

# Effect of various pretreatment methods on anaerobic mixed microflora to enhance biohydrogen production utilizing dairy wastewater as substrate

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Received 22 September 2006; received in revised form 30 November 2006; accepted 1 December 2006

Available online 23 January 2007

## Abstract

Influence of different pretreatment methods applied on anaerobic mixed inoculum was evaluated for selectively enriching the hydrogen ( $H_2$ ) producing mixed culture using dairy wastewater as substrate. The experimental data showed the feasibility of molecular biohydrogen generation utilizing dairy wastewater as primary carbon source through metabolic participation. However, the efficiency of  $H_2$  evolution and substrate removal efficiency were found to be dependent on the type of pretreatment procedure adopted on the parent inoculum. Among the studied pretreatment methods, chemical pretreatment (2-bromoethane sulphonic acid sodium salt (0.2 g/l); 24 h) procedure enabled higher  $H_2$  yield along with concurrent substrate removal efficiency. On the contrary, heat-shock pretreatment (100 °C; 1 h) procedure resulted in relatively low  $H_2$  yield. Compared to control experiments all the adopted pretreatment methods documented higher  $H_2$  generation efficiency. In the case of combination experiments, integration of pH (pH 3; adjusted with ortho-phosphoric acid; 24 h) and chemical pretreatment evidenced higher  $H_2$  production. Data envelopment analysis (DEA), a frontier analysis technique model was successfully applied to enumerate the relative efficiency of different pretreatment methods studied by considered pretreatment procedures as input and cumulative  $H_2$  production rate and substrate degradation rate as corresponding two outputs.

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**Keywords:** Biohydrogen; Pretreatment; Anaerobic mixed microflora; Dairy wastewater; Chemical treatment; Data envelopment analysis (DEA)

## 1. Introduction

With increasing gap between the energy requirement of the industrialized world and inability to replenish such needs from the limited sources of energy like fossil fuels, an ever increasing levels of green house pollution from the combustion of fossil fuels in turn aggravate the perils of global warming and energy crisis. Hydrogen ( $H_2$ ) is a promising green alternative to fossil fuels as a sustainable energy source with minimal or zero use of hydrocarbons and high-energy yield (122 kJ/g) (Mu et al., 2006). Availability of abundant amount of wastewater coupled with

acidophilic anaerobic treatment resulting in  $H_2$  generation is considered to be an ideal methodology to reduce pollution load apart from renewable energy generation. Recently international support for developing these relatively new sources of energy was increased due to their benefits one such benefit is the reduction in green house gas emissions (Chynoweth et al., 2001; Charters, 2001; Logan, 2004).  $H_2$  generation during anaerobic wastewater treatment involves hydrolysis, acidogenesis and solventogenesis of which hydrolysis is the rate limiting step (Li and Noike, 1992). At present a practical and efficient  $H_2$  generation process is the growing concern among the research fraternity (Logan, 2004; Hawkes et al., 2002). Pretreatment of parent anaerobic inoculum is one strategy which helps to accelerate the hydrolysis step reducing the impact of rate limiting step and augment the anaerobic digestion to

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enhance H<sub>2</sub> generation (Kim et al., 2003; Zhu and Béland, 2006). Thermal pretreatment, alkaline pretreatment, acidification, ultrasonic pretreatment, etc., are a few pretreatment methods employed to enhance H<sub>2</sub> production (Woodard and Wukasch, 1994; Sawayama et al., 1996; Penaud et al., 1999; Zhu and Béland, 2006). In this study we have made an attempt, to evaluate the efficiency of different pretreatment methods on anaerobic mixed microflora for selectively enriching H<sub>2</sub> producing mixed consortia to enhance biohydrogen evolution rate and substrate removal efficiency utilizing dairy wastewater as main substrate.

## 2. Experimental design

### 2.1. Dairy wastewater

Dairy wastewater was collected from AP Dairy Development Cooperative Federation Ltd., Hyderabad. The combined wastewater was having pH of 6.3, total alkalinity of 1.20 g/l, total solids (TS) of 2.34 g/l, volatile suspended solids (VSS) of 1.24 g/l, chemical oxygen demand (COD) of 10.4 g/l, biochemical oxygen demand (BOD<sub>5</sub>) of 5.9 g/l, total phosphorus of 0.67 g/l, total nitrogen – of 0.18 mg/l, total volatile fatty acids (VFA) – 0.59 g/l, oil and grease of 1.92 g/l and protein concentration of 0.277 g/l. After collection, the wastewater was immediately transferred to the laboratory and stored at 0 °C. The water was not corrected for deficiency of trace elements.

### 2.2. Anaerobic mixed consortia

Anaerobic mixed microflora acquired from an operating laboratory scale upflow anaerobic sludge blanket (UASB) reactor treating composite chemical wastewater for past three years in our laboratory was used as parent inoculum

for producing H<sub>2</sub> from dairy wastewater after the specified pretreatments.

