AN INVESTIGATION ON THE OPTIMIZED PRODUCTION OF A MICROALGAE-BASED BIOPOLYMER USING A NOVEL MEDIA COMPOSITION FOR CHLORELLA VULGARIS

MOHAMMED SAAD ABDULLAH AL-HAMMADI

Thesis for the Master's Program in Bioengineering

Graduate School

Izmir University of Economics

Izmir

2023

AN INVESTIGATION ON THE OPTIMIZED PRODUCTION OF A MICROALGAE-BASED BIOPOLYMER USING A NOVEL MEDIA COMPOSITION FOR CHLORELLA VULGARIS

MOHAMMED SAAD ABDULLAH AL-HAMMADI

THESIS ADVISOR: ASSOC. PROF. DR. MINE GÜNGÖRMÜŞLER

A Master's Thesis

Submitted to

the Graduate School of Izmir University of Economics

the Department of Bioengineering

Izmir

2023

ETHICAL DECLARATION

I hereby declare that I am the sole author of this thesis and that I have conducted my work in accordance with academic rules and ethical behavior at every stage from the planning of the thesis to its defense. I confirm that I have cited all ideas, information, and findings that are not specific to my study, as required by the code of ethical behavior, and that all statements not cited are my own.

Name, Surname: Mohammed Saad Abdullah AL-HAMMADI

Date: 23.08.2023

ABSTRACT

AN INVESTIGATION ON THE OPTIMIZED PRODUCTION OF A MICROALGAE-BASED BIOPOLYMER USING A NOVEL MEDIA COMPOSITION FOR CHLORELLA VULGARIS

Al- hammadi, Mohammed

Master's Program in Bioengineering

Advisor: Assoc. Prof. Dr. Mine Güngörmüşler

August, 2023

A novel culture media that contains commercial NPK fertilizer with urea was successfully made and optimized for *Chlorella vulgaris* and it showed a good potential for growing microalgae, and the maximum weight of the obtained biomass was 280 mg/L. Furthermore, the obtained biomass was successfully harvested by centrifugation and a modified way using synthesized magnetic particles with a harvesting efficiency of 92.9% ∓ 1.322 for 50 ml culture media and then recycled and utilized in bioplastic production. PLA powder was mixed with *Chlorella vulgaris* biomass in the solvent-casting method and for the first time, two steps of the annealing process were applied for *Chlorella vulgaris* biomass with PLA at 105° C, and compared to a method without the annealing process, the results showed improved tensile strength and the maximum average was 15.646 ±1.631 MPa, and as far as we know, this is the highest strength obtained by utilizing *Chlorella vulgaris* with PLA in solvent casting method.

Moreover, biodegradation was observed in seawater, and the produced polymer was significantly degraded in the seawater.

Keywords: Biomaterial, PLA/ *C. vulgaris* blend, Biopolymer, Sustainable development, Microalgae

ÖZET

CHLORELLA VULGARIS İÇİN YENİ BİR ORTAM BİLEŞİMİ KULLANARAK MİKROALG BAZLI BİYOPOLİMERİN OPTİMİZE EDİLMİŞ ÜRETİMİ ÜZERİNE BİR ARAŞTIRMA

Al-hammadi, Mohammed

Biyomühendislik Yüksek Lisans Programı

Tez Danışmanı: Doç. Dr. Mine Güngörmüşler

Ağustos, 2023

Üre ile ticari NPK gübresi içeren yeni bir kültür ortamı başarıyla yapıldı ve *Chlorella vulgaris* için optimize edildi ve mikroalg gelişimi için iyi bir potansiyel gösterdi ve elde edilen biyokütlenin maksimum ağırlığı 280 mg/L idi. Ayrıca, elde edilen biyokütle, 50 ml kültür ortamı için %92,9 ∓ 1,322 hasat verimliliğine sahip sentezlenmiş manyetik parçacıklar kullanılarak santrifüjleme ve modifiye edilmiş bir yöntemle başarılı bir şekilde toplandı ve daha sonra geri dönüştürülerek biyoplastik üretiminde kullanıldı. PLA tozu *Chlorella vulgaris* biyokütlesi ile solvent-döküm yönteminde karıştırılmış ve ilk kez 105°C'de PLA ile *Chlorella vulgaris* biyokütlesi için iki aşamalı tavlama işlemi uygulanmış ve tavlama işlemi yapılmayan bir yöntemle kıyaslanmıştır. Sonuçlar, çekme dayanımının iyileştiğini ve maksimum ortalamanın 15.646 ±1.631 MPa olduğunu gösterdi ve bu, bildiğimiz kadarıyla, solvent döküm yönteminde PLA ile birlikte *Chlorella vulgaris* kullanılarak elde edilen en yüksek dayanımdır. Ayrıca deniz suyunda biyolojik bozunma gözlenmiş ve üretilen polimer

deniz suyunda önemli ölçüde bozunmuştur.

Anahtar Kelimeler: Biomaterial, PLA/ *C. vulgaris* harmanı, Biyopolimer, Sürdürülebilir kalkınma, Mikroalgler

Dedicated to my magnificent family and fabulous friends

ACKNOWLEDGEMENTS

I would express my sincerest gratitude for my advisor Assoc. Prof. Dr. Mine Güngörmüşler for her patience with me and her constant support that has been tangible in all aspects, and you would not be reading this now without her support because simply, she is the secret of my success. Additionally, I would thank all my teachers who taught me at Izmir University of Economics for the precious information that provided me during my study, and special thanks to Zehranur Gıcır Tekin who was supporting me and helped me to operate the devices at the laboratory.

I am thankful for my parents and my brother who stood by my side during all stages of my life and I would not be able to complete my study further without their assistance which was unlimited from my childhood till the current day.

Absolutely, I would not and will never forget my gratitude to all my loyal friends and lovers

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

1.1. Plastic Impact on Human Health

Using plastic and plastic items for a long time and heating their products during the process of handling or open burning of plastic releases toxic substances into the air, water, and food leading to a risk in public health (Okunola A et al., 2019). In some countries, plastic trash is to blame for one fatality every 30 seconds. It has been discovered that living nearby poorly managed plastic waste areas causes between 400000 and a 1000000 people to die annually from illnesses including cancer. Rats, flies, and mosquitoes—all of which can spread dengue fever and malaria—breed on the plastic waste, which also serves as a breeding ground. Waterway flooding brought on by plastic garbage can transmit diseases that are carried by the water, and children who are exposed to plastic waste and pollution are twice prone to get serious and frequently fatal intestinal diseases like cholera (Sexton, 2019). Simply, plastic impact on human health is summarized in Figure 1 below:

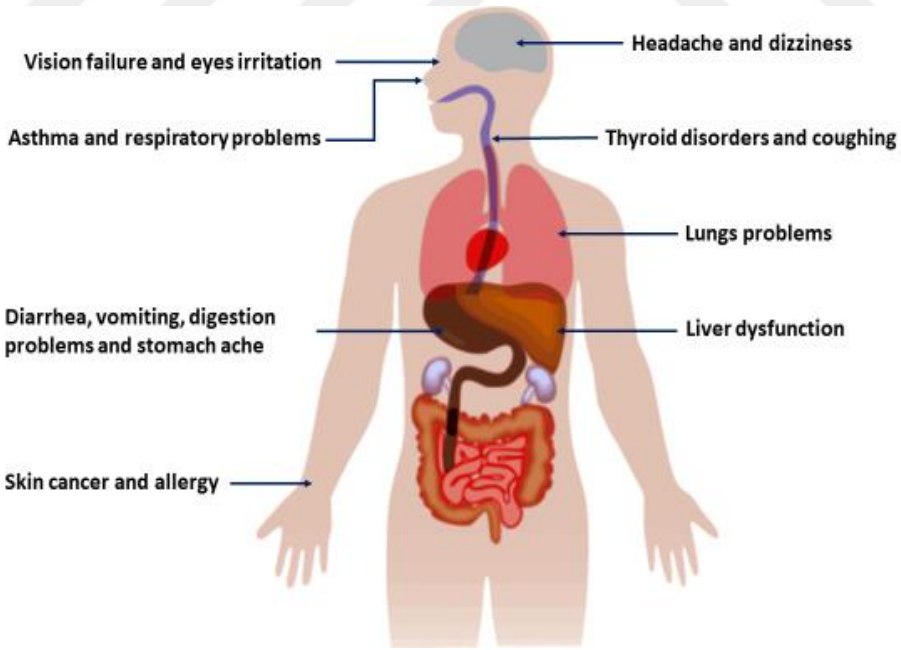


Figure 1. Plastic impact on human health (Source: Proshad et al., 2017).

1.2. Plastic Generation

Plastic manufacturing increased from 2 million tons in 1950 to 4 million tons in 2015, yet over 6.3 billion tons of the 8.3 billion tons created since 1950 are discarded, 9% of plastic garbage is recycled, and only 12% is burned. About 79% of plastic waste is found in the natural environment, and if current trends continue, this will be the equivalent of 12 billion tons by 2050 (Mullarkey, 2017). The plastic breaks down by a process called photodegradation when ultraviolet light breaks the plastic into smaller particles over time. However, landfills struggle to achieve this by their design. Every day new soil is added, giving the sun a chance to heat the waste before another layer is added. Oceanic photodegradation occurs more quickly, but the sun's rays also break down plastic into microscopic particles known as "microplastics," which harm millions of marine animals every year. (Gammage, 2022). The figure of the latest statistics about the highest countries that contribute to ocean pollution by plastic waste is inserted below (Figure 2), and a summary of plastic impact on marine life is shown below in Table 1. However, these wastes are not necessarily created by the consumption of these countries themselves, while they could be imported from different countries for a “recycling purpose” but they end up in the ocean because the capacity of recycling all these wastes is limited (Harrabin and Edgington, 2019).

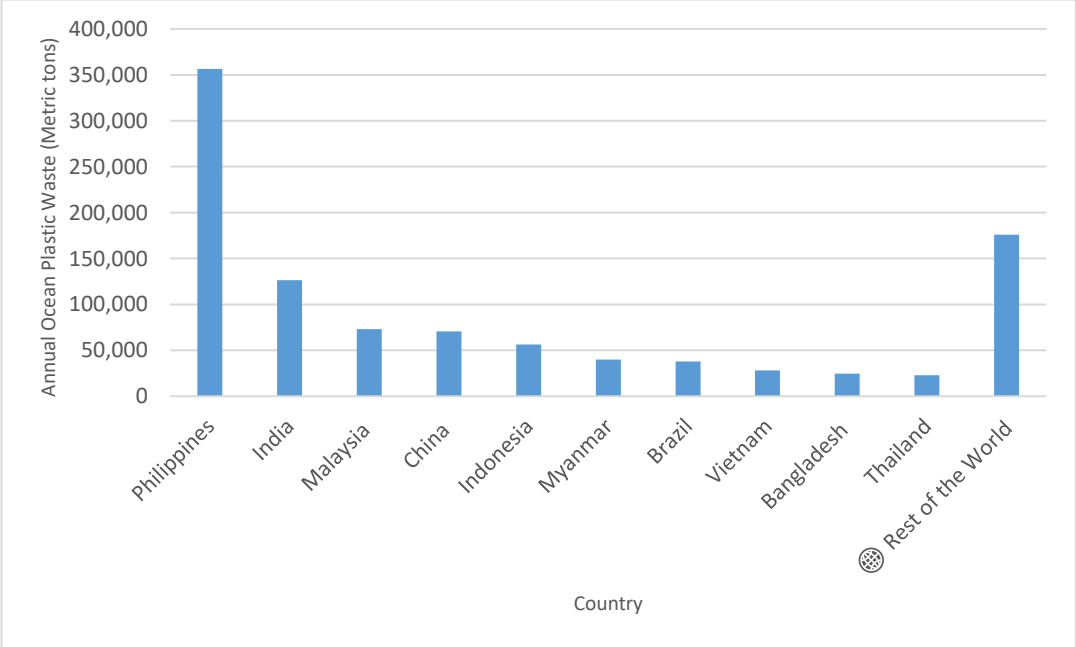


Figure 2. The latest statistics about the highest contributor to ocean pollution by plastic waste (Source: Wicaksono, 2023).

1.3. Plastics and Polymers

We all know that all types of plastics are considered "polymers". Nevertheless, not all polymers have to be plastics. Polymers and plastics are manufactured by many different methods and processes depending on many factors such as the cost (Toghyani, Matthews and Varis, 2020), processing time (Cenna and Mathew, 1997), and more importantly, the characteristics of the material itself (Kazmer, 2016) which might affect the degradation rate of the produced polymer (Maraveas, 2020). Simply, plastic manufacturing includes the polymerization process after preparing the raw material which involves producing long chains of polymers from small molecules or monomers to obtain a stable chain of a polymer (Hamielec, Macgregor and Penlidis, 1989), followed by additives addition to obtaining the desired properties (Ambrogi et al., 2017), and finally, molding the polymer to form a specific shape (Park and Lee, 2012).

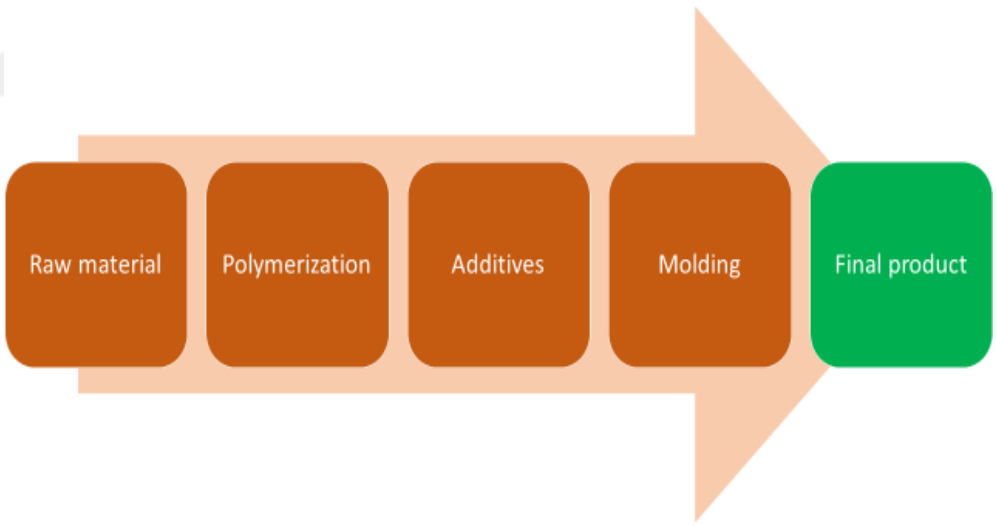


Figure 3. Polymer manufacturing process

A polymer can generally be classified as "biodegradable" if more than 60-70% of the specimen degrades within 6 months. There are different types of polymers, fossil-based non-biodegradable plastics like polyethylene terephthalate (PET) (Awaja et al., 2004), polypropylene (PP) (Tokiwa et al., 2009), polyvinyl chloride (PVC) (Zhang et al., 2015; ComaniţĂ et al., 2016), and polystyrene (PS) (Merrington, 2011),

fossil-based biodegradable like polybutylene succinate (PBS) (Gigli et al., 2016; Hongsriphan and Pinpueng, 2019) polybutylene adipate terephthalate (PBAT) (Ren et al., 2010), polycaprolactone (PCL) (Mohamed and Yusoh, 2015), and polyvinyl alcohol (PVA or PVOH) (Gajra et al., 2012; Singh et al., 2020). However, these plastics are not eco-friendly since fossil fuel is required for their production. Thus, bio-based plastics are preferred, especially biodegradable ones because not all bio-based plastics are biodegradable. In-store bio-based plastics including bio-polyamide (bio-PA), bio-polyethylene-terephthalate (bio-PET), bio-poly trimethylene terephthalate (bio-PTT), bio-polypropylene (bio-PP), and bio-polyethylene (bio-PE) are almost all non-biodegradable. (Rahman and Bhoi, 2021). While bio-based biodegradable plastics involve a microbial fermentation process to obtain biodegradable plastics such as polyhydroxyalkanoates (PHAs) (Lu, Tappel and Nomura, 2009; Chanprateep, 2010; Kourmentza et al., 2017; Costa et al., 2019), or utilizing renewable sources to obtain plastics like polylactic acid (PLA) such as corn starch (Rogers, 2015), or biomass (Talukder, Das and Wu, 2012). Plastic types and its life cycle are shown below in Figure 4 and 5, respectively.

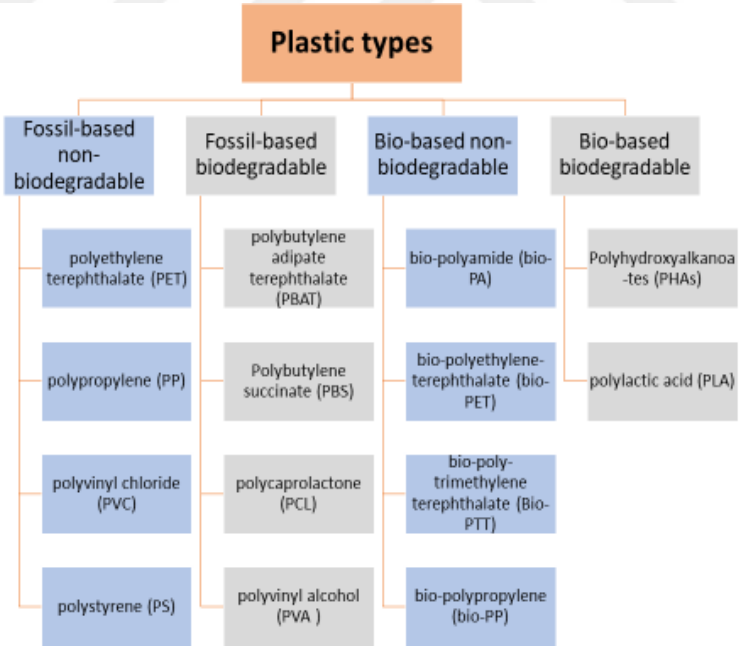


Figure 4. Plastic types according to its source and biodegradability.

Figure 5. Lifecycle of plastics (Source: Devasahayam et al., 2019).

1.4. Polylactic acid (PLA)

Polylactic acid (PLA), a plastic alternative made from fermented plant starch (typically maize), is quickly replacing traditional plastics made from petroleum. When certain countries and states follow the lead of Italy, South Africa, Turkey, Uganda, and San Francisco and ban plastic shopping bags, which are credited with causing so-called "white pollution" throughout the world, PLA is poised to play a large role as a workable, biodegradable replacement. Proponents also advocate using PLA as a way to reduce greenhouse gas emissions in a world that is fast warming because it is technically "carbon neutral" because it originates from renewable, carbon-absorbing plants. (West, 2020). When PLA is burned, no toxix gasses will be released, and it is considered as biocompatible and due to the fact that PLA is biodegradable and can be hydrolyzed into lactic acid, which is then introduced into the metabolic pathway, it is currently frequently employed in medical applications and has received FDA approval. (Rahman and Miller, 2017). However, there are still problems related to PLA usages like its slow rate of biodegradability, incompatibility with other polymers in recycling, and highly usage of genetically modified corn (although it can be one of the positive effects of PLA as it provides a good opportunity for using genetically engineered crops to do not compete with food sources). PLA may very well break down into its component parts (carbon dioxide and water) in a controlled setting, such as an

industrial composting facility heated to 140 F and fed to microorganisms, in less than three months. In a compost bin or landfill that is so tightly packed that there is little to no light or oxygen available to aid in the process, it will take much longer (West, 2020). Therefore, new aims were investigated to achieve a better degradation rate by different strategies such as inclusion of additives, molecular weight reduction, size reduction, and reactive blending are followed to accelerate the biodegradation rate (Bher, Cho and Auras, 2023). Nitrogen source is a critical factor that plays a direct role in polymer degradation since using a nitrogen source is necessary to feed the microorganisms (Bonifer et al., 2019), for instance, soytone addition to manure extract with *Pseudomonas geniculata* WS3 is a good option to accelerate the biodegradation of PLA because soytone provided nitrogen for the microorganisms which triggered microorganisms' activity (Boonluksiri, Prapagdee and Sombatsompop, 2021). Molecular weight is also important to accelerate the biodegradation of PLA by reducing the molecular weight of PLA films. Ultraviolet (UV) was also used to reduce the molecular weight before burying them in soil which was provided with manure, green yard waste, and sludge from, rice, dairy, and coconut milk companies to speed up the biodegradation process (Pattanasuttichonlakul, Sombatsompop and Prapagdee, 2018). Another strategy to accelerate the degradation of PLA is blending it with other biomaterials. (Lv et al., 2017) blended wood flour/starch with PLA and the results showed that biodegradation rate was increased when the produced polymer was buried in the soil because starch provided the microorganisms with carbon source (glucose) which activated the microorganisms.

