TEZ ŞABLONU ONAY FORMU
THESIS TEMPLATE CONFIRMATION FORM

1. Şablonda verilen yerleşim ve boşluklar değiştirilmemelidir.
2. **Jüri tarihi** Başlık Sayfası, İmza Sayfası, Abstract ve Öz'de ilgili yerlere yazılmalıdır.
3. İmza sayfasında jüri üyelerinin unvanları doğru olarak yazılmalıdır. Tüm imzalar **mavi pilot kalemle** atılmalıdır.
4. **Disiplinlerarası** programlarda görevlendirilen öğretim üyeleri için jüri üyeleri kısmında tam zamanlı olarak çalıştıkları anabilim dalı başkanlığının ismi yazılmalıdır. Örneğin: bir öğretim üyesi Biyoteknoloji programında görev yapıyor ve biyoloji bölümünde tam zamanlı çalışıyorsa, İmza sayfasına biyoloji bölümü yazılmalıdır. İstisnai olarak, disiplinler arası program başkanı ve tez danışmanı için disiplinlerarası program adı yazılmalıdır.
5. Tezin **son sayfasının sayfa** numarası Abstract ve Öz'de ilgili yerlere yazılmalıdır.
6. Bütün chapterlar, referanslar, ekler ve CV sağ sayfada başlamalıdır. Bunun için **kesmeler** kullanılmıştır. **Kesmelerin kayması** fazladan boş sayfaların oluşmasına sebep olabilir. Bu gibi durumlarda paragraf (¶) işaretine tıklayarak kesmeleri görünür hale getirin ve yerlerini **kontrol edin**.
7. Figürler ve tablolar kenar boşluklarına taşmamalıdır.
8. Şablonda yorum olarak eklenen uyarılar dikkatle okunmalı ve uygulanmalıdır.
9. Tez yazdırılmadan önce PDF olarak kaydedilmelidir. Şablonda yorum olarak eklenen uyarılar PDF dokümanında yer almamalıdır.
10. Tez taslaklarının kontrol işlemleri tamamlandığında, bu durum öğrencilere METU uzantılı öğrenci e-posta adresleri aracılığıyla duyurulacaktır.
11. Tez yazım süreci ile ilgili herhangi bir sıkıntı yaşarsanız, Sıkça Sorulan Sorular (SSS) sayfamızı ziyaret ederek yaşadığınız sıkıntıyla ilgili bir çözüm bulabilirsiniz.

1. Do not change the spacing and placement in the template.
2. Write **defense date** to the related places given on Title page, Approval page, Abstract and Öz.
3. Write the titles of the examining committee members correctly on Approval Page. **Blue ink** must be used for all signatures.
4. For faculty members working in **interdisciplinary programs**, the name of the department that they work full-time should be written on the Approval page. For example, if a faculty member staffs in the biotechnology program and works full-time in the biology department, the department of biology should be written on the approval page. Exceptionally, for the interdisciplinary program chair and your thesis supervisor, the interdisciplinary program name should be written.
5. Write **the page number of the last page** in the related places given on Abstract and Öz pages.
6. All chapters, references, appendices and CV must be started on the right page. **Section Breaks** were used for this. **Change in the placement** of section breaks can result in extra blank pages. In such cases, make the section breaks visible by clicking paragraph (¶) mark and **check their position.**
7. All figures and tables must be given inside the page. Nothing must appear in the margins.
8. All the warnings given on the comments section through the thesis template must be read and applied.
9. Save your thesis as pdf and Disable all the comments before taking the printout.
10. This will be announced to the students via their METU students e-mail addresses when the control of the thesis drafts has been completed.
11. If you have any problems with the thesis writing process, you may visit our Frequently Asked Questions (FAQ) page and find a solution to your problem.

☒ **Yukarıda bulunan tüm maddeleri okudum, anladım ve kabul ediyorum. / I have read, understand and accept all of the items above.**

Name : Nehir
Surname : Arık Kınalı
E-Mail : nehir.arik@metu.edu.tr
Date : 09.12.2022
Signature : _____

EVALUATION OF PERFORMANCE OF ARSENIC BIOREPORTER IMMOBILIZED ELECTROSPUN MEMBRANES FOR ARSENIC DETECTION IN WATER

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

NEHİR ARIK KINALI

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY
IN
MOLECULER BIOLOGY AND GENETICS

DECEMBER 2022

Approval of the thesis:

EVALUATION OF PERFORMANCE OF ARSENIC BIOREPORTER IMMOBILIZED ELECTROSPUN MEMBRANES FOR ARSENIC DETECTION IN WATER

submitted by **NEHİR ARIK KINALI** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** i**n Molecular Biology and Genetics, Middle East Technical University** by,

Prof. Dr. Halil Kalıpçılar
Dean, Graduate School of **Natural and Applied Sciences** _____

Prof. Dr. Ayşe Gül Gözen
Head of the Department, **Biological Science** _____

Prof. Dr. Hüseyin Avni Öktem
Supervisor, **Molecular Biology and Genetics,, METU** _____

Prof. Dr. Ayşen Tezcaner
Co-Supervisor, **Engineering Science, METU** _____

Examining Committee Members:

Prof. Dr. Füsun İnci Eyidoğan
Educational Sciences, Başkent University _____

Prof. Dr. Hüseyin Avni Öktem
Molecular Biology and Genetics**,** METU _____

Assoc. Prof. Dr. Çağdaş Devrim Son
Biology, METU _____

Prof. Dr. Dilek Keskin
Engineering Sciences, METU _____

Assoc. Prof. Dr. Nesrin Horzum Polat
Engineering Sciences, Izmir Katip Celebi University _____

Date: 09.12.2022

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name Last name : Arık Kınalı, Nehir

Signature :

ABSTRACT

EVALUATION OF PERFORMANCE OF ARSENIC BIOREPORTER IMMOBILIZED ELECTROSPUN MEMBRANES FOR ARSENIC DETECTION IN WATER

Arık Kınalı, Nehir
Doctor of Philosophy, Molecular Biology and Genetics
Supervisor: Prof. Dr. Hüseyin Avni Öktem
Co-Supervisor: Prof. Dr. Ayşen Tezcaner

December 2022, 150 pages

Heavy metal pollution, which is growing and threatening the health of all living things as well as human health, can lead to serious lifelong consequences. It is very important to detect and monitor these heavy metals that cause environmental pollution. In addition to traditional laboratory techniques, the use of bacterial biosensors to detect heavy metals has come into focus. In addition, it is known that the immobilization of these bacterial biosensors is more advantageous in many respects, such as being more stable and protected than the free ones.

In this thesis study, arsenic bioreporters whose expression of the reporter gene increased in the presence of arsenic and which we could record as fluorescent signal, were immobilized on 12.5% cellulose acetate (CA) and 10 wt% polycaprolactone (PCL) electrospun fibers. The characterization tests of the developed electrospun fibers were carried out by means of scanning electron microscopy (SEM), fourier-transform infrared (FTIR) spectroscopy and contact angle meter. It was observed that the developed fiber support membranes were beadless and homogeneous fibers (Diameters: 2.15 μm ±0.55 for CA; 0.61 μm ±0.20 for PCL) and both were

hydrophobic (143.6°±13.5 for CA, 106°±0.3 for PCL). The parameters of the immobilization studies were changed, and their optimization was tried to be achieved. For this, experiments were carried out in different conditions to determine the relationship between the fluorescent signal emitted in the presence of arsenic and the concentrations and growth phases of the immobilized cells. In addition, according to the viability test performed by AlamarBlue method, the viability of the bioreporters immobilized on CA fiber was 55.4%, while those immobilized-on PCL fiber were 91%.

Afterwards, the studies were continued on the PCL fiber and the effects of the growth medium and different immobilization times on the fluorescent signal were examined and the properties of the bioreporter immobilized on PCL system were tried to be determined.

Following the optimization studies, in order to determine the properties of the bioreporter immobilized on PCL system, the sensitivity, selectivity, response time to arsenic metal and storage life were determined by changing the storage conditions.

The study results show that the bioreporter immobilized on PCL system can be used to detect arsenic heavy metal at environmentally safe concentrations. In addition, it is thought that the study will fill the deficiencies in this field in the literature and shed light on future studies.

Keywords: Arsenic, Electrospun Fibers, Whole Cell Bioreporter, Adsorption, Bacteria Immobilization

ÖZ

SUDAKİ ARSENİK TESPİTİ İÇİN ARSENİK BİYORAPORTÖR İMMOBİLİZELİ ELEKTROSPUN MEMBRANLARIN PERFORMANSININ DEĞERLENDİRİLMESİ

Arık Kınalı, Nehir
Doktora, Moleküler Biyoloji ve Genetik
Tez Yöneticisi: Prof. Dr. Hüseyin Avni Öktem
Ortak Tez Yöneticisi: Prof. Dr. Ayşen Tezcaner

Aralık 2022, 150 sayfa

Gittikçe büyüyen ve insan sağlığının yanısıra diğer tüm canlıların da sağlığını tehdit eden ağır metal kirliliği yaşam boyu ciddi sonuçlara yol açabilir. Çevre kirliliğine yol açan bu ağır metallerin tespit edilmesi ve izlenmesi oldukça önem arz etmektedir. Geleneksel laboratuvar tekniklerine ek olarak, ağır metalleri tespit etmek amacıyla bakteriyel biyosensörlerin kullanımı odak haline gelmiştir. Ayrıca bu bakteriyel biyosensörlerin immobilizasyonunun serbest haldekilere nazaran daha stabil olması ve korunabilmesi gibi birçok açıdan daha avantajlı olduğu bilinmektedir.

Bu tez çalışmasında, arsenik varlığında içerdiği raportör genin ifadesi artan ve bunu da floresan sinyali olarak kaydedebildiğimiz arsenik biyoraportörlerinin, ağırlıkça %12.5 selüloz asetat (CA) ve ağırlıkça %10 polikaprolakton (PCL) electrospun liflerine immobilizasyonu gerçekleştirilmiştir. Geliştirilen elektrospun liflerin karakterizasyon testleri taramalı electron mikroskopi (SEM), fourier dönüşümlü kızıl ötesi (FTIR) spektroskopi ve temas açısı ölçer aracılığıyla yapılmıştır. Geliştirilen lif destek membranların boncuksuz ve homojen görüntüde lifler (Çapları, CA için: 2.15 µm ±0.55; PCL için: 0.61 µm ±0.20) olduğu ayrıca her ikisinin de hidrofobik özellikte (CA için 143.6°±13.5, PCL için 106°±0.3) olduğu görülmüştür.

İmmobilizasyon çalışmalarının parametreleri değiştirilerek optimizasyonu sağlanmaya çalışılmıştır. Bunun için arsenik varlığında yaydıkları floresan sinyali ile immobilize edilen hücrelerin konsantrasyonları ve büyüme fazları arasındaki ilişkiyi tespit etmek amacıyla farklı koşullarda denemeler yapılmıştır. Ayrıca AlamarBlue yöntemi ile yapılan canlılık deneyine göre CA lifine immobilize edilen biyoraportörlerin canlılıkları %55.4 iken PCL lifine immobilize edilenlerin % 91 olduğu bulunmuştur.

Daha sonra çalışmalara PCL lifi üzerinden devam edilmiş ve büyüme ortamının ve farklı immobilizasyon sürelerinin floresan sinyali üzerine etkisi incelenerek PCL üzerine immobilize edilmiş biyoraportör sistemin özellikleri belirlenmeye çalışılmıştır.

Optimizasyon çalışmalarını takiben, PCL üzerine immobilize edilmiş biyoraportör sistemin özelliklerini belirleyebilmek için de hassasiyeti, seçiciliği, arsenik metaline karşı verdiği yanıt süresi ve saklama koşulları değiştirilerek saklama ömrü tayini yapılmıştır.

Çalışma sonuçları göstermektedir ki PCL üzerine immobilize edilmiş biyoraportör sistem çevresel olarak güvenli konsantrasyonlardaki arsenik ağır metalini tespit etmek amacıyla kullanılabilirler. Ayrıca çalışmanın literatürdeki bu alandaki eksik yanları dolduracağı ve gelecek çalışmalara ışık tutacağı düşünülmektedir.

Anahtar Kelimeler: Arsenik, Elektrospun Lifler, Tam Hücre Biyoraportörü, Adsorpsiyon, Bakteri İmmobilizasyonu

Dedication to ***My Family*** ♡

ACKNOWLEDGMENTS

In this PhD journey of many challenges, I am grateful for the kind people around me supporting me in different ways.

First and foremost, I would like to express my truthful gratitude to my thesis supervisor Prof. Dr. Hüseyin Avni Öktem for his guidance, encouragement, and trust in me. He devoted his time to guide me and share his expertise and experiences not only academic education also life education. As a scientific mentor, he taught me to think analytically, to look outside the box, and many skills to succeed in research. In addition to being a supervisor, I am also grateful to him for treating us like a father.

Prof. Dr. Ayşen Tezcaner is acknowledged for accepting being a co-advisor and for the helpful comments during my thesis progress presentations. I am grateful for her unlimited time and energy to help me in my thesis. Above all, her patient, inspirational and accessible were priceless for me. I would like to thank both of my advisors once again for making my PhD process easier and supporting me in this adventure that was first steps to the success of this thesis. I am so grateful to have advisors with such kind personality and understanding.

I extend my special appreciation to the Prof. Dr. Dilek Keskin and Assoc. Prof. Dr. Çağdaş Devrim Son for serving as a member of my thesis committee and their academic guidance. Also, I am grateful to Prof. Dr. Füsun İnci Eyidoğan and Assoc. Prof. Dr. Nesrin Horzum Polat for serving on my jury for their time, interest, insightful questions, and helpful comments.

I have been lucky enough to meet many wonderful people who made the university a warm place for me. I would like to express my sincere appreciations to my senior and friend, Dr. Evrim Elçin for her helping in my thesis also I would like to thank Barış Çağrı Tosun, for his help at critical points and for stating that he is always ready to help.

I would like to take this opportunity to express my deepest sense of gratitude to my family, who has been with me throughout this journey. I could write a thesis length about them. My parents, Ahu – Timur Arık have always supported, encouraged, and trusted me with their unconditional love. Without my beautiful family's motivating support, I could never have accomplished so much. It was marvelous to see my dad as a rock, my mom as a shelter whenever I needed their help. Therefore, I dedicate this work to them. I also would like to thank my parents – in – law, Nimet – Mehmet Kınalı, whose contributions and supports cannot be ignored. Their prayer for me was what sustained me thus far. Special thanks go to my sisters, Nil, Nur and İlayda Arık and brothers, Halil İbrahim and Enes, for being with me. Their endless love and trust in me were what made me motivated all the time.

Lastly, but hero of my life, there is no word to describe my gratitude toward man who has been in my heart and mind, my love, and my best friend, Ömer Selçuk Kınalı, for his eternal trust, support, and love throughout my life. He was stay with me in every step of this study, in its difficulty and ease. I am in debt to very special thanks to him.

Of course, I owe to thank my dear little man, Göktuğ Eren Kınalı, who was included in my life later on, but whom I do not remember before. His journey, which started with the experiments in the laboratory we did together while he was in my womb, passed in the laboratories after he was born. He has been a constant source of joy in my life through the struggles and trials of this PhD adventure. My dear little son, who has made the most sacrifices by being without a mother at some moments, also deserves the biggest and most loving thanks...

This work is partially funded by METU Scientific Research Projects Coordination Unit under grant number ÇDAP-108-2021-10659.

TABLE OF CONTENTS

LIST OF TABLES

TABLES

LIST OF FIGURES

FIGURES

LIST OF ABBREVIATIONS

ABBREVIATIONS

AAS	Atomic absorption spectroscopy
Ace	Acetone
AFS	Atomic fluorescence spectroscopy
As	Arsenic
As (III)	Arsenite, Arsenous acid
As (V)	Arsenate, Arsenic acid
CA	Cellulose acetate
CA – IB	Immobilized arsenic bioreporter cell on CA fibers
CD	Cyclodextrin
CM	Confocal Microscopy
DNA	Deoxyribonucleic acid
DCM	Dichloromethane
DMF	N, N Dimethylformamide
dH_2O	Distilled water
E. coli	Escherichia coli
FB	Free arsenic bioreporter cell
FB – background	Background for free arsenic bioreporter cell
FB – control	Control for free arsenic bioreporter cell

FTIR	Fourier-transform infrared spectroscopy
GFP	Green fluorescent protein
h	Hour
HPLC/GC	Liquid chromatography
HSD	Honestly significant difference
IB	Immobilized bioreporter on fibers
IB – background fibers	Background for immobilized arsenic bioreporter cell on
IB – control	Control for immobilized arsenic bioreporter cell on fibers
ICP-MS	Inductively coupled plasma-mass spectroscopy
ICP-OES	Inductively coupled plasma atomic emission spectroscopy
LB Medium	Luria-Bertani medium
Min	Minute
MOPS	3-(N-morpholin-o) propanesulfonicacid
OD_{600}	Optical density at 600 nm
PCL	Polycaprolactone
PCL – IB	Immobilized arsenic bioreporter cell on PCL fibers
Pit	Inorganic phosphate transporter
PLA	Polylactic acid
Pst	Phosphate specific transporter
PSU	Polysulfone
RFU	Relative fluorescence unit

RT	Room temperature
SEM	Scanning electron microscopy
v/v	Volume per volume
w/v	Weight per volume
WCBs	Whole – cell biosensors

CHAPTER 1

INTRODUCTION

In recent years, heavy metals emanating from different anthropogenic sources had a direct or indirect negative impact on the environment. Heavy metals have hazardous impact on living organisms. Therefore, it is important to identify whole cell bioreporters that can be used to monitor their levels in environmental and biological samples, especially waters.

1.1 Pollution in Environment

In Turkey and in the world, the lack of sufficient number of waste treatment facilities and infrastructure inadequacies brought about by urbanization, industrialization and rapid population growth bring along the growing environmental pollution problem. The environment can be exposed to either contamination or pollution, which have distinct sense from each other even so, all pollutants are contaminants, but not all contaminants are pollutants that act malefic effect on the environment (Chapman, 2007). In the literature, pollution is described as contamination, in short, is the presence of substances that should not be or in higher concentrations than natural background. Besides, pollution is specified as the direct or indirect entry of matter or energy into the environment by humans or naturally occurring contaminants, which have the capacity to harm living resources and human health, hinder the environment and cause undesirable consequences such as deteriorating its quality. Pollution is contamination that results in or can result in adverse biological effects to living organisms.

1.2 Water Pollution

Water pollution, one of the types of pollution, has a very important place for the health of all living organisms. Despite this, existing water resources are a threat to future generations because they are used and polluted unconsciously. Pollution of water means that it becomes unsafe for humans with the increase in the amount of dangerous or toxic substances and disease-causing microorganisms in the water (Kılıç, 2021). Pollutants, the elements of pollution, may cause undesirable alterations in physical, chemical, and biological characteristics of air, water and soil which is malign for living communities and harmful for living organisms, including animals and plants, as well (Masindi & Muedi, 2018).

1.2.1 Heavy Metal Pollution

Heavy metals, with a density of at least 5 times greater than water and a high atomic mass are well-known elements due to their persistence in the environment and their bio accumulative nature. The sources that cause the formation of heavy metals, also called environmental pollutants, are divided into two as natural and anthropogenic. The former is known as the erosion of metal-containing rocks and volcanic eruptions, while the latter includes mining and various industrial, medical and technological applications and agricultural activities. Even for this reason, its potential effects on animal health and the environment are of concern (Ali et al., 2019). It is impossible for humans to survive on earth without toxic metals. These mentioned toxic metals, Arsenic (As), cadmium (Cd), chromium (Cr, +3, +6), copper (Cu), lead (Pb), zinc (Zn), and mercury (Hg), which have the potential to cause serious harm, are among the priority metals that are jeopardous for living health (Rashed, 2020; Tchounwou et al., 2012). The level of toxicity is closely related to many conditions such as dose, type of interaction, age, gender, genetics, and nutritional status of the individuals with whom it interacts. Some metals such as zinc, copper, iron, manganese, and cobalt are necessary for the human body, on the contrary, they have various harmful

effects on human health. However, these heavy metals are dose dependent they can also be toxic and carcinogenic, i.e. DNA damage and neurological abnormalities (Gorini et al., 2014), when subjugate to very high concentrations (Balali-Mood et al., 2021; Rehman et al., 2018). Concentrations of heavy metals in water allowed by EPA and WHO are given in Table 1.1.

Table 1.1 Limit heavy metal concentrations in drinking water (mg/L) allowed by EPA and WHO.

Heavy metals	WHO (mg/L)	EPA (mg/L)	References
Arsenic, $As^{3+,5+}$	0.01	0.01	(Binkley & Simpson, 2003; Umoh, 2013; Y. Zhang et al., 2014)
Barium, Br^{2+}	2.0	2.0	(Odobašić, 2019)
Cadmium, Cd^{2+}	0.003	0.005	(Kinuthia et al., 2020)
Calcium, Ca^{2+}	50	50	(Odobašić, 2019; Umoh, 2013)
Chromium, Cr^{3+}	0.05	0.1	(M. Bansal et al., 2009; O. P. Bansal, 2020)
Lead, Pb^{2+}	0.01	0.015	(Bahnasawy et al., 2011; Shamsollahi & Partovinia, 2019)

Table 1.1 (cont'd).			
Magnesium, Mg^{2+}	50	50	
Mercury, Hg^{2+}	0.001	0.002	(Binkley & Simpson, 2003; Kinuthia et al., 2020)
Nickel, Ni^{2+}	0.05	0.01	(Azadi et al., 2018; Kinuthia et al., 2020)
Zinc, Zn^{2+}	0.05	0.05	(Bahnasawy et al., 2011)

1.2.2 Arsenic Contamination and Toxicity

Arsenic is one of the most toxic metals derived from the natural environment. The source that people are most exposed to arsenic toxicity is neither due to mining nor agricultural resources (pesticides or fertilizers), but the contamination of natural geological resources affects them more. In other words, the movement of arsenic from geological sediments to sources such as underground and drinking water has the potential to seriously harm the health of living things (Sikdar & Kundu, 2018). Drinking water of even industrial or less industrial countries can be contaminated with arsenic. In addition, arsenic, which is number one in the 2001 priority list of dangerous substances and disease records defined by WHO, is described as the 'king of poison' because it is an extremely toxic element (Shaji et al., 2021). The amount of As in soil, water and living organisms can be found in concentrations varying from ppm to ppb. While the As concentration in sea water is around 2 μg/L, this concentration is generally 1-40 μg/g in soil. The toxicity of arsenic in natural waters depending upon both its chemical form and its doses. The allowable concentration of arsenic in drinking water, which was 50 μg/L until 1993, has been decreased to

10 µg/L since 1993 (Shaji et al., 2021). With the decision taken by the Environmental Protection Agency in 2001, the level of arsenic allowed in drinking water in the USA was reduced from 50 ppb to 10 ppb (Ratnaike, 2003). In Turkey, the amount of arsenic in drinking water has been determined as <10 µg/L with the standard numbered TSE 266 prepared by the Ministry of Health (Anonim, 2005). The most dangerous and frightening feature of arsenic has been shown by IARC to be carcinogenic (IARC, 2012), besides, it also has mutagenic and teratogenic properties. (Ratnaike, 2003). It is known that long-term exposure of living organisms to these metals can cause damage such as DNA mutations, cell cycle disorders, neurological problems, liver and kidney damage, disruption in the endocrine system, cardiovascular dysfunction and even apoptosis (Genchi et al., 2020; Jadoon & Malik, 2017).

Aqueous arsenic (0) commonly exists forms in either +3 (trivalent) or +5 (pentavalent), affiliated to local oxidation-reduction circumstances. Arsenic is found arsenate (As (V)) form in aerobic (surface waters), and arsenite (As (III)) form in anaerobic environment (deep groundwaters) (Queensland Fire and Emergency Services, 2018). It is considered that As (III) is more toxic than As (V) (Ferguson & Gavis, 1972; Raju, 2022). Arsenic in drinking water is severely harmful for living health due to it is carcinogenicity, hence, requires its concentration to be measured and monitored in real time (Ahmad & Bhattacharya, 2019; Bräuner et al., 2014).

1.3 Environmental Monitoring of Metal Contamination

Rapid and reliable detection of metal ions is very important in the pollution caused by the mentioned heavy metals (Sikdar & Kundu, 2018). Otherwise, they can cause irreversible problems by causing severe harm to the health of living things. Therefore, rapid analysis should be prioritized for the initial and on-site detection of contaminated environments (Rigo et al., 2019). There are two main techniques used for these purposes. These techniques detect heavy metals with two different approaches, physicochemical and biosensing analysis.

1.3.1 Physicochemical Techniques for Monitoring of Metal Ions

For physicochemical methods are the first of the heavy metal monitoring methods and instrumental devices working with chromatographic and spectroscopic sense are utilized. Although they have the same biological or toxic effect, many compounds with different chemical structures exist in nature. The devices described in this group make it easier to detect such compounds, but they cannot provide information about what kind of biological effect these compounds will have. These techniques are very sensitive for the simultaneous detection of different heavy metals, making them very versatile (Malik et al., 2019). The techniques utilized to monitor these toxic heavy metals are named chromatography and high performance liquid chromatography (GC and HPLC) (Okano et al., 2015; Sarzanini, 2000) with specific element detectors based either on atomic absorption spectroscopy (AAS) (Aquisman et al., 2019), or inductively coupled plasma atomic emission spectroscopy (ICP-OES) (Karami et al., 2004; Velitchkova et al., 2003), or inductively coupled plasma-mass spectroscopy (ICP-MS) (Mittal et al., 2017), atomic fluorescence spectroscopy (AFS) (Sánchez-Rodas Navarro et al., 2010). However, all these techniques need labor-intensive and equipment that is high price, complicated to operate, and consume several days for purification processes and to get results (L. Wang et al., 2019). Besides, in terms of their use in field studies, their operation is quite complex and they are not sufficiently equipped to detect the presence and amount of arsenic in rare earths (Pola-López et al., 2018).

AAS was utilized monitoring of arsenic with the detection limit of 0.19 mg L^{-1}, (Nie et al., 2008) unlike HPLC was used to measure arsenic in the range of $9x10^{-5}$ to $24x10^{-5}$ ppm (Lindemann et al., 2016). ICP-MS can be an optimum detection technique for As contamination analysis due to its high sensitivity (B'Hymer & Caruso, 2004).

1.3.2 Biological Sensing Techniques for Monitoring of Metal Ions

The second group includes bioassay-based analysis and biosensors. For former group, several living systems have been utilized to monitor and analyze water with aquatic organisms i.e., fish (Authman et al., 2015), mussels (Ng et al., 2013; I. Saleh et al., 2021), water flea (Cui et al., 2017), algae (F. Hussain et al., 2021; Wu et al., 2016), bacteria (Elcin & Öktem, 2020b) or plants (Y.-E. Chen et al., 2019; Çiftçi et al., 2021) to sense the poisonousness of materials.

Certain methods mentioned above (such as AAS or ICP – AES) involve a very tedious application procedure and require at least 48-96 hours of stimulus exposure. (Hussain et al., 2021; Latała et al., 2005). Much more attention has been given to the development of affordable and accurate methods that allow the determination of even very low concentrations of pollutants or contaminants (Eltzov et al., 2015). Hence, there is a vogue technique to monitor water in terms of simple, low-cost, mobilize, rapid and real-time measuring capability.

Biosensors have the potential to be a viable alternative to the above-mentioned features (Farré et al., 2005). By definition, biosensor is known as a powerful and innovative analytical technique with its biological sensing element, which has uses in many areas such as food safety and processing, drug discovery, disease diagnosis, biomedicine, biomedical research, environmental monitoring and security (Vigneshvar et al., 2016). They can also be used to analyze and continuously monitor drinking water and warn in the early stages. They are used to control water quality for water purification purposes, additionally, detect and monitor pharmaceuticals and bioactive compounds that are impossible or difficult to detect (Long et al., 2013; Sin et al., 2014). The first biosensor, which measures glucose levels, was laid out in 1960s by the pioneers Clark and Lyons (Clark Jr. & Lyons, 1962). Biosensors have many more outstanding features over conventional techniques such as basic structures, fast response, and enhanced sensitivity (R. Liu et al., 2008). Researchers are interested in developing biosensors with higher accuracy and sensitivity by taking advantage of the high specificity and strict interaction among bio – sensing

molecules and analytes (Park et al., 2013). They are classified into according to their bioreceptors (biological sensing elements) that are responsible for binding analyte of interest to biosensor for measurement. These bioreceptors comprise enzymes, antibodies/antigens, nucleic acids, and others (aptamers, peptides) (Chambers et al., 2008; P. Mehrotra, 2016; Tetyana, 2021). Another grouping of biosensors is according to their transducers. There are four main type of transducers converting the signal into an appropriate measurable response for the detection and analyze the biological compounds: optical (colorimetric, chemiluminescence, fluorescence, absorbance), piezoelectrical, electrochemical (potentiometric and amperometric), thermal and magnetic transducers based on type of their transduction signal (Majdinasab et al., 2019; Newman & Turner, 1992; Turdean, 2011; Yoo & Lee, 2010). Optical and electrochemistry bio-transducers are highly preferred in biosensors due to their characteristic features. Optical biosensors work on the principle of generating an optical signal by interacting in specific regions of the biosensor elements. (Khansili et al., 2018). There is working principle of biosensor with various bioreceptors that can relate to transducer elements (Figure 1.1).

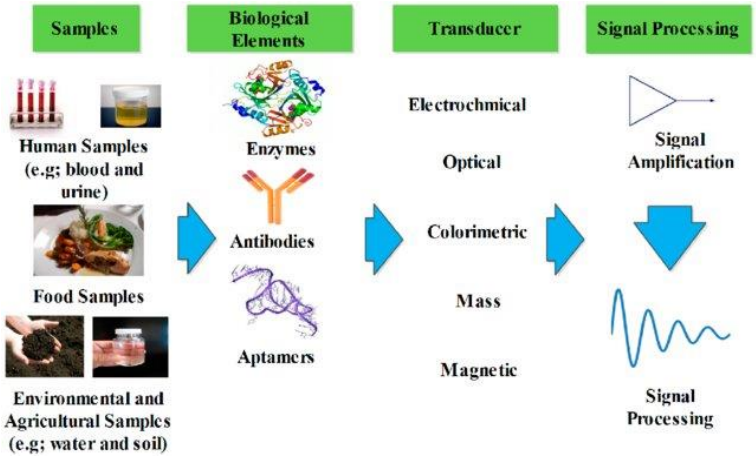

Figure 1.1 Schematic representation of working principle of biosensors with different inducers, receptors and transducers (Luka et al., 2015).

A disadvantage of biosensing techniques is that the reproducibility is very low, even near impossible since the measurements are made using living materials. In addition, the lack of validation of (bio)sensors for being used extensively in environmental monitoring or before their commercialization can be cited as another disadvantage, which requires inherent sensitivity and selectivity, (Ranveer et al., 2015). On the other hand, they not only detect certain chemical substances, but also identify various biological effects of these substances such as toxicity, cytotoxicity, genotoxicity (Odobašić, 2019). Another use of biosensors is environmental quality monitoring, that is, they take part in the evaluation of the biological or ecological quality of the environment or in the chemical monitoring of both inorganic and organic primary pollutants (Ranveer et al., 2015).

Biosensors that sense heavy metals can be grouped as protein-based (cell-free, i.e., enzymes, antibodies, aptamers) and whole cell-based systems (Verma & Singh, 2005). Enzymes used as bioreceptors are very selective and sensitive, which can be given the reason for their frequent use, but their cost and rather tedious purification processes are the handicap of enzyme-based biosensors. Unlike these, whole cells have the potential to be a very good alternative to other bioreceptors, especially to enzymes, because they are much cheaper, have better stability in harsh environments, and have the ability to reproduce very quickly in a short time because they have a very short doubling time (Lagarde & Jaffrezic-Renault, 2011). Cofactors are already present in whole cells, although enzymes need multienzymes or cofactors to generate measurable signals (D'Souza, 2001; Park et al., 2013; Su et al., 2011). These biosensors can be altered very easily with simple genetic engineering techniques, hence they can be converted in the next attempt to detect complex responses in a different living cell, despite the fact that cell-based biosensors are not as sensitive as much as protein-based biosensors and whole cell-based sensors may have limited detection range due to the toxicity and harmful effects of analytes on cells (Gui et al., 2017; K.-H. Lee & Kim, 2019).

1.4 Whole-Cell Biosensors

For environmental bio sensing purposes such as water quality monitoring or heavy metal detection, bacteria and yeasts are often preferred as whole cells, and algae are are less preferred (Lei et al., 2006). On the contrary, higher eukaryotic cells are used in the detection and analysis of basic cellular functions and disease pathogenesis (Gupta et al., 2019). Whole cell biosensors (or called microbial biosensors) allow to obtain information about various characteristics of a sample that cannot be given by molecule-based biosensors. In whole cell-based biosensors, the bio sensing element is integrated with the reporter gene. The working principle involves the reporter gene becoming active as a result of the treatment of the target analyte with the bioreceptor and emitting a measurable output signal in direct proportion to the heavy metal concentration (Odobašić, 2019).

Among the advantages of using a whole cell-based biosensor, the most important one, is to acquire information about the effect of a biological element on a living system by using only one component of the living cell. This system offers the possibility to compare the amount of biological element present with analytical information. Under favour of these biosensors, besides specific chemical analysis, rapid determination of general quality parameters and biological contamination can be evaluated. (Bousse, 1996).

A heavy metal specific regulatory elements are expressed from the resistance operon, which responds to the presence of heavy metals. When these elements are combined with appropriate optical and electrochemical bioreporters, they form a whole cell biosensor with a bioreceptor and/or transducer function.

1.4.1 Principles of Whole-Cell Bacterial Bioreporters

Whole cell-based biosensors which have been studied so far can be divided into two main classes according to the signal transmission strategy: electrochemical and optical biosensors. The first of these techniques, which is a widely used very

sensitive and fast sensing feature, functions by recording the change in conductivity or voltage across the cell membrane of the microbial cell, which is affected by the physiological conditions created by the heavy metal present in the environment. In other words, electroactive specimens produced/consumed by microorganisms are detected by methods such as conductimetric, amperometric, impedimetric or potentiometric. The second involves the use of natural bioluminescent bacterial strains or genetically modified organisms as detectors. That is, this technique works with the principle of measuring fluorescent, luminescent light production, colorimetric or other optical outputs emitted from living cells after treatment with heavy metals (Kannappan & Ramisetty, 2022; Olaniran et al., 2011; Su et al., 2011).

Many cell-based biosensors are used to detect and monitor heavy metals in water. It provides results by measuring quickly, its capacity to make continuous measurements, its ease of production, its ability to determine the concentrations of toxic substances, and its field test applicability to determine the effects of toxic heavy metals on living organisms (Odobašić, 2019; Teo & Ling Shing, 2014).

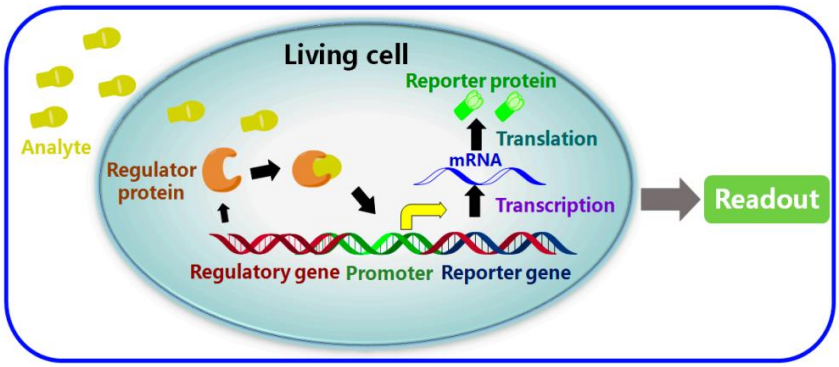

Figure 1.2 Schematic representation of whole cell based biosensor (Gui et al., 2017).

Whole cell-based biosensors, utilize the whole prokaryotic/eukaryotic cells as a single reporter, reuniting together bioreceptor and transducer elements in the same cell (Figure 1.2). They are based on using natural or genetically engineered biosensing cells, i.e. microorganisms, plant cells, algae, fungi, protozoa (Gavrilaș et al., 2022; Gutiérrez et al., 2015).

There are many advantages of using a whole cell as a biological element. For example, these cells are more resistant to various changes (i.e., pH and temperature) occurring in the environment. The cell includes all the enzymes and cofactors necessary to detect the analyte, indicating that a multistep reaction is possible. Its cultivation is easy, and its isolation and purification are not difficult. Since it allows the growth and maintenance of cells, it allows comfortable use *in situ*. Whole cell-based biosensor comprises of a transducer linked with immobilized living or non-viable microbial organisms. They are low-cost biosensing elements in both non-viable cells obtained after permeabilization and whole cells containing periplasmic enzymes that can be used instead of enzymes. However, contrast to enzyme, it can be considered as a disadvantage since it allows the entry of other analytes, although it is not pollutant/contaminant. In addition, they have relatively longer response times than biosensors developed with other biosensing elements (D'Souza, 2001; Odobašić, 2019).

Whole cell bacterial bioreporters (WCBs), developed according to classical recombinant DNA technology, are engineered by designing a plasmid vector (cloning vector). These vectors contain promoters that are sensitive to the presence of agents such as heavy metals that control the expression of the reporter gene (H. J. Kim et al., 2018; Martinez et al., 2019; Nourmohammadi et al., 2020). There are two main factors that determine the performance of WCBs. The first factor is the reporter gene selection. Another factor that is at least as important as the first is the selectivity and sensitivity of molecular recognition that eventuates when regulatory proteins bind to analytes (Gui et al., 2017).

Biological and specific responses of bacteria to toxic analytes via metal-sensitive transcription or bicomponent signal transduction arise in consequence of various interactions among bacteria and metals coexisting in the environment (H. J. Kim et al., 2018). Promoters in normal bacterial cells regulate genes that respond to stimuli. However, engineered bacterial bioreporters disrupt the association of promoters with the mentioned genes and these genes are replaced by reporter genes. After being supported by promoters, these modified reporter genes are transcribed and translated

into reporter protein that emits the signal such as bioluminescent, fluorescent, or colorimetric. (Cosansu & Ayhan, 2003; Ivask et al., 2009; Rawson et al., 1989; Xu et al., 2013). The major advantages of WCBs include the easy manipulation of bacteria and the production of large amounts of biomass in a very short time (Cerminati et al., 2015). Among the convenience of using bacterial cells, it has the potential to provide a stable environment against environmental conditions that will break down sensory elements and damage those (Odobašić, 2019).

