HYDROGEN PRODUCTION FROM STARCH WASTEWATER USING ANAEROBIC SLUDGE IMMOBILIZED ON MAGHEMITE NANOPARTICLE

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ABSTRACT

In this study, hydrogen production from starch wastewater using anaerobic sludge immobilized on maghemite nanoparticles (γ-Fe2O3) was investigated. Two anaerobic baffled reactors (ABR-1 and ABR-2) were operated at organic loading rate (OLR) of 3.63±0.43 g-COD/L.d and hydraulic retention time (HRT) of 10 h. The ABR-1 was inoculated with 3.5 g-VSS/L preheated sludge at 90°C for 30 minutes. The seeded sludge, in ABR-2, was immobilized on maghemite nanoparticles with a ratio of 25 mg-(γ-Fe2O3)/g-VSS. The maghemite nanoparticles were observed by scanning electron microscopy (SEM). Results obtained indicated that, chemical oxygen demand (COD) removal efficiency and hydrogen production rate were higher in ABR-2 as compared to ABR-1. COD removal efficiency of ABR-1 and ABR-2 were 46.6±8 and 63.4±4.9%, respectively. Moreover, 7±1 and 12±1.7% of the total influent COD, for ABR-1 and ABR-2 respectively, were converted to hydrogen gas. Based on these results, adding maghemite nanoparticle to the inoculated sludge would enhance the fermentation process and produce higher effluent quality.

Keywords: Maghemite nanoparticle, Hydrogen production, Starch wastewater, COD removal

INTRODUCTION

In Egypt, dependence on fossil fuels as a primary energy source leads to global climate change, environmental degradation, and human health problems. Moreover, the recent rise in oil and natural gas prices may drive the Egypt economy toward alternative energy sources. Therefore, it is worthwhile to find an alternative and clean source of energy.

Hydrogen energy has been recognized to be environmentally safe and alternative to fossil fuels (Singh et al. [1]); since it has triple the energy yield of conventional hydrocarbon fuels (Rifkin [2]). Hydrogen produces only water without carbon monoxide, carbon dioxide, hydrocarbons, or fine particles when combusted (Liu [3]). In the chemical industry, hydrogen is used for syntheses of ammonia, alcohols, aldehydes, hydrogen chloride and for the hydrogenation of edible oils, heavy oils or ammonia, for removal of oxygen traces in prevention against metal oxidation and corrosion processes (Nath & Das [4]; Logan [5]; Antoni et al. [6]; Piela & Zelenay, [7]).

Fermentation is an anaerobic type of metabolic process of low energy gain in which organic compounds are degraded in the absence of external electron acceptors and a mixture of oxidized and reduced products are formed. Products, namely organic compounds and gasses (hydrogen and carbon dioxide), determine the type of fermentation.

The theoretical maximum hydrogen yield during clostridial-type fermentation is 4 mole-H2/mole-glucose, when all of the substrate is converted to acetic acid. This gives the maximal possible level of hydrogen yield during dark fermentation. When the glucose is converted to butyrate the hydrogen yield drops to 2 mole-H2/mole-glucose.

Biohydrogen production requires essential micronutrients for the bacterial metabolism during fermentation. Sodium, magnesium, zinc and iron are all important trace metals affecting hydrogen production (Wang and Wan [8]). Among them iron is an important nutrient element to form hydrogenase or other enzymes, which almost all biohydrogen production fundamentally needs. A study by Wang and Wan [9] stated that iron is an important nutrient element to form hydrogenase or other enzymes, which almost all biohydrogen production fundamentally needs. Another study by Frey [10] reported that irons form the metal content at their active site of hydrogenase enzymes that catalyze the reduction of proton to hydrogen. On the other side, high iron concentrations can also inhibit the activity of hydrogen-producing bacteria (Zhang et al. [11], Zhang and Shen [12]; Yang and Shen [13]). The reason is probably that the high initial concentration of iron promoted the start-up of hydrogen production, but the excess soluble iron was harmful to the mixed microbe, and resulted in the decrease of hydrogen production rate.