### 2.3. Pretreatment experiments

Experiments were designed to evaluate the influence of various pretreatment methods on anaerobic inoculum to enhance H<sub>2</sub> production efficiency utilizing dairy wastewater as substrate. Dewatered anaerobic sludge (pH – 7.4, SS – 66.02 g/l, VSS – 27.82 g/l, and total organic carbon (TOC) – 1.127 g/l) acquired from UASB reactor was subjected to various pretreatment procedures to selectively enrich H<sub>2</sub> producing mixed consortia (Table 1). Experimental conditions employed for each pretreatment procedure was also described in table. A total of eight experimental sets were designed and performed. Acid pretreatment procedure involves harvesting of the anaerobic consortia at pH 5 (orthophosphoric acid) under anaerobic microenvironment for a period of 24 h. Chemical pretreatment procedure involves harvesting of anaerobic consortia in 2-bromoethane sulphonic acid sodium salt solution (BESA, 0.2 g/l) for a period of 24 h in anaerobic microenvironment. In heat-shock pretreatment procedure, the anaerobic inoculum was subjected to heating (maintained at 100 °C) for a period of 1 h. Control experiments were operated parallelly in a flask containing anaerobic inoculum without any pretreatment. All the experiments were performed in batch mode using a series of 250 ml conical flasks (working volume of 200 ml). Each flask prior to experimentation was inoculated with 30 ml of defined anaerobic mixed consortia (VSS – 7.6 g/l) under aseptic anaerobic conditions. Aqueous phase pH before feeding was adjusted employing either concentrated orthophosphoric acid or 3N NaOH solution to the desired initial level. After loading the inoculum, the flasks were flushed with oxygen free nitrogen gas for 30 s and capped tightly

Table 1  
Details of pretreatment methods used in the experiment

S.no	Experiment	Details	Pretreatment conditions adopted	Purpose
1	Control	Control	Without any pretreatment	To enumerate control mixed microflora efficiency in H <sub>2</sub> evolution
2	P	pH treatment	pH 3 adjusted with OPA under anaerobic environment for 24 h	To inhibit methanogenic bacteria in mixed consortia due to their restricted pH range when compared with H <sub>2</sub> producers
3	H	Heat treatment	100 °C for 1 h under anaerobic environment	To selectively enrich spore forming bacteria
4	C	Chemical treatment	0.2 g/l BEA under anaerobic environment for 24 h	To inhibit methanogenic bacteria in mixed consortia
5	PH	pH + heat treatment	pH 3 adjusted with OPA under anaerobic environment for 24 h + 100 °C for 1 h under anaerobic environment	–
6	CH	Chemical + heat treatment	0.2 g/l BEA under anaerobic environment for 24 h + 100 °C for 1 h under anaerobic environment	–
7	PC	pH + chemical treatment	pH 3 adjusted with OPA under anaerobic environment for 24 h + 0.2 g/l BEA under anaerobic environment for 24 h	–
8	PHC	pH + heat + chemical treatment	100 °C for 3 pH adjusted with OPA under anaerobic environment for 24 h + 1 h under anaerobic environment + 0.2 g/l BEA under anaerobic environment for 24 h	–

BEA – 2-bromoethane sulfonic acid; OPA – concentrated orthophosphoric acid.

with a rubber septum (butyl rubber) and placed in orbital shakers (110 rpm). All the experiments were performed at a constant mesophilic temperature of  $29 \pm 2$  °C. After monitoring H<sub>2</sub> gas stored in the head space and collection of sample for analyses, the contents in the flasks were safely discarded. Each flask was used only for single analysis and represents single point on the graph.

### 3. Analytical methods

H<sub>2</sub> gas generated during experiments was estimated using a microprocessor based pre-calibrated H<sub>2</sub> sensor (electrochemical sensor, FMK satellite 4–20 mA version, ATMI GmbH Inc., Germany). The output signal displayed % volume of H<sub>2</sub> in the headspace of flasks, which was further converted to mmol. The sensor has a measuring range of 0.01–10% H<sub>2</sub> with 5 s response time in a temperature range of 20–80 °C. The system was calibrated once in two days using calibration cap provided with the instrument. pH values were determined by a pH meter (Model 20, Denver instruments Ltd.). Total alkalinity, TSS, VSS, volatile fatty acids (VFA), oil and grease, soluble COD (closed refluxing-titration) and BOD<sub>5</sub> were determined according to the Standard Methods (APHA, 1998). Protein concentration was estimated by Lowry's method and ammonification was qualitatively determined by estimating proteolytic enzymes activity employing Nessler's reagent and peptone broth supplemented with organic nitrogen.

