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Ectoine production from biogas: A sensitivity analysis. Effect of local commodity prices, economy of scale, market trends and biotechnological limitations

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ABSTRACT

The utilization of methanotrophic haloalkaliphilic bacteria for the production of ectoine in waste treatment plants has demonstrated a great potential to upgrade the traditional use of biogas as energy vector and the current industrial routes for the production of this high added-value chemical (600–1000 €·kg^{-1}). However, the influence of socio-economic aspects such as the location of the plant, the economy of scale and the market fluctuations on the profitability of the process remains unknown. A techno-economic and sensitivity analysis of the bioconversion of CH_4 into ectoine in a bubble column bioreactor and the extraction and purification of ectoine via ionic exchange chromatography was herein conducted using Madrid as base-case scenario. The geographical assessment performed in 13 representative cities revealed high differences in the ectoine production costs, ranging from 158 to 231 €·kg^{-1}. The economy of scale analysis evidenced a high dependence of the ectoine production costs towards the production scale, amounting to 782 and 164 €·kg^{-1} when manufacturing 0.1 and 89.6 t ectoine·y^{-1}, respectively. The techno-economic study also showed a high robustness of CH_4-ectoine profitability towards future market fluctuations, with all the scenarios analyzed guaranteeing internal rates of return >15% and payback periods <10 y. Finally, the sensitivity analysis identified the improvement of CH_4 elimination capacity in bioreactors, the development of highly efficient microbial strains and the selection of the highest quality ionic exchange resins as key factors impacting the profitability of future biogas-to-ectoine biorefineries.

1. Introduction

During the last decade, the construction of new anaerobic digestion plants and the associated biogas production in Europe have declined, showing a marginal growth of 4.3% between 2015 and 2019 (European Biogas Association, 2021). This decline was likely induced by the decreasing profitability of biogas valorization into heat and energy caused by the high capital (400–1100 €·kW^{-1}) and maintenance costs (0.01–0.02 €·kWh^{-1}) of co-generation biogas engines (Wellinger et al., 2013). Additionally, the rapid decrease in production costs of competing solar and wind energies (82 and 39% drop between 2010 and 2019, respectively), has triggered the end of fiscal exemptions on the use of biogas as renewable energy vector, reducing dramatically the incomes associated to this technology (International Renewable Energy Agency, 2020; Pérez et al., 2020a).

In this context, the attention of policy-makers and academia has progressively shifted towards the use of the main biogas constituents, methane (CH_4) and carbon dioxide (CO_2), as raw materials for the chemical and biotechnology industry and towards the transformation of the current linear waste treatment plants into modern circular biorefineries (European Comission, 2020). As a result, in the last few years, different European demo-scale projects have incorporated in their biorefinery concepts the transformation of biogas into marketable products such as: biomethane (INCOVER and URBIOFIN), polyhydroxyalkanoates (URBIOFIN), biostimulants (CIRCULAR BIOCARBON) or ectoine (DEEP PURPLE). These recent investigations have demonstrated the technical feasibility and the enormous economic potential of using mixed methanotrophic bacterial cultures for the bioconversion of the CH_4 present in biogas into a portfolio of bulk commodities such as polyhydroxybutyrate

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Abbreviations

BCB	Bubble column bioreactor
CH_4-EC	Methane elimination capacity
CH_4-RE	Methane removal efficiency
CSTR	Continuous stirred tank reactor
EBRT	Empty bed residence time
H/D	Height to diameter ratio
HRT	Hydraulic residence time
H_2S-RE	Hydrogen sulfide removal efficiency
IEX	Ionic exchange chromatography
IRR	Internal rate of return
NPV_{20}	Net present value evaluated at 20 years
PEC	Purchased equipment cost
PP	Payback period
TIC	Total investment cost

(PHA), methanol or single cell protein (Cantera et al., 2018b; Pérez et al., 2020a; Strong et al., 2016; Toledo-Cervantes et al., 2017). Additionally, these studies have demonstrated that the development of cleaner biogas valorization technologies entails a significant reduction of the environmental impact measured in terms of emissions of greenhouse gases and release of toxic compounds to the atmosphere and water bodies (Pérez et al., 2020b; Toledo-Cervantes et al., 2017). However, these bioproducts cannot often compete in price against their petro-chemical counterparts, due to the high investment and operational costs derived from the poor solubility of methane and oxygen (O_2), the low productivity of methanotrophic bacteria and the low market price of these bulk chemicals (Pérez et al., 2020a).

The potential of haloalkaliphilic methanotrophic species capable of accumulating high contents of ectoine, a hyposmotick protector with a retail market value ranging from 600 to 1000 €·kg^{-1}, has recently shifted the attention of the waste treatment and industrial biotechnology sector to the production of fine and high added-value chemicals from biogas (Cantera et al., 2018a; Strong et al., 2015). Recent studies have reported a high profitability of the CH_4-to-ectoine process given the significant difference between the ectoine production costs (200–300 €·kg^{-1}) and the current market value of this chemical (600–1000 €·kg^{-1}) (Cantera et al., 2018a; Pérez et al., 2021). However, some socio-economic aspects such as the influence of commodity prices and the effect of the economy of scale on process profitability, or the feasibility of displacing the current industrial routes of ectoine production based on high-quality sugars, remain unknown.

To the best of the authors knowledge, this study constitutes the first comprehensive analysis assessing the profitability of biogas for the production of ectoine in waste treatment plants. For this purpose, a techno-economic analysis has been performed including the anoxic desulfurization of biogas, the bioconversion of CH_4 biogas into ectoine using a mixed culture of methanotrophic haloakaliphilic bacteria, the extraction of the ectoine via hyposmotick shock and the purification of the final product via ionic exchange chromatography and electrodialysis. Additionally, the sensitivity of ectoine production costs towards the commodity prices in 13 representative cities, the economy of scale, the market fluctuations and current biotechnological limitations were herein evaluated.

2. Materials and methods

2.1. Process design

As recommended by industrial waste managers, a medium-size municipal waste treatment plant with a treatment capacity of 300 t·d^{-1} and biogas production potential of 1000 Nm^3·h^{-1} was considered as a representative and common scenario in the European context for the production of ectoine from biogas. Madrid (Spain), which presents worldwide average values for energy, water and labor costs, was selected as the ideal location for the construction of the plant (Pérez et al., 2020a). The mass and energy balances were calculated considering a yearly ectoine production of 10 t, for which only 67 Nm^3 biogas·h^{-1} were required as raw material. The process was modeled including four different stages: (I) biogas desulfurization in an anoxic biotrickling filter, (II) ectoine biosynthesis from CH_4-biogas in a bubble column bioreactor (BCB), (III) ectoine extraction via hyposmotic shock and (IV) ectoine purification via ionic exchange chromatography (IEX) (Fig. 1). A more detailed process flow diagram, including all the auxiliary equipment, is included in the Supporting Information (Figures S1-S3).