In order for a biosensor to be highly effective and comprehensive, it must have certain features. These six basic features are given below (Bhalla et al., 2016; Dincer et al., 2019; Kalantar-zadeh, 2013).

i. Selectivity: It is the most important feature that a bioreceptor should have. Therefore, it is essential to consider when designing a biosensor. A selective bioreceptor has the capacity to detect the target analyte molecule from the environment where different pollutants coexist.

ii. Sensitivity: It is the capacity to detect target analytes in the sample or medium to be analyzed, even if they are in the lowest concentration (μg/L or fg/mL).

iii. Linearity: It is known that the higher the linearity, which is accepted as the accuracy criterion, the more accurate the detection of target analyte concentration by the biosensor. Linearity is the property mathematically represented as y=mc, which indicates the accuracy of the measured response (for sample containing different concentrations of analyte) to a straight line, where c is the concentration of the analyte, m is the sensitivity of the biosensor, and y is the output signal. Besides the analyte concentration tested, the resolution of the biosensor, expressed as the slightest change in the concentration of the analyte required to produce a change in the response of the biosensor, can be correlated with the linearity of a biosensor (Naresh & Lee, 2021).

iv. Response time: Time taken for the biosensor induced by the target analyte to identify and analyze the amount/concentration (about 95%) of that analyte.

v. Repeatability: This property is closely related to precision (ability to give the same results even when measured more than once) and accuracy (ability to produce an average value closer to the true value in each measurement). In other words, it can be defined as the biosensor giving the same results even if the same sample is measured more than once. However, this may not always be true for bacteria-based biosensors (Ranveer et al., 2015).

vi. Stability: It is the feature that the biosensor should have in studies where continuous monitoring is necessary. It can be defined as the degree of tenderness of the biosensor ambient disturbances in and around the biosensing system. These disturbances can cause a shift in the output signals of a biosensor responding in the presence of the analyte. This may cause an error in the measured concentration and affect the accuracy and reliability of the biosensor. The responses of transducers and electronics can be temperature sensitive, which can affect the stability of a biosensor. Therefore, the electronics used must be properly tuned in order for the designed/selected biosensor to give a stable response. Its limitations are the loss of the affinity of the bioreceptor (the degree of binding of the analyte to the bioreceptor) and the function of the bioreceptor over time.

The read signal in WCBs is produced by the expression of the reporter gene by the promoter. There are several reporter genes: the β-galactosidase gene (lacZ), the luciferase (bioluminescence) gene (luxAB and luc), and the fluorescent protein genes (GFP, YFP, CFP, and RFP).

Bacterial luciferase (lux) genes have the ability to respond very quickly, but it may not always be possible to use these genes as reporter genes in mammalian cells. Because these genes are thermally labile and suffer from dimeric protein interaction, they also need O_2. Instead of this gene, the researchers use the more sensitive and thermally stable gene, the firefly luciferase (luc) reporter, in mammalian cells. However, the limitations of this gene include its need for O_2 and ATP and its low permeability. Another reporter gene, β-Galactosidase (lacZ), is widely used in molecular biology as it is an excellent determinant and monitor of transfection

efficiency. Although the limitation of the lacZ gene is its low permeability and need for substrate, it is often preferred because it is simple to use, has a short response time, very high sensitivity capacity and low detection limit (Gui et al., 2017). The reporter gene, *gfp*, encoding green fluorescent protein (GFP), does not need the appendage of cofactors/substrates to emit light due to its autofluorescent. The autofluorescence source of GFP is associated with the internal covalent bonding of the imidazolinone chromophore. The formation of chromophores occurs by post – clastic modification. This means that the chromophore undergoes oxidation of three amino acids after cyclization. Also, the chromophore is the rate – limiting factor, that is, it determines the reporting rate of gene expression by the GFP. The disadvantages of GFP include the need for posttranslational modifications, mixing of light from biological systems with autofluorescence from GFP not being hypersensitive, and having cytotoxic properties for some cell types (Daunert et al., 2000). It is thought that this may be the reason why GFP – based biosensors are less sensitive than lux and lacZ – based biosensors. Fluorescent protein genes are suitable for visualizing WCBs in single cells, while luciferase genes are more useful in the use of quantitative detections (Song et al., 2012). Moreover, the linearity and stability of the fluorescent signals emitted by GFP require a longer time to be high, which negatively affects the maximum detection performance of GFP – based biosensors. Therefore, GFP – based WCBs are not suitable for use where analytes need to be detected in a short time. By radiation – free energy transfer from primary proteins (aequorin or luciferase) to GFP, the emission spectrum is converted from blue bioluminescence to green fluorescence. As this transition is made, GFP acts as an auxiliary protein to the primary proteins. The excitation spectrum of GFP has a maximum at 395 nm and a small peak at 475 nm, while its emission maximum occurs at 509 nm and a small shoulder at 540 nm (Brovko, 2010; Molinari et al., 2008; Ward, 1979).

To generate arsenic bioreporter which detects arsenite, ars is used as the promoter and arsR is used as the regulatory gene. Ars operons, which are frequently used in studies, were produced from the R773 and R46 plasmids of *E. coli*. In addition,

although bacteria have the chromosomally encoded *ars*RBC operon, which provides moderate resistance to arsenic, the most powerful resistance system for bacteria is the conjugative resistance factor R773, derived from *E. coli* (Elcin, 2019). With this technology, the arsR gene derived from *E. coli* and the operator/promoter region of the *ars* operon were used to develop the arsenic bioreporter plasmid in *E. coli* (Fujimoto et al., 2006). When arsenic is present in the medium, arsR dissociates from the promoter and regulates expression of the reporter gene. Expression of the reporter gene increases as the concentration of arsenic increases, so in this case WCB acts as an arsenic sensor. It is also very sensitive to detect arsenic concentration in μg/L range and has a high selectivity for arsenic even if other heavy metals and metalloids are present in the environment (Baumann & van der Meer, 2007; Loska et al., 2004).

Li et al. created the arsenite – inducible vector with GFP as the reporter gene, pUC18-*ars*-gfp and combined it with *E. coli* to design a whole cell biosensor. They noted that the As (III) detection limit capacity of this biosensor increased from 3 μg/L to 0.75 μg/L concentration after 1 hour of induction (Li et al., 2015).

Prévéral et al. formed the transcriptional fusion between the bacterial luciferase operon lux and arsenide controlled by the transcriptional repressor ArsR and an inducible promoter P*ars*. *E. coli* was used as the host for this construct. The whole-cell biosensors they designed were able to detect 7.5 μg/L – 15 μg/L As (III) concentrations in LB medium after 60 minutes (Prévéral et al., 2017).

Pola – López et al. created an arsenite – sensitive bacterial biosensor using *E. coli* as a host, made with three genetic modules called arsenic biosensor POLA (ABP). The detection capacity of this scheme was reported to be 1–h for 5 μg/L – 140 μg/L As (III) in LB medium (Pola-López et al., 2018).

Jia et al. developed an arsenic WCB with a positive feedback amplifier in Escherichia coli DH5α. The output signal from the mCherry reporter was significantly enhanced by the positive feedback amplifier. They showed that this designed whole-cell biosensor could detect 7.5 μg/L As (III) after 2–h (Jia et al., 2019).

Elcin and Oktem developed a *GFP* – based whole – cell bacterial biosensor using *E. coli* as host. They emphasized that this biosensor could detect arsenic forms (As (III) and As (V)) at a concentration of 10 µg/L after 2–h (Elcin & Öktem, 2020a).

1.4.2 Applications of Whole-Cell Bacterial Bioreporters

Whole-cell bacterial bioreporters are promising for use in a wide variety of fields in detecting pollutants and heavy metals in the environment according to it is simple, inexpensive, fast and not difficult to use (Roointan et al., 2015). After the enzymatic biosensor was developed to measure the sugar level in the blood, various biosensors have been developed that serve many purposes such as environmental and food monitoring and homeland security (Naresh & Lee, 2021).

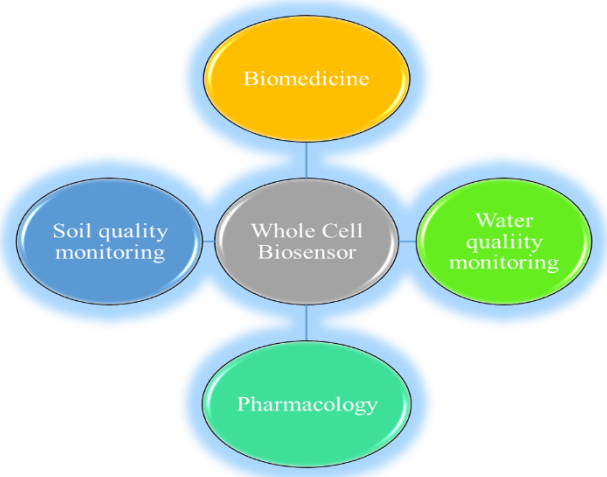

Figure 1.3 Main application fields of WCBs.

Biomedicine and Pharmacology

Another usage area of WCBs is medical/health fields. That is, they are suitable for use in applications such as cellular physiological analysis, pharmaceutical evaluation, and medical diagnosis in the field of biomedicine, thanks to their capacity to analyze biological metabolic disorders and other diseases at the cellular/molecular level (Gui et al., 2017).

Feng et al. (2007)., who generated a whole cell biosensor from a frog fibroblast cell line, aimed to evaluate the activity of G protein-coupled receptors and to determine the amount of adrenaline they secreted with this biosensor (Feng et al., 2007). The human hepatoma cell-based biosensor of Zager et al. (2010) can be given as a different example for the same field of use. For the simple and rapid detection of genotoxic agents, which is the aim of this study, they used a GFP encoding plasmid under the control of a p21 promoter to develop the biosensor (Zager et al., 2010). In their study, Riangrungroj et al. managed to develop a label – free optical whole cell biosensor using *E. coli* in order to determine the pyrethroid insecticide exposure with a limit of 3 ng/mL 3-phenoxybenzoic acid concentration in the linear range of 0.01–2 ng/mL (Riangrungroj et al., 2019).

Quantification of micronutrients can also be determined using WCBs. For example, excessive intake of vitamin B2 (Riboflavin), which has a very important place in human growth and development and causes serious diseases such as reproductive problems, metabolism disorders, anemia, skin disorders, visual impairment and even cancer in its deficiency in the body, is also harmful. In this case, it is inevitable to experience liver problems and skin problems against ultraviolet rays (Cardoso et al., 2012; Suwannasom et al., 2020). For the determination of this vital vitamin for humans, Si et al. developed a whole cell bio – electrochemical biosensor (Si et al., 2016). One of the most important steps in the diagnosis of diseases is the fast and reliable detection of the causative pathogens. Detection of these pathogens, which takes a very long time with traditional microbiological techniques, can be detected very quickly and with higher selectivity with whole cell biosensors (Shabani et al., 2008).

Soil quality monitoring

Soil is very important for all living things on earth. Soil, which is considered indispensable for living things to survive, has been polluted with various pollutants such as aromatic compounds and heavy metals brought about by developing industry and agriculture, which has seriously damaged the naturalness and sustainability of

the soil (Zeng et al., 2021). Detection and monitoring of soil pollutants is very important for the health and survival of living things in an ideal environment. In the light of recent studies, it is seen that the use of potential and promising WCBs in this regard allows the rapid determination and analysis of pollutants in the soil. WCBs recognize target analytes with their molecular recognition elements and emit a measurable signal. WCBs are very suitable for the detection and analysis of toxic substances in the soil, thanks to reasons such as simplicity, ease of use, portability, fast response, and cheapness (Francisco et al., 2019; X. Wang et al., 2014; Zeng et al., 2021). Frick et al. (2019) used a Cu and As (V) – responsive bioluminescent whole-cell biosensor to stabilize soil contaminated with chromated copper arsenates (CCA). Pcop/luxAB was used as Promoter/reporter construct, reporter was luminescent and host cell was *P. fluorescens*. (Frick et al., 2019). Yoon et al (2016) used *E. coli* based WCB in their study and tested the bioavailability of As in soil by measuring directly (*in vivo*) and indirectly (*in vitro*). WCB was used to evaluate the effectiveness of As-removal from As-regulated soils as well as where bioavailability could be achieved. According to the results obtained from the contaminated soil samples, it was observed that the amount of biologically usable arsenic increased, although the total amount of arsenic decreased. In the light of the results obtained, it is very important that the ecotoxicological aspects of soil improvement are important and WCBs provide information for soil recovery by determining the total amount of pollutants and bioavailability in the soil. WCBs were used to determine bioavailability in modified soil and soil solution samples. LUFA (modified soil) and field soil were added to the cultured cells and after waiting for certain times, they were centrifuged to remove the soil from the cells and finally to pick up the cells. The resulting cells were suspended and their GFP fluorescence was measured by spectrophotometer (Yoon et al., 2016).

Water quality monitoring

Heavy metals are inorganic pollutants that need to be studied and given importance because they have biological toxicity to the environment, humans, and other biota. According to the information obtained from the studies carried out up to this date, it

has become known that bacteria, in general, have defense mechanisms against the harmful effects of heavy metals. These bacteria can cope with metal toxicity through efflux carriers that remove harmful heavy metal ions from the cell or modification enzymes that can convert these toxic heavy metals into less harmful ones. Energy is spent for the expression of the proteins used in this process. Because the energy expended here imposes an additional metabolic load on the host cells, the defense against toxic substance becomes active only when heavy metal ions reach toxic concentrations. Inducing the defense mechanism in this way requires a specific transcriptional regulator that, upon metal binding, activates the transcription of genes in the downstream defense. As the defense against mercury (Hg) and arsenic (As) is activated by the transcriptional regulators MerR and ArsR, respectively. The developed metal bacterial bioreporter is formed by promoter/operator splicing in addition to the genes encoding the metal-specific transcriptional regulator. This promoter/operator is the defense related genes to a promoterless reporter gene (luc, gfp, yfp or cfp) or reporter genes (luxAB and luxCDABE). After WCBs are induced by the heavy metal, the transcriptional regulator becomes active, which then regulates the expression of the reporter gene(s), producing a measurable readout signal (Xu et al., 2013).

Roointan et al. (2015) developed WCB to detect mercury in water in their study. GFP gene, that was cloned downstream of the Pseudomonas pBS228 merR gene, was used as the reporter. They cloned merR promoter, gene, and GFP into the vector and transferred it to the *E. coli* BL21 (DE3) to use as a host cell. Engineered bacteria were used as WCBs for detecting mercury. They used microscopy and fluorescence to measure the mercury concentration in water. It has been noted that it can detect even concentrations of mercury of 10^{-8} M after 3h treatment with mercury (Roointan et al., 2015).

The study of King et al (1990) which reported WCB developed by to detect naphthalene, is one of the first studies to show the importance of genetic engineering and how to use it to turn bacteria into biosensors (King et al., 1990). Development of this WCB was accomplished by combining the bacterial bioluminescence gene

with an expression promoter induced by naphthalene and transferring this construct into a *Pseudomonas fluorescens*. Engineered *E.coli* is used in WCBs developed with the same logic and used to detect metals such as silver (Martinez et al., 2019), arsenic (Elcin & Öktem, 2020a), cadmium (Elcin & Öktem, 2020b; G. Zhang et al., 2021), copper (Chen et al., 2017; Costello et al., 2020; Kang et al., 2018) or zinc (Watstein & Styczynski, 2018). It was known that arsenic detecting WCBs have higher sensitivity than other chemical test kits even at low levels (Trang et al., 2005).

1.5 Support Materials for Bacterial Bioreporters

Immobilization of whole-cell bacterial bioreporters is of great importance in order to survive and preserve the response properties of bioreporters. WCBs have been immobilized on transducers or support materials using various techniques to obtain integrated microbial biosensors. Immobilized cells are more resistant to environmental changes (such as pH or temperature) and have the potential to be reused. (Berillo et al., 2021). These immobilization techniques are generally divided into 4 groups as encapsulation/entrapment, covalent bonding, crosslinking and adsorption (Bjerketorp et al., 2006; Vigués Frantzen, 2020). Encapsulation/entrapment is a physically immobilized and irreversible immobilization method, usually in small sizes of porous matrix or hollow fibered that provides a 3D environment to living cells. For a matrix to be defined as ideal for the immobilization of WCBs, it must be functional at room temperature, be biocompatible, prevent the escape of immobilized cells, allow nutrients, oxygen, and analytes to circulate by diffusion, and have protective barrier properties.

In principle, encapsulation/entrapment cells should be able to survive for a long time, that is, they should be stored under suitable conditions, but should not grow. In studies conducted with this principle, it has been reported that bacterial growth is limited in encapsulation/entrapment immobilization with inorganic polymers, which does not include carbon atoms in the backbone (Michelini & Roda, 2012). Covalent binding occurs when reactive groups on the cell are attached to reactive groups on

the support material surface, such as carboxyl or amine groups. In order to do this immobilization, a bifunctional cross-linker, either homo or hetero, is needed. Although very few, it has been reported that glutaraldehyde homobifunctional crosslinker (binder agent) is used for covalent immobilization in some studies. Some environmental parameters, temperature, pH or ion energy affect the success of immobilization. This technique, which is generally used for enzyme immobilization, is also rarely used for WCBs in some cases, but the binder agents used can impair cell viability or enzyme activity (Bayat et al., 2015; Shriver-Lake et al., 2002). Adsorption immobilization technique is divided into two groups, physically or chemically, according to the type of interactions between the bacterial cell and the support material surface. In physical adsorption with van der Waals forces, bacterial bioreceptor cells are deposited on the support material and after a certain period of incubation, the non-adsorbed ones are removed by washing with liquid solutions. This very easy immobilization technique can be performed under static or dynamic conditions. On the other hand, chemical adsorption by electrochemical or ionic interactions, electrostatic charges on the support material attract and immobilize the bacterial bioreceptor. Another group of bacteria immobilization techniques is the attaching bacteria bioreporter to a surface. The parameters that affect the success of this technique are the characteristics of the support material used, the bacterial cell surface, the adhesive substances secreted by the bacterial cells, and even having flagella/fimbriae (Kimkes & Heinemann, 2020; Michelini & Roda, 2012; Zheng et al., 2021).

Even if any support material is used in this immobilization, it does not mean that almost all of the bacterial cells will be successfully immobilized (Jesionowski et al. 2014). In such cases, the efficiency of immobilization increases, and they can be adhered more successfully, thanks to the use of organic/inorganic compounds such as positively charged poly – L – lysine, polyethyleneimine, chitosan and silanes. The positive charge of these molecules attracts negatively charged bacteria to the sensor surface for immobilization. No conclusion has yet been reached regarding which type of support material the bacterial cells adhere to better. While some researchers'

reports state that hydrophobic materials are more suitable for the adhesion of bacteria according to their wettability (Bruinsma et al., 2002; Parreira et al., 2011), the studies presented by some researchers show that materials with hydrophilic surfaces are more suitable for bacterial cells to adhere to and maintain their viability (Lassen et al., 1994; Yuan et al., 2017). It is thought that this difference may be related to the better spread of bacteria on hydrophilic surfaces due to the polar nature of the extracellular polymeric substance (Oh et al., 2018).

The selection of support materials to be used in the immobilization of bacterial bioreporters has a very important place. For this purpose, ideal support materials should be insoluble, non-biodegradable, harmless, non-polluting, light, flexible, robust, chemically stable, less preferred for other microorganisms, and affordable. Support materials are divided into two groups as inorganic material (zeolite, clay, anthracite, porous glass, activated charcoal and ceramic) and organic polymers. Inorganic materials are more resistant to microbial degradation and have thermostable properties. However, organic polymers are more common in nature and they are divided into two groups as natural and synthetic (Suzana et al., 2013).

Natural algal polysaccharides such as alginate, carrageenan, agar and agarose, chitosan, which is chitin-derived amino polysaccharide, and various synthetic (acrylamide, polyurethane, polyvinyl, resins) polymers have been used as support materials in bacterial immobilization. Although natural polymers such as alginate and carrageenan are the most commonly used polymers, synthetic polymers are more advantageous because natural polymers are unstable in wastewater (Suzana et al., 2013).

1.5.1 Electrospun Fiber Support Materials

Electrospinning technique can be used to produce a potential support material for the immobilization of microorganisms, especially bacteria, due to its unrivaled features such as simplicity, versatility, and cheapness technique and it makes possible tunable

fabrication, high porosity, large surface area of fibrous matrices, the morphology of fibers can be controlled by changing parameters (Horzum et al., 2017; Tlili & Alkanhal, 2019). Bacteria have been immobilized on matrices or substrates ranging from silica gels to alginate microcapsules or from glass beads to polymer microparticles, depending on their characteristics and application areas (Xie et al., 2016).

Salalha et al., in their study in 2006, subjected bacteria (*Escherichia coli* and *Staphylococcus albus*) and bacterial viruses (T7, T4 and λ) suspended in a poly (vinyl alcohol) (PVA) solution dissolved in water to electrospinning technique. It was stated that the bacteria and viruses encapsulated in this study managed to survive in the electrospinning technique and maintained their viability at very high levels (Salalha et al., 2006).

Gensheimer et al., *Micrococcus luteus* and *E. coli* bacterial organism suspensions and polyethylene oxide (PEO) polymer solution were subjected to electrospinning technique separately and produced biocomposites. The viability of these developed biocomposites was tested and they revealed that the PEO polymer is suitable as a bacterial carrier (Gensheimer et al., 2007).

In another study, a support material that was not used very often before was used. *Pseudomonas fluorescens* bacteria were physically adsorbed on this matrix by using the eggshell membrane, which has very high gas and water permeability, as a support material. As a result of this study, Yeni et al. suggested that immobilization by bacterial adsorption on the eggshell membrane is a sufficiently simple and rapid procedure for the preparation of stable, immobilized microorganisms. It has been demonstrated that this step, called preactivation, which is important to prevent the toxic effects of glutaraldehyde on existing healthy cells, is not needed thanks to the procedure proposed by them (Yeni et al., 2008).

Chu et al. used polyethyleneimine as a crosslinking agent for immobilization of *E. coli* bioreporter cells onto Cotton, polyester, rayon and silk fibers as the support

material and it was observed that *E. coli* bioreporters could remain alive and stimulated for 3 days in this immobilized structure (Chu et al., 2009).

Klein et al. encapsulated *Pseudomonas ADP* bacteria in polycaprolactone microtubes via coaxial electrospinning technique. When comparing encapsulated cells with free cells (unencapsulated), it was reported that encapsulated cells retained approximately 10% of phosphatase activity and 23% of β-galactosidase activity (S. Klein et al., 2009).

Liu and his team added a bacterial suspension (*Pseudomonas fluorescens, Zymomonas mobilis* and *E. coli*) into the hydrophobic FDMA/PEO polymer solution and subjected it to electrospinning technique to obtain water – insoluble fibrous polymeric material. It has been shown that the encapsulated bacteria survive more than 1 week at 4 °C and more than 2 months at –70 °C in their study, which aims to elucidate the conditions of the electrospinning and crosslinking process that allows encapsulation as well as preserving the viability of intact bacteria. It has been suggested that the integrity and viability of bacteria are maintained by the cross – linking process (Liu et al., 2009).

Lopez – Rubio et al. carried out the encapsulation of bifidobacterial strains by coaxial electropinning technique by using PVA polymer, which is water-soluble and has a high oxygen barrier, as a carrier. It has been shown that the integration of *Bifidobacterium animalis* Bb12 into the fiber leads to a decrease in the melting point and crystallinity of PVA fibers and an increase in the polymer glass transition temperature. As a result of the viability tests performed at different temperatures, it was found that they can survive up to 40 days at room temperature and 130 days at -20 °. In addition, no significant decrease was observed in the viability of non-encapsulated bacteria in both conditions (López-Rubio et al., 2009).

Fung et al. probiotic bacteria, *Lactobacillus acidophilus*, was incorporated into the spinning solution to fabricate bacteria encapsulated nanofibers. As encapsulators of bacteria, soluble dietary fiber (SDF) from agrowastes, okara (soybean solid waste), oil palm trunk (OPT), and oil palm frond (OPF) obtained via alkali treatment were

used. According to the results of the viability studies they gave, it is seen that after being subjected to the electrospinning technique, the bacteria maintain their viability at a rate of about 78.6 – 90% and they maintain their viability after 21 days of storage at +4°C. They claimed that this study was the first to use agricultural waste-based electrospuns used as carrier material for *Lactobacillus acidophilus* (Fung et al., 2011).

Sarioglu et al. successfully immobilized *Acinetobacter calcoaceticus* STB1 bacterial strain on electrospun porous CA fiber. The ammonium removal capacity of this developed biocomposite has been tested and is 100%, 98.5% and 72% within 48 hours for 50 mg/L, 100 mg/L and 200 mg/L, respectively. When their reusability was investigated, it was shown that the bacteria immobilized CA fiber system could be reused for at least 5 cycles (Sarioglu et al., 2013).

Keskin et al. showed in their study that CA electrospun fiber is a suitable support material for the immobilization of bacteria. Three different bacterial strains (*Aeromonas eucrenophila, Clavibacter michiganensis and Pseudomonas aeruginosa*) were immobilized and their potential for decolorization of methylene blue dye was evaluated. It was stated that 95% effective methylene blue dye removal was achieved within 24 hours and this rate was very close to that of free bacteria (non-immobilized). Their re – use capacity has been tested and it has been highlighted that there is a 45% dye removal potential after the fourth cycle (San et al., 2014).

Sarioglu et al. performed the immobilization of two different sodium dodecyl sulfate (SDS) biodegradable bacterial strains, *Serratia proteamaculans* STB3 and *Achromobacter xylosoxidans* STB4, on electrospun mats. They used porous and non-porous CA as electrospun support materials for bacterial immobilization. The effects of contact time required for immobilization were investigated and ended with a contact time of 25 days. It has been stated that porous electrospun mats are more suitable than non-porous for immobilization. By evaluating the SDS biodegradation capacities of porous CA – immobilized bacteria, it has been shown that the most

efficient results are obtained at concentrations up to 100 mg/L. It was emphasized that the biodegradation results were similar to the free cells (Sarioglu et al., 2015).

Keskin et al. identified a pollutant – resistant Lysinibacillus sp. NOSK bacterial strain was immobilized on polysulfone electrospun fiber to remove Reactive Black 5 (RB5) and Cr (VI). The researchers worked with several parameters such as initial pH value, temperature, shaking conditions, RB5 and Cr (VI) at different concentrations in order to determine the performance of this system they developed in their studies. In the shared results, it is given that the removal rate of RB5 was 99.7%, and Cr (vi) was 98.2%. In addition, according to the results of the reusability test, the developed bacteria immobilized PSU fiber system has 28.1 ± 0.6% and 66.7 ± 0.8% removal efficiency for RB5 and Cr (VI), respectively, thus at least 7 cycles. stated that they can be reused (Keskin et al., 2015).

In a study by Sarioglu et al. *Morganella morganii* STB5 bacteria were immobilized to electrospun polystyrene (PS) and polysulfone (PSU) fibers and the hexavalent chromium recovery performance of this immobilized system was evaluated. According to the results obtained, it was found that the bacteria adhered very strongly to the polymeric surfaces of the fibers. Also given are the removal efficiencies of hexavalent carbon at different concentrations over 72 hours: 93.60% and 93.79% for 10 mg/L, 99.47% and 90.78% for 15 mg/L, and 70.41% and 68.27% for 25 mg/L. According to the reusability test, it has been shown that it can be reused for at least 5 cycles. The storage results also proved that this biocomposite system can be stored without losing its bioremoval capacities (Sarioglu et al., 2016).

In a different study, the same team investigated the immobilization of specific bacteria (*Clavibacter michiganensis*) on electrospun mats (polycaprolactone (PCL) and polylactic acid (PLA)) in order to remove textile dyes (Cetazol Blue BRF-X). It has been proven that both PCL and PLA fibers are suitable support materials for bacterial immobilization, and it has been observed that this immobilized system cured the tested dye within 48 hours. It was emphasized that the results obtained were similar to those of free (not immobilized) bacteria, and their reusability was

tested, and good results were obtained even at the end of the 5th trial (Sarioglu et al., 2017b).

In another study conducted in the same year by Sarioglu et al., the fabricated polysulfone (PSU) electrospun fibers were used as support material and bacteria were immobilized on these fibers. At the end of the 21 – day immobilization period, the bioremediation performance of this developed immobilized system was evaluated and the effects of fiber morphology (random or aligned) and diameters (small and large diameter) on performance were investigated. Random oriented and thin (1065 ± 140 nm) fibers have been found to be the most suitable support material for the immobilization of bacteria, and according to the results of the trials performed on this system, it has been proven that they are promising for the simultaneous removal of ammonium and methylene blue dyes (Sarioglu, et al., 2017).

Keskin et al. encapsulated bacteria for bioremediation into cyclodextrins (CD), which can be converted into ultra – thin electrospun fiber form with the electrospinning technique. Within the scope of the study, the bacterial suspension in CD aqueous solution was subjected to electrospinning technique. They showed that this developed biocomposite system is suitable for wastewater treatment application, CD fibers act as an additional source of nutrition for bacteria and maintain cell viability for up to 7 days after storage conditions at +4° C. In addition, the performance of this system in removing heavy metals such as Nickel (II) and chromium (VI) and RB5 dye was evaluated, and it was stated that they were more efficient than unencapsulated bacteria (Keskin et al., 2017).

In another study conducted by Sarioglu et al., *Pseudomonas aeruginosa* bacterial strain was electrospinned with polyvinyl alcohol (PVA) and PEO aqueous solutions. They evaluated the methylene blue dye improvement performance of this biocomposite system they developed. Efficient results were obtained for both biocomposite systems (bacteria/PVA and bacteria/PEO) from methylene blue dye improvement trials. However, the bacteria/PEO biocomposite system gave much better results than the bacteria/PVA system. In addition, the viability test results

performed after storing at +4 degrees for 3 months showed that the bacteria preserved their viability (Sarioglu et al., 2017a).

Gordegir et al. combined polyethylenimine (PEI) polymer with polycaprolactone (PCL) to provide functional amino groups that are absent in the polycaprolactone polymer. *Gluconobacter oxydans* bacteria were immobilized with covalent bonds with the help of glutaraldehyde in this PCL/PEI nanofiber fabricated via electrospinning technique. While the characterization of this developed gluconosensor was done by SEM, its performance was tested with its glucose sensing capacity. It has been observed that the response time of the sensor is shortened and the limit of detection concentration for glucose is reduced (Gordegir et al., 2019).

Jayani et al. evaluated its potential as a support material for the development of bacterial cellulose nanofibers (BCNF) and the immobilization of probiotic bacteria *Lactobacillus acidophilus* 016 by adsorption method. Characterization test results such as tensile strength, surface area, pore properties and thermal analysis of cellulose fiber showed that these nanofibers can be used in the food industry. SEM results showed that the immobilized bacterial cells remained intact up to 24 days in storage conditions. In addition, 71% proved to survive when stored at 35 °C. The results of this study suggest the availability of BCNF-based probiotics for various commercial applications (Jayani et al., 2020).

Bacterial immobilization studies using CA and PCL fibers used in our study, which are available in the literature, are summarized in Table 1.2.

Table 1.2 Bacterial immobilization studies on CA and PCL electrospun fibers in the literature.

Polymer	Bacteria	Immobilization Technique	Application	References
CA	*Acinetobacter calcoaceticus* STB1	Adsorption	Ammonium removal	(Sarioglu et al., 2013)
	Aeromonas eucrenophila, Clavibacter michiganensis and Pseudomonas aeruginosa		Decolorization of methylene blue	(San et al., 2014)
	Serratia proteamaculans STB3 and *Achromobacter xylosoxidans*		SDS biodegradation	(Sarioglu et al., 2015)
PCL and PLA	*Clavibacter michiganensis*		Textile dye (Cetazol Blue BRF-X) removal	(Sarioglu, et al., 2017b)
PCL/PEI	*Gluconobacter oxydans*	Encapsulation	Gluconosensor development	(Gordegir et al., 2019)
BCNF	*Lactobacillus acidophilus*	Adsorption	Optimization study	(Jayani et al., 2020)

1.5.2 Applications of Electrospun Fiber Support Materials

The immobilization process, which limits the active area of the bacteria, allows them to be used safely. The use of electrospun fibrous mats as support material/carrier for the immobilization of bacteria is also very suitable since the electrospinning technique has the potential to be a technique that can fabricate these electrospun fibers in a versatile manner. That is, these fabricated electrospun fibers have large surface area, high porosity, and interconnected pores. Encapsulated/Adsorbed bacteria system can use drug delivery, wastewater treatment, water filtration, and bioremediation applications (Sarioglu et al., 2017). The increasing popularity of nanofiber – based technologies produced by electrospinning in the pharmaceutical distribution industry can be attributed to the high surface area/volume ratio that allows the controllable diffusion of active ingredients from the material (Diep & Schiffman, 2021). From another point of view, which supports the use of electrospun fibrous mats fabricated via electrospinning technique in drug delivery, these electrospun mats can be designed for localized drug delivery in order to alleviate systemic toxicity and provide drug accumulation in tumors. Compared to the current protein and gene delivery strategy, it has superior advantages in sustained release, bioactivity retention, and toxicity reduction (Xie et al., 2016).

1.5.2.1 Drug Delivery

Xie et al. who designed GFP – containing *Escherichia coli* bacteria in 2016, arrested these engineered bacteria in core - sheath fibers via coaxial electrospinning or grafted on the fiber surface by covalent binding or affinity adsorption. In this study, which was conducted for the first time until 2016, it was aimed to evaluate the potential of bacteria encapsulated in fibers to be used in drug delivery system. The pore sizes of the fibers obtained by using NaCl microparticles increased, thus facilitating protein secretion into the environment. In this study, optimization experiments were carried out by investigating the parameters of the production technique and the

immobilization methods on bacterial viability. In addition, growth and protein expression of immobilized bacteria were evaluated. It has been shown that approximately 48% of the bacteria in the fiber produced with optimum electrospinning parameters can survive. On the other hand, it was stated that bacteria immobilized on the fibers achieved a high bacterial viability over 92%. It was emphasized that the number of bacteria immobilized by affinity absorption was significantly higher than that of covalent bonding. The results obtained in this study show that the immobilization of engineered bacteria on electrospun fibers has the potential to be used for drug delivery purposes. In particular, they emphasized that their aim in this study is the immobilization of bacteria designed on electrospun fibers for efficient gene delivery and disease management (Xie et al., 2016).

1.5.2.2 Wastewater Treatment

Changing the properties of electrospun fiber mats by changing the parameters during production gives strong advantages in their use in various application fields such as water treatment. Fibrous mats used in wastewater treatment should not dissolve in water and should be easily recoverable (Nayl et al., 2022).

Biocompatible polystyrene (PS) and polysulfone (PSU) polymers can be given as examples of fibrous mats produced via the electrospun technique used for water filtration (Sarioglu et al., 2016).

The use of electrospun fiber mats, which are used as support material in bacterial immobilization, for the detection and removal of pollutants has become very popular and studies on this have recently started to take place in the literature. (Sarioglu et al., 2016)

Sarıoğlu et al. (2013) achieved the removal of ammonium in the aqueous medium by immobilizing *Acinetobacter calcoaceticus* STB1 cells on CA (Sarioglu et al., 2013).

In their another study, Sarıoğlu et al. (2017) utilized water-based and biocompatible polymers, PVA, and PEO, for the encapsulation of bacterial cells in order to maintain the viability of the bacteria and not be affected by the external environment. They tested the methylene blue dye removal capacity of these bacteria-immobilized electrospun fibers and showed that they provided bioremediation in water (Sarioglu et al., 2017).

Zamel et al. (2019) tested the methylene blue dye removal capacity of this system from aqueous media by producing a CA/PEO electrospun mat loaded with *Bacillus paramycoides*. In this process, adsorption and biodegradation strategies were used together. Moreover, it has been shown to be reusable and it has been noted that 44% methylene blue dye can be removed even after a fourth use (Zamel et al., 2019). While cyclodextrin electrospun fibers are a very suitable support material for bacterial encapsulation, they are also a very powerful nutritional source for bacteria.

And in order to test the bioremediation capacity of this biocomposite material in wastewater treatment, Keskin et al. tested some heavy metals, such as nickel and chromium, textile dye, reactive black 5, removal capacity. Their results show that the bioremediation capacity of bacteria-encapsulated cyclodextrin electrospun biocomposite material is promising in wastewater treatment (Keskin et al., 2017).

The poor adhesion between bacteria and electrospun fibers greatly limits the use of this laboratory-created structure on a real-life industrial scale. Moreover, when this structure is used in water applications, it is inevitable that bacteria that cannot adhere are easily separated from the electrospun fibers. For this reason, the encapsulation of bacteria in electrospun fibers has become a subject that has been focused on recently (Zamel et al., 2019). The rapid change in osmotic pressure, which occurs with the rapid evaporation of water during electrospinning, causes the bacteria to lose their viability. This is also disadvantages of bacterial encapsulation (Hirsch et al., 2021; Xie et al., 2016).

1.6 Aim of the Study

In this thesis study, our chief goal is to arrest fluorescent *E. coli* bioreporters with the ability to detect toxic metals on electrospun fibers by immobilization technique.

In the first part of the thesis, the fabrication of electrospun fibers via electrospinning technique from CA and PCL polymer solutions prepared at different concentrations and the immobilization of arsenic bioreporter cells on these fibers were done.

In the second part of the thesis, various characterization tests were carried out to determine the properties of the fabricated electrospun fibers and optimization of immobilized bioreporter systems. Morphological, chemical structure and wettability properties of the fabricated electrospun fibrous support materials were determined by different characterization tests such as SEM, FTIR and contact angle. The fluorescence performances of the bacteria immobilized CA and PCL electrospun fibers in the presence of arsenic were optimized under different conditions such as growth phase and bacterial cell concentrations. In addition, viability tests were performed to determine the percentage of live bacteria attached to the fibers. Continuing from the bacterial cell system immobilized on PCL, the effects of growth medium and immobilization time on the fluorescence performances emitted in the presence of arsenic were investigated.

In the last part of the study, arsenic detection limit, metal specificity, and response time experiments in the presence of arsenic were performed to characterize the properties of bioreporters immobilized on PCL. Additionally, shelf – life tests were conducted to evaluate the performances of bioreporter systems stored under certain conditions for certain periods.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

Commercial cellulose acetate (CA; Mn 30 000) and polycaprolactone (PCL; Mn 70 000–90 000) were purchased from Aldrich (Missouri, USA.) were utilized as fiber support material for bacterial immobilization. Dichloromethane (DCM; ≥99.9%) and acetone (Laboratory Reagent ≥99.5%) were purchased from Sigma Aldrich (USA) that were used as solvents of polymers utilized to fabricate fiber via electrospinning technique. Arsenic sensing pbR_arsR_sGFP in *E. coli* whole cell bacterial bioreporter was used as bacteria immobilized on fibers and utilized to detect arsenic in aqueous solution. M9 minimal medium and MOPS – supplemented medium (Appendix A) were used as the bacteria growth medium. Ampicillin (100 μg/mL) was added to bacterial culture to maintain plasmid stability and positive selection. Sodium arsenite ($NaAsO_2$) and sodium arsenate ($Na_2HAsO_4.7H_2O$) were supplied from Merck, Sigma-Aldrich and Applichem was used as an inducer. $AgNO_3$, $CdCl_2$, $CuSO_4$, $FeCl_3$, $MgCl_2$, $MnCl_2$, Na_2MoO_4, $NiCl_2$, and $ZnCl_2$ metal salts were supplied from Merck, Sigma – Aldrich and Applichem utilized for determining immobilized bioreporter system's selectivity characteristics. Resazurin sodium salt (known as AlamarBlue) were purchased from Serva (Heidelberg, Germany) for determining cell viability.

2.2 Methods

As shown in Figure 1.4, the optimization of fabrication of fibers used as support material for bioreporter bacterial cell from polymer solutions via electrospinning technique and the immobilization of bioreporter bacterial cell to these fabricated

fibers constitute the first part of the thesis. Characterization of the features of the fabricated fibers and optimization studies of the designed bioreporter immobilized on fibers are included in the second part of the thesis study. In the third and last part, the performance properties of the developed bioreporter immobilized fibers was investigated. The experimental part of the study is given below, respectively.

