Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2019.23014

149 (2019) 76–90
May

Sewage treatment by using green algae *Scenedesmus*, *Chlorella* and their combination

P. Rajasulochana*, V. Preethy

Bharath University, Selaiyur, Chennai 600073, Tamil Nadu, India, Tel: +91-44-2229 0742; emails: prsnellore@gmail.com (P. Rajasulochana), preethy.vijaykumar@gmail.com (V. Preethy)

Received 16 May 2017; Accepted 15 August 2018

ABSTRACT

Novel methodologies are essential to reduce the concentrations of nutrients, microbes and chemicals from sewage prior to discharge into the environment. These are required to preserve and maintain the environmental conditions and human health. Although several techniques exist for the reduction of the chemicals and microbes, the efficacy of the techniques is not significant due to significant physical and structural diversity. Treatment of sewage with microalgae has gained popularity over the past few years, and limited investigations were reported in the literature. The present study deals with the treatment of household sewage treatment plant by using green algae, namely, *Scenedesmus*, *Chlorella* and their combination. During the experiment, it was observed that the algae grow under adverse condition and utilised the available nutrient for their survival. Further, the advantage of algae treatment is that they are efficient, ecofriendly, renewable and very much cost-efficient. Thus, many disorders can be prevented by using this treatment. The new study on mechanism of degradation and isolation of commercially important enzymes will be very significant.

Keywords: Toxic chemicals; Personal care products chemicals; Sustainable; Algae remediation

1. Introduction

In the present scenario, the rate of population growth and human development is considerably high. In view of this, there is demand for human health wherein water-borne diseases and infections cause human mortality and morbidity globally. In view of industrialisation and urbanisation, the pollution levels are increasing and the consumption of this polluted water is also increasing. On the whole, sanitation problems are increasing day by day in most of the countries. The quality of wastewater effluents is responsible for the degradation of receiving water bodies such as lakes, rivers, streams and oceans. In order to preserve environmental and human health, improved treatment process is needed to reduce nutrients, microbes and emerging chemical contaminates from domestic wastewater prior to discharge into the environment.

The reduction or removal of impurities from wastewater or sewage is broadly classified into two categories, namely, chemical and biological treatments. The content of residual toxic metals in wastewater treatment plants influences the choice of the removal method to be employed. Conventional methods for removing metals are either becoming inadequate to address current stringent regulatory efficient limits or increased cost. Several methods including environmentally friendly technologies based on the micro-organisms and/or microbial aggregates are usually used to remove pollutants. The methods include simulation, adsorption, biodegradation, complexation, iron exchange, post-precipitation iron-exchange, reverse osmosis, electrochemical treatment, evaporation, flocculation, precipitation and predation by micro-organisms and pollutant depletion through complex biochemical reactions during micro-organism metabolism [1,2].

The major microbial populations found in wastewater treatment systems include bacteria, protozoa, viruses, fungi, algae and helminths. The presence of these organisms

* Corresponding author.

in water leads to spread of several diseases. The use of biological material, including living and non-living micro-organisms has gained populating to remove or recover toxic metals from wastewaters, it has been observed from the literature that the treatment with biological materials is excellent [2–5].

Recently, quiet few studies were reported on the use of green and red algae for the biosorption of heavy metals. The heavy metals include: lead, copper, cadmium, zinc and chromium [6–8]. The mechanism in removal of heavy metals is related to the biochemical constitutions of the algae, especially their cell wall, as well as water chemistry. Biological approach for the control of foaming in activated sludge process was proposed by Khairnar et al. [9]. The potential application of bacteriophage needs much attention for the control of foaming since it is ecofriendly and cost-effective; micro-organisms in aquatic ecosystems include bacteria, yeasts, molds, protozoa, algae and viruses. Microbial aggregates, such as river epilithic biofilms and periphytons, are composed of heterotrophic and or autotrophic micro-organisms, which exist in many ecosystems including water and soil systems. Microbial aggregates play a significant role in natural aquatic ecosystems and biological wastewater treatment systems by affecting primary production, food chains, organic matter and nutrient curing, in addition to the accumulation of contaminations such as pesticides and toxic metals [10]. Wei et al. [11] studied the effect of three sulfonylurea herbicides and their degradation products on the green algae *Chlorella pyrenoidosa*. It was found that low concentrations of the degradation products would significantly stimulate the algal growth, and they can be used as the only nitrogen sources.

Freshwater green microalgae, such as *Chlamydomonas reinhardtii*, *Scenedesmus obliquus*, *C. pyrenoidosa* and *Chlorella vulgaris*, have been employed in studying the removal of various contaminants due to their high removal efficiencies for pollutants and potential feedstock for bioenergy production or other high value-added products [12–19].

Algae can be found in wastewater because they are able to use solar energy for photosynthesis as well as nitrogen and phosphorus for their growth leading to eutrophication [20]. Some types of algae that can be found in wastewater include *Euglena* sp., *Chlamydomonas* sp. and *Oscillatoria* sp. Algae are significant organisms for the biological purification of wastewater because they can be able to accumulate plant nutrients, heavy metals and pesticides, organic and inorganic toxic substances.

A major requirement in wastewater treatment is the removal of nutrients and toxic metals to acceptable limits prior to discharge and reuse [21]. Algae are autotrophs, that is, they can synthesise organic molecules themselves from inorganic nutrients. A stoichiometric formula for the most common elements in an average algal cell is C10H181O45N16P, and the elements should be present in these proportions in the medium for optimal growth [22]. Microalgae have been proven to be efficient in removing nitrogen, phosphorus and toxic metals from a wide variety of wastewaters [12,23,24]. These are extensive studies of algae growth in municipal [25,26], agricultural [27] and industrial wastewaters [28,29]. Substantial amounts of nutrient removal and algae biomass production were obtained in these studies. Hence, controlled microalgae cultivation shows promise as a potential biological treatment method for wastewater.

The studies related to detection of estrogenic endocrine-disrupting compounds by biological methods such as whole organisms, cellular and noncellular assays was carried out by Campbell et al. [30]. Symonds et al. [31] developed the bench top electrocoagulation unit to treat the industrial wastewater specially to remove the personal care products and it was observed that reduction is moderate. Wu et al. [32] provided the information of the role of micro-organisms play a vital role in removing the pollutants by consuming the inorganic nutrients in the polluted environment. It was mentioned that the microbe cleans the pollutants by assimilation, consumption of organic material and adsorption. The biofilm formed by the microbes plays role in removing the heavy metals, organic matter, phenol, nitrates, pentachlorophenol, trichlorophenol, sulphates and quinoline. Soltmann et al. [33], Davis et al. [34] and Chankitkan et al. [35] used algae to remove heavy metals from the industrial wastes and municipal wastes. The potential application of microalgae in wastewater treatment was reviewed by Abdel-Raouf et al. [36] and Carlsson et al. [37]. Various aspects covered in the review include (1) algae culture, (2) applications of algae in industry, (3) production of biodiesel and other products and (4) phycoremediation.

Table 1 shows the typical effects and diseases that are caused by the toxic chemicals present in the personal care products.

In the present investigation, sewage was treated with green alga, *Scenedesmus*, *Chlorella* and their combination. Standard methods were employed for the studies. Growth rate was measured for about 20 d at different intervals. To confirm the results, many repetitive studies were carried out. Average of the data is presented.

2. Materials and methods

2.1. Sample collection and analysis

The samples were collected from various sewage treatment plants (STPs) of Chennai, India, and the analysis of toxic chemicals present in the wastewater on the same day is carried out in CVR Private Ltd, Chennai, India.

2.2. Algae culturing

The microalgae samples of *C. vulgaris* and *Scenedesmus quadricauda* were purchased from the Phycospectrum Environmental Research Lab, Chennai, Tamil Nadu, India. The species were morphologically identified in the microscope. These microalgae were cultured and maintained in the bold's basal medium (BBM) [38].

