International Journal of Applied and
Natural Sciences (IJANS)
ISSN(P): 2319-4014; ISSN(E): 2319-4022
Vol. 7, Issue 5, Aug - Sep 2018; 67-84

International Academy of Science,
Engineering and Technology
Connecting Researchers; Nurturing Innovations

BIOREMEDIATION OF INDUSTRIAL EFFLUENTS WITH HEAVY METALS USING IMMOBILISED MICROALGAE

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ABSTRACT

The present investigation was carried out to treat industrial effluents containing heavy metals and other toxic compounds using freshwater lake isolated algae. In the current study, two freshwater algal species C. vulgaris and Scenedesmus abundances were immobilized by encapsulation with sodium alginate with a measured pore size. These immobilized algae were subjected to industrial effluents for bioremediation. Parameters like temperature pH turbidity nitrates sulphates and heavy metals such as iron aluminum and copper were determined initially at day zero and on the 30th day of inoculation. Artificial wastewater with known parameters was used as a standard. Among the species studied C. vulgaris possessed the greater affinity for adsorption resulting in the higher uptake. C. Vulgaris showed more positive values reducing the concentration of nitrates, sulphates, metals like iron, aluminum, and copper which were seen to be treated in both the water samples i.e. industrial effluent and artificial wastewater. Scenedesmus abundans also showed positive results but lesser when it is compared with C. vulgaris.

KEYWORDS: *Bioremediation, Encapsulation, Industrial Effluent*

Article History

Received: *20 Jul 2018* **|** ***Revised:*** *27 Jul 2018* **|** ***Accepted:*** *09 Aug 2018*

INTRODUCTION

In today's world, water scarcity is a global concern. Only about 0.75% of the Earth's water is directly available for human use, and about 70% of the World's freshwater withdrawals go to agriculture (Sato et al., 2013). In parallel, about 3 million tons of human wastes and other toxic substances are disposed of in water sources each day. While developed countries treat >70-80 % of their wastewater, developing countries treat only 18% to 28% and undeveloped treat only about 8% of their wastewater (Sato et al., 2013). Wastewater can originate from a combination of industrial, domestic, commercial or agricultural activities, surface runoff or storm-water, and from sewer inflow or infiltration (Tilley et al., 2010). There can be significant health hazards related to using untreated wastewater in agriculture. Wastewater from cities can contain a mixture of chemical and biological pollutants. In low-income countries, there are often high levels of pathogens from excreta, while in emerging nations, where industrial development is outpacing environmental regulations there are increasing risks from inorganic and organic chemicals. Therefore it is becoming mandatory to treat the wastewater before releasing it into other water bodies.

Aerobic biological and physical technologies are used for most wastewater treatment today in the developed world, but stricter environmental regulations are forcing existing facilities to move to advanced methods. In addition, high costs and greenhouse gas emission are leading many to explore more sustainable alternatives (Rittmann *et al.,* 2015). Also, the detection of new pollutants, stricter environmental regulations, and advancements in treatment technologies are driving improvements in bioprocesses for treating wastewater. Specifically, the special concern is being placed on phosphorus and nitrogen forms, which spur eutrophication of water bodies, and emerging micro-pollutants such as pharmaceuticals and person-care products. The interplay of physical, chemical and biological properties of water most often leads to the production of phytoplankton, while their assemblage (composition, distribution, diversity, and abundance) is also structured by these factors.

One such phytoplankton is the algae which are the indicator of the polluted water body. They can have much higher biomass production rates (Per unit land area) than higher plants, representing a valuable source for biofuel production (biodiesel, bioethanol, and bio-oil). Microalgae comprise a large group of autotrophic microorganisms accounting for >50,000 species living in diverse freshwater, brackish water, seawater and wastewater environments. Their cells are composed of proteins, carbohydrates, lipids, fatty acids, pigments, vitamins, and enzymes that can have value for human use. Phytoplankton constitutes the basic components of the aquatic food chain. Microalgae also can be used to produce food and health supplements, support aquaculture, and to achieve wastewater treatment (L. Lewis 2016). Microalgae's basic cultivation requirements are light, a carbon source, macronutrients such as N and P, and some trace metals. N is involved in amino acids, proteins, and chlorophyll production, while P is used for energy transfer, photosynthesis, and nucleic acids formation (Kan *et al.,* 2016). While light can come from the sun and carbon from atmospheric carbon dioxide (CO_2), N and P (and trace metals) have to be supplied with the cultivation medium. However, adding inorganic salts (fertilizers) does not represent an economically or environmentally sustainable option (Rawat *et al.,* 2011).

Phytoremediation refers to wastewater treatment by macro and microalgae for the removal of organic and/or inorganic pollutants (Olguín, 2003). Here, the microalgae- algae and cyan bacteria assimilate or disintegrate organic and inorganic compounds (Carbon, Nitrogen or Phosphorus), metals and emerging contaminants in wastewater. In addition, added values come when the microalgae are harvested to become feedstock for biofuels such as biogas. P and N in wastewater originate from human feces and urine, food, detergents, pesticides, pharmaceuticals, industrial inputs and agricultural drainage (Rittmann *et al.,* 2015). Traditional wastewater treatments are energy intensive. Moreover, conventional aerobic and anaerobic treatments do not remove P from the effluent, leading to eutrophication problems if water is not used for other purposes than agriculture. Besides N and P in wastewater, other compounds such as metals and organic contaminants must be removed during wastewater treatments. Microalgae may enhance the removal of specific metals and organic contaminants, some of them cataloged as Emerging Contaminants (EC) due to their eco-toxicological effects (Smith *et al.*, 2006).

Microalgae cultivation represents an option to recover different compounds from wastewater, while wastewater represents a continuous source of water and nutrients for algae biomass production. In addition, biomass produces can be used for beneficial purposes, such as bioenergy production or fertilizers. Although removal of nutrients and contaminants by microalgae species has been studied since 1957, microalgae cultivation in wastewater still faces scale-up challenges (Hoh *et al., 2016* and Watson *et al.,* 1953). In this review, one of the easiest and economical methods of wastewater treatment using microalgae has been described along with their advantages.

MATERIALS AND METHODS

Sample Collection

A sample was collected in 10-litre pet jar from an outlet source contain the high rate of copper and aluminum from forging industry. The water was used for electrolysis of copper and aluminum sheets. The sample hence is supposed to contain the high concentration of copper and other heavy metals thereby this water is regarded as polluted industrial effluent and may cause environmental hazards if let off in the environment. Hence in the recent investigation, this water has been taken for the treatment and Phytoremediation.

Cultures Used

Algal cultures *Chlorella vulgaris (AS-4)* and *Scenedesmus abundans (AS-9)* were obtained from sustainable development and biofuel laboratory, Department of Microbiology and Biotechnology, Karnataka University, Dharwad.

Physical Parameters

Physico-chemical analysis of the sample was done according to standard methods followed by APHA, (1995) and parameters were considered for testing the industrial effluent sample and artificial water. Temperature (surface water) was recorded on the spot using a Centigrade thermometer. The pH of the water samples was measured by using the gun pH meter on the spot and later conferred in the laboratory. The pH was determined by the electrometric method and was measured using pH meter (ELICO LI120 Type 003). Turbidity was determined by the nephelometric method as per APHA 19th Edition 1995.TDS was determined by the Gravimetric method.

