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# Mathematical modeling of bio-hydrogen production from starch wastewater via up-flow anaerobic staged reactor

Mahmoud Nasr[a,*], Ahmed Tawfik[a], Masaaki Suzuki[a,b], Shinichi Ookawara[a,b]

[a]*Environmental Engineering Department, Egypt-Japan University of Science and Technology (E-JUST), P.O. Box 179, New Borg El Arab City, Alexandria 21934, Egypt*
*Tel./Fax: +2 03 4599520; email: mahmoud.said@ejust.edu.eg*
[b]*Department of Chemical Engineering, Tokyo Institute of Technology, 2-12-1, O-okayama, Meguro-ku, Tokyo 152-8552, Japan*

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ABSTRACT

Hydrogen production from starch wastewater industry via up-flow anaerobic staged reactor was investigated. The reactor was operated at an average organic loading rate of 13.17 ± 8.35 g COD/L d and hydraulic retention time of 8 h. The reactor achieved chemical oxygen demand (COD) and carbohydrate removal efficiencies of 84 and 92%, respectively. The total volatile fatty acids increased from 58.5 ± 30.0 (influent) to 235.6 ± 190.8 mg/L in the treated effluent, indicating that acidogenesis bacteria were dominant in the reactor. The system achieved maximum hydrogen production rate (HPR) and hydrogen yield of 2.48 L $H_2$/d and 8.8 mL $H_2$/g $COD_{removed}$, respectively. Simulated model tracks the experimental data with a correlation coefficient ($R^2 = 0.893$). Maximum substrate utilization rate ($\mu_{max,s}$) and maximum volumetric HPR ($\mu_{max,h}$) were calculated at different food to microorganisms ($F/M$) ratios of 0.15, 0.31, 0.46, 0.62, and 0.93 g COD/g VSS. Results showed that $\mu_{max,s}$ increased to −0.76 g COD/L h at $F/M$ ratio of 0.46, and then remained relatively constant at a value of −0.68 g COD/L h. Similar trends were observed for HPR, where it peaked ($\mu_{max,h}$ of 93.89 mL $H_2$/h) at $F/M$ ratio of 0.46.

*Keywords:* Starch wastewater; Up-flow anaerobic staged reactor; Hydrogen yield; Carbohydrate; COD

## 1. Introduction

Starch manufacturing factories discharge huge amount of wastewater which is rich in biodegradable organic matter. This wastewater has to be treated prior discharging into sewer network. Generally, the chemical oxygen demand (COD) levels of starch wastewater range from 6 to 10 g/L and it can impose heavy loads on the environment or be expensive in terms of sewer disposal [1]. Starch wastewater contains a relatively high percentage of carbohydrates, cellulose, protein, and nutrients, representing an important energy-rich resource, which can be potentially converted to a wide variety of useful products such as microbial biomass protein and biopesticide [2–5]. However, end users hesitate to use the microbial biomass protein because of its uncomfortable taste, high nucleic acid content, and slow digestion. The high production cost and technical barriers to large-scale implementation also limit the application of biopesticide production. Therefore, it is worthwhile to find a promising sustainable approach for

*Corresponding author.

simultaneous treatment and conversion of starch wastewater into renewable energy in the form of hydrogen.

Hydrogen energy has been recognized to be environmentally safe and alternative to fossil fuels. Moreover, hydrogen does not contribute to the greenhouse effect, it produces only water with no carbon monoxide, carbon dioxide, hydrocarbons, or fine particles when combusted, and has a high energy yield of 142 kJ/g, which is 2.75 times more than that of other hydrocarbons [6]. Hydrogen production from food industry wastewater via dark fermentation process has been extensively investigated for its advantages of low operation cost and effectiveness. Previous studies have proved the feasibility of converting starch wastewater to hydrogen gas by dark fermentation process [7]. However, most of them were conducted their research in batch cultivation pure $H_2$ producing bacteria [8–10]. Moreover, reports on continuous $H_2$ production from starch wastewater via dark fermentation processes were relatively scarce, although the simultaneous COD degradation and $H_2$ production from starch wastewater in a pilot-scale operation is highly recommended. Lay [11] obtained hydrogen yield (HY) of 1.29 L $H_2$/g starch using a chemostat reactor at a hydraulic retention time (HRT) of 17 h. Lower HY of 31 mL $H_2$/g wheat was found by Hawkes et al. [12] who used hydrolysate wheat feed as a sole substrate in completely stirred anaerobic reactor (CSTR).

