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Decolorization of mono azo dye methyl orange with Epilithon biofilm: effects of physico-chemical parameters

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Abstract

Azo dyes are one of the most widely used class of dyes in different industries. These dyes are generally recalcitrant under natural aquatic and conventional wastewater treatment systems. The study is innovative in demonstrating the role of aquatic biofilm, epilithon, in removal of an azo dye, methyl orange, under varying environmental conditions in a laboratory set up. Epilithon biofilm was collected by peeling it from the surface of submerged rocks in Xuan Wu Lake, Nanjing, China. The biofilm was immobilized on the surface of specific bio-carriers (AAM carriers). The attached biofilms showed 50->99.9% removal of dye at a concentration of 25-500 mg L^{-1} in 24-172 hrs. A maximum dye removal rate was observed at pH 7 using 0.8 mg L^{-1} biomass under mesophilic temperature (30 ˚C).

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Introduction

Industrial effluents are comprised of different kinds of contaminants and color is the most visible contaminant that can be detected with naked eye even in small amount. Colored industrial effluents are mainly comprised of synthetic dyes that are widely implemented in manufacturing various products such as paper, leather, food, textile etc. (Vieira, 2011). Overall, textile industry is the principal consumer of synthetic dyes around the world, consuming 10,000 tonnes of synthetic dyes per year. According to a rough estimate, textile industries annually dump 100 tonnes of dye wastewater into aquatic ecosystems (Yagub, 2012).

Structurally, synthetic dyes are divided into various types including acid, bas, disperse, azo, anthraquinone dyes. Among these groups, azo dyes comprised of 60% of the synthetic dyes and are most widely implemented (Fu, 2001) compared to other groups of dyes. Azo dyes can be characterized by their specific structure with one or more azo bonds (–N=N–) with sulphonic acid ($–SO_3$) substituents (Gupta, 2009). The aromatic structure of these dyes makes them resistant to biological degradation (Mahvi, 2009) resulting in accumulation of these noxious compounds in food chain. The persistence of these dyes in aquatic environments and food chains may result in drastic effects on the flora and fauna of aquatic environments. Methyl orange (MO) is an anionic dye that is widely implemented in many industries including textile, paper, printing, pharmaceutical and food industries and for research purposes (Mittal, 2007). MO is a mono azo dye and its specific mono azo structure along with sulphonic acid group gives it a characteristic color along with enhancing its water solubility (Han, 2015). Exposure to it can lead to allergy, and it is poisonous if swallowed (Chen, 2011). Therefore its removal from textile waste water is vital due to its toxicity.

A number of methods have been employed for the treatment of dye-contaminated industrial waste water. These methods include physico-chemical methods such as oxidation (El-Ashtoukhy, 2010), adsorption (Yagub, 2014), Ozonation (Dan, 2013), and photo-oxidation (Sohrabi, 2014). These methods are expensive, not efficient, and also produce secondary products such as sludge that needs further treatment (El-Desoky, 2010). Biological methods have proven to be more efficient and are preferred over other methods due to their low cost and environment-friendly nature. However, there are certain shortcomings in the biological methods; they require maintenance, produce large amounts of sludge and sometimes require an additional energy source (Liehr, 2004). Therefore, innovation and further improvement in biological systems are needed in order to get better efficiency and sustainability.

Epilithon is a type of periphyton biofilm that attaches to submerged rocks in aquatic environments. Epilithon biofilms are composed principally of algae, bacteria, protozoa and other smaller invertebrates (Cejudo, 2014) that are embedded in extracellular polymeric substance (EPS) including proteins, organic acids and minerals (Azim, 2005). The abundance and structure of these biofilms are dependent on the physico-chemical and environmental conditions (Sabater, 1998; Hillebrand, 2000). These factors also influence periphyton biofilms during their complex responses (Villeneuve, 2010).

