International Journal of Research in Engineering, Science and Management
Volume 4, Issue 2, February 2021
https://www.ijresm.com | ISSN (Online): 2581-5792

Formation of Bio-Based Polymer (Poly-Lactic Acid) From Potato Peel Waste and Blending with Chitosan Extracted from Fish Scales

Radhika Gune[1], Athang Sawant[2*], Neha Joglekar[3]
[1,2,3]*Department of Biotechnology, KET's V. G. Vaze College of Arts, Science and Commerce, Mulund, India*

***Abstract*: Most common forms of plastics are non-biodegradable and produced from petrochemicals. The synthesis of these plastics not only generate greenhouse gases but they also do not decompose for decades and are therefore difficult to dispose of. Polylactic acid (PLA) is a bio-based plastic made from polymerization of lactic acid. Bio-based plastics are produced from non-petrochemical sources and they can be biodegradable and compostable, therefore they are better for the environment. Commercially, the lactic acid used for polymerization is sourced from fermentation of corn. Corn fields get diverted to plastic production, whereas they could be used to produce food and therefore there is a vital need to replace the nutrient substrate used in fermentation. Recently, organic waste has been used to produce PLA but this has not been implemented commercially. In this study, a biodegradable bio-based polymer, polylactic acid was attempted to be produced. There is no standardized method for polymerization of lactic acid and therefore three different processes were tried out. These low molecular weight polymers were subjected to blending with chitosan, a polysaccharide that was extracted from fish scales. Blending of a biopolymer with another compound is a common process used to improve the structural properties of the polymer. Formation of a standard method for formation of polylactic acid can help replace conventional petrochemical plastics in the future and reduce the environmental impact of plastics without having to give up the luxury of single use plastics.**

***Keywords*: Biodegradable, biopolymer, chitosan, fermentation, lactobacilli, poly-lactic acid, waste.**

1. Introduction

Plastics are solid synthetic materials that can be molded into various objects. Due to its properties of malleability and plasticity, they are widely used - from shopping bags to space crafts [1].

Plastics can be divided into two categories based on their source of material for production. They could be made from non-renewable materials like petrochemicals or renewable materials like cellulose or lactic acid. The latter are known as bio-based plastics and even though they are far less common than conventional petrochemical plastics, they are better for the environment. Production of plastics from petrochemicals releases a large amount of greenhouse gases, significantly contributing to global warming and climate change. Most petrochemical plastics do not degrade after disposal, especially single use plastics (with exceptions of polyglycolic acid, polycaprolactone, polyvinyl alcohol etc.) which can choke natural environments like the oceans which further affects the organisms that are exposed to it.

Polylactic acid (PLA) is a bio-based plastic made from polymerization of lactic acid. Commercially, the lactic acid used for polymerization is sourced from fermentation of corn. Corn fields get diverted to plastic production while they could be used to produce food instead and therefore there is a vital need to replace the nutrient substrate used in fermentation. Organic waste has been used to produce PLA but not commercially

Companies like Starbucks and Frito lays have started incorporating PLA in their product packaging by using several layers of it along with a petrochemical plastic coating. Despite its popularity, it has not yet replaced petrochemical plastics, especially in the field of packaging that produces a lot of single use non degradable plastic waste.

In this study, a biodegradable bio-based polymer, polylactic acid was attempted to be produced. The lactic acid was synthesized by the process of fermentation where an isolated strain of Lactobacilli (Lactobacillus casei) was used with potato peel waste as its nutrient substrate. The lactobacilli were isolated from a commercial probiotic source and were tested biochemically to confirm its identity.

The fermentation was carried out until the pH dropped below 2 after which the lactic acid was separated out from the fermentation broth by whole broth separation techniques. This process required usage of chemicals to alter the pH of the broth for separation. The presence of lactic acid was tested using Kelling's test and the yield was determined by measuring the volume.

Chitosan was extracted from waste fish scales of Labeo rohita that were obtained from local fish vendors. The fish scales were then dried and subjected to demineralization and deproteinization to obtain chitin. The chitin was then subjected to de-acetylation for obtaining fine chitosan powder.

