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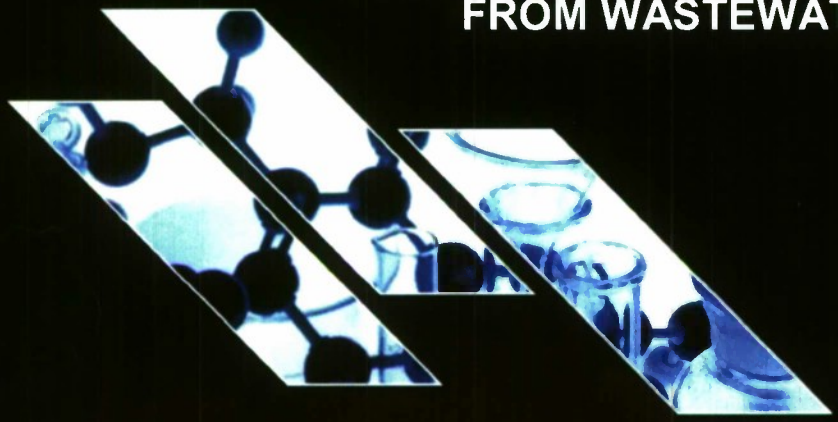
BIOLOGICAL HYDROGEN PRODUCTION: SIMULTANEOUS SACCHARIFICATION AND FERMENTATION WITH NITROGEN AND PHOSPHORUS REMOVAL FROM WASTEWATER EFFLUENT



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14. ABSTRACT

A simple anaerobic biodegradation process using wastewater treatment plant (WWTP) effluent, shredded paper, and a purge of nitrogen gas was used to produce hydrogen and simultaneously capture nitrogen and phosphorus. Two reactor configurations were tested as simultaneous saccharification and fermentation reactors. Using the classic batch reactor provided greater stability of hydrogen production and simplicity of operation, whereas using the sequencing batch reactor provided better nitrogen and phosphorus removal efficiencies. Evaluation of nuclear magnetic resonance analyses showed acetic acid to be the main product from both reactors. Experiments with an argon purge in place of nitrogen and with ammonium chloride spiking suggested that hydrogenase and nitrogenase enzymes contributed similarly to hydrogen production in the cultures. Analysis of a single fermentation showed that hydrogen production occurred relatively early in the course of total organic carbon removal, and that follow-on treatments might extract more energy from the products.

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PREFACE

The work described in this report was authorized under project No. 1WER37. The work was started in May 2010 and completed in May 2011.

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1. INTRODUCTION

1.1 Hydrogen Production

Hydrogen is potentially an ideal fuel because its only oxidation product is water. Fuel cells that use hydrogen to generate electricity are up to three times as efficient as internal combustion engines.[1] However, hydrogen production, primarily from steam reformation of natural gas at 700–1100 °C, is energy intensive and completely dependent on fossil fuel:

$$CH_4 + H_2O \rightarrow CO + 3H_2 \quad (1)$$

Hydrogen can also be produced by electrolysis, splitting water into its component gases (hydrogen and oxygen), although the electrical demand, and therefore cost, is high:

$$2H_2O\,(L) \rightarrow 2H_2\,(g) + O_2\,(g);\ E_0 = +1.229\ V \quad (2)$$

Biological hydrogen production, typically using anaerobic bacteria or photosynthetic algae, occurs catalytically at ambient temperature and pressure. Because hydrogen has little solubility in water (<0.0015 g of H_2 per kilogram of water at 30° C),[2] it quickly accumulates in the headspace of the reactor where it can be easily collected. If biological hydrogen production were developed into a stable and economically viable process, it may be possible to produce useful amounts of hydrogen from renewable or discarded materials.

Bacteria can catalyze the production of hydrogen with either hydrogenase or nitrogenase enzymes.[3] Recent research on hydrogenase enzymes has been reviewed by English et al.[4] The enzyme catalyzes the reversible oxidation of molecular hydrogen, and the reaction can be most simply written as

$$H_2 \leftrightarrow 2H^+ + 2e^- \quad (3)$$

1.2 Nitrogen Fixation

Nitrogenase enzymes catalyze the reduction of atmospheric nitrogen to ammonia and are found only in nitrogen-fixing bacteria. They are typically downregulated by the presence of ammonia, to avoid the energetically expensive fixation of nitrogen when not needed by the cell. Molybdenum-containing nitrogenases, the most common type found, catalyze the production of hydrogen in addition to ammonia at the rate of one mole of H_2 per mole of N_2 fixed:[5]

$$N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16\ P_i \quad (4)$$

Relatively few (perhaps 100) bacteria possess this capability, which is critical in nature because the supply of fixed nitrogen to the biosphere is rate-limiting for biological activity in

most areas of the planet. Both nucleic acids and proteins require nitrogen for their biosynthesis. Bacteria of the *Azotobacter* genus are frequently used as model organisms in fermentation studies.[6] Biological nitrogen fixation provides about 40% of the nitrogen found in the world's soil and water.[5]