Nanotechnology is a promising technology that deals with nano-meter sized objects. They are found to enhance the reaction because of their high surface area. They are capable of giving uniquely physical and chemical properties and are gaining importance in areas such as biomedical, optics, mechanics, catalysis and energy science (Mody et al. [14]; Mohanraj and Chen [15]; Salata [16]). However, in the case of fermentative hydrogen production, Zhang, and Shen [17] found that gold nanoparticles could remarkably improve the bioactivity of hydrogen-producing microbes and the enhancement effect strongly depended on the size of gold particles. Moreover, Han et al. [18] studied the role of hematite nanoparticles concentration and initial pH value on hydrogen production of sucrose-fed anaerobic mixed bacteria. The results showed that addition of hematite nanoparticles could remarkably improve the hydrogen production, modify the bacteria growth as well as their metabolites distribution. Mullai et al. [19] studied the optimisation and enhancement of biohydrogen production using nickel nanoparticles. It was found that the maximum cumulative biohydrogen production of 4400 mL and biohydrogen yield of 2.54 mol-H2/mol-glucose was achieved at optimum conditions, initial glucose concentration of 14.01 g/L at initial pH of 5.61 and nickel nanoparticles concentration of 5.67 mg/L. Mu et al. [20] investigated a long-term effect of zinc oxide nanoparticles on waste activated sludge anaerobic digestion, and found that impact of ZnO nanoparticles on methane production was dosage dependant. Yang et al. [21] studied the potential nanosilver impact on anaerobic digestion at moderate silver concentrations.

The main objective of this study is to assess the effect of maghemite nanoparticles on hydrogen production from starch wastewater anaerobic baffled reactor (ABR) inoculated with thermally pre-treated sludge. The size and morphology of the maghemite nanoparticles, and also the surfactant distribution on the surface of the nanoparticles were investigated with the scanning electron microscopy (SEM). Moreover, the SEM analysis has been applied in to investigate the adsorption of maghemite nanoparticles to sludge.

MATERIALS AND METHODS

Mesophillic anaerobic baffled reactors (ABRs) at a pilot scales

Continuous experiments were carried out in a two identical mesophillic anaerobic baffled reactors (ABR-1 and ABR-2) (Figure 1). Those reactors was designed and manufactured from Perspex material. The ABRs are rectangular shape consisting of five compartments of equal volume. Each reactor is provided with baffles to increase contact time between the hydrogen producing bacteria and the substrate, with total volume of 30 L. The excess sludge were periodically discharged from the reactors and analyzed in terms of total suspended solids (TSS) and volatile suspended solids (VSS).

The released gas was separately collected via porthole at the top of the reactors. During the experimental period, the gas volume was daily measured by a gas meter (drum type gas meter-thermometer-packing fluid).

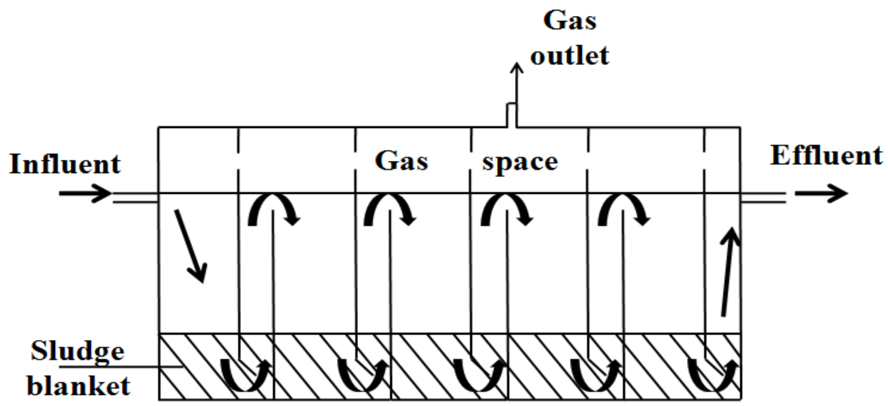

Figure1: Schematic diagram of the anaerobic baffled reactor (ABR) treating starch wastewater industry