Immobilization of microorganisms is a new and promising method to obtain larger amounts of active biomass by attachment to different physical and/or biological carriers. This technique provides a solution to overcome the problem of solid-liquid separation in microbial treatment systems. Furthermore, using immobilized cells is advantageous because attached biofilms are more resistant to environmental perturbations (Cheng *et al.*, 2012).

The aim of the study is to evaluate the ability of immobilized epilithon biofilms to remove of an azo dye methyl orange (MO) from a simulated wastewater. The effect of various environmental conditions (pH, temperature, initial dye concentration and initial biomass concentration) on

dye removal rate was also estimated. To date, no study has been reported on the role of immobilized epilithon biofilm in the removal of azo dyes.

Material and methods

Analytical grade chemicals were purchased from Sinopharm Chemical Reagents, Shanghai. Stock solution (500 mg L^{-1}) of methyl orange was prepared by dissolving the requisite quantity in deionized water, filter sterilizing and storing in the dark at 4˚C. This stock was diluted to make a range of concentrations (25, 50, 100 and 200 mg L^{-1}) used in the experiments.

Biofilm Collection and Identification

The epilithon biofilm was isolated by scraping submerged rocks from Xuan Wu Lake, China by using standard operating conditions. The biofilm was stored at 4 ˚C for transportation to the laboratory. The collected biofilm samples were grown in larger tanks, containing specific periphyton biocarriers, *viz.* artificial aquatic mats (AAM, cylinders, diameter 1 cm and length 9 cm). The tanks were charged with Woods-Hole Medium (WC) media to simulate natural waters. The WC media is composed of major minerals ($NaNO_3$, $CaCl_2{\cdot}H_2O$, $MgSO_4{\cdot}7H_2O$, $NaHCO_3$, $Na_2SiO_3{\cdot}9H_2O$, K_2HPO_4 and H_3BO_3) and trace elements/vitamins ($Na_2EDTA{\cdot}2H_2O$, $FeCl_3{\cdot}6H_2O$, $CuSO_4{\cdot}5H_2O$, $ZnSO4{\cdot}7H_2O$, $CoCl_2{\cdot}6H_2O$, $MnCl_2{\cdot}4H_2O$, $Na_2MoO_4{\cdot}2H_2O$, Na_3VO_4, VitaminB_{12}, Thiamine, and Biotin).

The tanks were placed in a greenhouse to minimize the effect of external environmental factors and allow for control. The biomass used for the experiments was isolated after 60 days growth, when the epilithon biofilm appeared to have evenly covered the biocarriers. Epilithon biofilm was scratched from the surface of the biocarriers with a sterilized knife and was examined under phase contrast microscopy to characterize the dominant microbial community. In order to determine the growth and amount of epilithon biomass grown on the AAM biocarriers, samples were collected from the tank each week for 6 weeks. The biocarriers were scraped with a sterilized razor and the biomass obtained was filtered through 1.2-µm Whatman GF/C fiberglass filters and the deposits on filter were used to determine the chlorophyll-a (Chl-a) content and Ash Free Dry Weight (AFDW) (APHA, 2005).

Decolorization Experiment

The decolorization experiment was conducted by introducing epilithon biofilm into 150 mL of MO solution (pH 7, 0.4 g biomass and 25 mg L^{-1} MO) under static conditions. These conditions were selected on the basis of growth condition of epilithon biofilms i.e. pH 7 and 30 ˚C. It was determined that each piece of AAM was home to 0.1 g of biofilm , therefore, 0.4 g epilithon was grown on total four pieces of AAM biocarrier that were added during screening and in the following experiment accordingly. The experimental set up was placed in a dark incubator at 30 ˚C to avoid photo-degradation. The control flask was filled with 150 mL MO solution but was not inoculated with biofilm. From the experimental samples, aliquots of 5mL were withdrawn at specific time intervals (after every 12 hrs) until complete decolorization was observed by naked eye. The aliquots were centrifuged at 4200 rpm for 15 minutes at 4 ˚C to separate the biofilm biomass from the solution. The cleared supernatants were analyzed to determine the decolorization of MO at λ_{max} = 484 nm (Shimadzu, UV2450, Japan).The experiment was performed in triplicate. The decolorization capability of epilithon was expressed in terms of removal rate (%) and was determined by following equation.

$$Removal\ rate\ (\%) = \frac{MO_f - MO_i}{MO_i} \times 100$$

where MO_f and MO_i were the initial and final absorbances of MO solutions as observed by spectrophotometer.

