DYE DECOLOURIZATION BY USING RICE HUSK AND WHITE ROT FUNGI

A DISSERTATION

Submitted in partial fulfilment of the requirements for the award of the degree of

MASTER OF TECHNOLOGY

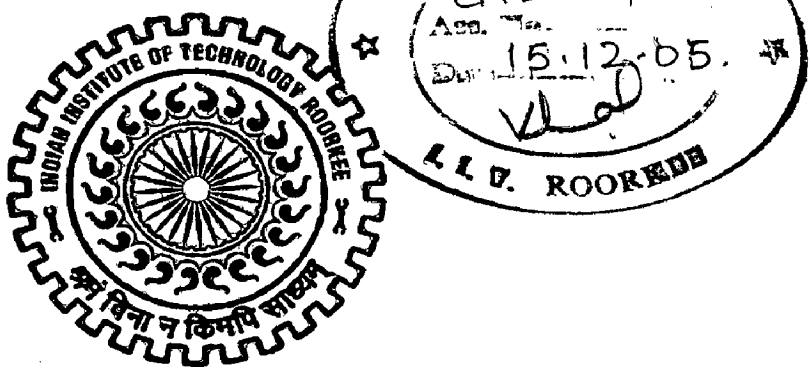
in

CHEMICAL ENGINEERING

(With Specialization in Industrial Pollution Abatement)

By

HARCHARAN SINGH



DEPARTMENT OF CHEMICAL ENGINEERING
INDIAN INSTITUTE OF TECHNOLOGY ROORKEE
ROORKEE-247 667 (INDIA)

JUNE, 2005

CANDIDATE'S DECLARATION

I hereby declare that the work which is being presented in this dissertation entitled **"DYE DECOLOURIZATION BY USING RICE HUSK AND WHITE ROT FUNGI"** in partial fulfillment of the requirements for the award of degree, Master of Technology in Chemical Engineering with specialization in Industrial Pollution Abatement (IPA) submitted in the Department of Chemical Engineering, Indian Institute of Technology Roorkee, is an authentic record of my work under the supervision of **Dr. C. Balomajumder**, Assistant Professor, Department of Chemical Engineering and **Dr. Ben M.J.Pereira**, Professor, Department of Biotechnology, Indian Institute of Technology, Roorkee, Roorkee during the period july-04 to june-05.

The matter presented in this dissertation has not been submitted by me for the award of any other degree of this or any other Institute/University.

Place: IIT Roorkee
Date: June 30, 2005

(HARCHARAN SINGH)

CERTIFICATE

This is to certify that the above statements made by the candidate are correct to the best of my knowledge and belief.

Ben. M. J. Pereira
30.06.05

(Dr. Ben M.J.Pereira)
Professor
Department of Biotechnology
Indian Institute of Technology Roorkee
Roorkee-247667

(Dr. C.Balomajumder)
Assistant Professor
Department of Chemical Engineering
Indian Institute of Technology Roorkee
Roorkee-247667

ACKNOWLEDGEMENT

I take the opportunity to pay my regards and a deep sense of gratitude to my guide **Dr. C. B. MAJUMDER**, Assistant Professor, Department of Chemical Engineering, Indian Institute of Technology Roorkee and **Dr. BEN M. J. PEREIRA**, Professor, Department of Biotechnology, Indian Institute of Technology Roorkee, for their valuable guidance, keen co-operation, and cheerful encouragement throughout my dissertation work.

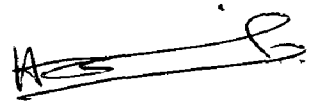
I am also thankful to **Dr. BIKASH MOHANTY**, Professor and Head, Department of Chemical Engineering and **Dr. I. M. MISHRA, Professor,** Indian Institute of Technology Roorkee, and all the faculty members (Department of Chemical Engineering, Indian Institute of Technology Roorkee), for providing me all the possible support and facilities for my dissertation work.

I wish to express my deep gratitude to **Dr. SOMA SEN**, Research Associate, Department of Biotechnology, Indian Institute of technology Roorkee for providing valuable guidance, tips and all kind of support in completion of this thesis.

I express my deep sense of gratitude to the staff of PDR lab, Department of Chemical Engineering, especially to **Mr. AKHILESH, Mr. MANGE RAM** for their instant help in all kinds of work. I am also thankful to the staff of PA lab, specially to **Mr. AYODHYA JI,** for their cooperation all through my dissertation work.

I am highly thankful to all my friends for their help and necessary support during my dissertation work.

Last but not the least, I wish to place on record my deep sense of gratitude to my **parents** without whose blessings I would not be joined in this prestigious department for higher studies to enrich myself with the latest knowledge.



HARCHARAN SINGH

ABSTRACT

Synthetic dyes are used in the textile, food, paper, printing, drug, cosmetics and leather industries. Major classes of synthetic dyes including Azo, Anthraquinone dyes constitute more than 50% of those used in industrial applications.

They are generally considered as xenobiotic compounds that are very recalcitrant against biodegradative processes. The ability of microorganism to decolourize and metabolize dyes has long been known, and the use of biodegradation based technologies for treatment of azo and Anthraquinone dyes have attracted interest. Within this study, we investigate the various techniques to decolourize the Azo and Anthraquinone dyes.

Adsorption and Biodegradation are two important techniques for treating the effluent containing these synthetic dyes. Both have significant advantages as well as disadvantages. The combined application of these above two techniques has got an extra edge over the two processes separately. In the present study Reactive Orange 16(494 nm), Reactive Black 5(597 nm) and Remazol Brilliant Blue R (592 nm) have been biodegraded by using White Rot Fungi (*Pleurotus Ostreatus*) in the presence of Rice Husk. Simultaneous Adsorption and Biodegradation (SAB) has been studied against Adsorption. The effects of initial concentration, pH and adsorbent dose have been studied for SAB as well as for adsorption and are compared.

The results shows that the degradation of Reactive Orange 16(494 nm) is 94.5039 %, Reactive Black 5(597 nm) is 92.2644 % and Remazol Brilliant Blue R (592 nm) is 93.2691 % is possible at 35°C with 3 gm of adsorbent (Rice Husk) with initial concentration of 200 mg/l in static condition and by use of SAB method.

Results also show that a pH value of 4.5 is favourable for the adsorption of Reactive Orange 16(494 nm), Reactive Black 5(597 nm) and Remazol Brilliant Blue R (592 nm) dyes. The isothermal data could be well described by the Langmuir and Freundlich equations.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

1.1. General Introduction

Synthetic dyes are used in the textile, food, paper, printing, drug, cosmetics and leather industries. Major classes of synthetic dyes including azo, anthraquinone, and triarylmethane dyes constitute more than 50% of those used in industrial applications. These synthetic dyes have stable chemical structures to meet various colouring requirements. Current environmental concern with these dyes revolves around the carcinogenic potential of these dyes and their intermediates. In many developing countries textile wastewater contaminated with these dyes is directly discharged without treatment to rivers, lakes or the ocean. Efficient and economical methods for colour removal and total dissolved organic carbon (DOC) reduction in textile wastewater before its discharge is needed. Common treatment processes, e.g. adsorption and flocculation, are not efficient methods because they result in solid wastes, thus creating other environmental problems requiring further treatment. To treat dye-containing wastewater, several methods such as adsorption, flocculation, ozonation and reverse osmosis have been suggested. However, these methods are not widely applied because of poor colour removal or high cost. Therefore, the microbial decolourization of dye-containing wastewater has been of considerable interest.

In this study we are discussing about the reactive azo and anthraquinone dyes. These dyes are the largest class of synthetic dyes used in the textile, food, paper, printing, drug, cosmetics and leather industries, characterized by the presence of one or more azo bonds (R-N=N-R') in association with one or more aromatic systems, which may also carry sulfonic acid groups and the anthraquinone dyes containing the carboxyl group. Many studies indicate that these dyes are toxic or carcinogenic. If these colourants come into contact with certain drugs (e.g. aspirin, benzoic acid) within the human body they can induce allergic and asthmatic reactions in sensitive people. Some natural microbial species, including bacteria, fungi, and algae, are capable of removing the colour of azo dye via biotransformation, biodegradation, or even mineralization (J.S. Chag et al 2001).

Many microorganisms capable of degrading azo dyes include bacteria (Wong and Yuen, 1998), fungi (Ozfer yesilada et al 1997;Shin and Kim 1998; Fu-ming Zhang et al 1998; Swamy and Ramsay 1999; Koichi harazono et al 2003, Weixiao liu et al 2004), yeasts (Martins et al., 1999), actinomycetes (Heiss et al., 1992) and algae (Dilek et al., 1999), *Escherichia coli and Pseudomonas sp.* (Mustafa et al 2002), *Trametes versicolour* (Maria Teresa Moreira et al 2003), *Basidiomycete Phanerochaete sordida* (Koichi harazono et al 2003) , PVA-immobilized microorganisms (Kuo-Cheng Chen et al 2003), *Phanerochaete chrysosporium and Pleurotus sajorcaju* (Eliana Pereira Chagas et al 2001), *Proteus mirabilis* (K-C Chen et al 1999) *P. chrysosporium* produces extracellular *lignin peroxidase* (LIP) and *manganese peroxidase* (MnP) during the degradation of lignin or xenobiotic compounds.

Lignin is the most abundant aromatic chemical on earth, comprising roughly 15% of all terrestrial biomass. Formed through the free radical polymerization of several *p*-hydroxycinnamyl alcohols, it is a heterogeneous, highly cross-linked polymer that encases the structural polysaccharides of higher plant cell walls. The inter-monomer linkages of lignin consist of alkyl aryl ether, aryl-propane, biphenyl, and other bonds that resist cleavage by most biochemical agents. These properties are consonant with the polymer's functions, which are to give vascular plants the rigidity they need to stand upright and to protect their structural polysaccharides (cellulose and hemicelluloses) from microbial degradation. Despite the chemical recalcitrance of lignin, it and the polysaccharides it protects are degraded in nature. The overall mass balance of this process remains unknown, but it is clear that lignocellulose is biologically converted to partially degraded polymers such as humic and fulvic acids, to low molecular weight organics, and to CO_2 .Were this not the case, photosynthetically fixed carbon would accumulate irreversibly into lignified biomass, eventually causing the terrestrial carbon cycle to halt. Lignocellulose degradation is thus an essential ecological process.

Only few organisms in nature belonging to the white-rot fungi are able to attack and destroy the lignin molecule. Both cellulose and lignin are rather rigid organic polymers which have been 'invented and optimized' by nature during the evolution process for constructive and long term preservation purposes. Harsh physico-chemical conditions have to be applied to attack or modify these two compounds. In principle the

defibration of wood is still based on inventions from the last century (mechanical grinding process, 1848; sulphite process, 1857; kraft (sulphate) process, 1879) which have been fine tuned. More than 150×10^6 tons of pulp are produced annually and about 50×10^6 of lignin together with the chemicals used are released from the P&P industry indicating that a lot of efforts have to be undertaken to handle the enormous amounts of hazardous potential. Comparing the strong chemical conditions in technical use and the mild reaction conditions of enzymatic catalysis, one cannot be surprised that it took the biochemists and enzymologists a long time to find an edge for their alternative approach. But again, nature had already 'developed' a process, the white- rot of wood. Bavendamm (1928) developed a diagnostic tool to determine the decay type of fungi. A positive reaction indicates a white-rot type fungus destroying carbohydrate and lignin, a negative test result is obtained for brown-rot fungi, which attack mainly the polysaccharide portion of wood and merely modify its lignin. Two of the most important and best examined lignin degrading microorganisms are the white rotting fungi *Phanerochaete chrysosporium* and *Trametes* (*Coriolus*) *Versicolour*, but also some other fungi (eg. *Plurotus Ostreatus*) had been proposed for treatment. Lignozym's idea was to find a technically feasible system which would reasonably simulate the situation in nature and which is at the same time compatible with the pulp technology of today.Starting in 1986 (Call, 1986; 1990a; 1991a) with the enzyme-mediator concept, Lignozym has recently improved the effectiveness of the mediator system for laccase from *Coriolus Versicolour* by changing and further fine-tuning the chemical nature of the compound (Call, 1993; Call and Gucke, 1994a).after degradation of lignin by various method mentioned above we get the lignolytic enzyme which is used for many purpose eg. decolourization of dyes etc.

Bioremediation is defined as the application of biological processes to the treatment of pollution. Most research within the field of bioremediation has focused on bacteria, with fungal bioremediation (mycoremediation) attracting interest just within the past two decades. The toxicity of many of the above- named pollutants limits natural attenuation by bacteria, but white rot fungi can withstand toxic levels of most organopollutants (Aust et al, 2004). White rot fungi is a physiological grouping of fungi that can degrade lignin (and lignin- like substances). Four main genera of white rot fungi

have shown potential for bioremediation: *Phanerochaete, Trametes, Bjerkandera,* and *Pleurotus* (Hestbjerg et al, 2003). These fungi cannot use lignin as a source of energy, however, and instead require substrates such as cellulose or other carbon sources. Thus, carbon sources such as corncobs, straw, and sawdust can be easily used to enhance degradation rates by these organisms at polluted sites. Also, the branching, filamentous mode of fungal growth allows for more efficient colonization and exploration of contaminated soil. The main mechanism of biodegradation employed by this group of fungi, however, is the lignin degradation system of enzymes. These extracellular ligninmodifying enzymes (LMEs) have very low substrate specificity so they are able to mineralize a wide range of highly recalcitrant organopollutants that are structurally similar to lignin (Cajthaml et al, 2002; Mansur et al, 2003; Pointing, 2003, Veignie, 2004). The fact that these fungal enzymes work extracellularly allows them to access many of the non-polar, non-soluble toxic compounds that intracellular processes (such as cytochrome P450) cannot (Reddy and Mathew, 2001; Levin et al, 2003). The three main LMEs are lignin peroxidase, Mn-dependent peroxidase, and laccase. All three of these enzyme groups are stimulated by nutrient limitation (Mansur et al, 2003; Aust et al, 2004). They are most effective at degrading lignin and lignin- like substances when certain nutrient levels, primarily nitrogen, are low. Conversely, activities of these enzymes are completely suppressed in media containing high levels of nitrogen (Reddy and Mathew, 2001). This characteristic is advantageous for the fungi inhabiting highly contaminated sites with very low productivity due to toxic levels of organopollutants.Lignin peroxidase is a glycosylated heme protein that catalyzes hydrogen peroxide-dependent oxidation of lignin-related aromatic compounds. They have a higher redox potential than most peroxidases and so are able to oxidize a wide range of chemicals, including some non-phenolic aromatic compounds (Reddy and Mathew, 2001). Mn-dependent peroxidase also requires hydrogen peroxide to oxidize Mn^{2+} to Mn^{3+}. The Mn^{3+} state of the enzyme then mediates the oxidation of phenolic substrates (Mester and Tien, 2000). Laccase, a multicopper oxidase enzyme, is the primary enzyme involved in the degradation process. It was first described in 1883, making it one of the oldest enzymes ever described (Mayer and Staples, 2002). It uses dioxygen as an oxidant, reducing it to water and it has the ability to catalyze the oxidation

of a widerange of dihydroxy and diamino aromatic compounds (Mester and Tien, 2000; Reddy and Mathew, 2001; Saito et al, 2003; Aust et al, 2004). It is most stable at a pH of 4-6 and temperature of 45°C. (Lau et al, 2003; Mansur et al, 2003; Saito et al, 2003; Baldrian, 2004). However, this enzyme is still active at pH levels as low as 4 and as high as 7 (Mansur et al, 2003). This is beneficial in contaminated field sites with very low pH levels.

The mechanism of biodegradation depends in part, on the compound being degraded, but there are some consistent steps in the process regardless of the substrate. The lignolytic enzymes in white rot fungi catalyze the degradation of pollutants by using a non-specific free radical mechanism (Pointing, 2001; Law et al, 2003). When an electron is added or removed from the ground state of a chemical it becomes highly reactive, allowing it to give or take electrons from other chemicals. This provides the basis for the non-specificity of the enzymes and the ability of the enzymes to degrade xenobiotics, chemicals that have never been encountered in nature (Reddy and Mathew, 2001; Pointing, 2001). The main reactions that are catalyzed by the lignolytic enzymes include depolymerization, demethoxylation, decarboxylation, hydroxylation and aromatic ring opening. Many of these reactions result in oxygen activation, creating radicals that perpetuate oxidation of the organopollutants (Reddy and Mathew, 2001). Once the peroxidases have opened the aromatic ring structures by way of introducing oxygen, other more common species of fungi and bacteria can mineralize the products intracellularly into products such as CO_2 and other benign compounds.

White rot fungi may play a large role in this search, providing an environmentally- friendly, economical approach that we are really just beginning to understand.

Recently, we attempted to determine the decolourization kinetics of a *pleurotus Ostreatus* strain able to decolourize the azo and anthraquinone dyes efficiently.

1.2. Dyes, history

Ever since the beginning of humankind, people have been using colourants for painting and dyeing of their surroundings, their skins and their clothes. Until the middle of the 19th century, all colourants applied were from natural origin. Inorganic pigments such as soot, manganese oxide, hematite and ochre have been utilized within living memory.

Palaeolithic rock paintings, such as the 30,000 year old drawings that were recently discovered in the Chauvet caves in France, provide ancient testimony of their application. Organic natural colourants have also a timeless history of application, especially as textile dyes. These dyes are all aromatic compounds, originating usually from plants (e.g. the red dye alizarin from madder and indigo (Figure 2.1) from woad) but also from insects (e.g. the scarlet dye kermes from the shield-louse *Kermes vermilio*), fungi and lichens. Synthetic dye manufacturing started in 1856, when the English chemist W.H. Perkin, inan attempt to synthesise quinine, obtained instead a bluish substance with excellent dyeing properties that later became known as aniline purple, Tyrian purple or mauveine. Perkin, 18 years old, patented his invention and set up a production line. This concept of research and development was soon to be followed by others and new dyes began to appear on the market, a process that was strongly stimulated by Kekule's discovery of the molecular structure of benzene in 1865. In the beginning of the 20th century, synthetic dyestuffs had almost completely supplanted natural dyes.

1.3 Lignolytic Enzyme, History

1.3.1 Application of the natural delignification process using microorganisms

Many experiments have been made and a lot of efforts have been invested in using fungi for technical lignin removal in the pulping process and for biobleaching. (Kirk and Yang 1979) reported for the first time that *P. chrysosporium* can partially delignify unbleached Kraft pulp. In 1982, ligninase was discovered (Tien and Kirk, 1983; Glenn et al., 1983) as a part of the lignolytic system in fungi, whereas laccase had been described already in the last century (Yoshida, 1883; Bertrand, 1894, 1896; Laborde, 1896). However, in order to attain a satisfactory lignin removal, extended incubation times of several days or weeks have to be accepted which are not at all compatible with state-of-the-art pulping and bleaching processes (Wall et al., 1993; Messner and Srebotnik, 1994; Katagiri et al., 1995). Nevertheless, due to the encouraging results a Biopulping Consortium was founded in 1987 and research was intensified in this field (Kirk et al., 1994; Akhtar et al., 1995). The knowledge about strains and their potential, as well as the effectiveness of the process on a small scale has been improved within the last few years and some patents (Kobe Steel, 1988; 1991) have been filed. Although considerable pulp delignification (kappa reductions of 50% or more), brightness increase,

saving of beating energy and improved paper quality can be achieved with intact fungi (Erikksson and Vallander, 1982; Kirkpatrick et al., 1989; Paice et al., 1989; Reid et al., 1990; Fujita et al., 1991, 1993; Murata et al., 1992; Akhtar et al., 1995; for more detailed information see Messner and Srebotnik, 1994), incubation time and conditions are still an obstacle to a broad commercial application. Today's goals of fungal pre-treatment have changed from the lignin removal (as much is possible) in the beginning, to a modification of lignin so that energy consumption during mechanical pulping is reduced or lignin removal will be enhanced in the digester already (Akhtar et al., 1992, 1995; Wall et al., 1996). For pitch control applications (Farrell et al., 1993) a commercial fungal inoculum preparation is available.

Table 1. 1. Study about the history of lignolytic enzyme (H.P. Call et al. 1997)

Generation	Enzyme	Mediator(S)	Comment
1. Generation (1986) (original system)	Lignolytic enzymes	+ Phenolics or non-phenolics + Reducing agents	Complex multicomponent reduction:
	Lignolytic enzymes	+ Oxidizing agents	oxidation-system
2. Generation (1990) (modified system)		+ Phenolics or non-phenolics + Chelated metals as 'mimic enzymes' (Mn^{2+}:Cu^{2+}) + Reducing agents + Oxidizing agents	Complex multicomponent reduction: oxidation cascade
3. Generation (1993) (Lignozym)	Lignolytic enzymes	+ A single compound (-N-OH;N-oxide; oxime- or hydroxamic acid)	Simple system

1.4 Objectives of the Thesis

The objective of this thesis is to carry out the adsorption alone, biodegradation alone and simultaneous adsorption and biodegradation, for the decolourization of Azo and Anthraquinone dyes by using rice husk and white rot fungi.

The effect of following parameters on color removal of dyes has been studied:

(1) Effect of pH.

(2) Effect of initial concentration of dyes.

(3) Effect of adsorbent doses.

(4) Effect of contact time.

(5) Effect of operating condition (shaking and static).

CHAPTER 2

DYES, WHITE ROT FUNGI AND ITS ENZYMES

2.1 Dye classification

All aromatic compounds absorb electromagnetic energy but only those that absorb light with wavelengths in the visible range (~350-700 nm) are coloured. Dyes contain *chromophores*, delocalized electron systems with conjugated double bonds, and *auxochromes*, electron-withdrawing or electron-donating substituents that cause or intensify the colour of the chromophore by altering the overall energy of the electron system. Usual chromophores are -C=C-, -C=N-, -C=O, -N=N-, $-NO_2$ and quinoid rings, usual auxochromes are $-NH_3$, -COOH, $-SO_3H$ and -OH. Based on chemical structure or chromophore, 20-30 different groups of dyes can be discerned. Azo (monoazo, disazo, triazo, polyazo), anthraquinone, phthalocyanine and triarylmethane dyes are quantitatively the most important groups. Other groups are diarylmethane, indigoid, azine, oxazine, thiazine, xanthene, nitro, nitroso, methine, thiazole, indamine, indophenol, lactone, aminoketone and hydroxyketone dyes and dyes of undetermined structure (stilbene and sulphur dyes).

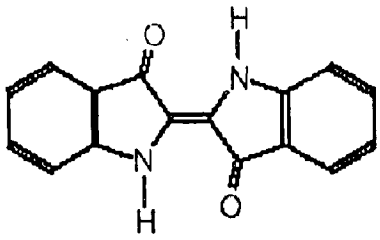


Figure 2.1 Indigo

The vast array of commercial colourants is classified in terms of colour, structure and application method in the Colour Index (C.I.) which is edited since 1924 (and revised every three months) by the Society of Dyers and Colourists and the American Association of Textile Chemists and Colourists. The Colour Index (3rd Edition, issue 2) lists about 28,000 commercial dye names, representing approx.10,500 different dyes,

45,000 of which are currently produced. Each different dye is given a C.I. generic name determined by its application characteristics and its colour.

The Colour Index discerns 14 different application classes:

2.1.1 Acid dyes

The largest class of dyes in the Colour index is referred to as *Acid dyes* (approx. 2300 different acid dyes listed, ~40% of them are in current production). Acid dyes are anionic compounds that are mainly used for dyeing nitrogen-containing fabrics like wool, polyamide, silk and modified acryl. They bind to the cationic NH_4^+-ions of those fibres. Most acid dyes are azo (yellow to red, or a broader range colours in case of metal complex azo dyes), anthraquinone or triarylmethane (blue and green) compounds. The adjective acid refers to the pH in acid dye dyebaths rather than to the presence of acid groups (sulphonate, carboxyl) in the molecular structure of these dyes.

2.1.2 Metal complex dyes

Among acid and reactive dyes, many *Metal complex dyes* can be found (not listed as a separate category in the Colour Index). These are strong complexes of one metal atom (usually chromium, copper, cobalt or nickel) and one or two dye molecules, respectively 1:1 and 1:2 metal complex dyes. Metal complex dyes are usually azo compounds. About 1/6 of all azo dyes listed in the Colour Index are metal complexes 37 but also phthalocyanine metal complex dyes are applied.

2.1.3 Direct dyes

Direct dyes are relatively large molecules with high affinity for especially cellulose fibres. Vander Waals forces make them bind to the fibre. Direct dyes are mostly azo dyes with more than one azo bond or phthalocyanine, stilbene or oxazine compounds. In the Colour Index, the direct dyes form the second largest dye class with respect to the amount of different dyes: About 1600 direct dyes are listed but only ~30% of them are in current production.

2.1.4 Reactive dyes

Reactive dyes are dyes with reactive groups that form covalent bonds with OH-, NH-, or SH-groups in fibres (cotton, wool, silk, nylon). The reactive group is often a heterocyclic aromatic ring substituted with chloride or fluoride, e.g. dichlorotriazine. Another common reactive group is vinyl sulphone. The use of reactive dyes has increased

ever since their introduction in 1956, especially in industrialised countries. In the Colour Index, the reactive dyes form the second largest dye class with respect to the amount of active entries: about 600 of the ~1050different reactive dyes listed are in current production. During dying with reactive dyes (Figure 2.2), hydrolysis (i.e. inactivation) of the reactive groups is an undesired side reaction that lowers the degree of fixation. In spite of the addition of high quantities of salt and ureum (up to respectively 60 and 200 g/l) to raise the degree of fixation, it is estimated that 10 to 50% will not react with the fabric and remain .hydrolysed in the water phase. The problem of coloured effluents is therefore mainly identified with the use of reactive dyes. Most (~80%) reactive dyes are azo or metal complex azo compounds but also anthraquinone and phthalocyanine reactive dyes are applied, especially for green and blue.

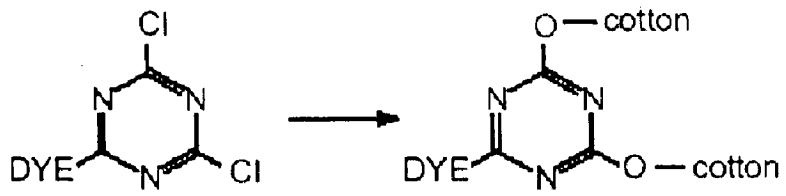


Figure 2.2 Principle of cotton dying with a triazyl reactive dye

2.1.5 Basic dyes

Basic dyes are cationic compounds that are used for dyeing acid-group containing fibres, usually synthetic fibres like modified polyacryl. They bind to the acid groups of the fibres. Most basic dyes are diarylmethane, triarylmethane, anthraquinone or azo compounds. Basic dyes represent ~5% of all dyes listed in the Colour Index.

2.1.6 Mordant dyes

Mordant dyes are fixed to fabric by the addition of a mordant, a chemical that combines with the dye and the fibre. Though mordant dyeing is probably one of the oldest ways of dyeing, the use of mordant dyes is gradually decreasing: only ~ 23% of the ~600 different mordant dyes listed in the Colour Index are in current production. They are used with wool, leather, silk, paper and modified cellulose fibres. Most mordant dyes are azo, oxazine or triarylmethane compounds. The mordants are usually dichromates or chromium complexes.

2.1.7 Disperse dyes

Disperse dyes are scarcely soluble dyes that penetrate synthetic fibres (cellulose acetate, polyester, polyamide, acryl, etc.). This diffusion requires swelling of the fibre, either due to high temperatures (>120 °C) or with the help of chemical softeners. Dying takes place in dyebaths with fine disperse solutions of these dyes. Disperse dyes form the third largest group of dyes in the Colour Index: about 1400 different compounds are listed, of which ~40% is currently produced. They are usually small azo or nitro compounds (yellow to red), anthraquinones (blue and green) or metal complex azo compounds (all colours).

2.1.8 Pigment dyes

Pigment dyes (i.e. organic pigments) represent a small but increasing fraction of the pigments, the most widely applied group of colourants. About 25% of all commercial dye names listed in the Colour Index are pigment dyes but these approx. 6900 product names stand for less than 800 different dyes. These insoluble, non-ionic compounds or insoluble salts retain their crystalline or particulate structure throughout their application. Pigment dyeing is achieved from a dispersed aqueous solution and therefore requires the use of dispersing agents. Pigments are usually used together with thickeners in print pastes for printing diverse fabrics. Most pigment dyes are azo compounds (yellow, orange, and red) or metal complex phthalocyanines (blue and green). Also anthraquinone and quinacridone pigment dyes are applied.

2.1.9 Vat dyes

Vat dyes are water-insoluble dyes that are particularly and widely used for dyeing cellulose fibres. The dyeing method is based on the solubility of vat dyes in their reduced (leuco) form. Reduced with sodium dithionite, the soluble leuco vat dyes impregnate the fabric. Next, oxidation is applied to bring back the dye in its insoluble form. Almost all vat dyes are anthraquinones or indigoids. Indigo itself is a very old example of a vat dye, with about 5000 years of application history. .Vat. refers to the vats that were used for the reduction of indigo plants through fermentation.

2.1.10 Anionic dyes and ingrain dyes

Azoic dyes and *Ingrain dyes* (naphthol dyes) are the insoluble products of a reaction between a coupling component (usually naphthols, phenols or

acetoacetylamides; listed in the Colour Index as C.I. azoic coupling components) and a diazotised aromatic amine (listed in the Colour Index as C.I. azoic diazo components). This reaction is carried out on the fibre. All naphthol dyes are azo compounds.

2.1.11 Sulphur dyes

Sulphur dyes are complex polymeric aromatics with heterocyclic S-containing rings. Though representing about 15% of the global dye production, sulphur dyes are not so much used in Western Europe. Dyeing with sulphur dyes involves reduction and oxidation, comparable to vat dyeing. They are mainly used for dyeing cellulose fibres.

2.1.12 Solvent dyes

Solvent dyes (lysochromes) are non-ionic dyes that are used for dyeing substrates in which they can dissolve, e.g. plastics, varnish, ink, waxes and fats. They are not often used for textile-processing but their use is increasing. Most solvent dyes are diazo compounds that underwent some molecular rearrangement. Also triarylmethane, anthraquinone and phthalocyanine solvent dyes are applied.

2.1.13 Fluorescent brighteners

Fluorescent brighteners (or bluing agents) mask the yellowish tint of natural fibres by absorbing ultraviolet light and weakly emitting visible blue. They are not dyes in the usual sense because they lack intense colour. Based on chemical structure, several different classes of fluorescent brighteners are discerned: stilbene derivatives, coumarin derivatives, pyrazolines, 1,2-ethene derivatives, naphthalimides and aromatic or heterocyclic ring structures. Many fluorescent brighteners contain triazinyl units and water-solubilising groups.

2.1.14 Other dye classes

Apart from the dye classes mentioned above, the Colour Index also lists *Food dyes* and *Natural dyes*. Food dyes are not used as textile dyes and the use of natural dyes (mainly anthraquinone, indigoid, flavenol, flavone or chroman compounds that can be used as mordant, vat, direct, acid or solvent dyes) in textile-processing operations is very limited.

2.2 Azo Dyes

The azo dues and pigments form the largest group of all the synthetic colourants and play a prominent part in almost every type of application. The chromophoric system

consists essentially of the azo group, -N=N-, in association with one or more then one azo group present in the dye molecule and thus one speaks of monoazo , disazo, trisazo tetrakisazo and polyazo dyes according to whether there are one, two three , four of more azo group present in the dye molecule. The range of hues covered by this group is very wide and includes yellows, a great number of reds and oranges, navy blues violets and blacks. Greens are known but the range is comparatively limited.

Azo dyes are made almost exclusively by the diazotization of a primary aromatic amine to give a Diazo or diazonium salt a reaction of fundamental importance to the dye industry discovered by Griess in 1862. The Diazo compound is then coupled with a second substance usually a phenol, an enolizable ketone or an aromatic amine and azo compound being formed. The participating molecules are known as diazaocomponent and coupling component respectively.

It is the practice to describe azo by the convention –

amine → coupling component E

→ coupling component E

diamine → coupling component E

and similarly for tris-, tetrakis- and polyazo dyes. In such cases the arrow indicates that the appropriate primary aromatic amine has been diazotized and coupled with a component, e.g. the dyestuff is represented as aniline →2 naphthol.

This system is used in the Colour Index to describe all the azo dyes of declared constitution and has been adopted generally.

2.2.1 Monoazo dyes

The monoazo dyes are a large group covering many important applications. They are of the type

A → E

When A=a Diazo component, i.e a diazotized arylamine,

E=a coupling component coupled with one molecule of a Diazo component.

It is convenient of consider first those dyes, the molecules of which contain no solubilizing groups.

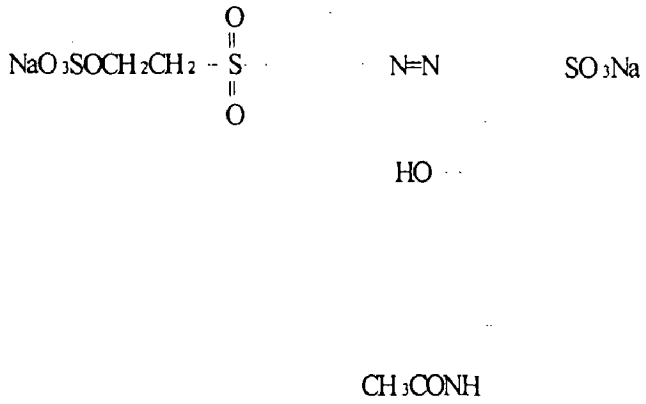


Fig. 2.3 Chemical structure of Reactive Orange 16, MW= 617

2.2.2 Disazo dyes

There are four subdivisions in the Colour Index among the disazo dyes, each of which can be assigned a general formula using the symbols introduced by Winther. Thus:

(1) A → Z → A'
E

(2) D

E'

(3) A → M → E

(4) A → Z.X.Z ← A'

where A is a diazo component ,

D is a tetrazo component

E is a coupling component coupling with one molecule of a diazo component,

M is an aromatic amine which after coupling with a Diazo component provides an amino group for further diazotization,

Z is a coupling component coupling with two or more molecules of a Diazo component of with one molecule of each of two or more different Diazo components.

In Z.X.Z, X may be –NH-, -NH.CO.NH- or more complex linkages.

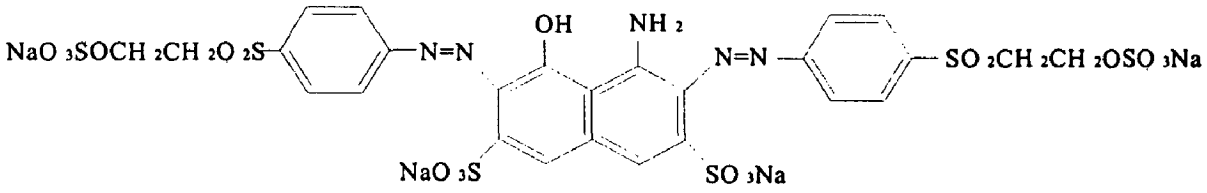


Fig. 2.4 Chemical structure of RB5

2.3 Anthraquinone dyes and related colourants

The characteristic chromophore of the anthraquinone series consists of one of more carbonyl group, their simple substitution derivatives such as alkylamino, arylamino, acylamino groups and more complex fused-ring compounds such as pyrathrone, violantrone and others the parent carbonyl compound is coloured although no auxochrome is present.

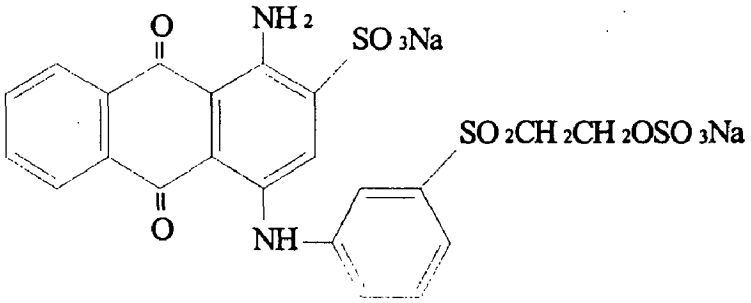


Fig. 2.5 Chemical structure of RBBR

Anthraquinone dyes and colourants make important contributions to a number of widely different usage groups, among them the acid direct disperse, mordant solvent vat pigment and reactive dyes.

2.4 White rot fungi

In order to improve the carbon recycling pathways a compartment of eight higher plants was added parallel to the IVth compartment of the MELISSA cycle. The liquefying compartment was not able to degrade the totally of the extra amount of fibres that this addition provoked. A new compartment colonised by white-rot fungi was proposed as solution to compensate the loss of balance of the cycle. These fungi have an

excellent ability to degrade organic fibres using lignolytic enzymes and could also contribute to improve the nutritional value of the crew diet. The FOOD project was aimed to investigate the efficiency of edible fungi in degrading the extra amount of fibres introduced in the MELISSA cycle after the addition of the plants. During the development of the FOOD project, various *Pleurotus* and *Lentinus* mushroom strains were cultivated using as substrate the output of the liquefying compartment (before addition of plants) and the non-edible parts of the plants that will be included in the photosynthetic compartment. *Lentinus* edodes grew very slow in those substrates but all the *Pleurotus* strains tested could colonise the substrates rather fast and utilise them as source of nutrients. Not all the *Pleurotus* strains showed the same development. Depending on the substrate some of them were better suited than others to grow, which indicated that their metabolic pathways differed (amount of lignolytic enzymes produced were not the same). A detailed study was done on the behaviour of a selected strain of *Pleurotus pulmonarius* that grew very fast on the output of the liquefying compartment. The fast growth was probably due to its production of lignolytic enzymes (mainly laccases and peroxidases) able

2.5 Enzymes

2.5.1. Enzymes in general:

Advantages and Disadvantages

Enzymes are proteins, widespread in nature. They are a fundamental part of life. Chemically, enzymes contain one or more chains of hundreds of amino acids in a highly complex three-dimensional structure, which is tremendously important for their action. They act as biological catalysts in biochemical reactions of all living organisms, but mean-while have also been established as reliable and convenient processing aids in many technical industries. As all catalysts a biochemical catalyst like an enzyme is defined as a reaction partner which increases the rate or velocity of a chemical reaction without itself being changed in the overall process. This definition implies that one single enzyme molecule is able to convert thousands of substrate molecules during its lifetime, which is dependent on the conditions. The conversion rate or velocity of the reaction is called the activity of an enzyme. Enzymes differ from ordinary chemical catalysts in

several important aspects such as: (i) higher reaction rates; (ii) milder reaction conditions and (iii) higher reaction specificity.

(i) Enzymatically catalysed reactions are typically accelerated by factors of 106–1012 compared to corresponding uncatalysed reaction and are often several orders of magnitude faster than the respective chemically catalysed reaction.

(ii) Most enzymatically catalysed reactions occur under relatively mild conditions: temperatures below 100°C, atmospheric pressure, pH values from 4 to 8, and without the need for aggressive chemicals or solvents. But because of its proteineous nature like all proteins enzymes are often sensitive to untypical, 'non-biological' conditions in pH and temperature which influence the correct three-dimensional structure. In contrast, efficient chemical catalysis often requires elevated temperatures and pressures as well as extremes of pH.

(iii) The most striking characteristic of enzymes is their high substrate and reaction specificity which also is a direct consequence of their complex three-dimensional structure. Most of the enzymes are able to use only a few very similar substrates, sometimes only one single substrate. In some cases these proteins can discriminate molecules differing only in their stereo chemical structure. But in the case of enzymes which can find an application in pulp bleaching (e.g. lignin peroxidase (LiP), manganese peroxidase (MnP) or laccase) these types are, untypically, unspecific in their choice of substrates (Higuchi, 1990; Popp and Kirk, 1991; Yaropolov et al., 1994).

The interaction of an enzyme with its substrate can be illustrated by the lock-and-key model. Only a substrate (the key), which can dock to the active centre (the lock), will be converted at the catalytic centre, which is a part of the active centre. Of course the reactions are much more complicated in detail and can be described only in very complex models. In contrast to chemical catalysts, one should keep in mind that enzymes can be finetuned for special reactions by a combination of highly sophisticated methods as genetic engineering and computer-aided protein-design. New products for the detergent industry, the most important user of technical enzymes today, have demonstrated that a realization is possible today. Based on such favorable properties enzymes are widely used

as catalysts and processing aids in many industries. Various industries including chemicals, fuel, food, brewery and wine, animal feed, textile and laundry, pulp and paper and agriculture which rely, A typical example for the replacement of former chemical catalysts by enzymes in established bulk processes is the corn wet-milling industry. More than 12 m tons of dextrose and sugar syrup are produced per year by enzymatic hydrolysis of starch. In the baking industry xylanases are used for improving desirable texture, loaf volume and shelf-life of bread. A xylanase, Novozyme 867, has shown excellent performance in the wheat separation process. Hemicellulases are used for pulping and bleaching in the pulp and paper industry where they are used to modify the structure of xylan and glucomannan in pulp fibres to enhance chemical delignification Laccases can degrade a wide variety of synthetic dyes making them suitable for the treatment of wastewater from the textile industry In dairy cows the milk yield increased in the range of 4 to 16% on various commercial fibrolytic enzyme treated forages.

2.5.2 Laccase

Laccase (benzenediol:oxygen oxidoreductase;*p*-diphenol oxidase, urishiol oxidase, EC 1.10.3.2), a blue copper-containing oxidase, was first discovered by Yoshida (1883) in plants deduced from the observation that the latex of the Chinese or Japanese lacquer trees (*Rhus sp.*) was rapidly hardened in the presence of air. The enzyme was named laccase about 10 years later after isolation and purification of the responsible catalyst (Bertrand, 1894). Fungal laccases already were discovered during this century (Bertrand, 1896; Laborde, 1896). Laccase has been extensively examined since the mid seventies and results are reviewed in a number of articles (Mayer and Harel, 1979; Malkin and Malmstro¨m, 1970; Malmstro¨m et al., 1975; Fee, 1975; Va¨nngard, 1972; Reinhammar, 1979, 1984; Holwerda et al., 1976; Reinhammar and Malmstro ¨m, 1981; Thurston, 1994; Yaropolov et al., 1994). More than 200 enzymes devided into oxygenases and oxidases are known which are using molecular oxygen (dioxygen) as one of their substrates (cosubstrates). Only six of them are able to reduce oxygen to two molecules of water. Besides cytochrome-*c* oxidase, a heme:Cu containing enzyme, only the blue oxidases (laccase,(EC 1.10.3.2), L-ascorbate oxidase (EC 1.10.3.3: L-ascorbate:oxygen oxidoreductase; ascor-base), ceruloplasmin (EC 1.16.3.1:Fe(II): oxygen oxidoreductase;

ferrooxidase) are able to achieve this reaction. Today bilirubin oxidase (EC 1.3.3.5, bilirubin:oxygen oxidoreductase) and phenoxazinone synthase (Hiromi et al., 1992; Freeman et al., 1993) are classified into the group of so-called multicopper oxidases, too. All these 'blue', copper as an important cofactor containing enzymes differ remarkably in their primary amino acid sequence and their biological function, but they are rather similar in their three dimensional structure (Messerschmidt and Huber, 1990; Messerschmidt et al., 1989, 1992) and their characteristic copper binding in three different types of sites.

2.5.3 Laccases—their biological function

2.5.3.1. Synthesis and lignification in plants

Laccases are widely distributed in the plant kingdom. This enzyme has been discovered already in the last century as the responsible catalyst in the wound healing mechanism of *Rhus* species. By oxidation of endogenous phenols to toxic quinones a role in a defense mechanism of plants against pathogens is discussed. Erdtmann (1933) proposed a mechanism of lignification based on enzyme catalyzed oxidation of phenolic precursors to radicals polymerizing spontaneously. Freudenberg's hypothesis was that laccases and peroxi- : 53 (1997) 163–202 dases cooperate in the lignin polymerization. As part of the lignin synthesizing system laccases and:or laccase-type polyphenol oxidases are localized in woody tissues and cell walls of herbaceous species. The biotechnological implications of a laccase participation in lignin biosynthesis in trees have been discussed (Dean and Eriksson, 1992; O'Malley et al., 1993).

2.5.3.2. Detoxification

Due to the polymerizing activity of laccase (as well as of LiP and MnP) in vitro an enzymatic protection mode of living cells is discussed. Potentially toxic, low molecular weight phenolic compounds are polymerized to reaction products of sufficient size hindered in penetration of cells. But the production of quinines instead of polymers will lead to the opposite due to the higher toxicity of the quinones. Laccase is considered as an efficient radical scavenger, a property widespread among proteins, in particular blue copper proteins. As first radicals interact with the polypeptide chain; targets are tyrosine, tryptophan, histidine, cystine and cysteine residues where OH· is able to form adducts with or to abstract hydrogen. In laccase only a limited number of amino acid side chains

(only one or two amongst his, trp or tyr, but no cys) are detected to form an adduct with OH·. By intramolecular electron transfer from the protein site to the copper type-1 site Cu-reduction can be observed.

2.5.3.3. Delignification

Laccase is particularly abundant in wood-destroying fungi which are able to degrade lignin (white-rot fungi, known as lignolytic fungi) and it is absent in brown-rot fungi, which are unable to decompose lignin. The fact that laccase-less mutants of a white-rot fungi cannot, but revertants can attack lignin very early led to the hypothesis that laccase may be involved in the natural delignification process. *Phanerochaete chrysosporium* is widely described as a white-rot fungus lacking laccase activity, but able to degrade lignin very well. The conclusion is that lignin degradation can occur in the absence of laccase, too. But recently Srinivasan et al. (1995) detected laccase activity in a strain of *Phanerochaete chrysosporium*. They demonstrated that the examined strain produces low but consistent levels of laccase in a defined culture medium. This raises the questions again whether all white-rot fungi are able to produce the whole set of lignolytic enzymes, the extent is dependent on the conditions, only.

