Indian Journal of Biochemistry & Biophysics
Vol. 56, August 2019, pp. 309-315

Coupling dye degradation and biodiesel production by *Geitlerinema* sp TRV27

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Received 18 March 2019; *revised 15 April 2019*

In this study, the dye degrading the ability of marine cyanobacteria, *Geitlerinema* sp TRV27 was tested against the textile dye Acid black 52. Optimum conditions like pH, temperature, dye concentration for acid black 52 dye degradation were studied and were found to be pH 7, 25±2°C. More than 50% of degradation was observed for the tested maximum dye concentration, 100 ppm. The degraded dye intermediate was found to be naphthalene by GC-MS analysis and their toxicity on seed germination was studied. The dye treated biomass was used for the production of biodiesel and the physicochemical properties of biofuel were found to be within the standard limits.

Keywords: Acid black 52, Biofuel, Cyanoabcteria, Degradation, Dye, *Geitlerinema* sp

The textile industry is one of the major industrial sectors of Tamil Nadu which releases large quantities of effluent. These effluents contain 10-15% of dye and have high COD, BOD, pH, colour, heavy metals, hence it is very difficult to treat such effluents. Several methods like flocculation, membrane filtration, adsorption, irradiation, ozonation, ion exchange have been used to treat these effluents. And these processes are energy intensive, less efficient, produces sludge and costly[1,2].

Bioremediation is an emerging technology, which uses microbes like bacteria, yeast, fungi, and algae to treat the textile wastes. Among these, microalgae have become promising bioremediation agent in recent decades because of its ability to degrade textile wastewater and also accumulate fatty acids which can be extracted and used for the production of biofuel[1].

Socioeconomic development of a country is greatly affected by the available energy factor. The present energy demand of the world is largely fulfilled by fossil fuels like petroleum, diesel, natural gas, oil, coal, lignite *etc*[3]. These fossil fuels are nonrenewable resources which get depleted massively on usage[4]. There is a need to find an alternate for fossil fuel in order to fulfill the future demand of the world. One such alternative is a biofuel[3].

Biofuels are any diesel or equivalent fuel extracted from the renewable feedstocks like plant material, plant oil, animal fat, and algal oil. These feedstocks are classified as first generation, second generation, and third generation feedstocks. First generation feedstocks are food feedstocks like edible oils of rapeseed, sunflower oil[5], palm oil[6]. Usage of first generation feedstocks will impact global food market and cause food scarcity[7]. Second generation feedstocks are nonfood feedstocks (energy crops) like jatropha[8], and animal fats like beef tallow, pork lard[9]. The third generation feedstocks are algal oil derived from the microalgae[10].

Compared to first and second generation feedstocks, the microalgae are well known for their rapid growth, high lipid content due to their high photosynthetic efficiency, increased biomass productivity, high efficiency in conversion of solar energy, easy to cultivate, can be cultivated using sea water and wastewater, increased oil yield, high oil content, environment factors will not affect the growth of the microalgae, produces valuable by products like protein, biopolymers, carbohydrates which can be used as a feed or biofertilizer, capable of fixing CO_2[11], facilitating the reduction of CO_2 level[10].

In this study, the ability of *Gielterienem* sp *TRV27* to degrade acid black 52, a widely used commercial textile azo dye and successive production of biofuel from the dye treated biomass.

Materials and Methods

The marine cyanobacterial isolate, *Geitlerinema* sp TRV27 (accession number KX710092) was grown in F/2 media prepared using sea water[12,13]. The obtained wet biomass was used as an inoculum for the

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degradation of textile dye acid black 52. Acid black 52 was obtained from CLRI, Chennai, India.

The decolorizing ability of *Geitlerinema* sp was studied by adding 50 ppm acid black 52 dye to 100 mL of F/2 media containing 1g of algal culture. The culture flask was incubated at 27±2°C for 15 days. Decolorization was noted by determining the absorbance of the culture supernatant at the 588 nm (λ_{max}) using UV-Vis spectrometer (Shimadzu). Sterile cell-free media containing dye was used as control[14].

$$\%\ Decoloriza\ tion = \frac{Initial\ absorbance\ -\ final\ absorbance}{Initial\ absorbance} \times 100$$

Effect of decolorization of acid black 52 by *Geitlerinema* sp was studied at varying pH (5-9), temperature (4°C, 16°C, 25°C, 37°C, and 45°C) and dye concentration (10-100 ppm). For the assay, the dye was added to 100 mL of F/2 media which was inoculated with 1gm of biomass and the decolourization was noted at an interval of 24 h.

