

MASS PRODUCTION OF BIODEGRADABLE COPOLYMERS FROM RENEWABLE RESOURCES

LOVELY



**DEPARTMENT OF BIOCHEMICAL ENGINEERING & BIOTECHNOLOGY
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**MASS PRODUCTION OF BIODEGRADABLE
COPOLYMERS FROM RENEWABLE
RESOURCES**

by

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Department of Biochemical Engineering and Biotechnology

*Submitted
in fulfillment of the requirements of the degree of Doctor of Philosophy*

to the



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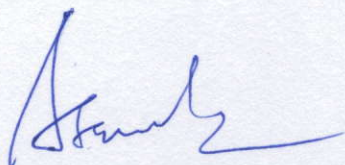
Dedicated

To

My Family

CERTIFICATE

This is to certify that the thesis entitled "*Mass production of biodegradable copolymers from renewable resources*" being submitted by **Ms. Lovely** to the **Indian Institute of Technology Delhi**, for the award of the degree of "**Doctor of Philosophy**" is a record of the bonafide research work performed by her, under my guidance and supervision and in conformity with rules and regulations of the Indian Institute of Technology Delhi. The research reports and results presented in the thesis have not been submitted in part or full to any other University or Institute for the award of any degree/diploma.



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Every worthwhile dream demands intense motivation, perseverance and dedication which doesn't let you fall at tough times as the journey brings failure and success hand-in-hand. Like success screams as joy when a significant momentous accomplished, failure breaks you down and you do not have the courage to carry things forward, so constant motivation and guidance from the supervisor, cooperation from the colleagues and a strong desire to reach the goals enlightens the strengths and keeps you moving throughout the journey. I would like to take this opportunity to offer my heartfelt gratitude to everyone who assisted me in attaining the research goals of my PhD thesis.

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Lovely

ABSTRACT

PHBV is the most extensively studied member of PHA family due to its similarity in physical and thermal properties with petroleum derived plastics. Therefore, mass production of the PHBV produced from different bacterial cultures was the main focus of the present investigation. The biodegradability and biocompatibility of PHBV featured more flexibility and improved processivity than PHB. Therefore the mass production protocols of PHBV from a variety of diverse groups of bacterial cultures (both gram +ve and -ve) were critically evaluated during the present investigation. The mass production of PHBV was particularly attempted in low cost substrate, glycerol (a byproduct of biofuel industry) as carbon source for economic production of PHB. This was followed by the addition of statistically optimized concentrations of selected electron acceptors (VA / PA) at appropriate cultivation time followed by subsequent addition of multiple instalments of electron acceptors at statistically optimized time intervals to produce PHBV. Independent studies were also done to further enhance the copolymer production by addition of statistically optimized concentrations of electron acceptors at different time intervals during the model based fed-batch cultivations wherein the pseudo steady state with respect to substrate (glycerol) was maintained for major period of their cultivation.

The primary goal of this research was to create optimized mass production protocols of few custom tailored PHBV from gram negative and gram positive bacteria so that the same can be used for diverse commercial and medical applications in society. The processing properties of these copolymers were significantly altered by the addition of different concentration of alternate electron acceptors (PA/VA) added to the exponentially growing cultures at different statistically optimized feeding time interval for the three microorganisms (*Ralstonia eutropha*, *Cupriavidus necator* and *Bacillus thuringiensis*). The optimized PHBV production was then experimentally verified in 7L bioreactor and scale up was attempted using various scale up criteria (P / V , k_{La} , Π , t_m , N_{Re}) from 7L to 15L/70L bioreactor. It emerged from the extensive calculations that constant P / V scale up criteria was the best scale up criteria therefore it was implemented for scale up of batch cultivations from 7L to 15L bioreactor by *R. eutropha* and also for both batch and fed-batch cultivations from 7L to 70L bioreactor by *C. necator* and *B. thuringiensis*. The intracellular PHBV produced was extracted from the biomass harvested after the completion of fermentation and was extensively characterized by GPC, XRD, FTIR NMR, DSC and TGA in order to map their specific characteristic properties to the biopolymers used for various societal and biomedical applications.