Industrially, nitrogen fixation is typically accomplished using the Haber-Bosch process, in which hydrogen is first produced from methane (eq. 1), then ammonia is produced from nitrogen and hydrogen:

$$N_2\,(g) + 3H_2\,(g) \rightleftharpoons 2NH_3\,(g) \qquad (5)$$

Agronomists have calculated that well over one-third of the world's present population is fed by virtue of the Haber-Bosch process.[7] The reaction is of great economic importance given that the world's industrial production of nitrogenous fertilizer increased 27-fold between 1950 and 1990, when it reached 8×10^7 tonnes of nitrogen per year.[8] Currently, 1% of the world's energy supplies are consumed in the industrial fixation of nitrogen through the Haber-Bosch process,[9] leading to a potential confluence of energy and fertilizer crises.

Biological nitrogen fixation provides a catalytic alternative to the commercial fixation of nitrogen, and its broader use could help decouple the price of fertilizer from the price of natural gas. A nitrogen-fixing, hydrogen-producing culture offers the potential to simultaneously produce organic ammonia and hydrogen from renewable materials. Used in conjunction with a relatively carbon-rich material such as paper, it could also be useful for the removal of nitrogen and/or phosphorus from wastewater effluent to prevent eutrophication in receiving waters.

1.3 Biological Nitrogen and Phosphorus Removal

The Chesapeake Bay in the eastern United States is an example of a body of water suffering from a high load of nitrogen and phosphorus. U.S. Executive Order 13508,[10] "Chesapeake Bay Protection and Restoration," dated 12 May 2009, describes action necessary to respond to a pollution crisis affecting the Chesapeake Bay. The order states, "The main contaminants affecting the Bay are nitrogen, phosphorus, and sediment. In 2008, the estimated loads of contaminants from the Bay watershed included 311 million pounds of nitrogen and 19 million pounds of phosphorus. EPA [U.S. Environmental Protection Agency] estimates that in order to achieve water quality standards for the Bay, the nitrogen load must be reduced by 44% and the phosphorus loading cut by 27%." The target date for these goals is 2025. This effort will be administered by a Federal Leadership Committee that includes the Department of Defense.

In this study, we sought to determine the efficiency with which shredded paper could be biologically converted to hydrogen using discharge effluent from the Aberdeen Proving Ground (APG) Edgewood, MD wastewater treatment plant (WWTP) as the medium and sole source of micronutrients and organisms. This plant treats low-strength waste consisting almost totally of human waste with essentially no industrial waste component. It discharges to a tributary of the Chesapeake Bay. Simultaneously, we sought to determine the efficiency with which nitrogen and phosphorus could be removed from the WWTP discharge waters using the same biological process as used to generate hydrogen.

Two reactor configurations were used, including a sequencing batch reactor (SBR) and a classic batch reactor (CBR). Paper, in addition to being carbon-rich, has the added advantage that it can be directly catalyzed to monosaccharides by cellulases without requiring thermochemical pretreatment, as would typically be required with lignocellulosic feedstocks. Therefore, it offers a readily processed and reproducible substrate with which to test various reactor conditions and configurations. Data from these tests may inform similar processes conducted with higher impact feedstocks such as corn stover or switchgrass.

1.4 Batch Reactor Configurations

The SBR is a periodically operated reactor that is commonly used in wastewater treatment operations. It offers the simplicity and control of a batch reactor with the kinetic advantages of a plug flow reactor followed by a continuously stirred tank reactor. Organisms are settled (concentrated) and retained after each cycle. The CBR is a tank that is filled, stirred for the duration of the cycle, and completely drained at the end of the cycle. It typically offers greater simplicity of operation but does not concentrate the organisms between cycles.

2. MATERIALS AND METHODS

2.1 Materials

Post-treatment wastewater effluent was collected from the discharge area at the APG Edgewood, MD WWTP and used as the sole source of organisms, medium, and nutrients (other than paper) in the bioreactors. Specifically, the organisms used were only those naturally occurring in the wastewater. No other inorganic nutrients were added; the experiments utilized only nutrients found naturally in the wastewater effluent, including nitrogen and phosphorus compounds, which were measured and reported for each experiment performed.

The only exogenously added organic nutrient was paper. Skilcraft brand recycled copy paper (national stock no. 7530-01-334-7817; Louisiana Association for the Blind; Shreveport, LA), which contains 30% post-consumer waste, was used.