Alga was cultured in two mediums namely BBM and Central Food Technological Research Institute (CFTRI) (one of the CSIR Laboratories, India). BBM is mostly used medium for freshwater species, and CFTRI is used for both freshwater and seawater as well as for outdoor culture. Alga in marine water is maintained at the constant temperature of 20°C and in the fresh water at 26°C. Cool-white fluorescent lighting to provide 4,304 lux was used at the liquid level with closure in place.

Table 1
List of occurrence of some common toxic chemicals from personal care products in wastewater from STP tanks

Chemical	Cosmetic application/usage in personal care products	Toxic effects in environment
Allylmethallyl ether	Masking agents Perfuming agents	Active ingredient of insecticides
Oxirane	Disinfectant cleaner, pump spray, professional use, pesticidal shampoo	Irritation of the eyes and skin, leukaemia, stomach and pancreatic cancer
Nonanoyl chloride	Soaps and detergents	Skin burns and respiratory damage
Methacrylamide	Shampoos and conditioners	Neurotoxicity and reproduction toxicity
Glycols, polyethylene	Shampoos, detergents, gels, etc.	Central nervous system depression, skin damage, etc.
Tetradecane, morpholine, pyrrolidine, oxazolidin-2-one	Shampoos, detergents, soaps, scents	Skin, respiratory damage, kidney and liver damage
7-Octenal, nonane, Citronellal	Scents, deodorants	Inhalation toxicity, respiration problems
1-Benzylindole, [(1-benzyl-1H-indol-3-yl) carbonyl]-3-hydroxyfuran-2(5H)-ones	Soaps, exfoliants, cleansers, facial masks, shaving creams, nail glues	Biological, cyto toxicity
Metoprolol	Commercial beauty care products	Beta-blocker toxicity, cardiovascular toxicity
7-Hydroxy-6-methoxy-2-methyl-2,4-hexadiyne	Skin care products	Toxicity to the aquatic organisms and skin irritations
Fumaronitrile	Sunscreen lotions, hair conditioning	Mutagenic activity and skin irritation and respiratory irritation
Indolizine	Deodorant and hair removal creams	Genotoxicity
Chalcone	Cosmetic and pet care products	Myotoxicity, oxidative damage, cytotoxicity
1,3,5-Cycloheptatriene	Dyes	Skin irritants
Diethyl phthalate	Perfumes, lotions, nail polishes	Insecticides, live damage

2.3. Screening

Screening was done to ensure that algae species can adapt to the sludge conditions and at which the concentration or ratio of culture of the algae degrades best. Usually screening was done in 10 mL (medium and sludge ratio), and 1 mL of culture was added equally to all 10 mL tubes with the initial cell count of 1.24×10^{-4} (per mL) approximately. The cell counting was done using the Haemocytometer. The growth rate was determined according to Levasseur et al. [39].

Growth rate: $K' = \text{Ln}\,(N_2/N_1) / (t_2 - t_1)$

where N_1 and N_2 = biomass at time 1 (t_1) and time 2 (t_2), respectively. It is done for both the species.

3. Wastewater treatment using algae

The treatment was started in small scale such as 100 mL of sludge in the conical flask with inoculums of 10 mL algae culture was added. The initial cell count of 17×10^{-4} (per mL) approximately of both the species was added equally to all the flaks. For the mixed culture total cell count was considered and it was 20×10^{-4} (per mL). It was done for both the species, and another flask of mixed strains was also done. The sludge treatment in 1 L of the sludge was initiated by adding the pellet taken from the 30 mL of the 100 mL grown culture in sludge and was centrifuged at 4,000 rpm for 10 min. It was done for both the species, and another flask of mixed strains was also done.

4. Discussion

The gas chromatography-mass spectrum (GCMS) of the wastewater analysis resulted in nearly hundreds of toxic chemicals, which are related to the triclosan and parabean chemicals. In the present study, about 200 chemicals of personal care products are found and are larger than the chemicals found in the biosolids of the wastewater. These compounds are directly flushed from each household septic tanks into sewage tanks. An analogous study was analyzing the pesticide and insecticide chemicals in wastewater. The highly toxic chemicals like oxirane, methacrylamide, metoprolol and diethyl phthalate were present in the wastewater, and these chemicals are not removed during the normal wastewater treatment, which was detailed by Ye et al. [40]. Ye et al. performed investigations to reduce the endocrine disturbing compounds by normal wastewater treatment technique, wherein the compounds are not removed effectively.

5. Comparison of growth rate of Chlorella, Scenedesmus and mixed culture

The pure cultures of the *Scenedesmus* and *Chlorella* have shown gradual increase in growth rate and decline after the nutrient is depleted over 20 d in 100 mL wastewater. The mixed culture at first shows the reduced growth and after some days it adapts and grows in the wastewater. In the mixed culture, growth of *Chlorella* is affected after few days but the growth of *Scenedesmus* keeps on increasing, which shows that *Scenedesmus* survives well in wastewater condition than *Chlorella*. The performance of the *Scenedesmus* in degrading the chemicals is found to be higher when compared with the *Chlorella*. Symonds et al. [31] carried out electrocoagulation treatment for reducing the chemicals and observed that it is efficient for certain level and cost of this treatment at industrial level is quite high and cannot be implemented in developing countries. Hence, in the present investigation, algae remediation, which is natural greener treatment, was used. These algae reduced these chemicals in the most efficient way than this electrocoagulation treatment with less energy and cost-efficient. It also reduced the triclosan related organic chemicals more effectively than other treatments. The phenolic compounds, antibiotics such as tetracycline, surfactants, benzoxazin, 2-trifluoro-pyrrolo[2,3-f] quinoline, indolizine and metoprolol are completely treated by the algae. *Scenedesmus* is found to be very efficient in reducing these compounds and it degraded mostly all the chemicals into lower molecular weight compound confirmed in the GCMS analysis. Only 40 chemicals related to triclosan are found after treatment with *Scenedesmus* algae. Though *Chlorella* has much efficiency to degrade and tolerate these chemicals but it has not grown in the wastewater even though sewage water is nutrient rich enough for growth of the algae. In the studies carried out by Zhou [12] by using *Chlorella* and *Scenedesmus* algal species, it was found that 28 chemicals were completely eliminated. In the present study, similar chemical degradation was obtained but the *C. vulgaris* could not grow on the chemical environment because of some other toxic chemicals such as metoprolol, oxrime, meracrylamide present in the sample.

During the wastewater analysis by GCMS, it was observed that most of the chemical compounds are high molecular weight of more than 100 due to its long chain. The occurrence of compounds with mass of 28 and 32 was higher. These compounds were highly complicated branched chain compounds. Hence, the microalga especially *Scenedesmus quadricauda* has amazingly broken these compounds into simpler compounds and also eliminated some of the compounds completely such that the mass of the chemicals has reduced to 30. The occurrence of compounds with mass of 22 and 24 was higher. The treatment with both *Chlorella* and *Scenedesmus* has shown more or less similar performance in reducing or degrading the chemicals when compared with the treatment with *Scenedesmus* and *Chlorella*. The occurrence of compounds with mass of 22 and 27 was higher. This has reduced the long chain into compounds of mass 32. This also has some complex chain compounds which are not present in the untreated sample which may be formed due to the degradation products and products released during their growth metabolism. Due to *Scenedesmus* treatment also, some unique compounds that are not present in the untreated samples are identified. Hence, these degraded products are not found to be toxic enough to the environment.

Through some common mechanism, the algae have ability to degrade the chemicals present in the wastewater and impart energy. Semple et al. [41] demonstrated the mechanism of reduction of the chemicals. The highly aromatic and phenolic compounds were degraded often to obtain carbon dioxide from that process. These are *ortho* and *meta* cleavage pathways and hence giving the clue that these algae may produce those enzymes for the degradation. Thus the degraded products were used as source of nutrients for the algal growth. The growth rate and decline are purely depending on the amount of degraded products released during the mechanism. The concentration of the degraded product influences the growth rate and metabolic rate of the algae. Table 2 presents the important chemicals identified in a sample collected from STP based on mass by charge ratio.

