

An Aerobic Bio-Hydrogen Production from Dairy Waste Water Treatment in Sequential Batch Reactor (ANSBR): Effect of Organic Loading Rate

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ABSTRACT: Dairy wastewater was evaluated for biological hydrogen (H₂) production in conjugation with wastewater treatment in a suspended growth sequencing batch reactor (AnSBR) employing sequentially pretreated [heat-shock (100 °C, 2 h) and acid (pH 3.0, 24 h)] mixed consortia. The bioreactor was operated at mesophilic (room) temperature (28±2°C) under acidophilic conditions (pH 6.0) with a total cycle period of 24 h consisting of FILL (15 min), REACT (23 h), SETTLE (30 min), and DECANT (15 min) phases at three different organic loading rates (OLR) of 1.85, 2.42, and 3.0 Kg COD/m³-day, respectively. H₂ evolution rate differed significantly with the substrate/OLR of wastewater used as substrate [OLR 1.85 Kg COD/m³-day - volumetric H₂ production rate: 0.270mmolH₂/m³-min; specific H₂ production rate: 0.0144 mmolH₂/min-g COD_L; OLR 2.42 Kg COD/m³-day - volumetric H₂ production rate:0.349 mmolH₂/m³-min; specific H₂ production rate: 0.144 mmolH₂/min-g COD_L and OLR 3.0 Kg COD/m³-day - volumetric H₂ production rate: 0.255 mmolH₂/m³-min; specific H₂ production rate: 0.085 mmolH₂/min-g COD_L]. Substrate (COD) removal efficiency of 76 (substrate degradation rate (SDR): 1.674 Kg COD/m³-day), 72 (SDR: 2.193 Kg COD/m³-day), and 65% (SDR-2.713 Kg COD/m³-day), respectively, was observed at operating OLR of 1.85, 2.42, and 3.0 KgCOD/m³-day, respectively. The system showed rapid stabilization tendency (1.85 Kg COD/m³-day: 43 days; 2.42 Kg COD/m³-day: 22 days; 3.0 Kg COD/m³-day: 19days) with respect to H₂ generation and COD reduction. A surge in pH values from 6.1 to 3.8 (1.87Kg COD/m³- day), 4.8 to 3.5 (2.42 Kg COD/m³-day), and 4.8 to 3.4 (3.0 Kg COD/m³-day) was observed during stabilized phase of operation.

KEYWORDS: Biohydrogen, Fermentation, Chemical oxygen demand (COD), Anaerobic mixed consortia, Suspended growth reactor, Anaerobic sequencing batch reactor (AnSBR).

I. INTRODUCTION

Nowadays, global energy requirements are mostly dependent on fossil fuels, which eventually lead to foreseeable depletion due to limited fossil energy resources. In recent times a great deal of attention is being paid to the usage of hydrogen (H₂) as alternative and eco-friendly fuel throughout the world Presently H₂ is produced mainly from fossil fuels, biomass, and water. About 90% of H₂ is produced by the reactions of natural gas or light oil fractions with steam at high temperatures. These methods mainly consume fossil fuels as energy source, and are considered to be energy intensive, and not always environmental friendly. Present utilization of H₂ is equivalent to 3% of the energy consumption, and is expected to grow significantly in the years to come. Biological production of H₂ is one of the alternative methods where processes can be operated at ambient temperatures and pressures, and are less energy intensive and more environmental friendly. Broadly, biological H₂ production processes can be classified as biophotolysis of water using algae and cyanobacteria, photodecomposition of organic compounds by photosynthetic bacteria, and fermentative H₂ production from organic compounds. So far H₂ production by photosynthetic microorganisms was extensively studied, while H₂ evolution by fermentation was treated with little attention. The fermentative evolution is more advantageous than photochemical evolution for mass production of H₂ by

