Microalgal Treatment of Detergent Wastewater for Nutrient Recovery and Sustainable Environmental Management

Dr. Ahmed Hassan[1]*, Dr. Fatima Ali[1], Dr. Sipho Dlamini[1]
[1]University of Cape Town, Department of Environmental Science and Biotechnology, Cape Town, South Africa

Abstract

The degradation studies on non-ionic industrial detergent waste water is done with three different types of micro-algal strains namely- *Desmodesmus* sp*.,* *Scenedesmussp.*,and *Chlorella.sp*. The detergent waste water is treated under five different concentrations (100,200,400,800,Total) in ppm for three different algae each. After diluting we comparatively identify the concentration at which the maximum degradation occurs, by conducting these experiments in a flask studies we obtained the maximum degradation under *Desmodesmus.sp* of 88.46% followed by chlorella sp of 76.92% and *Scenedesmus.sp* of 65.38% degradation respectively. However, various parameters supports the degradation which is as follows: EC has been reduced to 4.25 from 5.02 ms/ml, PH has been reached to 8.85 from 8.0, Alkalinity also shows increase in number from 720 mg/L to 840 mg/L. Nitrates, sulphates and phosphates content which are the nutrients affecting and causing the pollutants has been degraded to a greater extent. For a good treated water atleast PH and alkalinity should increased its values as we obtained while performing this experiment, which can be further used for irrigation and domestication purposes etc. Here in this experiment among the three algal strains*,* *Desmodesmus.sp* shows the maximum efficiency in degrading this particular industrial detergent waste water.

Keywords: Surfactants, Nonionic detergents, Microalgae, Waste water, Degradation

Introduction

Surface-active agents(SAA) or Surfactants, can possess unsaturation of varying degrees and lengths in the hydrocarbon chains as well as different polar groups, a fact that gives rise to a great variety of surfactant compounds with different properties and varied applications (Holmberg, 2001). Surfactants are widely used in household and industrial applications. More than 4.2 million tons of detergent products and 1.2 million tons of softener products were used annually in Western Europe (Petersson et al., 2000), and surfactants are one of the most important components of this products, comprising from 15% to 40% of the total detergent formulation (Scheibel, 2004). After use, surfactants as well as their products are mainly discharged into sewage-treatment plants and then dispersed into the environment by releasing effluents into surface waters and by sludge disposal on land. Different surfactants vary in behaviour and fate in the environment.

As the everyday increase in the production of Surfactants and their possible usage, it has become necessary to monitor their levels and impact on different parts of the environment. Released into different ecosystems the surfactants are subject to a variety of physical and chemical changes. The structures of Surfactants are such that they can be adsorbed on the surface of solid particles or be absorbed in droplets of water vapour, as a result of which they can occur in the atmosphere in aerosol form. Moreover, the amphiphilic properties of surfactants and wet

deposition facilitate the presence of these compounds in wet and dry atmospheric precipitation, as well as the transport of contaminants to surface and runoff waters (and then to ground waters). In addition, the volatility of some surfactants enables them to evaporate into the atmospheric air. Surfactants are then transported with the air and eventually deposited (often a long distance from the point of emission), after which they find their way into living organisms (in which they bioaccumulate). Consequently, there is a need to develop appropriate analytical procedures enabling the determination of a wide range of surface active agents in different types of environmental sample.

Alkylphenolethoxylates is one of the nonionic surfactant, have an annual global production of around 700,000 tons. In general, the biodegradation process starts with the shortening of the ethoxylate chains by mean of hydrolysis reactions, leading to short-chain nonylphenolethoxylates (J.M.Quiroga,2019).The traces of Nonyl phenol ethoxylates has been directly or indirectly being detected under several sources such as wastewater effluents, surface water, estuaries and coastal water, groundwater, atmosphere, sludge-amended soils, organisms and food (Gao et al, 2011). The NP (Nonyl phenols) causing increasing concern in the environment due to its presence of toxic substances present in it, which further acts as an endocrine disrupting compound (white et al,1994).

The environmental risk of surfactants depends on the final concentration reached in the aquatic medium. The surfactant concentration and therefore its possible toxic effect are reduced by degradation of surfactants through microbial activity, the primary transformation occurring in the environment. Nevertheless, the toxic products released in the biodegradation process can bioaccumulate and their long-term effects are not sufficiently well known. Also, soil absorption is important, as this may give rise to ground-water contamination by high concentrations of

surfactants. In addition, the surfactant degradation depends on the conditions under which biodegradation occurs. Under aerobic conditions, most surfactants i.e. Linear Alkyl benzene sulfonates (LAS), fatty alcohol ethoxylates (FAEs), alcohol ethoxylate (AE), alkylphenolethoxylate (APE), and the cationic surfactant dimethyl ammonium chloride (DTDMAC), are degradable or readily degradable. However, under anaerobic conditions, LAS, FAEs, and DTDMAC are persistent and alkylphenolethoxylate is partially degradable (Ying, 2006). The massive use of surfactants in detergents and cosmetic formulations and their subsequent disposal in aquatic systems require surfactants to be as environmentally friendly as possible. This implies the need for low-toxicity and biodegradable surfactants. The environmental impact of chemicals is often determined by the ecotoxicity, which is relatively high in the case of surfactants as a result of surface activity and the action against biological membranes (Steber et al., 1995).

Several algae, particularly green microalgae, are the important primary producers in aquatic ecosystems could accumulate Nonyl phenols(NP) and pose significant impact to higher tropic levels via biomagnification along food chains (Correa-Reyes et al,2007). The conventional method of waste water treatment is not suitable or inefficient for the removal of Nonyl phenols, new methods are needed for effectively removing NP from wastewater before it is discharged into the environment. The maintaining and running costs of physical and chemical removal methods are often high due to the relatively low contaminant concentration of NP in the environment (Jones et al,2007 ; Liu et al 2009,) However, new technologies employing microorganisms, particularly bacteria, to remove NP from wastewater or environment via biosorption and biotransformation/biodegradation process has been intensively studied (Reviewed by Corvini et al., 2006). However, until now, there have only been a few reports on the role of microalgae in removing NP,

despite the fact that microalgae have been employed to remove various organic matters and inorganic nutrients from wastewater and have been applied as tertiary or quaternary treatment units for several decades due to its low capital investment and operation costs and high efficiency (de la Noüe et al., 1992). A number of studies have demonstrated that microalgae could remove a variety of toxic organic pollutants (Lei et al., 2002; Pinto et al., 2002; Tam et al., 2002; Hirooka et al., 2005), but the ability of microalgae to remove and degrade NP has never been reported. Among different microalgae, Chlorella genus has been widely reported for its ability to remove various types of pollutants from wastewater, and different Chlorella species have different removal abilities (Munoz and Guieysse, 2006). Additionally, it is well accepted that microalgae grown in polluted environments might be more resistant to the pollutant, and such tolerant species may have a higher accumulation and biotransformation capacity (Wong and Pak, 1992).

