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Simultaneous Decolorization of Dye Contaminated Wastewater and Energy Production Using Algae

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Abstract

Use of dyes is increasing day by day due to its multiple applications in different industries. The result is dye contaminated rich colored water, which not only produces aesthetically unpleasing effect but also creates serious environmental concerns. In present study, competent algae were isolated from wastewater streams and were tested for their efficiency to remove color from the dye contaminated wastewater. It was observed that three strains were capable of removing color from the wastewater. Among these three strains two strains were found more effective, therefore wastewater treatment experiment was conducted by the strain ADS-1 and ADS-2. These potent strains decolorized water up to 100% in 4-5 days, and complete decolorizationwas observed in 15 days. The algal biomass that was produced was further extracted using chloroform: methanol extraction and was analyzed for its biofuel potential using GC-Ms analysis. It was observed that many significant bio-products like kerosene, Paraffin, waxes blends were produced by the algae during course of wastewater decolorization. Such type of treatment options provides dual benefits of wastewater treatment and bioenergy production. Therefore, it can be opted for the development of efficient technology to ensure environmental protection of pollution reduction.

Keywords: Fruit and Vegetable Waste, Enzyme Ionic Plasma, Soil Salinity, Soil Quality, Plant Growth.

Introduction

Environmental pollution and specifically the water pollution is becoming serious concern these days. Human activities such as industries and domestic uses are the main source of contaminant in the natural water resources such as lakes, rivers,oceans and groundwater aquifers (Alseroury,2018). Wastewater is usually categorized based on its source and toxicity. Water pollution caused by the discharged of colored water from textile, tanneries, paint and varnishes and food industry creates serious environmental issues (Yamjala et al., 2016; Salima et al., 2013). The decline in the environmental quality indirectly causes humans to be constantly faced with the problems related to the polluted environment (Astiti Asih, et al.,2018). The waste water of industrial activities contains a variety of potentially toxic and environmentally harmful compounds (Fahdil,et al.2018). Such type of wastewater pervades the water resources, and apart from producing aesthetically unpleasing effect also affects the penetration of light in the water and therefore effecting photosynthesis and over all ecosystem productivity. The discharge of dye-contaminated wastewater into open water channels cause stern impairment to the fresh water resources and are very toxic to the aquatic flora and fauna (Khataee et al., 2013). Among various colored compounds azo dyes accounts for almost 40% of the total dye types (Meng et al., 2014).

As azo dyes pose serious threats to environmental quality and water ecosystem, therefore strict legislations are needed to be followed before the discharge of such contaminated water to the open water channels (Soon and Hameed, 2011). Thus, for the treatment of wastewater different physical, chemical, and biological options are opted to ensure water safety (Meerbergen et al 2018). Among different biological treatment processes, bioaccumulation and biosorption are known to be very effective as these tend to produce lesser toxic byproducts and are also economically more feasible (Khouni*et al*., 2013).

Biological entities like microbes, fungi, algae and plants can give a broad choice of options to reduce water pollution (El-Sheekh*et al*., 2009). Previous studies have documented a wide number of bacterial and fungal strains capable of decolorizing the azo dyes, however only few studies have pointed out the potential of algae for the bio-sorption of azo dyes. Algae usually have edge over other biological options as being autotrophic, they also ensure lesser CO_2 emissions (Raino*et al*., 2011). Although the presence of dyes in the water exhibit high toxicity to aquatic life but still there are few species that are capable to grow on dye contaminated wastewater and correspondingly also decolorize the dye contaminated waste water (Solis *et al*., 2012). Several species

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of algae have capacity to tolerate the dyes toxicity, even degrade the azo dye compounds. As algae are photosynthetic therefore they receive their energy from the sunlight, oxygen from the air and nutrients from the wastewater (Solis *et al*., 2012). During the proliferation of algae, it absorbs colored water and thus can potentially decolorize the dye contaminated wastewater, where the obtained biomass can be processed further for the production of bioenergy and algal based bio products.

