reaction finished, the absorbance value was read from the photometer (Merck Spectroquant® – Ammonium Test Cat. No: 1.14752.0001).

3.6.6 Suspended Solid Matter Measurement

By filtering the samples through milipore filters (45 µm) and drying until constant weight at 105 °C, biomass concentrations were measured and calculated by using following equation (Greenberg, 1989);

$$M_x = (S_s - S_0) * 1000 / V \quad (3.1)$$

where, M is the concentration of the sample and the 'x' indice is the number of the used sample, S_s is the weight of filter with residue after drying, S_0 is the weight of filter after drying and V is the mL volume of the sample taken.

CHAPTER FOUR

RESULTS AND DISCUSSION

Nutrient removal was aimed with *Rhodobacter sphaeroides* in wastewater. The ability of this species growing on different environmental conditions favors the use of this organism. The adaptability of the culture to different nutrient concentrations is an advantage and adding activated sludge along with this culture can enhance nutrient uptake in waters which will be a low cost novel approach for nutrient rich wastewaters.

4.1 Batch Shake Flask Experiments

4.1.1 Determination of Environmental Conditions with *R. sphaeroides* maximizing COD removal

In these experiments, the optimal environmental conditions maximizing biomass growth and nutrient removal with *R. sphaeroides* was determined. COD removal efficiencies were investigated under four different environmental conditions as aerobic light, aerobic dark, anaerobic light and anaerobic dark. Light ambient conditions were applied under fluorescent lamp LTD36W/54-270 W/ m² light intensity in an incubator shaker at 30 °C, 150 rpm for 168 hours. For this purpose, biomass, ORP, pH, and COD under aerobic-light, aerobic-dark, anaerobic-light and anaerobic-dark environment conditions were analyzed and discussed.

Figure 4.1 depict the pH variations with time under different ambient conditions. The results showed a pH decrease at the first 24 hours under all four ambient conditions. The pH was adjusted to about 6.8 at regular intervals with NaOH. Under aerobic-light conditions, *R. sphaeroides* decreased to 5.8 levels in the first 24 hours and was around 6.3 till the end of the incubation time. In aerobic dark conditions, it was observed the pH tended to decrease in the first 48 hours and reached to 5.34. After 48 hours, the pH increased and reached to 6.5 hours at 120 hours, but it decreased to 5.8 at 168 hours. In anaerobic light conditions with *R. sphaeroides*, the pH decreased to 4.8 in the first 48 hours. Afterwards, it increased to 6.1 levels and

decreased to 4.7 at the end of the incubation time. Under anaerobic-dark conditions, the culture reached from pH 6 to pH 5.65 in 48 hours and increased in time to 6.42. However at the end of the incubation time the pH decreased again to 5.9.

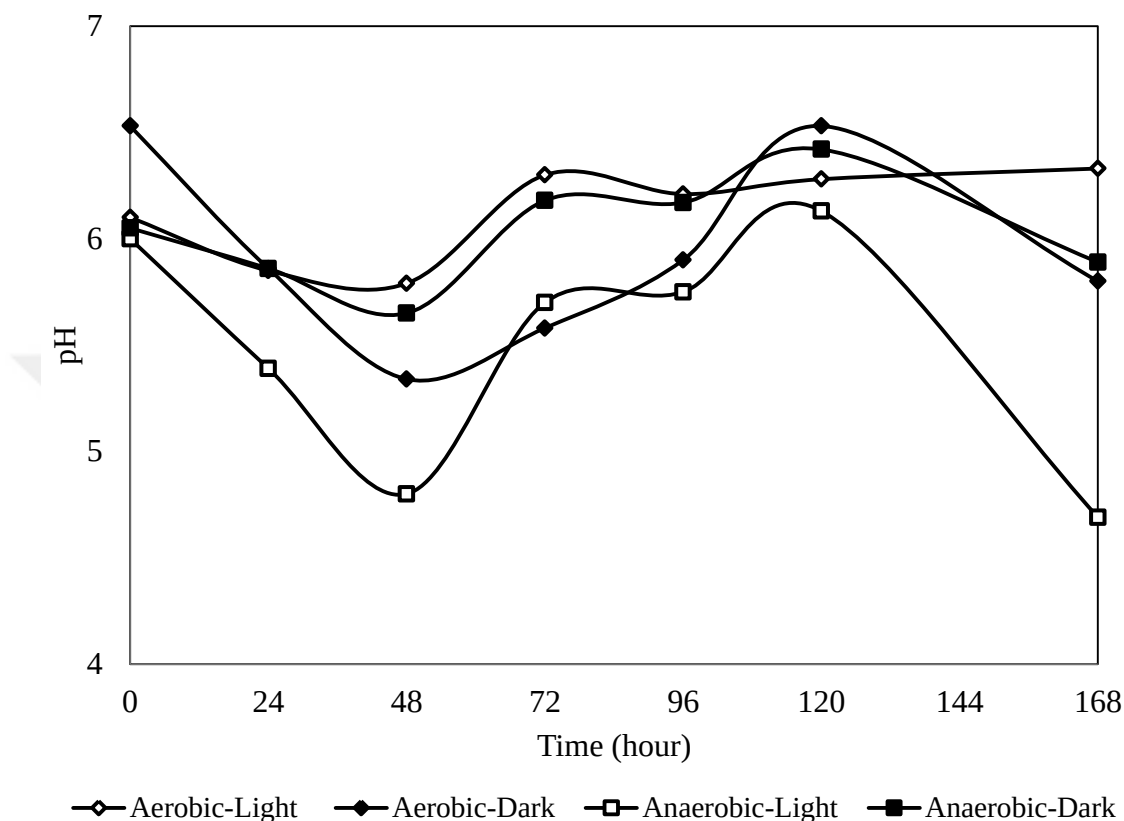


Figure 4.1 Variation of pH with time in different ambient conditions

Oxidation-reduction potential (ORP) measurements were performed for each ambient condition. Figure 4.2 shows the ORP variations with time under different environmental conditions of the microorganism. As a result of the measurements, it was observed that anaerobic conditions were close to 0 and aerobic conditions were higher than 150 mV. In aerobic light conditions, ORP, which was around 200 mV at the beginning of the experiment, decreased during the experiment and was measured as 155 mV at the end of the test. However, although it tends to decrease, it is understood that aerobic conditions are provided in the experimental environment. In aerobic dark conditions, it was observed that the ORP was around 150- 170 mV during aerobic-dark conditions and aerobic conditions were provided. In anaerobic-light conditions; the ORP was around 130 mV at the beginning of the experiment and

it decreased in time -50mV. In anaerobic-dark conditions; initial ORP was around 86 mV, and decreased after a while to around zero. Meng et al. (2019) investigated the effects of dissolved oxygen on enzymes of PSB in wastewater treatment. They demonstrated that oxygenation have an impact over PSB metabolism. They found out that under anerobic conditions hexokinase (might simultaneously regulate PSB growth and COD degradation), and photosynthetic enzyme RuBisCO (might fuction in PSB growth) and under aerobic conditions phosphofructokinase, and pyruvate kinase (both might be effective in COD degradation) are dominating the metabolism. They revealed that oxygen is beneficial for COD degradation and PSB growth. The same group in another study (Meng et al., 2018a) says that while strenght wastewaters have better removal efficiency in anaerobic conditions and wastewaters with 0- 500 mg/ L COD need aerobic environmental conditions to treat the wastewater effectively.

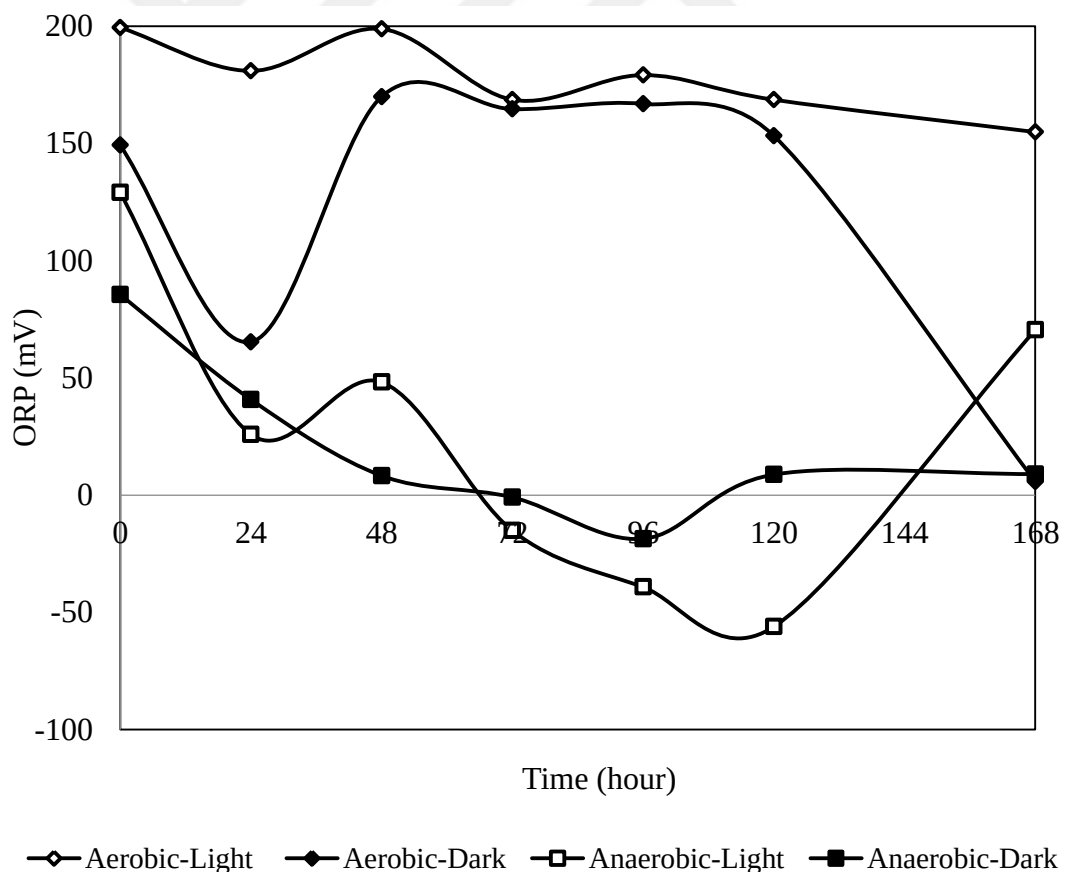


Figure 4.2 Variation of ORP with time in different ambient conditions

In Figure 4.3, COD variations with time in different environmental conditions of microorganisms are given. The initial COD of this experiment was chosen high to understand the behavior of the organism under strength wastewater conditions. In aerobic-light conditions 23 g/ L COD remained nearly constant in the incubation time. In aerobic-dark conditions, COD values decreased between 18- 20 g/ L during the incubation period and decreased to 6.5 g/ L at the end of the experiment. The COD removal efficiency obtained under these conditions was determined as 67.35%. In anaerobic light conditions, it is seen that COD tends to decrease at the first 48 hours and then increases again and reaches 21 g/ L. In anaerobic-dark conditions, the COD was around 20 g/ L where it remained nearly constant till the end of the incubation time. These results are quite different from Meng et al. (2018 a). They found a COD removal efficiency of nearly 20% (72 h) with an initial COD of 15000-25000 mg/ L where in or study the removal efficiency in the same range of initial COD was 67%. This might be because of the difference of the incubation time.

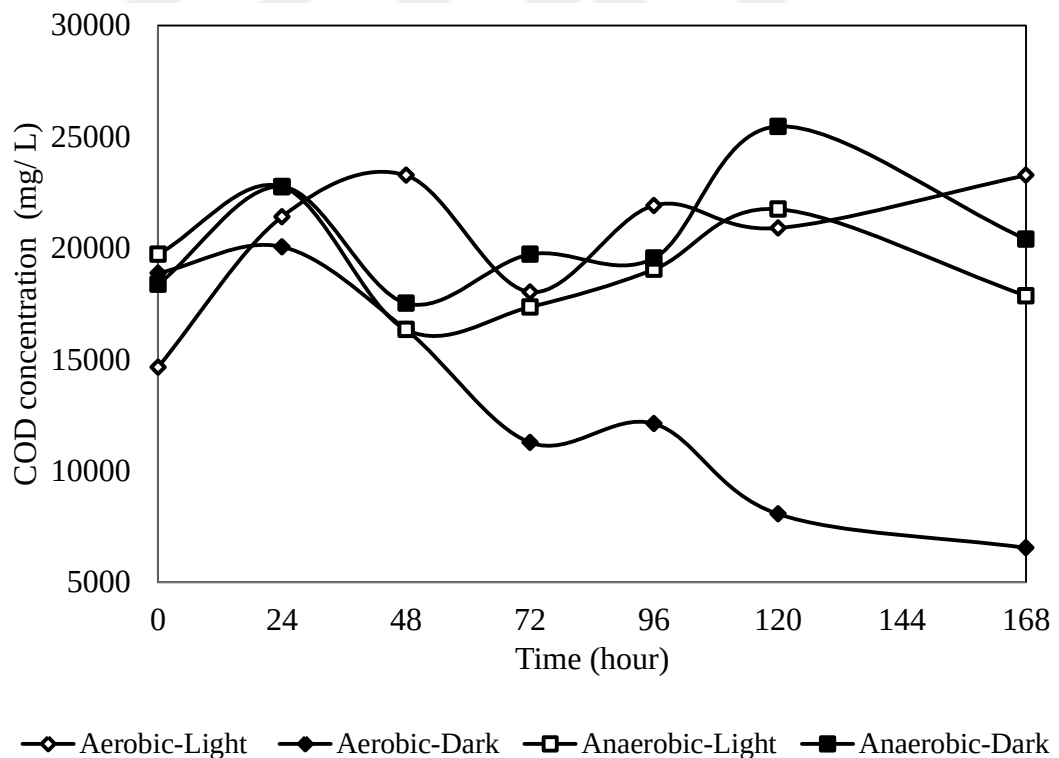


Figure 4.3 Variation of COD concentrations with time in different ambient conditions

Glucose as the sole carbon source in the experimental media was analyzed to understand the mechanism of the organism in different environmental conditions. In all conditions, the glucose concentration decreased. It is observed that the glucose concentration of 20 g/ L has decreased to 5- 6.7 g/ L in the first 24 hours. This shows that culture can maintain its vital functions by using the substrate that is present in all ambient conditions. Fig. 4.4 depicts the variation of glucose concentration with time. In aerobic light conditions, glucose decreased with time, the initial glucose concentration of 20 g/ L was reduced to 5.9 g/ L at the end of the experiment (70.5% glucose removal efficiency). *R. sphaeroides* is adapted well to aerobic light conditions, resulting biopolymers as products after the incubation period effecting COD results (Pattanamanee et al., 2012). In aerobic dark conditions, all the glucose was used for maintenance of the organism (100% glucose consumption). The adaptation of the culture to these ambient conditions is a sign that it will have the capacity to efficiently remove the desired nutrients in the wastewater via biomass uptake (Nath et al., 2005). The glucose concentration in anaerobic-light conditions decreased with time and was 3.2 g/ L in 168 hours. It is understood that the culture, which consumes approximately 84% glucose, can adapt to the environment but does not provide the desired level of COD removal due to the switch of the pathway of the organism to hydrogen production from organic acids in the fermentation media (Yetiş et al., 2000). In anaerobic-dark conditions the glucose concentrations also decreased in time and reached to 6 g/ L at the end of the incubation time.

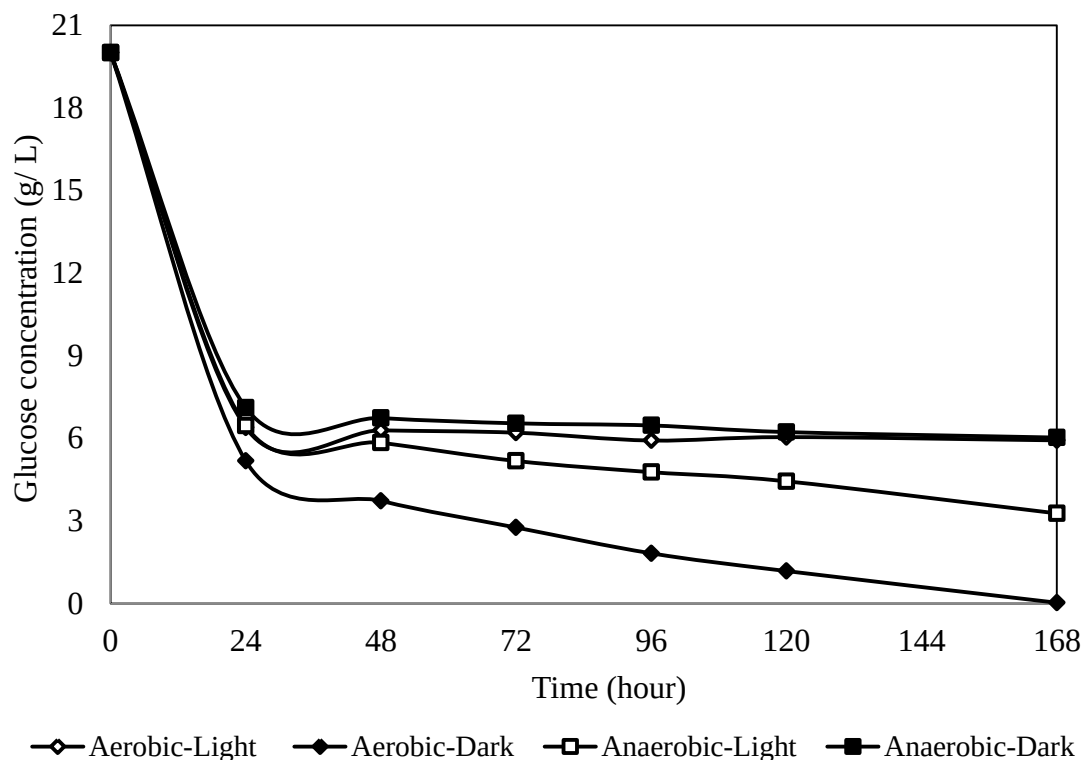


Figure 4.4 Variation of Glucose concentrations with time in different ambient conditions

The concentrations of acetic acid formed in different environmental conditions were shown in Figure 4.5. In aerobic-light conditions the acetic acid was produced in the early stages while sufficient aerobic conditions were not achieved as seen in ORP variations. In aerobic-dark conditions, it was observed that up to 72 hours acetic acid production occurred (1820 mg/ L) and after that time the culture consumed the produced acetic acid. Under anaerobic light conditions, about 500 mg/ L acetic acid was produced. Under anaerobic dark conditions, the acetic acid concentrations produced remained low at around 280 mg/ L.

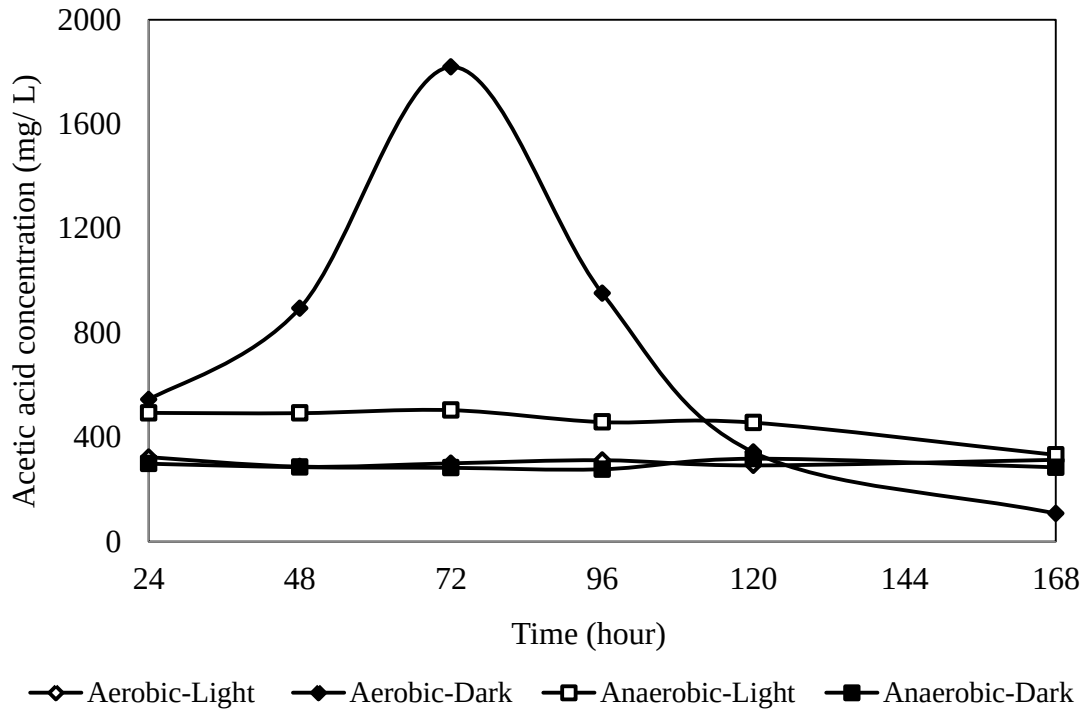


Figure 4.5 Variation of Acetic acid concentrations with time in different ambient conditions

Aerobic dark conditions gave the best COD and glucose removal, where the biomass was grown well and the nutrient uptake can be maximized.

