



**UNIVERSITI PUTRA MALAYSIA**

**PRODUCTION OF SOLVENT (ACETONE-BUTANOL-ETHANOL) IN  
BTACH AND CONTINUOUS FERMENTATION BY CLOSTRIDIUM  
SACCHAROBUTYLICUM DSM 13864 USING GELATINISED SAGO  
STARCH AS SUBSTRATE**

**LIEW SHIAU TSUEY.**

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**By**

**LIEW SHIAU TSUEY**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

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**May 2005**

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Study on the feasibility of using improved computer-controlled HPLC and GC systems was carried out to simplify the analysis method used in solvent fermentation. The use of HPLC system with a single injection to analyse the composition of culture broth (substrates and products) during solvent fermentation was achieved by raising the column temperature to 80°C. Although good separation of the components in the mixture was achieved, a slight peak overlapped for butyric acid and acetone was observed. However, improved GC system was developed and capable to measure the products of solvent fermentation (acetone, butanol, ethanol, acetic acid and butyric acid) within 22 min of analysis time. In order to obtain accurate quantification, GC was used to determine the products whereas HPLC was used to detect the substrates.

The effect of different sago starch concentrations on solvent fermentation by *Clostridium saccharobutylicum* DSM 13864 was studied in anaerobic condition



using 250 mL schott Duran bottle. The optimal sago starch concentration obtained was 50 g/L where the total solvent concentration, total solvent yield and total solvent productivity were 8.97 g/L, 0.20 g/g and 0.14 g/L.h, respectively. The performance of solvent fermentation was greatly improved when 2 L stirred tank fermenter was applied using 50 g/L sago starch. The fermentation time to reach the maximum total solvent concentration was shortened from 66 h to 28 h. The total solvent concentration, total solvent yield and total solvent productivity obtained was 10.89 g/L, 0.24 g/g and 0.39 g/L.h, respectively.

The total solvent production in 2 L stirred tank fermenter was significantly improved when glycerol was added to the medium. With the addition of 2 to 10 g/L glycerol to the medium, the production of total solvent was increased by about 3% to 50.4% as compared to fermentation without the addition of glycerol (10.89 g/L), respectively. Although there was a reduction in ethanol production, the production of acetone and butanol was significantly increased. Glycerol with concentration of 6 g/L was optimal for improvement of the total solvent production (16.38 g/L), total solvent yield (0.35 g/g) and total solvent productivity (0.59 g/L.h).

From the study it was found that the condition could be adjusted to suit for acids production (high dilution rate and high pH) or solvent production (low dilution rate and low pH) by manipulating the dilution rate and culture pH of single stage continuous fermentation. The highest solvent concentration in outflow (9.10 g/L) was obtained at pH 4.5 and dilution rate of  $0.05 \text{ h}^{-1}$ , which gave the overall productivity of 0.46 g/L.h. However, the highest total solvent productivity (0.85 g/L.h) was obtained at dilution rate of  $0.11 \text{ h}^{-1}$  at pH 4.5. Although the total solvent

productivity was greatly increased in continuous culture, the final solvent concentration attained in outflow was decreased by about 53% as compared to batch culture.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**PENGHASILAN PELARUT (ASETON-BUTANOL-ETANOL) SECARA  
FERMENTASI SEKELOMPOK DAN SELANJAR OLEH  
*CLOSTRIDIUM SACCHAROBUTYLICUM* DSM 13864 DENGAN  
MENGUNAKAN KANJI SAGU TERGELATIN  
SEBAGAI SUBSTRAT**

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Kajian feasibiliti berasaskan kemajuan Kromatografi Cecair Prestasi Tinggi (HPLC) kawalan berkomputer dan sistem Kromatografi Gas (GC) telah dijalankan untuk memudahkan kaedah analisis pelarut dalam proses fermentasi. Dalam kajian Kromatografi Gas, suntikan tunggal untuk menganalisis kesemua komponen (substrat dan produk) telah dicapai dengan meningkatkan suhu kolum kepada 80°C. Walau bagaimanapun, puncak pertindihan telah didapati bagi asid butirik dan aseton. Kaedah analisis bagi GC telah berjaya dimajukan dan berupaya untuk menganalisis produk fermentasi iaitu aseton, butanol, etanol, asid asetik dan asid butirik dalam masa 22 minit. Untuk memperolehi analisis yang tepat, produk fermentasi akan dianalisis menggunakan GC manakala substrat akan ditentukan dengan HPLC.

