Revised manuscript_ COBIOT_2017

Technologies for the bioconversion of methane into more valuable products

S. Cantera, R. Muñoz, R, Lebrero, J.C López, Y. Rodríguez, P A. García-Encina*

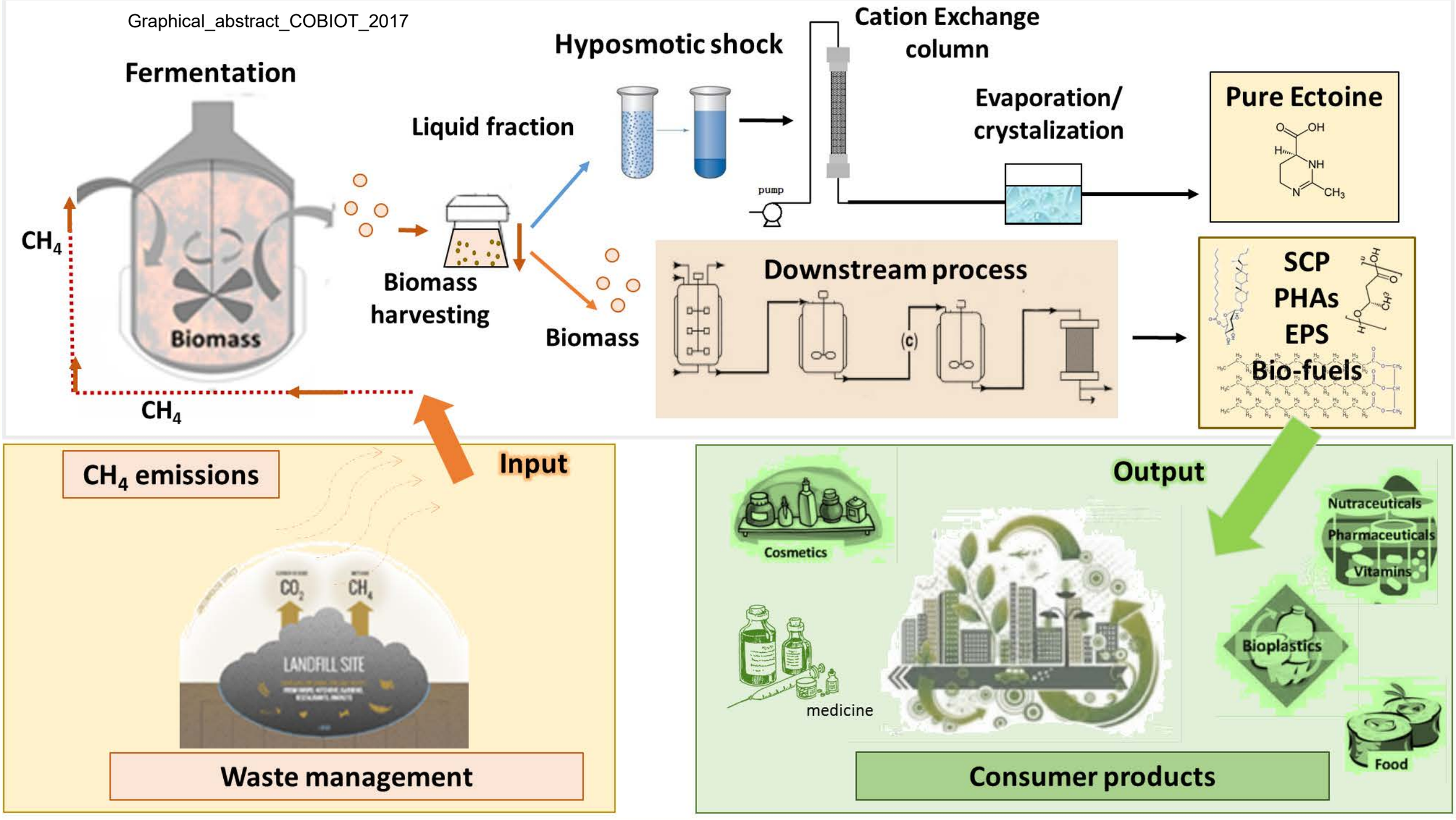
Department of Chemical Engineering and Environmental Technology, Valladolid University, Dr. Mergelina, s/n, Valladolid, Spain, Tel. +34 983186424, Fax: 983423013. pedro@iq.uva.es

Abstract

Methane, with a global warming potential twenty five times higher than that of CO_2 is the second most important greenhouse gas emitted nowadays. Its bioconversion into microbial molecules with a high retail value in the industry offers a potential cost-efficient and environmentally friendly solution for mitigating anthropogenic diluted CH_4-laden streams. Methane bio-refinery for the production of different compounds such as ectoine, feed proteins, biofuels, bioplastics and polysaccharides, apart from new bioproducts characteristic of methanotrophic bacteria, has been recently tested in discontinuous and continuous bioreactors with promising results. This review constitutes a critical discussion about the state-of-the-art of the potential and research niches of biotechnologies applied in a CH_4 biorefinery approach.

Keywords: CH_4 bio-refinery, methane treatment, bio-products, industrial approach

Graphical_abstract_COBIOT_2017



- Methanotrophs as a competitive platform for the generation of valuable products
- CH_4 emissions used as a feedstock in biorefinery reduces GHGs environmental impact
- Current biotechnological limitations and potential improvements are reviewed
- Environmental and economic analysis supports the feasibility of CH_4 revalorization

Technologies for the bioconversion of methane into more valuable products

S. Cantera, R. Muñoz, R, Lebrero, J.C López, Y. Rodríguez, P A. García-Encina*

Department of Chemical Engineering and Environmental Technology, Valladolid University, Dr. Mergelina, s/n, Valladolid, Spain, Tel. +34 983186424, Fax: 983423013. pedro@iq.uva.es

Abstract

Methane, with a global warming potential twenty five times higher than that of CO_2 is the second most important greenhouse gas emitted nowadays. Its bioconversion into microbial molecules with a high retail value in the industry offers a potential cost-efficient and environmentally friendly solution for mitigating anthropogenic diluted CH_4-laden streams. Methane bio-refinery for the production of different compounds such as ectoine, feed proteins, biofuels, bioplastics and polysaccharides, apart from new bioproducts characteristic of methanotrophic bacteria, has been recently tested in discontinuous and continuous bioreactors with promising results. This review constitutes a critical discussion about the state-of-the-art of the potential and research niches of biotechnologies applied in a CH_4 biorefinery approach.

