Journal of Environmental Chemical Engineering 13 (2025) 120093

Contents lists available at ScienceDirect

Journal of Environmental Chemical Engineering

journal homepage: www.elsevier.com/locate/jece

Toward complete and sustainable biogas valorization: Ectoine synthesis using a methanotroph-microalgae consortium

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ARTICLE INFO

Keywords:
Biogas valorization
Ectoine
GHG emissions
Methanotrophs
Microalgae
Sustainability analysis

ABSTRACT

To improve the economic viability and sustainability of anaerobic digestion plants, alternative biogas valorization routes are increasingly being sought. One promising strategy involves biosynthesizing high-value products, such as ectoine, from biogas, given their high market value and diverse industrial applications. This study presents the first techno-economic assessment and environmental impact evaluation of a newly established ectoine production pathway, which uses a consortium of methanotrophs and microalgae (methalgae) to simultaneously valorize both the CH_4 and CO_2 fractions of biogas. The techno-economic assessment showed that this process can achieve ectoine production costs of €186 kg^{-1} , with best- and worst-case scenario costs ranging from €115 to €342 kg^{-1} . This represents an average cost reduction of 39 % compared to traditional ectoine production routes, namely bacterial milking and methanotroph-only processes. Cost sensitivity analyses identified production scale and ectoine yield as the most influential economic drivers. The environmental impact assessment showed that the methalgae-based process can achieve important greenhouse gas emissions and water use reductions, reaching an average of 36 % and 22 %, respectively, compared to conventional ectoine production approaches. These findings demonstrate the potential of methalgae-based processes as economically attractive and environmentally sustainable alternatives for producing high-value products from biogas.

1. Introduction

The total European biogas production capacity has grown considerably over the last years, reaching 21 billion cubic meters produced in 21,000 anaerobic digestion (AD) facilities in 2023 [1]. This substantial increase is primarily driven by the versatility and environmental sustainability of biogas [2]. Biogas is typically valorized through combined heat and power (CHP) systems for electricity and heat production or upgraded into biomethane for natural gas grid injection [3]. While these pathways are well-established and provide immediate energy outputs, they are faced with several challenges. Cogeneration systems often have low efficiencies (around 40 % if only electricity is considered), while biomethane production requires energy-intensive and costly upgrading processes [4,5].

To maximize the potential of biogas, alternative valorization pathways are being explored, including its bioconversion into value-added products, such as polyhydroxyalkanoates (PHA) and methanol [6]. Nevertheless, synthesizing these bioproducts from biogas is still not financially competitive compared to conventional fossil- or agricultural feedstock-based processes [7]. In recent years, osmolytes have emerged as promising target products for biogas valorization through the use of methanotrophic bacteria, which is particularly interesting given their high market value and wide application range [8,9]. The production of these compatible solutes, including ectoine, from biogas can offer an attractive alternative to the currently used approaches. Such methods typically rely on halophilic bacteria, such as *Halomonas elongata*, to produce ectoine from glucose or other agricultural feedstocks through a process known as bacterial milking [10,11]. These approaches are, however, associated with high ectoine production costs and greenhouse gas (GHG) emissions, as a result of their reliance on high-quality carbon sources [12].

Recent techno-economic assessment (TEA) studies have highlighted

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https://doi.org/10.1016/j.jece.2025.120093
Received 15 September 2025; Received in revised form 31 October 2025; Accepted 1 November 2025
Available online 3 November 2025

the economic potential of producing ectoine from biogas using methanotrophs. Pérez et al. assessed ectoine synthesis using a mixed culture of haloalkaliphilic methanotrophs, and reported costs between €158 and €275 kg^{-1} for a scale of 10 tonnes per year [7]. Similarly, Jung et al. conducted a TEA on ectoine production using the haloalkaliphilic methanotrophic strain *Methylotuvimicrobium alcaliphilum 20Z* and reported production costs between €196 and €340 kg^{-1} for production scales ranging from 37 to 16 tonnes per year [13]. The same study evaluated the global warming potential (GWP) of ectoine production from methane, reporting a value of 0.71 kg CO_2-eq per kg CH_4, which is substantially lower compared to conventional methane utilization pathways, such as natural gas combined cycle (2.75 kg CO_2-eq per kg CH_4) and steam methane reforming (6.25 kg CO_2-eq per kg CH_4) [13].

While these studies show that ectoine can be produced at costs below its market price (€600 – €1000 kg^{-1}) and promising environmental benefits, conventional methanotroph-based approaches face several limitations [14]. First, these processes only utilize the methane fraction of the biogas, leaving the carbon dioxide fraction (typically around 30–50 %) unused [15]. Second, oxygen, which is essential for methanotrophic metabolism, is typically supplied externally, which increases energy consumption, operational costs, and GHG emissions [13]. Finally, methanotrophic processes often exhibit limited ectoine productivity potentials [16]. It is therefore important to address these challenges to improve the economic and environmental performance of biogas-based ectoine production processes.

A novel methalgae-based pathway for ectoine production from biogas was newly established to circumvent these limitations [15]. This process combines methanotrophic bacteria and microalgae in a co-culture system (methalgae), thus allowing simultaneous utilization of both the methane and carbon dioxide fractions of biogas. The microalgae fix CO_2 through photosynthesis and produce O_2, which is then consumed by methanotrophs for CH_4 metabolism. This eliminates the need for external oxygen supply and improves the overall efficiency of biogas valorization. Early experimental studies have demonstrated the feasibility of this system and the benefits of the synergistic interactions between methanotrophs and microalgae, showing high CH_4 removal efficiency, CO_2 uptake, and ectoine production under controlled conditions [15].

This study presents a comprehensive techno-economic assessment and environmental impact analysis of the methalgae-based ectoine production process (EctoMet process). The economic and environmental performances of this novel process were evaluated and compared against two benchmarks: the methanotroph-only approach and the conventional glucose-based production route (bacterial milking). A sensitivity analysis was performed to evaluate critical cost and GHG emissions drivers, process resilience to variations in key parameters, and areas for further optimization. To the authors' knowledge, this work presents the first techno-economic and environmental investigation of methalgae-based processes for ectoine production.

