

**BIODEGRADATION OF HIGH STRENGTH PHENOLIC
WASTEWATER IN A MODIFIED EXTERNAL LOOP
INVERSED FLUIDIZED BED AIRLIFT BIOREACTOR**

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INVERSED FLUIDIZED BED AIRLIFT BIOREACTOR**

THIN THIN AYE

(B.E., Yangon Technological University)

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SUMMARY

In an earlier study, a 4L working volume External Loop Inversed Fluidized Bed Airlift Bioreactor (EIFBAB) was constructed, characterized and tested for treating high-strength phenolic wastewater. Expanded polystyrene beads (EPS) were used as supporting materials for cell immobilization in the downcomer of the EIFBAB while gas was sparged in the riser. A unique feature of the novel bioreactor was the installation of a globe valve between the riser and the downcomer sections. By controlling the valve opening, liquid circulation velocity and bed fluidization were decoupled from the gas flow rate to enhance gas holdup. While the enhanced gas holdup and cell immobilization allowed for biodegradation of high strength phenolic wastewater up to 1200mg/L phenol, phenol concentrations in excess of 2000mg/L either experienced extended lag periods, or no degradation at all. This occurred because of substrate inhibition affects experienced by the cells at elevated phenol concentrations; cell immobilization could only provide a limited extension of the substrate inhibition threshold.

In order to extend the treatability range of phenol (which exerts strong substrate inhibition on the biofilm), a well-mixed tank reactor was placed in series with the EIFBAB. This modification provided for additional biodegradation via suspended and wall-growth bacteria. On the other hand, partially degraded wastewater was recycled back to the reactor with an optimized recycle flow rate to overcome substrate inhibition. Consequently, the highly concentrated phenol was diluted in the large-volume, well-mixed tank and therefore, inhibitory effects were reduced, resulting in higher phenol removal rates in addition to reducing the reactor size.

The hydrodynamic characteristics of the modified reactor were studied to optimize the reactor design and operation. It was observed that a low liquid recycle rate had no significant influence on the parameters studied. On the contrary, sharp changes of gas holdup and liquid circulation velocity were found at higher liquid recycle rates. Model predictions of hydrodynamic properties were also conducted. The experimental results obtained were correlated using linear regression. The constants in the correlation were related to the physical properties of the liquid used, the geometry of the reactor, solids loading in the downcomer and the sparger's location in the riser as well as the reactor's operating parameters. The correlation between the model and the experimental data for gas holdup and liquid circulation velocity was very good.

In the next part of this research, phenol degradation at high concentrations was investigated in the modified bioreactor under the batch mode, at varying liquid recycle rates ranging from 0 to 15L/h. With a total working volume of 10L, it was found that the new setup could handle up to 3000mg/L phenol at enhanced total and volumetric biodegradation rates. Compared to the EIFBAB (degradation rates of 28mg/h and 6.9 mg/L-h) in the batch mode, optimized operation at the 5L/h recycle rate in the modified system provided degradation rates of 91mg/h and 9mg/L-h, respectively.

NOMENCLATURE

Symbols

A_d	Area of downcomer
A_r	Area of riser
d	Particle diameter
dh_M	Height difference in manometer levels
dz	Height difference between point 1 and 2 in reactor
D	Column diameter
DO	Dissolved Oxygen
F	Feed rate (L/h)
g	Gravitational force
H	Height of reactor
h	Height difference between manometer level and specific point in reactor
K_f	Fraction factor
K_{La}	Mass transfer coefficient
P	Pressure
t	Time (h)
t_c	Average time for one complete circulation in the reactor
$d_{\text{electrode}}$	Distance between two conductivity electrode
t_{peaks}	Distance between two peaks on the recording chart and the moving speed of chart
V	Volume of reactor (L)
V_G	Gas superficial velocity (m/s)
V_L	Liquid circulation velocity (cm/s)

V_R Liquid recycle rate (L/h)

Greek letters

ε Gas holdup

θ Valve opening position

α Correction factor

β Exponent

ρ Density

Subscripts

D Liquid with dissolved gas

G Gas

L Liquid

S Solid

R Recycle

M Manometer

max Maximum

o Out of reactor

1 Pressure inlet point 1

2 Pressure inlet point 2

r Riser

d Downcomer

t Top section

Abbreviations

ALR	Airlift Reactor
IL-ALR	Internal Loop Airlift Reactor
EL-ALR	External-Loop Airlift Reactor
FBR	Fluidized Bed Bioreactor
EIFBAB	External Loop Inversed Fluidized Bed Airlift Bioreactor

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CHAPTER 1

INTRODUCTION

1.1 Background

Phenol and phenolic compounds are well known components in a wide variety of wastewaters including those from coal conversion process, coking plants, petroleum refineries and several chemical industries such as pharmaceuticals, resin, fertilizer and dye manufacturers (Morsen and Rehm, 1990; Bandyopadhyay et al., 1998). These organic compounds pose serious environmental concerns because of their widespread use and toxicity for aquatic species (Fava et al., 1995). Furthermore, they are troublesome contaminants in surface waters and add an objectionable taste to municipal drinking waters even at lower concentrations.

Different methods of treatment are available for the removal of phenol and its homologues, including ozonation, adsorption, solvent extraction, membrane process, coagulation, flocculation and biological treatment. However, physicochemical methods of treating phenolic wastewaters have inherent drawbacks because of their tendency to form secondary toxic materials, such as chlorinated phenols and hydrocarbons, etc. Moreover, the physicochemical treatment processes have proven to be costly. Thus, biological treatment systems are generally preferred to degrade these substances since they are effective and produce end-products that are environmentally benign (Hobson and Millis, 1990). Recently, airlift reactors have been shown to be efficient contactors for processes involving gases, liquids and solids. Their relatively simple mechanical design, low shear rate, high degradation capacity, good mixing and low costs make them a versatile type of bioreactor (Snape et al., 1995; Gavrilesco and Tudose, 1998).

However, certain disadvantages and limitations are also apparent when these reactors are applied to biological wastewater treatment involving microorganisms in a solid phase because gas sparging can damage cells, and shear stress due to turbulence of the three-phase flow, results in thinning of biofilms (Michaels et al., 1995).

At the same time, three phase fluidized bed bioreactors have created increased interest in wastewater treatment due to their high degradation efficiency (Holladay et al., 1978; Denac & Dunn, 1986; Shimodaira et al., 1981; Nikolov and Karamanev, 1990; Ibrahim et al., 1996; Farag et al., 1997). Moreover, these bioreactors have a number of advantages over conventional bioreactors, including a low washout of microorganisms, excellent contact between liquid and solid phases, and their ability to out-perform other reactor configurations, namely the activated sludge and the trickling filter. However, due to bubble dispersion, cells are sloughed off the bioparticles. Therefore, it is desirable to develop a new type of bioreactor to keep gas bubbles from contacting the solids, resulting in a good reactor performance.

By combining inversed fluidized bed reactors with airlift bioreactors, the desirable properties of both systems can be obtained (Garnier et al., 1990; Guo et al., 1997; Loh and Liu, 2001). In this setup, gas-liquid and solid-liquid contacts are separately accomplished. Flow reversal occurs only at two points, at the bottom bend and the top gas disengagement sections, and the resulting gas is effectively disengaged from the liquid and the liquid flowing into the downcomer was essentially bubble-free. This eliminates the potential damage caused by the bubbles to the cells in a biological operation.

Many studies have shown that numerous microbial species are capable of degrading phenol at low concentrations. However, at toxic concentration, phenol inhibits microbial growth (Hill and Robinson 1975; Fava et al., 1995; Chung et al., 1998; Loh and Wang, 1998; Loh and Liu, 2001) and can also cause cellular lysis (Ruiz-Ordaz et al., 1998). In order to protect microorganisms from being damaged as well as to maintain continuous cell growth and phenol degradation, it is necessary to construct a barrier between the highly toxic concentration of phenol and the microorganisms (Chung et al., 1998). Immobilization of cells has become an established technique in meeting this requirement. Much research has been conducted recently on phenol removal from wastewater using immobilized cells (Livingston and Chase, 1989; Ehrhardt and Rehm, 1989; Molin and Nilsson, 1985; Gonzalez et al., 2001a; 2001b; Loh and Liu, 2001). The immobilization of active cells creates many advantages such as high biomass concentration in the reactor, increased resistance to toxic shock loadings and low washout of microorganisms (Parkin and Spencer, 1984).

Current technology permits the use of these microorganisms in both the batch and continuous processes. However, the difficulty associated with the batch mode is that the initial substrate concentration must be lower than the value at which the organisms are inhibited. Moreover, biodegradation in the fluidized-bed biofilm system is known to be limited by oxygen transfer (Fan et al., 1987) and inhibitory levels of the substrate (Loh and Liu, 2001).

In an earlier study by Loh and Liu (2001), a 4L external loop inversed fluidized bed airlift bioreactor (EIFBAB) was constructed, characterized and tested for treating high strength phenolic wastewater. Expanded polystyrene beads (EPS) were used as supporting materials for cell immobilization in the downcomer of the EIFBAB. A

unique feature of the novel bioreactor was the installation of a globe valve between the riser and the downcomer sections. By controlling the valve opening, liquid circulation velocity and bed fluidization were decoupled from the gas flow rate to enhance gas holdup. While the enhanced gas holdup and cell immobilization allowed for biodegradation of high phenolic wastewater up to 1200mg/L phenol, phenol concentrations in excess of 2000 mg/L either experienced extended lag periods, or no degradation at all. This occurred because of substrate inhibition effects experienced by the cells at elevated phenol concentrations (Hill and Robinson, 1975) and cell immobilization could only provide a limited extension of the substrate inhibition threshold (Keweloh et al., 1989; Chung et al., 1998a; 1998b).

In the hollow fiber membrane cell immobilization studies conducted by Chung et al. (1998a, 1998b) and Loh et al. (2001), it was found that the membrane immobilized cells could degrade high phenol concentrations to a low enough level before suspension cells started to leak from the membranes. When this happened, subsequent high degradation rates resulted from both the suspended cells and the membrane-immobilized cells. Based on these observations, it was anticipated that the EIFBAB could benefit from the inclusion of a suspended cell reactor to extend the treatability range of the phenolic wastewater. This modification would provide additional biodegradation means via suspended and wall-growth bacteria in a mixed-tank reactor and at the same time provide for dilution of high phenol concentrations in the feed under continuous operation.

In this research, the EIFBAB was incorporated with a well-mixed reactor placed in series. The partially degraded phenol solution was withdrawn from the downcomer of the EIFBAB into the well-mixed reactor, and the effluent from this was recirculated

back to the EIFBAB through a port located at the bottom of the riser. With the presence of the well-mixed reactor, and consequential withdrawal of liquid from the downcomer of the EIFBAB, the hydrodynamics behavior in the modified EIFBAB was first investigated under cell-free condition to elucidate the effect of liquid recycle rate (V_R) on the gas holdup in the riser. It was found that for $0 < V_R < 15 \text{ L/h}$, the gas holdup was not significantly different regardless of the addition of the mixed-tank reactor.

1.2 Objectives and scope

The overall objective for this project was to study degradation of high strength phenolic wastewater in the modified external loop inversed fluidized bed airlift bioreactor (EIFBAB), which incorporated a well mixed tank in series for mixing the influent with the spent medium before recycling it back to the reactor. *Pseudomonas putida* ATCC 11172 was immobilized onto expanded polystyrene beads placed in the downcomer while gas was sparged in the riser. After the preliminary study of phenol degradation by the cell in batch culture, the bioreactor was to be tested for treating high concentrations of phenolic wastewater using first batch operation with recycling and then continuous operation. The primary objective of this study was to investigate the performance of the bioreactor under high loads of the inhibitory influent. Hence, the benefits of immobilization and operation mode were also important factors of evaluation.

The specific objectives of the research and its scope may be summarized as follows:

- 1) to modify External Loop Inversed Fluidized Bed Airlift Biorecotor (EIFBAB) by adding a well-mixed tank in series.

- 2) to investigate its hydrodynamic properties and to establish the mathematical model for the relationship between the gas holdup, gas flow rate, liquid circulation velocity, valve opening position and liquid recycle rate based on experimental data.
- 3) to perform a feasibility study on the biodegradation of high strength phenolic wastewater operating in different operation modes and comparing the respective performances.
- 4) to evaluate of the response of the system operating in the continuous mode to shock loadings.
- 5) to study the dilution effect on degradation efficiencies.

1.3 Structure of the thesis

This thesis consists of six chapters. Chapter 1 gives a brief introduction on this project. Chapter 2 provides a comprehensive review of literature relevant to this project. All the important information on previous research, their advantages, disadvantages and consequent objectives of this project are mentioned in this chapter. Chapter 3 describes details in the materials and experimental methods used in this project. The bioreactor setup, design and fabrication of the modified bioreactor system are also described in this chapter. Chapter 4 discusses the hydrodynamic studies in which the mathematical model is proposed and compared with the experimental data. Chapter 5 explains the kinetics studies of phenol biodegradation using *Pseudomonas putida* ATCC 11172 in this bioreactor. The result includes preliminary study in batch culture. This is followed by a study of recycling of the medium in the continuous modes. The high strength phenol degradation in the bioreactor is also summarized. Finally, the major

conclusions drawn from this research and the recommendations for future work are given in Chapter 6.

In summary, the contributions of this thesis comprise two areas: 1) to study the optimization of modified bioreactor's operating system in cell free condition; and 2) to study the performance of this bioreactor system on phenol degradation using different operation modes. According to the results, the degradation ability for high strength phenol in this bioreactor shows very promising application for future environmental biotechnology.

CHAPTER 2

LITERATURE REVIEW

2.1 Hydrodynamic studies of airlift bioreactors

The behavior of a bioreactor is determined not only by the reactor's geometry but also its hydrodynamic properties (Chisti, 1989). Therefore, knowledge of liquid velocity and gas holdup is essential for reliable predictions of mixing and mass transfer characteristics. In contrast to bubble columns, in an airlift reactor, the above mentioned hydrodynamic parameters predetermine each other, thus impeding a fundamental prediction of gas holdup and liquid velocity. Many empirical and semi-empirical relationships have been proposed in literature to link the two hydrodynamic parameters, i.e. ϵ_r , the gas hold up in the riser and V_L , the liquid circulation velocity (Chisti, 1989; Gavrilesco and Tudose, 1998; Hwang and Cheng, 1997). All these relationships can be applied in determined ranges of operating conditions and cannot be generalized to any geometry (Chisti, 1989). This is due to the fact that, for a given value of gas superficial velocity, any variation of the following parameters can modify the liquid velocity and gas holdup: 1) the physical properties of gas and liquid; 2) the geometry of the top and bottom connection sections between the riser and downcomer; 3) the ratio between the cross-sections of the downcomer and riser (A_d/A_r); 4) the condition for phase separation; 5) the heights of non-aerated liquid, i.e. the liquid level before aeration; 6) the height of the reactor and 7) the gas sparger.

Therefore, for optimum design and scale-up of loop bioreactors, it is essential to be able to predict the liquid circulation rate, the liquid phase physiological properties such

as viscosity, interfacial tension, density and rheological characteristics. In addition, the bioreactor geometry must also consider the relevant parameters for determining the optimum reactor performance. Several correlations which can serve as useful guidelines for design and scale-up are summarized and briefly discussed in the following sections.

2.1.1 Gas holdup

The gas holdup determines the gas residence time in the liquid and bubble size, which in turn influences the gas liquid interfacial area available for mass transfer (Chisti, 1989). At any specific gas flow-rate (for a given physico-chemical composition of the fermentation broth), there will be a given gas holdup in the riser and downcomer, which will dictate the liquid circulation velocity around the loop. In order to change the mass transfer or mixing in the airlift reactor, the influent gas flow rate must be changed, thus resulting in a new gas holdup and liquid circulation velocity. Hence, after the initial geometric parameters are established, the influent gas flow rate is usually the sole adjustable operating variable. Therefore, most of the equations can be reduced to simple power law type functions of gas velocity as follows (Chisti, 1989).

$$\varepsilon = \alpha V_G^\beta \quad (2.1)$$

where ε is the gas holdup at a particular gas velocity, V_G , α is the reactor's geometry and β is the exponent which varies depending upon the reactor's operation mode and other factors. Chisti (1989) also reported that the interfacial area may be enhanced by either increasing the gas holdup or decreasing the prevailing bubble size. These two are not mutually independent, however, because the bubble rise velocity on which the gas holdup depends, is itself, to some extent, dependent on the flow regime, as well as on the size of bubble. For instance, if the gas is in a very fine bubble, due to its long

residence time in the liquid, is in equilibrium with the bulk liquid, hence these bubbles contribute little to mass transfer (Kawase and Moo-Young, 1986).

