Studies on biosorption of textile dye from environment using a selected microbial biomass

Thesis submitted by

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CERTIFICATE FROM THE SUPERVISOR(S)

This is to certify that the thesis entitled **"STUDIES ON BIOSORPTION OF TEXTILE DYE FROM ENVIRONMENT USING A SELECTED MICROBIAL BIOMASS"**, submitted by Smt. Mukulika Bagchi who got her name registered on 27.06.2011 for the award of Ph. D. (Engineering) degree of Jadavpur University, is absolutely based upon her own work under the supervisions of Late Prof (Dr.) Lalitagauri Ray of Department of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata, Dr. Debabrata Bera of Department of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata and Dr. Sunita Adhikari (Nee Pramanik) of Department of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata and that neither her thesis nor any part of the thesis has been submitted for any degree/diploma of any other academic award anywhere before.

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Dedicated to my

Parents

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PREFACE

The research embodied in the present thesis entitled "**Studies on biosorption of a textile dye from environment using selected microbial biomass**" deals with the biosorptive removal of a tri-azine textile dye (Reactive Blue 4), a highly toxic environmental pollutant from its aqueous solution.

The present investigation had been carried out by the author in the Department of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata-700032, India during the period of 2011-2017 under the supervision of Late Prof. Lalitagauri Ray, Dr. Debabrata Bera, Dr. Sunita Adhikari (Nee Pramanik), Department of Biological Chemistry, Indian Association for the Cultivation of Science. The thesis has been presented in six chapters.

Chapter 1 describes the introduction part where the general topic and some background of the research were mentioned and a review of literature related to the topic was provided. The terms and scope of the proposed research topic were also defined.

Chapter 2 deals with the biosorptive removal of Reactive Blue 4 by dry biomass of the selected fungal strain *Rhizopus oryzae* (MTCC 262) and optimization of the process parameters.

Chapter 3 describes the physico chemical studies on the adsorption of Reactive Blue 4 on the biosorbent.

Chapter 4 describes removal of the dye using *Rhizopus oryzae* (MTCC 262) biomass immobilized in different carriers, selection of a potent carrier followed by optimization of the biosorption process.

Chapter 5 gives an account of desorption of dye from loaded dry and immobilized biomass of *Rhizopus oryzae* (MTCC 262).

Chapter 6 deals with the continuous removal of textile dye using dry cell as well as immobilized cell using a packed bed column reactor and Desorption of dye from the column and repeated use of the column reactor.

Each chapter (**Chapter 2 to 6**) begins with a short **Introduction** followed by **Materials & Methods**, **Results & Discussion** and **Conclusions**. For convenience, **References** are given at the end of each chapter and **summary of the thesis**, **list of Publications** have been appended to the end of the thesis.

LIST OF ABBREVIATIONS…….

µg	Microgram.
ºC	Degree Celsius.
cm	Centimeter.
CMC	Carboxy-methyl Cellulose.
SEM	Scanning Electron Microscope.
FTIR	Fourier Transform Infra Red.
g	Gram.
h	Hour.
IR	Infra Red.
L	Liter.
M	Molar.
N	Normal.
mg	Milligram.
min	Minute.
MTCC	Microbial Type Culture Collection.
nm	Nanometre.
R^2	Correlation Coefficient.
rpm	Rotation per minute.
UV	Ultra violet.
χ^2	Chi-square.

CHAPTER - 1

General introduction

1.0 Environment

Generally the term **environment** is used to describe "natural" environment. It includes the sum of living and non-living things which surround an organism or group of organisms. All elements, factors and conditions that have some impact on growth and developments of a certain organism are included in the environment. Environment encompasses both biotic and abiotic factors such as light, temperature, water, atmospheric gases that have influence on observed organism.

When some adverse effect is found in the environment due to the introduction of contaminants is known as pollution. Pollution is responsible for instability of the ecological balance and causes serious harm to the ecosystem i.e. physical system or living organisms. Pollution can take place in various forms such as chemical substances or energy, noise, heat or light. The pollutants, the components of pollution, can be either foreign substances/energies or naturally occurring contaminants. Here are some instances of different forms of environmental pollution:

1.1 Air pollution

Air pollution causes when particulates, biological molecules, or other harmful materials are introduced into Earth's atmosphere and thereby cause diseases, allergies, death to humans, damage to other living organisms such as animals and food crops, or the natural or built environment. Air pollution may happen from anthropogenic or natural sources. An air pollutant is a substance in the air that can have adverse effects on humans and the ecosystem. The substance can be solid particles, liquid droplets, or gases. A pollutant can be of natural origin or man-made. Pollutants are classified as primary or secondary pollutants. Major primary pollutants includes sulphur oxides (SO_x); nitrogen oxides (NO_x); Carbon monoxide (CO); Carbon dioxide (CO_x); volatile organic compound (VOC); particulate matter (PM); persistent free radicals; dyes, heavy metal, radioactive pollutants etc.

1.2 Noise pollution

Excessive noise produced by human, animal or mechanical activity causes noise pollution. The source of most outdoor noise worldwide is mainly construction and transportation systems, including motor vehicle noise, aircraft noise and rail noise. Noise pollution can cause annoyance and aggression, hypertension, high stress levels, tinnitus, hearing loss, sleep disturbances, and other harmful effects to human health.

1.3 Soil pollution

Soil pollution is caused by presence of xenobiotic (man-made) compounds or other alteration in the natural soil environment. This type of contamination typically arises from the failure caused by corrosion of underground storage tanks (including piping used to transmit the contents), application of pesticides, percolation of contaminated surface water to sub surface strata, oil and fuel dumping, disposal of coal ash, leaching of wastes from landfills or direct discharge of industrial wastes to the soil. The most common chemicals involved are petroleum hydrocarbons, carcinogenic dyes, poly-nuclear aromatic hydrocarbons, solvents, pesticides and other heavy metals. Thus occurrence of these phenomena is co-related to the degree of rapid industrialization and intensities of chemical usage.

1.4 Water pollution

So far, the Earth is the only planet in the whole universe, accredited to have life. The presence of water is one of the prime reasons which support life. Water pollution is a global concern and, it is the high time to realize the gravity of the situation. Removing pollutants from water is the crying need of the hour and developing a cost effective and environmentally safe method to achieve the same is a challenging task. After all, it is the future of mankind, which is at stake.

Water pollution happens when chemicals or harmful foreign substances are introduced to water, including chemicals, dyes, sewage, pesticides and fertilizers from agricultural runoff, or heavy metals like lead or mercury. It has been suggested that water pollution is the leading cause of deaths and diseases worldwide and that it accounts for the deaths if more than 14,000 people daily. Coliform bacteria are the invisible pollutant of water and also other microorganisms found at surface water causes health problem. The industrial effluent contains both organic (Detergent, Chloroform, Food processing waste, Insecticides, Herbicides, Petroleum hydrocarbons, Volatile organic compounds etc.) and inorganic (Ammonia, Fertilizer, Dyes, Heavy metals etc) chemicals. The contaminated water destroys lives and reduces reproductive ability of aquatic flora and fauna. Nowadays, pollution of water with dyes and heavy metals are thought to be a major problem.

There is another type of water pollution which is also called eutrophication. It is basically Nutrient pollution that cases harm to water body. When nutrients, such as nitrogen, are added

into bodies of water, the nutrient works like fertilizer and makes algae grow at excessive rates, according to NOAA. The algae blocks light from other plants. The plants die and their decomposition leads to less oxygen in the water. Less oxygen in the water kills aquatic animals.

1.4.1 Dye pollution

A dye is a colored substance that has an affinity to the substrate to which it is being applied. Dyes appear to be colored because they absorb some wavelengths of light more than others. The year 1856 witnessed a historic discovery of first synthetic dye, Mauvine, by Perkin [Hunger, 2003; Venkataraman, 1965]. In due course of time, these synthetic dyes gained huge popularity and began to be synthesized on a large scale. In fact, it has reached to a level of annually, over $7.0x10^5$ and nearly 1000 different types of dyes are produced worldwide.

Now-a-days, a large amount of waste water having color is generated by many industries like textile, leather, paper, printing, plastic and so on [Crini, 2006]. The presence of dye materials greatly influence the quality of water and the removal of this kind of pollutant is of prime importance. Owing to their complicated chemical structures, dyes are difficult to treat. Even a small quantity of dye does cause high visibility and undesirability. Moreover, the color produced by dyes in water makes it aesthetically unpleasant [Crini, 2006]. They can have acute or chronic effects on exposed organisms, which depend on the concentration of the dye and the exposed time. In addition to that, many dyes are considered to be toxic and even carcinogenic [Crini, 2006; Mittal et al., 2007; Chen et al., 2010]. An indication to the magnitude of this problem can be inferred from the fact that 2 % of dyes produced are directly discharged into aqueous effluents [Pearce et al., 2003; Robinson et al., 2001]. With the increased stringent laws on industrial discharge, it has become very important to treat this wastewater. Because of their detriment and large scale distribution in the ecological environment, their removal and separation has become one of the important studies of environmental analysis. Of prime importance is the need for clear information on the safety related properties of the colorants and the measure to be taken for lowering exposure. If all these elements are seriously considered, then the technical use of colorants and the handling involved might be possible without much health danger.

1.4.2 EFFLUENT DISCHARGE FROM TEXTILE INDUSTRIES




1.4.3 DEFINITION OF DYE: A **dye** is a coloured substance that has an affinity to the substrate to which it is being applied. The dye is generally applied in an aqueous solution, and may require a mordant to improve the fastness of the dye on the fibre.

Mordant or **dye fixative** is a substance used to set dyes on fabrics or tissue sections by forming a coordination complex with the dye which then attaches to the fabric or tissue. It may be used for dyeing fabrics, or for intensifying stains in cell or tissue preparations. A mordant is often a polyvalent dye ion. The resulting coordination complex of dye and ion is colloidal and can be either acidic or alkaline.

Mordant includes tannic acid, alum, urine, chrome alum, sodium chloride, and certain salts of aluminium, chromium, copper, iron, iodine, potassium, sodium, and tin.

A dye molecule consists of two key components: the chromophores, responsible for producing

the colour, and the auxochromes, which in addition to support the chromophore, also render the molecule soluble in water and give enhanced affinity toward the fibers. Dyes have high structural diversity and can be classified in many ways [Gupta and Suhas, 2009]. Some properties of dyes are based on their usage and can be broadly classified as [Christie, 2007]:

1.4.4 CLASSIFICATION OF DYE:

1.4.4.1 Acid dyes: This is a very large and important group of dyestuffs. While an acid dye is a salt the colour comes from the acidic component, while in the basic dye it's from the organic base. The first acid dyes were combinations of basic dyes with sulphuric or nitric acid.

Adding dyelic salts especially chrome to the dyed fabric in an after-treatment generally has increased colour fastness of acid dyes. Acid dyes cannot be used for wool tops but are used in dyeing wool piece goods, silk, nylon, and some of the other manmade fibers. If a mordant is used they will successfully dye cotton and linen, though this is seldom done today. The ordinary type of acid dye is reserved largely for apparel fabrics and for knitting and rug yarns. A great deal of it is used on nylon carpeting.

1.4.4.2 Predyeized dyes: This is an important group of acid dyes, which have been complexed with dyelic ions to improve light fastness on wool and nylon.

1.4.4.3 Basic dyes: This group was the first of the synthetic dyes to be taken out of coal-tar derivatives. As textile dyes, they have been largely replaced by later developments. However, they are still used in discharge printing, and for preparing leather, paper, wood, and straw. More recently they have been successfully used with some readymade fibers, especially the acrylics. The name means that these are dyes with an organic material, which is soluble in a simple acid. Basic dyes were originally used to color wool, silk, linen, hemp, etc., without the use of a mordant, or using agent. With a mordant like tannic acid they were used on cotton and rayon. Basic dyes give brilliant colors with exceptional fastness to acrylic fibers. They can be used on basic dyeable variants of nylon and polyester.

Nowadays basic dyes are no longer used to any great extent on cotton or linen and seldom on wool. Since they are cheap, however, they are used for hemp, jute and similar fibers. Their most important use today is on acrylics. They can also be used on basic dyeable variants of nylon and polyester.

1.4.4.4 Direct dyes: Historically, the direct dyes followed the basic dyes and were widely hailed because they made it unnecessary to use a mordant or binder in dyeing cotton. The colors are not as brilliant as those in the basic dyes but they have better fastness to light and washing, and such fastness can be measurably improved by after treatments (diazotized and developed.) Direct dyes can be used on cotton, linen, rayon, wool, silk and nylon. These dyes usually have azo linkage –N=N- and high molecular weight. They are water soluble because of sulfonic acid groups.

1.4.4.5 Disperse dyes: These are substantially insoluble non-ionic dyes. These are used mainly on polyester and also applied on nylon, cellulose, acrylic fibers etc.

1.4.4.6 Sulphur dyes: The sulphur dyes provide very deep shades, which have excellent resistance to washing but poor resistance to sunlight. They will dye cotton, linen, and rayon, but not brightly. A problem with sulphur dyes especially the black colors is that they make the fabric tender, or weaken its structure, so that it breaks easily. Sulphur dyed fabrics therefore usually must be treated with alkalis to neutralize the acids, which have formed.

1.4.4.7 Azoic dyes: These dyes are used primarily for bright red shades in dyeing and printing since most other classes of fast dyes are lacking in good red dyes. Azoic dyes, called Naphthols in the industry, are actually manufactured in the fabric by applying one half of the dye. The other half is then put on and they combine to form the finished color. Unless they are carefully applied and well washed, they have poor fastness to rubbing or crocking.

1.4.4.8 Reactive dyes: This group have a chromophore attached to a substituent that is capable of directly reacting with the fibre substrate. The prime reason to make them classified among the most permanent of dyes is the covalent bond that attaches reactive dyes to natural fibers. They find their uses for cotton, wool and nylon.

1.4.4.9 Solvent dyes: These type of dyes are generally non-polar or little polar and thus, are water insoluble. They are used for plastics, gasoline, lubricants, oils etc.

1.4.4.10 Vat dyes: These are perhaps the best known group of dyes in use today because of their all round fastness to washing and sunlight on cotton and rayon.

The term vat comes from the old indigo method dyeing in a vat: indigo had to be reduced to light form. Vat dyes are made from indigo, anthraquinone and carbazole. They are successfully used

on cotton, linen, rayon, wool, silk, and sometimes nylon. Vat dyes are also used in the continuous piece of dying process sometimes called the pigment application process. In this method the dyes are not reduced before application, but after they have been introduced into the fabric. This makes for a dyeing superior appearance and economy. There are no light red vat dyes.

1.4.4.11 Collective dyes: Collectives are the latest dyestuff and because they react chemically with cotton, viscose, linen, wool and silk they are very fast to washing treatments. They can be dyed and printed by many methods and for the first time, the whole spectrum of colour can be put onto cloth using just one class of dyes. Substituting a reactive group on a direct dye produces these dyes.

1.4.4.12 Food dyes: Food dyes are water soluble and non-toxic to human life. The role of food dyes are classed as food additives and are produced under good manufacturing conditions. Food dye can be direct, mordant and vat dyes, and their use is strictly controlled by legislation. Some naturally occurring dyes are also used as food dyes.

Table 1.1 CONVENTIONAL TREATMENTS FOR THE REMOVAL OF TEXTILE DYE

NAME OF THE TREATMENT	ADVANTAGE	DISADVANTAGE
Coagulation, flocculation	➢ Simple ➢ Economically feasible.	➢ Large amount of sludge production. ➢ Disposal problems.
Membrane separation	➢ Remove all dye types ➢ Produce a high quality treated effluents.	➢ Expensive ➢ Incapable of treating large volume.
Adsorption with activated carbon	➢ Very efficient. ➢ Produce a high quality treated effluents.	➢ High cost. ➢ Ineffective against disperse and vat dyes.
Advanced oxidation process	➢ No sludge production, ➢ Little or no consumption of chemicals.	➢ Efficiency for recalcitrant dyes.
Ion exchange	➢ Effective ➢ No loss of sorbent on regeneration.	➢ Expensive
Treatment with Fentos reagent	➢ Effective decolourization for soluble and insoluble dyes.	➢ Sludge generation
Ozonation	➢ Applied in gaseous state.	➢ no alteration of volume ➢ short half life (20 mins)

1.5 BIOSORPTION

- **The adsorption of organic and inorganic toxicants from their aqueous solution with the help of a suitable adsorbent of biological origin covering microbial biomass is known as biosorption.**

- Biosorption is the process performed by all biomasses either live or dead. But the mechanism of uptake by living materials (bioaccumulation) and removal by dead ones (biosorption) are entirely different.

1.5.1 ADVANTAGES OF BIOSORPTION

- The biosorbent can be found easily as wastes or byproducts and at almost no cost.
- The conditions of the process are not limited by the living biomass, no aseptic conditions required.
- There is no need of costly growth media.
- The process is independent of physiological constrains of living cells.
- Process is very rapid, as non-living materials behaves as ion exchange resin, dye loading is very high.
- Process is reversible and adsorbate can be desorbed easily thus recycling of the materials is quite possible
- Chemical or biological sludge is minimized.
- Effective when initial concentration of the targeted toxicant is low.

TABLE 1.2. REMOVAL OF DIFFERENT DYES USING DIFFERENT MICROBIAL BIOMASSES

Biomass	Dye	References
Aspergillus niger	Acid Blue 29	Fu and Viraraghaban, 2001.
Rhizopus arrhizus	Reactive Blue 19	O'Mahony et al., 2002.
Activated sludge biomass	Basic Red 18	Gulnaz et al., 2004.
Chlorella vulgaris	Reactive Red 5	Aksu and Tezer, 2005.
Phanerochete chrysosporium	Reactive Blue 4	Bramoglu et al., 2006a.
Rhizopus oryzae	Rhodamine B	Das et al., 2006.
Streptomyces rimosus	Methylene Blue	Nacera and Aicha, 2006.
Azolla rongpong	Acid Green 3	Padmesh et al., 2006.
Corynebacterium glutamicum	Reactive Black 5	Won et al., 2006.
Trametes versicolor	Direct Blue 1, Direct Red 128	Bayramoglu and Arica, 2007
Thuja orientalis	Acid Blue 40	Akar et al., 2008.
Penicillium chrysogenum	Acid orange 8, Acid Blue 45, Reactive Orange 16	Low et al., 2008
Corynebacterium glutamicum	Reactive Yellow 2	Won and Yun, 2008.
Bithophora sp.	Malachite Green	Beksi et al., 2009.

Biomass	Dye	References
Corynebacterium glutamicum	Basic Blue 3	Won et al., 2009
Baker's yeast	Basic magenta	Yu et al., 2009.
Bacillus cereus **M^{1}_{16}**	Malachite Green	Nath et al., 2015
Bacillus subtilis	Reactive Blue 4	Binupriya et al., 2010
Aspergillus fumigatus	Reactive Brilliant Red K-2BP	Wang et al., 2008
Trametes versicolor	Direct Blue 1 and Direct Red 128	Bayramoglu and Arıca, 2007
Pinus sylvestris	Reactive Red 195	Aksakala and Ucun, 2010
Pseudomonas sp. **strain DY1**	Acid Black 172	Du et al., 2012
Agaricus bisporus, Thuja orientalis	Reactive Red 45	Akar et al., 2008
Corynebacterium glutamicum	Reactive Black 5	Vijayaraghavan et al., 2008
Citrus sinensis	Reactive Yellow 42 and Reactive Red 45	Asgher and Bhatti, 2010
Corynebacterium glutamicum	Reactive Red 4, Reactive Orange 16 and Basic Blue 3	Vijayaraghavan and Yun, 2008
Neurospora sitophila	Reactive Blue 49	Akar and Celik, 2011
Rhizopus arrhizus	Brilliant Red 3B, Brilliant Blue R, Brilliant Orange 3R	O'Mahony et al., 2002
Aspergillus foetidus	Reactive Black 5	Patel and Suresh, 2008

Paenibacillus macerans	Acid Blue 225 (AB 225) and Acid Blue 062 (AB 062)	Colak et al., 2009
Waste beer yeast slurry	Reactive Red 239 (RR239) Reactive Black B (RBB) and Direct Blue 85 (DB85)	Castro et al., 2017
Aspergillus fumigatus	Methylene blue (MB)	Kabbout et al., 2014
Rhizopus arrhizus	Azo dyes	Salvi and Chattopadhyay, 2017

1.5.2 Adsorption Principles

Adsorption is a process that occurs when a gas or liquid solute accumulates on the surface of a solid or a liquid, forming a molecular or atomic film. In other words, adsorption is the adhesion of atoms, ions, biomolecules or molecules of gas, liquid, or dissolved solids to a surface and this process creates a film of the adsorbate (the molecules or atoms being accumulated) on the surface of the adsorbent. It is a surface phenomenon and a consequence of surface energy. The atoms on the surface of the adsorbent are not wholly surrounded by the other atoms and thus, can attract adsorbates. The exact nature of the bonding depends on the details of the species involved, but the adsorption process is generally classified as follows:

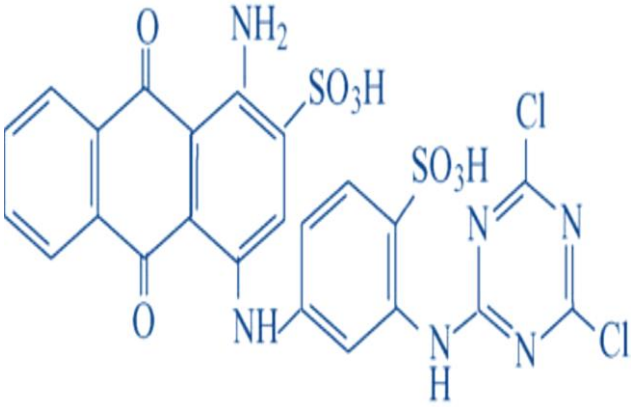
- **Physisorption**: It is a type of adsorption in which the adsorbate adheres to the surface through Van der Walls (weak intermolecular) interactions.
- **Chemisorption**: It is a type of adsorption whereby a molecule adheres to a surface through the formation of a chemical bond.

Adsorption takes place primarily on the walls of the pores or at specific sites inside the particle. As the pores are generally small, the internal surface area is greater than the external area. Separation occurs because differences in molecular weight, shape or polarity cause some molecules to be held more strongly on the surface than others. In many cases, the adsorbate is held strongly enough to allow complete removal of that component from the fluid [McCabe et al., 2005].

1.5.3 Toxicological aspects of azo dyes:

Textile dyeing industry effluents are one of the most contaminated wastewaters to be treated. Azo dyes are known to be the largest and most versatile class of synthetic dyes (Pandey et al., 2007) for their extensive use in textile dyeing, paper, printing, colour photography, pharmaceutics, cosmetics and other industries. These are the important class of environmental contaminants, characterized by the presence of one or more azo bonds (−N = N−) in their molecular structure. Azo dye containing effluents resist many types of treatments due to their molecular complexity. Approximately, 40,000 different dyes and pigments are used industrially presumably more than 2,000 different azo dyes are currently used and over 7×10^5 tons of these are produced annually worldwide. Approximately 10–15% of the dyes are released into the environment during manufacturing and usage (Puvaneswari et al., 2006). Azo dye-containing wastes pose an important environmental problem as several commonly used azo dyes have been reported to be carcinogenic and mutagenic (Gurulakshmi et al., 2008). Furthermore, their discharge into streams can be extremely deleterious and they hinder light penetration and oxygen transfer into bodies of water; hence threaten aquatic living organisms (Pinheiro et al., 2004). Moreover, it is very difficult to treat textile industry effluents because of their high BOD, COD value, colour, pH and the presence of metal ions (Gogate and Pandit, 2004).

CHEMICAL STRUCTURE OF REACTIVE BLUE 4, A REACTIVE TRI-AZINE DYE



- **GENERAL CHARACTERISTICS OF REACTIVE BLUE 4:**

Chemical formula: $C_{22}O_8H_{22}N_6S_2Cl_2$

Molar mass: **637.4**

λ_{max} (nm): **595**

1.5.4 Mechanism of Biosorption:

The mechanism of biosorption is a complex one which includes a combination of ion exchange, physical adsorption, surface complexation and surface micro-precipitation process, (Beolchini et al., 2006; Veglio & Beolchini, 1997) such as diverse is the structure of biological materials (Veglio & Beolchini, 1997). In the past, physical adsorption process was reported to be the domination mechanism. However, present studies (Diniz & Volesky, 2005; Davis et al., 2003; Binupriya et al., 2010) proved that the mechanism is quite similar to ion-exchange process with the functional groups (amino, carboxyl, phosphate, sulphate, hydroxyl groups) present on the cell surface and therefore adsorbents can be considered as weak acidic cation-exchangers (Lodeiro et al., 2005).

1.5.5 Factors affecting biosorption:

The study of the parameters influencing biosorption process was usually focused during batch experiments. Thus, batch studies are very important in the evaluation of the total biosorption potential of any biomaterial including microbial strains. The most important physico-chemical factors include:

- Solution pH.
- Temperature.
- Initial solute concentration.
- Biosorbent dosage.

Of these, solution pH plays the most important role in total adsorption process. It influences the solution chemistry of dyes and the participation of the functional groups present on the adsorbent surface. The activity of the binding sites can also be altered by the adjustment of the pH. In case of dye biosorption, the solution dye chemistry, surface charge of the adsorbent, the degree of ionization of the material present in the solution and the dissociation of functional groups on the active sites of the adsorbent is governed by the pH of the dye solution (Crini et al., 2007) and

therefore, different dye categories require different pH ranges. For example, basic dyes require alkaline or neutral conditions (Farah at al, 2007); whereas, reactive dyes demand strong acidic conditions (O'Mahony et al., 2002) for their optimum biosorption.

The biosorption process using microbial biomass was found to be dependent (Veglio and Beolchini, 1997; Diniz and Volesky, 2005) or independent (Nath et al., 2015) with incubation temperature. It affects biosorption ony to a lesser extent within the range from 20 to 35 ◦C (Nath et al., 2015). With increase in temperature surface activity increases and kinetic energy of the solute (Sag and Kutsal, 2000; Vijayraghaban and Yun, 2007) usually enhance the adsorption capacity of a biomaterial. However, higher temperature can cause physical damage to the biosorbent. Although an increase in temperature has been reported to reduce the adsorption capacity of the biomass as some of the adsorption processes are exothermic in nature. But it is always desirable to conduct/evaluate biosorption at normal room temperature, as this condition is easy to replicate.

The initial solute concentration seems to have impact on adsorption process, with a higher solute concentration resulting in a high solute uptake (Ho and McKay, 1999; Ho and McKay, 2000; Binupriya et al., 2007). The reason can be explained as at lower initial solute concentrations, the ratio of the initial moles of solute to the available surface area is low; subsequently, the functional sorption becomes independent of the initial concentration. However, at higher concentrations, the sites available for adsorption become fewer compared to the moles of solute molecules present and hence, the removal of solute is strongly dependent upon the initial solute concentration.

The biosorbent dosage also acts as a factor during biosorption phenomenon. In many cases, lower biosorbent dosages yield higher uptakes and lower percentage removal efficiencies (Aksu and Cagatay, 2006). On the other hand, an increase in the biomass concentration generally increases the amount of solute adsorbed, due to the increased surface area of the biosorbent, which in turn increases the number of binding sites (Esposito et al., 2011). Conversely, the quantity of adsorbed solute per unit weight of biosorbent decrease with increasing biosorbent dosage, which may be due to the complex interaction involving several parameters. At higher biosorbent dosage, the available solute molecules completely cover the available exchangeable sites on biosorbent, usually resulting in low uptake of solute (Tangaromsuk et al., 2002). It is also suggested that the interference between binding sites due to increased adsorbent dosages cannot be overruled, as this will result in a low specific uptake capacity.

1.6 QUANTIFYING DYE-BIOMASS INTERACTIONS

The case of a biosorption process involves a solid phase (sorbent) and a liquid phase (solvent normally water) containing a dissolved species to be adsorbed (sorbate, e.g. textile dyes). Due to the higher 'affinity' of the sorbent for the sorbate species the latter is attracted into the solid and bound there by different mechanisms. This process takes place until an equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in solution (at a residual, final or equilibrium concentration C_f).

The degree of the sorbent 'affinity' for the sorbate determines its distribution between the solid and liquid phases. The quality of the sorbent material is judged according to how much sorbate it can attract and retain in an 'immobilized' form. For this purpose it is customary to determine the dye uptake (q) by the biosorbent as the amount of sorbate bound by the unit of solid phase (by weight, volume, etc.). The calculation of the dye uptake [mg g^{-1} (dry) sorbent] is based on the material balance of the sorption system. Correspondingly, the amount of dye molecule bound by the sorbent which 'disappeared' from the solution can be calculated based on the mass balance for the sorbent in the system:

$$q = \frac{[(C_i - C_f)V]}{1000\,W} \quad \text{..... (1.1)}$$

Where, V is the volume of the dye particle-bearing solution contacted (batch) with the sorbent [L]; C_i and C_f are the initial and equilibrium (residual) dye concentrations in the solution, respectively. They have to be analytically determined [mg L^{-1}]; W is the amount of the added biosorbent on the dry basis [g].

1.7 SORPTION ISOTHERM MODELS

Analysis of equilibrium data is important for developing an equation that can be used to compare different biosorbents under different operational conditions and to design and optimize an operating procedure. To examine the relationship between biosorption and aqueous concentration at equilibrium, various biosorption isotherm models are widely employed for fitting the data.

1.7.1 Two parameter isotherm model

Langmuir and Fruendlich isotherm model

Most mathematical biosorption models used in the literature describe simple Langmuir (Holan et al, 1993; Crist et al, 1992; Kuyucak et al, 1989) or Fruendlich (Tsezos and Volesky 1981; Tsezos et al, 1990) sorption isotherms, where the dye binding is determined as a function of the

equilibrium concentration of that dye ions in the solution, without reference to pH or other ions in the same solution system. The Fruendlich isotherm (Fruendlich, 1907) is originally of empirical nature but has later been interpreted as sorption to sites with an affinity distribution, i.e. it is assumed that the stronger binding sites are occupied first and that the binding strength decreases with increasing degree of site occupation. More specifically, the Fruendlich isotherm is obtained when a log-normal affinity distribution (i.e. a normal distribution of log (K) is assumed (Stumm, 1992). The Fruendlich isotherm is defined by the following (Fruendlich, 1907).

$$\mathbf{q_{eq} = K_F C_{eq}^{1/n} \quad .....(1.2)}$$

where q_{eq} is the uptake of dye per unit weight of biosorbent (mg/g), C_e the equilibrium concentration of dye ions in solution (mg/L), K_f the Fruendlich constants denoting adsorption capacity (mg g^{-1}) and n is the empirical constant, indicating adsorption intensity (L mg^{-1}). The Langmuir isotherm (Langmuir, 1918) is based on considering sorption as a chemical phenomenon. It is assumed that the forces exerted by chemically unsaturated surface atoms (total number of dye binding sites) do not extend further than the diameter of one adsorbed molecule and that therefore adsorption is restricted to monolayer. In the simplest case the following assumptions are made:

- All sorption sites are uniform (i.e. constant heat of adsorption);
- There is only one sorbate
- One sorbate molecule reacts with one active site;
- There is no interaction between adsorbed species.

The Langmuir isotherm relationship is of a hyperbolic form:

$$\mathbf{q_e = q_{max}(bC_{eq})^{1/n}/\ 1+(bC_{eq})^{1/n} \quad .....(1.3)}$$

where, q_{max} is the maximum sorbate uptake under the given conditions; b is a coefficient related to the affinity between the sorbent and sorbate.

A further analysis of the Langmuir equation can be made on the basis of a dimensionless equilibrium parameter, RL (Gupta et al, 2009) also known as the separation factor, given by Eq. (5):

$$\mathbf{R_L = 1/1 + bC_0 \quad .....(1.4)}$$

The value of R_L lies between 0 and 1 for a favorable adsorption, while $R_L > 1$ represents an unfavorable adsorption, and $R_L = 1$ represents the linear adsorption, while the adsorption operation is irreversible if $R_L = 0$.

1.7.2 Three parameter isotherm model

1.7.2.1 Langmuir-Fruendlich Model

The single-component Langmuir-Fruendlich model is

$$q_e = q_{max}(bC_{eq})^{1/n}/\ 1+(bC_{eq})^{1/n} \ldots\ldots (1.5)$$

where 'b' is the apparent dissociation constant that includes contributions from ligand binding to monomer, monomer-dimer, and more highly associated forms of proteins; q_{max} is the maximum binding capacity; and n is the Langmuir-Fruendlich coefficient.

The Langmuir-Fruendlich model was analyzed by Sips (Sips, 1948) who found that the energy distribution function corresponds to a symmetrical quasi-Gaussian function. At low concentrations, the model reduces to the Fruendlich model and in the case of a homogeneous surface, it reduces to the Langmuir model. By analogy with protein-multiple ligand interactions it has been suggested that equation (1.5) works well to model adsorption co-operativity (Jiang and Hearn, 1996; Sharma and Agarwal, 2001). For purely independent non-interacting sites, the value of n is 1. For positive co-operativity of the protein binding sites, n is greater than 1, while when $0 < n < 1$ negative co-operativity in the binding process is indicated. The value of n can thus be employed as an empirical coefficient, representing the type and the extent of co-operativity present in the binding interaction.

Since the Langmuir-Fruendlich model has more than two adjustable parameters, it is not easily fitted to the experimental data by linear regression or graphical means. In this case, it is necessary to apply non-linear least-squares analysis.

1.7.2.2 Redlich–Peterson isotherm model

Redlich–Peterson isotherm contains three parameters and is an improvement over the Langmuir and Freundlich isotherms (Redlich and Peterson, 1959). It has a linear dependence in the numerator and an exponential dependence in the denominator on concentration which makes it an overall complex and non-linear dependence on Ce. It can be described by Eq(1.6).

$$q_e = ACe/1 + BCeg \ldots\ldots\ldots (1.6)$$

where A, B and g ($0 < n < 1$) are the Redlich–Peterson parameters. Needless to say, for different sorption systems and mechanisms outside the scope of these assumptions the application and fit of the model equations is a matter of chance. They become just mathematical relationships which happen to be capable of following the experimental data. The usual concept of the solid-phase sorbent with physical pores and 'surface area' etc. may not be so close to the real structure,

appearance and behavior of biosorbent materials. Particularly in conjunction with dye ions as sorbate species, biosorbents may appear as gels, very transparent for the minute ions and protons. Actually, when it comes to an ion exchange process, which apparently plays a very important role in biosorption, at least one ion from within the molecular structure of the sorbent is exchanged for another one coming from outside. This leads to ever-changing conditions in sorption system due to the stream of 'exchanged ions also leaving the sorbent into the liquid environment. That is until the sorption equilibrium is established.

1.8 Application of Packed bed column reactor in the process of Biosorption

Large-scale application of biosorption in industry is ultimately the main goal for biosorption research. Some dye sequestering biosorbents have been commercialized. For instance, the algal biosorbent AlgaSORB which employed a fresh water algae *Chlorella vulgaris* and AMTBioclaim which employed *Bacillus* biomass were developed for wastewater treatment and dye recovery (Gupta et al, 2000).

The process of dye recovery by biosorbent materials is basically a solid-liquid contact process consisting of the dye uptake cycle and the dye desorption cycle. Appropriate contact between the solution and the solid phase can be accomplished by the fixed packed-bed contactor. The contactor is represented by a column arrangement where the biosorbent is packed into a solid-bed, which does not normally move. The liquid usually trickles through the bed in down flow or up flow arrangement. Continuous flow systems have some process application advantages over batch type operations. In packed bed systems, fluid flows continuously through a column a adsorbent allowing its more efficient application. The design of a fixed bed reactor involves estimation of the shape of the breakthrough curve and the appearance of the breakpoint. When contaminated water is passed through a long enough bed of adsorbent (sufficient contact time), there would be no contaminant in the effluent until the entire contaminant break through at once and all of the adsorbent is exhausted. Zulfadhly et al (2001) studied the ability of the macro fungus *Pycnoporus sanguineus* to adsorb lead, copper and Reactive Blue 4 in a fixed-bed column. They studied the effect of column bed height, flow rate and initial dye concentration on the breakthrough profile of the bed. Biomass of any kind cannot be used directly in the standard sorption process. Usually if it is very soft, and without reinforcement and granulation, it cannot be used in column operations. Continuous packed bed sorption system has a number of process engineering advantages. Including:

- ✓ It is a simple process.
- ✓ It is a high yield operation
- ✓ Relatively easily scaled up from laboratory scale procedure.
- ✓ The stages in the operation protocol can be automated and high degree of purification can often be achieved in a single step process.
- ✓ A large volume of waste water can be continuously treated using defined quantity of sorbent in the column.
- ✓ Reuse of microorganism is also possible.

1.9 Desorption of adsorbed dye and Regeneration of the biomass

If the biosorption process were to be used as an alternative in the wastewater treatment scheme, the regeneration of the biosorbent may be crucially important for keeping the process costs down and for opening the possibility of recovering the dye extracted from the liquid phase. For this purpose it is desirable to desorb the sorbed dye molecules and to regenerate the biosorbent material for another cycle of application. The desorption process should:

- ✓ yield the dyes in a concentrated form;
- ✓ restore the biosorbent close to the original condition for effective reuse with:
  - • Undiminished dye uptake and
  - • No physical changes or damage.

The desorption and sorbent regeneration studies might require somewhat different methodologies particularly for fluidized-bed arrangements. While the regeneration of the biosorbent may be accomplished by washing the dye-laden one with an appropriate solution, the type and strength of this solution would depend on just how the deposited dye has been bound. Screening for the most effective regenerating solution is the beginning. In batch tests of desorbing solutions, one has to realize that the desorbed adsorbate dye stays in the solution and a new equilibrium is established between that and the one remaining still fixed on the biosorbent. This leads to the concept of a desorption isotherm where the equilibrium is strongly shifted towards the adsorbate dissolved in the solution. However, some residual adsorbate may still be retained by the biosorbent to a various degree.

Due to different affinities of dye ions for the predominant sorption site under the solution conditions, there will be a certain degree of dye selectivity by the biosorbent on the uptake. Similarly, selectivity may be achieved upon the elution–desorption operation. Advantage could be taken of this selectivity on the desorption side of the operation which can contribute to the separation of dye molecules from one another if desirable.

1.10 SCOPE AND OBJECTIVE OF THE PRESENT INVESTIGATION

Rapid industrialization and increase in human population has led to many fold increase in the release of toxic chemicals including textile dyes in the environment. Traditional treatment technologies for the removal of harmful textile dyes from wastewater are either inadequate or costly besides generating huge quantity of toxic sludge. In this regard, technically and economically viable pre-treatment procedures using biomaterials are gaining importance in recent years. In view of the environmental, ecological and societal health issues, it is considered necessary to develop suitable cost effective methods for the removal of textile dyes from wastewater. The use of microorganisms as biosorbents is an attractive alternative to the existing methods for toxicity reduction and removal of dyes from industrial effluents because of their good performance and low cost. On review of literature, a stark revelation of lack of research in fungal adsorbents compared to other microorganisms is seen. Unfortunately, the use of native biomass (such as bacteria, yeast, fungi and algae) in freely suspended state is limited owing to their inherent disadvantages such as small particle size, possible clogging and low mechanical strength of the biomass. In addition, a density similar to that of the suspending medium may complicate biomass/effluent separation. Immobilized biomass overcomes some of these problems and offers greater potential applications. Benefits being better control of particle size, easy separation of biomass and effluent, capability of regeneration, high biomass loadings and minimal clogging under continuous flow. The efficacy of any biosorbent depends on the micro-environment of the targeted toxicant and also on the availability of the material. Thus there is always a need for search of new biosorbents. Further an intimate understanding of the adsorption mechanism is also a prerequisite for improving the efficiency of the process. Although there are few reports of dye biosorption by bacterial extracellular polymeric material, but research in this area is lacking in the field of dye removal with fungal biomass.

The present study deals with the adsorptive removal of Reactive Blue 4 by dry as well as immobilized and pre-treated cells of a *Rhizopus oryzae* (MTCC 262). The model dye used asadsorbate was Reactive Blue 4, which is an anthraquinone based chloro-triazine dye very important in dyeing of cellulosic fabrics. Physicochemical parameters that influence the adsorption process have been included in this investigation. Equilibrium adsorption isotherm and kinetics of adsorption data will be analyzed in the light of different models.

The investigation will focus on the elucidation of the mechanism of interaction between adsorbate and adsorbent employing Fourier transform infrared spectroscopy, Scanning electron microscopic study. The relative role of the functional groups in the overall binding process will also be investigated by applying some physical pre-treatment procedure. Removal of Reactive Blue 4 by the biosorbent immobilized in a suitable matrix is also included in the present investigation. Adsorption experiments will also be carried out in a continuous packed bed reactor using both dry and immobilized biomass. In order to assess the reusability of the selected biosorbent desorption studies are planned using various desorbing agents in four consecutive cycles.

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CHAPTER - 2

Biosorption of Reactive Blue 4, by dry biomass of Rhizopus oryzae (MTCC 262) and optimization of the process parameters

2.1. INTRODUCTION

Aquatic ecosystem is vigorously affected by the presence of textile dyes as dye is a visible toxic pollutant and the presence of very minute amount of dye makes it undesirable due to its appearance. Industries such as textile, tannery, food, paper and pulp, printing, carpet and mineral processing use dyes and pigments to color their products. There are greater than 10,000 different commercial dyes and pigments and over 7 x 10^5 tons are produced annually worldwide [Patel and Vashi, 2012]. Around 5–10% of these dyes are discharged into water as wastes [Liu et al., 2014]. The present scenario is very much alarming as dye also reduces photosynthesis by restricting sunlight penetration into the stream.

Reactive Blue 4 is a reactive tri-azine dye, used heavily in textile industries for coloring different cloth materials, which is known to be highly toxic and potentially carcinogenic, mutagenic and allergenic and sometimes causes damage not only to aquatic life but also on exposed organisms [Heiss et al., 1992; Ozer et al., 2005; Leing, 1991; Aksu and Tezer, 2000]. Most of the tri-azine dye components are resistant to chemical and photochemical degradation [Aksu and Cağatay, 2006; O'Mahony et al., 2002; Fu and Viraraghavan, 2002]. Hence, the effluent containing textile dyes must be treated properly before releasing into the receiving field or aqueous phase.