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 1, January 2014

microorganisms, where various wastewaters can be used as substrates. Of late, H₂ production through anaerobic fermentation using wastewater as substrate has been attracting considerable attention. Exploitation of wastewater as substrate for H₂ production with concurrent wastewater treatment is an attractive and effective way of tapping clean energy from renewable resources in a sustainable approach. This provides dual environmental benefits in the direction of wastewater treatment along with sustainable bioenergy (H₂) generation. However, the microbial conversion of substrate by anaerobic fermentation is a complex series of biochemical reactions manifested by diverse group of selective bacteria. Dairy wastewater contains complex organics, such as polysaccharides, proteins and lipids, which on hydrolysis form sugars, amino acids, and fatty acids. In subsequent acidogenic reaction, these intermediate products are converted to volatile fatty acids (VFA), which are further degraded by acetogens, forming acetate, CO₂, and H₂. Lastly, both acetate and H₂/CO₂, are converted by methanogens to CH₄. To harness H₂ as end product from anaerobic process instead of CH₄, inhibition of specific biochemical reaction (methanogenic) and enhancement for specific biochemical reaction (acidogenic) are important prerequisites. Also optimized operating conditions can result in good H₂ yield. In this direction, we have made an attempt to harvest H₂ from dairy wastewater treatment through anaerobic fermentation in suspended growth bioreactor using anaerobic mixed consortia, by restricting the methanogenic activity and manipulating operating conditions of the reactor.

II. EXPERIMENTAL SET-UP

2.1 H₂ producing mixed consortia

Anaerobic mixed microflora acquired from an operating laboratory scale upflow anaerobic sludge blanket (UASB) reactor treating chemical wastewater for the past three years was used as parent inoculum. Prior to inoculation, Cow dung acquired from UASB reactor was subjected to cyclic pretreatment sequences (four times) changing between heat-shock (100 °C, 2 h) and acid [pH 3 adjusted with *ortho*-phosphoric acid (88%), 24 h] treatment to restrain the growth of methanogenic bacteria (MB), at the same time to selectively enrich the H₂ producing acidogenic bacteria (AB). The resulting enriched mixed culture was used as inoculum to startup the suspended reactor.

2.2 Dairy wastewater

Dairy wastewater (suspended solids (SS), 2415 mg/l; total dissolved solids (TDS), 1200 mg/l; total solids (TS), 3615 mg/l; COD, 4400 mg/l; BOD, 2020 mg/l, and pH 7.2) collected from Sivaganga Aavin Cooperative Dairy Society, Karaikudi, was used as substrate. The wastewater can be considered as complex in nature (BOD/COD~0.45) due to the presence of proteins, carbohydrates, and lipids content. After collection, the wastewater was transferred immediately to the laboratory and stored at 4°C, and the wastewater was not corrected for trace elements deficiency. So Synthetic Wastewater was used requisite organic loading rate (OLR) prior to feeding and pH adjustment.

2.3 Reactor design and operation

Bench scale anaerobic sequencing batch reactor (AnSBR) with suspended growth configuration as designed and fabricated in the laboratory using Borosil[®] glass material with a working volume of 34.65 l (gas holding capacity, 6.93 l; liquid volume, 20.79 l) as depicted in Fig. 1. The reactor was fabricated using leak proof sealing along with proper inlet and outlet arrangements. Feed was introduced from top of the reactor, and operated in suspended mode employing magnetic stirrer mechanism for keeping biomass in suspension during operation. The reactor was operated in sequencing/periodic discontinuous batch mode with a total cycle period of 24 h (hydraulic retention time (HRT)) consisting of 15 min of FILL, 23 h of REACT (anaerobic), 30 min of SETTLE and 15 min of DECANT phases (Table 1). At the beginning of each cycle, immediately after withdrawal (earlier sequence), a predefined volume (20.79 l) was fed to the reactor during FILL phase. The reactor was kept in suspension during the REACT phase. The reactor was operated at three OLRs (1.85 Kg COD/m³-day, 2.42 Kg COD/m³-day, and 3.00 Kg COD/ m³-day). Constant COD removal and H₂ production were considered as indicator for stabilized performance of the bioreactor, subsequently the reactor was shifted to higher OLR. The influent pH was adjusted to 6.0, before feeding using *ortho*-phosphoric acid. Reactor was operated at mesophilic (room) temperature (29±2 °C). Substrate degradation rate (SDR, Kg COD/m³-day) was calculated to study the rate and pattern of COD removal during the cycle operation according to Eq. (1), where, COD₀ and COD_T represent COD (mg/l) at t_0 and t_c times, respectively, FR represents