1.5. PLA and Microalgae

PLA is now a commercial bioplastic, and because it is made of 100% biological materials like organic materials such as corn and sugar beet, it was indicated by several authors that PLA is an ideal candidate for mixing with microalgal biomass and producing biodegradable plastic (Rahman and Miller, 2017; Rajpoot et al., 2022; Cheah et al., 2023). One of these species of microalgae, *Chlorella vulgaris* is an important microalga due to its easy to cultivate, and fast growth (Ma'mun, Wahyudi and Raghdanesa, 2022). Algae was analyzed in very few studies to obtain bioplastic by blending its biomass with different biopolymers such as PVA (Dianursanti and Khalis, 2018) and PLA (Adli et al., 2018a) and it was found that blends are necessary

for commercial scales compared to 100% produced bioplastics from microalgae (Cinar et al., 2020). Furthermore, microalgae blending with PLA can play as an accelerator for the degradation of PLA/algae plastic because the biomass is a good source of nitrogen (Kalita et al., 2021). However, the main challenge of growing microalgae is the cost of the process and this is why a novel media was proposed in this study to overcome this challenge. Very few studies indicated the ability to grow microalgae by utilizing organic fertilizer (Lam and Lee, 2012) such as NPK fertilizer which is suitable to grow microalgae (Abdulsamad, Varghese and Thajudeen, 2019) including *C. vulgaris* (Mtaki, Kyewalyanga and Mtolera, 2021; Sadak Turhan and Şensoy, 2022). Therefore, NPK+TE 20.20.20 was optimized with urea as a nitrogen source and a strategy for urea treatment and investigating the tolerance of *C. vulgaris* to grow in high concentrations of urea which can be utilized further to treat the urine which was studied by (Jaatinen, Lakaniemi and Rintala, 2016; Zou et al., 2020) by supplementing the urine with NPK fertilizer since it has the required trace elements for *C. vulgaris* (see the composition of NPK below in Table 2). Moreover, the obtained biomass was utilized further to produce bioplastic after harvesting the biomass by centrifugation and by a modified method of magnetic particles. The method of producing PLA/algae blend is very critical, for example, melt mixing for PLA/algae was negative in terms of flexibility, while solvent casting was a proper way to produce PLA/ algae composite (Adli et al., 2018). Solvent casting has also better clarity and better uniformity of thickness than extrusion (Milind et al., 2013) and it was not investigated widely. Thus, the solvent casting method was followed to produce the biopolymer by two steps of an annealing process which was investigated for the first time- study for PLA/ *C. vulgaris* blend, and how the annealing process with biomass concentration affected the tensile strength with biodegradation rate.

Table 1. Plastic impact and generation worldwide

Plastic Impact and Generation	Number	Unit	References
Created Plastics yearly	300	Million ton	(CondorFerries, 2021)
Plastics end up in the ocean yearly	4.8-12.7	Million ton	(CondorFerries, 2021)
Animals death yearly	100	Million	(CondorFerries, 2021)
Ingested Plastics with animals, yearly	12	Thousand Ton	(CondorFerries, 2021)
Ingestible microplastics yearly	236	Thousand Ton	(CondorFerries, 2021)
Floating Plastics yearly	269	Thousand Ton	(CondorFerries, 2021)
Ingested microplastics by Fin whales	3-10	Million	(Garthwaite, 2021)
Consumed microplastic by a human yearly	78-211	Thousand	(Armstrong, 2022)
Recycled Plastics	9	Percentage	(Oecd, 2022)
Plastic that becomes waste	75	Percentage	(Vuleta, 2022)
Estimated microscopic pieces in the ocean	51	Trillion	(Bonastre and Lopez, 2022)
Approximate weight of the microplastic in the ocean	269	Thousand Ton	(Bonastre and Lopez, 2022)
Estimated covered area by plastic waste in the ocean	580	Thousand per km^2	(Wilcox et al., 2015)

CHAPTER 2: METHODS

2.1. Media Preparation for C. vulgaris

Commercial NPK+TE fertilizer and Urea were purchased from TerraOrga® and Alokrais® from Turkey, respectively. The chemical composition is shown below in Table 2. Different concentrations were optimized according to Box Behnken design, Design Expert (see Table 4). They were dissolved using ultra-pure water since NPK+TE and urea are water soluble and they do not change the color of the water in a case that can affect how the light passes through the media, and eventually, affect the photosynthesis process due to the low light intake by microalgae. The media was mixed with a magnetic stirrer for 30 minutes till the media became completely homogenous and no big particles can be seen. After that, the media was autoclaved and cooled down to reach room temperature. Finally, the obtained results of biomass in both media (BG-11 and NPK with urea) were compared under the same conditions at the lab scale.

Table 2. Chemical composition of NPK fertilizer

Chemical	W/W %
Total nitrogen (N)	20
Nitrate nitrogen (N0-N)	5.5
Ammonium nitrogen (NH-N)	4
Urea nitrogen	10.5
Neutral ammonium citrate and phosphorus pentoxide (P_2O_5)	20
Phosphorus pentoxide (P_2O_5)	20
Potassium oxide (K_2O)	20
Boron (B)	0.02
Copper (Cu)	0.02
Manganese (Mn)	0.02
Molybdenum (Mo)	0.002
Zinc (Zn)	0.02

2.2. Microalgae Strain and Culture Condition

2.2.1. Lab scale:

Chlorella vulgaris (SAG 211-12) culture was received from Ege University which is located in Izmir-Turkey and cultivated in BG-11 media. After that, the microalgae were subcultured utilizing commercial NPK+TE fertilizer with urea, by using Erlenmeyer flasks with 250 ml capacity and the working area was 100 ml which had 20% inoculum. The initial pH was changeable according to the experimental design, which was set by utilizing 0.1M NaOH and 0.1M HCl and measured by (Milwaukee, MW106). The flasks were kept in controlled conditions at 25 ± 2° C in a shaker incubator (Shaker, IKA-KS 4000 ic control, IKA Turkey Laboratuvar ve Proses Teknolojileri A.Ş./İstanbul, IKA, German) with rotation speed 120 rpm and it was provided with two LED lights with 5 watts and 2800-3000 lux in total and this range is enough to grow *C. vulgaris* (Rai et al., 2014; Gammanpila, Rupasinghe and Subasinghe, 2015; Kendirlioglu, Agirman and Cetin, 2015; Febrieni, Sedjati and Yudiati, 2020) to simulate the sunshine with light: dark cycle 16:8 h for five days followed by two days of continuous light, because the dark cycle was investigated in some species of microalgae, and it was indicated that dark cycle leads to losing or lowering the biomass and lipids (Han et al., 2013; Edmundson and Huesemann, 2015), while air pumping was not provided to check the ability of fertilizer in growing the algae at a low cost. Afterward, the microalgae were maintained in NPK media for a month before starting the analysis, and then the analysis was conducted to obtain the exact results of NPK's ability since taking an inoculum directly from BG-11 and cultivating it in a new media to check the ability of that media is misleading because the results were completely different when an inoculum was taken from BG-11 compared to NPK media.

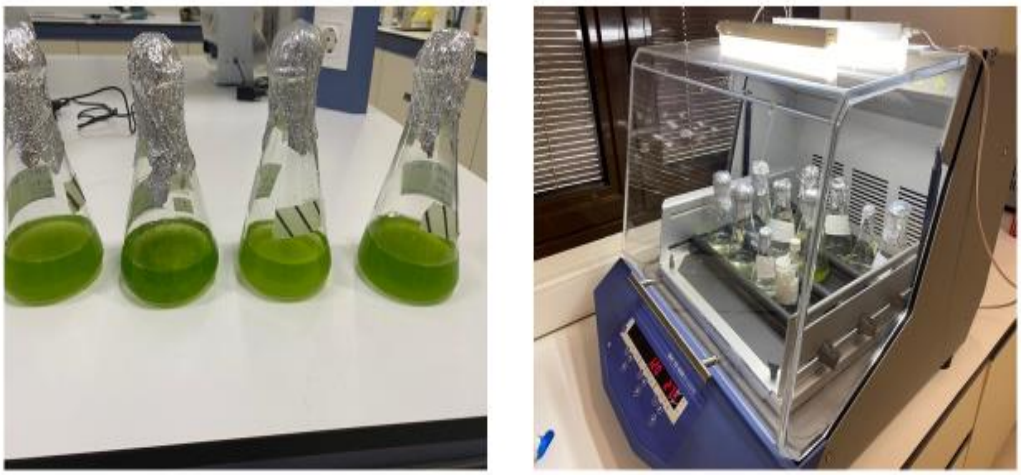


Figure 6. Microalgae cultivation on a lab scale

2.2.2. Non-sterilized conditions:

The microalgae were also cultivated without sterile conditions using plastic bottles with 5 L capacity and two and a half litter working area. The cultivation was conducted without special conditions or controlled conditions; it was at room temperature (20-28° C) with the same LED lights that were provided for the lab scale. Air pumping was provided in this method since it can function as a mixer (instead of the shaker incubator) as well as providing compressed air. The air compressor that was used is the same one for the aquarium (SERA 110 PLUS, 5 watts, Germany) which was sufficient for two photobioreactors by providing it with air stones that play a role as a sparger. The duration time of operating air pumping was continuously 9 hours per day, and the initial pH was 9.

Figure 7. Microalgae cultivation without sterilization conditions. The first day of the cultivation appears on the right side and the last day on the left side.

2.3. Statistical Design:

Culture media composition was designed according to Box Behnken design by using Design Expert® software (activated version 7) and t-test for tensile strength analysis by using IBM® SPSS® Statistics (activated version 25). Both software were provided by Izmir University of Economics.

2.4. Growth Profiling of C. vulgaris:

2.4.1. Absorbance:

The growth was measured by (PerkinElmer, Lambda 750, UK) after its calibration according to the manual of the manufacturer. There was a big difference in the literature to determine the best wavelength for *C. vulgaris* detection. It was mentioned in the literature 550 nm (Schagerl et al., 2022), 650 nm (Deniz, 2020), 700 nm (El-Naggar et al., 2020), 750 nm (Chioccioli, Hankamer and Ross, 2014) are the best for microalgae detection. However, it was unclear which wavelength was the best. Therefore, the microalgae were scanned by different wavelengths (550, 650, 700, 750) after waiting for 30 minutes since the device was turned on to warm up the detectors to determine the best wavelength for this strain of microalgae. The scanning was

performed in two ways, automatically and manually to compare the results and the differences. Automatic analysis was performed by placing quartz cuvettes in the device and then the scanning was done automatically by selecting the wavelength range, while manual scanning was performed by repeating the procedure of the absorbance in every selected wavelength multiple times and the average was calculated to eliminate any error.

2.4.2. Cell Counting:

Cell counting is considered the best way to figure out cell growth, even though it might require time (Lund, Kipling and Le Cren, 1958). Thus, cell counting was performed by using Thoma Counting Chamber (Figure 8 below) with and without dilution of the samples to recognize if there is any effect or error. The microscope which was used for cell counting is (OPTIKA B 150, Italy). A 10x lens was used to locate the chamber at first, and then 40x was used for counting.

Figure 8. Thoma cell counting chamber

2.4.3. Biomass Measurment:

The biomass was calculated according to Total Suspended Solids (T.S.S.) standard. The filters that were used are (DiaFilter, India) for the experiments with a 47 mm diameter and 0.45 micron pore size. The filters were dried for one hour at 105° C

in the oven and then they were placed in the desiccator for 30 minutes, and the procedure was repeated until a steady weight was obtained, then the sampling was taken place and followed by drying at the same duration and temperature that was done previously before sampling. The duration of drying can be changed from time to time according to the lab conditions and humidity. The formula below was used to determine the biomass:

Formula 1. Total Suspended Solids formula for biomass calculation

$$\frac{\text{mg}}{\text{L}}\text{ of TSS} = \frac{\text{Final Weight (g)} - \text{Initial Weight (g)} \times 1000000}{\text{Sample Volume (ml)}}$$

2.4.4. Chlorophyll and Pigment Measurement

Chlorophyll and carotenoids were measured by harvesting 5 ml of microalgae at 4000 rpm for 5 minutes and 5 ml of pure methanol (100%) was added to the obtained biomass and vortexed for 3 minutes. Afterward, the samples were kept in the oven at 60 ° C for 30 minutes and vortexed again and followed by centrifugation at 4100 rpm for 10 minutes. The supernatant which was obtained after the centrifugation was used for spectrophotometer analysis by using different wavelengths, 470 nm, 645 nm, and 662 nm according to the followed method by (Mhatre et al., 2019).

2.5. Harvesting and Drying:

Microalgae were mainly harvested by centrifugation (Combi-514R, Korea) with 5000 rpm for 15 minutes using falcon tubes. According to (Hosseinizand, Sokhansanj and Lim, 2018), the optimal drying temperature is 60- 80° C, and accordingly, the harvested biomass was vortexed and dried in the oven after placing it onto flat glass plates in 60 ° C for two hours and then it was placed into the desiccator for 30 minutes to reach the room temperature and to absorb the leftover humidity. The dried biomass then was collected and saved by using a household food vacuum packaging machine (Vestal, China). Moreover, synthesized magnetic nanoparticles were also examined. With constant stirring, one gram of iron (II) sulfate (FeSO4_7H2O) was diluted in 50 ml of distilled water. The pH of the solution was

adjusted by 1M of NaOH to 12, afterwards, the solution was radiated in the microwave for 2 minutes at 700 W and the magnetic particles were isolated from the solution by using magnet N35, 11,800-12,100 G, with scales 50 mm X 40 mm X 20 mm which was purchased from (Magnet Market, Co.Ltd., Turkey). The magnetic particles were exposed to a 60° C oven drying process overnight before being utilized for the harvesting. The harvested volume was a replica of 50 ml which contained NPK fertilizer and urea with the microalgae. For the detachment of magnetic particles, the same procedure as (Savvidou et al., 2021) did, except without sonication. The following formula was used to determine the harvesting efficiency:

$$Harvesting\ Efficiency\% = \frac{OD_0 - OD_I}{OD_0}.100$$

Figure 9. Synthesized magnetic particles for harvesting

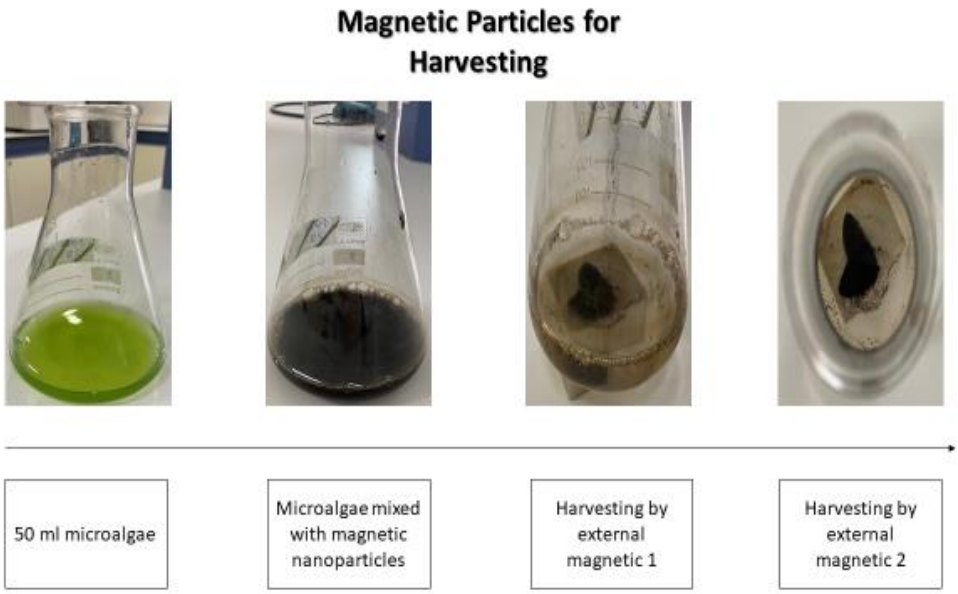


Figure 10. Microalgae harvesting process by magnetic particles

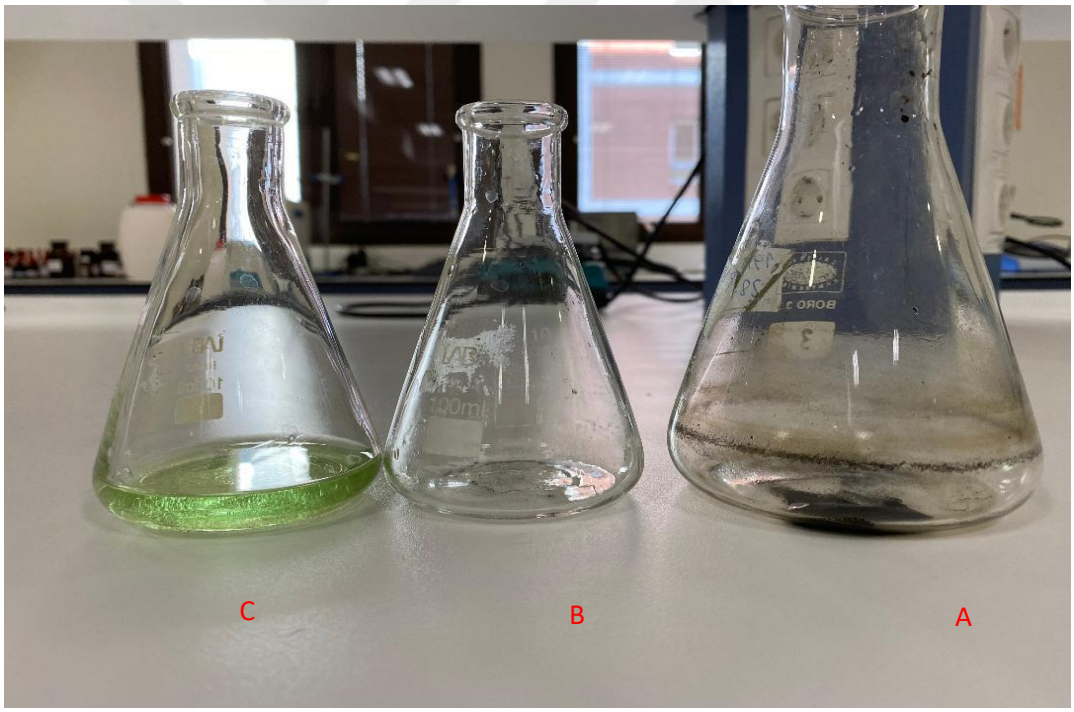


Figure 11. Detached biomass from the magnetic particles. A: The first application of the magnetic field. B: The second application of the magnetic field to separate the left particles after drawing the content into a flask. C: Microalgae after the full separation of the particles

2.6. Polymer Production:

The production of the polymer was performed by using the solvent casting method. Biodegradable biobased PLA powder 100 mesh was purchased from (Keyue Material Store; China) and placed in the desiccator for 30 minutes to come to room temperature after being placed in the oven overnight at 40° C. Afterward, PLA powder was dissolved in chloroform with continuous stirring and biomass was added gradually followed by glycerol. The stirrer operated for 10 minutes at ambient temperature with keeping the beaker partially closed to delay the evaporation of chloroform since fast evaporation leads to minimizing the duration of stirring because chloroform evaporates quickly. Afterward, the solution was poured on two glass plates, the scales of the plate are 19.5 cm x 19.5 cm x 3 mm while the solution was not high density to control the poured solution. The plate was placed in the oven for 10 minutes at 105° C after drying at ambient temperature for two hours. The plate was then heated once more the next day and let to cool to ambient temperature before being dried out entirely overnight at room temperature.

