Extraction, production and purification of added value products from urban wastes - Part 1: Production and purification of ectoine obtained from biogas

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European foreword

This CEN Workshop Agreement (CWA 17897-1:2022) has been developed in accordance with CEN-CENELEC Guide 29 “CEN/CENELEC Workshop Agreements– A rapid way to standardization” and with the relevant provision of CEN/CENELEC Internal Regulations – Part 2. It was approved by a Workshop of representatives of interested parties on 2019-01-29, the constitution of which was supported by CEN following the public call for participation made on 2022-04-17. However, this CEN Workshop Agreement does not necessarily reflect the views of all stakeholders who may have an interest in its subject matter.

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Introduction

The treatment of wastewater and organic fraction of municipal solid waste is responsible for the annual generation of up to 138 million tonnes of bio-waste in the EU. It has been estimated that almost 75% of this waste is currently sent to incineration or landfilling, with an extraordinary environmental and economic cost associated. Moreover, a high percentage of this waste holds a great potential as a source of recycled materials and recovered valuable components. Wastewater contains cellulose and nutrients that could be used as feedstock for many applications while the organic fraction of municipal solid waste constitutes an interesting source of materials for added-value applications (e.g. ectoine, polyhydroxyalkanoates (PHA), biomethane, etc.) to complement their conventional valorisation routes into fertilisers and biogas.

Nowadays, bio-waste is usually processed by means of methods such as anaerobic digestion and composting for the production of biogas or compost to be used as fertiliser. Similarly, domestic wastewater treatment is often conducted in activated sludge systems, which present high operating costs and energy demand. These methods present a low performance, high carbon footprint and low recovery of nutrients and valuable components.

The European R&I project DEEP PURPLE (Conversion of diluted mixed urban bio-wastes into sustainable materials and products in flexible purple photobiorefineries funded by the European Union under the Horizon 2020 program with Grant Agreement No 837998) has developed innovative processes for the production and purification of different added-value products. These processes are based on the recovery of bioproducts from the treatment of urban bio-waste as an inexpensive and sustainable carbon or/nutrient source for biomass growth to be further transformed into high added-value products for different industrial sectors.

This workshop faces the standardisation of the methodology extraction, production and purification of two added-value products from urban waste: ectoine and PHA.

Europe is nowadays the region with the largest number of anaerobic digesters in the world (approx. 18,000 units in 2017), followed by China (7,000 units by 2015) and USA (2,200 in 2015).

Europe’s leadership in biogas production has been triggered by the urgent need to reduce its dependence on imported natural gas and valorise the organic waste from the domestic. For instance, 16.1 million tonnes of oil equivalent, corresponding to an electricity production of 62.5 TWh and sales to heat district systems of 643,000 tonnes of oil equivalent, were produced in 2016 in Europe. The anaerobic digestion of energy crops, urban solid waste and livestock waste accounted for almost 12 Mtoe, landfill gas for 3 Mtoe and wastewater treatment for 1.5 Mtoe in Europe in 2016. The regulatory limit of 60% in the use of energy crops in Germany (the largest producer of biogas) has slowed down the exponential growth of biogas production occurred in the past decade. However, the recent European commitment at COP25 to achieve net-zero greenhouse gas emissions by 2050, along with the increase in the price of fossil fuels and in the number of political initiatives to implement a circular economy in Europe, foresees a steady increase in biogas production. Indeed, an increase in the annual production of biogas up to 41 Mtoe by 2030 is expected according to the European Biogas Association. The rapid reduction in the cost of solar and wind energy production in the past decade is causing a gradual reduction in feed-in tariffs and fiscal incentives for the in-situ generation of electricity from biogas worldwide. This recent fact is triggering research in alternative uses of biogas, which would ultimately improve the final economic balance of anaerobic digestion. In this context, biogas can be used as a feedstock for the generation of products with higher added-value than biogas such as biomethane via biogas purification, chemical building blocks via catalysis and commodities and fine chemicals via biotechnologies. In order to be competitive and sustainable, the future urban biorefineries need to offer a wider portfolio of bioproducts that can combine the production of bioenergy but also added-value products such as biofertilizers, biopolymers, construction materials or in this case fine chemicals such as ectoine.

