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Full Length Article

Biohydrogen production by co-digesting whey and glycerin in an AnSBBR: Performance optimization, metabolic pathway kinetic modeling and phylogenetic characterization

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ABSTRACT

The co-digestion of cheese whey with glycerin for biohydrogen production, in an anaerobic sequencing batch biofilm reactor (AnSBBR) with recirculation of the liquid phase (at 30 °C with 3.5 L of working volume and treating 1.5 L per cycle), was assessed. The feeding time (1.5 h) was equal to half of the cycle length (3 h). Two variables were studied: the ratio between whey and glycerin (100:0; 75:25; 50:50; 25:75; 0:100% on a COD basis) and the organic loading rate (from 10.1 to 23.9 kgCOD m^{-3} d^{-1}) by altering the influent concentration (from 3 to 7 kgCOD m^{-3}). The highest hydrogen productivity (129.0 molH_2 m^{-3} d^{-1}) and yield (5.4 molH_2 kgCOD^{-1}) with complete inhibition of methanogenisis were achieved when the reactor was fed with 75% cheese whey and 25% glycerin with an applied volumetric organic load of 23.9 kgCOD m^{-3} d^{-1}; an increase in productivity of almost 145% and 27% when compared to the anaerobic digestion of cheese whey and glycerin separately, respectively, showing the significant benefit of glycerin addition for anaerobic whey digestion, most likely due to its buffering capacity. A kinetic metabolic model was efficiently fitted to the process to help understand the metabolic routes. Cloning analyses targeting 16S rRNA genes indicated the dominant microorganisms were phylogenetic affiliated to *Ethanoligenens* and *Megasphaera* genera.

1. Introduction

Energy production from renewable resources and from low energy demand processes has an important role in the fight against climate change and in creating a more sustainable world. Transition to a fully sustainable energy system has been estimated around year 2050 to utilize a major part of renewable energy without affecting normal life activities that employ energy sources [1,2].

Biogas as clean energy source, from anaerobiosis of various degradable substrates, can effectively reduce greenhouse emissions and replace fossil energy sources that are used in household and commercial activities [2]. The biogases generated by anaerobic digestion that are suitable as biofuels are methane and hydrogen. The latter, with only water as combustion product and an energy yield of 121 kJ g^{-1} (around 2.7 times as high as that of hydrocarbon fuels), is one of the most promising sustainable energy resources.

Different wastewaters have been used as substrate for hydrogen production. Nonetheless, anaerobic digestion of single substrates (mono-digestion) presents some drawbacks regarding substrate properties. These problems can be solved by adding a co-substrate in what has been recently called anaerobic co-digestion.

Cheese whey, or milk whey, is the watery part of milk that is separated from the curd in the cheese-making process. It is the major by-product of dairy industries, and because of the high organic load, it has strong pollution potential, causing excess of oxygen consumption when directly disposed of in water bodies. Moreover, cheese whey can cause impermeabilization, eutrophication, toxicity etc. in receiving environments. Generally, cheese whey presents high organic load (up to 80 gCOD L^{-1}), low alkalinity content (<2500 mg$CaCO_3$ L^{-1}), high TKN content (up to 1700 mg L^{-1}) and very high biodegradability. On a global scale, only 50% of all produced cheese whey is used in industries and, on average, 873 mL of whey is generated per liter cow milk, resulting in approximately 5 million tons of whey per year [3–5].

Several researchers have been using whey for biohydrogen production, but due to its high biodegradability and low alkalinity

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Notation

AnSBBR	Anaerobic stirred batch biofilm reactor
AVOL	Applied volumetric organic load based on organic matter (kgCOD m^{-3} d^{-1})
C_C	Carbohydrates concentration (mgCarbohydrate L^{-1})
C_G	Gas concentration (mmol L^{-1})
C_G	Glycerin concentration (mgGlycerin L^{-1})
COD	Chemical oxygen demand
C_{OM}	Organic matter concentration (mgCOD L^{-1})
EtOH	Ethanol
F	Influent flow (1 L h^{-1})
H	Hydrogen
HAc	Acetic acid
HBu	Butyric acid
HPr	Propionic acid
HVa	Valeric acid
LDPE	Low-density polyethylene
M	Methane
MPr	Daily molar productivity of hydrogen (molH_2 m^{-3} d^{-1})
M_{TVS}	Biomass in the reactor expressed in total volatile solids (gTVS)
MYAL	Molar hydrogen yield per applied load based on organic matter (molH_2 kgCOD^{-1})
MYAS	Molar hydrogen yield per applied substrate (molH_2 molSubstrate^{-1})
OTU	Operational taxonomic unit
rRNA genes	Ribosomal ribonucleic acid
RVOL	Removed volumetric organic load based on organic matter (kgCOD m^{-3} d^{-1})
S	Substrate, which is considered as the molar combination of glycerin and lactose
SMPr	Daily specific molar productivity of hydrogen (molH_2 kgTVS^{-1} d^{-1})
TA	Total alkalinity (mg$CaCO_3$ L^{-1})
TKN	Total Kjeldahl nitrogen
TS	Total solids (gTS)
TVA	Total volatile total acids expressed in acetic acid mass base (mgHAc L^{-1})
TVS	Total volatile solids (gTVS)
VFA	Volatile fatty acids (mmolVFA L^{-1}),
V_G	Total biogas volume per cycle (NmL cycle^{-1})
V_{H2}	Hydrogen volume per cycle (NmL cycle^{-1})
$\varepsilon_{C,F}$	Removal efficiency based on carbohydrates for filtered samples (soluble) (%)
$\varepsilon_{G,F}$	Removal efficiency based on glycerin for filtered samples (soluble) (%)
$\varepsilon_{OM,F}$	Removal efficiency based on organic matter for filtered samples (soluble) (%)