The bioreactors were continuously fermented by starch wastewater industry with an organic loading rate (OLR) of 3.6±0.43 g-COD/L.d. Steady-state conditions reached when the product concentrations such as hydrogen gas content, digestion gas volume, and effluent volatile fatty acid (VFA) concentration were stable (less than 10% variation). The bioreactors were operated at a temperature of 30 °C, a pH of 6.4-6.8 and operation HRT of 10 h. Influent COD, carbohydrates, NH4-N, total Kjeldahl nitrogen and total phosphorus were 1510, 1270, 33.5, 50.3 and 14.2 mg/L, respectively.

2.2 Inoculums sludge

A thermally pretreated and digested anaerobic granular sludge was collected, after the thickening process, from a domestic wastewater treatment plant (WWTP). Afterwards, the sludge was allowed to settle for 24 h. The supernatant was withdrawn, and the settled sludge was pre-heated at 90 ºC for 30 minutes to inactivate non spore forming methanogens (Hafez et al. [22]). The preheat-treated sludge produces a more stable and efficient inoculum for hydrogen production. The heated sludge's pH and VSS concentration were 7.4 and 16 g/L respectively. The ABR-1 was inoculated with 3.5 g-VSS/L, while ABR-2 was seeded with sludge immobilized on maghemite nanoparticles with a ratio of 25 mg-(γ-Fe2O3)/g-VSS.

2.3 Immobilization of sludge on maghemite nano particles

Magnetite nano-particles were prepared in two beakers. In beaker-1, 6.2 gm FeCl3.6H2O was added to 4 gm FeCl2.4H2O, and then dissolved in 25 ml deionized water with 1 ml HCl (12 mol/L). In beaker-2, 250 ml of 1.5 mol/L NaOH solution was heated to 80oC. After that, Argon was inserted in beaker-2 for 10 minutes with vigorous stirring. Then drops from beaker-1 were added to the former solution drop-wise under vibration for a few minutes leading to smaller and homogenized particles. A black precipitate was quickly formed, which was allowed to crystallize completely after 30 min.
Hematite nano-particles were prepared by dissolving 3.2 gm FeCl3 in 500 ml distilled water, and then was left in the oven at 100oC for 72 h. After mixing the solutions, the color of the solution slowly changed from light yellow to red.

Maghemite nano particles were washed by distilled water, more than four times, until the pH of the suspension was in range of 7-7.5. The maghemite nanoparticles were observed by scanning electron microscope (SEM) (Figure 2).

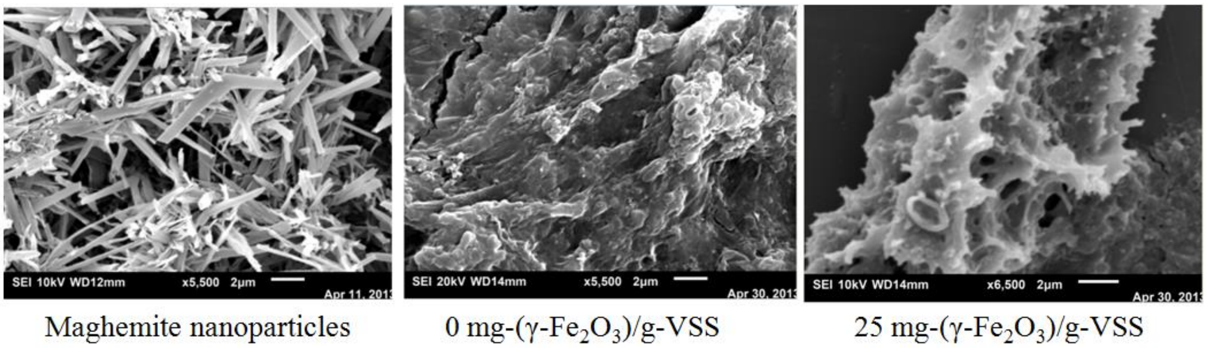

Figure 2: Maghemite nano particles

The SEM analysis has been applied in literature to investigate the adsorption of nanoparticles to sludge (Kiser et al. [23]). As seen in Figure 2, there were large numbers of maghemite nanoparticles on the surface of sludge. The same observations were reported by other researchers when the behavior of NPs in wastewater treatment system was studied (Kiser et al. [24]; Limbach et al. [25]).