## 4. Results and discussion

### 4.1. Comparison of pretreatment methods

The aim of this study is to evaluate the feasibility of various pretreatment methods on the H<sub>2</sub> evolution rate and substrate removal efficiency by utilizing dairy wastewater as substrate. In this study various pretreatment methods viz., acid (P), chemical treatment (C), heat treatment (H) and their possible combinations (PC, PH, HC and PHC) were performed on the anaerobic inoculum. It is apparent from Fig. 1a, that the pretreatment methods applied on the parent anaerobic inoculum have evidenced significant influence on the H<sub>2</sub> generation. Experimental data enumerated the necessity of pretreating the parent inoculum for effective H<sub>2</sub> production. Among the studied pretreatment methods, chemical pretreatment of inoculum (C) evidenced relatively higher H<sub>2</sub> yield (0.0317 mmol/g COD), while the control (Ctrl) experiment resulted in low H<sub>2</sub> yield (0.0018 mmol/g COD). About 18 times enhancement in the H<sub>2</sub> production rate was observed with chemical pretreatment procedure compared to control experiment. Among the pretreatment methods studied, the acid pretreated sludge (P) showed lowest H<sub>2</sub> (0.0079 mmol/g COD) enhancement (four times more than the control). Eventhough H<sub>2</sub> could be produced without any treatment (Ctrl), application of pretreatment procedure resulted in considerable enhancement in H<sub>2</sub> yield. In combination,

pH and chemical pretreatment (PC) procedure established higher H<sub>2</sub> yield compared to other combinations. It has resulted in 16.1 times enhancement over the control accounting for a maximum H<sub>2</sub> yield of 0.029 mmol H<sub>2</sub>/g COD. Combination of pH and heat shock pretreatment (pH) established 11.5 times enhancement over the control and combinations of all three pretreatment procedures (PHC) evidenced 6 times enhancement (0.0108 mmol H<sub>2</sub>/g COD). All the experimental variations studied except chemical pretreatment (6 h) resulted in higher H<sub>2</sub> yield after 18 h of fermentation period (Fig. 1a). H<sub>2</sub> production as resulted from the pretreatment studies (enhancement with reference to control) are depicted below

$$C(17.6) > PC(16.1) > PH(11.5) > H(6.77) > PHC(6) \\ > HC(4.66) > P(4.38) > Ctrl(0)$$

However, when cumulative H<sub>2</sub> production was considered, the combination of pH and chemical pretreatment (PC) procedure evidenced higher H<sub>2</sub> yield and can be considered as ideal pretreatment methodology over the individually chemical treatment (C) procedure. Other combination of pretreatments viz., HC and PH also resulted in better performance than the individual pretreatments procedures (H, P), PHC and Ctrl. The order of cumulative H<sub>2</sub> production is summarized in the descending order as shown below.

$$PC > C > PH > HC > H > PHC > P > Ctrl$$

Experimental data revealed the possibility of molecular H<sub>2</sub> generation utilizing dairy wastewater as a primary carbon source/substrate through anaerobic metabolic reaction. This is evident from reduction in substrate (COD) observed during the H<sub>2</sub> generation in all the experimental variations studied (Fig. 1b). A steady decrease in the COD values were observed irrespective of the experimental variations studied. Among the studied pretreatment methods, chemical pretreatment procedure dominated revealing maximum efficiency with respect to substrate removal (87%) as well as H<sub>2</sub> yield (0.0317 mmol H<sub>2</sub>/g COD). In combination studies, PC (0.029 mmol H<sub>2</sub>/g COD; 83%), PH (0.0207 mmol H<sub>2</sub>/g COD; 86%), PHC (0.0108 mmol H<sub>2</sub>/g COD; 83%) and HC (0.0084 mmol H<sub>2</sub>/g COD; 81%) showed comparatively superior performance over the other methods studied with respect to both H<sub>2</sub> yield and substrate removal efficiency. Individually, H (0.122 mmol H<sub>2</sub>/g COD, 69%) and AP (0.079 mmol H<sub>2</sub>/g COD, 63%) procedures yielded moderate H<sub>2</sub> production and substrate degradation efficiency. Among all the pretreatments studied, P showed inferior performance with respect to H<sub>2</sub> yield and substrate removal efficiency. However, in the case of control, low H<sub>2</sub> yield (0.0018 mmol H<sub>2</sub>/g COD) inspite of effective substrate removal efficiency (79%) was documented. This could be attributed to the onset of the solventogenesis in control supported by cessation in H<sub>2</sub> production from 18 h onwards coupled with increased substrate removal efficiency after 24 h leading

