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Editorial

Recent Trends in Biogenic Gas, Waste and Wastewater Fermentation

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Abstract: In recent years, the optimization of bioprocesses for the removal of pollutants from industrial biogenic gas emissions, waste and wastewater has been the focus of intensive research. Recently developed technologies not only aim to remove such pollutants, but also to valorize them, whenever possible, through their bioconversion into useful added-value products. In this domain of progressive research, lab-, pilot-, and demonstration-scale studies are dealing with the fermentation of biogenic gases (e.g., CO_2, CO, and CH_4), waste or wastewater to produce a range of biofuels and valuable products, based on the activity of pure or mixed cultures of native or recombinant aerobic and anaerobic bacteria, algae, or yeasts as biocatalysts. Waste can also be converted to syngas, which can subsequently be fermented as well. A broad range of bioproducts can be obtained, e.g., biofuels and several other platform chemicals and products. This environmentally-friendly biorefinery approach addresses the need to build modern societies according to the concept of a circular economy, and yields products of commercial interest. Different examples of such approaches are described in this collection of scientific reports.

Keywords: acetogenic bacteria; biomass; biorefinery; carbon dioxide; constructed wetlands; greenhouse gas; syngas; waste; wastewater; oleaginous yeast

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1. Introduction

Conventional substrate (e.g., carbohydrates) fermentation processes have occurred naturally for centuries in the environment, e.g., biogas and methane production resulting from the biodegradation of organic matter. For many years, human beings have also taken advantage of the metabolic ability of microorganisms to ferment and produce specific foods and beverages with improved preservation properties, e.g., beer, wine, and sauerkraut. More recently, since a few decades ago, as a result of growing concern about environmental issues, research began on the valorization of biogenic gases, wastes or wastewaters in order to bioconvert their fermentable substrates content into valuable biofuels or bioproducts. This allows an environmental problem to be solved while simultaneously generating useful products. Besides the abovementioned pollutants, renewable and sustainable feedstocks, mainly biomass, have also been considered as interesting substrates for their bioconversion to biobased fuels and products.

2. Water and Wastewater Valorization

Water and wastewater treatment and, later on, their valorization have been studied for several decades. Of all the different types of the most common pollutants (i.e., water, solid waste, gas and greenhouse gas emissions), wastewater is probably the most extensively studied source. After many years focused on the development and optimization of wastewater treatment technologies, recent trends are now more focused on converting soluble pollutants into valuable products, yielding both environmental and economic benefits.

Upon efficient treatment, polluted water and wastewaters may allow pure water to be obtained, which can be reused, leading to the recovery of resources such as nutrients or bioproducts. A recent approach in this field is producing fatty acids [1] and, later on, converting those acids into biopolymers, e.g., polyhydroxyalkanoates [2], and other possible metabolites.

Besides the production of short- and medium-chain fatty acids, as well as biopolymers, some other products have recently been considered. The accumulation of intracellular lipids, by either oleaginous bacteria or yeasts, is one example. It was recently demonstrated that phenolic wastewaters generated during the conversion of lignocellulose can be valorized with the oleaginous yeasts *Rhodotorula kratochvilovae* EXF7516 and *Cutaneotrichosporon oleaginosum* ATCC 20,509 to produce lipids efficiently and cost-effectively [3]. In this study, the authors identified two causes of the limited production of lipids: (i) the phenolic waste stream contains only 1.5 g C/L of monomeric carbon and 8–12.5 g C/L of non-monomeric carbon, and (ii) lipids were consumed for the majority of each operational cycle. Thus, to increase lipid production, the proposed repeated batch process must be further optimized.

These lipids, also known as microbial oils, are precursors of biofuels, mainly biodiesel. An alternative to the use of yeasts consists of cultivating algae that have also been identified as potential lipid accumulators. This was recently tested and demonstrated with freshwater green algae, such as *Chlorella* spp. and *Chlamydomonas* spp., grown in non-sterile malting effluent used for biomass production and lipid accumulation [4]. This work isolated the native freshwater microalgae and incorporated them for the treatment of wastewater from the malting process, which was followed by the generation of biomass and lipids. Microalgae that were cultured in the diluted malting effluent reached their maximum cell number at 50 ME for *Chlorella* sp. and at 70 ME for *Chlamydomonas* sp., respectively. According to the findings of this study, using diluted malting effluent as a nutrient medium for large-scale algae cultivation also contributes to bioremediation and biofuel production.

