DEVELOPMENT OF BACTERIAL CULTURE FOR ODOUR CONTROL

CHIN SIAT PEI

Universiti Sains Malaysia

August 2011

DEVELOPMENT OF BACTERIAL CULTURE FOR ODOUR CONTROL

by

CHIN SIAT PEI

Thesis submitted in fulfilment of the

requirements for the degree of

Master of Science

August 2011

ACKNOWLEDGEMENT

This project would not have been possible without the assistance of many people. First and foremost, I would like to express my deep gratitude to my supervisors, Dr. Ahmad Ramli bin Mohd Yahya and Associate Professor Dr. Amirul Al-Ashraf Balakrishnan bin Abdullah for their comments, criticism and constant support throughout this project.

Special thanks to Mr. Segaran, Puan Hamizah, Puan Nurul and Puan Asma (School of Chemical Sciences) for their precious guidance and assistance during laboratory work. Besides that, I would like to express my appreciation to all my laboratory mates and friends for their support and invaluable advices during my 2-3 years of master study. In addition, help from Dr. H'ng Tiang Chuan and Mr. Teong Tiek Wah in assisting my thesis writing is much appreciated.

Last but not least, I would like to acknowledge the endless support from my family members, who gave me the strength and motivation to complete this study.

Thank you.

CHIN SIAT PEI

October 2010

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

DEVELOPMENT OF BACTERIAL CULTURE FOR ODOUR CONTROL

ABSTRACT

Volatile fatty acids particularly butyric acid is known as the major component of odour, associated with livestock farming, agricultural activities and wastewater treatment system. Thirteen butyric acid-degrading bacteria were isolated from the rivers and cow farm in Malaysia. Screening with specific growth rates and butyric acid degradation rates had selected four strains of highest values. These four bacteria were identified as *Cupriavidus* sp. strain B3, *Burkholderia* sp. strain C2, *Acinetobacter* sp. strain C4 and *Cupriavidus* sp. strain C5. Among the four, *Acinetobacter* sp. strain C4 showed the highest values of specific growth rate at (0.321±0.002) h^{-1}, and degradation rate at 54 ppm butyric acid/h. Further studies revealed that *Acinetobacter* sp. strain C4 was able to perform butyric acid degradation at pH 6-9 and temperature of 25-40ºC. Additionally, this bacterium was found degrading other volatile fatty acids such as acetic acid, propionic acid, valeric acid and caproic acid, which were reported to deplete air quality extensively. In the cells production course, cultivation medium was formulated with 15.50 g/L yeast extract. *Acinetobacter* sp. strain C4 was cultivated through exponential feeding at 0.086 h^{-1} specific growth rate, and the cells concentration achieved was (8.729±0.352) g/L at 12 h of fed. A significant decline in growth thereafter was presumed to be correlated with the generation of inhibitory metabolites. Hence, continuous culture at different dilution rates (0.086, 0.20, 0.26, 0.35, 0.43 h^{-1}) were implemented to eliminate substrate limitation and metabolites inhibition. The highest cells concentration, (5.206±0.050) g/L was obtained at 0.086 h^{-1}, while the maximum cells productivity, (0.926±0.034) g/L/h was achieved at 0.20 h^{-1}. The fermentation studies showed that continuous culture demonstrated the feasibility to achieve high cells productivity. The produced cells were then immobilized in polyvinyl alcohol and incorporated in the

biofilter development. The biofilter was constructed with a glass column of 24×2 cm (height × diameter) and packed with 24 g polyvinyl alcohol cubes containing 0.24 g dry cells *Acinetobacter* sp. strain C4. Under aerobic condition, the propionic acid (452±2 ppm), butyric acid (1371±4 ppm) and valeric acid (1399±4 ppm) in cow farm wastewater were removed at (63.4±2.2)%, (66.2±0.6)% and (61.2±1.1)%, respectively at 96 h.

PERKEMBANGAN KULTUR BAKTERIA UNTUK KAWALAM BAU

ABSTRAK

Asid lemak mudah meruap terutamanya asid butirik dikenali sebagai komponen utama bau, berkaitan dengan penternakan, aktiviti pertanian dan sistem pemprosesan air kumbahan. Tiga belas bakteria mengurai asid butirik dipencil dari sungai dan kawasan penternakan lembu di Malaysia. Saringan dengan kadar pertumbuhan spesifik dan kadar mengurai asid butirik menentukan empat bakteria dengan nilai tertinggi. Keempat-empat bakteria dikenalpasti sebagai *Cupriavidus* sp. strain B3, *Burkholderia* sp. strain C2, *Acinetobacter* sp. strain C4 dan *Cupriavidus* sp. strain C5. Di antara empat bakteria tersebut, *Acinetobacter* sp. strain C4 menunjukkan nilai tertinggi dalam kadar pertumbuhan spesifik (0.321±0.002) jam^{-1}, dan kadar degradasi 54 bpj asid butirik/jam. Kajian lanjutan mendapati *Acinetobacter* sp. strain C4 mampu melakukan penguraian asid butirik pada pH 6-9 dan suhu 25-40ºC. Selain itu, bakteria ini ditemui turut menguraikan asid lemak lain seperti asid asetik, asid propionik, asid valerik dan asid caproik, yang dilaporkan menjejaskan kualiti udara. Dalam penghasilan sel bakteria, media pengkulturan telah dirumuskan dengan 15.50 g/L ekstrak yis. *Acinetobacter* sp. strain C4 dikulturkan melalui suapan eksponen pada kadar 0.086 jam^{-1}, dan kepekatan sel pada 12 jam adalah (8.729±0.352) g/L. Penurunan ketara dalam pertumbuhan selanjutnya diduga berkorelasi dengan kehadiran perencatan metabolit. Dengan itu, pengkulturan berterusan pada kadar pencairan berbeza (0.086, 0.20, 0.26, 0.35, 0.43 jam^{-1}) dilaksanakan untuk menghilangkan substrat penghad dan perencatan metabolit. Kepekatan sel tertinggi (5.206±0.050) g/L diperolehi pada 0.086 jam^{-1}, manakala produktiviti sel maksimum (0.926±0.034) g/L/jam dicapai pada 0.20 jam^{-1}. Keputusan fermentasi menunjukkan bahawa pengkulturan berterusan boleh digunakan untuk mencapai produktiviti sel yang tinggi. Sel yang dihasilkan kemudian disekatgerakkan

