CHAPTER I

INTRODUCTION

Nowadays, lactic acid has become a large volume chemical commodity due to its various applications such as feedstock for polymerization to biodegradable polymers (PLA), oxygenated chemicals, plant growth regulators, green solvents and specialty chemical intermediates. Lactic acid is a versatile chemical used as an acidulant, flavour and preservative in the food, pharmaceutical, leather, and textile industries [1 – 3].

Lactic acid is an organic acid that exists as two optical isomers, D(–) and L(+)lactic acid. Both isomeric forms of lactic acid can be polymerized to be obtained polymers with different properties which these properties can be produced depending on the composition. Lactic acid can be produced by chemical synthesis and microbial fermentation [2 – 4]. Moreover, the mentioned chemical synthesis occupied the 10% production of 80,000 tons of lactic acid per year and the rest is produced by bacterial fermentation [4]. Fermentative production has the advantage that by choosing a strain of microorganism which produces only one of the isomers, an optically pure lactic acid can be obtained, whereas synthetic production always results in a racemic mixture of lactic acid [1 – 3, 5]. Regarding to the strain selection, it is found that *lactobacillus salivarius* subsp. *salivarius* ATCC 11741 is known as a high-yield L(+)lactic acid producer [5 – 6].

Lactic acid production through free cell fermentation occupies about 50% of the world supply, but the productivity is very low in conventional processes. Cell immobilization method can maintain high cell concentration and enhanced fermentation productivity, cell stability and lower costs of recovery and recycling and downstream processing [7 – 10]. Immobilization often mimics what occurs naturally when cells grow on surfaces or within natural structures. Many microorganisms own the ability to adhere to different kinds of surfaces in nature.

Several techniques and support materials have been proposed to localize of intact cells to the certain region of space with preservation of some desired catalytic activity. These techniques can be divided into four major categories based on the physical mechanism employed: (a) attachment or adsorption on solid carrier surfaces, (b) entrapment within a porous matrix, (c) self-aggregation by natural flocculation or with cross-linking agents (artificially induced), and (d) cell containment behind barriers [7, 8, 10 – 14]. Many researches work on immobilization by cell entrapment method have been carried out to produce lactic acid using alginate [15 – 18] and κ-carageenan [19, 20]. However, there are many disadvantages of gel immobilization. First, gel beads can be disrupted because of the built up pressure of dividing cells. The product cannot easily diffuse through gel layer and is accumulated in gel beads. Gels can solubilize gradually and has low mechanical strength. Moreover, the production of gel beads in large amount for industrial scale is uneconomical, complicated and difficult.

Immobilization on solid carrier surfaces by physical adsorption can overcome the problems of cell entrapment, including mass transfer limitations. Cells are adhered to the solid support surfaces by vander waals forces, ionic or covalent interactions [7]. System using immobilized cells on a surface is popular due to the relative ease of carrying out this type of immobilization. The type of carrier is an important factor. The good carrier matrix should be non reactive, non toxic, cheap, available in large amounts, simple to use, high stability, high capacity with effective yield and preservation. Examples of solid carriers used in this type of immobilization are cellulosic materials (DEAE-cellulose, wood, sawdust, delignified sawdust), inorganic materials (polygorskite, montmorilonite, hydromica, porous porcelain, porous glass), etc. Solid materials like glass or cellulose can also be modified with polycations, chitosan or other chemicals (pre-formed carriers) to enhance their adsorption ability [7]. Some researchers used synthetic or polymer support matrix for cell binding in lactic acid fermentation [12, 21 – 23]. However, most of them have closed structure and are not naturally disposable. Thus natural biodegradable support matrix is an alternative choice of interest.

Agricultural residues have been used for immobilization and production starting materials. Reusing and recycling these residues can minimize the environmental problems associated with their build-up reducing the use of noble materials. This trend has contributed to the reconsideration of the use of traditional biomaterials such as natural fibres instead of abundance. Normally, agricultural residue composes of cellulose, the most abundant of all naturally occurring substances, hemicellulose and lignin. Cellulose is the principal structural cell wall component of all major plants. It is non-toxic, renewable, biodegradable and modifiable, and has great potential as an excellent industrial material. Thus, there has been a surge in the attempts towards finding out a renewable and biodegradable carrier which essentially in not synthetic, easy to use, cheaper and available naturally [4].

Chitosan, a flocculating agent, was then used for better adhesion of the cells to the solid support [24 – 26]. Chitosan acts not only as flocculating agent but also as polycations in solid support treatment for better adsorption ability of support surface to cells [23].

From the text above, it can be concluded that no research had been working on the immobilization of *L. salivarius* for lactic acid production. Hence, immobilization of *L. salivarius* on agricultural residues is a challenging alternative to make the new biocatalyst for lactic acid production. The present work is aimed towards evaluation of the suitable immobilization support material for lactic acid fermentation.

1.1 Objectives

1. To develop immobilized cell carrier that made from agricultural residue for lactic acid fermentation.
2. To investigate lactic acid fermentation by immobilized cell on agricultural residue in repeated batch system.

1.2 Working scopes

The fermentation in this work was carried out in shake flask mode at shaking frequency of 50 rpm and temperature of 37°C. In addition, pH of the fermentation system is controlled by addition of $CaCO_3$ in medium.

1. Study effects of various parameters on lactic acid production as follows

    1.1 $CaCO_3$ concentration (2.5% and 5.0%) at glucose 20 g/L

    1.2 Initial glucose concentration (20, 40, 50, 70, 90 and 100 g/l)

2. Construct the immobilized cell carrier from agricultural residues namely loofa sponge, sugarcane bagasse, tamarind fruit fibre, and coconut fibre with and without surface modification.
3. Surface modification of the support material is divided into 2 steps. Firstly, the material is pre-treatment with hydrogen peroxide in order to remove waxy and gummy substances [27]. After that the pre-treatment materials are incubated in various molecular weight chitosan, 83,000, 185,000, 380,000, and 800,000 Da.
4. Compare the lactic acid production of the prepared biocatalyst.
5. Verify the reusability of the carrier by repeated batch fermentation.

1.3 Expected benefit

1. To reveal suitable agricultural residue immobilized carrier which can applied to lactic acid fermentation.
2. To obtain useful information for a better understanding of immobilized cell technology.