POLY(3-HYDROXYBUTYRATE) BY RECOMBINANT *PHAEODACTYLUM TRICORNUTUM* VIA LIGHT-EMITTING DIODE CULTIVATION AND MICROWAVE-ASSISTED EXTRACTION

LEE WAH HOCK

A thesis submitted in fulfilment of the
requirements for the award of the degree of
Master of Philosophy

School of Chemical and Energy Engineering
Faculty of Engineering
Universiti Teknologi Malaysia

JANUARY 2019

DEDICATION

To my beloved mother and father

ACKNOWLEDGEMENT

In course of completing my master study, I had received a lot of assistance from my sources. First and foremost, I would like to express my deepest gratitude to my supervisor, Prof. Dr. Ani Idris for her patient guidance, enthusiastic encouragement, cooperation and useful advices. At the same time, I would also like to express my appreciation to her for her useful experience and instructions. She has no hesitation upon answering any questions in my master study.

I take immense pleasure in thanking Prof. Uwe Maier and Dr. Franziska Hempel from LOEWE Research Centre for Synthetic Microbiology (SYNMIKRO) for providing the genetically modified *Phaeodactylum tricornutum* strain. Furthermore, I would like to thank also Dr. Ehsan in assisting me on statistical analysis of the extraction data.

With this wonderful opportunity, my gratitude is extended to all my lab mates especially Effaliza, Nurdiana, Farahah, and Maryam under Advanced Bioprocessing and Separation (ABS) research group. I would like express my appreciation to all the people who involved and gave support to me directly or indirectly in completing my research work.

Last but not least, I not forget also to express a sense of gratitude and love to my beloved parents and family members as giving me their manual support and helped me without hesitation and require no rewards.

ABSTRACT

Bioplastic such as poly(3-hydroxybutyrate) (PHB) is an alternative approach to replace petroleum-derived plastic. However, high substrate cost and slow extraction process have hindered wide application of PHB. In this research, genetically modified *Phaeodactylum tricornutum* was used as an alternative PHB producer because it is able to utilize carbon dioxide. Ammonium and nitrate were used as nitrogen sources in the cultivation to suppress and activate PHB synthesis of this strain, respectively. The first phase of this study aimed to simplify the cultivation process by substituting the ammonium at its complete depletion point on day 5 with nitrate and enhance PHB productivity by different light wavelengths strategy. In the simplified cultivation method or known as one-step cultivation (OSC) for PHB synthesis, the steps in cell harvesting were removed when switching the nitrogen sources. Findings revealed that OSC is feasible as no sign of PHB synthesis suppression was present. In fact, the PHB productivity has improved to 9.75±0.64 (117.02±7.73 mg/L) from 7.40±0.52 mg/L/day (85.26±1.83 mg/L) which was achieved via multiple-step cultivation. In different light wavelength studies, red light was determined as better wavelength where the culture revealed higher specific growth rate and approximately 1.45-fold higher PHB productivity against white light culture. In the second phase of the study, PHB was extracted from wet biomass using microwave-assisted extraction (MAE) and propylene carbonate/isopropanol (PC/IPA). PC/IPA has high PHB solubility of 94.8±1.5%, boiling point of 99.1 °C, good dielectric properties, and miscible with water. Two-level full factorial design was used to evaluate the effect of the parameters that were A: extraction temperature (65-85 °C), B: extraction duration (5-15 min), and C: solvent-to-biomass ratio (5-15 mL/g) in PHB MAE. The results revealed that factor A and C significantly influenced the PHB recovery. The PHB MAE was optimized using central composite design. Based on prediction, the optimum PHB recovery of 97.89% can be achieved at 88 °C for 15 min with solvent-to-biomass ratio of 6.4 mL/g. The experimental PHB recovery of 95.63±0.70% with purity of 75±4% achieved by MAE method were significantly higher than conventional heating extraction (CHE) using chloroform (recovery: 79.53±2.87%, purity: 97±2%). The recovered PHB by MAE has high molecular weight of 1.4×10^6 Da. However, the melting point, melting enthalpy, and crystallinity were lower than PHB recovered from CHE using chloroform. The outcomes revealed the MAE system was excellent for PHB extraction as it offers high PHB recovery, cell breaking feature, safe processing conditions, wet biomass extraction and less hazardous compared to chloroform.

ABSTRAK

Bioplastik seperti poli(3-hidroksibutirat) (PHB) adalah pendekatan alternatif bagi menggantikan plastik berasaskan petroleum. Walau bagaimanapun, kos substrat yang tinggi dan proses pengekstrakan yang perlahan menghalang aplikasi PHB secara meluas. Dalam kajian ini, *Phaeodactylum tricornutum* terubah suai genetik telah digunakan sebagai penghasil PHB alternatif memandangkan ia boleh menggunakan karbon dioksida. Ammonium dan nitrat masing-masing telah digunakan sebagai sumber nitrogen dalam penanaman untuk menghalang dan mengaktifkan sintesis PHB oleh mikroorganisma ini. Fasa pertama kajian bertujuan untuk mempermudahkan proses penanaman dengan menggantikan ammonium pada titik penghabisannya pada hari ke-5 dengan nitrat dan meningkatkan produktiviti PHB melalui strategi gelombang cahaya yang berlainan. Dalam kaedah penanaman mudah atau dikenali sebagai penanaman satu langkah (OSC) bagi penghasilan PHB, langkah penuaian sel telah dikeluarkan semasa penukaran sumber nitrogen. Hasil kajian menunjukkan OSC boleh dilaksanakan kerana tiada tanda halangan yang wujud pada sintesis PHB. Malah, produktiviti PHB telah meningkat kepada 9.75±0.64 (117.02±7.73 mg/L) daripada 7.40±0.52 mg/L/hari (85.26±1.83 mg/L) yang telah dicapai melalui penanaman langkah berganda. Dalam kajian gelombang cahaya yang berbeza, cahaya merah merupakan gelombang cahaya lebih baik yang mana kultur menunjukkan kadar pertumbuhan spesifik yang lebih tinggi dan produktiviti PHB lebih tinggi sebanyak 1.45 kali ganda lebih tinggi berbanding kultur di bawah cahaya putih. Dalam fasa kedua kajian, PHB telah diekstrak daripada biojisim basah menggunakan pengekstrakan berbantukan gelombang mikro (MAE) dan propilena karbonat/isopropanol (PC/IPA). PC/IPA mempunyai kelarutan PHB yang tinggi iaitu 94.8±1.5%, takat didih 99.1 °C, sifat dielektrik yang baik dan larut dalam air. Reka bentuk dua peringkat faktorial penuh telah diguna untuk menilai kesan parameter A: suhu pengekstrakan (65-85 °C), B: tempoh pengekstrakan (5-15 minit) dan C: nisbah pelarut kepada biojisim (5-15 mL/g) di MAE. Keputusan menunjukkan bahawa faktor A dan C mempengaruhi perolehan PHB secara signifikan. MAE bagi PHB telah dioptimumkan menggunakan reka bentuk komposit sentral. Berdasarkan ramalan, perolehan PHB optimum sebanyak 97.89% boleh dicapai pada 88 °C, 15 minit dengan nisbah pelarut kepada biojisim 6.4 mL/g. Perolehan PHB secara ujikaji adalah 95.63±0.70% dengan ketulenan 75±4% dicapai oleh kaedah MAE dan nilai ini jauh lebih tinggi daripada pengekstrakan pemanasan konvensional (CHE) menggunakan kloroform (perolehan: 79.53±2.87%, ketulenan: 97±2%). PHB yang diperoleh melalui MAE mempunyai berat molekul yang tinggi iaitu 1.4×10^6 Da. Namun, takat lebur, entalpi lebur dan kekristalan lebih rendah daripada PHB yang diperoleh daripada CHE menggunakan klorofom. Hasil kajian menunjukkan sistem MAE ini amat baik untuk pengekstrakan PHB kerana ia menawarkan perolehan PHB yang tinggi, ciri pemecahan sel, pemprosesan yang selamat, pengekstrakan menggunakan biojisim basah, dan kurang berbahaya berbanding dengan penggunaan klorofom.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