4.1.2 Effects of initial COD/ TN Ratios

4.1.2.1 Effects of initial COD/ TN ratios on COD removal ranging between 100/ 0 and 100/ 8

In this study, the growth of *Rhodobacter sphaeroides* with different COD/ TN ratios under aerobic dark conditions was investigated for COD removal. In the first set of COD/ TN ratio experiments 100/ 0, 100/ 1, 100/ 2, 100/ 4, 100/ 6 and 100/ 8 ratios were tested. The initial COD was kept high to mimic a strength industrial wastewater discharge. The behavior of the organism under these conditions will give an important overview for applications to different surface water characteristics. The nitrogen fixing capability under these conditions will also be investigated along with these COD/ TN ratios.

Figure 4.6 depicts the variation of COD concentrations at different COD/ TN ratios ranging between 100/ 0 to 100/ 8. The strength wastewater containing around 13- 15 g/ L COD at the beginning of the incubation period was removed at the end in all COD/ TN ratios. In 264 hours 1.5- 0.2 g/ L COD remained in all COD/ TN ratios that were tested. COD/ N/ P ratio needs to be 100/ 6/ 2 for in a nutritionally balanced wastewater. *Rhodobacter sphaeroides* was able to fix nitrogen from the environment in low COD/ TN ratios to remove carbon substances from the media. The COD removal efficiencies with 100/ 0, 100/ 1, 100/ 2, 100/ 4 were 93%, 97%, 98%, 94%, respectively. Also the culture adapted well to higher COD/ TN ratios and degraded the carbon sources for its maintenance. The COD removal efficiencies of 100/ 6 and 100/ 8 were 96% and 88%. There was nitrogen deficiency in the medium. RS was successful to adapt to both severe nitrogen limitations and nitrogen source concentrated conditions.

Figure 4.7 shows the pH variation with time at different COD/ TN ratios. In all ratios the pH dropped to around 5.4 ± 0.1 at the first 24 hours where ammonia may be consumed caused the pH drops in high COD/ TN ratios. The pH was adjusted daily to obtain a pH of 7 everyday via adding NaOH or H₂SO₄ into the media. The pH increased in time and reached to 8.7 in all bottles, and then fluctuated with different COD/ TN ratios. The pH increased in time with increasing COD/ TN ratios. At the end of the incubation time the pH ranged between, 7- 8 in different COD/ TN ratios.

Figure 4.8 depicts the ORP data in time with different COD/ TN ratios. In the first 144 hours the ORP was over zero however low at COD/ TN ratios of 100/ 0, 100/ 1, 100/ 4, 100/ 8. In COD/ TN ratios of 100/ 2 and 100/ 6 the ORP was low at first 72 hours and increased after this time. The ORP increased after 144th hour to approximately 100- 130 mV in all experimental bottles. The low pH in the first 24 hours was due to the switch of the metabolic pathway to anaerobic conditions which is verified with ORP data. However after some time ORP reached over 100 mV obtaining aerobic conditions and increasing the pH. The ORP in all bottles ranged between 100- 160 mV at the end of the incubation time.

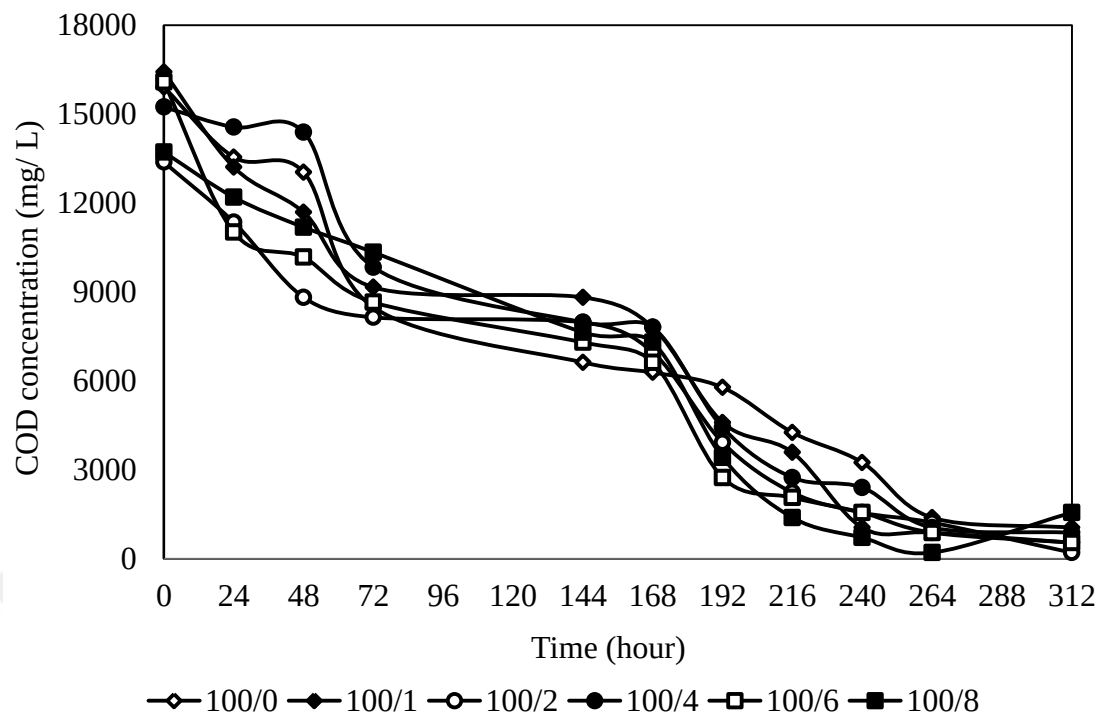


Figure 4.6 Variation of COD concentrations at different COD/ TN ratios with time

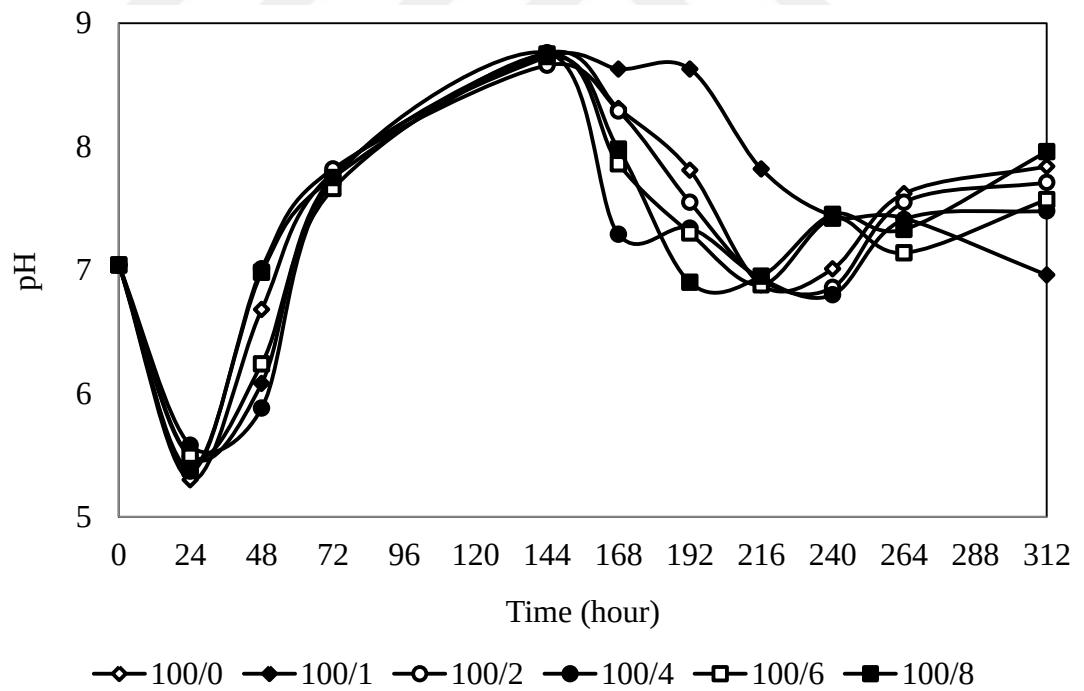


Figure 4.7 Variation of pH with time in different COD/ TN ratios

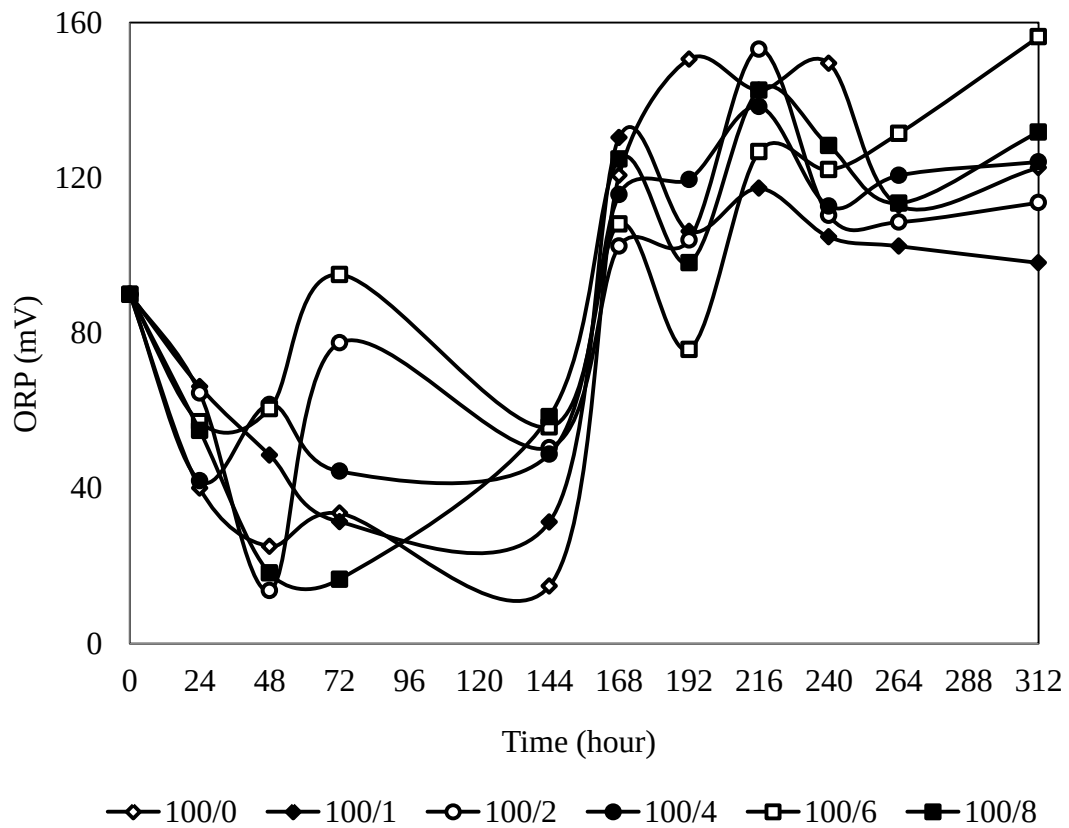


Figure 4.8 Variation of ORP with time at different COD/ TN ratios

4.1.2.2 Effects of initial COD/ TN ratios on COD removal ranging between 100/ 5 and 100/ 25

In this set of experiments, the growth of *Rhodobacter sphaeroides* with higher COD/ TN ratios, having excessive nitrogen contents, under aerobic dark conditions were investigated for COD removal. In the second set of COD/ TN ratio experiments; 100/ 5, 100/ 10, 100/ 15, 100/ 20, 100/ 23 and 100/ 25; COD/ TN ratios were tested. The initial COD was kept high (approximately 14 ± 0.4 g/ L COD) to mimic a strength wastewater discharge. The behavior of the culture under excessive carbon and nitrogen concentrations will outline the nutrient uptake performance of the organism. The culture with a 10% inoculation to 150 mL working volume and initial pH of 7 was incubated for 96 hours in an incubator shaker at 30 °C and 150 rpm.

Figure 4.9 gives the COD concentration data with time at different COD/ TN ratios. The COD concentrations decreased in time in all tested bottles. However the COD removal efficiency was not satisfying with *Rhodobacter sphaeroides* alone. The organism gave the highest COD removal efficiency with the COD/ TN ratio of 100/ 23 as 42%. COD/ TN ratios of 100/ 5, 100/ 15, 100/ 20 gave a COD removal efficiency of 27 to 29% while 100/ 10 and 100/ 25 were 41.6%. The low COD removal efficiencies are because of high substrate concentrations causing substrate inhibition.

Figure 4.10 depicts the variation of pH with time at different COD/ TN ratios. The pH dropped in the first 24 hours to around 5.8 in all bottles except 100/ 25. The pH in all tested ratios increased after 24 hours and was around 8 at the end of the incubation period. Figure 4.11 shows the variation of ORP data with time and Figure 4.12 represents the variation of dissolved oxygen concentrations (DO) versus time at different COD/ TN ratios. The reaction in the bottles started with 150 mV in all bottles giving a DO of 8.31 mg/ L. However the ORP dropped under zero after sometime and varied between 120- 224 mV in the bottles which resulted as a nearly zero DO concentration. In a strength wastewater with high COD/ TN ratios the incubation turned to anaerobic conditions affecting the metabolic pathway of the pure culture. It is understood that the growth of the organism was not enough to uptake the nutritional ingredients in the media. The ORP rose above zero after 72 hours however it was not enough to obtain aerobic conditions which were also verified with the DO concentration that was nearly zero at the end of the incubation period. The pH increases which are sycronize with ORP drops may refer to nitrate uptake while previously they were produced in the first hours of the incubation period causing drop of pH at aerobic conditions.