2.1.2 Liquid circulation velocity

In a biodegradation reaction, the dissolved oxygen concentration typically depends on the riser since this has the higher fractional gas holdup and consequently, this is where most of the gas liquid mass transfer takes place. The gas-free liquid from the gas disengagement section flows into the downcomer, then re-enters the riser. Thus the liquid phase circulates continuously around the loop. This circulation is an effect caused by the difference in the fractional gas holdup that exists between the riser and the downcomer. In turn, this creates a difference in hydrostatic pressure between the bottom of the riser and the bottom of the downcomer, which in turn acts as the driving force for the fluid circulation. Furthermore, the liquid circulation velocity, while itself is controlled by the gas holdups in the riser and downcomer, in turn affects these holdups by either enhancing or reducing the superficial gas velocity of bubble rise. In addition, the circulation affects turbulence, the fluid-reactor wall heat transfer coefficients, the gas-liquid mass transfer, and the shear forces to which the microorganisms are exposed. Figure 2.1 is a detailed chart of these interrelationships, which was produced by Chisti and Moo-Young (1988).

In this chart, the liquid circulation velocity in the airlift reactor originates from the differences in the bulk densities of the fluids in the riser and downcomer. The fluids circulate along well-defined paths: an upflow in the riser and a downflow in the downcomer. Hence, a mean liquid circulation velocity is defined as:

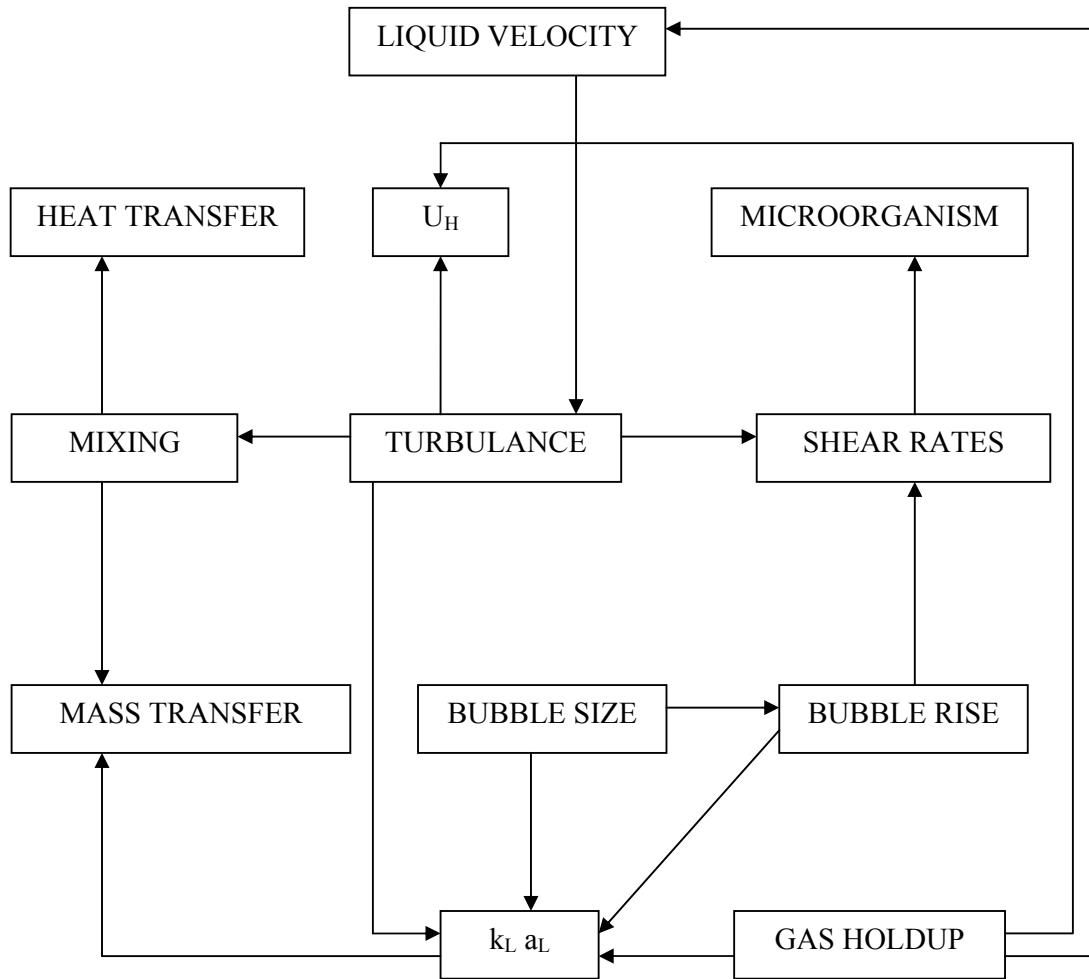


Figure 2.1 The inter-relationships between the liquid circulation velocity and other bioreactor performance characteristics (Chisti and Moo-Young, 1988).

$$V_L = \frac{X_c}{t_c} \quad (2.2)$$

where X_c is the circulation path's length and t_c is the average time for one complete circulation (Blenke, 1979).

Moreover, many studies (Bello et al., 1985; Onken and Weiland, 1983; Siegel et al., 1986) have shown that liquid circulation depends on the superficial gas velocity in the form of an equation, in which ω is a function of the reactor's geometry and of the properties of the liquid, whereas β is determined by the flow regimes as well as by reactor geometry. The equation is defined as:

$$U_{Lr} = \omega U_{Gr}^\beta \quad (2.3)$$

Numerous investigators have developed models of the fluid hydrodynamics which demonstrate the theoretical relationship between the gas holdup, circulation pressure drop and liquid velocity in the different types of airlift reactors (Chisti et al., 1988; Joshi et al., 1990). These models were derived using either an energy balance on the gas input due to the isothermal expansion of the sparged gas, macroscopic momentum balances and/or steady state mechanical energy balance, usually in conjunction with the Zuber and Findlay (1965) drift-flux model.

A key parameter in these models is the total fractional resistance to fluid flow in the circulating liquid loop, including the flow reversals at the top and bottom. For Newtonian systems, friction coefficients (K_f) have been estimated by applying standard relationships for single-phase flow in pipes and bends (Verlaan et al., 1986); two phase flow (Joshi et al., 1990); or by treating K_f as a fitting parameter (Chisti et al., 1988). The studies on the K_f in the internal-loop airlift reactor have shown that K_f

is mainly related to the cross-sectional area of downcomer A_d and the free area below baffle/draft-tube A_b . In the external-loop, however, it is far more complicated getting a unified, quantitative expression. Experimental verification of these models generally has been restricted to bench or pilot scale airlifts and to the air-water systems. Without an estimation of K_f , Bello et al. (1985) derived the following relationship for the linear liquid velocity in the riser:

$$V_L = (2gH_d U_G / K_f)^{1/3} \quad (2.4)$$

where V_L is liquid circulation velocity in the riser, U_G is gas superficial velocity and K_f is fraction coefficient.

Verlaan (1986) developed a model and was able to estimate K_f using only single-phase (liquid) flow relationships, as had been previously shown by Wallis (1969). Their model can predict the hydrodynamic parameters such as V_L, ϵ_r , reactor geometry and the gas sparging rate. The two-phase drift flow model of Zuber and Findlay (1965) was used in the gas holdup estimation to count the effective slip velocity between the bubbles and the liquid arising from the non-uniformity between the holdup and velocity profile in the radial direction. Young et al. (1991) developed a differential, two-fluid hydrodynamic model for two-phase flow in airlift risers, using point equations of continuity and motion. Macroscopic energy balance equations were used to describe flow in the downcomer and gas liquid separator. To describe the flow in the riser, differential separated flow type models were used. The models have the advantage that the only empirical parameters are those related to fraction effects in the reactor. The comparison between experimental results and predicted model values showed an agreement of approximately 10%.

When investigating the effects of the distributing plate geometry on gas holdup and liquid velocity, different results were obtained. Oken and Weiland (1987), for instance, found the gas holdup in the reactor to be independent of the initial bubble size generated by the gas sparger. In contrast, Merchuk and Stein (1981) reported that gas holdup depends on the geometry of the gas sparger and on the fraction of gas in the reactor. Snape et al. (1995) found that a plate with 0.5 mm orifices had a markedly different behavior from plates with higher orifice diameters, while only a slight increase of gas holdup and liquid velocity was observed with decreasing orifice diameters in the range of 1.0-3.0mm. Thus, parameters of the gas distributing system, in particular, the free plate area and the orifice diameter, have been shown to strongly influence the gas holdup in bubble columns.

Working with low-density solids and with high amounts of solids (up to 30% v/v), Freitas and Teixeira (1997) reported that solids loading and density had a considerable influence on gas holdup, liquid velocity and mixing time of an internal-loop airlift reactor with a degassing zone. The gas holdup was found to increase with the increased solids loading and particle density, while the liquid circulation velocity showed a reversed trend. Popovic and Robinson (1984) investigated gas holdup by installing a low-pressure drop throttling valve in one of the horizontal conduits linking the riser and downcomer to regulate the liquid velocity independently of the gas sparging rate in their reactor. Siegel and Robinson (1992) proposed an external loop airlift reactor and also established a mathematical model for predicting the hydrodynamic behavior of the reactor. This reactor has special benefits for large reactor volumes in the economic production of single-cell protein or for wastewater treatment at high aspect ratios without sacrificing mixing. Control over liquid circulation in the reactor enables good

liquid-phase mixing and good temperature control. This particular design enables higher rates of absorption per unit volume and rapid removal of unwanted gas. The main difference between conventional bubble columns and the riser section of the external loop airlift reactor is that the range of liquid velocities in the latter is one to two orders of magnitude higher than that in bubble column.

2.2 Biodegradation studies in bioreactor

2.2.1 Biodegradation studies of phenolic wastewater

Some industrial wastewaters, especially those coming from the production processes involving phenolic resins, contain high concentrations (>10,000mg/L) of phenolic compounds. These compounds pose serious environmental concerns because of their toxicity to aquatic species and their pollution of the environment. Several physico-chemical and biological treatments have been suggested in the last two decades to remove these compounds efficiently. In fact, adsorption with bone char or zeolites, stripping with air (Zilli et al., 1993), wet air oxidation (Lin and Chung, 1994) and biological treatments with pure or mixed cultures of microorganisms (Molin and Nilsson, 1985; Loh and Liu, 2001) have been used.

In a biological treatment system, the degradation process is based on cell growth since the cells are capable of optimizing the contact between the cells and organic substances through certain metabolic processes. This system is generally preferred for degrading phenolic compounds because the cells are effective and produce end-products that are innocuous (Hobson and Mills, 1990; Bandyopadhyay et al., 1998). Depending on the materials and methods used, such a system can be classified into different groups such as aerobic or anaerobic degradation, pure or mixed culture degradation, and

degradation of single or multiple substrates. The most rapid and complete biodegradation of organic compound occurs under aerobic conditions.

Although there are many conventional bioreactors for degrading phenol such as stirred tank and bubble column, airlift bioreactors (Chisti, 1989) and fluidized bed bioreactors (Fan et al., 1987) could yield a remarkably high biodegradation rate because of the high oxygen transfer. However, phenol is not readily biodegradable and it is very toxic to most types of microorganisms at sufficiently high concentrations. Phenol can inhibit the growth rate of even those species that have the metabolic capability of using it as a growth substrate. Therefore, the optimal design and operation criteria of these bioreactors require further exploration. The activated sludge process, which is known to be sensitive to fluctuations in the phenol load, has been introduced for many years (Soda et al., 1999). However, this traditional biological treatment process causes many serious problems especially that of solid waste disposal. Immobilization techniques have proved to be very effective with little sludge production (Ehrhardt and Rehm, 1989). Various reports dealing with phenol degradation by immobilized cells have appeared in the literature in the last two decades.

Immobilized cells generally show better degradation rates and can be exposed to higher phenol concentrations without loss of cell viability compared with free cells. It may give some protection for reactive sites on cells against denaturation, and thus provides easy access for co-enzymes and substrates. Moreover, in wastewater treatment, bioreactors with immobilized microbial cells may offer several advantages over processes with suspended cells. These include:

- (1) favorable environment conditions (i.e. cell-to-cell contact, nutrient-product gradients) for cells, resulting in better performance of biocatalysts (Molin and Nilsson, 1985).
- (2) higher cell concentration per unit volume of reactor (Tang and Fan, 1987)
- (3) cell reuse and eliminating the costly processes of cell recovery and cell recycle (Hallas and Heitkamp, 1992)
- (4) eliminated cell washout problems at high dilution rates (Kierstan and Coughlan, 1985)
- (5) cell protection against shear damage and high toxicity (Edwards et al. 1994; Heitkamp et al., 1992, 1993)

Normally, substrate availability and disposal of waste are not hindered by surface immobilization. However, the optimization of an aerobic process with immobilized cells may be complicated due to the problem of providing sufficient oxygen and nutrients to the cells. In addition, after prolonged immobilization and repeated use of a granular carrier in biological process, cell lysis has been found to be serious in the center of the carrier granule (Ruiz-Ordaz et al., 1998). It may be rationalized that poor transport of nutrients is less conducive to cell survival in the center of the granules. As the diameter of carrier is increased, the problems of mass and gas transfers in the matrix become increasingly pertinent. Therefore, bead size should be considered as an important parameter in determining the immobilized bioreactor's performance.

Moreover, the morphology of carrier particles plays an important role. Different types of particles have been used for cell immobilization, including glass beads (Koide et al., 1985), sand (Molin and Nilsson, 1985), polyethylene granules (Nikolov and

Karamanev, 1987), activated carbon particles (Fan et al., 1987; Wisecarver and Fan, 1988), plastic beads (Miyahara and Kawate, 1993), calcium-alginate beads (Lu et al., 1995), polystyrene cylinders (Hwang and Lu, 1997), calcined diatomaceous earth (Livingston and Chase, 1989), as well as expanded polystyrene beads (Loh and Liu, 2001). Furthermore, microenvironments can develop within the beads and these may alter intraparticle growth, metabolism and product formation (Doherty et al., 1995).

Numerous immobilized microorganisms have been used for the aerobic degradation of phenol including *Candida tropicalis* (Ruiz-Ordaz et al., 1998), *Fusarium flocciferum* (Anselmo et al., 1985 and 1989), *Cryptococcus elinovii* (Morsen and Rehm, 1990), *Aspergillus fumigatus* (Jones et al., 1993), and *Pseudomonas putida* (Ehrhardt and Rehm, 1989; Wang and Loh, 1998; Loh and Liu, 2001). Of these microorganisms, *Pseudomonas putida* has been reported to be capable of degrading high concentration of phenol and also has a strong ability to form biofilms.

Of the many immobilization methods, one simple approach is that of natural attachment to a surface, biofilm. Molin and Nilsson (1985) systematically studied the biofilm formation ability of *P. putida* ATCC 11172 at different environmental parameters. They indicated that although cell concentration, oxygen tension or temperature did not have any significant effect on the biofilm build-up, pH levels affected the biofilm formation. They pointed out that a pH of 6.7 is the optimal condition for biofilm formation. Gjaltema et al. (1997) reported that the physicochemical surface characteristics of the carrier surface prove to be less important due to the turbulent conditions in airlift reactors. However they found that hydrodynamics conditions and particle collisions control the biofilm formation. Some

other researchers have reported that biological removal rate is greatly affected by the specific surface area of the biofilm (Molin and Nilsson, 1985, Hirata et al., 1998). Their results showed that the phenol degradation rate increased with increased ratio of the biofilm surface area to culture volume. The fluidized bed bioreactor (FBR) has a larger biofilm support surface than several different types of biofilm reactors such as rotating disk contactors, and fixed bed reactors (Holladay et al., 1978; Denac and Dunn, 1986). However, the biofilm system in the FBR requires a long startup time to reach a desired removal rate (Wisecarver and Fan, 1988). In addition, the cells can slough off easily in the event of strong shear and attrition force between the particles. Therefore, it is necessary to make detailed studies of biodegradation efficiency in different types of bioreactors.

2.2.2 Airlift bioreactor

The airlift reactor was first patented for use as a bioreactor by Lefrancois in 1955 (Siegel and Robinsin, 1992). Airlift reactors have been extensively used in aerobic fermentation, wastewater treatment and other process areas due to the mildness and uniformity of turbulence, compared to conventional pneumatic reactors such as the stirred tanks and the bubble column. In addition, airlift bioreactors have a higher mass transfer rate in the biological reaction (Verschoor, 1985), better mixing and comparatively lower power inputs (Chisti, 1989). In addition to their simplicity of design and construction (Moresi, 1981), they also offer the added advantage of overcoming the drawback of high shear stress, which occurs commonly in conventional reactors (Chisti et al., 1986).

Airlift reactors are reaction vessels divided into two sections - the riser, where the gas is injected, and the downcomer -are classified according to the way in which the loop for circulating the liquid is arranged. Two categories of airlift can be encountered: airlifts built with internal or with external loop. In the internal-loop airlift reactor (IL-ALR), the riser and the downcomer are in the same column, whereas in the external-loop airlift reactor (EL-ALR), the downcomer and the riser are separate tubes put up side-by-side and connected at the top and at the bottom. In IL-ALR, the gas liquid separator is usually simply an un baffled extension above the riser and downcomer, allowing for little gas disengagement. Wu et al. (2003) reviewed the performance of a special tower-type bioreactor, the airlift bioreactor with net draft tube, which is equipped with a wire-mesh draft tube, resulting in better liquid circulation and larger gas liquid interfacial areas and therefore produces much higher mixing efficiency and oxygen transport capacity in several microbial fermentation situations. However, the internal loop inversed fluidized bed airlift reactor has some inherent problems namely: (1) some level of gas entrainment in the downcomer; (2) the adherence of light particles to the bubbles and (3) subsequent disengagement and bursting of the bubbles resulting in cellular damage (Garnier et al., 1990). More importantly, flow cycling that exists in internal loop reactors results in an unstable liquid circulation in these reactors though it eliminates cellular damage resulting from the limitations of a packed bed reactor (Chisti, 1989; Chiou et al., 1991).

