

# Synchronous Use of Hindakia Sp. For Electricity Generation and Dairy Wastewater Treatment

Sikkandhar WasimRaja (✉ [wasimrajaatr@gmail.com](mailto:wasimrajaatr@gmail.com))

Tamil Nadu Agricultural University <https://orcid.org/0000-0002-7320-1044>

Kalyanasundaram Geetha Thanuja

Tamil Nadu Agricultural University

Subburamu Karthikeyan

Tamil Nadu Agricultural University <https://orcid.org/0000-0003-1003-3546>

Subramanian Marimuthu

Tamil Nadu Agricultural University

---

## Research Article

**Keywords:** Electrogenic, microalgae, waste water treatment, cyclic voltammetry

**Posted Date:** May 27th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-545507/v1>

**License:** © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

# Abstract

This paper illustrates the potential of microalgae in electricity production in concurrence with wastewater treatment. In order to explore the potential biogenic algal strains, our study focused on the isolation of microalgal strains from various aquatic sources. Cyclic voltammetry was performed to detect the electrogenic activity and out of 18 algal isolates, six algal strains were screened. The cyclic voltammograms of *Hindakia* sp from the culture collection revealed the well-defined redox peak in contrast to the other algal strains. The electrogenic *Hindakia* sp. was also analyzed for their potential to remove nutrients in wastewater treatment. A fifteen days trial under lab scale race way pond was conducted to evaluate the performance of electrogenic algae. A significant decrease in N, P, BOD, COD, and TOC was observed. The removal efficiency of NH<sub>4</sub>-N, NO<sub>3</sub>-N, P, BOD, COD and TOC were 90.38, 90.24, 66.75, 67.15, 69.44, and 83.51 respectively. *Hindakia* sp was able to produce 13.79 mg/ml of EPS which paves way to a hydrated biofilm matrix that aids in better electrogenicity. To the best of our knowledge, EPS production, electrogenic activity and their utility in waste water treatment are reported for the first time in *Hindakia* sp. The results of our study demonstrated the combined beneficial traits of microalgae towards electricity production and waste water treatment.

## Introduction

Hitherto, the global energy demand is coped up by fossil fuels which are estimated to be exhausted in the ensuing years(1). There is a pressing need for alternative energy sources and the reliability of renewable energy extends their demand around the globe in the ever-expanding energy crisis scenario. Various renewable energy viz., solar, wind, and geothermal are well-rounded and ready to be offered at the commercial level(2). However, the energy generation from biomass products with the growth of plants and crops, algae, organic waste would be economically sound and environmentally safe. In a bio-based economy both macro and micro algae have gained worthwhile interest as a potential feedstock(3). Microalgae are efficient converters of solar energy, thereby holds a wide application in mass production for high value products. The photosynthetic productivity of algae on earth is well acknowledged contributing more than 50%. The promising technology for the production of electricity viz algae has been evolved in microbial fuel cells. The metabolic activity of the algae with the potential to transfer electrons extracellularly to interact with conductive material results in the electric current (4). They are effectively used in biological photovoltaics to generate sustainable long-term energy. Various green algae are reported in the exploitation of bio-electrochemical fuel cells such as *Chlorella vulgaris*, *Dunaliella tertiolecta* (5), *Chlamydomonas*, *Geobacter*(6), *Synechocystis* (7) etc. The different electron transfer strategies reported in various organisms namely direct electron transfer and mediated electron transfer have been an object of inquisitiveness in microbial bioelectrochemical systems.

Energy production employing wastewater as a resource further assists in ameliorating the burden on other technologies. The electron-producing catalytic process in photosynthetic algae to generate electricity is a promising technology(8). The use of bio-electrochemical systems has set steps to treat wastewater with the support of electro-active microorganisms(9). The photosynthetic machinery

endowed with the algae serves as a potential alternate for diverse forms of energy including biohydrogen, biodiesel, bioethanol etc., Bioelectrochemical systems assisting electro-active microbes are gaining interest in the recent days due to their ability to afford the power and to treat wastewater. The recruitment of microalgae in BES offers simultaneous consumption of CO<sub>2</sub> and organic matter production. The environmental challenge has posed numerous threats besides increasing global population and one amongst is wastewater production. The conventional tertiary treatment can be altered with the growth of microalgae for the removal of nitrogen and phosphorus(10). Several studies have described the potential of several species of microalgae including *Chlorella*, *Chlamydomonas*, *Botryococcus*, *Chlamydomonas*, *Phormidium* in wastewater treatment(11, 12). Various pollution including nitrogen, phosphorus, and other nutrients in the sewage causes concern in the environmental safety(13). The untreated wastewater contains nutrients like nitrogen and phosphorus, whose discharge poses threat to various forms of life. The treatment of industrial wastewater is crucial in protection against health risk, freshwater demand, eutrophication of lakes etc., Waste water treatment (WWT) assisted with microalgae ensures a key solution for nutrient removal due to their uptake and desolating ammonia(14). The technology of employing algae in WWT is utterly natural and the biomass produced can be reused for extracting products to clinch a circular economy. Many studies have employed dairy waste wastewater for cultivating algae as they could efficiently remove complex organics and high contents of COD, which are distinct characteristics of dairy wastewater

The main aim of the study is to i) demonstrate the electrogenic potential of microalgae ; ii) investigate the potential of electrogenic microalgae in WWT by nutrient removal.

## Materials And Methods

### Sample collection

The algal samples were collected from aquatic regions of various districts. The stock algal strains from the culture collection of the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore were also used.

### Isolation of microalgal cultures

The collected samples were serially diluted from 10<sup>1</sup> to 10<sup>5</sup> times and plated on sterile plates containing BG11 medium. The plates were incubated at an algal growth chamber supplying 16h: 8h of light: dark (1400 l), with a temperature of (28 ± 2°C) for 7–10 days. Cold fluorescent lamps were used for illuminating the growth chamber. The colonies grown were picked and continuously sub-cultured in BG11 agar plate, supplemented with ampicillin (100 µg/ml) and kanamycin (100 µg/ml), cycloheximide (100 µg/ml) ensuring the absence of bacterial and fungal contamination respectively. The purified colonies were cultured in BG11 broth and used for further analysis.

## Screening for electrogenic activity

Cyclic voltammetry (CV) method, under electrochemical work station (AMETEK, scientific instruments, USA) was performed using three-electrode system, namely glassy carbon electrode (3mm diameter) as a working electrode, a platinum wire and Ag/AgCl (3M KCl) as a counter and reference electrodes respectively. The electron transfer mechanism was analyzed using a working electrode covered with a concentrated microalgal coat. The coated electrode was shade dried and incubated at room temperature ( $28 \pm 2^\circ\text{C}$ ) for two days to induce the interaction between electrode and algae. CV traces were determined in the potential range of -0.8 to +0.8 V at a scan rate of  $10 \text{ mVs}^{-1}$ . Deoxygenated, deionized water was used as an electrolyte to ensure the absence of oxidation reduction.

## Authentication of electrogenic activity

*Hindakia* sp. was grown in BG 11broth and waste water separately along with stainless steel mesh (anode) and incubated in above-mentioned specifications of the light chamber. One end of the anode was let outside the rubber cork to measure the electron flow. They were connected to the cathode of the multimeter (MASTECH, MAS830L) and the circuit is closed by connecting its anode to a graphite plate (air cathode) as depicted in Fig. 1. The power density was recorded on alternate days up to the 25th day. The study level current and voltage were calculated using the formula  $I = V/R$ , where I is the current, V is the voltage measured and R is external resistance. The power density P was calculated from the measured voltage ( $P = V^2/RA$ ). The cathode area in our study used is  $64 \text{ cm}^2$  and the resistance provided is  $500 \Omega$ .