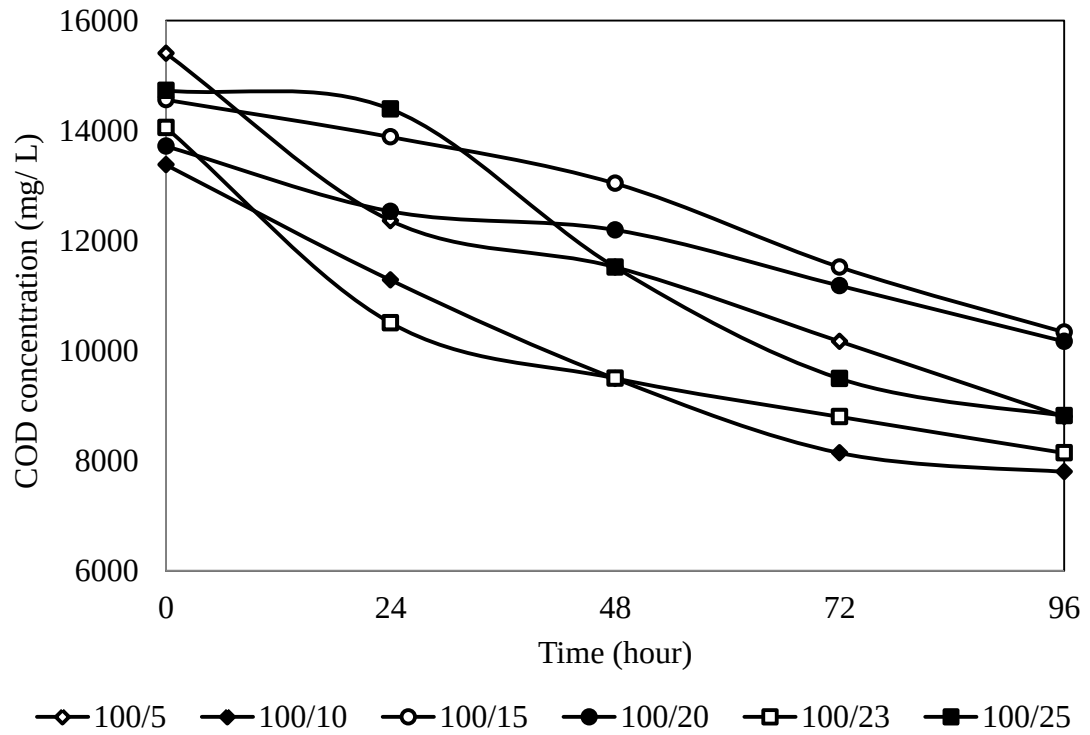


Figure 4.9 Variation of COD with time at different COD/ TN ratios

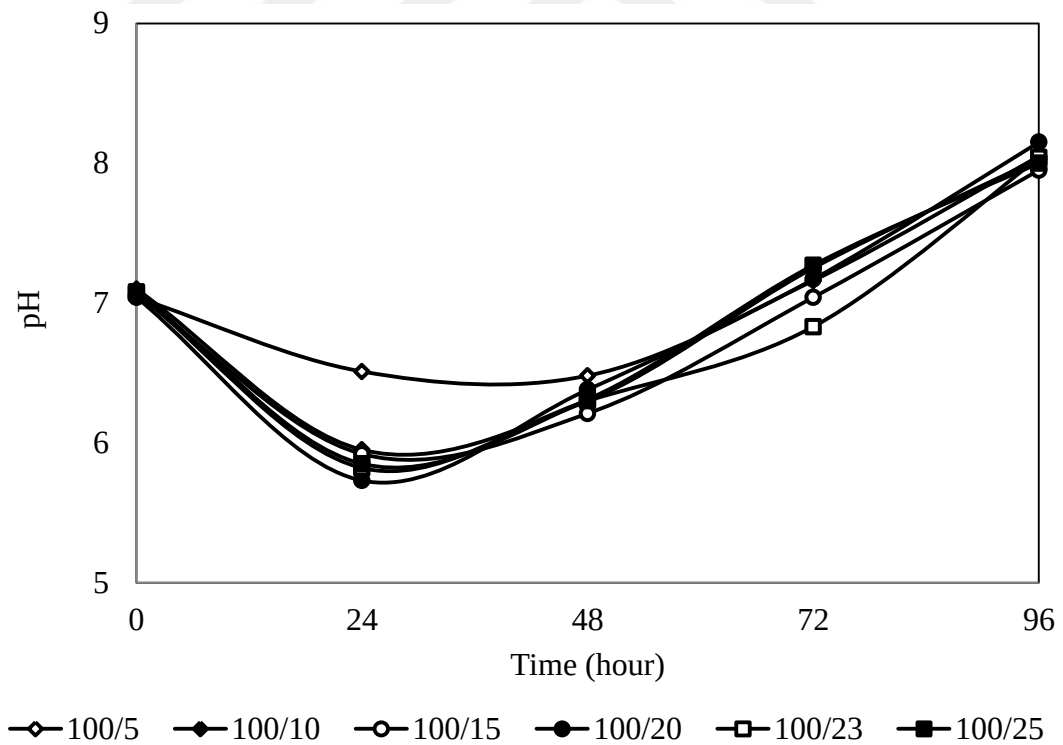


Figure 4.10 Variation of pH with time at different COD/ TN ratios

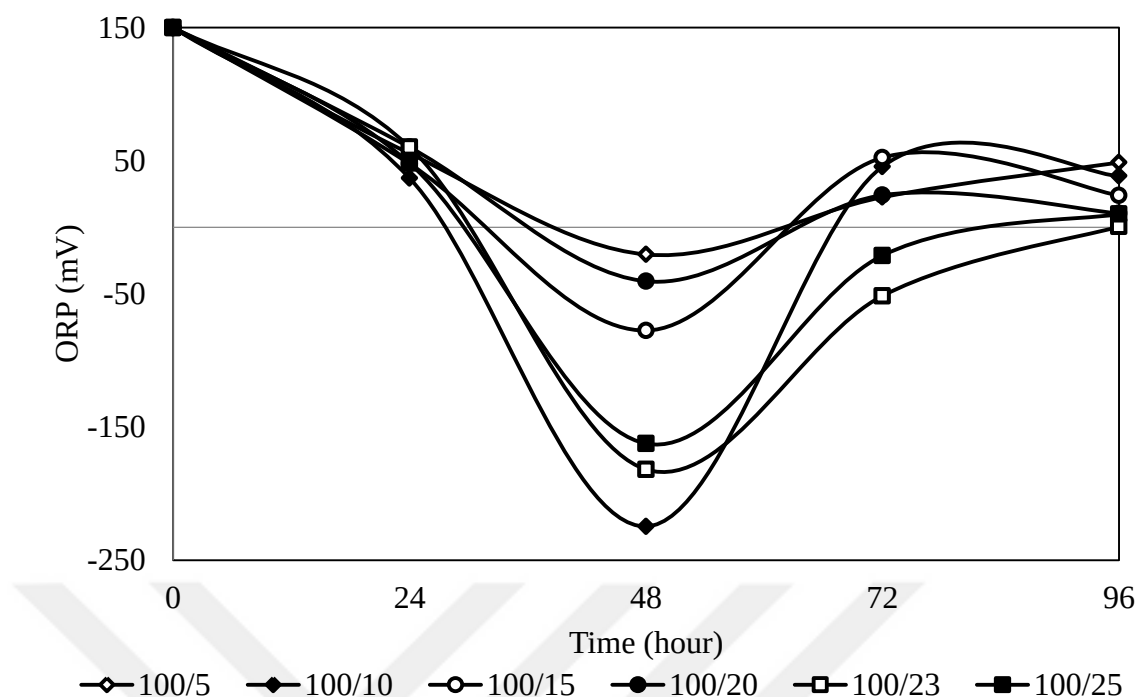


Figure 4.11 Variation of ORP with time at different COD/ TN ratios

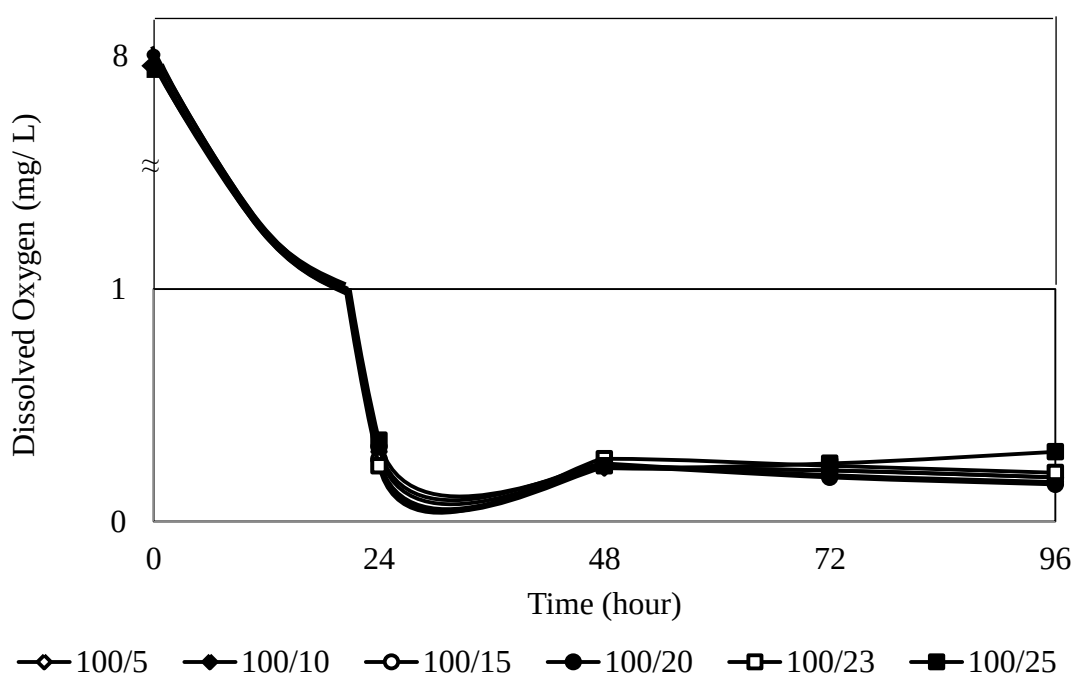


Figure 4.12 Variation of Dissolved Oxygen with time at different COD/ TN ratios

Furthermore; DO and ORP due to high initial COD concentrations effected the probable organism growth and may lowered the COD removal efficiencies.

4.1.3 Effects of COD/ TN Ratio in a Model Synthetic Wastewater

In this set of experiments a synthetic wastewater was prepared to simulate a model wastewater taken from the Porsuk River. The data taken from the literature and the characteristic of the sampled surface water was used. The COD/ TN/ TP ratio of the model wastewater was determined as 100/ 20-23/ 1.4. The sampled wastewater was in a characteristic of a sewer discharge and had a COD concentration of around 1000 mg/ L. This COD concentration is very high for the characteristic of a surface water. However the high COD concentration of the model wastewater will allow getting knowledge on the nutrient uptake capacity of the organism in excessive concentrations. Also the chosen organism may be used in this area to lower the nutrient concentrations before the wastewater stream of the village will interfere the next levels of Porsuk River. The nutrient concentrations in the river change annually. It increases after fertilizer, pesticide and herbicide applications or with livestock wastewater discharges. The model wastewater was taken after these applications to mimic the highest concentrations that can be obtained in the sampling point. The maximizing excessive carbon and nitrogen sources in the wastewater were tested in this set of experiments by changing the COD/ TN ratios. 100/ 5, 100/ 10, 100/ 15, 100/ 20, 100/ 23 and 100/ 25 of COD/ TN ratios were chosen.

Figure 4.13 depicts the variation of COD concentration with time in a model synthetic wastewater at different COD/ TN ratios. The COD concentration decreased in time and in all bottles at the end of the incubation period. The COD removal efficiencies were higher than 95%. In the control experiments no COD removal was obtained. Despite strength wastewater with an initial COD concentration of around 15 g/ L, where max. 42% of COD removal efficiency was obtained with a COD/ TN ratio of 100/ 23; *Rhodobacter sphaeroides* was successful to remove the COD from the wastewater with an initial COD of 1000 mg/ L. These results are also verified by Meng et al. (2018a) saying PBS's are successful at aerobic dark conditions at lower COD characterized wastewaters.

Figure 4.14 presents the variation of pH at different COD/ TN ratios in the model synthetic wastewater. The pH dropped to 6.35- 6.75 in all tested experiments and increased within 48 hours. At the end of the incubation the pH was found to be around 6.7- 7.1 in all COD/ TN ratios.

Figure 4.15 depicts the variation of ORP and Figure 4.17 gives the dissolved oxygen concentration with time at different COD/ TN ratios in a model synthetic wastewater. The initial ORP was 270- 300 mV in all tested bottles giving a dissolved oxygen concentration of 6 g/ L. At COD/ TN ratio of 100/ 5 the ORP decreased slightly within 24 hours but remained around 220- 270 mV through the incubation period. The DO concentration in 24 hours remained at 6 g/ L with this COD/ TN ratio. The DO concentration after 48 hours was higher than 7.4 g/ L in all experimental bottles. The ORP decreased slightly in time till 48 hours and then increased in all tested COD/ TN ratios with lasting ORP's around 220- 300 mV at the end of the incubation period.

The synchronized drops of pH, ORP and DO shows that nitrite nitrogen may be removed by RS within 24- 48 hours and then the pH, ORP and DO increased, due to the uptake capacity of RS where it might release nitrite again to the media.

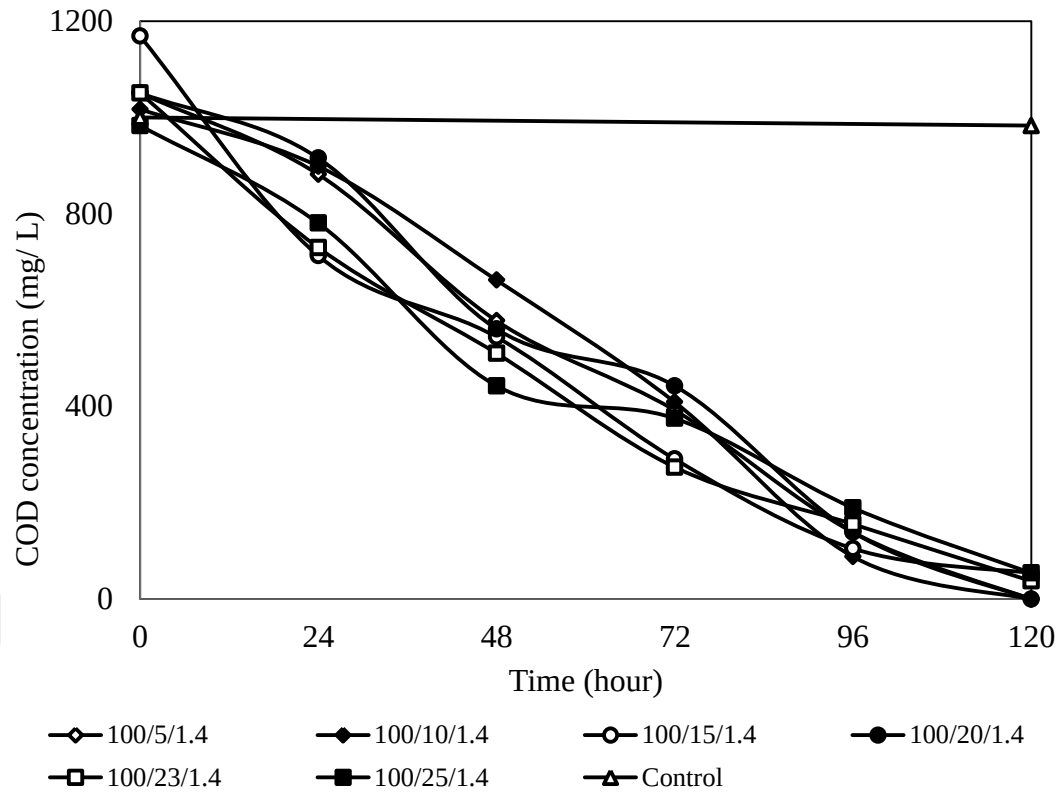


Figure 4.13 Variation of COD concentrations with time at different COD/ TN ratios in a model synthetic wastewater

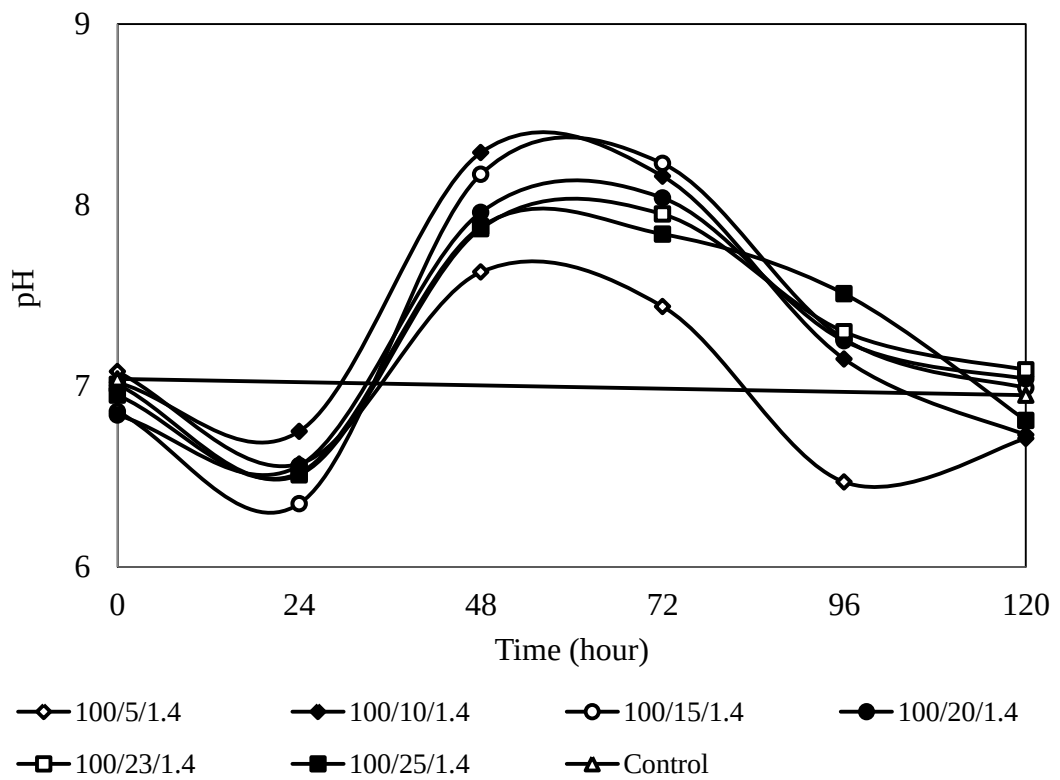


Figure 4.14 Variation of pH with time at different COD/ TN ratios in a model synthetic wastewater

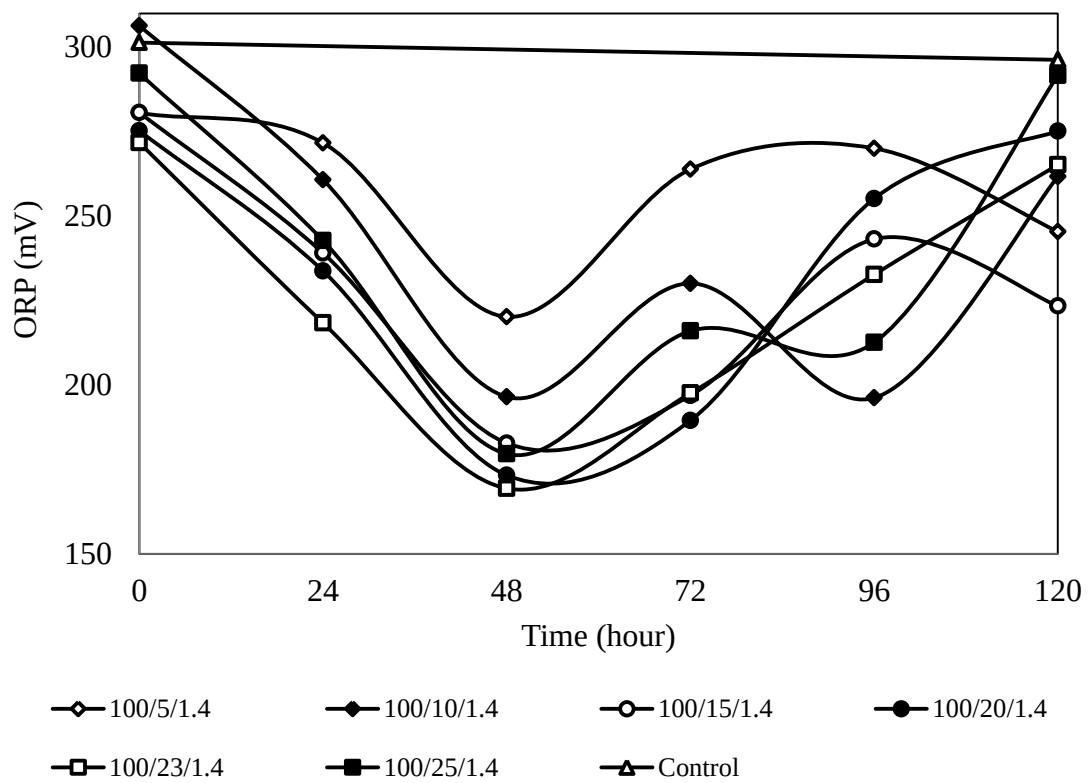


Figure 4.15 Variation of ORP with time at different COD/ TN ratios in a model synthetic wastewater

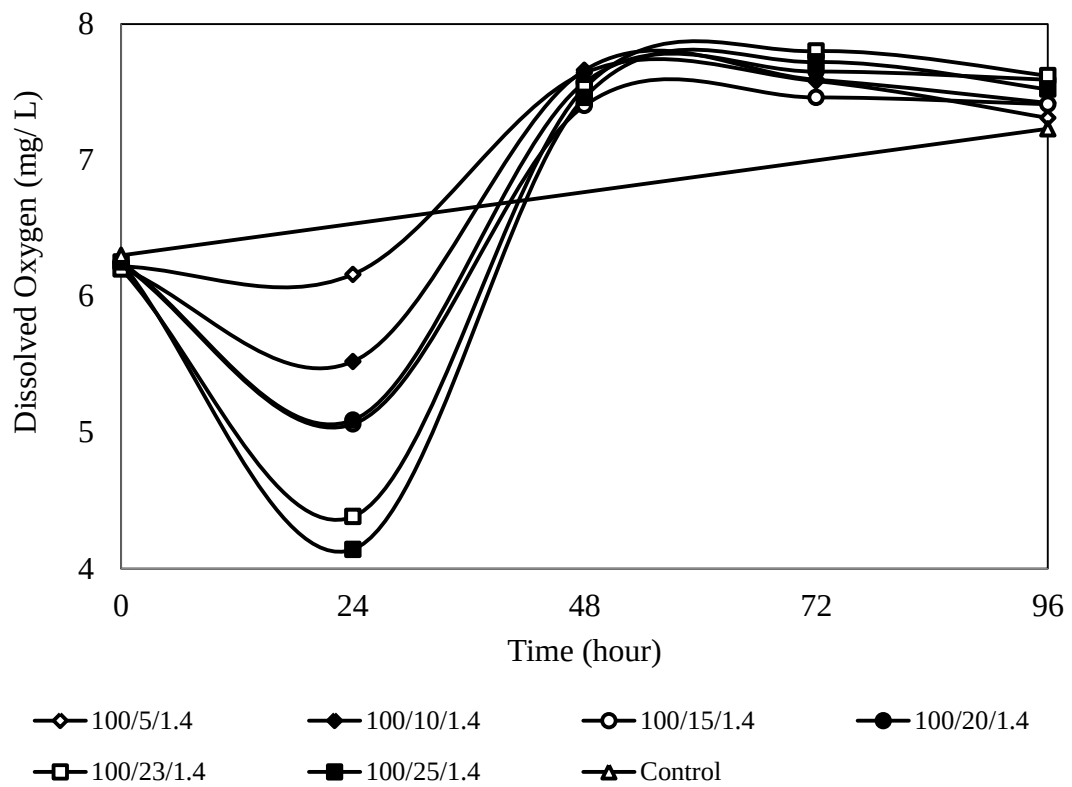


Figure 4.16 Variation of dissolved oxygen concentration with time at different COD/ TN ratios in a model synthetic wastewater

Figure 4.17 depicts the data of total phosphorus (TP) concentrations with time at different COD/ TN ratios in the model syntetic wastewater. The phosphorus concentration was kept constant to have a COD/ TP ratio of 100/ 1.4 which was taken from the charecteristic of the sampled wastewater. The total phosphorus concentration decreased in time. The initial TP concentration was 63 ± 2 mg/ L in the tested COD/ TN ratios. All the tested bottles gave a similar trend of TP removal due to having the same TP concentration. At the end of the incubation time TP removal efficiencies were around $54 \pm 3\%$ in the tested COD/ TN ratios. At the end of the incubation time there was a slight release of total phosphorus to the media in batch conditions. The remaining TP concentration was around 29 ± 1 mg/ L which was not meeting the surface water discharge standards which are 0.02 mg/ L. TP for Class I waters.

Figure 4.18 gives the total nitrogen concentrations with time at different COD/ TN ratios. The tested COD/ TN ratios ranged between 100/ 5-100/ 25. At COD/ TN ratio of 100/ 20 the removal efficieny was the highest with a percentage of 82% followed by the COD/ TN ratio of 100/ 5 (80%). At COD/ TN ratio of 100/ 23, which was the simulation of th the model wastewater, the TN removal efficieny was found to be 53% with a concentration of 205 mg/ L at the end of the incubation time. The final TN concentration of COD/ TN ratio of 100/ 20 was also high as 220 mg/ L. The other concentration in the tested COD/ TN ratios were lower but still not in surface water discharge standards which are 5.5 mg/ L TN for Class I waters.

Figure 4.19 represents the data of the biomass concentration with time at different COD/ TN ratios at a model syntetic wastewater. The biomass increased with time which shows a good maintance of the organism with high uptake capacity. The highest biomass growth was seen with a COD/ TN ratio of 100/ 15. The biomass concentration reached to 4 g/ L at this ratio. The lowest growth was seen at COD/ TN ratio of 100/ 20 with a biomass concentration of 1.8 g/ L at the end of the incubation period. However this is also a sufficient concentration for nutrient uptake.

