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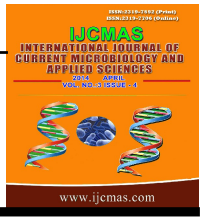

Original Research Article

Degradation of malachite green and congo red using *Aloe barabadensis* Mill. Extract

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A B S T R A C T

Degradation of trimethyl dye malachite green and azo dye congo red was investigated using water extract of *Aloe barbadensis* with different parameters such as buffer systems, temperature, dye and enzyme concentration and pH. Phyto-toxicity study was carried out to detect the effect of dye, plant extract treated dye on seed germination and seedling growth. Decolorization assay was performed to calculate percent degradation by taking absorbance using UV-visible spectrophotometer. Congo red showed a maximum of 27.33% decolorization with plant extract using citrate buffer and skin extract produced 30% decoloration. Maximum pH for highest decoloration varied from 6-8 for both congo red and malachite green and temperature was 40˚C - 50˚C. With different dye concentrations 0.1% exhibited higher decoloration. The time for decoloration varied from 30 minutes to 2 hours. There was a marked difference in the toxic effect of dyes on seedling compared with plant extract treated dye products.

K e y w o r d s

Aloe vera,
A. barbadensis,
malachite green,
congo red,
decoloration,
degradation

Introduction

Colors gives delightful pleasure to eyesight but at the same time they may act as serious pollutants when their origin is dyes and dyestuffs. Textile industries have been using dyes intensively because of their ease and cost effectiveness in synthesis most widely used in textile, rubber, and enamel, plastic, cosmetic and many other industries (Cerniglia, 1997). It is estimated that about 10-15% dyes are released into processing water during this procedure. There are different class of organic compounds characterized by the presence of unsaturated groups (chromophores) such as ─C═C─, ─N═N─ and ─C≡N─, which are responsible for the dye colours, and of functional groups responsible for their fixation to fibres, for example, ─NH_2, ─OH, ─COOH and ─SO_3H. These dyes are chemically diverse and divided into azo, anthraquinone, heterocyclic polymers and triphenylmethane dyes. Most of these are stable against light, temperature and biodegradation and have therefore accumulated in the environment as

recalcitrant compounds. Conventional waste water treatment is not efficient to remove recalcitrant dyestuffs from effluents. Several physical and chemical methods are effective but have high operating costs and limited applicability (Gupta and Manisha, 2012). Approximately 10,000 different dyes and pigments are produced worldwide and used extensively in the dye and printing industries. It is estimated that about 10-14% of the total dye used in the dyeing process may be found in wastewater. These dyes are considered to be recalcitrant, and toxic. They resist microbial biodegradation and are therefore not easily degraded in wastewater treatment plant. Thus, treatment of dye is yet one of the challenging tasks in environment field. Currently available methods such as chemical oxidation, reverse osmosis, adsorption, etc., suffer from disadvantages such as high cost, regeneration problems and secondary pollutants/sludge generation. Recently, Researchers have been focusing their attention to enzymatic treatment (Tezcanli-Guyer, 2003). A major class of synthetic dyes includes the azo, anthroquinone and triphenylmethane dyes. Dyes are difficult to degrade biologically, so that degradation of dyes has received considerable attention. About 10-15% of all dyes are directly lost to wastewater in the dyeing process.[1-2] Thus, the wastewater must be treated before releasing into the natural environment. The Food and Drug Administration nominated MG as a priority chemical for carcinogenicity testing by the National Toxicology Program 1993. MG and its reduced form, leucomalachite green, may persist in edible fish tissues for extended periods of time.[5] Therefore, there are both environmental and human health concerns about bioaccumulation of MG and leucomalachite green in terrestrial and aquatic ecosystems (Puvaneswari et al., 2006).

The popularity and widespread use of azo dyes is due to several factors. As a group, they are color-fast and encompass the entire visible spectrum, and many are easily synthesized from inexpensive and easily obtained starting materials. Also azo dyes are typically amenable to structural modification, and can be made to bind to most synthetic and natural textile fibers. Because azo dyes are highly colored, they are readily apparent and can create a significant environmental problem by affecting water transparency as well as aesthetic problems. Dyes in wastewater not only present aesthetic objections, but they can pose threats to public health. Some of the dyes, their precursors, or their biotransformation products such as aromatic amines, have been shown to be carcinogenic (Razo-Flores et al., 1997). Most textile effluents have to be treated to remove these dyes to meet increasingly stringent legislation or to meet local citizenry objections.