## Estimation of Extracellular polymeric substances (EPS)

The algae at the log phase were harvested on the 20th day and the cells were heated at  $45^\circ\text{C}$  for 20 min. It was then centrifuged at 8000 rpm for 20 min at room temperature. The supernatant was collected and added with three volumes of acetone and stored at  $-20^\circ\text{C}$  for 24h. After 24h, it was spun down at  $4^\circ\text{C}$  for 20 min. Along the sides of the tube, the precipitated polysaccharides were dried to measure the EPS yield gravimetrically(15).

## Microscopic analysis

The screened algal cultures were observed in a light microscope and their morphological characteristics were studied. Further, to analyze the live and dead cells, algal strains were stained with trypan blue(16).

## Effluent collection

The raw industrial effluents from the processing plant were collected from Aavin Dairy Plant, Coimbatore District Co-operative Milk Producers Union Ltd., Pachapalayam, Perur, Coimbatore ( $10.9755^\circ \text{ N}$ ,  $76.9153^\circ \text{ E}$ ).

## Experimental setup

The screened algal strain was inoculated into wastewater at lab-scale reactor. The preliminary trials were carried out with lab scale raceway pond fabricated for the growth of microalgae of 190 L capacity made up of acrylic sheet. It is a transparent plastic material of 5mm thickness. The laboratory setup was fabricated with a length of 1.2m, width 0.41 m and height 0.3 m. The pond was assembled over a steel structure to the height of 0.62 m from the ground level. A paddle wheel of acrylic sheet and fixed to the axle. The axle is supported by two bearings in the structure. The gearbox is used for regulating the speed of an electric motor, which in turn maintains the speed of the paddle wheel. The operating speed is maintained in the range of 10 to 40 rpm throughout the study. The movement is transmitted from the motor to the axle by a drive chain. The operational view of constructed lab-scale raceway pond is given in Fig. 2.

The quantity of wastewater was maintained up to 20 cm height throughout the experiment. The experimental setup was maintained under sunlight ( $360 \times 10^2$  l). The trial was conducted for 15 days.

### Effluent treatment with algae

The screened algal isolate was treated with wastewater. The raw wastewater was fed into raceway pond solely without dilution. To 100 l of wastewater, 2% of *Hindakia* cultures at the optical density of 0.5 at  $A_{680}$  nm were inoculated.

### Measurements

The change in characteristics of the effluents namely pH, EC(17), Total dissolved solids (18), Total suspended solids(18), Dissolved oxygen(19) biological oxygen demand(18), chemical oxygen demand(18), total nitrogen(20), ammoniacal ( $\text{NH}_4\text{-N}$ ) and nitrate nitrogen ( $\text{NO}_3\text{-N}$ ) (18) total phosphorus total organic carbon(21) and chlorophyll(22) was assessed using standard protocols at a periodic interval of 3 days for 15 days.

## Statistical analysis

The dataset generated from our study were subjected to one-way ANOVA, in SPSS (Version 20, IBM, N, USA). Statistically significant differences between algal treatments were analyzed using Tukey HSD. The graphs were plotted using Origin, 2018.

## Results

### Light microscopic observation of electrogenic microalgae

The brief notes on the algal isolates are presented in Table 1. The morphology of algal isolates screened with electrogenic activity was observed under the light microscope (Fig. 3).

Table 1  
Brief description on the algal strains

Isolate code	Source	Geographical location	Morphological characteristics
KWU	Well water	Keeripatti (11.532937 ° N/ 78.485444 ° E)	Unicellular, spiral, solitary and curved, non-motile
PMU	Mangrove (brackish water)	Pichavaram (11.417586 ° N/ 79.772133 ° E)	Unicellular, spherical, arranged in clusters, non-motile
SSU	Sea water	Samiyarpettai (11.551264 ° N/ 79.759134 ° E)	Unicellular, spherical shape, non-motile and solitary
CLU	Lake	Chidambaram (11.406645 ° N/ 79.691559 ° E)	Unicellular, flattened, non-motile, 4 cells arranged in parallel
CRU	River	Chengam (12.308555 ° N/ 78.796766 ° E)	Unicellular, non-motile, spherical with vacuole like structure inside the cell
KPU	Pond	Kayambattu (12.305423 ° N/ 78.773261 ° E)	Unicellular, oval, non-motile with folding like structure inside the cell
OSU	Sewage	Orathanadu (10.624915 ° N/ 79.250908 ° E)	Unicellular, spherical, non-motile with sac-like structure
KLU	Lake	Kulichapattu (10.760682 ° N/ 79.190935 ° E)	Unicellular, spherical and non-motile
KPU	Pool	Karungulam ( 8.634334 ° N/ 77.854799 ° E)	Unicellular, spherical, non-motile with triad arrangement of cells
MDU	Dam outlet	Mettur (11.784609° N/ 77.802816 ° E)	Unicellular, oval, non-motile with thick outer covering

Isolate code	Source	Geographical location	Morphological characteristics
ULU	Lake	Ukkadam (10.982817 ° N/ 76.961144 ° E)	Unicellular, spherical and non-motile
PPU	Pond	Poosaripalayam (11.004039 ° N/ 76.932391 ° E)	Unicellular, oval shaped, arranged in either pair or triads
NPU	Pond	Nagarajapuram (11.002473 ° N/ 76.912199 ° E)	Unicellular, spherical, with oil-like outer covering
WPU	Paddy field	Wetland (11.002288 ° N/ 76.926175 ° E)	Unicellular, oval curved, arranged in tetrads
KLU	Lake	krishnampathy lake (11.004363 ° N/ 76.925233 ° E)	Unicellular, spherical and solitary
PPU	Pond	Perur (10.964691 ° N/ 76.930098 ° E)	Unicellular, spherical, 8 cells arranged parallelly to other 8 cells
<i>Hindakia</i> sp	Culture collection, Department of Agricultural Microbiology, TNAU, Coimbatore		Unicellular, spherical, solitary colonies, non-motile with smooth cell wall
<i>Chlorococcum</i> sp			Unicellular, tetrad arrangement of cells
<i>Anabena azollae</i>			Chain like arrangement of cells, heterocystous
<i>Chlorella</i> sp			Unicellular, spherical, non-motile

#### EPS production

Among the isolates analyzed, *Hindakia* displayed higher EPS production (13.79 mg/ml) followed by *Anabena azollae* (9.83 mg/ml) and *Chlorella* sp. (8.21 mg/ml) (Fig. 4). EPS production of *Chlorococcum* sp, ULU and MDU were on par ranging from 3.12 to 5.34 mg/ml

#### Electrogenic activity of the algal isolates

To assess the electrogenic activity of the algal isolates, CV measurements of the microalgae were carried out on the glassy carbon. Among the 21 isolates, six were found to have electrogenic activity. It could be observed the supernatant of the culture (control) displayed only background current elucidating their electro inactivity of the broth. However, the electrogenic activity was observed in *Chlorella* sp. *Chlorococcum* sp. *Anabena azollae* and unidentified isolates of our study (Fig. 5), but distinct redox peak was obtained only in *Hindakia* sp. over a potential range of -0.8 to 0.4V versus Ag/Ag Cl<sub>2</sub> underpinning their redox activity. Among the screened strains *Hindakia* displayed the oxidation peak at the potential range of + 100 to + 200 mV under anaerobic conditions, while no distinct peaks were observed in other strains. Cyclic voltammograms studies on *Hindakia* supernatant, recorded nearly negotiable electrochemical response and in contrast, significant response was observed in pellets. Hence following electrochemical analysis, the *Hindakia* strain was chosen for further investigation.