2.4 Analytical techniques

Chemical oxygen demand (COD), volatile fatty acids (VFA), ammonium nitrogen (NH4–N), total Kjehldahl Nitrogen (TKj-N), mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were analyzed in the influent and the effluents twice per week. All analysis was carried out according to (APHA [26]). Soluble components were filtered using 0.45 µm filter paper (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 shimadzu HPLC system (Kyoto, Japan). The chromatographic system consists of: degasser (Model 20A5), pump (Model LC-20AT), column oven (Model CTO-20A) and prominences Diode Array Detector (model SPD-M20A). The biogas constituents (H2, CO2 and CH4) were analyzed by gas chromatogram (GC, Agilent 4890D) with thermal conductivity detector (TCD) and a 2.0 m stainless column packed with porapak TDS201 (60/80 mesh). Statistical analysis was determined by using least significant difference values calculated at $p < 0.05$ (Tukey's test).

3 RESULTS AND DISCUSSION

3.1 COD removal efficiency

Higher efficiency of treatment would be achieved by increasing the MLVSS, since the more organisms that are present in the mixed liquor; the faster the COD should be ingested. Accordingly, the rate of COD removal in the bioreactors is related to sludge biomass.

The results presented in Figures 3a and 3b show the effect of adding maghemite nanoparticles on the performance of anaerobic baffled reactor (ABR) for COD removal. ABR-1 and ABR-2 achieved average removal efficiencies of 46.6±8% and 63.4±4.9%, 36.6±7.2% and 46.9±6.7%, and 65.2±14.7% and 93.4±6.6% for COD-total, COD-soluble, and COD-particulate, respectively. Removal efficiency of COD products was higher in ABR-2 due to increasing the VSS by 31.25%. Moreover, due to the high entrapment/adsorption efficiency of the maghemite nanoparticles, ABR-2 can remove more than 93% of the particulate COD within 10 h of contact time.

In order to measure the amount of feed available to a unit of biomass, the COD is divided by the MLVSS. The value obtained is the so-called the F/M ratio or the food/microorganism ratio. F/M ratio was 1.03 and 0.72 g-COD/g-VSS.d for ABR-1 and ABR-2, respectively.