There is no standardized method for polymerization of lactic acid6 and therefore three different processes were tried out. The catalyst used was tin octoate and the co-catalyst used was

*Corresponding author: athangsawant911@gmail.com

isoamyl alcohol. The temperature and reaction time in the three processes were varied. Bubbling nitrogen was supplied to provide an inert atmosphere. Two out of the three processes that were tried provided a low molecular weight polymer while the other process failed to show any polymerization. A separate batch of PLA was blended using chloroform with chitosan obtained from the processes that exhibited successful polymerization earlier during the study. The blending of chitosan with the PLA was done to achieve a more stable and durable polymer. Addition of such compounds to the PLA significantly increases their physical properties such as tensile strength, melting point which are important parameters for any material to be used commercially and are aimed to replace conventional plastic products. Formation of a standard method for formation of polylactic acid can help replace conventional petrochemical plastics in the future and reduce the environmental impact of plastics without having to give up the luxury of single use plastics.

2. Methodology

A. Isolation of Suitable Bacterial Strain

A suitable strain of lactobacilli bacterial culture was isolated from a commercially available product and grown on MRS (De Man, Rogosa and Sharpe) media agar due to its selectivity for supporting the growth of lactobacilli species. Biochemical tests for confirming the species of bacteria were done which included Sugar fermentation test, Indole test, Citrate utilization test, Gram staining and observing the cells.

B. Fermentation of Kitchen Waste

Organic kitchen waste which included vegetable waste, fruit peels and food stuffs were collected. Only raw waste was included for fermentation due to the potential chances of introducing chemicals present in processed foods which could possibly affect the fermentation process. A makeshift fermentation apparatus was assembled. To this apparatus, one liter of kitchen waste slurry was added and equal amounts of water was added to dilute the substrate and allow for proper mixing. To the above mix, 200 mL of MRS media broth was added which was inoculated with the lactobacilli strain isolated earlier in the study. Fermentation was carried out at room temperature with agitation provided to it. The process was carried out till the pH of fermentation media eventually reached below three.

C. Separation of Lactic Acid from Broth

The lactic acid produced during fermentation was separated by first adding calcium hydroxide to the fermentation media till it reached the pH of around 10 to 11 while supplying heat to the broth using a boiling water bath set at 100^{o}C, which produced calcium salt of the acid, calcium lactate. This extract was then filtered and activated carbon was added to it to remove any impurities and colored components. It was then heated at 70^{o}C to remove excess water. The extract was then treated with sulphuric acid to precipitate calcium sulphate whereas organic lactic acid is filtered through the filtrate. After collecting the filtrate, it is evaporated to obtain pure lactic acid. The yield of lactic acid was later calculated along with performing a confirmatory test (Kelling's test) for the presence of lactic acid.

D. Chitosan Extraction

Fish scales of Labeo rohita were obtained from local fish markets. These were cleaned thoroughly and dried under sunlight. The weight of the dried scales was noted and these were mixed with 2% HCl in 1:5 (W/V) ratio at room temperature for 16 hours. The scales were then cleaned with water till their pH reached neutral levels, after which they were immersed in 4% NaOH in 1:5 (W/V) ratio at room temperature till for 20 hours. Wash the obtained residue (chitin) till it reaches a neutral pH. Subject the chitin to 4% NaOH with 1:10 (W/V) ratio at 60^{o}C for 20 hours after which, wash the residue and dry it at 40^{o}C for 4 hours. Collect the dried chitosan and store in air-tight containers at 4^{o}C.

E. Polymerization of Lactic Acid

This study aimed at comparing the final results and yield of the poly-lactic acid obtained from fermented lactic acid and standard lactic acid and its effects on varying concentration of chitosan blends. But since there is no standardized process for polymerization, three different protocols were followed. Each protocol set different parameters for the reactions.

1) Method-I (Lee Tin Sin et al)

In this, 100mL of lactic acid was measured in a measuring cylinder and poured into the double neck RB flask. One of the mouths of the RB flask was closed with the help of a cork which had been connected to a source of nitrogen gas. The RB flask was then placed in a heating mantle. In this protocol a stepwise increase in the temperature was maintained. The flask was held at a particular temperature for a fixed amount of time and then increased in a linear manner. The stage one temperature started at 135 degrees Celsius, stage two was maintained at 150, stage three was kept at 160, stage four at 180 and finally stage five temperature was set at 200 degrees Celsius. After lactic acid was concentrated enough and the moisture loss was apparent, 600 microliters of the catalyst (tin octoate) was added and the reaction was maintained at 200 degrees for a few minutes. After that the polymer that was formed was poured into petri dishes and was left for air drying. Similar method was carried out for the natural lactic acid obtained from fermentation of waste.

