Biological Hydrogen Production from Starch Wastewater Using a Novel Up-flow Anaerobic Staged Reactor

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Continuous and batch tests were conducted to evaluate fermentative biohydrogen production from starch wastewater via a mesophillic up-flow anaerobic staged reactor (UASR). The effects of organic loading rate (OLR) and food to micro-organisms ratio (F/M) on hydrogen yield (HY) and hydrogen production rate (HPR) were investigated. The bioreactor was continuously operated at a constant hydraulic retention time (HRT) of 6.7 h. The optimal OLR and F/M ratios were 54 g-COD/L.d and 1.4 g-COD/g-VSS.d, respectively. The maximum HY and HPR were 1.87 mol-H_2/mol-glucose and 246 mmol-H_2/L.d, respectively. Batch experimental results indicated that the optimal initial cultivation pH ranged from 5.5 to 6.5 with a hydrogen potential (P) of 1435-1420 mL-H_2, while the initial substrate concentration of 20 g-starch/L showed a maximum HPR (R_m) of 300 mL-H_2/h. Zero, 1st and 2nd order kinetic studies were used to develop a model of the experimental data. The 2nd order model adequately fitted with the experimental results (R^2>0.97) better than those for a zero- or a 1st order kinetic reaction.

Keywords: Bio-energy; High strength organic wastewater; Optimum condition for anaerobic treatment; F/M ratio

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INTRODUCTION

The starch processing industry consumes a lot of water, resulting in a huge amount of industrial wastewater. Unfortunately, this wastewater is mainly discharged into sewerage networks without any treatment, which causes serious environmental pollution problems. The chemical oxygen demand (COD) levels of starch wastewater range from 10 to 30 g/L, and it undoubtedly imposes heavy loads on the environment, causing high expenses in terms of sewer disposal (Jin *et al*. 2002). Fortunately, starch wastewater is rich with biodegradable organic matter and its temperature is relatively high (35 to 40 °C). Moreover, starch wastewater contains a high percentage of carbohydrates, cellulose, protein, and nutrients, representing an important energy-rich source that can potentially be converted to a wide variety of useful products. A bioconversion process is an advantaged way to recover useful resources from starch wastewater, especially to produce more valuable products, such as microbial biomass protein (Jamuna and Ramakrishna 1989) and biopesticide (Lu *et al*. 2007). However, end users hesitate to use the microbial biomass protein because of its unappetizing taste, high nucleic acid content, and slow rate of digestion. The high production cost and technical barriers to large-scale implementation also limit the application of biopesticides (Lee *et*

al. 2008). Therefore, it is worthwhile to find a promising sustainable approach for simultaneous treatment and conversion of starch wastewater into renewable energy in the form of H_2. Hydrogen energy has been recognized to be environmentally safe and an alternative to fossil fuel (Singh *et al*. 1994). It has triple the energy yield of conventional hydrocarbon fuels per unit mass (Rifkin 2002). In the course of its combustion, hydrogen produces only water without harmful emissions such as CO, CO_2, hydrocarbons, or fine particles (Liu 2008). The dark fermentation process for hydrogen production of starch wastewater has been extensively investigated for its advantageous low operational cost and effectiveness. The rate of hydrogen production using an anaerobic fermentation process depends on several parameters such as pH, substrate concentration, and hydraulic retention time (HRT) (Kumar and Das 2001; Wu *et al*. 2006). The optimum conditions for maximum hydrogen and acetic acid production from starch processing wastewater using a mixed culture of anaerobes have been reported as 0.56 day HRT, pH 5.9, and 36.1 °C (Lee *et al*. 2004). Zhang *et al*. (2003) found that an increase in starch concentration of the influent resulted in a decrease of acetic acid but an increase in butyric acid production. Arooj *et al*. (2008) concluded that only butyrate concentration instead of butyrate to acetate ratio (B/A) governs the trend of hydrogen yield (HY) at short HRTs. Moreover, the study stated that the highest HY of 0.92 mol-H_2/mol-glucose-added was achieved from starch wastewater at a HRT of 12 h. Due to the accumulation of VFAs in the reactors, the pH decreased from 8 to 5, inhibiting the production of methane and enhancing the hydrogen production (Tawfik and Salem 2012). The optimum pH, iron concentration, and nitrogen concentration for hydrogen production from starch wastewater at a substrate concentration of 15 g/L were 7.0 to 8.0, Fe^{2+} 10 mg/L, and NH_4HCO_3 5.64 g/L, respectively (Liu and Shen 2004). Lo *et al*. (2008) found that combining enzymatic hydrolysis, dark fermentation, and photo-fermentation led to a marked improvement of overall H_2 yield (up to 16.1 mmol-H_2/g-COD or 3.09 mol-H_2/mol-glucose) and COD removal efficiency (54.3%), suggesting the potential of using the proposed integrated process for efficient and high-yield biohydrogen production from starch feedstock.

This study presents anaerobic degradation of starch wastewater using an up-flow anaerobic staged reactor (UASR). This reactor is economically feasible, simple, and applicable for hydrogen energy production from wastes (Tawfik *et al*. 2013; Nasr *et al*. 2012). Moreover, the main advantages of this bioreactor are rapid biodegradation, low yields of sludge, excellent process stability, reduced land area required, and easy construction. Therefore, the aim of this investigation is to assess the performance of the UASR for continuous hydrogen production from starch wastewater with emphasis on the COD removal and carbohydrate conversion. Factors affecting on hydrogen production rate were extensively investigated.

EXPERIMENTAL

Seed Microorganisms

The bacterial culture (sludge) used in this study was enriched from the thickener of a sewage treatment plant situated in Alexandria, Egypt. The harvested sludge was allowed to settle for 24 h. The supernatant was withdrawn and the concentrate was boiled at 90 ºC for 30 min to inhibit the bioactivity of hydrogen consumers (Hafez *et al*. 2010). The organisms responsible for hydrogen production were dominated by *Clostridium*

pasteurianum, which is resistant to high temperatures. The pH and volatile suspended solids (VSS) concentration were 7.4 and 38 g/L, respectively for the inoculums sludge. The UASR was inoculated with 10 L sludge, which represented 40% of the total reactor volume.

Bioreactor and substrate

Figure 1 shows the schematic of the up-flow anaerobic staged reactor (UASR) used in this study. The working volume of the reactor was 28 L. The reactor dimensions were 19.5 cm in length, 19.5 cm in width, and 75 cm in height. The reactor was manufactured from Perspex material with a pyramid shape at the bottom. The reactor was provided by inclined baffles to increase the contact time between the H_2 producing bacteria and the influent substrate. Five sampling ports were distributed along the height of the reactor in order to assess the intermediate products, pH, and the residual COD concentrations. The gas volume was daily measured by a gas meter (Drum type – thermometer – packing fluid). The wastewater was continuously stirred to avoid precipitation of coarse suspended solids, and the temperature was maintained at 35 °C using a controlled thermostatic heater.