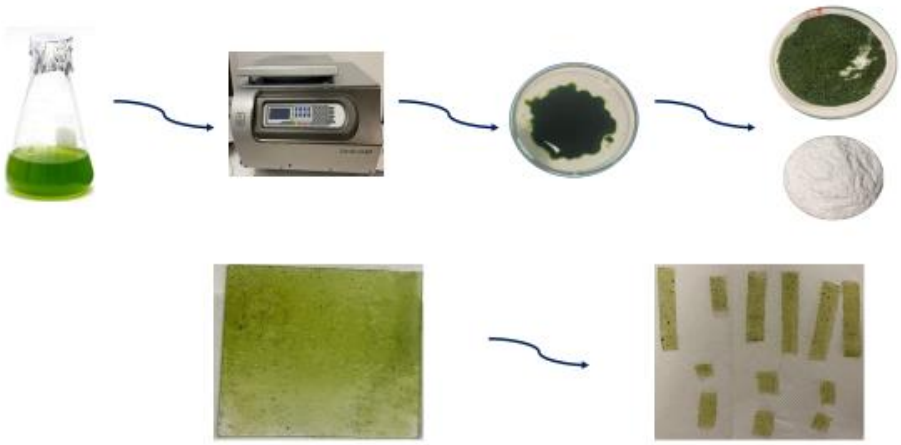


Figure 12. Flow chart of biopolymer production

Figure 13. Biopolymer films and sheet

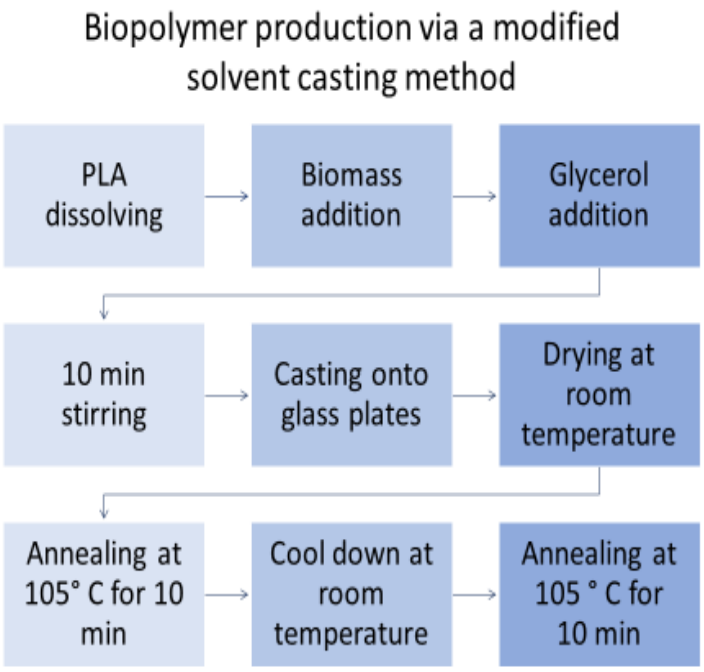


Figure 14. Biopolymer production process

2.7. Tensile Strength:

After being calibrated in accordance with the manufacturer's handbook, the (Stable Micro Systems, TA. XT-Plus texture analyzer, UK) was used to test the tensile

strength in accordance with ASTM D882 with a one-gram applied force.

2.8. Thermal analysis:

The thermal analysis was performed by using Differential Scanning Calorimetry (DSC 4000, PerkinElmer, Netherlands) to determine glass transition temperature (Tg), melting temperature (Tm), and finally cold crystallization temperature (Tcc). The conditions of the test were set initially from 25° C to 190° C since the purchased PLA has 150° C – 165° C, at heating- cooling- heating cycle with a rate of 10° C/ min. The cooling-heating rate was also 10° C/ min. Afterwards, removing thermal history was done by scanning the sample at 180° C and a hold for 2 minutes followed by cooling to 25° C and a second heat at 180° C. The data of thermal analysis were obtained from Pyris Manager software.

2.9. Biodegradation Test:

The produced films were dipped in seawater at the lab (a replica of each) after measuring the weight of each film. The seawater was taken from the Aegean coast which is located in Karantina, Izmir, Turkey (the map is inserted in Figure 15). The seawater was taken from an average area in its cleanness because the activity of microorganisms could be completely different from one area to another one depending on location (Elmanama et al., 2005; Rozen and Belkin, 2005). The seawater was changed every three days to make sure the microorganisms were keeping their activity. The atmospheric temperature of the place where the seawater was gathered was almost the same as the lab temperature of 20°-28° C and the light was on during the daytime and dark during the night. The biodegradation test was taken place using seawater because it is known that PLA by itself takes a long time in marine water (Green, 2012) and due to the problems and impacts that have already been mentioned previously.

Figure 15. Seawater location utilized to test the biodegradation of the produced biopolymer

CHAPTER 3: RESULTS

3.1. Growth Profiling of C. vulgaris and Statistical Results

Microalgae have been grown successfully by the novel media composition and growth detection was observed in spectrophotometer, cell counting, T.S.S. analysis and chlorophyll and carotenoid measurement along with pH observation. The manual scanning of the samples by spectrophotometer showed the ideal wavelength for microalgae detection, on the other hand, the automatic scanning was negative. The results showed that 550 nm is the best wavelength for microalgae detection and these results were obtained from a replicate for each wavelength reading and the average was calculated with the standard deviation as shown in Table 3 below.

Table 3. The average value of different wavelengths for *C. vulgaris* detection

Wavelength (nm)	Absorbance (nm)
550	0.3363 ∓ 0.0014
650	0.2179 ∓ 0.0038
700	0.2234 ∓ 0.0002
750	0.1476 ∓ 0.0016

The results were significant statistically in the Box-Behnken design- quadratic model in terms of biomass weight and cell number as shown in ANOVA analysis in Figure 17 and 18, respectively. The minimum/maximum concentrations of N.P.K. fertilizer and urea were 100/ 1000 and 500/2000 mg/L, respectively. While the minimal and maximal pH value was 7 and 9, respectively. Depending on the added concentration of the culture media, the number of the cells and the size of the cells were significantly different, when only the fertilizer was added (550 mg/L) the cell size was obviously smaller than in another culture where urea was added (550 mg/L Fertilizer and 1200 mg/L urea) as shown in Figure 23 below , and the obtained number of cells took a longer time (one to two weeks) to reach the equivalent number of cells, and this is another reason to add urea in order to accelerate the growth.

The experimental design is shown in Table 4 below, while the obtained results are shown in Table 5. The maximum cell number was more than 2000000 cells per ml after 6 days of growing with an initial number 620000 per ml, while the maximum biomass weight was 280 mg/L with an initial biomass weight 55.55 mg/L for the central point of statistical design and the central points were validated and the growth curve for all central points is shown in Figure 16 below. Chlorophyll and carotenoid measurement was also an indication for the growth. The observation from Table 6 indicates that the concentration of chlorophyll a+ b and carotenoid were maximum 5.953845, and 474.111146 mg/L with an initial concentration 3.2669, and 220.3212 mg/L, respectively. While it was 0.555 mg/L in BG-11.

Chlorophyll a and b, and carotenoid changes during the growth day by day is shown in Figure 22 below. Moreover, pH association with microalgae growth was also observed. When microalgae started to grow, the culture media became alkali and was increasing from the initial pH value8 till the growth phase ended reaching 8.5. All central points showed an association between the cell number and pH value as shown in Figure 21.

Table 4. Box Behnken design setup

Run	Factor 1/ pH	Factor 2/ Fertilizer (mg/L)	Factor 3/ Urea (mg/L)
1	7	1000	1250
2	9	1000	1250
3	8	550	1250
4	8	550	1250
5	9	550	2000
6	8	1000	2000
7	8	550	1250
8	8	550	1250
9	8	100	2000
10	8	1000	500
11	9	550	500
12	8	100	500
13	8	550	1250
14	7	100	1250
15	7	550	500
16	7	550	2000
17	9	100	1250

Table 5. The obtained results in Box Benhnken design. R stands for Response. R1-R6 represent the cell count (cells/ml). R7-R12 represent the Absorbance (nm). R13-R14 represent the biomass weight (mg/L). E+006 refers that the numbers are in millions.

Run	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14
1	197250	434000	187500	98750	93750	165000	0.4369	0.4283	0.2941	0.3267	0.3665	0.4138	66.66	333.33
2	276250	198000	201250	108750	585000	175000	0.2901	0.2938	0.2985	0.3425	0.4588	0.6326	66.66	277.77
3	620000	760000	750000	1.17E+006	1.2E+006	2.44E+006	0.2885	0.3092	0.3276	0.338	0.3535	0.3725	55.55	280
4	680000	650000	740000	950000	1.33E+006	1.55E+006	0.2892	0.3015	0.3091	0.3347	0.3515	0.4251	44.44	200
5	86250	11875	130000	290000	675000	178750	0.2246	0.2202	0.2478	0.3394	0.5843	0.502	22.22	177.77
6	102500	165000	153750	312500	662500	98750	0.2221	0.2591	0.3739	0.6883	0.7074	0.4816	33.33	55.55
7	660000	660000	760000	810000	1.75E+006	1.48E+006	0.2879	0.3021	0.3141	0.3276	0.3427	0.3517	44.44	200
8	810000	530000	940000	1.19E+006	1.2E+006	1.3E+006	0.289	0.3178	0.3155	0.3291	0.3478	0.3526	44.44	200
9	35000	32500	55000	200000	52500	111250	0.1448	0.1593	0.2876	0.7146	0.6364	0.4236	11.11	22.22
10	260000	342500	416250	750000	500000	887500	0.4844	0.5438	0.7559	1.1501	0.9568	0.9091	33.33	188.88
11	273750	295000	321250	412000	487500	500000	0.5062	0.5823	0.6652	0.7061	0.749	0.8294	22.22	322.22
12	231250	326250	445000	1.075000	1.112500	837500	0.4988	0.5655	0.7418	1.4654	1.3592	1.2837	33.33	277.77
13	650000	660000	740000	950000	1.35E+006	1.75E+006	0.2879	0.3026	0.3099	0.3356	0.3501	0.3579	55.55	250
14	228750	201250	153750	212500	350000	287500	0.4391	0.5456	0.5543	0.5821	0.6061	0.6616	44.44	311.11
15	191250	235000	172500	325000	225000	387500	0.4611	0.561	0.596	0.6213	0.6938	0.7669	55.55	322.22

16	187500	201250	270000	350000	262500	100000	0.4143	0.5176	0.5055	0.5332	0.6287	0.5999	55.55	88.88
17	277500	157500	227500	162500	162500	250000	0.4129	0.5242	0.5086	0.5148	0.5398	0.5445	11.11	144.44

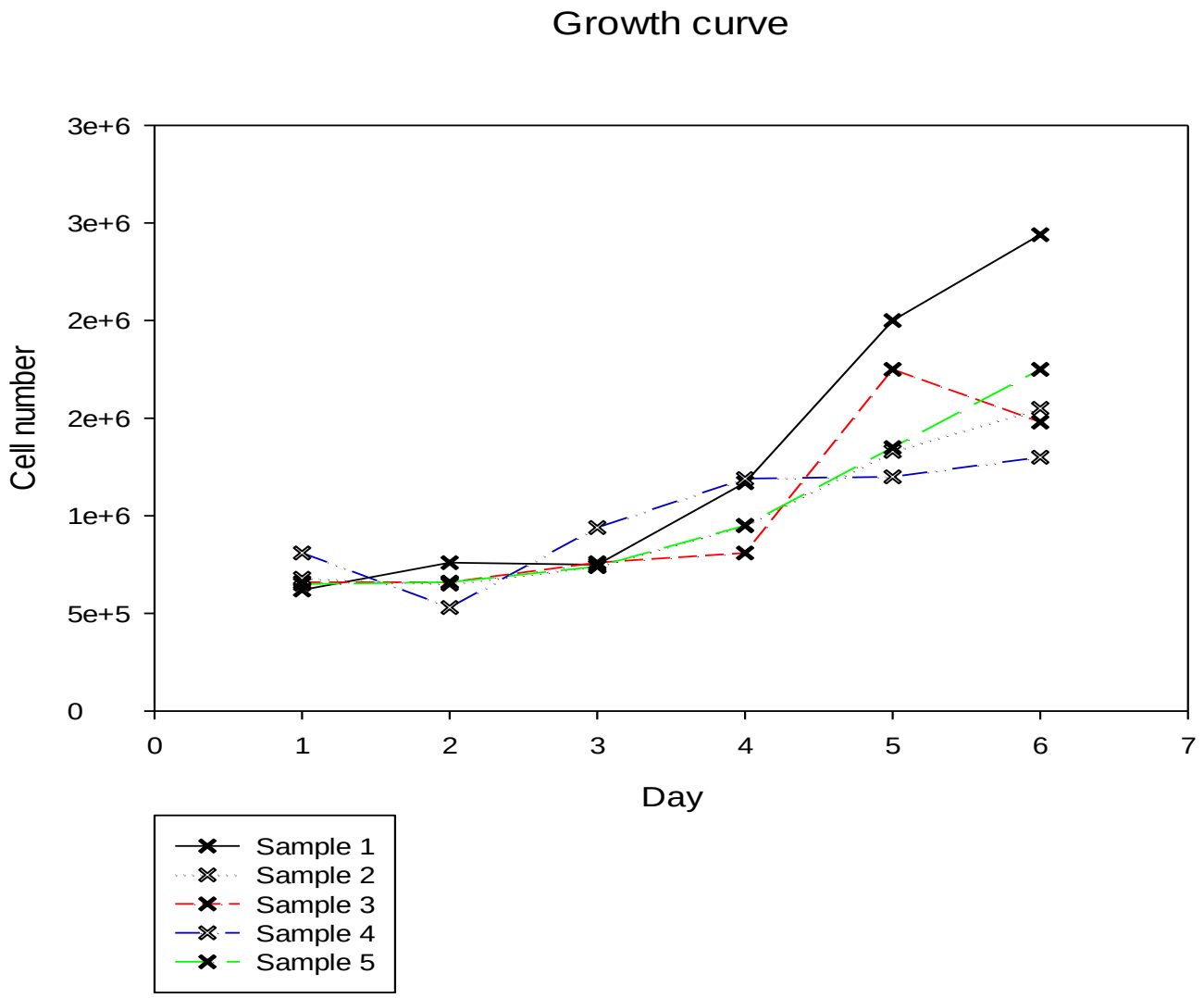

Growth curve
Cell number
3e+6
3e+6
2e+6
2e+6
1e+6
5e+5
0
0
1
2
3
4
5
6
7
Day
Sample 1
Sample 2
Sample 3
Sample 4
Sample 5

Figure 16. Growth curve by utilizing the fertilizer with urea and the maximum obtained cell count. 1e+6 stands for a million.

ANOVA for Response Surface Quadratic Model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1.271E+005	9	14123.01	13.25	0.0013	significant
A-ph	*387.25*	*1*	*387.25*	*0.36*	*0.5657*	
B-fertilizer	*985.46*	*1*	*985.46*	*0.92*	*0.3683*	
C-urea	*73472.86*	*1*	*73472.86*	*68.93*	*< 0.0001*	
AB	*22483.50*	*1*	*22483.50*	*21.09*	*0.0025*	
AC	*1975.36*	*1*	*1975.36*	*1.85*	*0.2156*	
BC	*3734.43*	*1*	*3734.43*	*3.50*	*0.1034*	
A^2	*7619.59*	*1*	*7619.59*	*7.15*	*0.0318*	
B^2	*10162.15*	*1*	*10162.15*	*9.53*	*0.0176*	
C^2	*6997.85*	*1*	*6997.85*	*6.56*	*0.0374*	
Residual	7461.86	7	1065.98			
Lack of Fit	*1941.86*	*3*	*647.29*	*0.47*	*0.7199*	*not significant*
Pure Error	*5520.00*	*4*	*1380.00*			
Cor Total	1.346E+005	16				

Std. Dev.	32.65	R-Squared	0.9445
Mean	203.72	Adj R-Squared	0.8733
C.V. %	16.03	Pred R-Square	0.7050
PRESS	39694.82	Adeq Precisior	12.701

Figure 17. Biomass results after performing ANOVA analysis

ANOVA for Response Surface Quadratic Model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	7.370E+012	9	8.188E+011	6.39	0.0115	significant
A-ph	*3.352E+009*	*1*	*3.352E+009*	*0.026*	*0.8761*	
B-fertilizer	*3.200E+009*	*1*	*3.200E+009*	*0.025*	*0.8789*	
C-urea	*5.638E+011*	*1*	*5.638E+011*	*4.40*	*0.0741*	
AB	*5.641E+008*	*1*	*5.641E+008*	*4.404E-003*	*0.9489*	
AC	*2.848E+008*	*1*	*2.848E+008*	*2.224E-003*	*0.9637*	
BC	*9.766E+008*	*1*	*9.766E+008*	*7.625E-003*	*0.9329*	
A^2	*2.960E+012*	*1*	*2.960E+012*	*23.11*	*0.0019*	
B^2	*1.758E+012*	*1*	*1.758E+012*	*13.73*	*0.0076*	
C^2	*1.387E+012*	*1*	*1.387E+012*	*10.83*	*0.0133*	
Residual	8.965E+011	7	1.281E+011			
Lack of Fit	*1.155E+011*	*3*	*3.852E+010*	*0.20*	*0.8933*	*not significant*
Pure Error	*7.809E+011*	*4*	*1.952E+011*			
Cor Total	8.266E+012	16				

Std. Dev.	3.579E+005	R-Squared	0.8915
Mean	7.352E+005	Adj R-Squared	0.7521
C.V. %	48.67	Pred R-Square	0.6287
PRESS	3.069E+012	Adeq Precisior	6.157

Figure 18. Cell count results after performing ANOVA analysis

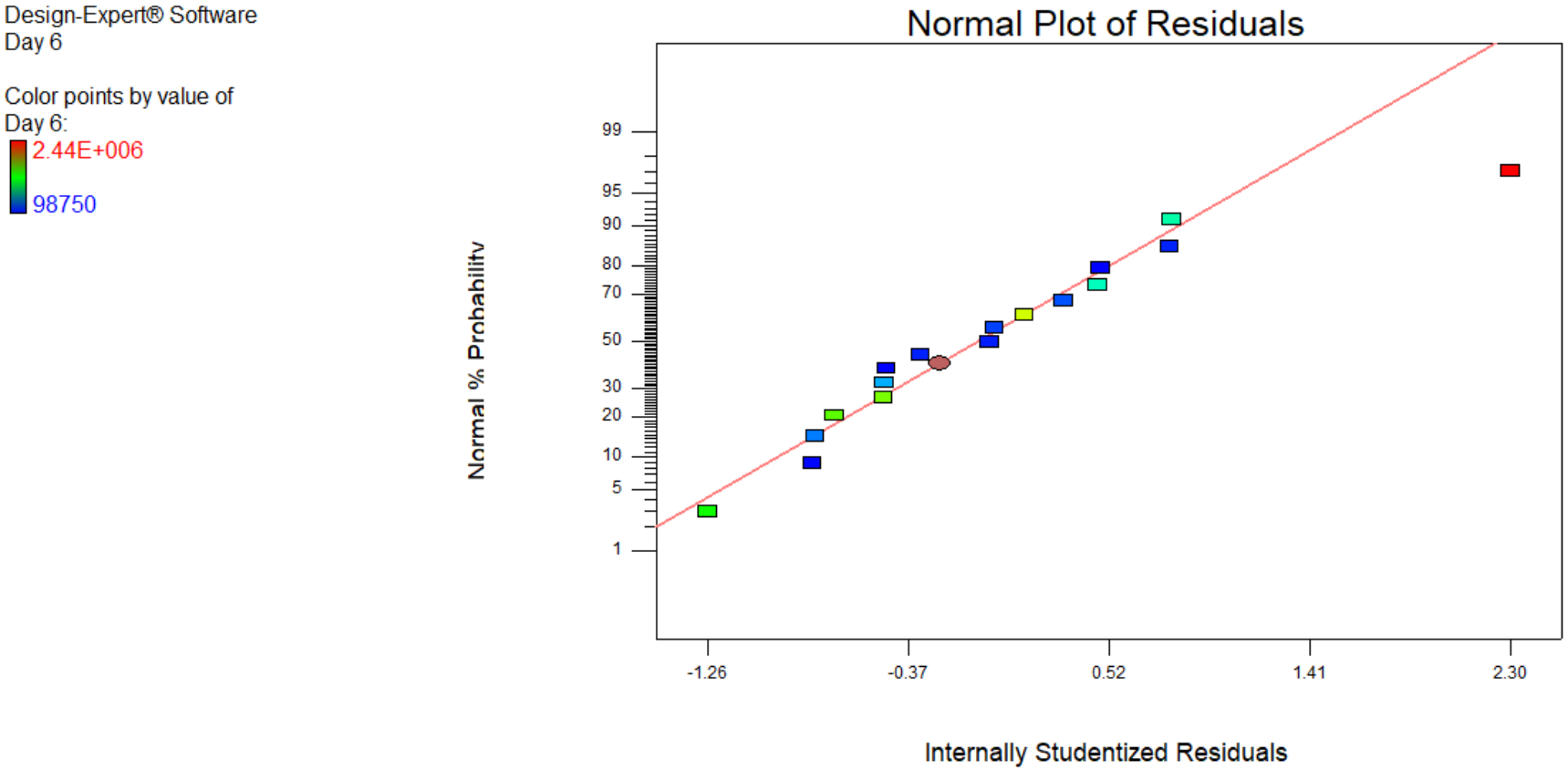
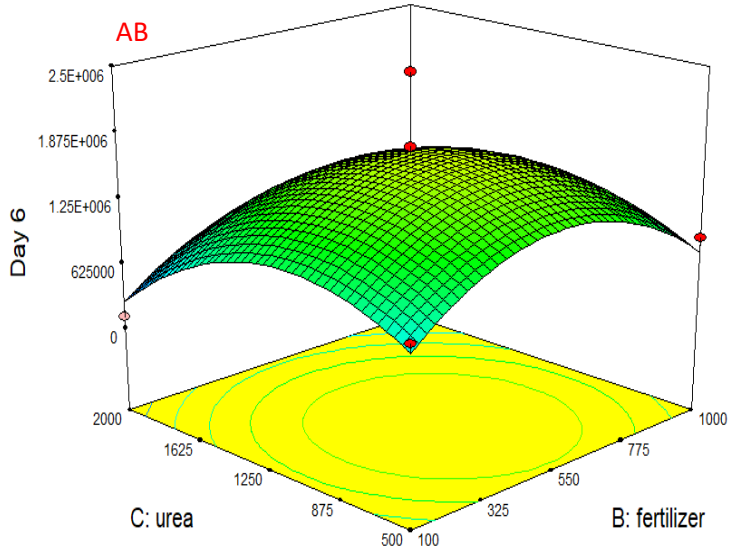
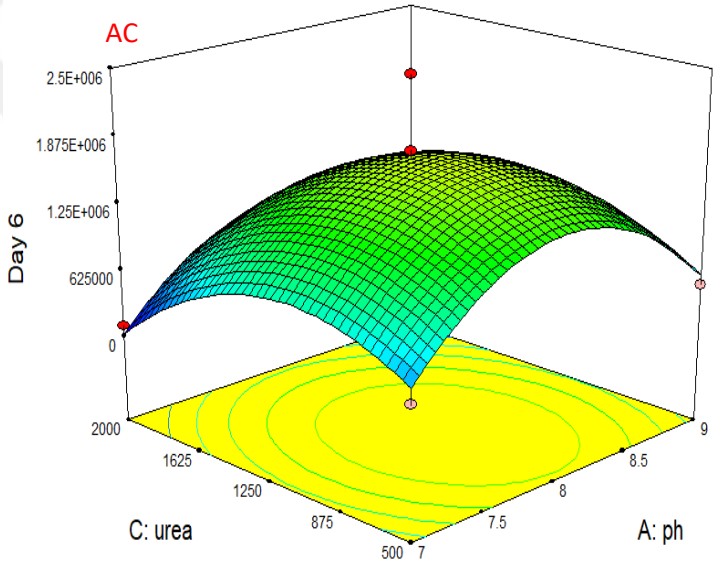


Figure 19. Normal Plot of Residuals on Box Behnken design for the cell number.