Conversely, EL-ALR, the gas liquid separator has a clear region of horizontal flow from either a closed horizontal connection pipe or an open reservoir between the riser and downcomer conduits, which allows for either partial or total gas disengagement. Therefore, EL-ALR has frequently been used in investigating the reactor behavior in

laboratories, bench-scales and pilot-plant installations, apparently because of the well-defined conditions in the system. In the study on the characteristic properties of EL-ALR, Onken and Weiland (1983) found that in their reactor there was: (a) a complete degassing of the liquid at the top, which prevents accumulation of CO₂ in the fermentation of liquid and the reduction of the driving force for oxygen transfer due to entrainment of bubbles depend on oxygen; (b) an absence of zones of irregular flow at the top and bottom of the fermenter; (c) easy removal of the heat from the fermenter; and (d) easy measurement and control of the liquid circulation rate in the downcomer without complications arising from the gas content.

The applications of airlift reactors in the biodegradation process have been reviewed previously by many researchers (Onken and Weiland, 1983; Siegel et al., 1986). In airlift reactors, fluid motion is induced by differences in the mean densities between the riser and downcomer sections of the reactor, whereas in a bubble column, the energy source inducing fluid motion is focal. Therefore, the primary advantage of an airlift reactor over a conventional bioreactor is related to the shear stress imposed by the turbulent field on the cells or pellets suspended in the medium. This is because the lack of uniformity in the shear field in bubble columns and the stirred tank reactors exposes the organisms to varying shear stresses and environments as they pass through the reactor which in turn can adversely affect the growth and degradation ability of the microorganism.

Recently, many bench and pilot-scale applications of airlift reactors have been studied for a variety of microorganism and cell cultures, focusing on growth kinetics rather than transport phenomena during the fermentation. However, little research has been

conducted on optimizing airlift reactor design and operation during actual fermentations. Consequently, little basic hydrodynamic and mass transfer information is available for optimum reactor design and scale-up.

Frohlich et al. (1991a, 1991b) examined the cultivation of *S. Cerevisiae* in laboratory airlift reactors using both the batch and continuous modes. They examined the global axial mixing and local gas holdup, the bubble diameter and bubble velocity during the yeast cultivation. It was found that the local gas holdup, bubble size and the bubble velocity changed only slightly along the length of the riser. Huppe et al. (1990) used a two-stage pilot plant using immobilized cell for the biological treatment of effluents from a coal tar refinery. Tyagi et al. (1990) used an external-loop airlift reactor to study mesophilic and thermophilic aerobic digestion of primary and secondary municipal sludges. A cost analysis showed that the autoheated airlift reactor-digester would represent significant savings in both capital and operating costs compared to conventional two-stage aerobic digesters using pure oxygen to achieve the required oxygen transfer rate.

2.2.3 The inverse fluidized bed bioreactor

The fluidized bed biofilm reactor, in which the biomass is fixed on particles of a bed, is the most promising and effective bioreactor for treating high strength organics wastewater. Basically, with classic three-phase fluidization, particles of a larger density than the liquid are fluidized by the upward concurrent flow of liquid and gas; the liquid is the continuous phase in which the gas bubbles move up through the bed (Fan et al., 1989). Denac and Dunn (1987) showed that the biofilm in such a reactor has a higher volumetric degradation efficiency than that in packed bed reactors. A large biofilm

interfacial area and a good mass transfer are the main advantages of this type of reactor. Fan et al. (1989) studied phenol degradation by a biofilm on activated carbon particles in a draft-tube, three-phase, fluidized-bed reactor in a fed-batch mode. They pointed out that the overall rate of phenol consumption by the biofilm depends on three steps: (1) diffusion of phenol from the bulk of the liquid to the biofilm; (2) diffusion of phenol within the biofilm; and (3) biodegradation reaction.

Many researchers found one significant problem in practically all types of biofilm reactors (including fluidized bed reactors) and that is uncontrolled biofilm growth. The uncontrolled growth leads to substrate mass transfer limitations, lysis and detachment of biofilm from the support. Furthermore, in the case of fluidized bed bioreactors, the uncontrolled biofilm growth results in over-expansion of the fluidized bed with subsequent elutriation of the particles (Livingston et al., 1993). Some researchers have attempted to control the biofilm by removing some of the solid phase and replacing them with biomass free particles (Fan et al., 1989; Nikolov and Karamanev, 1987). The control of biofilm thickness within a narrow range can be achieved in the inverted fluidized bed biofilm reactor (Nikolov et al., 1987; Nikolov and Karamanev, 1990). They set up a compact inverse fluidized bed bioreactor with a high oxygen concentration in the reaction zone in which the hydrodynamic conditions are suitable for bacteria growth. To control the biofilm thickness, a bed of heavy inert particles supported on a grid placed in the draft tube above the air sparger, was introduced. In their reactor setup, the particles have a lower density than the liquid. The bed expands downward, dragged down by the flow of the liquid against the net buoyancy force of the particles. The third phase is the gas phase which is presented as bubbles at the bottom of the reactor and rises counter-currently to the liquid flow. Then the solids can

be fluidized at a low liquid velocity, requiring little energy expenditure and with minimum attrition of solids. This type of reactor was first recognized in 1970 (Page, 1970). Later on, Shimodaira et al. (1981) obtained a patent for its application in wastewater treatment. To date, it has attracted increasing attention for its promising application in cell cultures and other areas of wastewater treatment because it is expected that the gas-phase holdup and mean residence time in the reactor would be significantly higher than in any other configuration due to the drag action of the liquid phase on the gas bubble. However, there are still some limitations in using this type of reactor. Since the three phases (G-L-S) are in direct contact in the reactor, the gas bubbles can cause damage to the cells due to power dissipation (Michaels et al., 1995; Cherry and Hulle, 1992). The shear force created by the turbulence of a three-phase flow can break the soft biofilm on the surface of carriers, resulting in the problem of controlling the biofilm thickness in the bioreactor. Moreover, in the countercurrent mode, if the liquid velocity is too high, the gas bubbles become entrained by the liquid and are not able to rise. This becomes a limitation of the liquid flow rate.

To overcome these limitations, it is desirable to develop a new type of bioreactor to keep gas bubbles from the solid in order to maintain good reactor performance.

2.2.4 The external loop inversed fluidized bed airlift bioreactor

A novel bioreactor which combines the advantages of both the airlift reactor and the fluidized bed reactor into a single reactor has been investigated (Garnier et al., 1990; Guo et al., 1997; Loh and Liu, 2001). This reactor has the benefits of mechanical simplicity, good mixing and lower liquid shear rates as well as the features of good liquid-solid contact and good heat and mass transfer. It also eliminates simultaneous

contact of the gas-liquid-solid phases and can operate in two stages, i.e. gas-liquid contact in the riser and solid-liquid contact in the downcomer during the three-phase operation.

Ramsay et al. (1991) used a draught-tube inverse fluidized bed airlift bioreactor to produce penicillin and obtained promising results. In that study, the beads used for inverse fluidization were specially treated expanded polystyrene. They were 1.3mm in diameter and had a density of 0.21g/cm^3 . The same reactor setup as that developed by Garnier et al. (1990) was used to control the thickness of the biofilm due to the rapid growth. On the other hand, Guo et al. (1997) proposed the use of an external-loop airlift fluidized bed reactor. In this setup, gas-liquid and solid-liquid contacts were separately accomplished. Flow reversal occurred only at two points - at the bottom bend and at the top gas disengagement section. However, to increase gas holdup and enhance favorable fluidization, it was necessary to increase the height of the riser. However, Joshi et al. (1990) reported that an external-loop airlift fluidization reactor of $H/D = 40$ can enhance mass transfer since higher concentrations of dissolved solute gas are desirable in biodegradation.

Loh and Liu (2001) studied the external loop inverse fluidized bed airlift bioreactor (EIFBAB) for treating of high strength phenol operating in the batch mode. The highlight of this study was the installation of a globe valve between the riser and downcomer to control the liquid circulation velocity. By controlling the valve opening, liquid circulation velocity and bed fluidization were decoupled from the gas flow rate to enhance gas holdup. The bioreactor was tested for degrading up to 1200mg/L successfully.

2.3 Bioreactor operation mode

2.3.1 Batch mode

Phenol degradation can be achieved in either batch or continuous mode. Batch operation refers to a partially-closed system in which most of the materials required are loaded into the fermentor and subsequently removed at the end of the degradation. Under certain conditions (temperature, pH, aeration, etc.), the bacteria go through all the growth phases (lag, exponential, stationary, etc.). Therefore, conditions are continuously changing with time, and the fermentor is an unsteady-state system, despite the fact that in a well-mixed reactor, conditions are supposed to be uniform throughout. Since microbial conversions are autocatalytic, the rate of conversion increases with cell concentration. In order to keep the cell concentration higher than the normal steady state level and to get an effective dilution, the effluent can be recycled back to the reactor. The reactor allows for the mixing of the recycle streams with solutions of existing high concentrations so that a diluted medium is achieved for further processing to yield higher degradation efficiency (Shuler and Kargi, 1992) and for increasing the stability of systems by minimizing the effects of process perturbation due to the dilution process. Dilution could increase growth rate in the bioreactor, improve the mass transfer rate and reduce the characteristic lag phase, resulting in better reactor performance.

The batch reactor is versatile and this means it can be used for different reactions daily and with little risk of infection or strain mutation as it is properly sterilized. However, the main disadvantage of batch processing is unavoidable. This includes the high proportion of unproductive time (down-time) between batches, time spent on the feeding and discharging the fermentor vessel, as well as the cleaning, sterilization and

re-starting of processes. Furthermore, interpretation of results is difficult for batch culture because of changing concentrations of products and reactants, varying pH and redox potentials, and a complicated mix of growing, dying, and dead cells. Data from continuous cultures are much less complex because of dynamic equilibria from the steady state.

2.3.2 Continuous mode

The continuous mode enables the substance of continuous cell growth rate and constant effluent quality. Moreover, due to the autocatalytic nature of microbial reactions, the efficiency can be high. The main advantages of a continuous process over a batch process are the ease of automation and control. These modern engineering measures can lead to reduce operational cost and more consistent effluent quality. Normally, the culture environment changes continually in a batch culture. Growth, product formation and substrate utilization end after a certain time interval, whereas in continuous culture, fresh nutrient medium is continually supplied to a well-stirred culture and cells are simultaneously withdrawn. After a certain period of time, the system usually reaches a steady state where cell and substrate concentrations remain constant. Continuous culture is an important tool in determining the response of microorganisms to their environment and to produce the desired efficiency under optimal environmental conditions.

In comparing of batch and continuous cultures in terms of their suitability for large-scale operations, it has been found that the continuous culture can thus be operated for long periods of time without having to be shut down (Metcalf and Eddy, 1991). Therefore, the continuous mode can be many times more productive than batch mode

in any reactor. This is partly due to the fact that the growth rate of the bacteria in the continuous culture can be more easily controlled and optimized. Generally, in both batch and continuous operations, the major determinant of the rate of the degradation is cell numbers (Mordocco et al., 1999). In a continuous culture, the influent concentration and dilution rate control the cell numbers. When the influent concentration is high, this in turn means that the degradation rates will be high at steady state. To maintain a high cell number, continuous cultures need to be operated at low dilution rates when the influent concentration is high. In this case, the use of immobilized cells increases the maximum dilution rate that can be achieved before cell washout occurs. Thus, under such conditions, the treatment of high concentrations of phenol wastewater will be typified by small hydraulic throughputs. Hence, continuous cultures with immobilized cells should be capable of degrading phenol at either low or high concentrations because cell numbers potentially can be maintained through the use of various dilution rates. Pai et al. (1994) studied continuous phenol degradation by *Rhodococcus sp* immobilized on calcium alginate and granular activated carbon as a supporting material. However, it has been found that some limitations occurring in continuous production are due to infection and spontaneous mutation of microorganisms to non-productive strains.

Ultimately, although the continuous mode has some disadvantages, it can outperform the batch mode by eliminating the inherent turnout time for cleaning and sterilization as well as the long lags before the organisms enters a brief period of high productivity.

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental setup

A schematic diagram of the modified EIFBAB is shown in Figure 3.1. The Perspex EIFBAB (Appendix A) had a working volume of 4L and the PVC constructed mixed-tank reactor a 6L working volume. The riser and the downcomer had the same inner diameter of 0.03m and height of 0.786m. Air was introduced in the riser through a circular perforated plate sparger of mesh 40 orifice sizes. A stainless steel screen was placed between the gas-liquid separator and the downcomer. This screen prevented the solid particles from rising from the downcomer to the gas-liquid separator. A standard 0.0254m globe valve was installed at the bottom of the reactor, connecting the riser and the downcomer. The recycled liquid between the mixed-tank reactor and the EIFBAB was delivered by a peristaltic pump (Nikkiso Seven Pump CPL-551G).

3.2 Hydrodynamics study

Tap water and air were used as the liquid and gas phases, respectively, for the hydrodynamics study. The gas flow rate was measured using a rotameter, and inverted U-tube Manometer setup was used to determine the gas holdup (Appendix B). The distance of two pressure inlets on the outside wall of riser is 500mm. The tracer response technique was applied to obtain the liquid circulation velocity, the tracer used was saturated NaCl solution. The injection point was set up at the top and bottom of the downcomer. The distance between two conductivity electrodes mounted on the wall of downcomer section is also 500mm, and the two conductivity meters were

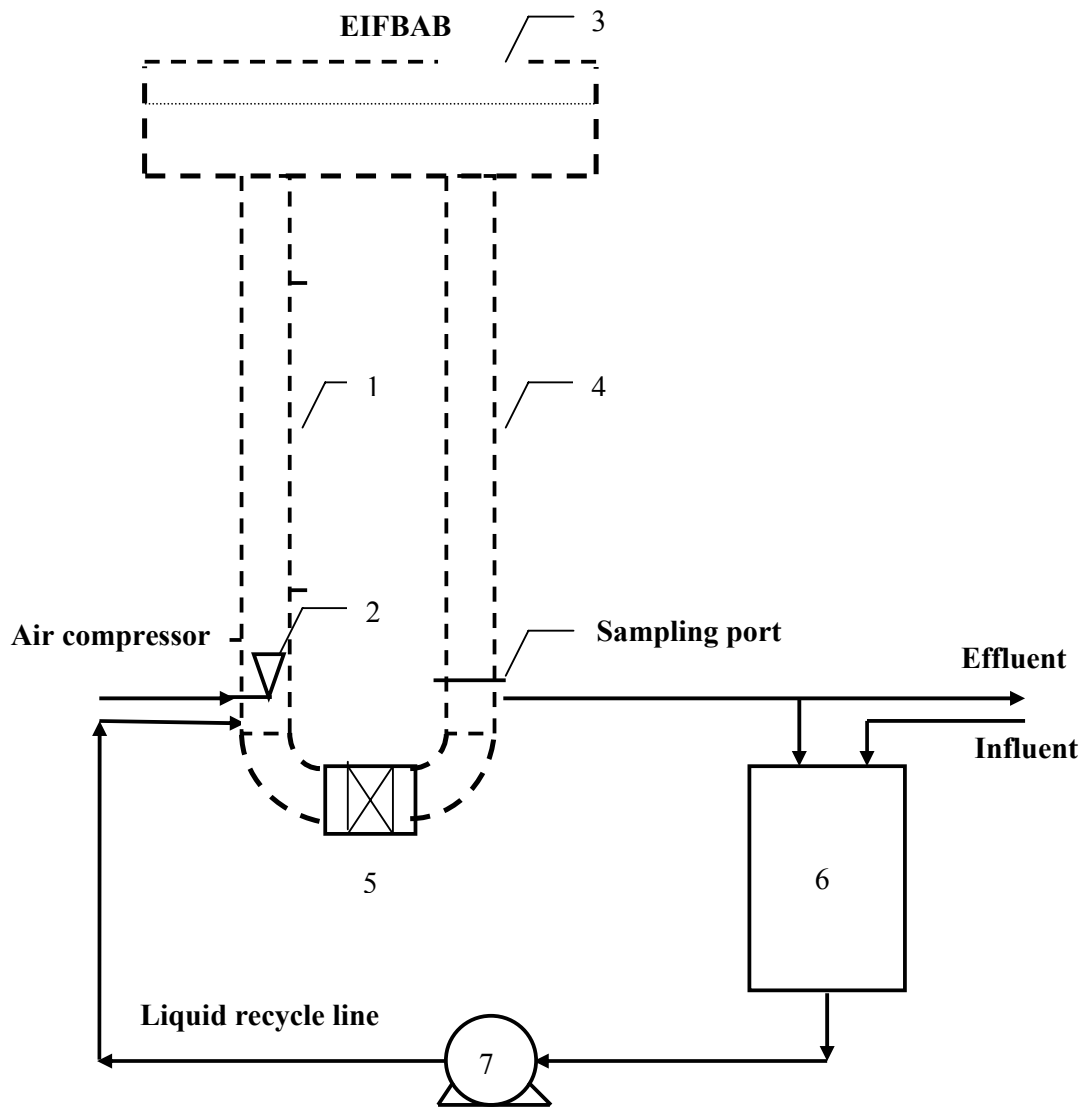


Figure 3.1 Schematic diagram of external-loop inverted fluidized bed airlift bioreactor (shown in dotted line) and its modification (shown in solid line).
 1.riser; 2.gas sparger; 3. gas disengagement section; 4. downcomer; 5. globe valve; 6. well-mixed tank reactor; 7. liquid recycle pump.

connected to the electrode and the signals were transferred to the recorder.