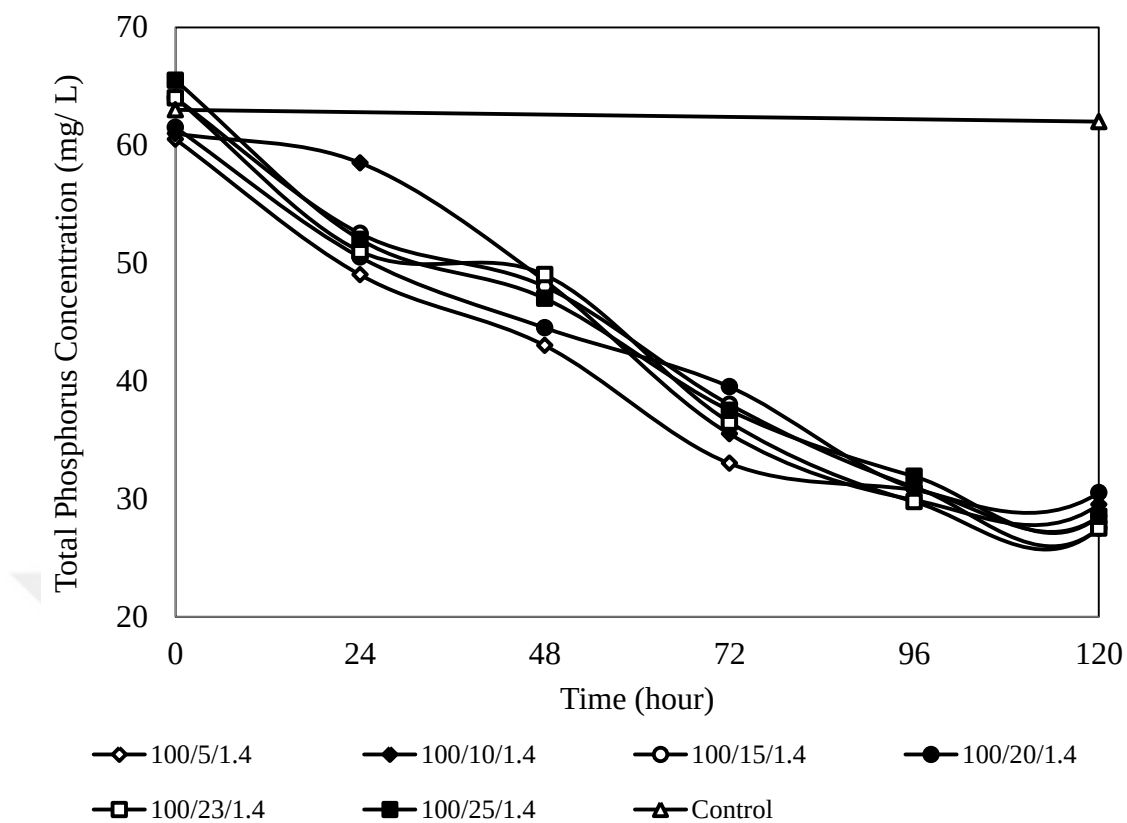


Figure 4.17 Variation of total phosphorus concentration with time at different COD/ TN ratios in a model synthetic wastewater

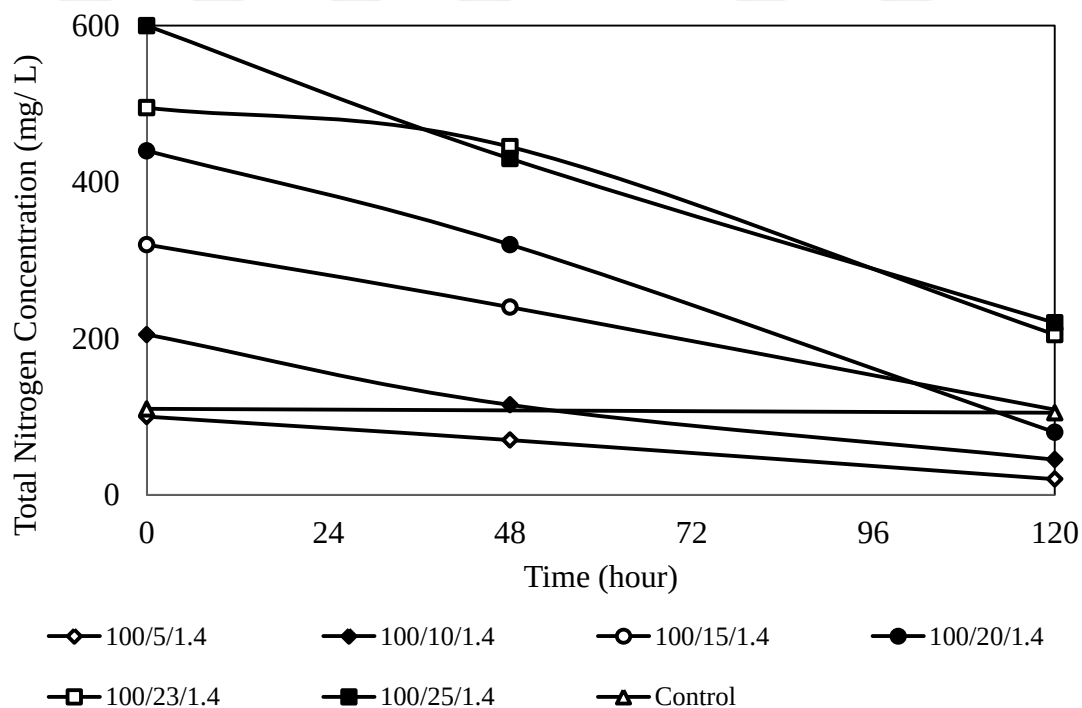


Figure 4.18 Variation of total nitrogen concentration with time at different COD/ TN ratios in a model synthetic wastewater

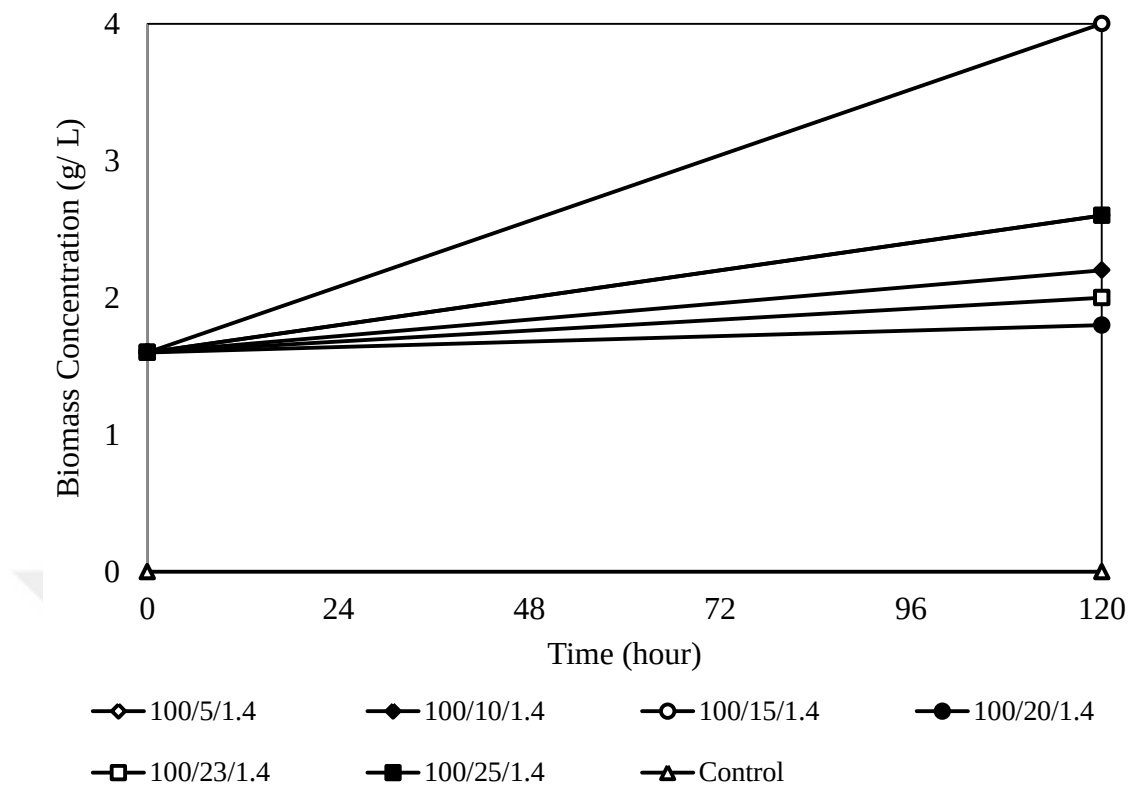


Figure 4.19 Variation of biomass concentration with time at different COD/ TN ratios in a model synthetic wastewater

4.1.4 Effects of COD/ TP Ratio in a Model Synthetic Wastewater

In this set of experiments as explained in 4.1.3 the synthetic wastewater was prepared to simulate a model wastewater taken from the Porsuk River. The COD/ TN ratio was kept constant to be 100/ 20 and the COD/ TP ratios changed as 100/ 0.5, 100/ 0.8, 100/ 1, 100/ 1.4, 100/ 2. TP ratios were kept in these ranges to mimic the model wastewater. The characteristic of Porsuk River gives a TP concentration range of 0.04- 1.7 g/ L.

The COD concentration data with time was given in Figure 4.20. The initial COD concentration was 1000 mg/ L. The COD concentration decreased with time in all tested COD/ TP ratios and the removal efficiency was 100%.

At different COD/ TP ratios in the synthetic wastewater, with an initial pH of 7; the pH dropped to 4.8 ± 0.1 in all experimental bottles except COD/ TP ratios of 100/ 1.4 and 100/ 2 (Figure 4.21). The pH increased within 48 hours in all bottles. Only in the COD/ TP ratio of 100/ 1.4 the pH was seen around 8 at time point of 72 hours and decreased in time to 7 at the end of the incubation time. At COD/ TP ratio of 100/ 2 the pH ranged between 6.4- 7.2, and at the other ratios the trend of pH changes were nearly similar lasting with pH range of 6.43- 6.78 at 120 hours.

Figure 4.22 depicts the variation of ORP with time in different COD/ TP ratios. The initial ORP was around 270- 300 mV in the experimental bottles. The ORP decreased in time till 48 hours in all bottles, however was over 170 mV. At all COD/ TP ratios the ORP was around 190 mV at 72 hours. Only at COD/ TP ratio of 100/ 1.4 the ORP increased to 275 mV at the end of the incubation period. At the other tested ratios the ORP data changed slightly, having an ORP range of 160- 190 mV at 120 hours.

The variation of dissolved oxygen concentrations were given in Figure 4.23 with time for the model wastewater. The initial DO concentration was 6.3 mg/ L. The DO decreased in the first 24 hours in all tested COD/ TP ratios. The DO concentration

increased in time after 24 hours. The highest DO concentrations were obtained with the COD/ TP ratio of 100/ 1.4 at was nearly constant after 48 hours (7.6 mg/ L). At the other COD/ TP ratios the increase of the DO concentrations were lower than COD/ TP ratio of 100/ 1.4, but was still high ending with a DO concentration range of 6.6 ± 0.2 mg/ L.

Figure 4.24 represents the variation of total phosphorus concentrations with time at different COD/ TP ratios. At COD/ TP ratios of 100/ 0.5, 100/ 0.8, 100/ 1, 100/ 1.4 and 100/ 2; the TP removal efficiencies were 66, 59, 47, 50, and 52%, respectively. Despite COD removal, TP removal was lower and it is understood that *Rhodobacter sphaeroides* could not uptake phosphate from the wastewater sufficiently to meet the surface water discharge standards.

The nitrogen concentrations were kept constant in these experiments and the decrease of TN concentrations were shown in Table 4.1 at different COD/ TP ratios. The initial TN concentration was 440 mg/ L in all tested bottles. The TN removal efficiencies decreased with increasing COD/ TP ratios. The removal efficiencies were 91, 87.5, 83.5, 81.8, 79.5 at COD/ TP ratios of 100/ 0.5, 100/ 0.8, 100/ 1, 100/ 1.4, 100/ 2, respectively. The TN removal efficiencies were high in these conditions however it was not sufficient to reach the surface water discharge standards. Also the initial NO_3^- and NH_4^+ concentrations and the concentrations taken at the end of the incubation period were given in Table 4.2. The end point NO_3^- and NH_4^+ concentrations were also not meeting the surface water discharge standards.

The biomass concentration increased in time at different COD/ TP ratios (Figure 4.25). The lowest biomass growth was seen with a COD/ TP ratio of 100/ 0.5 having a biomass concentration of 1.3 g/ L at the end of the incubation period. The highest growth was obtained with a COD/ TP ratio of 100/ 1.4 with a biomass concentration of 1.8 g/ L. It is understood that the deficiency of phosphate effects slightly the culture growth.

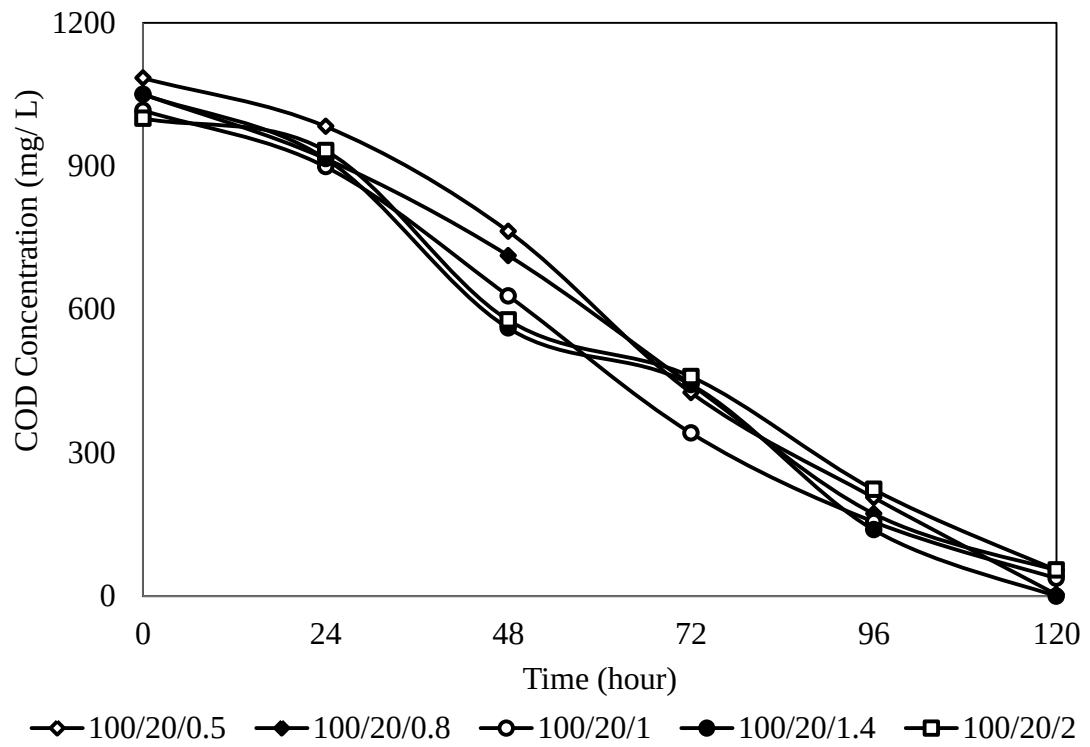


Figure 4.20 Variation of COD concentrations with time at different COD/ TP ratios in a model synthetic wastewater

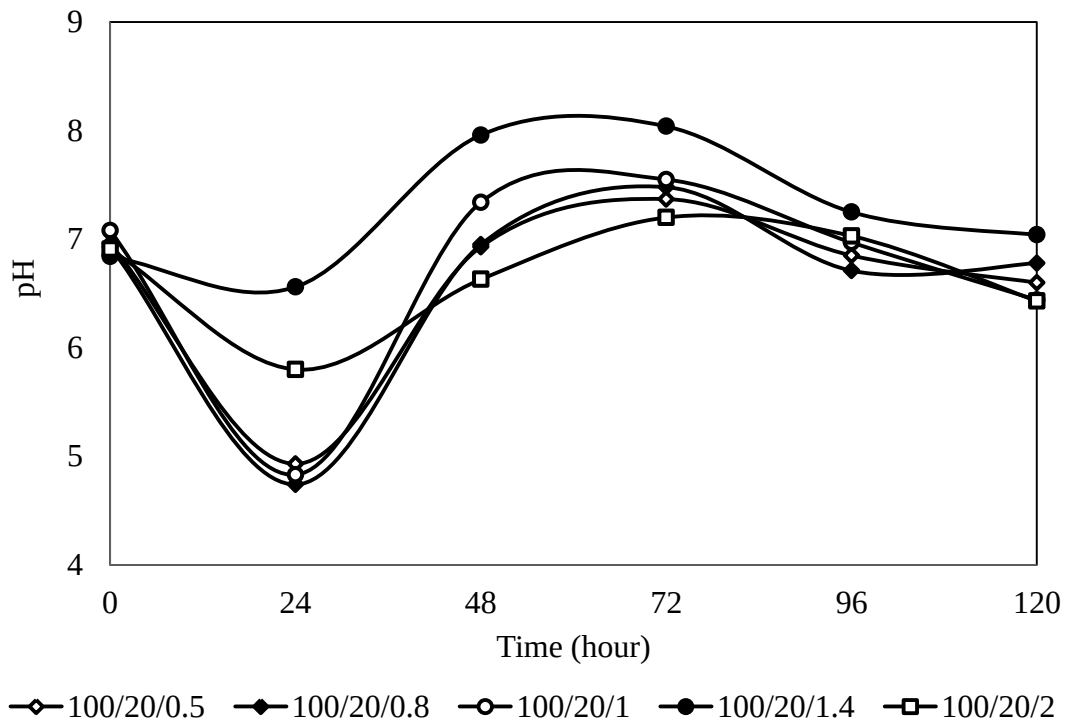


Figure 4.21 Variation of pH with time at different COD/ TP ratios in a model synthetic wastewater

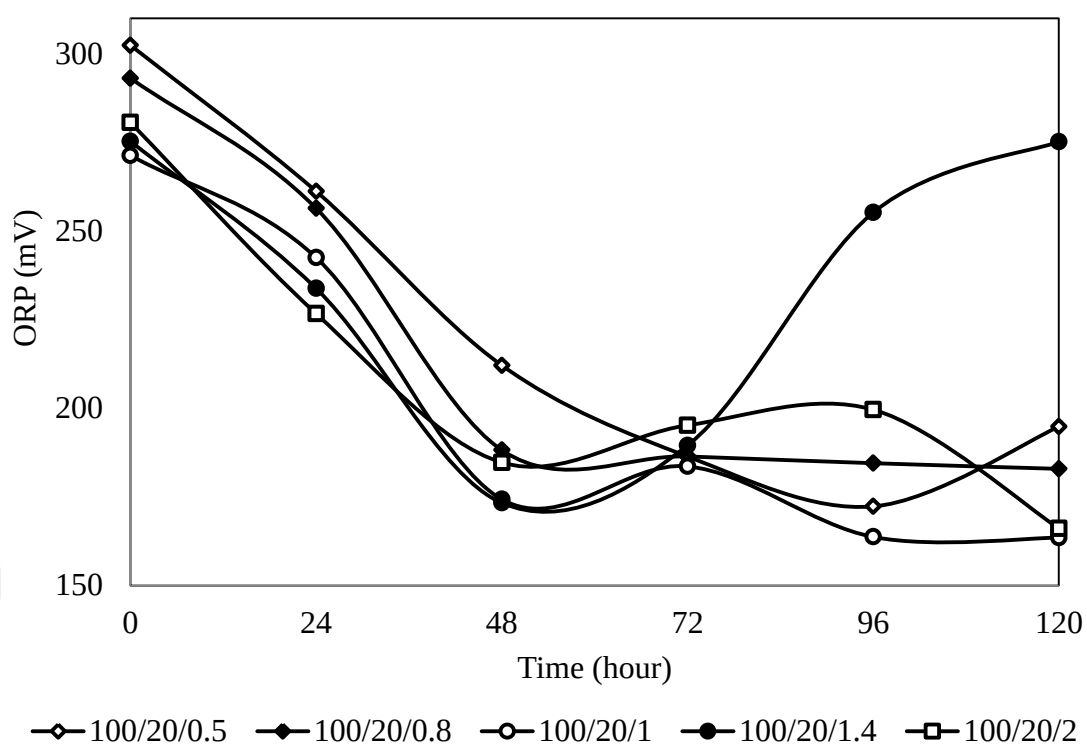


Figure 4.22 Variation of ORP with time at different COD/ TP ratios in a model synthetic wastewater

