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Acetogenic inoculum selection for acetate production from waste biomasses via thermal shock treatment

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Innovative treatment and utilization of waste biomass streams are crucial for increase environmental sustainability of human activities. Sewage sludge from the biological degradation of biomass can be valorized for the selection of biocatalysts capable to convert CO2 into valuable products. Indeed, chemoautotrophic microorganisms, like methanogens and acetogens, respectively, are able to convert CO2 into CH4 or acetate by using hydrogen as electron donor, i.e., their utilization for several bio-based CO2 reutilization processes has been widely proposed by several authors. Chemoautothrophic acetogens are widely present in waste streams deriving from the organic matter degradation, however, due to the syntrophic relationship between acetogens and acetoclastic methanogens in anaerobic environments, autothropihc acetate results immediately converted into methane. Therefore, the selection of an acetogenic inoculum which allow to obtain CO2 reduction into acetate, requires methanogens inhibition. Among the different methanogen’s inhibition strategies, the most common method is the use of BES (bromo-ethane sulphonate) which results a not scalable technique for large scale application. A most promising and sustainable approach is offered by the adoption of a thermal treatment which allows to the selection of an acetogenic inoculum, thanks ot the sporogenous capacity of acetogenic bacteria. This work presents the results obtained in the thermal pre-treatment of different type of waste biomasses coming from pilot and full-scale biological processes for the selection of an acetogenic inoculum able to convert CO2 into acetate. Each waste biomass was treated by a thermal shock procedure that consisted in the treatment of the dried biomass at 120°C for 2 hours. Acetogenic inoculums obtained by the thermal pre-treatment of an acidogenic fermentate, an activated sludge and a mesophilic anaerobic digestate, were tested under hydrogenophilic conditions in comparison with blank tests and raw inoculums. The results clearly indicate the effectiveness of the thermal pre-treatment in the selection of the acetogenic microorganisms



* 1. Introduction

The application of a circular economy concenpt is becoming more and more important in recent years, considering waste management as a possible source of income (Smol et al 2015). The utilization of sewage sludge or other organic waste streams as raw materials is becoming an attractive strategy considering the limited landfill availability (Gherghel et al 2019).The annual production of sewage sludge from wastewater treatment plants accounted for 3 million tonnes in Europe, which are typically disposed through landfill and incineration at a cost of 30-100 per ton (Wang et al 2017). In the treatment line, the sludge can be subjected to various treatments, such as heat treatment, electrical treatment, chemical, mechanical. Sludge stabilization through the anaerobic digestion is the most adopted strategy. Despite the conversion of sewage sludge into biogas, anaerobic digestion also produces digestate which usually requires further treatment and stabilization (Angenent et al 2004). Both the activated sludge and the anaerobic digestate are functionally complete from a biochemical point of view, as they constitute real mixed cultures that include a wide range of microorganisms. Very interesting microorganisms from a biotechnological point of view are those able to reach CO2 in products with added values, that is methanogens and acetogens, which are ubiquitous in the sludge due to the close syntrophy between the metabolic pathways involved in the degradation of the organic substance (Gomez Camacho et al 2018). Being the CO2 removal and recycle major challenge to reduce emissions by 2030 an interesting approach is the development of technologies that allowed the CO2 capture and conversion into valuable products. Recycling the CO2 into valuable products like fuels and chemicals represent an attractive strategy in the industrial sector in which concentrated CO2 flue gasses are available such as cement and steel production or the wastewater treatment. Among different strategies which involved thermochemical processes, the use of bio-based technologies for CO2 reduction offers a more sustainable and resilient alternatives because of the utilization of mild temperature and pressure conditions, the use of non-sterile conditions, and the low cost of the microorganisms. Trough the different biological carbon capture and utilization technologies, the fixation of CO2 into acetate performed by acetogenic microorganisms, also named homoacetogens (or acetogens), results particularly attractive due to the possibility to perform the conversion of CO2 into acetate which can be considered as the building block for further biotechnological transformation (Zeppilli et al 2016). A valuable integration of the biomass valorization is the adoption of waste organic materials as biocatalysts source through for biological reactions. Indeed, chemoautotrophic microorganisms, such as methanogens and acetogens, respectively, reduce CO2 to CH4 or acetate using hydrogen as an electron donor and their use has been proposed for several bio-based CO2 reduction applications, including biogas upgrading (Villano et al 2010 , Angelidaki et al 2018, Cristiani et al 2022) and chemicals production (Gildemyn et al 2018). In particular, acetate is a value-added product with many commercial and industrial uses and its production from the autotrophic pathway, named Acetyl CoA Pathway (or Wood-Ljungdahl pathway) constituted the reaction for the CO2 fixation into a first organic building block for further biotechnological application (Valentino et al 2021). Being acetate a fundamental substrate for methane production via acetoclastic methanogenesis in anaerobic environments, the methanogenesis inhibition is therefore necessary to obtain a net acetate production. Several strategies for methanogenesis inhibition have been proposed, such as the use of chemical compounds such as 2-bromo-ethane sulfonate or chloroform, pH control, ultrasonication pretreatment, aeration and thermal shock (et al). The 2-bromo-ethane sulfonate, which is a structural analogous of M-coenzyme necessary for the methanogenesis (Scholten et al 2006), is currently the most effective solution on a laboratory scale, however, its adoption in a scaled up and continuous flow process results not scalable due to the high cost of the BES and to its degradation linked to the release of sulphate.but not on an industrial scale. In terms of a process scaling up perspective, heat treatment is an attractive alternative, as it inhibits methanogens which are susceptible to heat, and selects spore-forming acetogens, whose spores are very resistant at high temperatures (Leguérinel et al 2007). This work presents the results obtained by testing the heat treatment of different biomasses. In this study, activated sludge, mesophilic anaerobic digestate and an acidogenic fermentate from full scale plants were thermally treated to obtain the methanogenesis inhibition for the selctio of an acetogenic inoculum. The acetogenic test were conducted under hydrogenophilic condition at two different level of pH (i.e. 7.5 and 5.5) considering the possibility to combine the pH and thermal treatment of the inoculum as combined strategies for the selection of an acetogenic inoculum from waste biomass streams.