Table 2
Important chemical identified in a sample of typical STP

S. no.	Compound	RT (min)	*m/z*	S. no.	Compound	RT (min)	*m/z*
1.	Ethane, 1-chloro-2-isocyanato	7.675	281	33.	4-(3-Dimethylaminopropoxy) benzaldehyde	13.732	72
2.	2-1-Phenyl ethylidene-hydrazono-3-methyl-2,3-dihydrobenzothiazole	7.675	105	34.	6-Nitro-8-methoxy-2H-chromene	13.732	176
3.	1-Benzylindole	7.952	281	35.	Pyridine	13.876	79
4.	Piperidine	7.952	207	36.	*N*-Isobutyl-11-(3,4-methylenedioxyphenyl)-2E,4E,10E-undecatrienoic amide	14.063	355
5.	Carbamic acid	8.102	281	37.	3′-Amino-6-methoxyaurone	14.226	267
6.	3-Methylindole-2-carboxylic acid	8.102	207	38.	Metoprolol	14.226	72
7.	Adenosine	8.102	207	39.	1(2H)-Isoquinolinone	15.852	136
8.	1,5-Hexadiyne	8.471	281	40.	2,4-Hexadiyne	15.965	78
9.	Benzene	8.471	78	41.	Fumaronitrile	15.965	51
10.	Propanedinitrile	8.471	78	42.	1,1′-Bicyclopropyl	17.466	82
11.	Titanium	8.859	105	43.	Thiazolidine-2,5-dione	19.462	74
12.	Indolizine	8.859	179	44.	2,1-Benzisoxazole	20.894	118.8
13.	Cyclohexane-1,3-dione	8.859	179	45.	1-(2-Cyanovinyl)aziridine-2-carbonitrile	20.894	119
14.	Morpholine	9.147	191.1	46.	Bicyclo[2.2.1]hept-5-ene-2-carbonitrile	20.894	66

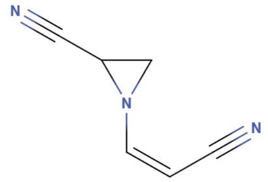
Table 2 *Continued*

S. no.	Compound	RT (min)	*m*/*z*	S. no.	Compound	RT (min)	*m*/*z*
15.	9-Octadecenamide	9.147	266	47.	6-[*N*-Aziridyl]hexadiene-1,3	25.404	123
16.	Methyl-2,3-dihydrobenzothiazole	9.147	264	48.	5,6-Epoxy-2,2-dimethyl-3-heptyne	25.404	108
17.	*N*-Benzoylglycine ethyl ester	9.434	206.9	49.	3-Aminocrotononitrile	27.162	105
19.	5H-Tetrazol-5-amine	9.710	85.1	50.	Furan	27.162	67
20.	Azetidine	9.710	69	51.	1H-Imidazole	27.162	82
21.	Acetic acid	9.935	90.9	52.	1,1,3,3,5,5,7,7,9,9-Decamethyl-9-(2-methylpropoxy)pentasiloxan-1-ol	28.657	401
22.	*N*-Chloro-2-methylaziridine	9.935	91	53.	Amodiaquine	28.675	355
23.	Benzaldehyde	10.091	91	54.	1,3,5,7,9-Pentaethylbicyclo[5.3.1] pentasiloxane	28.675	282
24.	Diaminomethylidenhydrazone	10.091	206.9	55.	2,5,8-Triphenyl benzotristriazole	32.197	429
25.	*m*-Nitrobenzaldehydeacetylhydrazone	10.191	192	56.	Acetamide	32.197	91
26.	Ethane	10.410	44	57.	Thiocyanic acid	35.169	87
27.	Guanidine	10.604	91	58.	(Methylthio)-acetonitrile	35.169	87
28.	Propanenitrile	11.094	90.9	59.	*O*-methyloxime	35.169	87
29.	1-Methyl-3,5-dinitro-1H-[1,2,4]triazole	11.094	54	60.	2-Propanone	35.169	87
30.	3,4-Methylenedioxy-3-methylamino-butane	11.392	91	61.	2-1-Phenyl ethylidene-hydrazono-3-methyl-2,3-dihydrobenzothiazole	44.789	206.9
31.	Carbamic acid	13.113	58	62.	4-(Benzoylmethyl)-6-methyl-2H-1, 4-benzoxazin-3-one	44.789	281
32.	2-Propenamide	13.300	71	63.	Benzamide	44.789	148
33.	4-(3-Dimethylaminopropoxy) benzaldehyde	13.732	72	64.	4-(Benzoylmethyl)-6-methyl-2H-1,4-benzoxazin-3-one	45.295	207
65.	Pentafluorobenzoic acid	45.295	148	73.	Chalcone	49.648	207
66.	Morpholide	45.295	176	74.	Malonic acid	49.825	176
67.	2-Amino-7-methyl-5-oxo-4-pyridin-3-yl-4H,5H-pyrano[4,3-b] pyran-3-carbonitrile	45.295	195	75.	Pentafluorobenzoic acid	49.825	148
68.	Pyrrolo[2,3-f]quinoline	47.573	110	76.	Morpholide	49.825	176
69.	4a,5,6,7,8,8a,10,10a-Octahydro-2H-1-oxa-9a-azaanthracen-9-one	47.836	189	77.	4-Piperidinecarboxylic acid	57.206	105
70.	6-Azaspiro[2.5]octa-4,7-diene-6-carboxylic acid	47.832	192	78.	l-Histidine	57.206	203
71	4-Trimethylsilylmethylbenzamide	47.832	192	79.	Carbonic acid	57.203	203
72.	Gamma-Cyano-3-methyl-5,10-dihydrobenzo[f]indolizine	49.648	191	80.	Benzamide	57.203	148

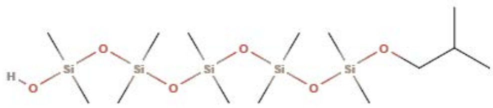
The corresponding chemical structures are presented in Fig. 1.

Samples were collected from several STPs of Chennai, India, at different timings. Fig. 2 shows the overall average growth rate of *Chlorella* in wastewater containing toxic chemicals, and Fig. 3 presents the typical growth of the algae in wastewater containing toxic chemicals. Fig. 4 gives the overall average growth rate of *Scenedesmus* in wastewater containing toxic chemicals, and the corresponding typical growth of the algae in wastewater containing toxic chemicals is shown in Fig. 5.

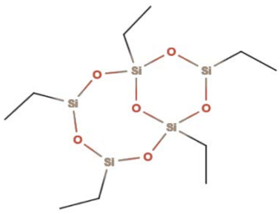
Fig. 6 presents growth rate of combined *Chlorella* and *Scenedesmus* in wastewater containing toxic chemicals, and Fig. 7 shows the corresponding growth of the algae in wastewater containing toxic chemicals. Table 3 presents average algae growth rate which was determined by the cell counting and change in the pH of the water in the pilot scale bottles containing the wastewater with toxic chemicals. Fig. 8 shows the colour change after treatment indicating the growth of the algae (*Chlorella*, *Scenedesmus*, mixed cultures) in wastewater containing toxic chemicals and thereby reducing the chemicals in the wastewater. Table 4 presents the consolidated chemicals found in the treated sample with *Chlorella*, *Scenedesmus* and combined. From Tables 2 and 4, it can be noted that the algae are very efficient in removing the most of the chemicals including toxic in nature. All the three methods found to be very effective in treating the sewage or wastewater. Kabir [42] carried out studies on performance of microalgae *C. vulgaris* and *S.* obliquus in wastewater treatment of Gomishan (Golestan-Iran) shrimp farms. Their results showed that among the various treatments, mix treatment shows the best result in the removal of organic and inorganic compounds from Gomishan shrimp farms. Koreivienė et al. [43] performed similar studies for remediation of wastewater by using *Chlorella*/*Scenedesmus*



