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Executive Summary

Rhamnolipids, biosurfactants produced from bacterial fermentation, are gaining attention due to their biodegradability, low toxicity, and effectiveness across various industries, from bioremediation to personal care. The sustainable production of rhamnolipids is, therefore, critical both from an environmental perspective and for meeting the market's growing demand for green products. Life Cycle Assessment (LCA) and Life Cycle Costing (LCC), combined with rigorous process engineering, have been instrumental in evaluating the environmental and economic profiles of the rhamnolipid production process, enabling the identification of hotspots and the development of strategies for improvement.

The LCA conducted highlighted key environmental hotspots: Media Preparation was the leading contributor to global warming potential and terrestrial ecotoxicity, largely due to the reliance on energy-intensive agricultural inputs. Downstream processing, particularly purification and extraction, also had significant impacts on global warming potential and fossil resource scarcity. Fermentation was a major factor in water consumption. Addressing these issues could potentially lower the environmental impact, with strategies including the optimization of resource use, the adoption of renewable energy, and the implementation of water recycling processes.

Economic analysis through LCC revealed that labour costs constituted the most substantial portion of operational expenses (OPEX), pinpointing the potential efficiency gains through automation. Product purification processing emerged as another significant cost driver due to its resource-intensive nature. Utility costs and solvent recovery processes also presented opportunities for cost reduction, primarily through energy management and improved resource recovery systems.

In synthesizing these analyses, process engineering offers a roadmap to enhancing the production of rhamnolipids. It suggests that scalability may be an effective lever for reducing both environmental impact per unit and OPEX, provided that it is managed to ensure proportional benefits in efficiency and sustainability.

In conclusion, the joint use of LCA and LCC has provided a comprehensive view of the rhamnolipid production process, elucidating the interplay between environmental sustainability and economic feasibility. The insights gained point towards targeted interventions for each identified hotspot, including the advancement of sustainable agricultural practices, process optimization, energy and resource efficiency improvements, and scaling up production. Implementing these strategies could lead to a more sustainable and economically viable rhamnolipid production process, positioning it favourably in the market and contributing positively to the shift towards green chemistry and sustainable industrial practices.

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1. Introduction

In recent years, the quest for sustainable industrial processes has intensified, driven by growing environmental concerns and the imperative to reduce human impacts on the planet. Among the various areas of focus, the production of biosurfactants, particularly rhamnolipids, has garnered significant attention due to their potential to replace petroleum-based surfactants with more environmentally friendly alternatives. Rhamnolipids, biosurfactants produced by certain strains of bacteria, such as *Pseudomonas putida*, offer promising applications across a range of industries, including bioremediation, pharmaceuticals, cosmetics, and food processing, owing to their biodegradability, low toxicity, and effectiveness in diverse conditions [1].

Despite the clear benefits, the production of rhamnolipids is not without its environmental and economic challenges. Environmental concerns include the consumption of resources, the generation of waste, and use of chemicals in the production process. Concurrently, economic considerations encompass the costs associated with raw materials, energy, labour, and technology, which can influence the feasibility and sustainability of rhamnolipid production. Identifying and addressing the hotspots—stages or aspects of the production process with significant environmental impacts and economic costs—is essential for enhancing the process's overall sustainability [2–4].

This report aims to explore these hotspots through an integrated Life Cycle Assessment (LCA) and Life Cycle Costing (LCC) approach. LCA provides a framework for assessing the environmental impacts of rhamnolipid production from cradle to grave, encompassing raw material extraction, production, distribution, use, and disposal. LCC complements this analysis by evaluating the economic aspects, offering a comprehensive view of the costs associated with each stage of the life cycle. By combining these methodologies, the report seeks to identify areas where improvements can lead to more environmentally sustainable and economically viable rhamnolipid production.

The scope of this report includes a detailed analysis of the rhamnolipid production process, highlighting environmental and economic hotspots. The objectives are to conduct a holistic assessment of the production process using LCA and LCC methodologies, identify key areas for improvement, and propose strategies to mitigate environmental impacts while optimizing economic outcomes.

The following sections will delve into the theoretical background of rhamnolipids, environmental and economic hotspots, and the principles of LCA and LCC. The methodology section will outline the integrated approach to LCA and LCC analysis, followed by a presentation of the results, a discussion of their implications, and conclusions drawn from the study.

2. Theoretical Background

The pursuit of sustainable industrial processes necessitates an understanding of both the technical aspects of production and the frameworks used to evaluate environmental and economic impacts. This section provides the theoretical foundation necessary to assess the sustainability of rhamnolipid production, focusing on the environmental and economic dimensions. It lays the groundwork for applying Life Cycle Assessment (LCA) and Life Cycle Costing (LCC) methodologies to identify and analyse hotspots within the production process.

2.1.Rhamnolipids

Rhamnolipids are a class of biosurfactants, which are surface-active substances produced by microorganisms. These biosurfactants have garnered attention for their potential to replace synthetic surfactants due to their eco-friendly profile and effective surface-active properties. Produced primarily by the bacterium *P. aeruginosa*, rhamnolipids are composed of one or two rhamnose sugar molecules bonded to one or two fatty acid chains [5–7]. This structure is responsible for their surface activity, enabling them to reduce surface and interfacial tension between liquids, solids, and gases.

2.1.1. Production Processes

The production of rhamnolipids involves fermentation, where *P. aeruginosa* is cultured in a nutrient-rich medium [5,8]. This process can be performed in various fermentation configurations, including batch, fed-batch, and continuous processes. The choice of process affects the yield, purity, and economic viability of rhamnolipid production. Following fermentation, rhamnolipids are extracted and purified through a series of steps, including solvent extraction, precipitation, and chromatography, to achieve the desired purity for specific applications.

2.1.2. Applications

Rhamnolipids have a wide range of applications due to their biodegradability, low toxicity, and effectiveness in various environments. They are used in environmental bioremediation to break down pollutants, in the pharmaceutical and cosmetic industries as ingredients in products due to their antimicrobial properties, and in the food industry as emulsifiers and preservatives. The versatility of rhamnolipids makes them a valuable alternative to synthetic surfactants, aligning with the goals of sustainable development.

2.1.3. Market Relevance

The market for rhamnolipids is expanding as industries seek sustainable alternatives to synthetic chemicals. The demand is driven by increasing regulatory pressures to reduce environmental impact and consumer preference for eco-friendly products. However,

the economic viability of producing rhamnolipids at a commercial scale depends on optimizing production processes to reduce costs and increase yields [9].