2.1.1. Biogas desulfurization

Firstly, a hydrogen sulfide (H_2S) removal step was included for preventing the formation of sulfuric acid (H_2SO_4) in the presence of water and the associated corrosion and damage in CH_4 bioconversion and downstream equipment. Biological biogas desulfurization in an anoxic biotrickling filter was selected as the best platform technology, as suggested elsewhere, given its high H_2S removal efficiency (H_2S-RE) and low operational costs (Muñoz et al., 2015). The biotrickling filter was dimensioned assuming a biogas empty bed residence time (EBRT) of 3 min and a H_2S-RE of 99%. This desulfurization technology is based on the utilization of a culture of sulfur-oxidizing bacteria capable of using nitrate (NO_3^-) as electron acceptor for the oxidation of H_2S into sulfate (SO_4^{2-}) (Lebrero et al., 2016). The packing media was sprayed at a trickling liquid velocity of 10 m·h^{-1} with a sodium nitrate ($NaNO_3$) and micronutrients solution for guaranteeing a high nitrogen-to-sulfur molar ratio of 2.5 (Almenglo et al., 2016). A 5 M sodium hydroxide (NaOH) solution was added for maintaining a constant pH value of 7.

2.1.2. CH_4-biogas bioconversion into ectoine

A BCB equipped with fine bubble diffusers was selected as model bioreactor for the cultivation of haloalkaliphilic methanotrophic bacteria, responsible for the bioconversion of CH_4-biogas into ectoine. Atmospheric air was supplied for guaranteeing a constant O_2:CH_4 molar ratio of 1.5. A BCB with a biogas EBRT of 1.2 h and a height-to-diameter ratio (H/D) of 10 was designed for an enhanced gas-liquid mass transfer of CH_4 (biogas) and O_2 (air), resulting in a CH_4 removal efficiency (CH_4-RE) of 90%. A CH_4-elimination capacity (CH_4-EC) of 148 g CH_4·m^{-3}·h^{-1} was assumed given the high turbulence in the liquid phase and the high H/D of the bioreactor. This value was extrapolated from commercial BCBs treating 30 %v·v^{-1} carbon monoxide (CO) streams, which have consistently supported CO-EC up to 1 kg CO·m^{-3}·h^{-1} (Humbird et al., 2017; Lantatech, 2021; Muñoz et al., 2018; Yu et al., 2006). Detailed calculations regarding CH_4-EC and gas-liquid volumetric mass transfer coefficients (kl_a) in large-scale bioreactors are included in the Supporting Information (Figure S4).

A mixed culture of haloalkaliphilic methanotrophic bacteria was selected for the bioconversion of CH_4-biogas into ectoine. Recent work at laboratory scale has demonstrated the ability of these cultures to support long-term and efficient CH_4 removal and ectoine productivity under high salinity conditions. These studies have shown a predominance of ectoine producing methanotrophic species such as *Methylomicrobium japanense* and *Methylomicrobium buryatense* in these mixed cultures (Carmona-Martínez et al., 2021; Rodero and Muñoz, 2021). The BCB was operated under a fed-batch strategy with a dilution rate of 0.4 d^{-1}. A mineral medium solution containing $NaNO_3$, sodium chloride (NaCl) and trace amounts of micronutrients was used to support haloalkaliphilic methanotrophic bacteria growth and ectoine biosynthesis (Cantera et al., 2017a). A NaCl concentration in the mineral medium solution of 6 %w·w^{-1} was selected as the optimal salinity for the accumulation of ectoine in haloalkaliphilic methanotrophic cultures as suggested by Cantera and colleagues (Cantera et al., 2017a;

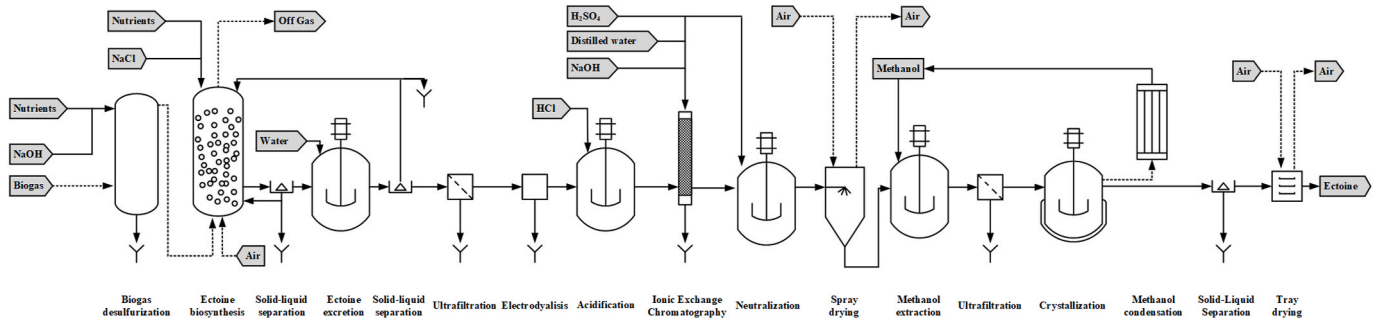

Fig. 1. Simplified process flow diagram for CH_4-biogas bioconversion into ectoine.

Carmona-Martínez et al., 2021). Microbial kinetics for the oxidation of CH_4 and the production of biomass ($C_4H_8O_2N$), ectoine ($C_6H_{10}N_2O_2$) and carbon dioxide (CO_2) were modeled according to equations (1)–(3). A biomass (X) yield of 0.4 g X·g CH_4^{-1}, an ectoine yield of 70 mg ectoine·g X^{-1} and a mineralization ratio of 0.7 mol CO_2·mol CH_4^{-1} were herein considered (Carmona-Martínez et al., 2021).

$$\text{Biomass production:}\quad CH_4 + \frac{3}{8}\,O_2 + \frac{1}{4}\,NO_3^- \rightarrow \frac{1}{4}\,C_4H_8O_2N + H_2O \qquad (1)$$

$$\text{Ectoine production:}\quad CH_4 + \frac{1}{4}\,O_2 + \frac{1}{3}\,NO_3^- \rightarrow \frac{7}{6}\,H_2O + \frac{1}{6}\,C_6H_{10}N_2O_2 \qquad (2)$$

$$\text{Mineralization:}\quad CH_4 + 2\,O_2 \rightarrow 2\,H_2O + CO_2 \qquad (3)$$