It is necessary to clarify the concepts of decolorization, degradation, and mineralization of dyes in that these words often occur in the literature and sometimes were misused. Decolorization is simply the disappearance of the color in wastewater without the actual breaking apart of the dye molecules, which does not necessarily mean degradation of the complex dye molecules. Degradation is the destruction of the large dye molecule to smaller components, along with the breakdown of the chromophores. While chromophore groups of dyes may be destroyed, the intermediate produced may be more toxic than the original compounds and could present significant problems for

receiving water bodies. Mineralization means organic compounds are converted to inorganic compounds, i.e., nitrate, carbon dioxide, and water. In this case, a complete detoxification is achieved and no secondary pollution will be introduced.

The aloe plant *Aloe barbadensis* Miller (*Aloe vera* L.) in the family Liliaceae is one of the most researched and used plant among 300 species of aloe. Aloe has been used medicinally for several thousands of years in many cultures from ancient Egypt, Greece, and Rome to China and India. The plant has many common names and is often referred to as *Aloe vera*, burn plant, first-aid plant, or medicine plant. Aloes are thought to have originated in tropical Africa but are now cultivated in warm climate areas of Asia, Europe and America.

The aloe plant is the source of two herbal preparations: *Aloe* gel (AG) and *Aloe* latex. *Aloe* gel is often called "aloe vera" and refers to the clear gel or mucilaginous substance produced by parenchymal cells located in the central region of the leaf. Diluted aloe gel is commonly referred to as "aloe vera extract". The gel is composed mainly of water (99%) and mono- and polysaccharides (25% of the dry weight of the gel). The most prominent monosaccharide in AG is mannose-6-phosphate, and the most common polysaccharides are called gluco-mannans (beta-(1,4) acetylated mannan). They are long-chain sugars containing glucose and mannose. A prominent gluco-mannan named acemannan has been isolated and is being marketed as Carrisyn. Recently a glycoprotein with antiallergic properties, called alprogen, was isolated from AG. In addition, a novel anti-inflammatory compound, C-glucosyl chromone, has recently been isolated from AG.

Aloe gel also contains lignan, salicylic acid, saponins, sterols, and triterpenoids. The fresh gel contains the proteolytic enzyme carboxypeptidase (which breaks down bradykinin), glutathione peroxidase, as well as several isozymes of superoxide dismutase. The gel also contains vitamins A, C, E, B_{12}, thiamine, niacin and folic acid, as well as the minerals sodium, potassium, calcium, magnesium, manganese, copper, zinc, chromium, and iron.

The *A. vera* plant is made up of fibrous roots, short stem and a spiral greenish leaves. The leaf is made of a gel, which is colourless, viscous liquid consisting primarily of water and ploysaccharides and has a bitter taste (Brinelon, 1995). Over 250 species of the genus *Aloe* exist, with only two species grown on commercial basis (*Aloe barbadensis* and *Aloe arborescens*). Regarding chemical constituents, *A. vera* contains amino acids, lipids, sterols, tannins, enzymes flavonoids and mannose –6–phosphate (Brinelon, 1995; Davis and Robson, 1999). Therefore, this plant had been found useful in the treatment of wound, burns, skin disorders and anti-inflammatory activity.

Materials and Methods

Preparation of crude *Aloe barbadensis* Extract

Aloe plant was procured from Alva's Shobhavana, Moodbidri. Three different extracts were prepared namely *Aloe* pulp extract, *Aloe* skin extract and *Aloe* whole plant extract. *Aloe* plant was cleaned and pulp was separated from skin. About 200gm of the pulp as weighed and crushed in a wet grinder with the addition of 200ml

of water. The mixture was filtered using muslin cloth and the filtrate was diluted with distilled water and made up to 500ml and the final concentration of 40% was obtained. In the same way *Aloe* skin was cleaned and 40gms of the cleaned skin was crushed with 60ml of distilled water in a grinder. The mixture was filtered using muslin cloth and the filtrate was collected. 200gms of the whole plant was cleaned and crushed in a wet grinder. The extract was filtered using muslin cloth and the filtrate was diluted and made up to 500ml using distilled water giving the final concentration of 40gms/100ml. All the 3 extracts were centrifuged at 8000rpm for 12 minutes. Supernatant was collected and stored at 4˚C. It was brought to room temperature before use.