2.5.3.4. Distribution in nature

Laccase is widely distributed in higher plants and fungi like *Agaricus*, *Podospora*, *Rhizoctonia*, *Trametes* (*syn. Polyporus* (*syn. Dichomitus*) *syn. Coriolus*), *Pholiota*, *Pleurotus*, *Neurospora*, *Aspergillus*, *Fusarium*, *Schinus Rigidoporus*, *Botrytis*, *Schizophyllum*, *Cerrena*, *Phlebia* (*syn. Merulius*), *Pyricularia*, *Pycnoporus*, *Junghuhnia*, *Lentinus*, *Armillaria*, *Monocillium*, *Fomes*, *Russula*, *Leptoporus*. The comparison of primary sequences of fungal laccases indicate that different laccase-families exit, e.g. the enzyme from *Aspergillus* or *Neurospora* is more similar to *Cucumis sati6us* ascorbate oxidase than to *Trametes* laccase. Up to now laccase-type phenol oxidase activity has been seldom detected in bacteria, in *Streptomyces galbus* and *Azospirillum lipoferum* only.

2.5.4. Properties and reactions of laccase

2.5.4.1. Substrates and inhibitors of laccase

In contrast to the high specificity of enzymes ingeneral, one of their remarkable properties, all lignolytic enzymes are rather unspecific. Laccase is an important oxidant

for aromatic rings substituted with electron withdrawing groups. The enzyme reacts with many substrates under H-atom abstraction (i.e. 1 H^++1 e^-) and formation of radicals, which may undergo further non-enzymatic reactions leading to the often observed polymerized products. As substrates laccase can convert *o*- and *p*-diphenols, aminophenols, polyphenols, polyamines, lignin, some inorganic ions, aryl diamines and is not subjected to product inhibition. It can catalyze decarboxylation, demethylations and demethoxylations, a reaction often observed in fungal delignification. All known laccases catalyze with equally high efficiencies both the oxidation of ascorbic acid and phenol substrates. Despite their different biological functions the substrate specificity of laccase is close to that of ceruloplasmin. The one or two magnitude higher catalytic rates indicate the better adaptation of laccase to their oxidative function compared to ceruloplasmin which had been used in determination of aromatic diamines and aminophenols, too. Kinetic data of laccases from different sources are discussed by Yaropolov et al. (1994). *K*m values, the Michaelis constants, are similar for the cosubstrate oxygen (about 10^{-5} M), but *V*max varies with the source of laccase (50–300 $M \cdot s^{-1}$). The turnover is heterogeneous over a broad range dependent on source of enzyme and substrate: type of reaction. The kinetic constants differ in their dependence on pH, the *K*m is pH-independent for both the substrate and the cosubstrate, the catalytic constant is pH-dependent. For activity determinations of laccase a modified syringaldazine based method was used which was described for qualitative detection of laccase in fungi and used by Ander and Eriksson (1976) and by Leonowicz and Grzywnowicz (1981) for quantification of enzyme activity in different fungi. Syringaldazine is a nonautooxidizable (in the absence of H_2O_2) laccase-specific, very sensitive substrate (ε525-65 000 M^{-1} cm^{-1}). The highest reaction rates and specific catalytic activities for laccase from Phlebia were determined using the phenolic compounds such as syringaldazine or vanillalacetone. With the non- phenolic ABTS activity was measured as twice as high, another non-phenolic (VA) cannot be converted due to its high redox potential. Oxidation rates of laccase were 10–100 fold higher compared to LiP or MnP tested with the same substrate. Independent of the source, laccase can be very strongly inhibited by many anions which are able to interact with the copper sites like azide, cyanide, thiocyanide and fluoride. Complexing agents removing copper from the active

site inhibit the activity reversible. In Table 5 some important properties of laccase in general and from *Trametes* in detail are summarized.

2.5.4.2. Reactions catalyzed by laccase

In principle an oxidoreductive-like activity such as 'VA-oxidase' or 'laccase' can be obtained rather simple by artificial systems:

(i) CBH I, a cellulolytic enzyme was chemically modified by attachment of penta ammine ruthenium (III), which causes a peroxide-dependent oxidation of veratryl alcohol, (ii) copper (II) amine complexes cleaved bonds in lignin model compounds comparable to the enzyme. The conversion rates are, not surprisingly, very low in comparison to reactions supported by an 'optimized catalyst,' the enzyme. Both structure and redox potential of a compound seems to define what is a substrate of lignolytic enzymes. Ligninases are able to oxidize substrates of extremely high electropotential up to $E_{1/2}$=1.49 V, e.g. veratryl alcohol ($E_{1/2}$= 1,22 V vs. a saturated calomel electrode), MnP and HRP up to $E_{1/2}$=1,12 V whereas laccase cannot oxidize non-phenolics with a higher electropotential than $E_{1/2}$=1,06 V assumed that the redox potential of LiP compound I is pH dependent comparable to those of CCP and HRP. The redox potential of compound I increases with decreasing pH, i.e. the lower the pH the better the oxidizing agent which can be an explanation for the low pH optimum of LiP for oxidations of non-phenolics. For laccase, in more detail for the fungal enzymes, such data are not available.

For a long time it was recognized that laccase catalyzed reactions can be modulated. For example, the oxidation of a worse laccase-substrate (2, 4-dichlorophenol) can be prevented by addition of a good substrate (catechol). In contrast syringic acid results in a threefold enhancement of the conversion, i.e. 'crosscoupling' of substrates can alter the reaction of, e.g. this chlorinated substrate. Products of these reactions are polymerizates as expected from the knowledge about laccases. But in the presence of ABTS, a very good substrate for laccase, a conversion of the non-phenolic compound veratryl alcohol, which is not a substrate of this enzyme, to veratrylaldehyde was observed. Is this case not only a modulation but a formerly impossible reaction was described.

Besides these, laccase is able to catalyze enzymatic and electrochemical reactions even in the absence of a low-molecular-weight carrier of electrons, too. The reaction is

called mediator-less electroreduction (of oxygen to water) which runs without formation of hydrogen peroxide as an intermediate.

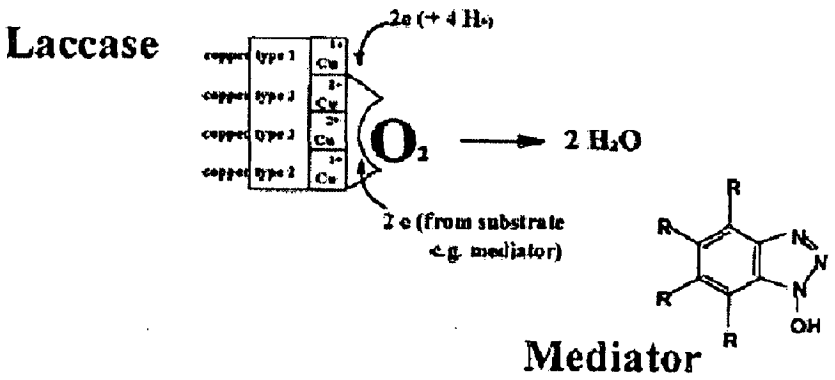
The electrode potential established as a result of the reaction

$$O_2 + 4\,H^+ + 4e \xrightarrow{\text{laccase}} 2\,H_2O$$

is close to the equilibrium oxygen potential. The most effective inorganic catalyst for this reaction is specially treated platinum (equilibrium oxygen potential: 1.23 V). In contrast laccase immobilized on electrodes of various materials is able to change the potentials in the range of 1.2–0.6 V, depending on the amount of enzyme fixed. Potentiometric sensors based on this principle have been developed. Negative results with apoenzyme, i.e. copper depleted molecules, and with fluoride or azide (interaction with Cu type-2) inhibited enzyme indicates that copper and not the protein moiety is responsible for the reaction. Electrons were taken by the active site of the enzyme directly from the electrode. At potentials higher than 1.2 V laccase is reversibly inactivated by overoxidation of catalytically important groups.

2.5.4.3. The catalytic site of laccase

In laccase-catalysed reactions one-electron substrate oxidations are coupled with one four-electron reduction of oxygen to water, i.e. electrons from the oxidation steps has to be 'stored' as in a battery in order to reduce molecular oxygen.



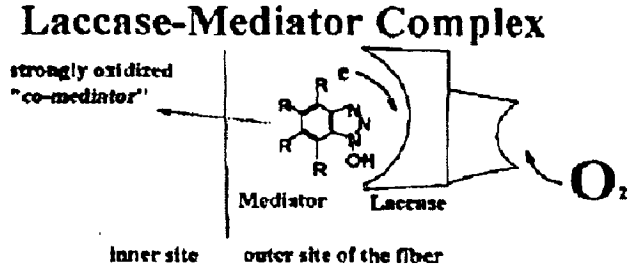


Fig. 2.6 Laccase and Laccase mediator

2.5.4.4 Crystal structure of the laccase (*Trametes versicolour*)

Laccase (*p-diphenol:oxygen oxidoreductase*, EC 1.10.3.2) is multi-copper oxidase that catalyzes the oxidation of various aromatic compounds and also some inorganic compounds with the concomitant reduction of dioxygen to water. The laccases can be divided into two categories, plant and fungal, although diphenol-oxidizing enzymes which are thought to be laccases have also been identified in insects and eubacteria. Due to its biochemical, catalytical and electrochemical properties laccase has gained considerably interest because of its potential biotechnological applicability.

The quaternary structure of laccase has been solved only for a limited number of enzymes, e.g. *Coprinus cinereus*, *Melanocarpus albomyces*, *Pycnoporus cinnabarinus*, and *Trametes versicolour*. It was shown that laccases have a minimum of one mononuclear copper site containing one type-1 Cu, and a trinuclear copper site containing one type-2 Cu and two type-3 Cu. Oxidation of simple organic substrates occurs via a ping-pong type mechanism. Substrates are oxidized near the mononuclear site, and the electrons are transferred to the trinuclear site, where the molecular oxygen is reduced. Neither the electron transfer mechanism nor the oxygen reduction to water is fully understood. Nevertheless, a number of mechanistic schemes satisfactory describing the set of kinetic and structural data has been proposed.

2.6 Manganese Peroxidase(MnP)

Manganese peroxidase first detected in the culture fluid of *Phanerochaete chrysosporium*, catalyzed the oxidation of MnP(II) to MnP(III), which in turn can oxidized phenolic structures in lignin. Such reaction may lead to polymer fragmentation but the role of MnP is not an apparent as that of lignin peroxidase. MnP from *Coriolus*

versicolour plays an important role in delignification of kraft pulp, decolourization of bleach plant effluent and degradation of several dyes.

2.7 Lignin Peroxidase(LiP)

The mechanism by which lignin peroxidase (Lip) interacts with the lignin polymer is discussed. Veratryl alcohol (Valc), a secondary metabolite of white rot fungi, acts as a cofactor for the enzyme. The Lip-redox cycle is discussed in terms of Marcus theory of electron transfer. It is proposed that reaction of a nucleophile in the active site channel with the incipient Valc'. is an essential event in the cycle. Subsequently, outside the channel the Valc'. is regenerated and will oxidize the lignin polymer. Further it is postulated that a carbohydrate residue from a β-glycosylation site at Ser-334 may act as the nucleophile in this process.

2.7.1. Lignin Peroxidase Catalyzed Veratryl Alcohol Oxidation

Ever since the discovery of lignin peroxidase, veratryl alcohol has played a pivotal role in the study of the lignin biodegradation process. Veratryl alcohol is used as an assay for enzyme activity due to the easily detectable absorbance of the product veratraldehyde at 310 nm. The optimum pH of the enzyme is rather low (pH = 2.5) and the fact that lignin peroxidase is capable of oxidizing non-phenolic substrates has attracted considerable attention . The fact that lignin peroxidase will oxidize (non-phenolic) compounds with a relatively high redox potential has been interpreted by many authors as the result of an unusually high redox potential of the oxidized enzyme intermediates, lignin peroxidase Compound I (LiPI) or Compound Il (LiPII). Alternatively, it has been argued that the enzyme is capable of stabilizing the initial product of the veratryl alcohol oxidation, the veratryl alcohol radical cation ($Valc^+$). The redox cycle of the enzyme is schematically depicted in scheme 1.

$LiP + H_2O_2$	$\rightarrow$	H_2O +LiP I	(Compound I)
Lip I + Valc	$\rightarrow$	$Valc^+$ + LiP II	(Compound II)
LiP II + Valc + 2H+	$\rightarrow$	$Valc^+ + H_2O$ + LiP	(native enzyme)

Scheme 1 The lignin peroxidase redox cycle

Thus, Lip1 will oxidize the first molecule of veratryl alcohol to the corresponding radical cation ($Valc^+$), which is liberated from the active site. Subsequently, the second substrate molecule is oxidized by LiPII to form the second $Valc^+$. In the process LiPII is

converted to native enzyme. Veratryl alcohol binds in the active site channel close to the heme. This binding is followed by an electron transfer process in the ender-gonic region of the driving force, which is rendered essentially irreversible due to a concomitant reaction with a nucleophile, which can be either solvent or a part of the enzyme (protein or carbohydrate, *vide infra).* In this way the highly reactive $Valc^+$ is temporarily neutralized both in charge and in activity and as such transported away from the active site. Outside the active site channel protonation of the enzyme-product or the solvent-product complex will result in the regeneration of the very strong oxidant, $Valc^+$, which subsequently might oxidize any recalcitrant chemical present, like the lignin polymer.

CHAPTER 3

LITERATURE REVIEW

3.1. Adsorption and Biodegradation:

With the viewpoint of this thesis report, various literatures published have been studied and critically analyzed for understanding the importance of the present work on the removal of various dyes from aqueous solution by adsorption, biodegradation and by both has been reported.

The adsorption of impurities from solution onto solids materials is an attractive method of waste water treatment. Recently a number of successful systems have been developed using adsorption technique. Activated carbon is the most commonly used adsorbent. However, its cost is high and about 10-15% is loss during regeneration. Unconventional adsorbents like fly ash, peat, lignite, baggase, pith, wood saw dust etc. have attracted significant attention, and their adsorption characteristics have been widely investigated for the removal of clour dyes, heavy metals, phenol compounds and other refractory organics.(K. Vasanth 2002)

Considerable amount of work has been available in literatures on colour removal from waste water using adsorption technique.

The removal of congo red from baggase and observed that adsorption rate was more at concentration of less than 100 mg/l. (subanandam, 1999)

The removal of malachite green from carbonized coconut shell and conclude that the external mass transfer resistance was the sole rate controlling step during the adsorption. (Vasanth and krishnaswamy, 2001)

Removal of basic dyes from carbonized coconut shell adsorbents and conclude that the rate of adsorption of dyes particles was controlled by the operating variables like initial concentration, adsorbent dosage, and agitation speed. (Vasanth and subanandam, 2002)

The removal of methylene blue and malachite green using low and high carbon content fly ash and they found that the colour removal rate was more with high carbon content fly ash. (Mall and Upadhyay, 1998)

Removal of crystal violet colour by use of low cost material Rice Husk from its dilute solution. (Vasanth and subanandam, 2003)

Two strictly related laccase isoenzymes (POXA3a and POXA3b), produced by *Pleurotus ostreatus* in copper supplemented cultures, have been purified and characterised. Both the native proteins were found to be constituted by a large subunit (67 kDa) and a small subunit (18 or 16 kDa). Peptide mapping of the 18 and 16 kDa polypeptides from POXA3a and POXA3b suggests the identity of the 18 kDa subunits and the generation of the 16 kDa polypeptides from the 18 kDa ones. Structural data on POXA3a and POXA3b do not allow ascertaining significant differences between the two isoenzymes. On the other hand, dissociation of POXA3a complex is observed in 3M urea, whilst POXA3b complex is not dissociated even in 6M urea. Evidences are reported on the role played by extracellular proteases in the activation of these isoenzymes. The sequence of a unique gene and of the corresponding cDNA, encoding the 67 kDa POXA3 subunit, has been determined. (Gianna Palmieri, Giovanna Cennamob, Vincenza Faraco, Angela Amoresanob, Giovanni Sannia , Paola Giardina 2003)

In this study purified and investigated the catalytic properties of a manganese peroxidase isoenzyme produced by the fungus *Pleurotus ostreatus* in liquid medium with peptone as nitrogen source. The isoenzyme was purified to homogeneity by chromatography on Bio-Rad Q-cartridge, Sephacryl S-200 and Mono-Q with activity yield of 59% and a purification factor of 36. The *P. ostreatus* MnP obtained had the same p*I*_3.75.and N-terminal sequence as MnP-1 of *Pleurotus eryngii* produced in the same medium _both exhibiting Mn-independent activities on phenolic and non-phenolic substrates. However, the N-terminal sequence of this *P. ostreatus* isoenzyme differed from a previous published sequence of MnP from this fungus. The results obtained show the importance of media composition in the production of different isoenzymes within the same fungal species. We have also demonstrated by Southern blots that the different isoenzymes are probably encoded by different genes, and that the MnP genes in both *Pleurotus* species are similar but different to those of *Phanerochaete chrysosporium.* (Sovan Sarkar, Angel T. Martinez, Maria Jesus Martinez, 1996)

The rate and efficiency of decolourization of poly R-478- or Remazol Brilliant Blue R (RBBR)-containing agar plates (200 μg g−1) were tested to evaluate the dye degradation activity in a total of 103 wood-rotting fungal strains. Best strains were able to completely decolourize plates within 10 days at 28 °C. *Irpex lacteus* and *Pleurotus ostreatus* were selected and used for degradation of six different groups of dyes (azo, diazo, anthraquinone-based, heterocyclic, triphenylmethane, phthalocyanine) on agar plates. Both fungi efficiently degraded dyes from all groups. Removal of RBBR, Bromophenol blue, Cu-phthalocyanine, Methyl red and Congo red was studied with *I. lacteus* also in liquid medium. Within 14 days, the following colour reductions were attained: RBBR 93%, Bromophenol blue 100%, Cu-phthalocyanine 98%, Methyl red 56%, Congo red 58%. The ability of *I. lacteus* to degrade RBBR spiked into sterile soil was checked, the removal being 77% of the dye added within 6 weeks. The capacity of selected white rot fungal species to remove efficiently diverse synthetic dyes from water and soil environments is documented. (Cenek Novotny, Bhavin Rawal, Manish Bhatt, Milind Patel Vaclav Sasek, Hans Peter Molitoris 2001)

The results of the cultivation of six strains of *Pleurotus* (*P. djamor* (2), *P. ostreatus* (2) and *P. pulmonarius* (2)) on coffee pulp and wheat straw are presented. Metabolic activity associated with biomass of each strain was determined, as well as changes in lignin and polysaccharides (cellulose and hemicellulose), phenolic and caffeine contents in substrate samples colonized for a period of up to 36 days. Analyses were made of changes during the mycelium incubation period (16 days) and throughout different stages of fructification. Greater metabolic activity was observed in the wheat straw samples, with a significant increase between 4 and 12 days of incubation.A decrease was observed in caffeine content of the coffee pulp samples during fruiting stage, which could mean that some caffeine accumulates in the fruiting bodies. (Dulce Salmones , Gerardo Mata, Krzysztof N. Waliszewsk 2004)

A screening using several fungi (*Phanerochaete chrysosporium, Pleurotus ostreatus, Trametes versicolour and Aureobasidium pullulans*) was performed on the degradation of syringol derivatives of azo dyes possessing either carboxylic or sulphonic groups, under optimized conditions previously established by us. *T. versicolour* showed the best biodegradation performance and its potential was confirmed by the degradation

of differently substituted fungal bioaccessible dyes. Enzymatic assays (lignin peroxidase, manganese peroxidase, laccase, proteases and glyoxal oxidase) and GC-MS analysis were performed upon the assay obtained using the most degraded dye. The identification of hydroxylated metabolites allowed us to propose a possible metabolic pathway. Biodegradation assays using mixtures of these bioaccessible dyes were performed to evaluate the possibility of a fungal wastewater treatment for textile industries. (M. Adosinda M. Martins, Nelson Lima, Armando J.D. Silvestre, M. Joao Queiroz, 2003)

Immobilization of laccase by *Trametes versicolour* on silica chemically modified with imidazol groups, amberlite IRA-400, glass–ceramic chemically modified with carbodiimide/glutaraldehyde and by aminoprolyltriethoxysilane/glutaraldehyde and montmorillonite modified by aminoprolyltriethoxysilane/glutaraldehyde were afforded. These supports were used in the decolourization of textile reactive dyes (Remazol Brilliant Blue R, Remazol Black B, Reactive Orange 122 and Reactive Red 251). One of the most efficient supports was studied in a more detailed way; silica modified by imidazole SiIm led us to obtain almost total immobilization of laccase at contact times lower than 45 min. The immobilized laccase showed high decolourization efficiency toward aqueous solutions of the dyes. In the first stages of the process, the decolourization is mainly due to adsorption of the dyes onto the support surface. However, when working in a successive dye addition system, the adsorption capacity of the supports decrease (saturation) and the enzymatic decolourization process is clearly evidenced. The use of a brief photochemical pre-treatment permitted a significantly increase on the efficiency of the enzymatic decolourization process. (Patricio Peralta-Zamora 2002)

White rot fungus *Irpex lacteus* was grown in both stationary and agitated submerged liquid cultures as well as in cultures immobilized on polyurethane foam (PUF) and pine wood (PW) to study the effect of growth conditions on degradation of commercial dyes. The respective values of decolourization of the anthraquinone-based Remazol Brilliant Blue R (RBBR) dye used at 150μg ml^{-1} were 100, 95, 97 and 100% within 6–10 days. Stationary cultures exhibited higher levels of lignin peroxidase (LiP), manganese-dependent peroxidase (MnP) and laccase than submerged cultures and selective inhibition by NaN_3 and *n*-propylgallate brought evidence that MnP played a

major role in the decolourization of RBBR by *I. lacteus*. When comparing PUF- and PW-immobilized cultures decolourizing RBBR, five-fold MnP levels were detected in the former cultures whereas laccase activities were similar. No LiP was detected in either immobilized culture. Both immobilized cultures were able to rapidly decolourize not only RBBR but also various textile-industry, colour bath effluents. Reusability and regenerative capacity of the immobilized cultures, important for application to water bioremediation, were documented. (Aparna Kasinath, Cenek Novotny, Katerina Svobodova, Kamlesh C. Patel Vaclav Sasek 2002)

Biological decolourization of textile dyestuff Reactive Orange 16 was investigated in a fed-batch reactor by using facultative anaerobic bacterial consortium called PDW. Effects of different operating parameters like flow rate (Q = 50–300 ml/h), dyestuff concentration (50–600 mg/l) and yeast extract concentration (1–5 g/l) on decolourization efficiency were studied. Reaction cycle was varied between 2 and 7 h depending on the feeding rate. System was operated at room temperature (T = 19 ± 1 °C) and at pH = 7. The experimental results indicated that over 90% efficiency can be obtained up to 350 mg/l dyestuff concentration and 200 ml/h feeding rate. Three grams per liter yeast extract concentration as sole carbon source was enough to obtain around 95% colour removal. (Ilgi Karapinar Kapdan, Rukiye Oztekin2003)

This study was taken up to enrich and isolate bacterial strains capable of decolourizing azo dyes present in soil/sludge samples collected from waste disposal sites of local textile industries. Four bacterial isolates identified as *Bacillus cereus* (BN-7), *Pseudomonas putida* (BN-4), *Pseudomonas fluorescence* (BN-5) and Stenotrophomonas acidaminiphila (BN-3) capable of completely decolourizing C.I. Acid Red 88 (AR-88), were used to develop consortium designated HM-4. The concerted metabolic activity of these isolates led to complete decolourization of AR-88 (20 mg L^{-1}) in 24 h, whereas individual cultures took more than 60 h to achieve complete decolourization of the added dye. The consortium was screened for its ability to decolourize different concentrations of other commonly used azo dyes in addition to AR-88. It was able to decolourize 78% of C.I. Acid Red 88, 99% of C.I. Acid Red 119, 94% of C.I. Acid Red 97, 99% of C.I. Acid Blue 113 and 82% of C.I. Reactive Red 120 dyes at an initial concentration of 60 mg L^{-1} of mineral salts medium (MSM) in 24 h. This consortium will be used to develop

bioreactor for achieving effective decolourization of textile industry effluent containing mixture of azo dyes. (Manjinder Singh Khehraa, Harvinder Singh Sainia, Deepak Kumar Sharmaa, Bhupinder Singh Chadhaa, Swapandeep Singh Chimni, 2005)

An investigation was conducted to explore the lignin-degrading capacity of attached-growth white-rot fungi. Five white-rot fungi, *Phanerochaete chrysosporium, Pleurotus ostreatus, Lentinus edodes, Trametes versicolour and S22*, grown on a porous plastic media, were individually used to treat black liquor from a pulp and paper mill. Over 71% of lignin and 48% of chemical oxygen demand (COD) were removed from the wastewater. Several factors, including pH, concentrations of carbon, nitrogen and trace elements in wastewater, all had significant effects on the degradation of lignin and the removal of COD. Three white-rot fungi, *P. chrysosporium, P. ostreatus* and *S22*, showed high capacity for lignin degradation at pH 9.0–11.0. The addition of 1 g l^{-1} glucose and 0.2 g l^{-1} ammonium tartrate was beneficial for the degradation of lignin by the white-rot fungi studied. (Juan Wu, Ya-Zhong Xiao, Han-Qing Yu, 2005)

Submerged culture conditions for laccase production by *Pleurotus ostreatus* 1804 were optimized by Taguchi orthogonal array (OA) experimental design (DOE) methodology. This approach facilitates the study of interaction of a large number of variables spanned by factors and their settings with a small number of experiments leading to considerable saving in time and cost for the process optimization. The proposed Taguchi DOE methodology consists of four phases viz., planning, conducting, analysis and validation, which were connected sequence wise to achieve the overall process optimization. Eight factors viz., carbon, nitrogen source (urea), 2,5-xylidine (inducer), wheat bran (ligninocellulosic material), inoculum size, fermentation broth pH, yeast extract and phosphate source (KH_2PO_4) at three levels with a OA layout of L18 (21 ×37) were selected for the proposed experimental design. Laccase yield obtained from the 18 sets of fermentation experiments performed with the selected factors and levels were further processed with Qualitek-4 software at bigger is better as quality character. The optimized conditions showed an enhanced laccase expression of 32.9% (from 538.8 to 803.3 U). The optimal combinations of factors obtained from the proposed DOE methodology was further validated by conducting fermentation experiments and the obtained results revealed an enhanced laccase yield of 28.3%. Taguchi approach of DOE

resulted in evaluating the main and interaction effects of the factors individually and in combination. This methodology facilitated analysis of the experimental data to establish the optimum conditions for the process, understand the contribution of individual factors and to evaluate the response under optimal conditions. Application of Taguchi approach appears to have potential usage in bioprocess optimization. (K. Krishna Prasad, S. Venkata Mohana, R. Sreenivas Rao, Bikas Ranjan Pati , P.N. Sarma , 2005)

Decolourisation of the recalcitrant dye Remazol Brilliant Blue R (RBBR) by the fungus basidiomycete *Pleurotus ostreatus* was investigated. *P. ostreatus* is able to decolourise RBBR on agar plate. When grow in liquid media supplemented with veratryl alcohol, the fungus completely decolourises RBBR in 3 days. In these conditions, *P. ostreatus* produces among other enzymes, laccases, veratryl alcohol oxidase and dye decolourising peroxidase but only laccases seem to be responsible of RBBR transformation. Two purified laccases (POXC and POXA3) were found able to degrade RBBR in vitro, in the absence of any redox mediators. These laccases differ significantly in their efficiency of decolourisation of the tested dye, as suggested by comparison of their catalytic efficiency (*k*cat/*K*m values) towards RBBR. Furthermore, using a mixture of both POXC and POXA3 a remarkable improvement in the reaction rate and in the final level of dye decolourisation was observed. The extent of RBBR decolourisation by laccase mixture also depends on incubation temperature and enzyme concentration. The dye is decolourised by laccase isoenzymes most efficiently under acidic conditions. Treatment of RBBR with the laccase mixture reduced its toxicity by 95%. (G. Palmieria,G. Cennamo, G. Sanniab,*2004)*

White-rot fungi were studied for the decolourization of 23 industrial dyes. Laccase, manganese peroxidase, lignin peroxidase, and aryl alcohol oxidase activities were determined in crude extracts from solid-state cultures of 16 different fungal strains grown on whole oats. All *Pleurotus ostreatus* strains exhibited high laccase and manganese peroxidase activity, but highest laccase volumetric activity was found in *Trametes hispida*. Solid-state culture on whole oats showed higher laccase and manganese peroxidase activities compared with growth in a complex liquid medium. Only laccase activity correlated with the decolourization activity of the crude extracts. Two laccase isoenzymes from *Trametes hispida* were purified, and their decolourization

activity was characterized. Elizabeth Rodriguez, Michael A. Pickard, Rafael Vazquez-Duhalt, 1998

Fungi of *Pleurotus* genus present a high content of proteins and vitamins and low content of fat. Based on these facts, this work aimed to evaluate some nutritional characteristics of fruiting bodies of *Pleurotus ostreatus* and *Pleurotus sajor-caju* after the first harvest, using rice straw and banana straw as substrates. Both species presented a higher content of ash in the rice straw substrate (5.86% average) than in the banana straw (5.36%). P. sajor-caju CCB 019 showed higher moisture and fibre content when cultivated in rice straw (88.08% and 9.60%, respectively) than in banana straw (83.17% and 7.60%, respectively). The other parameters were not influenced by the cultivation substrate. The protein content (from 1.54% to 3.10%) of Pleurotus fresh fruiting bodies proved to be similar to, or even higher than, the values observed in various vegetables but lower than the protein contents of eggs, meat and cheese. (M. Bonatti, P. Karnopp, H.M. Soares, S.A. Furlan 2004)

Therefore, the adsorption and the biodegradation successfully supplement each other in the various schemes of wastewater treatment. The Rice Husk adsorption can possibly allow the removal of dyes from the wastewater and thus ensure a stable biological post treatment of the wastewater.

The adsorption and biodegradation can be modeled by using isotherms. Since biodegradation is isothermal it can be modeled by any of the isotherms available in literature (Annadurai et al, 2000). Biodegradation is firstly modeled by Manod (1902) as

$$\mu = \frac{\mu_{max} S}{K_S + S}$$

Where,

μ = Specific growth rate

μ_{max} = maximum specific growth rate

S = Substrate concentration in the solution

K_S = Manod Half saturation constant

The above equation is in the form of popular Langmuir isotherm, which is in the form of

$$Q = \frac{Q_{max} C}{K + C}$$

Where,

Q = mass of solute adsorbed / mass of adsorbent.

Q_{max} = mass of solute adsorbed /mass of adsorbent for complete monolayer.

C = Concentration of solute in solution, mass/volume

K = Constant related to enthalpy of adsorption.

So both the processes can be modeled in one form and can be compared with normal adsorption to find the effect of biodegradation (Annadurai et al, 2000).

Besides Langmuir isotherm model there are so many models, which are well popular and are given in the literature. One of the most important is Freundlich isotherm, which is applicable for heterogeneous surfaces, where as Langmuir isotherm takes monolayer into account. Freundlich isotherm is given by the following equation

$$Q_e = K_f C_e^{\frac{1}{n}}$$

Where,

Q_e = Equillibrium specific uptake (mg/g)

K_f = Freundlich constant

n = Freundlich adsorption parameter

Apart from the above all the parameters, which are applicable for adsorption, can be attributed for simultaneous adsorption and biodegradation like the intraparticle diffusion studies, rate constant study (Legergren equation).

3.2. Rate constant study:

The rate constant for the adsorption and Simultaneous Adsorption and Biodegradation (SAB) for solution of different amount of initial concentrations, pHs and particle sizes can be studied by using Lagergren's equation, which is

$$\log(q_e - q) = \log q_e - \frac{K_{ad} t}{2.303}$$

A plot of log (q_e-q) Vs (t/2.303) gives the applicability of the above equation. The average of the rate constant (K_{ad}) can be calculated from the slope of the curves

representing different conditions, namely initial concentrations, pHs, and different particle sizes for adsorption as well as biodegradation.

Table 3.1 Literature Review of various parameters

Ref.no	Dyes with Optical density (nm)	Materials /Strain	Parameters					
			pH	% Decolourization	Temp (°C)	Dye conc.(mg/l)		Time (days)
						Initial	Final	
1	4-phenylazophenol,4-phenylazo-2-methoxyphenol,dispese yellow, solvent yellow,4-phenylazoanilin	phanerochaete chrysoporium	NR	48	NR	NR	NR	30
2	OrangeII,TropaeolinO,Co-ngo red,AzureB	phanerochaete chrysoporium	3.5-5	95	39	NR	NR	2
3	Remazol brilliant blue R(595), Poly R-478	Bjerkandera sp. (B33/3)	3.5	96	30	NR	NR	11
4	C.I. reactive red 22	Escherchia coli NO3	5-9	NR	37	NR	200	0.42
5	Orange G(479) Congo red(497) Amido black(618)	Laccase, LiP MnP Thelephora sp.	4.5 4.5 7	33.5 97.1 98.8	37	NR	5 10 15	6
6	Bordeaux S(520), Indigoid, Acid blue74(611.5)	Aeromonas sp. B-5	NR	93	NR	NR	NR	1
7	Reactive red 120(469 & 310)	Phanerochaete sordida YK-624, MnP, Lipid peroxidation	4.5	90.6	37	NR	200	7

8	Congo red28, Direct black38,	Escherchia coli, Pseudomonas sp.,aromatic amine	8-9.31	98	35	100	1100	9
9	Solvent yellow 7, Solvent blue 11	Micro per oxidase-11	8-10	NR	25	NR	NR	NR
10	C.I.Reactive red 22(510)	P.Luteola	5-9	85	22 - 47	0	200	5
11	C.I.Reactive red 22(510)	P.Luteola	7-9	99	37	0	200	NR
12	Orange II(488), bromphenol blue(591), Methyline blue (662) RBBR (590)	C.Versicolour, CCF of C.Versicolour	NR	73	30	NR	NR	2
13	Orange II(480)	White rot fungus	NR	97	25	NR	NR	30-60
14	Reactive black 5	Trametes villosa laccase	5	79	45	NR	40	1
15	Reactive black 5 (583)	H_2O_2 UV	7	90	25	NR	400	NR
16	Reactive red 3.1	Activated sludge, coloured effluents	5-7	93	27	NR	400	0.75
17	Reactive black 5(600)	Aerobic CSTR/anaerobic USB	NR	95	37.5	NR	NR	1.25
18	RED RBN(515)	Phosphoryladed polyvinyl alcohol	4-10	75	30	50	500	0.833
19	Dye stuff Reactive brilliant red X-3B(538)		5-9	NR	NR	50	500	NR
20	Congo red ,amaranth	TiO_2/UV	NR	90	NR	NR	NR	NR
21	Acid yellow 36(414)	Rice husk carbon, saw dust carbon	6.5	90	30	NR	100	.083

NR = Data not available

Table3.2 Recent reports on strains of *Pleurotus Ostreatus* and *Phanerochaete chrysosporium* capable of dye decolourization

Dye and concentration	Percent removal/time	Reference
Diazo dyes(50mg/l)	10% (7 days)	Martins et al (2003)
Poly R-478 (unknown concentration)	24% (15min.)	Moldes et al (2002)
RBBR	30 % (20 min.)	Grac, M.B. Soares (2001)
Tropaeolin O(20 mg/l),Remazol Orange(60 mg/l), Remazol B Blue R(60 mg/l), Reactive Blue(20 mg/l).	Unknown (24 hrs)	J. Swamy and J. A. Ramsay(1999)
Remazol Black(60 mg/l)	90% (3 days)	G. Palmieri, G. Cennamob, G. Sannia,(2004)
Amaranth(50 mg/l)	94 %(3 days)	Min Chen (1998)
RBBR	92 % (14 days)	Ivana Eichlerova(2001)
Reactive Orange 16 (150 mg/l)	96 % (14 days)	Patricia R. Moreira(2003)
RBBR (100 µm) RBBR (200 mg/l), Orange G (200 mg/l)	90 % (2 days) 90 % (10 days) 96 % (11 days)	Cristina Máximo, Maria Costa-Ferreira (2003)
Congo Red (76 µm) Polymeric B-411 (200 mg/l) Polymeric R-481 (200 mg/1) Polymeric Y-606 (200 rag/l)	93% (24 h) unknown (8 days) unknown (6 days) unknown (14 days)	Glenn and Gold (1983)

Table 3.3 The main characteristics of the lignolytic fungal strain are shown below (Waliszewsk et al.2004)

Physiological	Nitrogen Unregulated
	Manganese Regulated
	Organic Acid Induction
Lignolytic Enzymes	Manganese Independent Peroxidase (MIP)
	Lignin Peroxidase (LiP)
	Laccase
	Aryl Alcohol Oxidase (AAO)
Secondary Aryl Metabolites	Veratryl alcohol
	3-chloro-*p*-anisaldehyde
	2-chloro-1,4-dimethoxybenzene

Table 3.4 Types of lignocellulosic materials and their current uses (Howard R.L. et al. 2003)

Lignocellulosic material	**Residues**	**Competing use**
Grain harvesting: Wheat, rice, oats barley and corn	Straw, cobs, stalks, husks	Animal feed, burnt as fuel,compost, soil conditioner
Processed grains: Corn, wheat, rice, soybean	Waste water, bran	Animal feed
Fruit and vegetable harvesting	Seeds, peels,husks,stones,rejected whole fruit and juice	Animal and fish feed, someseeds for oil extraction
Fruit and vegetable processing	Seeds, peels, waste water, husks, shells, stones, rejected whole fruitand juice	Animal and fish feed, someseeds for oil extraction
Sugar cane other sugar products	Bagasse	Burnt as fuel
Animal waste	Manure, other waste	Soil conditioners
Forestry-paper and pulpHarvesting of logs	Wood residuals, barks, leaves etc.	Soil conditioners, burnt
Saw-and plywood waste	Woodchips, wood shavings, sawdust	Pulp and paper industries, chipand fibre board
Pulp & paper mills	Fibre waste, sulphite liquor	Reused in pulp and boardindustry as fuel

Table 3.5 Recent reports on various fungi capable of dye decolourization (I.M.Banat et al .1996)

Culture	Dye and concentration	Percent removal/time	Mechanism	Reference
Aspergillus sojae B-10	Amaranth (10 mg/l) Sudan III (10 mg/l) Congo Red (10 mg/l)	97.8%(5 days) 97.4%(5 days) 93.0%(5 days)	NR NR NR	Ryu & Weon (1992)
Myrothecum verrucaria	Orange II (200 mg/l) 10B (Blue) (200 mg/l) RS (Red) (200 mg/l)	70.0% (5 h) 86.0%(5 h) 95.0%(5 h)	adsorption adsorption adsorption	Brahimi-Horn *et al.* (1992)
Myrothecum sp.	Orange II (100 mg/l) 10B (Blue) RS (Red) (100 mg/l)	25-91% (24h) 58-98% (24 h) 81-98% (24h)	adsorption adsorption adsorption	Mou *et al.* (1991)
Neurospora crassa	Vermelho Reanil P8B (16-32 mg/l)	89-91% (24h)	adsorption	Corso *et al.* (1981)
Pycnoporus cinnabarinus	Pigment plant effluent(unknown concentration)	90% (3 days)	extracellular oxidases	Schliephake *et al.* (1993)
Trichoderma sp.	Hardwood extraction effluent (unknown concentration)	85% (3 days)	lignolytic enzymes	Prasad & Joyce (1991)

Candida sp.	Procyon Black SPL (100 mg/l)	93.8% (2h)	adsorption	De Angelis & Rodrigues (1987)
	Procyon Blue MX2G (100 mg/l)	96.8% (2h)	adsorption	
	Procyon Red HE7B (100 mg/l)	98.9%(2h)	adsorption	
	Procyon Orange HER (100 mg/l)	96.8% (2h)	adsorption	

NR = Data not available

Table 3.6 Basic biochemical properties of the laccases from different source (Han-Qing Yu et al. 2005)

Laccase	MW, kDa	Activity, units	pI	pH-optimum	Carbohydrate content, %	Inactivation half time at 50 °C, h
Trametes ochracea	60	370	4.6	4.9	10	56
Trametes hirsuta	69	350	4.05	4.5	12	72
Cerrena maxima	57	340	3.50	4.5	13	52
Coriolisimus fulvocinerea	54	350	3.50	5.2	32	64

Spectral studying of the laccases from *Trametes ochracea* (a), *Trametes hirsuta* (b), *Coriolopsis fulvocenerea* (c) and *Cerrena maxima* (d)

Table 3.7 List of fungi with the highest specific activity (μmol.min^{-1}.mg^{-1}) for hemi-cellulases (Han-Qing Yu et al. 2005)

Enzyme	Organism	Substrate	Specific activity	Opt. temp. (oC)	Opt. pH
Feruloyl esterase	*Aspergillus niger*	Methyl sinapinate	156	55	5
Endo-1,4-β -xylanase	*Trichoderma longibrachiatum*	1,4-β-D-xylan	6630	45	5
β-1,4-xylosidase	*Aspergillus nidulans*	p-nitrophenyl-β-D-xylopyranoside	107.1	50	5
Exo-β-1,4-mannosidase	*Aspergillus niger*	β-D-Man-(1-4)-β-D-GlcNAc-(1-4)-β-DGlcNAc-Asn-Lys	188	55	3.5
Endo-β-1,4-mannanase	*Sclerotium rolfsii*	Galactoglucomannan/mannans galactomannans/glucomannans/	380	72-74	2.9/3.3
Endo-β-1,5-arabinanase	*Aspergillus niger*	1,5-α-L-arabinan	90.2	50-55	4.5-5.0
α-L-arabinofuranosidase	*Aspergillus niger*	1,5-α-L-arabinofuranohexaose/ 1,5-α-L-arabinotriose/ 1,5-L-arabinan/ α-Larabinofuranotriose	396.6	50-60	3.4-4.5
α-Glucuronidase	*Phanerochaete chrysosporium*	4-O-methyl-glucuronosyl-xylobiose	4.5	50	3.5
α-Galactosidase	*Mortierella vinacea*	melibiose	2000	60	4
Endo-galactanase	*Aspergillus niger*	NA	6593	50-55	3.5
β-glucosidase	*Humicola insolvens*	(2-hydroxymethylphenyl)-β-Dglucopyranoside	266.9	50	5
Acetyl xylan esterase	*Schizophyllum commune*	4-methylumbelliferyl acetate/ 4-nitrophenyl acetate	227	30	7.7

Table 3.8 Lignocellulose contents of common agricultural residues and wastes (Howard R.L. et al. 2003)

Lignocellulosic materials	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwood stems	40-55	24-40	18-25
Softwood stems	45-50	25-35	25-35
Nut shells	25-30	25-30	30-40
Corn cobs	45	35	15
Paper	85-99	0	0-15
Wheat straw	30	50	15
Rice straw	32.1	24	18
Sorted refuse	60	20	20
Leaves	15-20	80-85	0
Cotton seeds hairs	80-95	5-20	0
Newspaper	40-55	25-40	18-30
Waste paper from chemical pulps	60-70	10-20	5-10
Primary wastewater solids	8-15	NA	24-29
Fresh bagasse	33.4	30	18.9
Swine waste	6	28	NA
Solid cattle manure	1.6-4.7	1.4-3.3	2.7-5.7
Coastal Bermuda grass	25	35.7	6.4
Switch grass	45	31.4	12.0
S32 rye grass (early leaf)	21.3	15.8	2.7
S32 rye grass (seed setting)	26.7	25.7	7.3
Orchard grass (medium maturity)	32	40	4.7
Grasses (average values for grasses)	25-40	25-50	10-30

Table 3.9 Lignolytic enzyme maxima of various white-rot fungi are given as mean activities±standard deviation (n = 3), (G. Palmieria et al. 2004)

Culture	LiP activity (units ml^{-1} min^{-1})	MnP activity (units ml^{-1} min^{-1})	Laccase activity (CU ml^{-1})
T. versicolour	0:39 ± 0:031	2:00 ± 0:514	2:40 ± 0:172
D. Flavida	0:85 ± 0:07	ND	0:45 ± 0:038
D. squalens	ND	0:16 ± 0:015	0:15 ± 0:003
P. chrysosporium	0:94 ± 0:075	1:40 ± 0:718	ND
P. fascicularia	0:41 ± 0:19	0:19 ± 0:003	8:50 ± 0:369
P. floridensis	0:50	0:95 ± 0:006	6:72 ± 0:48
P. radiata	0:48 ± 0:078	29:80 ± 1:296	5:53 ± 0:017

Table 3.10 Selected spectrophotometric enzyme assays (S.A. Furlan et al. 2004)

Assay	Substrates	PH	Wavelenght (Nm)	Molar Extinction Coefficient C ($M^{-1}cm^{-1}$)
Phenoloxidases Lacca.scs ECl.10.3.2	2.6dimethoxyphenol (19 PM) ABTS (0.54 mM) TMPD (I mM) syringaldazine)' (0. I mM)	4.5 4.5 6.0 6.0	468 420 525 525	14,800 36,000 14,685 65,000
Monophenol monooxygenases ECl.14.18.1	chlorogenic acid (0. I 11~). EDTA (ImM). DL-DOPA (14 mM) 4.4 mM H_2O_2 for plant	6.0 6.0	326 475	3,600
Peroxidases ECl.11.1.7	perosidases or 0.44 mM H_20_2 for fungal peroxidases pyrogallol (0.4 mM)	4.5 7.0	430 436	2,470 6,400
Mn dependcnt peroxidases EC1.11.1.13	MnSO H_20 (0.2 mM), H_20_2(0.2 mM) Na-malonate (50 mM)	4.5	270	11,590
Lignin perosidnses ECl.II.I.IJ	verattyl alcohol (3.3 mM), H_20_2 (0.44 mM)	3	310	9,300
Bleaching of dye molecules	Crystal violet)' (12.5pM). H,OI (0.44mM) Azure B (32 pM). H_20_2 (0.44mM) Poly R-478 (0.093 g/l). H_20_2 (0.44mM) RBB-R (0. I mM) H_20_2 (0.44mM)	4.5 4.5 4.5 4.5	590 651 520 595	80,658 48,800 10.725 6,050

CHAPTER 4

EXPERIMENTAL PROCEDURE

Batch studies (in static and shaking condition) were carried out to understand the effect of adsorbent dose, initial concentration, pH of the adsorbent on the removal of the dyes Reactive Orange 16; Reactive Black 5 and Remazol Brilliant Blue R were done for adsorption and simultaneous adsorption & biodegradation.