2) Method-II (A. Bakibaey et al)

100mL of lactic acid was measured in a measuring cylinder and poured into the double neck RB flask. One of the necks was blocked using a cork which had a passage for a needle to introduce nitrogen in the system. This apparatus was improvised for nitrogen bubbling required for dehydration of lactic acid. The RB flask was then placed in a heating mantle. The removal of water from the lactic acid was carried out at temperature between 120-140 degrees Celsius for 60 minutes with bubbling nitrogen. After sufficient viscosity was achieved, a mixture of 600 microliters consisting of the catalyst (tin octoate) and the co-catalyst (isoamyl alcohol) in equal quantities was added to the viscous fluid. After that the polymer that was formed was poured into petri dishes and was left for air drying and a similar method was carried out for the natural

lactic acid obtained from fermentation of waste.

3) Method-III (Milena S. Lopes et al)

100mL of lactic acid was measured in a measuring cylinder and poured into the double neck RB flask. As showcased in the above two methods this method also employed nitrogen bubbling for removing water in the system. The RB flask was then placed in a heating mantle. 100 mL of lactic acid standard was measured and dispensed into the RB flask and kept on the mantle for heating. The lactic acid was dehydrated to produce oligomers at 160 degrees Celsius for half an hour. The removal of water in this protocol was assisted by using a Clevenger in combination with nitrogen bubbling. These conditions were maintained for 45 mins. After the initial dehydration step, the temperature was raised to 220 degrees Celsius so that lactide could be recovered in the condensate flask. The condenser was maintained 90 degrees Celsius so that the product did not solidify in the condenser itself. The lactide which was then obtained by distillation in the distillation flask was washed with cold water, separated by filtration and then dried overnight at 40 degrees Celsius. In the final step, lactide produced was mixed with catalyst (Tin octoate) and co catalyst (isoamyl alcohol) in 1%w/w concentration at 140 degrees Celsius for 30 mins. The polymer was then poured into petri dishes and allowed to air dry.

F. Blending of Chitosan

The blending of chitosan was done by first producing poly-lactic acid by the third method mentioned above, as this showed the maximum yield. The prepared PLA polymer was then transferred to three fresh petri dishes in equal quantities and dissolved in chloroform. To these beakers a set quantity of chitosan was added, which were 1%, 3% and 5% respectively. The PLA was then obtained by drying the chloroform.

G. Purification of Poly-Lactic Acid

The partially polymerized product was collected in a test tube and ethyl acetate was added to it. The test tube was carefully heated on a burner until the solution looked homogenous. This solution was passed through a funnel containing filter paper. The filtrate that contained soluble impurities passed through the filter paper and was collected in a beaker. The remaining solid particles that were collected on the filter paper were air dried overnight. The process was repeated with the filtrate until a satisfactory yield was achieved.

H. Confirmatory Tests for Poly-Lactic Acid

1) Melting point of PLA

The apparatus for determination of melting point includes a Thiele's tube containing paraffin oil, a thermometer, a fusion tube and a thread – a burner was used to supply heat intermittently. The compound to be tested was added to the fusion tube which was sealed from one end. The fusion tube was tied to the bulb of the thermometer using a thread and the thermometer was submerged in the Thiele's tube in such a way that the bulb and the sealed end of the capillary tube were dipped in the paraffin oil. Heat was supplied using a burner at the side arm of the Thiele's tube. The heat was supplied until the compound melted inside the fusion tube. The temperature at which the compound melted was noted.

2) Dissolution of PLA in suitable solvent

1 ml of our polymerized product was added to a test tube to which chloroform was added dropwise until the product dissolved. Dissolution was observed which indicated the possibility of presence of PLA.

