Evaluation of substrates for biopolymer processing

Evaluación de sustratos para procesamiento de biopolímeros

REYES-CABRERA, Estefanía Guadalupe†*, REYES-ESPINO, Jacqueline, MURGUIA-FIERRO, Salma Verónica and PEREZ-GARCIA, Laura Andrea

TecNM - Instituto Tecnológico de La Laguna, Laboratorio de Energías Renovables, México.

ID 1st Author: *Estefania Guadalupe, Reyes-Cabrera* / **ORC ID:** 0009-0005-4925-7397**, CVU CONAHCYT ID:** 1288239

ID 1st Co-author: *Jacqueline, Reyes Espino* / **ORC ID:** 0009-0000-9202-4066, **CVU CONAHCYT ID:** 1288140

ID 2nd Co-author: *Salma Verónica Murguia-Fierro* / **ORC ID:** 0009-0001-5331-9125, **CVU CONAHCYT ID:** 1165081

ID 3rd Co-author: *Laura Andrea, Perez-Garcia* / **ORC ID:** 0000-0002-5880-6192 **CVU CONAHCYT ID:** 887623

DOI: 10.35429/P.2023.1.71.78

Abstract

The substitution by biodegradable plastics through the implementation of lignocellulosic polymers based on lignocellulose represents a potential idea to reduce their impact and take advantage of these wastes, decreasing their cost for final disposal and the environmental damage they generate. In this project, substrates based on banana and mango peels were evaluated for *Pseudomona* to obtain a biopolymer with the objective of determining which substrates generate a greater amount of polymers by comparing mango, banana and potato peels with known growth media. The process is composed of a mechanical pretreatment and acid hydrolysis, for the delignification of the peels, compared with the basal medium and the LMC for the culture of *Pseudomona* at 35°C under sterile conditions, neutral PH and the addition of some elements of the basal medium, cell extraction by centrifugation and a method of extraction of the polymer based on chloroform. Obtaining that the biopolymer produced in gr/L 25.86, 19.53, 6.63, 4.55, 15, for Mango, Banana, Potato, LMC and Basal medium, having better yields than the commercial media.

Biopolymers, Lignocellulosic residues, *Pseudomona*

7 Introduction

Plastic waste pollution is one of the main environmental problems. Technological development and industrialization generate more and more waste. An average of 8 million tons of plastic are dumped into the oceans every year, which is equivalent to emptying a garbage truck full of plastics every minute. (Plastic pollution. One of the greatest environmental challenges of the 21st century, n/d).

Biopolymers present a very promising alternative for the replacement of plastics because they are completely biodegradable and are produced from renewable carbon sources. (Carlos, 2023) They also have an important application as biocompatible materials in the biomedical and pharmaceutical area, in addition to the fact that their production does not generate dependence on oil as is the case of conventional plastics, a resource that is becoming increasingly expensive and scarce, in addition to being highly polluting (García et al., 2013).

Lignocellulosic biomass has a high potential for obtaining high value-added bioproducts, among which bioplastics stand out. Lignocellulosic materials are abundant and generally low priced, being the current challenge to produce valuable products with high selectivities and yields at economic costs. This type of biomass is considered a low-cost source of carbon. (Bioplastics and high value-added bioproducts from lignocellulosic biomass - Chemical Industry, n.d.)

The project consists of the combination of biotechnology and the use of waste rich in carbon source for the production of biopolymers, through the synthesis carried out by some bacterial genera under nutritional stress, within these bacterial genera are species of Pseudomonas that are capable of generating a substitute product for petroleum-derived plastics, by completely biodegradable bioplastics.

7.0.1 *Pseudomonas*

The member species of the genus *Pseudomonas* spp. belong to the phylum of Proteobacteria, of the subclass gamma, group of aerobic Gram-negative bacteria measuring 0.5 to 0.8 μm by 1.5 to 3.0 μm, are highly diverse morphologically and physiologically their motility is by a single polar flagellum, they have a great capacity for adaptability to different environments, finding from animal and plant pathogens, to soil colonizers.