Kesan pelbagai kepekatan sagu kanji terhadap fermentasi pelarut menggunakan *Clostridium saccharobutylicum* DSM 13864 telah dikaji dalam botol schott Duran

berisipadu 250 mL di bawah keadaan anaerobik. Kepekatan sagu kanji yang optimum adalah 50 g/L di mana jumlah kepekatan pelarut, angkali hasil pelarut berasaskan sumber karbon digunakan, dan jumlah produktiviti pelarut adalah 8.97 g/L, 0.20 g/g dan 0.14 g/L.j masing-masing. Prestasi fermentasi pelarut dalam 50 g/L sagu kanji telah ditingkatkan apabila fermenter tangki pengaduk yang berisipadu 2 L digunakan. Masa fermentasi untuk mencapai kepekatan pelarut tertinggi telah dikurangkan dari 66 j kepada 28 j. Nilai untuk kepekatan pelarut, angkali hasil pelarut berasaskan sumber karbon digunakan dan jumlah produktiviti pelarut adalah 10.89 g/L, 0.24 g/g dan 0.39 g/L.j masing-masing.

Penambahan gliserol ke dalam medium telah meningkatkan penghasilan pelarut di dalam fermenter tangki pengaduk berisipadu 2 L. Penambahan sebanyak 2 g/L-10 g/L gliserol telah meningkatkan 3% -50.4% kepekatan pelarut berbanding dengan fermentasi tanpa penambahan gliserol (10.89 g/L). Walaupun terdapat pengurangan dari segi penghasilan etanol, tetapi peningkatan penghasilan aseton dan butanol adalah signifikan. Kepekatan 6 g/L gliserol merupakan kepekatan optimum dalam peningkatan nilai kepekatan pelarut (16.38 g/L), angkali hasil pelarut berasaskan sumber karbon digunakan (0.35 g/g) dan jumlah produktiviti pelarut (0.59 g/L.j).

Dalam kajian kultur selanjar, didapati keadaan fermentasi boleh diubah kepada penghasilan asid (kadar pencairan tinggi dan pH tinggi) ataupun penghasilan pelarut (kadar pencairan rendah dan pH rendah) dengan memanipulasi kadar pencairan dan pH. Kepekatan pelarut tertinggi pada aliran keluar (9.10 g/L) dengan jumlah produktiviti pelarut sebanyak 0.46 g/L.j telah dicapai pada pH 4.5 dan kadar pencairan 0.05 j<sup>-1</sup>. Nilai jumlah produktiviti pelarut tertinggi (0.85 g/L.j) telah

dicapai pada kadar pencairan sebanyak  $0.11 \text{ j}^{-1}$  dan pH 4.5. Walaupun jumlah produktiviti pelarut telah ditingkatkan secara drastik dalam kultur selanjar tetapi kepekatan pelarut yang dicapai pada aliran keluar adalah 53% lebih rendah berbanding dengan kultur sekelompok.



