CHAPTER 1

INTRODUCTION

1.1 INDUSTRIAL WASTEWATER POLLUTION

Wastewater released from various industries is the major concern for environmentalists in recent times. Industrial effluent contains various toxic metals, harmful gases and several organic and inorganic compounds. Both the quality and quantity of effluent result in various impacts on the availability of good quality water as well as on marine environment. Due to the discharge of this toxic effluent, there has been a major loss in the ecological, social and economic perspective. The long-term consequences of exposure cause fatal diseases like cancer, delayed nervous responses, mutagenic changes, neurological disorders etc (Wahaab 2000).

1.2 PHENOL - A MODEL POLLUTANT

Phenol ($C_6H_5OH$) is one among the major organic pollutants included in the list of Environmental Pollution Act (EPA) and is found mainly in effluent stream. Aqueous phenolic effluent are relatively common industrial wastes, being produced in several industries and operations such as petroleum refineries, gas and coke oven industries, phenolic resins, explosive manufacture, plastic and varnish industries, textiles units using organic dyes, smelting and related metallurgical operations (Pattersom 1985).
Phenol exhibits a moderate solubility of about 83 g/L in water. Acute exposure of phenol can cause central nervous system disorders, myocardial depression and hepatic damage to humans. Phenol is found to be a powerful disinfectant and bactericide and is used in pharmaceutical products like lotions and ointments. However, it is highly corrosive and toxic and causes a burning effect on skin. Phenol attributes carbolic odor to river water and can be toxic to living bodies. High concentration of phenol (up to 1200 mg/L) is expected to be found in the industrial effluent. In these perspectives central pollution control board set the minimum permissible level for phenol in environment as 0.05 to 0.1 mg/L.

Phenols and phenolic compounds have by far been recognized as one of the major recalcitrant, resistant to natural degradation and hence persist in environment. They represent the basic structural unit in a wide variety of synthetic organic compounds. It imparts objectionable taste to municipal drinking water at a much lower level. Phenol is either toxic (reduces enzyme activity) or lethal to fish even at relatively low levels, e.g. 5-25 mg/L, depending on the temperature and state of maturity of rainbow trout (Brown et al 1967). In this regard, industrial effluent containing phenol requires proper treatment before being discharged into the environment.

Traditionally, high concentration of phenol in industrial wastewater require the use of expensive chemical and physical processes for their removal like ozonization, adsorption, ion exchange, membrane filtration, chemical oxidation etc (Wang & Chirwa 1998; Aksu & Gonen 2006; Tziotzios et al 2008; Liu et al 2008). These conventional methods often suffer from serious drawbacks including high cost, high energy consuming, non economic and formation of hazardous by-products which again pollute the environment (Atlow et al 1984). United State Environmental Protection Agency (USEPA) has thus set a water purification standard of phenol less
than 1.0 µg/L in surface waters. Hence, removal of phenol from industrial effluent is an important practical problem. The current treatment methods often produce other toxic end products, requiring further processing steps. To overcome the drawbacks of these physical and chemical methods recently biotechnological processes have been reported as an alternative to these complex and expensive treatment methods.

The biological treatment of phenol waters is thus getting more and more popular in terms of pollution prevention and an environmentally friendly method. Owing to the high removal efficiency of phenols from waste discharges, many studies have been done in Pseudomonas species like *Pseudomonas aeruginosa* (*P.aeruginosa*), *Pseudomonas fluorescens* (*P.fluorescens*) and *Pseudomonas putida* (*P.putida*) and several model studies have also been done (Zilli et al 1993; Hannaford & Kuek 1999; Mordocco et al 1999). However the problem such as solid disposal is associated with biological treatment of wastewater (Chung et al 2003). In microbial degradation of phenol under aerobic conditions, the degradation is initiated by oxygenation to form catechol. Catechol is the main intermediate resulting from metabolism of phenol by different microbial strains. Depending upon the type of strain the catechol then undergoes ring cleavage which can occur either at the ortho position or at the meta position (as in the case of *Pseudomonas fluorescens*) initiating the meta pathway that leads to the formation of pyruvate and acetaldehyde (Figure 1.1). *P.fluorescens* is a non-pathogenic organism and was found to be effective in biodegrading phenolic industrial effluent as it use phenol as the sole carbon and energy source.
Figure 1.1 Different stages of phenol degradation

