Bioconversion of Cheese Whey to Methane in an Upflow Anaerobic Packed Bed Bioreactor

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Upflow anaerobic packed bed (UAPB) reactor is an upflow fixed film packed bed bioreactor that is used for rapid biotransformation of organic matter to methane. In this study, biofilm was established on seashell, packed in an UAPB bioreactor. The start-up duration for the bioreactor was 3 to 5 days while the major problem associated with normal UASB reactors is long start-up. The reactor was operated at room temperature (25 °C) with various HRT of $\tau = 6$, 9, 10, 13, 16, 20 and 24 h. The organic loading was gradually increased from 1.6 to 9.9 g L^{-1} h^{-1} COD. The UAPB reactor was continuously operated for 65 d. The treatment of high organic load dairy wastewater at HRT of 6 h was conducted. Maximum biogas production of 12.4 L h^{-1} (6.57 mol h^{-1}) was achieved. At HRT of 16 h, a 94.5 % of COD removal was obtained. Methane yield of 0.12 g CH_4 per g lactose at the highest OLR was achieved.

Key words:
Anaerobic treatment, UAPB, cheese whey, methane, COD and fixed film

Introduction

Dairy wastes are abundant in dairy industries. The wastes contain high organic matters and the disposal of the effluents may cause serious environmental pollution.[1–3] The dairy industry, like most other agro-industries, generates residues from which whey is the most important wastewater produced, with an extremely high organic load. Cheese whey as a by-product of the dairy industry consists of 5, 3, 1, 1, and 0.5 % carbohydrates mainly lactose, salts, lactic acid, proteins and fat, respectively.[4–6]

World annual production of whey is estimated to be 115 million tons; approximately 47 % of the produced whey is disposed into the environment.[7] Due to the high organic content of whey, anaerobic digestion processes are recommended.[8–9] Since the whey naturally contains lactose and biodegradable organic matter, biological treatment is a practical process.[1–5] Among biological treatment processes, treatment in ponds, activated sludge plants and anaerobic treatment are commonly employed for dairy wastewater treatment.[10] Whey is initially hydrolyzed and converted to organic acids by acidogenic microorganisms then the degradation is followed by the methanosarcina and methanogenic bacteria.[11]

In cheese production plants, full recovery of by-products from whey may not be possible. Whey may contain valuable substrate for bioconversion; whey has high organic matters or high chemical oxygen demands (COD) ($\gamma = 60-80$ g L^{-1}).[12] More than 90 % of the total COD of the whey accounted for lactose, lactate, protein and fat.[13,14]

Anaerobic digestion of cheese whey offers an excellent solution in terms of both energy saving and pollution control.[15–16] The anaerobic process has a number of advantages; one of them is the production of methane as an energy source which consists of 50 to 80 % methane.[17–18] Despite these advantages, anaerobic digestion is not extensively used in the dairy industry, largely due to the problem of slow reaction, which requires longer HRT, and rapid acidification.[19–24] The problem of anaerobic digestion is slow reaction. It was overcome by novel hybrid systems such as upflow anaerobic sludge fixed film bioreactor and upflow packed bed biofilters.[22– 29]

The main purpose of this research was to explore the performance and stability of the UAPB bioreactor in treatment of whey from the local dairy industry. Bioconversion of high organic load (lactose) to biogas was investigated. In UAPB reactor experiments, major operational parameters such as yield of methane production, HRT, influent COD concentration and loading rate were determined.

Materials and methods

Cheese whey

The cheese whey was supplied from the "Gela Factory" (Amol, Iran), which is benefitted from

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Ultra filtration process for production of cheese. The whey samples provided from the factory, were collected in 20 L containers and transported daily to the laboratory and stored at $\vartheta = 4$ °C to avoid acidification of the cheese whey. During the adaptation phase, diluted whey at pH of 6.5 was fed into the reactor. Based on necessity of the experiment, diluted cheese whey with variable concentrations was prepared using distilled water. The pH of the feed was adjusted to 6.5, using a concentrated sodium hydroxide solution (6 mol L^{-1}). The characteristic and chemical composition of the cheese whey is shown in Table 1. The notable characteristic of this effluent was the high COD content.