Algae are autotrophic entities and thus don't requisite carbon sources, whereas other microbes like fungi and bacteria demand continuous supply of external carbon sources (Solis *et al*., 2012). Algae obtain energy from photosynthesis, air and nutrients and are more economical for large scale cultivation of algal biomass. The nutrients source and water prerequisite can be achieved from different wastewater like textile industry and thus make them effective bio-treatment of coloured effluents (Omar, 2008). Algae are capable of decolorizing colored wastewater through mechanisms of enzymatic pathways as well as adsorption on algal biomass. In degradation process it was found that an azo-reductase enzyme is involved in break of azo bond of dye (McMullan*et al*., 2010). Where in few cases oxidative enzymes also play their role in degradation (Solis *et al*., 2012).Clarens*et al.,* in 2014 found that *Chlamydomonasreinhardtii*, *Scenedesmusobliquus*, *Chlorella pyrenoidosa* and *C. vulgaris* have great efficiency for the nutrient removal form the wastewater and also produce efficient biomass for bio-energy. Both organic and inorganic quotas of wastewater can support as nutrient augmentation for algal biomass production (Devi e*t al.*, 2012).

Different algal strains have already established their efficacy in dye degradation potential (El-Sheekh*et al*., 2009). The azo dyes in wastewater do not ominously hinder the progression of certain algal species in spite of their harmfulness to fish. Where, few other algal strains are highly subtle to azo dyes in water (Novotny *et al*., 2006). Liu *et al*., 2013 found higher efficacy of *Shewanella* algae isolated form sea water as compared to bacteria in decolorizing azo dyes even under saline conditions.

However, algal growth is extremely subtle to some toxic substances (Raino*et al*., 2011). In microalgae cultivation on wastewater the key restraint in development is high content of NH_3 in wastewater. Similarly, the scarce amount of light also slows down the phosynthetic activity and limits the algal growth (Gonzalez *et al.*, 2008). Temperature is additional essential constraint disturbing algal biomass synthesis. For the optimal growth of algae temperature range of 15 to 30 ºCis found to be ideal. Lower temperatures may cause decrease in metabolic reaction rates whereas higher temperature could decrease the metabolic activities. Similarly, reduction in solubility of these gases solubility may be of serious concern for algal growth (Gonzalez-Fernandez *et al*., 2011).

The present study is designed in light of the fact that colored water is highly toxic to the different life forma therefore complete decolorization is essential to ensure water quality. In this regard Treatment of wastewater using biological treatment technology option, where the autotrophic nature of algae provides a competent option for the biosorption of azo dyes from dye contaminated wastewater to reduce the pollution load.

Materials and Method

Sampling Algal Strains

Algal strains were collected from the wastewater streams; each bulk biomass was collected from different waste streams (n=30). All the algal biomass was properly washed with distilled water and was stored at 4°C and was transported to the lab.

- *Preparation Algal Media*

Algal strains were enriched using modified liquid COMBO media. The COMBO media could be used for isolation of cyanobacteria, cryptophytes, green algae and diatoms form both brackish and saline water (Mutanda*et al*., 2011). For the separation of individual strain COMBO algal medium containing 2.0% agar was used. The detailed description of COMBO media is given in table 1.

Table 1: Algal COMBO media Recipe

Nutrient	Quantity (mM)
NaNO3	10
CaCl2•2H2O	2.5
MgSO4•7H2O	1.5
NaHCO3	1.5
Na2SiO3•9H2O	1
K2HPO4	0.5
H3BO3	3.9
KCl	1
Algal vitamins	Traces
Algal trace metals	Traces

Isolation and Screening of Dye degrading Algae

After 4 days these enriched and separated algal biomass were tested on dye containing media. For sanitization of algae from bacterial adulteration, the centrifuge and antibiotic treatment (James, 2012) was applied to the enriched cultures. Further, the pure colonies of algae were separated using streak plate methods. The screening of algae from purified colonies was performed using artificial textile wastewater prepared in the laboratory. For dye degrading strains total 12 strains were tested. The decolorizationwas tested on modified COMBO media. 1 mM of azo dyes were added to the media. Three efficient algal species were screened on the basis of dyes degradation (Riano et al., 2012).