Their ability to produce and accumulate pha has been highly studied in different conditions, mainly the production of medium-chain PHAs,(Carreiro 3-5) these microorganisms grow at near neutral pH and mesophilic temperatures, up to 43°C. It has been reported that many species of Pseudomonas grow efficiently in chemically defined media containing different aliphatic compounds (carbohydrates, fatty acids, dicarboxylic and tricarboxylic acids, alcohols) and aromatics as carbon source.(Martínez, 2008).

Among the growth media for pseudomonas is King's Agar B in a solid medium that allows obtaining bacterial colonies, the presence of magnesium sulfate in its composition provides the necessary cations for the activation of pioverdine, which gives the culture medium a fluorescent greenish-yellow color. The presence of phosphate inhibits the production of pyocyanin, a specific pigment of Pseudomonas aeruginosa (King B Agar | Pseudomonas | Bioser, n.d.).

Among the liquid media, the LMC medium is characterized by providing the necessary nutrients to achieve maximum growth of Pseudomonas, its composition is shown in Table 7.1. While a basa medium constitutes simple media that favor the growth of undemanding bacteria, under stress conditions, covering the minimum nutritional requirements shown in Table 7.2.

Table 7.1 LMC medium

Formula	gr / L
Mannitol (substituted by glucose)	10.00
Yeast extract	10.00
Dipotassium phosphate	00.50
Magnesium sulfate	00.10
Sodium chloride	00.20

Table 7.2 Basal medium

Formula	gr / L
Yeast extract	1.00
Peptone	5.00
Disodium hydrogen phosphate	1.00
Magnesium sulfate	0.20
Glucose	10.00

7.0.2 Lignocellulosic biomass

The lignocellulosic material is the agro-industrial product of greater abundance, it is a source of renewable raw material, by constituting a structural part in the plant kingdom, are waste a low economic valuation, and a great economic and environmental impact for its final disposal, is composed mainly of cellulose, hemicelluloses and lignin compounds that stand out for their numerous applications. (René Rafael Gallego-Domínguez, 2017)

7.0.3 Banana peel

The main by-product of the banana industrial process, is the peel which represents approximately 30% of the weight of the fruit, Banana peel is rich in dietary fiber, proteins, essential amino acids, polyunsaturated fatty acids and potassium; among the efforts to use the peel, proteins, methanol, ethanol, pectins and enzymes have been obtained. (Gómez Montaño et al., 2019)

7.0.4 Mango peel

Mango is a fruit appreciated and highly consumed around the world, and in Mexico, mango is among the 3 most consumed fruits, after banana and apple Mango peel is the residue of multiple products such as juices, pickles, sauces, preserves, jams, jellies and dehydrated products, the peel represents about 32% of the total weight of the fruit which is discarded generating pollution in the environment. (Use of mango and its byproducts in animal production, n.d.)

7.0.5 Potato skin

Potato production is the main source of income due to the diversity of varieties that can be grown. It is estimated that about a quarter of the potato waste generated during industrial processing is discarded. In addition, agro-industrial waste has a considerable starch content that can be used industrially and in biotechnological processes as a substrate. (Sánchez-Castelblanco & Heredia-Martín, 2020).

7.0.6 Pretreatment

This stage is carried out with the objective of reducing the crystallinity of the cellulose, breaking the lignin-cellulose complex, increasing the surface area of the material, minimizing the presence of substances that may hinder the subsequent stages and minimizing the loss of the original material. (Antonio & Jiménez, n/d).

7.0.7 Hydrolysis

Hydrolysis is a process that consists of breaking complex carbohydrates into simple sugars to be used as a substrate for subsequent fermentation. Cellulose is hydrolyzed into D-glucose monosaccharides, while hemicellulose is hydrolyzed into pentoses and hexoses (mannose, glucose, xylose, etc.) (Oviedo, 2017).(Oviedo, 2017)

7.0.8 Cell separation methods

The various separation techniques are based on the existing differences between different cell types based on their differences in size, density, their antibody affinity towards certain cell surface epitopes, light scattering, fluorescence emission. Centrifugation is a technique to separate cells based on their density. This method is ideal for separating cells whose densities differ by more than 0.02 g/ml and is performed by conventional centrifuges (TOPIC 2 Cell Isolation and Purification Techniques - 1st Edition This document contains - Studocu, n.d.).