3.3 Organism and culture conditions

Pseudomonas putida ATCC 11172, which is able to grow on phenol and capable of forming biofilms, was used for the experiments. The bacteria were maintained by periodic sub-transfer on nutrient agar (Oxide, Hampshire, UK) slants, which were stored at 4°C in the refrigerator.

The mineral medium contained (g/L): Na₂HPO₄·2H₂O, 0.4; KH₂PO₄, 0.37; MgSO₄, 0.5; FeSO₄·7H₂O, 0.1; (NH₄)₂SO₄, 0.25; MnSO₄·H₂O, 0.05; CaCl₂, 0.1; ZnSO₄·7H₂O, 0.005; CuSO₄·5H₂O, 0.005; citric acid, 0.02 and pH was adjusted to 6.7 before use. Cells were activated in the mineral medium containing 500mg/L phenol as the sole carbon substrate. The phenol-grown cells in the exponential growth phase were used as inoculum for the bioreactor. All activation cultures were grown in 500mL Erlenmeyer flasks containing 250mL medium at 30°C with shaking at 200rpm on a GFL rotary shaker.

All media (except phenol), pipette tips and Erlenmeyer flasks fitted with cotton plugs were autoclaved at 121°C for 20min before use. Culture transfers and sampling were conducted aseptically around a Bunsen flame to minimize contamination.

3.4 Preparation of expanded polystyrene beads (EPS)

The polystyrene beads (diameter, 0.3mm; density, 9kg/m³, porosity, 44(%V)) were expanded to different sizes and densities in a hot water bath. Once expanded, the particles lost their smooth spherical shape, showing an irregular surface due to the

close cell structure of polystyrene foams. A desired density and size were obtained by varying the heating time while boiling. In order to obtain the uniform diameter, expanded beads were screened by using sieves plates. The cell immobilized beads are identical for each batch.

3.5 Reactor startup (Cell immobilization)

A 4L synthetic wastewater was made up of 3.75L of mineral medium containing 500 mg/L phenol mixed with 250mL of the activated bacteria. The EIFBAB was operated batch-wise at a gas velocity of 0.071m/s to establish on the biofilm on the expanded polystyrene (EPS) beads in the downcomer. The biofilm was deemed established when all the phenol had been depleted. The spent medium was removed and the beads were rinsed with sterilized deionized water. The cell-immobilized EPS beads were used for all the subsequent biodegradation experiments.

3.6 Bioreactor operation

The modified EIFBAB was operated under both batch mode and continuous mode. The batch mode operation was optimized for degradation rate with respect to the recycle rate from the mixed-tank to the EIFBAB (V_R) while the continuous mode operation was optimized with respect to the feed rate (F). Details of each of the operation modes are outlined as follow.

3.6.1 Batch mode operation

Four liters of phenol solution was placed in the EIFBAB and 6L in the mixed-tank reactor. The cell-immobilized beads (11%v/v solids loading based on downcomer volume) were placed in the downcomer and the system was operated at a gas velocity

of 0.071m/s and globe-valve opening of 5-10%. Under these conditions, the gas-holdup in the riser was about 0.4-0.45. The 10L working volume of liquid was circulated around the system such that liquid from the EIFBAB was withdrawn from the downcomer to the mixed tank and recirculated to the EIFBAB riser for V_R ranging from 0 (EIFBAB alone) to 15L/h. The content in the mixed tank was kept in circulation by means of a magnetic stirrer. The liquid level in the mixed tank was maintained constant by means of a pump (maintained at the same rate as V_R) to control the return stream to the tank. This operation was tested on two initial phenol concentrations - 2400mg/L and 3000mg/L.

3.6.2 Continuous mode operation

The system (modified EIFBAB) was initiated by batch operation using 500mg/L phenol. When the phenol was completely depleted, a continuous feed (Influent in Figure 3.1) of phenol solution was introduced into the mixed-tank reactor. This feed solution was aerated by direct air sparging before introduction. At the same time, an effluent stream (at the same flow rate as the feed) was taken off the recycle line (Effluent in Figure 3.1). Phenol feed was introduced at two different concentrations – 2500mg/L and 3500 mg/L. At each phenol concentration, the feed rate (F) was varied from 80mL/h to 320mL/h, corresponding to hydraulic retention times (HRT) of 125h to 32h, respectively. The system was then investigated for sustained continuous operation for gradually increasing phenol concentrations ranging from 500mg/L to 4500 mg/L, and at HRTs of 125h and 62.5h.

3.7 Analytical methods

Samples were taken periodically for analysis. Cell density was monitored spectrophotometrically by measuring the absorbance at a wavelength of 620 nm using a Shimadzu model UV-1601 spectrophotometer with 1-cm-path square quartz cuvettes. The measurement was made such that the optical density (OD) of the sample was less than 0.70 by diluting the samples. This is to ensure that the Beer-Lambert law applies. For determining phenol concentration, sample was immediately acidified to pH 2 with 6N sulphuric acid. The acidified samples were extracted with 3 ml of methylenechloride (GC grade, Merck, Darmstadt, Germany), which contained 100mg/l *o*-cresol (Merck, Darmstadt, Germany) as internal standard. The extract was analyzed for phenol by gas chromatography (GC). GC analysis was carried out using a capillary GC (Perkin Elmer, Model 8700) equipped with a split injector and flame ionization detector (FID). The injector and detector temperatures were both 250 °C. The column was a Hewlett-Packard fused silica capillary column, 30 m long, 0.25 mm internal diameter, coated with 0.25 µm thickness of 5% phenyl methyl silicone. Injection volume was 1 µl and sample split ratio was 10:1. The oven-temperature profile started with maintaining at 100 °C for 1 minute before ramping at 10 °C/min to 160 °C after which the program was halted. Under these conditions, the retention times, in minutes for phenol and *o*-cresol, were 2.28 and 2.82 respectively.

CHAPTER 4

HYDRODYNAMIC STUDIES OF MODIFIED EIFBAB

4.1 Summary

In this study, the hydrodynamic characteristic of a modified EIFBAB was studied to optimize the reactor design and operation. All experiments were conducted under cell free conditions. At the liquid recycle rates ($0\text{L/h} < V_R < 5\text{L/h}$), the decrease in gas holdup was not so significant compared with that in the original EIFBAB. However, a reversed trend was observed at liquid recycle rates ($5\text{L/h} > V_R > 15\text{L/h}$) because the gas retention time was obviously decreased with an increasing liquid recycle rate. Because of this increasing liquid recycle rate, liquid downflow in the downcomer was increased, indicating that good fluidization could be achieved at a low valve opening position (5-10%). As a result, the low liquid circulation velocity was attributed to the fluidised bed. Consequently, a higher gas holdup in the riser was accomplished in the modified reactor. In addition, the experimental results were correlated using linear regression. From the results presented, the values predicted by the developed model were in good agreement with the experimental values of gas holdup and liquid circulation velocity.

4.2 Introduction

The behavior of a bioreactor is determined not only by the reactor's geometry but also by its hydrodynamic properties. For reliable prediction of mixing and mass transfer characteristics, knowledge of liquid velocity and gas holdup is essential. Model predictions of the hydrodynamics of external loop airlift reactors have been studied by

a number of researchers (Chisti and Moo-Young, 1988; Hwang and Cheng, 1997; Guo et al., 1997; Loh and Liu, 2001). Such models are based, usually, on momentum or energy balances, taking into account the energy losses along the total circulation loop.

Loh and Liu (2001) developed a model for the estimation of gas holdup and liquid circulation velocity in the external loop inverted fluidized bed airlift bioreactor (EIFBAB). With the valve fully-opened, the reactor became a typical airlift reactor. Conversely, the reactor became a bubbly column when the valve was in a fully-closed position. By controlling the valve opening, liquid circulation velocity and bed fluidization were decoupled from the gas flow rate to enhance gas holdup. This advantage suggested a great potential application for the existing reactor in wastewater treatment and cell fermentation, as the gas holdup was a controlled parameter in culture conditions. In the model of Loh and Liu (2001), the riser gas holdup estimation was made using a modification of the Zuber and Findlay's model (1965). An energy balance was used for the prediction of the liquid circulation velocity. By using the Langmuir-Hinshelwood kinetics, liquid circulation velocity was investigated along with the effects of valve opening positions and gas superficial velocity.

The present study was conducted for a better understanding of reactor performance in different reactor setup and operating conditions. The model developed by Loh and Liu (2001) was used as a basis on which the liquid velocity and gas holdup could be predicted in relation to the gas input rates and valve opening positions. New model was developed to account for the effect of liquid recycle rates on gas holdup and liquid circulation velocity.

4.3 Gas holdup

In an airlift bioreactor, gas holdup is one of the most importance parameters characterizing bubble bed hydrodynamics. Its values determine the fraction of gas formed in the bubble bed and hence the residence time of the gas in the liquid for given values of gas flow rates. Moreover, the difference between gas holdup in the riser and downcomer is responsible for the resulting circulation in the reactor. Therefore, detailed studies of gas holdup were investigated in relation with gas superficial velocity, valve opening positions and liquid recycle rates.

Figure 4.1 shows the correlation between gas holdup and gas superficial velocity in the riser as a function of the liquid recycle rate, where the valve opening is at fully-opened/closed position. Regardless of the effect of valve opening position, the gas holdup is increasing with the increase of gas superficial velocity. As verified by other researchers (Guo et al., 1997; Loh and Liu, 2001), it is due to the fact that with a higher gas superficial velocity, more gas fraction is detected inside the riser resulting in more gas being retained in the liquid and consequently, an increasing gas holdup. Moreover, at the same gas superficial velocity and liquid recycle rate, gas holdup at valve fully-closed position is much higher than that at valve fully-opened position. This is due to the fact that when the valve is fully opened, the induced liquid circulation enhances the bubble rise velocity relative to those at closed-valve position, hence, the lower gas holdup in the former. Moreover, the effect of liquid recycle rate on gas holdup is less at the low liquid recycle rate because liquid circulation velocity was mainly achieved by gas superficial velocity. However, when liquid recycle rate increases, gas holdup decreases significantly because higher liquid recycle rate creates higher liquid flow in the riser section resulting in increasing liquid circulation velocity.

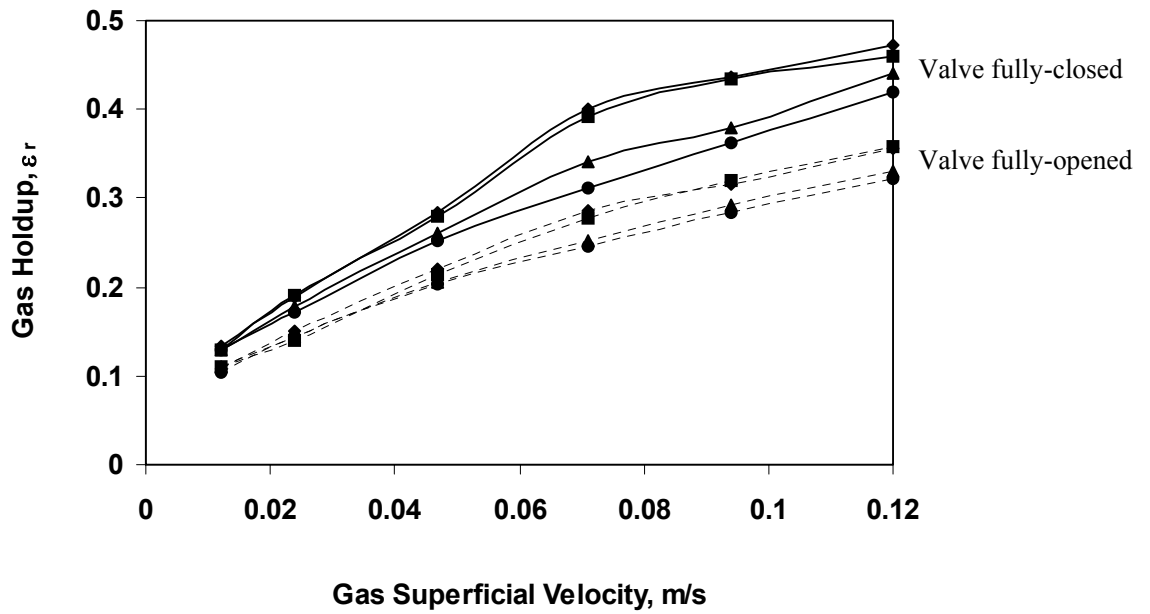


Figure 4.1 Effect of gas superficial velocity on gas holdup for various liquid recycle rates: \blacklozenge 2.5L/h; \blacksquare 5L/h; \blacktriangle 10L/h; \bullet 15L/h (gas holdup data for valve fully-closed are connected by solid lines, whereas these data for valve fully-opened are connected by dotted lines)

As shown by Loh and Liu (2001), gas holdup (ϵ_r) and gas superficial velocity (V_G) were related as a power law, $\epsilon_r = \alpha V_G^\beta$, where V_G is in m/s. By plotting $\ln(\epsilon_r)$ Vs $\ln(V_G)$ for each liquid recycle rate (V_R), the exponents (β) were obtained and these are tabulated in Table 4.1, with the corresponding correlation coefficients..

Table 4.1 Exponent for the power law relationship at valve fully-opened/closed positions.

Valve Position	Exponent β (R^2)			
	V_R (L/h) = 2.5	V_R (L/h) = 5	V_R (L/h) = 10	V_R (L/h) = 15
Fully-closed	0.576 (0.992)	0.603 (0.989)	0.557 (0.986)	0.529 (0.978)
Fully-opened	0.515 (0.990)	0.529 (0.993)	0.533 (0.980)	0.521 (0.994)

From Table 4.1, β values can compare with 0.571 for fully-closed valve and 0.526 for fully-opened valve by Loh and Liu (2001), 0.56 by Bello et al. (1985) and 0.603 by Chisti et al. (1989).

Figure 4.2 shows the observed gas holdups as functions of valve opening position and the different series of plots shown are as functions of various liquid recycle rates. In that case gas superficial velocity is fixed at 0.071m/s for all experiments. As shown in figure, at each liquid recycle rate, gas holdup is seen to steeply decrease at slightly-opened valve positions due to the high-pressure difference between the riser and downcomer. However, gas holdup curve tend to flatten gradually at increasingly open valve positions because high liquid circulation causes a lower pressure difference, resulting in the same low gas holdup. It is obvious that the greater the valve opening position, the lower the gas holdup.

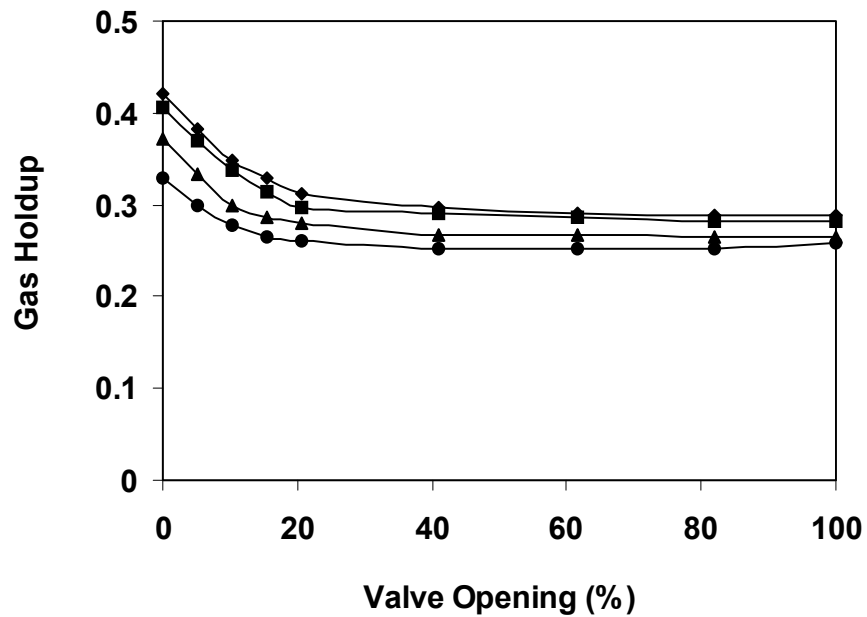


Figure 4.2 Effect of valve opening position on gas holdup as a function of liquid recycle rates: ■ 2.5L/h; ◆ 5L/h; ▲ 10L/h; ● 15L/h (gas superficial velocity $V_G = 0.071$ m/s)

Moreover, in accordance with Loh and Liu (2001), the power law relationships between gas holdup and gas superficial velocity were obtained for each valve opening position. By plotting $\ln(\epsilon_T)$ Vs $\ln(V_G)$ at particular valve opening position for different liquid recycle rate (V_R), the exponent (β) of gas superficial velocity can be obtained and tabulated in Table 4.2 with the corresponding linear regression coefficients.