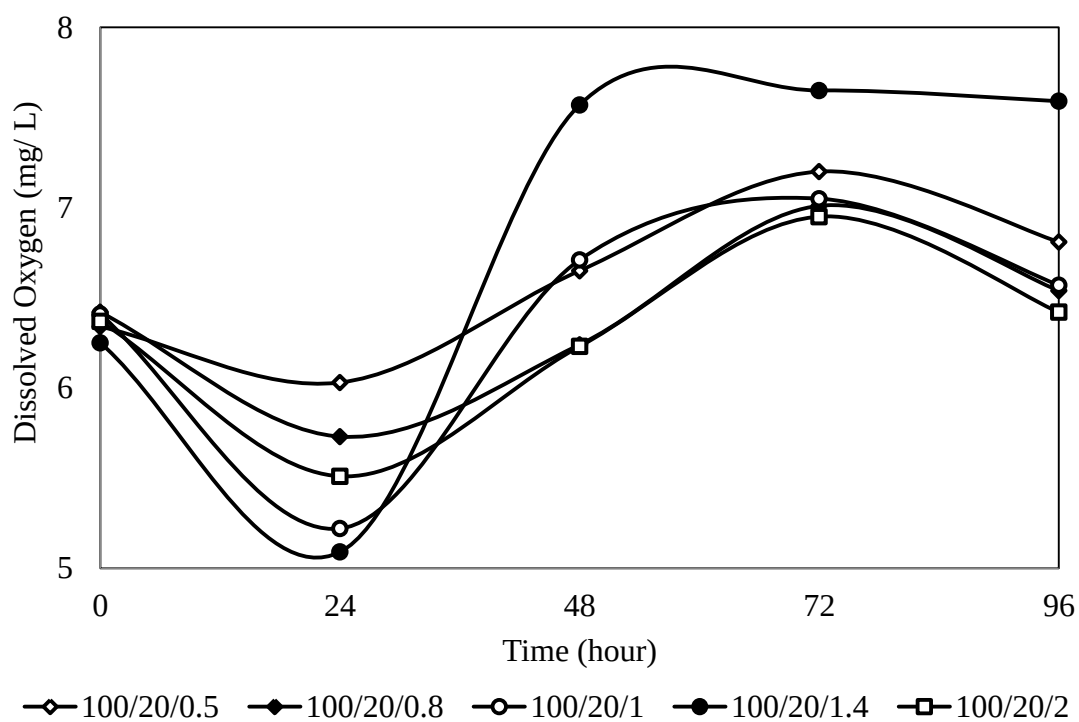


Figure 4.23 Variation of dissolved oxygen concentration with time at different COD/ TP ratios in a model synthetic wastewater

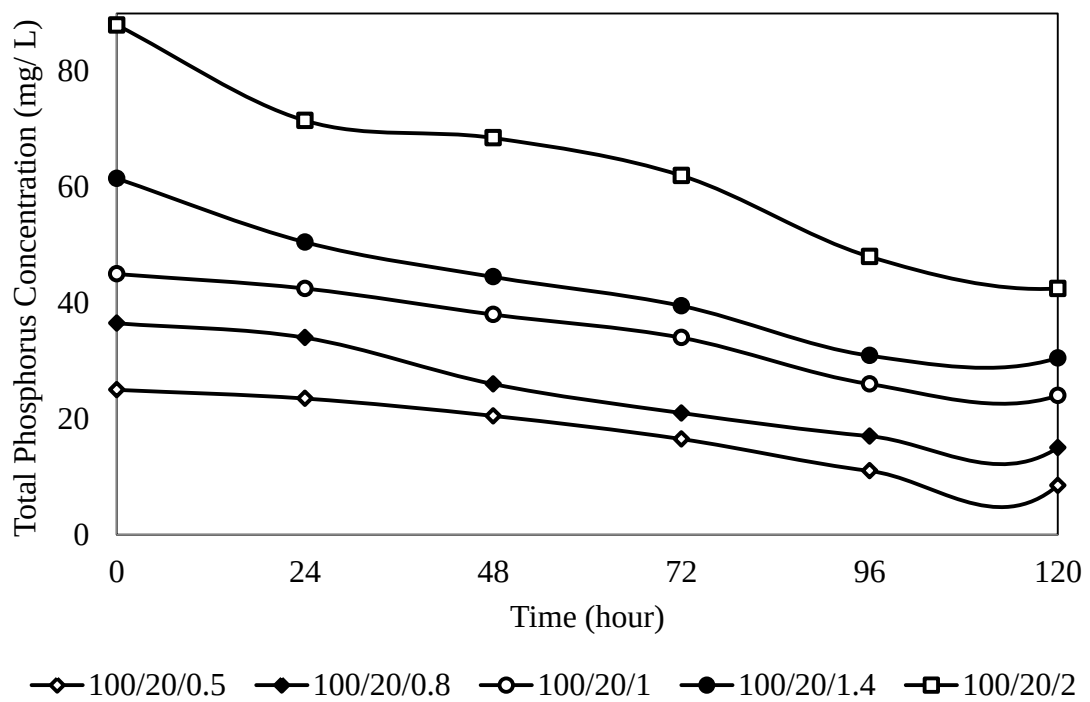


Figure 4.24 Variation of total phosphorus concentration with time at different COD/ TP ratios in a model synthetic wastewater

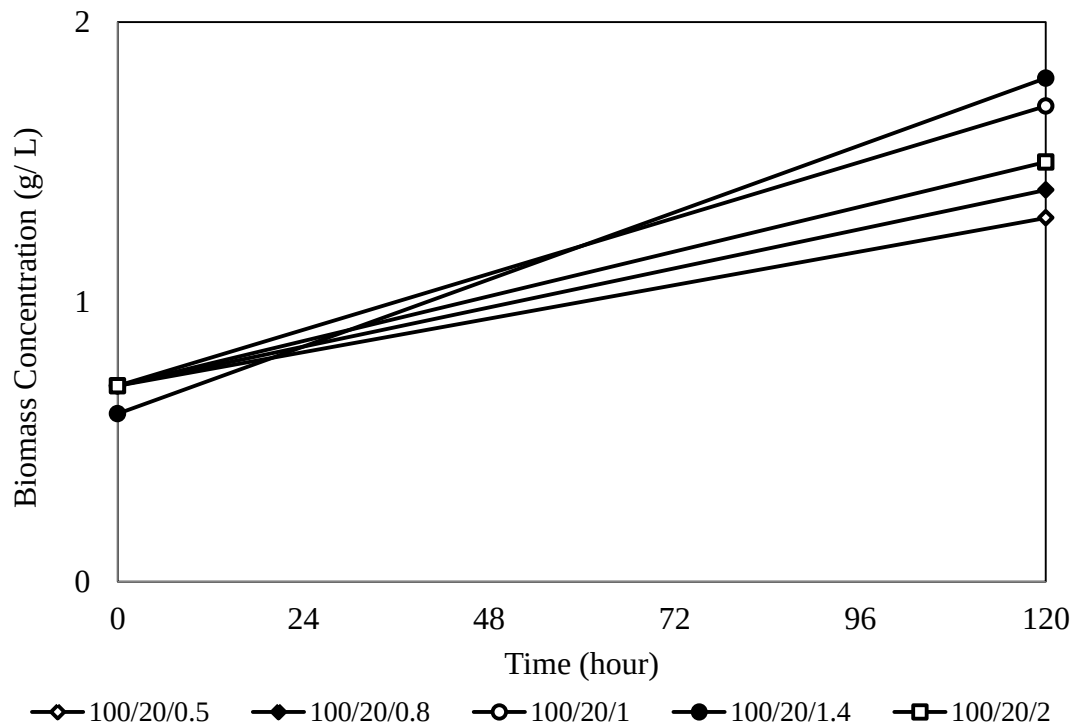


Figure 4.25 Variation of biomass concentration with time at different COD/ TP ratios in a model synthetic wastewater

Table 4.1 Variation of total nitrogen concentration with time at different COD/ TP ratios in a model synthetic wastewater

Total Nitrogen Concentration		
Hours	0	120
100/ 20/ 0.5	435	40
100/ 20/ 0.8	435	55
100/ 20/ 1	437.5	72.5
100/ 20/ 1.4	440	80
100/ 20/ 2	432.5	90
Control	440	440

Table 4.2 Variation of NO_3^- , NH_4^+ concentrations at the end of the incubation time

Hours	NO_3^- Concentration		NH_4^+ Concentration	
	0	120	0	120
100/ 20/ 0.5	17.50	0.50	60.00	26.00
100/ 20/ 0.8	17.50	2.00	58.00	29.50
100/ 20/ 1	17.50	1.50	59.00	30.50
100/ 20/ 2	17.50	1.50	64.00	37.00
Control	17.50	15.00	61.50	59.00

4.1.5 Evaluation of *R. sphaeroides* and Activated Sludge Co-Culture Ratios with a Model Synthetic Wastewater

Rhodobacter sphaeroides was used alone in previous experiments having good removal efficiencies on carbon and nitrogen. However the final C, N, P concentrations were not sufficient enough to maintain a discharge appropriate for surface waters. A co-culture consisted of *Rhodobacter sphaeroides* and activated sludge can enhance C, N, P removal in aerobic conditions which will be easy to apply as a low cost treatment method in areas having agricultural applications or having industrial wastewater discharges with high C/ N/ P ratios. For this purpose, *Rhodobacter sphaeroides* with an initial biomass concentration of 3 g/ L, and activated sludge with an initial biomass concentration of 7 g/ L were mixed in appropriate ratios to form a co-culture. The co-culture ratios were 1/ 1, 1/ 2, 1/ 5, 1/ 10 and 1/ 20. Also pure cultures were added to the experiments to understand the nutrient removal efficiencies of the cultures of activated sludge (AS) and *Rhodobacter sphaeroides* (RS) alone in the same conditions. The C/ N/ P ratio was set to 100/ 23/ 1.4 in the experiments to simulate the sampled wastewater taken from Porsuk River. Experiments were carried out in an incubator shaker at 30°C, 150 rpm at 500 mL erlenmayer flasks with a working volume of 200 mL. The initial pH was set to 7.

Figure 4.26 depicts the variation of COD concentration with time at different co-culture ratios. The initial COD concentration in all tested bottles was around 1200 mg/ L. The final COD concentrations in these experiments gave a concentration of 20- 100 mg/ L at the end of the incubation period. The COD removal efficiency for the tested Activated Sludge was 91%, while for *Rhodobacter sphaeroides* it was found to be 97%. The co-culture ratio of 1/ 5 was able to convert all the COD concentration with a removal efficiency of 100%.

Figure 4.27 shows the variation of pH with time in different co-culture ratios. The initial pH of 7 increased in all bottles except RS and co-culture 1/ 20 in the first 24 hours. After 24 hours all bottles showed an increase in pH except AS. At 48 hours the

pH was 8 in all tested experiments except RS with a pH of 7.8 and AS with a pH of 7.3. At the bottles with culture used alone the final pH was lower than the co-culture ratio experiments and was 7.2. In the co-culture experiments the final pH of all co-culture ratios were nearly same and was around 7.7.

Figure 4.28 gives the ORP data with time and Figure 4.29 depicts the DO concentrations with time in different co-culture ratios. At the beginning of the experiments the ORP was around 220- 265 mV. It increased or was constant in some bottles in 24 hours and then decreased to around 150- 200 mV at 48 hours. At the bottle with AS it was nearly constant at 180- 190 mV and in RS it decreased to 100 mV at time 96 hours and increased at the end of the incubation period to 160 mV. At the co-culture ratios the ORP ranged between 180- 210 mV till the end of the reaction. At dissolved oxygen concentrations the initial DO concentration was 6.5 mg/ L and decreased a little bit in the co-culture experiments to around 5 mg/ L. In the control experiments with AS and RS the DO concentrations at 24 hours were 6 and 5.7 mg/ L, respectively. After 48 hours RS was constant till the end of the incubation time at 6.8 mg/ L and at AS the DO concentration dropped to 0.8 mg/ L at time 48 hours and then increased to around 3.5 mg/ L. At the co-culture ratios the DO concentrations were around 3.7- 5.6 mg/ L maintaining aerobic conditions in the incubation media.

Figure 4.30 depicts the data of total phosphorus concentration with time in different co-culture ratios. The initial phosphorus concentration was kept constant in all tested bottles (62.5 mg/ L) The total phosphate removal efficiencies in experimental bottles of RS, AS, 1/ 1, 1/ 2, 1/ 5, 1/ 10, 1/ 20 were 61.6, 59, 62, 70, 72, 61.6, 60%, respectively. The highest total phosphorus removal efficiency was obtained with a co-culture ratio (AS/ RS) of 1/ 5. The final total phosphorus concentration in this experiment was 17.5 mg/ L. Total phosphorus removal efficiencies were better in tested co-cultures when compared with cultures alone.

Figure 4.31 represents the variation of total nitrogen concentration with time at different co-culture ratios. The nitrogen concentrations in all tested experiments were

kept constant at 502.5 mg/ L. The total nitrogen decreased with time in all bottles. However the final total nitrogen concentrations of the tested bottles were around 57.5- 70 mg/ L, which were high and need more treatment. The highest total nitrogen removal was obtained with the co-culture ratio of 1/ 5 and was 89.5%. At the control experiments of AS and RS the total nitrogen removal efficiencies was 86.5% and 83%, respectively. Nitrate concentrations also decreased with time having a final nitrate concentration of approximately 2.5 mg/ L in all tested bottles. The decrease of ammonium concentrations in the tested experiments reached to around 9.5- 15.5 mg/ L. The pH drop and the nitrogen removal of RS showed that ammonium was removed from the incubation media at the first 24 hours followed by nitrate uptake. At AS and at tested the co-cultures, the pH rise and the decrease of DO at the first 48 hours give a hint of slight nitrite removal. After 72 hours at AS shows that nitrification of ammonium took place where it is also been seen in the co-cultures.

Biomass concentrations with time in the co-culture experiments were depicted in Figure 4.32. The biomass concentration in all tested bottles increased with time. At AS and RS the biomass concentrations increased to 1.6 g/ L and 1.8g/ L, respectively from an initial biomass concentration of 0.6 g/ L. At the co-culture ratios of 1/ 5 and 1/ 10 the biomass concentrations reached to 2 g/ L. The lowest increase was obtained with the co-culture ratio of 1/ 20 with a biomass concentration of 1.4 g/ L at the end of the incubation period.

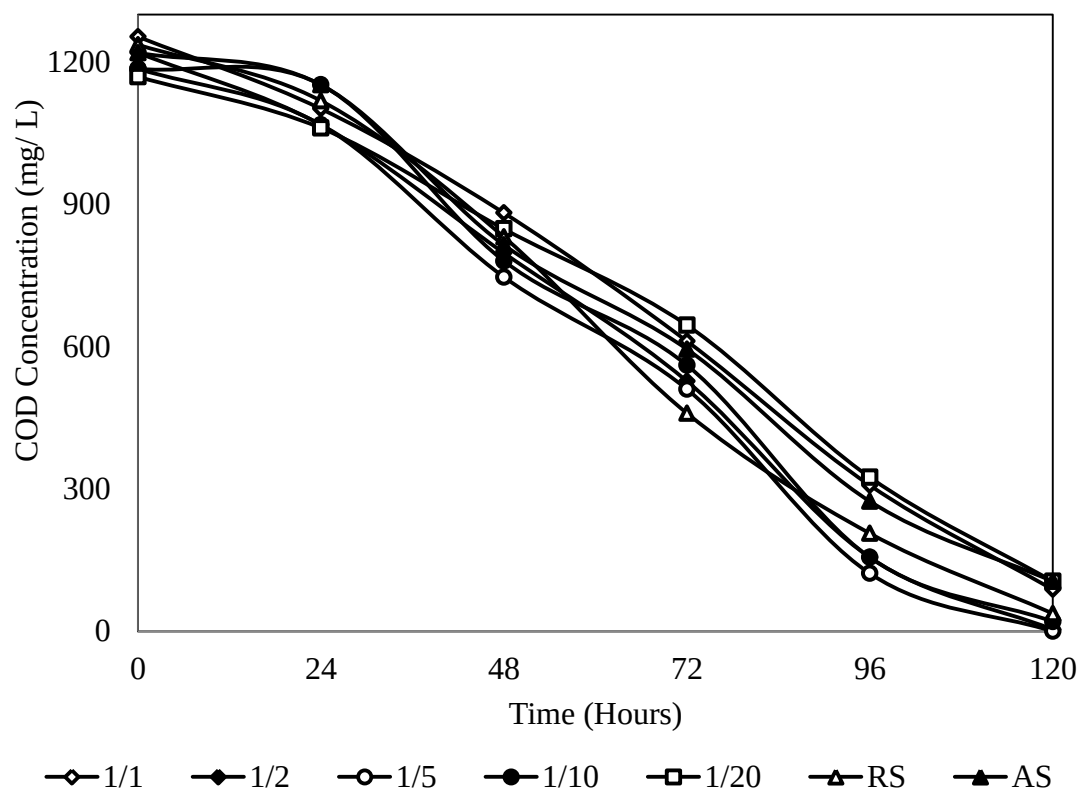


Figure 4.26 Variation of COD concentrations with time at different co-culture ratio