2. Methodology

2.1. Process description

Three ectoine production processes were evaluated. The Bacterial Milking process uses glucose as a carbon source for fermentation using halophilic bacteria. The Methanotroph-based process involves the use of methanotrophic bacteria to produce ectoine from biogas. These processes served as the basis of comparison with the novel EctoMet process, where ectoine is biosynthesized from biogas using a consortium of methanotrophs and microalgae (methalgae).

2.1.1. Bacterial milking process

This process corresponds to the conventionally used pathway for ectoine production developed in 1998 [11]. This process involves using a high-salt medium (typically 15 – 20 % salinity) to stimulate ectoine biosynthesis in halophilic bacteria, such as *Halomonas elongata* [10,11]. The bacteria then excrete ectoine into a low-salt medium following an osmotic shock. To purify the ectoine solution, it undergoes intensive downstream processing (DSP), including desalination, ion exchange chromatography, crystallization, and drying [11,17].

The desalination of the aqueous solution containing ectoine is performed through ultrafiltration (flux of 15 $L/m^2/h$ and a biomass recovery rate of 99 %), followed by electrodialysis (flux of 45 $L/m^2/h$) [7]. The subsequent concentration and purification of ectoine are carried out using ion exchange chromatography (IEX) and water crystallization. An acidification step (using 10 M HCl) is needed prior to the IEX to improve the selective adsorption of ectoine. The IEX was sized based on an adsorbing capacity of 100 g of ectoine per kg of ion exchange resin (IER) and an ectoine recovery rate of 90 % [7]. Washing with H_2SO_4 and distilled water followed by elution with NaOH are also performed to remove impurities and extract the ectoine. Neutralization using H_2SO_4 is required to bring the pH of the ectoine stream close to neutral. Water crystallization, centrifugation, and tray drying are finally carried out to obtain a high-quality ectoine end product.

2.1.2. Methanotroph-based process

This process exploits the ability of haloalkaliphilic methanotrophic bacteria to synthesize ectoine from CH_4. The process begins with sulfur removal from the biogas feed using a biological anoxic desulfurization unit, with an H_2S removal efficiency of 99 % [7]. This step is crucial, given that the presence of sulfur may inhibit the methanotrophs and could cause corrosion to downstream piping and equipment [18]. Ectoine production is carried out in a bubble column bioreactor to improve the gas−liquid mass transfer of CH_4 [19]. Air is also introduced to the bioreactor to provide the O_2 required by the methanotrophs.

The methanotrophic bacteria, containing the generated ectoine, are centrifuged to concentrate the biomass. The biomass then undergoes a hypoosmotic shock to promote the release of ectoine. After the excretion of most of the intracellular ectoine (around 85 %), the biomass is centrifuged and reintroduced to the bubble column bioreactor [7]. The ectoine solution then undergoes desalination and IEX, following similar steps and conditions to the Bacterial Milking process explained previously. The ectoine solution stream is then dried (using a spray dryer), and the solid product is dissolved in methanol. The ectoine-containing solution then undergoes ultrafiltration to remove insoluble solids. Next, the ectoine is crystallized from methanol, centrifuged, and dried to obtain a high-quality end product. Due to the high quantity of methanol used for crystallization, solvent recovery (including a boiler and a condenser) is considered, which requires a flow of steam and cooling water.

2.1.3. EctoMet process

This process involves the biosynthesis of ectoine using biogas through the synergistic action of methalgae, a consortium of methanotrophs and microalgae (Fig. 1). This process is similar to the Methanotroph-based process, with some specific differences. Microalgae require a light source to be able to achieve photosynthesis, necessitating the use of an artificial lighting system. Additionally, due to the microalgae's ability to produce oxygen (by product of photosynthesis), external air supply is not needed in this process.

2.2. Economic assessment

Techno-economic assessment (TEA) is a widely used approach for analyzing the economic performance of processes [20]. The chances of successfully introducing innovative processes to the market are greatly improved when this assessment is carried out early. A TEA usually follows 4 main steps: (1) market analysis, (2) process flow diagram and mass and energy balance, (3) economic analysis, and (4) sensitivity analysis. More details about the TEA methodology are given in [21].