Decolorization assay

All decolorization experiments were performed in two sets. The change in absorbance was monitored spectrophotometrically at their maximum wavelength of each dye. The set without the addition of enzyme in the respective dye served as control. The decolorization efficiency was determined using the following equation:

$$\% \text{ Decolourization} = \frac{\text{Initial absorbance} - \text{observed absorbance}}{\text{Initial absorbance}} \times 100$$

The percentage decolorisation/ degradation was studied at different parameters like maximum part of plant showing degradation, effect of buffers, effect of pH, effect of temperature, effect of dye concentration, effect of enzyme quantity and effect of time period.

Use of appropriate buffers for each dye

A buffer is needed for the activity of enzyme. Three buffers (Acetate buffer, phosphate buffer and citrate buffer) of pH 7 were used. Four different dyes (Congo red, Brilliant blue, crystal violet and Malachite green) were initially used. Each dye was examined with all the three buffers. Each set contains one tube with 5ml of 0.1% dye solution, next tube contains 3ml of buffer: 0.5ml of 0.1% dye solution, the third tube contains 3ml of buffer: 0.5ml of 0.1% dye solution: 1ml of enzyme extract was added. Temperature and time was kept constant at 30˚C and 1hr respectively. All the set was performed in duplicates. Decolorations were examined using different buffers.

Effect of different *Aloe* extract

Different part of the plant has different composition. The effect of the different *Aloe* extracts was examined with the two dyes. For the study each set contains one tube with 5ml of 0.1% dye solution, next tube contains 3ml of buffer: 0.5ml of 0.1% dye solution, the third tube contains 3ml of buffer: 0.5ml of 0.1% dye solution: 1ml of each enzyme extract was added. Temperature and time was kept constant at 30˚C and 1hr respectively. All the set was performed in duplicates. Decolorations using different buffers were examined.

Effect of pH

The pH factor was optimized to obtain maximum decoloration. pH range from 4-9 was studied. Buffers of different pH range were prepared. 6 tubes with 3ml of buffer of different pH were taken in 2 tubes. To each tube 0.5ml of 0.1% dye solution was added. In one of the tube 1ml of enzyme extract was added according to previously observed result. The same was repeated for both the dye solution. Temperature and time was kept constant at 30˚c and 1hr respectively. All the set was

performed in duplicates. Decolorations at different pH were examined.

Effect of temperature

Temperature plays an important role in the enzyme activity. Temperature range for 30˚C- 60˚C was taken. 5 tubes with different temperature were taken with uninoculated enzyme taken as control and the tube with extract as test. Other parameters were kept constant according to previously observed results. All the set was performed in duplicates. Decolorations at different temperature were examined.

Effect of dye concentration

Dye concentration from 0.1ml- 1ml/ 100ml was used for the studies. Optimal temperature, pH was kept constant. All the set was performed in duplicates. Decolorations at different dye concentration were examined.

Effect of enzyme quantity

40% enzyme concentration was previously prepared. Hence decolorisation rate at different enzyme quantity from 0.5ml to 3ml was conducted. Each tube contains buffer and dye in control and buffer, dye and enzyme was taken. Optimal temperature, pH and dye concentration was kept constant. All the set was performed in duplicates. Decolorations at different enzyme quantity were examined.

Effect of time

Decoloration rate was studied at the time interval of 30 min, 1hr. 1.5hr and 2hrs. Each tube contains buffer and dye in control and buffer, dye and enzyme was taken. Optimal temperature, pH dye concentration and enzyme quantity was kept constant. All the set was performed in duplicates. Decolorations at different time interval were examined.

Toxicity study

Phytotoxicity studies were carried out with 1gms/L of each dye and its extracted metabolites using seeds of *Triticum aestivum*, and *Lens esculenta*. The seeds were surface sterilized with 1.2% sodium hypochlorite solution to discourage fungal growth. Five seeds of each plant species were placed in each pot and watered separately with 5ml samples of each dye and its degradation product per day.. The glass were kept in the dark and observed for germination. Seeds with radical (>1mm) were considered germinated (Chimezie and Thomson, 2011). The germinated seeds were then exposed to day and night cycle length of 10/14 h, respectively. The length of plumule (shoot) and radical (root), and the germination rate (%) were recorded after 7 days.