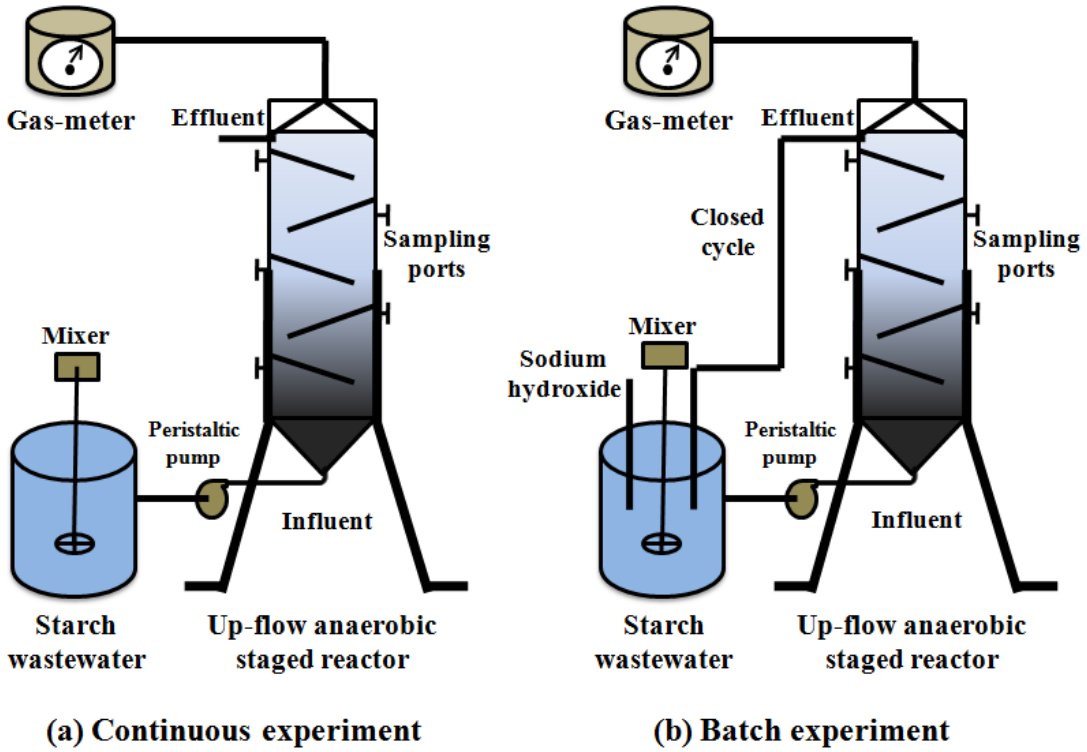

Fig. 1. Schematic diagram of an up-flow anaerobic staged reactor (UASR) used for (a) continuous and (b) batch experiments

Starch wastewater was used as the sole substrate throughout the study. Wastewater was collected from a starch manufacturing company situated in 10th Ramadan city, Egypt. The substrate was diluted with tap water to attain COD concentrations in a range from 5 to 30 g/L. The characteristics of starch wastewater used in the experiments are presented in Table 1.

Table 1. Characteristics of the Starch Processing Wastewater Used in the Experiments

Parameters	COD (g/L)		Carbohydrates (g/L)		Nitrogen (mg/L)		TSS (g/L)	pH
	Total	soluble	Total	soluble	NH_4-N	TKj-N		
Values	5–30	1–6	4.25–25.5	0.85–5.1	20–60	82–148	2.8–18	6.4–6.8
COD: Chemical oxygen demand; NH_4–N: Ammonium nitrogen; TKj-N: Total Kjeldahl nitrogen; TSS: Total suspended solid								

Experimental Procedures

Continuous experiments

Continuous experiments were conducted to investigate the effect of different organic loading rates (OLRs) of 18, 36, 54, 72, and 108 g-COD/L.d and food to microorganism (F/M) ratios of 0.5, 0.9, 1.4, 1.9, and 2.8 (g-COD/g-VSS.d) on the hydrogen production from starch wastewater using UASR.

The bioreactor was operated at a constant hydraulic retention time (HRT) of 6.7 h. Steady-state operating conditions were reached after 3 months when hydrogen gas content and effluent volatile fatty acids (VFAs) concentration were stable (less than 10% variation).

Batch experiments

After operating the reactor continuously for 6 months, the influent wastewater was stopped. The supernatant was withdrawn leaving hydrogen-producing bacteria in the reactor. Afterwards, the reactor was operated without an acclimatization period in a closed cycle for 24 h. Two series of experiments were conducted in the UASR at a temperature of 35 °C.

Series **1** examined the effect of pH (from 4.5 to 8.5 in increments of 1.0) on the treatment of wastewater containing 15 g-starch/L.

Experiments in series **2** investigated hydrogen production at different substrate concentration of 5, 10, 15, 20, and 30 g-starch/L and a constant pH of 6.5.

Calculations

The daily volumetric and specific hydrogen production rate and hydrogen yield were calculated by the following equations (1), (2), and (3),

$$VHPR = V_{H_2} / V \quad (1)$$

where VHPR is the volumetric hydrogen production rate (mL-H_2/L.d), V_{H2} is the daily hydrogen gas production (mL-H_2/d), and V is the volume of inocula (L)

$$SHPR = V_{H_2} / X.V \quad (2)$$

In Eq. 2, SHPR is the specific hydrogen production rate (mL-H_2/g-biomass.d), and X is the biomass concentration in the inocula at steady-state (g-VSS/L),

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

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

Kinetic studies

The modified Gompertz equation was used to fit the cumulative hydrogen production in each batch experiment (Zwietering *et al*. 1990). This was found to be a suitable equation for describing the progress of cumulative hydrogen production obtained from a batch experiment, where H represents the cumulative hydrogen production, P is the hydrogen potential (mL-H_2), R_m is the maximum hydrogen production rate (mL-H_2/h), t is the incubation time (h), and λ is the duration of the lag phase (h). The values of P, R_m, and λ for each batch experiment were determined by best fitting the hydrogen production data for equation (4).

$$H(t) = P.\exp\{-\exp\left(\frac{R_m.e}{P}(\lambda - t) + 1\right)\} \quad (4)$$

Kinetic modeling is an analytical approach to describe the specific parameters affecting HY and HPR. The results of kinetic studies obtained from experimental data can be certainly used for estimating treatment efficiencies of full-scale reactors at the same operational conditions of HRT and OLR (Debik and Coskun 2009). Moreover, reaction rates describe the reactor performance and its design. Therefore, in this study three reaction kinetic models (zero, 1st, and 2nd order) were used to predict organics removal and conversion rates in the reactor (Tchobanoglous *et al*. 2003). In order to determine the kinetic coefficients, a graph was plotted with time (h) on x-axis and S, -ln(S/S_o), and 1/S on the y-axis for zero, 1st, and 2nd order reaction, respectively.

