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Optimization of substrate composition for biohydrogen production from buffalo slurry co-fermented with cheese whey and crude glycerol, using microbial mixed culture

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ABSTRACT

Biohydrogen production from buffalo slurry (BS) co-fermented with cheese whey (CW) and crude glycerol (CG) was investigated using a suitable microbial community (F210) as inoculum. Mixture Design was used to find the optimal composition (%) of the three substrate components and to investigate the effect of the mixing ratio on Bio-H_2 yields. Maximum H_2 yield estimated through the model was around 117 mL H_2/g VS_{added}, while the maximum experimentally detected was 111.6 ± 21.8 mL H_2/g VS_{added}, obtained for a mixing ratio of substrate composition of 66% BS and 33% CW ($R^2 = 0.962$; p-value = 0.0001). CW was clearly the most suitable substrate (with a relative contribution higher than 46%), but led to a rapid drop in pH from 6.5 to 4, while BS showed high buffering capacity by maintaining the pH above 6. Interestingly, the co-digestion of the different substrates decreased the H_2 production lag phase λ; in particular the presence of BS shortened the lag period ($\lambda < 3$ h) and increased the degradation efficiency of CG.

The results demonstrate the usefulness of the mixture design for finding the optimal substrate composition, using BS as co-fermentation substrate to obtain high H_2 production yields. Moreover the response surface shows the possibility of mixing the substrates in different ways, while maintaining H_2 production within an optimum range: 105–117 mL H_2/g VS_{added}. This might offer a considerable advantage in the effective management of systems or processes, in which the substrates availability may change over the time.

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Introduction

With the fast growth of animal industry in Europe and around the world, the increasing amount of manure produced from animal activity makes this currently one of the largest waste streams in the livestock industry. Three main types of animal manure can be distinguished: urinary waste i.e. slurry or liquid manure from livestock or poultry; solid manure or farm yard manure; and wastewater which is a collection of process water in farms, feedlot runoff, silage juices, beddings, disinfectants and liquid manure. More than 1500 million tons of animal manure is produced yearly, including 128 million tons of cattle manure and 295 million tons of pig manure across the 27 member states of the European Union [1]. Animal waste is one of the most polluting agro-industrial wastewaters. The problem of proper effluent management has run place with the development of intensive farming and the concentration of livestock on limited operating space, possibly "landless", which produces large amount of manure. Due to its high organic matter, nitrogen and phosphorous contents, when improperly stored or used, this waste, contributes to negative environmental and human health impacts. Therefore, the primary issue with dairy manure, both now and for the future, is development of management systems that use the resource without adverse environmental impacts [2].

Biohydrogen production by dark fermentation (DF) of organic wastes has received significant attention in recent years, since it not only treats organic wastes but also produces clean energy [3–6]. Typically, the main suitable substrates for DF are rich in carbohydrates [7]. Effective H_2 production often requires supplementation of an adequate amount of pH buffer and minerals, which will inevitably increase the cost of production [8]. However co-digestion of several wastes with complementary characteristics is a widely applied procedure for increasing biogas production [9], since it can provide balanced nutrient and the required buffering capacity, thereby reducing the cost for pH control or nutritional supplements. Although animal manure may not be considered the ideal substrate for the DF, it could be used as an amendment to carbohydrate-rich and promptly degradable materials to improve the H_2 yield. Indeed, it has been demonstrated that cattle manure addition to carbohydrate-rich substrates increases the hydrogen production [10,11], since it can provide macro and micronutrients (NH_3, P, K, metals) required for bacterial growth [12], buffering capacity necessary to maintain an optimal pH of 4.5–6 for DF [13] and also serves as co-substrate, due to its organic content. In this perspective, cattle manure could supply an almost inexhaustible renewable biomass source for potential H_2 fermentation. Therefore, research effort in this area is crucial for building a more sustainable economy and environment. Besides, according to a techno-economic evaluation by Sarma and colleagues [14], the cost of synthetic growth media in DF can determine up to 50% of the total operation and equipment costs. Therefore, different and less expensive materials should be evaluated as supplementary nutrient for bioconversion processes [14].

The Design of Experiments (DOE) approach offers several advantages when compared to the conventional optimization methods, because it helps to improve process yields, reducing variability and development time, as well as overall costs. Statistical methods such as central composite design and response surface methodology (RSM) have been already successfully employed for optimization in several bioprocesses [15,16], including biohydrogen production [17–20]. Among different DOE methods, mixture designs represent a special class of response surface designs, where the total amount of material is kept constant (100%) and the response depends on the relative proportions of the components in the mixture, and not on the total amount.

The objective of this research was to explore the feasibility of using buffalo slurry (BS), co-fermented with de-proteinized cheese whey (CW) and crude glycerol (CG), in mesophilic (37 °C) fermentative biohydrogen production in batch experiments. The substrates used in this experimentation are typical agro-industrial wastes and livestocks, usually destined to disposal, with high costs and environmental impacts. Buffalo livestock farming plays a central role in the economy of some areas of central and southern Italy, through the production of milk that is used to make mozzarella cheese. The number of animals per farm is often exceeding 100 in some regions; with about 250,000 heads per utilizable agricultural area (equal to 107,400 ha), the ratio per surface of usable agricultural surface exceeds 10 head/ha [21]. Therefore the intensive livestock husbandry makes the management of BS in these areas very complex.

CW, a high organic strength by-product, is a wastewater rich in lactose and proteins, with a COD concentration typically ranging between 50 and 102 g/l. It has been estimated that the world CW production is over than 160 million tons per year. Approximately half of this total CW production is discarded directly to the environment, thus representing a significant loss of resources and a major pollution problem [22]. CG is the main by-product of biodiesel industry. Due to the exponential growth of biodiesel production in the last ten years there has been a concomitant exponential growth in CG availability, thus generating a large amount of waste glycerol surplus [23]. In fact, the estimated production of glycerol is expected to reach 5.8 billion pounds in 2020 [24].

Since the aim of the research project (that financed this work) was the valorization of BS, we chose it as the target substrate. To support BS fermentation, we chose to add CW and CG, two highly fermentable substrates, which have already shown to have good potentials for hydrogen production [20,25]; they were thus considered to be beneficial for improving H_2 production performances, when used in co-digestion with other substrates [11,26].

Mixture Design was used to investigate the optimal mixing ratio (%) and to verify how the H_2 production yields are affected by the variation of the composition of the three substrates. A stable community of microorganisms, all known as good hydrogen producers, was used as inoculum (F210) with the aim of improving the hydrogen yield and stability of the fermentation process with the different substrates.

Material and methods

Substrates

The substrates used in this study were: 1) buffalo slurry (BS) supplied by the experimental farm of CRA (Italian National