STUDIES ON THE REMOVAL OF MALACHITE GREEN DYE FROM AQUEOUS PHASE USING LYOPHILLISED FUNGAL BIOMASS, *Aspergillus versicolor*.

A Thesis submitted for the partial requirement for the degree of
Master of Technology
In
Food Technology and Biochemical Engineering
2014-16

By

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This project is dedicated to my parents and

our beloved Late Dr. Lalitagauri Ray …

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Declaration of Originality and Compliance of Academic Ethics

I hereby declare that the thesis contains survey and original research work by the undersigned candidate, as part of her Master of Technology in Food Technology and Biochemical Engineering studies.

All information in this document have been obtained and presented in accordance with academic rules and ethical conduct.

I also declare that, as required by these rules and conduct. I have fully cited and referenced all materials and results that are not original to this work.

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Certificate of Recommendation

I hereby recommend the thesis entitled, **"Studies on the removal of Malachite Green dye from aqueous phase using Lyophilised fungal biomass, *Aspergillus versicolor*"** prepare by Ananya Bardhan, (M4FTB1606) student of M.tech 2nd year, under the guidance and supervision of Late Dr. Lalitagauri Ray has been evaluated thus found satisfactory. It is therefore accepted and submitted as a dissertation for the partial fulfilment for the requirement for awarding the degree of Masters of Technology in Food Technology and Biochemical Engineering.

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This is to certify that Miss Ananya Bardhan (M4FTB1606) has carried out her research work entitled **"Studies on the removal of Malachite Green dye from aqueous phase using Lyophilised fungal biomass, *Aspergillus versicolor*."** under the direct supervision of Late Dr. Lalitagauri Ray, at Department of Food Technology and Biochemical Engineering, Jadavpur University. We are satisfied that she has carried out this work independently with proper care and confidence. We hereby recommend that this dissertation be accepted in the partial fulfilment of the requirements of the degree of Maters of Technology in Food Technology and Biochemical Engineering.

We are very much pleased to forward this thesis for evaluation.

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ACKNOWLEDGEMENT

I would like to take this opportunity to express my sincere thanks to each and every individual whose contribution has been invaluable for the successful completion of thesis work entitled **"Studies on removal of Malachite Green dye from aqueous phase using Lyophilised fungal biomass, *Aspergillus versicolor*".**

I am grateful to Dr. Runu Chakraborty, Head of Department Food Technology and Biochemical Engineering, Jadavpur University for giving me the opportunity to work and also for her precious suggestion and constant motivation during my project tenure.

I express my deepest regard and unbound gratitude with sincerest thanks to my guide respected, Late Dr. Lalitagauri Ray, Professor, Department of Food Technology and Biochemical Engineering, Jadavpur University for her constant inspiration, suggestion, active support and patience.

I am also thankful to other respected faculty members of Department Food Technology and Biochemical Engineering, Dr. Utpal Ray Chaudhuri, Dr. Uma Ghosh, Dr. Paramita Bhattacharya, Dr. Dipankar Halder, Dr. Debabrata Bera, Dr. Prashanta Kumar Biswas and Dr. Sunita Adhikari for their valuable guidance. I would also like my sincere thanks to all Research scholars, non-teaching staff and sub-staff of this department.

I would like to thanks Mr. Surajit Bag for his valuable guidance and tremendous assistance throughout the project work by proving me fruitful suggestion and cooperation. I would also like to thank Mr. Animesh Naskar, Mrs Arundhati Das Sinha, Mrs. Mukulika Bagchi, Mrs. Priyangi Charaborti, Ms. Roopkotha Banerjee for their kind cooperation.

Lastly, I would like to express my heartfelt gratitude to my parents for their constant inspiration and belief.

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Chapter 1

Introduction

1.1.Introduction

Environmental pollution is one of the major and most urgent problems of the modern world. Industries are the greatest polluters, with textile industries generating high liquid effluent pollutants due to the large quantities of water used in fabric processing. These industries, generate wastewaters differing in composition are produced and the coloured water released during the dyeing of fabrics may be the most problematic since even a trace of dye can remain highly visible. Other industries such as paper and pulp mills, dyestuff, distilleries, and tanneries are also producing highly coloured wastewaters. Textile industries generates the largest quantities of aqueous wastes and dye effluents from the dyeing process, with both strong persistent colour and a high biological oxygen demand (BOD), both of which are aesthetically and environmentally unacceptable [1].The textile industry plays a major role in the economy of Asian and other countries. In India, it accounts for the largest consumption of dyestuffs approximately 80% [2], accounting every type of dye and pigment produced, this amounts to close to 80,000 tonnes. India is the second largest exporter of dyestuffs, after China. Worldwide, 106 tons of synthetic dyes are produced annually; of which 1-1.5 x 10^5 tonnes are released into the environment in wastewaters [3]. This release is because not all dye binds to the fabric during the dyeing processes; depending on the class of the dye, the losses in wastewaters can vary from 2% for basic dyes to as high as 50% for reactive dyes, leading to severe contamination of surface and ground waters in the vicinity of dyeing industries [4]. It is estimated that globally 280,000 tons of textile dyes are discharged in textile industrial effluent every year [5]. Apart from the aesthetic point of view, dyes are undesirable because they can affect living creatures in the water discharged as effluent into the environment. Industrial effluents containing synthetic dyes reduce light penetration in rivers and thus affect the photosynthetic activities of aquatic flora, thereby severely affecting the food source of aquatic organisms. The thin layer of discharged dyes that can form over the surfaces of the receiving waters also decreases the amount of dissolved oxygen, thereby affecting the aquatic fauna. Furthermore, dye-containing effluents increase biochemical oxygen demand (BOD). Dyes are generally stable organic pollutants that persist in the environment and concern has been raised that such artificial compounds are xenobiotic. Therefore, methods for their degradation have been increasingly explored and development.

1.2. Basics on Dyes

A dye may be defined as ―A coloured substance which when applied to the fabrics imparts a permanent colour and the colour is not removed by washing with water, soap or an exposure to sunlight. The evolution of dyes began in the year 1856 with the discovery and industrial production of Mauve, by W.H. Parkin. Due to toxicity and mutagenicity of dyes, their removal from effluents has been an urgent challenge. Nowadays, research attention has been focused on biological methods for the treatment of effluents and bio adsorption is one of the processes that do not require energy and huge amount of synthetic chemical. Major pollutants in textile wastewaters are high-suspended solids, chemical oxygen demand, heat, colour, acidity, and other soluble substances. The removal of colour from textile industry and dyestuff manufacturing industry wastewaters represents a major environmental concern [14-19]. Although, dye constitutes a very small portion of total volume of discharged waste and the colour removal is the major and the most urgent problem of textile industry because of the following reasons:

- Due to high tinctorial value of dye, the presence of even a small portion of dye is highly visible.

- Dyes cannot be readily removed by conventionally used waste treatment because most dye have complex aromatic structure which makes them resistant to biological activity, ozone, light and other degrading environment.

- Few dyes and their by-product formed due to degradation have possible long term effect.

- Dyes also affect rate of photosynthesis in aquatic life by reducing penetration of sunlight and due to presence of metal and chloride in them they may also be toxic to certain form of aquatic life.

The release of coloured effluents into the environment is undesirable, not only because of their colour, but also because many dyes from wastewater and their breakdown products are toxic and/or mutagenic to life [20]. Without adequate treatment these dyes are stable and can remain in the environment for an extended period of time. For instance, the half-life of hydrolysed Reactive Blue 19 (RB19) is about 46 years at pH 7 and 25℃ [21]. In addition to the environmental problem, the textile industry consumes large amounts of potable water. In many countries where potable water is scarce, this large water consumption has become intolerable and wastewater recycling has been recommended in order to decrease the water requirements.

Dye's molecules are consisted of chromophores and auxochromes components where chromophores (OH, NH_2, NHR, NR_2, Cl and COOH) are responsible for the production of colours and auxochromes (NO_2, NO, N=N) enhance the affinity of the dye toward the fibres [9]. Dye bearing effluents from these industries are characterized by its high colour, organic content and hazardous as well. Dyes can be produced from natural or synthetic sources as shown below:

- **Natural Dye-** Natural dyes are organic compounds used to colour various products. In Prior to the year of 1856, natural dyes are extracted from plants, animals, insects and minerals sources. Natural dyes are such as Turmeric, Weld, Onion, Jackfruit, henna, eucalyptus are used in the early textile industry. Due to the increase in population and industrial activities, natural dyes do not meet the industrial demand and their applications have been limited to food industries only.

- **Synthetic Dye-** The first synthesis dye was discovered by William Henry Perkin in 1856. Dye based effluents can cause a serious hazards to the water stream and environment due to their synthetic origin and complex molecular structures which decrease their ability to biodegrade. There are various types of dyes used in various industries such as acid dyes, reactive dyes, basic dyes, azo dyes, direct dyes, vat dyes and disperse dyes [10]. All dyes are water soluble except disperse dyes and vat dyes. All dyes contain traces of metals such as copper, zinc, lead, chromium and cobalt in their aqueous solution except vat and disperse dyes. Dye bearing effluents from these

industries are characterized by its high colour, organic content and hazardous as well. Dyes are broadly classified into cationic, anionic and non-ionic dyes. Anionic dyes include various dyes' groups such as acid dyes, reactive dyes, azo dyes and direct dyes while cationic dyes are the basic dyes.

Some properties of dyes are based on their usage and thus can be broadly classified as:

- **Acid dyes:** It is a sodium salt of a sulfonic, carboxylic or phenol organic acid. It is soluble in water and possesses affinity for amphoteric fibres. It is used for nylon, wool, paper, leather, food, cosmetics etc. Most synthetic food colours fall in this category.

- **Basic dyes:** They yield coloured cations in solution and this is the reason for calling them cationic dyes. This type of dye finds uses in paper, modified nylons and polyesters, medicine etc.
- **Direct dyes:** These are water soluble anionic dyes. Dyeing is normally carried out in a neutral or slightly alkaline dye bath or at near boiling point, with addition of either sodium chloride or sodium sulphate or sodium carbonate. These are used for dyeing of cotton and rayon, paper, leather etc. They are also used as pH indicators and as biological stains.
- **Disperse dyes:** These are substantially insoluble non-ionic dyes. These are used mainly on polyester and also applied on nylon, cellulose, acrylic fibres etc.
- **Mordant dyes:** These dyes use a mordant, which improves the fastness of the dye against water, light and perspiration. The choice of mordant is very important as different mordants can change the final colour significantly. These are basically used for wool.
- **Sulphur dyes:** These types of dyes have intermediate structures. They form a relatively small group of dyes, but the low cost and good wash fastness properties make it very important from the economic point of view. Its main uses are for cotton and rayon.
- **Reactive dyes:** They 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 fibres. They find their uses for cotton, wool and nylon.

- **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.

- **Vat dyes:** This type of dye is water insoluble and incapable of dyeing fibres directly. But, reduction in alkaline liquor produces the water soluble alkali metal salt of the dye, which, in this leuco form, has an affinity for the textile fibre. These are used mainly for cotton, wool and nylon.

1.3.Methods of Dye removal :

Wastewater containing dyes is very difficult to treat, since the dyes are recalcitrant organic molecules, resistant to biological degradation and are stable to light. A synthetic dye in wastewater cannot be efficiently decolorized by traditional methods. This is because of the high cost and disposal problems for treating dye wastewater at large scale in the textile and paper industries [14-19].

1.3.1. Physicochemical Technique-

This technique includes Coagulation/Flocculation, Ion-exchange, Adsorption and Membrane separation. These techniques remove or separate colour physically.

❖ **Coagulation and flocculation technique**

Coagulation-flocculation is a frequently used physicochemical treatment method employed in textile wastewater treatment plants to decolourize textile effluent and reduce the total load of suspensions and organic pollutants [22]. Coagulation and flocculation occur in successive steps intended to overcome the forces stabilizing the suspended particles, allowing particle collision and growth of floc [23].

Advantages of Coagulation and flocculation technique:

Surfactant and dyes with high molecular weight can be successfully removed by coagulation and flocculation method followed by sedimentation, flotation and filtration respectively.

Limitations of Coagulation and flocculation technique [24, 25]:

- In coagulation and flocculation process, relatively large amount of coagulant (i.e. chemical) is required which may cause problem related to sludge.
- The use of electricity in some cases may prove to be expensive
- Gelatinous hydroxide may tend to solubilise in some case.
- High conductivity of waste-water suspension is required in some case.

❖ **Ion-exchange technique**

Ion exchange is a process in which the mobile ions from external solutions are exchanged for ions that are electrostatically bound to the functional groups contained within a solid matrix [26].

Advantage of ion exchange technique:

- Most widely used treatment methods for waste water stream.
- Ion exchange process can be employed in variety of ways such as in batch, column, and continuous loop or in combination with membrane technique.
- This is a cost effective technique and on re-generation there is no loss of sorbent.

