UNIVERSITI PUTRA MALAYSIA

THE OPTIMISATION OF CARBON SOURCES IN SUSTAINABLE BIOSURFACTANT PRODUCTION USING GENERAL ALGEBRAIC MODELLING SYSTEM

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APPROVAL SHEET

This project report, entitled "The Optimisation of Carbon Sources in Sustainable Biosurfactant Production" was prepared and submitted by Amirah binti Manab in partial fulfilment of the requirement for the Bachelor of Agricultural and Biosystems Engineering is hereby accepted.

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ABSTRACT

Biosurfactants are surface active agents that been synthesized by microorganisms naturally. They are consisting of both hydrophilic and hydrophobic parts in their general structures which can decrease the surface tension between two distinct stages or the liquid interfacial tension between water and oil. By having this feature, these natural surfactant have proven that they are more advantageous compared to the synthetic surfactants. However, these compounds unable to beat synthetic surfactants in term of production cost. In order to boost the output of biosurfactants, there are several options that can be carried out such as developing more economical manufacturing method and use the cheaper substrates during the process. The use of raw materials from agro-waste, hydrocarbon or by-products of process is one of the alternatives in offering a culture medium with carbon sources and, at the same moment, can be cost-effective in generating the biosurfactants. From this research, the potential carbon sources were evaluated to be used in biosurfactant production. To achieve the purposes of experiment, a mixed integer linear programming (MILP) model was created to select the suitable carbon origin. The software General Algebraic Modeling System (GAMS) is chosen as an optimization instrument in developing the desired models. As a result of modelling, rhamnolipid is verified as the easiest biosurfactant to be produced. While, the soapstock is highly potential to be utilized as carbon source in synthesizing the biosurfactant.

ABSTRAK

Biosurfaktan adalah agen aktif permukaan yang disintesis oleh mikroorganisma secara semulajadi. Mereka terdiri daripada kedua-dua bahagian hidrofilik dan hidrofobik dalam struktur umum mereka yang dapat mengurangkan ketegangan permukaan antara dua peringkat yang berbeza atau ketegangan pada permukaan cecair antara air dan minyak. Dengan mempunyai ciri ini, surfaktan semulajadi ini telah dibuktikan lebih berfaedah berbanding dengan surfaktan sintetik. Walau bagaimanapun, sebatian ini tidak dapat mengalahkan surfaktan sintetik dari segi kos pengeluaran. Untuk meningkatkan pengeluaran biosurfaktan, terdapat beberapa pilihan yang boleh dilakukan seperti membangunkan kaedah pembuatan yang lebih ekonomi dan menggunakan substrat yang lebih murah semasa proses tersebut. Penggunaan bahan mentah dari bahan buangan agrokimia, hidrokarbon atau produk sampingan merupakan salah satu alternatif dalam menawarkan medium dengan sumber karbon dan pada masa yang sama, boleh menjadi kos efektif dalam menjana biosurfaktan. Dari kajian ini, potensi sumber karbon dinilai untuk digunakan dalam pengeluaran biosurfaktan. Untuk mencapai tujuan eksperimen, model pengaturcaraan linear integer bercampur (MILP) dicipta untuk memilih sumber karbon yang sesuai. Sistem Pemodelan Algebra Umum (GAMS) dipilih sebagai alat pengoptimuman dalam membangunkan model yang dikehendaki. Akibat pemodelan, rhamnolipid disahkan sebagai biosurfaktan yang paling mudah untuk dihasilkan. Sementara itu, sabun sangat berpotensi digunakan sebagai sumber karbon dalam mensintesis biosurfaktan.

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LIST OF ABBREVIATIONS

GAMS	General Algebraic Modelling System
LP	Linear Programming
MSM	Mineral salt medium

LIST OF SYMBOLS

a	The flow of biosurfactant process
b	The amount of the type of biosurfactant conversion (litres)
c	The flow of biosurfactant demand
i	The type of carbon sources used
k	The type of produced biosurfactant
m	The market
res (i)	The amount of the type of carbon sources used (*litres*)
con (k,m)	The amount of produced biosurfactant from conversion to the market
demand (k,m)	The demand for produced biosurfactant (*litres)*
COST (i)	The price for each carbon sources used per litres (*RM/L*)
PRICE (k)	The price for produced biosurfactant per litres (*RM/L)*
PROFIT	The total profit of biosurfactant production (*RM*)

Greek Letters

$\sum$	Summation
$\forall$	All belong to

CHAPTER 1

INTRODUCTION

1.1 BIOSURFACTANT

Biosurfactants are naturally occuring surfactant which been extracted by microorganisms like bacteria and yeast (Vigneshwaran et al. 2016). The production of biosurfactant was introduced because of increasing problems in detrimental of environment ecology which been created by chemical surfactants such as sodium cocoyl isethionate and nonylphenol polyethoxylate (Jiang et al 2012). These issues arise from illegal practices or policies that give negative impacts on the environment.

In recent years, the scientific and industrial communities interested into the microbial surfactants because of their beneficial advantages (Yeh et al 2005; Cameotra et al 2010). For example, capability to degrade naturally with help of bacteria or other living organisms, renewability and maintenance of functionality under extreme operating conditions. From past research studies, the biosurfactants show more potential benefits over their synthetic counterparts in many areas of environmental, food, biomedical and other industrial applications.

The microbial surfactants have amphipathic nature as they are capable to separate two different surface tensions like air – water or water – oil interface spontaneously. In their general components, they contain the molecules that consisting of a hydrophobic part and hydrophilic part which can reduce surface or interfacial tensions as shown in Figure 1.1.

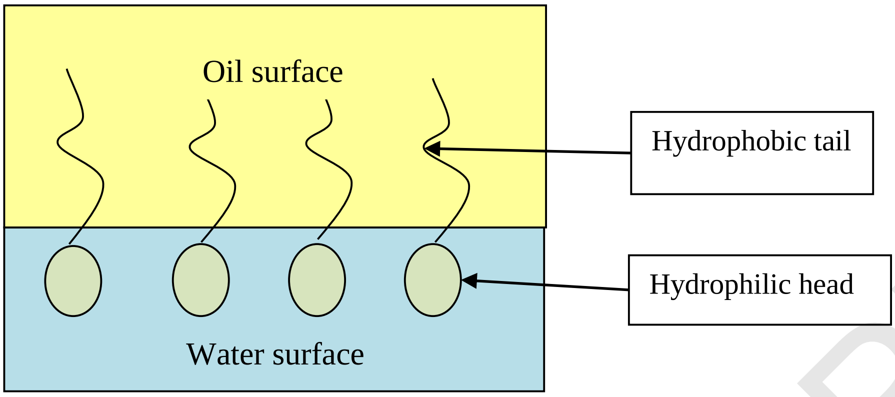

Figure 1.1 The schematic illustration of a biosurfactant.