Other less reported alternatives have also been considered, such as the nitrification of high-ammonium wastewaters. The latter are generated by several industrial sectors, including fertilizer and anaerobic-digestion plants [5]. It was reported that the complete conversion of ammonium to nitrate could be achieved in a batch setup, as well as a conversion of 93% in a continuous stirred tank reactor, in addition to being a sustainable alternative for fertilizer production. According to the authors, for the proper installation and operation of the CSTR, it is necessary to have knowledge about the ammonium concentration in the wastewater. If this concentration is unknown or varies while the product is being produced, the batch control system could be considered as a more suitable alternative.

Besides the treatment and valorization of wastewaters in aerobic (e.g., activated sludge) and anaerobic (e.g., upflow anaerobic sludge bed) bioreactors, another approach that has recently gained increased interest is the so-called constructed wetlands, based on the use of vegetation for the removal of pollutants from some wastewaters [6]. They have been used, among other functions, as tertiary systems for plant biomass production, i.e., *Typha latifolia* and *Canna hybrids*, from the treatment of swine wastewater in tropical climates. In this study, the total biomass production of both aboveground and belowground biomass from a large-scale horizontal subsurface-flow constructed wetland that treats swine wastewater from a farm located in a tropical region was demonstrated. The results indicate that the constructed wetland, as a secondary (post-treatment) system for swine wastewater treatment, significantly reduced the concentrations of COD, TSS, TN, TP and TOC.

Alongside research focusing on the bioconversion of wastewater components into value-added products, efforts have also been dedicated to the modeling of wastewater treatment and wastewater fermentation processes [7]. Recently, besides performing pilot-scale experimental research on the anaerobic treatment of printing and dyeing wastewater, the system's performance was also predicted based on support vector regression as an efficient tool, contributing to the design and optimal operation of such anaerobic reactors.

3. Waste and Biomass Valorization

Waste and biomass valorization often uses a similar approach to wastewater treatment and valorization, and thus generates similar products, e.g., carboxylic acids [8] or biopolymers [9]. Algae cultivation, considered for wastewater treatment and described in the above section, can also be applied to waste; for example, for the production of microalgae based on the minimal processing of oil palm biomass ash [10]. Such ashes were proved to provide suitable phosphorus supplements in microalgae cultivation with, e.g., *Arthrospira platensis Paracas*, *Neochloris oleoabundans* UTEX 1185, and *Dunaliella salina* SAG 184.

Waste and biomass can also be valorized through different strategies than those applied to wastewaters. However, due to their complex structure, and contrary to wastewaters, effective waste and biomass valorization often depends on preliminary pretreatments. Some such pretreatments, such as lipase addition, hydrothermal pretreatment, or a combination of both methods, were applied to food waste to hydrolyze organic matter, resulting in improved methane production compared to raw, untreated feedstocks [11]. The co-digestion of different types of wastes or biomass is also an efficient alternative that improves the fermentation process and was also applied to the aforementioned food waste. In that sense, adding low concentrations of crude glycerol (e.g., 10%) resulted in higher methane production, while a higher amount (e.g., 15%) negatively affected the anaerobic digestion performance.

Similarly as reported above for wastewater treatment, waste and biomass valorization can also be optimized through modeling approaches. In a study focused on hydrogen production through the water–gas shift reaction from biomass gasification in a downdraft gasifier, artificial neural network modeling appeared to be an effective modeling tool [12]. Elemental and proximate analysis compositions, as well as the operating parameters, were the factors considered as inputs to the models, using an input layer, a hidden layer and an output layer for the model structure. In order to train the artificial neural network model, one thousand eight hundred samples derived from the simulation of 50 various feedstocks in different operating situations were used. Another study applied a mathematical analysis based on an updated ADM1 model for biomethane production by anaerobic digestion in order to assess and implement possible improvements in terms of biomethane yields [13]. MATLAB® and Simulink Toolboxes were used to modify the ADM1 model based on the modification of several parameters (e.g., substrate consumption yields in equations describing microbial growth), resulting in possible process improvements.