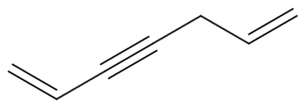
1-(2-Cyanovinyl)aziridine-2-carbonitrile



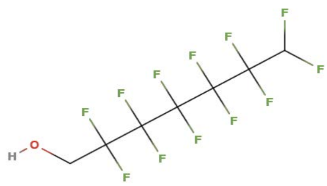
1,1,3,3,5,5,7,7,9,9-Decamethyl-9-(2- methylpropoxy) pentasiloxan-1-ol



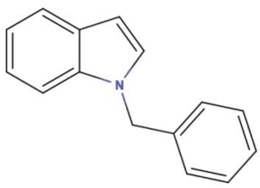
1,3,5,7,9-Pentaethylbicyclo [5.3.1]pentasiloxane



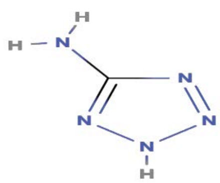
1,6-Heptadien-3-yne



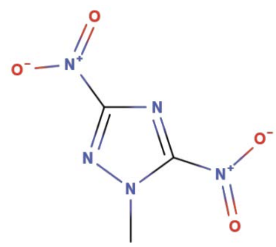
1h,1h,7h-dodecafluoro-1-heptanol



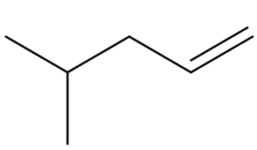
1-benzylindole



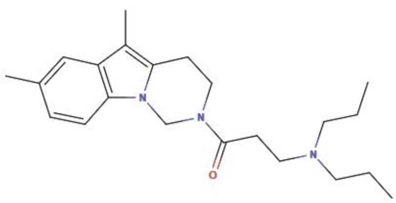
1H-Tetrazol-5-amine



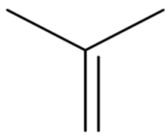
1-Methyl-3,5-dinitro-1H-[1,2,4] triazole



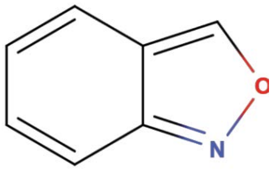
1-Pentene, 4-methyl-



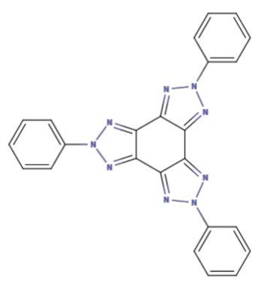
2-(.beta.-Dipropylaminopropionyl)-5,7-dimethyl1,2,3,4-tetrahy dropyrimido(3,4-a)indole



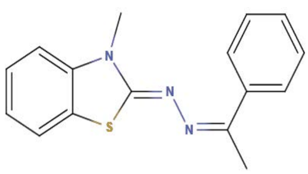
1-propene, 2-methyl-



2,1-Benzisoxazole

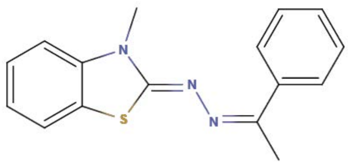


2,5,8-Triphenyl benzotristriazole

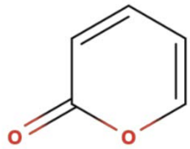


2-1-Phenylethylidene-hydraZono -3-methyl-2,3-DihydroBenzo thiazole

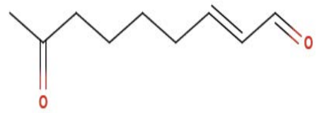
Fig. 1. Chemical structures corresponding to the identified chemicals of Table 2.



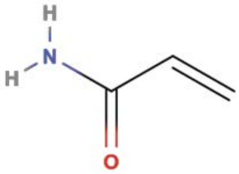
2-1-Phenylethylidene-hydrazono-3-methyl-2,3-dihydrobenzothiazole



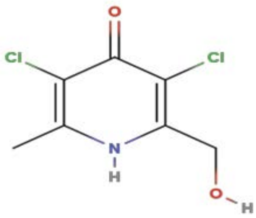
2h-pyran-2-one



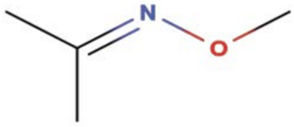
2-Nonenal, 8-oxo



2-Propenamide

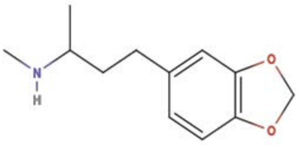


2-Pyridinemethanol3,5-ichloro-4-hydroxy-6-methyl

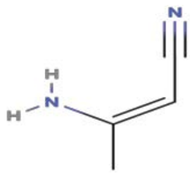


2-Propanone, O-methyloxime

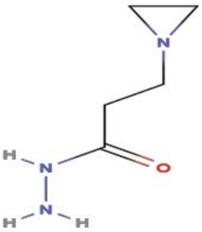
2-Undecene, (E)



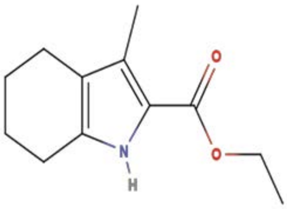
3,4-Methylenedioxy-3-methylaminobutane



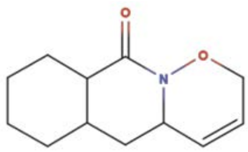
3-Aminocrotononitrile



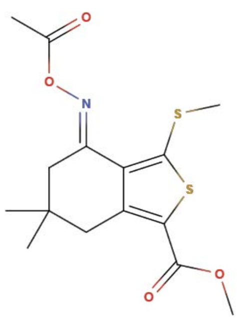
3-[N-Aziridyl]propionylhydrazide



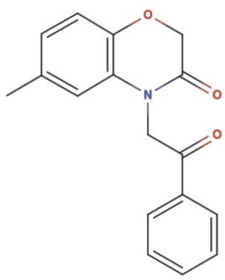
3-Methylindole-2-carboxylicacid, 5,6,7-tetrahydro-, ethyl ester



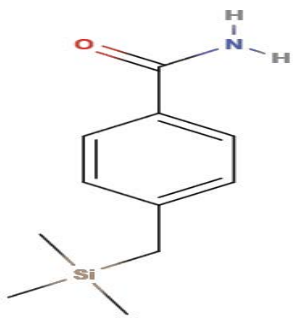
4a,5,6,7,8,8a,10,10a-Octahydro-2H-1-oxa-9a-azaanthracen-9-one



4-Acetyloxyimino-6,6-dimethyl-3-methylsulfanyl-4,5,6,7-tetrahydrobenzo[c]thiophene-1-carboxylic acidmethyl ester

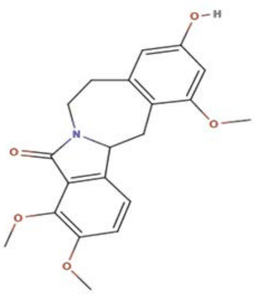


4-(Benzoylmethyl)-6-methyl-2H-1,4-benzoxazin-3-one

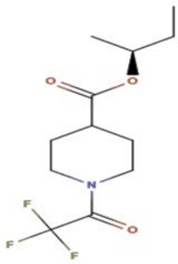


4-Trimethylsilylmethylbenzamide

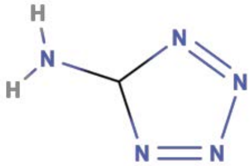
Fig. 1. *(continued)*



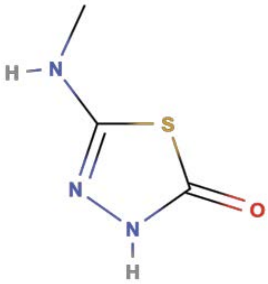
5H-Isoindolo[1,2-b][3] benzazepin-5-one,7,8,13,13a-tetrahydro-10-hydroxy-3,4,12-trimethox