Desai and Banat (1997) indicated that the biosurfactants could be divided into five main groups based on their natural chemical structure and bacterial origins as mentioned in Figure 1.2. For instances, lipopeptides, glycolipids, phospholipids, neutral lipids and polymeric compounds.

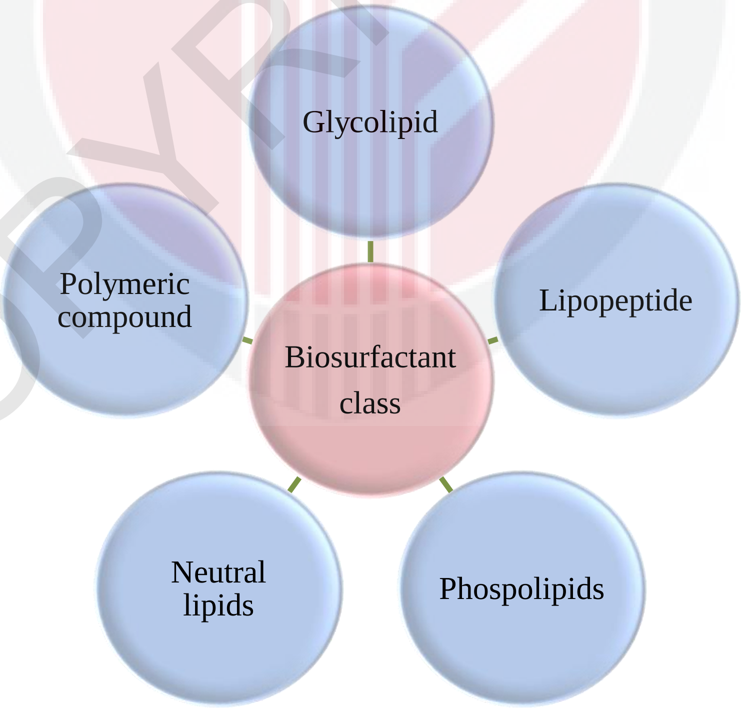

Figure 1.2 List of main group for biosurfactants (Desai and Banat, 1997).

1.2 PROBLEM STATEMENT

In the chemical industry, surfactants are categorized as an important class due to its high demand in usage. Today, alkyl polyglycosides (APGs) is the one of synthethic surfactants that produced from the petroleum. But, the consumers demand for the utilization of renewable resources and environmentally friendly products despite of the sources for petroleum do not endured for the long period of time.

As a results, there are a lot of research in bio-based surfactant were done for time being. For example, the production of rhamnolipids by *Pseudomonas aeruginosa* and the synthesization of surfactin from *Bacillus subtilis.* Because of their outstanding characteristics and possible applications, these products become the most analyzed biosurfactants (Henkel et al 2017). Microbial biosurfactants are actually more beneficial compared to the chemical surfactants. It is because, under intense circumstances, their ability to perishable to the surrounding and practicable.

The large scale manufacturing of biosurfactant is constrained by the dilemma of expensive commercial surcharges and low production output even though their application in bioremediation operation becomes a highly favorable value. Within this basic research, the best carbon sources were figured out to be used in the production of biosurfactant while at the same time, cost-effective resource.

1.3 RESEARCH QUESTION

In order to model the environmentally sustainable biosurfactant production, research topic must be tackled. This study's research question is as follow:

1) What are the absolute best carbon resource which can be used in producing biosurfactant to achieve minimum production cost?

1.4 RESEARCH OBJECTIVES

The major purpose of this study is to construct a new systematic structure by synthesizing a potential carbon source in biosurfactant production using structural optimization. The specific objectives of this research are as follows:

i. To create and modify a mathematical model capable of identifying the best carbon source to minimize the production cost.

ii. To create and modify a mathematical model capable of identifying the best biosurfactant to produce.

1.5 SCOPE OF RESEARCH

The structure of the research study has been recognized as follows in terms of achieving the expected research interests.

a) The study about existing and current issues of biosurfactant manufacturing situation, as well as for mechanisms, constraints and possible improvements, which gained from other global sources.

b) There is no data collection from the site since the information input was acquired from the sequence of research.

CHAPTER 2

LITERATURE REVIEW

2.1 BIOSURFACTANT INDUSTRY

In 2011, a worldwide microbial surfactant economy entered USD 1,735.5 million and projected to grow 3.5 percent of compound annual return which equivalent to USD 2,210.5 million as well from 2011 to 2018 as reported by Transparency Market Research (2012). The global market for biosurfactant recorded 476,512.2 ton in total volume by 2018. From 21% of this outstanding consumption, the great contribution from the established Asian regions, South America, and African countries leading the market to grow robustly.

European region was placed in the first position with regarding to international biosurfactant trade as it conquered almost 53.3% of global biosurfactant market revenue and volume in 2018. Then, North America come after that (Transparency Market Research, 2012).

From the previous research study, the international market for biosurfactants is divided into several groups; which are can be categorized into type of by-product, application and geographical location. The trade for the by-product mainly consists of glycolipids (sophorolipids and rhamnolipids), lipopeptides, phosposlipids, polymeric surfactants and others.

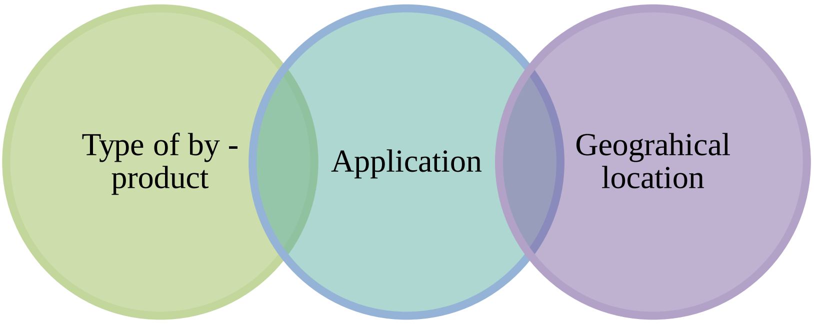

Figure 2.1 The factors of demand in the international market of biosurfactants (Transparency Market Research, 2012).