2.2 Equipment

Bioreactor studies were conducted in New Brunswick BioFlo 110 5 L vessels. Nitrogen (5 mL/min) was sparged through the bioreactor to maintain anaerobicity. Hydrogen detection was accomplished with a HY-OPTIMA 700 in-line process hydrogen monitor (H2scan, Valencia, CA), which was sealed in a separate vessel connected to the reactor headspace. The monitor was factory-calibrated with 0.5 to 100% hydrogen standards traceable to the National Institute of Standards and Technology. Reactor pH was controlled throughout all fermentations by automatic addition of 0.1 N NaOH. Total organic carbon (TOC), ammonia nitrogen, nitrate nitrogen, nitrite nitrogen, and phosphorus analyses were conducted with the respective kits from Hach (Loveland, CO).

2.3 SBR and CBR Methods

The SBR was established using WWTP effluent and shredded paper as described in Section 2, Materials and Methods. The reactor was initially seeded with effluent, which was collected and analyzed in batches, and was periodically fed paper and cellulose (2–4 times per week) as appropriate until the mixed liquor suspended solids (MLSS) levels were greater than 3000 mg/L. Initial batches of wastepaper feed increased the pH of the solution and required adjustment with HCl to pH 5.0. However, as the reactor equilibrated, an effective buffer was established (presumably because of the organic acid products of paper biodegradation) and the pH changed very little upon paper addition. Once the reactor MLSS level was established above 3000 mg/L, paper was added in 20 g batches along with 9 mL of Accellerase 1500 enzyme solution (a kind gift from Genencor International). Conditions were those of simultaneous saccharification and fermentation (SSF, meaning that enzymatic degradation and fermentation were conducted simultaneously in the same vessel). Fermentation of the Accellerase 1500 solution alone (without paper) produced no detectable hydrogen when tested at the maximum concentration used and under optimal conditions determined for hydrogen production.

The CBR was operated on a periodic basis by filling the 5 L reactor with fresh effluent (used within three days of collection), adding 20 g of paper and 9 mL of Accellerase 1500 enzyme (also SSF conditions), reacting for several days until hydrogen production was complete, draining the reactor completely, then repeating the cycle.

Cellulase concentrations were in accordance with the manufacturer's recommendations (0.15 to 0.45 mL enzyme solution per gram of substrate). In our studies, the best results were obtained with a 0.45 mL/g concentration, which yielded almost three times more hydrogen than a 0.15 mL/g concentration. Cultures of shredded paper alone with no enzyme produced very little hydrogen.

2.4 Nuclear Magnetic Resonance (NMR) Methods

Analysis of solution-state reaction products was carried out at 25 °C using Bruker Avance DRX-300 MHz and DRX-500 MHz NMR spectrometers. The DRX-300 MHz spectrometer was equipped with a quadruple nucleus probe (QNP), and the DRX-500 MHz was equipped with a cryogenic triple resonance inverse (TCI) detection CryoProbe with enhanced detection of ^{1}H and ^{13}C. The NMR experiments performed were as follows: ^{1}H zg with and without solvent presaturation pulse, ^{13}C attached proton test (Bruker jmod pulse program), ^{1}H–^{13}C heteronuclear multiple quantum coherence, and ^{13}C–{^{1}H} zgdc.

3. THEORY/CALCULATIONS

The central hypothesis of this work was that it would be possible to run an anaerobic bioreactor using only shredded paper, commercially available cellulase enzymes, and WWTP effluent to simultaneously produce significant amounts of hydrogen while removing nitrogen and phosphorus from the effluent.

Assuming that paper is comprised of 100% cellulose, its molecular formula would be $(C_6H_{10}O_5)_n$, corresponding to 44% carbon by weight:

$$(12.01 \text{ g/mol C} \times 6)/[(12.01 \text{ g/mol C} \times 6) + (1.01 \text{ g/mol H} \times 10) + (16.00 \text{ g/mol O} \times 5)] \times 100 = 44.44\% \text{ C}$$

This would yield 888 lb of carbon per ton of paper:

$$0.4444 \text{ lb C/lb paper} \times 2000 \text{ lb/ton} = 888 \text{ lb C per ton of paper}$$

Assuming a carbon-nitrogen-phosphorus biological demand ratio of 100:10:1, biodegradation of 1 ton of paper could remove 88 lb of nitrogen and 8.8 lb of phosphorus that would otherwise be discharged to receiving waters. As a practical example, a WWTP discharging one million gallons per day of effluent containing 10 mg/L nitrogen (83 lb nitrogen per day) would require 0.85 ton of paper per day to remove about 90% of its nitrogen from the effluent and partition that nitrogen to the solid phase, where it could be separated with typical solids handling equipment and subsequently recycled as fertilizer.