Results and Discussion

Buffers play a very important role in the activity of the enzyme. In the present study, among the three buffers solutions used for the four dyes, congo red showed a maximum decoloration with citrate buffer with 27.73%. In case of Brilliant blue maximum degradation was seen with acetate buffer. On the other hand, Crystal violet did not showed decoloration with any of the used buffers but Malachite green gave maximum degradation with phosphate buffer (Fig. 1). There were several reports on degradation of malachite green dyes and congo red using enzymes from fungal cultures and the discoloration was in the range of 80-90% (Elias Abdulla et al., 2000;

Daneshvar. 2005; Poonam Dayramam and Debjani, 2008; Hazrat et al., 2009; Saranraj et al., 2010).

As different part of *Aloe* plant has different composition, it showed different degree of decoloration. Congo red showed a maximum decoloration with skin extracts of *Aloe vera* and decoloration was up to 30% whereas Malachite green decoloration was maximum with pulp extract of *Aloe barbadensis* (Fig. 2). Vasanthy et al. (2011) showed phytodegradation of textile dyes by water hyacinth from aqueous dye solution. The dyes were Red RB and Black B. Batch type experiment were done using the hydrophytes and its dye removal capacity was studied. The used material was subjected to GC-MS analysis for phytochemical analysis. The experiment has proved the efficiency of water hyacinth to remove the colour and degrade the dye by about 95% -Red RB, Black B-99.5. The phytochemical component analysis indicates the increased production of hexadeconoic acid, which may be a promising result, the reduction in phytol content was recorded which showed significant reduction in the chlorophyll content.

The effect of pH on degradation and decoloration of various dyes by aloe extract was given in figure 3. For malachite green a maximum degradation was found at pH 8 up to 32%, whereas for congo red it was at pH 6 with 24% degradation. Daneshvar et al. (2005) in his paper showed the decolorization of triphenymethane dye, malachite green by *Cosmarium* species which dependent on specific pH on decolorization rate and dye concentration as it followed Michaelis-Menten model. On the other hand a pH of 8.5 removed 99% of malachite green dye kinetic model (Anabia and Ghaffari, 2011).

In the present study, malachite green exhibited a maximum decoloration at 50˚C but it was found to be less than the control as showed in figure 4. Hence a negative value was observed. At Malachite green and congo red showed a maximum decoloration at 30˚C and 40˚C respectively. This may be attributed to the enzyme activity which may be very less.

Dyes of different concentration were tested with the corresponding enzyme. Dye concentration from 0.1gms to 1gms /100ml of water was taken. In case of malachite green maximum degree was found at 0.1% dye concentration up to 78%. But decoloration was found up to 1% also. In case of congo red decoloration was seen up to 0.7%, but maximum decoloration was observed at 0.1 % (Fig. 5). In the earlier studies by Kavita Vasdev (2011) using white rot fungi 6gms/l of malachite green was decolorized. But in the present investigation, in the presence of enzyme 10gms/l of dye was degraded.

The studies on the quantity of enzymes, malachite green exhibited a maximum decoloration at 2.5ml of enzyme, whereas for congo red maximum decoloration was seen at 3ml up to 95.22% and 27% respectively (Fig. 6).

Malachite green showed a maximum degradation with 30 minutes of incubation whereas congo red showed maximum decoloration in 2hrs as represented in figure 7. This result was comparable to the result of Chimezie and Thomson (2011), where they achieved the decolorization of triarylmethane dyes using *Aeromonas hydrophila* within 24hrs with color removal in the range of 72% to 96%.

Poonam Dayaram and Debjani (2008) identified *Polyporous subidus* and isolated its enzyme and used for decoloration of Reactive Blue, Reactive Orange, Ramazol Black and Congo red. Treatment of effluents was done in a laboratory scale bioreactor constructed with laccase immobilized Na alginate beads. More than 80% dye was degraded within 5 days under stationary incubation condition. 17.1U of enzyme activity was found maximum after 3 days. Kavita Vasdev (2011) in her work showed the ability of six white rot fungi to decolorize three Triphenyl methane dyes (crystal violet, Bromophenol blue and Malachite green). All six-fungi showed high decolorization capacity and were able to decolorize all three dyes within 72 hours. All the six fungal strains not only decolorized the dyes, but also showed varied levels of laccase production during decolorization. Their growth was not affected much by presence of dyes in the medium. Three of these fungi were found to have capacity to decolorize as high as 6g/L concentration of these dyes. Out of the three dyes tested, malachite green was decolorized fastest. With the addition of sucrose and peptone to PDA enhanced the rate of decolorization of congo red by *Mucor mucedo* by taking 3 days compared to 8 days in PDA itself (Gupta and Manisha, 2012). There are other reports supports our results (Ganesh et al., 2006; Liu et al., 2006).