#### Proof of electrogenic activity

The stainless steel (SS) mesh in the conical flask holds dimensions of radius and area of 3.5 cm and 38.48 cm<sup>2</sup> respectively. The microscopic view of algae in SS (Fig. 6A) and without algae is given in the Fig. 6B. SS supported the growth of the algae and one sq. cm of the mesh yielded 3.1x 10<sup>5</sup> cells. The indirect electron flow of *Hindakia* sp was studied as a measure of power density and their assessment in wastewater and BG 11 broth are depicted in Fig. 6C

#### Wastewater treatment by electrogenic algae

The physical and chemical properties of wastewater taken for the study are illustrated in Table 2. The algal strains were inoculated into the wastewater and routine measurements taken are provided in supplementary Fig. 1. The results of nutrient removal displayed no significant difference upon various days. The multiple comparisons between the parameters were performed in Tukey HSD and the results are furnished in Table 3. Algae inoculated with wastewater exhibited a significant reduction of nutrients. *Hindakia* sp removed 90.38, 90.24, 66.75 of NH<sub>4</sub>-N, NO<sub>3</sub>-N, P during 15 days (Fig. 7). A removal percentage of 67.15, 69.44, and 83.51 were observed for BOD, COD and total organic carbon respectively.

Table 2  
 Characteristics of the  
 effluent taken for the study

<b>Parameters</b>	<b>Values</b>
pH	8.65
EC (mS/cm <sup>3</sup> )	6.058
DO (mg/L)	3.72
COD (mg/L)	1970
BOD (mg l <sup>-1</sup> )	968
TS (mg/L)	1510
TSS (mg/L)	619.5
TDS (mg/L)	890.5
VS (mg/L)	120
TOC (mg/L)	722
TN (mg/L)	103.6
NH <sub>4</sub> -N (mg/l)	39.3
NO <sub>3</sub> -N (mg/l)	64.1
TP (mg/L)	8.0

Table 3

Multiple comparisons of analyzed parameters in wastewater treatment using Tukey HSD

Parameters	pH	OD	Chl	BOD	COD	DO	TN	TP	TOC	Biomass
pH					*				*	
OD				*	*				*	
Chl					*				*	
BOD		*		*	*			*	*	*
COD	*	*	*	*	*	*	*	*		*
DO									*	
TN					*				*	
TP				*	*				*	
TOC	*	*	*	*		*	*	*	*	*
Biomass				*	*				*	

## Discussion

### Electrogenicity in algae

Electrochemical techniques are the key tools for the analysis and interpretation of the electrode reactions occurring in the microalgal fuel cells. Since the constructions of fuel cells are cumbersome, electrochemical analysis at a lab scale is the reliable and beneficial method for preliminary screening(15). The redox activity of the isolates and the stains from the culture collections were evaluated by CV measurements on glassy carbon (GC) electrode, as it is highly conductive(23). The distinct redox peak in *Hindakia* sp. were observed and might be attributed to the EPS production of cells indicating the viable cells for active transfer of electrons, as depicted in Fig. 8. Similar results were obtained by(15) in *Scenedesmus* sp. whose enhanced EPS production displayed electrogenic activity witnessing their plausible use in photosynthetic algal microbial fuel cells. (24) demonstrated cathodic reduction for generation of electricity in *Scenedesmus obliquus*, in which the quantity of available oxygen was claimed to be essential for the reduction peak. In analogy, the redox peak observed might be due to the oxygen produced from the algal strains for electrogenicity.

The photosynthetic activity of microalgae generates electrical current due to the release of electrons from them, which could be employed in biophotovoltaic devices. Studies by (25) reported NADPH oxidase activity in *Phaeodactylum tricomutum* and *Thalassiosira pseudonona* responsible for long-term output power. The mediated electron transfer has been reported in *Shewanellaceae* which exploits melanin, menaquinone, riboflavin and their derivatives as redox shuttles (26, 27). (28) have interpreted the importance of EPS matrix in contact of microorganisms with the electrode. The result of our study

correlates the EPS production with electrogenecity, which beholds a corresponding relation. Among the strains in our study, *Hindakia* sp expressed higher EPS production (Fig. 4), which is selfsame for electrogenecity. The reason could be attributed to the mediator role of EPS for electron transfer, rendering more electrogenic activity to *Hindakia* sp. Similarly, (29) have demonstrated EPS as a crucial site for binding of c-type cytochromes which are indeed essential for electron transfer to the electrode.

### Current density analysis

The assessment of the current density of *Hindakia* sp. in multimeter confirms their electricity production potential. (30) have investigated the electricity production of *Chlorocystis* sp. using a sensitive current collector in which the translated photochemical signal is obtained. The polarization curves of the anode under the influence of *Hindakia* grown in BG11 broth and wastewater are represented in Fig. 3B. The experiment was carried out at 1400 lux and the maximum current recorded grown on BG11 broth and wastewater was  $26.4 \text{ mW m}^{-2}$  and  $31.4 \text{ mW m}^{-2}$  respectively which is significantly higher than that of the control. The photosynthetic algal MFC produced a maximum current density of  $539 \text{ mA/m}^2$  with at power production of  $110 \text{ mW/m}^2$  (31). (32) have revealed the correlation between MFC grown on cathode and their COD degradation potential. In analogy, the culture used in our study had potential to grow on the anode and also COD removal. The results were in analogy to the study conducted by (33) using photo microbial fuel cells of *Desmodesmus* sp. whose recorded power intensity was  $99.09 \text{ mW m}^{-2}$  at 3000 lux. It could be interpreted as with the increase in light intensity, the power density could be enhanced. The electron flow from algae passes through the anode, gets neutralized on the graphite plate where oxygen from air serves as electron acceptor. The efficient current production of algal isolate on wastewater over BG11 broth confirms its potential in photovoltaics.

### Removal of nutrients in wastewater

The results of algal inoculation on wastewater show that *Hindakia* sp. could able to grow on milk processing waste water. The effect of microalgal treatment nutrients by removal of wastewater with respect to days was measured. Microalgae can assimilate nutrients for its growth from wastewater and convert into biomass constituents. pH value is crucial criterion for the growth of microalgae (34, 35). pH increased from 8.6 to 9.5 at the 5th day which gradually decreased and was found significant with COD and TOC (Tukey HSD,  $P < 0.05$ ). Studies by (36) on *C. vulgaris* treatment in piggery wastewater showed the negative impact upon alkaline pH. Similarly, in our study the initial pH was slightly alkaline, which became neutral upon algal growth. The crucial necessitate of waste water treatment is the removal of BOD as it deteriorates the dissolved oxygen. The average BOD reduction under current experimental condition displayed 67.15. (37) have reported 82.92% of BOD removal rate upon treatment with *Chlorella vulgaris*. (38) reported 29.52% of COD removal efficiency by *Chlorella* sp. in meat processing wastewater, while the current study displayed 69.44%. The COD removal efficiency displays that algae in wastewater are prone to utilize organic carbon. The consistent studies were performed by (39) in the treatment of Cassava processing wastewater using electrogenic and biomass production potential of *Spirulina platensis* for COD reduction. Among all the observed measurements, TOC displayed significant

differences (Tukey HSD,  $P < 0.05$ ) between all parameters except COD. On the other hand COD is found significant except DO and TOC. From Table 3, it can be interpreted as COD and TOC are effectively reduced followed by BOD. As depicted in supplementary Fig. 1, the initial concentration of total nitrogen in the wastewater was  $103.6 \text{ mg L}^{-1}$  and it was decreased upon algal inoculation. The decline in amount of total nitrogen indicates that *Hindakia* used wastewater as a source of nitrogen for growth. Studies have reported the  $\text{NH}_4\text{-N}$  removal efficiency of *Chlorella* sp. in municipal wastewater was 81%. (40). In analogy, *Hindakia* sp. have also utilized  $\text{NH}_3$  from 39.3 to  $3.75 \text{ mg l}^{-1}$  contributing to the removal rate of 90.38%. During the test period, phosphates in the wastewater reduced gradually. Fate of P in the bioreactors followed the same trend as that of N with the removal from  $8.0 \text{ mg L}^{-1}$  to  $2.66 \text{ mg L}^{-1}$ . The result was comparable to the removal rate of phosphorus with the study conducted by (41) by *Galidieria sulphuraria* in primary effluent. The C, N and P removal were higher in waste water upon algal inoculation and the same in uninoculated were lower as depicted in Fig. 7 This in turn can be correlated with higher electrogenecity in wastewater. The study conducted by (42) in dairy wastewater with cultivation of *Chlorella vulgaris* yielded  $0.45 \text{ g}^{-1} \text{ l}^{-1} \text{ day}^{-1}$  at 4th day of cultivation.. Correspondingly, in our study the biomass productivity gradually increased upon time and reached maximum at 15th day recording  $4.61 \text{ g}^{-1} \text{ l}^{-1} \text{ day}^{-1}$  witnessing their probable use in large scae cultivation.