Several factors affecting on the hydrogen production from wastewater i.e. Lin et al. [13] found that the optimal initial cultivation pH was 5.5 with peak HY of 1.1 mol $H_2$/mol hexose. Nevertheless, Zhang et al. [14] found maximum HY of 92 mL $H_2$/g starch added at pH 6.0 under thermophilic condition. Chen et al. [15] achieved a HY of 12.52 mmol $H_2$/g starch from hydrolyzed starch at a HRT of 12 h. Hussy et al. [16] observed an increase of HY by 1.36 times when shortened the HRT from 18 to 12 h, in a CSTR reactor fed with starch wastewater. However, if HRT was continuously shortened, the washout of fermentative hydrogen producing bacteria would occur in the reactor. As a matter of fact, organic loading rate (OLR) is an important parameter that may affect the metabolic routes of hydrogen production from wastewater via dark fermentation processes [17]. However, there is disagreement in the literature as to whether higher HY is achieved with lower or higher OLRs [17–19].

Modified Gompertz equation [20] has been widely used for batch fermentative biohydrogen production. Still, it does not take into consideration the effect of several parameters such as the substrate concentration, pH, volatile fatty acids (VFAs) generation, and food to micro-organisms ($F/M$) ratio. Modeling of fermentative hydrogen production process is essential to simulate and predict the degradation of organics and metabolite products from wastewater, and certainly provides information on the different factors affecting the production processes [21]. The experiments reported in this investigation have been carried out in order to assess the potentials of using up-flow anaerobic staged reactor (UASR) for hydrogen production from starch wastewater. Emphasis is afforded to the removal efficiency of the COD, carbohydrate, VFAs generation, and HY. Mathematical modeling equations were formulated to simulate the correlation between OLR, VFA, pH, food to micro-organism ratio, and hydrogen production rate (HPR).

## 2. Material and methods

### 2.1. Starch wastewater

Starch processing wastewater was collected from a starch manufacture plant located at 10th of Ramadan, in which corn was used as raw materials. The treatment system was installed and operated at the main starch wastewater source of National Company for Maize Products. The starch wastewater was collected and continuously pumped to the reactor. The organic content of the wastewater was mainly in a particulate form (80%) and only 20% was in a soluble fraction (Table 1).

### 2.2. Up-flow anaerobic staged reactor

Fig. 1 shows the schematic diagram of the UASR fed with starch wastewater. The working volume of the

Table 1
Mean characteristics of the starch wastewater used in the experiments

| | |
|---|---|
| COD (mg/L) | |
| Total | 4,390 |
| Soluble | 878 |
| Particulate | 3,512 |
| Carbohydrates (mg/L) | |
| Total | 3,880 |
| Soluble | 1,115 |
| $NH_4$-N (mg/L) | 2.12 |
| TKj–N (mg/L) | 12.2 |
| pH | 6.6 |
| VFAs (mg/L) | 58.8 |
| Conductivity (μS/cm) | 837.17 |

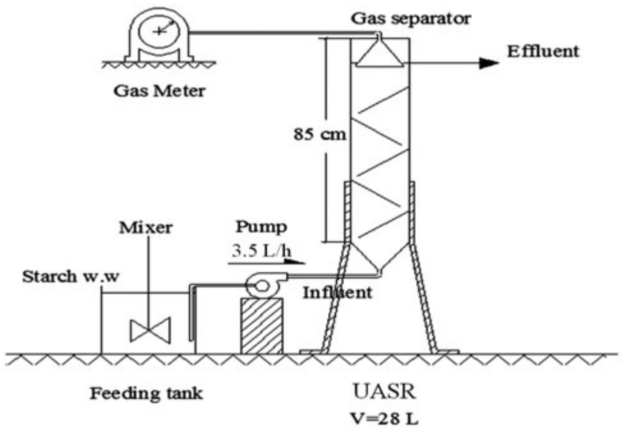


Fig. 1. Schematic diagram of the UASR treating starch wastewater industry.