AB
Day 6
2.5E+006
1.875E+006
1.25E+006
625000
0
2000
1625
1250
875
500
C: urea
100
325
550
775
1000
B: fertilizer


AC
Day 6
2.5E+006
1.875E+006
1.25E+006
625000
0
2000
1625
1250
875
500
C: urea
7
7.5
8
8.5
9
A: ph

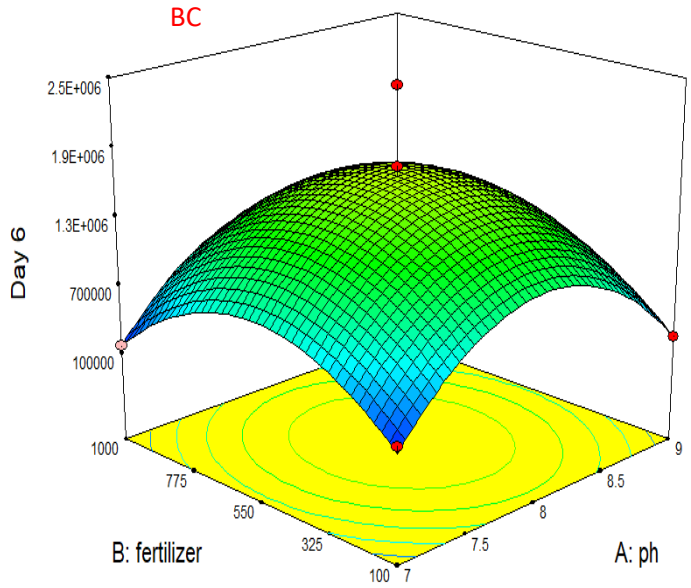


Figure 20. 3D response surface for AB, AC, and BC. A: pH. B: Fertilizer. C: Urea.

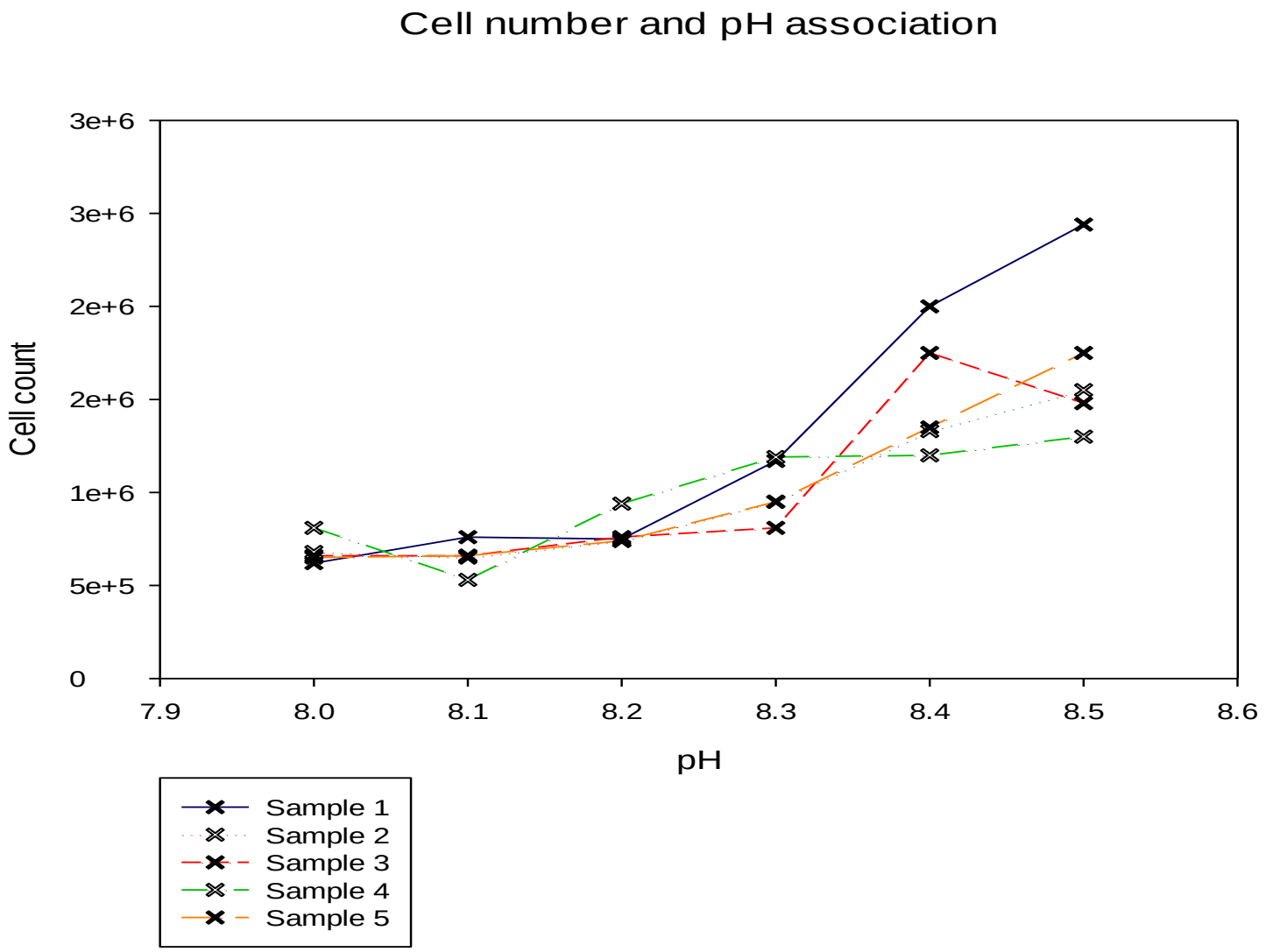

Cell number and pH association
Cell count
3e+6
3e+6
2e+6
2e+6
1e+6
5e+5
0
7.9
8.0
8.1
8.2
8.3
8.4
8.5
8.6
pH
Sample 1
Sample 2
Sample 3
Sample 4
Sample 5

Figure 21. pH association with microalgae growth. 1e+6 stands for a million.

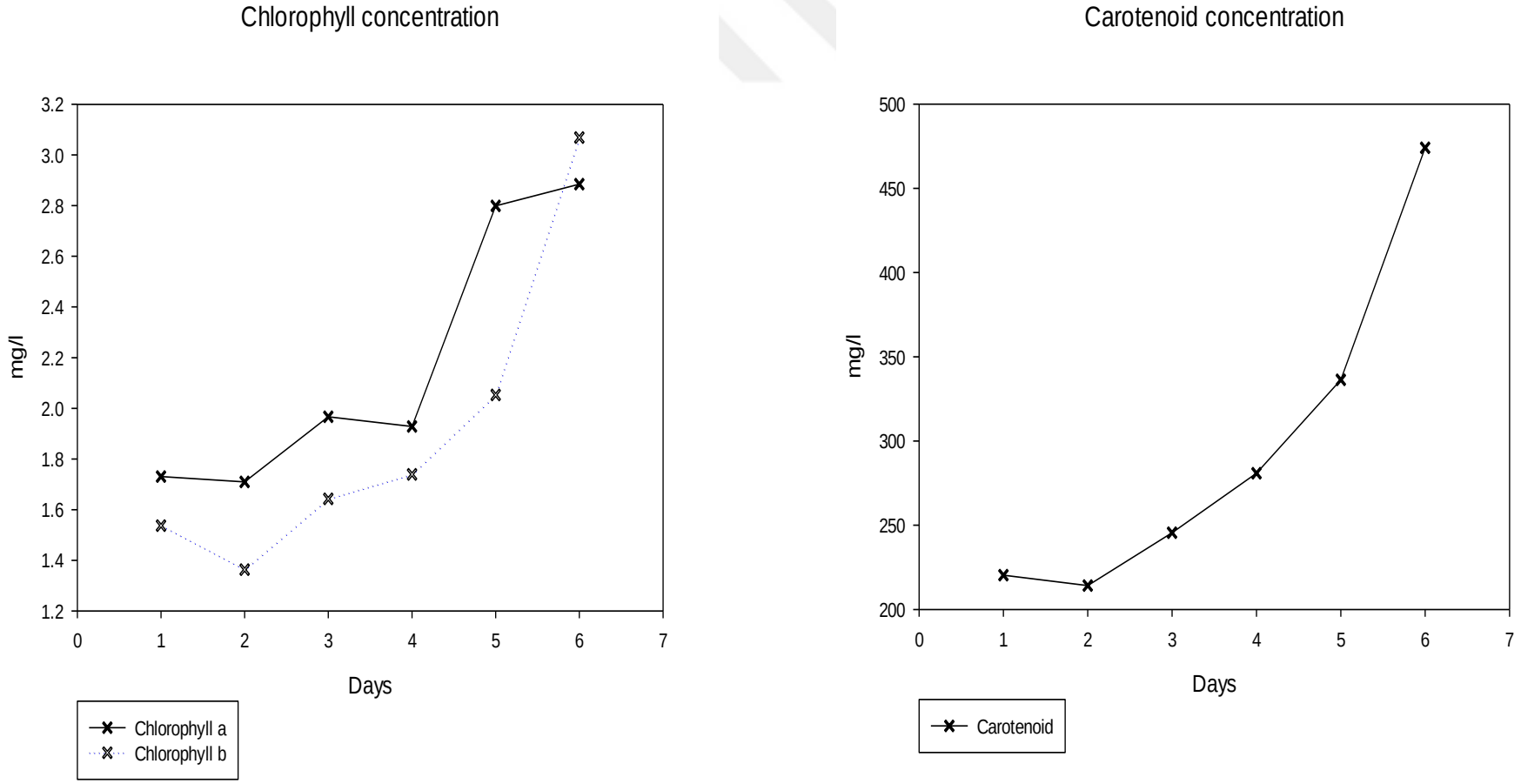


Figure 22. Chlorophyll a and b, and carotenoid concentration.

Table 6. Chlorophyll and carotenoid values for the first and the last day for each sample. Chl. a refers to Chlorophyll a. Chl. b refers to chlorophyll b.

Sample	Chl. a (mg/L)	Chl. b (mg/L)	Chl. a + Chl. b (mg/L)	Carotenoid (mg/L)
1	1.730305- 2.88471	1.536595- 3.069135	3.2669- 5.953845	220.3212- 474.111146
2	1.508935- 2.411805	1.543341- 1.940874	3.052276- 4.352679	187.72129- 304.329224
3	2.110065- 2.671245	2.389012- 2.185596	4.499077- 4.856841	260.753476- 335.15254
4	1.458645- 2.3453	1.14777- 1.781136	2.606415- 4.126436	186.477297- 291.737471
5	1.643622- 2.688621	1.543199- 2.02294	3.186821- 4.711561	200.95821- 343.197601

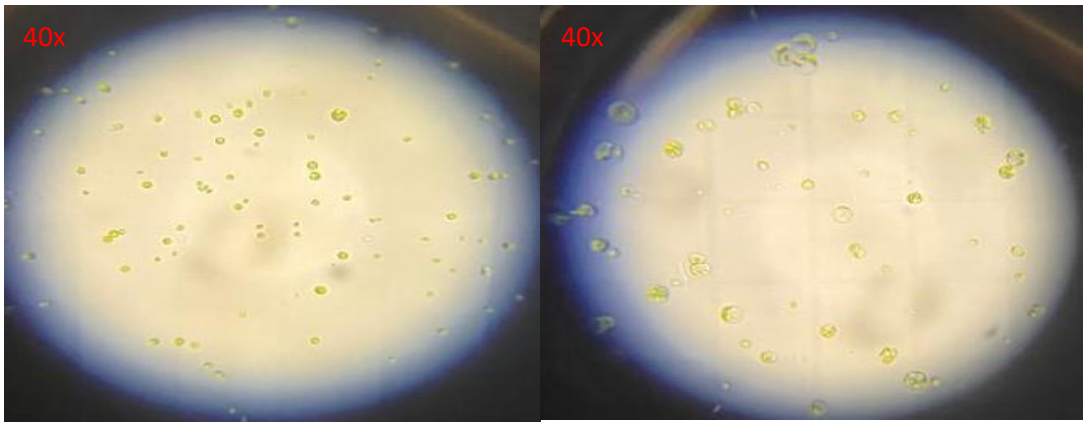


Figure 23. *C. vulgaris* cells under the microscope. The right side with urea addition and the left side without.

3.2. Harvesting and Drying

The harvesting by centrifuge was done using 5000 rpm for 15 minutes since it was highlighted by (Zhu, Li and Hiltunen, 2018; Deniz, 2020) as an efficient way for harvesting *C. vulgaris*. Afterward, the biomass was dried in the oven for two hours at 60 °C and it was sufficient for the drying for two hours. However, if the used glass plates were not flat and the volume was large, more time is required for the complete drying and whether the oven has a vacuum or not.

The synthesized magnetic nanoparticles had high efficiency to harvest the microalgae. The harvesting efficiency was 92.9% ∓ 1.322 for 50 ml culture media. Cell lysis and different phases were appeared (as shown in Figure 24 below) in the detachment process due to the chloroform: methanol addition with NaOH since they can break the electrostatic forces between the particles and microalgae (Savvidou et al., 2021)

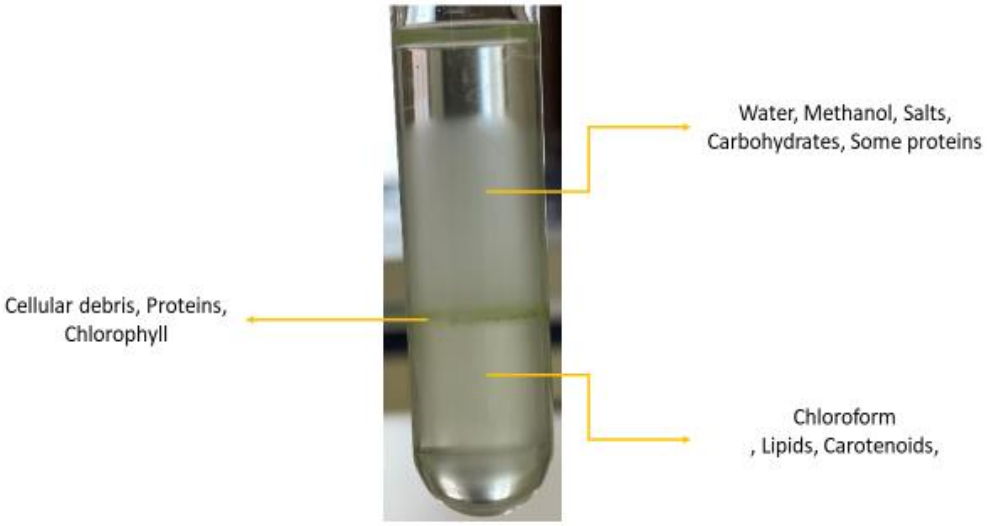


Figure 24. The obtained phases after biomass treatment with sodium hydroxide, methanol, and chloroform for the detachment process of magnetic particles

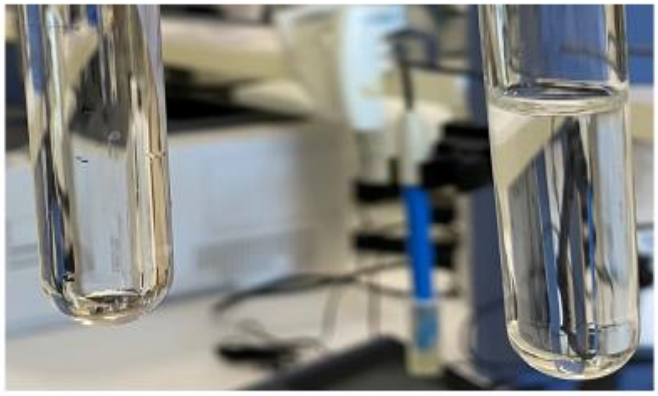


Figure 25. The comparison between the blank (on the right side) and the supernatant (on the left side) after harvesting microalgae by magnetic particles

3.3. Polymer Casting

The PLA powder must be dry before adding the solvent to it, otherwise, the solvent will not dissolve the PLA and the PLA will end up in aggregates as shown below in Figure 26 and it will be impossible to cast it. The chloroform and glycerol were optimized as well, the chloroform amount (30 ml) was enough for the process to give enough time for the stirrer to mix the solution for 10 -15 minutes when a less amount of the chloroform was used, the stirring time was not enough to mix the solution since chloroform evaporates quickly before mixing the solution properly as well as forming bubbles in the polymer as shown in Figure 26 below. On the other hand, when a bigger amount of the chloroform was used, the process took a long time till the chloroform was partially evaporated. Moreover, a large amount of chloroform is impractical due to its cost.

Two and a half ml of the glycerol was also enough for the process since a larger amount of glycerol made the material oily, on the other hand, a less amount was not enough to make the material flexible enough and it becomes hard to remove the material from the mold without losing some of it. There are other factors that play a vital role in the characteristics of the final product. These factors are the stirring time and the type of mold. 10-15 minutes were enough to mix the solution, when less time was used for stirring it, the polymer showed two separate layers, the upper side was the biomass and the lower side was PLA with a very fragile product as shown below in Figure 27. The scales of the mold are really important for the process, when a petri dish was used as a mold, the obtained polymer was thicker and stronger. However, it was not flexible enough as shown in Figure 28 and its color was darker due to the concentrated solution that was poured into the dish. Moreover, it took more than two days to be dried.