2.1.3. Extraction of ectoine via hyposmotic shock

The methanotrophic biomass containing intracellular ectoine was centrifuged to a biomass concentration of 200 g X·L^{-1}. An aliquot of 10% of the liquid volume in the bioreactor was continuously wasted from the system in order to avoid the accumulation of secondary metabolites. Then, 85% of the intracellular ectoine was naturally excreted by subjecting the concentrated biomass stream to a hyposmotic shock in a non-saline medium, in a process known as "bio-milking" (Cantera et al., 2017b). The *bio-milking* process was carried out in a continuous stirred tank reactor (CSTR) designed with a hydraulic retention time (HRT) of 5 min. After the *bio-milking* process, the biomass containing the remaining intra-cellular ectoine was centrifuged to a biomass concentration of 200 g X·L^{-1} and recycled to the BCB. Similarly to the industrial process using *Halomonas Elongata*, an aliquot of this concentrated biomass stream was daily wasted from the system in order to guarantee a maximum biomass age of 9 d. Process operation at a low biomass residence time has been shown as an effective method for preventing biomass activity decay and promoting high ectoine accumulation yields (Strong et al., 2016). The ectoine liquid stream resulting from the *bio-milking* process was subsequently filtered and desalinized in a sequential ultrafiltration and electrodialysis process. In this study, ultrafiltration membranes were designed with a typical permeate flux of 15 L·m^{-2}·h^{-1}, a pressure drop of 300 mbar and a solid recovery efficiency of 99%. Similarly, electrodialysis systems were calculated with a permeate flux of 45 L·m^{-2}·h^{-1}, a pressure drop of 200 mbar and a salt recovery efficiency of 96%.

2.1.4. Ectoine purification

A two-step IEX and methanol crystallization process was simulated for the purification of the final product. This method has been reported in literature as the most scalable and cost-effective process for ectoine concentration and purification, achieving a product recovery of 62% and an ectoine purity of 97% (Chen et al., 2017; Fülberth et al., 2002). Prior to the adsorption of ectoine on the ionic exchange resin, the liquid stream was acidified to pH 2 via addition of 10 M hydrochloric acid (HCl) in a CSTR designed with a HRT of 1 h. Then, the acidified liquid stream containing ectoine was selectively adsorbed in an IEX column with a 500 L bed volume (BV) packed with a high performance ion exchange resin (DOWEX 50w × 8). This ionic exchange resin exhibits an adsorption capacity of 0.1 kg ectoine·kg $resin^{-1}$, a density of 800 kg·m^{-3} and an ectoine recovery efficiency of 90% (Sauer and Galinski, 1998). The adsorbed ectoine was first washed with 2 BV of 98 %w·w^{-1} H_2SO_4 and then washed with 2 BV distilled water, in order to remove impurities. Then, the adsorbed ectoine was eluted with 6 BV of 1.3 M NaOH, of which 4 BV were discarded. Finally, the liquid stream was neutralized to pH 7 via addition of 98 %w·w^{-1} H_2SO_4 in a CSTR designed with an HRT of 1 h. After the neutralization process, the ectoine broth was dried to a solid content of 95 %w·w^{-1} via spray drying. The spray drying system was operated at 300 mbar and heated with low pressure steam (2 bar), assuming a specific evaporation rate of 100 kg water·m^{-3}·h^{-1}.

Ectoine solubilization into methanol was performed in a CSTR operated at a HRT of 1 h and a methanol-to-ectoine ratio of 10 kg·kg^{-1}. Prior to ectoine crystallization, an ultrafiltration step was required for removing the insoluble sodium sulfate (Na_2SO_4) formed during the neutralization step. Ectoine crystallization was carried out in a continuously stirred crystallizer operated at a HRT of 1 h. An evaporation-condensation cycle using low pressure steam (2 bar) and cooling water (15 °C) was used for recovering 99% of the methanol. The final product was obtained after removal of the remaining methanol via centrifugation and subsequent tray drying using warm air (20 °C).

2.2. Economic analysis

The economic analysis evaluated the profitability of biogas bioconversion into ectoine based on the net present value evaluated at 20 years (NPV_{20}), the internal rate of return (IRR) and the payback period (PP). The NPV_{20} was calculated according to equation (4):

$$NPV_{20} = \sum_{t=0}^{t=20} \frac{FCF_t}{(1+r)^t} \qquad (4)$$

Where t stands for the time period in years, *FCFt* represents the free cash flow in the period t and r is the interest rate (considered 5% in this study). *FCFt* was calculated attributing the total investment cost (TIC) to year 0 and a circulating capital over the TIC of 5% to year 1. A tax rate of 30% and a linear depreciation of 20 years were considered for the calculation. The IRR was calculated according to equation (5), as the value of r to obtain a $NPV_{20} = 0$.

$$NPV_{20} = \sum_{t=0}^{t=20} \frac{FCF_t}{(1+IRR)^t} = 0 \qquad (5)$$

The PP was calculated as the first t necessary to obtain a positive *NPVt*, according to equation (6).

$$NPV_t = \sum_{t=0}^{t=PP} \frac{FCF_t}{(1+r)^t} \geq 0 \qquad (6)$$

A median ectoine selling price of 600 €·kg^{-1} was selected for the calculation of NPV_{20}, IRR and PP in this article. The ectoine production costs (or minimum ectoine selling price) were estimated according to the

break-even price which is the value of sales that guaranteed a NPV_{20} equal to zero.

2.2.1. Capital costs

Lang's method was used for estimating the TIC in this study. This method is based on a series of multiplying factors, allowing the estimation of the total equipment installation costs based on the individual price of equipment (PEC) (Ulrich and Vasudevan, 2006). A Lang's factor of 4.1 was calculated for the base-case scenario (Madrid), in agreement to the recommended factor for solid-liquid processes like the one presented in this paper (Table 1) (Levett et al., 2016). Most PEC was obtained from Matches' online estimation tool, a database that compiles purchase equipment prices for more than 275 types of equipment (Matches, 2021). The PEC not included in Matches was obtained from literature and from quotations with international companies and experts. Detailed equipment costs for the base-case scenario can be found in the Supporting Information (Table S1).