Effect of physico-chemical conditions on MO removal

The dye removal in can be affected by many physical and physiological parameters. Therefore, a traditional approach to optimizing the parameters was adopted by changing one parameter at a time. The parameters chosen, were; temperature, pH, initial biomass of

periphytic biofilm and initial concentration of dye. All the experiments were performed in triplicate, with control flasks having no biofilm.

Effect of temperature

In order to examine the effect of temperature, the decolorization of dye was observed at different temperatures (10, 15, 20, 25, 30, 35, 40 and 45 ˚C). Each flask was charged with 100 mL of dye (25 mg L^{-1}), inoculated with epilithon biofilm and incubated for 7 days at the specified temperatures. Dye decolorization was monitored every 24 hrs.

Effect of pH

Batch experiments were performed to find the optimum pH required for the decolorization of MO by epilithon. The experiment was performed at pH 3, 5, 7, 9 and 11 in individual sets of flasks containing 150 mL of MO (25 mg L^{-1}). The pH of the experimental solution was adjusted using 0.1M HCl (aq) and 0.1M NaOH (aq). The flasks were inoculated with 0.4 g epilithon and incubated at 30 ˚C for 7 days. Abiotic control flasks (without biofilm) were prepared with each experimental set of flasks. The samples were withdrawn at specific time intervals (after every 24 hrs) and the decolorization was determined by observing change in absorbance as described above.

Effect of initial biomass dosage

The degradation capability of epilithon was also evaluated at different amounts of epilithon biomass (0 g L^{-1}, 0.1 g L^{-1}, 0.2 g L^{-1}, 0.4 g L^{-1}, 0.8 g L^{-1} and 1.2g L^{-1}). The extent of decolorization was determined by withdrawing 5 mL aliquots at regular intervals and processing them in the way described above. All experiments were performed in triplicate and the mean results are shown with standard deviations (±SD).

Effect of initial concentration of dye

The effect of initial concentration was evaluated by exposing epilithon to different initial concentrations of MO (25 mg L^{-1}, 50 mg L^{-1}, 100 mg L^{-1}, 200 mg L^{-1} and 500 mg L^{-1}). The experiment was conducted in 250 mL flasks, inoculated with 0.4 g epilithon and incubated for 84 hrs under optimized conditions of pH and temperature. Aliquots were withdrawn every 12 h and were analyzed in the way described above.

Results and discussion

In this short report we are not concerned with the mechanism of decolorization of MO. Rather, we are concerned primarily whether the epilithon biofilm can function as an efficient removal agent of an example azo dye from a simulated industrial wastewater. Only brief comments relating to the mechanism of dye removal are presented here.

Epilithon biofilm

Epilithon biofilm covering the biocarriers uniformly (Fig. 1a) was observed to be dominated by an autotrophic microbial community consisting mainly of green algae and cyanobacteria together with unicellular diatoms as observed by phase contrast microscopy (Fig. 1b and 1c). The biomass abundance was increased with the passage of time. Biomass of the epilithon was determined in terms of chlorophyll-a (Chl-a) and AFDW contents. The contents of the epilithon varied significantly ($p < 0.005$) with time.