Rice Husk is used for the physical adsorption and white rot fungi and its enzyme is used for removal of colour.

4.1 Materials

4.1.1. Adsorbate

The various dyes Reactive Orange 16(494 nm), Reactive Black 5(597 nm) and Remazol Brilliant Blue R (592 nm) supplied by sigma pvt. Limited.

4.1.2. Adsorbent

The Rice Husk from the local market of Roorkee was used in the present study. Initially, the Rice Husk was cleaned with distilled water to remove the dust etc. and then dried in the oven for 24 hrs at 100 ^{0}C.

4.1.3. Stock solution

Stock solution of the various dyes Reactive Orange 16, Reactive Black 5 and Remazol Brilliant Blue R with a concentration of 200 mg/l was prepared by dissolving 200 mg of the respective dye in 1 liter distilled water respectively.

4.1.4. Microorganisms

A pure culture of *Pluerotus Ostreatus* (MTCC 1804) was procured from Microbial Type culture collection and Gene Bank, Institute of Microbial Technology, (IMTECH) Chandigargh.

4.1.5. Growth Medium

Apart from the carbon source, the bacteria require many macro and micronutrients for their Nutrient agar medium was used whose composition is given in table 4.1

4.1.6. Revival of culture

As the freeze-dried culture/ lyophilized culture can't be used directly for the degradation studies, this obtained culture from IMTECH was revived according to the procedure prescribed by the, IMTECH, Chandigargh, India.

The optimum culture growth conditions and composition of nutrient agar medium prescribed by IMTECH are stated below in table 4.1 and 4.2 respectively

Table 4.1: Optimum Condition of Growth Culture

Culture	*Pluerotus Ostreatus* (MTCC 1804)
Growth conditions	Aerobic
Temperature	35 ^{0}C
Incubation Time	7 days

Table 4.2: Composition of nutrient agar (PDA) medium

Nutrient	Concentration (gm/lt)
Potatoes Infusion form	200.0
Dextrose	20.0
Agar (for solid medium)	15.0

4.1.7. Basal Salt Medium (BSM)

The basal salt medium used for the present study is given in table 4.3. The final volume of the BSM after addition of salts was 1liter. The pH of the medium was adjusted to 4.5 $\pm$.2

Table 4.3. Composition of BSM

K_2HPO_4	0.2 %
Glucose	1.3 %
$MgSO_4$	0.05 %
$CaCl_2$	0.01 %
Yeast extract	0.05%
Ammonium Tartrate	0.05 %
Dimethyl Succinate	0.22 %

4.2. Methods

4.2.1. Determination of various dyes Reactive Orange 16(494 nm), Reactive Black 5(597 nm) and Remazol Brilliant Blue R (592 nm)

The concentration of the various dyes Reactive Orange 16(494 nm), Reactive Black 5(597 nm) and Remazol Brilliant Blue R (592 nm) in the solutions were measured by using UV/VIS Spectrophotometer [Model Perkin Elmer]

The samples were 10 times diluted to bring the concentration of compound to the straight line portion of the absorbance plot. Dilution does not affect the absorbance.

4.3. Isolation of pure culture by plating method

Pure culture is obtained by one of the modification of the plating method for the microorganisms that form discrete colonies on solid media. This method basically involves the separation and immobilization of the individual organisms on or in nutrient medium solidified with agar or some other appropriate jelling agent. Each viable organism gives rise to a colony from which transfers can be readily made.

4.4. Sterility

All the glassware along with the solid and liquid medium were sterilized in a autoclave at 15 psi for 20 min. Transfer of bacteria and media were all done in an inoculation chamber

4.5. Method of Transfer

Liquid medium containing composition of nutrient growth medium was prepared in distilled water in two 250 ml conical flasks. For solid medium appropriate quantity of agar was added to one of the flask containing liquid medium. Both these flasks were autoclaved for 20 min at 15 psi. While hot, the conical flask containing agar medium was poured into sterilized Petri dishes. On cooling, the liquid medium with agar was solidified. Inoculation was done in the inoculation chamber.

4.6. Acclimatization of the culture

Acclimatization of the culture is done to bring the dyes degrading culture to the dyes environment. The acclimatization of culture was performed in the batch mode in a 250 ml conical flask. The stock solution of dyes was added to the flask along with the appropriate quantity of BSM and the culture. The culture, which is to be used for the experiment to find the effect of initial concentration, was kept aside with acclimatization

of various dyes Reactive Orange 16(494 nm), Reactive Black 5(597 nm) and Remazol Brilliant Blue R (592 nm) at 200 mg/l only. Now this culture, containing 200 mg/l dyes, along with rice husk dose was used to degrade 200mg/l of various dyes Reactive Orange 16(494 nm), Reactive Black 5(597 nm) and Remazol Brilliant Blue R (592 nm) respectively.

4.7. White Rot Fungi Growth

The White Rot Fungi growth was monitored by weighing the digital weighing machine. To measure the growth of white rot fungi after particular interval (24 hrs), filtered the growth solution and put it into the oven at 70 °C and then take weight.

4.8. pH of the culture

The pH of the culture was determined periodically for the degradation of phenol by using digital pH meter.

4.9. Experiment

4.9.1. Batch studies

4.9.1.1. Biological study

The batch studies using rice husk, white rot fungi and its enzymes were conducted to get the bio-kinetic parameters and to find the percentage removals with time.

4.9.1.2. Adsorption study

Batch studies were conducted to find the affect of mass of adsorbent, initial concentration, pH on percentage removal of various dyes Reactive Orange 16(494 nm), Reactive Black 5(597 nm) and Remazol Brilliant Blue R (592 nm).

4.9.1.3. Simultaneous adsorption and biodegradation study

Batch studies were conducted by using various adsorbent doses, initial concentrations, pH on the percentage removal of various dyes Reactive Orange 16(494 nm), Reactive Black 5(597 nm) and Remazol Brilliant Blue R (592 nm).

CHAPTER 5

RESULTS AND DISCUSSIONS

The main aim of the present study is to compare adsorption and simultaneous adsorption and biodegradation (SAB) on the removal of various dyes separately. Experiments conducted in the present study include the adsorption experiments for different adsorbent doses, different pHs of the sample and different concentrations of the dyes. The same experiments have been repeated for simultaneous adsorption and biodegradation. The experiments conducted in the study include the initial growth of white rot fungi with time. The graphs shown in figure 5.1 and figure 5.2 represent the growth rate of white rot fungi. Batch study includes the growth of white rot fungi in basalt salt medium (BSM).

5.1. BATCH STUDY

5.1.1. Biodegradation

5.1.1.1. Growth study

Batch studies were conducted to study the growth of white rot fungi. Figure 5.1 shows the curves for white rot fungi growth Vs time. The curves are similar to the shape discussed in the literature. The curves are drawn for initial dye concentration of 200mg/l. The growth rate was determined by measuring the weight of white rot fungi. And the degradation rate was measured by optical density at 494nm for RO16, 597nm for RB5 and 592nm for RBBR.

5.1.1.2. Rate of biodegradation of dyes with time

Batch experiments have been conducted to find the biodegradation of various dyes with time separately with initial concentration of 200 mg/l. For the biodegradation of dyes, up to 12hrs, the percentage removal is very less, but later it increases with higher rate. Maximum removal was achieved for 10% rice husk within 72 hrs and was 84.79% for RO16, 73.38% for RB5 and 70.00% for RBBR. Specific growth rate is also found out

for various dyes in the above study. The absorbance is found out periodically and by using the formula stated below. Maximum specific growth rate has also been found out.

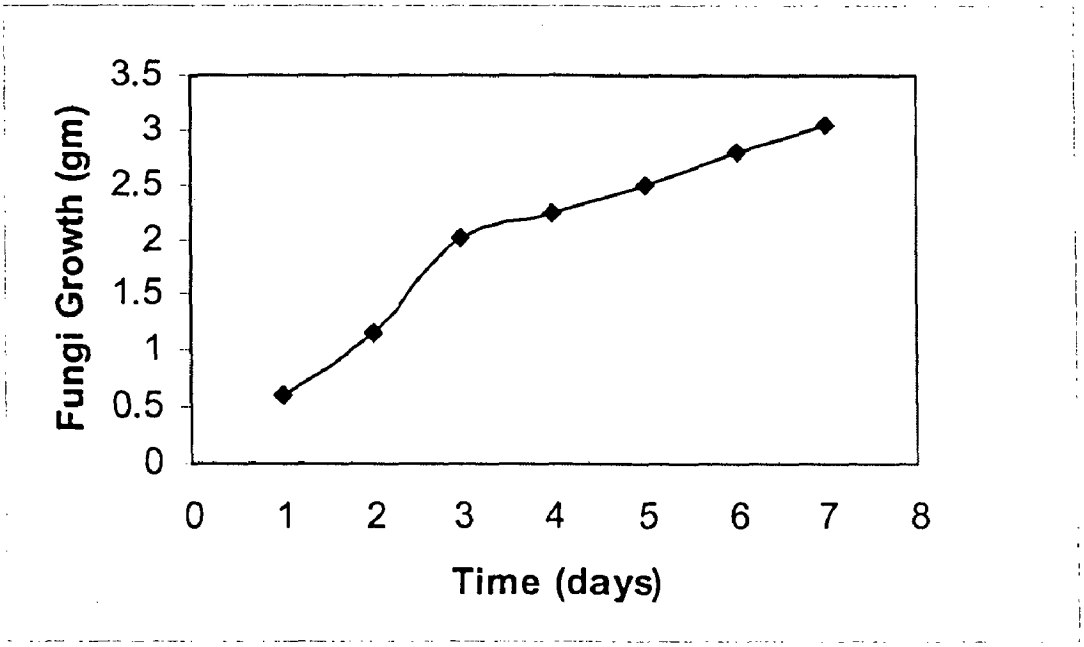


Fig.5.1 Growth of white rot fungi

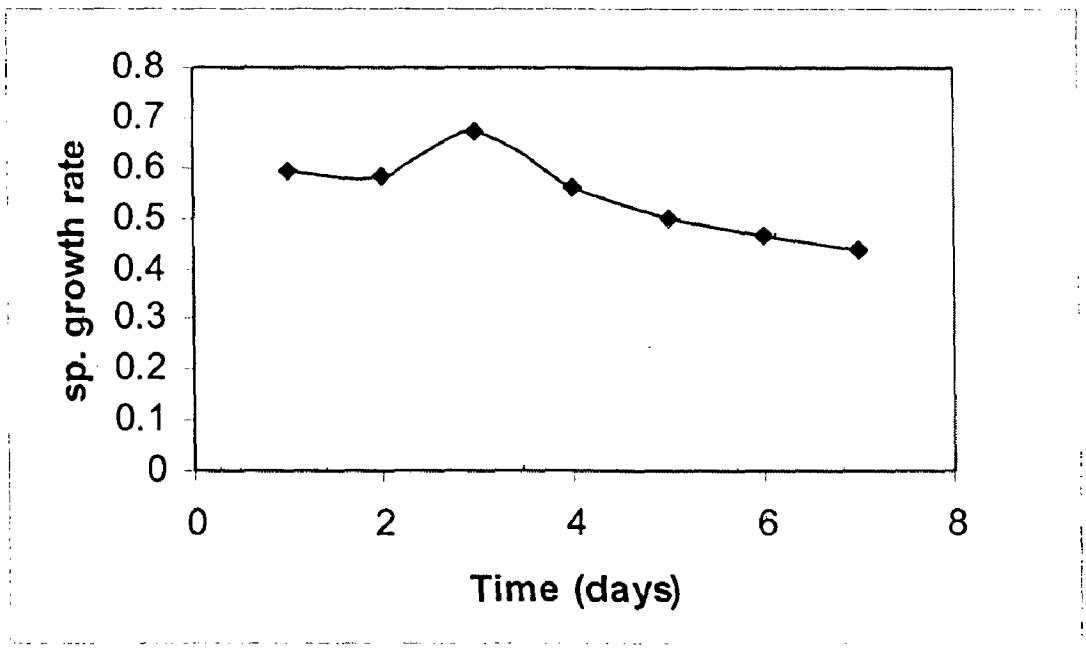


Fig. 5.2 Specific growth rate v/s time (days)

CENTRAL LIBRARY
612194
Acc. No.
Date 15.12.05
I.I.T. ROORKEE

$$\mu = \frac{1}{t} \ln\left(\frac{OD_t}{OD_0}\right)$$

Where

OD_t = Optical Density at time t

OD_0 = Initial Optical density

t = time

The specific growth rate curves for various dyes are shown in fig. 5.2

5.1.2. Comparison of adsorption and Simultaneous Adsorption and Biodegradation (SAB)

Separate experiments were conducted for adsorption and simultaneous adsorption and biodegradation. The experimental parameters were kept constant for both the experiments. The conditions are temperature: 35^0C, adsorbent dose 3 gm/100ml, initial concentration: 50mg/l, other wise mentioned. Optimum conditions have been found out in the experimental range and are used in the subsequent study. . Proper justification is given at proper places.

5.1.2.1. Effect of Mass of Adsorbent

Different amounts of adsorbent doses are taken in conical flasks separately to find the optimum dose for the removal of various dyes. Fig shows the removal of various dyes for different adsorbent doses. For various dyes the optimum dose is 3gm/100ml (10%). So for the entire study the adsorbent dose has been fixed at 3gm/l.

Apart from the above studies, rate studies have also been done for other adsorbent doses of 1.5gm/100ml. Separate graphs are shown for individual dose for Adsorption and Simultaneous Adsorption and Biodegradation (SAB)

Figs 5.3 to 5.8 show the percentage removal of various dyes for 1.5gm/100ml and 3gm/100ml for adsorption and simultaneous adsorption and biodegradation. For the initial period of 1 hr, there is no variation in percentage between the removal for adsorption and simultaneous adsorption and biodegradation. But after one hour it varies according to the adsorbent dose. In case of 3gm/100ml as adsorbent dose, the simultaneous adsorption and biodegradation has significant percentage removal when compared to adsorption alone. It is due to the predominance of biodegradation over adsorption. As the adsorbent reaches its saturation early because of its low adsorption

capacity the biodegradation increases the rate of removal, which clearly states that in simultaneous adsorption and biodegradation, biodegradation takes over after adsorption equilibrium is reached.

In case of 1.5gm/100ml adsorbent dose, the variation in adsorption and simultaneous adsorption and biodegradation on percentage removal of various dyes is low. The above justification holds good for this also.

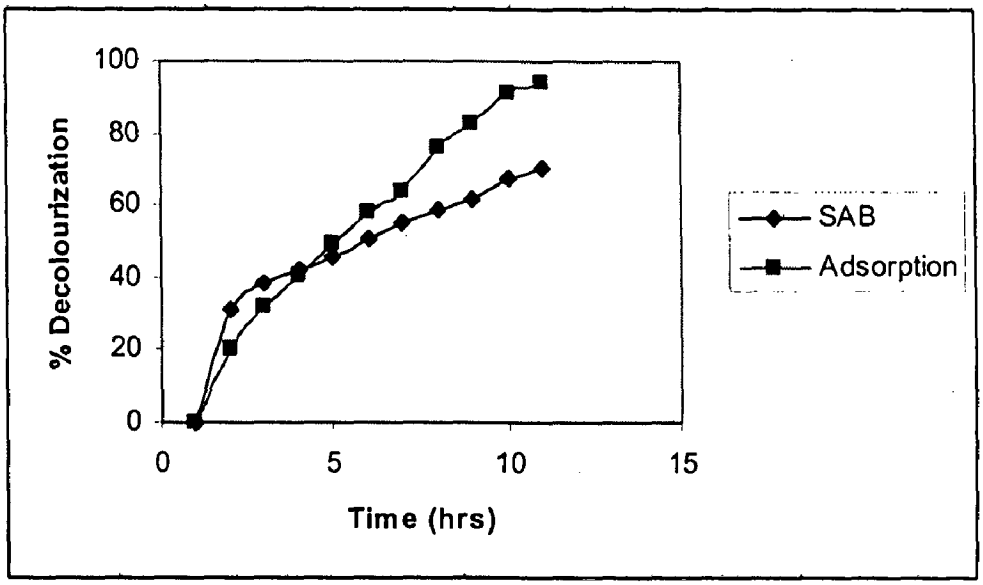


Fig.5.3 Percentage Decolourization v/s Time (hrs) for adsorbent doses 1.5 gm/100ml, for RO 16 dye

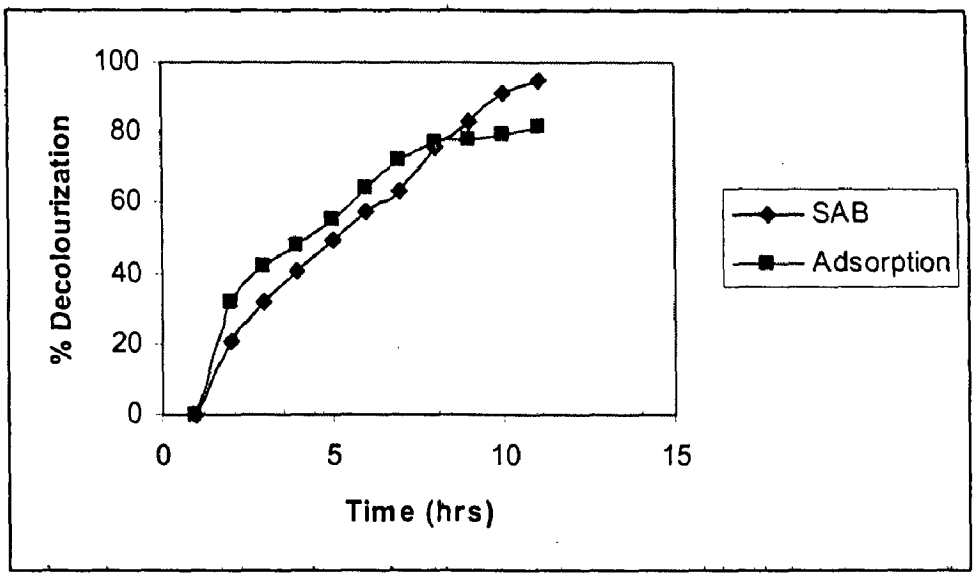


Fig.5.4 Percentage Decolourization v/s Time (hrs) for adsorbent doses 3gm/100ml, for RO 16 dye

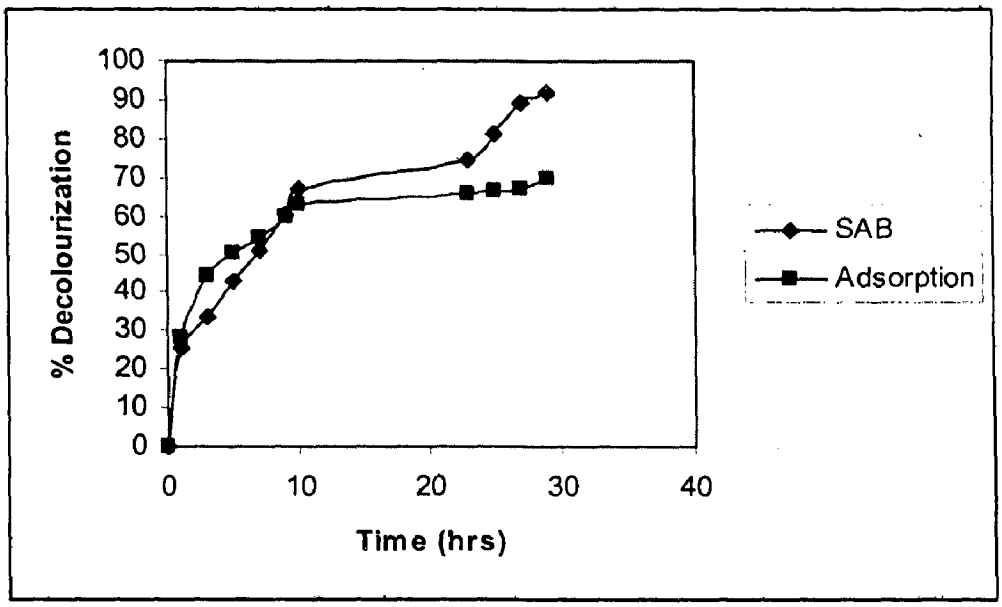


Fig.5.5 Percentage Decolourization v/s Time (hrs) for adsorbent doses 1.5 gm/100ml, for RB 5 dye

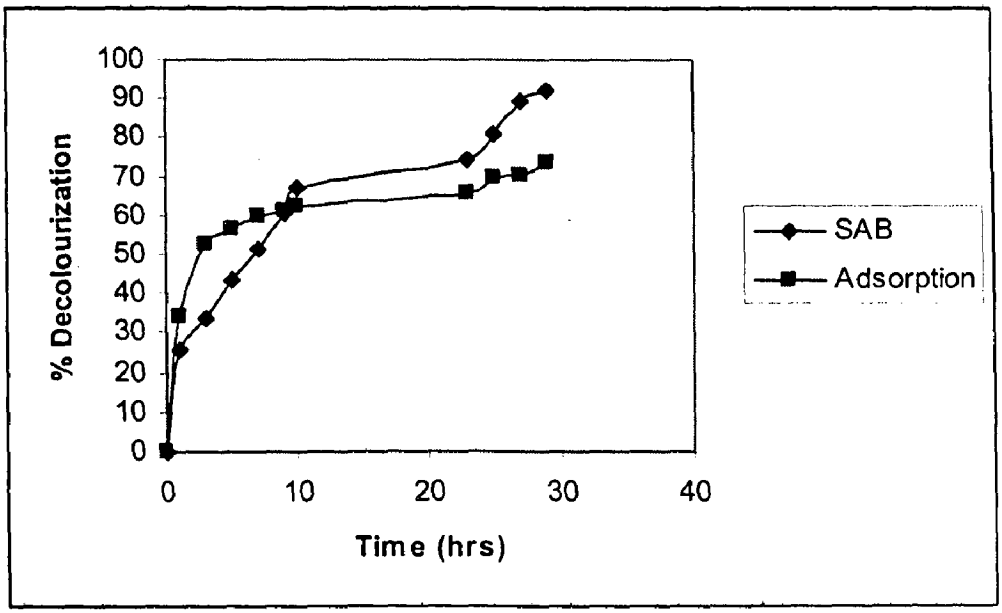


Fig.5.6 Percentage Decolourization v/s Time (hrs) for adsorbent doses 3 gm/100ml, for RB 5 dye

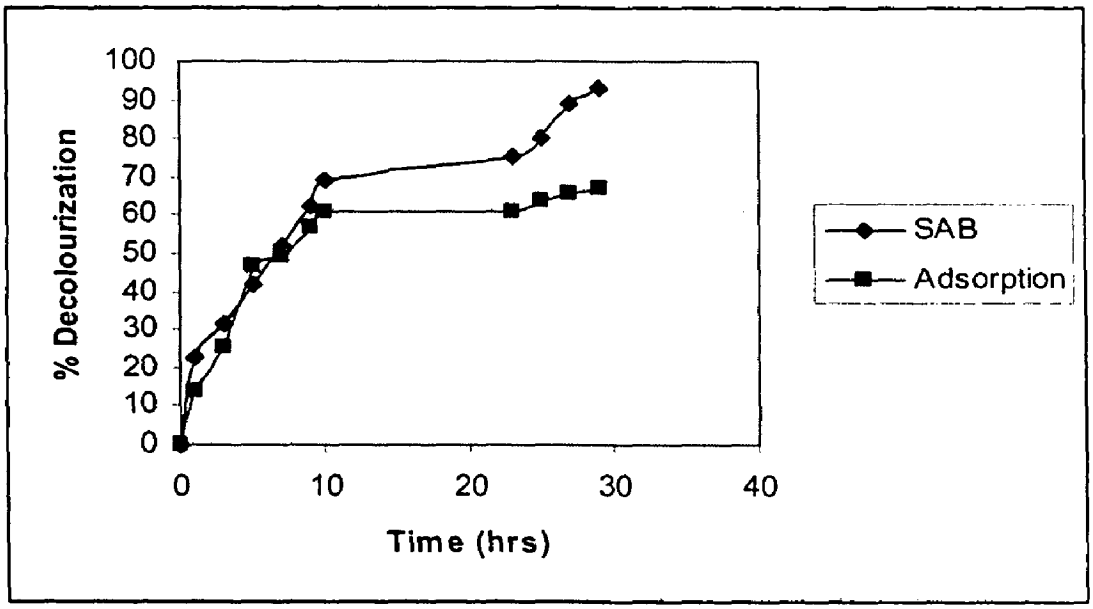


Fig.5.7 Percentage Decolourization v/s Time (hrs) for adsorbent doses 1.5 gm/100ml, for RBBR dye

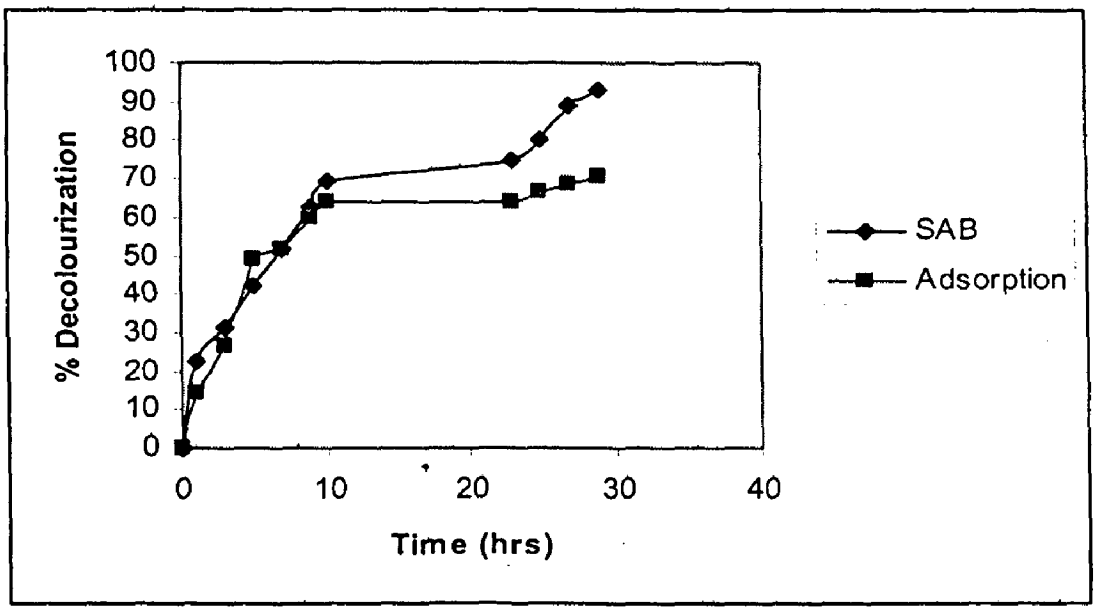


Fig. 5.8 Percentage Decolourization v/s Time (hrs) for adsorbent doses 3 gm/100ml, for RBBR dye

the adsorbate is adsorbed to maximum extent and biodegradation has very less effect in the percentage removal of various dyes.

Table 5.1 gives the percentage removals [illegible] for adsorption and simultaneous adsorption and biodegradation (SAB) for above three dyes.

Table 5.1. Physical Adsorption Simultaneous Adsorption and Biodegradation (SAB) of Reactive Orange 16, Reactive Black 5 and Remazole Brilliant Blue R by Rice Husk

S.No.	Condition	Dye Conc. (mg/l)	Dyes			Amount of Rice Husk
			RO 16	RB 5	RBBR	
1	Shaking(150 rpm)	200	63.52219	30.19502	55.03834	5 %
2	Shaking(150 rpm)	200	65.47586	37.92872	59.63855	10 %
3	Static	50 100 150 200	87.3069 85.2003 83.6921 82.3492	75.7355 74.8635 73.7435 67.81608	75.26114 73.69562 71.43248 66.66924	5 %
4	Static	50 100 150 200	88.5831 86.3174 85.7311 84.7918	80.8826 79.8362 76.4922 73.3793	79.0242 77.3804 75.0041 70.0027	10 %
5	SAB	200	94.5039	92.2644	93.2691	10 %

5.1.2.2. Effect of pH

[illegible] different pHs have been tested for adsorption of various dyes. For adsorption experiments 2, 3.5, 4, 4.5, 5, 6, 7 are considered. For adsorption of various dyes, lower pHs favour higher rate of adsorption and higher adsorption capacity. The optimum value

of pH was 4.5 at which we get maximum % decolourization was occur. Table 5.2 gives the details of the percentage removal various dyes for adsorption with variation in pH values.

Table 5.2. Effect of pH on Dye Decolourization

S.No.	pH	% Decolourization		
		Dyes		
		RO 16	RB 5	RBBR
1	2	56.179	34.074	47.4903
2	3.5	66.890	57.1973	59.9276
3	4	91.828	73.1024	63.8028
4	4.5	94.361	85.4725	87.4172
5	5	80.893	79.5291	76.3801
6	6	65.426	68.3375	64.4009
7	7	50.84	53.8926	49.5728

Fig 5.9 represents the percentage removal of various dyes with various values of pH. Since at low pH bacteria can't sustain, a pH near to 4.5 is taken for simultaneous adsorption and biodegradation studies. pH 2, 3.5 4, 5, 6, and 7 are taken for further studies.

As shown in fig. 5.9 we saw that if the pH is below or higher than 4.5 the % decolourization is reduced.

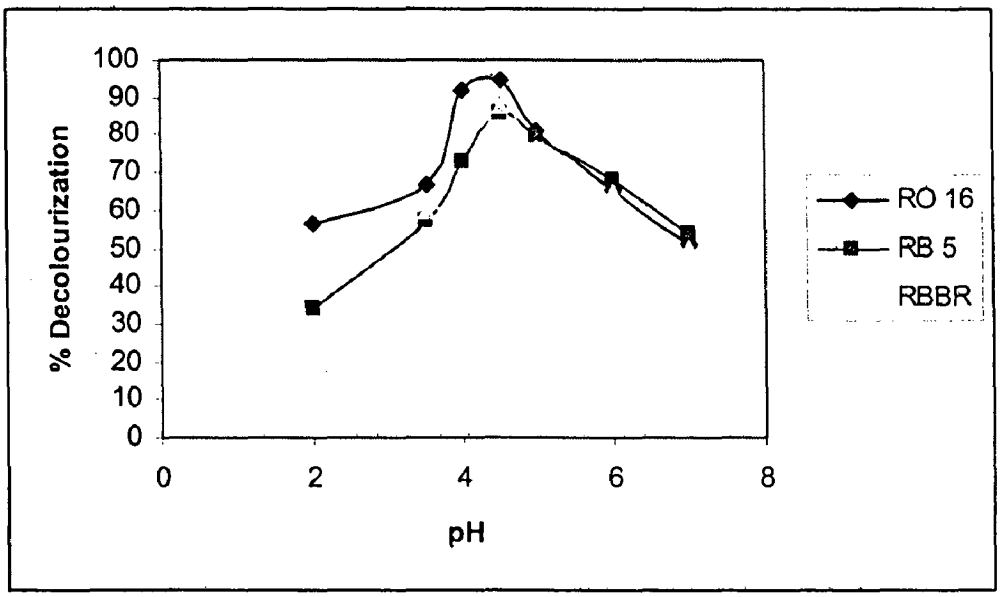


Fig.5.9 Effect of pH values on decolourization of different dyes

5.1.2.3. Effect of initial concentration

Four different initial concentrations were used for the study of adsorption and simultaneous adsorption and biodegradation of RO16, RB5, RBBR. Figs 5.10 to 5.20 give the percentage removal of Dyes with time for various concentrations. For all the cases the percentage removal in SAB is much higher than the adsorption. In 50mg/l, 100mg/l and 150mg/l, 200 mg/l removal is identified with in 72 hrs. And an important result in this experiment is that the percentage removal of Dyes for initial concentration of 50mg/l quickly approaches 94.5039%. This is due to the synergetic combination of adsorption and biodegradation. As increase in the concentration of dye we saw that the % removal is reduced.

Here we use both static and shaking condition. The % removal is higher in static condition this is because in shaking condition the contact time is less. The Fig. 5.10 to 5.20 shows the % decolourization under various conditions.

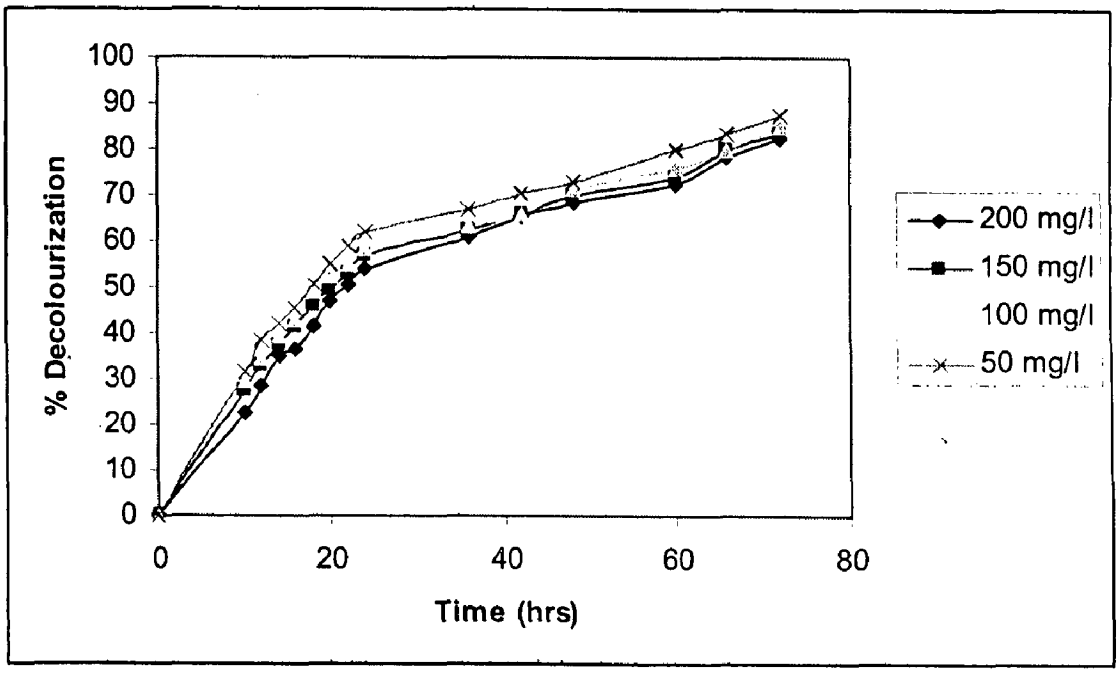


Fig.5.10 Percentage removal of Reactive Orange 16 in static condition with 5 % Rice Husk and different dye concentration

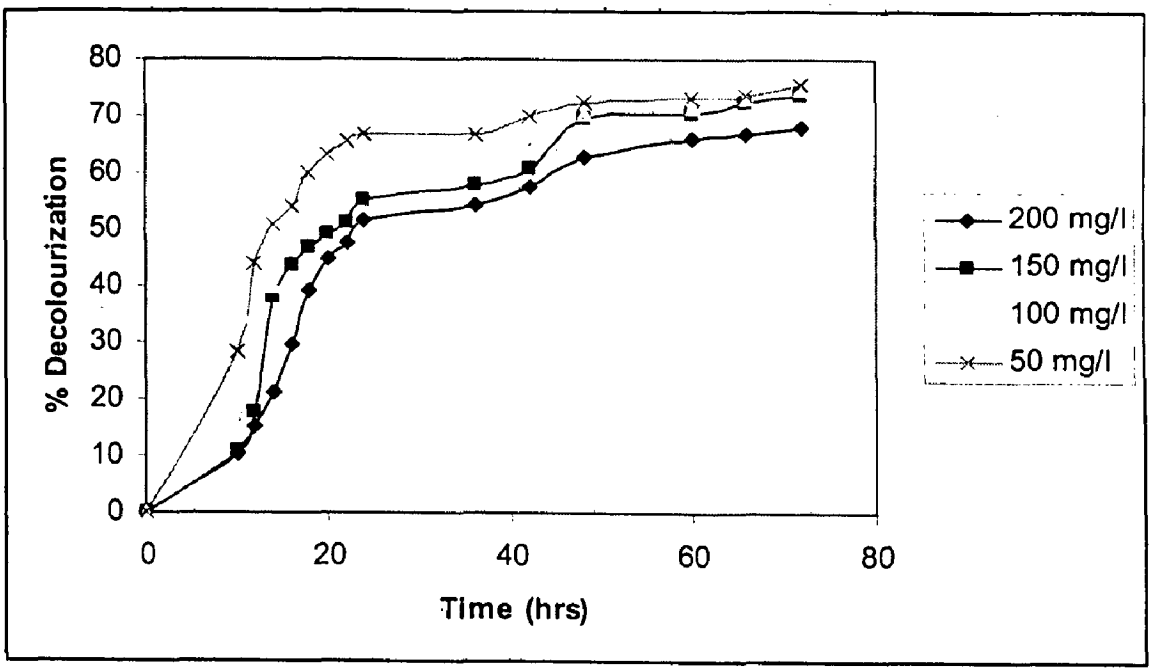


Fig.5.11 Percentage removal of Reactive Black 5 in static condition with 5 % Rice Husk and different dye concentration

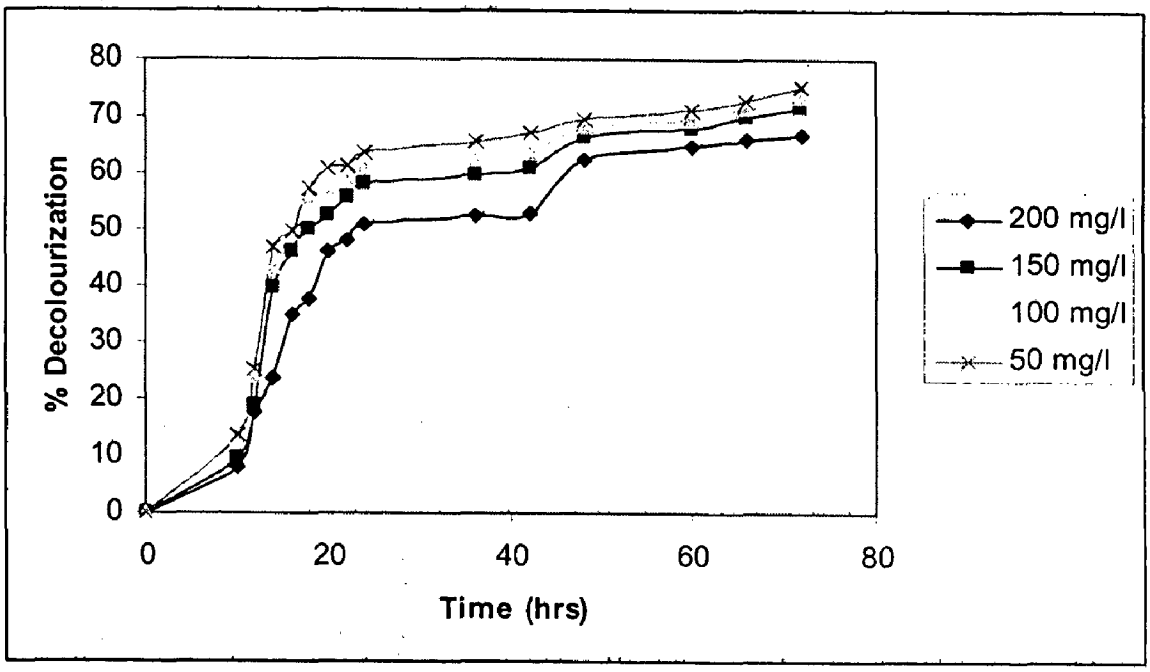


Fig.5.12 Percentage removal of Remazole Brilliant Blue R in static condition with 5 % Rice Husk and different dye concentration

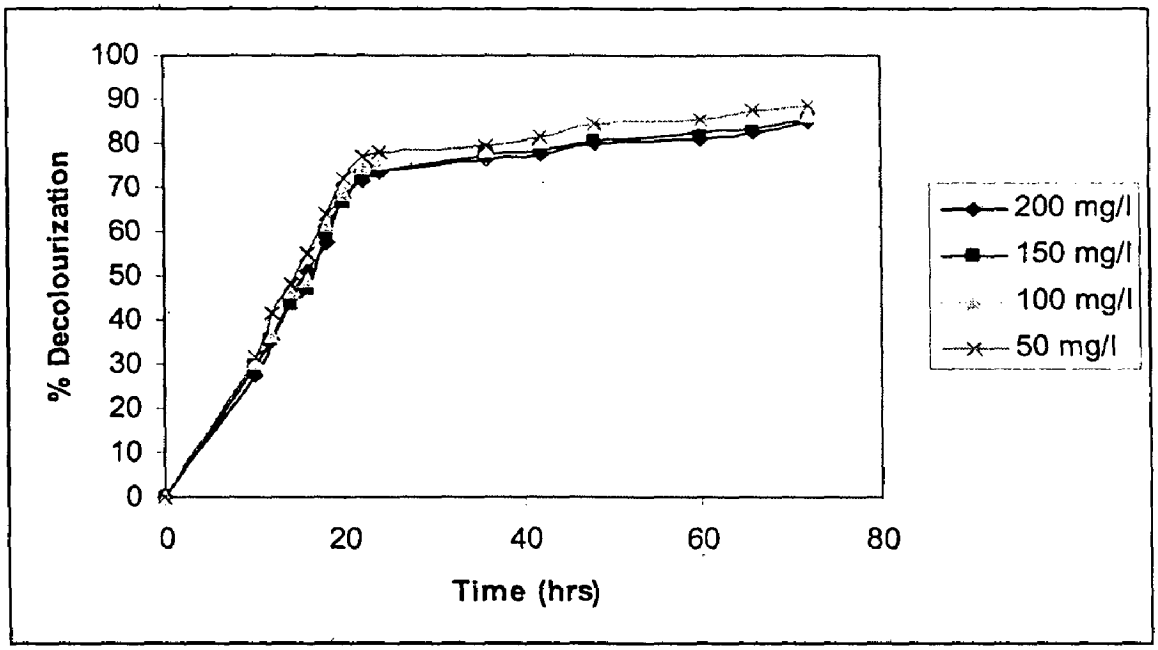


Fig.5.13 Percentage removal of Reactive Orange 16 in static condition with 10 % Rice Husk and different dye concentration

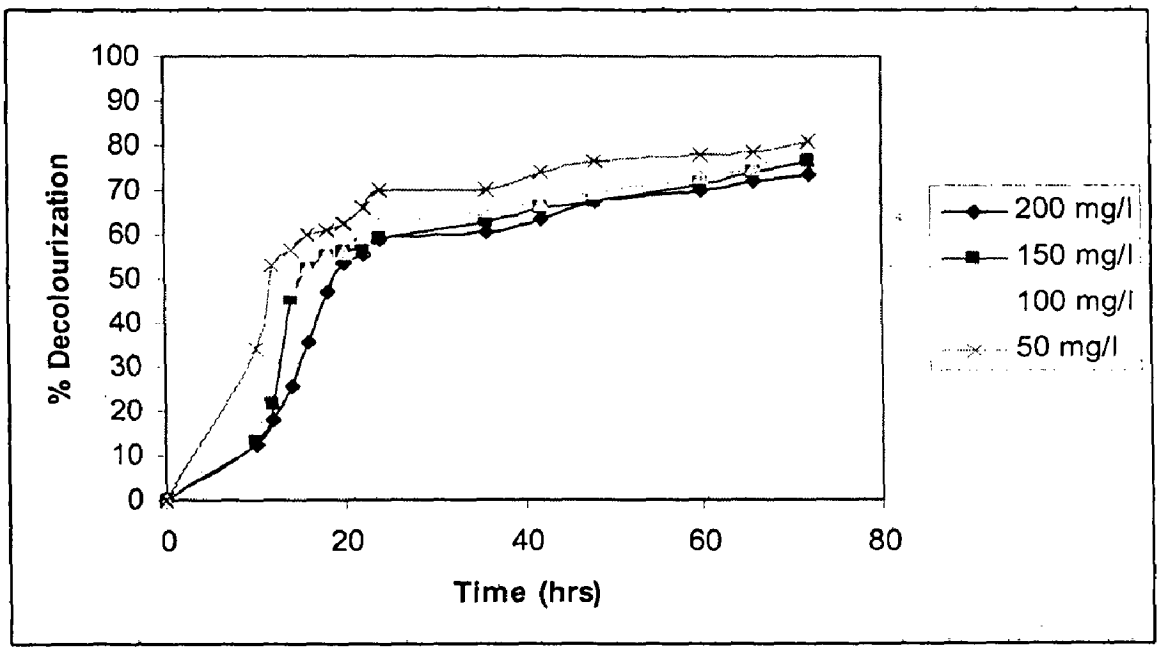


Fig.5.14 Percentage removal of Reactive Black 5 in static condition with 10 % Rice Husk and different dye concentration

