



e-ISSN: 2319-8753 | p-ISSN: 2347-6710

# IJRSET

International Journal of Innovative Research in  
**SCIENCE | ENGINEERING | TECHNOLOGY**

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# INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH

IN SCIENCE | ENGINEERING | TECHNOLOGY

Volume 10, Issue 11, November 2021

**ISSN** INTERNATIONAL  
STANDARD  
SERIAL  
NUMBER  
INDIA

Impact Factor: 7.569

 9940 572 462

 6381 907 438

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# Production of Biohydrogen from Water Lettuce (*Pistia stratiotes*)

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**ABSTRACT:** Water lettuce (*Pistia stratiotes*) plant were collected from various ponds in and around Dindigul (Dt), TamilNadu, India. The plants were washed the roots and leaves were separated, dried, powered and stored. The dried samples were analyzed for their physical and chemical properties. The bacterial strains were isolated from sewage sample. The predominant bacterial strain was identified based on morphological and biochemical characteristics *E.coli*. The selective or test organisms was conformed as *E.coli*. 10 gm of pretreated water lettuce were fermented by *E. coli*. 500ml of water displaced at acid and alkali (5%) microwave, steam using pretreated water lettuce in 5 days by hydrogen gas produced by *E.coli*. More amount of water displaced in the acid and alkali (5%) microwave, steam pretreated water-lettuce when compared to the control. The estimation of hydrogen by water displacement done upto 12 days.

**KEYWORDS:** *Pistia stratiotes*, Pretreatment, *E.coli*, Biohydrogen

## I.INTRODUCTION

Water lettuce is also free floating aquatic plant native to Asia, America and equatorial America. There is some debate as to whether the northern territory forms part of its native range. One established, it potential to quickly spread and forma dense mat that can cover on entire body of water. It is through have been introduced to NSW Rivers and dams via eel traps from queensland and as an aquarium plant and water garden specimen sold in nurseries. Rivers, wetland, lakes, reservoirs and slow moving stream are most at risk form this weed, especially in the subtropical region of the world. Under favorable condition, water lettuce will produce abundant growth, expand rabidly and form obstructive mats these large dance floating mat can have impacts on native aquatic plants and animals. They can also interfere with irrigation, boating and water sports activities by Rahman and Hasegawa [1]. Water lettuce grows best on still or slow moving bodies of fresh water such as farm dams, reservoirs, lakes, rivers and creeks. It will tolerate temperature between 15°C and 35°C; however optimum temperature for growth range between 22°C and 30°C, water lettuce is frost sensitive and growth is limited in temperature zone by long cool winder. It can survive for long periods on mud banks or in other damp locations such as road side culverts water lettuce is free floating plant than can grow up to 15 cm tall and 30 cm wide. Mother and daughter plants are attached by solons (white root like structure which link plants together) up to 60cm long. Each plant produced a number of solons, enabling the plant to increase rapidly once shed, the seeds will float to the bottom. They germinate in early summer once shed, the seeds will float on the water before sinking to the bottom. The germinate in early summer once temperatures rice above 20°C and then float to the surface as seedling. flowering and reproduction can occur as early as the four to five leaf stage of development. When condition for growth are good, the plant can quickly reproduced and cover an entire body of water with a thick mat of connected rosettle. Plate green leaves are ribbed, wedge –shaped and small thick hairs that cover them Singhal and Rai [2].



## II.METHODOLOGY

### Collection of samples:

Water hyacinth (*Pistia stratiotes*) plants were collected from various ponds in and in around Dindigul(dt), TamilNadu, India. Harvested water lettuce was washed manually with tap water and leaves and roots were separated. The leaves and roots were dried at 60°C and then powdered using 100nm-mesh for further analysis and experiments.

### Analysis of organic materials:

Analysis of Physical properties such as moisture content, pH, ash content total solids, volatile solids, temperature and chemical properties such as estimation of carbon, estimation of nitrogen, estimation of phosphorus, estimation of potassium and Pretreatments like acid treatment, alkali treatment, steam treatment, microwave treatment, isolation of bacterial strain from sewage sample, hydrogen production in fermentor, hydrogen production at various treatment and collection of hydrogen were done using the standard methods.

## III. EXPERIMENTAL RESULTS AND DISCUSSION

### Analysis of physical and chemical characteristic:

Physico - chemical characteristics of water lettuce plant were analysed at the initial stage of digestion given in Table-1.

