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Effect of pH on the anaerobic acidogenesis of agroindustrial wastewaters for maximization of bio-hydrogen production: A lab-scale evaluation using batch tests

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HIGHLIGHTS

- The maximum biohydrogen production and total VFAs yield was achieved at pH 6.0.
- Different pH values contribute to different distribution of end-products.
- Hydrogen is exclusively produced via bioconversion of lactic acid.
- A linear correlation exists between hydrogen and butyric acid production.

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ABSTRACT

The aim of this study was to investigate the impact of pH on the production of bio-hydrogen and end-products from a mixture of olive mill wastewater, cheese whey and liquid cow manure (with a ratio of 55:40:5, v/v/v). Batch experiments were performed under mesophilic conditions (37 °C) at a range of pH from 4.5 to 7.5. The main end-products identified were acetic, propionic, butyric, lactic acid and ethanol. The highest hydrogen production yield was observed at pH 6.0 (0.642 mol H_2/mol equivalent glucose consumed), whereas the maximum VFAs concentration (i.e. 13.43 g/L) was measured at pH 6.5. The composition of acidified effluent in acetic and butyric acid was similar at pH 6.0 and 6.5, albeit an increase of propionic acid was observed in higher pH. Lactic acid was identified as a major metabolite which presented an intense accumulation (up to 11 g/L) before its further bioconversion to butyric acid and hydrogen.

1. Introduction

Olive mills, cheese factories and cow farms are agro-industries that represent a considerable share of the worldwide economy with particular interest focused in the Mediterranean region. These industries generate millions of tons of wastewaters and large amounts of by-products, which are in many cases totally unexploited and thus dangerous for the environment. More specifically, approximately 5.4×10^6 m^3 of olive mill wastewater is produced annually worldwide (Basheer et al., 2007), while 180–190 $\times 10^6$ tons per year of cheese whey are generated (Mollea et al., 2013) and 55×10^6 dry tons of animal manure are collected every year for subsequent disposal (Liao et al., 2006).

The liquid by-product of olive oil production using the three-phase centrifugation process, i.e. olive mill wastewater (OMW), is recognized in the whole Mediterranean, Aegean and Marmara region as a severe environmental problem because of its high organic content and recalcitrance to biodegradation which is particularly due to the presence of phenolic compounds. The total concentration of phenols in OMW, which contribute to a high toxicity and antibacterial activity (Capasso et al., 1995) can reach up to 24 g/L (Paraskeva and Diamadopoulos, 2006). Its chemical oxygen demand (COD) and biological oxygen demand (BOD_5) range from 25 to 220 kg O_2/L and 9 to 100 kg O_2/L respectively (Paraskeva and Diamadopoulos, 2006), contributing to a significantly high bioenergy content. On the other hand, nitrogen, which is one of the essential macronutrients required for anaerobic bioprocesses, is usually low in OMW. The carbon-to-nitrogen-to-phosphorus ratio is around 100:1.77:0.94, respectively, for OMW.

Cheese manufacturing industry generates large amounts of high strength wastewater, with associated high biological (BOD_5) and chemical oxygen demand (COD). Cheese whey (CW) is a by-product of cheese manufacturing which mainly contains a significant

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Nomenclature

OMW	olive mill wastewater	SOC	soluble organic carbon, g/L
CW	cheese whey	BOD_5	biochemical oxygen demand, g/L
LCM	liquid cow manure	TKN	total Kjeldahl nitrogen, g/L
CSTR	continuous stirred tank reactor	SHPP	specific H_2 production potential, mL/gCOD
HRT	hydraulic retention time, days	$SHPR_m$	specific H_2 production rate, mL/gVSS h
COD	chemical oxygen demand, g/L	STP	standard temperature and pressure
VFA	volatile fatty acid, g/L	H	cumulative H_2 production, mL
TVFA	total volatile fatty acid, g/L	P	maximum H_2 production potential, mL
TS	total solids, g/L	R_m	maximum H_2 production rate, mL/h
VS	volatile solids, g/L	λ	lag-phase duration, h
TSS	total suspended solids, g/L	e	Euler's number (2.71828)
VSS	volatile suspended solids, g/L		
TOC	total organic carbon, g/L		

amount of carbohydrates (4–5%), mainly lactose (45–50 g/L), proteins (6–8 g/L), lipids (4–5 g/L) and mineral salts (8–10% of dried extract); mineral salts include NaCl and KCl (>50%), calcium salts and others. CW also contains appreciable quantities of lactic (0.5 g/L) and citric acid, non-protein nitrogen compounds and B-group vitamins (Venetsaneas et al., 2009). Despite the high carbohydrate content of CW which is suitable for biological processing, the anaerobic treatment of raw CW is quite problematic due to its low bicarbonate alkalinity (50 meq/L), high COD concentration (up to 70 g COD/L) and tendency for rapid acidification (Mawson, 1994).

