A biorefinery case study based on the valorization of *Opuntia Ficus Indica* for biofuels and bio-compounds productions

F. Rizzioli, F. Battista, D. Bolzonella

Department of Biotechnology, University of Verona, Strada Le Grazie 15, 37134 Verona, Italy
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Presenting author email: fabio.rizzioli@univr.it

Opuntia ficus-indica, known also as nopal or prickly pear, is a crassulacean acid metabolism (CAM) plant, which is capable to live in environments that are usually hostile to other plants, such as arid and semi-arid lands. For this reason, *Opuntia ficus-indica* gained interest as alternative to classic energy crops in arid soils, such as southern Italy. As energy crop, the main purpose of *Opuntia ficus-indica* cultivation is to provide energy, commonly as biomethane, through Anaerobic Digestion (AD) process. Garcia et al. (2019) reported a potential biomethane yield of 345 LCH_4 per kgVS, which is comparable to maize, wheat and triticale which yields ranged between 425 to 260 LCH_4 per kg VS. AD is a renewable form of energy generation and is seen as a key factor for the European Union (EU) for the reaching of an energetic independence that can contrast the energetic crisis sparked by the outbreak of Russian-Ukraine war. With the plan "REPowerEU", the EU set an increase of the biomethane production from 3 bcm (billion cubic meters) of the 2021 to 30 bcm by 2030. This ten-fold increase requires an optimization of the over 19,000 biogas AD plants currently operating in the EU countries and, most importantly, a new land exploitation, especially the uncultivated ones like the semi-arid lands of southern Europe, in particular Italy, Greece and Spain, where the *Opuntia ficus-indica* plants naturally grows.

In addition, with AD is also possible to obtain Volatile Fatty Acids (VFAs) which are short-chain carboxylic acids that are used as "building blocks" in chemical, pharmaceutical, food, cosmetic and bioplastic industries. VFAs are thus high-value compound that are currently produces through non-renewable methods (Rizzioli et al., 2021). The global market for caproic acid is estimated to be valued 176.6 million US$ in 2020, with a projected increase of 7.0% by 2027, when will reach an estimated value of 283.6 million US$.

Another product of AD is the digestate, which is the remaining part of the substrate after the digestion process. The digestate is composed by recalcitrant lignocellulosic material and, most importantly, can be rich in nutrients such as nitrogen and phosphorus, that can be recovered to produce a soil conditioner and fertilizer (Rizzioli et al., 2023).

On behalf of the numerous applications mentioned above, AD is a promising application not only for reaching an energetic independence with biomethane production, but also to improve the market of biobased products, such as VFAs, and fertilizers. In this work, we evaluated the feasibility of *Opuntia ficus-indica* cladodes for a biorefinery loop, evaluating the VFAs, biomethane and nutrient recovery yields.

Materials and methods

The performance of the AD of *Opuntia ficus-indica* cladodes was evaluated through bio-methanation potential tests (BMP). The minced cladodes used as substrate are characterized by a high apparent viscosity that can influence the mixing of the bioreactor and, consequently, can reduce the biogas production yields. For this reason, the first test regarded the evaluation of AD at different apparent viscosity of the substrate. The different apparent viscosities of the substrate were obtained modifying the water to substrate ratio (0; 5; 13; 24; 51; 64 % w/w). The second test concerned the effect of acid and basic pretreatments to the AD performance, which could improve the biomethane yield through solubilization of the lignocellulosic material of the substrate. For the acid and basic pretreatment, the substrate was treated over-night at pH 2 through HCl addition and at pH 13 through NaOH addition, respectively. All the BMP tests were performed accordingly to Angelidaki et al. (2009).

The best conditions obtained with the BMP tests were evaluated in continuous mode with a laboratory-scale bioreactor. The tests were performed at different Hydraulic Retention Times (HRT) (1.05; 2.5; 5; 10; 20; 30; 40 days) in order to evaluate the productivity for VFAs, in particular caproic acid, and biomethane. All tests were performed at 37°C. The biogas volume was evaluated through water-displacement method; the biomethane concentration was determined with a portable gas analyzer (Geotech Biogas 5000); the VFAs concentration was analysed by an ion-chromatography system (Dionex ICS 1100 with AS23column).