During the period of study, the epilithon biomass varied greatly, as determined by the two measures chosen. Chl-a increased from 17.6 to 990.03 mg m^{-2}. This 56-fold increase reflects the growth of the photosynthetic organisms, while for AFDW, the change from 1.14 to 3.04 mg m^{-2}, suggests a 2.7-fold increase in the bulk of the diatoms, with the dry weight remaining after combustion being due principally to their siliceous skeletal remains (Fig. 1d). The combination plot shows that the two parameters parallel each other in terms of growth. It reflects a healthy community.

Screening of Epilithon for MO removal

Epilithon biofilm is comprised of a complex community of microorganisms and has been reported as an important bioindicator of primary producers in aquatic environments. Therefore, their role in the transformation of pollutants is well understood with reference to pesticides, pharmaceuticals and nutrients

(Tlili, 2011; Du, 2015; Lu, 2014; Lu, 2014). There are also some previously reported studies for the decolorization of MO by individual microbial species (Mnif, 2016; Parshetti, 2010; Shah, 2013; Seyis, 2008). However, the decolorization capability of MO by an epilithon community is reported for the first time in this study. The azo dye, MO used in this study, might have acted as an electron donor in this enzymatic system.

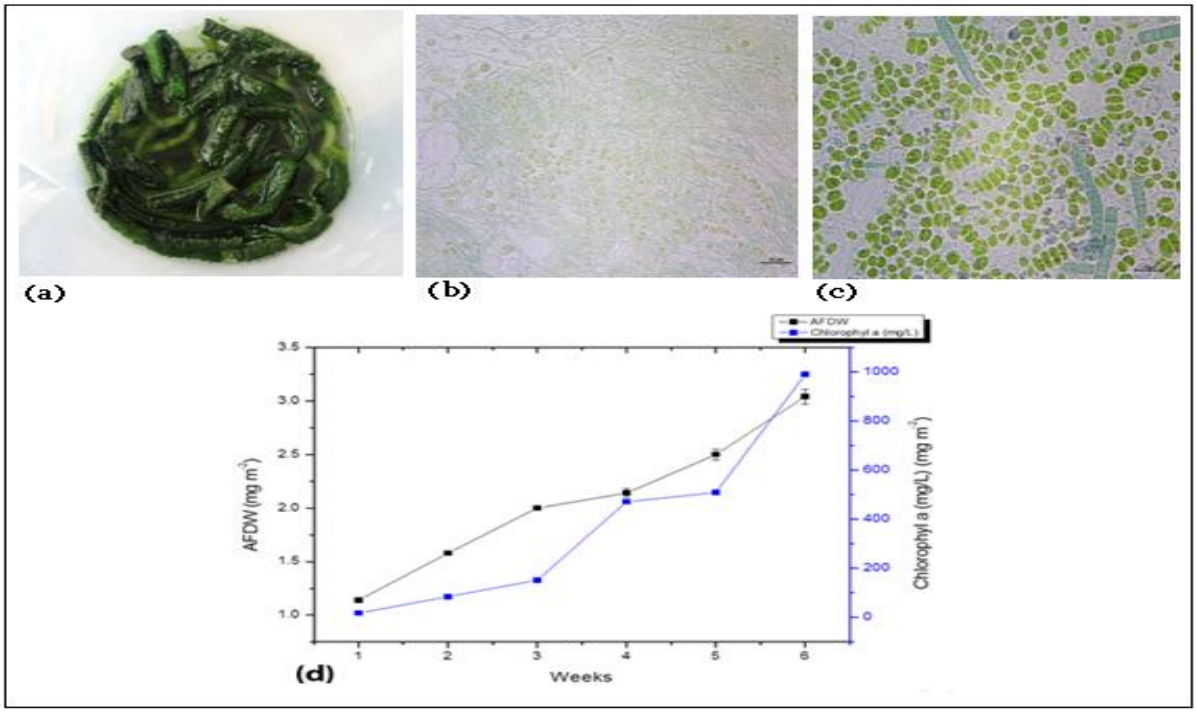

Fig. 1. Immobilization of epilithon on AAM biocarriers (a); Phase contrast microscopy of epilithon (b and c) and comparison of Chl-a and AFDW of biofilm during 6 weeks of growth (d).