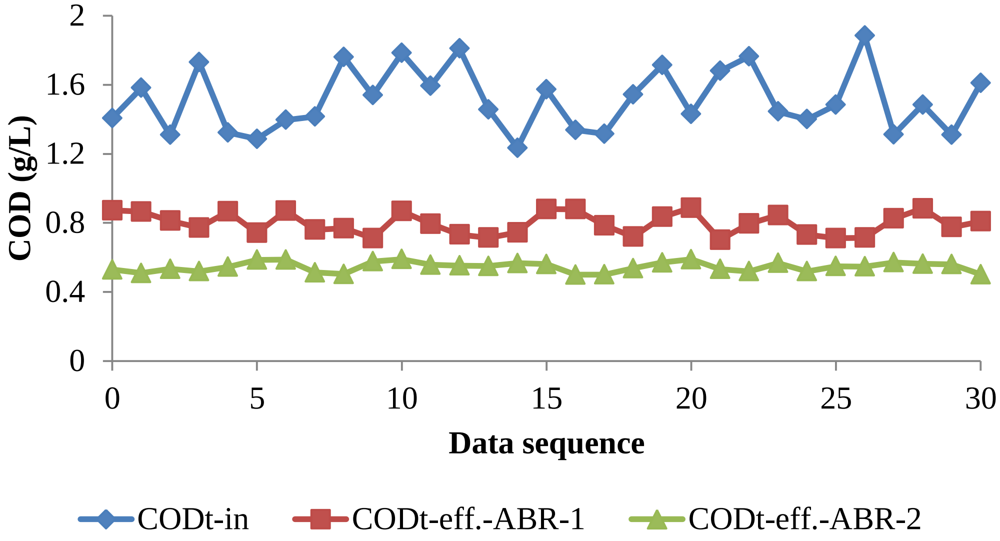

Figure 3a: Diurnal variation of total COD in ABR-1 and ABR-2

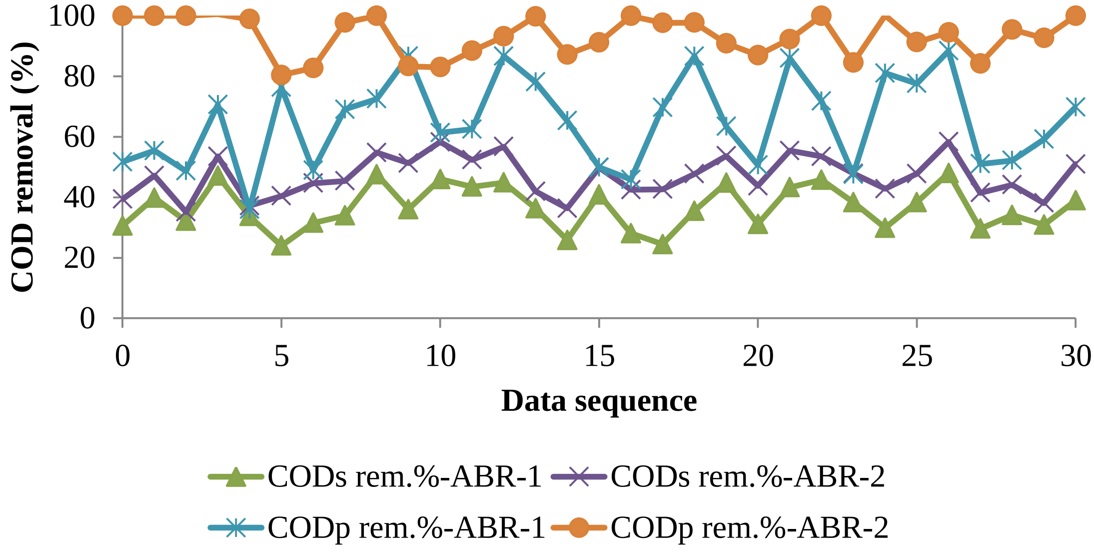

Figure 3b: Effect of maghemite nanoparticles on COD (soluble and particulate) removal efficiencies from starch wastewater industry

3.2 Hydrogen production rate (HPR)

The anaerobic fermentation produced a biogas only containing hydrogen and carbon dioxide, without any detectable methane or hydrogen sulfide, suggesting that methanogens and sulphate-reducing bacteria were absent during the experiments. Figure 4 illustrates the effect of maghemite nanoparticles on hydrogen production during the course of anaerobic fermentation. The results showed that immobilization of the anaerobic sludge on maghemite nanoparticles significantly improved the hydrogen production rate (HPR). The HPR in ABR-1 and ABR-2 were 0.47 and 0.8 mol-H2/d (corresponds to volumetric HPR of 15 and 25 mL-H2/L.h, respectively). Moreover, maghemite nanoparticles achieved hydrogen yield of 2.23 mol-H2/mol-glucose which was 26% higher ABR-1.