Sulphates

Sulphate was determined by the Nepheloturbidometric method. 20 ml of clear water sample in 100 ml STD flask was taken and 1 ml of 1:9 hydrochloric acid followed by 1ml conditioning agent and 0.5 gm of barium chloride was added further the preparation was mixed thoroughly for 30 sec. The absorbance was taken at 420 nm after 10 mins of incubation. Calibration curve: Prepare a series of standards and blank (0.0, 5.0, 10.0, 15.0 and 20.0 ml of stock sulphate solution and the above procedure was repeated. This is 0.0, 10.0, 20.0, 30.0 and 40.0 mg/L of sulphate). Calibration curve of standards mg/L vs absorbance prepared.

Calculation:

$$\text{Sulphate as } SO_4 \text{ mg/L} = \frac{M}{\text{Volume of sample}}$$

Where,

M = mg/L of sulphate sample directly from the calibration graph.

Nitrates (Chromotrophic Acid Method)

Standard nitrate was prepared in the range of 0.10-5.0 mg/L by diluting 0.0, 0.1, 5.0, 10.0, 25.0, 40.0 and 50.0 ml of standard nitrate solution to 100 ml. 2 ml of each of these portions was pipette out and water blank into dry 10 ml volumetric flask. One drop of sulphite urea was added to each flask. Flasks were kept in cold water and then 2 ml of antimony reagent was added and mixed. After four minutes1 ml of chromotrophic acid reagent was added, swirled allowed to stand in cooling bath for 3 mins. Then concentrated sulphuric acid was added to make up the volume to 10 ml. Mixed

well by inverting the stoppered flask slowly. After 15 mins absorbance was read at 410 nm.

Calculation:

$$\text{Nitrate nitrogen (as nitrate) mg/L} = \frac{\mu\text{g of nitrate nitrogen in 10 ml final volume}}{\text{Volume in ml of sample taken for test}}$$

Determination of Heavy Metals

Iron

Iron was determined by Phenanthroline method. Approximate portions of the standard were pipette into the conical flask and diluted to 50ml by adding water. 1ml hydroxylamine hydrochloride solution and 2ml conc. HCl was added along with few boiling chips and the solution was boiled until reduced to 20 ml. After cooling to room temperature, it was transferred to the 250ml conical flask and ammonium acetate buffer was added after which 10ml of 1, 10 Phenanthroline solutions were added. Again it was diluted to 100ml with water and allowed to stand for 10-15min. Later, the absorbance was measured at 510nm against the reagent blank. A calibration curve was constructed by plotting absorbance values against micrograms of iron in100ml of the final solution.

Calculation

$$\text{Iron, mg/L} = \frac{\mu\text{g of Iron (in 100 ml of the final solution)}}{V}$$

Where,

V = Volume in ml of the sample taken for the test

Copper

Copper was determined using Neocuproine method (Ref: IS: 3025(PT-42)). The sample was acidified with HCl and boiled and cooled. The pH was maintained between 4-6. 50ml of the sample was transferred to a separating funnel and 5ml hydroxylamine hydrochloride was added along with 10ml of Neocuproine solution and 10 ml of sodium citrate solution. The above mixture was shaken well and 20ml chloroform was added and shaken for a minute. Chloroform layer as collected in a dry flask and extraction process was repeated with 20ml chloroform and the volume was made up to 50ml using Isopropyl alcohol. A reagent blank was prepared the same way. 50ml portions were treated as 1.25, 2.5 and 12.5. A calibration graph of Absorbance versus copper concentrations (mg/l) for standards was plotted and the concentrations of copper in the sample were read using the calibration graph.

Calculation

$$\text{Copper, mg/L} = \frac{M}{V} \times 1000$$

Where,

V = Volume in ml of sample taken for test

Aluminum

Aluminum was tested using Erichrome Cyanine R Method. A series of aluminum standards were prepared by accurately measuring calculated volumes of STD aluminum solution into 50 ml volumetric flask. Total volume was made up to 50ml by using distilled water. 1ml of 0.02N Sulphuric acid and Ascorbic acid solution were added to the volumetric flask and mixed. 10ml of buffer solution was added and 5ml of the working dye reagent was pipette and mixed. The volume was adjusted to 50ml with water and it was allowed to stand for 5-10 mins. The absorbance of the aluminum complex was measured using reagent blank as reference solution and a calibration curve was plotted for absorbance values against micrograms of aluminum in 50ml of the final solution.

Calculation

$$\text{Aluminium, mg/L} = \frac{M}{V}$$

Where,

M = Mass of aluminum present in microgram in 50ml of the final solution

V = Volume in ml of the sample taken

RESULTS

Sample Collection

A sample was collected in 10 -litre pet jar from an outlet source contain the high rate of copper and aluminum from forging industry. The water was used for electrolysis of copper and aluminum sheets. The sample hence is supposed to contain the high concentration of copper and other heavy metals thereby this water is regarded as polluted industrial effluent and may cause environmental hazards if let off in the environment. Hence in the recent investigation, this water has been taken for the treatment and Phytoremediation. The artificial wastewater sample was also prepared in the laboratory and was inoculated with *Chlorella vulgaris* and *Scenedesmus abundans*. The composition of artificial wastewater is depicted in the table. 10 which was used as the control.

Table 1: Composition of Artificial Waste Water (AW)

Compound	Conc^{n} in g/ liter
Nitrates	0.1
Sulphates	0.1
Calcium	0.1
Magnesium	0.2
Iron	0.03
Lead	0.03
Zinc	0.03
Aluminum	0.03
Chloride	0.16

Preparation of Culture and Immobilisation of Cells

The pure algal culture of *Chlorella vulgaris* AS-4 (Figure 1) was inoculated in to the fresh BG-11 media that was kept for incubation for about 3-4 months. The culture *Scenedesmus abundans*AS-9 (Figure1) was also cultured using BG-11 media and was incubated for 3-4 months. After it was observed with the maximum algal mass the culture was used to

prepare the alginate beads and used further as inoculants to water samples. The pure cultures were immobilized using 2% sodium alginate and 10% calcium chloride solution. (Figure 2).

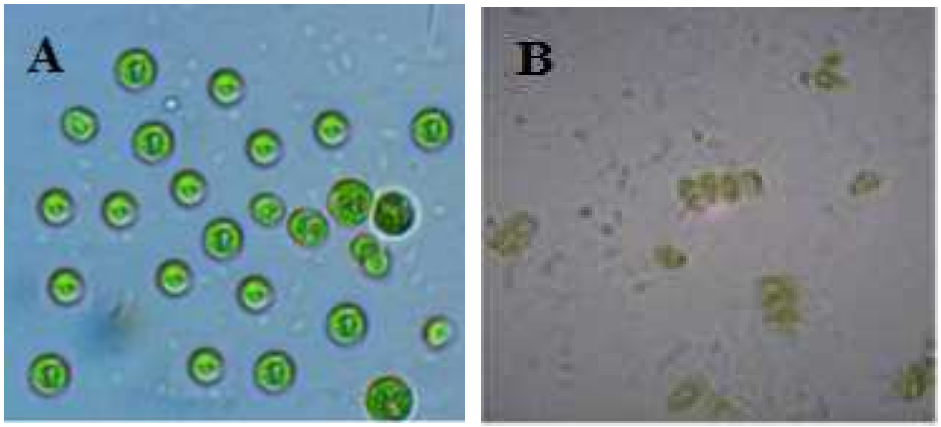


Figure 1: Microscopic Image of (A) *Chlorella Vulgaris* AS-4 and (B) *Scenedesmus Abundans* AS-9.