4. RESULTS

4.1 Reactor Setup and Operation

The SBR was initially established as described in Section 2.3 and was operated for a total of 100 days. Hypothetically, using the concentration of organisms resulting from the repeated settling of the reactor could lead to a more efficient process for hydrogen production. However, because biological hydrogen production in mixed cultures is in equilibrium with biological hydrogen consumption (often by methanogens), it was also possible that overall hydrogen yields in the reactor would decrease.

The WWTP effluent that was used as influent to the SBR was analyzed for TOC, ammonia, nitrate, nitrite, and phosphorus concentrations. The SBR effluent was periodically analyzed for MLSS, effluent suspended solids (ESS), TOC, ammonia, nitrate, nitrite, phosphorus, and hydrogen production. Figure 1 shows the MLSS on the basis of individual measurements and calculated as a 10 day moving average. Solids concentrations ranged between about 3000 and 9000 mg/L. Effluent TOC averaged 856 mg/L, and ESS averaged 266 mg/L.

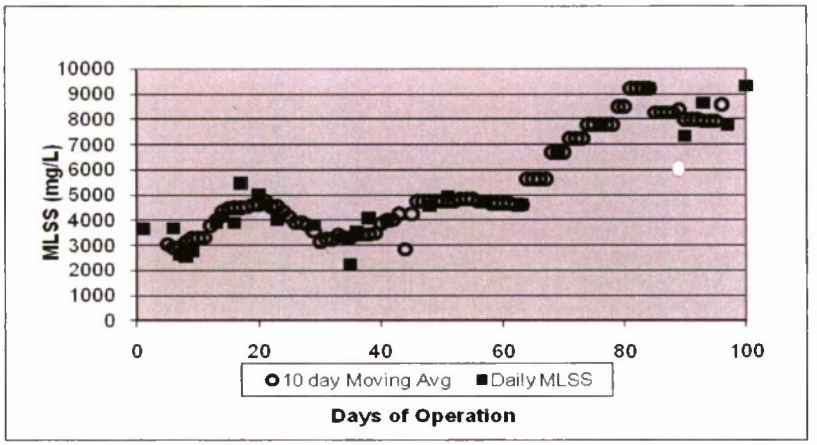

Figure 1. SBR MLSS.

CBR operation was much simpler because it involved no concentration of biomass via settling. The tank was simply filled with WWTP effluent, and paper and enzyme were added. When hydrogen production was complete, the tank was drained and refilled.

4.2 Operational Comparison: SBR and CBR

The reactors were compared with regard to hydrogen production, nitrogen and phosphorus removal, and final products, as detected by NMR. Initial hydrogen production levels were similar in the two reactors. In the SBR, hydrogen production levels declined over time as MLSS increased. In the CBR, however, initial levels were maintained over the course of repeated batch operations (Figure 2).

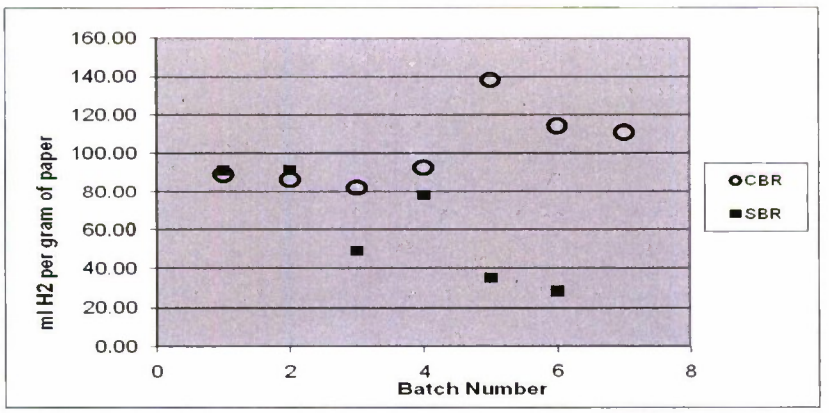

Figure 2. Stability of hydrogen production: SBR vs. CBR.

Total nitrogen (nitrate, nitrite, and ammonia combined) and phosphorus concentrations were periodically determined for the influent and the effluent of both reactors (Table 1). The SBR performed slightly better in terms of total nitrogen removal efficiency (95 vs. 92%) and

significantly better in terms of phosphorus removal efficiency (97 vs. 56%). Part of the difference in efficiency was attributable to the higher starting values for the SBR feed, especially with regard to phosphorus (1.08 vs. 0.63 mg/L), although the final average concentrations were also lower in the SBR.

