RESEARCH ARTICLE

Screening Potential of Electrogenic Algae for Bio Power Production from Aquatic Habitats of Tamil Nadu

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ABSTRACT

. This paper illustrates the potential ofmicroalgae in electricity production.The present study focuses on searching for, indigenous microalgal strains from various aquatic sources exhibiting electrogenic activity. Sixteen microalgal strains were obtained from the diverse water bodies and assessed for electron transfer ability between cell and electrode. Six algal strains were screened out of eighteen for potential electrogenicity, based on cyclic voltammetry assay. In contrast to other algal strains, the cyclic voltammograms of *Hindakia* sp. isolated from paddy fields displayed an oxidation peak under anaerobic conditions in the potential range of +100 to +200 mV, while no distinct peaks were observed in other strains. This research broadened the exoelectrogen and identified model microalgae for investigating the extracellular electron transport process.

Keywords: *Microalgae; Electrogenic; Cyclic Voltammetry;Hindakia sp.*

INTRODUCTION

The continuous demand for energy across the globe coupled with the contribution to global warming deserves an alternate sustainable energy source. The discoveries of microbes that produce electricity from wastes represent an emerging technology of interest. The microbial systems are being employed in the production of electricity from various organic wastes with the simultaneous production of valuable chemicals and are termed as Bio-Electrochemical Systems (BES). It can use light energy to provide power and treat wastewater with the support of electro-active microorganisms (Oh and Logan, 2005). Microalgae have been widely recruited as BES in light of phototrophic microorganisms serves as *in-situ* generators of oxygen that ease reaction in the electrochemical process. Further, they are potential feedstock for a wide range of hydrocarbons (Rajesh *et al.,* 2020) including biofuels, including methane, bio-oil, methanol, bio-hydrogen (Medipally *et al.,* 2015; Zhou *et al.,* 2015)

As one of the most common microorganisms, microalgae utilize solar energy to divide the water and provide electrons and oxygen. As BES, microalgae can utilize organic matter upon consuming carbon dioxide on an electrode surface yielding energy by metabolic activity (Lovley and Nevin 2011). Several potential activities including nitrogen and phosphorus absorption (Li *et al.,* 2010), biodiesel synthesis, and biomass supply (Chen *et al.,* 2012) enlarge microalgae's application for renewable energy generation and wastewater treatment. In BES microalgae and cyanobacteria perform oxygenic photosynthesis, which might be a viable alternative to mechanical aeration. Oxygen is produced in this process by direct biophotolysis, coupled with the simultaneous generation of reduced equivalents, which aids in CO_2 sequestration in the presence of light. Electrogenesis, wastewater treatment (through anodic oxidation and cathodic reduction), and CO_2 sequestration are added advantages upon algal employment (Rosenbaum *et al*., 2010). Understanding extracellular electron transport will aid in the optimization of practical applications and the development of innovative BES functionalities. The electron transfer mechanism of novel microalgae strain, *Desmodesmus* sp. isolated from wastewater was studied in terms of electron transfer mechanisms and applications to improve current production under a variety of circumstances (Wu *et al.,* 2013). With the growing popularity of photosynthetic microalgae, a better understanding of the electron transfer process between the microalgae and the electrode is crucial. Further, a pure algal model is necessary to investigate the interaction for energy generation. In this context, a microalgal strain from the paddy field was characterized in terms of their electrogenic activity in this study to disclose the potentiality of unicellular algae.

MATERIAL AND METHODS

Sampling

Algal samples were collected from various aquatic bodies in different districts of Tamil Nadu (Table 1). The water samples collected were immediately transported to the laboratory and proceeded with isolation and the rest were stored at 4 ºC.

Isolation and purification of microalgal cultures

The water samples were serially diluted from 10^1 to 10^5 before being plated on sterile BG11 agar medium. The plates were incubated in an algal growth chamber for 7-10 days, which supports 16h: 8h of light: dark (1400 L) at a temperature of 28±2 ºC. The growing chamber was illuminated with cold fluorescent lights. To confirm the absence of bacterial and fungal contamination, the colonies were selected and subsequently sub-cultured in BG11 agar plate supplemented with ampicillin (100 g/mLL) and kanamycin (100 g/mL), cycloheximide (100 g/mL). For further analysis, the individual microalgal colonies were cultured in liquid BG 11 media.