Synthetic wastewater Preparation

For dye decolorizationstudies synthetic WW was prepared using Direct-blue dye. A stock solution of 1000ppm of direct blue dye was prepared and was diluted from 100ppm to 500 ppm. The three selected strains were used separately to check their effect on WW treatment.

Dye Degradation studies

Dye degradation studies were carried out on the synthetic wastewater. Two efficient algal strains capable of degrading the direct blue dye that is ADS-1 and ADS-2 were tested for further degradation studies. The studies were carried out for 15 days and dye degradation percentage was estimated using spectrophotometric absorbance at 700 nm.

- *Wastewater Analysis*

Wastewater was analyzed for its Physico-chemical properties. Estimated parameters include pH, EC (electrical conductivity) and total dissolved solids (TDS), using standard procedure of American Public Health Association (APHA, 2007).

Lipid Analysis

After 15 days the algal biomass was filtered from the waste water, dried and pressed. The extraction of lipids from the algal biomass was carried out using chloroform: methanol extraction using Bligh and Dyer method (1959). The extracted lipids were then analyzed using GC-Ms. The conditions of the GC-Ms were; Capillary column (50m, 0.321 D.) with helium as gas carrier at 0.7 psi.

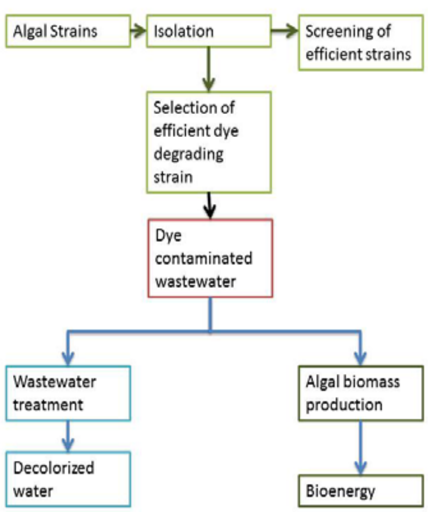

Figure 1: Complete layout of experimental setup

Results and Discussion

The results regarding decolorization efficiency of algae and production biofuel byproduct is described as follows.

Dye wastewater treatment efficiency

- *Removal direct black dyes*

The results regarding decolorization of direct blue dye contaminated wastewater using selected algal strains are described in Figure 2. As the time passed the algae achieved more efficiency in degradation of the dyes where algae ADS-1 degraded the dye as low as 6.2 % after 15 days using 200 mg/L of initial dye concentration (Figure 2A). As the concentration of dye increased the dye degradation efficiency decreased where at highest dye concentration (500 mg/L) the decolorization efficiency was lowest (78% dye remaining). In case of using second algal strain ADS-2 (Figure 2B), a slight lower efficiency of dye removal was found where at lowest dye concentration (200 mg/L) the highest dye removal lower the dye concentration to 16.2 % followed by 30.89% at 300 mg/L dye solution. Overall, both algal strains showed an efficient removal of dye thus can be used for treatment of dye contaminated wastewater. Reportedly microalgae are well-organized in removing nitrogen, phosphorus, and toxic metals from wastewater under controlled conditions. Therefore, if the key nutrients in the wastewater are used by the microalgae for growth, the nutrients can be removed from wastewater (Ting 2013). In the current study algae utilized the dyes in wastewater as their nutrient source, therefore the color of the contaminated water declined from 100 % to <10 %.