FTIR analysis of cyanobacterial biomass of control and dye treated biomass was done to arbitrate whether the surface of the cell is affected by the using Shimadzu FTIR spectrometer[14]. *Geitlerinema* sp which was grown on the F/2 media without dye was considered as a control and with dye was consider as a dye-treated test sample.

The degraded dye intermediates were extracted after 15 days of incubation by adding twice the volume of ethyl acetate which was evaporated and used for further study like GC-MS analysis and phytotoxicity study. The GC-MS analysis was carried out using GCMS-QP2010 Plus, Shimadzu at SRM Institute of Science and Technology, Chennai, India.

The phytotoxicity of the dye and the dye intermediates was tested on the mustard seed (*Brassica juncea* L.) by measuring the seed germination. For the study, seeds were surface sterilized using 2.5% of sodium hypochlorite with 0.1% Tween for 15 min and subsequently washed with sterile distilled water. 15 healthy seeds were placed in 9 cm petri plates on top of three layers of whatman filter paper. 1 mL of dye or extracted degraded dye intermediate was added to whatman filter paper every day and petriplates were incubated at 27°C. After 6 days, % germination, relative shoot and root length were measured. Triplicates were maintained and average results were noted. Sterile drinking water was used to grow the control seeds[15].

The effect of dye on the fatty acid profile of *Geitlerinema* sp was evaluated by comparing the chemical profile of dye treated biomass with control. The biomass was collected after 15 days and was extracted with hexane solvent extraction. The obtained hexane extract of the dye treated biomass was transesterified by alkali catalyst method, using sodium methoxide to extract biofuel. Physicochemical characterization of the obtained biofuel was characterized in Chennai petroleum corporation limited, Manali, Chennai, India and the chemical composition of biofuel was analysed by GC-MS analysis.

Results and Discussion

Dye-containing effluent from the Textile industries causes major environmental problems. Among the textile dye, azo dyes are one of the most detrimental class due to their chemical composition and high persistence in the water environment. They are proved to have a cytotoxic, genotoxic, mutagenic and carcinogenic effect on different organisms. In this study, an attempt has been made to study the degradation of Acid black 52, a mono-azo dye used for dyeing leather, nylon, wool, and silk by *Geitlerinema* sp[16].

The absorption spectrum of the control acid black 52 and spent media of test sample after 15 days was shown in (Fig 1A). The spectrum of textile dye had shown peaks at 204 nm and 588 nm whereas the degraded dye metabolites had shown peak at 378 nm and 676 nm. The change in the spectra and disappearance of peak at 588 nm confirmed the degradation of dye.

Cyanoabcteria posses additional outer membrane molecules mostly polysaccharide in nature are responsible for the removal of a pollutant from the environment[17,18]. FTIR spectrum of control biomass (Fig 1B) and treated biomass (Fig 1C) was found to have similar stretching in both the sample. But dye treated biomass sample had shown more stretching vibration in the range 1762 cm^{-1} and 1490 cm^{-1}. This may due to the involvement of cell surface structure in the dye degradation[19]. Omar[19] has also reported the change in the spectra of the biomass of *S. crassifolium*, *G. corticata*, *U. reticula* of before and after malachite green dye degradation and involvement of the corresponding functional group in dye degradation.

Figure 1D shows the effect of dye concentration on dye decolourization by *Geitlerinema* sp 100% decolourization was observed for lower concentrations 10 ppm and 20 ppm and more than

90% decolourization was observed for 30, 40, 50, and 60 ppm. For the tested maximum concentration of 100 ppm maximum of 50.66% decolourization was observed during the study. For further study, 50 ppm dye concentration was used.

Figure 1E indicates the effect of Temperature on decolourization of dye. Maximum decolourization was observed to be 90.56% and 84.90% at 25°C and 16°C, respectively. Change in the temperature may affect the linkage between dye and active site of the biomass or change in the active site of the biomass[19]. This may be the reason for the decrease in the dye degradation ability of *Geitlerinema* sp

Figure 1F indicates the effect of pH on decolourization and maximum degradation was found to be 88.67% at pH 7. At another pH less than 50% decolourization was observed. Change in the pH may affect the dye degradation either due to the degree of ionization of dye or active sites of the biomass[20].