Figure 2: Bottles Containing Waste Water Samples Inoculated with Immobilise d *Scenedesmus Abundans* and *Chlorella Vulgaris* Cultures

Physico-Chemical Analysis

Physico-chemical analysis of the sample was done according to standard methods followed by APHA, (1995) and the following parameters were considered for testing the industrial effluent sample in accordance with the Cultures *Chlorella vulgaris AS-4*and *Scenedesmus abundans* and artificial wastewater sample in accordance with the cultures *Chlorella vulgaris AS-4*and *Scenedesmus abundans* (CV-IE, CV-AW, SA-IE, and SA-AW).

Temperature

The physical and chemical parameters were observed in varying values, which was initiated by Temperature. The temperature of industrial effluent sample inoculated with entrapped *Chlorella vulgaris AS-4*culture noted at Day-0 was 18 ^{0}C, Day-10 was 18 ^{0}C, Day-20 was 20 ^{0}C, and Day-30 was 21 ^{0}C. Similarly, temperature noted for artificial waste water sample inoculated with entrapped *Chlorella vulgaris AS-4* culture at Day-0 was 20 ^{0}C, Day-10 was 20 ^{0}C, Day-20 was 19 ^{0}C, and Day-30 was 18 ^{0}C. Simultaneously the temperature of industrial effluent sample inoculated with entrapped *Scenedesmus abundans* culture noted at Day-0 was 18 ^{0}C, day-10 was 19 ^{0}C, Day-20 was 18 ^{0}C, and Day-30 was 18 ^{0}C.Similarly, temperature noted for Artificial wastewater sample inoculated with entrapped *Scenedesmus abundans* culture at Day-0 was 20 ^{0}C, Day-10 was 21 ^{0}C, Day-20 was 19 ^{0}C, and Day-30 was 20 ^{0}C (Table 2). The temperature was taken as a parameter as change is temperature is observed with the increase or decrease in metabolic activity of the organism.

Table 2: Temperature (^{0}C) Values for *Chlorella Vulgaris* against Industrial Effluent and Artificial Waste Water and *Scenedesmus Abundans* against Industrial Effluent and Artificial Waste Water from Day-0 to Day-30

Temperature (^{0}c) Cultures/Samples.	Day-0	Day-10	Day-20	Day-30
CV-IE	18	18	20	21
CV-AW	20	20	19	18
SA-IE	18	19	18	18
SA-AW	20	21	19	20

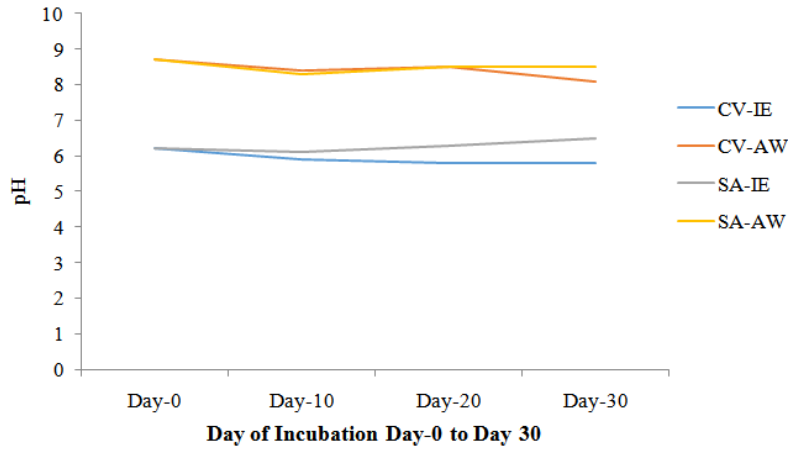
CV-IE-*Chlorella vulgaris in industrial effluent*

CV-AW- *Chlorella vulgaris in artificial water*

SA-IE-*Scenedesmus abundans in industrial effluent*

SA-AW- *Scenedesmus abundans in artificial water*

Temperature (^{0}C) versus day of incubation (Day-0 to Day-30) graph was plotted using the respective samples CV-IE, CV-AW, SA-IE, and SA-AW (Graph 1).



Graph 1: Temperature (^{0}C) Values for *Chlorella Vulgaris* against Industrial Effluent and Artificial Waste Water and *Scenedesmus Abundans* Against Industrial Effluent and Artificial Waste Water from DAY-0 to Day-30

pH

The alkalinity of water samples was measured as neutralized acids. The pH of the industrial effluent sample inoculated with entrapped *Chlorella vulgaris AS-4* culture noted at Day-0 was 6.2 ± 0.05 Day-10 was 5.9 ± 0.03, Day-20 was 5.8 ± 0.05, and Day-30 was 5.8 ± 0.05. Similarly, pH of artificial wastewater sample inoculated with entrapped *Chlorella vulgaris AS-4* culture at Day-0 was 8.7 ± 0.05, Day-10 was 8.4 ± 0.00, Day-20 was 8.5 ± 0.05, and Day-30 was 8.1± 0.05. Simultaneously the pH of industrial effluent sample inoculated with entrapped *Scenedesmus abundans* at Day-0 was 6.2 ± 0.05, day-10 was 6.1 ± 0.03, Day-20 was 6.3 ± 0.05, and Day-30 was 6.5 ± 0.02. Similarly pH of artificial waste water sample inoculated with entrapped *Scenedesmus abundans* culture at Day-0 was 8.7 ± 0.05, Day-10 was 8.3 ± 0.05, Day-20 was 8.5 ± 0.02, and Day-30 was 8.5 ± 0.03 (Table 3).

PH versus day of incubation (Day-0 to Day-30) graph was plotted using the respective samples CV-IE, CV-AW, SA-IE, and SA-AW (Graph.2).