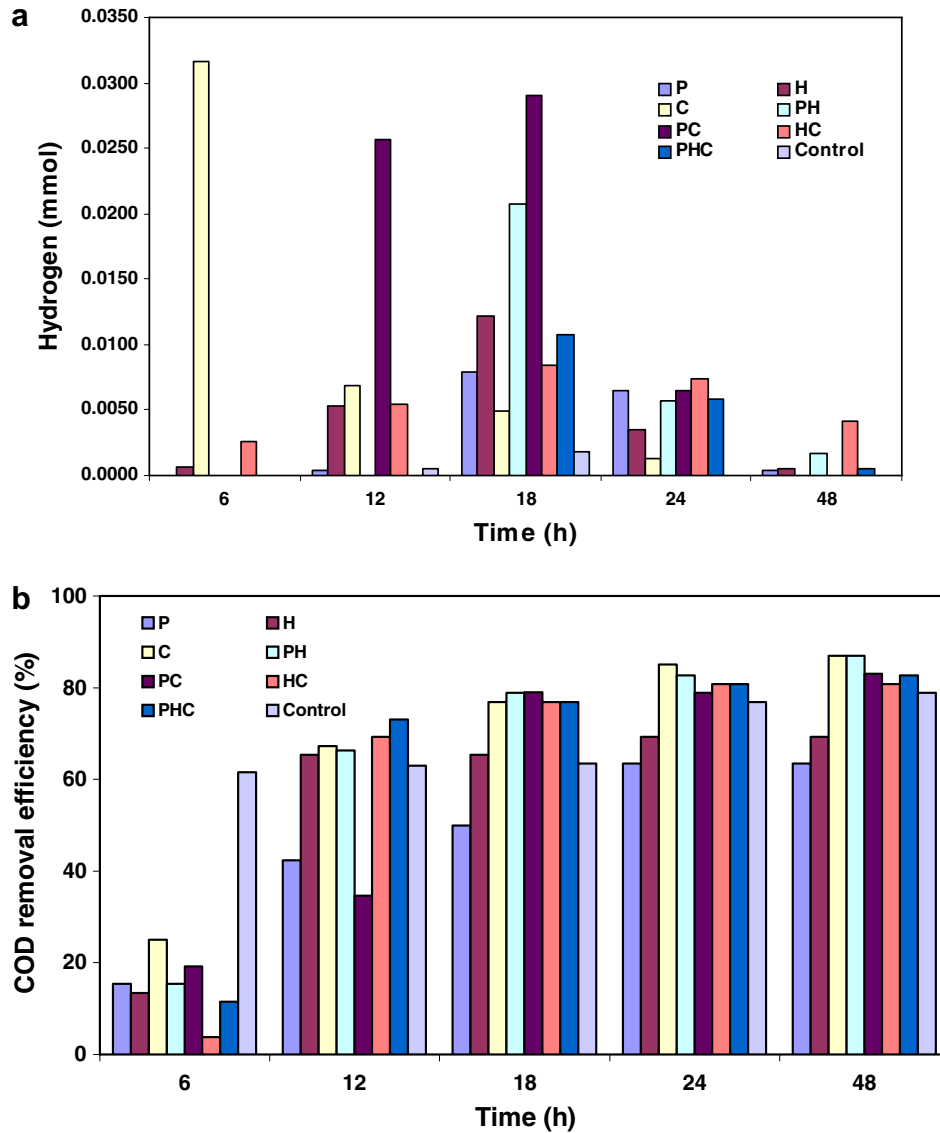


Fig. 1. Relative performance of pretreatment method studied: (a) hydrogen production and (b) substrate (COD) removal efficiency.

to possibility of methane formation due to the presence of methanogenic group of bacteria. An optimum HRT, with respect to substrate reduction was found to be 24 h for all the experimental variations studied. Thereafter a minimum increment in the COD removal efficiency (4% in C, 3% in PH, 2% in C, PHC and Ctrl and 0% in P, H and HC) was observed till 48 h. Based on the COD removal efficiency all pretreatment methods except P and H were found to be effective. The overall substrate removal efficiency by the studied experimental variations is summarized as follow:

C > PH > PC > PHC > HC > Ctrl > H > P

#### 4.2. Data envelopment analysis (DEA)

Data envelopment analysis (DEA) model was used to analyze the performance of different pretreatment proce-

dures studied in terms of H<sub>2</sub> production and substrate degradation rate (SDR). DEA is a frontier analysis technique normally used to measure the performance by evaluating the relative efficiency adopting graphical approach (Charnes et al., 1994). This method takes input and converts them into output (Ram Mohan, 2005). Ratio, calculated by taking output divided by the input is used to measure and compare the performance of the given system. All the process variations are compared with the system that has got the highest ratio. In this method, 2 outputs and 1 input are considered at a time and plotted and the positions on the graph provides a system having a level of performance. A straight line can be drawn from the Y-axis through points of system with superior performance to the X-axis. This line is called 'Efficient Frontier' and it envelops all the data. The model gives relative efficiency and not absolute efficiency. The relative efficiency of the system can be calculated by comparing the current perfor-

mance of the system to the best possible performance that the system could be reasonably expected to achieve as per the equation.

$$\text{Relative efficiency of the system} = [(X/Y) * 100]$$

where,  $X$  represents length of the line from the origin to the point obtained by plotting two ratio of the system and  $Y$  denotes length of the line from the origin through the point obtained by the system to the efficient frontier. This model offers an easy approach to analyze results obtained by interpreting different ratio. The relative efficiency of any system would indicate that other systems adopting process which would enable it to improve its performance. The best possible performance that a system could be expected to achieve is given by the point labeled best, the point where the line from the origin through the point of that system meets the efficient frontier.