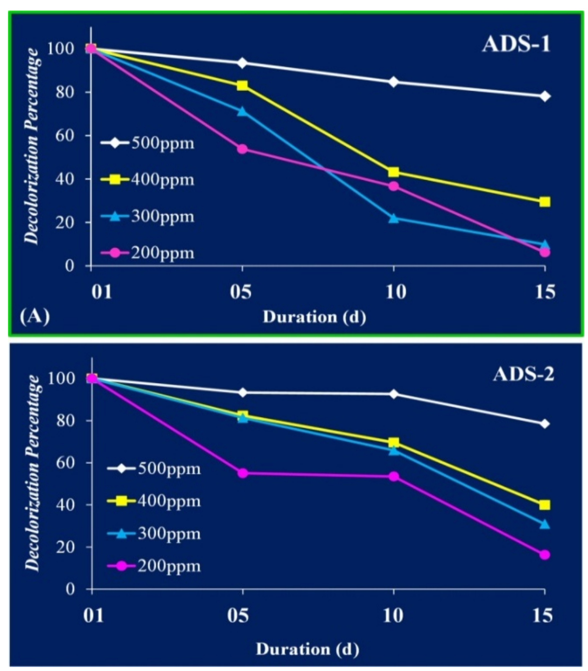

Figure 2: Decolorization efficiency of selected algal strain: (A) Decolorization with algae ADS-1, (B) Decolorization with algae ADS-2

- *Total Dissolved Solids (TDS)*

Total dissolved solid is another important parameter used to analyses the treatment efficiency of wastewater. The results explaining TDS concentration using two algal strains at various dye concentration are explicit in Figure 3. It can be seen that the as time passed both algal strains showed an increase in TDS concentration, however, the increasing comparison among the treatment is non-significant. In case of using Algal strain ADS-1 the highest increase in TDS content was observed in 500 mg/L dye solution after 15 days of algae cultivation. However, a slight lower value was found with other dye concentration solution (Figure 3A). The highest increase in TDS at 500 mg/L dye solution was due to the more available dye molecules to algae which are degraded into dissolved byproducts. On the other hand, the algae ADS-2 provided a greater increase in TDS content as compared to algae ADS-1 (Figure 3B). The highest increment in TDS was 103 mg/L form initial value of 35 mg/L. The following treatments showed an increase in TDS content as 94.1 mg/L.

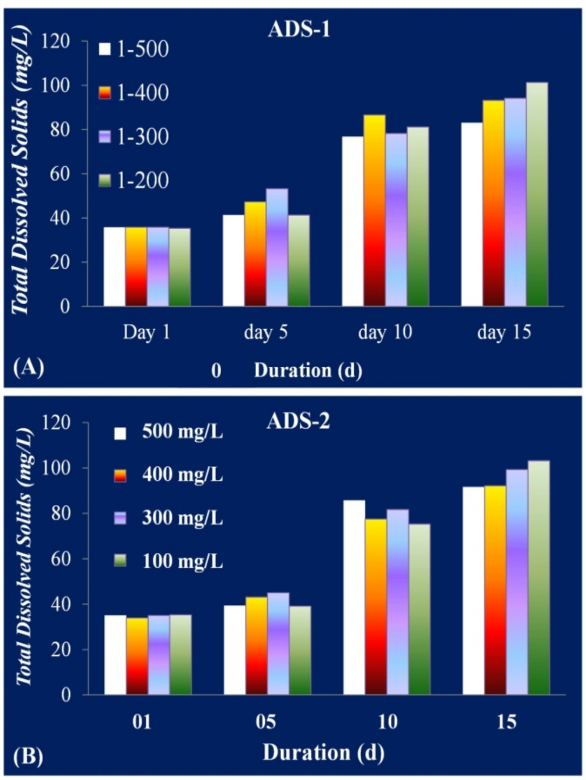

Figure 3: TDS concentration of dye in wastewater using selected algal strain: (A) TDS with algae ADS-1, (B) TDS with algae ADS-2

- *Electrical Conductivity (EC)*

Electric conductivity represents the total salts and ions concentration in the wastewater. The EC of the dye contaminated wastewater was analyzed at various intervals during algal removal of dyes from wastewater and the results are illustrated in Figure 4.