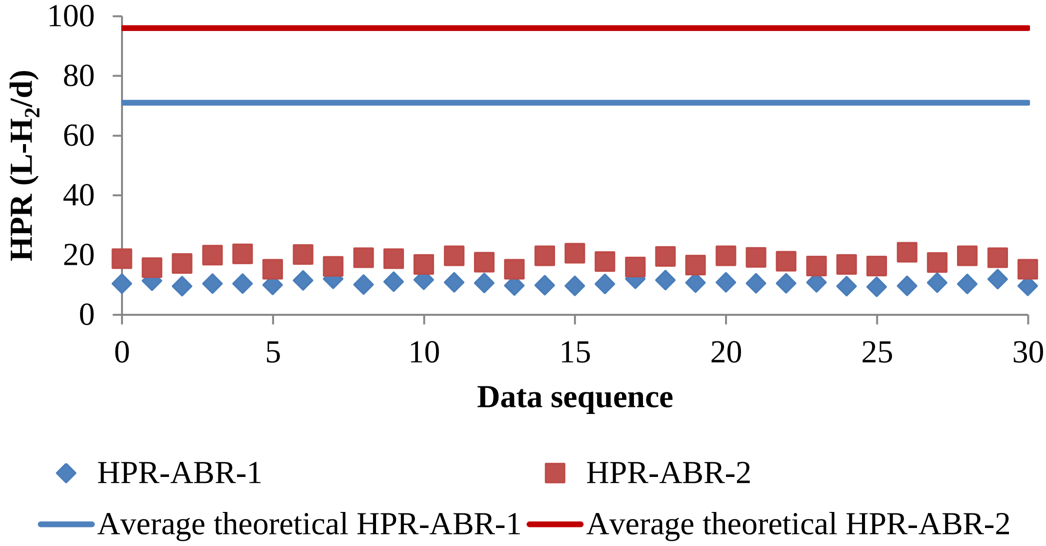

Figure 4: Effect of maghemite nanoparticles on hydrogen production rate

This result was lower than a study by Han et al. [18] who found that the hematite nanoparticles with a concentration of 200 mg/L provided maximum hydrogen yield of 3.21 mol-H2/mol-sucrose, which was 32.64% higher than the blank test. This indicates that the addition of nanoparticles plays a key role to enhance hydrogen production by activating the hydrogenase enzymes inside the reactor. Moreover, Yang and Shen [13] found that the mixed bacteria cell was immobilized on FeCO3 sediment by sorption and enhanced chances of bacteria to meet substrate, thereby improved biohydrogen production. The proper irons meet the need of bacteria growth. The propagation of bacteria resulted in the increase of turbidity.

3.3 Metabolites products and COD balance

Aside from hydrogen and carbon dioxide, the major metabolites produced by the anaerobic culture were solvents and VFAs. In the nanoparticle bioreactor, although hydrogen production was accompanied by acid formation, the pH values were always within a range of 6.4–6.6, due to the buffering capacity of the medium. The acid-forming pathway dominated the metabolic flow; acetic acid (HAc) and butyric acid (HBu) were the major metabolites of the hydrogen-producing bacterial microorganisms.

The results in Figure 5 show that at ABR-1, 63% and 37% of the hydrogen produced were through the acetate and butyrate pathways, respectively. For ABR-2, the steady-state acetate concentration was

260 mg/L while the butyrate was 160 mg/L, with acetate and butyrate pathways contributing 70%, 30% of the hydrogen production.

Since the theoretical hydrogen yield from glucose with acetate formation of 4 mol- H2/mol-glucose is twice that of butyrate formation, previous studies indicated that the hydrogen yield increases with the molar ratio of acetate/butyrate (Wang et al. [9]). The steady-state average molar ratios of acetate/butyrate were 1.17 and 1.63 for ABR-1 and ABR-2, respectively.