reactor is 28 L. The reactor is manufactured from Perspex material with a pyramid shape at the bottom with dimensions of 19.5 cm for width and 85 cm for height. The reactor consists of six chambers and provided by baffles to increase the contact time between the $H_2$ producing bacteria and the substrate. Moreover, gas–solid separator was situated along the reactor height and on the top of the reactor in order to maximize the gases collection resulted from the anaerobic conversion processes. The total biogas volume of the reactor was daily measured by a gas meter (Drum-type gas meter—thermometer—packing fluid). The wastewater flows from the feeding tank to the reactor using peristaltic pump with a head of 60 cm and a power of 7.66E-6 horsepower. Prior starting the experiments, the UASR was inoculated with sludge harvested from El-Agamy wastewater treatment plant. The sludge was collected after the thickening process. Afterwards, the harvested sludge was allowed to be settled for one day. The sludge was preheated at 90˚C for 20 min to inactivate methanogenic bacteria [17]. The reactor was inoculated with 14 L of the pretreated sludge. The UASR was continuously fed with starch wastewater at a flow rate of 3.5 L/h, HRT of 8.0 h, and operated at a temperature of 30˚C. During the whole experimental period of 85 d, pH value was not artificially controlled.

### 2.3. Calculations

The daily volumetric and specific HPR and HY were calculated by the following Eqs. (1)–(3) [2];

$$\mathrm{VHPR} = V_{\mathrm{H_2}}/V \tag{1}$$

VHPR: the volumetric HPR (mL $H_2$/L d), $V_{\mathrm{H_2}}$: the daily hydrogen gas production (mL $H_2$/d), and $V$: the volume of inocula (L).

$$\mathrm{SHPR} = V_{\mathrm{H_2}}/X \cdot V \tag{2}$$

SHPR: the specific HPR (mL $H_2$/g VSS d) and $X$: the biomass concentration in the inocula at steady-state (g VSS/L).

$$\mathrm{HY} = V_{\mathrm{H_2}}/Q(S_0 - S) \tag{3}$$

where HY: the HY (mL $H_2$/g COD or mol $H_2$/mol glucose); $Q$: the feed flow rate (L/d), and $S_0$ and $S$: the influent and effluent of total COD concentrations (g/L), respectively. HY was calculated based on the glucose/COD ratio (0.94) and the molecular weight of glucose (180 g/mol).

### 2.4. Mathematical modeling

A system of linear equations is considered over determined. These mainly are in case of more equations than unknowns. Each equation introduced into the system can be viewed as a constraint that restricts one degree of freedom. From the experimental data, mathematical equations were formulated to correlate the relationship between HPR and OLR, VFAs, and pH.

$$\begin{aligned}&|\mathrm{HPR}_1 \quad \mathrm{HPR}_2 \ \cdots \ \mathrm{HPR}_3|_{(1\times n)}\\ &= a \times |\mathrm{OLR}_1 \quad \mathrm{OLR}_2 \ \cdots \ \mathrm{OLR}_n|_{(1\times n)}\end{aligned} \tag{4}$$

$$\begin{aligned}&|\mathrm{HPR}_1 \quad \mathrm{HPR}_2 \ \cdots \ \mathrm{HPR}_3|_{(1\times n)}\\ &= b \times |\mathrm{VFA}_1 \quad \mathrm{VFA}_2 \ \cdots \ \mathrm{VFA}_n|_{(1\times n)}\end{aligned} \tag{5}$$

$$\begin{aligned}&|\mathrm{HPR}_1 \quad \mathrm{HPR}_2 \ \cdots \ \mathrm{HPR}_3|_{(1\times n)}\\ &= c \times |\mathrm{pH}_1 \quad \mathrm{pH}_2 \ \cdots \ \mathrm{pH}_n|_{(1\times n)}\end{aligned} \tag{6}$$