## Conclusion

*Hindakia* sp. displayed a reduction peak in the potential range of -0.4V to -0.2V and oxidation peak in 5–10 in  $10^{-6} \text{ mA}$ . The result of our study depicts the feasibility of using *Hindakia* sp. in wastewater sampled from a dairy products processing plant, which effectively removes nutrients and roughly produces  $314 \text{ mW m}^{-2}$  per liter of wastewater. Elimination of nutrients and BOD, COD of the wastewater was evaluated in our study with subsequent electricity production. Their power density production can further be enhanced by optimizing several parameters like light intensity, cell load, selection of anode, etc, in future studies. Additionally, progress in design and operation for algal photovoltaic cell can still augment the electro genesis, which could be exploited in wastewater treatment plants.

## Declarations

### Acknowledgement

Authors are thankful to The Manager, Aavin Milk Industry, Perur, Coimbatore for giving permission to acquire waste water sample; Dr.Pon Pandiyan, Professor and Head, Department of Nanoscience, Bharathiyar University, Coimbatore for providing instrumental help for electrogenic analysis, Dr.P.Tamilarasan, Scientist& Assistant Professor, Central Electrochemical Research Institute, Karaikudi for result interpretation and Dr. D. Ramesh, Associate Professor for rendering ideas in preparing draft.

### Ethical Approval

Not applicable

## Consent to Participate

Not applicable

## Consent to Publish

Not applicable. However informed consent was obtained from all the authors included in the study.

## Authors Contributions

SWR collected the data, performed the analysis and wrote the paper; KGT contributed data and analysis tools and revised the paper; SK conceived and designed the analysis and edited the paper; SM supported in data analysis, CV analysis and manuscript editing & revising. All authors read and approved the final manuscript.

## Funding

Not applicable.

## Competing Interests

The authors declare that they have no competing interests.

## Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article and/or its supplementary materials.

## References

1. Kumar, R., Ghosh, A. K., & Pal, P. (2020). Synergy of biofuel production with waste remediation along with value-added co-products recovery through microalgae cultivation: a review of membrane-integrated green approach. *Science of the Total Environment*, *698*, 134169.
2. Qazi, A., Hussain, F., Rahim, N. A. B. D., Hardaker, G., Alghazzawi, D., Shaban, K., et al. (2019). Towards sustainable energy: a systematic review of renewable energy sources, technologies, and public opinions. *IEEE access : practical innovations, open solutions*, *7*, 63837–63851.
3. Jones, C. S., & Mayfield, S. P. (2012). Algae biofuels: versatility for the future of bioenergy. *Current opinion in biotechnology*, *23*(3), 346–351.
4. Borole, A. P., Reguera, G., Ringeisen, B., Wang, Z.-W., Feng, Y., & Kim, B. H. (2011). Electroactive biofilms: current status and future research needs. *Energy & Environmental Science*, *4*(12), 4813–4834.
5. McCormick, A. J., Bombelli, P., Scott, A. M., Philips, A. J., Smith, A. G., Fisher, A. C., et al. (2011). Photosynthetic biofilms in pure culture harness solar energy in a mediatorless bio-photovoltaic cell

- (BPV) system. *Energy & Environmental Science*, 4(11), 4699–4709.
6. Nishio, K., Hashimoto, K., & Watanabe, K. (2013). Light/electricity conversion by defined cocultures of *Chlamydomonas* and *Geobacter*. *Journal of bioscience and bioengineering*, 115(4), 412–417.
  7. Pinhassi, R. I., Kallmann, D., Saper, G., Larom, S., Linkov, A., Boulouis, A., et al. (2015). Photosynthetic membranes of *Synechocystis* or plants convert sunlight to photocurrent through different pathways due to different architectures. *PloS one*, 10(4), e0122616.
  8. Rosenbaum, M., He, Z., & Angenent, L. T. (2010). Light energy to bioelectricity: photosynthetic microbial fuel cells. *Current opinion in biotechnology*, 21(3), 259–264.
  9. Min, B., Kim, J., Oh, S., Regan, J. M., & Logan, B. E. (2005). Electricity generation from swine wastewater using microbial fuel cells. *Water research*, 39(20), 4961–4968.
  10. Molazadeh, M., Ahmadzadeh, H., Pourianfar, H. R., Lyon, S., & Rampelotto, P. H. (2019). The use of microalgae for coupling wastewater treatment with CO<sub>2</sub> biofixation. *Frontiers in bioengineering and biotechnology*, 7, 42.
  11. Stephens, E., Ross, I. L., Mussnug, J. H., Wagner, L. D., Borowitzka, M. A., Posten, C., et al. (2010). Future prospects of microalgal biofuel production systems. *Trends in plant science*, 15(10), 554–564.
  12. Pittman, J. K., Dean, A. P., & Osundeko, O. (2011). The potential of sustainable algal biofuel production using wastewater resources. *Bioresource technology*, 102(1), 17–25.
  13. Blackall, L. L., Crocetti, G. R., Saunders, A. M., & Bond, P. L. (2002). A review and update of the microbiology of enhanced biological phosphorus removal in wastewater treatment plants. *Antonie van Leeuwenhoek*, 81(1), 681–691.
  14. Aslan, S., & Kapdan, I. K. (2006). Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. *Ecological engineering*, 28(1), 64–70.
  15. Angelaalincy, M., Senthilkumar, N., Karpagam, R., Kumar, G. G., Ashokkumar, B., & Varalakshmi, P. (2017). Enhanced extracellular polysaccharide production and self-sustainable electricity generation for PAMFCs by *Scenedesmus* sp. SB1. *ACS omega*, 2(7), 3754–3765.
  16. Song, Y. S., Kang, C., Jeong, J., Kim, K.-O., & Lim, E. (2018). Rheological analysis of live and dead microalgae suspensions. *Journal of the Korean Physical Society*, 72(8), 858–862.
  17. Jackson, M. L. Soil chemical analysis prentice. Hall of India Private Limited, New Delhi. 1967;498.
  18. American Public Health, A., American Water Works, A., Water Pollution Control, F., & Water Environment, F. (1912). *Standard methods for the examination of water and wastewater*. American Public Health Association.
  19. Winkler, L. W. (1888). The determination of dissolved oxygen in water. *Berlin DeutChem Gas*, 21, 2843–2855.
  20. Kjeldahl, C. (1883). A new method for the determination of nitrogen in organic matter. *Z Anal Chem*, 22, 366.