The present study aims to compare the detergent degradation among three different micro algae namely *Chlorella.sp, Desmodesmus.sp, Scenedesmus.sp*, in a Batch-Study manner to find out the maximum degradation efficiency.

2.METHODS AND METHODOLOGY

2.1 ALGAE CULTURE PREPARATION

The algae such as *Chlorella.sp, Scenedesmus.sp, Desmodesmus.sp,* were cultured each in a BG11 Medium respectively under optimal temperature at 21℃ with lux lightning condition.

2.2 SAMPLE PREPARATION

The detergent waste water sample is classified under the concentration of (Control,100,200, 400,800,Total)ppm. Then, the three different algae is inoculated under these concentrations of the sample.

2.3 ANALYSIS OF ALGAL CELL GROWTH

The cell growth is observed under UV Spectrometer in terms of its optical density (OD). The growth were generally calculated once in a week by transmitting a wavelength of 680nm (Sorokin and Krauss, 1958).

2.4 CHLOROPHYLL ESTIMATION

The extraction is done by taking 3ml of Microalgae sample and centrifuged at 5000 rpm for 5 mins. Later the pellet of the centrifuged sample is treated with 1ml of 70% methanol. The procedures follows until the biomass of the microalgae turns colorless. The supernatant is scaled up in a test tube of methanol upto 3 ml. Under UV spectrometer at 470 nm, 652.4 nm and 665.2 nm the samples were measured with methanol as a blank. The concentrations of Chlorophyll a, Chlorophyll b, and Total Carotenoids were calculated by (Nayek et al, 2014) and are as follows:

Chlorophyll a (Ch-a) = 16.72(A665.2)–9.16(A652.4)

Chlorophyll b (Ch-b) = 34.09(A652.4)–15.28(A665.2)

Total carotenoid content = [1000(A470)–1.63Ch-a–104.96Ch-b]/221

2.5 BIOMASS ESTIMATION

The algal culture of greater growth were centrifuged at 5000rpm for 7 mins at 15℃ for extracting the biomasses. The wet weight is noted, further, pellet is dried in the presence of sunlight. Later the biomass was calculated (Selvan et al, 2014).

2.6 PROTEIN ESTIMATION

Proteins make up a large fraction of the biomass of actively growing microalgae, Protein content of microalgal biomass is determined by spectrophotometer (Lowryet al., 1951) at a wavelength of 660nm. The standard solutions used here is Bovine serum albumin (BSA) and the Reagents A,B and C are prepared using standard solutions and Folin's Reagent for determining the presence of the protein in the sample.

2.7 PHYSIO-CHEMICAL PARAMETERS

The detergent sample were subjected to analyze various parameters initial and final respectively to determine the degradation rates. The parameters analyzed are as follows: PH, Electrical conductivity (EC), Total dissolved solids (TDS), Biological oxygen demand (BOD), Salinity, Alkalinity,Nitrates, Sulphates and Phosphates. For analyzing PH and Salinity PH-4500 H^+B was used. TDS was determined using 2540C method and TDS was dried at 180℃. Electrical conductivity was analyzed using 2510B laboratory method. 4500-O C method was used to analyze Biological oxygen demand. The incubation was at 27℃ for three days. Initial Dissolved oxygen (DO) was measured for both sample and blank. And later the third day DO was observed. The difference in the final and initial DO is the BOD. Chemical oxygen demand was analyzed using 5220B method. COD was performed by Open reflux manner. The treated COD sample was stirred up with ferroin indicator and titrated against using ferrous ammonium sulphate (FAS). The variation in the volume of blank and sample on titration is the measure of COD. Alkalinity was performed by Standard EPA method. Nitrates test was performed using 4500-NO3-B method. For the determination of Phosphates 4500-P E method of UV Spectrophotometer screening protocol is used. The content of Sulphates present is obtained by Absorbic acid method of 4500-SO_4^{2-}. The wavelength of UV for

Sulphates is 420 nm, for Nitrates it is 220 nm and 275 nm and for Phosphates 880nm is used (Eaton et al, 2017). UV Spectrophotometer (Systronics model 404) is used throughout the experiment for analyzing.

2.8 DETERGENT DEGRADATION

The density of the sample was founded initially. The concentration of the detergent waste water was calculated by using Titration methodwith the reference of winter-halter method of surfactant concentration. It was performed by adding two drops of phenolphthalein indicator to the sample which gives a pink colour to the solution and titrated against 0.1N Hydrochloric acid until the sample turns out to be colorless. Later the titrated sample is once again added with two drops of Phenolphthalein indicator in order to confirm the sample solution doesn't form a pink colour again. Later with the help of the chart, density and titration value is compared to find out the value of the concentration. The overall degradation is calculated by using the formula:

Overall degradation = (final conc- initial conc)/initial conc × 100

3. RESULTS AND DISCUSSION

3.1 CELL GROWTH

Successful treatment of wastewater with microalgae requires good growth, and understanding of the factors that affect growth is therefore essential. The growth rate of algae is influenced by physical, chemical and biological factors .Examples of physical factors are light and temperature. Chemical factors can be availability of nutrients and carbon dioxide, and biological factors are e.g. competition between species, grazing by animals and virus infections. Operational factors

affect the factors mentioned above, and basically concerns bioreactor design, mixing and dilution rate. The growth of micro algae was monitered for eight week time period. The cell growth under *Desmodesmus.sp*(fig1.a)was higher at 200,400ppm respectively and at Total concentration the algal growth was lasted upto 5 weeks, after the fifth week cells in the total concentration started to attain death phase. In *Scenedesmus.sp*(fig1.b) the growth was seen to be higher at 100 & 800 ppm. The growth over here lasted upto eighth week. Under chlorella sp (fig1.c) 100,200 ppm the maximum growth rate is obtained, even here the growth lasted upto the eighth week. However, here *Desmodesmus.sp* was found to more effective in growth under the detergent waste water than the *Scenedesmus.sp* followed by *Chlorella.sp.*