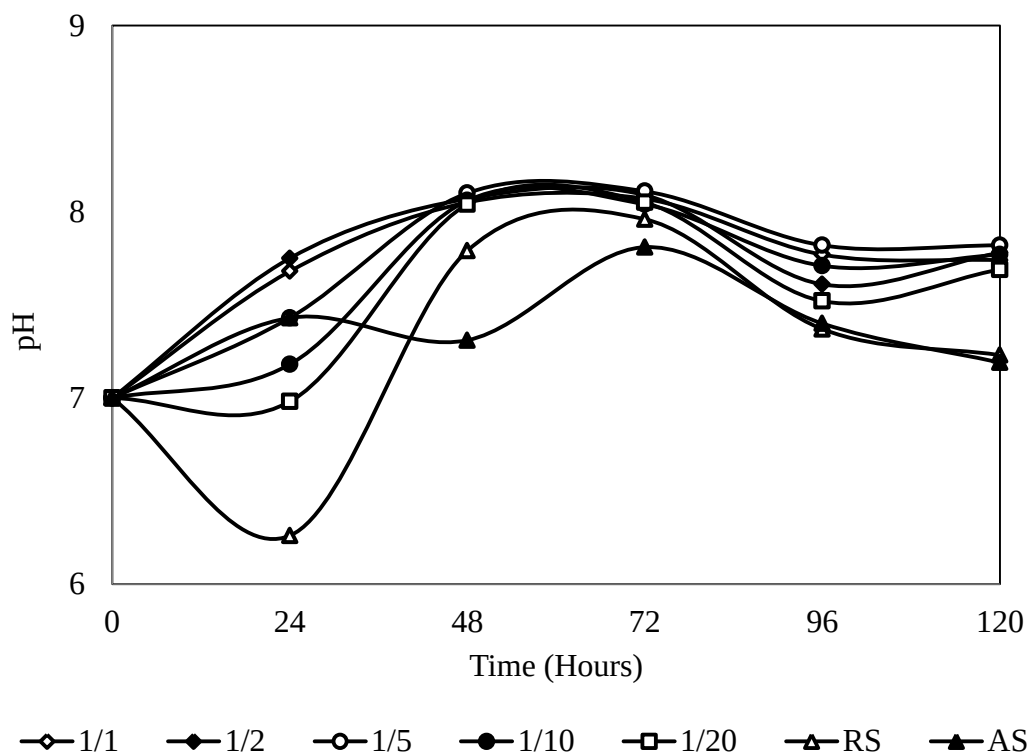


Figure 4.27 Variation of pH with time at different co-culture ratio

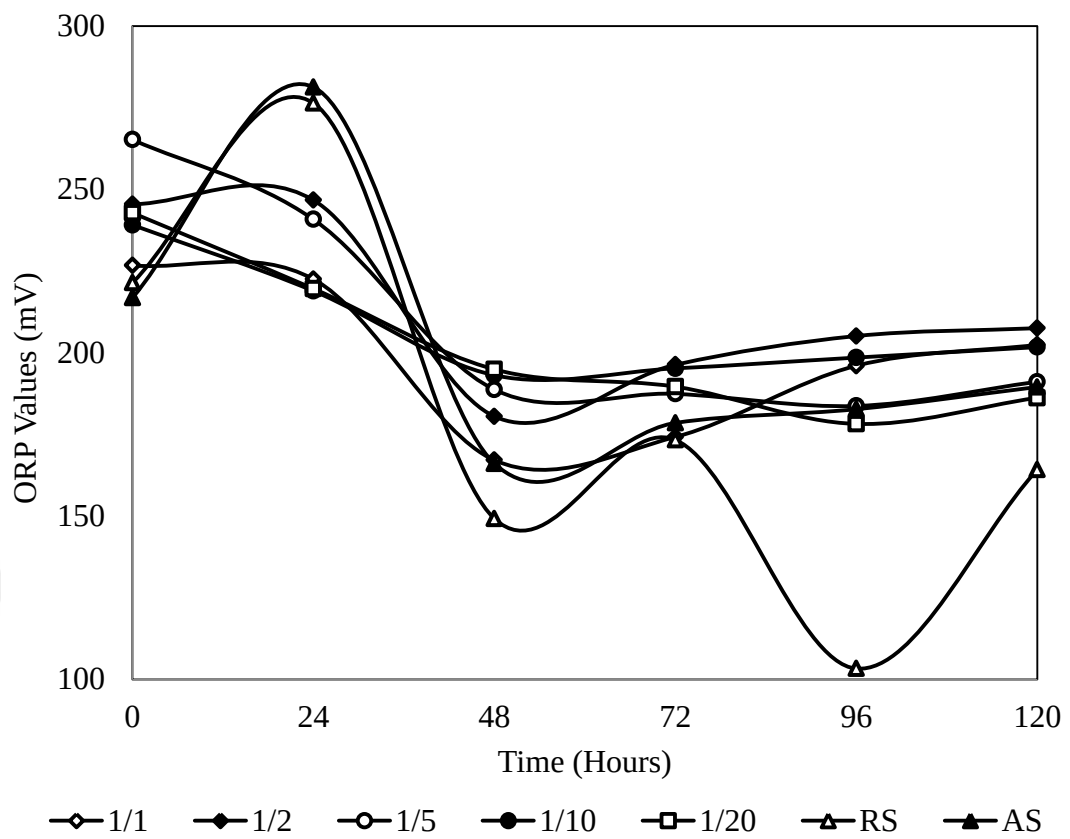


Figure 4.28 Variation of ORP with time at different co-culture ratio

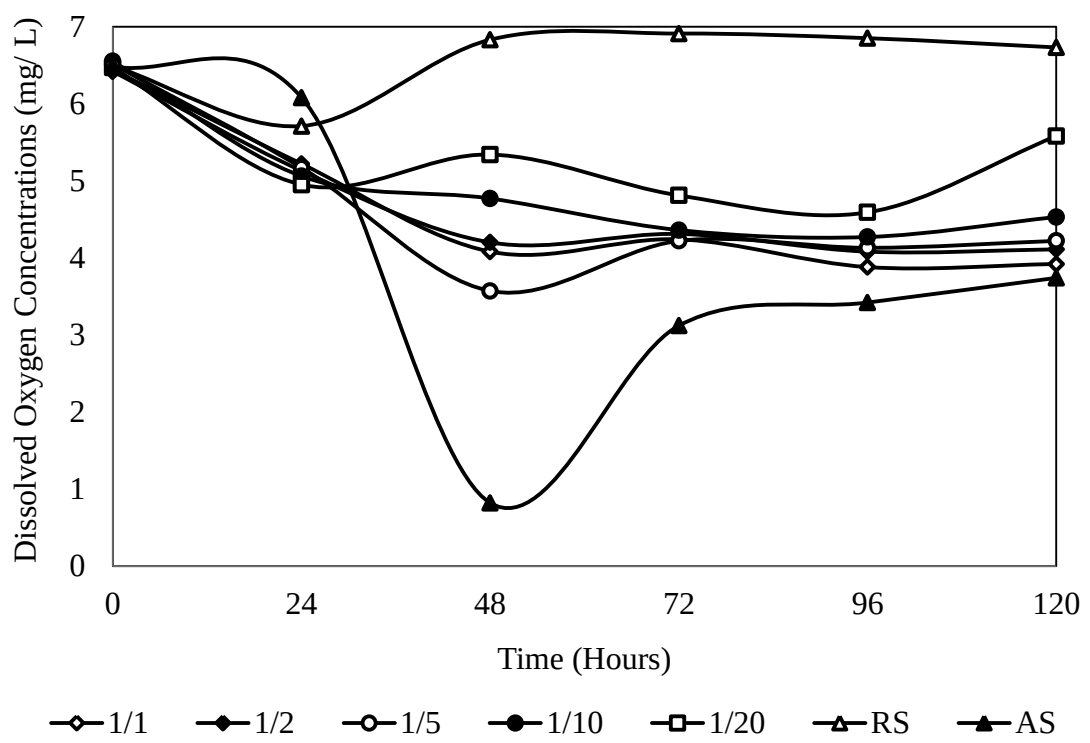


Figure 4.29 Variation of dissolved oxygen concentration with time at different co-culture ratio

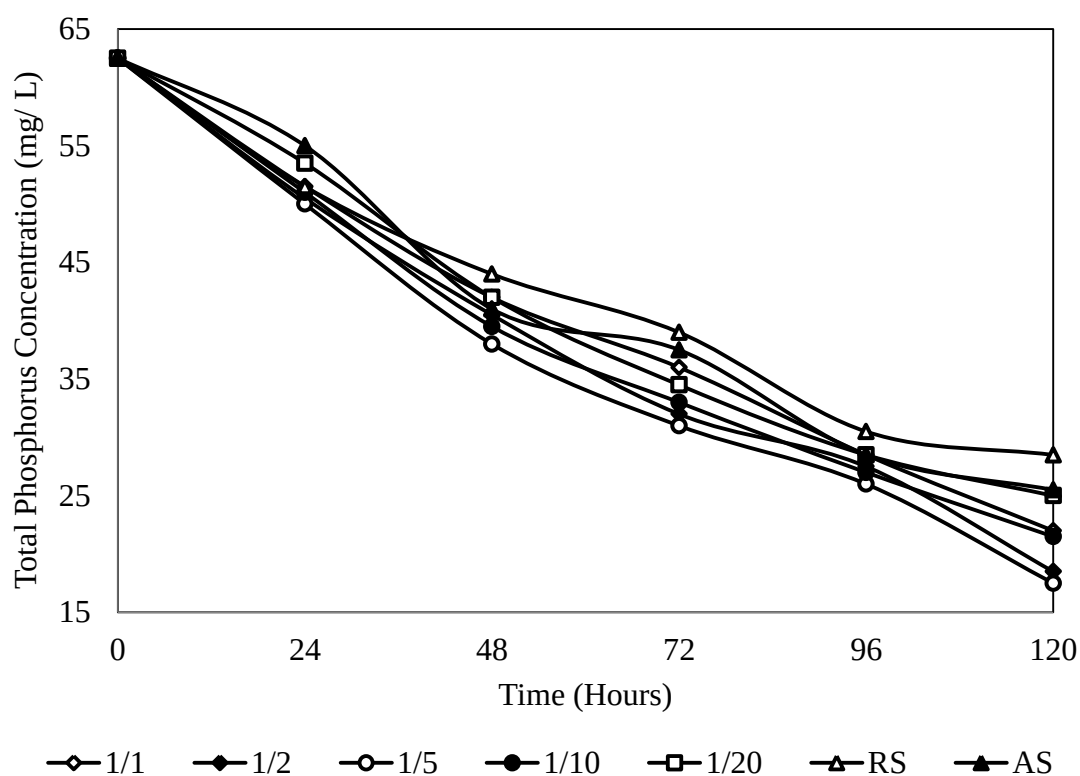


Figure 4.30 Variation of total phosphorus concentration with time at different co-culture ratio

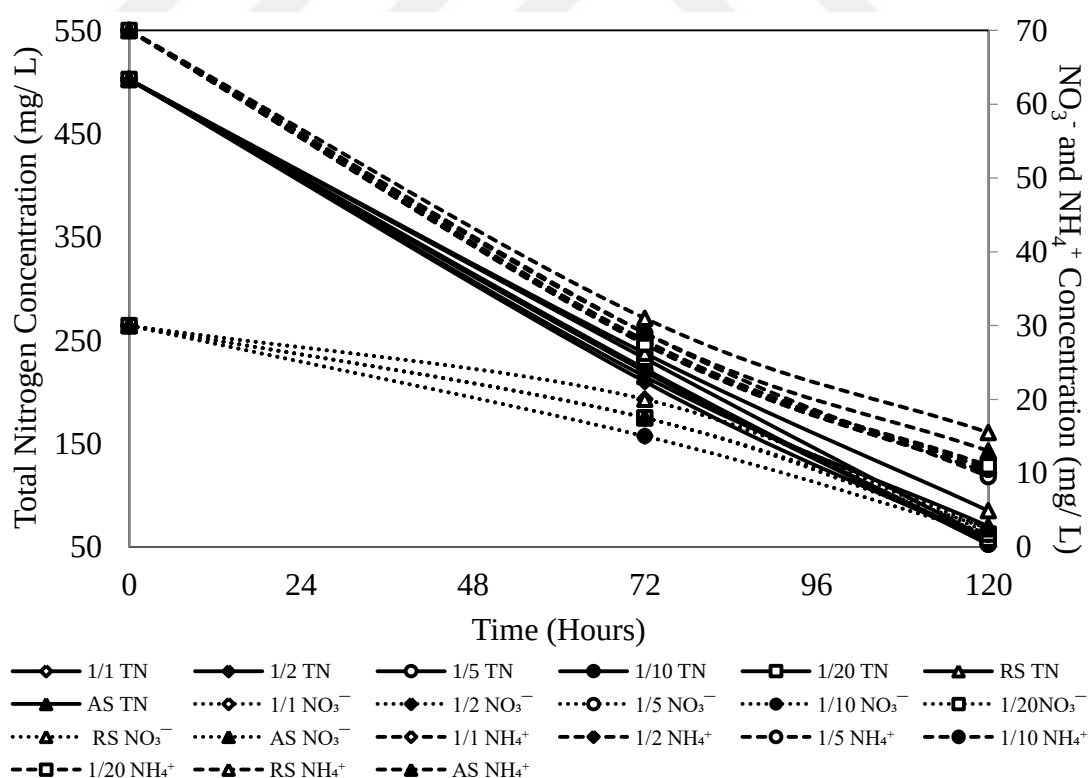


Figure 4.31 Variation of TN, NO_3^- and NH_4^+ with time at different co-culture ratios

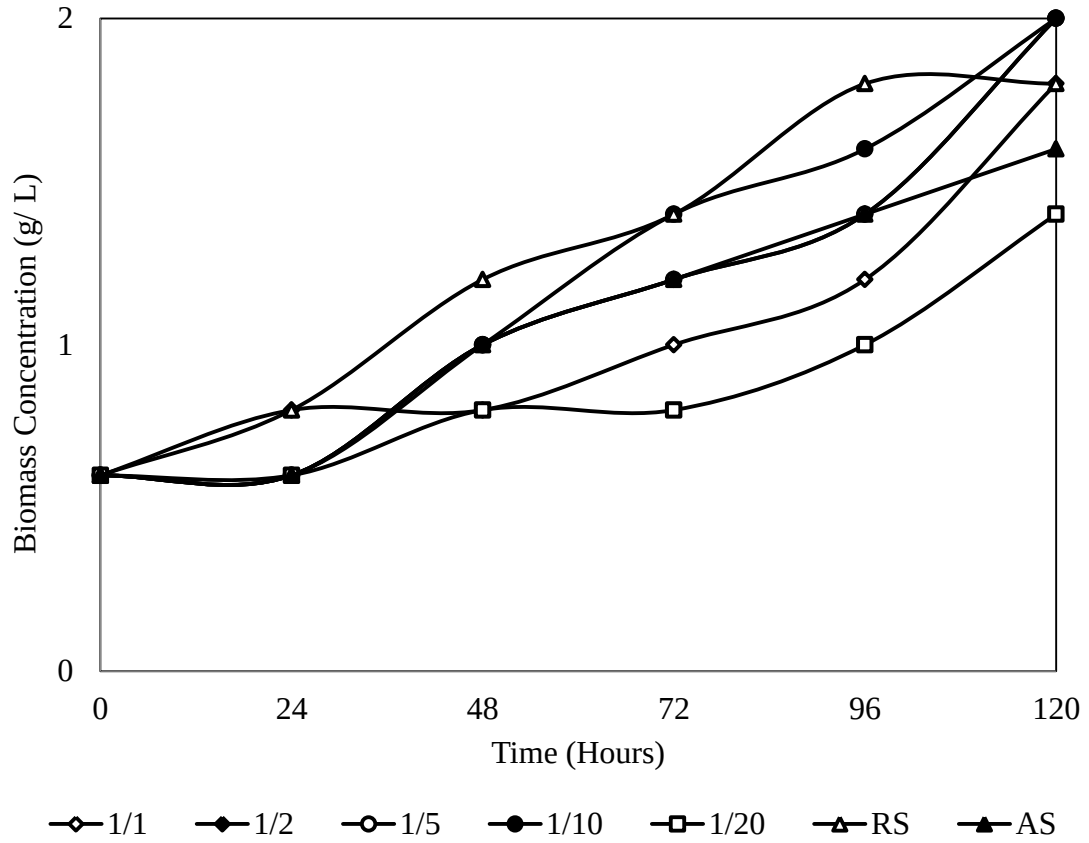


Figure 4.32 Variation of biomass concentration with time at different co-culture ratio

4.2 Continuous Experimental Studies in activated Sludge System

4.2.1 Effects of Hydraulic Retention Time on nutrient Removal with a co-culture of AS/ RS

Continuous experiments were performed in an activated sludge unit in order to investigate the COD, nitrogen and phosphorus removal performance at different hydraulic retention times (Θ_H). Θ_H varied between 5.94- 15.54 h, sludge age (Θ_c) was kept constant at 12 days, and feed COD concentration was constant at 1270.3 mg/ L. A co-culture consisted of activated sludge (AS) and *Rhodobacter sphaeroides* (RS) with a ratio of 1/ 5 (AS/ RS) was used in the experimetments. pH varied between 7- 7.5.

Figure 4.33 depicts pH, dissolved oxygen (DO) contents, and biomass concentrations at different HRT. The results depicted that the initial pH of 7 increased in time. After the reactor reached to steady state the pH in all HRT's varied

between 8- 8.41. Figure 4.33 (b) giving the final DO concentrations at different HRT's showed that the DO varied between 2.86- 3.37 in all tested HRT's. Figure 4.33 (c) shows the variation of biomass concentrations at different HRT's where it increased in time. The initial biomass content was 0.6 g/ L and after the system reached to steady state the biomass concentrations in all tested HRT's reached to 2- 2.7 g/ L.

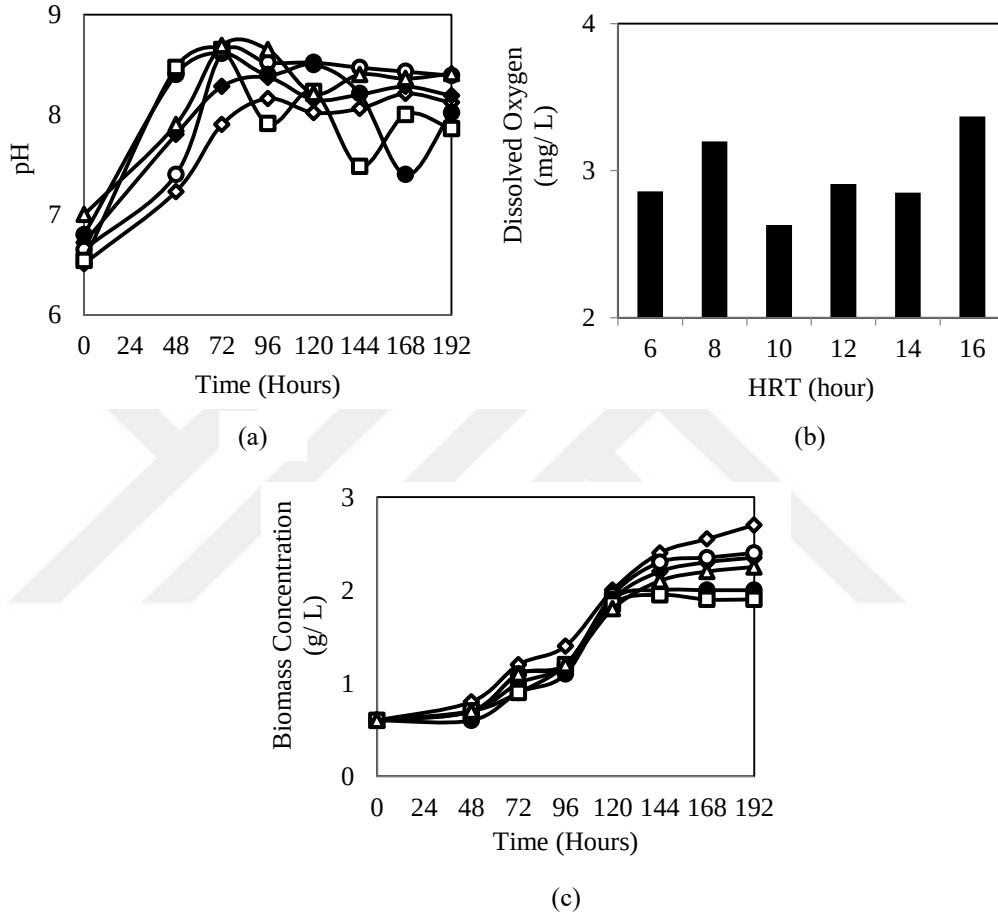


Figure 4.33 (a) Variation of pH with time at different HRT. (b) Final dissolved oxygen concentration in the reactor at different HRT. (c) Biomass concentration variations at different HRT. (HRT's: $\diamond$ 6h, $\blacklozenge$ 8h, $\circ$ 10h, $\bullet$ 12 h, $\square$ 14 h, Δ 16h).

The results of this set of experiments in terms of COD effluent and COD removal efficiency with different hydraulic retention times are depicted in Figure 4.34. Effluent COD concentrations increased with increasing HRT's till 11.87 hours and then decreased with increasing HRT's. The highest COD removal efficiency was depicted with the lowest HRT that was 5.9 hours. The co-culture was successful to remove COD in low HRT's. However at HRT of 11.87 hours the COD removal

efficiency dropped to approximately 60%. At HRT's of 14.42 h and 15.5 hours the effluent COD and COD removal efficiencies were higher than at HRT 11.8 hours with data of 67% and 82% respectively. These results show that low HRT's can be used for COD removal. The decrease in higher HRT's may be due to inhibition effects of excess nutrient concentrations which was kept constant at C/ N/ P of 100/ 23/ 1.4.