R. eutropha was grown using optimized media in shake flasks wherein a statistically optimized feeding of VA concentration of 0.76 g/L at 24 h, 30 h and 36 h was implemented. The batch cultivation was then experimentally verified during scale up from 7L to 15L bioreactor employing constant P / V scale up criteria to obtain biomass, HV and PHBV concentrations of 6.07 g/L, 0.734 g/L and 1.98 g/L respectively in 15L bioreactor which were quite close to the results obtained during 7L bioreactor cultivation. Similar set of RSM experiments were conducted to optimize PA concentration (1.27 g/L) and its addition time (24 h, 30.43 h and 36.86 h) which featured prediction of high PHBV production. These shake flask batch cultivations were experimentally verified in 7L bioreactor and then scaled up to 15L bioreactor using constant P / V scaling criteria to produce 10.29 g/L, 0.946 g/L and 4.53 g/L of biomass, HV and PHBV concentration respectively.

C. necator was grown using optimized media for shake flask experiments and statistical optimization studies were performed to identify the appropriate concentration and feeding time of electron acceptors, VA & PA addition to facilitate enhanced PHBV production. The model predicted the optimum biomass and HV concentration of 7.96 g/L and 0.444 g/L respectively when 0.5 g/L of VA was fed at 24 h, 34.6 h and 45.2 h which was thereafter experimentally verified in shake flask and 7L bioreactor cultivation. This batch cultivation was then scaled up to 70L bioreactor using constant P / V scale up criteria for PHBV production (VA) to obtain an HV, PHBV and biomass concentration of 0.587 g/L, 5.97 g/L and 10.23 g/L respectively which were quiet closer to the values obtained in 7L bioreactor thereby demonstrating successful scale up. Fed-batch cultivation featuring maintenance of Pseudo steady state w.r.t major substrate glycerol was employed wherein statistically optimized concentrations of VA were added at different time intervals in 7L bioreactor for PHBV production which resulted in HV, PHBV and biomass concentration of 3.19 g/L, 10.9 g/L and 17.11 g/L respectively. This fed-batch cultivation of PHBV production was thereafter scaled up to 70L bioreactor cultivation using constant P / V scale up criteria wherein the culture featured HV, PHBV and biomass concentration of 3.25 g/L, 11.49 g/L and 16.65 g/L establishing successful scale up. PHBV production was also explored using PA as electron acceptor wherein, statistically optimized PA concentration of 0.85 g/L at feeding time of 24 h, 33.88 h and 43.76 h was identified by RSM. The model predicted an HV concentration and biomass growth of 0.79 g/L and 10.84 g/L respectively which was experimentally validated in shake flask cultivation and 7L bioreactor cultivation. This batch cultivation was then scaled up to 70L bioreactor employing constant P / V scale up criteria and the culture exhibited an accumulation of 0.793 g/L, 8.25 g/L and 11.23 g/L of HV, PHBV and biomass concentration respectively. PSS

fed batch cultivation strategy was explored for improved PHBV production in 7L bioreactor by *C. necator* using PA wherein the culture exhibited an HV, PHBV and biomass concentration of 4.7 g/L, 13.33 g/L and 21.21 g/L respectively. This fed batch cultivation was scaled up to 70L bioreactor using constant P / V scale up criteria to obtain HV, PHBV and biomass concentration of 4.12 g/L, 12.88 g/L and 19.82 g/L respectively, thereby demonstrating successful scale up.

B. thuringiensis was grown using optimized media in shake flasks wherein attempt was made to statistically optimize PA concentration and its feeding interval during the fermentation media to promote enhanced PHBV synthesis. Statistical optimization of PA concentration and its feeding interval was conducted, which indicated the predicted optimum PA concentration of 1.2 g/L to be fed at 0 h, 14.86 h and 19 h. These optimization experiments were validated in shake flask and 7L bioreactor cultivation wherein the culture featured 3.98 g/L, 0.592 g/L and 8 g/L, 0.805 g/L of biomass and HV concentration respectively which might be due to the more profound agitation and aeration conditions in 7L bioreactor. The batch cultivation was thereafter scaled up using constant P / V scale up criteria to 70L bioreactor wherein the culture showed an accumulation of 0.622 g/L, 3.57 g/L and 7.15 g/L of HV, PHBV and biomass concentrations respectively. PSS fed batch cultivation w.r.t glycerol as the best identified nutrient feeding strategy was employed to augment PHBV production wherein the culture featured 0.706 g/L, 4.36 g/L and 11.61 g/L of HV, PHBV and biomass respectively. This fed batch cultivation was scaled up to 70L bioreactor employing constant P / V scale up criteria wherein the culture exhibited an HV, PHBV and biomass concentration of 0.636 g/L, 4.56 g/L and 10.74 g/L respectively, demonstrating successful scale up.

