The Role of Thermophilic Bacterial Strains in Aerobic Wastewater Treatment

BY

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This thesis, written by BASSAM ABUBAKER under the direction of his thesis advisor and approved by his thesis committee, has been presented and accepted by the Dean of Graduate Studies, in partial fulfilment of the requirements for the degree of MASTER OF LIFE SCIENCES IN LIFE SCIENCES.

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Dedication

I would like to express my heartfelt gratitude especially to my dearest mother, father and to my sisters and brothers for their love, care and encouragements.

I would like to convey my heartfelt gratitude to my ever-loving wife Ala Abubaker and to my dear son Yamen, who are always there for me no matter what, always encouraging me towards success, coping with my never ending complaints and chatter, and giving me so much love.

I would like to dedicate my thesis to the holy land “Palestine”. The land of peace, love and forgiveness.

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COD: Chemical Oxygen Demand

TOC: Total Organic Compound

ABSTRACT

Full Name: Bassam Hussein Ibrahim Abubaker

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The present work was carried out to assess the efficiency of thermophilic bacteria in wastewater treatment. A consortium of four thermophilic bacterial strains was used in this study. At 55 °C and 50 °C, the thermophilic consortium showed a very high efficiency in removing total organic carbon (TOC), reaching approximately 90.2% in 36 hours and 79% in 60 hours, respectively, while the removal of nitrogen was approximately 92% and 83%, respectively. The removal of phosphorous was relatively low at 15.4% and 37% in the same set of experiments. The consortium of thermophilic bacteria grown at 37 °C exhibited substantially different performance. After 60 hours of incubation at 37 °C, only 58.5% of TOC, 75.9% of nitrogen and 13.2% of phosphorous were removed. Properties such as the fast growth of thermophilies may explain the exceptional ability of these thermophilic organisms in waste removal.

Keywords: thermophilic, mesophilic, bacteria, wastewater, treatment, nutrient

ملخص الرسالة

الإسم الكامل: بسام حسين ابراهيم أبوبكر.

عنوان الرسالة: الدور الذي تقوم به سلاسلات من البكتيريا المحبة للحرارة في معالجة مياه الصرف الصحي بوجود الأكسجين.

التخصص: العلوم الحياتية.

تاريخ الدرجة العلمية: ١٢ / ٢٠٢٠

البحث التالي تم تنفيذه لقياس فعالية البكتيريا المحبة للحرارة في معالجة مياه الصرف الصحي. في هذا البحث تم استخدام مزيج من أربع سلالات من البكتيريا المحبة للحرارة. عند درجة حرارة ٥٥ و ٥٠ درجة مئوية، البكتيريا المحبة للحرارة أظهرت فعالية عالية في التخلص من مجمل الكربون العضوي يصل إلى ٩٠,٢% في ٣٦ ساعة و ٧٩% في ٦٠ ساعة على التوالي. بينما التخلص من النيتروجين كان حوالي ٩٢% و ٨٣% على التوالي. التخلص من الفسفور كان نوعاً ما ضعيف بحدود ١٥,٤ و ٣٧% في نفس التجارب السابقة.

تم تجربة تكاثر مزيج من البكتيريا المحبة للحرارة على درجة حرارة ٣٧ درجة مئوية أظهر أداء مختلف تماماً. بعد ٦٠ ساعة من بدء التجربة على درجة حرارة ٣٧ درجة مئوية، ٥٨,٥ من مجمل الكربون العضوي و ٥٨,٩ من النيتروجين و ١٣,٢ من الفسفور تم التخلص منهم.

خصائص البكتيريا المحبة للحرارة مثل سرعة التكاثر قد تفسر هذه القدرة الإستثنائية على التخلص من الفضلات.

كلمات مفتاحية: البكتيريا المحبة للحرارة، البكتيريا المعتدلة، البكتيريا، مياه صرف صحي، معالجة، عناصر غذائية

CHAPTER 1

INTRODUCTION

Since the 1980s, water use has increased significantly (1% per year worldwide). It is expected to increase at the same rate until 2050 (United Nations, 2018). The increased use of water for municipal purposes has led to an increase in the volume of wastewater generated. Wastewater poses various challenges, such as the spread of diseases and risks to the environment, water availability, and food security. Removing pollutants and safely treating wastewater provides an opportunity to reuse the water produced in different fields, including human consumption, industry, and agriculture.

According to statistics from the World Bank and Food and Agriculture Organization (FAO) of the United Nations, the annual average volume of reused water is up to 25% in the USA, China, Japan, Spain, and Australia. In Singapore, reused water is used for approximately 30% of the nation's current water needs, and they are planning to increase this to 55% by 2060 (Angelakis & Snyder, 2015).

Wastewater treatment techniques involve several physical, chemical, and biological processes. The secondary stage in the treatment process relies on the biodegradation of wastes by microorganisms. Thus, studying the conditions of the biological waste biodegradation process is a key aspect of optimizing the efficiency of waste removal from wastewater.

For the sustainable use of such valuable water resources, there should be mitigation techniques that enhance the performance of wastewater treatment. Thermophilic wastewater treatment is a growing technology that has attracted scientific research and industrial applications. Moreover, the comparison between thermophilic and mesophilic conditions has indicated that thermophilic conditions have many advantages over mesophilic conditions in terms of high biodegradation rate, low sludge yield, and removal of pathogens. In contrast, thermophilic conditions present many challenges, and further investigation is required to overcome these challenges. Different techniques have been used to increase the efficiency of thermophilic bacteria in wastewater treatment. (Rozich & Bordacs, 2002).

The aim of this work is to investigate the efficiency of thermophilic bacteria in wastewater treatment and identify the optimum temperature and pH for achieving the maximum rate of treatment.

1.1 Bacterial growth and biodegradation process

Bacterial growth requires sufficient sources of carbon (organic compounds) and inorganic compounds to synthesize new cellular components. Optimizing the environmental conditions for bacterial growth enhances the biodegradation process of waste in wastewater treatment (Henze et al., 2019). Biological wastewater treatment is based on the role of bacteria in removing the waste (C, N, P) within certain conditions. Furthermore, select the best microorganism’s strains that adapt to grow significantly under such conditions. These conditions include the availability of electron doners (organic components), electron acceptors (such as oxygen and nitrate), temperature, pH, oxygen rate and other conditions (Henze et al., 2019).

1.2 Mesophilic and Thermophilic Bacterial strains

Based on their growth temperature, bacteria can be classified as mesophiles or thermophiles. Mesophiles grow in the moderate temperature range, from approximately 20 °C to 45 °C, while thermophiles are heat-loving, with an optimum growth temperature of 50 °C to 70 °C, a maximum of up to 70 °C-120 °C (hyperthermophiles) (Figure 1).

This study concentrates on utilizing thermophilic bacteria in wastewater treatment, and several previous studies have reported efficient wastewater treatment using thermophilic bacteria (Henze et al., 2019).

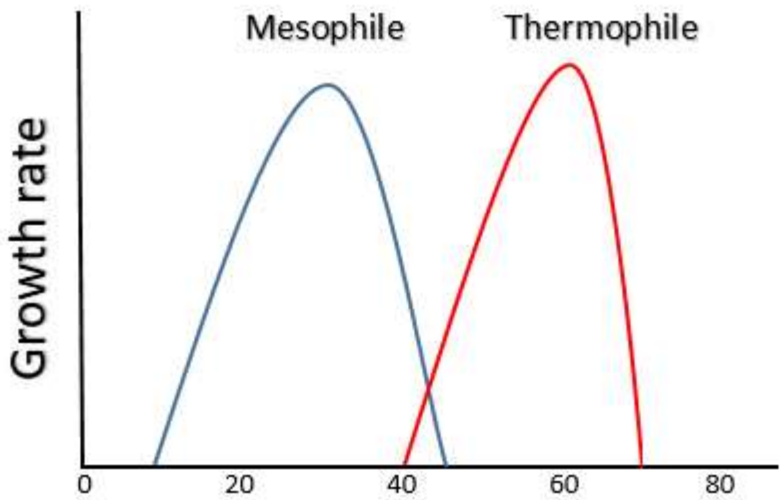


Figure 1 Effect of temperature on the microbial growth rate

1.3 Waste removal

The efficiency of microbial wastewater treatment is associated with the removal of soluble organic and inorganic compounds from its environment. A number of aerobic bacterial strains denitrify the wastewater by removing nitrogenous sources as well as removing phosphorus from its environment (Grady et al., 2011).

This study shows a significant removal rate of carbon sources, nitrogenous compounds and phosphorous by a consortium of thermophilic bacteria.

1.4 **Statement of the Problem**

As the human population continues to grow, the worldwide need for water increases significantly. The challenges concerning securing water resources and the reuse of wastewater will become increasingly important for sustainable life. Accordingly, wastewater treatment technologies must meet the massive increase in global water use through the development of new methods.

Moreover, poor water quality poses risks to public health and ecosystem services/functions. Wastewater contaminates the environment, causes the spread of various diseases, and damages ecosystems. Therefore, utilization of thermophilic bacteria, which are environmentally friendly, to biodegrade wastes is one of the most effective treatment processes. A consortium of thermophilic bacteria was used under different physical and chemical conditions to enhance the process of wastewater treatment. The optimum conditions for thermophilic wastewater treatment processes were investigated.

1.5 Study Significance

Water reuse offers tremendous potential to conserve water for humanity. Maintaining healthy reusable water requires much work and attention, and one of the attractive methods to achieve this is the use of thermophilic microorganisms to increase the rate of wastewater treatment.

This research used novel strains of thermophilic bacteria isolated from hot springs in

Saudi Arabia. These strains showed high efficiency in the waste biodegradation process in terms of decreasing the time, cost and energy consumption (A. Khalil, 2011).

The acceptable range of high temperatures in the kingdom of Saudi Arabia and the presence of previously isolated thermophilic bacteria from the same environment were important factors for investigating these thermophilic bacteria at the laboratory scale of synthetic wastewater treatment and possibly later in the field at a large scale .

