# OPTIMIZATION OF CULTIVATION MEDIUM FOR LEVAN PRODUCTION USING *Bacillus subtilis* IN SEMI-INDUSTRIAL SCALE

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To Allah S.W.T the most gracious and merciful.

To my beloved husband, Munir; my daughters, Alya&Aqasyah, my parents &my siblings.

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# ABSTRAK

Levan merupakan polisakarida luarsel fruktosa, dan boleh ditemui pada tumbuhan dan produk mikrobial. Kajian tentang levan baru-baru ini menampilkan peranannya sebagai antitumor, antioksidan, antivirus, kesan terapeutik, agen antikanser dan tindakan fibrinolitik yang dihasilkan daripada pelbagai jenis mikroorganisma. Kajian ini memberi tumpuan kepada mengoptimumkan media pengkulturan *Bacillus subtilis* dalam kelalang goncang terhadap pertumbuhan sel and penghasilan levan. Kesan parameter bioproses juga turut di kaji terhadap penggunaan dalam bioreactor 16-L berskala industri. Kajian terbaru melaporkan bahawa jumlah tertinggi levan dihasilkan daripada media menggunakan sukrosa komersial, dengan terdapat sedikit kuantiti mendakan alkohol. Media belum di optimasikan menghasilkan ketumpatan sel sebanyak 1.95 g $L^{-1}$ dan levan sebanyak 13.5 g $L^{-1}$ dari kajian kelalang goncang, dan selepas pengoptimuman mengikut kaedah OFAT dan statistikal dilakukan ketumpatan sel meningkat kepada 2.26 g $L^{-1}$ dan levan juga meningkat kepada 22.1 g $L^{-1}$ dan 21.8 g $L^{-1}$, masing-masing. Oleh itu, terdapat peningkatan 38.07 % dalam levan yang dihasilkan daripada media dioptimumkan secara statistik berbanding media tidak di optimumkan. Pengkulturan dalam bioreactor 16-L telah dijalankan dikeadaan pH yang tidak dikawal dan pH dikawal dengan menggunakan medium yang telah di optimumkan secara statistik. Formulasi baru terdiri daripada (g $L^{-1}$): sukrosa, 130.0, ekstrak yis, 6.3, $K_2HPO_4$, 5.0, dan $MgSO_4.7H_2O$, 0.2. Hasilnya menunjukkan bahawa bioreaktor tanpa kawalan pH adalah terbaik berbanding bioreactor dengan kawalan pH dalam mendapatkan jumlah ketumpatan jisim sel dan penghasilan levan. Dari bioreaktor tanpa kawalan pH menunjukkan jisim ketumpatan sel yang tertinggi iaitu 5.06 g $L^{-1}$ dan levan sebanyak 26.65 g $L^{-1}$. Ini adalah hampir 49.34 % lebih tinggi daripada yang di perolehi dalam pengkulturan menggunakan kelalang goncang. Walau bagaimanapun hasilan produk iaitu levan bagi setiap jisim sel adalah 5.64 g $L^{-1}$ berbanding 11.54 g $L^{-1}$ dari bioreaktor dengan kawalan pH kerana ketumpatan selnya rendah. Kesimpulannya, kajian ini telah mencadangkan kaedah dan strategi pengkulturan efektif untuk menghasilkan ketumpatan sel dan pengeluaran levan yang tinggi dalam skala semi-industri.

## ABSTRACT

Levan is extracellular polysaccharides of fructose, and it can be found in plants and microbial products. Recent studies of levan showing characteristics as antitumor, antioxidants, antiviral, therapeutic effects and anticancer agents with fibrinolytic action and were produced from various types of microorganisms. This study focused on medium optimization study of *B. subtilis* in the shake flask levels on the cell growth and levan production. The effects of bioprocessing parameters was also studied in the semi-industrial scale 16-L bioreactor. Recent studies reported that the highest amounts of levans are produced from the medium with commercial sucrose, with minor quantity of alcohol precipitate. The un-optimized medium yielded the cell mass production of 1.95 g $L^{-1}$ and 13.5 g $L^{-1}$ of levan from the shake flasks studies. Then, the cell mass increased to 2.26 g $L^{-1}$ and the production of levan also increased to 22.1 g $L^{-1}$ and 21.8 g $L^{-1}$, respectively after medium optimization using OFAT method and statistical approach. Thus, showed that there was 38.07% increment in levan produced from statistical optimized medium compared to un-optimized medium. Batch cultivations in 16-L stirred tank bioreactors were carried out under un-controlled pH and controlled pH condition by using statistically optimized production medium generated from statistical tool. The new formulation was composed of (g $L^{-1}$): sucrose, 130.0, yeast extract, 6.3, $K_2HPO_4$, 5.0, and $MgSO_4.7H_2O$, 0.2. The result showed that bioreactor under uncontrolled pH was favorable compared to controlled pH condition in obtaining the highest amount of cell mass and levan production. From the cultivation under un-controlled pH in the 16-L bioreactor showed high cell mass production of 5.06 g $L^{-1}$ and 26.65 g $L^{-1}$ of levan production was produced. This was almost 49.34 % higher than those obtained in shake flask cultivation. However the yield of levan per cell mass was 5.64 g $L^{-1}$ compared to 11.54 g $L^{-1}$ from bioreactor with controlled pH due to the low cell mass production. In conclusion, this study proposed a reliable industrial production medium and an effective cultivation strategy for the cell mass and levan production in semi industrial scale.