2.2.Environmental and Economic Hotspots

Understanding the concept of hotspots is crucial in the sustainability assessment of industrial processes. Hotspots are defined as stages or aspects of a process that have significant environmental or economic impacts. Identifying these hotspots is the first step toward mitigating negative consequences and enhancing the overall sustainability of the process.

2.2.1. Environmental Hotspots

Environmental hotspots in the production of rhamnolipids, or any other product, are those stages in the life cycle that contribute most significantly to the product's total environmental impact. These impacts can be diverse, including greenhouse gas emissions, water usage, energy consumption, pollution, and depletion of natural resources. Identifying environmental hotspots requires a comprehensive analysis of the entire life cycle of the product, from raw material extraction through to production, use, and disposal. This analysis helps in pinpointing areas where interventions can yield the most significant environmental benefits. For rhamnolipid production, environmental hotspots might include the fermentation process, which can be energy-intensive, or the extraction and purification stages, which may involve the use of hazardous chemicals or generate significant waste.

2.2.2. Economic Hotspots

Economic hotspots, on the other hand, are identified by analysing the cost implications throughout the product's life cycle. This includes the costs associated with raw materials, energy, labour, maintenance, waste management, and any other expenses incurred during the production, use, and end-of-life phases. Economic hotspots are critical for understanding where the most significant cost drivers lie and where cost reduction efforts can be most effectively applied. For instance, in rhamnolipid production, high raw material costs or energy-intensive processes may be identified as economic hotspots, suggesting areas for potential cost savings or efficiency improvements.

2.2.3. Integrating Environmental and Economic Assessments

The integration of environmental and economic assessments provides a holistic view of a product's sustainability. It allows for the identification of trade-offs or synergies between environmental and economic objectives. For example, a process modification that reduces energy consumption (an environmental benefit) may also lower operating costs (an economic benefit), addressing both environmental and economic hotspots simultaneously. Conversely, a strategy that reduces costs by using cheaper but more

environmentally harmful materials would highlight a trade-off between economic gains and environmental impacts.

The goal of identifying environmental and economic hotspots is not merely to document these impacts but to inform strategies for improvement. By understanding where the greatest impacts occur, decision-makers can prioritize interventions, invest in research and development, and implement changes that lead to more sustainable and economically viable production processes.

This understanding of environmental and economic hotspots lays the groundwork for the application of Life Cycle Assessment (LCA) and Life Cycle Costing (LCC) methodologies in the subsequent analysis of rhamnolipid production.

2.3.Life Cycle Assessment (LCA)

Life Cycle Assessment (LCA) is a standardized methodological framework used to assess the environmental aspects and potential impacts associated with a product, process, or service throughout its life cycle. From raw material extraction (cradle) through production, use, and disposal (grave), LCA provides a comprehensive view of environmental impacts, enabling the identification of opportunities for improvement. The International Organization for Standardization (ISO) provides guidelines for LCA under ISO 14040 and 14044, ensuring consistency and comparability of assessments.

2.3.1. Principles of LCA

LCA is built on several key principles:

- **Holistic Approach:** LCA considers the entire life cycle of the product or process, avoiding the shifting of environmental burdens between life cycle stages or environmental compartments (e.g., air, water, soil).
- **Transparency:** The LCA process is conducted in a transparent and systematic manner, with clear documentation of data sources, assumptions, and methodologies.
- **Comparability:** Standardized methods allow for the comparison of environmental performance between products or processes.
- **Iterative Nature:** LCA is an iterative process, where findings from one phase may necessitate revisiting earlier steps for refinement.

2.3.2. Stages of LCA

The LCA methodology comprises four interconnected stages:

1. **Goal and Scope Definition:** This initial stage clarifies the purpose, the system boundaries, and the functional unit (a quantified performance of a product system

for use as a reference unit). It sets the stage for the analysis, defining what will be assessed and to what extent.

2. **Inventory Analysis (Life Cycle Inventory, LCI):** This stage involves the compilation and quantification of inputs and outputs for a product system. Inputs include raw materials and energy, while outputs encompass emissions to air, water, and soil, as well as other environmental releases. The inventory is the foundation for assessing potential impacts.

3. **Impact Assessment (Life Cycle Impact Assessment, LCIA):** LCIA translates inventory data into potential environmental impacts using specific categories, such as global warming potential, eutrophication, acidification, and resource depletion. This stage evaluates the significance of the identified impacts.

4. **Interpretation:** The final stage analyses the results, addressing the initial goals and reporting the conclusions. It identifies significant issues, evaluates the results, and provides recommendations for reducing environmental impacts.

2.3.3. Importance of LCA in Environmental Impact Assessment

LCA is crucial for understanding the environmental impacts of products or processes. It provides a data-driven basis for identifying environmental hotspots, informing decisions on product design, materials selection, process optimization, and policy development. By assessing the life cycle, LCA helps stakeholders make informed choices that lead to the reduction of environmental impacts and the promotion of sustainability in industrial practices.

In the context of rhamnolipid production, LCA can pinpoint stages in the production process that are most detrimental to the environment, guiding efforts towards mitigating these impacts. Whether it's reducing energy use, minimizing waste, or selecting less harmful raw materials, LCA serves as a valuable tool for making rhamnolipid production more sustainable.

By incorporating LCA, this report aims to thoroughly evaluate the environmental aspects of rhamnolipid production, offering insights into how the process can be optimized to reduce its ecological footprint while maintaining or enhancing its economic viability.

2.4. Life Cycle Costing (LCC)

Life Cycle Costing (LCC) is a methodological approach used to assess the total cost of ownership of a product, process, or service over its entire life cycle. LCC encompasses all costs from initial acquisition through operation, maintenance, and disposal, providing a comprehensive understanding of the economic aspects of sustainability. By evaluating these costs, LCC helps identify economic hotspots and opportunities for cost

reduction, complementing environmental assessments conducted through Life Cycle Assessment (LCA).

2.4.1. Principles of LCC

LCC is guided by principles aimed at ensuring a thorough and accurate economic analysis:

- **Comprehensiveness:** LCC considers all relevant costs associated with the life cycle of a product or process, including initial capital costs, operating costs, maintenance costs, and end-of-life costs.
- **Long-term Perspective:** LCC focuses on the total cost over the life span of the product or process, which encourages the consideration of long-term costs and benefits, rather than just initial expenditures.
- **System Boundary Definition:** Similar to LCA, LCC requires clear definition of the system boundaries to determine which costs are included in the analysis. This definition is crucial for ensuring the accuracy and relevance of the assessment.