Table 1 – *Characteristics and chemical composition of cheese whey*

Characteristic	Unit	Value
COD	mg L^{-1}	60 000
lactose	g L^{-1}	50
TS	g L^{-1}	55
VS	g L^{-1}	49
proteins	g L^{-1}	2.2
phosphate	g L^{-1}	0.6
Ca	g L^{-1}	0.02
pH		5.5–6.6

Experimental set up

The schematic diagram of the pilot scale UAPB bioreactor is shown in Fig. 1. The actual system of the operated pilot scale UAPB bioreactor is shown in Fig. 2. The Plexiglas reactor was fabricated with an internal diameter of 19.4 cm and height of 60 cm. The total volume of the reactor

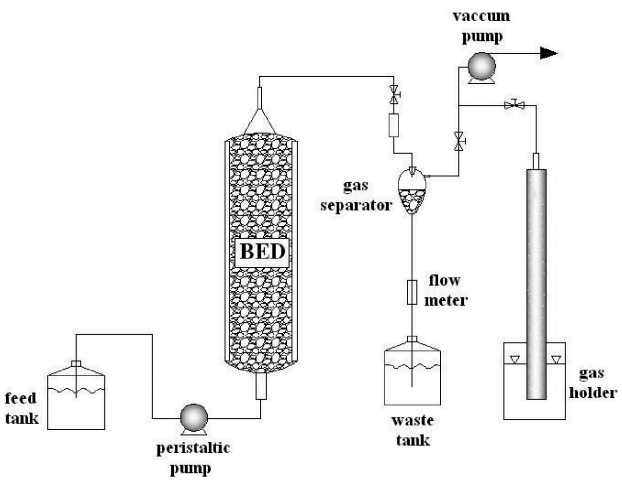


Fig. 1 – *Schematic diagram of the UAPB bioreactor*

Fig. 2 – *Pilot scale of the operated UAPB bioreactor*

was 17.667 L. The column was randomly packed with seashell. The voidage of the packed bed reactor was 65 %. A 1000 mL funnel shaped gas separator was used to liberate the generated biogas from the effluent, and then the gas was led to the gas collector tank. The gas tank was a cylindrical glass pipe with an internal diameter of 80 mm and 1 m length. The liberated gas was frequently measured for the selected fixed HRT and the gas volume was recorded with respect to time. The UAPB reactor was operated at room temperature (25 °C). Cheese whey as a suitable substrate was continuously fed to the reactor using a peristaltic pump (SR25 adjustable flow rate, Thomas, Germany). The feed was introduced from the bottom of the column and it was distributed through the column using a perforated plate. The effluent was collected from the top of the column in a 20 L polyethylene container.

Reactor operation

The reactor was inoculated with 3 L of seed culture originated from anaerobic sludge of the wastewater treatment plant, Gela Factory. In order to develop a biofilm, a sticky surface on a seashell as packed material was created. A 2 L solution of $\gamma = 1$ g L^{-1} nutrient agar (Merck, Germany) was introduced from the top of the column for fast development of biofilm. In order to acclimate the sludge with cheese whey, the reactor was operated in batch mode with recirculating feed of diluted cheese whey ($\gamma = 7-20$ g L^{-1} COD). For the first three days of operation, the bioreactor was continuously operated in full recycle mode. Then the feed tank was gradually loaded with fresh whey. For start-up of the bioreactor, it was fed with fresh whey containing supplementary nutrients and carbohydrate. The system was in full recycle operation

and the cell was also recycled for 3 to 5 d till the biofilm developed on the seashell. Additional time of 5 d was given to ensure steady state condition. Replicated data were collected. After a short period of start-up, the bioreactor was maintained at HRT of 24 h. At each selected HRT, daily samples were taken for a duration of 5 d. The reactor was continuously fed with an initial organic loading rate (OLR) of 0.66 g L^{-1} h^{-1} COD and HRT of 24 h. The influent COD concentration was 15 g L^{-1} for the first 5 days. The COD concentration was increased stepwise to $\gamma = 60$ g L^{-1} (2.47 g L^{-1} h^{-1} COD) for a duration of 15 d. The entire experiments were operated continuously for 65 d.

