Fermentative hydrogen production from ricemill effluent by *Klebsiella oxytoca 18132*

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Abstract:

Biohydrogen is one of the sustainable sources of energy. Microbial production of the biohydrogen by microbial method is one the promising method. In the present study, production of biohydrogen from wastewater from rice mills by *Klebsiella oxytoca 18132* is presented in this paper. Physiochemical properties of effluent was analysed as soon as it was brought into the laboratory. Inoculums age and volume was optimised. At 20 hrs of inoculums age the total biohydrogen production was 68.00±3.56 ml with a highest conversion efficiency of 95.33%. At 30% inoculums size was best for the production of 80.62 ml biohydrogen with maximum carbohydrate conversion efficiency of 95.32 %. With these parameters the bacterial culture were immobilized with different concentrations of sodium alginate. With 6% alginate maximum production of 101.67±3.2 ml with conversion efficiency of 96.67±1.48 % was observed.

Keywords: Biohydrogen, Ricemill effluent, *Klebsiella oxytoca 18132,* Immobilization, Sodium alginate.

Introduction:

Over consumption of non renewable energy sources has resulted in depletion of fossil fuel and release of green house gases in to the atmosphere which is causing some serious environmental issues. All these problems have led to the search of an alternative and sustainable energy source (Engliman et al., 2017). Hydrogen which is derived from renewable and sustainable energy sources is one of the alternative energy sources. Hydrogen is a sustainable energy source as it produces only water and energy after combustion (Poleto et al., 2016). A number of studies used industrial wastewater from the sugar, beverage, chemical, palm oil, and distillery industries as a substrate for the generation of biohydrogen (Boodhum et al., 2017). Thermo-chemical reactions like pyrolysis, steam reforming/gasification, and supercritical water gasification have all produced H_2. Because of its inherent benefits—renewable, carbon-neutral, sustainable, having the maximum energy density, and being an environmentally responsible fuel to meet the growing need for energy—biohydrogen production has recently attracted a lot of attention worldwide. When it burns, biohydrogen releases water vapor, emits no emissions, and has three times the energy conversion efficiency (with an energy yield of 122 kJ/g) of other hydrocarbon fuels, making it a promising alternative fuel for the future (Mishra et al., 2019). Because it uses less energy and produces hydrogen at room temperature, biological methods are regarded as one of the sustainable ways to fulfill future energy demands. In addition to producing waste treatment credits, the synthesis of biohydrogen can make use of a variety of organic waste and wastewater types (Keskin et al. 2011). Choosing an appropriate raw material is crucial to the generation of biohydrogen. The substrates with a high organic content that provide a high net energy are considered suitable raw materials for the synthesis of biohydrogen for this purpose.

Furthermore, because they are widely available, wastewater from many sectors can be used as a feasible option for the production of biohydrogen. Biodegradable organic materials can be found in wastewater. This makes it an inexpensive choice for producing biohydrogen (Garça-Depraect et al. 2019; Veeramalini et al. 2019). Numerous industrial wastewaters have been documented in the literature for the production of biohydrogen, including cheese whey, paper mills (Hay et al. 2015), rice mills (Ramprakash and Muthukumar 2014), beverages (Sivagurunathan et al. 2015), cassava starch processing (Intanoo et al. 2016), palm oil, food processing industries (Gupta and Pawar 2018), distilleries (Laurinavichene et al. 2018), and sugar industry (Jayabalan et al. 2019). The microbial fermentative pathway is the most straightforward and economical of the different bio-H2 production processes; it just mimics the natural process but in the desired circumstances. Additionally, recent studies have focused closely on employing microorganisms to produce bio-H2 from trash. Because they produce bio-H2, anaerobic microbes are essential to dark fermentation. Numerous studies have been conducted on the synthesis of bio-H2 through dark fermentation using bacterial strains, including those of Bacillus, Klebsiella, Clostridium, Escherichia, and Enterobacter. In the meantime, it is essential to pretreat wastewater in order to collect potential H2-producing microorganisms. Currently, a number of pretreatment methods are widely employed to extract and enrich H2-producing bacteria. Heat-based pretreatment is one of those that effectively eliminates spore-forming microorganisms that use H2 and is thought to completely inhibit methanogenic activity. In addition to ensuring that methanogenic bacterial activity is suppressed, acid pretreatment is effective in driving out spore-forming microorganisms that produce H2. The pretreatments supported by the inoculum allow for the specific enrichment of acidogenic bacteria that produce H2 as well as the inhibition of hydrogenotrophic methanogens. The majority of research on biohydrogen production uses suspended cells, which are vulnerable to washout in continuous operations. As a result, there is operational instability and lowers its yield. To avoid this issue, immobilized microbial cells are utilized.In the present study, biohydrogen production was done by using *Klebsiella oxytoca* 18132 strain and operating parameters were also optimized.