DEA methodology was adopted in the study to evaluate the relative efficiency of each pretreatment method studied.

Table 2  
Cumulative  $H_2$  evolution rate and substrate degradation rate (SDR) vis a vis pH

Pretreatment	Relative efficiency (%)	H/pH	SDR/pH
P (6.0)	70	0.005	1.85
H (6.2)	74	0.0035	0.96
C (6.1)	100	0.0073	1.26
PH (5.8)	100	0.0048	0.76
PC (5.9)	100	0.0103	1.24
HC (6.0)	94	0.0046	1.19
PHC (6.0)	93	0.0028	1.22
Ctrl (6.3)	85	0.0003	1.16

H – cumulative  $H_2$  evolution rate (mmol  $H_2$ /g COD); SDR – substrate degradation rate (kg COD/cum-day).

In this study, pretreatment procedures studied were considered as input and cumulative  $H_2$  production rate and substrate degradation rate (SDR) were considered as corresponding two outputs in the model analysis. To get quantifiable figures for input, pH used to treat the inoculum was considered to represent pretreatment methods. The data pertaining to the inputs and outputs are used are depicted in Table 2. The resultant graphical output from DEA analysis is depicted in Fig. 2. It is evident from the figure that the pretreatments methods viz. C, PH and PC showed superior performance. Efficient frontier line was drawn as a horizontal line from the Y-axis enveloping PH and PC and a vertical line from PC to X-axis. It represents a standard of performance that pretreatments which are not on efficient frontier, could try to achieve. It can be interpreted that pretreatments methods PC, C and PH yield 100% efficiency  $H_2$  production along with substrate degradation. The best possible performance that P could achieve is given by the point, where the line from the origin through P meets the efficient frontier. Relative efficiency was calculated by 100 (length of line from origin to P/length of line from P to efficient frontier) and it was observed to be 70%. Similarly relative efficiencies of H, HC, PHC and Ctrl pretreatment methods were found to be 74%, 94%, 93% and 85% respectively. It was observed that PH, C and PC are the best performing systems with ideally good  $H_2$  production capability coupled with substrate removal efficiency followed by HC and PHC. Control experiments without any pretreatment produced  $H_2$  and effectively removed the substrate compared to H and P. Interestingly the order of substrate removal efficiency for experimental variations in study almost matched with the relative efficiency order obtained by adopted DEA

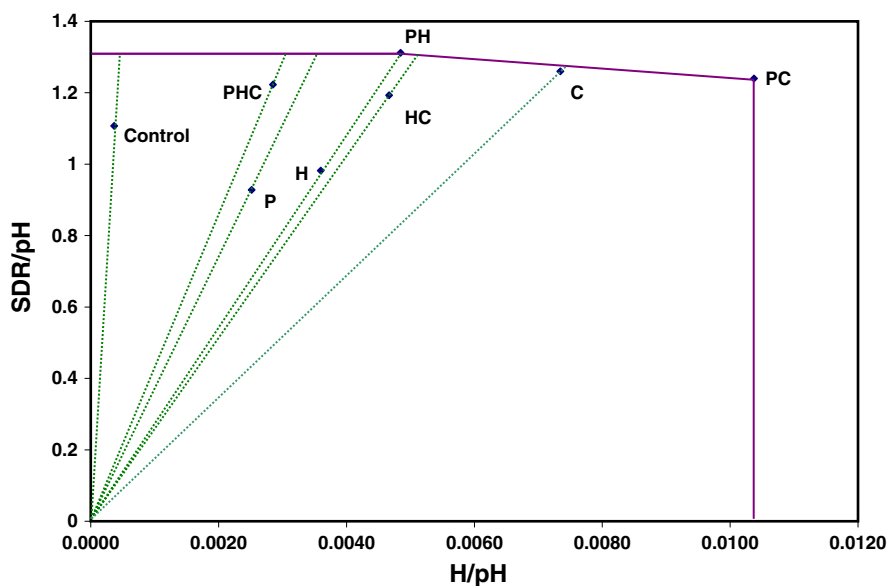


Fig. 2. Experimental data expressed in DEA format to calculate the relative efficiency (H – cumulative  $H_2$  production rate (mmol  $H_2$ /g COD); SDR – substrate degradation rate (kg COD/cum day).

methodology, while, order of H<sub>2</sub> production varied subtly with the relative efficiency order.