Limitations of ion exchange technique [27]:

- The generated wastes are highly concentrated and thus require careful disposal.
- This technique is generally not effective against low pH and high concentration of Iron, Manganese and Aluminium.
- This technique is generally not effective for complex mixture of metal
- This treatment requires pre-treatment for removal of suspended solids prior treatment.
- The on-going operational cost is high.

❖ **Adsorption:**

The process of adsorption involves the ions, atoms or molecules of the adsorbate to transfer and adhere to the surface of the adsorbent creating a thin film. Adsorption technique can be divided into physical and chemical adsorption. Physical adsorption process is controlled by physical forces such as Van der Waals forces, hydrophobicity, hydrogen bond, polarity, static interaction, dipole–dipole interaction, Π- Π interaction etc. In the physical adsorption, pollutants get accumulated on adsorbent surface by the above mentions interactions while chemical adsorption (Chemisorption or Langmuir adsorption) is defined when the adsorbate is chemically bound to the adsorbent's surface due to the exchange of electrons [28]. The extent of adsorption depends on the nature of adsorbate such as molecular weight, molecular structure, molecular size, polarity and solution concentration. It is also depends on the surface properties of adsorbent such as particle size, surface area, surface charge etc. [29]. Adsorption process is an effective separation technique and it is considered to be superior compared to other available techniques for wastewater treatment in terms of initial cost, simplicity of design, ease of operation and insensitive to toxic substances [30, 31]. Adsorbent's selectivity is based on the adsorption capacity, surface area, availability and total cost. Following are some adsorbent commonly used in textile industries:

- **Activated charcoal**- Activated charcoal is most widely used absorbent, as this extensively acts among wide range of dyes including acid, base, disperse etc. Disperse dye, vat dye and pigment has low solubility in water and their rate of absorption on carbon is slow at room temperature whereas water soluble dye such as acid, basic, direct, metallised mordant are also not readily absorbed on carbon. Apart from the fact that use of activated charcoal is although a reasonably efficient process following are some limitation which also needs to be considered :
    i. On reactivation, 10-15% adsorption takes place.
    ii. This process of adsorption is costly than conventional.
- **Inorganic adsorbent-** The inexpensive inorganic adsorbents such as peat, fly-ash, bentonite, calcium meta-sulphite, activated aluminium, clay and bauxite. The use of betonite for basic dyes and anthracite charcoal for acid yellows are also known. The use of in-organic adsorbent such as high surface area silica, cinder ash and clay can also be used.

❖ **Membrane Separation**

Membrane Separation technology is extensively used to concentrates, purifies and improve the final product [34]. The application of membrane separation process in textile industry includes improvement in the quality of finished product, increased yield, recovery of product from waste. The membrane separation process can be further subdivided to:

Reverse Osmosis and Ultra filtration: Regardless of the type of dye used reverse osmosis and ultrafiltration are very effective technique for the removal of colour from Dye-house effluent.

Advantage of Membrane separation technique:

- The main advantage of membrane separation process is that the concentration can be achieved without any input of thermal energy or a change of state thus making the process efficient.
- The waste-water can be efficiently treated to the level required for recycle and reuse.

Dis-advantage of Membrane separation technique:

- High cost of this technique is mainly associated with the high cost of membrane and other filtration equipment, depending on the size of plant, operation condition.
- Lower rate of productivity.
- Membrane fouling because of the deposition of the precipitated dyestuff.
- Membrane process results highly concentrated waste.

Table 1: Advantages and Dis-advantages of conventional methods [42]:

Physicochemical methods	**Advantages**	**Disadvantages**
Activated	Carbon removes wide variety of dyes	Very expensive, ineffective against disperse and vat dyes
Non-conventional adsorbents (agricultural and industrial by-product)	Effective adsorbent, inexpensive, widely available, operation is easy, simple process design.	Transfer of pollutant from liquid phase to solid matrix(adsorbent)
Membrane filtration	Filtration removes all types of dye, quick method and requires less space	Concentrated sludge production, membrane fouling
Ion- exchange	Regeneration; no adsorbent loss	Cost is high, incapable to treat large volume
Coagulation and flocculation	Economically feasible, process design is simple	Generation of sludge

1.3.2. Chemical technique

Chemical oxidizing agents such as Chlorination, Chlorine-dioxide treatment, ozonation, use of hydrogen peroxide with other salt (Fenton's reagent), permanganate etc. have the ability to effectively de-colourises dyes[35,36].

- **Ozonation technique**

Ozone is one of the most powerful oxidant than chlorine and oxidizing agent. Then fading of dye occurs by oxidative cleavage of conjugated system of the molecule. Ozone is also useful for removal of many toxic chemicals from wastewater, as it is capable to decompose detergent, chlorinated hydrocarbon, phenol, pesticide and aromatic hydrocarbon. Ozone treatment has successfully used to remove colour from dyeing wastewater [37-40]. Ozonation process does not form sludge because of complete decomposition of dyes thus reduce the toxicity of by-products [41]. However, the half-life of ozone is very short and it requires a high voltage to run a continuous ozonation process thus increases the capital cost and limits its uses in the industrial scale [42].

Advantages of ozonation technique:

- Oxidation of reactive dye occurs more readily when treated with ozone than when treated with other oxidative agent.
- Ozonation decreases COD and increases the bio- degradability of waste stream.

Limitation of ozonation technique:

- This process is less effective in reducing TOC.
- Since, ozone is hazardous; this process requires an additional ozone destructive unit to prevent ozone from escaping and damaging the environment.
- This is a high cost and low efficiency process.

❖ **Chlorination**

Chlorination using chlorine gas or sodium hypochlorite is found to be effective in colour removal [43]. Chlorine rapidly decolorizes acid and reactive dyes but even large dose of fail to completely decolorize direct and disperse dye rather a persistent yellow decomposition product forms

Advantage of chlorination technique:

- Decolorisation using sodium hypo-chlorite is an in-expensive technique.
- This technique is very effective method of decolorisation. At a chlorine level of 150mg/L, colour reduces to 77%.

Dis-advantage of chlorination technique:

Chlorination technique has the potential of generating toxic chlorinated compound such as absorbable organo-halides that are harmful to human as well as environment.

❖ **Chlorine di-oxide**

Chlorine di-oxide is found to be less effective than chlorine. It does not decolourise dye waste efficiently and has no effect on some dye classes such as vat dyes. Chlorine di-oxide is highly effective against reactive, direct, disperse and anionic pre-metallised dyes.

❖ **Hydrogen peroxide (photochemical oxidation)**

Hydrogen per-oxide is the main oxidising agent used for decolourisation by chemical means and removes the dye from the dye containing effluent by oxidation resulting in aromatic ring cleavage of the dye molecule. This agent needs to be activated by some means such as UV-light, inorganic salt, ozone and ultrasound.

- Fenton's Reagent: Fenton's reagent is also known as hydrogen peroxide and it is more effective if applied at acidic solution. Iron ions such as Fe^{+2} and Fe^{+3} are the most common reagents used in Fenton activation. Fenton's reagent is cheap and easy to

handle compared to other reagents. The removal efficiency of this process depends on the production of the oxidant, hydroxyl radicals which exhibit higher removal percentage at higher dyes concentration. This process has its own limitations as these reagents are toxic and may cause more harm to the biological treatment system used for the post treatment than the original textile dyes [44]. Previous studies of Fenton and Fenton-like reagents are used to remove textile dyes such as reactive red, acid blue and direct blue [45], acid orange and reactive blue [44] and Reactive Black 5, Reactive Orange 16 and Reactive Blue 2 [46].

- H_2O_2/UV: Decolourisation of dyes using H_2O_2/UV photochemical oxidation [47-49], this method degrade dye molecule to CO_2 and H_2O by UV treatment in presence of H_2O_2. UV light activates the destruction of H_2O_2 into two-hydroxyl radicals, which causes chemical oxidation of organic material. The rate of dye-removal is influenced by the intensity of UV radiation, pH and dye-structure and dye-bath composition. The major advantage of photo-chemical treatment of dye containing effluent is:
    i. No sludge is produced
    ii. The foul odour can be greatly reduced.

- H_2O_2-UV-Ultrasound: Feng et. al. [50], studied decolourisation and degradation kinetics of reactive dye waste-water by H_2O_2- UV Ultrasound system. It was observed that the ultrasound in combination with UV-light dramatically improves the initial reaction rate and the overall dye removal efficiency. Ultrasound may increase the oxygen uptake and transfer rates which enhance the oxidation processes due to the hydroxyl radical.

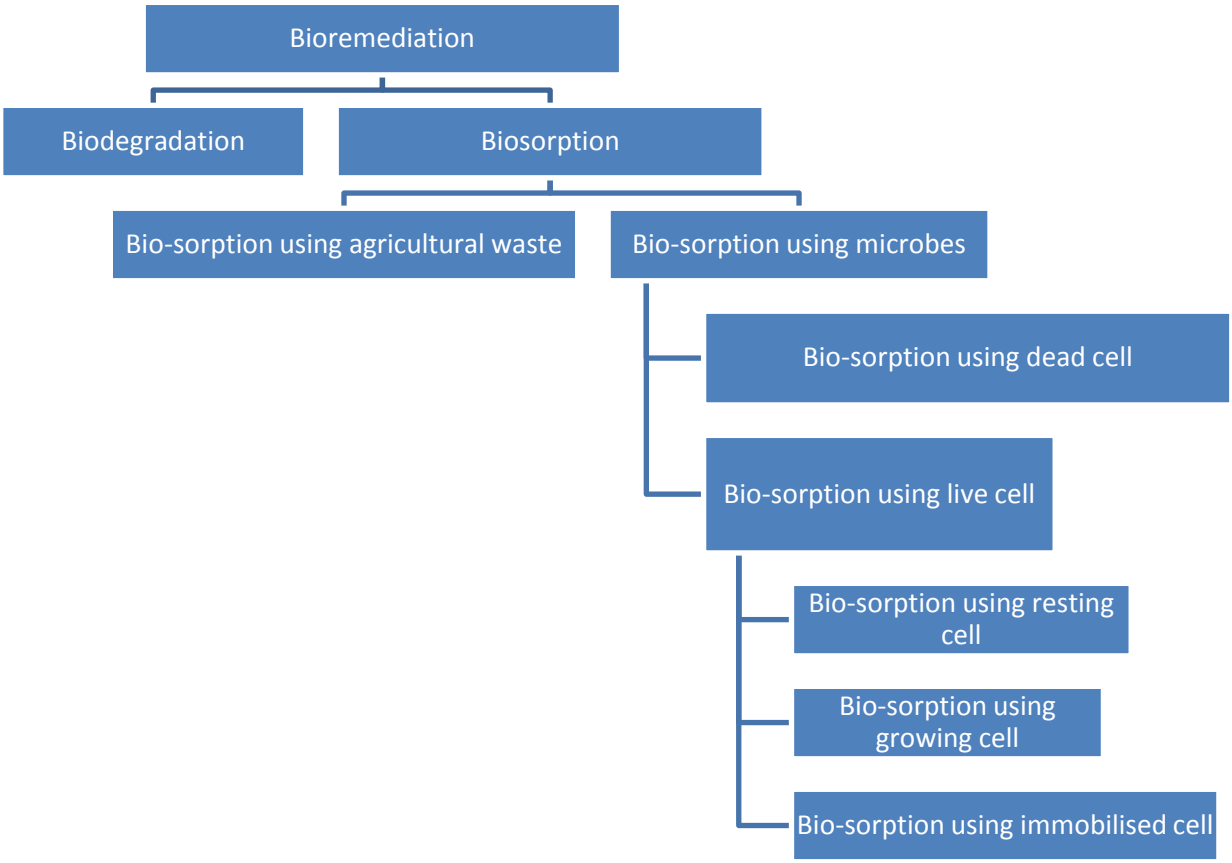
- H_2O_2-ozone: De-colourisation by means of H_2O_2-ozone combination is applied for direct dyes, metal complex or blue disperse dyes but there are some problem with de-colourisation of acid, red disperse dye and their mixture.

Table 2: ***Advantages and disadvantages of chemical methods*** **[42]:**

Chemical Methods	**Advantages**	**Disadvantages**
Fenton's reagent	Effective de-colourisation of both soluble and insoluble dyes	Sludge production
Ozonation	Applied in gaseous state; no alteration of volume	Short half-life of ozone
Photochemical	No sludge production	Formation of by-product
NaOCl	Initiates and accelerates azo-bond cleavage	Release of aromatic amines
Electrochemical destruction	Breakdown compounds are non-hazardous	High cost electricity

1.3.3. Bio-removal of dyes:

Biological treatment is the most economical methods compared to other physical and chemical processes. Bio-removal methods such microbial degradation, adsorption by (living or dead) microbial biomass and bioremediation systems are commonly applied to the treatment of industrial effluents because many microorganisms such as bacteria, yeast, algae and fungi are able to accumulate and degrade different pollutants. Although many organic molecules are degraded, many others are recalcitrant due to their complex chemical structure and synthetic organic origin. In particular, due to their xenobiotic nature, azo dyes are not totally degraded.



