

**BIO-BASED PRODUCTION AND DOWNSTREAM
PROCESSING OF LACTIC ACID FOR
APPLICATION IN POLYLACTIC ACID
SYNTHESIS**

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**DEPARTMENT OF CHEMISTRY
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by

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DEPARTMENT OF CHEMISTRY

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CERTIFICATE

This is to certify that the thesis entitled “**Bio-based production and downstream processing of lactic acid for application in polylactic acid synthesis**” being submitted by **Ms. NEERJA YADAV** to the Indian Institute of Technology Delhi for the award of the degree of *Doctor of Philosophy* in Chemistry is a record of bonafide research work carried out by her. Ms. NEERJA YADAV has worked under my guidance and supervision, and has fulfilled the requirements for the submission of the thesis, which, to my knowledge, has reached the requisite standard.

The results contained in this dissertation have not been submitted in part or full to any other University or Institute for the award of any degree or diploma.

Date:
Place:

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Abstract

The implementation of a sustainable bio-based economy is today's priority. Lignocellulose is a suitable alternative for the production of high added-value products. Lactic acid is being abundantly used worldwide for industrial and biotechnological applications. Lactic acid can be produced by microbial fermentation and chemical synthesis. But recent studies show that lactic acid production through microbial fermentation is more significant compared to chemical synthesis because of high purity, low energy consumption, and environmental concerns, etc. Production of biopolymer polylactic acid requires the pure D & L form of lactic acid for high stability and durability. Efficient downstream processes are essential that drive 50% of production costs. Therefore, extensive researches are required to get pure lactic acid with optimal yield and recovery by different downstream processing routes. The thesis assesses different lactic acid fermentation technologies and downstream processing. The main challenge currently employed for lactic acid recovery are precipitation, solvent extraction, distillation, and separation with membranes. Drawbacks that limit the application of these technologies are also presented.

Lignocellulosic biomass is promising renewable resource for the production of biofuels and platform chemicals. The present study encompasses the screening and isolation of lactic acid bacteria (LAB), stable in the presence of ionic liquids ([EMIM][OAc], [BMIM][MeSO₄], and [BMIM][Cl]) and lignocellulosic inhibitors (HMF and furfural), and explores them for effective bio-based lactic acid production. Nineteen LAB producing isolates were identified based on their morphological, biochemical, and 16S rRNA sequence analysis. Among these five LAB strains namely *Pediococcus pentosaceus* SKL-7, *Leuconostoc fallax* SKL-15, *Lactobacillus plantarum* SKL-19, *Lactobacillus paracasei* SKL-21, and *Lactobacillus plantarum* SKL-22 grew well in presence of ILs [EMIM][OAc], [BMIM][MeSO₄], and

[BMIM][Cl]. The *L. plantarum* SKL-22 exhibited relatively high tolerance with the highest specific growth rates in presence of 0.5% and 1% [BMIM] [MeSO₄] and [BMIM][Cl]. Overall, *L. plantarum* SKL-22 produced a reasonable good amount of lactic acid i.e. 34.26 g/L. It appears a promising strain for one-pot production of lactic acid from lignocellulosic biomass.

Production of platform chemicals has been advocated as a sustainable option to tackle the problems associated with agro-waste management. Efforts were made to effectively produce second- generation lactic acid from rice straw pretreated with imidazolium ionic liquid [EMIM][OAc] and subsequently fermented with a promising *Lactobacillus plantarum* SKL-22 strain saccharified with a commercial cellulase enzyme. Medium optimization was carried out to enhance the lactic acid (LA) yield by response surface methodology. In a 5 L bioreactor, the process was further upscaled, and a yield increment of 1.11% was observed. The process using rice straw as substrate led to a LA yield of 36.75 g/L from *L. plantarum* SKL-22 in a single pot bioprocess.

Lactic acid is one of the essential platform chemicals, and despite the availability of a range of downstream processes, its effective recovery is still elusive. A phase partitioning process using n-butanol and a chaotropic salt ammonium sulphate was developed to recover lactic acid from the fermentation broth of *Lactobacillus pentosus* SKL-18. During the optimization of various process parameters, the extraction medium's pH was found to be critical, with 2.5 being the best. The optimized process resulted in a lactic acid yield of 86% and found it to be 93% pure. The purity and characteristics of lactic acid were confirmed by FTIR and NMR spectra. This solvent- based extraction procedure is an economical and straightforward downstream process for purifying lactic acid produced from agro- and bakery-residues. The pure lactic acid can further be used for enzymatic synthesis of high value-added product

PLA, a biodegradable and biocompatible plastic.