From the study, it can be concluded that the optimum temperature and pH for decolourization were found to 25°C and pH 7 which were also the optimum growth condition for this cyanobacteria. This optimum growth condition helps the growth of the *Geitlerinema* sp in a positive level which further helps in degrading the textile dye. Change in the condition may affect the growth of the cyanobacteria which in turn may affect the dye degradation process. Park *et al.*[21] have observed 90% of decolorization acid black 52 using immobilized *Funalia trogii.*

Priscila *et al.*[22] have reported that the *Phormidium*, a cyanobacteria have decolorized an indigo azo more effectively than *Anabaena* and the intermediate was reported to be anthranilic acid and isatin. *Synechococcus* have decolorized sulphur black, RBRR dye and indigo dye. Shah *et al.*[23] have reported *Phormidium valderianum* have discoloured 90% of acid red 19, acid red and direct black 155 dyes. Queiroz and Stefanelli[24] have reported 81% decolourization of blue drin dye by *Anabaena*.

Figure 1G shows the GC-MS analysis of the degraded metabolite which was eluted at 8.477 min and it was found to contain naphthalene. This intermediate compound might have formed due to the azo bond breakage of acid black 52. Further study is required to confirm the pathway of complete degradation of the acid black 52 azo dye. This intermediate naphthalene can be further degraded into salicylic acid and then into CO_2 by bacteria like *Staphylococcus* sp, *Corynebacterium* sp, *Pseudomonas* sp, *Bacillus* sp, *and Micrococcus* sp[25]. Complete degradtion can be achieved by co-culturing or carrying out in 2 step degradation process.

Phytotoxicity study of dye and degraded dye metabolites on mustard seed revealed that there was 80% germination of the seed which was exposed to the degraded dye metabolites and 40% seed germination was observed on dye treated seeds. The percentage germination, shoot and root length of the seeds which was grown in the presence of dye metabolite were comparatively better than the seed which were grown on the dye (Table 1). Tallika *et al.*[26] have also studied phytotoxicity of dye effluent and dye treated effluent on *V. radiate* and *T. foenumgraecum* and reported that there was a decrease in the toxicity of the treated dye on seed germination.

GCMS analysis of the hexane extract of the control biomass (*Gielterinema* sp) and degraded dye biomass of *Geitlerinema* sp revealed a decrease in the saturated fatty acid especially dodecanoic acid percentage and presence of more amount of other alkanes, alkenes in the dye treated biomass (Table 2).

Hexane extract of degraded dye biomass was used for biofuel production and the yield was found to be 75 mL/L. The GC-MS analysis of biofuel has revealed the presence of n-alkanes, alcohol, and methyl esters of the fatty acids which were listed (Table 2).

The physicochemical property like density, kinematic viscosity, flash point, acid value, moisture content, calorific value, distillation temperature, cetane number, pour point, cloud point of the biofuel were studied and compared with the Indian standard as well as ASTM biodiesel standard (US) (Table 3). The physicochemical property of the extracted biofuel was found to be within the standard limits. Density and kinematic viscosity were found to be 0.849 g/cc and 3.36 mm^2/s. When compared to the biodiesel extracted from sorghum, jatropha[27], *lyngbya* sp and *synechococcus* sp[3], the biofuel extracted from *Geitlerinema* sp had shown the better density of 0.849 g/cc. Kinematic viscosity of biodiesel of cyanobacteria, *Lyngbya* sp and *Synechococcus* sp was reported to be 3.07 and 3.13 $mm^{2/}s$, these values were quite similar to the extracted biofuel[28,29].