**Table 1: Physico properties of water lettuce:**

S. no	Parameters observed	Water lettuce
1	pH	6.8
2	Moisture content (%)	10.2
3	Total solid (%)	11.20
4	Ash content (%)	16.8
5	Volatile solids (%)	78.5
6	Carbon (%)	37.30
7	Nitrogen (%)	2.52
8	Potassium ppm	61.23ppm
9	Phosphorus (OD)	0.079

### Isolation of *Escherichia coli*:

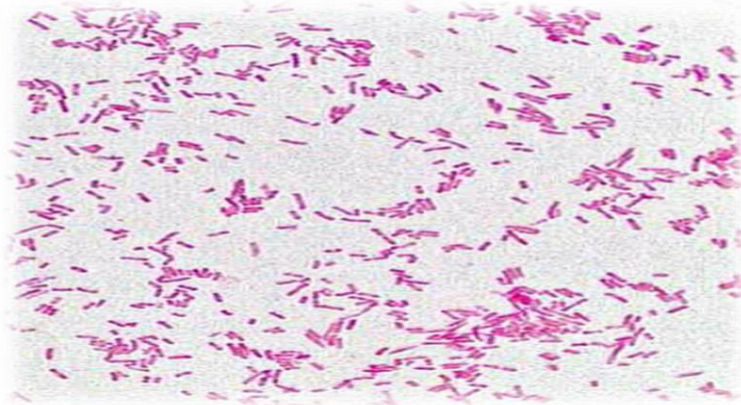
The *E.coli* was isolated from sewage sample using the standard methods.

### Identification of *E.coli*:

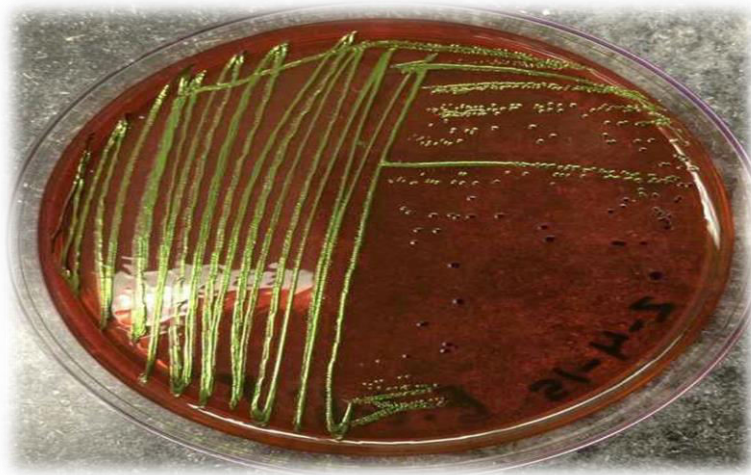
The *E.coli* strain was identified based on the Gram staining, growth on selected media (EMB) and biochemical test. The results were shown in plate 1 and 2 and table 2 and 3.



**Plate 1: Gram's staining**



**Plate 2: Growth of *E. coli* on EMB agar plate**



**Table 2: Biochemical characteristic of *E. coli***

S. no	Identification test	Colour change observed	Result
1	Gram staining	Pink and rod shape	Gram negative
2	Streaking on EMB plate	Metallic green seen	Positive

**Table 3: Biochemical characteristics of *E. coli*:**

Biochemical test	Results
Indole	Positive
Methyl red	Positive
Voges proskauer test	Negative
Citrate utilization	Negative
Nitrate reductase	Negative
Urease	Negative
Starch hydrolysis	Positive

**Estimation of hydrogen produced by *E. coli* using water displacement method:**

The total gas produced by *E. coli* from water lettuce was allowed to pass through 10%



NaOH solution. After absorption of other gases remaining hydrogen gas collected in another bottle. The collection of gas is shown in plate 3.

**Plate 3: Hydrogen production in various pretreatments(acid, alkali, microwave, steam)**

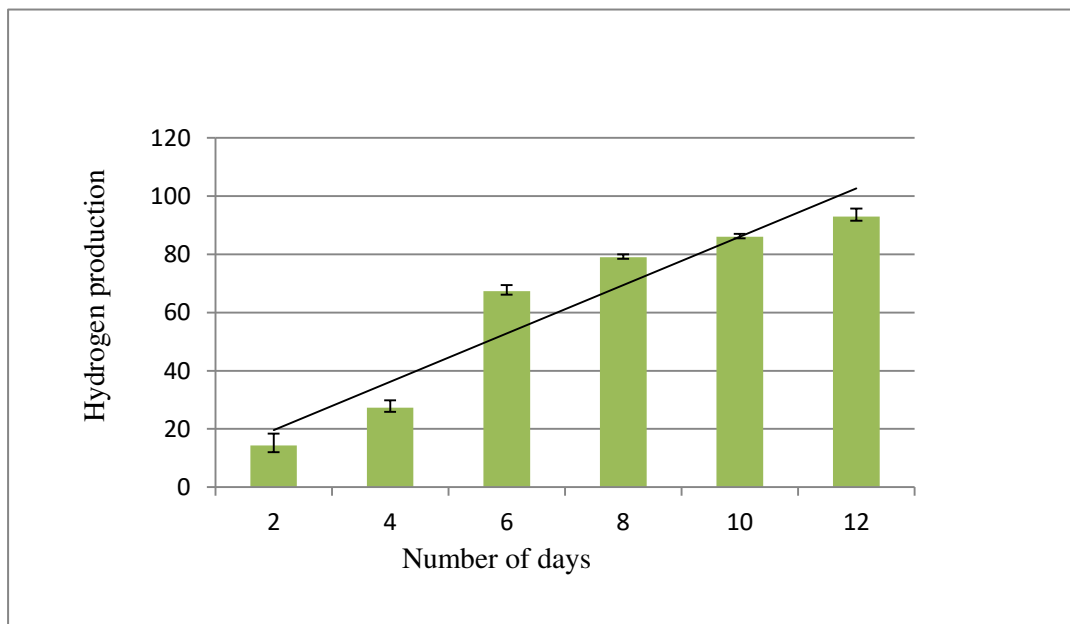


A – 0.5% pretreatment of acid, alkali.  
B – 1% pretreatment of acid, alkali.  
C - 2% pretreatment of acid, alkali.