Polylactic acid (PLA) is becoming one of the most paramount polymers due to its eco-friendly, biocompatible, and biodegradable nature. The monomer employed for PLA synthesis is the lactic acid produced through fermentation from starchy and cellulosic materials. The most popular PLA processing techniques are ring-opening polymerization and direct condensation reactions. Mostly, polycondensation reaction uses a metal catalyst (Zn/Sn oxides), which is not safe for biomedical applications as it contains metal contamination. The present study focused on developing novel bioplastic to solve the environmental plastic problems. A bioprocess was developed for manufacturing high molecular weight PLA using an enzymatic polycondensation process. The degradation studies on the metal-free synthesized polymer suggested its environmental safety and biocompatibility. Lactide formation was analyzed by FTIR and NMR study. The polymerized product's efficiency and structural changes that occurred before and after degradation were evaluated using FTIR, NMR, GPC, XRD, DSC, TGA, and SEM. After 10 months of decay, FTIR spectra exhibited a slight shift in the carbonyl peak and a decrease in peak intensity, and the degree of crystallinity dropped by 18.23%, and after one month in compost, it dropped by 11.12%. The GPC and TGA analysis confirmed the maximum M_w loss and lowered T_{max} by 46 °C after being buried in compost. The SEM analysis showed the existence of the cracks and voids after degradation. This new approach for the synthesis of metal-free degradable PLA would encourage its use for plastic and biomedical products in the future.

एक सतत जैव-आधारित अर्थव्यवस्था का कार्यान्वयन आज की प्राथमिकता है। उच्च वर्धित मूल्य वाले उत्पादों के उत्पादन के लिए लिग्नोसेल्यूलोज एक उपयुक्त विकल्प है। दुनिया भर में औद्योगिक और जैव प्रौद्योगिकी अनुप्रयोगों के लिए लैक्टिक एसिड का बहुतायत से उपयोग किया जा रहा है। लैक्टिक एसिड का उत्पादन माइक्रोबियल किण्वन और रासायनिक संश्लेषण द्वारा किया जा सकता है। लेकिन हाल के अध्ययनों से पता चलता है कि उच्च शुद्धता, कम ऊर्जा खपत और पर्यावरण संबंधी चिंताओं आदि के कारण रासायनिक संश्लेषण की तुलना में माइक्रोबियल किण्वन के माध्यम से लैक्टिक एसिड का उत्पादन अधिक महत्वपूर्ण है। बायोपॉलिमर पॉलीलैक्टिक एसिड के उत्पादन के लिए उच्च के लिए लैक्टिक एसिड के शुद्ध डी एंड एल फॉर्म की आवश्यकता होती है। स्थिरता और स्थायित्व। कुशल डाउनस्ट्रीम प्रक्रियाएं आवश्यक हैं जो उत्पादन लागत का 50% चलाती हैं। इसलिए, विभिन्न डाउनस्ट्रीम प्रसंस्करण मार्गों द्वारा इष्टतम उपज और पुनर्प्राप्ति के साथ शुद्ध लैक्टिक एसिड प्राप्त करने के लिए व्यापक शोध की आवश्यकता है। थीसिस विभिन्न लैक्टिक एसिड किण्वन प्रौद्योगिकियों और डाउनस्ट्रीम प्रसंस्करण का आकलन करती है। लैक्टिक एसिड रिकवरी के लिए वर्तमान में नियोजित मुख्य चुनौती वर्षा, विलायक निष्कर्षण, आसवन और झिल्ली के साथ पृथक्करण है। इन प्रौद्योगिकियों के अनुप्रयोग को सीमित करने वाली कमियां भी प्रस्तुत की गई हैं। लिग्नोसेल्यूलोसिक बायोमास जैव ईंधन और प्लेटफॉर्म रसायनों के उत्पादन के लिए अक्षय संसाधन का वादा कर रहा है। वर्तमान अध्ययन में लैक्टिक एसिड बैक्टीरिया (एलएबी) की जांच और अलगाव शामिल है, जो आयनिक तरल पदार्थ ([ईएमआईएम] [ओएसी], [बीएमआईएम] [मेसो 4], और [बीएमआईएम] [सीएल]) और लिग्नोसेल्यूलोसिक इनहिबिटर की उपस्थिति में स्थिर है। एचएमएफ और फरफुरल), और प्रभावी जैव-आधारित लैक्टिक एसिड उत्पादन के लिए उनकी खोज करता है। उन्नीस एलएबी उत्पादक आइसोलेट्स की पहचान उनके रूपात्मक, जैव रासायनिक और 16S rRNA अनुक्रम विश्लेषण के आधार पर की गई थी। इन पांच एलएबी उपभेदों में पेडियोकोकस पेंटोसेअस एसकेएल -7, ल्यूकोनोस्टोक फालैक्स एसकेएल -15, लैक्टोबैसिलस प्लांटारम एसकेएल -19, लैक्टोबैसिलस पैरासेसी एसकेएल -21, और लैक्टोबैसिलस प्लांटारम एसकेएल -22 आईएल [ईएमआईएम] [ओएसी], [की उपस्थिति में अच्छी तरह से विकसित हुए। बीएमआईएम [MeSO₄], और बीएमआईएम [सीएल]। एल. प्लांटारम एसकेएल-22 ने 0.5% और 1% [बीएमआईएम] [मेसो4] और [बीएमआईएम] [सीएल] की उपस्थिति में उच्चतम विशिष्ट विकास दर के साथ अपेक्षाकृत उच्च सहनशीलता का प्रदर्शन किया। कुल मिलाकर, एल. प्लांटारम एसकेएल-22 ने उचित मात्रा में लैक्टिक एसिड यानी 34.26 ग्राम/लीटर का उत्पादन किया। यह लिग्नोसेल्यूलोसिक बायोमास से लैक्टिक एसिड के एक-पॉट उत्पादन के लिए एक