Table 4.2 Exponent for the power law relationship at different valve opening positions.

Valve Opening (%)	Exponent β (R^2)			
	V_R (L/h) = 2.5	V_R (L/h) =5	V_R (L/h) =10	V_R (L/h) =15
0	0.576 (0.982)	0.603 (0.975)	0.557 (0.979)	0.529 (0.927)
5	0.531 (0.979)	0.582 (0.991)	0.566 (0.988)	0.512 (0.949)
10	0.535 (0.989)	0.567 (0.984)	0.561 (0.949)	0.513 (0.971)
15	0.539 (0.973)	0.557 (0.982)	0.547 (0.953)	0.509 (0.959)
20	0.546 (0.979)	0.552 (0.979)	0.546 (0.976)	0.518 (0.974)
40	0.549 (0.983)	0.549 (0.968)	0.551 (0.981)	0.521 (0.943)
60	0.539 (0.987)	0.546 (0.977)	0.562 (0.984)	0.504 (0.972)
80	0.533 (0.983)	0.546 (0.943)	0.539 (0.952)	0.501 (0.990)
100	0.515 (0.979)	0.529 (0.989)	0.533 (0.923)	0.521 (0.967)
Average	0.540	0.559	0.551	0.513

The average exponents β value for each liquid recycle rate (V_R) are slightly different and not able to take an average. Hence, in order to find a good relationship between liquid recycle rate and gas holdup, exponent (β) Vs liquid recycle rate (V_R) were replotted. The result is shown in Figure 4.3. A 2nd order polynomial is the best-fitted curve and the correlation found is;

$$\beta = - 0.001V_R^2 + 0.010V_R + 0.522 \quad (4.1)$$

Statistical analysis of the curve gave a R^2 value of 0.984.

As shown in Figure 4.3, at low V_R , the V_L value only depends on V_G . However, at high V_R , the effect of V_G on V_L is found to be lower in comparison with that of V_R .

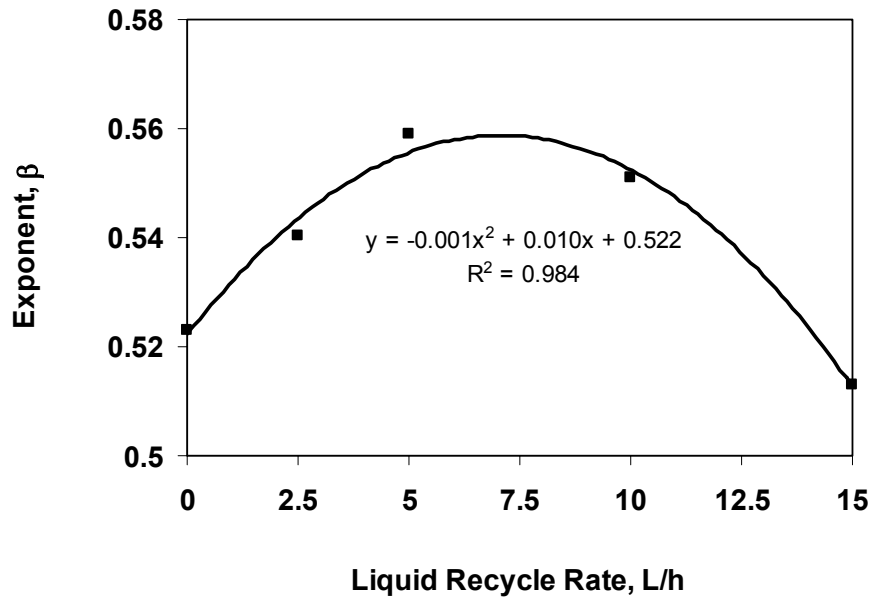


Figure 4.3 Relationship between exponent β and liquid recycle rate.

As According to the equation developed by Loh and Liu (2001), $\varepsilon_r = \alpha \theta^\beta$, $\ln(\varepsilon_r)$ Vs $\ln(\theta)$ are plotted at certain gas superficial velocity and the results are tabulated in Table 4.3.

Table 4.3 Exponent for the power law relationship between gas holdup and valve opening positions.

V_G (m/s)	Exponent β			
	V_R (L/h) = 2.5	V_R (L/h) =5	V_R (L/h) =10	V_R (L/h) =15
0.012	-0.037	-0.047	-0.037	-0.042
0.024	-0.072	-0.055	-0.068	-0.059
0.047	-0.057	-0.056	-0.058	-0.064
0.071	-0.085	-0.089	-0.053	-0.059
0.094	-0.070	-0.079	-0.081	-0.081
0.12	-0.057	-0.063	-0.079	-0.079
Average	-0.063	-0.065	-0.063	-0.064

As seen in Table 4.3, for each liquid recycle rate, the average exponent (β) value can be taken as an average value of -0.064 .

Hence, by combining the two relationship, we found that a power law relationship was fitted to the data for the gas holdup (ε_r) as a function of valve opening (θ in %), gas superficial velocity based on the riser (V_G in m/s) and liquid recycle rate (V_R in L/h).

$$\varepsilon_r = 1.33 \theta^{-0.064} V_G^\beta \tag{4.2}$$

where β is $-0.001V_R^2 + 0.010V_R + 0.522$. It is anticipated that the pre-exponential constant 1.33 is a function of the reactor geometry (Chisti, 1989). Figure 4.4 shows the comparison of the measured values of gas holdup with the predicted values from equation (4.2) are in good agreement with a correlation coefficient of $R^2 = 0.983$. Evidence suggests that the proposed model can predict the riser gas holdup with high degree accuracy in the modified EIFBAB while it is working in different operation modes, gas superficial velocity and valve opening position.

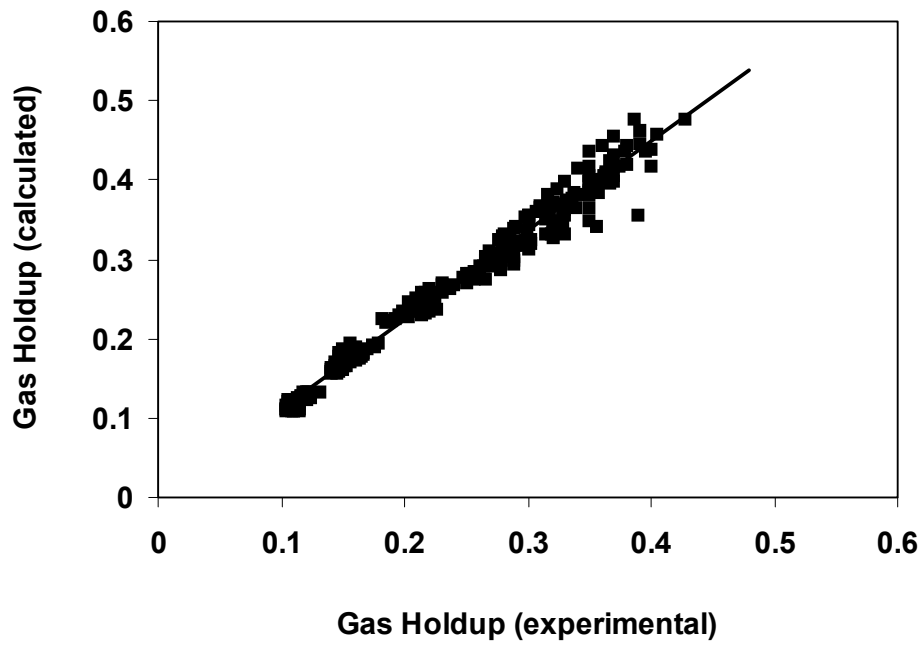


Figure 4.4 Comparison of experimental data and the model predictions using equation 4.2.

4.4 Liquid circulation velocity

The liquid circulation in airlift reactors originates from the difference in the bulk densities of the fluids in the riser and downcomer. This circulation affects turbulence, the reactor wall heat transfer coefficient, the gas liquid mass transfer and the shear forces to which the microorganisms are exposed. Detailed studies of liquid circulation velocity with the effect of gas superficial velocity, valve opening position and liquid recycle rate are discussed in the following sections.

Figure 4.5 shows liquid circulation velocity is plotted against gas superficial velocity using liquid recycle rate as a variable parameter. At the beginning, liquid circulation velocity is steeply increased with increasing gas superficial velocity due to larger driving force between the riser and the downcomer. However, at relatively high value of gas superficial velocity, the increasing rate of liquid circulation velocity is gradually decreased and only a minor increment is observed. This is because the increased rate of the driving force for liquid circulation (density difference between the dispersion phase in the riser and the downcomer) decreases. In addition, it is clear that increasing liquid recycle rate favours the increase in liquid circulation velocity due to decrease in average time for one complete recirculation in the reactor. As many researchers (Livingston and Zhang, 1993; Lu et al., 1994; Guo et al., 1997; Loh and Liu, 2001) reported, we found that there is a power law relationship between liquid circulation velocity (V_L in cm/s) and gas superficial velocity (V_G in m/s), $V_L = \alpha V_G^\beta$, in which the correction factor is 4.128 and exponent β is variable for different liquid recycle rate.

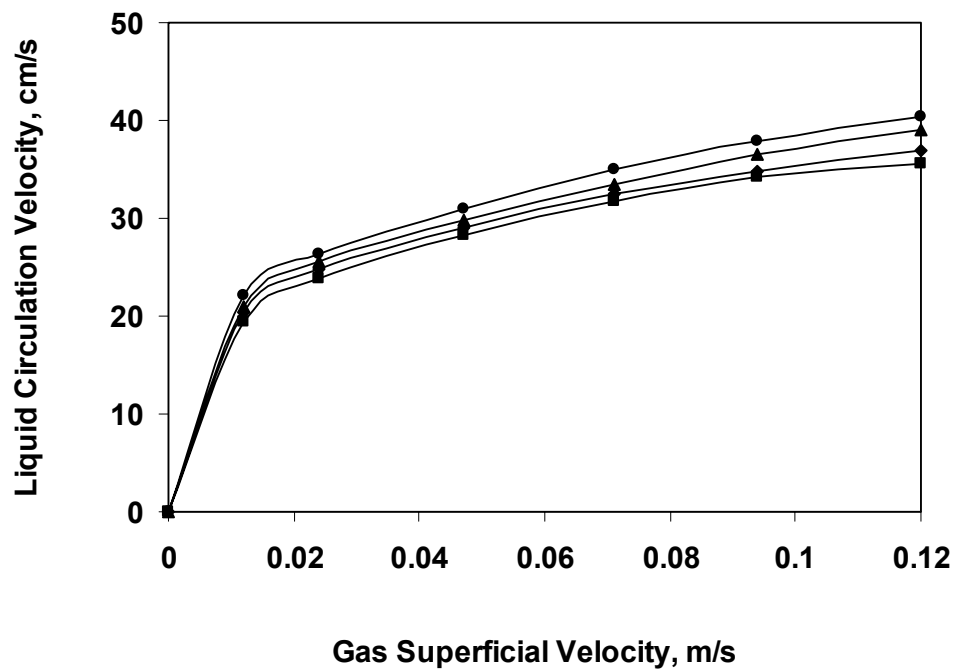


Figure 4.5 Effect of gas superficial velocity on liquid circulation velocity as a function of liquid recycle rates: ■ 2.5L/h; ◆ 5L/h; ▲ 10 L/h; ● 15 L/h.

Exponent β is, therefore, appeared to be a function of liquid recycle rate i.e. $\beta = f(V_R)$. Hence, in order to find a good relationship between liquid circulation velocity and gas superficial velocity, exponent (β) Vs liquid recycle rate (V_R) is replotted. The result is shown in Figure 4.6 and the correlation found is:

$$\beta = - 0.002V_R + 0.257 \quad (4.3)$$

where correlation coefficient gave an R^2 value of 0.98.

In conclusion, the new power law correlation is developed and can be written in the form;

$$V_L = 4.128 V_G^\beta \quad (4.4)$$

where $\beta = -0.002V_R + 0.257$; the liquid circulation velocity (V_L in cm/s); gas superficial velocity (V_G in m/s) and liquid recycle rate (V_R in L/h).

This equation is similar to that of Loh and Liu (2001), Choi and Lee (1993) except V_R term which represents the liquid recycle rate. The predicted data using equation (4.4) is fitted with the experimental data and shown in Figure 4.7. Excellent agreements are observed with regression factor of $R^2 = 0.99$.

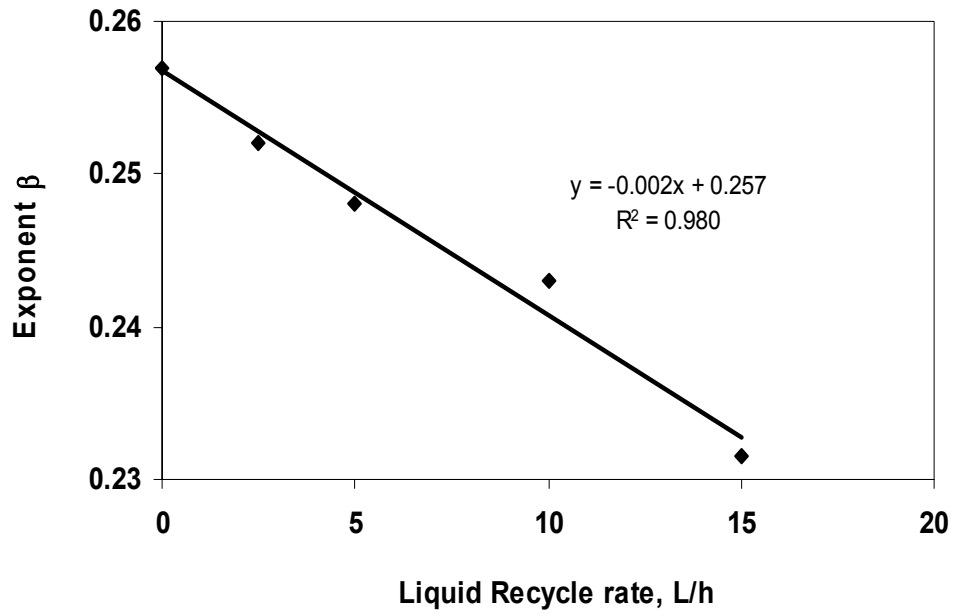


Figure 4.6 Relationship between Exponent β and liquid recycle rate V_L .

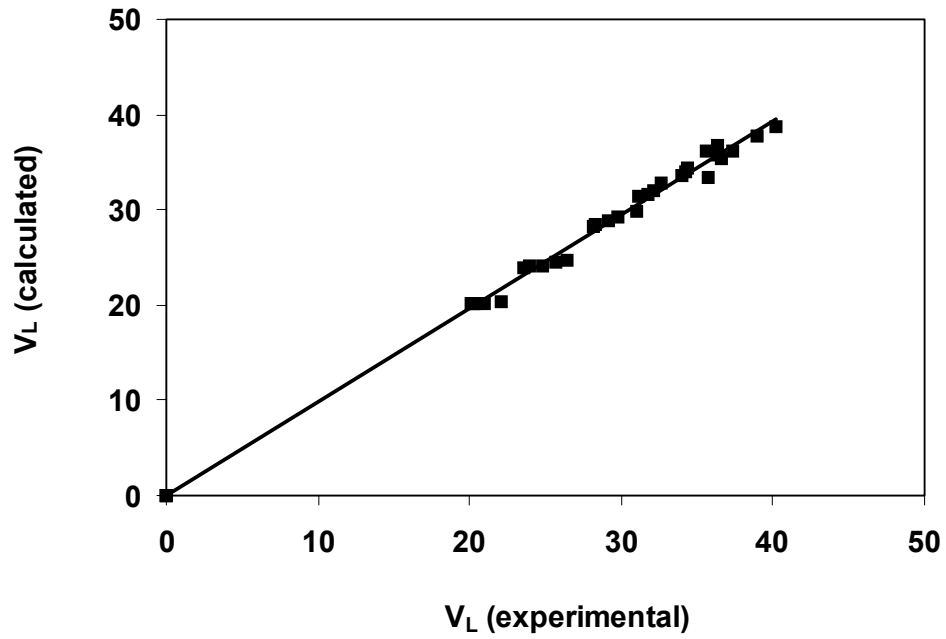


Figure 4.7 Comparison of experimental data and model predictions using equation (4.4).

Figure 4.8 illustrates the effect of valve opening on liquid circulation velocity as a function of gas superficial velocities. For each gas superficial velocity, it is observed that by closing the valve, the liquid circulation velocity decreased sharply and consequently increased gas holdup. However, liquid circulation curve tends to flatten at high valve opening position because of reducing density difference between the riser and downcomer.

Therefore, based on the behaviour of the experimental data (Similar to the Langmuir Hinshelwood kinetics), the liquid circulation velocity (V_L) was modelled in terms of gas superficial velocity (V_G in cm/s), valve opening position (θ in %) and liquid recycle rate (V_R in L/h). The following expression is obtained;

$$V_L = \left[\frac{\theta}{0.018 + (-0.0003V_R + 0.052)\theta} \right] V_G^\beta \quad (4.5)$$

where $\beta = -0.002V_R + 0.257$ (Ref to equation 4.3).

