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# Production of Biohydrogen from Water Hyacinth (*Eichhornia crassipes*)

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**ABSTRACT:** Water hyacinth (*Eichhornia crassipes*) plant were collected from various ponds in and around Dindigul (Dt), TamilNadu, India. The plants were washed the roots and leaves were separated, dried, powdered 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 hyacinth 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 hyacinth when compared to the control. The estimation of hydrogen by water displacement done upto 12 days. The data were reported as mean, standard deviation and standard error.

**KEYWORDS:** *Eichhornia crassipes*, Pretreatment, Biohydrogen

## I. INTRODUCTION

Many substrates have been used for fermentative hydrogen production; glucose sucrose and starch have been the most widely used. In recent years, however, a few studies have begun to use organic waste as a substrate for hydrogen production by Kapdan and Kargi [1]. Unused lignocellulosic waste biomass from forestry, agriculture, and municipal source is a potential feedstock for the synthesis of bio fuels like hydrogen, which could replace fossil fuels and reduced green house gas emission by Das et al.[2]. Lignocellulosic biomass refers to plant biomass that is composed of cellulose, hemicelluloses, and lignin.

Water hyacinth (*Eichhornia crassipes*) is a free floating aquatic weed by Polprasert et al. [3]. It is a fast growing plant under all climate conditions. Due to this fast growing and the robustness of it's seeds. It cause many problems in the river area i.e reduction of fish etc, by Gunnarsson and Peterson [4]. A wide variety of microorganism were used for the production of hydrogen. Biohydrogen produced by facultative anaerobes and obligate anaerobes. *Clostridium*, *E. coli* was the facultative anaerobes that produce the hydrogen in harder environmental condition by Sivasankari et al.[5].

## II. METHODOLOGY

### Collection of samples:

Water hyacinth (*Eichhornia crassipes*) plants were collected from various ponds in and in around Dindigul(dt), TamilNadu, India. Harvested water hyacinth 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 hyacinth plant were analysed at the initial stage of digestion given in Table-1.

**Table 1: Physical and chemical properties of water hyacinth**

S.No	Parameters observed	Water hyacinth
1.	pH	6.8
2.	Moisture content (%)	10.4
3.	Total solid (%)	15.20
4.	Ash content (%)	17.10
5.	Volatile solids (%)	79.5
6.	Carbon (%)	38.40
7.	Nitrogen (%)	2.54
8.	Potassium (ppm)	62.24
9.	Phosphorus (OD)	0.082

**Isolation of *Escherichia coli*:**

The *E.coli* was isolated from sewage sample.

**Identification of *E.coli*:**

The *E.coli* strain was identified based on the Gram staining, growth on selected media (EMB) and biochemical test.

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

The total gas produced by *E.coli* from water hyacinth 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

**Hydrogen production in various treatment:**

The result for production of hydrogen gas by *E.coli* using water hyacinth at various pretreatment like acid, alkali (0.1%, 2%, 3%, 4%, 5%), microwave and steam for 12 days in figure 1.