The equipment costs, reference capacities, scaling exponents, and

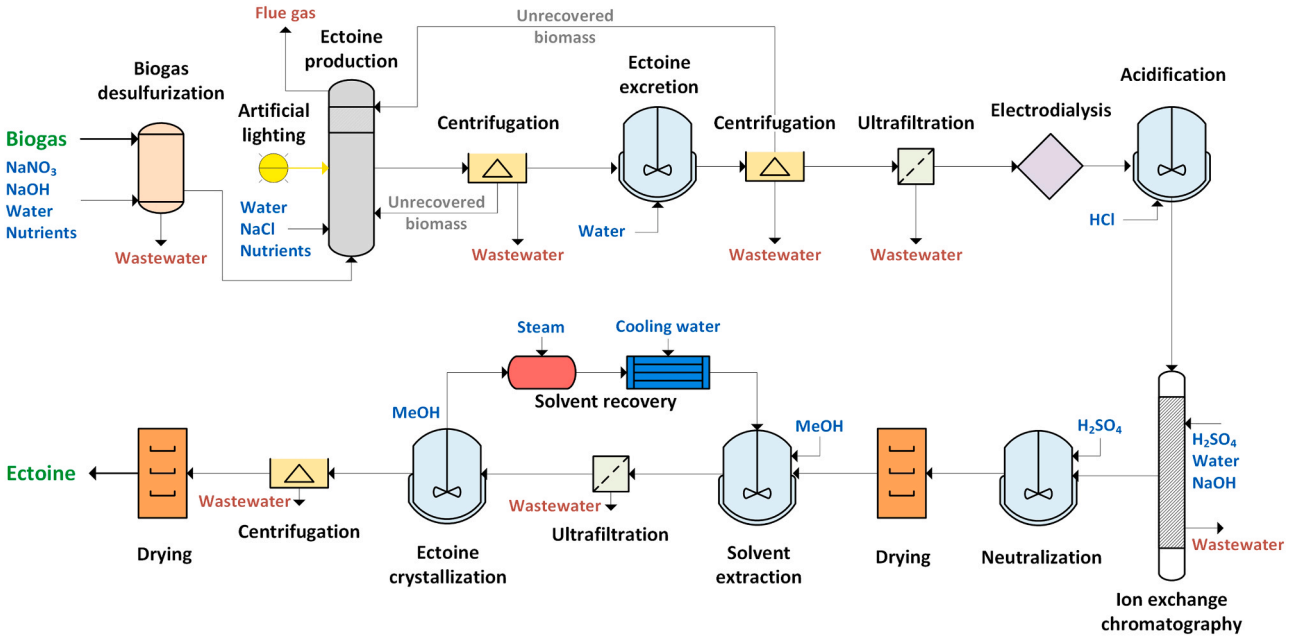

Fig. 1. Process flow diagram of the EctoMet process.

installation factors were obtained from a combination of sources: literature, modeling (using Aspen Plus), equipment quotes, and assumptions as listed in Table S1 (Supplementary Materials). The total capital cost of each of the considered equipment was calculated using the "scaling law" following Eq. (1), where *IF* is the installation factor and *n* is the scaling exponent. The costs of the equipment were adjusted to the year of the study using the chemical engineering plant cost indices (CEPCI).

$$CAPEX = IF \times CAPEX_{ref} \times \left(\frac{Capacity}{Capacity_{ref}}\right)^{n} \quad (1)$$

The present TEA was based on the year 2023, with an assumed economic lifetime of 20 years for the evaluated plant. An operation time of 7884 h/y (continuous) was considered, accounting for a 10 % downtime for equipment cleaning and maintenance. The operational expenditure (OPEX) needed for running and maintaining the plant was estimated based on the unit prices given in Table S2 (Supplementary Materials). The minimum selling price (MSP) of ectoine was selected as the main economic performance indicator in the present assessment. The MSP, also known as the total production cost, is calculated using Eq. (2).

$$MSP\ \left(€ \Big/ kg\right) = \frac{\text{Annualized CAPEX } (€/y) + \text{OPEX } (€/y)}{\text{Product } (kg/y)} \quad (2)$$

2.3. Environmental impact assessment

This assessment quantified the global warming potential (GWP) of the involved processes. This metric was calculated by multiplying the mass and energy flows (in t/y and MWh/y, respectively) by their associated GHG emission factors (in $t_{CO2\text{-}eq}$/t and $t_{CO2\text{-}eq}$/MWh, respectively) [22]. The GHG emission factors of the streams involved in the considered processes are listed in Table S3 (Supplementary Materials), which were mainly obtained from the Ecoinvent database and literature.

Besides GWP, water use and wastewater generation were also evaluated. Fresh water is a finite and increasingly scarce resource, which makes its efficient use a critical aspect of economically sustainable industrial practices. Similarly, quantifying wastewater generation is important for evaluating environmental impact, as untreated effluents are well-known contributors to pollution.

2.4. Sensitivity analysis

The economic and environmental data used in this work mainly originated from literature, which resulted in deterministic (fixed) rather than stochastic (probabilistic) values. To account for potential uncertainties in the input data, a Monte Carlo simulation was carried out using Oracle Crystal Ball following a triangular distribution (10,000 trials). This also allowed the identification of economic and environmental "hotspots" that need further optimization [23].

The baseline values and ranges used in this work are listed in Table S4 (Supplementary Materials). The variation ranges selected for the sensitivity analysis were based on realistic technical, economic, and environmental considerations. The most influential parameters were subsequently investigated in more detail through a local sensitivity analysis, which allowed understanding how each key variable affects the system in isolation.

3. Results and discussion

3.1. Experimental input

Part of the data used for modeling the EctoMet process, in terms of operating conditions and reactor design, was based on the experimental findings reported in our previous work [15]. This study explored the potential of methanotrophic–microalgal co-cultures to achieve complete biogas valorization by converting both CH_4 and CO_2 fractions into ectoine.

The methalgae consortium considered in this study was dominated by the photosynthetic microalgae *Picochlorum oklahomense*, with *Nannochloris sp.* present at a lower abundance. The prokaryotic community was more diverse, comprising various halophilic heterotrophic and methylotrophic microorganisms, primarily *Methylobacter marinus/whittenburyi, Methylophaga marina, Labrenzia aggregata, Labrenzia sp.,* and *Hyphomonas adhaerens*. The enriched culture exhibited strong performance in terms of ectoine accumulation and CH_4 removal efficiency (Table 1).

Key parameters explored in this study include salinity levels, which were varied to evaluate their impact on ectoine synthesis and CH_4 removal. Temperature was maintained within a range suitable for both microbial growth and ectoine accumulation. Irradiance intensity was set

Table 1
Experimental inputs used in the present work.

Parameter	Value	Unit
Salinity (NaCl)	4.5	%
Temperature	28 ± 2	°C
Irradiation intensity*	120	μmol/m^2.s
Ectoine accumulation	51.3 ± 1.1	mg/g_{VSS}
CH_4 removal efficiency	88 ± 16	%

* Full-spectrum LED

to promote microalgal photosynthesis and support O_2 production for CH_4 oxidation by methanotrophs. An overview of the experimental parameters used in the present study is provided in Table 1.