Currently, ectoine (1,4,5,6-tetra-2-methyl-4-pyrimidinecarboxylic acid) is one of the most profitable products produced by microorganisms. Due to its high effectiveness as stabiliser of enzymes, DNA-
protein complexes and nucleic acids, ectoine has a value in the pharmaceutical industry of approximately 1,000 US$/kg and a global consumption of 20 tonnes/year. Nowadays, industrial bacterial processes for the production of ectoine only use the γ-Proteobacteria *Halomonas elongata*. This strain that can accumulate ~160 mg ectoine/g biomass, has a broad salt tolerance and is able to rapidly synthesise and excrete ectoine to the medium. Industrial ectoine production, also known as *bio-milking*, consists of a long fed-batch fermentation (~120 h) with two steps at different salt concentrations (12 % and 0 %), to obtain first a high culture density (25 g/L) and subsequently induce a hypo-osmotic shock. The sudden decrease in media salinity results in the excretion of ectoine from the cell to the culture broth, where the product is collected for its downstream purification. The upstream processing is still inefficient due to the high amount of nutrients, oxygen and time required, besides entailing a complex and expensive downstream processing. These limitations represent a challenge to its commercial large-scale production. In this context, the use of biogas as an inexpensive feedstock livestock and industrial sector for ectoine biosynthesis by halotolerant methanotrophs has been demonstrated. Also, the utilization of biogas for the production of ectoine provides a significant improvement of the environmental impacts when compared to the current industrial ectoine production routes.

In summary, the motivation of this innovative technology is aligned with the circular economy principles and is focused on the transition of current linear waste management schemes towards more circular biorefinery schemes, supporting a more sustainable (environmental, economic and social) economy.

This Workshop Agreement has been proposed by the DEEP PURPLE consortium ([https://deep-purple.eu/](https://deep-purple.eu/)), which is developing a Horizon 2020 project to move forward in the valorization of municipal biowaste into high value products.

The secretariat of the CEN Workshop that developed this CWA was the Spanish Association for Standardisation (UNE).
1 Scope

This CEN Workshop Agreement specifies an operational process for biogas bioconversion into ectoine, the extraction of the ectoine from the resulting solution and its purification.

2 Normative references

There are no normative references in this document.

3 Terms, definitions and abbreviations

3.1 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp
— IEC Electropedia: available at https://www.electropedia.org/

3.1.1 bubble column bioreactor

A bubble column bioreactor (BCB) is a type of gas-liquid bioreactor characterised by a large height-to-diameter ratio and by the absence of internal elements. Typically, in BCBs dedicated to gas-liquid operations, fine bubble diffusers are installed at the bottom of the bioreactor for guaranteeing an enhanced gas-liquid mass transfer. Perfect mixture is considered in the liquid phase given the high turbulence induced by the upcoming gas bubbles. This type of bioreactor is generally recommended for the operation with poorly soluble gas compounds (namely oxygen or methane) and for suspended growth cultivation of microorganisms.

3.1.2 bed volume

Bed volume (BV) in column chromatography, is the total volume of material, both solid and liquid, in the column.

3.1.3 continuous stirred tank reactor

A continuous stirred tank reactor (CSTR) is a type of bioreactor characterised by a continuous stirring (typically mechanical) and continuous inlet and product streams. Typically, perfect mixture is assumed in this type of bioreactor given the high stirring rate, therefore, the conditions of the outlet stream are considered identical to the bulk liquid contained in the bioreactor.

3.1.4 ectoine

Ectoine is a compatible solute (1,4,5,6-tetrahydro-2-methyl-4-pyrimidinecarboxylic acid) highly valued in the cosmetic and pharmaceutical industries. Ectoine constitutes the highest added-value bioproduct that is being currently synthesised by bacteria, with a market value ranging 600-1000 €/kg. Current industrial processes for ectoine production struggle with the use of high-cost carbon sources (e.g. glucose), long fermentation processes (up to 120 h) and an expensive downstream processing. Methanotrophic haloalkaliphilic bacteria constitute an opportunity for reducing ectoine production costs given their ability to produce ectoine using the methane contained in biogas as a low-cost carbon substrate.
3.1.5 ectoine recovery ratio

The ectoine recovery ratio ($E_{Re}$) is defined as the percentage of ectoine that is adsorbed and subsequently eluted during the ionic exchange chromatography step. The $E_{Re}$ can be calculated with the following equation:

$$E_{Re} = \frac{Q_{eluted} \cdot C_{eluted}}{Q_{in} \cdot C_{in}}$$

where

- $E_{Re}$ is the ectoine recovery ratio;
- $Q_{in}$ and $Q_{eluted}$ represent the inlet and outlet stream flowrate (m$^3$/h);
- $C_{in}$ and $C_{eluted}$ stand for the ectoine concentration in the inlet and outlet streams (g/m$^3$).