3. Results and Observations

A. Isolation and Enrichment of Bacterial Culture

The bacterial culture was grown on MRS media for 48 hours at 37^{o}C under anaerobic conditions after which bacterial colony characteristics were observed.

Table 1
Colony characteristics of isolated bacteria

S. No.	Parameter	Observation
1	Size	0.2 cm
2	Shape	Circular
3	Color	Pale white
4	Opacity	Opaque
5	Elevation	Concave
6	Border	Smooth
7	Consistency	Sticky
8	Gram nature	Gram positive

B. Fermentation of Kitchen Waste

The fermentation was successfully carried out over a span of 12 days with an average temperature of 28 degree Celsius. The pH reached 3 after 10-12 days of fermentation, which was tested using a pH paper.

C. Biochemical Testing of Bacteria

Biochemical tests were performed to confirm whether the isolated strain belonged to the Lactobacilli species which could be used for fermentation of the waste.

Table 2
Biochemical testing of bacteria

S. No.	Test	Observation	Inference
1	Gram Staining	Bacteria observed in violet color and exhibited rod-shaped morphology	The bacteria were gram-positive bacillus
2	Sugar fermentation		
A	Glucose	Red coloration in Durham's tube	Bacteria is glucose positive
B	Lactose	Red coloration in Durham's tube	Bacteria is lactose positive
C	Fructose	Red coloration in Durham's tube	Bacteria is fructose positive
D	Maltose	Red coloration in Durham's tube	Bacteria is maltose positive
3	Indole utilization	No red ring observed	Indole negative
4	Citrate utilization	No color change observed	Citrate negative
5	Methyl red test	Solution turned red	Methyl red negative
6	Voges Proskauer test	No color change observed	VP negative

D. Separation of Lactic Acid from Broth

50 mL of the fermented broth was used for finding the percentage yield of lactic acid obtained after the fermentation was completed. After separation processes were conducted on this sample of fermented broth 15 mL lactic acid was recovered. Thus, the percentage yield of the lactic acid obtained was calculated to be 30.0%.

By interpreting the above data, it was estimated that 750mL of lactic acid could potentially be obtained from the total fermentation reaction that was set up which contained a total 2.5L of fermentation broth.

1) Confirmatory Tests for Lactic Acid

Kelling's test was performed to confirm the presence of lactic acid. Three reactions were set up in three different test tubes which were used as positive control, test and negative control. Both test tubes with reactions set for positive control and test exhibited bright yellow coloration whereas the tube marked negative control exhibited pale coloration. Thus, the coloration displayed in the tube which contained the test sample was a positive indication of presence of lactic acid.

E. Chitosan Extraction

The total weight of fish scale used for extraction of chitosan was about 240g and the weight of chitosan obtained after the whole process was 11.86g. Thus, the percentage yield of chitosan obtained was calculated to be 4.941%.

F. Polymerization of Lactic Acid

Polymerization of both standard commercial lactic acid and lactic acid generated from the fermentation broth was carried out. 100 mL of lactic acid was used for each of the three different methods mentioned earlier for the polymerization of lactic acid. After the polymerization was carried out, the contents of the reaction flask were distributed equally among four petri dishes and the average weight of the plates were measured.

Table 3
Weight and yield of polymerization of the lactic acid

Method No.	Plate	Initial weight (g)	Final weight (g)	Yield	Percentage Yield
1	Standard	25.56	20.41	0.798	79.85%
	Test	26.25	18.09	0.689	68.91%
2	Standard	25.56	14.21	0.555	55.59%
	Test	26.25	13.28	0.505	50.59%
3	Standard	25.56	6.12	0.239	23.94%
	Test	26.25	7.89	0.312	31.25%
Chitosan	1%	25.56	16.75	0.655	65.53%
	3%	25.56	13.89	0.543	54.34%
	5%	25.56	11.38	0.446	44.62%

It was observed that even though second and third methods of polymerization showed better visual results in terms of product formed. It did not mean that these were better but it indicated that the amount of unpolymerized lactic acid present in the second and third protocol were less as compared to the first protocol.

Thus, in order to find the actual yield of the polymerized PLA, the polymerized and the unpolymerized fractions needed to be separated. This was achieved by recrystallizing the obtained PLA.