3.2 CHLOROPHYLL ESTIMATION

Algae capture light energy through photosynthesis and convert inorganic substances into simple sugar using the captured energy. Algae range from single – celled organism to multicellular organisms, Algae are made up of eukaryotic cells. These are cells with nuclei and organelles. All algae have plastids, the bodies with chlorophyll that carry out photosynthesis. But the various strains of algae have different combinations of chlorophyll molecules. Some have only Chlorophyll A, some only have chlorophyll B (Byung-Hwan Uma et al., 2009). Even here chlorophyll a is considered. Unlike normal cell growth analysis where the active and inactive is mixed together, here the active cell can be differentiated from that of inactive cell, that is the reason behind to perform this estimation. The chlorophyll estimation was performed for three different types of algal strains as shown in fig 2a,b and c. Here the chlorophyll was performed for the time period of seven week. Under *Desmodesmus.sp*, the maximum chlorphyll content was found, in the 5^{th} week of the total and 400ppm at 6^{th} week. For *Scenedesmus.sp*, the

maximum chlorophyll content was found in 7th week of total and followed by 400ppm respectively. Under chlorella sp, the maximum content was seen in 200ppm within two weeks, however, the same range of chlorophyll content was seen later at the 7th week of 400,800 and Total ppm respectively. Hence it is concluded that the maximum number of active cells grown was found to be more under chlorella sp, than that of desmodemus and scenedesmussp respectively in detergent waste water sample

3.3 BIOMASS ESTIMATION

Algae are good source of organic carbon. They uptake inorganic carbon (CO2), transform it into organic carbon and store it as cellular components of biomass which can be converted to energy (Bruton et al., 2009; Singh et al., 2011). The biodegradable components (i.e. carbohydrates, proteins and, lipids) representing most of the cellular constituents of the algal biomass, usually contribute to more than 70% of the dry cell mass and contains approximately 50% carbon by dry weight (Chisti, 2007; Sanchez Miron et al., 2000). The mineral composition of algae favorably meets the nutrient requirements of the anaerobic microflora. Along with the carbon, nitrogen and phosphorus (major components of algal biomass) several micronutrients such as iron, cobalt, zinc etc. are also found. These are known to stimulate methanogenesis and growth of anaerobic microflora (Sialve et al., 2009). Depending upon the species, algal biomass can either be enriched in any of the sugars, proteins or lipids or may have a balanced composition of these biomolecules.. Alternatively, microalgal biomass can be employed in the production of animal feed and fertilizers, as well as in various nutraceutical applications (Barba et al., 2014; Taelman et al., 2015). Apart from extensive use in cosmetics, fine chemicals, and value added products, microalgae can be also used

for energy generation, as biodiesel, bioethanol, or bio-hydrogen fuels, and in photosynthetic microbial fuel cells (Wang et al., 2015a; Zhu, 2015).

The biomass estimation of three algae strains at different detergent concentration were shown in table 1 a, b, and c. Higher biomass were found in 200ppm of chlorella, 400ppm of *Desmodesmus.sp* and 800ppm of *Scenedesmus.sp* respectively. Whereas the lower amount of biomass were found in total of chlorella, 100ppm of *Desmodesmus.sp* and 100ppm of *Scenedesmus.sp* respectively.

3.4 PROTEIN ESTIMATION

Proteins represent a large portion of organic nitrogen and carbon in wastewater treatment effluents, but their detailed characteristics and their role and fate in receiving waters are virtually unknown.(westgate,2010).

Figure 7 shows the protein estimation in three different algal strains of different detergent concentrations. The protein concentrations for *Desmodemus.sp* is higher at the total concentration i.e 2600ppm. For *Scenedesmus.sp*, it is the same like *Desmodesmus.sp*, at total it shows the maximum protein content followed by the 100ppm to its next. Under chlorella sp the higher amount of protein is found in total ppm concentration. However, among the three micro algal strains the maximum amount of protein content was found in chlorella than that of *Scenedesmus.sp* and *Desmodemus sp.*

3.5 PHYSIO-CHEMICAL PARAMETERS

This parameter is purposely done for comparing the degradation efficiencies of the detergent waste water sample. The PH value of the sample comes to 8.8 from 8.0,(Table 2) which a good PH for a treated water sample. Electrical conductivity of the sample comes to 4.25ms/ml from 5.02ms/ml (Table 2), the Salinity of the

sample is reduced to 2.15 from 2.74 ppm(parts per million) (Table 2). The initial Alkalinity value was 720 mg/L whereas the treated sample shows the value of 840 mg/L(Table 2), after the treatment process the PH and Alkalinity should increase to some extent which is good for the treated water for domestication purposes. The Biological oxygen demand (BOD) is the amount of dissolved oxygen needed by aerobic biological organisms to break down organic material present in a given water sample at certain temperature over a specific time period. Here the value of the Initial BOD sample was 54 mg/L(fig 3), however, after the algal treatment of *Desmodesmus.sp* it shows 5.25 mg/L, for *Scenedesmus.sp* it shows 11.25 mg/L and for chlorella it shows 9 mg/L. Morever reduction in the BOD values can be inferred after the algal treatment of detergent sample. The total dissolved solids (TDS) was reduced to 2.75 ppm from 3.6 ppm. The Nitrates test was performed to find out the reduction content after the treatment. In the initial, the amount of Nitrates was 11.7 g/L(fig 4) for detergent WW(waste water) sample. By treating with *Desmodesmus.sp*(fig 4a) the amount of nitrates was found to be 3.1016 g/L in the 6^{th} week at 200ppm and for *Scenedesmus,sp*(fig 4b) it comes to 2.6087 g/L in 6^{th} week at 200ppm. For chlorella sp(fig 4c) the value is around 2.8936 g/L in the 6^{th} week at 100ppm. In nitrate test we could observe that at lower ppm the degradation is high and it takes six weeks to perform this process.

The phosphate content in the initial detergent sample is 3.4344 g/L(fig 6). Later after the sample is subjected to the algal treatment the values are as follows: for *Scenedesmus.sp* under 400ppm the value is about 0.9426 g/L, for chlorella sp under 200ppm the value is about 0.01 g/L and for *Desmodesmus.sp* the value is about 0.03 g/L at 400ppm is the maximum reduction content found respectively. The initial sulphate value is 1647.5 g/L(fig 5) and later after the treatment under algae the values are as follows: for *Desmodesmus.sp* (fig 5a) at 200ppm the

maximum reduction value is obtained at 6th week and which is 137.57 g/L. For *Scenedesmus.s*p (fig 5b) at 200ppm the maximum reduction value is obtained of 317.2 g/L at 6thweek and for chlorella sp (fig 5c) at 6th week of 200ppm the maximum reduction value is obtained and that is 106.18 g/L.

3.6 DETERGENT DEGRADATION

The initial detergent concentration was calculated and noted as 2600 g/L (fig 8). Later after inoculating the algal strains into the detergent ww sample and by comparing the efficieny among the three, the *Desmodemus.sp* shows the maximum degradation content of 300 g/L, followed by chlorella of 600 g/L and eventually the *Scenedesmus.sp* which shows of about 900 g/L. Hence in this experiment we came to conclude that *Desmodesmus.sp* is the most effective degradating strains among the three(fig 8), particulary in this type of detergent waste water sample.