Figure 4.9 represents the comparison of the experimental data with the model (equation 4.5). The agreement between the predicted modelled values and the experimental values is very good with $R^2 = 0.99$.

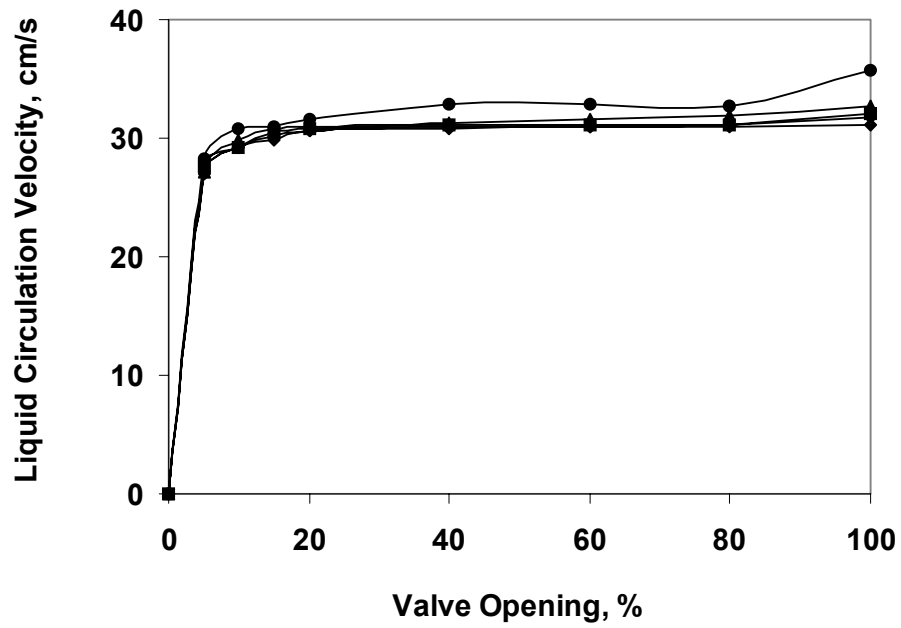


Figure 4.8 Effect of valve opening position on liquid circulation velocity for different liquid recycle rates: ■ 2.5L/h; ◆ 5L/h; ▲ 10L/h; ● 15L/h (gas superficial velocity $V_G = 0.071\text{m/s}$)

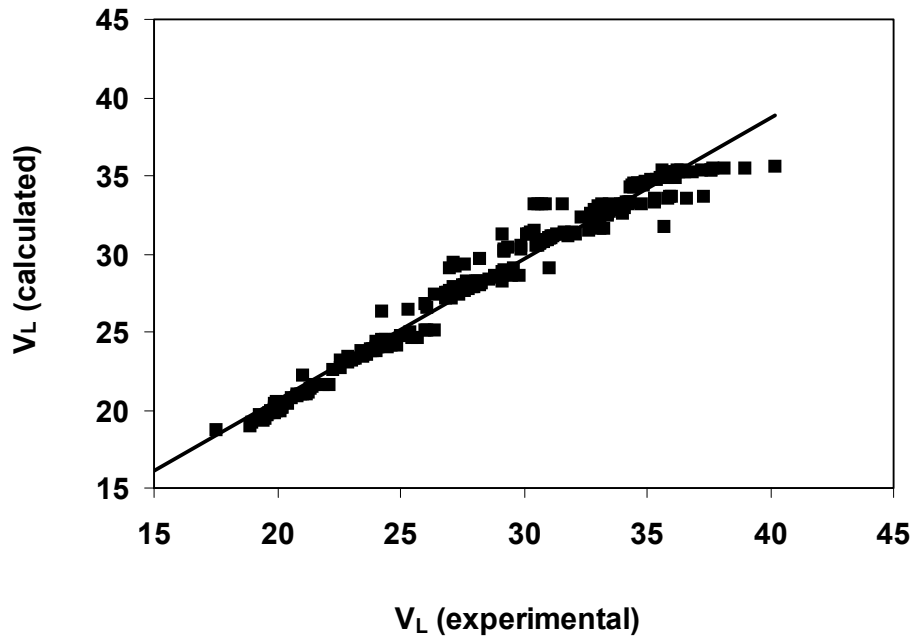


Figure 4.9 Comparison of experimental data for liquid circulation velocity and model predictions using equation (4.5).

CHAPTER 5

BIODEGRADATION STUDIES IN MODIFIED EIFBAB

5.1 Summary

In this study, an EIFBAB was modified with a mixed-tank reactor placed in series to extend the phenol treatability range. *Pseudomonas putida* ATCC 11172, which is able to degrade phenol and has a strong surface-attaching ability to form a biofilm, was used. In order to reduce the toxic effects of phenol on cells, cell immobilization on high porosity EPS beads was proposed in this work. Phenol degradation at high concentrations was investigated at recycle rates ranging from 0 to 15L/h under batch operation. With a total working volume of 10L, the new setup could handle 3000mg/L phenol at an enhanced total biodegradation rate of 90mg/h at an optimized operation of 5L/h recycle rate, compared to 46mg/h in the original EIFBAB. Continuous operation was ascertained for complete degradation up to 3500mg/L phenol and feed flow rates up to 160mL/h. The recoverability of the system under shock loading of phenol up to 3500mg/L was also assessed.

5.2 Introduction

In an earlier study by Loh and Liu (2001), a 4L external loop inversed fluidized bed airlift bioreactor (EIFBAB) was constructed, characterized and tested for treating high strength phenolic wastewater. Expanded polystyrene beads (EPS) were used as supporting materials for cell immobilization in the downcomer of the EIFBAB. With enhanced gas holdup and cell immobilization, the novel bioreactor allowed for biodegradation of high strength phenolic wastewater up to 1200mg/L phenol. Phenol

concentrations in excess of 2000mg/L either experienced extended lag periods, or no degradation at all. This occurred because of substrate inhibition effects experienced by the cells at elevated phenol concentrations, a fact well established in the literature (Hill and Robinson, 1975). Furthermore, cell immobilization could only provide a limited extension of the substrate inhibition threshold (Keweloh et al., 1989; Chung et al., 1998a; 1998b).

In the hollow fiber membrane cell immobilization studies conducted by Chung et al. (1998a, 1998b) and Loh and Liu (2001), it was found that the membrane immobilized cells could degrade high phenol concentrations to a sufficiently low enough level before suspension cells started to leak from the membranes. When this happened, subsequent high degradation rates resulted from both the suspended cells and the membrane immobilized cells. Based on these observations, it was anticipated that the EIFBAB could benefit from the inclusion of a suspended cell reactor to extend the treatability range of the phenolic wastewater. This modification would provide additional biodegradation means via suspended and wall-growth bacteria in a mixed tank reactor, and at the same time provide for dilution of high phenol concentrations in the feed under continuous mode.

In the present study, the EIFBAB was incorporated with a well-mixed reactor placed in series. The partially degraded phenol solution was withdrawn from the downcomer of the EIFBAB and recycled into the well-mixed reactor, and the effluent from this was recirculated back to the EIFBAB through a port located at the bottom of the riser. With the presence of the well-mixed reactor, and consequential withdrawal of liquid

from the downcomer of the EIFBAB, the biodegradation efficiencies of this reactor under different operation mode were investigated.

5.3 Baseline study of phenol degradation in EIFBAB

To establish a basic study for comparison between the original EIFBAB and the current system, the formal EIFBAB was operated under batch mode for biodegradation using initial phenol concentration of 2400mg/L. In both experiment, valve-opening position is between 5–10 % in order to get a good fluidization in the downcomer. As it was discussed before, the gas holdup is around 0.45.

Figure 5.1 shows the total degradation and cell growth profile of initial phenol concentration of 2400mg/L. There was a significant lag phase of 60h before degradation of phenol was detected. After the lag period, phenol was steadily degraded for the next 70h, during which there was no dramatic increase in suspended cells in solution. When the phenol concentration dropped to about 1500mg/L, cells began to grow in suspension, and these rapidly ensued an exponential growth rate. During this rapid cell growth period, phenol degradation was enhanced and phenol was completely depleted in 210h from the start of the operation. The overall degradation rate of this experiment was therefore 11.4mg/L-h.

Figure 5.2 shows the biodegradation and cell mass profile of initial phenol concentration of 3000mg/L. There was no phenol degradation was occurred for 100h. After significant lag phase, phenol started to degrade slowly until suspended cell density increases. The exponential growth phase is attained after 200h whereas phenol concentration reached 1000mg/L. Phenol was totally degraded after

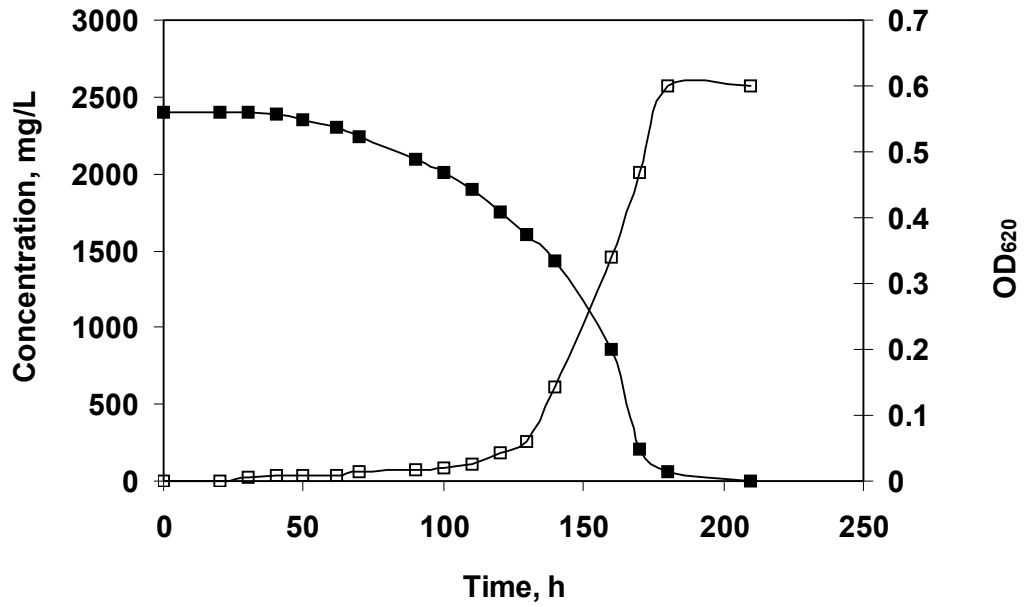


Figure 5.1 Biodegradation profile of initial phenol concentration of 2400mg/L:
■ Phenol degradation profile; □ cell density profile

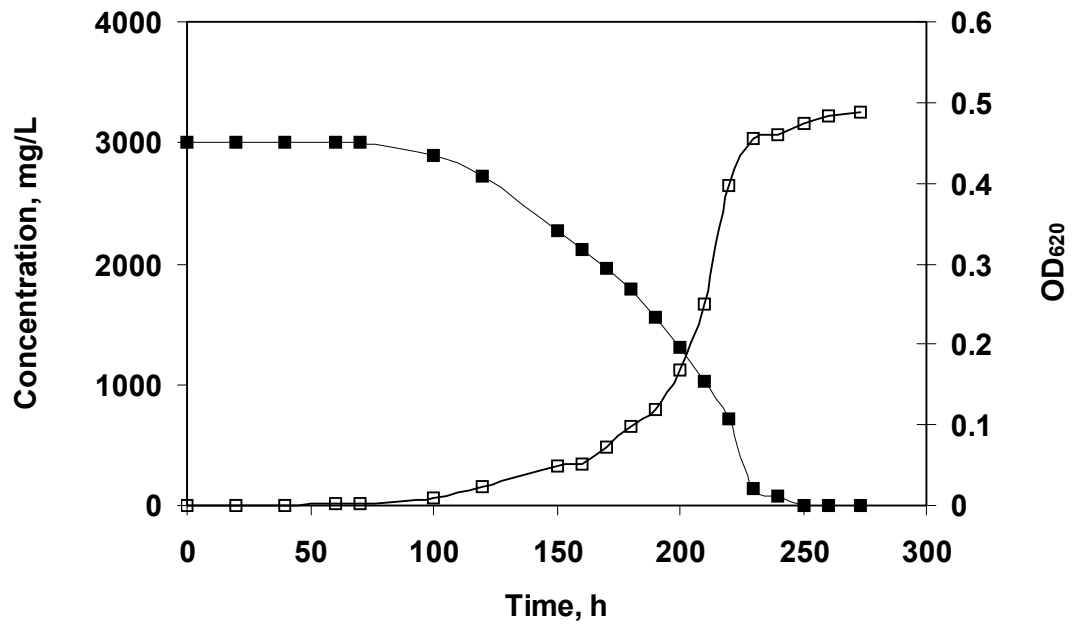


Figure 5.2 Biodegradation profile of initial phenol concentration of 3000mg/L:
■ Phenol degradation profile; □ Cell density profile

250h with overall degradation rate of 10.5mg/L-h.

In comparison with experiments recorded in Figure 5.1 and 5.2, the lower initial phenol concentration, the larger the degradation efficiency is observed. This is due to the fact that toxicity to cell growth and inhibition of substrates increase with increasing initial phenol concentration resulting in lower degradation rate. At the end of the experiment, the lower initial phenol concentration has higher suspended cell density because the use of higher phenol concentrations leads to a reduction of the cells liberated from the EPS beads into the medium, which may be due to the toxic effects of phenol on the cells inside the beads.

Furthermore, without the addition of microorganisms into the system, an abiotic test is conducted using 2400mg/L phenol to determine if any physical and chemical reactions occurred. The control experiment shows no change in phenol concentration up to 3 days (data not shown) indicating that the phenol does not otherwise removed by volatilization or other physical reactions.

5.4 Phenol degradation in modified EIFBAB under batch operation

The modified EIFBAB reactor was operated under batch operation to test its performance in terms of enhanced degradation rate, as well as to optimize the recycle rate between the EIFBAB and the mixed-tank reactor. The recycle rate was varied from 2.5L/h to 15L/h, and the system was tested on 2400mg/L and 3000mg/L phenol. Moreover, to establish a basis for comparison between the EIFBAB and the current system, the EIFBAB alone was also operated under batch mode for biodegradation of

2400mg/L and 3000mg/L phenol, i.e. recycle rate of 0L/h. All biodegradation experiments in this section are summarized in Table 5.1.

Table 5.1 Summary of biodegradation experiments in modified EIFBAB

Experiments	Initial concentration (mg/L)	Recycle rate (L/h)
B1	2400	2.5
B2	2400	5.0
B3	2400	10
B4	2400	15
B5	3000	2.5
B6	3000	5.0
B7	3000	10
B8	3000	15

For both phenol concentrations, the results were similar, and representative results for the runs performed at 2400mg/L are shown in Figure 5.3 which plots the phenol concentration in the exit stream from the downcomer of the EIFBAB, as well as the corresponding suspended cell concentration for the various recycle rate studied (i.e. Experiment B1 to B4). For batch operation ($V_R= 0L/h$), there was a significant lag phase of 60h before degradation of phenol was observed. After the lag period, phenol was steadily degraded for the next 70h, during which time there was no dramatic increase in suspended cells in solution. It is clear that phenol depletion was accomplished by the immobilized cells during this period. When the phenol concentration dropped to about 1500mg/L, cells began to grow in suspension, and these rapidly ensued an exponential growth rate. During this rapid cell growth period, phenol degradation was enhanced and phenol was completely depleted in 210h from the start of the operation. The overall degradation rate of this experiment was therefore 11mg/L.h.

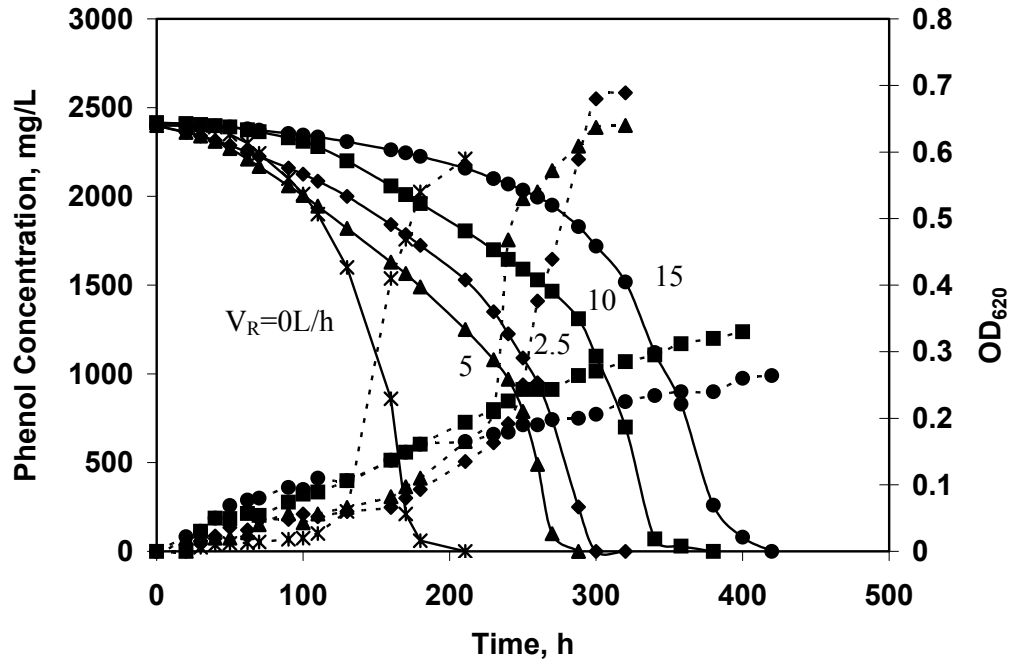


Figure 5.3 Biodegradation and biomass profile for 2400mg/L phenol at different recycle rates in modified EIFBAB. (Phenol concentration data are connected by solid line, while biomass data are connected by dotted lines. * 0L/h; ◆ 2.5L/h; ▲ 5L/h; ■ 10L/h; ● 15L/h.