1.3.3.1. Bio-degradation – Slokar and LeMarechal, indicated that biodegradation is promising method for dye removal [49]. Biodegradation is a biologically mediated breakdown of chemical compounds. When biodegradation is complete the process is called mineralization i.e. the total breakdown of organic molecules into water, carbon dioxide and/or any other inorganic end products. These processes have potential to mineralize dyes to harmless inorganic compounds like carbon di- oxide, water and the formation of a lesser quantity of relatively insignificant amount of sludge. The fungal de-colourization of dye present in wastewaters is turning into a promising alternative to replace or supplement for present treatment processes [51]. The ability of white rot fungi to degrade synthetic chemicals, such as dyes, is well known, and a white rot fungus, *Phanerocheate chrysosporium* has been reported to de-colourise dyes with enzymes involved in lignin degradation, such as lignin peroxidase (Lip), manganese peroxidase (MnP) and laccase [42].

1.3.3.2. Bio-sorption- Bio-sorption has been demonstrated to possess good potential to replace conventional methods for the removal of dyes/metals [52]. The uptake or accumulation of chemicals by microbial mass has been termed as bio-sorption. It mainly takes place in the cell surface, will differ according to the biomass type. Both bacterial and fungal cells were reported for their capability for partial or complete removal of dye through the process of adsorption. In white-rot fungi both adsorption and degradation can occur simultaneously or sequentially. Bio-sorption was not a practical approach for treating industrial effluents because of the problems associated with disposal of the large volumes of biomass after bio-sorption. The bio sorption is one of the processes which involve a solid phase (bio sorbent or adsorbent) and a liquid phase (solvent) containing a dissolved species to be biosorbed (adsorbate, dye or metal). It occurs naturally in certain biomass which allows it to passively concentrate and bind onto their cellular structure. If physical forces are involve then dyes will be adsorbed and if chemical forces are involved then they will be removed. The effectiveness of bio-sorption depends on: pH, temperature, ionic strength, time of contact, adsorbent and dye concentration, dye structure and type of microorganism.

Advantages of bio-sorption process:

- For Bio-sorption process, the land area required is comparatively less than that for a biological system.
- The bio-sorption process has lower sensitivity to diurnal variations.
- Not affected by presence of toxic substances.
- This process can be considered has potential for significant heavy metal removal.
- This process has greater flexibility in design and operation.
- This process can be considered as very effective against the removal of organic waste constituents.

❖**Bio-sorption using Agricultural waste:**

Agricultural waste such as mango peel, banana peel, coconut bunch waste, wheat husk, tree barks, tea leaf powder etc. B.H. Hameed et. al. studied the ability of coconut bunch waste (CBW), an agriculture waste to remove basic dye (methylene blue) from aqueous solution by adsorption [53]. The use of sugarcane bagasse, an agro industry waste, to remove MR dye from the waste water was studied by H. S. Ashoka and S. S. Inamdar [54]. They investigated the potential use of sugarcane bagasse pre-treated with formaldehyde and sulphuric acid.

❖ **Bio-sorption using Microbial biomass:**

Microbial de-colourisation and degradation of dyes have been found to be a cost effective and eco-friendly technique for removal of dyes from textile effluents. In this technique waste-water is treated against microorganisms (such as yeast, fungi, bacteria, algae etc.) [8].

Advantages of Bio-sorption using Microbial biomass:

- Biological treatment can be carried out in-situ.
- The running or operational cost is low.
- Biological de-colourisation technique has no harmful side effect, thus microbial. Bio-degradation of dyes can be considered as a better alternative.
- This technique is effective against wide range of dyes since microorganisms, such as bacteria, fungi and algae, can degrade or absorb a wide range of dyes

- This method is more economical and leads to less accumulation of relatively less harmful sludge.
- This technique is eco-friendly and it causes mineralization of dyes to simpler inorganic compounds which are relatively not lethal to life forms.

❖ **Bio-sorption using live cell**-

- Bio-sorption using growing cell- Bio-sorption using growing cell was reported by Yetis et al. [71]. In this process the biosorption takes place in the growth medium containing adsorbate (i.e. dye)
- Bio-sorption using resting cell- In the year 2000, Yetis et al. from turkey reported removal of Pb(II) using resting cell of *Phanerochaete chrysosporium* and the comparison of ability of resting cell with live and dead cell. The highest capacity of bio-sorption was reported with resting cell [71]. The biosorption of Cr (VI) with resting cells of *Aspergillus* sp. and *Fusarium solami* [72].

❖**Biosorption using dead cell**:

Dead cells remove dyes through the mechanism of bio-sorption which involves physico-chemical interactions such as adsorption, deposition and ion exchange. In case of *Aspergillus niger,* Y. Fu, T. Viraraghavan observed different functional groups in the fungal biomass play different roles in bio-sorption of different dyes [73]. K. Nathkumar et. al. examined the bio-sorption equilibria and kinetics of Reactive blue 140 using dead fungal biomass of *Aspergillus niger* HM11 by, and by their study they concluded that dead biomass can be considered as a good sorbent material for Reacive blue 140 solution [74]. Further, Aksu and Tezer, studied with dried *R. arrhizus* for the removal of Remazol Black B, a reactive anionic dye from aqueous solution [75].

1.4. CONCERN WITH THE USE OF DYES – TOXICOLOGICAL EFFECT

Synthetic dyes are extensively used in textile, paper, printing industries and dye houses. Dyeing industry effluents are one of the most hazardous wastewaters to be treated not only for their high chemical oxygen demand, but also for high BOD, suspended solids, turbidity, and toxic constituents. Colour is a visible pollutant and the presence of even very minute quantity is detectable and undesirable due to its appearance. Dyes have a tinctorial value is high; hence concentration less than 1ppm produces obvious colouration [76]. Dyes may significantly affect photosynthetic activity in aquatic life because of interference in transmission of light. They upset the biological metabolism process which causes the destruction of aquatic communities present in ecosystem. Further, the dyes have a tendency to sequester metal and may cause micro toxicity to fish and other organisms.

Many dyes and their metabolites have been shown to be toxic as well as have chromosomal disorders, respiratory problems, carcinogenic, mutagenic and teratogenic effects on marine life and humans [77]. The presence of non-biodegradable organics and inorganics in the dye effluent poses considerable problem in wastewater treatment. Metal complex dyes pose even magnified problems due to presence of metals which are often carcinogenic.

Through the formation, in 1974 of the Ecological and Toxicological Association of the Dyestuffs Manufacturing Industry (ETAD), aims were established to minimise environmental damage, protect users and consumers and to cooperate fully with government and public concerns over the toxicological impact of their products. Over 90% of studied 4000 dyes tested in an ETAD survey had LD_{50} values greater than 2×10^3 mg/kg. The highest rates of toxicity were found amongst basic and di-azo direct dyes.

1.5.**ADSORBATE:**

Malachite Green, a tri-aryl-methane dye (C_{23} $H_{26}N_2OCl$, M.W. 927.2) is a dark green and crystalline solid prepared by condensing one part of benzaldehyde with two parts of di-methyl-aniline in the presence of concentrated sulphuric acid or zinc chloride. Neutral water solutions are bluish green in colour with an absorption maximum of 616.5 nm. Malachite Green hydrochloride is an industrial grade variety which, during its manufacture, is precipitated by the addition of zinc chloride and is, therefore, produced as a double zinc salt. This dye, like other tri-phenyl-methane, can exist in two ionic forms- as the dye salt and as the carbinol or pseudo-base.

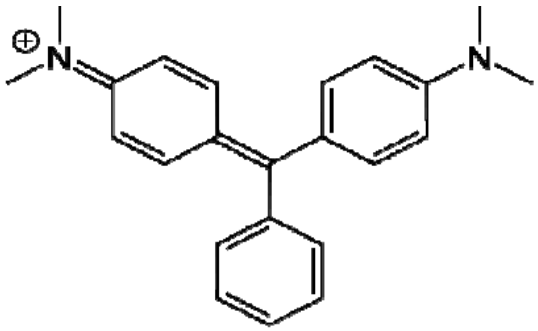


Fig 1: Malachite Green

❖ **Toxicological Effect Of Malachite Green On Mammals And Other Animals**

Malachite Green is environmentally persistent and acutely toxic to a wide range of terrestrial and aquatic animals. It causes serious public health hazards and poses potential environmental problems. Both clinical and experimental observation reveals that MG is a multi-organ toxin. Desciens and Bablet (1994) found renal changes in rabbit following repeated oral dosing of this dye. It decreases food intake, growth and fertility rates; causes damage to liver, spleen, kidney and heart; inflicts lesions on skin, eyes, lungs and bones; and produces teratogenic effects in rats and mice (Werth and Boiteaux, 1967, 1968; Culp et al., 1999). Apoptosis in the transitional epithelium of the urinary bladder and thyroid follicles of MG fed mice. Malachite Green has been found to be mutagenic in rats and mice; and it causes significant developmental abnormalities in pregnant New Zealand white rabbits *(Oryctolagus cuniculus)*. Malachite Green is highly cytotoxic to mammalian cells (Fessard et al., 1999) and carcinogenic to liver, thyroid and other organs of experimental animals (Rao, 1995; Rao and Fernandes, 1996; Doerge et al., 1998b; Mahudawala et al., 1999; Sundarrajan

et al., 2000). Incidences of tumours in lungs, breast and ovary have also been reported from rats exposed to Malachite Green (Werth, 1958).Toxicity of Malachite Green is greatly affected by the presence of calcium ions and humic substances. Therefore survivals of MG exposed fishes are minimum in high Ca^{2+} and humic substance containing solution.

❖ **Concern over the Use of Malachite Green**

The preceding account of MG reveals that this dye has now become one of the most debated and controversial compounds used in aquaculture, due to the risks it poses to the consumers, including its effects on the immune system and reproductive system as well as its genotoxic and carcinogenic potentials. It has also been identified as class 2 health hazards. Although the use of MG for controlling fungal infections and eco-parasites in the Aqua-culture industry is prohibited in the USA because of its carcinogenic nature but it is still used in some areas of the world because of its low cost. This triphenylmethane dyes are xenobiotic compounds and recalcitrant molecules. Inspite the fact that the use of this dye is banned since 2002 due to its carcinogenic and toxicological effects [78], this may persist in the environment [79]. Due to this significant health risks to human and fish, it is essential to establish a proper method to remove this substance from water or industrial effluent.

1.6. Biosorption:

Biosorption or passive uptake is defined as a non-directed physico-chemical interaction that may occur between dyes species and cellular compounds. The uptake of dye can take place by entrapment in cellular structure and subsequent sorption onto the binding sites present in the cellular structure. This method of uptake may be independent or dependent of the biological metabolic cycle.

Different types of biosorbents:

a. Bacterial culture- *Bacillus subtilis* [55], *Acromonas hydriphi* [56], *Bacillus cereus* [57] are few isolates of bacterial culture capable of degrading azo-dyes. An azo dye reductase enzyme is responsible for the initial degradation of orange-11 dye but substituting any of the groups near the azo group in the chemical structure of the dye hindered the degradation. Haun et. al. described a bacterial consortium capable of

mineralizing the sulphonated azo dye mordant yellow [58]. Bacteria are able to culture and grow more quickly than fungi as they are able to metabolize chlorinated and other organic contaminants and use them as carbon or energy source [59]. Bacteria are classified as mono-oxygenase or di-oxygenase enzyme and they are used to catalyse the incorporation of oxygen from O_2 into the aromatic ring of organic compounds such as azo dyes and reactive dyes [60]. Zissi et. al., showed that *Bacillus subtilis* could be used to breakdown p-amino azo benzene, a specific azo dye [61].

b. Algae culture- *Chlorella* and *Oscillatoria* sp. [62] are capable of degrading azo dyes to their aromatic amines to simple organic compound and CO_2. Such algae can be used for stabilization of ponds as they can play a role in aromatic amines. The mechanisms of algae decolouration can involve enzymatic degradation, adsorption, or both [63]. Similar to bacteria, algae are capable of degrading azo dyes through an induced azo- reductase to break the azo bond, resulting in the production of aromatic amines.

c. Yeast culture- Yeast such as, *Klyveromyces marxians* are capable of decolourising Ramazol black B dyes by 78-98% [62]. The mechanisms of decolouration by yeast can involve adsorption [64], enzymatic degradation, or a combination of both. Adsorption on yeast biomass is more efficient at low pH [65] as in the case of maximal accumulation of Direct Violet 51 in *Candida albicans* occurs at pH 2.5, Violet 3 by *Candida tropicalis* occurs at pH 4.0 [66] and acid red B by *Pichia sp.* TCL [67]. Fungi have high capacity of biodegradation of dyes as they are able to deplete complex organic compounds by producing extracellular ligninolytic enzymes including laccase, manganese peroxidase and lignin peroxidise [68].

d. Filamentous fungi- The application of filamentous fungi in the de-colouration process is an attractive alternative due to low cost and the possibility of total mineralisation of the dye according to Erden et. al. [69]. Adsorption is enhanced at pH 2–3, which is probably due to electrostatic attractions between charged dye molecules and the charged cell surface. De-colourisation is decreased at very high temperatures, which is possibly due to the deactivation of the adsorbent surface or the destruction of some active sites. White-rot fungi such as *Phanerochatete chrysosporium, Dichomitus squalens, Daedalea flavida* and *Irpex flavus* have been used widely in the

decolourization and degradation of textile waste of many chromophoric groups of dyes [70]. White rot fungi are those organisms that are capable of degrading lignin, the structural component found in woody plants.