dalam alkohol polivinil dan dimasukkan dalam pembangunan biopenuras. Biopenuras dibina dengan turus gelas 24×2 sm (panjang × diameter) dan diisi dengan 24 g ketulan alkohol polivinil yang mengandungi 0.24 g sel kering *Acinetobacter* sp. strain C4. Pada keadaan aerobik, asid propionik (452±2 bpj), asid butirik (1.371±4 bpj) dan asid valerik (1.399±4 bpj) daripada air kumbahan ladang lembu masing-masing diuraikan sebanyak (63.4±2.2)%, (66.2±0.6) % dan (61.2±1.1)% pada 96 jam.

1.0 INTRODUCTION

1.1 Research background

Within the past four decades, the medical and scientific communities have begun to comprehend the lethal chronic effects of environmental pollution resulting from rapid industrialization and urbanization. The environmental problems such as industrial emissions, poor sanitation, inadequate waste management, contaminated water supplies and exposures to indoor air pollution are affecting every individual in the communities (Briggs, 2003). The drive for further development with a substantial population increment will enhance these environmental problems (McMichael *et al*., 1998). These environmental pollutions play a significant role in a number of health outcomes, and in several cases these add up to a serious public health concern (Briggs, 2003). However, among all the environmental issues, the emission of odorous compounds has become one of the biggest nuisances for human. The negative impacts of odour emission on human are serious and thus require more attention.

Odorous gases are released from various human activities such as livestock and agriculture, waste treatment system, municipal and industries (Both, 2001). Most of the odorous substances are categorized into classes such as volatile fatty acids, phenols, nitrogen derivatives and sulphur compounds, in which these components are highly irritants and odorous (Hobbs *et al*., 1995). Among these substances, volatile fatty acids such as propionic acid, butyric acid and valeric acid are recognized as the main components of odours associated with livestock farming and wastewater from human activities (Hamano *et al*., 1972). Zahn and co workers (1997) reported that the volatile fatty acids with carbon numbers from two to nine show the greatest potential for air deterioration, as these acids exhibited the highest transport coefficients and highest airborne concentrations. Hence, the emission of volatile fatty acids to the atmosphere was

restricted, particularly by the Ministry of Environmental Management in Japan (Kono, 1993).

Studies by some research team reported that butyric acid is the main volatile fatty acids generating from animal industries, agricultural operations and waste treatment system (Hartung, 1987; Fang *et al*., 1995; Spinhirne and Koziel, 2003). Butyric acid having a low odour threshold value of 0.4 – 3.6 μg [butyric acid] m^{-3} [air] contributes significantly to the odour threshold concentration and odour intensity (O'Neill and Philips, 1992; Tamminga, 1992; Sheridan *et al*., 2003). The low odour threshold indicates that butyric acid is a highly odour nuisance, which is detectable at very low concentrations. The offensiveness of butyric acid is reported to be at least 30 and 35 times more compared to hydrogen sulphide and propionic acid, respectively, in accordance with Japanese offensive odour control laws (Tanaka, 2000). The emission of butyric acid does not only degrade air quality and depreciate property values, but also affect public comfort and health. Long term exposure to this compound can trigger irritation, nausea, headache, loss of appetite and emotional disorder.

In order to reduce the odour emissions to the atmosphere, conventional techniques used including incineration, chemical oxidation and condensation (Walsh, 1967; Fleming, 1973; Snow, 1975; Rolfe, 1980). These techniques are proven to be effective and reliable in treating air contaminants. However, these techniques are of high capital and operating costs, high maintenance fees, no mineralization and often generate secondary waste streams at the end of treatments, requiring further treatment upon releasing to the environment (Devinny *et al*., 1999; Schlegelmilch *et al*., 2005; Sercu *et al*., 2006). Therefore, biological method offers an alternative technique for odour control at comparable low of investment and operating costs without producing secondary wastes (Mohseni and Allen, 2000; Nevin and Barford, 2000; Rappert and Muller, 2005).

Biofiltration is the most common biological odour treatment process (Luo and Lindsey, 2006), and it is a proven technology for odour reduction from industrial and commercial sources (Nicolai and Janni, 2000). Most of the research in biofiltration has been carried out in other parts of the world such as Australia, Ireland and Poland (Otten *et al*., 2002; Sheridan *et al*., 2002; Tymczyna *et al*., 2007), but very few, if any, has been conducted in Malaysia. Considering the fact that cost effectiveness is a determining factor for application in the local industries and environment, such low intensity has created the opportunity for research on biofilters in reducing volatile fatty acids to be explored locally. Therefore, the opportunity to explore cell immobilization using strains extracted from the environment in Malaysia and to further improve the immobilization techniques on biofiltration technologies is still growing. Furthermore, it is also worthwhile to mention that different biological properties of support matrices such as calcium alginate and PVA provide an exciting area for investigation for the cell immobilization.