Agricultural wastewaters, including liquid animal manure (LCM), are characterized by high organic content with high amounts of total solids, ammonia and pathogens (Rico et al., 2011). Insufficient or uncontrolled handling and disposal of such highly polluting agro-wastes encounter imminent danger to environment and thus to public health.

Multiple waste streams of organic substrates can be anaerobically co-digested to generate a homogeneous mixture increasing both process and equipment performance. Co-digestion of different types of organic by-products such as agro-industrial wastewaters has been increasingly applied in order to enhance the biogas production and overcome a number of problems such as nutrient imbalance, rapid acidification and presence of inhibiting compounds, among other factors (Paraskeva and Diamadopoulos, 2006; Dareioti et al., 2009). Two-stage anaerobic digestion (AD) for integrated bio-hydrogen and bio-methane production from organic materials has been reported to promise higher process efficiency and energy recoveries as compared to traditional one-stage AD (Schievano et al., 2012). If correctly operated, the first stage of these systems can achieve several steps including hydrolysis, acidification, and hydrogen gas production. The performance of an acidogenic reactor is of paramount importance especially during the two-phase anaerobic stabilization of wastes, since the acid reactor should provide the most appropriate substrate for the subsequent methanogenic one. Since many different types of bacteria are involved in the fermentation of organic wastes, anaerobic acidification of agroindustrial wastes may produce volatile fatty acids (VFAs), alcohols, H_2, CO_2 and other intermediate products (i.e. lactic acid). For sustainable bio-hydrogen production the feedstock has to meet certain criteria. For example, carbohydrate-rich feed stocks produced from sustainable resources in large quantities are of paramount importance, since they can be easily fermented favoring thus energy recovery, require minimum pretreatment and are of low cost. Different substrates such as solid wastes and food industry wastewaters can be easily fermented to produce hydrogen (Kapdan and Kargi, 2006) although pure carbohydrates (e.g. glucose, sucrose) have been most commonly used (Wang and Wan, 2009).

Enhancing biological production of hydrogen gas is of great interest nowadays, because it is a promising alternative to fossil fuels due to its clean and high-energy yield. However, very little information is available even on pilot-scale (Supplement Table 1) and practically none on full-scale. A pilot-scale study is critical to testify the productivity before a new biotechnological process is put into full-scale operation. In larger than lab-scale, the use of mixed microbial cultures is a cost-effective and promising approach to achieve bio-hydrogen production.

Not only hydrogen gas itself is a beneficial energy source but also VFAs can be used further for methane production by methanogenesis. The reactions involved in bio-hydrogen production are rapid and can be effectively used for treating large quantities of organic wastes. The behavior of acidogens in a two-phase process plays a primary role in producing major substrates, such as short-chain organic acids and hydrogen. A series of operating parameters including pH, temperature and hydraulic retention time are known to influence the performance of fermentation and the formation of intermediate fermentative products such as hydrogen, organic acids and ammonia (Wang and Wan, 2009). Among these factors, pH has been found to be crucial to the distribution of acidogenic products (Ren et al., 1997). Although a substantial number of studies have been conducted on the optimal pH range for fermentative hydrogen production, the results are often inconsistent due to differences in substrate and seed type and other operating conditions adopted (Wu et al., 2010). Furthermore, many studies have been conducted to look into the effect of initial pH on fermentative hydrogen production, whereas the importance of pH control has rarely been investigated (Wang and Wan, 2009). It is acknowledged, for example, that low pH values result in inhibition of the hydrogenase activity, which is regarded to as a key factor explaining the influence of pH on fermentative hydrogen production (Khanal et al., 2004; Mohd Yasin et al., 2011). The metabolic pathways involving acetic and butyric acid production appear to be favored at pH ranging from 4.5 to 6.0, while neutral or higher pH are believed to promote ethanol and propionate production (Guo et al., 2010). Meanwhile, the reported optimal pH values for different substrates differed substantially from 4.0 to 6.5, but for each specific situation, the optimal pH range was quite narrow (usually within 0.5). For instance, an optimal pH value of 6.0 was obtained using cheese whey (Ferchichi et al., 2005), food wastes (Jiang et al., 2013) and kitchen wastes (Zhang et al., 2013), a lower pH value of 5.5 was considered as optimum using glucose (Fang and Liu, 2002), whereas the initial pH of 4.5 gave the highest specific hydrogen