Keywords: CH_4 bio-refinery, methane treatment, bio-products, industrial approach

1. Introduction

Methane (CH_4), with a global warming potential 25 times higher than that of carbon dioxide (CO_2), is the second most important greenhouse gas (GHG) worldwide, currently representing 18 % of the total EU-28 GHG emissions. This current scenario has increased concern about global warming, has encouraged the development of political initiatives for GHGs abatement and has promoted intensive research on novel biotechnological strategies for CH_4 treatment.

Current biotechnological processes could be the best solution for methane abatement due to their cost-effectiveness and their low environmental impact; however the implementation of bio-reactors for CH_4 treatment is still limited. This limitation is mainly based on the low water solubility of CH_4, which hampers the transport of this GHG to the microbial community and increases the cost of CH_4 treatment biotechnologies [1]. In this context, the development of a CH_4 biorefinery based on using CH_4-laden emissions as raw materials to bio-synthesize high added value products represents a cost-effective solution for the mitigation of this GHG [2–6]. Although optimization from a micro and macroscopic perspective is still required to enhance microbial CH_4 bioconversion, this innovative CH_4 biorefiney can turn GHG abatement into a sustainable, profitable and competitive process.

This review will critically discuss the state-of-the-art of innovative CH_4 treatment biotechnologies adapted to the production of key added value products. The main current biotechnological limitations and potential improvements will also be reviewed together with an environmental and economic analysis of this novel CH_4 biorefinery.

2. Microbiology of CH_4 treatment

Biotechnologies for the treatment of CH_4 are based on the biocatalytic action of microorganisms, mainly aerobic methane-oxidizing bacteria called methanotrophs, which transform methane into carbon dioxide and water using oxygen as electron acceptor. Methanotrophs belong to the methylotrophic bacterial group, which consists on organisms that utilize reduced one-carbon substrates for their metabolism. Within this physiological group, methanotrophs were classified and considered the only group able to use CH_4 as their single energy and carbon source [7]. However, recent findings demonstrated that some methanotrophs are also able to utilize multicarbon compounds as their carbon source in some environments [8]. The current classification of known aerobic methanotrophic genera based

on 16S rRNA gene encloses a wide phylogenetic distribution within the three general groups: *Alphaproteobacteria*, *Gammaproteobacteria* and *Verrucomicrobia* [9,10]. The alpha-proteobacterial methanotrophs can be further divided into the *Beijerinckiaceae* and *Methylocystaceae* families, while the gamma-proteobacterial methanotrophs belong to the *Methylococcaceae* family and the verrucomicrobial methanotrophs to the family *Methylacidiphilacecae*[11]. Furthermore, even if aerobic oxidation is the main implemented process in biotechnologies treating methane, recent findings have demonstrated that some anaerobic archaea and bacteria are responsible for 7-25% of the total methane oxidation worldwide[12]. The anaerobic oxidation of methane (AOM) is carried out by bacteria belonging to NC10 phylum (*Candidatus* "*Methylomirabilis oxyfera*")which couple methane oxidation with denitrification and three groups of archaea: ANME-1 (distantly related to the *Methanosarcinales* and *Methanomicrobiales* spp.), ANME-2 (within the *Methanosarcinales* sp.), and ANME-3 (closely related to the *Methanococcoides* sp.) [13]. ANME-1 and ANME-2a, 2b and 2c oxidize methane with sulfate as electron acceptor, forming consortia with sulfate reducing bacteria, while some ANME-2 (ANME-2d) belong to a cluster that couples AOM to nitrate reduction through the archaea of the order Methanosarcinales, related to 'Ca. Methanoperedens nitroreducens'. Moreover, some ANME-2 are able to oxidize methane with metals as iron or humic acids as acceptor, also without a syntrophic partner. In the particular case of ANME-3, little is known about its metabolism [14–16]. On the other hand, some fungal genera such as *Graphium* have been reported as methane oxidizers [17]. However, knowledge on both fields is still scarce and further research is necessary to understand these processes before their implementation as methane treatment technologies [18].

3. CH_4 based commercial bio-products

During the last 20 years, a wide range of high added value compounds produced by methanotrophic bacteria has been described (Table 1).

Ectoine

Ectoine is a cyclic imino acid that provides osmotic balance to a wide number of halotolerant bacteria [5,19,20]. Due to its high effectiveness as stabilizer of enzymes, DNA-protein complexes and nucleic acids, ectoine is used in medicine, cosmetology, dermatology and nutrition [21]. In this regard, this osmolyte is probably one of the most valuable products synthesized by microorganisms, retailing in the pharmaceutical industry at approximately

US$1000 kg^{-1} (global consumption of 15000 tones $year^{-1}$) [5]. Despite its potential, ectoine is only currently biotechnologically produced by *Halomonas elongate* through a long fed-batch fermentation process called *biomilking* (total duration ~120 h), which consists of sequential hypo and hyper osmotic shocks [22]. However, this process is costly due to the high quality substrates required, besides entailing a complex and expensive downstream processing [19,20,23]. Current investigations [24–26] have shown that halotolerant methanotrophs belonging to the genera *Methylomicrobium, Methylobacter* and *Methylohalobius* are able to produce ectoine in batch and in continuous bioreactors reaching contents of 3-10 % (g/g) depending on the concentration of methane and NaCl. To date, the feasibility of the ectoine production in continuous bioreactors has been tested at laboratory scale. In 2016 a study demonstrated for the first time the continuous production of ectoine by *M. alcaliphilum 20Z* with average contents of 37.4 mg ectoine (g biomass)$^{-1}$)[27], while in 2017 a CH_4-based *biomilking* process resulted in extracellular concentrations of 253.4 ± 55.1 mg L^{-1}, corresponding to a recovery of ~ 70 % of the total intra-cellular ectoine accumulated [28]. Although the productivities achieved by *M. alcaliphilum* are still lower than those reported in literature by other organisms using sugars as a carbon source, *M. alcaliphilum 20Z* exhibits a superior environmental performance (associated to climate change mitigation) based on its ability to produce ectoine from dilute CH_4 emissions.