2.4.2. Stages of LCC

The LCC process typically involves the following stages:

1. **Goal and Scope Definition:** This stage establishes the purpose of the LCC analysis, the system boundaries, and the study period. It sets the framework for what costs will be considered and over what timeframe.
2. **Inventory of Costs:** This stage involves identifying and quantifying all costs associated with the product or process throughout its life cycle. It includes direct costs (e.g., materials, energy, labour) and indirect costs (e.g., overheads, environmental compliance costs).
3. **Cost Assessment:** In this stage, the collected cost data are analyzed and aggregated to assess the total life cycle cost. This may involve discounting future costs to present value to account for the time value of money.
4. **Interpretation:** The final stage interprets the results of the cost assessment, identifying economic hotspots and providing insights into potential cost-saving measures. It involves comparing different options or scenarios to guide decision-making towards more economically sustainable solutions.

2.4.3. Importance of LCC in Sustainability Assessments

Integrating LCC with environmental assessments like LCA provides a more holistic view of sustainability, addressing both economic and environmental dimensions. This integrated approach is particularly valuable in decision-making processes, as it allows

for the identification of strategies that are not only environmentally beneficial but also economically viable. For industries and processes where, economic considerations significantly influence sustainability outcomes, such as in the production of rhamnolipids, LCC plays a critical role in ensuring that sustainable practices are also financially sustainable.

In the context of rhamnolipid production, LCC can reveal economic hotspots, such as high energy consumption or expensive raw material inputs and offer insights into where cost efficiencies can be achieved without compromising environmental objectives. By systematically evaluating the costs associated with different stages of the production process, stakeholders can make informed decisions that optimize both economic and environmental performance.

The incorporation of LCC into this report aims to provide a comprehensive analysis of the economic aspects of rhamnolipid production, identifying opportunities for cost reduction and efficiency improvements. This, combined with the environmental insights provided by LCA, will offer a robust foundation for recommendations aimed at enhancing the sustainability of rhamnolipid production from both an environmental and economic perspective.

3. Methodology – Rhamnolipids production scale-up

3.1. Process Description Overview

- **Objective**: To economically evaluate the production of rhamnolipids, focusing on material requirements, process equipment, utilities consumption, and production costs.
- **Sections Divided for Reporting and Analysis**:
  - Media Preparation
  - Fermentation
  - Product Purification
  - Solvent Recovery and Reuse

Figure 1 showcases a schematic overview of the rhamnolipids production process, segmented into bioproduction and separation/purification phases. In the bioproduction phase, two bacterial strains, *P. putida* and *P. gessardii*, utilize a substrate to produce biomass, which then undergoes fermentation to generate rhamnolipids. The subsequent separation/purification phase begins with membrane separation to remove biomass, followed by solvent extraction to separate rhamnolipids from the residual biomass [10,11]. A mixture of rhamnolipids and solvent is obtained from which rhamnolipids are finally isolated through a solvent recovery process, yielding pure rhamnolipids and residual water. The diagram indicates additional process intricacies, such as the

possibility of residual biomass influencing subsequent extraction steps and the potential recirculation within the biomass production. The solid lines denote the primary process flow, while dashed lines suggest inputs, secondary flows, or byproducts [12,13].

The calculations, kinetics, and yields presented in this document are based on the data and results obtained by the WP6 of the SECRETed project. Specifically, the contributions from Igor de las Heras and Manuel Salvador from IDE have been instrumental in establishing the quantitative aspects of this study [14,15]. Their work encompasses detailed analysis and verification of process parameters, ensuring that the modelled outcomes closely reflect the actual production capabilities and efficiencies.

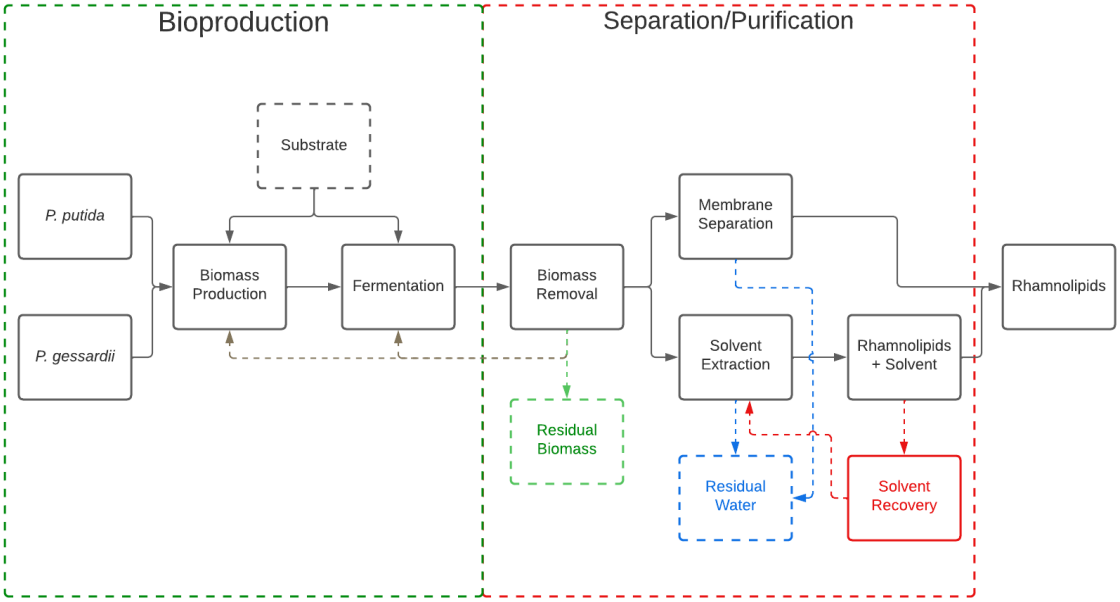

Figure 1: Rhamnolipids production general process flowchart.

3.2. Media Preparation and Fermentation

3.2.1. Media Preparation

- **Operation Mode**: Continuous for media preparation units, contrasting with the batch mode operation of fermentation units.
- **Process Steps**:
  - **Carbon Substrate Preparation**:
    - Components are mixed to prepare the carbon substrate, facilitating the initial step in the preparation of the media.
    - The mixture is stored in an agitated tank to ensure uniformity.
    - A sterilization process is employed to ensure the solution is free from any contamination. This involves preheating, sterilization at high temperatures, and subsequent cooling to 31 °C for optimal microbial growth conditions.