Pollutants generated in some processes may sometimes result in the accumulation of both waste and wastewater together. For example, the dewatering of wood by mechanical pressing, which is an innovative method to reduce the moisture content prior to thermal drying, generates a liquid effluent as a by-product, known as press water or wood juice [14]. This press water has been tested in anaerobic digestion experiments with digested sewage sludge as inoculum, which showed that press water from wood fuel preparation has potential as an interesting co-substrate for anaerobic digestion processes. Another link between waste and wastewater is the valorization of sludge as a waste from wastewater treatment plants. A recent study assessed its valorization through hygienization through electron beam treatment [15]. Different irradiation methods were used, allowing the identification of the optimal doses necessary for the elimination of pathogens.

4. Waste Gas and Greenhouse Gases Valorization

The third main source of pollutants of concern, besides water and solid wastes, is waste gases and, because of their effect on climate change, greenhouse gases have been a major concern in recent years. This is mainly the case for CO_2 and other related gases, such as CO, as well as H_2. The presence of hydrogen is generally necessary for the biological removal of carbon dioxide, while mixtures of CO_2, CO and H_2 are typically found in syngas and in some industrial gas emissions such as in emissions from steel industries. Such gases can also be treated and valorized through anaerobic fermentation with acetogenic bacteria, to produce carboxylic acids and ethanol or higher alcohols, among others [16,17].

Since short-chain and medium-chain carboxylic acids can be produced [18], similarly to wastewaters and waste, the latter can then also be converted to biopolymers [19]. On the other hand, lipids or microbial oils for the production of biofuels (e.g., biodiesel) can also be products from gases such as CO and CO_2, through the carboxylate platform [20]. Yet another alternative consists of producing biomethane from the biomethanation of CO or CO_2, generally studied with mixed or co-cultures [21]. Though methane is a greenhouse gas, contrary to carbon dioxide, methane can be used as a biofuel.

Besides the anaerobic fermentation of greenhouse gases, such as CO_2, other authors have also been dealing with its aerobic bioconversion. In this case, the most studied organism is the facultative chemolithoautotroph species *Cupriavidus necator*. It has mainly been studied for the production of biopolymers, e.g., polyhydroxyalkanoates (PHA). It generally needs the presence of hydrogen, but was recently shown to be able to grow under mixotrophic conditions, co-metabolizing volatile fatty acids and CO_2 as sources of carbon [22]. It was observed that the production of PHA was enhanced under mixotrophic growth and in the presence of hydrogen as an additional energy source compared to heterotrophic or mixotrophic growth conditions, without hydrogen.

5. Conclusions

The papers published in this Special Issue on the recent trends in biogenic gas, waste and wastewater fermentation covered the following broad themes: (i) lignocellulosic wastewater valorization, (ii) the cultivation of microalgae for enhanced biomass production and lipid productivity, (iii) the anaerobic treatment of printing and dyeing wastewater, press water and food wastes, (iv) the application of pH-based control strategies, artificial neural networks and ADM1 models in waste-to-energy conversion technologies, (v) WWTP sludge valorization, (vi) the valorization of greenhouse gases, e.g., through biomethanation of carbon monoxide or conversion of CO_2 to biopolymers, and (vii) the application of constructed wetlands for wastewater treatment. In the field of waste valorization through fermentative pathways, one of the major criteria for industrial-scale application is to ensure a high degree of quality control to guarantee that the produced conversion products meet all requirements and product specifications. Besides the techno-economics of the process, the compatibility of the product with the existing infrastructure components such as storage, transportation, and distribution should also be considered, in order to meet the guidelines and regulations stipulated by local authorities.

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