

CHAPTER I

INTRODUCTION

1.1 Overview

Lactic acid is an organic acid that is known to have two stereoisomers, including L(+) and D(-) forms. It has been applied in several industries, including foods, pharmaceutical, cosmetic, and chemical industries. It is generally used as a precursor of biodegradable and biocompatible polylactic acid (PLA) polymers for producing packaging, foam, and fibers (Rodrigues et al., 2017). The production of biocompatible PLA products that require D(-)-lactic acid as raw materials is considered as an early stage of development compared to those derived from petrochemical plastics in industrial scale (Razali and Abdullah, 2017). The market size of D(-)-lactic acid was estimated at \$3.37 billion USD in 2023 and is expected to gain 8.0% of CAGR by 2030. Lactate can be produced by both chemical synthesis and microbial fermentation. Compared to chemical synthesis, biological fermentation of D(-)-lactic acid is more preferable due to the ability to use renewable resources and lignocellulosic biomass as a feedstock to reduce production costs as well as environment pollution (Yaashikaa et al., 2022).

Considering environmental pollution and production cost savings, biofuel and biochemical production from lignocellulosic biomass have been significantly increased. Lactic acid is so far produced from lignocellulosic biomass generated from agricultural and agro-industrial sectors, including corn stover (Zhang and Vadlani, 2013), waste wood (Hama et al., 2015), and sorghum stalks (Zhang et al., 2016a) using various lactic acid bacteria (LAB). Lactic acid production is performed using different kinds of biomass by various microorganisms, including *Lactobacillus rhamnosu* (Bernardo et al., 2016), *Rhizopus oryzae* (Thongchul et al., 2010), *Enterobacter aerogenes* (Thapa et al., 2017), and metabolically engineered strains of *Escherichia coli* (Utrilla et al., 2009) and

Klebsiella oxytoca (In et al., 2020).

Alternatively, pineapple is known as the most active crop in the global tropical fruit trade, which is mainly distributed in Asia, America, and Africa. With the total global area of pineapple cultivation exceeding 400,000 hectares, pineapple has been produced about 25.4 million metric tons annually (Li et al., 2022). Pineapple is a valuable and non-climacteric tropical fruit and can be eaten fresh or processed as canned, juiced, jams, and dried pineapple based on its maturity (Dhar et al., 2023). Along with pineapple processing and consumption, pineapple crown, peel, core, and stem are left as wastes, with 50% (w/w) of the pineapple weight. Subsequently, the pineapple wastes are disposed of directly in landfills, contributing to environmental pollution due to microbial spoilage and the growing volume of waste associated with increased pineapple production (Hikal et al., 2021). Therefore, among pineapple wastes, pineapple crown is weight about 10-25% of total weight and consists of cellulose and hemicellulose, making it ideal as lignocellulosic biomass (Choquechua et al., 2020; Prado and Spinace, 2019). To mitigate environment impacts and enhance value creation, the pineapple crown (PIC) presents strong potential for biofuel and biochemical production, owing to its rich carbohydrate content and cost-effectiveness as an agro-industrial waste. To achieve this, the conversion of lignocellulosic fibers in PIC into fermentable sugars requires appropriate pretreatment processes to breakdown the structure of cellulose and hemicellulose, remove lignin, reduce inhibitor accumulation, and increase surface area for subsequent enzyme digestion (Yankov, 2022). However, there are very few research studies on the biochemical conversion of PIC into valuable bioproducts via fermentation due to its complicated lignocellulosic pattern and multiple cellular structure (Arib et al., 2006). Therefore, using pineapple crown as lignocellulosic substrate for D-(-)-lactic acid production is still limited, making its challenges for a proper preparation of PIC to be used for D-(-)-lactic acid production.

Klebsiella oxytoca KIS004-91T strain was previously engineered to efficiently produce high levels of D-(-)-lactic acid from glucose with minimal by-product

formation. This strain achieved a D-(-)-lactic acid concentration of 100 g/L, with a yield of 0.96 g/g and a productivity of 2.1 g/L/h (In et al., 2020). To enhance its industrial feasibility and cost effectiveness for D-(-)-lactic acid production, this study aimed to develop efficient processes for the pretreatment and enzymatic saccharification of PIC. D-(-)-lactic acid production by *K. oxytoca* KIS004-91T strain was optimized using separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) strategies, including batch and fed-batch processes, in a low-cost medium containing fermentable sugars derived from the pretreated PIC.

1.2 Research objectives

This research aims to valorize pineapple crown, which is the waste from dried and canned fruit factories, promoting an effective process of D-(-)-lactic acid production using *K. oxytoca* KIS004-91T strain. This work focused on:

1.2.1 Optimizing the suitable concentration of alkali solution for pretreating pineapple crown into pretreated lignocellulosic structures.

1.2.2 Optimizing the appropriate enzymatic treatment and duration of hydrolysis to obtain the maximum level of sugar conversion from pretreated pineapple crown.

1.2.3 Optimizing the maximum production of D-(-)-lactic acid from pretreated pineapple crown by metabolically *K. oxytoca* KIS004-91T strain.

1.3 Scope and limitations

Fermentation of D-(-)-lactic acid from pineapple waste was conducted under different conditions, including simultaneous saccharification and fermentation (SSF) and separate hydrolysis and fermentation (SHF) processes in batch and fed-batch fermentation by *K. oxytoca* KIS004-91T strain. Accordingly, the concentration of alkaline solution during the pretreatment of pineapple crown and enzymatic loading for enzymatic saccharification of pretreated-pineapple crown were also optimized. Different concentrations of pretreated PIC were investigated to obtain the highest

productivity and yield of D-(-)-lactic acid closed to the theoretical maximum (1 g/g) as appeared using glucose as a substrate.