Table 1. Nitrogen and Phosphorus Removal Efficiencies

	SBR			CBR		
	Starting Conc. (mg/L)	Average Final Conc. (mg/L)	Average Removal (%)	Starting Value (mg/L)	Average Final Value (mg/L)	Average Removal (%)
Nitrate	8.7	0.24	97	8.0	0.19	98
Nitrite	0.035	0.01	58	0.19	0.01	92
Ammonia	0.53	0.18	66	0.13	0.48	–269
Total nitrogen	9.27	0.44	95	8.32	0.69	92
Phosphorus	1.08	0.03	97	0.63	0.28	56

NMR analyses of the products from both reactors showed compounds frequently associated with anaerobic fermentation. Both reactors produced acetic acid as their primary product. The CBR had two products representing either isopropyl alcohol or an ether compound, although they could not be clearly distinguished from each other analytically (Table 2).

Table 2. NMR Analyses of SBR and CBR Products

SBR		CBR	
Compound	Mole %	Compound	Mole %
Acetic acid	75.7	Acetic acid	36.2
Propionic acid	14.9	Isopropyl alcohol or ether	24.1
Ethanol	7.2	Ethanol	22.6
Propanol, etc.	2.1	Butyric acid	10.6
Methanol (tentative)	0.09	Propionic acid	4.1
		Isopropyl alcohol or ether	2.55

4.3 Effect of pH and Loading on Hydrogen Production

CBR reactors were operated at various pH levels to determine the optimum pH value. At least two reactors were operated at each pH value, and the optimum was about pH 5 (Figure 3). This value is generally consistent with that determined for other hydrogen-producing systems.[9]

Various paper loadings were tested to approximate the level producing the most hydrogen per gram of paper. The optimum loading was around 4 g of paper per liter of WWTP effluent (Figure 4).

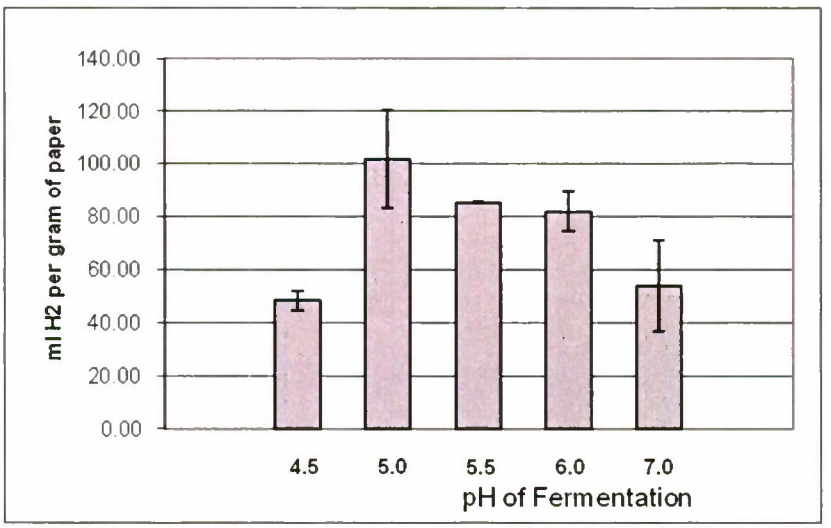

Figure 3. Effect of pH on hydrogen production.

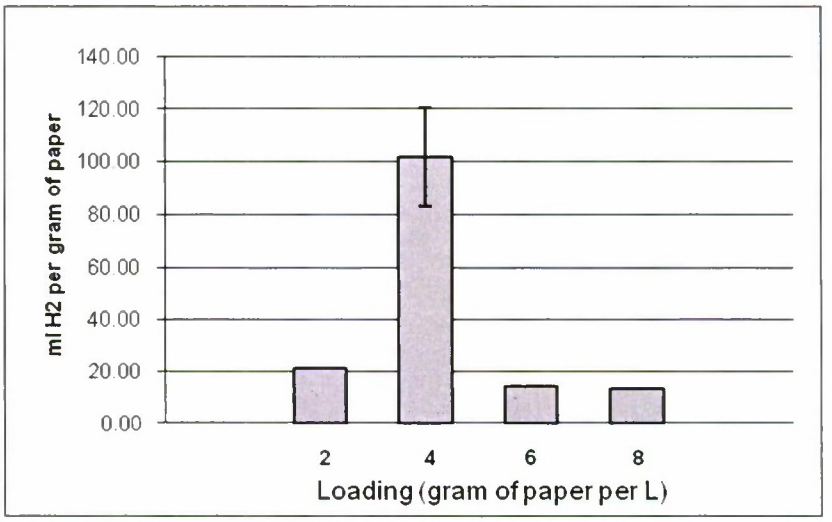

Figure 4. Effect of loading on hydrogen production.