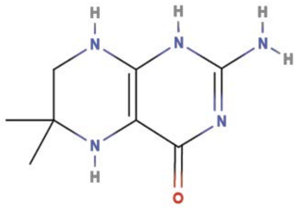
4-Piperidinecarboxylicacid,1-(trifluoroacetyl)-,1-ethylpropyl ester,(S)



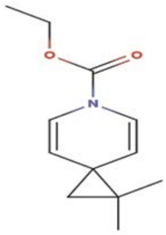
5h-tetrazol-5-amine



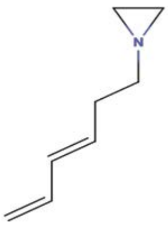
5-Methylamino-1,3,4-thiadiazole-2-one



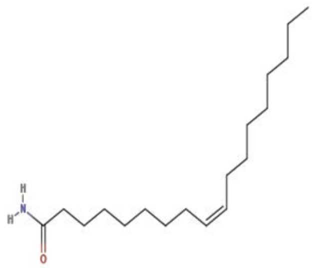
6,6-Dimethyltetrahydropterin



6-Azaspiro[2.5]octa-4,7-diene-6-carboxylicacid,2,2-dimethyl-, ethyl ester

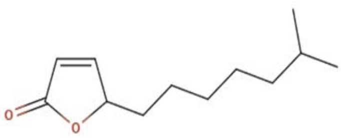


6-[N-Aziridyl]hexadiene-,3

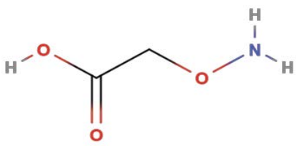


9-octadecenamide, (z)

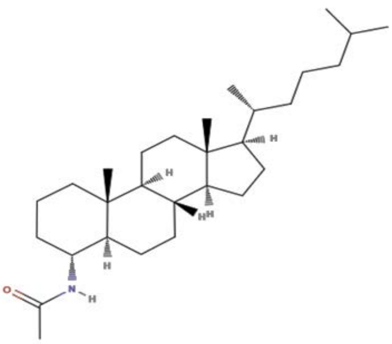
9-Octadecene, (E)



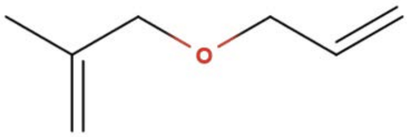
10-Methylundec-2-en-4-olide



Acetic acid, (aminooxy)-

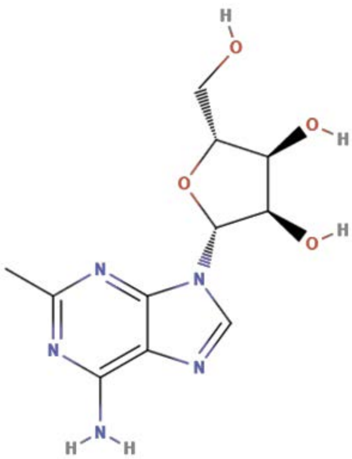


Acetamide, N-[(4.alpha.,5.alpha.)-cholestan-4-yl

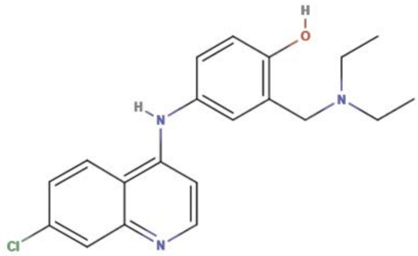


Allylmethallyl ether

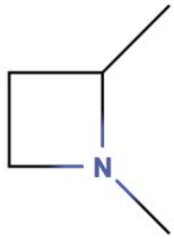
Fig. 1. *(continued)*



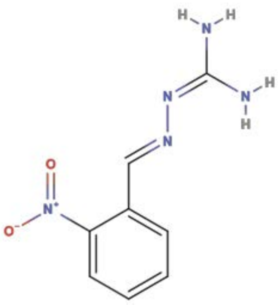
Adenosine, 2-methyl



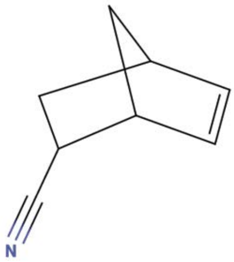
Amodiaquine



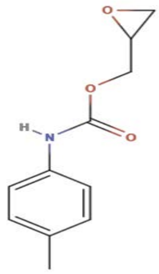
Azetidine, 1,2-dimethyl



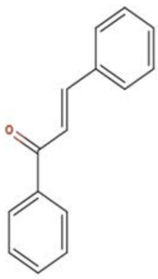
Benzaldehyde,2-nitro-diaminomethylidenhydrazone



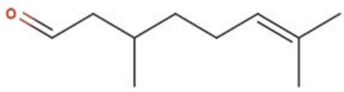
Bicyclo[2.2.1]hept-5-ene-2-carbonitrile



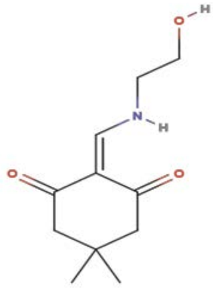
Carbamicacid,N-(4-methyl phenyl)-,oxiranylmethylester



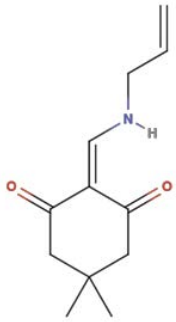
Chalcone



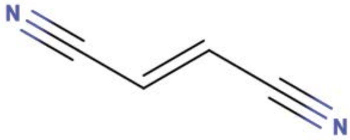
Citronellal



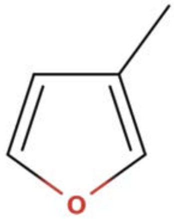
Cyclohexane-1,3-dione,2-(2-hydroxyethylaminomethylene)-5,5-dimethyl



Cyclohexane-1,3-dione,2-allylaminomethylene-5,5-dimethyl

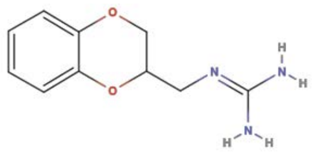


Fumaronitrile

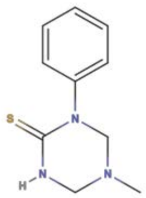


Furan,3-methyl-

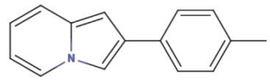
Fig. 1. *(continued)*



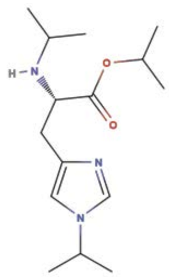
Guanidine,(1,4-benzodioxan
-2-ylmethyl)-



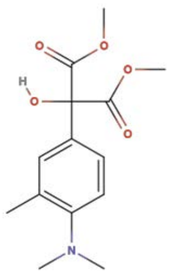
Hexahydro-5-methyl-1-phenyl-
1,3,5-triazine-2-thione