While for agriculture chemicals, detergents, food processing, personal care and medicine are categorized into end-use market. However, the household cleaning products and personal care portion covered 56.8 percent of the market share. The geographical locations (including Africa, Asia/Pacific, Europe, Middle East, North America and Latin America) also importance for analysis of worldwide microbial surfactant economy (Transparency Market Research, 2012).

In industry, the rising issue of huge biosurfactant production costs has gained a great deal of concern that can have an impact on their future development. However, they are becoming a match for chemical surfactants in the contexts of functionality and production capacity to handle demand because of their advantageous characteristics.

Indeed, biosurfactants are still acceptable in certain premium items, for example, beauty products and pharmaceuticals. This is due to application of biosurfactants as low-volume but high-priced components. In contrast with the end product like oil recovery, the high cost of production is not proved where high-volume and low-cost surfactants are needed. Therefore, it is importance to ensure that commercial uses are environmentally biocompatible.

2.2 FACTORS AFFECTING BIOSURFACTANT PRODUCTION

2.2.1 Effect of carbon sources

Microbes used in the biosurfactant production utilise variety of carbon sources and energy for their growth. The microorganisms utilise water soluble carbon source such as glycerol, mannitol, glucose and ethanol for natural surfactant production (Robert et al, 1989). Among the different carbon sources, glycerol behaves differently in such a way that when glycerol concentration is higher than 2%, the biosurfactant level sharply decreases. Safi et al *(*2007) reported that 3% glycerol can produce for 2 g/L rhamnolipids with fermentation. He also reported that biosurfactant can be generated by using grape seed oil and sunflower oil as another carbon origins.

Soybean lecithin and crude oil were also identified as suitable carbon sources for biosurfatant production. Changjun Zoua et al (2014) proved through his study that soybean lecithin was well utilized for biosurfactant production than crude oil with a slight difference. But crude oil also proved to be an efficient carbon source in case of Acenitobacter-related bacteria as reported by Huy et al (1999). The use of hydrocarbons such as n-hexadecane and paraffin were also were also attempted as carbon source by Jorge F.B. Pereira (2013) and found that only water soluble carbon sources could be easily utilized for biosurfactant production than paraffin and n-hexadecane.

2.2.2 Effect of nitrogen sources

Usually, the nitrogen sources that involved in the production of biosurfactant consist of 2 different types: organic and inorganic origin. The organic nitrogen sources are urea, peat hydrolysate, tryptone, pancreatic digest of casein, casein hydrolysate, beef extract and yeast extract (Fonseca et al 2007; Das Neves et al 2007). While for inorganic sources including sodium nitrate, potassium nitrate, ammonium nitrate, ammonium chloride, ammonium bromide, ammonium carbonate and ammonium sulfate (Abdel-Mawgoud et al 2008).

Based on the research by Abdel-Mawgoud et al (2008), they carried out the experiment with various sources of nitrogen whether from organic or inorganic origin. For their experimental result, sodium nitrate is concluded as the best nitrogen sources to be used.

2.2.3 Effect of temperature

Cultivation condition of temperature plays a critical role in this biosurfactant production through affecting the cellular growth of microorganism used. Naturally, biosurfactant can be produced at temperature between 25 to 37 ^{o}C (Sen and Swaminathan 1997). If there is any sudden changes occur from specified temperature, the composition of generated of biosurfactant could be destroyed. An optimum temperature of 37.4 ^{o}C was suggested through investigation performed by Sen and Swaminathan (2005). At this temperature, the cell growth was promoted and yielded higher production output.

2.2.4 Effect of pH

pH is the another important factor that needed for the biosurfactant production. Sen and Swaminathan (1997) have been evaluated that a series of pH value started from 6.0 to 9.0. To be more specific, pH of 6.75 is found to be ambient because of the natural surfactant was yield at high quantity.

Abdel-Mawgoud et al (2008) stated that the production tend to decrease when pH is above 8.0. This is due to the reason that the bacterium is unable to reduce the surface tension of culture medium. Therefore, the pH value should be constant in order to reach a large amount of microbial surfactant production (Wei and Chu 1998).

2.2.5 Effect of aeration and agitation

During synthesizing the biosurfactant, aeration and agitation also included as the one of factors that influencing the production. As for aeration, it is more related to the accumulation of foam which formed along the fermentation process (Shaligram and Singhal 2010). The agitation refers to the stirring activity that increase the efficiency of oxygen transfer in the medium component.

The production can reached at maximum yield when supported by rapid agitation (Yeh et al 2005; Fonseca et al 2007). As the agitation rate increases, the dissolved oxygen concentration level become high and thus, greatly influenced the cell growth of microbes (Wei et al 2005).

To obtain the best yield of biosurfactant, the recommended agitation speed is around 200 – 250 rpm (Yeh et al 2005). Shalingram and Singhal (2010) specified that the foam started to accumulate when the speed is more than 350 rpm and reduced the production. This happen because the presence of foam will decrease the oxygen transfer rate. This problem causes the fermentation period become shorten as there is less amount of biomass left (Davis et al 1999).

2.3 TYPE OF CARBON SOURCES

The composition of the carbon substrate influences biosurfactant quality and production amount was tabulated in Table 2.1. By using the sources of carbon in synthesizing the biosurfactant, the microbes in the culture media can grow effectively.

2.3.1 Carbohydrates

In producing the biosurfactant, there are some of carbohydrate-based media that have been experimented as the carbon sources. They are including sucrose, glucose, galacatose, maltose, mannitol, soluble starch and dextrin (Al-Ajlani et al 2007; Liu et al 2012).