1.6 Study Objectives

The main objective of the study is to demonstrate the efficiency of thermophilic bacteria in the synthetic municipal wastewater treatment process. The specific objectives are as follows:

1. Investigate the efficiency of wastewater treatment using a consortium of thermophilic bacteria, including *Anoxybacillus flavithermus AK1* and *Brevibacillus borselensis AK1*, in a lab-scale system. This was conducted by monitoring the removal of N and P and reducing dissolved oxygen
2. Determine the optimum temperature and pH for the treatment process.

CHAPTER 2

LITERATURE REVIEW

The theory behind this study is discussed in the following sections, starting from the process of wastewater treatment, characteristics of thermophilic bacteria, utilization of thermophilic bacteria in the treatment process, removal of nutrients (N, P), and bacterial community.

2.1 **Wastewater treatment**

Wastewater treatment is an ancient method for reusing water. This process has been developed throughout human history, leading to improvements in the efficiency of treating and distributing water (Paranychianakis et al., 2015). The main objective of the treatment process is to convert wastewater into a usable effluent, one with no side effects on either human health or the environment. Thus, the produced water can be used or returned to the water cycle (Ameta & Ameta, 2018).

According to statistics from the United Nations (United Nations, 2018), water use is increasing significantly, and a large amount of wastewater needs to be treated.

The use of thermophilic bacteria during this process will increase biodegradation at high temperatures, which will increase the speed of biological transformation, remove wastes in a shorter amount of time, and reduce costs (Jahren et al., 2002; Collivignarelli et al., 2014). Another study reported that temperature is the most important factor affecting the biodegradation process in biological wastewater treatment (Alisawi, 2020).

Ab Halim et al. (2016) studied the effect of temperature on the efficiency of organic and nutrient removal during the biological treatment of synthetic wastewater. Three (3) L sequencing batch reactors operated at different temperatures (30, 40 and 50 °C) for simultaneous COD, phosphate and ammonia removal. The statistical comparison of COD, phosphate and ammonia for the effluent revealed that there was a significant difference between groups of all of the working temperatures of the bioreactors. At high temperatures, the results exhibited a positive correlation with the removal of waste.

2.2 Thermophilic bacteria

Thermophilic microorganisms grow and thrive in high-temperature environments (55–60 °C) (Brock 1986). The main habitats of thermophilic bacteria are hot springs, geothermal vents, and volcanic vents (A. Khalil, 2011; El-Gayar et al., 2017). Thermophiles can be classified into three groups depending on their survival and growth temperatures: (i) thermophiles (optimum growth at 50 – 60 °C), (ii) extremophiles (optimum growth at 60 – 80 °C), and (iii) hyperthermophiles (optimum growth at 80 – 110 °C). Many studies in wastewater treatment consider temperatures above 45 °C as thermophilic conditions (Timothy M. Lapara & Alleman, 1999).

The ability of these microorganisms to stay alive in harsh environments related to many features of these organisms, including structural and biological factors. Thermophilic organisms play a key role in industrial applications, including pharmaceutical and industrial processes (Duncan et al., 2017; Jawad & Aqma, 2018), and livestock wastes (Juteau, 2006). These microorganisms can also be used effectively in pollutant degradation, e.g., wastewater treatment (A. Khalil et al., 2018b).

2.2.1 **Novel thermophilic strains**

The novel thermophilic bacteria *Anoxybacillus flavithermus* AK1 and *Brevibacillus borstelensis* AK1, isolated from hot springs in Saudi Arabia, have a small genome size but high GC content. The strains possess putative genes for the degradation of a wide range of substances. The genomic features supposedly enhance the biodegradation process of wastes (A. Khalil et al., 2018a).

2.3 **Utilizing thermophilic bacteria in wastewater treatment**

A comparison study between a full-scale conventional treatment and thermophilic treatment of wastewater reported many potential benefits of thermophilic conditions, such as saving energy, decreasing the cost, and advanced nutrient management of the effluent (De Vrieze et al., 2016).

The use of thermophilic bacteria during the treatment process is an advanced treatment technology due to the removal efficiency of organic and inorganic wastes by reducing the chemical oxygen demand (COD) at a high rate and minimizing the generation of sludge compared with other wastewater treatment technologies (Kurian et al., 2005).

A thermophilic wastewater system is characterized by an increasing removal rate of organic and inorganic waste components. High rates of chemical reactions and specific thermostable enzymes determine the efficiency of this process (Suvilampi & Rintala, 2003).

Many studies concentrate on both enhancing the performance of wastewater treatment and comparing different methods of the treatment process. Thermophilic wastewater treatment has many advantages over conventional mesophilic conditions. The advantages include a high efficiency rate, fast biodegradation, low amount of remnant sludge, and inhibition of pathogens (Abeynayaka & Visvanathan, 2011). Timothy M. Lapara & Alleman (1999) reported a drastic reduction in the COD from 760 mg COD/L to 160 mg COD/L. In contrast, the process has many disadvantages, such as the need for a huge aeration tank, high oxygen requirements because of the high biodegradation rate, low sludge yield, and foam formation (Rozich & Bordacs, 2002). Furthermore, the effluent from thermophilic treatment is usually of poorer quality (Suvilampi & Rintala, 2003).

Table 1 Biological performance (removal of the chemical oxygen demand) of a thermophilic aerobic membrane bioreactor

Type of wastewater	Temp (°C)	COD removal %	Reference
Synthetic (lactose and gelatin)	55	93-98	(Timothy M. Lapara et al., 2000)
Pulp and paper wastewater	55	70	(Lopetegui & Sancho, 2003)
Kraft pulp condensate	55	87	(Dias et al., 2005)
Pharmaceutical wastewater	48-50	98	(Collivignarelli et al., 2014)
Sewage sludge	55	80	(Collivignarelli et al., 2015)
Municipal sewage sludge	54.1-54.9	85	(Collivignarelli et al., 2017)

A recent study reported efficient removal of pollutant COD (78% - 97%) and total phosphorous (>60%) within a hydraulic retention time of 5 days. The study was conducted at 48 °C using a thermophilic aerobic membrane bioreactor (Collivignarelli et al., 2020).

2.4 Phosphorous and nitrogen removal

The presence of phosphorous and nitrogen in wastewater causes one of the most serious environmental problems because of its role in the eutrophication process that leads to algal blooms. The consequences of algal blooms include low dissolved oxygen, fish kill, and depletion of fauna and flora.

Many studies have shown the significance of using thermophilic bacteria to remove nitrogen from wastewater. Henze et al. (2019) showed that many environmental and operational advantages, including low energy consumption, economic operating costs and preservation of more water. Lopez-Vazquez et al. (2014) investigated the removal of nitrogen from industrial wastewater with an operating temperature of 50 °C, and nitrite- and ammonium-oxidizing bacteria were identified, such as *Nitrosomonas halotolerant*, *Nitrospira* sub lineage, and *Nitrosomonas oligotrophy*.

Several advantages regarding the use of aerobic thermophiles for wastewater treatment were reported. In this process, nutrient uptake is very fast, the conversion rate is highly efficient, and some organic and organic byproducts can be used as fertilizers, in addition to that , high removal rates of ammonia, sulfur and phosphorus were reported (Juteau, 2006).

2.5 Thermophilic wastewater treatment in industrial applications

Jahren et al. (2002) studied the treatment process in the pulp and paper industry under thermophilic conditions using a continuous lab-scale reactor (moving bed biofilm reactor). They noted that 60-65% of the total solid COD was removed from the wastewater. There was a high percentage of removal from the carbohydrate components (greater than 90%), and the material with low biodegradability was lignin (30-35%).

J. Nowakl et al. (2002) studied the use of thermophilic microorganisms in the potato starch industry, showing a comparison between the inclusions of a single strain of bacteria and using the complete mixture. The results showed that a mixture of bacteria has more metabolic activity than a single strain due to the reduction of the COD in the effluent that results from the treatment process. This process characterized by a high oxygen demand. The use of shake flask systems decreased the COD compared to the bioreactor system (fermenter).

Dias et al. (2005) investigated the treatment of waste from a Kraft pulp mill at different temperatures (45 and 50 °C) using a continuous membrane bioreactor to treat the foul. The authors focused on three components: COD, total reduced sulfur (TRS), and methanol. The loading COD was 5.12 kg, and the hydraulic retention time was 19 h. The results show a relationship between increased temperature and higher removal efficiency of COD and methanol.

Collivignarelli et al. (2014) investigated the biodegradation process of high-strength liquid waste from the pharmaceutical industry using the thermophilic biological process. At high temperatures (49 °C), a pilot plant was used in the treatment process. The results show a high removal rate of COD (approximately 98%), very low sludge production, and removal of 99% of the phosphorus.

A combination of two thermophilic treatment mechanisms was studied by Collivignarelli et al. (2018), a thermophilic membrane reactor and conservative activated sludge, by designing semi-industrial pilot plants to simulate the full-scale plant for the treatment of heavy-strength waste. This study yielded a high COD removal of 85% for the conservative activated sludge and 78% for the thermophilic treatment plants.

2.6 **Bacterial community**

The microbial community structure, physiology, and maintenance requirements (the total energy consumed by cells for all functions, excluding growth) in a continuous flow bioreactor, with thermophilic aerobic conditions (55 °C), using synthetic wastewater with a COD of approximately 3100 mg/L, at different hydraulic residue times (HRT), were investigated. The analysis of the results using PCR–DGGE shows a similarity between the thermophilic aerobic biological wastewater treatment processes and conventional mesophilic systems concerning the bacterial maintenance requirements. Moreover, HRT is an important factor that affects the microbial community (T. M. Lapara et al., 2000a).

Timothy M. Lapara et al. (2000b) studied the phylogenetic diversity of the microbial community in seven-stage wastewater treatment plants with mesophilic and thermophilic reactors. PCR-DGGE of the 16S rRNA gene was used to complete the nucleotide sequence for the gene. A quantitative study of the microbial communities under thermophilic conditions (50-58 °C) and mesophilic conditions (28-32 °C) was conducted. The results show that an increasing temperature is inversely proportional to the microbial communities in the wastewater treatment.