3.2. Mass balance

The mass balance of the designed EctoMet process was based on large-scale production of ectoine (10 t/y) from biogas. The process started with the introduction of 42.3 kg/h of biogas to an anoxic desulfurization unit, along with 27.7 kg/h of water, 0.01 kg/h of nutrients, 1.3 kg/h of $NaNO_3$, and 0.2 kg/h of NaOH. The desulfurized biogas was fed to a bubble column bioreactor, together with 0.04 kg/h of nutrients, 4.1 kg/h of NaCl, and 90.8 kg/h of water (ectoine yield of 191.5 g/kg of CH_4 at 28°C and 4.5 % salinity). The liquid fraction was centrifuged, and the unrecovered biomass was recycled back to the bioreactor. The ectoine was subsequently excreted in a continuous stirred tank reactor (CSTR) through the addition of 527.9 kg/h of water. Another centrifugation was performed, which generated three streams: ectoine-containing stream (541 kg/h), unrecovered biomass (64.8 kg/h, recycled back to the bioreactor), and wastewater (62.1 kg/h).

The ectoine-containing stream was desalinized and then acidified through the addition of 43.3 kg/h of diluted HCl (10 M). An IEX was performed (requiring 21.9 kg/h of H_2SO_4, 21.4 kg/h of water, and 66.7 kg/h of 1.3 M NaOH), followed by neutralization (requiring 1.3 kg/h of H_2SO_4). Ectoine was subsequently dried in a spray dryer and dissolved in methanol. Ultrafiltration was necessary to filter out the insoluble matter, mainly the Na_2SO_4 previously generated during neutralization, thus producing 3.9 kg/h of wastewater. Ectoine was then crystallized from methanol, while the methanol was recovered using an evaporator (requiring 4.2 kg/h of steam at 2 bar) and a condenser (requiring 51.7 kg/h of cooling water at 15°C). The crystallized ectoine subsequently underwent a final centrifugation (0.2 kg/h of wastewater) and tray drying to remove any remaining methanol and obtain the purified ectoine end product (1.27 kg/h or 10 t/y).

3.3. Energy use

The energy use of a particular process is a critical parameter that influences both its economic performance and environmental impact. The total energy consumption (electricity and heat) of the EctoMet process was quantified and compared to the considered benchmark processes, as shown in Figure S1 (Supplementary Materials). The EctoMet and Methanotroph-based processes consume respectively 20 % and 54 % more energy compared to the Bacterial Milking process. This higher energy consumption was mainly attributed to the larger feedstock requirements of these processes, which respectively used 42.3 and 84.6 kg/h of biogas, compared to 11.5 kg/h of glucose for the Bacterial Milking process. Consequently, the latter required less energy for heating the fermentation bioreactor and operating DSP equipment. The need for biogas desulfurization in the EctoMet and Methanotroph-based processes contributed further to increasing their energy consumption.

Another important finding is related to the 35 % lower energy consumption of the EctoMet process compared to its Methanotroph-based counterpart. This reduction was mainly attributed to the reduced material flow through the equipment (associated with higher yields), which decreased the energy required for processing at various stages. The internal O_2 production by the microalgae contributed to energy savings by eliminating the need for external air supply via energy-intensive blowers. The electricity required for the artificial lighting system to support microalgal growth was minimal, contributing only to 1.7 % of the total energy expenditure, and therefore did not have a noticeable impact on the overall energy consumption of the EctoMet process.

In terms of individual equipment energy use, the ectoine production reactor, desalination unit (ultrafiltration and electrodialysis), and evaporator (for solvent recovery) account for more than 80 % of the total energy expenditure of the EctoMet process (Figure S1). The high energy consumption of the bioreactor can be reduced by using thermal insulation, which would minimize heat losses and improve energy efficiency [24–26]. Similarly, the considerable energy use of desalination may be lowered by adopting more energy-efficient technologies, such as reverse osmosis [27]. The energy demand of the solvent recovery step could be brought down by including waste heat reuse or mechanical vapor recompression [28].

3.4. Techno-economic assessment

The capital expenditure (CAPEX) breakdowns of the considered value chains are presented in Fig. 2. The assessment shows that the combined use of methanotrophs and microalgae can reduce the CAPEX by 49 % compared to the use of methanotrophs alone. This is a result of the higher ectoine production yield of the methalgae consortium, which required smaller ectoine production and DSP equipment sizes. Although the EctoMet process requires an artificial lighting system to promote microalgae photosynthesis, its cost is offset by no longer needing air blowers (since oxygen is supplied internally). The CAPEX of the EctoMet process is also lower by 39 % compared to the Bacterial Milking process. This cost advantage is mostly attributed to the lower salinity levels required by the EctoMet process (4.5 %) compared to the Bacterial Milking process (15 %), thus requiring less costly equipment, especially reactors, desalination units, and IEX [29].

The ectoine production step is the largest contributor to the total CAPEX. This is mostly due to the high costs of bubble column reactors, which need to be constructed using corrosion-resistant materials to withstand high salinity levels. These bioreactors must also comply with ATEX safety standards for handling biogas-air mixtures, further increasing their cost [7].

The individual contributions of capital and operating expenditures to the ectoine production cost are shown in Fig. 3. The EctoMet process can produce ectoine at a cost 33 % lower than its Methanotroph-based counterpart. This is a result of the former's improved yield, which contributed to lowering both its CAPEX (smaller equipment capacities) and OPEX (lower consumption of feedstock, utilities, chemicals, etc.). The EctoMet process also financially outperforms the conventional ectoine production route (bacterial milking), achieving a cost reduction of 45 %. This is mostly attributed to the former's lower investment costs coupled with its lower demand for chemicals. The lower cost of feedstock used in the EctoMet process (€114 t^{-1} for biogas compared to €1590 t^{-1} for glucose) further improved its economic attractiveness [30, 31].