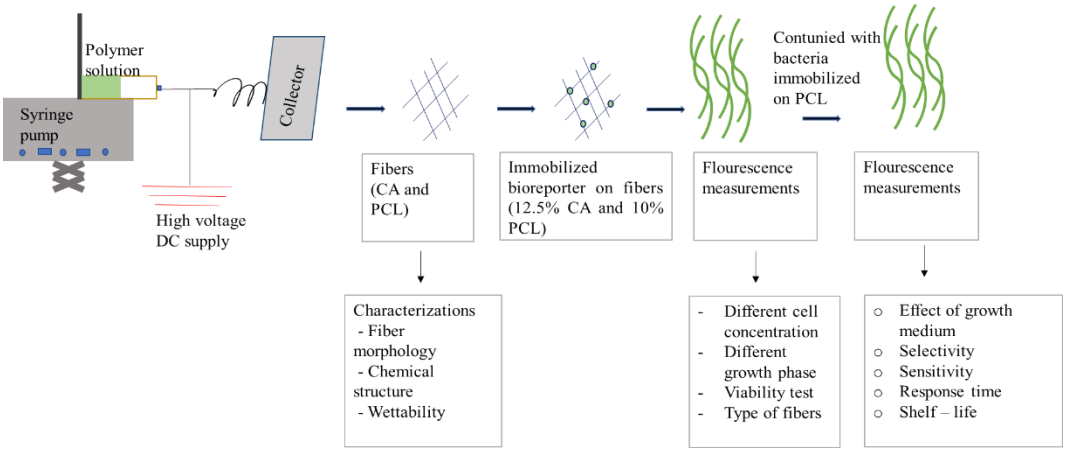

Figure 2.1 An illustration of a summary of the experimental studies.

2.2.1 Fabrication Support Materials and Their Characterizations

2.2.1.1 Preparation of Polymer Solutions

Various concentrations of CA (5 – 7.5 – 10 wt% and 12.5 wt%) and PCL (7.5 wt% and 10 wt%) solutions were prepared for electrospinning. CA polymer was dissolved in dichloromethane:acetone (DCM:Ace, 2:1 v/v) (San et al., 2014) solvent system. PCL polymer dissolved in different solvent systems, such as dichloromethane (Qin and Wu, 2012), tetrahydrofuran (Katsogiannis et al., 2015), and combinations of DCM: dimethylformamide (DCM:DMF, 1:1 v/v) (Mochane et al., 2019) was then subjected to electrospinning technique, but fiber could not be obtained. Eventually, fiber could be obtained from the PCL solution prepared in acetone (Malikmammadov et al., 2019).

2.2.1.2 Electrospinning Set Up

The experimental setup for conventional electrospinning experiments consisted of NE-1000 syringe pump (New Era Pump Systems, Inc., New York, USA), Gamma High Voltage Source ES30 (Gamma High Voltage 25 Research, Inc., Florida, USA), static metal collector (Gözer Elektronik, Ankara, Turkey) and plexiglass cabinet (Section Plexi, Ankara, Turkey) made up of PMMA. During conventional electrospinning, the system is set up on the horizontal axis to ensure that the formed jet is equally affected by the gravitational forces along the way. The static metal collector was covered with an aluminum sheet and all the components of the system were placed in the cabinet according to the specified configuration, distance, and angle (Figure 13).

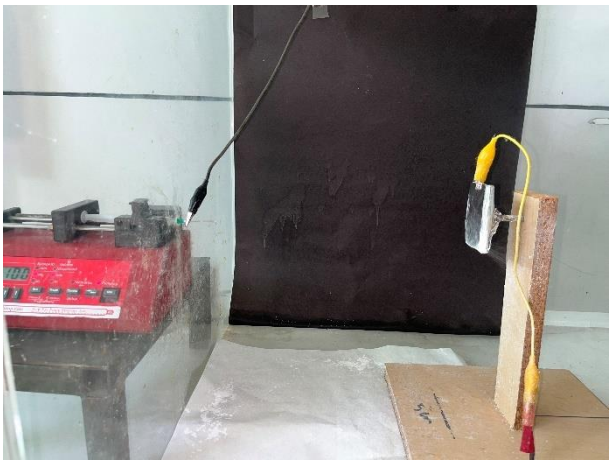

Figure 2.2 Electrospinning set-up.

Eventually, the solution loaded into the syringe pump was electrospun at the adjusted flow rate, electrical voltage provided by the connected power supply and tip-to-the-collector distance to fabricate the fibrous webs. Electrospining parameters tested for the fabrication of fiber support materials are given in Table 2.1. CA and PCL fibers were fabricated with the final parameters decided. For CA, the flow rate was 1.0 mL/h, the applied voltage was set to 15 kV, and the tip to collector distance was set to 10 cm. For PCL, on the other hand, flow rate was set as 6.0 mL/h, applied voltage 15 kV, and tip to collector distance 30 cm.

Table 2.1 Prepared polymer:solvent system concentrations and electrospinning fabrication parameters.

Polymer	Polymer concentration (wt%)	Solvent systems (v/v)	Voltage (kV)	Flow rate (mL/h)	End to tip distances (cm)
PCL	10 – 15 – 20 – 25	DCM:DMF (1:1)	25	0.5	15
	7.5 – 10	Ace	15	6.0	30
CA	5 – 7.5 – 10 – 12.5	DCM:Ace (2:1)	15	1.0	10

2.2.1.3 Characterization of Support Materials

2.2.1.3.1 Morphology of Fabricated Electrospun Fibers

The morphology of the CA and PCL fibers was investigated using scanning electron microscopy (SEM; Stereoscan S4-10, Cambridge, UK and JSM-6400 Electron Microscope, Jeol Ltd., UK in Center of Excellence in Biomaterials and Tissue Engineering, BIOMATEN at METU, Ankara, Turkey) operating at an accelerating voltage of 5 kV. The fibrous mats fabricated were cut in 1x1 cm^2 dimensions. Prior to the imaging, a gold coating (682 PECS, Gatan, Inc., USA) was applied to the fibrous samples to enhance the fibers conductivity. Fiber morphologies and fiber diameter distributions of electrospun fibrous support materials were examined from various images obtained from different regions on the surface of the samples. From three different SEM micrographs at least 100 fiber segments of each micrograph were randomly selected, to calculate the average diameter (via using ImageJ software) and fiber diameter distribution graphs were created via using Origin software.

Pore size diameters of 12.5 wt% CA and 10 wt% PCL fibers were also calculated from obtained SEM micrographs analyzed in ImageJ software and distribution histogram graphs were constructed via using Origin software.

2.2.1.3.2 Wettability of Fabricated Electrospun Fibers

The wettability of the fabricated fiber support materials (CA and PCL) was determined via a contact angle measuring device (One Attension, Biolin Scientific, Sweden, METU). Water contact angle measurements of samples were made with sessile drop method using goniometer at room temperature. dH_2O dripped on each fiber, the process was repeated with three different samples. Average contact angle values were reported.

2.2.1.3.3 Chemical Structure of Fabricated Electrospun Fibers

To identify molecular structure, such as functional group and bond of these fabricated fiber membranes (CA and PCL) was done via Fourier transform infrared spectrometry (FTIR; PerkinElmer L1050002 series, PerkinElmer, Inc., UK) in METU, BIOMATEN using spectrum 100/100N software program in transmission mode. The analysis was performed within the wavelength range of 400 – 4000 cm^{-1}, with a resolution of 4 cm^{-1}, and a total of 50 scans per sample.

2.2.2 Immobilization and Optimization Studies

2.2.2.1 Immobilization of Bioreporter Bacterial Cells on Fibers

For the immobilization study, arsenic sensing bacterial bioreporter cells previously developed in our laboratory (Elcin & Öktem, 2020a) were used. In their study, Elçin and Öktem created a DNA sequence to detect arsenic from the promoter region of the arsR operon and the arsR gene derived from *E. coli* plasmid R773. DNA fragments were amplified by PCR and the sticky ends were ligated with BamHI after digesting the promoterless plasmid pBR_sGFP with EcoRI. After transforming this resulting product into *E. coli* DH5a cells, they transformed the sensor plasmid,

named pBR_arsR773 into *E. coli* MG1655 cells. These engineered arsenic sensitive bioreporters was referred to as pBR_ars773_sGFP in *E. coli* in this thesis.

Cultivation of Bioreporter Bacterial Cells

E. coli bacterial bioreporter cells were kept in Luria – Bertani (LB) agar – ampicillin (Appendix A) petri dishes for storage at +4°C and in 20% glycerol and stored at – 80 °C as freezer stocks. M9 (minimal) medium and MOPS – supplemented medium (Appendix A) were used for growth and immobilization of bacteria. Plasmid – selective antibiotics, ampicillin (Amp, 100 μg/mL), was added to the bacterial suspension. Before starting the experimental procedures, stock bacteria stored at 4°C were cultured in M9 minimal medium or MOPS – supplemented medium, then the inoculated flasks were incubated at 37 °C on a rotary shaker at 150 rpm. After growing in the incubator for 18 h, experimental procedures were started.

Immobilization

The fabricated fiber support materials were prepared by cutting in a circle shape to fit into the wells of a 96-well microplate. For sterilization, after being treated with 70% ethanol for 5 minutes, they were left under UV for 2 hours. Then, sterile fibers were placed into the 96-well microplate (black and opaque) and 100 μL of each bacterial cell culture was transferred to the wells onto the fibers for absorption (immobilization) for 3h. Thus, at least 4 repetitions were made for each sample. Many parameters were tested for optimization and characterization of cell immobilization and fluorescence studies.

2.2.2.2 Optimization of Bioreporter System Parameters

Cell concentration and cell growth phase parameter trials were conducted for the optimization of designing arsenic bioreporter systems using –PCL and –CA fiber support materials. In this optimization experiment, the effects of growth phase and cell concentration on the fluorescent signals emitted by the bioreporter system in the presence of arsenic were investigated. After the immobilization process, 90 μL of

medium and 10 μL of arsenic were added to the bacteria immobilized fibers in the wells. Experimental groups used for fluorescence measurement experiments are given in Table 2.2. Emitted fluorescent signals were recorded for 18h on the SpectraMax® iD3 Multi-Mode Microplate Reader (Molecular Devices, USA). The fluorescent microplate reader was set at the following parameters to take readings at 395 nm/509 nm $\lambda_{excitation}$/ $\lambda_{emission}$ wavelength; recorded fluorescence values every 30 minutes for 18–h with 15 seconds of orbital pulsed shaking before every measurement. According to the tried parameters, in which cell concentration and in which growth phase the highest fluorescent signal is obtained in the presence of arsenic, it will be decided that that parameter is more appropriate, and the studies will proceed over it.

Table 2.2 Experimental groups used in fluorescence measurement experiments

Group Name	Definition
IB	Immobilized bioreporter on fibers
CA – IB	Immobilized arsenic bioreporter cell on CA fibers
PCL – IB	Immobilized arsenic bioreporter cell on PCL fibers
IB – background	Background for immobilized arsenic bioreporter cell on fibers (Neat fiber + 90 μL medium + 10 μL arsenic)
IB – control	Control for immobilized arsenic bioreporter cell on fibers (Neat fiber + 90 μL medium + 10 μL sterile dH_2O)
FB	Free arsenic bioreporter cell

Table 2.2 (cont'd)	
FB – background	Background for free arsenic bioreporter cell (100 µL medium + 20 µL arsenic)
FB – control	Control for free arsenic bioreporter cell (100 µL free bioreporter cell + 20 µL sterile dH_2O)

2.2.2.2.1 Effect of Growth Phase

To examine the effect of the growth phase on the fluorescent signal to be obtained from the immobilized bioreporters, the cells were grown up to lag phase, log phase, and stationary phase. Overnight liquid cultures in M9 minimal medium of arsenic bioreporter were transferred to fresh M9 minimal medium at a ratio of 1:50 (v/v) and left to grow at 37 °C, 150 rpm till they reached OD_{600} = 0.1 (early growth phase), OD_{600} = 0.5 (mid – exponential growth phase) and OD_{600} = 1.5 (stationary phase). Cells grown up to the determined growth phase were transferred onto the fibers (12.5 wt% CA and 10 wt% PCL) in the wells of the 96 well plate also 100 µL of free arsenic bioreporters were also placed in other empty wells and incubated at 32° for 3 h. Adsorption studies of arsenic bioreporters on CA and PCL fibers were explained in detail in Section 2.2.2.1 At the end of the immobilization, the fluorescent signals emitted by the arsenic bioreporters immobilized on the fibers and the free bioreporter cells in the presence of 0 µg/L (uninduced, no arsenic), 50 µg/L and 100 µg/L arsenic were recorded. Designated 0 µg/L, 10 µL of sterile dH_2O was added on the IB and 20 µL sterile dH_2O was added on the FB instead of the arsenic. For 50 µg/L and 100 µg/L final As (III) concentrations, 10 µL and 20 µL As (III) were added for IB and FB, respectively. Fluorescence measurement studies were carried out as mentioned above in Section 2.2.2.2.

2.2.2.2.2 Effect of Cell Concentration

The effect of cell growth phase study conducted in the previous Section 2.2.2.2.1 showed that the cells in the mid exponential expressed more GFP when induced by arsenic. Hence, to investigate the cell concentration effect, OD_{600} values of overnight liquid cultures in M9 supplemented medium, were measured after being transferring to fresh M9 supplemented medium at a ratio of 1:50 (v/v) and left to grow at 37 °C, 150 rpm till reaching the mid – exponential growth phase. The arsenic bioreporter cell concentration was then adjusted to OD_{600} = 0.1 (~$8.0x10^7$ cells/mL), OD_{600} = 0.5 (~$4x10^8$ cells/mL) and OD_{600} = 1.5 (~$1.2x10^9$ cells/mL) (Yap & Trau, 2019). 100 µL of the bioreporter cells, whose cell concentrations were adjusted, was either placed on the sterile fibers (12.5 wt% and 10 wt% PCL) in the wells or to the empty wells and left for 3 h at 32° C. At the end of the incubation, bacterial suspension placed on the fibers or wells was removed and washed twice with 1X PBS using 150 µL each time of to remove the cells that could not adhere to the fibers. Then, 90 µL of M9 supplemented medium and 10 µL of As (III) (from 50 µg/L and 100 µg/L arsenic concentration) were added to the wells with IB. 20 µL of As (III) was added to the wells with FB, and the final arsenic concentration in the well was adjusted to be 50 µg/L and 100 µg/L. The same amount of water was added instead of arsenic to the uninduced (indicated as 0 µg/L) wells, that is, the control group. Immobilization studies of bacteria by adsorption on fibers are detailed in Section 2.2.2.1, and the measurement methods of fluorescence emitted from bioreporters are retailed in Section 2.2.2.2.

2.2.2.2.3 Evaluation of As (V) Detection Performance

CA – IB and PCL – IB groups were induced with the +5 – valence form (arsenate) to test their ability to detect different arsenic forms. Accordingly, in these experiments, arsenic bioreporter cells grown in MOPS – supplemented medium until mid – exponential growth phase and cell concentration adjusted to OD_{600} = 0.5 were

separately immobilized to CA and PCL electrospun fibers with 3–h incubation. Afterwards, they were washed with 1X PBS to remove non – adherent cells. All the steps applied regarding the immobilization studies are given in Section 2.2.2.1. Following the completion of the immobilization studies, the CA – IB and PCL – IB groups were induced with As (V) at different concentrations (50 µg/L and 100 µg/L), 0 µg/L represents the control group, 10 µL of dH_2O was added instead of As (V) on the uninduced IB. The values recorded in the fluorescence reading taken during 18h were accepted as an indicator of the responses of these two developed groups to As V. All details about fluorescence reading studies are given in Section 2.2.2.2.

2.2.2.2.4 Cell Viability

Resazurin sodium salt (Resazurin-Na-salt; Serva 34226.02), known as AlamarBlue, was utilized to analyze metabolic activities of bioreporters immobilized on the fibers. Resazurin salt was prepared according to the reagent instruction (Lee, 2017). Briefly, Resazurin stock solution (100x) was prepared by dissolving 0.50 g Resazurin sodium salt into 100 ml 1x PBS. Then, the stock solution was diluted 1:100 of the stock solution with 1X PBS to prepare the working solution. 100 µL of bacterial culture (concentration adjust to $1*10^4$ cells/mL (Scientific, 2008) for each sample was added to the black and opaque 96 – well microplate including fiber and left in the incubator at 32 °C for 3h for immobilization. At the end of the immobilization period, the bacterial suspensions on the fibers were withdrawn and the fibers were washed with PBS to fend off non-adherent bacteria. 10 µL of resazurin sodium solution and 90 µL growth medium (LB medium) was added to the wells. After incubation for 2 h and 4 h at 37° C, the cell viability was measured by a fluorescence spectrophotometer at 590 nm (emission) and 550 nm (excitation), SpectraMax® iD3 Multi-Mode Microplate Reader (Molecular Devices, USA) pursuant to reagent instruction (Eilenberger et al., 2018; Grzywaczyk et al., 2021). Results were calculated as the percent difference in reduction of reagent compared to control samples according to

Equation 1 and as the percent of viability of immobilized bioreporters to the 12.5% CA and 10% PCL electrospun support materials according to Equation 1.

Equation 1. % Viability (Scientific, 2008)

$$\frac{\text{Experimental RFU value with test compound x 100}}{\text{Untreated Control RFU value}}$$

where:
Untreated Control RFU value = Free bioreporter RFU value
Experimental RFU value with test compound = Bioreporter immobilized on fiber RFU value

Theoretically, non-fluorescent resazurin can be used as a fluorogenic oxidation-reduction indicator in a variety of cells, i.e., bacteria, yeast and eukaryotes by flow cytometry, fluorescence microscopy and high-throughput screening. Briefly, resazurin tests are based on the reduction of blue dye to resorufin by living cells. Alamar Blue, which is initially nonfluorescent, is reduced to a pink, fluorescent dye by cell activity, in other words, there is a direct relationship between the reduction of resazurin (Alamar Blue) in the growth medium and the amount or proliferation of living organisms (Borra et al., 2009; Garcia et al., 2019; Mcgaw et al., 2014; O'Brien et al., 2000).

2.2.2.2.5 Morphology of Immobilized Bioreporters on Fabricated Fibers

Morphology of arsenic bioreporter on –CA and –PCL electrospun fibers were visualized of via SEM (Quanta 400F field emission SEM in Central Laboratory at METU, Ankara, Turkey) and confocal microscopy (CM, Leica DMI4000B automated inverted epifluorescence microscope equipped with confocal attachment Andor AMH200 Metal Halide lamp), for visualization of immobilized bioreporters

on fibers. In SEM analysis CA – IB and PCL – IB groups were examined, and their images were taken at 10 000X and 20 000X magnifications. The developed IB groups were cut in 1x1 cm^2 dimensions. Prior to the imaging, a gold coating (682 PECS, Gatan, Inc., USA) was applied to the samples to enhance their conductivity. In confocal imaging, bacteria had auto fluorescence due to their GFP, so staining was not needed. However, due to the auto fluorescent nature of PCL fiber, background fluorescence from the fibers could only be blocked at $\lambda_{excitation}$ =405 nm and imaging was obtained. A flat surface for imaging could not be obtained from the bacteria immobilized on the CA fiber. The reason for this can be given that the fiber structure was fluffy. As a result of all these, the image showing the presence of bacteria could not be recorded.

2.2.2.2.6 Effect of Growth Medium

M9 minimal medium and MOPS–supported medium were used to examine the effect of growth medium on fluorescent signals emitted from immobilized arsenic bioreporter systems. Based on the results obtained in the previous experiments, it was decided with which fiber support material to continue the next experiments. PCL was considered to be suitable for our purpose and was included in the scope of the thesis, since the fluorescent signals obtained as a result of cell concentration and cell growth phase parameters were high and consistent, as well as obtaining higher fluorescent signals than free bioreporters, and having the highest bacterial viability percentage according to cell viability results. PCL – IB group continued in other studies. Accordingly, bioreporters grown in M9 minimal medium and MOPS–supported medium were adjusted to OD_{600} = 0.5 (at mid – exponential growth phase) and immobilized on PCL fiber support material for 3h at 32° C in incubator. As mentioned in Section 2.2.2.1 in detail, at the end of the 3h immobilization period, the bacterial suspensions in the wells were removed and washed twice with 1X PBS to ensure that the cells that could not adhere to the fibers were removed. Then, 90 µL of M9 minimal medium/MOPS – supplemented medium and 10 µL of As (III)/As

(V) were added to the PCL – IB group and the fluorescent signals they emitted were recorded via using Varioskan Lux multimode microplate reader (Thermo Fisher Scientific, USA) at $\lambda_{excitation}$: 395 nm and $\lambda_{emission}$: 509 nm with a bandwidth value of 12 nm. As a result of this experiment, it will be found that more GFP is expressed than immobilized bioreporters induced in which arsenic form and in which growth medium. Fluorescence measurements studies were carried out as mentioned above in Section 2.2.2.2.

2.2.2.2.7 Effect of Immobilization Time

The fluorescent signals emitted by the arsenic bioreporters immobilized at various times (1h, 3h and 7h) were recorded at the end of the immobilization period. The effect of immobilization time on the response of bioreporters adsorption (immobilized) on PCL to arsenic in terms of the fluorescence value emitted was investigated. Accordingly, 100 µL of arsenic bioreporter cell suspension, grown till mid – exponential growth phase (OD_{600} set to 0.5), planted on PCL fibers were left in the incubator for adsorption at 32° C for different immobilization times. At the end of the immobilization period, the bacterial suspensions on the fiber in the well plates were removed and washed twice with PBS. Then, 90 µL of M9 minimal medium and 10 µL of As (III) were added. For fluorescence measurements, Varioskan Lux multimode microplate reader (Thermo Fisher Scientific, USA) was used at $\lambda_{excitation}$: 395 nm and $\lambda_{emission}$: 509 nm with a bandwidth value of 12 nm. Immobilization studies and fluorescence measurements in detail were performed as mentioned above in Section 2.2.2.1 and Section 2.2.2.2, respectively.

2.2.3 Determination of the Performance Characteristics of The Bioreporter System

Sensitivity, selectivity, response time and shelf-life features were evaluated in order to evaluate the performance of the developed PCL – immobilized arsenic bioreporter systems.

2.2.3.1 Sensitivity

In order to assess the sensitivity performance of the immobilized arsenic bioreporter system was induced with several concentrations of arsenic forms. Accordingly, arsenic bioreporters grown up to the mid exponential phase in M9 minimal medium, were immobilized on PCL fiber after 3h immobilization period. Then the bacterial suspensions were removed and washed with PBS. To measure their sensitivity, they were induced with As (III) and As (V) forms at different concentrations (10, 20, 30, 50, 75 and 100 μg/L). More detailed descriptions of immobilization and fluorescence measurements studies were given above in Section 2.2.2.1 and Section 2.2.2.2, respectively.

2.2.3.2 Selectivity

In order to determine the selectivity performance of the immobilized arsenic bioreporter system, with different metals (Ag (I), As (III), As (V), Cd (II), Fe (II), Mg (II), Mn (II), Mo (IV), Ni (II)) were induced. Final metal ion concentrations of all heavy metals were 100 μg/L. For M9 minimal medium, cells grown until stationary phase were adjusted to OD_{600} = 1.5 and incubated in well plate at 32 °C for 3 – h to be immobilized on PCL fibers. At the end of the immobilization period, the necessary procedure was followed to remove non – adherent bacteria as detailed in Section 2.2.2.1 Then, 90 μL of M9 minimal medium and 10 μL of metal were added onto the immobilized arsenic bioreporter system. No metal was used in the

control group. After 8–h of induction, the viability of the immobilized bioreporters was determined by the AlamarBlue method. Non – treatment group (metal – free fiber immobilized bioreporters) was used as the control. The detailed explanation of the viability test is given in Section 2.2.2.2.4. After 8 hours of induction with metals, the response of immobilized bioreporters to metals used was measured as a fluorescent signal. Fluorescence measurement studies were taken using the procedure described Section 2.2.2.2 above via using Varioskan Lux multimode microplate reader (Thermo Fisher Scientific, USA) at $\lambda_{excitation}$: 395 nm and $\lambda_{emission}$: 509 nm with a bandwidth value of 12 nm. The fluorescent signal values they emitted were corrected for 100 % cell viability, based on viability results PCL immobilized bioreporters after induction with metals

2.2.3.3 Response Time

This experiment was carried out so as to determine the time of the first signals received from the immobilized arsenic bioreporter systems induced by As (III) at different concentrations, in other words, the speed of the response given at these arsenic concentrations. After removing the cells that could not adhere at the end of the 3 h immobilization period, 90 µL of M9 minimal medium and 10 µL of As (III) were added onto the PCL – IB. Accordingly, the responses of the bioreporter system induced by As (III) at different concentrations (10, 30, 50 and 100 µg/L) were recorded as fluorescent signals for 18 h. More detailed descriptions of immobilization and fluorescence measurements studies were given above in Section 2.2.2.1 and Section 2.2.2.2, respectively.

2.2.3.4 Shelf–life Experiments

In this experiment, the shelf life of immobilized arsenic bioreporter systems was determined by studying the performance of fiber immobilized bioreporters which were incubated at different temperatures (+4 °C and room temperature (RT)) and for

various times (0, 7, 14, 21 and 28 days). The immobilization procedure detailed in Section 2.2.2.1 was followed. Accordingly, 200 µL of 1 X PBS was added to those that were kept at +4°C and room temperature after the lids are closed and wrapped with parafilm. Then, the first measurement was taken on day 0, they were removed from their environments at the same time at certain days, the medium on it was removed, 90 µL M9 minimal medium and 10 µL 100 µg/L As (III) was added. The fluorescence measurement process as mentioned above in Section 2.2.2.2 was followed via using Varioskan Lux multimode microplate reader (Thermo Fisher Scientific, USA) at $\lambda_{excitation}$: 395 nm and $\lambda_{emission}$: 509 nm with a bandwidth value of 12 nm. The viability of these immobilized bioreporter systems kept at +4 °C and room temperature was then measured with the AlamarBlue assay as mentioned above in Section 2.2.2.2.4. When calculating for both relative fluorescence and relative viability, 1st day (Day1) group accepted as control group, 100%.

CHAPTER 3

RESULTS AND DISCUSSION

PCL and CA fibers were used to create bioreporters immobilized on them. By changing the parameters, their effects on the emitted fluorescent signals were evaluated, so these systems were optimized and compared with each other. Accordingly, performance evaluation experiments were carried out by deciding on the bioreporter system immobilized on PCL fiber.

3.1 Fabrication Support Materials and Immobilized Systems

3.1.1 Fabrication of Fiber Support Materials

Within the scope of the thesis, CA and PCL electrospun fibers with uniform, beadless, and water – stable characteristics were fabricated successfully. The homogeneity of these fibers depends not only on the process parameters, but also on the properties of the polymer solution. However, desirable fiber production could not be observed in some solvents initially tried to dissolve the PCL polymer, i.e. PCL/DCM, PCL/THF, PCL/DCM:DMF. It was observed that when PCL/DCM and PCL/THF solutions were subjected to electrospinning technique, fiber jets did not form, and the polymer solution reached the top of the collector without evaporation of the solvent. After obtaining a wet and sticky form, although the fabrication parameters were changed, the fibers in the desired form could not accumulate on the collector. 10 – 15 – 20 – 25 wt% PCL polymer prepared in DCM:DMF solvent combination was subjected to electrospinning technique and fiber was obtained but later it was seen that fiber could not be obtained by imaging in SEM. Then, PCL polymer solutions of different concentrations (7.5 wt% and 10 wt%) were prepared in acetone and fiber could be also obtained. 5 – 7.5 – 10 – 12.5 wt% CA polymer

solutions in DCM:Ace was subjected to electrospinning technique and fibers were obtained.

3.2 Characterization of Fabricated Fiber Support Materials and Optimization of Immobilized Bioreporter Systems

The results of the SEM, FTIR, and wettability tests for the characterization of fiber support materials and the results of the experiments carried out within the scope of the optimization of the bioreporters immobilized on fibers are given below, respectively.

3.2.1 Characterization of Fabricated Fiber Support Materials

3.2.1.1 SEM

CA and PCL electrospun fibers collected in aluminum foil were examined in terms of fiber morphology by SEM. It is known from the literature that the morphology of electrospun fibers is of great importance in order to provide a suitable environment for the attachment and growth of bacterial cells (Hemmatian et al., 2021; Varshney et al., 2020).

SEM micrographs in 5 000 X magnification and fiber diameter distributions of fabricated CA fibers at different concentrations are given in Figure 3.1, respectively. As seen in Figure 3.1a – b, the CA/DCM:Ace solution, prepared at 5 and 7.5 wt %, bead structures were formed instead of continuous fibers. It can be deduced that the polymer concentration is low as the reason for the inability to form fiber. Because it is known that viscosity and concentration are directly related to each other (Hong, 2016). When a solution of extremely low concentration is subjected to electrospinning, it is quite possible to observe bead morphology as a result of the applied electric field (Şenol & Akkoyun, 2020). It can be seen in Figure 3.1c – d, that as the concentration increases (when 10 wt% and 12.5 wt%) the bead – like

morphology was replaced by elongated fibers. Because with the increase in concentration, the surface tension is overcome and homogeneous fibers without beads are obtained (Unnithan et al., 2015).

The diameters of the fibers were calculated by analyzing the SEM micrographs using Fiji software, and a histogram graph of the diameters was plotted (Figure 3.1d and 3.1e). Since fiber could not be obtained from 5 wt% and 7.5 wt% CA, fiber diameters could not be calculated. However, as the polymer concentration in the solution was increased (10 and 12.5 wt %), the diameters of the fibers also increased. The reason for this situation can be associated with the increase in viscosity (Ramakrishna et al., 2006).

The increase in the viscosity of the solution as a result of the adhesion of the polymer chains in the solution causes the liquid to disperse in the electric field and accumulates continuously on the collector (Arik et al., 2022), thus the fiber diameter has increased. In addition, the reason for the observed bead – like structures (5 and 7.5 wt % CA) may be due to the greater surface tension of the solvent in the solution (Rieger et al., 2013). The diameters of the fabricated fibers at 10 and 12.5 wt % CA/DCM:Ace (2:1 v/v) solutions were 1.27 μm ±0.17 and 2.15 μm ± 0.55, respectively. The histogram plot was constructed from at least 100 measurements taken at different sites of CA (Figure 3.1c, 3.1d). Also, for 10 wt % CA and 12.5 wt % CA, fiber diameter distributions range from 1.037 μm – 1.4498 μm and 0.773 μm – 3.15 μm, respectively.

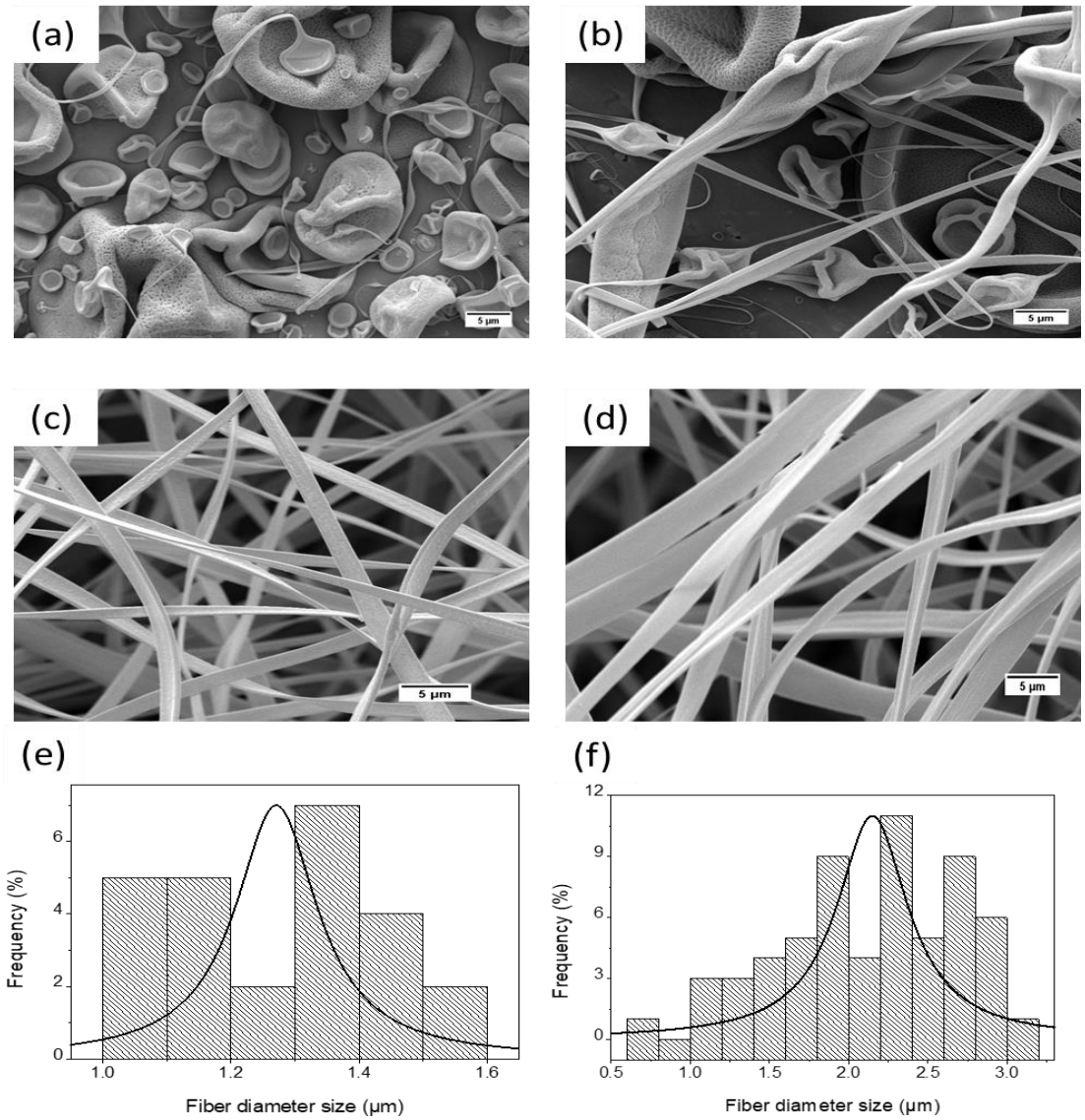

Figure 3.1 Typical SEM micrograph of (a) 5 wt% CA fibrous support materials and (b) 7.5 wt% CA (c) 10 wt% CA and (d) 12.5 wt% CA; Fiber diameter distribution histogram, (e) 10% CA fibrous support materials and (f) 12.5% CA (Voltage: 15 kV, Flow rate: 1.0 mL/h, End to tip distance: 10 cm, Solvent system: DCM:Acetone (v/v 2:1)).

10 – 15 – 20 – 25 wt % PCL solutions prepared by dissolving in DCM:DMF solvent system were subjected to electrospinning. Fibers could not be obtained at the end of fabrication process (Figure 3.2). The bead – like structures obtained can be seen in Figure 3.2a. Although smooth fiber morphology appeared as the concentration

increased, the amount of bead – like structures remained in excess (Figure 3.2b-d). This is thought to be due to the high surface tension of the DCM:DMF solvent system. Surface tension is the cohesive force between the same molecules that brings the surface molecules of the liquid closer together. The liquid, which wants to minimize the surface area it covers and therefore forces itself to shrink, causes Rayleigh instability (Korycka et al., 2018; H. Zhao, 2018). Many studies have shown that surface tension is very effective in the formation of bead or bead – on – string fibers. It is argued that this problem can be eliminated by changing the solvent type or solvent ratios in the system (Fong et al., 1999; Zuo et al., 2005).

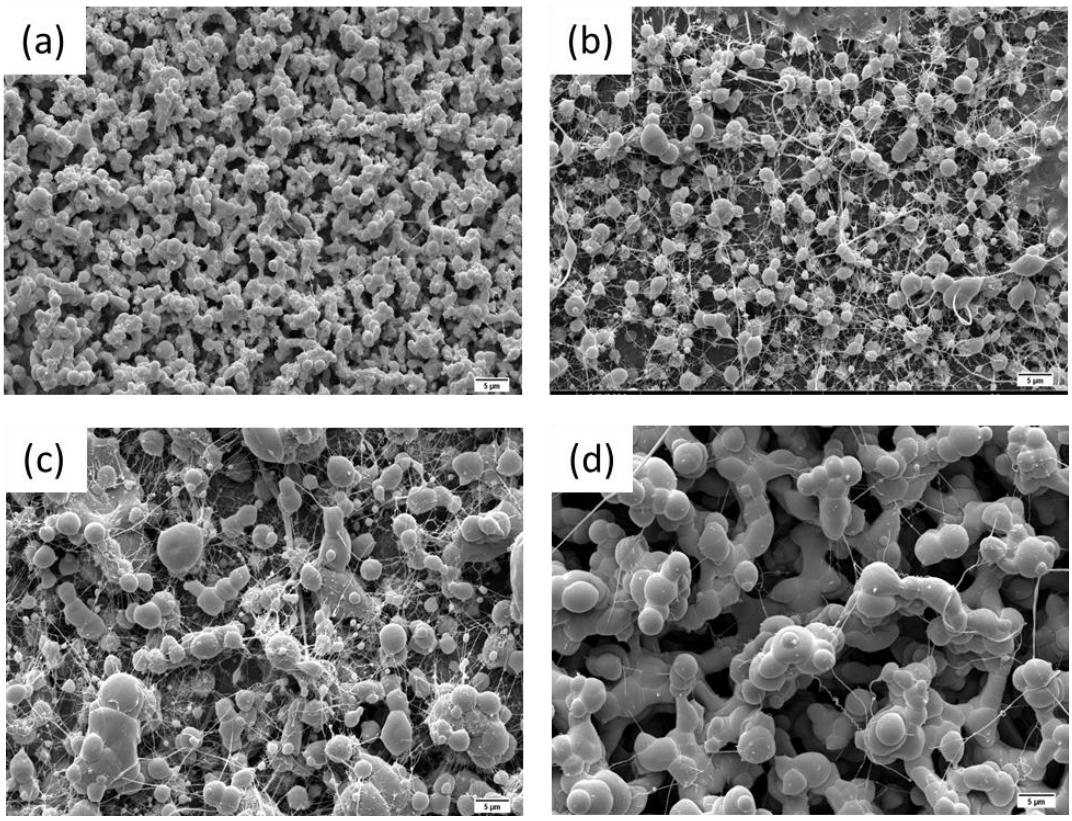

Figure 3.2 Typical SEM micrograph of (a) 10% PCL, (b) 15% PCL , (c) 20% PCL and (d) 25% PCL electrospun support material (Solvent system: DCM/DMF (1/1 v/v),Voltage: 25 kV, Flow rate: 0.5 mL/h, End to tip distance: 15 cm).

Afterwards, it was tried to fabricate fiber from PCL polymer dissolved in acetone via electrospinning technique and the fabrication was carried out successfully. SEM

micrographs of 7.5 and 10 wt% PCL fibers are given in Figure 3.3. Beadless structure and continuous fiber image was obtained. The diameters of 7.5 wt% PCL and 10 wt% PCL fibers were calculated, and histogram graphs are given in Figure 3.3c – d. The fiber diameters increased as the polymer concentration was increased. The increase in fiber diameters can be attributed to the increase in viscosity (Ramakrishna et al., 2006). Because as the polymer concentration in the solution increases, the viscosity of the solution increases, and accordingly, the diameter of the fibers increases and thicker fibers are obtained (Bagbi et al., 2019; Petras et al., 2011). The diameters of the fibers obtained from 7.5 wt % and 10 wt % PCL:Ace solutions were 0.24 ±0.08 and 0.61 ±0.20 μm, respectively. The diameter distributions of the fibers ranged from 0.116 μm – 0.406 μm for 7.5 wt % PCL and 0.306 μm – 1.136 μm for 10 wt % PCL (Figure 3.3c, 3.3d). In addition, it has been reported that electrospun membranes containing bead are not suitable for the adhesion of bacteria (Chen et al., 2009).