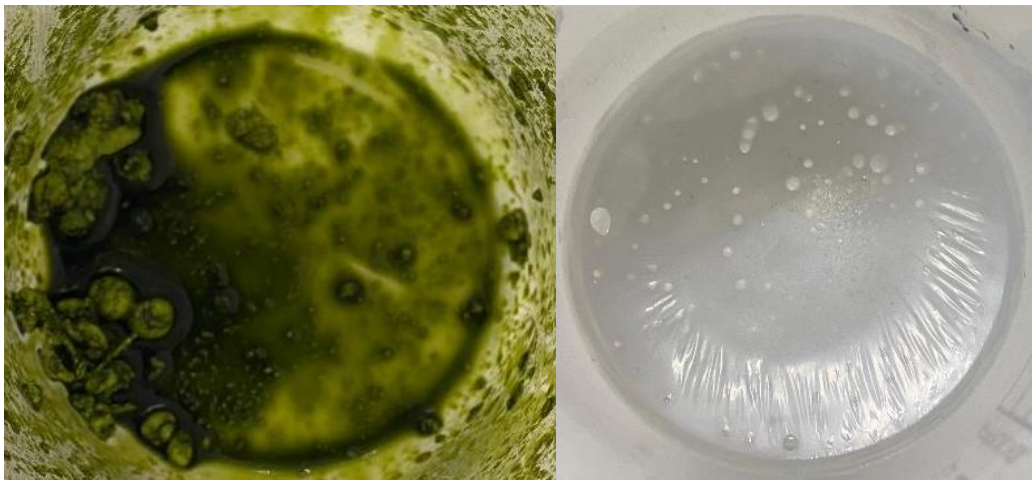


Figure 26. PLA aggregates (on the left side) due to the undried PLA powder and bubbles formation (on the right side) due to the fast evaporation of chloroform

Figure 27. Inappropriate duration for the stirring

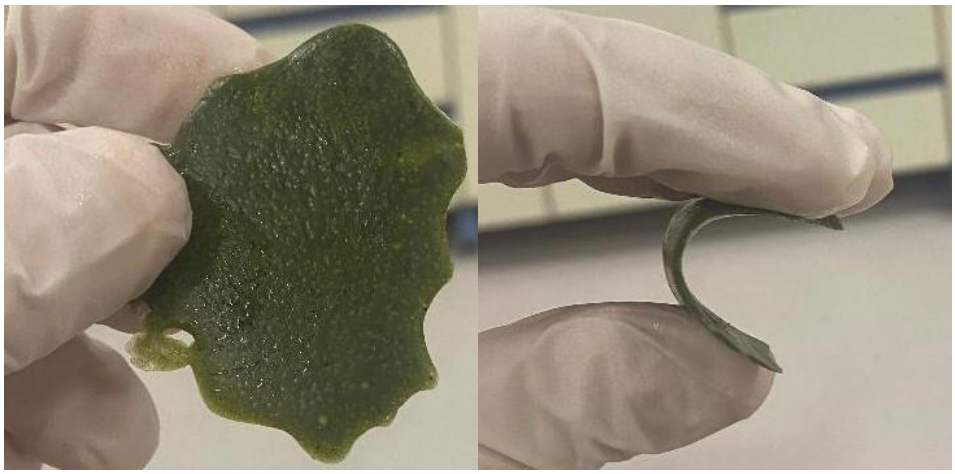


Figure 28. The produced polymer that has been produced with the same process with changing the mold

3.4. Mechanical Properties of the Polymer

Obviously, two steps of the annealing process were very effective as shown in Table 7, 8, and 9. The same concentration of biomass was compared before and after annealing it, the tensile strength was notably improved from 2.209 MPa to 9.809 MPa with improved breaking strain from 51.089 ∓ 0.638 to 56.205 ∓ 0.089 and toughness from 0.006 ∓ 0.003 to 0.021 ∓ 0.007. Another concentration (200 mg) was examined to improve the tensile strength further, the results showed improvement in tensile strength up to 15.646 MPa and improvement in toughness reaching 0.039 ∓ 0.005. However, the breaking strain was decreased to 50.624 ∓ 0.069. Moreover, the texture of the polymer was changed after annealing process as shown in Figure 29 below:

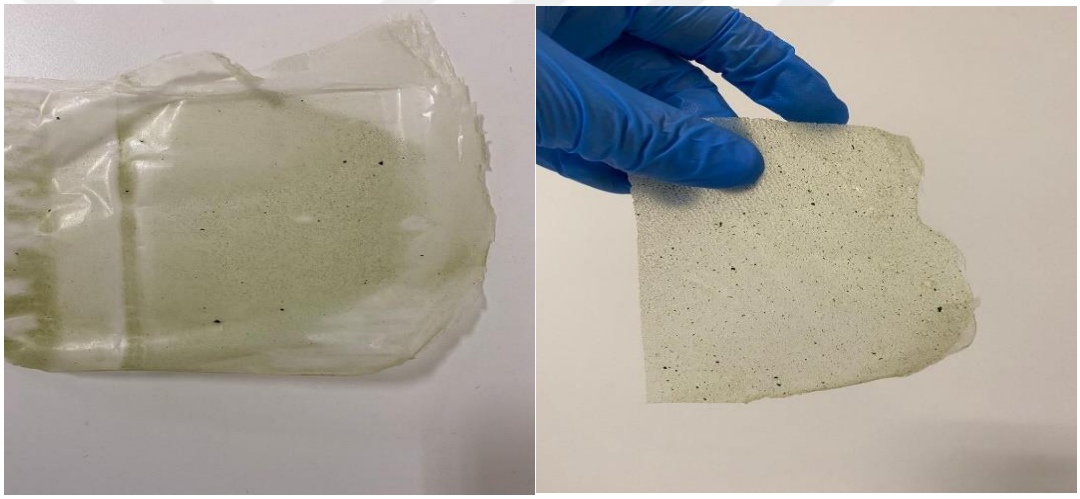


Figure 29. Annealed polymer (on the right side) and non-annealed polymer (left side) leading to various textures of the polymer

Furthermore, Figure 30 below can show up the variation of the produced samples which indicates the stability of stress-strain value for each sample. When annealing was not taking place, the difference in the tensile strength along with breaking strain was unstable from one sample to another (higher variation), while it was more stable with the annealing process with less variation when 200 mg of biomass was utilized with only 10.423% of coefficient of variation in terms of tensile strength and 0.137 and 11.674% in breaking strain value and toughness, respectively. In addition to that, t-test analysis between annealed and non-annealed polymer using SPSS software, and the results are shown in Figure 31 showing the results are

statistically different.

Table 7. Toughness results for the different produced polymers

	Toughness (MJ/m^3) 100 mg without annealing	**Toughness (MJ/m^3) 100 mg with annealing**	**Toughness (MJ/m^3) 200 mg with annealing**
	0.004	0.015	0.045
	0.003	0.022	0.039
	0.009	0.015	0.043
	0.011	0.027	0.036
	0.007	0.014	0.036
	0.002	0.029	0.034
	0.008	0.026	
Average	0.006 ∓ 0.003	0.021 ∓ 0.007	0.039 ∓ 0.005
Coefficient of variation%	53.332	30.337	11.674

Table 8. Tensile strength results for the different produced polymers

	Tensile strength (MPa) (MPa) 100 mg with annealing	**Tensile strength (MPa) 100 mg with annealing**	**Tensile strength (MPa) 200 mg with annealing**
	1.423	7.340	17.656
	0.629	10.018	14.172
	3.237	8.893	15.980
	4.340	10.809	16.615
	2.984	7.909	16.202
	0.590	12.364	13.253
	2.265	11.331	
Average	2.209 ∓ 1.410	9.809 ∓ 1.845	15.646 ∓ 1.631
Coefficient of variation%	63.831	18.814	10.423

Table 9. Breaking strain results for the different produced polymers

	Breaking strain% 100 mg without annealing	Breaking strain% 100 mg with annealing	Breaking strain% 200 mg with annealing
	50.862	56.089	50.663
	52.456	56.254	50.580
	50.646	56.108	50.679
	50.762	56.273	50.513
	50.779	56.144	50.613
	51.310	56.310	50.696
	50.812	56.254	
Average	51.089 ∓ 0.638	56.205 ∓ 0.089	50.624 ∓ 0.069
Coefficient of variation%	1.250	0.158	0.137

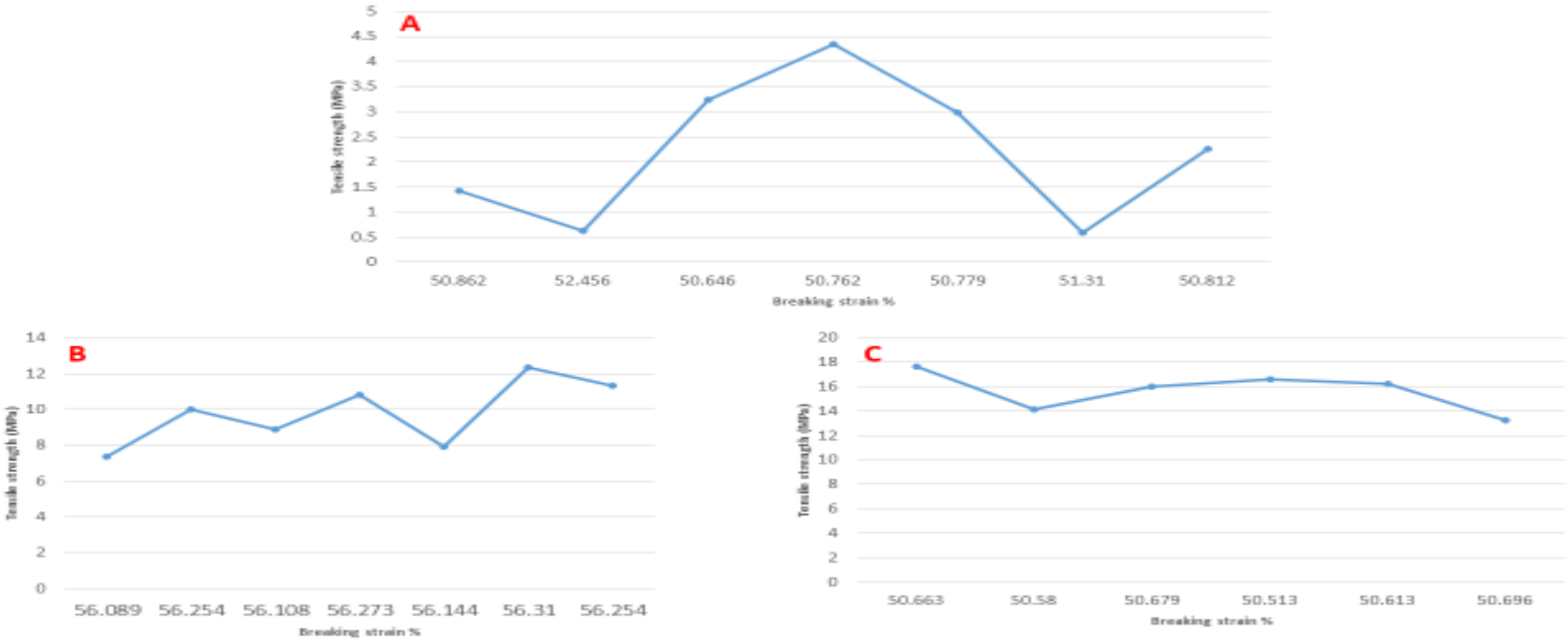


Figure 30. Stress-strain comparison among the utilized concentrations to produce biopolymer. A: 100 mg of biomass without annealing process. B: 100 mg of biomass with annealing process. C: 200 mg of biomass with annealing process

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
Results	Equal variances assumed	.789	.392	-8.656	12
	Equal variances not assumed			-8.656	11.227

		t-test for Equality of Means			
		Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference Lower
Results	Equal variances assumed	.000	-7.59943	.87791	-9.51222
	Equal variances not assumed	.000	-7.59943	.87791	-9.52694

		t-test for Equality of Means 95% Confidence Interval of the Difference Upper
Results	Equal variances assumed	-5.68663
	Equal variances not assumed	-5.67192

Figure 31. t-test analysis showing a significant difference between annealed and non-annealed polymers

3.5. Thermal analysis

DSC analysis was successfully performed for the sample with 200 mg of

biomass and 2 g of PLA since it was the best tensile strength with the lowest coefficient of variation and all expected results were obtained. Glass transition temperature (Tg) which is known as transition temperature when the polymer starts to be transferred from a rigid state or known as glossy state to a soft state or a rubbery state by heating (Becker and Locascio, 2002) (a higher Tg temperature means a better resistance to extreme heat, long-term delamination resistance) appeared during the first heating process at 59.03° C, while the first melting temperature (Tm) appeared at 159.48° C. The cold crystallization (Tcc) appeared during cooling cycle (exothermic reaction) between Tm and Tg after the first heating process at 108.27° C because Tcc appears when exothermic reaction takes place due to the mobility of the chain which happens when the temperature exceeds Tg point that softens the polymer (Ishino et al., 2021; Yoganandam et al., 2021).

It was noticed that PLA/algae polymer has two melting peaks because PLA has a low rate of crystallinity (Jamshidian et al., 2010) and due to the presence of crystals that form and recrystallization during the first heating (Liu et al., 2019). Moreover, the second heat can sometimes be used to differentiate various batches of a material. The second melting temperature appeared at the second heating process at 154.55° C, followed by the degradation point at 161.38° C during the second heating as well.

Table 10. Thermal properties of PLA/microalgae blend. Tg: Glass transition temperature. Tm_1 and Tm_2 : The first and second melting temperature, respectively. Tcc: Cold crystallization temperature. Td: Degradation temperature.

Heating flow cycle (° C)	Tg (° C)	Tm_1(° C)	Tcc (° C)	Tm_2 (° C)	Td (° C)
25-190	59.03	159.48			
190-25			108.27		
25-190				154.55	161.38

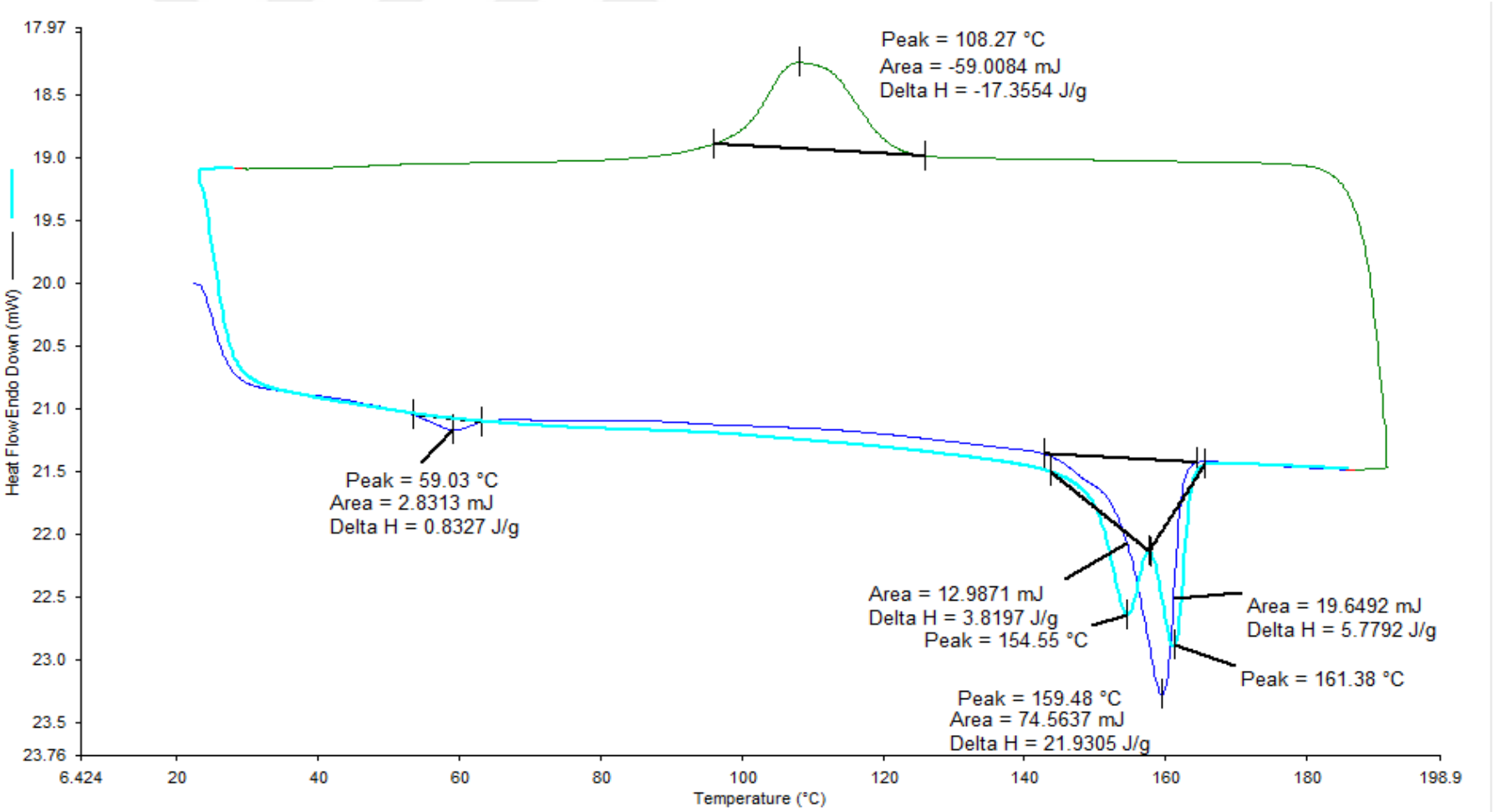


Figure 32. DSC analysis for PLA/microalgae blend (200 mg biomass/2 g PLA).

3.6. Biodegradation Test

The results of the degradation were obtained after 20 days, the weight of PLA films had no change in their weight, while PLA with biomass were changed. For the 100 mg of biomass with the PLA, weight decreased by 3.5 mg, while it decreased by 0.9 mg with 200 mg biomass. In addition to that, one of the specimens showed cracking as shown below in the red area in Figure 33.

Figure 33. The degraded films after dipping them in seawater. B stands for before adding seawater and A for after adding it

CHAPTER 4: DISCUSSION

The novel media showed a good potential for *C. vulgaris* to grow, urea is a good source of nitrogen, while it lacks the essential trace elements (Hsieh and Wu, 2009; Altın et al., 2018; Krausfeldt et al., 2019; Ribeiro et al., 2020; Rosa et al., 2023). Therefore, urea was supplemented with NPK fertilizer because it has the necessary trace elements for the microalgae. Urea was selected to be an essential nutrient (larger amount) and supplemented with the fertilizer (less amount) to open a new door for urine treatment by tolerating a high amount of urea that exists in the urine (Ahmad et al., 2014) and also to avoid the competition with the plants since NPK is widely used for the plants (Agbede, Adekiya and Eifediyi, 2017; Khalofah et al., 2022) by increasing plant dry matter (Adeniyan et al., 2011). Urea could be applied to improve some crops like rice as well (Ismael, Ndayiragije and Fangueiro, 2021). Moreover, utilizing urea for microalgae gives another advantage which is bio-cement production (Ariyanti, 2012) as well as wastewater treatment for urea factories (Hidayahtullah, Hermawan and Gofar, 2019) which is considered important because urea can affect water quality and stimulate toxicity (Swarbrick, Bergbusch and Leavitt, 2022) with a possibility in changing the community of the microorganisms (Lu et al., 2020), and changing the acidity of water with forming carbonic acid according to the following reaction (A, Brinkman and Margaria, 1933):

$$H_2O + CO_2 \rightleftharpoons H_2CO_3$$

and dissolved urea in water leads to CO_2 formation (Yu et al., 2021):

$$CO(NH_2)_2 + H_2O \longrightarrow CO_2 + 2NH_3$$

Hence, there was a study about utilizing NPK 15.15.15 with urea to stimulate the microorganisms to treat the water and it indicated the ability of these combined fertilizers to stimulate the microorganisms to treat the domestic water (N. Amenaghawon, A. Asegame and O. Obahiagbon, 2013). Therefore, tackling urea is a considerate choice to be treated or utilized.

Considering the price of the media, the media is very cheap, commercial NPK fertilizer and urea cost in US dollars 9.76 and 9.29 for one kilogram, respectively.

Considering the best concentrations according to the optimized ones, one litter media costs 0.017 US dollars. If we assume we need a maximum of five liters to reach the biomass weight of BG-11, it means the media will cost only 0.085 US dollars. The current cost of the prepared standard media for microalgae, BG-11 is listed below:

Table 11. BG-11 medium cost for one liter. Note that the cost is dynamically changing and the current price can be changed 5-10% according to the inflation status.

