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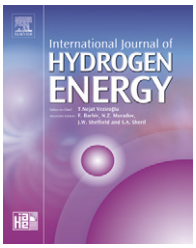

Integration of acidogenic and methanogenic processes for simultaneous production of biohydrogen and methane from wastewater treatment

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ABSTRACT

Feasibility of integrating acidogenic and methanogenic processes for simultaneous production of biohydrogen (H_2) and methane (CH_4) was studied in two separate biofilm reactors from wastewater treatment. Acidogenic bioreactor (acidogenic sequencing batch biofilm reactor, AcSBBR) was operated with designed synthetic wastewater [organic loading rate (OLR) 4.75 kg COD/m^3-day] under acidophilic conditions (pH 6.0) using selectively enriched acidogenic mixed consortia. The resultant outlet from AcSBBR composed of fermentative soluble intermediates (with residual carbon source), was used as feed for subsequent methanogenic bioreactor (methanogenic/anaerobic sequencing batch biofilm reactor, AnSBBR, pH 7.0) to generate additional biogas (CH_4) utilizing residual organic composition employing anaerobic mixed consortia. During the stabilized phase of operation (after 60 days) AcSBBR showed H_2 production of 16.91 mmol/day in association with COD removal efficiency of 36.56% (SDR_A—1.736 kg COD/m^3-day). AnSBBR showed additional COD removal efficiency of 54.44% (SDR_M—1.071 kg COD/m^3-day) along with CH_4 generation. Integration of the acidogenic and methanogenic processes enhanced substrate degradation efficiency (SDR_T—4.01 kg COD/m^3-day) along with generation of both H_2 and CH_4 indicating sustainability of the process.

1. Introduction

Increasing gap between the energy requirements, inability to replenish the depleting fossil fuels, ever increasing green house pollution from the combustion of fossil fuels and energy crisis is stimulating the need for alternative eco-friendly and sustainable fuels worldwide. In recent times a great deal of attention is focused on hydrogen (H_2) production by biological route as an alternative and viable method by research fraternity [1–15]. H_2 has been recognized as a promising, green and ideal energy carrier of the future due to its high energy yield (122 kJ/g) and clean, efficient, renewable, sustainable and recyclable nature [16]. H_2 has a higher gravimetric energy density which can be used itself or blended with other fuels such as methane (CH_4) and is compatible with electrochemical and combustion processes for energy conversion without producing carbon-based emissions [17–21]. In fuel cell applications, use of H_2 is considered to be superior to CH_4 and alcohol combustion due to its higher energy efficiency [21,22].

Among biological H_2 production processes, fermentative production has been considered as a viable and effective method. This process occurs at ambient temperatures

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Nomenclature

H_2	biohydrogen gas
CH_4	methane gas
AcSBBR	acidogenic sequencing batch biofilm reactor
AnSBBR	anaerobic sequencing batch biofilm reactor
PDBR	periodic discontinuous batch reactor
SBR	sequencing batch reactor
COD	chemical oxygen demand (mg/l)
COD_R	chemical oxygen demand removed (mg/l)
BOD_5	five days biological oxygen demand at 20 °C (mg/l)
VFA_A	volatile fatty acids of observed in AcSBBR (mg/l)
VFA_M	volatile fatty acids of observed in AnSBBR (mg/l)
OLR	organic loading rate (kg COD/m^3-day)
SDR_A	substrate degradation rate (kg COD/m^3-day) of acidogenic process
SDR_M	substrate degradation rate (kg COD/m^3-day) of methanogenic process
SDR_T	aggregated substrate degradation rate (kg COD/m^3-day) from acidogenic and methanogenic processes
UASB	upflow anaerobic sludge blanket reactor
MB	methanogenic bacteria
AB	acidogenic bacteria
ξ_{COD}	COD removal efficiency (%)
C_0	COD (mg/l) concentration at '0' time
C_T	COD (mg/l) concentration at 'T' time
F_R	feed rate (m^3/day)
R_v	reactor volume (m^3)
KOH	potassium hydroxide

and pressures, which is less energy intensive and more environmental friendly [23,24]. Exploitation of wastewater as substrate for H_2 production with simultaneous wastewater treatment is gaining importance and further leads to open a new avenue for the utilization of renewable and inexhaustible energy sources [1–4,6–10,13,25–36]. Combined with the wastewater treatment, this process is capable of solving two problems: the reduction of pollution of waste and the generation of a clean alternative fuel [18,37].

At present, development of a practical and efficient H_2 generation process is the growing concern among the research fraternity. Fermentative conversion of substrate to H_2 is generally manifested by diverse group of specific anaerobic bacteria by a complex series of biochemical/metabolic reactions and requires considerable optimization prior to scaling up. Low substrate conversion efficiency to H_2 is one of the significant problems encountered in the fermentative process and most of the organic fraction remains as soluble fermentation products. Typical H_2 yield range from 1 to 2 mol of H_2/mol of glucose, and results in 80–90% of the initial chemical oxygen demand (COD) remaining in the wastewater in the form of various volatile organic acids (VFAs) and solvents, such as acetic, propionic, butyric acids and ethanol [1]. Even under optimal conditions about 60–70% of the original organic matter remains in solution [12–15]. In spite of theoretical conversion efficiency of 33%, only 15% of the energy from the organic source is typically obtained in the form of H_2 [1,11,38,39]. According to Logan, there are no known naturally occurring biochemical routes for achieving 60–80% conversion efficiency. He suggested to find an alternative for the use of the remaining 67–85% of the unused substrate [1]. One way to utilize/recover the remaining organic matter in a usable form for energy production is to produce methane [1].

Therefore, in the present study, an attempt was made to investigate the feasibility of integrating acidogenic process of H_2 generation with anaerobic/methanogenic process of methane production to utilize residual organic composition present in wastewater generated from acidogenic process.

2. Experimental design

2.1. *Parent mixed cultures*

Anaerobic mixed microflora from an operating laboratory scale upflow anaerobic sludge blanket (UASB) reactor treating chemical wastewater for the past three years was used as parent inoculum for the startup of both the bioreactors. However, for acidogenic reactor, prior to inoculation, dewatered sludge 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) and at the same time to selectively enrich the H_2 producing acidogenic bacteria (AB) [13,35,36]. The resulting enriched mixed culture was used as parent inoculum to startup the AcSBBR.