आशाजनक तनाव प्रतीत होता है। कृषि-अपशिष्ट प्रबंधन से जुड़ी समस्याओं से निपटने के लिए एक स्थायी विकल्प के रूप में प्लेटफॉर्म रसायनों के उत्पादन की वकालत की गई है। इमिडाजोलियम आयनिक तरल [ईएमआईएम] [ओएसी] के साथ उपचारित चावल के भूसे से दूसरी पीढ़ी के लैक्टिक एसिड को प्रभावी ढंग से उत्पादित करने के प्रयास किए गए थे और बाद में एक वाणिज्यिक सेल्युलेस एंजाइम के साथ पवित्र किए गए एक आशाजनक लैक्टोबैसिलस प्लांटारम एसकेएल -22 तनाव के साथ किण्वित किया गया था। प्रतिक्रिया सतह पद्धति द्वारा लैक्टिक एसिड (एलए) उपज बढ़ाने के लिए मध्यम अनुकूलन किया गया था। 5 लीटर बायोरिएक्टर में, प्रक्रिया को और बढ़ाया गया था, और 1.11% की उपज वृद्धि देखी गई थी। सबस्ट्रेट के रूप में चावल के भूसे का उपयोग करने की प्रक्रिया ने एल प्लांटारम एसकेएल-22 से एक एकल पॉट बायोप्रोसेस में 36.75 ग्राम/ली की एलए उपज प्राप्त की। लैक्टिक एसिड आवश्यक प्लेटफॉर्म रसायनों में से एक है, और डाउनस्ट्रीम प्रक्रियाओं की एक शृंखला की उपलब्धता के बावजूद, इसकी प्रभावी वसूली अभी भी मायावी है। लैक्टोबैसिलस पेंटोसस एसकेएल-18 के किण्वन शोरबा से लैक्टिक एसिड को पुनर्प्राप्त करने के लिए एन-ब्यूटेनॉल और एक कैओट्रोपिक नमक अमोनियम सल्फेट का उपयोग करके एक चरण विभाजन प्रक्रिया विकसित की गई थी। विभिन्न प्रक्रिया मापदंडों के अनुकूलन के दौरान, निष्कर्षण माध्यम का पीएच महत्वपूर्ण पाया गया, जिसमें 2.5 सबसे अच्छा था। अनुकूलित प्रक्रिया के परिणामस्वरूप 86% लैक्टिक एसिड की उपज हुई और यह 93% शुद्ध पाया गया। लैक्टिक एसिड की शुद्धता और विशेषताओं की पुष्टि FTIR और NMR स्पेक्ट्रा द्वारा की गई थी। यह विलायक-आधारित निष्कर्षण प्रक्रिया कृषि और बेकरी-अवशेषों से उत्पादित लैक्टिक एसिड को शुद्ध करने के लिए एक किफायती और सीधी डाउनस्ट्रीम प्रक्रिया है। शुद्ध लैक्टिक एसिड का उपयोग उच्च मूल्य वर्धित उत्पाद के एंजाइमी संश्लेषण के लिए किया जा सकता है। पीएलए एक बायोडिग्रेडेबल और बायोकंपैटिबल प्लास्टिक है। पॉलीलैक्टिक एसिड (पीएलए) अपने पर्यावरण के अनुकूल, जैव-संगत और बायोडिग्रेडेबल प्रकृति के कारण सबसे सर्वोपरि पॉलिमर में से एक बन रहा है। पीएलए संश्लेषण के लिए नियोजित मोनोमर स्टार्च और सेल्युलॉसिक सामग्री से किण्वन के माध्यम से उत्पादित लैक्टिक एसिड है। सबसे लोकप्रिय पीएलए प्रसंस्करण तकनीक रिंग-ओपनिंग पोलिमराइजेशन और प्रत्यक्ष संघनन प्रतिक्रियाएं हैं। अधिकतर, पॉलीकॉंडेंशन प्रतिक्रिया धातु उत्प्रेरक (जेएन/एसएन ऑक्साइड) का उपयोग करती है, जो जैव चिकित्सा अनुप्रयोगों के लिए सुरक्षित नहीं है क्योंकि इसमें धातु प्रदूषण होता है। वर्तमान अध्ययन पर्यावरणीय प्लास्टिक समस्याओं को हल करने के लिए उपन्यास बायोप्लास्टिक विकसित करने पर केंद्रित है। एक एंजाइमैटिक पॉलीकंडेंसेशन प्रक्रिया का उपयोग करके उच्च आणविक भार पीएलए के निर्माण के लिए एक बायोप्रोसेस विकसित किया गया था। धातु मुक्त संश्लेषित बहुलक पर गिरावट के अध्ययन ने इसकी पर्यावरणीय सुरक्षा और जैव-अनुकूलता का सुझाव दिया। एफटीआईआर और एनएमआर अध्ययन द्वारा लैक्टोइड गठन का विश्लेषण किया गया था।