Using algal strain ADS-1 it was recorded that the EC values were increased under all dye solutions (Fig. 4A). The EC value was increased from 52.6 µS/cm to the maximum of 171 µS/cm after 15 days at 500 mg/L dye solution. The increase in EC values was due the production of various ions during degradation of dye under influence of algae cultivated under natural sunlight. Moreover, it is also due to total oxidation by aerobic process and decomposition of organic matter into simple nutrients (Iqbal and Mehta,1998). The algal strain ADS-2 showed the similar trend as observed in algal ADS-1 (Figure 4B). However, the greatest increase in EC (161 µS/cm) was attained by 400 mg/L dyes solution unlikely to ADS-1. The followed EC value was shown under 400 mg/L dye concentration where after 15 days of cultivation the EC value was increased up to 99 µS/cm.

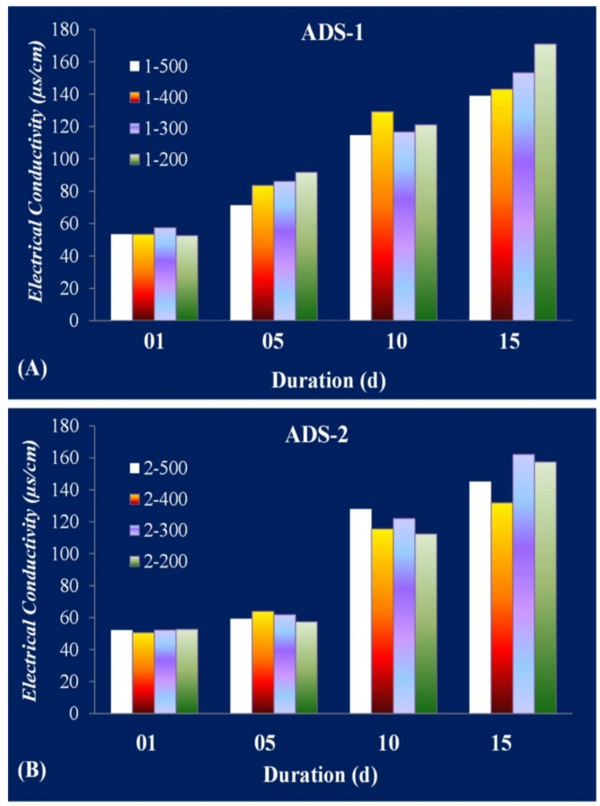

Figure 4: EC values in dye wastewater using selected algal strain: (A) EC with algae ADS-1, (B) EC with algae ADS-2

Algal biomass Analysis

- *Component of Algal lipids*

After treatment of dye contaminated wastewater the algal biomass of both strains was harvested and dried to form a algal biomass. The biomass was let to extraction for its identification of crude oil profile. The results are explained in Table 2. It can be observed that the high proportion of straight chain alkanes was found mostly in biomass of algal ADS-1 where no components were detected in case of ADS-2. The crude components were divided in to four fraction classed on the basis of alkane chain length as fuel oil fraction. Highest number of paraffin and wax components was found in ADS-1 which is followed by lubricant oil and kerosene oil. However, in case of ADS-2 only single component of fuel blended ethyl benzene was detected. Overall, the algal strain ADS-1 showed highest fuel conversion potential as compared to ADS-2 algae cultivated in dye contaminated wastewater.