A number of conventional methods (viz., electro-floatation, precipitation, electro-kinetic coagulation, ion exchange, membrane filtration, ozonation, adsorption through activated carbon, chemical oxidation, electrochemical treatment, reverse osmosis, hydrogen peroxide catalysis, etc.) have been used for removal of textile dyes from the effluent [Vandevivere et al., 1998; Lucarelli et al., 2000; Correia et al., 1994; Lorimer et al., 2000]. But these methods are costly, generate huge amount of toxic sludge and inefficient for very dilute solution. On the contrary, biosorption is an efficient eco-friendly alternative process for dye removal as compared to other conventional processes. The use of fungal biomass as biosorbent in dry or inactivated form is found to have more advantages over live biomass. The dry biomass may be stored for long time [Adhikari et al., 2010] and chances of contamination are low. Although, different textile dyes can be removed using microbial biomass including bacteria, fungi and algae by biosorption and biodegradation [Carliell, 1995] which consume less chemicals and energy. Some low cost fungal biosorbent has been developed for the removal of dye and metal ions from wastewater, which included *Trametes versicolor* [Bayramoglu et

al., 2003], *Corynebacterium glutamicum* [Won et al., 2004], *Aspergillus versicolor* [Bairagi et al., 2011], *Lentinus sajor caju* [Arıca and Bayramoğlu, 2005; Radha et al., 2005], *Rhizopus nigricans* [Kogej and Pavko, 2001], *Aspergillus niger* [Fu and Viraraghavan, 2002], *Aspergillus fumigates* [Wang et al., 2008], and *Phanerocheate chrysosporium* [Baldrian, 2003; Juan and Han-Qing, 2006]. However, a few studies have been focused on utilization of the fungal biomass for the biosorption of Reactive Blue 4.

The earlier reports describe the sorption process as economic and having good performance for toxicity reduction and removal of dyes from industrial effluents. The process may be more economic if the sorbent is inexpensive and eliminates the requirement of any expensive pretreatment of waste water.

Fungal biomasses have very high potential for biosorption of Reactive Blue 4 from aqueous solutions (Saraf and Vaidya, 2015). Fungi in dried form serve as inert biosorbent for removal of pollutants like textile dyes, heavy metals etc from aqueous solutions. Cell surface of dry biosorbent have high affinity for ionic molecules such as azo dyes. Working with dry, inert biomass offers several advantages such as no nutrient requirement for cell growth, high decolorizing efficiency of colored effluents, longer shelf life of the biosorbent which can be regenerated or stored without direct contact with the environment and lack of toxicity constraints (Adhikari et al., 2010).

The present investigation was undertaken to evaluate the biosorption capacity of dry cells of *Rhizopus oryzae* (MTCC 262) for the removal of Reactive Blue 4 from its aqueous solution. The uptake capacity of the selected mold was studied as a function of pH, temperature, dose of biosorbent, initial dye concentration and time of contact. The conspicuous changes in the surface morphology of the biomass as a result of dye adsorption were demonstrated by scanning electron micrograph (SEM) study. The possible mechanisms involved in the dye biosorption were discussed on the basis of FTIR spectroscopic studies.

2.2. MATERIALS AND METHODS

2.2.1. Microorganisms

Rhizopus oryzae (MTCC 262), *Aspergillus versicolor* (MTCC 280) and *Aspergillus niger* (MTCC 281) were procured from Institute of Microbial Technology, Chandigarh, India.

Termitomyces clypeatus was kindly provided by Dr. S. Sengupta, Indian Institute of Chemical Biology, Kolkata, India. The organisms were maintained on potato dextrose agar slant by monthly sub-culturing at 30°C for 120 h and stored at 4°C for further use.

2.2.2. Chemicals and dye

All the chemicals and ingredients of microbiological media used in the present study were purchased from E. Merck, Germany and Hi Media, India, respectively. Reactive Blue 4 [Chemical formula: $C_{22}O_8H_{22}N_6S_2Cl_2$, molecular weight: 637.4 and λ_{max} (nm): 595] was procured from Sigma-Aldrich Chemical Co., St. Louis, MO, USA.

2.2.3. Medium composition and biomass production

Composition of maintenance medium (potato dextrose agar) was as follows: (g L^{-1}); potato extracts: 200.0, dextrose: 20.0, agar: 20.0, pH 5.0. Composition of inoculum and growth medium was (g L^{-1}); potato extracts: 200.0, dextrose: 20.0, pH 5.0. Inoculum was prepared by transferring one loopful of biomass from slant culture to 50 mL inoculum medium in 250 ml Erlenmeyer flask and incubated at 30°C, 120 rpm for 48 h. 1 mL of inoculum was then added to 250 mL Erlenmeyer flask containing 50 ml of growth medium and the flasks were incubated for 72 h at the above mentioned conditions for biomass production.

2.2.4. Preparation of dry cells

The fungal mycelia were harvested by centrifugation at 5000 rpm for 15 min, washed thrice with distilled water and dried by lyophilization. Afterwards the dried biomass was ground using a mixer grinder and used for adsorption study.

2.2.5. Cell size measurement

Size and shape of dry powdered biomass were determined under optical microscope using stage and ocular micrometer [Ramana et al., 2013].

2.2.6. Preparation of dye solution

A stock solution of dye (1000 mg L^{-1}) was prepared by dissolving the required amount of Reactive Blue 4 in distilled water and diluted to get desired concentrations.

2.2.7. Biosorption experiment

0.1 g of each fungal adsorbent (dry biomass of *A. niger*, *A. versicolor*, *R. oryzae*, *T. clypeatus*) was added separately to each of 250 mL Erlenmeyer flasks containing 50 mL dye solution (100 mg L^{-1}) having different pH viz., 3.0, 5.0, 7.0, and 9.0 and incubated at 30°C and 120 rpm for 24 h unless stated otherwise. The flask containing only dye solution (without biomass) served as control. After incubation, the fungal biomass was separated by centrifugation at 5000 rpm for 15 min and the concentration of residual dye in the supernatant was measured.

2.2.8. Estimation of dye concentration

The concentration of dye in the solution was determined using a UV-visible spectrophotometer (HITACHI U-2000) at maximum absorption wavelength, (λ_{max}) 595 nm, which was determined by spectrophotometrical scanning of the dye over a range of wavelength (200-800 nm). A standard curve was prepared using known concentration of dye solution and used as the reference standard for determination of dye concentration.

2.2.9. Determination of dye uptake capacity

The uptake of dye by the biomass was calculated using the following mass balance equation:

$$q_e = \frac{[(C_0 - C_f)V]}{1000W} \qquad \textbf{(2.1)}$$

where q_e is the amount of dye uptake by the biomass in mg g^{-1}. C_0 and C_f are the initial and final dye concentrations in mg L^{-1} in the solution, respectively. V and W represent the volume of solution (L) and weight of the biomass (g) used respectively.

2.2.10. Effect of pH

To determine the effect of pH on biosorption of dye, the dried biomass (0.1 g) of the selected strain were suspended in 50 mL dye solution (100 mg L^{-1}) having different pH (range 3.0-9.0) in 250 mL Erlenmeyer flasks separately and incubated at 30°C and 120 rpm for 24 h. After harvesting the cell mass by centrifugation at 5000 rpm for 15 min, residual dye concentration in the supernatant was estimated.

2.2.11. Effect of temperature

To study the effect of temperature on biosorption experiment was carried out at optimized pH and at different temperatures (viz., 20°, 25°, 30° 35°C), other conditions remaining the same. Residual dye concentration was measured as usual after dye adsorption.

2.2.12. Effect of dry biomass concentration

Dried (lyophilized) cells at different concentration (2 g L^{-1} to 8 g L^{-1}) were added to 50 mL dye solution (100 mg L^{-1}) in 250 mL Erlenmeyer flask separately and incubated for 24 h at 30°C and 120 rpm. Residual dye concentration was measured as usual.

2.2.13. Kinetic study

Kinetic studies were carried out with three initial dye concentrations viz. 50, 100 and 200 mg L^{-1} under optimized pH, temperature and biomass concentration. The solute uptake rate by the biosorbent from solid-solution interface is described by the kinetic study of adsorption. The adsorption kinetics of Reactive Blue 4 were investigated using Lagergren's pseudo first (Equation: 2.2) and pseudo second-order (Equation: 2.3) rate models. The pseudo first-order kinetic model of Lagergren is based on solid capacity [Lagergren and Zur 1898; Ho and McKay, 1999; Aksu, 2001]. It is expressed as follows:

$$log(q_e - q_t) = log q_e - \frac{k_1 t}{2.303} \qquad (2.2)$$

Where q_e, q_t, represent the amount of dye adsorbed at equilibrium (mg g^{-1}) and dye adsorbed (mg g^{-1}) at time t respectively and k_1 is the first order rate constant (min^{-1}). It has been reported that as pseudo-first-order equation is not applicable to several observations, in that case the use of pseudo-second-order equation has been suggested [Ho and McKay, 2000] and it has been considered the rate-limiting step through the formation of chemisorptive bond between the adsorbate and the adsorbent. The generalized equation of pseudo second-order kinetic model is given as:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \qquad (2.3)$$

Where q_e and q_t are the amount of dye adsorbed at equilibrium (mg g^{-1}) and dye adsorbed

(mg g^{-1}) at time t respectively, and k_2 is the pseudo second-order rate constant.

The plot of $\frac{t}{q_t}$ versus t yields very good straight lines from where k_2 value can be calculated.

Adsorption kinetic data was further analyzed to understand the role of intraparticle diffusion in the present experiment. Intraparticle diffusion model (Eq 2.4) (Weber and Moris, 1963) can be characterized by the relationship between specific sorption capacity at time't' and the square root of the time.

$$q_t = K_p t^{1/2} + C \qquad (2.4)$$

Where q_t (mg g^{-1}) is the amount of dye adsorbed at time t'. K_p is the intraparticle diffusion rate constant (mg g^{-1} $min^{½}$) and C is the intercept.

2.2.14. Adsorption isotherm

The efficiency of an adsorbent depends on its capacity to adsorb a particular adsorbate. Therefore, it is necessary to design an efficient operating system to analyze the experimental data from the angle of different isotherm models to understand the adsorbate-adsorbent interaction. The biosorption equilibrium defines the distribution of a solute phase between the liquid phase and solid phase after the sorption reaction reached equilibrium condition [Binupriya et al., 2010]. In the present study equillibrium data were analysed using three of the most commonly used isotherm equation viz., Freundlich, Langmuir and Redlich-Peterson isotherm models. The isotherm expressions of the respective models are given by Eq. (2.5), (2.6), (2.7), as follows:

Langmuir: $q_e = \frac{q_0 K_L C_e}{(1+K_L C_e)}$ q_e **(2.5)**

Freundlich: $q_e = K_F C_e^{1/n}$ **(2.6)**

Redlich-Peterson: $q_e = \frac{K_{RP} C_e}{(1+\alpha C_e^{\beta})}$ **(2.7)**

Where, C_e is the equilibrium dye concentration (mg L^{-1}) and q_e is the mg of dye adsorbed per gram of the biomass at equilibrium (mg g^{-1}). K_L, q_0 and K_F, are the Langmuir and Freundlich

constants, respectively, whereas 'n' is the heterogeneity factor. The Redlich-Peterson model deals with three parameters: where K_{RP} (L g^{-1}), α (L $mg^{-1})^g$ is the Redlich-Peterson constant and 'g' is the Redlich–Peterson isotherm exponent. The value of 'g' lies between 0 and 1, and when g = 1, this model converts to the Langmuir model [Bhatt et al., 2000].

2.2.15. FTIR Spectra analysis

The Fourier Transform Infrared Spectra of pristine and dye laden biomass of *R. oryzae* (MTCC 262) were obtained by using IR spectrophotometer (SHIMADZU CORPORATION, IR-Prestige 21, resolution 4 cm^{-1}). For FTIR spectra analysis, approximately 0.01 g of fungal biomass was mixed with KBr (0.1 g) and the mixture was pressed into a tablet form by pressing the ground mixed material with the aid of a bench press [Lo et al., 1999]. The FTIR spectrum was analyzed in the region of 4000–400 cm^{-1} with a scanning frequency of 200 kHz (i.e. 0.0805 s/scan, 40 s total for an experiment).

2.2.16. Scanning electron microscopy study

Scanning electron micrographs of the pristine and dye loaded biomass of *R. oryzae* were obtained using a JEOL, JMS 5600 (Japan) scanning electron microscope after coating with thin layer platinum under reduced pressure.

2.2.17. Model fitting and statistical analysis

Using Origin 6.0 Professional adsorption isotherm experimental data were fitted and analyzed in some standard isotherm models viz., Langmuir isotherm, Fruendlich isotherm and Redlich-Peterson isotherm model.

2.3. RESULTS AND DISCUSSIONS

2.3.1. Screening of biosorbent

The four fungal strains used in the present experiment, adsorbed dye from the aqueous solution to the extent of 16.84~98.95% depending on the type of the species and pH of the solution (**Figure 2.1**). *Rhizopus oryzae* biomass was found to be the most efficient and adsorbed 98.95% of dye (initial concentration 100 mg L^{-1}) at pH 3.0. The noted difference in adsorption capacity may be attributed to difference in surface structure and functional groups

present on the cell wall of the fungi [Lo et al., 1999]. The average particle volume of dry *Rhizopus oryzae* biomass was found to be 0.00645 mm^3.

Table 2.1. Screening of biosorbent using different pH range.

pH	Dye removal (%)			
	A. niger	***A. versicolor***	***R. oryzae***	***T. clypeatus***
3	50.00	53.42	98.95	48.55
5	16.87	25.51	31.72	27.07
7	16.84	24.25	30.59	24.19
9	19.92	21.58	36.98	20.76

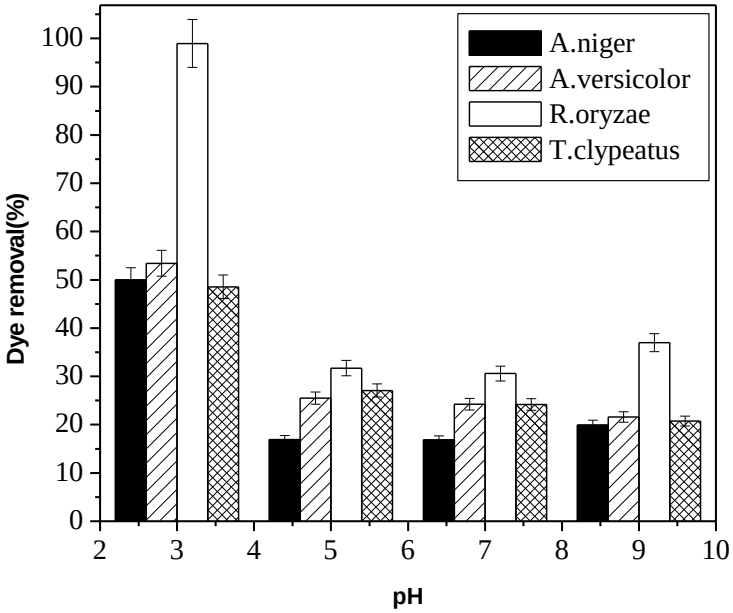

Figure 2.1. Screening of biosorbent using different pH range.

2.3.2. Effect of pH on biosorption

To study the effect of pH on dye biosorption, experiment was conducted at different pH (3.0-9.0). From **Figure 2.2**, it was observed that biosorption of dye on *Rhizopus oryzae* biomass increased with decrease in pH and maximum dye uptake capacity was found 47.66 mg g^{-1} at

pH 3.0. Reactive Blue 4 dye molecule has two sulfonate and a primary amino groups. The p*K*a values of the sulfonate and amino groups of the dye molecule were around 0.8 and 7.0, respectively. These functional groups could be easily dissociated and thus, the dye molecule had negative and positive charges in the working experimental conditions. Therefore, the positive and negative sites of the fungal biomass such as protonated form of amino groups (i.e., $–NH_3^+$; p*K*a values between 7.0 and 10.0), and deprotonated form of carboxylic and phosphate groups (the p*K*a values around 4.0 and 6.5, respectively) [Glu et al., 2006] could play a role in Reactive Blue 4 biosorption. Therefore, with decreasing pH, the binding sites increased, and thereby the biosorption of Reactive Blue 4 increased. Other researchers also reported the same results. Hu reported that the maximum adsorption of different reactive dyes on *Aeromonas* sp. biomass was observed at pH 3.0 [Hu, 1992]. O'Mahony et al. also studied biosorption of different reactive dyes on *Rhizopus arrhizus* biomass and the maximum dye biosorption was observed at pH 2.0 [O'Mahony et al, 2002].

Table 2.2. Effect of pH on Reactive Blue 4 adsorption by dry cells of *Rhizopus oryzae* (MTCC 262)

pH	q (mg g^{-1})
3	47.66
4	27.24
5	19.54
6	13.78
7	11.97

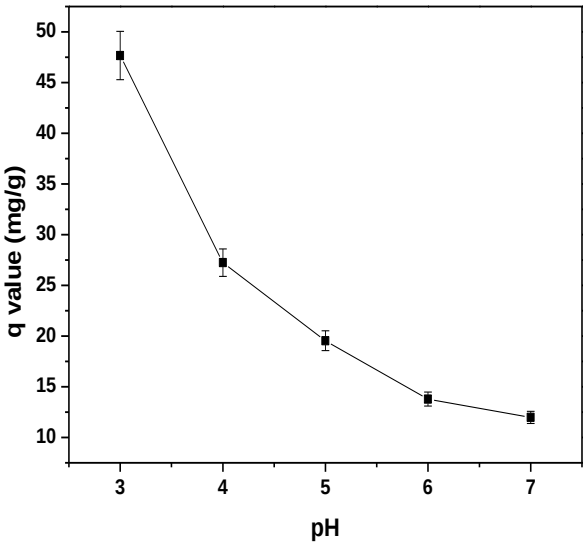

Figure 2.2. Effect of pH on biosorption of Reactive Blue 4 by dry cells of *Rhizopus oryzae* (MTCC 262).

2.3.3. Effect of temperature

As shown in **Figure 2.3** temperature had no significant effect on biosorption. The present study showed slight increase in uptake capacity (q) from 41.32 mg g^{-1} (at 20°C) to 43.80 mg g^{-1} (at 35°C) due to increased surface activity of cells and kinetic energy of solute molecules at higher temperature (Carliell et al., 1995). Aksu and Cagatay also reported that the adsorption capacity of *Chlorella vulgaris* for Ramazol Black B dye was increased a little as the temperature increased (Aksu and Cagatay, 2006). Thus, an economically feasible temperature within the above range may be suitably used for the biosorption process.

Table 2.3. Effect of temperature on Reactive Blue 4 adsorption by dry cells of *Rhizopus oryzae* (MTCC 262)

Temperature (°C)	q (mg g^{-1})
20	41.32
25	42.62
30	43.12
35	43.80

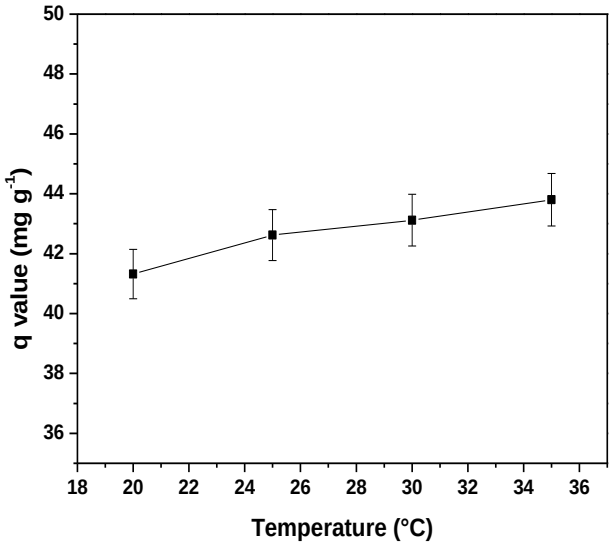

Figure 2.3. Effect of temperature on biosorption of Reactive Blue 4 by dry cells of *Rhizopus oryzae* (MTCC 262).

2.3.4. Effect of dry cell mass concentration

Reactive Blue 4 biosorption by *Rhizopus oryzae* biomass was studied at different biomass concentration (range 2.0-8.0 g L^{-1}), other conditions remaining the same. It is evident from **Figure 2.4** that with increase in biomass load, uptake capacity (q value) was decreased (43.27-12.18) while percentage removal of dye was increased (86.54-97.44). As the number of biosorption sites (or total surface area) increased with the increase in biomass load, percentage removal increased. On the other hand, at higher cell concentration the agglomeration of cells results in shortage of bare adsorption sites leading to decrease in uptake capacity (Nath and Ray, 2015). Thus, from the above study, biomass load of 2.0 g L^{-1} was selected as optimum for removal of Reactive Blue 4 by *Rhizopus oryzae* biomass.

Table 2.4. Effect of biomass concentration on Reactive Blue 4 adsorption by dry cells of *Rhizopus oryzae* (MTCC 262).

Biomass concentration (g L^{-1})	Percentage of removal (%)	q (mg g^{-1})
2.0	86.54	43.27
4.0	93.38	23.34
6.0	95.09	15.84
8.0	97.44	12.18

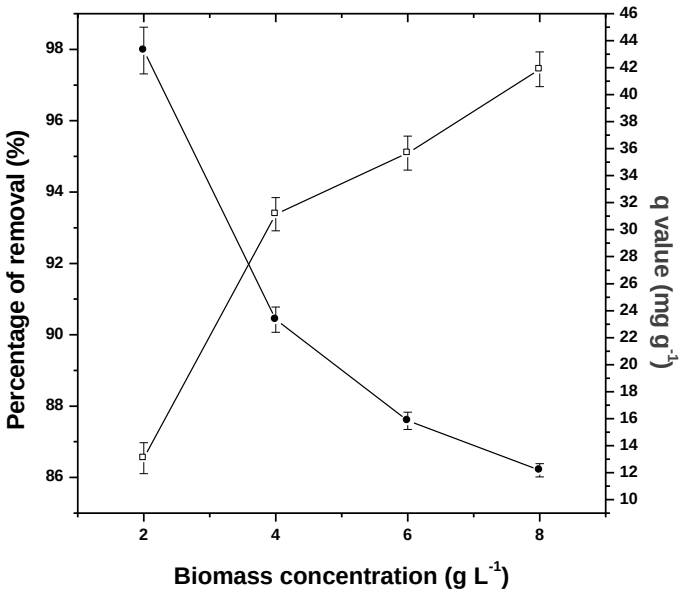

Figure 2.4. Effect of biomass load on biosorption of Reactive Blue 4 by dry cells of *Rhizopus oryzae* (MTCC 262).

2.3.5. Adsorption isotherm analysis

In the present work, adsorption isotherm studies were carried out at a range of 25-600 mg L^{-1} concentration of dye solution. **Figure 2.5** showed the equilibrium plot for biosorption of Reactive Blue 4 by dry *Rhizopus oryzae* biomass (0.1 g) using 50 mL dye solution individually ranging from 25-600 mg L^{-1}, at pH 3.0 and temperature 30°C. All the model parameters are described in Table 3.5 as the "goodness of fit" of the experimental data with the calculated data from the isotherm model can be assessed by R^2 (linear coefficient) and χ^2 (nonlinear coefficient) value. The value of R^2 varies from 0 to 1 and will be very low or zero, if the experimental data differs from the data obtained from the model, whereas the perfect matching of these values yields a coefficient of 1.0. And in case of χ^2, the coefficient value will be small if the experimental data is very close to the obtained data from the model and will be higher if they differ. Therefore, it is essential to evaluate the data considering both for R^2 and χ^2 values.

From the Table 2.5 it was observed that the adsorption data were very well represented by Redlich-Peterson isotherm with an higher correlation coefficient R^2 of 0.95 followed by Langmuir and Freundlich isotherms with correlation coefficient R^2 of 0.93 and 0.82

respectively and the nonlinear regression coefficient (χ^2) of 44.97, 57.17, 155.89, respectively. Therefore, the present sorption data were found to be best fitted to Redlich–Peterson model (Redlich and Peterson, 1959) [**Figure 2.5 (a)]** in comparison with the other two models considering high correlation coefficient (R^2 = 0.95) and low χ^2 value (44.97). This indicated that the adsorption mechanism was a hybrid one and did not follow the ideal monolayer adsorption behavior [Pan et al., 2006]. The value of Freundlich exponent 'n' was 2.85 and in the range of 1-10, indicated the favourable adsorption. Also the higher adsorption capacity, q_0(>>1) indicated the strong electrostatic force of attraction between adsorbent and the dye solution [Freundlich, 1906]. The graphical presentation of experimental q value versus theoretical q value **[Figure 2.5 (b)]** yields a very good straight line with R^2 value of 0.996 and this indicates that the adsorption isotherm strictly follows the Redlich–Peterson isotherm model (Redlich and Peterson, 1959).

Table 2.5. Table for adsorption isotherm study of Reactive Blue 4 adsorption using dry cells of *Rhizopus oryzae* (MTCC 262)

Initial dye concentration (mg L^{-1})	**Equilibrium dye concentration (C_{eq}) (mg L^{-1})**	**q (mg g^{-1})**
25	09.32	6.76
50	12.24	21.25
100	29.52	56.82
200	36.12	74.56
300	63.71	75.72
400	91.68	77.34
500	144.86	78.85
600	191.44	81.17

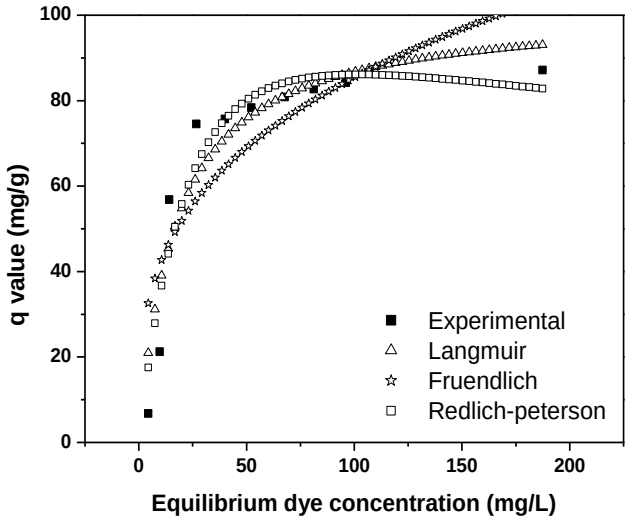

Figure 2.5 (a). Equilibrium adsorption isotherm for removal of Reactive Blue 4 by dry cells of *Rhizopus oryzae* (MTCC 262).

Table 2.5 (a). Table for Experimental q Value Vs Theoretical q Value of adsorption isotherm study of Reactive Blue 4 adsorption using dry cells of *Rhizopus oryzae* (MTCC 262)

Experimental q Value	Theoretical q Value
6.76	6.619
21.25	20.84
56.82	55.32
74.56	76.41
75.72	75.66
77.34	81.74
78.85	80.14
81.17	85.01

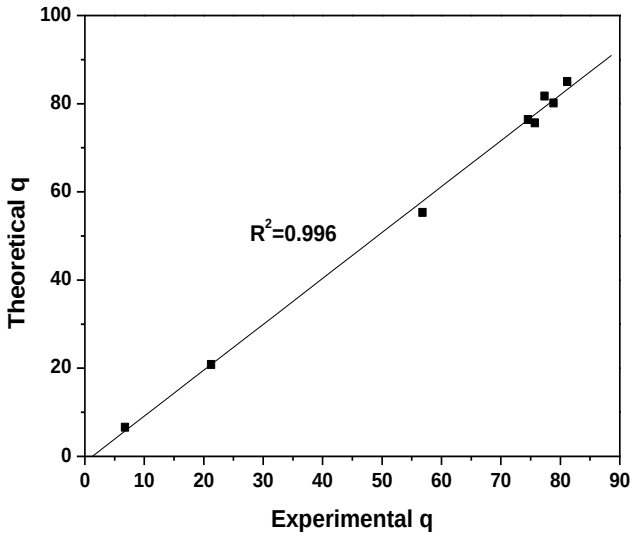

Figure 2.5 (b). Experimental q value Vs theoretical q value of adsorption isotherm study of Reactive Blue 4 by dry cells of *Rhizopus oryzae* (MTCC 262).

Table 2.6. Sorption isotherm coefficients correlation and constants for removal of Reactive Blue 4 by dry biomass of *Rhizopus oryzae* (MTCC 262).

Langmuir				**Freundlich**				**Redlich-Peterson**				
q_0 (mg g^{-1})	K_L (L mg^{-1})	R^2	χ^2	1/n	K_F (L g^{-1})	R^2	χ^2	K_{RP} (L g^{-1})	α (L mg^{-1})	β(g)	R^2	χ^2
101.1	0.009	0.935	57.17	0.35	8.491	0.82	155.89	0.664	0.01	0.87	0.95	44.97

2.3.6. Incubation time and adsorption kinetics

It is evident from **Figure 2.6** that removal of Reactive Blue 4 was comparatively rapid initially and gradually decreased with lapse of time until equilibrium. The surface of the biosorbent was bare in initial stage, the biosorption rate was faster which normally governed by the diffusion process from the bulk solution to the surface. The equilibrium time was achieved within 6 h for all used initial dye concentrations. From figure 2.6, it is clear that increase in incubation time after 6 h, adsorption capacity did not increase, rather it reached equilibrium state as change in adsorption capasity was found negligible after 6 h (Table 2.7). On changing the initial dye concentration (50-200 mg L^{-1}), dye uptake capacity (q value) increased from 10.12 to 67.74 mg g^{-1}. Biosorption was also found to increase with increase in initial dye concentration upto 200 mg L^{-1}. The overall adsorption rate showed that the kinetics of the adsorption of Reactive Blue 4 on the *Rhizopus oryzae* biomass (MTCC 262) was best described by the pseudo-second order model [**Figure 2.7.(b)**]. Further increase in initial dye concentration resulted in slight reduction in biosorption efficiency due to saturation of the dye adsorption sites on the biomass (Nath and Ray, 2015).

The non-linear nature of the curves and deviation of plots from the origin (**Figure 2.8**) indicate that the intraparticle diffusion is not the rate limiting step for the Reactive Blue 4 adsorption by dry biomass of *R. oryzae* (Sahmoune et al., 2009).

Table 2.7. Table for adsorption kinetics on removal of Reactive Blue 4 by dry cells *R. oryzae* (MTCC 262) beads.

Time (min)	q_e (mg g^{-1}) [50 mg L^{-1}]	q_e (mg g^{-1}) [100 mg L^{-1}]	q_e (mg g^{-1}) [200 mg L^{-1}]
1	10.12	14.53	39.21
2	11.75	16.35	39.42
4	12.12	18.06	40.17
6	14.86	18.48	41.35
8	15.16	18.59	43.8
10	15.94	19.87	44.55

15	16.24	20.62	45.51
20	17.18	22.01	48.93
30	17.68	25.48	50.21
45	18.76	28.42	53.96
60	19.22	29.91	56.39
90	20.67	32.59	58.46
120	21.64	35.16	60.91
150	22.78	35.9	62.48
180	23.46	36.75	63.32
240	23.85	37.67	65.77
300	24.12	38.46	66.88
360	24.42	38.68	67.52
420	24.64	38.97	67.74

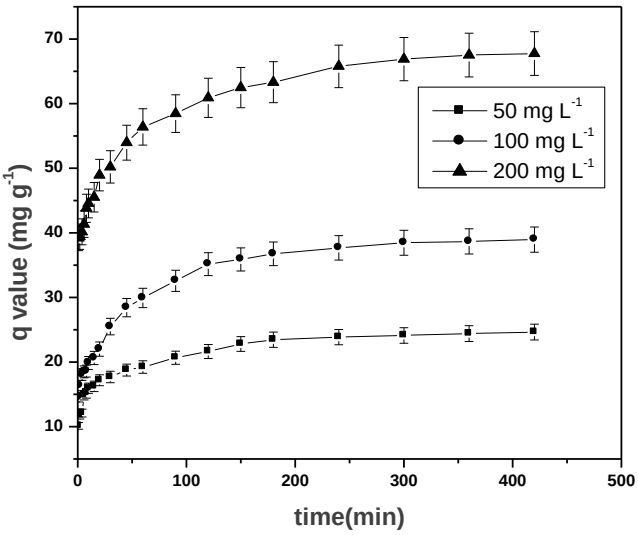

Figure 2.6. Adsorption kinetics of Reactive Blue 4 using dry cells of *Rhizopus oryzae* (MTCC 262)

Table 2.8. Pseudo-first order rate values at 50, 100, 200 mg L^{-1}

Time (min)	$\log (q_e-q_t)$(g.min mg^{-1})		
	On removal of Reactive Blue 4 at dye concentration		
	50 (mg L^{-1})	100 (mg L^{-1})	200 (mg L^{-1})
1	1.161	1.388	1.455
2	1.11	1.354	1.452
4	1.097	1.32	1.44
6	0.99	1.311	1.421
8	0.976	1.309	1.379
10	0.939	1.281	1.365
15	0.924	1.263	1.346
20	0.872	1.229	1.274
30	0.842	1.13	1.243
45	0.769	1.023	1.139
60	0.733	0.957	1.056
90	0.598	0.804	0.967
120	0.477	0.58	0.834
150	0.269	0.487	0.72
180	0.071	0.346	0.645

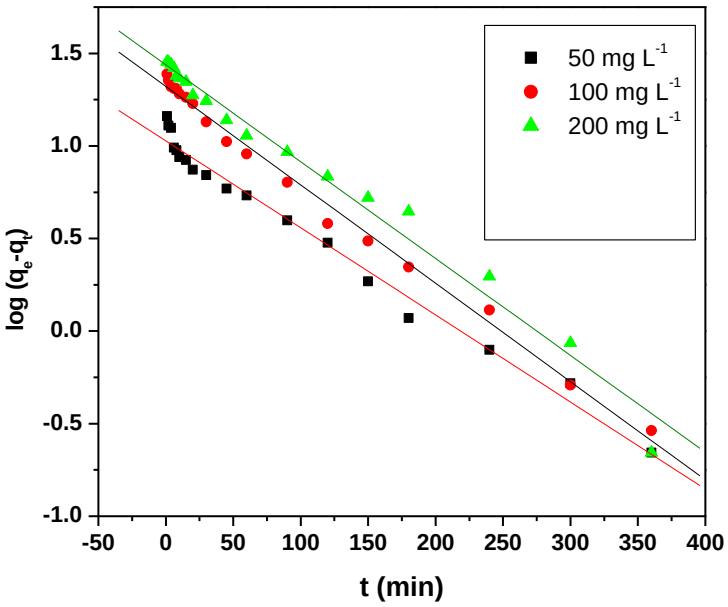

Figure 2.7. (a) Pseudo-first order rate kinetic model.

Table 2.9. Pseudo-second order rate values at different dye concentrations.

Time (min)	t/q_t (g-min mg^{-1})		
	On removal of Reactive Blue 4 dye concentration		
	50 (mg L^{-1})	100 (mg L^{-1})	200 (mg L^{-1})
2	0.17	0.122	0.05
4	0.33	0.221	0.099
6	0.403	0.324	0.145
8	0.527	0.43	0.182
10	0.627	0.503	0.224
15	0.923	0.727	0.329
20	1.164	0.908	0.408
30	1.696	1.177	0.597
45	2.398	1.583	0.833
60	3.121	2.006	1.064
90	4.35	2.761	1.539
120	5.545	3.412	1.97
150	6.584	4.178	2.4
180	7.672	4.897	2.842
240	10.06	6.371	3.649
300	12.437	7.80	4.485
360	14.742	9.307	5.331
420	17.045	10.77	6.2

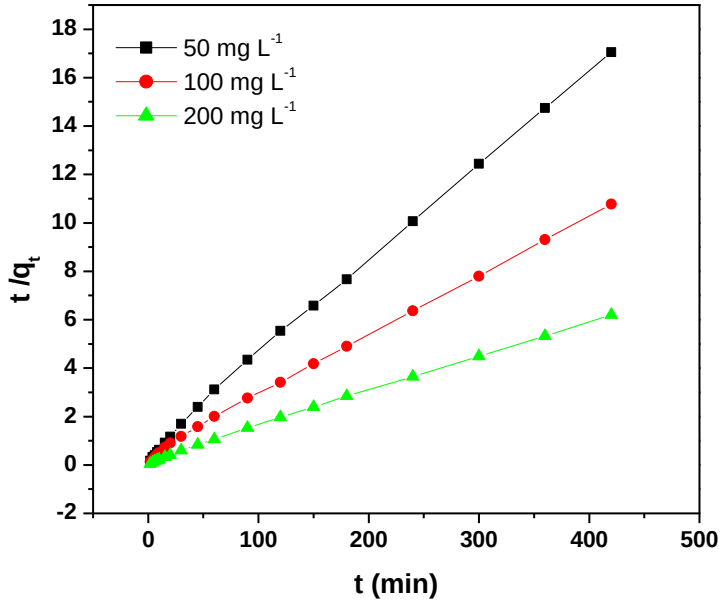

Figure 2.7. (b) Pseudo-second order rate kinetic model.

Table 2.10. Effect of initial dye concentration on kinetic parameters for sorption on dry cells of *Rhizopus oryzae* (MTCC 262)

Initial dye Concentration (mg L^{-1})	Pseudo-first order kinetic model		Pseudo-second order kinetic model		
	k_1 (min^{-1})	R^2	q_e (mg g^{-1})	K_2 (g mg min^{-1})	R^2
50	0.004016	0.992	24.64	0.040	0.999
100	0.005104	0.996	38.97	0.025	0.999
200	0.005022	0.991	67.74	0.014	0.999

Table 2.11. Intraparticle diffusion rate values at 50, 100, 200 mg L^{-1} dye concentrations

$t^{1/2}$ (min ½)	q_t mg g^{-1} [50 mg L^{-1})	q_t mg g^{-1} [100 mg L^{-1})	q_t mg g^{-1} [200 mg L^{-1})
1.000	10.12	14.53	39.21
1.414	11.75	16.35	39.42
2.000	12.12	18.06	40.17
2.449	14.86	18.48	41.35
2.828	15.16	18.59	43.8
3.162	15.94	19.87	44.55
3.872	16.24	20.62	45.51
4.472	17.18	22.01	48.93
5.477	17.68	25.48	50.21
6.708	18.76	28.42	53.96
7.745	19.22	29.91	56.39
9.486	20.67	32.59	58.46
10.95	21.64	35.16	60.91
12.24	22.78	35.9	62.48
13.41	23.46	36.75	63.32
15.49	23.85	37.67	65.77
17.32	24.12	38.46	66.88
18.97	24.42	38.68	67.52
20.49	24.64	38.97	67.74

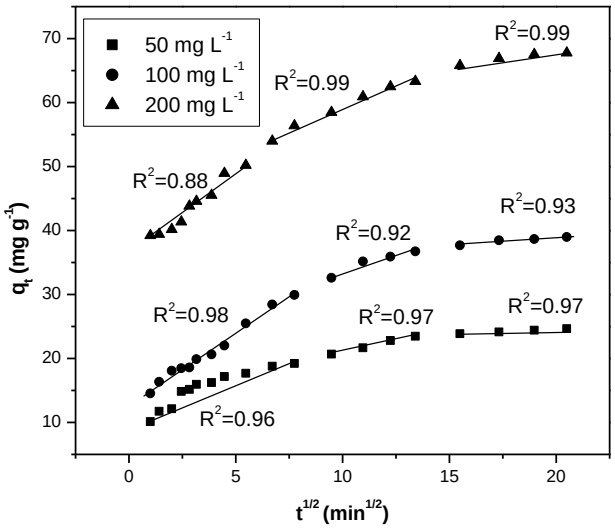

Figure 2.8. Intraparticle diffusion rate kinetic model on removal of Reactive Blue 4 using dry cell of *R. oryzae* (MTCC 262).

The intra-particle diffusion model was employed for manifestly examining the nature of adsorption process. The results (**Figure 2.8**) exhibited that there was three adsorption stages. The first one was instantaneous stage related to the external diffusion of the dye ions from aqueous phase to the external surface of the biomass (Salvi and Chattopadhyay, 2017). The second slower stage is involved in the gradual adsorption of the dye ions on the internal surface of the biomas, which was mainly controlled by pore diffusion and finally achieved the equilibrium state. Therefore, deviation of plots from the origin and the non-linear nature of the curve indicated that the intraparticle diffusion was not the rate limiting step for the entire adsorption process.

2.3.7. FTIR Spectroscopic studies

Fourier transform infrared spectroscopy was employed to get an idea about the possible mechanism of adsorption by identifying the functional groups present on the cell surface of *Rhizopus oryzae* biomass because, each group has a unique energy absorption band [Ganguly et al., 2011]. FTIR spectrum of pristine *Rhizopus oryzae* biomass (**Figure 2.9**) exhibited distinct peaks suggesting the presence of amine, carbonyl, phosphate and hydroxyl groups. The broad mixed stretching vibrations frequency of N–H and O–H were observed in the region of 3500–3300 cm^{-1} and those for alkyl chains were found around 2920–2850 cm^{-1}. The sharp peak at 1646 cm^{-1} can be attributed to C=O stretching of carboxyl or amide

groups. The band at 1550 cm^{-1} was assigned to N–H bending. The presence of COO− of the carboxylate could be attributed to the peak positions at 1452 cm^{-1} and 1400 cm^{-1} on the biomass. The complex amide III band was located near 1370 cm^{-1}. The wave number at 1027 cm^{-1} arises due to the presence of P–O–C link of the organo phosphorous groups on the biomass. The shift of around 4 cm^{-1} in the FTIR analysis may be due to the resolution of the device. In the present case the shift of more than 4 cm^{-1} was only considered. It is evident from the FTIR spectra (Figure 3.8) that peak at about 3404 cm^{-1} due to amino group has been shifted to 3409 cm^{-1} after dye adsorption. Similarly, peak due to carboxylate group in pristine biomass at 1738 cm^{-1} had been shifted to 1748 cm^{-1} and the peak shape had also been altered. Peak of 1646 cm^{-1} in pristine biomass had been shifted to 1635 cm^{-1} due to involvement of C=O of carboxyl or amide group and these indicated the role of amino and carboxylate group on dye adsorption process. The peaks due to alkyl chains at about 2922 cm^{-1} to 2891 cm^{-1} as expected remained unaltered. There were also noticeable changes in the fingerprint zone (at about 1500 cm^{-1}) but it was difficult to interpret these changes.

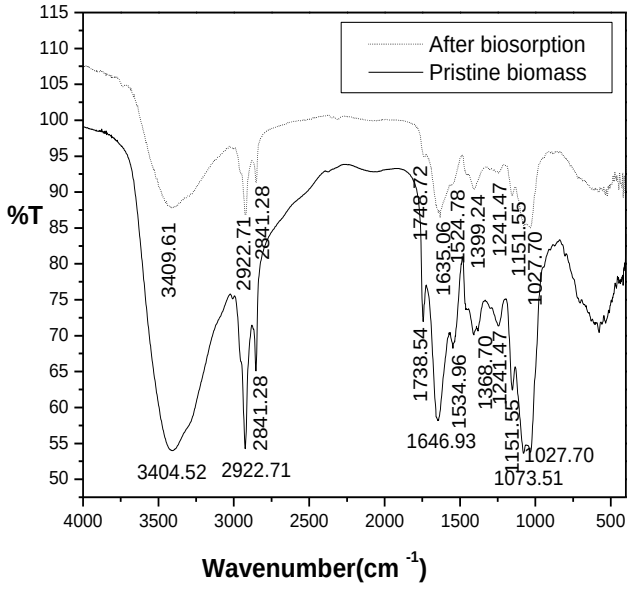

Figure 2.9. FTIR spectroscopy study of pristine biomass and dye loaded biomass of *Rhizopus oryzae* (MTCC 262).