2.2. *Bioreactors*

Two bioreactors were designed and operated separately to evaluate H_2 production [acidogenic sequencing batch biofilm reactor (AcSBBR)] and CH_4 production [methanogenic/anaerobic sequencing batch biofilm reactor (AnSBBR)]. Schematic details of the experimental setup including bioreactors used in this study are depicted in Fig. 1. Both the bench scale bioreactors were designed and fabricated in the laboratory using 'perplex' material with biofilm configuration having a working volume of 1.4 l (AcSBBR)/1.3 l (AnSBBR) and gas holding capacity of 0.35 l (AcSBBR)/0.3 l (AnSBBR). Design and operation details of both the reactors are depicted in Table 1. Both the bioreactors were filled with inert stone chips (2.5 cm × 1.5 cm, void ratio ~0.49) as fixed bed packing material to support the growth of H_2 producing/anaerobic mixed microflora. Outlet was collected from the overflow of the gas–liquid–solid separator (GLSS) provided at the top of the bioreactors. Biogas generated during the reactor operation was collected by water displacement method through an outlet provided at the top of the reactor. Bioreactors were operated in the upflow mode at mesophilic (room) temperature

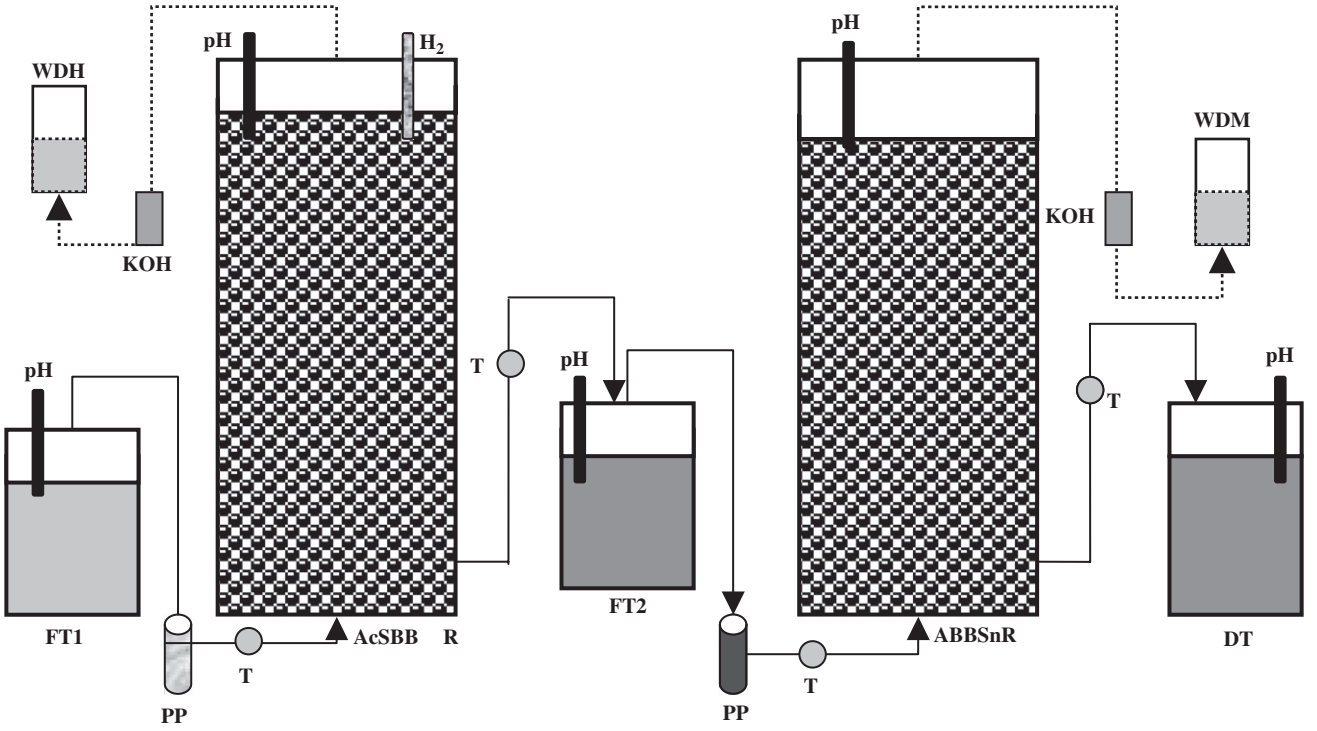

Fig. 1 – Schematic details of experimental setup [AcSBBR—Acidogenic sequencing batch biofilm reactor; AnSBBR—Anaerobic sequencing batch biofilm reactor; WDH—Water displacement for H_2; WDM—Water displacement for CH_4; pH—pH monitoring probe; H_2—Hydrogen monitoring probe; FT1—Feeding tank to AcSBBR (pH 6.0); FT2—Feeding tank to AnSBBR (pH 7.0); DT—decant storing tank from AnSBBR outlet; T—preprogrammed timer; PP—peristaltic pump; KOH—2 N KOH solution].

(28±2 °C). Both the bioreactors were covered with aluminum foil during operation to prevent exposure to sunlight.

2.3. *Operation of bioreactors*

AcSBBR was operated in periodic discontinuous batch reactor (PDBR)/sequencing batch reactor (SBR) mode with a total cycle period (hydraulic retention time) of 24 h consisting of 15 min of FILL, 23 h of REACT (anaerobic), 30 min of SETTLE and 15 min of DECANT phases (Table 1). AcSBBR was operated under acidophilic conditions (pH 6.0) by feeding designed synthetic wastewater at organic/volumetric loading rate of 4.75 kg COD/m^3-day. AnSBBR was also operated in PDBR/SBR mode with a total cycle period of 24 h (Table 1). However, the reactor was fed with outlet generated from AcSBBR after adjusting pH to 7 to sustain methanogenic activity. The performance of reactor was evaluated at variable organic loading rate (OLR) ranging between 0.975 and 2.08 kg COD/m^3-day. At the beginning of each cycle, immediately after withdrawal (earlier sequence), a pre-defined volume [1.4 l (AcSBBR)/1.3 l (AnSBBR)] was fed to the reactors during FILL phase. During REACT phase operation of both the bioreactors, the reactor volume was circulated with outlet in closed loop at recirculation rate (recirculation volume to feed volume ratio) of 2 to achieve a homogeneous distribution of the substrate. Peristaltic pumps controlled by preprogrammed electronic timer were used to regulate the feeding, recirculation, and decanting operations in both the reactors.

