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# Bioreactor design for continuous dark fermentative hydrogen production

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ABSTRACT

Dark fermentative $H_2$ production (DFHP) has received increasing attention in recent years due to its high $H_2$ production rate (HPR) as well as the versatility of the substrates used in the process. For most studies in this field, batch reactors have been applied due to their simple operation and efficient control; however, continuous DFHP operation is necessary from economical and practical points of view. Continuous systems can be classified into two categories, suspended and immobilized bioreactors, according to the life forms of $H_2$ producing bacteria (HPB) used in the reactor. This paper reviews operational parameters for bioreactor design including pH, temperature, hydraulic retention time (HRT), and $H_2$ partial pressure. Also, in this review, various bioreactor configurations and performance parameters including $H_2$ yield (HY), HPR, and specific $H_2$ production rate (SHPR) are evaluated and presented.



## 1. Introduction

Fossil fuels have played a fundamental role in industrial development and are responsible for fulfilling 80% of energy demand globally. However, this current energy system is now facing two fundamental problems: gradual depletion and environmental pollution. This lack of sustainability has led to extensive research on new alternative energy sources (Brey et al., 2006). Among various alternative energy sources, $H_2$ is regarded as the most promising future energy carrier, since it produces only water when combusted, generating a 2.75 times higher energy yield (122 kJ/g) than hydrocarbon fuels. Furthermore, $H_2$ fuel cells and related $H_2$ technologies provide an essential link between renewable energy sources and sustainable energy services (Levin et al., 2004).

$H_2$ production methods can be broadly divided into physico-chemical and biological processes. Recently, more than 90% of $H_2$ production has been achieved via steam reforming of hydrocarbons and coal gasification, due to the low costs involved in these processes. However, these methods have been criticized by the public and specialists, because they entail the use of fossil fuels, thus emitting a significant amount of greenhouse gases (Ewan and Allen, 2005). Given these perspectives, biological $H_2$ production assumes paramount importance as an alternative energy resource. Despite relatively lower yields of $H_2$ compared with photo-driven processes, dark fermentative $H_2$ production (DFHP) is a promising method due to its higher rate of $H_2$ evolution in the absence of light sources and the transformation of waste into environmentally sound materials.

Therefore, this article presents an up-to date overview of current knowledge on important operation parameters and various reactor configurations in DFHP.

## 2. Reactor design parameters

The operational parameters such as inoculum preparation and start-up, pH, temperature, HRT, substrate concentration and liquid product inhibition, feedstock, $H_2$ partial pressure and nutrients are considered to have significant effects on the performance of DFHP. Defining their optimal ranges would provide important information in determining reactor and system size, materials, additional equipment, chemical reagents, and so on.

### 2.1. Inoculum preparation and start-up

It is more practical to use mixed cultures than pure cultures in engineering point of view, because they are simpler to operate and easier to control, and may have broader choice of feedstock (Valdez-Vazquez et al., 2005). Thus, numerous methods have been made to obtain $H_2$ producing inocula from various seeding sources such as anaerobic digester sludge, sewage sludge, compost, manure and soil. Physico-chemical attack was generally used to get the inocula on the basis that main $H_2$ producing bacteria, *Clostridium* sp., are spore-forming bacteria (Li and Fang, 2007).

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At first, toxic chemical addition was applied to inhibit $H_2$-consuming reaction, especially killing methanogens. 2-Bromoethanesulfonate (BES), acetylene and chloroform were added to anaerobic digester sludge and showed successful performances (Liang et al., 2002). However, it is seldom used today due to the high cost of chemicals and their ineffectiveness on non $H_2$-producing bacteria such as lactic and propionic acid bacteria.

Acid/base treatment was also effective in obtaining $H_2$ producing inocula because microbial activity is hindered at low and high pH. Acid (pH 3) and base (pH 10) enrichment of sewage sludge increased the $H_2$-production potential by 200 and 333 times compared to control, respectively (Chen et al., 2002). Similarly, successful continuous operation using acid (pH 3–4) enriched microflora has been reported (Lin and Chou, 2004).

Heat treatment of seed sludge has mostly been used for screening of $H_2$ producing bacteria. It is reported that heat treatment not only reduces the non-spore-forming bacteria but also activates clostridia spores to commence germination by altering the germination receptor (Hawkes et al., 2007). The heating temperature and time was varied from 75 °C to 121 °C and 15 min to 12 h, respectively (Li and Fang, 2007). Sometimes sequential pretreatment (heat shock at 100 °C for 2 h and acid treatment at pH 3.0 for 24 h) was applied for the perfect extermination of non $H_2$-producing bacteria (HPB) in the seeding source (Mohan et al., 2007). In addition to above main pretreatment methods, aeration was applied to compost sludge (Ueno et al., 1996).