21. Walkley, A., & Black, I. A. (1934). Chromic acid titration for determination of soil organic matter. *Soil Sci*, 63, 251.
22. Şükran, D., GÜNeŞ, T., & Sivaci, R. (1998). Spectrophotometric determination of chlorophyll-A, B and total carotenoid contents of some algae species using different solvents. *Turkish Journal of Botany*, 22(1), 13–18.
23. Zittel, H. E., & Miller, F. J. (1965). A Glassy-Carbon Electrode for Voltammetry. *Analytical Chemistry*, 37(2), 200–203.
24. Kakarla, R., & Min, B. (2014). Evaluation of microbial fuel cell operation using algae as an oxygen supplier: carbon paper cathode vs. carbon brush cathode. *Bioprocess and biosystems engineering*, 37(12), 2453–2461.
25. Laohavisit, A., Anderson, A., Bombelli, P., Jacobs, M., Howe, C. J., Davies, J. M., et al. (2015). Enhancing plasma membrane NADPH oxidase activity increases current output by diatoms in biophotovoltaic devices. *Algal research*, 12, 91–98.
26. Hong, Y.-G., Guo, J., Xu, Z.-C., Xu, M.-Y., & Sun, G.-P. (2007). Humic substances act as electron acceptor and redox mediator for microbial dissimilatory azoreduction by *Shewanella decolorationis* S12. *Journal of microbiology and biotechnology*, 17(3), 428–437.
27. Turick, C. E., Tisa, L. S., & Caccavo, F. Jr. (2002). Melanin production and use as a soluble electron shuttle for Fe (III) oxide reduction and as a terminal electron acceptor by *Shewanella algae* BrY. *Applied and environmental microbiology*, 68(5), 2436–2444.
28. Harnisch, F., & Rabaey, K. (2012). The diversity of techniques to study electrochemically active biofilms highlights the need for standardization. *ChemSusChem*, 5(6), 1027–1038.
29. Rollefson, J. B., Stephen, C. S., Tien, M., & Bond, D. R. (2011). Identification of an extracellular polysaccharide network essential for cytochrome anchoring and biofilm formation in *Geobacter sulfurreducens*. *Journal of bacteriology*, 193(5), 1023–1033.
30. Cevik, E., Tombuloglu, H., Anil, I., Senel, M., Sabit, H., AbdulAzeez, S., et al. (2020). Direct electricity production from Microalgae *Choricystis* sp. and investigation of the boron to enhance the electrogenic activity. *International Journal of Hydrogen Energy*, 45(19), 11330–11340.
31. Strik, D. P., Terlouw, H., Hamelers, H. V. M., & Buisman, C. J. N. (2008). Renewable sustainable biocatalyzed electricity production in a photosynthetic algal microbial fuel cell (PAMFC). *Applied microbiology and biotechnology*, 81(4), 659–668.
32. Juang, D. F., Lee, C. H., & Hsueh, S. C. (2012). Comparison of electrogenic capabilities of microbial fuel cell with different light power on algae grown cathode. *Bioresource Technology*, 123, 23–29.
33. Wu, Y., Wang, Z., Zheng, Y., Xiao, Y., Yang, Z., & Zhao, F. (2014). Light intensity affects the performance of photo microbial fuel cells with *Desmodesmus* sp. A8 as cathodic microorganism. *Applied energy*, 116, 86–90.
34. Kumar, A., Ergas, S., Yuan, X., Sahu, A., Zhang, Q., Dewulf, J., et al. (2010). Enhanced CO<sub>2</sub> fixation and biofuel production via microalgae: recent developments and future directions. *Trends in biotechnology*, 28(7), 371–380.

35. Hu, B., Zhou, W., Min, M., Du, Z., Chen, P., Ma, X., et al. (2013). Development of an effective acidogenically digested swine manure-based algal system for improved wastewater treatment and biofuel and feed production. *Applied Energy*, *107*, 255–263.
36. Zheng, H., Liu, M., Lu, Q., Wu, X., Ma, Y., Cheng, Y., et al. (2018). Balancing carbon/nitrogen ratio to improve nutrients removal and algal biomass production in piggery and brewery wastewaters. *Bioresource technology*, *249*, 479–486.
37. Choi, H.-J., & Lee, S.-M. (2012). Effects of microalgae on the removal of nutrients from wastewater: various concentrations of *Chlorella vulgaris*. *Environmental Engineering Research*, *17*(S1), 3–8.
38. Lu, Q., Zhou, W., Min, M., Ma, X., Chandra, C., Doan, Y. T. T., et al. (2015). Growing *Chlorella* sp. on meat processing wastewater for nutrient removal and biomass production. *Bioresource technology*, *198*, 189–197.
39. Hadiyanto, H., Christwardana, M., & da Costa, C. Electrogenic and biomass production capabilities of a microalgae–microbial fuel cell (MMFC) system using tapioca wastewater and *Spirulina platensis* for COD reduction. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects*. 2019:1–12.
40. Zhang, E., Wang, B., Ning, S., Sun, H., Yang, B., Jin, M., & Hou, L. (2012). Ammonia-nitrogen and orthophosphate removal by immobilized *Chlorella* sp. isolated from municipal wastewater for potential use in tertiary treatment. *African Journal of biotechnology*, *11*(24), 6529–6534.
41. Tchinda, D., Henkanatte-Gedera, S. M., Abeysiriwardana-Arachchige, I. S. A., Delanka-Pedige, H. M. K., Munasinghe-Arachchige, S. P., Zhang, Y., et al. (2019). Single-step treatment of primary effluent by *Galdieria sulphuraria*: Removal of biochemical oxygen demand, nutrients, and pathogens. *Algal Research*, *42*, 101578.
42. Qin, L., Shu, Q., Wang, Z., Shang, C., Zhu, S., Xu, J., ...Yuan, Z. (2014). Cultivation of *Chlorella vulgaris* in dairy wastewater pretreated by UV irradiation and sodium hypochlorite. *Applied biochemistry and biotechnology*, *172*(2), 1121–1130.