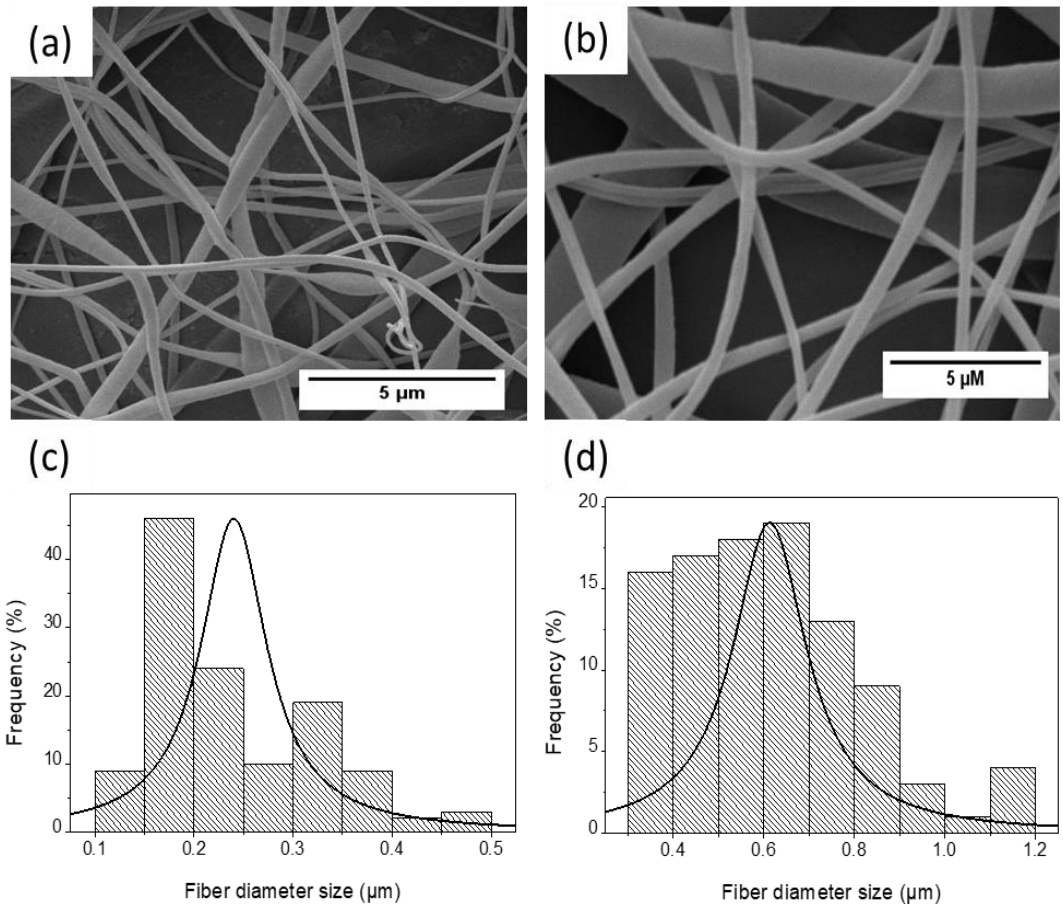

Figure 3.3 Typical SEM micrograph of (a) 7.5% PCL fibrous support materials and (b) 10% PCL; Fiber diameter distribution histogram, (c) 7.5% PCL fibrous support materials and (d) 10% PCL (Voltage: 15 kV, Flow rate: 6.0 mL/h, End to tip distance: 30 cm).

Fiber pore size distribution

After the images obtained from SEM micrographs were analyzed with ImageJ software and fiber diameters were determined, the pore sizes of 12.5 wt% CA and 10 wt% PCL electrospun fibers were also calculated. The pore diameter of PCL electrospun fiber was significantly higher than that of 12.5 % CA (0.65 µm ±0.02 > 0.30 µm ±0.03). The pore size diameters were in the range of 0.151 – 1.12 µm for CA, while those of PCL were in the range of 0.035 – 1.11 µm (Figure 3.4). It is thought that the higher porosity obtained in PCL fibers compared to CA fibers may

be due to the rapid evaporation of the highly volatile solvent (acetone) and as a result, the solidification of the polymer chains (Anitha et al., 2016). Bacterial cells usually range in size from 0.5 to 3 μm, but mostly around 1 – 2 μm (Jiang et al., 2014). Except in some cases, it has been stated that it is very difficult for microorganisms to penetrate narrow pores below <10 μm (Gaveau et al., 2017). In materials with <1 μm and smaller pore sizes, cells only grow on the outer surface, but it is an undeniable fact that such small pore sizes increase the surface roughness and encourage the growth of cells (Champigneux et al., 2018; Chong et al., 2019). However, it is foreseen as an inconvenient situation because small – sized pores can become clogged over time. However, long – term experiments (18 – 25 days) by Karthikeyan et al., Zhao et al., and Yang et al. proved that it does not cause pore clogging when the pore size is close to the bacterial cell size (Karthikeyan et al., 2015; Y. Yang et al., 2016; S. Zhao et al., 2015).

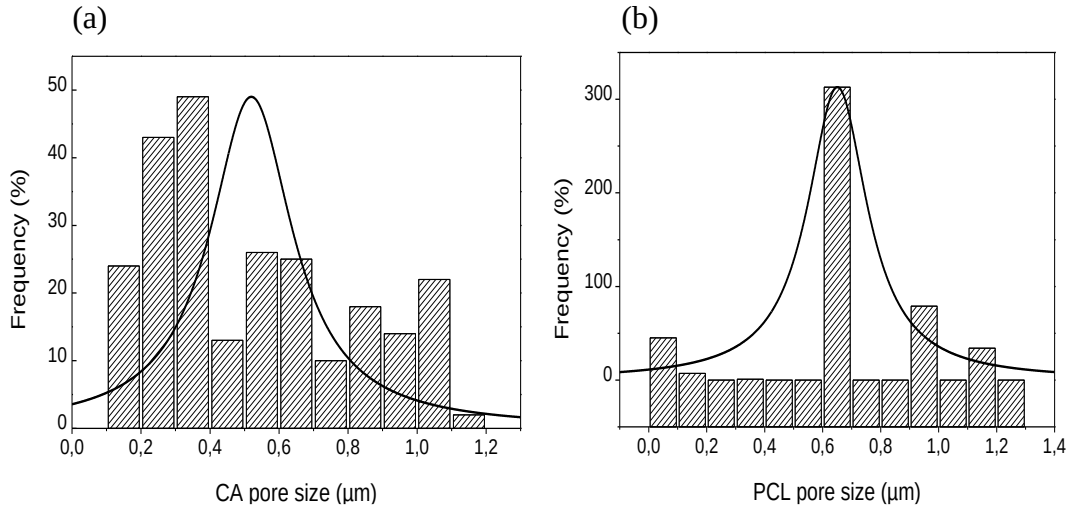

Figure 3.4 Pore size diameters of (a) CA and (b) PCL electrospun fibers (at least 245 pore diameters were measured for drawing the pore size distribution of the fibers).

3.2.1.2 FTIR

FTIR Spectroscopy is based on the principle of measuring the vibration of chemical bonds by the absorption of infrared radiation (IR), the energy available to excite vibrations in molecules. (Almeida & Marques, 2016). IR radiation is absorbed by different vibrational movements of chemical bonds such as stretching, shrinking, and bending. Changes in the vibrations of chemical bonds in the IR region and their absorption properties lead to the formation of spectral peaks. Each functional group has its own vibration frequency, and each IR spectrum is specific (Griffiths, 2007). Bacteria-surface interaction takes place under the influence of Van der Waals forces (Mohamed Zuki et al., 2021). From the FTIR results, the chemical bonds of the fibers were determined and characterized whether they are suitable for the adhesion of bacteria. FTIR spectra of 10 wt% CA fiber samples show characteristic peak absorptions in Figure 3.5a. According to the figure; a band (O–H stretch) at 3477 cm^{-1} and a wide band at about 1753 cm^{-1} (C=O stretch), peak (C=C stretch) at about 1631 cm^{-1}, a band at 1428 cm^{-1} (CH_2 vibration), a sharp band at 1041 cm^{-1} (C–O stretch) (Ibrahim et al., 2015). Figure 3.5b shows FTIR spectrum of 10 wt% PCL fiber characteristic peak absorptions. Asymmetric and symmetric CH_2 (methylene group) stretching vibrations were detected at 2936 and 2861 cm^{-1}, respectively, and there was a slightly displaced band of ester carbonyl group (C=O stretching) at 1724 cm^{-1} due to the presence of carbonyl. In – plane carboxylic acid bending band (C–O–H) was observed at 1462 cm^{-1} and the out-of-plane one at 933 cm^{-1}, respectively. C–O, C–C, and asymmetric/symmetric C–O–C strong stretch bands were detected at approximately 1300–1000 cm^{-1}. A specific band located at 732 cm^{-1} was found corresponding to the scissor – like bending mode of the methylene groups. The results found were similar to those of other researchers (Azizi et al., 2018; Elzein et al., 2004; Rocha, 2019; Turşucular et al., 2018).

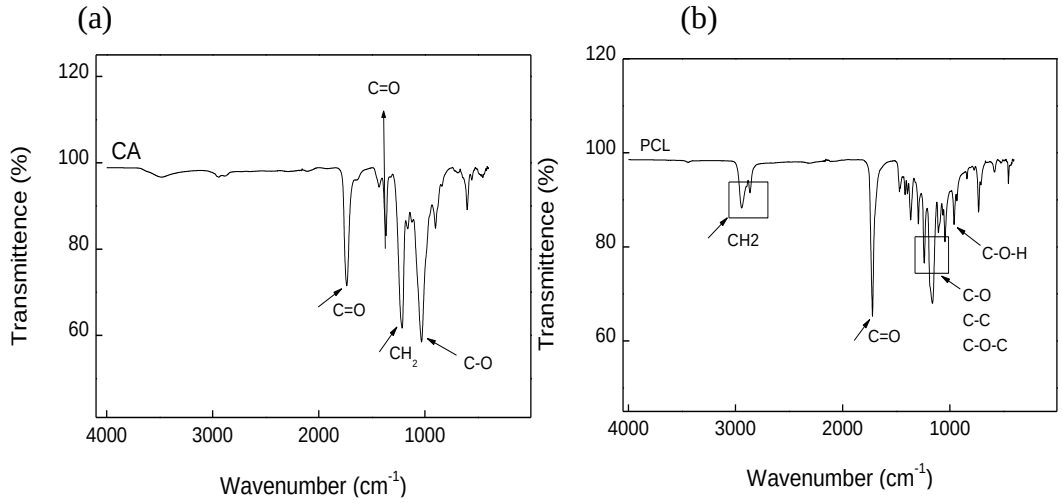

Figure 3.5 FTIR spectra of (a) 10 wt% CA and (b) 10 wt% PCL electrospun fibers.

3.2.1.3 Contact Angle Measurements

To determine the wettability of the fiber support materials, static contact angles were measured using the constant drop method given in Figure 3.6. It has been shown in the literature that CA is hydrophobic, it was expected that our results would be consistent with this. As a matter of fact, we obtained similar results shown in Figure 3.6a that the contact angle of 10 wt% CA fiber was found to be 123.4°±9.9, while the contact angle of 12.5 wt% CA fiber was calculated as 106°±0.3. It was figured out that there was a significant change in hydrophobicity as a result of concentration differences ($p<0.00$). It was mentioned in literature that the contact angle values for PCL are in the range of 115° – 136° (De Cesare et al., 2019), and according to our results, the contact angle values for 7.5 wt % and 10 wt % PCL are 136.9°± 10.5 and 143.6°±13.5, respectively (Figure 3.6b). The adhesion of bacteria to a surface is due to the extensions they have. The pili of *Escherichia coli* (*E. coli)* are very suitable for adhesion to surfaces. It has been emphasized that this may be due to the tendency of pili/flagella to hydrophobic surfaces. When the bacterium approaches the surface, it is attracted first by long – range Van der Waals and then by short – range repulsive electrostatic forces (Kimkes & Heinemann, 2020). Friedlander et al. in their study,

they evaluated the adhesion performance of *E. coli* cells by using self – assembled monolayers (SAMs) of thiol – bearing molecules on gold films. At the end of the study, they concluded that flagella are more prone to adhere to hydrophobic surfaces (Friedlander et al., 2015).

E. coli was used as host cell in genetically designed arsenic bioreporters used in our study. In a study, it was stated that the contact angle values of all *E. coli* cells were in the range of 17 – 36°, that is, the *E. coli* cell shows hydrophilic properties (Hamadi et al., 2008). Some studies show that the increased hydrophobicity of abiotic surfaces and the corresponding decrease in surface energy increase the affinity of hydrophilic bacteria to adhere to these surfaces (Oh et al., 2018; Parreira et al., 2011). Thus, it is supported that the hydrophobicity of the fibers we have chosen as the support material will increase the adhesion of arsenic bioreporter cells. It is clearly seen that the contact angle values of the fibers increase as the concentration increases, so fiber mats with the highest polymer concentration (12.5 wt% CA and 10 wt% PCL) were selected.

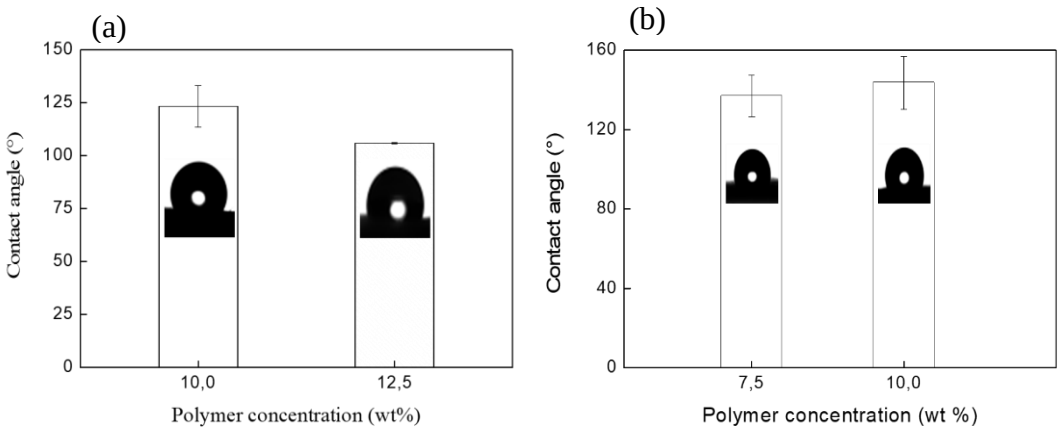

Figure 3.6 Contact angle values of fibers obtained from (a) CA electrospun fibers produced using different polymer concentrations (10 and 12.5 wt%); (b) PCL electrospun fibers using different polymer concentrations (7.5 and 10 wt%), n=3.

3.2.2 Optimization of Arsenic-Sensing Properties of Bioreporter System

3.2.2.1 Effect of Growth Phase

In order to measure the effect of cell growth phase on the sensitivity performance of arsenic bioreporter, bioreporter cells of different growth stages immobilized on mats obtained with 12.5% CA and 10% PCL obtained by electrospinning were tested. Fluorescence emitted by As (III) induced immobilized bioreporters on 12.5 wt% CA fibrous support material, which were grown in M9 minimal medium and in three different growth phases (early growth phase, mid – exponential growth phase and stationary phase) was given in Figure 3.7. In general, GFP expression in bioreporter cells increased as the cells continues to grow (Shachrai et al., 2010) and the arsenic bioreporter yielded higher fluorescence signals with increasing As (III) concentrations. As can be seen in Figure 3.7, the highest response signals generated by the bioreporters immobilized on 12.5% CA fibers in all three growth phases were obtained at the highest induction concentration of As (III) (100 μg/L) ($p<0.05$). As expected, bioreporters were induced more in the presence of high concentrations of As (III), thus emitting more fluorescence. At 50 μg/L of As (III), cells in the stationary phase showed higher response which can be explained by the higher cell concentration (OD_{600}=1.5) compared to the cells in mid – exponential growth (OD_{600}=0.5) and early growth (OD_{600}=0.1) phase.

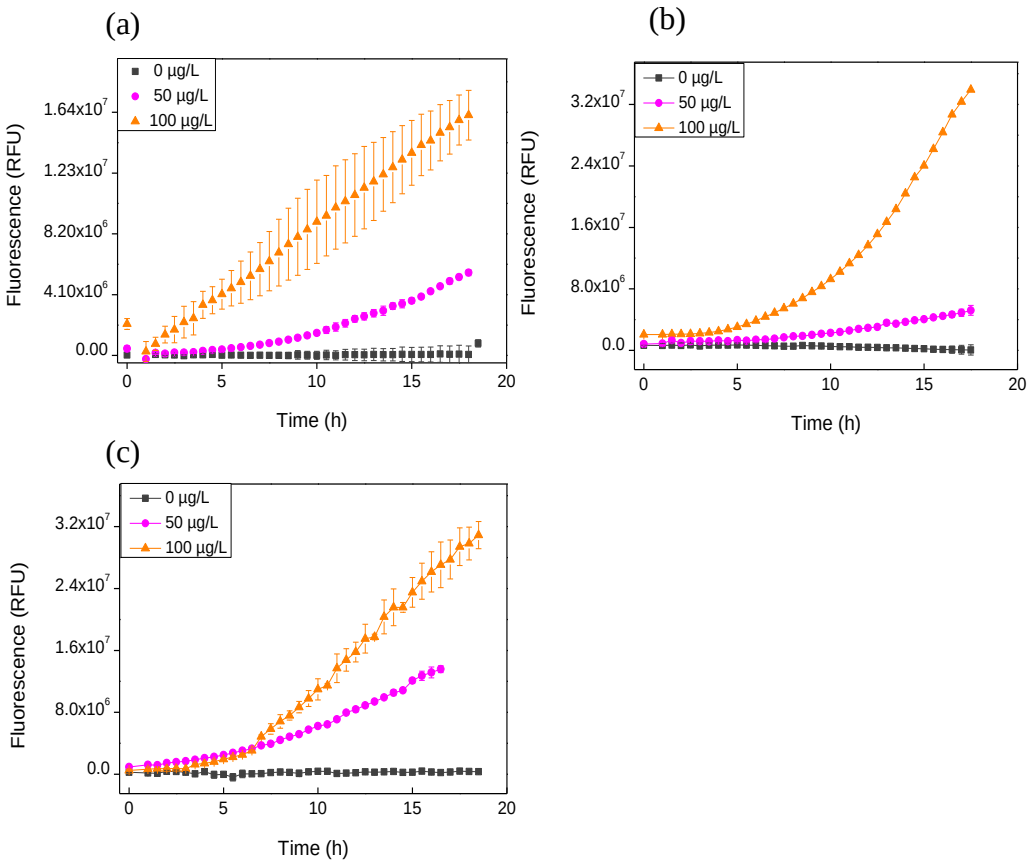

Figure 3.7 Fluorescence emission kinetics of 12.5 wt% CA-immobilized arsenic bioreporter induced with varying As (III) concentrations (uninduced (0 µg/L), 50 µg/L and 100 µg/L). (a) Early growth (b) mid – exponential (c) stationary phase. M9 minimal medium was used as the immobilization and growth media, n=4.

The fluorescence responses recorded for 10% PCL – immobilized bioreporters, which were grown in M9 minimal medium, in three different growth phases (early growth phase, mid – exponential growth phase and stationary phase) are given in Figure 3.8. In the light of these results, it was seen that the highest signal was obtained from the immobilized bioreporter induced with 100 µg/L As (III) ($p<0.05$). As expected, the bioreporters emitted more fluorescence in the presence of higher concentrations of As (III). In the mid – exponential growth phase, the presence of 50 µg/L As (III) resulted in significantly higher GFP expression in cells and thereby higher emission of fluorescent signals, as the growth medium was fresher than in the

other growth phases (p=.000). Besides, it was thought that the increase in hydrophobicity of the cell wall of metabolically active bacterial cells in the mid-exponential phase may also help the cells to adhere to the membranes better (Żur et al., 2016).

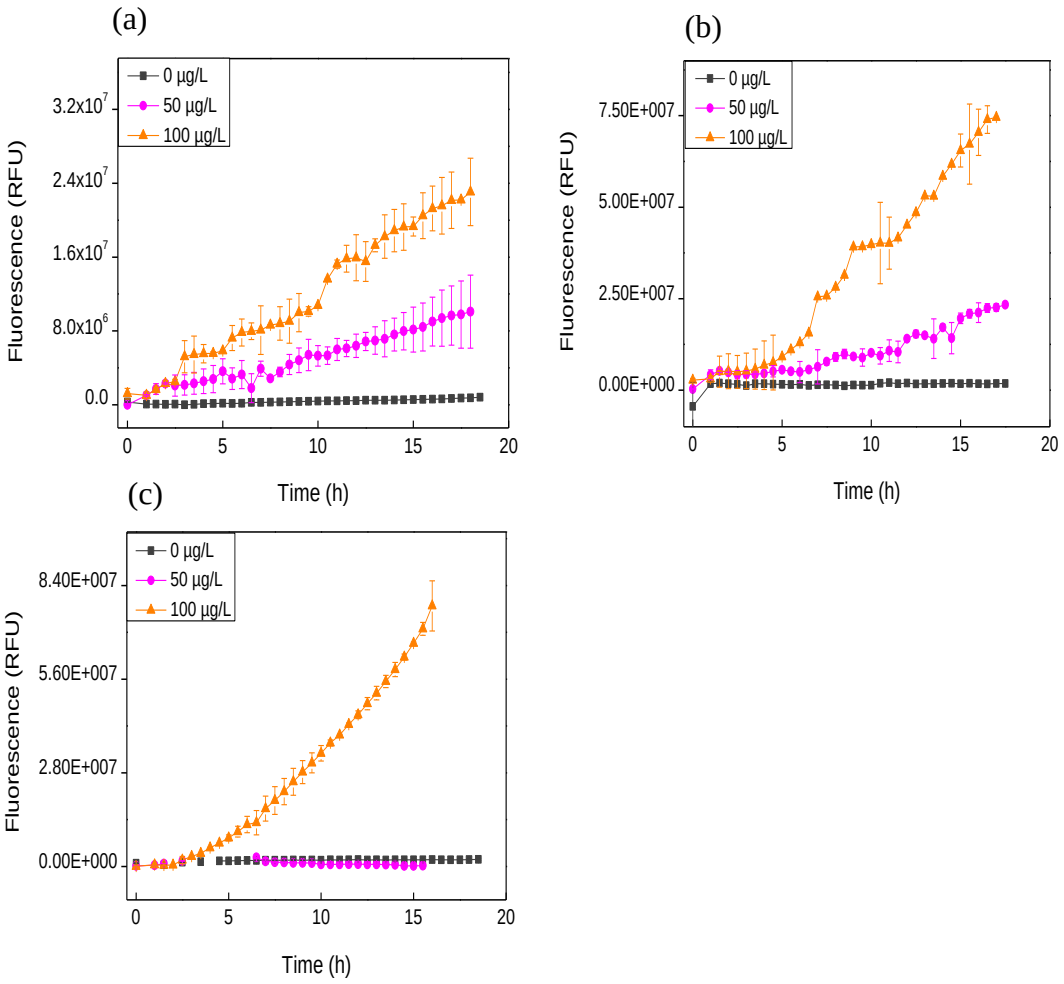

Figure 3.8 Fluorescence emission kinetics of 10 wt% PCL-immobilized arsenic bioreporter induced with varying As (III) concentrations (uninduced (0 µg/L), 50 µg/L and 100 µg/L). (a) Early growth (b) mid – exponential (c) stationary phase. M9 minimal medium was used as the immobilization and growth media, n=4.

The bar graph of the fluorescence values measured at 8 hours in 3 different growth phases (early growth phase, mid – exponential growth phase and stationary phase)

of –CA and –PCL immobilized bioreporters grown and immobilized in M9 minimal medium and induced by several As (III) concentrations are given in Figure 3.9. In Figure 3.9a, the fluorescence values of CA – IB were given, it is clearly seen that the intensity of the emitted fluorescent signal surged with ascending As (III) concentration. However, the best results were obtained in the early growth phase in the CA – IB system induced by 100 µg/L As (III). Furthermore, as the inducer concentration was increased, there was an augmentation in the fluorescent signals from the immobilized bioreporters in all three growth phases ($p<0.05$). In the Figure 3.9b, the bar graph of the fluorescent signal values from the GFPs expressed by the induction of the PCL – IB system at different As (III) concentrations was shown. Statistically the highest fluorescent signal was obtained by immobilizing cells grown up to the mid–exponential phase ($p<0.05$). It was observed that the emitted fluorescence value increased significantly as the arsenic concentration was increased ($p<0.05$). While the highest response was obtained by the cells in the mid – exponential growth phase, cells in the stationary phase also showed a higher response with a higher standard deviation compared with the cells in the early growth phase. As expected, the highest fluorescence value was reached at the highest inducer concentration for all growth phases.

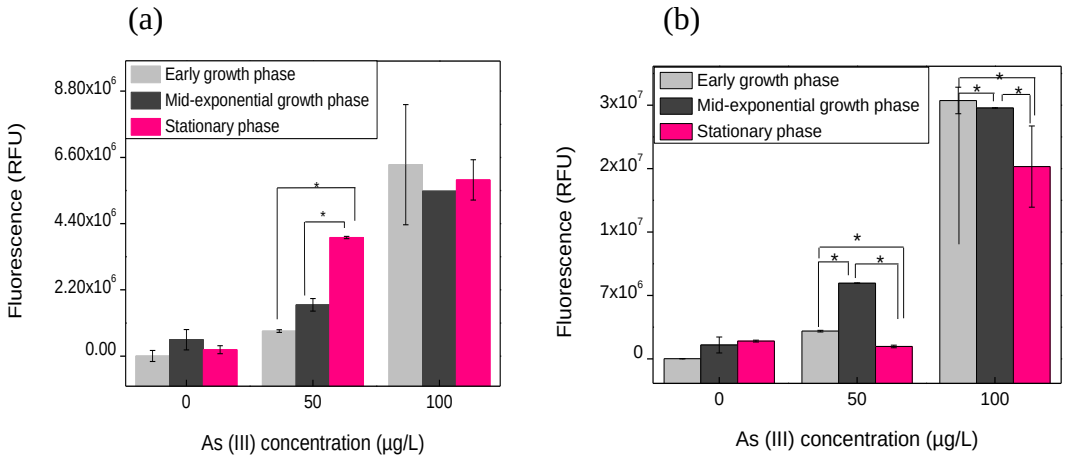

*Figure 3.9 Fluorescence emission kinetics of immobilized arsenic bioreporter at different cell growth phases (early growth phase (light gray); mid–exponential growth phase (hard coal); and stationary phase (pink)) induced with varying As (III) concentrations (uninduced (0 µg/L)), 50 µg/L and 100 µg/L). a) 12.5% CA and b) 10% PCL were used as fiber support materials, M9 was used as the growth and immobilization media, n=4, t=8h, * means p<0.05.*

Figure 3.10 shows the graph drawn from the signals received by inducing As (III) with the free bioreporter cells (non – immobilized) and the fiber immobilized bioreporters. Despite using the same number of bioreporter cells in the same growth phase, a significant difference (p=0.000) was observed between the free and immobilized cells. The reason for this may be the fact that some of the bacteria could not adhere and maintain their viability during immobilization. In Figure 3.10a, free bioreporter had the highest fluorescence signal value for after being exposed to both 50 µg/L and 100 µg/L As (III) concentration (p<0.05). In addition, when the PCL – IB group was induced for 8–h with both 50 µg/L and 100 µg/L arsenic concentrations, the results obtained were found to be significantly higher (p<0.05) than the CA – IB group. As shown in Figure 3.10b, PCL – IB had more GFP expression than free bioreporter and 10% CA – immobilized bioreporters when bioreporter cells grown till mid – exponential growth phase (p<0.05). Among the disadvantages of free cells are that they can be easily affected by the environmental

factors and can be removed from the environment during use. In Figure 3.10c, where the fluorescent signals emitted by FB, CA – IB and PCL – IB groups in the stationary phase are given, it was the free bioreporter that emits the significantly most fluorescent signal after being treated with both 50 μg/L and 100 μg/L ($p<0.05$). On the other hand, the use of arsenic bioreporter in the field is quite difficult compared to the immobilized one. Immobilized bioreporters are more sheltered and not easily affected by environmental factors, and they have the potential to be reused without loss of activity (Mehrotra et al., 2021).

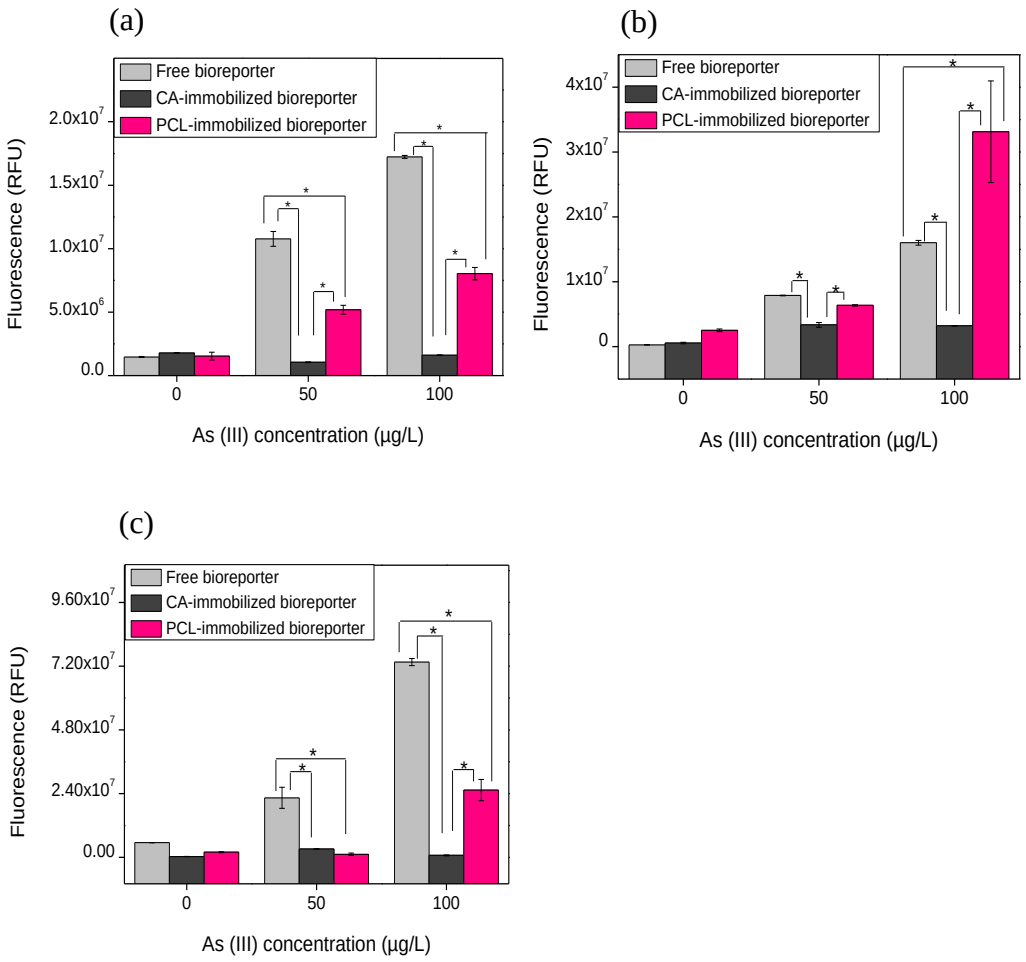

*Figure 3.10 Fluorescence emission kinetics of free bioreporters (light gray), 12.5 wt% CA–immobilized bioreporters (hard coal), and 10 wt% PCL–immobilized bioreporters (pink) induced with varying As (III) concentration (0, 50 and 100 µg/L). M9 was used as the growth and immobilization media, cells at (a) early growth phase (b) mid – exponential growth phase and (c) stationary phase, n=4, t=8h, * means $p<0.05$.*

Moreover, when we compared the two types of bioreporter immobilized electrospun fibers, 10% PCL group had the highest fluorescence signal and thus can be considered more sensitive than the 12.5% CA fiber. When we examined the results, it is evident that the bioreporter cells collected and assayed at the mid – exponential growth phase provides both high fluorescence as well as high reproducibility and

reliability with the lowest standard deviation. Thus, mid – exponential cells were used for further experiments. To evaluate the significance levels of growth phase groups, one of the post hoc tests, Tukey's HSD (honestly significant difference) test was run. The descriptive statistics table, ANOVA table and post hoc test results are given in Appendix C.

3.2.2.2 Effect of Cell Concentrations

The effect of concentrations of the bioreporter cells to be immobilized to the fibers, on the fluorescence intensity emitted was investigated. In order to adjust the density of bacterial cells which were left to grow in the incubator overnight and taken after 18 hours, the OD_{600} values were set to 0.1, 0.5, and 1.5. Then, the immobilization process was performed as mentioned above, and the fluorescence intensities were measured. 12.5% CA fiber support material was utilized, and the results are given in Figure 3.11. According to the results, the most intense fluorescent signal was obtained from arsenic bioreporters immobilized on 12.5% CA fibrous support material when OD_{600} = 1.5. The most intense fluorescent signal obtained by adjusting the bacterial density to OD_{600}= 0.5 (p=0.00).

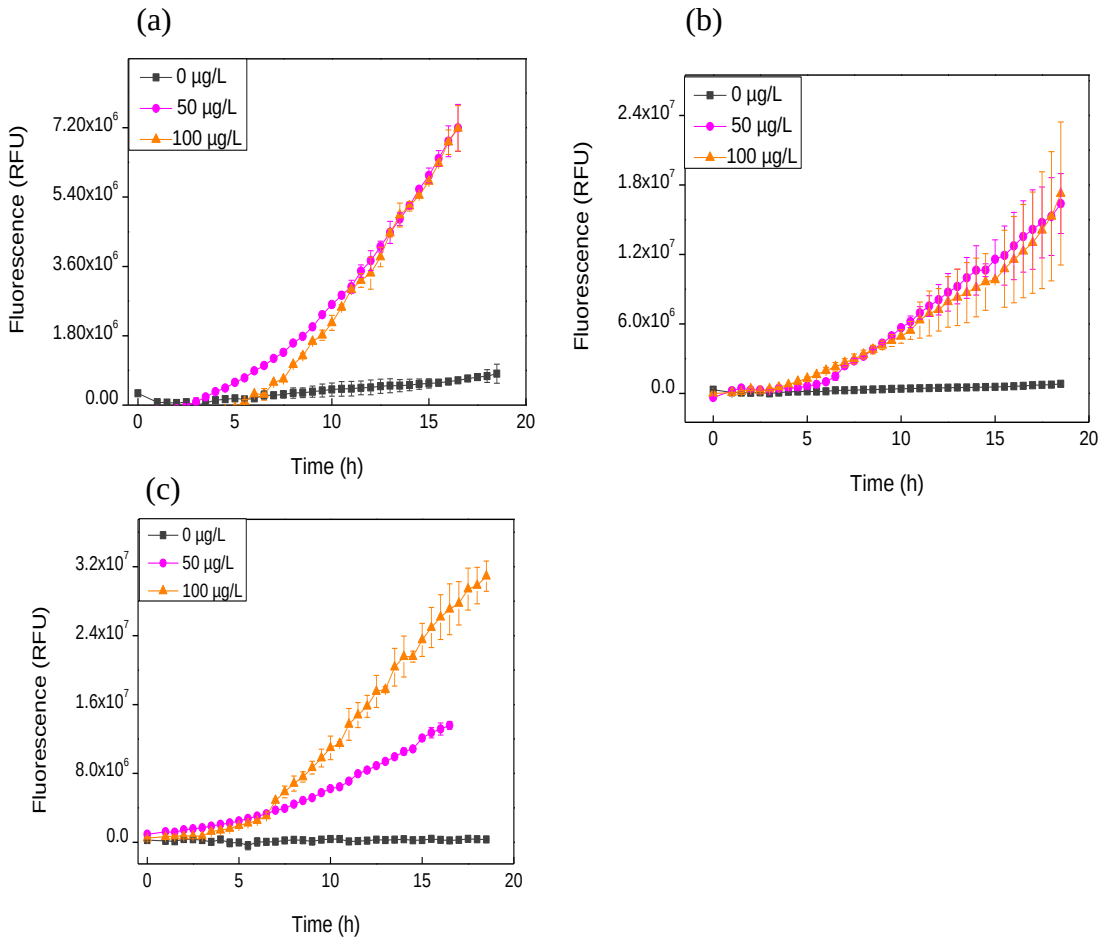

Figure 3.11 Fluorescence emission kinetics of immobilized arsenic bioreporter with varying As (III) doses at different cell concentrations (a) OD_{600} = 0.1, (b) OD_{600}=0.5, (c) OD_{600} = 1.5. 12.5% CA was used as the fibrous support material, M9 minimal medium was used as the growth and immobilization media, n=4.

The fluorescent signals emitted by the bioreporters immobilized on PCL fibers at different concentrations (OD_{600} = 0.1, 0.5 and 1.5) at various As (III) concentrations (50 µg/L and 100 µg/L) are given in Figure 3.12. Accordingly, when the As (III) concentration (100 µg/L) was the highest in all three cell concentrations, the fluorescent signal was also found to be the highest. However, when the cell concentration was adjusted to OD_{600} = 0.5, the PCL – IB system was found to be more sensitive to arsenic and emit a higher fluorescent signal.

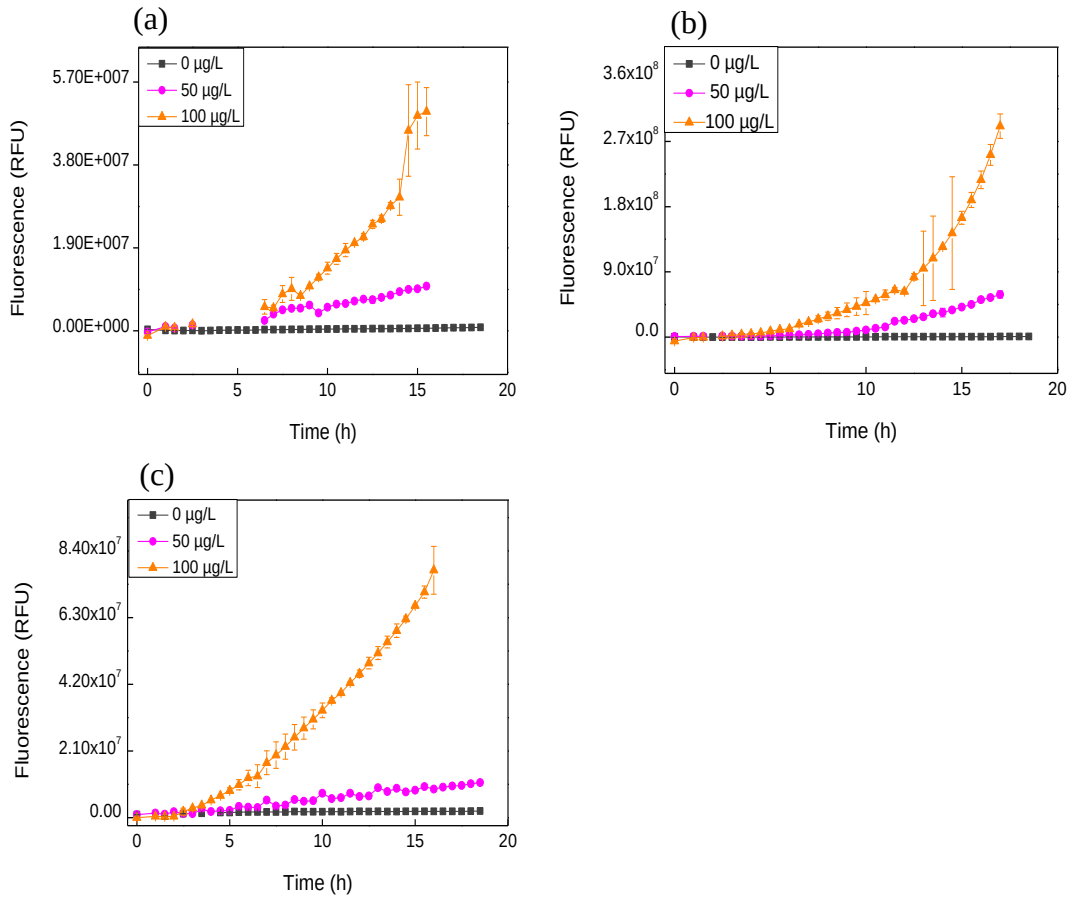

Figure 3.12 Fluorescence emission kinetics of immobilized arsenic bioreporter with varying As (III) doses at different cell concentrations (a) OD_{600} = 0.1, (b) OD_{600} = 0.5, (c) OD_{600} = 1.5. 10% PCL was used as the fibrous support material, M9 minimal medium was used as the growth and immobilization media, n=4.