Effect of different environmental conditions on MO Removal

Results show a significant effect for the different parameters on removal of MO. However, the pH and temperature are more vital to determine the effect of environmental parameters in the removal of MO lead by initial dye and biomass concentration.

Temperature

The biofilm did not show any thermal inactivation at higher or lower temperature ranges, therefore, epilithon are potentially effective for removal of azo dyes over a wide range of temperatures. Generally mesophilic conditions supported the removal dyes with 30 ˚C proving to be the optimum temperature. High temperature might have escalated the mobility of the dye ions giving them sub-adequate conditions to interact with the active sites on the surface of the epilithon biofilm and hence result in lower dye-removal efficiency. The percentage dye decolorization observed for MO was 82.2 % for at 30 °C (Fig. 3). Clearly the temperatures between 25 and 35 °C were the most efficient.

Effect of initial pH

It is already reported that pH can affect the transport of dye across the membrane (ref??). Therefore it is important to determine the effect of pH on the removal capability of epilithon for MO by exposing it to different pH values (Fig. 4). The optimal pH for the complete removal of MO within 7 days was close to neutral. Not surprisingly, pH 7 also proved to be the optimum pH for the growth of epilithon biofilms. Therefore, dye decolorization was highest at this value due to higher metabolic activity. These results were consistent with previous studies where pH 7 was found to be optimum for removal of MO (Thao, 2013; Mnif, 2016).

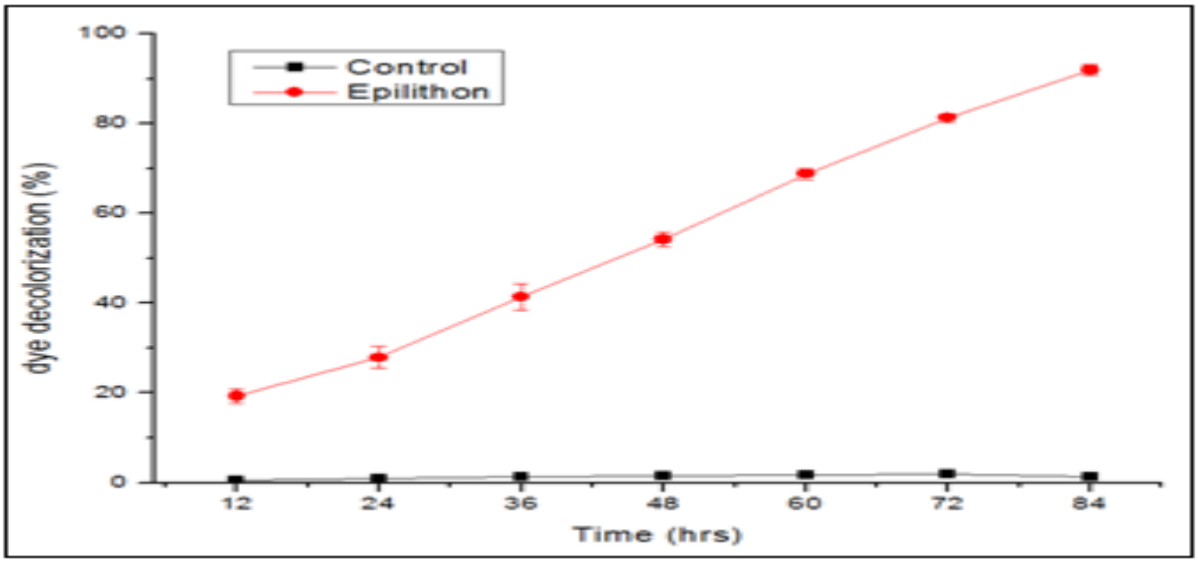

Fig. 2. Decolorization of MO by epilithon under optimal conditions (pH = 7 and t = 30 °C).