Table 3: pH Values for *Chlorella Vulgaris* against Industrial Effluent and Artificial Waste Water and *Scenedesmus Abundans* against Industrial Effluent and Artificial Waste Water from Day-0 to Day-30

pH of Cultures/Samples.	Day-0	Day-10	Day-20	Day-30
CV-IE	6.2 ±0.05	5.9 ±0.03	5.8 ±0.05	5.8 ±0.05
CV-AW	8.7 ±0.05	8.4 ±0.00	8.5 ±0.05	8.1 ±0.05
SA-IE	6.2 ±0.05	6.1 ±0.03	6.3 ±0.05	6.5 ±0.02
SA-AW	8.7 ±0.05	8.3 ±0.05	8.5 ±0.02	8.5 ±0.03

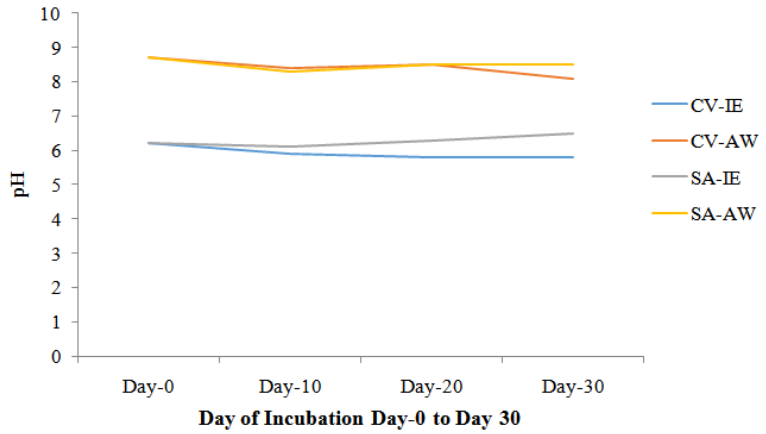
CV-IE-*Chlorella vulgaris in industrial effluent*

CV-AW- *Chlorella vulgaris in artificial water*

SA-IE-*Scenedesmus abundans in industrial effluent*

SA-AW- *Scenedesmus abundans in artificial water*

pH versus day of incubation (Day-0 to Day-30) graph was plotted using the respective samples CV-IE, CV-AW, SA-IE, and SA-AW (Graph 2).



Graph 2: pH Values for *Chlorella Vulgaris* against Industrial Effluent and Artificial Waste Water and *Scenedesmus Abundans* against Industrial Effluent and Artificial Waste Water from Day-0 to Day-30

Turbidity

Suspension of particles in water interfering with the passage of light is called turbidity. Measuring unit for turbidity is Nephelometric Turbidity Unit (NTU). Low levels of turbidity were observed in industrial effluent sample inoculated with entrapped *Chlorella vulgaris AS-4* culture at Day-0 was 8.1 ± 0.05 NTU, Day-10 was 7.3 ± 0.05 NTU, Day-20 was 7.1 ± 0.05 NTU, and Day-30 was 6.5 ± 0.03 NTU. Similarly, turbidity of artificial wastewater sample inoculated with entrapped *Chlorella vulgaris AS-4* culture at Day-0 was 5.0 ± 0.02NTU, Day-10 was 4.1 ± 0.03 NTU, Day-20 was 4.0 ± 0.02 NTU, and Day-30 was 3.0 ± 0.02 NTU. Simultaneously turbidity of industrial effluent sample inoculated with entrapped *Scenedesmus abundans* at Day-0 was 8.1 ± 0.05 NTU, Day-10 was 7.6 ± 0.03 NTU, Day-20 was 7.1 ± 0.05 NTU, and Day-30 was 6.7 ± 0.02 NTU. Similarly, turbidity of artificial wastewater sample inoculated with entrapped *Scenedesmus abundans* culture at Day-0 was 5.0 ± 0.02 NTU, Day-10 was 4.1 ± 0.02 NTU, Day-20 was 4.2 ± 0.05 NTU, and Day-30 was 3.1 ± 0.03 NTU (Table 4).

Table 4: Turbidity (NTU) Values for *Chlorella Vulgaris* against Industrial Effluent and Artificial Waste Water and *Scenedesmus Abundans* against Industrial Effluent and Artificial Waste Water from Day-0 to Day-30

Turbidity (NTU) Cultures/Samples.	Day-0	Day-10	Day-20	Day-30
CV-IE	8.1 ±0.05	7.3 ±0.05	7.1 ±0.05	6.5 ±0.03
CV-AW	5.0 ±0.02	4.1 ±0.03	4.0 ±0.02	3.0 ±0.02
SA-IE	8.1 ±0.05	7.6 ±0.03	7.1 ±0.05	6.7 ±0.02
SA-AW	5.0 ±0.02	4.1 ±0.02	4.2 ±0.05	3.1 ±0.03

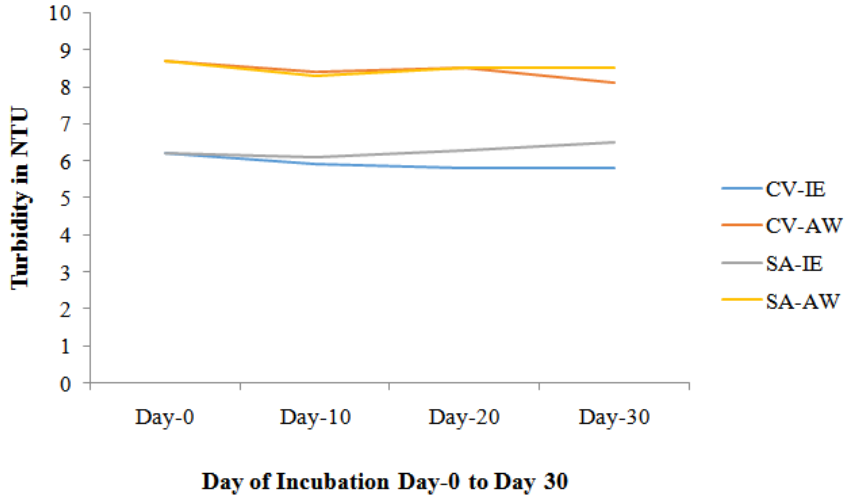
CV-IE-*Chlorella vulgaris in industrial effluent*

CV-AW- *Chlorella vulgaris in artificial water*

SA-IE-*Scenedesmus abundans in industrial effluent*

SA-AW- *Scenedesmus abundans in artificial water*

Turbidity (NTU) versus day of incubation (Day-0 to Day-30) graph was plotted using the respective samples CV-IE, CV-AW, SA-IE, and SA-AW (Graph.3).



Graph 3: Turbidity (NTU) Values for *Chlorella Vulgaris* against Industrial Effluent and Artificial Waste Water and *Scenedesmus Abundans* against Industrial Effluent and Artificial Waste Water from Day-0 to Day-30

Total Dissolved Solids

Total dissolved solids refer to matter suspended or dissolved in water or wastewater. The algal cells inorganic and organic components are detected by FTIR spectroscopy which is known for its high sensitivity. The affinity of *Chlorella vulgaris AS-4* towards treating the wastewater samples was found to be more as compared to that of *Scenedesmus abundans.* The TDS has resulted in low levels, industrial effluent sample inoculated with entrapped *Chlorella vulgaris AS-4*culture results at Day-0 was 432 ± 0.02 mg/L, Day-10 was 396 ± 0.02 mg/L, Day-20 was 387 ± 0.00 mg/L, and Day-30 was 381 ± 0.02 mg/L. Similarly, TDS of artificial wastewater sample inoculated with entrapped *Chlorella vulgaris AS-4* culture at Day-0 was 153 ± 0.05 mg/L, Day-10 was 149 ± 0.03 mg/L, Day-20 was 144 ± 0.02 mg/L, and Day-30 was 138 ± 0.02 mg/L. Simultaneously TDS of industrial effluent sample inoculated with entrapped *Scenedesmus abundans* at Day-0 was 432 ± 0.02 mg/L, Day-10 was 415 ± 0.02 mg/L, Day-20 was 408 ± 0.02 mg/L, and Day-30 was 390 ± 0.02 mg/L. Similarly, TDS of artificial wastewater sample inoculated with entrapped *Scenedesmus abundans* culture at Day-0 was 153 ± 0.05 mg/L, Day-10 was 145 ± 0.05 mg/L, Day-20 was 140 ± 0.03 mg/L, and Day -30 was 138 ± 0.03 mg/L (Table 5).