$$\begin{aligned}&|\mathrm{HPR}_1 \quad \mathrm{HPR}_2 \cdots \mathrm{HPR}_3|_{(1\times n)}\\ &= |d \quad e|_{(1\times 2)} \begin{vmatrix} \mathrm{OLR}_1 & \mathrm{OLR}_2 & \ldots & \mathrm{OLR}_n \\ \mathrm{VFA}_1 & \mathrm{VFA}_2 & \ldots & \mathrm{VFA}_n \end{vmatrix}_{(2\times n)}\end{aligned} \tag{7}$$

$$\begin{aligned}&|\mathrm{HPR}_1 \quad \mathrm{HPR}_2 \cdots \mathrm{HPR}_3|_{(1\times n)}\\ &= |k \quad l \quad m|_{(1\times 3)} \begin{vmatrix} \mathrm{OLR}_1 & \mathrm{OLR}_2 & \ldots & \mathrm{OLR}_n \\ \mathrm{VFA}_1 & \mathrm{VFA}_2 & \ldots & \mathrm{VFA}_n \\ \mathrm{pH}_1 & \mathrm{pH}_2 & \ldots & \mathrm{pH}_n \end{vmatrix}_{(3\times n)}\end{aligned} \tag{8}$$

where constants $a$, $b$, $c$, $d$, $e$, $k$, $l$, and $m$ were determined by creating M-file in MATLAB software and solved as a matrix equation.

Batch experiments concerning the effect of $F/M$ ratios of 0.15, 0.31, 0.46, 0.62, and 0.93 g COD/g VSS on the hydrogen production from starch wastewater were tested [2]. The food (*F*) was calculated based on the COD of the influent starch wastewater, while the micro-organisms (*M*) were estimated by the volatile suspended solids (VSS) of the retained sludge. Monod-type kinetic model was used for simulation of HPR from starch wastewater at different $F/M$ ratios.

$$\frac{dS}{dt} = \frac{\mu_{\max,s}}{k+s} \times S \tag{9}$$

$$\frac{dH_2}{dt} = \frac{\mu_{\max,h}}{k+S} \times S \tag{10}$$

where $\mu_{\max,s}$: the Monod maximum uptake rate (g COD/L h), $k$: the half saturation constant (g COD/L), $S$: the concentration of substrate (g COD/L), $\mu_{\max,h}$: the maximum volumetric HPR (mL $H_2$/h). Genetic algorithm technique was used for estimating the parameters, and sum of square error performance function was applied to fit the experimental data.

### 2.5. Analytical methods

Samples of the starch wastewater and the treated effluent were collected twice a week in a clean container and immediately analyzed for COD, TSS, VSS, ammonia, TKj–N, pH, VFAs, conductivity, sludge, and carbohydrate. All analysis was carried out according to APHA [22] except carbohydrate which was measured according to the phenol–sulfuric acid method, using glucose as the standard. To determine the filtrate COD and carbohydrate, 0.45 μm sterile membrane filter paper was used. The $COD_{particulate}$ and $carbohydrate_{particulate}$ was calculated by the difference between $COD_{total}$ and $COD_{filtered}$, and $carbohydrate_{total}$ and $carbohydrate_{filtered}$, respectively. The biogas constituents ($H_2$, $CO_2$, and $CH_4$) were analyzed by gas chromatography (GC, Agilent 4890D) with thermal conductivity detector and a 2.0 m stainless column packed with porapak TDS201 (60/80 mesh).