After inoculating the bioreactors with respective parent cultures (AcSBBR—selectively enriched acidogenic mixed culture; AnSBBR—anaerobic mixed culture) were operated with designed synthetic wastewater as feed [(g/l) glucose—3.0, NH_4Cl—0.5, KH_2PO_4—0.25, K_2HPO_4—0.25, $MgCl_2.6H_2O$—0.3, $FeCl_3$—0.025, $NiSO_4$—0.016, $CoCl_2$—0.025, $ZnCl_2$—0.0115, $CuCl_2$—0.0105, $CaCl_2$—0.005 and $MnCl_2$—0.015] at OLR of 1.5 kg COD/m^3-day to facilitate the biofilm formation on the supporting medium at respective pHs (AcSBBR—6; AnSBBR—7). The characteristics of the substrate are depicted in Table 2. Constant COD removal and biogas production (±4% variation) were taken as indicators to assess satisfactory formation of biofilm. After stable performance was achieved, the AcSBBR was further operated by feeding with designed synthetic wastewater at OLRs of 4.75 kg COD/m^3-day (pH was adjusted to 6.0) to evaluate molecular H_2 production along with substrate degradation. The outlet generated from AcSBBR was fed to AnSBBR after adjusting pH to 7.0. The pH of wastewater was adjusted with 0.1 N NaOH.

2.4. *Biochemical analysis*

The performance of the bioreactors was assessed by monitoring COD (COD-closed refluxing titrimetric method) throughout the cycle operation. Alkalinity (total), volatile suspended solids (VSS), pH, volatile fatty acids (VFA), COD (closed refluxing method) and BOD_5 were also monitored. Analyses were performed according to standard methods [40].

Table 1 – Design criteria and dimensions of bioreactors

	Acidogenic reactor (AcSBBR)	Methanogenic/ anaerobic reactor (AnSBBR)
Design flow (l/day)	4.2/upflow	3.9/upflow
Reactor volume (l), total/working	1.75/1.4	1.6/1.3
Gas holding capacity (l)	0.35	0.30
Depth of reactor (cm), total/liquid	64/54	66/59
Diameter of reactor (cm), reactor/gas holding portion	9/11	9/11
Biofilm supporting material (size/void ratio)	Stone chips (2.5 cm × 1.5 cm/ 0.49)	Stone chips (2.5 cm × 1.5 cm/ 0.49)
Recirculation rate (feed: recirculation) (R/F)	1:2	1:2
Upflow velocity (m/day) at R/F of 2	0.165	0.153
Hydraulic loading rate (HLR) at R/F of 2 (m^3(liq)/m^3-day)	1.4	2.5
Volumetric organic loading rate (kg COD/m^3-day)	4.75	1.812[a]
Operating pH	6.0	7.0
Mode of operation	PDBR/SBR	PDBR/SBR
Hydraulic retention time (HRT) at R/F of 2 (h)	24 (FILL—15 min; REACT—23 h ; SETTLE—30 min; DECANT—15 min)	24 (FILL—15 min; REACT—23 h ; SETTLE—30 min; DECANT—15 min)
Microenvironment	Acidophilic-Anaerobic	Anaerobic
Operating temperature	28 ± 2 °C	28 ± 2 °C

[a] Average value varied between 0.975 and 2.08 kg COD/m^3-day depending on efficiency of AcSBBR.

Oxidation–reduction potential (ORP) and pH values were determined using combination pH/ORP electrodes (pH products Co., Hyderabad, India).

The separation and quantitative determination of the composition of soluble metabolites was performed by high performance liquid chromatography [HPLC; UV–VIS detector; C18 reverse phase column—250 mm × 4.6 mm and 5 μ particle size; flow rate—0.5 ml/h; wavelength—210 nm; mobile phase—40% of acetonitrile in 1 mN H_2SO_4 (pH 2.5–3.0); sample injection—20 μl]. Along with substrate degradation, AcSBBR and AnSBBR were also monitored for H_2 and CH_4, respectively, using water displacement method. H_2 gas generated during the bioreactor operation was estimated using a microprocessor-based pre-calibrated H_2 sensor (FMK satellite 4–20 mA version, ATMI GmbH Inc., Germany). The output signal displayed the % volume of H_2 and the system was calibrated once in two days using calibration cap provided with the instrument.

Table 2 – Average characteristics of wastewaters used as feed for acidogenic and methanogenic bioreactors

Parameters	Design synthetic wastewater[a]	Acidogenic treated wastewater[b]
pH	7.6	3.2–4.4[c]
TDS (mg/l)	960	780[c]
COD (mg/l)	3800	2047[c]
BOD_5 (mg/l)	1600	860[c]
Chlorides (mg/l)	46	46
Total nitrogen (TKN) (mg/l)	112	84
Volatile fatty acids (mg/l)	0	609[c]

[a] Feed to acidogenic bioreactor (AcSBBR).
[b] Feed to methanogenic bioreactor (AnSBBR, outlet from AcSBBR).
[c] Average values.

3. Results

3.1. *Biohydrogen production—acidogenic process*

After inoculation with the selectively enriched acidogenic mixed consortia, AcSBBR was operated initially with designed synthetic wastewater at OLR of 1.5 kg COD/m^3-day after adjusting the influent feed pH to 6 for a period of 29 days. Constant COD removal efficiency and biogas production were considered as indicators for satisfactory formation of the biofilm. Subsequently, the bioreactor was shifted to higher OLR 4.75 kg COD/m^3-day with the same wastewater for a period of 65 days at acidophilic pH (6.0). Experimental data documented the feasibility of fermentative H_2 production along with substrate degradation during operation (Figs. 2 and 3). Fig. 2a illustrates significant variation in the H_2 production rate [16.91 mmol H_2/day to 4.38 mmol H_2/day] during 65 days of operation. Inconsistent H_2 production was observed during the initial phase of operation (53 days after startup) (Fig. 2a). After 66 days of startup, maximum H_2 production (16.91 mmol H_2/day) was documented and subsequently, the production gradually leveled off and stabilized in a narrow range (11.2–13.2 mmol H_2/day). In terms of H_2 production on hourly basis, a reasonably good production (3.12 mmol H_2/h) was observed during the initial phase of operation and gradually approached maximum after 12 h of operation (3.6 mmol H_2/h) prior to stabilization (16 h, 4.8 mmol H_2/h) (data not shown). During this phase of operation, cumulative production of 6.52 mmol H_2/day was observed.