2.2.2. Operational costs

Operational costs were calculated including consumables (raw materials, chemical reagents and utilities), transportation, maintenance, labor and wastewater treatment costs. Mass and energy balances were used for the calculation of commodity and consumable requirements (energy, water, utilities, reagents and raw materials). The energy balances were based on the estimation of individual equipment energy consumption rates. For pumps, the power consumption was calculated according to equation (7), where P_{pump} stands for the power consumption, Q represents the volumetric flow, ΔP is the pressure drop and 0.7 is the electrical efficiency of pumps.

$$P_{Pump} = \frac{Q \cdot \Delta P}{0.7} \tag{7}$$

Power requirements for blowers and compressors were estimated according to equations (8) and (9), where P_{Blower} stands for the power requirements, P_{is} is the isentropic power, 0.7 represents the electrical blower efficiency, γ refers the adiabatic coefficient, T_{out} is the gas isentropic outlet temperature, T_{in} stands for the gas inlet temperature, Pm represents the gas molecular weight and Q stands for the inlet volumetric flow.

$$P_{Blower} = \frac{P_{is}}{0.7} \tag{8}$$

$$P_{is} = 2.31 \cdot \frac{\gamma}{\gamma - 1} \cdot \frac{T_{out} - T_{in}}{Pm} \cdot Q \tag{9}$$

Energy requirements for the less common pieces of equipment such as centrifuges (1 $kWh \cdot m^{-3}$), mixers (0.2 $kWh \cdot m^{-3}$) and electrodialysis membranes (7 $kWh \cdot m^{-3}$) were calculated as suggested in specific literature (Cantera et al., 2018a; Szepessy and Thorwid, 2018; Turek, 2003). A detailed summary of the individual energy requirements in the base-case scenario can be found in the Supporting Information (Table S2).

Maintenance costs were estimated as 3.5% over the TIC (Eti and Ogaji, 2006). As recommended by industrial waste operators, labor costs were calculated assuming a total of 192 person-h·$week^{-1}$. This value was calculated assuming that 2 full-time operators with 8 h-shift during week days and 2 part-time operators during the evening and night shifts during the whole week were required. The average salary in Madrid (14.5 €·person-h^{-1}) was used for the estimation of direct labor costs in the base-case scenario (Worlddata.info, 2021). Given the low organic load of the wastewater produced in this process, wastewater treatment costs were considered comparable to domestic wastewater treatment costs (0.2 €·m^{-3}) (Pérez et al., 2020b). The transportation costs for finished products and raw materials were assumed similar to those of ordinary petrochemical products (60 €·t^{-1}).

Table 1
Lang's factor calculation in New Delhi (lowest price level), Madrid (base-case scenario) and Doha (highest price level).

	New Delhi	Madrid	Doha
Equipment	1.00	1.00	1.00
+ Equipment installation labor[a]	0.03	0.38	0.94
+ Instrumentation and controls	0.12	0.12	0.12
+ Piping	0.31	0.31	0.31
+ Electrical installations	0.10	0.10	0.10
+ Buildings	0.29	0.29	0.29
+ Yard improvements[a]	0.01	0.10	0.25
+ Service facilities	0.54	0.54	0.54
+ Land	0.06	0.06	0.06
Direct plant cost	2.45	2.90	3.60
+ Engineering and supervision[a]	0.02	0.32	0.79
+ Construction expenses[a]	0.02	0.34	0.84
Direct and indirect costs	2.50	3.56	5.23
+Contractor's fee	0.12	0.18	0.26
+Contingency	0.25	0.36	0.52
Total depreciable costs (Lang Factor)	2.87	4.09	6.01

[a] Wage dependent parameters.

2.3. Sensitivity analysis

2.3.1. Influence of combined commodity prices: geographical analysis

A geographical analysis was performed in order to study the combined influence of commodity prices (electricity, water and wage) on the ectoine production costs. This approach allows a more sensitive cost analysis given the high geographical variability of local wages, energy and water selling prices (Estrada et al., 2012; Pérez et al., 2020a). The influence of commodity prices on the fixed and amortization costs, and on the operational costs of ectoine production was assessed individually. The geographical analysis focused on 13 representative cities of the world (Table 2). Energy and water industrial selling prices were obtained from national suppliers (Pérez et al., 2020a). The influence of wage was estimated using a price level factor. The price level factor was calculated from the annual average wage (Aw) expressed in €·y^{-1} in each city, using Madrid as a reference city, according to equation (10) (Worlddata.info, 2021). In this context, the variability in the average wage induced a significant change not only on the direct labor costs but also on the calculation of the Lang's Factor, given the wage-dependent nature of some of the factors considered (Table 1).

$$Price\ level = \frac{Aw_i}{Aw_{Madrid}} \tag{10}$$

2.3.2. Influence of the economy of scale

An economy of scale analysis was performed for assessing the influence of process scale on the ectoine production costs. The effect of the economy of scale on the fixed and amortization costs, and on the

Table 2
Summary of electricity and water prices and average wage selected for the geographical analysis.

City	Energy (€·kWh^{-1})	Water (€·m^{-3})	Wage (€·y^{-1})	Price level
New Delhi	0.07	0.58	1945.0	0.07
Johannesburg	0.07	1.95	5541.3	0.20
Singapore	0.16	0.43	5090.8	0.18
Shanghai	0.07	0.19	9532.1	0.34
Sofia	0.08	1.00	8779.8	0.32
Sao Paulo	0.16	2.13	8376.1	0.30
Madrid	**0.10**	**1.89**	**27853.2**	**1.00**
Toronto	0.08	2.28	39779.8	1.43
Tokyo	0.17	1.90	38266.1	1.37
Sydney	0.21	1.27	50550.5	1.81
Copenhagen	0.08	5.59	58715.6	2.11
Los Angeles	0.12	2.70	60412.8	2.17
Doha	0.02	1.21	68557.8	2.46

operational costs, was assessed individually using Madrid (Spain) as base-case scenario. Medium-size waste treatment plants, like the one used in this paper, typically present an average biogas production of 1000 $Nm^3 \cdot h^{-1}$ of which 40% are typically transformed into heat and energy in co-generation engines for internal provision of energy (Pérez et al., 2020b). Hence, only the remaining 600 $Nm^3 \cdot h^{-1}$ were considered available for other valorization alternatives. Thus, in this study, the effect of the economy of scale on the ectoine production costs has been assessed considering biogas processing capacities ranging from 1 $Nm^3 \cdot h^{-1}$ to 600 $Nm^3 \cdot h^{-1}$. The PEC at the different scenarios was calculated using the same method described in the capital cost estimation section, with a combination of online estimation tools, literature review and quotations from experts and international companies. The workforce, measured in person$\cdot h^{-1}$, required for the supervision and operation of the plant was considered to follow a potential growth associated with biogas processing capacity, similar to the growth of equipment cost associated with equipment size.