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## TABLE OF CONTENTS

		<b>Page</b>
<b>ABSTRACT</b>		ii
<b>ABSTRAK</b>		v
<b>ACKNOWLEDGEMENTS</b>		viii
<b>APPROVAL</b>		ix
<b>DECLARATION</b>		xi
<b>LIST OF TABLES</b>		xv
<b>LIST OF FIGURES</b>		xvii
<b>LIST OF ABBREVIATIONS</b>		xix
<b>CHAPTER</b>		
<b>1</b>	<b>INTRODUCTION</b>	<b>1</b>
<b>2</b>	<b>LITERATURE REVIEW</b>	<b>5</b>
	2.1 Application of Acetone, Butanol and Ethanol (ABE)	5
	2.2 <i>Clostridium</i> Species in Solvent Production	6
	2.2.1 <i>Clostridium saccharobutylicum</i> DSM 13864	8
	2.3 Biochemistry of ABE Fermentation	9
	2.3.1 Acid-Producing Pathways	11
	2.3.2 Solvent-Producing Pathways	13
	2.4 Starch as a Substrate for ABE Fermentation	13
	2.4.1 Pre-treatment of Starch	16
	2.4.2 Degradation of Starch	16
	2.5 Amylolytic Enzymes	17
	2.5.1 $\alpha$ -amylase	19
	2.5.2 Glucoamylase	19
	2.6 Medium Composition on ABE Fermentation	20
	2.6.1 Carbon Source	20
	2.6.2 Nitrogen Source	24
	2.6.3 The Effects of Other Nutrient	25
	2.7 The Potential of the Addition of Glycerol in Sago Starch	28
	2.8 Culture Conditions on ABE Fermentation	28
	2.8.1 Oxygen	29
	2.8.2 Temperature and pH	30
	2.9 Fermentation Techniques	31
	2.9.1 Batch Fermentation	31
	2.9.2 Continuous Fermentation	34
	2.10 Concluding Remarks	37
<b>3</b>	<b>GENERAL MATERIALS AND METHOD</b>	<b>39</b>
	3.1 Microorganism and Strain Maintenance	39
	3.2 Inoculum Preparation	39
	3.3 Medium Composition	40
	3.4 Preparation of Strict Anaerobic Medium in Air Tight Bottle	41

3.5 Experimental Design	42
3.6 Fermenter	45
3.7 Analytical Procedures	48
3.7.1 Measurement of Dry Cell Weight	48
3.7.2 Determination of Solvent and Organic Acids	49
3.7.3 Determination of Sugars and Glycerol	49
3.7.4 Determination of Starch	50
3.7.5 Extracellular Enzyme Assay	51
<b>4 IMPROVED SOLVENT (ACETONE-BUTANOL-ETHANOL) ANALYSIS USING GAS CHROMATOGRAPHY AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY</b>	<b>53</b>
4.1 Introduction	53
4.2 Materials and Methods	54
4.2.1 GC and HPLC Analysis	54
4.2.2 Standards Preparation	55
4.3 Results and Discussion	55
4.3.1 Determination of Substrates, Solvent and Organic acids Using HPLC	55
4.3.2 Determination of Solvent Fermentation Products Using GC	59
4.4 Conclusions	61
<b>5 DIRECT FERMENTATION OF GELATINISED SAGO STARCH TO SOLVENT (ACETONE-BUTANOL-ETHANOL) BY <i>C. saccharobutylicum</i> DSM 13864 IN BATCH CULTURE</b>	<b>62</b>
5.1 Introduction	62
5.2 Materials and Methods	64
5.2.1 Microorganism and Medium	64
5.2.2 Fermentation in 250 mL Schott Duran Bottle	64
5.2.3 Fermentation in 2 L Stirred Tank Fermenter	64
5.2.4 Analytical Procedure	65
5.2.5 Determination of Rheological Properties	65
5.2.6 Statistical Procedure	66
5.3 Results and Discussion	66
5.3.1 Rheological Characteristic of Different Sago Starch Concentrations	66
5.3.2 Growth Characteristics of <i>C. saccharobutylicum</i> DSM 13864	71
5.3.3 Effects of Different Sago Starch Concentrations	75
5.3.4 Comparison of Fermentation Performance Using Schott Duran Bottle and 2 L Stirred Tank Fermenter	78
5.3.5 Effects of the Addition of Different Glycerol Concentrations	82
5.4 Conclusions	88
<b>6 ANAEROBIC FERMENTATION OF SOLVENT BY <i>C. saccharobutylicum</i> DSM 13864 USING GELATINISED SAGO STARCH IN A SINGLE STAGE CONTINUOUS CULTURE</b>	<b>90</b>
6.1 Introduction	90

