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Sequential Evolution of Bio-hydrogen from Rubber Industrial Effluent and its Microbial Interaction to Fermentation Kinetics

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Summary

Rubber industrial (RI) effluent was evaluated for the biological hydrogen (H_2) production in 1 litre capacity bio reactor employing the pretreated mixed micro flora at temperature (37°C) under neutral conditions (pH 7.0) for 20 days. The physicochemical characteristic of the RI effluent was analyzed. The cumulative hydrogen production reached the maximum values with 447.2 ml/l at the end of 20th day and the maximum hydrogen potential of 72ml/l was reached on 7th day. The hydrogen production rate of RI effluent was found to be 0.608ml/gVSS/h. At the end of the fermentation, n-butyrate was obtained as the end product. The dominant microorganisms in RI effluent were *Clostridium* sp., which is responsible for n-butyrate fermentation in hydrogen production. The maximum production rate of 5.934ml/gVSS/h was reached at the present study. The effect of cell biomass and hydrogen production was predicted using (R^2=0.9561) with the modified Luedeking-Piret model. The gas production was predicted using the model modified logistic and gompertz. Therefore the finding of this study can be applied in the design of high rate bio hydrogen in the bioreactor

1 Introduction

Hydrogen is considered as a viable alternative fuel and energy carrier of the future with wide range of applications. It is a clean fuel with no CO_2 emission and used in fuel cells for electricity generation. Bioconversion of biomass to hydrogen has been demonstrated using the anaerobic fermentation from wastewater [1], solid waste [2], glucose [3], crystalline cellulose [4], peptone [5]. Moreover organic wastes like sugary effluent and starch manufacturing effluent [6] has been reported as the substrates for hydrogen production with an advantage of cost reduction and waste disposal.

Rubber industrial effluent is an important renewable biomass energy source with high nutrient content makes an ideal consideration as fermentative substrate for biological hydrogen production. Fermentative hydrogen production was studied by using organic wastes such as high strength wastewater, lingo cellulosic waste [7], food manufacturing waste and waste activated sludge [8]. The cumulative hydrogen production in the batch experiment was analyzed by using the gompertz model [9] and by the modified logistic model [10]. The present study employs pretreated rubber industrial effluent as the primary substrate for the microbial hydrogen production. This endeavour would lead to a great extent to solve the problem of rubber industrial effluent disposal and concurrently attend several limitations of biological hydrogen production.

2 Experimental Work

Effluent sample was collected at the disposal point (Rubber factory, Nagarkoil, India) and the physico chemical and biological characteristics were analyzed [11]. The culture medium was supplemented with the ingredients (1g, K_2HPO_4; 0.05mg, $MgCl_2 \cdot 6H_2O$; 0.75g, KH_2PO_4; 5ml) and trace element solution (5mg, H_3BO_4; 0.5mg, $ZnCl_2$; 30 mg, $CuCl_2$; 500 mg, $MnSO_4 \cdot H_2O$, 50 mg; (NH_4)6Mo7O_2 4·4H_2O, 50 mg; $AlCl_3$; 50 mg, $CoCl_2 \cdot 6H_2O$; 50 mg, $NiCl_2$; 1 ml, HCl (36 %) and 0.1% resazurin) was used for the present study. Batch fermentation was conducted at 37ºC with initial pH 7. The evolved gas was collected in a gas collection bag (Mylor bag). Gas composition was measured by GC and the samples from the supernatant were taken to analyze pH and organic concentrations. Various unstructured models were proved to be sufficient for characterizing the fermentation kinetics along with the growth kinetics [10]. The cumulative hydrogen production in batch experiment was analyzed using the gompertz model [9] and by the modified logistic model [10].

3 Results and Discussion

RI effluent contains rubber hydrocarbons, fresh latex, non rubber constituents such as resins, sugars, glycosides, tannins, alkaloids, mineral salts, waxes and crystals. Table 1 explained the result of physicochemical characterization of RI effluent taken for the hydrogen production. Total solids and dissolved solids were found to be 16000mg/l and 4800mg/l respectively. BOD and COD of the RI effluent were found to be 26100mg/l and 450mg/l respectively.

Table 1: Physico chemical characterization of the rubber industrial effluent.