- The sterilized solution is then distributed to the fermentation process, ensuring a sterile environment for microbial growth.

– **Fermentation Water Sterilization**:

- Water used in the fermentation process undergoes sterilization at high temperatures to eliminate any microorganisms, ensuring the purity of the fermentation environment.
- After sterilization, the water is stored and similarly distributed to support the fermentation process.

– **Fed-Batch Media Solution Preparation**:

- A specific media composition is prepared for the fed-batch phase of fermentation, which differs slightly from the initial batch phase, catering to the different nutritional requirements during this phase.
- This involves preparation, agitation, sterilization, and storage before being supplied to the fermenters, ensuring the media supports optimal microbial growth and product formation.

Table 1 provides a comprehensive list of materials required for the inoculum and fermentation stages in a bioproduction process. Each material is checked for its availability in environmental databases, specifically Ecoinvent 3.8 and the Agri-footprint database.

For components not directly available, proxies or stoichiometric quantities of other substances are suggested; for example, $Na_2EDTA.2H_2O$ can be substituted with EDTA, and for $CoCl_2.6H_2O$, a combination of $CoCO_3$ and HCl is recommended. Similarly, $MnCl_2.4H_2O$ and $Na_2MoO_4.2H_2O$ can be represented using stoichiometric amounts of MnO_2 and MoO_3 with HCl and NaOH, respectively.

Materials such as KH_2PO_4 and Spectinomycin dihydrochloride pentahydrate are not available in the database and need to be sourced from literature or replaced with proper proxies. For Toluic acid, the database suggests using benzoic acid as a proxy. The Agri-footprint database offers alternatives for certain biological materials like Tryptone, Yeast extract, Tryptic soy broth, Bacto-peptone, Soy peptone, and Malt extract, suggesting the use of lactose, fodder yeast, soybean meal, and generic enzymes as proxies. Soluble starch is available from various plant sources such as maize, potato, wheat, and pea.

Furthermore, the table indicates the use of dextrose in place of glucose, and for Mannitol, glucose is again used as a proxy. The consistency in database availability for most materials underscores the thoroughness of the sourcing strategy and facilitates the life cycle assessment of the bioproduction process.

Table 1: Raw material data availability for Media Preparation and Fermentation

Material Input	Availability in Databases
Glucose	yes, in Ecoinvent 3.8
Glycerol	yes, in Ecoinvent 3.8
$(NH_4)2SO_4$	yes, in Ecoinvent 3.8
$(NH_4)Cl$	yes, in Ecoinvent 3.8
Na_2HPO_4	yes, in Ecoinvent 3.8
KH_2PO_4	No, needs to be obtained from literature
$Na_2EDTA.2H_2O$	use EDTA as proxy, in Ecoinvent 3.8
$ZnSO_4.7H_2O$	yes, in Ecoinvent 3.8
$CoCl_2.6H_2O$	use stoichiometric quantities of $CoCO_3$ and HCl, in Ecoinvent 3.8
$MnCl_2.4H_2O$	use stoichiometric quantities of MnO_2 and HCl, in Ecoinvent 3.8
H_3BO_3	yes, in Ecoinvent 3.8
$Na_2MoO_4.2H_2O$	use stoichiometric quantities of MoO_3 and NaOH, in Ecoinvent 3.8
$FeSO_4.7H_2O$	yes, in Ecoinvent 3.8
$CuSO_4.5H_2O$	yes, in Ecoinvent 3.8
$MgSO_4.7H_2O$	yes, in Ecoinvent 3.8
$CaCl_2.2H_2O$	yes, in Ecoinvent 3.8
Spectinomycin dihydrochloride pentahydrate	No, needs to be obtained from literature
Toluic acid	use benzoic acid as proxy, in Ecoinvent 3.8
Tryptone	use co-product lactose as proxy, in Agri-footprint data base
Yeast extract	use fodder yeast as proxy, in Agri-footprint data base
Tryptic soy broth	use soybean meal as proxy, in Agri-footprint data base
Bacto-peptone	use generic enzymes, in Ecoinvent data base
Tris Base	use diethanolamine as proxy, in Ecoinvent data base
KCl	yes, in Ecoinvent data base
Dextrose	use glucose, in Ecoinvent data base
Soluble starch	yes (from maize, potato, wheat and pea available Agri-footprint data base)
Soy peptone	use soybean meal as proxy, in Agri-footprint data base
Soybean flour	yes (soybean meal) in Agri-footprint data base
NaCl	yes, in Ecoinvent data base
$NaSO_4$	yes, in Ecoinvent data base
$CaCO_3$	yes, in Ecoinvent data base
Mannitol	use glucose as proxy, in Ecoinvent data base
Malt extract	use fodder yeast as proxy, in Agri-foodprint data base

3.2.2. Fermentation

In the design of batch reactors, particularly for biological processes such as the cultivation of microorganisms or the production of biochemicals, several theoretical aspects must be considered, including reactor design, kinetics, and volume calculations.

Batch Reactor Design:

A batch reactor is a closed system where a reaction takes place over a period without any addition or removal of materials until the reaction is complete. In bioprocessing, this typically involves an initial inoculation of cells or microorganisms, followed by a period of growth and product formation, and finally, reactor harvesting. The main advantage of batch reactors in biological systems is their relative simplicity and the high degree of control over the process environment.

- **Operation Mode**: Batch mode to facilitate specific microbial growth and product formation phases.
- **Key Steps and Equipment**:
  - Utilization of seed fermenters for the growth of inoculum, ensuring a robust start to the fermentation process.
  - Main production fermenters are used for the bulk of the fermentation, where the actual product formation occurs.
  - Oxygen is supplied to support aerobic microbial growth, with specific equipment employed for air filtration, compression, and cooling.
- **Operations**:
  - Various operations including sterilization of equipment before use, substrate and inoculum addition, and controlled fermentation conditions (temperature, air supply) to optimize product formation.
  - Post-fermentation processes like the transfer of culture to subsequent batches and cleaning of the fermenters to maintain a sterile environment for each batch.

Table 2 details the chemical reactions and their conversion efficiencies involved in the fermentation process to produce rhamnolipids based on the available literature, this values, however, will be adjusted as the project develops [7]. The first reaction described is biomass growth with molar stoichiometry, where glucose reacts with oxygen and is facilitated by yeast extract and peptone to yield biomass, carbon dioxide, and water, achieving a high conversion efficiency of 95%. The second reaction focuses on the product formation with mass stoichiometry, where glucose and oleic acid are converted into rhamnolipids, glycerol, and water. This process is remarkably efficient, with a conversion rate of 99%, indicating that almost all the reactants are turned into the desired end products.