## Figures

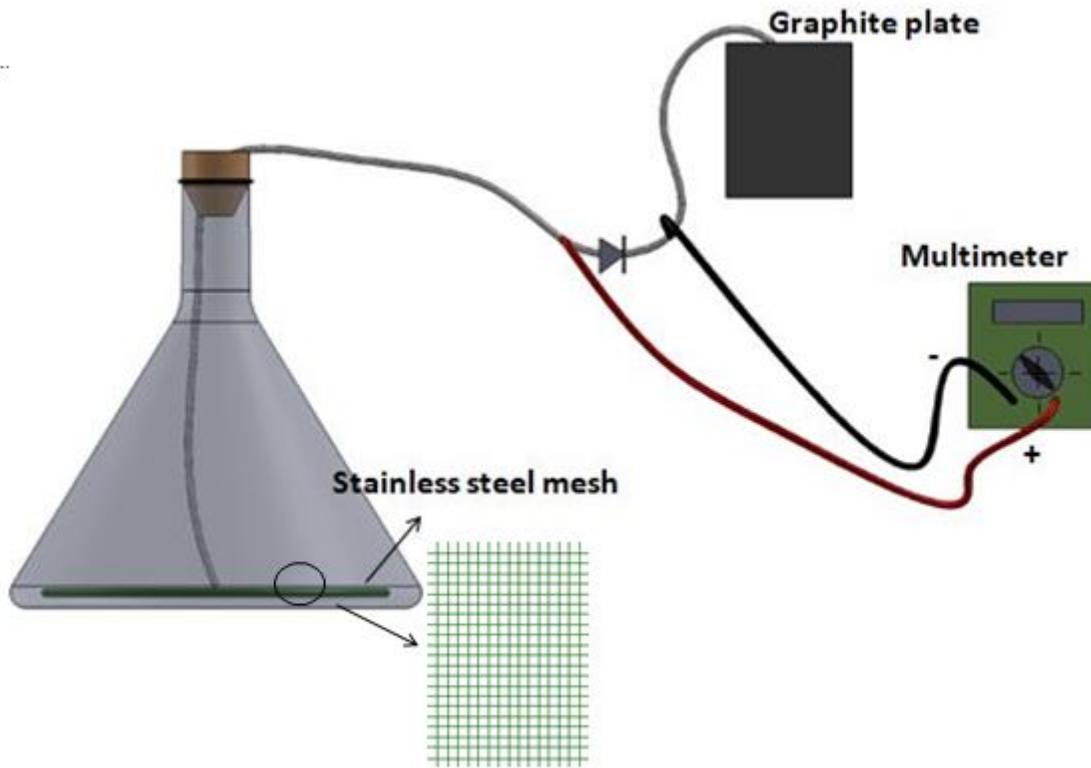


Figure 1

Experimental setup for electrogenic activity confirmation

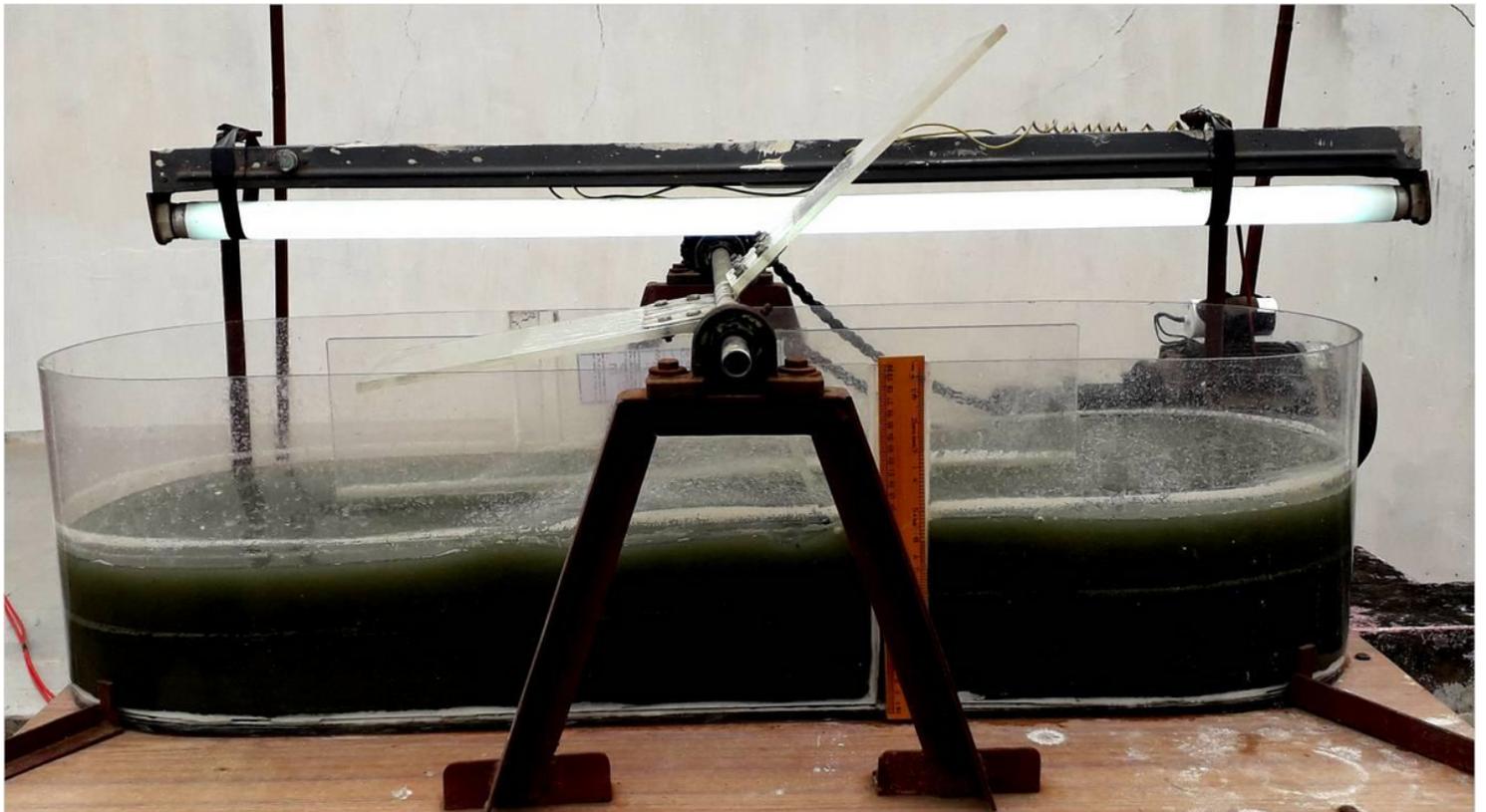
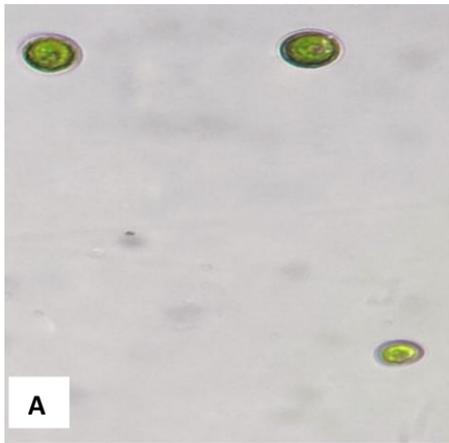
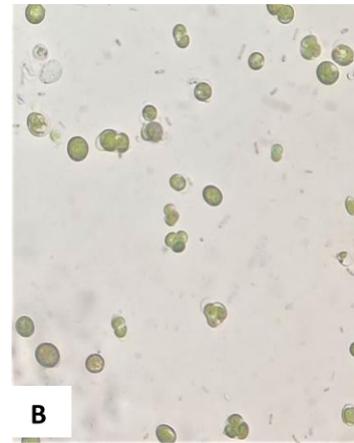


Figure 2

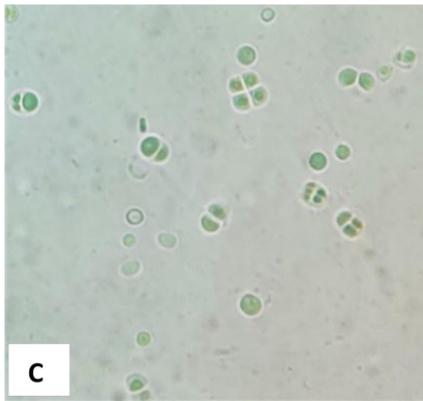
Operational view of lab-scale race way pond