Parameters	Unit	Mean ± SD
pH	-	7.09 ±0.2
Colour	-	Brown/Black
Total suspended solids	mg/l	11700± 2.9
Total dissolved solids	mg/l	4800 ± 2.3
Total solid	mg/l	16000 ± 0.22
Alkalinity	mg/l	4200 ± 0.42
Biochemical oxygen demand	mg/l	26100± 1.79
Dissolved oxygen demand	mg/l	4500± 5.68
Phosphate	mg/l	1.200±0.05

Total bacterial count from RI effluent was found to be 24 x 10^6 Cfu/ml whereas in heat treated industrial effluent (100ºC - 20 minutes), the total count was 5 x 10^6 x Cfu/ml, which indicated the presence of spore formers. Hydrogen production stops by the growth of methanogens in continuous flow systems and the coexistence of hydrogen consuming microorganisms [12]. Single batch experiment was conducted using the mixed culture without heat treatment to study the hydrogen fermentation [13]. So in this study, batch experiments were conducted using the heated RI effluent for the hydrogen production to reduce the process of methanogenesis.

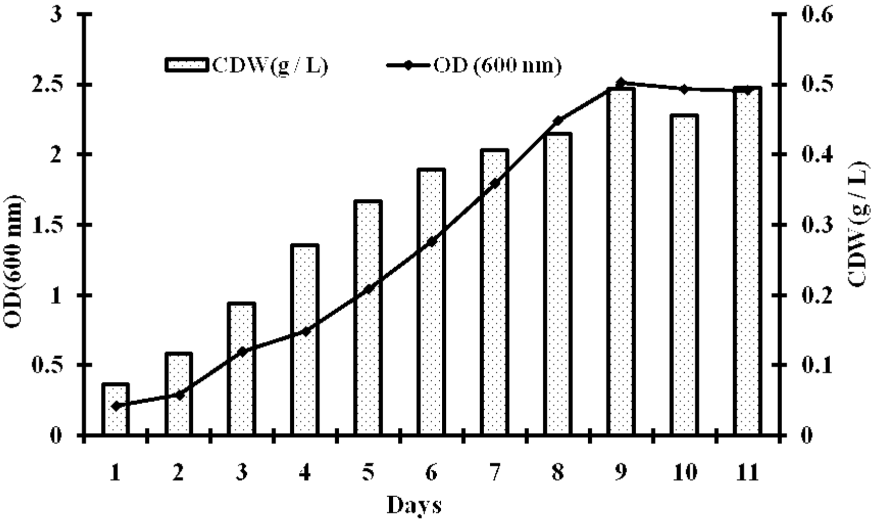

Figure 1: Growth profile of the hydrogen producing pretreated mixed micro flora: CDW (g/l) and OD (600nm).

Growth profile of the mixed micro flora taken from pretreated RI effluent was given in Figure 1. At the end of the study, the hydrogen production stopped which was due to the accumulation of the metabolites in the fermentor. The optimum growth range of methanogens was 30-37°C [14]. In this batch experiment, the gas production showed remarkable increase on 7th day with 72 ml/day (Figure 2).

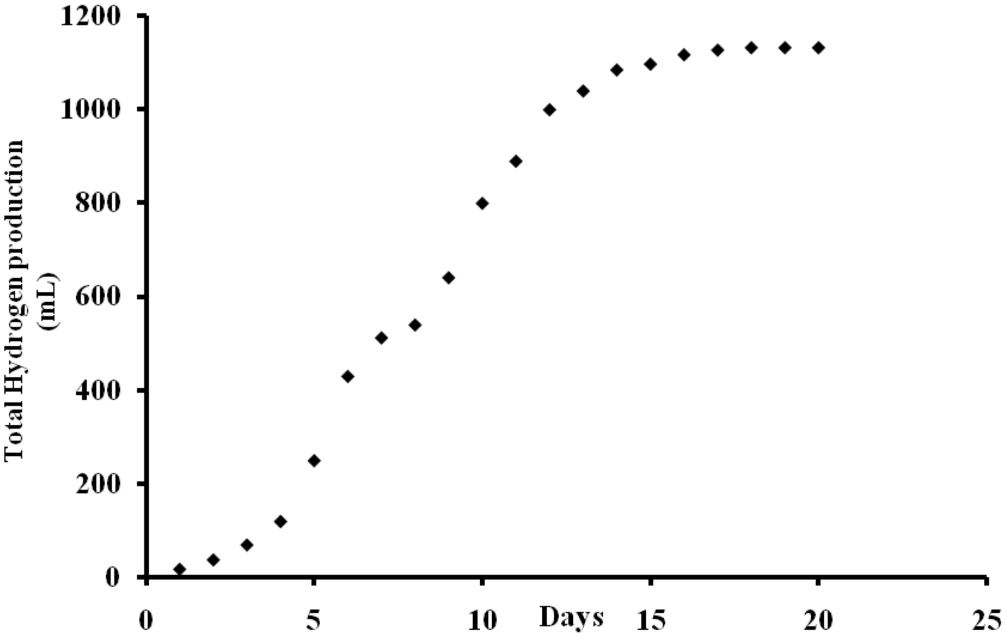

Figure 2: Total hydrogen production (ml/l) of the pretreated mixed micro flora from the rubber industrial effluent.