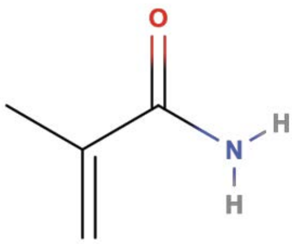
Indolizine,2-(4-methylphenyl)-



L-Histidine,N,1-bis(1-methylethyl)-
,1-methylethyl ester

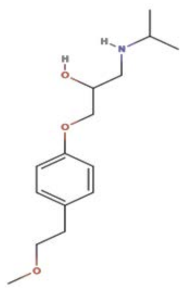


Malonicacid,2-hydroxy-2-(4-
dimethylamino-3-ethylphenyl)-,
dimethyl ester

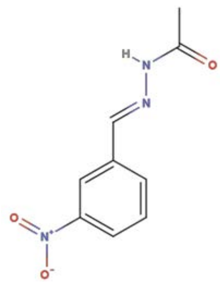


Methacrylamide

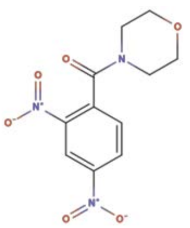
Methyl 10,12-pentacosadiynoate



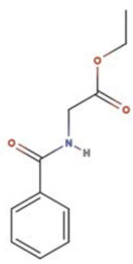
Metoprolol



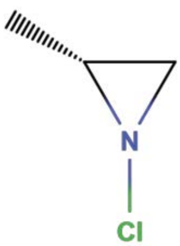
M-
Nitrobenzaldehydeacetylhydrazone



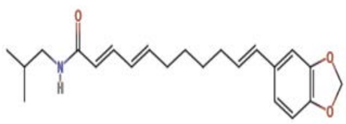
Morpholine,4-(2,4-dinitro
Phenylbenzoyl)



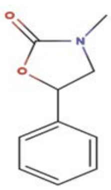
N-Benzoylglycineethylester



N-Chloro-2-methylaziridine, 1,2-cis



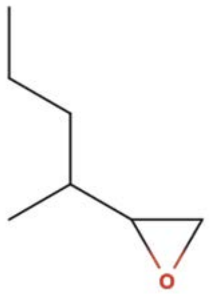
N-Isobutyl-11-(3,4-methylene
dioxyphenyl)-E,4E,10E-
undecatrienoic amide



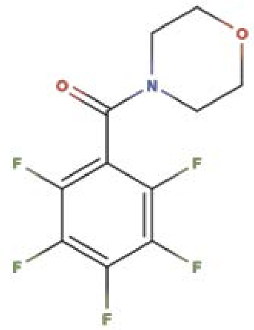
Oxazolidin-2-one,3-methyl-5-
phenyl-

Oxirane

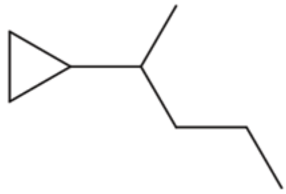
Fig. 1. *(continued)*



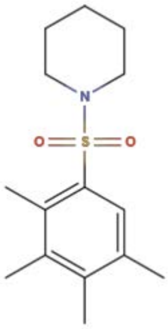
Oxirane, (1-methylbutyl)-



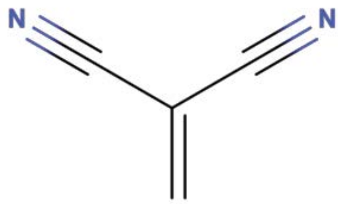
Pentafluorobenzoicacidmorpholide



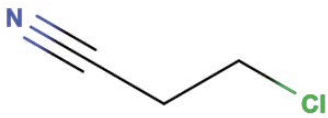
Pentane, 2-cyclopropyl-



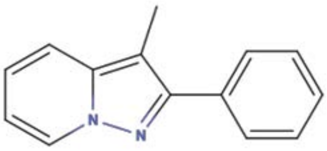
Piperidine,1-[(2,3,4,5-tetramethylphenyl)sulfonyl]



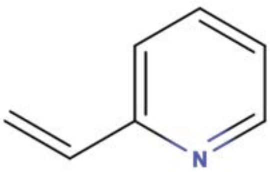
Propanedinitrile,methylene-



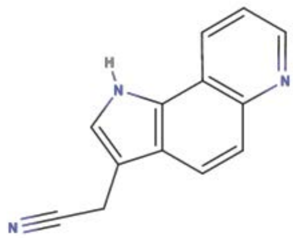
Propanenitrile,3-chloro-



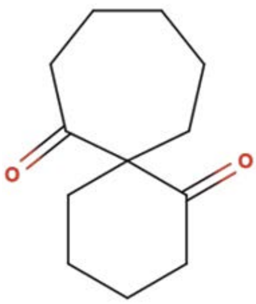
Pyrazolo[1,5-a]pyridine,3-methyl-2-phenyl



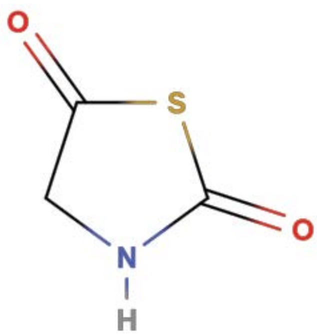
Pyridine, 2-ethenyl-



Pyrrolo[2,3-f]quinoline,3-acetonitrile-



T-Butyl cyclopentane peroxycarboxylate [loading]



Thiazolidine-2,5-dione

Thiocyanicacid,ethylester

Fig. 1. Chemical structures corresponding to the identified chemicals of Table 2.

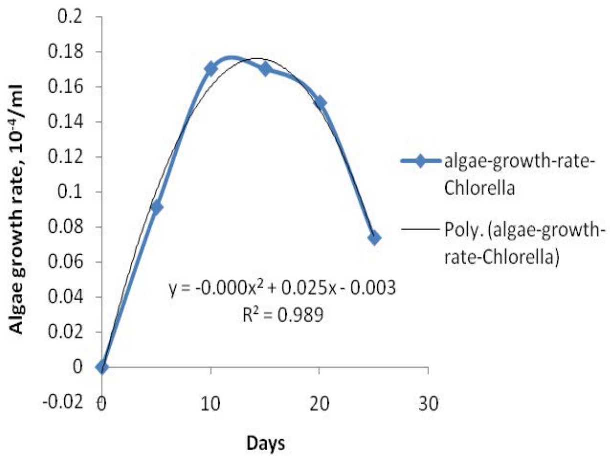


Fig. 2. Average growth rate of *Chlorella* in wastewater.

Fig. 3. Typical growth of the algae in wastewater.

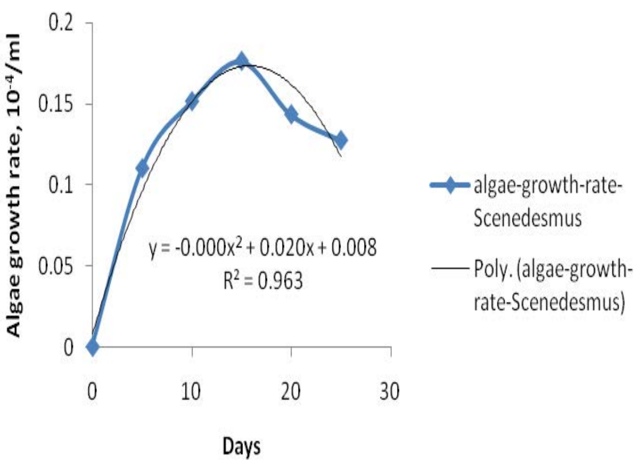


Fig. 4. Average growth rate of *Scenedesmus* in wastewater.

Fig. 5. The growth of the *Scenedesmus* algae in wastewater.

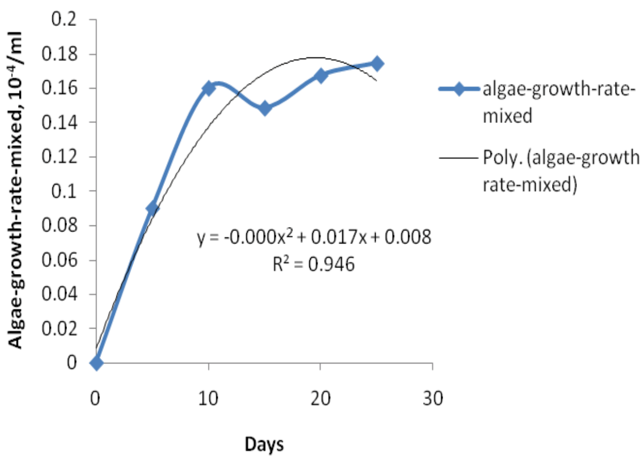


Fig. 6. Average growth rate of combined (*Scenedesmus* + *Chlorella*) in wastewater.