1.6.1 Mechanism of biosorption

The mechanism of biosorption is complex, mainly ion exchange, chelation, adsorption by physical forces, entrapment in inters and intra-fibrilliar capillaries and spaces of the structural poly-saccharide network as a result of the concentration gradient and diffusion through cell walls and membranes.

There are several chemical groups that would attract and sequester the metals in biomass: acetamido groups of chitin, structural polysaccharides of fungi, amino and phosphate groups in nucleic acids, amido, amino, sulphhydryl and carboxyl groups in proteins, hydroxyls in polysaccharide and mainly carboxyls and sulphates in polysaccharides of marine algae that belong to the divisions *Phaeophyta*, *Rhodophyta and Chlorophyta*. However, it does not necessarily mean that the presence of some functional group guarantees biosorption, perhaps due to steric, conformational or other barriers.

The complex structure of microorganisms implies that there are many ways for the metal to be taken up by the microbial cell. The biosorption mechanisms are various and are not fully understood. They may be classified according to various criteria.

According to the dependence on the cell's metabolism, biosorption mechanisms can be divided into:

- Metabolism dependent
- Non -metabolism dependent.

According to the location where the metal removed from solution is found, biosorption can be classified as:

- Extra cellular accumulation/ precipitation
- Cell surface sorption/ precipitation
- Intracellular accumulation.

Transport of the metal across the cell membrane yields intracellular accumulation, which is dependent on the cell's metabolism. This means that this kind of biosorption may take place only with viable cells. It is often associated with an active defence system of the microorganism, which reacts in the presence of toxic metal.

During non-metabolism dependent biosorption, metal uptake is by physico-chemical interaction between the metal and the functional groups present on the microbial cell surface. This is based on physical adsorption, ion exchange and chemical sorption, which is not dependent on the cells' metabolism. Cell walls of microbial biomass, mainly composed of polysaccharides, proteins and lipids have abundant metal binding groups such as carboxyl, sulphate, phosphate and amino groups. This type of biosorption, i.e., non-metabolism dependent is relatively rapid and can be reversible (Kuyucak and Volesky, 1988). In the case of precipitation, the metal uptake may take place both in the solution and on the cell surface (Ercole, et al. 1994). Further, it may be dependent on the cell metabolism if, in the presence of toxic metals, the microorganism produces compounds that favour the precipitation process. Precipitation may not be dependent on the cells' metabolism, if it occurs after a chemical interaction between the metal and cell surface.

1.6.2. Two phase microbial uptake of dyes

The first rapid phase is the metabolism independent phase which can occur in both living and non-living cells. The dye molecules are quickly adsorbed and they bind onto the external surfaces of the biomass. Adsorption involves the accumulation or concentration of substances, the adsorbate at the surface or interface of the adsorbent. Electrostatic force of attraction, Vander Waal’s force and chemical interaction are mainly cause of such adsorption. The succeeding slower phase is metabolism dependent which involves the migration of dye molecules across the cellular membrane and thus is controlled by a number of parameters temperatures, presence of metabolic inhibitors and un-couplers. Rates of intracellular uptake may also be influenced by physiological state of cells and the nature and composition of the growth medium.

Both these phases may not be seen in all living cells. In growing cells, either or both phases of uptake may be enhanced or obscured by additional aspects of metabolism, such as changes in the chemical nature of the growth medium, and excretion of metabolites that may combine with dyes.

1.6.3 Factors affecting biosorption

In the decolourisation process by fungi, bacteria and yeast there are various factors that influence the efficiency. They can be grouped into two categories- the ones that affect the microbial growth conditions and the other that affect the characteristics of dye waste water. pH, dye concentration, temperature, biomass load, contact time are certain basic parameters affecting the process.

1.6.4 Prospect of biosorption for use on industrial scale

- The removal of hazardous toxicants of dyes and heavy metals for detoxification of industrial effluent can be achieved with minimum cost, recovery of dye/ heavy metals and reuse of substrate or biosorbent.
- No sludge generation thus avoiding the problem of waste disposal.
- Eco-friendly emerging technology that is likely to be most effective in treating waste water in near future with newer and more stringent environmental laws and regulations.

Since long time some solids have been used to remove dye from aqueous solution. Various scientists Brunauer, Emmet and Teller, Langmuir, McBain and Barrer in early twentieth century studied ways to remove contaminants from liquids and gases using solids. Despite the number of successful systems employing various physicochemical and biological processes, economical removal of colour from effluents remains a major problem. Since these concerns about the environment are gaining momentum, it is necessary to develop better economically and environmentally friendly treatment technologies. Dye wastewaters are usually treated by physical or chemical treatment processes. These includes physical-chemical flocculation combined with Flotation, Electro-flotation, Flocculation with Fe(II)/$Ca(OH)_{2,}$ Membrane filtration, Electrochemical destruction, Ion exchange, Irradiation, Precipitation, Ozonation and Katox treatment method involving the use of Activated charcoal and air mixture [8]. However, these technologies are usually in-efficient in the removal of colour and are little adaptable to wide range of dye wastewaters.

It is obvious that each process has its own constraints in terms of cost, feasibility, practicability, reliability, stability, environmental impact, sludge production, operational difficulty, pre-treatment requirements and the extent of the organic removal and potential toxic by-products. Also, the use of any single process may not completely decolourise the wastewater and degrade the dye molecules. Even when some processes are reported to be successful in decolourising a particular wastewater, the same may not be applicable to other types of coloured wastewaters. Though there are various ways to remove dyes from wastewater discharges like coagulation, electrochemical process, membrane separation process, chemical oxidation, reverse osmosis and aerobic and anaerobic microbial degradation. Many of these processes are not so popular due to their economic disadvantages and inefficiency. Coagulations and chemical and electrochemical oxidations have low feasibility on large scale plants. Adsorption has been observed to be an effective process for colour removal from dye wastewater. Though absorption using activated charcoal is effective but it is quiet an expensive method. Many studies have been undertaken to find low cost adsorbent which includes peat, bentonite, steel plant slag, fly ash, china clay, maize cob, wood shaving and silica [9-13]. Moreover as the adsorption capacities of the various adsorbents are not very large, the new adsorbents which are more economical, easily available and highly effective are still needed so the work is still going on. Fungal biomass thus can be proved to be a low cost effective adsorbent. Adsorption is preferred over these processes and is widely used due to low cost and high performance. Common adsorbents are activated carbon, alumina silica and metal hydroxides. Among the current pollution control technologies, biodegradation of synthetic dyes by various microbes is emerging as an effective and promising approach. The bioremediation potential of microbes and their enzymes acting on synthetic dyes has been demonstrated, with others needing to be explored in the future as alternatives to conventional physicochemical approaches [6, 7]. Economic advantages, performance efficiencies and environment are the main concerns when selecting an adsorbent, thus researchers generally goes for using low-cost adsorbents, such as lyophilised dried fungal biomass.

1.2.Objective

- To determine effectiveness of removing malachite green dye using lyophilised fungal biomass and to study effects of various experimental parameters on adsorption.
- To carry out adsorption kinetic and isotherm studies of removal of dye using lyophilised fungal biomass.
- To study desorption efficiency using various eluent

Chapter 2

Literature Review

B.H. Hameed et. al. (2005) studied adsorption of Methylene blue by bamboo-based activated carbon. Bamboo was used to make activated carbon by physiochemical activation with potassium hydroxide and carbon dioxide. Adsorption model and kinetic studies were also conducted and it was found that adsorption of Methylene blue followed pseudo-second-order kinetics [90].

I.D. Mall et. al. (2005) studied the removal of malachite green dye from aqueous solution using bagasse fly ash and analysed kinetic and adsorption isotherm models. Adsorption was carried out in batch processes and the effects of various parameters were analysed. Adsorption of dye followed pseudo-second-order kinetics [95].

In 2007, Shawabkeh and Abu-Nameh performed studied on adsorption of phenol and methylene blue by activated carbon from pecan shells. Activated carbon is prepared from pecan shells by chemical activation with phosphoric acid. Then it is treated with sodium dodecyl sulphate to prepare the surface for adsorption. The results indicated that phenol and methylene blue removal efficiency was high by activated charcoal prepared from pecan shells [96].

Hameed et. al. (2008), studied on the adsorption of Malachite green dye by rattan sawdust at 30^{o}C. Results indicated that rattan sawdust can be used as low cost adsorbent source. Two isotherms Freundlich and Langmuir were analysed and the best fit model was found to be Langmuir isotherm model. Adsorption kinetics was predicted as pseudo-first –order model. For a short period, the adsorption rate was controlled by film diffusion at longer adsorption period pore-diffusion controlled the rate of adsorption when time is long [58].

The feasibility of malachite green dye removal using degreased coffee beans as adsorbent was evaluated by HwaBaek et. al. (2010). Rate kinetics, adsorption isotherms and thermodynamic properties were also evaluated for this purpose [97].

In 2010, removal of Malachite Green dye from wastewater was studied by Anbia and Ghaffari using meso-porous carbon adsorbent. Meso-porous carbon was synthesized and effects of various conditions such as dye concentration, adsorbent dose, contact time and pH were observed, the adsorption isotherms and rate kinetics were also determined [98].

Samiey and Toosi conducted studies on the adsorption of MG on silica gel in the year 2010. The adsorption process obeys Langmuir isotherm for low concentration of Malachite Green. As the MG concentration increases, reverse desorption occurs in MG molecules. The adsorption process happens through chemical bonding [99].

Kushwaha et. al. studied the removal of Malachite green(MG) and Methylene blue(MB) using waste materials of Daucus carrot plant (carrot stem powder: CSP and carrot leaves powder: CLP) in the year 2011. The maximum absorption was found at pH 7 and temperature 303 K was found to be optimum for removal of MB and MG by CSP and CLP. The equilibrium of adsorption of MB/MG onto CSP/CLP was suitably described by the Langmuir and Freundlich isotherm models. Monolayer adsorption capacities of CLP were higher suggested that CLP is better adsorbent than CSP. The adsorption process was best described by the pseudo-second order kinetics and intraparticle diffusion model. The dye uptake process was found to be controlled by both surface and pore diffusion, with surface diffusion at the earlier stages followed by pore diffusion at later stages. The thermodynamics parameters indicated that the adsorption was spontaneous, exothermic and physical in nature [100].

In 2011 Bairagi et. al., studied the Adsorption profile of lead on *Aspergillus versicolor*. The results establish that 1.0 g of the biomass adsorbs 45.0mg of lead and the adsorption process is found to depend on the pH of the solution with an optimum at pH 5.0. The rate of adsorption of lead by AVB (*Aspergillus versicolor* biomass) is very fast initially attaining equilibrium within 3 h following pseudo second order rate model. The adsorption process can better be described by Redlich–Peterson isotherm model compared to other ones tested. Scanning electron micrograph demonstrates conspicuous changes in the surface morphology of the biomass as a result of lead adsorption. Zeta potential values, chemical modification of the functional groups and Fourier transform infrared spectroscopy reveal that binding of lead on AVB occurs through complexation as well as electrostatic interaction [94].

In 2012 Singh et al., studied the degradation of textile dye orange 3R by *Aspergillus* strain (MMF3) and their culture optimization. Different parameters such as various carbon source, nitrogen source, temperature, pH and salinity concentrations were optimized for decolourisation of textile dye Orange 3R by using fungal isolates. *Aspergillus* strain (MMF3) showed maximum dye decolourisation of 96% at the end of 5th day under optimum condition

and found to be more efficient in dye decolourisation. High decolourisation extent and facile conditions show the potential for this fungal strain to be used in the biological treatment of dyeing mill effluents [93].