Total hydrogen production was found to be 447.2 ml/l at 20th day. The effect of pretreated mixed micro flora on hydrogen production profile (ml/h/day) was given in Figure 3.

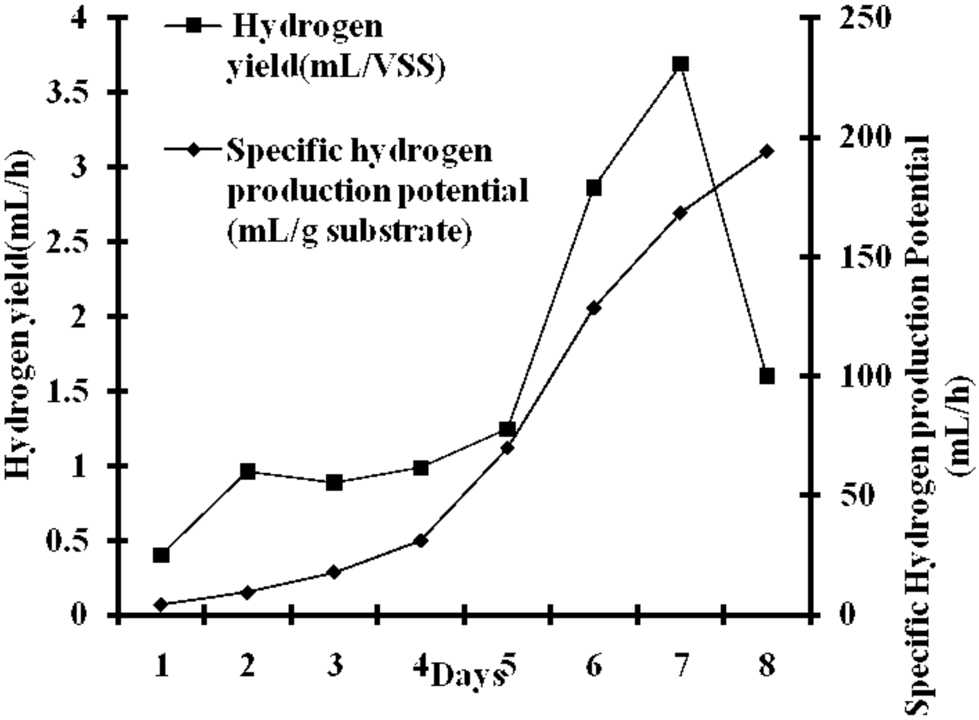

Figure 3: **Effect of pretreated mixed micro floral concentration (OD 600nm) on hydrogen production (ml/day).**

The modified logistic model predicted for the hydrogen production (ml/h/l) using the pretreated mixed micro flora (Figure 4) yields high (R^2=0.95). The total hydrogen yield (ml/l) was determined by Luedeking-Piret model and given in (Figure 5) with R^2, 0.94.The optimum temperature for hydrogen production was 50-60°C [19]. CREST compost and sludge compost produce high amount of hydrogen at 60°C with rate 0.07l/h and 0.125 l/h respectively [15]. The anaerobic spore forming bacteria forms an important part of the acidogenic population in hydrogen production [16]. The capability of the hydrogen producing mixed micro flora using the RI effluent was found to have maximum hydrogen production potential with 72ml/day on 7th day. Hydrogen production per unit biomass was constant [17]. Thus this study also confirmed the relation of the hydrogen productions and the cell concentration as growth associated.