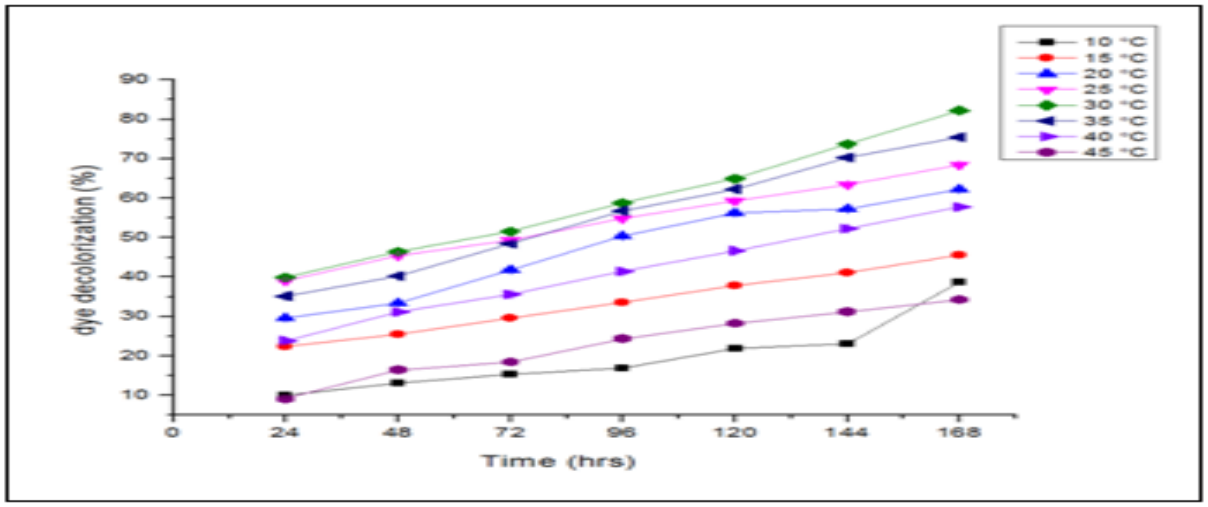

Fig. 3. Effect of different temperatures on the removal of MO.

Additionally, MO is an anionic dye; therefore at higher pH conditions the rate of removal of MO is very low due to competition between the dye and hydroxyl ions and vice versa (Alqaragully, 2014).

Initial biomass concentration

Generally, greater removal of any contaminant is linked with the density and diversity of living organisms in aquatic environment. Similar pattern of dye removal was observed when biomass concentration was increased. The removal rate of MO significantly varied with an increase in biomass from 0.2-1.2 g L^{-1} in distinct sets of experiments. It was found that MO 0.8 g/L biomass was sufficient to completely decolorize 100 mg L^{-1} of dye within 120 hrs (Fig. 5). A higher amount of biomass gives an increased surface area and hence the number of active sites on biofilm for the removal and biodegradation of MO. It was observed that 0.2 g L^{-1} biomass showed just 31.98% decolorization after 24 h, whereas 0.8 g L^{-1} biomass shows ~59% decolorization after 24 h. The results differed non-significantly after 120 h for both 0.8 and 1.2 g L^{-1} biomass showing 98 and 99.9% decolorization of MO, respectively, suggesting that there is no need to further increase the biomass.

Effect of Initial concentration of MO

The rate of removal of dye is also dependent on the concentration of the dye (Chen, 2015); therefore the dye removal capability of epilithon was tested at

different concentrations ranging from 50-500 mg L^{-1} (Fig. 6). The rate of removal was faster compared to previous results because all the parameters had been optimized before this experiment. Initially, the rate of removal of dye was rapid with a gradual diminution in the process until the equilibrium conditions were attained as observed by kinetic (data not shown here). Almost all the concentrations of dyes were completely removed within 84 hrs of treatment by epilithon biofilms. However, increasing the initial concentration of MO increases the time for complete decolorization to occur.