A	-	Extraction temperature
Ace	-	Acetone
Adeq.	-	Adequate
Adj. R^2	-	Adjusted R-squared
ANOVA	-	Analysis of variance
ATP	-	Adenosine triphosphate
B	-	Extraction duration
C	-	Solvent-to-biomass ratio
CCD	-	Central Composite Design
CHE	-	Conventional heating extraction
Conc.	-	Concentration
C.V.	-	Coefficient of variation
dcw	-	Dry cell weight
DF	-	Degree of freedom
DOE	-	Design of experiment
E. coli	-	*Escherichia coli*
e.g.	-	For Example
EPA	-	Eicosapentaenoic acid
EtOH	-	Ethanol
FabG	-	3-ketoacyl-CoA reductase
FDA	-	Food and Drug Administration
FID	-	Flame ionization detector
GC	-	Gas chromatography
GM	-	Genetically modified
GP	-	Growth phase
GPC	-	Gel permeation chromatography
GSK	-	GlaxoSmithKline
HPLC	-	High performance liquid chromatography
IPA	-	Isopropanol
LED	-	Light-emitting diodes

MAE	-	Microwave-assisted extraction
Max.	-	Maximum
MSC	-	Medium shift cultivation
M_w	-	Molecular weight
N/A	-	Not applicable
NADPH	-	Nicotinamide adenine dinucleotide phosphate
No.	-	Number
NPCM	-	Non-PHA cellular mass
OD	-	Optical density
OSC	-	One-step cultivation
PBS	-	Phosphate buffered saline
PC	-	Propylene carbonate
PEA	-	Propylene carbonate:Ethanol:Acetone
PHA	-	Polyhydroxyalkanoate
PhaA	-	β-ketothiolase
PhaB	-	Acetoacetyl-CoA reductase
PhaC	-	PHA synthase
PhaG	-	3-hydroxyl-ACP-CoA transferase
PhaJ	-	(*R*)-enoyl-CoA hydratase
Prob	-	Probability
Prod.		Productivity
PS	-	Photosystem
P. tricornutum	-	*Phaeodactylum tricornutum*
Pred R^2	-	Predicted R-squared
R	-	PHB recovery
R^2	-	R-squared
RL	-	Red light
RSM	-	Response surface methodology
SEM	-	Scanning electron microscopy
SP	-	PHB-synthesis phase
Std	-	Standard
Std. Dev.	-	Standard deviation
Temp.	-	Temperature

US	-	United states
UV	-	Ultraviolet
v/v	-	Volume per volume
WBL	-	White to blue light
WL	-	White light
WRL	-	White to red light
WM	-	Wet mass
w/v	-	Weight per volume
3D	-	Three dimension

LIST OF SYMBOLS

α	-	Axial point
A_i, B_i, C_i	-	Antoine constants of component i
β	-	Beta
β_0, β_i, β_{ii}, β_{ij}	-	Regression coefficients for intercept, linear, quadratic and interaction terms of quadratic regression model
C_0	-	Total number of disrupted cells
C_t	-	Total cell disruption after treatment
C	-	Cell density (cells/mL)
Da	-	Dalton
ε'	-	Dielectric constant
ε''	-	Dielectric loss
ε	-	Statistical error
ΔH_m	-	Melting enthalpy (Jg^{-1})
Hz	-	Hertz
k	-	Rate of cell division (day^{-1})
k	-	Number of factor
$\acute{k}$		Number of the coded independent variables
mm	-	Millimeter
M	-	Molar
$M_{biomass}$	-	Mass of biomass (mg)
M_{PHB}	-	Total mass of PHB recovered from a biomass (mg)
m_{gcPHB}	-	Mass of PHB quantified by GC (mg)
m_{PHB}	-	Mass of extracted PHB (mg)
mm HG	-	Millimeter of mercury
n	-	Degree of polymerization
nm	-	Nanometre
N	-	Concentration of ammonium (μM)
N_0	-	Total number of intact cell for non-treated sample
N_t	-	Intact cells number after treatment
ρ_i	-	Vapour pressure of component i (mm HG)

ρ_{total}	-	Total vapour pressure (mm HG)
pH	-	Potential of hydrogen
R	-	Alkyl group
R^2	-	Regression coefficient
rpm	-	Rotation per minute
t	-	Time interval (day)
T	-	Temperature (°C)
T_m	-	Melting temperature (°C)
$\tan \delta$	-	Loss tangent
µmol $m^{-2}s^{-1}$	-	Micromoles per square meter per second
μ	-	Specific growth rate (day^{-1})
$U_{NH_4^+}$	-	Uptake rate of ammonium (µM N/cell/day)
wt%	-	Weight percentage
x	-	Number of repeating units
Δx	-	Step size for the significant factor
χ_c	-	Crystallinity (%)
X_i	-	Mole fraction of component *i* in the solution
$\hat{Y}$	-	Response
Y_i	-	Mole fraction of component *i* in gas phase
Y_{yield}	-	Percentage of PHB recovery yield (%)

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Research Background

Plastics are organic polymer commonly synthesized using petrochemicals such as polyethylene, polyvinyl chloride, and polystyrene. These petrochemicals are obtained by catalysis of their monomers through polymerization and polycondensation process. Due to its outstanding properties which can be moulded into desired shape and performance better than metal and wood, plastics have wide variety of industrial applications (Álvarez-Chávez *et al.*, 2012). Hence, the demand for plastics is increasing while the petroleum reserves are declining. According to Qualman in 2017, over 400 million tons of plastics were produced by 2017. However, the wide applications of plastics have raised abundance of environmental issues. The high molecular weight of plastics which ranged from 50 to 1,000 kDa has made the plastics non-biodegradable and persist in the environment for a long time (Kumaravel *et al.*, 2010). This eventually led to severe pollutions, landfill problem, and climate change (Barker *et al.*, 2007; Law *et al.*, 2010; Philp *et al.*, 2013). In order to reduce the use of petrochemical-derived plastics, attempts were made to develop potential sustainable green alternatives.

Bioplastics are advance materials derived from renewable sources and can be biodegraded. These materials were developed to replace problematic petroleum-based plastics. The bioplastics can be classified into starch-based plastics, chemically synthesized bioplastics, and polylactic acid plastics (Jabeen *et al.*, 2015). There is another group of bioplastics known as organism-based bioplastics which are synthesized by microorganisms using starch or glucose (Tan *et al.*, 2014). Polyhydroxyalkanoates (PHAs) are a type of saturated poly-α-hydroxy esters synthesized by bacteria. Under stress condition, bacteria produce enzymes that convert acetyl-CoA to PHAs. These polyesters have physical properties which are

similar to polypropylene but are 100% biodegradable and biocompatible (Singh, 2015). Due to these properties, PHAs received attention from the industry and can be potentially used in medical applications.

One of the applications of PHAs is in food packaging material. Besides being biodegradable, these polyesters have low water vapour permeability which is an advantage over other biopolymers. The most common type of PHAs is poly(3-hydroxybutyrate) (PHB), which is the mostly used PHA in food packaging. This polyester is applied in bulk shrink packaging and flexible intermedia bulk containers (Jabeen *et al.*, 2015). Furthermore, PHB has also been utilized in bone scaffold development in tissue engineering. In medical industry, PHB has been utilized in sustained drug delivery systems and medical devices. In tissue engineering, scaffolding materials should satisfy several criteria such as biocompatible which are non-immunogenic, non-cytotoxic, and non-inflammatory. Besides, the materials should possess great mechanical properties, particularly for bone scaffold to support bone tissue regeneration and prevent structural failure during patient's normal activities. Hence, PHB is a potential candidate for achieving ideal bone scaffold (Shrivastav *et al.*, 2013).

To increase the availability and reduce the production cost of PHB, several organisms had been genetically modified to synthesize the polymer. Besides bacterial expression system, plant-based expression systems are also used to produce PHAs. Plant-based expression systems utilize photosynthesis process and do not require external organic source in PHAs synthesis. This low-cost expression system is very attractive when applied in large scale production as it reduces the production cost. Despite its advantages, this expression system is not ideal for application as it competes the land use with subsistence crops, disperses uncontrollably, and has extremely slow growth rate (Daniell *et al.*, 2009). Another expression system which uses microalgae (*Phaeodactylum tricornutum*) was introduced to solve the problems faced while sharing the advantages of plant-based expression systems. Hence, the microalgae expression system is relatively desirable to act as a low cost platform for PHB production (Hempel *et al.*, 2011a). To the best of our knowledge, the enhancement of cultivation for this genetically modified microalgae strain has yet to

be proposed. Since microalgae are strongly influenced by light quality, the growth and PHB synthesis of genetically modified *P. tricornutum* were enhanced by subjecting the cells to different light wavelengths.