Materials and Methods:

1. **Collection and maintenance of ricemill effluent:** The effluent from the ricemill was collected from the Khandelwal ricemill, Raipur, Chhattisgarh. The sample was collected in clean plastic containers and kept in low temperature.
2. **Physicochemical Analysis of effluent sample:** Various physicochemical properties of the effluent sample like pH, turbidity, conductivity, dissolved oxygen, chemical oxygen demand, total suspended solids, and total dissolved solids was observed before and after hydrogen production (APHA 2005).
3. **Bacterial culture:** The bacterial culture *Klebsiella oxytoca* 18132 was revived in fresh nutrient agar medium (NAM peptone 5.0g, beef extract 3.0g, NaCl 5.0 g, pH 7.0).
4. **Immobilization of bacterial cell:** Bacterial cells were immobilized in sodium alginate in different concentrations ranging from 1-4%.
5. **Age of inoculums:** Bacterial cells were incubated between 18 to 24 hrs, with the intervals of 2 hrs the inoculums were inoculated into the effluent sample.
6. **Experimental setup for biohydrogen production:** Hydrogen production feasibility was assessed in a 250 ml conical flask. A single 200 ml conical flask was used as a batch fermentor and placed on a hot plate magnetic stirrer. It was sealed with a rubber cork. Using a pipe, this flask was joined to another flask that contained 20% KOH. An empty glass measuring cylinder was once again attached to this flask. Using the gas displacement method, the gas collection was measured. (Zanchetta *et al.,* 2007).

Results and discussion: As soon as the sample was brought into the laboratory different physiochemical parameters of the ricemill effluent were analyzed as mentioned in table 1.

Table 1. Physiochemical properties of rice mill effluent.

S.No.	**Parameters**	**Value**
1	pH	8.00±0.12
2	Conductivity (mS)	0.81±0.06
3	Turbidity (NTU)	479.34±25.44
4	TDS (mg/l)	750.20±16.00
5	COD (mg/l)	1214.32±61.33
6	DO (mg/l)	0.85±0.52
7	BOD (mg/l)	52.84±2.65

At a particular age the bacteria are more metabolically active to produce the biohydrogen. The concentration of bacteria also plays an essential role. Age and volume of the inoculums was optimized for increased biohydrogen production. At an intervals of 2 hrs the inoculums age was optimized from 18-24 hrs. Table 2 shows the pattern of biohydrogen production rate with increasing age of bacteria. Best biohydrogen production was observed at 20hrs old culture (Fig 1). The production was 68.00±3.56 ml with a highest conversion efficiency of 95.33% suggesting that at this stage the bacteria were more metabolically active as they utilized the substrate at higher percentage. When the inoculums age was further increased to 22 hrs, and 24 hrs a slight decrease in the production was observed which were 65.22±2.2 ml and 65.00±2.5 ml respectively. When effect of inoculums size was determined it was found that 30% inoculums size was best for the production of biohydrogen with maximum carbohydrate conversion efficiency (Fig 2). Kotay and Das (2007) obtained maximum hydrogen yield of 2.28 molH2/mol glucose by using *Bacillus coagulans* strain IIT-BT S1 with inoculums age of 14hrs and 10% inoculums volume. Similarly, Seengenyoung et al., (2014) worked on palm oil effluent with T. thermosaccharolyticum PSU-2 and found that at the concentration of 10, 20, and 30% of inoculums which the production of 78.5, 82.4, and 82.6% respectively.

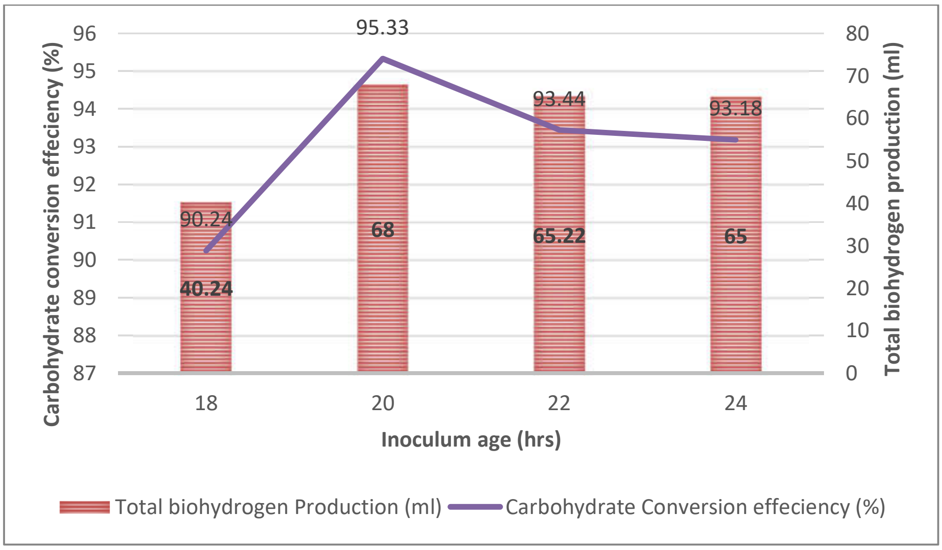

Fig 1. Effect of Inoculums age on biohydrogen production.