4. CONCLUSION

In this study various parameters such as PH, Electrical conductivity (EC), Total dissolved solids (TDS), Biological oxygen demand (BOD), Salinity, Alkalinity, Nitrates, Sulphates, Phosphates are analysed as well as Biomass, Protein Content and Chlorphyll content were estimated. However, by comparing with three different algal strains such as *Desmodesmus.sp, Scenedesmus.sp, Chlorella.sp* the effectiveness of the degradation was calculated in flask studies. For Industry, Textile effluents degradation in flask studies isn't enough, hence we have to scale up in a Reactor. Our future work is also based on comparative studies in the reactor with the same protocol. Moreover our desired product is the treated water sample which can be further used for several purposes including domestication etc., Later the algae which is the by-product is further harvested and treated for several purposes such as nutraceutical products, Bio-fuels and animal feed stock. The algal

byproduct which is subjected to run SDS-PAGE in order to find out the protein molecular weight present in it. Based on the protein molecular weight of the algal strains its applications is designed.

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Tables

Table 1: Biomass concentration of microalgae in various concentration of detergent treatment

Table 1a: Biomass concentration of Desmodesmus sp.,

S.NO	CONCENTRATION (mg)	BIOMASS (g/L)
1.	0	0.4175
2.	100	0.3553
3.	200	0.6185
4.	400	0.3029
5.	800	0.3115
6.	2600	0.2712

Table 1b: Biomass concentration of Scenedesmus sp.,

S.NO	CONCENTRATION (mg)	BIOMASS (g/L)
1	0	0.6266
2	100	0.2616
3	200	0.3308
4	400	0.5315
5	800	0.6908
6	2600	0.4833

Table 1c: Biomass concentration of Chlorella sp.,

S.NO	CONCENTRATION (mg)	BIOMASS (g/L)
1	0	0.8963
2	100	0.3708
3	200	0.3843
4	400	0.4715
5	800	0.4810
6	2600	0.1073

Table 2: Various Physio-Chemical Parameters

S.NO	PARAMETERS	INITIAL VALUE	FINAL VALUE
1.	PH	8.0	8.85
2.	ELECTRICAL CONDUCTIVITY(ms/ml)	5.02	4.25
3.	TOTAL DISSOLVED SOLIDS (ppm)	3.6	2.75
4.	SALINITY(ppm)	2.74	2.15
5.	ALKALINITY(mg/L)	720	840