3.5. Environmental assessment

The breakdown of the GHG emissions of the three considered value chains is shown in Fig. 4. The ectoine production pathways using biogas as a feedstock, namely the EctoMet and Methanotroph-based value chains, have considerably lower GHG emissions (by 42 % and 16 %, respectively) compared to Bacterial Milking. This difference is mainly attributed to the latter's higher reliance on chemical inputs, particularly sodium chloride (NaCl), which is needed to maintain the required high-salinity conditions [32]. The GHG emissions of the EctoMet value chain are 31 % lower than those of the Methanotroph-based process. This

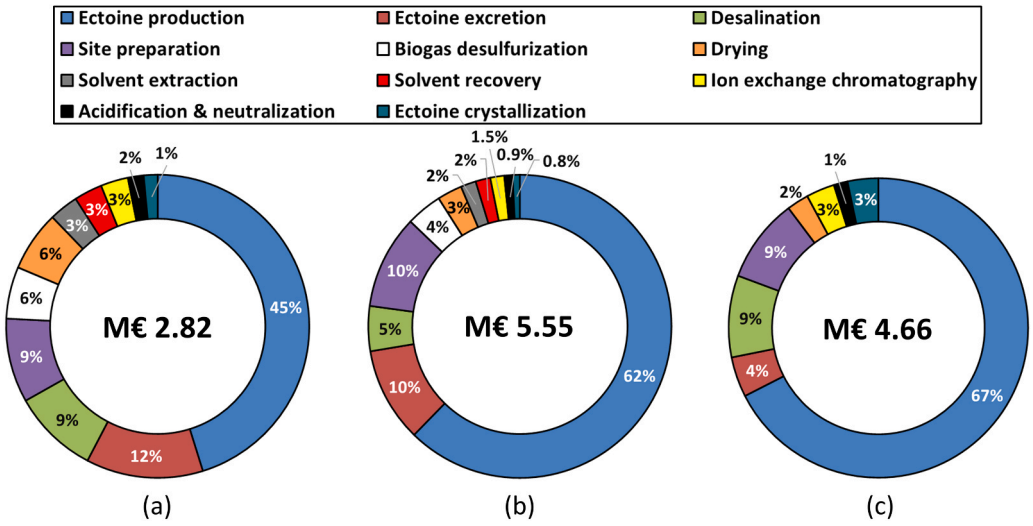

Fig. 2. CAPEX breakdown for the (a) EctoMet process, (b) Methanotroph-based process, and (c) the Bacterial Milking process.

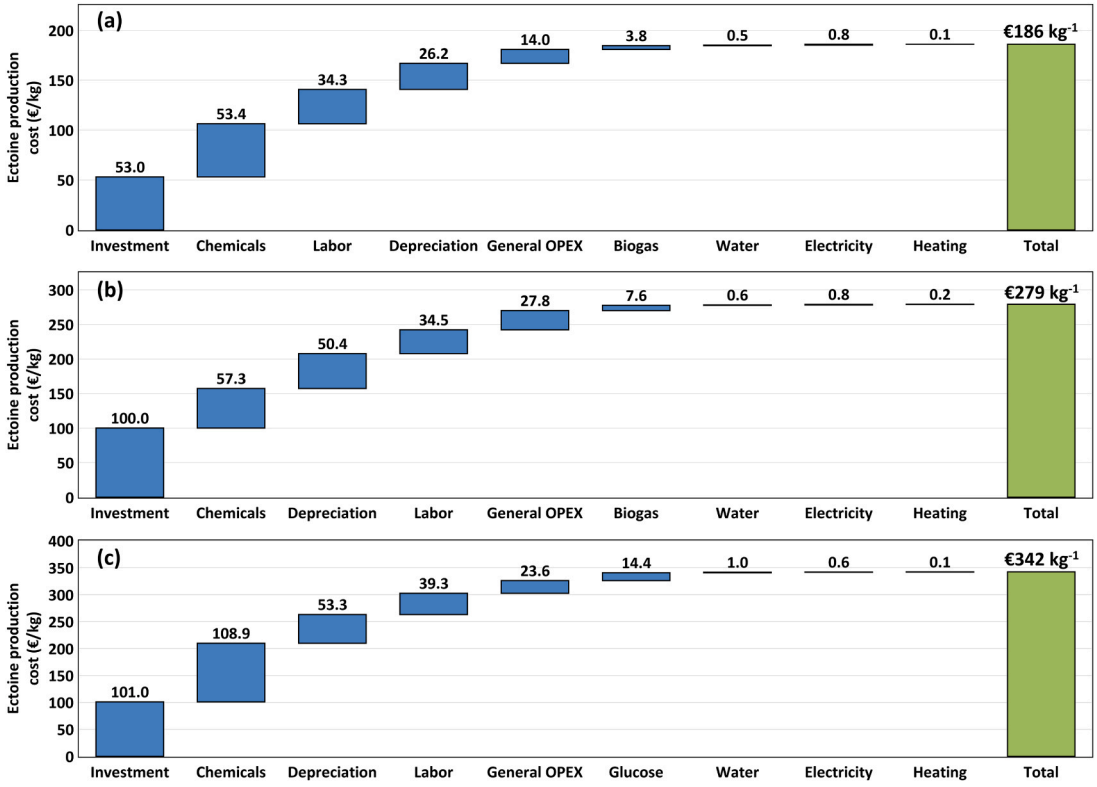

Fig. 3. Breakdown of the ectoine production cost for the (a) EctoMet process, (b) Methanotroph-based process, and (c) Bacterial Milking process.

reduction is due to the higher ectoine yield associated with the use of methalgae, which results in decreased consumption of feedstock (biogas), chemicals, and utilities, as well as reduced release of flue-gases.

The water consumption of the EctoMet, Methanotroph-based, and Bacterial Milking processes is shown in Figure S2 (a) (Supplementary Materials). The EctoMet process uses 15 % less water compared to its Methanotroph-based counterpart. This is mostly due to the higher ectoine production yield of methalgae, which considerably lowers water use per unit of produced ectoine. Figure S2 (a) also highlights that the ectoine excretion step accounts for the majority of the water consumption of the EctoMet value chain (63 %), making it a key target for optimization to further reduce its water footprint. This water use could be further reduced by an additional 4 % if biogas desulfurization is no longer needed.