Analytical Methods

Chemical oxygen demand (COD), ammonium nitrogen (NH_4–N), total Kjehldal nitrogen (TKj-N), and volatile suspended solids (VSS) were analyzed in the influent and the effluent twice a week. All analyses were carried out according to APHA (1998). Soluble components were determined by filter paper (0.45 μm-Whatman, 7141-104, Japan).

The carbohydrate was measured according to the phenol–sulfuric acid method, using glucose as the standard. Analysis of VFAs in terms of acetate (HAc) and butyrate (HBu) were performed on a Shimadzu HPLC system (Kyoto, Japan). The chromatographic system consisted of: degasser (20A5), pump (LC-20AT), column oven (CTO-20A), and prominences Diode Array Detector (SPD-M20A). The biogas constituents (H_2, CO_2, and CH_4) were analyzed by gas chromatogram (GC, Agilent 4890D) with a thermal conductivity detector (TCD) and a 2.0 m stainless column packed with Porapak TDS201 (60/80 mesh).

RESULTS AND DISCUSSION

Results of Continuous Experiments

Effect of organic loading rate (OLR)

Figure 2 shows the effect of organic loading rate (OLR) on the hydrogen yield (HY). A HY of 1.88 mol-H_2/mol-glucose (11.5 mmol-H_2/g-starch) was almost constant at increasing the OLR from 18 to 54 g-COD/L.d. To determine the optimum OLR that maximizes HY, the OLR was further increased from 72 to 108 g-COD/L.d. The average HY fell to 1.34 and 0.83 mol-H_2/mol-glucose (8.21 and 5.11 mmol-H_2/g-starch) for 72 and 108 g-COD/L.d, respectively. Thus, increasing the OLR up to 72 g-COD/L.d is negatively affecting the HY. This can be due to accumulation of volatile fatty acids at higher OLRs. A study by Akutsu *et al*. (2009) showed a lower HY (1.7 mol-H_2/mol-glucose) from starch-wastewater with bio-granules. However, Cakır *et al*. (2010) found a higher HY of 2.4 mol-H_2/mol-glucose from hydrolyzed wheat straw. The maximum HY of 260 mL-H_2/g-starch registered here is greater than the results obtained by Wei *et al*. (2010), who achieved 186 mL-H_2/g-starch at 37 ^{o}C, pH 6.5, and a substrate concentration of 5 g-starch/L. Nevertheless, Sen and Suttar (2012) achieved a higher HY of 323.4 mL-H_2/g-starch in two-step processes consisting of hydrolysis and dark fermentation. This indicates that, introducing of the hydrolysis step prior to dark fermentation of starch wastewater would improve the hydrogen yield.

Likewise, a maximum volumetric hydrogen production rate (HPR) of 5.5 L-H_2/L.d was achieved at loading rate not exceeding 54 g-COD/L.d. These results are higher than those obtained by Guo *et al*. (2008), who obtained 1.64 L-H_2/L.d at an OLR of 1.0 g-starch/L.d, pH of 4.42, and HRT of 4 h in an expanded granular sludge bed reactor, but lower than those recorded (12.48 L-H_2/L.d) by Chen *et al*. (2009) at a HRT of 12 h using starch hydrolysate as a substrate.

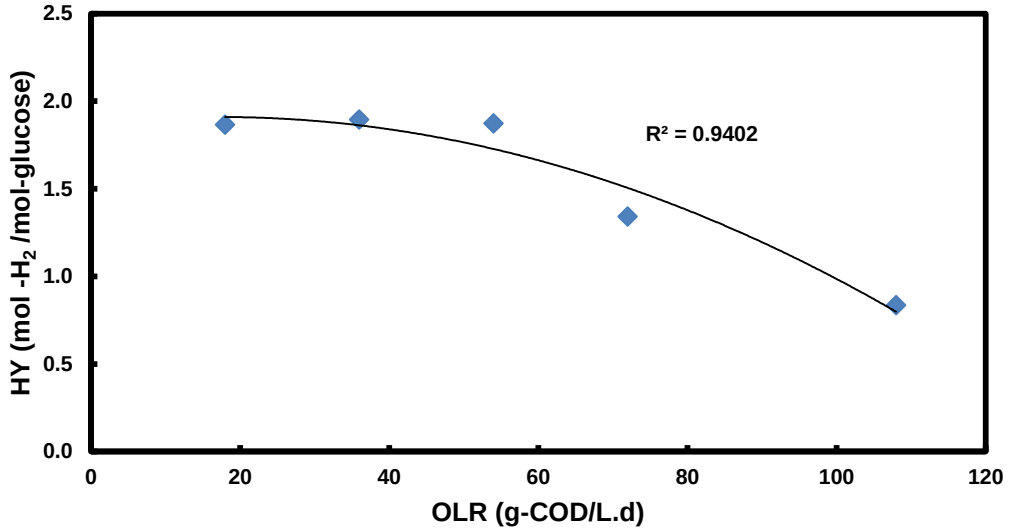

Fig. 2. Steady-state hydrogen yields (HY) from starch processing wastewater at different organic loading rates (OLRs)

The acetate and butyrate pathways were deduced by using the following stoichiometric equations,

$$C_6H_{12}O_6+2H_2O\rightarrow 2CH_3COOH+2CO_2+4H_2, \quad (5)$$

$$C_6H_{12}O_6\rightarrow CH_3CH_2CH_2COOH+2CO_2+2H_2, \quad (6)$$

where production of 1 mol of acetate or butyrate is accompanied by the production of 2 mol of hydrogen.

The results in Table 2 show that, at an OLR of 18 g-COD/L.d, 64% and 36% of the hydrogen was produced through the acetate and butyrate pathways, respectively. The acetate (HAc) concentrations increased in the treated effluent from 860 to 1480 mg/L, while the butyrate varied from 600 to 1110 mg/L, at increasing the OLR from 36 to 54 g-COD/L.d respectively. Acetate (HAc) and butyrate (HBu) pathways contributed 68 and 32% of the hydrogen production at OLR of 36 g-COD/L.d, and 66 and 34% with OLR of 54 g-COD/L.d, respectively. Furthermore, in OLR of 72 and 108 g-COD/L.d, the acetate concentration significantly dropped to 780 and 530 mg/L, respectively, and both acetate and butyrate pathways equally contributed to hydrogen production at 50% each. This indicates that the generation of volatile fatty acids (VFAs) is OLR dependent. Likely, Lee *et al*. (2004) found that acetic acid concentration was increased up to 1681 ± 49 mg/L in the treated effluent of starch wastewater using a mixed culture of anaerobes at HRT of 0.56 d.