In 2013 Sartape et al., studied the removal of MG using, *Mimonia acidissima* shell or commonly known as wood apple shell as adsorbent. The effects of different parameters were investigated and optimal conditions were found out. The Langmuir isotherm model was found to be more suitable than Freundlich model. The maximum adsorption capacity was found to be 80.645mg/g at 299K.

In 2015 Dey and Mukhopadhyay studied, Adsorptive Removal of Methylene Blue by Biomass of *Aspergillus versicolor*. The optimum pH and temperature for adsorption was found to be 7.0 and 28° C respectively. Scanning electron microscopy (SEM) of the biomass suggested changes in surface topology following MB adsorption, while FTIR studies indicated chemical interaction between the surfaces of the biomass with MB. The kinetics study suggested the adsorption rate was fast initially and reached equilibrium at 4 h following a pseudo-second-order-kinetics. The adsorption isotherm follows Freundlich isotherm model. [91]

Nath and Ray studied the Biosorption of Malachite green from aqueous solution by dry cells of *Bacillus cereus* M^1_{16} (MTCC 5521) in the year 2015. The optimum biosorption of Malachite green by B. cereus M^1_{16} was obtained at pH 5.0, biomass concentration 0.5 g/L, initial dye concentration 400 mg/L and contact time 360 min. The kinetics of the overall adsorption process was best described by pseudo-second order kinetic model and sorption data fit best to both Langmuir and Redlich–Peterson isotherm model indicating a hybrid and not ideal monolayer adsorption behaviour. Maximum biosorption (saturation) capacity was found to be 485 mg/g of biomass. FTIR spectroscopic analysis and chemical modification of the functional groups of *B. cereus* M^1_{16} suggested the major involvement of carboxylate groups of *B. cereus* M^1_{16} in Malachite green adsorption. Study of cell morphology of *B. cereus* M^1_{16} using atomic force microscopy showed structural changes of *B. cereus* M^1_{16} after interaction with dye. *B. cereus* M^1_{16} dry biomass was also found capable of removing Malachite green from dye solutions containing ingredients that may normally be present in actual industrial effluent efficiently up to 400 mg/L dye concentration [92].

Tehrani and Zare-Dorabei studied removal of removal of malachite green (MG) and methylene blue (MB) dyes from aqueous solutions using second order derivative spectrophotometric method (SODS) which was applied to resolve the overlap between the spectra of these dyes in the 2016. The optimized experimental conditions were set as pH 7.78, contact time 5 min, initial MB concentration 22 mg L^{-1}, initial MG concentration 12 mgL^{-1} and adsorbent dosage 0.0055 g. The equilibrium data was fitted to isotherm models such as Langmuir, Freundlich and Tempkin and the results revealed the suitability of the Langmuir model. The maximum adsorption capacity of 666.67 and 153.85 mg g−1 was obtained for MB and MG removal respectively. Kinetics data fitting to pseudo-first order, pseudo-second order and Elovich models confirmed the applicability of pseudo-second order kinetic model for description of the mechanism and adsorption rate. Dye-loaded MIL-68(Al) can be easily regenerated using methanol and applied for three frequent sorption/desorption cycles with high performance. The impact of ionic strength on removal percentage of both dyes in binary mixture was studied by using NaCl and KCl soluble salts at different concentrations. According to our findings, only small dosage of the proposed MOF (metal–organic framework) is considerably capable to remove large amounts of dyes at room temperature and in very short time that is a big advantage of MIL-68(Al) as a promising adsorbent for adsorptive removal processes.

Chapter 3

Material and Methods

3.1. Chemicals and glass-wares

All the reagents used during this including Malachite Green Oxalate dye (chemical formula, $C_{52}H_{54}N_4O_{12}$ and molecular weight of 927.03) were of analytical grade and Distilled Water was used to prepare solution. Glass-wares used for the experiments volumetric flasks, pipette, weighing cylinder etc. are all made up of Borosil. All the glass-wares were acid washed using HCl and rinsed thoroughly with water several times (tap water) followed with distilled water.

3.2. Growth Medium

The growth medium had the following composition (L^{-1}): Dextrose 20 g, Potato extracts 300g; same as the maintenance medium except agar. The pH was maintained at 5.0 using 1(N) HCl or 1(N) NaOH solution.

3.3. Biosorbent (lyophilized fungal biomass)

In present study lyophilised fungal biomass was used as biosorbent. In a 250 mL Erlenmeyer flask, 70 mL of sterile growth medium was incubated at 30℃ at 120rpm speed in a shaker.After 72hours of period of incubation, the harvested biomass was washed three times with distilled water and centrifuged (REMI centrifuge, India) at 5000rpm for 10mins. After centrifugation the biomass collected was lyophilized and stored in desiccators until use.

3.4. Preparation of stock solution

Stock dye solution (1000 mg/L) was prepared by dissolving 0.5g of Malachite green dye in 500 mL of distilled water (DW). For further preparation, dye solutions of different concentrations were prepared by adequate dilution of the stock solution with DW.

3.5. Estimation of Dye Concentration

Dye concentration in solution was measured using UV-Visible spectrophotometer (HITACHI U-2000) at particular maximum wavelength value, after required dilution. Firstly the dye solution is scanned in the visible region (400-800 nm) to find the maximum wavelength. Bio-sorption of dye was calculated by the difference in dye concentration in the initial and final solution. Prior to the measurement, a calibration curve was obtained by using the standard Malachite Green solution with concentrations ranging from 1-7ppm.

The percentage adsorption and the amount of dye adsorbed by the biomass were calculated using the following equation:

% adsorption = $[(C_0-C_f)/ C_0] \times 100$

Amount of dye adsorbed, $q = \frac{(C_0 - C_f)V}{1000 \times W}$

where,

q is the amount of dye adsorbed by biomass (mg/g)
C_0 is the initial concentration of dye (mg/L),
C_f is the concentration of dye at equilibrium (mg/L)
V is the initial volume of dye solution (L), and
W is the weight of the biomass (g).

All experiments were conducted in triplicate and mean values used in the analysis of data.

3.6. Screening of Microorganism

Before conducting absorption experiments, screening was conducted in which *Aspergillus versicolor, Aspergillus acculeatus, Rhizopus oryzae, Aspergillus oryzae, T. clypeatus* were subjected to similar condition to study absorption efficiencies. The fungal biomass exhibiting maximum absorption efficiencies was subjected for further experimental studies.

3.7. Biosorption Experiments

The dye solutions of various concentrations varying from 1.0-7.0 ppm were prepared and their absorbance was measured by UV-Visible spectrophotometer at 617nm. The results were plotted to get the standard curve. This curve is the basis for all the future calculations done during this project work.

Table 3: Standard curve for Malachite Green

Concentration of dye (mg/L)	Adsorbance at 617 nm
1	0.101
2	0.227
3	0.339
4	0.492
5	0.642
6	0.794
7	0.908

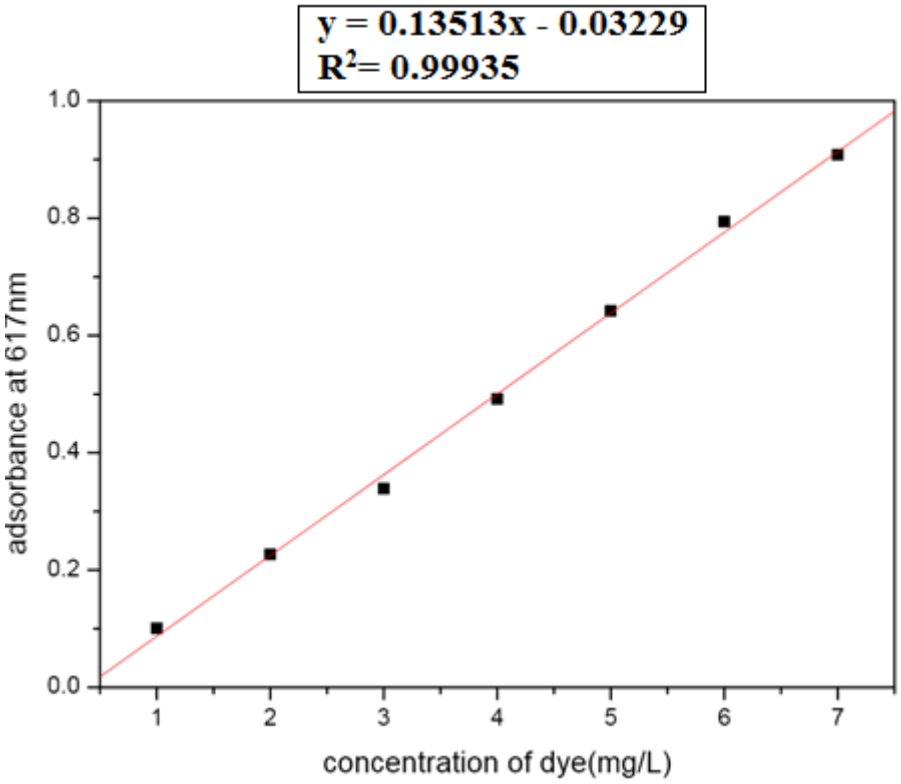


Fig 2: Standard curve of Malachite Green

3.7.1. Effect of pH

The effect of pH was studied by varying the pH of experimental solution (50ppm) from pH 4.0-6.0. After fixing pH, 0.1g of biomass was suspended into different pH solutions (pH 4.0, 5.0 and 6.0) for 24hours under shaking condition (120 rpm) at 30℃. After 24hrs of incubation, the dye solution is centrifuged at a speed of 5000rpm for 10mins. The supernatant was analysed to determine the percentage adsorption and the amount of dye adsorbed (q mg/g), using UV-Visible spectrophotometer at 617nm.

3.7.2. Effect of Temperature

To investigate the effect of incubation temperature, a 50ml 50ppm dye solution (pH 5.0) was taken in a 250ml Erlenmeyer flask, 0.1gmLyophilised biomass was added and the suspension was kept for shaking at 120 rpm for 24 hour but at different temperatures i.e. 25℃, 30℃, 35℃ and 40℃. After 24hrs of incubation, the suspension was centrifuged at a speed of 5000rpm for 10mins. The supernatant was analysed to determine the percentage adsorption and the amount of dye adsorbed (q mg/g), using UV-Visible spectrophotometer at 617nm.

3.7.3. Effect of Biomass dose

The effect of biomass load on removal was studied with varying quantities of biomass (0.05 - 0.3 g/ 50ml). At first 50ml dye solution of 50ppm was taken in a 250ml Erlenmeyer flask then the pH of the solution was adjusted to 5.0 using 1(N) NaOH or 1(N) HCl. Then lyophilized biomass (0.05 - 0.3 g/50ml) was added to dye solutions and incubated at 30℃ for 24 hours under shaking condition (120rpm). After 24hrs of incubation, the dye solution is centrifuged at a speed of 5000rpm for 10mins. The supernatant was analysed to determine the percentage adsorption and the amount of dye adsorbed (q mg/g) using UV- Visible spectrophotometer at 617nm.

3.7.4. Effect of Initial dye concentration

The effect of initial dye concentration on dye uptake by the lyophilized biomass was studied using varying dye concentrations 25-900 mg/L and then the pH of the solution was adjusted to 5.0 using 1(N) NaOH or 1(N) HCl and incubated under shaking condition at 120 rpm for 24 hours at 30^0C.After 24hours of incubation, the dye solution is centrifuged at a speed of 5000rpm for 10mins. The supernatant was analysed to determine the percentage adsorption and the amount of dye adsorbed (q mg/g), using UV-Visible spectrophotometer at 617nm.

3.7.5. Effect of contact time

When biomass was placed in a solution containing dye and agitated for an adequate time, biosorption of dye occurs. The dye concentration decreases from an initial value to an equilibrium value provided the contact time is sufficient. The time needed to reach equilibrium is defined as “equilibrium time”. Batch kinetic studies were conducted to determine the equilibrium time for biosorption of dye. At first 50ml dye solution was taken in a 250ml Erlenmeyer flask of 50ppm was prepared then the pH of the solution was adjusted to 5.0 using 1(N) NaOH or 1(N) HCl then 0.1g of lyophilised fungal biomass was added to dye solutions and incubated under shaking condition at 120rpm for 24 hours at 30℃. The samples were then collected after 1-330 min. Then the dye solutions collected were centrifuged at a speed of 5000 rpm for 10 min. The supernatant was analysed to determine the percentage

adsorption and the amount of dye adsorbed (q mg/g), using UV-Visible spectrophotometer at 617nm.