The advantages of the EctoMet process compared to the Bacterial Milking process, in terms of water use, become even more considerable when also considering the water footprint associated with producing the used carbon sources. Producing 1 tonne of glucose through hydrolysis of amylaceous feedstock under acid conditions, which represents the most industrially used glucose production route [33], can use up to 122 m^3 of freshwater (Ecoinvent database). On the other hand, producing an equivalent amount of biogas (3.7 tonnes) through AD necessitates around 34 m^3 of freshwater (Ecoinvent database). This represents a

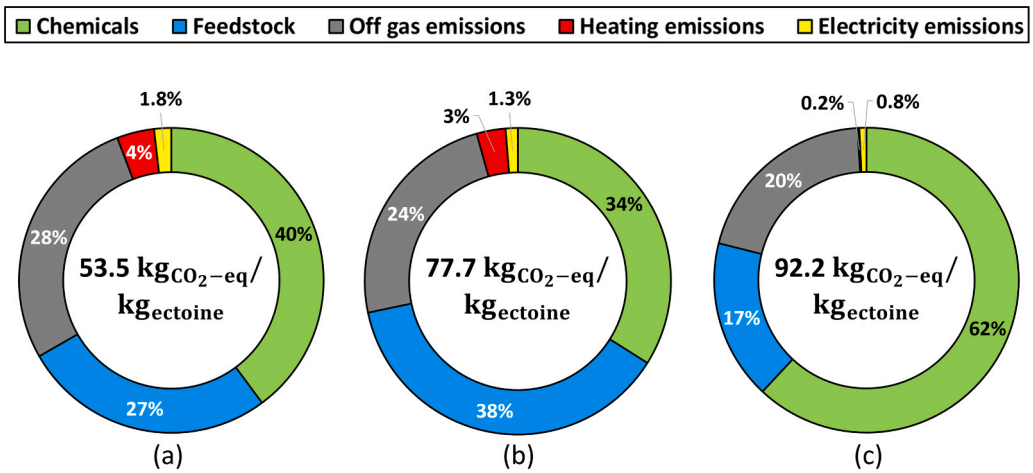

Fig. 4. GHG emissions of the (a) EctoMet process, (b) Methanotroph-based process, and (c) Bacterial Milking.

potential decrease in freshwater use of approximately 72 %.

The EctoMet value chain generates, respectively, 26 % and 58 % less wastewater compared to the Methanotroph-based and Bacterial Milking processes, as shown in Figure S2 (b). This is a direct result of the lower water and chemicals usage of the former, owing to its higher ectoine production yield. The use of microalgae, which are known to uptake nutrients, can also lead to lowering the nutrient loads of the generated waste streams of the EctoMet value chain, thus reducing risks of aquatic

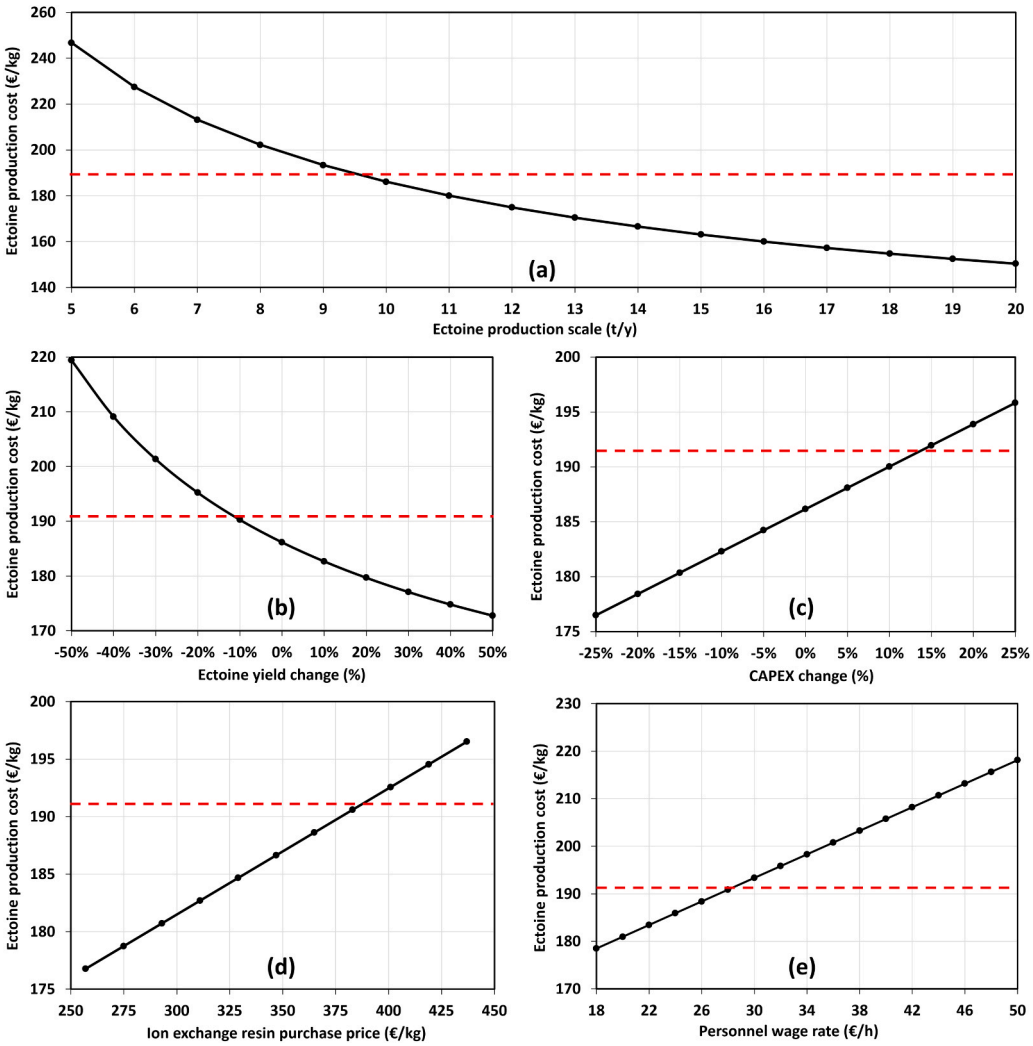

Fig. 5. Impact of the most influential parameters –(a) ectoine production scale, (b) ectoine yield, (c) CAPEX, (d) IER purchase price, and (e) personnel wage rate– on the ectoine production cost of the EctoMet value chain (red line corresponds to the best-case production cost reported by Pérez Martínez (2022) for a methanotroph-only process).

systems eutrophication [34].