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# LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| CDW | - | Cell dry weight |
| DO | - | Dissolved Oxygen |
| OD | - | Optical density |
| OD600 | - | Optical density at 600 nm |
| sp. | - | Species |
| $K_2HPO_4$ | - | Di-potassium hydrogen phosphate |
| $MgSO_4.7H_2O$ | - | Magnesium sulfate heptahydrate |
| OFAT | - | One Factor At Time |
| RSM | - | Response Surface Methodology |
| $Lev_{max}$ | - | Maximum levan production |
| $X_{max}$ | - | Maximal Dry weight |
| $Q_{lev}$ | - | Levan production rate |
| μ | - | Specific growth rate |
| $Y_{p/x}$ | - | Maximum product produce at maximum cell mass |

# CHAPTER 1

# INTRODUCTION

## 1.1 Research Background

Levan is a biopolymer of fructose with link by β(2-6) glycosidic bond. It also have some β(2-1) linked branched. Levan can be found in plants or microbial products but their molecular weights are different. Levan in plants are low molecular weight compared to levan obtained from microbial products (Moosavi-Nasab *et al.*, 2010). Levan and inulin are two common types of fructan. Levan is polyfructose with larger branch while inulin is much smaller branch, with β-2, 1 linkages. Levan and inulin are totally different in terms of characteristic, where levan is water soluble compound while inulin is robust adhesive properties than levan. Levan is different compared to other polysaccharides that had been used as emulsifier and thickener, because it does not swell in water.

Nowadays, the industrial production of levan is carried out by different types of microorganisms. Levan potentials as emulsifier, stabilizer, thickener, surface-finishing agents, carrier for flavour and fragrances had been proposed by many research and experiments. Its value in medical, pharmaceutical, food industry has shown to be successful through many research and journals. Regardless of many

interesting characteristics and advantages showed by levan, the requirements of producing it in large scale increased dramatically.

In this study the cultivation of *B. subtilis* in the shake flask and semi industrial scale 16L bioreactor was investigated to study the growth kinetics of *B. subtilis* and levan production in different medium composition and under different bioprocessing parameters.

This study generally focused to optimize the production of levan that contribute to the potentially wide application of levan in pharmaceutical and food industry.

## 1.2 Problem Statement

Recent studies of levan were motivated for its role as antitumor, antioxidants, antiviral and anti-cancer agents with fibrinolytic action. Many researchers worldwide have published data on production of levan, but their researches are focusing on the use of different carbon sources, yield, and comparison between different microbes producing levan, including purification and characterization of of levan.

However, there are so little information on producing levan at higher cell density cultivation strategy. Several studies conducted for decades in producing of levan using various microorganisms, but most of them are facing disadvantages of low yield of levan (Shih *et al*., 2010). Through optimizing medium composition in this work, the optimized condition for levan production was studied to formulate production medium towards industrial applications in the shake flasks levels. This followed by study of bioprocessing condition in the semi industrial scale 16L bioreactor that gave high impact to the cell growth and production of levan.

## 1.3 Research Objectives

This study was focused on the development of an industrial production medium and a cultivation strategy for the cell growth and levan production by *B. subtilis* in semi industrial scale bioreactor.

## 1.4 Research Scope

The scopes of the research were applied in order to achieve the objective of this study which are;

(i) Medium optimization for shake flask cultivation of *B. subtilis* using One Factor At Time (OFAT) approach.
(ii) Medium optimization for shake flask cultivation of *B. subtilis* using statistical approach (RSM).
(iii) Study the growth kinetics and levan production before and after medium optimization in shake flask levels.
(iv) Batch cultivation of *B. subtilis* in 16-L pilot scale stirred tank bioreactor to investigate the effect of pH (controlled vs uncontrolled) on the levan production in 16-L bioreactor.