Screening for electrogenic activity

Under an electrochemical work station (AMETEK, scientific instruments, USA), a three-electrode system in Cyclic voltammetry (CV) method was used, with a glassy carbon electrode (3mm diameter) as a working electrode, a platinum wire as a counter electrode, and Ag/AgCl (3M KCl) as a reference electrode (Wu *et al.,* 2013). A working electrode coated in a concentrated microalgal coat (cell pellet) along with 1% nafion (binder) was used to investigate the electron transfer process. To stimulate electrode-algae contact, the coated electrode was shade dried and incubated at room temperature (28 ±2 ºC) for two days. At a scan rate of 10 mVs^{-1}, CV traces were obtained in the potential range of -0.8 to +0.8 V. To verify the lack of oxidation-reduction, deionized water was deoxygenated and utilized as an electrolyte.

RESULTS AND DISCUSSION

Light microscopic observation of electrogenic microalgae

Water from various aquatic sources was sampled onto agar plates and the clones were picked after several cycles of agar plate spreading. Sixteen species were isolated and their morphology was observed with light microscope as depicted in figure 1. A brief description of the isolated algal isolates from various sources is presented in table 1. Isolates with various colony morphology were obtained.

Cyclic Voltammogram analysis

To assess the redox activity of the algal isolates, CV measurements were carried out on glassy carbon, as they are highly conductive Zittel and Miller, (1965) and measure the current that flows between two electrodes. The pellet from the harvested microalgae on 20th day after inoculation was coated on the glassy carbon electrode along with nafion solution to observe the redox peak. CV profile evinced significant variation in electron discharge and generation of energy among the isolates analyzed. In general, nafion serves as cation-exchange polymer through which the single ions or multiple charged ions can pass (Naji *et al*., 2013) and were reported to promote the actuation response of the electrode (Naji *et al.,* 2016). The electrons from algae could be transferred with the assistance of nafion membrane ensuing the electric power display. The comparable results were obtained by Wang *et al.*, (2014) with the use of nafion membrane in the dual chambered microbial fuel cell.

Six of the 16 isolates were positive for electrogenic activity. The electrogenic activity was observed in *Chlorella* sp., *Chlorococcum* sp., *Anabena azollae*, isolate MDU and isolate ULU from our investigation. However only *Hindakia* sp. produced a clear redox peak throughout a potential range of -0.8 to 0.4V vs Ag/Ag Cl_2, reflecting their redox activity (Figure 2). The electrochemical experiment performed in the culture supernatant (control) displayed only the background current indicating the electrical inactivity, while on the contrary pellets showed a significant response. This represents that no components were exclusively present contributing to the electrogenicity in the cell-free supernatant. Akin to our study, Cereda *et al.,* (2014) have demonstrated the negative response of photocurrent in the fresh medium in which no mediators are contributing to the donation of electrons to the electrode.

The electric response gives forth the current peaks (10^{-6} A) of electro active microalgal strains, *Hindakia* sp. which produced higher potential peak as shown in Figure 3. In analogy with the results, Wu *et al.,* (2018) have demonstrated the enhanced current generation by electrogenic microalgae *Desmodesmus* sp. A8. Exoelectrogens are distinguished by their capacity to transport electrons at electrode/biofilm interactions (Meitl *et al.,* 2009), indirect transfer via flavin and direct transfer via proteins (Patil *et al.,* 2012). In electron transfer processes, certain cytochromes of terminal reductases are involved. Wu *et al.,* (2013) reported there was no electrochemical reaction in the supernatant of *Desmodesmus* sp, certain proteins on the outer membrane, such as cytochromes, may be engaged in direct electron transfer involving in that strain A8 and also reported that *Desmodesmus* sp. was able to act as a cathodic microorganism. In a cyclic voltammogram *Hindakia* sp displayed an oxidation peak in the potential range of +100 to +200 mV which describes its electron releasing capability. The results demonstrate that *Hindakia* sp. may transfer electrons to an electrode via electro-active proteins on the cellular surface or secreted oxygen. The exploitation of *Hindakia* sp for electricity generation can be taken up by constructing algal fuel cells. Similarly, in our study, there would be several mechanisms underpinning the electrogenic activity of *Hindakia* which have to be explored further. Hence following electrochemical analysis, the *Hindakia* sp. was chosen for further investigation to ensure the power generation.