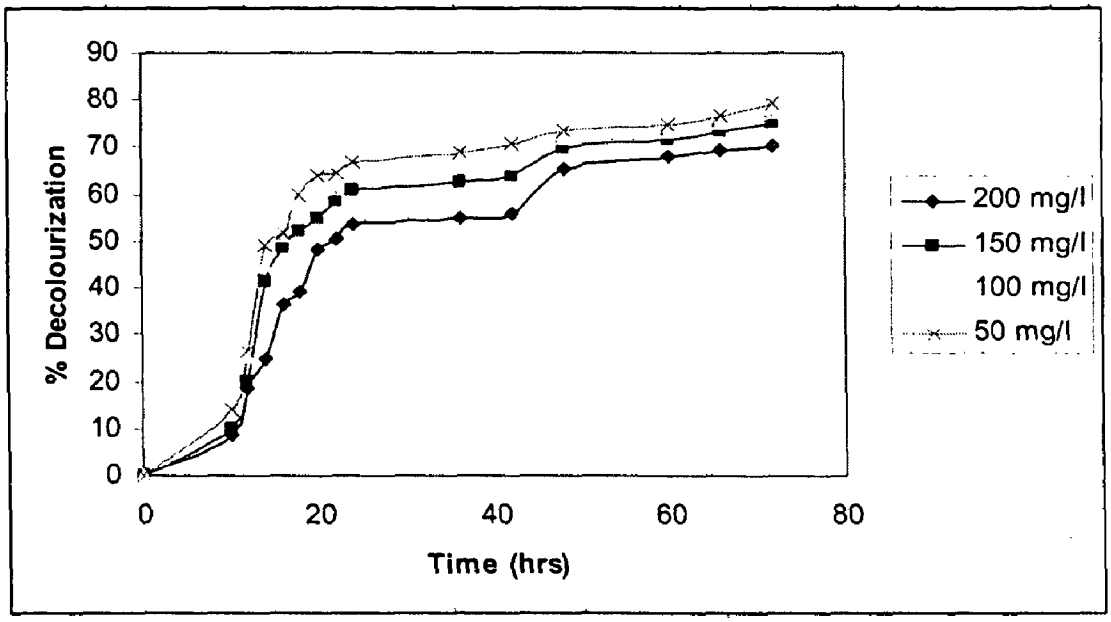


Fig.5.15 Percentage removal of Remazole Brilliant Blue R in static condition with 10 % Rice Husk and different dye concentration

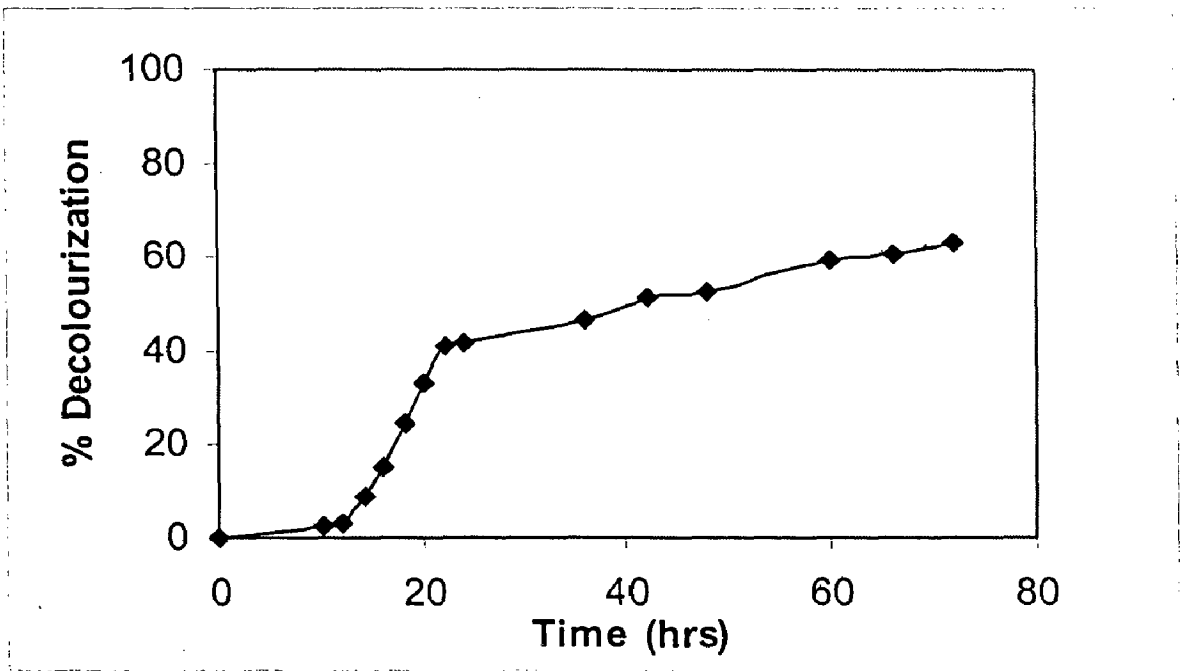


Fig.5.16 Percentage removal of Reactive Orange 16 in shaking condition with 5 % Rice Husk and different dye concentration

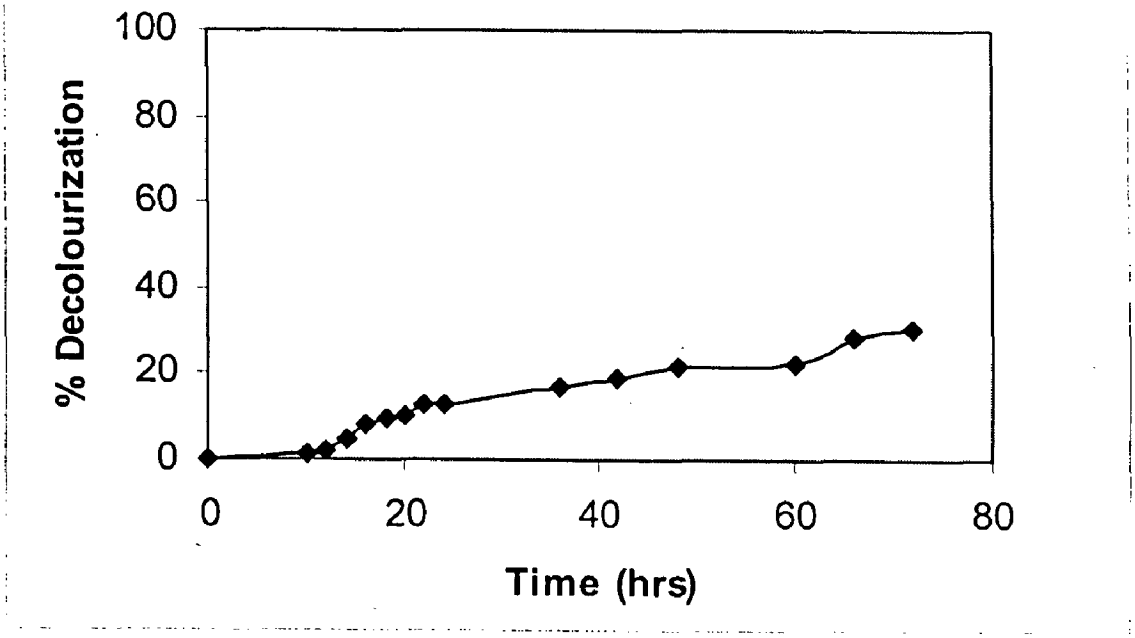


Fig.5.17 Percentage removal of RB 5 in shaking condition with 5 % Rice Husk and different dye concentration

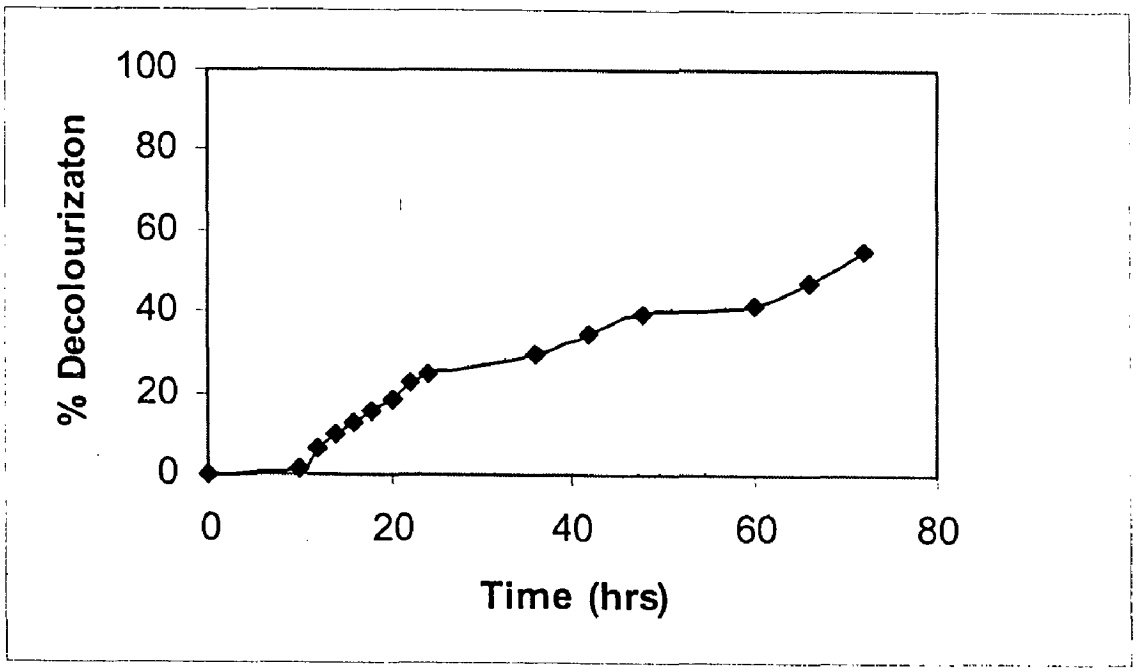


Fig.5.17 Percentage removal of RBBR in shaking condition with 5 % Rice Husk and different dye concentration

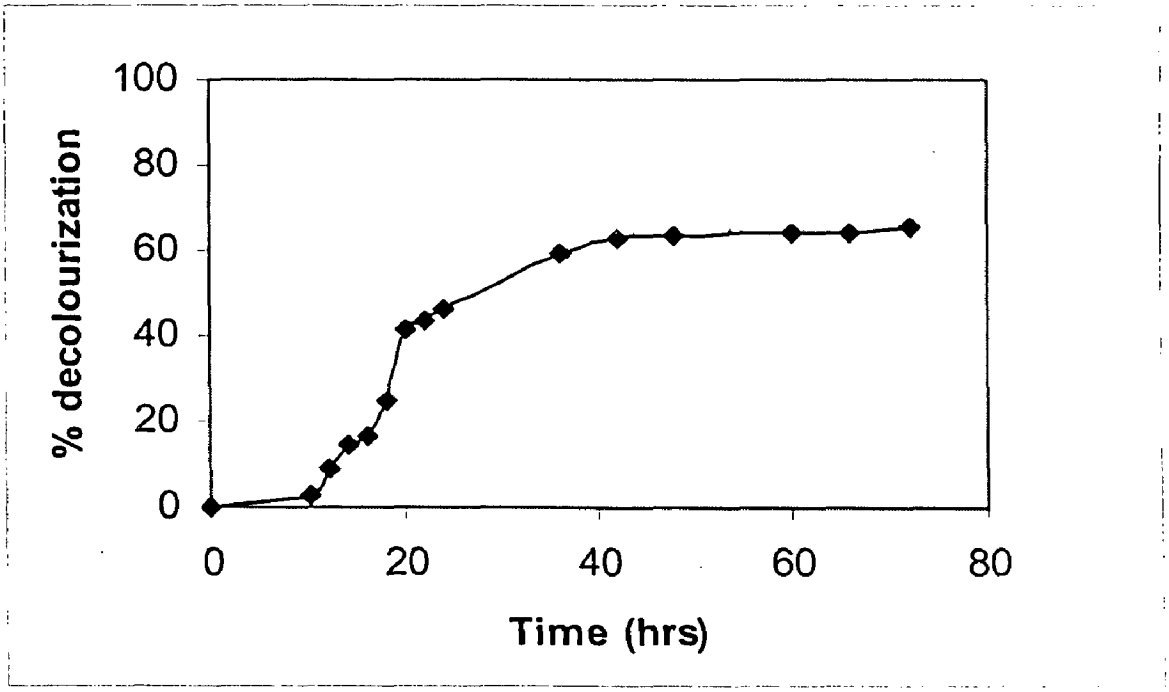


Fig.5.18 Percentage removal of RO 16 in shaking condition with 10 % Rice Husk and different dye concentration

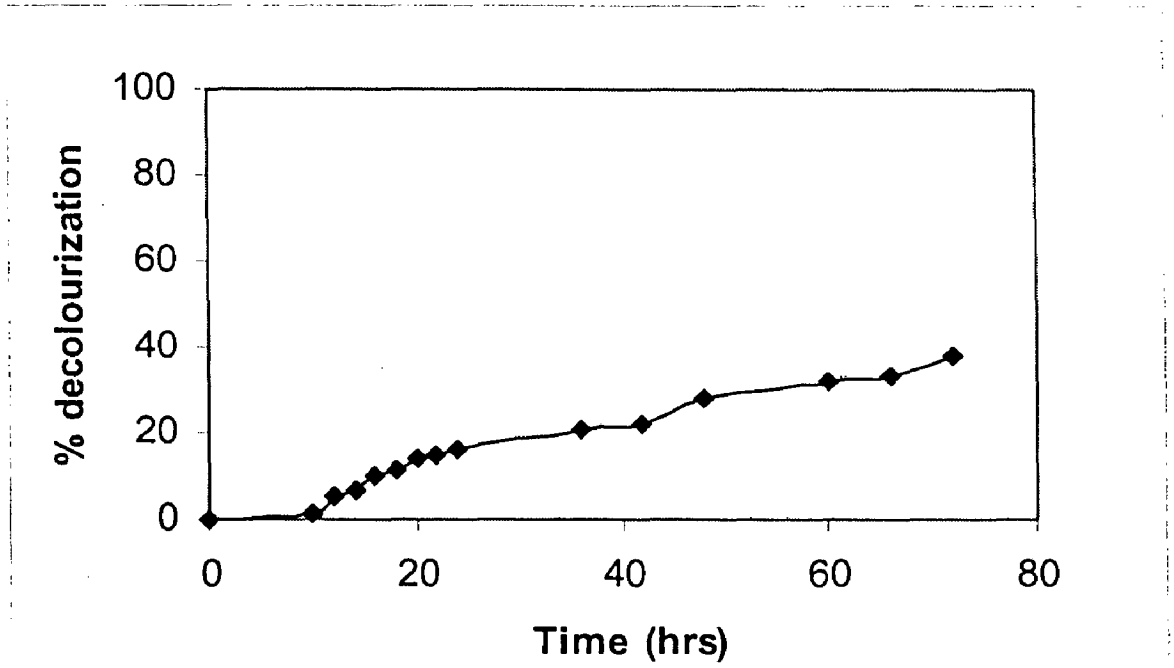


Fig.5.19 Percentage removal of RB 5 in shaking condition with 10 % Rice Husk and different dye concentration

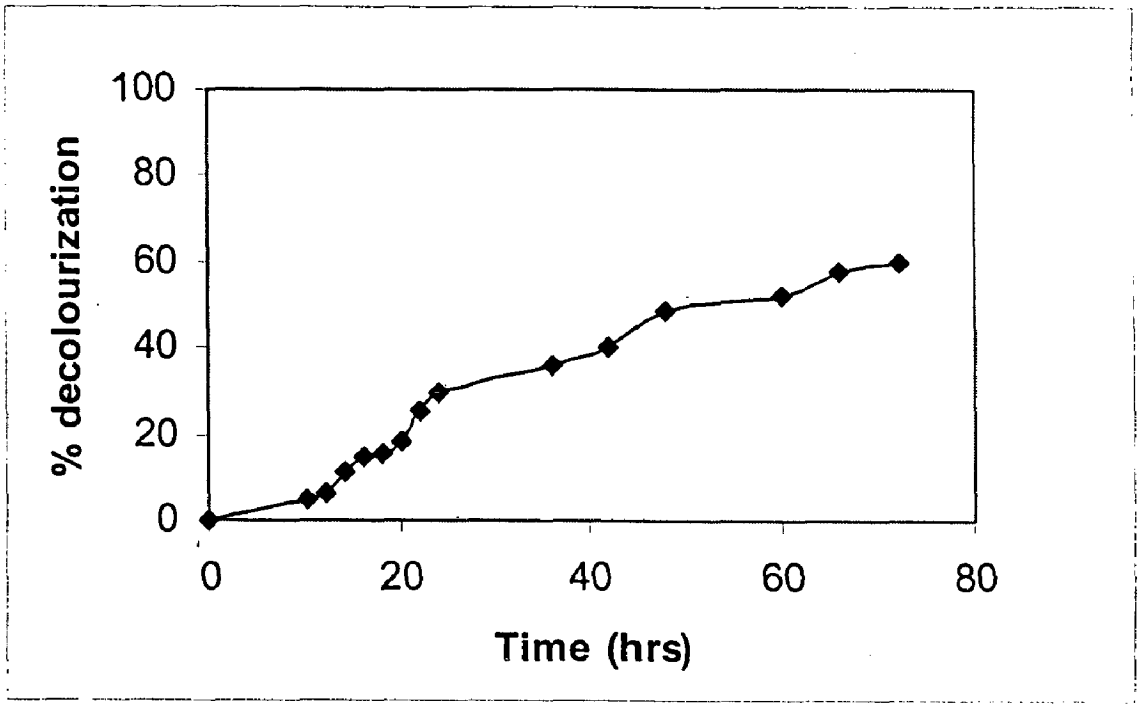


Fig.5.20 Percentage removal of RBBR in shaking condition with 10 % Rice Husk and different dye concentration

Since RB 5 is hardly adsorbable the biodegradation effect is very much higher in simultaneous adsorption and biodegradation. The variation in percentage removals is higher at low concentrations. But for higher concentrations substrate inhibition or blockage of pores or increase in pH (More substrate, more time to degrade, so increase in pH is more) may be the reasons for lower rate of removals.

The **Langergren plots** for different conc. of RO 16, RB 5and RBBR dyes for various adsorbent doses and under different conditions shown below:

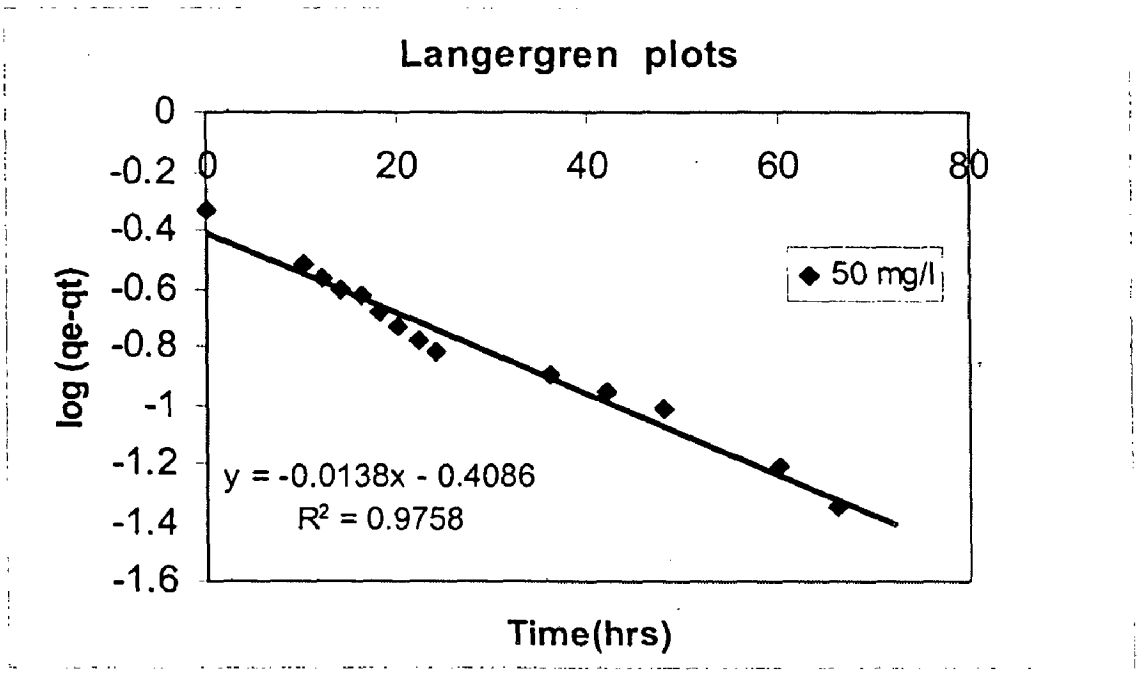


Fig. 5.21 Langergren Plots for 50 mg/l RO 16 dye conc. and for 5% RH

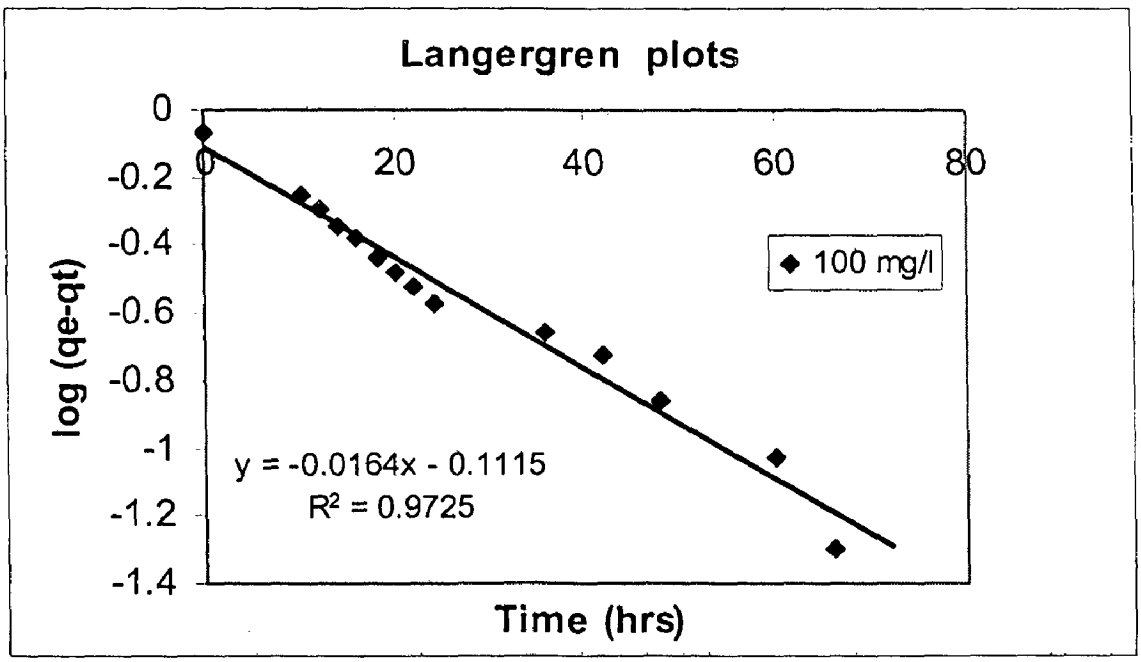


Fig. 5.22 Langergren Plots for 100 mg/l RO 16 dye conc. and for 5% RH

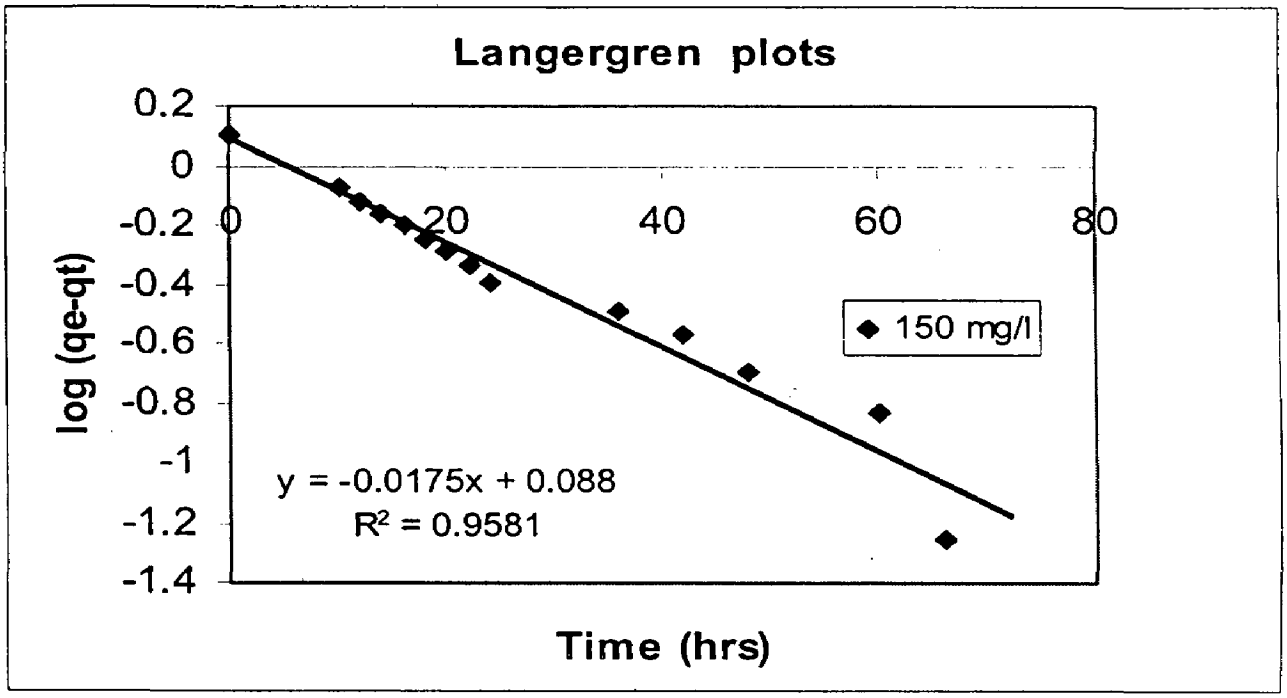


Fig. 5.23 Langergren Plots for 150 mg/l RO 16 dye conc. and for 5% RH

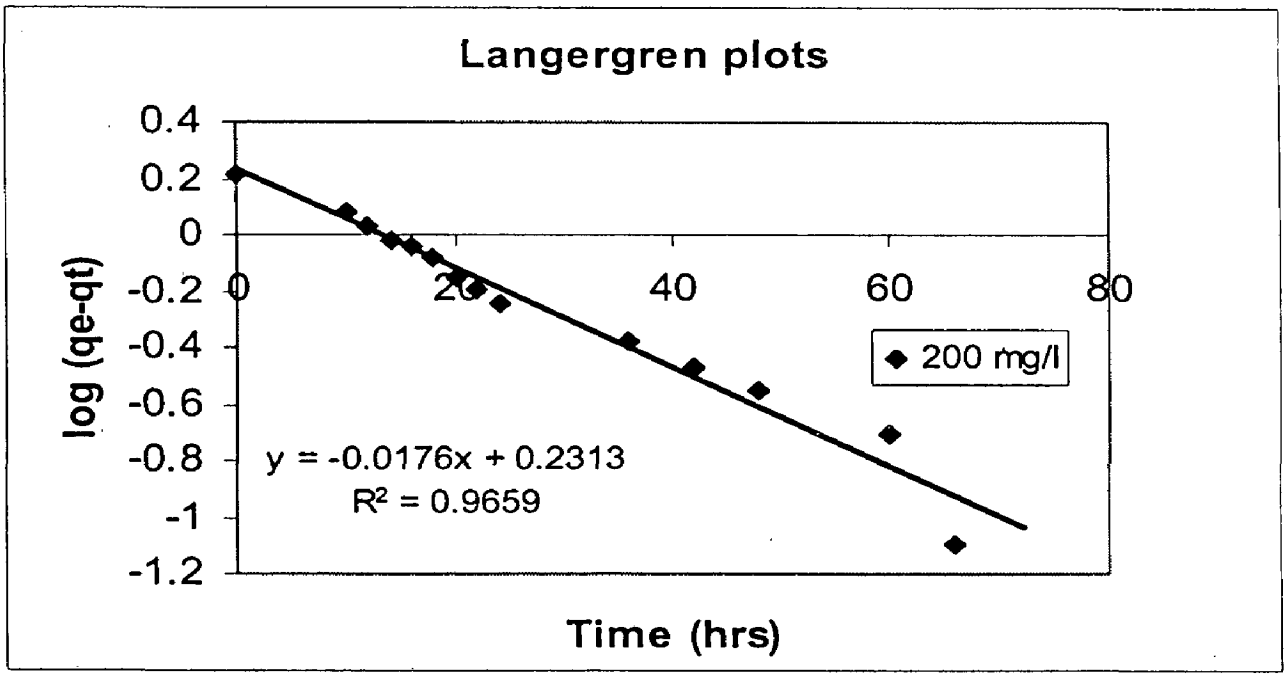


Fig. 5.24 Langergren Plots for 200 mg/l RO 16 dye conc. and for 5% RH

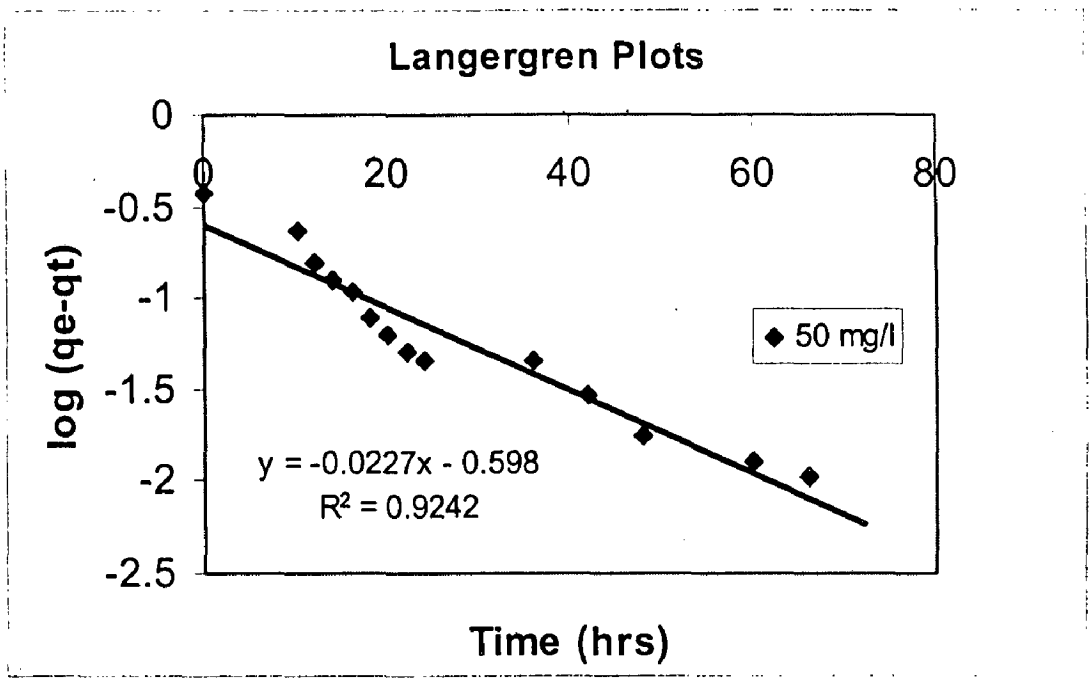


Fig. 5.25 Langergren Plots for 50 mg/l RB 5dye conc. and for 5% RH

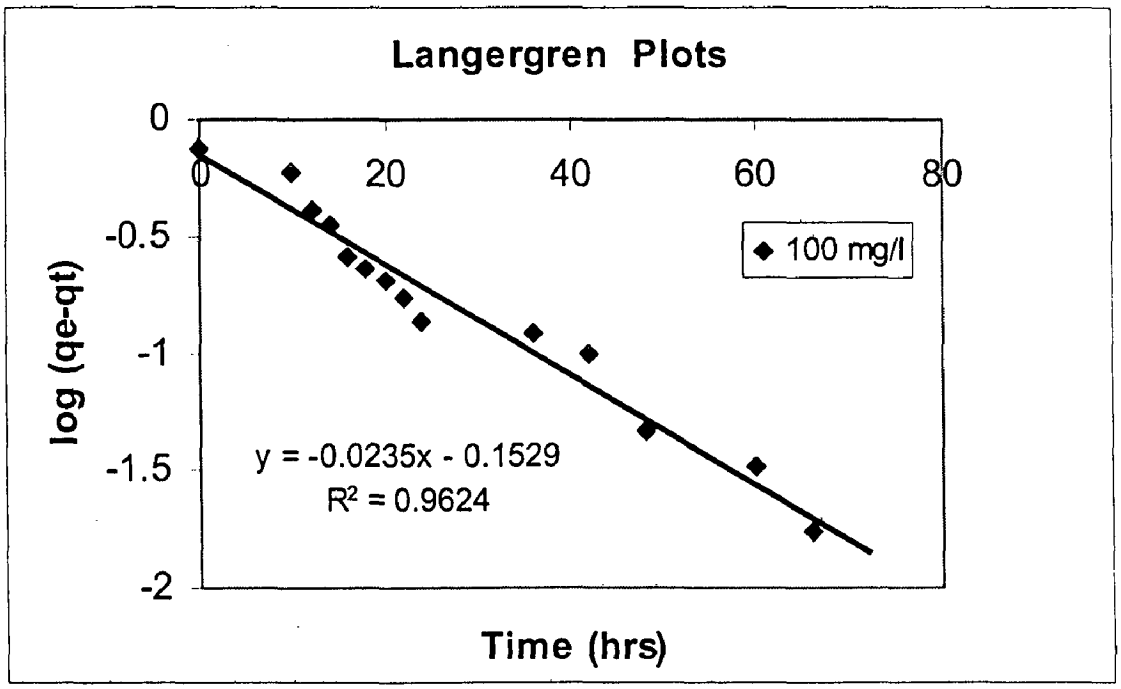


Fig.5.26 Langergren Plots for 100 mg/l RB 5 dye conc. and for 5% RH

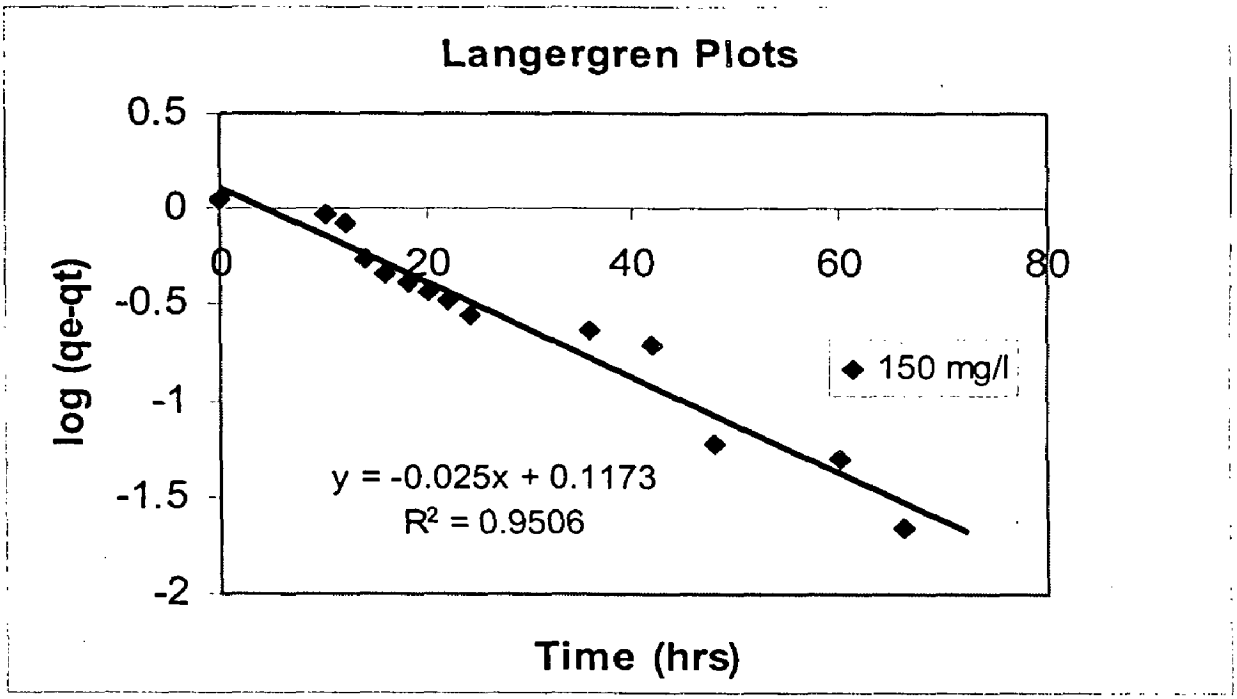


Fig. 5.27 Langergren Plots for 150 mg/l RB 5 dye conc. and for 5% RH

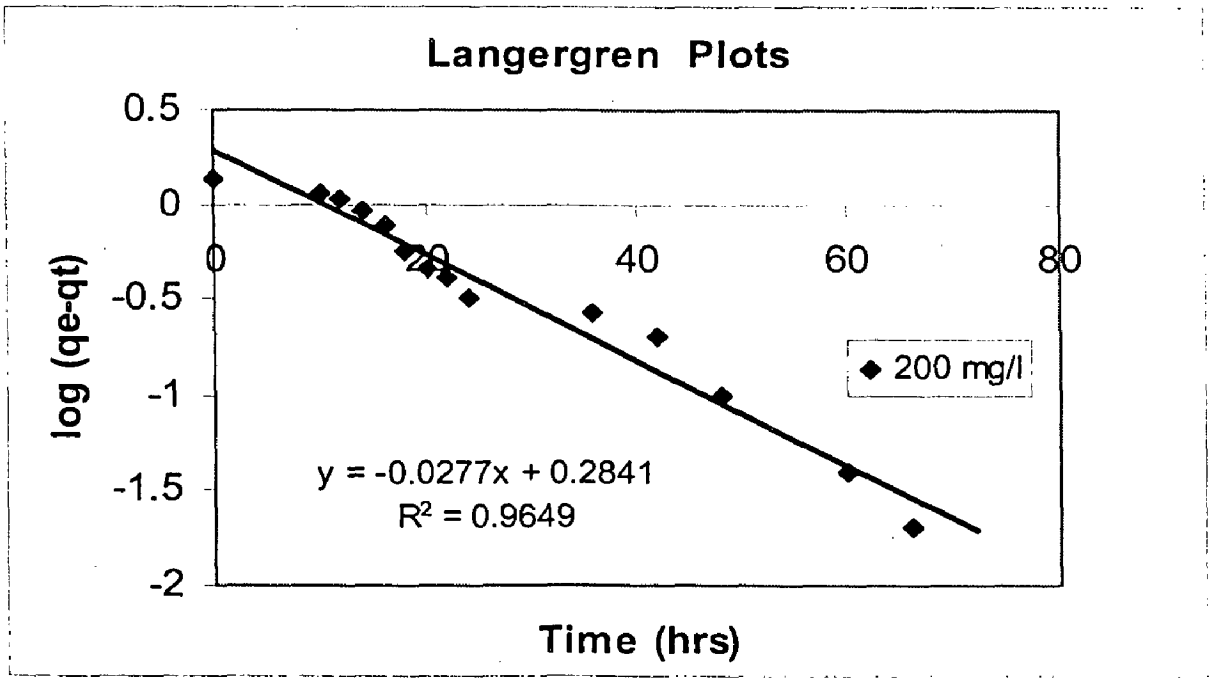


Fig. 5.28 Langergren Plots for 200 mg/l RB 5 dye conc. and for 5% RH

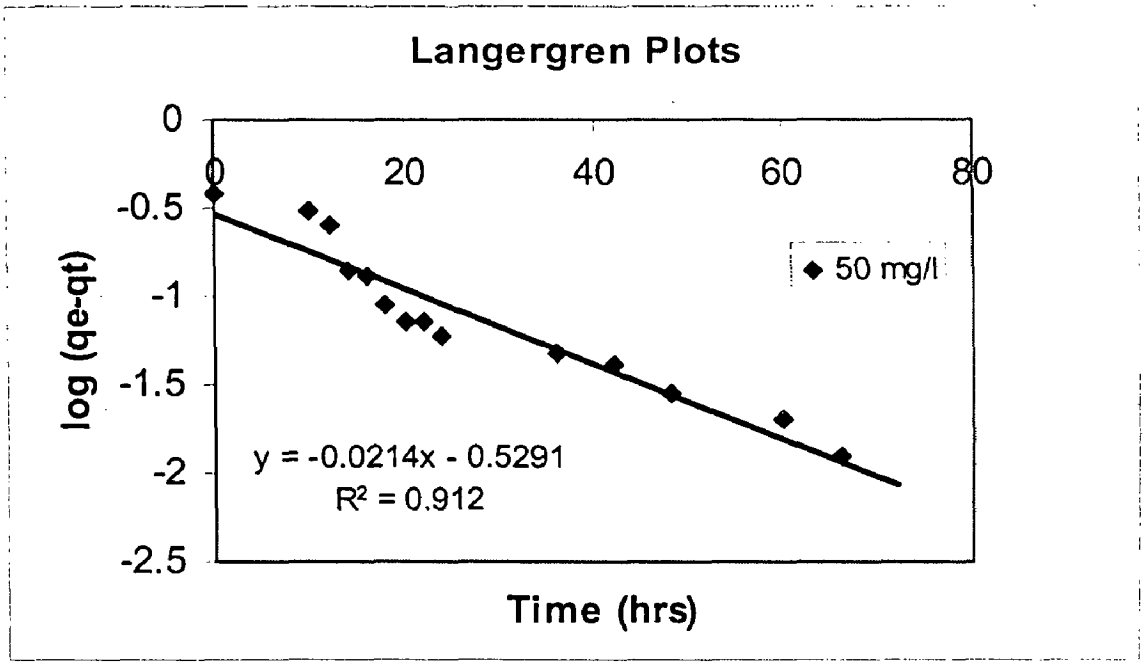


Fig. 5.29 Langergren Plots for 50 mg/l RBBR dye conc. and for 5% RH

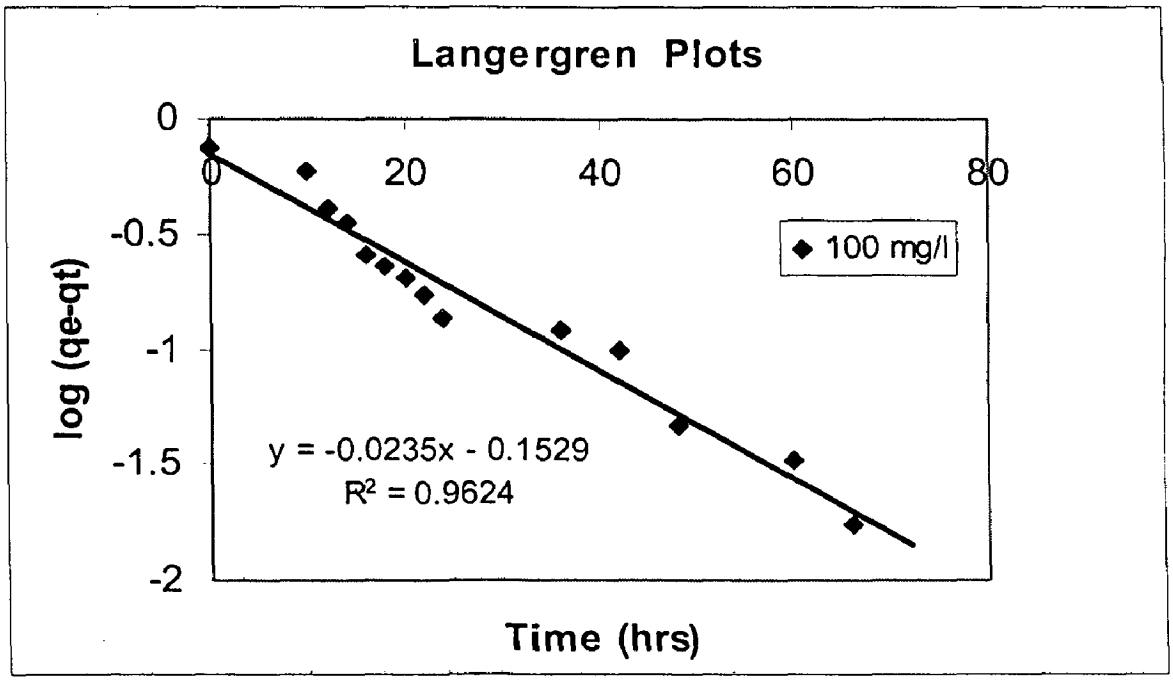


Fig. 5.30 Langergren Plots for 100 mg/l RBBR dye conc. and for 5% RH

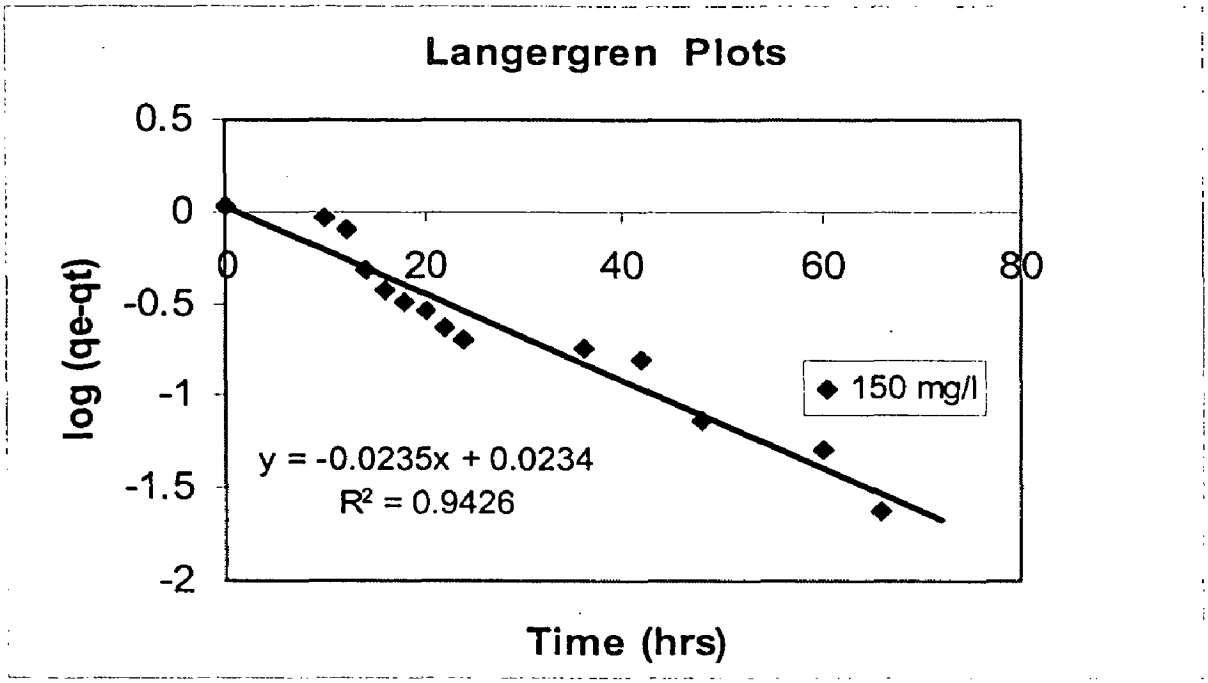


Fig. 5.31 Langergren Plots for 150 mg/l RBBR dye conc. and for 5% RH

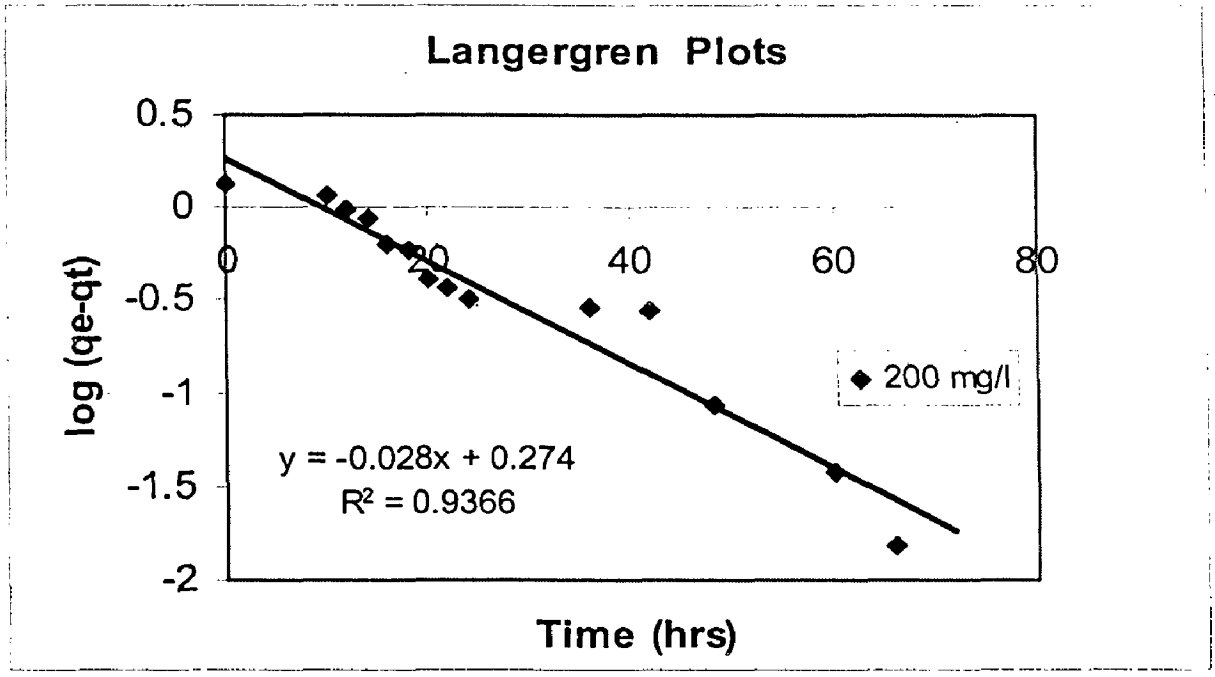


Fig. 5.32 Langergren Plots for 200 mg/l RBBR dye conc. and for 5% RH

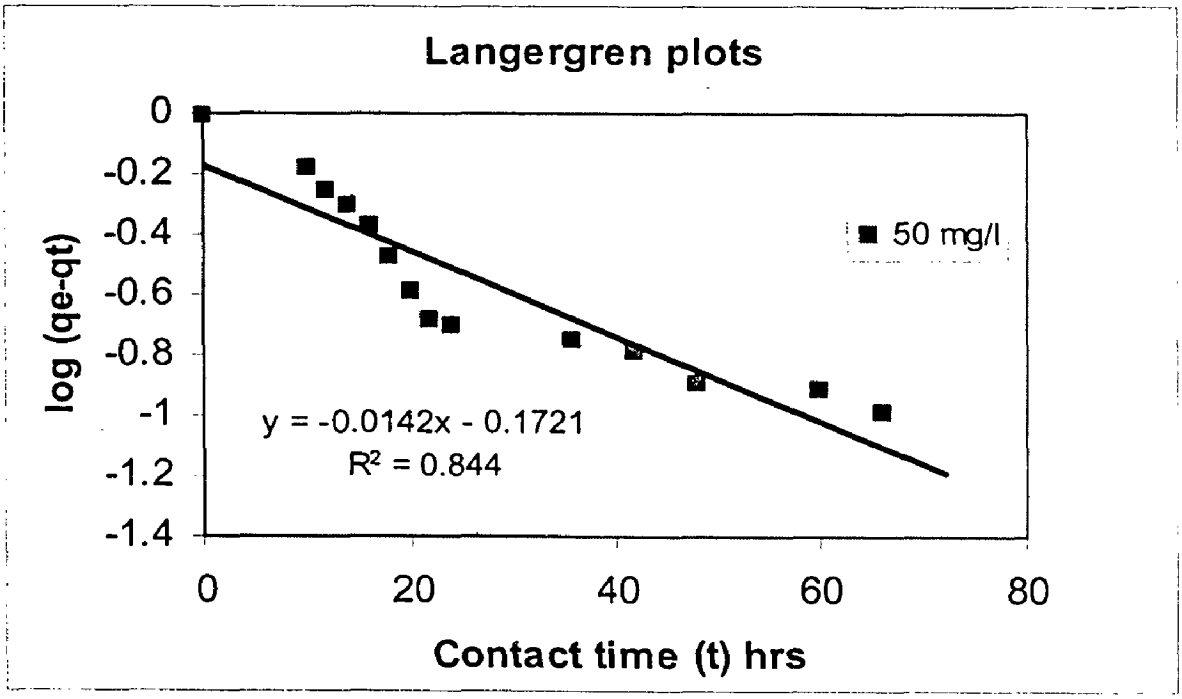


Fig. 5.33 Langergren Plots for 50 mg/l RO 16 dye conc. and for 10 % RH

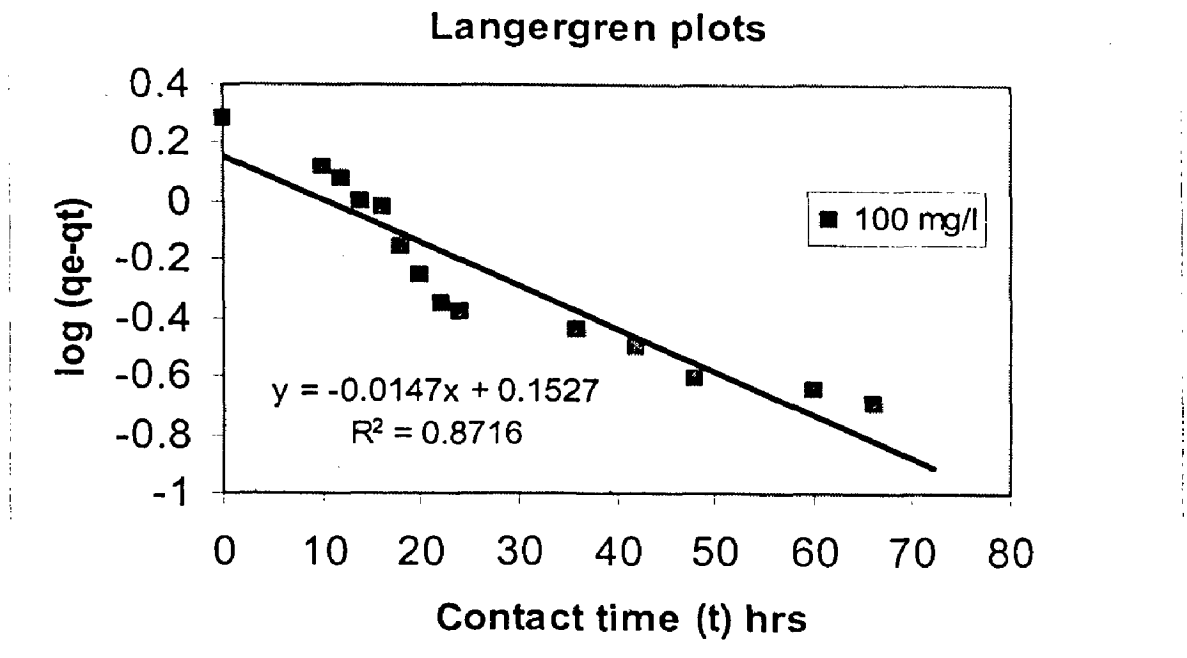


Fig. 5.34 Langergren Plots for 100 mg/l RO 16 dye conc. and for 10 % RH

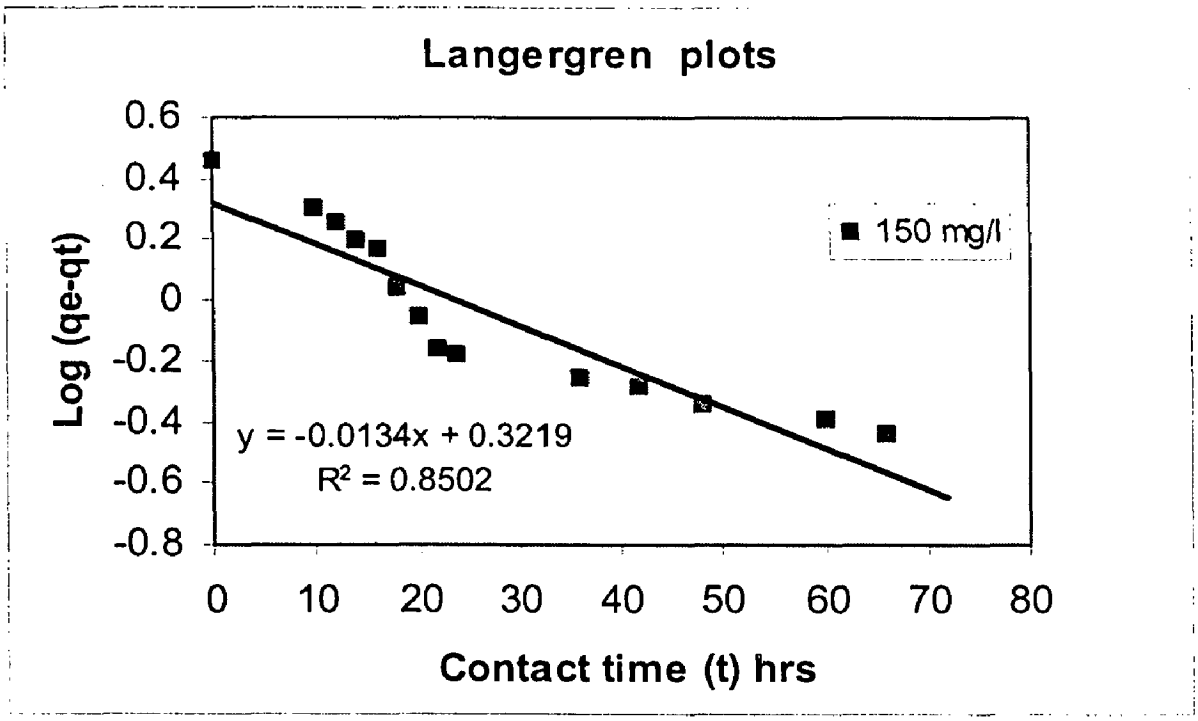


Fig. 5.35 Langergren Plots for 150 mg/l RO 16 dye conc. and for 10 % RH

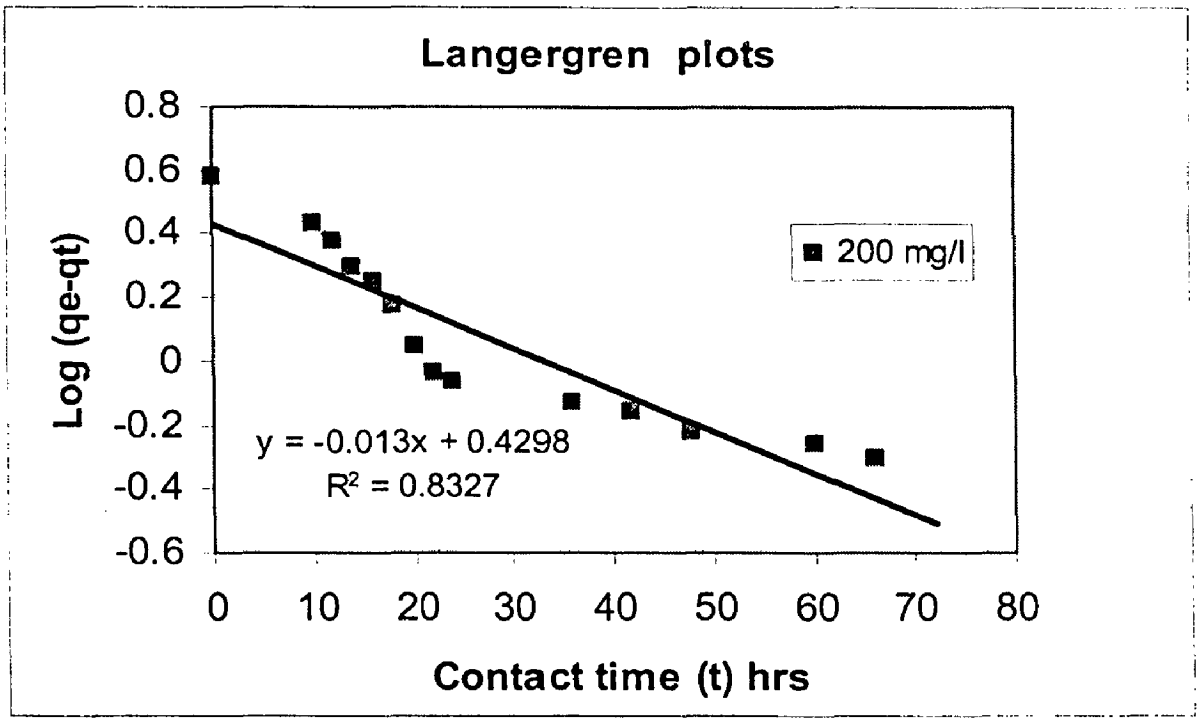


Fig. 5.36 Langergren Plots for 200 mg/l RO 16 dye conc. and for 10 % RH

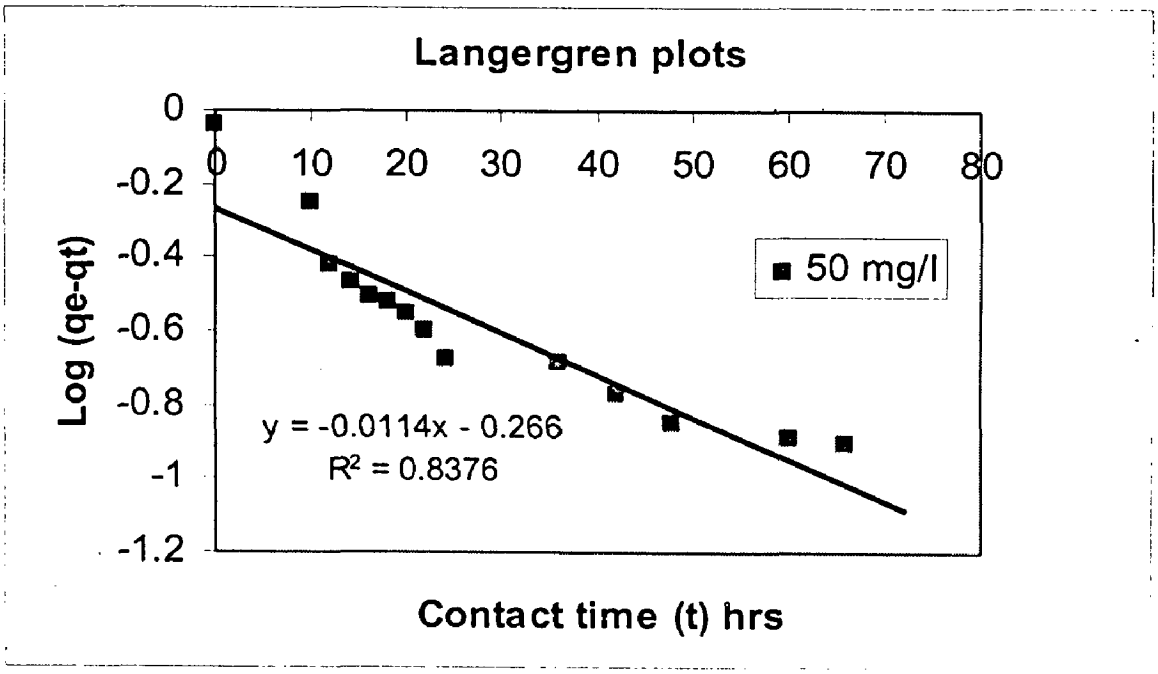


Fig. 5.37 Langergren Plots for 50 mg/l RB 5 dye conc. and for 10 % RH

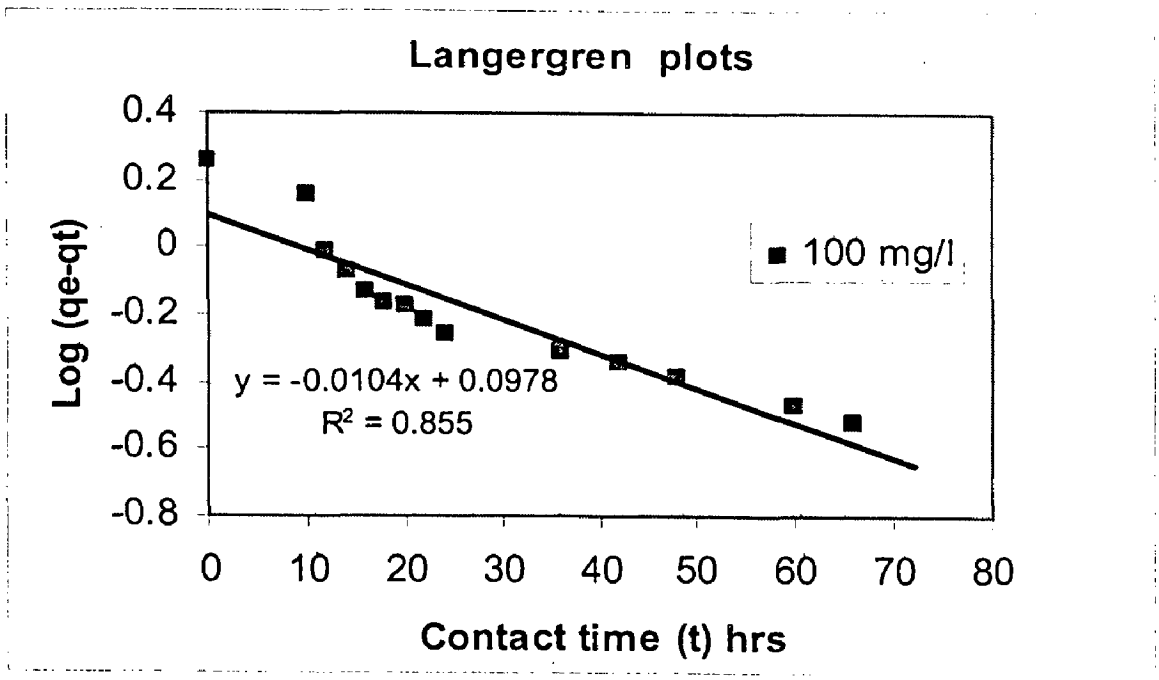


Fig. 5.38 Langergren Plots for 100 mg/l RB 5 dye conc. and for 10 % RH

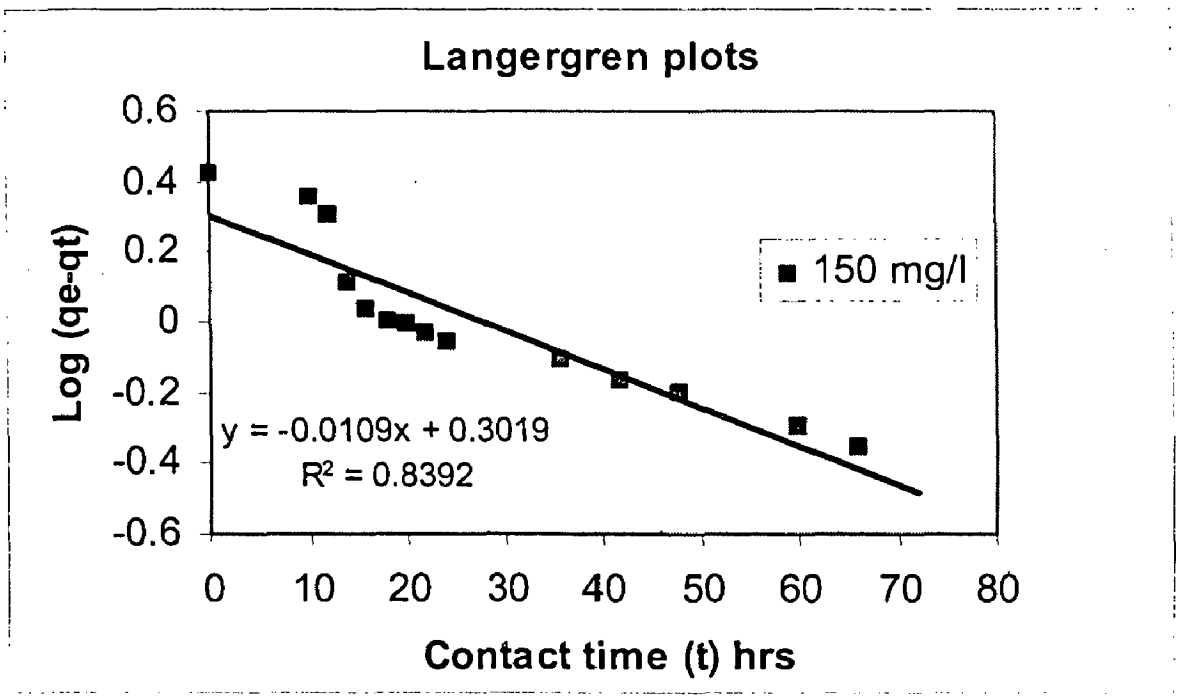


Fig. 5.39 Langergren Plots for 150 mg/l RB 5 dye conc. and for 10 % RH

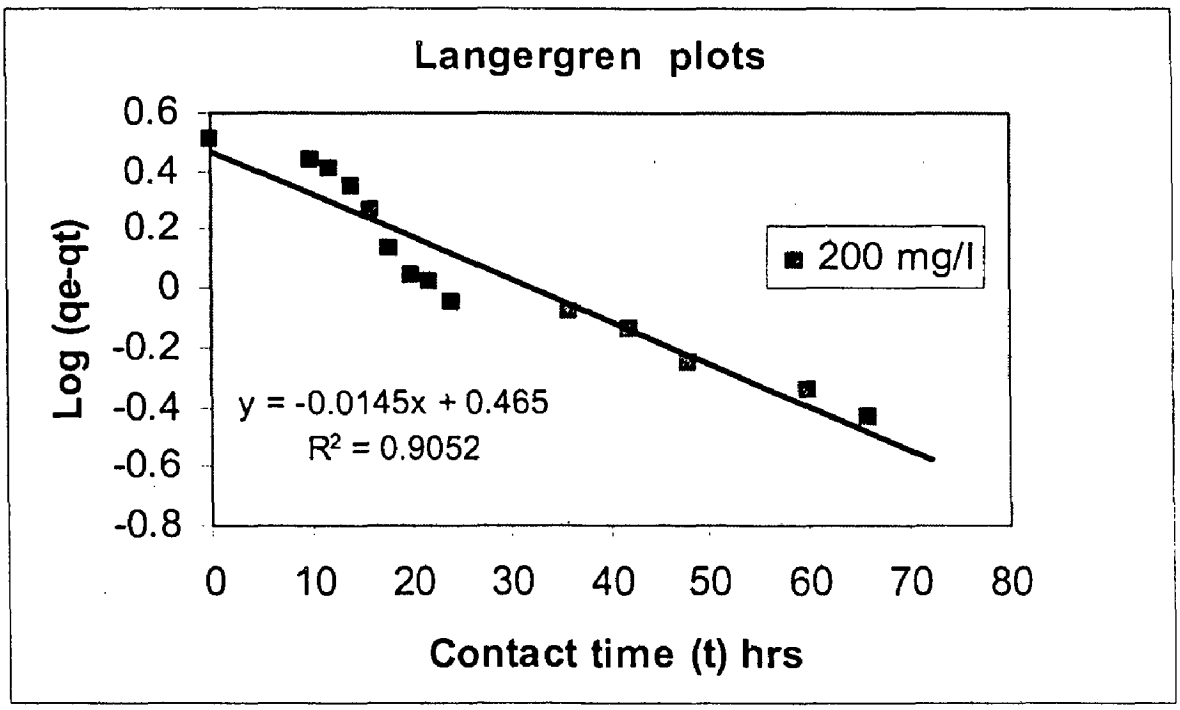


Fig. 5.40 Langergren Plots for 200 mg/l RB 5 dye conc. and for 10 % RH

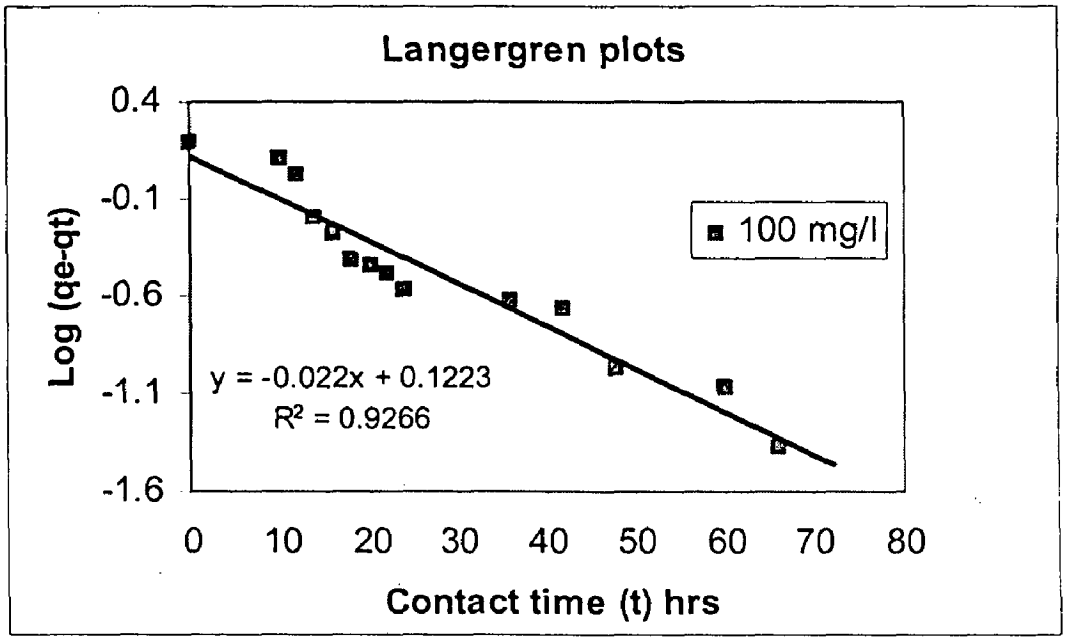


Fig. 5.41 Langergren Plots for 100 mg/l RBBR dye conc. and for 10 % RH

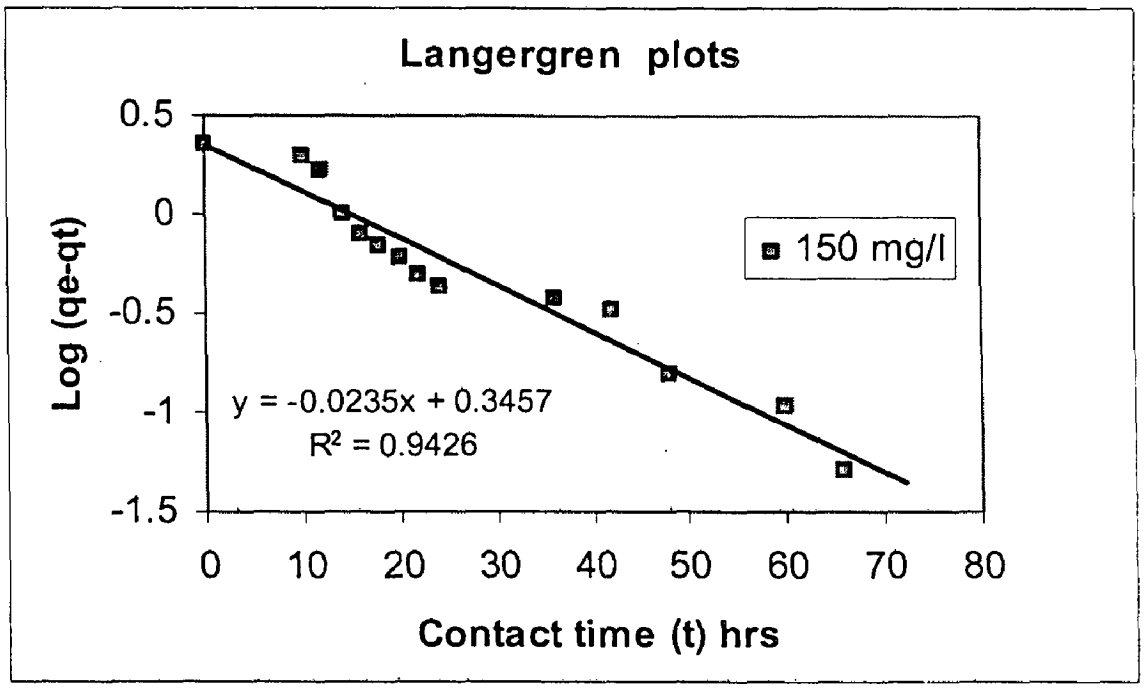


Fig. 5.42 Langergren Plots for 150 mg/l RBBR dye conc. and for 10 % RH

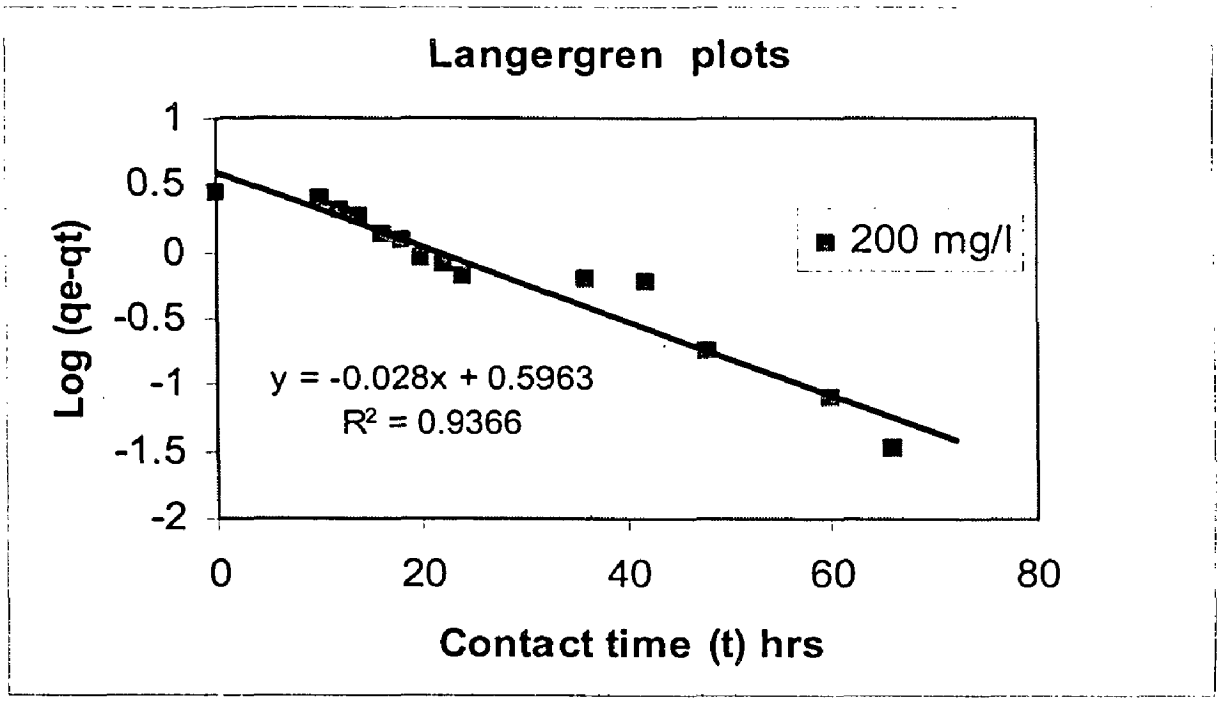


Fig. 5.43 Langergren Plots for 200 mg/l RBBR dye conc. and for 10 % RH

Langmuir Isotherm at different time intervals between **Ce/qe v/s Ce** for various dyes and various adsorbent doses