## 3. Results and discussion

### 3.1. Performance of UASR for $H_2$ production from starch wastewater

The results presented in Fig. 2 show that the UASR is effective for COD removal at an HRT of 8 h. Although the influent COD was largely fluctuated from 1.09 to 8.75 g/L, the reactor achieved almost constant effluent quality of 0.69 ± 0.39 g COD/L. Those results were corresponding to an average COD removal efficiency of 84%. The low discrepancy in the effluent COD values i.e. min, max, and standard deviation of 0.23, 1.48, and 0.39 g/L, respectively, could be attributed to the excellent process stability maintained in the UASR. Moreover, the results recorded here are higher than those obtained by Guo et al. [23] who found that maximum COD removal rate of 38% was achieved from anaerobic acidogenesis of starch wastewater in an expanded granular sludge bed reactor at a HRT of 24 h. However, better results for COD removal efficiency of 93.8 and 98.7% from starch wastewater was achieved by Wang et al. [24] and Rajasimman and Karthikeyan [25], respectively. The lower removal efficiency of COD in this investigation is mainly due to the increase of the soluble microbial products including VFA in the hydrogen fermentative reactor. Those results can be supported by Arooj et al. [26] who found that the major portion of the influent substrate is consumed for producing VFAs in the acidogenesis reactor. Fig. 3 shows the effect of OLR on HPR. The results obtained indicated that HPR was slightly increased from 0.26 to 1.91 L $H_2$/d, when OLR

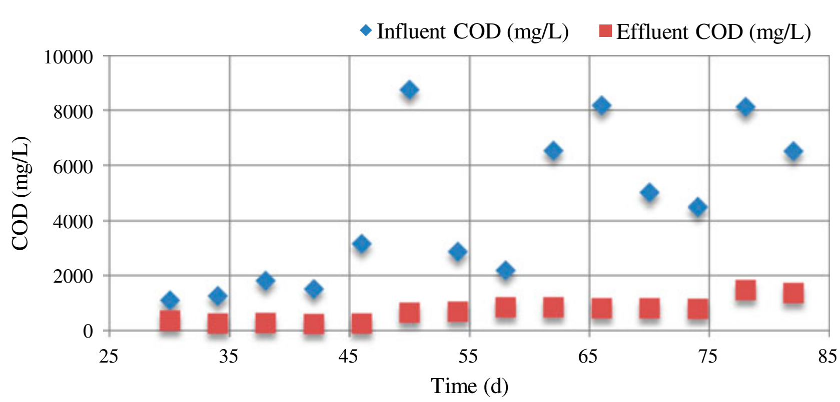


Fig. 2. Time course of COD concentrations in the UASR fed with starch wastewater at a HRT of 8 h.

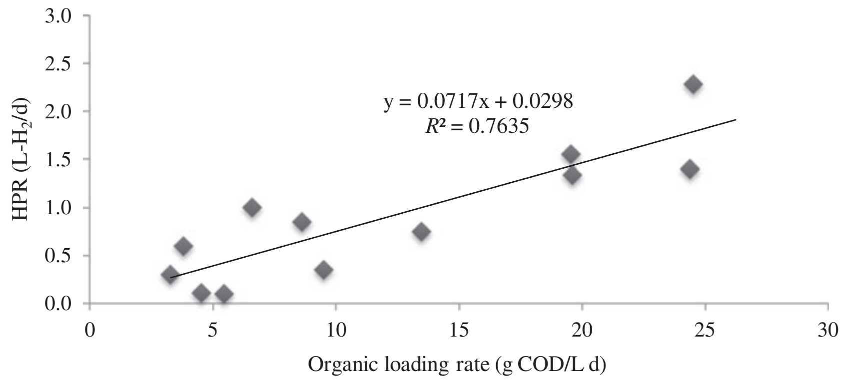


Fig. 3. Effect of OLR on HPR in UASR treating starch processing wastewater industry.

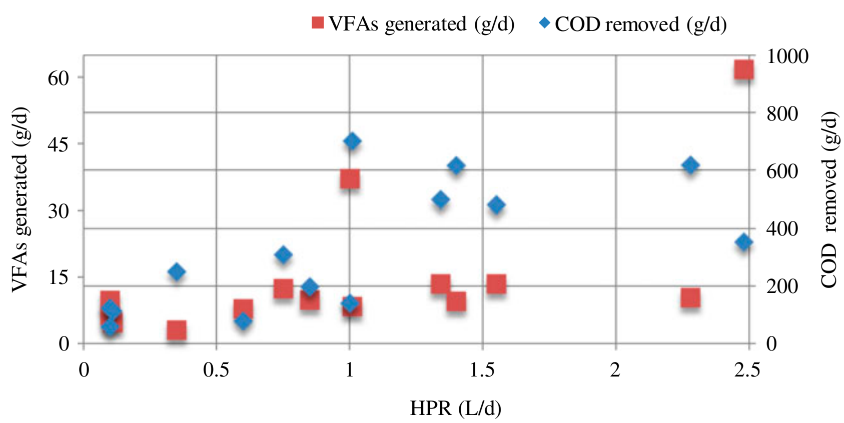


Fig. 4. The relationship between $H_2$ production rate HPR, $COD_{removed}$, and $VFAs_{generated}$.