1.3  *Pseudomonas fluorescens*

*Pseudomonas fluorescens* is a gram negative rod shaped bacterium which has an extremely versatile metabolism, and can be found in soil and in water (Adams & Cox 1985). *P. fluorescens* is an aerobe and is oxidase positive. *P. fluorescens* is unable to grow under anaerobic conditions. It can produce certain enzymes such as heat stable lipases and proteases which are involved in the spoiling of milk (Rajmohan et al 2002). Despite their commercial nature, *P. fluorescens* are nonpathogenic and lack virulence factors of other plant pathogens. It grows at an optimum temperature of 25°C but can also survive in temperatures as low as 0°C. Therefore, it is rarely pathogenic in humans making it an effective microbe for the biodegradation of phenol.

*P. fluorescens* is an important food spoilage organism, usually found in the form of biofilms. In dairy industry, it is one of the most commonly isolated psychrotropic bacteria that dominate the microflora of raw or pasteurized milk at the time of spoilage. Furthermore, *P. fluorescens* is recognized to be a model organism for biofilm studies as it can easily form biofilms in different laboratory simulators. Another advantage of *P. fluorescens* over other biofilm forming bacteria is that it involves relatively less risk while handling. Its ability to use phenol as a sole carbon source in its
growth medium makes it an important choice of biodegradation of phenolic industrial effluent. Many species of pseudomonas had been experimented with the treatment of wastewater: *P. putida, P. aeruginosa* showed good results for the same (Hinsa & O'Toole 2006). However their inefficiency in forming stable biofilms and relatively high risk while handling makes them unsuitable for use in our work.

1.4 BIOFILM

Biofilm is a multispecies, immobilized cell community and can be found in a wide range of different systems. The formation of biofilm is a multi step process, and physiochemical and biological factors are involved. The ability of a bacterium to attach to a surface and form a biofilm is thought to be important for its survival in a variety of environments. None of the individual species in the biofilm may be capable of completely degrading influent wastes. Complete degradation of industrial waste involves a complex series of interaction between the resident species. The morphological characteristics of biofilms (biofilm thickness, biofilm density, bioparticle density and attached biomass concentration) are very important for the stability and performance of the biofilm. These factors strongly affect the biomass hold-up and mass transfer in a biofilm reactor (Garrido et al 1997; Tijhuis et al 1994).

To begin the formation of a biofilm, bacteria need to interact with the substratum. Each individual bacterium first undergoes reversible attachment, which involves contact of the pole of the cell with the surface. This interaction is relatively weak and can be easily disrupted. Eventually the cell attaches by its long axis, so-called irreversible attachment, in which the bacterium is very firmly attached to the surface, resulting in the formation of a monolayer of cells. Subsequent steps result in the formation of microcolonies
leading to the development of a ‘mature’ biofilm. Thus the biofilm development has 5 stages. Stage 1 is the initial attachment; stage 2 is the irreversible attachment; stage 3 is the maturation I phase; stage 4 is the maturation II phase; stage 5 is the dispersion phase (Pakula et al 1999). The genetic analyses suggest that biofilm formation can proceed via multiple, convergent signaling pathways, which are regulated by various environmental signals.

Microbial biofilms on surfaces are responsible for huge monetary losses each year in industrial sector due to both product and equipment damage, e.g. pipe plugging, affecting heat exchange. Conversely, microbial biofilm processes at surfaces also offer opportunities for positive industrial and environmental effects, such as bioremediating hazardous waste sites, biofiltering industrial water, and forming biobarriers to protect soil and groundwater from contamination. Hence biofilm reactors are being used in wastewater and industrial effluent treatment.