3.8. Desorption Experiment

The experimental conditions for desorption of dye on biomass were similar to that of the batch adsorption tests (biomass dosage: 0.1 g/50 ml, dye concentration 50 ppm mixing speed: 120 rpm, temperature: 30 °C). After being subjected to adsorption process for 24 hour, the reaction mixture was centrifuged and collected fungal biomass was then dried. The desorption studies were performed using different eluent such as, 0.1(M) H_2SO_4, 0.1 (M) HNO_3, 0.1(M) EDTA, 0.1(M) NaCl, 20% Ethanol, 20% Methanol, DDW (pH 2-7). Other conditions remained the same as during desorption (30°C, 120 rpm shaking). The supernatant resulting from centrifugation of the samples was analysed for dye concentration using UV-Visible spectrophotometer at 617nm.

Chapter 4

Result and Discussion

4.1. Screening of Microorganism

Before conducting absorption experiments, screening was conducted in which *Aspergillus versicolor, Aspergillus acculeatus, Rhizopus oryzae, Aspergillus oryzae, T. clypeatus* were subjected to similar condition to study absorption efficiencies. The fungal biomass exhibiting maximum absorption efficiencies was subjected for further experimental studies.

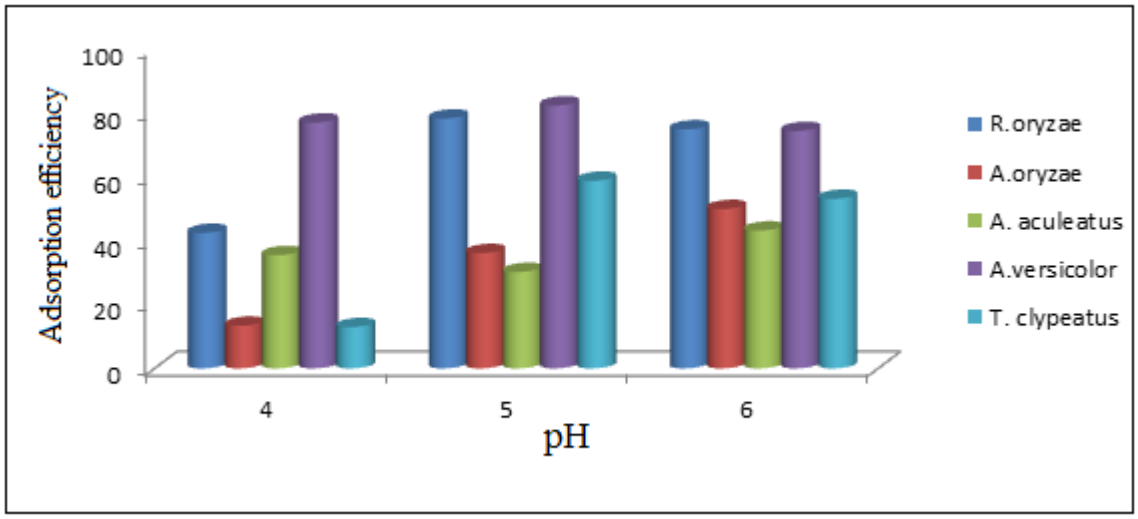


Fig 3: Screening of microorganism (pH against adsorption efficiency)

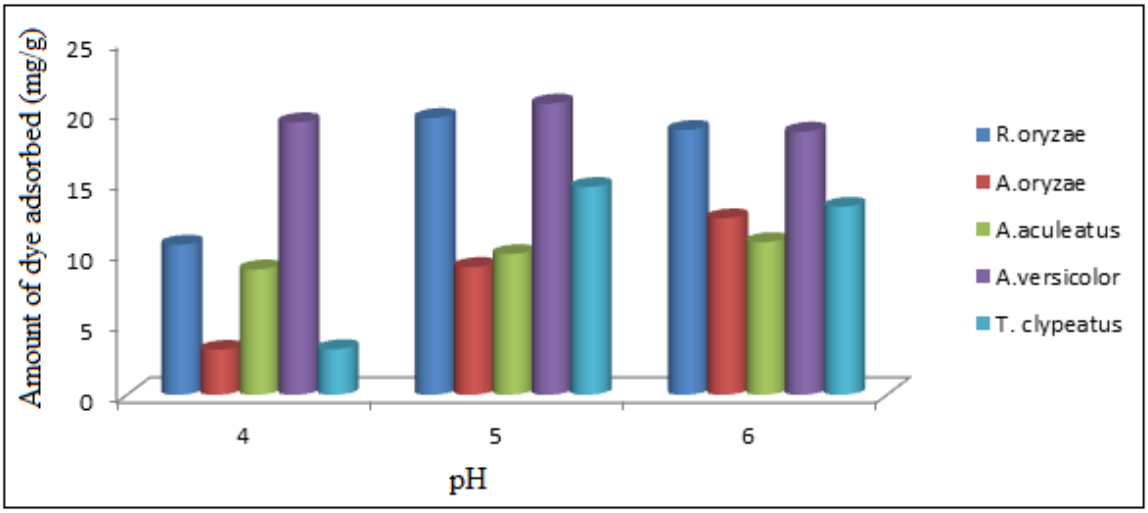


Fig 4: Screening of microorganism (pH against amount of dye adsorbed)

At pH 5.00, *Aspergillus versicolor* exhibited the maximum adsorption efficiency (%) and q (mg/g) i.e. 82.18% and 20.54 mg/g respectively. Hence, the influence of several parameters, such as, pH treatment, biosorbent dosage, temperature, initial dye concentration and contact time on biosorption kinetics of malachite green (Basic dye) by *Aspergillus versicolor* were studied.

4.2. Effect of pH

The interaction between the dye and the functional groups of the biomass depends on the nature of the adsorbent as well as on the solution chemistry of the adsorbate, which in turn depends on the pH considerably influencing dye speciation, sequestration, and/ or mobility. It is well documented that pH is an important variable governing the uptake of dye by biosorption process as it not only affects dye in solution, but also influences the surface properties of biosorbents in terms of dissociation of binding sites and surface charge. The effect of pH on the biosorption process can be varied from the type of biomass to the type of dye being studied.

pH	**Adsorption Capacity(mg/g)**
4	19.22
5	20.54
6	18.56

Table 4: Effect of pH on dye biosorption

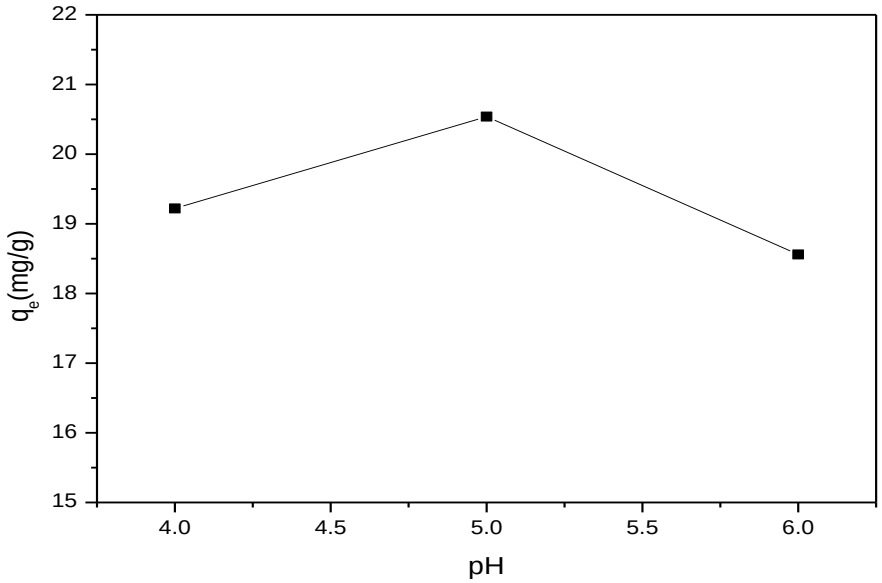


Fig 5: Effect of pH on dye biosorption (Initial dye concentration: 50 mg/L, biomass dosage 0.1g/50ml, temperature: 30°C, time 24 hours at 120rpm).

In this experiment, the dye removal percentage increased with an increase in solution pH from 4.0 to 5.0. The relatively low biosorption values at strong acidic conditions can be attributed to proton-dye ion competition for occupancy of the binding sites on the biosorbent surface. In other words at highly acidic conditions biosorbent surface becomes saturated with protons thus reducing the interaction between biosorbent and dye ions. At lower pH, the number of positively charged adsorbent surface sites increased at the expense of the number of negatively charged surface sites. The carboxylic groups of MG (pKa = 10.3) were protonated and had high positive charge density at a lower pH [82]. Therefore, for pH below 5, electrostatic repulsion exists between the positively charged surface and the positively charged dye molecule. This illustrates as to why adsorption increases with increase in pH. When the pH of the initial solution was further increased above 5.0, excess hydroxyl ions may compete for the active sites on the biosorbents and resulted in less dye removal efficiency [103].

4.3. Effect of Temperature

Temperature (℃)	Adsorption capacity(mg/g)
25	18.87
30	20.63
35	20.55
40	18.84

Table 5: Effect of Temperature on dye biosorption.

The variation of the biosorption capacity with temperature is presented in Fig.3.

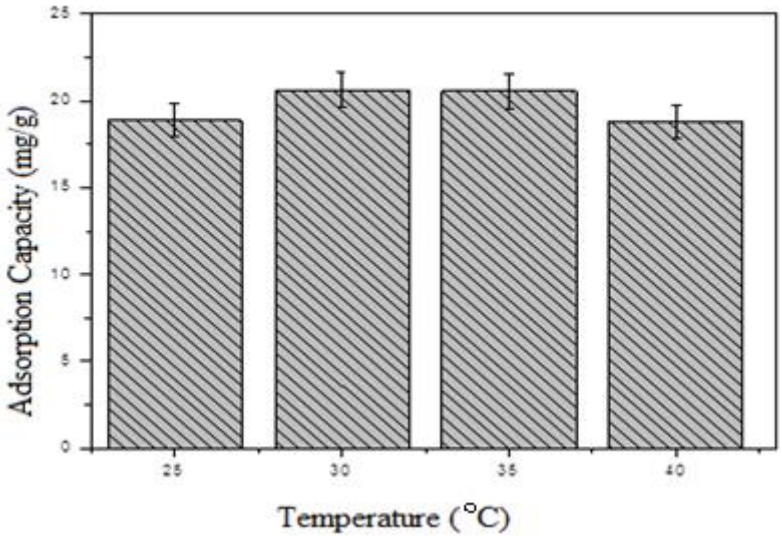


Figure 6: Effect of temperature on dye biosorption (pH 5.0, Initial dye concentration: 50 mg/L, biomass dosage: 0.1 g/50 ml, time 24 hours at 120 rpm)

As seen from Fig.4, the dye bio-sorption by *Aspergillus versicolor* appears to be temperature dependent in the temperature range studied. When the temperature was increased from 25°C to 30°C the dye loading capacity of biosorbent increased and after that when the temperature was again increased from 30°C to 40°C, the adsorption of dye decreased slightly. Further increase in temperature from 30°C may alter the surface nature of biomass result in a decrease in removal value, indicating that this process is exothermic in nature. The exothermic nature of dye biosorption has also been reported for the biosorption of Remazol

Black B and Acid Red 274 dyes by *R. arrhizus* and *E. prolifera*, respectively [80,81]. The present results showed essentially no thermal deactivation of bio-sorption activity under operational temperatures. Therefore, the used organism could adsorb dye in broad range of temperature.

4.4. Effect of biomass dose

When the biosorbent load increased from 0.05 g/50 ml to 0.15 g/50 ml the percentage of dye biosorption as well as the biosorption capacity significantly increased. This was attributed to binding sites increment. However, the biosorption capacity of *Aspergillus versicolor* showed a reduced trend as biosorbent concentrations further increased from 0.15g/50ml to 0.3g/50ml. This may be due to several causes, including partial cell aggregation causing a decrease of the availability of active dye binding sites, availability of solute, electrostatic interactions, and interface between binding sites. The decrease in q value may be due to the splitting effect of flux (concentration gradient) between adsorbate and adsorbent with increasing biomass concentration causing a decrease in amount of dye adsorbed onto unit weight of biomass. At higher biomass concentration colour removal (%) is increased as there is a very fast superficial adsorption onto the cell that produces a lower solute concentration in the solution than when cell concentration is low [83].