Table 5: TDS (mg/L) Values for *Chlorella Vulgaris* against Industrial Effluent and Artificial Waste Water and *Scenedesmus Abundans* against Industrial Effluent and Artificial Waste Water from Day-0 to Day-30

TDS (mg/l) Cultures/Samples.	Day-0	Day-10	Day-20	Day-30
CV-IE	432 ±0.02	396 ±0.02	387 ±0.00	381 ±0.02
CV-AW	153 ±0.05	149 ±0.03	144 ±0.02	138 ±0.02
SA-IE	432 ±0.02	415 ±0.02	408 ±0.02	390 ±0.02
SA-AW	153 ±0.05	145 ±0.05	140 ±0.03	138 ±0.03

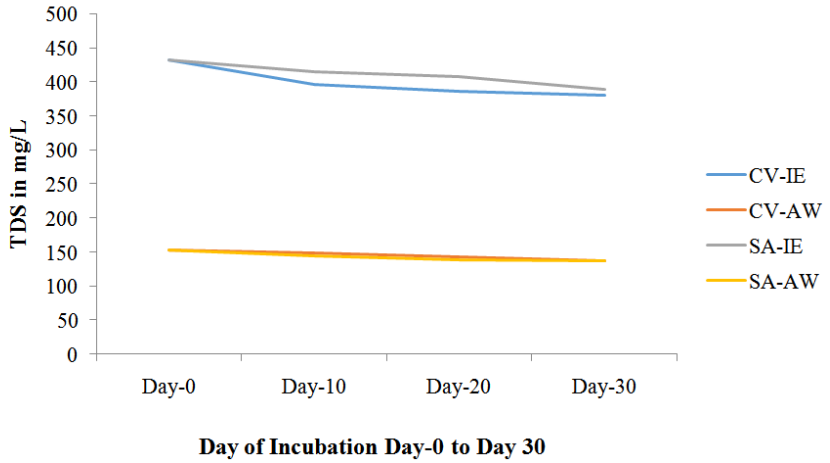
CV-IE-*Chlorella vulgaris in industrial effluent*

CV-AW- *Chlorella vulgaris in artificial water*

SA-IE-*Scenedesmus abundans in industrial effluent*

SA-AW- *Scenedesmus abundans in artificial water*

TDS (mg/L) versus day of incubation (Day-0 to Day-30) graph was plotted using the respective samples CV-IE, CV-AW, SA-IE, and SA-AW (Graph 4).



Graph 4: TDS (mg/L) Values for *Chlorella Vulgaris* against Industrial Effluent and Artificial Waste Water and *Scenedesmus Abundans* against Industrial Effluent and Artificial Waste Water from Day-0 to Day-30

Sulphates

High sulphates levels were observed in industrial Effluent. The treated values of sulphates in industrial effluent sample inoculated with entrapped *Chlorella vulgaris AS-4* culture, at Day-0 was 42.9±0.05 mg/L, Day-10 was 32.1±0.02 mg/L, Day-20 was 23.7±0.03 mg/L, and Day-30 was 20.0±0.02 mg/L. Similarly, sulphates noted inartificial wastewater sample inoculated with entrapped *Chlorella vulgaris AS-4* culture at Day-0 was 0.002±0.02 mg/L, Day-10 was 0.001± 0.02 mg/L, Day-20 was 0.001±0.02 mg/L, and Day-30 was 0.000±0.00 mg/L. Simultaneously sulphates of industrial effluent sample inoculated with entrapped *Scenedesmus abundans* at *Day*-0 was 42.9±0.05 mg/L, Day-10 was 38.3±0.02 mg/L, Day-20 was 36.2±0.02 mg/L, and Day-30 was 35.1±0.03 mg/L. Similarly, sulphates noted in artificial wastewater sample inoculated with entrapped *Scenedesmus abundans* culture at Day-0 was 0.002±0.02 mg/L, Day-10 was 0.001±0.00 mg/L, Day-20 was 0.001±0.02 mg/L, and Day-30 was 0.000± 0.02 mg/L (Table 6).

Table 6: Sulphate Values for *Chlorella Vulgaris* against Industrial Effluent and Artificial Waste Water and *Scenedesmus Abundans* against Industrial Effluent and Artificial Waste Water from Day-0 to Day-30

Sulphate (mg/l) Cultures/Samples.	Day-0	Day-10	Day-20	Day-30
CV-IE	42.9 ±0.05	32.1 ±0.02	23.7 ±0.03	20.0 ±0.02
CV-AW	0.002 ±0.02	0.001 ±0.02	0.001 ±0.02	0.000 ±0.00
SA-IE	42.9 ±0.05	38.3 ±0.02	36.2 ±0.02	35.1 ±0.03
SA-AW	0.002 ±0.02	0.001 ±0.00	0.001 ±0.02	0.00 ±0.02

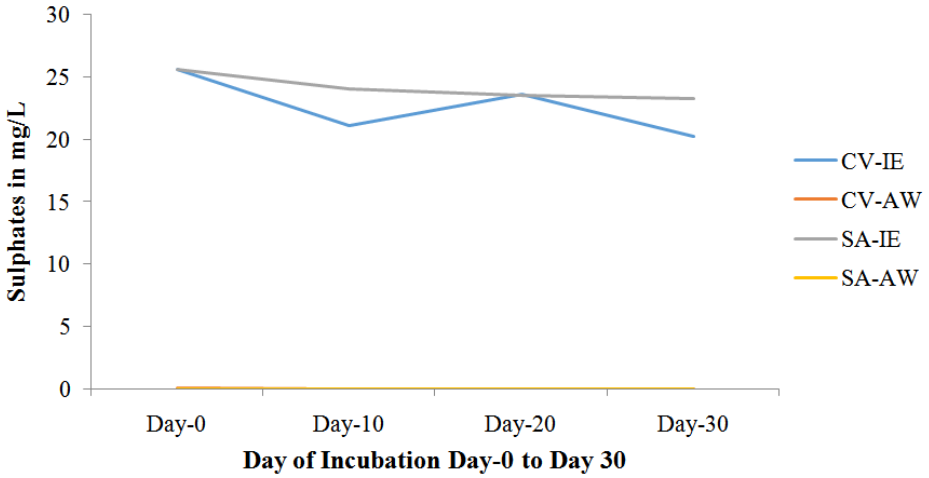
CV-IE-*Chlorella vulgaris in industrial effluent*

CV-AW- *Chlorella vulgaris in artificial water*

SA-IE-*Scenedesmus abundans in industrial effluent*

SA-AW- *Scenedesmus abundans in artificial water*

Sulphates estimated versus day of incubation (Day-0 to Day-30) graph was plotted using the respective samples CV-IE, CV-AW, SA-IE, and SA-AW (Graph 5).