1.5 BIOFILM REACTORS

A reactor which involves the growth of biofilm in it for a specific purpose is called as biofilm reactor. Biofilm reactor has been frequently applied in wastewater treatment. The feasibility and efficiency of biofilm reactors have been studied for removing biodegradable matter, nitrogen and phosphate from municipal and industrial wastewater (Liu & Capdeville 1996; Selivanovskaya et al 1997; Rusten et al 2006). Biofilm reactors are desirable in biological treatment processes because a very high number of highly concentrated organisms can be maintained and treated in the reactor which takes up a small amount of space. According to Souza et al (2004) conditions under which aerobic microorganisms grow better are in the temperature ranging from 25° – 40°C and pH between 4 and 9, and showed that this
temperature and pH range was enough to ensure good degradation inside the biofilm reactor.

Biofilm reactors can be classified into two types: tank biofilm reactors (e.g. continuous stirred tank reactors) and tubular biofilm reactors (e.g. packed bed reactor, fluidized bed reactors). Tubular biofilm reactors have a high volumetric unit conversion and hence provide better efficiency. Packed bed biofilm reactors are useful in maintaining low pressure drops but they result in poor temperature control. On the other hand fluidized biofilm bed reactors are used where a good uniformity in temperature is required.

Fluidized bed biofilm bioreactor has several advantages over other conventional reactors used for the treatment of wastewaters. This reactor retains high biomass concentration attached on an inert particle, shows no bed clogging problems, requires small reactor volume, and exhibits a low external mass transfer resistance when compared to other reactors. Phenol biodegradation experiments have usually been conducted in fluidized bed biofilm reactors with bacteria attached to a solid substratum. However the main limitation of the fluidized bed biofilm reactor is the uncontrolled biofilm thickness. There is a problem of increase in biofilm thickness when the microorganisms in the biofilm multiply. This limits diffusion of oxygen and organic substrates to the deeper layers of the biofilm. Starvation of the microorganisms at the base of the biofilm causes pieces of the biofilm to detach and leads to ineffective bioreactor operation. The uncontrolled biofilm growth results in over expansion of the fluidized bed with subsequent elutriation of the particles.

In order to maintain a constant biofilm thickness, the particles are taken out to remove excess biofilm and then sent back to the reactor. The use of inverse fluidized bed bioreactor can solve this problem and maintains
uniform biofilm thickness due to particle-particle collision and particle wall collision inside the reactor.

1.6 INVERSE FLUIDIZED BED BIOFILM REACTOR

Inverse fluidized bed biofilm reactor (IFBBR) works on the principle of inverse fluidization using low density support particles whose density is less than that of the liquid phase. The reactor plays a significant role in the bio-treatment of wastewater.

1.6.1 Inverse Fluidization

Inverse fluidization is a multiphase liquid – solid or gas – liquid – solid system. The difference between the ordinary fluidization and the inverse fluidization lies in the density of the solid particles used in the reactor. If the density of the particles used is greater than the fluid, it is ordinary fluidization. If it is less than the fluid it is called inverse fluidization. Inverse bio-fluidization is relatively a new technique where the low density bioparticles are fluidized by either upward cocurrent flow of gas and liquid or by a downward flow of liquid and countercurrent upward flow of gas. In the former case, fluidization is achieved by upward flow of gas making the bed to expand downwards whereas in the latter case fluidization is done by the downward flow of liquid counter to the net buoyancy force of the particles. When the liquid flow is small and not sufficient to counter the net buoyancy force of the particles, inverse fluidization can also be achieved by upward flow of gas phase (Sókóól & Woldeyes 2011).
1.6.2 **Significance of IFBBR**