Biomass Dose (g/50ml)	Adsorption Efficiency (%)	Adsorption Capacity (mg/g)
0.05	65.73	32.89
0.10	82.12	20.53
0.15	87.15	14.53
0.20	86.90	10.86
0.25	85.76	8.58
0.30	83.43	6.96

Table 6: Effect of biomass dose on dye biosorption

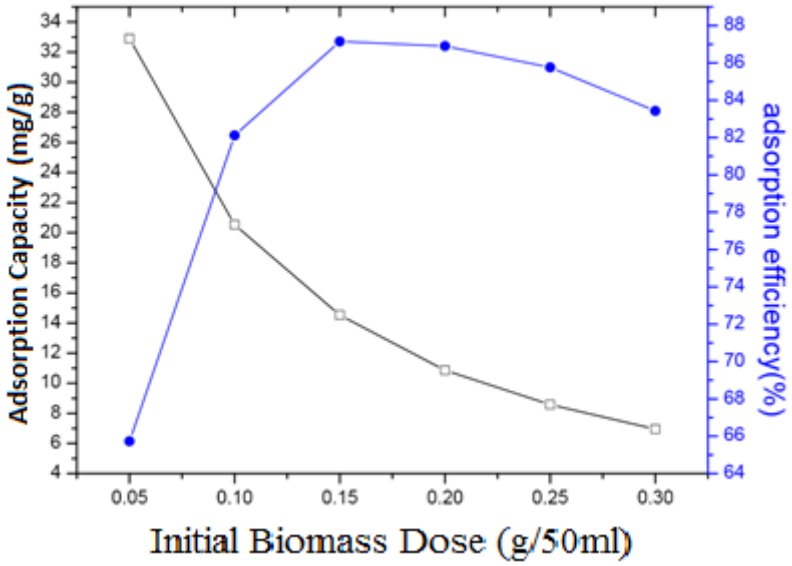


Fig 7: Effect of Initial Biomass Dose (g/50ml) on dye biosorption (Initial dye concentration: 50 mg/L, Temperature 30℃, pH 5.0, Time 24 hours at 120 rpm).

4.5. Adsorption Isotherm

The dye removal capacity of lyophilized *Aspergillus versicolor* biomass is presented as a function of the initial concentration of dye in the aqueous solution is represented in table below.

Initial Dye concentration (ppm)	**C_e(ppm)**	**q_e(mg/g)**
25	3.72	10.64
50	8.53	20.73
100	17.01	41.49
200	33.45	83.29
300	62.34	118.83
400	87.31	156.35
500	107.89	196.06
600	148.21	225.89
700	187.44	256.27
800	236.28	281.86
900	336.56	281.72

Table 7: Effect of Initial Dye concentration on dye biosorption

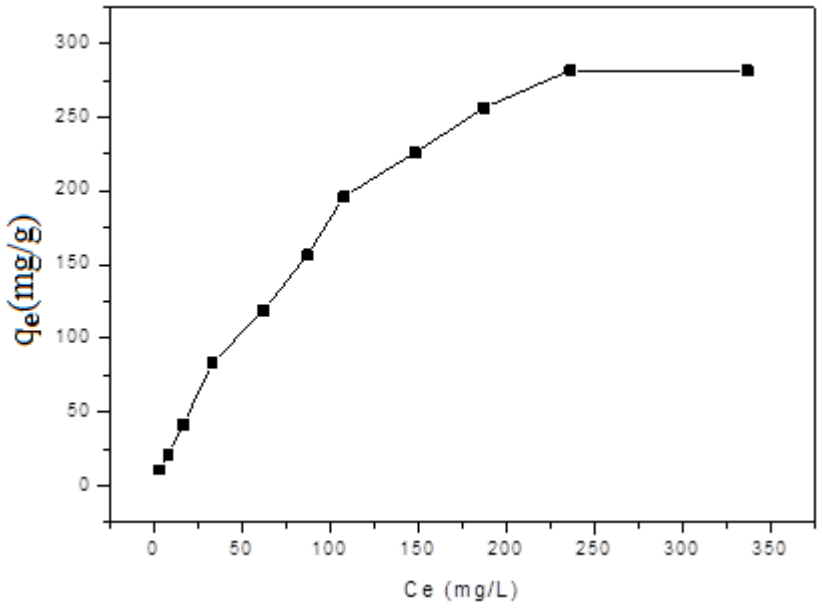


Fig 8: Effect of Initial Dye concentration (mg/L) on dye biosorption (Temperature 30℃, Biomass dose 0.1g/50ml, pH 5.0, Time 24 hours at 120rpm)

It can be seen that the biosorption capacity increased as initial dye concentration increased. The observed enhancement of dye uptake could be due to an increase in electrostatic interactions (relative to covalent interactions) which involve sites of progressively lower affinity for dye. Dursun (2006) reported that increasing dye concentration provides a driving force to overcome mass transfer resistance between adsorbent and its medium, thus, higher initial dye concentration allows obtaining higher bio-sorption capacity.

Adsorption isotherms help to describe how molecules of adsorbate interact with adsorbent surface. The adsorption processes are generally described by the Langmuir and the Freundlich isotherm models.

The Langmuir equation is based on the fact that there is no interaction between the adsorbate molecules and that the adsorption process is localized in a monolayer. Langmuir isotherm model assumes uniform energies of adsorption onto the surface without transmigration of adsorbate in the plane of the surface [85]. The Langmuir equation is commonly expressed as in the linear form [84]:

$$C_e/q_e = (1/q_o K_L) + (C_e/q_o)$$

Where C_e is the equilibrium concentration of dye solution (mg/L), q_e is the equilibrium adsorbing capacity of dye on the adsorbent surface (mg/g), q_o is the monolayer adsorption capacity of the adsorbent (mg/g), and K_L is the Langmuir adsorption constant (L/mg) and is related to the free energy of adsorption. A plot with C_e/q_e vs C_e gives the value of q_o from its slope and K_L from its intercept.

The Freundlich adsorption model assumes that adsorption takes place on heterogeneous surfaces. Its linear form can be written as [86]:

$$\log q_e = \log K_f + (1/n) \log C_e$$

Where, K_f and n (dimensionless constants) are the Freundlich adsorption isotherm constant, which indicates the capacity and intensity of the adsorption, respectively. If we plot log q_e vs log C_e we get a straight line with slope 1/n and intercept log K_f.

Analysis of Adsorption isotherm

Analysis of the obtained equilibrium data is essential to develop an equation which precisely represents the results and can be used for design purposes. Experimental equilibrium sorption data are usually represented by Langmuir and Freundlich isotherm models. These isotherms have been widely applied since they are simple, give a good description of experimental behaviour in a large range of operating conditions and re-characterized by a limited number of adjustable parameters.

The biosorption isotherms of lyophilized *Aspergillus versicolor* were studied at 30°C, biomass dosage of 0.1 g/50 ml, and agitation rate of 120 rpm for 24 hours. In these studies, while other parameters were kept constant, the initial dye concentration was varied from 25 to 900 mg/L.

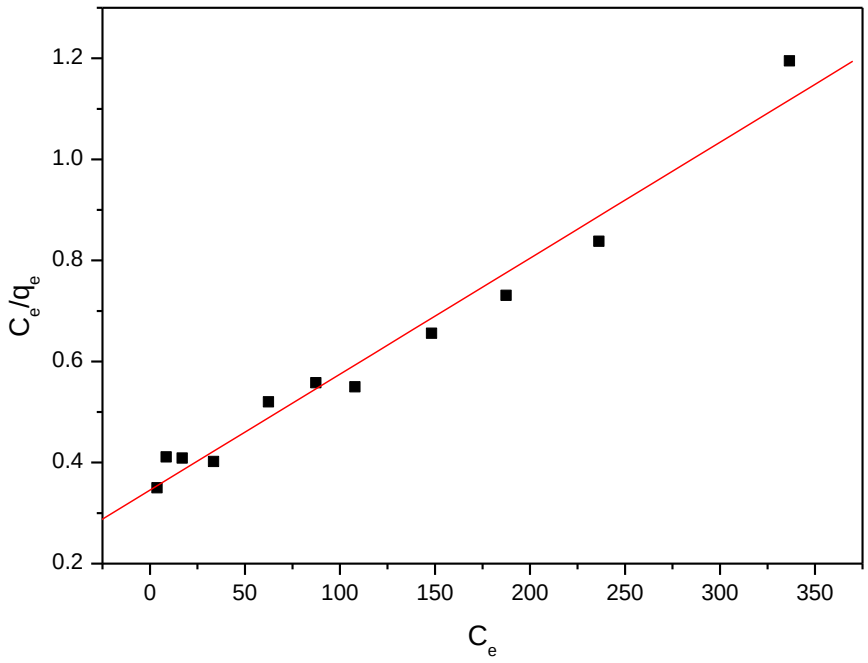


Fig 9: Langmuir isotherm curve

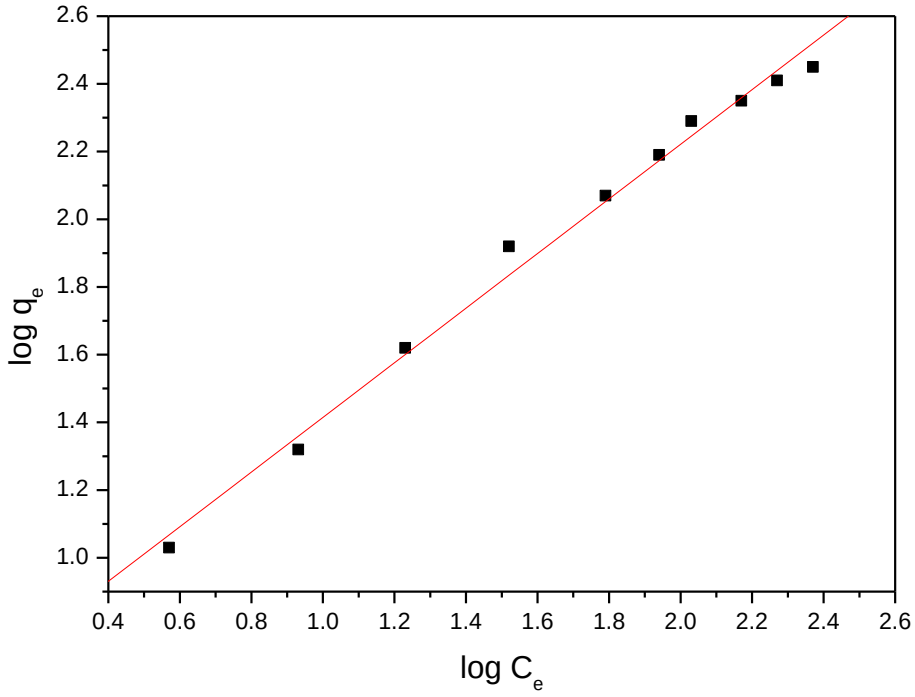


Fig 10: Freundlich Isotherm curve

Table 8: Langmuir and Freundlich isotherm model constant

Langmuir Constant			Freundlich Constant		
q_{max}(mg/g)	k_L(L/mg)	R^2	K_f	1/n	R^2
436.68	6.6 $X10^{-3}$	0.986	4.549	0.768	0.996

Langmuir isotherm model denotes chemisorption and monolayer adsorption procedure. On the contrary Freundlich isotherm denotes multilayer sorption of sorbate on sorbent. The experimental data were plotted to obtain Langmuir and Freundlich curve. From the data obtained from table above, it is evident that adsorption of Malachite green on Lyophilised fungal biomass *Aspergillus versicolor* followed Freundlich Isotherm model since the value of coefficient correlation (R^2) was found higher in Freunlich than in Langmuir isotherm model.

4.6. Kinetic study

The malachite green dye removal capacity of lyophilized *Aspergillus versicolor*, biomass as a function of time is presented as below:

Time (min)	q_t (mg/g)
1	10.49
3	10.79
5	11.22
7	11.49
10	12.09
12	12.14
15	12.31
20	12.56
25	12.71
30	12.87
45	13.39
60	13.87
90	14.92
120	15.73
150	16.68
180	17.38
210	18.14
240	18.76
270	19.14
300	19.22
330	19.37

Table 9: Effect of Contact time on dye biosorption

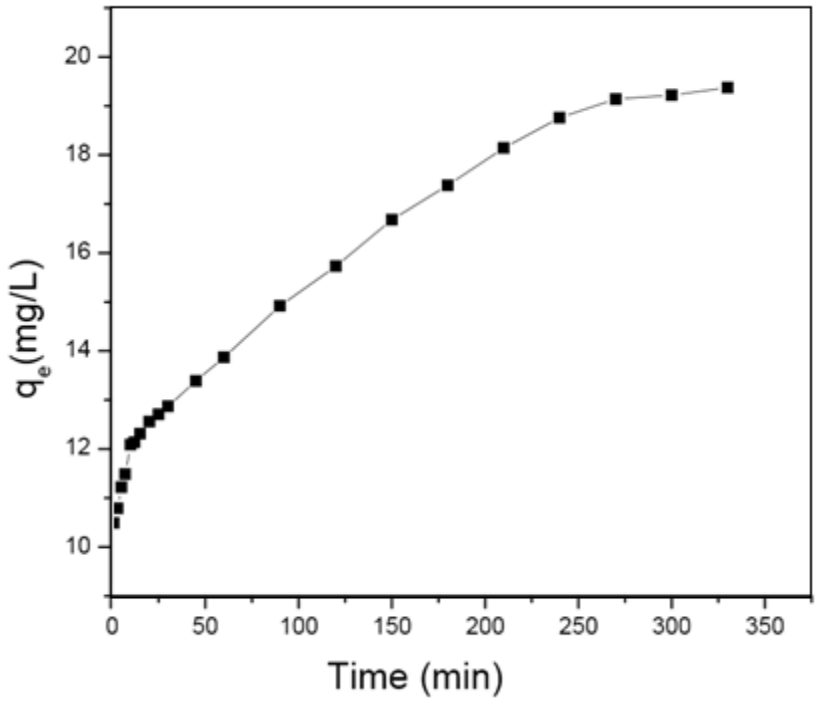


Fig 11: Effect of Contact time on a dye biosorption (Initial dye concentration 50 mg/L, Biomass dose 0.1g/50ml, pH 5.0, Temp 30℃ at 120rpm)

It can be seen that the reaction was very fast initially and attained saturation within 6 hours. After the equilibrium period no further increase in the amount of dye adsorbed occurs. The similar fast biosorption trends have also been reported by other researchers. The surface of cells is bare in the initial stage; the biosorption rate is fast and normally governed by the diffusion process from the bulk solution to the surface. In the later stage, the biosorption is likely an attachment- controlled process due to less available biosorption sites. The time profile of Malachite Green uptake is a single, smooth and continuous curve leading to saturation, suggesting the possible monolayer coverage of Malachite Green on the surface of the biosorbent. The rate of dye-sorption is of great significance for developing a microbial origin biosorbent based water treatment technology and practical application of the process.