Table 2: Expected reactions for the fermentation steps

Fermentation Reactions	Conversion
Biomass Growth (Molar Stoichiometry): Glucose + $5O_2$ + Yeast Extract + 0.94Peptone → 3Biomass +5CO2 + $5H_2O$	95%
Product Formation (Mass Stoichiometry): 108Glucose + 180Oleic acid → 240Rhamnolipids + 30Glycerol + $18H_2O$	99%

3.3.Product Purification

Process Flow:

a. Initial separation of biomass from the fermentation broth, employing centrifugation for effective removal.

b. Additional purification steps to remove remaining biomass and unwanted materials, including filtration and hydrolyzation of proteins, to improve the purity of the rhamnolipids.

c. Extraction processes using ethyl acetate to separate rhamnolipids from the aqueous phase, followed by phase adjustments to recover the product efficiently.

3.3.1. Principles of Centrifugation

Centrifugation is a technique utilized to separate components in a mixture by applying centrifugal force, significantly speeding up the process of sedimentation that would naturally occur due to gravity. It's a critical operation in various fields, from biochemistry and molecular biology to industrial production and waste management.

The underlying principle of centrifugation is the differential sedimentation of particles under the influence of a radial force. This force causes particles of higher density than the solvent to move outward to the bottom of the tube or container, while less dense particles remain closer to the center.

Types of Centrifugation:

- **Differential Centrifugation:** This type separates components based on differences in their sedimentation rate due to size and density.
- **Isopycnic Centrifugation:** Here, particles are separated based on their buoyant density, typically using a density gradient within the centrifuge tube.
- **Rate-Zonal Centrifugation:** This is used for separating particles that might be similar in size or density but differ in shape or frictional properties.

3.4.Solvent Recovery and Reuse

- **Process Summary**:
  - A distillation process recovers ethyl acetate from the mixture, allowing for its reuse in the extraction process, highlighting the emphasis on sustainability and cost-efficiency.
  - Management of wastewater and recovery of solvents to minimize environmental impact and improve process sustainability.
  - The recycling of ethyl acetate back into the extraction process, reducing the need for fresh solvent and lowering production costs.

3.5.Ethyl Acetate in Rhamnolipids Production Process

Ethyl acetate is a widely used solvent in the biotechnology and pharmaceutical industries due to its favourable chemical properties, including its ability to dissolve a wide range of organic compounds, moderate volatility, and relatively low toxicity. Its role in the rhamnolipids production process, particularly in the product purification phase, is pivotal due to several key attributes that make it particularly suitable for this application.

3.5.1. Properties of Ethyl Acetate

- **Chemical Compatibility**: Ethyl acetate is an excellent solvent for many organic compounds, especially lipophilic substances like rhamnolipids. Its ability to efficiently dissolve these compounds facilitates their separation from the aqueous phase of the fermentation broth.
- **Volatility**: Its moderate volatility allows for easy recovery and reuse through distillation processes. This property is crucial in minimizing solvent losses and reducing the environmental impact of the process.
- **Safety and Environmental Impact**: Compared to other organic solvents, ethyl acetate is less toxic and poses lower risks to health and the environment. It is considered a green solvent in many applications due to its faster biodegradability and lower toxicity.
- **Economic Efficiency**: The relatively low cost of ethyl acetate, combined with its effectiveness and recyclability, contributes to the economic efficiency of the rhamnolipids production process.

3.5.1.1. Role in Rhamnolipids Purification

- **Selective Extraction**: Ethyl acetate's selective solubility properties make it ideal for extracting rhamnolipids from the fermentation broth. It can effectively separate rhamnolipids based on their amphiphilic nature, pulling them into the organic phase while leaving behind aqueous components.

- **Phase Separation Efficiency**: The distinct difference in solubility of rhamnolipids in ethyl acetate versus water enables a clear phase separation, facilitating the recovery of purified rhamnolipids from the organic phase.
- **Recovery and Reuse**: After extraction, ethyl acetate can be easily separated from the rhamnolipids through distillation due to its favourable boiling point. This property allows the solvent to be recycled and reused in subsequent extraction processes, significantly reducing waste and operational costs.

3.5.2. Sustainability Considerations

The use of ethyl acetate aligns with sustainability practices in industrial bioprocessing by reducing the environmental footprint of the purification process. Its recyclability minimizes solvent consumption and waste generation, contributing to the overall sustainability of the rhamnolipids production process. Moreover, its lower toxicity and biodegradability compared to other solvents used in similar applications enhance the process's environmental compatibility.

Table 3: Sustainability considerations of using Ethyl Acetate

Consideration	Description
Recyclability	Ethyl acetate can be recycled and reused in the process, minimizing solvent waste.
Reduced Environmental Footprint	Its lower toxicity and faster biodegradability contribute to a reduced environmental footprint of the production process.
Economic Sustainability	The solvent's cost-effectiveness and efficiency in rhamnolipids extraction support the economic sustainability of the process.

4. Process modelling results.

4.1.Process description.

4.1.1. Fermentation

Figure 2 showcases the biotechnological process for the continuous production of rhamnolipids through the fermentation of sugars by *P. putida* or *P. gessardii*. The process is methodically laid out in several key sections: media preparation, fermentation broth preparation, biomass production, and main fermentation.

The initial stage, media preparation, involves the mixing of vegetable oil, yeast extract, glucose, peptone, and water, which after being combined in a mixing tank, undergoes pasteurization to ensure sterility and to prepare the media for inoculation. In parallel, the fermentation broth preparation follows a similar flow, where vegetable oil, glucose, and water are processed for the subsequent fermentation stages.

Both the media and fermentation broth preparation stages operate continuously with a residence time of 16 hours, which synchronizes with the residence time required for the downstream processes. This continuity is crucial for maintaining a steady flow of resources for the growth of biomass and the production of rhamnolipids.

The seed fermenters for biomass production consist of three sets of four bioreactors of various sizes. This stepped configuration is designed to optimize the growth of microbial biomass, which is essential for the main fermentation stage. Each bioreactor operates over a 50-hour reaction duration, but because there are four in each set, they operate in a staggered manner, allowing for continuous inoculation into the main fermenters.