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feed rate (m^3/day) and R_v denotes reactor volume (m^3).
 $SDR = [(COD_0 - COD_T) \times FR] / R_v$

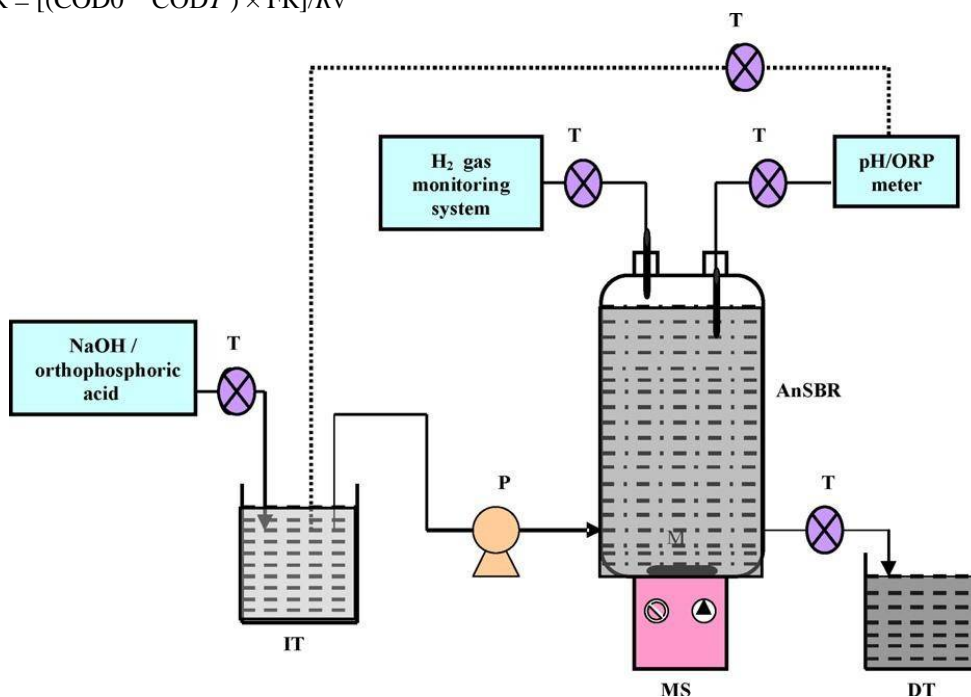


Fig. 1. Schematic details of the experimental set up [AnSBR, anaerobic sequencing batch reactor; T, preprogrammed timer; P, peristaltic pump; IT, influent storing tank; DT, decanting tank; MS, magnetic shaker; M, magnetic pellet]

Table 1. Details of reactor operation

Mode of operation	Sequencing/Periodic discontinuous batch mode
Reactor microenvironment	Anaerobic
HRT	24 h (single cycle period-FILL-15 min, REACT-23 h, SETTLE-30 min, DECANT-15 min)
Total Volume	34.65 l
Feed Volume	20.79 l
Sludge volume	6.93 l
Gas Holding Capacity	6.93 l
Operating temperature	28 ± 2 °C
Feeding pH	6.0 ± 0.1
OLR 1	1.85 kg COD/ m^3 day, 43days
OLR 2	2.42 kg COD/ m^3 day, 22days
OLR 3	3.00 kg COD/ m^3 day, 19days

2.4. Analysis

H_2 gas generated in the bioreactor was analyzed pH change. H_2 gas was collected in cladder. In Normal water pH was measured (8.0). After adding bleaching powder in that water pH is 6.9. Now H_2 gas mixed with that water, pH reduced

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

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(6.5). In this experimental Analysis H₂ gas estimated, on 20% H₂ gas observed in the total gas collection. Another way to observed H₂ gas, the match stick react with H₂ gas, producing a BIB sound