3.1.6 empty bed residence time

Empty bed residence time ($EBRT$) is defined as the residence time of the gas phase in a reactor in the absence of packing material. In bubble column reactors the $EBRT$ refers to the residence time the gas would have in the vessel in the absence of liquid phase and can be calculated with the following equation:

$$EBRT = \frac{V_L}{Q_G}$$

where

- $EBRT$ represents the empty bed residence time (h);
- $V_L$ indicates the reactor liquid volume (m$^3$);
- $Q_G$ is the inlet gas flowrate (m$^3$/h).

3.1.7 haloalkaliphilic methanotrophic bacteria

Methanotrophic bacteria are aerobic microorganisms characterised by the ability of using methane as their only carbon source. More particularly, in high salinity environments, haloalkaliphilic methanotrophic bacteria are able to produce large amounts of ectoine as a protection mechanism. These bacteria are also able to excrete the ectoine, when the salinity conditions are reduced. This 2-stage process, which is typically known as bacterial bio-milking, can be exploited industrially for the production of ectoine.

3.1.8 hydraulic retention time

The hydraulic retention time ($HRT$) is defined as the residence time of the liquid phase in a reactor in the absence of packing material or internal elements and can be calculated with the following equation:

$$HRT = \frac{V_R}{Q_L}$$

where

- $HRT$ represents the hydraulic residence time (h);
$V_R$ indicates the reactor working volume (m$^3$);

$Q_L$ is the inlet liquid flowrate (m$^3$/h).

### 3.1.9 Methane elimination capacity

The elimination capacity represents the amount removed of a certain compound per unit of time and per unit of reactor volume. Methane elimination capacity ($CH_4 - EC$) can be calculated with the following equation:

$$CH_4 - EC = \frac{Q_{in} \cdot C_{CH_4 in} - Q_{out} \cdot C_{CH_4 out}}{V_R}$$

where

- $CH_4 - EC$ is the methane elimination capacity (g/m$^3$·h);
- $Q_{in}$ and $Q_{out}$ represent the inlet and outlet gas flowrate (m$^3$/h);
- $C_{CH_4_{in}}$ and $C_{CH_4_{out}}$ stand for the methane concentration in the inlet and outlet streams (g/m$^3$);
- $V_R$ indicates the working reactor volume (m$^3$).

### 3.1.10 Methane removal efficiency

The removal efficiency indicates the fraction of a compound that is removed in a gas-liquid system. The methane removal efficiency ($CH_4 - RE$) can be calculated with the following equation:

$$CH_4 - RE = \frac{Q_{in} \cdot C_{CH_4 in} - Q_{out} \cdot C_{CH_4 out}}{Q_{in} \cdot C_{CH_4 in}}$$

where

- $CH_4 - RE$ is the methane removal efficiency (%);
- $Q_{in}$ and $Q_{out}$ represent the inlet and outlet gas flowrate (m$^3$/h);
- $C_{CH_4_{in}}$ and $C_{CH_4_{out}}$ stand for the methane concentration in the inlet and outlet streams (g/m$^3$).

### 3.1.11 Salt concentration factor

The salt concentration factor ($S_f$) indicates the ratio of salt concentration between the inlet stream and the permeate stream. The $S_f$ can be calculated with the following equation:

$$S_f = \frac{Q_{in} \cdot C_{in}}{Q_{permeate} \cdot C_{permeate}}$$

where

- $S_f$ indicates the salt concentration factor (adim.).
Q_{in} and Q_{permeate} stand for the inlet and permeate streams flowrate (m\(^3\)/h);
C_{in} and C_{permeate} represent the salt concentration in the inlet and permeate streams (g/m\(^3\)).