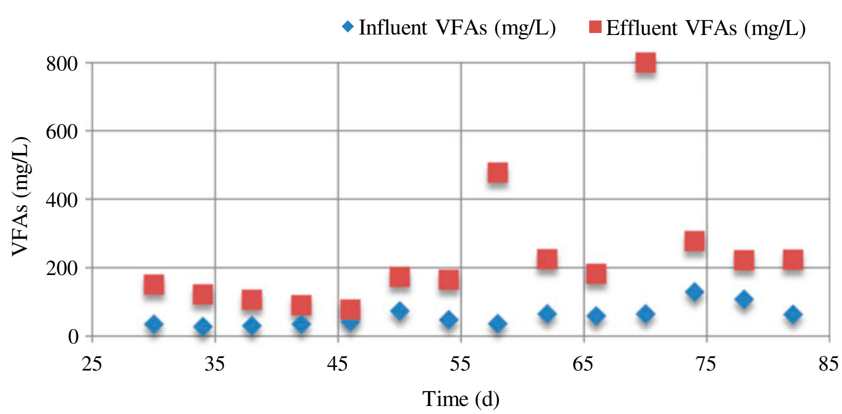


Fig. 5. VFAs generation in UASR treating starch wastewater.

was raised from 3.27 to 26.25 g COD/L d, respectively ($R^2$: 0.7635). The trends observed in this study are comparable to those reported by Tawfik and Salem [18]. The results presented in Fig. 4 show that $H_2$ production was strongly dependant on COD conversion and VFAs generation. Maximum $H_2$ production of 2.48 L/d was achieved at $COD_{removed}$ of 352 g/d and maximum $VFAs_{generated}$ of 62 g/d. On the contrary, maximum $COD_{removed}$ of 680 g/d produced only 1.0 L $H_2$/d. This was mainly due to less VFAs generation of

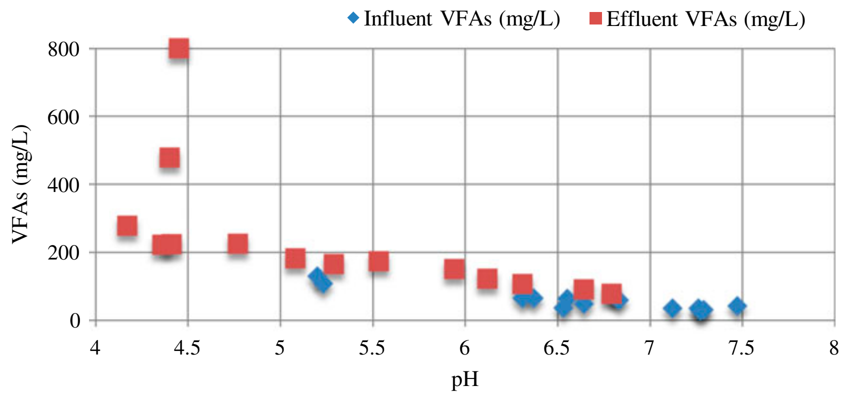


Fig. 6. Correlation between pH and VFAs generation in UASR treating starch wastewater.