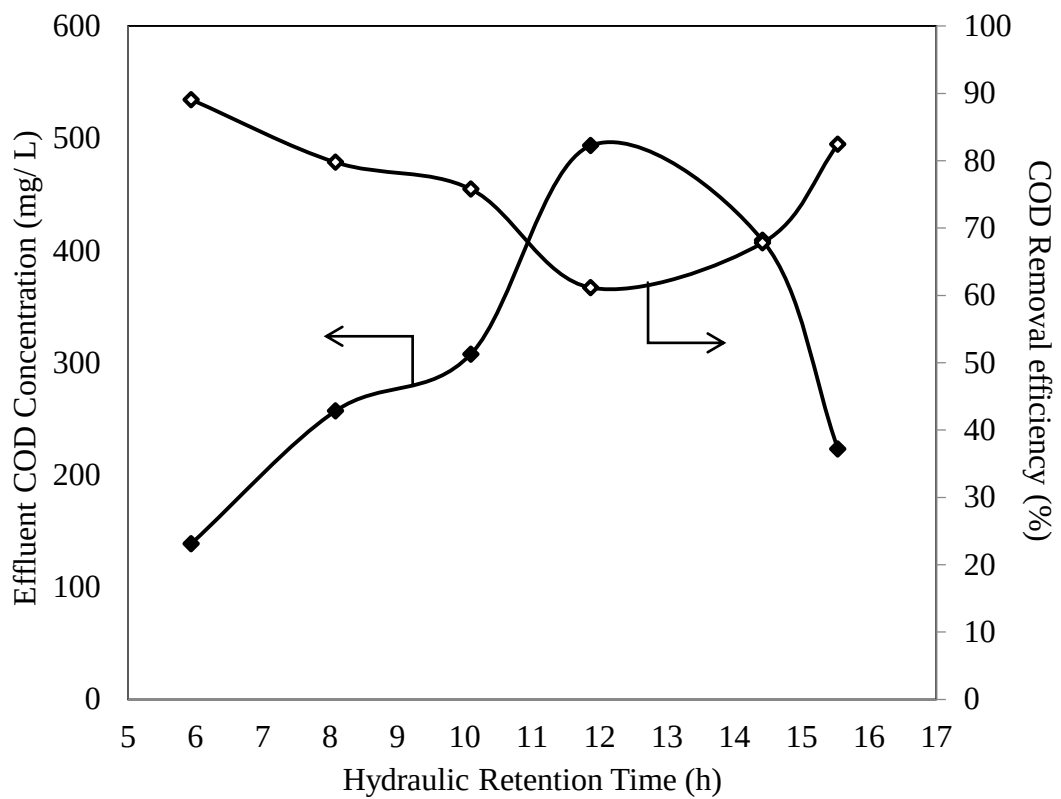


Figure 4.34 Variation of effluent COD and COD removal efficiency at steady state with different HRT. ($\Theta_c=12$ d, $COD_0=1270$ mg/ L)

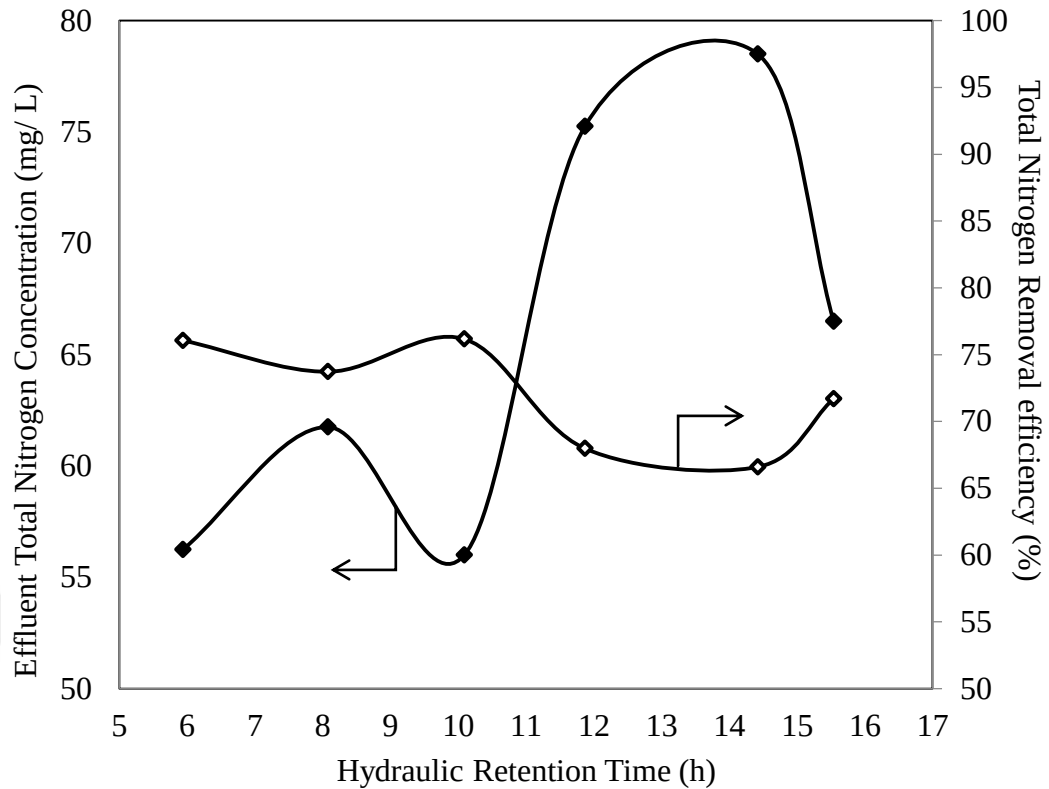


Figure 4.35 Variation of effluent Total Nitrogen and Total Nitrogen removal efficiency at steady state with different HRT's. ($\Theta_c=12$ d, $COD_0=1270$ mg/L)

Figure 4.35 shows the effluent total nitrogen and total nitrogen removal efficiencies with different HRT's at steady state conditions. The effluent total nitrogen concentrations fluctuated with increasing HRT's. At HRT of 11.87 hours and 14.4 hours the effluent total nitrogen concentration was approximately 75 mg/L having a removal efficiency of approximately 67%. The highest total nitrogen removal efficiency was obtained with HRT's of 5.9 and 10.1 hours. The nitrogen source which was applied in urea form represents the natural composition of the wastewater characteristic that is seen in livestock applications and wastewaters coming from croplands due to fertilizers. Nitritation-Anammox process is the emerging biological nutrient removal technology used widely nowadays for wastewaters including high nitrogen and low biodegradable organic materials. The shortcut conversion of NH_4 to NO_2 and then to N_2 brings an advantage for energy saving when compared with conventional nitrification-denitrification processes. The consortium used in the experiments with RS and AS have an advantage to uptake and convert nitrogen sources, respectively. AS including ammonium oxidizing bacteria may oxidize

partially NH_4 to NO_2 and may enhance nitrogen removal with annomox bacteria by converting these substances into N_2 gas. The dissolved oxygen concentrations ($\text{DO}_0=8 \text{ mg/L}$) decreased in time at all HRT's giving a hint of nitrogen removal via annomox bacteria that prefers anoxic environment. $\text{NO}_3\text{-N}$ is generated as a by-product in the entire process through further oxidation of NO_2 or via the annomox process. Annomox bacteria are difficult to cultivate because of their long generation time and they have a low tolerance to differentiations in environmental conditions. However, these reactions may be present in our experiments due to high sludge age ($\Theta_c=12$ days). Reports depict that annomox bacteria have a doubling time of 11 days (Isaka et al., 2006). The Total Nitrogen, $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$ removal efficiencies with different HRT's were depicted in Figure 4.36. Uptake of nitrogen via Rhodobacter and removal of nitrogen via nitrification-annomox reactions may explain the fluctuations in removal efficiencies of nitrogen source with increasing HRT's. The highest removal efficiencies in total nitrogen was seen with the lowest HRT (same with 10 hours of HRT) where uptake mechanism may be dominated with a removal efficiency of 76% ($\text{NH}_4\text{-N}$: 64%, $\text{NO}_3\text{-N}$: 84%). When RS and AS was used alone in batch mode the TN, $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ removal efficiencies were slightly higher at AS with a reaction time of 5 days which is not enough for growth of bacteria with high doubling time. The maximum nitrogen removal efficiencies were obtained at 10.1 hour. The TN, $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ removal efficiencies were nearly 76, 67, 86% respectively. The removal efficiencies were very close to HRT 5.9 hours. Probably uptake of nitrogen sources influenced the reaction mechanism in the co-culture. The lowest nitrogen removal was prevailed at HRT of 11.9 hours. The TN, $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ removal efficiencies were 67, 41 and 64%, respectively. The bacteria that needs high generation time in AS may be dominated in co-culture consortium which resulted with low nitrogen removal and high COD removal. Further increases of HRT allowed to increase nitrogen removal. Probably the nitrification and annomox reactions took place at HRT's of 14.4 and 15.5 hours. The TN, $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ removal efficiencies were 72, 51, and 78% at HRT of 15.5 hours.

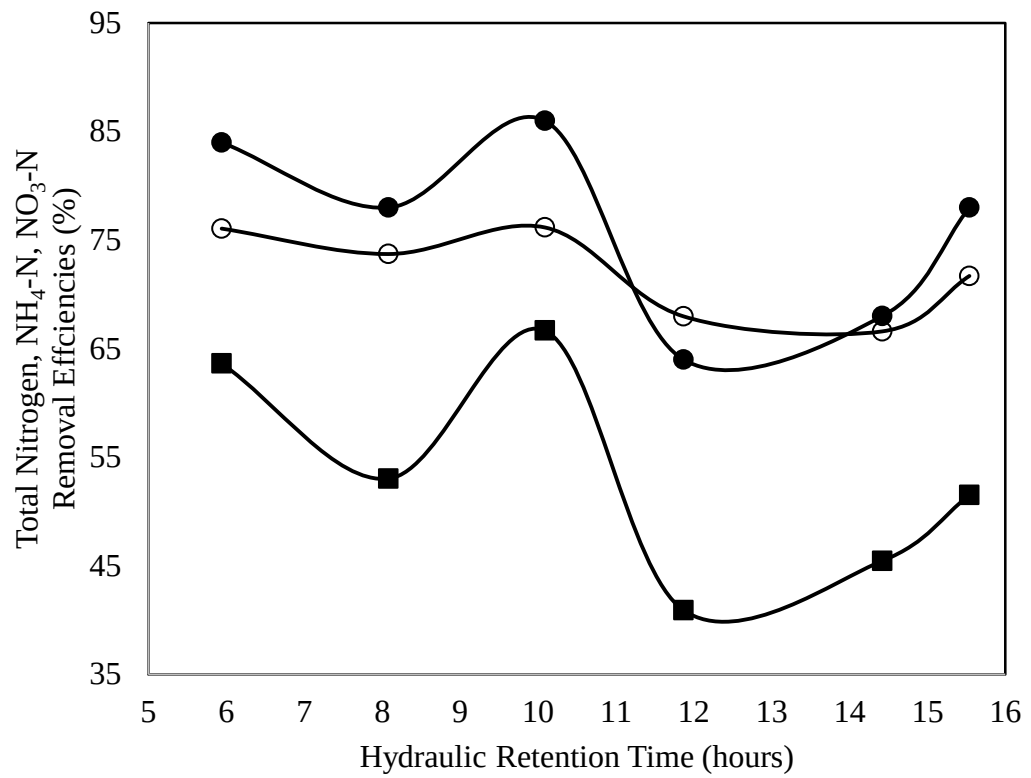


Figure 4.36 Variation of TN, NH₄-N, NO₃-N removal efficiency at steady state with different HRT's.
 ○TN, ■ NH₄-N, ●NO₃-N ($\Theta_c=12$ d, COD₀=1270 mg/L)

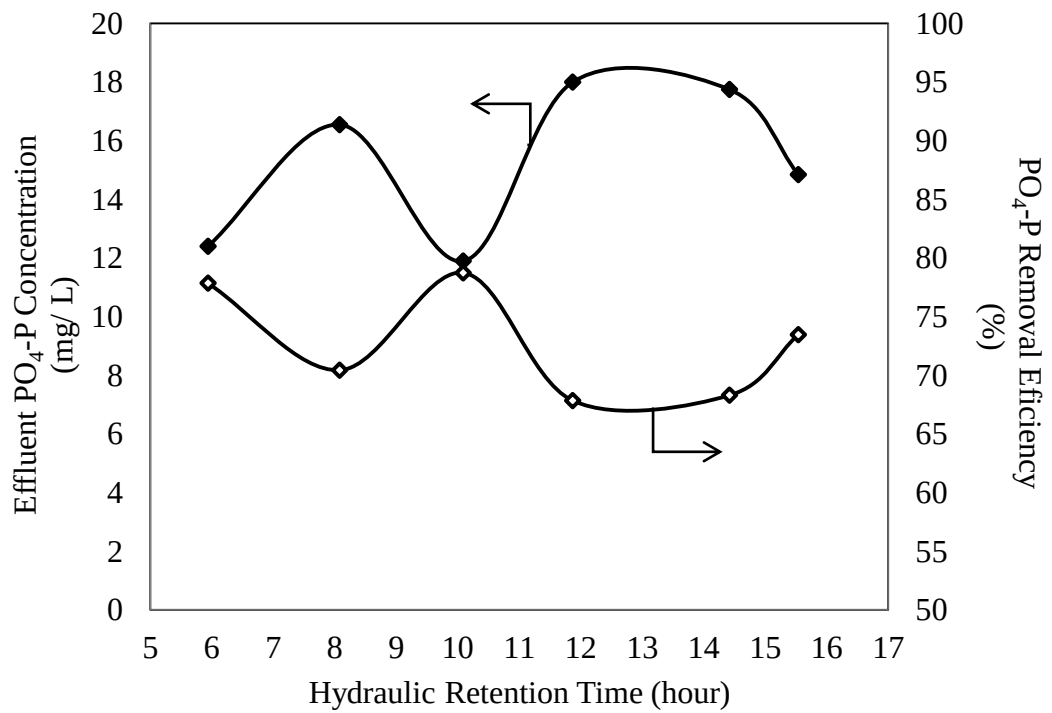


Figure 4.37 Variation of PO₄-P removal efficiency at steady state with different HRT's

The P-PO₄ removal efficiency (Figure 4.37) was above ~68% in all tested HRT's, which were higher than in batch experiments. The effluent PO₄ concentration varied between 12.4- 18.0 mg/ L. The PO₄ removal efficiencies fluctuated with increasing HRT's. The P-PO₄ concentrations were assimilated by biomass which was shown in Figure 4.33.c. The biomass concentrations showed that at HRT 11.9 and 14.4 h were around 2 g/ L. At HRT 15.5 h, it was 2.25 g/ L. At the remaining HRT's, the biomass concentration increased with decreasing HRT's. In higher biomass concentrations (>2.25 g/ L), the P-PO₄ uptake was higher which explains higher P-PO₄ removal efficiencies.

The effluent concentrations were not meeting the water quality standards according to regulations. However, it has to be considered that the simulated surfacewater characteristic is very close to domestic wastewater which can be classified as open sewage. Besides, Porsuk River has much lower nutrient concentrations than the chosen sampling point of wastewater which can be treated well to meet the receiving water discharge standards. It can be concluded that using a co-culture of activated sludge and *R. sphaeroides* to treat nutrients may be used as a natural treatment method.

CHAPTER FIVE

CONCLUSIONS

The target of this thesis is nutrient uptake with a special organism from wastewaters. As an alternative treatment method to conventional treatment technologies for excessive nutrients can be a novel approach using *Rhodobacter sphaeroides* for nutrient removal. This method can be applied especially to surface waters containing excessive nutrients due to agricultural applications in certain basins. The benefits of this method over conventional ones can be; lower investment and operational costs, and may be applied directly into water streams in special conditions. The main purpose of this thesis is to develop a treatment method for surface waters contaminated by nutrients in agricultural basins. A model wastewater taken from Porsuk River with high excessive nutrient conditions was simulated and used to remove the nutrients.

Different experiments were conducted with *Rhodobacter sphaeroides* to evaluate the nutrient uptake capacity of the organism. First environmental conditions to enhance nutrient uptake by *Rhodobacter sphaeroides* was evaluated by comparing aerobic-anaerobic and light-dark conditions. Then a strength wastewater with high COD contents and low C/ N ratios were tested to understand the COD removal capacity of the organism. The same experiments were conducted with high COD/ TN ratios for COD removal. Then the model wastewater taken from Porsuk River was simulated to have the same COD concentration. COD/ TN and COD/ TP ratios were changed to evaluate the nutrient uptake capacity of the culture. The COD/ TN/ TP ratio of the model wastewater was applied to a co-culture of activated sludge and *Rhodobacter sphaeroides* to enhance nutrient uptake. The optimized co-culture of activated sludge and *R. sphaeroides* was used in an activated sludge system to investigate the effects of hydraulic retention time (HRT) on nutrient removal.

The experiments except investigation of effects of HRT on nutrient removal was carried out in shake flask experiments to understand the nutrient removal performance of the culture and the co-culture. Shake flask experiments were carried

out in an incubator shaker at 30 °C. 150 rpm. at an initial pH of 7 with a working volume of 200 mL. Continuous experiments were carried out in a 1 L reactor in different HRT's.

In the first experiment environmental conditions were tested to optimize the nutrient uptake capacity of *R. sphaeroides*. In different environmental conditions *Rhodobacter sphaeroides* metabolic pathway switches into different ways to produce hydrogen, polymers or different end products effecting the COD removal efficiency. Aerobic dark conditions were favored for COD removal. As a strength wastewater the initial COD concentration was chosen 23 g/ L. In these conditions 67.35% COD removal with an ORP of around 150- 170 mV was obtained. The sole carbon source of glucose was used in all environmental conditions. and all the glucose was consumed under aerobic dark conditions.

R. sphaeroides was used to evaluate its COD removal efficiency with low COD/ TN ratios ranging between 100/ 0- 100/ 8. A strength wastewater with an initial COD concentration of 13- 15 g/ L was applied to the experimental bottles. The nitrogen fixation capacity of the culture was high and the COD removal efficiencies in low COD/ TN ratios were over 93%. The highest removal efficiency was obtained with a COD/ TN ratio of 100/ 2. The pH varied between 7- 8 along the experiments and the ORP ranged between 100- 160 mV in all experimental bottles.

The same experiments were carried out with high COD/ TN ratios ranging between 100/ 5- 100/ 25. The behavior of the culture under excessive carbon and nitrogen concentrations outlined the nutrient uptake performance of the organism. The highest COD removal efficiency was obtained with a COD/ TN ratio of 100/ 23 with a COD removal efficiency of 42%. The pH was nearly same in all tested bottles and was 8 at the end of the incubation period while the DO was in low concentrations and the ORP was near to zero. The DO and ORP due to high initial COD concentrations effected the probable organism growth and lowered the COD removal efficiencies.

In the next experiments a surface water sample taken from Sevinç, Eskişehir where one of the branches of Porsuk River passes. was simulated. COD/ TN (100/ 5-100/ 25) and COD/ TP (100/ 0.5- 100/ 2) ratio effects were investigated with a synthetic wastewater mimicking the sample. *R. sphaeroides* removed all the COD in a COD/ TN ratio of 100/ 23. The pH, ORP and DO ranged between 6.7- 7.1, 220-300 mV, 7.4 mg/ L, respectively in all tested conditions. The total phosphorus removal efficiency in COD/ TN ratio experiments was around 54±3%. At COD/ TN ratio of 100/ 20 the total nitrogen removal efficiency was the highest with a percentage of 82%. The remaining TP and TN concentration were not meeting the surface water discharge standards which are respectively SSM, COD, TN and TP for 500, 4000, 0, 0 mg/ L (Regulation on Water Pollution Control, 2004, 25687), needing additional nutrient treatment. All the COD was removed in COD/ TP ratio experiments. The pH was nearly in neutral within the experiments. The ORP and DO was 275 mV and 7.6 mg/ L. respectively in the COD/ TP ratio of 100/ 1.4 which is representing the model wastewater. Despite COD removal TP removal was low and it is understood that *Rhodobacter sphaeroides* could not uptake phosphate from the wastewater sufficiently. The TN removal efficiencies decreased with increasing COD/ TP ratios but were over 82%. In these experiments the final TP, TN, NO₃⁻ and NH₄⁺ concentrations couldn't meet the surface water discharge standards. The highest biomass growth was obtained with a COD/ TP ratio of 100/ 1.4 (1.8 g/ L). It is understood that the deficiency of phosphate effects slightly the culture growth.

A co-culture consisted of *Rhodobacter sphaeroides* and activated sludge with a high C/ N/ P ratio was used for nutrient removal. The characteristic of the sampled wastewater was used for different co-culture ratio experiments. The co-culture ratio of 1/ 5 was able to convert all the COD concentration with a removal efficiency of 100%. The pH was 7.7 and the ORP was 180- 220 mV and the DO concentrations were 3.7- 5.6 mg/ L in the co-culture experiments. The highest total phosphorus removal efficiency was obtained with a co-culture ratio (AS/ RS) of 1/ 5 (72%). The highest total nitrogen removal was obtained with the co-culture ratio of 1/ 5 and was 89.5%. Nitrate and ammonium concentrations also decreased with time. The tested final nutrients could not meet the surface water discharge standards.

Continuous experiments were performed in an activated sludge unit in order to investigate the COD, nitrogen and phosphorus removal performance at different hydraulic retention times (Θ_H). Θ_H varied between 5.94- 15.54 h, sludge age (Θ_c) was kept constant at 12 days and feed COD concentration was constant at 1270.3 mg/ L. A co-culture consisted of activated sludge (AS) and *Rhodobacter sphaeroides* (RS) with a ratio of 1/ 5 (AS/ RS) was used in the experiments. The optimum HRT throughout the experiments was 10.1 h with COD, TN, NO_3^- , NH_4^+ and PO_4 removal efficiencies of 80, 76, 86, 67 and 79%, respectively. At different HRT's the reaction mechanisms changed due to probable changing co-culture consortium. The effluent concentrations were not meeting the water quality standards according to regulations. However, it has to be considered that the simulated surfacewater characteristic is very close to domestic wastewater which can be classified as open sewage. Besides, Porsuk River has much lower nutrient concentrations than the chosen sampling point of wastewater which can be treated well to meet the receiving water discharge standards. It can be concluded that using a co-culture of activated sludge and *R. sphaeroides* to treat nutrients may be used as a natural treatment method.

RECOMMENDATION FOR THE FUTURE STUDIES

A co-culture of activated sludge and *R. sphaeroides* to treat nutrients may be used as a natural treatment method. However meeting the receiving water discharge standards is very difficult with a wastewater that can be classified as domestic wastewater. Following recommendations may be followed for nutrient removal in wastewaters:

1. Different operational conditions such as biomass concentration might be tested in the activated sludge system,
2. Lower COD/ TN/ TP ratios might be tried, simulating different regions of Porsuk River; that can easily meet receiving water discharge standards,
3. Fed-batch process, sequential batch reactor may be tried to enhance nutrient removal.
4. Researches with immobilized systems can be applied to increase C, N and P removal.