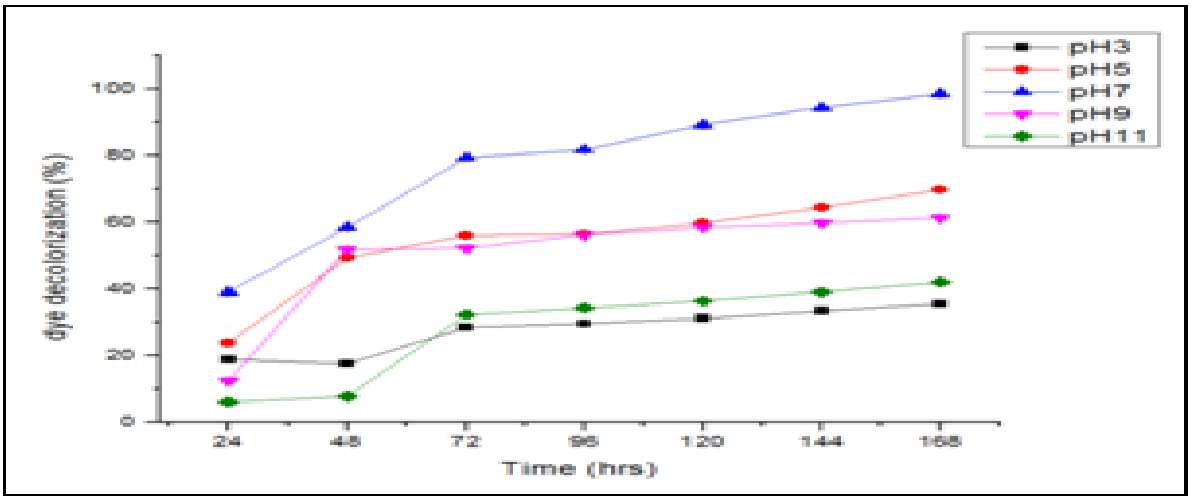

Fig. 4. Effect of various pH on the decolorization of MO.

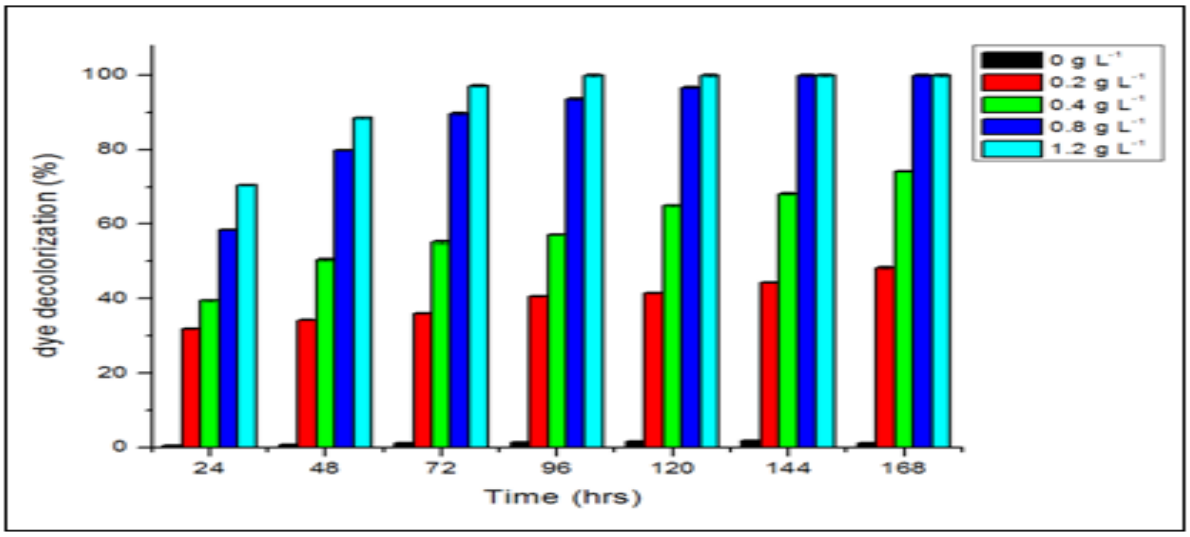

Fig. 5. Effect of different biomass on the removal of MO.