When *Pseudomonas Putida* ATCC 11172 was cultivated in suspension, due to substrate inhibition effects from phenol, the bacteria could not grow at phenol concentrations exceeding about 800mg/L. It is clear from this experiment that immobilization of the bacteria on the EPS beads allowed the cells to acclimate to a higher phenol concentration. This is consistent with that reported by Keweloh (1989). Moreover, when the phenol concentration decreased to a low level (1500mg/L in this case), the cells leaked from the biofilm to grow in suspension. In suspension, due to the absence of diffusional limitations, the cells were directly exposed to the nutrient substrate, and therefore cell growth was accompanied by a high rate of phenol disappearance.

It can be seen that the degradation profiles for low recycle rates (2.5L/h and 5L/h) were similar, with immediate decrease of phenol from the beginning. The total degradation time was however slightly shorter at 5L/h, being at 280h compared to 300h at 2.5L/h. It is important to note here that the experiments have been performed in duplicates to maintain reproducibility. However, for clarity, error bars were not included in the figure, but it suffices to mention that in the duplicate experiments, variations in phenol concentrations never exceeded 10% while cell density measurements never differed by more than 7%. The reduced degradation time could be due to the slightly higher gas holdup observed at 5L/h. It happened that in order for the bed expansion in the downcomer to remain uniform, the globe-valve had to be closed slightly (from 10% valve opening to about 5% valve opening) resulting in the slightly higher gas holdup (0.45 vis-à-vis 0.4). The overall effect of this is a mere 7% increase in the overall degradation rate.

The degradation profiles at higher recycle rates (10L/h and 15L/h) were more interesting. Both profiles witnessed a lag phase of about 100h before phenol was steadily depleted. This could be due to the sloughing of the biofilm at higher liquid circulation velocity (hence higher liquid shear) in the EIFBAB as a result of the high increase in the recycle rate, which more than overweighed the slight increase in gas holdup. At all recycle rates, there was a slow, but steady increase in suspended cell concentration during the first 200h, which was even higher in the cases of 10L/h and 15L/h recycle rates. These suspended cells were not capable of degrading the high phenol concentration and therefore much degradation capacity was dependent on the remainder of the biofilm on the EPS beads. Consequently, operating the system at 10L/h gave total phenol depletion in 380h while operation at 15L/h took 420h to complete. Despite the higher liquid shear generated from adding a recycle stream, the overall degradation rate was enhanced because of the presence of the mixed-tank reactor. Cells sloughed off the biofilm (at all the recycle rates studied) eventually grew in suspension in the mixed-tank reactor when the phenol concentration dropped to a manageable level, and this contributed significantly to the overall degradation given that the working volume in the mixed-tank reactor was 6L and that in the EIFBAB was 4L.

For degrading 2400mg/L phenol, results in the batch mode operation show that the volumetric degradation rate in the EIFBAB (4L working volume) was 11mg/L-h while the current system (10L working volume) operated at 5L/h recycle rate achieved a comparable volumetric degradation rate of 9mg/L-h. However, what's important is that the current system did not experience a significant lag period (for $V_R < 5L/h$), even when operated in the batch mode, and as will be shown in the later experiments that

the modified EIFBAB could handle phenol concentrations exceeding 3000mg/L under continuous operation, at comparable volumetric degradation rates.

Figure 5.4 shows the results obtained by measuring substrate concentrations corresponding to time for initial phenol concentration of 3000mg/L (experiments B4 to B8) combined with cell mass profile. Degradation profiles for each recycle rate (5L/h to 15L/h) were similar to Figure 5.3 with no phenol degradation from the beginning to 100h. The complete degradation time decreases when liquid recycle rate is increased from 2.5L/h having a 400h degradation time compared to 380h at 5L/h because of getting better mass transfer in both EIFBAB and well-mixed tank. During the first 250h, the biomass concentration change slightly because of the toxicity of high concentration to the cell. Although phenol could be degraded at 2400mg/L and 3000mg/L in the EIFBAB, the bioreactor could not operate at phenol concentrations beyond 3000mg/L.

The degradation rate as a function of recycle rate for both phenol concentrations – 2400mg/L and 3000mg/L conducted in the current system are summarized in Table 5.2.

Table 5.2 Effect of recycle rate on volumetric degradation rate for different initial phenol concentration.

Initial phenol concentration (mg/L)	2400	3000
Recycle rate (L/h)	Volumetric degradation rate (mg/L-h)	
0	11	12
2.5	8	8
5	9	8
10	6	7
15	6	6

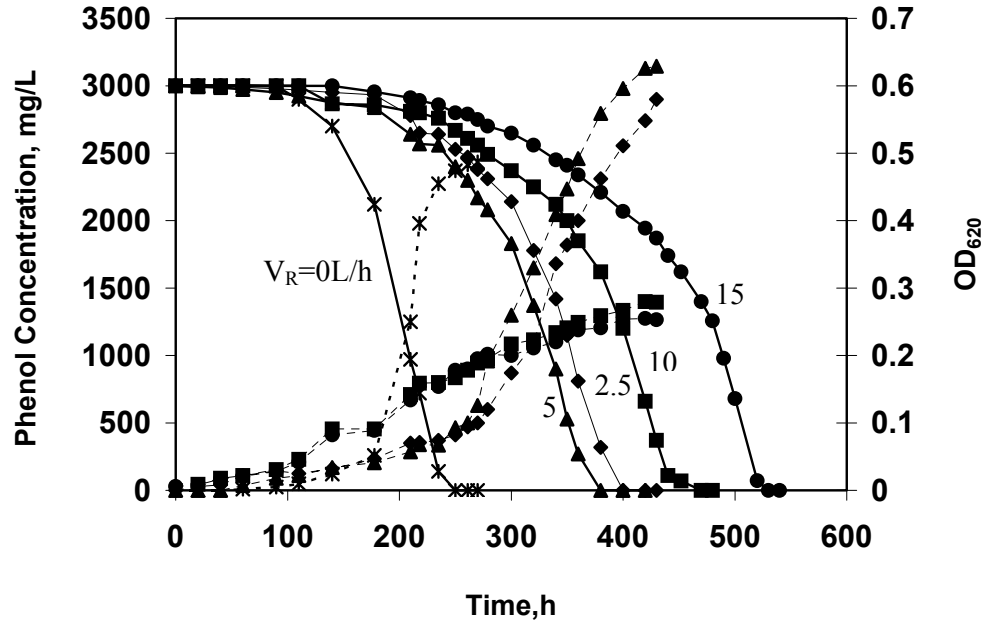


Figure 5.4 Biodegradation and biomass profile for 3000mg/L phenol at different recycle rates in modified EIFBAB. (Phenol concentration data are connected by solid line, while biomass data are connected by dotted lines. * 0L/h; ◆ 2.5L/h; ▲ 5L/h; ■ 10L/h; ● 15L/h.

However, liquid recycle rate ranging from 5L/h to 15L/h, the higher the liquid recycle rate, the longer the degradation time is required to get complete degradation. As a consequence, lower degradation rate is observed. Hence, it is clear that there exists an optimum recycle rate for operation, which is at about 5L/h.

5.5 Effect of feed rate under continuous operation

The treatment performance of toxic wastes can be destroyed by high substrate phenol loading which are caused by the improper operations or concentration fluctuation. Once a shock load occurs, the system requires a long-term adaptation and hence, to avoid washout, suitable dilution rate (suitable inlet feed rate for constant volume) is vital.

To investigate the maximum inlet feed rate that could be degraded high initial phenol concentration, the system was operated under continuous operation after achieving total degradation of 500mg/L phenol under batch operation at V_R of 5L/h. Feed solution containing 2500mg/L and 3500mg/L were fed separately in two experimental runs at feed rates ranging from 80mL/h to 320mL/h to elucidate the effect of feed rate on the steady state phenol concentration in the effluent. Table 5.3 shows the results obtained from these experiments. For the run at 2500mg/L phenol, feeding continuously up to 160mL/h gave complete phenol degradation and the reactor effluent was maintained at 0mg/L phenol. However, when the feed rate was increased to 240mL/h and 320mL/h, there was gradual breakthrough of phenol and new steady state phenol concentrations were obtained, at 870mg/L and 1800mg/L, respectively. For completely degrading 2500mg/L phenol, therefore, the maximum feed rate that can be used was limited to 160mL/h. For the case of 3500mg/L, this limit was further

diminished. Breakthrough of phenol in the effluent occurred at a feed rate of 160mL/h, although a new steady state phenol concentration of 860mg/L could be achieved.

Table 5.3 Effect of feed rate on steady state effluent phenol concentration

Initial [phenol] (mg/L)	2500	3500
Feed rate (mL/h)	Steady state [phenol] (mg/L)	
80	0	0
160	0	860
240	870	1830
320	1800	2820

When the feed rate was high, biodegradation rates in the EIFBAB and the mixed-tank reactor could not cope especially when the feed phenol concentration was high. That there exists an optimum operating feed rate in the system is evident from results of the overall degradation rate, which are summarized in Table 5.4.

Table 5.4 Effect of feed rate on overall degradation rate for different initial phenol concentration in continuous operation.

Initial phenol concentration (mg/L)	2500	3500
Feed rate (mL/h)	Overall degradation rate (mg/L-h)	
80	20	28
160	40	42
240	39	40
320	22	22

The overall degradation rate was obtained from the product of the amount of phenol removed at steady state and the dilution rate of the system (or 1/HRT). From Table 5.4, at low feed rates, because phenol was almost completely depleted, the overall degradation rate was dependent only on the dilution rate. This therefore increased with increased feed rate. At high feed rates, phenol was not completely degraded at steady

state, and despite the increase in dilution rate, the overall degradation rate decreased. The maximum feed rate was found to be about 180mL/h for degrading both phenol concentrations investigated, although in the case of 3500mg/L, the steady state phenol concentration at that feed rate was not 0mg/L.

5.6 Sustained continuous operation

To evaluate the system performance under long term continuous operation at high phenol concentrations, the system was operated at two feed rates 80mL/h and 160mL/h (HRT of 125h and 62.5h, respectively) for feed concentrations ranging from 500mg/L to 4500mg/L. The methodology used in all tests was the same as described in baseline study and phenol degradation in modified bioreactor. Furthermore, all subsequent continuous runs were performed at 5L/h recycle rate.

The results obtained for both of these runs were similar, and representative data are shown in Figure 5.5. At each phenol loading, the system was operated at the steady state of 0mg/L phenol in the effluent for at least 50h before increasing to the next phenol loading level. This ensured that the biofilm was fully acclimated at each phenol concentration. It can be seen that the system was very stable for phenol loading of up to 56mg/L-h. When the loading was increased further to 72mg/L-h, there was gradual breakthrough of phenol in the effluent over time. In order to assess the recoverability of the system, phenol loading was lowered back to 56mg/L-h before a new steady state of effluent phenol was achieved, for fear that the system might fail if we had continued at the higher loading. With a lowering of the phenol concentration back to 3500mg/L, we found that the system recovered, and a steady state effluent with no phenol present was once again obtained. This occurred because the biofilm was already adapted to the

high phenol concentration, and there was no significant damage done to it when the concentration was increased to 4500mg/L (72mg/L-h phenol loading) over the 50h period.

5.7 Response under shock loading

In the presence of shock loading and/or toxic compound in feed wastewater, system performance drops quite significantly as a result of partial loss of microbial activity. Hence, In order to study the capacity of the system to withstand sudden increase in phenol loading, the system was operated at an HRT of 125h, and phenol concentration was increased gradually from 500mg/L to 5000mg/L after every 25h of operation. During each time period, it was envisaged that the biofilm did not have sufficient time to acclimatize. Figure 5.6 shows that no phenol breakthrough in the effluent occurred right from the beginning, and even when the phenol concentrations were suddenly increased, the effluent remained clear of phenol until the feed concentration reached 4500mg/L phenol. At that concentration, the system could not absorb the shock, and phenol breakthrough was detected. The feed concentration was increased further to 5000mg/L and it can be seen that despite a breakthrough in phenol in the effluent, the breakthrough rate was gradual, and the effluent concentration did not increase beyond 1000mg/L phenol.

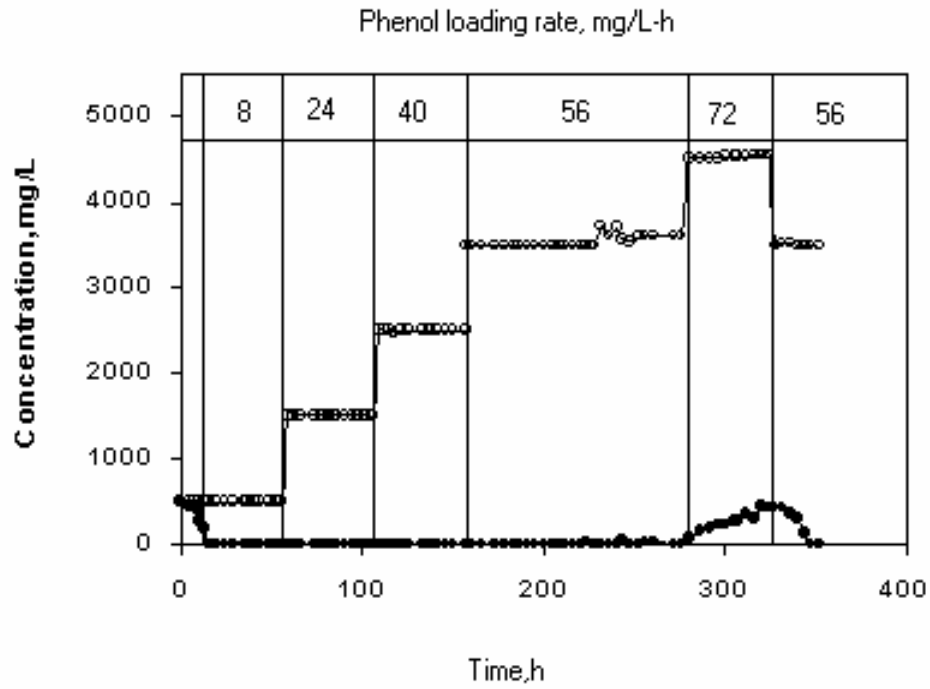


Fig 5.5 Influent and effluent phenol concentrations under sustained continuous operation of modified EIFBAB at fixed HRT of 62.5 hours.
○ phenol concentration in the influent; ● phenol concentration in the effluent

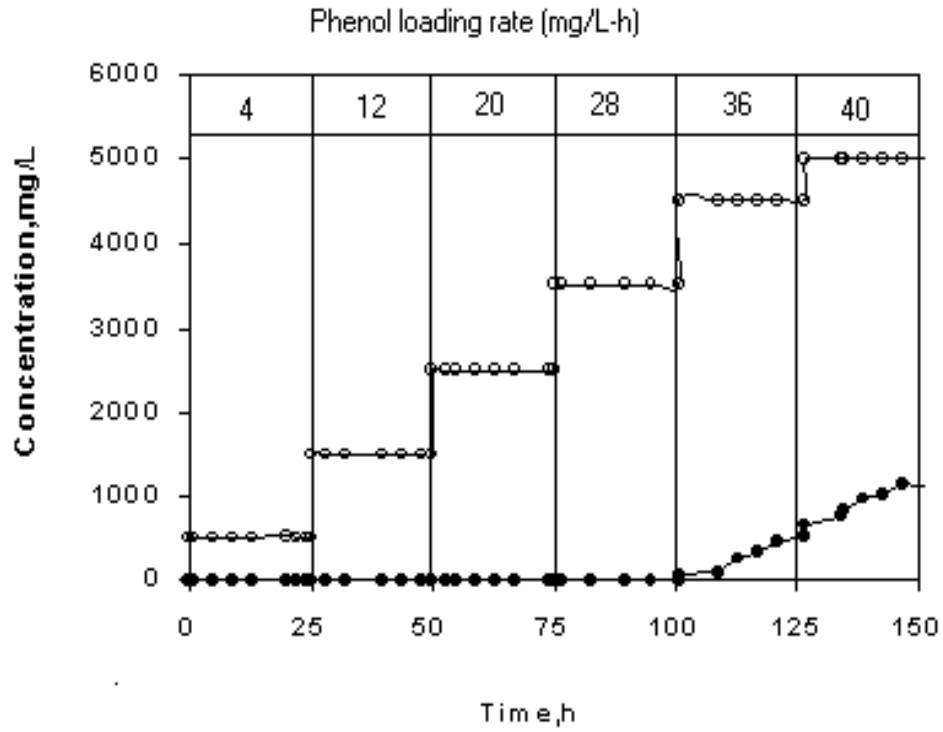


Figure 5.6 Influent and effluent phenol concentrations under shock loading in the modified EIFBAB operated at fixed HRT of 125 hours.
 ○ phenol concentration in the influent; ● phenol concentration in the effluent

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Hydrodynamics studies

This study set out to establish a design basis for the proposed reactor, and hence experiments were conducted to investigate the effects of liquid recycle rates, the airflow rates, and valve opening positions on the riser gas holdup and liquid circulation velocity under cell-free conditions. It was found that liquid recycle rate did not have a significant influence on the parameters studied and was observed as slight changes of gas holdup and liquid circulation velocity for high superficial velocities. Any increase in the liquid recycle rate in an airlift reactor enhanced bubble rise velocity and consequently reduced gas holdup. On the contrary, superficial gas velocities have a great effect on gas holdup and liquid circulation velocity. Such an increase in gas superficial velocity led to an increase in gas holdup and liquid circulation velocity while circulation time consequently decreased. The experimental results were correlated using linear regression. The correlations are summarized in Table (6.1).

