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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/and 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, DNAprotein 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/), 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