While the current environmental assessment primarily focused on GHG emissions, water use, and wastewater generation, future studies should evaluate additional indicators such as human toxicity, land use, and resource depletion. Including more categories will enable a more comprehensive life cycle assessment of the EctoMet process and its integration potential in circular bioeconomy frameworks.

3.6. Sensitivity analysis

3.6.1. Economic sensitivity

Results of the economic uncertainty analysis for the EctoMet value chain are presented in Figure S3 (Supplementary Materials). The most impactful parameters on the variance of the ectoine production cost are the production scale, ectoine yield, CAPEX variation, IER price, and personnel wage. These key parameters were varied within specific ranges in the local sensitivity analysis to reflect realistic best- and worst-case scenarios (Fig. 5).

The ectoine production scale was varied between 5 and 20 t/y to account for potential changes in market demand for ectoine [35]. The ectoine production yield was varied by ±50 % to account for the impact of yield variations, which could result from changes in operating conditions or differences in feedstock composition [36]. The CAPEX was varied by ±50 % to capture uncertainties in equipment prices and installation costs. The IER price was varied in the range of €257 – €437 kg^{-1} to reflect potential changes in supply chain dynamics or raw material costs. Labor wage rates were varied between €18 and €50 h^{-1} to account for differences in regional wage fluctuations and degree of process automation.

The ectoine production scale is the most critical factor impacting the economic performance of the EctoMet process. As shown in Fig. 5(a), the ectoine production cost decreased considerably, and non-linearly, as the production scale increased. This trend reflects the well-established economy of scale effect, in which larger facilities achieve more efficient use of equipment and reduced per-unit operating expenses [37]. It is therefore crucial to optimize plant capacity to maximize economic viability.

Scaling up the EctoMet process may introduce several challenges related to maintaining stable methanotroph–microalgae interactions, ensuring efficient gas–liquid mass transfer, and achieving uniform light distribution within photobioreactors [38]. These aspects can considerably impact process stability and overall productivity. Such challenges can be mitigated by adopting modular reactor configurations and optimizing mixing and lighting strategies [38].

The production scale must not be chosen only to maximize economic viability, but also to align with the market demand for ectoine, reaching around 20 t/y globally [39]. Overestimating demand and constructing facilities with excessive capacity can lead to underutilized equipment, reducing the economic benefits of scaling up and raising fixed costs relative to production output [40]. On the other hand, underscaling production capacity can lead to diminished revenues and reduced competitiveness in the market. It is therefore essential to strike a balance between production capacities, market needs, and openness to new opportunities.

Such new opportunities include the potential to convert biogas into a variety of other high-value products besides ectoine, including carotenoids, long-chain fatty acids, microbial protein, and other osmolytes, by adapting the microbial consortium. This product diversification strategy can mitigate risks associated with market fluctuations or seasonal demand variations for ectoine [41]. For instance, if ectoine demand declines, due to market saturation or changes in industry focus, the plant can transition to producing other products that are in higher demand, maintaining a profitable operation.

Another important parameter that considerably impacts the production cost is the ectoine production yield. This is evidenced by the substantial production cost reductions associated with increased yields, as portrayed in Fig. 5(b). This is a result of the direct relationship between ectoine yield and the plant's CAPEX and OPEX. Higher yields reduce the capacity requirements and, consequently, costs of various processing steps, including biogas pretreatment (desulfurization), ectoine production and excretion, and downstream processing (mainly dewatering, purification, and drying). Higher yields also decrease operational expenses by reducing the use of feedstock, chemicals, and utilities (water and energy) per unit of produced ectoine.

Enhancing the ectoine yield is, therefore, essential for improving the economic feasibility of the EctoMet value chain, which can be achieved through several strategies. First, it is essential to optimize growth conditions for the methalgae consortium. Temperature plays an important role in promoting both methanotrophic and microalgal activity, with optimal ranges between 26 and 30°C [42]. Maintaining pH levels between 6.5 and 7.5 ensures favorable conditions for microbial activity and enzymatic processes essential for ectoine production [42]. Controlling biomass ratios and ensuring balanced growth of both methanotrophs and microalgae can enhance yields. This can be achieved through light regulation, which has been shown to effectively control activity and pH without requiring external chemicals addition [42]. Adequate availability of nutrients, particularly nitrogen, carbon, and trace minerals, is also crucial for supporting microbial growth and promoting ectoine synthesis [43]. Salinity levels also play an important role in determining the ectoine yield, as moderate salinity levels (around 3–6 % NaCl) have been shown to enhance ectoine production using methalgae [15].

The ectoine yield could also be improved by selecting highly performant and stress-resilient strains of methanotrophs and microalgae. Genetically modifying these strains can further enhance their metabolic pathways, thus increasing the efficiency of biogas conversion to ectoine and improving their tolerance to environmental stresses [44]. At the process level, process intensification strategies can be adopted to improve biogas delivery and utilization and increase ectoine yield. Optimizing gas-liquid mass transfer for instance is important to ensure that the microorganisms can efficiently consume CH_4 and CO_2 [43]. This can be achieved by optimizing several parameters of the bubble column bioreactor, such as improving gas delivery systems by adjusting diffuser pore size to increase the gas-liquid interface [19].

Variations in the CAPEX also impact the ectoine production cost of the EctoMet process, as shown in Fig. 5(c). Evidently, higher investments directly increase ectoine production costs, due to higher equipment purchase, installation, depreciation, and maintenance costs. CAPEX can be reduced by optimizing plant design to minimize the number of required process steps and equipment.