Analytical methods

The COD was determined by closed reflux method as described in Standard Methods.[30] Lactose and COD values were measured via colorimetric method using spectrophotometer, UNICO 2100 (New Jersey, USA). A gas-tight syringe (Hamilton CO., Reno, Nevada, USA) was used to take the samples from the gas sampling port. Gas Chromatograph (Perkin Elmer, Auto system XL), equipped with thermal conductivity detector (TCD) and was used for gas composition analysis. A GC column, Carboxen 1000, with 100/120 mesh (Supelco, Park, Bellefonte, PA, USA) was used. The column temperature was initially maintained at $\vartheta = 40$ °C for $t = 3.5$ min, followed by temperature programming with an increasing rate of 20 °C min^{-1} until it reached 180 °C. The injector and detector temperatures were $\vartheta = 150$ and 200 °C, respectively. Flow rate of the carrier gas (He) was set at $Q = 30$ mL min^{-1}.

Scanning Electron Microscopy (SEM) was used to examine the external structure of the biofilm built on packing. A specimen is bombarded with a scanning beam of electrons and then the slowly moving "secondary electrons" are collected, amplified and displayed on the cathode ray tube. The electron beam and the cathode ray tube scanned synchronously so that an image of the specimen's surface was formed. Specimen preparation for SEM included fixation with 5 % glutaraldehyde and 1 % osmium tetroxide, followed by dehydration with 50−100 % ethanol before drying, finally making the specimen to become conductive to electricity. The sample was examined using a Leo Supra 50 VP Field emission SEM (UK) equipped with Oxford INCA 400 energy dispersive X-ray microanalysis system.[22]

Results and discussion

In this research, the UAPB was continuously operated with HRT of $\tau = 6$ to 24 h. The biofilm was fully established on the natural packing (seashell). Sample of the biofilm was scanned and the image was taken by SEM. Fig. 3 shows the SEM

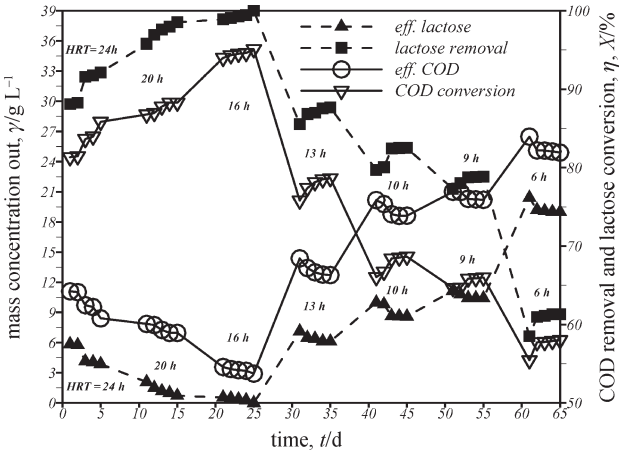
Fig. 3 – *Biofilm of the microorganisms built on the surface of seashells as packing*

micrographs of the biofilm created by the anaerobic microbial consortia. The magnification scale is from 500 to 5000. The microbial core and brush shape are clearly shown. In these images, the support surfaces are fully covered by the active biofilm.

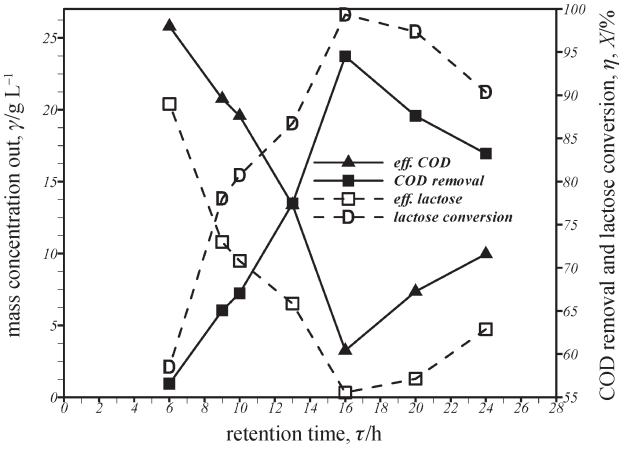
Fig. 4 depicts the effluent COD concentration and lactose utilization with respect to operation time. The dashed and solid lines are related to lactose and COD concentrations, respectively. The bioreactor was successfully started with HRT of 24 h, and then the removal rate was gradually increased. While the HRT was decreased stepwise to 16 h, maximum film was built on the surface of packing. The lactose concentration at downstream drastically dropped to zero. The COD and lactose in the effluent gradually increased as the retention time decreased stepwise. Table 2 represents the categorized data for the performance of UAPB bioreactor at various HRT, under steady state condition.