Since PHB is accumulated as intracellular granules, it has to be extracted and it typically involves cell rupture and dissolution of PHB granules. Generally, the extraction methods can be classified into solvent extraction methods, chemical-based, and enzyme-based digestions methods. Solvent extraction is the most well-established and commonly used among all the methods due to the high purity of PHB obtained (Tan *et al.*, 2014). The solvent helps to improve the permeability of the cell wall and dissolves the polymer. In order to increase the recovery of PHB, additional mechanical treatments such as bead milling and high pressure homogenization were also supplemented to the extraction process (Kunasundari and Sudesh, 2011). Beside these treatments, several types of mechanical method such as microwave and ultrasonic had also been applied to extract intracellular components.

Microwave-assisted extraction (MAE) is an alternative of conventional heating method utilizing microwave radiation as the heating source for extraction. The application of this radiation helps to accelerate the extraction process where the heat and mass transfer occur from inside to outside of substrate. Besides short processing time, this method offers high recovery, lesser solvent consumption, and product degradation (Veggi *et al.*, 2013). All the pros make the extraction method widely applied to extract natural products, for instance flavonoids from *radix astragali* (Xiao *et al.*, 2008), solanesol from tobacco leaves (Zhou and Liu, 2006), and zerumbone from *Zingiber zerumbet* (Ghasemzadeh *et al.*, 2017). However, there is no report on the application of MAE in PHB recovery. Hence, the very first extraction of PHB using microwave radiation was demonstrated in this study.

1.2 Problem Statements

Poly(3-hydroxybutyrate) (PHB) possesses commercial potential for the mass production of thermoplastic (Sudesh *et al.*, 2000). Despite the advantages of PHB, the

application of this bioplastic is still in its infant stage in the industry. This was due to the high cost of PHB which has hampered their commercial applications. In 2014, the cost of PHA was in the range of $ 4.6 to 9.3/kg (Bolck, 2014). The high cost of PHB was caused by the high purity substrate price which accounts for 45% of the total cost and high downstream processing cost (Kourmentza *et al.*, 2017).

According to Yousuf in 2017, the cost for PHB extraction was predicted to occupy half of the production cost. Besides the high fermentation cost, PHB extraction is also time consuming and tedious (Chen and Wu, 2005; Kunasundari and Sudesh, 2011). Majority of the reported solvent extraction methods of PHB take few hours or even one day (Dalcanton, 2006; Fei *et al.*, 2016; Manangan and Shawaphun, 2010; Ramsay *et al.*, 1994). Moreover, large amount of solvent is required in the extraction process. The additional step for cells rupture increased the complexity of extraction process and energy consumption which make the step not economically feasible (Kunasundari and Sudesh, 2011). Hence, the extraction process might be a challenge to a cost effective industrial upscale production for large amounts of PHB.

Solvent extraction is capable to achieve high recovery and purity of PHB, but negative environmental impact caused by the generation of hazardous waste is the greatest current concern. Most of the developed solvent extraction methods involve the use of chlorinated organic solvents such as methylene chloride (Ramsay *et al.*, 1994), 1,2-dichloroethane (Holmes and Lim, 1990), and chloroform which possess high efficiency in extraction of PHB. But, these chlorinated organic solvents are toxic, hazardous towards the environment and human, expensive, and may cause PHB degradation (Hahn *et al.*, 1994). Although several green solvents such as 1,2-propylene carbonate (Fiorese *et al.*, 2009), and butyl acetate (Aramvash *et al.*, 2015) had been reported in previous PHB extraction studies, most of them were used without much evaluation in the aspect of waste issues, degree of environmental and health impact, and processing safeness. In addition, the extraction process conditions using green solvents are still lengthy and involved high extraction temperature which are not desirable. Moreover, the recovery yield has not been optimized yet.

Thus, in this extraction study, solvent extraction was performed under microwave radiation to extract PHB from biomass and this method is known as microwave-assisted extraction (MAE). The significant parameters and effects of microwave radiation on the extraction of PHB were determined and the process was further optimized.

1.3 Objectives

The main objective of this study is to enhance the overall production of PHB by improving both the cultivation technique and extraction method. In order to achieve the ultimate goal, the following integrated objectives need to be fulfilled:

(i) To improve the PHB productivity of genetically engineered *Phaeodactylum tricornutum* in a single step (without transferring it from the medium with NH_4^+ which inhibits PHB synthesis to another medium with NO_3^- for gene expression) under blue and red LED light wavelengths.

(ii) To select solvent system based on dielectric properties, boiling point and PHB solubility.

(iii) To extract PHB using microwave-assisted extraction method under the selected green solvent and optimize MAE parameters.

1.4 Research Scopes

The scope of research are as follows:

1. (i) The one-step cultivation for PHB production by genetically modified (GM) *Phaeodactylum tricornutum* through direct shifting nitrogen source from ammonium chloride to sodium nitrate was performed. The complete depletion day of ammonium in culture medium was determined. The growth and PHB accumulation by *P. tricornutum* cultivated under one-step and medium shift cultivations were compared.

(ii) Different light wavelengths were used to enhance the PHB productivity of *P. tricornutum*. The blue, red, and white wavelengths were used as the treatment in the study. The growth and PHB accumulation by *P. tricornutum* cultivated under different light wavelengths were determined.

2. Development of green solvent system for recovery of PHB through microwave-assisted extraction. Several solvent systems such as acetone (Ace), ethanol (EtOH), isopropanol (IPA), propylene carbonate (PC), PC:Ace, PC:EtOH, PC:IPA, and PC:EtOH:Ace were evaluated in the aspects of dielectric properties, boiling point and PHB solubility.

3. (i) The factors varied in the microwave-assisted extraction are extraction temperature (65-85 °C), extraction duration (5-15 minutes), and solvent-to-biomass ratio (5.00-15.00 mL/g) on PHB extraction and experiments were performed using full factorial design. The significance of the factors and their interactions were determined.

(ii) The extraction parameters were optimized using research surface methodology to recover maximum amount of PHB. The central composite design was utilized to develop the response surface. A polynomial model for the prediction of PHB recovery in MAE was developed. The model was verified by various diagnostic plots and the error percentages between the predicted and experimental PHB recovery.

(iii) Recovered PHB was characterized by determining its molecular weight, melting point, and melting enthalpy.

(iv) The effects of different solvent systems and extraction methods on PHB recovery percentage, purity percentage and properties of extracted PHB were compared.

(v) The effects of microwave radiation on cells disruption were determined. The cells disruption percentages by conventional and microwave heating using PC:IPA were determined and compared. The cell morphological changes under different treatments were investigated by scanning electron microscope.

1.5 Significance of Study

The outcomes of this study are redounded for cost effective PHB production by solving the problems in the microalgae cultivation and PHB extraction. A simplified cultivation method for PHB production by GM *P. tricornutum* was developed. Light wavelength was used as the factor affecting the PHB productivity of *P. tricornutum*. Besides that, microwave-assisted extraction (MAE) was reported for the first time in PHB extraction. In the extraction study, a new green solvent system with high PHB solubility, high boiling point, good dielectric properties, and soluble in water was developed for PHB MAE. The MAE capable to recover most of the PHB from biomass in 15 minutes and below solvent's boiling point. The microwave radiation was proved to improve the dissolution of PHB and no sign of PHB degradation was detected where the recovered PHB possesses high molecular weight.

REFERENCES

Abiusi, F., Sampietro, G., Marturano, G., Biondi, N., Rodolfi, L., D'Ottavio, M., and Tredici, M. R. (2014). Growth, Photosynthetic Efficiency, and Biochemical Composition of Tetraselmis Suecica F&M-M33 Grown with LEDs of Different Colors. *Biotechnology and Bioengineering*. 111(5), 956–964.

Acierno, D., Barba, A. A., and D'Amore, M. (2004). Heat Transfer Phenomena during Processing Materials with Microwave Energy. *Heat and Mass Transfer*. 40(5), 413–420.

Al-Harahsheh, M., and Kingman, S. W. (2004). Microwave-Assisted Leaching-A Review. *Hydrometallurgy*. 73(3–4), 189–203.

Alfaro, M. J., Bélanger, J. M. R., Padilla, F. C., and Paré, J. R. J. (2003). Influence of Solvent, Matrix Dielectric Properties, and Applied Power on the Liquid-Phase Microwave-Assisted Processes (MAP^{TM}) Extraction of Ginger (Zingiber Officinale). *Food Research International*. 36(5), 499–504.

Álvarez-Chávez, C. R., Edwards, S., Moure-Eraso, R., and Geiser, K. (2012). Sustainability of Bio-Based Plastics: General Comparative Analysis and Recommendations for Improvement. *Journal of Cleaner Production*. 23(1), 47–56.