Conversion of starch wastewater to hydrogen gas by dark-fermentation process occurs in two steps. The first step is the enzymatic hydrolysis of starch containing wastewater to glucose and maltose. The second reaction is the fermentation of glucose into volatile fatty acids (VFAs), H_2, and CO_2 by *Clostridium* bacteria. Since the theoretical hydrogen yield (HY) from glucose with acetate formation of 4 mol-H_2/mol-glucose is twice that of butyrate formation, previous studies indicate that the HY increases with the molar ratio of HAc/HBu (Wang *et al*. 2008). The steady-state average molar ratios of HAc/HBu were 1.2, 1.43, and 1.33 for OLRs of 18, 36, and 54 g-COD/L.d, respectively, but dropped to 0.68 at OLRs of 72 and 108 g-COD/L.d. This certainly confirmed a peak (HY) of 1.89 mol-H_2/mol-glucose at the highest HAc/HBu ratio of 1.43. Maximum HY of 0.92 mol-H_2/mol-glucose-added was observed by Arooj *et al*. (2008) in an anaerobic continuous stirred-tank reactor (CSTR) using corn starch as a substrate at 12 h HRT with HBu/HAc ratio of 4.3. These results suggest the superiority of UASR for HY from starch wastewater at a short HRT of 6.7 h, as compared to CSTR.

Effect of food to microorganisms (F/M) ratio

The data illustrated in Fig. 3 show the effect of F/M ratio on the hydrogen production rate (HPR) from starch wastewater using UASR. The results revealed that an increase in the volumetric HPR from 75 to 229 mL-H_2/L.h was associated with an increase in the F/M from 0.5 to 1.4 g-COD/g-VSS.d. During the same range of F/M, specific HPR (computed from dividing the volumetric HPR by the biomass 38 g-VSS/L) increased from 47 to 145 mL-H_2/g-VSS.d. Further increase in F/M ratio up to a value of 2.8 g-COD/g-VSS.d not only decreased the volumetric HPR to 117 mL-H_2/L.h, but also reduced the specific HPR up to 74 mL-H_2/g-VSS.d. Those results are lower than the 388 mL-H_2/g-VSS.d obtained by Sreethawong *et al*. (2010) at a COD loading rate of 30 g/L.d, from cassava starch wastewater using an anaerobic sequencing batch reactor (ASBR). However, the maximum volumetric HPR of 229 mL-H_2/L.h, is higher as compared to the

value of 144.5 mL-H_2/L.h registered by Sen and Suttar (2012), who used enriched mixed cultures for fermentative hydrogen production from starch-processing wastewater.

The bioreactor operated at an F/M ratio of 1.4 g-COD/g-VSS.d provided the highest cell yield of 303 mg-VSS/g-starch. The high cell yield may lead to more electron flow toward the biomass (Lee *et al*. 2008). This might explain why an F/M ratio of 1.4 g-COD/g-VSS.d had a higher HPR of 145 mL-H_2/g-VSS.d (corresponding to 229 mL-H_2/L.h), but a lower HY of 1.87 mol-H_2/mol-glucose when compared to an F/M ratio of 0.9 g-COD/g-VSS.d (1.89 mol-H_2/mol-glucose). It was also found that an F/M ratio of 0.5-1.4 g-COD/g-VSS.d attained better substrate utilization efficiency (44-46.7%) and cell yield (256-303 mg-VSS/g-starch) than that having an F/M ratio of 1.9-2.8 g-COD/g-VSS.d (40-26.7%, and 235-118 mg-VSS/g-starch, respectively), suggesting that the F/M ratio range (0.5 to 1.4 g-COD/g-VSS.d) was a better condition for the cells to utilize starch for growth.

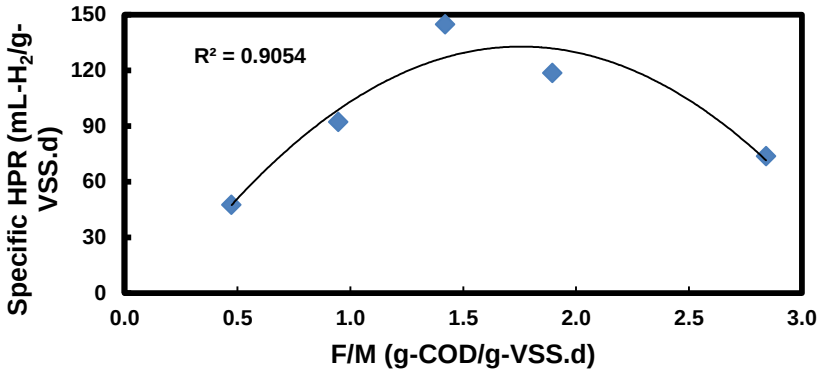

Fig. 3. Steady-state specific biomass hydrogen production rates from starch processing wastewater at different F/M ratios

The substrate balance model developed by Borja *et al*. (2002) defines the total chemical oxygen demand (TCOD) balance of the reactor based on two hypotheses: (i) the anaerobic reactor is operated under steady state at all the OLRs applied and (ii) the coarse suspended solids in the feeding are readily biodegradable and the volatile suspended solids (VSS) in the effluent corresponds to the biomass generated (Wang *et al*. 2009).

Table 2. Metabolites Products and COD Mass Balance for UASR Fed with Starch Processing Wastewater

Parameters	Organic loading rate (OLR)				
	18 g-COD/L.d	36 g-COD/L.d	54 g-COD/L.d	72 g-COD/L.d	108 g-COD/L.d
F/M (g-COD/g-VSS.d)	0.5	0.9	1.4	1.9	2.8
Cell yield (mg-VSS/g-starch)	256	267	303	235	118
Acetate (mg/L)	420	860	1480	780	530
Butyrate (mg/L)	350	600	1100	1150	790
VSS-out (g-COD/d)[a]	25.6	51.1	92	81.8	40.9
sCOD-out (g/d)	97.2	201.6	288	432	792
Hydrogen yield (g-COD/d)[b]	12.96	25.2	39.6	32.4	20.2
COD balance (%)[c]	75	77	78	76	79

[a]Based on 1.42 g-COD/g-VSS; [b]Based on 8 g-COD/g-H_2; [c]COD balance (%) = (VSS_{out} + H_2 + $sCOD_{out}$)/$TCOD_{in}$

The COD mass balance for the UASR (Table 2) was computed considering all the metabolites products, the hydrogen gas produced, and the equivalent COD for the biomass produced. Due to neglecting the fraction of $TCOD_{in}$ consumed for cell maintenance, the COD mass balance was in the range of 75 to 79%.