Fig. 5 presents the concentration of the effluent lactose and COD with respect to HRT. The biofilm was gradually developed on the solid support and the reactor performance was also improved with respect to time. After the start-up period was completed, HRT of 24 to 6 h in descending order was selected for the system. Although the HRT was in a reducing trend, the bioreactor performance was progressively improved. After 20 days of continued operation the biofilm was fully developed. Minimum concentrations of lactose and COD were obtained at HRT of 16 h. The reactor was started with HRT of 24 h. At the beginning, the reactor performed poorly, which resulted from insufficient biofilm. Therefore, the results for HRT of 24 h were less effective than other HRTs.

The COD removal and lactose conversion of $X = 94.5$ and 99.3 % was obtained at HRT of $\tau = 16$ h, respectively. Gannoun and coworkers stated that the most easily biodegradable substrates



F i g . 4 – *Performance of UAPB bioreactor, COD removal and lactose utilization*



F i g . 5 – *COD removal and lactose conversion*

are mainly sugars and some proteins, whereas the second one corresponded to volatile fatty acid degradation.[21] It should be noted that the applied HRTs in this work were always less than 1 day which is much smaller than HRTs reported in the literature.[2,3,21,27,29]

T a b l e 2 – *Performance of UAPB bioreactor at various HRT under steady state condition*

HRT/h	Influent lactose, γ/g L^{-1}	Lactose conversion, X/%	Influent COD, γ/mg L^{-1}	COD removal, η/%	Biogas production rate, ζ/L h^{-1}	Methane production rate, ζ/L h^{-1}	Methane production rate, ζ/mol h^{-1}	Exp. yield/ g biogas g^{-1} lactose	Theo. yield/ g CH_4 g^{-1} lactose	Exp. yield/ g CH_4 g^{-1} lactose
24	49.17	90.37	59419.64	83.24	4.58	3.25	0.133	0.175	0.28	0.099
20	49.17	97.37	59419.64	87.62	5.59	4.41	0.180	0.170	0.28	0.111
16	49.17	99.34	59419.64	94.51	6.93	5.89	0.240	0.159	0.28	0.118
13	49.17	86.73	59419.64	77.46	8.61	5.59	0.230	0.172	0.28	0.091
10	49.17	80.72	59419.64	67.05	10.78	6.36	0.260	0.176	0.28	0.080
9	49.17	78.03	59419.64	65.06	11.40	6.50	0.266	0.165	0.28	0.073
6	49.17	58.51	59419.64	56.58	12.40	6.57	0.269	0.129	0.28	0.049

Fig. 6 and Table 3 show the performance of the UAPB bioreactor in a wide range of OLR (γ = 15.855 − 59.420 g L^{-1}) for HRT of 6, 8 and 10 h. For all HRTs, the COD removal and lactose conversion increased as the OLR decreased, which is supported by the findings in the literature.[3,26] As the COD in the form of OLR increased from 1.98 to 7.43 g L^{-1} h^{-1} COD, COD removal decreased from 98 to 62.5 %; lactose removal efficiency dropped from 100 to 73 %, while lactose as OLR increased from 1.5 to 6.14 g L^{-1} h^{-1} lactose.

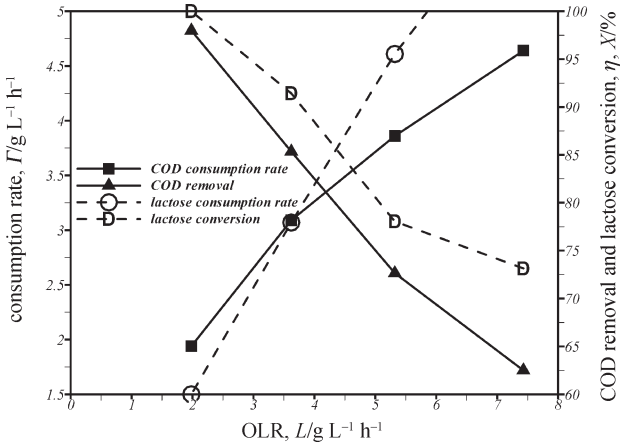


Fig. 6 – *Lactose conversion and COD removal efficiency for HRT of 8 h*

Fig. 7 depicts the biogas production rate, as well as the lactose and COD utilization rate with respect to HRT at room temperature (25 °C) and atmospheric pressure. The data show that, as the HRT increased, the production rate and utilization rate decreased.[26] The rate of biogas and methane production gradually decreased from 44.17 to 16.31 mmol L^{-1} h^{-1}, while the HRT increased from 6 to 24 h. However, at high HRT the methane was enriched. The biogas production rate increased from 53 to 85 % for HRT of 6 and 16 h, respectively.