4.4 Enzymatic Source of Hydrogen Production: Hydrogenase vs. Nitrogenase

Given that bacteria can produce hydrogen from reactions catalyzed by either hydrogenase or nitrogenase enzymes, an effort was made to estimate the relative contributions of the products of the two enzymes to the overall hydrogen yield. Reactors were operated under similar conditions and purged with either nitrogen or argon. Argon-purged cultures, which were not provided nitrogen gas as a substrate for nitrogenase, averaged 55% of the hydrogen output of the nitrogen-purged cultures (56.08 ± 2.8 mL H_2 per gram of paper vs. 101.98 ± 18.63 mL H_2 per gram of paper). The most straightforward explanation of these results could be that about half of the hydrogen that evolved from the cultures was produced as a byproduct of the fixation of

nitrogen by nitrogenase, which did not occur in the argon-purged cultures. This tentative conclusion was further supported by the results of a fermentation conducted under the same conditions in the presence of nitrogen gas but with 50 mM ammonium chloride. Nitrogenase activity is normally repressed in the presence of ammonia because of the metabolic cost in ATP to fix nitrogen: that fermentation produced 46.78 mL H_2 per gram of paper (46% of that measured in the absence of ammonium chloride). Generally similar results were obtained with argon-purged cultures and in the presence of excess ammonium chloride, which suggests a strong role for nitrogenase (perhaps 50%) in the production of hydrogen in this system.

4.5 Cycle Analysis: Hydrogen, TOC, Nitrogen, and Phosphorus

To better understand the overall process chemistry, a longer CBR cycle was performed with periodic sampling to allow measurement of TOC, nitrogen, and phosphorus levels in addition to hydrogen production. Figure 5 shows a comparison between hydrogen production, which occurred during approximately the first two days of the cycle, and TOC removal, which continued for about a week. The initial, lower TOC value was apparently due to the heterogeneity of the system before degradation of the paper by the added cellulase. Theoretically, the 4 g/L of added paper added would have a calculated TOC value of 1778 mg/L. Because of the nature of the SSF reactor, in which hydrogen production and cellulose hydrolysis occur simultaneously, the reactor never reached this level. In addition to operation within a single tank, an advantage of this type of system is that microbial fermentation activity continually removes the enzyme reaction product. This pulls the reaction equilibrium toward the monosaccharide products, which are quickly utilized. The TOC profile in Figure 5 shows that TOC continues to be removed several days after the apparent cessation of hydrogen production. It is possible, of course, that hydrogen production may continue at a greatly reduced rate to yield a headspace concentration below the detection limit of the HY-OPTIMA 700 hydrogen monitor (0.5% hydrogen). Potentially, such lower production levels could be determined with use of a lower nitrogen gas flow rate.

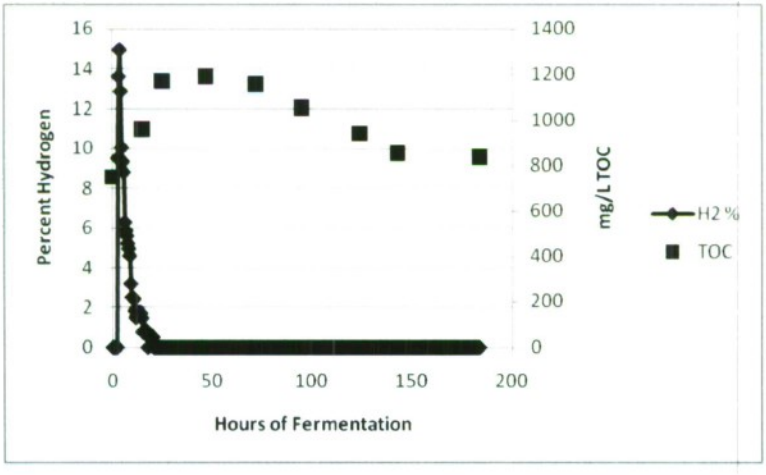

Figure 5. Comparison of hydrogen production and TOC profiles from a single CBR cycle. The conditions for the cycle were 38 °C, pH 5.0, 4 g/L shredded paper, and 0.45 mL/g Accellerase 1500.

Figure 6 shows the nitrate, nitrite, ammonia, total nitrogen, and phosphorus profiles from the same cycle as shown in Figure 5. Nitrogen content consisted mainly of nitrate and ammonia (starting concentrations of 8.4 and 2.64 g/L, respectively). Nitrate and ammonia values were reduced to around 1 mg/L or less within the first day, while nitrite stayed relatively constant at around 0.2 to 0.3 mg/L. Phosphorus levels showed a similar magnitude of decline, going from 6.6 mg/L to around 1 mg/L (Figure 7).