2.3.3. Influence of ectoine selling price

The low global ectoine demand and the limited number of companies responsible for its commercialization have resulted in a scarce number of publications addressing the actual industrial production costs of ectoine (Cantera et al., 2018a; Pérez et al., 2021; Strong et al., 2015, 2016). In this scenario, whether ectoine follows a cost-based or a market-based pricing-strategy remains unknown. Thus, the recently reported high profitability of biogas to ectoine process, supported by the wide difference between the operational costs and the current ectoine selling price, opens the debate of how the implementation of this technology would impact future ectoine prices and vice versa (Cantera et al., 2018a; Pérez et al., 2021). In this study, the sensitivity of biogas-based ectoine process profitability (NPV_{20}, IRR and PP) towards the ectoine selling price has been studied using Madrid as base-case scenario. For this sensitivity analysis, ectoine selling prices ranging from the ectoine production costs ($NPV_{20} = 0$) to the maximum reported selling price for ectoine (1000 €$\cdot kg^{-1}$) were evaluated.

2.3.4. Influence of biotechnological limitations

The design of enhanced gas-liquid mass transfer bioreactors, the increase of product yields and the development of cost-effective and highly efficient extraction and purification processes have been identified in literature as the main biotechnological limitations of CH_4-based bioproducts (Choi and Lee, 1999; López et al., 2019; Pérez et al., 2020a). The influence of these performance parameters on ectoine production costs was assessed individually in this work. Table 3 summarizes the minimum and maximum parameter values considered in the sensitivity analysis. Minimum parameter values for the sensitivity analysis were determined as −50% of the values originally considered for the base-case scenario. Maximum parameter values were determined as +25% or +50% of the values considered for the base-case scenario, when feasible.

Table 3
Summary of the minimum and maximum values considered for the sensitivity analysis of ectoine production costs towards changes in the design parameters.

Biotechnological limitation	Parameter	Min	Base	Max
BCB performance	RE-CH_4 (%)	45	90	100
	CH_4-EC ($g \cdot m^{-3} \cdot h^{-1}$)	74	148	184
Microbial yields	Biomass yield (g X$\cdot g^{-1}$ CH_4)	0.2	0.4	0.6
	Ectoine accumulation (mg ectoine$\cdot g^{-1}$ X)	35	70	105
	Ectoine excretion (%)	42.5	85	100
IEX resin characteristics	Ectoine recovery (%)	45	90	100
	Resin capacity (kg ectoine$\cdot kg^{-1}$ resin)	0.05	0.1	0.125
	Resin density (kg resin$\cdot m^{-3}$ resin)	400	800	1000
	Resin lifespan (d)	40	80	100

2.3.4.1. Influence of gas-liquid mass transfer bioreactor performance.

The low solubility of CH_4 (biogas) and O_2 (air) in methanotrophic cultures entails process operation at high gas EBRT and the installation of large gas-liquid contactors in order to ensure an effective gas-liquid mass transfer. Indeed, the commissioning and operation of gas-liquid bioreactors have been reported in previous techno-economic assessments as the primary investment and operational cost of CH_4-based bioproducts such as PHA and ectoine (Levett et al., 2016; Pérez et al., 2020b, 2021). More specifically, Pérez and colleagues reported significant effects of CH_4-RE and CH_4-EC on biogas-based PHA productivity and TIC, respectively (Pérez et al., 2020a). The influence of the design and performance of BCBs on the ectoine production costs was assessed in terms of CH_4-RE and CH_4-EC in this work. CH_4-RE was defined as the fraction of the inlet CH_4 mass flowrate being oxidized in the bioreactor by the action of haloalkaliphilic methanotrophic bacteria (%), while CH_4-EC was determined as the mass flowrate of CH_4 oxidized by volumetric unit of bioreactor (g $CH_4 \cdot m^{-3} \cdot h^{-1}$).

2.3.4.2. Influence of methanotrophic bacterial yields.

The lower bacterial productivities of methanotrophs compared to other bacterial cultures has been reported as the main disadvantage of using CH_4 and O_2 for the production of bioproducts at industrial scale (Pieja et al., 2017; Strong et al., 2015). The sensitivity of ectoine production costs towards changes in the microbial yields was herein evaluated in terms of biomass growth rate, ectoine accumulation capacity and ectoine excretion efficiency during the *bio-milking* process. The biomass yield was defined as the mass ratio between biomass concentration and CH_4 oxidized in the bioreactor (g X$\cdot g^{-1}$ CH_4). The ectoine yield accounted for the mass of intracellular ectoine that haloalkaliphilic bacteria can accumulate intracellularly (mg ectoine$\cdot g^{-1}$ X). The ectoine excretion efficiency was measured as the percentage of intracellular ectoine that haloalkaliphilic bacteria excrete during the *bio-milking* process under non-saline conditions (%).

2.3.4.3. Influence of IEX purification performance.

Downstream costs have been reported to account for 30–50% of the total production costs of certain low-cost bioproducts such as PHA, single cell protein or methanol (Ling et al., 1997; López-Abelairas et al., 2015). The utilization of expensive ionic exchange resins has been also reported as the main cost of current industrial production of ectoine via sugar-based fermentation with *Halomonas Elongata* (Pastor et al., 2010; Strong et al., 2016). The influence of the IEX downstream process performance on the final production costs of biogas-based ectoine was evaluated in terms of ectoine recovery, resin capacity, resin density and resin lifespan. The ectoine recovery during the IEX step was calculated as the percentage of ectoine being selectively adsorbed on the ionic exchange resin (%). The resin capacity represents the mass ratio of ectoine adsorbed per unit of ionic exchange resin (kg ectoine$\cdot kg^{-1}$ resin). The resin density was measured as the resin weight per cubic meter of adsorbent material (kg resin$\cdot m^{-3}$ resin). The re-usability of the ionic exchange resin was measured in terms of lifespan (d).