content it may be hard to stabilize biogas production, since peaks of volatile acids are easily formed and may inhibit the process. It should also be mentioned that hydrogen is an intermediate product in the anaerobic digestion process and may be consumed in the liquid medium by sulfate-reducing and nitrate-reducing bacteria and hydrogenotrophic microorganisms [6–10]. Hence, using a co-substrate may be an interesting approach to overcome process instability. Glycerol, which is the major by-product of the biodiesel industry, may be a good co-substrate for cheese whey digestion. In general, for every 100 kg of biodiesel produced, approximately 10 kg of crude glycerol is generated and the global biodiesel market is estimated to reach 37 billion gallons by 2016 with an average annual growth of 42%. Crude glycerol, generated by homogeneous base-catalyzed transesterification, contains approximately 50–60% glycerol, 12–16% alkalis (especially in the form of alkali soaps and hydroxides), 15–18% methyl esters, 8–12% methanol, and 2–3% water. In addition to methanol and soaps, crude glycerol also contains a variety of elements such as Ca, Mg, P, and S as well as other components [11]. Therefore, glycerol is an attractive co-substrate because it is readily biodegradable, has a pH suitable for anaerobic processes and its high C content (36g-C/92g-glycerin) increases the C:N ratio in the mixture, impeding process inhibition by excess N.

The main objective of this study is to assess the hydrogen production by co-digestion of whey and glycerin in an anaerobic sequencing batch biofilm reactor, with recirculation of the liquid phase that contains immobilized biomass (AnSBBR). The mixture of these wastewaters is expected to generate an effluent with balanced nutrients and good buffering capacity. The influence of mixture ratio and organic loading rate on reactor stability, efficiency, conversion factor (between removed organic matter and produced hydrogen) and hydrogen productivity was assessed. Moreover, a metabolic kinetic model and the phylogenetic characterization of the microbial consortium present in the reactor, when hydrogen production was highest, were assessed to better understand the biotechnological viability of this system.

The reason for choosing this bioreactor is to study the technological options for discontinuous operation as alternative to commonly employed continuous operations. The point is to assess the main advantages related to improved operational control (load-reaction-discharge), feeding mode flexibility (different cycle lengths) because of the different periods of production stops, and suitability for different wastewater concentrations available for biogas generation from relatively small production units.

2. Material and methods

2.1. AnSBBR (Anaerobic sequencing batch biofilm reactor)

Fig. 1 shows a scheme of the system used for the hydrogen production by co-digestion of whey with glycerin (Adapted from Lovato [12]). The system consisted of an acrylic tubular reactor and a recirculation unit. The latter comprised a diaphragm pump and a 2.1 L acrylic tubular side reservoir containing 1.5 L of liquid medium. The reactor was 540 mm high and the side reservoir 300 mm. Both had an external diameter of 100 mm and a wall thickness of 3.5 mm.

The interstitial recirculation velocity was 0.2 cm s^{-1}. Hydrodynamic analysis of the AnSBBR was performed by Camargo [13] and showed that the liquid phase achieves perfect mixing around 7 min.

The total volume of the reaction medium (2.0 L contained in the reactor and 1.5 L in the side tank) was 3.5 L. The chamber in which the reactor was operated was kept at 30 ± 1 °C.

2.2. Support for immobilization and inoculum

The support used for biomass immobilization consisted of low-density polyethylene (LDPE) pellets obtained from recycled plastic waste, with length of 5 mm and diameter of 3 mm. The bed formed with these LDPE particles had an apparent and real density of 470 and 825 g L^{-1}, respectively, and a bed porosity of 43%. The LDPE pellets were immersed for 2 h in 2 L of inoculum previously crushed through a sieve (2 mm mesh). The excess sludge was removed and the support material was introduced in the reactor (Manssouri [14]). LDPE was chosen as support in accordance with Fernandes [15].

The inoculum used in all experiments came from an up-flow anaerobic sludge blanket reactor treating wastewater from a poul-