2.3.8. Scanning Electron Microscopy

The surface morphology of a biosorbent can be characterized extensively using scanning electron microscopy as a tool [Mogollon et al., 1998]. **Figure 2.10**, paneled A and C, showed the surface morphology of the pristine *Rhizopus oryzae* biomass, which appeared to be rough and irregular structure with large area of dye surface interaction. Significant changes in surface morphology were noted after dye adsorption (**Figure 2.10.B**). SEM image of the dye adsorbed biomass at higher magnification (**Figure 2.10.D**) depicted that the dye had been adsorbed throughout the surface, especially along the edge of the cell boundary.

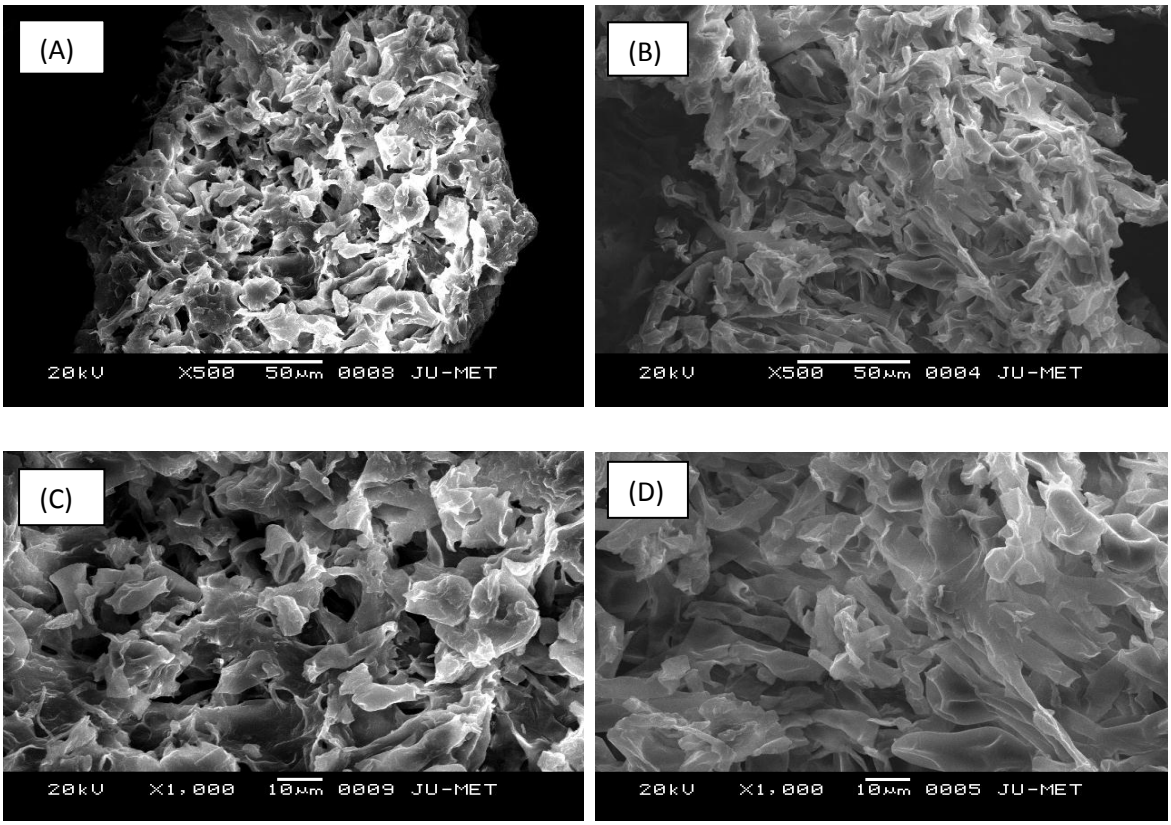

Figure 2.10. Scanning electron micrographs of *Rhizopus oryzae* (MTCC 262) biomass: **(A)** pristine biomass, **(B)** dye loaded biomass, **(C)** pristine biomass at higher magnification and **(D)** dye loaded biomass at higher magnification.

2.4. CONCLUSIONS

From the present investigations, it might be concluded that *Rhizopus oryzae* biomass was capable of removing the carcinogenic, tri-azine textile dye Reactive Blue 4 from aqueous solution efficiently. The biosorption process was found to be influenced by environmental factors viz., initial pH of the solution, biomass load, initial dye concentration and contact time between the dry fungal biomass and dye solution. Dye solution having pH 3.0 and biomass load of 2 g L^{-1} was found to be optimum for removal of the dye by *Rhizopus oryzae* biomass. Temperature showed no significant effect on biosorption. The experiment achieved equilibrium within 6 h. The kinetics of the overall adsorption process was best described by pseudo-second order kinetic model. At equilibrium, experimental results of isotherm study were found to be fitted best to Redlich-Peterson isotherm model. FTIR spectroscopy study showed amino and carboxyl groups were the functional groups responsible for the dye adsorption process. Scanning electron microscopy showed significant changes in surface morphology of *Rhizopus oryzae* biomass after adsorption. Thus, removal of Reactive Blue 4 using the biomass of *Rhizopus oryzae* may be viewed as a considerable alternative process in near future.

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CHAPTER - 3

Physico-chemical studies on the adsorption of dye on the biosorbent

3.1. INTRODUCTION

Pollutants can be described as the substances or energy that causes hazards to living resources and human health or damage ecological systems. Concerns of their interference with the legitimate uses of the environment have never been alleviated over years due both to the high amount and variety of such substances in use. Dyes, for example, being not an exemption among many others, are represented by more than 10,000 chemically different types and the estimated production of them is 7×10^8 kg/year [Fu and Viraragvahan, 2002; Toh et al., 2003; Radha et al., 2005]. Dye related effluents are discharged from a wide variety of industries such as textile, tannery, packed food, pulp and paper, paint and electroplating, threaten the life in much aspects.

The reactive tri-azine dyes are very useful in textile industry for coloring different cloth materials and their metabolites (e.g. aromatic amines) can be potentially carcinogenic, mutagenic and highly toxic and allergenic on exposed organisms [Aksu andTezer, 2000]. The dye components of most of the tri-azine dyes are resistant to physicochemical or biological methods for degradation. Chemical, photochemical and biological degradation methods are also not appeared as a useful method [Aksu and Cagatay 2006; Fu and Viraraghavan, 2002].

Color that inherently occurs in water bodies after receiving dye contaminated effluents can significantly affect photosynthetic activity in aquatic life due to reduced penetration ofsunlight and may also be harmful to some aquatic flora and fauna due to the presence of aromatics, metals, chlorides, etc., in them [Bayramoglu et al., 2006;Lowry et al., 1980; Ozer et al., 2006]. Having usually synthetic origin and complex aromatic molecular structures, dye components are hardly degradable [Fu and Viraraghavan,2002; Radha et al., 2005]. The majority of technologies based on physicochemical processes such as dilution, adsorption, coagulation and flocculation, chemical precipitation, oxidation, ion-exchange, reverse osmosis and ultra filtration [Banat et al., 1996]are presently employed for color removal from the aquatic media. However, high cost, formation of hazardous by-products, intensive energy requirements and inefficient reusability of adsorbents are still limitations commonly countered during the application of these techniques [Arica and Bayramoglu, 2005; Gupta et al., 2000; Couto et al., 2004]. Among treatment technologies, adsorption has been shown to be the most promising option for the removal of non-biodegradable organics from aqueous effluents, activated carbons being themost common

adsorbent for this process due to its effectiveness and versatility [Aksu, 2005]. But, activated carbon has also some disadvantages. Both chemical and thermal regeneration of used carbon is expensive, impractical on a large scale and produces additional effluent and results in considerable loss of the adsorbent. Over the past three decades, biosorption has been continuously studied for the removal of textile dyes and other pollutants including heavy metals [Veglio and Beolchini, 1997; Wu and Yu, 2007]. Among several biosorbent examined previously, fungal biomass has been reported as a very efficient and inexpensive adsorbent that can be produced at low cost byusing cheap growth media. It is also available as a by-product or waste material from various industrial processes [Maurya et al., 2006]. Itwas found that some fungus could adsorb dyes efficiently [Zhang et al., 2003;Aksu and Cagatay, 2006]. Among these useful fungal strains, chemically and physically treated *Rhizopusoryzae* (MTCC 262) was selected as biosorbent in our present experiment.

In this study, the heat-, acid-, alkali-treated and autoclaved (dead) biomasses of *R. oryzae*(MTCC 262) were used for the biosorption of Reactive Blue 4 in batch system. The effects of experimental conditions such aspH, temperature and adsorption isotherm are investigated to obtain information on dye removal properties of the fungal biomass. The possible mechanisms involved in the dye biosorption are discussed on the basis of the treatment of the fungal biomass followed by SEM and FTIR spectroscopic studies. Finally, autoclaved fungal biomasswas found to be the most efficient among all treatment for removal of the dye Reactive Blue 4.

In order to handle the present pollution load, this investigation was designed to eliminate the problems associated with the use of native *R. oryzae* biomass as biosorbentfor the removal of Reactive Blue 4 and the decolorizing efficiency of physically and chemically treated*R. oryzae* from its aqueous solutions. The effect of initial dye concentration, initial pH, incubation temperature, and concentration of biomass were also examined. Langmuir, Freundlich and Redlich-Peterson adsorption isotherm models were applied to the experimental data. The pseudo first-order, pseudo second-order, and intraparticle diffusion models were used for interpretation of the adsorption kinetics.

3.2. MATERIALS AND METHODS

3.2.1. Microorganism

The fungal strain *Rhizopusoryzae* (MTCC 262), used in the present studywasobtained from the Institute of Microbial Technology, Chandigarh, India. The organism was maintained on potato dextrose agar by monthly sub-culturing at 30°C for 120 h and preserved at 4°C for further use.

3.2.2. Medium composition and biomass production

Potato dextrose agar and potato dextrose broth were used as maintenance and growth medium respectively. The composition of maintenance medium was as follows (g L^{-1}); potato extracts: 200.0, dextrose: 20.0, agar: 20.0, and pH 5.0. Potato dextrose broth was prepared without adding agar and other composition mentioned above remaining same. Inoculum was prepared by transferring one loopful of spore from slant culture to 50 mL of inoculum medium in 250 mL Erlenmeyer flask and incubating at 30 °C, 120 rpm for 48 h. 0.1 mL of inoculums was added to 250 mL Erlenmeyer flask containing 50 ml of growth medium and the flasks were incubated for 72 h under the above mentioned conditions for biomass production. The fungal mycelia were harvested by centrifugation at 5000 rpm for 15 min, washed thrice with double distilled water, and dried by lyophilization.

3.2.3. Chemicals and dye

All the chemicals and ingredients of microbiological media used in the study were procured from E. Merck, Germany and Hi Media, India.Reactive Blue 4 [Chemical formula: $C_{22}O_8H_{22}N_6S_2Cl_2$, molecular weight: 637.4 and λ_{max} (nm): 595] was purchased from Sigma-Aldrich Chemical Co., St. Louis, MO, USA.CMC-sodium salt (CMC-Na) (high viscosity; 1.0% in H_2O at 20°C: 300–600 mPa s; content of sodium: 6.5-6.8%) was supplied byLobaChemie Pvt. Ltd.

3.2.4. Pretreatment of fungal biosorbents

The native fungal biomass of *R. oryzae*(about 10.0 g) was transferred into 50 mL 0.1 M HCl andNaOH solutions separately and the mixture was stirred at 250 rpm for 1.0 h at ambient temperature and, hereafter they were called acid- and base-treated fungal biomass respectively.Heat-treated form of *R. oryzae*(MTCC 262) was prepared in physiological saline solution by heating at 100°C for 10 min. Dead biomass was prepared by autoclaving *R. oryzae*

biomass at 121°C for 15 min. Each treated fungal biomass was centrifuged at 5000 rpm for 15 min, washed with distilled waterrepeatedly and dried in a hot air oven at 50°C [Fu and Viraraghavan, 2003].

3.2.5. Chemical modification of the functional groups of *Rhizopusoryzae* (MTCC262)

The functional groups of *Rhizopusoryzae* were modified by chemical treatments as described below. The modified biomass was dried and used for biosorption studies.

Formaldehyde–formic acid: Dried (lyophilized) *R. oryzae* biomass (1.0 g) was shaken (130 rpm) with a mixture of 20 mL formaldehyde and 40 mL formic acid for 6 h at30˚C.

Triethyl phosphate-nitromethane: dried (lyophilized) *R. oryzae*biomass (1.0 g) was refluxed with 40 mL of triethyl phosphate and 30 mL nitro-methane for 6 h.

Acetic anhydride: Dried (lyophilized) *R. oryzae* biomass (1.0 g) was refluxed with acetic anhydride at 80◦C for10 h.

Methanol-hydrochloric acid: Lyophilized biomass of *R. oryzae*(1.0 g) was stirred with 70 mL of anhydrous CH_3OH and 0.6 mL of concentrated HCl for 6 h at room temperature (30˚C).

3.2.6. Preparation and estimation of dye solution

A dye stock solution of 1000 mg L^{-1} was prepared by dissolving Reactive Blue 4 in distilled water and diluted to get the desired dye concentration during experiments. The concentration of Reactive Blue 4 in solution was measured using UV–vis spectrophotometer (HITACHI U-2000) at 595 nm (λ_{max}).

3.2.7. Screening of pretreated biomass

0.1 g of all the four different pre-treated (heat treated, acid treated, base treated and autoclaved biomass) fungal strains used in the present experiment were added separately having different pH (range 3.0, 5.0,7.0, 9.0) in each of 250 mL Erlenmeyer flasks separately and incubated at

30°C and 120 rpm for 24 h. After harvesting the cell mass by centrifugation at 5000 rpm for 15 min, residual dye concentration in the supernatant was estimated.

3.2.8. Effect of pH

To determine the effect of pH on biosorption, 0.1 g ofthe selected biomassof *R oryzae* (MTCC 262) was suspended in 50 mL dye solution (100 mg L^{-1}) having different pH (range 3.0-7.0) in 250 mL Erlenmeyer flasks separately and incubated at 30°C and 120 rpm for 24 h. After harvesting the cell mass by centrifugation at 5000 rpm for 15 min, residual dye concentration in the supernatant was estimated.

3.2.9. Effect of temperature

To study the effect of temperature on biosorption experiment was carried out at optimized pH and at different temperatures (viz., 20°, 25°, 30° 35°C), other conditions remaining the same. Residual dye concentration was measured as usual after dye adsorption.

3.2.10. Effect of dry biomass concentration

The selected biomass at different concentration (2 g L^{-1} to 8 g L^{-1}) were added to 50 mL dye solution (100 mg L^{-1}) in 250 mL Erlenmeyer flask separately and incubated for 24 h at 30°C and 120 rpm maintaining other parameters same. Residual dye concentration was measured as usual.

3.2.11. Adsorption isotherm study

Equilibrium adsorption isotherm of Reactive Blue 4 was studied for selectedbiomass of*R. oryzae*(MTCC 262). Adsorption isotherm study describes the distribution of a solute between liquid and solid phases after the adsorption process reached equilibrium. In order to understand the adsorbate–adsorbent interaction, the adsorption equilibrium data were analyzed with different

isotherm models viz. Langmuir, Freundlich and Redlich–Petersonisotherm using Equations (3.1), (3.2) and (3.3), respectively [Langmuir, 1918; Freundlich, 1906;Redlich and Peterson,1959].

$$q_e = \frac{q_0 K_L C_e}{(1 + K_L C_e)} \qquad \textbf{(3.1)}$$

$$q_e = K_F C_e^{1/n} \qquad \textbf{(3.2)}$$

$$q_e = A C_e (1 + B C_e^g) \qquad \textbf{(3.3)}$$

Where C_e is the equilibrium dye concentration (mg L^{-1}) and q_e is the amount of dye adsorbed per gram of biomass at equilibrium. q_0 indicates the monolayer sorption capacity of adsorbent (mg g^{-1}), K_L is the Langmuir constant related to the energy of adsorption (L mg^{-1}), K_F is the Freundlich constant related to adsorption capacity of adsorbent (mg g^{-1}), n is the Freundlich exponent related to adsorption intensity (dimensionless). A (L g^{-1}) and B (L mg^{-1}) are Redlich–Peterson isotherm constants, 'g' is the exponent reflecting the heterogeneity of the sorbent whose value lies between 0 and 1.

3.2.12. Adsorption kinetics

The rate of dye uptake as well mechanism can be divulged from kinetic studies. In general, microorganisms accumulate dye molecule by a process that includes two phases. The first one is (i) initial rapid, reversible or irreversible metabolism independent surface binding phase followed by (ii) intra particle diffusion by specific carrier systems which is a relatively slow energy dependent process. In order to understand the rate controlling and mass transfer mechanism, the experimental kinetic data were analyzedaccording to linear forms of first order and pseudo-second order rate models given by the equations (3.4) and (3.5) respectively.

$$\boldsymbol{log(q_e - q_t) = logq_e - \frac{k_1 t}{2.303}} \qquad \textbf{(3.4)}$$

$$\boldsymbol{\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e}} \qquad \textbf{(3.5)}$$

where q_e (mg g^{-1}) is the concentration of dye adsorbed at equilibrium, q_t (mg g^{-1}) is the concentration of dye adsorbed at time 't', k_1 and k_2 are the rate constants of the first and second orderrate equations, respectively.

3.2.13.SEMstudy

Pristine and dye adsorbed dead *R. oryzae*(MTCC 262) biomass were coated with platinum by a vacuum electric sputter coater to finest thickness (Bai and Li, 2005). Surface morphology was examined with a field emission scanning electron microscope (JEOL JSM-6700F).

3.2.14.The FTIR spectra

The FTIR spectra of dead, acid, base and heat-treated *R. oryzae*were obtained by using a FTIR spectrophotometer(SHIMADZU CORPORATION, IR-Prestige 21). For FTIR spectra, approximately0.01 g fungal biomass was mixed with KBr (0.1 g) andpressed into a tablet form by pressing the ground mixed materialwith the aid of a bench press. The FTIR spectrum was thenrecorded.

3.3. RESULTS AND DISCUSIONS

3.3.1 Screening of pre-treated biomass of *R. oryzae* (MTCC 262)

All the four different pre-treated (heat treated, acid treated, base treated and autoclaved biomass) fungal strains used in the present experiment adsorption of Reactive Blue 4 from the aqueous solution to the extent of 38.16–98.94% depending on the type of physical treatment and pH of the solution (**Figure 3.1**).

All the tested fungal preparations gave different contact angle values depending on the surface properties. Physical and chemical treatments of the fungal biomass resulted in increase in the hydrophilicity of the Fig. 2. Representative SEM micrograph of the fungus.fungal biomass compared to native form. The same trend was observed for the dye adsorbed fungal preparations compared to dye-free counterparts. The native form of the fungus was hydrophobic, $\theta > 90$. As seen from the table, after heat, acid or base treatment most of the hydrophobic entities of the fungal cell surfaces were removed as shown by contact angle measurement. It should be noted that physical and chemical treatments change the surface properties with respect to native form. Such changes cause contact angles and later adsorption capacity changes too. The base-treated fungal preparation had lower adsorption capacity for the dye than those of the native, heat- and acid-treated forms (Table 2).

Dead biomass of *R. oryzae* was found to be the most efficient at highly acidic pH (pH 3.0) with respect to pH range (3.0-9.0) tested. Dead *R. oryzae* (MTCC 262**)** adsorbed 99% of dye molecules at pH 3.0 under the experimental conditions. The difference found in adsorption capacity may be attributed to the difference in physical modification treatment, surface structure

and functional groups present on the cell wall of the fungi [Kumar et al., 2005]. It may be explained with the SEM photographs where surface morphology of the dead biomass was significantly changed after autoclaving and it appears to be rough and having large surface area for the binding of dye molecule.So, it can be concluded that out of four treated fungal biomass dead biomass of *R. oryzae* was found to be the most efficient adsorbentand hence it was used for further study.

Table 3.1.Selection of most efficient form of pre-treated biomass of *R. oryzae* (MTCC 262)

pH	Dye removal (%)			
	Heat treated Biomass	**Acid treated Biomass**	**Base treated biomass**	**Dead biomass**
3	82.72	87.86	60.92	98.94
5	60.35	64.26	48.66	62.68
7	54.44	52.32	42.46	50.92
9	50.41	47.5	38.16	48.44

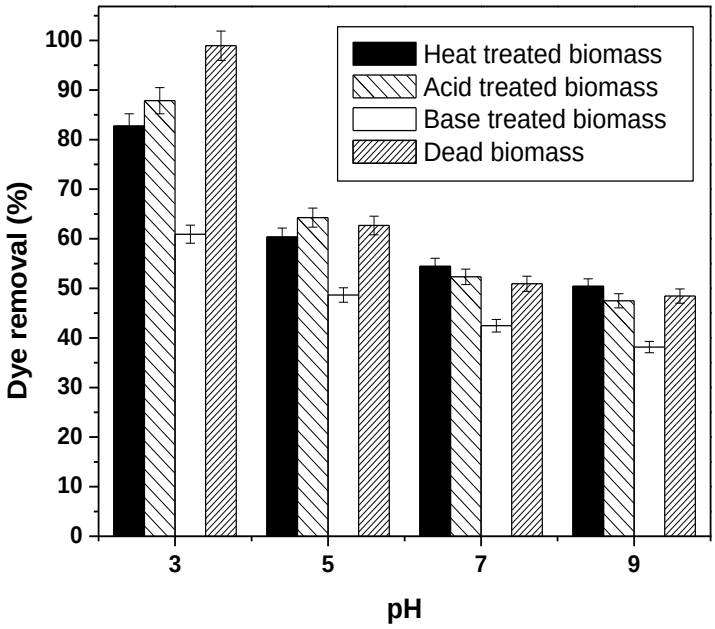

Figure: 3.1.Screening of pre-treated biomass at different pH.

3.3.2. Effect of pH

The effect of pH on biosorption of Reactive Blue 4 by dead cells of *R. oryzae* (MTCC 262) was studied by varying the pH over a range of 3.0–7.0 using initial dye concentration 200 mg L^{-1} at 30°C and 120 rpm. It is evident from **Figure 3.2** that with increase in pH the uptake capacity (q value) was decreased and pH 3.0 was found to be the most favorable with maximum dye adsorption (98.94%, q value 74.66) for the removal of Reactive Blue 4. It might be due to presence of sulfonate and primary amino groups, present in the dye surface were dissociated in lower pH and thus, the dye molecule had negative and positive charges under the working experimental conditions. Therefore, the positive and negative sites of the fungal biomass such as protonated form of amino groups (i.e. $-NH^{+3}$; p*K*a values between 7.0 and 10.0), and deprotonated form of carboxylic and phosphate groups (the p*K*a values around 4.0 and 6.5,respectively) [Weber and Moris, 1963] might play a role in Reactive Blue 4 biosorption at acidic pH.

Table 3.2.Effect of pH on removal of Reactive Blue 4 by dead *R. oryzae*(MTCC 262) biomass.

pH	q (mg g^{-1})
3	**74.66**
4	50.92
5	39.06
6	31.56
7	28.45

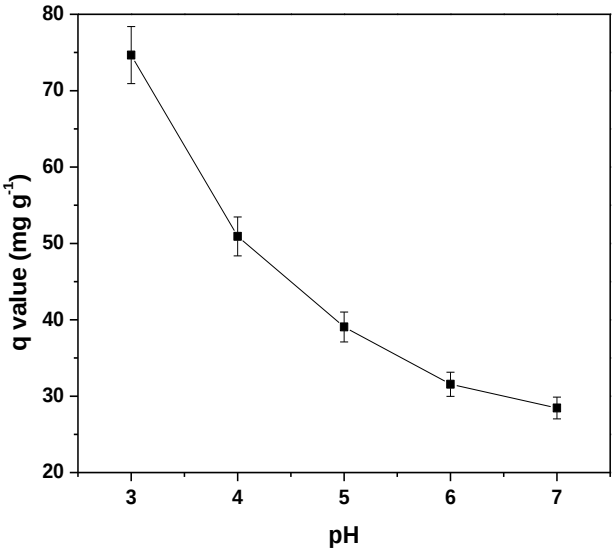

Figure3.2.Effect of pH on removal of Reactive Blue 4 by dead *R. oryzae*(MTCC 262) biomass.

3.3.3. Effect of temperature

Effect of temperature on biosorption wasstudied at pH 3.0 by varying the temperature in the range of 20–35°C, other conditions remaining the same.**Figure 3.3**show that temperature has no significant effect on biosorption. But over the range of 20–35°C,with increase in temperature a little increase in adsorption capacity (q value were increased from 72.86 to 74.15 mg g^{-1} by varying the temperature from 20-35°C) was observed. Sight increase in uptake capacity may be due to the increased kinetic energy that was increased with the temperature.

Table 3.3.Effect of temperature on biosorption using dead of *R. oryzae* (MTCC 262) biomass.

Temperature (◦C)	q (mg g^{-1})
20	72.86
25	73.29
30	73.72
35	74.15

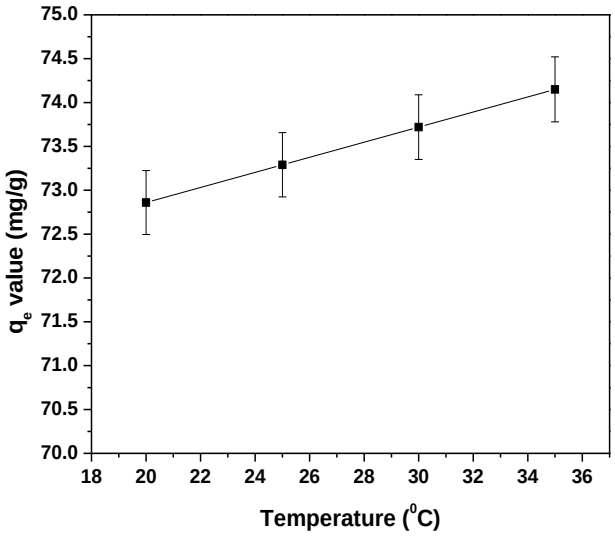

Figure 3.3.Effect of temperature on biosorption using dead of *R. oryzae* (MTCC 262) biomass.

3.3.4. Effect of biomass concentration

Effect of biomass concentration was investigated using 2.0–8.0 g L^{-1} concentration in 50 mL dye solution (200 mg L^{-1}, pH 3.0) taken in 250 mL Erlenmeyer flask and incubated at 30°C, 120 rpm for 20 h. For an increase in biomass concentration from 2.0 to 8.0 g L^{-1}, dye uptake was found to decrease from 73.18 to 18.58 mg g^{-1} (**Figure 3.4**). Percentage removal was found to increase from 97.58% to 99.14% with increase in biomass concentration. It may be explained as the number of biosorption sites (or total surface area) increased with the increase in biomass concentration percentage removal increased. Therefore, biomass concentration of 2.0 g L^{-1} was found optimum for removal of Reactive Blue 4 by *R. oryzae* (MTCC).

Table 3.4.Effect of biomass concentration on biosorption of Reactive Blue 4 using dead biomass of *R. oryzae* (MTCC 262).

Biomass concentration (g L^{-1})	Percentage Removal (%)	q (mg g^{-1})
2	97.58	73.18
4	97.95	36.73
6	98.32	24.58
8	99.14	18.58

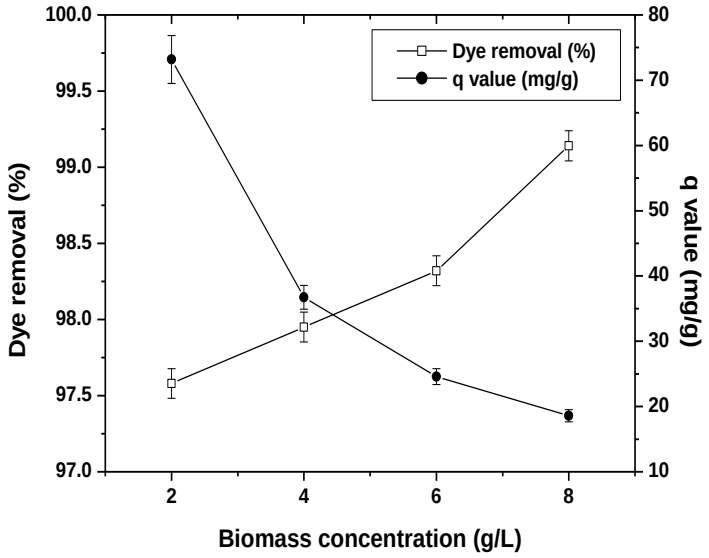

Figure 3.4.Effect of biomass concentration on biosorption of Reactive Blue 4 using dead biomass of *R. oryzae* (MTCC 262).

3.3.5. Adsorption isothermstudy

Adsorption isotherm studies were carried out with both dead *R. oryzae* biomass and at a range of 100–800 mg L^{-1} concentration of dye solution. **Figure 3.5 (a)**shows the equilibrium plot for biosorption of Reactive Blue 4 by dead biomass (biomass dose 2 g L^{-1}) using 50 mL of dye solution (concentration range 100–800 mg L^{-1}) in 250 mL Erlenmeyer flask, at pH 3.0 and temperature 30 °C. All the model parameters are described in **Table 3.6**as the'goodness of fit' of the experimental data with the calculated data from the isotherm model can be assessed by R^2 (linear coefficient) and χ^2 (nonlinear coefficient) values.

From the **Table 3.6**, it is observed that the adsorption data of dead biomass are very well represented by Redlich-Peterson isotherm with an average higher correlation coefficient R^2 of 0.957 followed by Langmuir and Freundlich isotherms with correlation coefficient R^2 of 0.945 and 0.915, respectively, and the nonlinear regression coefficient(χ^2) of 136.41, 149.14, 232.30, respectively. Therefore, the present sorption data are found to be best fitted toRedlich-Peterson model [Das et al., 2006] [**Figure 3.5(a)]** in comparison with the other two models considering high correlation coefficient (R^2 = 0.95) and low χ^2 value (136.41). This indicates that the adsorption mechanism is a hybrid one and does not follow the ideal monolayer

adsorptionbehavior.[Ganguly et al., 2011] The value of Freundlich exponent n is 2.08 and, in the range of 1–10, indicates the favorable adsorption. Also, the higher adsorption capacity, q_0 (>1) indicates the strong electrostatic force of attraction [Prakasham et al., 1999].The graphical presentation of experimental q value versus theoretical q value **[Figure 3.5 (b)]** yields a very good straight line with R^2 value of 0.999 and this indicates that the adsorption isotherm strictly follows the Redlich–Peterson isotherm model (Redlich and Peterson, 1959).

Table 3.5.Adsorption isothermstudy using dead cells of *R. oryzae* (MTCC 262).

Initial dye concentration ($mg\ L^{-1}$)	Equilibrium concentration ($mg\ L^{-1}$)	q ($mg\ g^{-1}$)
100	13.24	68.38
200	30.76	84.62
300	38.03	105.98
400	43.8	128.1
500	52.99	148.5
600	63.88	168.06
700	73.93	188.03
800	83.97	208.01

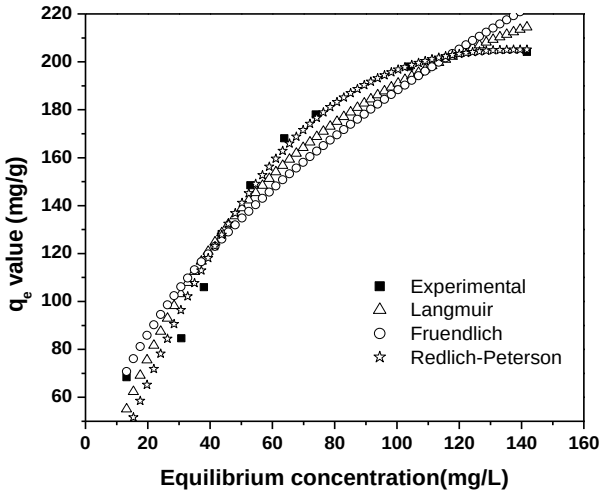

Figure 3.5 (a).Adsorption isotherm study using dead biomass of *R. oryzae* (MTCC 262).

Table 3.5 (a). Table for Experimental q value Vs Theoretical q Value of adsorption isotherm on removal of Reactive Blue 4 by dead biomass of *R. oryzae* (MTCC 262).

Experimental q Value	Theoretical q Value
68.38	68.61
84.62	83.98
105.98	106.16
128.1	127.74
148.5	146.38
168.06	169.71
188.03	192.67
208.01	211.39

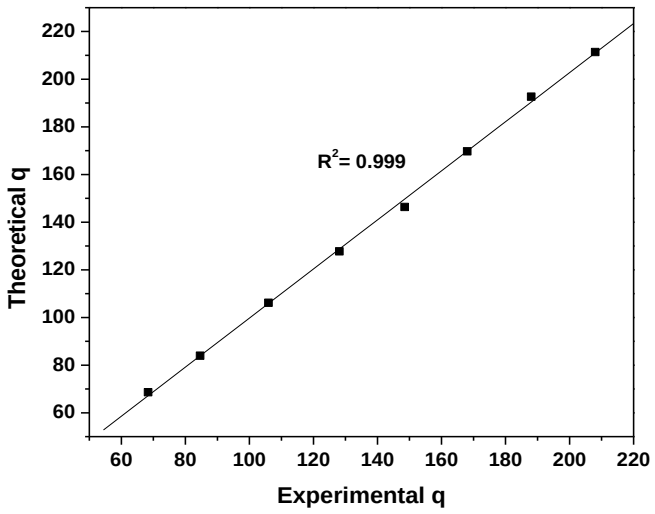

Figure 3.5 (b)Experimental q value Vs theoretical q value of adsorption isotherm study of Reactive Blue 4 by dead biomass of *Rhizopus oryzae* (MTCC 262).

Table3.6. Adsorption Isotherm coefficients correlation and constants for removal of Reactive Blue 4 by dead cells of*Rhizopusoryzae*(MTCC262).

Adsorbents	Langmuir				Fruendlich				Redlich-Peterson				
	q_0 mg g^{-1}	K_L Lmg^{-1}	R^2	χ^2	1/n	K_F Lg^{-1}	R^2	χ^2	K_{RP}(L g^{-1})	α (L mg^{-1})	β(g)	R^2	χ^2
Dead cell	317.25	0.016	0.945	149.14	0.48	20.27	0.915	232.30	3.44	0.008	0.73	0.957	136.41

3.3.6. Adsorption kinetic plot of Reactive Blue 4 removal using dead cells

Adsorption kinetic study of dead cells was carried out using initial dye concentration 100, 150, 200 mg L^{-1}respectively, contact time 2-420 min were varied using 2.0 g L^{-1} biomass concentrationother conditions remaining the same. The rate of biosorption was rapid initially but gradually decreased with time until equilibrium was reached (**Figure 3.6**). The process of biosorption was also found to increase with increase in initial dye concentration up to 200 mg L^{-1}. Further increase in initial dye concentration showed negative effect on biosorption efficiency. Equilibrium dye uptake capacity of 38.96, 43.34 and 57.14 mg g^{-1} was achievedin 360 min.

Table 3.7.Adsorption kinetics study values by dead biomass of *R. oryzae* on dye adsorption

Time (min)	q_e (mgg^{-1}) (100 mg L^{-1})	q_e (mg g^{-1}) (150 mg L^{-1})	q_e (mg g^{-1}) (200 mg L^{-1})
2	11.14	13.32	16.02
4	11.34	13.66	16.51
6	11.86	14.08	17.55
8	12.38	15.14	18.7
10	13.41	16.3	19.94
15	14.44	17.68	21.04
30	23.74	25.98	32.1
45	25.22	29.86	36.05
60	26.92	31.38	37.8
90	28.26	33.86	40.3
120	30.12	34.76	41.87
150	34.77	37.16	48.74
180	35.99	38.49	52.26
240	36.41	39.17	54.37
300	37.08	41.26	56.46
360	38.33	43.07	56.98
420	38.96	43.34	57.14

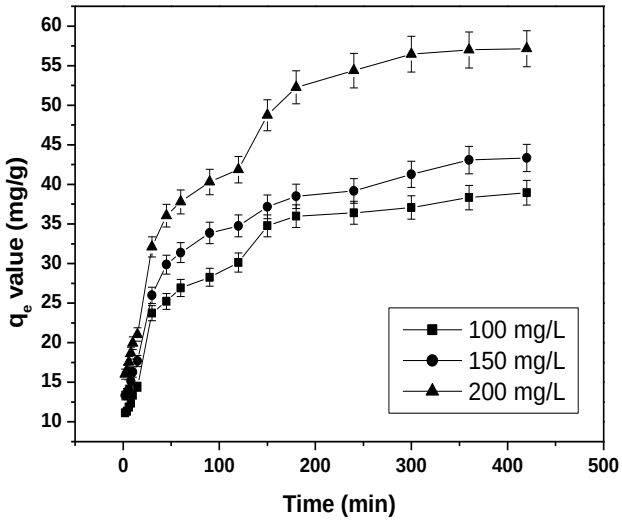

Figure3.6.Adsorption kinetics study using dead cell of *R. oryzae* (MTCC 262) on dye adsorption.

Table 3.8.Pseudo-first order rate values at 100, 150 and 200 mg L^{-1}

Time (min)	$\log (q_e-q_t)$(g-min mg^{-1})		
	On removal of Reactive Blue 4 at dye concentration		
	(100 mgL^{-1})	(150 mgL^{-1})	(200 mgL^{-1})
2	1.444	1.477	1.614
4	1.441	1.472	1.608
6	1.432	1.466	1.597
8	1.424	1.45	1.584
10	1.407	1.432	1.57
15	1.389	1.409	1.557
30	1.182	1.239	1.398
45	1.137	1.129	1.324
60	1.08	1.077	1.286
90	1.029	0.976	1.226
120	0.946	0.933	1.183
150	0.622	0.79	0.924
180	0.472	0.685	0.688
240	0.406	0.62	0.442

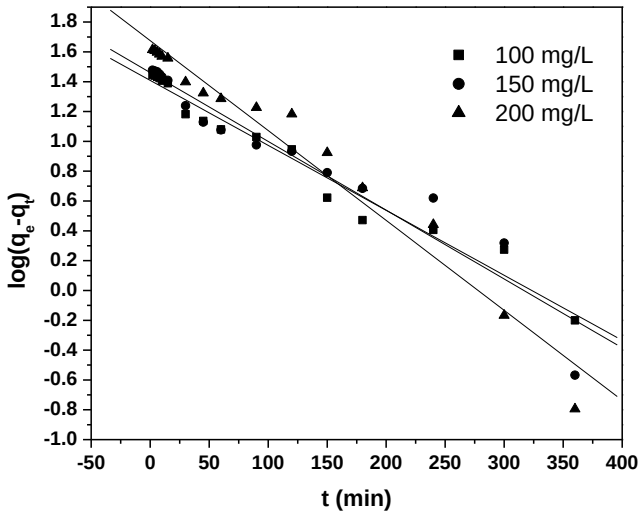

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Figure 3.6 (a) Pseudo-first order rate model at 100, 150 and 200 mg L^{-1}

Table 3.9.Pseudo-second order rate values at different dye concentrations

Time (min)	t/q_t(g-min mg^{-1})		
	On removal of Reactive Blue 4 at dye concentration		
	(100 mgL^{-1})	(150 mgL^{-1})	(200 mgL^{-1})
2	0.179	0.15	0.124
4	0.352	0.292	0.242
6	0.505	0.426	0.341
8	0.646	0.528	0.427
10	0.745	0.613	0.501
15	1.038	0.848	0.712
30	1.263	1.154	0.964
45	1.784	1.507	1.248
60	2.228	1.912	1.587
90	3.184	2.658	2.233
120	3.984	3.452	2.866
150	4.314	4.036	3.077
180	5.001	4.676	3.444
240	6.591	6.127	4.414
300	8.09	7.27	5.313
360	9.392	8.358	6.318
420	10.78	9.69	7.35

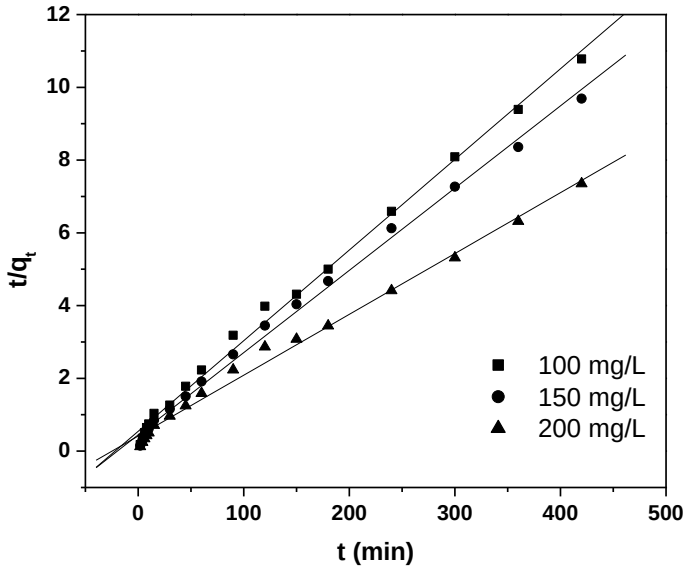


Figure 3.6 (b) Pseudo-second order rate model at different dye concentrations.

Table 3.10. Intraparticle diffusion model on dye adsorption by dead *R. oryzae* (MTCC 262) biomass.

$t^{1/2}$ (min ½)	q_t mg g^{-1}(100 mg L^{-1})	q_t mg g^{-1}(150 mg L^{-1})	q_t mg g^{-1}(200 mg L^{-1})
1.414	11.14	13.32	16.02
2.0	11.34	13.66	16.51
2.417	11.86	14.08	17.55
2.771	12.38	15.14	18.7
3.125	13.41	16.3	19.94
3.82	14.44	17.68	21.04
5.577	23.74	25.98	32.1
6.708	25.22	29.86	36.05
7.853	26.92	31.38	37.8
9.486	28.26	33.86	40.3
10.954	30.12	34.76	41.87
12.24	34.77	37.16	48.74
13.65	35.99	38.49	52.26
15.49	36.41	39.17	54.37
17.32	37.08	41.26	56.46
19.09	38.33	43.07	56.98
20.49	38.96	43.34	57.14

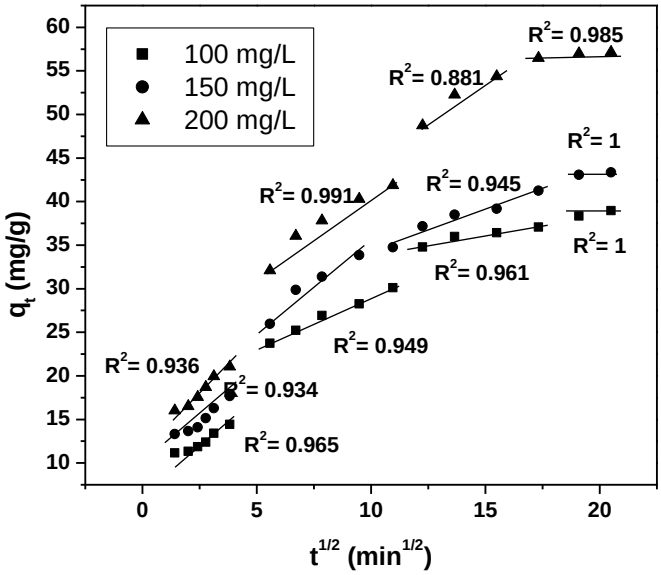


Figure3.6 (c) Intraparticle diffusion models on dye adsorption by dead *R. oryzae* (MTCC 262) biomass.

