Institutul pentru Cercetări în Economie Circulară și Mediu „Ernest Lupan”

Microorganism and process for obtaining some biosurfactants by biological synthesis

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INTRODUCTION

The present invention refers to a process for obtaining biosurfactants of the rhamnolipid type, by biological synthesis, using the microorganism Pseudomonas putida ICCF 421, isolated from the nature.
The technical problem that the invention solves consists in a process for obtaining rhamnolipid-type biosurfactants, by microbial synthesis, using the newly isolated bacterial strain Pseudomonas putida, on a mixed substrate that contains, as a carbon source, used sunflower oil, waste from food preparation, and glycerin, a byproduct of the biodiesel industry, resulting in biosurfactants containing rhamnolipid compounds.
By applying the invention, the following advantages are obtained:
- the isolation from nature of a new rhamnolipid-type biosurfactant-producing microorganism, identified by the Rep-PCR method as the bacterial species Pseudomonas putida, included in the Collection of Microorganisms of Industrial Importance CMII-ICCF-WFCC 232, with the identification number ICCF 421, as well as within the International Depository Authority NCAIM in Budapest, Hungary (National Collection of Agricultural and Industrial Microorganisms, Institute of Food Science and Technology), with registration number NCAIM P (B) 001516.
- obtaining by biological synthesis rhamnolipids with biosurfactant activity, using the microorganism Pseudomonas putida ICCF 421, which contain rhamnose (1-3%) and lipids (10-12%).
- the utilization of sunflower oil waste resulting from food preparation, as well as glycerin resulting as a by-product from the biodiesel industry.
Therefore, the present invention describes obtaining biosurfactants of the rhamnolipid type by
utilization of waste vegetable oils and glycerin, using the newly isolated microorganism Pseudomonas putida ICCF 421.

MATERIALS & METHODS

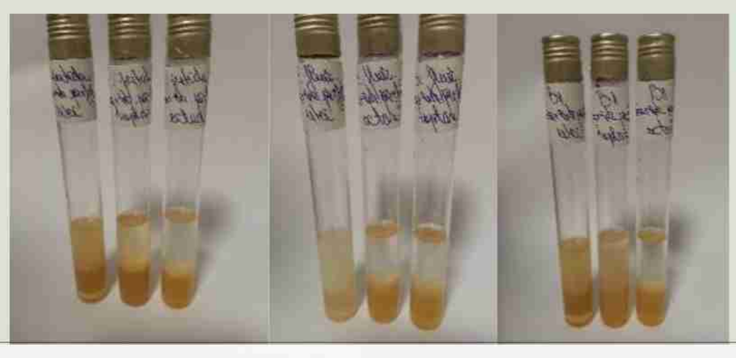
The process of the invention involves the growth and production of biosurfactants using the strain Pseudomonas putida ICCF 421, and consists of:

Pre-inoculation phase:

Cultivation of the stem on an agarized nutrient medium.

Inoculum phase:

Cultivation of the strain on a mixed substrate containing spent sunflower oil, glycerin, yeast extract, bacto-peptone and monopotassium phosphate as carbon and nitrogen sources.Bioprocess phase:



Submerged fermentation with a composition similar to the inoculum.

Post-biosynthesis:

Separation of the cell-free solution by centrifugation to obtain the supernatant.Precipitation with ethanol (1:3 v/v ratio), followed by separation by centrifugation.A second precipitation of the supernatant with concentrated hydrochloric acid.

Extraction of the precipitate with ethyl acetate for 5 hours, with stirring, followed by concentration of the organic phase under vacuum until the separation of the product.

Highlighting the production of biosurfactants:

Analysis of emulsifying activity for heptane, octane and sunflower vegetable oil, with 24-hour emulsification indices (E24) of 65.09% for heptane, 66.35% for octane and 63.74% for vegetable oil, indicating emulsifying properties and emulsion stability.

Characterization of biosurfactants:

Purified biosurfactants were shown to contain rhamnose (1-3%) and lipids (10-12%) by high-performance liquid chromatography (HPLC).

Potential applications:

Emulsifiers, foaming, dispersing, softening or antimicrobial (antibacterial, antifungal and antiviral) agents in the food or pharmaceutical industry.Combating phytopathogenic agents in agriculture.Bioremediation for crude oil recovery and heavy metal removal from contaminated soils.

Example of realization:

Pre-inoculation: Growth of the microorganism in tubes with nutrient agar medium, incubated at 30°C for 48-72 hours.

Inoculum:

Cultivation of the strain in Erlenmeyer flasks with medium containing spent sunflower oil, glycerin, yeast extract, bacto-peptone and monopotassium phosphate at 30°C for 24 hours with shaking.