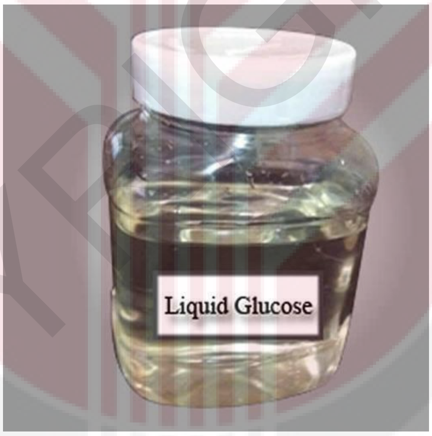

Figure 2.2 Pure liquid glucose

2.3.2 Hydrocarbon and Fatty Acids

The usage of vegetable oils (like sunflower oils, olive oil, paraffin and corn oil) and hydrocarbons (such as benzene, diesel, kerosene, and heptane) in synthesizing biosurfactant are possible in order to gain the carbon origin from those sources (Chen et al 2015). These compounds will be break down by biosurfactant as nutrient supply in the culture medium and thus, improving the biosurfactant production (Abdel-Mawgoud et al 2008).

Figure 2.3 The example for fatty acid source (sunflower oil)

Figure 2.4 The example for hydrocarbon source (kerosene)

2.3.3 Renewable Resources

Renewable resources are recognized as acceptable low-cost carbon sources as they can be gained from industrial wastewater and food processing waste. For the industrial wastewater, the examples are cassava wastewater (Nitschke and Pastore 2004) and soapstock (Benincasa et al 2004). The potato waste (Noah et al 2005) and soybean oil refinery waste (Abalos et al 2001) are categorized in food processing waste. By using these renewable origin, the cost of production can be cut below than predicted production cost.

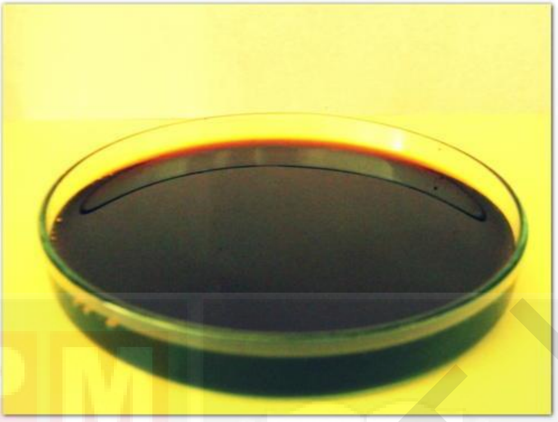

Figure 2.5 The soapstock (Benincasa et al 2004).

Types of carbon sources		Biosurfactant production (mg/L)	References
Carbohydrate	MSM plus D-glucose	800	Abdel-Mawgoud et al (2008)
	MSM plus D-fructose	480	
	MSM plus sucrose	700	
Fatty acids and hydrocarbons	MSM plus 2% olive oil	1000	Ghribi and Ellouze-Chaabouni (2011)
	MSM plus 2% sunflower oil	1100	
	MSM plus 2% corn oil	1100	
Renewable resources	MSM plus cassava wastewater	3000	Nitschke and Pastore (2004)
	MSM plus apple juice from cashew	123	Giro et al (2009)
	MSM plus effluent of processing potato	900	Noah et al (2005)

Table 2.1 The types of carbon sources used in production of biosurfactants.

2.4 APPLICATION OF BIOSURFACTANT

2.4.1 Environmental application

Today, many pollutants are released into the environment due to vast industrialization. Those contaminants consisting organic and inorganic compound are flushed out either by accidents or with intentions. This problem contributes to the major cause of the on-going pollutions nowadays. For example, oil spills in the marine environment.

As for process in cleaning up the oil spills at sea and on shorelines, the biosurfactants become potential alternative for bioremediation. Because of their excellent surface activity and emulsifying properties, those amphiphiles will alter the physico-chemical conditions at the interphase (Tiehm 1994).

Rosenberg and Ron (1999) and Cavalcante Barros et al (2008) examined that surfactin is the one of successful biosurfactants which been applied to environment remediation. This can be seen through evidence that carried out by Lai et al (2009) where the process of biodegradation the hydrocarbon can be fasten by surfactin. This natural surfactant exhibits a higher total petroleum hydrocarbon (TPH) removal efficiency compared to the synthetic surfactants.

2.4.2 Industrial application

For industrial application, the biosurfactants are normally involved in the field of oil recovery and processing. Microbial enhanced oil recovery (MOER) is considered as the most cost effective potential in solving poor oil recovery problem in oil wells.

This tertiary oil recovery technology uses biosurfactants such as surfactin and rhamnolipids in mobilizing the residual oil in oil reservoirs that trapped in porous rocks via capillary forces (Khire 2010). They able to reduce the oil-water interfacial tension and form stable emulsions as for improving the efficiency of oil recovery process (Sarafzadeh et al 2013; Al-Sulaimani et al 2012).

Banat et al (2010) categorized that the surfactin is the better promising candidate for MOER because of its effectiveness. This microbial surfactant can maintain activities under harsh conditions during recovering sand trapped oil. Also, be active in extreme environment with sodium chloride concentration of 0% - 10%, pH 3– 10 and temperature of 21 – 70 °C (Schaller et al 2004). Makkar and Cameotra (1999) reported that synthesized surfactin by B. subtilis could emulsify diesel with 90% E_{24} and recover 62% of oil entrapped in sand core even at high temperature.

2.5 MATHEMATICAL MODELLING

The mathematical model is consisting of several categories such as linear programming (LP), nonlinear programming (NLP), mixed integer linear programming (MILP), and mixed integer nonlinear mixed integer programming (MINLP). LP or also be called "linear optimization" is defined as the mathematical method that involving the determination of the best potential outcome from the objective function into one or more constraints portrayed in linear relationships. But for NLP, it does not require sequential or straightforward variables in order to obtain a possible alternative in the allocation of scarce resources. Both MILP and MINLP refers to extension problems with continuous and discrete variables and functions which whether some or all of the decision variables are only allowed to be integers. The differences between both programming are types of function used (linear or nonlinear).

The optimization technique is a useful analytical method in identifying the ideal approach or unrestrained maximum amount or minimum amount of simultaneous and distinguishable functions. The significance value of performance measurement in a problem can be acquired by using the differential calculus way.

Probably, most of the problems are connected to the process of designing, constructing, operating and analyzing the process plants where there are numerous of alternatives that capable to be problem solutions. Compared to the performance measure, it only related to profitability. In gaining the perfect performance measure, a designer can utilize this kind of technique to discover the type and magnitude of variables even confronting the limitation of issues.

Through this research, the application of General Algebraic Modelling System (GAMS) is important in solving the optimization problems. This specialized design software contained a combination of modelling systems like linear, nonlinear and mixed integer programming where the optimization technique become easier by inserting all collected data. In this research, GAMS version 24.0.1 is preferred to be used.