From Table 6.1, the constant in the correlation are related to the physical properties of liquid, the geometry of the reactor, the solid loading in the downcomer and the sparger location in the riser, as well as the reactor's operating system. From the results presented, it was found that the predicted values using the model are in good agreement with the experimental values of gas holdup and liquid circulation velocity obtained, for all experiments.

Table 6.1 Summary of empirical correlations.

Correlation	Equation	Remarks
$\varepsilon_r = f(V_G)$	$\varepsilon_r = 1.13 V_G^{0.522}$	V_G (m/s)
$\varepsilon_r = f(V_G, \theta)$	$\varepsilon_r = 1.13 \theta^{-0.064} V_G^{0.522}$	θ (%), V_G (m/s)
$\varepsilon_r = f(V_G, \theta, V_R)$	$\varepsilon_r = 1.13 \theta^{-0.064} V_G^\beta$ where $\beta = -0.001V_R^2 + 0.010V_R + 0.522$	θ (%), V_G (m/s), V_R (L/h)
$V_L = f(V_G)$	$V_L = 4.128 V_G^{0.248}$	V_G (m/s)
$V_L = f(V_R)$	$V_L = 4.128 V_G^\beta$ where $\beta = -0.002V_R + 0.257$	V_G (m/s), V_R (L/h)
$V_L = f(V_G, V_R, \theta)$	$V_L = \left[\frac{\theta}{0.018 + (-0.0003V_R + 0.0523)\theta} \right] V_G^\beta$ where $\beta = -0.002V_R + 0.257$	θ (%), V_G (m/s), V_R (L/h)

In summary, the gas holdup increases with the increase in gas superficial velocity but decreases with the increasing liquid recycle rate and valve opening positions, according to the power law relationship. With a higher gas superficial velocity, there is a higher gas fraction inside the riser and more gas is retained in the liquid, enhancing a higher gas holdup. It is obvious that by increasing the valve opening position, the gas holdup can be decreased at each gas superficial velocity because of the higher liquid circulation rate. On the other hand, whenever recycle rate is increased, gas holdup decreases because of the lower residence time inside the reactor. With liquid recycle

rates ranging from ($0 < V_R < 5$ L/h), a decrease in gas holdup is not as significant as that in the original EIFBAB. A different trend was observed for liquid recycle rates ranging from ($5 > V_R > 15$ L/h) because the gas retention time was manifestly decreased. As a consequence, a decrease in liquid circulation velocity is attributed to the fluidised bed, resulting in an increase of bubble residence time in the reactor. This resulted in the lowering of the liquid circulation and hence, a higher gas holdup in the riser at the same gas superficial velocity.

Before universal design and operation correlations could be developed, the following recommendations for additional work are in order:

- 1) The hydrodynamic regime and flow patterns at the exit of the riser, in the liquid separator and at the entrance of the downcomer need to be examined. The flow patterns in these regions are very complex and their understanding would allow the development of local friction factors and dispersion coefficients. A better understanding of these regions would also allow for better manipulation of any flexibility in the reactor during operations.
- 2) A better understanding is needed for hydrodynamic flow regimes, flow regime transitions as well as bubble coalesce and redispersion, in all the different sections of the reactor.
- 3) Although methods and techniques to measure local sectional mass transfer coefficients have been presented (Merchuk and Seigel, 1988), no method of measuring local $K_{L,a}$ values nor sufficient reliable data for local dispersion coefficients currently

exists. More information is therefore needed on the contribution of the mass transfer to airlift reactor behaviour.

4) The greatest gap in airlift reactor knowledge which still exists is a thorough understanding of the transport phenomena in these reactors under actual fermentation conditions.

5) Much more work is needed on the scale-up of airlift reactors before conclusive statements can be made about this process. For example, in short reactors, where kinetic energy input from the gas injection and gas sparger design will be significant, geometrically similar designs may not give similar results during scale-up.

6.2 Biodegradation studies

This work studies the effects of different operation modes in the biodegradation of phenol. In the preliminary tests, batch operation mode involving different initial phenol concentrations was studied. In addition, the influence of liquid recycle rate on the process performance of the EIFBAB reactor incorporated with well-mixed tank at different initial phenol loading conditions was investigated. Using experimental results, the optimum liquid recycle rate was developed. Finally, the optimum recycle rate was used for predicting the reactor performance in continuous mode.

According to the results of this study, the following conclusions can be drawn;

1) Phenol was shown to have an inhibitory effect on *P. Putida* ATCC 11172 above a concentration of 800 mg/L. Increased phenol concentration was also shown to increase

the lag-phase before exponential growth of the bacteria. At a phenol concentration of 2400-3500mg/L, the lag-phase extended over more than four days.

2) Initially, cell concentration and degradation efficiency were higher along with higher liquid recycle rates, resulting in a higher rate of substrate consumption till optimum liquid recycle rate was reached. Conversely, it was found that the higher the recycle rate, the lower the degradation efficiency obtained due to cell washout and short residence time inside the reactor.

3) Initial investigations into the continuous breakdown of phenol proved successful with complete degradation of phenol along with a gradual increase in concentration from 500mg/L to 4500mg/L in the system. It was shown that complete breakdown of phenol was dependent largely on the retention time of the system.

4) The most favorable flow rate through the system was to found to be 160 ml/hr which in the system set up in the laboratory, resulted in a retention time of 65 hours. This was equivalent to 54 mg of phenol/ L-h.

5) The ability of the system to withstand a shock load i.e., a sudden increase in the concentration of phenol, indicated that the system could not cope with sharp increases of the influent into the system. It was found that this was not due to the nutrients becoming limited as they were present in large quantities. It was thought that there might be a lag-phase in the system due to the sudden increase in phenol concentration.

6) The experimental results showed that it was possible to treat industrial effluents containing high phenol concentrations.

Based on the results obtained in the current work, some recommendations for future work are suggested.

1) EPS beads are good supporting carriers in an inversed fluidized bed for biofilm formation because of their high porosity. According to experimental results, the cells were easily detached from the biofilm, especially when liquid velocity is high. However, because of cell preferences and their initial attachment onto hydrophilic surfaces, EPS beads could be made of a hydrophobic material. Surface modification of the EPS beads is recommended to change the physical and chemical properties of the surface. The suggested methods could be chemical oxidation or plasma etching, etc.

2) The surface area-to-volume ratio of a bead and the number of beads are critical determinants of the degradation capacity of the bioreactor. According to the results, with an increased distance in the matrix and the decrease in the surface area-to-volume ratio of the beads, degradation rate could certainly decrease. Hence, either decreasing bead diameter or increasing solids loading is recommended.

3) To understand the biological degradation in the EIFBAB more systematically, some modifications of the reactor are also recommended. For instance, DO probes can be installed to obtain more accurate values of gas holdup, while mass transfer behavior can also be studied indepth. Other parameters such as the ratio of riser area over

downcomer (A_d/A_r), ratio of height over area (H/D), as well as culture volume are areas for further study.

4) The whole system was found to be capable of running over a long period of time but one of the main problems was maintaining the integrity of the system. This study was on the ability of *Pseudomonas putida* ATCC 11172 to degrade phenol. However, contamination proved to be one of the problem encountered. Even though strict measures were taken to prevent contamination, i.e. autoclaving of influent, attachment of sterilizing filters to the air supply and into the continuous flow system, contamination inevitably still occurred. This contamination led to a loss in the overall rate of phenol breakdown and it also increased significantly the rate of clogging of the beads. These contamination problems could be the areas for future studies.

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Appendix A

EXPERIMENTAL SETUP FOR EIFBAB

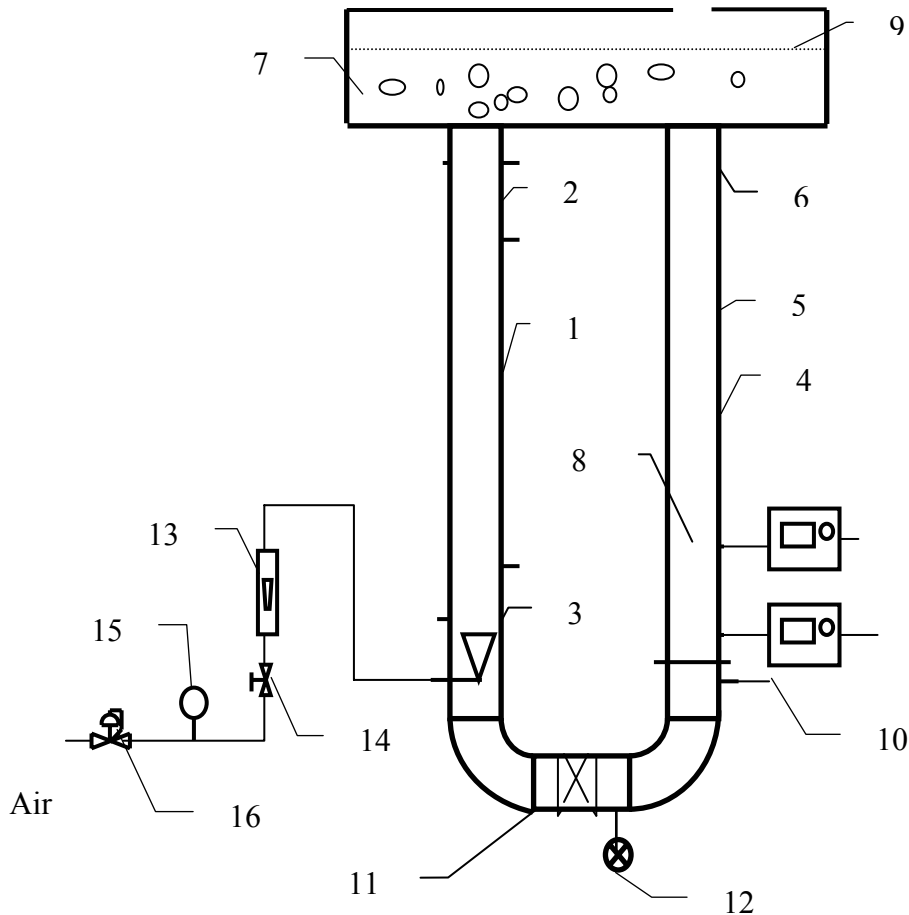


Figure A.1 Experimental setup for External Loop Inversed Fluidized Bed Airlift Bioreactor (Loh and Liu, 2001).

1.riser; 2.bubble; 3.gas sparger; 4.downcomer; 5.EPS beads; 6. Screen; 7.gas disengagement section; 8. Conductivity probe; 9.liquid level; 10.conductivity meter; 11. Controlling valve; 12.drainage valve; 13.rotameter; 14.valve; 15.pressure gauge; 16.pressure regulator and filter

Appendix B

MANOMETRIC DETERMINATION OF THE FRACTIONAL GAS

HOLDUP

The Inverted U-tube Manometer

The inverted U-tube manometer arrangement is depicted in Figure A.1. The pressure difference between points 1 and 2 is given as

$$P_2 - P_1 = \rho_D g dz \quad (1)$$

But

$$P_1 = \rho_M g h_1 + P_m \quad (2)$$

And

$$P_2 = \rho_M g h_2 + P_m \quad (3)$$

Therefore

$$P_2 - P_1 = \rho_M g (h_2 - h_1) \quad (4)$$

But

$$dz + h_1 = h_2 + dh_M \quad (5)$$

Therefore

$$h_2 - h_1 = dz - dh_M \quad (6)$$

The substitution of equation(6) in eq.(4) leads to

$$P_2 - P_1 = \rho_M g (dz - dh_M) \quad (7)$$

Equating eq. (1) and eq. (7) followed by rearrangement yields

$$\rho_D = \frac{\rho_M (dz - dh_M)}{dz} \quad (8)$$

Since

$$\rho_D = \rho_L(1-\varepsilon) + \rho_G\varepsilon \quad (9)$$

We get

$$\varepsilon = \frac{\rho_L}{\rho_L - \rho_G} \frac{dh_M}{dz} \quad (10)$$

Since $\rho_G \ll \rho_L$

$$\varepsilon = \frac{dh_M}{dz}$$

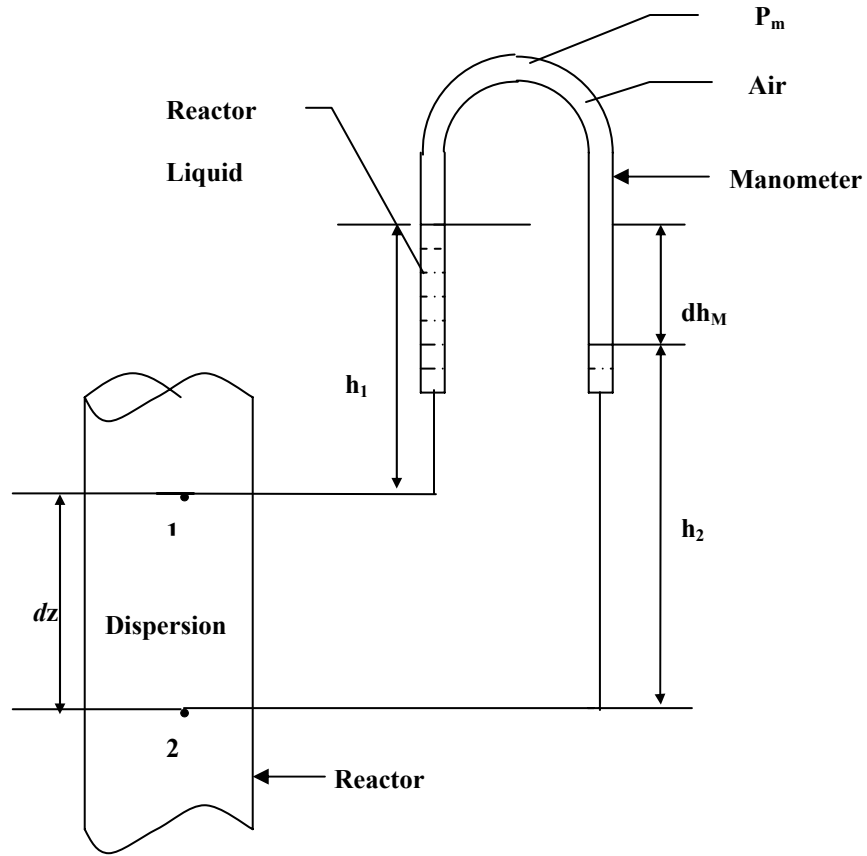


Figure B.1 The inverted U-tube manometer arrangement.

Appendix C

LIQUID CIRCULATION VELOCITY

A tracer method was employed for accurate measurement of the liquid circulation velocity. Concentrated NaCl solution was used as a tracer which was injected instansaneously into the top of the downcomer and the conductivity was followed at two downstream locations by identical conductivity probes placed some distance apart in the downcomer. From the measured time interval between the tracer peaks from the two conductivity probes and the known vertical distance between them, the linear liquid circulation velocity could be be calculated:

$$V_{Ld} = \frac{d_{electrode}}{t_{peaks}}$$

where $d_{electrode}$ is the distance between two conductivity electrode, and t_{peaks} is obtained from the distance between two peaks on the recording chart and the moving speed of chart.

Appendix D

Publication

- 1) Aye, T. T and K. C. Loh (2003). Biodegradation of high strength phenolic wastewater in a modified external loop inversed fluidized bed airlift bioreactor (EIFBAB). Can. J. Chem. Eng. in press.
- 2) Aye, T. T and K. C. Loh (2003). Biodegradation of high strength phenolic wastewater in a modified external loop inversed fluidized bed airlift bioreactor (EIFBAB). 6th internal conference of Gas-Liquid and Gas-Liquid-Solid Reactor Engineering (GLS-6), 17-20 August 2003, University of British Columbia, Vancouver, Canada.