The following equations were used for computing acidogenic fermentation balance [41,42].

$$\text{Acetic acid}: \quad C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2, \tag{1}$$

$$\text{Propoinic acid}: \quad C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O, \tag{2}$$

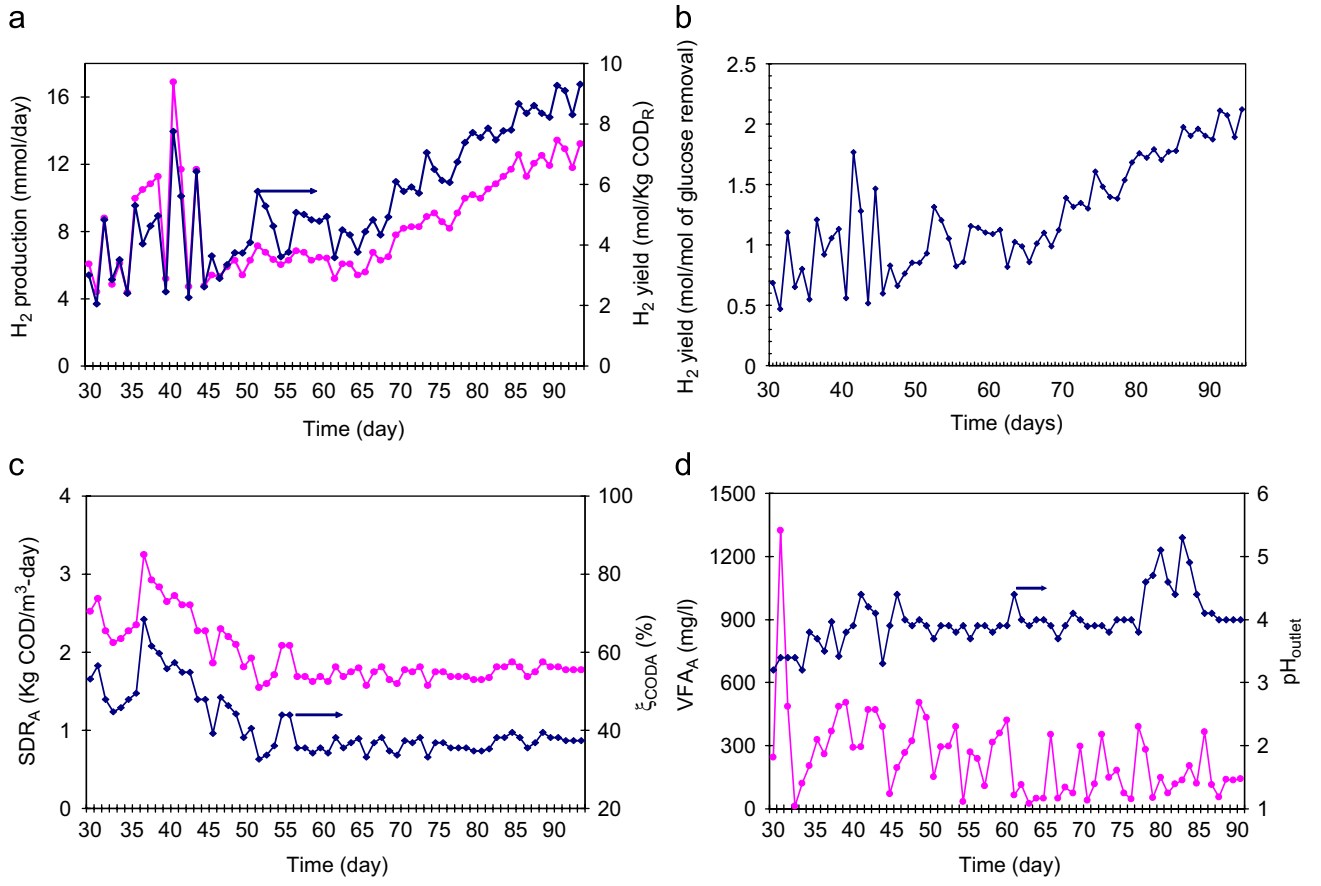

Fig. 2 – (a) Performance of AcSBBR with respect to H_2 production and yield during operation [●—H_2 production; ■—H_2 yield]. (b) H_2 yield with respect to glucose consumed. (c) Substrate degradation rate (SDR_A) and COD removal efficiency (ξCOD_M (%)) during AcSBBR operation [●—SDR; ■—ξ_{COD} (%)]. (d) Variation of VFA_A and pH (outlet) during AcSBBR operation [●—VFA; ■—pH].

$$\text{Butyric acid}: \quad C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2. \tag{3}$$

Fig. 2b depicts H_2 yield with the function of glucose removed. It is evident from profile that maximum yield of 2.122 mol H_2/mol of glucose removal was observed during operation. Even though the yield was lower than the theoretical yield (4 mol H_2/mol of glucose removal), the obtained values are reasonably good and agreeing with the reported literature [43–46].