1.2 Objectives

The objectives of this research are:

(i) Isolation and screening for potential butyric acid-degrading bacteria from the Malaysia environment

(ii) Identification and characterization of the preferred isolated degrading bacteria (e.g. biochemical tests, microscopic tests, degradation characteristics at different pH and temperature)

(iii) Scouting of suitable growth conditions for production of butyric acid-degrading bacteria

(iv) Cells production of butyric acid-degrading bacteria through shake flasks and stirred tank reactor cultivations

(v) Development of the biofiltration system with immobilized cells for wastewater treatments

2.0 LITERATURE REVIEW

2.1 Odorous gases

Air quality status depends mainly on the gaseous emission from municipal, industrial and agricultural activities, such as composting plants, waste treatment system, livestock farming, food processing and chemical production (Both, 2001). Waste gas is one of the main emission pathways of these industries and production processes containing toxic, in particular odour forming off-gases of high noxiousness. The emissions of waste gases are associated with a wide variety of odorous compounds including sulphur-containing compounds, nitrogen derivatives (such as ammonia, amines), volatile fatty acids, phenols, alcohols, aldehydes and ketones (Curtis, 1993; Ramel and Nomine, 2000; Mahin, 2001). Many of these compounds have low odour thresholds, thus these compounds are perceived as odour nuisances even when their concentration in the air is very low (Persaud *et al*., 1996; Sunesson *et al*., 2001).

Odours are usually a complex mixture of up to several hundred single odorous compounds that vary widely between diverse industries and facilities (Schlegelmilch *et al*., 2005). The emission of these odorous gases has become a universal environmental problem, which has attracted national and international concern (Frechen, 2001). Odour emission causes serious annoyance in the neighborhood of the sources as the malodorous chemicals are affecting health and comfort of personal in the communities (Leuch *et al*., 2003). As a result, complaints related to odours have increased around the world.

There are a number of reasons for the increase in complaints including: a) the increase in the size and number of industries and processing facilities, b) the increase in residential development near industrial areas, and c) the increase in sensitivity and demand of the general public for a clean and pleasant environment (Both, 2001; Mahin, 2001). For instance, the Department of Environment (DOE) under the Ministry of Natural Resources and Environment, Malaysia received more than 1000 cases of

complaints related to odour in 2006, as shown in Table 2.1 (Othman *et al*., 2008). From the analysis, the government has predicted that the ongoing trend involving odor problem is becoming more and more acute each year.

Table 2.1 Complaints regarding odorous emissions in 2006 (Othman *et al*., 2008)

No	Classification	Total
1	Animal base	141
2	Chemical base industry	114
3	Rubber base industry	101
4	MSM relate	96
5	Waste water treatment	89
6	Palm oil factory relate	26
7	Plastic industry	22
8	Metal industry	17
9	Fish industry	11
10	Food industry	10
11	Wood base industry	7
12	Housing	8
13	Small shop	4
14	Printer	2
15	Other	424
	Total	1082

In industries such as animal farming, landfills, wastewater treatment and composting plants particularly, the offensive odours are caused by the production of intermediate volatile odorous compounds during the anaerobic microbial breakdown of organic matter. Microbial activities are considered to be responsible for malodour generation from these industries. The odorous compounds originate from the breakdown of proteins (producing ammonia, hydrogen sulphide, mercaptans), carbohydrates (generating volatile fatty acids, alcohols, aldehydes, ketones) and fats (generating acetic

acids, alcohols) (Barth and Polkowski, 1974). These simplified compounds then react to form tertiary products, for example amines are produced from ammonia and alcohols, while hydrogen sulphide and alcohols react to form mercaptans (McGinn, 2001).

2.1.1 Sulphur-containing compounds

This group of compounds is mainly composed of sulphides, methyl and ethyl mercaptans, which are formed at the occurrence of anaerobic breakdown of proteins and sulphur amino acids. The smell of these compounds is characterized as an unpleasant, irritative, nauseating, rotten egg odour (hydrogen sulphide), garlic smell (sulphides and disulphides) or sauerkraut smell (mercaptans). These compounds are highly toxic and can affect the respiratory and the central nervous system at high concentrations (Schiffman *et al*., 1995).

Sulphur-containing compounds are produced by microorganisms through two main processes, the reduction of sulfate and metabolism of sulphur containing amino acids (cysteine and methionine). The sulfate reduction proceeds via two different pathways, either assimilatory or dissimilatory. In the assimilatory process, bacteria produce sufficient reduced sulphur for cell biosynthesis of cysteine and methionine. On the other hand, sulphate is utilized in the dissimilatory process as terminal electron acceptor, and produces large quantities of toxic sulphide (Hao *et al*., 1996). The chemical equation showing this process is given below. In these activities, the involving bacterial genera include *Megasphaera, Desulfovibrio desulfuricans, Veillonella* and enterobacteria (Zhu, 2000).

$$4\,H_2 + SO_4^{2-} + 4\,H^+ \longrightarrow HS^- + 4\,H_2O$$

Besides that, deamination reactions of sulphur-containing amino acids such as cysteine and methionine also give rise to sulphides and mercaptans, respectively (Rappert and Muller, 2005).