Bioplastics (PHAs)

PHAs such as poly-3-hydroxybutyrate (PHB) and the copolymer poly (3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) are intracellular biopolyesters produced under nutrient-limiting and carbon-excess conditions by a wide range of microorganisms as carbon and energy storage resources [29]. Their outstanding mechanical properties, similar to those of polypropylene and polyethylene, along with their biodegradability and biocompatibility make PHAs an attractive and potential alternative to oil-based plastics [30,31]. As a result, PHAs are manufactured nowadays by nearly 30 companies, with Meredian Inc. (annual production of 300 kt) and Bio-On (annual production ~10 kt) being the leading manufacturers in U.S. and Europe, respectively[30]. *Ralstonia eutropha, Bacillus megaterium* and *Alcaligenes latus* are the main industrial PHA-producing heterotrophic organisms, with the most common feedstocks utilized being glucose and fructose. Nevertheless, the high cost of these carbon sources, which accounts for 30-40% of the total production costs, still hinders PHAs

commercialization due to their uncompetitive market price (4-20 € kg_{PHA}^{-1})[32,33]. In this context, the methane contained in diluted industrial gas emissions (≤ 5 % *v/v*) has recently emerged as a potential feedstock for PHA production. The use of residual methane as a carbon source will significantly decrease PHAs production costs, while reducing the environmental impacts of GHG emissions [31,34]. Under nutrient-limited conditions (i.e N-, P- or Mg-limitation), *Methylocystis*, *Methylosinus and Methylocella* are considered the main methanotrophic PHA producer genera, achieving PHBVs contents ranging from 20 to 51 % (wt) under batch [35,36] and continuous operation in suspended growth reactors [37–40]. In this regard, Mango Materials and Newlight Technologies (U.S.) are the pioneering companies in the development of methanotrophic-based technologies devoted to PHB production using CH_4 emissions [11].

Single cell protein (SCP)

Microbial protein is generally referred to as a single cell protein. Although microbial protein provides a relatively small proportion of current human nutrition, the growing global demand for protein is likely to make SCP increasingly important. Bacterial SCP generally contains 50–80% protein on a dry weight basis and the essential amino acid content is expected to be comparable to or higher than the FAO recommendations[4,41]. Imperial Chemical Industries produced SCP (Pruteen) for animal feed from methanol. However, they could not compete with cheaper animal feeds that were available at the end of the 1970s and production was discontinued. Nevertheless, methane is now gaining interest as a substrate for SCP. UniBio A/S and Calysta Inc. have both developed fermentation technologies to convert natural gas to animal feed protein by using methanotrophic bacteria [5,42]. UniBio A/S uses a U-loop fermenter to achieve a productivity of 4 kg biomass m^{-3} h^{-1} with ~ 70% protein (approved as animal feed). In 2017, Calysta Inc. started to distribute commercial samples of its CH_4-based protein, FeedKind®, for animal feed. Calysta plans to open a larger facility with Cargill Inc. in the U.S.A [41,43]. Additionally, due to the increased interest in methane mitigation in recent years, new companies such as VTT Ltd. are investigating new reactor designs to use the methane produced from cattle and pig farming (considered dilute CH_4 emissions) for SCP production for animal nutrition.

Biofuels

Nonpolar lipids are mainly in the form of triacylglycerides and can be transesterified to produce biodiesel. In methanotrophs, these lipids represent an important fraction because they host methane monooxygenase (the microbial lipid fraction in the biomass can be more than 20%). Moreover, the conversion of methane into other liquid biofuels, such as ethanol or 1-butanol, is thermodynamically favourable and can be accomplished through chemical or biological methods. Currently, the biotechnological production of fuels depends mainly on costly sugars, such as glucose, the cost of which is estimated to be about 50% of the final products. Therefore, considerable efforts to identify alternative carbon sources in order to minimize the production cost of fuels are currently under way. In 2012, a $4 million Advanced Research Projects Agency — Energy (ARPA-E) grant was awarded to a group from the University of Washington (UW, Seattle, WA, USA) to develop microbes capable of converting methane into liquid diesel fuel for transportation. Moreover, the National Renewable Energy Laboratory (NREL, Golden, CO, USA), the biofuel company LanzaTech, Inc. (Chicago, IL, USA) and the chemical company Johnson Matthey (London, UK) have joined with UW to develop a more efficient bioconversion process for liquid fuel production. Additionally, the company Oberon has recently started to convert methane and carbon dioxide from various methane feedstocks to Dimethyl ether.