The biomass of *R. eutropha*, *C. necator* & *B. thuringiensis* was harvested at 57 h, 57 h and 36 h respectively, followed by its pretreatment with SDS and NaOCl, extracted in chloroform and the recovered copolymer was characterized. NMR and FTIR studies confirmed the molecular structure of the copolymer while XRD analysis identified the crystal structure of PHBV extracted by all the cultures to be orthorhombic. DSC analysis determined the melting temperature of PHBV in the range between 127-167°C for different samples while TGA studies analyzed the degradation temperature to be between 245-290°C. GPC analysis of various PHBV samples indicated the molecular weight in the range of 40.2 kDa and 131.9 kDa. These properties indicate the copolymer produced by different bacteria was suitable for food, chemical, materials as well as biomedical industries in society.

सार

पीएचबीवी पेट्रोलियम व्युत्पन्न प्लास्टिक के साथ भौतिक और तापीय गुणों में समानता के कारण PHA परिवार का सबसे व्यापक रूप से अध्ययन किया गया सदस्य है। इसलिए, विभिन्न जीवाणु संस्कृतियों से उत्पादित पीएचबीवी का बड़े पैमाने पर उत्पादन वर्तमान जांच का केंद्र बिंदु था। पीएचबीवी की बायोडिग्रेडेबिलिटी और बायोकम्पैटिबिलिटी में पीएचबी की तुलना में अधिक लचीलापन और बेहतर प्रक्रियात्मकता है। इसलिए वर्तमान जांच में बैक्टीरिया संस्कृति के विभिन्न समूहों (ग्राम पॉजिटिव और नेगेटिव दोनों) से पीएचबीवी के बड़े पैमाने पर उत्पादन का व्यापक रूप से पता लगाया गया था। पीएचबीवी के बड़े पैमाने पर उत्पादन को विशेष रूप से कम लागत वाले सबस्ट्रेट, ग्लिसरॉल (जैव ईंधन उद्योग उपोत्पाद) में कार्बन स्रोत के रूप में पीएचबी का आर्थिक उत्पादन करने का प्रयास किया गया था। इसके बाद उपयुक्त उत्पादन के समय पर चयनित इलेक्ट्रॉन स्वीकर्ता (वीए/पीए) के सांख्यिकीय रूप से अनुकूलित सांद्रता को डाला गया, इसके बाद सांख्यिकीय रूप से अनुकूलित समय अंतराल पर इलेक्ट्रॉन स्वीकर्ता की कई किस्तों को डाला। मॉडल निर्देशित फेड-बैच उत्पादन के दौरान अलग-अलग समय अंतराल पर इलेक्ट्रॉन स्वीकर्ता के सांख्यिकीय रूप से अनुकूलित सांद्रता के अलावा कोपोलिमर उत्पादन को और बढ़ाने के लिए स्वतंत्र अध्ययन भी किया गया था, जिसमें सबस्ट्रेट (ग्लिसरॉल) के संबंध में छद्म स्थिर अवस्था को प्रमुख अवधि के लिए कायम रखा गया था।

वर्तमान अध्ययन का प्राथमिक उद्देश्य ग्राम पॉजिटिव और ग्राम नेगेटिव बैक्टीरिया से कुछ के अनुकूलित बड़े पैमाने पर उत्पादन प्रोटोकॉल विशेष रूप के पीएचबीवी विकसित करना था ताकि समाज में विविध व्यावसायिक और चिकित्सा अनुप्रयोगों के लिए इनका उपयोग किया जा सके। वैकल्पिक इलेक्ट्रॉन स्वीकर्ता (पीए/वीए) के अलग-अलग सांद्रण को डालकर कॉपोलीमर के गुणों में काफी बदलाव किया गया था, जो तीन बैक्टीरिया (*राल्स्टोनिया यूट्रोफा क्यूप्रियाविडस नेकेटर*, और *बैसिलस थुरिंजिएन्सिस*) के लिए अलग-अलग सांख्यिकीय रूप से अनुकूलित समय अंतराल पर तेजी से बढ़ते बैक्टीरियल संवर्धन में डाला गया था। अनुकूलित उत्पादन को तब 7L बायोरिएक्टर में प्रयोगात्मक रूप से सत्यापित किया गया था और 7L से 15L/70L बायोरिएक्टर तक विभिन्न स्केल-अप मानदंड (पी/वी, मास ट्रांसफर गुणांक, मिश्रण समय, रेनॉल्ड्स संख्या) का उपयोग करके पीएचबीवी का स्केल-अप का प्रयास किया गया था। कठोर गणनाओं के बाद लगातार पी/वी स्केल-अप मानदंड को इष्टतम स्केल अप मानदंड पाया गया था, इसलिए इसका उपयोग आर. यूट्रोफा के बैच उत्पादन को 7L से 15L बायोरिएक्टर तक स्केल-अप करने के साथ में, सी. नेकेटर और बी. थुरिंजिएन्सिस के बैच और फेड-बैच उत्पादन को 7L से 70L बायोरिएक्टर तक स्केल-अप करने के लिए किया गया। उत्पादित इंटरसेल्युलर पीएचबीवी किण्वन के पूरा होने के बाद एकत्रित किये गए बायोमास से निकाला गया था और विभिन्न सामाजिक और बायोमेडिकल अनुप्रयोगों के लिए उपयोग किए जाने वाले बायोपॉलिमर के लिए उनके विशिष्ट गुणों को मैप करने के लिए जीपीसी, एक्सआरडी, डीएससी, टीजीए, एफटीआईआर और एनएमआर द्वारा व्यापक रूप से विशेषताएँ पता लगायी गईं।