FIGURES

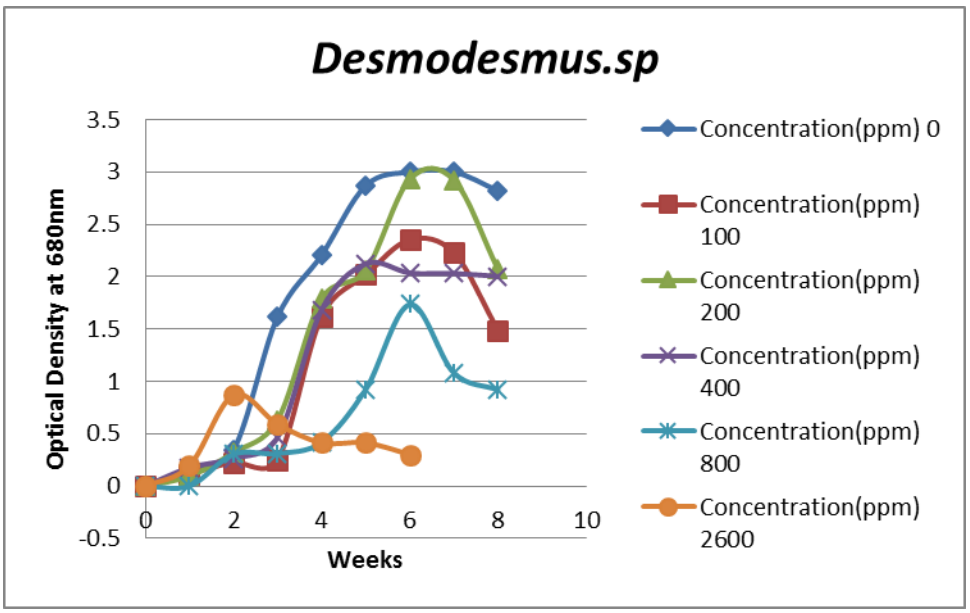

Fig 1a: Growth analysis of Detergent WW of Desmodesmus sp.,

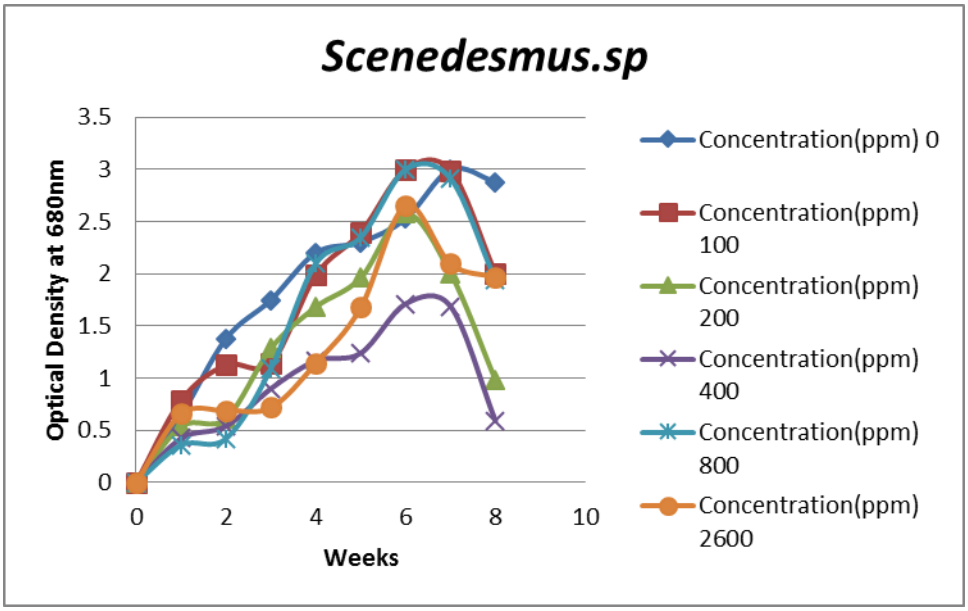

Fig 1b: Growth analysis of Detergent WW of Scenedesmus sp.,

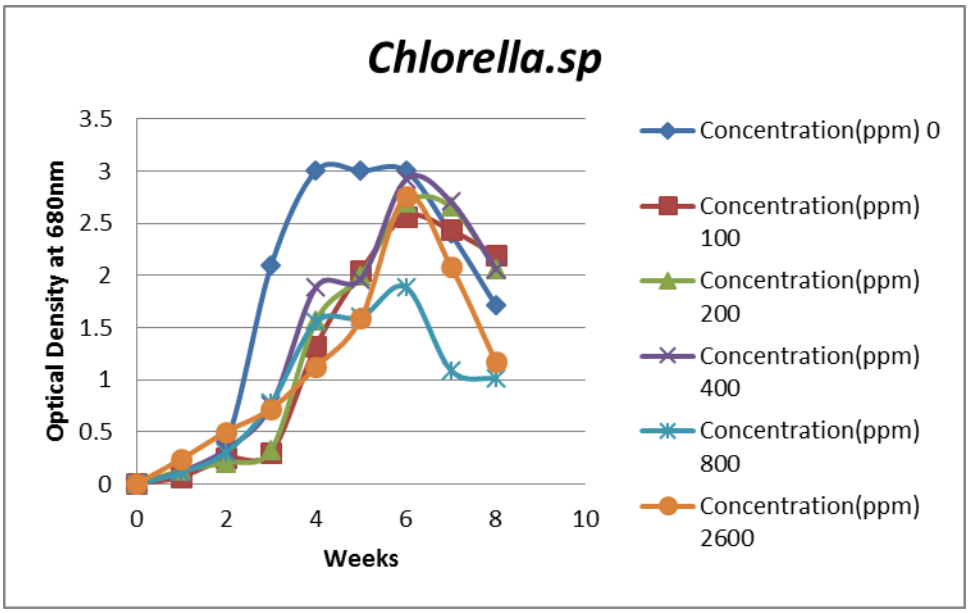

Fig 1c: Growth analysis of Detergent WW of Chlorella sp.,

Fig1: shows the Micro algal Growth trend at 680nm in UV spectrophotometer.