Amarni, F., and Kadi, H. (2010). Kinetics Study of Microwave-Assisted Solvent Extraction of Oil from Olive Cake Using Hexane: Comparison with the Conventional Extraction. *Innovative Food Science & Emerging Technologies*. 11(2), 322–327.

Amaro, H. M., Guedes, A. C., and Malcata, F. X. (2011). Advances and Perspectives in Using Microalgae to Produce Biodiesel. *Applied Energy*. 88(10), 3402–3410.

Apt, K. E., Grossman, A. R., and Kroth-Pancic, P. G. (1996). Stable Nuclear Transformation of the Diatom Phaeodactylum Tricornutum. *Molecular and General Genetics*. 252(5), 572–579.

Aramvash, A., Gholami-Banadkuki, N., Moazzeni-Zavareh, F., and Hajizadeh-Turchi, S. (2015). An Environmentally Friendly and Efficient Method for Extraction of PHB Biopolymer with Non-Halogenated Solvents. *J. Microbiol. Biotechnol.* 25(11), 1936–1943.

Baba, M., Kikuta, F., Suzuki, I., and Shiraiwa, Y. (2012). Wavelength Specificity of Growth, Photosynthesis, and Hydrocarbon Production in the Oil-Producing Green Alga Botryococcus Braunii. *Bioresource Technology*. 109, 266–270.

Baethgen, W. E., and Alley, M. M. (1989). A Manual Colorimetric Procedure for Measuring Ammonium Nitrogen in Soil and Plant Kjeldahl Digests. *Communications in Soil Science and Plant Analysis*. 20(9–10), 961–969.

Barham, P. J. (1990). Physical Properties of Poly(Hydroxybutyrate) and Poly(Hydroxybutyrate-Co-Hydroxyvalerate). In Dawes, E. A. (Ed.) *Novel Biodegradable Microbial Polymers* (pp. 81–96). Dordrecht: Springer.

Barker, T., Bashmakov, I., Bernstein, L., Bogner, J. E., Bosch, P. R., Dave, R., … Zhou, D. (2007). Technical Summary. In Metz, B., Davidson, O. R., Bosch, P. R., Dave, R., and Meyer, L. A. (Eds.) *Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, United Kingdom and New York, NY, USA.: Cambridge University Press.

Berg, J. M., Tymoczko, J. L., and Stryer, L. (2002). *Biochemistry*. (5th ed.). New York: W. H. Freeman and Company.

Berger, E., Ramsay, B. A., Ramsay, J. A., Chavarie, C., and Braunegg, G. (1989). PHB Recovery by Hypochlorite Digestion of Non-PHB Biomass. *Biotechnology Techniques*. 3(4), 227–232.

Bhandari, B. R. (2010). Beyond Water: Waterlike Functions of Other Biological Compounds in a Waterless System. In Reid, D. S., Sajjaanantakul, T., Lillford, P. J., and Charoenrein, S. (Eds.) *Water Properties in Food, Health, Pharmaceutical and Biological Systems: ISOPOW 10* (pp. 157–164). Bangkok, Thailand: John Wiley & Sons, Inc.

Blanken, W., Cuaresma, M., Wijffels, R. H., and Janssen, M. (2013). Cultivation of Microalgae on Artificial Light Comes at a Cost. *Algal Research*. 2(4), 333–340.

Bolck, C. (2014). The Revival of Microbial PHA Polymers - Are PHA's Back to Stay? Retrieved From http://edepot.wur.nl/305141.

Bohmert, K., Balbo, I., Steinbüchel, A., Tischendorf, G., and Willmitzer, L. (2002). Constitutive Expression of the β-Ketothiolase Gene in Transgenic Plants. A Major Obstacle for Obtaining Polyhydroxybutyrate-Producing Plants. *Plant Physiology*. 128(4), 1282–1290.

Bohmert-Tatarev, K., McAvoy, S., Daughtry, S., Peoples, O. P., and Snell, K. D. (2011). High Levels of Bioplastic Are Produced in Fertile Transplastomic Tobacco Plants Engineered with a Synthetic Operon for the Production of Polyhydroxybutyrate. *Plant Physiology*. 155(4), 1690–1708.

Breil, C., Meullemiestre, A., Vian, M., and Chemat, F. (2016). Bio-Based Solvents for Green Extraction of Lipids from Oleaginous Yeast Biomass for Sustainable Aviation Biofuel. *Molecules*. 21(2), 196.

Brglez Mojzer, E., Knez Hrnčič, M., Škerget, M., Knez, Ž., and Bren, U. (2016). Polyphenols: Extraction Methods, Antioxidative Action, Bioavailability and Anticarcinogenic Effects. *Molecules*. 21(7), 901.

Bury, D., Jelen, P., and Kaláb, M. (2001). Disruption of Lactobacillus Delbrueckii Ssp. Bulgaricus 11842 Cells for Lactose Hydrolysis in Dairy Products: A Comparison of Sonication, High-Pressure Homogenization and Bead Milling. *Innovative Food Science & Emerging Technologies*. 2(1), 23–29.

Byrom, D. (1992). Production of Poly-Hydroxyvalerate Copolymers. *FEMS Microbiology Letters*. 103, 247–250.

Camel, V., and Bermond, A. (1999). Un Gain de Temps Notable Pour L'analyse Chimique: L'utilisation Des Micro-Ondes Dans Le Traitement de L'échantillon. *Ann Fals Exp Chim*. 92, 117–135.

Carvalho, A. P., Silva, S. O., Baptista, J. M., and Malcata, F. X. (2011). Light Requirements in Microalgal Photobioreactors: An Overview of Biophotonic Aspects. *Applied Microbiology and Biotechnology*. 89(5), 1275–1288.

Chan, C.-H., Yusoff, R., Ngoh, G.-C., and Kung, F. W.-L. (2011). Microwave-Assisted Extractions of Active Ingredients from Plants. *Journal of Chromatography A*. 1218(37), 6213–6225.

Chan, C. H., Kummerlöwe, C., and Kammer, H. (2004). Crystallization and Melting Behavior of Poly(3-hydroxybutyrate)-Based Blends. *Macromolecular Chemistry and Physics*. 205(5), 664–675.

Chang, D. C., and Reese, T. S. (1990). Changes in Membrane Sructure Induced by Electroporation as Revealed by Rapid-Freezing Electron Microscopy. *Biophysical Journal*. 58(1), 1–12.

Chao, Y., and Zhang, T. (2011). Optimization of Fixation Methods for Observation of Bacterial Cell Morphology and Surface Ultrastructures by Atomic Force Microscopy. *Applied Microbiology and Biotechnology*. 92(2), 381–392.

Chen, C.-Y., Chen, Y.-C., Huang, H.-C., Huang, C.-C., Lee, W.-L., and Chang, J.-S. (2013). Engineering Strategies for Enhancing the Production of Eicosapentaenoic Acid (EPA) from an Isolated Microalga Nannochloropsis Oceanica CY2. *Bioresource Technology*. 147, 160–167.

Chen, C.-Y., Yeh, K.-L., Aisyah, R., Lee, D.-J., and Chang, J.-S. (2011). Cultivation, Photobioreactor Design and Harvesting of Microalgae for Biodiesel Production: A Critical Review. *Bioresource Technology*. 102(1), 71–81.

Chen, G.-Q., and Wu, Q. (2005). The Application of Polyhydroxyalkanoates as Tissue Engineering Materials. *Biomaterials*. 26(33), 6565–6578.

Chen, G., Zhang, G., Park, S., and Lee, S. (2001). Industrial Scale Production of Poly(3-Hydroxybutyrate-Co-3-Hydroxyhexanoate). *Applied Microbiology and Biotechnology*. 57(1), 50–55.

Chen, L., Song, D., Tian, Y., Ding, L., Yu, A., and Zhang, H. (2008). Application of On-Line Microwave Sample-Preparation Techniques. *TrAC Trends in Analytical Chemistry*. 27(2), 151–159.

Chisti, Y. (2007). Biodiesel from Microalgae. *Biotechnology Advances*. 25, 294–306.

Choi, J. S., and Park, W. H. (2004). Effect of Biodegradable Plasticizers on Thermal and Mechanical Properties of Poly(3-Hydroxybutyrate). *Polymer Testing*. 23(4), 455–460.

Cleaver, G., and Agilent Tehcnologies Inc. (2015). GPC/SEC Analysis of Biodegradable Polyhydroxyalkanoate on Agilent PLgel 10 Mm MIXED-B Columns. 1–4.