6.1.1 Theory of A Single Stage Continuous Culture	91
6.2 Materials and Methods	96
6.2.1 Microorganism and Medium	96
6.2.2 Fermentation Condition	96
6.2.3 Analytical Procedure	99
6.2.4 Statistical Procedure	99
6.3 Results and Discussion	99
6.3.1 Effects of Different Dilution Rates	99
6.3.2 Effects of Different Culture pHs	104
6.3.3 Estimation of $\mu_{max}$ and $K_s$	107
6.3.4 Comparison Between Batch and Continuous Culture	109
6.4 Conclusions	111
<b>7 GENERAL DISCUSSION, CONCLUSIONS AND SUGGESTIONS FOR FUTHER WORK</b>	<b>112</b>
7.1 General Discussion	112
7.2 Conclusions	117
7.3 Suggestions for Further Work	118
<b>REFERENCES</b>	<b>119</b>
<b>APPENDICES</b>	<b>128</b>
<b>BIODATA OF THE AUTHOR</b>	<b>144</b>

## LIST OF TABLES

<b>Table</b>	<b>Page</b>
2.1 Industrial solvent-producing Clostridia	8
2.2 Typical amylose and amylopectin content of starches	15
2.3 Raw materials used in solvent fermentation	23
2.4 Influence of the concentration of compounds on the growth and solvent production on <i>C acetobutylicum</i> ATCC 824	27
2.5 pH and temperature used in previous solvent fermentation	31
2.6 The performance of batch solvents fermentation using different types of strain and substrate	33
2.7 Comparison of kinetic parameters for different strains of <i>Clostridium</i> in single stage continuous culture	36
3.1 Composition of medium for solvent fermentation by <i>C. saccharobutylicum</i> DSM 13864	41
3.2 Dimension and variables for stirred tank fermenter used in this study	45
4.1 Retention time achieved for substrates and products using HPLC in different columns temperature, flow rate 0.8 ml/min and 3 mM H <sub>2</sub> SO <sub>4</sub>	58
4.2 Retention times in minutes obtained for the solvent fermentation products that analysed using GC	59
5.1 Rheological properties of sago starch concentrations	67
5.2 Density, Reynolds number and mixing time calculated for different sago starch concentrations	71
5.3 The performance of direct fermentation of different sago starch concentrations to solvent by <i>C. saccharobutylicum</i> DSM 13864	77
5.4 Comparison of kinetic performance of direct fermentation of sago starch to solvent by <i>C. saccharobutylicum</i> DSM 13864 during batch culture using Schott Duran bottle and 2 L stirred tank fermenter	79
5.5 The performance of direct fermentation of different glycerol concentrations to solvent by <i>C. saccharobutylicum</i> DSM 13864	83
6.1 Steady state of kinetic solvents fermentation by <i>C. saccharobutylicum</i> DSM 13864 using gelatinised sago starch at different dilution rates in	

single stage continuous culture at pH 4.5	103
6.2 Steady state of kinetic solvent fermentation by <i>C. saccharobutylicum</i> DSM 13864 using gelatinised sago starch at different pH in single stage continuous culture at dilution rate $0.11 \text{ h}^{-1}$	106
6.3 Experimental data for single stage continuous culture in solvent production operated at different dilution rates	107
6.4 Comparison between batch culture and continuous culture	111
7.1 The performance of batch solvents fermentation using different types of strain and substrate	115
7.2 Comparison of kinetic parameters for different strains of <i>Clostridium</i> in single stage continuous culture	116

## LIST OF FIGURES

<b>Figure</b>	<b>Page</b>
2.1 A typical time course of batch solvent fermentation by <i>C. acetobutylicum</i> ATCC 824 using 50 g/L glucose	10
2.2 Metabolic pathways and enzymes involved in the conversion of starch to acids and solvent	12
2.3 Amylose structure	14
2.4 Amylopectin structure	15
2.5 Starch hydrolysis amylolytic enzymes such as $\alpha$ -amylase, glucoamylase and pullulanase	17
3.1 The apparatus used for the preparation of strict anaerobic medium	42
3.2 Experimental design of solvent fermentation by <i>C. saccharobutylicum</i> DSM 13864 using sago starch as a carbon source	44
3.3 Schematic diagram of a 2 L stirred tank fermenter	46
3.4 A photograph of 2 L stirred tank fermenter	47
4.1 HPLC chromatograms of a mixture of standards at flow rate 0.8 mL/min and mobile phase 3mM H <sub>2</sub> SO <sub>4</sub>	56
4.2 GC chromatogram	61
5.1 The time course of solvent production using 50 g/L sago starch in 250 mL Schott Duran bottle	73
5.2 Three phases that involved in direct fermentation of sago starch to solvent by <i>C. saccharobutylicum</i> DSM 13864	74
5.3 The time course of solvent production using 50 g/L sago starch in 2 L stirred tank fermenter	81
5.4 The time course of solvent production using 50 g/L sago starch and 6 g/L glycerol in 2 L stirred tank fermenter	87
6.1 Schematic diagram of a single stage continuous for solvent production by <i>C. saccharobutylicum</i> DSM 13864 using sago starch as a carbon source	91