For this work, the substrate for AD, *Opuntia ficus-indica* cladodes, were provided by AssoBiometano company (Catania, Italy). The inoculum for AD batch and continuous tests was originated from a full-scale anaerobic digestor located in Isola della Scala (Italy) which treats agricultural residues and animal manure. The substrate and inoculum characterization are listed in Table 1.

Table 1. Substrate and inoculum characterization

	Substrate	Inoculum (Digestate)
Total Solids (%w/w)	7.37 ± 0.26	6.86 ± 0.37
Volatile Solids (%w/w)	5.01 ± 0.20	4.53 ± 0.19
VS/TS (%)	68.05 ± 0.38	66.02 ± 0.28

COD	503.96 ± 13.60 mgO_2/gTS	48.29 ± 0.91 gO_2/L
TKN	14.50 ± 0.39 mgN/gTS	6.97 ± 0.42 gN/L

Results and discussion

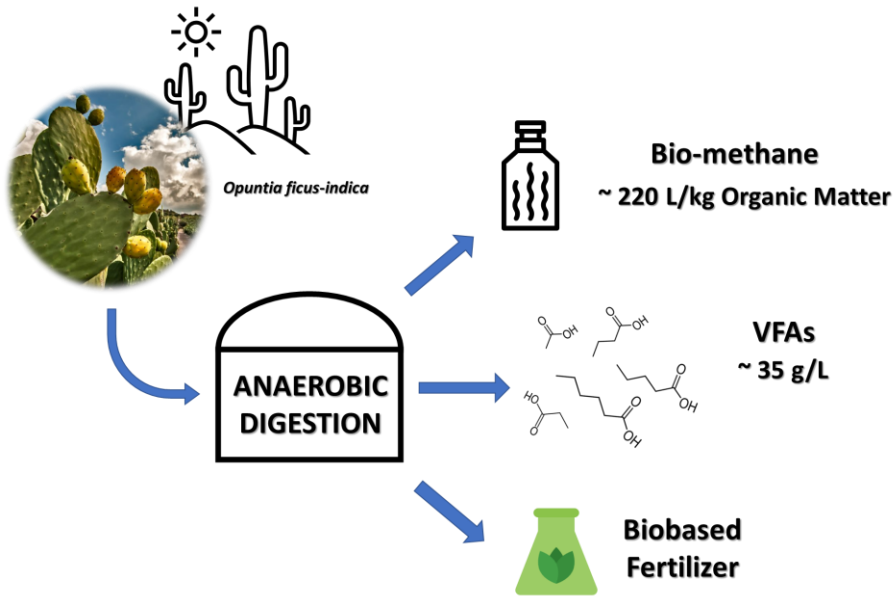
The first test, regarding the AD evaluation at different apparent viscosity, reported no significative difference on biomethane production yields between the different water to substrate ratio, with a maximum of 300 LCH_4 per kgVS. Thus, we selected 5 % water to substrate ratio for the next tests because this ratio is the minimum amount of water needed to reduce the viscosity to an acceptable level for the bioreactor mixing.

Regarding the evaluation of the acid and basic pretreatment, the BMP tests reported no difference for the overall biomethane production yield, compared to the non-pretreated reference. Moreover, the reaction rates of the pretreated tests can suggest a slight inhibition of the reaction at the initial days, probably due to the formation of inhibitors during the decomposition of the lignocellulosic content (Wang et al., 2020). For this reason, no pretreatment of the *Opuntia ficus-indica* cladodes was performed for the successive tests.

The continuous test for the evaluation of the productivity for VFAs and biomethane at different HRT indicated the HRT 5 as the best for VFAs and caproic acid accumulation, while HRT 20 was the best regarding the biomethane production. These results are expected and consistent with the reaction times of the AD process, allowing to modulate the reaction duration and, consequently, to selective obtain different compounds.

These results indicate that *Opuntia ficus-indica* cladodes are a promising substrate for AD. However, further analysis are needed to optimize VFAs and biomethane yield.

Graphical Abstract



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