Cooper, W. J., Krasicky, P. D., and Rodriguez, F. (1986). Dissolution Rates of Poly(Methyl Methacrylate) Films in Mixed Solvents. *Journal of Applied Polymer Science*. 31(1), 65–73.

Dalcanton, F. (2006). *Produção, Extração e Caracterização de Poli(3-Hidroxibutirato) Por Ralstonia Eutropha Em Diferentes Substratos*. Universidade Federal de Santa Catarina.

Daniell, H., Singh, N. D., Mason, H., and Streatfield, S. J. (2009). Plant-Made Vaccine Antigens and Biopharmaceuticals. *Trends in Plant Science*. 14(12), 669–679.

Das, P., Lei, W., Aziz, S. S., and Obbard, J. P. (2011). Enhanced Algae Growth in Both Phototrophic and Mixotrophic Culture under Blue Light. *Bioresource Technology*. 102(4), 3883–3887.

Datta, A. K., and Anantheswaran, R. C. (2000). *Handbook of Microwave Technology for Food Applications*. New York: Marcel Dekker Inc.

Dawes, E. A., and Senior, P. J. (1973). The Role and Regulation of Energy Reserve Polymers in Microorganisms. *Advances in Microbial Physiology*. 10, 135–266.

Delgadillo-Mirquez, L., Lopes, F., Taidi, B., and Pareau, D. (2016). Nitrogen and Phosphate Removal from Wastewater with a Mixed Microalgae and Bacteria Culture. *Biotechnology Reports*. 11, 18–26.

Demesmay, C., and Olle, M. (1993). Utilisation Des Micro-Ondes Dans Les Laboratoires D'analyse. *Spectra Analyse*. 175, 27–32.

Eskilsson, C. S., and Björklund, E. (2000). Analytical-Scale Microwave-Assisted Extraction. *Journal of Chromatography A*. 902(1), 227–250.

Eskilsson, C. S., Björklund, E., Mathiasson, L., Karlsson, L., and Torstensson, A. (1999). Microwave-Assisted Extraction of Felodipine Tablets. *Journal of Chromatography A*. 840(1), 59–70.

FDA. (2017). *Q3C — Tables and List Guidance for Industry*. (Vol. 9765).

Fei, T., Cazeneuve, S., Wen, Z., Wu, L., and Wang, T. (2016). Effective Recovery of Poly-β-Hydroxybutyrate (PHB) Biopolymer from Cupriavidus Necator Using a Novel and Environmentally Friendly Solvent System. *Biotechnology Progress*. 32(3), 678–685.

Fidalgo, P., Cid, A., Abalde, J., and Herrero, C. (1995). Culture of the Marine Diatom Phaeodactylum Tricornutum with Different Nitrogen Sources : Growth, Nutrient Conversion and Biochemical Composition. *Cahiers de Biologie Marine*. 36, 165–173.

Fiorese, M. L., Freitas, F., Pais, J., Ramos, A. M., Aragao, G. M. F. de, and Reis, M. A. M. (2009). Recovery of Polyhydroxybutyrate (PHB) from Cupriavidus Necator Biomass by Solvent Extraction with 1,2-Propylene Carbonate. *Eng. Life Sci.* 9(6), 454–461.

Fortunato, A. E., Jaubert, M., Enomoto, G., Bouly, J.-P., Raniello, R., Thaler, M., … Falciatore, A. (2016). Diatom Phytochromes Reveal the Existence of Far-Red-Light-Based Sensing in the Ocean. *The Plant Cell*. 28(3), 616–628.

Ganzler, K., Szinai, I., and Salgó, A. (1990). Effective Sample Preparation Method for Extracting Biologically Active Compounds from Different Matrices by a Microwave Technique. *Journal of Chromatography A*. 520, 257–262.

Ghasemzadeh, A., Jaafar, H. Z. E., Rahmat, A., and Swamy, M. K. (2017). Optimization of Microwave-Assisted Extraction of Zerumbone from Zingiber Zerumbet L. Rhizome and Evaluation of Antiproliferative Activity of Optimized Extracts. *Chemistry Central Journal*. 11(1), 5.

Gopi, K., Balaji, S., and Muthuvelan, B. (2014). Isolation Purification and Screening of Biodegradable Polymer PHB Producing Cyanobacteria from Marine and Fresh Water Resources. *Iranica Journal of Energy & Environment*. 5(1), 94–100.

Hahn, J. J., Eschenlauer, A. C., Sleytr, U. B., Somers, D. A., and Srienc, F. (1999). Peroxisomes as Sites for Synthesis of Polyhydroxyalkanoates in Transgenic Plants. *Biotechnology Progress*. 15(6), 1053–1057.

Hahn, S. K., Chang, Y. K., Kim, B. S., and Chang, H. N. (1994). Optimization of Microbial Poly (3-Hydroxybutyrate) Recover Using Dispersions of Sodium Hypochlorite Solution and Chloroform. *Biotechnology and Bioengineering*. 44(2), 256–261.

Hahn, S. K., Chang, Y. K., and Lee, S. Y. (1995). Recovery and Characterization of Poly(3-Hydroxybutyric Acid) Synthesized in Alcaligenes Eutrophus and Recombinant Escherichia Coli. *Applied and Environmental Microbiology*. 61(1), 34–39.

Halim, R., Danquah, M. K., and Webley, P. A. (2012). Extraction of Oil from Microalgae for Biodiesel Production: A Review. *Biotechnology Advances*. 30(3), 709–732.

Hempel, F., Bozarth, A. S., Lindenkamp, N., Klingl, A., Zauner, S., Linne, U., … Maier, U. G. (2011). Microalgae as Bioreactors for Bioplastic Production. *Microbial Cell Factories*. 10(1), 81.

Hempel, F., Lau, J., Klingl, A., and Maier, U. G. (2011). Algae as Protein Factories: Expression of a Human Antibody and the Respective Antigen in the Diatom Phaeodactylum Tricornutum. *PLoS ONE*. 6(12), e28424.

Hemwimon, S., Pavasant, P., and Shotipruk, A. (2007). Microwave-Assisted Extraction of Antioxidative Anthraquinones from Roots of Morinda Citrifolia. *Separation and Purification Technology*. 54(1), 44–50.

Henderson, R. K., Jimenez-Gonzalez, C., Constable, D. J. C., Alston, S. R., Inglis, G. G. A., Fisher, G., Curzons, A. D. (2011). Expanding GSK's Solvent Selection Guide - Embedding Sustainability into Solvent Selection Starting at Medicinal Chemistry. *Green Chemistry*. 13(4), 854–862.

Holmes, P. A., and Lim, G. B. (1990). Separation Process. US.

Hoseinabadi, A., Rasooli, I., and Taran, M. (2015). Isolation and Identification of Poly β-Hydroxybutyrate Over-Producing Bacteria and Optimization of Production Medium. *Jundishapur Journal of Microbiology*. 8(7), e16965.

Huie, C. W. (2002). A Review of Modern Sample-Preparation Techniques for the Extraction and Analysis of Medicinal Plants. *Analytical and Bioanalytical Chemistry*. 373(1), 23–30.

Jabeen, N., Majid, I., and Nayik, G. A. (2015). Bioplastics and Food Packaging: A Review. *Cogent Food & Agriculture*. 1(1), 1117749.

Jacquel, N., Lo, C.-W., Wei, Y.-H., Wu, H.-S., and Wang, S. S. (2008). Isolation and Purification of Bacterial Poly(3-Hydroxyalkanoates). *Biochemical Engineering Journal*. 39(1), 15–27.

Janigová, I., Lacík, I., and Chodák, I. (2002). Thermal Degradation of Plasticized Poly(3-Hydroxybutyrate) Investigated by DSC. *Polymer Degradation and Stability*. 77(1), 35–41.

Jassie, L., Revesz, R., Kierstead, T., Hasty, E., and Metz, S. (1997). Microwave-Assisted Solvent Extraction. In Kingston, H. M. S. and Haswell, S. J. (Eds.) *Microwave-Enhanced Chemistry: Fundamentals, Sample Preparation, and Applications* (pp. 569–609). Washington: American Chemical Society.

Jungandreas, A., Costa, B. S., Jakob, T., Von Bergen, M., Baumann, S., and Wilhelm, C. (2014). The Acclimation of Phaeodactylum Tricornutum to Blue and Red Light Does Not Influence the Photosynthetic Light Reaction but Strongly Disturbs the Carbon Allocation Pattern. *PLoS ONE*. 9(8), e99727.