6.2	A photograph of the bioreactor system and its accessories used for a single stage continuous culture	98
6.3	Effect of dilution rate on the performance of continuous solvent fermentation by <i>C. saccharobutylicum</i> DSM 13864 using sago starch as carbon source	102
6.4	Lineweaver-Burk plot for determination of $\mu_m$ and $K_s$	108
6.5	The fitness of the continuous models to the experimental data	109



**LIST OF ABBREVIATIONS**

ABE	acetone-butanol-ethanol
rpm	rotation per minute
$\mu_{\max}$	maximum specific growth rate ( $\text{h}^{-1}$ )
$X_m$	maximum cell concentration (g/L)
$P_m$	maximum total solvent concentration (g/L)
$Y_{p/x}$	total solvent yield (g/g)
$P_r$	total solvent productivity (g/L.h)

# CHAPTER 1

## INTRODUCTION

The anaerobic fermentation of carbohydrates to solvent (acetone-butanol-ethanol/ ABE) by *Clostridium* species has been well documented and commercially applied for several decades World War I and II. However, with the advent of cheaper petrochemical-based production of solvent, the production through fermentation process becomes economically unattractive and unfavorable during 1960s and, as a result, almost all the industry-scale fermentation facilities have been closed (Jones and Woods, 1986; Jones, 2001).

The quantities of acetone, butanol and ethanol produced worldwide are extremely large and almost all production is via petrochemical synthesis. As a result, the price of these products are to a large extent dependent on the price of crude oil and prices can fluctuate considerably on the open market from year to year (Jones, 2001). In addition, known crude oil reserves could be depleted in less than 50 years at the present rate of consumption (Crabbe *et al.*, 2001). According to Petronas president and chief executive officer Tan Sri Mohamad Hassan Marican, Malaysia's oil reserves were expected to last another 18 years (Lee and Kamiso, 2004). Thus, this situation represents an opportunity for the fermentation-derived process technology based on an alternative feedstock whose supply is not limited, i.e., renewable resources (biomass).

The acetone, butanol and ethanol have many commercial applications in various industries. These solvents have been used as a chemical feedstock and liquid fuels

since 1940s. During World War I, the fermentation was first aimed at the production of acetone for the manufacture of munitions by the British army and later at the production of butanol for the manufacture of lacquer in automobile industry (Jones and Woods, 1986). ABE fermentation process has remained of interest due to its potential application in biotechnology and, thus attempting to improve the production is still being intensively studied worldwide (Ishizaki *et al.*, 1999; Jones, 2001; Ezeji *et al.*, 2004).

In recent years, considerable work has been conducted towards the improvement of the traditional batch fermentation process and the development of some novel fermentation technologies. One of the problems hindering commercial development of ABE fermentation is the fact that it suffers severely from product inhibition caused principally by butanol. Therefore, various type of fermentation mode integrated with product removal system such as pervaporation, adsorption, liquid-liquid extraction, perstraction and gas stripping has been reported (Qureshi *et al.*, 2001). Besides, the immobilization of *Clostridium* strain using different types of immobilization support as an approach to enhance solvent production has also been well established. Cell recycling using ultrafiltration was also demonstrated as a successful method for retaining biomass and increasing productivity in solvent fermentation (Jones, 2001). Another possible approaches to improve solvent production is the development of strain that manipulated at the genetic level (Dürre, 1998).