Company	Cost (USD)	Website (28th of July, 2023)
Sisco Research Laboratories Pvt. Ltd.	112.14	Sisco Research Labortories
Utex	55	Utex culture collection of algae
Gibco™	67.89	Fisher Scientific
Sigma Al-drich®	239.12	Sigma Al-drich
Wild Chemicals-Ebay	36	Ebay

Furthermore, the media could be recycled further for soil and plant application since it is a fertilizer (Malghani et al., 2010; Agbede, Adekiya and Eifediyi, 2017). Growing microalgae in wastewater could be also cheap compared to the standard media (Fazal et al., 2018; Goswami, Makut and Das, 2019; Spennati, Casazza and Converti, 2020; Amenorfenyo et al., 2022; Kumar et al., 2022), and supplementing the wastewater with NPK fertilizer is also cheaper to cultivate C. vulgaris (Mtaki, Kyewalyanga and Mtolera, 2021). However, utilizing wastewater has also some considerations sparked by some significant drawbacks of conventional treatment methods, including (a) variable efficiency depending on the nutrient to be removed, (b) high operating costs, (c) frequent secondary pollution, and (d) loss of valuable potential nutrients (N, P) (Abdel-Raouf, Al-Homaidan and Ibraheem, 2012) and the availability of the wastes (Pacheco et al., 2015).

Media preparation utilizing tap water was also investigated as well. The tap water was unclean and once the chemicals were dissolved, it became turbid as shown in Figure 34, and the addition of the NaOH made the water more turbid, which may be because of the high amount of calcium carbonate that tap water has because NaOH can readily absorb carbon dioxide from the air resulting in calcium carbonate precipitation by sodium carbonate.

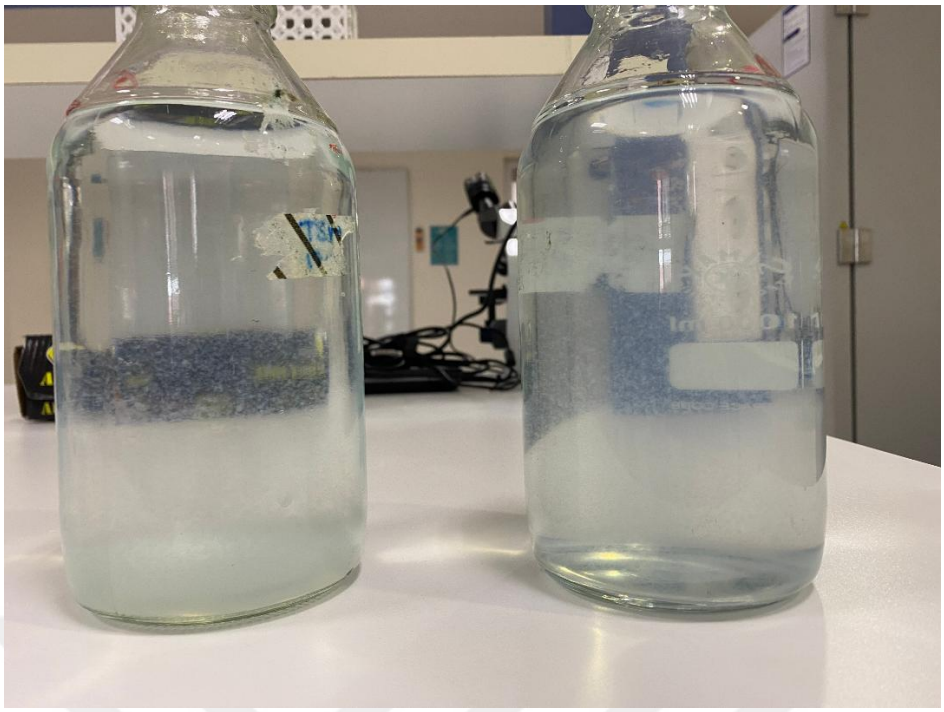


Figure 34. Tap water interaction with other chemicals

For microalgae analysis, the absorbance for microalgae growth is not reliable compared to cell counting and biomass calculation because it depends on the homogeneity of the samples. Moreover, the variance in microalgae detection by the spectrophotometer (wavelengths) is due to the pigments and chlorophyll absorption which might interfere with the analysis (Griffiths et al., 2011), Therefore, scanning the samples was critical to determine the best wavelength for microalgae detection. When automatic scanning was performed to detect microalgae, the results were unreliable completely due to the long time of the scanning which lead to cell sedimentation in the cuvette, on the other hand, the manual scanning was accurate which indicated that 550 nm is the best wavelength and these results are in line with other studies (Mohseni and Moosavi Zenooz, 2022; Schagerl et al., 2022).

The cell counting is very accurate and the best way to describe the growth of microalgae. However, the homogeneity of the sample can affect the results and these findings are in line with (Richmond, 1986). When specific chambers were counted to obtain the final cell number, it was found that the number can be changed after each loading of the same culture and the cell number was unequal in each square on Thoma counting chamber. Therefore, all chambers were counted after agitation and pipetting the samples thoroughly to obtain a homogenous sample as it was referred to in the literature (Xu and Mi, 2011). The dilution of the sample was avoided since the cell

number was countable without dilution to avoid any error because it was noticed that cell number can be slightly different than in undiluted samples if there were too few cells in the samples or if the samples were not homogenous enough and these findings are in line with (Sarrafzadeh et al., 2015).

Biomass measurement is also one of the followed methods to describe microalgae growth and when it comes to biomass measurement, the selected photobioreactor for biomass calculation is important (Nava et al., 2022). If the selected photobioreactor showed the potential of biomass to be attached on the walls or on the internal surface of the reactor, then the real biomass weight that could be obtained would not be expressed typically due to the loss of biomass. For instance, when plastic photobioreactor was used for microalgae cultivation, biomass attachment on the walls or on the internal surface of the bioreactor showed a high significance when the air pump was not operating for a long time, thus, mixing was essential for plastic bioreactor. Glass flasks also showed a potential for microalgae attachment if the shaker incubator was not operating. However, the detachment was simpler than in plastic photobioreactor by a simple agitation of the samples.

Growth measurement was tracked for 14 days initially for cell count, OD, and biomass measurement. However, the results were insignificant and the variance was really big among the replicates after the 7^{th} day due to the high concentration of urea which might stop the metabolism in some cultures while the others carry on with growing. Therefore, the analysis was carried out and tracked for 6 days.

One more indicator for microalgae growth which was significantly associated with the growth is pH, since air was not provided for the microalgae, and it is well known that microalgae absorb carbon dioxide and leading to alkaline environment (Jena et al., 2012; Wu et al., 2022). Therefore, pH was observed during the growth and it is a good indicator to microalgae growth. Furthermore, the chlorophyll and carotenoid were measured to support the growth profile of the followed methodology, as well as they are considered as promising alternative to dye the polymers (Orona-Navar et al., 2021; Benbelkhir and Medjekal, 2022). The measurement was tracked for 6 days in parallel with growth measurement experiments (cell count, OD, biomass). However, the results are very low compared to other studies. It was stated that 5.52 g/l of chlorophyll was obtained (Schüler et al., 2020) while the amount of carotenoid was

52000 mg/L (Bazarnova et al., 2022) by C. vulgaris. Many parameters can affect the chlorophyll and carotenoid concentration in microalgae, for instance, when the red light was used for C. vulgaris cultivation, it improved the concentration of chlorophyll a, b, and c, while the nitrogen reduction lead to chlorophyll reduction (da Silva Ferreira and Sant'Anna, 2017). Carotenoid concentration in microalgae also can be affected by light, nutrients, pH, and temperature (Ivanova et al., 2023). Therefore, new optimization is required in the future for chlorophyll and carotenoid analysis by primarily the light type, light intensity, and nutrients and secondly other factors such as pH and temperature.

Harvesting and drying were taking place when microalgae reached the maximum growth rate. The biomass was obtained by centrifugation and testing a modified method of magnetic particles was also performed. The harvesting efficiency of the magnetic particles was around 92%, while it was stated to reach 99%. However, more energy consumption was required in addition to the sonication process (Savvidou et al., 2021). Therefore, a modified method was tested by avoiding sonication process and less energy consumption by decreasing the radiation time of magnetic particles during the synthesize. Magnetic nanoparticles can be used for reducing the overall volume of the culture by harvesting the microalgae from the main media, and since the chloroform and methanol are used for the detachment of the magnetic nanoparticles, then, it can be used also to extract the lipid from the microalgae, and for the second step, the centrifuge can be used to obtain the biomass with saving energy since the overall volume was already reduced by the magnetic harvesting. However, the obtained microalgae after the harvesting process utilizing the magnetic nanoparticles were not utilized for biopolymer production because it was noticed that this process adds additional cost to the overall process of biopolymer production and it tends to be more practical in bio-refinery. Moreover, changes the properties of the biopolymer since the detachment process involves solvents addition that leads to the cell disruption (Karim et al., 2019; Yew et al., 2019), and this process separates the lipid, carbohydrates, and protein in different phases and the protein and carbohydrates are necessary for biopolymer production since a large amount of protein could improve the value of the elongation for blended biopolymers (Kim, Chang and Kim, 2022). Nevertheless, this method of harvesting was modified and tested because it was addressed that de-oiled or treated biomass with solvents can be utilized to produce

biopolymers (Naresh Kumar et al., 2020; Kalita et al., 2021; Arun et al., 2022). Moreover, when urea was combined with NPK, it was noticed that the cell size was increased by microscope detection, and cell size could play a significant role in the harvesting (Mata, Martins and Caetane, 2012) and it was investigated in co-culture harvesting process by pellets formation with fungi which could lead to a cheaper process for harvesting due to their ease separation (Zhang and Hu, 2012).

For the biopolymer production, chloroform was selected to be the solvent because it has a lower vapor pressure and slower evaporation compared to other solvents, namely, hexafluoro-2-propanol (HFIP) and dichloromethane (DCM) (Smita Mohanty, 2015) and it showed its compatibility with both PLA and algae (Adli et al., 2018b). However, the residue of the chloroform can be found in the produced plastic, but it was stated that a simple process to decrease the amount of the residue by washing the material with water-methanol and by annealing process at 80° C (Teske et al., 2016), and the study indicated to the importance of the utilized temperature. However, the results showed that this annealing temperature is not enough to meet the requirements of The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) (European Medicines Agency, 2022). Thus, a higher degree of temperature was chosen for the annealing process. Moreover, annealing is very crucial for the polymers because it can lead to the crystallinity in the polymers and improving mechanical properties (Song, Feng and Wu, 2011; Gleadall, 2015; Taki, Suenaga and Ito, 2018; Liao et al., 2019; Beauson et al., 2022). Annealing degree of PLA was indicated to be better if the temperature was above glass transition temperature (Tg) which is known to be usually around 55-60° C (Chuensangjun, Pechyen and Sirisansaneeyakul, 2013; Habib, 2023) and few studies indicated that PLA could be improved by annealing it at 105° C in extrusion, 3D printing and fabrication (Yang et al., 2010; Li, Hashaikeh and Arafat, 2013; Butt and Bhaskar, 2020). However, the duration time of the annealing process was varied in the studies. Considering the cost of the process, a long duration for annealing is not preferred as well (Yeo et al., 2014). Moreover, a long heating process could affect the quality of the biomass (Aljabri et al., 2023). Therefore, the polymer was annealed at 105 ° C for 20 minutes totally and in two steps (heating-drying-heating) because 10 minutes was not enough to obtain the desired strength. Compared to other polymers that were produced, PHA was stated to have a tensile strength ranging from 14-27 MPa

with a very low strain (El-Hadi et al., 2002; Thellen et al., 2008). Additionally, it was stated that different types of bio-based biopolymers made from banana peel flour, corn starch with eggplant flour, ulluco starch, had a tensile strength ranging from 0.14- 15.1 MPa, and 27-28 MPa from fruits and vegetable residue (Galus et al., 2020) (the tensile strength of different bio-based biopolymers is mentioned below). Nevertheless, algae are a better option than utilizing food sources to produce the biopolymers to avoid the competition with food sources (Cinar et al., 2020). PLA was also mixed with macroalgae (green, red, brown) to produce biopolymer, the tensile strength was 30 MPa by processing the materials in a mixer (Bulota and Budtova, 2015). Algae was also blended with PLA in the solvent casting method (Adli, Ali and Azmi, 2018), and the obtained tensile strength was maximum 1.23 MPa. The produced polymer tends to be suitable for agricultural applications. Seaweed was already suggested as an excellent candidate for mulching, and soil improvement as a fertilizer (Hodgson, 2021), due to the existence of nitrogen (Kumar and Bera, 2020) which is considered a very important substance for the plant and soil (Harvesto Group, 2020). Moreover, it was stated that C. vulgaris is the most species that are used as a bio-fertilizer (Ammar et al., 2022) because it improves the crops (Sadak Turhan and Şensoy, 2022) by increasing plant growth and nutrition while lowering the demand for artificial fertilizers (Alvarenga et al., 2023).

Thermal properties of the biopolymer were very close to the obtained results by (Adli, Ali and Azmi, 2018). However, the authors did not state anything about the thermal degradation. It was stated in the literature that thermal degradation of PLA starts at 150° C in air and in one heating process (Gupta and Deshmukh, 1982), while it can be above 160° C depending on the number of heating cycles, the atmosphere if the samples were exposed to air or nitrogen, and the structure of PLA. It was reported that PLLA starts to degrade in 180-280°C under nitrogen and air, while PDLLA at 170°C-200°C under air or nitrogen with two or three heating cycles when the sample processed over 160° C or over the melting temperature of PLA (Rasselet et al., 2014). The biopolymer showed a significant biodegradation rate in seawater, although the samples were annealed to improve the tensile strength and the crystallinity decreases the degradation rate of PLA, especially if the sample was high in density (Pantani and Sorrentino, 2013). Interestingly, the films that contained 100 mg of biomass showed a higher degradation in spite of they were at the same weight of others, probable due

to the amount of added nitrogen by the biomass to the seawater since nitrogen is well known about its effect on the microorganisms in marine life (Goldman and Dennett, 2000; Fiore et al., 2010; Sun et al., 2018; Birnstiel, Sebastián and Romera-Castillo, 2022) or because of the density of the biopolymer since more biomass was incorporated with PLA made the density higher and a higher density prevents water diffusion into the polymer (Pantani and Sorrentino, 2013).

Table 12. Tensile strength of different bio-based biopolymers

Biopolymer composition	Tensile strength (MPa)	Reference
Chia-seed mucilage and starch	6.7-7.5	(Tosif et al., 2021)
Nopal mucilage and Rice starch	2.80-3.96	(Tosif et al., 2021)
Banana starch and peel fibers	8.9–11.1	(Tosif et al., 2021)
Corn starch and cellulose nanofibers	21.90–28–87	(Tosif et al., 2021)
Potato starch and coconut fiber nanocrystals	4.09–8.20	(Tosif et al., 2021)
Oriented collagen	0.9-7.4	(Onar, 2014)
PLLA	28-50	(Onar, 2014)
Polyglycolide	70	(Onar, 2014)
D- and L-lactide	29-35	(Onar, 2014)
Low density polyethylene	9–17	(Galus et al., 2020)
Methylcellulose	69.0	(Galus et al., 2020)
PLA	39–42	(Arrieta et al., 2017)
PHB	35–50	(Arrieta et al., 2017)
PLA-PHB 85:15	31.0 ∓ 5.0	(Arrieta et al., 2017)
PLA-PHB 75:25	16–50	(Arrieta et al., 2017)
PLA-PHB 50:50	8 ∓ 1	(Arrieta et al., 2017)
PLA-PHB 25:75	2.5 ∓ 1	(Arrieta et al., 2017)
Seaweed- empty fruit bunch 50%	81 ∓ 1	(Abdul Khalil et al., 2017)
Seaweed- Microcrystalline Cellulose (MCC)	20.21– 29.76	(Hasan et al., 2019)
Seaweed- Oil palm shell nanofiller	31.4– 44.8	(Hasan et al., 2019)

CHAPTER 5: CONCLUSION AND FUTURE WORK

A novel media which contains NPK fertilizer with urea was optimized and showed a good potential to grow microalgae, namely *C. vulgaris* in lab scale and non-lab scale with a very cheap price which does not exceed 0.017 US dollars per litter with a maximum weight of biomass 280 mg/L and the results were statistically significant according to Box-Behnken design. While the maximum weight using BG-11 under the same conditions was 555 mg/L. The novel media has a lower growth rate obviously. However, the novel composition is much cheaper and the water which was obtained after the harvesting might be utilized for further applications for the soil and plants. Therefore, the future work should involve the chemical composition of the media after microalgae cultivation and its effect on the soil and crops, as well as the potential of utilizing the media after harvesting to re-cultivate the microalgae to save water. Moreover, the feasibility of utilizing the composition for biodiesel production should be studied along with media improvement to enhance the growth.

The microalgae were harvested to obtain the biomass by centrifugation and a modified method of synthesizing magnetic particles successfully. Harvesting efficiency using the particles was 92.9% $\mp$ 1.322 with energy saving by minimizing the radiation time in the microwave and without the sonication process for the detachment. However, the most challenging part of using magnetic particles for the harvesting is not the synthesis of the particles themselves, it is the detachment process, and this process seems suitable for biofuel production due to the usage of chloroform-methanol for the detachment or for a bio-refinery like obtaining the lipids for biodiesel and utilization of cell debris for fermentation. New studies should be investigated for the detachment process of magnetic nanoparticles beside PLA/de-oiled microalgae blend should be investigated in the future after the detachment process and compared to the current results when the whole biomass is utilized for biopolymer production. Furthermore, the obtained biomass was utilized to produce biopolymer by blending it with PLA powder by the solvent casting method with two steps of annealing at 105° C for ten minutes which its effect on the chloroform residue might be studied in the future. The maximum tensile strength was notably improved (compared to other studies by using the solvent casting method) reaching 15.646 $\mp$ 1.631 MPa with a

significant biodegradation rate in seawater by losing its weight by 0.9 mg during 20 days when 200 mg of biomass was blended with 2 g of PLA powder. While the degradation rate was too much better when 100 mg of biomass was blended with the same weight of PLA by losing 3.5 mg from its original weight sacrificing the tensile strength which average was 9.809 ∓ 1.845 MPa. Therefore, future studies may focus on the correlation between the biomass concentration and the degradation rate. Thermal analysis by DSC was successfully performed and the results indicated that two steps of annealing process at 105° C did not change the thermal properties of PLA, regardless of improving the crystallinity along with mechanical properties of the biopolymer (tensile strength). However, new studies should be investigated regarding the impact of annealing process on the residue of the chloroform.

REFERENCES

A, S., Brinkman, R. and Margaria, R. (1933) '*The kinetics of the carbon dioxide-carbonic acid reaction*', Philosophical Transactions of the Royal Society of London. Series A, Containing Papers of a Mathematical or Physical Character, Vol. 232(707–720), pp. 65–97.

Abdel-Raouf, N., Al-Homaidan, A.A. and Ibraheem, I.B.M. (2012) '*Microalgae and wastewater treatment*', Saudi Journal of Biological Sciences. Elsevier, Vol. 19(3) pp. 257–275.

Abdul Khalil, H.P.S., Tye, Y.Y., Chow, S.T., Saurabh, C.K., Tahir, P.M., Dungani, R. and Syakir, M.I. (2017) '*Cellulosic pulp fiber as reinforcement materials in seaweed-based film*', BioResources, Vol. 12(1), pp. 29–42.

Abdulsamad, J.K., Varghese, S.A. and Thajudeen, J. (2019) '*Cost effective cultivation and biomass production of green microalga desmodesmus subspicatus mb. 23 in NPK fertilizer medium*', Journal of Microbiology, Biotechnology and Food Sciences, Vol. 9(3), pp. 599–604.

Adeniyan, O.N., Ojo, A.O., Akinbode, O.A. and Adediran, J.A. (2011) '*Comparative study of different organic manures and NPK fertilizer for improvement of soil chemical properties and dry matter yield of maize in two different soils*', Journal of Soil Science and Environmental Management, Vol. 2(1), pp. 9–13.

Adli, S.A., Ali, F., Azmi, A. and Azmi, A.S. (2018a) '*Preparation and Characterization of PLA/Algae Nanocomposite for Packaging Industry Plasticizing PLA View project Preparation and Characterization of PLA/Algae Nanocomposite for Packaging Industry*', Journal of Advanced Research in Materials Science, Vol. 50(1), pp. 19–25.

Adli, S.A., Ali, F., Azmi, A. and Azmi, A.S. (2018b) '*Preparation and Characterization of PLA/Algae Nanocomposite for Packaging Industry Plasticizing PLA View project Preparation and Characterization of PLA/Algae Nanocomposite for Packaging Industry*', Journal of Advanced Research in Materials Science, Vol. 50(1), pp. 19–25.

Adli, S.A., Ali, F. and Azmi, A.S. (2018) '*Preparation and Characterization of PLA / Algae Nanocomposite for Packaging Industry*', Journal of Advanced Research in Materials Science, Vol. 1(1), pp. 19–25. Available at:

www.akademiabaru.com/arms.html (Accessed: 1 May 2023).