The main fermentation, where rhamnolipids production mainly occurs, has a duration of 128 hours per cycle. With eight fermentation units working concurrently, the process is designed to align with the continuous input from the media preparation and fermentation broth preparation stages, thus achieving a seamless operation.

Auxiliary streams, including treated water and air, are also integral to the process. Water is subjected to pasteurization before being used in the mixing tanks, while air is filtered and compressed, providing a controlled environment necessary for microbial fermentation.

The overall design emphasizes a meticulous balance between the batch-based approach for biomass production and the continuous process for media and broth preparation. This hybrid approach leverages the advantages of both systems, ensuring a constant production flow while also allowing for the scaling of biomass to meet the demands of the main fermenter units. The output is then directed to downstream processing, where the rhamnolipids will be further purified and refined for use.

4.1.2. Rhamnolipids purification and Solvent Recovery

Figure 3 depicts a process flow diagram for the purification of rhamnolipids and recovery of solvent, following the output from the fermentation process. The process can be described in the following steps:

1. **Biomass Separation**: Initially, the output stream from fermentation undergoes biomass separation through centrifugation to remove the biomass. The solid biomass is then washed with water and centrifuged again. The supernatants from both centrifugations are subsequently directed through microfiltration to remove any remaining biomass, ensuring a clearer liquid stream.

2. **Drying of Biomass**: The separated and washed biomass proceeds to a rotary dryer where it is dried, resulting in dried biomass and an airstream that exits the process.

3. **Protein Hydrolysis**: The stream that is rich in rhamnolipids, which is the focus of the process, enters a plug flow reactor (PFR) where it is treated with the enzyme alcalase to hydrolyse any proteins present in the stream.

4. **pH Adjustment**: After hydrolysis, the pH of the stream is adjusted using hydrochloric acid (HCl) to prepare for the extraction phase.

5. **Liquid-Liquid Extraction**: This step involves the extraction of rhamnolipids using ethyl acetate as a solvent. This process helps separate rhamnolipids from the aqueous phase into the solvent phase.

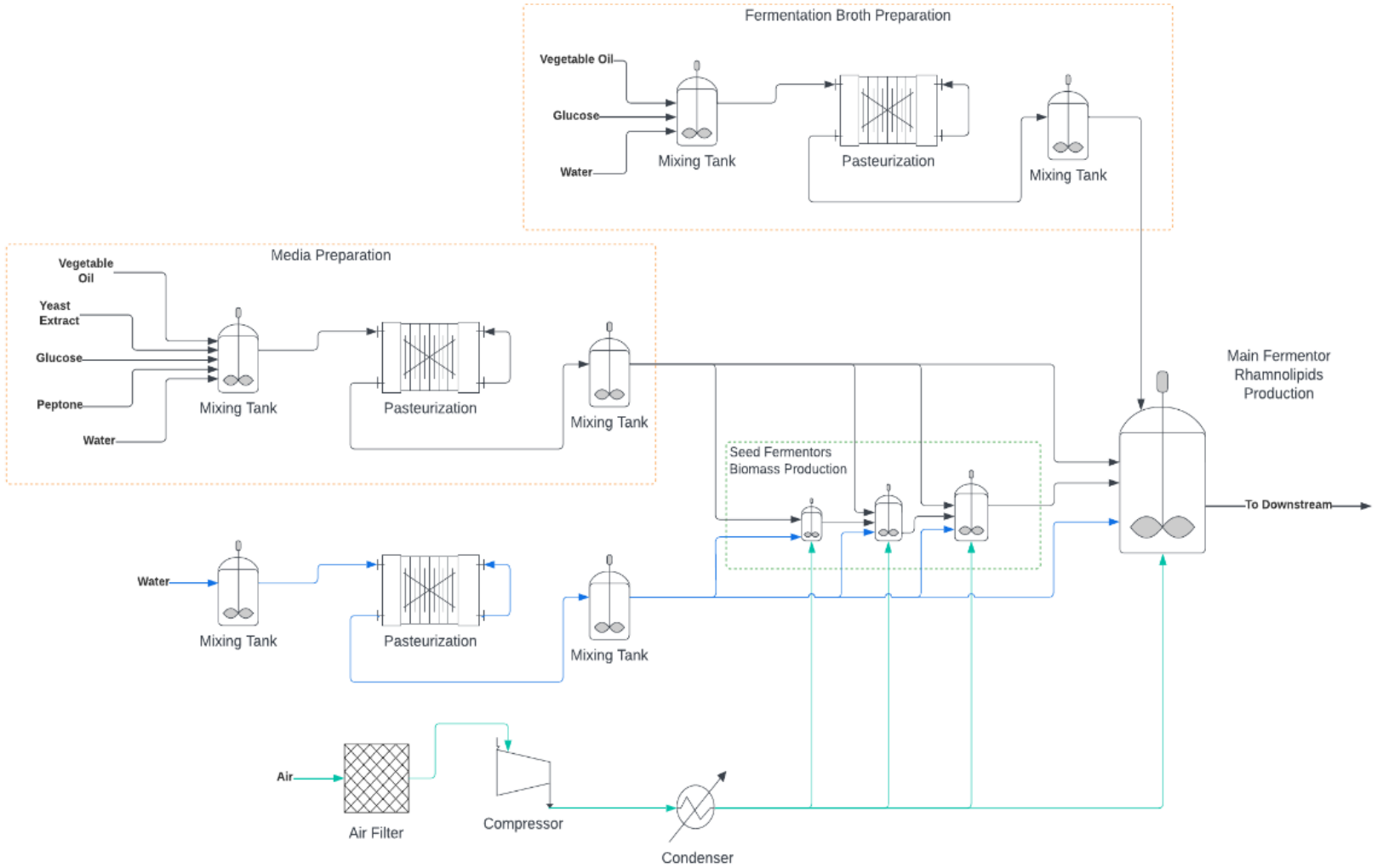

Figure 2: "Process Flowchart for the Continuous Production of Rhamnolipids via Fermentation Using *P. putida* or *P. gessardii*.