Performance of bioreactor was also evaluated for substrate degradation potential as COD removal efficiency (ξ) using Eq. (4), where, C_{SO} represents the initial COD concentration (mg/l) in the feed and C_S denotes COD concentration (mg/l) in the reactor outlet.

$$\xi_{COD} = [(C_{SO} - C_S)/C_{SO}] \times 100. \tag{4}$$

Substrate degradation rate (SDR—kg COD/m^3-day) was calculated to study the pattern of COD removal according to Eq. (5), where, C_0 and C_T represent COD (mg/l) at '0' and 'T' times, respectively, F_R represents feed rate (m^3/day) and R_v denotes reactor volume (m^3).

$$SDR = \{[(C_0 - C_T) \times F_R]/R_v\}. \tag{5}$$

COD removal efficiency varying between 32.6% and 68.4% accounting for SDR of 1.55–3.25 kg COD/m^3-day was observed during fermentative H_2 production in AcSBBR (Fig. 2c). Substrate (COD) removal in concurrence with the molecular H_2 production indicates the participation of wastewater as primary carbon source in the metabolic reactions. Irregular pattern of substrate degradation (SDR: 1.55–3.25 kg COD/m^3-day; ξ_{COD}: 38.63–68.42%) was observed during the initial phase of operation up to 57 days after startup (where maximum efficiency was observed). Subsequently, substrate removal efficiency leveled off in a narrow range [SDR: 1.575–1.875 kg COD/m^3-day; ξ_{COD}: 33.16–39.47%] indicating system stabilization with respect to substrate degradation. Low substrate removal might be due to the persistent acidophilic microenvironment due to generation of soluble metabolites during the fermentative process. Substrate degradation with the function of single cycle period (24 h cycle) showed more or less uniform substrate removal pattern prior to approaching maximum almost at the end of the cycle period (Fig. 3a). A steady decrease in the COD concentration was observed with the function of cycle period. Specific H_2 yield varied between 2.06 and 9.31 mol H_2/kg COD_R during 65 days of reactor operation phase (Fig. 2a). Maximum H_2 yield (1.59 mol H_2/kg COD_R) was observed after 1 h of cycle operation and subsequently dropped and stabilized around 0.2 mol H_2/kg COD_R after 12 h of operation when monitored with the function of single cycle operation (Fig. 3a).

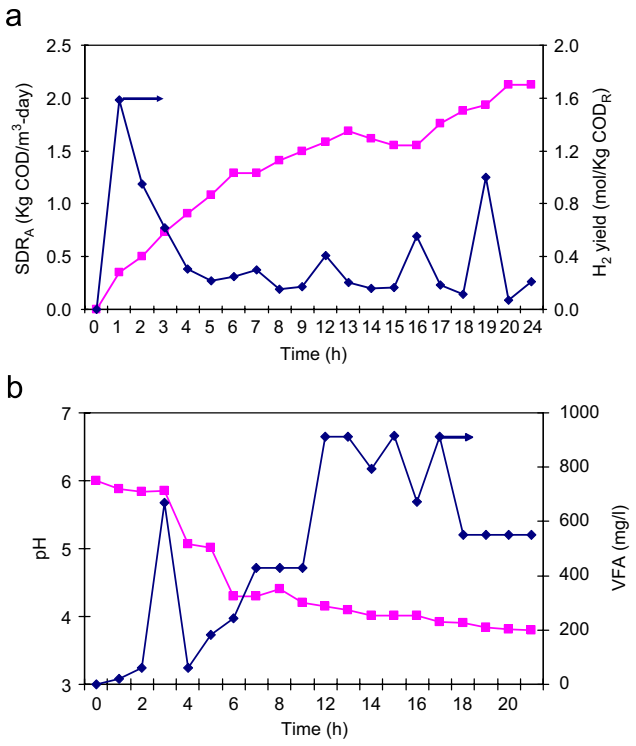

Fig. 3 – (a) Performance of AcSBBR with respect to H_2 yield and substrate degradation rate (SDR_A) during single cycle operation [●—SDR; ■—H_2 yield]. (b) Variation of VFA_A and pH during single cycle of AcSBBR operation [●—pH; ■—VFA].

3.2. *Methane production—methanogenic process*

After inoculation of anaerobic mixed consortia, AnSBBR was operated with designed synthetic wastewater at OLR of 1.5 kg COD/m^3-day initially by adjusting the feed pH to 7 for a period of 29 days. Subsequently, the reactor was operated with outlet generated from AcSBBR as feed at an average OLR of 1.81 kg COD/m^3-day after adjusting pH to 7 for a period of 65 days. The reactor was operated at neutral pH microenvironment to enumerate the function of MB activity. Experimental data documented the feasibility of utilizing VFA bound wastewater as substrate for the subsequent production of biogas (CH_4) and additional reduction of substrate (COD) (Fig. 4a–c and 5a). Bioreactor performance data illustrated significant variation in the CH_4 production and substrate degradation during the operation. CH_4 production varied between 1.79 and 11.89 mmol CH_4/day over 65 days of operation. Inconsistency in CH_4 generation during the initial phase of operation was observed up to 49 days after startup. Maximum CH_4 production was observed on the 37th day after startup and subsequently, the production gradually leveled off prior to stabilization. Insignificant variation in CH_4 production [10–11 mmol CH_4/day] was observed after 84th day of startup indicating stabilized performance of the system with respect to biogas generation. With the function of single cycle operation, biogas production (56th day after startup) is depicted in Fig. 5a. In terms of hourly cumulated CH_4 production (data not shown), maximum values of biogas were observed after 2 h of cycle operation which gradually reduced and approached zero after 16 h.

The following equations were used for computing methanogenic fermentation balance consuming H_2 and VFA generated from the primary acidogenic process [41,42,47].

$$CH_3COOH \rightarrow 2H_2 + CO_2, \quad (6)$$

$$CH_3CH_2CH_2COOH + 2H_2O \rightleftharpoons 2CH_3COOH + 2H_2, \quad (7)$$

$$CH_3CH_2CH_2CH_2COOH + H_2O \rightleftharpoons CH_3CH_2COOH^- + CH_3COOH^- + 2H_2, \quad (8)$$

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O. \quad (9)$$

Fig. 4b shows volumetric CH_4 yield with the function of COD removal during reactor operation. Volumetric CH_4 yield varied in between 0.059 and 0.363 m^3 CH_4/kg COD_R during reactor operation. The obtained biogas yield was relatively on lower side [48,49]. The lower CH_4 yield obtained in this study may be attributed to the two reasons. The first one is due to the persistence of acidophilic conditions due to presence of VFA. The second reason might be that the extraction of H_2 gas formed during acidogenic process (in the first stage of treatment) which is essential for CH_4 formation in subsequent methanogenic step as depicted in Eqs. (6)–(9).