According to the Figure 3.13a, the best results were obtained when OD_{600} was 1.5 and As (III) value was 100 µg/L. The reason for this could be due to immobilization of more cells and the inductor expresses more bioreporters due to the high concentration. In Figure 3.13b when OD_{600} was set to 1.5, significantly higher value was obtained in the presence of 100 µg/L As (III) ($p<0.05$) however, when the concentration was 0.5, a high signal was obtained even when bioreporters were

induced with 50 µg/L. This situation showed that the bioreporters were more and rapidly expressed when induced with 50 µg/L As (III) in the first 8 hours, however, as more As (III) entered the cell later (100 µg/L As (III)), the fluorescence signal from the bioreporters became higher ($p<0.05$).

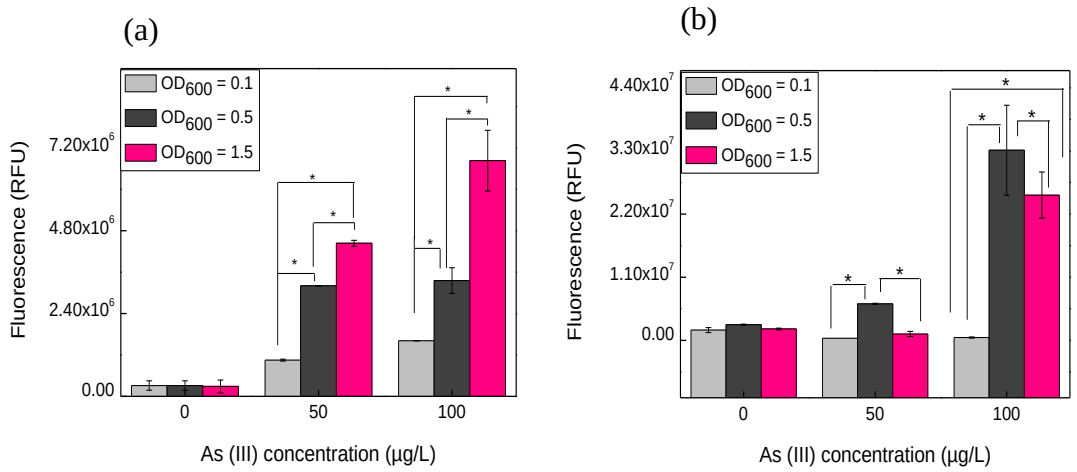

*Figure 3.13 Fluorescence emission kinetics of imm obilized arsenic bioreporter at different cell concentrations OD_{600} = 0.1 (dark gray bar), OD_{600} = 0.5 (light gray bar) and OD_{600} = 1.5 (pink bar) induced with varying As (III) concentrations (uninduced (0 µg/L)), 50 µg/L and 100 µg/L). a) 12.5% CA and b) 10% PCL were used as the fibrous support material, M9 minimal medium was used as the growth and immobilization media, n=4, t=8h, * means p<0.05.*

In Figure 3.14, the responses of free (non-immobilized) and immobilized bioreporters at different cell concentrations in the presence of arsenic are shown in bar graphs as the fluorescent signal value. Accordingly, the responses of free bioreporters at all three cell concentrations (OD_{600} = 0.1, 0.5 and 1.5) induced with different arsenic concentration (50 µg/L and 100 µg/L) were higher, except for the fluorescence value emitted by the PCL – IB system set to OD_{600}= 0.5 and induced by 100 µg/L As (III) ($p<0.05$). In Figure 3.14b, when the PCL – IB system, whose cell concentration was adjusted to OD_{600} = 0.5, and the free bioreporters were induced with 50 µg/L, there was no statistically significant difference ($p>0.05$) in

terms of the fluorescent signal they emitted. Although, when the As (III) concentration was increased to 100 μg/L, it was found that the PCL – IB system's response to arsenic was higher than both free bioreporter and CA – IB system ($p<0.05$). The reason for the difference between the fluorescent signal values emitted by the arsenic – induced CA – IB and the PCL – IB system is thought to be due to the fact that the cells could not adhere due to the fluffy structure of the CA fiber (Sinha et al., 2020). It is also known that thin electrospun membranes with a smaller fiber diameter facilitate the attachment of cells due to their larger surface area (Ko and Yang, 2008; Sarioglu et al., 2017).

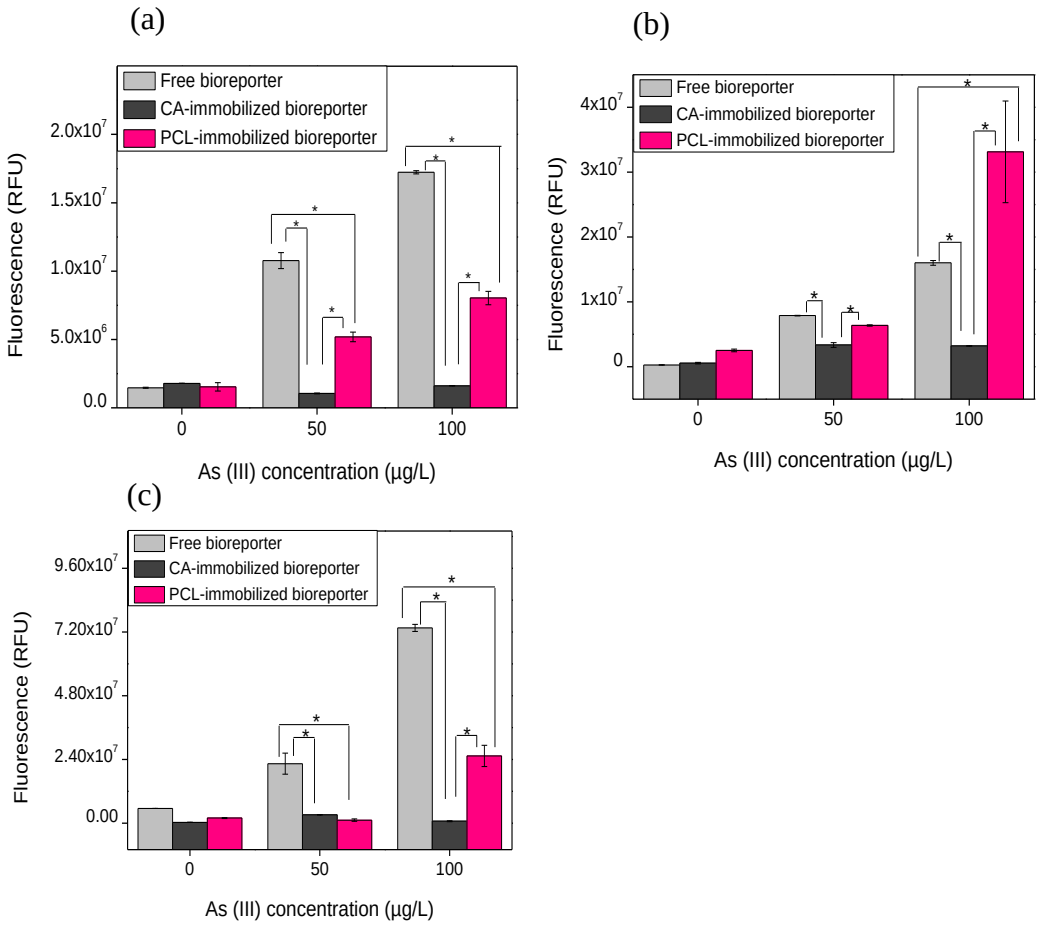

*Figure 3.14 Fluorescence emission kinetics of free bioreporters (light gray), 12.5 wt% CA–immobilized bioreporters (hard coal), and 10 wt% PCL–immobilized bioreporters (pink) and induced with varying As (III) concentration (0, 50 and 100 µg/L). M9 minimal medium was used as the growth and immobilization media, cells at (a) OD_{600} = 0.1 (b) OD_{600} = 0.5 and (c) OD_{600} = 1.5, n=4, t=8h, * means p<0.05.*

3.2.2.3 Evaluation of As (V) Detection Performance

The fluorescent signal values of the responses of the CA – IB and PCL – IB groups we developed, when induced with As (V), a different form of arsenic, are given in Figure 3.15. When the graph was examined, it was deduced that the PCL – IB system was more sensitive than the CA – IB system when induced with both 50 and 100

µg/L As (V) ($p<0.05$). However, there was no statistically significant difference between the fluorescence signal value emitted when CA – IB group was induced with 100 µg/L As V and the emitted fluorescence signal value when PCL – IB group was induced with 50 µg/L As V ($p=0.651$). This showed that the PCL – IB group was more sensitive than the CA – IB group. Due to the fact that it is a biological – based study, different results were obtained in each trial, and as a result, the calculated standard deviation values were quite high. Both systems expressed more GFP when the As (V) concentration was increased, from 50 µg/L to 100 µg/L, and the intensity of the fluorescent signal they emitted increased.

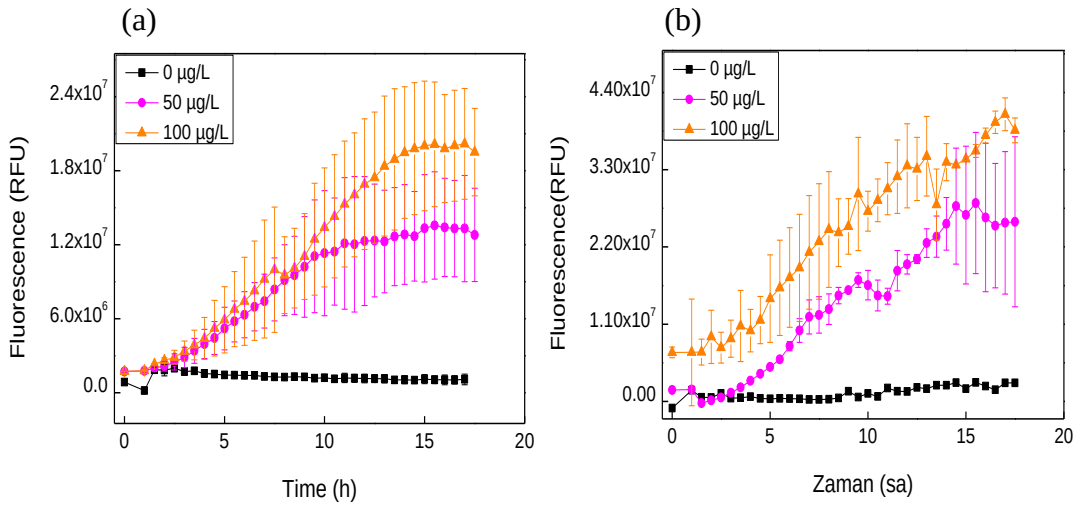

Figure 3.15 Fluorescence emission kinetics of immobilized arsenic bioreporter induced with varying As (V) concentrations (uninduced (0 µg/L)), 50 µg/L and 100 µg/L). a) 12.5% CA and b) 10% PCL were used as the fibrous support materials, MOPS – supplemented medium was used as the growth and immobilization media; cells were grown till mid – exponential growth phase, OD_{600} was set as 0.5 n=4.

In the bar graph in Figure 3.16, the fluorescence values of the non – immobilized (free) and immobilized bioreporters read at 8 hours after being induced with As (V) at different concentrations (50 µg/L and 100 µg/L) are given. The fluorescence values emitted by the PCL – IB group at 50 µg/L and 100 µg/L concentrations were statistically different from the other two groups, free bioreporter and CA – IB system. It was observed that the emitted fluorescent signal value increased as the As (V)

concentration increased. As with As (III), the PCL – IB system emitted more fluorescent signals in the presence of As (V). However, the emitted fluorescent signal value was less than when induced by As (III) ($p<0.05$). As (III) is a water – soluble and movable polyol similar in structure to glycerol. The bidirectional channels that play a role in the entrance to the cell allow small and uncharged molecules such as water and glycerol to enter the cell, just as they allow for As (III). However, the situation is different with As (V) since the rate of absorption of As (V) by the cell is much lower than that of As (III), due to the fact that the role of phosphate transporters (Pit and Pst) in the uptake of As (V) is very limited. Therefore, it is better understood why the As (III) – induced IB system emits more fluorescent signals than the As (V) – induced despite being induced even at the same concentrations (Fernández et al., 2005; Hirano, 2020). To evaluate the statistically significant differences of each group, one of the post hoc tests, Tukey's HSD test was run. The descriptive statistics table, ANOVA table and post hoc test results were given in Appendix C.

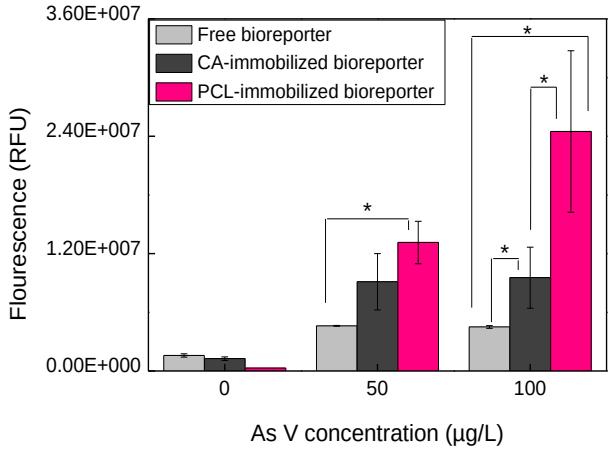

*Figure 3.16 Fluorescence emission kinetics of free bioreporters (light gray), 12.5 wt% CA–immobilized bioreporters (hard coal), and 10 wt% PCL–immobilized bioreporters (pink) and induced with varying As (V) concentration (0, 50 and 100 µg/L). MOPS supplemented medium was used as the growth and the immobilization media, cells were grown till mid – exponential growth phase, OD_{600} was set as 0.5, n=4, t=8h, * means $p<0.05$.*

3.2.2.4 Cell Viability

In order to clarify and confirm the results made with fluorescence experiments, the viability of bacterial bioreporter cells immobilized on fibers was compared by using the AlamarBlue test. Relative viability graph showing the percentage of reduction of the AlamarBlue by immobilized bioreporter cells according to the fluorescence readings taken at the end of the 4th incubation hours with AlamarBlue was drawn in Figure 3.17. In favorable fabrication conditions, up to 55.4% of bacteria were kept alive on CA fibers. In other respects, bacteria were grafted on PCL fibers and a high bacterial viability was achieved at over 91%. When relative viability of PCL – immobilized bioreporters and CA – immobilized bioreporters was compared, a significant statistical difference was observed (p = 0.005).

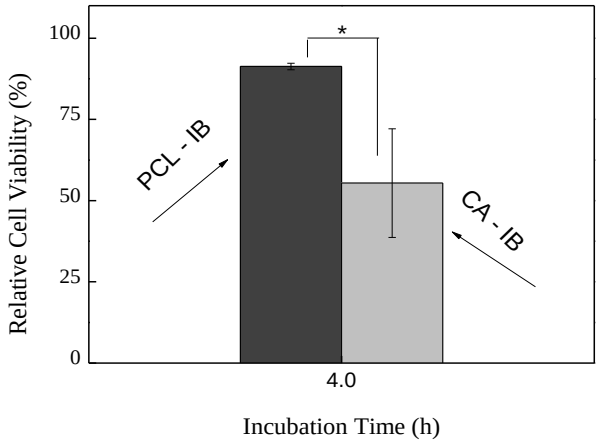

*Figure 3.17 Relative cell viability (%) results of immobilized bacterial bioreporter cells on –CA and (light gray bar) and –PCL (dark gray bar) fibrous support materials. (LB was used as immobilized and growth medium, n=4, * means p<0.05), free bioreporter cells serve as the control group).*

Fung et al. fabricated soluble dietary fibers from okara, palm oil stem and palm oil obtained by alkali treatment from agricultural wastes via electrospinning technique. They investigated the potential of these fibers to be used as carriers in the encapsulation of *Lactobacillus acidophilus*, and electrospinned by adding bacteria to

each organic waste solution. They observed that the encapsulated bacteria could survive at a rate of approximately 78.6–90 % (Fung et al., 2011).

Xie et al. immobilized genetically engineered *E. coli* (GFP) on poly (ethylene glycol)-polylactide (PELA) fibers. They showed that in their study, around 92% and 95% of the viability of bacteria was observed after covalent binding and affinity absorption, respectively (Xie et al., 2016). The authors tested effect of chemical modifications on fibers and immobilization method it is therefore difficult to compare their results with ours.

Jayani et al. showed that 71.1 % of *Lactobacillus acidophilus* 016, which they immobilized on bacterial cellulose, maintained their viability (Jayani et al., 2020).

Grzywaczyk et al. evaluated the viability of the arrested bacterial cells in the polystyrene/sodium alginate – bacteria/polystyrene sandwich model they developed. According to the results they found, while the free bacteria were viable around 60%, up to 81% of the immobilized bacteria were viable (Grzywaczyk et al., 2021).

3.2.2.5 Morphology of Bioreporter Immobilized on Fibers

SEM was used to display the bioreporters immobilized onto the fibers. The images of bacteria immobilized fibers at 10 000X magnification are shown in Figure 3.18. Rod – shaped bacteria were observed on the fibers. The number of bacteria seen on PCL fiber was greater than that observed on CA. The diameters of the bacteria attached to the fibers were calculated as approximately 0.91 μm ± 0.14.

In the imaging performed with a confocal microscope, a CA – IB group image could not be obtained because it has a floating structure and therefore cannot be fixed on a flat surface. In imaging with PCL – IB group despite attempts to block the background fluorescence, the green color of the fibers is due to the intrinsic autofluorescence of the PCL material at selected excitation wavelengths. Living bacteria that bridge and spread between the fibers glow green fluorescent thanks to

the *gfp* in their structure. The bacteria not only adhered to the fiber surface, but also spread to the spaces within the fibrous mesh.

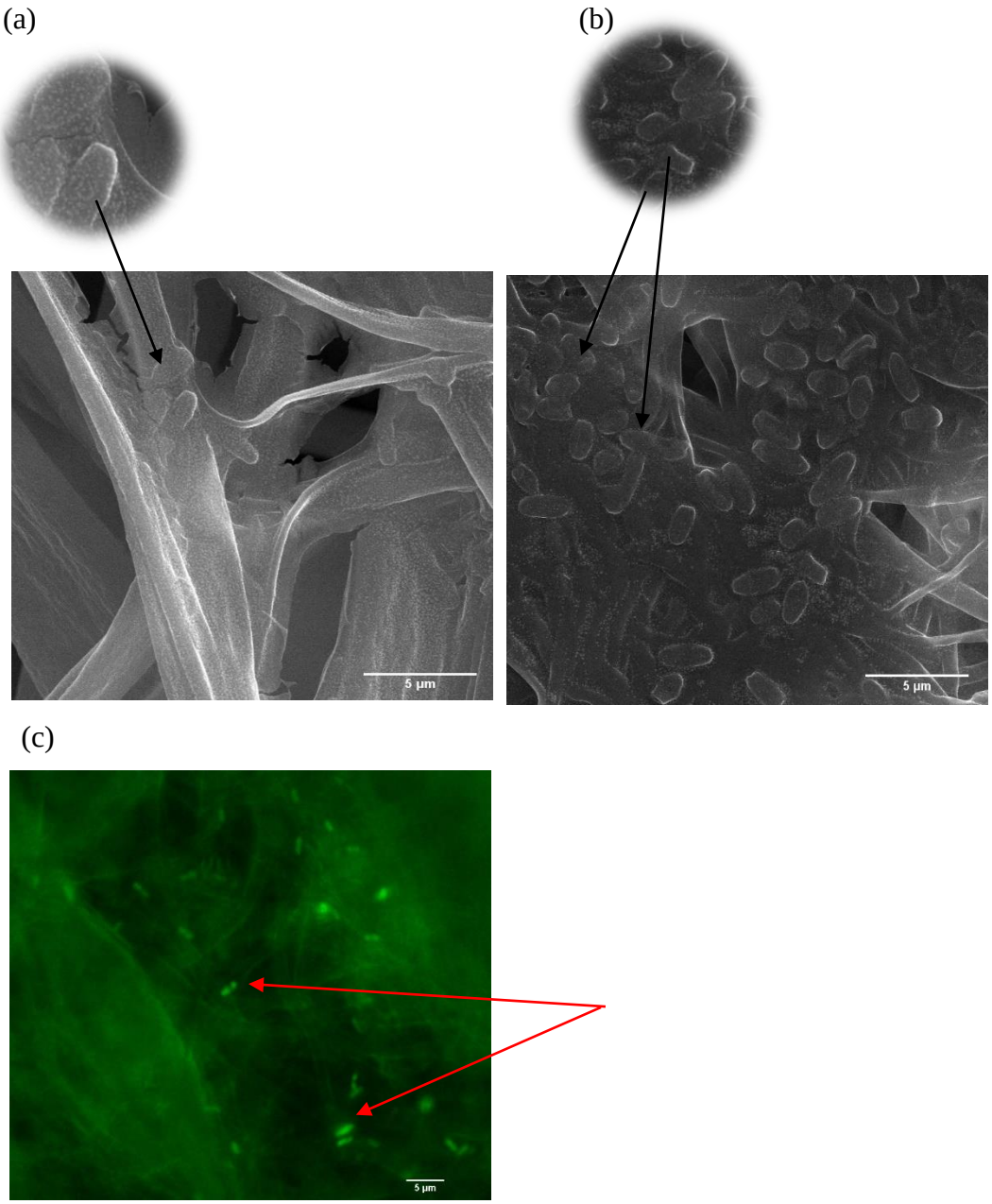

Figure 3.18 SEM micrographs of bacteria immobilized on (a) CA fibers and (b) PCL fibers at 10 000X magnification and (c) confocal microscopy of PCL – IB groups after 3h immobilization. Arrows show the bioreporters adhered on the fibers.

When the fiber diameters are larger than the *E. coli* bacteria size, the bacteria accept each fiber surface as a flat substrate. On the other hand, it has been reported that when the fiber diameters are smaller than the bacteria diameter, it is not always possible for the cells to change shape and form colonies by building a bridge between the fibers (De Cesare et al., 2019). Therefore, fibers with a diameter greater than that of 1.0 – 2.0 μm in length and 0.5 μm in diameter, known as *E. coli* cells (Knoll et al., 1999) are considered to be suitable for bacterial immobilization.

In the trials conducted so far, PCL and CA electrospun fibers were used as the support materials to immobilize arsenic bioreporters. When the fiber support material characterization tests and then the optimization results of the immobilized bioreporter systems are examined, it is clearly seen that the fluorescent signals from the PCL immobilized bioreporters were higher additionally, in the viability test, the viability percentages of the bacteria attached to the PCL fiber were found to be higher than IB – CA systems. Sarioglu et al, in their study to show the effect of fiber diameter and morphology on the immobilization of bacteria, immobilized *Acinetobacter calcoaceticus* STB1 bacteria on four different groups of PSU fiber, namely aligned and random, thin, and thick. As a result of their study, they emphasized that random oriented thin fibers are more suitable for bacteria to adhere to because they have enough space (Sarioglu, et al., 2017). In our study, it can be deduced that the PCL – IB group emits more fluorescent signal than the CA – IB group in the presence of As (III), because they have a thinner and larger pore diameter, so that the bacteria adhere better. This means that PCL electrospun fiber was a more suitable support material for bacteria to survive and adhere. Accordingly, following studies was continued with PCL – immobilized arsenic bioreporters.

3.2.2.6 Effect of Growth Medium

In order to show the change in the response of immobilized arsenic bioreporters to arsenic when grown in different growth media (M9 minimal medium and MOPS – supplemented medium), the fluorescent signals emitted at the 8th h were recorded

(Figure 3.19). It was seen that arsenic bioreporters grown and immobilized in M9 minimal medium expressed more GFP in the presence of As (III) than those induced by As (V), therefore emit more fluorescence ($p<0.05$). Similarly, it is also clear that the signal received from bioreporters induced by As (III), but this time grown and immobilized in MOPS – supplemented medium, was considerably lower than those grown in M9 minimal medium. The reason may be that the fluorescence performance of immobilized bioreporters in MOPS – supplemented medium was significantly lower than that of M9 minimal medium ($p<0.05$), which is presumably owing to the presence of inorganic phosphates in M9 minimal medium. Because inorganic phosphate is one of the basic nutrients of bacterial cells (Li et al., 2019). In addition, *E. coli* cell carries out the uptake of As (V) into the cell via the non – specific phosphate transporter, inorganic phosphate transporter (Pit), which is known to play the biggest and main role (Yang et al., 2012). In some cases, phosphate specific transport (Pst) is also expressed, which is more specific but less efficient than Pit, which carries phosphate. However, if the cell is continually subject to large amounts of As (V), it will only express Pst in such cases to minimize As (V) uptake. This may mean that the cell emits less fluorescence in the presence of As (V). In addition, it has been reported that *E. coli* cells have water glyceroporins, such as glycerol facilitator GlpF, to facilitate the entry of As (III) into the cell and to ensure its entry in greater amounts (Garbinski et al., 2019; Krueger et al., 2013; Kumari & Jagadevan, 2016). This may be another explanation proving that it expresses more GFP in the presence of As (III) because As (III) enters the cell more easily.

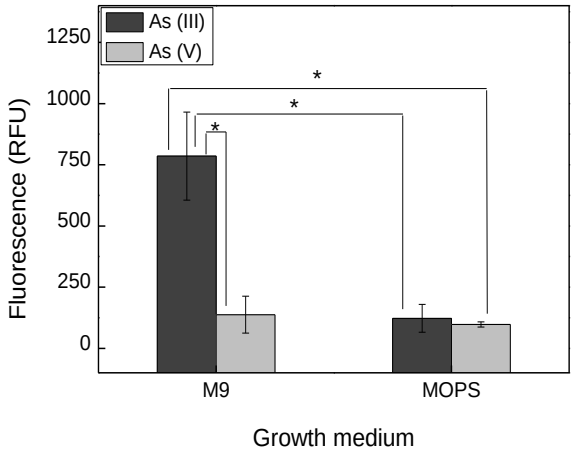

*Figure 3.19 Fluorescence emission kinetics of immobilized bioreporter induced with As (III) (dark coal bar) and As (V) (light gray bar) concentrations (100 µg/L) in different growth medium. 10% PCL was used as the fibrous support materials, MOPS and M9 were used as the growth and immobilization media, respectively. Cells at mid-exponential growth phase were used during the study, n=4, t=8h, * means p<0.05.*

In another experiment, the fluorescent signals emitted by arsenic bioreporters induced by arsenic forms at different concentrations were recorded (Figure 3.20). However, it is seen that although the As (V) concentration was increased, the fluorescent signals they emitted did not increase as in As (III) ($p<0.05$). There was no statistically significant difference between the fluorescent signal emitted by the PCL – IB group when induced with 50 µg/L As (V) and the fluorescent signal emitted when induced with 100 µg/L As (V) ($p>0.05$). As (V) enters the cell via phosphate transporters and replaces phosphate, but as phosphate is already present in the M9 – minimal medium, it may be difficult for As (V) to enter the cell and may not induce immobilized arsenic bioreporters as much as As (III) (Cavalca et al., 2013) It is known that As (V) is particularly sensitive to pH depending on biological and chemical conditions and cannot maintain its stability (Darma et al., 2022; Edmundson & Horsfall, 2015) which might explain the results given in Figure 3.18. Furthermore, bacterial periplasmic phosphate binding proteins responsible for As

(V) uptake are 500 to 850 times more selective for phosphate than As (V), and it is known to be 4500 times more in arsenic – resistant species (Hirano, 2020).

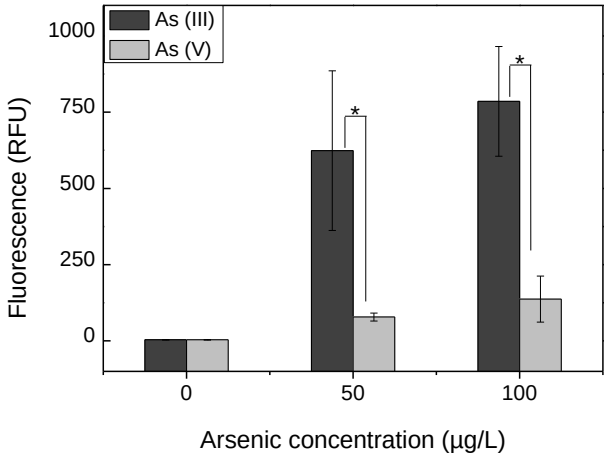

*Figure 3.20 Fluorescence emission kinetics of immobilized bioreporter induced with varying As (III) (dark coal bar) and As (V) (light gray bar) concentrations (0, 50 and 100 µg/L). 10% PCL was used as the fibrous support material, M9 minimal medium was used as the growth and immobilization medium, cells at mid – exponential growth phase, n=4, t=8h, * means p<0.05.*

In Figure 3.21, the graph of fluorescent signals emitted by inducing arsenic bioreporter cells grown and immobilized in MOPS – supplemented medium with different concentrations of arsenic forms is given. Statistically significant difference ($p<0.05$) was found between different arsenic concentrations (0 µg/L, 50 µg/L and 100 µg/L). With increasing arsenic concentration, more GFP was expressed in bioreporters, causing an increase in signal intensity.

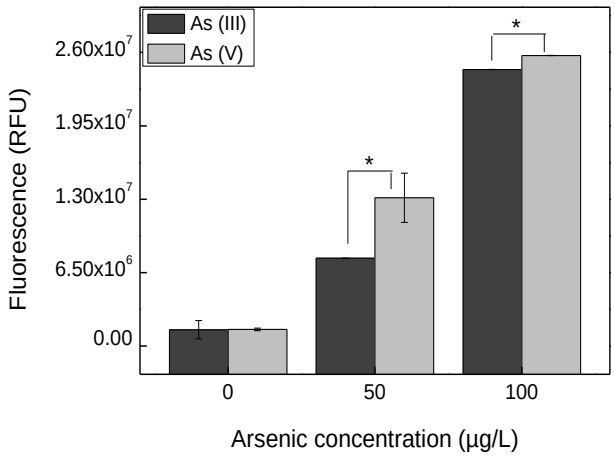

*Figure 3.21 Fluorescence emission kinetics of immobilized bioreporter induced with varying As (III) (dark coal bar) and As (V) (light gray bar) concentrations (0, 50 and 100 µg/L). 10% PCL was used as the fibrous support material, MOPS supplemented media were used as the growth and immobilization media. Cells at mid – exponential growth phase were used during the study, n=4, t=8h, there is a statistically significant difference between arsenic concentrations, * means p<0.05.*

The fluorescent signals (RFU) emitted by the PCL – IB system induced by different As (V) concentrations (50 µg/L and 100 µg/L) in different growth mediums (M9 minimal and MOPS – supplemented medium) are given in Figure 3.22. When the PCL – IB system was treated with 50 µg/L As (V) in MOPS supplemented medium, it gave significantly higher fluorescent signal than that in M9 minimal medium, an analogous situation was observed when the concentration was 100 µg/L ($p<0.05$). The reason for the PCL – IB system to express more GFP in the presence of As (V) in MOPS-supported media may be that arsenate is chemically like phosphate. Basically, the ability of As (V) to enter the cell relies on competitive inhibition. That is, if the cell is saturated with phosphate, it may be insensitive to As (V). It is known that As (V) uptake systems in *E. coli* are made by the phosphate transporters Pst and Pit. Pst is a high – affinity, low – capacity system caused by phosphate starvation, while Pit is a low – affinity, high – capacity constituent system that is also the main uptake system for As (V) (Yang et al., 2012). Therefore, the PCL – IB system in the

phosphate – rich M9 minimal medium was not phosphate-starved, so it took up relatively less As (V) into the cell, thus emitting less fluorescent signal.

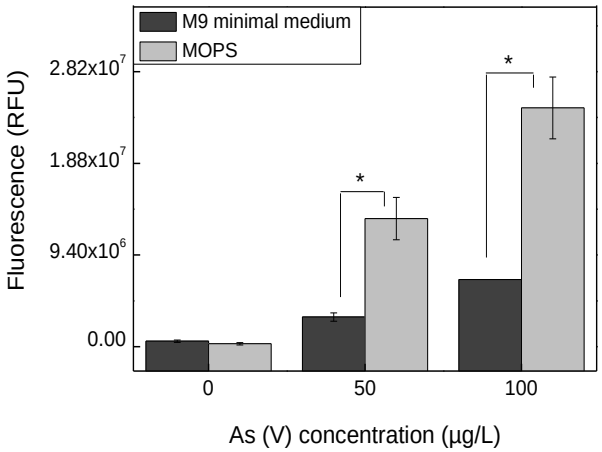

Figure 3.22 Fluorescence emission kinetics of immobilized bioreporter grown in M9 minimal medium (dark coal bar) and MOPS supplemented medium (light gray bar) induced with varying As (V) concentrations (0, 50 and 100 µg/L). Fibrous support materials produced with 10% PCL were used for immobilization of cells at mid – exponential growth phase, t=8h, n=4.

3.2.2.7 Effect of Immobilization Time

Different immobilization times (1 h, 3 h and 7 h) were tried to examine the effect of the time taken for the immobilization of arsenic bioreporters to the PCL electrospun fiber on the fluorescent signal they emit. Accordingly, at the end of the immobilization period, the immobilized bioreporters were induced with As (III) at concentrations of 50 µg/L and 100 µg/L and the fluorescence signal emitted was recorded. The emitted fluorescence value was found to be the highest after 3 hours of immobilization pursuant to the bar graph ($p<0.05$) (Figure 3.23). After 3–h incubation for immobilization, PCL – IB emitted statistically higher fluorescence than those oberved for the other immobilization periods (1–h and 7–h) after they were induced with both 50 µg/L As (III) and 100 µg/L As (III) ($p<0.05$). PCL – IB system subjected to 7–h immobilization time emitted a high fluorescent signal with a high standard deviation when induced with 100 µg/L As (III). However, it was

decided that 3–h would be sufficient because it emits more fluorescent signals, so 7–h waiting would not be necessary. It was predicted that the longer the waiting period, the older the cells and the lower their vitality.

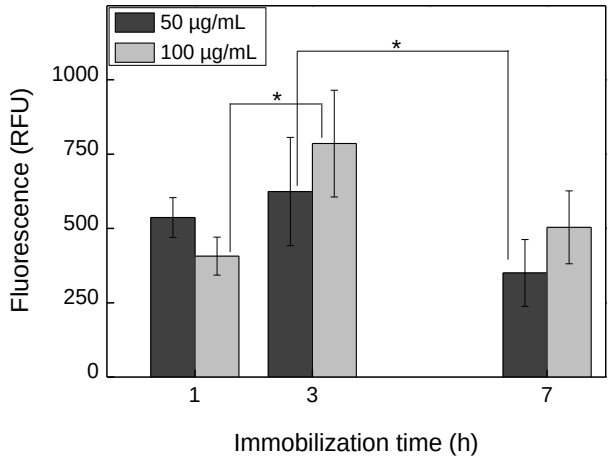

*Figure 3.23 Fluorescence emission kinetics of arsenic bioreporters immobilized for different immobilization periods (1h, 3h and 7h) induced with varying As (III) concentrations (50 µg/L (dark coal bar, and 100 µg/L (light gray bar). 10% PCL fibrous mats were used as the support materials, M9 minimal medium was used as the growth and immobilization media, n=4, t=8h, * means p<0.05.*

Parreira et al. used self-assembled monolayers (SAMs) of alkanethiols on gold in order to procure several functional groups such as OH, CH_3 and ethylene glycol (EG_4) in their studies. As a result of their experiments, they stated that *Helicobacter pylori* (*H. pylori*) bacteria adhered to the surfaces they developed after 2 hours. (Parreira et al., 2011).

San et al. immobilized *Aeromonas eucrenophila*, *Clavibacter michiganensis* and *Pseudomonas aeruginosa* bacteria on CA to evaluate the methylene blue decolorization performance. By figuring out the immobilization time of 7 days, they left the fibers to incubate in the growth medium with bacteria at a rotational speed of 100 rpm at 30 °C (San et al., 2014).

Abrigo et al. used polystyrene (PS) electrospun fibers fabricated in several diameters as support material to immobilize *Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus* bacterial cells by adsorption method. After growing the

bacterial cells on LB agar, electrospun fibers were placed on the agar and incubated for 1 hour at 37°C. At the end of 1–h, they removed the fibers and continued their work by washing with PBS and removing the bacteria that could not adhere (Abrigo et al., 2015).

Sarioglu et al. *Serratia proteamaculans* STB3 and *Achromobacter xylosoxidans* STB4 bacteria immobilized on electrospun fibers which are non – porous CA and porous CA. The aim of this study is to investigate the effect of contact time and fiber porosity on bacterial adhesion and to evaluate their performance in terms of SDS remediation in water. They started a 25 – day incubation at 25°C 180 rpm by including the fibers in the liquid medium with the bacteria. They evaluated the SDS remediation performance of the system they created by renewing the liquid medium with fresh medium every 7 days. As a result, they concluded that 25 days was the optimum incubation period (Sarioglu et al., 2015).

Sarioglu et al. developed *Morganella morganii* STB5 immobilized on PS or PSU fiber mats for hexavalent chromium remediation in water. In their immobilization studies, bacterial cells were incubated in M1 growth medium with fiber mats (PS or PSU) for 30 days (Sarioglu et al., 2016).

Sarioglu et al. used *Clavibacter michiganensis* immobilized on PCL and PLA fibers for remediation of textile dyes in water. They incubate bacteria and fiber for 7 days (Sarioglu, et al., 2017b).