The application of low density support particles in inverse fluidized bed biofilm reactor overcomes the problem of conventional upflow fluidized bed biofilm reactor; the biofilm thickness can be easily maintained in inverse fluidized bed biofilm reactor throughout the operation due to particle-particle collision and particle-wall collision inside the reactor. This resulted in lesser mass transfer resistance, greater contact of solid-liquid-gas phases, large specific support surface area, fast biofilm formation and hence greater biodegradation effect. Low density particles require low fluid velocity for their expansion and hence low power requirement. Thus a three phase inverse fluidized bed biofilm reactor can be successfully employed in all aerobic bio-treatment of wastewater due to its high energy performance, low pressure drop, high gas holdup and high heat and mass transfer rates (Sókól 2012).

1.7 **RATIONALE AND PROBLEM DEFINITION**

Several different types of biofilm reactor have been studied for the biodegradation of toxic phenolic compounds including rotating disk bioreactor, fixed bed reactor and three-phase fluidized bed bioreactor (Kaymaz et al 2012). Fluidized bed biofilm reactors (FBBR) are considered superior because of large biofilm support surface, appropriate hydrodynamic conditions, high mass transfer rates of both oxygen and substrate and excellent contact between the liquid and solid phases. However, one significant problem practically in all types of biofilm reactors, including fluidized bed reactors, is uncontrolled biofilm growth which resulted in greater mass transfer resistance. The inverse fluidized bed biofilm reactor (IFBBR) can overcome this problem by maintaining constant biofilm thickness throughout the process (Kryst & Karamanev 2001).
Literature studies reported that considerable efforts has been made in exploring and understanding the hydrodynamic characteristics of fluid flow, heat and mass transfer effects in three phase inverse fluidized bed biofilm systems. The detachment force resulting from hydrodynamic shear and/or particle-particle collision is a key factor that influences the formation, structure and stability of biofilm under hydrodynamic conditions (Kwok 1998). Thus the optimization of hydrodynamic conditions in relation to detachment force is necessary in future engineering design of biofilm reactors (Chang et al 1991). However, most of the data available are concerned with hydrodynamic factors of the reactor and is limited in studying the biodegradation effects of the industrial effluent. It is therefore essential to study and correlate the optimum hydrodynamic operating parameters of the reactor with the biodegradation of hazardous toxic chemicals in industrial effluent for achieving more efficient biodegradation (Ochieng et al 2002).

The successful design of any fluidized bed biofilm systems requires the knowledge of mass transfer characteristics of the reactor. Unfortunately studies on gas-liquid and liquid-solid mass transfer effects are very limited in inverse fluidized biofilm reactors (Beyenal & Tanyolaç 1998). The performance evaluation of IFBBR often requires the calculation of external mass transfer resistance to the biofilm. In literature, it has been generally assumed that this external mass transfer resistance can be neglected in case of high fluidization rates. However, some studies have shown that it influences the biofilm performance significantly even at low fluidization rates (Livingston & Chase 1989). Thus the knowledge of external mass transfer resistance on biofilm system is much essential for the design and performance evaluation of IFBBR which operates at low fluidization rates.

By keeping all the above limiting cases, IFBBR with draft tube arrangement was developed in the present work to investigate the
performance of the reactor for the biodegradation of phenol using *P. fluorescens*. The biodegradation kinetics of phenol was studied for the suspended biomass and biofilm system. The present study analyzed the hydrodynamic characteristics of IFBBR by optimizing the hydrodynamic operating parameters. The study has also been attempted to optimize the effect of hydrodynamic detachment force on the structure and behavior of biofilm in degrading phenol. Studies were done to analyze the effects of gas-liquid volumetric oxygen mass transfer coefficient. The diffusion effects of biofilm on liquid-solid external mass transfer coefficient have also been studied and discussed for various particle sizes in this thesis.