Flash point, distillation temperature, pour point and cloud point were found to be 126, 366, 2, and 1°C, respectively. When compared to Tallow, palm oil and biofuel extracted from *Geitlerinema* sp had shown less cloud point, which may be due to the

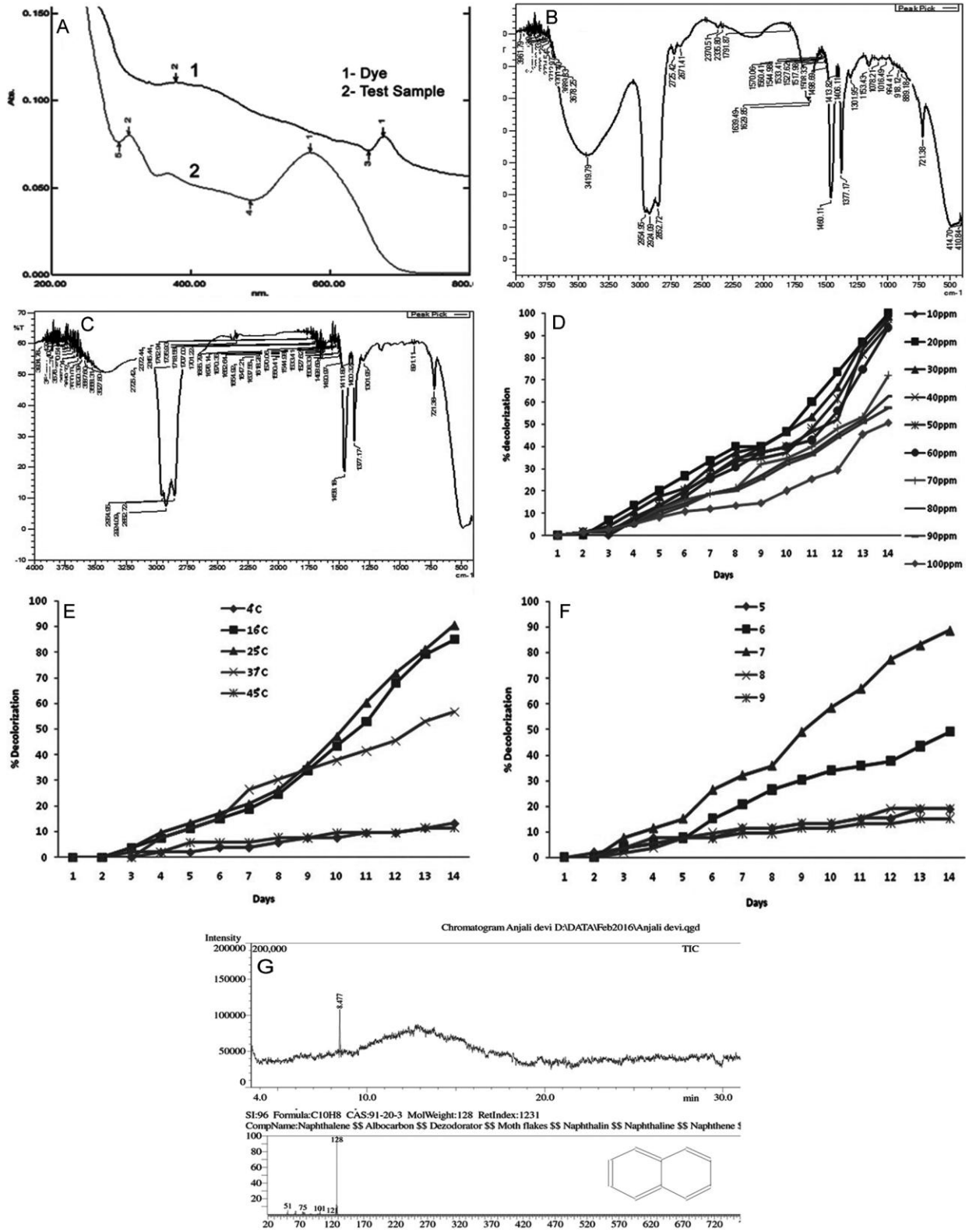

Fig. 1 — (A) UV spectral analysis of the dye and test sample; (B) FTIR analysis of untreated biomass; (C) FTIR analysis of dye treated biomass; (D) Effect of dye concentration; (E) Effect of temperature; (F) Effect of pH on dye degradation; and (G) GC-chromatogram of degraded dye intermediates

Table 1 — Effect of dye and degraded metabolite on the germination of *Brassica juncea* L.