3.1.12 solid recovery efficiency
the solid recovery efficiency (S_{Re}) indicates the percentage of solids recovered in the thickened stream and accounts for the overall performance of the solid-liquid separation. The S_{Re} can be calculated with the following equation:

\[
S_{Re} = \frac{Q_{out} \cdot C_{out}}{Q_{in} \cdot C_{in}}
\]

where

S_{Re} represents the solid recovery efficiency (%);
Q_{in} and Q_{out} stand for the inlet and outlet liquid flowrate (m\(^3\)/h);
C_{in} and C_{out} indicate the solid concentration in the inlet and outlet streams (g/m\(^3\)).

3.1.13 water recovery efficiency:
the water recovery efficiency (W_{Re}) indicates the percentage of water recovered in the permeate stream of ultrafiltration membranes and accounts for the overall performance of the ultrafiltration membrane performance. The W_{Re} can be calculated with the following equation:

\[
W_{Re} = \frac{Q_{permeate}}{Q_{in}}
\]

where

W_{Re} represents the water recovery efficiency (%);
Q_{permeate} indicates the permeate flowrate (m\(^3\)/h);
Q_{in} stands for the inlet liquid flowrate (m\(^3\)/h).

3.2 Abbreviations

BCB Bubble column bioreactor
BV Bed volume
CH\(_4\) Methane
CH\(_4\) – EC Methane elimination capacity
CH\(_4\) – RE Methane removal efficiency
CO\(_2\) Carbon dioxide
CSTR Continuous stirred tank reactor
EBRT Empty bed residence time
HCl Hydrochloric acid
HRT Hydraulic retention time
4 Materials and equipment

4.1 Materials

**Biogas**: 67.0 Nm$^3$/h with an average composition of 60 % v/v CH$_4$, 35 % v/v CO$_2$, 2.5 % v/v N$_2$, 0.5 % v/v O$_2$, 0.4 % v/v H$_2$S and 1.6 % v/v other compounds.

**Cooling water (15 °C)**: 2,136.8 kg/d.

**Distilled water**: 780.9 kg/d.

**Haloalkaliphilic methanotrophic bacteria consortium**: composed mainly by the genera *Methylomicrobium*.

**HCl 32 % v/v**: 19.32 kg/d.

**H$_2$SO$_4$ 98 % v/v**: 89.8 kg/d.

**Strong cation exchange resin**: 5 kg/d.

**Low pressure steam (2 bar)**: 2,817.8 kg/d.

**Methanol**: 34.1 kg/d. Purity ≥ 99 %.

**Micronutrients**: 3.4 kg/d. The micronutrient composition is depicted in Table 1. All compounds should present a purity ≥ 99 %.
Table 1 — Micronutrients distribution based on DSMZ 921 (DSMZ, 2007)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Daily consumption</th>
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<tr>
<td>MgSO₄</td>
<td>1.6 kg/d</td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>1.1 kg</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>0.4 kg/d</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.3 kg/d</td>
</tr>
<tr>
<td>Na₂WO₄·2-H₂O</td>
<td>4.6 g/d</td>
</tr>
<tr>
<td>CuSO₄</td>
<td>1.6 g/d</td>
</tr>
<tr>
<td>FeSO₄</td>
<td>0.8 g/d</td>
</tr>
<tr>
<td>ZnSO₄</td>
<td>0.6 kg/d</td>
</tr>
<tr>
<td>Fe-EDTA</td>
<td>0.6 g/d</td>
</tr>
<tr>
<td>Na₂MoO₄</td>
<td>0.4 g/d</td>
</tr>
<tr>
<td>EDTA-disodium</td>
<td>0.4 g/d</td>
</tr>
<tr>
<td>CoCl₂</td>
<td>78.3 mg/d</td>
</tr>
<tr>
<td>MnCl₂</td>
<td>31.3 mg/d</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>23.5 mg/d</td>
</tr>
<tr>
<td>NiCl₂</td>
<td>15.7 mg/d</td>
</tr>
</tbody>
</table>

NaCl: 546.2 kg/d. Purity ≥ 99 %.
NaNO₃: 331.5 kg/d. Purity ≥ 99 %.
NaOH: 67.72 kg/d. Purity ≥ 99 %.
Water: 31.7 m³/d.

4.2 Equipment

Biogas desulphurisation stage: The biogas concentration prior injection into the bubble column bioreactor shall present a H₂S concentration below 100 ppm.