G. Purification of Poly-Lactic Acid

After recrystallisation, the amount of polylactic acid formed was weighed in each process. Even though 100ml of lactic acid was used in each case, the product was divided into 25 ml each and poured into petri plates. Only one of the four petri plates were used further in each method for weighing Here, in the first method virtually no polymer was formed as there was no polylactic acid formed after recrystallisation. This concludes that there was no polymerization in the first method in either case. The second and third method showed relatively better results but the yield was very less. This confirmed that most of the yield obtained before recrystallisation was the unpolymerized lactic acid or lactide.

Table 4
Percentage yield of PLA obtained after recrystallization

Method No.	Plate	Initial weight (g)	Final weight (g)	Yield	Percentage Yield
1	Standard	25.56	0.0	0.0	0.0%
	Test	26.25	0.0	0.0	0.0%
2	Standard	25.56	1.58	0.061	6.18%
	Test	26.25	0.78	0.029	2.97%
3	Standard	25.56	2.50	0.097	9.78%
	Test	26.25	2.07	0.078	7.89%
Chitosan	1%	25.56	1.38	0.053	5.39%
	3%	25.56	1.79	0.070	7.00%
	5%	25.56	2.12	0.082	8.29%

H. Confirmatory Test for PLA

1) Melting point of poly-lactic acid

The melting point of lactic acid is 140 degrees Celsius. The melting point of the polylactic acid which was isolated from the kitchen waste fermentation was found to be at 134 degrees Celsius. The melting point of the PLA bended with chitosan was found to be 139 degrees Celsius. This confirms the fact that chitosan could provide more physical toughness to the polymer. The decrease in the melting point is suspected to be a result of the lower molecular weight PLA formed.

2) Dissolubility of poly-lactic acid

The polymer formed a homogeneous solution when dissolved in chloroform. The product dissolved completely and was recovered when the solution was gradually heated, resulting in the vaporization of chloroform and the formation of a polymer similar to that dissolved in chloroform.

4. Discussion and Conclusion

Biopolymers and bio-based polymer development technology is extremely useful for plastic industries as conventional petrochemical plastics need to be phased out of production due to their environmental impact. As of now only a few companies have successfully standardized and patented their own method of polylactic acid production and their use has been very limited compared to the widespread use of petrochemical plastics.

In this study, the polymerization process was not only time consuming but it also resulted in a low yield and low molecular weight product. Companies that are interested in production of biopolymers like polylactic acid have to consider the costs and

time required to mass produce them. The process of fermentation took 12 days in this study. An industry producing this polymer at a large scale can speed up the fermentation process by controlling the environmental conditions inside the fermentation tank. Agitation and aeration have also been found to speed up the reaction. The substrate used for fermentation in industries to produce lactic acid for polymerization is generally corn. Being a food crop, corn is not only an expensive source but usage of corn and corn fields would much rather be used to feed populations of people. Therefore, alternate substrates that have low economical value can be used.

Potato peel waste is generated from industries that produce potato chips. These industries also use single use plastics for their packaging. The potato peel waste they generate can be used for fermentation and formation of biopolymers like polylactic acid which can then be incorporated in their packaging. This cyclical usage of their waste will also substantially reduce the cost of packaging and waste disposal on their part. The sludge generated after fermentation can always be composted. The fish scales that are used for chitosan extraction are just waste products and thus, their use can be directed towards production of such novel compounds.

The separation of lactic acid from the fermentation broth can be carried out using chromatographic and solvent recovery methods. These methods are much more expensive than the one carried out in this experiment but they have higher yields. Better purity also confirms better polymerization. As there are no standardized processes available in the public domain for the polymerization of lactic acid, three different methods of polymerization were carried out in this study. The processes could be developed further for better yield. The production can be further sped up as the processes which were used in this study such as fermentation, polymerization and chitosan extraction can be modified and standardized to give optimum yields in significantly less time. Ring opening method used here can be changed for another process such as direct condensation polymerization or azeotropic dehydration condensation. The catalysts can also make a significant change in results.

These steps can be then incorporated by corporations for successfully mass-producing biopolymers. Due to practical constraints, low pressure conditions could not be provided to the system. This significantly affected the yield of polymerization and the final quality of the product. Large industries that produce single use packaging need to develop better technology for production of PLA. This information needs to be shared in the public domain so that the production of biopolymers increases.