Results of Batch Experiments

Effect of initial pH

Figure 4 shows the time course of cumulative hydrogen production from fermentation of starch wastewater at various initial cultivation pH values. The results showed that hydrogen production is strongly pH-dependent. Initial pH ranging from 5.5 to 6.5 was found to be favorable for hydrogen production. At pH values of 7.5 to 8.5, lower levels of hydrogen production were generated, which suggests that those environments are not favorable for hydrogen production. It has previously been reported that pH 5 and 6.5 are two optimal ranges favorable to fermentative hydrogen production from hexose (Fang *et al*. 2002 and Lay and Noike 1999).

Table 3 summarizes the kinetic parameters, plus the maximum specific HPR at pH values ranging from 4.5 to 8.5. The results showed that the initial pH significantly affected all of the kinetic parameters. The lag-phase time decreased with pH from 5.5 to 7.5, and then increased to 4.5 h at pH 8.5. The short lag-phase in this investigation was mainly due to using the UASR reactor, which was operated for long enough periods (6 months) for acclimatization of H_2-producing bacteria prior starting batch experiments. Results indicated that no hydrogen was produced at pH 4.5, which implies that hydrogen production from starch wastewater was inhibited at low pH. This is in agreement with previous results (Lee *et al*. 2002 and Zhang *et al*. 2003). Initial pH values ranging from 5.5 to 6.5 were found to be effective for hydrogen production (1435 to 1420 mL-H_2) from starch wastewater. At pH values of 7.5 to 8.5, lower levels of hydrogen production (615 to 550 mL-H_2, respectively) were generated, which suggests that those environments are not preferable for hydrogen production from starch wastewater.

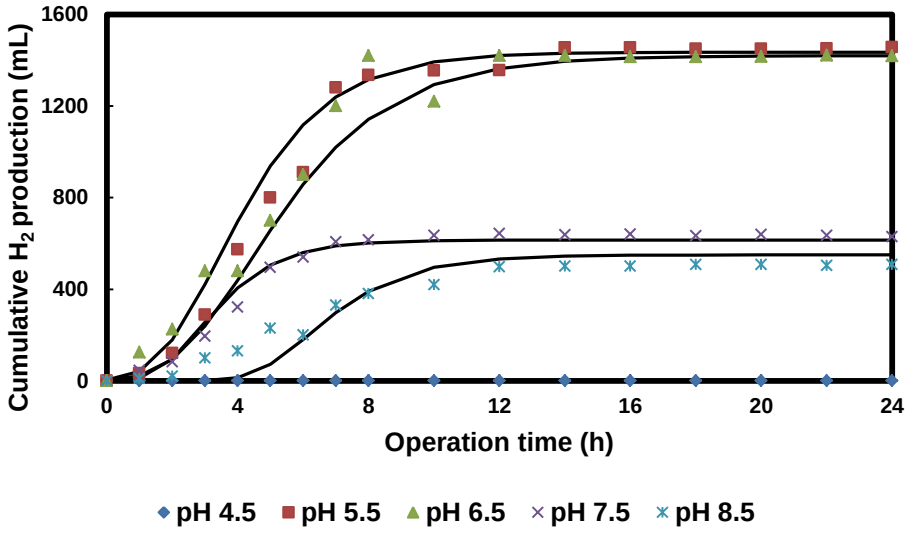

Fig. 4. Hourly variations of accumulative hydrogen production at various initial cultivation pH values (substrate: 15 g-starch/L)

The R_m value varied with pH and peaked at pH 5.5 (280 mL-H_2/h) as shown in Table 3. The increment of R_m value at pH 5.5 relative to that at pH 6.5 was 27%. Maximum specific HPR of 17.68 mL-H_2/g-VSS.d is lower than that obtained by Liu *et al*. (2004), who observed 146 mL-H_2/g-VSS.d at pH value of 7.0–8.0. Additionally, a higher specific HPR of 365 mL-H_2/g-VSS.d was achieved by Zhang *et al*. (2003) at pH=7. Such a discrepancy could be due to many factors, including differences in microbial population and mixing of culture medium (Lamed *et al*. 1988).

Table 3. Modified Gompertz Equation Parameters for Various Initial Cultivation pH Values at an Initial Substrate Concentration of 15 g-Starch/L

pH initial	P (mL-H_2)	R_m (mL-H_2/h)	Volumetric HPR (mL-H_2/L.d)	Specific HPR (mL-H_2/g-VSS.d)	λ (h)	R^2
4.5	0	0	0	0	--	--
5.5	1435	280	672	17.68	1.5	0.983
6.5	1420	220	528	13.89	2	0.972
7.5	615	170	408	10.74	1.5	0.984
8.5	550	120	288	7.58	4.5	0.957

Effect of substrate concentration

Figure 5 depicts the hourly variation of accumulated hydrogen production at various starch concentrations. Table 4 summarizes the results of initial starch concentration experiments. Hydrogen production did not increase incrementally with substrate concentration.

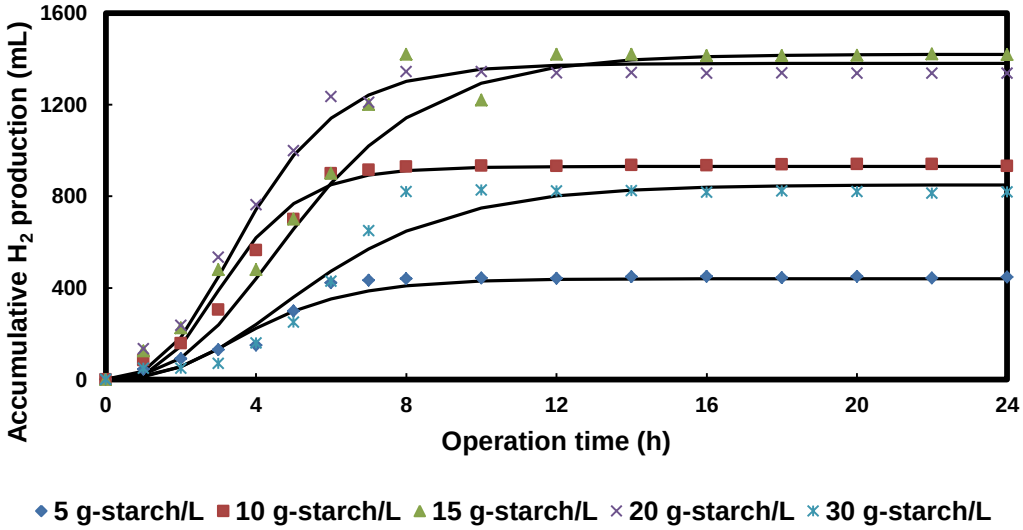

Fig. 5. Hourly variations of accumulative hydrogen production at various initial starch concentrations (pH: 6.5)