2 Material and methods

2.1 Inoculum and thermal treatment

Three potential acetogens sources have been compared in the following study, an activated sludge from a wastewater treatment plant, a digestate from a full scale mesophilic anaerobic diestor and an acidogenic fermentate from a pilot-scale fermenter fed with agro-zootechnic waste. Each sludge was thermally treated by the following procedure (Zeppilli et al 2020), drying the sludge in a stove at 70 ° C, grounded with a mortar and sieved at 500 µm, and treated in a muffle at 120 °C for 2 hours. The treated powder was re-suspended in a mineral medium and washed three times for the soluble organic molecules removal. The composition of the mineral medium at pH 7.5 was 2.5 g/L K2HPO4, 4.0 g/L, NH4Cl, 0.125 g/L, CaCl2 0.05 g/L, MgCl2 0.1 g/L 1.0 ml vitamin solution and 10 ml metals solution. At pH 5.5, on the other hand, 8.0 g / L K2HPO4 and 156 g/L KH2PO4 were used to obtain a buffer solution at the desired value.

2.2 Hydrogenophilic tests operation

The experiments were carried out in a 245 ml volume borosilicate glass serum bottle. The serums were filled with 150 ml of liquid medium consisting of the raw inoculum or to the thermal treated inoculum suspencion obtained by the dispersion of 125 mg of powdwer in 150 mL mineral medium. The serum bottles were closed with rubber stoppers and flushed with a mixture of N2/CO2 (70-30 v/v) to ensure anaerobic conditions. Experiments were conducted at two different pH levels, 7.5 and 5.5, with both thermal treated and non-pretreated sludge. Each trial was tested under hydrogenphilic conditions and under endogenous control conditions (i.e. no H2 addition). For each hydrogenphilic test 2.04 mmol (50 ml) of H2 (partial pressure equal to 0.52 atm) was initially added, and it was added whenever its concentration came close to zero, to ensure that it was always present. in the system.

2.3 Analytical methods and calculations

Analytical methods for CH4, H2, CO2, and CH3COO- determination has been already described (Zeppilli et al 2020). Main calculation related to the anodic and cathodic bioelectrochemical reactions are summarized in Table 1.

*Table 1. Main parameters calculations*

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| --- | --- |
| **Conversion of concentration into meq** | $$meq=mM\*ne^{-}\*V$$ |
| **CH3COO-/CH4 production rate (rCH3COO-/CH4 )** | $$\frac{rCH\_{3}COO^{-}}{CH4} =\frac{mmol\_{\frac{CH\_{3}COO^{-}}{CH\_{4}}}}{V\*Δt} \*8 = \frac{meq\_{\frac{CH\_{3}COO^{-}}{CH\_{4}}}}{V\*Δt}$$ |
| * **rCH3COO-**(meq/Ld): daily milliequivalents in acetate per liter
* **8 meq/mmolCH3COO-:** conversion factor
 |
| **Hydrogen consumption efficiency (**η%**)** | $$η\_{CH\_{3}COO^{-}/CH4}=\_{ \frac{meq\_{CH\_{3}COO^{-}/CH\_{4}}}{meq\_{H\_{2}}}}$$ |
| * **meq*H2*:** calculated with conversion factor of 2 meq/mmol
 |

3. Results and discussion

3.1 Effect of the thermal treatment on the selected inouculums

Figure 1 shows the results obtained for the activated sludge inoculum tested at pH of 7.5. The thermal treatment effect results evident by the comparison of Figure 1-A and Figure 1-C which reported the methane and acetate time course for the hydrogenophilic tests conducted on raw activated sludge and thermal treated activated sludge, respectively. Indeed, while the use of raw activated sludge allowed for a net production of acetate for the first 20 days while, after day 20 the acetoclacist methanogenesis acclimatation allowed for the conversion of acetate into methane. On the contrary the use of the thermal treatment of the activated sludge allowed for the complete inhibition of the methanogenesis until day 21, which produced methane at a negligible rate. Figure 1-B and Figure 1-D, which constituted the endogeneous test conducted without addition of hydrogen, showed similar beahaviuor with respect the corresponding hydrogenophilic tests with a lower activity, indeed, the residual activity probably is related to the availability of organic carbon which can be used as an electron donor through the synthrophy with fermentative microorganisms.