Use of a combined treatment system consisting of thermophilic aerobic digestion as a pretreatment followed by mesophilic anaerobic digestion resulted in an increase in the microbial activity and shortened the residence time from 4 days to 1 day. The bacterial community in the thermophilic bioreactor was dominated by *Ureibacillus* and *Thermus* bacteria (Jang et al., 2013).

CHAPTER 3

MATERIALS AND METHODS

Overview:

This work intended to investigate the performance of thermophilic bacteria in wastewater and their ability to carry out the process under different temperatures and pH. The effect of high temperature on the wastewater process by enhancing the biodegradation rate and reducing the duration of the treatment process was also investigated.

The stages of the experimental work were as follows:

Media preparation, culturing and growth of the bacterial consortium, operating the bioreactor and different experimental conditions, monitoring bacterial growth and analysis of TOC, N, and P.

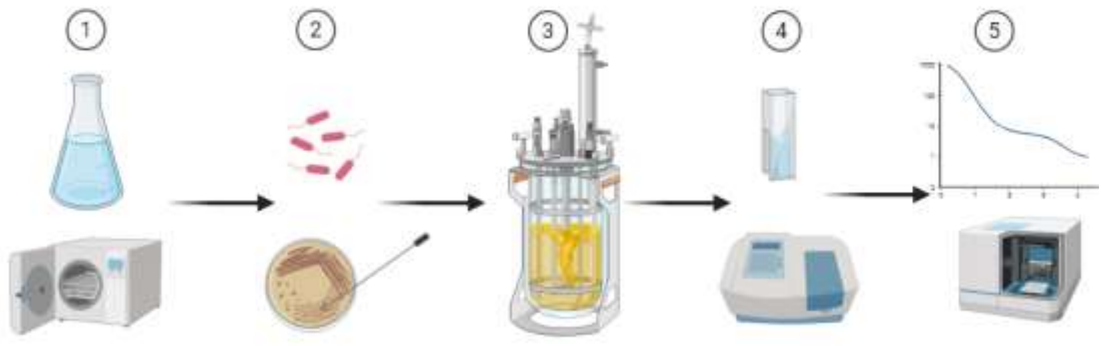


Figure 2 Steps of the lab work. The figure was created by Biorender.com

3.1 **Synthetic wastewater media**

The media was prepared by the addition of the following ingredients (in mg/L): sucrose (10,000), K_2HPH_4 (2500), KH_2PH_4 (2500), $(NH_4)_2HPO_4$ (1000), $MgSO_4 \cdot 7H_2O$ (200), $FeSO_4 \cdot 7H_2O$ (10) and $MnSO_4 \cdot 7H_2$ (7). The ingredients were added to 1 L distilled water in a 2-L flask and autoclaved at 121 °C for 4 h.

3.2 **Bacterial consortium**

The synthetic media were added to a fully automated control bioreactor with controlled pH, temperature, aeration and mixing rate (model number: Bio. 0919. India) and inoculated by a consortium of thermophilic bacteria. In the first set of experiments, the temperature was adjusted to 50 °C, and the pH was adjusted to 8. The culture was aerated at a rate of 0.5 L/min and mixed at 100 rpm. The optical density at 600 nm was measured at zero time to monitor bacterial growth.

The same set of experiments was repeated at 37 °C and a pH of 8. The culture was aerated at a rate of 0.5 L/min and mixed at 100 rpm.

The bioreactor cultures were operated until a steady state was reached with respect to biomass concentration (stationary phase), as determined by periodic measurement of the optical density (OD600) using a UV-Visible recording (spectrophotometer, V-760-Japan). Once the steady state was reached, it was defined by a <5% change in OD600.

Different conditions were investigated to increase the efficiency of thermophilic bacteria in wastewater treatment.

3.2.1 Thermophilic consortium under thermophilic conditions

The bioreactor was inoculated with a consortium of thermophilic bacteria. In the first set of experiments, the temperature was adjusted to 50 and 55 °C, and the pH was adjusted to 8. The culture was aerated at a rate of 0.5 L/min and mixed at 100 rpm. The optical density at 600 nm was monitored every 12 hours to measure bacterial growth. Another set of experiments was conducted at 50 °C with different pH values, one experiment at a pH of 4 and another experiment at a pH of 10 (to see the effect of pH on the wastewater treatment process).

3.2.2 Thermophilic consortium under mesophilic conditions

The same set of experiments was repeated at 37 °C and a pH of 8. The culture was aerated at a rate of 0.5 L/min and mixed at 100 rpm. Samples were collected every 12 hours to measure bacterial growth and to analyze TOC, N, and P.

3.2.3 Mesophilic consortium under mesophilic conditions

To perform a comparative study between the efficiency of thermophilic and mesophilic bacteria, another set of experiments was conducted using a mesophilic bacterial consortium at 37 °C and a pH of 8.

3.3 Monitoring bacterial growth

To monitor bacterial growth, samples were taken every 6 hours to measure the OD600 and for viable cell count analysis. Viable cell counts were performed by the serial dilution method to count bacterial colonies on agar plates.

3.3.1 Sampling

The sampling was performed daily. For this, 100 ml of the experimental media and 100 ml of the control media were taken and centrifuged for 5 min at 6000 rpm (Centrifuge, Heraeus, D-37520, Germany). The supernatant was transferred to another sterile tube for analysis of TOC, nitrogen and phosphorous.

3.4 Nutrient analysis, nitrogen, phosphorous and TOC (total organic carbon)

Nitrate and nitrite in the collected samples were analyzed using a nutrient autoanalyzer (SAN^{++} Skalar System) at zero time (before adding the bacteria) and 24, 48 and 72 hours (after adding the bacteria). Briefly, for nitrate and nitrite, the sample was diluted 10 times and passed through a reduction column to reduce nitrate to nitrite through copper-cadmium (analysis of nitrite does not require this step). Then, the nitrite ions reacted with sulfanilamide and N-naphthylethylenediamine to form a red-colored product, and the intensity of the produced color was measured at 540 nm.

3.4.1 Total organic carbon (TOC) analysis

Total organic carbon (TOC) analysis was performed by employing the catalytic combustion of organic compounds in the sample to CO_2 in the presence of oxygen. Carbon dioxide (CO_2) was determined by a direct nondispersive infrared detector (NDIR). Briefly, the sample was diluted 400 times and acidified by adding a few drops of concentrated H_2SO_4. Twenty milliliters of the diluted and acidified sample was introduced into the TOC analyzer (Shimadzu TOC-V CPN) (APHA, 2005).

CHAPTER 4

RESULTS

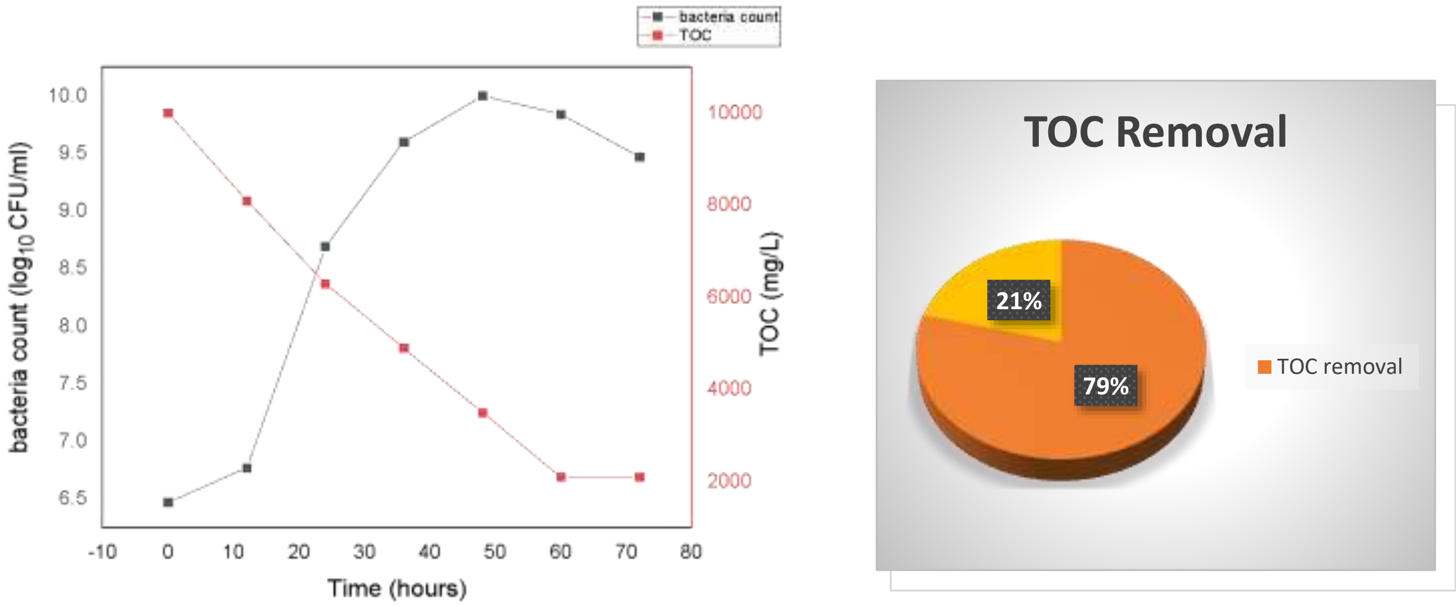
4.1 Thermophilic bacterial consortium under thermophilic conditions

4.1.1 Thermophilic bacterial consortium at 50 °C

A consortium of four thermophilic bacterial strains isolated from hot springs in Saudi Arabia was used in this study. At 50 °C, the thermophilic consortium showed a very high efficiency in removing TOC, which reached approximately 79% in 60 hours of incubation (Figure 3). The removal of phosphorous was relatively low at 15.4% (Figure 4), while the removal of nitrogen was approximately 83% (Figure 5) within 60 hours. Dissolved oxygen was significantly decreased from 3.43 ppm to 0.13 ppm in the same time frame (Figure 6).