Apart from CH_4 generation, AnSBBR also showed good amount of substrate (COD) removal efficiency varying between 35.95% and 66.20% accounting for an SDR_M of 1.1–0.42 kg COD/m^3-day (Fig. 4c). In concurrence with biogas production, irregular pattern of substrate degradation was also documented during the first 56 days of operation. This might be attributed to the inconsistency in the feed concentration (outlet of AcSBBR) and the adaptation time required for anaerobic consortia in the reactor to new feed composition. Outlet generated from AcSBBR varied in the range of 1200–2560 mg/l and 3.2–5.3 in terms of COD and pH, respectively. After providing sufficient adaptation time (56 days after startup), consistent substrate removal was observed. More or less uniform outlet concentration from AcSBBR was also noticed during this phase. A steady decrease in the COD concentration was observed with the function of cycle period (Fig. 2). Biogas yield varied between 2.67 mol CH_4/kg COD_R and 16.23 mol H_2/kg COD_R during the 94 days of bioreactor operation. Inconsistent biogas yield was observed during the initial phase of operation (up to 54 days) prior to stabilization around 7.0 mol CH_4/kg COD_R (Fig. 4a). Initial phase of cycle period evidenced high values of CH_4 yield, which gradually approached zero after 16 h of cycle operation (Fig. 5a).

4. Discussion

Summarized experimental data pertaining to biogas generation (both H_2 and CH_4) and total substrate degradation (SDR_T) aggregated from both the acidogenic and methanogenic processes are shown in Fig. 6. Experimental data supported the efficacy of integrating acidogenic H_2 production process with anaerobic methanogenic process in enhancing substrate degradation efficiency along with both H_2 and CH_4 generation as renewable by-products. In terms of substrate removal, on

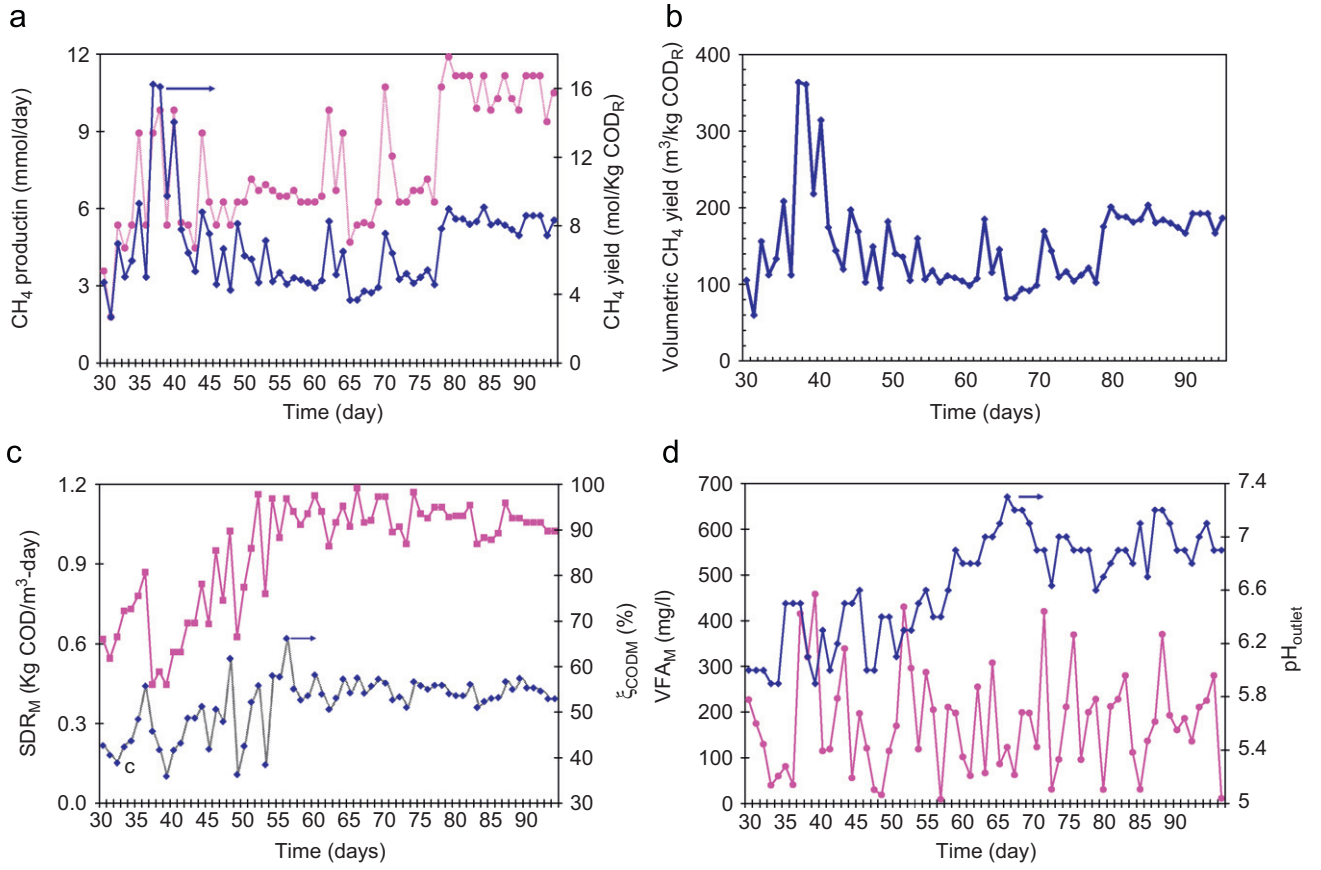

Fig. 4 – (a) Performance of AnSBBR with respect to CH_4 production and yield during operation [●—CH_4 production; ■—CH_4 yield]. (b) Volumetric CH_4 yield. (c) Substrate degradation rate (SDR_M) and COD removal efficiency (ξCOD_M (%)) during AnSBBR operation [●—SDR; ■—ξ_{COD} (%)]. (d) Variation of VFA_M and pH (outlet) during AnSBBR operation [●—VFA; ■—pH].

totality, acidogenic and methanogenic processes resulted in total substrate degradation rate (SDR_T), ranging between 3.2 and 3.4 kg COD/m^3-day during stabilized operation phase (65–94 days) at operating OLR of 4.75 kg COD/m^3-day accounting for a total COD removal efficiency of 67–72%. Integration of acidogenic and methanogenic processes appeared to be a feasible option for sustainable H_2 production utilizing wastewater as substrate.