Hence when all the optimum conditions were given it was seen that *Aloe vera* has the ability to decolorize and degrade the triphenylmethane dye of concentration up to 10gms/L almost up to >96%.

The disposal of untreated and treated textile dye wastewater on land may have a direct impact on soil fertility and by extension agricultural productivity. Thus, it was pertinent to assess the phytotoxicity of the dyes before and after degradation as environmental safety demands both pollutant removal and their detoxification. Phytotoxicity studies were carried out by evaluating the relative sensitivities of *Triticum aestivum*, toward the dyes and their degradation products using seed germination and plant growth assays. Germination (%) of plant seeds was less with the raw dye treatment when compared to the treatment with degradation metabolites (Table. 1). The lengths of shoot and root were also significantly affected by the two dyes than by their degradation metabolites, indicating less toxic nature of degradation metabolites as compared to dyes. Amongst the dyes, treatments with Malachite green good results were seen with malachite green. Toxicity exerted by the treated samples was generally lower than that obtained for the untreated samples. Pallavi Chaube et al. (2010) and Chimezie and Thomson (2011) made similar observations on effect of dyes and their degraded products on seed germination and seedling growth..

Before now, most of the decolorization projects have concentrated mainly on color removal while neglecting the fact that sometimes biological processes are connected with formation of toxic intermediates. Hence, it is required that the evaluation of decolorization effect be carried out with relation to ecotoxicity assessment. In this study, phytotoxicity studies have revealed that the biodegradation of triarylmethane dyes by *Aloe barbadensis* resulted in their detoxification and generation of nontoxic

Table.1 Effect of dyes on the germination, shoot length and root length of *Triticum aestivum*

Test parameter	control	malachite green		congo red	
		dye	treated	dye	treated
Germination (%)	100	58	90	65	87
Shoot length (cm)	12.4	7.5	10.5	7	11
root length (cm)	10.8	6.1	9.2	5.8	9.6