Agbede, T.M., Adekiya, A.O. and Eifediyi, E.K. (2017) '*Impact of Poultry Manure and NPK Fertilizer On Soil Physical Properties and Growth and Yield of Carrot*', Journal of Horticultural Research, Vol. 25(1), pp. 81–88.

Ahmad, S.I., Syed, I.A., Ravi Prasad, P. and Ahmad, A. (2014) '*Quantitation of urea in urine by Fourier transforms infrared spectroscopy*', Der Pharma Chemica, Vol. 6(1), pp. 90–96.

Aljabri, H., Cherif, M., Siddiqui, S.A., Bounnit, T. and Saadaoui, I. (2023) '*Evidence of the drying technique's impact on the biomass quality of Tetraselmis subcordiformis (Chlorophyceae)*', Biotechnology for Biofuels and Bioproducts, Vol. 16(1), pp. 1–11.

Altın, N., Kutluk, T., Uyar, B. and Kapucu, N. (2018) '*Effect of Different Nitrogen Sources on the Growth and Lipid Accumulation of Chlorella variabilis*', Journal of Applied Biological Sciences, Vol. 12(2), pp. 38–40.

Alvarenga, P., Martins, M., Ribeiro, H., Mota, M., Guerra, I., Cardoso, H. and Silva, J.L. (2023) '*Evaluation of the fertilizer potential of Chlorella vulgaris and Scenedesmus obliquus grown in agricultural drainage water from maize fields*', Science of The Total Environment, Vol. 861, p. 160670.

Ambrogi, V., Carfagna, C., Cerruti, P. and Marturano, V. (2017) '*Additives in Polymers*', in Modification of Polymer Properties. William Andrew Publishing, pp. 87–108. Available at: https://www.sciencedirect.com/science/article/abs/pii/B978032344353100004X?via%3Dihub (Accessed: 5 June 2023)

Amenorfenyo, D.K., Li, F., Zhang, Y., Li, C., Zhang, N. and Huang, X. (2022) '*Effects of Microalgae Grown in Membrane Treated Distillery Wastewater as Diet on Growth and Survival Rate of Juvenile Pearl Oyster (Pinctada fucata martensii)*', Water (Switzerland), Vol.14(17), p. 2702.

Ammar, E.E., Aioub, A.A.A., Elesawy, A.E., Karkour, A.M., Mouhamed, M.S., Amer, A.A. and EL-Shershaby, N.A. (2022) '*Algae as Bio-fertilizers: Between current situation and future prospective: The role of Algae as a Bio-fertilizer in serving of ecosystem*', Saudi Journal of Biological Sciences, Vol. 29(5), pp. 3083–3096.

Ariyanti, D. (2012) '*Feasibility of Using Microalgae for Biocement Production through Biocementation*', Journal of Bioprocessing & Biotechniques, Vol.2(1), p. 1000111.

Arrieta, M.P., Samper, M.D., Aldas, M. and López, J. (2017) '*On the use of PLA-PHB*

blends for sustainable food packaging applications', Materials, Vol. 10(9), pp. 10–12.
Arun, J., Vigneshwar, S.S., Swetha, A., Gopinath, K.P., Basha, S., Brindhadevi, K. and Pugazhendhi, A. (2022) '*Bio-based algal (Chlorella vulgaris) refinery on de-oiled algae biomass cake: A study on biopolymer and biodiesel production*', Science of The Total Environment, Vol. 816, p. 151579.
Awaja, F., Daver, F., Kosior, E. and Cser, F. (2004) '*The effect of chain extension on the thermal behaviour and crystallinity of reactive extruded recycled pet*', in Journal of Thermal Analysis and Calorimetry, Vol. 78, pp. 865–884.
Bazarnova, J., Smyatskaya, Y., Shlykova, A., Balabaev, A. and Đurović, S. (2022) '*Obtaining Fat-Soluble Pigments—Carotenoids from the Biomass of Chlorella Microalgae*', Applied Sciences (Switzerland), Vol. 12(7), p. 3246.
Beauson, J., Schillani, G., Van der Schueren, L. and Goutianos, S. (2022) '*The effect of processing conditions and polymer crystallinity on the mechanical properties of unidirectional self-reinforced PLA composites*', Composites Part A: Applied Science and Manufacturing, Vol. 152, p. 106668.
Becker, H. and Locascio, L.E. (2002) '*Polymer microfluidic devices*', Talanta, Vol. 56(2), pp. 267–287.
Benbelkhir, F.Z. and Medjekal, S. (2022) '*Microalgal carotenoids: A promising alternative to synthetic dyes*', Algal Research, Vol. 66, p. 102823.
Bher, A., Cho, Y. and Auras, R. (2023) '*Boosting Degradation of Biodegradable Polymers*', Macromolecular Rapid Communications, Vol. 44(5), p. 2200769.
Birnstiel, S., Sebastián, M. and Romera-Castillo, C. (2022) '*Structure and activity of marine bacterial communities responding to plastic leachates*', Science of the Total Environment, Vol. 834(4), p. 155264.
Bonifer, K.S., Wen, X., Hasim, S., Phillips, E.K., Dunlap, R.N., Gann, E.R., DeBruyn, J.M. and Reynolds, T.B. (2019) '*Bacillus pumilus B12 Degrades Polylactic Acid and Degradation Is Affected by Changing Nutrient Conditions*', Frontiers in Microbiology, Vol. 10(11), pp. 1–13.
Boonluksiri, Y., Prapagdee, B. and Sombatsompop, N. (2021) '*Promotion of polylactic acid biodegradation by a combined addition of PLA-degrading bacterium and nitrogen source under submerged and soil burial conditions*', Polymer Degradation and Stability, Vol. 188(6), p. 109562.
Bulota, M. and Budtova, T. (2015) *'PLA/algae composites: Morphology and mechanical properties*', Composites Part A: Applied Science and Manufacturing, Vol.

73(6), pp. 109–115.

Butt, J. and Bhaskar, R. (2020) '*Investigating the effects of annealing on the mechanical properties of FFF-printed thermoplastics*', Journal of Manufacturing and Materials Processing, Vol. 4(2), pp. 1–20.

Cenna, A.A. and Mathew, P. (1997) '*Evaluation of cut quality of fibre-reinforced plastics - A review*', International Journal of Machine Tools and Manufacture, Vol. 37(6), pp. 723–736.

Chanprateep, S. (2010) '*Current trends in biodegradable polyhydroxyalkanoates*', Journal of Bioscience and Bioengineering, Vol. 110(6), pp. 621–632.

Cheah, W.Y., Er, A.C., Aiyub, K., Mohd Yasin, N.H., Ngan, S.L., Chew, K.W., Khoo, K.S., Ling, T.C., Juan, J.C., Ma, Z. and Show, P.L. (2023) '*Current status and perspectives of algae-based bioplastics: A reviewed potential for sustainability*', Algal Research, Vol. 71(4), p. 103078.

Chioccioli, M., Hankamer, B. and Ross, I.L. (2014) '*Flow cytometry pulse width data enables rapid and sensitive estimation of biomass dry weight in the microalgae Chlamydomonas reinhardtii and Chlorella vulgaris*', PLoS ONE, Vol. 9(5), pp. 1–12.

Chuensangjun, C., Pechyen, C. and Sirisansaneeyakul, S. (2013) '*Degradation behaviors of different blends of polylactic acid buried in soil*', Energy Procedia, Vol. 34(12), pp. 73–82.

Cinar, S.O., Chong, Z.K., Kucuker, M.A., Wieczorek, N., Cengiz, U. and Kuchta, K. (2020) '*Bioplastic production from microalgae: A review*', International Journal of Environmental Research and Public Health, Vol. 17(11), pp. 1–21.

ComaniţĂ, E.D., Ghinea, C., Roşca, M., Simion, I.M., Petraru, M. and Gavrilescu, M. (2016) '*Environmental impacts of polyvinyl chloride (PVC) production process*', 2015 E-Health and Bioengineering Conference, EHB 2015 [Preprint]. Available at: https://ieeexplore.ieee.org/document/7391486 (Accessed 21 August 2023).

CondorFerries (2021) Marine & Ocean Pollution Statistics & Facts 2020-2021, CondorFerries. Available at: https://www.condorferries.co.uk/marine-ocean-pollution-statistics-facts (Accessed: 13 December 2022).

Costa, S.S., Miranda, A.L., de Morais, M.G., Costa, J.A.V. and Druzian, J.I. (2019) '*Microalgae as source of polyhydroxyalkanoates (PHAs) — A review*', International Journal of Biological Macromolecules. Elsevier, Vol. 131(6), pp. 536–547.

Deniz, İ. (2020) '*Determination of Growth Conditions for Chlorella vulgaris*', Marine Science and Technology Bulletin, In Press, Vol. 9(2), pp. 114–117.

Devasahayam, S., Raman, R.K.S., Chennakesavulu, K. and Bhattacharya, S. (2019) '*Plastics-Villain or hero? Polymers and recycled polymers in mineral and metallurgical processing-A review*', Materials, Vol. 12(4), p. 655.

Dianursanti and Khalis, S.A. (2018) '*The Effect of Compatibilizer Addition on Chlorella vulgaris Microalgae Utilization as a Mixture for Bioplastic*', E3S Web of Conferences, Vol. 67, pp. 2–6.

Edmundson, S.J. and Huesemann, M.H. (2015) '*The dark side of algae cultivation: Characterizing night biomass loss in three photosynthetic algae, Chlorella sorokiniana, Nannochloropsis salina and Picochlorum sp*.', Algal Research, Vol. 12(11), pp. 470–476.

El-Hadi, A., Schnabel, R., Straube, E., Müller, G. and Henning, S. (2002) '*Correlation between degree of crystallinity, morphology, glass temperature, mechanical properties and biodegradation of poly (3-hydroxyalkanoate) PHAs and their blends*', Polymer Testing, Vol. 21(6), pp. 665–674.

El-Naggar, N.E.A., Hussein, M.H., Shaaban-Dessuuki, S.A. and Dalal, S.R. (2020) '*Production, extraction and characterization of Chlorella vulgaris soluble polysaccharides and their applications in AgNPs biosynthesis and biostimulation of plant growth*', Scientific Reports, Vol. 10(1), pp. 1–19.

Elmanama, A.A., Fahd, M.I., Afifi, S., Abdallah, S. and Bahr, S. (2005) '*Microbiological beach sand quality in Gaza Strip in comparison to seawater quality*', Environmental Research, Vol. 99(1), pp. 1–10.

European Medicines Agency (2022) *ICH guideline Q3C (R8) on impurities: guideline for residual solvents*, ICH. Available at: www.ema.europa.eu/contact (Accessed: 5 June 2023).

Fazal, T., Mushtaq, A., Rehman, F., Ullah Khan, A., Rashid, N., Farooq, W., Rehman, M.S.U. and Xu, J. (2018) '*Bioremediation of textile wastewater and successive biodiesel production using microalgae*', Renewable and Sustainable Energy Reviews, Vol. 82(2), pp. 3107–3126.

Febrieni, V.N., Sedjati, S. and Yudiati, E. (2020) '*Optimization of light intensity on growth rate and total lipid content of Chlorella vulgaris*', IOP Conference Series: Earth and Environmental Science, Vol. 584(1), p. 012040.

Fiore, C.L., Jarett, J.K., Olson, N.D. and Lesser, M.P. (2010) '*Nitrogen fixation and nitrogen transformations in marine symbioses*', Trends in Microbiology, Vol. 18(10), pp. 455–463.

Gajra, B., Pandya, S.S., Vidyasagar, G., Rabari, H., Dedania, R.R. and Rao, S. (2012) '*Poly vinyl alcohol hydrogel and its pharmaceutical and biomedical applications: A review*', International Journal of Pharmaceutical Research, Vol. 4(2), pp. 20–26.

Galus, S., Kibar, E.A.A., Gniewosz, M. and Kraśniewska, K. (2020) 'Novel materials in the preparation of edible films and coatings-A review', *Coatings*, Vol. 10(7), pp. 1–14.

Gammage, E. (2022) *How long does it take for plastic to biodegrade?*, *SaveMoneyCutCarbon*. Available at: https://www.savemoneycutcarbon.com/learn-save/how-long-does-it-take-for-plastic-to-biodegrade/ (Accessed: 13 December 2022).

Gammanpila, A.M., Rupasinghe, C.P. and Subasinghe, S. (2015) '*Light Intensity and Photo Period Effect on Growth and Lipid Accumulation of Microalgae Chlorella Vulgaris and Nannochloropsis Sp for Biodiesel Production 1 Anjala M*', Proceedings of 12th ISERD International Conference, Vol. 16(9), pp. 51–55.

Gigli, M., Fabbri, M., Lotti, N., Gamberini, R., Rimini, B. and Munari, A. (2016) '*Poly(butylene succinate)-based polyesters for biomedical applications: A review*', European Polymer Journal, Vol. 75(2), pp. 431–460.

Gleadall, A. (2015) '*Mechanical properties of biodegradable polymers for medical applications*', Modelling Degradation of Bioresorbable Polymeric Medical Devices, pp. 163–199. Available at: https://www.sciencedirect.com/science/article/abs/pii/B9781782420163500098?via%3Dihub (Accessed 21 August 2023).

Goldman, J.C. and Dennett, M.R. (2000) '*Growth of marine bacteria in batch and continuous culture under carbon and nitrogen limitation*', Limnology and Oceanography, Vol. 45(4), pp. 789–800.

Goswami, G., Makut, B.B. and Das, D. (2019) '*Sustainable production of bio-crude oil via hydrothermal liquefaction of symbiotically grown biomass of microalgae-bacteria coupled with effective wastewater treatment*', Scientific Reports, Vol. 9(1), pp. 1–12.

Green, J. (2012) '*Report Topic: PLA and PHA biodegradation in the marine enviornment*', CalRecycle: California Department of Resources Recycling and Recovery, p. 38.

Griffiths, M.J., Garcin, C., van Hille, R.P. and Harrison, S.T.L. (2011) '*Interference by pigment in the estimation of microalgal biomass concentration by optical density*',

Journal of Microbiological Methods, Vol. 85(2), pp. 119–123.

Gupta, M.C. and Deshmukh, V.G. (1982) '*Thermal oxidative degradation of poly-lactic acid*', Colloid & Polymer Science, Vol. 260(5), pp. 514–517.

Habib, S. (2023) *PLA Glass Transition Temperature | The Definitive Guide*, *plasticrange*. Available at: https://plasticranger.com/pla-glass-transition-temperature/ (Accessed: 9 June 2023).

Hamielec, A.E., Macgregor, J.F. and Penlidis, A. (1989) '*Copolymerization*', in Comprehensive Polymer Science and Supplements. Pergamon, Vol. 3, pp. 17–31.

Han, F., Wang, W., Li, Y., Shen, G., Wan, M. and Wang, J. (2013) '*Changes of biomass, Lipid content and fatty acids composition under a light-dark cyclic culture of Chlorella pyrenoidosa in response to different temperature*', Bioresource Technology, Vol. 132(3), pp. 182–189.

Harrabin, R. and Edgington, T. (2019) *Recycling: Where is the plastic waste mountain?*, BBC. Available at: https://www.bbc.com/news/science-environment-46566795 (Accessed: 15 June 2023).

Harvesto Group (2020) *Importance of Nitrogen in Soil & Plants*, *Harvesto*. Available at: https://www.harvestogroup.com/post/importance-of-nitrogen-in-soil-plants (Accessed: 15 June 2023).

Hasan, M., Chong, E.W.N., Jafarzadeh, S., Paridah, M.T., Gopakumar, D.A., Tajarudin, H.A., Thomas, S. and Khalil, H.P.S.A. (2019) '*Enhancement in the physico-mechanical functions of seaweed biopolymer film via embedding fillers for plasticulture application-A comparison with conventional biodegradable mulch film*', Polymers, Vol. 11(2), p. 210.

Hidayahtullah, F.S., Hermawan, A. and Gofar, N. (2019) '*Microalgae Potential To Decreasing Dissolved Amonia Concentration (Nh3) on Liquid Waste of Urea Fertilizer Factory*', Techno: Jurnal Penelitian, Vol. 8(1), p. 224.

Hodgson, L. (2021) *Using Seaweed as Mulch and Fertilizer*, *Laidback Gardener*. Available at: https://laidbackgardener.blog/2021/09/18/using-seaweed-as-mulch-and-fertilizer/#:~:text=Answer%3A Seaweed%2C composed of both,an equally good soil amendment. (Accessed: 7 June 2023).

Hongsriphan, N. and Pinpueng, A. (2019) '*Properties of Agricultural Films Prepared from Biodegradable Poly(Butylene Succinate) Adding Natural Sorbent and Fertilizer*', Journal of Polymers and the Environment, Vol. 27(2), pp. 434–443.

Hosseinizand, H., Sokhansanj, S. and Lim, C.J. (2018) '*Studying the drying*

mechanism of microalgae Chlorella vulgaris and the optimum drying temperature to preserve quality characteristics', Drying Technology, Vol. 36(9), pp. 1049–1060.
Hsieh, C.H. and Wu, W.T. (2009) '*Cultivation of microalgae for oil production with a cultivation strategy of urea limitation*', Bioresource Technology, Vol. 100(17), pp. 3921–3926.
Ishino, K., Shingai, H., Hikita, Y., Yoshikawa, I., Houjou, H. and Iwase, K. (2021) '*Cold Crystallization and the Molecular Structure of Imidazolium-Based Ionic Liquid Crystals with a p-Nitroazobenzene Moiety*', ACS Omega, Vol. 6(48), pp. 32869–32878.
Ismael, F., Ndayiragije, A. and Fangueiro, D. (2021) '*New fertilizer strategies combining manure and urea for improved rice growth in mozambique*', Agronomy, Vol. 11(4), pp. 1–18.
Ivanova, N., Gugleva, V., Dobreva, M., Pehlivanov, I., Stefanov, S. and Andonova, V. (2023) '*We are IntechOpen , the world ' s leading publisher of Open Access books Built by scientists , for scientists TOP 1 %*', Intech, i, p. 13. Available at: https://www.intechopen.com/chapters/74904 (Accessed 21 August 2023).
Jaatinen, S., Lakaniemi, A.M. and Rintala, J. (2016) '*Use of diluted urine for cultivation of Chlorella vulgaris*', Environmental Technology (United Kingdom), Vol. 37(9), pp. 1159–1170.
Jamshidian, M., Tehrany, E.A., Imran, M., Jacquot, M. and Desobry, S. (2010) '*Poly-Lactic Acid: Production, applications, nanocomposites, and release studies*', Comprehensive Reviews in Food Science and Food Safety, Vol. 9(5), pp. 552–571.
Jena, J., Nayak, M., Sekhar Panda, H., Pradhan, N., Sarika, C., Ku. Panda, P., V. S. K Rao, B., B. N. Prasad, R. and Behari Sukla, L. (2012) '*Microalgae of Odisha Coast as a Potential Source for Biodiesel Production*', World Environment, Vol. 2(1), pp. 12–17.
Kalita, N.K., Damare, N.A., Hazarika, D., Bhagabati, P., Kalamdhad, A. and Katiyar, V. (2021) '*Biodegradation and characterization study of compostable PLA bioplastic containing algae biomass as potential degradation accelerator*', Environmental Challenges, Vol. 3(2), p. 100067.
Karim, A., Amirul Islam, M., Khalid, Z. Bin, Faizal, C.K.M., Khan, M.M.R. and Yousuf, A. (2019) '*Microalgal cell disruption and lipid extraction techniques for potential biofuel production*', Microalgae Cultivation for Biofuels Production, Vol. 11, pp. 129–147.

Kazmer, D. (2016) '*Design of Plastic Parts*', in Applied Plastics Engineering Handbook: Processing, Materials, and Applications: Second Edition. William Andrew Publishing, pp. 617–634. Available at: https://www.sciencedirect.com/science/article/abs/pii/B9780323390408000286?via%3Dihub (Accessed 21 August 2023).

Kendirlioglu, G., Agirman, N. and Cetin, A.K. (2015) '*The effects of photoperiod on the growth, protein amount and pigment content of Chlorella vulgaris*', Turkish Journal of Science & Technology, Vol. 10(2), pp. 7–10.

Khalofah, A., Ghramh, H.A., Al-Qthanin, R.N. and L'taief, B. (2022) '*The impact of NPK fertilizer on growth and nutrient accumulation in juniper (Juniperus procera) trees grown on fire-damaged and intact soils*', PLoS ONE, Vol. 17(1), pp. 1–14.