पॉलीमराइज़्ड उत्पाद की दक्षता और गिरावट से पहले और बाद में हुए संरचनात्मक परिवर्तनों का मूल्यांकन FTIR, NMR, GPC, XRD, DSC, TGA, और SEM का उपयोग करके किया गया था। 10 महीनों के क्षय के बाद, FTIR स्पेक्ट्रा ने कार्बोनिल शिखर में मामूली बदलाव और शिखर तीव्रता में कमी का प्रदर्शन किया, और क्रिस्टलीयता की डिग्री में 18.23% की गिरावट आई, और खाद में एक महीने के बाद, यह 11.12% गिर गई। जीपीसी और टीजीए विश्लेषण ने अधिकतम मेगावाट नुकसान की पुष्टि की और खाद में दफन होने के बाद टीमैक्स को 46 डिग्री सेल्सियस तक कम कर दिया। एसईएम विश्लेषण ने गिरावट के बाद दरारों और रिक्तियों के अस्तित्व को दिखाया। मेटल-फ्री डिग्रेडेबल पीएलए के संश्लेषण के लिए यह नया दृष्टिकोण भविष्य में प्लास्टिक और बायोमेडिकल उत्पादों के लिए इसके उपयोग को प्रोत्साहित करेगा।

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List of Abbreviations

[BMIM][MeSO ₄]	1-butyl-3-methylimidazolium methane-sulfonate
[BMIM][Cl]	1-butyl-3-methylimidazolium chloride
[EMIM][OAc]	1-ethyl-3-methylimidazolium acetate
CCD	Central composite design
CD	Circular dichroism
CFU	Colony forming unit
CMC	Carboxymethylcellulose
DES	Deep eutectic solvent
DSC	Differential scanning calorimetry
EPS	Exopolysaccharides
FPU	Filter paper unit
FT-IR	Fourier Transform Infrared Spectroscopy
GPC	Gel permeation chromatography
HMF	5-hydroxymethylfurfural
IL	Ionic liquid
LAB	Lactic acid bacteria
LDH	Lactate dehydrogenase
NMR	Nuclear Magnetic Resonance
PBS	Poly(butylene succinate)
PCL	Poly(caprolactone)
PEG	Polyethylene glycol
PHA	Polyhydroxyalkanoates
PLA	Poly(lactic acid)

PLGA	Poly lactide glycolic acid
RSM	Response surface methodology
SEM	Scanning electron microscope
SHF	Separate hydrolysis and fermentation
SSF	Simultaneous saccharification and fermentation
SSCF	Simultaneous saccharification and co- fermentation
TEM	Transmission electron microscope
TGA	Thermogravimetric Analysis
XRD	X-ray diffraction