आर. यूट्रोफा को शेक फ्लास्क में अनुकूलित मीडिया का उपयोग करके उपजाया गया, जिसमें 24 घंटे, 30 घंटे और 36 घंटे में 0.76 ग्राम/ली के वीए सांद्रता की सांख्यिकीय रूप से अनुकूलित फीडिंग लागू की गई। 15L बायोरिएक्टर में क्रमशः 6.07 ग्राम/ली, 0.734 ग्राम/ली और 1.98 ग्राम/ली के बायोमास, एचवी और पीएचबीवी सांद्रता प्राप्त करने के लिए निरंतर पी/वी स्केल अप मानदंड का उपयोग करते हुए बैच उत्पादन को 7L से 15L बायोरिएक्टर के पैमाने के दौरान प्रयोगात्मक रूप से सत्यापित किया गया। जो 7L बायोरिएक्टर उत्पादन के दौरान प्राप्त परिणामों के काफी करीब

थो पीए सांद्रता (1.27 ग्राम/ली) और इसको डालने का समय (24 घंटे, 30.43 घंटे और 36.86 घंटे) को अनुकूलित करने के लिए आरएसएम प्रयोगों के समान सेट आयोजित किए गए थे, जिनमें उच्च पीएचबीवी उत्पादन की भविष्यवाणी थी। इस शेक फ्लास्क बैच के उत्पादन को 7L बायोरिएक्टर में प्रयोगात्मक रूप से सत्यापित किया गया था और फिर क्रमशः 10.29 ग्राम/ली, 0.946 ग्राम/ली और 4.53 ग्राम/ली बायोमास, एचवी और पीएचबीवी सांद्रता का उत्पादन करने के लिए निरंतर पी/वी स्केल अप मानदंड का उपयोग करके 15L बायोरिएक्टर तक बढ़ाया गया।

