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Title	Efficient Microbial Production of Lactate-based Polymers Using Hemicellulose-derived Carbon Sources [an abstract of dissertation and a summary of dissertation review]
Author(s)	NDUKO, JOHN MASANI
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Efficient Microbial Production of Lactate-based Polymers Using Hemicellulose-derived
Carbon Sources
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The biopolymer, polylactic acid (PLA), which is produced from renewable carbon
resources, can be used as a plastic replacement for the petrochemical-derived plastics that
cause global warming and petroleum depletion problems. The aim of this study was there-
fore to produce the useful LA-based polyester using the inexpensive carbon sources derived
from lignocellulosic biomass. The utilization of lignocellulosic biomass has advantages over
the most used starch and sugar-based carbon sources in that lignocellulosic biomass is ined-
ible and inexpensive. These advantages make lignocellulosic biomass an attractive carbon
source in establishing a ' biorefinery' for the production of LA-based polymers. PLA is a
biocompatible and biodegradable material employed in various applications. Recently, LA-
based polyester production system has been established in microorganisms using a lactate-
polymerizing enzyme (LPE). This system was achieved by cultivating cells expressing LPE to
produce LA-based polymers from refined glucose.

In this context, the general introduction including the research theme is described
in chapter 1.

In chapter 2, the application of the major lignocellulosic biomass sugar; xylose,
for the production of the LA-based polymer,P(lactate-co-3-hydroxybutyrate) [P(LA-co-3HB)]
was reported for the first time in *Escherichia coli* expressing an evolved LPE. The monomer
composition and polymer yields of P(LA-co-3HB) from xylose were compared with those from
glucose. Furthermore, P(LA-co-3HB) productivity was compared with that of the most char-
acterized microbial polymer, P(3HB). Analyses of carbon flux and intracellular cofactor level
were carried out to understand the differences between the two polymers and between xylose
and glucose. The study revealed that xylose could be utilized more efficiently than glucose for
the production of P(LA-co-3HB) (7.3 g/L) compared to P(3HB) production (4.1 g/L) at flask
scale level. This showed that lactate polymerization conserves carbon than 3HB polymer-
ization, resulting into higher polymer yields. Moreover, xylose was found to give higher LA
fractions (60 mol%) in P(LA-co-3HB) compared to glucose (47 mol%), which was attributed
to the differences in the metabolism of the two sugars that affects the regeneration of the
reduced cofactors, NADPH and NADH that are both essential for P(LA-co-3HB) synthesis.
The results showed the production of P(LA-co-3HB) as a potent target for xylose utilization.

In chapter 3, because xylose was demonstrated to be efficiently utilized for the pro-
duction of LA-enriched P(LA-co-3HB), I sought to exploit xylose by optimizing the metabolic

pathways. To achieve this, two distinct strategies were employed. First, because xylose up-take consumes ATP, the xylose transport system was engineered by overexpressing an ATP-independent galactitol transporter (GatC), which has also been demonstrated to transport xylose. By overexpressing GatC, P(LA-*co*-3HB) yields of 14.4 g/L were attained, which were the highest polymer yields obtained from xylose or other sugars for the shake-flask scale. Additionally, *E. coli* mutants which have enhanced lactic acid production capacity were employed for P(LA-*co*-3HB) production. This led to the production of P(LA-*co*-3HB) having 73 mol% LA at 6.4 g/L, which was the highest yield for the P(LA-*co*-3HB) with LA-enriched fractions ever reported.

In chapter 4, the utilization of lignocellulosic biomass for the production of polyesters is discussed. Cellulose was hydrolyzed by the recyclable ruthenium catalyst into glucose. However, the hydrolysis reaction formed 5-hydroxymethylfurfural (5-HMF) as a by-product, which was toxic to *E. coli*. Screening of *E. coli* strains identified a 5-HMF resistant strain that was applied for the production of P(3HB) and P[(3HB-*co*-3-hydroxyvalerate (3-HV)]. The strain produced polymers from the hydrolysate with yields that were comparable to those of pure glucose, demonstrating that the strain was resistant against 5-HMF and lignocellulosic biomass could be utilized for polymer production without detoxification steps.

Chapter 5 is the summary of the thesis. In short, for the first time, the major lignocellulosic biomass sugar, xylose, was demonstrated to be efficiently used for P(LA-*co*-3HB) production. Xylose was found to be superior to glucose in giving LA-enriched P(LA-*co*-3HB). Furthermore, the P(LA-*co*-3HB) production system was advanced by engineering the xylose transport system and lactic acid formation-competing pathways to achieve P(LA-*co*-3HB) productivities and LA fractions never achieved before. Finally, the application of lignocellulosic biomass for the production of polymers was demonstrated in a 5-HMF-resistant strain, indicating that lignocellulosic biomass could be utilized as good carbon sources thus, replacing pure sugars for the production of polymers.

Based on the review and interview of the doctor thesis, this study can be judged to be very significant and valuable from the viewpoints of academic research and industrial potential. Ph.D degree of Engineering should be awarded to the candidate.