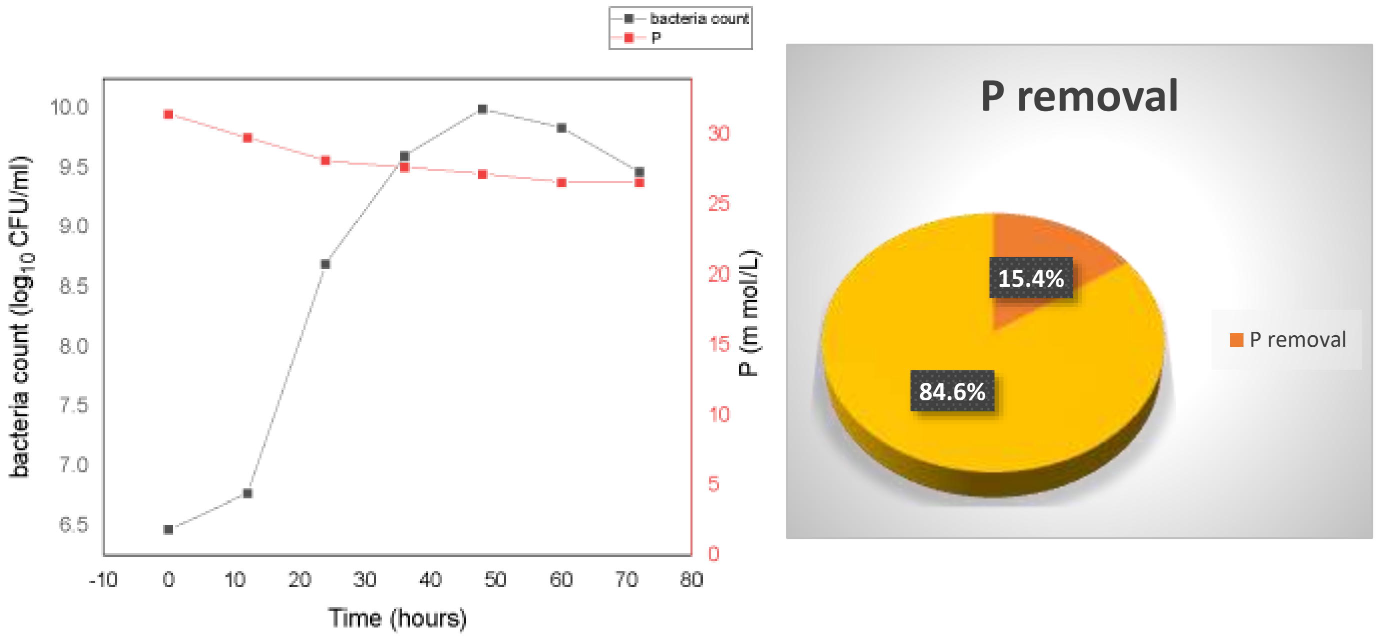
Table 2 Waste removal during biological wastewater treatment using a thermophilic consortium under thermophilic conditions

Time (hours)	OD	Bacterial count CFU/ml	Bacterial count Log_{10} CFU/ml	TOC mg/l	P m mol/L	NH_3 m mol/L	DO ppm
0	0.043	3×10^6	6.47	10000	31.49	22.5	3.43
12	0.431	6×10^6	6.77	8100	29.8	14.5	2.6
24	0.86	5×10^8	8.69	6300	28.16	8.4	0.65
36	1.51	4×10^9	9.6	4900	27.7	5.1	0.15
48	2.73	1×10^{10}	10	3500	27.21	4.3	0.13
60	3.81	7×10^9	9.84	2100	26.63	3.8	0.13
72	3.81	3×10^9	9.47	2100	26.63	3.8	0.13



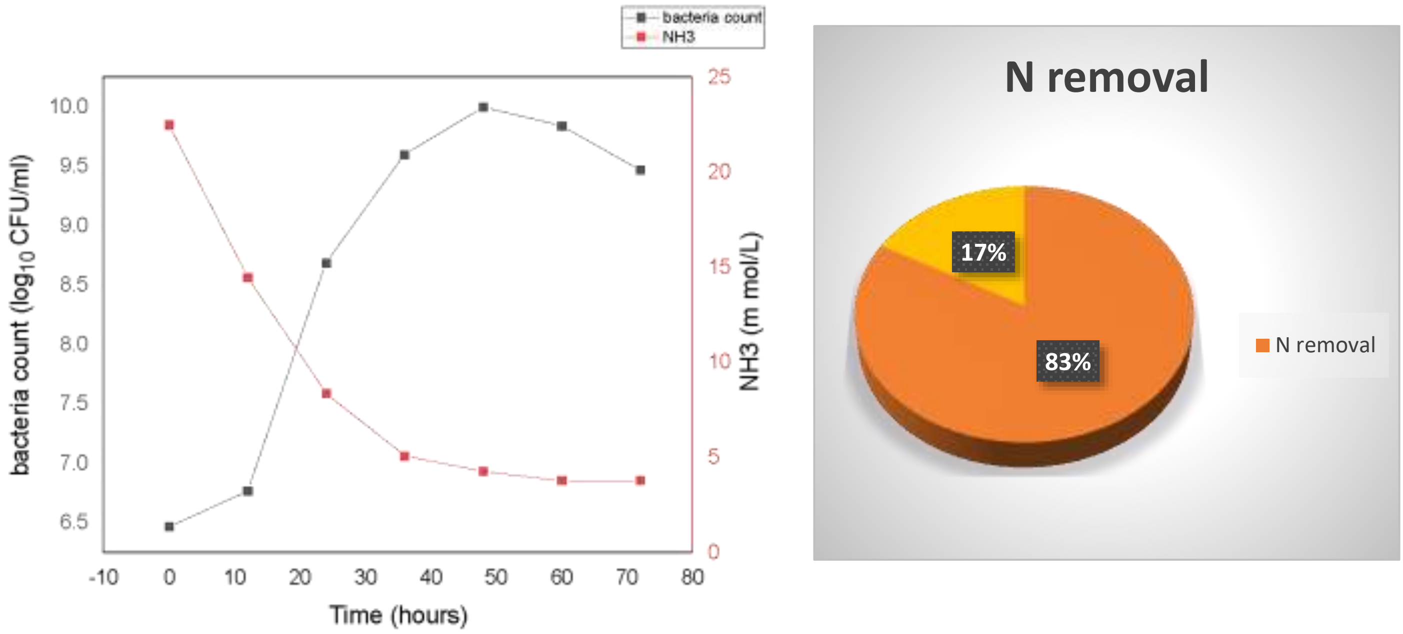
(a) (b)

Figure 3 (a) Removal of total organic carbon (TOC) by a thermophilic bacterial consortium at 50 °C. (b) Removal percentage of TOC at 50 °C.



(a) (b)

Figure 4 (a) Phosphorus removal by a thermophilic bacterial consortium at 50 °C. (b) Removal percentage of P at 50 °C.



(a) (b)

Figure 5 (a) NH_3 removal by a thermophilic bacterial consortium at 50 °C. (b) Removal percentage of N at 50 °C.

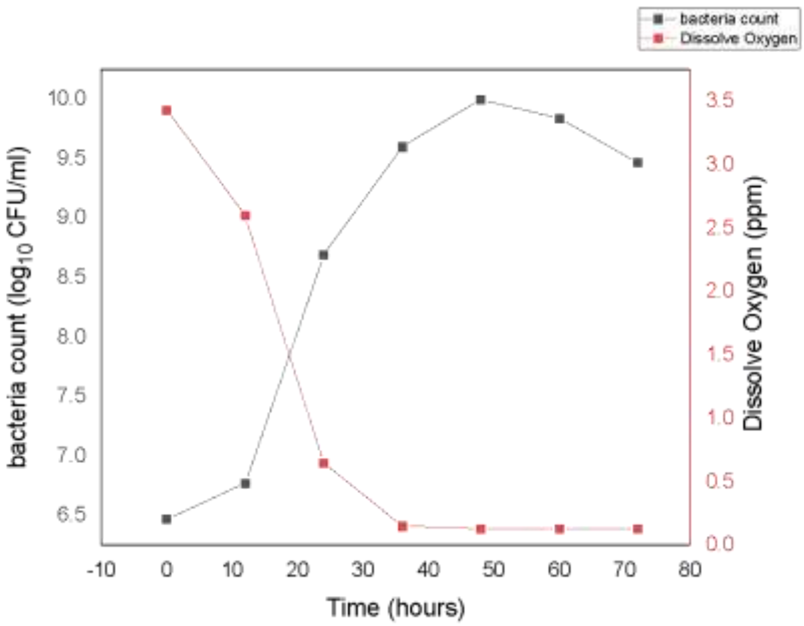


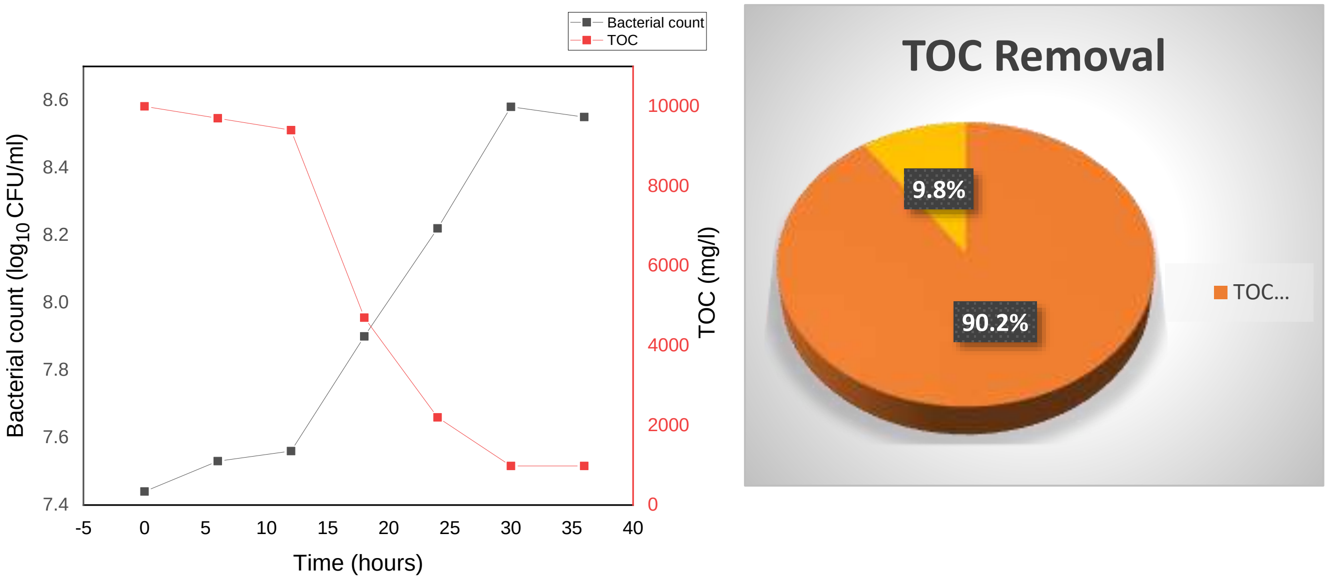
Figure 6 Dissolved oxygen (DO) consumption by a thermophilic bacterial consortium at 50 °C