Owing to its high economic value, IER purchase price has an influence on the ectoine production cost. The IER is a critical input for the downstream processing, particularly for the IEX step needed for ectoine purification [7]. Given its indispensable role and high price, it is important to keep costs associated with IER purchase reasonable. For instance, using resin regeneration technologies can reduce the amount of virgin resin that needs to be purchased [45].

Labor is an equally important factor given its high contribution to operating expenses. Higher wages directly increase the total OPEX and, consequently, raise the ectoine production cost. Locating the plant in regions with competitive wage rates can reduce these expenses. Another option is automating plant operations, which can reduce labor costs. However, a higher level of automation may increase CAPEX, due to the need for advanced control systems, in addition to higher OPEX, due to increased energy consumption [46].

The local sensitivity analysis results were further interpreted by comparing the ectoine production cost of the EctoMet process to a reference value, defined as the best-case ectoine production cost reported in [47] (Fig. 5). This reference cost, representing ectoine synthesis from biogas using methanotrophs, was adjusted to the year of the study (€191.5 kg^{-1}). This specific case was chosen as a basis for comparison since it shares similarities with the EctoMet process in terms of

feedstock type, plant design, and geographic location. Using the best-case ectoine production cost as the reference allowed comparing the economic performance of the EctoMet process to fully optimized methanotroph-only pathways. This comparison provides insights into the targets that the EctoMet process must achieve to maintain its economic competitiveness.

The EctoMet value chain has an economic advantage over the reference case (methanotroph-based process) at production capacities higher than 9300 kg of ectoine per year, which is particularly interesting given that the global demand for ectoine (and osmolytes in general) is considerably higher than this capacity. Despite a 12 % loss in ectoine yield, the EctoMet process maintains economic competitiveness, indicating its resilience to moderate fluctuations in productivity. This process can also overcome increases in its CAPEX by up to 12.5 % without losing its economic advantage, providing reasonable flexibility in terms of higher investment requirements. The purchase price of IER should stay below €387 kg^{-1}, while personnel wages must not exceed €28 h^{-1} to maintain financial competitiveness. By meeting these targets, the EctoMet value chain has the potential to offer a reliable and economically viable alternative for ectoine production from biogas.

3.6.2. GHG emissions sensitivity

The results of the GHG emissions uncertainty analysis for the EctoMet process are shown in Figure S4 (Supplementary Materials). The parameters with the highest impact on the variance of the GHG emissions are biogas emissions, yield change, and flue gas emissions. A local sensitivity analysis was performed on these parameters, as shown in Figure S5 (Supplementary Materials). Emissions from biogas have the greatest impact on the total GHG emissions of the EctoMet process, mainly due to the relatively high amount of biogas used, coupled with the wide variability in its GHG emission factor [48]. This variability is attributed to differences in plant designs and operational conditions across AD facilities, including variations in feedstock type, reactor technology, and energy source.

Additional uncertainty is introduced by the difficulty of accurately measuring emissions of AD plants [49]. Fugitive biogas emissions, which can occur during gas storage and handling, are particularly difficult to measure reliably [50]. Emissions from digestate storage and application, another potential source of GHG emissions, also add to the variability, due to differences in storage and application practices and environmental conditions [51].

To minimize emissions from biogas and improve the environmental performance of the EctoMet value chain, it is essential to source biogas from sustainable AD plants that operate under optimized conditions and utilize renewable energy sources [52]. These AD plants rely on optimized reactor designs and improved digestate storage conditions to minimize methane leaks [53,54]. Incorporating renewable energy sources, such as wind or solar, to satisfy their energy requirements can further reduce the carbon footprint of the generated biogas [55].

The environmental performance of ectoine production facilities can be improved by constructing them near existing AD plants, thus eliminating the need for long-distance biogas transport, which is associated with higher methane losses and increased energy consumption (for compression and storage) [56,57]. Proximity also enables the direct use of raw biogas, reducing the need for extensive upgrading or treatment typically needed before transportation and thus lowering associated emissions.

Improving ectoine production yields can equally lower the GHG emissions. Higher yields enhance process efficiency by reducing the consumption of feedstock, chemicals, and utilities per unit of ectoine produced. Higher yields also result in less waste generation, which further contributes to reducing the total carbon footprint.

4. Conclusions

This study presents the first techno-economic assessment and environmental impact evaluation of a novel biogas-based ectoine production process using a methanotroph–microalga consortium. This process has the potential of producing ectoine at costs lower by 45 % and 33 % compared to the bacterial milking and methanotroph-based processes, respectively. These cost reductions are mostly driven by higher ectoine yields, which reduce equipment size and operational expenses. The use of biogas as a low-cost feedstock further improves its economic competitiveness. Sensitivity analysis identified production scale and ectoine yield as the most influential parameters affecting economic performance. The process also achieves considerable GHG emissions reductions, averaging 36 % compared to competing technologies. Water consumption and wastewater generation are also lower by an average of 28 %, reducing the process' overall water footprint and minimizing the risk of pollution. Overall, the novel process offers an economically viable and environmentally sustainable alternative for ectoine production from biogas.

Funding

This research was funded by the Research Foundation of Flanders (FWO Project Bio Economy "EctoMet" G0E0723N).

CRediT authorship contribution statement

Mohammed El Ibrahimi: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mohammed Nazeer Khan:** Writing – review & editing, Visualization, Validation, Software, Methodology, Funding acquisition, Conceptualization. **Patricia Ruiz-Ruiz:** Writing – review & editing, Validation, Data curation, Conceptualization. **Jo De Vrieze:** Writing – review & editing, Resources, Funding acquisition, Data curation, Conceptualization. **Miet Van Dael:** Writing – review & editing, Software, Resources, Methodology, Funding acquisition, Conceptualization.

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.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2025.120093.

Data availability

Data will be made available on request.

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