D - 3% pretreatment of acid, alkali.  
E - 4% pretreatment of acid, alkali.  
F - 5% pretreatment of acid, alkali.

**Hydrogen production in various treatment:**

The result for the production of hydrogen gas by *E.coli* using water lettuce at various Pre-treatment like acid, alkali (5%) microwave and steam for 12 days is shown in figure 1



**Figure 1: Hydrogen production at microwave, steam, acid and alkali (5%) pretreatment**



Biohydrogen is conventionally produced from substrate containing high amount of carbohydrates such as sugarcane, starch and corn by Sivasankari et al.[4]. Lignocellulosic biomass which primarily contains wood, grass, weed, agricultural by products or crops is an ideal inexpensive, renewable and abundant resource for hydrogen production by Demirbas [5]. Water-hyacinth is a free floating aquatic weed and it cause many problems in the whole river area. It is a lignocellulosic biomass and consist of cellulose, hemicellulose and lignin by Cathryn et al. [6]; Richen Lin et al.,[7] and Sivasankari and David Ravindran [8]. Cellulose is a unbranched polymer chain constituted by glucose units. Hemicellulose is a group of different branched polysaccharides that comprise both pentoses ( xylose and arabinose) and hexoses (glucose ,mannose and galactose). Lignins are regarded as complex, amorphous, branched polymer that comprise different phenyl propane units, which give the structural support. The mixed hydrogen producing bacteria and methane producing bacteria were obtained from a gas plant in Zhejiang province, China. The dominating species in hydrogen producing bacteria was *clostridium butyricum*, and dominating species in methane producing bacteria were *Methanosarcina* and *Methanothrix*. The bacteria isolation enrichment processes have been described in previous studies by Sivasankari et al.[3]. In the present study the attempt was made to estimate the hydrogen production using *E.coli* because enterobacteriaceae group of microorganisms is efficient for the production of hydrogen. The organism were isolated from the sewage sample and identified the morphological and biochemical characters. The hyacinth were pre-treated with some methods like acid, alkali, microwave and steam pretreatment. Effective pretreatment methods include the preservation of pentoses (hemicelulose) fractions, avoidance of fermentative production inhibitors, minimisation of energy demand and savings in process costs by Manikandan et al.[9]. Several pretreatment methods have been developed such as acid, alkali, microwave and steam pretreatment. The effective microwave pretreatment time on reducing sugar yields were investigated and maintaining the temperature at 190°C ,the measured reducing sugar yields increased. xylose is the main source after the microwave pretreatment along with small amount of manmade, glucose and galactose. This was due to the degradation of hemicellulose in water hyacinth during microwave treatment. The acid and alkali pretreatment was done in different concentrations (0.5,1,2,3,4,5) and the microwave treatment was done in 190°C at 5-30 minutes. The steam treatment was done in 15 lbs at 20 minutes. This method helps for the degradation of lignocellulosic waste into cellulose and hemicellulose. The pretreated samples produce higher amount of hydrogen. The xylose and mannose is decreased when the pretreatment time was extended to 20 minutes ,which was attributed to the degradation of monosaccharide into other byproducts (eg., acetic acid and furfural ) at a longer treatment. Microwave heated alkali pretreatment can effectively disrupt lignocellulosic structures and improve the enzymatic digestibility of water hyacinth, resulting in enhanced hydrogen and methane production by Richards et al.[10]. At 5% ( acid and alkali) pretreated sample produce higher amount of hydrogen. The pretreatment having the capability of distrust the lignocellulosic structure and hydrolyse the cellulose and hemicelluloses structure that were easily utilized by hydrogen producing bacteria for the production of hydrogen. At lower pretreated sample there could be the degradation of lignocellulose is lesser and the yield of reducing sugar is decreased ,resulting in lowering of intracellular level of Adenosine triphosphate (ATP) and inhibiting the glucose production.

#### IV. CONCLUSION

The bacterial strains were isolated from sewage sample. The predominant bacterial strain was identified based on morphological and biochemical characteristics *E.coli*. The selective or test organisms was conformed as *E.coli*. 10 gm of pretreated water lettuce were fermented by *E. coli*. 500ml of water displaced at acid and alkali(5%) microwave, steam using pretreated water hyacinth in 5 days by hydrogen gas produced by *E.coli*. More amount of water displaced in the acid and alkali(5%) microwave, steam pretreated water lettuce when compared to the control.

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