- *Protein, Carbohydrate and Fats of algal biomass*

The organic composition of algal biomass was analyzed for protein, carbohydrate and fat content and results are explained in Figure 5. The ADS-1 showed comparatively higher content of carbohydrate (12.12%) and fat content (75.37 %) compared to that in ADS-2 where 8.33% of carbohydrates and 69.24% fats content were measured. The higher fat content of ADS-1 represents the more biofuel potential because the fatty compounds are the main components which act as fuel precursor. Overall, it can be found that both algal strains performed well in treating dye contaminated wastewater, however, the algal ADS-1 gain the promising potential to utilize for biofuel energy production.

Table 2: Algal crude oil profile using algal biomass extract.

Type of Algae	**S No.**	**Components of algal crude oil**			
		Paraffin and waxes >C20	**Lubricant Oil C15-C20**	**Kerosene (C4-C15)**	**Fuel Blend Ethylbenzene**
Algae ADS-1	1	Tritericontane ($C_{43}H_{88}$)	Heptadecane ($C_{17}H_{36}$)	Pentadecane ($C_{15}H_{32}$)	x
	2	Tritericontane ($C_{44}H_{90}$)	Hexadecane ($C_{16}H_{34}$)	Tetradecane ($C_{14}H_{30}$)	x
	3	Octacosane ($C_{28}H_{58}$)	Nonadecane ($C_{19}H_{40}$)	Decane, 4-ethyl-4-Ethyldecane ($C_{12}H_{26}$)	x
	4	Tetratetracontane ($C_{44}H_{90}$)	Eicosane ($C_{20}H_{42}$)	Tetradecane ($C_{14}H_{30}$)	x
	5	Pentatricontane ($C_{35}H_{72}$)	di-n-Decyl ether / 1-(Decycloxy) decane ($C_{20}H_{42}O$)		x
	6	Tetratriacontane ($C_{34}H_{70}$)			x
	7	1,2-Benzenedicarboxylic acid / diisooctyl ester ($C_{24}H_{38}O_4$)			x
	8	Tetratricontane ($C_{34}H_{90}$)			x
	10	1,2-Benzenedicarboxylic acid / diisooctyl ester ($C_{34}H_{58}O_4$)			x
Algae ADS-2	1	x	x	x	Ethylbenzene (C_8H_{10})

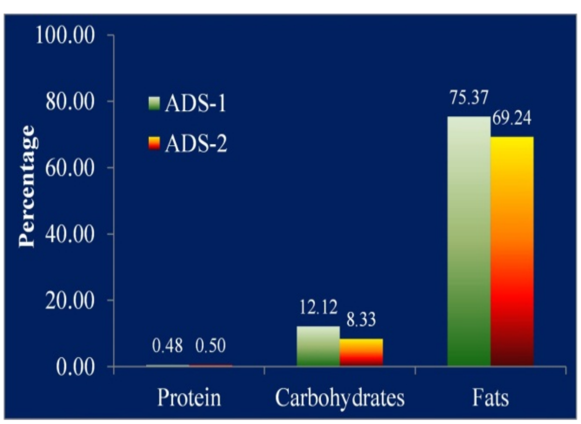

Figure 5: Carbohydrate, Protein and fat content of algal biomass strains

Conclusion

Form the findings of the present study following points are drawn.

- The highest decolorization of dye wastewater was achieved within fifteen days of algal cultivation especially for ADS-1 algae.
- The algae ADS-1 showed higher fat content in its biomass, thus having more biofuel potential for energy production.
- The algal based cured oil was found to be detected with efficient components of biofuel/ethanol precursor compounds.

Our overall study clearly shows that the treatment of dye contaminated wastewater through microalgae is a competent technology because of its secondary benefit of biofuel production. The technology is highly economical in terms of energy recovery and cultivation of algae under sunlight, thus no additional energy source is required.

Recommendations and Future Prospects

- The algae-based treatment of dye contaminated wastewater could be integrated in existing treatment plans in future.
- Field application of algal based technology should be conducted.
- Algal cultivation using wastewater should be optimized in order to get the maximum benefit of algae biofuel potential.

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