CHAPTER 3

MATERIALS AND METHODS

3.1 RESEARCH METHODOLOGY

Figure 3.1 indicates the eight phases that involved in constructing the suitable model for biosurfactant production. Each phase explained in detail on how to develop the mathematical model by using the GAMs. Step 1 describes the ongoing problems in synthesizing natural surfactant. This step has already been explained in Chapter 1 through the section 1.2. Next, the mathematical model is recommended as the problem solver for Step 2. In the Step 3, the collection of data is obtained from related reported data, historical data and statistic. After extraction of data is completed, proceed with Step 4 where the superstructure diagram was proposed in order to find out the potential equations and solutions to be apply. Mathematical model for formulating the equations corresponding to the superstructure features and GAMS is used as the optimization instrument for applying the Step 5 and Step 6 solutions methods. Once the optimal solution was reached in Step 7, to draw a conclusion, data analysis was conducted. Last but not least, a case study was performed to check the model as the final step.

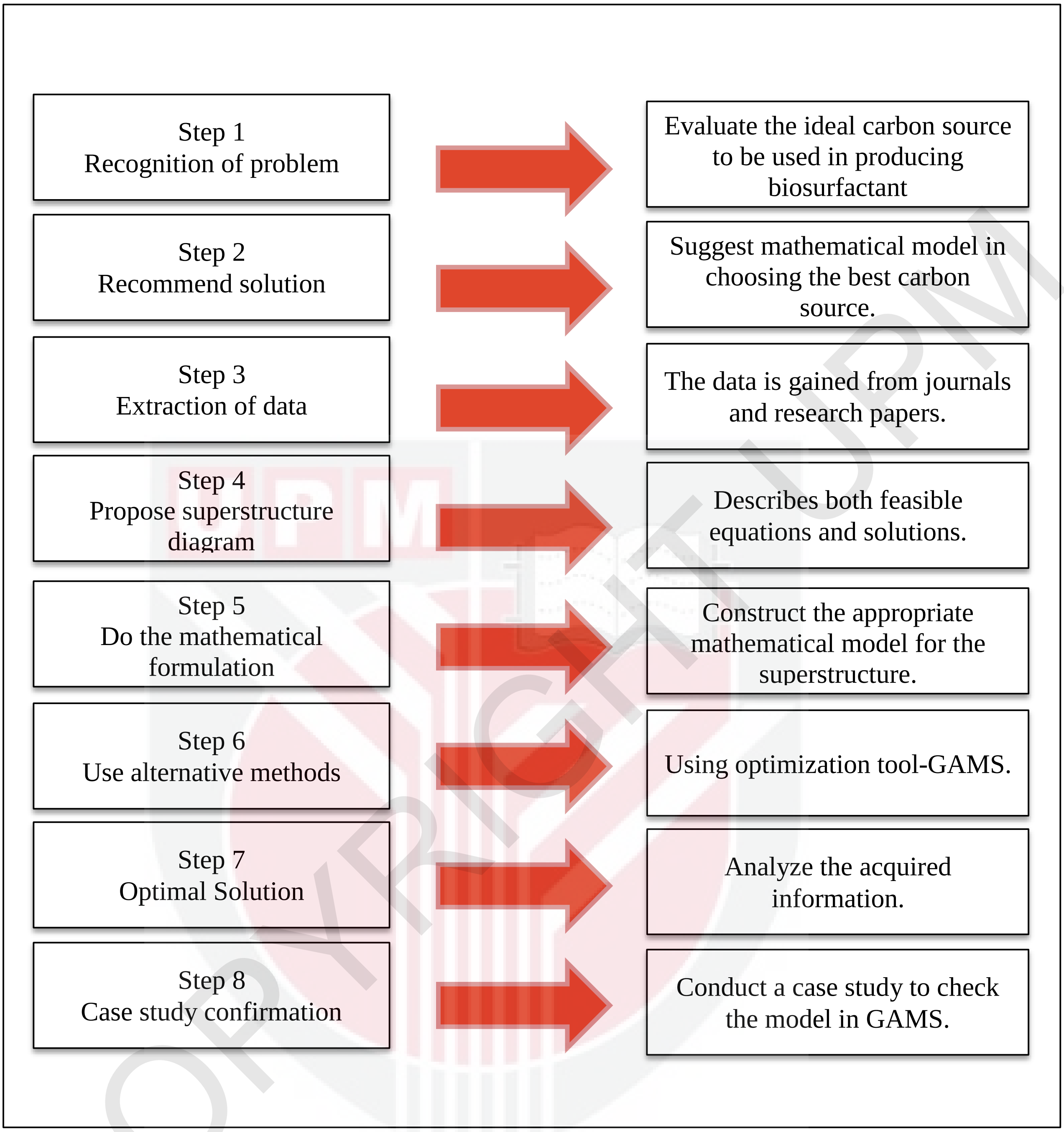

Figure 3.1 The stages of research methodology

3.1.1 Mathematical Model Generation

From the simplified superstructure diagram, the mathematical model is created. The developed mathematical model focuses on searching the ideal types of carbon sources in making microbial surfactant. The constructed linear programming model (LP) figured out most cost-effective carbon origin for biosurfactant production.

Table 3.1 The phase of mathematical model.

Phase	Selection of carbon sources
Objective function	Maximize profit of biosurfactant production
Decision variable	Carbon sources cost, biosurfactant price, biosurfactant demand and the production amount of biosurfactant.
Constraint	The amount of carbon sources used
Model	LP model

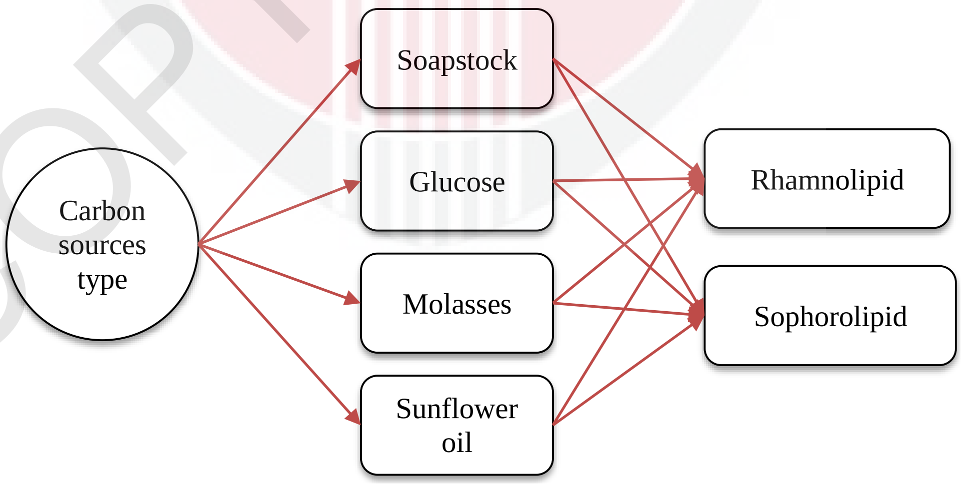

Figure 3.2 Structure diagram for biosurfactant production.

