Optimisation and Scale-up of a Biotechnological Process for Production of L(+)-Lactic Acid from Waste Potato Starch by *Rhizopus arrhizus*

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ABSTRACT

L(+)-Lactic acid is a commonly occurring organic acid, which is valuable due to its wide use in food and food-related industries, and its potential for the production of biodegradable and biocompatible polylactate polymers.

The aim of this study was to optimize and scale-up a biotechnological process of L(+)-lactic acid production by suspended cells of *R. arrhizus* DAR 36017 with waste potato starch as the substrate. Commonly used inorganic and organic nitrogen sources, including ammonium sulphate, ammonium nitrate, urea, yeast extract and peptone, were assessed in conjunction with various ratios of carbon to nitrogen (C:N). Fermentation media with a low C:N ratio enhanced the production of lactic acid, biomass and ethanol, while a high C:N ratio led to production of more fumaric acid as a by-product. The use of organic nitrogen sources (yeast extract, peptone and urea) resulted in a significant reduction of lactic acid yields by 15 % - 34 % with a decrease of C:N from 168 to 28. The use of inorganic nitrogen sources (ammonium nitrate and ammonium sulphate) led to a high lactic acid yield of 84 % - 91 % at a C:N below 168. Therefore, ammonium nitrate and ammonium sulphate were considered to be better nitrogen sources for lactic acid production.

Small pellets are the favoured morphological form for many fermentation processes by filamentous fungi. However, to control filamentous *Rhizopus* sp in the pellet form in a submerged fermentation system is difficult due to its filamentous characteristics. An acid-adapted preculture technique was developed to induce the formation of the pellet form in bioreactors. Using the acid-adapted precultures, the fungal biomass can be controlled in small dispersed pellets as a dominant morphological form. With these small pellets, a lactic acid yield of 86-89%, corresponding to a concentration of 86-89g/L, was obtained in a laboratory scale process using a stirred tank reactor (STR) and a bubble column reactor (BCR). A batch bioprocess for lactic acid production was successfully scaled-up from shake flasks to laboratory scale bioreactors. Results from a simulated scale-up process revealed that the concentration and productivity of lactic acid decreased with the increase of the scale-up steps because of increased pellet size. This suggested that a one-step scale-up process using the acid-adapted preculture may be feasible in an industrial-scale bioreactor system.

A comprehensive investigation of the impact of cultivation parameters on the morphology of *R. arrhizus* and lactic acid production was carried out in the BCR. The results showed that the fungal morphology was significantly influenced by carbon sources, pH, starch concentrations, sparger designs and aeration rates. The favoured morphology for lactic acid production was freely dispersed small pellets, which could be retained as a dominant morphology under operation conditions at pH 5.0 – 6.0, starch concentrations of 60 – 120 g/L and aeration rates of 0.2 – 0.8 vvm, using a sintered stainless steel disc sparger. The optimal cultivation conditions at pH 6.0 and aeration rate of 0.4 vvm resulted in the formation of the freely dispersed small pellets and production of 103.8 g/L lactic acid, with a yield of 87 %, from 120 g/L liquefied potato starch in 48 h.

This study shows a technically feasible and economically promising process for the production of lactic acid from waste potato starch. The use of waste potato starch instead of pure glucose or starch as substrate can significantly reduce the production cost, making this technology environmentally and economically attractive.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

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1. Z.Y. Zhang, B. Jin, J.M. Kelly. 2007. Production of lactic acid from renewable materials by *Rhizopus* fungi (review). *Biochemical Engineering Journal* 35:251-263. Copyright for this paper belongs to Elsevier B.V..

2. Z.Y. Zhang, B. Jin, J.M. Kelly. 2007. Production of lactic acid and byproducts from waste potato starch by *Rhizopus arrhizus*: role of nitrogen sources. *World Journal of Microbiology and Biotechnology* 23:229-236. Copyright for this paper belongs to Springer Science & Business Media.

3. Z.Y. Zhang, B. Jin, J.M. Kelly. 2008. Production of L(+)-lactic acid using acid-adapted precultures of *Rhizopus arrhizus* in a stirred tank reactor. *Applied Biochemistry and Biotechnology*. D.O.I., 10.1007/s12010-007-8126-7. Copyright for this paper belongs to Humana Press.

4. Z.Y. Zhang, B. Jin, J.M. Kelly. 2007. Effects of cultivation parameters on the morphology of *Rhizopus arrhizus* and the lactic acid production in a bubble column reactor. *Engineering in Life Sciences* 7:490-496. Copyright for this paper belongs to WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

An additional publication which employs similar research methodologies, not included in this thesis directly but presented as an appendix of this thesis,

Z.Y. Zhang, B. Jin, Z.H. Bai, X.Y. Wang. 2008. Production of fungal biomass protein using microfungi from winery wastewater treatment. *Bioresource Technology* 99: 3871-3876. Copyright for this paper belongs to Elsevier Ltd.

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Signed………………………………………

Date…………………………………………

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PREFACE

This thesis contains eight chapters, of which five chapters (Chapter 2, 4, 5, 6 and 7) comprise the main body. In Chapter 1, a general introduction to this project and thesis is outlined. Chapter 2 contains a comprehensive literature review, which has been published. Chapter 3 contains a general introduction to the experimental materials and methods used in this study. Specific details of the methods are given in the relevant chapters. This project aimed to optimize and scale-up the lactic acid production process using waste potato starch by *Rhizopus arrhizus*. The research outcomes and findings are presented thoroughly in Chapters 4 to 7. In Chapter 4, the focus is on identification of the role of nitrogen sources, and optimization of the ratio of C:N for lactic acid production in shake flasks. In Chapter 5, a newly developed inoculation strategy using acid-adapted precultures as the inoculum is described. With this strategy, the lactic acid production process was successfully scaled up from shake flasks to a stirred tank reactor (STR). Furthermore, the newly developed inoculation strategy was applied to a self-designed bubble column reactor (BCR) resulting in successful process scale-up, described in Chapter 6. In addition, the scale-up between reactors was simulated in this BCR to determine the ideal scale-up steps for lactic acid production. The results from Chapter 5 and Chapter 6 also proved that the BCR is more suitable for lactic acid production in terms of yield and productivity. Therefore, in Chapter 7, the results of further comprehensive optimization of cultivation conditions carried out in the BCR are presented. Chapter 8 draws the conclusions from each individual published paper and discusses the possible prospects of this project.

Chapters of 2, 4, 5 and 7 have been published or accepted for publication in refereed academic journals. Chapter 6 will be submitted to a refereed academic journal. All the papers are closely related to the research field of this work.