3.3 Determination of the Performance Characteristics of The Bioreporter System

3.3.1 Sensitivity

A dose – dependent experiment was performed to understand the sensitivity of the PCL – immobilized bioreporter in the presence of As (III) and As (V). Different concentrations (10, 20, 30, 50, 75 and 100 μg/L of As (III) form and 0 μg/L

(uninduced; control group)) were used for this experiment. In the Figure 3.24, as expected the intensity of the emitted fluorescent signal increased as the As (III) and As (V) concentration (from 0 µg/L to 100 µg/L) was increased (Brutesco et al., 2017; Yuvaraj et al., 2016). However, there was no significant difference between fluorescence intensity at 50 µg/L and 75 µg/L As (III) concentrations (Figure 3.24a). The fluorescence values emitted by PCL – immobilized bioreporters induced by As (V) are also given in Figure 3.24b. Accordingly, as in As (III), with increasing concentration, more arsenic was introduced and more GFP was expressed. However, looking at the two graphs, it is clear that the fluorescent signal from As (V) induced immobilized bioreporters was significantly lower than that of As (III) induced ones. This is because, under anoxic conditions, As (III) has the ability to bind slightly to less absorbent surface regions, and after bonding it diffuses into the aqueous phase, increasing its mobility, that is, it moves faster than As (V). The results obtained can be explained by the fact that As (III) moves faster inside the cell and expresses GFP in a shorter time (Darma et al., 2022).

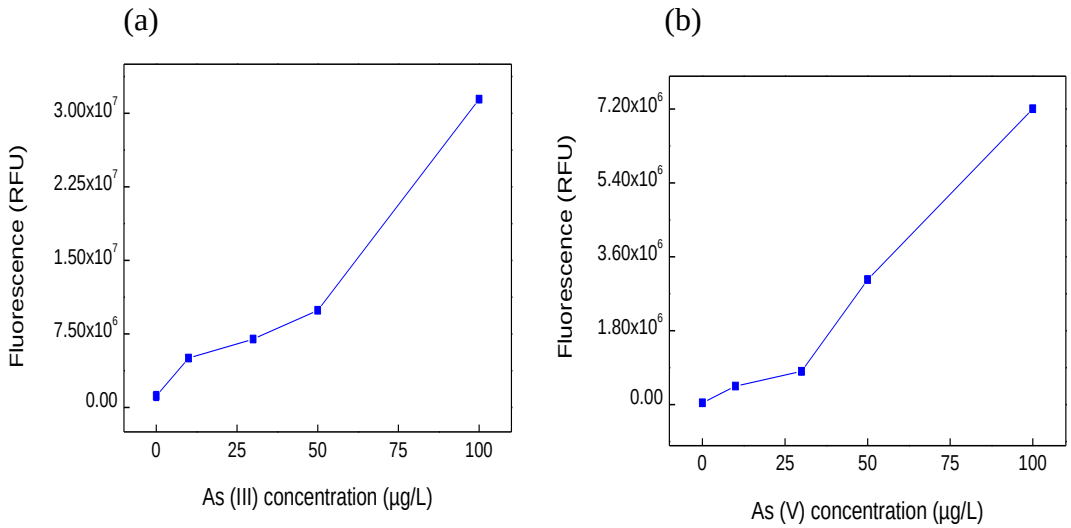

Figure 3.24 Fluorescence emission kinetics of immobilized bioreporters induced with varying arsenic concentration (0, 10, 20, 30, 50, 75 and 100 µg/L) (a) As (III) (b) As (V). 10% PCL was used as fiber support materials, M9 was used as a growth and immobilized medium, cells at mid-exponential growth phase, n=4, t=8h. Error bars are shown only when they exceed the size of the symbols.

3.3.2 Selectivity

There may be other metal ions in drinking water or natural spring water as well as arsenic (Moiseenko et al., 2013; Payal & Tomer, 2021). Selectivity is one of the most important characteristics for determining the performance of a biosensor. Selectivity, in other words, metal specificity is defined as the capability of a bioreceptor to detect a particular analyte despite the presence of different contaminants in the environment (Bhalla et al., 2016). In order to evaluate the selectivity of the immobilized arsenic bioreporter, they were induced with arsenic forms (As (III) and As (V)) and other metals (threatening human health) at a concentration of 100 µg/L. First, viability analysis of bacterial cells in PCL – IB system induced with different metals at 100 µg/L concentration for 8 hours was performed by AlamarBlue method. The results obtained in the viability analysis are given in Figure 3.25. According to Figure 3.25, although it was induced with a very low metal concentration (100 µg/L), a decrease in the viability of bacteria treated with some metals was observed according to the AlamarBlue test results at the end of the 8–hour induction period.

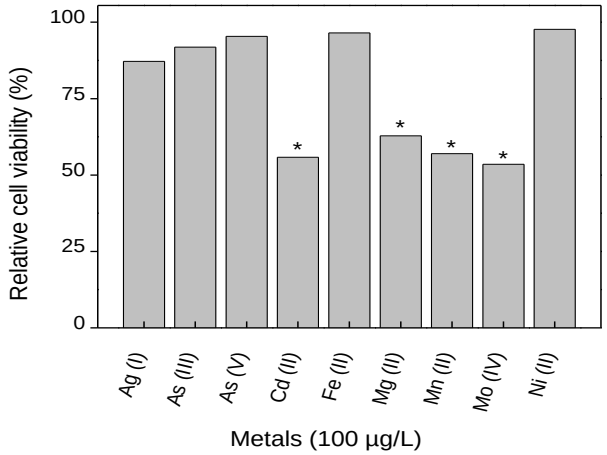

*Figure 3.25 Relative cell viability (%) results of immobilized arsenic bioreporters which were induced with different metals (100 µg/L). Bioreporter cells were grown in M9 minimal medium (till stationary phase, OD_{600}=1.5). The viability of the no metal addition group was accepted as 100%. n=3, t=8h, * shows the groups significantly different than the others, p<0.05.*

The viability results of PCL – IB group after 8-hour treatment with metals are given in Figure 3.25. It was observed that there was a significant decrease in the viability of the immobilized bioreporter cells treated with some metals ($p<0.05$). The relative cell viabilities of the PCL – IB system induced by Cd (II), Mg (II), Mn (II) and Mo (IV) were significantly lower than the uninduced group ($p<0.05$). The pH value of the M9 minimal medium used in the experiment was adjusted to around 7.0, but it has been reported in the literature that Cd (II) is more toxic at neutral pH than acidic environment (Worden et al., 2009).

In their study, Kalantari et al. suggested that Fe (II) metal ion completely inhibited *E. coli* bacteria cells at a concentration of 55840 µg/L (1 mM/L). In addition, it was stated that chromium metal at 193.350 µg/L (0.5 mM/L) and 3867 µg/L (0.1 mM/L) concentrations decreased the viability of *E. coli* bacterial cells to 77% and 38% (Kalantari, 2008).

Vijayadeep and Sastry reported in their study that *E. coli* treated with heavy metals at different concentrations showed a very high tolerance. In the study, it was reported that the viability of the cells treated with 2000 µg/L (2 ppm) Cd (II) remained constant, but their viability was greatly reduced when the metals dose was increased (Sastry & Vijayadeep, 2014).

According to the results of their study, Terzi and Civelek showed that *E. coli* isolates treated with 750 µg/mL arsenic concentration maintained their viability. They also remarked that these cells could survive at concentrations of 112 µg/mL Cd (II) (Terzi & Civelek, 2021).

According to the viability results of PCL – IB system after induction with different metals (100 µg/L), the fluorescent signal values they emitted were corrected for 100 % cell viability. Accordingly, the extrapolated fluorescence values are given in Figure 3.26.

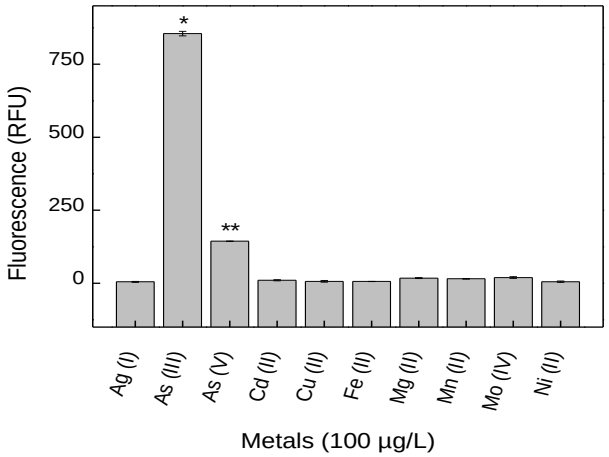

*Figure 3.26 Metal selectivity of the PCL – immobilized arsenic bacterial bioreporter. Bioreporter cells grown in M9 minimal medium (till stationary phase, OD_{600}=1.5). Control refers to no metal addition, n=3, t=8h, * means the significantly the highest group, ** means the significantly the higher group than others, p<0.05. Error bars are shown only when they exceed the size of the symbols. The fluorescent signal values the bioreporters emitted were corrected for 100 % cell viability.*

It can be deduced from Figure 3.26, development PCL – immobilized arsenic bioreporter system did not give worthy of note fluorescence response to the assessed metals. The highest fluorescence was measured from the As (III) – induced PCL – IB system ($p<0.05$). It is thought that the reason why it was higher than those induced by As (V) was due to the growth the growth media effect as explained above in Section 3.2.2.6. Although, the arsenic bacterial bioreporter appeared to be sensitive to arsenic forms (As (III) and As (V) forms tested here), it was not sensitive to the other metals and did not respond even if induced by them ($p<0.05$) (Kim et al., 2020). Of all tested metals, only As (III) and As (V) showed high fluorescence response. This result can be explained that arsR protein consist of a strikingly high – affinity ligand and a very specific binding site toward arsenic and it can differentiate arsenic than other metals (Kostal et al., 2004). Arsenic bioreporters induced by Ag (I), Fe

(II) and Ni (II) metals did not emit fluorescent signal, although they remained viable (87.2%, 96.5% and 97.8%), respectively. According to the results, it has been proven that the bioreporters maintain their viability, but the arsR protein in its structure does not react to metals used other than arsenic forms (Elcin & Öktem, 2020a).

3.3.3 Response Time

The results of the experiment conducted to determine the response time of the immobilized bioreporter system to different concentrations of As (III) are given in Figure 3.27. The detection limit was decided using statistically significant changes ($p < 0.05$) in fluorescence signal values compared with fluorescent signal value emitted from the control group. According to the One – way ANOVA result for M9 minimal medium, it was found that a response could be obtained 3.5 hours after the PCL – IB group induced by all As (III) concentrations tested compared to the initial ($p<0.05$). These results showed us that the As (III) detection limit of the developed PCL – IB system was initially higher but decreased over time. In addition, GFP tends to accumulate in the cell (Leveau & Lindow, 2002), so it can be deduced from the Figure 3.27, as well as the recorded fluorescent signal value increased continuously over time. Fluorescent signals from the no metal – induced PCL – IB system did not have any statistically significant changes over time ($p>0.05$). It was mentioned that so as to short response time besides high growth rates under aerobic and/or anaerobic conditions bacterial cells may be utilized as biosensors (Roointan et al., 2015). Since there is no study in the literature where we can compare the detection limit of immobilized bioreporters used in metal detection within the scope of the thesis, previous studies with free bioreporters are given below and the superior to or equal of immobilized bioreporters to free bioreporters is shown. This difference in response time between immobilized and non-immobilized bioreporters can be attributed to direct exposure of free bioreporters to the changing environment so that immobilized bioreporters can better tolerate environmental stresses (such as nutrient depletion and pH changes) (Ge et al., 2017; Hussain et al., 2017).

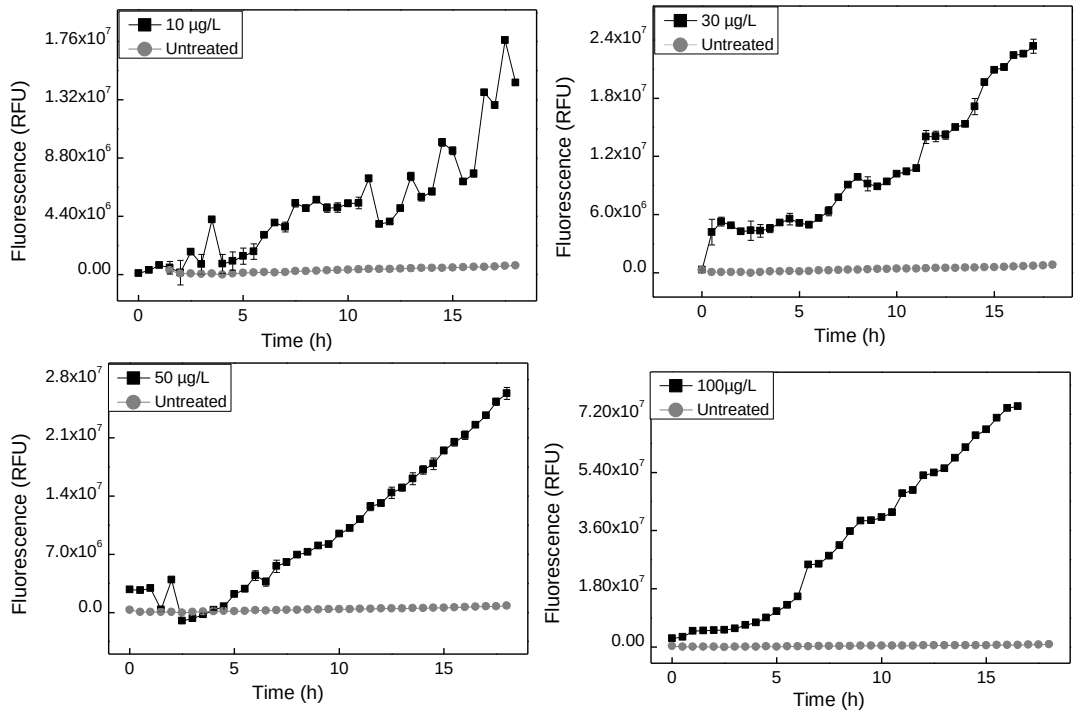

Figure 3.27 Fluorescence emission kinetics of immobilized arsenic bioreporter cells in response to As (III). Bioreporter cells grown in M9 minimal medium (till mid – exponential growth phase, OD_{600}=0.5) induced with various As (III) concentrations.

Liao and Ou developed an *E. coli* whole cell biosensor based on GFP expression of *Staphylococcus aureus* plasmid pI258 under the control of *ars*R gene in order to detect As (III), As (V) and Sb (III). In their studies conducted in LB medium, it was reported that the engineered biosensor could detect 30 µg/L As (III) and 75 µg/L As (V) concentrations after 2 hours of induction, and when the induction time was increased to 8 hours, the lowest detection limit for As (III) and As (V) was 7.5 µg/L (Liao & Ou, 2005). In our study, the limit of detection (LOD) concentration of the developed PCL – IB system was 10 µg/L As (III), and it was able to detect arsenic at this concentration after 3.5 hours. When induced with a concentration of 30 µg/L As (III), the response time decreased to 1.5 hours. Tani et al. designed a whole – cell biosensor to detect As (III) using *E. coli* DH5α. The biosensor they developed reduced the detection limit from 20 µg/L to 7.5 µg/L As (III) concentration at the end of the 6–h induction period (Tani et al., 2009). The PCL– IB system developed

in the study responded after 3.5 h in the presence of 20 µg/L As (III). Theytaz et al. designed a microfluidic chip using *E. coli* DH5α and evaluated this design for its response in the presence of arsenic and recorded the fluorescent signals emitted by GFP. According to the results they gave, it was seen that the biosensor system should be induced for 90 minutes in order to detect 50 µg/L As (III) in minimal medium (Theytaz et al., 2009). According to the test results of the response time of the PCL – IB system developed in the presence of As (III), a significant response was obtained 30 minutes after it was induced with 50 µg/L As (III) according to the control group. Hu et al. introduced the reporter protein gene *phi*YFP into *E. coli* DH5a to convert it into a whole – cell biosensor. They reported that they could detect 375 µg/L As (III) and As (V) after 2 hours of induction in LB medium, according to their detection study (Hu et al., 2010). In our study, the developed PCL – IB system's detection limit was reduced from 100 µg/L to 30 µg/L As (III) concentration in a 1.5 – hour induction period.

3.3.4 Shelf – life

To determine the shelf life of the PCL – IB group, they were stored at different temperatures (+4 °C and RT) for 28 days. The fluorescence measurements of the immobilized bioreporters, which were removed from their storage environments after a certain period, were recorded at 8th hour after the PBS was removed and treated with 100 µg/L As (III). After removing the PBS buffer on the immobilized bioreporters removed from the storage media for certain periods (0 – 7 – 14 – 21 – 28 days), they were treated with 100 µg/L As (III) for 8 hours. The response of the PCL – IB system to arsenic on the first day (day 0) was accepted as 100% in terms of fluorescent signal, and the fluorescent signal values measured on other days were calculated accordingly and are given in Figure 3.28. According to Figure 3.27a, the PCL – IB system stored at +4 °C and RT for 7 days decreased by 52% and 54%, respectively, when the PCL – IB system was induced with arsenic (100 µg/L) for 8h. After continuing the experiment, the decrease in the fluorescent signals emitted by

the PCL – IB system, which was stored for 14 days, was significantly lower than that of Day7 ($p<0.05$). As expected, the fluorescent signals they emitted continued to decrease as the cells continued to be stored at RT and +4 °C. It was observed that the decrease in fluorescent signal in all groups was higher in the PCL – IB system stored at room temperature. It was thought that the reason for this was that the cells were not in an environment that limited their metabolic activities because they were not at low temperature, but that there was a decrease in their viability due to the lack of sufficient nutrients in the environment, and therefore they could not respond to the arsenic. This situation can also be explained by the adaptation of *E. coli* to its natural habitat, because when *E. coli* live in the intestines, they are not accustomed to lack of nutrient because they are fed from the host at high temperatures. However, it is thought that when they were outside at the same temperatures, they could not adapt and maintain their vitality (Nowroth et al., 2016).

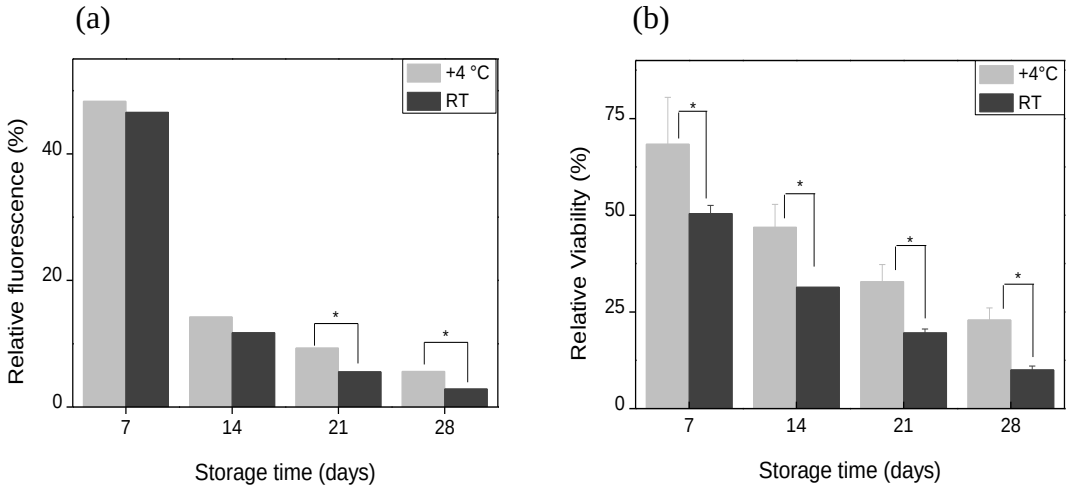

*Figure 3.28 PCL – IB system stored at different temperatures (+4 °C and RT) (a) comparative emitted fluorescent signals induced with 100 µg/L As (III) at t=8h, and (b) their relative cell viability. Bioreporter cells grown in M9 minimal medium (till stationary phase, OD_{600}=1.5), n=3, * means p<0.05, there is also a significant difference between days, 1st day (Day 0) group is accepted as control, and cell viability for the control was taken as 100%.*

The relative cell viability (%) of the PCL – IB system kept at different temperatures (+4 °C and RT) is shown in Figure 3.28b. Figure 3.28b shows that as with the

fluorescent signal values emitted by the PCL – IB system, a decrease in their viability was observed when stored for a long time. At the end of 7, 14 and 21 days, the relative cell viability of the PCL – IB system stored at room temperature was significantly lower than that those stored at +4 °C ($p<0.05$).

It is evident that the relative fluorescence value and relative viability values did not decrease to the same extent (for example, in Day7 the relative fluorescence value was 34.56% (Figure 3.28a) while the relative viability was 46.87%, (Figure 3.28b)). It was thought that the reason for this might be that they aim to increase the survival time of the cells by reducing their metabolic activities (Nowroth et al., 2016). In addition, lyophilization of immobilized bioreporters and keeping them at – 80 °C using cryoprotectants can be tried to improve shelf life. Although cryoprotectants are used to protect cells from damage caused by freeze – drying and to increase cell viability, they have the feature of being easily removed. In other words, non – toxic and biocompatible cryoprotectants help to prevent/reduce the damage caused by ice crystallization, membrane rupture and/or osmotic pressure to the cells desired to be stored at deep cryogenic temperatures (Kar et al., 2019; Nguyen et al., 2020). Cryoprotectants to be used in freeze – drying bacteria can be dimethyl sulfoxide (Bağiş et al., 2005; Goss, 2013), ethylene glycol (Chi et al., 2002; Kresna et al., 2019), glycerol (Kim et al., 2009), polyethylene glycol (Bhattacharya, 2018; Mi et al., 2004), skimmilk (Cody et al., 2008; Saeki et al., 2015), sucrose (Jong-Bum et al., 2004), and trehalose (Li & Ricke, 2004).

Since no study was found about the fluorescence signal value and viability of immobilized bioreporters similar to the one in our study, we compared our results on the viability of free bacterial cells and immobilized bacterial cells. In the study of Suo et al., *Salmonella typhimurium* cells immobilized on CFA/I fimbriae were able to survive for 6 h in PBS buffer at +4 °C. In addition, it has been stated that immobilized cells stored in growth medium at +4 °C can last for at least 3 days and that they can survive even longer if growth factors are present in the medium (Suo et al., 2008). Since the viability of the immobilized bioreporters we developed was investigated instead of dividing and multiplying, they were kept in PBS buffer, not

in the growth medium, and they were able to survive similar to this study. Fung et al. reported that *Lactobacillus acidophilus* bacteria encapsulated into electrospun dietary fibers obtained from organic wastes maintain their viability for up to 21 days when stored at +4 °C. It was stated that bacteria encapsulated in OPF nanofiber remained viable at a rate of 72% after 21 days, but bacteria encapsulated in OPT and okara nanofibers maintained their viability at a rate of 49% and 37.7%, respectively (Fung et al., 2011). In our study, however, it was observed that the bacteria were more affected by environmental factors (Nussinovitch, 1997), and their viability decreased by 32.8%, since the bacteria were not encapsulated but were immobilized by adsorption method. Since this study is different from our immobilization method, it is very difficult to compare our results.

Sarioglu et al. immobilized STB5 bacterial cells on PS and PSU by adsorption method. They then stored these immobilized STB5/PS and STB5/PSU biocomposites in a closed moist environment at +4 °C for 15 days. They stated that at the end of 15 days, STB5/PS and STB5/PSU retained their Cr (VI) removal capacity about 72.79% and 68.65%, respectively (Sarioglu et al., 2016). Since the value that we will compare with this study in our study did not match, when looked in terms of viability, it is seen that its vitality is 46.87% at +4 °C at the end of 15 days, that is, it maintains its vitality.

Jayani et al. immobilized *Lactobacillus acidophilus* 016 probiotic bacterial cells to bacterial cellulose electrospun fibers in their study. When they tested the storage conditions, they stated that these immobilized bacteria remained viable (71.1 %) for up to 24 days at 35°C (Jayani et al., 2020). *Lactobacillus acidophilus* probiotic bacteria is known to be more resistant to high temperatures than *E. coli*. It is suggested that this is due to the fact that *Lactobacillus acidophilus* is a Gram – positive bacteria, that is, because it has a thicker cell wall. In addition, it has been reported that the presence or absence of nutrients in the environment at high temperature has a large share in affecting the viability of the cells (Kiepś & Dembczyński, 2022). In our study, it was observed that the viability of immobilized

bacteria stored in PBS at room temperature had 19.55% vitality at the end of the 21st day.

CHAPTER 4

CONCLUSION

In this thesis, a biosensor system was developed by immobilizing bioreporters on the electrospun fiber mats to detect arsenic in aqueous media for the first time in the literature. Firstly, CA and PCL fibers were fabricated via electrospinning technique as the support material and compared. Immobilization studies conducted with arsenic bioreporters at different concentrations and different growth phases on CA and PCL electrospun membranes showed that PCL – IB group was found more suitable substrate for the biosensor.

Selectivity, sensitivity, response time and shelf life tests, in which the performance of the PCL – IB system as a biosensor was tested, showed that the biosensor system was able to detect the required arsenic concentration in safe drinking water limits. PCL – IB system does not respond to other metals tested, but only to arsenic forms.

The results of this study will lead to further research to develop a fast, specific, and cost – effective mobile biosensor device for the detection of arsenic content in environmental specimens. They can be used by anyone for on – site applications to figure out the bioavailable content of the heavy metal along with its forms, giving an idea of the toxicity risk and planning of treatment or removal processes.

REFERENCES

Abrigo, M., Kingshott, P., & McArthur, S. L. (2015). Electrospun polystyrene fiber diameter influencing bacterial attachment, proliferation, and growth. *ACS Applied Materials & Interfaces*, *7*(14), 7644–7652. https://doi.org/10.1021/acsami.5b00453

Ahmad, A., & Bhattacharya, P. (2019). Arsenic in Drinking Water: Is 10 μg/L a Safe Limit? *Current Pollution Reports*, *5*(1), 1–3. https://doi.org/10.1007/s40726-019-0102-7

Ali, H., Khan, E., & Ilahi, I. (2019). Environmental Chemistry and Ecotoxicology of Hazardous Heavy Metals: Environmental Persistence, Toxicity, and Bioaccumulation. *Journal of Chemistry*, *2019*, 6730305. https://doi.org/10.1155/2019/6730305

Almeida, R. M., & Marques, A. C. (2016). *Characterization of Sol–Gel Materials by Infrared Spectroscopy BT - Handbook of Sol-Gel Science and Technology* (L. Klein, M. Aparicio, & A. Jitianu (Eds.); pp. 1–31). Springer International Publishing. https://doi.org/10.1007/978-3-319-19454-7_33-1

Anitha, S., Balusamy, B., Celebioglu, A., & Uyar, T. (2016). Nanotraps in porous electrospun fibers for effective removal of lead(II) in water. *J. Mater. Chem. A*, *4*. https://doi.org/10.1039/C5TA09166G

Anonim. (2005). İnsani Tüketim Amaçlı Sular Hakkındaki Yönetmenlik. *Resmi Gazete*, 1–68.

Aquisman, A. E., Assim, Z. Bin, Wahi, R. B., Kwabena, D. E., & Festus, W. (2019). Validation of the Atomic Absorption Spectroscopy (AAS) for Heavy Metal Analysis and Geochemical Exploration of Sediment Samples from the Sebangan River. *Advances in Analytical Chemistry*, *2019*(2), 23–33. https://doi.org/10.5923/j.aac.20190902.01

Arik, N., Horzum, N., & Truong, Y. B. (2022). Development and Characterizations of Engineered Electrospun Bio-Based Polyurethane Containing Essential Oils.

Membranes, *12*(2), 1–16. https://doi.org/10.3390/membranes12020209

Arsenic, metals, fibres, and dusts. (2012). *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, *100*(Pt C), 11–465.

Authman, M., Zaki, M., Khallaf, E., & Abbas, H. (2015). Use of Fish as Bio-indicator of the Effects of Heavy Metals Pollution. *J Aquaculture Research and Development, J Aquacult*, 328. https://doi.org/10.4172/2155-9546.1000328

Azadi, F., Saadat, S., & Karimi-Jashni, A. (2018). Experimental Investigation and Modeling of Nickel Removal from Wastewater Using Modified Rice Husk in Continuous Reactor by Response Surface Methodology. *Iranian Journal of Science and Technology, Transactions of Civil Engineering*, *42*(3), 315–323. https://doi.org/10.1007/s40996-017-0090-z

Azizi, M., Azimzadeh, M., Afzali, M., Alafzadeh, M., & Mirhosseini, S. (2018). *Characterization and optimization of using calendula officinalis extract in fabrication of polycaprolactone-gelatin electrospun nanofibers for wound dressing applications*. *6*, 31–38.

B'Hymer, C., & Caruso, J. A. (2004). Arsenic and its speciation analysis using high-performance liquid chromatography and inductively coupled plasma mass spectrometry. *Journal of Chromatography. A*, *1045*(1–2), 1–13. https://doi.org/10.1016/j.chroma.2004.06.016

Bagbi, Y., Pandey, A., & Solanki, P. R. (2019). Chapter 10 - Electrospun Nanofibrous Filtration Membranes for Heavy Metals and Dye Removal. In S. Thomas, D. Pasquini, S.-Y. Leu, & D. A. B. T.-N. M. in W. P. Gopakumar (Eds.), *Micro and Nano Technologies* (pp. 275–288). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-12-813926-4.00015-X

Bağiş, H., Odaman Mercan, H., & Kumtepe, Y. (2005). Effect of three different cryoprotectant solutions in solid surface vitrification (SSV) techniques on the development rate of vitrified pronuclear-stage mouse embryos. *Turkish Journal of Veterinary and Animal Sciences*, *29*(3), 621–627.

Bahnasawy, M., Khidr, A.-A., & Dheina, N. (2011). Assessment of heavy metal concentrations in water, plankton, and fish of Lake Manzala, Egypt. *Turkish Journal of Zoology*, *35*, 271–280. https://doi.org/10.3906/zoo-0810-6

Balali-Mood, M., Naseri, K., Tahergorabi, Z., Khazdair, M. R., & Sadeghi, M. (2021). Toxic Mechanisms of Five Heavy Metals: Mercury, Lead, Chromium, Cadmium, and Arsenic. *Frontiers in Pharmacology*, *12*. https://doi.org/10.3389/fphar.2021.643972

Bansal, M., Garg, U., Singh, D., & Garg, V. K. (2009). Removal of Cr(VI) from aqueous solutions using pre-consumer processing agricultural waste: A case study of rice husk. *Journal of Hazardous Materials*, *162*(1), 312–320. https://doi.org/10.1016/J.JHAZMAT.2008.05.037

Bansal, O. P. (2020). Health Risks of Potentially Toxic Metals Contaminated Water. In J. K. Nduka & M. N. Rashed (Eds.), *Heavy Metal Toxicity in Public Health*. IntechOpen. https://doi.org/10.5772/intechopen.92141

Baumann, B., & van der Meer, J. R. (2007). Analysis of Bioavailable Arsenic in Rice with Whole Cell Living Bioreporter Bacteria. *Journal of Agricultural and Food Chemistry*, *55*(6), 2115–2120. https://doi.org/10.1021/jf0631676

Bayat, Z., Hassanshahian, M., & Cappello, S. (2015). Immobilization of Microbes for Bioremediation of Crude Oil Polluted Environments: A Mini Review. *The Open Microbiology Journal*, *9*, 48–54. https://doi.org/10.2174/1874285801509010048

Berillo, D., Al-Jwaid, A., & Caplin, J. (2021). Polymeric Materials Used for Immobilisation of Bacteria for the Bioremediation of Contaminants in Water. *Polymers*, *13*(7). https://doi.org/10.3390/polym13071073

Bhalla, N., Jolly, P., Formisano, N., & Estrela, P. (2016). Introduction to biosensors. *Essays in Biochemistry*, *60*(1), 1–8. https://doi.org/10.1042/EBC20150001

Bhattacharya, S. (2018). *Cryoprotectants and Their Usage in Cryopreservation Process* (Y. Bozkurt (Ed.); p. Ch. 2). IntechOpen.

https://doi.org/10.5772/intechopen.80477

Binkley, J., & Simpson, J. A. (2003). *35 - Heavy metals in wastewater treatment processes* (D. Mara & N. B. T.-H. of W. and W. M. Horan (Eds.); pp. 597–610). Academic Press. https://doi.org/https://doi.org/10.1016/B978-012470100-7/50036-4

Bjerketorp, J., Håkansson, S., Belkin, S., & Jansson, J. K. (2006). Advances in preservation methods: keeping biosensor microorganisms alive and active. *Current Opinion in Biotechnology*, *17*(1), 43–49. https://doi.org/https://doi.org/10.1016/j.copbio.2005.12.005

Borra, R. C., Lotufo, M. A., Gagioti, S. M., Barros, F. de M., & Andrade, P. M. (2009). A simple method to measure cell viability in proliferation and cytotoxicity assays. *Brazilian Oral Research*, *23*(3), 255–262. https://doi.org/10.1590/s1806-83242009000300006

Bousse, L. (1996). Whole cell biosensors. *Sensors and Actuators, B: Chemical*, *34*(1–3), 270–275. https://doi.org/10.1016/S0925-4005(96)01906-5

Bräuner, E. V., Nordsborg, R. B., Andersen, Z. J., Tjønneland, A., Loft, S., & Raaschou-Nielsen, O. (2014). Long-term exposure to low-level arsenic in drinking water and diabetes incidence: a prospective study of the diet, cancer and health cohort. *Environmental Health Perspectives*, *122*(10), 1059–1065. https://doi.org/10.1289/ehp.1408198

Brovko, L. (2010). The Family of Fluorescent Proteins. *Bioluminescence and Fluorescence for In Vivo Imaging*, 23–42. https://doi.org/10.1117/3.862866.ch2

Bruinsma, G. M., van der Mei, H., & Busscher, H. (2002). Bacterial adhesion to surface hydrophilic and hydrophobic contact lenses. *Biomaterials*, *22*, 3217–3224. https://doi.org/10.1016/S0142-9612(01)00159-4

Brutesco, C., Prévéral, S., Escoffier, C., Descamps, E. C. T., Prudent, E., Cayron, J., Dumas, L., Ricquebourg, M., Adryanczyk-Perrier, G., de Groot, A., Garcia, D.,

Rodrigue, A., Pignol, D., & Ginet, N. (2017). Bacterial host and reporter gene optimization for genetically encoded whole cell biosensors. *Environmental Science and Pollution Research International*, *24*(1), 52–65. https://doi.org/10.1007/s11356-016-6952-2

Cardoso, D. R., Libardi, S. H., & Skibsted, L. H. (2012). Riboflavin as a photosensitizer. Effects on human health and food quality. *Food Funct.*, *3*(5), 487–502. https://doi.org/10.1039/C2FO10246C

Cavalca, L., Corsini, A., Zaccheo, P., Andreoni, V., & Muyzer, G. (2013). Microbial transformations of arsenic: perspectives for biological removal of arsenic from water. *Future Microbiology*, *8*(6), 753–768. https://doi.org/10.2217/fmb.13.38

Cerminati, S., Soncini, F., & Checa, S. (2015). A sensitive whole-cell biosensor for the simultaneous detection of a broad-spectrum of toxic heavy metal ions. *Chem. Commun.*, *51*. https://doi.org/10.1039/C5CC00981B

Chambers, J., Arulanandam, B., Matta, L., Weis, A., & Valdes, J. (2008). Biosensor recognition elements. *Current Issues in Molecular Biology*, *10*, 1–12. https://doi.org/10.1007/978-0-387-75936-4_1

Champigneux, P., Delia, M.-L., & Bergel, A. (2018). Impact of electrode micro- and nano-scale topography on the formation and performance of microbial electrodes. *Biosensors and Bioelectronics*, *118*, 231–246. https://doi.org/https://doi.org/10.1016/j.bios.2018.06.059

Chapman, P. M. (2007). Determining when contamination is pollution — Weight of evidence determinations for sediments and effluents. *Environment International*, *33*(4), 492–501. https://doi.org/https://doi.org/10.1016/j.envint.2006.09.001

Chen, M., Patra, P. K., Lovett, M. L., Kaplan, D. L., & Bhowmick, S. (2009). Role of electrospun fibre diameter and corresponding specific surface area (SSA) on cell attachment. *Journal of Tissue Engineering and Regenerative Medicine*, *3*(4), 269–279. https://doi.org/10.1002/term.163

Chen, P.-H., Lin, C., Guo, K.-H., & Yeh, Y.-C. (2017). Development of a pigment-based whole-cell biosensor for the analysis of environmental copper. *RSC Adv.*, *7*(47), 29302–29305. https://doi.org/10.1039/C7RA03778C

Chen, Y.-E., Wu, N., Zhang, Z.-W., Yuan, M., & Yuan, S. (2019). Perspective of Monitoring Heavy Metals by Moss Visible Chlorophyll Fluorescence Parameters . In *Frontiers in Plant Science* (Vol. 10). https://www.frontiersin.org/article/10.3389/fpls.2019.00035

Chi, H.-J., Koo, J.-J., Kim, M.-Y., Joo, J.-Y., Chang, S.-S., & Chung, K.-S. (2002). Cryopreservation of human embryos using ethylene glycol in controlled slow freezing. *Human Reproduction (Oxford, England)*, *17*(8), 2146–2151. https://doi.org/10.1093/humrep/17.8.2146

Chong, P., Erable, B., & Bergel, A. (2019). Effect of pore size on the current produced by 3-dimensional porous microbial anodes: A critical review. *Bioresource Technology*, *289*, 121641. https://doi.org/https://doi.org/10.1016/j.biortech.2019.121641

Chu, Y.-F., Hsu, C.-H., Soma, P. K., & Lo, Y. M. (2009). Immobilization of bioluminescent Escherichia coli cells using natural and artificial fibers treated with polyethyleneimine. *Bioresource Technology*, *100*(13), 3167–3174. https://doi.org/10.1016/j.biortech.2009.01.072

Çiftçi, H., Çalışkan, Ç. E., Aslanhan, E., & Aktoklu, E. (2021). Monitoring of heavy metal pollution by using populus nigra and cedrus libani. *Journal of Engineering and Natural Sciences – Sigma Mühendislik ve Fen Bilimleri Dergis*, *39*(December), 367–373. https://doi.org/10.14744/sigma.2021.00025

Clark Jr., L. C., & Lyons, C. (1962). Electrode systems for continuous monitoring in cardiovascular surgery. *Annals of the New York Academy of Sciences*, *102*(1), 29–45. https://doi.org/https://doi.org/10.1111/j.1749-6632.1962.tb13623.x

Cody, W., Wilson, J., Hendrixson, D., Mciver, K., Hagman, K., Ott, C., Nickerson, C., & Schurr, M. (2008). Skim Milk Enhances the Preservation of Thawed -80°C Bacterial Stocks. *Journal of Microbiological Methods*, *75*, 135–138.

https://doi.org/10.1016/j.mimet.2008.05.006

Cosansu, S., & Ayhan, K. (2003). Bacterial bioluminescence and its applications. *Journal of Gıda*, *28*, 69–75.