3. Results and discussion

3.1. Geographical analysis

The geographical analysis showed notable differences in ectoine production costs, ranging from 158 €$\cdot kg^{-1}$ in New Delhi to 231 €$\cdot kg^{-1}$ in Doha (Fig. 2). In all the scenarios studied, the fixed cost and amortization represented between 31.5% and 45.4% of the total production costs, while the remaining 54.6–68.5% were allocated to the operational costs. Fixed and amortization costs ranged from 50.2 €$\cdot kg^{-1}$ in New Delhi to 104.9 €$\cdot kg^{-1}$ in Doha. This variability was strictly related to the

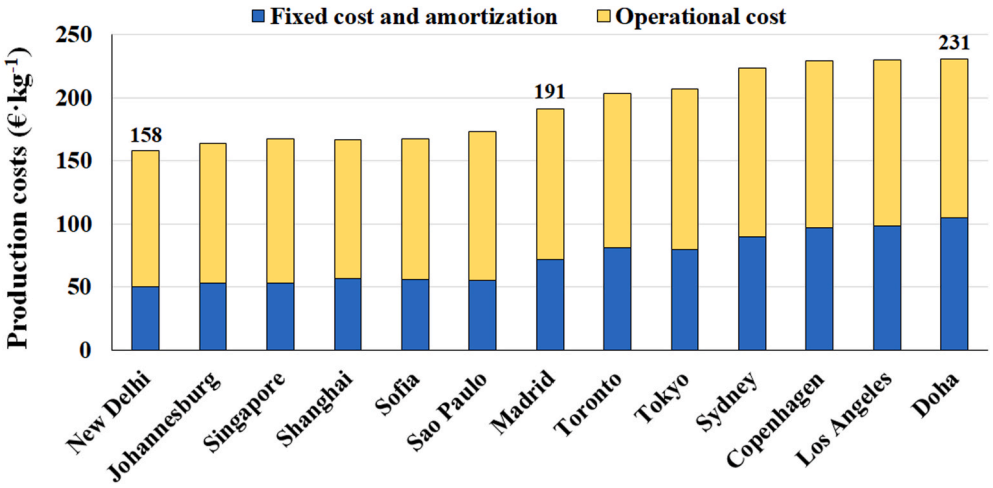

Fig. 2. Influence of the geographical location of the plant on the total ectoine production costs evaluated in 13 representative cities of the world. Fixed and amortization costs (blue bars) and operational costs (yellow bars) were assessed individually.

changes in the price levels, with values ranging from 0.07 to 2.46 in New Delhi and Doha, respectively. Variations in the price levels induced significant changes on the wage dependent parameters identified in the calculation of the Lang's Factor (from 2.9 to 6.0 in New Delhi and Doha, respectively), which incurred in significant changes in the total capital investment from 2.9 to 6.2 M€ in New Delhi and Doha, respectively.

A moderate variability in the operational costs was observed, ranging from 108.2 €·kg^{-1} ectoine in New Delhi to 133.4 €·kg^{-1} ectoine in Sydney. The variability in operational costs was attributed to the differences in energy, water and price level. Energy consumption was calculated as 65.6 kWh·kg^{-1} ectoine, thus resulting in energy costs ranging from 1.3 €·kg^{-1} to 14.1 €·kg^{-1} in Doha and Sydney, respectively, given the high difference in energy prices (0.02–0.21 €·kWh^{-1}). Water consumption was estimated at 1.2 m^3·kg^{-1} ectoine, which considering the differences in water prices revealed a low influence in the associated water costs, moving from 0.2 €·kg^{-1} ectoine in Shanghai to 6.5 €·kg^{-1} ectoine in Copenhagen. Given the high differences between the actual ectoine selling prices (600-1000 €·kg^{-1}) and the production costs herein reported, the influence of energy and water prices might not be of significance for the selection of the geographical location of the plant. In addition, changes in the price level entailed minor changes in the direct labor costs associated to the supervision and operation of the plant, ranging from 0.3 €·kg^{-1} in New Delhi to 9.6 €·kg^{-1} in Doha. However, indirect labor costs associated to the maintenance of the equipment were highly influenced by the changes that price level induced in the Lang's factor calculation, with values ranging between 10.3 €·kg^{-1} and 21.6 €·kg^{-1} in New Delhi and Doha, respectively. Besides, in all the scenarios studied, the dominant operational cost was the purchase of consumables, that was considered constant at 80.1 €·kg^{-1} ectoine, accounting for 60–74% of the total operational costs.

The results of this geographical analysis have also indicated that Madrid was a convenient location for the selection of base-case scenarios for bioproducts production in waste treatment plants, given the average scenario in terms of energy, water and labor costs. In this study, the estimated production costs in Madrid were 191 €·kg^{-1} at a10 t ectoine·y^{-1} production capacity.

3.2. Influence of the economy of scale

The influence of the economy of scale was assessed by varying the biogas flow treated between 1 Nm^3·h^{-1} and the maximum biogas available in medium size waste treatment facilities, 600 Nm^3·h^{-1}. Interestingly, the resulting ectoine production varied linearly between 0.1 and 89.6 t·y^{-1}, respectively (Fig. 3). This result can be explained because gas-liquid mass transfer of methane is considered the limiting

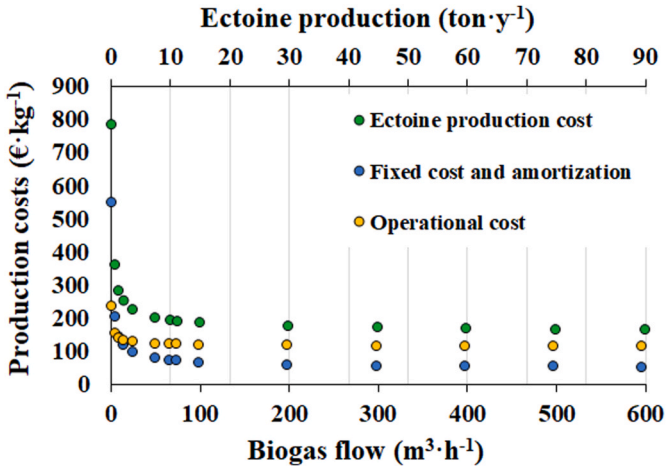

Fig. 3. Influence of the economy of scale on the ectoine production costs. Total ectoine production costs (green), fixed and amortization costs for ectoine production (blue) and operational costs for ectoine production (yellow).

step of this bioprocess. Therefore, under constant conditions of CH_4-EC (148 g CH_4·m^{-3}·h^{-1}) and CH_4-RE (90%), the ectoine productivity remained constant at a value of 17 mg·Nm^{-3} biogas. The ectoine production costs showed a high dependence on the size of the plant, with ectoine production costs ranging between 782 and 164 €·kg^{-1}, for 0.1 and 89.6 t·y^{-1}, respectively. Under all the scenarios evaluated, the ectoine production cost remained below 1000 €·kg^{-1}, a typical price for ectoine in the market. However, given the ectoine selling price of 600 €·kg^{-1} selected in this article, a production capacity of 0.1 t·y^{-1} exhibited a negative NPV_{20}, which represented the only non-profitable scenario studied. The most realistic scenarios entailed ectoine yearly productions below 20 t·y^{-1}, given the actual ectoine global demand in the order of 10–20 t·y^{-1} (Strong et al., 2015). Fixed and amortization costs showed the highest sensitivity towards the economy of scale with values ranging between 204 and 51 €·kg^{-1} at production capacities of 0.7 and 89.6 t·y^{-1}, respectively. These results can be explained by the non-linearity of equipment purchase cost, which followed a potential growth associated to equipment size. The operational costs exhibited a lower influence of the economy of scale, ranging from 113 €·kg^{-1} to 154 €·kg^{-1} in all the profitable scenarios studied. The decrease in the operational costs was also explained by the non-linearity of direct labor costs and of equipment purchase cost, which induced significant changes in

the maintenance costs.