4.1.2 Thermophilic bacterial consortium at 55 °C

A consortium of four thermophilic bacterial strains isolated from hot springs in Saudi Arabia was used in this study. At 55 °C, the thermophilic consortium showed a very high efficiency in removing TOC, which reached approximately 90.2% in 36 hours of incubation time (Figure 7). The removal of nitrogen was approximately 92% (Figure 8) within 36 hours. The removal of phosphorous was relatively low at 37% (Figure 9), while dissolved oxygen was significantly decreased from 4.96 ppm down to 0.25 ppm in the same time frame (Figure 10).

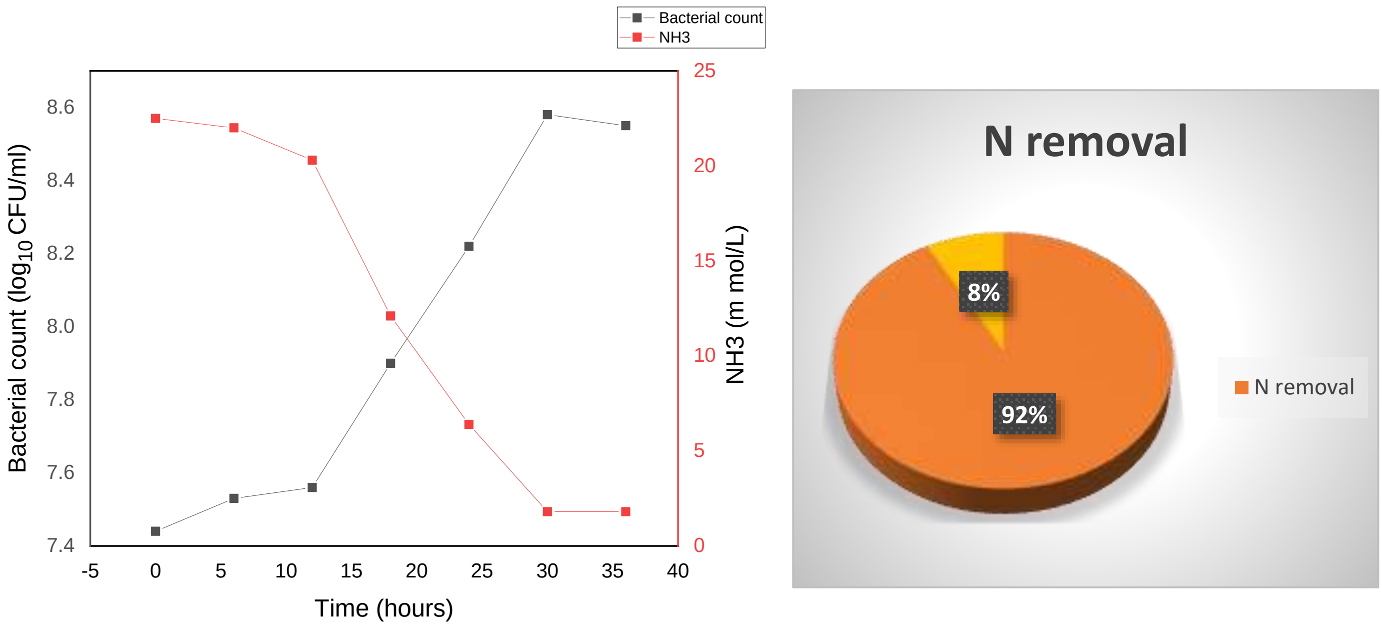
Table 3 Waste removal during biological wastewater treatment using a thermophilic consortium under thermophilic conditions (temp. at 55 °C)

Time (hours)	OD	CFU/ml	Bacterial count Log_{10} CFU/ml	TOC	P mmol/L	NH_3 mmol/L	DO ppm
0	0.139	2.78×10^7	7.44	10000	30.2	22.5	4.96
6	0.17	3.4×10^7	7.53	9700	30.1	22	4.87
12	0.184	3.68×10^7	7.56	9400	28.5	20.3	3.47
18	0.4	8×10^7	7.9	4700	25.0	12.1	1.82
24	0.84	1.68×10^8	8.22	2200	22.7	6.40	0.83
30	1.9	3.8×10^8	8.58	980	19.3	1.8	0.5
36	1.8	3.6×10^8	8.55	980	19.3	1.8	0.25



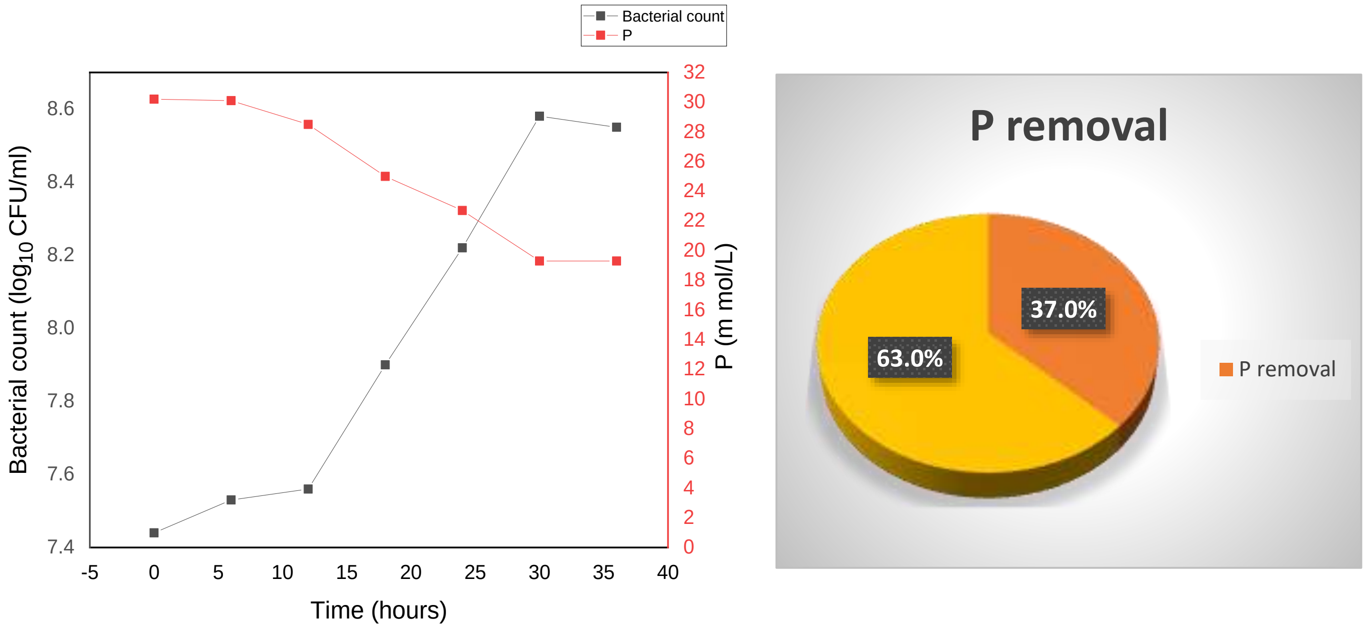
(a) (b)

Figure 7 (a) Removal of total organic carbon (TOC) by a thermophilic bacterial consortium at 55 °C. (b) Removal percentage of TOC at 55 °C.



(a) (b)

Figure 8 (a) NH_3 removal by a thermophilic bacterial consortium at 50 °C. (b) Removal percentage of N at 55 °C



(a) (b)

Figure 9 (a) Phosphorus removal by a thermophilic bacterial consortium at 55 °C. (b) Removal percentage of P at 55 °C.