Further studies need to be conducted around these biopolymers to understand their properties thoroughly and to find more ways to improve their physical properties so as to increase the speed in which these can replace the conventional plastics. Already established plastic industries, corporations and even governments should spear-head these programs and take initiatives to try and bring these into market. The production and utilization of bio plastics is extremely important for the plastic industry so that the environmental damage by plastics is curbed while not compromising the luxury of plastics. An increase in production and utilization of bio plastic technology will lower the prices of the product. Plastic industries need to take responsibility for the environmental damage they have caused over the years by switching to bio plastics. The sooner plastic industries replace conventional petrochemical plastics to bioplastics, the better we have a chance at preventing extinction of our environment.

References

[1] https://packaging360.in/insights/polylactic-acid---a-sustainable-bioplastics-packaging-option

[2] https://plasticoceans.org/the-facts/

[3] https://www.weforum.org/agenda/2016/10/every-minute-one-garbage-truck-of-plastic-is-dumped-into-our-oceans/

[4] https://www.economist.com/asia/2019/06/15/south-east-asian-countries-are-banning-imports-of-waste-for-recycling

[5] https://www.onegreenplanet.org/environment/marine-species-extinction-and-plastic-pollution/

[6] Y. Tokiwa, B. P. Calabia, C. U. Ugwu, and S. Aiba, "Biodegradability of plastics," in *Int J Mol Sci.*, vol. 10, no. 9, pp. 3722-3742, 2009.

[7] K. Madhavi, A. D. Reddy, and N. Tambireddy, "Plastics in marine environment," 2014.

[8] US Environmental Protection Agency. "Reducing Wasted Food & Packaging: A Guide for Food Services and Restaurants." EPA, 2014. Retrieved March 7, 2019, https://www.epa.gov/sites/production/files/2015-08/documents/reducing_wasted _food_pkg_tool.pdf

[9] US Environmental Protection Agency. "Advancing Sustainable Materials Management: Facts and Figures." EPA, November 2017. https://www.epa.gov/facts-and-figures-about-materials-waste-and-recycling/advancing-sustainable-materials-management-0

[10] US Energy Information Administration. "Frequently Asked Questions: How much oil is used to make plastic?" EIA. https://www.eia.gov/tools/faqs/faq.php?id=34&t=6

[11] Global Tragedy for Our Oceans and Sea Life, Center for Biological Diversity, http://www.biologicaldiversity.org/campaigns/ocean_plastics/

[12] United Nations Environment Programme. "Plastic planet: How tiny plastic particles are polluting our soil," UN Environment, April 3, 2018, https://www.unenvironment.org/news-and-stories/story/plastic-planet-how-tinyplastic-particles-are-polluting-our-soil

[13] Center for Biological Diversity. "Oceans Plastic Pollution: A Global Tragedy for Our Oceans and Sea Life," Center for Biological Diversity, http://www.biologicaldiversity.org/campaigns/ocean_plastics/

[14] L. T. Sin, A. R. Rahmat, and W. A. W. A. Rahman, "Overview of Poly (lactic Acid)," in *Plastics Design library*, Polylactic Acid, William Andrew Publishing, 2013.

[15] L. Shen, J. Haufe, and M. K. Patel, "Product overview and market projection of emerging bio-based plastics," PRO-BIP 2009, Final report, report commissioned by European Polysaccharide Network of Excellence (EPNOE) and European Bioplastics, Group Science, Technology and Society, Universiteit Utrecht, the Netherlands, 2009.

[16] G. Reddy, M. Altaf, B. J. Naveena, M. Venkateshwar, E. V. Kumar, "Amylolytic bacterial lactic acid fermentation - A review," in *Biotechnol. Adv*., vol. 26, pp. 22-34, 2008.

[17] S. Andreas, M. Bullet, A. Saldaña, and D. Marlenym "Potato Peels: A Source of Nutritionally and Pharmacologically Interesting Compounds–A Review," in *Food*, vol. 3, 2009.

[18] D. Garlotta, "A literature review of poly (lactic acid)," in *J. Polymer Environ*., vol. 9, pp. 63-84, 2001.