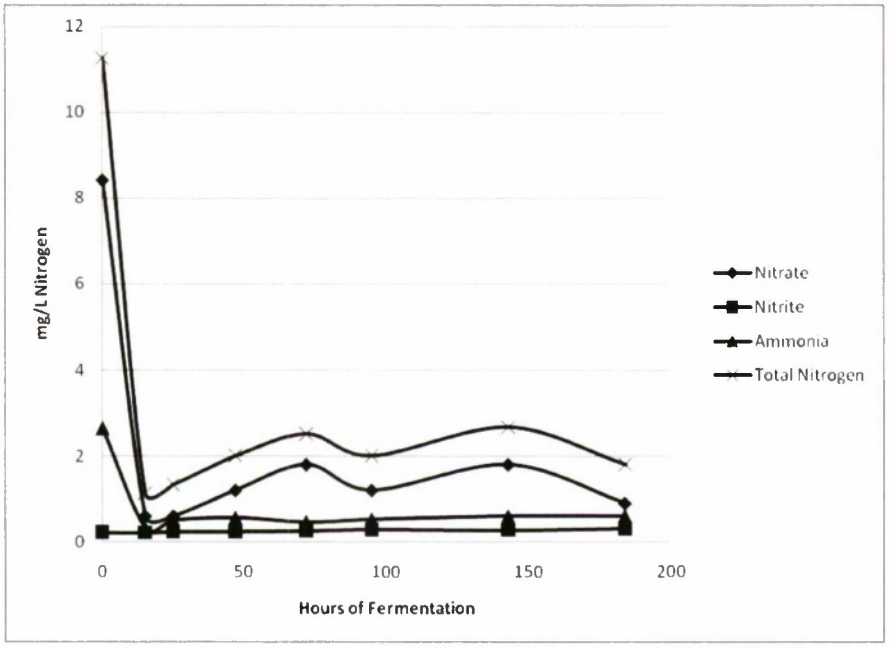

Figure 6. Nitrogen profiles from extended run.

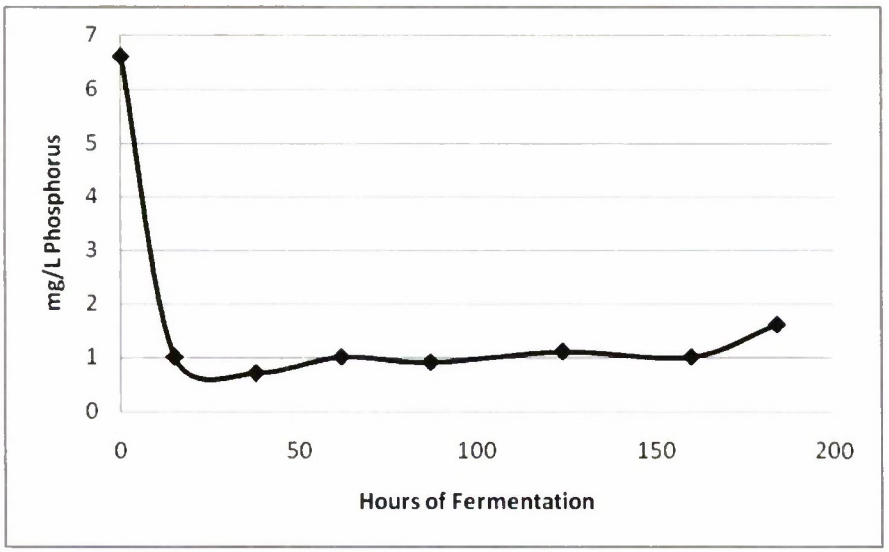

Figure 7. Phosphorus levels from extended run.

4.6 Hydrogen Production Rate

Using the data on headspace hydrogen concentrations over time, it is possible to determine hydrogen production rates for the system. The headspace volume was about 2 L for a reactor volume of 5 L, so the instantaneous changes in hydrogen production rates were necessarily somewhat averaged by the headspace volume and carrier gas flow rate (5 mL/min nitrogen). Hydrogen production rates over the course of the reaction were calculated for the three highest producing CBR runs (138, 114, and 110 mL hydrogen per gram of paper, respectively) (Figure 8). The maximum observed rate of hydrogen production was about 14 mL per liter of WWTP effluent per hour, which was also observed with the culture yielding the greatest overall volume of hydrogen.

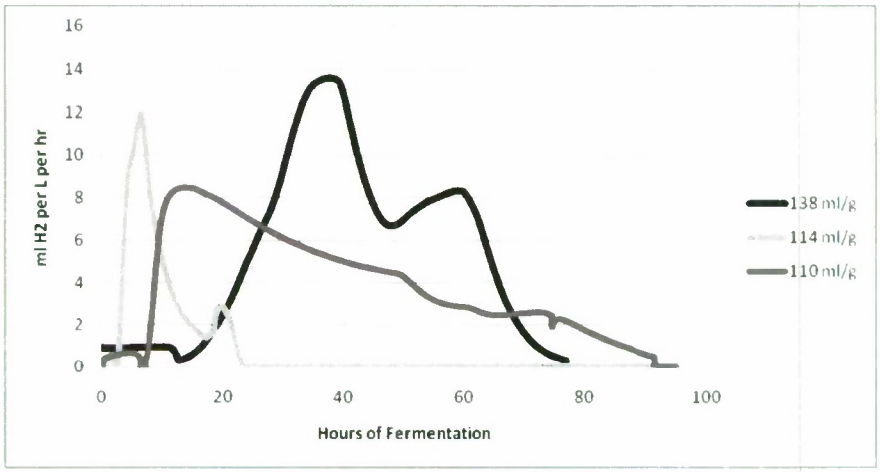

Figure 8. Hydrogen production rates.