III. RESULTS AND DISCUSSIONS

3.1 Biohydrogen production

After inoculating with selectively enriched mixed consortia, the bioreactor was initially operated with dairy wastewater as feed at OLR of 1.85 Kg COD/m³-day by adjusting the influent pH to 6.0 for a period of 43 days. Subsequent to stable operation, the reactor was shifted to higher OLR of 2.42 Kg COD/m³-day, and operated for a period 22 days, until the reactor attained stable performance. The reactor was then operated at OLR of 3.0 Kg COD/m³-day for a period of 19 days. The experimental data depicted the feasibility of molecular H₂ production by utilizing dairy Wastewater as substrate along with COD reduction (Fig. 2). However, the performance with respect to H₂ generation and substrate removal was found to depend on the operating OLR (Fig. 4). The H₂ evolution rate differed significantly with the strength of wastewater used as substrate and experimental data (Figs. 2 and 3) illustrate the influence of substrate/organic loading on process. At operating OLR of 1.85 Kg COD/m³-day, reactor showed stable performance with respect to H₂ generation and carbon removal after 35 days of startup H₂ production was first observed from 14 days after startup, and subsequently increased gradually, and approached maximum on 39th day, it remained more or less uniform after that (Fig. 2). H₂ production rate for the period of a single cycle operation at stabilized performance is depicted in Fig. 3. During the cycle operation, maximum H₂ yield of 0.372 mmol/min after 16 h was observed which gradually reduced to zero at the end of the cycle period. The system showed cumulative H₂ yield of 42.83 mmolH₂/day at the end of the cycle period, which accounts for a volumetric H₂ production rate of 0.270 mmolH₂/m³-min and specific H₂ production of 0.144 mmolH₂/gCOD_L.

At operating OLR of 2.42 Kg COD/m³-day, the reactor showed a maximum H₂ yield of 0.493 mmol/min after 12 h of cycle operation accounting for a volumetric H₂ production rate of 0.349 mmolH₂/m³-min and specific H₂ production rate of 0.144 mmolH₂/g COD_L. H₂ production showed a gradual increase up to 720 min of the cycle operation and a later decrease was observed reaching almost zero at the end of the cycle period. The reactor showed stable performance after 22 days of operation with respect to H₂ generation and substrate removal. In the case of operating OLR of 3.0 Kg COD/m³-day, the reactor showed a maximum H₂ yield of 0.353 mmol/min after 12 h of cycle operation, and registered volumetric H₂ production rate of 0.255 mmolH₂/m³-min and specific H₂ production of 0.085 mmolH₂/gCOD_L. H₂ production showed a gradual increase up to 720 min of cycle operation, and further increase in cycle period showed a marked decrease in production. Stabilized performance with respect to H₂ generation and substrate removal was observed after 24 days of operation. It is evident from the experimental data that, dairy wastewater showed relatively high and rapid hydrogen yield (12 h) at operating OLR 2.42 and 3.0 Kg COD/m³-day, respectively, compared to 1.85 Kg COD/m³-day (16 h). This might be attributed to sufficient adaptation time for the suspended microbial inoculum with the dairy wastewater and higher substrate loading rate, which obviously showed higher availability of substrate with increase in OLR resulting in active substrate metabolism leading to an early and higher H₂ yield. Early H₂ production (1 h) observed at higher OLR (3.0 Kg COD/m³-day) revealed the active microbial substrate metabolism contrary to 4 h observed at OLR 1.85 and 2.42 Kg COD/m³-day. This is indicative of the fact that the effective metabolic activity is the key for effective H₂ yield 2.193 Kg COD/m³-day. The system showed 65% of COD removal efficiency at operating OLR of 3.0 Kg COD/m³-day accounting for SDR of 2.713 Kg COD/m³-day.

3.3 Bioprocess evaluation

Parameters such as VFA (represented as the total of all acids generated during acidogenic fermentation step), pH and alkalinity were also monitored along with COD during the cycle operation of the reactor. H₂ production is generally accompanied by acid and solvent production due to acidogenic metabolism. Generation of the acidic intermediates causes changes in the metabolic pathway of the microorganisms involved, and provides a better knowledge, which can be used to improve the conditions favourable for H₂ production. The pH drop in the bioreactor system especially in anaerobic microenvironment was considered as an index of VFA with the existing buffering capacity (alkalinity) of the system. VFA production was always associated with conversion of organic fraction to acid intermediates in the anaerobic microenvironment with the help of specific group of bacteria. H₂ was the important product released along