	Germination %	Root length in cm	Shoot length in cm
Control	100%	3.5±0.5	3.3±0.3
Acid black 52 dye-50 ppm	40%	2±0.5	1.2±0.4
Degraded dye metabolite-50 ppm	90%	2.7±0.4	2.3±0.4

presence of less saturated fatty acid esters percentage in the algal oil. The pour point of the biofuel extracted from *Geilternima* sp is similar to the petrol diesel and is less than the palm oil, jatropha oil and tallow[30,31].

The acid value was found to be 0.222 mg of NaOH/g of oil, which is less when compared to sorghum and jatropha oil diesel, which were 0.434

Table 2 — GC-MS analysis: Hydrocarbon fractions of hexane extract of the *Geitlerinema* sp and the methyl esters of fatty acids, alkanes, alkenes of the biofuel

Hydrocarbon	List of the hydrocarbon	Area % of hexane extract of dye treated biomass	Area % of biofuel extract	Area % of hexane extract of control biomass
Alkane	n-Nonane	-	0.55	-
	n-Decane	0.19	1.31	-
	n-Undecane	-	0.31	-
	n-Dodecane	1.73	0.65	-
	n-Tetradecane	0.57	0.22	-
	n-Pentadecane	0.6	1.22	0.41
	n-Hexadecane	0.21	2.54	0.57
	n-Heptadecane	0.28	2.56	2.38
	n-Octadecane	0.64	5.6	0.64
	n-Nonadecane	0.47	2.43	0.47
	n-Eicosane	0.57	8.1	5.99
	n-Heneicosane	3.34	1.55	1.81
	n-Docosane	0.55	2.19	0.86
	n-Tetracosane	-	1.31	-
	n-Hexacosane	0.36	1.14	0.40
	n-Octacosane	-	3.07	-
	n-nonacosane	-	0.77	-
	n-Dotriacontane	3.46	0.22	-
	n-Tetratriacontane	2.74	1.01	0.98
Alkene	Cyclohexene	-	0.13	-
	Hexadecene	5.69	0.36	-
	Squalene or tetracosahexaene	1.49	0.43	-
Saturated fatty acid	Butanoicacid	0.23	0.27	-
	Pentanoic acid	0.87	0.51	-
	Hexanoicacid	-	0.19	-
	Heptenoicacid	-	0.36	-
	Tridecanoic acid	0.34	-	-
	Tetradecanoic acid	0.73	0.97	0.39
	Dodecanoic acid	0.43	1.93	64.28
	Hexadecanoic acid	0.33	0.56	4.23
	Octadecanoic acid	2.3	0.72	1.52
	Eicosanoic acid	0.43	0.89	-
	Docosanoic acid,	1.07	0.52	-
	Tetracosanoic acid	0.53	1.52	-
Unsaturated fatty acid	Nonenoic acid	0.76	-	-
	Nonadecenoic acid	-	0.17	-
Alcohol	Cyclopentanol	-	0.39	-
	Pentanol	-	0.25	-
	Hexanol	-	0.14	-
	Cycloheptanol	0.34		-
	Octanol	0.54	1.7	-
	Dodecanol	-	0.76	-
	Tridecanol	1.48	0.35	-
	Hexadecanol	-	2.55	-
	Tetracosanol	-	0.75	-
Alkanone	Hexanone	-	1.26	-
	Heptanone	-	0.75	-

Table 3 — Physicochemical property of the standard diesel and biofuel

Physicochemical property	Biofuel extracted from *Geitlerinema* sp	Diesel standard	Indian standard-IS 15607-05	ASTM biodiesel standard (US)
Density	0.849 g/cc	0.838 g/cc	0.860-0.900 g/cc	0.86-0.9 g/cc
Kinematic viscosityat40C	0.336/3.36 mm^2/s	1.9-4.1 mm^2/s	2.5-6.0 mm^2/s	1.9-6.0 mm^2/s
Flash point	126°C	75°C	Min 120°C	Min 130°C and Min 93°C
Acid value	0.222	Max 0.5	Max 0.50	Max 0.8
Calorific value	37.27 MJ/kg	40-45MJ/kg	-	-
Distillation temperature	366°C	372°C	<366 °C	360°C
Cetane number	52	45	Min 51	Min 47
Pour point	2°C	−6°C	-	-
Cloud point	1°C	-	-	-

and 5.31, respectively[29,30]. The calorific value and cetane value were found to be 37.27 MJ/Kg and 52. Sivaramkrishan and Ravikumar[32] have reported the cetane value of biodiesel of vegetable oil of babassu, palm, soyabean sunflower has shown 63 and 62, 45 and 49, respectively, and rapeseed, peanut, had shown 54.