3.3. Influence of ectoine selling price

The results from the ectoine selling price sensitivity analysis showed that PP increased dramatically with a decreasing market price of ectoine. Most authors agree that it would be difficult to invest in biogas valorization projects with PP longer than 10 years (U S Environmental Protection Agency, 2011; Water Environment Research Foundation, 2012). In this context, a minimum selling price of ectoine of 220.0 €·kg^{-1} would make biogas bioconversion of ectoine still profitable in the scenarios herein presented (Fig. 4A). The sensitivity analysis showed a linear increase of the IRR with increasing ectoine market prices. Successful investments in novel bioproducts should present a minimum IRR of 15% (Levett et al., 2016; National Renewable Energy Laboratory, 2011). In this context, an ectoine selling price of 240.0 €·kg^{-1} could still support an effective implementation of ectoine production from biogas in waste treatment plants (Fig. 4B).

These results are of paramount importance for the potential implementation of biogas-based biorefineries, given the low production costs (3–6 times lower than the current market price of ectoine) and the high profitability of biogas-based ectoine production herein reported. In this context, if ectoine is a cost-based product, the production of ectoine from biogas would potentially displace the current production route via sugar-based fermentation with *Halomonas Elongata*. On the contrary, if ectoine is a market-based product, the inclusion of new technologies with a reduced price, could result in a product price drop in the near future. In view of these results, it could be stated that whether ectoine is currently following a cost-based or a market-based pricing strategy, biogas bioconversion into ectoine constitutes a present and future profitable opportunity for biogas producers to invest in circular economy concepts and anticipate the incoming stricter environmental policies.

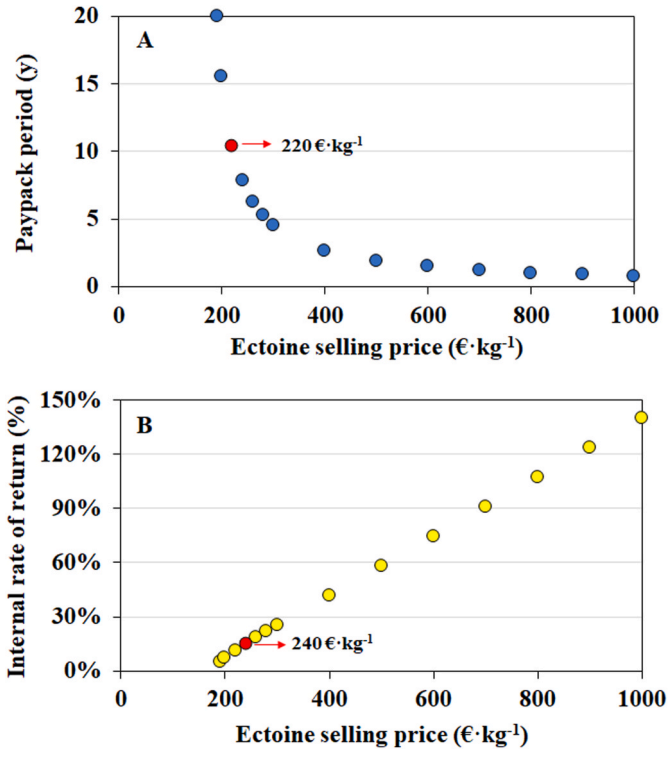

Fig. 4. Influence of the ectoine selling price on the economic feasibility of the process: (A) payback period of the investment and (B) internal rate of return of the investment.

3.4. Influence of biotechnological limitations

3.4.1. Influence of gas-liquid mass transfer bioreactor performance

The sensitivity analysis showed a slight decrease in ectoine production costs ranging from 224 €·kg^{-1} to 188 €·kg^{-1}, with increasing values of CH_4-RE in the BCB from 45% to 100%, respectively (Fig. 5A). This was explained by the concomitant increase in CH_4-to-ectoine productivity from 8.5 to 18.9 g ectoine·m^{-3} biogas, which entailed an increase in ectoine production from 5.0 to 11.1 t·y^{-1}, respectively. The results also indicated that an improved CH_4-RE of 100% represented only a marginal 1.8% decrease in the production costs compared to the base-case scenario with CH_4-RE of 90%.

On the contrary, an increase in the CH_4-EC from 74 to 184 g CH_4·m^{-3}·h^{-1} entailed a significant decrease in the ectoine production costs from 278 to 171 €·kg^{-1}, respectively (Fig. 5A). The high influence of CH_4-EC on the final ectoine production costs was supported by the associated decrease in bioreactor volumes (from 389.7 m^3 to 155.9 m^3) concomitant to a decrease in the TIC (from 6.8 M€ to 3.7 M€). These results were in well agreement with previous studies on biogas