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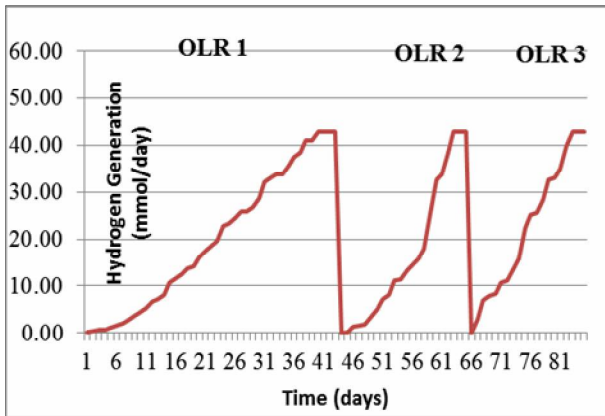
with VFA during anaerobic fermentation of wastewater during acidogenic phase. Acidogens grow relatively faster and are less sensitive to pH variation than acetogens/methanogens. This usually results in the accumulation of organic acids and lowering of pH, leading to the suppression of methanogenic activities, and in some cases, even process failure. Fig. 2.(g) illustrates VFA production during reactor operation and it is interesting to note that VFA production varied consistently with the substrate concentration. VFA varied drastically during the initial days of reactor operation. At operating OLR of 1.85 Kg COD/m³-day, VFA concentration showed a steady increase from 2550 to 1780 mg/l at the end of the cycle period. At OLR of 2.42 Kg COD/m³-day, VFA approached 1760 mg/l at the end of the cycle period from 390 mg/l. While at operating OLR of 3.0 Kg COD/m³-day, VFA production was found to vary between 690 and 470 mg/l (at the end of cycle period). At lower OLR (1.85 Kg COD/m³-day) operated, comparatively low yield of VFA production was observed which might be attributed to the low substrate availability leading to retarded substrate metabolism resulting in comparatively poor specific and cumulative H₂ production. Conversion of organic substrate to fatty acids was observed at relatively shorter cycle period (6 h) at OLR 2.42 and 3 Kg COD/m³-day. While at 2.4 Kg COD/m³-day relatively delayed (8 h) and lower efficiency in VFA production was observed. Alkalinity plays a vital role in restricting the organic acid accumulation leading to a balanced pH level within the reactor to enable hydrogen production coupled with substrate removal during the reaction. Alkalinity during the bioreactor operation was monitored to understand the buffering activity of the reactor system (Fig.2.d). Initially at 2.4 Kg COD/m³-day OLR, the variations of alkalinity were high. The alkalinity concentration varied from a maximum of 2040 mg/l to a minimum of 330 mg/l. Variation in alkalinity was restricted between 2040 and 420 mg/l after 39th day after startup. Wastewater loading was increased to an OLR of 2.42 Kg COD/m³-day on 43th day, after attaining stable H₂ production. During this phase of operation alkalinity values varied from a maximum of 850 mg/l to a minimum of 330 mg/l. Finally the OLR was increased to 3.0 Kg COD/m³-day on 66th and at this loading rate it was observed that alkalinity values varied from a maximum of 870 mg/l to a minimum of 780 mg/l. On 85th day, after reaching steady state condition, variation in alkalinity was restricted to 870 mg/l till the end indicating stabilized acid production and utilization within the system. In spite of major variations in alkalinity during the initial days of every OLR studied, alkalinity was found to have attained a better balance among the acid producers and acid utilizers, leading to higher COD reduction and lower VFA accumulation during the stabilization phase. The experimental conditions thus prevented complete acidification of the reactor and higher H₂ production. Production of acids gradually reduced the buffering capacity (alkalinity) that resulted in concomitant decline in the system pH with the wastewater studied (Fig.2.(g)). Concomitant values of pH and ORP were observed in all the experimental variations studied (Figs. 2(f) and (h)). pH drop showed a distinct trend towards acidification. During the reactor operation, due to acid production a gradual reduction in the buffering capacity (total alkalinity) was observed, which resulted in a concomitant decline in the system pH. pH and ORP variation during stabilization phase of reactor operation at different operating OLR are 6.1 to 3.8 (60–180mV, 1.85 Kg COD/m³-day), 4.8–3.5 (90–195mV, 2.42 Kg COD/m³-day), and 4.8–3.4 (110–195mV, 3.0 Kg COD/m³-day). The variation in pH was observed in a narrow range indicating a stable system. Higher pH drop resulted in rapid H₂ production with concomitant increase in VFA production. Optimum pH for methanogenic bacteria is between 6.0 and 7.5, while AB functions well below pH 6. It is reported that conversion efficiency (of H₂ production) was increased by maintaining the operating pH in and around 6 compared to a near neutral. Constant drop in pH except during initial hours (2–4 h) leading to acidification followed by lower VFA values was observed at OLR 1.85 Kg COD/m³-day. Lower H₂ production could be attributed for this phenomenon. The pH drop from 6 to 4.5 was considered to be the ideal pH range for the functioning of the AB and at the same time for inhibiting the activity of MB. The basic aim to maintain reactor operating environment at pH 6.0 was to facilitate the inhibition of MB at the same time promoting the AB indicated by stabilized acid production and utilization within the system. The pH range of 5.5–6 was considered to be ideal to avoid both methanogenesis and solventogenesis, and could be considered optimum pH range for effective H₂ generation. The observed pH drop during the reactor operation can be considered to be favourable for effective H₂ yield and for the effective functioning of the AB coupled with the inhibition of MB. The basic aim to maintain reactor- operating environment at pH 6.0 was to facilitate the inhibition of MB at the same time to create conducive environment for effective functioning of AB. The most effective way to enhance H₂ production from the anaerobic culture is to restrict or terminate the methanogenesis process by allowing H₂ to become an end product in the metabolic flow. In this study, it was observed that adoption of low operating pH inhibited MB. This facilitated generation of H₂ as terminal product of anaerobic fermentation due to suppression of the formation of CH₄. The adopted HRT of 24 h further helped to control the methanogenic reaction. Sequencing batch operation mode of the