$$\text{Cysteine} \longrightarrow \text{Pyruvate} + NH_3 + H_2S$$

$$\text{Methionine} \longrightarrow \text{Pyruvate} + NH_3 + CH_3SH$$

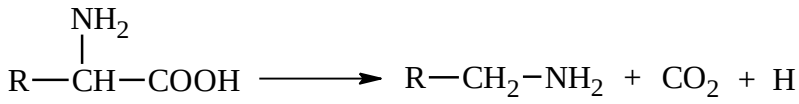
2.1.2 Nitrogenous compounds

The nitrogenous compounds are produced in high temperature processes, by microbial decomposition of amino acids. This type of compound is mainly composed of ammonia and amines such as methyl amine, dimethylamine, trimethylamine, ethylamine, butylamine, putrescine and cadaverine. These odorous contaminant originating from various sources, including landfills, wastewater treatment plants, composting operations, livestock farming, food preparation and metal manufacturing (Chung *et al.*, 1997 and Bucsa and Pistarino, 2003). However, livestock operations are known to be prominent sources of atmospheric ammonia (Isermann, 1994).

Ammonia is a colorless, toxic, reactive and corrosive gas with a very sharp odour. The emission of ammonia has always been a concern of the public, as it is harmful to the environment. Ammonia possesses the ability to react with acids such as acid nitric and acid hydrochloric, forming aerosols once it volatized. In addition, ammonia may also cause acidification of the ecosystems, by capturing sulfur dioxide in clouds, and then deposited on land and in water (ApSimon *et al.*, 1987). Ammonia which deposited on land eventually cause damage to vegetation (van der Eerden *et al.*, 1998), hence reduces plant biodiversity in natural ecosystem (Sutton *et al.*, 1993).

On the other hand, amines are compounds with unpleasant odour and are known to be highly toxic. In livestock facilities, emissions of amine are often associated with ammonia. Potential problems related to with methylamines in particular, are speculative with possible atmospheric reactions that lead to the formation of more hazardous compounds such as nitrous oxide and hydrogen cyanide (Schade and Crutzen, 1995).

In livestock industries, amino acids are most likely to undergo decarboxylation, as shown in the following equation, in which amines and ammonia are produced during the storage of fresh manure.



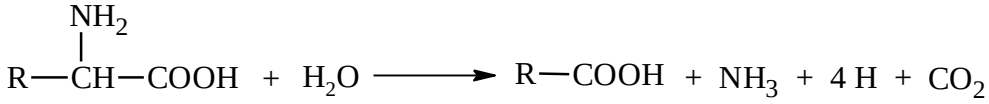
Besides that, ammonia can also be produced from urea and nitrates (Spoelstra, 1980; Mackie *et al*., 1998). Urea is hydrolyzed to ammonia by ureolytic bacteria with enzyme urease (Rappert and Muller, 2005). In this activity, the involving bacteria genera include *Peptostreptococcus*, *Streptococcus* and *Bacteroides*, *Bifidobacterium*, *Selenomonas* and the enterobacteria (Zhu, 2000).

2.1.3 Volatile fatty acids (VFAs)

Typical acids associated with odour consist of acetic, propionic, butyric, iso-butyric, valeric, iso-valeric, caproic and capric acid. These compounds are generated from agricultural activities, landfills, wastewater treatment plants and composting operations. VFAs such as the *n*- and *iso*-isomers of butyric and valeric acids are some of the main malodour compounds from animal excreta in livestock farming as well as wastewater caused by human activities. Barth and Polkowski (1974) reported that volatile fatty acids (VFAs) correlated best with odour intensity. In addition, several studies indicated VFAs as the major indicators of swine odour offensiveness (Williams, 1994; Zhu *et al*., 1998).

Acids with long alkyl chains (such as butyric, valeric, caproic and caprylic acid) and branching (such as *iso*-butyric, *iso*-valeric, *iso*-caproic and *iso*-caprylic acid) are reported to be more offensive odour compared to acids with short alkyl chains (such as formic, acetic and propionic acid) (Mackie, 1994; Zhu *et al*., 1999). Due to the structural properties of acid, the high concentration of VFAs may not necessarily cause high intensity of malodour as a large portion of the VFAs can be composed of short chain acids with less odour potential (Zhu, 2000).

VFAs originate from the microbial decomposition of carbohydrates and proteins under the anaerobic conditions. In carbohydrate catabolism, sugars are converted into pyruvate which then be fermented to various VFAs. Sugar fermentation usually yields short and straight chain fatty acids such as formic, acetic, propionic, butyric, succinic and lactic acids. However, protein and amino acids catabolism are recognized as the major sources of VFAs production. The general equation showing the formation of VFAs from amino acids is given as:



The protein degradation produces both the straight and branched chain fatty acids (Rappert and Muller, 2005). Bacterial genera involve in this activity include *Eubacteria*, *Peptostreptococcus*, *Bacteroides*, *Streptococcus*, *Escherichia*, *Megasphaera*, *Propionibacterium, Lactobacilli* and *Clostridium* (Zhu, 2000).