Fig. 7. The growth of the typical combined algae in wastewater.

Fig. 8. Colour formation after treatment indicating the growth of the algae (*Chlorella*, *Scenedesmus*, mixed cultures).

microalgae and found that the combination is a promising tool for nutrients elimination from the wastewater.

6. Summary and conclusion

Efficiency of green algae, namely, *Scenedesmus*, *Chlorella*, and their combination, has been employed to treat sewage. Household collected sewage from various STPs of Chennai, India, has been collected at different times. Standard protocols have been employed to identify chemicals available in the untreated and treated samples. Most of the chemicals and their structures are identified. Several experiments have been carried out to observe the growth of algae in sewage at different intervals such as 1st day, 5th day, 10th day, 15th day, 20th day and 25th day. Average growth is determined

Table 3
Algae growth rate and corresponding pH

Algae species	Initial day 1×10^{-4} (per mL)	Growth rate $\times 10^{-4}$ (per mL)	pH	Day 5×10^{-4} (per mL)	Growth rate $\times 10^{-4}$ (per mL)	pH	Day 10×10^{-4} (per mL)	Growth rate $\times 10^{-4}$ (per mL)	pH	Day 15	Growth rate $\times 10^{-4}$ (per mL)	pH	Day 20×10^{-4} (per mL)	Growth rate $\times 10^{-4}$ (per mL)	pH	Day 25×10^{-4} (per mL)	Growth rate $\times 10^{-4}$ (per mL)	pH
Chlorella (10)	17	0	6.85	21.75	0.0911	7.80	55.25	0.1700	8.85	102	0.1700	7.70	141	0.1506	7.7	61	0.0737	7.6
Senedesmus (10)	12	0	6.85	19.5	0.110	7.60	34.25	0.1513	8.75	76.75	0.1761	7.86	78	0.1432	7.8	109	0.1273	7.8
Chlorella + *Senedesmus* (5:5)	20.5 + 12.25 = 32.25	0	6.85	14.75 + 7.25 = 22	0.0349 + 0.0551 = −0.09	7.78	23.25 + 19 = 42.25	0.0699 + 0.0901 = 0.16	8.83	40 + 30 = 70	0.0634 + 0.0850 = 0.1484	8.10	45 + 52 = 97	0.0559 + 0.1114 = 0.1673	7.9	43 + 120 = 163	0.0427 + 0.1317 = 0.1744	7.9

Table 4
Chemicals found in the treated samples

	Senedesmus treated sample	Mixed algae treated sample	*Chlorella* treated sample
1.	4-Methyl-2H-Pyran-2-one	Isopropyl isothiocyanate	7-Methyl-Z-tetradecen-1-ol acetate
2.	2-Cyclopropyl-1-Pentene	4-Pentenal	4-Methyl-1-heptyn-3-ol
3.	Propanedinitrile	Dicyclopropylcarbinol	Isobutyl nonyl carbonate
4.	Oxirane	1,3,5-Cycloheptatriene	Carbonic acid
5.	Tetrahydro-6,6-dimethyl-1-propene	Toluene	Butyl 2-ethylhexyl ester
6.	2-Methyl-1-Propene	Silacyclobutane	(S)(+)-Z-13-methyl-11-pentadecen-1-ol acetate
7.	1,6-Heptadien-3-yne	4-(Hexyloxy)-1,3-Cyclopentanedione	1H-1,2,4-Triazole-1-acetic acid
8.	Molybdenum	6-Oxabicyclo[3.1.0] oxirane	3,4-Furandicarboxylic acid
9.	dl-arginine	(1-Methylbutyl)-4-pentenal	Nonane
10.	Allylmethallyl ether	4-Butyl-allylmethallyl ether 1-Hexene	2-Methyl-decane,octane
11.	Methacrylamide	3,5-Dimethyl-2H-Pyran-2-one	Cyclohexanecarboxylic acid
12.	Oxazolidin-2-one	1-Acetyl-4-[4,5-dihydro1-Propene	Dodecyl acrylate
13.	9-Octadecene	2-Trifluoroacetoxytetradecane	Diethyl phthalate
14.	Carbonic acid	2-Nonenoic acid	Hexanoic acid
15.	2-Propen-1-amine	1,2-Dimethyl cyclopropene	*N*-glycyl-dl-valine
16.	*N*-2-propenyl-Nonanoyl chloride	4-Pentenal	3-Butenenitrile
17.	2-(2-Nitrovinyl)furan	Cyclobutanone	
18.	Allyltrifluoroacetate	2-Methyl-2-oxiranyl	
19.	6-Tridecene	3-(Prop-2-enoyloxy)dodecane	
20.	Ethyl ester Citronellal	3-[*N*-Aziridyl]propionylhydrazide	

and the rate of growth is predicted in the polynomial form. The curve fit equation is proposed to predict the rate of algae growth. The chemicals available in the sample after treatment are tabulated, and it is confirmed that the treatment with *Scenedesmus*, *Chlorella* and their combination are found to be effective. The algae treatment is beneficial in terms of cost-effective, ecofriendly and renewable.

Acknowledgement

Authors thank the management of Bharath University, Chennai, India, for their financial support to carry out the investigation. Authors also thank Prof. Ponvaiko, Vice-Chancellor, Prof. Hameed Hussain, Dean Engineering, Prof. Thoyamani, Pro-Vice Chancellor, Mr. Rajasekharan, Administrative Office, Bharath University for their continuous support in execution of the work.

References

[1] I. Bakkaloglu, T.J. Butter, L.M. Evison, F.S. Holland, I.C. Hancockt, Screening of various types biomass for removal and recovery of heavy metals (Zn, Cu, Ni) by bio-sorption, sedimentation and desorption, Water Sci. Technol., 38 (1998) 269–277.
[2] N. Matsumoto, H. Uemoto, H. Saiki, Case study of electrochemical metal removal from actual sediment, sludge, sewage and scallop organs and subsequent pH adjustment of sediment for agricultural use, Water Res., 41 (2007) 2541–2550.
[3] S.S. Ahluwalia, D. Goyal, Microbial and plant derived biomass for removal of heavy metals from wastewater, Bioresour. Technol., 98 (2007) 2243–2257.
[4] H. Benaissa, M.A. Elouchdi, Removal of copper ions from aqueous solutions by dried sunflower leaves, Chem. Eng. Process., 46 (2007) 614–622.
[5] S. Bunluesin, M. Kruatrachue, P. Pokethitiyook, S. Upatham, G.R. Lanza, Batch and continuous packed column studies of cadmium biosorption by *Hydrilla verticillata*, J. Biosci. Bioeng., 103 (2007) 509–513.
[6] J.P. Cazón, C. Bernardelli, M. Viera, E. Donati, E. Guibal, Zinc and cadmium bio-sorption by untreated and calcium-treated *Macrocystis pyrifera* in a batch system, Bioresour. Technol., 116 (2012) 195–203.
[7] E. Romera, F. González, A. Ballester, M.L. Blázquez, J.A. Muñoz, Comparative study of bio-sorption of heavy metals using different types of algae, Bioresour. Technol., 98 (2007) 3344–3353.
[8] Y.-C. Lee, S.-P. Chang, The biosorption of heavy metals from aqueous solution by *Spirogyra* and *Cladophora filamentous* macroalgae, Bioresour. Technol., 102 (2011) 5297–5304.
[9] K. Khairnar, P. Pal, R.H. Chandekar, W.N. Paunikar, Isolation and characterization of bacteriophages infecting nocardioforms in wastewater treatment plant, Biotechnol. Res. Int., 2014 (2014) 5.
[10] I. Oller, S. Malato, J.A. Sánchez-Pérez, Combination of advanced oxidation processes and biological treatments for wastewater decontamination – a review, Sci. Total Environ., 409 (2011) 4141–4166.
[11] L. Wei, H. Yu, J. Cao, Y. Sun, J. Fen, L. Wang, Determination and prediction of partition coefficient and toxicity for sulfonylurea herbicides and their degradation products, Chemosphere, 38 (1999) 1713–1719.
[12] G.J. Zhou, F.Q. Peng, L.J. Zhang, G.G. Ying, Biosorption of zinc and copper from aqueous solutions by two freshwater green microalgae *Chlorella pyrenoidosa* and *Scenedesmus obliquus* environment, Sci. Pollut. Res., 19 (2012) 2918–2929.
[13] G.J. Zhou, F.Q. Peng, B. Yang, G.G. Ying, Cellular responses and bio removal of nonylphenol and octylphenol in the fresh water green microalga *Scenedesmus obliquus*, Ecotoxicol. Environ. Saf., 87 (2013) 10–16.
[14] R. Munoz, B. Guieysse, Algal–bacterial processes for the treatment of hazardous contaminants: a review, Water Res., 40 (2006) 2799–2815.
15] DOE, US National Algal Biofuels Technology Roadmap, US Department of Energy, Office of Energy Efficiency and Renewable Energy, Biomass Program, USA, 2010.