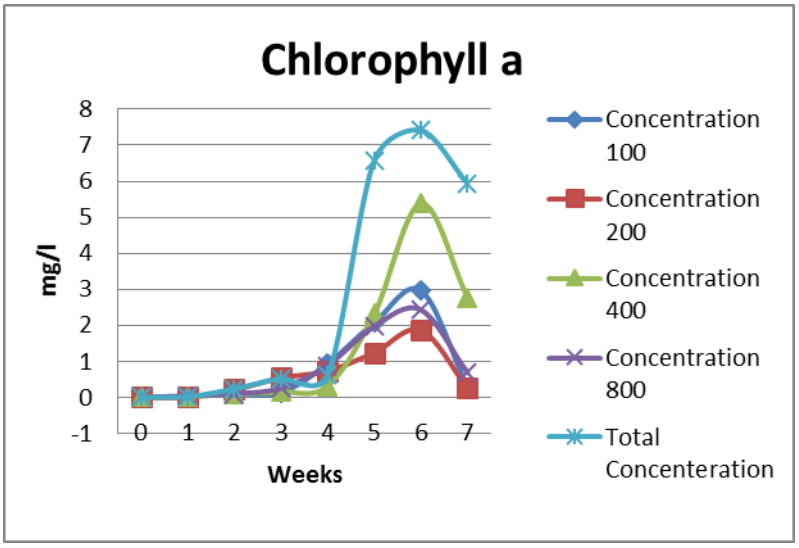

Fig 2a: Chlorophyll estimation of Desmodesmus sp.,

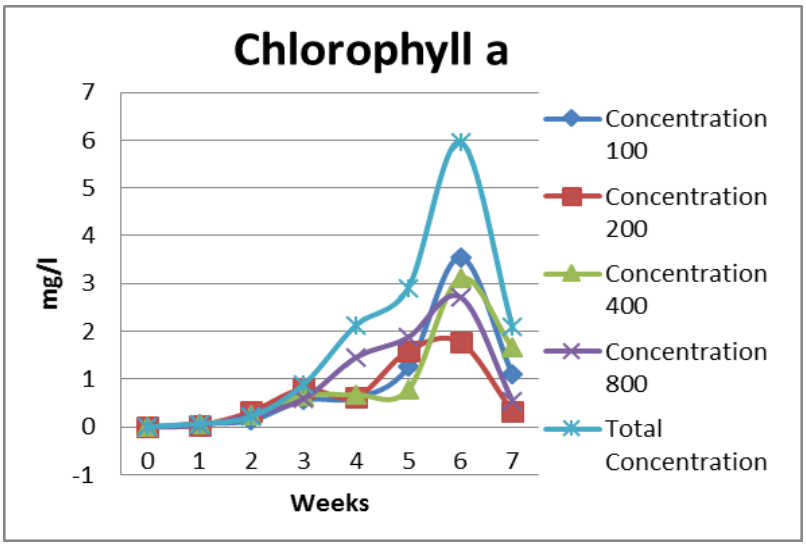

Fig 2b: Chlorophyll estimation of Scenedemus sp.,

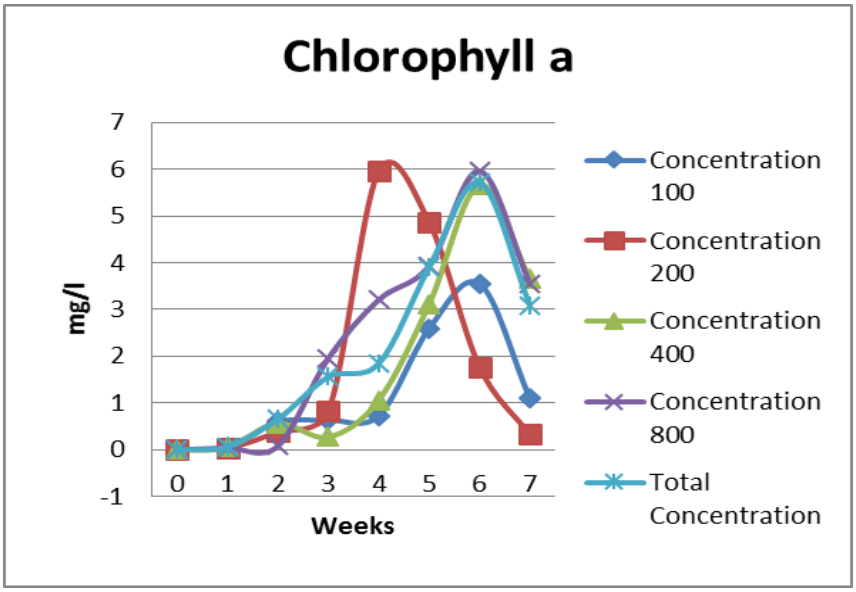

Fig 2c: Chlorophyll estimation of Chlorella sp.,

Fig 2: shows the Chlorophyll estimation of the three algal strains

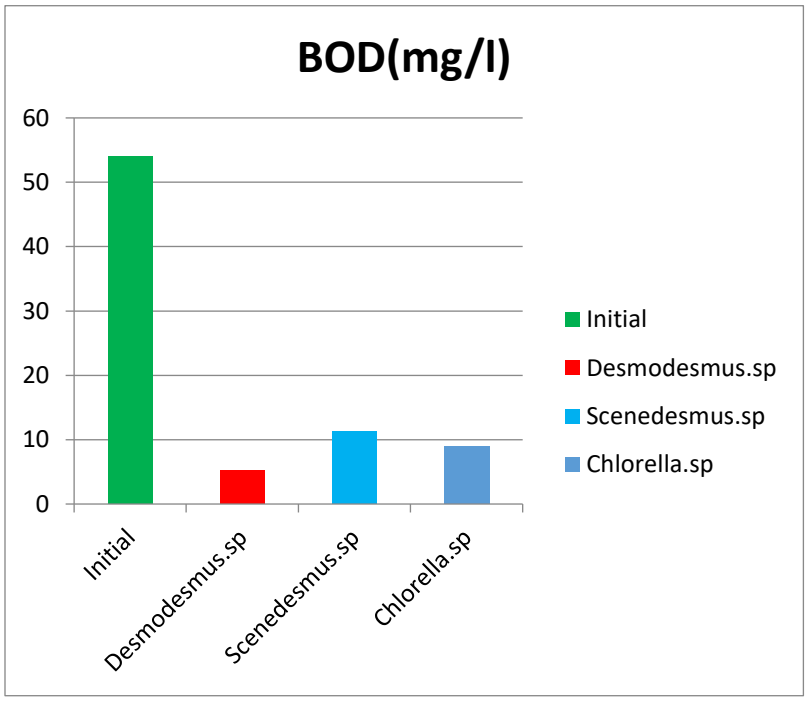

Fig 3: BOD values of detergent ww sample under the three algal strains