2.1.4 Aromatic compounds

Major compounds belonging to this group are indole, skatole, phenol, *p*-cresol and 4-ethylphenol. These compounds are produced through the metabolism of aromatic amino acids, mainly by the members of genera *Bacteroides, Bifidobacterium*,

Clostridium, *Escherichia*, *Eubacteria* and *Propionibacterium* (Mackie *et al*., 1998; Zhu, 2000). In addition, microbial degradation of tyrosine also produces phenolic compounds such as phenol, *p*-cresol and 4-ethylphenol (Macfarlane and Macfarlane, 1995; Mackie *et al*., 1998; Zhu, 2000). On the other hand, phenyl propionate and phenyl acetate are produced from the degradation of phenylalanine, while indole and indoleacetate results from the metabolism of tryptophan (Macfarlane and Macfarlane, 1995).

2.2 Environmental-related advances over last three decades

The impact of human activities and issues of environmental health has increased globally in scale and extent, and has led to numerous global, national and regional environmental odour control regulations which were developed with the aim to maintain a healthy environment. Despite of whether a country has specific regulations for odour control, two basic principles for controlling odours are the reduction of odours at the generation sources and the removal of odours from collected gaseous streams before they are discharged in to the environment (Rappert and Muller, 2005). Various methods to tackle odour issues have been proposed. However, the stringent regulations have given the communities few options but to rely on technologies to reduce the environmental damage. Many of these issues have led to the discovery of various techniques and technologies which were never before possible.

2.2.1 Chemical methods

One of the environmental issues which have triggered rigorous research is the waste gas contaminants mainly from industrial and human activities. As early as 1981, the treatment for malodorous process emissions has already been developed (Pope *et al*., 1981). In this treatment, sodium hypochlorite was introduced as the main component of a multi-stage absorption system. Absorption, in this context, removes the waste gas

contaminants with water, the most used scrubbing solution. The gas enters a large contactor where the gaseous pollutants are transferred to a liquid phase (Devinny *et al*., 1999). The pH was adjusted with additional reagents such as dilute sulphuric acid, sodium hydroxide and sodium hydrogen sulphide to increase the solubility of acidic or basic gases for effective odour control (Pope *et al*., 1981). As the technology develops, sulphuric acid scrubber was found capable to remove ammonia successfully, particularly from the swine farming (Hahne and Vorlop, 2001).

Another chemical-based technique developed to tackle the environmental issues was adsorption. Adsorption is the process whereby the contaminants attach to solid surfaces like activated carbon, activated alumina, silica gels and zeolites (Buonicore, 1992a). This method was used for controlling volatile organic compounds (VOCs) with low vapor pressure and high molecular weights released from industries (Devinny *et al*., 1999). Among all the mentioned materials, activated carbon was often used as a modulator, to adsorb relatively high concentrations of toxic compounds. In the 1980s, researchers incorporated indigenous microorganisms on activated carbon for degradation of phenol (Ehrhardt and Rehm, 1985; Morsen and Rehm, 1987).

Twenty years later, adsorption with photocatalytic effect was studied for the decomposition of odorous sulfur compounds. Dimethylsulphide (DMS) and dimethyl disulphide (DMDS) were degraded using an improved type of silica bead inner supported with titanium (IV) oxide (TiO2) or silicon dioxide (SiO2) (Nishikawa and Takahara, 2001). In this photocatalytic degradation, activated carbon was used as a supporting material for TiO2, to increase the adsorptive activity of contaminants around the TiO2, resulting in a rapid degradation. At the same time, another process was developed for VOC adsorption on activated carbon fibre cloths to clean the waste air streams containing VOCs and other odorous substances. In this frame work, electric current was used to directly heat the cloth for desorption purposes (Schippert *et al*., 2002). Most recently,

several studies have been conducted on the performance of various granular activated carbons for the removal of VOC from the waste stream (Haghighat *et al*., 2008).

Chemical methods using various oxidizing agents like $KMnO_4$ (Faith, 1964; Emanuel, 1965), H_20_2 (Hollenback, 1971; Kibble *et al*., 1972) and O_3 (Watkins *et al*., 1997; Wu *et al*., 1998; Kim *et al*., 2005) have been developed much earlier, and they were known to be effective in reducing malodours. Ye and co workers (2009) reported that horseradish peroxide with hydrogen peroxide or calcium peroxide was effective in controlling the release of malodour compounds from swine industry. The deodorization effect of the peroxides was reported to last for at least 48 h (Ye *et al*., 2009). Furthermore, biological additives such as essential oils (Varel and Miller, 2001), soybean oil (Zhang *et al*., 1996; Jacobson *et al*., 2000) and microbial additives (Mackie *et al*., 1998; Zhu *et al.*, 1999) were also utilized in controlling odour.

Technologies mentioned above are proven to be effective and reliable in treating air contaminants. However, these methods are found to have several disadvantages. Among them are high capital and operating costs, high maintenance fee, no mineralization and possible generation of secondary waste streams (Sercu *et al*., 2006).

2.2.2 Biological methods

Biological method, also known as bioremediation uses the biological systems to reduce environmental pollution which threatens public health (Rastogi, 2007). Bioremediation employs living organisms, primarily bacteria and fungi to degrade the environmental contaminants into less toxic or non-toxic forms, through reactions which take place as parts of their metabolic processes (Vidali, 2001). The microorganisms feed on pollutant substances and oxidize them, producing carbon dioxide, mineral and water. As such, biological treatment is often environmentally benign and less complicated, without producing secondary waste. In addition, the investment and operational costs for

biological waste treatments systems are relatively low (Devinny *et al*., 1999; Ergas and Cárdenas-Gonzáles, 2004).