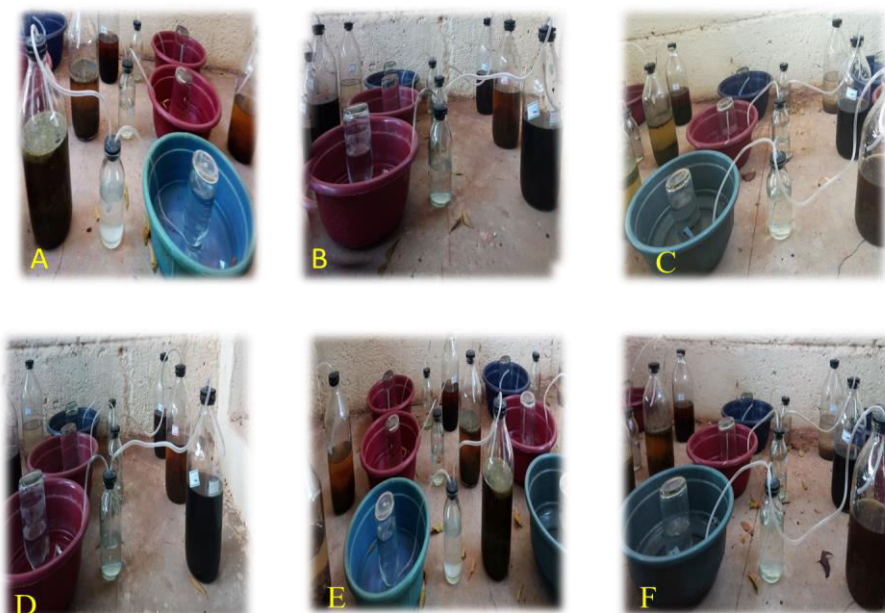


Figure 1:hydrogen production in various treatment (acid, alkali, micro wave, steam)

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.

At 5% ( acid and alkali) pretreated sample produce higher amount of hydrogen. Results are given in figure 2.

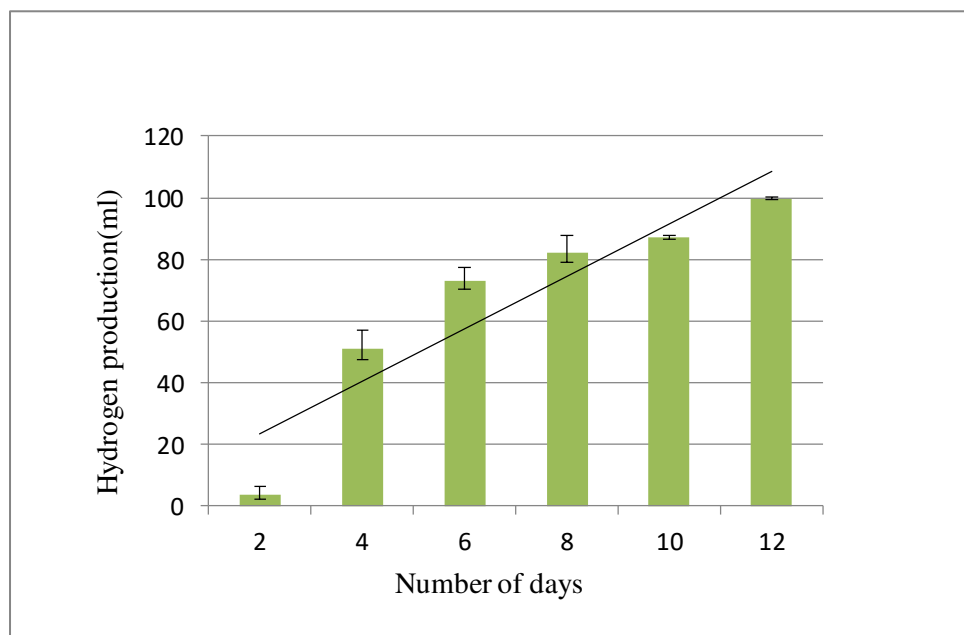


Figure2: Hydrogen production in microwave, steam, acid and alkali (5 %) pretreatment

Biohydrogen is promising alternative fuel to replace fossil fuels due to the increasing of renewable energy, damaging of climate and environmental, depletion of fossil or petroleum fuel. Biohydrogen is conventionally produced from substrate containing high amount of carbohydrates such as sugarcane, starch and corn by



Sivasankari et al.[6]. 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 [7]. 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. [8]; Richen Lin et al.,[9] and Sivasankari and David Ravindran [10]. 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.[5]. 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 (hemicellulose) fractions, avoidance of fermentative production inhibitors, minimisation of energy demand and savings in process costs by Manikandan et al.[11]. 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.[12]. At 5% ( acid and alkali) pretreated sample produce higher amount of hydrogen. The pretreatment having the capability of disrupt 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.

All the values and readings are the result of mean of three replicates data is reported as mean, standard deviation and statistical error.

#### 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 hyacinth 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 hyacinth when compared to the control

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