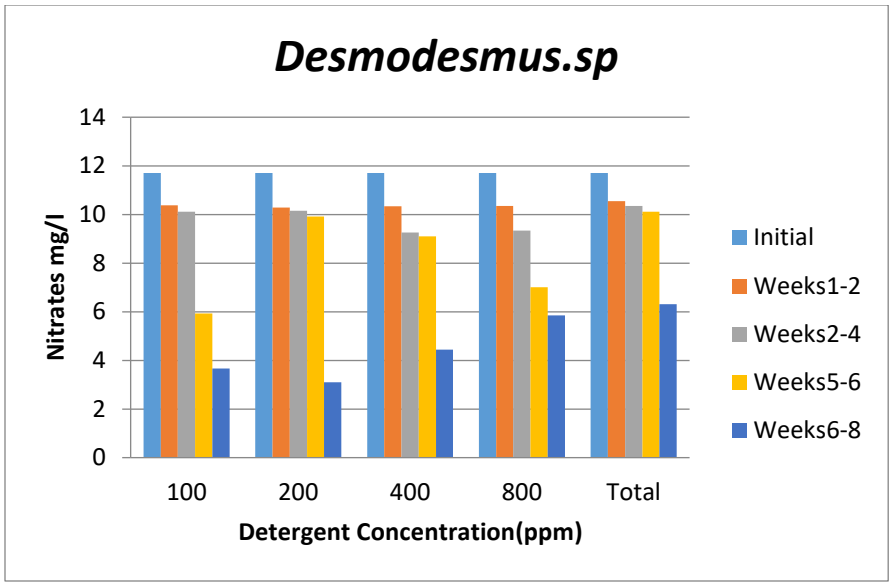

Fig 4a: Nitrates value of Desmodesmus Sp.,

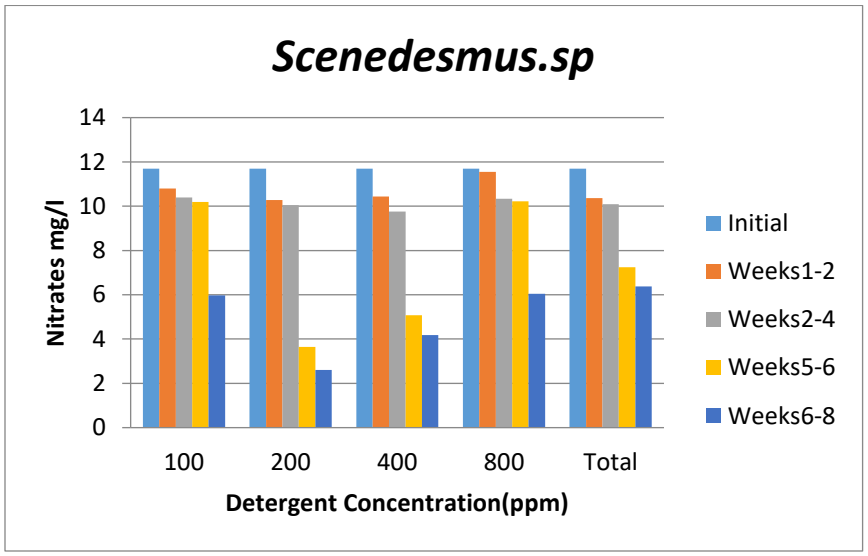

Fig 4b: Nitrates value of Scenedesmus sp.,

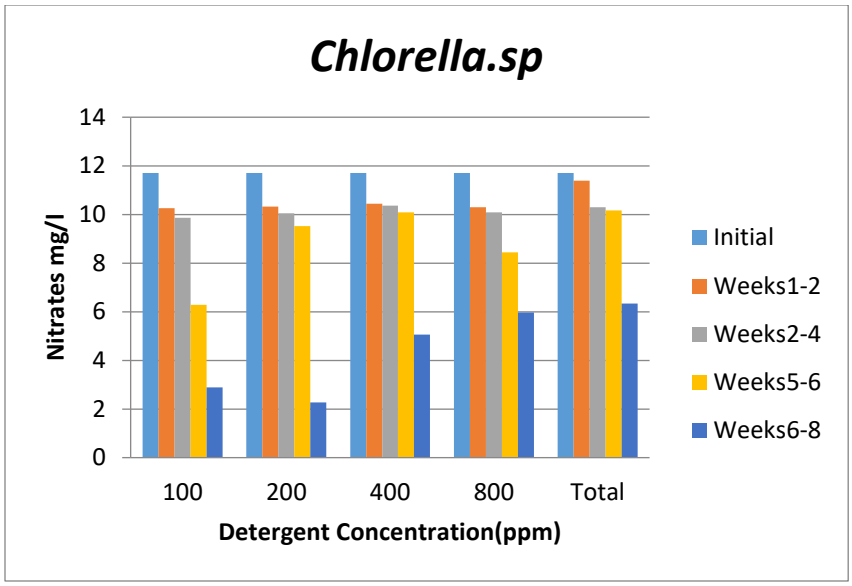

Fig 4c: Nitrates value of Chlorella sp.,

Fig 4: shows the Nitrates reduction in the three algal strains

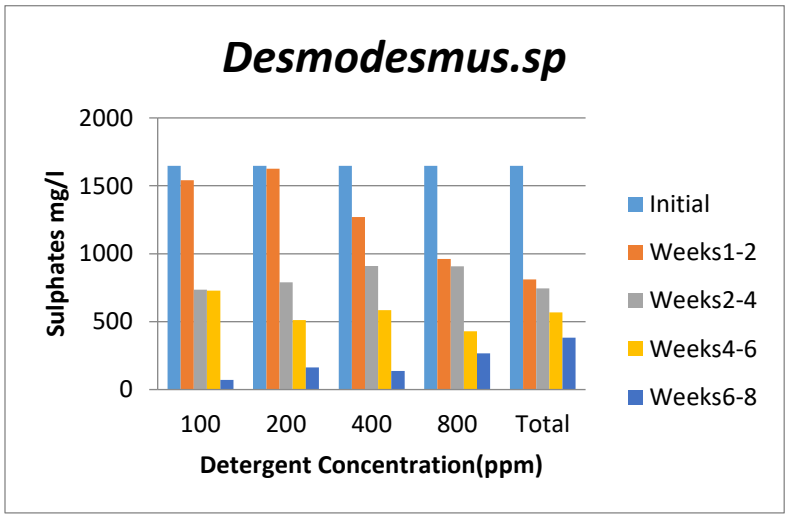

Fig 5a: sulphates value of Desmodesmus sp.,

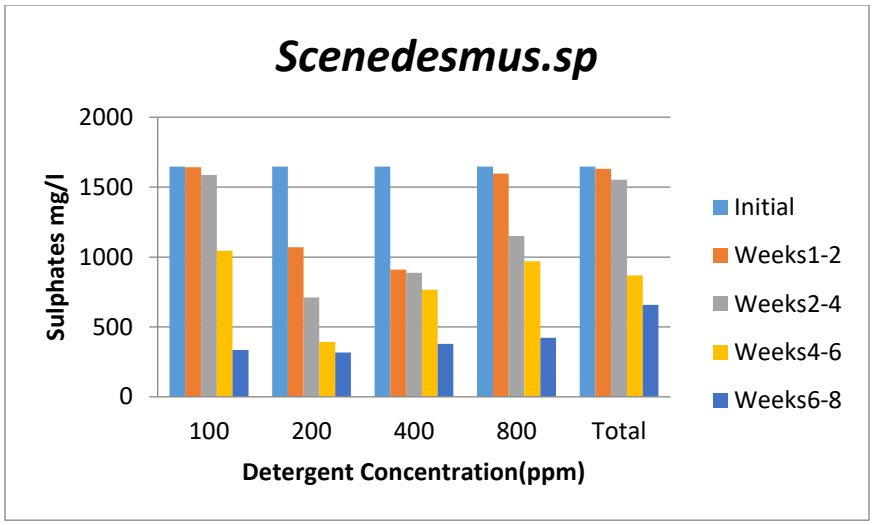

Fig 5b: sulphates value of Scenedesmus sp.,