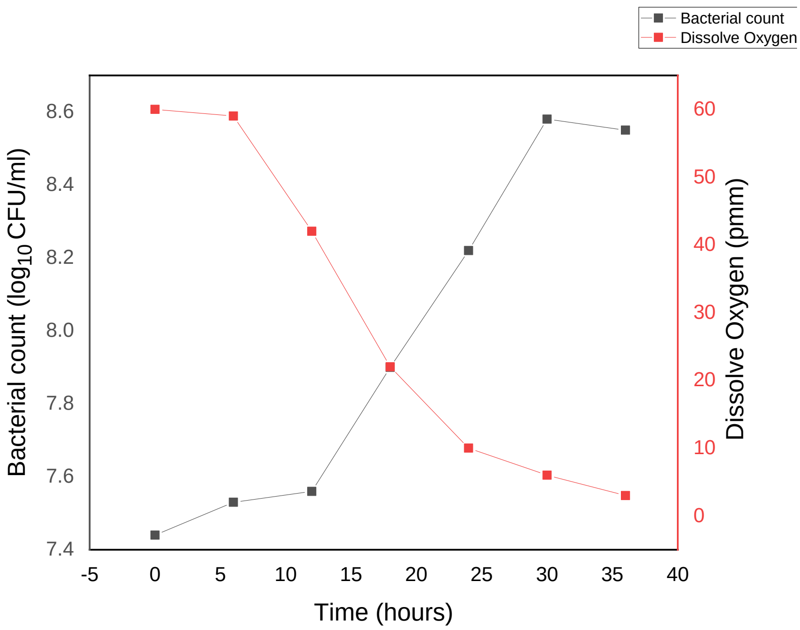


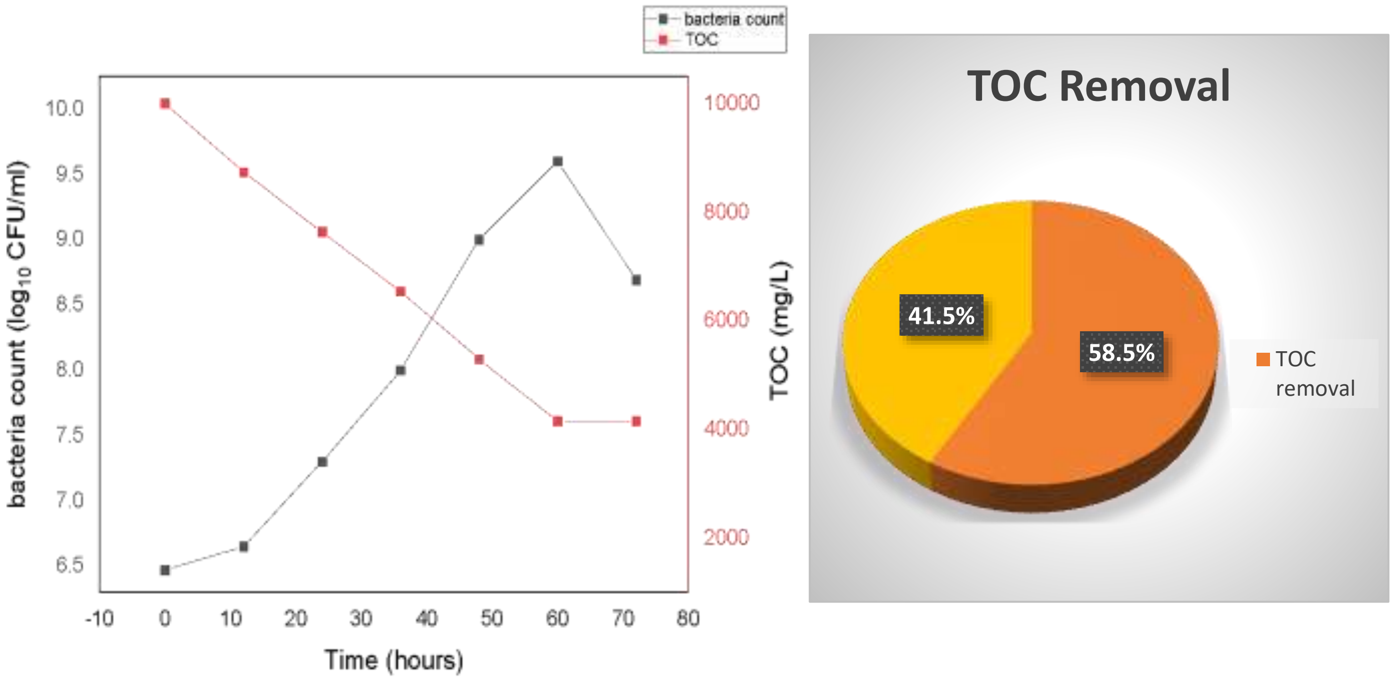
Figure 10 Dissolved oxygen (DO) consumption by a thermophilic bacterial consortium at 55 °C

4.2 Thermophilic bacterial consortium under mesophilic conditions

The consortium of thermophilic bacteria grown at 37 °C exhibited behavior and results that were substantially different from those of the consortium grown at 50 °C. By incubation for 60 hours at 37 °C, 58.5% of TOC (Figure 11), 13.2% of phosphorous (Figure 12), and 75.9% of nitrogen were removed (Figure 13). Dissolved oxygen was decreased from 3.43 ppm down to 0.28 ppm in the same time (Figure 14).

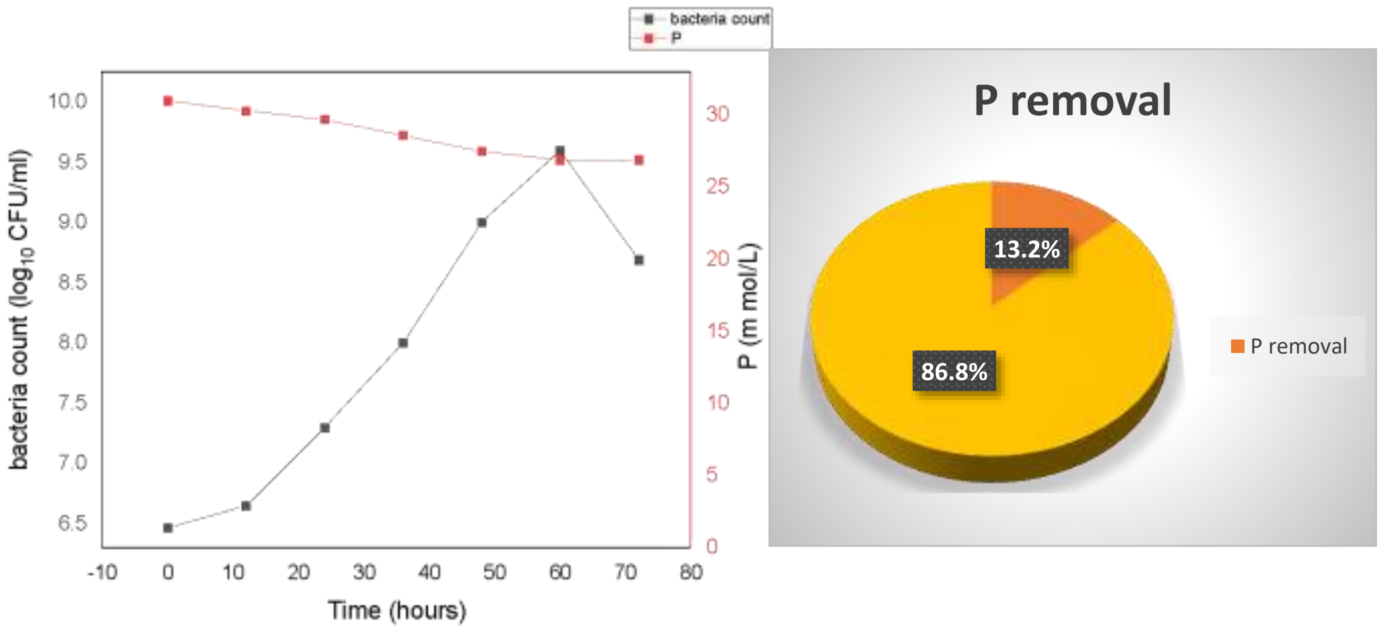
Table 4 Waste removal during biological wastewater treatment using a thermophilic consortium under mesophilic conditions

Time (hours)	OD 600 nm	Bacterial count CFU/ml	Bacterial count Log_{10} CFU/ml	TOC mg/l	P m mol/L	NH**3** m mol/L	DO ppm
0	0.032	3×10^6	6.47	10000	31	22	3.41
12	0.26	4.5×10^6	6.65	8734	30.3	16.2	3.05
24	0.51	2×10^7	7.3	7632	29.7	11.4	1.15
36	1.06	1×10^8	8	6538	28.6	7.7	0.33
48	1.97	1×10^9	9	5289	27.5	6.4	0.28
60	2.4	4×10^9	9.6	4147	26.9	5.3	0.28
72	2.4	5×10^8	8.69	4147	26.9	5.3	0.28



(a) (b)

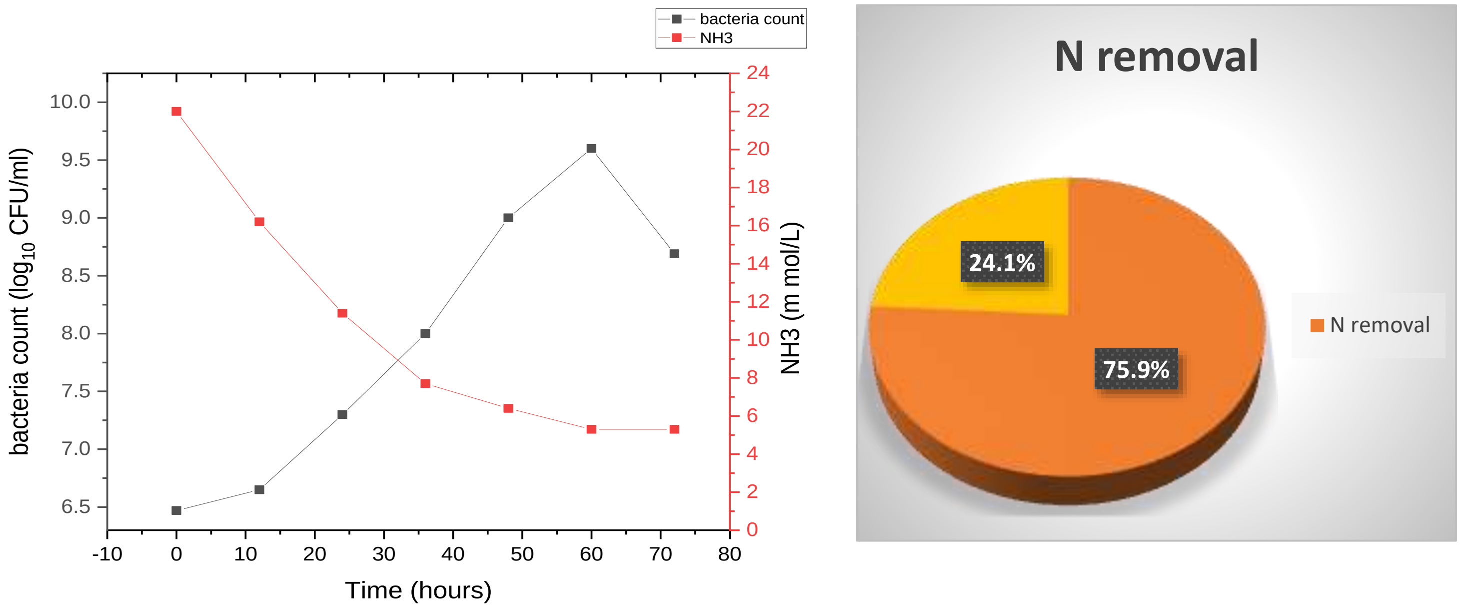
Figure 11 (a) Removal of total organic carbon (TOC) by a thermophilic bacterial consortium at 37 °C. (b) Removal percentage of TOC at 37 °C.