Extracellular polysaccharides (EPS)

EPS are biopolymers in which biofilms are embedded and comprise a wide variety of proteins, glycoproteins, glycolipids and polysaccharides [44]. EPSs provide the microorganisms an effective protection against high or low temperature, salinity and predators [2, 37]. These bioproducts are of interest due to their colloidal and adhesive properties and their effects on liquid rheology in the food, pharmaceutical, textile and oil industries[45]. CPKelco, Merck, Pfizer and Prasinotech Ltd are companies currently focused on producing polysaccharides such as xanthan (4-13 € kg^{-1}) using *Xanthomonas campestris* and dextran using *Leuconostoc mesenteroides* and *Streptococcus mutans* (30-50 € kg^{-1}), though productivities and costs derived from the supplementation of the carbon source and the down streaming still hamper their industrial production [46]. In this sense, CH_4 represents an alternative feedstock to reduce costs, since EPS productions of 300-430 mg g $biomass^{-1}$ in type I methanotrophs (*Methylobacter, Methylomonas*) have been reported [47]. Overall, extreme conditions of salinity is one of the best strategies to promote EPS formation [48]. In this context, an study carried out recently in our lab (data non published) showed that under a

high alkalinity and salinity medium (pH=9 and 6 % NaCl) EPS reached concentrations of 1833 ± 87.0 mg EPS g biomass^{-1} using *M. alcaliphilum* in bubble column bioreactors treating diluted methane emissions, which allowed coupling ectoine generation with high rate EPS production.

New bio-products

Recent studies have focused on alternative bio-products that can be naturally generated by methanotrophs and are of interest in the chemical, food, pharmaceutical and environmental industries [5,6]. Two products that are receiving special attention are the bacterial Surface-Layers and methanobactins, both proper of methanotrophic bacteria. These compounds are copper-binding extracellular proteins that can be applied in environmental remediation (as a metal chelator or reducing agent for metal recovery from mine leachates) [49] and for the treatment of the Wilson disease [50]. Membrane lipids in methanotrophs may have an alternative high-value application as a health supplement. There is a current patent for using methanotrophic lipids to manufacture an oral administration to reduce plasma cholesterol levels or lower the ratio of LDL to HDL in animal subjects [51,52] . On the other hand, soluble metabolic intermediates such as methanol, formaldehyde and organic acids are all potential products from methanotrophs with multiple industrial applications. In this regard, Calysta and NatureWorks have an R&D collaboration to transform methane into lactic acid. Moreover, there is a wide number of gas fermentation companies – such as, Kiverdi, Coskata, INEOS Bio or LanzaTech – that produce a wide range of commercially useful molecules using methanotrophic microbes.

4. Current biotechnological limitations

Although the perspective of a near future methane based bio-refinery is promising, the implementation of these CH_4 bioconversion processes is still scarce due to the occurrence of physical and biological limitations. Thus, even if CH_4 treatment biotechnologies have been studied for the last 50 years, conventional bioreactors are still limited by the poor mass transport of CH_4 from the gas to the microbial community. CH_4 is a hydrophobic gas pollutant with a low aqueous solubility (dimensionless Henry's law constant $H_{CH4} = C_g/C_{aq}$ = 30 at 25 ºC) [53–55]. This entails a low gas-liquid concentration gradient and therefore process operation at high empty bed gas residence times, which significantly increases both the investment and operating costs of conventional biotechnologies [55]. Moreover, this low

CH_4 solubility limits process performance as a result of the low elimination rates and low biomass concentrations present in the bioreactors. For example, systems operated with sugars as a carbon source achieved biomass concentrations up to 30 g L^{-1} and therefore, higher fermenter productivities. However, pure methanotrophic strains achieved average biomass concentrations of 1.0 g L^{-1} and bioproduct productivities 10 to 100 times lower depending on the product [20,28,38,39,41,56–58]. In this context, mass transfer limitations during EPS and ectoine production can be even more pronounced as a result of the lower CH_4 solubility at higher cultivation broth salinity [59], Nowadays, the process implemented at industrial scale using *Halomonas elongate* involves the cyclic increase and decrease of the salt concentration in the cultivation broth up to 12 % NaCl, which severely limits O_2 mass transport and also triggers reactor corrosion. However, recent studies conducted for the production of ectoine with methanotrophs were carried out at an optimum salt concentration of 6 % NaCl, which could eventually limit the above mentioned limitations [28,60]. On the other hand, methanotrophic bacteria are limited by a low growth rate mediated by the slow kinetics of methane uptake. For example, MMO oxidizes methane to methanol in the first step of methane assimilation, but requires a high-energy electron donor (i.e NADPH) as an energy input to functionalize the otherwise inert methane molecule[61]. Additionally, most methanotrophic bacteria discovered to date are sensitive to mechanical stress, which requires reducing the agitation rate of the system used, thus hampering the mass transfer of CH_4 to the microbial community[62].

5. Potential improvements

One of the major challenges to improve the CH_4 conversion yields is the enhancement of the gas-liquid mass transfer through the design of novel bio-reactors [63]. In this regard, suspended-growth bioreactors are the most suitable configurations for the bioconversion of CH_4-laden gas streams into valuable byproducts and their subsequent recovery. The most popular approach for the production of high added value bioproducts in industrial biotechnology has been fed-batch cultivation in mechanically stirred fermenters with controlled nutrient feeding[22,64], since this operation mode allows to achieve high cell densities. However, this approach would entail prohibitive operating costs during CH_4 bioconversion as a result of the intensive stirring required. One of the most innovative industrial bio-reactors designed to improve methane abatement and the production of SCP has been the new U-Loop fermentor patented by UniBio A/S[43]. This bioreactor is a

modified airlift capable of handling a large biomass concentration while providing a high gas to liquid mass transfer in comparison with those obtained in stirred tank reactors or in previous tubular loop reactors. Other novel concepts for enhanced mass transport are suspended-growth membrane diffusion and pressurized bioreactors, which operate at low-moderate energy demands. In addition, the performance of these innovative high-mass transfer bioreactors can be boosted via internal gas recirculation, which allows decoupling the $K_la_{G/A}$ from the gas residence time [65].

On the other hand, the development of a bioprocess maximizing the production of a target product requires the standardization of culture conditions. The most important environmental variables affecting the cultures of methanotrophs are pH, temperature, dissolved O_2 concentration, the ratio of methane and O_2, and the time of cultivation [10,61]. However, the composition of the cultivation medium often has a key influence in the synthesis of specific bio-products. For instance, the starvation of an essential nutrient under a sustained CH_4 supply can trigger the production of PHAs and copper concentration has been identified as a factor inducing the natural excretion of ectoine to the extracellular medium [11]. In addition, selective conditions such as pH values and high salt contents can minimize and control microbial contamination in the process. Moreover, the effect of co-substrates addition during methane bioconversion must be investigated in order to tailor the characteristics of the bioproducts synthesized.