Adsorption kinetics

In order to investigate the controlling mechanisms of the adsorption processes like mass transfer and chemical reactions, pseudo first-order and pseudo second-order kinetic studies are used to test the experimental data.

The kinetic data were treated with the following Lagergren's pseudo first-order rate equation [87]:

$$\frac{dq}{dt} = k_1(q_e - q_t)$$

After integrating we get

$$\log(q_e - q_t) = \log q_e - k_1 t$$

Where q_t is the adsorption capacity at time't' (mg/g) and k_1 (min^{-1}) is the rate constant of the pseudo-first adsorption. A plot of log (q_e-q_t) vs t, gives a straight line with negative slope k_1 and intercept log q_e.

A plot of Ho's pseudo-second-order kinetic model is based on the assumption that the sorption follows second order chemisorption.

Pseudo-second-order kinetic model is given by [88, 89]:

$$\frac{dq}{dt} = k_2(q_e - q_t)^2$$

After integration,

$$\frac{t}{q_t} = \frac{1}{k_2 {q_e}^2} + \frac{t}{q_e}$$

Where k_2 (g /mg-min) is the rate constant of pseudo-second order adsorption and q_t is the adsorption capacity at time 't' (mg/g).

The plots of (t/q_t) vs t gives a straight line with slope $1/q_e$ and intercepts $(1/k_2q_e^2)$. Hence from the value of slope and intercept we can calculate the value k_2.

Analysis of the model

In order to investigate the mechanisms of the present biosorption process and the potential rate controlling steps such as mass transport, pore diffusion and chemical reaction processes, kinetic models have been used to fit experimental data.

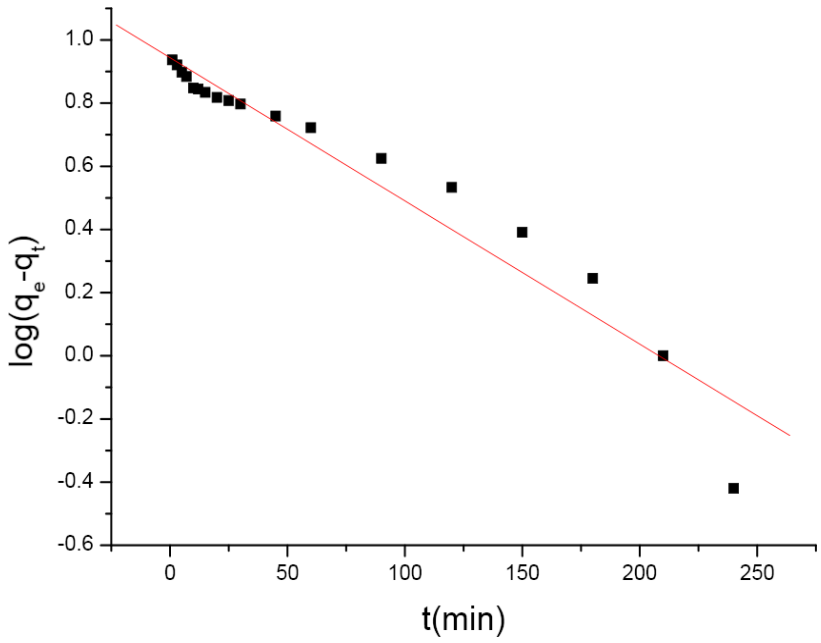


Fig 12: Pseudo First order kinetic plot

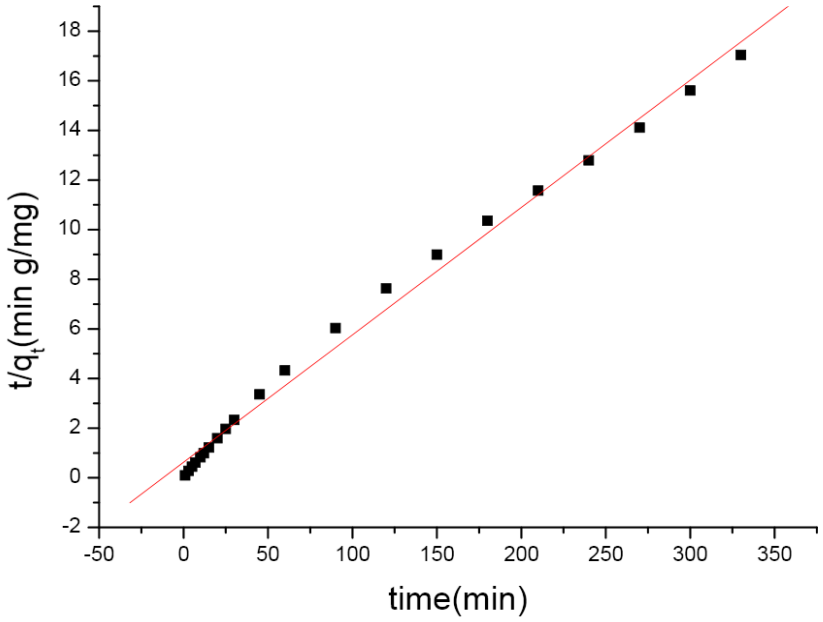


Fig 13: Pseudo second order kinetic plot

Initial dye concentration (mg/L)	**Pseudo first order**			**Pseudo second order**		
50	$k_1(min^{-1})$	q_e(mg/g)	R^2	k_2(g/mg-min)	q_e(mg/g)	R^2
	0.0103	2.5699	0.9679	0.0041	19.608	0.997

Table 10: Pseudo first order and second order rate constant

From a mechanistic point of view to interpret the kinetic experimental data, prediction of the rate-limiting step is an important factor to be considered in the sorption process (Vadivelan and Kumar, 2005). Although kinetic studies help to identify the sorption process, predicting the mechanisms is required for design purposes. For a solid-liquid sorption process, the solute transfer is usually characterized by either external mass transfer (boundary layer diffusion) for non-porous media or intra-particle diffusion for porous matrices, or both combined. According to the kinetic model results shown in the Table, the correlation coefficients for first-order model obtained at the studied initial dye concentrations were low and the theoretical q_e did not give acceptable values when compared to the experimental ones.

Therefore, the reaction involved in the present biosorption system is not of the first-order. On the other hand, the pseudo-second-order model shows the best fit to the experimental data related to the biosorption of Malachite green onto *Aspergillus versicolor* with the highest correlation coefficients. In addition, as shown in Table, the pseudo-second order predicted qe are the most agreeing values with the experimental data. These results indicate that the biosorption of Malachite green by *Aspergillus versicolor* follows the pseudo-second-order kinetics model, which relies on the assumption that the rate-limiting step may be biosorption involving valence forces through the sharing or exchange of electrons between biosorbent and sorbate.

4.7. Desorption Experiment

The biosorbed dye was eluted from fungal biomass using various eluant, such as: 0.1(M) H_2SO_4, 0.1(M) HNO3, 0.1(M) EDTA,0.1(M) NaCl, 20% Ethanol, 20% methanol, DDW(pH 2-7) to investigate the removal of biosorbed dye from dye-loaded biomass, i.e. to investigate the possibility of regenerating the biosorbent.

Eluent used (50ml)	**Desorption efficiency (%)**
0.1 (M) H_2SO_4	21.41
0.1 (M) HNO_3	36.41
0.1 (M) EDTA	52.42
0.1 (M) NaCl	36.18
20% Ethanol	39.51
20% Methanol	29.76
DDW (pH 2.0)	82.55
DDW (pH 3.0)	73.81
DDW (pH 4.0)	57.92
DDW (pH 5.0)	26.32
DDW (pH 6.0)	25.84
DDW (pH 7.0)	18.46

Table 11: Desorption efficiencies of various eluent

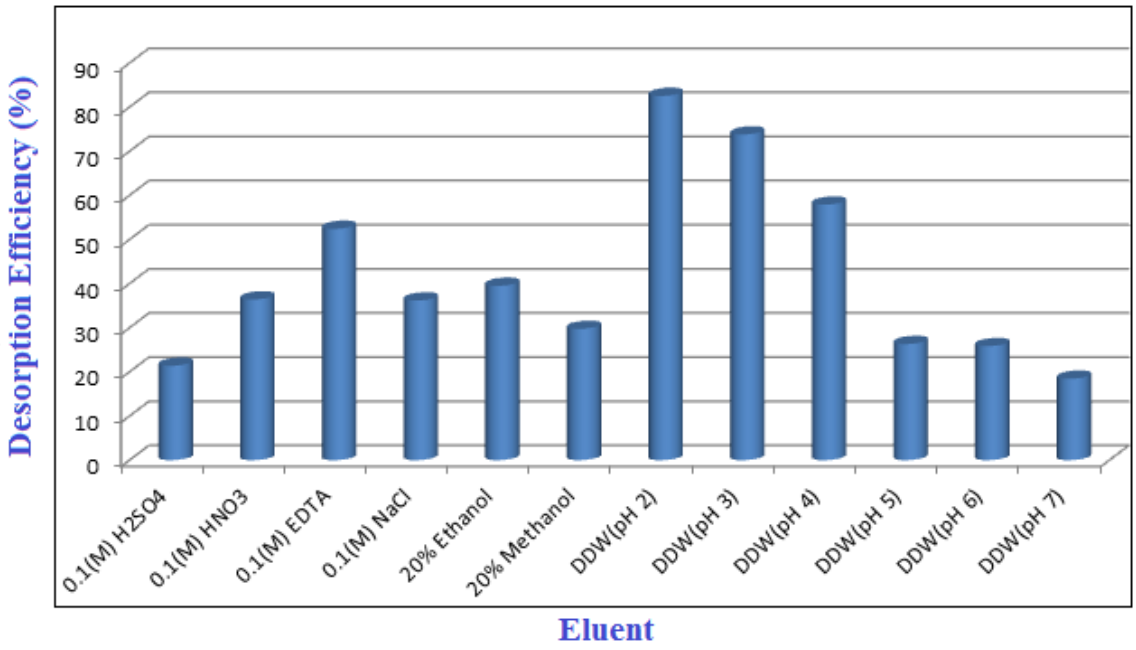


Fig14: Desorption efficiencies of various eluent (Elent volume 50ml, Temp 30℃ at 120rpm).

From the above figure it can be clearly stated that DDW at pH 2.00 can be used efficiently for desorption of Malachite Green with desorption efficiency of 82.55%.The use of fungal biomass as a potential biosorbent depends not only on the biosorption capacity, but also on how well the biomass can be regenerated and reused. The high percentage of recovery of dye allows the reuse of biomass and is an important feature for its possible use in continuous systems in industrial processes and in analytical procedures for pre-concentration of dye. The results indicated that *Aspergillus versicolor* appears to be potential adsorbent for malachite green dye removal from aqueous solution.

5. Conclusion

It was observed from present study the bio-sorption of Malachite green was found to be maximum at pH 5.0 at temperature 30℃ and 0.1g/50ml was found to be the optimum biosorbent dose. The rate of adsorption was found to be very fast initially and equilibrium was reached at 270 min following the pseudo-second rate kinetics. The adsorption process followed Freundlich Isotherm curve. The desorption efficiency DDW maintained at pH 2.0 was found to be maximum i.e. 82.55% thus it can be efficiently used for the desorption of Malachite Green.

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