The need for effective and economical waste gas treatment technologies has led to the development of biological air pollution control processes. Over the years, applications of different biotreatment processes in air pollution control strategies have increased, primarily because the method relies on the activity of microorganisms to degrade pollutants from air stream (Schegelmilch *et al*., 2005). In particular, biological treatments are believed to be the most economical options for treating odorous compounds. For instance, in the 1990s, biological methods of odour treatment have gained much attention in Europe and they accounted for 78% of odour treatment in Germany (Frechen, 1994).

The two biological systems commonly used for the treatment of odorous emissions are bioscrubbers/biotrickling filters and biofilters. The odorous compounds diffuse into the liquid phase where microorganisms degrade them into simple compounds. According to Brauer (1986), the transformation process can be expressed by:

$$\text{odorous gas} + \text{oxygen} \xrightarrow{\text{via bacteria}} \text{more bacteria cells} + \text{carbon dioxide} + \text{water}$$

2.2.2.1 Biofilter

Biofilter consists of a layer of biologically active media, which provides a large surface area, nutrients and moisture for the microbial activity, and adsorption/absorption of the odorous molecules (Schlegelmilch *et al*., 2005). As the contaminated air passes through the media, the immobilized microorganisms degrade the odorous compounds, to simple and less toxic forms. The critical properties of biofilter media are particle size uniformity, high porosity, large surface areas, pH buffering capability and ability to support microorganisms. Many different materials have been used as biofilter media,

including soil, peat, wood chips, domestic rubbish (Tang *et al*., 1996), digested sewage sludge (Morgenroth *et al*., 1996), forest subproducts and wastewater biosolids (Veir *et al*., 1996). Among these materials, soil is a common choice due to its porosity, particle size distribution and existing microbial flora (Burgess *et al*., 2001).

However, immobilization of microorganisms in support matrix such as alginate or suitable polymeric materials has gained popularity in the last few years. This technique involves entrapping the cells in a capsule volume where the nutrients, substrate and metabolite transportation takes place through diffusion of the capsule membrane. The immobilization technique in biofilter offers several advantages, including high cells concentrations, improve genetic stability, protect cells from shear destruction and enhance favorable microenvironment for microorganisms provision (nutrient gradients and pH) (Kim *et al*., 2007). This technique has been studied in wastewater treatment (Sekhar *et al*., 2004; Georgiou *et al*., 2005), however the application in waste gases treatment is often limited and sparse (Chung *et al*., 2001; Chan and Zheng, 2005). Immobilization of aerobic microorganisms is only applicable to the operational condition where oxygen is not limiting (Kim *et al*., 2007).

Biofilter has been developed in the 1950s (Sercu *et al*., 2006) to treat off-gases from sewage treatment plants, composting facilities, rendering plants (Sabo et al., 1994 and Schlegelmilch *et al*., 2005), food and tobacco producing as well as processing industries (Eitner, 1992 and Kersting, 1992). For instance, biofiltration system has been studied in the laboratory for the removal of butyric acid from waste exhaust air (Otten, 2002 and Sheridan *et al*., 2003). This system was also utilized in the treatment of waste gases containing industrial solvents and other volatile organic compounds (Fell, 2002).

Biofilration system is proven to be effective for the removal of various odorous compounds, such as aldehydes, organic acids, sulphur dioxide, nitrous oxides, hydrogen sulphide and methane with simple operating procedure and low capital costs. However,

this system has several disadvantages which require further development and improvement. Until today, the system requires large land area, regular media replacement (Burgess *et al*., 2001) and long gas residence times, as the dissolution of gas into liquid is the rate limiting step (Kennes and Thalasso, 1998).

2.2.2.2 Biotrickling filter

Biotrickling filter is a single unit operation reactor (for both capture and destruction), built with plastic, steel or ceramic material (Sercu *et al*., 2006). In this system, a packed column containing particles of biotrickling filter is inoculated with microorganisms. Biofilm grows using nutrients supplied by the contaminated airstream and by liquid flow that trickles down the packing. The packed column allows the mass transfer between gaseous and aqueous phase to take place, hence facilitating the diffusion of odorous substances to the biofilm. The degradation process of odorous compounds takes place in the biofilm that grows on the packing materials (Schlegelmilch *et al*., 2005).

Several studies have been performed to investigate on the efficiency of biotrickling filter. Recently, Tsang and co-workers developed a biotrickling filter with synthetic fibrous packing medium for the removal of malodorous volatile fatty acids. The study showed that the bioreactor was effective in removing volatile fatty acids, with efficiency remained above 99% and long term operation stability (Tsang *et al*., 2008). Even though the system is proven to be useful, several weaknesses have also been identified. Biotrickling filter requires regular media replacement which increases further the operating and maintenance costs and gas residence times. Another disadvantage of the system is the accumulation of excess biomass in the media reduced the specific surface area and bed volume and caused the pressure to drop, resulting in the performance fall off (Kennes and Thalasso, 1998).

2.3 Fermentation

The success of bioremediation relies on the application of microorganisms to the environment. Microorganisms applied to the environment can be in the form of either suspended or immobilized cells. Cells immobilized in gel matrix is often been utilized in bioreactors such as biofilter and biotrickling filter to enhance the efficiency of biodegradation. In order to perform any of these applications, cells of extracted microorganisms need to be produced in high concentrations, which can be achieved through fermentation processes.