Table 3.11.Effect of initial dye concentration on kinetic parameters for sorption of Reactive Blue 4 on dead biomass of*Rhizopusoryzae*(MTCC262)

Adsobents	**Initial dye concentration (mg L^{-1})**	**Pseudo first-order kinetic model**			**Pseudo-second order kinetic model**		
		q_e (mg g^{-1})	$k_1 min^{-1}$	R^2	q_e (mg g^{-1})	K_2 (g mgmin^{-1})	R^2
Dead cell	100						
	150	229.35	0.0000104	0.970	3.497	0.024	0.994
	200	229.35	0.0000106	0.999	2.781	0.226	0.996
		166.66	0.0000201	0.964	2.592	0.016	0.992

3.3.7. Effect of functional group modification on biosorption

Adsorption of dye mainly involves the functional groups present on the microbial cell surface. The functional groups Such as, amino, hydroxyl carboxyl and phosphate groups of *R. oryzae* were chemically modified to understand their role in dye adsorption process. Formaldehyde and formic acid methylates the primary and secondary amines present on the cell surface of *R. oryzae* biomass according to the reaction scheme shown below:

$$\mathbf{R–NH_2 + 2\ HCHO + 2\ HCOOH \rightarrow R–N\ (CH_3)_2 + 2CO_2 + 2H_2O}$$

The methylation of amino group reduced the adsorption capacity by21.67%, indicating that the amino groups present on *R. oryzae* biomass play a crucial role in Reactive Blue 4 adsorption on *R. oryzae*(MTCC 262).

Refluxing with acetic anhydride causes the acetylation of the hydroxyl group present on *R. oryzae*. The general reaction scheme is:

$$\mathbf{R–CH_2OH + (CH_3CO)_2 \rightarrow R–CH_2COCH_3 + CH_3COOH}$$

Blocking of hydroxyl groupswere found to have no significant effect on the adsorption capacity of Reactive Blue 4 suggesting that hydroxyl groups of *R. oryzae*(MTCC 262) are not the binding sites for Reactive Blue 4.

Treatment of *R. oryzae* with anhydrous methanol and HCl results in the esterification of the carboxyl group. The general reaction scheme is shown below:

$$\mathbf{R–COOH + CH_3OH \xrightarrow[H^+]{} R–COOCH_3 + H_2O}$$

The reduction of dye adsorption capacity due to esterification of the carboxyl group of *R. oryzae* by 57.27 % suggests the major involvement of carboxyl group on the process.

R. oryzae on refluxing with tri-ethyl phosphate and nitro-methane blocks the phosphate group of ortho-phosphoric acid present on the *R. oryzae* cell surface. Since no change in adsorption capacity of Reactive Blue 4 is noted due to blocking of phosphate group, it is evident that this group is not involved in the adsorption process.

Table 3.12.Adsorption behavior of Reactive Blue 4 in relation to chemical modification of the functional groups present on the surface of *R. oryzae* (MTCC 262) biomass.

Type of biomass	q_e(mg g^{-1})	Percent change in adsorption
Pristine biomass	49.52	_
Carboxyl modified	21.16	57.27% reduction
Amino modified	38.79	21.67% reduction
Hydroxyl modified	48.97	_
Phosphate modified	46.75	_

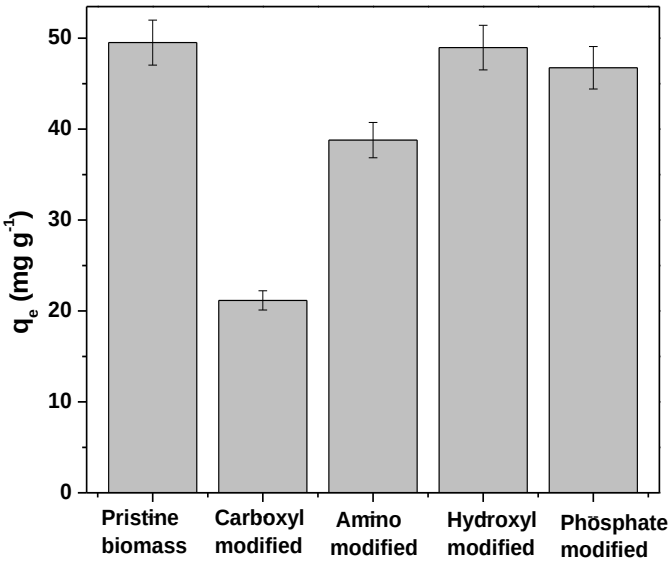


Figure 3.7 Effect of chemical group modification on Reactive Blue 4 adsorption by *R. oryzae* biomass at an initial solute concentration of 100 mg L^{-1}, incubation temperature 30°C and pH 3.0.

3.3.8. SEM analysis

SEM analysis was used to identify the morphological changes due to adsorption of textile dyes on adsorbent cells (Mogollon et al., 1998). Examination of dead*R. oryzae*biomass before and after dye adsorption by scanning electron microscopy was carried out to locate the active sorptive areas of biosorbents. Surface morphology of the dead biosorbent appeared to be rough and irregular with a large area for dye surface interaction [**Figure 3.8(a**)]. Significant changes in the surface morphology of dead *R. oryzae* biomasswere noted which became compact after Reactive Blue 4 adsorption [**Figure 3.8(b**)].**Figure 3.8(c)** and **3.8(d)** shows the morphological changes of dead biomass in higher magnification using scanning electron microscopy before and after dye adsorption respectively.Thus, it can be assumed that the dye attached to the functional groups present on the autoclaved biomass (dead) surface was the reason for the morphological changes found on dead cell surfaces[Prakasham et al., 1999]. It is evident from the micrograph of higher magnification that the light colored fungal cells were also changed into blackish due to adsorption of Reactive Blue 4.

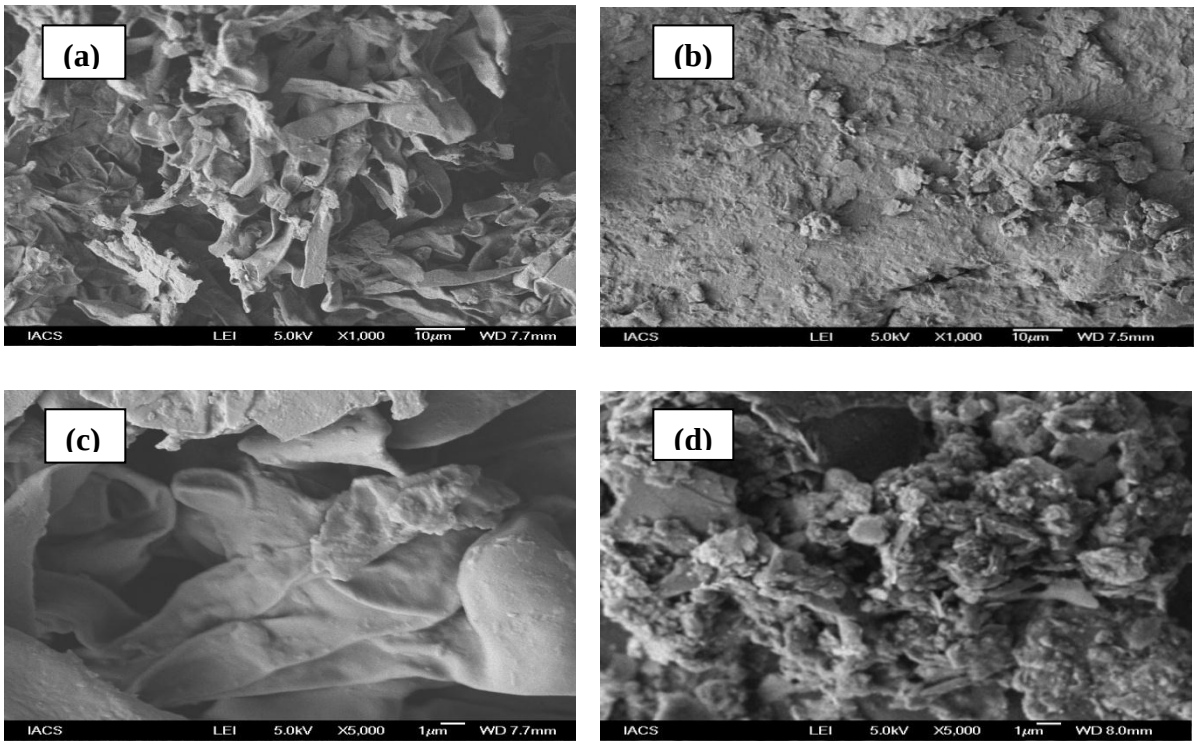


Figure 3.8 Scanning electron micrograph of **(a)** pristine dead (autoclaved) *R. oryzae***(b)**dye loaded dead*R. oryzae* (MTCC 262) biomass**(c)** pristine dead biomass of *R. oryzae* at higher magnification (5000X) **(d)** dye loaded dead*R. oryzae* (MTCC 262) biomass at 5000X.

3.3.9. FTIR study

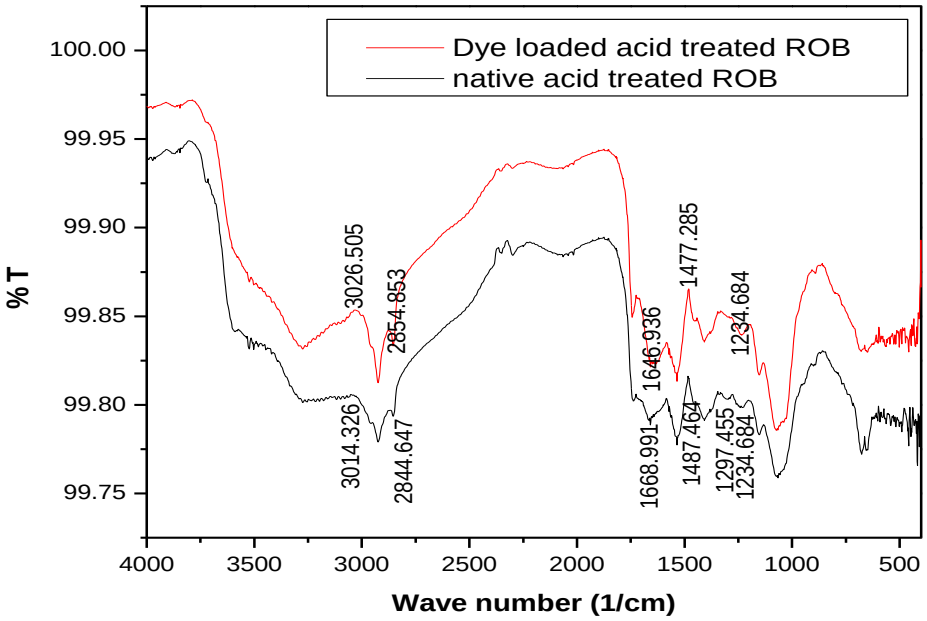
Fourier transform infrared spectroscopy can be employed to identify the functional group present on adsorbent surface as each functional group has a unique energy absorption band. FTIR analysis of pre-treated biomass was taken before and after dye (Reactive Blue 4) uptake. FTIR spectrum of pre-treated dry biomass exhibits distinct peaks suggesting the presence of amine, carboxyl, hydroxyl, phosphate groups etc. The broad mixed stretching vibrations frequency of N–H and O–H were observed in the region of 3014–3026 cm^{-1} in case of dye loaded acid treated biomass[**Figure 3.9(a)]**and those for alkyl chains at around 2844–2854cm^{-1}. The peak shifting from 1646–1668 cm^{-1} can be attributed to C=O stretching of carboxyl or amide groups. The strong peaks at around 1650, 1400and 1240 cm^{-1} are caused by the C=O stretching band of carbonylgroups and peak shifting around those region indicates that C=O group of carbonyl and amide group is playing a vital role in the adsorption process using acid treated biomass.

In case of base treated biomass**[Figure 3.9(b)]**, peak shifting were observed only in the region at around 1650, 1400 and 1240 cm^{-1} were caused by the C=O stretching band of carbonyl group or amide group. Peak shifting from 1538-1529 cm^{-1} and 1309-1292 cm^{-1} indicated that carbonyl or amide group may play an important role in case of adsorption with base treated biomass.

The spectrum of the dye-free dead biomass **[Figure 3.9(c)]**showed a broad and strong peak at 3289 cm^{-1}, which was attributed to the NH_2 stretching vibrations in polysaccharides and proteins. In the spectrum of dye-loaded dead biomass, this peak was shifted to the 3268 cm^{-1} region and significantly decreased compared to that of the dye free dead biomass, indicating the binding of the dye with NH_2 groups. The peak at 1339–1319 cm^{-1} was mainly due to the C-H bending, -CH_3 stretch, or COO^- symmetric stretch.

Aliphatic C-H stretching (fatty acids) was observed in the region at around 2842–2834 cm^{-1} in FTIR analysis of dye free and dye loaded heat treated biomass**[Figure 3.9(d)]**.

(a)


Dye loaded acid treated ROB
native acid treated ROB
% T
Wave number (1/cm)
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99.95
99.90
99.85
99.80
99.75
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3500
3000
2500
2000
1500
1000
500
3026.505
2854.853
1477.285
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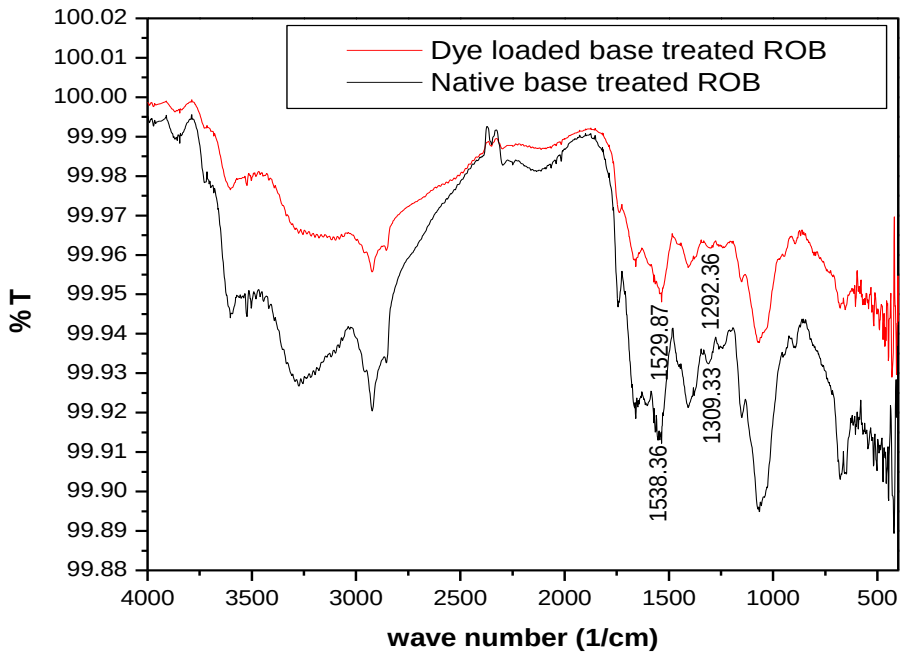
1668.991
1487.464
1297.455
1234.684

(b)


Dye loaded base treated ROB
Native base treated ROB
% T
wave number (1/cm)
100.02
100.01
100.00
99.99
99.98
99.97
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99.93
99.92
99.91
99.90
99.89
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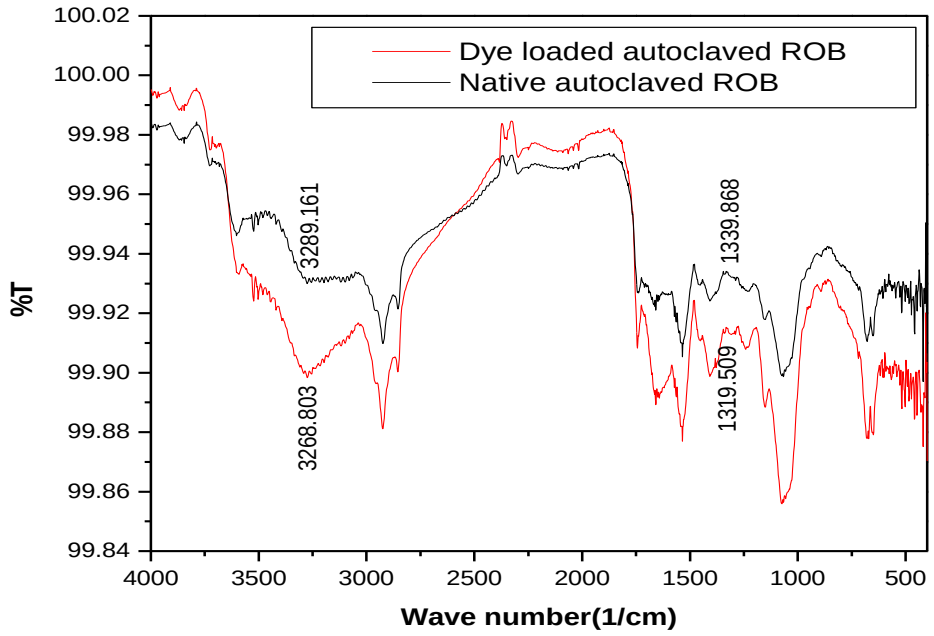
1529.87
1292.36
1309.33
1538.36

(c)



(d)

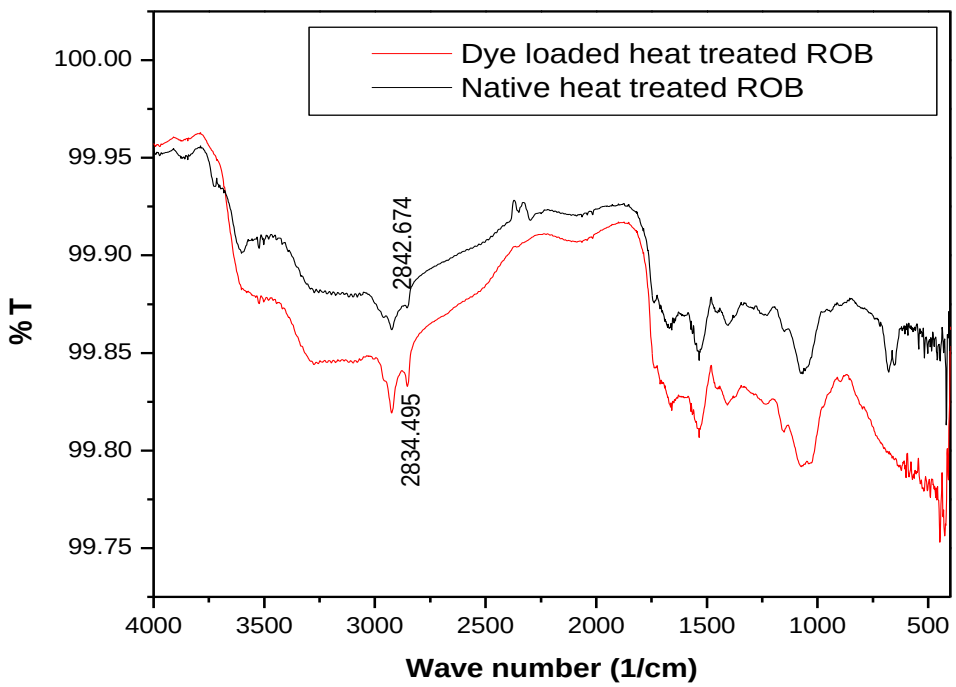


Figure3.9 FTIR spectroscopic studies of **(a)** acid treated **(b)** base treated **(c)** dead and **(d)** heat treated biomass.

3.4. CONCLUSIONS

The present study shows that Reactive Blue 4 solutions can be effectively removed by adsorption using dead biomass of *R. oryzae*(MTCC 262). Dead (autoclaved) biomass of *R. oryzae* was observed to adsorb Reactive Blue 4 more efficiently than other pre-treated biomass.Physico-chemical properties indicate that the optimum pH for adsorption is 3.0 and it is not significantly affected by incubation temperature. The process is very fast initially, reaches equilibrium within 360 min and the kinetics of the overall adsorption process was best described by pseudo-second order kinetic model. Experimental results with dead biomass at equilibrium were found to fit best to Redlich–Peterson isotherm model with higher correlation coefficient. The results of chemical modification study proved the involvement of amino and carboxyl groups in the dye adsorption process.FTIR spectroscopic study reveals the active involvement of different functional groups of acid, base, heat treated and dead biomass in dye adsorption. Cell surface study of dead *R. oryzae*showed prominent morphological changes after interaction with dye. The results of this study clearly showed that physical surface modification treatments can be used to maximize the dye removal efficiency of the fungal biomass.

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CHAPTER - 4

Removal of the dye using selected microbial biomass immobilized in different carriers selection of a potent carrier followed by optimization of the biosorption process

4.1. INTRODUCTION

There are extensive uses of synthetic dyes in textile dyeing, paper, printing, colour photography, pharmaceutics, cosmetics and other industries. Among these, azo dyes represent the largest and most versatile class of synthetic dyes [Pandey et al., 2007]. Azo dyes are characterized by the presence of one or more azo bonds (—N=N—) in their molecular structure and represent an important class of environmental contaminants. Azo dye containing effluents resist many types of treatments due to their molecular complexity. Approximately, 40,000 different dyes and pigments are used industrially presumably more than 2,000 different azo dyes are currently used and over 7×10^5 tons of these are produced annually worldwide. Approximately 10–15% of the dyes are released into the environment during manufacturing and usage [Puvaneswari et al., 2006]. Since some of the dyes are harmful, dye-containing wastes pose an important environmental problem as several commonly used azo dyes have been reported to be carcinogenic as mutagenic [Gurulakshmi et al., 2008]. Furthermore, their discharge into streams can be extremely deleterious and they hinder light penetration and oxygen transfer into bodies of water; hence threaten aquatic living organisms [Pinheiro et al., 2004]. Moreover, it is very difficult to treat textile industry effluents because of their high BOD, COD, colour, pH and the presence of dye ions [Gogate and Pandit, 2004].

Over the past three decades, biosorption has been continuously studied for the removal of textile dyes and other organic pollutants including heavy metals [Veglio and Beolchini, 1997; Aksu, 2005; Wu and Yu, 2007]. Among several biosorbent examined previously, fungal biomass has been reported as a very efficient and inexpensive sorbent that can be produced at low cost with the use of cheap growth media. It is also available as a by-product or waste material from various industrial processes [Maurya et al., 2006]. It was found that some fungus could adsorb dyes efficiently [Zhang et al., 2003; Aksu and Cagatay, 2006]. Among these fungal biomass, *Rhizopus oryzae* (MTCC 262) was used in our present study.

Unfortunately, the use of native biomass (such as bacteria, yeast, fungi and algae) in freely suspended state is limited owing to their inherent disadvantages such as small particle size, possible clogging and low mechanical strength of the biomass. In addition, a density similar to that of the suspending medium may complicate biomass/effluent separation. Immobilized biomass overcomes some of these problems and offers greater potential applications. Compared

with freely suspended cells, immobilized microbial cell systems could provide additional advantages, which include efficient and effective regeneration and reuse of the biomass, easier solid–liquid separation and minimal clogging in continuous flow systems [Bayramoglu et al., 2003]. The use of dead biomass appears to be more attractive as compared with live biomass, since heavy metals or other toxic pollutants have few influences on the biosorption process. Besides, there is no requirement for the supplement with any nutrients for maintaining growth of dead biomass. In terms of pollutant removal efficiency, it was reported that biosorption of heavy metals on immobilized dead *Trametes versicolor* was more effective than that on immobilized live *T. versicolor* [Bayramoglu et al., 2003]. Moreover, using immobilized dead cells as the biosorbent has several advantages because the mass of biosorbent used in a sorbing system is known and the sorption kinetics are not complicated by active growth of cells [Konti et al., 2016]. In terms of applicability of immobilized dead cells, it was found that immobilized fungal cells were far more stable during continuous operation in a bioreactor than the fungal cells in free forms [Arıca et al., 2003; Fu and Viraraghavan, 2003] immobilized pretreated powdered fungus, *Aspergillus niger* in a polysulphone matrix and used these immobilized cells in columns to remove dyes by biosorption from aqueous solutions. They proposed that treatment systems using the immobilized *Aspergillus niger* beads could be developed for the removal of some dyes from aqueous solutions. Hence, immobilized dead cells have a potential applicability for the removal of dyes from aqueous solutions whereas there are some undiscovered influences which may affect the dye removal (Nadeem et al., 2016). Recently, on the basis of the fungal dye biosorbent, *R. oryzae*, we have performed entrapment immobilization methods by comparing dye removal using immobilized *R. oryzae* beads entrapped in different matrix carriers viz., sodium alginate, carboxylmethyl cellulose (CMC) and agarose. The natural polymer carboxylmethyl cellulose (CMC) was found as an excellent support for immobilization. In this study, we have examined both the biosorption equilibrium and kinetics of an azo dye (Reactive Blue 4) using the inactive sodium CMC immobilized *R. oryzae* beads as the biosorbent.

In order to handle the present pollution load, the present investigation was designed to eliminate the problems associated with the use of native *R. oryzae* biomass as biosorbent for the removal of Reactive Blue 4 and the decolorizing efficiency of CMC immobilized *R. oryzae* from its aqueous solutions. The effect of initial dye concentration, initial pH, incubation temperature, and concentration of biomass were also examined. Langmuir, Fruendlich and Redlich-Peterson

adsorption isotherms were applied to the experimental data. The pseudo first-order, pseudo second-order, and intraparticle diffusion models were used for determination of the adsorption kinetics.

4.2. MATERIALS AND METHODS

4.2.1. Fungal Strain and Maintenance

The fungal strain *Rhizopus oryzae* (MTCC 262), used in this study was procured from the Institute of Microbial Technology, Chandigarh, India. Potato dextrose slant was used as the maintenance medium for the organism by sub culturing at 30°C for 120 h maintaining a regular interval of 30 days.

4.2.2. Chemicals and dye

All the chemicals and ingredients of microbiological media used in this study were obtained from E. Merck, Germany and Hi Media, India, respectively. Reactive Blue 4 [Chemical formula: $C_{22}O_8H_{22}N_6S_2Cl_2$, molecular weight: 637.4 and λ_{max} (nm): 595] was procured from Sigma-Aldrich Chemical Co., St. Louis, MO, USA The immobilization carrier, CMC sodium salt (CMC-Na) high viscosity; 1.0% in H_2O at 20°C: 300–600 mPa s; content of sodium: 6.5–6.8%) was procured from Loba Chemie Pvt. Ltd.

4.2.3. Preparation of biosorbent (dry biomass)

For immobilization, dried cells of *R. oryzae* were obtained by growing the cells in potato dextrose broth for 72 h at 30°C under continuous shaking (120 rpm). Cells were harvested by centrifugation at 5,000 rpm for 15 min. Then it was washed 2–3 times with distilled water (pH 7.0), and dried by lyophilization and stored in the desiccators until used for immobilization. Sodium alginate [Johnsen and Flink, 1986], carboxyl methyl cellulose (CMC) [Bao et al., 2008], and agarose [Lopez et al., 1997] were used as cell immobilization matrices, and the following methods were adopted for immobilization.

4.2.4. Immobilization in Alginate

Dry cells of *R. oryzae* (MTCC 262) were immobilized in calcium alginate following the method of Johnsen and Flink (1986). A 10 mL of 2% sodium alginate solution was prepared by taking the required amount of sodium alginate in distilled water and melted using a boiling water bath. Then 1 % of *R. oryzae* (MTCC 262) was added to it. The biomass alginate slurry was mixed properly to make the homogeneous solution and then added drop wise into 0.5 M $CaCl_2$ solution using a hypodermic syringe. Instantaneous spherical bead formation occurred due to cross linkage formation by Ca^{2+} at the interface of the drop solution. The beads were kept at the same solution for 2 h at 4°C for increasing its mechanical strength and then harvested from the $CaCl_2$ solution by filtration and washed thoroughly with distilled water. After that the beads were air dried and used for dye adsorption studies.

4.2.5. Immobilization in carboxyl methyl cellulose (CMC)

CMC solution (10 mL 2.5%) and 1 % of dried biomass of *R. oryzae* was mixed until homogeneous. The mixture was injected drop-wise to a $FeCl_3$ solution (0. 05 mol L^{-1}) using an injector with a 16-sized pinhead to form beads. The cell-immobilized beads were cured in the $FeCl_3$ solution for 1 h to enhance their mechanical stability. Thereafter, the freshly immobilized *R. oryzae* beads were harvested and rinsed with distilled water thrice. After that the beads were air dried and transferred into the dye solution with an initial concentration of 200 mg L^{-1} after maintaining the pH at 3.0.

4.2.6. Immobilization in agarose

A 10 mL 2% agarose solution was prepared by taking the required amount of agarose in distilled water and melted using a boiling water bath and cooled to 37° C. Required amount of dried biomass (1%) was added to it, homogenized and then poured in petriplate. After solidification on the petriplate, 3×3×3-mm^3 cubes were cut and washed with distilled water, air dried and used for the adsorption experimental purpose (Lopez et al., 1997).

4.2.7. Preparation and estimation of dye solution

A dye stock solution of 1000 mg L^{-1} was prepared by dissolving Reactive Blue 4 in distilled water and diluted to get the desired dye concentration during experiments. The concentration of Reactive Blue 4 in solution was measured with UV–VIS spectrophotometer (HITACHI U-2000) at 595 nm (λ_{max}).

4.2.8. Batch dye removal procedure by Immobilized Cell Beads

A series of batch adsorption experiment were carried out to determine the optimized adsorption condition for the removal of Reactive Blue 4 by immobilized biomass of *Rhizopus oryzae* using a 250 mL Erlenmeyer flask containing 50 mL of dye solution at 30°C, 120 rpm by varying one parameter at a time. The environmental factors influencing the adsorption experiment and the range of the parameters used in the experiment are as follows, pH: 3.0–7.0, biosorbent concentration: 1.0–8.0 g L^{-1}, initial dye concentration: 200–500 mg L^{-1} and contact time 2–420 min. Flasks containing only dye solution (without fungal biomass) were also incubated under the same conditions to serve as control. Biomass was separated from the dye solution after biosorption through centrifugation (REMI R8C) at 5000 rpm for 15 min. The residual dye concentration in the clear supernatant was determined using spectrophotometer (HITACHI U-2000) at 595 nm. The amount of dye uptake by the biosorbent was calculated using the following mass balance Eq. (4.1):

$$\mathbf{q = (C_i - C_f)\ V / 1000W} \text{..... (4.1)}$$

Where, q is the Reactive Blue 4 uptake capacity of the biosorbent (mg g^{-1}), C_i and C_f are the initial and final Reactive Blue 4 concentrations in the solution (mg L^{-1}), respectively, V is the total volume (L), and W (gm) is the amount of biosorbent, respectively.

4.2.9. Equilibrium adsorption isotherm

Adsorption isotherm study is a prerequisite for understanding the adsorbate–adsorbent interaction and to optimize of the use of the adsorbent. The efficiency of an adsorbent can be determined by its capacity to absorb a particular adsorbate. In order to understand the adsorption isotherm process, it is necessary to analyze the equilibrium adsorption data to different isotherm

models such as Langmuir, Fruendlich and Redlich–Peterson models using Eqs. (4.2) – (4.4), respectively [Inbaraj et al., 2006; Pan et al., 2006; Kumar et al., 2005].

$$q_e = q_0 K_L C_e/(1 + K_L C_e) \text{..... (4.2)}$$

$$q_e = K_F C_e^{1/n} \text{ ..... (4.3)}$$

$$q_e = AC_e(1 + B C_e^g) \text{ .....(4.4)}$$

Where C_e is the equilibrium dye concentration and q_e is the amount of dye adsorbed per gram of biomass at equilibrium. q_0 indicates the monolayer sorption capacity of adsorbent (mg g^{-1}), K_L is the Langmuir constant related to the energy of adsorption (L mg^{-1}), K_F is the Fruendlich constant related to adsorption capacity of adsorbent (mg g^{-1}), n is the Fruendlich exponent related to adsorption intensity (dimensionless). A (L g^{-1}) and B (L mg^{-1}) are Redlich–Peterson isotherm constants, 'g' is the exponent reflecting the heterogeneity of the sorbent whose value lies between 0 and 1.

The Langmuir isotherm is valid for monolayer adsorption onto a surface with a finite number of identical sites. Langmuir equation assumes that the surface is homogenous. The fitting of experimental data to Langmuir isotherm model confirms the monolayer coverage of solute onto biosorbent surface and also the homogenous distribution of solute on active sites. To determine if biosorption process is favourable or unfavourable, for the Langmuir type biosorption process, separation factor (R_L) is studied from following equation [Dahri et al., 2014]:

$$R_L = 1/ (1 + K_L C_0) \text{..... (4.5)}$$

Where C_0 (mg g^{-1}) is the highest initial dye concentration, R_L is a dimensionless constant which indicates whether isotherm is unfavourable ($R_L > 1$) or linear ($R_L = 1$) or favourable ($0 < R_L < 1$) or irreversible ($R_L = 0$) [Dahri et al., 2014].

The Fruendlich isotherm assumes that the adsorption process takes place on heterogeneous surfaces and considers the multilayer adsorption phenomenon. It assumes that adsorption capacity is related to the concentration of dye at equilibrium. The Redlich–Peterson isotherm has three parameters and has features of both Langmuir and Fruendlich isotherm equations. It is versatile and applicable for wide range of dye concentrations. It approaches the Fruendlich model at high concentrations and also suits the low concentration limit of the Langmuir equation.

4.2.10. Adsorption kinetics

The rate of uptake as well as mechanism can be divulged from kinetic studies. In general, microorganisms adsorb dye molecule by a process that includes two phase. The first one is initial rapid, reversible or irreversible metabolism independent surface binding phase followed by intra particle diffusion by specific carrier systems which is a relatively slow energy dependent process (Baldi et. al., 1990). Adsorption of Reactive Blue 4 on CMC immobilized *R. Oryzae* biomass is very fast initially and then slows down to reach equilibrium at about 360 min. The initial rapid rate was probably due to the availability of abundant binding sites on the cell surface, which became saturated after a certain period of time resulting in the decreased rate of uptake. Transportation of the dye from the solution phase into the pores of the adsorbent may also be considered as the rate controlling stage in batch experiments under rapid stirring condition (Das et al., 2006). In order to understand the rate controlling and mass transfer mechanism, the experimental kinetic data were analysed according to linear forms of first order and pseudo-second order rate models given by the Equations (4.6) and (4.7) respectively.

$$\mathbf{Log\ (q_e - q_t) = log\ q_e - k_1.t/2:303 \ldots\ldots\ (4.6)}$$

$$\mathbf{t/qt = 1/k_2 q_e^2 + t/qe \ldots\ldots\ldots\ldots\ldots\ (4.7)}$$

where q_e (mg g^{-1}) is the concentration of dye adsorbed at equilibrium, q_t (mg g^{-1}) is the concentration of dye adsorbed at time 't', k_1 and k_2 are the rate constants of the first and second order rate equations, respectively.

These kinetic rate models could not identify the diffusion mechanism; hence the obtained results were further analyzed by using the intraparticle diffusion (Weber and Morris, 1963) model presented by Eq. (4.8.).

$$\mathbf{q_t = K_D\, t^{1/2} \ldots\ldots\ldots\ldots\ (4.8)}$$

Where q_t (mg g^{-1}) and K_D represent the amount of Reactive Blue 4 adsorbed per unit weight of biomass at a time ''t'' and diffusion coefficient, respectively. Thus, the plot of q_t versus $t^{1/2}$ would be a straight line according to diffusion controlled transport mechanism.

4.2.11. SEM study

Pristine and dye laden beads of fungal biomass were coated with platinum by a vacuum electric sputter coater to finest thickness before glue mounted on it (Bai and Li, 2005). Surface morphology was examined with a field emission scanning electron microscope (JEOL JSM-6360F).

4.2.12. Model fitting and statistical measurement

Using Origin 6.0 Professional adsorption isotherm experimental data were fitted and analysed in some standard isotherm models viz., Langmuir isotherm, Fruendlich isotherm and Redlich-Peterson isotherm model.

4.3. RESULTS AND DISCUSSIONS

4.3.1. Selection of matrix

Dry cells of *Rhizopus oryzae*, immobilized in sodium alginate, carboxymethyl cellulose (CMC) and agarose were used and compared for their efficiencies in Reactive Blue 4 removal. Bead integrity and leaching of cells from immobilized matrices were also compared among these three different carriers. Experiments were carried out in 250 mL flasks and incubated at 30°C, 120 rpm for 20 h. Samples were withdrawn at regular intervals and residual dye in the solution was measured. It was evident from the screening experiment [**Table 4.1(a)]** that the CMC immobilized *R. oryzae* cells was the most effective biosorbent for dye removal. Using this combination, about 99% of 200 mg L^{-1} dye was removed without any leakage of cells or visible disintegration of beads. While *R. oryzae* biomass immobilized in other two carriers showed poor efficiencies for the removal of the same dye.

4.3.2. Effect of the concentration of CMC

Carboxymethyl cellulose at different concentrations viz., 2.0%, 2.5%, and 3.0% were used to determine the suitable concentration. It was observed from **Table 4.1 (b)** that with decrease in the concentration of CMC, the adsorption of dye was increased due to larger pore size and greater cell-dye interaction but the concentration of CMC less than 2.5% results into poor bead integrity and lesser mechanical strength of beads (Bao et al., 2008). Therefore, recycling of the beads as biosorbent was not possible with the concentration of CMC below 2.5%.

4.3.3. Effect of bead size on dye adsorption

During the time of immobilizing the *R. oryzae* cells using CMC as a carrier, the needle size was varied to prepare the beads with different diameters. Therefore, the 2.1, 2.2 and 2.3 sized beads were prepared using different injector. It was observed **[Table 4.1(c)]** that dye removal increased from 2.1±0.2 mm bead size to 2.2±0.2 mm, then it was found to decrease. Available surface area was increased with increase in bead size and hence number of pore was also increased.

Table 4.1(a). Removal of Reactive Blue 4 by immobilized *R. oryzae* (MTCC 262) using different matrices, viz, calcium alginate, agarose and carboxymethyl cellulose (CMC) (Bead size 2.5 ±0.2).

Carrier matrix	q (mg g^{-1})
calcium alginate (A)	28.85
agarose (B)	22.86
carboxymethyl cellulose (C)	55.89

Table 4.1(b). Removal of Reactive Blue 4 by immobilized *R. oryzae* (MTCC 262) using different CMC concentrations (Bead size 2.5 ±0.2).

CMC concentration (%)	q [CMC with biomass] (mg g^{-1})	q [CMC](mg g^{-1})
2.0	62.44	25.72
2.5	55.22	23.08
3.0	49.10	20.95
3.5	44.36	18.85

Table 4.1(c). Removal of Reactive Blue 4 by immobilized *R. oryzae* (MTCC 262) using various bead sizes: 2.4 ± 0.2, 2.5 ± 0.2 and 2.6 ± 0.2 mm.

Bead size (mm.)	q (mg g^{-1})
2.4±0.2 (A)	53.62
2.5±0.2 (B)	55.78
2.6±0.2 (C)	49.37

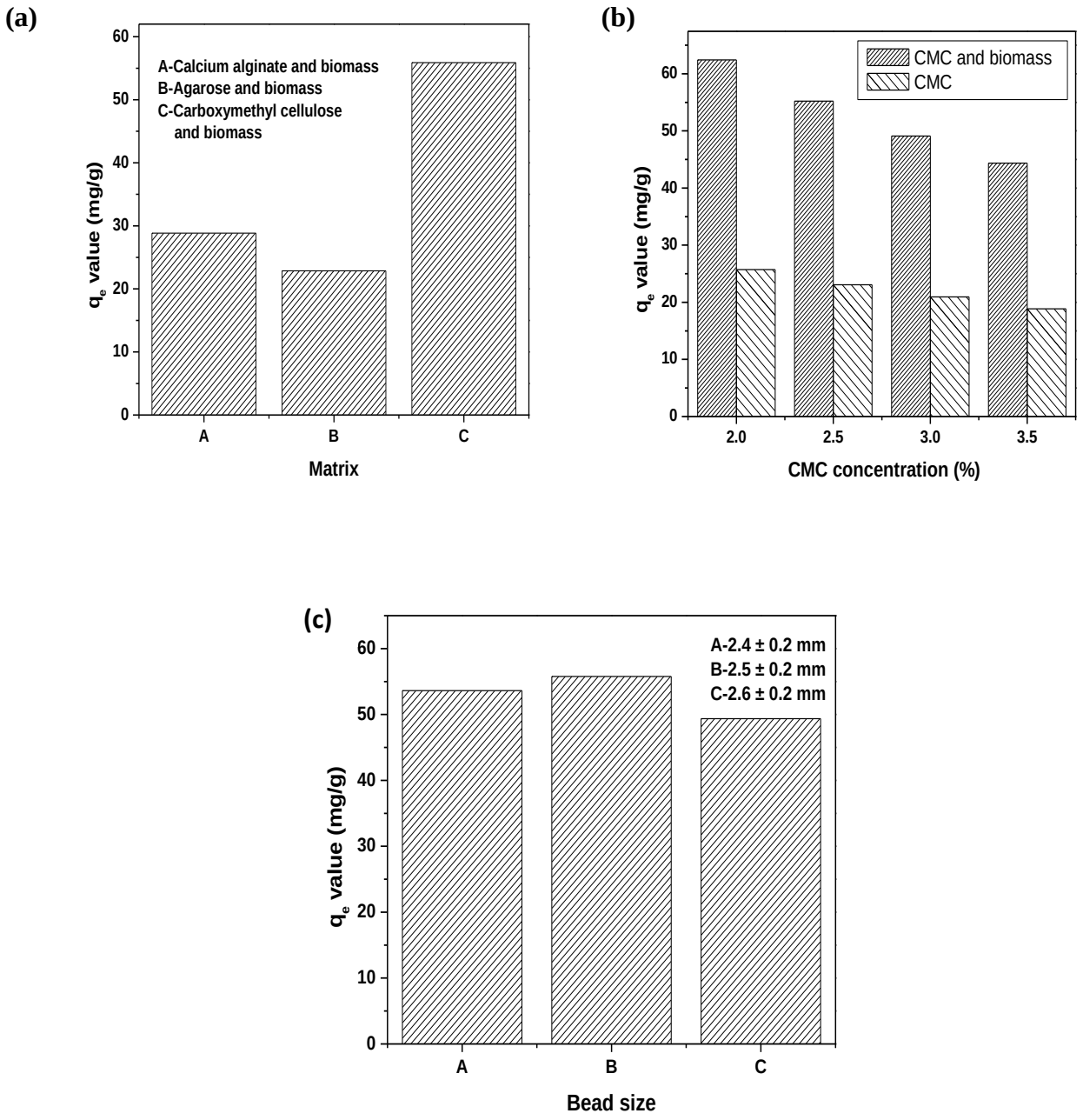

Figure 4.1. Removal of Reactive Blue 4 by immobilized *R. oryzae* (MTCC 262) using **(a)** different matrices: calcium alginate, agarose and carboxymethyl cellulose (CMC); **(b)** different CMC concentrations and **(c)** various bead sizes: 2.4 ± 0.2, 2.5 ± 0.2 and 2.6 ± 0.2 mm.