The initial starch concentration affected hydrogen production with 15 g-starch/L (corresponding to F/M ratio of 0.46 g-COD/g-VSS) having the maximum *P* value of 1420 mL-H_2. However, the R_m value varied with starch concentration and peaked at 20 g-starch/L (300 mL-H_2/h). Substrate concentrations of 5 to 10 g-starch/L provided a low hydrogen production, as shown in Fig. 5. Moreover, the results indicated that, when starch concentrations were exceeding 20 g-starch/L, hydrogen production decreased. This

suggests that hydrogen production is strongly affected by the substrate concentration via the bacterial metabolism, the microbial community structure, and the sludge loading rate (Akutsu *et al*. 2009). Those results are higher than a study of Wang *et al*. (2007), who obtained (43.1 mL-H_2/h) from hydrolyzed starch by anaerobic mixed micro-flora with phosphate-buffered medium. Similarly, Yang and Shen (2006) reported that a high starch concentration of 20 to 40 g/L had no remarkable effect on hydrogen production, and obtained a maximum P value of 260.5 mL-H_2 at a starch concentration of 20 g/L. Maximum volumetric HPR of 720 mL-H_2/L.d is lower than those obtained by Thanwised *et al*. (2012) who showed 883 mL-H_2/L.d at 6 h HRT from tapioca starch using anaerobic mixed cultures in an anaerobic baffled reactor.

Once again, this discrepancy with previous investigations might be due to the different microbial communities resulting from differences in the inocula, in the various pretreatments methods used, in the start-up period of the inoculums sludge, and in substrates and/or the reactor operational conditions in terms of HRT and OLR.

Table 4. Modified Gompertz Equation Parameters for Various Initial Starch Concentrations at an Initial pH of 6.5

Starch (g/L)	P (mL-H_2)	R_m (mL-H_2/h)	Volumetric HPR (mL-H_2/L.d)	Specific HPR (mL-H_2/g-VSS.d)	λ (h)	R^2
5	440	90	216	5.68	1.5	0.969
10	930	260	624	16.42	1.5	0.987
15	1420	220	528	13.89	2	0.972
20	1380	300	720	18.95	1.5	0.993
30	850	120	288	7.58	2	0.964

Kinetic studies

Table 5 shows the kinetic parameters and the corresponding R^2 values. According to the simulated results, the 2nd order kinetic constant, k_2, related to substrate concentrations of 5, 10, 15, 20, and 30 g-starch/L were 0.022, 0.011, 0.007, 0.004, and 0.002, respectively. This indicates that the bioreactor is capable of reducing organics of 5 g-starch/L more than 10, 15, 20, and 30 g-starch/L. This phenomenon needs further research for examining the suitability of 2nd order kinetics to assess HPR.

Table 5. Results of Kinetics Model for Batch Experiments at Various Starch Concentrations

Kinetic model	Parameter	5 g-starch/L	10 g-starch/L	15 g-starch/L	20 g-starch/L	30 g-starch/L
Zero order	k_0	0.306	0.629	0.933	1.089	1.53
	R^2	0.915	0.923	0.907	0.939	0.945
1st order	k_1	0.081	0.084	0.082	0.07	0.064
	R^2	0.944	0.944	0.922	0.96	0.967
2nd order	k_2	0.022	0.011	0.007	0.004	0.002
	R^2	0.964	0.96	0.933	0.975	0.982

Function ode45 in MATLAB was applied to simulate the ordinary differential equations of the biochemical conversion processes. Model validation was corroborated when plotting the experimental and modeled data, resulting in the correlation regression (R^2 value). The equations obtained confirmed that a second order model for substrate kinetics fit adequately with the experimental results obtained (R^2>0.97) (data are shown

in the Appendix). Consequently, the 2nd order can simulate the substrate removal in the bioreactor more efficiently than that of zero and 1st orders.

CONCLUSIONS

1. Biohydrogen was successfully produced from starch wastewater via an up-flow anaerobic staged reactor (UASR).
2. Hydrogen yield (HY), hydrogen production rate (HPR), and specific HPR (SHPR) peaked at OLR of 54 g-COD/L.d and F/M of 1.4 g-COD/g-VSS.d, resulting 1.87 mol-H_2/mol-glucose, 246 mmol-H_2/L.d, and 145 mL-H_2/g-VSS.d, respectively.
3. The initial cultivation pH and substrate concentration markedly affected hydrogen-producing bioactivity.
4. Starch wastewater had an optimal initial cultivation pH of 5.5 with a maximum HPR of 1.25 mmol-H_2/L.h and initial substrate concentration of 20 g-starch/L with a maximum specific HPR of 0.85 mmol-H_2/g-VSS.d.
5. For all substrate concentrations, the 2nd order kinetic was effectively able to track the measured data with $R^2 > 0.97$.

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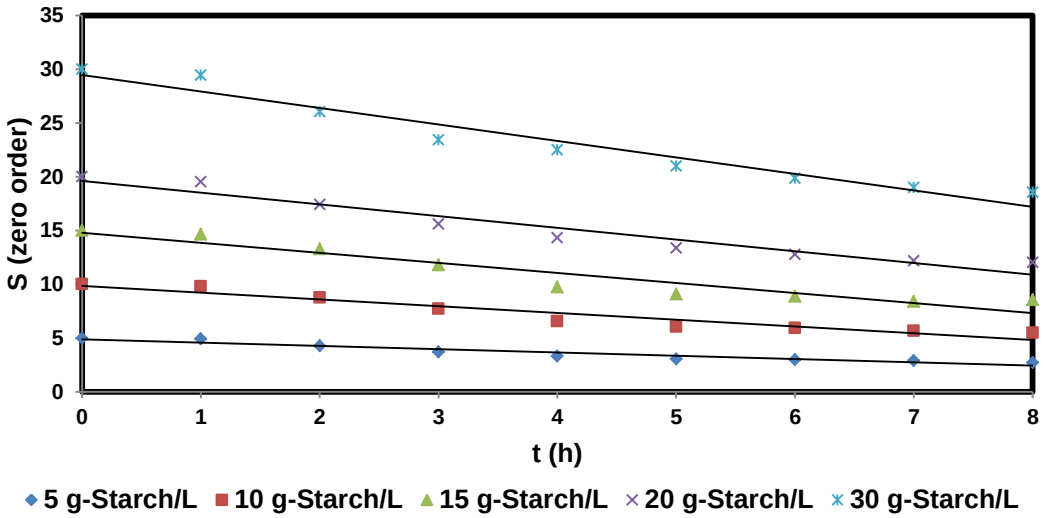
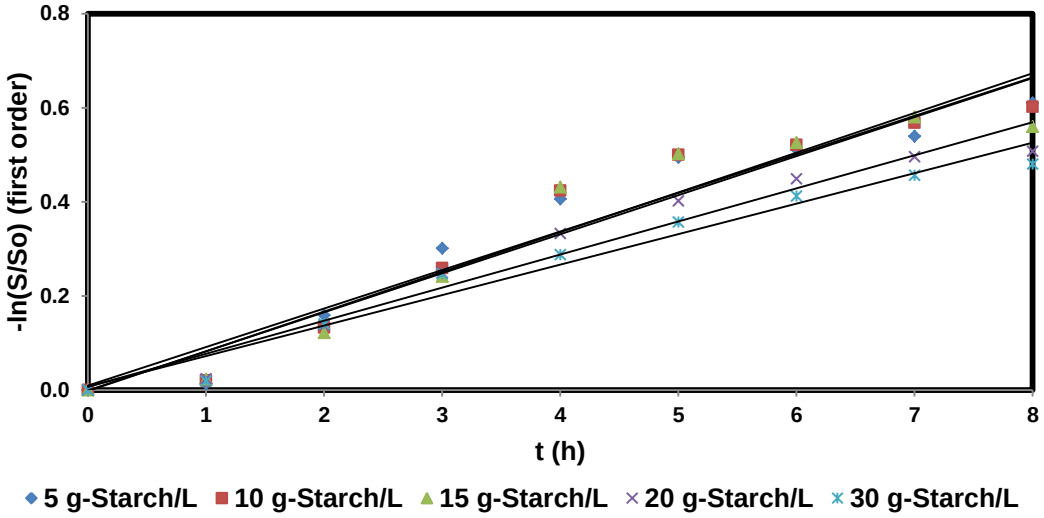
APPENDIX