Finally, methane-activation strategies using a dioxygenase-like enzyme capable of oxidizing two methane molecules simultaneously have been also envisaged to boost process performance [61]. However, such an enzyme has not been found or engineered successfully to date. In this regard, '-omics' technologies will play a key role in the near future to improve and realize a profitable methane bio-refinery. Companies like Intrexon and Calysta are deeply investing into finding more competitive modified methanotrophs capable of generating multiple products from a single methanotroph process in order to improve the economic viability of the process.

6. Perspectives

Provided that the current economic and technical barriers for the operation of methane biorefineries are solved, these climate-change mitigating technologies can be a cost-effectiveness solution for the continuous abatement of methane dilute emissions (Figure 1).

In this regard, a brief environmental and economic viability analysis of a methane bio-refinery producing ectoine, SCP, PHAs and EPS from a 50 000 m^3 h^{-1} landfill emission is shown in Table 2.

References

1. Cantera S, Estrada JM, Lebrero R, García-Encina PA, Muñoz R: **Comparative performance evaluation of conventional and two-phase hydrophobic stirred tank reactors for methane abatement: Mass transfer and biological considerations**. *Biotechnol Bioeng* 2016, **113**:1203–1212.

2. Cantera S, Lebrero R, Sadornil L, García-Encina PA, Muñoz R: **Valorization of CH4 emissions into high-added-value products: Assessing the production of ectoine coupled with CH4 abatement**. *J Environ Manage* 2016, **182**:160–165.

3. Cal AJ, Sikkema WD, Ponce MI, Franqui-Villanueva D, Riiff TJ, Orts WJ, Pieja AJ, Lee CC: **Methanotrophic production of polyhydroxybutyrate-co-hydroxyvalerate with high hydroxyvalerate content**. *Int J Biol Macromol* 2016, **87**:302–307.

4. Strong PJ, Xie S, Clarke WP: **Methane as a resource: Can the methanotrophs add value?** *Environ Sci Technol* 2015, **49**:4001–4018.

5. Strong PJ, Kalyuzhnaya M, Silverman J, Clarke WP: **A methanotroph-based biorefinery: Potential scenarios for generating multiple products from a single fermentation**. *Bioresour Technol* 2016, **215**:314–323.

6. Khmelenina VN, Rozova N, But CY, Mustakhimov II, Reshetnikov AS, Beschastnyi AP, Trotsenko YA: **Biosynthesis of secondary metabolites in methanotrophs: biochemical and genetic aspects (review)**. *Appl Biochem Microbiol* 2015, **51**:150–158.

7. Hanson RS, Hanson TE: **Methanotrophic bacteria.** *Microbiol Rev* 1996, **60**:439–471.

8. Semrau JD: **Bioremediation via methanotrophy: Overview of recent findings and suggestions for future research**. *Front Microbiol* 2011, **2**.

9. Pieja AJ, Morse MC, Cal AJ: **Methane to bioproducts: the future of the bioeconomy?** *Curr Opin Chem Biol* 2017, **41**:123–131.

10. Semrau JD, Dispirito AA, Yoon S: **Methanotrophs and copper**. *FEMS Microbiol Rev* 2010, **34**:496–531.

11. Cantera S, Frutos OD, López JC, Lebrero R, Torre RM: **Technologies for the bio-conversion of GHGs into high added value products: Current state and future prospects**. In *Carbon footprint and food cycel book. Green Energy and Technology ed. (Springer ed.)*. 2017:359–388.

12. Morris BEL, Henneberger R, Huber H, Moissl-Eichinger C: **Microbial syntrophy: Interaction for the common good**. *FEMS Microbiol Rev* 2013, **37**:384–406.

13. Knittel K, Boetius A: **Anaerobic Oxidation of Methane: Progress with an Unknown Process**. *Annu Rev Microbiol* 2009, **63**:311–334.

14. Timmers PH, Suarez-Zuluaga DA, van Rossem M, Diender M, Stams AJ, Plugge CM: **Anaerobic oxidation of methane associated with sulfate reduction in a natural freshwater gas source**. *ISME J* 2015, **10**:1–13.

15. Timmers PHA, Welte CU, Koehorst JJ, Plugge CM, Jetten MSM, Stams AJM: **Reverse Methanogenesis and Respiration in Methanotrophic Archaea**. *Archaea* 2017, **2017**.

16. Gao Y, Lee J, Neufeld JD, Park J, Rittmann BE, Lee H-S: **Anaerobic oxidation of methane coupled with extracellular electron transfer to electrodes**. *Sci Rep* 2017, **7**:5099.

17. Lebrero R, Lopez JC, Lehtinen I, Perez R, Quijano G, Muñoz R, Lopez JC, Lehtinen I, Perez R, Quijano G, et al.: **Exploring the potential of fungi for methane abatement: Performance evaluation of a fungal-bacterial biofilter**. *Chemosphere* 2016, **144**:97–106.

18. Wang D, Wang Y, Liu Y, Ngo HH, Lian Y, Zhao J, Chen F, Yang Q, Zeng G, Li X: **Is denitrifying anaerobic methane oxidation-centered technologies a solution for the sustainable operation of wastewater treatment Plants?** *Bioresour Technol* 2017, **234**:456–465.

19. Lang Y jun, Bai L, Ren Y nan, Zhang L hua, Nagata S: **Production of ectoine through a combined process that uses both growing and resting cells of**

Halomonas salina DSM 5928T. *Extremophiles* 2011, **15**:303–310.