Figure 1: Time course of the batch tests conducted at pH 7.5 on activated sludgewiht and without thermal treatment under hydrogenophilic (A, C) and endogenous condtion (B, D).

3.2 Acetate production in the different inoculums adopted

It was possible to appreciate the effect of the thermal treatment and of the adopted pH on acetate production by the Figure 2 which reported the net acetate production rate and the hydrogen utilization efficiency (i.e. the ratio between the acetate produced and the provided hydrogen ). Among the different inoculum the thermal treatment positive effect on acetate production at pH 7.5 is clearly showed in the figure 2, more in details, thermal treated activated sludge showed the higher production rate of 20 mg/Ld with an efficiency of 45 %. On the other hand, despite a slightly lower acetate production rate of 15 mg/Ld, thermal treated digestate at pH 7.5 showed a higher efficiency of 65 %, representing the higher hydrogen consuming efficiency determined among the different test. Thermal treated acidogenic fermentate showed a negligible production rate of 0.5 mg/Ld, with a hydrogen consuming efficiency of 22 %. The diffent tests conducted at pH 5.5 showed in general lower acetate production rate and hydrogen consuming efficiencies, however, it is interesting to highlight a different behaviour of the tests considering the type of selected inoculum. Indeed, while activated sudge and digestate showed higher performances at pH 7.5, the acidogenic fermentate showed higher acetate production rate and hydrogen consuming efficiency at pH 5.5. This evidence are well explained by considering the physiological pH of the selected inocuum, i.e. while the physiological pH of the activated sludge and digestate resulted 7.2 and 7.5 after their sampling, the acidogenic fermentate operated at a pH of 5.5. Moreover, acidogenic fermentate showed a higher acetate production rate without thermal treatment, indicating a negative effect performed by the heat shock strategy.

3.3 Methane production in the different inoculums adopted

The different results in terms of methane production rate and hydrogen consuming efficicency have been reported in Figure 3. The tests conducted by the three different inoculums are in accordance with the effects described for the acetate production, indeed, methane production rate resulted higher in the raw inoculum wich was not thermal treated. The raw activated sludge and the digestate showed the same methane production rate of 7 mg/Ld despite a higher hydrogen consuming efficiency of 64 showed by the digestate at pH 7.5. Moreover, as previously showed in Figure 1 for the thermal treated activated sludge, methane production was considerably reduced by the thermal treatment. The adoption of a pH of 5.5 also considerably reduced the methane production rate in the raw activated sludge and digestate while, the combination of thermal treatment and pH 5.5 completely inhibited the methanogenic activity. The acidogenic fermentate showed a negligible methane production rate in all the conducted tests with the exception of the thermal treated test conducted at pH 7.5 which showed a methane production rate of 4 mg/Ld. The latter evidence probably derived by the acclimatation of new acetoclastic methanogens ata favourable pH of 7.5.



Figure 2: Production performance of acetate of production rate and efficiency. AS = activated sludge; D = digestate; AF= acidogenic fermentate; TT = thermal treatment.



Figure 3: Production performance of methane in terms of production rate and efficiency. AS = activated sludge; D = digestate; AF= acidogenic fermentate; TT = thermal treatment.

4. Conclusions

In this study the possible utilization of waste biomass streams coming from wastewater or organic waste treatment has been demonstrated. Throuhg the comparative study of three different waste biomass streams (e.g., activated sludge, anaerobic digestate and an acidogenic fermentate) it was shoed how a thermal treatment allowed for the production of biological catalysts for the CO2 conversion into acetate trough the acetogenesis reaction. Thermal treatment of the biomass and pH tuning in the adopted test can significantly improved the acetogenic capability of the microrganisms present in the waste stream. Inded, through the thermal treatment activated sludge and digestate was possible to obtain, at pH 7.5, a net production of acetate with the respect the raw corresponding inoculums in which acetate was rapidly converted into methane due to the acclimatation of acetolastic methanogens. Under the pH of 7.5, the activated sludge showed the higher acetate production rate of 20 mg/Ld with a corresponding hydrogen consuming efficiency of 45 %, moreover, digestate showed a slightly lower acetate production rate of 15 mg/Ld showing a considerably higher hydrogen consumption efficiency of 65 %. Regarding the tests at pH 5.5, due to the non-optimal pH condition for the activated sludge and digestate, the production rates were lower than pH 7.5 for acetate and methane in presence or not of the thermal treatment. According to the starting physiological pH of the inoculum, acidogenic fermentate hydrogenophilic tests conducted at pH 7.5 were considerably lower in terms of acetate production than the activated sludge or digestate, the latter evidence was clearly attributed to the difference in terms of microoranisms composistion. Methanogenesis inhibition was effective at this pH in aerobic sludge and digestate, except in the acidogenic fermented, in which no trace of methane was found at pH 5.5.

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