MDU



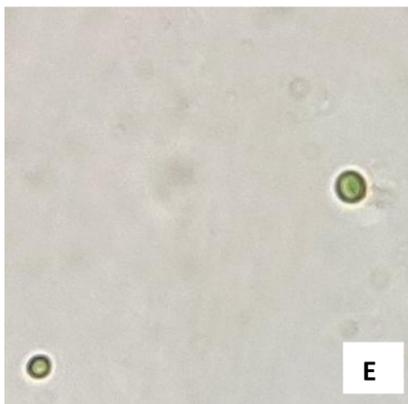
*Hindakia* sp



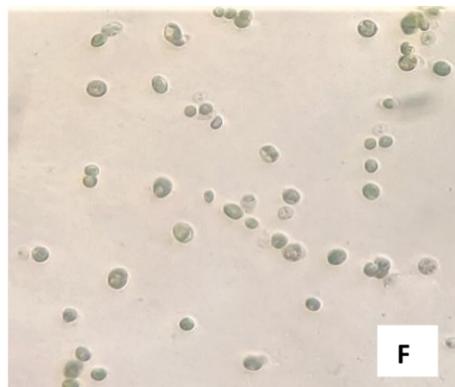
*Chlorococcum* sp



*Anabena azollae*



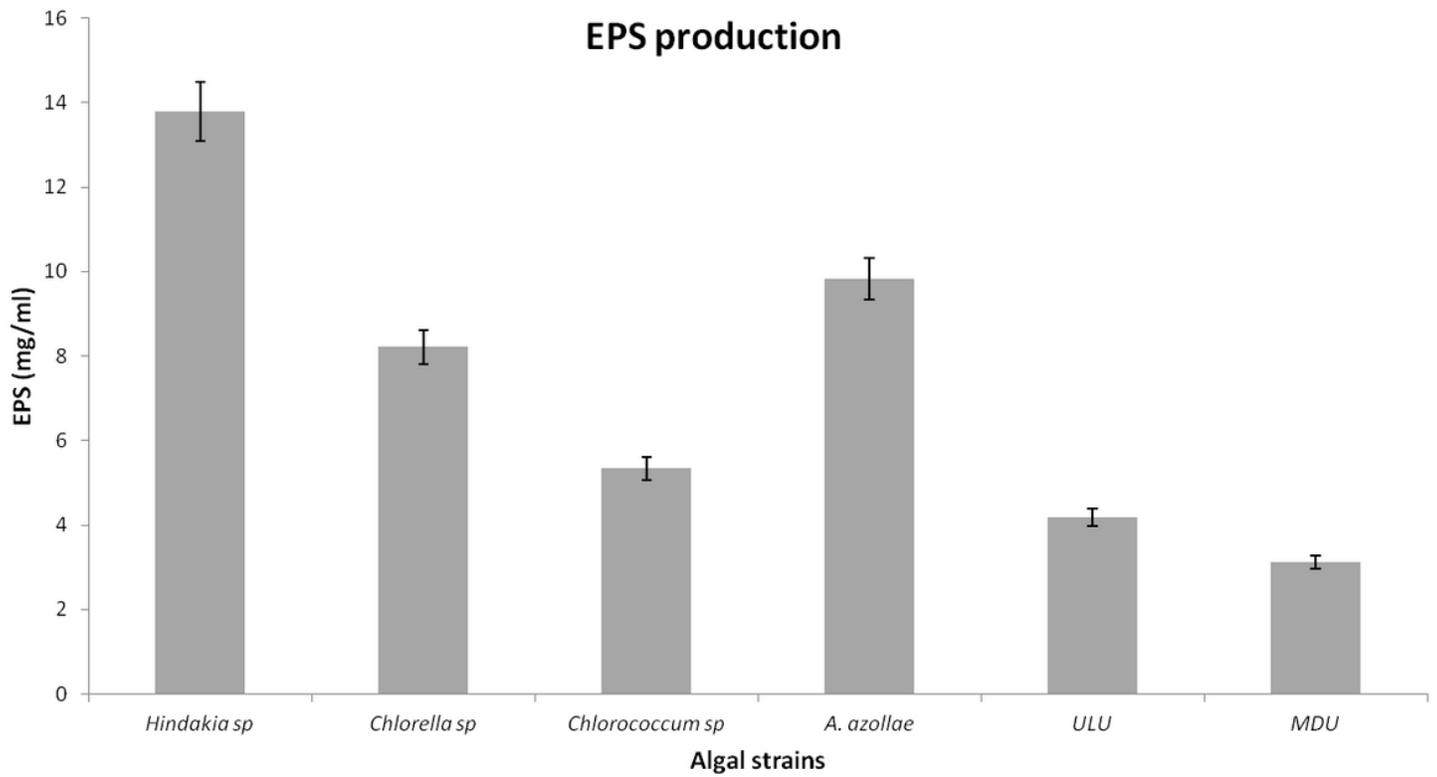
ULU



*Chlorella* sp

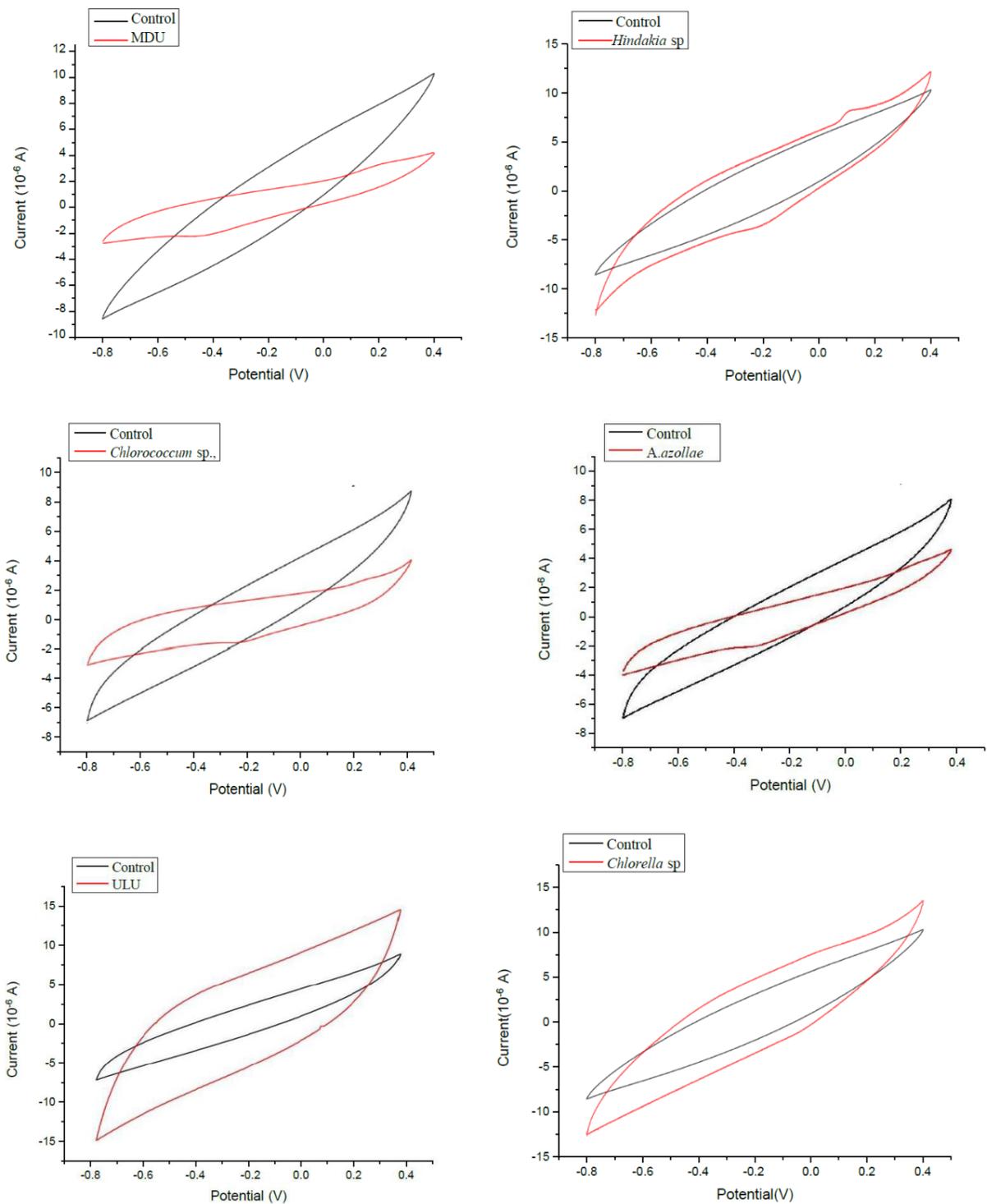
**Figure 3**

Microscopic images of A.MDU, B. *Hindakia* sp. C. *Chlorococcum* sp. D. *Anabena azollae* E.ULU, F. *Chlorella* sp