Costello, A., Parker, J., Clynes, M., & Murphy, R. (2020). Development of whole-cell and cell-free biosensors for the detection and differentiation of organic and inorganic forms of copper. *Metallomics*, *12*(11), 1729–1734. https://doi.org/10.1039/D0MT00146E

Cui, R., Kwak, J. Il, & An, Y.-J. (2017). A novel method for preventing surface film entrapment of water fleas and its application for toxicity testing with heavy metals. *Environmental Science and Pollution Research*, *24*. https://doi.org/10.1007/s11356-016-8091-1

D'Souza, S. F. (2001). Microbial biosensors. *Biosensors and Bioelectronics*, *16*(6), 337–353. https://doi.org/https://doi.org/10.1016/S0956-5663(01)00125-7

Darma, A., Yang, J., Zandi, P., Liu, J., Możdżeń, K., Xia, X., Sani, A., Wang, Y., & Schnug, E. (2022). Significance of Shewanella Species for the Phytoavailability and Toxicity of Arsenic—A Review. *Biology*, *11*(3), 472. https://doi.org/10.3390/biology11030472

Daunert, S., Barrett, G., Feliciano, J. S., Shetty, R. S., Shrestha, S., & Smith-Spencer, W. (2000). Genetically engineered whole-cell sensing systems: coupling biological recognition with reporter genes. *Chemical Reviews*, *100*(7), 2705–2738. https://doi.org/10.1021/cr990115p

De Cesare, F., Di Mattia, E., Zussman, E., & Macagnano, A. (2019). A study on the dependence of bacteria adhesion on the polymer nanofibre diameter. *Environmental Science: Nano*, *6*(3), 778–797. https://doi.org/10.1039/c8en01237g

Diep, E., & Schiffman, J. (2021). Encapsulating bacteria in alginate-based electrospun nanofibers. *Biomaterials Science*, *9*. https://doi.org/10.1039/D0BM02205E

Dincer, C., Bruch, R., Costa-Rama, E., Fernández-Abedul, M. T., Merkoçi, A., Manz, A., Urban, G. A., & Güder, F. (2019). Disposable Sensors in Diagnostics, Food, and Environmental Monitoring. *Advanced Materials*, *31*(30), 1806739. https://doi.org/https://doi.org/10.1002/adma.201806739

Edmundson, M. C., & Horsfall, L. (2015). Construction of a Modular Arsenic-Resistance Operon in E. coli and the Production of Arsenic Nanoparticles . In *Frontiers in Bioengineering and Biotechnology* (Vol. 3). https://www.frontiersin.org/articles/10.3389/fbioe.2015.00160

Eilenberger, C., Kratz, S. R. A., Rothbauer, M., Ehmoser, E. K., Ertl, P., & Küpcü, S. (2018). Optimized alamarBlue assay protocol for drug dose-response determination of 3D tumor spheroids. *MethodsX*, *5*, 781–787. https://doi.org/10.1016/j.mex.2018.07.011

Elcin, E. (2019). *Development of fluorescent whole-cell bacterial bioreporters for detection of inorganic arsenic and cadmium*. Middle East Technical University (Tez no 577910).

Elcin, E., & Öktem, H. A. (2020a). Immobilization of fluorescent bacterial bioreporter for arsenic detection. *Journal of Environmental Health Science and Engineering*. https://doi.org/10.1007/s40201-020-00447-2

Elcin, E., & Öktem, H. A. (2020b). Inorganic Cadmium Detection Using a Fluorescent Whole-Cell Bacterial Bioreporter. *Analytical Letters*, *53*(17), 2715–2733. https://doi.org/10.1080/00032719.2020.1755867

Eltzov, E., Yehuda, A., & Marks, R. (2015). Creation of a new portable biosensor for water toxicity determination. *Sensors and Actuators B Chemical*, *221*. https://doi.org/10.1016/j.snb.2015.06.153

Elzein, T., Nasser-Eddine, M., Delaite, C., Bistac, S., & Dumas, P. (2004). FTIR study of polycaprolactone chain organization at interfaces. *Journal of Colloid and Interface Science*, *273*(2), 381–387. https://doi.org/10.1016/j.jcis.2004.02.001

Farré, M., Brix, R., & Barceló, D. (2005). Screening water for pollutants using biological techniques under European Union funding during the last 10 years. *TrAC Trends in Analytical Chemistry*, *24*(6), 532–545. https://doi.org/https://doi.org/10.1016/j.trac.2005.03.008

Feng, X., Castracane, J., Tokranova, N., Gracias, A., Lnenicka, G., & Szaro, B. G. (2007). A living cell-based biosensor utilizing G-protein coupled receptors: Principles and detection methods. *Biosensors and Bioelectronics*, *22*(12), 3230–3237. https://doi.org/https://doi.org/10.1016/j.bios.2007.03.002

Ferguson, J. F., & Gavis, J. (1972). A review of the arsenic cycle in natural waters. *Water Research*, *6*(11), 1259–1274. https://doi.org/https://doi.org/10.1016/0043-1354(72)90052-8

Fernández, P., Sommer, I., Cram, S., Rosas, I., & Gutiérrez, M. (2005). The influence of water-soluble As(III) and As(V) on dehydrogenase activity in soils affected by mine tailings. *Science of The Total Environment*, *348*(1), 231–243. https://doi.org/https://doi.org/10.1016/j.scitotenv.2004.12.065

Fong, H., Chun, I., & Reneker, D. H. (1999). Beaded nanofibers formed during electrospinning. *Polymer*, *40*(16), 4585–4592. https://doi.org/https://doi.org/10.1016/S0032-3861(99)00068-3

Francisco, R., Branco, R., Schwab, S., Baldani, I., & Morais, P. V. (2019). Two plant-hosted whole-cell bacterial biosensors for detection of bioavailable Cr(VI). *World Journal of Microbiology and Biotechnology*, *35*(8), 129. https://doi.org/10.1007/s11274-019-2703-0

Frick, H., Tardif, S., Kandeler, E., Holm, P. E., & Brandt, K. K. (2019). Assessment of biochar and zero-valent iron for in-situ remediation of chromated copper arsenate contaminated soil. *Science of The Total Environment*, *655*, 414–422. https://doi.org/https://doi.org/10.1016/j.scitotenv.2018.11.193

Friedlander, R. S., Vogel, N., & Aizenberg, J. (2015). Role of Flagella in Adhesion of Escherichia coli to Abiotic Surfaces. *Langmuir : The ACS Journal of Surfaces and Colloids*, *31*(22), 6137–6144.

https://doi.org/10.1021/acs.langmuir.5b00815

Fujimoto, H., Wakabayashi, M., Yamashiro, H., Maeda, I., Isoda, K., Kondoh, M., Kawase, M., Miyasaka, H., & Yagi, K. (2006). Whole-cell arsenite biosensor using photosynthetic bacterium Rhodovulum sulfidophilum. Rhodovulum sulfidophilum as an arsenite biosensor. *Applied Microbiology and Biotechnology*, *73*(2), 332–338. https://doi.org/10.1007/s00253-006-0483-6

Fung, W.-Y., Yuen, K.-H., & Liong, M.-T. (2011). Agrowaste-Based Nanofibers as a Probiotic Encapsulant: Fabrication and Characterization. *Journal of Agricultural and Food Chemistry*, *59*(15), 8140–8147. https://doi.org/10.1021/jf2009342

Garbinski, L. D., Rosen, B. P., & Chen, J. (2019). Pathways of arsenic uptake and efflux. *Environment International*, *126*, 585–597. https://doi.org/10.1016/j.envint.2019.02.058

Garcia, E., Shinde, R., Martinez, S., Kaushik, A., Chand, H. S., Nair, M., & Jayant, R. D. (2019). Chapter 12 - Cell-Line-Based Studies of Nanotechnology Drug-Delivery Systems: A Brief Review. In S. S. Mohapatra, S. Ranjan, N. Dasgupta, R. K. Mishra, & S. B. T.-N. for D. D. Thomas (Eds.), *Micro and Nano Technologies* (pp. 375–393). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-12-814033-8.00012-6

Gaveau, A., Coetsier, C., Roques, C., Bacchin, P., Dague, E., & Causserand, C. (2017). Bacteria transfer by deformation through microfiltration membrane. *Journal of Membrane Science*, *523*, 446–455. https://doi.org/https://doi.org/10.1016/j.memsci.2016.10.023

Gavrilaș, S., Ursachi, C. Ștefan, Perța-Crișan, S., & Munteanu, F.-D. (2022). Recent Trends in Biosensors for Environmental Quality Monitoring. *Sensors (Basel, Switzerland)*, *22*(4), 1513. https://doi.org/10.3390/s22041513

Ge, X., Yang, L., & Xu, J. (2017). Cell Immobilization: Fundamentals, Technologies, and Applications. In *Industrial Biotechnology* (pp. 205–235). https://doi.org/https://doi.org/10.1002/9783527807833.ch7

Genchi, G., Sinicropi, M. S., Lauria, G., Carocci, A., & Catalano, A. (2020). The Effects of Cadmium Toxicity. *International Journal of Environmental Research and Public Health*, *17*(11). https://doi.org/10.3390/ijerph17113782

Gensheimer, M., Becker, M., Brandis-Heep, A., Wendorff, J. H., Thauer, R. K., & Greiner, A. (2007). Novel Biohybrid Materials by Electrospinning: Nanofibers of Poly(ethylene oxide) and Living Bacteria. *Advanced Materials*, *19*(18), 2480–2482. https://doi.org/https://doi.org/10.1002/adma.200602936

Gordegir, M., Oz, S., Yezer, I., Buhur, M., Unal, B., & Demirkol, D. O. (2019). Cells-on-nanofibers: Effect of polyethyleneimine on hydrophobicity of poly-Ɛ-caprolacton electrospun nanofibers and immobilization of bacteria. *Enzyme and Microbial Technology*, *126*, 24–31. https://doi.org/10.1016/j.enzmictec.2019.03.002

Gorini, F., Muratori, F., & Morales, M. A. (2014). The Role of Heavy Metal Pollution in Neurobehavioral Disorders: a Focus on Autism. *Review Journal of Autism and Developmental Disorders*, *1*(4), 354–372. https://doi.org/10.1007/s40489-014-0028-3

Goss, C. A. (2013). *Chapter 43 - Frozen Blood Products* (B. H. Shaz, C. D. Hillyer, M. Roshal, & C. S. B. T.-T. M. and H. (Second E. Abrams (Eds.); pp. 283–286). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-12-397164-7.00043-4

Griffiths, P. R. (2007). *Introduction to vibrational spectroscopy*. *1*, 33–43.

Grzywaczyk, A., Zdarta, A., Jankowska, K., Biadasz, A., Zdarta, J., Jesionowski, T., Kaczorek, E., & Smułek, W. (2021). New Biocomposite Electrospun Fiber/Alginate Hydrogel for Probiotic Bacteria Immobilization. In *Materials* (Vol. 14, Issue 14). https://doi.org/10.3390/ma14143861

Gui, Q., Lawson, T., Shan, S., Yan, L., & Liu, Y. (2017). The application of whole cell-based biosensors for use in environmental analysis and in medical diagnostics. *Sensors (Switzerland)*, *17*(7), 1–17. https://doi.org/10.3390/s17071623

Gupta, N., Renugopalakrishnan, V., Liepmann, D., Paulmurugan, R., & Malhotra, B. D. (2019). Cell-based biosensors: Recent trends, challenges and future perspectives. *Biosensors and Bioelectronics*, *141*, 111435. https://doi.org/https://doi.org/10.1016/j.bios.2019.111435

Gutiérrez, J. C., Amaro, F., & Martín-González, A. (2015). Heavy metal whole-cell biosensors using eukaryotic microorganisms: an updated critical review. *Frontiers in Microbiology*, *6*, 48. https://doi.org/10.3389/fmicb.2015.00048

Hamadi, F., Latrache, H., Zahir, H., Elghmari, A., Timinouni, M., & Ellouali, M. (2008). The relation between Escherichia coli surface functional groups' composition and their physicochemical properties. *Brazilian Journal of Microbiology : [Publication of the Brazilian Society for Microbiology]*, *39*(1), 10–15. https://doi.org/10.1590/S1517-83822008000100003

Hemmatian, T., Lee, H., & Kim, J. (2021). Bacteria Adhesion of Textiles Influenced by Wettability and Pore Characteristics of Fibrous Substrates. *Polymers*, *13*(2). https://doi.org/10.3390/polym13020223

Hirano, S. (2020). Biotransformation of arsenic and toxicological implication of arsenic metabolites. *Archives of Toxicology*, *94*(8), 2587–2601. https://doi.org/10.1007/s00204-020-02772-9

Hirsch, E., Pantea, E., Vass, P., Domján, J., Molnár, M., Suhajda, Á., Andersen, S. K., Vigh, T., Verreck, G., Marosi, G. J., & Nagy, Z. K. (2021). Probiotic bacteria stabilized in orally dissolving nanofibers prepared by high-speed electrospinning. *Food and Bioproducts Processing*, *128*, 84–94. https://doi.org/https://doi.org/10.1016/j.fbp.2021.04.016

Hong, Y. (2016). *19 - Electrospun fibrous polyurethane scaffolds in tissue engineering* (S. L. Cooper & J. B. T.-A. in P. B. Guan (Eds.); pp. 543–559). Woodhead Publishing. https://doi.org/https://doi.org/10.1016/B978-0-08-100614-6.00019-6

Horzum, N., Arik, N., & Truong, Y. B. (2017). Nanofibers for fiber-reinforced composites. In *Fiber Technology for Fiber-Reinforced Composites* (pp. 251–

275). Woodhead Publishing.

Hu, Q., Li, L., Wang, Y., Zhao, W., Qi, H., & Zhuang, G. (2010). Construction of WCB-11: A novel phiYFP arsenic-resistant whole-cell biosensor. *Journal of Environmental Sciences (China)*, *22*, 1469–1474. https://doi.org/10.1016/S1001-0742(09)60277-1

Hussain, A., Kangwa, M., & Fernandez-Lahore, M. (2017). Comparative analysis of stirred catalytic basket bio-reactor for the production of bio-ethanol using free and immobilized Saccharomyces cerevisiae cells. *AMB Express*, *7*(1), 158. https://doi.org/10.1186/s13568-017-0460-8

Hussain, F., Eom, H., Ali Toor, U., Lee, C. S., & Oh, S.-E. (2021). Rapid assessment of heavy metal-induced toxicity in water using micro-algal bioassay based on photosynthetic oxygen evolution. *Environmental Engineering Research*, *26*(6), 200390–200391. https://doi.org/10.4491/eer.2020.391

Ibrahim, M. M., Fahmy, T. Y. A., Salaheldin, E. I., Mobarak, F., Youssef, M. A., & Mabrook, M. R. (2015). Role of tosyl cellulose acetate as potential carrier for controlled drug release. *Life Science Journal*, *12*(10), 127–133. https://doi.org/10.7537/marslsj121015.16

Ivask, A., Rõlova, T., & Kahru, A. (2009). A suite of recombinant luminescent bacterial strains for the quantification of bioavailable heavy metals and toxicity testing. *BMC Biotechnology*, *9*(1), 41. https://doi.org/10.1186/1472-6750-9-41

Jadoon, S., & Malik, A. (2017). DNA Damage by Heavy Metals in Animals and Human Beings: An Overview. *Biochemistry & Pharmacology: Open Access*, *06*(03). https://doi.org/10.4172/2167-0501.1000235

Jayani, T., Sanjeev, B., Marimuthu, S., & Uthandi, S. (2020). Bacterial Cellulose Nano Fiber (BCNF) as carrier support for the immobilization of probiotic, Lactobacillus acidophilus 016. *Carbohydrate Polymers*, *250*, 116965. https://doi.org/https://doi.org/10.1016/j.carbpol.2020.116965

Jia, X., Bu, R., Zhao, T., & Wu, K. (2019). Sensitive and Specific Whole-Cell

Biosensor for Arsenic Detection. *Applied and Environmental Microbiology*, *85*(11), e00694-19. https://doi.org/10.1128/AEM.00694-19

Jong-Bum, C., Yong-Woo, S., Nam-Soo, P., & Young-Man, K. (2004). Enfluence of Herbal Extract on Lactic Acid Bacteria Growth and Cryoprotectants. *The Korean Journal of Food And Nutrition*, *17*.

Kalantar-zadeh, K. (2013). *Sensors Characteristics BT - Sensors: An Introductory Course* (K. Kalantar-zadeh (Ed.); pp. 11–28). Springer US. https://doi.org/10.1007/978-1-4614-5052-8_2

Kalantari, N. (2008). Evaluation of Toxicity of Heavy Metals for Escherichia coli Growth. *Iranian Journal of Environmental Health Science & Engineering*, *5*.

Kang, Y., Lee, W., Kim, S., Jang, G., Kim, B.-G., & Yoon, Y. (2018). Enhancing the copper-sensing capability of Escherichia coli-based whole-cell bioreporters by genetic engineering. *Applied Microbiology and Biotechnology*, *102*(3), 1513–1521. https://doi.org/10.1007/s00253-017-8677-7

Kannappan, S., & Ramisetty, B. C. M. (2022). Engineered Whole-Cell-Based Biosensors: Sensing Environmental Heavy Metal Pollutants in Water—a Review. *Applied Biochemistry and Biotechnology*, *194*(4), 1814–1840. https://doi.org/10.1007/s12010-021-03734-2

Kar, M., Chourasiya, Y., Maheshwari, R., & Tekade, R. K. (2019). Chapter 2 - Current Developments in Excipient Science: Implication of Quantitative Selection of Each Excipient in Product Development. In R. K. B. T.-B. F. of D. D. Tekade (Ed.), *Advances in Pharmaceutical Product Development and Research* (pp. 29–83). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-817909-3.00002-9

Karami, H., Mousavi, M. F., Yamini, Y., & Shamsipur, M. (2004). On-line preconcentration and simultaneous determination of heavy metal ions by inductively coupled plasma-atomic emission spectrometry. *Analytica Chimica Acta*, *509*(1), 89–94. https://doi.org/10.1016/j.aca.2003.12.022

Karthikeyan, R., Wang, B., Xuan, J., Wong, J. W. C., Lee, P. K. H., & Leung, M. K. H. (2015). Interfacial electron transfer and bioelectrocatalysis of carbonized plant material as effective anode of microbial fuel cell. *Electrochimica Acta*, *157*, 314–323. https://doi.org/https://doi.org/10.1016/j.electacta.2015.01.029

Katsogiannis, K. A. G., Vladisavljević, G. T., & Georgiadou, S. (2015). Porous electrospun polycaprolactone (PCL) fibres by phase separation. *European Polymer Journal*, *69*, 284–295. https://doi.org/https://doi.org/10.1016/j.eurpolymj.2015.01.028

Khansili, N., Rattu, G., & Krishna, P. M. (2018). Label-free optical biosensors for food and biological sensor applications. *Sensors and Actuators B: Chemical*, *265*, 35–49. https://doi.org/https://doi.org/10.1016/j.snb.2018.03.004

Kiepś, J., & Dembczyński, R. (2022). Current Trends in the Production of Probiotic Formulations. *Foods (Basel, Switzerland)*, *11*(15). https://doi.org/10.3390/foods11152330

Kim, H. J., Jeong, H., & Lee, S. J. (2018). Synthetic biology for microbial heavy metal biosensors. *Analytical and Bioanalytical Chemistry*, *410*(4), 1191–1203. https://doi.org/10.1007/s00216-017-0751-6

Kim, H., Jeon, Y., Lee, W., Jang, G., & Yoon, Y. (2020). Shifting the Specificity of E. coli Biosensor from Inorganic Arsenic to Phenylarsine Oxide through Genetic Engineering. *Sensors (Basel, Switzerland)*, *20*(11). https://doi.org/10.3390/s20113093

Kim, S.-J., Park, H. W., Shin, C.-H., & Kim, C.-W. (2009). Establishment of a cryopreservation method for the industrial use of D-amino acid oxidase-overexpressing Escherichia coli. *Bioscience, Biotechnology, and Biochemistry*, *73*(2), 299–303. https://doi.org/10.1271/bbb.80507

Kimkes, T. E. P., & Heinemann, M. (2020). How bacteria recognise and respond to surface contact. *FEMS Microbiology Reviews*, *44*(1), 106–122. https://doi.org/10.1093/femsre/fuz029

King, J. M., Digrazia, P. M., Applegate, B., Burlage, R., Sanseverino, J., Dunbar, P., Larimer, F., & Sayler, G. S. (1990). Rapid, sensitive bioluminescent reporter technology for naphthalene exposure and biodegradation. *Science (New York, N.Y.)*, *249*(4970), 778–781. https://doi.org/10.1126/science.249.4970.778

Kinuthia, G. K., Ngure, V., Beti, D., Lugalia, R., Wangila, A., & Kamau, L. (2020). Levels of heavy metals in wastewater and soil samples from open drainage channels in Nairobi, Kenya: community health implication. *Scientific Reports*, *10*(1), 8434. https://doi.org/10.1038/s41598-020-65359-5

Kılıç, Z. (2021). Water Pollution: Causes, Negative Effects and Prevention Methods. In *İstanbul Sabahattin Zaim Üniversitesi Fen Bilimleri Enstitüsü Dergisi* (Vol. 3, Issue 2, pp. 129–132). İstanbul Sabahattin Zaim Üniversitesi. https://doi.org/10.47769/izufbed.862679

Klein, S., Kuhn, J., Avrahami, R., Tarre, S., Beliavski, M., Green, M., & Zussman, E. (2009). Encapsulation of bacterial cells in electrospun microtubes. *Biomacromolecules*, *10*(7), 1751–1756. https://doi.org/10.1021/bm900168v

Knoll, A., Osborn, M. J., Baross, J., Berg, H. C., Pace, N. R., & Sogin, M. (1999). Size Limits of Very Small microorganisms: Proceedings of a Workshop. In *Proceedings*. papers2://publication/uuid/BEDA218D-276A-40FD-A1FF-1DA2AAA5C982

Ko, F., & Yang, H. (2008). Functional Nanofibre: Enabling Material for the Next Generation Smart Textiles. *Journal of Fiber Bioengineering and Informatics*, *1*, 81–92. https://doi.org/10.3993/jfbi09200801

Korycka, P., Mirek, A., Kramek-Romanowska, K., Grzeczkowicz, M., & Lewińska, D. (2018). Effect of electrospinning process variables on the size of polymer fibers and bead-on-string structures established with a 23 factorial design. *Beilstein Journal of Nanotechnology*, *9*, 2466–2478.

Kostal, J., Yang, R., Wu, C. H., Mulchandani, A., & Chen, W. (2004). Enhanced arsenic accumulation in engineered bacterial cells expressing ArsR. *Applied and Environmental Microbiology*, *70*(8), 4582–4587.

https://doi.org/10.1128/AEM.70.8.4582-4587.2004

Kresna, A., Widjiati, W., & Damayanti, T. (2019). Cryoprotectant combination ethylene glycol and propanediol on mice blastocyst viability post vitrification. *Journal of Physics: Conference Series*, *1146*(1). https://doi.org/10.1088/1742-6596/1146/1/012028

Krueger, M., Bertin, P., Heipieper, H., & Arsène-Ploetze, F. (2013). Bacterial metabolism of environmental arsenic - Mechanisms and biotechnological applications. *Applied Microbiology and Biotechnology*, *97*. https://doi.org/10.1007/s00253-013-4838-5

Kumari, N., & Jagadevan, S. (2016). Genetic identification of arsenate reductase and arsenite oxidase in redox transformations carried out by arsenic metabolising prokaryotes – A comprehensive review. *Chemosphere*, *163*, 400–412. https://doi.org/10.1016/j.chemosphere.2016.08.044

Lagarde, F., & Jaffrezic-Renault, N. (2011). Cell-based electrochemical biosensors for water quality assessment. *Analytical and Bioanalytical Chemistry*, *400*(4), 947. https://doi.org/10.1007/s00216-011-4816-7

Lassen, B., Holmberg, K., Brink, C., Carlén, A., & Olsson, J. (1994). Binding of salivary proteins and oral bacteria to hydrophobic and hydrophilic surfaces in vivo and in vitro. *Colloid and Polymer Science*, *272*(9), 1143–1150. https://doi.org/10.1007/BF00652384

Latała, A., Stepnowski, P., Nedzi, M., & Mrozik, W. (2005). Marine Toxicity Assessment of Imidazolium Ionic Liquids: Acute Effects on the Baltic Algae Oocystis submarina and Cyclotella meneghiniana. *Aquatic Toxicology (Amsterdam, Netherlands)*, *73*, 91–98. https://doi.org/10.1016/j.aquatox.2005.03.008

Lee, A. (2017). Measuring Cell-Viability By Resazurin (Alamarblue ®) Assay Using Photopette ®. *Life Science*, 1–3. http://www.sigmaaldrich.com/catalog/product/sigm

Lee, K.-H., & Kim, D.-M. (2019). In Vitro Use of Cellular Synthetic Machinery for Biosensing Applications. *Frontiers in Pharmacology*, *10*, 1166. https://doi.org/10.3389/fphar.2019.01166

Lei, Y., Chen, W., & Mulchandani, A. (2006). Microbial biosensors. *Analytica Chimica Acta*, *568*(1), 200–210. https://doi.org/https://doi.org/10.1016/j.aca.2005.11.065

Leveau, J., & Lindow, S. (2002). Predictive and Interpretive Simulation of Green Fluorescent Protein Expression in Reporter Bacteria. *Journal of Bacteriology*, *183*, 6752–6762. https://doi.org/10.1128/JB.183.23.6752-6762.2001

Li, L., Liang, J., Hong, W., Zhao, Y., Sun, S., Yang, X., Xu, A., Hang, H., Wu, L., & Chen, S. (2015). Evolved Bacterial Biosensor for Arsenite Detection in Environmental Water. *Environmental Science & Technology*, *49*(10), 6149–6155. https://doi.org/10.1021/acs.est.5b00832

Li, X., & Ricke, S. C. (2004). Comparison of Cryoprotectants for Escherichia Coli Lysine Bioavailability Assay Culture. *Journal of Food Processing and Preservation*, *28*(1), 39–50. https://doi.org/https://doi.org/10.1111/j.1745-4549.2004.tb00536.x

Li, Y., Zhang, J., Zhang, J., Xu, W., & Mou, Z. (2019). Characteristics of Inorganic Phosphate-Solubilizing Bacteria from the Sediments of a Eutrophic Lake. *International Journal of Environmental Research and Public Health*, *16*(12). https://doi.org/10.3390/ijerph16122141

Liao, V. H.-C., & Ou, K.-L. (2005). Development and testing of a green fluorescent protein-based bacterial biosensor for measuring bioavailable arsenic in contaminated groundwater samples. *Environmental Toxicology and Chemistry*, *24*(7), 1624–1631. https://doi.org/10.1897/04-500r.1

Lindemann, T., Hamester, M., Hinrichs, J., Wills, J. D., Electron, T., & Gmbh, B. (2016). High Sensitivity Arsenic Speciation : HPCL Sector Field ICP-MS. *Thermo Scientific*, 1–4.

Liu, R., Wu, P., Xu, K., Lv, Y., & Hou, X. (2008). Highly sensitive and interference-free determination of bismuth in environmental samples by electrothermal vaporization atomic fluorescence spectrometry after hydride trapping on iridium-coated tungsten coil. *Spectrochimica Acta Part B: Atomic Spectroscopy*, *63*(6), 704–709. https://doi.org/https://doi.org/10.1016/j.sab.2008.03.010

Liu, Y., Rafailovich, M. H., Malal, R., Cohn, D., & Chidambaram, D. (2009). Engineering of bio-hybrid materials by electrospinning polymer-microbe fibers. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(34), 14201–14206. https://doi.org/10.1073/pnas.0903238106

Long, F., Zhu, A., & Shi, H. (2013). Recent advances in optical biosensors for environmental monitoring and early warning. *Sensors (Basel, Switzerland)*, *13*(10), 13928–13948. https://doi.org/10.3390/s131013928

López-Rubio, A., Sanchez, E., Sanz, Y., & Lagaron, J. M. (2009). Encapsulation of living bifidobacteria in ultrathin PVOH electrospun fibers. *Biomacromolecules*, *10*(10), 2823–2829. https://doi.org/10.1021/bm900660b

Loska, K., Wiechuła, D., & Korus, I. (2004). Metal contamination of farming soils affected by industry. *Environment International*, *30*(2), 159–165. https://doi.org/https://doi.org/10.1016/S0160-4120(03)00157-0

Luka, G., Ahmadi, A., Najjaran, H., Alocilja, E., DeRosa, M., Wolthers, K., Malki, A., Aziz, H., Althani, A., & Hoorfar, M. (2015). Microfluidics Integrated Biosensors: A Leading Technology towards Lab-on-a-Chip and Sensing Applications. *Sensors*, *15*, 30011–30031. https://doi.org/10.3390/s151229783

Majdinasab, M., Mitsubayashi, K., & Marty, J. L. (2019). Optical and electrochemical sensors and biosensors for the detection of quinolones. *Trends in Biotechnology*, *37*(8), 898–915. https://doi.org/https://doi.org/10.1016/j.tibtech.2019.01.004

Malik, L., Bashir, A., Qureashi, A., & Pandit, A. (2019). Detection and removal of heavy metal ions: a review. *Environmental Chemistry Letters*, *17*.

https://doi.org/10.1007/s10311-019-00891-z

Malikmammadov, E., Tanir, T. E., Kiziltay, A., & Hasirci, N. (2019). Preparation and characterization of poly(ε-caprolactone) scaffolds modified with cell-loaded fibrin gel. *International Journal of Biological Macromolecules*, *125*, 683–689. https://doi.org/10.1016/j.ijbiomac.2018.12.036

Martinez, A. R., Heil, J. R., & Charles, T. C. (2019). An engineered GFP fluorescent bacterial biosensor for detecting and quantifying silver and copper ions. *Biometals : An International Journal on the Role of Metal Ions in Biology, Biochemistry, and Medicine*, *32*(2), 265–272. https://doi.org/10.1007/s10534-019-00179-3

Masindi, V., & Muedi, K. L. (2018). Environmental Contamination by Heavy Metals. In H. E.-D. M. Saleh & R. F. Aglan (Eds.), *Heavy Metals*. IntechOpen. https://doi.org/10.5772/intechopen.76082

Mcgaw, L., Elgorashi, E., & Eloff, J. (2014). Cytotoxicity of African Medicinal Plants Against Normal Animal and Human Cells. In *Toxicological Survey of African Medicinal Plants* (pp. 181–233). https://doi.org/10.1016/B978-0-12-800018-2.00008-X

Mehrotra, P. (2016). Biosensors and their applications – A review. *Journal of Oral Biology and Craniofacial Research*, *6*(2), 153–159. https://doi.org/https://doi.org/10.1016/j.jobcr.2015.12.002

Mehrotra, T., Dev, S., Banerjee, A., Chatterjee, A., Singh, R., & Aggarwal, S. (2021). Use of immobilized bacteria for environmental bioremediation: A review. *Journal of Environmental Chemical Engineering*, *9*(5), 105920. https://doi.org/https://doi.org/10.1016/j.jece.2021.105920

Mi, Y., Wood, G., & Thoma, L. (2004). Cryoprotection mechanisms of polyethylene glycols on lactate dehydrogenase during freeze-thawing. *The AAPS Journal*, *6*(3), e22. https://doi.org/10.1208/aapsj060322

Michelini, E., & Roda, A. (2012). Staying alive: new perspectives on cell

immobilization for biosensing purposes. *Analytical and Bioanalytical Chemistry*, *402*(5), 1785–1797. https://doi.org/10.1007/s00216-011-5364-x

Mittal, M., Kumar, K., Anghore, D., & Rawal, K. R. (2017). ICP-MS: Analytical Method for Identification and Detection of Elemental Impurities. In *Current Drug Discovery Technologies* (Vol. 14, Issue 2, pp. 106–120). https://doi.org/http://dx.doi.org/10.2174/1570163813666161221141402

Mochane, M. J., Motsoeneng, T. S., Sadiku, E. R., Mokhena, T. C., & Sefadi, J. S. (2019). Morphology and properties of electrospun PCL and its composites for medical applications: A mini review. *Applied Sciences (Switzerland)*, *9*(11), 1–17. https://doi.org/10.3390/app9112205

Mohamed Zuki, F., Edyvean, R. G. J., Pourzolfaghar, H., & Kasim, N. (2021). Modeling of the Van Der Waals Forces during the Adhesion of Capsule-Shaped Bacteria to Flat Surfaces. *Biomimetics (Basel, Switzerland)*, *6*(1). https://doi.org/10.3390/biomimetics6010005

Moiseenko, T., Dinu, M., Gashkina, N., & Kremleva, T. (2013). Occurrence forms of metals in natural waters depending on water chemistry. *Water Resources*, *40*. https://doi.org/10.1134/S009780781304009X

Molinari, P., Casella, I., & Costa, T. (2008). Functional complementation of high-efficiency resonance energy transfer: a new tool for the study of protein binding interactions in living cells. *The Biochemical Journal*, *409 1*, 251–261.

Naresh, V., & Lee, N. (2021). A Review on Biosensors and Recent Development of Nanostructured Materials-Enabled Biosensors. In *Sensors* (Vol. 21, Issue 4). https://doi.org/10.3390/s21041109

Nayl, A. A., Abd-Elhamid, A. I., Awwad, N. S., Abdelgawad, M. A., Wu, J., Mo, X., Gomha, S. M., Aly, A. A., & Bräse, S. (2022). Review of the Recent Advances in Electrospun Nanofibers Applications in Water Purification. *Polymers*, *14*(8). https://doi.org/10.3390/polym14081594

Newman, J. D., & Turner, A. P. (1992). Biosensors: principles and practice. *Essays*

in Biochemistry, *27*, 147–159.

Ng, E., Yap, Zakaria, M., Tan, M., & Tan, S. (2013). Assessment of Heavy Metal Pollution in the Straits of Johore by Using Transplanted Caged Mussel, Perna viridis. *Pertanika Journal of Science and Technology*, *21*, 75–96.

Nguyen, T. H., Kim, Y., Kim, J.-S., Jeong, Y., Park, H. M., Kim, J. W., Kim, J.-E., Kim, H., Paek, N.-S., & Kang, C.-H. (2020). Evaluating the Cryoprotective Encapsulation of the Lactic Acid Bacteria in Simulated Gastrointestinal Conditions. *Biotechnology and Bioprocess Engineering*, *25*(2), 287–292. https://doi.org/10.1007/s12257-019-0406-x

Nie, L., Jin, H., Wang, G., Tian, J., & Lin, R. (2008). Study of determination method for heavy metals and harmful elements residues in four traditional Chinese medicine injections. *Zhongguo Zhong yao za zhi = Zhongguo zhongyao zazhi = China journal of Chinese materia medica*, *33*(23), 2764–2767.

Nourmohammadi, E., Hosseinkhani, S., Nedaeinia, R., Khoshdel-Sarkarizi, H., Nedaeinia, M., Ranjbar, M., Ebrahimi, N., Farjami, Z., Nourmohammadi, M., Mahmoudi, A., Goli, M., Ferns, G. A., & Sadeghizadeh, M. (2020). Construction of a sensitive and specific lead biosensor using a genetically engineered bacterial system with a luciferase gene reporter controlled by pbr and cadA promoters. *BioMedical Engineering OnLine*, *19*(1), 79. https://doi.org/10.1186/s12938-020-00816-w

Nowroth, V., Marquart, L., & Dieter, J. (2016). Low temperature-induced viable but not culturable (VBNC) state of Ralstonia eutropha and its relationship to accumulated polyhydroxybutyrate (PHB). *FEMS Microbiology Letters*, *363*. https://doi.org/10.1093/femsle/fnw249

Nussinovitch, A. (1997). *Immobilization and encapsulation BT - Hydrocolloid Applications: Gum technology in the food and other industries* (A. Nussinovitch (Ed.); pp. 247–264). Springer US. https://doi.org/10.1007/978-1-4615-6385-3_15

O'Brien, J., Wilson, I., Orton, T., & Pognan, F. (2000). Investigation of the Alamar

Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. *European Journal of Biochemistry*, *267*(17), 5421–5426. https://doi.org/10.1046/j.1432-1327.2000.01606.x

Odobašić, A. (2019). *Biosensors for Determination of Heavy Metals in Waters* (I. Šestan (Ed.); p. Ch. 10). IntechOpen. https://doi.org/10.5772/intechopen.84139

Oh, J. K., Yegin, Y., Yang, F., Zhang, M., Li, J., Huang, S., Verkhoturov, S. V, Schweikert, E. A., Perez-Lewis, K., Scholar, E. A., Taylor, T. M., Castillo, A., Cisneros-Zevallos, L., Min, Y., & Akbulut, M. (2018). The influence of surface chemistry on the kinetics and thermodynamics of bacterial adhesion. *Scientific Reports*, *8*(1), 17247. https://doi.org/10.1038/s41598-018-35343-1

Okano, G., Igarashi, S., Yamamoto, Y., Saito, S., Takagai, Y., Ohtomo, T., Kimura, S., Ohno, O., & Oka, Y. (2015). HPLC-spectrophotometric detection of trace heavy metals via 'cascade' separation and concentration. *International Journal of Environmental Analytical Chemistry*, *95*(2), 135–144. https://doi.org/10.1080/03067319.2014.994619

Olaniran, A. O., Hiralal, L., & Pillay, B. (2011). Whole-cell bacterial biosensors for rapid and effective monitoring of heavy metals and inorganic pollutants in wastewater. *Journal of Environmental Monitoring : JEM*, *13*(10), 2914–2920. https://doi.org/10.1039/c1em10032g

Park, M., Tsai, S.-L., & Chen, W. (2013). Microbial biosensors: engineered microorganisms as the sensing machinery. *Sensors (Basel, Switzerland)*, *13*(5), 5777–5795. https://doi.org/10.3390/s130505777

Parreira, P., Magalhães, A., Gonçalves, I. C., Gomes, J., Vidal, R., Reis, C. A., Leckband, D. E., & Martins, M. C. L. (2011). Effect of surface chemistry on bacterial adhesion, viability, and morphology. *Journal of Biomedical Materials Research. Part A*, *99*(3), 344–353. https://doi.org/10.1002/jbm.a.33178

Payal, R., & Tomer, T. (2021). *Determination of Heavy Metal Ions From Water* (pp. 255–272). https://doi.org/10.1002/9781119725237.ch9

Petras, D., Slobodian, P., Pavlínek, V., Sáha, P., & Kimmer, D. (2011). The effect of PVAc solution viscosity on diameter of PVAc nanofibres prepared by technology of electrospinning. *AIP Conference Proceedings*, *1375*(2011), 312–319. https://doi.org/10.1063/1.3604491

Pola-López, L. A., Camas-Anzueto, J. L., Martínez-Antonio, A., Luján-Hidalgo, M. C., Anzueto-Sánchez, G., Ruíz-Valdiviezo, V. M., Grajales-Coutiño, R., & González, J. H. C. (2018). Novel arsenic biosensor "POLA" obtained by a genetically modified E. coli bioreporter cell. *Sensors and Actuators B: Chemical*, *254*, 1061–1068. https://doi.org/https://doi.org/10.1016/j.snb.2017.08.006

Prévéral, S., Brutesco, C., Descamps, E. C. T., Escoffier, C., Pignol, D., Ginet, N., & Garcia, D. (2017). A bioluminescent arsenite biosensor designed for inline water analyzer. *Environmental Science and Pollution Research*, *24*(1), 25–32. https://doi.org/10.1007/s11356-015-6000-7

Queensland Fire and Emergency Services. (2018). *Queensland Prevention, Preparedness, Response and Recovery Disaster Management Guideline PREPARED BY THE Queensland Fire and Emergency Services*. *2018*(1), 92–100. https://www.disaster.qld.gov.au/dmg/Documents/QLD-Disaster-Management-Guideline.pdf

Raju, N. J. (2022). Arsenic in the geo-environment: A review of sources, geochemical processes, toxicity and removal technologies. *Environmental Research*, *203*, 111782. https://doi.org/https://doi.org/10.1016/j.envres.2021.111782

Ramakrishna, S., Fujihara, K., Teo, W. E., Yong, T., Ma, Z., & Ramaseshan, R. (2006). Electrospun nanofibers: Solving global issues. *Materials Today*, *9*(3), 40–50. https://doi.org/10.1016/S1369-7021(06)71389-X

Ranveer, A., Nasipude, S., & Bagwan, S. (2015). Biosensor for environmental monitoring. *Internation Journal of Innovations in Engineering Research and Technology (IJIERT)*, *2*.