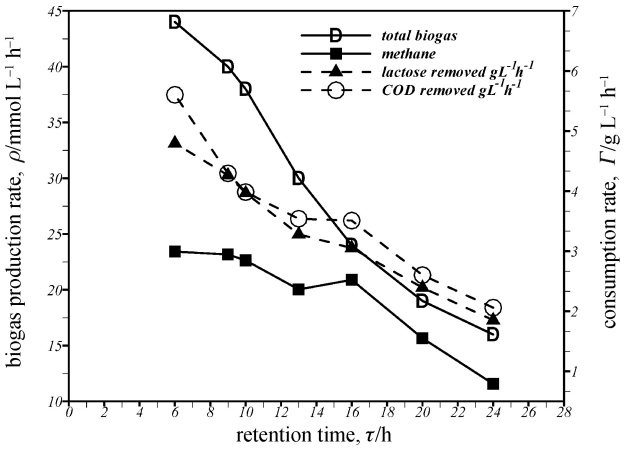


Fig. 7 – *Biogas production and utilization rate for COD and lactose at 25 °C and 1 bar*

The biogas production rate at atmospheric pressure and room temperature (25 °C) with respect to OLR is presented in Fig. 8. As the OLR increased, the biogas production rate also increased which is justified with the reported data.[21,26] The plotted data for all HRTs of 6, 8 and 10 h and OLR range of 1.5 to 10 g L^{-1} h^{-1} COD were linearly fitted.

Table 3 – *Performance of UAPB bioreactor at various OLR under steady state condition*

HRT, *t*/h	Lactose consumption rate, γ/g L^{-1} h^{-1}	OLR, COD/ g L^{-1} h^{-1}	Influent COD, γ/mg L^{-1}	COD removal, η/%	Influent lactose, γ/g L^{-1}	Lactose conversion, *X*/%	Methane production rate, ζ/L h^{-1}	Methane production rate, ζ/mol h^{-1}	Biogas production rate, ζ/L h^{-1}	Exp. yield/ g biogas g^{-1} lactose	Theo. yield/ g CH4 g^{-1} lactose	Exp. yield/ g CH_4 g^{-1} lactose
10	4.90	5.90	59419.64	67.05	49.17	80.72	6.360	0.260	10.70	0.176	0.28	0.080
	3.70	4.20	42564.56	76.86	36.90	90.20	4.484	0.183	7.60	0.166	0.28	0.075
	2.46	2.90	29012.50	92.30	24.59	98.40	3.009	0.123	5.10	0.167	0.28	0.076
	1.20	1.60	15854.91	99.83	12.04	100.00	1.357	0.056	2.30	0.154	0.28	0.070
8	6.14	7.43	59419.64	62.50	49.17	73.14	6.450	0.264	11.72	0.157	0.28	0.065
	4.61	5.32	42564.56	72.65	36.90	78.05	4.560	0.186	8.30	0.148	0.28	0.061
	3.07	3.62	29012.50	85.34	24.59	91.42	3.080	0.126	5.60	0.150	0.28	0.062
	1.50	1.98	15854.91	97.98	12.04	100.00	1.540	0.063	2.80	0.153	0.28	0.063
6	8.20	9.90	59419.64	56.58	49.18	58.51	6.570	0.269	12.40	0.129	0.28	0.049
	6.15	7.09	42564.56	64.84	36.90	69.86	4.664	0.191	8.80	0.122	0.28	0.047
	4.10	4.83	29012.50	73.43	24.59	80.20	3.127	0.128	5.90	0.123	0.28	0.047
	2.00	2.64	15854.91	86.10	12.04	93.70	1.696	0.069	3.20	0.136	0.28	0.052