1.8 OBJECTIVES OF THE PRESENT INVESTIGATION

The present work aims to study the feasibility and performance of three phase inverse fluidized bed biofilm reactor for the biodegradation of phenol. Based on this, the objectives are outlined as follows:

- To study the biodegradation of phenol in inverse fluidized bed biofilm reactor using *Pseudomonas fluorescens*
- To analyze the biodegradation kinetic studies and performance evaluation of biomass and biofilm characteristics
- To determine the optimum hydrodynamic operating parameters - superficial air velocity, ratio of settled bed height volume to reactor volume
- To study the influence of hydrodynamic effects on the performance of biofilm of various particle sizes
- To optimize the particle size for efficient biodegradation of phenol with better hydrodynamic effects and biofilm morphology
- To study the effect of biofilm characteristics and the influence of hydrodynamic parameters on gas–liquid volumetric mass transfer coefficient
- To study the diffusion effects of biofilm on liquid-solid external mass transfer coefficient
- To experiment the optimized hydrodynamic and mass transfer conditions in treating industrial effluent of leather and tannery

1.9 THESIS LAYOUT

This thesis is organized into nine chapters. A brief description of the contents that are covered in the following chapters is presented below. The present chapter explains the recalcitrant characteristics of phenolic effluent from various sources, importance of biofilm reactors and significance of inverse fluidized bed biofilm reactor in the biodegradation of phenol. The chapter talks about the rationale behind the research work, problem definition and illustrates the organization of thesis.

Chapter 2 covers the extensive literature survey on biodegradation of phenol using biofilm reactors and deliberates the significance of inverse fluidized bed biofilm reactor for the bio-treatment of toxic effluent. The wide literature report entails the importance of hydrodynamic aspects of IFBBR and reviews the studies on gas-liquid mass transfer coefficient. The chapter assesses the effects of external mass transfer resistance due to film diffusion effects.
Chapter 3 deals with the materials used and the experimental methods adopted in the present work. Description about bacterial strain, physical properties of the support particles, experimental set up and reactor design configuration, experimental procedure, fluid flow regimes, analytical techniques had been explained in detail.

Chapter 4 discusses the feasibility and performance of three phase (solid-liquid-gas) inverse fluidized bed biofilm reactor (IFBBR) in biodegrading synthetic wastewater containing phenol. The chapter explains the biodegradation kinetic studies of the suspended biomass and biofilm culture with respect to their specific growth rate and specific phenol consumption rate.

Chapter 5 explicates the hydrodynamic characteristic performance of inverse fluidized bed biofilm reactor for the aerobic biodegradation of phenol. The chapter finds way to evaluate the optimum hydrodynamic operating parameters such as superficial air velocity and ratio of settled bed height volume to reactor volume for effective biodegradation of phenol in IFBBR.

Chapter 6 discusses the influence of some hydrodynamic effects on the performance of biofilm for phenol biodegradation in IFBBR with support particles of various sizes under optimized fixed bed height condition. The particle size is optimized in this chapter for efficient biodegradation of phenol with better hydrodynamic effects and biofilm morphology.

Chapter 7 reveals the effect of gas-liquid and liquid-solid mass transfer characteristics in IFBBR for the biodegradation of phenol. The chapter discusses the effect of biofilm characteristics and the influence of hydrodynamic parameters on volumetric mass transfer coefficient. The mass transfer film diffusion effect in biofilm system has been studied by
determining external liquid – solid mass transfer resistance and effectiveness factor. The dependence liquid-solid mass transfer coefficient on surface properties of the support particles has been explained.

Chapter 8 deliberates the performance of IFBBR in treating industrial effluent from leather and tannery industry under the optimized hydrodynamic and mass transfer experimental conditions of synthetic phenolic effluent. The chapter reveals the physio – chemical characterization of the effluent before and after treatment and explains the biodegradation mechanism of phenol using *P. fluorescens*.

Chapter 9 elucidates the summary of the work and conclusion of the obtained results from biodegradation kinetic studies, hydrodynamic and mass transfer studies in IFBBR along with the possible avenues of further work.