[16] W. Zhou, M. Min, Y. Li, B. Hu, X. Ma, Y. Cheng, Y. Liu, P. Chen, R. Ruan, A hetero-photoautotrophic two-stage cultivation process to improve wastewater nutrient removal and enhance algal lipid accumulation, Bioresour. Technol., 110 (2012) 448–455.
[17] J.K. Pittman, A.P. Dean, O. Osundeko, The potential of sustainable algal biofuel production using wastewater resources, Bioresour. Technol., 102 (2011) 17–25.
[18] G.W. Roberts, M.-O.P. Fortier, B.S.M. Sturm, S.M. Stagg-Williams, A promising pathway for algal biofuels through wastewater cultivation and hydrothermal conversion, Energy Fuels, 27 (2013) 857–867.
[19] A.F. Clarens, E.P. Resurreccion, M.A. White, L.M. Colosi, Environmental life cycle comparison of algae to other bioenergy feedstocks, Environ. Sci. Technol., 44 (2010) 1813–1819.
[20] J. Noue, N. Pauw, The potential of micro algal biotechnology: a review of production and uses of microalgae, Biotechnol. Adv., 6 (1988) 725–770.
[21] T. Cai, S.Y. Park, Y. Li, Nutrient recovery from wastewater streams by microalgae: status and prospects, Renew. Sust. Energy Rev., 19 (2013) 360–369.
[22] W.J. Oswald, Micro-algae and Waste-water Treatment, M.A. Borowitzka, L.J. Borowitzka, Eds., Micro-algal Biotechnology, Cambridge University Press, Cambridge, 1988.
[23] N.C. Boelee, H. Temmink, M. Janssen, C.J.N. Buisman, R.H. Wijffels, Scenario analysis of nutrient removal from municipal wastewater by microalgal biofilms, Water, 4 (2012) 460–473.
[24] B.S.M. Sturm, S.L. Lamer, An energy evaluation of coupling nutrient removal from wastewater with algal biomass production, Appl. Energy, 88 (2011) 3499–3506.
[25] Y. Li, Y.F. Chen, P. Chen, M. Min, W. Zhou, B. Martinez, J. Zhu, R. Ruan, Characterization of a microalga *Chlorella* sp. well adapted to highly concentrated municipal waste-water for nutrient removal and biodiesel production, Bioresour. Technol., 102 (2011) 5138–5144.
[26] Z. Chi, Y. Zheng, A. Jiang, S. Chen, Lipid production by culturing oleaginous yeast and algae with food waste and municipal wastewater in an integrated process, Appl. Biochem. Biotechnol., 165 (2011) 442–453.
[27] W. Mulbry, S. Kondrad, C. Pizarro, E. Kebede-Westhead, Treatment of dairy manure effluent using fresh water algae: algal productivity and recovery of manure nutrients using pilot-scale algal turf scrubbers, Bioresour. Technol., 99 (2008) 8137–8142.
[28] S. Chinnasamy, A. Bhatnagar, R.W. Hunt, K.C. Das, Microalgae cultivation in a wastewater dominated by carpet mill effluents for bio fuel applications, Bioresour. Technol., 101 (2010) 3097–3105.
[29] G. Markou, D. Georgakakis, Cultivation of filamentous cyano bacteria (blue-green algae) in agro-industrial wastes and wastewaters: a review, Appl. Energy, 88 (2011) 3389–3401.
[30] C.G. Campbell, S.E. Borglin, F. Bailey Green, A. Grayson, E. Wozei, W.T. Stringfellow, Biologically directed environmental monitoring, fate, and transport of estrogenic endocrine disrupting compounds in water: a review, Chemosphere, 65 (2006) 1265–1280.
[31] E.M. Symonds, M.M. Cook, S.M. McQuaig, R.M. Ulrich, R.O. Schenck, J.O. Lukasik, E.S. Van Vleet, M. Breitbart, Reduction of nutrients, microbes, and personal care products in domestic wastewater by a bench top electrocoagulation unit, Scientific reports, 5, paper No. 9380, 2015, doi: 10.1038/srep09380.
[32] Y. Wu, T. Li, L. Yang, Mechanisms of removing pollutants from aqueous solutions by microorganisms and their aggregates: a review, Bioresour. Technol., 107 (2012) 10–18.
[33] U. Soltmann, S. Matys, G. Kieszig, W. Pompe, H. Bottcher, Algae-silica hybrid materials for biosorption of heavy metals, JWARP, 2 (2010) 122–115.
[34] T.A. Davis, B. Volesky, A. Mucci, A review of the biochemistry of heavy metal bio-sorption by brown algae, Water Res., 37 (2003) 4311–4330.
[35] P. Chankitkan, Modeling for metals contaminated wastewater by algae adsorption, Southeast Asian J. Sci., 2 (2013) 1–10.
[36] N. Abdel-Raouf, A.A. Al-Homaidan, I.B.M. Ibraheem, Microalgae and wastewater treatment, Saudi J. Biol. Sci., 19 (2012) 257–275.
[37] A.S. Carlsson, Micro and Macro Algae: Utility for Industrial Applications, D. Bowles, Ed., Outputs from the EPOBIO Project, CPL Press, Tall Gables, The Sydings, Speen, Newbury, Berks RG14 1RZ, UK, 2007.
[38] H.W. Bischoff, H.C. Bold, Phycological Studies IV, Some Soil Algae from Enchanted Rock and Related Algal Specie, University of Texas, Austin, 1963, pp. 1–95.
[39] M. Levasseur, P.A. Thompson, P.J. Harrison, Physiological acclimation of marine phytoplankton to different nitrogen sources, J. Phycol., 29 (1993) 87–595.
[40] X. Ye, X. Guo, X. Cui, X. Zhang, H. Zhang, M.K. Wang, L. Qiu, S. Chen, Occurrence and removal of endocrine-disrupting chemicals in wastewater treatment plants in the Three Gorges Reservoir area, Chongqing, China, J. Environ. Monit., 14 (2012) 2204.
[41] K.T. Semple, R.B. Cain, S. Schmidt, Biodegradation of aromatic compounds by microalgae, FEMS Microbiol. Lett., 170 (1999) 291–300.
[42] M. Kabir, S.A. Hoseini, R. Ghorbani, H. Kashiri, Performance of microalgae *Chlorella vulgaris* and *Scenedesmus obliquus* in wastewater treatment of Gomishan (Golestan-Iran) shrimp farms, AACL Bioflux, 10 (2017) 622–632.
[43] J. Koreivienė, R. Valčiukas, J. Karosienė, P. Baltrėnas, Testing of *Chlorella/Scenedesmus* micro algae consortia for remediation of wastewater, CO_2 mitigation and algae biomass feasibility for lipid production, J. Environ. Eng. Landsc. Manage., 22 (2014) 105–114.