सी. नेकेटर को शेक फ्लास्क में अनुकूलित मीडिया का उपयोग करके उपजाया गया और बेहतर पीएचबीवी उत्पादन को सुविधाजनक बनाने के लिए इलेक्ट्रॉन स्वीकर्ता, वीए और पीए की उचित सांद्रता और फीडिंग समय का अनुमान लगाने के लिए सांख्यिकीय अनुकूलन अध्ययन किए गए थे। मॉडल ने इष्टतम बायोमास और एचवी सांद्रता क्रमशः 7.96 ग्राम/ली और 0.444 ग्राम/ली की भविष्यवाणी की, जब वीए के 0.5 ग्राम/ली को 24 घंटे, 34.6 घंटे और 45.2 घंटे पर डाला गया था जिसे शेक फ्लास्क और 7L बायोरिएक्टर के उत्पादन में प्रयोगात्मक रूप से सत्यापित किया गया था। इस बैच उत्पादन को पीएचबीवी उत्पादन (वीए) के लिए निरंतर पी/वी स्केल अप मानदंड का उपयोग करके क्रमशः 0.587 ग्राम/ली, 5.97 ग्राम/ली और 10.23 ग्राम/ली की एचवी, पीएचबीवी और बायोमास सांद्रता प्राप्त करने के लिए 70L बायोरिएक्टर तक बढ़ाया गया था। 7L बायोरिएक्टर में प्राप्त करीब मूल्य सफल स्केल-अप को दर्शाता है। प्रमुख सबस्ट्रेट ग्लिसरॉल के संबंध में छद्म स्थिर अवस्था के रखरखाव के विशेषता वाले फेड-बैच उत्पादन को नियोजित किया गया था जिसमें पीएचबीवी उत्पादन के लिए 7L बायोरिएक्टर में अलग-अलग समय अंतराल पर वीए के सांख्यिकीय रूप से अनुकूलित सांद्रता को डाला गया था, जिसके परिणामस्वरूप क्रमशः 3.19 ग्राम/ली, 10.9 ग्राम/ली और 17.11 ग्राम/ली एचवी, पीएचबीवी और बायोमास सांद्रता पाई गई। पीएचबीवी के इस फेड-बैच उत्पादन को इसके बाद निरंतर पी/वी स्केल अप मानदंड का उपयोग करके 70L बायोरिएक्टर तक बढ़ाया गया, जिसमें बैक्टीरियल संवर्धन में एचवी, पीएचबीवी और बायोमास की सांद्रता 3.25 ग्राम/ली, 11.49 ग्राम/ली और 16.65 ग्राम/ली सफल पैमाने पर प्राप्त की गई। पीए का उपयोग करके पीएचबीवी उत्पादन का भी पता लगाया गया था, जिसमें आरएसएम द्वारा 24 घंटे, 33.88 घंटे और 43.76 घंटे के फीडिंग समय पर 0.85 ग्राम/ली की सांख्यिकीय रूप से अनुकूलित पीए सांद्रता की पहचान की गई थी। मॉडल ने क्रमशः 0.79 ग्राम/ली और 10.84 ग्राम/ली की एचवी सांद्रता और बायोमास वृद्धि की भविष्यवाणी की, जिसे प्रयोगात्मक रूप से शेक फ्लास्क और 7L बायोरिएक्टर उत्पादन के लिए मान्य किया गया था। इस बैच उत्पादन को निरंतर पी/वी स्केल-अप मानदंड का उपयोग करके 70L बायोरिएक्टर तक बढ़ाया गया था और बैक्टीरियल संवर्धन में क्रमशः 0.793 ग्राम/ली, 8.25 ग्राम/ली और 11.23 ग्राम/ली के एचवी, पीएचबीवी और बायोमास सांद्रता का संचय दिखाया गया। पीए का उपयोग करते हुए 7L बायोरिएक्टर में पीएचबीवी उत्पादन में सुधार के लिए पीएसएस फेड बैच उत्पादन की रणनीति का पता लगाया गया था और बैक्टीरियल संवर्धन में क्रमशः 4.7 ग्राम/ली, 13.33 ग्राम/ली और 21.21 ग्राम/ली के एचवी, पीएचबीवी और बायोमास सांद्रता का प्रदर्शन किया। इस फेड बैच उत्पादन को क्रमशः 4.12 ग्राम/ली, 12.88 ग्राम/ली और 19.82 ग्राम/ली की एचवी, पीएचबीवी और बायोमास सांद्रता प्राप्त करने के लिए निरंतर पी/वी स्केल-अप मानदंड का उपयोग करके 70L बायोरिएक्टर तक बढ़ाया गया था, जो सफल पैमाने को प्रदर्शित करता है।

बी. थुरिंजिएन्सिस को शेक फ्लास्क में अनुकूलित मीडिया का उपयोग करके उपजाया गया था जिसमें संवर्धित पीएचबीवी संश्लेषण को बढ़ावा देने के लिए क्रिप्वन मीडिया के दौरान पीए सांद्रता और इसके डालने का अंतराल सांख्यिकीय रूप से अनुकूलित करने का प्रयास किया गया था। पीए सांद्रता