Bubble column bioreactor: The bubble column bioreactor should provide a CH₄-RE higher than 90 %.

Solid-liquid separator (1): The solid-liquid separator shall have a solid recovery efficiency (SRe) of at least 95 % and the outlet stream a solid concentration of at least 20 % w/w.

Continuous stirred tank reactor (1): The continuous stirred tank reactor (CSTR) shall have a hydraulic retention time (HRT) of at least 5 minutes.

Solid-liquid separator (2): The solid liquid separator shall have a SRe of at least 95 % and the outlet stream a solid concentration of at least 20 % w/w.

Ultrafiltration membrane (1): The ultrafiltration membrane shall have a water recovery efficiency (WRe) of at least 90 % and a SRe of at least 99 %.

Electrodialysis system: The electrodialysis system membrane shall present an ectoine recovery rate (ERe) of at least 90 % and a desalination efficiency of 90 %.

Continuous stirred tank reactor (2): The CSTR shall have a HRT of at least 2 h.
Ionic exchange chromatography column: The ionic exchange chromatography column shall have an ectoine recovery rate \((E_{Re})\) of at least 90 %.

Continuous stirred tank reactor (3): The CSTR shall have a HRT of at least 1 h.

Spray drying: The spray drying shall provide a solid product with a moisture content lower than 5 % w/w.

Continuous stirred tank reactor (4): The CSTR shall have a HRT of at least 1 h.

Microfiltration membrane (1): The microfiltration membrane shall have a \(W_{Re}\) of at least 90 % and a \(S_{Re}\) of at least 99 %.

Vacuum crystalliser: The crystalliser shall have a vacuum system and a jacket connected to heating and cooling.

Microfiltration membrane (2): The microfiltration membrane shall have a \(W_{Re}\) of at least 90 % and a \(S_{Re}\) of at least 99 %.

Tray-dryer: The tray-dryer shall provide a solid product with a moisture content lower than 5 % w/w.

Ancillary equipment: Pumps, flowmeters and compressors are required for the circulation of gas and liquid streams throughout the process.

4.3 Laboratory equipment for monitoring

pH meter: The pH in the cultivation broth shall be monitored and controlled around a value of 8.

Conductivity meter: The conductivity shall be monitored in order to corroborate the salt concentration in the medium.

System for determination of TSS and VSS: The biomass growth will be followed by the analysis of VSS. The following equipment shall be available: analytical balance, vacuum filtration system and oven.

Gas chromatography: The composition of the inlet and outlet gas in the bubble column bioreactor shall be analysed by gas chromatography coupled with Thermal Conductivity Detection.

High performance liquid chromatography: The ectoine concentration in the cultivation broth shall be analysed using high performance liquid chromatography coupled with a UV Dual λ Absorbance detector.

5 Process design

A process for the production of 10 t/y of ectoine from biogas in waste treatment plants is herein presented. This type of plant does not present of any especial requirements and given the low amount of biogas required (67 Nm³/h) compared to the median biogas production in medium-scale waste treatment facilities (500-1 000 Nm³/h), could be placed in all sort of waste treatment facility.

Figure 1 shows the simplified process flow diagram for CH₄-biogas bioconversion into ectoine.

For clarification purposes, the process can be divided into two different stages:

- Ectoine biosynthesis from biogas (I).
- Ectoine extraction and purification (II).
6 Ectoine biosynthesis from biogas

Prior to biogas valorisation into ectoine, a biogas desulphurisation stage shall be considered for preventing corrosion in downstream piping and equipment. All kind of desulphurisation technology is acceptable while it reduces H$_2$S concentration in the BCB injection below 100 ppm.

A BCB shall be inoculated with a mixed culture of haloalkaliphilic methanotrophic bacteria.

The BCB shall operate under continuous mode at a dilution rate of 0.1-0.4 l/d.

A mineral medium solution containing NaNO$_3$, NaCl and micronutrients (Table 1) shall be continuously added to support haloalkaliphilic methanotrophic bacteria growth and ectoine synthesis.

Copper concentration in the mineral medium shall be reduced to the minimum, as a high copper concentration would promote ectoine excretion in the BCB.

A tungsten concentration of 0.07 mg/L should be present in the liquid medium in order to prevent the formation of formic acid, which typically exhibits a significant inhibitory effect on the CH$_4$ – EC.