5. DISCUSSION

With regard to reactor design, use of the CBR offers simpler operation with superior hydrogen yields over time, although with somewhat less efficient nitrogen and phosphorus removal. The decline in hydrogen production in the SBR when compared with the CBR can probably most simply be explained by the accumulation of hydrogen-consuming organisms such as methanogens. These cultures were largely grown under nitrogen fixation conditions (with the exception of the initial consumption of the approximately 10 mg/L nitrogen found in the starting effluent), and nitrogen fixation is widespread in methanogenic organisms. Hydrogen-producing acidogenic bacteria are fast growers compared to methanogens,[11] and the steady accumulation of biomass in the SBR may have gradually increased the predominance of methanogens in the culture. The CBR reactor, on the other hand, produced hydrogen at a fairly steady level, averaging a little over 100 mL H_2 per gram of paper.

The average hydrogen yield under optimal conditions (pH 5, 4 g/L loading, 0.45 mL/g Accellerase 1500, 38 °C) of 101.98 ± 18.63 mL H_2 per gram of paper equates to 4.52 ± 0.83 lb of hydrogen per ton of paper.

One potentially significant variable not controlled for in the experiments described here is the concentration of hydrogen in the headspace of the reactor. Acidogenic bacteria, particularly those that produce acetic acid as a byproduct, are subject to feedback inhibition when hydrogen accumulates to even very low levels in the headspace.[12] This feedback might at least partially explain the relatively high variability in hydrogen yields observed under the most efficient conditions (4 g/L, pH 5) that typically provided for the overall highest concentrations of hydrogen in the headspace at any one time. A total of seven fermentation runs were performed under those conditions and yields ranged from 82.10 to 138.2 mL/g. These reactors were operated with about 2 L headspace volume; by minimizing that volume without changing the nitrogen gas flow rate, hydrogen would be swept from the headspace more rapidly and could be collected in a separate vessel for quantitation by the same means used here.

Regarding the removal of nitrogen and phosphorus, part of the significance of the role of nitrogen fixation in the biological production of hydrogen is that it could help supplant the need for fossil fuel in fertilizer production. There may, however, be some trade-offs between nitrogen removal and nitrogen fixation/hydrogen production, in that the former largely utilizes nitrogen already found in the WWTP effluent. However, confirmation would require testing of the process at lower paper loadings than were used in this study.

The observed temporal difference between the hydrogen production peak and the TOC peak (Figure 5) suggests the possibility of at least two different energy-yielding processes that might be conducted on the residual organic compounds (mainly organic acids) found in the spent bioreactor medium. First, it should be possible to use the residual organic acids as substrates for organic acid-consuming, hydrogen-producing, photosynthetic bacteria.[13,14] A second approach of potential interest could be the use of a microbial fuel cell to directly produce electricity from the residual organics. Both of these approaches would combine the benefits of energy production with enhanced waste treatment.

Other than the small amount of nitrogen used to purge the headspace of these reactors, the only feedstock material not available for free are the cellulase solutions. However, recent research on cellulases has been promising with regard to substrate range and potential cost reductions.[15,16]

6. CONCLUSIONS

Dark fermentation of paper in WWTP effluent offers the potential to simultaneously produce hydrogen and remove nitrogen and phosphorus from wastewater. Used in the CBR configuration, the process is particularly simple in concept, requiring no sterilization or added nutrients. The optimized conditions developed with paper as a substrate may also convey to the use of a similar process with lignocellulosic biomass, although such biomass would likely require thermochemical pretreatment prior to enzymatic digestion and fermentation. TOC removal data suggest the feasibility of linking this approach with a second stage, possibly using photosynthetic bacteria or a microbial fuel cell.

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ABBREVIATIONS

APG	Aberdeen Proving Ground
CBR	classic batch reactor
EPA	U.S. Environmental Protection Agency
ESS	effluent suspended solids
MLSS	mixed liquor suspended solid
NMR	nuclear magnetic resonance
QNP	quadruple nucleus probe
SBR	sequencing batch reactor
SSF	simultaneous saccharification and fermentation
TCI	triple-resonance inverse detection
TOC	total organic carbon
WWTP	wastewater treatment plant