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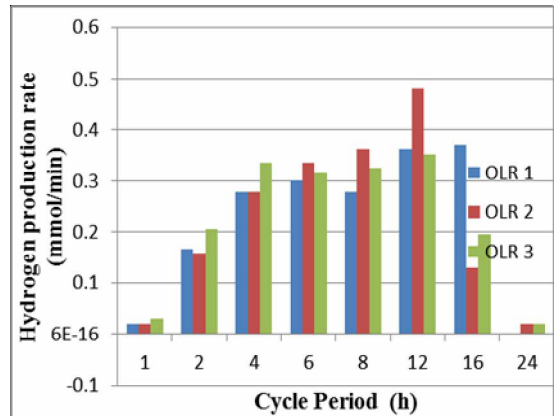
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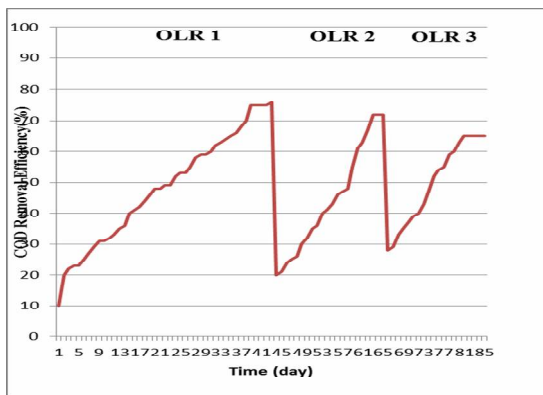
reactor used might also have influenced the H₂ evolution. The sequencing/periodic discontinuous batch mode operation facilitates controlled unsteady-state conditions and exposure time, frequency of exposure and substrate concentration can be set independent of inflow condition. This facilitated the microorganisms to periodically expose to defined process conditions and helped to select organisms that were generally more robust and able to withstand shock loads.