CONCLUSION

In this study, the electrogenic activity of *Hindakia* sp. was analyzed by voltammogram analysis using cyclic voltammetry, which provided a qualitative report on its redox process. This study serves as a step for further construction of bio-electrochemical fuel cell in which *Hindakia* can be coated in anode which produces electricity by utilization of light energy. Furthermore, since it is a candidature alga their possibility of wastewater utilization as a medium in electrogenesis shall also be explored.

Funding and Acknowledgment

The authors acknowledge Dr. Pon Pandian, Professor and Head, Department of Nanoscience, Bharathiyar University, Coimbatore for rendering Electrochemical workstation assistance for the analysis, and this research does not hold any funding assistance.

Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

Originality and plagiarism

Authors declare that the contents are original and plagiarism free.

Consent for publication

All the authors agreed to publish the content.

Competing interests

There were no conflict of interest in the publication of this content

Data availability

All the data of this manuscript are included in the MS. No separate external data source is required. If anything is required from the MS, certainly, this will be extended by communicating with the corresponding author through corresponding official mail; skarthy@tnau.ac.in

Author contributions

Idea conceptualization, Guidance, reviewing and editing –SK, Experiments and writing original draft – SW, writing original draft- KGT

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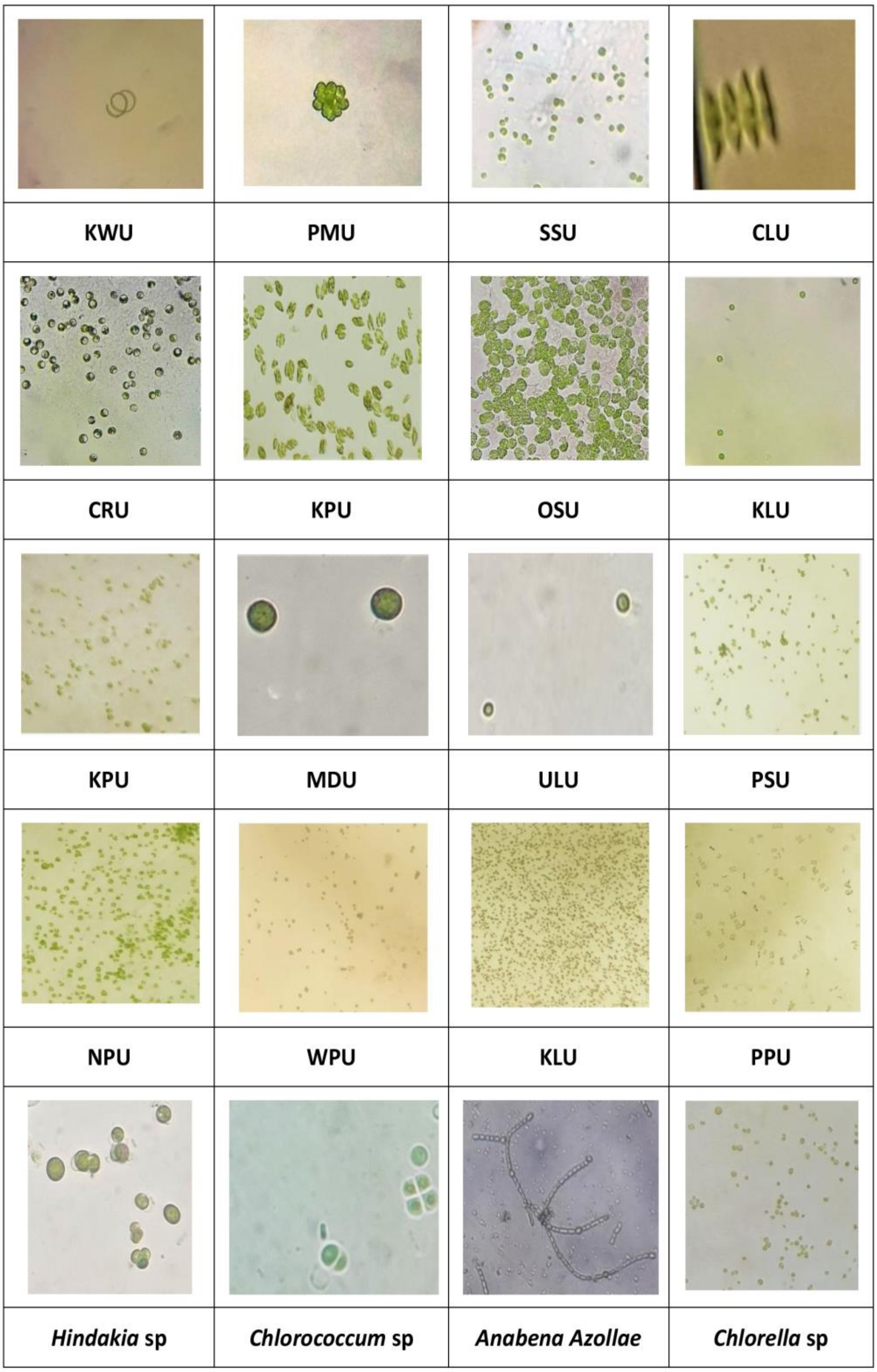