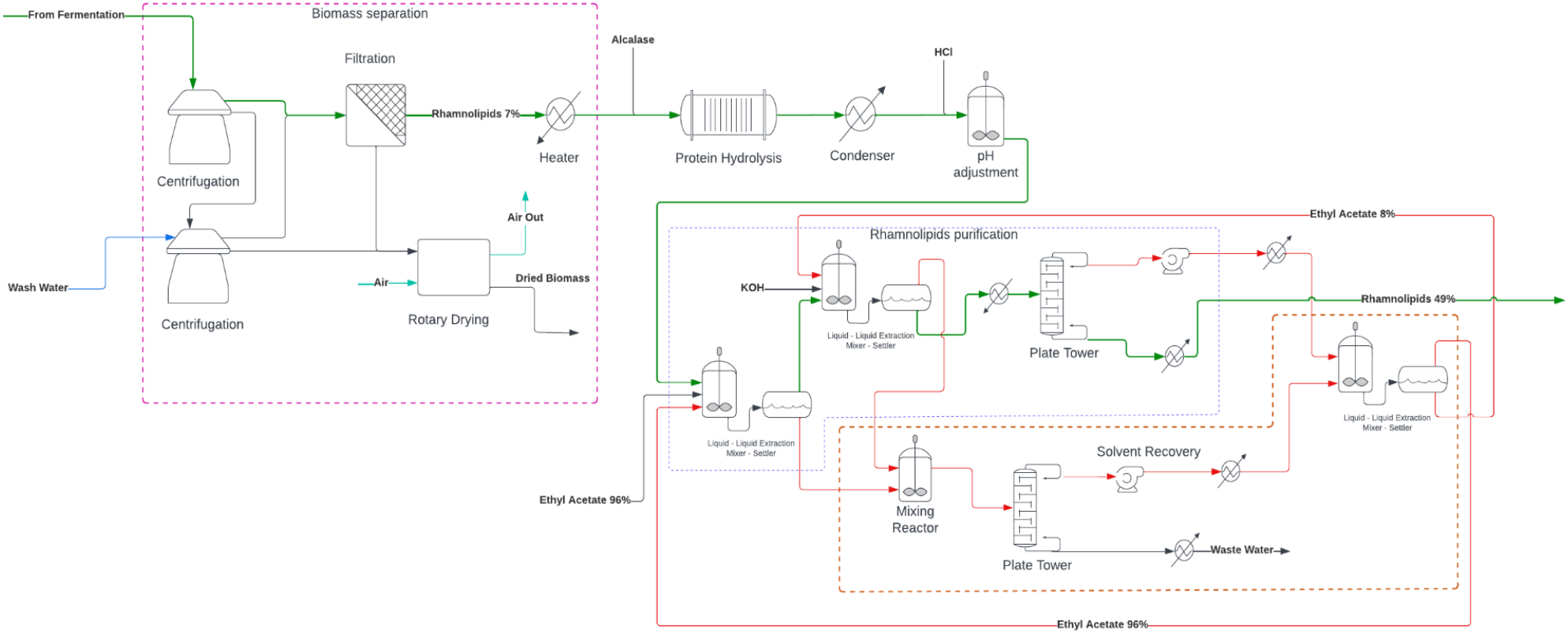

Figure 3: Rhamnolipids purification and Solvent Recovery

6. **Distillation for Rhamnolipids Purification**: The ethyl acetate-rhamnolipids mixture then undergoes distillation at a reduced pressure of 0.2 bar and a temperature of 42°C. From the bottom of the distillation setup, a solution rich in rhamnolipids (49% concentration) is collected

7. **Solvent Recovery**: The top stream primarily consisting of ethyl acetate from the distillation step is fed into a solvent recovery system. This system includes additional liquid-liquid extraction units followed by a distillation column (plate tower).

8. **Final Separation**: The distillation in the solvent recovery section is designed to recover ethyl acetate, which is taken off from the top of the column. The bottom stream of this column results in wastewater, concluding the process

4.2.Mass Balances

The Table 4 presents an overview of the mass balances for a combined process involving fermentation and subsequent rhamnolipids purification. The table lists various components involved in the process along with their respective input and output masses in arbitrary units.

Table 4: Overall Mass Balances for the Combined Fermentation and Rhamnolipids Purification Process

Component	Input [kg/batch]	Output [kg/batch]
Alcalase	0.18	0.18
Biomass	0	223.78
Carb. Dioxide	0	666.41
Ethyl Acetate	0.29	0.29
Glucose	1047.77	3.45
Glycerol	0	138.49
HCl	0.3	0.3
KOH	1.14	1.14
Nitrogen	81687.49	81687.55
Oxygen	24798.74	24314.22
Peptone	64.63	5.65
Proteins	0	0.07
Rhamnolipids	0	1107.96
Sodium Hydroxide	29.14	29.14
Vegetable Oil	864.03	32.93
Water	20454.33	20811.04
Yeast Extract	78.23	3.67
Total	129026.27	129026.27

This table provides a detailed account of the mass balance of inputs and outputs for each component used in the fermentation and rhamnolipids purification process. Inputs include substances such as glucose, which serves as a feedstock for fermentation, and KOH and HCl, which are used for pH adjustment. Outputs capture the products and by-products of the process, such as the produced rhamnolipids and biomass, as well as waste materials like carbon dioxide and excess water.

4.2.1. Raw Material Cost

Table 5 details the costs associated with the raw materials for each kilogram of rhamnolipids produced at different stages of the production process. Each section lists the materials used, their unit cost in Euros, the required amount per kilogram of rhamnolipids, the resulting cost per kilogram of rhamnolipids, and the percentage contribution of each material to the total cost of its respective stage.

Table 5: Cost Analysis of Raw Materials for Rhamnolipid Production Process

Media Preparation					
Material Name	Unit Cost (€)	Amount per kg of rhamnolipids		Cost (€/kg rhamnolipids)	%
Glucose	0.58[16]	1.04736	kg	0.61	38.82
Peptone	3.60[17]	0.06460	kg	0.23	14.73
Vegetable Oil	0.45[18]	0.86369	kg	0.39	24.62
Water	4.50	0.01401	MT	0.06	3.99
Yeast Extract	3.60[17]	0.07820	kg	0.28	17.84
TOTAL				1.58	100.00
Fermentation					
Material Name	Unit Cost (€)	Amount per kg rhamnolipids		Cost (€/kg rhamnolipids)	%
Air	0.00	71.28619	kg	0.00	0.00
Biomass	0.00	0.00000	kg	0.00	0.00
NaOH (2%)	0.00621[19]	1.45550	kg	0.01	35.38
Water	4.50	0.00367	MT	0.02	64.62
TOTAL				0.03	100.00
Product Purification					
Material Name	Unit Cost (€)	Amount per kg rhamnolipids		Cost (€/kg rhamnolipids)	%
Air	0.00	35.09633	kg	0.00	0.00
Alcalase	45.00[20]	0.00018	kg	0.01	55.80
HCl	0.09[21]	0.00030	kg	0.00	0.18
KOH	0.45[22]	0.00114	kg	0.00	3.48
Water	4.50	0.00133	MT	0.01	40.54
TOTAL				0.01	100.00
Solvent Recovery and Reuse					
Material Name	Unit Cost (€)	Amount per kg rhamnolipids		Cost (€/kg rhamnolipids)	%
Ethyl Acetate	1.07[23]	0.00016	kg	0.00	99.99
Water	4.50	0.00000	MT	0.00	0.01
TOTAL				0.00	100.00

- **Media Preparation**: The costs reflect the quantity and cost of glucose, peptone, vegetable oil, water, and yeast extract necessary to produce one kilogram of rhamnolipids. Glucose is the most significant cost factor, accounting for 38.82% of the media preparation expenses, while the total cost for this stage is €1.58 per kilogram of rhamnolipids.