VFA (represents total of all organic acids) and pH were also monitored during process operation in both the bioreactors. Fig. 2c illustrates the pattern of VFA_A produced and outlet pH during the operation of AcSBBR. Generally, VFA production was associated with conversion of organic fraction to acid intermediates in the anaerobic/acidogenic microenvironment with the help of specific group of anaerobic bacteria [50–52]. Fermentative H_2 production is associated with acid and solvent generation as metabolic intermediates due to the acidogenic metabolism under acidophilic microenvironment. VFA_A concentration varied between 52 and 780 mg/l during the operation. Lower concentration of soluble metabolite production was observed during the initial phase of the cycle operation (Fig. 3b). VFA_A concentration showed a steady increase with time prior to stabilization at the end of the cycle period. Increase in VFA_A concentration during H_2 production and substrate degradation enumerates the effective function of acidogenic metabolic process.

Inlet pH of feed in acidogenic reactor was adjusted to 6 prior to feeding. Optimum pH range for the growth of MB was reported in the range of 6.0–7.5, while AB functions well below 6 pH [6,53–55]. The pH range of 5.5–6 was considered to be ideal to avoid both methanogenesis and solventogenesis [39,56] in addition to effective H_2 generation. By maintaining the pH around 6 compared to a near neutral pH the conversion efficiency (of fermentative H_2 production) can be increased [6,13–14,55]. AcSBBR outlet pH varied between 3.2 and 4.4 during the operation which might be attributed to the production of acid (Fig. 2d). Acid accumulation causes sharp drop in the pH. Shift in pH values towards acidic range was considered as an index of volatile acid generation in the anaerobic microenvironment. In terms of cycle operation, system pH showed a marked decline in the system pH from 6 to 3.8 (Fig. 3b). The observed pH drop during H_2 production was considered to be a favorable microenvironment for effective H_2 yield by inhibiting the MB. However, pH below 6 reduces MB activity which has considerable influence on the substrate degradation efficiency. This might be the reason for relatively low substrate degradation efficiency observed in the acidogenic process.

The influent to AnSBBR was adjusted to pH 7 prior to feeding, to provide susceptible environment for the effective functioning of MB. Each of the microbial groups involved in anaerobic degradation had a specific pH optimum and

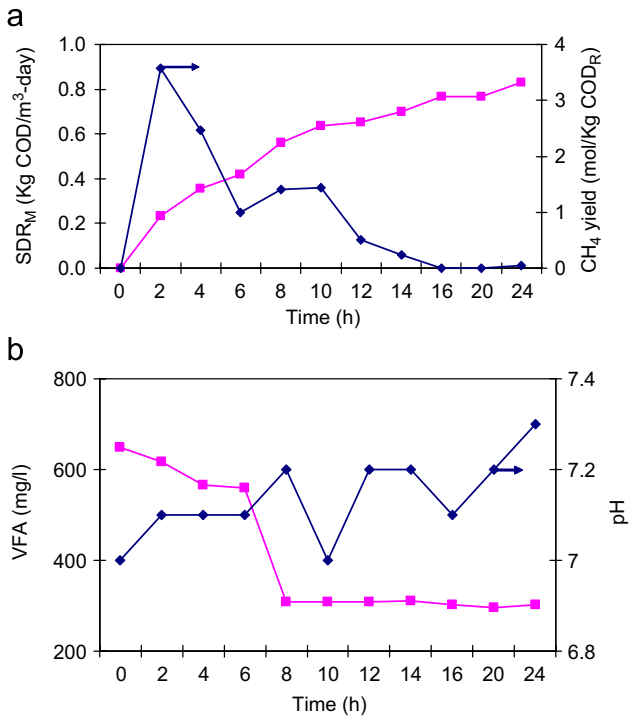

Fig. 5 – (a) Performance of AnSBBR with respect to CH_4 yield and substrate degradation rate (SDR_M) during single cycle operation [●—SDR; ■—Methane yield]. (b) Variation of VFA_M and pH during single cycle of AnSBBR operation [●—VFA; ■—pH].

functioned well in a specific pH range. The optimum range for all MB was between 6.0 and 8, with an optimum near pH 7.0, while AB had lower pH optimum around 6.0, but the optimum value for the group as whole was close to 7.0 and a pH value outside the range could lead to imbalance [53–55]. Figs. 4c and 5b depict outlet pH and VFA_M variation during AnSBBR operation with the function of reactor and cycle operations, respectively. During the initial phase (up to 65 days after startup), the system documented alkaline microenvironment (above 7). On 65th day after startup, the outlet pH fluctuated between 6.6 and 7.3. The observed persistent alkaline microenvironment during the initial phase of feeding, and subsequent transition to near neutral conditions might be attributed to the acclimatization phase taken by native anaerobic mixed culture with respect to feeding of VFA bound wastewater. Outlet pH showed a gradual rise in the pH values from 7.0 to 7.3 with the exhaustion of the cycle period (Fig. 5b).

After feeding soluble fermentative metabolite bound substrate, VFA_M varied inconsistently in the range of 39–458 mg/l (Fig. 4d). Consistent decrease in VFA_M concentration observed with the cycle period (Fig. 5b). Initially, VFA concentration was around 450 mg/l which was gradually decreased and approached 296 mg/l at the end of the cycle period. VFA_M composition present in the wastewater is generally utilized by MB in the process of CH_4 generation under anaerobic microenvironment. At the end of the cycle period, the VFA_M concentration attained low values which might be indicative