[19] HIMEDIA technical data, lactobacillus MRS agar

[20] http://www.farmersjoint.com/blog/livestock-farming/how-to-convert-food-waste-to-nutritious-feed-via-lactic-acid-fermentation/

[21] M. Battcock, and S. Azam-Ali, "Bacterial Fermentations". Fermented Fruits and Vegetables: A Global Perspective. Food and Agriculture Organization of the United Nations. ISBN 92-5-104226-8, 1998.

[22] http://www.farmersjoint.com/blog/livestock-farming/how-to-convert-food-waste-to-nutritious-feed-via-lactic-acid-fermentation/

[23] Evangelista and R. Lagman, "Recovery and purification of lactic acid from fermentation broth by adsorption," Retrospective Theses and Dissertations, 11252, 1994.

[24] Sood, "Textbook of Medical Laboratory Technology," Jaypee Brothers Publishers. p. 444. ISBN 978-81-8061-591-7, 2006.
[25] K. Masutani and Y. Kimura, Chapter 1: PLA Synthesis. From the Monomer to the Polymer, in Poly (lactic acid) Science and Technology: Processing, Properties, Additives and Applications, 2014.
[26] A. A. Bakibaev, A. M. Gazaliev, S. K. Kabieva, V. I. Fedorchenko, G. Guba, Ya, E. I. Smetanina, I. R. Dolgov, and R. O. Gulyaev, "Polymerization of Lactic Acid Using Microwave and Conventional Heating," in *Procedia Chemistry*, vol. 15, pp. 97-102. 2015.
[27] M. S. Lopes, A. Jardini, R. M. Filho, "Synthesis and haracterizations of poly (lactic acid) by ring opening polymerization for biomedical applications," in *Chemical Engineering Transactions*, vol. 38, pp. 331-336, 2014.
[28] L. James, "Large-scale production, properties and commercial applications of polylactic acid polymers," in Polymer Degradation and Stability, vol. 59, no. 1–3), pp. 145–152, January 1998.
[29] https://www.sciencedirect.com/topics/engineering/petroleum-based-polymer
[30] Tullo and H. Alexander, "Global Top 50 Chemical Companies," in *Chemical & Engineering News*, American Chemical Society, July 2015.
[31] https://www.statista.com/statistics/282732/global-production-of-plastics-since-1950/
[32] https://microbeonline.com/carbohydratefermentation-test-uses-principle-procedure-results
[33] https://microbeonline.com/indole-test-principle-procedure-results
[34] https://microbeonline.com/citrate-utilization-test-principle-procedure-expected-results-and-positive-organisms
[35] https://serc.carleton.edu/microbelife/research_methods/microscopy/gramstain.html
[36] https://www.frontiersin.org/articles/10.3389/fmicb.2017.02285/full
[37] http://silverstripe.fkit.hr/cabeq/assets/Uploads/Cabeq-2005-02-7.pdf
[38] https://bioresources.cnr.ncsu.edu/wpcontent/uploads/2017/08/BioRes_12_3_6885_Komesu_MF_REVIEW_Separation_Purif_Technol_Lactic_Acid_Review_12165.pdf
[39] https://www.mt.com/in/en/home/applications/L1_AutoChem_Applications/L2_Crystallization/recrystallizeation.html
[40] http://www.chem.ucalgary.ca/courses/351/laboratory/meltingpoint.pdf
[41] https://bioresources.cnr.ncsu.edu/wp-content/uploads/2017/08/BioRes_12_3_6885_Komesu_MF_REVIEW_Separation_Purif_Technol_Lactic_Acid_Review_12165.pdf
[42] http://downloads.hindawi.com/journals/amse/2013/629092.pdf
[43] https://3dinsider.com/what-is-PLA
[44] https://en.wikipedia.org/wiki/Glycolysis
[45] https://en.wikipedia.org/wiki/Lactic_acid_fermentation
[46] http://textbookofbacteriology.net/
[47] https://www.nationalgeographic.com/environment/2018/11/are-bioplastics-made-from-plants-better-for-environment-ocean-plastic/
[48] https://www.britannica.com/science/plastic-pollution