Kaplan, D., Richmond, A. E., Dubinsky, Z., and Aaronson, S. (1986). Algal Nutrition. In Richmond, A. (Ed.) *Handbook of Microalgal Mass Culture* (pp. 147–198). Florida: CRC Press.

Kapritchkoff, F. M., Viotti, A. P., Alli, R. C. P., Zuccolo, M., Pradella, J. G. C., Maiorano, A. E., … Bonomi, A. (2006). Enzymatic Recovery and Purification of Polyhydroxybutyrate Produced by Ralstonia Eutropha. *Journal of Biotechnology*. 122(4), 453–462.

Kaufmann, B., and Christen, P. (2002). Recent Extraction Techniques for Natural Products: Microwave-Assisted Extraction and Pressurized Solvent Extraction. *Phytochemical Analysis*. 13, 105–113. http://doi.org/10.1002/pca.631

Khanna, S., and Srivastava, A. K. (2005). Recent Advances in Microbial Polyhydroxyalkanoates. *Process Biochemistry*. 40(2), 607–619.

Koller, M., Bona, R., Chiellini, E., and Braunegg, G. (2013). Extraction of Short-Chain-Length Poly-[(R)-Hydroxyalkanoates] (Scl-PHA) by the ``anti-Solvent'' Acetone under Elevated Temperature and Pressure. *Biotechnology Letters*. 35(7), 1023–1028. http://doi.org/10.1007/s10529-013-1185-7

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*. 4(2). http://doi.org/10.3390/bioengineering4020055

Kourtz, L., Dillon, K., Daughtry, S., Peoples, O. P., and Snell, K. D. (2007). Chemically Inducible Expression of the PHB Biosynthetic Pathway in Arabidopsis. *Transgenic Research*. 16(6), 759–769.

Kumaravel, S., Hema, R., and Lakshmi, R. (2010). Production of Polyhydroxybutyrate (Bioplastic) and Its Biodegradation by *Pseudomonas Lemoignei* and *Aspergillus Niger*. *E-Journal Chem*. 7, S536-542.

Kunasundari, B., and Sudesh, K. (2011). Isolation and Recovery of Microbial Polyhydroxyalkanoates. *Express Polymer Letters*. 5(7), 620–634.

Law, K. L., Morét-Ferguson, S., Maximenko, N. A., Proskurowski, G., Peacock, E. E., Hafner, J., and Reddy, C. M. (2010). Plastic Accumulation in the North Atlantic Subtropical Gyre. *Science*. 329(5996), 1185–1188.

LearnChemE. (2014). Boiling Point from Heat of Vaporization (Example). Retrieved From https://www.youtube.com/watch?v=HjK9IfWY2qg&t=27s

Lee, J.-Y., Yoo, C., Jun, S.-Y., Ahn, C.-Y., and Oh, H.-M. (2010). Comparison of Several Methods for Effective Lipid Extraction from Microalgae. *Bioresource Technology*. 101(1), S75–S77.

Lee, S. Y. (1996). Bacterial Polyhydroxyalkanoates. *Biotechnology and Bioengineering*. 49(1), 1–14.

Leonelli, C., Veronesi, P., and Cravotto, G. (2013). Microwave-Assisted Extraction: An Introduction to Dielectric Heating. In Chemat, F. and Cravotto, G. (Eds.) *Microwave-Assisted Extraction for Bioactive Compounds: Theory and Practice* (pp. 1–14). Boston, MA: Springer US.

Li, M., Wang, J., Geng, Y., Li, Y., Wang, Q., Liang, Q., and Qi, Q. (2012). A Strategy of Gene Overexpression Based on Tandem Repetitive Promoters in *Escherichia Coli*. *Microbial Cell Factories*. 11, 19.

Lide, D. R. (2009). *CRC Handbook of Chemistry and Physics*. (87th ed.). Boca Raton, Florida: CRC Press.

Liggat, J. J., and Brien, G. O. (1996). Melt Processing of BIOPOLs Polymers—Recent Advances. In Eggink, G., Steinbuchel, A., Poirier, Y., and Witholt, B. (Eds.) *International Symposium on Bacterial Polyhydroxyalkanoates* (pp. 151–160). Davos, Switzerland: NRC Research Press.

Loo, C.-Y., and Sudesh, K. (2007). Biosynthesis and Native Granule Characteristics of Poly(3-Hydroxybutyrate-Co-3-Hydroxyvalerate) in Delftia Acidovorans. *International Journal of Biological Macromolecules*. 40(5), 466–471.

Luengo, J. M., García, B., Sandoval, A., Naharro, G., and Olivera, E. R. (2003). Bioplastics from Microorganisms. *Current Opinion in Microbiology*. 6(3), 251–260.

Luque de Castro, M. D., and Fernández-Peralbo, M. A. (2013). The Role of Microwaves in Omics Disciplines. In Chemat, F. and Cravotto, G. (Eds.) *Microwave-Assisted Extraction for Bioactive Compounds: Theory and Practice* (pp. 127–180). Boston, MA: Springer US.

Madison, L. L., and Huisman, G. W. (1999). Metabolic Engineering of Poly(3-Hydroxyalkanoates): From DNA to Plastic. *Microbiology and Molecular Biology Reviews*. 63(1), 21–53.

Malik, M. R., Yang, W. , Patterson, N. , Tang, J. , Wellinghoff, R. L., Preuss, M. L., Burkitt, C. , Sharma, N. , Ji, Y. , Jez, J. M., Peoples, O. P., Jaworski, J. G., Cahoon, E. B. and Snell, K. D. (2015). Production of High Levels of Poly-3-hydroxybutyrate in Plastids of *Camelina sativa* Seeds. *Plant Biotechnol J.* 13, 675-688.

John, M. E. (1997). Cotton Crop Improvement Through Genetic Engineering. *Critical Reviews in Biotechnology*. 17(3), 185–208.

Manangan, T., and Shawaphun, S. (2010). Quantitative Extraction and Determination of Polyhydroxyalkanoate Accumulated in Alcaligenes Latus Dry Cells. *ScienceAsia*. 36(3), 199–203.

Mandal, S. C., Mandal, V., and Das, A. K. (2015). *Essentials of Botanical Extraction: Principles and Applications*. (1st ed.). USA: Academic Press.

Mandal, V., Mohan, Y., and Hemalatha, S. (2007). Microwave Assisted Extraction - An Innovative and Promising Extraction Tool for Medicinal Plant Research. *Pharmacognosy Reviews*. 1(1), 7–18.

Manjkow, J., Papanu, J. S., Soong, D. S., Hess, D. W., and Bell, A. T. (1987). An in Situ Study of Dissolution and Swelling Behavior of Poly-(Methyl Methacrylate) Thin Films in Solvent/Nonsolvent Binary Mixtures. *Journal of Applied Physics*. 62(2), 682–688.

Martino, A. De, Meichenin, A., Shi, J., Pan, K., and Bowler, C. (2007). Genetic and Phenotypic Characterization of Phaeodactylum Tricornutum (Bacillariophyceae) Accessions 1. *Journal of Phycology*. 43(5), 992–1009.

Mata, T. M., Martins, A. A., and Caetano, N. S. (2010). Microalgae for Biodiesel Production and Other Applications: A Review. *Renewable and Sustainable Energy Reviews*. 14(1), 217–232.

Mehrotra, S. C., Lokhande, M. P., Mirgane, S. R., Arbad, B. R., and Helambe, N. S. (1998). Study of Interactions between Drugs and Methanol at Microwave Frequency by Time Domain Dielectric Spectroscopy. *Indian Journal of Pure and Applied Physics*. 36, 138–143.

Menzel, G., Harloff, H.-J., and Jung, C. (2003). Expression of Bacterial Poly(3-Hydroxybutyrate) Synthesis Genes in Hairy Roots of Sugar Beet (*Beta Vulgaris L.*). *Applied Microbiology and Biotechnology*. 60(5), 571–576.

Meseck, S. L., Alix, J. H., and Wikfors, G. H. (2005). Photoperiod and Light Intensity Effects on Growth and Utilization of Nutrients by the Aquaculture Feed Microalga, Tetraselmis Chui (PLY429). *Aquaculture*. 246(1–4), 393–404.

Metaxas, A. C. (1996). *Foundation and Electroheat: A Unified Approach*. New York: John Wiley & Sons.