3.2 CODED INTO GAMS

Optimization is a process where the ideal solution is pointed out in gaining the optimum value in a specified problem. Here, the mathematical equation is created and coded into the Generalized Algebraic Modeling System (GAMS) as an optimization instrument. GAMS is a modeling software for linear, nonlinear and mixed integer optimization. Then, the constructed model is been fixed by the GAMS solver through providing the optimum results. GAMS functions based on the specific elements as below:

1) To illustrate sizeable and complicated models, it applies high-level algebraic language.
2) The user can use straightforward and comprehensible coding and alter them based on requirement with ease.
3) The limited data set could be integrated into a big data set whereas there is an extensible modeling setting which owing to subscription-based upgradeability.
4) An accessible platform which can help the users in identifying the solution to the required situation.

In the Appendices, the coded GAMS input files of models are attached as the reference (refer to Appendix A).

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 RESULTS

4.1.1 Problem Definition and Assumptions

Based on the several requirements by industries and community, there are carbon sources and biosurfactants that appropriate to be utilize in this model. From there, the optimum value of consumption of carbon sources in the production of bio – based surfactants can be determined. Therefore, each resource contains unknown flow rate and must be decided.

For further understanding, a demonstration is given. In the industry, 4 different type of carbon sources was chosen with amount of 1 000 000 litres for each type and only produced 2 different type of biosurfactant which are rhamnolipid and sophorolipid. Those carbon sources were came from 4 separate groups which are carbohydrates, fatty acids, hydrocarbon and renewable resources simultaneously. Rhamnolipid and sophorolipid are included into same main class of biosurfactant, glycolipid.

Following assumptions and limitations were made:

a. The carbon sources used is limited into 4 different types.

b. Amount of carbon sources for each type is 1 000 000 litres.

c. Biosurfactant production is only limited to 2 different type (rhamnolipid and sophorolipid).

d. Production cost is calculated for carbon sources cost only.

4.1.2 Superstructure Development

There are three sets that required in this developed superstructure diagram. They are including types of carbon sources (set i), biosurfactant type (set k) and market (set m). In the case study, Figure 4.1 indicated the superstructure of the biosurfactant production and Figure 4.2 described the simplified structure.

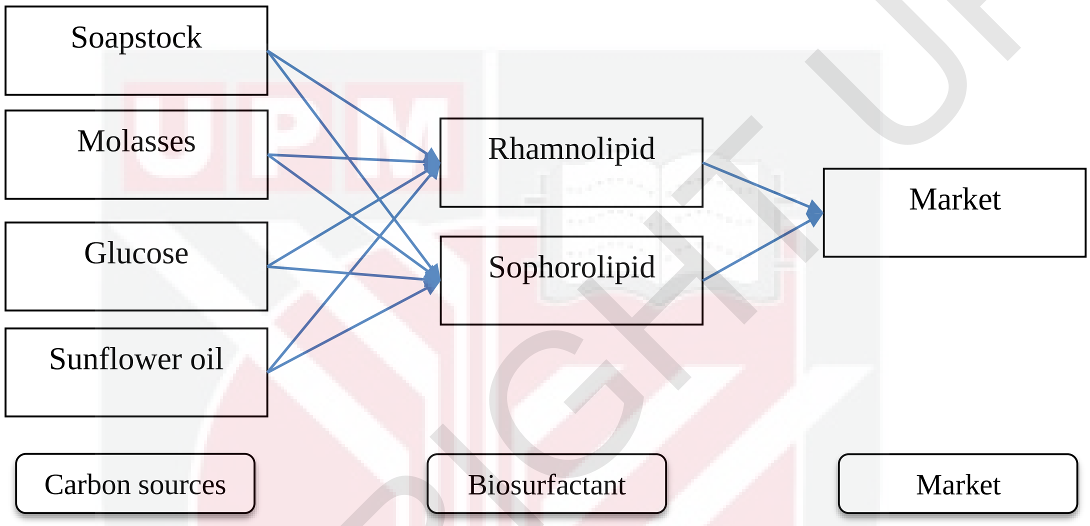

Figure 4.1 The superstructure of the biosurfactant production

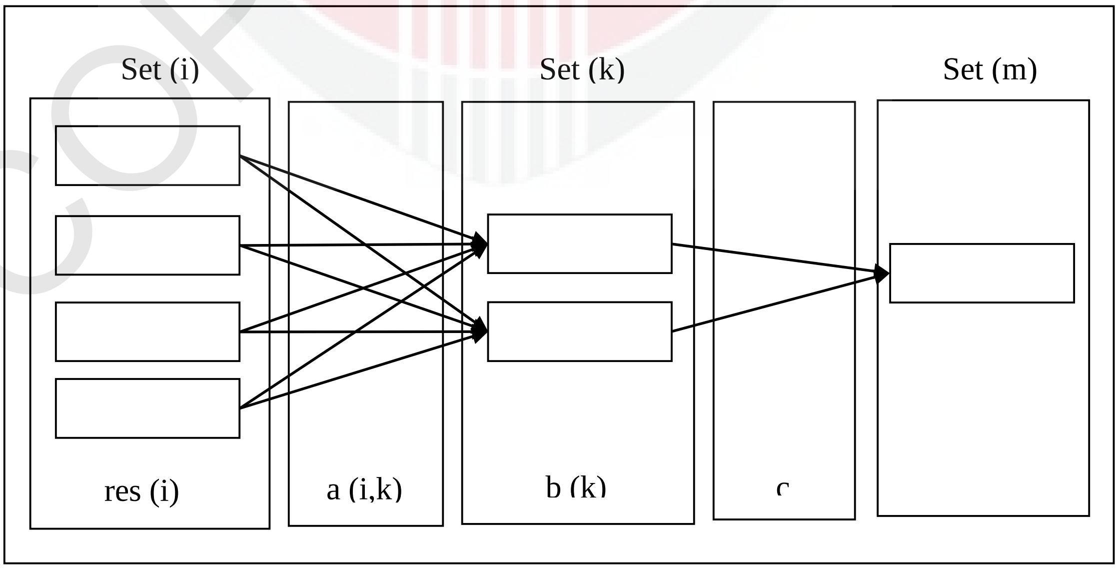

Figure 4.2 Simplified structure of biosurfactant

4.1.3 Model Formulation

During the decision-making process, several main issues must be addressed:

1) What is the profitable carbon sources can be employed by the industry?
2) What is the profit for each biosurfactant production to the industry?