(a)

(b)

Figure 12 Phosphorus removal by a thermophilic bacterial consortium at 37 °C. (b) Removal percentage of P at 37 °C



(a) (b)

Figure 13 (a) NH3 removal by a thermophilic bacterial consortium at 37 °C. (b) Removal percentage of N at 37 °C.

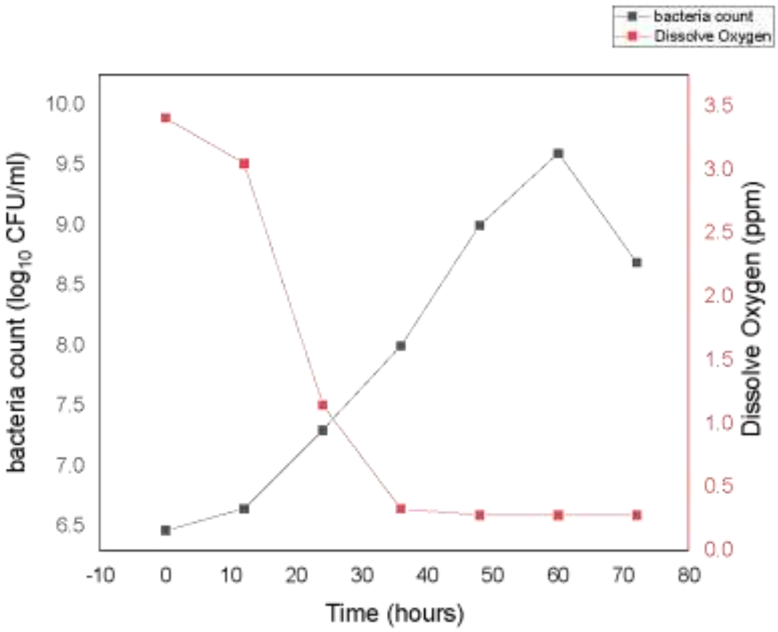


Figure 14 Dissolved oxygen (DO) consumption by a thermophilic bacterial consortium at 37 °C

Bacterial growth (CFU/ml) at temperatures of 50 °C, 55 °C, and 37 °C showed very significant consistency in relation to the removal of TOC, N and dissolved oxygen. Exponential growth of the thermophilic consortium was reflected directly in the fast removal of TOC and nitrogen within 60 hours of incubation at all three incubation temperatures, 50 °C, 55 °C and 37 °C. Phosphorous removal was slower at these temperatures.

The wastewater treatment process using a thermophilic bacterial consortium and synthetic wastewater at 55 °C showed much higher efficiency and removal capacity (removal of TOC, N, P and dissolved oxygen) compared to the treatment at 50 °C and 37 °C.

Control experiments without bacterial inoculums were carried out to determine the effect of different temperatures on the wastewater treatment process. The results from these control experiments showed no removal of TOC, N, P and dissolved oxygen.

Table 5 compares the removal efficiency of TOC, N, P and dissolved oxygen of thermophilic bacteria at 50 and 55 °C vs. a thermophilic bacterial consortium at 37 °C.

Table 5 Summary of differences between thermophilic bacteria wastewater treatment at 50 °C and 55 °C vs. a thermophilic bacterial consortium at 37 °C

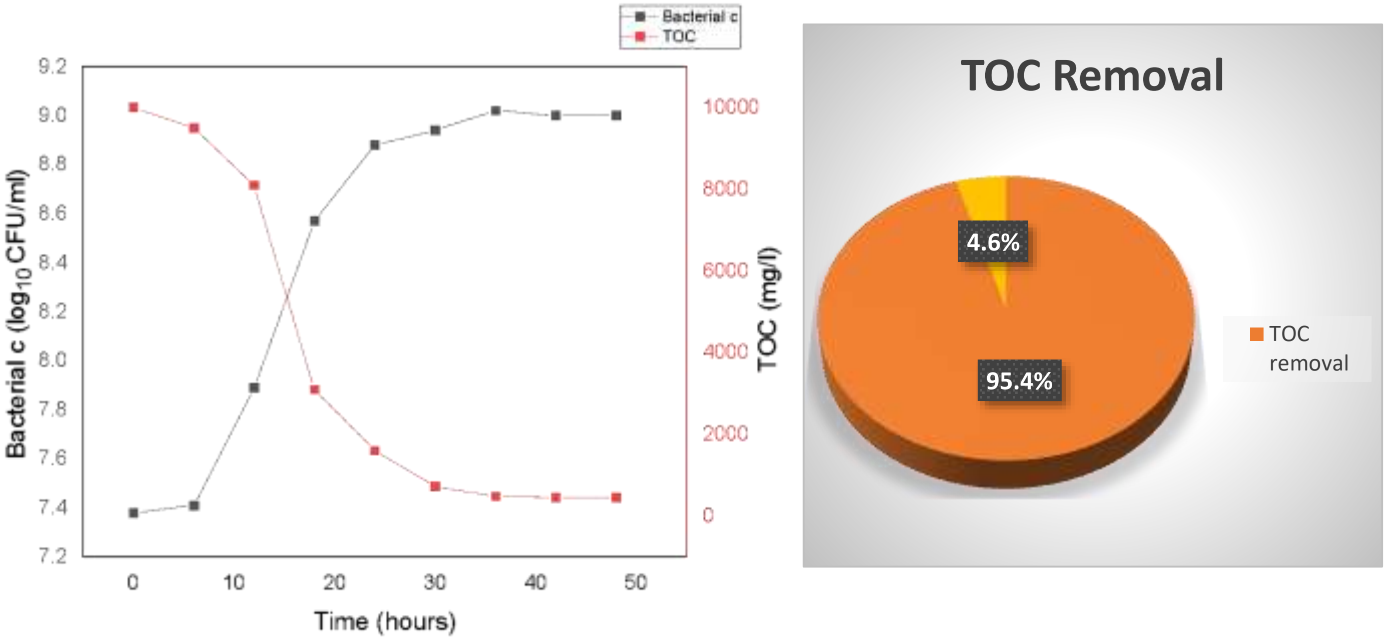
Bacterial consortium	TOC removal %	Dissolved oxygen removal %	Nitrogen removal %	Phosphorous removal %
Thermophilic bacteria (37 °C)	58.5	91.7	75.9	13.2
Thermophilic bacteria (50 °C)	79	96	83	15.4
Thermophilic bacteria (55 °C)	90.2	95	92	37

4.3 Mesophilic bacteria and mesophilic conditions

To perform a comparison study, an experiment was conducted using a mesophilic consortium under mesophilic conditions at 37 °C and a pH of 8. The results show significant efficiency of the mesophilic consortium used in this experiment (Table 6). Within 48 hours at 37 °C, the removal rate of TOC was 95.4% (Figure 15), that of nitrogen was 54.5% (Figure 16), and that of phosphorous was 27.2% (Figure 17).

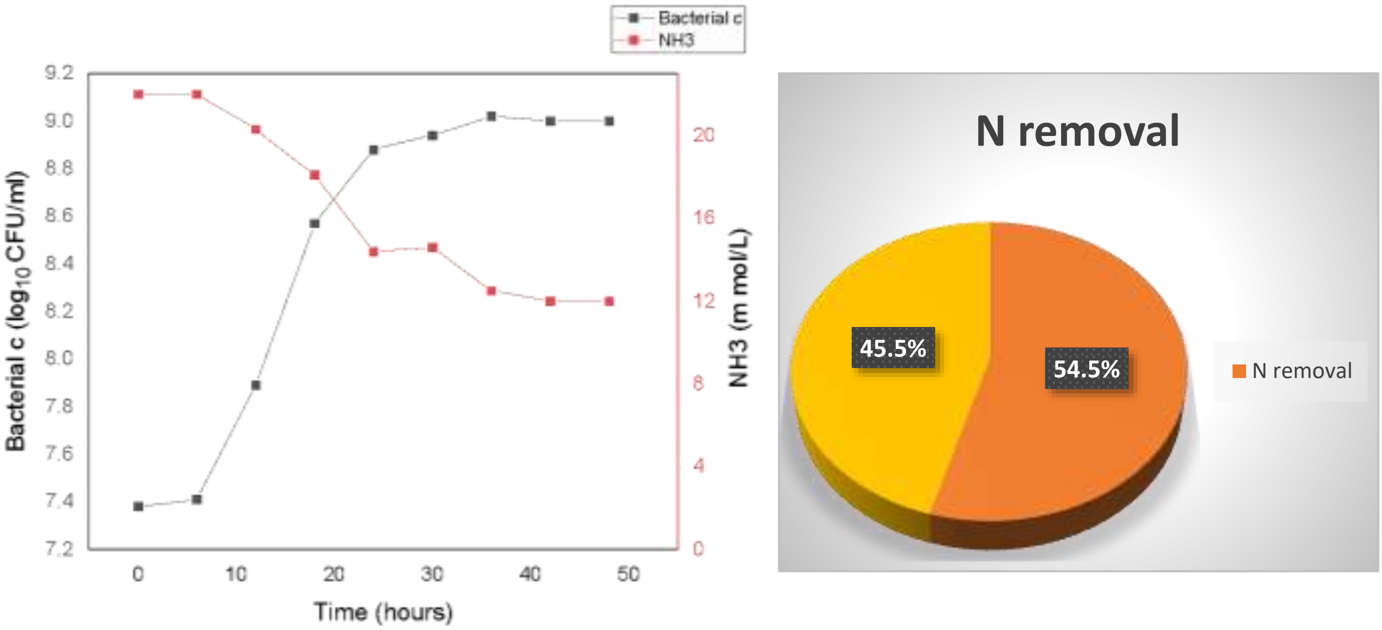
Table 6 Waste removal during biological wastewater treatment using a mesophilic consortium under mesophilic conditions