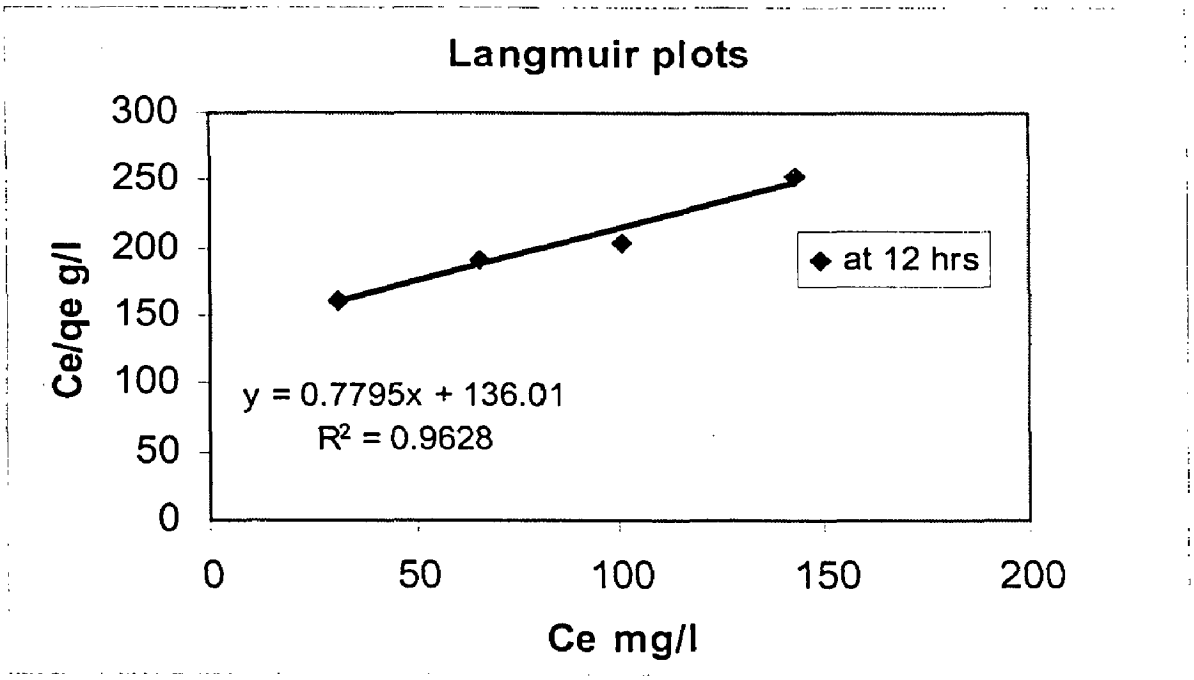


Fig. 5.44 Langmuir Plots at 12 hrs time interval, for RO 16 and 5% RH

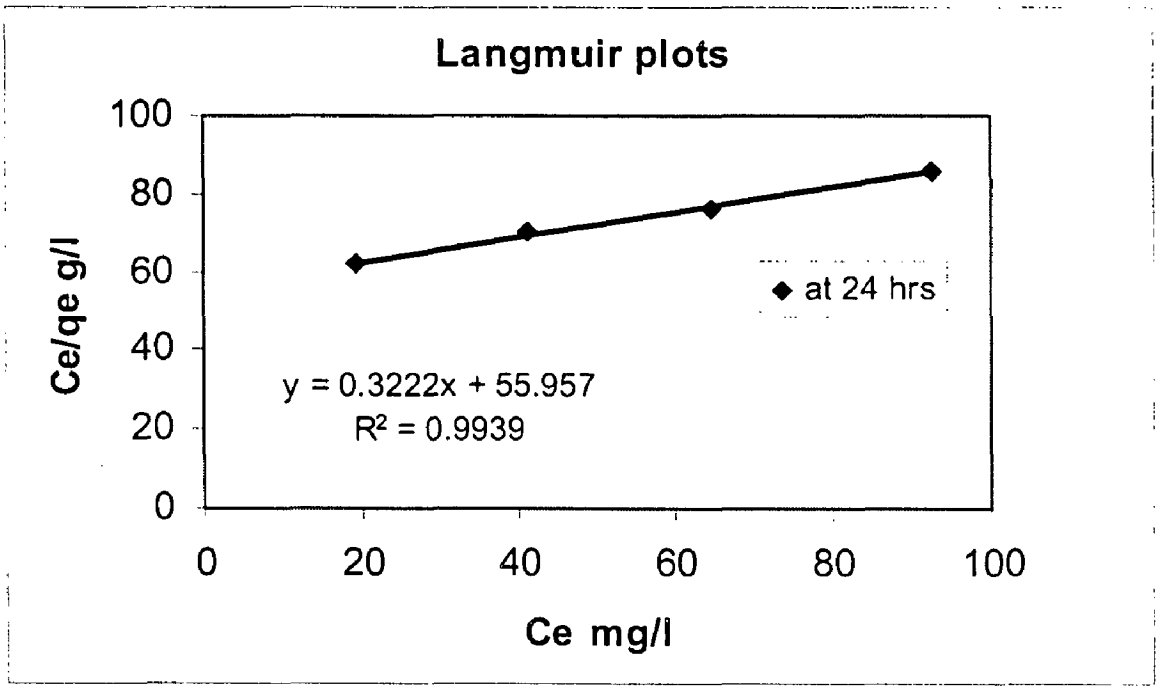


Fig. 5.45 Langmuir Plots at 24 hrs time interval, for RO 16 and 5% RH

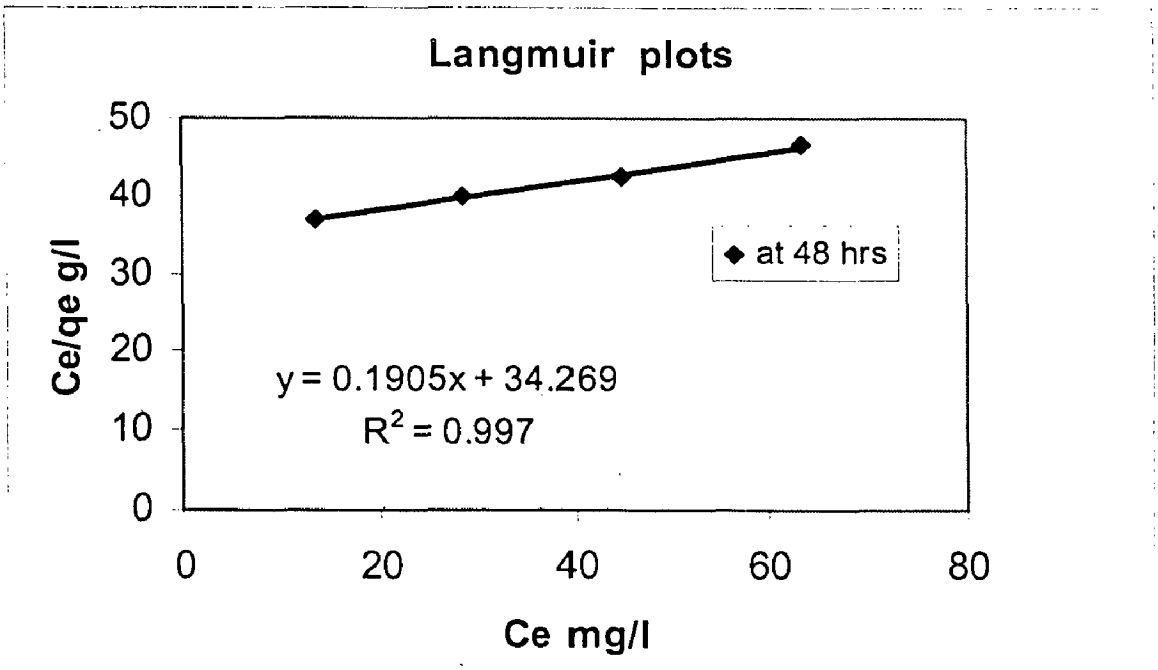


Fig. 5.46 Langmuir Plots at 48 hrs time interval, for RO 16 and 5% RH

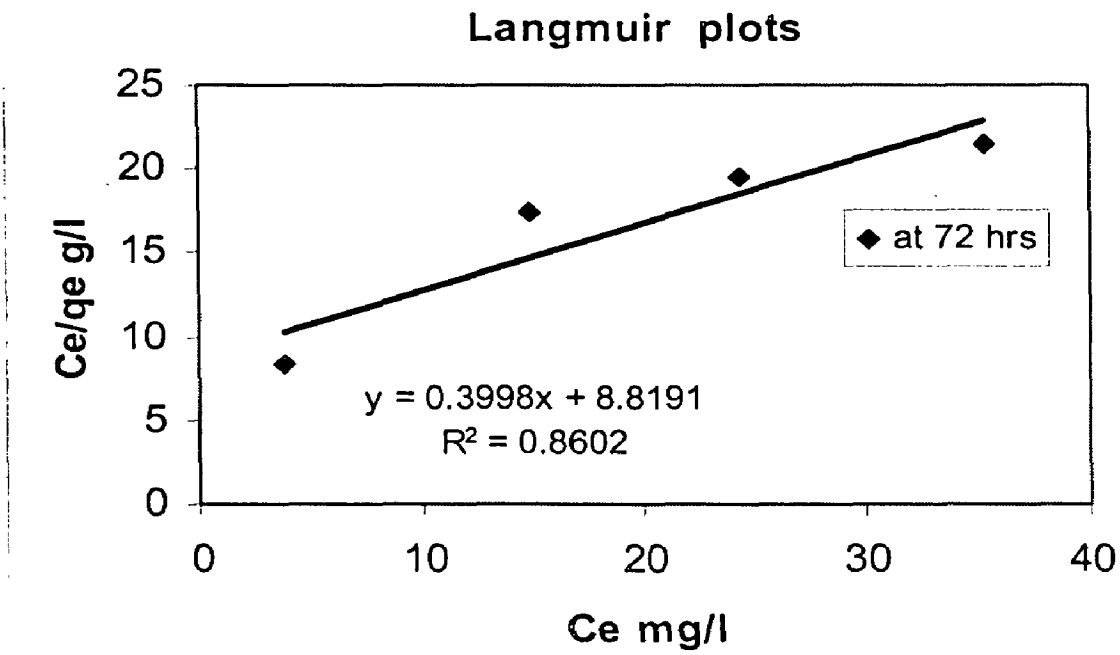


Fig. 5.47 Langmuir Plots at 72 hrs time interval, for RO 16 and 5% RH

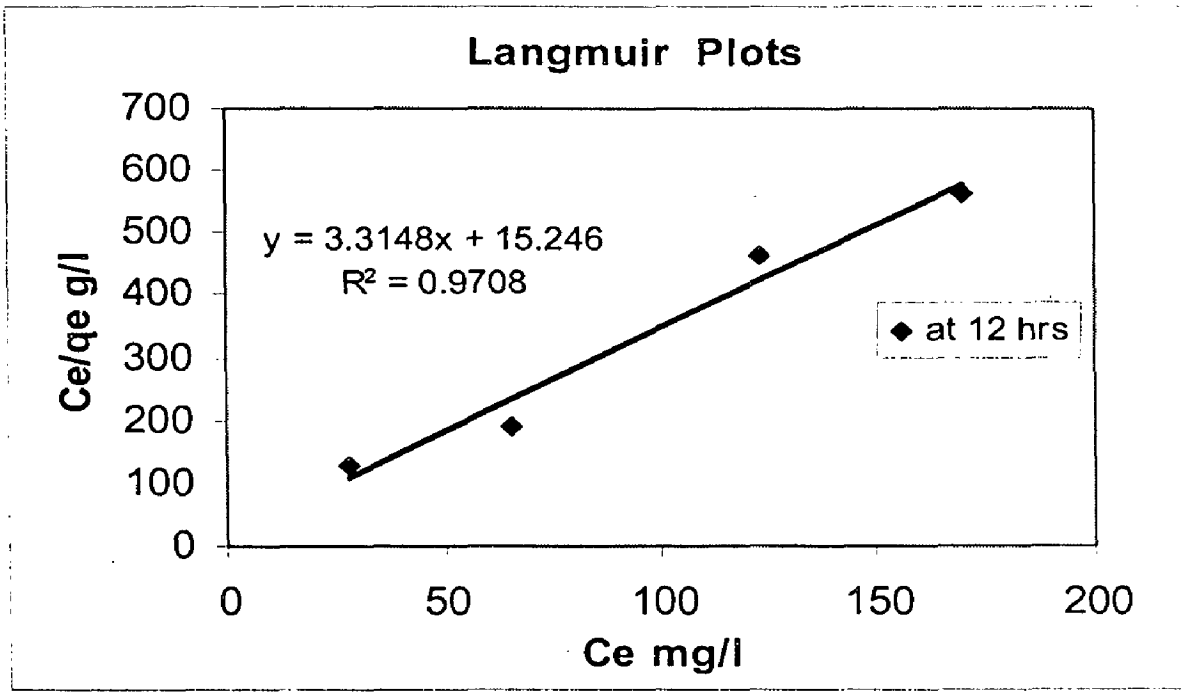


Fig. 5.48 Langmuir Plots at 12 hrs time interval, for RB 5 and 5% RH

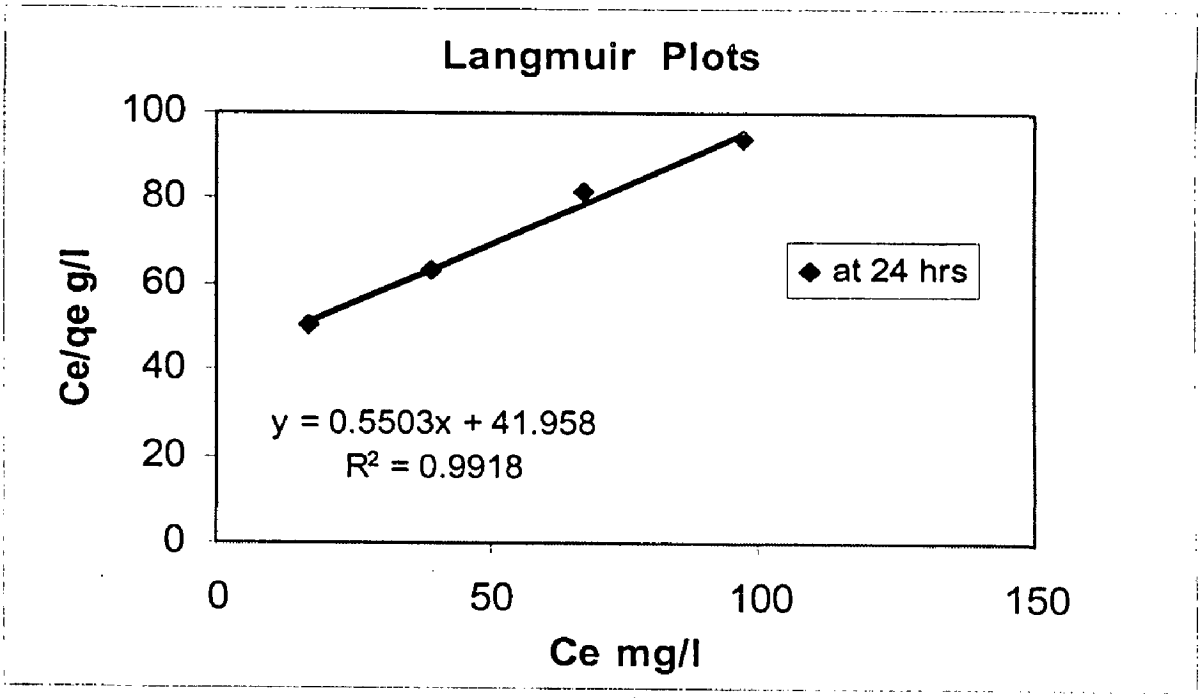


Fig. 5.49 Langmuir Plots at 24 hrs time interval, for RB 5 and 5% RH

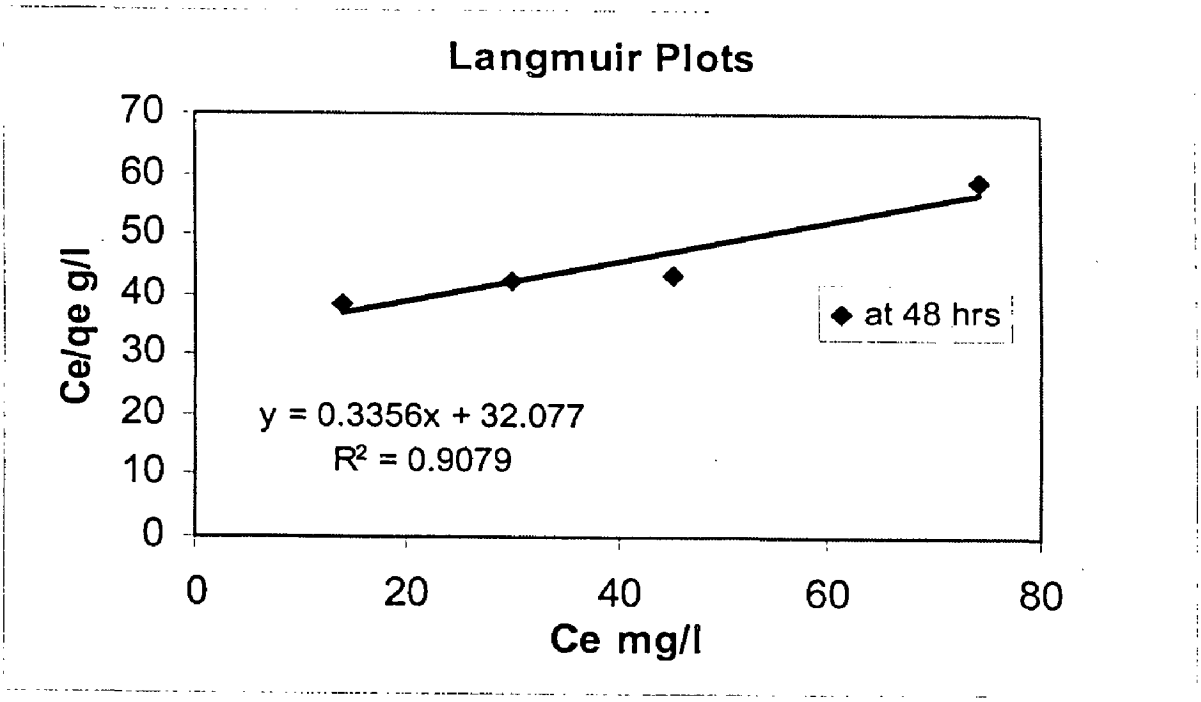


Fig. 5.50 Langmuir Plots at 48 hrs time interval, for RB 5 and 5% RH

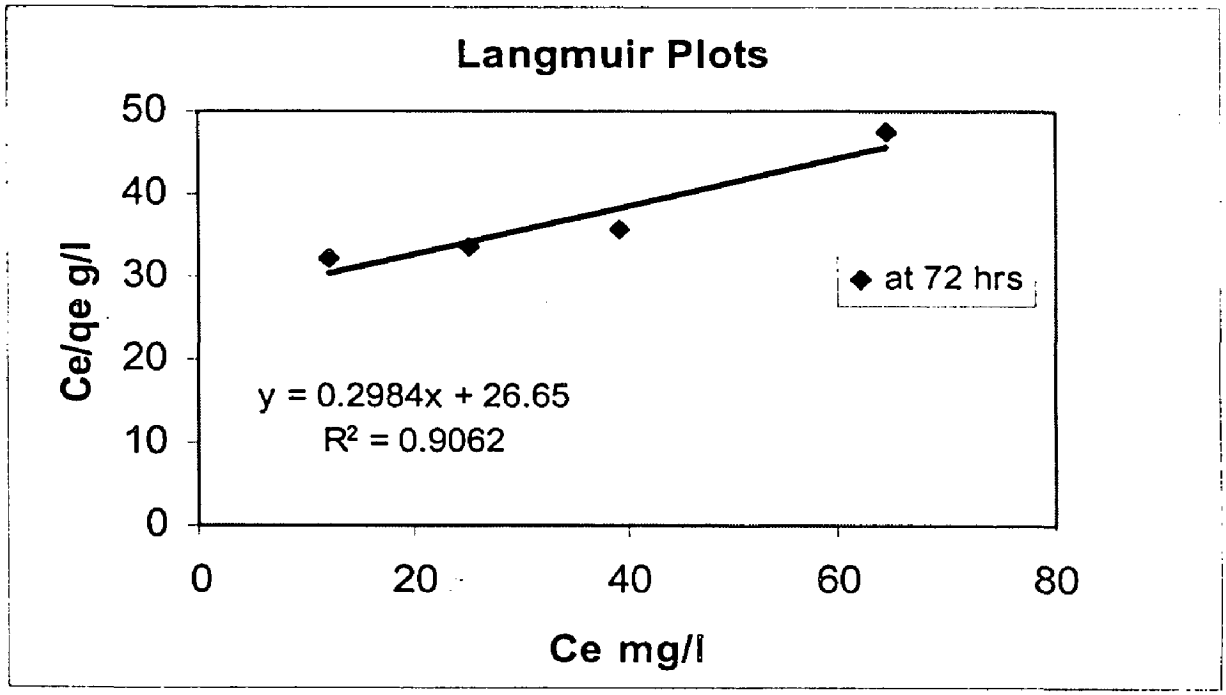


Fig. 5.51 Langmuir Plots at 72 hrs time interval, for RB 5 and 5% RH

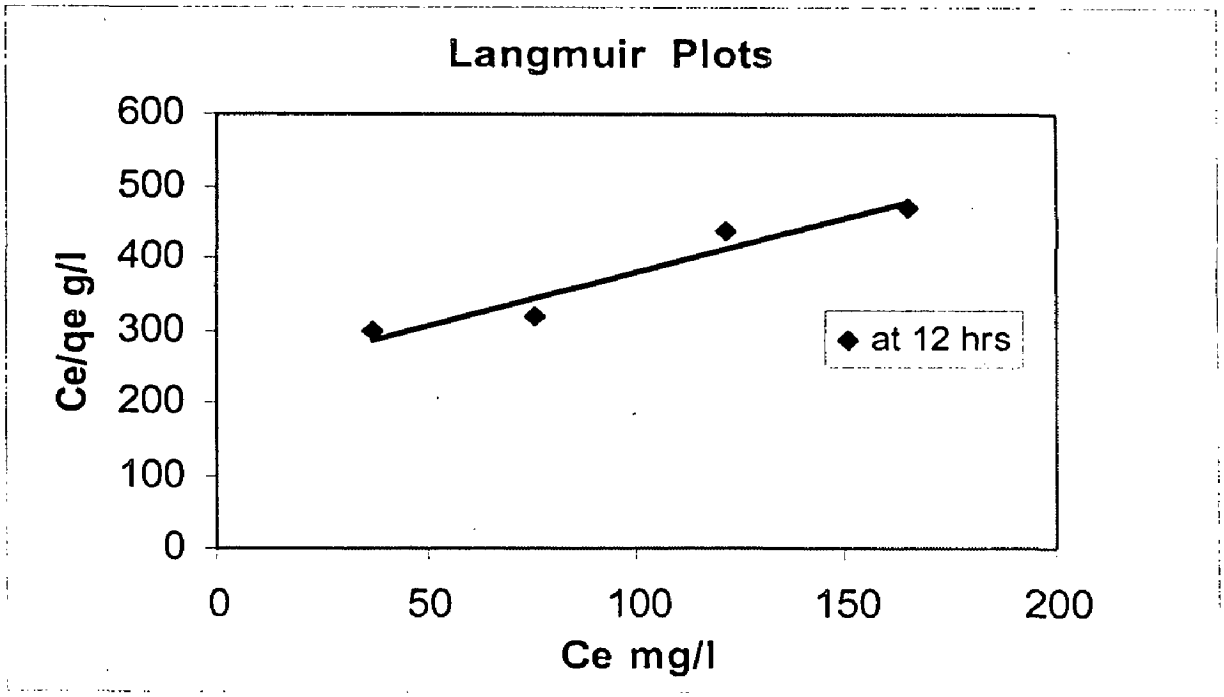


Fig. 5.52 Langmuir Plots at 12 hrs time interval, for RBBR and 5% RH

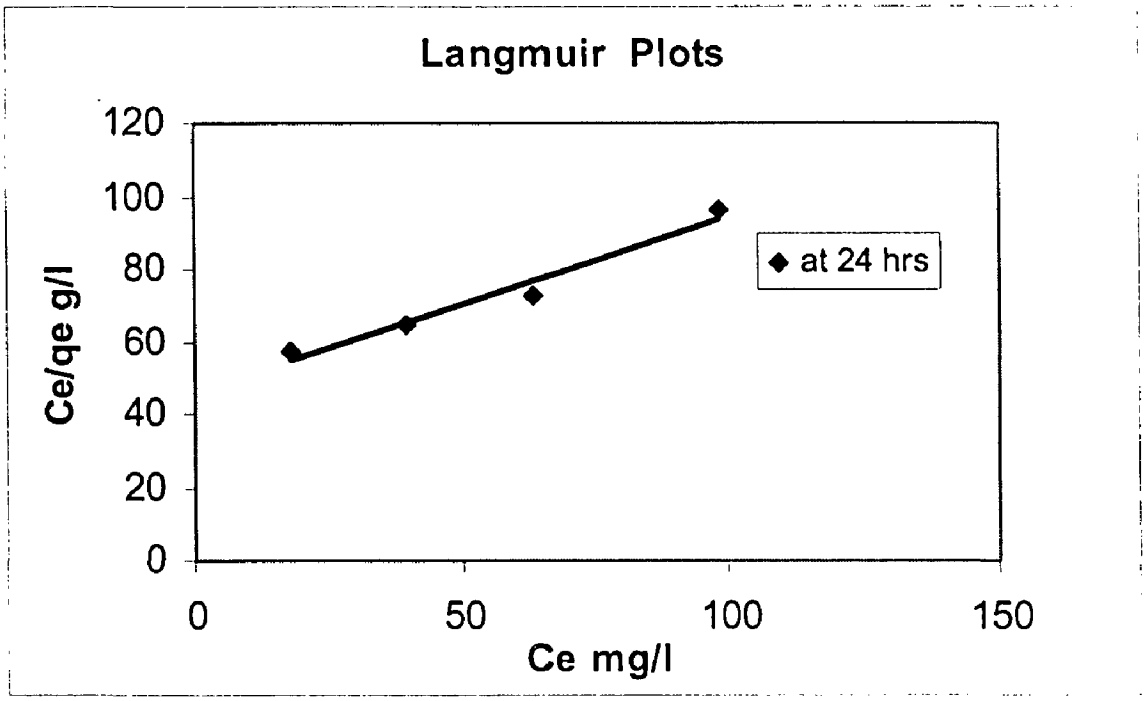


Fig. 5.53 Langmuir Plots at 24 hrs time interval, for RBBR and 5% RH

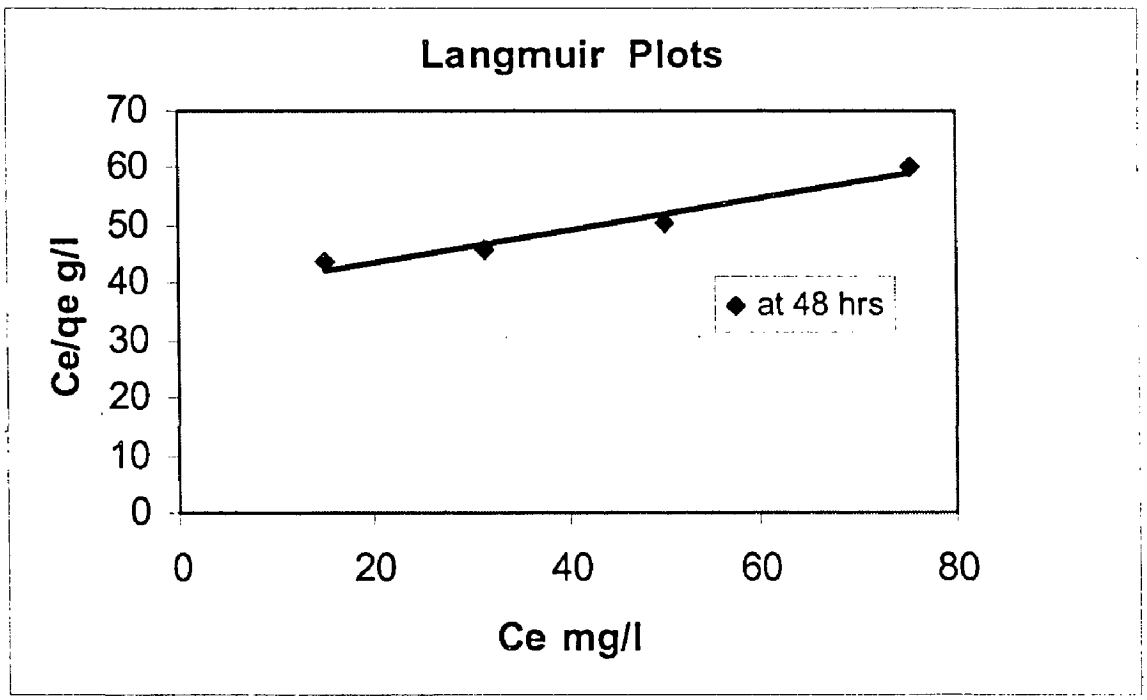


Fig. 5.54 Langmuir Plots at 48 hrs time interval, for RBBR and 5% RH

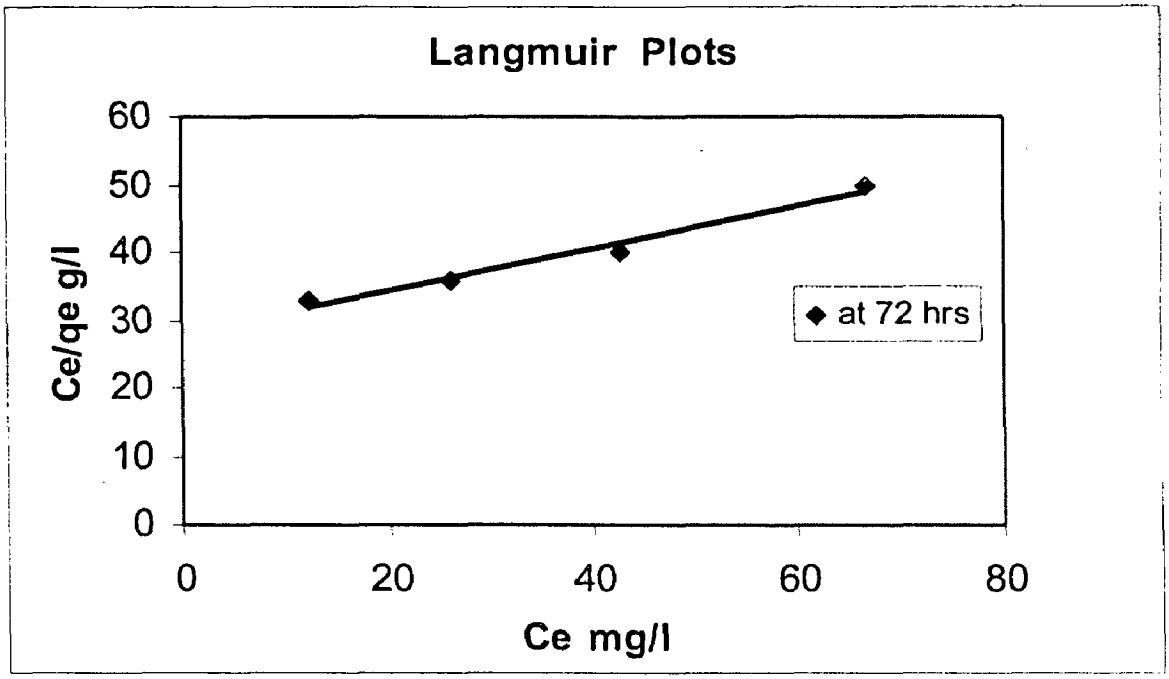


Fig. 5.55 Langmuir Plots at 72 hrs time interval, for RBBR and 5% RH

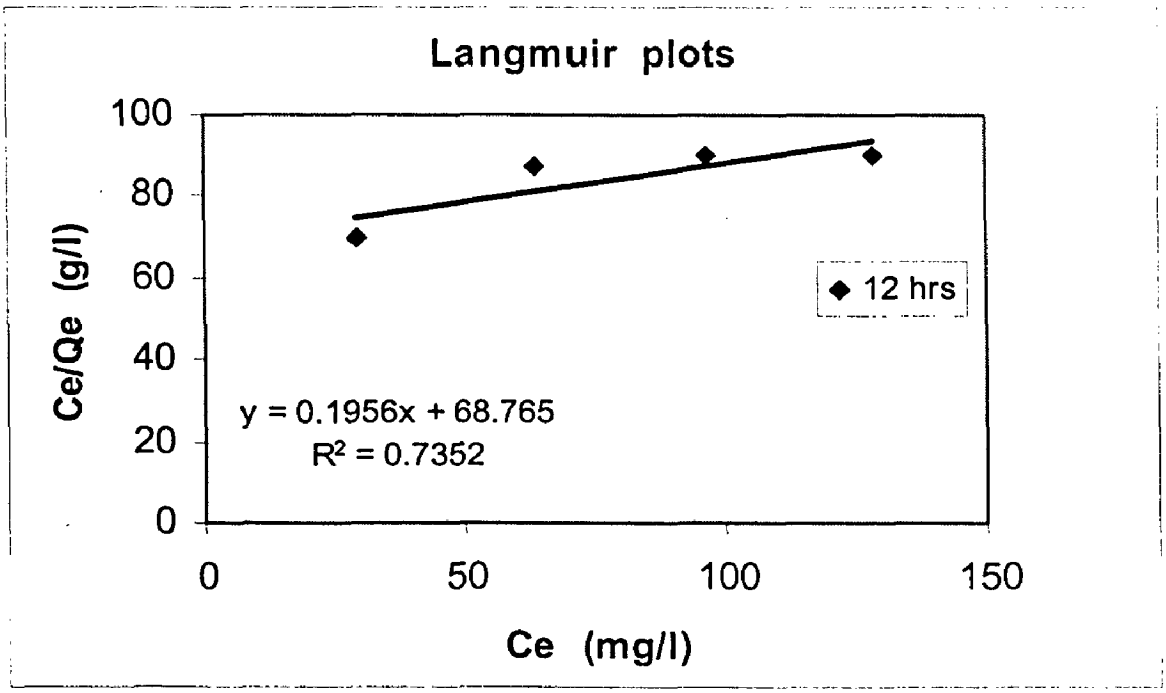


Fig. 5.56 Langmuir Plots at 12 hrs time interval, for RO 16 and 10% RH

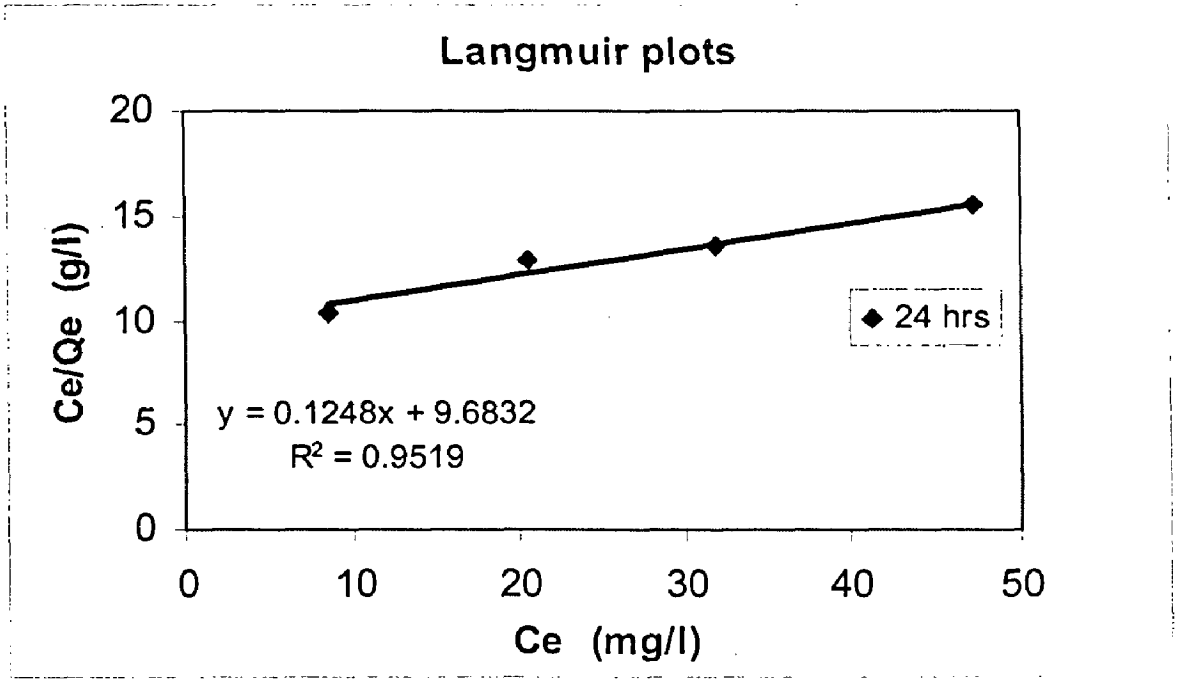


Fig. 5.57 Langmuir Plots at 24 hrs time interval, for RO 16 and 10% RH

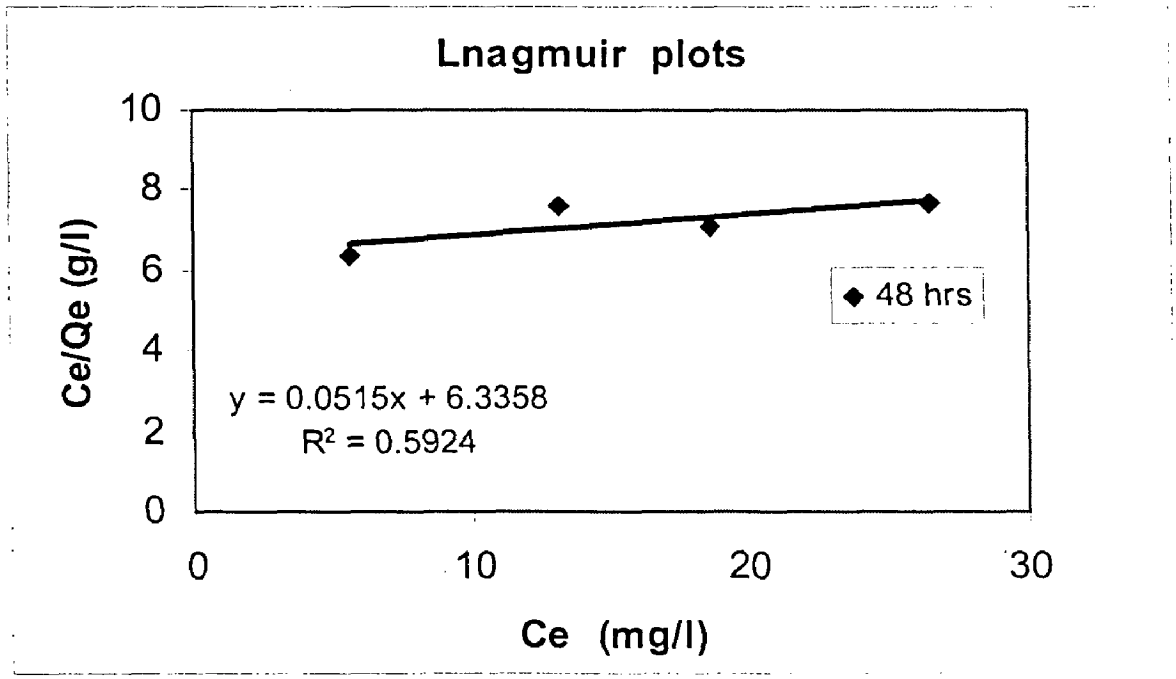


Fig. 5.58 Langmuir Plots at 48 hrs time interval, for RO 16 and 10% RH

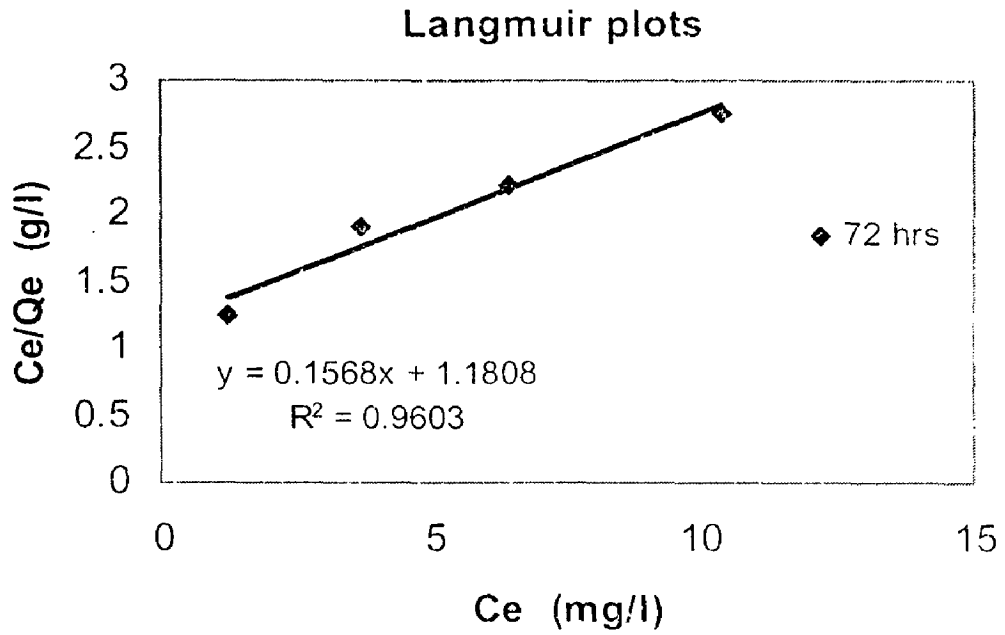


Fig. 5.59 Langmuir Plots at 72 hrs time interval, for RO 16 and 10% RH

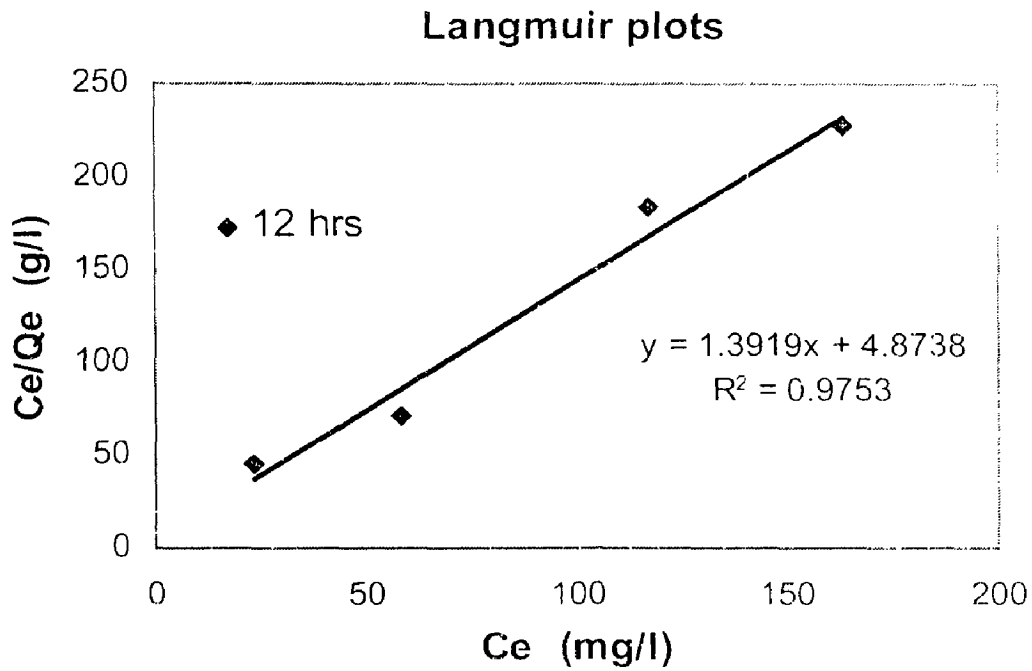


Fig. 5.60 Langmuir Plots at 12 hrs time interval, for RB5 and 10% RH

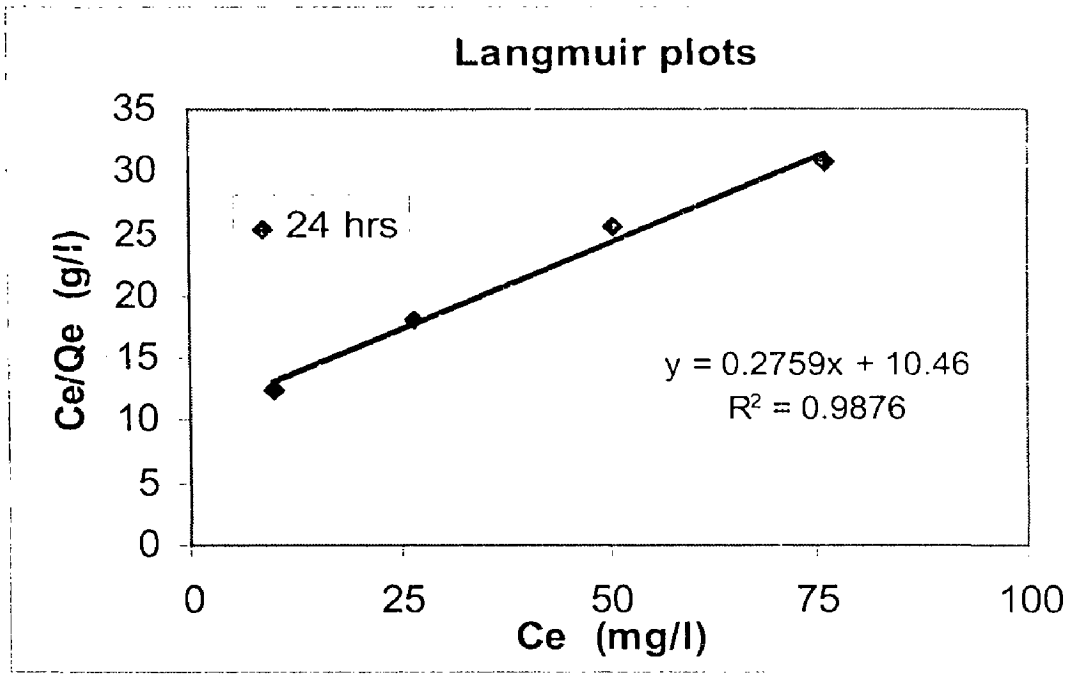


Fig. 5.61 Langmuir Plots at 24 hrs time interval, for RB5 and 10% RH

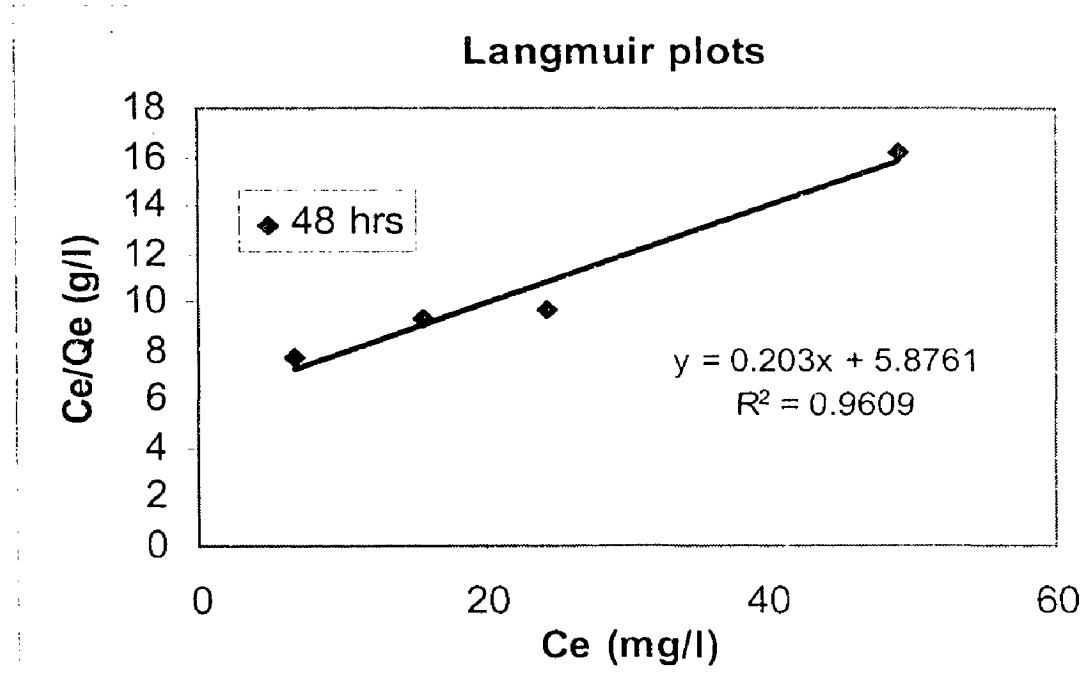


Fig. 5.62 Langmuir Plots at 48 hrs time interval, for RB5 and 10% RH

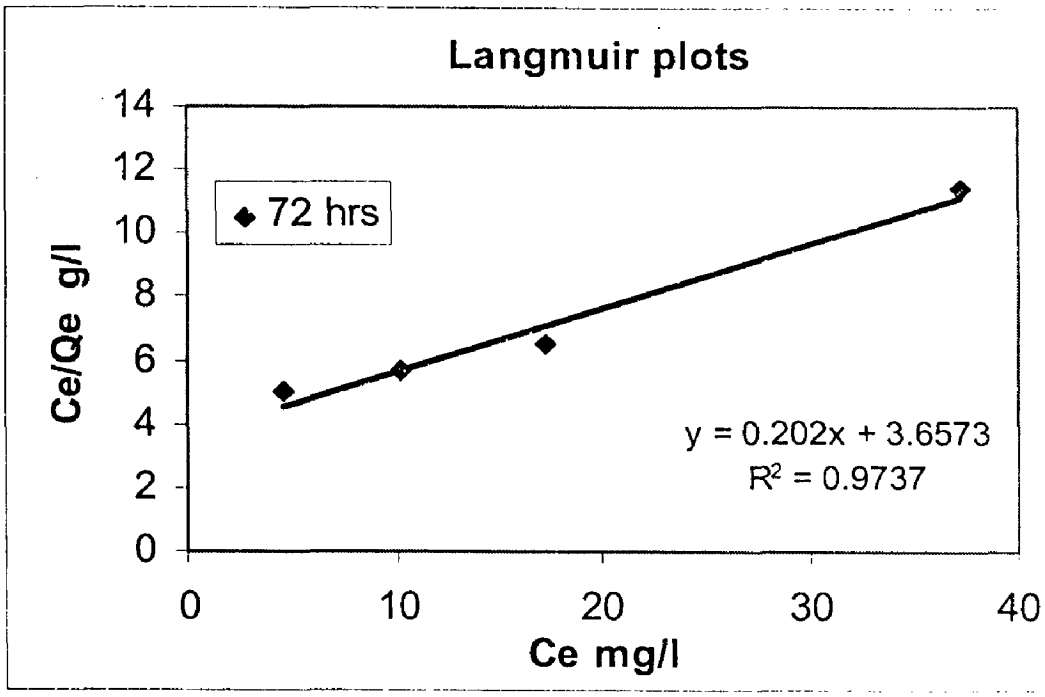


Fig. 5.63 Langmuir Plots at 72 hrs time interval, for RB5 and 10% RH

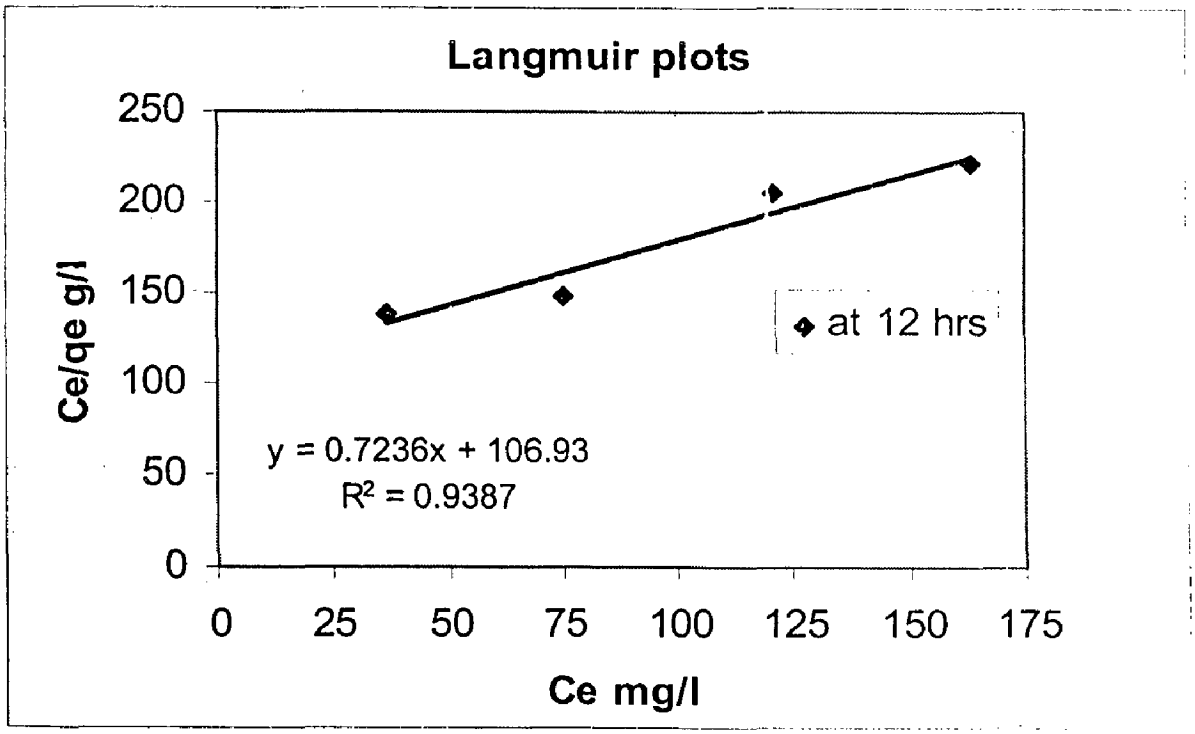


Fig. 5.64 Langmuir Plots at 12 hrs time interval, for RBBR and 10% RH