It was observed that for the epilithon 0.8 mg L^{-1} biomass samples, the MO (25 mg L^{-1}) was completely removed in 36 h, whereas only ~ 69% removal was observed for the 500 mg L^{-1} samples in the same time. Similar results have been reported in previous studies (Chen, 2015; Aracagök, 2013; Saratale, 2006).

The authors reported reductions in the removal rates with increases in initial concentrations of the dyes. The initially higher rates of removal at higher concentrations are suggestive of diffusion-controlled and/or first order kinetics because of the higher driving force due to the concentration gradient created by the higher initial concentrations of the dyes (Ezechi, 2015). This driving force compels the dye molecules to the micropores on the surface of the biofilm resulting in a transient higher removal of MO.

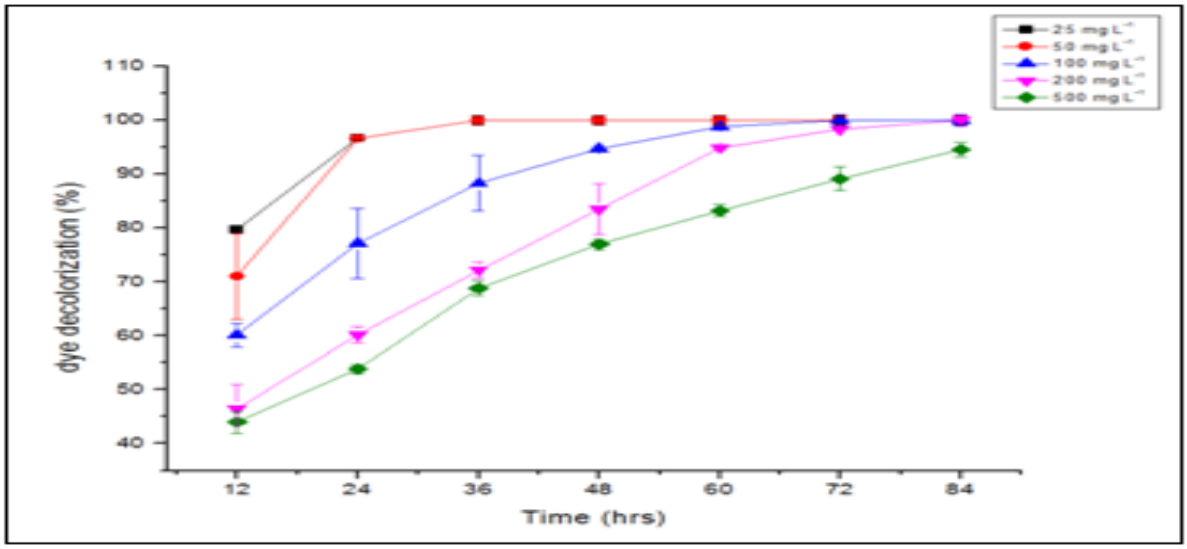

Fig. 6. Effect of different concentrations on the decolorization of MO.

Conclusion

This is the first report highlighting the importance of immobilized epilithon biofilms in the removal of the azo dye methyl orange under laboratory scale conditions. Overall, dye (25-500 mg L^{-1}) removal varied from 50-95% within 48 h under mesophilic conditions (at pH 7 and 30 ˚C). Further studies quantifying the kinetics and mechanisms for a range of industrially important dyes have been completed and are being analyzed (unpublished results). Additionally, the roles of specific organisms and their application at pilot scale for the removal of recalcitrant aromatic compounds need to be carried out.

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