20. Pastor JM, Salvador M, Argandoña M, Bernal V, Reina-Bueno M, Csonka LN, Iborra JL, Vargas C, Nieto JJ, Cánovas M: **Ectoines in cell stress protection: Uses and biotechnological production**. *Biotechnol Adv* 2010, **28**:782–801.

21. Poli A, Finore I, Romano I, Gioiello A, Lama L, Nicolaus B: **Microbial Diversity in Extreme Marine Habitats and Their Biomolecules**. *Microorganisms* 2017, **5**:25.

22. Sauer T, Galinski EA: **Bacterial Milking : A Novel Bioprocess for Production of Compatible Solutes**. *Biotechnolnogy Bioeng* 1998, **57**:306–313.

23. Kunte HJ, Lentzen G, Galinski EA: **Industrial Production of the Cell Protectant Ectoine: Protection Mechanisms, Processes, and Products**. *Curr Biotechnol* 2014, **3**:10–25.

24. Reshetnikov AS, Khmelenina VN, Mustakhimov II, Trotsenko YA: **Genes and enzymes of Ectoine biosynthesis in halotolerant methanotrophs**. *Methods Enzymol* 2011, **495**:15–30.

25. Khmelenina VN, Kalyuzhnaya MG, Starostina NG, Suzina NE, Trotsenko Y a.: **Isolation and characterization of halotolerant alkaliphilic methanotrophic bacteria from Tuva soda lakes**. *Curr Microbiol* 1997, **35**:257–261.

26. Stępniewska Z, Goraj W, Kuźniar A, Pytlak A, Ciepielski J, Frączek P: **Biosynthesis of ectoine by the methanotrophic bacterial consortium isolated from Bogdanka coalmine (Poland)**. *Appl Biochem Microbiol* 2014, **50**:594–600.

27. Cantera S, Lebrero R, Rodríguez E, García-Encina PA, Muñoz R: **Continuous abatement of methane coupled with ectoine production by Methylomicrobium alcaliphilum 20Z in stirred tank reactors: A step further towards greenhouse gas biorefineries**. *J Clean Prod* 2017, **152**:134–141.

28. Cantera S, Lebrero R, Rodríguez S, García-Encina PA, Muñoz R: **Ectoine bio-milking in methanotrophs: A step further towards methane-based bio-refineries into high added-value products**. *Chem Eng J* 2017, **328**:44–48.

29. Castilho LR, Mitchell DA, Freire DMG: **Production of polyhydroxyalkanoates (PHAs) from waste materials and by-products by submerged and solid-state**

fermentation. *Bioresour Technol* 2009, **100**:5996–6009.

30. Chen GQ, Hajnal I, Wu H, Lv L, Ye J: **Engineering Biosynthesis Mechanisms for Diversifying Polyhydroxyalkanoates**. *Trends Biotechnol* 2015, **33**:565–574.

31. Strong P, Laycock B, Mahamud S, Jensen P, Lant P, Tyson G, Pratt S: **The Opportunity for High-Performance Biomaterials from Methane**. *Microorganisms* 2016, **4**:11.

32. Bugnicourt E, Cinelli P, Lazzeri A, Alvarez V: **Polyhydroxyalkanoate (PHA): Review of synthesis, characteristics, processing and potential applications in packaging**. *Express Polym Lett* 2014, **8**:791–808.

33. Koller M, Maršálek L, de Sousa Dias MM, Braunegg G: **Producing microbial polyhydroxyalkanoate (PHA) biopolyesters in a sustainable manner**. *N Biotechnol* 2017, **37**:24–38.

34. Rostkowski KH, Criddle CS, Lepech MD: **Cradle-to-gate life cycle assessment for a cradle-to-cradle cycle: Biogas-to-bioplastic (and back)**. *Environ Sci Technol* 2012, **46**:9822–9829.

35. Pieja AJ, Sundstrom ER, Criddle CS: **Cyclic, alternating methane and nitrogen limitation increases PHB production in a methanotrophic community**. *Bioresour Technol* 2012, **107**:385–392.

36. Zhang T, Zhou J, Wang X, Zhang Y: **Coupled effects of methane monooxygenase and nitrogen source on growth and poly-??-hydroxybutyrate (PHB) production of Methylosinus trichosporium OB3b**. *J Environ Sci (China)* 2017, **52**:49–57.

37. Rahnama F, Vasheghani-Farahania E, Yazdian F SS: **PHB production by Methylocystis hirsuta from natural gas in a bubble column and a vertical loop bioreactor.** *Biochem Eng J* 2012, **65**:51–56.

38. García-pérez T, López JC, Passos F, Lebrero R, Revah S: **Simultaneous methane abatement and PHB production by Methylocystis hirsuta in a novel gas-recycling bubble column bioreactor**. *Chem Eng J* 2018, **334**:691–697.

39. López JC, Arnáiz E, Merchán L, Lebrero R, Muñoz R: **Biogas-based polyhydroxyalkanoates production by Methylocystis hirsuta : A step further in**

anaerobic digestion biorefineries. *Chem Eng J* 2018, **333**:529–536.

40. Myung J, Flanagan JCA, Waymouth RM, Criddle CS: **Expanding the range of polyhydroxyalkanoates synthesized by methanotrophic bacteria through the utilization of omega-hydroxyalkanoate co-substrates**. *AMB Express* 2017, **7**:118.

41. Ritala A, Häkkinen ST, Toivari M, Wiebe MG: **Single Cell Protein—State-of-the-Art, Industrial Landscape and Patents 2001–2016**. *Front Microbiol* 2017, **8**.

42. Silverman J: **BioGTL platform for the conversion of natural gas to fuels and chemicals** in *2014 AIChE Spring National Meeting*, Calysta Inc. 2012.

43. Petersen LAH, Villadsen J, Jørgensen SB, Gernaey K V: **Mixing and mass transfer in a pilot scale U-loop bioreactor**. *Biotechnol Bioeng* 2017, **114**:344–354.