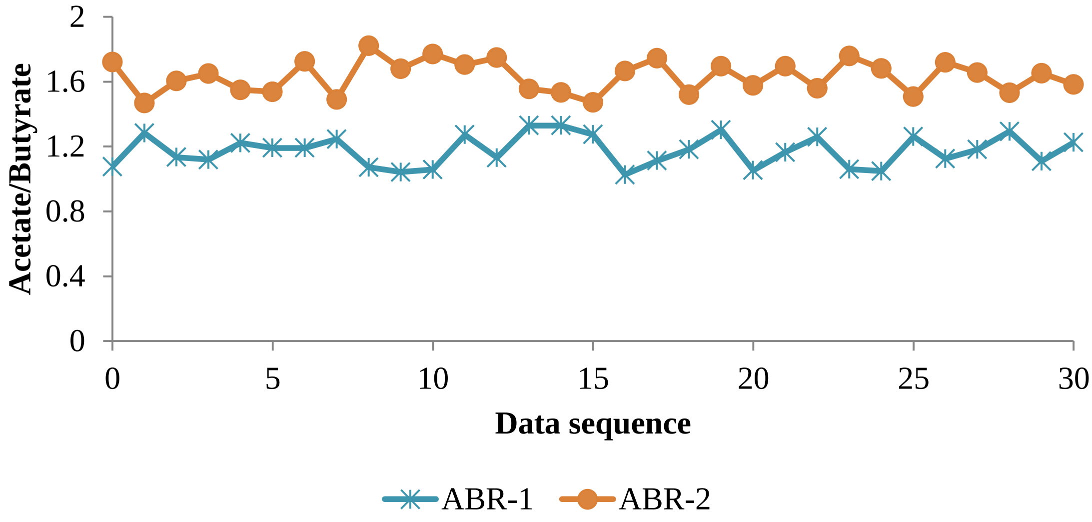

Figure 5: Diurnal variation of Acetate/Butyrate for ABR-1 and ABR-2

The substrate balance model developed by Borja et al. [27] 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 organic loading rates applied; and (ii) the suspended solids in the feeding are readily biodegradable and the volatile suspended solids in the effluent corresponds to the biomass generated (Wang et al. [8]). The COD mass balance for the UASR (Table 1) was computed considering all the metabolites products, the hydrogen gas produced and the equivalent COD for the biomass produced.

Table 1: Total COD mass balance for ABR fed with starch processing wastewater

	ABR-1	**ABR-2**
%H2/TCOD a	7±1	12±1.7
%VSS-out/TCOD b	14±3	9±2
% CODs-eff./TCOD	41±5	34±4
TCOD balance (%) c	62±6	55±4

[a] Based on 8 g-COD/g-H2

[b] Based on 1.42 g-COD/g-VSS

[c] TCOD balance (%) = (VSS-out + H2 + sCOD-eff.)/TCOD-in

The sludge retention time (SRT) is a fundamental design and operating parameter that significantly affects both COD conversion and sludge yield. It is assumed that the effluent VSS had the same SRT (d) as the excess sludge. The results in Table 1 revealed that, the effluent VSS as percentage to TCOD in case of ABR-1(e.g. 14±3%) is higher than those obtained by ABR-2 (e.g. 9±2). This mainly can be due to the high entrapment efficiency of the maghemite nanoparticles that causes agglomeration of

VSS on the particles surface, preventing the sludge from leaving the reactor. Consequently, SRT increased in the nanoparticle bioreactor by 27% contributing to higher COD removal efficiency.

4 CONCLUSIONS

The addition of maghemite nanoparticles to the preheated sludge enhanced the biohydrogen production in mesophillic ABRs fed with starch wastewater industry. Under the operational conditions; initial glucose concentration of 1.41 g/L at initial pH of 6.4-6.8 and maghemite nanoparticles concentration of 25 mg-(γ-Fe2O3)/g-VSS, the experimental hydrogen yield was 2.23 mol-H2/mol-glucose (corresponds to 26% higher than the classic ABR-1). Moreover, addition of maghemite nanoparticles effectively directed the bacterial metabolism to produce more VFAs (mainly in terms of acetic and butyric acids). Additionally, maghemite nanoparticles increased COD-t, COD-s and COD-p removal efficiencies by 36, 29 and 43% respectively. Accordingly, it is recommended to use anaerobic sludge immobilized on maghemite nanoparticle for enhancement of fermentative hydrogen.

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