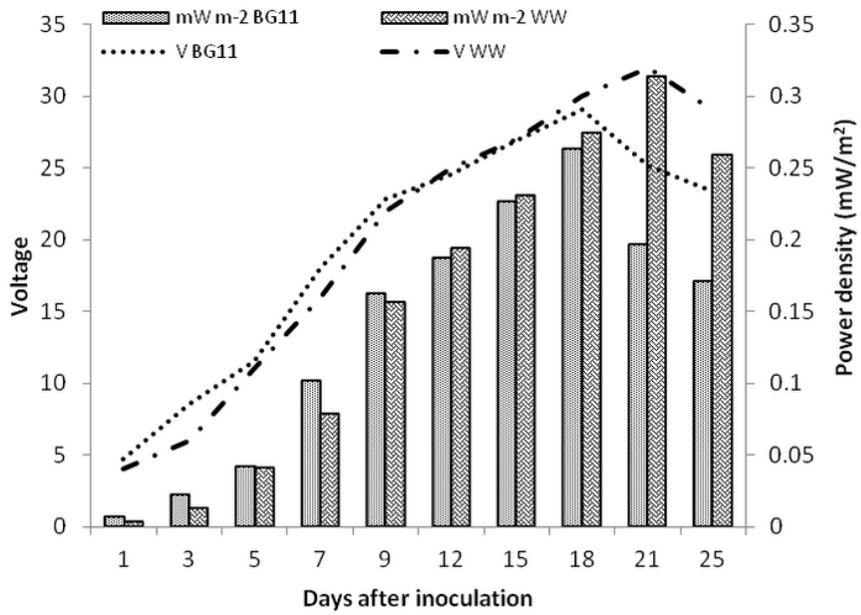
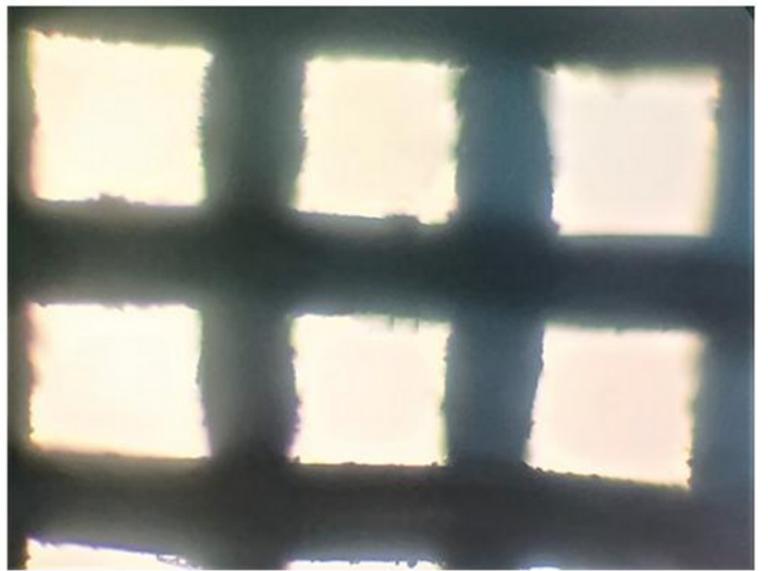
**Figure 4**

Extracellular polymeric substance production by the algal strains



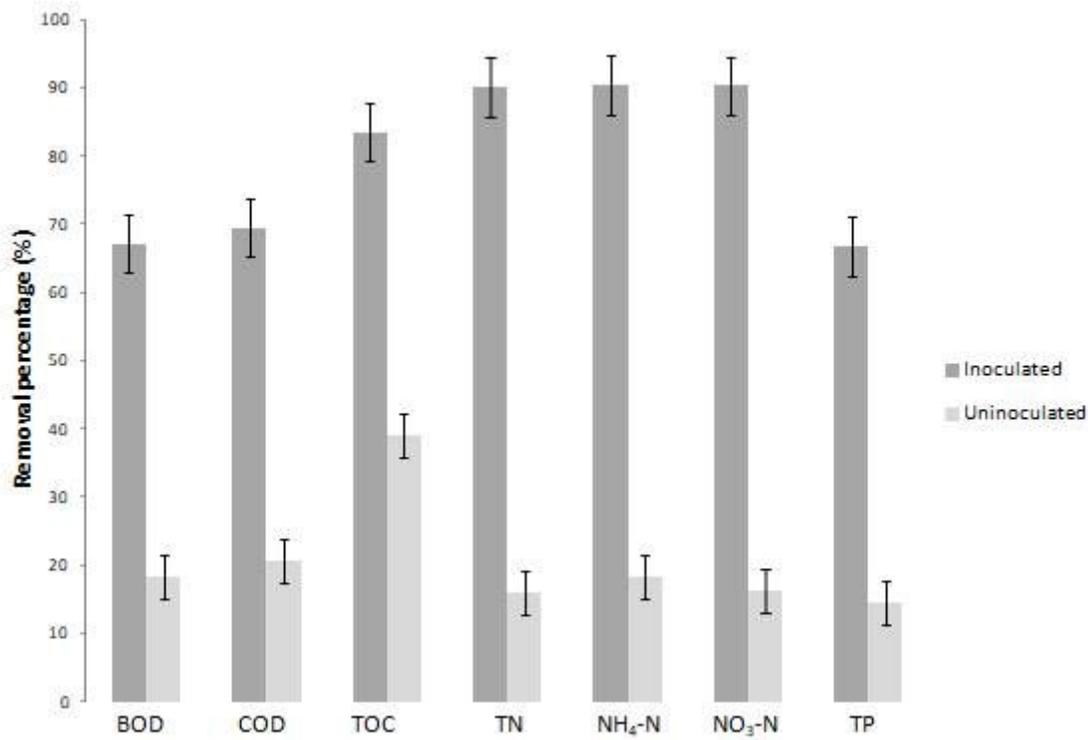
**Figure 5**

Cyclic voltammograms of the micro algal strains (A.MDU, B. Hindakia sp. C. Chlorococcum sp. D. Anabena azollae E.ULU, F. Chlorella sp)



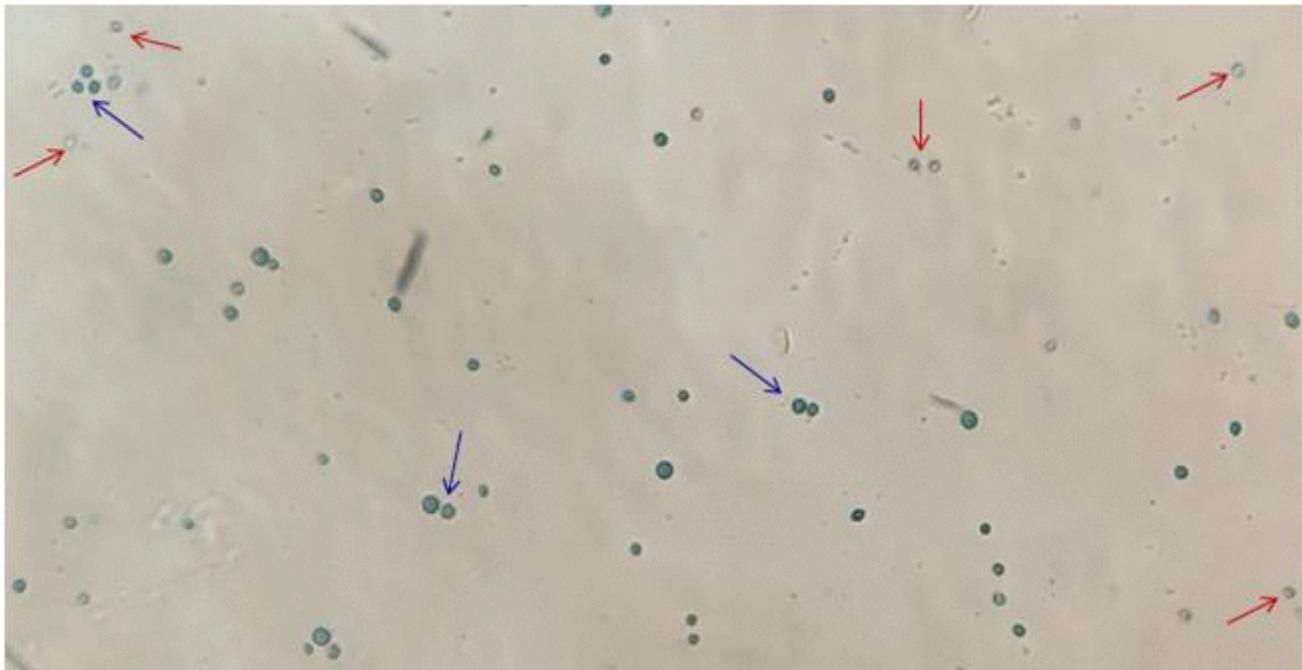
**Figure 6**

A & B. Micro algal growth in mesh vs control and their C. Power density measured in multimeter of *Hindakia* sp grown in wastewater and BG11 broth (Control)



**Figure 7**

Removal percentage of nutrients upon inoculation of algae vs uninoculated



**Figure 8**

Trypan blue staining of algal cells from the electrode. Red arrows indicate the live cells and blue arrows indicate the dead cells.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Graphicalabstract.tif](#)
- [Suppfig.tif](#)