44. Flemming H, Wingender J: **The biofilm matrix**. *Nat Rev Microbiol* 2010, **8**:623–33.

45. Nwodo UU, Green E, Okoh AI: **Bacterial exopolysaccharides: Functionality and prospects**. *Int J Mol Sci* 2012, **13**:14002–14015.

46. Morris G, Harding S: **Polysaccharides, microbial.** in Encyclopedia of Microbiology, ed. Schaechter M., editor. (Amsterdam: Elsevier;) 2009:482–494.

47. Malashenko IP, Pirog TP, Romanovskaia V a, Sokolov IG, Gringerg T a: **Search for methanotrophic producers of exopolysaccharides**. *Prikl Biokhim Mikrobiol* 2001, **37**:702–705.

48. Qurashi AW, Sabri AN: **Bacterial exopolysaccharide and biofilm formation stimulate chickpea growth and soil aggregation under salt stress**. *Brazilian J Microbiol* 2012, **43**:1183–1191.

49. DiSpirito AA, Semrau JD, Murrell JC, Gallagher WH, Dennison C, Vuilleumier S: **Methanobactin and the Link between Copper and Bacterial Methane Oxidation**. *Microbiol Mol Biol Rev* 2016, **80**:387–409.

50. Summer KH, Lichtmannegger J, Bandow N, Choi DW, DiSpirito AA, Michalke B: **The biogenic methanobactin is an effective chelator for copper in a rat model for Wilson disease**. *J Trace Elem Med Biol* 2011, **25**:36–41.

51. Scolari S, Müller K, Bittman R, Herrmann A, Müller P: **Interaction of mammalian**

seminal plasma protein PDC-109 with cholesterol: Implications for a putative CRAC domain. *Biochemistry* 2010, **49**:9027–9031.

52. Muller H., Skrede A. KG: **Lipids from methanotrophic bacteria for cholesterol reduction: Patent. Ep 1 556 475 b1 (12)**. 2007, **1**:1–25.

53. Muñoz R, Villaverde S, Guieysse B, Revah S: **Two-phase partitioning bioreactors for treatment of volatile organic compounds**. *Biotechnol Adv* 2007, **25**:410–422.

54. Kraakman NJR, Rocha-Rios J, Van Loosdrecht MCM: **Review of mass transfer aspects for biological gas treatment**. *Appl Microbiol Biotechnol* 2011, **91**:873–886.

55. Estrada JM, Rodríguez E, Quijano G, Muñoz R: **Influence of gaseous VOC concentration on the diversity and biodegradation performance of microbial communities**. *Bioprocess Biosyst Eng* 2012, **35**:1477–1488.

56. Salar-García MJ, Bernal V, Pastor JM, Salvador M, Argandoña M, Nieto JJ, Vargas C, Cánovas M: **Understanding the interplay of carbon and nitrogen supply for ectoines production and metabolic overflow in high density cultures of "Chromohalobacter salexigens".** *Microb Cell Fact* 2017, **16**:23.

57. Fei Q, Chang HN, Shang L, Choi JDR: **Exploring low-cost carbon sources for microbial lipids production by fed-batch cultivation of Cryptococcus albidus**. *Biotechnol Bioprocess Eng* 2011, **16**:482–487.

58. Wei X-M, Su Y, Zhang H-T, Chen M, He R: **Responses of methanotrophic activity, community and EPS production to CH4 and O2 concentrations in waste biocover soils**. *Waste Manag* 2015, **42**:118–127.

59. Duan Z, Mao S: **A thermodynamic model for calculating methane solubility, density and gas phase composition of methane-bearing aqueous fluids from 273 to 523 K and from 1 to 2000 bar**. *Geochim Cosmochim Acta* 2006, **70**:3369–3386.

60. Cantera S, Lebrero R, Rodríguez E, García-Encina PA, Muñoz R: **Continuous abatement of methane coupled with ectoine production by Methylomicrobium alcaliphilum 20Z in stirred tank reactors: A step further towards greenhouse gas biorefineries**. *J Clean Prod* 2017, **152**:134–141.

61. James C. Liao, Luo Mi SP& SL: **Fuelling the future: microbial engineering for the**

production of sustainable biofuels. *Nat Rev Microbiol* 2016, **14**:288–304.

62. Cantera S, Lebrero R, Sadornil L, García-encina PA, Mu R: **Valorization of CH 4 emissions into high-added-value products : Assessing the production of ectoine coupled with CH 4 abatement**. *J Environ Manage* 2016, **182**:160–165.

63. Muñoz R, Meier L, Diaz I, Jeison D: **A review on the state-of-the-art of physical/chemical and biological technologies for biogas upgrading**. *Rev Environ Sci Bio/Technology* 2015, **14**:727–759.

64. Harding KG, Dennis JS, von Blottnitz H, Harrison STL: **Environmental analysis of plastic production processes: Comparing petroleum-based polypropylene and polyethylene with biologically-based poly-??-hydroxybutyric acid using life cycle analysis**. *J Biotechnol* 2007, **130**:57–66.

65. Estrada JM, Lebrero R, Quijano G, Pérez R, Figueroa-González I, García-Encina PA, Muñoz R: **Methane abatement in a gas-recycling biotrickling filter: Evaluating innovative operational strategies to overcome mass transfer limitations**. *Chem Eng J* 2014, **253**:385–393.