Fig.1 Effect of buffers on decoloration

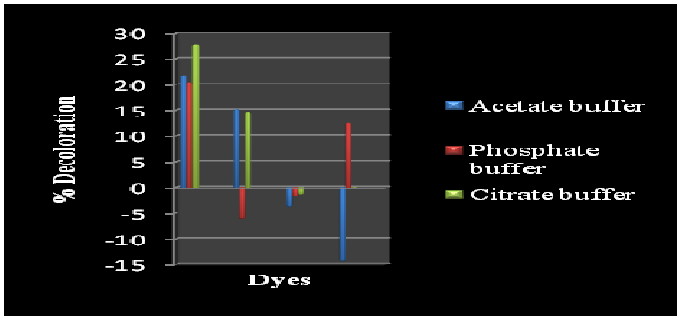


Fig.2 Effect of plant extract on decoloration

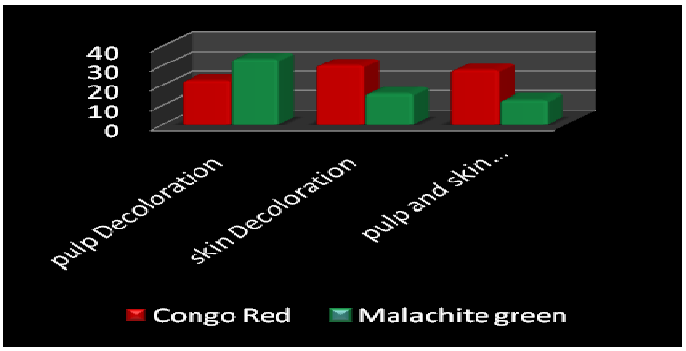


Fig.3 Effect of pH on decoloration

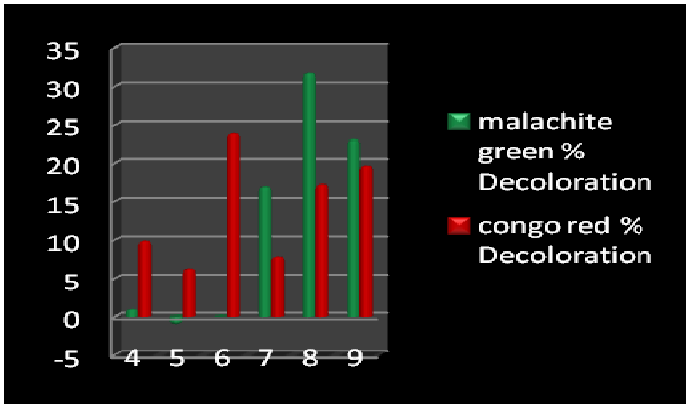


Fig.4 Effect of temperature on decoloration

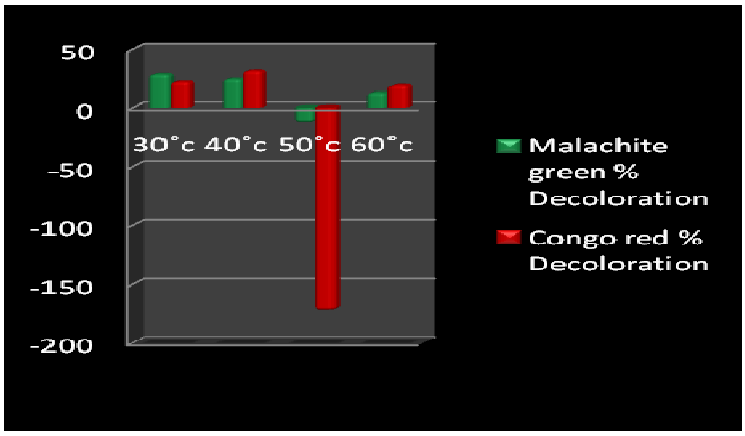


Fig.6 Effect of enzyme quantity on decoloration

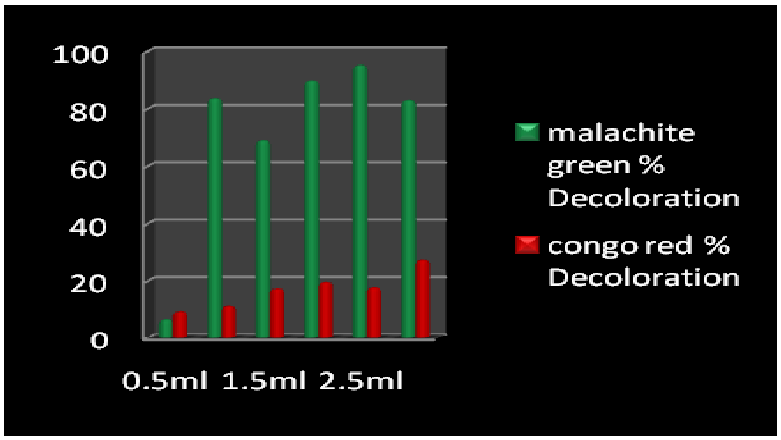
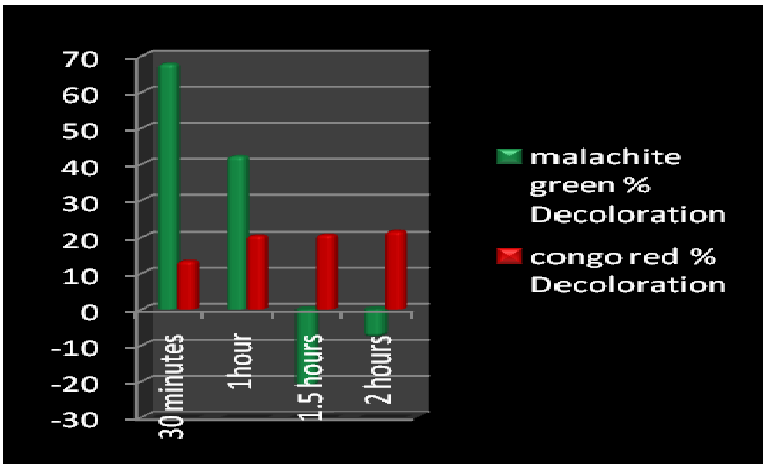


Fig.7 Effect of time on decoloration



metabolites thus suggesting biotreated dye wastewater can be used for irrigation.

Application of traditional waste water treatment requires enormous cost and continuous input of chemicals which becomes uneconomical and causes further environmental damage. Hence, economical and eco-friendly techniques using plant extracts can be applied for fine tuning of waste water treatment. Biotreatment offers easy, cheaper and effective alternative for colour removal of textile dyes. Thus, by this present study I concluded that the *Aloe barbadensis* extracts proved very well in the decoloration ability to decolorize/degrade Malachite green and Congo red and can be used in waste water treatment.

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