Kim, G.M., Chang, W.S. and Kim, Y.K. (2022) '*Biocomposites using whole or valuable component-extracted microalgae blended with polymers: A review*', Catalysts, Vol. 12(1), pp. 1–10.

Kourmentza, C., Plácido, J., Venetsaneas, N., Burniol-Figols, A., Varrone, C., Gavala, H.N. and Reis, M.A.M. (2017) '*Recent advances and challenges towards sustainable polyhydroxyalkanoate (PHA) production*', Bioengineering, Vol. 4(2), p. 55.

Krausfeldt, L.E., Farmer, A.T., Castro Gonzalez, H.F., Zepernick, B.N., Campagna, S.R. and Wilhelm, S.W. (2019) '*Urea Is Both a Carbon and Nitrogen Source for Microcystis aeruginosa: Tracking 13C incorporation at bloom pH conditions*', Frontiers in Microbiology, Vol. 10(5), pp. 1–12.

Kumar, A. and Bera, S. (2020) '*Bioresource Technology Reports Revisiting nitrogen utilization in algae : A review on the process of regulation and assimilation*', Bioresource Technology Reports, Vol. 12(10), p. 100584.

Kumar, N., Banerjee, C., Chang, J.S. and Shukla, P. (2022) '*Valorization of wastewater through microalgae as a prospect for generation of biofuel and high-value products*', Journal of Cleaner Production, Vol. 362, p. 132114.

Lam, M.K. and Lee, K.T. (2012) '*Potential of using organic fertilizer to cultivate Chlorella vulgaris for biodiesel production*', Applied Energy, Vol. 94, pp. 303–308.

Li, L., Hashaikeh, R. and Arafat, H.A. (2013) '*Development of eco-efficient micro-porous membranes via electrospinning and annealing of poly (lactic acid)*', Journal of Membrane Science, Vol. 436, pp. 57–67.

Liao, Y., Liu, C., Coppola, B., Barra, G., Di Maio, L., Incarnato, L. and Lafdi, K. (2019) '*Effect of porosity and crystallinity on 3D printed PLA properties*', Polymers,

Vol. 11(9), pp. 1–14.

Liu, Y., Li, K., M. Mohideen, M. and Ramakrishna, S. (2019) '*Fiber membranes obtained by melt electrospinning for drug delivery*', in Melt Electrospinning. Academic Press, pp. 173–195. Available at: https://www.sciencedirect.com/science/article/abs/pii/B9780128162200000099?via%3Dihub (Accessed 21 August 2023).

Lu, J., Tappel, R.C. and Nomura, C.T. (2009) '*Mini-Review: Biosynthesis of Poly(hydroxyalkanoates)*', Polymer Reviews, Vol. 49(3), pp. 226–248.

Lu, Z., Xu, Z., Kong, L., Shen, H. and Aschenbach, J.R. (2020) '*Functional Changes of the Community of Microbes With Ni-Dependent Enzyme Genes Accompany Adaptation of the Ruminal Microbiome to Urea-Supplemented Diets*', Frontiers in Microbiology, Vol. 11(12).

Lund, J.W.G., Kipling, C. and Le Cren, E.D. (1958) '*The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting*', Hydrobiologia, Vol. 11(2), pp. 143–170.

Lv, S., Zhang, Y., Gu, J. and Tan, H. (2017) '*Biodegradation behavior and modelling of soil burial effect on degradation rate of PLA blended with starch and wood flour*', Colloids and Surfaces B: Biointerfaces, Vol. 159, pp. 800–808.

Ma'mun, S., Wahyudi, A. and Raghdanesa, A.S. (2022) '*Growth rate measurements of Chlorella vulgaris in a photobioreactor by Neubauer-improved counting chamber and densitometer*', IOP Conference Series: Earth and Environmental Science, Vol. 963(1). p. 012015.

Malghani, A.L., Malik, A.U., Sattar, A., Hussain, F., Abbas, G. and Hussain, J. (2010) '*RESPONSE OF GROWTH AND YIELD OF WHEAT TO NPK*', Sci.Int.(Lahore), Vol. 24(2), pp. 185–189.

Maraveas, C. (2020) '*Production of sustainable and biodegradable polymers from agricultural waste*', Polymers, Vol. 12(5), p. 1127.

Merrington, A. (2011) '*Recycling of Plastics*', Applied Plastics Engineering Handbook: Processing and Materials, pp. 177–192. Available at: https://www.sciencedirect.com/science/article/abs/pii/B978143773514710011X?via%3Dihub (Accessed: 21 August 2023).

Mhatre, A., Patil, S., Agarwal, A., Pandit, R. and Lali, A.M. (2019) '*Influence of nitrogen source on photochemistry and antenna size of the photosystems in marine green macroalgae, Ulva lactuca*', Photosynthesis Research, Vol. 139(1–3), pp. 539–

551.

Milind, L., Santosh, P., Zaki, T. and John, D. (2013) '*Polymer Based Wafer Technology: A Review*', International Journal of Pharmaceutical & Biological Archives, Vol. 4(6), pp. 1060–1074.

Mohamed, R.M. and Yusoh, K. (2015) '*A Review on the Recent Research of Polycaprolactone (PCL)*', Advanced Materials Research, Vol. 1134, pp. 249–255.

Mohseni, F. and Moosavi Zenooz, A. (2022) '*Flocculation of Chlorella vulgaris with alum and pH adjustment*', Biotechnology and Applied Biochemistry, Vol. 69(3), pp. 1112–1120.

Mtaki, K., Kyewalyanga, M.S. and Mtolera, M.S.P. (2021) '*Supplementing wastewater with NPK fertilizer as a cheap source of nutrients in cultivating live food (Chlorella vulgaris)*', Annals of Microbiology, Vol. 71(1), p. 7.

Mullarkey, M. (2017) *Just 9% of discarded plastic recycled since 1950s*, IEMA. Available at: https://www.iema.net/articles/just-9-of-discarded-plastic-recycled-since-1950s#media-enquiry (Accessed: 13 December 2022).

N. Amenaghawon, A., A. Asegame, P. and O. Obahiagbon, K. (2013) '*Potential Application of Urea and NPK 15:15:15 Fertilizers as Biostimulants in the Bioremediation of Domestic Wastewater*', American Journal of Environmental Protection, Vol. 1(4), pp. 91–95.

Naresh Kumar, A., Chatterjee, S., Hemalatha, M., Althuri, A., Min, B., Kim, S.H. and Venkata Mohan, S. (2020) '*Deoiled algal biomass derived renewable sugars for bioethanol and biopolymer production in biorefinery framework*', Bioresource Technology, Vol. 296, p. 122315.

Nava, V., Matias, M.G., Castillo-Escrivà, A., Messyasz, B. and Leoni, B. (2022) '*Microalgae colonization of different microplastic polymers in experimental mesocosms across an environmental gradient*', Global Change Biology, Vol. 28(4), pp. 1402–1413.

Okunola A, A., Kehinde I, O., Oluwaseun, A. and Olufiropo E, A. (2019) '*Public and Environmental Health Effects of Plastic Wastes Disposal: A Review*', Journal of Toxicology and Risk Assessment, Vol. 5(2).

Onar, N. (2014) '*Usage of Biopolymers In Medical Applications*', Conference Paper, Vol. 3, pp. 2011–2013.

Orona-Navar, A., Aguilar-Hernández, I., Nigam, K.D.P., Cerdán-Pasarán, A. and Ornelas-Soto, N. (2021) '*Alternative sources of natural pigments for dye-sensitized*

solar cells: Algae, cyanobacteria, bacteria, archaea and fungi', Journal of Biotechnology, Vol. 332, pp. 29–53.

Pacheco, M.M., Hoeltz, M., Moraes, M.S.A. and Schneider, R.C.S. (2015) '*Microalgae: Cultivation techniques and wastewater phycoremediation*', Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering, Vol. 50(6), pp. 585–601.

Pantani, R. and Sorrentino, A. (2013) '*Influence of crystallinity on the biodegradation rate of injection-moulded poly(lactic acid) samples in controlled composting conditions*', Polymer Degradation and Stability, Vol. 98(5), pp. 1089–1096.

Park, C.H. and Lee, W.I. (2012) '*Compression molding in polymer matrix composites*', in Manufacturing Techniques for Polymer Matrix Composites (PMCs). Woodhead Publishing, pp. 47–94. Available at: https://www.sciencedirect.com/science/article/abs/pii/B9780857090676500031?via%3Dihub (Accessed 21 August 2023).

Pattanasuttichonlakul, W., Sombatsompop, N. and Prapagdee, B. (2018) '*Accelerating biodegradation of PLA using microbial consortium from dairy wastewater sludge combined with PLA-degrading bacterium*', International Biodeterioration and Biodegradation, Vol. 132 (8), pp. 74–83.

Rahman, A. and Miller, C.D. (2017) '*Microalgae as a Source of Bioplastics*', Algal Green Chemistry: Recent Progress in Biotechnology, pp. 121–138. Available at: https://www.sciencedirect.com/science/article/abs/pii/B9780444637840000060?via%3Dihub (Accessed: 21 August 2023).

Rahman, M.H. and Bhoi, P.R. (2021) '*An overview of non-biodegradable bioplastics*', Journal of Cleaner Production, Vol. 294(4), p. 126218.

Rai, U., Deshar, G., Rai, B., Bhattarai, K., Dhakal, R. and Rai, S. (2014) '*Isolation and Culture Condition Optimization of Chlorella vulgaris*', Nepal Journal of Science and Technology, Vol. 14(2), pp. 43–48.

Rajpoot, A.S., Choudhary, T., Chelladurai, H., Nath Verma, T. and Shende, V. (2022) '*A comprehensive review on bioplastic production from microalgae*', Materials Today: Proceedings, Vol. 56(1), pp. 171–178.

Rasselet, D., Ruellan, A., Guinault, A., Miquelard-Garnier, G., Sollogoub, C. and Fayolle, B. (2014) '*Oxidative degradation of polylactide (PLA) and its effects on physical and mechanical properties*', European Polymer Journal, Vol. 50(1), pp. 109–116.

Ribeiro, D.M., Roncaratti, L.F., Possa, G.C., Garcia, L.C., Cançado, L.J., Williams, T.C.R. and dos Santos Alves Figueiredo Brasil, B. (2020) '*A low-cost approach for Chlorella sorokiniana production through combined use of urea, ammonia and nitrate based fertilizers*', Bioresource Technology Reports, Vol. 9, p. 100354.

Richmond, A. (1986) *HANDBOOK of microalgae culture*. Blackwell. Available at: https://books.google.com.tr/books?hl=en&lr=&id=OEoPEAAAQBAJ&oi=fnd&pg=PA117&dq=cell+counting+for+microalgae&ots=4j26pa0GXG&sig=yB_CpuAMId7uD4Op61sNNpKZICY&redir_esc=y#v=onepage&q=cell counting for microalgae&f=false (Accessed: 21 August 2023).

Rogers, T. (2015) *Everything You Need To Know About Polylactic Acid (PLA), CREATIVEMECHANISMS*. Available at: https://www.creativemechanisms.com/blog/learn-about-polylactic-acid-pla-prototypes (Accessed: 16 September 2022).

Rosa, R.M., Machado, M., Vaz, M.G.M.V., Lopes-Santos, R., Nascimento, A.G. do, Araújo, W.L. and Nunes-Nesi, A. (2023) '*Urea as a source of nitrogen and carbon leads to increased photosynthesis rates in Chlamydomonas reinhardtii under mixotrophy*', Journal of Biotechnology, Vol. 367, pp. 20–30.

Rozen, Y. and Belkin, S. (2005) '*Survival of enteric bacteria in seawater: Molecular aspects*', Oceans and Health: Pathogens in the Marine Environment, Vol. 25, pp. 93–107.

Sadak Turhan, A. and Şensoy, S. (2022) '*Utilization of microalgae [Chlorella vulgaris Beyerinck (Beijerinck)] on plant growth and nutrient uptake of garden cress (Lepidium sativum L.) grown in different fertilizer applications*', International Journal of Agriculture, Environment and Food Sciences, Vol. 6(2), pp. 240–245.

Sarrafzadeh, M.H., La, H.J., Seo, S.H., Asgharnejad, H. and Oh, H.M. (2015) '*Evaluation of various techniques for microalgal biomass quantification*', Journal of Biotechnology, Vol. 216, pp. 90–97.

Savvidou, M.G., Dardavila, M.M., Georgiopoulou, I., Louli, V., Stamatis, H., Kekos, D. and Voutsas, E. (2021) '*Optimization of microalga chlorella vulgaris magnetic harvesting*', Nanomaterials, Vol. 11(6), p. 1614.

Schagerl, M., Siedler, R., Konopáčová, E. and Ali, S.S. (2022) '*Estimating Biomass and Vitality of Microalgae for Monitoring Cultures: A Roadmap for Reliable Measurements*', Cells, Vol. 11(15), p. 2455.

Schüler, L., Greque de Morais, E., Trovão, M., Machado, A., Carvalho, B., Carneiro,

M., Maia, I., Soares, M., Duarte, P., Barros, A., Pereira, H., Silva, J. and Varela, J. (2020) '*Isolation and Characterization of Novel Chlorella Vulgaris Mutants With Low Chlorophyll and Improved Protein Contents for Food Applications*', Frontiers in Bioengineering and Biotechnology, Vol. 8(5)

Sexton, C. (2019) *Plastic waste kills a person every 30 seconds in developing countries,* earth.com. Available at: https://www.earth.com/news/plastic-waste-kills-person/ (Accessed: 10 June 2023).

da Silva Ferreira, V. and Sant'Anna, C. (2017) '*Impact of culture conditions on the chlorophyll content of microalgae for biotechnological applications*', World Journal of Microbiology and Biotechnology, Vol. 33(1), p. 20.

Singh, R., Kumar, N., Mehrotra, T., Bisaria, K. and Sinha, S. (2020) *Environmental hazards and biodegradation of plastic waste: Challenges and future prospects*, Bioremediation for Environmental Sustainability: Toxicity, Mechanisms of Contaminants Degradation, Detoxification and Challenges. INC. pp. 193-214

Smita Mohanty, M.C. (2015) '*Effect of Different Solvents in Solvent Casting of Porous caffolds – In Biomedical and Tissue Engineering Applications*', Journal of Tissue Science & Engineering, Vol. 6(01), pp. 1–7.

Song, S., Feng, J. and Wu, P. (2011) '*Annealing of melt-crystallized polyethylene and its influence on microstructure and mechanical properties*: *A comparative study on branched and linear polyethylenes*', Journal of Polymer Science, Part B: Polymer Physics, Vol. 49(19), pp. 1347–1359.

Spennati, E., Casazza, A.A. and Converti, A. (2020) '*Winery wastewater treatment by microalgae to produce low-cost biomass for energy production purposes*', Energies, Vol. 13(10), p. 2490.

Sun, X., Chen, B., Li, Q., Liu, N., Xia, B., Zhu, L. and Qu, K. (2018) '*Toxicities of polystyrene nano- and microplastics toward marine bacterium Halomonas alkaliphila*', Science of the Total Environment, Vol. 642(11), pp. 1378–1385.

Swarbrick, V.J., Bergbusch, N.T. and Leavitt, P.R. (2022) '*Spatial and temporal patterns of urea content in a eutrophic stream continuum on the Northern Great Plains*', Biogeochemistry, Vol. 157(2), pp. 171–191.

Taki, K., Suenaga, H. and Ito, H. (2018) '*Effect of thermal annealing on crystallinity and mechanical strength of textile glass and carbon fiber reinforced in situ polymerized ϵ-caprolactam parts*', Microsystem Technologies, Vol. 24(1), pp. 663–668.

Talukder, M.M.R., Das, P. and Wu, J.C. (2012) '*Microalgae (Nannochloropsis salina) biomass to lactic acid and lipid*', Biochemical Engineering Journal, Vol. 68(9), pp. 109–113.

Teske, M., Arbeiter, D., Schober, K., Eickner, T. and Grabow, N. (2016) '*Systemic analysis about residual chloroform in PLLA films*', Current Directions in Biomedical Engineering, Vol. 2(1), pp. 49–52.

Thellen, C., Coyne, M., Froio, D., Auerbach, M., Wirsen, C. and Ratto, J.A. (2008) '*A processing, characterization and marine biodegradation study of melt-extruded polyhydroxyalkanoate (PHA) films*', Journal of Polymers and the Environment, Vol. 16(1), pp. 1–11.

Toghyani, A., Matthews, S. and Varis, J. (2020) '*Forming challenges of extruded wood plastic composite products in a post-production process*', in Procedia CIRP. Elsevier, Vol. 93, pp. 502–507.

Tokiwa, Y., Calabia, B.P., Ugwu, C.U. and Aiba, S. (2009) '*Biodegradability of plastics*', International Journal of Molecular Sciences, Vol. 10(9), pp. 3722–3742.

Tosif, M.M., Najda, A., Bains, A., Zawiślak, G., Maj, G. and Chawla, P. (2021) '*Starch–mucilage composite films: An inclusive on physicochemical and biological perspective*', Polymers, Vol. 13(16). p. 2588.

West, L. (2020) *Pros and Cons of PLA: Corn-Based Plastic, Treehugger*. Available at: https://www.treehugger.com/pros-cons-corn-based-plastic-pla-1203953 (Accessed: 17 June 2023).

Wilcox, C., Van Sebille, E., Hardesty, B.D. and Estes, J.A. (2015) '*Threat of plastic pollution to seabirds is global, pervasive, and increasing*', Proceedings of the National Academy of Sciences of the United States of America, Vol. 112(38), pp. 11899–11904.

Wu, M., Wu, G., Lu, F., Wang, H., Lei, A. and Wang, J. (2022) '*Microalgal photoautotrophic growth induces pH decrease in the aquatic environment by acidic metabolites secretion*', Biotechnology for Biofuels and Bioproducts, Vol. 15(1), pp. 1–13.

Xu, R. and Mi, Y. (2011) '*Simplifying the process of microalgal biodiesel production through in situ transesterification technology*', JAOCS, Journal of the American Oil Chemists' Society, Vol. 88(1), pp. 91–99.

Yang, Z., Peng, H., Wang, W. and Liu, T. (2010) '*Crystallization behavior of poly(ε-caprolactone)/layered double hydroxide nanocomposites*', Journal of Applied

Polymer Science, Vol. 116(5), pp. 2658–2667.

Yeo, J., Kim, G., Hong, S., Kim, M.S., Kim, D., Lee, J., Lee, H.B., Kwon, J., Suh, Y.D., Kang, H.W., Sung, H.J., Choi, J.H., Hong, W.H., Ko, J.M., Lee, S.H., Choa, S.H. and Ko, S.H. (2014) '*Flexible supercapacitor fabrication by room temperature rapid laser processing of roll-to-roll printed metal nanoparticle ink for wearable electronics application*', Journal of Power Sources, Vol. 246 (1), pp. 562–568.

Yew, G.Y., Chew, K.W., Malek, M.A., Ho, Y.C., Chen, W.H., Ling, T.C. and Show, P.L. (2019) '*Hybrid liquid biphasic system for cell disruption and simultaneous lipid extraction from microalgae Chlorella sorokiniana CY-1 for biofuel production*', Biotechnology for Biofuels, Vol. 12(1), pp. 1–12.

Yoganandam, K., Shanmugam, V., Vasudevan, A., Vinodh, D., Nagaprasad, N., Stalin, B., Karthick, A., Malla, C. and Bharani, M. (2021) '*Investigation of Dynamic, Mechanical, and Thermal Properties of Calotropis procera Particle-Reinforced PLA Biocomposites*', Advances in Materials Science and Engineering, Vol. 2021, p. 7.

Yu, B.S., Sung, Y.J., Hong, M.E. and Sim, S.J. (2021) '*Improvement of photoautotrophic algal biomass production after interrupted co2 supply by urea and kh2 po4 injection*', Energies, Vol. 14(3), p. 778.

Zhang, M., Buekens, A., Jiang, X. and Li, X. (2015) '*Dioxins and polyvinylchloride in combustion and fires*', Waste Management and Research, Vol. 33(7), pp. 630–643.

Zhu, L., Li, Z. and Hiltunen, E. (2018) '*Microalgae Chlorella vulgaris biomass harvesting by natural flocculant: Effects on biomass sedimentation, spent medium recycling and lipid extraction*', Biotechnology for Biofuels, Vol. 11(1), pp. 1–10.

Zou, G., Liu, Y., Zhang, Q., Zhou, T., Xiang, S. and Gu, Z. (2020) '*Pond for Ammonium and Phosphorus Removal from Pretreated Pig Urine*', Energies, Vol. 13, p. 1644.