और इसके डालने का अंतराल का सांख्यिकीय अनुकूलन आयोजित किया गया था, जो 1.2 ग्राम/ली पीए सांद्रता की अनुमानित इष्टतम 0 घंटे, 14.86 घंटे और 19 घंटे पर डाले जाने का संकेत देता है। इन अनुकूलन प्रयोगों को शेक फ्लास्क और 7L बायोरिएक्टर उत्पादन में मान्य किया गया था और बैक्टीरियल संवर्धन में क्रमशः 3.98 ग्राम/ली, 0.592 ग्राम/ली और 8 ग्राम/ली, 0.805 ग्राम/ली बायोमास और एचवी सांद्रता अधिक गहन आंदोलन और वातन के कारण पाए गए थे। 7L बायोरिएक्टर की स्थितियों को बैच उत्पादन स्केल-अप करने के लिए पी/वी स्केल-अप मानदंड का उपयोग करके 70L तक बढ़ाया गया था, जिसमें बैक्टीरियल संवर्धन में क्रमशः 0.622 ग्राम/ली, 3.57 ग्राम/ली और 7.15 ग्राम/ली का एचवी, पीएचबीवी और बायोमास का संचय दिखाया गया था। पीएचबीवी उत्पादन को बढ़ाने के लिए सबसे अच्छी पहचान वाली पोषक तत्व फीडिंग रणनीति के रूप में ग्लिसरॉल के संबंध में पीएसएस फेड बैच उत्पादन को पी/वी स्केल-अप मानदंड का उपयोग करते हुए 70L बायोरिएक्टर तक बढ़ाया गया था, जिसमें बैक्टीरियल संवर्धन में क्रमशः 0.636 ग्राम/ली, 4.56 ग्राम/ली और 10.74 ग्राम/ली की एचवी, पीएचबीवी और बायोमास सांद्रता प्रदर्शित की, जो सफल पैमाने का प्रदर्शन करती है।

आर. यूट्रोफा, सी. नेकेटर और बी. थुरिंजिएन्सिस के बायोमास को क्रमशः 57 घंटे, 57 घंटे और 36 घंटे पर एकत्रित किया गया, इसके बाद एसडीएस और सोडियम हाइपोक्लोराइट के साथ इसका पूर्व उपचार किया गया, जिसे क्लोरोफॉर्म में निकाला गया और बरामद कॉपोलीमर की विशेषताएँ पता लगायी गईं। एनएमआर और एफटीआईआर अध्ययनों ने कॉपोलीमर की आणविक संरचना की पुष्टि की, जबकि एक्सआरडी विश्लेषण ने सभी बैक्टीरियल संवर्धनों द्वारा निकाले गए पीएचबीवी के क्रिस्टल संरचना को ऑर्थोरोम्बिक होने की पहचान की। डीएससी विश्लेषण ने विभिन्न नमूनों के लिए पीएचबीवी के पिघलने के तापमान को 127-167 डिग्री सेल्सियस के बीच निर्धारित किया, जबकि टीजीए अध्ययनों ने 245-290 डिग्री सेल्सियस के बीच गिरावट तापमान का विश्लेषण किया। विभिन्न पीएचबीवी नमूनों के जीपीसी विश्लेषण ने आणविक भार को 40.2 किलो डाल्टन और 131.9 किलो डाल्टन की सीमा में दर्शाया। इन गुणों से संकेत मिलता है कि विभिन्न जीवाणुओं द्वारा उत्पादित कोपोलिमर समाज में भोजन, रसायन, सामग्री के साथ-साथ जैव चिकित्सा उद्योगों के लिए उपयुक्त साबित होगा।

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ANOVA: Analysis of Variance
ATR: Attenuated total reflectance
CCD: Central composite Design
CoA: Coenzyme A
Conc.: Concentration
DCE: Dichloroethane
DCW: Dry cell weight
DNS: Dinitrosalicylic acid
DO: Dissolved oxygen
DOE: Design of experiments
DSC: Differential scanning calorimetry
DTG: derivative thermogravimetry
FID: Flame ionization detector
FTIR: Fourier transform infrared spectroscopy
GC: Gas chromatography
GPC: Gel permeation chromatography
HBA: 3-Hydroxy butyric acid
HVA: 3-Hydroxy valeric acid
HPLC: High performance liquid chromatography
LAF: Laminar Air Flow
Lcl: Long chain length
Mcl: Medium chain length
h: hours
HCl: Hydrochloric acid
Min: minutes
N: Nitrogen
NA: Nutrient agar
NB: Nutrient broth
NaOCl: Sodium hypochlorite

NaOH: Sodium hydroxide
NMR: Nuclear magnetic resonance
NPCM: non PHA cellular mass
OD: Optical density
OVAT: One variable at a time
PA: Propionic acid
PHA: polyhydroxyalkanoates
PHB: Poly-3-hydroxybutyrate
PHBV: Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) or poly (3HB-co-3HV)
PSS: Pseudo steady state
PTFE: Polytetrafluoroethylene
P / V: Power input per unit volume
RI: Refractive index
RSM: Response surface methodology
RO: Reverse osmosis
S: Substrate
s: seconds
Scl: Short chain length
SDS: Sodium dodecyl sulfate
TES: Trace element solution
TGA: Thermogravimetric analysis
TGY: Tryptone-glucose-yeast extract
TKN: total kjeldahl nitrogen
VA: Valeric acid
X: biomass
XRD: X-ray diffraction