Moire, L., Rezzonico, E., and Poirier, Y. (2003). Synthesis of Novel Biomaterials in Plants. *Journal of Plant Physiology*. 160(7), 831–839.

Montsant, A., Allen, A. E., Coesel, S., Martino, A. De, Falciatore, A., Mangogna, M., Bowler, C. (2007). Identification and Comparative Genomic Analysis of Signaling And Regulatory Components in the Diatom Thalassiosira Pseudonana 1. *Journal of Phycology*. 43(3), 585–604.

Myers, R. H., Montgomery, D. C., and Anderson-Cook, C. M. (2016). *Response Surface Methodology: Process and Product Optimization Using Designed Experiments*. (4th ed.). Hoboken, New Jersey: John Wiley & Sons, Inc.

Navarro, M. T., Prieto, R., Fernández, E., and Galván, A. (1996). Constitutive Expression of Nitrate Reductase Changes the Regulation of Nitrate and Nitrite Transporters in Chlamydomonas Reinhardtii. *The Plant Journal*. 9(6), 819–827.

Nikel, P. I., de Almeida, A., Melillo, E. C., Galvagno, M. A., and Pettinari, M. J. (2006). New Recombinant *Escherichia coli* Strain Tailored for The Production of Poly(3-hydroxybutyrate) from Agroindustrial By-products. *Applied and Environmental Microbiology*. 72(6), 3949–54.

Olle, M., and Viršile, A. (2013). The Effects of Light-Emitting Diode Lighting on Greenhouse Plant Growth and Quality. *Agricultural and Food Science*. 22, 223–234.

Pachekoski, W. M., Agnelli, J. A. M., and Belem, L. P. (2009). Thermal, Mechanical and Morphological Properties of Poly(hydroxybutyrate) and Polypropylene Blends After Processing. *Mat. Res*. 12(2), 159–164.

Pan, X., Niu, G., and Liu, H. (2003). Microwave-Assisted Extraction of Tea Polyphenols and Tea Caffeine from Green Tea Leaves. *Chemical Engineering and Processing*. 42(2), 129–133.

Pantazaki, A. A., Papaneophytou, C. P., Pritsa, A. G., Liakopoulou-Kyriakides, M., and Kyriakidis, D. A. (2009). Production of Polyhydroxyalkanoates from Whey by *Thermus Thermophilus* HB8. *Process Biochemistry*. 44(8), 847–853.

Papanu, J. S., Hess, D. W., Bell, A. T., and Soane, D. S. (1989). Dissolution of Thin Poly(Methyl Methacrylate) Films in Ketones, Binary Ketone/Alcohol Mixtures, and Hydroxy Ketones. *Journal of the Electrochemical Society*. 136(10), 3077–3083.

Parveez, G. K. A., Bahariah, B., Ayub, N. H., Masani, M. Y. A., Rasid, O. A., Tarmizi, A. H., and Ishak, Z. (2015). Production of Polyhydroxybutyrate in Oil Palm (*Elaeis Guineensis Jacq.*) Mediated by Microprojectile Bombardment of PHB Biosynthesis Genes into Embryogenic Calli. *Frontiers in Plant Sci*. 6, 598.

Pasquet, V., Chérouvrier, J.-R., Farhat, F., Thiéry, V., Piot, J.-M., Bérard, J.-B., … Picot, L. (2011). Study on the Microalgal Pigments Extraction Process: Performance of Microwave Assisted Extraction. *Process Biochemistry*. 46(1), 59–67.

Patil, P. D., Gude, V. G., Mannarswamy, A., Cooke, P., Munson-McGee, S., Nirmalakhandan, N., … Deng, S. (2011). Optimization of Microwave-Assisted Transesterification of Dry Algal Biomass Using Response Surface Methodology. *Bioresource Technology*. 102(2), 1399–1405.

Philp, J. C., Ritchie, R. J., and Guy, K. (2013). Biobased Plastics in a Bioeconomy. *Trends in Biotechnology*. 31(2), 65–67.

Poirier, Y., Dennis, D. E., Klomparens, K., and Somerville, C. (1992). Polyhydroxybutyrate, a Biodegradable Thermoplastic, Produced in Transgenic Plants. *Science*. 256(5056), 520–523.

Pötter, M., and Steinbüchel, A. (2005). Poly(3-Hydroxybutyrate) Granule-Associated Proteins: Impacts on Poly(3-Hydroxybutyrate) Synthesis and Degradation. *Biomacromolecules*. 6(2), 552–560.

Prieto, M. A., Bühler, B., Jung, K., Witholt, B., and Kessler, B. (1999). PhaF, a Polyhydroxyalkanoate-Granule-Associated Protein of Pseudomonas Oleovorans GPo1 Involved in the Regulatory Expression System for Pha Genes. *Journal of Bacteriology*. 181(3), 858–868.

Qin, L.-F., Gao, X., Liu, Q., Wu, Q., and Chen, G.-Q. (2007). Biosynthesis of Polyhydroxyalkanoate Copolyesters by Aeromonas Hydrophila Mutant Expressing a Low-Substrate-Specificity PHA Synthase PhaC2Ps. *Biochemical Engineering Journal*. 37(2), 144–150.

Qualman, D. (2017). Global Plastics Production, 1917 to 2050. Retrieved From https://www.darrinqualman.com/global-plastics-production/

Radmann, E., Oliveira, C. F., Zanfonato, K., and Vieira, J. A. (2009). Cultivo Mixotrófico Da Microalga Spirulina Sp. LEB18 Com Adição Noturna de Diferentes Fontes de Carbono Orgânico. In *XVII Simpósio Nacional de Bioprocessos*. Natal, Brazil.

Ramsay, J. A., Berger, E., Voyer, R., Chavarie, C., and Ramsay, B. A. (1994). Extraction of Poly-3-Hydroxybutyrate Using Chlorinated Solvents. *Biotechnology Techniques*. 8(8), 589–594.

Raner, K. D., Strauss, C. R., Vyskoc, F., and Mokbel, L. (1993). A Comparison of Reaction Kinetics Observed under Microwave Irradiation and Conventional Heating. *The Journal of Organic Chemistry*. 58(4), 950–953.

Reddy, C. S. K., Ghai, R., Rashmi, and Kalia, V. C. (2003). Polyhydroxyalkanoates: An Overview. *Bioresource Technology*. 87(2), 137–146.

Refaat, A. A., El Sheltawy, S. T., and Sadek, K. U. (2008). Optimum Reaction Time, Performance and Exhaust Emissions of Biodiesel Produced by Microwave Irradiation. *International Journal of Environmental Science & Technology*. 5(3), 315–322.

Renoe, B. W. (1994). Microwave Assisted Extraction. *Am Lab*. 26, 34–40.

Riedel, S. L., Brigham, C. J., Budde, C. F., Bader, J., Rha, C., Stahl, U., and Sinskey, A. J. (2012). Recovery of Poly(3-Hydroxybutyrate-Co-3-Hydroxyhexanoate) from Ralstonia Eutropha Cultures with Non-Halogenated Solvents. *Biotechnology and Bioengineering*. 110(2), 461–470.

Rosengart, A., Cesário, M. T., de Almeida, M. C. M. D., Raposo, R. S., Espert, A., de Apodaca, E. D., and da Fonseca, M. M. R. (2015). Efficient P(3HB) Extraction from Burkholderia Sacchari Cells Using Non-Chlorinated Solvents. *Biochemical Engineering Journal*. 103, 39–46.

Sayyed, R. Z., Gangurde, N. S., and Chincholkar, S. B. (2009). Hypochlorite Digestion Method for Efficient Recovery of PHB from Alcaligenes Faecalis. *Indian Journal of Microbiology*. 49(3), 230–232.

Schellenberger Costa, B., Jungandreas, A., Jakob, T., Weisheit, W., Mittag, M., and Wilhelm, C. (2013). Blue Light Is Essential for High Light Acclimation and Photoprotection in the Diatom Phaeodactylum Tricornutum. *Journal of Experimental Botany*. 64(2), 483–493.

Shamis, Y., Taube, A., Mitik-Dineva, N., Croft, R., Crawford, R. J., and Ivanova, E. P. (2011). Specific Electromagnetic Effects of Microwave Radiation on Escherichia Coli. *Applied and Environmental Microbiology*. 77(9), 3017–3022.

Sheu, D.-S., and Lee, C.-Y. (2004). Altering the Substrate Specificity of Polyhydroxyalkanoate Synthase 1 Derived from Pseudomonas Putida GPo1 by Localized Semirandom Mutagenesis. *Journal of Bacteriology*. 186(13), 4177–84.