Figure 1. Microscopic images of screened algal isolates under light microscope (250 X)

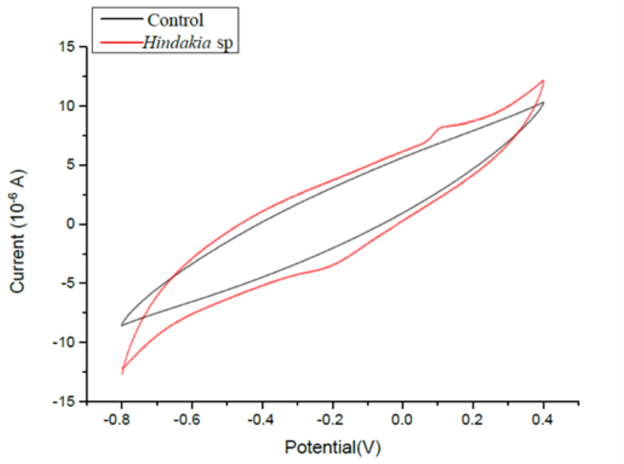

Figure 2. Cyclic voltammograms of Hindakia sp. on glassy carbon under anaerobic condition

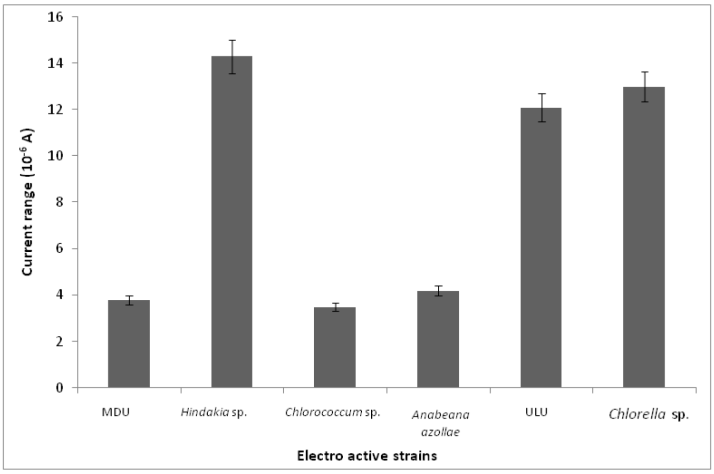

Figure 3. Current peak ranges (10^{-6} A) of electro-active algal strains shown in cyclic voltammograms

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

Isolate code	Source	Geographical location	Morphological characteristics
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
ULU	Lake	Ukkadam (10.982817° N/76.961144 ° E)	Unicellular, spherical and non-motile
PSU	Pond	Poosaripalayam (11.004039 ° N/76.932391 ° E)	Unicellular, ovalshaped, 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
Hindakia sp	Paddy field	Wetland paddy fields (11.002288° N/76.926175 ° E)	Unicellular, spherical, solitary colonies, non-motile with smooth cell wall
Chlorococcum sp			Unicellular, tetrad arrangement of cells
Anabena azollae			Chain like arrangement of cells, heterocystous
Chlorella sp.			Unicellular, spherical and non-motile