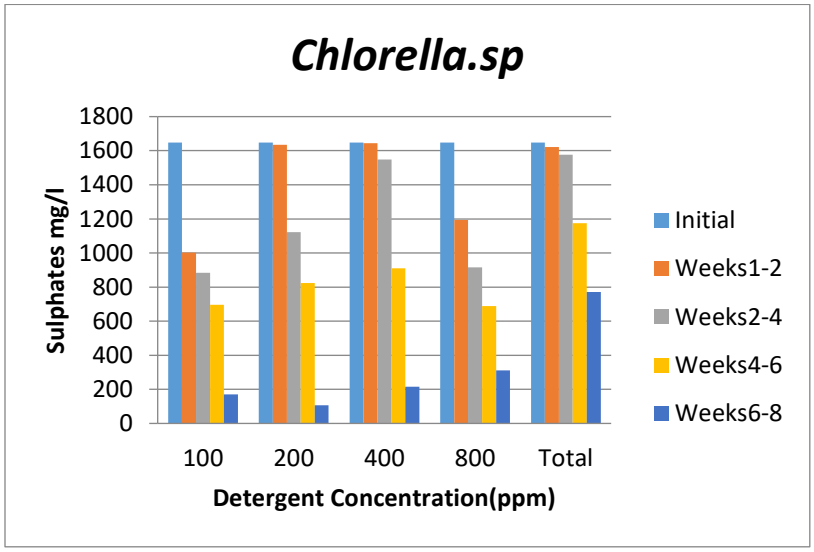

Fig 5c: sulphates value of Chlorella sp.,

Fig 5: shows the sulphates reduction under the three algal strains

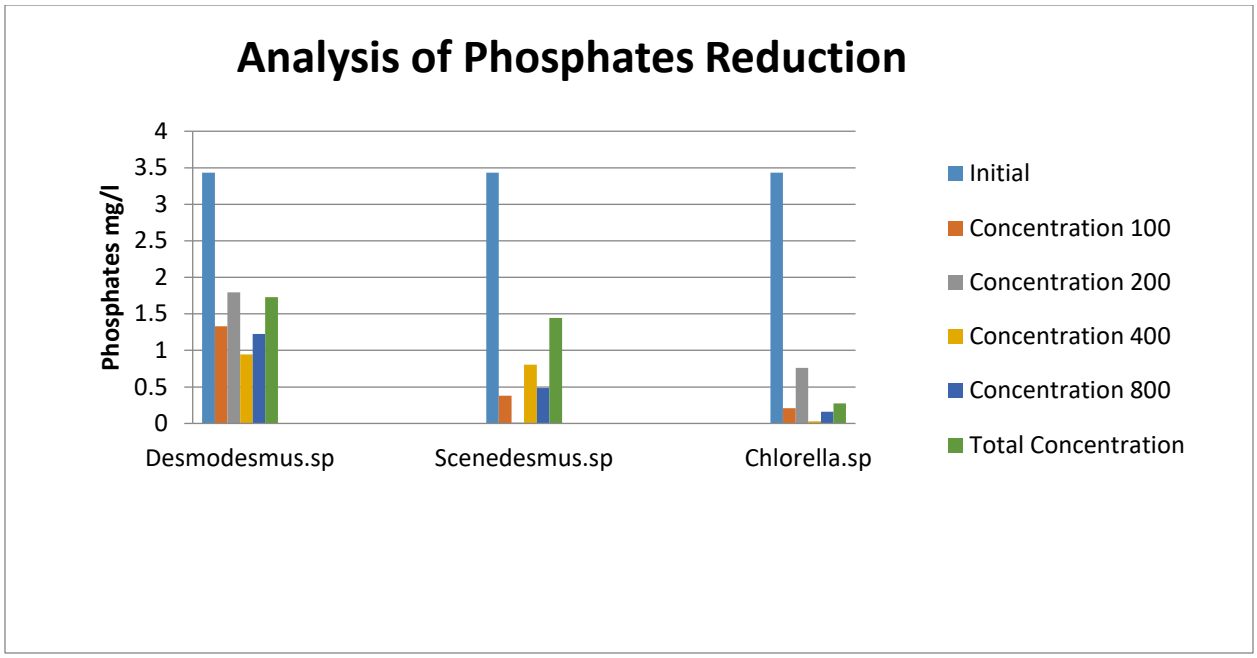

Fig 6: shows the phosphate reduction in the three algal strains

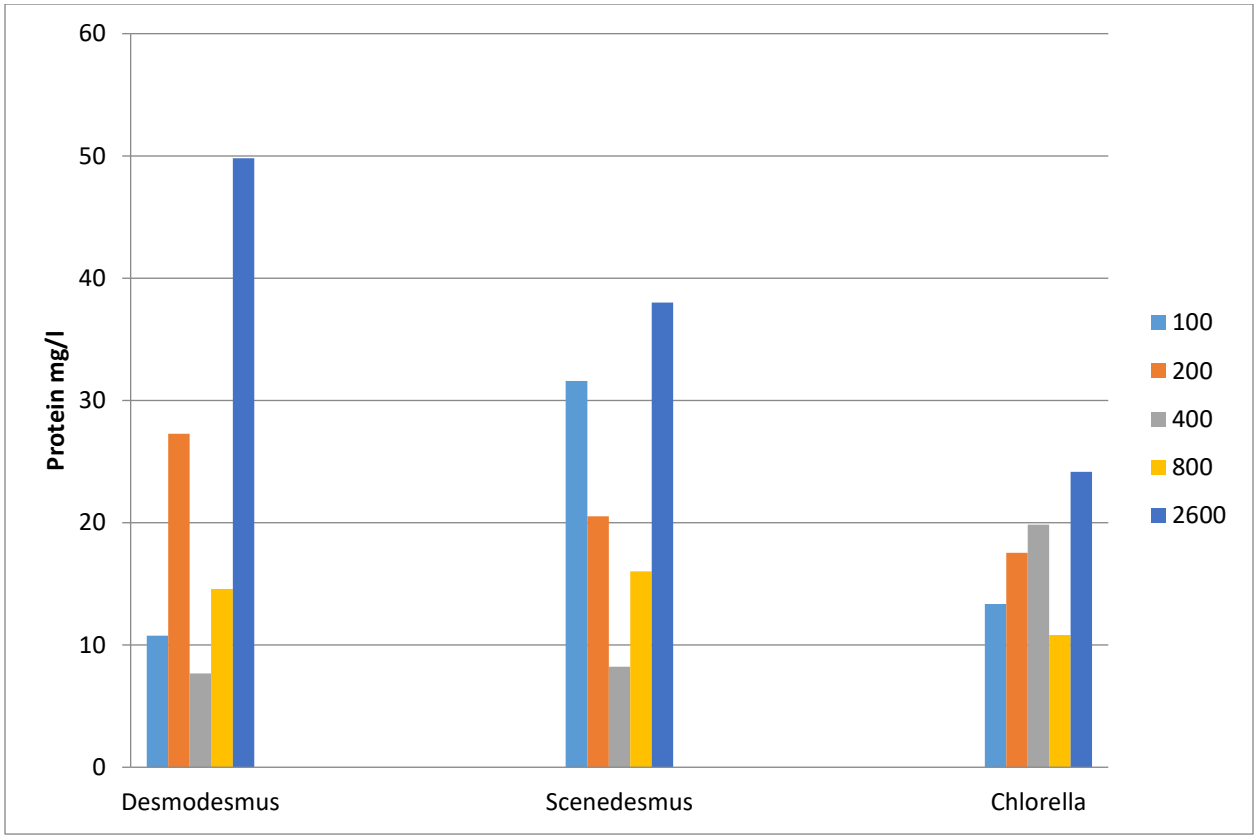

Fig 7: shows the Total Protein estimation in the three algal strains

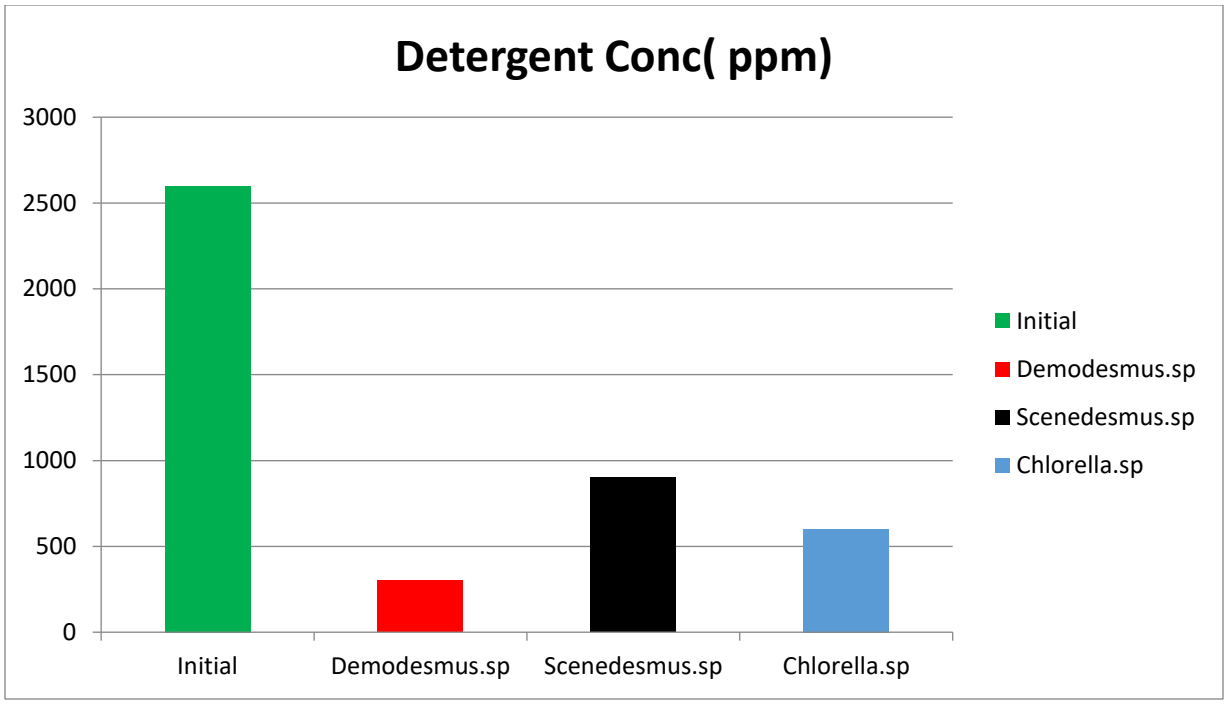

Fig 8: Total detergent degradation in the three algal strains