Shrivastav, A., Kim, H.Y., Kim, Y.R. (2013). Advances in the Applications of Polyhydroxyalkanoate Nanoparticles for Novel Drug Delivery System. *Biomed Res. Int.* 2013, 581684.

Sim, S. J., Snell, K. D., Hogan, S. A., Stubbe, J., Rha, C., and Sinskey, A. J. (1997). PHA Synthase Activity Controls the Molecular Weight and Polydispersity of Polyhydroxybutyrate in Vivo. *Nature Biotechnology*. 15, 63.

Singh, R. V. (2015). Polyhydroxybutyrate (PHB): Biodegradable, Bioplastics Produced by Microorganisms. *International Journal of Pharmaceutical Research*. 7(2), 17–23.

Sinquin, A., Görner, T., and Dellacherie, E. (1993). L'utilisation Des Micro-Ondes En Chimie Analytique. *Analusis*. 21, 1–10.

Spigno, G., and De Faveri, D. M. (2009). Microwave-Assisted Extraction of Tea Phenols: A Phenomenological Study. *Journal of Food Engineering*. 93(2), 210–217.

Spyros, A., Kimmich, R., Briese, B. H., and Jendrossek, D. (1997). 1H NMR Imaging Study of Enzymatic Degradation in Poly(3-Hydroxybutyrate) and Poly(3-Hydroxybutyrate-Co-3-Hydroxyvalerate). Evidence for Preferential Degradation of the Amorphous Phase by PHB Depolymerase B from Pseudomonas Lemoignei. *Macromolecules*. 30(26), 8218–8225.

Steinbüchel, A., and Lütke-Eversloh, T. (2003). Metabolic Engineering and Pathway Construction for Biotechnological Production of Relevant Polyhydroxyalkanoates in Microorganisms. *Biochemical Engineering Journal*. 16(2), 81–96.

Sudesh, K. (2013). *Polyhydroxyalkanoates from Palm Oil: Biodegradable Plastics*. Berlin Heidelberg: Springer-Verlag.

Sudesh, K., Abe, H., and Doi, Y. (2000). Synthesis, Structure and Properties of Polyhydroxyalkanoates: Biological Polyesters. *Progress in Polymer Science*. 25(10), 1503–1555.

Sudesh, K., and Doi, Y. (2005). Polyhydroxyalkanoates. In Bastioli, C. (Ed.) *Handbook of Biodegradable Polymers* (pp. 219–256). United Kingdom: RAPRA Technology Ltd.

Talebi, M., Ghassempour, A., Talebpour, Z., Rassouli, A., and Dolatyari, L. (2004). Optimization of the Extraction of Paclitaxel from Taxus Baccata L. by the Use of Microwave Energy. *Journal of Separation Science*. 27(13), 1130–1136.

Tamer, I. M., and Moo-Young, M. (1998). Optimization of Poly(β-Hydroxybutyric Acid) Recovery from Alcaligenes Latus: Combined Mechanical and Chemical Treatments. *Bioprocess Engineering*. 19(6), 459–468.

Tan, G.-Y. A., Chen, C.-L., Li, L., Ge, L., Wang, L., Razaad, I. M. N., … Wang, J.-Y. (2014). Start a Research on Biopolymer Polyhydroxyalkanoate (PHA): A Review. *Polymers*. 6(3), 706–754.

Tatke, P., and Jaiswal, Y. (2011). An Overview of Microwave Assisted Extraction and Its Applications in Herbal Drug Research. *Research Journal of Medicinal Plants*. 5, 21–31.

Teraoka, I. (2002). *Polymer Solutions: An Introduction to Physical Properties*. New York: John Wiley & Sons.

The Pennsylvania State University. (2017). Lesson 11: Response Surface Methods and Designs. Retrieved From https://onlinecourses.science.psu.edu/stat503/node/57

Thuéry, J. (1992). Microwaves and Matter. In Grant, E. H. (Ed.) *Microwaves: Industrial, Scientific, and Medical Applications* (pp. 83–125). London: Artech House.

Tsuji, H., and Ikada, Y. (1996). Blends of Aliphatic Polyesters. I. Physical Properties and Morphologies of Solution-Cast Blends from Poly(DL-lactide) and Poly(E-caprolactone). *Journal of Applied Polymer Science*. 60(13), 2367–2375.

Ueberreiter, K. (1968). The Solution Process. In Crank, J. and Park, G. S. (Eds.) *Diffusion in Polymers* (pp. 219–257). New York: Academic Press.

USF Health. (2017). Processing Cells for SEM. Retrieved From http://health.usf.edu/~/media/Files/Medicine/Core/PROCESSINGCELLSFORSEM.ashx?la=en

Veggi, P. C., Martinez, J., and Meireles, M. A. A. (2013). Fundamentals of Microwave Extraction. In Chemat, F. and Cravotto, G. (Eds.) *Microwave-Assisted Extraction for Bioactive Compounds: Theory and Practice* (pp. 15–52). Boston, MA: Springer US.

Virot, M., Tomao, V., Ginies, C., Visinoni, F., and Chemat, F. (2008). Microwave-Integrated Extraction of Total Fats and Oils. *Journal of Chromatography A*. 1196, 57–64.

Visakh, P. M. (2015). Polyhydroxyalkanoates (PHAs), Their Blends, Composites and Nanocomposites: State of the Art, New Challenges and Opportunities. In Roy, I. and Visakh, P. M. (Eds.) *Polyhydroxyalkanoate (PHA) Based Blends, Composites and Nanocomposites* (pp. 1–17). Cambridge, UK: The Royal Society of Chemistry.

Wahidin, S., Idris, A., and Shaleh, S. R. M. (2014). Rapid Biodiesel Production Using Wet Microalgae via Microwave Irradiation. *Energy Conversion and Management*. 84, 227–233.

Wahlen, B. D., Willis, R. M., and Seefeldt, L. C. (2011). Biodiesel Production by Simultaneous Extraction and Conversion of Total Lipids from Microalgae, Cyanobacteria, and Wild Mixed-Cultures. *Bioresource Technology*. 102(3), 2724–2730.

Wallen, L. L., and Rohwedder, W. K. (1974). Poly-Beta-Hydroxyalkanoate from Activated Sludge. *Environmental Science & Technology*. 8(6), 576–579.

Wang, Y., Leng, F., Liu, X., Zhang, W., and Yang, M. (2014). Optimization of Microwave-Assisted Extraction of Water-Soluble Polysaccharides from Piteguo Fruit by Response Surface Methodology. *Food Science and Technology Research*. 20(4), 755–764.

Xiao, W., Han, L., and Shi, B. (2008). Microwave-Assisted Extraction of Flavonoids from Radix Astragali. *Separation and Purification Technology*. 62(3), 614–618.

Yadav, G. D., and Devendran, S. (2015). Microwave Assisted Enzyme Catalysis: Practice and Perspective. In Coelho, M. A. Z. and Ribeiro, B. D. (Eds.) *White Biotechnology for Sustainable Chemistry* (pp. 52–103). London: The Royal Society of Chemistry.

Yeh, N., and Chung, J.-P. (2009). High-Brightness LEDs—Energy Efficient Lighting Sources and Their Potential in Indoor Plant Cultivation. *Renewable and Sustainable Energy Reviews*. 13(8), 2175–2180.

Yousuf, R. G. (2017). Novel Polyhydroxybutyrate (PHB) Production Using A Waste Data Seed Feedstock. The University of Manchester.

Zhang, S., Fang, L., Li, Z., Guo, Y., and Chen, G.-Q. (2016). Enhanced Production of Poly-3-Hydroxybutyrate by Recombinant *Escherichia Coli* Containing NAD Kinase and PhbCAB Operon. *Science China Chemistry*. 59(11), 1390–1396.

Zhao, P., Gu, W., Wu, S., Huang, A., He, L., Xie, X., … Wang, G. (2014). Silicon Enhances the Growth of Phaeodactylum Tricornutum Bohlin under Green Light and Low Temperature. *Scientific Reports*. 4, 3958.

Zhou, H.-Y., and Liu, C.-Z. (2006). Microwave-Assisted Extraction of Solanesol from Tobacco Leaves. *Journal of Chromatography A*. 1129(1), 135–139.

Zinn, M., Witholt, B., and Egli, T. (2001). Occurrence, Synthesis and Medical Application of Bacterial Polyhydroxyalkanoate. *Advanced Drug Delivery Reviews*. 53(1), 5–21.