4.3.4. The effect of initial pH on biosorption

In aquatic systems, Initial pH of dye solution is considered to be a main variable as pH is an important environmental factor influencing biosorbent site dissociation as well as the solution chemistry of the dyes (Maurya et al., 2006). The biosorption of Reactive Blue 4 in the initial pH range of 3.0–7.0 was carried out for 20 h in the study. It is evident from **Figure 4.2** that biosorption percentage of dye on *Rhizopus oryzae* biomass increased with decrease in pH values and maximum biosorption was occurred at pH 3.0. The biosorption percentage was decreased with increasing initial pH values, and it was 99.42% (q value 71.25 mg g^{-1}) at initial pH value 3.0 and 83.78% (q value 54.24 mg g^{-1})at pH 7 **(Table-4.2)**. Therefore, pH 3.0 was used in the further experiments.

At low pH values, the surface of biomass would be surrounded by hydronium ions which may lead to a net positive charge on CMC immobilized *R. oryzae* beads surface (Maurya et al., 2006). It was expected that nitrogen-containing functional groups such as amines or imadazoles in the biomass would also be protonated at acidic pH values (Aksu and Cagatay, 2006). On the other hand, Reactive Blue 4 has its two sulphonate groups which could be ionized into negatively charged sulfonate anions in an aqueous solution. Therefore, high biosorption capacities obtained at low pH values may result from the electrostatic attractions between these negatively charged dye anions and positively charged CMC immobilized *R. oryzae* beads. The decrease of biosorption capacity of dye with increasing initial pH (**Figure 4.2**) values can be attributed to the change in surface characteristics and charge. Nevertheless, it seems very difficult to quantitatively describe the biosorption mechanisms with respect to initial pH due to a large number of variables involved in the biosorption of dye by the biomass entrapped in CMC immobilized *R. oryzae* beads and the complexity of the surface and water chemistry (Aksu and Tezer, 2005).

Table 4.2. Effect of pH on removal of Reactive Blue 4 by CMC immobilized *R. oryzae* (MTCC 262) beads.

pH	q (mg g^{-1})
3	71.25
4	65.54
5	61.89
6	57.56
7	54.24

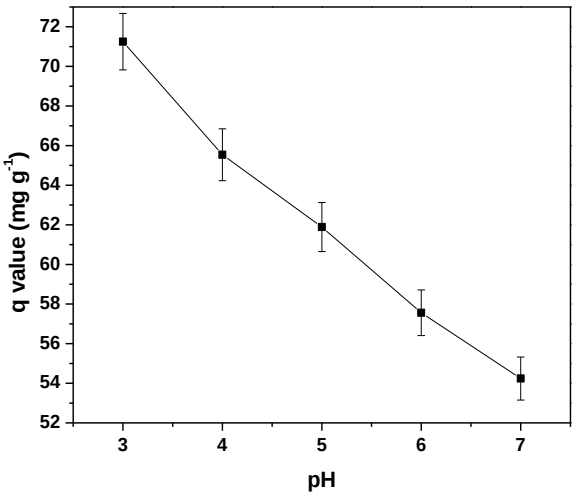

Figure 4.2. Effect of pH on removal of Reactive Blue 4 by CMC immobilized *R. oryzae* (MTCC 262) beads.

4.3.5. Effect of Temperature

To determine the optimum temperature for Reactive Blue 4 adsorption using immobilized *Rhizopus oryzae*, temperature was varied from 20˚C to 35˚C with other conditions remaining the same. As shown in **Figure 4.3**, temperature has a very little effect on biosorption over the range 20° to 35°C. However, higher temperature slightly enhances the biosorption capacity due to

increased surface activity of cells and kinetic energy of solute molecules (Binupriya et al., 2010). The present study also showed slight increase in uptake capacity (q value) from 68.86 mg g^{-1} (at 20°C) to 70.94 mg g^{-1} (at 35°C). Thus, an economically feasible temperature within the above range may be suitably used for the biosorption process.

Table 4.3. Effect of temperature on removal of Reactive Blue 4 by CMC immobilized *R. oryzae* (MTCC 262) beads.

Temperature (°C)	q (mg g^{-1})
20	68.86
25	69.44
30	70.12
35	70.94

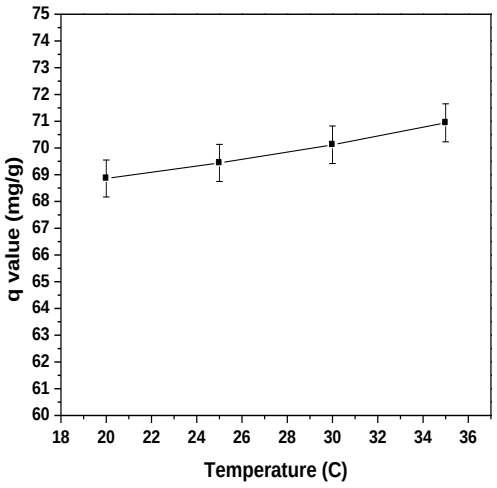

Figure 4.3. Effect of temperature on biosorption of Reactive Blue 4 using immobilized cell of *R. oryzae* (MTCC 262).

4.3.6. Effect of biomass concentration in beads

Effect of biomass concentration using immobilized biomass was investigated using 1.0–8.0 g L^{-1} concentration of dry fungal biomass (immobilized in CMC) in 50 mL dye solution separately in each of 250 mL Erlenmeyer flask (at pH 3.0, temperature 30◦C, incubation time 20 h). From **Figure 4.4**, it was observed that using carboxymethyl cellulose as immobilization carrier, removal percentage increased from 96.75 to 99.83. At the same time, the q value decreased from 69.11-23.76. Availability of dye adsorption sites increases with increasing cell mass concentration, but due to agglomeration of biomass, total adsorption sites are not all available and hence the q value decreases [Selatnia et al., 2004].

Table 4.4. Effect of biomass concentration on removal of Reactive Blue 4 by CMC immobilized *R. oryzae* (MTCC 262) beads.

Biomass load (g L^{-1})	Percent removal (%)	q (mg g^{-1})
1	96.75	69.11
2	98.54	54.74
4	98.8	38.00
6	99.32	29.21
8	99.83	23.76

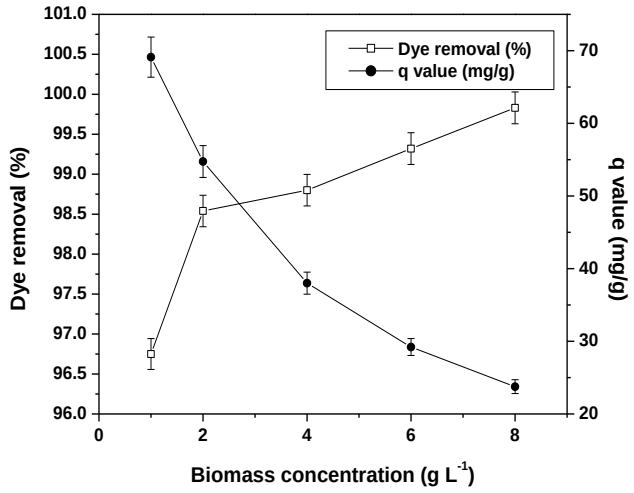

Figure 4.4. Effect of biomass concentration on biosorption of Reactive Blue 4 using immobilized biomass of *R. oryzae* (MTCC 262).

4.3.7. Equilibrium adsorption isotherm

It is observed that the dye adsorption capacity increases with increasing the dye concentration and ultimately attains equilibrium value [Volesky and Prasetyo, 1994] and the maximum adsorption capacity is found to be 175.97 mg g^{-1} (**Figure 4.5**). **Figure 4.5** shows the adsorption equilibrium plot of Reactive Blue 4. Isotherm model parameters were obtained from the slope and intercept of their respective equilibrium plots. All the values of isotherm parameters and linear regression correlation coefficient (R^2) for all three isotherm models have been represented in Table 1 for comparison. It was observed that the adsorption data were very well represented by both Langmuir isotherm and Redlich–Peterson isotherm models with correlation coefficient (R^2) value 0.988 and 0.986 respectively. Fruendlich isotherm showed a correlation coefficient (R^2) of 0.972. The process of adsorption was considered favourable as the value of Langmuir separation factor R_L = 0.128 lies between 0 and 1, also the Fruendlich exponent, n = 2.19 was in the range 1–10 and value of adsorption capacity (q_0) was also reasonably high, all of which indicated a strong electrostatic force of attraction between the adsorbate and adsorbent. Based on linear regression correlation coefficient (R^2) values of all the three studied isotherm models, the present sorption data were found to be described best by both Langmuir and Redlich–Peterson isotherm model **[Figure 4.5.(a)].** These observations indicate that the adsorption mechanism is a hybrid one and does not follow the ideal monolayer adsorption behaviour [Pan et al., 2006; Weber and Moris, 1963; Kumar et al., 2005; Dahri et al., 2014]. The graphical presentation of experimental q value versus theoretical q value **[Figure 4.5 (b)]** yields a very good straight line with R^2 value of 0.996 and this indicates that the adsorption isotherm strictly follows the Redlich–Peterson isotherm model (Redlich and Peterson, 1959)

Table 4.5. Table for adsorption isotherm on removal of Reactive Blue 4 by CMC immobilized *R. oryzae* (MTCC 262) beads.

Equilibrium concentration	q_e (mg g^{-1})
3.24	56.21
3.96	70.29
5.02	84.28
6.14	98.24
9.22	111.65
14.46	124.44
27.08	135.12
52.84	156.33
107.84	169.18
184.1	175.97

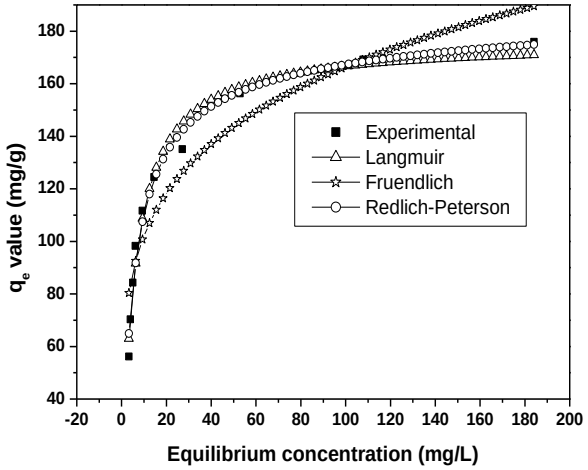

Figure 4.5 (a). Adsorption isotherm of Reactive Blue 4 using immobilized cell of *R. oryzae* (MTCC 262).

Table 4.5 (a). Table for Experimental q value Vs Theoretical q Value of adsorption isotherm on removal of Reactive Blue 4 by CMC immobilized *R. oryzae* (MTCC 262) beads.

Experimental q value	Theoretical q Value
56.21	53.78
70.29	68.44
84.28	84.80-
98.24	100.56
111.65	116.91
124.44	125.14
135.12	142.45
156.33	159.25
169.18	179.32

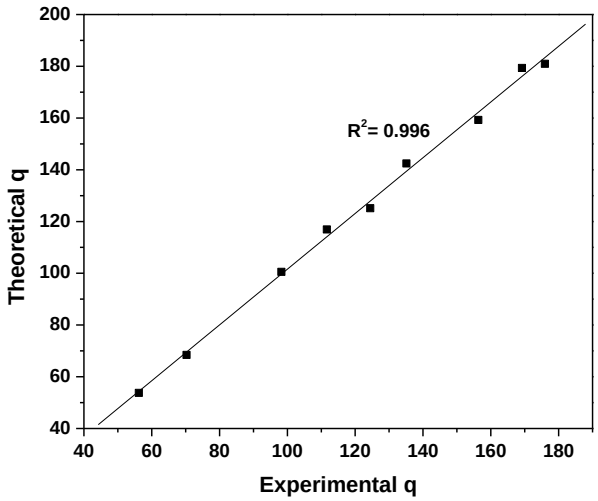

Figure 4.5 (b). Experimental q value Vs theoretical q value of adsorption isotherm study of Reactive Blue 4 by immobilized biomass of *Rhizopus oryzae* (MTCC 262).

Table 4.6. Adsorption Isotherm coefficients correlation and constants for removal of Reactive Blue 4 by dead and immobilized cells of *Rhizopus oryzae* (MTCC262).

Adsorbent	Langmuir				Fruendlich				Redlich-Peterson				
	q_0 mg g^{-1}	K_L L mg^{-1}	R^2	χ^2	n^{-1}	K_F L g^{-1}	R^2	χ^2	K_{RP}(L g^{-1})	α (L mg^{-1})	B (g)	R^2	χ^2
Immobilized cell	49.46	0.128	0.988	143.81	0.455	17.57	0.972	332.55	1.28	2.15	0.04	0.986	178.26

4.3.8. Biosorption kinetics

Experimental data show high degree of nonlinearity (**Figure 4.7**) and poor correlation coefficients for first-order model. On the other hand, linearity (**Figure 4.8)** with high degree of correlation coefficient indicates that the present sorption process follows pseudo-second order rate model (**Table 4.11**).

The results presented in **Figure 4.9** shows a multi linearity of three steps. Maximum adsorption occurs at the external surface of the adsorbent, and the initial sharp portion of the observed curve may be assigned to the instantaneous adsorption stage (Konti et al., 2016). The dye adsorption behaviour at latter stages does not follow the model given in Eq. (6). This suggests that intraparticle diffusion (McKay et al., 1980) governs the successive adsorption stages since most of the available binding sites are occupied. The third portion corresponds to the ultimate equilibrium stage where the rate is inhibited due to the non availability of the binding sites and the process would probably exhibit transition from diffusion to attachment control characteristics (Das et al., 2006).

Table 4.7. Table for adsorption kinetics on removal of Reactive Blue 4 by CMC immobilized *R. oryzae* (MTCC 262) beads

Time (min)	q_e (mg g^{-1}) [200 mg L^{-1}]	q_e (mg g^{-1}) [400 mg L^{-1}]	q_e (mg g^{-1}) [500 mg L^{-1}]
1	15.63	31.35	39.17
2	15.81	32.02	39.72
4	16.72	33.74	41.96
6	17.83	36.08	44.67
8	18.01	37.86	45.22
10	19.29	38.94	48.52
15	19.96	39.72	50.09
20	21.97	43.45	54.98
30	30.39	61.26	75.87
45	33.55	66.94	83.92
60	35.93	72.16	90.02
90	42.75	85.36	106.88
120	45.42	91.6	113.75
150	47.51	95.42	118.82
180	49.26	99.16	123.25
240	50.97	102.66	127.52
300	51.84	103.94	129.7
360	52.01	105.58	130.12
420	52.26	105.89	130.65

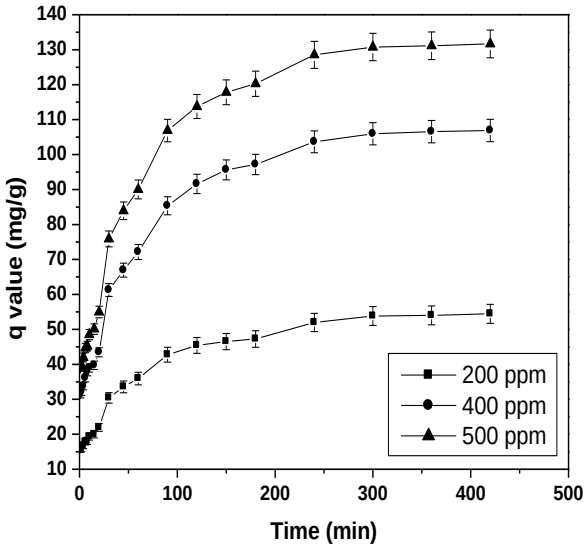

Figure 4.6. Adsorption kinetics model on removal of Reactive Blue 4 using immobilized cell of *R. oryzae* (MTCC 262).

Table 4.8. Pseudo first order rate values at 200, 400, 500 mg L^{-1}

Time (min)	$\log (q_e-q_t)$(g-min mg^{-1})		
	On removal of Reactive Blue 4 at dye concentration		
	200(mg L^{-1})	400(mg L^{-1})	500(mg L^{-1})
2	1.587	1.874	1.963
4	1.576	1.864	1.952
6	1.563	1.85	1.939
8	1.561	1.839	1.936
10	1.546	1.832	1.919
15	1.537	1.827	1.911
20	1.511	1.802	1.884

30	1.381	1.659	1.746
45	1.32	1.601	1.678
60	1.267	1.54	1.619
90	1.068	1.333	1.393
120	0.956	1.184	1.252
150	0.9	1.051	1.14
180	0.857	0.988	1.056
240	0.396	0.509	0.495

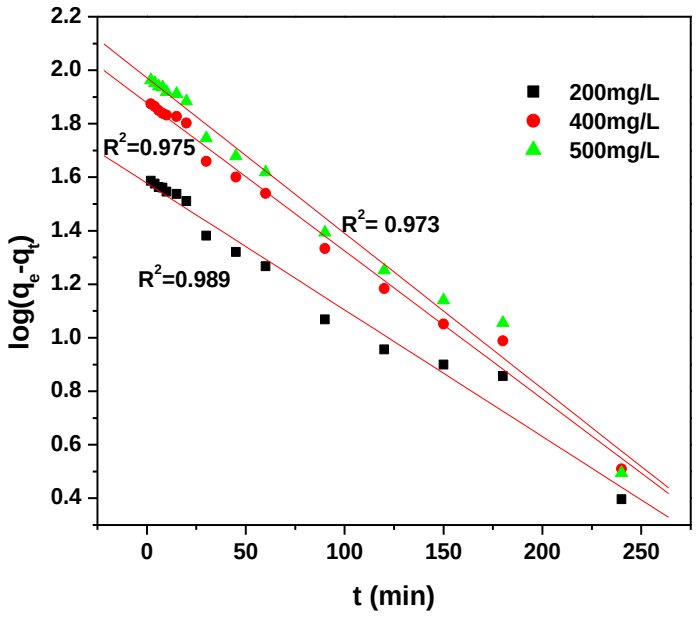

Figure 4.7. Pseudo first order rate kinetic model on removal of Reactive Blue 4 using immobilized biomass of *R. oryzae* (MTCC 262).

Table 4.9. Pseudo second order rate values at 200, 400, 500 mg L^{-1} dye concentrations

Time (min)	t/q_t (g-min mg^{-1})		
	On removal of Reactive Blue 4 at dye concentration		
	200 (mg L^{-1})	400 (mg L^{-1})	500 (mg L^{-1})
2	0.126	0.062	0.05
4	0.239	0.118	0.095
6	0.336	0.166	0.134
8	0.444	0.211	0.176
10	0.518	0.256	0.206
15	0.751	0.377	0.299
20	0.91	0.46	0.363
30	0.987	0.489	0.395
45	1.341	0.672	0.536
60	1.669	0.831	0.666
90	2.105	1.054	0.842
120	2.642	1.31	1.054
150	3.225	1.568	1.273
180	3.808	1.852	1.496
240	4.618	2.315	1.867
300	5.572	2.831	2.294
360	6.665	3.377	2.745
420	7.712	3.929	3.19

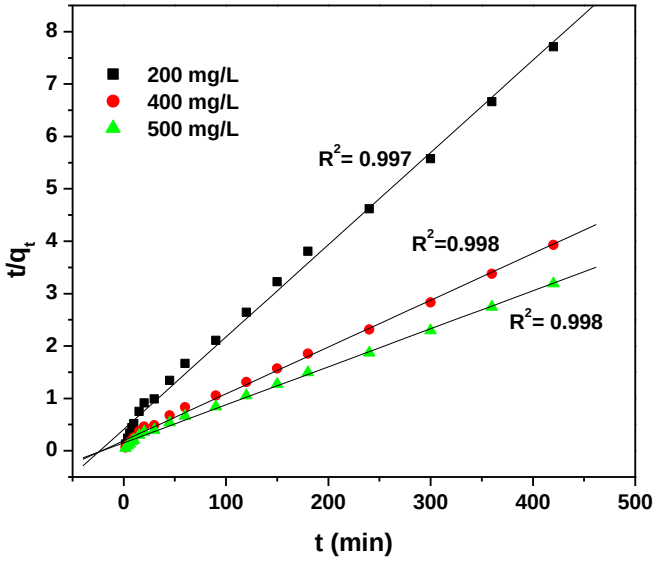

Figure 4.8. Pseudo second order rate kinetic model on removal of Reactive Blue 4 using immobilized biomass of *R. oryzae* (MTCC 262).

Table 4.10. Intraparticle diffusion rate values at 200, 400, 500 mg L^{-1} dye concentrations

$t^{1/2}$ (min ½)	q_t mg g^{-1} [200 mg L^{-1})	q_t mg g^{-1} [400 mg L^{-1})	q_t mg g^{-1} [500 mg L^{-1})
1.41	15.81	32.02	39.72
2.00	16.72	33.74	41.96
2.41	17.83	34.93	44.67
2.77	18.01	36.02	45.22
3.12	19.29	37.63	48.52
3.82	19.96	39.72	52.33
4.61	24.03	45.75	61.54
5.57	30.39	55.55	75.87
6.70	33.55	66.94	83.92
7.85	37.05	73.46	93.05
9.48	42.75	85.36	106.88
10.95	45.42	91.60	113.75
12.24	46.51	95.62	117.82
13.65	49.04	99.56	123.47
15.49	51.97	103.66	128.52
17.32	53.84	105.94	130.74
19.09	55.03	106.58	131.12
20.49	56.13	106.89	131.65

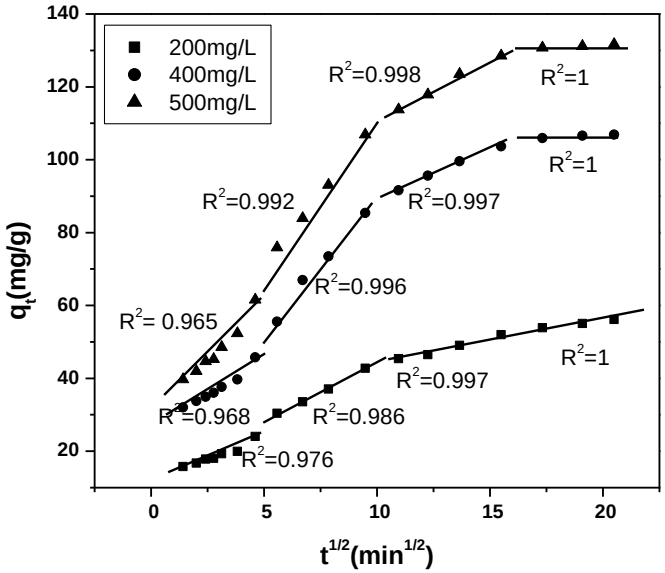

Figure 4.9. Intraparticle diffusion rate kinetic model on removal of Reactive Blue 4 using immobilized cell of *R. oryzae* (MTCC 262).

Table 4.11. Effect of initial dye concentration on kinetic parameters for sorption of Reactive Blue 4 on dead and immobilized cells of *Rhizopus oryzae* (MTCC262).

Adsobent	Initial dye concentration (mg L^{-1})	Pseudo-first order kinetic model			Pseudo-second order kinetic model		
		q_e (mg g^{-1})	k1(min^{-1})	R^2	q_e (mg g^{-1})	K_2 (g mg min^{-1})	R^2
Immobilized cell	200	6.28	0.08	0.989	11.25	0.04	0.997
	400	9.08	0.16	0.975	13. 32	0.06	0.998
	500	12.76	0.21	0.973	16.12	0.22	0.998

4.3.9. SEM analysis

SEM analysis was used to identify the morphological changes due to adsorption of textile dyes on adsorbent cells. Examination of CMC immobilized *R. oryzae* immobilized in CMC before and after dye sorption by scanning electron microscopy was carried out to locate the active adsorptive areas of these sorbents. The electron micrograph of *R. oryzae* (whole bead) after Reactive Blue 4 adsorption (**Figure 4.10. b**) was denser, and the surface had started to fracture in comparison with the smooth surface of control experiment (**Figure 4.10. a**) that were not in contact with the dye molecule. The changes in structure of the immobilized cells were probably influenced by the dye ions. Thus, it can be assumed that the dye attached to the functional groups present on the immobilized biomass surface was the reason for the morphological changes found on CMC immobilized cell surfaces [Nadeem et al., 2016]. When immobilized *R. oryzae* biomass was introduced to higher magnification at 3000 X and 7000 X, [Figure 4.10. (c); (d); (e); (f)] the shape of the cells found on the surface of the beads became irregular and more folded, and they started to rupture and shrink. They were also attached to each other [Mehta et al., 2002]. It is evident from the micrograph of higher magnification that the white coloured fungal cells were also changed into blackish due to adsorption of Reactive Blue 4.

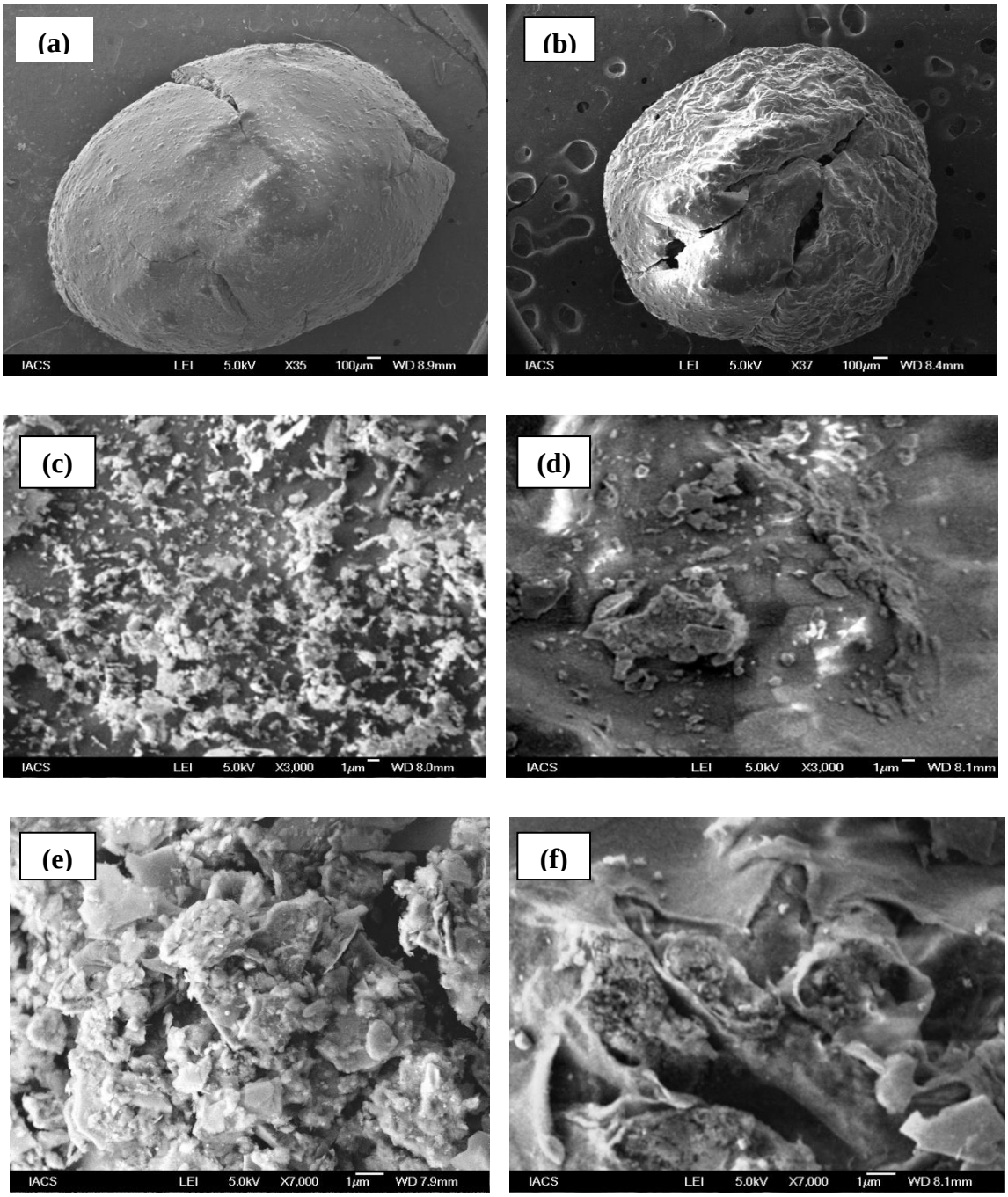

Figure 4.10. Scanning Electron micrograph of (a) pristine CMC immobilized *R. oryzae* (whole bead) (b) dye loaded CMC immobilized *R. oryzae* (whole bead) (c) pristine CMC immobilized *R. oryzae* at 3000X (d) dye loaded CMC immobilized *R. oryzae* at 3000X (e) pristine CMC immobilized *R. oryzae* at 7000X (f) dye loaded CMC immobilized *R. oryzae* (MTCC 262) biomass at 7000X.

4.4. CONCLUSIONS

The present study shows that Reactive Blue 4 solutions can be effectively decolorized by adsorption using immobilized *R. oryzae* beads. 2.5 % Carboxymethyl cellulose was found to be the most effective immobilization carrier for dye removal. Physico-chemical properties indicate that the optimum pH for adsorption is 3.0 and it is not affected by incubation temperature. The process is very fast initially, reaches equilibrium within 360 min and the kinetics of the overall adsorption process was best described by pseudo-second order kinetic model. Experimental results at equilibrium were found to fit best to both Langmuir and Redlich-Peterson isotherm model. Cell surface study of CMC immobilized (MTCC 262) using scanning electron microscopy showed morphological changes of *R. oryzae* beads after interaction with dye. On the whole, it can be concluded that among the various carrier used in this study for immobilization purpose *R. oryzae* (MTCC 262), immobilized in CMC were found most efficient due to high availability of adsorption sites for the removal Reactive Blue 4.

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CHAPTER - 5

Desorption of dye from loaded dry and immobilized biomass of Rhizopus oryzae (MTCC 262)

5.1. INTRODUCTION

Recovery of dye from the loaded microbial biomass from their aqueous solution for repeated use is of great significance for any successful biosorption process development. Regeneration of biosorbent for repeated use is important to make the process cost effective, eco-friendly and thereby decrease the dependency of the process on the continuous supply of the biosorbent. Desorption studies help in elucidating the mechanism of dye removal and recovery from dye loaded adsorbent and also for the regeneration and recycling of spent adsorbents, which in turn may reduce operational cost and protect the environment (Karim et al., 2017). Several researchers investigated the efficiency of desorption of loaded biosorbent with various mineral acids and strong complexion agents such as EDTA (Panda et al., 2006; Bunluesin et al., 2007; Robinson et al., 2002). For successful regeneration, the selection of an appropriate eluant is important, which strongly depends on the type of biosorbent and the mechanism of biosorption. Desorption studies were carried out with four desorption media *viz*., 1(N) NaOH solution, 70% ethanol, 70% acetone, and distilled water separately (Binupriya et al., 2010).

We have already seen that *R. oryzae* was proved to be an efficient Reactive Blue 4 remover from its aqueous solution in dry and immobilized form. In the present study a useful effort has been made to desorb the Reactive Blue 4 from the adsorbent and reuse of the adsorbent for few cycles.

5.2. METHODOLOGY

5.2.1. Micro-organism

R. oryzae (MTCC 262) was maintained on potato dextrose agar slant by monthly sub-culturing at 30 °C for 120 h and stored at 4 °C for further use.

5.2.2. Chemicals and dye

All the chemicals and ingredients of microbiological media used in the present study were purchased from E. Merck, Germany and Hi Media, India, respectively. Reactive Blue 4 [Chemical formula: $C_{22}O_8H_{22}N_6S_2Cl_2$, molecular weight: 637.4 and λ_{max} (nm): 595] was procured from Sigma-Aldrich Chemical Co., St. Louis, MO, USA.

5.2.3. Medium composition and biomass production

The procedure of biomass production has been described in chapter 2 & 3.

5.2.4. Preparation of dry cells and immobilized cells

The dry biomass of *Rhizopus oryzae* and carboxymethyl cellulose (CMC) immobilized *R. oryzae* biomass was prepared as described in chapter 2 and chapter 4 respectively.

5.2.5. Preparation of dye solution

The stock solution of Reactive Blue 4 (1000 mg L^{-1}) was prepared by the procedure as described in chapter 3.

5.2.6. Estimation of dye concentration

The concentration of dye in the solution was determined using a UV-visible spectrophotometer (HITACHI U-2000) at maximum absorption wavelength, (λ_{max}) 595 nm, which was determined by spectrophotometrical scanning of the dye over a range of wavelength (200-800 nm).

The uptake of dye by the adsorbent was calculated by the procedure described in chapter 3.

5.2.7. Adsorption studies

For the adsorption study 0.1 g of *Rhizopus oryzae* (MTCC 262) in dry and immobilized form were added separately to each of 250 mL Erlenmeyer flasks containing 50 mL dye solution (100 mg L^{-1}) having a pH value of 3.0, and incubated at 30°C in a shaker (120 rpm) for 8 h. After incubation, the biomass was separated by centrifugation at 5000 rpm for 15 min and the residual concentration of Reactive Blue 4 in the supernatant was measured by UV-Visible spectrophotometer.

5.2.8. Desorption studies

Desorption studies were performed using different type of four eluant including polar and non-polar solvents having different chemical structure and properties. Among polar solvents one organic and another inorganic solvent was chosen as eluant to find out the proper explanation regarding the dye desorption mechanism. As dye adsorption was found the best under acidic pH, it can be assumed that the solvent having alkaline properties may be suitable for dye desorption. After the adsorption experiment, the dye laden *R. oryzae* biomass was taken in different 250 mL Erlenmeyer flasks and 50 mL of four different eluant i.e., 1(N) NaOH solution, 70% ethanol, 70% acetone, and distilled water were added separately in each of the flasks. The flasks were agitated at 120 rpm for 12 h at 30°C and the concentration of the dye in the supernatant was measured. In case of immobilized biomass 0.1 (N) NaOH was used as desorbing agents instead

of 1 (N) NaOH for the stability of CMC beads and other agents used in same concentration mentioned above.

After the desorption process, the adsorbents were collected from the solutions by centrifugation, washed with distilled water, and reused in the next cycle of the adsorption experiment. The adsorption-desorption experiments were conducted upto four cycles for free dry biomass and five cycles for immobilized biomass. The concentration of desorbed dye was estimated using a spectrophotometer (HITACHI U-2000) at 595 nm (Bhatt et al., 2000).

The desorption efficiency was calculated by the following equation **(5.1)**:

$$\textbf{Desorption efficiency (\%)} = \frac{\textbf{Amount of dye desorbed}}{\textbf{Amount of dye adsorbed}} \times \textbf{100} \qquad \textbf{(5.1)}$$

5.3. RESULTS AND DISCUSIONS

Desorption of Reactive Blue 4 from the dye laden biomass for repeated use is extremely important for any useful biosorption process development (Ganguly et al., 2011). Desorption not only attempts to reuse the spent adsorbent for next adsorption cycle but also tries to recover the adsorbed dyes. It not only makes the entire process more eco-friendly, but also helps to determine the mechanism of adsorption (Fu and Viraraghavan, 2003).

In the present study, it was observed that 98.92% desorption was possible using 1(N) NaOH (**Table 5.1** and **Figure 5.1**) as eluant from dry biomass of *R. oryzae*. The removal and recovery efficiencies of the dry fungal biomass of *Rhizopus oryzae* in four cycles was shown in **Table 5.2** and **Figure 5.2**. It shows that biomass is reusable up to four cycles with 60.48% adsorption. After that the percent desorption was found below 40 % (37.77).

In case of CMC immobilized *R oryzae* beads about 96.52% desorption was observed using 0.1 (N) NaOH solutions as eluant. The adsorption-desorption efficiencies of immobilized biomass of *R oryzae* was shown in **Figure 5.4**. It indicated that the immobilized biomass is re-usable upto five consecutive cycles with 66.76% desorption and after that the percent desorption decreases below 60 % (52.22).

When the biomass was treated with NaOH solution under strong alkaline conditions, the adsorbent attains a negative charge and repels the anionic dye resulting in desorption (Binupriya

et al., 2010). Desorption results also revealed that no degradation has occurred during the biosorption process as the percent adsorption and desorption almost matched.

Table 5.1. Effect of the type of desorption agents on the desorption of Reactive Blue 4 from dry biomass of *Rhizopus oryzae* (MTCC 262).

Desorption agents	Desorption efficiency (%)
70% Ethanol	71.06
1(N) NaOH	98.85
70% Acetone	88.64
Distilled water	9.15

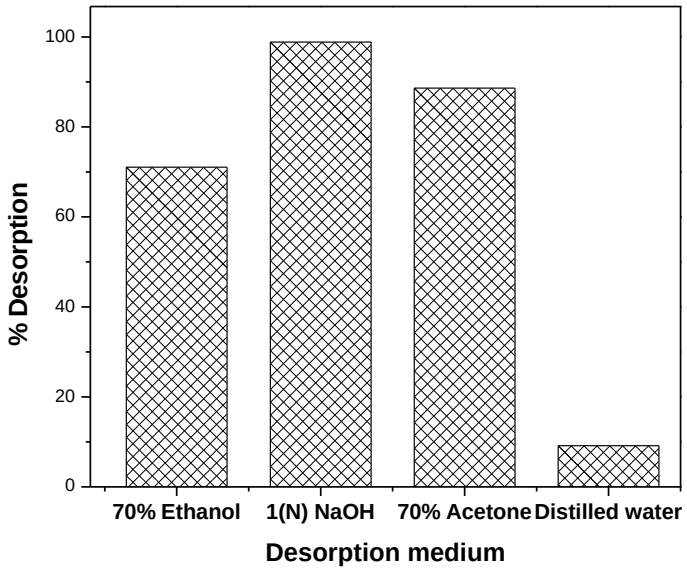

Figure 5.1. Effect of different desorption agents on desorption of Reactive Blue 4 from dry biomass of *Rhizopus oryzae* (MTCC 262).

Table 5.2 Adsorption-Desorption cycle of Reactive Blue 4 by dry biomass of *Rhizopus oryzae* (MTCC 262).

Cycle Number	Percent adsorption	Percent desorption
1	99.35	98.92
2	97.01	94.96
3	83.34	78.74
4	68.26	60.48
5	49.94	37.77

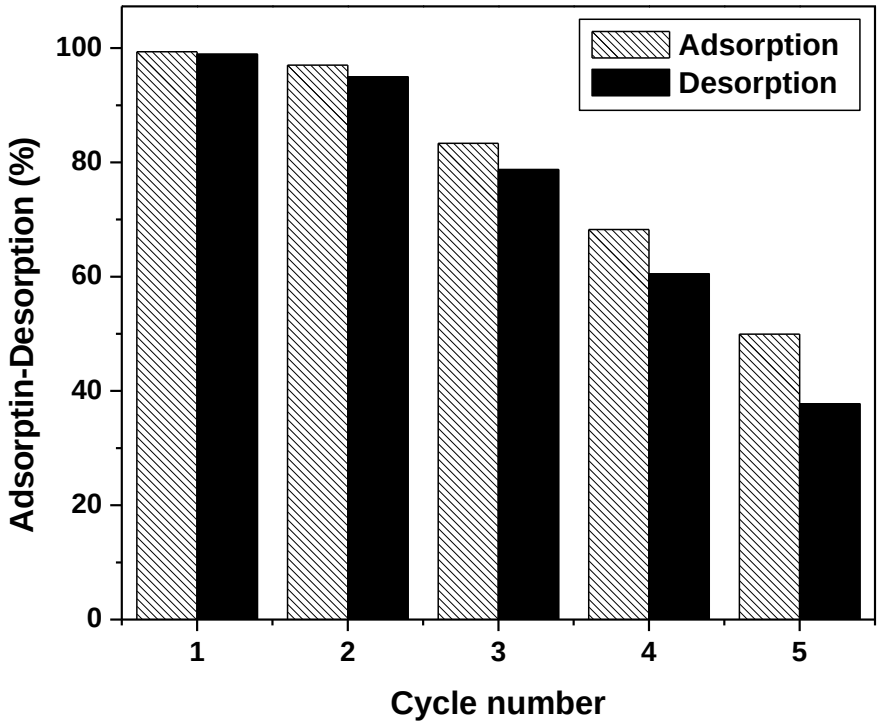

Figure 5.2 Adsorption-Desorption cycle of Reactive Blue 4 by dry biomass of *Rhizopus oryzae* (MTCC 262).

Table 5.3 Effect of the type of desorption agents on the desorption of Reactive Blue 4 from immobilized biomass of *Rhizopus oryzae* (MTCC 262).

Desorption agents	Desorption efficiency (%)
70% Ethanol	70.76
0.1(N) NaOH	96.35
70% Acetone	84.69
Distilled water	18.15

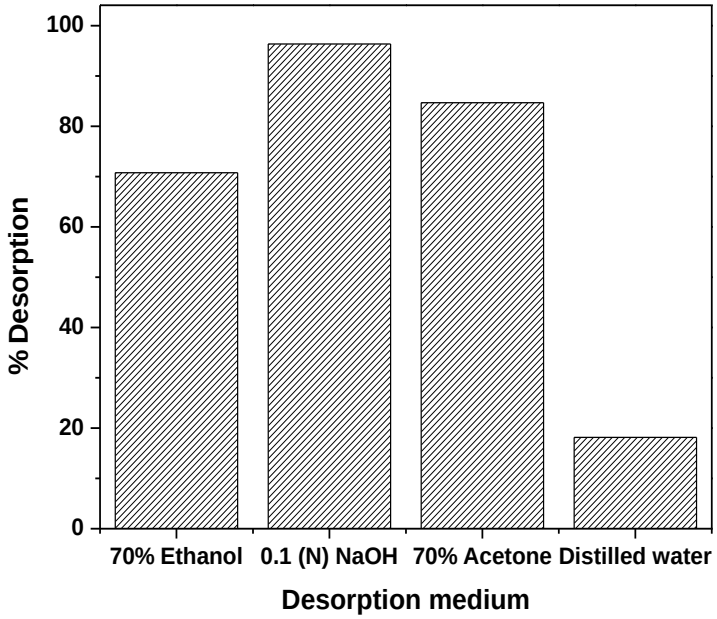

Figure 5.3. Effect of different desorption agents on the desorption of Reactive Blue 4 from immobilized biomass of *Rhizopus oryzae* (MTCC 262).