5. Different types of PSB's can be subjected to test the C, N and P removal efficiency in wastewaters.



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APPENDICES

APPENDIX A – Data of determination of environmental conditions with *R. sphaeroides* maximizing COD removal experiment

Table A.1 Variation of pH with time in different ambient conditions

pH				
	Aerobic-Light	Aerobic-Dark	Anaerobic-Light	Anaerobic-Dark
0	6.10	6.53	6.00	6.05
24	5.85	5.86	5.39	5.86
48	5.79	5.34	4.80	5.65
72	6.30	5.58	5.70	6.18
96	6.21	5.90	5.75	6.17
120	6.28	6.53	6.13	6.42
168	6.33	5.80	4.69	5.89

Table A.2 Variation of ORP with time in different ambient conditions

ORP (mV)				
	Aerobic-Light	Aerobic-Dark	Anaerobic-Light	Anaerobic-Dark
0	199.5	149.3	129.1	85.6
24	181.0	65.4	25.9	40.8
48	198.9	170.0	48.2	8.3
72	168.7	164.7	-15.1	-0.9
96	179.2	166.9	-39.2	-18.6
120	168.7	153.3	-56.1	8.8
168	154.9	5.8	70.5	9.0

Table A.3 Variation of COD concentrations with time in different ambient conditions

COD (mg/ L)				
	Aerobic-Light	Aerobic-Dark	Anaerobic-Light	Anaerobic-Dark
0	14656	18878	19723	18372
24	21411	20060	22763	22763
48	23269	16345	16345	17527
72	18034	11278	17358	19723
96	21918	12123	19047	19554
120	20905	8070	21749	25465
168	23269	6550	17865	20398

Table A.4 Variation of Glucose concentrations with time in different ambient conditions

Glucose (mg/ L)				
	Aerobic-Light	Aerobic-Dark	Anaerobic-Light	Anaerobic-Dark
0	20000	20000	20000	20000
24	6398	5183	6457	7117
48	6293	3735	5846	6740
72	6207	2765	5181	6548
96	5924	1829	4775	6471
120	6048	1184	4443	6230
168	5930	35	3279	6031

Table A.5 Variation of Acetic acid concentrations with time in different ambient conditions

Acetic Acid (mg/ L)				
	Aerobic-Light	Aerobic-Dark	Anaerobic-Light	Anaerobic-Dark
24	324	544	493	298
48	287	894	492	285
72	300	1819	504	283
96	312	952	458	277
120	292	342	456	317
168	313	107	332	285

APPENDIX B – Data of effects of initial COD/ TN ratios on COD removal ranging between 100/ 0 and 100/ 8 experiment

Table B.1 Variation of COD concentrations at different COD/ TN ratios with time

COD (mg/ L)												
	Hours	0	24	48	72	144	168	192	216	240	264	312
COD/ N	100/ 0	15912	13548	13041	8481	6623	6286	5779	4259	3246	1388	1050
	100/ 1	16419	13210	11690	9157	8819	7806	4597	3584	1050	881	544
	100/ 2	13379	11352	8819	8143	7975	6961	3921	2232	1557	1219	206
	100/ 4	15237	14561	14392	9832	7975	7806	4428	2739	2401	1050	881
	100/ 6	16081	11014	10170	8650	7299	6623	2739	2064	1557	881	544
	100/ 8	13717	12197	11183	10339	7637	7299	3415	1388	713	206	1557

Table B.2 Variation of pH with time in different COD/ TN ratios

pH												
	Hours	0	24	48	72	144	168	192	216	240	264	312
COD/ N	100/ 0	7.04	5.30	6.68	7.79	8.76	8.31	7.81	6.88	7.01	7.62	7.84
	100/ 1	7.04	5.49	6.08	7.76	8.73	8.63	8.63	7.82	7.44	7.42	6.96
	100/ 2	7.04	5.37	7.01	7.82	8.66	8.29	7.55	6.92	6.86	7.55	7.71
	100/ 4	7.04	5.58	5.88	7.73	8.76	7.29	7.34	6.93	6.80	7.41	7.48
	100/ 6	7.04	5.49	6.24	7.66	8.75	7.86	7.30	6.88	7.42	7.14	7.57
	100/ 8	7.04	5.39	6.98	7.75	8.73	7.98	6.90	6.95	7.45	7.33	7.96

Table B.3 Variation of ORP with time at different COD/ TN ratios

ORP (mV)												
	Hours	0	24	48	72	144	168	192	216	240	264	312
COD/ N	100/ 0	90.0	40.1	25.1	33.6	14.8	120.6	150.6	142.6	149.5	112.9	122.6
	100/ 1	90.0	66.2	48.6	31.4	31.3	130.4	106.2	117.3	104.8	102.4	98.1
	100/ 2	90.0	64.6	13.7	77.5	50.5	102.4	104.0	153.1	110.3	108.6	113.6
	100/ 4	90.0	42.0	61.6	44.4	48.8	115.7	119.6	138.4	112.7	120.6	124.1
	100/ 6	90.0	57.1	60.5	95.1	55.8	108.1	75.8	126.7	122.1	131.4	156.3
	100/ 8	90.0	54.9	18.2	16.6	58.5	124.8	98.1	142.6	128.3	113.5	131.8

APPENDIX C – Data of effects of initial COD/ TN ratios on COD removal ranging between 100/ 5 and 100/ 25 experiment

Table C.1 Variation of COD with time at different COD/ TN ratios

COD (mg/ L)						
	Hours	0	24	48	72	96
COD/ N	100/ 5	15405	12366	11521	10170	8800
	100/ 10	13379	11285	9495	8143	7800
	100/ 15	14561	13886	13041	11521	10339
	100/ 20	13717	12534	12197	11183	10170
	100/ 23	14054	10508	9500	8800	8143
	100/ 25	14730	14392	11521	9495	8819

Table C.2 Variation of pH with time at different COD/ TN ratios

pH						
	Hours	0	24	48	72	96
COD/ N	100/ 5	7.0	6.5	6.5	7.2	8.1
	100/ 10	7.1	6.0	6.3	7.3	8.0
	100/ 15	7.1	5.9	6.2	7.0	8.0
	100/ 20	7.0	5.7	6.4	7.2	8.2
	100/ 23	7.1	5.8	6.3	6.8	8.0
	100/ 25	7.1	5.9	6.3	7.3	8.0

Table C.3 Variation of ORP with time at different COD/ TN ratios

ORP (mV)						
	Hours	0	24	48	72	96
COD/ N	100/ 5	150.0	55.6	-20.3	22.7	48.7
	100/ 10	150.0	37.0	-224.5	45.7	38.6
	100/ 15	150.0	47.8	-77.4	52.4	24.0
	100/ 20	150.0	60.2	-40.4	24.2	10.5
	100/ 23	150.0	60.4	-181.7	-51.4	0.6
	100/ 25	150.0	48.2	-162.3	-21.1	10.0

Table C.4 Variation of Dissolved Oxygen with time at different COD/TN ratios

Dissolved Oxygen (mg / L)						
	Hours	0	24	48	72	96
COD/N	100/5	8.31	0.30	0.24	0.22	0.19
	100/10	8.31	0.26	0.23	0.22	0.19
	100/15	8.31	0.27	0.24	0.19	0.16
	100/20	8.31	0.32	0.25	0.20	0.17
	100/23	8.31	0.24	0.27	0.24	0.21
	100/25	8.31	0.35	0.24	0.25	0.30

APPENDIX D – Data of effects of COD/ TN Ratio in a model synthetic wastewater experiment

Table D.1 Variation of COD concentrations with time at different COD/ TN ratios in a model synthetic wastewater

COD (mg/ L)							
	Hours	0	24	48	72	96	120
COD/ N/ P	100/ 5/ 1.4	1051	882	578	392	139	0
	100/ 10/ 1.4	1017	899	662	409	88	0
	100/ 15/ 1.4	1169	713	544	291	105	54
	100/ 20/ 1.4	1051	916	561	443	139	0
	100/ 23/ 1.4	1051	730	510	274	156	37
	100/ 25/ 1.4	983	781	443	375	189	54
	Control	1000	-	-	-	933	983

Table D.2 Variation of pH with time at different COD/ TN ratios in a model synthetic wastewater

pH							
	Hours	0	24	48	72	96	120
COD/ N/ P	100 /5/ 1.4	7.08	6.57	7.63	7.44	6.47	6.71
	100 /10/ 1.4	7.02	6.75	8.29	8.16	7.15	6.73
	100 /15/ 1.4	6.86	6.35	8.17	8.23	7.26	6.99
	100 /20/ 1.4	6.84	6.56	7.96	8.04	7.25	7.04
	100 /23/ 1.4	7.01	6.51	7.87	7.95	7.30	7.09
	100 /25/ 1.4	6.95	6.52	7.88	7.84	7.51	6.81
	Control	7.04	-	-	-	6.83	6.95

Table D.3 Variation of ORP with time at different COD/ TN ratios in a model synthetic wastewater

ORP (mV)							
	Hours	0	24	48	72	96	120
COD/ N/ P	100/ 5/ 1.4	280.6	271.7	220.2	263.9	270.1	245.4
	100/ 10/ 1.4	306.4	260.8	196.5	230.1	196.2	261.7
	100/ 15/ 1.4	280.7	239.1	182.8	196.9	243.2	223.4
	100/ 20/ 1.4	275.3	233.8	173.3	189.5	255.2	275.2
	100/ 23/ 1.4	271.8	218.4	169.4	197.6	232.7	265.2
	100/ 25/ 1.4	292.3	242.8	179.6	216.0	212.6	291.7
	Control	301.4	-	-	-	291.5	296.3

Table D.4 Variation of dissolved oxygen concentration with time at different COD/ TN ratios in a model synthetic wastewater

Dissolved Oxygen (mg/ L)						
	Hours	0	24	48	72	96
COD/ N/ P	100/ 5/ 1.4	6.22	6.16	7.64	7.58	7.31
	100/ 10/ 1.4	6.21	5.52	7.66	7.59	7.42
	100/ 15/ 1.4	6.24	5.06	7.40	7.46	7.41
	100/ 20/ 1.4	6.25	5.09	7.57	7.65	7.59
	100/ 23/ 1.4	6.20	4.38	7.53	7.80	7.62
	100/ 25/ 1.4	6.25	4.14	7.46	7.72	7.52
	Control	6.30	-	-	-	7.23

Table D.5 Variation of total phosphorus concentration with time at different COD/ TN ratios in a model synthetic wastewater

TP (mg/ L)							
	Hours	0	24	48	72	96	120
COD/ N/ P	100/ 5/ 1.4	60.5	49.0	43.0	33.0	30.8	28.5
	100/ 10/ 1.4	61.0	58.5	48.5	35.5	29.9	29.5
	100/ 15/ 1.4	64.0	52.5	48.0	38.0	31.0	27.5
	100/ 20/ 1.4	61.5	50.5	44.5	39.5	30.9	30.5
	100/ 23/ 1.4	64.0	51.0	49.0	36.5	29.8	27.5
	100/ 25/ 1.4	65.5	52.0	47.0	37.5	31.9	28.5
	Control	63.0	-	-	-	62.5	62.0

Table D.6 Variation of total nitrogen concentration with time at different COD/ TN ratios in a model synthetic wastewater

TN (mg/ L)				
	Hours	0	48	120
COD/ N/ P	100/ 5/ 1.4	100	70	20
	100/ 10/ 1.4	205	115	45
	100/ 15/ 1.4	320	240	65
	100/ 20/ 1.4	440	320	80
	100/ 23/ 1.4	495	445	205
	100/ 25/ 1.4	600	430	220
	Control	110		105

Table D.7 Variation of biomass concentration with time at different COD/ TN ratios in a model synthetic wastewater

SSM (mg/ L)			
	Hours	0	120
COD/N/ P	100/ 5/ 1.4	1.60	2.60
	100/ 10/ 1.4	1.60	2.20
	100/ 15/ 1.4	1.60	4.00
	100/ 20/ 1.4	1.60	1.80
	100/ 23/ 1.4	1.60	2.00
	100/ 25/ 1.4	1.60	2.60
	Control	0.00	0.00

APPENDIX E – Data of effects of COD/ TP ratio in a model synthetic wastewater experiment

Table E.1 Variation of COD concentrations with time at different COD/ TP ratios in a model synthetic wastewater

COD (mg/ L)							
	Hours	0	24	48	72	96	120
COD/N/ P	100/ 20/ 0.5	1084.6	983.2	763.7	425.9	206.4	3.7
	100/ 20/ 0.8	1050.8	915.7	713.0	442.8	172.6	54.4
	100/ 20/ 1	1017.0	898.8	628.6	341.5	155.7	37.5
	100/ 20/ 1.4	1050.8	915.7	561.0	442.8	138.8	0.0
	100/ 20/ 2	1000.1	932.6	577.9	459.7	223.2	54.4

Table E.2 Variation of pH with time at different COD/ TP ratios in a model synthetic wastewater

pH							
	Hours	0	24	48	72	96	120
COD/N/ P	100/ 20/ 0.5	6.96	4.93	6.93	7.37	6.85	6.6
	100/ 20/ 0.8	6.93	4.74	6.95	7.48	6.71	6.78
	100/ 20/ 1	7.08	4.83	7.34	7.55	6.97	6.44
	100/ 20/ 1.4	6.84	6.56	7.96	8.04	7.25	7.04
	100/ 20/ 2	6.91	5.8	6.63	7.2	7.03	6.43

Table E.3 Variation of ORP with time at different COD/ TP ratios in a model synthetic wastewater

ORP (mV)							
	Hours	0	24	48	72	96	120
COD/ N/ P	100/ 20/ 0.5	302.4	261.2	212.1	186.4	172.3	194.8
	100/ 20/ 0.8	293.1	256.4	188.2	186.4	184.5	182.9
	100/ 20/ 1	271.3	242.5	174.3	183.6	163.7	163.5
	100/ 20/ 1.4	275.3	233.8	173.3	189.5	255.2	275.2
	100/ 20/ 2	280.6	226.7	184.7	195.2	199.6	166.1

Table E.4 Variation of dissolved oxygen concentration with time at different COD/ TP ratios in a model synthetic wastewater

Dissolved Oxygen (mg/ L)						
	Hours	0	24	48	72	96
COD/ N/ P	100/ 20/ 0.5	6.34	6.03	6.65	7.2	6.81
	100/ 20/ 0.8	6.42	5.73	6.24	7.01	6.54
	100/ 20/ 1	6.41	5.22	6.71	7.05	6.57
	100/ 20/ 1.4	6.25	5.09	7.57	7.65	7.59
	100/ 20/ 2	6.37	5.51	6.23	6.95	6.42

Table E.5 Variation of total phosphorus concentration with time at different COD/ TP ratios in a model synthetic wastewater

TP (mg/ L)							
	Hours	0	24	48	72	96	120
COD/ N/ P	100/ 20/ 0.5	25.0	23.5	20.5	16.5	11.0	8.5
	100/ 20/ 0.8	36.5	34.0	26.0	21.0	17.0	15.0
	100/ 20/ 1	45.0	42.5	38.0	34.0	26.0	24.0
	100/ 20/ 1.4	61.5	50.5	44.5	39.5	30.9	30.5
	100/ 20/ 2	88.0	71.5	68.5	62.0	48.0	42.5

Table E.6 Variation of biomass concentration with time at different COD/ TP ratios in a model synthetic wastewater

SSM (mg/ L)			
	Hours	0	120
COD/ N/ P	100/ 20/ 0.5	0.7	1.3
	100/ 20/ 0.8	0.7	1.4
	100/ 20/ 1	0.7	1.7
	100/ 20/ 1.4	0.6	1.8
	100/20/ 2	0.7	1.5

APPENDIX F – Data of evaluation of *R. sphaeroides* and activated sludge co-culture ratios with a model synthetic wastewater experiment

Table F.1 Variation of COD concentrations with time at different co-culture ratio

COD (mg/ L)							
	Hours	0	24	48	72	96	120
AS/ RS	1/ 1	1253	1101	882	612	308	88
	1/ 2	1220	1068	797	527	156	4
	1/ 5	1186	1068	747	510	122	0
	1/ 10	1186	1152	781	561	156	21
	1/ 20	1169	1061	848	645	325	105
	RS	1237	1118	831	460	206	37
	AS	1220	1152	814	595	274	105

Table F.2 Variation of pH with time at different co-culture ratio

pH							
	Hours	0	24	48	72	96	120
AS/ RS	1/ 1	7.00	7.68	8.05	8.07	7.77	7.74
	1/ 2	7.00	7.75	8.07	8.09	7.61	7.78
	1/ 5	7.00	7.43	8.10	8.11	7.82	7.82
	1/ 10	7.00	7.18	8.06	8.04	7.71	7.77
	1/ 20	7.00	6.98	8.04	8.05	7.52	7.69
	RS	7.00	6.26	7.79	7.96	7.37	7.23
	AS	7.00	7.43	7.31	7.81	7.40	7.19

Table F.3 Variation of ORP with time at different co-culture ratio

ORP (mV)							
	Hours	0	24	48	72	96	120
AS/ RS	1/ 1	226.7	222.5	167.1	174.2	196.0	202.4
	1/ 2	245.4	246.7	180.5	196.3	205.1	207.5
	1/ 5	265.2	2408	188.7	187.4	183.7	191.1
	1/ 10	239.1	218.9	193.1	195.2	198.5	201.7
	1/ 20	242.8	219.6	194.9	189.6	178.2	186.2
	RS	221.4	276.4	149.2	173.3	103.3	164.2
	AS	216.8	281.3	166.1	178.5	182.7	189.5

Table F.4 Variation of dissolved oxygen concentration with time at different co-culture ratio

Dissolved Oxygen (mg/ L)							
	Hours	0	24	48	72	96	120
AS/ RS	1/ 1	6.44	5.22	4.08	4.24	3.88	3.92
	1/ 2	6.42	5.13	4.20	4.31	4.08	4.11
	1/ 5	6.51	5.17	3.57	4.22	4.13	4.22
	1/ 10	6.55	5.06	4.77	4.36	4.27	4.53
	1/ 20	6.47	4.95	5.34	4.81	4.59	5.58
	RS	6.49	5.71	6.83	6.91	6.85	6.73
	AS	6.48	6.08	0.82	3.12	3.42	3.74

Table F.5 Variation of total phosphorus concentration with time at different co-culture ratio

TP (mg/ L)							
	Hours	0	24	48	72	96	120
AS/ RS	1/ 1	62.5	51.5	42.0	36.0	28.5	22.0
	1/ 2	62.5	50.5	40.5	32.0	27.5	18.5
	1/ 5	62.5	50.0	38.0	31.0	26.0	17.5
	1/ 10	62.5	51.0	39.5	33.0	27.0	21.5
	1/ 20	62.5	53.5	42.0	34.5	28.5	25.0
	RS	62.5	51.5	44.0	39.0	30.5	28.5
	AS	62.5	55.0	41.0	37.5	28.5	25.5

Table F.6 Variation of TN, NO_3^- and NH_4^+ with time at different co-culture ratios

		TN (mg/ L)			NO_3^- (mg/ L)			NH_4^+ (mg/ L)		
	Hours	0	72	120	0	72	120	0	72	120
AS/ RS	1/ 1	502.50	220.00	55.00	30.00	20.00	2.00	70.00	27.50	10.00
	1/ 2	502.50	210.00	55.00	30.00	15.00	1.75	70.00	27.50	10.00
	1/ 5	502.50	222.50	52.50	30.00	17.50	1.75	70.00	29.00	9.50
	1/ 10	502.50	220.00	52.50	30.00	15.00	1.75	70.00	28.00	10.50
	1/ 20	502.50	232.50	57.50	30.00	17.50	1.75	70.00	27.50	11.00
	RS	502.50	237.50	85.00	30.00	20.00	2.50	70.00	31.00	15.50
	AS	502.50	215.00	70.00	30.00	17.50	2.50	70.00	29.00	13.00

Table F.7 Variation of biomass concentration with time at different co-culture ratio

SSM (mg/ L)							
	Hours	0	24	48	72	96	120
AS/RS	1/ 1	0.60	0.80	0.80	1.00	1.20	1.80
	1/ 2	0.60	0.60	1.00	1.20	1.40	2.00
	1/ 5	0.60	0.60	1.00	1.20	1.40	2.00
	1/ 10	0.60	0.60	1.00	1.40	1.60	2.00
	1/ 20	0.60	0.60	0.80	0.80	1.00	1.40
	RS	0.60	0.80	1.20	1.40	1.80	1.80
	AS	0.60	0.60	1.00	1.20	1.40	1.60