8.4 g/d in the treated effluent. The maximum $H_2$ production obtained in this study is comparable to those of other studies [9,12,13,27–30]; however, interestingly, the operational conditions to maximize the $H_2$ yield differ drastically in those investigations. This might be due to the different microbial consortium caused by the differences of inocula, the various pretreatments methods, substrates and/or the reactor operational conditions. HY of 31.97 ± 21.36 mmol $H_2$/mol glucose (0.19 ± 0.13 mmol $H_2$/g starch) was achieved. Those results are corresponding to a volumetric and specific HPR of 36.0 ± 26.5 mL $H_2$/L d and 3.67 ± 2.7 mL $H_2$/g VSS d, respectively. The HY based on COD removal was 3.73 ± 2.49 mL $H_2$/g $COD_{removed}$ which was quite low as compared to the theoretical value of 1,390 mL $H_2$/g $COD_{removed}$. This can be attributed to the presence of high percentage of particulate starch in the influent (reached up to 80%) that is accumulated in the lower zone of the reactor and depress the hydrolysis process [2,18,19,31]. Indeed, increasing the HRT would increase the HY i.e. Argun and Kargi [29] found that the lowest and the highest HYs were 33 and 90 mL $H_2$/g starch for HRT of one and six days, respectively.

VFAs generation was always associated with the conversion of COD and degradation of carbohydrate in the UASR. Influent and effluent carbohydrates concentration recorded 3,880 and 310 mg/L, respectively, with a removal efficiency of 92%. Tawfik and El-Qelish [19] found that the fermentative bacteria can utilize carbohydrate in the dark fermentation process but the process remains effective only at substrate concentration below 36 g COD/L which implied that microbial activity was inhibited at high substrate condition (>36 g COD/L) and resulted in limited $H_2$ production and carbohydrate degradation. The VFAs was substantially increased from 58.5 to 235.6 mg/L, in the treated effluent as shown in Fig. 5. Results in Fig. 6 show the relationship between pH and VFAs generation in UASR. The results showed that the drop in pH values from 6.6 in the influent to 5.3 in the treated effluent was associated by increase in VFAs in the treated effluent. pH of the range 5.5–6.0 is considered as an optimum range for effective $H_2$ generation [16,20,31–35]. However, the optimum pHs for $H_2$ production in the comparable processes differ significantly among studies (pH 4–7) [27]. This discrepancy may be due to the different substrates used for $H_2$ production.

### 3.2. Simulation of the mathematical modeling

By solving the over-determined system of linear equations, the constants *a*, *b*, *c*, *d*, *e*, *k*, *l*, and *m* were estimated. The results showed that the mathematical model equations track the experimental results efficiently, and the correlation coefficient $R^2$ reached up to 0.893 (Table 2). Consequently, this high value

Table 2
The estimated $R^2$ values for parameters (OLR: g COD/L d, VFA: g/d, and pH) affecting HPR: L $H_2$/d

| Equation | Parameters | Estimated constants | $R^2$ value |
|---|---|---|---|
| Eq. (4) | OLR | $a = 0.0725$ | 0.692 |
| Eq. (5) | VFA | $b = 0.0431$ | 0.654 |
| Eq. (6) | pH | $c = 0.1698$ | 0.693 |
| Eq. (7) | OLR and VFA | $d = 0.0457$, $e = 0.023$ | 0.893 |
| Eq. (8) | OLR, VFA, and pH | $k = 0.0527$, $l = 0.0252$, $m = -0.0344$ | 0.893 |

Table 3
The estimated parameters: $\mu_{max,s}$, $k$, and $\mu_{max,h}$ for starch degradation via dark fermentation at different $F/M$ ratios

| $F/M$ ratios | 0.15 | 0.31 | 0.46 | 0.62 | 0.93 |
|---|---|---|---|---|---|
| $\mu_{max,s}$ (g COD/L h) | −0.26867 | −0.44842 | −0.76003 | −0.67515 | −0.69806 |
| $k$ (g COD/L) | −1.60208 | −3.88545 | −4.64671 | −7.22625 | −13.8484 |
| $\mu_{max,h}$ (mL $H_2$/h) | 31.49421 | 64.90144 | 93.89961 | 96.66862 | 33.16347 |
| $R^2$ for substrate degradation | 0.949 | 0.970 | 0.936 | 0.946 | 0.898 |
| $R^2$ for $H_2$ production | 0.993 | 0.996 | 0.992 | 0.971 | 0.982 |