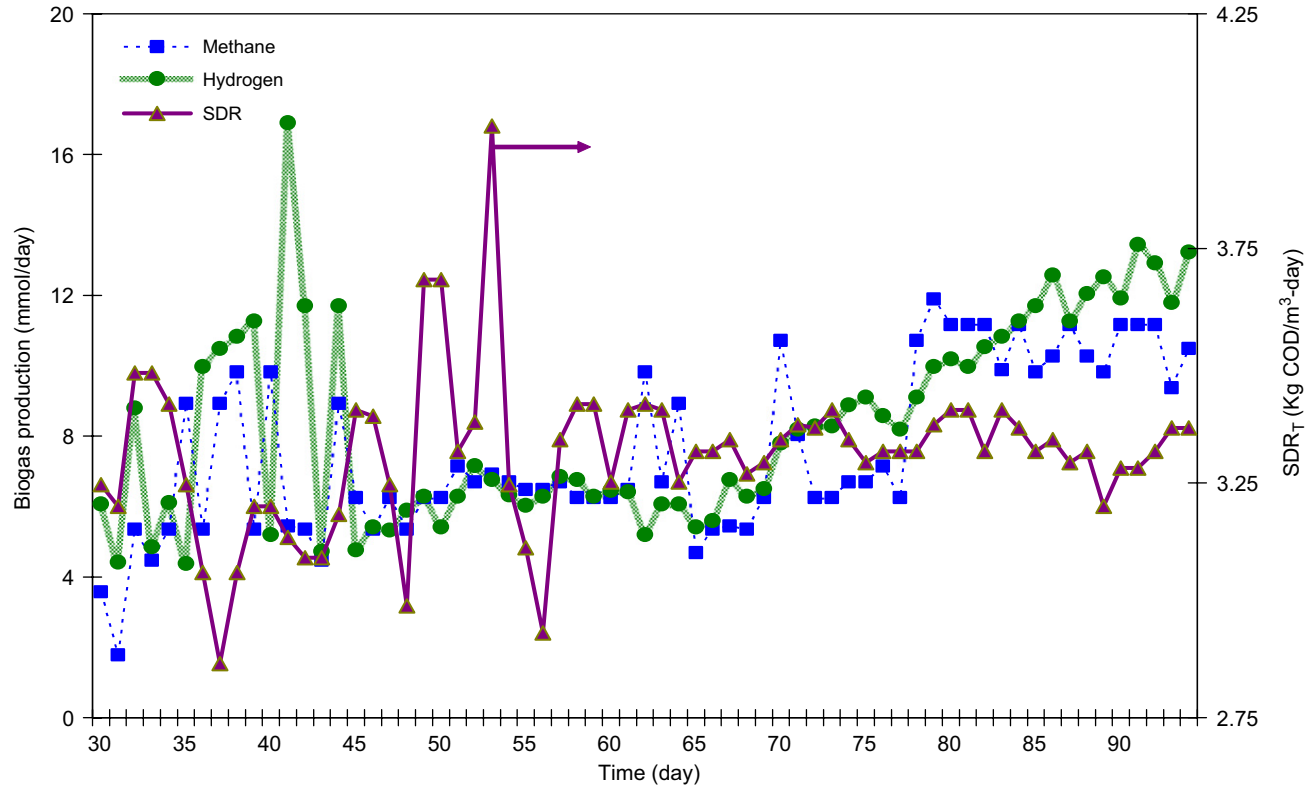

Fig. 6 – Integrated performance of AcSBBR and AnSBBR reactors with respect to biogas production (H_2 and CH_4) and total substrate degradation rate (SDR_T).

of effective functioning of the MB. Reduction in COD concentration in concurrence with the biogas production suggested the fact that the residual carbon source composed with soluble fermentative metabolites generated from acidogenic H_2 producing process had participated in methanogenic metabolic process.

The distribution of metabolites formed during H_2 and CH_4 generation were often considered as a crucial signal in assessing the metabolic pathway of the biochemical process. Samples during the course of experiments were analyzed for VFA composition viz., (HAc) to butyrate (HBu), propionic acid (HPr) and ethanol (HEt) to have understanding of the change in the metabolic pathway. Chromatography data revealed the presence of higher fraction of HAc (76.9%) along with relatively lower concentrations of HBu (13.8%), HPr (7.9%) and HEt (1.4%) during H_2 production. HAc was the major metabolite observed and suggested the persistence of acid forming pathway, which is considered to be important for efficient H_2 production by acidogenic bacteria. On the contrary, during methanogenic process, composition of metabolites varied significantly. A marked variation in HAc (40.7%) concentration was observed along with increase in HBu (24.5%), HPr (30.3%) and HEt (4.5%) concentrations. The variation observed in soluble metabolites concentration suggested that of VFA was consumed under methanogenic microenvironment in the process of CH_4 generation. Visible reduction in VFA concentration observed in methanogenic process as compared to acidogenic process corroborates the above statement (Figs. 3 and 5).

VFA and pH are integral expressions of the acid–base conditions of any anaerobic process as well as intrinsic index of the balance between two of the most important microbial groups viz., AB and the MB. Shift of pH to basic conditions correlated well with the documented VFA consumption. This also enumerated the fact that, residual generated from acidogenic process was consumed by MB in association with substrate degradation and generating biogas as metabolic byproduct. The utilization of VFA_M along with substrate degradation and biogas production in the anaerobic metabolic reaction is considered to be positive aspects of AnSBBR in the direction of sustainable H_2 generation.

It is evident from the study, that the acclimatization period to attain stable performance was relatively short for AnSBBR (16 days, Fig. 4), in spite of feeding VFA bound wastewater outlet from acidogenic process which was having fluctuating concentration of VFA and COD. This might be attributed to the adapted periodic discontinuous batch mode operation conditions coupled with biofilm configuration. Biofilm reactor configuration coupled with periodic discontinuous batch process has dual operational advantages and helps to maintain high biomass concentration. The system encourages the culture of slow growing organisms and can achieve homogeneous biomass distribution. This leads to improved reaction potential for stable and robust systems which is well suited for treating highly variable wastewater [12,15,56–61]. Further, the selection of effective biomass is possible in this system and the biomass concentration could be uniformly maintained along the height of the bed [57,62]. Moreover, biofilm configured systems are generally more resistant to shock loads [63] and protects slowly growing organisms with special metabolic capacities from washout [62,64].

5. Conclusions

Experimental data illustrated the feasibility of simultaneous integration of acidogenic hydrogen production process with anaerobic methanogenic process for enhancing substrate removal efficiency by utilizing residual organic fraction present in wastewater composed of fermentative soluble metabolites from acidogenic process in addition to H_2 and CH_4 generation. The process integration facilitated utilization of residual carbon source along with generated volatile fatty acids from acidogenic process as primary substrate in methanogenic process involving methane generation associated with additional substrate degradation. The process of integration appears to be a promising approach for sustainable H_2 generation with wastewater as substrate. The adapted process parameters [acidogenic (pH 6.0) and methanogenic (pH 7); cycle period—24 h], parent inoculum, reactor configuration (biofilm) and operation mode (periodic/sequencing batch) used for reactors operation had also significant influence on the efficiency of process integration.

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