Graph 5: Sulphate (mg/L) Values for *Chlorella Vulgaris* against Industrial Effluent and Artificial Waste Water and *Scenedesmus Abundans* against Industrial Effluent and Artificial Waste Water from Day-0 to Day-30

Nitrate

Nitrate the sewage contaminant, show a higher concentration of ammonia nitrogen, which is an aerobic environment is converted into nitrites and then nitrates. Nitrates in industrial effluent sample inoculated with entrapped *Chlorella vulgaris AS-4* culture at Day-0 was 32.6±0.03 mg/L, Day-10 was 26.3±0.03 mg/L, Day-20 was 22.7±0.00 mg/L, and Day-30 was19.5 ± 0.02 mg/L. Similarly, nitrates inartificial wastewater sample inoculated with entrapped *Chlorella vulgaris AS-4* culture at Day-0 was 0.002±0.02 mg/L, Day-10 was 0.001±0.03 mg/L, Day-20 was 0.001±0.02 mg/L, and Day-30 was 0.000±0.00 mg/L. Simultaneously nitrates in industrial effluent sample inoculated with entrapped *Scenedesmus abundans* at Day-0 was 32.6±0.03 mg/L, Day-10 was 31.2± 0.02 mg/L, Day-20 was 31.1±0.02 mg/L , and Day-30 was 31.0±0.00 mg/L. Similarly, nitrates inartificial wastewater sample inoculated with entrapped *Scenedesmus abundans* culture at Day-0 was 0.002±0.02 mg/L, Day-10 was 0.001±0.03 mg/L, Day-20 was 0.001±0.02 mg/L, and Day-30 was 0.000± 0.02 (Table 7).

Table 7: Nitrate (mg/L) Values for *Chlorella Vulgaris* against Industrial Effluent and Artificial Waste Water and *Scenedesmus abundans* against Industrial Effluent and Artificial Waste Water from Day-0 to Day-30

Nitrate (mg/l) Cultures/Samples.	Day-0	Day-10	Day-20	Day-30
CV-IE	32.6 ±0.03	26.3 ±0.03	22.7 ±0.00	19.5 ±0.02
CV-AW	0.002 ±0.02	0.001 ±0.03	0.001 ± 0.02	0.000 ±0.00
SA-IE	32.6 ±0.03	31.2 ±0.02	31.1 ±0.02	31.0 ±0.00
SA-AW	0.002 ±0.02	0.001 ±0.03	0.001 ±0.02	0.00 ±0.02

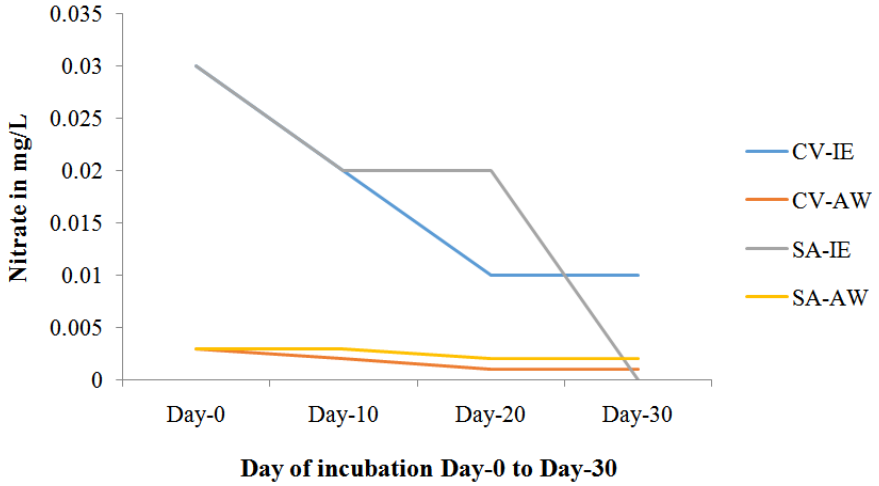
CV-IE-*Chlorella vulgaris in industrial effluent*

CV-AW- *Chlorella vulgaris in artificial water*

SA-IE-*Scenedesmus abundans in industrial effluent*

SA-AW- *Scenedesmus abundans in artificial water*

Nitrates estimated (mg/L) versus day of incubation (Day-0 to Day-30) graph was plotted using the respective samples CV-IE, CV-AW, SA-IE, and SA-AW (Graph 6).



Graph 6: Nitrate (mg/L) Values for *Chlorella Vulgaris* against Industrial Effluent and Artificial Waste Water and *Scenedesmus Abundans* against Industrial Effluent and Artificial Waste Water from Day-0 to Day-30

Iron

As the sample collected was from a metal-based industry, iron was a component present in the effluent sample causing the metallic pollution to the effluent discharged from the industry. It was estimated to be treated well by *Chlorella vulgaris AS-4* then *scenedesmus abundans*. Iron in industrial effluent sample inoculated with entrapped *Chlorella vulgaris AS-4* culture at Day-0 was 12.1±0.05 mg/L, Day-10 was 10.5±0.05 mg/L, Day-20 was 8.6±0.03 mg/L, and Day-30 was 5.1± 0.05 mg/L. Similarly, iron inartificial wastewater sample inoculated with entrapped *Chlorella vulgaris AS-4* culture at Day-0 was 0.004±0.05 mg/L, Day-10 was 0.002±0.03 mg/L, Day-20 was 0.001±0.03 mg/L, and Day-30 was 0.000±0.03 mg/L. Simultaneously iron in industrial effluent sample inoculated with entrapped *Scenedesmus abundans* culture at Day-0 was 12.1±0.05 mg/L, Day-10 was 10.6±0.03 mg/L, Day20 was 9.9±0.05 mg/L, and Day-30 was 9.1±0.05 mg/L. Similarly, iron inartificial wastewater sample inoculated with entrapped *Scenedesmus abundans* culture at Day-0 was 0.004±0.05 mg/L, Day-10 was 0.003±0.004 mg/L, Day-20 was 0.003±0.03 mg/L, and Day-30 was 0.002± 0.02 mg/L (Table 8).