After obtaining mixed cultures of $H_2$ producing bacteria by various pretreatments, continuous operation has to be postponed until $H_2$ production from batch operation reaches some extent in order to germinate $H_2$ producing bacteria and to block their wash-out. Continuous operation was preceded when the $H_2$ yield (HY) reached 0.5 mol $H_2$/mol hexose or after 48 h by batch mode (Kim et al., 2006a,b). Interestingly, Kim et al. (2008) observed the successful start-up only when the continuous feeding started after HY reached 0.2 mol $H_2$/mol hexose by batch mode at 12 h of HRT using continuously stirred tank reactor (CSTR). $H_2$ production ceased within 10 d when the operation mode was changed from batch to continuous after 0.5 mol $H_2$/mol hexose of HY was achieved by batch mode and concluded that it was due to the regrowth of propionic acid bacteria which were inhibited by heat shock (90 °C for 20 min) but not totally exterminated (Kim et al., 2008). Generally, HRT decreased gradually for the successful acclimation of $H_2$ producing bacteria in the reactor. It decreased from 5 d to 12 h in 4 steps, each taking 20 d (Lin and Jo, 2003), 120 to 12 h (Lin and Chou, 2004).

### 2.2. pH

Among various operational parameters, it has been widely accepted that pH has the most significant effect on DFHP, since it directly affects the hydrogenase activity, metabolic pathway, and dominant species (Lay, 2000; Fang et al., 2002). However, the optimal initial and operational pH values vary extensively from 4.5 to 9.0.

Some studies have shown that pH lower than 5.0 is preferable for $H_2$ production. $H_2$-consuming methanogenic activity has been detected at pH 5.0 in studies by Kim et al. (2004) and Hwang et al. (2004). They concluded that a weakly acidic condition around 5.0 is not sufficient to exterminate methanogens, and therefore pH should be lowered to 4.5 so as to prohibit $H_2$-consuming reactions. Based on equilibrium of the $NADH/NAD^+$ ratio inside the cell, it was proposed that an acetate–ethanol fermentation type induced at pH 4.5 is a better and more stable metabolic pathway than acetate–butyrate or acetate–propionate metabolic pathways induced at pH between 5.0 and 7.5 (Ren et al., 1997).

Several studies concluded that the proper pH range is above 7.0. The maximum $H_2$ production was detected at initial pH values of 9.0 and 7.5 by Lee et al. (2002) and Wang et al. (2006), respectively, using glucose as a substrate. Also, a maximum HY of 68.1 mL $H_2$/g TVS was observed at an initial pH 7.0 in treating wheat straw waste (Fan et al., 2006b). However, in the three aforementioned studies on the effects of initial pH, the pH was not controlled during fermentation; it was allowed to drop without any buffer addition. This experimental condition could lead to wrong conclusions, as the pH change is highly dependent on the substrate concentration and the amount of buffer capacity. For example, if there is an insufficient amount of buffer in the medium in treating a high-strength substrate, the pH drop will be drastic, and hence it is important to find the optimum pH at relatively higher ranges. However, if sufficient buffer is provided to this substrate and the buffer is also diluted, then a neutral initial pH also could yield high $H_2$ production. Thus, in a batch process, it is necessary to separately define the roles of initial and operational pH and find the optimal values with consideration of the substrate concentration and buffer capacity.

The main anaerobic HPB, *Clostridium* sp., have several metabolic pathways, and hydrogenic reactions are dominant at pH 5.0–6.5, while non-hydrogenic reactions are triggered outside of this range (Jones and Woods, 1986). Therefore, the pH of recent $H_2$ producing reactors is generally controlled at pH 5.0–6.5. In batch studies reported by Lay (2000) and Fan et al. (2006a), symmetric graphs were shown with pH 5.2 and 6.0 at the center peak, respectively. The alcohol production rate was greater than the $H_2$ production rate (HPR) if the pH was lower than 4.3 or higher than 6.1 (Lay, 2000). A number of reports on continuous operation studies have also revealed that pH around 5.5 is optimal. Fang and Liu (2002) and Yu et al. (2002), concluded that the optimal pH was 5.5 in treating glucose and rice winery wastewater, respectively. Increased microbial diversity was observed at high pH (Fang and Liu, 2002), and the compositions of propionate and ethanol were increased at lower pH (Yu et al., 2002).

The addition of an alkaline solution to control pH is essential in DFHP, but presents an economic burden. To date, to our knowledge, the required amount of alkaline solution has never been quantified and an efficient way to reduce the amount has not been reported. Kraemer and Bagley (2005) attempted to decrease the alkaline solution requirement by recycling the effluent in a methane fermenter; however, this resulted in 87% decrease of $H_2$ production, resulting from $H_2$-consuming methanogenic activity. We speculate that anaerobic co-digestion with a high buffer containing feedstock such as sewage sludge or livestock waste can be an economical solution. As these wastes are protein rich, a large amount of hydroxide ions along with ammonia ions ($NH_4^+$) would be supplied during the fermentation, which would help to mitigate pH drop.

### 2.3. Temperature

Temperature affects the activity of microorganisms and the conversion rate of fermentation products, and is closely related to economic benefit. Zhang and Shen (2006) obtained results indicating that the sensitivity of mixed bacteria to temperature was significantly high and the optimal temperature was found to be around 35 °C. Mu et al. (2006a,b) examined the effect of temperature by varying the temperature from 33 to 41 °C and found that $H_2$ production and microbial growth rate were increased with increased temperature, accompanied by a change of the metabolic product distribution.

In spite of placing an economic burden, $H_2$ fermenters are often operated in a thermophilic (50–60 °C) or hyper-thermophilic (70–80 °C) range, since it is believed that operation at high