The tested physico chemical property of the extracted biodiesel was found to be within the acceptable limits. The cold flow property, cloud point and pour point were found to be better which may be due to the presence of less percentage of saturated fatty acid in the *Geitlerinema* sp Further study, is required to standardize and purify the biofuel of degraded dye *Geitlerinema* sp in order to bring out an alternate for the diesel or to use as a blend for better performance of the existing fuel[33].

Conclusion

Textile dye effluents from the dyeing industries are the major environmental problems. Among the textile dye, azo dyes are difficult to degrade and persist in the environment for a long period. In this study, the ability of marine cyanobacteria *Geitlerinema* sp TRV27 to degrade acid black 52 was studied with successive production of biofuel. It was found that the cyanobacteria were able to degrade 50% of 100 ppm dye concentration. The optimum pH, Temperature for dye degradation was found to be 7 and 25±2°C. The optimum condition for the dye degradation was the same as the growth condition of the *Geitlerinema* sp The GC-MS analysis of the dye metabolite confirms the presence of naphthalene. When compared to acid black dye, degraded dye intermediate was found to be less toxic on mustard seed germination. The biofuel was extracted from the dye treated biomass and the physicochemical parameter was found to within the standard limits.

References

1 Fazal T, Azeem M, Fahad R, Asad UK, Naim R, Wasif F, Muhammad SUR & Jian X, Bioremediation of textile wastewater and successive biodiesel production using microalgae. *Renew Sust Energ Rev*, 82 (2018) 3107.

2 Jadhav UU, Dawkar VV, Ghodake GS & Govindwar SP, Biodegradation of Direct Red 5B, a textile dye by newly isolated *Comamonas* sp UVS. *J Hazard Mater*, 158 (2008) 507.

3 Sani, S, Kaisan MU, Kulla DM, Obi AI, Jibrin A & Ashok B, Determination of physico chemical properties of biodiesel from Citrullus lanatus seeds oil and diesel blends. *Ind Crops Prod*, 122 (2018) 702.

4 Sokoto MA, Hassan LG, Salleh MA, Dangoggo SM & Ahmad HG, Quality assessment and optimization of biodiesel from Lagenaria vulgaris (Calabash) seeds oil. *Int J Pure Appl Sci Technol*, 15 (1) (2013) 55.

5 Lang X, Dalai AK, Bakhshi NN, Reaney MJ & Hertz PB, Preparation and characterisation of biodiesels from various bio oils. *Bioresour Technol*, 80 (2001) 53.

6 Canakci M, Ozsezen AN, Arcaklioglu E & Erdil A, Prediction of performance and exhaust emissions of a diesel engine fueled with biodiesel produced from waste frying palm oil. *Expert Sys Appl*, 36 (2009) 9268.

7 Brennan L & Owende P, Biofuels from microalgae – a review of technologies for production, processing, and extractions of biofuels and co-products. *Renew Sust Energy Rev*, 14 (2010) 557.

8 Foidl N, Foidl G, Sanchez M, Mittelbach M & Hackel S, *Jatropha curcas* L. as a source for the production of biofuel in Nicaragu. *Bioresour Technol*, 58 (1996) 77.

9 Canakci M, The potential of restaurant waste lipids as biodiesel feedstocks. Bioresour Technol, 98 (2007) 183.

10 Ahmad AL, Mat Yasin NH, Derek CJC, & Lim JK, Microalgae as a sustainable energy source for biodiesel production- A review. *Renew Sust Energ Rev*, 15 (2011) 584.

11 Rodolfi L, Zittelli GC, Bassi N, Padovani G, Biondi N, Bonini G & Tredici MR, Microalgae for oil, strain selection, induction of lipid synthesis and outdoor mass cultivation in a low cost photobioreactor. *Biotechnol Bioeng*, 102 (2009) 100.

12 Renugadevi K, Valli CN, Panna S & Kumar N, Biopotential Activity of Marine Microalgae Extracts. *Int J Chem Tech Res*, 6 (2014) 5101.