Table 5.4 Adsorption-Desorption cycle of Reactive Blue 4 by immobilized biomass of *Rhizopus oryzae* (MTCC 262).

Cycle Number	Percent adsorption	Percent desorption
1	98.25	96.52
2	93.05	91.84
3	87.34	81.74
4	76.46	72.22
5	70.88	66.76
6	63.45	52.22

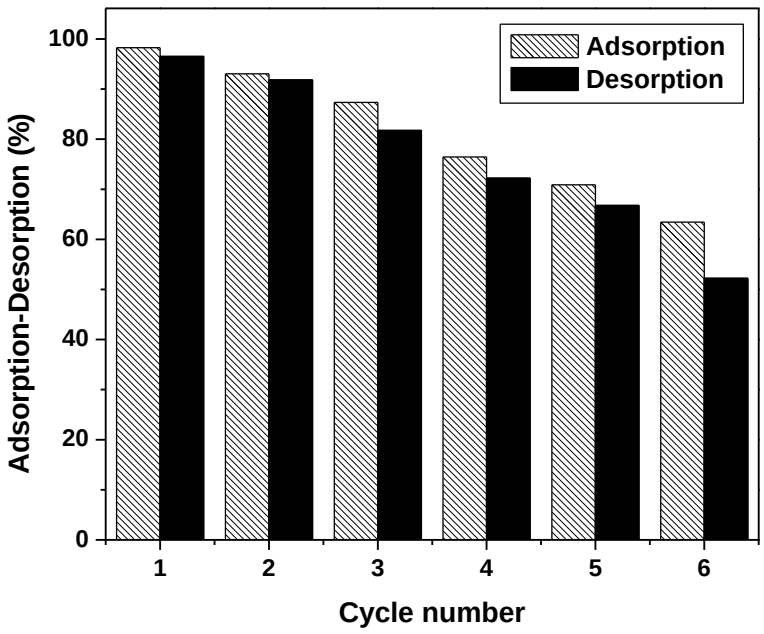

Figure 5.4 Adsorption-Desorption cycle of Reactive Blue 4 by immobilized biomass of *Rhizopus oryzae* (MTCC 262).

5.4. CONCLUSIONS

The desorption experiments proved that Reactive Blue 4 could be easily eluted from dry and CMC immobilized beads of *Rhizopus oryzae* (MTCC 262) respectively. Of the different desorption agents 1 (N) NaOH was found to be best (98.92 % desorption) for Reactive Blue 4 desorption for dry biomass and 0.1 (N) NaOH showed the best desorption efficiency (96.52 % desorption) for Reactive Blue 4 using immobilized biomass of *Rhizopus oryzae* (MTCC 262) respectively.

The regeneration studies indicated that the dry *Rhizopus oryzae* biomass can be used repeatedly for four consecutive adsorption-desorption cycles to treat the Reactive Blue containing effluent as some amount of biomass were lost during the phase alteration (adsorption $\rightleftharpoons$ desorption) of each cycle while *R. oryzae* biomass in immobilized form can be used successfully for five consecutive adsorption-desorption cycles (as immobilized biomass is more stable than dry biomass) to treat the Reactive Blue 4 contaminated waste water discharged from the textile industry in near future.

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CHAPTER - 6

Continuous removal of textile dye using dry cell as well as immobilized cell using a packed bed column reactor and desorption of dye from the column and repeated use of the column reactor

6.1. INTRODUCTION

Among all industrial sectors, one of the most significant users of dyes are textile industries. The textile industrial processing is very water-consuming which involves extensive use of synthetic dyes for dyeing process [Spagni et al., 2010]. Apart from textile industries, dyes have wide applications in other industries viz., tannery, food, paper and pulp, printing, carpet and mineral processing etc (Bagchi and Ray, 2015). Dyes are common contaminants in industrial wastewater, which are potentially carcinogenic, mutagenic and allergenic to aquatic flora and fauna. Due to their characteristic color the presence of dyes in industrial wastewater is easily visible even when present in small concentrations [Nath and Ray, 2015; Nigam et al., 2000]. Moreover, a large number of these dyes are non-biodegradable due to their synthetic origin and complex aromatic structure. It can remain in the environment for an extended period of time [Safa et al., 2011]. Hence, to consider the removal of dyes from textile effluent is a major environmental issue.

In a large-scale industrial effluent treatment, packed bed column with continuous flow operations are more effective. It makes best use of the concentration difference which is known to be a driving force for adsorption. Thus it allows more efficient utilization of the capacity of selected microbial adsorbent [Adhikari et al., 2012; Adhikari et al., 2013]. Passing the industrial effluent through packed bed column, results in a light colored or almost no colored, better quality of effluent for discharge into aquatic streams. Also large volume of wastewater can be continuously treated using a defined quantity of sorbent in a packed bed column [Aksu et al., 1998]. However, only little effort has been focused on employing packed bed columns for adsorption of dyes by a few researchers [Chen et al., 2003; Fu and Viraraghavan, 2003; Padmesh et al., 2006; Saeed et al., 2009; Vijayaraghavan and Yun, 2008]. A packed bed is also effective for cyclic sorption and desorption for maximum utilization of the biosorbent. Methods using dry cells suffer from washing out and cell damage during continuous flow operations whereas immobilized cells have several advantages such as minimal clogging, more stability during continuous flow and easier solid–liquid separation thus have higher possibility of reuse [Kathiravan et al., 2010]. Aksu et al. [Aksu et al., 1998] have reported the use of calcium alginate immobilized *C. vulgaris* in packed bed column for biosorption of cupper (II). Maity et al. [Maity et al., 2009] have reported calcium alginate immobilized *Bacillus cereus* $M^{1}{}_{16}$ in packed bed column for biosorption of Cr (VI). Kathiravan et al. [Kathiravan et al., 2010] have also reported calcium alginate immobilized *Bacillus* sp. in packed bed column for reduction of Cr (VI).

In the present study, the treatment of Reactive Blue 4 solution using carboxymethyl cellulose immobilized *Rhizopus oryzae* (MTCC 262) was investigated in an up-flow packed bed column reactor.

Experiments were conducted as a function of bed height, flow rate and dye concentration. The performance of the packed bed column was described through a plot of the ratio of final and initial effluent dye concentration versus time, which is usually referred to as breakthrough curve [Adhikari et al., 2013; Cheng and Wang, 2000; Ekpete et al., 2011]. The experimental data obtained were fitted to Adam- Bohart model, Yoon and Nelson model and the modified Thomas model in order to predict the breakthrough curve of the adsorption process.

Desorption of the loaded dye from the adsorbent in a regular interval is important for making the column operation successful. Desorption ensures the utilization of the same packed bed for a number of times; therefore it minimizes the operational cost, excessive sludge generation. It also enhances the column efficiency. As performed in batch process, here also desorption studies in continuous flow system was carried out using a base sodium hydroxide. Desorption of Reactive Blue 4 from dry *R. oryzae* biomass was carried out using 1 (N) NaOH and desorption of the same dye from CMC immobilized *R. oryzae* (MTCC 262) biomass packed bed was also performed using 0.1 (N) NaOH as desorbing agent.

Packed bed adsorption has a number of advantages on the basis of its process engineering. It is a high yield simple operation and can be easily scaled up from a laboratory scale to a large scale industrial procedure. The separation protocol stages can be automatically operated. In a single step process, a high degree of purification can often be achieved. Studies carried out using packed column reactor are necessary for the design of continuous flow adsorption-desorption processes, which allow a more efficient utilization of the sorbent repeatedly. Packed bed columns are used with dry and immobilized cells which provide a better quality of the effluent water for dye biosorption. A number of authors has been reported the use of continuous packed bed column reactor using dry and immobilized biomass. Many mathematical models were developed to study packed bed system. The dynamic behaviour has been well described the objective of this study is to investigate the removal of Reactive Blue 4 from aqueous solution by dry as well as immobilized *Rhizopus oryzae* (MTCC 262) in a packed bed up-flow column reactor. The effects of design parameters, such as flow rate, bed height, and inlet dye concentration, have been studied. In addition, kinetics of biosorption and rate-limiting step for removal of Reactive Blue 4 from solution has been investigated.

6.2. THEORY

The breakthrough curve for adsorption of a solute from its solution into packed bed column under fixed operational conditions is expressed in terms of adsorbed dye concentration ($C_{ad}=C_0-C$) or ration of final and initial dye concentration (C/C_0) as a function of time or volume of effluent, V_{eff} (Aksu and Gonen, 2004).Where, C_0 is the dye concentration and C is the final dye concentration at time (t).

Volume of effluent can be calculated from equation (6.1).

$$\mathbf{V_{eff} = Q_{total} \quad \ldots\ldots\ldots\ldots (6.1)}$$

Where, Q is the flow rate of the feed solution (mL min^{-1}) and t_{total} is the total flow time (min).

In the plot of adsorbed dye concentration (C_{ad}) versus time, the area under the curve (A) in mg/ min/L can be obtained by integrating C_{ad} (mg/L) against t (min). The total adsorbed dye quantity (q total in mg at a given dye concentration and flow rate can be calculated from equation (6.2).

$$\mathbf{q_{total} = QA/1000 \int_{t=0}^{t=t_{total}} C_{ad}\, dt \quad \ldots\ldots\ldots\ldots (6.2)}$$

Total amount of dye passed through the column (m_{total}) in mg can be calculated from equation (6.3).

$$\mathbf{m_{total} = (C_0 Q_{total}) / 1000 \ldots\ldots\ldots\ldots (6.3)}$$

Total dye removal percentage (% removal) can be calculated from the following equation (6.4).

$$\mathbf{\% \text{ removal} = (q_{total} / m_{total}) \times 100 \ldots\ldots\ldots\ldots (6.4)}$$

Void fraction of the column (ω) can be calculated from volume of water eluted out of the column after the beads of the packed bed are saturated with water (W) and total volume of effective column (W_h), expressed in the following equation (6.5).

$$\mathbf{\omega = W / W_h \ldots\ldots\ldots\ldots\ldots\ldots (6.5)}$$

Where, W_h can be calculated from equation (6.6)

$$W_h = \pi d^2_{column} H / 4 \text{ …….. (6.6)}$$

d_{column} is the internal diameter of the column and H is effective bed height.

6.3. MATERIALS AND METHODS

6.3.1. Fungal Strain and Maintenance

The fungal strain *Rhizopus oryzae* (MTCC 262), used in this study was procured from the Institute of Microbial Technology, Chandigarh, India. Potato dextrose slant was used as the maintenance medium for the organism by sub culturing at 30°C for 120 h maintaining a regular interval of 30 days.

6.3.2. Chemicals

Reactive Blue 4 used in the present study was obtained from Sigma-Aldrich Chemical Co., St. Louis, MO, USA. Other chemicals including ingredients of the microbiological media were procured from E. Merck, Germany and Hi Media, India and CMC sodium salt (CMC-Na) high viscosity; 1.0% in H_2O at 20°C: 300–600 mPa s; content of sodium: 6.5–6.8%) was obtained from Loba Chemie Pvt. Ltd..

6.3.3. Preparation and estimation of dye solution

Synthetic dye solution used in this study was prepared by dissolving Reactive Blue 4 in distilled water. The concentration of Reactive Blue 4 in solution was measured spectrophotometrically as described in Chapter 3.

6.3.4. Preparation of immobilized biomass

CMC solution (10 ml 2.5%) and 0.1 gm of dried biomass of *R. oryzae* (MTCC 262) was mixed until homogeneous. The mixture was injected drop-wise to a $FeCl_3$ solution (0. 05 mol L^{-1}) using an injector with a 16-sized pinhead to form beads. The cell-immobilized beads were cured in the $FeCl_3$ solution for 1 h to enhance their mechanical stability. Thereafter, the freshly immobilized *R. oryzae* beads were harvested and rinsed with sterilized water thrice (Wang et al., 2008) and used for column study.

6.3.5. Experimental Design

In this study the dye removal process was investigated with a packed bed up-flow column reactor on a laboratory scale. The experimental setup of the reactor is schematically shown in **Figure 6.1** Experiments were carried out in a glass column (35 cm in length internal diameter 0.95 cm) varying the effective bed height 20, 15 and 10 cm using immobilized biomass. The solution of Reactive Blue 4 with different initial concentration (C_0) was introduced continuously over the immobilized bead at different flow rate using a variable speed peristaltic pump. Glass wool was placed at the bottom of the column to ensure homogeneous distribution of the feeding solution and to protect the immobilized beads. The residual concentration (C) of dye in the effluent was measured at regular time intervals.

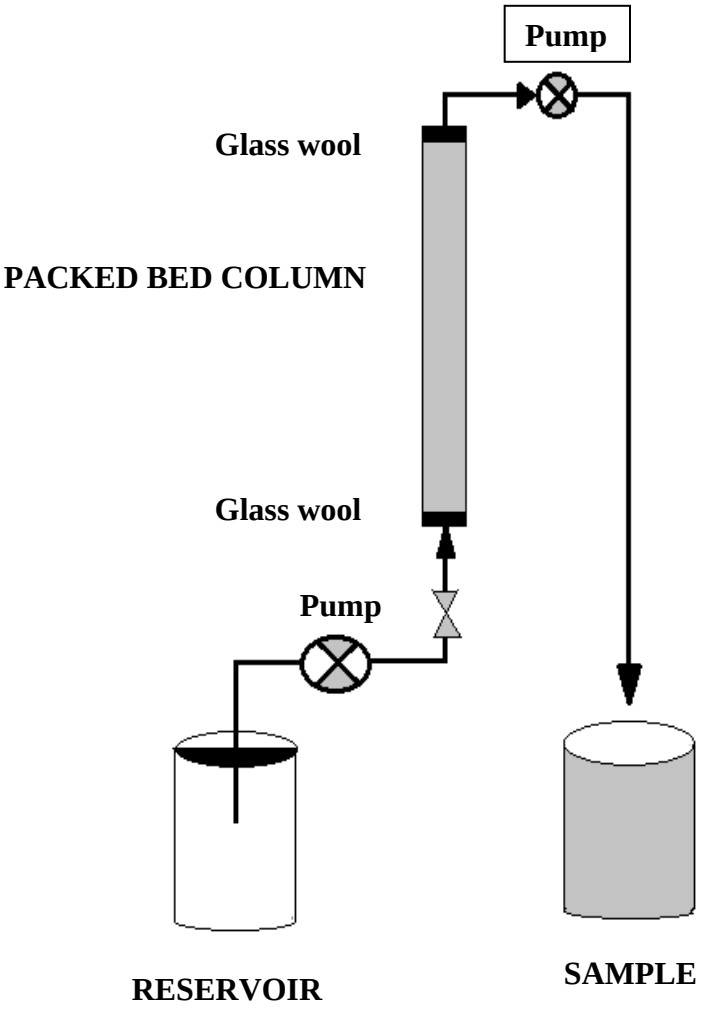

Figure 6.1. Schematic diagram of packed bed column reactor.

6.3.6. Operation of packed bed column reactor

The packed bed column reactor was operated on a laboratory scale. Packed bed of variable height were prepared with dry and CMC immobilized *R. oryzae* (MTCC 262) biomass in a glass column and dye removal process was carried out by feeding the dye solution from bottom of the bed at a continuous flow rate. Dye stock solution of 1000 mg L^{-1} was prepared initially and diluted to get the desired concentration of dye during experiments. The effect of important parameters, such as void fraction, inlet flow rate, influent dye concentration and effective bed height were studied. The experiments were performed at effective bed height of 10 cm, 15 cm & 20 cm where the inlet flow rate was varied from 2 mL min^{-1} to 4 mL min^{-1}. Experiments were performed at influent dye concentration of 50 mg L^{-1}, 100 mg L^{-1} and 200 mg L^{-1}. All the experiments were carried out in triplicate and an average of three independent experiments were provided in the "Results and discussion" section. Adsorption of dye by the packed bed was constantly monitored by measuring the concentration of dye in the effluent at fixed interval of time and by prediction of the breakthrough curve. The concentration of Reactive Blue 4 in solution was measured with UV-visible spectrophotometer (HITACHI U-2000) at 595 nm (λmax).

6.3.7. Desorption experiments

The adsorption experiment was carried out using previously mentioned glass column in the same way as described in **Chapter 6** at optimized flow rate (2 mL min^{-1}) and at the bed heights of 10 cm, 15 cm and 20 cm for dry and CMC immobilized *R. oryzae* packed bed respectively and at initial dye concentration of 100 mg L^{-1}.

The desorption experiment was performed by passing 1 (N) NaOH and 0.1 (N) NaOH solution through the column packed with dry *Rhizopus oryzae* cells and carboxymethyl cellulose beads respectively in the upflow mode at a speed of 2 mL min^{-1}. After each adsorption desorption the bed of the column was washed with distilled water. The adsorption- desorption was repeated for four consecutive cycles.

The regeneration studies (chapter 5) indicated that the dry *Rhizopus oryzae* biomass can be used repeatedly for four consecutive adsorption-desorption cycles to treat the Reactive Blue 4 containing effluent as some amount of biomass were lost during the phase alteration (adsorption

- desorption) of each cycle while *R. oryzae* biomass in immobilized form also can be used successfully for four consecutive adsorption-desorption cycles for packed bed column study as after fourth cycle, the adsorption percentage reach below 50 percent.

6.3.8. Estimation of dye (Reactive Blue 4)

The samples were collected at regular intervals during adsorption and desorption experiments and residual concentrations of dye were measured by UV-spectrophotometer (HITACHI U-2000) at 595 nm.

6.4. RESULTS AND DISCUSSION

6.4.1. Void fraction

Void fraction is uniform throughout the column as it is assumed that carboxymethyl cellulose beads are porous, spherical and of uniform size. Void fraction of the packed bed column used in the present study was 0.36.

6.4.2. Effect of flow rate

The effect of flow rate was studied by varying the flow rate of feed solution (Q) from 2 to 4 mL min^{-1} at a bed height of 15 cm for dry biomass and 20 cm for immobilized biomass and dye concentration of 50 mg L^{-1} (pH 3.0). The flow of dye solution through the column was continued for 360 min 420 min for dry and immobilized biomass respectively within which the bed became saturated with dye and the concentration of solute in the effluent attained near about C_0 i.e., the system had reached the breakpoint. The adsorption of dye using dry and immobilized biomass was found to decrease with increasing flow rate [**Figure 6.2 (a); 6.3 (a)]. Figure 6.2 (b) and 6.3 (b)**], shows the breakthrough curve (C/C_0 versus t) at different flow rate respectively. The breakpoint time decreases with increasing flow rate at a fixed bed height. Much sharper breakthrough curves were obtained at higher flow rate as the residence time of dye in the column was not long enough for adsorption equilibrium to be attained [Maity et al., 2009; Robinson et al.,2003]. Dye removal was found to decrease with increase in flow rate of dye solution into the column and maximum dye removal 67.26% (dry biomass) and 68.45% (immobilized biomass) was observed at 2 mL min^{-1} flow rate as shown in Table 6.1 (a) and 6.2 (a) for dry and immobilized biomass respectively.

Table 6.1 (a). Effect of flow rate on adsorption of Reactive Blue 4 using dry biomass of *R. oryzae* (MTCC 262) in a continuous packed bed column reactor (Bed height: 15 cm, dye concentration: 100 mg/L, pH: 3.0)

Time (min)	Q=2 mL/min				Q=3 mL/min				Q=4 mL/min			
	V_{eff} (mL)	C (mg/L)	C_{ad} (mg/L)	C/C_0	V_{eff} (ml)	C (mg/L)	C_{ad} (mg/L)	C/C_0	V_{eff} (mL)	C (mg/L)	C_{ad} (mg/L)	C/C_0
0	0	0	0	0	0	0	0	0	0	0	0	0
2	4	3.1	96.9	0.031	6	3.9	96.1	0.039	8	4.6	95.4	0.046
5	10	3.8	96.2	0.038	15	4.4	95.6	0.044	20	5.1	94.9	0.051
10	20	5.2	94.8	0.052	30	6.2	93.8	0.062	40	6.9	93.1	0.069
15	30	8.4	91.6	0.084	45	10.12	89.88	0.1012	60	13.06	86.94	0.1306
20	40	12.88	87.12	0.1288	60	15.55	84.45	0.1555	80	18.88	81.12	0.1888
30	60	17.72	82.28	0.1772	90	21.04	78.96	0.2104	120	24.66	75.34	0.2466
40	80	22.07	77.93	0.2207	120	25.98	74.02	0.2598	160	30.69	69.31	0.3069
50	100	28.56	71.44	0.2856	150	33.37	66.63	0.3337	200	35.26	64.74	0.3526
60	120	33.7	66.3	0.337	180	38.68	61.32	0.3868	240	41.94	58.06	0.4194
70	140	39.38	60.62	0.3938	210	44.78	55.22	0.4478	280	46.62	53.38	0.4662
80	160	45.46	54.54	0.4546	240	49.94	50.06	0.4994	320	52.76	47.24	0.5276
90	180	48.97	51.03	0.4897	270	54.56	45.44	0.5456	360	56.18	43.82	0.5618
120	240	52.16	47.84	0.5216	360	57.78	42.22	0.5778	480	60.38	39.62	0.6038
150	300	56.42	43.58	0.5642	450	60.85	39.15	0.6085	600	62.7	37.3	0.627
180	360	59.74	40.26	0.5974	540	62.37	37.63	0.6237	720	64.42	35.58	0.6442
210	420	61.48	38.52	0.6148	630	64.45	35.55	0.6445	840	65.98	34.02	0.6598
240	480	64.07	35.93	0.6407	720	65.22	34.78	0.6522	960	66.67	33.33	0.6667
270	540	65.84	34.16	0.6584	810	66.69	33.31	0.6669	1080	67.15	32.85	0.6715
300	600	66.24	33.76	0.6624	900	67.19	32.81	0.6719	1200	67.87	32.13	0.6787
330	660	67.81	32.19	0.6781	990	68.07	31.93	0.6807	1320	68.24	31.76	0.6824
360	720	68.02	31.98	0.6802	1080	68.34	31.66	0.6834	1440	68.56	31.44	0.6856

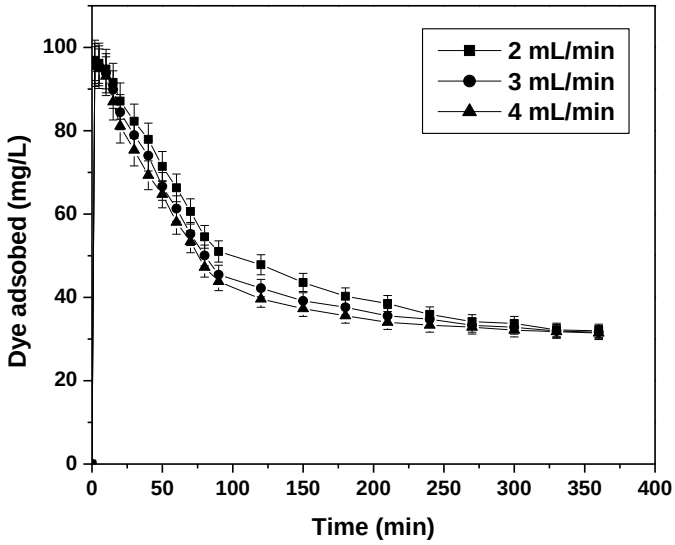

Figure 6.2 (a). Effect of flow rate on adsorption of Reactive Blue 4 by dry biomass of *R. oryzae* using packed bed column

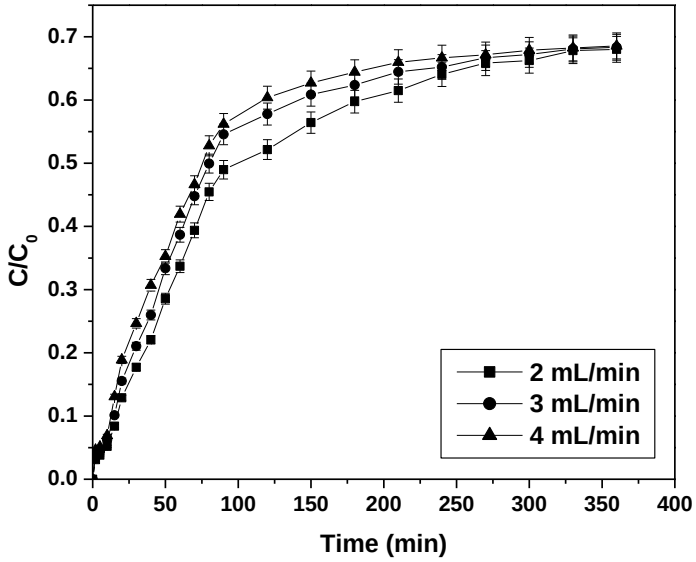

Figure 6.2 (b). Breakthrough curve of effect of flow rate on adsorption of Reactive Blue 4 by dry biomass of *R. oryzae* using packed bed column

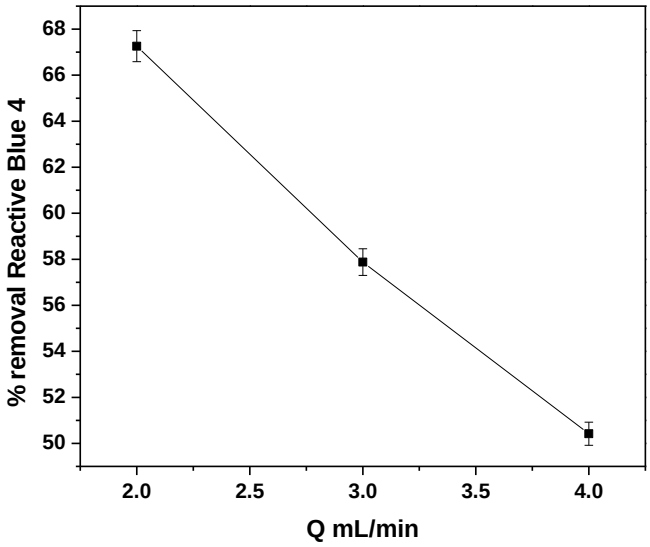

Figure 6.2 (c). Effect of flow rate on percent removal of Reactive Blue 4 using dry biomass of *R. oryzae* (MTCC 262) with packed bed column

Table 6.1(b). Effect of flow rate on percentage removal of Reactive Blue 4 using dry biomass (Bed height: 15 cm, dye concentration: 100 mg L^{-1}, pH: 3.0).

Flow rate (mL/min)	A (mg/min/L)	q_{total} (mg)	m_{total} (mg)	% removal
2	13910.04	26.78	41	67.26
3	10774.26	33.46	62	57.88
4	9894.32	39.88	85	50.42

Table 6.2(a). Effect of flow rate on adsorption of Reactive Blue 4 using immobilized biomass of *R. oryzae* (MTCC 262) in a continuous packed bed column reactor (Bed height: 20 cm, dye concentration: 100 mg/L, pH: 3.0).

Time (min)	Q = 2 mL/min				Q = 3mL/min				Q = 4 ml/min			
	Veff (mL)	C (mg/L)	Cad (mg/L)	C/Co	Veff (mL)	C (mg/L)	Cad (mg/L)	C/Co	Veff (mL)	C (mg/L)	Cad (mg/L)	C/Co
0	0	0	0	0	0	0	0	0	0	0	0	0
2	4	1.62	98.38	0.0162	6	1.94	98.06	0.0194	8	2.6	97.4	0.026
5	10	4.78	95.22	0.0478	15	4.96	95.04	0.0496	20	5.48	94.52	0.054
10	20	8.52	91.48	0.0852	30	23.54	76.46	0.2354	40	35.96	64.04	0.359
15	30	14.38	85.62	0.1438	45	27.72	72.28	0.2772	60	42.66	57.34	0.426
20	40	18.02	81.98	0.1802	60	32.4	67.6	0.324	80	44.12	55.88	0.441
30	60	20.98	79.02	0.2098	90	33.9	66.1	0.339	120	44.89	55.11	0.448
40	80	22.18	77.82	0.2218	120	34.86	65.14	0.3486	160	46.16	53.84	0.461
50	100	22.3	77.7	0.223	150	36.12	63.88	0.3612	200	46.76	53.24	0.467
60	120	22.97	77.03	0.2297	180	36.48	63.52	0.3648	240	47.08	52.92	0.470
70	140	23.12	76.88	0.2312	210	38.42	61.58	0.3842	280	47.5	52.5	0.475
80	160	23.88	76.12	0.2388	240	40.36	59.64	0.4036	320	47.8	52.2	0.478
90	180	24.02	75.98	0.2402	270	40.66	59.34	0.4066	360	48.02	51.98	0.480
120	240	26.95	73.05	0.2695	360	41.14	58.86	0.4114	480	48.2	51.8	0.482
150	300	30.46	69.54	0.3046	450	41.74	58.26	0.4174	600	49.14	50.86	0.491
180	360	31.42	68.58	0.3142	540	42.6	57.4	0.426	720	49.96	50.04	0.499
210	420	32.22	67.78	0.3222	630	44.98	55.02	0.4498	840	50.12	49.88	0.501
240	480	33.06	66.94	0.3306	720	45.18	54.82	0.4518	960	51.14	48.86	0.511
270	540	36.84	63.16	0.3684	810	46.99	53.01	0.4699	1080	51.97	48.03	0.519
300	600	40.64	59.36	0.4064	900	47.12	52.88	0.4712	1200	54.68	45.32	0.546
330	660	44.94	55.06	0.4494	990	48.42	51.58	0.4842	1320	55.16	44.84	0.551
360	720	46.82	53.18	0.4682	1080	50.04	49.96	0.5004	1440	56.42	43.58	0.564
390	780	47.18	52.82	0.4718	1170	52.48	47.52	0.5248	1560	57.08	42.92	0.570
420	840	48.02	51.98	0.4802	1260	53.12	46.88	0.5312	1680	57.96	42.04	0.579

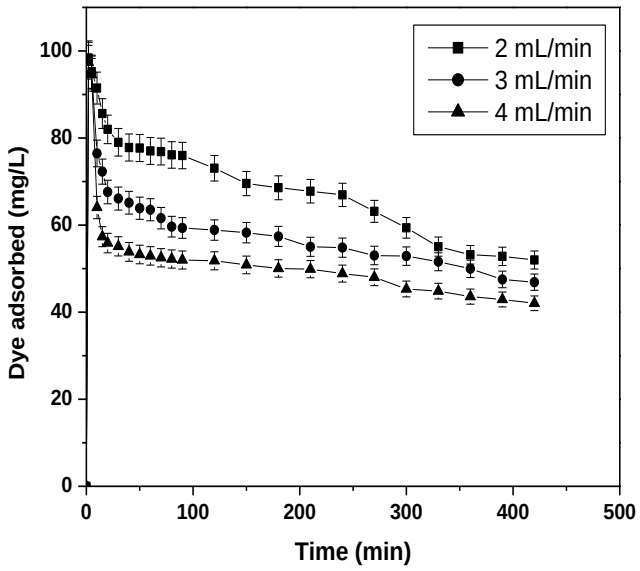

Figure 6.3 (a). Effect of flow rate on adsorption of Reactive Blue 4 by immobilized biomass using packed bed column

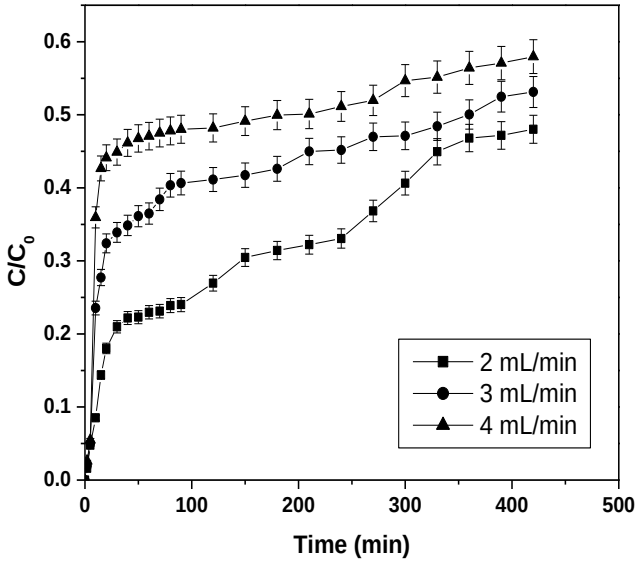

Figure 6.3 (b). Breakthrough curve of Effect of flow rate on adsorption of Reactive Blue 4 by immobilized biomass using packed bed column

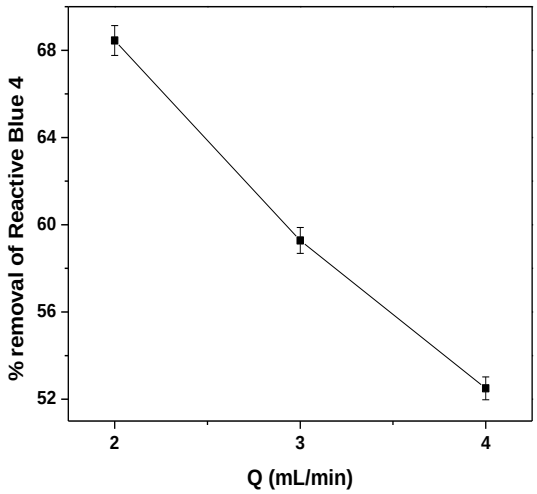

Figure 6.3 (c). Effect of flow rate on percent removal of Reactive Blue 4 with packed bed column reactor

Table 6.2 (b). Effect of flow rate on percentage removal of Reactive Blue 4 using immobilized biomass (Bed height: 20 cm, dye concentration: 100 mg L^{-1}, pH: 3.0).

Flow rate (mL/min)	A (mg/min/L)	q_{total} (mg)	m_{total} (mg)	% removal
2	14126.04	28.26	43	68.45
3	11988.26	35.96	65	59.28
4	10584.32	42.33	86	52.5

6.4.3. Effect of column bed height

Bed height of the column (H) was varied from 5 to 15 cm for dry biomass and 10 to 20 cm for immobilized biomass at a flow rate of 2 mL min^{-1}, other conditions remaining the same. Maximum sorption of dye was observed at 15 cm for dry biomass and 20 cm bed height [**Figure 6.4 (a) and 6.5 (a)** respectively]. **Figure 6.4 (b) and 6.5 (b)** shows the breakthrough curve as a function of time at different bed height. The breakpoint time increases with increasing bed height at a fixed flow rate. As the bed height increases, the amount of biomass increases and consequently the total number of binding sites also increases which enhances the adsorption process [Adhikari et al., 2012; Maity et al., 2012]. Maximum dye removal of 72.96% and 75.18% using dry and immobilized biomass was observed at 20 cm bed height as shown in Table 6.3 (b) and 6.4 (b) respectively.

Table 6.3 (a). Effect of bed height on adsorption of Reactive Blue 4 using dry biomass of *R. oryzae* (MTCC 262) in packed bed column (Flow rate: 2 mL/min, dye concentration: 100 mg/L, pH: 3.0)

Time (min)	H = 5 cm				H = 10 cm				H = 15 cm			
	V_{eff} (mL)	C (mg/L)	C_{ad} (mg/L)	C/C_0	V_{eff} (mL)	C (mg/L)	C_{ad} (mg/L)	C/C_0	V_{eff} (mL)	C (mg/L)	C_{ad} (mg/L)	C/C_0
0	0	0	0	0	0	0	0	0	0	0	0	0
2	4	3.5	96.5	0.035	4	2.9	97.1	0.029	4	2.4	97.6	0.024
5	10	5.5	94.5	0.055	10	4.2	95.8	0.042	10	3.1	96.9	0.031
10	20	8.72	91.28	0.0872	20	6.9	93.1	0.069	20	5.4	94.6	0.054
15	30	10.78	89.22	0.1078	30	8.7	91.3	0.087	30	7.26	92.74	0.0726
20	40	13.56	86.44	0.1356	40	11.26	88.74	0.1126	40	10.2	89.8	0.102
30	60	16.76	83.24	0.1676	60	14.45	85.55	0.1445	60	12.86	87.14	0.1286
40	80	18.52	81.48	0.1852	80	17.07	82.93	0.1707	80	16.66	83.34	0.1666
50	100	20.34	79.66	0.2034	100	19.09	80.91	0.1909	100	18.04	81.96	0.1804
60	120	23.97	76.03	0.2397	120	21.96	78.04	0.2196	120	20.85	79.15	0.2085
70	140	26.68	73.32	0.2668	140	24.65	75.35	0.2465	140	23.43	76.57	0.2343
80	160	31.44	68.56	0.3144	160	28.56	71.44	0.2856	160	26.96	73.04	0.2696
90	180	36.75	63.25	0.3675	180	34.55	65.45	0.3455	180	32.4	67.6	0.324
120	240	42.65	57.35	0.4265	240	39.9	60.1	0.399	240	37.74	62.26	0.3774
150	300	49.28	50.72	0.4928	300	45.4	54.6	0.454	300	42.9	57.1	0.429
180	360	53.32	46.68	0.5332	360	49.93	50.07	0.4993	360	47.61	52.39	0.4761
210	420	56.17	43.83	0.5617	420	53.23	46.77	0.5323	420	52.14	47.86	0.5214
240	480	59.19	40.81	0.5919	480	57.39	42.61	0.5739	480	55.94	44.06	0.5594
270	540	62.09	37.91	0.6209	540	60.6	39.4	0.606	540	57.07	42.93	0.5707
300	600	63.13	36.87	0.6313	600	61.02	38.98	0.6102	600	57.89	42.11	0.5789
330	660	63.88	36.12	0.6388	660	61.4	38.6	0.614	660	58.4	41.6	0.584
360	720	64.42	35.58	0.6442	720	61.67	38.33	0.6167	720	58.86	41.14	0.5886

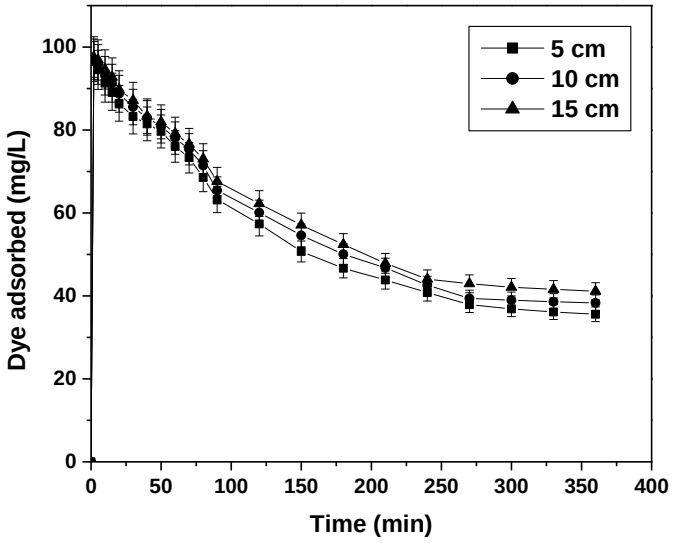

Figure 6.4 (a). Effect of bed height on adsorption of Reactive Blue 4 by dry biomass of *R. oryzae* using packed bed column

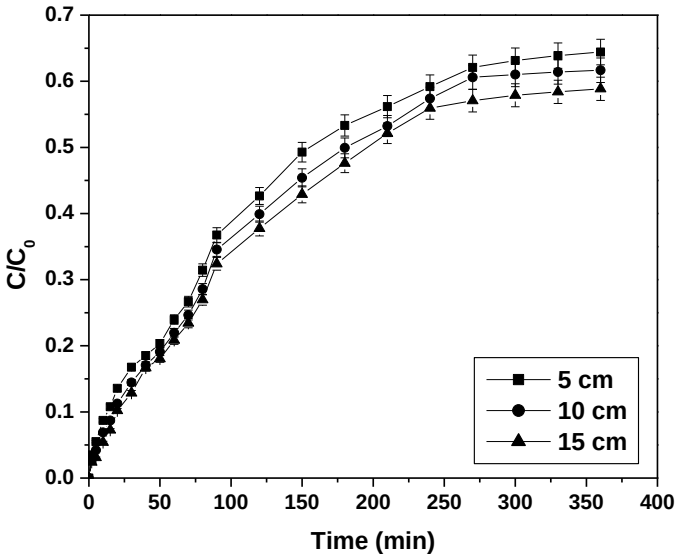

Figure 6.4 (b). Breakthrough curve of effect of bed height on adsorption of Reactive Blue 4 by dry biomass of *R. oryzae* using packed bed column

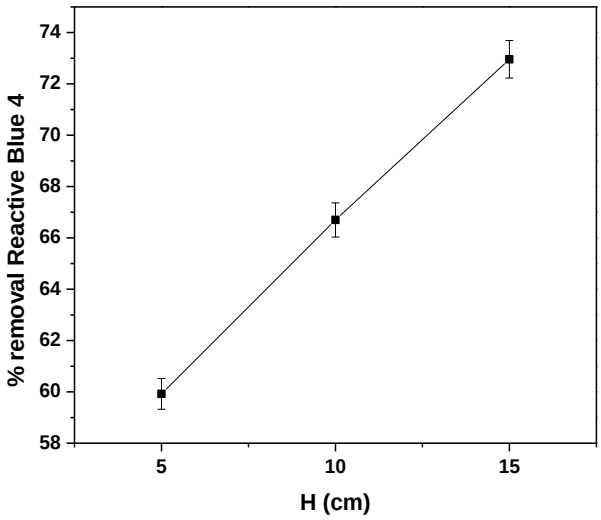

Figure 6.4 (c). Effect of bed height on percent removal of Reactive Blue 4 using dry biomass with packed bed column reactor

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Table 6.3 (b). Effect of Bed height on percentage removal of Reactive Blue 4 using dry biomass of *R. oryzae* (MTCC 262) (flow rate: 2 mL min^{-1}, dye concentration: 100 mg L^{-1}, pH: 3.0)

Bed height (cm)	A (mg/min/L)	q_{total} (mg)	m_{total} (mg)	% removal
5	12268.26	24.66	40	59.92
10	12067.19	27.04		66.7
15	14108.42	28.92		72.96

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Table 6.4(a). Effect of bed height on adsorption of Reactive Blue 4 using immobilized biomass of *R. oryzae* (MTCC 262) in packed bed column (Flow rate: 2 mL/min, dye concentration: 100 mg/L, pH: 3.0)