Effective H<sub>2</sub> production observed with the chemical pretreatment procedure may be attributed to the selective inhibition of methanogenic activity without disturbing the H<sub>2</sub> production. Co-enzyme M reductase complex a chief component for methanogenesis was reported to be inhibited by addition of BESA and acid pretreatment facilitates repression of methanogenic activity (Zhu and Béland, 2006). The reduced efficiency in H<sub>2</sub> yield by the heat-shock treated consortia could be due to the destruction of other non-spore forming H<sub>2</sub> producing bacteria which results in reduced conversion of substrate into H<sub>2</sub> (Zhu and Béland, 2006). The seed obtained by combined heat and acid pretreatments resulted in a simplified microbial population while the seed obtained by the chemical treatment had a relatively complex bacterial community which also suppressed methanogenic activity (Hawkes et al., 2002).

Chemical treatment method also had an advantage of being readily applied as and when needed basis (Zhu and Béland, 2006).

4.3. *Bioprocess evaluation*

H<sub>2</sub> production is habitually accompanied with acid production coupled with solvent production. To enumerate the possible relation among H<sub>2</sub> yield with VFA (represented as a total of all acids) production, a plot was constructed against fermentation time and experimental variation interrelating the pretreatment methods studied (Fig. 3a). VFA production showed distinct trend with the experimental variations studied. Irrespective of the pre-treatment procedure adopted, initially VFA production was high and stabilized at the end of the fermentation period. Higher concentration of VFA was observed up to 12 h of fermentation period, subsequently, VFA utilization was evidently

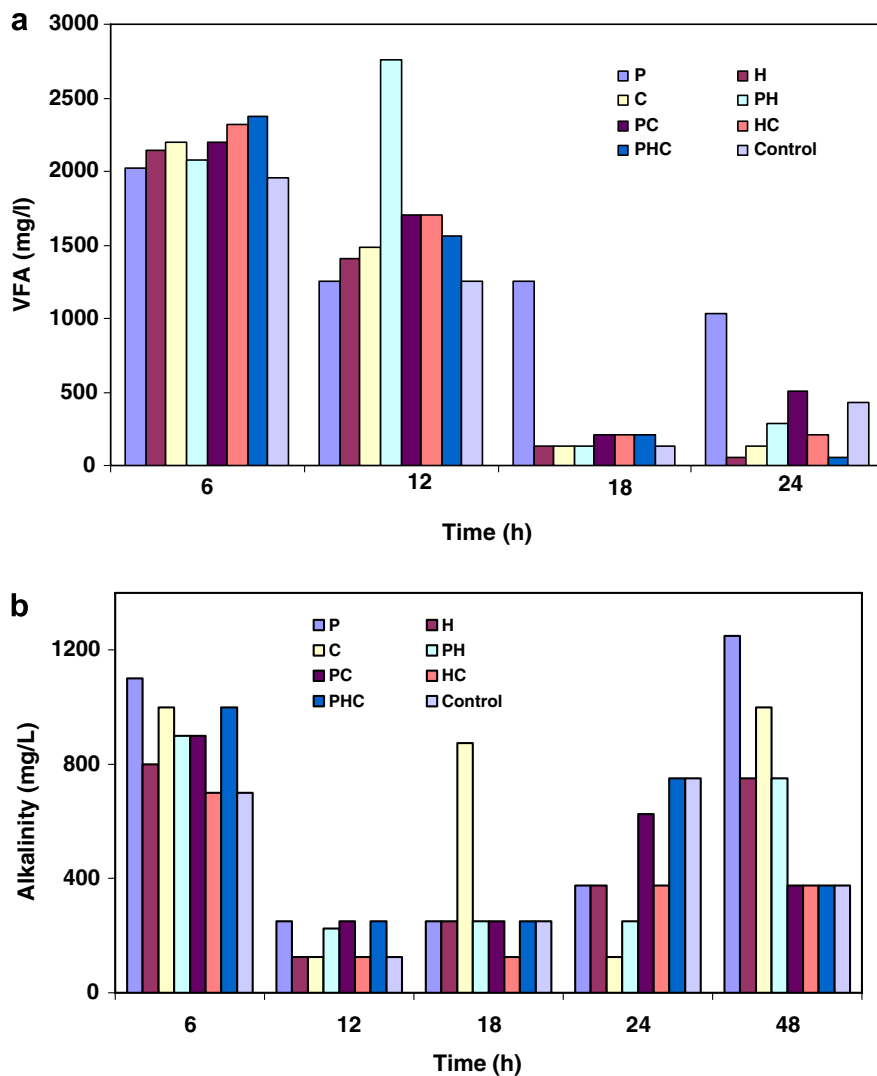


Fig. 3. VFA (a) and alkalinity (b) variation with the function of pretreatment method studied.