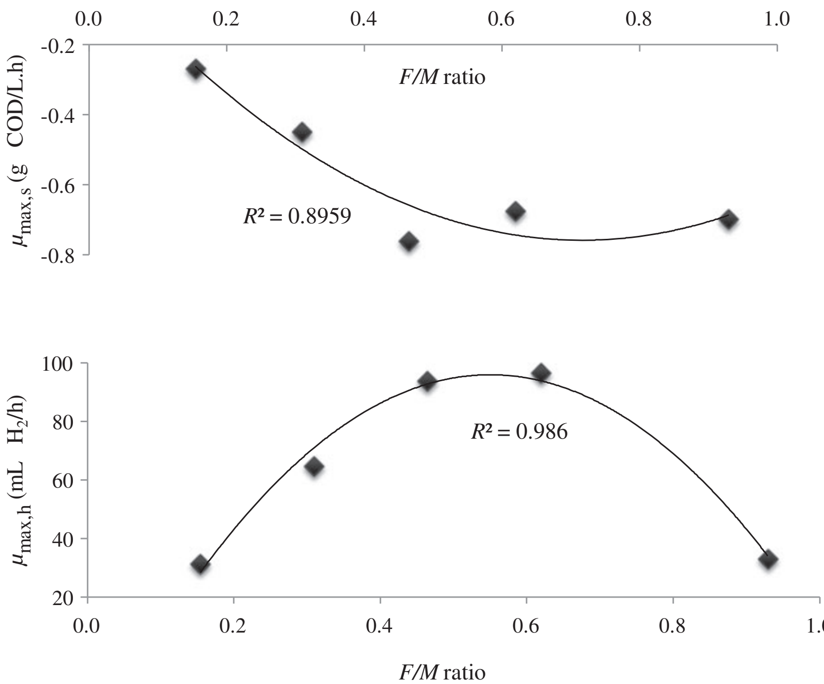


Fig. 7. Effect of $F/M$ ratios on (a) maximum substrate utilization rate ($\mu_{max,s}$): and (b) maximum volumetric HPR ($\mu_{max,h}$).

revealed the strong correlation between $H_2$ production rate, COD conversion, VFAs generation, and pH drop.

Table 3 summarizes the calculated parameters $\mu_{max,s}$, $k$, and $\mu_{max,h}$, and the corresponding $R^2$ values for HPR at various $F/M$ ratios. As shown in Fig. 7(a) and (b), maximum substrate utilization rate ($\mu_{max}$) increased up to −0.76 (g COD/L h) at increasing the $F/M$ ratio from 0.15 to 0.46. Further increase in $F/M$ ratio to 0.93 did not affect seriously on the $\mu_{max}$, and remained constant at a value of −0.68 g COD/L h. Simultaneously, to those results, the maximum volumetric HPR ($\mu_{max,h}$) of 93.89 mL $H_2$/h peaked at $F/M$ ratio of 0.46, and then decreased with further increase of $F/M$ ratio to 0.93. This mainly can be due to accumulation of VFAs at higher $F/M$ ratios. Moreover, substrate utilization rate and HPR are strongly affected by the $F/M$ ratio.

## 4. Conclusions

The results obtained in this investigation showed that UASR is economic, simple, and applicable for $H_2$ production from starch wastewater. The advantages of this bioreactor include rapid biodegradation process at a HRT of 8.0 h, and excellent process stability at OLR not exceeding 13.17 ± 8.35 g COD/L d. The reactor achieved a residual COD value of 690 mg/L in the treated effluent which compiles the Egyptian standards for discharge into sewerage network. Moreover, the reactor showed removal efficiencies of 84% for COD and 92% for carbohydrate. Furthermore, the drop in pH values from 6.6 to 5.3 was associated by increase in VFAs from 58 to 236 mg/L, respectively. Additionally, $H_2$ production rate was significantly affected by the conversion of COD, VFAs generation, and pH. HY of $31.97 \pm 21.36$ mmol $H_2$/mol glucose ($0.19 \pm 0.13$ mmol $H_2$/g starch) was achieved. A mathematical modeling showed a good correlation ($R^2 = 0.893$) between the experimental and modeled data.

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