7.1 Methodology to be developed

The production of PHA by lignocellulosic matter requires additional processes for the release of the carbon source for microorganisms such as Pseudomona, three raw materials were selected: banana peel, mango peel and potato peel, in which the same procedure was carried out.

7.1.1 Biomass preparation

To facilitate the storage of the raw material to be used, the water particles were eliminated from the material by means of a solar dryer, in which the peels were placed with the smallest possible thickness and in defined amounts in gr, remaining there until reaching constant weight, and then they were stored in separate containers.

7.1.2 Pretreatment

A mechanical pretreatment was carried out in order to reduce the size of the particles and improve the solubilization of sugars in the hydrolysis, crushing each of the three types of husks in a food processor until pulverized and then using a strainer the larger particles were removed to achieve a uniform consistency.

7.1.3 Hydrolysis

For the delignification of the pulverized husk, an acid pretreatment was carried out using a 2.5% solution of sulfuric acid at a ratio of 1:10 with respect to the biomass solution for 40 min at 125°C.

7.1.4 Preparation of the medium

After the chemical hydrolysis, a vacuum filtration with coffee filters was performed to remove the solid remains and reduce the time of a second filtration with 40 um paper. The solid remains were discarded and the filtered solution was kept to continue with the process.

Subsequently, the pH was adjusted to 7 of each of the three solutions of the hydrolyzed banana, mango and potato peels, by means of a solution of sodium hydroxide at .5 N, and the elements for the preparation of the basal medium were added (Table 2), in which glucose was replaced by the sugar present in the hydrolyzed solutions, for later comparison, a basal medium with glucose was prepared as indicated in Table 7.2. After the addition of the chemical compounds, each of the samples was sterilized at boiling point for 15 min.

7.1.5 *Pseudomonas* culture

King B medium was prepared, these media were stored in Petri dishes and after solidification they were seeded using a streaking technique with pseudomonas and allowed to grow at 35 ºC until visible growth was observed.

7.1.6 Culture media

For the preinoculum, a medium known for MCL growth (Table 1) was used for 24 hours at 35°C. The previously sterilized medium was inoculated with a bacteriological loop of the sample sown in solid King B medium, taking an absorbance measurement every hour for the analysis of its growth,

It was inoculated in a ratio of 1:10, pre-inoculated the same pre-inoculum 5 different samples, the one prepared with the basal medium, the LMC solution, and the three hydrolyzed solutions with banana peel, mango, potato 35°C for 72 HOURS using an Erlenmeyer flask with Bach type bireactor and monitoring the growth by measuring absorbance at 450 nm every 2 hours.

7.1.7 Extraction of the solute

Each sample was centrifuged at 2000 rpm for 20 min and the supernatant and solute were removed, the latter was extracted by washing with distilled water.

7.1.8 Extraction of PHA

The extracted solute was dried at 60 degrees for 24 hours for the determination of the total bacterial weight, sodium hypochlorite was added at 37 degrees for 2 hours.
Subsequently, the dried samples were placed in a water bath with chloroform and the resultant after evaporation was weighed.

7.2 Results

7.2.1 Preparation of the pre-treatment biomass

Three values of non-dried husk (wet weight) and their respective dry weight were recorded, and based on the following equation, the percentage of moisture was calculated. These values were averaged by type of husk to obtain a concrete value of moisture, which was compared with the theoretical values cited in other projects.

$$\%\,\boldsymbol{Humidity} = \frac{\boldsymbol{Weight_{initial} - Weight_{final}}}{\boldsymbol{Weight_{initial}}} \times \mathbf{100} \quad (1)$$

Equation 1 Percentage of moisture (Gestión de RSU Propiedades 16, n.d.).