In order to reintroduce an economically competitive biological process, the major drawbacks that must be overcome first, is the high cost of the substrate. About 60%

of the overall production cost is the cost of substrate (Jones and Woods, 1986). Since solventogenic *Clostridia* are able to utilise a wide range of carbohydrate substrates, considerable research into the use of substrates cheaper than molasses (the traditional substrate for ABE production) such as potato (Linden *et al.*, 1985), corn, maize (McNeil and Kristiansen, 1986), jerusalem artichoke (Marchal *et al.*, 1985), whey permeate (Ennis and Maddox, 1985), apple pomace (Voget *et al.*, 1985), peat (Forsberg *et al.*, 1986), potato waste (Grobben *et al.*, 1993), palm oil mill effluent (Lee *et al.*, 1995) and domestic organic waste (López-Contreras *et al.*, 2000) have been reported.

Malaysia has a lot of potential substrate that has not been exploited for its usage as substrate for fermentation for example sago starch. Recently, some works on direct fermentation of sago starch to solvent by *C. saccharobutylicum* (formerly known as *C. acetobutylicum*) P262 using batch culture and the ability of this bacterium to secrete amylolytic enzymes have been reported by Madihah *et al.* (2001a and b), however, the performance of the process is still not acceptable for industrial application. Thus, further improvement of the process either through microbiology or engineering approach is required.

Higher level of intracellular ATP and NADH obtained in *C. butyricum* that grown on glycerol-glucose mixture was reported (Saint-Amans *et al.*, 2001). The increased level of ATP and NADH are associated with increased solvent production in *Clostridium acetobutylicum* (Meyer and Papoutsakis, 1989). Therefore, the feasibility of adding glycerol into the medium might be employed in order to

improve the performance of direct fermentation of sago starch to solvent using *C. saccharobutylicum* DSM 13864.

The use of fermentation technique such as continuous culture for the improvement of solvent fermentation has been well established. However, the performance of direct fermentation of sago starch to solvent using continuous culture has not been reported elsewhere. In general, most of the reports on continuous solvent fermentation focused on the use of glucose as a carbon source (Bahl *et al.*, 1982a; Monot and Engasser, 1983a; Afschar *et al.*, 1985; Fick *et al.*, 1985; Soni *et al.*, 1987a; Mollah and Stuckey, 1992).

The objectives of this study are,

- i. To improve and simplify the method based on gas chromatography and high performance liquid chromatography for rapid quantification of substrates, intermediate acids and solvent produced during ABE fermentation using sago starch as a substrate.
- ii. To study the effect of different sago starch concentrations on the performance of ABE fermentation using *C. saccharobutylicum* DSM 13864.
- iii. To investigate the effect of the addition of glycerol into culture on the production of ABE by *C. saccharobutylicum* DSM 13864 using sago starch as a substrate.
- iv. To investigate the effect of dilution rate and culture pH on ABE production in a single stage continuous fermentation of *C. saccharobutylicum* DSM 13864.

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Application of Acetone, Butanol and Ethanol (ABE)

The solvent (ABE) has a great commercial value and has been used in various industries such as for fuel, reagents, feedstock and antibiotics (Badr *et al.*, 2001). During World War I acetone was widely used in aircraft wing dopes as a fuel. Besides, smokeless powder produced from acetone has been used by British Army as the ingredient in munitions manufacture (Jones and Woods, 1986). Acetone is a good solvent and important organic raw material, mainly used to produce plexyglass, phenolics, acetic acid fiber, epoxy in chemical application field and to produce antibiotic, hormone and vitamin in pharmaceutical industry (Beijing Yanshan Petrochemical, 2004).

Butanol is used primarily in the manufacture of lacquers, rayon, detergents, brake fluid and amine additive. In addition, butanol has also been applied to chemical industry as a general solvent for fats, waxes, resins, shellac and varnish (Linden *et al.* 1985). Butanol has many characteristics, which make it a better fuel extender and now is used in the formulation of gasohol (Mollah and Stuckey, 1993) and gasoline additive (Park *et al.*, 1989). Butanol has potential to be used as a cosurfactant and enhance the release of oil from the underground water (Krouwel *et al.*, 1982).