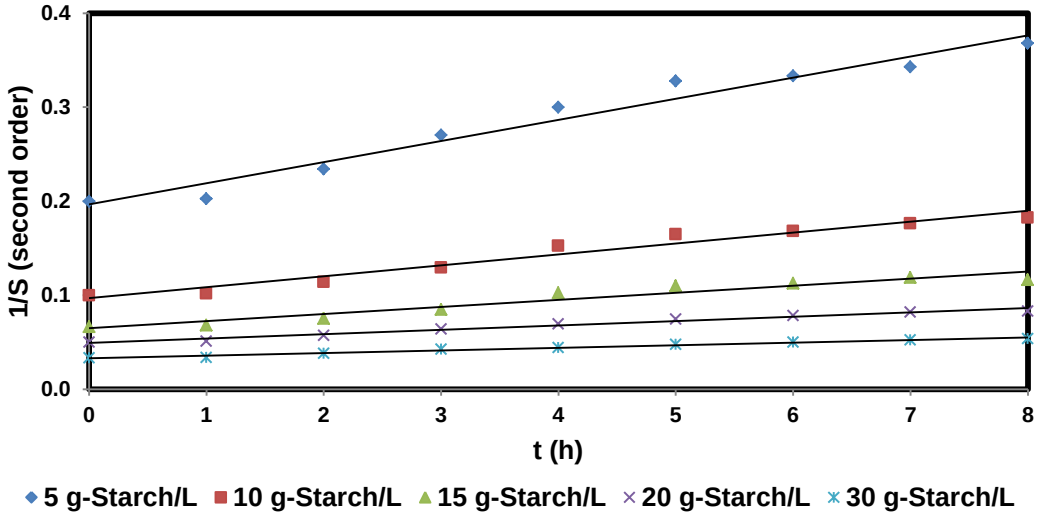
The following curves show the calculations of Zero, 1st and 2nd order at concentrations of 5, 10, 15, 20 and 30 g-starch/L



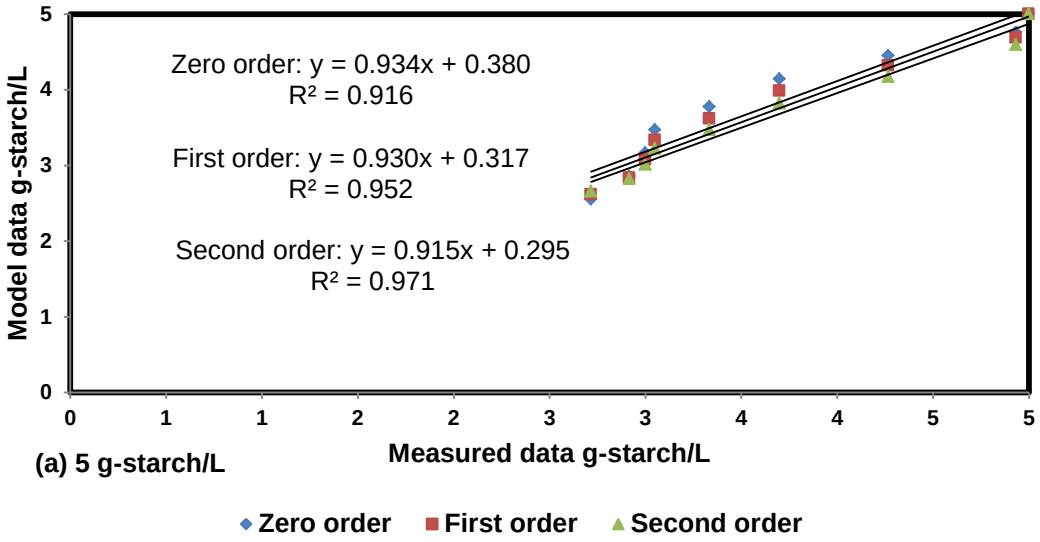
Correlation between time on x-axis and *S* on the y-axis for zero order at 5, 10, 15, 20, and 30 g-starch/L



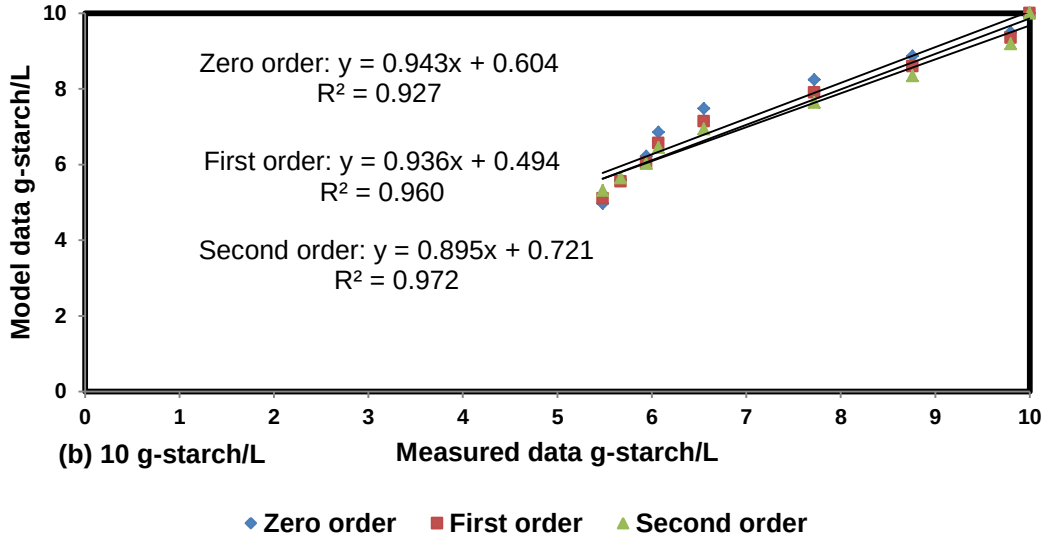
Correlation between time on x-axis and -ln(S/S_o) on the y-axis for first order at 5, 10, 15, 20, and 30 g-starch/L