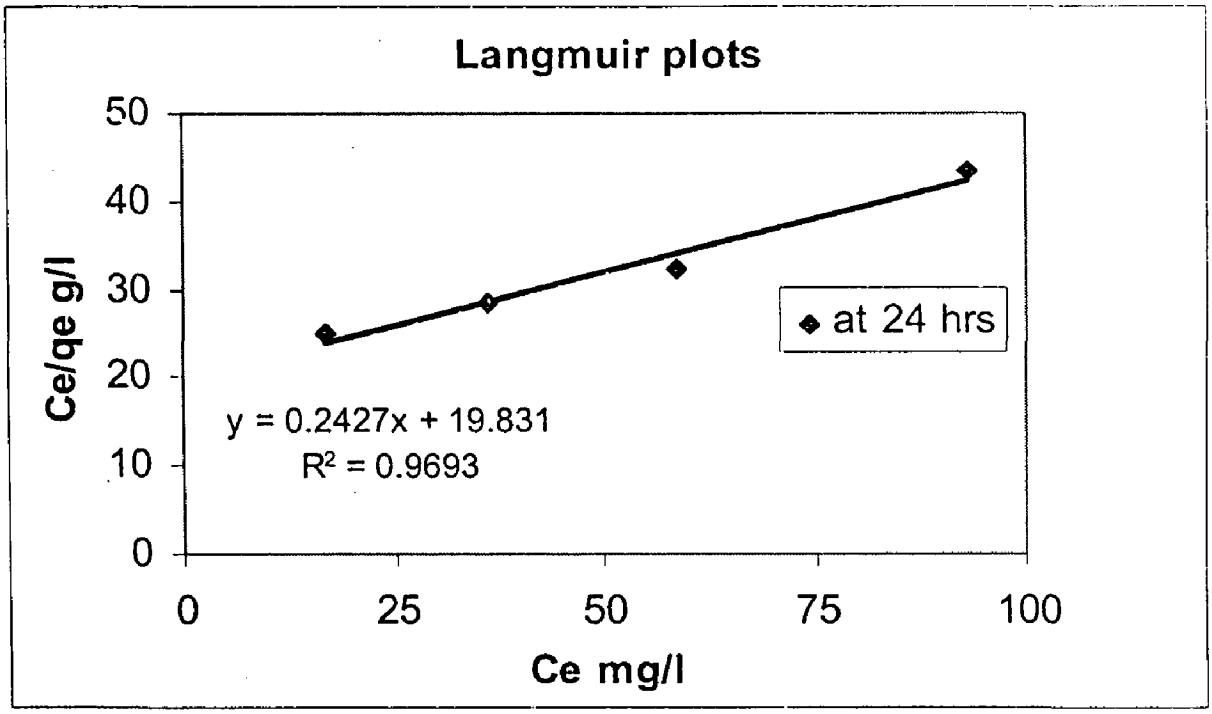


Fig. 5.65 Langmuir Plots at 24 hrs time interval, for RBBR and 10% RH

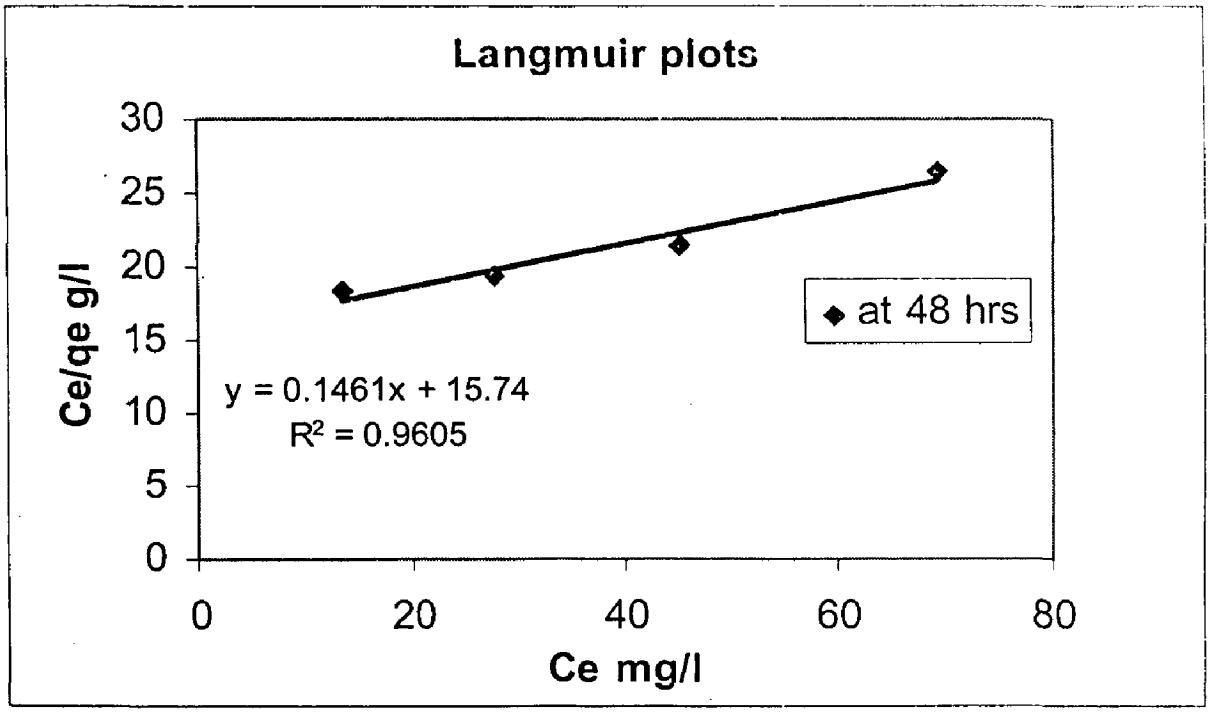


Fig. 5.66 Langmuir Plots at 48 hrs time interval, for RBBR and 10% RH

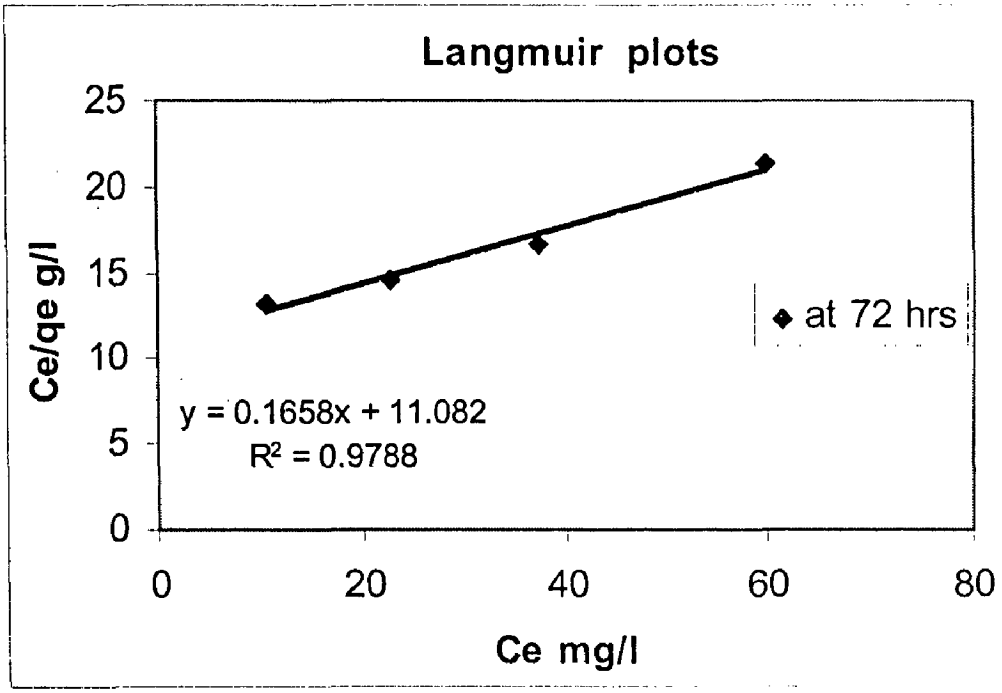


Fig. 5.67 Langmuir Plots at 72 hrs time interval, for RBBR and 10% RH

Freundlich Isotherm at different time intervals between **log qe** v/s log Ce and for various adsorbent doses

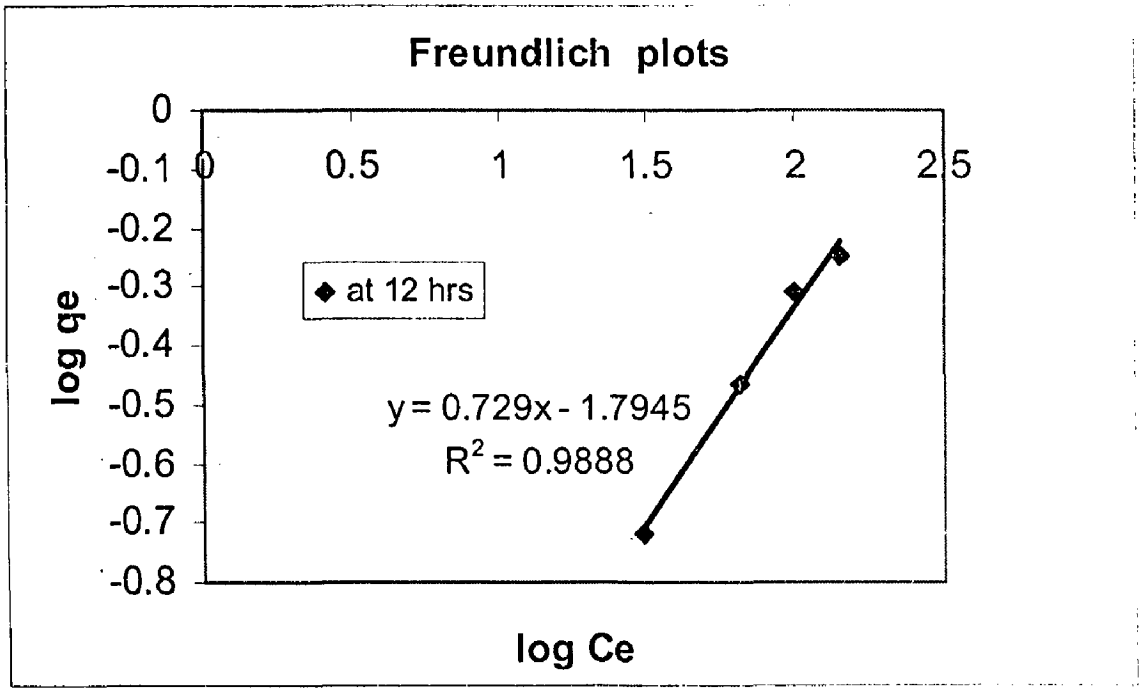


Fig. 5.68 Freundlich Plots at 12 hrs time interval, for RO 16 and 5% RH

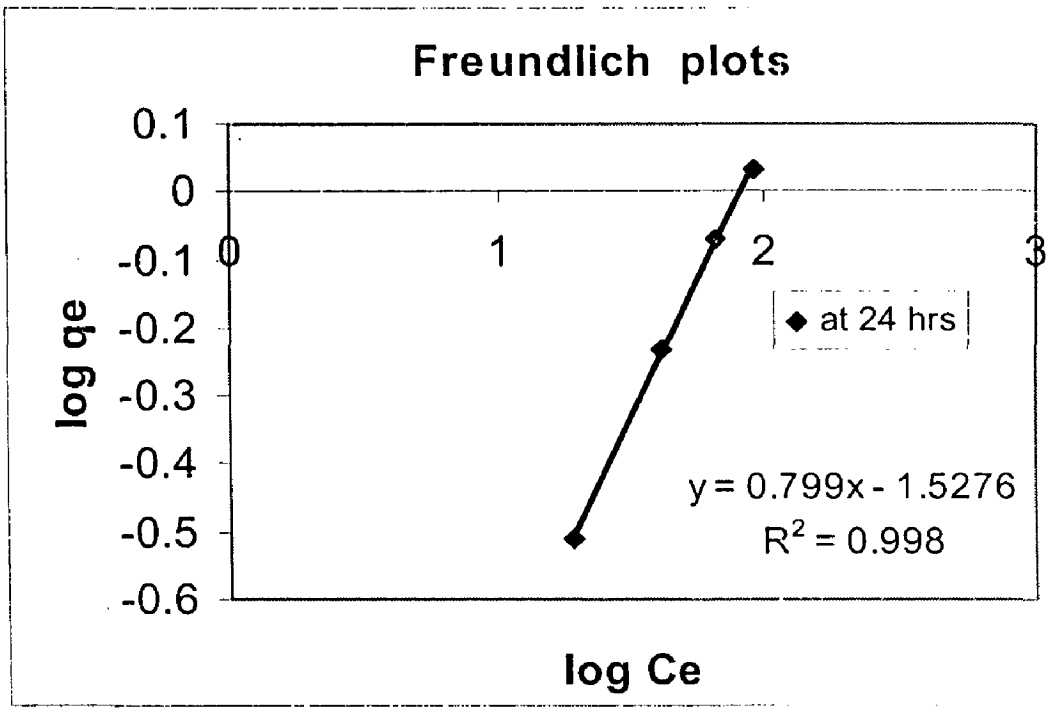


Fig. 5.69 Freundlich Plots at 24 hrs time interval, for RO 16 and 5% RH

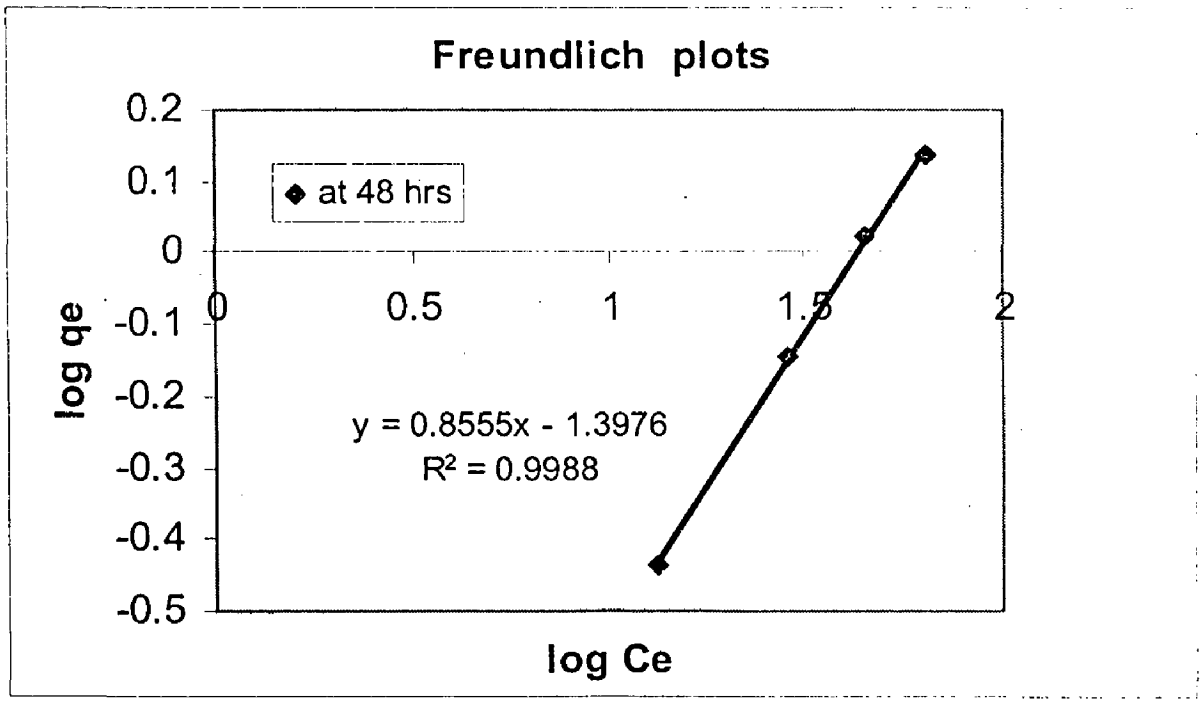


Fig. 5.70 Freundlich Plots at 48 hrs time interval, for RO 16 and 5% RH

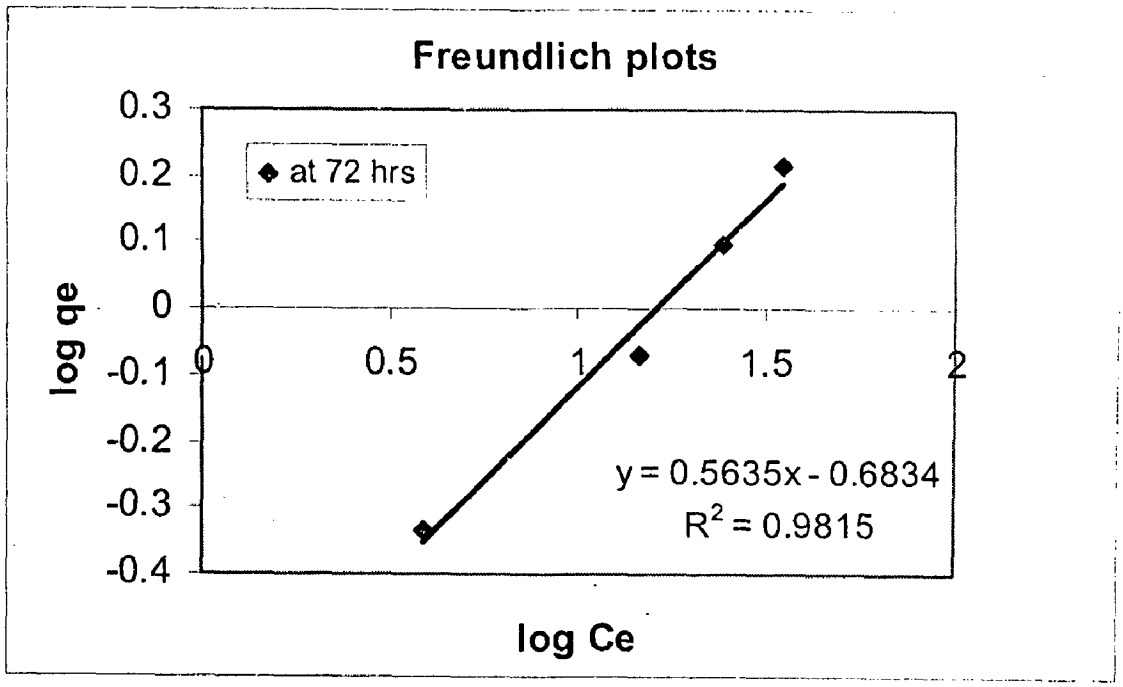


Fig. 5.71 Freundlich Plots at 72 hrs time interval, for RO 16 and 5% RH

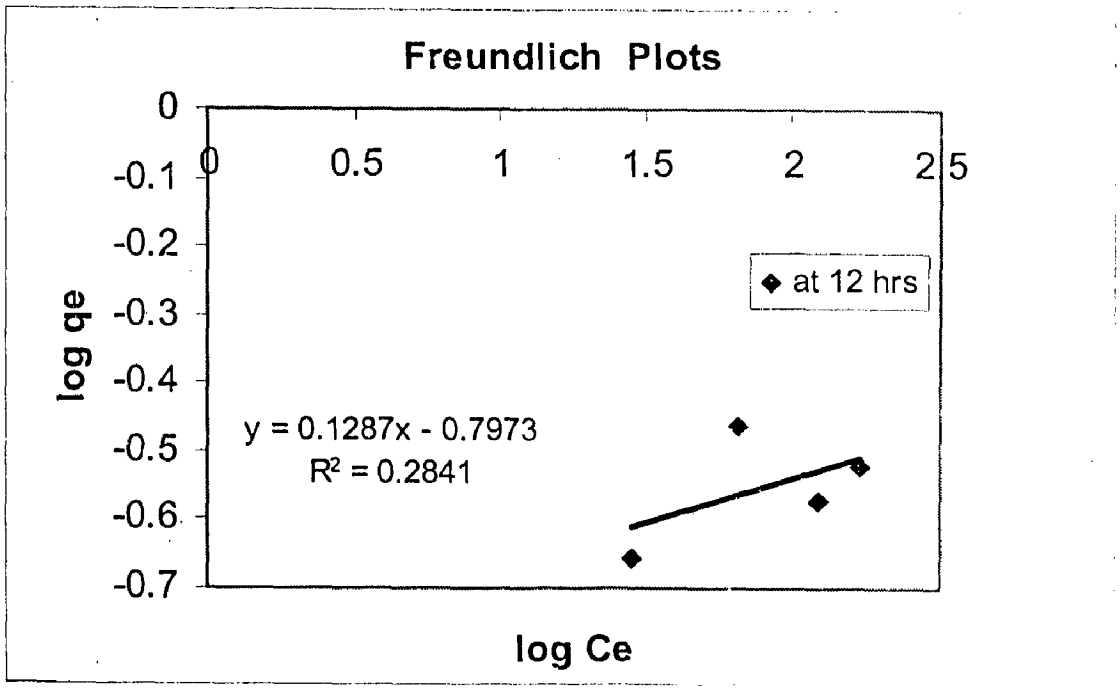


Fig. 5.72 Freundlich Plots at 12 hrs time interval, for RB5 and 5% RH

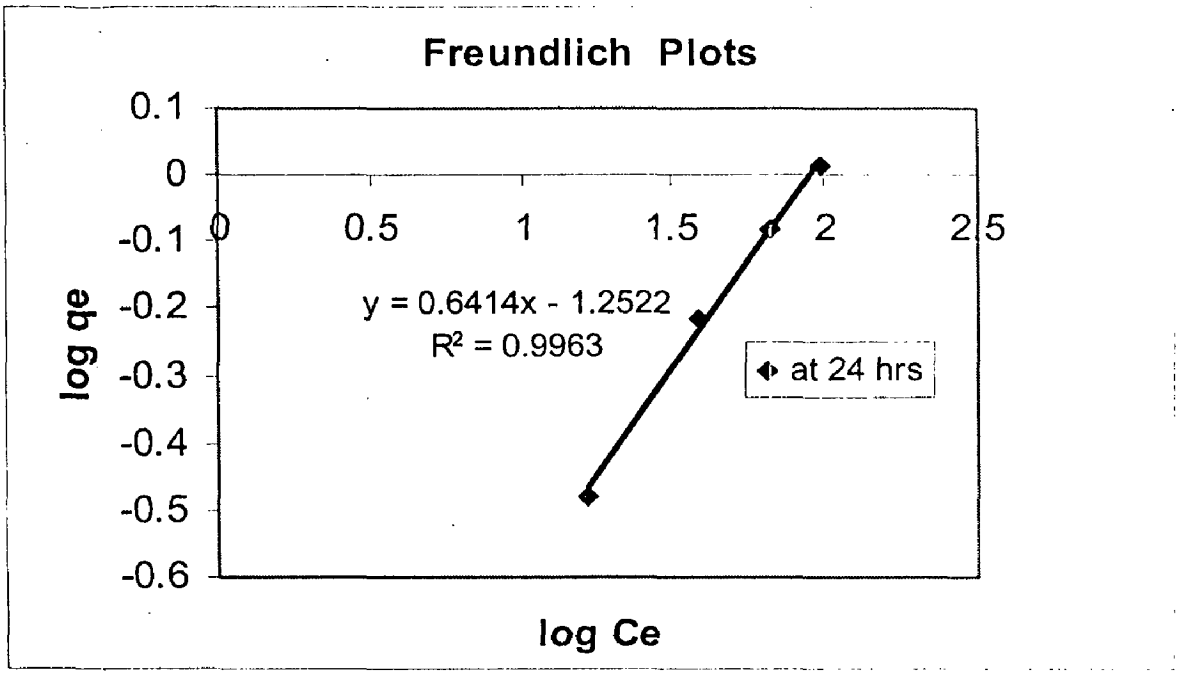


Fig. 5.73 Freundlich Plots at 24 hrs time interval, for RB5 and 5% RH

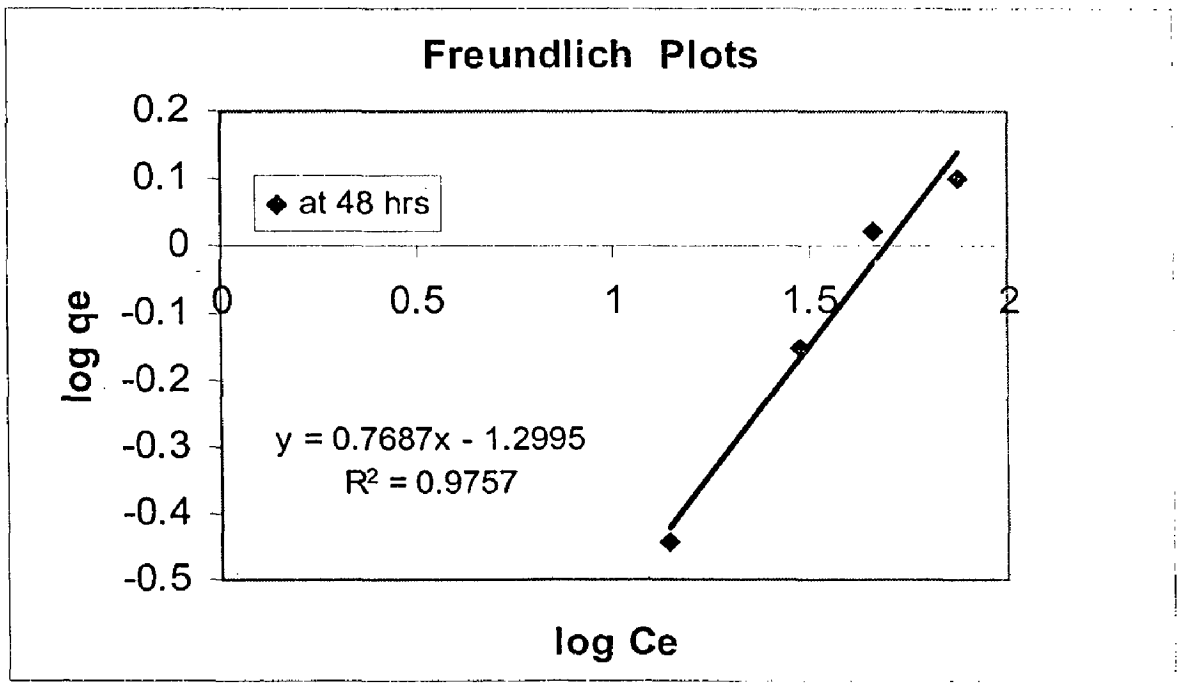


Fig. 5.74 Freundlich Plots at 48 hrs time interval, for RB5 and 5% RH

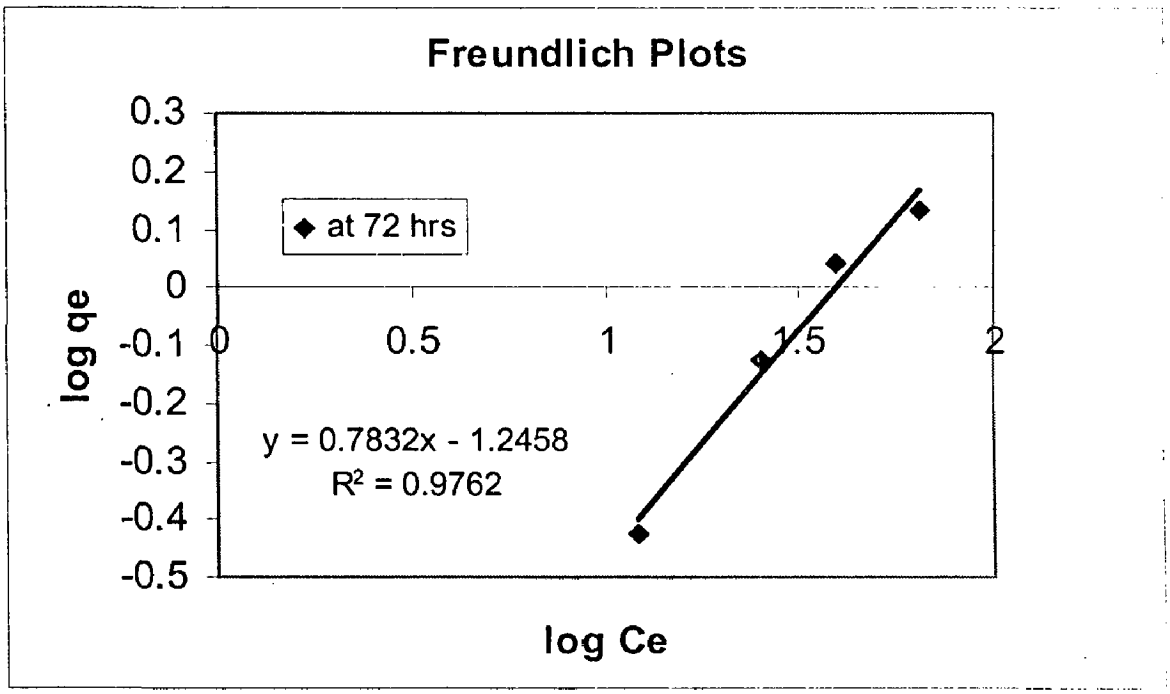


Fig. 5.75 Freundlich Plots at 72 hrs time interval, for RB5 and 5% RH

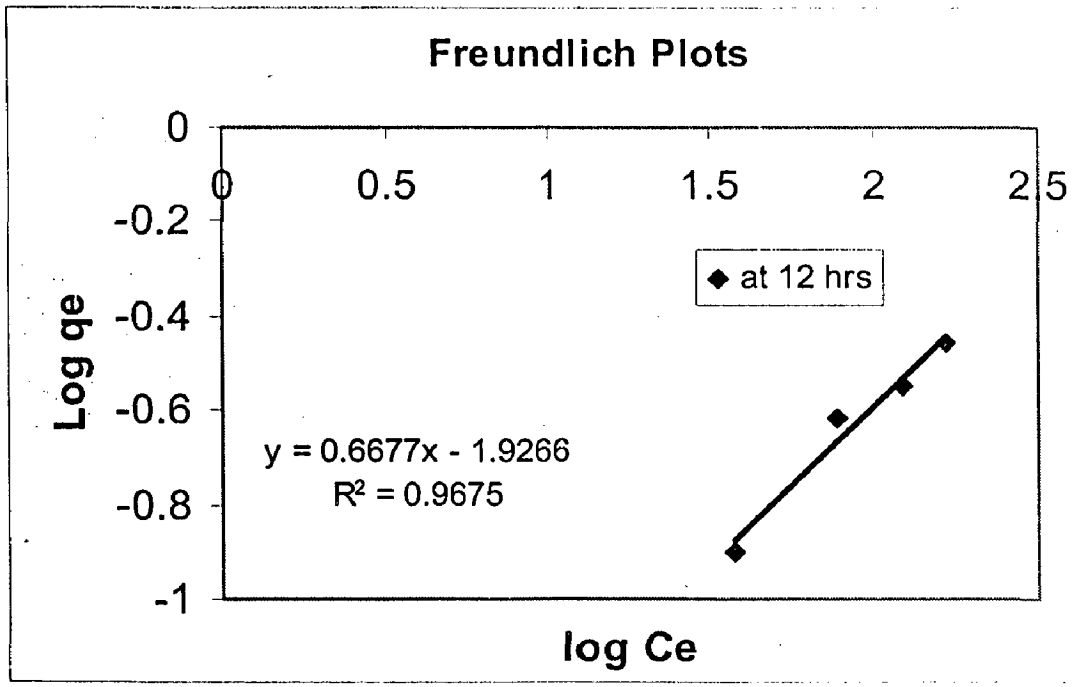


Fig. 5.76 Freundlich Plots at 12 hrs time interval, for RBBR and 5% RH

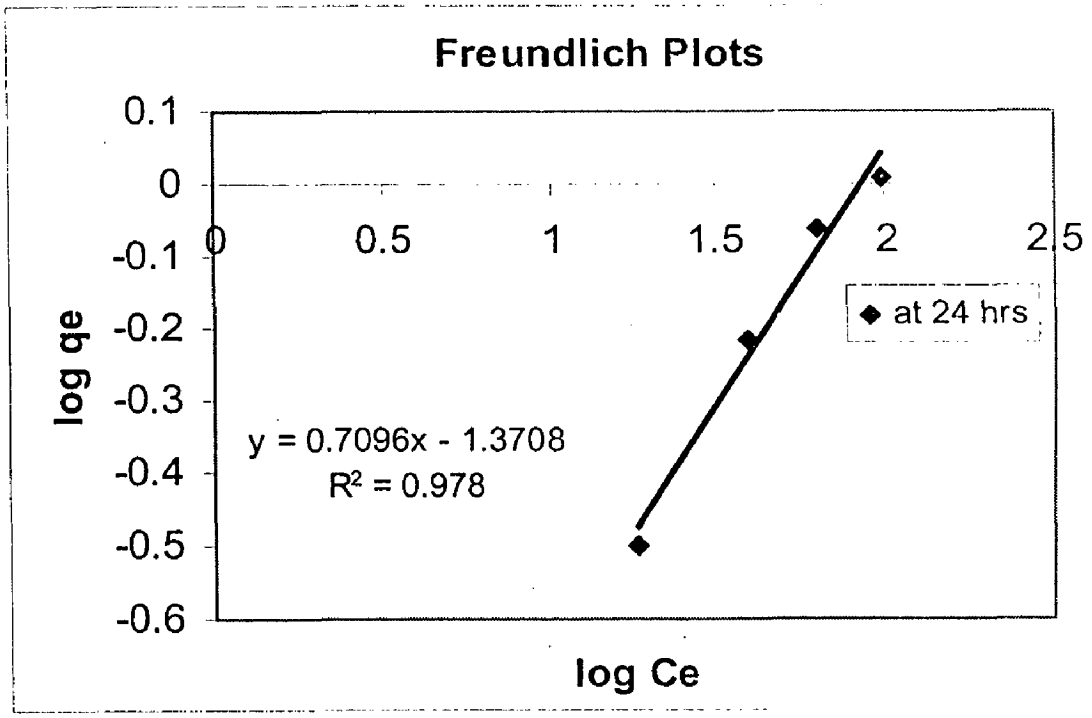


Fig. 5.77 Freundlich Plots at 24 hrs time interval, for RBBR and 5% RH

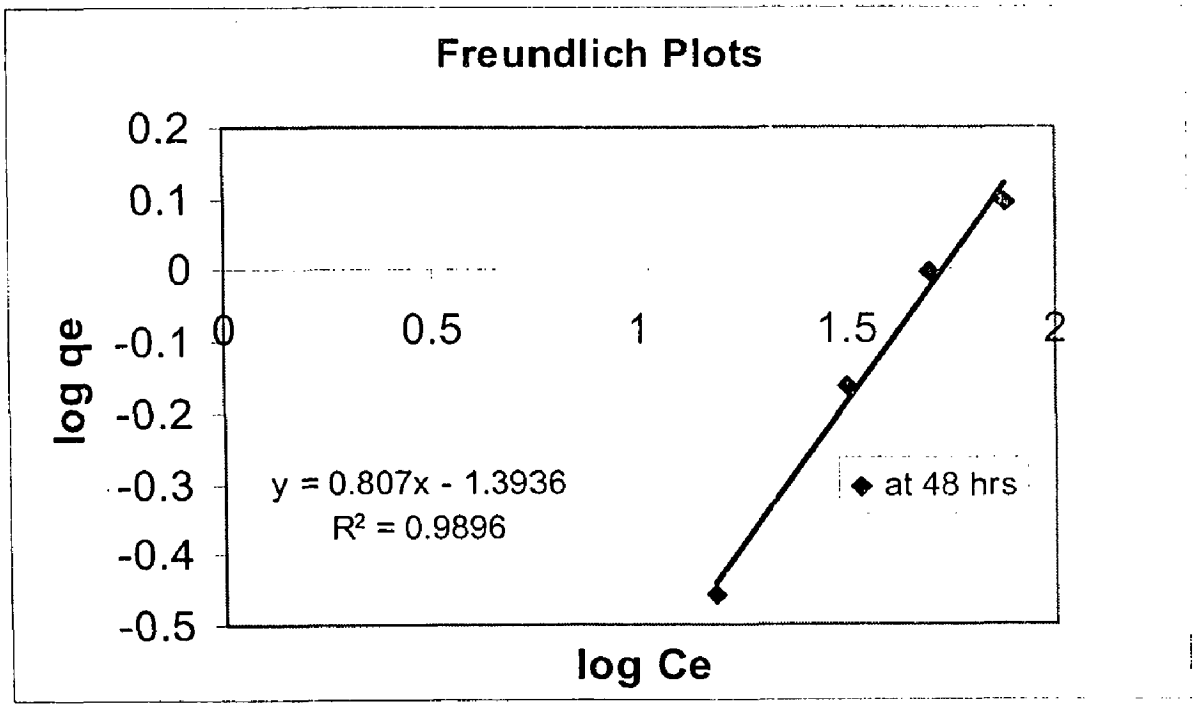


Fig. 5.78 Freundlich Plots at 48 hrs time interval, for RBBR and 5% RH

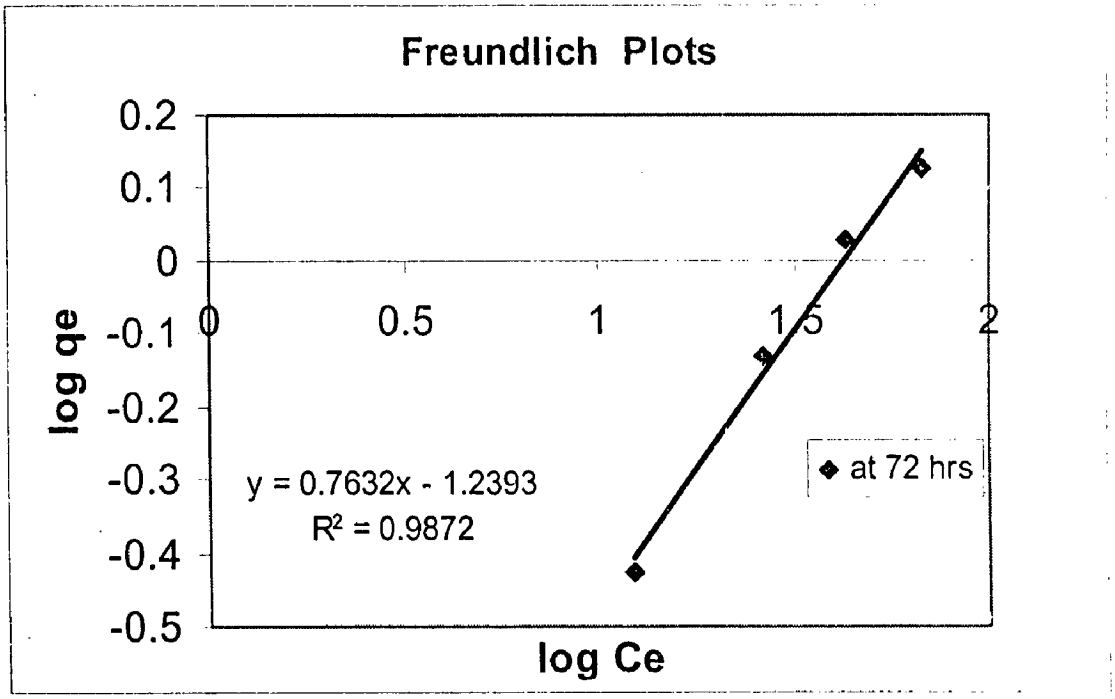


Fig. 5.79 Freundlich Plots at 72 hrs time interval, for RBBR and 5% RH

Table 5.3 Freundlich parameters for Rice Husk for Physical Adsorption

Dye	Time (hrs)	Physical adsorption			
		5 % static cond.		10 % static cond.	
		Kf	1/n	Kf	1/n
RO 16	12	0.0161	0.729	0.0255	0.8204
	24	0.0297	0.799	0.1563	0.7747
	48	0.0400	0.8555	0.1862	0.8917
	72	0.2073	0.5635	0.8604	0.636
RB 5	12	0.1595	0.1287	0.4053	0.118
	24	0.0559	0.6414	0.2271	0.5548
	48	0.0502	0.7687	0.2750	0.645
	72	0.0568	0.7832	0.3958	0.6168
RBBR	12	0.0118	0.6677	0.0255	0.6645
	24	0.0426	0.7096	0.0999	0.694
	48	0.0404	0.807	0.0983	0.7896
	72	0.0576	0.7632	0.1467	0.7371