The term fermentation is used to describe any process which involves the production of organic products by the mass culture of microorganisms. The few major groups of commercially important fermentations are production of microbial cell, production of microbial enzyme and production of microbial metabolites (Stanbury *et al.*, 1995). The fermentation process is carried out in an aseptic fermentation vessel, also known as the fermentor or bioreactor which is capable in providing a controlled environment for the growth of microorganisms to obtain desired products. Regardless of the designs, the fermentor must be constructed to promote optimal formation of desired organism and to eliminate contamination by unwanted organism (Rastogi, 2007).

The growth of microorganisms is directly associated with the physico-chemical environment. Under suitable conditions of pH and temperature, microorganism extracts nutrients from the medium and converts these nutrients into biological compounds. The availability of nutrients such as carbon, nitrogen, hydrogen and phosphorus is essential for the growth of microorganism (Rastogi, 2007). Reactions in the fermentor can occur with static or agitated cultures and in the presence or absence of oxygen (Smith, 1996). These parameters can be managed and controlled easily during the fermentation processes to achieve and maintain desired optimum culture conditions throughout the

processes. Fermentation may be carried out in several different ways including the batch, fed-batch and continuous processes (Stanbury *et al*., 1995).

2.3.1 Batch fermentation

Batch culture is a closed system containing limited amount of nutrients. During the batch fermentation, no additional nutrient is added, except oxygen (for aerobic microorganisms), antifoam agent and acid or base to control the pH. Through the batch fermentation process, the cells metabolism causes the changes in culture medium composition, metabolite concentration and biomass concentration (Rastogi, 2007). Eventually, cells multiplication ceases due to nutrients exhaustion and toxic waste products accumulation. Cell in the batch process is subjected to four typical phases of growth; the phases are lag phase, exponential phase, stationary phase and death phase (Smith, 1996).

The batch fermentation has several advantages over other cultivation methods. It requires low capital investments and provides more flexibility with different products and biological systems (Rastogi, 2007). In the industry, batch fermentation is operated to optimize the production of biomass and end-products such as amino acids, enzymes and antibiotics. These valuable products are optimally formed during the stationary phase of the growth cycle (Smith, 1996). In order to maximum biomass production, cultural conditions which support the highest growth rate and maximum cells population will be applied (Stanbury *et al*., 1995).

2.3.2 Fed-batch fermentation

The fed-batch fermentation is performed with one or more nutrients being supplied continuously or sequentially during cultivation without removal of the culture fluid (Yoshida *et al*., 1973). Gradual addition of nutrient into the culture results in the

increment of the culture volume as well as cells densities (Smith, 1996). The nutrients can be fed into the culture at different feed rates, either as variable volume or fixed volume (Stanbury *et al*., 1995). To maximize biomass production and yields, the feed can be manipulated by varying the feed composition and flow rate to suit the physiological state of the cells (Rastogi, 2007).

One of the main advantages of fed-batch fermentation is the production of high cells densities. The system allows more control over the production of by-products or catabolite repression effects as the concentration of substrates required for product and cells formation is low. The fed-batch system also gives control over the growth rate of the organism, which is well related to the specific oxygen uptake rate, to allow monitoring of oxygen demand during the fermentation (Stanbury *et al*., 1995).

2.3.3 Continuous fermentation

In continuous fermentation, an open system is set up, in which nutrient solution is added in continuously to the fermentor. At the same time, an equivalent amount of spent medium containing microorganisms is removed from the system, thus keeping the culture volume constant (Rastogi, 2007). In contrast to batch fermentation, continuous fermentation gives near-balanced growth, with little fluctuation of nutrients, metabolites and biomass. Factors such as pH, oxygen demand and the concentrations of nutrients and metabolites can be held near constant during the continuous cultivation. Continuous culture allows the organisms to grow under steady state conditions with constant growth rate in a constant environment (Smith, 1996).

There are two types of continuous fermentation, the chemostat and the turbidostat system. The chemostat system controls the cells growth by the availability of the growth limiting substrates in the nutrient solution. This system relies on the specific constant

flow rate (*F*) and constant volume (*V*), which defines the dilution rate, $D = F/V$. Under steady state conditions, D is found to be equal to the growth rate, μ. Hence, the growth rate can be controlled by adjusting the flow rate of the nutrient solution into the culture vessel (Calcott, 1981). On the other hand, turbidostat keeps the cells concentration at constant level to maintain the turbidity of the culture within certain limit by controlling the flow of nutrient solution. Turbidostat can be achieved by monitoring the biomass with a photoelectric cell. However, the chemostat is the more commonly used system because it does not require complex control systems to maintain a steady state (Stanbury *et al.*, 1995).

Continuous fermentation provides a higher degree of control than any other fermentation. Growth rate can be regulated and maintained for extended periods, with the biomass concentration and metabolite production remained constant throughout the cultivation period (Rastogi, 2007). Besides that, continuous fermentation can be operated for a very long duration (several weeks or months), in which the negative contribution of the unproductive time will be minimal (Stanbury *et al.*, 1995). Time consuming tasks such as cleaning and sterilizing are reduced with continuous system, thus results in higher productivity per unit volume. The ability to automate the process makes continuous fermentation more cost-efficient and less sensitive to the impact of human errors (Rastogi, 2007).

2.4 Cell immobilization technologies

Immobilization technologies have been developed for the removal of malodorous gases (Chung *et al.*, 1997) and pollutants such as phenol and hexavalent chromium from wastewater (Bandhyopadhyay *et al.*, 2001; Humphries *et al.*, 2005). The success of bioremediation depends on the introduction of specific microorganisms capable of

degrading toxic pollutants. However, the biodegradation process is often restricted by the indigenous predators, parasites and toxicants (Mallory *et al*., 1983; Murakami and Alexander, 1989; Prabu and Thatheyus, 2007). In terms of technology, immobilized cells offer several advantages over non-immobilized cells for the bioremediation of waste materials (Dwyer *et al*., 1986; Sathesh Prabu and Thatheyus, 2007).