observed (after 18 h of fermentation period). The higher  $H_2$  production rate was observed during utilization of VFA. In the case of chemical pretreatment, substrate utilization and VFA generation was in good agreement ( $R^2 < 0.92$ ) compared to other methods. The integration experiment methods showed higher  $H_2$  production along with higher substrate utilization up to 18 h of fermentation period. This could be due to rapid utilization of VFA towards  $H_2$  production resulting in lower VFA values and a simultaneous increase in pH. During the course of experiments samples were collected and analyzed for the composition of VFA. The distribution of metabolites formed during  $H_2$  fermentation was often a crucial signal in assessing the efficiency of  $H_2$ -producing cultures. Determination of the composition of VFA, revealed the presence of higher concentration of acetate along with relatively lower concentration of butyrate (propionate and alcohol was not found) implying that the acid-forming pathway dominated the metabolic flow. Low bio-available lipids normally present in the dairy wastewater degraded into long chain fatty acids (LCFAs) and glycerol followed by  $\beta$ -oxidation to form acetate and hydrogen (McInerney, 1988; Demirel et al., 2005). The inhibitory effects of lipids can mainly be correlated to the presence of LCFAs which cause retardation of methane production (Koster, 1987). Unsaturated LCFAs (present in dairy waste water) are more potential inhibitors than saturated ones (feed back inhibition) (Demirel et al., 2005). The metabolic phenomena observed in the present study might be associated with the acidogenesis inspite of solventogenesis which was considered as optimum environment for  $H_2$  generation. Alkalinity (as

buffering capacity) values were found to be high initially and subsequently showed gradual decrease (Fig. 3b). The alkalinity values varied between 50 and 1200 mg/l. Initially, the buffering capacity was in the range of 700–1100 mg/l, which eventually dropped due to production of VFA. With utilization of VFA and subsequent protein ammonification, the alkalinity values showed a sharp increase.

Dairy wastewater normally composed of higher concentrations of carbohydrates along with proteins (casein) and lipids. Sugar contributes to 97% of the total COD present in the dairy wastewater (Hwang and Hansen, 1998). The concentration of protein variation during  $H_2$  production was also evaluated (Fig. 4) to assess the metabolic mechanism. High sugar concentration in wastewater generally inactivates the proteolytic enzymes, thereby, decreasing the protein degradation (Fang and Yu, 2000). Recently, anaerobic degradation of proteins and effects of ammonium on the anaerobic mechanism were investigated in detail (Pavlostathis and Giraldo-Gomez, 1991; Gallert et al., 1998; Rittmann and McCarty, 2001; Ramsay and Pullammanappallil, 2001; Gavala and Lyberatos, 2001; Tommaso et al., 2003; Demirel et al., 2005). In the present study, after 18 h of fermentation, a significant reduction in sugar concentration (as COD reduction) along with VFA production followed by utilization was observed. From this point (after 48 h), due to activation of proteolytic enzymes visualized by ammonification, the protein degradation pathway instigated along with concomitant increase in total alkalinity (buffering capacity) due to ammonia generation as the end product from protein degradation. This observation also correlates well with the increase and