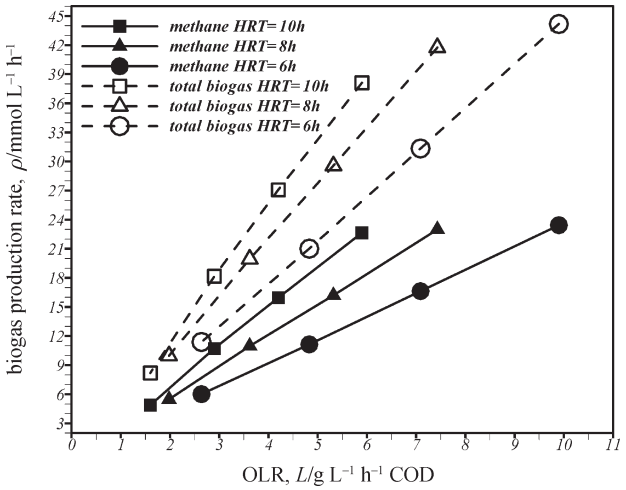


Fig. 8 – *Biogas production rate with respect to OLR at 25 °C and 1 bar*

Table 4 compares the data obtained for HRT and COD removal in the present study with data reported in the literature.[2–4,7,8,10,15,17,21,25,27–29] Biological treatment of cheese whey wastewater in a laboratory-scale UASB reactor was reported. Maximum COD removal rate was around 90 % in an OLR range from 6.5 to 28.5 g L^{-1} d^{-1} COD.[3] Patel and coworker investigated anaerobic treatment of cheese whey with COD of γ = 60 to 80 g L^{-1}, using an upflow fixed film reactor with various supports as packing media. They obtained a maximum COD removal of 81.5 %.[29] Recently, the feasibility of using various UASB reactors for dairy wastewater treatment was explored by two types of UASB hybrid reactors.[10] The reactors were operated at HRT of 10 days, loading rates of 0.5 to 13.3 kg m^{-3} d^{-1} COD and temperatures in the range of 12 to 20 °C. Maximum COD removal of 91.9 % was achieved for both types of reactors.[10] More recently, an upflow anaerobic filter reactor, treating dairy and cheese wastewater with OLR of 7.9 to 45.4 g L^{-1} d^{-1} COD, yielded an average of 80 % COD removal.[28] In the present research, at HRT of 16 h, maximum COD removal of 99.3 % was achieved. Comparing the HRT of the UAPB bioreactor with other systems showed that the present bioreactor was 5 times faster than other systems.[15]

Table 4 – *Comparison of experimental values with summary of dairy wastewater treatment cited in the literature*

Reactor type*	HRT, τ/d	COD in, γ/g L^{-1}	Max COD removal/%	References
UAPB	0.67	60	99.3	present study
UASB	2–10	77	95	3
UASB	6–40	37–60	80–92	2
UASB	2.06–4.95	42.7–55.1	95–97	25
UASB	5	64–67	97	15
UASFF	1.5–2	50–70	97.5	28
DAFF	5–10	56–62	88–95	27
ARBC	2–11	64–69.8	76–93	8
ARBC + SBR	7–10	37.4–65.7	96.2	4
UAF	1–4	55–60	72–92	21
UAF	1–5	60–80	67.5–81.5	29
CSTR + UAF	0.75–4	20	90	17
AHR	0.75–2	10	91.9	10
SAR	1–4	68.6	98.5	7

*UASB = upflow anaerobic sludge blanket
UASFF = upflow anaerobic sludge-fixed film bioreactor
ARBC = anaerobic rotating biological contact
SAR = stirred anaerobic reactor
AHR = anaerobic hybrid reactor
UAF = upflow anaerobic filter
CSTR = continuous stirred tank reactor
DAFF = downflow anaerobic fixed film reactor

Conclusions

The present research investigated the treatability of whey and biogas production rate using UAPB bioreactor. The novel anaerobic bioreactor with high performance was able to handle the high organic load. The UAPB reactor was highly efficient in treatment of whey with high COD loading rate in a short HRT. High COD and lactose removals of 94.5 and 99 % at HRT of 16 h were achieved. At HRT of 6 h, maximum biogas production rate of 44 mmol L^{-1} h^{-1} was obtained.

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List of Symbols

m – mass, g
Q – volume flow rate, mL min^{-1}
t – time, min, h, d
X – conversion, %

Greek letters

γ – mass concentration, g L^{-1}
Γ – consumption rate, g L^{-1} h^{-1}
ζ – production rate, mol h^{-1}, L h^{-1}
η – removal efficiency, %
ϑ – temperature, °C

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