Immobilization of microorganisms is defined as the restriction of the free cells migration in a defined region of space (Leenen *et al*., 1996). This technique has gained popularity as the microbial cells immobilized in a hydrogel matrix can be protected from harsh environmental conditions such as pH, temperature, organic solvent and poison. In addition, immobilized cells can be handled easily and recovered from the solution without difficulty (Park and Chang, 2000). Other advantages of immobilized cells include enhanced survival and improved physiological activity of the cells, increased cells densities and preferential cells growth in various internal aerobic and anaerobic zones of gel matrix (Moslemy *et al*., 2002).

The cells immobilization can be divided into two main processes, which are the attached growth and the artificial immobilization. The attached growth immobilization is defined as the self attachment of microorganisms upon submerged surfaces in aquatic ecosystems (Cohen, 2001). On the other hand, artificial immobilization is built by mankind to trap microbial cells within porous gel. There are several types of artificial immobilization, including the bead entrapment and encapsulation (Park and Chang, 2000).

2.4.1 Bead entrapment

This method involves the trapping of microorganisms in gel matrix in which the substrates and products diffuse in and out easily (Park and Chang, 2000). The general procedure of this method consists of suspending an amount of microorganisms in a liquid

solution containing macromolecule monomers. The solution is then gelated by the linkage of the macromolecules in order to entrap the microbial cells within the matrix. The pores of the matrix are smaller than the microbial cells, allowing the penetration of substrates through the matrix towards the trapped cells (Cohen, 2001).

Cells entrapment enables the attainment of high viable cells concentration, high resistance to toxic compounds within the waste materials, the possibility to entrap different species of microorganisms together and greater stability of plasmid with genetically engineered microorganisms. Several types of polymer have been used for the entrapment of microbial cells, which can be divided into natural and synthetic polymers. However, it is important that the polymers be hydrophilic in order to allow the diffusion of substrates into the beads (Sumino *et al*., 1992; Cohen, 2001).

The commonly used natural matrices are composed of agar, agarose, kappa-carrageenan, collagen, alginate and cellulose (Park and Chang, 2000). These polymers are usually brought to gelation by either cooling or contact with solution containing different ions (Cohen, 2001). On the other hand, the frequently used synthetic polymers are polyvinyl alcohol (PVA), polyacrylamide (PAM), polycarbamoyl sulphonate (PCS) and polyethylene glycol (PEG), which gel via a wide range of chemical or photochemical reactions. Generally, natural polymers have weaker mechanical strength compared to synthetic polymers. Hence, natural polymers are easily dissolved in wastewater and more susceptible to biodegradation, while synthetic polymers remain stable in the similar conditions. Furthermore, diffusion is higher in natural polymers and the preparation of beads is usually less hostile than the synthetic polymers (Leenen *et al*., 1996). A comparison between some natural and synthetic polymers for microbial entrapment in the application of domestic wastewater is presented in Table 2.2.

Table 2.2 Comparison between natural polymers (carrageenan and alginate) and synthetic polymers (PVA, PCS and PEG) used for microbial entrapment, for the application in domestic wastewater (Leenen *et al*., 1996)

Characteristics	Natural polymers		Synthetic polymers		
	Carrageenan	Ca-alginate	PVA	PCS	PEG
Solubility	High	High	Low/not	Low/not	Low/not
Biodegradability	Possible	Possible	Low	Low	Low
Stability	Low	Low	High	High	Medium
Diffusivity	Very good	Very good	Good	Moderate	Not determined
Growth	Good	Good	Moderate	Moderate	Good
Immobilization procedure	Simple	Simple	Laborious	Laborious	Laborious

2.4.2 Encapsulation

The encapsulation method consists of enveloping droplets containing microorganisms within a thin membrane. Unlike entrapment which is based on maintaining physical or chemical forces necessary for immobilization, encapsulation method maintains a solution environment around the cells. The membrane formed through this method allows free diffusion of substrate and product, while keeping the cells within the polymeric membrane (Klei *et al*., 1985; Chang *et al*., 1998). The encapsulated microorganisms can freely move within their own capsule, and metabolize substrates that penetrate via the membrane cover (Cohen, 2001). Materials used to construct the capsules are usually combinations of chemicals and materials, such as alginate, chitosan, cellulose nitrate, nylon, poly-L-lysine, polyamide, polyethyleneimine, polyacrylate and poly(vinylamine).

The encapsulation method offers several advantages in comparison with freely suspended cells and entrapment method. In the entrapment method, cells loading are limited due to the inadequate surface area available to the cells. Furthermore, the cells proliferate only in the periphery of the bead due to limitation of substrate and oxygen. For these reasons, only cells on the periphery of the bead are exposed to high concentrations of substrate, thus establishing a concentration gradient. On the other hand, dry cells densities in encapsulation can reach the value as high as 310 g/L, based on the inner volume of the capsule (Park and Chang, 2000).

However, the use of encapsulation method has been limited by several practical problems. The toxicity of the membranes causes the loss of catalytic activity within the immobilized microorganisms. Moreover, the growth, cells division and gas production during metabolism may lead to the mechanical rupture of the encapsulation membrane (Tampion and Tampion, 1987; Cohen, 2001).