Table 8: Iron (mg/L) Values for *Chlorella Vulgaris* against Industrial Effluent and Artificial Waste Water and *Scenedesmus Abundans* against Industrial Effluent and Artificial Waste Water from Day-0 to Day-30

Iron (mg/l) Cultures/Samples.	Day-0	Day-10	Day-20	Day-30
CV-IE	12.1 ±0.05	10.5 ±0.05	8.6 ±0.03	5.1 ±0.05
CV-AW	0.004 ±0.05	0.002 ±0.03	0.001 ±0.03	0.000 ±0.03
SA	12.1 ±0.05	10.6 ±0.03	9.9 ±0.05	9.1 ±0.05
SA-AW-IE	0.004 ±0.05	0.003 ±0.04	0.003 ±0.03	0.002 ±0.02

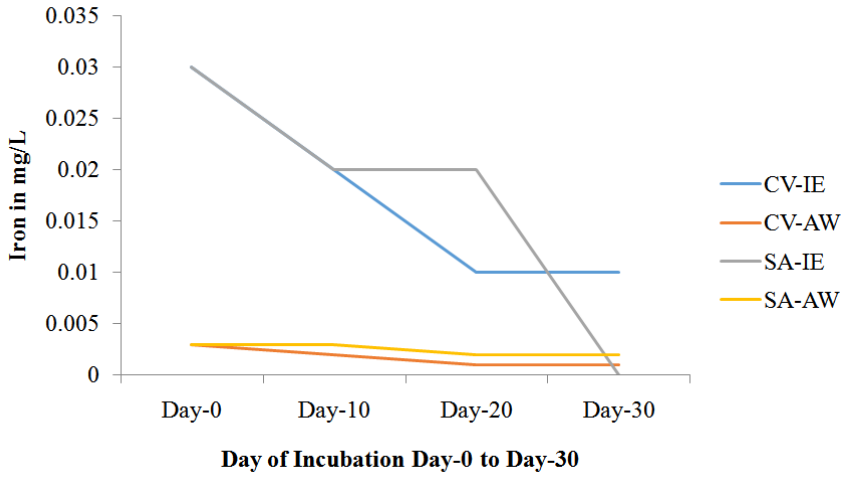
CV-IE-*Chlorella vulgaris in industrial effluent*

CV-AW- *Chlorella vulgaris in artificial water*

SA-IE-*Scenedesmus abundans in industrial effluent*

SA-AW- *Scenedesmus abundans in artificial water*

Iron estimated (mg/L) versus day of incubation (Day-0 to Day-30) graph was plotted using the respective samples CV-IE, CV-AW, SA-IE, and SA-AW (Graph 7).



Graph 7: Iron (mg/L) Values for *Chlorella Vulgaris* against Industrial Effluent and Artificial Waste Water and *Scenedesmus Abundans* against Industrial Effluent and Artificial Waste Water from Day-0 to Day-30

Copper

Same as that of Iron the Copper is also a metallic element obtained from a metal-based industry from where the sample was collected was estimated to be treated well by *Chlorella vulgaris AS-4* then *scenedesmus abundans*. Copper estimated in industrial effluent sample inoculated with entrapped *Chlorella vulgaris AS-4* culture atDay-0 was 88.9± 0.05 mg/L. Day-10 was 86.5±0.03 mg/L, Day-20 was 85.1±0.03 mg/L, and Day-30 84.0± 0.05 mg/L. Similarly, copper inartificial wastewater sample inoculated with entrapped *Chlorella vulgaris AS-4* culture at Day-0 to Day-30 was 0.000±0.002 mg/L. simultaneously copper in industrial effluent sample inoculated with entrapped *Scenedesmus abundans* culture at Day-0 was 88.9±0.05 mg/L, Day-10 was 87.1±0.03 mg/L, Day-20 was 86.4±0.03 mg/L, and Day-30 was 86.1±0.03 mg/L. Similarly, copper inartificial wastewater sample inoculated with entrapped *Scenedesmus abundans* culture at Day-0 to Day-30 was 0.000± 0.002 mg/L (Table 9).

Table 9: Copper (mg/L) Values for *Chlorella Vulgaris* against Industrial Effluent and Artificial Waste Water and *Scenedesmus Abundans* against Industrial Effluent and Artificial Waste Water from Day-0 to Day-30

Copper (mg/l) Cultures/Samples.	Day-0	Day-10	Day-20	Day-30
CV-IE	88.9 ±0.05	86.5 ±0.03	85.1 ±0.03	84.0 ±0.05
CV-AW	0.000 ±0.02	0.000 ±0.02	0.000 ±0.02	0.000 ±0.02
SA-IE	88.9 ±0.05	87.1 ±0.03	86.4 ±0.03	86.1 ±0.03
SA-AW	0.000 ±0.02	0.000 ±0.02	0.000 ±0.02	0.000 ±0.02

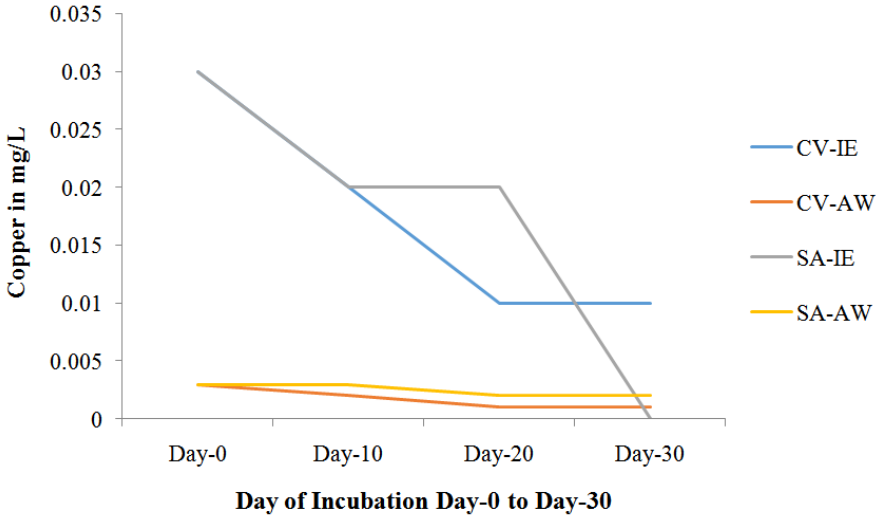
CV-IE-*Chlorella vulgaris in industrial effluent*

CV-AW- *Chlorella vulgaris in artificial water*

SA-IE-*Scenedesmus abundans in industrial effluent*

SA-AW- *Scenedesmus abundans in artificial water*

Copper estimated (mg/L) versus day of incubation (Day-0 to Day-30) graph was plotted using the respective samples CV-IE, CV-AW, SA-IE, and SA-AW (Graph 8).



Graph 8: Copper (mg/L) Values for *Chlorella Vulgaris* against Industrial Effluent and Artificial Waste Water and *Scenedesmus Abundans* against Industrial Effluent and Artificial Waste Water from Day-0 to Day-30

Aluminum

Same as that of Iron and Copper, Aluminum is also a metallic element obtained from a metal-based industry from where the sample was collected was estimated to be treated well by *Chlorella vulgaris AS-4* then *Scenedesmus abundans*. Aluminum estimated in industrial effluent sample inoculated with entrapped *Chlorella vulgaris AS-4* culture at Day-0 was0.03±0.03 mg/L, Day-10 was 0.02±0.03 mg/L, Day-20 was 0.01±0.02 mg/L, and Day-30 was 0.01±0.02 mg/L. Similarly, aluminium inartificial wastewater sample inoculated with entrapped *Chlorella vulgaris AS-4* culture at Day-0 was 0.003±0.03 mg/L, Day-10 was 0.002± 0.02 mg/L, Day-20 was 0.001±0.03 mg/L, and Day-30 was 0.001±0.03 mg/L. Simultaneously aluminum in industrial effluent sample inoculated with entrapped *Scenedesmus abundans* culture at Day-0 was 0.03±0.03 mg/L, Day-10 was 0.02±0.03 mg/L, Day-20 was 0.02±0.01 mg/L, and Day-30 was 0.00±0.02 mg/L. Similarly, aluminum inartificial wastewater sample inoculated with entrapped *Scenedesmus abundans* cultureatDay-0 was 0.003±0.03 mg/L, Day-10 was 0.003±0.05 mg/L, Day-20 was 0.002±0.2 mg/L, and Day-30 was 0.002± 0.02 mg/L (Table 10).