4.1.4 Objective function

For this study, the objective function of model is to determine the profit that can obtained from overall biosurfactant production whereas equation 4.1 represents the variable PROFIT. The element of PRICE (k) explained the price of biosurfactant per litre and the element of COST (i) describes the price of carbon source per litre. In this equation, the profit is calculated when the total production cost of biosurfactant minus with the total cost of carbon sources used.

$$\text{PROFIT} = [\sum c(k,m) \times \text{PRICE}(k)] - [\sum a(i,k) \times \text{COST}(i)] \quad (4.1)$$

4.1.5 Material Balance Contraints

Through this section, every constraint involved in the superstructure was interpreted into mathematical equations. For example, Equation 4.2 is formed as to restrict the amount of carbon sources from being exceeded to the corresponding conversion of biosurfactant. The types of biosurfactant conversion as shown in Equation 4.3 describes total consumption of used carbon origins in synthesizing the biosurfactant. Next, Equation 4.4 is when the types of biosurfactant conversion is met by the conjunction of biosurfactant demand. For the linearization purposes, Equation 4.5 is created.

$$res\ (i) \geq \sum a(i,k) \qquad \forall\ k \qquad (4.2)$$

$$b\ (k) = \sum a(i,k) \qquad \forall\ i \qquad (4.3)$$

$$c\ (k,m) = b\ (k) \times con\ (k,m) \qquad \forall k\ \ \forall m \qquad (4.4)$$

$$c\ (k,m) \geq demand\ (k,m) \qquad \forall k\ \ \forall m \qquad (4.5)$$

4.1.6 Case study and data collection

From the biosurfactant production, the model is created to satisfy the industry's maximum gain. It is possible to obtain the high quantity of biosurfactant manufacturing from each carbon source as well as the capability to choose the suitable carbon sources is important. This is due to gain the maximum profit in synthesizing biosurfactant. In Table 4.1, the data extraction for the price of each types of carbon sources are obtained from the assumption guided by Alibaba.com (2018).

Table 4.1 The price for each available carbon sources type that been used (Alibaba.com, 2018)

Types of Carbon Sources used	Price (RM/litre)
Soapstock	1.70
Molasses	16.59
Glucose	16.59
Sunflower oil	11.10

Table 4.2 The production amount by each type of biosurfactant

Types of Biosurfactant	Production amount (g/L)	Reference
Rhamnolipid	16	Benincasa et al (2004)
Sophorolipid	39	Nagarajan & Narayana (2010)

For Table 4.2, Benincase et al (2004) recorded that rhamnolipid can produced up to 16 g/L which is lower than production amount of sophorolipids. Because of low output production, this leads to the high price of rhamnolipid in the industry as shown in Table 4.3. Each natural surfactant type has different price from each other based on their applications in the industry. For example, the price for for sophorolipid is RM 83.00 per litre which is cheaper than rhamnolipid's price. The lower price of microbial surfactant, the higher demand in the market. As described in Table 4.4, the demand of sophorolipid can be achieved until 17 816 009 litre whereas quite higher compared to the rhamnolipid's demand.

Table 4.3 The price for each biosurfactant type that been produced (Alibaba.com, 2018)

Types of Biosurfactant	**Price (RM/litre)**
Rhamnolipid	4150
Sophorolipid	83

Table 4.4 The biosurfactant demand in industry

Types of Biosurfactant	**Demand (L)**	**Reference**
Rhamnolipid	47085	Transparency Market Research (2018)
Sophorolipid	17 816 009	Transparency Market Research (2017)

4.1.7 Overall Model Performances

The LP model was coded in GAMS and the model is solved using CPLEX 12.6.3.0 solver which uses the branch and cut algorithm to maximize the general profit. The findings of this model were defined as in Table 4.5 for each type of biosurfactant generated.

Based on Table 4.5, 1 000 000 litres of soapstock is divided into 2 parts which are 543 180 litres (for sophorolipid production) and 456 820 litres (for rhamnolipid production). Compared to molasses, glucose and sunflower, all of them provided 1000000 litres from each sources into rhamnolipid synthesization and not for sophorolipid. From this results, soapstock can be concluded as the optimum carbon origin in making any type of biosurfactants.

Other than that, Table 4.5 also shows that rhamnolipid is the most effective biosurfactant since it can be produced by using various sort of carbon sources. In addition, the production volume of rhamnolipid is higher than sophorolipid.

Table 4.5 The result output

Type of carbon sources used	Amount of volume used (L)	Type of produced biosurfactant	Production volume (L)
Soapstock	543 180	Sophorolipid	17 816 000
	456 820	Rhamnolipid	65 591 000
Molasses	1 000 000		
Glucose			
Sunflower oil			

4.2 DISCUSSION

The model mainly shows that the biosurfactant conversion of rhamnolipid produced more high volume compared to sophorolipid. The reason leading to this outcome is the abundant availability of carbon sources that can be used in rhamnolipid production. Besides that, the ideal carbon source also been revealed through this model. It is soapstock as this source capable to be used in either rhamnolipid or sophorolipid production. By using all the listed carbon sources, the overall profit for producing both biosurfactant types is obtained.