A specific biomass production yield of 0.4 g biomass/g CH$_4$ should be achieved.

An ectoine accumulation of 70 mg ectoine/g biomass should be achieved.

A mineralisation ratio of 0.7 mol CO$_2$/mol CH$_4$ should be achieved.

Biogas and air shall be supplied in order to guarantee a ratio of 1.5 mol O$_2$/mol CH$_4$ in the BCB.

The BCB shall be equipped with fine bubble diffusers in order to guarantee an enhanced gas-liquid mass transfer.
A BCB with a gas EBRT of 1.2 h and a height-to-diameter ratio of 10 should be designed to support an effective gas-liquid mass transfer of CH₄ (biogas) and O₂ (air).

A CH₄ – RE of at least 90 % should be achieved in the BCB.

7 Ectoine extraction and purification

Haloalkaliphilic methanotrophic bacteria containing ectoine shall be harvested and concentrated to a biomass concentration of at least 200 g biomass/L [solid-liquid separator (1)] in a centrifugation equipment or similar.

An aliquot of the liquid fraction of 10 % shall be daily wasted from the system to avoid the accumulation of secondary metabolites.

The concentrated biomass stream shall be subjected to a hypo-osmotic shock in a non-saline medium to promote the excretion of intracellular ectoine.

The excretion process shall be carried out in a CSTR with a HRT of at least 5 min (CSTR (1)).

The excretion of intracellular ectoine should be at least 85 %.

The biomass containing only 15 % of the initial intracellular ectoine should be concentrated to at least 200 g biomass/L in a centrifugation equipment or similar (Solid-Liquid separator (2)).

The biomass concentrated stream shall be recirculated to the bioreactor.

A fraction of the concentrated stream should be wasted to maintain an average biomass residence time of 9 d, preventing biomass activity decay.

The ectoine-containing liquid stream shall be subjected to an ultrafiltration step or similar (Ultrafiltration membrane (1)) to remove biomass traces and solid impurities. A solid recovery of at least 99 % should be achieved.

The aqueous solution containing the extracted ectoine should be desalinised via electrodialysis up to 95 % of the initial conductivity.

The liquid stream containing ectoine shall be acidified to pH 1.5-2 by addition of 10 M HCl.

The acidification process shall be performed in a CSTR operated at a HRT of 1 h (CSTR 2).

The acidified liquid stream containing ectoine shall be pumped into an ionic exchange chromatographic column designed with an ectoine recovery rate of at least 90 %.

This column should be packed with a high-performance cation exchange resin in sodium form such as Amberchrom 50WX8 or similar.

The cation exchange resin shall present an adsorbing capacity of at least 0.05-0.1 kg ectoine/kg resin.

The adsorbed ectoine should be washed with 2 BV of 98 % w/w H₂SO₄ diluted 1:2 in distilled water and up to 10 BV of distilled water to remove impurities.

Ectoine should be eluted with 2 BV of 2 M NaOH, of which 1 BV should be discarded.

The eluted liquid containing ectoine shall be neutralised to pH 7 via addition of an acid, preferable 98 % w/w H₂SO₄.

The neutralisation process shall be performed in a CSTR operated at a HRT of 1 h (CSTR 3).

The liquid stream shall be dried to a maximum moisture content of 5 % w/w in spray dryer system or similar (Spray drying). The solid stream shall be completely dissolved into methanol (approx. 10 g methanol per g ectoine) in a CSTR operated at a HRT of at least 1 h (CSTR 4). The methanol-dissolved ectoine stream shall be subjected to a microfiltration step for removing the precipitated salts (mainly Na₂SO₄). A solid retention efficiency of at least 99 % should be achieved (Microfiltration membrane 1).
Ectoine crystallisation shall be performed by concentrating the methanol solution to 1/3 of the initial volume and cooling down the solution to 4 °C (Vacuum crystalliser).

The crystals shall be filtered (Microfiltration membrane 2).

The final product should be obtained after a drying step in a tray dryer system with warm air (20 °C) or similar (Tray dryer).

The latter two steps can be repeated for a higher purity product.

A product recovery of at least 62 % should be achieved during the purification process.

An ectoine purity of at least 97 % should be obtained.
Bibliography