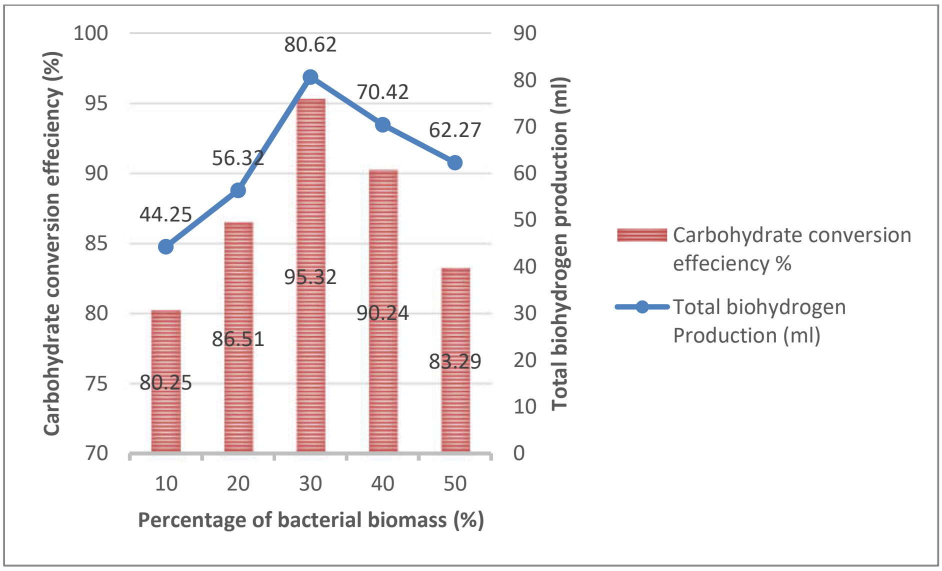

Fig. 2. Effect of concentration of bacterial biomass on biohydrogen production.

Effect of immobilization of bacteria with 20 hrs age and at concentration of 6% was studied. Maximum production of 101.67±3.2 ml with conversion efficiency of 96.67±1.48 % was observed (Fig 3). Further increase in the concentration of sodium alginate resulted in decreased production of biohydrogen. Wu et al., 2006 reported maximum hydrogen production of 21.3 mmol/l/h at 35 ^{0}C which was three times higher than the suspended bacterial culture. The bacterial culture was immobilized on calcium alginate matrix with titanium oxide and chitosan as a carriers. Liu et al., 2011 reported that immobilized Rhodopseudomonas faecalis RLD-53 exhibited the highest hydrogen production yield of 3.15 mol H2/mol acetate under various conditions like, diameter of agar granule 2.5mm, 24 h of inoculum age, and biomass concentration of 4mg/ml in agar.

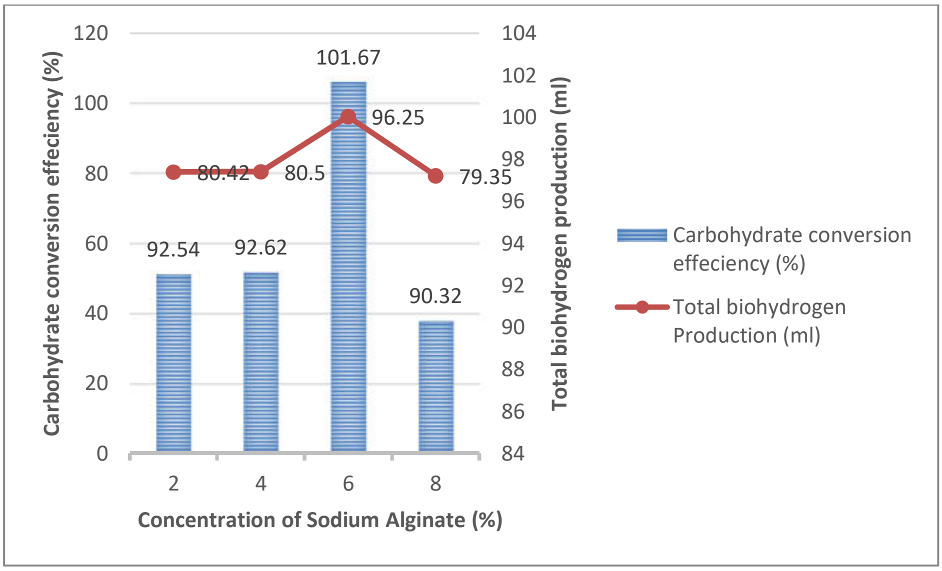

Fig. 3. Effect of immobilization on biohydrogen production.

Conclusion:

Ricemill waste water was successfully utilized for the biohydrogen production. At a particular age and at particular concentration of bacteria the production was found to be maximum. Therefore, the Total biohydrogen production and the utilization of the substrate were maximum. Then with the optimized age and volume, the bacteria were immobilized using various concentrations of sodium alginate. As suspended bacterial cultures are washed out easily immobilized bacterial culture gave the better yield.

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