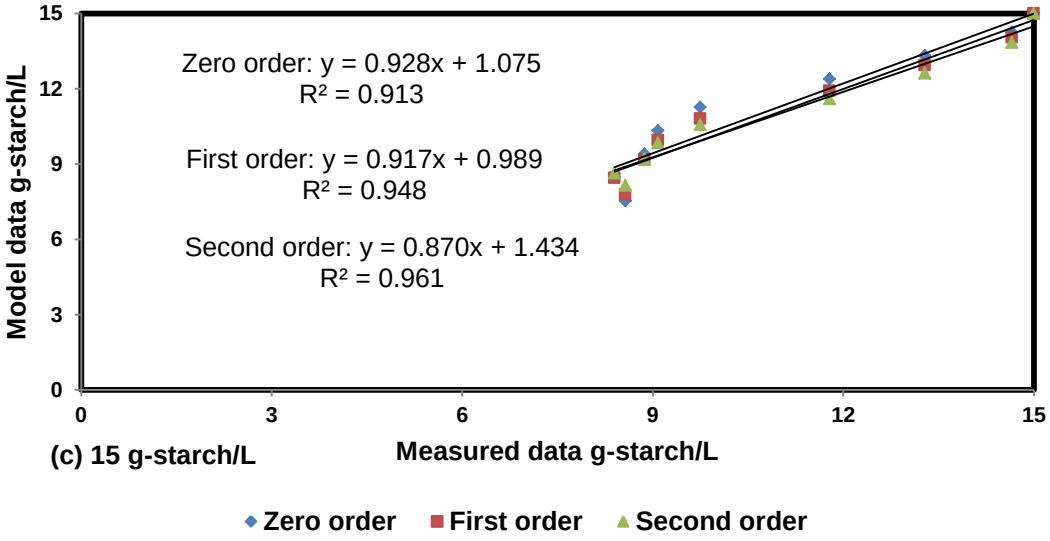
Correlation between time on x-axis and 1/*S* on the y-axis for second order at 5, 10, 15, 20, and 30 g-starch/L



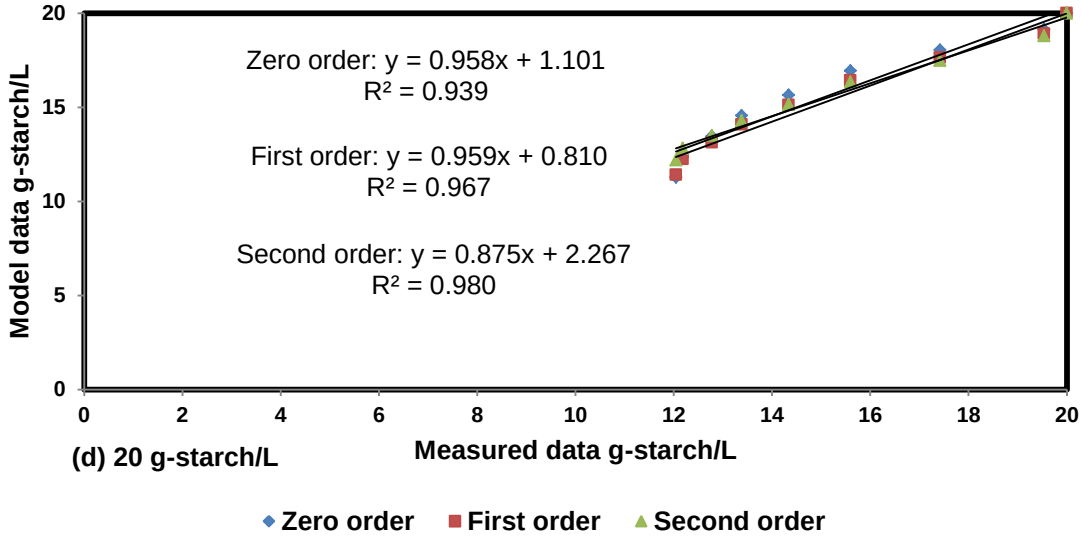
R^2 values between measured and modeled data for zero, 1st and 2nd orders at 5 g-starch/L



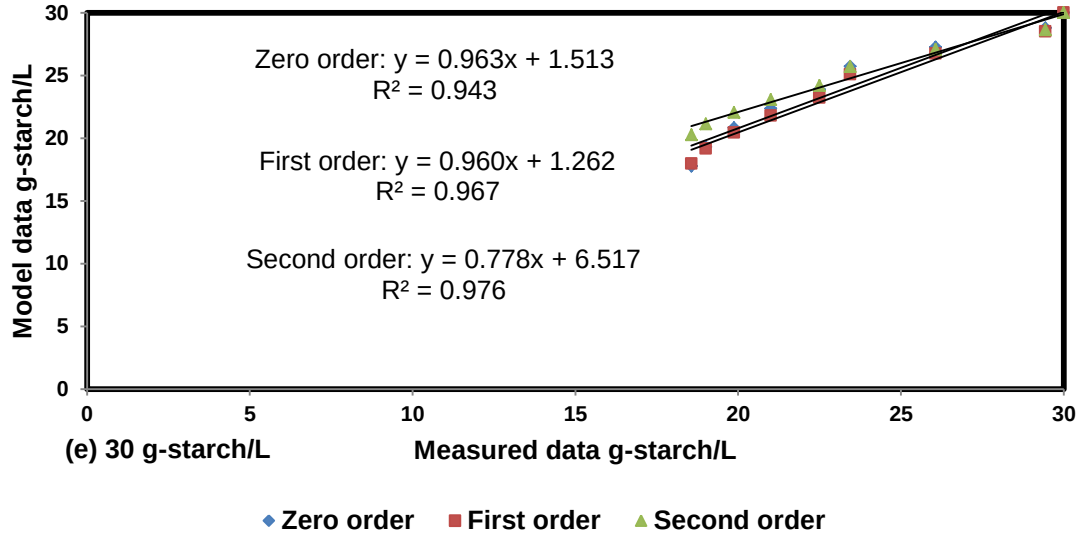
R^2 values between measured and modeled data for zero, 1st and 2nd orders at 10 g-starch/L



R^2 values between measured and modeled data for zero, 1st and 2nd orders at 15 g-starch/L



R^2 values between measured and modeled data for zero, 1st and 2nd orders at 20 g-starch/L



R^2 values between measured and modeled data for zero, 1st and 2nd orders at 30 g-starch/L

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