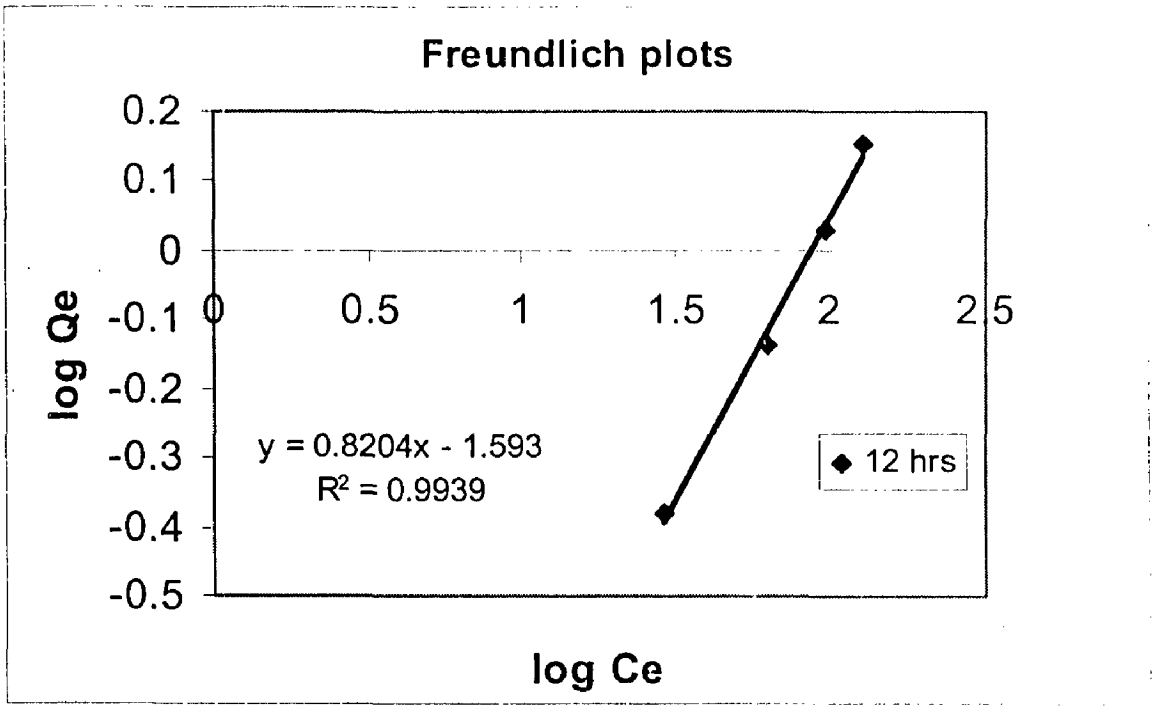


Fig. 5.80 Freundlich Plots at 12 hrs time interval, for RO 16 and 10% RH

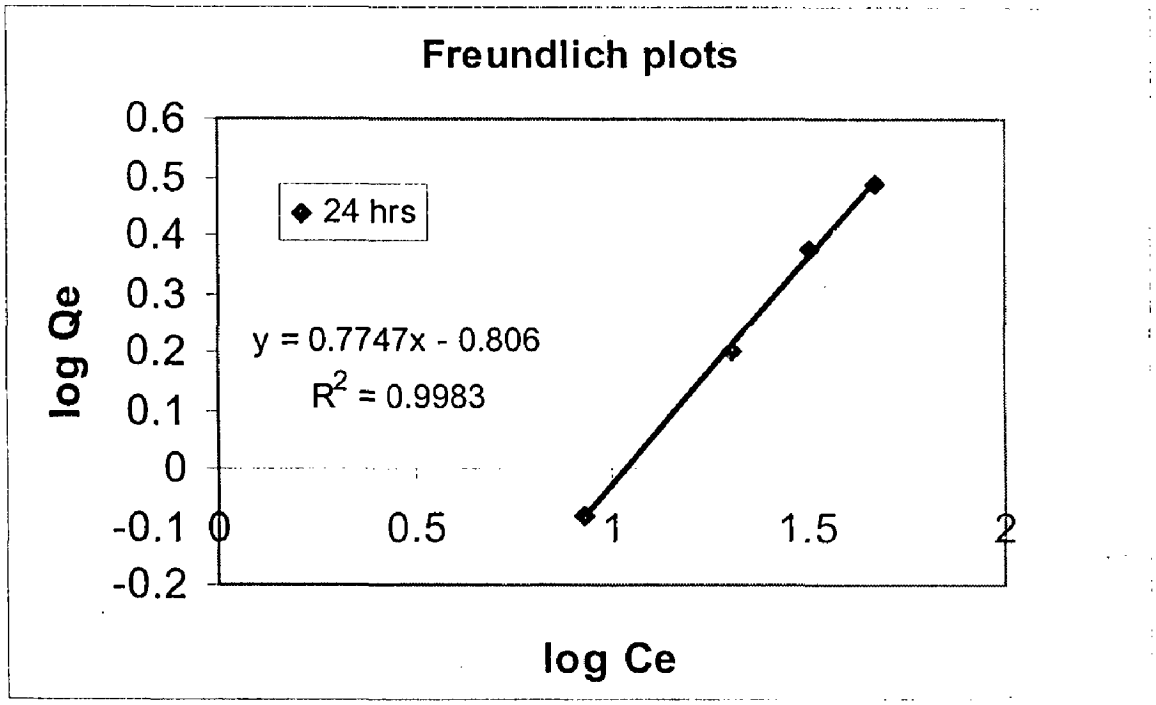


Fig. 5.81 Freundlich Plots at 24 hrs time interval, for RO 16 and 10% RH

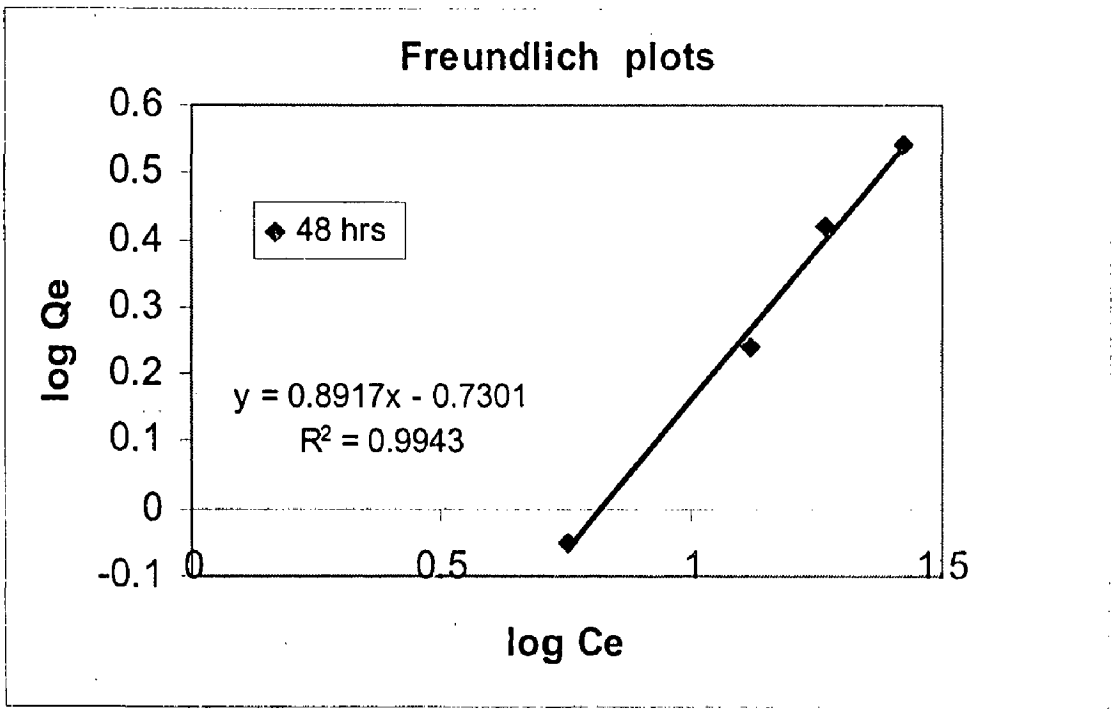


Fig. 5.82 Freundlich Plots at 48 hrs time interval, for RO 16 and 10% RH

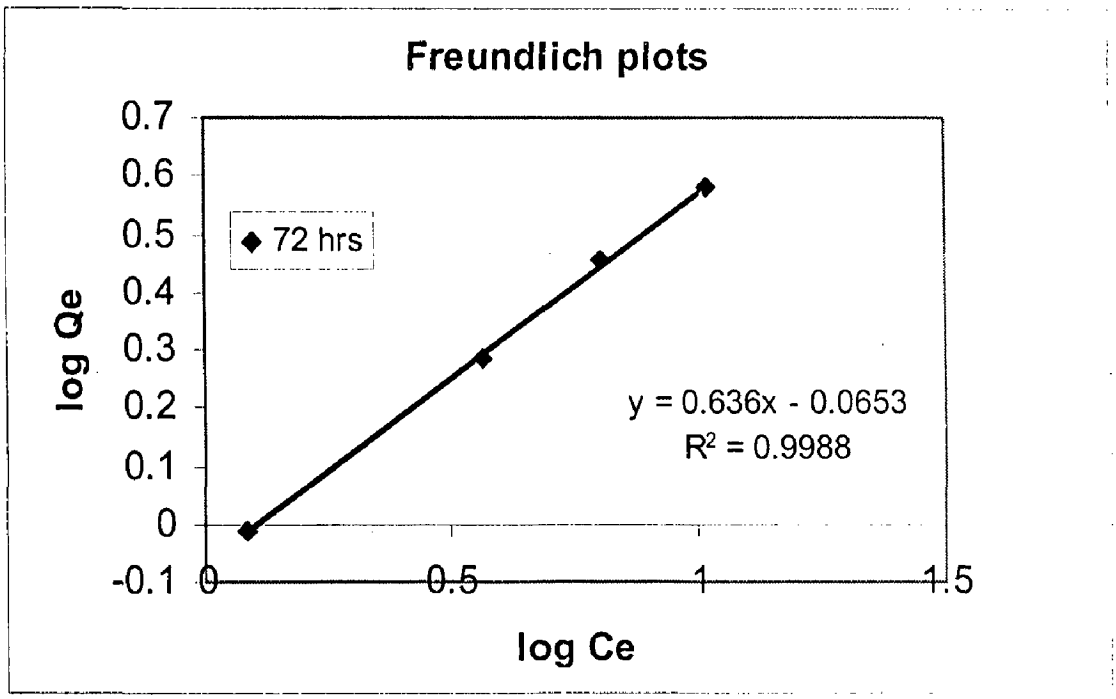


Fig. 5.83 Freundlich Plots at 72 hrs time interval, for RO 16 and 10% RH

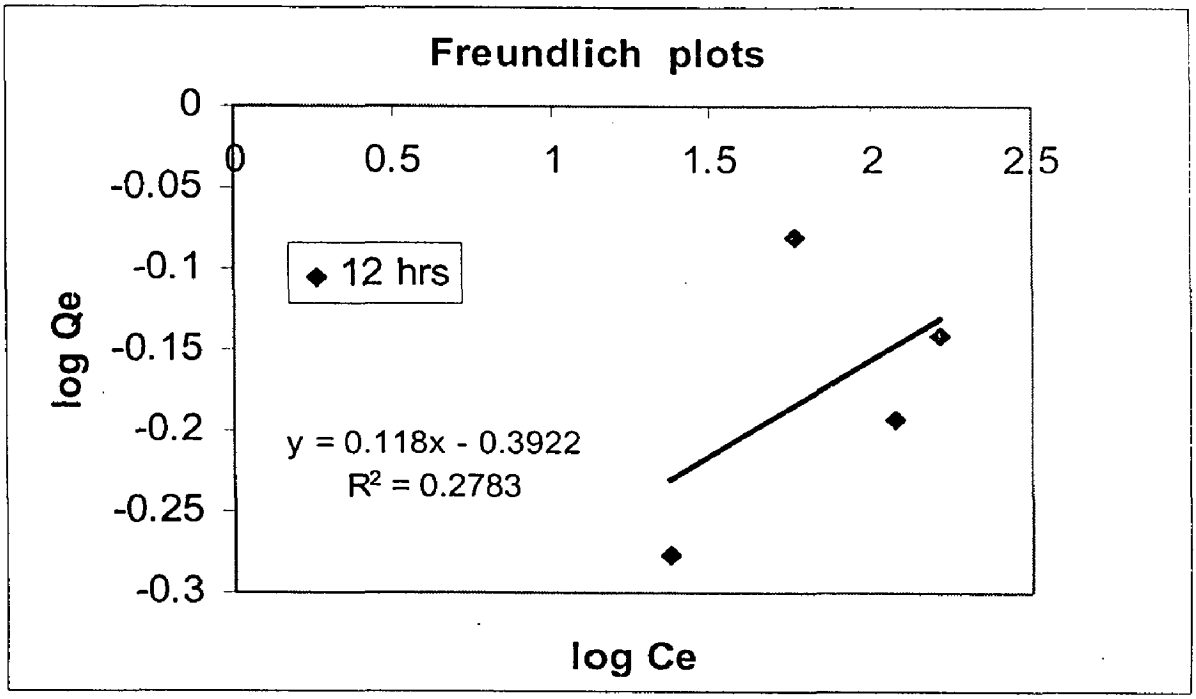


Fig. 5.84 Freundlich Plots at 12 hrs time interval, for RB5 and 10% RH

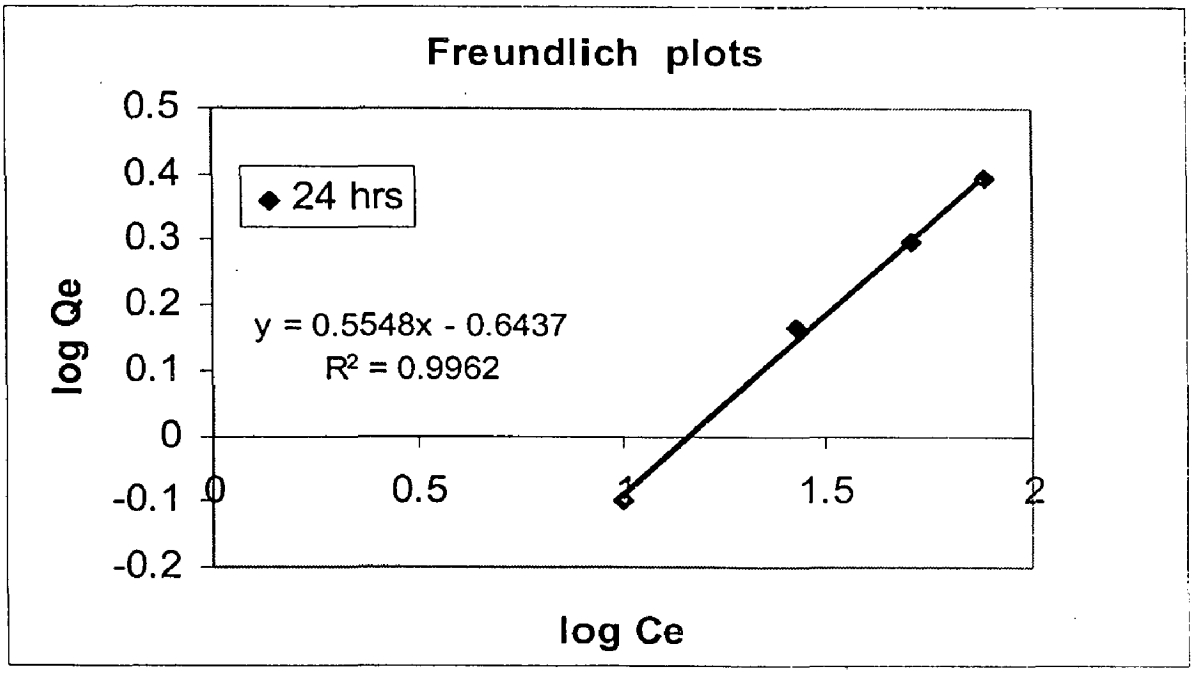


Fig. 5.85 Freundlich Plots at 24 hrs time interval, for RB5 and 10% RH

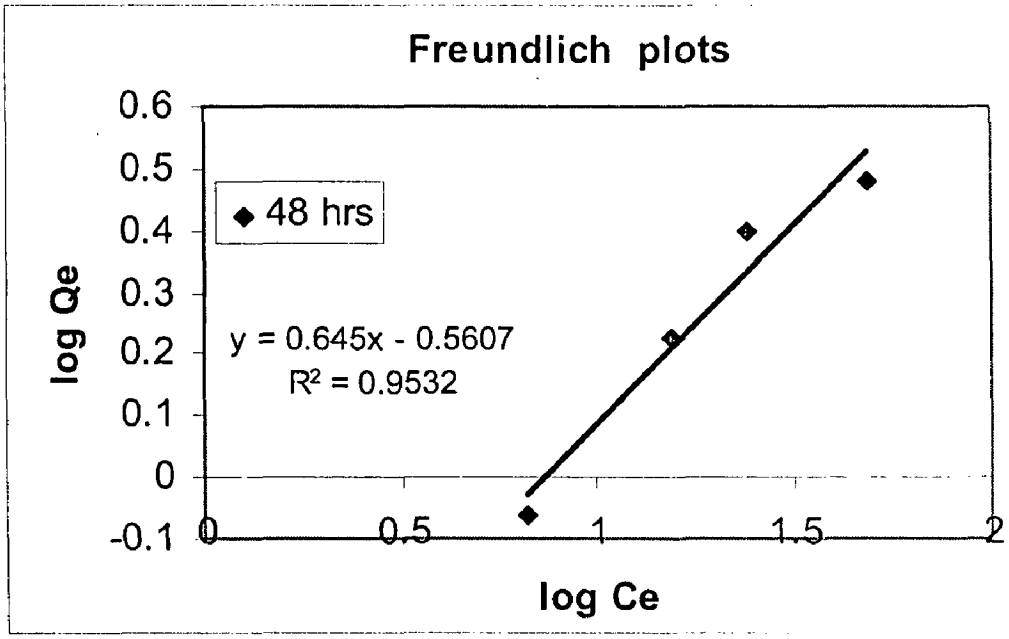


Fig. 5.86 Freundlich Plots at 48 hrs time interval, for RB5 and 10% RH

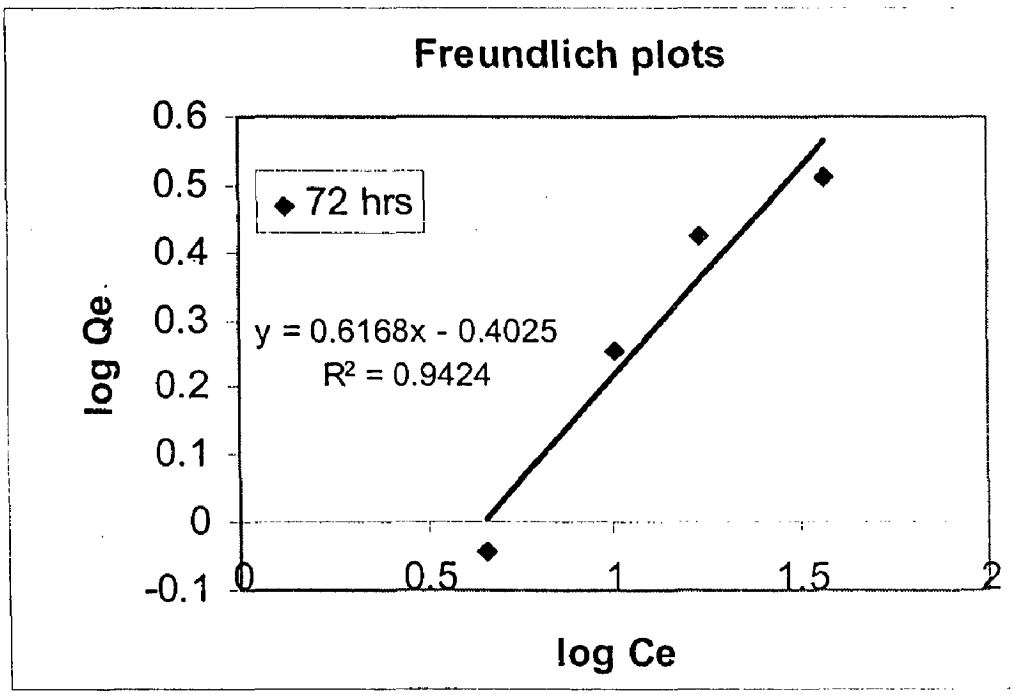


Fig. 5.87 Freundlich Plots at 72 hrs time interval, for RB5 and 10% RH

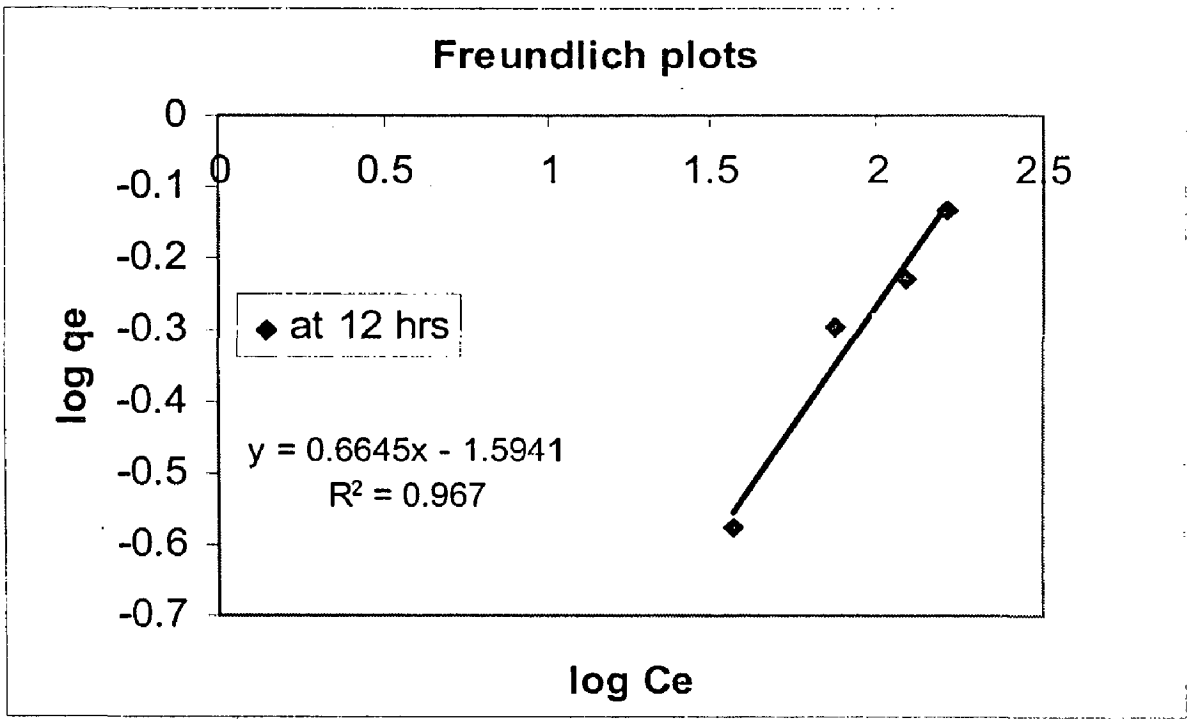


Fig. 5.88 Freundlich Plots at 12 hrs time interval, for RBBR and 10% RH

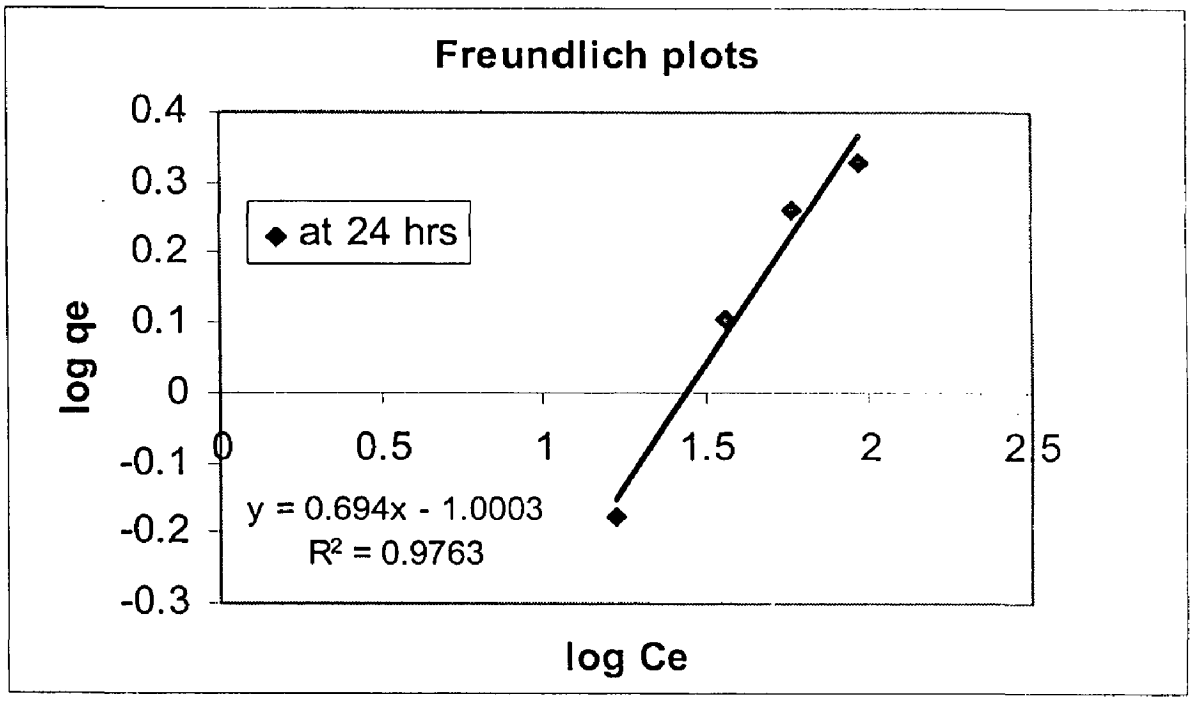


Fig. 5.89 Freundlich Plots at 24 hrs time interval, for RBBR and 10% RH

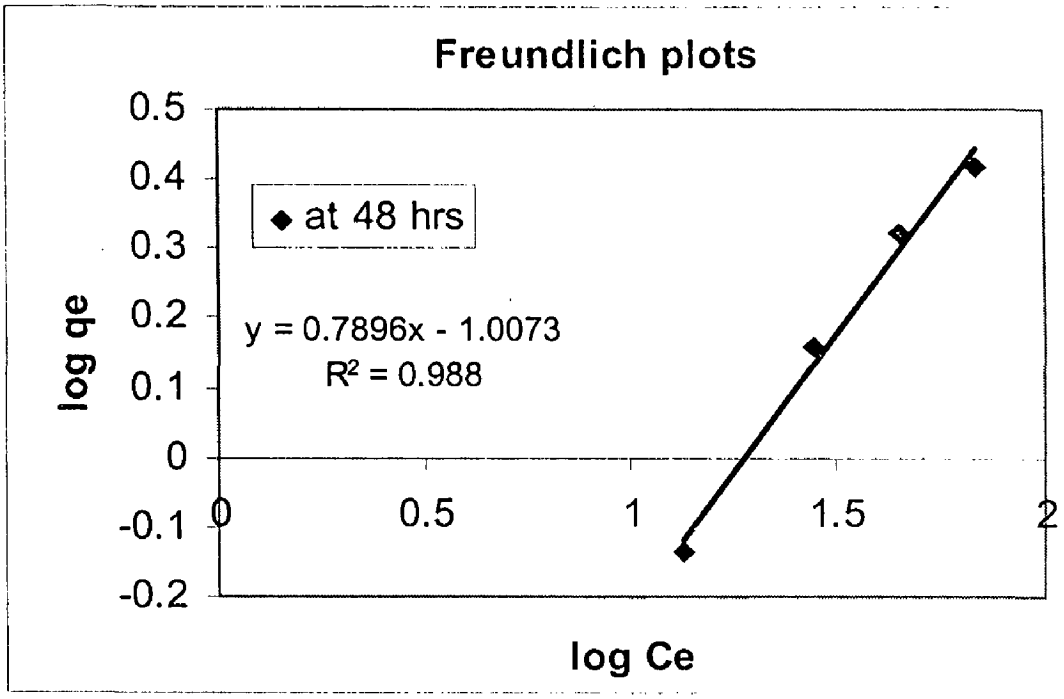


Fig. 5.90 Freundlich Plots at 48 hrs time interval, for RBBR and 10% RH

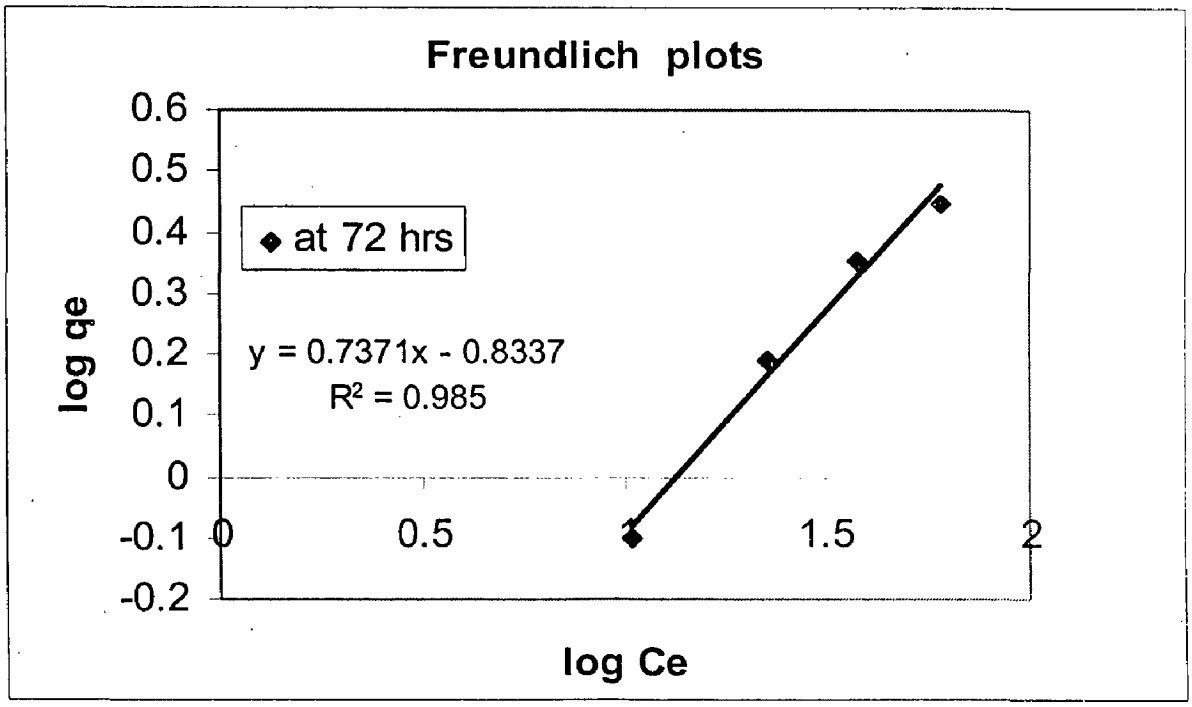


Fig. 5.91 Freundlich Plots at 72 hrs time interval, for RBBR and 10% RH

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

1.1. Conclusions

In the present study, the batch study has been carried out. Results from the chapter 4 show that for Reactive Orange 16, Reactive Black 5 and Remazol Brilliant Blue R Simultaneous Adsorption and Biodegradation (SAB) is a better treatment technique when compared to adsorption or biodegradation alone.

The following conclusions were drawn from the present study

- The present study shows that the Rice Husk, an agro based waste biomaterial can be used as an adsorbent for the removal of various dyes.
- Batch studies were conducted to observe the effect of adsorbent concentration and to find the optimum amount of adsorbent dose for the removal of Reactive Orange 16, Reactive Black 5 and Remazol Brilliant Blue R. Graph shows that 10 % (3 gm/100 ml) of rice husk is the optimum amount of adsorbent dose for the removal.
- Batch studies were conducted to study the kinetics of the removal of Reactive Orange 16, Reactive Black 5 and Remazol Brilliant Blue R for various adsorbent doses. It is shown that the variation in percentage removals between adsorption and Simultaneous Adsorption and Biodegradation (SAB) is very much higher in the case of 1.5 mg/100 ml. This variation decreases as adsorbent dose increases.
- Batch studies were conduced to study the kinetics of the removal of Reactive Orange 16, Reactive Black 5 and Remazol Brilliant Blue R for various pH. It is shown that for Simultaneous adsorption and Biodegradation optimum pH lies around 4.5. For SAB the rate of removal is highest at pH 4.5.
- Batch studies were conducted to study the kinetics of the removal of Reactive Orange 16, Reactive Black 5 and Remazol Brilliant Blue R for various initial concentrations (50 mg/l, 100 mg/l, 1500 mg/l, and 200 mg/l) respectively. It is found that the rate of removal of dyes incase of 50 mg/l is greater than that of remaining concentrations. It is due to the synergetic combination of adsorption and biodegradation.

- Biokinetic parameters have been found out and are compared for adsorption and Simultaneous Adsorption and Biodegradation (SAB)
- The biodegradation of Reactive Orange 16, Reactive Black 5 and Remazol Brilliant Blue R by using white rot fungi has been studied. Bio-kinetic parameters have been found out.
- Freundlich parameters have been determined for fresh rice husk adsorption, Simultaneous Adsorption and Biodegradation (SAB).
- Percentage removal of various dyes found as RO16 (94.5039 %), RB5 (92.2644%), RBBR (93.2691 %)

1.2. Recommendations

- Studies can be done to determine the effect of temperature on Simultaneous Adsorption and Biodegradation (SAB).
- Aerobic/anaerobic sequential process can be used for the removal of various dyes.
- Solid state fermentation can also be used for study the removal of various dyes.

- Freundlich parameters have been determined for fresh rice husk adsorption.
- Percentage removal of various dyes found as RO16 (94.5039 %), RB5 (92.2644%), RBBR (93.2691 %)

1.2. Recommendations

- Studies can be done to determine the effect of temperature on Simultaneous Adsorption and Biodegradation (SAB).
- Aerobic/anaerobic sequential process can be used for the removal of various dyes.
- Solid state fermentation can also be used for study the removal of various dyes.

REFERENCES

[1] **Annette Heinling, Francisco Javier Ruiz-Duenla, Mar|ea J. Martenez, Matthias Bergbauer, Ulrich Szewzyk, Angel T. Martlenez** : A study on reducing substrates of manganese-oxidizing peroxidases from Pleurotus eryngii and Bjerkandera adusta , FEBS Letters 428 (1998) 141-146

[2] **A. Mohey El-Dein , J.A. Libra, U. Wiesmann** : Mechanism and kinetic model for the decolorization of the azo dye Reactive Black 5 by hydrogen peroxide and UV radiation, Chemosphere 52 (2003) 1069–1077

[3] **Austen, George T., R. N. Shreve, and Joseph A. Brink.** Shreve's Chemical Process Industries. New York: McGraw-Hill. 1984.

[4] **Andrea Zille**:Immobilized laccase for Decolorization of reactive black-5 dyeing effluent,2003

[5] **A. Mohey El-Dein , J.A. Libra, U. Wiesmann**: Mechanism and kinetic model for the Decolorization of azo dye reactive black-5 by H_2O_2 & UV radiation, Chemosphere 52 (2003) 1069–1077

[6] **Akin DE, Rigsby LL, Sethuraman A, et al. (1995)**. Alterations in the structure, chemistry, and biodegradation of grass lignocellulose treated with white rot fungi *Ceriporiopsis subvermispora* and*Cyathus stercoreus*. Appl. Environ. Microbiol. 61:1591-1598.

[7] **Amann RI, Ludwig W, Schleifer K-H (1995)**. Phylogenetic identification and *in situ* detection of individual microbial cells with-out cultivation.Microbiol. Rev. 143-169.

[8] **Arora DS, Chander M, Gill PK (2002)**. Involvement of lignin peroxidase, manganese peroxidase and laccase in the degradation and selective ligninolysis of wheat straw. Int. Bioterior. Biodegrad. 50:115-120.

[9] **Aylward JH, Gobius KS, Xue G-P, et al. (1999)**. The *Neocallimastrix patriciarum* cellulase, CelD, contains three almost identical catalytic domains with high specific activity on Avicel. Enzyme Microb. Technol. 24:609-614.

[10] **Baldrian T, Gabriel J (2003)**. Lignocellulose degradation by *Pleurotus ostreatus* in the presence of cadmium. FEMS Microbiol. Lett. 220:235-240.

[11] **Bao W, Renganathan V (1991)**. Triiodide reduction by cellobiose:quinine xidore-ductase of *Phanerochaete chrysosporium*.FEBS 279(1):30-32.

[12] **Beldman G, Voragen AGJ, Rombouts FM, et al. (1987)**. Adsorption and kinetic behaviour of purified endoglucanases and exoglucanases from *Trichoderma viride*. Biotechnol. Bioeng. 30:251-257.

[13] **Beauchemin KA, Colombatto D, Morgavi DP, Yang WZ (2003)**. Use of exogenous fibrolytic enzymes to improve animal feed utilisation by ruminants. J. Anim. Sci. 81(E. Suppl.2): E37-E47.

[14] **Beauchemin KA, Morgavi DP, Mcallister TA, Yang WZ, Rode LM (2001)**. The use of enzymes in ruminant diets. In Wiseman J,Garnsworthy PC (eds) Recent Advances in Animal Nutrition.Nottingham University Press, pp 296-322.

[15] **Beauchemin KA, LM Rode, VJH Sewalt. 1995.** Fibrolytic enzymes increase fibre digestibility and growth rate of steers fed dry forages.Can. J. Anim. Sci. 75:641-644.

[16] **Beg QK, Kapoor M, Mahajan L, Hoondal GS (2001).** Microbial xylanases and their industrial applications: A review. Appl.Microbiol. Biotechnol. 56:326-338.

[17] **Béguin P (1990).** Molecular biology of cellulose degradation. Annu. Rev. Microbiol. 44:219-248.

[18] **Betts WB, Dart RK, Ball AS, Pedlar SL (1991).** Biosynthesis and Structure of lignocellulose. In Betts (eds) Biodegradation: Natural and Synthetic Materials. Springer-Verlag, Berlin, Germany, pp. 139-155.

[19] **Bhat MK (2000).** Research review paper: Cellulases and related enzymes in biotechnology. Biotechnol. Adv. 18:355-383.

[20] **Borris R, Hofmeister J, Thomsen KK, Olsen O, Von Wettstein D. Patent** Abstracts 5470725 pp 81.

[21] **Bosco F, Ruggeri B, Sassi G (1999).** Performances of a trickle bed reactor (TBR) for exoenzyme production by *Phanerochaete chrysosporium*: influence of a superficial liquid velocity. Chem. Eng. Sci. 54:3163-3169.

[22] **Bourbonnais R, Paice MG (1988).** Veratryl alcohol oxidases from the lignin-degrading basidiomycete *Pleurotus sajor-caju*. Biochem. J.255:445-450.

[23] **Broda P, Sims PFG, Mason JC (1989).** Lignin biodegradation: a molecular biological approach. Essays Biochemistry 24:82-114.

[24] **Brun E, Johnson PE, Creagh AL, et al. (2000).** Structure and binding specificity of the second N-terminal cellulose-binding domain from *Cellulomononas fimi* endoglucanase C. Biochemistry 39:2445-258.

[25] **Carliell CM, Barclay SJ, Naidoo N, Buckley CA, Mulholland DA, Senior E.** Microbial decolorization of a reactive dye under anaerobic conditions. Water SA 1995;21(1):61–9.

[26] **Cohen, H.**: The use of ultrafiltration membranes in treatment of textile dye-house waste. 2nd Nat. Conf. on Complete Water Reuse. Chicago, p. 992-995. Amer. Inst. Chem. Eng. and US Envronmental Protection Agency, Washington, D.C. (1996)

[27] **C.Cripps,John and Steven**:Biodegradation of azo and heterocyclic dyes by phanerochaete chrysoporium, Applied and Environment Microbiology,Vol 56, 1989

[28] **Call HP, Mücke I (1997).** History, overview and applications of mediated lignolytic systems, especially laccase-mediator-systems (Lignozyme®-process). J. Biotechnol. 53:163-202.

[29] **Campbell CJ, Laherrere JH (1998).** The end of cheap oil. Sci. Am. 3:78-83.
Carrard G, Linder M (1999). Widely different off rates of two closely related cellulose-binding domains from *Trichoderma reesei*. Eur. J. Biochem. 262:637-643.

[30] **Chahal DS (1992).** Bioconversions of poysaccharides of lignocellulose and simultaneous degradation of lignin. In Kennedy et al. (eds) Lignocellulosics: Science, Technology, Development and Use. Ellis Horwood Limited, England, pp. 83-93.

[31] **Chahal PS, Chahal DS, Le GBB (1996).** Production of cellulose in solid – state fermentation with *Trichoderma reesei* MCG 80 on wheat straw. Appl. Biochem. Biotechnol. 57/58:433-442.

[32] **Christopherson C, Anderson E, Jokobsen TS, Wagner P (1997).** Xylanases in wheat separation. Starch 49:5-12.

[33] **D.T. Sponza., M. I sik** :Decolorization & azo dye degradation by anaerobic/aerobic sequential process, Enzyme and Microbial Technology 31 (2002) 102–110

[34] **Dourado F, Bastos M, Mota M, Gama FM (2002).** Studies on the properties of Celluclast/Eudragrit L-100 conjugate. J. Biotechnol. 99:121-131.

[35] **Eliana Pereira Chagas, Lucia Regina Durrant**: Decolorization of azo dyes by *Phanerochaete chrysosporium* and *Pleurotus sajorcaju,* Enzyme and Microbial Technology 29 (2001) 473–477

[36] **E. Puzenat, H. Lachheb, M. Karkmaz, A. Houas, C. Guillard and J. M. Herrmann:**

Fate of nitrogen atoms in the photocatalytic degradation of industrial(congo red) & alimentary(amaranth) azo dyes, International journal of photoenergy Vol. 05 2003

[37] **Esterbauer H, Steiner W, Labudova I, et al. (1991).** Production of *Trichoderma* cellulase in laboratory and pilot scale. Biores. Technol. 36:51-65.

[38] **Fu-ming Zhang, Jeremy S. Knapp, and Kelvin N. Tapley**:Development of bioreactor systems for Decolorization of orangeII by white rot fungus, Department of Microbiology and Department of Color Chemistry, University of Leeds , United Kingdom

[39] **Fairweather JK, Faijes M, Driguez H, Planas A (2002).** Specific studies of *Bacillus* 1,3-1-4- beta-glucanase and application to glycosynthase-catalyzed transglycosylation. Chembiochem. 3:866-873.

[40] **Falcon MA, Rodríguez A, Carnicero A, et al. (1995).** Isolation of microorganisms with lignin transformation potential from soil of Tenerife Island. Soil Biol. Biochem. 27(2):121-126.

[41] **Galbe M, Zacchi G (1993).** Simulation of processes for conversion of lignocellulosics. In Saddler (eds) Bioconversion of Forest and Agricultural Plant Residues, CAB International, UK, pp. 291-342.

[42] **Gengenbach A, Syn S, Wang XT, Lu Y (1999).** Redesigning of a cytochrome c peroxidase into a manganesė peroxidase: role of tryptophans in peroxidase activity. Biochemistry (USA): 38:11425-11432.

[43] **Gold MH, Alic M (1993).** Molecular biology of the lignin-degrading basidiomycetes *Phanerochaete chrysosporium.* Microbiol. Rev. 57(3):605-622.

[44] **Goyal A, Ghosh B, Eveleigh D (1991).** Characterisation of fungal cellulases. Biores. Technol. 36:37-50.

[45] **Grethlein HE, Converse AO (1991).** Common Aspects of acid prehydrolysis and steam explosion for pretreating wood. Biores. Technol. 36:77-82.

[46] **Gupta R, Beg QK, Lorenz P (2002).** Bacterial alkaline proteases: molecular approaches and industrial applications. Appl. Micobiol. Biotechnol. 59:15-32.

[47] **Gingell R, Walker R.**:Mechanisms of azo reduction by Streptococcus faecalis II, the role of soluble flavins. Xenobiotics 1971;1(3):231–9.

[48] **H. Zollinger**:Color Chemistry—Syntheses, Properties and Applications of Organic Dyes and Pigments, VCH Publishers, New York, 1987, pp. 92–102.

[49] **Haug W, Schmidt A, Nortemann B, Hempel DC, Stolz A, Knackmuss HJ.** Mineralization of the sulfonated azo dye Mordant Yellow 3 by a 6-aminonaphthalene 2-sulfonate-degrading bacterial consortium. Appl Environ Microbiol 1991;57(11):3144–9.

[50] **Haltrich D, Nidetzky B, Kulbe KD, et al. (1996).** Production of fungal xylanases. Biores. Technol. 58:137-161.

[51] **Jo-Shu Chang , Chien Chou, Shan-Yu Chen**:Decolorization of azo dyes with immobilized Pseudomonas luteola, Process Biochemistry 36 (2001) 757–763

[52] **Jo-Shu Chang, Chien Chou, Yu-Chih Lin, Ping-Jei Lin, Jin-Yen Ho and Tai Lee Hu:** Kinetic characteristics of bacterial azo-dye decolorization by Pseudomonas luteola, Wat. Res. Vol. 35, No. 12, pp. 2841–2850, 2001

[53] **Jeffrey K. Glenn and Michael H. Gold**: Decolorization of Several Polymeric Dyes by the Lignin-Degrading Basidiomycete Phanerochaete chrysosporium, Applied and Environmental Microbiology, June 1983, p. 1741-1747 Vol. 45, No. 6

[54] **Jo-Shu Chang , Tai-Shin Kuo**:Kinetics of bacterial decolorization of azo dye with Escherichia coli NO3, Bioresource Technology 75 (2000) 107-111

[55] **J. P. Lorimer, T. J. Mason, M. Plattes, S. S. Phull, and D. J.Walton**: Degradation of dye effluent, Pure Appl. Chem., Vol. 73, No. 12, pp. 1957–1968, 2001.

[56] **K.C.A. Bromley-Challenor, J. S. Knapp, Z. Zhang, N. C.C. Gray, M. J. Hetheridge and M. R. Evans:** Decolorization of azo dyes by Unacclimated activated sludge under anaerobic conditions, Wat. Res. Vol. 34, No. 18, pp. 4410±4418, 2000

[57] **Koichi Harazono, Yoshio Yatanabe, and Kazunori Nakamura:**Decolorization of azo dye by the white-rot basidiomycete phanerochaete sordida and by its manganese peroxidase, journal of bioscience and bioengineering vol. 95,No. 5. 455459.2003

[58] **K.Selvam**: Decolorization of azo dyes and a dye industry effluent by a white rot fungus Thelephora sp., Biosourse technology 88(2003) 115-119

[59] **Kuo-Cheng Chen , Jane-Yi Wu, Chang-Cheng Huang, Yu-Min Liang , Sz-Chwun John Hwang:** Decolorization of azo dye using PVA-immobilized microorganisms, Journal of Biotechnology 101 (2003) 241_252

[60] **K.Vasanth and K.Subanandam**:Removal of basic dye from its aqueous sol. By adsorption process using rice husk adsorbent,2003

[61] **Kirk, Raymond E., and Donald F. Othmer.** Kirk-Othmer Encyclopedia of Chemical Technology. 3d ed. New York: John Wiley and Sons. 1980.

[62] **Li Fan., Fenglin Yang, Weishen Yang** :Performance of the Decolorization of azo dye with bipolar packed bed cell, Separation and Purification Technology 34 (2004) 89–96

[63] **M.A.M. Martins**:Comparative studies of fungal degradation of single/mixed bioaccessible reactive azo dyes,Chemosphere, 2003

[64] **Mustafa Isik, Delia Teresa Sponza**:Effect of oxygen on decolorization of azo dyes by Escherichia coli and Pseudomonas sp.and fate of aromatic amines, Process Biochemistry 38 (2003) 1183-1192

[65] **María Teresa Moreira, Cesar Viacava and Gladys Vidal,** Fed-batch Decolorization of Poly R-478 by *Trametes Versicolor*, brazilian archives of biology and technology Vol.47, n. 2 : pp. 179-183, June 2004

[66] **Nobuki Hayase,Kazuko Kouno, Kazutoshi Ushio**:Isolation and characterization of aeromonas sp. B-5 capable of decolorize various dyes, Journal of Bioscience and Bioengineering, vol. 90, no. 5, 570-573. 2000

[67] **Ozfer, Birgul:**Decolorization of orange II dye with crude culture filtrate of white rot fungus, Coriolus versicolor, Tr. J. of Biology 22 (1998) 463-476

[68] **O'Neill C, Lopez A, Esteves S, Hawkes FR, Hawkes DL, Wilcox S.:** Azo-dye degradation in an anaerobic–aerobic treatment system operating on simulated textile effluent. Appl Microbiol Biotechnol 2000;53:249–54.

[69] **Panswed, J. and Wongehaisuwan, S.**: Mechanism of dye wastewater color removal by Magnesium carbonate-hydrate basic Water. Sci. Technol., 18, 139-144 (1986).

[70] **Paszezynski and Ronald**:Degradation of azo compounds by ligninase from phanerochaete chrysoporium,Biochemical and Biophsycal research Communication, Vol 178, 1991

[71] **P.R.Moreira**: Decolorization of remazol brilliant blue R via a novel bjerkandera sp. Strain,Journal of Biotechnology 89(2001) 107-111 **[24] P.K. Malik:**Use of activated carbon prepared from sawdust & rice-husk for adsorption acid dyes: Acid yellow 36, Dyes and Pigments 56 (2003) 239–249

[72] **Perkowski, J., Kos, L., and Ledakowicz, S.**: Application of ozone in textile wastewater treatment. Ozone Sci. & Eng., 18, 73-85 (1996).

[73] **P. Nigam, G. Armour, I.M. Banat, D. Singh, R. Marchant**, Physical removal of textile dyes and solid state fermentation of dye-adsorbed agricultural residues, Bioresour. Technol. 72 (2000) 219–226.

[74] **Raziye Ozturk Urek , Nurdan Kasikara Pazarliojglu** :Production and stimulation of manganese peroxidase by immobilized *Phanerochaete chrysosporium*, Process Biochemistry (2004)

[75] **Spadaro**: Degradation of azo dyes by lignin-degrading fungus phanerochaete chrysoporium, Applied and Environment Microbiology,1992

[76] **Shin-ya Okazaki , Shin-ichiro Nagasawa , Masahiro Goto , Shintaro Furusaki , Hiroyuki Wariishi , Hiroo Tanaka** : Decolorization of azo and antraquinone dyes in hydrophobic organic media using microperoxidase-11 entrapped in reversed micelles, Biochemical Engineering Journal 12 (2002) 237–241

[77] **W. Liu Y. Chao X. Yang H. Bao S. Qian** : Biodecolorization of azo, anthraquinonic and triphenylmethane dyes by white-rot fungi and a laccase-secreting engineered strain, J Ind Microbiol Biotechnol (2004) 31: 127–132