Table 10: Aluminium (mg/L) Values for *Chlorella Vulgaris* against Industrial Effluent and Artificial Waste Water and *Scenedesmus Abundans* against Industrial Effluent and Artificial Waste Water from Day-0 to Day-30

Aluminium (mg/l) Cultures/Samples.	Day-0	Day-10	Day-20	Day-30
CV-IE	0.03 ±0.03	0.02 ±0.03	0.01 ±0.02	0.01 ±0.02
CV-AW	0.003 ±0.03	0.002 ±0.02	0.001 ±0.03	0.001 ±0.03
SA-IE	0.03 ±0.03	0.02 ±0.03	0.02 ±0.01	0.00 ±0.02
SA-AW	0.003 ±0.03	0.003 ±0.05	0.002 ±0.02	0.002 ±0.02

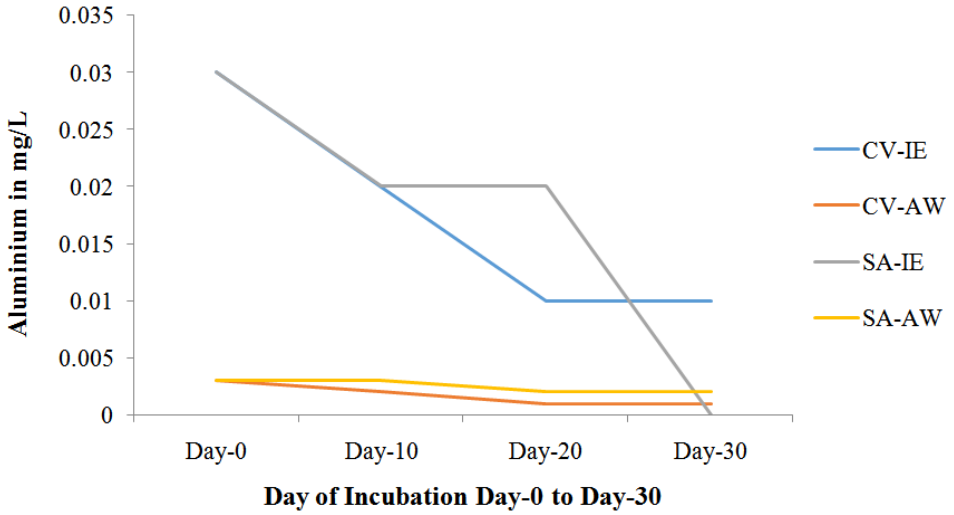
CV-IE-*Chlorella vulgaris in industrial effluent*

CV-AW- *Chlorella vulgaris in artificial water*

SA-IE-*Scenedesmus abundans in industrial effluent*

SA-AW- *Scenedesmus abundans in artificial water*

Aluminium estimated (mg/L) versus day of incubation (Day-0 to Day-30) graph was plotted using the respective samples CV-IE, CV-AW, SA-IE, and SA-AW (Graph 9).



Graph 9: Aluminium (mg/L) Values for *Chlorella Vulgaris* Against Industrial Effluent and Artificial Waste Water and *Scenedesmus Abundans* against Industrial Effluent and Artificial Waste Water from Day-0 to Day-30

DISCUSSIONS AND CONCLUSIONS

Sewage contains almost all the nutrient elements required for growth of algae. In Phytoremediation, the main problem identified is the harvesting of microalgae after treating the wastewater (James, 1998; Mallick, 2002; Aslan and Kapdan, 2006). Immobilization of microalgae is one of the solutions for harvesting problem (Vılchez*et al*., 2001; and Jimnez-Perez *et al*., 2004). Carrageenan, chitosan, and alginate are commonly used polymers for the microalgae immobilization. Immobilized microalgae beads are easy to harvest and reuse. The change in pH was observed in the treated water which gradually decreased. It was due to the removal of various salts or metallic ions. This lowering of pH was also due to the microbial activity which in turn increases the decomposition of organic matter. Klein (1972) stated that pH change causes a shift in the relative abundance of various genera in the aquatic system, which is in conformity with the present study where along with the decrease in pH i.e. from 6.3 ± 0.05 to 5.8 ± 0.05 Cause the change in the abundance of microalgae. Bokil and John (1981) recorded 78-83% reduction of nitrates from domestic sewage by mixed algae. Further,

the removal of removal of nitrogen was more pronounced. In the present investigation, the Nitrate was almost found in negligible quantities on the 30th day of treatment of the industrial effluent sample. In case of nitrogen uptake, immobilized microalgae had higher nitrogen uptake than the free cell in both the types of wastewater but in phosphorus removal efficiency, immobilized cultures removed more phosphorus in artificial wastewater than in urban wastewater (Ruiz-Marin *et al*., 2010). Since these nitrates are the best sources of nitrogen to algae hence there were manifold increases in algal biomass which in turn increase the removal of various pollutants. Algae appear to offer the most easily exploited biological system for extracting phosphorous from domestic sewage. In the present study there was a cent percent removal of phosphorus on the 30th day which is in conformity with the data presented by Lakshmi *et al.*, (1990). Iron in the form of Phosphorus is used by microalgae for the synthesis of cellular constituents such as phospholipids, nucleic acids synthesis and associated reactions with cell division (Martinez *et al*., 1999; Richmond, 2004). So, after 9 days of culture, the removal efficiency of *Chlorella vulgaris* microalgae in synthetic wastewater was 99.2%. Similar results were obtained by Wang *et al*., (2010) achieving up to 99% of Iron removal. On the other hand, Martinez *et al*., (1999) reported that *Scenedesmus obliquus* microalgae were able to remove 97% of Iron. The gradual reduction in phosphorus levels of the culture medium is due to the fact that this nutrient has been absorbed of wastewater by *Chlorella vulgaris* microalgae, the nutrient necessary for its growth. Thus, Iron concentration in the medium is directly related to the growth of the microalgae, as demonstrated earlier Xin *et al*., (2010). Furthermore, it can be said that Iron concentration is often a limiting nutrient in microalgae growth (Elser *et al*., 1990) and the cells can assimilate and store this nutrient diminishing the amount of Iron in the wastewater. Hence, with the present study, it can be concluded that among the species studied *C. vulgaris* possessed the greater affinity for adsorption resulting in the higher uptake. *C. Vulgaris* showed more positive values reducing the concentration of nitrates, sulphates, metals like iron, aluminum, and copper which were seen to be treated in both the water samples i.e. industrial effluent and artificial wastewater. *Scenedesmus abundans* also showed positive results but lesser when it is compared with *C. vulgaris.*

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