Time (min)	V_{eff} (mL)	H = 10 cm			H = 15 cm			H = 20 cm		
		C (mg/L)	C_{ad} (mg/L)	C/Co	C (mg/L)	C_{ad} (mg/L)	C/Co	C (mg/L)	C_{ad} (mg/L)	C/Co
0	0	0	0	0	0	0	0	0	0	0
2	4	3.28	96.72	0.0328	1.7	98.3	0.017	1.28	98.72	0.0128
5	10	6.24	93.76	0.0624	4.78	95.22	0.0478	2.47	97.53	0.0247
10	20	11.08	88.92	0.1108	8.42	91.58	0.0842	6.15	93.85	0.0615
15	30	25.92	74.08	0.2592	14.39	85.61	0.1439	11.04	88.96	0.1104
20	40	30.28	69.72	0.3028	18.04	81.96	0.1804	12.7	87.3	0.127
30	60	32.6	67.4	0.326	21.48	78.52	0.2148	14.88	85.12	0.1488
40	80	34.04	65.96	0.3404	22.26	77.74	0.2226	16.22	83.78	0.1622
50	100	34.2	65.8	0.342	22.28	77.72	0.2228	17.96	82.04	0.1796
60	120	34.3	65.7	0.343	22.82	77.18	0.2282	18.66	81.34	0.1866
70	140	34.56	65.44	0.3456	23.14	76.86	0.2314	19.76	80.24	0.1976
80	160	34.78	65.22	0.3478	23.92	76.08	0.2392	20.1	79.9	0.201
90	180	35.02	64.98	0.3502	24.06	75.94	0.2406	20.84	79.16	0.2084
120	240	35.32	64.68	0.3532	27.5	72.5	0.275	23.7	76.3	0.237
150	300	35.85	64.15	0.3585	30.52	69.48	0.3052	24.65	75.35	0.2465
180	360	36.26	63.74	0.3626	31.45	68.55	0.3145	27.81	72.19	0.2781
210	420	36.69	63.31	0.3669	32.25	67.75	0.3225	27.96	72.04	0.2796
240	480	37.18	62.82	0.3718	33.17	66.83	0.3317	28.75	71.25	0.2875
270	540	38.4	61.6	0.384	36.92	63.08	0.3692	30.44	69.56	0.3044
300	600	43.12	56.88	0.4312	40.64	59.36	0.4064	30.48	69.52	0.3048
330	660	46.96	53.04	0.4696	45.66	54.34	0.4566	30.55	69.45	0.3055
360	720	50.54	49.46	0.5054	47.35	52.65	0.4735	31.95	68.05	0.3195
390	780	51.02	48.98	0.5102	47.76	52.24	0.4776	32.4	67.6	0.324
420	840	51.52	48.48	0.5152	48.04	51.96	0.4804	33.28	66.72	0.3328

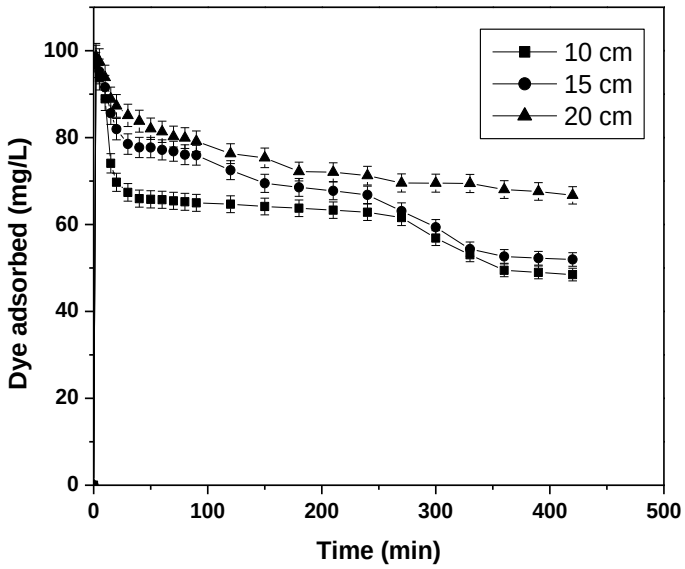


Figure 6.5 (a). Effect of bed height on adsorption of Reactive Blue 4 by immobilized biomass using packed bed column

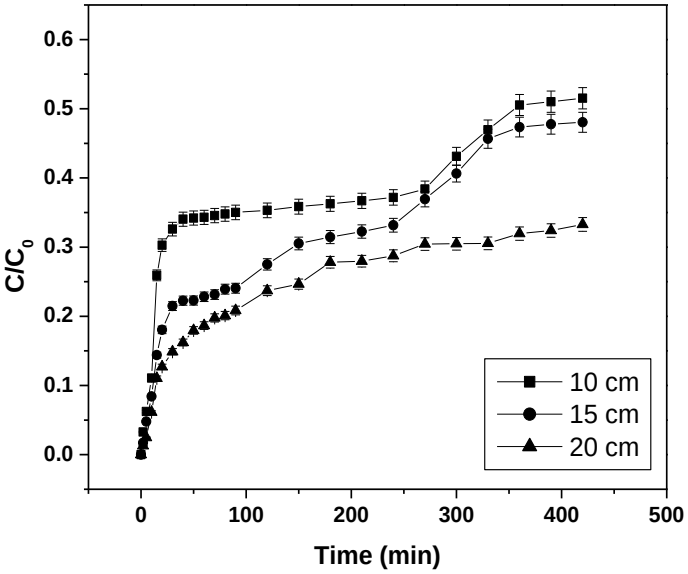


Figure 6.5 (b). Breakthrough curve of effect of bed height on adsorption of Reactive Blue 4 by immobilized biomass using packed bed column

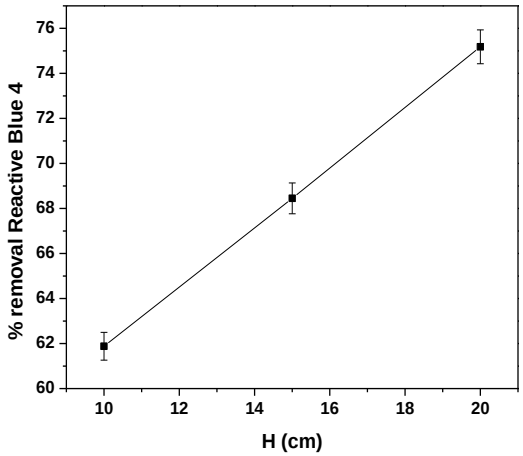


Figure 6.5 (c). Effect of bed height on percent removal of Reactive Blue 4 with packed bed column reactor

Table 6.4(b). Effect of Bed height on percentage removal of Reactive Blue 4 using immobilized biomass of *R. oryzae* (MTCC 262) (flow rate: 2 mL min^{-1}, dye concentration: 100 mg L^{-1}, pH: 3.0)

Bed height (cm)	A (mg/min/L)	q_{total} (mg)	m_{total} (mg)	% removal
10	13182.96	26.36	43	61.88
15	14126.04	28.26		68.45
20	15724.48	31.44		75.18

6.4. Effect of dye concentration in the feed solution

The initial dye concentration of feed solution (C_0) was varied from 50 to 200 mg L^{-1} at a bed height of 15 cm for dry biomass and 20 cm for immobilized biomass, other conditions remaining the same. At lower influent dye concentration, the packed bed was saturated after comparatively a long period of time. The curve of dye adsorbed (mg g^{-1}) versus time (t) at lower dye concentration was found to be dispersed in nature **[Figure 6.6 (a) and 6.7 (a)**]. At higher inlet dye concentration, the curves were much sharper. **Figure 6.6 (b) and 6.7 (b)** shows the breakthrough curve for different dye concentration as a function of time for dry and immobilized biomass respectively. The breakpoint time decreased with increase in initial dye concentration, because higher concentration difference between dye solution and solid matrix provides a rapid driving force for quick saturation of binding sites in the column [Maity et al., 2009]. Maximum dye removal of 71.81% and 76.88% was observed at 200 mg L^{-1} of influent dye concentration shown in Table 6.5 (b) and 6.6 (b) for dry and immobilized biomass respectively.

Table 6.5 (a). Effect of dye concentration on adsorption of Reactive Blue 4 using dry biomass of *R. oryzae* (MTCC 262) in packed bed column (Flow rate: 2 mL min^{-1}, bed height: 20 cm, pH: 3.0)

Time (min)	Veff (mL)	C_0 = 50 mg/ L			C_0 = 100 mg/ L			C_0 = 200 mg/ L		
		C (mg/L)	C_{ad} (mg/L)	C/Co	C (mg/L)	C_{ad} (mg/L)	C/Co	C (mg/L)	C_{ad} (mg/L)	C/Co
0	0	0	0	0	0	0	0	0	0	0
2	4	0.76	49.24	0.015	0.89	99.11	0.008	1.82	198.18	0.0091
5	10	1.78	48.22	0.035	1.94	98.06	0.019	2.15	197.85	0.0107
10	20	2.96	47.04	0.059	2.57	97.43	0.025	4.04	195.96	0.0202
15	30	4.6	45.4	0.092	5.02	94.98	0.050	9.96	190.04	0.0498
20	40	7.54	42.46	0.150	9.1	90.9	0.091	14.52	185.48	0.0726
30	60	9.42	40.58	0.188	12.92	87.08	0.129	22.47	177.53	0.1123
40	80	11.77	38.23	0.235	17.07	82.93	0.170	31.9	168.1	0.1595
50	100	13.06	36.94	0.261	22.24	77.76	0.222	40.71	159.29	0.2035
60	120	15.75	34.25	0.315	27.67	72.33	0.276	49.59	150.41	0.2479
70	140	17.47	32.53	0.349	32.25	67.75	0.322	61.6	138.4	0.308
80	160	18.88	31.12	0.377	37.18	62.82	0.371	72.36	127.64	0.3618
90	180	19.14	30.86	0.382	41.82	58.18	0.418	81.94	118.06	0.4097
120	240	20.17	29.83	0.403	48.65	51.35	0.486	90.5	109.5	0.4525
150	300	21.7	28.3	0.434	54.4	45.6	0.544	99.18	100.82	0.4959
180	360	22.81	27.19	0.4562	57.51	42.49	0.575	109.04	90.96	0.5452

210	420	23.5	26.5	0.47	61.04	38.96	0.610	118.4	81.6	0.592
240	480	24.35	25.65	0.487	65.5	34.5	0.655	127.25	72.75	0.63625
270	540	25.88	24.12	0.5176	68.8	31.2	0.688	136.5	63.5	0.6825
300	600	27.55	22.45	0.551	70.43	29.57	0.704	141.08	58.92	0.7054
330	660	28.3	21.7	0.566	71.36	28.64	0.713	142.16	57.84	0.7108
360	720	28.46	21.54	0.5692	71.9	28.1	0.719	142.87	57.13	0.71435

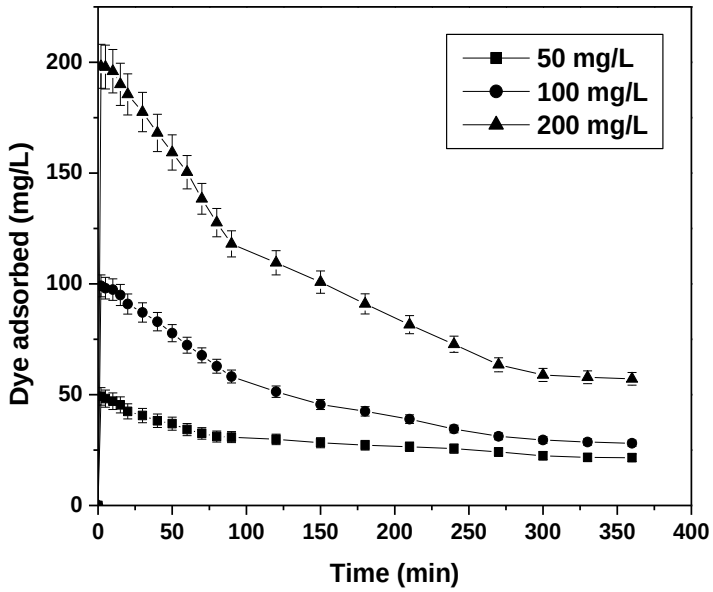


Figure 6.6 (a). Effect of initial dye concentration on adsorption of Reactive Blue 4 by dry biomass using packed bed column

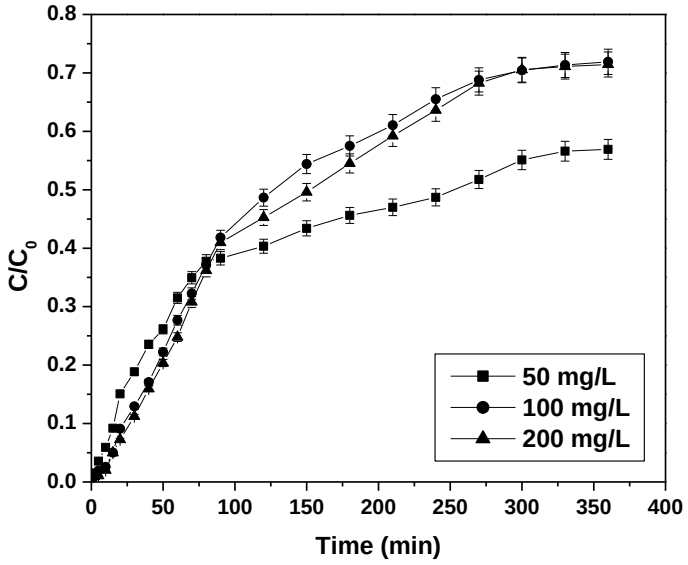


Figure 6.6 (b). Breakthrough curve of effect of initial dye concentration on adsorption of Reactive Blue 4 by dry biomass using packed bed column

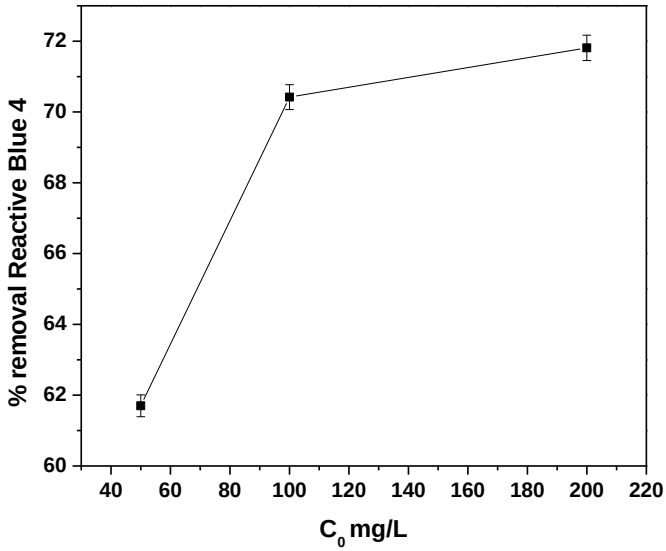


Figure 6.6 (c). Effect of initial dye concentration on percent removal of Reactive Blue 4 by dry biomass of *R. oryzae* using packed bed column

Table 6.5 (b). Effect of dye concentration on percentage removal of Reactive Blue 4 with dry biomass of *R. oryzae* (Flow rate: 2 mL min^{-1}, bed height: 15 cm, pH: 3.0)

Dye concentration (mg/L)	A (mg/min/L)	q_{total} (mg)	m_{total} (mg)	% removal
50	7671.57	23.69	39	61.7
100	10198.17	45.74	85	70.42
200	118994.29	64.45	97	71.81

Table 6.6(a). Effect of dye concentration on adsorption of Reactive Blue 4 using immobilized biomass of *R. oryzae* (MTCC 262) in packed bed column (Flow rate: 2 mL min^{-1}, bed height: 20 cm, pH: 3.0)

Time (min)	Veff (mL)	C_0 = 50 mg/ L			C_0 = 100 mg/ L			C_0 = 200 mg/ L		
		C (mg/L)	C_{ad} (mg/L)	C/Co	C (mg/L)	C_{ad} (mg/L)	C/Co	C (mg/L)	C_{ad} (mg/L)	C/Co
0	0	0	0	0	0	0	0	0	0	0
2	4	0.62	49.38	0.0124	0.886	99.114	0.00886	0.996	199.004	0.00498
5	10	1.22	48.78	0.0244	1.62	98.38	0.0162	2.28	197.72	0.0114
10	20	3.02	46.98	0.0604	2.825	97.175	0.02825	3.78	196.22	0.0189
15	30	5.48	44.52	0.1096	7.86	92.14	0.0786	9.72	190.28	0.0486
20	40	6.34	43.66	0.1268	10.32	89.68	0.1032	13.66	186.34	0.0683
30	60	7.42	42.58	0.1484	11.14	88.86	0.1114	16.24	183.76	0.0812
40	80	8.08	41.92	0.1616	14.64	85.36	0.1464	20.18	179.82	0.1009
50	100	8.94	41.06	0.1788	15.16	84.84	0.1516	25.22	174.78	0.1261
60	120	9.28	40.72	0.1856	17.78	82.22	0.1778	28.64	171.36	0.1432
70	140	9.86	40.14	0.1972	18.22	81.78	0.1822	32.6	167.4	0.163
80	160	10.01	39.99	0.2002	19.86	80.14	0.1986	34.97	165.03	0.17485
90	180	10.39	39.61	0.2078	20.48	79.52	0.2048	37.02	162.98	0.1851
120	240	11.77	38.23	0.2354	22.66	77.34	0.2266	40.98	159.02	0.2049
150	300	12.24	37.76	0.2448	23.6	76.4	0.236	43.04	156.96	0.2152
180	360	13.88	36.12	0.2776	24.41	75.59	0.2441	45.84	154.16	0.2292
210	420	13.99	36.01	0.2798	25.37	74.63	0.2537	47.72	152.28	0.2386
240	480	14.36	35.64	0.2872	25.66	74.34	0.2566	49.14	150.86	0.2457
270	540	15.2	34.8	0.304	25.95	74.05	0.2595	49.87	150.13	0.24935
300	600	15.24	34.76	0.3048	27.76	72.24	0.2776	51.88	148.12	0.2594

330	660	15.31	34.69	0.3062	28.39	71.61	0.2839	52.77	147.23	0.26385
360	720	15.96	34.04	0.3192	30.32	69.68	0.3032	54.6	145.4	0.273
390	780	16.18	33.82	0.3236	30.38	69.62	0.3038	55.18	144.82	0.2759
420	840	16.63	33.37	0.3326	30.58	69.42	0.3058	55.58	144.42	0.2779

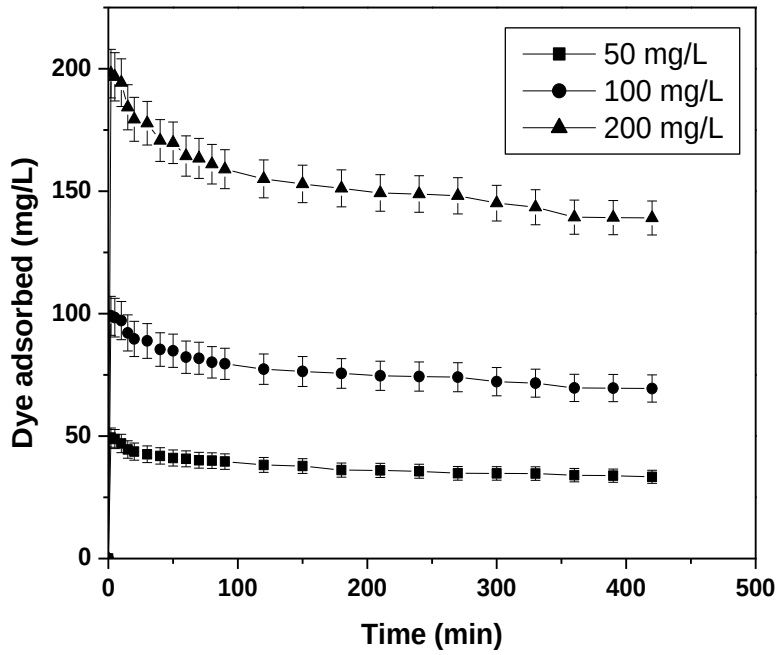


Figure 6.7 (a). Effect of initial dye concentration on adsorption of Reactive Blue 4 by immobilized biomass using packed bed column

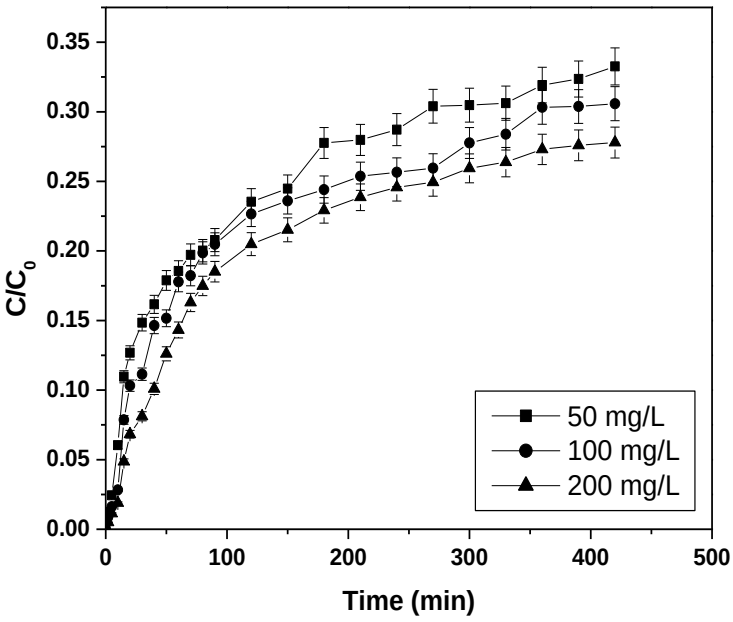


Figure 6.7 (b). Breakthrough curve of effect of initial dye concentration on adsorption of Reactive Blue 4 by immobilized biomass using packed bed column

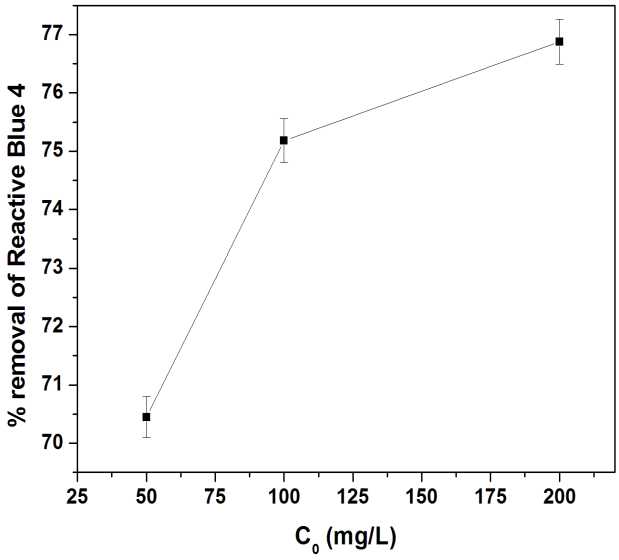


Figure 6.7 (c). Effect of initial dye concentration on percent removal of Reactive Blue 4 by immobilized biomass using packed bed column

Table 6.6 (b). Effect of dye concentration on percentage removal of Reactive Blue 4 with immobilized biomass of *R. oryzae* (Flow rate: 2 mL min^{-1}, bed height: 20 cm, pH: 3.0).

Dye concentration (mg/L)	A (mg/min/L)	q_{total} (mg)	m_{total} (mg)	% removal
50	7846.44	15.69	23	70.45
100	14126.04	28.26	43	75.18
200	32675.68	65.35	87	76.88

6.4.5. Application of Adam-Bohart model

The relationship between C/C_0 and t was first established by Adams and Bohart for a gas charcoal adsorption system [Bohart and Adams, 1920]. However, this concept can be used for description of any other adsorption processes. Adam Bohart model is used to describe the initial part of the breakthrough curve. Diffusion of solute takes place from bulk solution to the core of bead through the bead material. Initially the solute concentration in core region is nil or negligible, so diffusion rate follow linear relationship with time. Gradually the solute concentration increases in the core zone, thus the diffusion rate decreases and ultimately it gets saturated. The mass transfer rates for this model obey the following Equation (6.7).

$$\ln C/C_0 = K_{AB}C_0t - K_{AB}N_0 (H/Q) \quad \text{.....(6.7)}$$

Where, C_0 and C are the inlet and outlet dye concentration (mg L^{-1}) respectively, K_{AB} is the kinetic constant (L mg^{-1} min^{-1}) and N_0 is the saturation concentration (mg L^{-1}). H and Q are the bed height (cm) and flow rate (mL min^{-1}) respectively.

Parameters describing the characteristic operation of the column (K_{AB} and N_0) were obtained for linear regression analysis. From linear plot of ln C/C_0 against time (t), values of K_{AB} and N_0 were

determined from the slope and intercept. After applying Equation (6.7), to the experimental data for varying flow rates, bed height and inlet dye concentration, a linear relationship between C/C_0 and t was obtained [**Figure 6.8(a-e); 6.9(a-e)**] from which the respective values of K_{AB} and N_0 for all flow rates, bed heights and influent dye concentration were calculated and presented in Table 6.7 (a) and 6.7 (b) for dry and immobilized biomass respectively. The value of kinetic constant (K_{AB}) decreased with increase in initial dye concentration as well as the flow rate but N_0 increased with increase in flow rate and dye concentration. This indicated that the overall system kinetics was dominated by external mass transfer during initial stage of adsorption in the column [Aksu and Gonen, 2004]. Adam-Bohart model provides a simple approach for evaluating the adsorption process, however its validity is limited to the range of conditions used (Bohart and Adams, 1920; Chu and Hashim, 2007). The correlation coefficient (R^2) values were found between 0.972 and 0.982 [Table 6.7 (b)] for dry biomass and 0.983 and 0.997 [Table 6.8 (b)] for immobilized biomass for initial part of the breakthrough curve.

Table 6.7(a). Data for fitting Adam-Bohart model (dry biomass)

Time (min)	Q= 2 mL/min						Q= 3 mL/min		Q= 4 mL/min	
	C_0= 50mg/L		C_0= 100mg/L		C_0= 200mg/L		C_0= 100mg/L		C_0= 100mg/L	
	C mg/L	ln C/C_0	C mg/L	ln C/C_0	C mg/L	ln C/C_0	C mg/L	ln C/C_0	C mg/L	ln C/C_0
0	0	0	0	0	0	0	0	0	0	0
2	0.76	-4.186	0.89	-4.721	1.82	-4.699	3.9	-3.244	4.6	-3.079
5	1.78	-3.335	1.94	-3.942	2.15	-4.532	4.4	-3.123	5.1	-2.975
10	2.96	-2.826	2.57	-3.661	4.04	-3.902	6.2	-2.780	6.9	-2.673
15	4.6	-2.385	5.02	-2.991	9.96	-2.999	10.12	-2.290	13.06	-2.035
20	7.54	-1.891	9.1	-2.396	14.52	-2.622	15.55	-1.861	18.88	-1.667
30	9.42	-1.669	12.92	-2.046	22.47	-2.186	21.04	-1.558	24.66	-1.399
40	11.77	-1.446	17.07	-1.767	31.9	-1.835	25.98	-1.347	30.69	-1.181
50	13.06	-1.342	22.24	-1.503	40.71	-1.591	33.37	-1.097	35.26	-1.042
60	15.75	-1.155	27.67	-1.284	49.59	-1.394	38.68	-0.949	41.94	-0.868
70	17.47	-1.051	32.25	-1.131	61.6	-1.177	44.78	-0.803	46.62	-0.763
80	18.88	-0.973	37.18	-0.989	72.36	-1.016	49.94	-0.694	52.76	-0.639
90	19.14	-0.960	41.82	-0.871	81.94	-0.892	54.56	-0.605	56.18	-0.576
120	20.17	-0.907	48.65	-0.720	90.5	-0.792	57.78	-0.548	60.38	-0.504
150	21.7	-0.834	54.4	-0.608	99.18	-0.701	60.85	-0.496	62.7	-0.466
180	22.81	-0.784	57.51	-0.553	109.04	-0.606	62.37	-0.472	64.42	-0.439
210	23.5	-0.755	61.04	-0.493	118.4	-0.524	64.45	-0.439	65.98	-0.415
240	24.35	-0.719	65.5	-0.423	127.25	-0.452	65.22	-0.427	66.67	-0.405
270	25.88	-0.658	68.8	-0.373	136.5	-0.381	66.69	-0.405	67.15	-0.398
300	27.55	-0.596	70.43	-0.350	141.08	-0.348	67.19	-0.397	67.87	-0.387
330	28.3	-0.569	71.36	-0.337	142.16	-0.341	68.07	-0.384	68.24	-0.382
360	28.46	-0.563	71.9	-0.329	142.87	-0.336	68.34	-0.380	68.56	-0.377

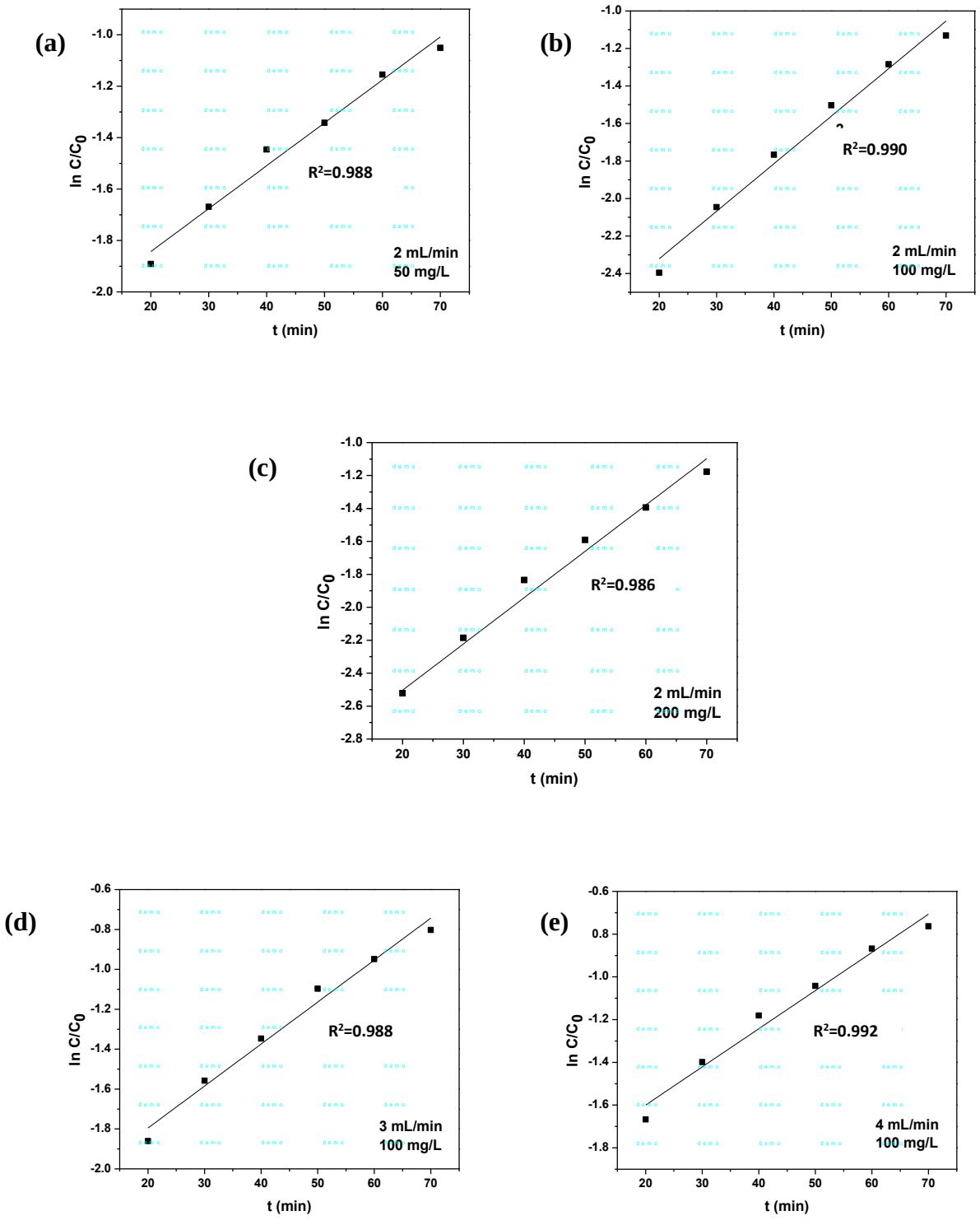


Figure 6.8. (a-e). Linear Regression Fit for Adam-Bohart model using dry biomass at 15 cm bed height with different flow rate (2-4 mL/min) and dye concentration (50-200 mg/L).

Table 6.7 (b). Adam-Bohart parameters at different conditions using linear regression analysis (dry biomass).

Flow rate (mL/min)	Dye concentration (mg/L)	K_{AB} (L/mg/min)	N_0	R^2
2	50	0.00012	2145.68	0.988
	100	0.000048	3569.17	0.990
	200	0.000078	5684.14	0.986
3	100	0.00006	3767.34	0.988
4	100	0.000034	5805.94	0.992

Table 6.8 (a). Data for fitting Adam-Bohart model (immobilized biomass)

Time (min)	Q= 2 mL/min						Q= 3 mL/min		Q= 4 mL/min	
	C_0= 50mg/L		C_0= 100mg/L		C_0= 200mg/L		C_0= 100mg/L		C_0= 100mg/L	
	C mg/L	ln C/C_0	C mg/L	ln C/C_0	C mg/L	ln C/C_0	C mg/L	ln C/C_0	C mg/L	ln C/C_0
0	0	0	0	0	0	0	0	0	0	0
2	0.62	-4.390	0.886	-4.726	0.996	-5.302	1.94	-3.942	2.6	-3.649
5	1.22	-3.713	1.62	-4.122	2.28	-4.474	4.96	-3.003	5.48	-2.904
10	3.02	-2.806	2.825	-3.566	3.78	-3.968	23.54	-1.446	35.96	-1.022
15	5.48	-2.210	7.86	-2.543	9.72	-3.024	27.72	-1.283	42.66	-0.851
20	6.34	-2.065	10.32	-2.271	13.66	-2.683	32.4	-1.127	44.12	-0.818
30	7.42	-1.907	11.14	-2.194	16.24	-2.510	33.9	-1.081	44.89	-0.800
40	8.08	-1.822	14.64	-1.921	20.18	-2.293	34.86	-1.053	46.16	-0.773
50	8.94	-1.721	15.16	-1.886	25.22	-2.070	36.12	-1.018	46.76	-0.760
60	9.28	-1.684	17.78	-1.727	28.64	-1.943	36.48	-1.008	47.08	-0.753
70	9.86	-1.623	18.22	-1.702	32.6	-1.814	38.42	-0.956	47.5	-0.744
80	10.01	-1.608	19.86	-1.616	34.97	-1.743	40.36	-0.907	47.8	-0.738
90	10.39	-1.571	20.48	-1.585	37.02	-1.686	40.66	-0.899	48.02	-0.733
120	11.77	-1.446	22.66	-1.484	40.98	-1.585	41.14	-0.888	48.2	-0.729
150	12.24	-1.407	23.6	-1.443	43.04	-1.536	41.74	-0.873	49.14	-0.710
180	13.88	-1.281	24.41	-1.410	45.84	-1.4731	42.6	-0.8533	49.96	-0.693
210	13.99	-1.273	25.37	-1.371	47.72	-1.432	44.98	-0.798	50.12	-0.690
240	14.36	-1.247	25.66	-1.360	49.14	-1.403	45.18	-0.794	51.14	-0.670
270	15.2	-1.190	25.95	-1.348	49.87	-1.388	46.99	-0.755	51.97	-0.654
300	15.24	-1.188	27.76	-1.281	51.88	-1.349	47.12	-0.752	54.68	-0.603
330	15.31	-1.183	28.39	-1.259	52.77	-1.332	48.42	-0.725	55.16	-0.594
360	15.96	-1.141	30.32	-1.193	54.6	-1.298	50.04	-0.692	56.42	-0.572
390	16.18	-1.128	30.38	-1.191	55.18	-1.287	52.48	-0.644	57.08	-0.560
420	16.63	-1.100	30.58	-1.184	55.58	-1.280	53.12	-0.632	57.96	-0.545

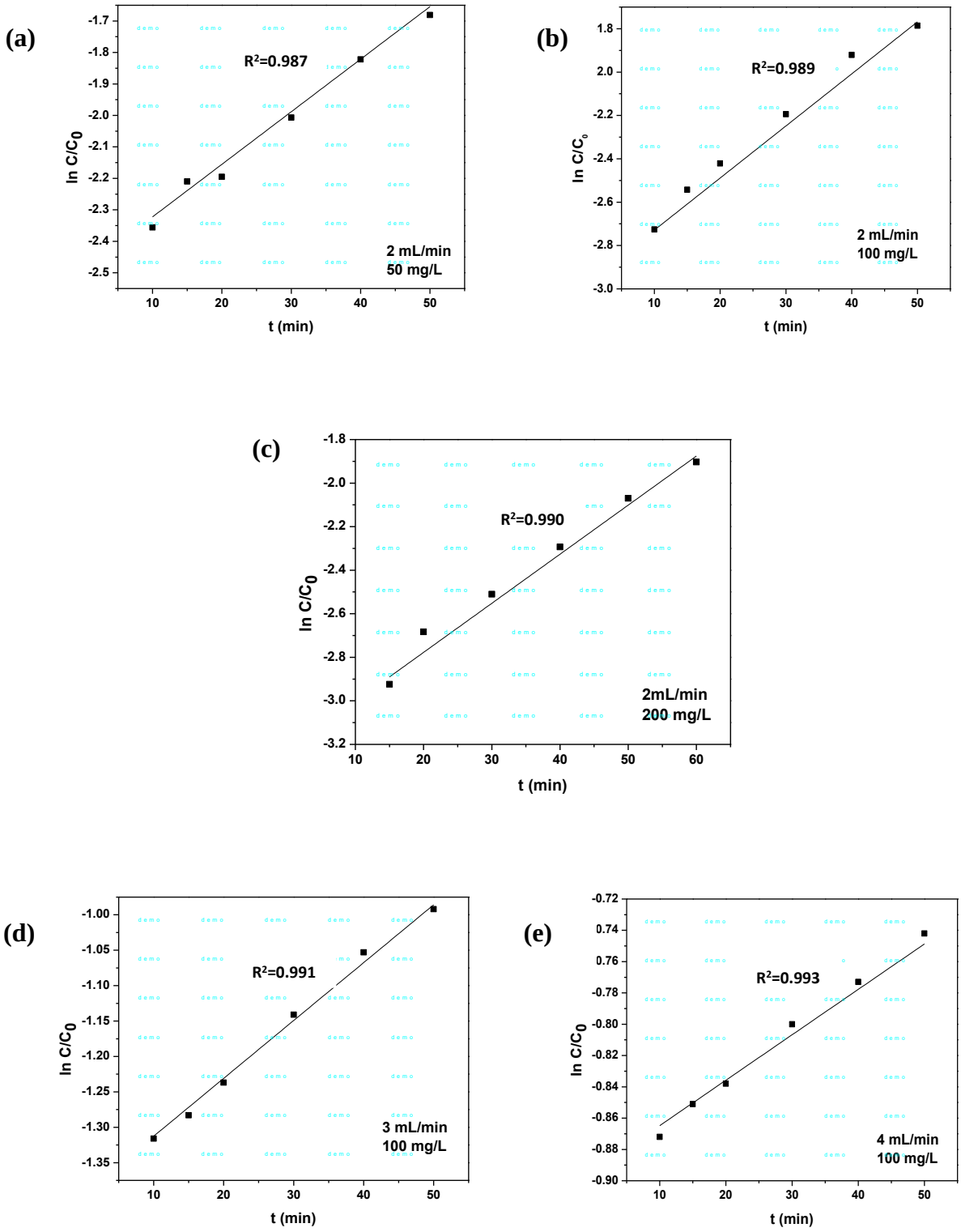


Figure 6.9. (a-e). Linear Regression Fit for Adam-Bohart model using immobilized biomass at 20 cm bed height with different flow rate (2-4 mL/min) and dye concentration (50-200 mg/L).

Table 6.8 (b). Adam-Bohart parameters at different conditions using linear regression analysis (immobilized biomass).

Flow rate (mL/min)	Dye concentration (mg/L)	K_{AB} (L/mg/min)	N_0	R^2
2	50	0.00009	2042.65	0.987
	100	0.000045	3429.38	0.989
	200	0.000068	5420.88	0.990
3	100	0.00005	3676.46	0.991
4	100	0.000027	5628.32	0.993

6.4.6. Application of Yoon and Nelson model

Yoon and Nelson, [Yoon and Nelson, 1984] developed a relatively simple model concerning gas-charcoal adsorption. This model is based on the assumption that the rate of decrease in the probability of adsorption of adsorbate molecule is proportional to the probability of the adsorbate adsorption and the adsorbate breakthrough on the adsorbent. The Yoon and Nelson model is less complicated than other models and also do not require detailed data concerning the characteristic of adsorbate, the type of adsorbent and the physical properties of the bed. This single component model is expressed in the form of Equation (6.8).

$$\ln [C/(C_0-C)] = K_{YN}t - \tau K_{YN} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (6.8)$$

Where, K_{YN} is the rate constant (min^{-1}) and τ is the time required for 50 % adsorbate breakthrough time.

From a linear plot of ln $[C/(C_0-C)]$ against sampling time (t), values of K_{YN} and τ were determined from slope and intercept of the respective linear regression fit [**Figure 6.10 (a-e); Figure 6.11 (a-e)**] for dry and immobilized biomass respectively] and presented in Table 6.9 (b) (dry biomass) and 6.10 (b) (immobilized biomass). It was observed that the value of K_{YN} decreased with increase in flow rate but increased for increase in influent dye concentration.