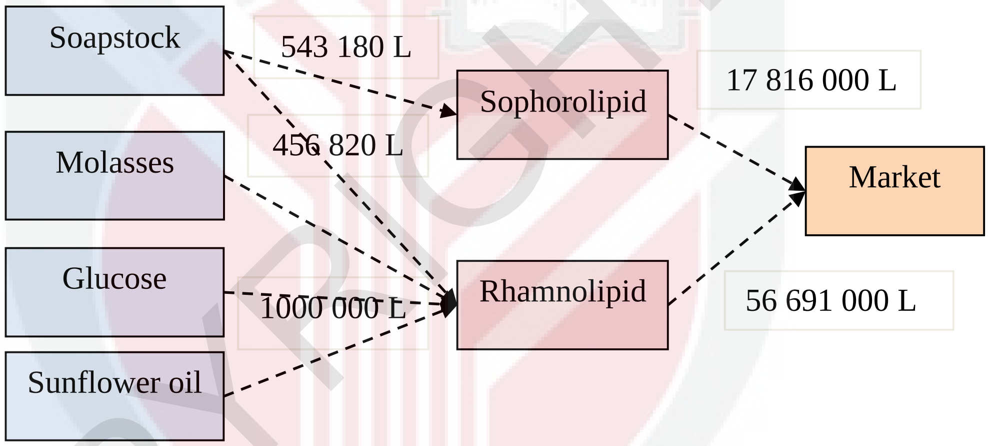

Figure 4.3 The mass balance of the optimal result

Based on Figure 4.3, the production amount of sophorolipid to the market is 17 816 000 litres which only used 543 180 litres of soapstock as carbon sources. Contrast with the rhamnolipid production, 3 456 820 litres from 4 different carbon sources such as soapstock, molasses, glucose and sunflower oil were used to produce 56 691 000 litres of rhamnolipid. The estimated amount of rhamnolipid to be produced for each type carbon source is 14 172 750 litres. Even though the low output amount of rhamnolipid for each carbon source, the best type biosurfactant to be synthesize in large-scale is still lead to the rhamnolipid. It is because the price of rhamnolipid is higher than sophorolipid which can contributes more profit. The high demand of sophorolipid unable to compete with rhamnolipid whereas there is huge gaps in comparison of their price.

From the mass balance, the overall profit for both biosurfactant production is RM 236 700 000 000. The production of sophorolipid gains RM 1 478 635 659 for profit as it only used one type carbon source – soapstock. However, the rhamnolipid production gains total profit of RM 235 223 292 300 with profit of RM 58 805 820 800 per each types of carbon sources used.

By estimation, the profit that gained from both type biosurfactant production using soapstock is RM 60 284 456 460 which can be concluded that soapstock is the ideal carbon source to be used for synthesizing the natural surfactant.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

To increase the yield of the biosurfactant, there are various factors that can affect the production. They are including carbon sources, temperature, nitrogen sources, pH and aeration. However, this research is more focused on the factor of carbon sources. It is because the microbes in the culture medium need carbon origin for their growth.

Through this study, 4 different types of carbon sources were selected which are soaptock, molasses, glucose and sunflower oil. Each of them represents the unrelated group of carbon sources such as carbohydrates, fatty acids, hydrocarbons and renewable resources. Refer to the developed model, the soaptock is described as potential carbon sources in producing biosurfactant. This source can be used in both rhamnolipid and sophorolipid production unlike the others. Besides that, the results showed that the biosurfactant conversion of rhamnolipid is the most profitable way as capable to use any kind of available carbon sources such as soapstock, glucose, molasses and sunflower oil.

Here, the outcomes presented in this research can give a good indication for Malaysia's economy with introduction of biosurfactant industry. By obtaining more profit from manufacturing of biosurfactants, this can increase the country's economy.

5.2 RECOMMENDATIONS

a) Another operation costs such as labour cost and technology cost should be included into the production cost. This can be integrated through the formulated models.

b) Use the real data collection from the industries. Since this biosurfactant industry is still new in Malaysia, not all data can be recorded. Therefore, a few of data were estimated based on the global biosurfactant production data. The real data can made the results more accurate compared to the estimated data.

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APPENDICES

Appendix A

Gam Input File for the Model

```
Sets

i   /Soapstocks, molasses, glucose, sunflower_oil /
k  /rhamnolipid , sophorolipid/
m /market /
;

Parameters

res (i) /Soapstocks 1000000, molasses  1000000, glucose 1000000, sunflower_oil  1000000/

price(k) / rhamnolipid 4150 , sophorolipid 83 /

cost(i) / Soapstocks 1.7, molasses  16.59, glucose 16.59, sunflower_oil  11.1 /
;

Table con (k,m)

                    market
rhamnolipid          16
sophorolipid         39
;

Table demand (k,m)

                    market
rhamnolipid         47085
sophorolipid        17816009
;

Variable

profit
;
```

```
Positive variable

a (i,k)
b (k)
c (k,m)
;


Equations

eq1
eq2
eq3
eq4
eq5
;

eq1..
Profit=e= sum ((k,m)  , c(k,m) * price (k)) - sum ((i,k) , a(i,k) * cost (i) );

eq2(i)..
res(i)=g= sum (k, a(i,k))    ;

eq3(k)..
b(k) =e= sum (i ,a(i,k))      ;

eq4(k,m)..
b(k)*con(k,m) =e= c(k,m)   ;

eq5(k,m)..
c(k,m)=g=demand(k,m)   ;

model biosurfactant /all/;
solve biosurfactant using LP maximise profit;
```

Appendix B

Gam Output File for the Model

```
---- VAR profit          -INF  2.367E+11     +INF       .

---- VAR a

                              LOWER     LEVEL     UPPER   MARGINAL

Soapstocks   .rhamnolipid       .     5.4318E+5    +INF       .
Soapstocks   .sophorolipid      .     4.5682E+5    +INF       .
molasses     .rhamnolipid       .     1.0000E+6    +INF       .
molasses     .sophorolipid      .         .        +INF      EPS
glucose      .rhamnolipid       .     1.0000E+6    +INF       .
glucose      .sophorolipid      .         .        +INF      EPS
sunflower_oil.rhamnolipid       .     1.0000E+6    +INF       .
sunflower_oil.sophorolipid      .         .        +INF      EPS

---- VAR b

                 LOWER     LEVEL     UPPER   MARGINAL

rhamnolipid        .     3.5432E+6    +INF       .
sophorolipid       .     4.5682E+5    +INF       .

---- VAR c

                        LOWER     LEVEL     UPPER   MARGINAL

rhamnolipid .market       .     5.6691E+7    +INF       .
sophorolipid.market       .     1.7816E+7    +INF       .
```