13 Renugadevi K, Valli CN, Sowmiya P & Swetha S, Antioxidant activity of phycocyanin pigment extracted from

marine filamentous cyanobacteria *Geitlerinema* sp TRV57. *Biocatal Agric Biotechnol*, 16 (2018) 237.

14 Mostafa M. El-Sheekh, Gharieb MM & Abou-El-Souod GW, Biodegradation of dyes by some green algae and cyanobacteria. *Int Biodeter Biodegr*, 63 (2009) 699.

15 Rathod MC, Senjaliya B & Dhale DA, Effect of textile dye on seed germination of chickpea. *Int J Recent Sci Res*, 6 (2015) 2938.

16 Bruna de Campos Ventura-Camargo & Maria Aparecida Marin-Morales, Azo Dyes, Characterization and Toxicity–A Review. *Textiles Light Ind Sci Technol*, 2 (2013) 85.

17 Bertocchi C, Navarini L, Cesaro A & Anastasio M, Polysaccharides from cyaanobatceria. *Carbohydr Polym*, 12 (1990) 127.

18 De Philippis R, Sili C, Paperi R & Vincenzini M, Exopolysaccharide producing cyanoabcteria and their possible exploitation. A review. *J Appl Phycol*, 13 (2001) 293.

19 Omar H, Adel E & Khairia A, Bioremoval of toxic dye by using different marine macroalgae. *Turk J Bot*, 42 (2018) 15.

20 Nandi BK, Goswami A & Purkait MK, Removal of cationic dyes from aqueous solutions by kaolin, kinetic and equilibrium studies. *Appl Clay Sci*, 42 (2009) 583.

21 Park C, Byunghwan L, Eun-Jung H, Jinwon L & Sangyong K, Decolorization of acid black 52 by fungal immobilization. *Enzyme Microb Technol*, 39 (2006) 371.

22 Priscila MD, Maria ES, Luiz ABM, Marli FF & Regina TRM, Degradation of textile dyes by cyanobacteria. *Braz J Microbiol*, 48 (2017) 25.

23 Shah V, Garg N & Madamwar D, An integrated process oftextile dye removal and hydrogen evolution using cyanobacterium *Phormidium valderianum. World J Microbiol Biotechnol*, 17 (2001) 499.

24 Queiroz BPV & Stefanelli T, Biodegradation de corantes Têxteis por *Anabaena flos-aquae. Eng Amb*, 8 (2011) 026

25 Shivendra S & Hardik P, Pseudomonas in Biodegradation. *Int J Pure Appl Biosci*, 2 (2014) 213.

26 Tallika LP, Bhargavi CP, Chetana R, Devayani RT & Shailesh RD, cost effective bioprocess for actual dye manufacturing industry wastewater using statistical tool, *Indian J Biochem Biophys*, 52 (2015) 316.

27 Knothe G & Steidley RK, Kinematic viscosity of biodiesel fuel components and related compounds. Influence of compound structure and comparison to petrodiesel fuel components. *Fuel*, 84 (2005) 1059.

28 Kumar V & Kant P, Study of Physical and Chemical Properties of Biodiesel from Sorghum Oil. *Res J Chem Sci*, 3 (2013) 64.

29 Selvan BK, Revathi M, Piriya PS, Vasan PT, Prabhu DI & Vennison SJ, Biodiesel production from marine cyanobacteria cultured in plate and tubular photobioreactors. *Indian J Exp Biol*, 51 (2013) 262.

30 Moser BR, Influence of blending canola, palm, soybean, and sunflower oil methyl esters on fuel properties of biodiesel. *Energy Fuels*, 22 (2008) 4301.

31 Vyas AP, Subrahmanyam N & Patel PA, Production of biodiesel through transesterification of Jatropha oil using KNO3/Al2O3 solid catalyst. *Fuel*, 88 (2009) 625.

32 Sivaramakrishnan K & Ravikumar P, Determination of cetane number of biodiesel and it's influence on physical properties. *ARPN J Eng Appl Sci*, 7 (2012) 205.

33 Yeniffer PC, Israel HO, Ángel DGD & Viatcheslav K, Environmental assessment of microalgae biodiesel production in colombia, comparison of three oil extraction systems. *Ciencia Tecnología y Futuro*. 5 (2013) 95.