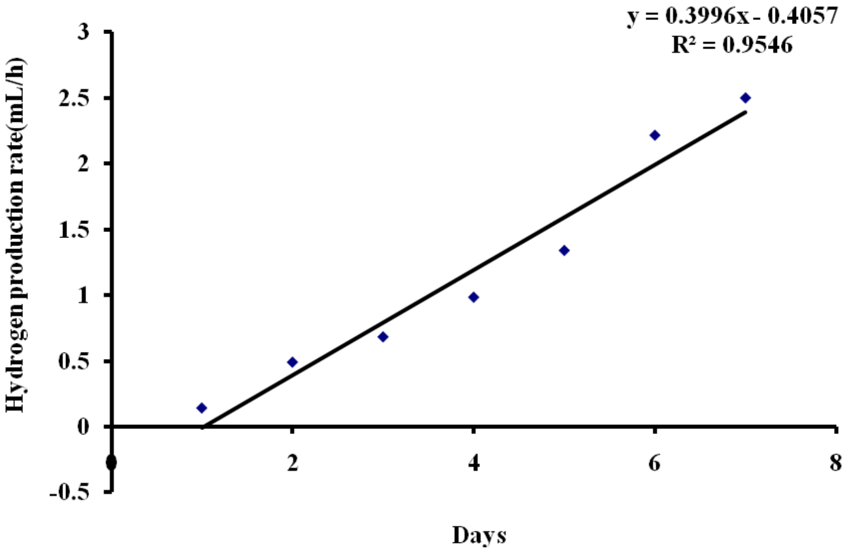

Figure 4: **Modified logistic model parameters for the hydrogen production (ml/h/l) using the pretreated mixed micro flora from the rubber industrial effluent.**

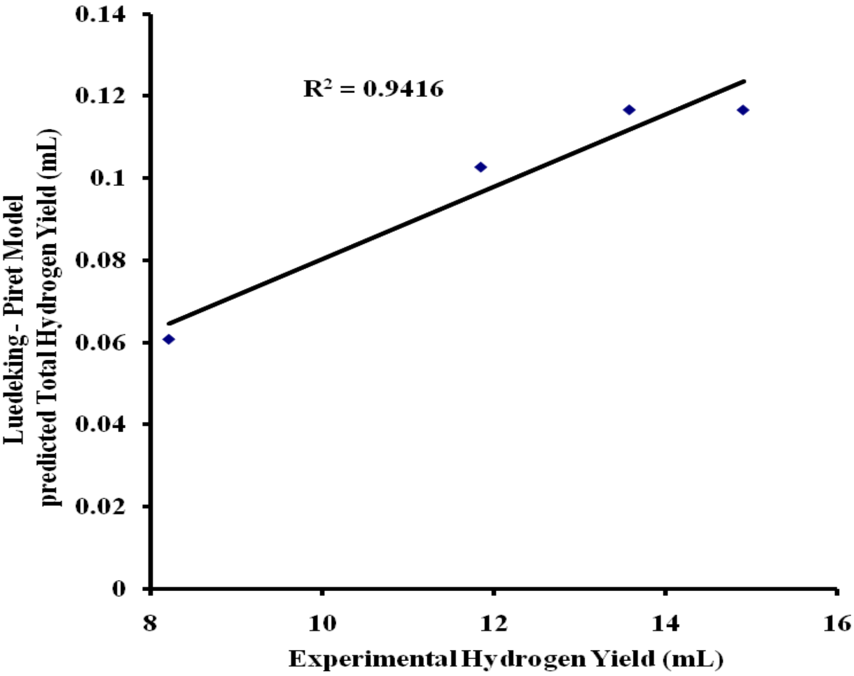

Figure 5: **Comparison of the observed and predicted values of the total hydrogen yield by Luedeking-Piret model.**

Table 2 shows the estimated parameters for both the modified gompertz model for cell growth and total hydrogen yield using the rubber industrial effluent. The values for the kinetic parameters, μ_o, α, β and Xo were found to be 2.476 (h), 0.0088 (ml/gcell biomass), 0.0078 (ml/gcell biomass/h) and 0.364g/l respectively. The modified gompertz model and modified logistic model were widely used to describe the cumulative hydrogen production in batch experiment [18]. The fermentative kinetics for the anaerobic culture taken from CREST compost at 50ºC and 60ºC exhibited gradual hydrogen gas evolution after 10h with 27.7mmol/l and 53.4mmol/l [16]. Similar results were noticed in the study as earlier [19] conducted at 60ºC with hydrogen evolution after 48 h. In this study, the maximum hydrogen production potential of 447.2ml/l was obtained on 20th day with the estimated final glucose concentration of 0.06g/l at the end of fermentation. The other end products were the trace concentrations of acetate and propionate. In this study, hydrogen production occurred in higher amount than the other end products. Because it was suspected that the metabolisms of hydrogen producing acidogenes shifted to form hydrogen rather than other products. The present study also confirmed the hydrogen production with similar phenomenon which yielded higher amount of hydrogen. The hydrogen production was found to be around pH 6 at the end of the present study. The optimum pH for the hydrogen production was observed in the pH 5 – 6 [19]. The proposed model also interpreted the trends of experimental data. The maximum specific growth rate of the micro flora (µo), and its yield coefficient (Y*x/s*) were estimated with 0.44 (h), 0.100 ml/gVSS/h, and 0.99 respectively.