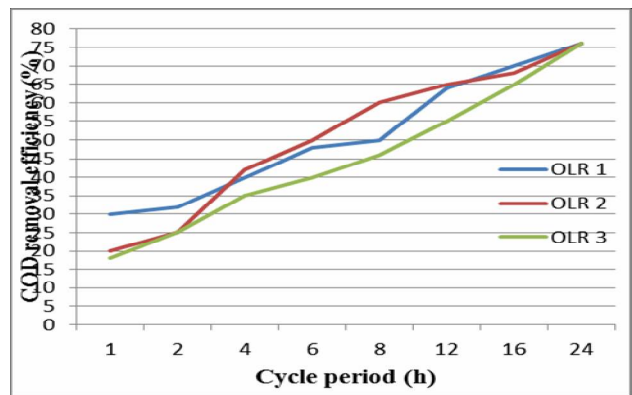
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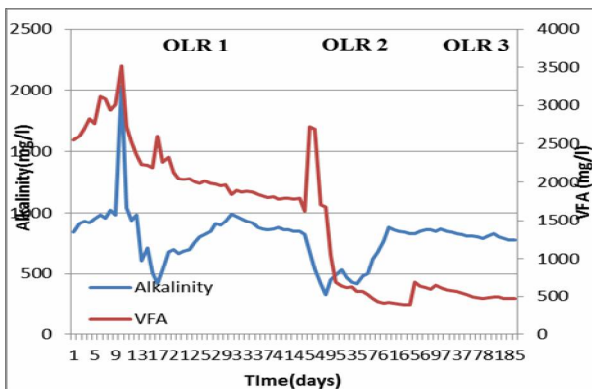
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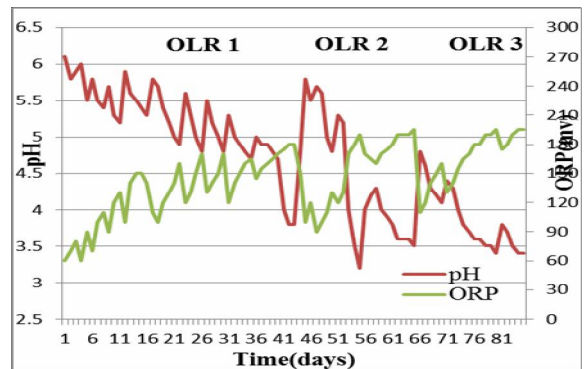
(c)



(d)



(e)



(f)