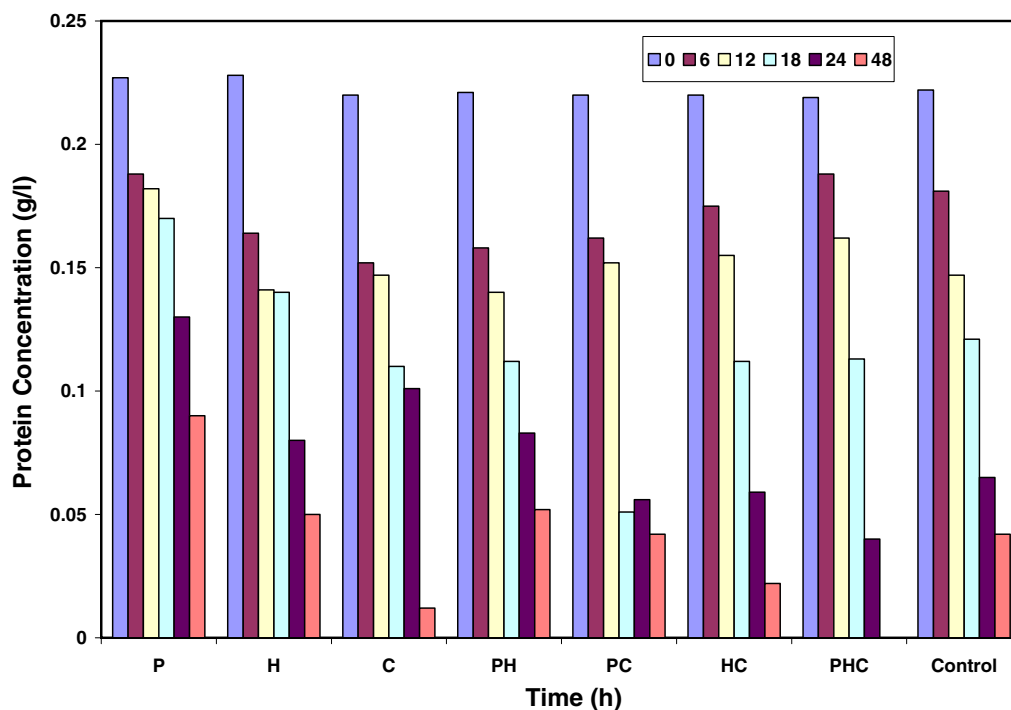


Fig. 4. Protein concentration variation during biohydrogen production.

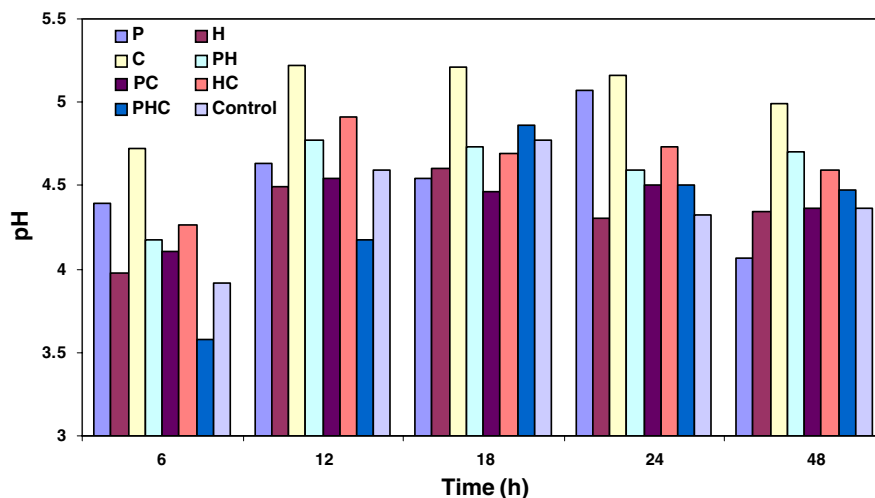


Fig. 5. pH variation with the function of pretreatment experiments.

decrease in pH during higher substrate utilization (COD) phase (up to 18 h).

Throughout the experiments pH was found to be in the acidic range (varied between 3.6 and 5.2) in all the experimental variations studied (Fig. 5). The acidic pH was considered to be ideal for effective H<sub>2</sub> production due to repression in methanogenic activity thus indirectly promoting the H<sub>2</sub> producers within the system (Zhu and Béland, 2006). However, highly acidic pH is also considered to be detrimental to H<sub>2</sub> production as it inactivates the H<sub>2</sub> producing bacteria (Bahl et al., 1986; Roy Chowdhury et al., 1988; Dabrock et al., 1992; Zhu and Béland, 2006). A sharp decline in pH along with lower H<sub>2</sub> and high VFA generation was documented during the initial hours (6 h) in all the experiments. Relatively low H<sub>2</sub> yield was observed at lower pH values (3.98) could be the result of VFA accumulation was observed during the same time interval. After 48 h a sharp raise in pH due to low VFA accumulation or utilization was observed probably due to lower production of VFA or its higher utilization or both resulting in increase of pH as well as H<sub>2</sub> generation.

## 5. Conclusions

The batch anaerobic fermentation studies performed on anaerobic mixed inoculum demonstrated the feasibility of H<sub>2</sub> generation utilizing dairy wastewater as substrate. The pretreatment methods (chemical treatment, heat-shock treatment and acid treatment and their possible combinations) used for selective enrichment of H<sub>2</sub> producing anaerobic consortia showed considerable influence on the overall H<sub>2</sub> production rate and substrate removal efficiency. All pretreatment methods showed positive influence on the overall H<sub>2</sub> production rate and substrate removal efficiency compared to the control experiments. Individually, chemical pretreatment procedure demonstrated higher H<sub>2</sub> yield and substrate removal efficiency. In combination experiments, pH and chemical pretreatment integration evidenced higher H<sub>2</sub> production efficiency. Data envelope

analysis (DEA) model was successfully used in this study to evaluate the relative performance of pretreatment procedures used.

## Acknowledgements

The authors gratefully acknowledge the financial support of Department of Biotechnology (DBT) [BT/PR/4405/BCE/08/312/2003], Government of India in carrying out this research work.

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