Table 1: Bio-compounds production

Product	***Production yield using sugar*** *(mg g biomass^{-1})*	***Main organism producer***	***Production yield using methane*** *(mg g biomass^{-1})*	***Main methanotrophic producer***
Ectoine	120-270[1]	*Halomonas* *Brevibacterium*	*70-230*[2,3]	*Methylomicrobium*
Bioplastics	300-850[4,5]	*Ralstonia* *Alcaligenes* *Azotobacter, Pseudomonas*	100-500[[6,7]	*Methylocystis* *Methylosinus*
SCP	680-710[8]	*Bacillus* *Ralstonia, Brevibacillus, Aneurunibacillus*	690-730[8]	*Methylomonas* *Methylococcus*
Biofuel	300-510[9]	*Botrioococcus brauni*	200-500[10,11]	*Methylococcus* *Methylosinus* *Methylocystis*
EPS	600-2775[12]	*Pseudomonas* *Enterobacter* *Leuconostoc* *Lactobacillus*	300-1800[13]	*Methylobacter* *Methylomonas* *Methylomicrobium*

Table 2: Economic and environmental analysis of a methane bio-refinery producing ectoine, SCP, PHAs and EPS from a landfill emission

REACTOR OPERATIONAL PARAMETERS

Landfill emission (4.5%) (g CH_4 m^{-3})	**Q_{gas}** (m^3 h^{-1})	**Internal Gas Recirculation**	**Total Gas Sparged Q_{gas}** (m^3 h^{-1})	**Methane feed** (Kg CH_4 h^{-1})	**Biomass concentration** (g L^{-1})	**Biomass produced from CH_4 ($\psi_{biomass}$=0.6)** (kg h^{-1})	**Q_{liquid}** (m^3 h^{-1})
30	*50.000*	*x10*	*550000*	*1500*	*50*	*900*	*18*

REACTOR ENERGY CONSUMPTION

Liquid pressure (bar)	**Total Gas Sparged** (m^3 s^{-1})	**ΔP** (kPa)	**Blower efficiency**	**Air flow Energy consumption** (KWh h^{-1})	**Centrifuge Energy Consumption** (KWh m^{-3} h^{-1})	**Centrifugation Energy Consumption** (KWh h^{-1})	**Total Energy consumption** (KWh h^{-1})
0.4	*153*	*38.7*	0.7	*8451*	3.2	58.1	*8509*

BIOCOMPOUNDS PRODUCED

Ectoine (10 % of CH_4) (kg h^{-1})	**SCP (50% of CH_4)** (kg h^{-1})	**EPS (40% of CH_4)** (kg h^{-1})	**PHA (40% of CH_4)** (kg h^{-1})	**Ectoine** ($ h^{-1})	**SCP** ($ h^{-1})	**EPS** ($ h^{-1})	**PHA** ($ h^{-1})
90	450	360	360	90000	450	1800	5400

ECONOMICAL BALANCE

Operating cost ($ h^{-1})	**Bioproduct value** ($ h^{-1})
1021	**100000**

ENVIRONMENTAL BALANCE

Kg CO_2 removed (Kg h^{-1})	**CO_2 from CH_4 (ψ=0.4)** (Kg h^{-1})	**CO_2 from energy consumption** (Kg h^{-1})	**Total CO_2 produced** (Kg h^{-1})
35250	1650	3157	**4807**

Ψ was obtained from García-Pérez et al. (2018); The energy consumption [kw] for gas circulation was calculated as Q [m^3 s^{-1}] × ΔP [kPa] / Blower efficiency (~0.7) according to Estrada et al. (2012); The cultivation broth was obtained from Strong et al. (2016); The centrifuge energy consumption (Kwh m^{-3}) was calculated according to Acien et al. (2012).

Figure 1

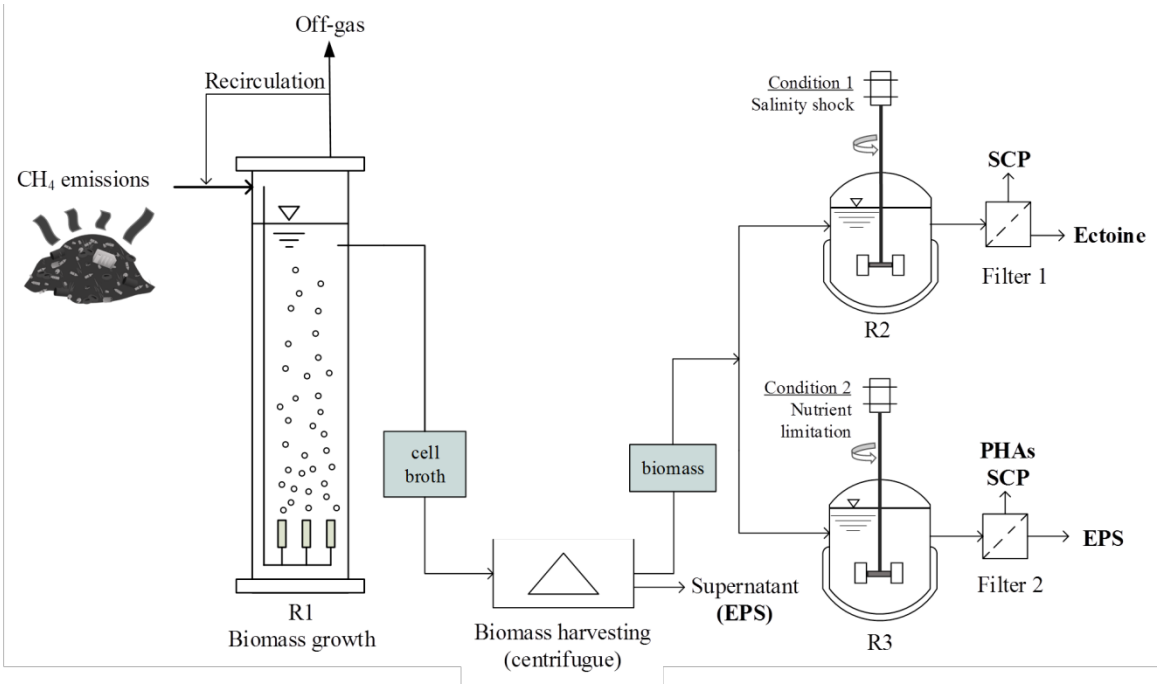


Figure 1. Process diagram for a methane bio-refinery producing ectoine, SCP, PHAs and EPS from a landfill emission.