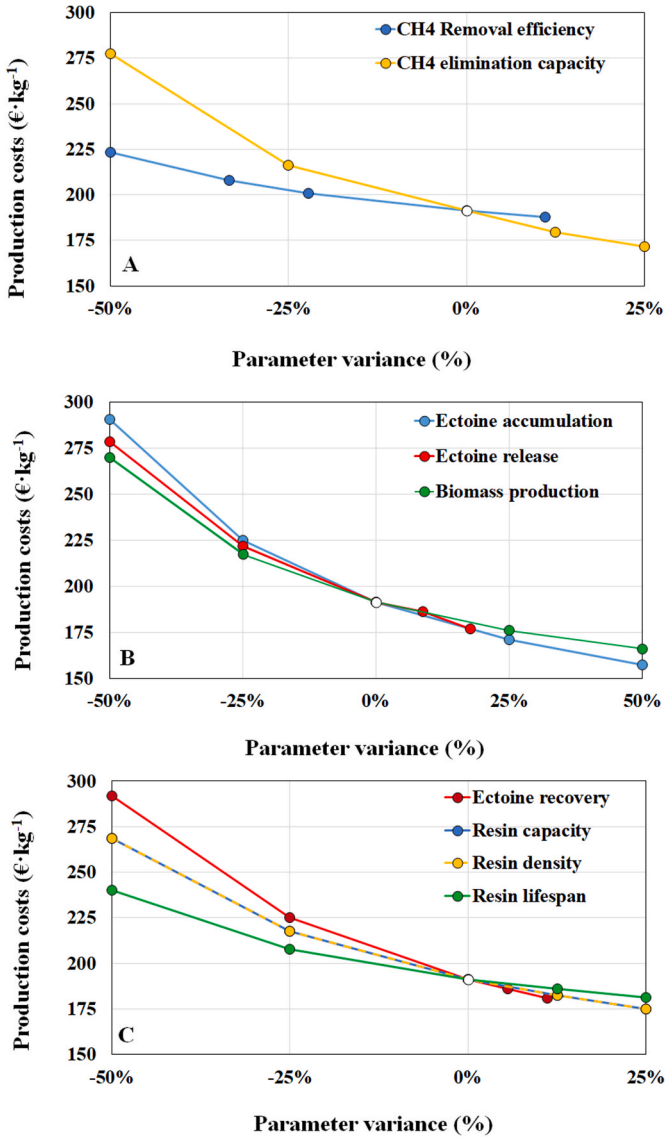

Fig. 5. Sensitivity analysis on the biotechnological limitations of ectoine production from biogas. (A) Bubble column bioreactor performance sensitivity analysis, (B) Bacterial yields sensitivity analysis, (C) Ionic exchange chromatography sensitivity analysis.

valorization in BCBs, which have shown that CH_4-EC constitutes the main biotechnological limitation given its large influence on the capital investment costs (TIC), accounting in some cases for more than 60% of the total equipment cost (Levett et al., 2016; Pérez et al., 2020a). Overall, it can be concluded that improvements in CH_4-RE would not result in a significant enhancement of process performance, but in contrast, the development of more advanced and compact BCBs would have a significant positive impact on biogas-to-ectoine cost-effectiveness.

3.4.2. Influence of methanotrophic bacterial yields

The minimum values considered for ectoine accumulation, ectoine excretion and biomass yield resulted in dramatic increases in ectoine production costs of +51.9, +41.1 and + 45.4% compared to the base-case scenario, respectively (Fig. 5B). On the other hand, the maximum values considered for these parameters entailed moderate reductions in ectoine production costs of −17.7, −13.2 and −7.5% compared to the base-case scenario, for ectoine accumulation, ectoine excretion and biomass yield, respectively. These results can be explained by the changes in ectoine productivity, which resulted in increased ectoine production rates with a marginal increase in the amortization and fixed costs and operational costs.

The ectoine production costs showed a similar trend towards changes in all the bacterial performance parameters herein studied, ectoine accumulation capacity exerting the most significant impact on ectoine production costs. This is especially relevant given the recent discovery of highly efficient bacterial species with outstanding ectoine accumulation capacities up to 230 g ectoine·kg^{-1} X, which would incur in ectoine production costs as low as 120 €·kg^{-1} (Khmelenina et al., 2000). These results demonstrated the paramount importance of the selection of highly efficient methanotrophic cultures for the manufacturing of market-competitive bioproducts, given the high cost-sensitivity towards a decrease in bacterial process efficiency. In this context, bacterial control strategies such as maintaining biomass age in the bioreactor below 9 d or carefully controlling salinity, copper and tungsten concentrations in the culture media, have been reported in literature as key aspects for maintaining optimal methanotrophic productivities (Akberdin et al., 2018; Cantera et al., 2017a; Carmona-Martínez et al., 2021).

3.4.3. Influence of IEX purification performance

The product recovery efficiency was identified as the most relevant parameter in the selection of ionic exchange resins for ectoine adsorption given its high influence on the final ectoine production costs, ranging from 291.9 to 181.1 €·kg^{-1} for recovery efficiencies of 45% and 100%, respectively (Fig. 5C). The high influence on ectoine price was attributed to the enhanced ectoine production caused by a better selectivity of the purification process, at similar fixed and amortization and operational costs. Besides, the selection of highly active (high resin capacity) and compact (high density) resins largely impacted the final ectoine production costs, inducing from a −8.5% reduction to a +40.4 increase compared to the base-case scenario. This cost-influence was explained by the higher column BV, which resulted in increased purchased equipment and operational costs due to the lower compactness and activity of the resins. A −50% reduction of resin capacity or density entailed a +50% increase in IEX column volume and therefore a +50% increase in the ionic exchange resin requirements.

Finally, the resin lifespan showed a moderate but still relevant influence on the final ectoine production costs, with values ranging from 240.3 €·kg^{-1} at a resin lifespan of 40 d to 181.5 €·kg^{-1} at a resin lifespan of 100 d. Ionic exchange resin lifespan impacts directly on resin re-usability and therefore resin requirements. In summary, the advantages of selecting a highly efficient ionic exchange resin in terms of selectivity, product quality and re-usability outcompeted clearly the disadvantage of high resin prices (342 €·kg^{-1}), which in the base case-scenario was estimated as the main consumable cost with 48.8 €·kg^{-1} ectoine.

4. Conclusions

The utilization of CH_4-biogas as a feedstock for the production of high-added value products such as ectoine has been demonstrated as a highly profitable alternative to energy production in waste treatment facilities, in terms of economic sustainability. Ectoine, typically produced via sugar-based fermentation and with a market price ranging from 600 to 1000 €·kg^{-1}, represents up-to-date the most valuable bioproduct that can be synthesized with methanotrophic bacteria. The results of this techno-economic assessment predicted the substitution of the current industrial ectoine production processes by the production of ectoine with methanotrophic haloalkaliphilic bacteria, given the 3–6 lower production costs herein reported. The sensitivity analysis showed a high profitability regardless of the commodity prices, the economy of scale and the fluctuations in the ectoine retail market. The analysis of the current biotechnological limitations showed that the improvement in CH_4 elimination capacity in high-mass transfer bioreactors, the research on highly efficient microorganisms and the selection of the highest quality ionic exchange resins are critical parameters for the future development of biogas-based biorefineries.

CRediT authorship contribution statement

Víctor Pérez: Data curation, Investigation, Methodology, Visualization, Writing – original draft. **Jose Luis Moltó:** Investigation, Methodology, Writing – review & editing. **Raquel Lebrero:** Methodology, Supervision, Writing – review & editing. **Raúl Muñoz:** Funding acquisition, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2022.133440.

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