Rashed, M. E. A. E.-J. K. N. E.-M. N. (2020). *Heavy Metal Contamination and Remediation of Water and Soil with Case Studies From Cyprus* (p. Ch. 1). IntechOpen. https://doi.org/10.5772/intechopen.90060

Ratnaike, R. N. (2003). Acute and chronic arsenic toxicity. *Postgraduate Medical Journal*, *79*(933), 391 LP – 396. https://doi.org/10.1136/pmj.79.933.391

Rawson, D. M., Willmer, A. J., & Turner, A. P. P. (1989). Whole-cell biosensors for environmental monitoring. *Biosensors*, *4*(5), 299–311. https://doi.org/https://doi.org/10.1016/0265-928X(89)80011-2

Rehman, K., Fatima, F., Waheed, I., & Akash, M. S. H. (2018). Prevalence of exposure of heavy metals and their impact on health consequences. *Journal of Cellular Biochemistry*, *119*(1), 157–184. https://doi.org/10.1002/jcb.26234

Riangrungroj, P., Bever, C. S., Hammock, B. D., & Polizzi, K. M. (2019). A label-free optical whole-cell Escherichia coli biosensor for the detection of pyrethroid insecticide exposure. *Scientific Reports*, *9*(1), 12466. https://doi.org/10.1038/s41598-019-48907-6

Rieger, K., Birch, N., & Schiffman, J. (2013). Designing electrospun nanofiber mats to promote wound healing - a review. *Journal of Materials Chemistry B*, *1*, 4531–4541. https://doi.org/10.1039/C3TB20795A

Rigo, A., de Cezaro, A., Muenchen, D., Martinazzo, J., Manzoli, A., Steffens, J., & Steffens, C. (2019). Heavy metals detection in river water with cantilever nanobiosensor. *Journal of Environmental Science and Health. Part. B, Pesticides, Food Contaminants, and Agricultural Wastes*, *55*, 1–11. https://doi.org/10.1080/03601234.2019.1685318

Rocha, M. (2019). Electrospun Polycaprolactone Fibrous Membranes Containing Ag, TiO2 and Na2Ti6O13 Particles for Potential Use in Bone Regeneration. *Membranes*, *9*, 12. https://doi.org/10.3390/membranes9010012

Roointan, A., Shabab, N., Karimi, J., Rahmani, A., Alikhani, M., & Saidijam, M. (2015). Designing a bacterial biosensor for detection of mercury in water

solutions. *Turkish Journal of Biology*, *Turk J Bio*, 550–555. https://doi.org/10.3906/biy-1411-49

Saeki, E., Farhat, L. P., & Pontes, É. A. (2015). *Efficiency of cryoprotectants glycerol and skimmed milk for microorganisms freezing*. *9*, 195–198.

Salalha, W., Kuhn, J., Dror, Y., & Zussman, E. (2006). Encapsulation of bacteria and viruses in electrospun nanofibres. *Nanotechnology*, *17*(18), 4675–4681. https://doi.org/10.1088/0957-4484/17/18/025

Saleh, I., Syamsir, S., Pramaningsih, V., & Hansen, H. (2021). The use of green mussel as bioindicator of heavy metal pollution in Indonesia: a review. *Environmental Analysis, Health and Toxicology*, *36*(4), e2021026-0. https://doi.org/10.5620/eaht.2021026

San Keskin, N. O., Celebioglu, A., Sarioglu, O. F., Uyar, T., & Tekinay, T. (2017). Encapsulation of living bacteria in electrospun cyclodextrin ultrathin fibers for bioremediation of heavy metals and reactive dye from wastewater. *Colloids and Surfaces. B, Biointerfaces*, *161*, 169–176. https://doi.org/10.1016/j.colsurfb.2017.10.047

San Keskin, N. O., Celebioglu, A., Sarioglu, O., Ozkan, A., Uyar, T., & Tekinay, T. (2015). Removal of a reactive dye and hexavalent chromium by a reusable bacteria attached electrospun nanofibrous web. *RSC Adv.*, *5*, 86867–86874. https://doi.org/10.1039/C5RA15601G

San, N. O., Celebioglu, A., Tümtaş, Y., Uyar, T., & Tekinay, T. (2014). Reusable bacteria immobilized electrospun nanofibrous webs for decolorization of methylene blue dye in wastewater treatment. *RSC Advances*, *4*(61), 32249–32255. https://doi.org/10.1039/c4ra04250f

Sánchez-Rodas Navarro, D. A., Corns, W. T., Chen, B., & Stockwell, P. B. (2010). *Atomic fluorescence spectrometry : a suitable detection technique in speciation studies for arsenic, selenium, antimony and mercury*. https://doi.org/10.1039/b917755h

Sarioglu, O. F., Celebioglu, A., Tekinay, T., & Uyar, T. (2015). Evaluation of contact time and fiber morphology on bacterial immobilization for development of novel surfactant degrading nanofibrous webs. *RSC Advances*, *5*(124), 102750–102758. https://doi.org/10.1039/C5RA20739H

Sarioglu, O. F., Celebioglu, A., Tekinay, T., & Uyar, T. (2016). Bacteria-immobilized electrospun fibrous polymeric webs for hexavalent chromium remediation in water. *International Journal of Environmental Science and Technology*, *13*(8), 2057–2066. https://doi.org/10.1007/s13762-016-1033-0

Sarioglu, O. F., Celebioglu, A., Tekinay, T., & Uyar, T. (2017). Evaluation of fiber diameter and morphology differences for electrospun fibers on bacterial immobilization and bioremediation performance. *International Biodeterioration & Biodegradation*, *120*, 66–70. https://doi.org/10.1016/j.ibiod.2017.02.010

Sarioglu, O. F., San Keskin, N. O., Celebioglu, A., Tekinay, T., & Uyar, T. (2017a). Bacteria encapsulated electrospun nanofibrous webs for remediation of methylene blue dye in water. *Colloids and Surfaces B: Biointerfaces*, *152*, 245–251. https://doi.org/10.1016/j.colsurfb.2017.01.034

Sarioglu, O. F., San Keskin, N. O., Celebioglu, A., Tekinay, T., & Uyar, T. (2017b). Bacteria immobilized electrospun polycaprolactone and polylactic acid fibrous webs for remediation of textile dyes in water. *Chemosphere*, *184*, 393–399. https://doi.org/10.1016/j.chemosphere.2017.06.020

Sarioglu, O. F., Yasa, O., Celebioglu, A., Uyar, T., & Tekinay, T. (2013). Efficient ammonium removal from aquatic environments by Acinetobacter calcoaceticus STB1 immobilized on an electrospun cellulose acetate nanofibrous web. *Green Chemistry*, *15*(9), 2566–2572. https://doi.org/10.1039/C3GC40885J

Sarzanini, C. (2000). Metals Analysis by High Performance Liquid Chromatography. In A. Gianguzza, E. Pelizetti, & S. Sammartano (Eds.), *Chemical Processes in Marine Environments* (pp. 339–356). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-662-04207-6_19

Sastry, C., & Vijayadeep, P. (2014). Effect of Heavy Metal Uptake by E. coli and Bacillus sps. *Journal of Bioremediation & Biodegradation*, *05*. https://doi.org/10.4172/2155-6199.1000238

Scientific, T. (2008). alamarBlue® Cell Viability Assay Protocol. *Thermo Fisher Scientific*, *Figure 3*, 1–8. http://www.lifetechnologies.com/my/en/home/references/protocols/cell-and-tissue-analysis/cell-profilteration-assay-protocols/cell-viability-with-alamarblue.html

Şenol, N., & Akkoyun, S. (2020). Effect of Processing Conditions on the Electrospinning Behavior of Polyvinylpyrrolidone with Lower Toxicity Solvents. *European Journal of Science and Technology*, 121–128.

Shabani, A., Zourob, M., Allain, B., Marquette, C. A., Lawrence, M. F., & Mandeville, R. (2008). Bacteriophage-Modified Microarrays for the Direct Impedimetric Detection of Bacteria. *Analytical Chemistry*, *80*(24), 9475–9482. https://doi.org/10.1021/ac801607w

Shachrai, I., Zaslaver, A., Alon, U., & Dekel, E. (2010). Cost of Unneeded Proteins in E. coli Is Reduced after Several Generations in Exponential Growth. *Molecular Cell*, *38*(5), 758–767. https://doi.org/10.1016/j.molcel.2010.04.015

Shaji, E., Santosh, M., Sarath, K. V, Prakash, P., Deepchand, V., & Divya, B. V. (2021). Arsenic contamination of groundwater: A global synopsis with focus on the Indian Peninsula. *Geoscience Frontiers*, *12*(3), 101079. https://doi.org/https://doi.org/10.1016/j.gsf.2020.08.015

Shamsollahi, Z., & Partovinia, A. (2019). Recent advances on pollutants removal by rice husk as a bio-based adsorbent: A critical review. *Journal of Environmental Management*, *246*, 314–323. https://doi.org/https://doi.org/10.1016/j.jenvman.2019.05.145

Shriver-Lake, L. C., Gammeter, W. B., Bang, S. S., & Pazirandeh, M. (2002). Covalent binding of genetically engineered microorganisms to porous glass beads. *Analytica Chimica Acta*, *470*(1), 71–78.

https://doi.org/https://doi.org/10.1016/S0003-2670(02)00540-8

Si, R.-W., Yang, Y., Yu, Y.-Y., Han, S., Zhang, C.-L., Sun, D.-Z., Zhai, D.-D., Liu, X., & Yong, Y.-C. (2016). Wiring Bacterial Electron Flow for Sensitive Whole-Cell Amperometric Detection of Riboflavin. *Analytical Chemistry*, *88*(22), 11222–11228. https://doi.org/10.1021/acs.analchem.6b03538

Sikdar, S., & Kundu, M. (2018). A Review on Detection and Abatement of Heavy Metals. *ChemBioEng Reviews*, *5*(1), 18–29. https://doi.org/10.1002/cben.201700005

Sin, M. L. Y., Mach, K. E., Wong, P. K., & Liao, J. C. (2014). Advances and challenges in biosensor-based diagnosis of infectious diseases. *Expert Review of Molecular Diagnostics*, *14*(2), 225–244. https://doi.org/10.1586/14737159.2014.888313

Sinha, R., Janaswamy, S., & Prasad, A. (2020). Enhancing Mechanical Properties of Electrospun Cellulose Acetate Fibers Upon Potassium Chloride Exposure. *SSRN Electronic Journal*. https://doi.org/10.2139/ssrn.3604714

Song, Y., Zhang, D., Polizzi, K., Zhang, E., Li, G., Huang, W., & W.E., H. (2012). *Bacterial Whole Cell Bioreporters in Environmental Health*. https://doi.org/10.1201/b12775-9

Su, L., Jia, W., Hou, C., & Lei, Y. (2011). Microbial biosensors: A review. *Biosensors and Bioelectronics*, *26*(5), 1788–1799. https://doi.org/https://doi.org/10.1016/j.bios.2010.09.005

Suo, Z., Avci, R., Yang, X., & Pascual, D. W. (2008). Efficient immobilization and patterning of live bacterial cells. *Langmuir : The ACS Journal of Surfaces and Colloids*, *24*(8), 4161–4167. https://doi.org/10.1021/la7038653

Suwannasom, N., Kao, I., Pruß, A., Georgieva, R., & Bäumler, H. (2020). Riboflavin: The Health Benefits of a Forgotten Natural Vitamin. *International Journal of Molecular Sciences*, *21*(3). https://doi.org/10.3390/ijms21030950

Suzana, C. udia S. M., Claudia, M., a, M., Larissa Guedes Fiuacute za, M. C. o, &

ra, T. dde S. (2013). Immobilization of microbial cells: A promising tool for treatment of toxic pollutants in industrial wastewater. *African Journal of Biotechnology*, *12*(28), 4412–4418. https://doi.org/10.5897/ajb12.2677

Tani, C., Inoue, K., Tani, Y., Harun-ur-Rashid, M., Azuma, N., Ueda, S., Yoshida, K., & Maeda, I. (2009). Sensitive fluorescent microplate bioassay using recombinant Escherichia coli with multiple promoter-reporter units in tandem for detection of arsenic. *Journal of Bioscience and Bioengineering*, *108*(5), 414–420. https://doi.org/10.1016/j.jbiosc.2009.05.014

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy metal toxicity and the environment. *Experientia Supplementum (2012)*, *101*, 133–164. https://doi.org/10.1007/978-3-7643-8340-4_6

Teo, S., & Ling Shing, W. (2014). Whole Cell-based Biosensors for Environmental Heavy Metals Detection. *Annual Research & Review in Biology*, *4*, 2663–2674. https://doi.org/10.9734/ARRB/2014/9472

Terzi, E., & Civelek, F. (2021). Cu, Cd, As and Hg resistance levels in Escherichia coli isolated from Mediterranean mussel and sea snail in the Southeastern Black Sea. *Marine Science and Technology Bulletin*, *10*, 36–41. https://doi.org/10.33714/masteb.776902

Tetyana, P. (2021). *Biosensors: Design, Development and Applications* (P. M. Shumbula (Ed.); p. Ch. 3). IntechOpen. https://doi.org/10.5772/intechopen.97576

Theytaz, J., Braschler, T., van Lintel, H., Renaud, P., Diesel, E., Merulla, D., & van der Meer, J. (2009). Biochip with E. coli bacteria for detection of arsenic in drinking water. *Procedia Chemistry*, *1*(1), 1003–1006. https://doi.org/10.1016/j.proche.2009.07.250

Tlili, I., & Alkanhal, T. A. (2019). Nanotechnology for water purification: electrospun nanofibrous membrane in water and wastewater treatment. *Journal of Water Reuse and Desalination*, *9*(3), 232–248. https://doi.org/10.2166/wrd.2019.057

Trang, P. T. K., Berg, M., Viet, P. H., Van Mui, N., & Van Der Meer, J. R. (2005). Bacterial bioassay for rapid and accurate analysis of arsenic in highly variable groundwater samples. *Environmental Science & Technology*, *39*(19), 7625–7630. https://doi.org/10.1021/es050992e

Turdean, G. L. (2011). Design and Development of Biosensors for the Detection of Heavy Metal Toxicity. *International Journal of Electrochemistry*, *2011*, 343125. https://doi.org/10.4061/2011/343125

Turşucular, Ö., Cerkez, I., Orhan, M., & Aykut, Y. (2018). Preperation And Antibacterial Investigation of Polycaprolactone/Chitosan Nano/Micro Fibers By Using Different Solvent Systems. *Tekstil ve Konfeksiyon*, *28*, 221–228.

Umoh, E. O. (2013). *Physico - Chemical Analysis of Effluents from Jacbon Chemical Industries Limited, Makers of Bonalux Emulsion and Gloss Paints*.

Unnithan, A. R., Arathyram, R. S., & Kim, C. S. (2015). *Chapter 3 - Electrospinning of Polymers for Tissue Engineering* (S. Thomas, Y. Grohens, & N. B. T.-N. A. for T. E. Ninan (Eds.); pp. 45–55). William Andrew Publishing. https://doi.org/https://doi.org/10.1016/B978-0-323-32889-0.00003-0

Varshney, S., Sain, A., Gupta, D., & Sharma, S. (2020). Factors Affecting Bacterial Adhesion on Selected Textile Fibres. *Indian Journal of Microbiology*, *61*. https://doi.org/10.1007/s12088-020-00903-5

Velitchkova, N., Pentcheva, E., & Daskalova, N. (2003). *ICP – AES investigation on heavy metal water and soil pollution in Plovdiv Region (Bulgaria)*.

Verma, N., & Singh, M. (2005). Biosensors for heavy metals. *Biometals*, *18*(2), 121–129. https://doi.org/10.1007/s10534-004-5787-3

Vigneshvar, S., Sudhakumari, C. C., Senthilkumaran, B., & Prakash, H. (2016). Recent Advances in Biosensor Technology for Potential Applications – An Overview . In *Frontiers in Bioengineering and Biotechnology* (Vol. 4). https://www.frontiersin.org/article/10.3389/fbioe.2016.00011

Vigués Frantzen, N. (2020). *High-density bacterial immobilization strategies for the*

development of microbial biosensors. https://widgets.ebscohost.com/prod/customerspecific/ns000545/customproxy.php?url=https://search.ebscohost.com/login.aspx?direct=true&db=edstdx&AN=edstdx.10803.670963&amp%0Alang=pt-pt&site=eds-live&scope=site

Wang, L., Pang, S., & Zhou, G. (2019). Recent Advances in Spectroscopy Technology for Trace Analysis of Persistent Organic Pollutants. *Applied Sciences*, *9*(17). https://doi.org/10.3390/app9173439

Wang, X., Lu, X., & Chen, J. (2014). Development of biosensor technologies for analysis of environmental contaminants. *Trends in Environmental Analytical Chemistry*, *2*, 25–32. https://doi.org/https://doi.org/10.1016/j.teac.2014.04.001

Ward, W. W. (1979). *Energy Transfer Processes in Bioluminescence BT - Photochemical and Photobiological Reviews: Volume 4* (K. C. Smith (Ed.); pp. 1–57). Springer US. https://doi.org/10.1007/978-1-4684-3551-1_1

Watstein, D. M., & Styczynski, M. P. (2018). Development of a Pigment-Based Whole-Cell Zinc Biosensor for Human Serum. *ACS Synthetic Biology*, *7*(1), 267–275. https://doi.org/10.1021/acssynbio.7b00292

Worden, C. R., Kovac, W. K., Dorn, L. A., & Sandrin, T. R. (2009). Environmental pH affects transcriptional responses to cadmium toxicity in Escherichia coli K-12 (MG1655). *FEMS Microbiology Letters*, *293*(1), 58–64. https://doi.org/10.1111/j.1574-6968.2009.01508.x

Wu, M. N., Wang, X. C., & Ma, X. Y. (2016). Phytotoxicity comparison of organic contaminants and heavy metals using Chlorella vulgaris. *Desalination and Water Treatment*, *57*(44), 20809–20816. https://doi.org/10.1080/19443994.2015.1110537

Xie, S., Tai, S., Song, H., Luo, X., Zhang, H., & Li, X. (2016). Genetically engineering of: Escherichia coli and immobilization on electrospun fibers for drug delivery purposes. *Journal of Materials Chemistry B*, *4*, 6820–6829. https://doi.org/10.1039/c6tb01165a

Xu, T., Close, D. M., Sayler, G. S., & Ripp, S. (2013). Genetically modified whole-cell bioreporters for environmental assessment. *Ecological Indicators*, *28*, 125–141. https://doi.org/10.1016/J.ECOLIND.2012.01.020

Yang, H.-C., Fu, H.-L., Lin, Y.-F., & Rosen, B. P. (2012). Pathways of arsenic uptake and efflux. *Current Topics in Membranes*, *69*, 325–358. https://doi.org/10.1016/B978-0-12-394390-3.00012-4

Yang, Y., Liu, T., Zhu, X., Zhang, F., Ye, D., Liao, Q., & Li, Y. (2016). Boosting Power Density of Microbial Fuel Cells with 3D Nitrogen-Doped Graphene Aerogel Electrode. *Advanced Science*, *3*(8), 1600097. https://doi.org/https://doi.org/10.1002/advs.201600097

Yap, P. Y., & Trau, D. (2019). Direct E.Coli Cell Count At Od600. *Tip Biosystems*, *1*(March), 1–3.

Yeni, F., Odaci, D., & Timur, S. (2008). Use of Eggshell Membrane as an Immobilization Platform in Microbial Sensing. *Analytical Letters*, *41*(14), 2743–2758. https://doi.org/10.1080/00032710802363594

Yoo, E.-H., & Lee, S.-Y. (2010). Glucose biosensors: an overview of use in clinical practice. *Sensors (Basel, Switzerland)*, *10*(5), 4558–4576. https://doi.org/10.3390/s100504558

Yoon, Y., Kim, S., Chae, Y., Jeong, S.-W., & An, Y.-J. (2016). Evaluation of bioavailable arsenic and remediation performance using a whole-cell bioreporter. *The Science of the Total Environment*, *547*, 125–131. https://doi.org/10.1016/j.scitotenv.2015.12.141

Yuan, Y., Hays, M., Hardwidge, P., & Kim, J. (2017). Surface characteristics influencing bacterial adhesion to polymeric substrates. *RSC Adv.*, *7*, 14254–14261. https://doi.org/10.1039/C7RA01571B

Yuvaraj, R., Nadarajan, S. P., Lee, C.-S., Jung, S., & Bae, D.-H. (2016). FMN-Based Fluorescent Proteins as Heavy Metal Sensors Against Mercury Ions. *Journal of Microbiology and Biotechnology*, *26*(3), 530–539.

https://doi.org/10.4014/jmb.1510.10040

Zager, V., Cemazar, M., Hreljac, I., Lah, T., Sersa, G., & Filipic, M. (2010). Development of human cell biosensor system for genotoxicity detection based on DNA damage-induced gene expression. *Radiology and Oncology*, *44*(1), 42–51. https://doi.org/doi:10.2478/v10019-010-0010-3

Zamel, D., Hassanin, A. H., Ellethy, R., Singer, G., & Abdelmoneim, A. (2019). Novel Bacteria-Immobilized Cellulose Acetate/Poly(ethylene oxide) Nanofibrous Membrane for Wastewater Treatment. *Scientific Reports*, *9*(1), 1–12. https://doi.org/10.1038/s41598-019-55265-w

Zeng, N., Wu, Y., Chen, W., Huang, Q., & Cai, P. (2021). Whole-Cell Microbial Bioreporter for Soil Contaminants Detection. *Frontiers in Bioengineering and Biotechnology*, *9*. https://doi.org/10.3389/fbioe.2021.622994

Zhang, G., Hu, S., & Jia, X. (2021). Highly Sensitive Whole-Cell Biosensor for Cadmium Detection Based on a Negative Feedback Circuit. *Frontiers in Bioengineering and Biotechnology*, *9*, 799781. https://doi.org/10.3389/fbioe.2021.799781

Zhang, Y., Zheng, R., Zhao, J., Ma, F., Zhang, Y., & Meng, Q. (2014). Characterization of Treated Rice Husk Adsorbent and Adsorption of Copper(II) from Aqueous Solution. *BioMed Research International*, *2014*, 496878. https://doi.org/10.1155/2014/496878

Zhao, H. (2018). *Electrospun Bead-on-String Fibers: Useless or Something of Value?* (H. C. E.-T. Lin (Ed.); p. Ch. 5). IntechOpen. https://doi.org/10.5772/intechopen.74661

Zhao, S., Li, Y., Yin, H., Liu, Z., Luan, E., Zhao, F., Tang, Z., & Liu, S. (2015). Energy Resources: Three-dimensional graphene/Pt nanoparticle composites as freestanding anode for enhancing performance of microbial fuel cells. *Science Advances*, *1*(10), 1–9. https://doi.org/10.1126/sciadv.1500372

Zheng, S., Bawazir, M., Dhall, A., Kim, H.-E., He, L., Heo, J., & Hwang, G. (2021).

Implication of Surface Properties, Bacterial Motility, and Hydrodynamic Conditions on Bacterial Surface Sensing and Their Initial Adhesion. *Frontiers in Bioengineering and Biotechnology*, *9*, 643722. https://doi.org/10.3389/fbioe.2021.643722

Zuo, W., Zhu, M., Yang, W., Yu, H., Chen, Y., & Zhang, Y. (2005). Experimental study on relationship between jet instability and formation of beaded fibers during electrospinning. *Polymer Engineering & Science*, *45*(5), 704–709. https://doi.org/https://doi.org/10.1002/pen.20304

Żur, J., Wojcieszyńska, D., & Guzik, U. (2016). Metabolic Responses of Bacterial Cells to Immobilization. *Molecules (Basel, Switzerland)*, *21*(7). https://doi.org/10.3390/molecules21070958

APPENDICES

A. COMPOSITIONS OF BACTERIAL CELL CULTURE MEDIA

Luria-Bertani Agar (1 L)

Agar	15 grams
Tryptone	10 grams
NaCl	10 grams
Yeast extract	5 grams

Distilled water (dH_2O) is added up to 1 L volume. The pH of the medium is adjusted to 7.0 and autoclaved at 121°C for 20 minutes.

M9 supplemented medium (1 L)

Stock M9 Minimal salts, 5X (1 L)

Na_2HPO_4	30 g
KH_2PO_4	15 g
NaCl	2.5 g
NH_4Cl	5 g

Autoclaved at 121°C for 20 minutes. Then,

5X Stock M9 Minimal Salts	200 mL

Filter sterilized stock solutions added,

$MgSO_4.7H_2O$ stock, 1 M	2 mL
$CaCl_2.2H_2O$ stock, 1M	1 mL
Casaminoacids stock, 10% (v/v)	10 mL
Glucose stock, 20% (v/v)	20 mL

The pH of the medium is adjusted to 7.0. Complete to 1 L with sterile dH_2O.

Mops supplemented medium (1 L)

MOPS sodium salt	9.8 g
NaCl	0.5 g
NH_4Cl	1 g
Na_2HPO_4	0.05 g
KH_2PO_4	0.05 g

The pH is adjusted to 7.0 by addition of NaOH and completed to 967 mL with dH_2O.
Autoclaved at 121°C for 20 min.

Filter sterilized stock solutions added,

$MgSO_4.7H_2O$ stock, 1 M	2 mL
$CaCl_2.2H_2O$ stock, 1M	1 mL
Casaminoacids stock, 10% (v/v)	10 mL
Glucose stock, 20% (v/v)	20 mL

B. BUFFER SOLUTION FOR IMMOBILIZATION STUDIES AND ALAMARBLUE

1 X Phosphate-buffered saline (PBS) (1 L)

Compound	Final molarity
NaCl	137 mM
KCl	2.7 mM
Na_2HPO_4	10 mM
KH_2PO	1.8 mM

The pH of the solution is adjusted to 7.4 with HCl. Autoclaved at 121°C for 20 min and performed filter sterilization.

Resazurin Sodium Salt – AlamarBlue (Working solution)

Stock solution (100X)

Resazurin sodium salt	0.50 gram
1X PBS	100 mL

Then, stock solution diluted 1:100 with 1X PBS.

C. ONE-WAY ANOVA RESULTS

Effect of Growth Phase

Multiple Comparisons

Dependent Variable: Early growth phase, CA-immobilized

Tukey HSD

(I) CA_Early	(J) CA_Early	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0 µg/L	50 µg/L	-1866100,12500*	706624,00290	,026	-3546031,8130	-186168,4366
	100 µg/L	-8209795,27800*	706624,00290	,000	-9889726,9660	-6529863,5890
50 µg/L	0 µg/L	1866100,12500*	706624,00290	,026	186168,4366	3546031,8130
	100 µg/L	-6343695,15300*	706624,00290	,000	-8023626,8410	-4663763,4640
100 µg/L	0 µg/L	8209795,27800*	706624,00290	,000	6529863,5890	9889726,9660
	50 µg/L	6343695,15300*	706624,00290	,000	4663763,4640	8023626,8410

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons

Dependent Variable: Mid-exponential growth phase, CA-immobilized

Tukey HSD

(I) CA_MİD	(J) CA_MİD	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0 µg/L	50 µg/L	-1950959,73300	1399907,36200	,348	-5280511,8330	1378592,3660
	100 µg/L	-10949644,92000*	1399907,36200	,000	-14279197,0200	-7620092,8190
50 µg/L	0 µg/L	1950959,73300	1399907,36200	,348	-1378592,3660	5280511,8330
	100 µg/L	-8998685,18600*	1399907,36200	,000	-12328237,2900	-5669133,0860
100 µg/L	0 µg/L	10949644,92000*	1399907,36200	,000	7620092,8190	14279197,0200
	50 µg/L	8998685,18600*	1399907,36200	,000	5669133,0860	12328237,2900

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons

Dependent Variable: Stationary phase, CA-immobilized

Tukey HSD

(I) Stat CA	(J) Stat CA	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0 µg/L	50 µg/L	-5572674,82400*	1561850,49600	,002	-9286338,8410	-1859010,8070
	100 µg/L	-11855356,89000*	1516569,81500	,000	-15461355,5200	-8249358,2610
50 µg/L	0 µg/L	5572674,82400*	1561850,49600	,002	1859010,8070	9286338,8410
	100 µg/L	-6282682,06800*	1561850,49600	,000	-9996346,0850	-2569018,0510
100 µg/L	0 µg/L	11855356,89000*	1516569,81500	,000	8249358,2610	15461355,5200
	50 µg/L	6282682,06800*	1561850,49600	,000	2569018,0510	9996346,0850

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons

Dependent Variable: Early growth phase, PCL-immobilized

Tukey HSD

(I) Early PCL	(J) Early PCL	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0 µg/L	50 µg/L	-4908422,59700*	1018853,75200	,000	-7330650,9640	-2486194,2300
	100 µg/L	-11479391,85000*	1018853,75200	,000	-13901620,2100	-9057163,4800
50 µg/L	0 µg/L	4908422,59700*	1018853,75200	,000	2486194,2300	7330650,9640
	100 µg/L	-6570969,25000*	1018853,75200	,000	-8993197,6170	-4148740,8830
100 µg/L	0 µg/L	11479391,85000*	1018853,75200	,000	9057163,4800	13901620,2100
	50 µg/L	6570969,25000*	1018853,75200	,000	4148740,8830	8993197,6170

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons

Dependent Variable: Mid-exponential growth phase, PCL-immobilized

Tukey HSD

(I) Mid PCL	(J) Mid PCL	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0 µg/L	50 µg/L	-9110263,92900*	3434691,47900	,025	-17280559,6400	-939968,2204
	100 µg/L	-31835489,05000*	3459854,39000	,000	-40065641,2100	-23605336,9000
50 µg/L	0 µg/L	9110263,92900*	3434691,47900	,025	939968,2204	17280559,6400
	100 µg/L	-22725225,12000*	3459854,39000	,000	-30955377,2800	-14495072,9700
100 µg/L	0 µg/L	31835489,05000*	3459854,39000	,000	23605336,9000	40065641,2100
	50 µg/L	22725225,12000*	3459854,39000	,000	14495072,9700	30955377,2800

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons

Dependent Variable: Stationary phase, PCL-immobilized

Tukey HSD

(I) Stat PCL	(J) Stat PCL	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0 µg/L	50 µg/L	995532,30490	3880739,12300	,964	-8259877,9650	10250942,5700
	100 µg/L	-26374018,27000*	3540282,49500	,000	-34817452,8600	-17930583,6700
50 µg/L	0 µg/L	-995532,30490	3880739,12300	,964	-10250942,5700	8259877,9650
	100 µg/L	-27369550,57000*	3929369,18700	,000	-36740941,6300	-17998159,5100
100 µg/L	0 µg/L	26374018,27000*	3540282,49500	,000	17930583,6700	34817452,8600
	50 µg/L	27369550,57000*	3929369,18700	,000	17998159,5100	36740941,6300

*. The mean difference is significant at the 0.05 level.

Effect of Cell Concentration

Multiple Comparisons

Dependent Variable: OD600=0.1, CA-immobilized

Tukey HSD

(I) 0.1 CA	(J) 0.1 CA	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0 µg/L	50 µg/L	-1631225,14900*	482684,06900	,003	-2780119,9740	-482330,3228
	100 µg/L	-2039633,31000*	482684,06900	,000	-3188528,1360	-890738,4844
50 µg/L	0 µg/L	1631225,14900*	482684,06900	,003	482330,3228	2780119,9740
	100 µg/L	-408408,16160	486272,84760	,679	-1565845,0740	749028,7507
100 µg/L	0 µg/L	2039633,31000*	482684,06900	,000	890738,4844	3188528,1360
	50 µg/L	408408,16160	486272,84760	,679	-749028,7507	1565845,0740

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons

Dependent Variable: OD600=0.5, CA-immobilized

Tukey HSD

(I) 0.5 CA	(J) 0.5 CA	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0 µg/L	50 µg/L	-5623814,18900*	974837,01140	,000	-7940473,6260	-3307154,7520
	100 µg/L	-5324584,74300*	974837,01140	,000	-7641244,1800	-3007925,3060
50 µg/L	0 µg/L	5623814,18900*	974837,01140	,000	3307154,7520	7940473,6260
	100 µg/L	299229,44590	974837,01140	,949	-2017429,9910	2615888,8830
100 µg/L	0 µg/L	5324584,74300*	974837,01140	,000	3007925,3060	7641244,1800
	50 µg/L	-299229,44590	974837,01140	,949	-2615888,8830	2017429,9910

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons

Dependent Variable: OD600=1.5, CA-immobilized

Tukey HSD

(I) 1.5 CA	(J) 1.5 CA	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0 µg/L	50 µg/L	-5572674,82400*	1561850,49600	,002	-9286338,8410	-1859010,8070
	100 µg/L	-11855356,89000*	1516569,81500	,000	-15461355,5200	-8249358,2610
50 µg/L	0 µg/L	5572674,82400*	1561850,49600	,002	1859010,8070	9286338,8410
	100 µg/L	-6282682,06800*	1561850,49600	,000	-9996346,0850	-2569018,0510
100 µg/L	0 µg/L	11855356,89000*	1516569,81500	,000	8249358,2610	15461355,5200
	50 µg/L	6282682,06800*	1561850,49600	,000	2569018,0510	9996346,0850

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons

Dependent Variable: OD600=0.1, PCL-immobilized

Tukey HSD

(I) 0.1 PCL	(J) 0.1 PCL	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0 µg/L	50 µg/L	-3849297,51700	2073467,82500	,158	-8804599,7670	1106004,7330
	100 µg/L	-15386050,66000*	2073467,82500	,000	-20341352,9100	-10430748,4100
50 µg/L	0 µg/L	3849297,51700	2073467,82500	,158	-1106004,7330	8804599,7670
	100 µg/L	-11536753,14000*	2264719,02800	,000	-16949119,4100	-6124386,8850
100 µg/L	0 µg/L	15386050,66000*	2073467,82500	,000	10430748,4100	20341352,9100
	50 µg/L	11536753,14000*	2264719,02800	,000	6124386,8850	16949119,4100

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons

Dependent Variable: OD600=0.5, PCL-immobilized

Tukey HSD

(I) 0.5 PCL	(J) 0.5 PCL	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0 µg/L	50 µg/L	-14019771,48000	11484974,29000	,444	-41360976,4100	13321433,4400
	100 µg/L	-64221147,70000*	11484974,29000	,000	-91562352,6300	-36879942,7700
50 µg/L	0 µg/L	14019771,48000	11484974,29000	,444	-13321433,4400	41360976,4100
	100 µg/L	-50201376,22000*	11484974,29000	,000	-77542581,1400	-22860171,2900
100 µg/L	0 µg/L	64221147,70000*	11484974,29000	,000	36879942,7700	91562352,6300
	50 µg/L	50201376,22000*	11484974,29000	,000	22860171,2900	77542581,1400

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons

Dependent Variable: OD600=1.5, PCL-immobilized

Tukey HSD

(I) 1.5 PCL	(J) 1.5 PCL	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0 µg/L	50 µg/L	995532,30490	3880739,12300	,964	-8259877,9650	10250942,5700
	100 µg/L	-26374018,27000*	3540282,49500	,000	-34817452,8600	-17930583,6700
50 µg/L	0 µg/L	-995532,30490	3880739,12300	,964	-10250942,5700	8259877,9650
	100 µg/L	-27369550,57000*	3929369,18700	,000	-36740941,6300	-17998159,5100
100 µg/L	0 µg/L	26374018,27000*	3540282,49500	,000	17930583,6700	34817452,8600
	50 µg/L	27369550,57000*	3929369,18700	,000	17998159,5100	36740941,6300

*. The mean difference is significant at the 0.05 level.

As (V) Detection

Multiple Comparisons

Dependent Variable: As (V) detection

Tukey HSD

(I) Ca_Pcl	(J) Ca_Pcl	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Ca_0,5_50	Ca_0,5_100	-2917431,514000000	1990803,656000000	,461	-8095671,49500000	2260808,46600000
	Pcl_0,5_50	-5233808,286000000*	1990803,656000000	,047	-10412048,27000000	-55568,30504000
	Pcl_0,5_100	-15150810,700000001*	1990803,656000000	,000	-20329050,68000000	-9972570,71900000
Pcl_0,5_50	Ca_0,5_50	5233808,286000000*	1990803,656000000	,047	55568,30504000	10412048,27000000
	Ca_0,5_100	2316376,771000000	1990803,656000000	,651	-2861863,20900000	7494616,75200000
	Pcl_0,5_100	-9917002,414000000*	1990803,656000000	,000	-15095242,39000000	-4738762,43400000

*. The mean difference is significant at the 0.05 level.

Response time_viability

Multiple Comparisons

Dependent Variable: Uninduced PCL – IB

Paired – Sample Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Control - Zn	71,725	,783	,452	69,780	73,669	158,681	2	,000
Pair 2	Control - Mg	37,317	5,045	2,913	24,783	49,851	12,810	2	,006
Pair 3	Control - Mn	40,070	1,377	,795	36,649	43,490	50,404	2	,000
Pair 4	Control - Mo	42,191	,887	,627	34,224	50,157	67,290	1	,009
Pair 5	Control - Fe	16,111	13,528	9,566	-105,431	137,653	1,684	1	,341
Pair 6	Control - Cu	64,068	3,076	2,175	36,429	91,708	29,453	1	,022
Pair 8	Control - Ag	19,992	6,264	4,429	-36,289	76,274	4,514	1	,139

*. The mean difference is significant at the 0.05 level.

CURRICULUM VITAE

Surname, Name: Arık Kınalı, Nehir

EDUCATION

Degree	Institution	Year of Graduation
MS	IKCU Biocomposite Engineering	2017
BS	Ege University Bioengineering	2015
High School	Bornova Science High School, İzmir	2010

Year	Place	Enrollment
2021-Present	Turkish Patent and Trademark Office	Engineer
2017-2021	Akdeniz University, Bioengineering	Research Assistant
2014, June-September	National Hellenic Research Foundation, Athens, Greece	Intern

FOREIGN LANGUAGES

Advanced English

PUBLICATIONS

1. MD Chatzidaki, MD., Arik, N., Monteil, J., Papadimitriou, V., Leal-Calderon, F., Xenakis, A. "Microemulsion versus emulsion as effective carrier of hydroxytyrosol", Colloids and Surfaces B: Biointerfaces, 137, 146-151 (2016).

2. Horzum, N., Arik, N., Truong, YB. "Nanofibers for fiber-reinforced composites", Fiber Technology for Fiber-Reinforced Composites, 251-275 (2017).

3. Arik, N., Inan, A., Ibis, F., Demirci, EA., Karaman, O., Ercan, UK., Horzum, N. "Modification of electrospun PVA/PAA scaffolds by cold atmospheric plasma:

alignment, antibacterial activity, and biocompatibility", Polymer Bulletin 76 (2), 797-812 (2019).

4. Polat, NH., Kınalı, NA. "Curcumin-loaded bio-based electrospun polyurethane scaffolds", Cumhuriyet Science Journal 40 (1), 125-135, (2019).

5. Arik, N., Horzum, N., Truong, YB. "Development and Characterizations of Engineered Electrospun Bio-Based Polyurethane Containing Essential Oils" Membranes 12 (2), (2022).

PROJECTS and SCHOLARSHIPS

Researcher in IKCU Scientific Research Projects Coordination Unit under grant number 2016-TYL-FEBE-0036, 2016-2017.

Student in METU Scientific Research Projects Coordination Unit under grant number ÇDAP-108-2021-10659, 2021-2023.