Table 7.3 Records of initial and final weights of different shells

Cascara	Wet weight (gr)	Dry weight (gr)	% Moisture	% Average humidity
Mango 1	290.043	41.906	86%	88%
Mango 2	213.000	25.780	88%	
Mango 3	183.010	18.055	90%	
Plantain 1	146.582	31.848	78%	81%
Plantain 2	74.564	11.158	85%	
Plantain 3	228.610	44.540	81%	
Papaya 1	98.818	6.918	93%	93%
Papaya 2	56.030	3.330	94%	
Pope 3	74.080	6.600	91%	

7.2.2 Pretreatment

A nutribullet was used for this process, grinding each type of peel separately (mango, potato and banana) and storing them in plastic containers.

After grinding, the larger particles were eliminated with a strainer and then regrinded until the desired size was reached.

7.2.3 Hydrolysis

For hydrolysis, the brix degrees were recorded to identify the percentage of sugars released at the end of the process; this was done only for the media in which the sugars were not free.

7.2.4 Preparation of the medium

It was recorded that for a hydrolyzed solution of 500 ml after filtration, a variable final volume was obtained for each of the substrates, the results are shown in the following table.

7.2.5 Growth media

For the preinoculum, a known medium was used for its MCL growth for 24 hours at 35°, taking absorbance measurements every hour in order to identify which pseudomonads were still in the growth phase and shorten the latency phase. At the end of the process the Brix degrees of each solution were recorded.

7.2.6 Extraction of the solute

Each sample was centrifuged at 2000 rpm for 20 min and the supernatant and solute were removed, the latter was extracted by washing with distilled water.

Table 7.4 Records of supernatant obtained after centrifugation

SUSTRATE	SUPERNATANT PER LITER
Mango	100 ml
Banana	83 ml
Potato	30 ml
MCL	22 ml
Basal	55 ml

7.2.7 Extraction of PHA

The extracted solute was dried at 60 degrees for 24 hours for the determination of the total bacterial weight, sodium hypochlorite was added at 37 degrees for 2 hours.

Subsequently, the dried samples were placed in a water bath with chloroform and the resultant was weighed after evaporation.

Table 7.5 Grams of pha per liter as a function of substrate type

SUBSTRATE	PHA (gr) liter
Mango	25.86 gr
Banana	19.53 gr
Potato	6.63 gr
CML	4.55
Basal	15.53

7.3 Discussions

It is concluded that higher amounts of PHA were obtained from mango peel because its sugar content is higher, exceeding the amount of production of the original basal medium with glucose, it was determined that providing the minimum requirements to the microorganism allows the production of PHA, while a medium rich in nutrients (LMC), generates a lower production.
The amount of PHA produced was 25.86 gr, 19.53 gr, 6.63 gr, 4.55, 15.53 for the Mango, Banana, Potato, LMC and Basal media.

7.4 Conclusions

It is concluded that greater amounts of PHA were obtained from mango peel because its sugar content is higher, exceeding the amount of production of the original basal medium with glucose, it was determined that providing the minimum requirements to the microorganism allows the production of PHA, while a medium rich in nutrients (LMC), generates a lower production.

The amount of PHA produced was 25.86 gr, 19.53 gr, 6.63 gr, 4.55, 15.53 for the Mango, Banana, Potato, LMC and Basal media.

7.5 References

Antonio, J., & Jiménez, P. (s/f). *UNIVERSIDAD DE JAÉN FACULTAD DE CIENCIAS EXPERIMENTALES DEPARTAMENTO DE INGENIRÍA QUÍMICA, AMBIENTAL Y DE LOS MATERIALES TESIS DOCTORAL ESTUDIO DEL PRETRATAMIENTO CON AGUA CALIENTE EN FASE LÍQUIDA DE LA PAJA DE TRIGO PARA SU CONVERSIÓN BIOLÓGICA A ETANOL PRESENTADA POR*.