Table 2: Modified Gompertz parameters for the hydrogen production using the natural micro flora from the rubber industrial effluent.

Temperature	37ºC
λ h	48
R_m (ml / h)	7.5
P (ml)	180
Maximum specific production rate (ml/gVSS/h)	3.04
Specific Hydrogen production (ml/g VSS)	29.67
Hydrogen yield (ml/g substrate)	55.6
R^2	0.9711

The result from this present study concludes that the substrate utilization, growth rate of micro flora, glucose utilization and by product formation has the remarkable influence on increased hydrogen production. Therefore under appropriate conditions natural micro flora from RI effluent were considered as the best choice of hydrogen production at cheap cost. The results from the present study can be also applied in the design experiment of the hydrogen production.

4 Conclusion

In this study, RI effluent was used as the substrate for the hydrogen production. Bio hydrogen production from renewable substrates is a promising element in the sustainable hydrogen economy. The statistical design offers efficient methods to identify the significant variables and optimized factors for hydrogen yield from RI effluent. Maximum hydrogen production potential was found to be 447.2 ml/l at the end of fermentation. The modified Gompertz and modified logistic model for hydrogen production also depicted the hydrogen production profile using the RI effluent. There was no formation of methane and the evolved gas was only hydrogen and carbon dioxide at 37ºC under pH 7. Very little work has been done on bio hydrogen production from renewable substrates using defined or complex microbial consortia. Therefore natural pretreated mixed micro flora from the RI effluent as the main substrate seems feasible way to produce hydrogen.

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Sequential Evolution of Bio-hydrogen from Rubber Industrial Effluent and its Microbial Interaction to Fermentation Kinetics. Article. A.G. Murugesan.Â As a clean energy source and industrial material, hydrogen is very valuable. Electrolysis of water and chemical methods are well-known for producing hydrogen, however, all of these methods need additional energy supply. Besides highly energy cost, the chemical methods will lead to serious environment pollution. Compared with traditional methods, biological production of hydrogen has showed significant advantages. Bio-hydrogen can be produced by anaerobic and photosynthetic microorganisms during treatment of organic waste. Sequential dark and photo-fermentation process is a rather new approach for bio-hydrogen production. One of the major problems in dark and photo-fermentative hydrogen production is the raw material cost. Carbohydrate rich, nitrogen deficient solid wastes such as cellulose and starch containing agricultural and food industry wastes and some food industry wastewaters such as cheese whey, olive mill and bakers yeast industry wastewaters can be used for hydrogen production by using suitable bio-process technologies. Utilization of aforementioned wastes for hydrogen production provides inexpensive There is not any fermentation product playing dominant role absolutely in hydrogen production fermentation. The pH value in effluent was about 4.7~4.9, the average utilizing rate of sugar reached 92.1%, most of the sugar in molasses wastewater was utilized.Â Evolution of biofuel feedstocks from edible oils to algae have been addressed in the initial section of the manuscript to provide insights on the different generation of biofuel. Different configuration of photobioreactor systems used to reduce contamination risk and improve biomass productivity were extensively discussed.Â Thermophilic biomethane production from hydrogen fermentation effluent provided the maximum methane yield of 310.77 mL CH 4 /g COD at initial pH 7.0. Microbial hydrogen production. Biohydrogen is H2 that is produced biologically.[1] Interest is high in this technology because H2 is a clean fuel and can be readily produced from certain kinds of biomass.[2]. Many challenges characterize this technology, including those intrinsic to H2, such as storage and transportation of a noncondensible gas. Hydrogen producing organisms are poisoned by O2. Yields of H2 are often low. Biohydrogen production has become important because of its potential to become inexhaustible, low-cost and renewable source of clean energy. With the use of appropriate technologies, biohydrogen would be the desired clean product of the microbial process. Fermentative route of hydrogen production from carbohydrate-rich renewable sources such as biomass or waste materials is a feasible approach. Fermentative hydrogen can be produced either by dark fermentation or by photofermentation or by a combination of both (sequential and combined dark and photofermentation). Both sequential and combined d