Bioprocess:

Fermentation of the submerged medium similar to the inoculum at 30°C for 72 hours, with agitation.

Post-biosynthesis processing:

Centrifugation, ethanol precipitation, pH adjustment with HCl, extraction with ethyl acetate, filtration and concentration in vacuo. The end result is a product containing rhamnose (1.87%) and lipids (10.98%).

CONCLUSIONS

The invention proposed by IRCEM in collaboration with ICCF represents an innovative biosynthesis process of biosurfactants, utilizing a specific strain of Pseudomonas putida (ICCF 421) for the production of rhamnolipids. This invention significantly contributes to sustainable development efforts and the efficient use of renewable resources.

The central innovation of the patent is the utilization of organic waste, specifically used sunflower oil and glycerin from the biodiesel industry, for producing biosurfactants. This process not only reduces production costs associated with traditional raw materials but also mitigates the environmental impact of oily waste. By using renewable substrates, the patent aligns with the principles of the circular economy, promoting the recycling and reuse of resources in an ecologically and economically efficient manner.

A notable aspect of the invention is the efficiency of the strain Pseudomonas putida ICCF 421, which, according to the documentation, produces biosurfactants with superior properties compared to chemical surfactants. These biosurfactants exhibit excellent biodegradability and are effective under various conditions, making them ideal for multiple industrial applications, including food, cosmetics, and pharmaceuticals.

Another significant advantage of the process is its ability to operate at temperature and pH conditions that allow adaptability in various industrial contexts. Moreover, the specific characteristics of the obtained rhamnolipids—rhamnose and lipid content—suggest their potential for extensive industrial applications, from emulsification to antimicrobial applications.

The detailed methodology, including the pre-inoculation, inoculum, and bioprocess steps, highlights a clear and replicable approach essential for large-scale adoption of the technology. Additionally, the purification and valorization processes of the final product are described with precision, emphasizing the commercial potential of the invention.

In conclusion, the described patent demonstrates a significant advancement in biosynthetic technologies with direct applications in supporting sustainability and resource efficiency. Its contribution to the field of biosurfactants not only promotes ecological innovations but also sets a precedent for the efficient use of industrial waste, reinforcing the principles of the circular economy in industrial practices.

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SIRACE-ROMD: Studies and investigations on the interplay between regenerative agriculture and the circular economy in Romania and the Republic of Moldova

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The project

Explores **regenerative agriculture** and the **circular economy** in **Romania** and **the Republic of Moldova**, providing innovative solutions to environmental problems and the sustainability of food systems.
It is a collaboration between the **Academy of Economic Studies of Moldova** and the National Center for Research and Seed Production "Selecția", under the coordination of the Ministry of Research, Innovation and Digitalization of Romania, funded under the "Collaborative Projects with the Republic of Moldova" program.

Main activities

- Analysis and comparison of regenerative agriculture practices in Romania and the Republic of Moldova.
- Mapping good practices for regenerative agriculture in both countries.
- Developing a circular economy framework for regenerative agriculture.
- Generate a policy brief to promote circular-regenerative agriculture.
- Organize a research visit to study regenerative agriculture practices.

Key findings and recommendations

- **Romania:** Regenerative agriculture focuses on soil health and biodiversity. Uptake is slow due to lack of knowledge, economic constraints and limited political support.
- **Republic of Moldova:** Agricultural land is degraded due to unsustainable practices and climatic stress. Modernization of agriculture includes promotion of organic farming and sustainable soil management.
- **Both countries:** Collaboration between governments, local communities and the private sector is essential to implement sustainable practices.
- **Policy:** The lack of a clear legislative framework for regenerative agriculture is a major challenge.
- **Financial support:** Subsidies and funding for regenerative practices are needed, alongside support for research and development.
- **Awareness:** Information campaigns and training programs are essential to increase awareness and knowledge about regenerative agriculture.
- **Integration of incentives and pilot projects:** There is a need to integrate incentives and implement pilot projects to promote sustainable agricultural practices.

Project results

- **A comparative study** on regenerative agriculture in Romania and the Republic of Moldova.
- **Identifying and mapping good practices** of regenerative agriculture in both countries.
- **Developing a framework** that integrates the principles of the circular economy with regenerative agriculture.
- **Policy recommendations** to facilitate circular-regenerative agriculture in Romania and Moldova.
- **The organized research visit** provided an opportunity to compare the potential of regenerative agriculture in Romania and Moldova, with the participation of researchers from institutions in both countries.




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