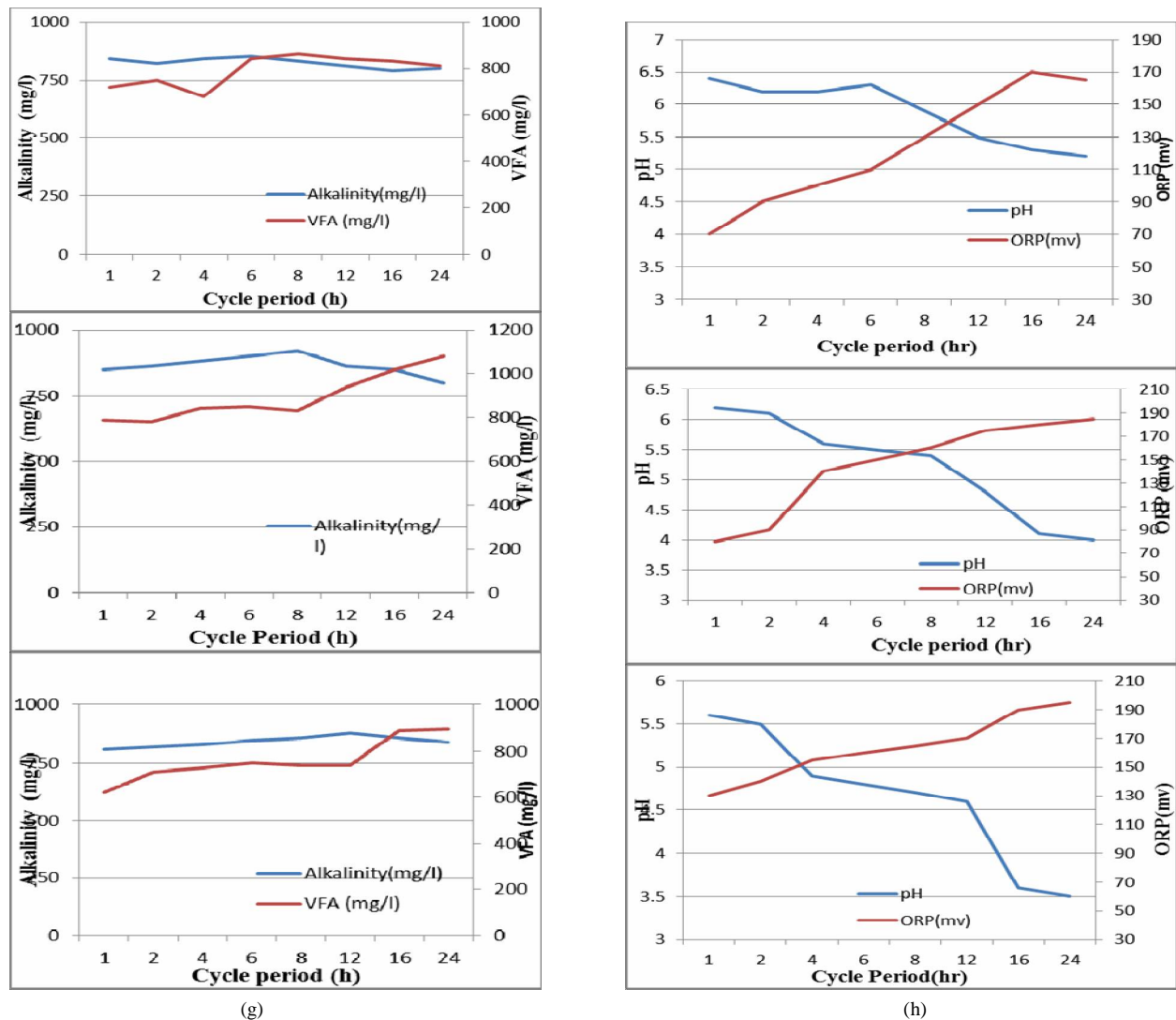


Fig. 2. (a) Hydrogen production profile at different during total operation phase of reactor (OLR1, 1.85 Kg COD/m³-day; OLR2, 2.42 Kg COD/m³-day; OLR3, 3.0 Kg COD/m³-day). (b) Hydrogen production profile during single cycle operation (OLR1, 1.85 Kg COD/m³-day; OLR2, 2.42 Kg COD/m³-day; OLR3, 3.0 Kg COD/m³-day). Substrate degradation (COD reduction) profile during reactor operation ((c) during total operation phase of reactor; (d) during single cycle operation) (OLR1, 1.85 Kg COD/m³-day; OLR2, 2.42 Kg COD/m³-day; OLR3, 3.0 Kg COD/m³-day). (e) Variation of VFA and alkalinity during total operation phase of reactor (OLR1, 1.85 Kg COD/m³-day; OLR2, 2.42 Kg COD/m³-day; OLR3, 3.0Kg COD/m³- day). (f) Variation of pH and ORP during Total operation phase reactor (OLR1, 1.85Kg COD/m³-day; OLR2, 2.42 Kg COD/m³-day; OLR3, 3.0 Kg COD/m³-day). (g) Variation of VFA and alkalinity during single cycle operation of reactor (OLR1, 1.85Kg COD/m³-day; OLR2, 2.42 Kg COD/m³-day; OLR3, 3.0 Kg COD/m³- day). ((h) Variation of pH and ORP during single cycle operation of reator(OLR1, 1.85Kg COD/m³-day; OLR2, 2.42 Kg COD/m³-day; OLR3, 3.0 Kg COD/m³-day).

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IV. CONCLUSION

The study demonstrated the feasibility of H₂ generation from dairy wastewater treatment by anaerobic fermentation in suspended growth bioreactor using anaerobic mixed inoculum. However, the process of H₂ generation was found to be dependent on the OLR applied. The pretreatment steps adopted for enumerating the H₂ production from anaerobic inoculum were found to be effective. The selected reactor operating conditions (acidophilic pH 6) were found to be optimum for effective H₂ yield. Integration of suspended configuration with sequencing/ periodic discontinuous batch operation was found to be highly flexible, and has a great potential to provide the possibilities of influencing the microbial system by selectively enriching the specific group of microflora. The system is comparatively easy to operate and cost efficient. Using mixed microbial cultures is considered to be a practical, cost-effective and promising approach to achieve H₂ production in large scale. The described process has a dual benefit of H₂ production with simultaneous wastewater treatment in an economical, effective, and sustainable way.

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