Time (hours)	OD	Bacterial count CFU/ml	Bacterial count Log_{10} CFU/ml	TOC mg/l	NH_3 m mol/L	P m mol/L	DO ppm
0	0.12	2.4×10^7	7.38	10000	22	30.2	4.96
6	0.13	2.6×10^7	7.41	9500	22	30.1	4.87
12	0.39	7.8×10^7	7.89	8100	20.3	28.5	3.47
18	1.9	3.8×10^8	8.57	3100	18.1	27	1.82
24	3.8	7.6×10^8	8.88	1600	14.4	25.7	0.83
30	4.4	8.8×10^8	8.94	720	14.6	24.3	0.5
36	5.2	10.4×10^8	9.02	480	12.5	22.1	0.25
42	5.1	10.2×10^8	9	460	12	22	0.17
48	5.1	10.2×10^8	9	460	12	22	0.17



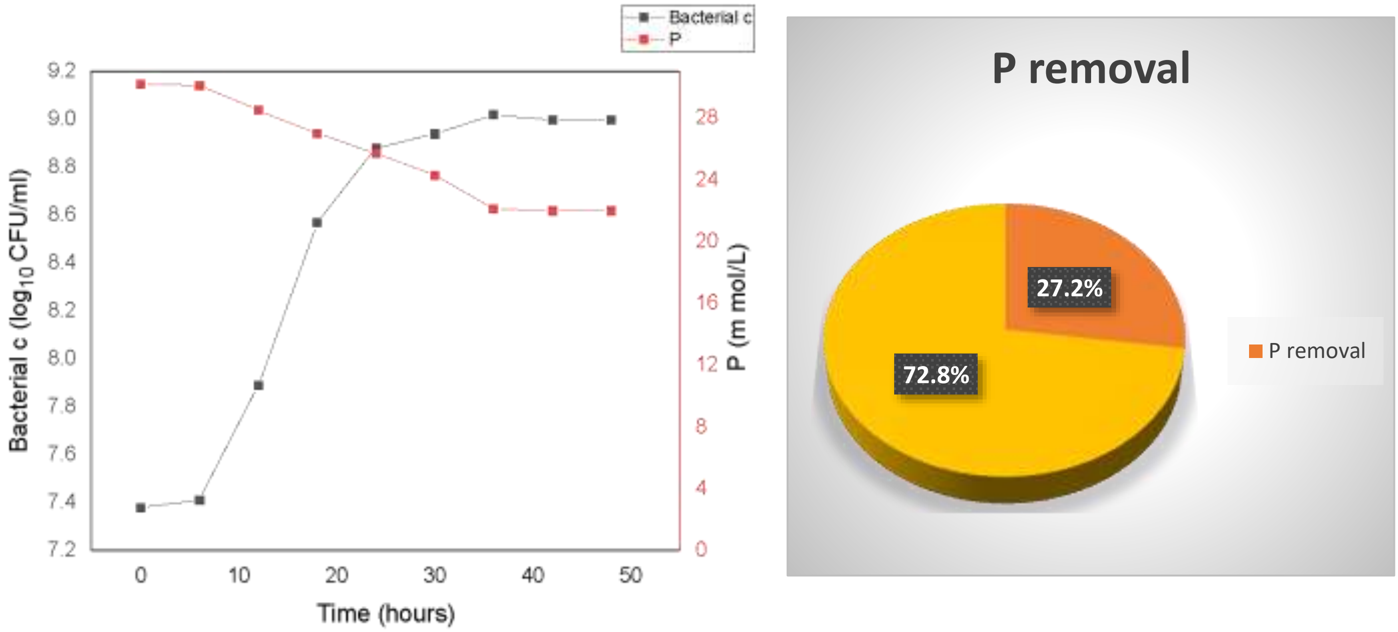
(a) (b)

Figure 15 (a) Removal of total organic carbon (TOC) by mesophilic bacterial consortium at 37 °C. (b) Removal percentage of TOC at 37 °C.



(a) (b)

Figure 16 (a) NH3 removal by mesophilic bacterial consortium at 37 °C. (b) Removal percentage of N at 37 °C.



(a) (b)

Figure 17 (a) Phosphorus removal by mesophilic bacterial consortium at 37 °C. (b) Removal percentage of P at 37 °C.

CHAPTER 5

DISCUSSION

The results show that there is significant efficiency (a high rate) of waste removal at high temperature. At 55 °C and 50 °C, the thermophilic consortium exhibited a very high efficiency in removing total organic carbon (TOC), reaching approximately 90.2% in 36 hours and 79% in 60 hours, respectively, while the removal of nitrogen was approximately 92% and 83%, respectively, in the same period. The removal of phosphorous was relatively low at 15.4 and 37% in the same set of experiments. The consortium of thermophilic bacteria grown at 37 °C exhibited substantially different performance. For example, after 60 hours of incubation at 37 °C, only 58.5% of TOC, 75.9% of nitrogen and 13.2% of phosphorous were removed.

The removal of phosphorus was limited, and this could be due to an excess source of phosphorus, which comes from the phosphate buffer used to adjust the pH during the reaction process and could be due to the high rate of metabolism carried out by the thermophilic consortium (Cheng et al., 2006).

Using a thermophilic consortium in wastewater treatment under thermophilic conditions showed advantages over mesophilic conditions, such as a higher growth rate, higher waste removal, less sludge formation and less operational time. A very important factor is the ability to deactivate pathogenic microorganisms, which can also be a primary consideration during the treatment of many types of wastewater, such as liquid manure from livestock production (Kim & Choi, 2002).

These findings (the removal efficiency by thermophilic bacteria) can be of major importance in contributing to the reduction of the operational time and costs for high-temperature treatment of industrial wastewaters. These findings agree with many previous studies that mentioned the advantages of utilizing thermophiles in wastewater treatment at high temperature. Timothy M. Lapara & Alleman (1999) reported that 79% of COD was removed under thermophilic conditions. A recent study (Collivignarelli et al., 2020) conducted using a thermophilic membrane bioreactor reported the efficient removal of pollutant COD (78% - 97%) and total phosphorous (>60%) within a hydraulic retention time of 5 days.

Thermophilic aerobic treatment appears to be a feasible option for the treatment of different wastewaters under different process conditions. Several studies have shown that thermophilic treatment at 50 °C is the best operating temperature (Kim & Choi, 2002).

It is very important to study the optimization of the most important operational aspects, such as increasing aeration rate (more oxygen), higher temperatures, and different carbon sources such as starch and cellulose. Testing real wastewater is another suggestion for future work. Combining thermophilic with mesophilic treatment is another approach suggested to achieve higher removal efficiency if thermophilic conditions alone are used.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

Sets of experiments were conducted at 50°C with different pH values, one experiment at pH 4 and the other at pH 10, there was no growth at these pH values. The optimum pH for the used thermophilic bacterial consortium was at pH 8. Sets of experiments were done at pH 8 and temperatures at 37, 50 and 55°C. The most efficient waste removal rate was at 55 °C and pH 8 within 36 hours.

Thermophilic wastewater treatment exhibited high efficiency in removal TOC, N and P. These findings very important to optimize the wastewater treatment process in term of operational time, cost and waste removal rate. Furthermore, many studies reported other advantages including low remnants yield and inhibition of pathogens (Abeynayaka & Visvanathan, 2011).

The high temperature ranges in Saudi Arabia and the Gulf region an important factor to investigate wastewater treatment at the thermophilic condition. Optimization of the most important operational aspects, such as increasing aeration rate (more oxygen), higher temperatures, and different carbon sources such as starch and cellulose. Testing real wastewater is another suggestion for future work.

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Baker, B.A., Tawabini, B., Nazal, M. et al. Efficiency of Thermophilic Bacteria in Wastewater Treat
https://doi.org/10.1007/s13369-020-04830-x – 2020

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11/2019 – King Fahd University of Petroleum and Minerals
Seminar: CRISPR Technology

04/2019 – King Fahd University of Petroleum and Minerals
Seminar: Microarray Technology Principle and Applications

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- The best employee in 2012, 2013, and 2016.

02/2020
Full scholarship to attend the Conference: Emerging Themes in Epi-Genome Architecture and Function – King Abdullah University of Science and Technology

ORGANISATIONAL SKILLS

Collaboration and Leadership

- Organize the collaboration with my colleagues.
- Leadership (currently I am a leader for a team of 17 teachers and technicians)
- Good managerial skills and ability to promote the events.

Planning and Prioritizing

- Ability to create an applicable plan with smart goals.
- Monitoring the achievement of the goals and solving the problems.
- Ability to give priority to important tasks.

COMMUNICATION AND INTERPERSONAL SKILLS

Teamwork

- Support people with a challenging workload by offering to aid them on some of their tasks.
- Achieve the tasks in cooperation with the other team members.
- Give constructive feedback to the team members to let them take advantage of their strengths and eliminate their weaknesses

Effective Communication

- Excellent verbal and written communication.
- Understanding and respecting points of view through engagement and interest.
- Using relevant knowledge, know-how, and skills to explain and clarify thoughts and ideas.

SELECTED WORKSHOPS

11/2020
G20 youth summit

04/2020
European social circles (Erasmus exchange program)

Climate change challenge

11/2019
Life matter Ithraa lab

06/2012 – 06/2013
Aramco science labs