Table 6.9 (a). Data for Yoon and Nelson model (dry biomass)

Time (min)	Q= 2 mL/min						Q= 3 mL/min		Q= 4 mL/min	
	C_0= 50mg/L		C_0= 100mg/L		C_0= 200mg/L		C_0= 100mg/L		C_0= 100mg/L	
	C mg/L	ln C/(C_0-C)	C mg/L	ln C/(C_0-C)	C mg/L	ln C/(C_0-C)	C mg/L	ln C/(C_0-C)	C mg/L	ln C/(C_0-C)
0	0	0	0	0	0	0	0	0	0	0
2	0.76	-4.171	0.89	-4.713	1.82	-4.690	3.9	-3.204	4.6	-3.032
5	1.78	-3.299	1.94	-3.923	2.15	-4.522	4.4	-3.079	5.1	-2.924
10	2.96	-2.766	2.57	-3.635	4.04	-3.882	6.2	-2.717	6.9	-2.602
15	4.6	-2.289	5.02	-2.940	9.96	-2.949	10.12	-2.184	13.06	-1.896
20	7.54	-1.728	9.1	-2.301	14.52	-2.547	15.55	-1.692	18.88	-1.458
30	9.42	-1.460	12.92	-1.908	22.47	-2.067	21.04	-1.323	24.66	-1.117
40	11.77	-1.178	17.07	-1.581	31.9	-1.662	25.98	-1.047	30.69	-0.815
50	13.06	-1.040	22.24	-1.252	40.71	-1.364	33.37	-0.691	35.26	-0.608
60	15.75	-0.777	27.67	-0.961	49.59	-1.110	38.68	-0.461	41.94	-0.325
70	17.47	-0.622	32.25	-0.742	61.6	-0.809	44.78	-0.210	46.62	-0.135
80	18.88	-0.500	37.18	-0.525	72.36	-0.568	49.94	-0.002	52.76	0.111
90	19.14	-0.478	41.82	-0.330	81.94	-0.365	54.56	0.183	56.18	0.248
120	20.17	-0.391	48.65	-0.054	90.5	-0.191	57.78	0.314	60.38	0.421
150	21.7	-0.266	54.4	0.176	99.18	-0.016	60.85	0.441	62.7	0.519
180	22.81	-0.176	57.51	0.303	109.04	0.181	62.37	0.505	64.42	0.594
210	23.5	-0.120	61.04	0.449	118.4	0.372	64.45	0.595	65.98	0.662
240	24.35	-0.052	65.5	0.641	127.25	0.559	65.22	0.629	66.67	0.693
270	25.88	0.070	68.8	0.791	136.5	0.765	66.69	0.694	67.15	0.715
300	27.55	0.205	70.43	0.868	141.08	0.873	67.19	0.717	67.87	0.748
330	28.3	0.266	71.36	0.913	142.16	0.899	68.07	0.757	68.24	0.765
360	28.46	0.279	71.9	0.940	142.87	0.917	68.34	0.769	68.56	0.780

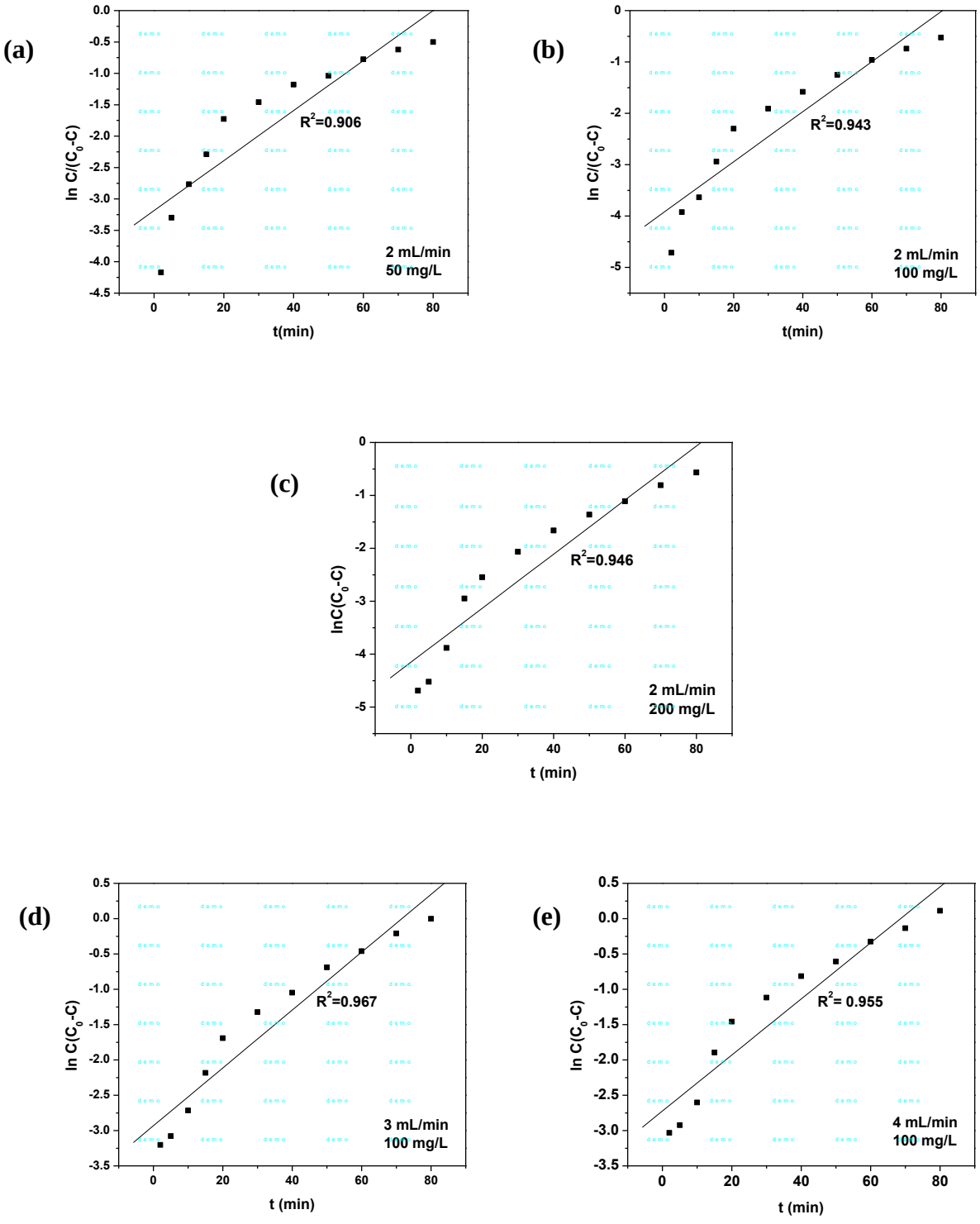


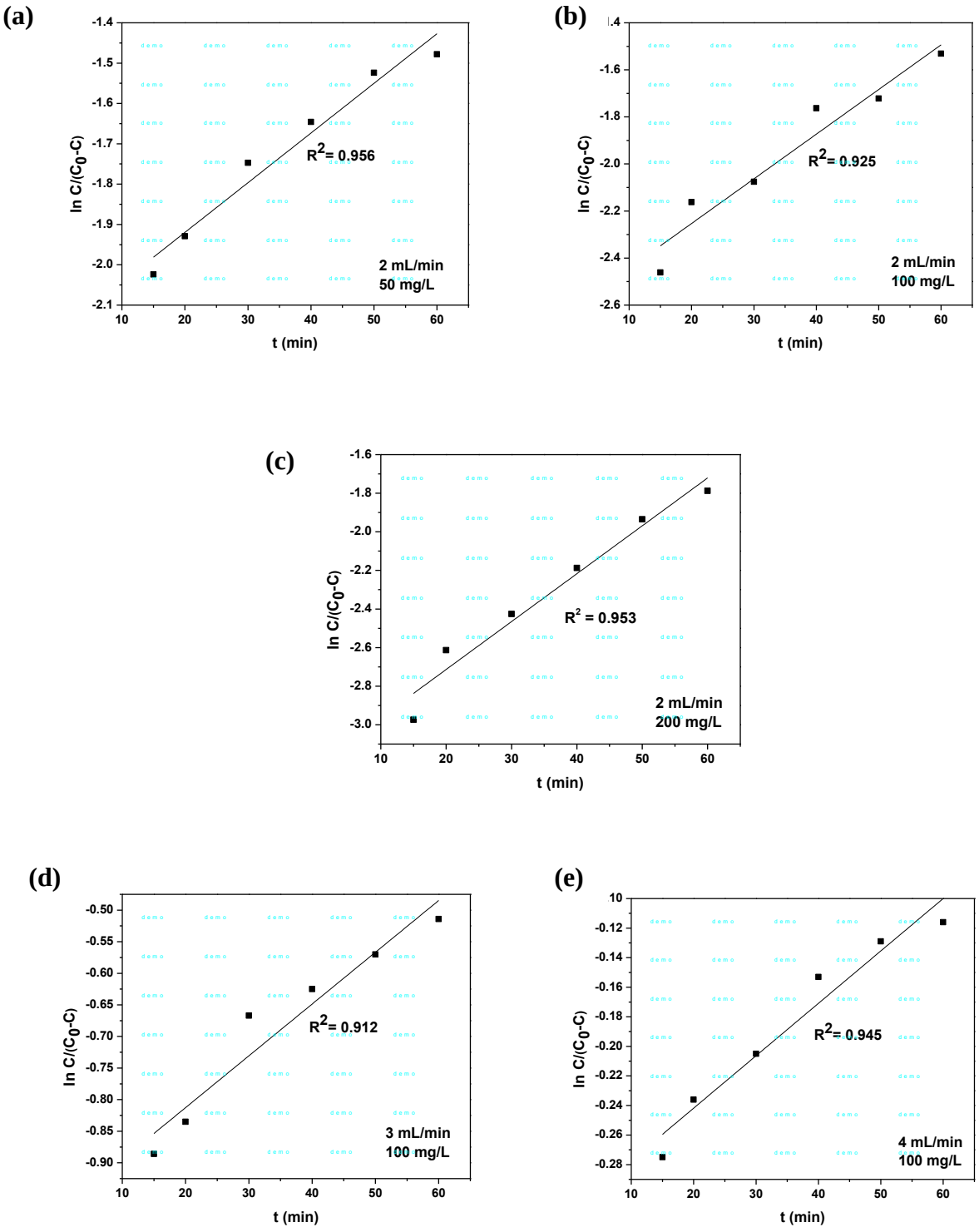
Figure 6.10. (a-e). Linear Regression Fit for Yoon and Nelson model using dry biomass at 20 cm bed height with different flow rate (2-4 mL min^{-1}) and dye concentration (50-200 mg L^{-1}).

Table 6.9 (b). Yoon and Nelson parameters at different conditions using linear regression analysis (dry biomass).

Flow rate (mL/min)	Dye concentration (mg/L)	K_{YN} (min^{-1})	τ (min)	R^2
2	50	0.0044	472.4	0.906
	100	0.0065	466.2	0.943
	200	0.0088	510.6	0.946
3	100	0.0042	398.5	0.967
4	100	0.008	361.2	0.955

Table 6.10 (a). Data for Yoon and Nelson model (immobilized biomass)

Time (min)	Q= 2 mL/min						Q= 3 mL/min		Q= 4 mL/min	
	C_0= 50mg/L		C_0= 100mg/L		C_0= 200mg/L		C_0= 100mg/L		C_0= 100mg/L	
	C mg/L	ln C/(C_0-C)	C mg/L	ln C/(C_0-C)	C mg/L	ln C/(C_0-C)	C mg/L	ln C/(C_0-C)	C mg/L	ln C/(C_0-C)
0	0	0	0	0	0	0	0	0	0	0
2	0.62	-4.377	0.886	-4.717	0.996	-5.297	1.94	-3.922	2.6	-3.623
5	1.22	-3.688	1.62	-4.106	2.28	-4.462	4.96	-2.952	5.48	-2.847
10	3.02	-2.744	2.825	-3.538	3.78	-3.949	23.54	-1.178	35.96	-0.577
15	5.48	-2.094	7.86	-2.461	9.72	-2.974	27.72	-0.958	42.66	-0.295
20	6.34	-1.929	10.32	-2.162	13.66	-2.613	32.4	-0.735	44.12	-0.236
30	7.42	-1.747	11.14	-2.076	16.24	-2.426	33.9	-0.667	44.89	-0.205
40	8.08	-1.646	14.64	-1.763	20.18	-2.187	34.86	-0.625	46.16	-0.153
50	8.94	-1.524	15.16	-1.722	25.22	-1.935	36.12	-0.570	46.76	-0.129
60	9.28	-1.478	17.78	-1.531	28.64	-1.788	36.48	-0.554	47.08	-0.116
70	9.86	-1.403	18.22	-1.501	32.6	-1.636	38.42	-0.471	47.5	-0.100
80	10.01	-1.385	19.86	-1.395	34.97	-1.551	40.36	-0.390	47.8	-0.088
90	10.39	-1.338	20.48	-1.356	37.02	-1.482	40.66	-0.378	48.02	-0.079
120	11.77	-1.178	22.66	-1.227	40.98	-1.355	41.14	-0.358	48.2	-0.072
150	12.24	-1.126	23.6	-1.174	43.04	-1.293	41.74	-0.333	49.14	-0.034
180	13.88	-0.956	24.41	-1.130	45.84	-1.212	42.6	-0.298	49.96	-0.001
210	13.99	-0.945	25.37	-1.079	47.72	-1.160	44.98	-0.201	50.12	0.004
240	14.36	-0.909	25.66	-1.063	49.14	-1.121	45.18	-0.193	51.14	0.045
270	15.2	-0.828	25.95	-1.048	49.87	-1.102	46.99	-0.120	51.97	0.078
300	15.24	-0.824	27.76	-0.956	51.88	-1.049	47.12	-0.115	54.68	0.1875
330	15.31	-0.817	28.39	-0.925	52.77	-1.026	48.42	-0.063	55.16	0.207
360	15.96	-0.757	30.32	-0.832	54.6	-0.979	50.04	0.001	56.42	0.258
390	16.18	-0.737	30.38	-0.829	55.18	-0.964	52.48	0.099	57.08	0.285
420	16.63	-0.696	30.58	-0.819	55.58	-0.954	53.12	0.124	57.96	0.321



6.11. (a-e). Linear Regression Fit for Yoon and Nelson model using immobilized biomass at 20 cm bed height with different flow rate (2-4 mL min^{-1}) and dye concentration (50-200 mg L^{-1}).

Table 6.10 (b). Yoon and Nelson parameters at different conditions using linear regression analysis (immobilized biomass).

Flow rate (mL/min)	Dye concentration (mg/L)	K_{YN} (min^{-1})	τ (min)	R^2
2	50	0.0047	468.6	0.956
	100	0.0058	462.2	0.925
	200	0.0082	502.4	0.953
3	100	0.0036	382.5	0.912
4	100	0.006	358.7	0.945

6.4.7. Thomas model

Thomas model assumes plug flow behavior in the bed, and uses Langmuir isotherm for equilibrium, and second-order reversible reaction kinetics. This model is suitable for adsorption process where the external and internal diffusion limitations are absent. Also the maximum adsorption capacity of an adsorbent is needed in successful design of column adsorption process. Traditionally, Thomas model is used to fulfill the purpose. The linearized form of Thomas model (Thomas, 1948) is expressed in Equation (6.9).

$$\ln [(C_0/C)-1] = (K_{Th}q_0M - k_{Th}C_0V_{eff})/Q \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (6.9)$$

Where, K_{TH} is the Thomas rate constant (mL min^{-1} mg^{-1}), q_0 (mg g^{-1}) is the equilibrium dye uptake capacity of the adsorbent, C_0 and C (mg L^{-1}) are the inlet and outlet dye concentration, M (g) is the mass of adsorbent, Q (mL min^{-1}) is the flow rate.

The experimental data were fitted to the modified Thomas model to obtain the respective linear regression fit for varied flow rates, bed height and influent dye concentration [**Figure 6.12 (a-e); 6.13 (a-e)**]. The determined coefficients and relative constants were obtained using linear regression analysis according to Equation (6.9) and the results are listed in Table 6.11 (a-b) for dry biomass and Table 6.12 (a-b) for immobilized biomass. The value of K_{TH} decreases as the influent dye concentration increases. These findings could be attributed to the higher driving force of the high influent dye concentration (Padmesh et al., 2006).

Table 6.11 (a). Data for fitting Thomas model (dry biomass)

Time (min)	V_{eff} (mL)	Q= 2 mL/min					
		C_0= 50mg/L		C_0= 100mg/L		C_0= 200mg/L	
		C mg/L	ln [(C_0/C)-1]	C mg/L	ln [(C_0/C)-1]	C mg/L	ln [(C_0/C)-1]
0	0	0	0	0	0	0	0
2	4	0.76	4.171	0.89	4.713	1.82	4.690
5	10	1.78	3.299	1.94	3.923	2.15	4.522
10	20	2.96	2.766	2.57	3.635	4.04	3.882
15	30	4.6	2.289	5.02	2.940	9.96	2.949
20	40	7.54	1.728	9.1	2.301	14.52	2.547
30	60	9.42	1.460	12.92	1.908	22.47	2.067
40	80	11.77	1.178	17.07	1.581	31.9	1.662
50	100	13.06	1.040	22.24	1.252	40.71	1.364
60	120	15.75	0.777	27.67	0.961	49.59	1.110
70	140	17.47	0.622	32.25	0.742	61.6	0.809
80	160	18.88	0.500	37.18	0.525	72.36	0.568
90	180	19.14	0.478	41.82	0.330	81.94	0.365
120	240	20.17	0.391	48.65	0.054	90.5	0.191
150	300	21.7	0.266	54.4	-0.176	99.18	0.016
180	360	22.81	0.176	57.51	-0.303	109.04	-0.181
210	420	23.5	0.120	61.04	-0.449	118.4	-0.372
240	480	24.35	0.052	65.5	-0.641	127.25	-0.559
270	540	25.88	-0.070	68.8	-0.791	136.5	-0.765
300	600	27.55	-0.205	70.43	-0.868	141.08	-0.873
330	660	28.3	-0.266	71.36	-0.913	142.16	-0.899
360	720	28.46	-0.279	71.9	-0.940	142.87	-0.917

Table 6.11 (b). Data for Thomas model (continuation).

Time (min)	Q = 3 mL/min			Q = 4 mL/min		
	C_0 = 100 mg/L			C_0 = 100 mg/L		
	V_{eff} (mL)	C (mg/L)	ln [(C_0/C)-1]	V_{eff} (mL)	C (mg/L)	ln [(C_0/C)-1]
0	0	0	0	0	0	0
2	6	3.9	3.204	8	4.6	3.032
5	15	4.4	3.079	20	5.1	2.924
10	30	6.2	2.717	40	6.9	2.602
15	45	10.12	2.184	60	13.06	1.896
20	60	15.55	1.692	80	18.88	1.458
30	90	21.04	1.323	120	24.66	1.117
40	120	25.98	1.047	160	30.69	0.815
50	150	33.37	0.691	200	35.26	0.608
60	180	38.68	0.461	240	41.94	0.325
70	210	44.78	0.210	280	46.62	0.135
80	240	49.94	0.002	320	52.76	-0.111
90	270	54.56	-0.183	360	56.18	-0.248
120	360	57.78	-0.314	480	60.38	-0.421
150	450	60.85	-0.441	600	62.7	-0.519
180	540	62.37	-0.505	720	64.42	-0.594
210	630	64.45	-0.595	840	65.98	-0.662
240	720	65.22	-0.629	960	66.67	-0.693
270	810	66.69	-0.694	1080	67.15	-0.715
300	900	67.19	-0.717	1200	67.87	-0.748
330	990	68.07	-0.757	1320	68.24	-0.765
360	1080	68.34	-0.769	1440	68.56	-0.780

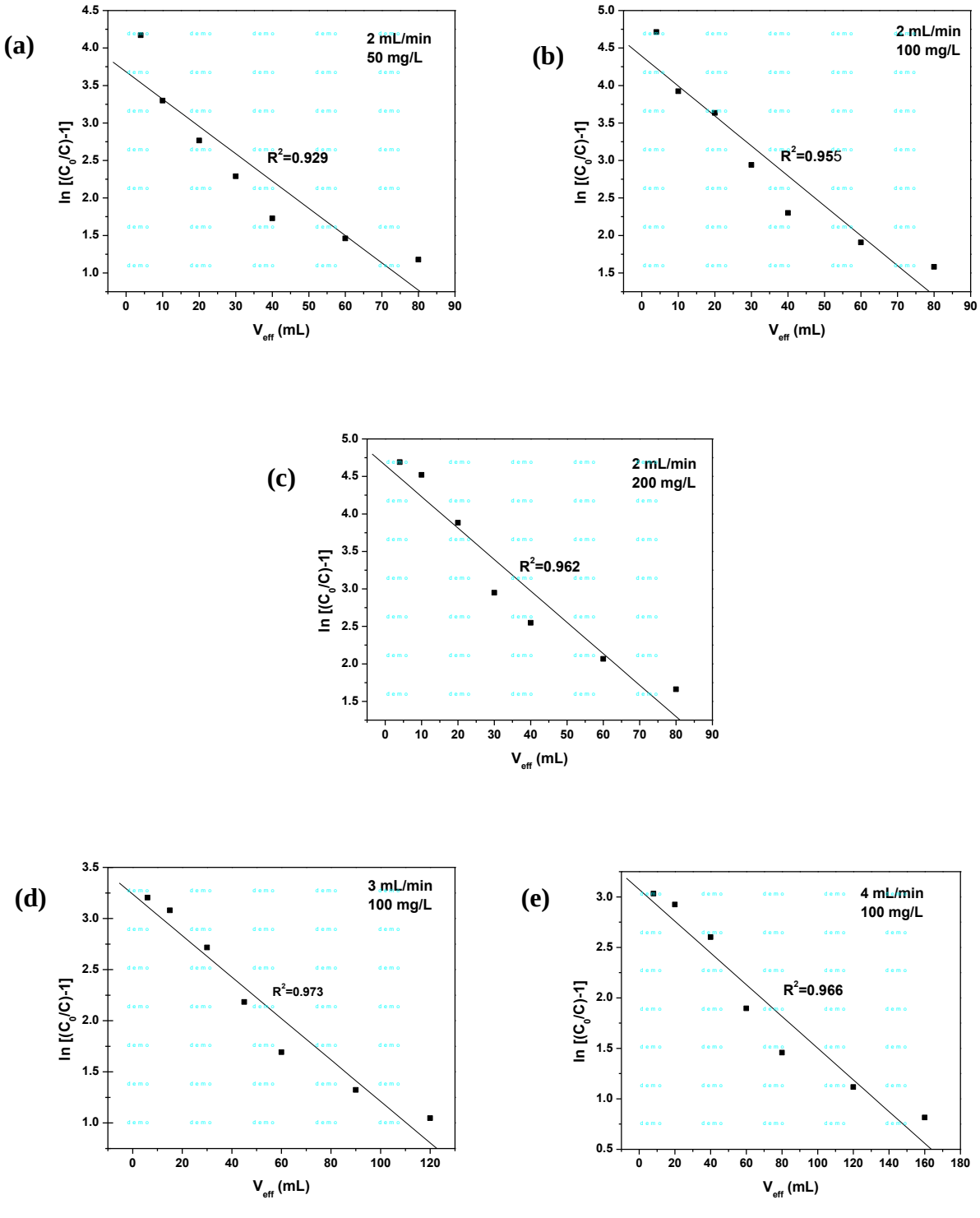


Figure 6.12 (a-e). Linear Regression Fit for Thomas model using dry biomass at 15 cm bed height with different flow rate (2-4 mL/min) and dye concentration (50-200 mg/L).

Table 6.11 (c). Thomas model parameters at different conditions using linear regression analysis (dry biomass).

Flow rate (mL/min)	Dye concentration (mg/L)	K_{TH} (mL/mg/min)	q_0 (mg/g)	R^2
2	50	0.097	20.44	0.929
	100	0.062	42.65	0.955
	200	0.045	66.87	0.962
3	100	0.547	10.14	0.973
4	100	0.425	11.24	0.966

Table 6.12 (a). Data for fitting Thomas model (immobilized biomass)

Time (min)	Q= 2 mL/min						
	V_{eff} (mL)	C_0= 50mg/L		C_0= 100mg/L		C_0= 200mg/L	
		C mg/L	ln[(C_0/C)-1]	C mg/L	ln[(C_0/C)-1]	C mg/L	ln[(C_0/C)-1]
0	0	0	0	0	0	0	0
2	4	0.62	4.377	0.886	4.717	0.996	5.297
5	10	1.22	3.688	1.62	4.106	2.28	4.462
10	20	3.02	2.744	2.825	3.538	3.78	3.949
15	30	5.48	2.094	7.86	2.461	9.72	2.974
20	40	6.34	1.929	10.32	2.162	13.66	2.613
30	60	7.42	1.747	11.14	2.076	16.24	2.426
40	80	8.08	1.646	14.64	1.763	20.18	2.187
50	100	8.94	1.524	15.16	1.722	25.22	1.935
60	120	9.28	1.478	17.78	1.531	28.64	1.788
70	140	9.86	1.403	18.22	1.501	32.6	1.636
80	160	10.01	1.385	19.86	1.395	34.97	1.551
90	180	10.39	1.338	20.48	1.356	37.02	1.482
120	240	11.77	1.178	22.66	1.227	40.98	1.355
150	300	12.24	1.126	23.6	1.174	43.04	1.293
180	360	13.88	0.956	24.41	1.130	45.84	1.212
210	420	13.99	0.945	25.37	1.078	47.72	1.160
240	480	14.36	0.909	25.66	1.063	49.14	1.121
270	540	15.2	0.828	25.95	1.048	49.87	1.102
300	600	15.24	0.824	27.76	0.956	51.88	1.049
330	660	15.31	0.817	28.39	0.925	52.77	1.026
360	720	15.96	0.757	30.32	0.832	54.6	0.979
390	780	16.18	0.737	30.38	0.829	55.18	0.964
420	840	16.63	0.696	30.58	0.819	55.58	0.954

Table 6.12 (b). Data for Thomas model (continuation).

Time (min)	Q = 3 mL/min			Q = 4 mL/min		
	C_0 = 100 mg/L			C_0 = 100 mg/L		
	V_{eff} (mL)	C (mg/L)	ln [(C_0/C)-1]	V_{eff} (mL)	C (mg/L)	ln [(C_0/C)-1]
0	0	0	0	0	0	0
2	6	1.94	2.942	8	2.6	2.649
5	15	4.96	2.003	20	5.48	1.904
10	30	23.54	0.446	40	35.96	0.022
15	45	27.72	0.283	60	42.66	-0.148
20	60	32.4	0.127	80	44.12	-0.181
30	90	33.9	0.081	120	44.89	-0.199
40	120	34.86	0.053	160	46.16	-0.226
50	150	36.12	0.018	200	46.76	-0.239
60	180	36.48	0.008	240	47.08	-0.246
70	210	38.42	-0.043	280	47.5	-0.255
80	240	40.36	-0.092	320	47.8	-0.261
90	270	40.66	-0.100	360	48.02	-0.266
120	360	41.14	-0.111	480	48.2	-0.270
150	450	41.74	-0.126	600	49.14	-0.289
180	540	42.6	-0.146	720	49.96	-0.306
210	630	44.98	-0.201	840	50.12	-0.309
240	720	45.18	-0.205	960	51.14	-0.329
270	810	46.99	-0.244	1080	51.97	-0.345
300	900	47.12	-0.247	1200	54.68	-0.396
330	990	48.42	-0.274	1320	55.16	-0.405
360	1080	50.04	-0.307	1440	56.42	-0.427
390	1170	52.48	-0.355	1560	57.08	-0.439
420	1260	53.12	-0.367	1680	57.96	-0.454

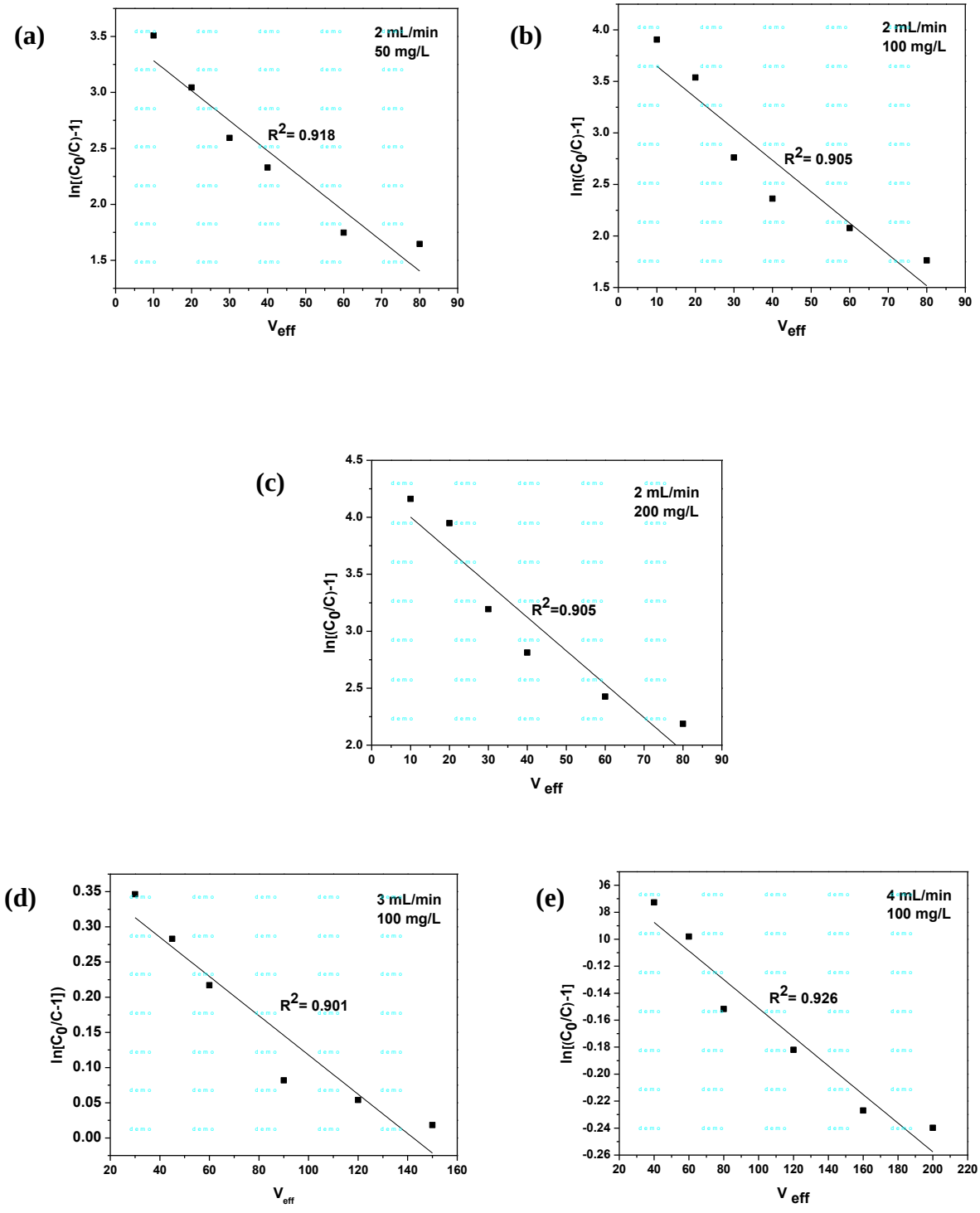


Figure 6.13 (a-e). Linear Regression Fit for Thomas model using immobilized biomass at 20 cm bed height with different flow rate (2-4 mL/min) and dye concentration (50-200 mg/L).

Table 6.12 (c). Thomas model parameters at different conditions using linear regression analysis (immobilized biomass).

Flow rate (mL/min)	Dye concentration (mg/L)	K_{TH} (mL/mg/min)	q_0 (mg/g)	R^2
2	50	0.094	18.66	0.918
	100	0.058	38.24	0.905
	200	0.036	62.74	0.905
3	100	0.548	7.80	0.901
4	100	0.416	8.12	0.926

6.4.8. Desorption of Reactive Blue 4 from column reactor packed with dry biomass of *R. oryzae*:

In this case it is seen that after the 4th adsorption-desorption cycle, 57.78% of dye can be desorbed (**Figure 6.14**) using dry biomass of *R. oryzae* (MTCC 262) in packed bed column reactor. Desorption efficiency of the bed for Reactive Blue 4 desorption is shown in the Table 6.13.

Table 6.13. Dye adsorbed and desorbed from dry biomass of *R. oryzae* packed bed column:

Cycle No.	Dye adsorption (%)	Dye desorption with respect to dye adsorption (%)
Cycle 1	**99.26**	**93.47**
Cycle 2	**94.12**	**90.62**
Cycle 3	**86.38**	**77.76**
Cycle 4	**62.21**	**57.78**

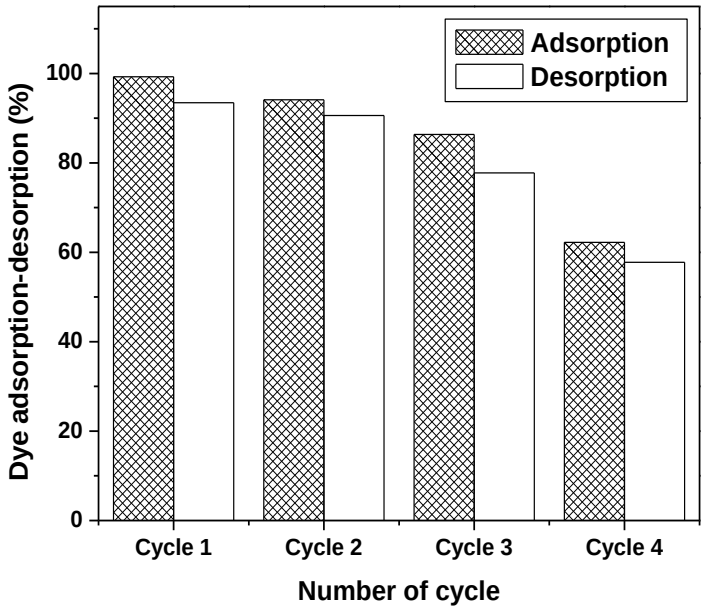


Figure 6.14. Adsorption-desorption cycle of Reactive Blue 4 using dry biomass of Rhizopus oryzae (MTCC 262).

6.4.9. Desorption of Reactive Blue 4 from packed bed column reactor with biomass of *R. oryzae* immobilized in carboxymethyl cellulose:

In present study it was found that after the 4th adsorption-desorption cycle, 61.28% of dye can be desorbed (**Figure 6.15**) using immobilized biomass of *R. oryzae* (MTCC 262) in packed bed column reactor. Desorption efficiency of the bed for Reactive Blue 4 desorption is shown in the Table 6.14.

Table 6.14. Dye adsorbed and desorbed from CMC immobilized *R. oryzae* (MTCC 262) biomass packed bed column:

Cycle No.	Dye adsorption (%)	Dye desorption with respect to dye adsorption (%)
Cycle 1	**98.02**	**92.26**
Cycle 2	**90.54**	**87.42**
Cycle 3	**86.28**	**81.72**
Cycle 4	**72.46**	**61.28**

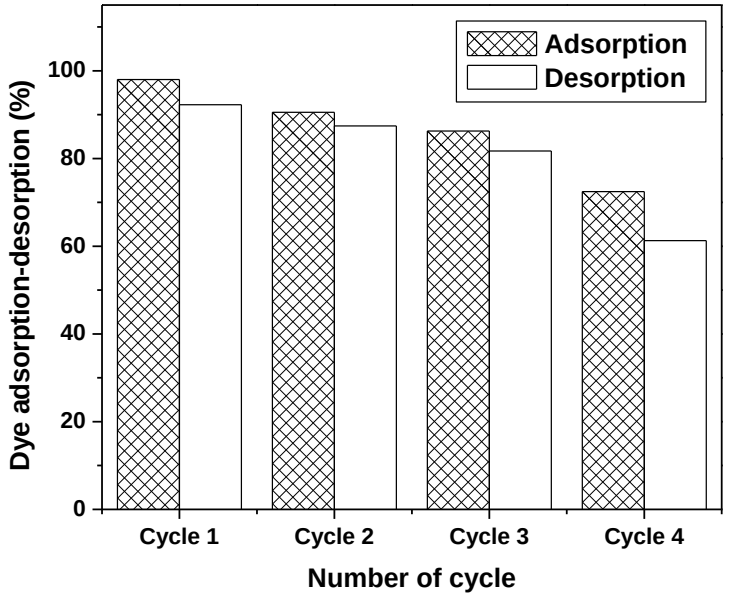


Figure 6.15. Adsorption-desorption cycle of Reactive Blue 4 using immobilized biomass of Rhizopus oryzae (MTCC 262).

6.5. CONCLUSIONS

Optimization of some process parameters such as the bed height, flow rate and influent dye concentration were found essential using both dry and immobilized biomass for optimum removal of dye through packed bed column. The data obtained in a series of experiments by varying bed height, flow rate and dye concentration were fitted to different models viz., Adam-Bohart model, Yoon and Nelson model and the modified Thomas model in order to predict the breakthrough curve of the adsorption. The packed bed adsorption system was found to perform better with lower dye concentration, lower feed flow rate and higher bed height. Reactive Blue 4 removal of 71.81% and 76.88 % was obtained using dry and immobilized biomass respectively at 2 mL min^{-1} flow rate, 15 cm (dry biomass) and 20 cm (immobilized biomass) bed height and 200 mg L^{-1} influent dye concentration. Adam–Bohart model fitted well to the initial part of breakthrough curve. Yoon–Nelson and Thomas models showed poor fitting to the breakthrough curve.

To perform a successful dye removal procedure it is not only necessary to develop a continuous packed-bed column bioreactor system with a powerful adsorbent material for onsite operations, but also to determine the dye recovery percentage with an efficient desorbent solution. Adsorbed dye can be successfully recovered from dry and CMC immobilized *R. oryzae* (MTCC 262) packed bed column reactor by running 1 (N) NaOH and 0.1 (N) NaOH respectively through the bed and it showed up to 57.78 and 61.28 % respectively of dye recovery after four adsorption-desorption cycles. The eluants desorb dye without significant deterioration of adsorption capacity of the bed. As recovery of the adsorbed dye molecules is possible to a considerable extent from both the beds so it can be concluded that dry and CMC immobilized *R. oryzae* packed bed column may be repeatedly utilized for practical purposes for the removal of Reactive Blue 4.

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SUMMARY OF THE THESIS

From the present investigations, it might be concluded that *Rhizopus oryzae* biomass was capable of removing the carcinogenic, tri-azine textile dye Reactive Blue 4 from aqueous solution efficiently. The biosorption process was found to be influenced by environmental factors viz., initial pH of the solution, biomass load, initial dye concentration and contact time between the dry fungal biomass and dye solution. Dye solution having pH 3.0 and biomass load of 2 g L^{-1} was found to be optimum for removal of the dye by *Rhizopus oryzae* biomass. Temperature showed no significant effect on biosorption. The experiment achieved equilibrium within 6 h. The kinetics of the overall adsorption process was best described by pseudo-second order kinetic model. At equilibrium, experimental results of isotherm study were found to be fitted best to Redlich-Peterson isotherm model. FTIR spectroscopy study showed amino and carboxyl groups were the functional groups responsible for the dye adsorption process. Scanning electron microscopy showed significant changes in surface morphology of *Rhizopus oryzae* biomass after adsorption. Thus, removal of Reactive Blue 4 using the biomass of *Rhizopus oryzae* may be viewed as a considerable alternative process in near future.

The present study shows that Reactive Blue 4 solutions can be effectively removed by adsorption using dead biomass of *R. oryzae* (MTCC 262). Dead (autoclaved) biomass of *R. oryzae* was observed to adsorb Reactive Blue 4 more efficiently than other pre-treated biomass. Physico-chemical properties indicate that the optimum pH for adsorption is 3.0 and it is not significantly affected by incubation temperature. The process is very fast initially, reaches equilibrium within 360 min and the kinetics of the overall adsorption process was best described by pseudo-second order kinetic model. Experimental results with dead biomass at equilibrium were found to fit best to Redlich–Peterson isotherm model with higher correlation coefficient. The results of chemical modification study proved the involvement of amino and carboxyl groups in the dye adsorption process. FTIR spectroscopic study reveals the active involvement of different functional groups of acid, base, heat treated and dead biomass in dye adsorption. Cell surface study of dead *R. oryzae* showed prominent morphological changes after interaction with dye. The results of this study clearly showed that physical surface modification treatments can be used to maximize the dye removal efficiency of the fungal biomass.

The present study shows that Reactive Blue 4 solutions can be effectively decolorized by adsorption using CMC immobilized *R. oryzae* beads. Physico-chemical properties indicate that the optimum pH for adsorption is 3.0 and it is not affected by incubation temperature. The process is very fast initially, reaches equilibrium within 360 min and the kinetics of the overall adsorption process was best described by pseudo-second order kinetic model. Experimental results at equilibrium were found to fit best to both Langmuir and Redlich-Peterson isotherm model. Cell surface study of CMC immobilized (MTCC 262) using scanning electron microscopy showed morphological changes of *R. oryzae* beads after interaction with dye. On the whole, it can be concluded that among the various carrier used in this study for immobilization purpose *R. oryzae* (MTCC 262), immobilized in CMC were found most efficient due to high availability of adsorption sites for the removal Reactive Blue 4.

The desorption experiments proved that Reactive Blue 4 could be easily eluted from dry and CMC immobilized beads of *Rhizopus oryzae* (MTCC 262) respectively. Of the different desorption agents 1 (N) NaOH was found to be best (98.92 % desorption) for Reactive Blue 4 desorption for dry biomass and 0.1 (N) NaOH showed the best desorption efficiency (96.52 % desorption) for Reactive Blue 4 using immobilized biomass of *Rhizopus oryzae* (MTCC 262) respectively. The regeneration studies indicated that the dry *Rhizopus oryzae* biomass can be used repeatedly for four consecutive adsorption-desorption cycles to treat the Reactive Blue containing effluent while *R. oryzae* biomass in immobilized form can be used successfully for five consecutive adsorption-desorption cycles to treat the Reactive Blue 4 contaminated waste water discharged from the textile industry in near future.

Optimization of some process parameters such as the bed height, flow rate and influent dye concentration were found essential using both dry and immobilized biomass for optimum removal of dye through packed bed column. The data obtained in a series of experiments by varying bed height, flow rate and dye concentration were fitted to different models viz., Adam-Bohart model, Yoon and Nelson model and the modified Thomas model in order to predict the breakthrough curve of the adsorption. The packed bed adsorption system was found to perform better with lower dye concentration, lower feed flow rate and higher bed height. Reactive Blue 4 removal of 71.81% and 76.88 % was obtained using dry and immobilized biomass respectively

at 2 mL min^{-1} flow rate, 15 cm (dry biomass) and 20 cm (immobilized biomass) bed height and 200 mg L^{-1} influent dye concentration. Adam–Bohart model fitted well to the initial part of breakthrough curve. Yoon–Nelson and Thomas models showed poor fitting to the breakthrough curve.

To perform a successful dye removal procedure it is not only necessary to develop a continuous packed-bed column bioreactor system with a powerful adsorbent material for onsite operations, but also to determine the dye recovery percentage with an efficient desorbent solution. Adsorbed dye can be successfully recovered from dry and CMC immobilized *R. oryzae* (MTCC 262) packed bed column reactor by running 1 (N) NaOH and 0.1 (N) NaOH respectively through the bed and it showed up to 57.78 and 61.28 % respectively of dye recovery after four adsorption-desorption cycles. The eluants desorb dye without significant deterioration of adsorption capacity of the bed. As recovery of the adsorbed dye molecules is possible to a considerable extent from both the beds so it can be concluded that dry and CMC immobilized *R. oryzae* packed bed column may be repeatedly utilized for practical purposes for the removal of Reactive Blue 4.