Bioplásticos y bioproductos de alto valor añadido a partir de biomasa lignocelulósica - Industria Química. (s/f). Recuperado el 31 de agosto de 2023, de https://www.industriaquimica.es/articulos/20210713/bioplasticos-bioproductos-alto-valor-anadido-partir-biomasa-lignocelulosica

Contaminación por plásticos. Uno de los mayores desafíos ambientales del siglo XXI. (s/f). Recuperado el 31 de agosto de 2023, de https://ecodes.org/hacemos/cultura-para-la-sostenibilidad/salud-y-medioambiente/observatorio-de-salud-y-medio-ambiente/contaminacion-por-plasticos-uno-de-los-mayores-desafios-ambientales-del-siglo-xxi

MARTINEZ, D. (2008)EFECTO DEL GEN fadH1 EN LA PRODUCCION DE PHA CONTENIENDO MONOMEROS INSATURADOS POR Pseudomonas putida . (s/f). Recuperado el 31 de agosto de 2023, de https://repository.javeriana.edu.co/bitstream/handle/10554/8224/tesis219.pdf

García, Y. G., Carlos, J., Contreras, M., Reynoso, G., Antonio, J., & López, C. (2013). Síntesis y biodegradación de polihidroxialcanoatos: plásticos de origen microbiano. *Revista internacional de contaminación ambiental*, *29*(1), 77–115. http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S0188-49992013000100007&lng=es&nrm=iso&tlng=es

Gestión de RSU Propiedades 16. (s/f). Recuperado el 31 de agosto de 2023, de https://aulagaasociacion.files.wordpress.com/2015/03/4_propiedades_rsu.pdf

Gómez Montaño, F. J., Bolado García, V. E., Blasco López, G., Gómez Montaño, F. J., Bolado García, V. E., & Blasco López, G. (2019). Compositional and antioxidant analysis of peels from different banana varieties (Musa spp.) for their possible use in developing enriched flours. *Acta universitaria*, *29*, 1–14. https://doi.org/10.15174/AU.2019.2260

King B Agar | Pseudomonas | Bioser. (s/f). Recuperado el 31 de agosto de 2023, de https://www.bioser.com/productos/king-b-agar-260p/

Oviedo, U. DE. (2017). *"OBTENCIÓN DE BIOETANOL A PARTIR DE HIDROLIZADOS DE RESIDUOS DE FRUTA" TRABAJO FIN DE MASTER POR CELIA HERNÁNDEZ GALINDO*.

René Rafael Gallego-Domínguez, R. Z.-Z. M. E. de L.-O. R. M.-R. (2017). Respuesta de la caña de azúcar ante la aplicación de una mezclade fitoestimulantes. *ICIDCA. Sobre los Derivados de la Caña de Azúcar*, *3*. https://www.redalyc.org/pdf/2231/223158039001.pdf

Carlos, M. C. J. (2023). *Repositorio Institucional de la Universidad San Gregorio de Portoviejo:Uso factible del bloque sintético tipo Lego para mampostería no estructural de viviendas de interés social en la ciudad de Portoviejo.* Recuperado el 31 de agosto de 2023, de http://repositorio.sangregorio.edu.ec/handle/123456789/3004

Sánchez-Castelblanco, E. M., & Heredia-Martín, J. P. (2020). Evaluation of potato peel wastes to produce amylases from bacillus amyloliquefaciens A16. *Revista de la Academia Colombiana de Ciencias Exactas, Fisicas y Naturales*, *44*(172), 794–804. https://doi.org/10.18257/RACCEFYN.1122

TEMA 2 Técnicas DE Aislamiento Y Purificación Celular - 1ª Edición Este documento contiene - Studocu. (s/f). Recuperado el 31 de agosto de 2023, de https://www.studocu.com/es/document/universidad-de-sevilla/biologia-celular/tema-2-tecnicas-de-aislamiento-y-purificacion-celular/21188664

Utilización del mango y sus subproductos en producción animal. (s/f). Recuperado el 31 de agosto de 2023, de https://nutrinews.com/utilizacion-del-mango-y-sus-subproductos-en-produccion-animal/