- **Fermentation**: At this stage, only sodium hydroxide (NaOH) and water have associated costs. The total expense for the fermentation process is €0.03 per kilogram of rhamnolipids, with the major cost contributor being water at 64.62% of the fermentation costs.

- **Product Purification**: This stage includes the cost of alcalase, HCl, KOH, and water. Alcalase, though used in small quantities, represents the majority of the purification costs at 55.80%. The total cost for purification is €0.01 per kilogram of rhamnolipids.

- **Solvent Recovery and Reuse**: For this final stage, the cost is associated mainly with the use of ethyl acetate, accounting for 99.99% of the costs, even though the amount used is minimal. The overall cost is close to negligible, indicating the cost-efficiency of the solvent recovery system.

4.2.2. Waste production and cost.

Table 6 provides an assessment of the waste products generated during the rhamnolipids production process, across three stages: Fermentation, Product Purification, and Solvent Recovery and Reuse. It also outlines the costs associated with the disposal of these wastes, when applicable.

Table 6: Waste Generation and Disposal Costs per Kilogram of Rhamnolipids in Production Process

Fermentation						
Waste Type	Average Unit Cost		Amount (/kg rhamnolipids)		Cost (€/kg rhamnolipids)	%
Aqueous Waste	0.00447[24]	€/kg	5.12419	kg	0.02288	100
Emissions	0	€/kg	71.48822	kg	0	0
Subtotal			76.61241	kg	0.02288	100
Product Purification						
Waste Type	Average Unit Cost		Amount (/kg rhamnolipids)		Cost (€/kg rhamnolipids)	%
Emissions	0	€/kg	36.23608	kg	0	0
Subtotal			36.23608	kg	0	0
Filter Cartridge	0	€/item	0.00011	item	0	0
Subtotal					0	0
TOTAL					0	0
Solvent Recovery and Reuse						

Waste Type	Average Unit Cost		Amount (/kg rhamnolipids)		Cost (€/kg rhamnolipids)	%
Aqueous Waste	0.0045	€/kg	13.71994	kg	0.06174	100
Subtotal			13.71994	kg	0.06174	100

- **Fermentation**: Here, aqueous waste is generated at a rate of 5.12419 kg per kilogram of rhamnolipids produced, with an associated disposal cost of €0.02288 per kilogram of rhamnolipids produced, accounting for 100% of the waste disposal costs at this stage. Emissions are noted as well, with 71.48822 kg of emissions per kilogram of rhamnolipids; but an estimation of the cost incurred here will depend on the location of the production facilities, which is not within the scope of the current analysis.
- **Product Purification**: This stage lists emissions as the only waste type, with 36.23608 kg of emissions per kilogram of rhamnolipids produced. Similar to fermentation, there is no associated cost for these emissions. Additionally, the table mentions the filter Cartridge with a negligible amount per kilogram given its cycles of reuse.
- **Solvent Recovery and Reuse**: The only waste generated in this stage is aqueous waste, at 13.71994 kg per kilogram of rhamnolipids, with a disposal cost of €0.06174 per kilogram of rhamnolipids produced, which again accounts for 100% of the waste disposal costs for this stage.

4.3.Utility needs and cost.

Table 7 provides a detailed summary of utility usage and the associated costs for each unit operation in the rhamnolipid production process. It specifies the consumption of electricity, high-pressure steam, cooling water, chilled water, and low-pressure steam (Steam LP) per kilogram of rhamnolipids produced and the corresponding costs.

Table 7: Utility Consumption and Costs for Rhamnolipid Production Process

Electricity				
Section Name	Unit Cost (€/kW-h)	Amount (kW-h/kg rhamnolipids produced)	Cost (€/kg rhamnolipids produced)	%
Media Preparation	0.063[25]	0.01525	0	0.28
Fermentation	0.063	4.77079	0.3	87.81
Product Purification	0.063	0.64571	0.04	11.89
Solvent Recovery and Reuse	0.063	0.00109	0	0.02
TOTAL		5.43285	0.34	100
Steam				

Section Name	Unit Cost (€/MT)	Amount (MT/kg rhamnolipids produced)	Cost (€/kg rhamnolipids produced)	%
Media Preparation	27	3.60E-04	0.01	2.67
Fermentation	27	5.00E-05	0	0.37
Product Purification	27	7.97E-03	0.22	59.46
Solvent Recovery and Reuse	27	5.02E-03	0.14	37.49
TOTAL		1.34E-02	0.36	100
Cooling Water				
Section Name	Unit Cost (€/MT)	Amount (MT/kg rhamnolipids produced)	Cost (€/kg rhamnolipids produced)	%
Media Preparation	0.045	0.01923	0	1.12
Fermentation	0.045	0.51337	0.02	29.82
Product Purification	0.045	0.60811	0.03	35.32
Solvent Recovery and Reuse	0.045	0.58106	0.03	33.75
TOTAL		1.72177	0.08	100
Chilled Water				
Section Name	Unit Cost (€/MT)	Amount (MT/kg rhamnolipids produced)	Cost (€/kg rhamnolipids produced)	%
Fermentation	0.36	0.51378	0.18	100
TOTAL		0.51378	0.18	100
Steam LP				
Section Name	Unit Cost (€/MT)	Amount (MT/kg rhamnolipids produced)	Cost (€/kg rhamnolipids produced)	%
Solvent Recovery and Reuse	18	7.30E-04	0.01	100
TOTAL		7.30E-04	0.01	100

- **Electricity**: This utility is used throughout the process with the highest consumption during Fermentation (4.77079 kW-h/kg), which incurs a cost of €0.30 per kilogram of rhamnolipids and represents 87.81% of the total electricity cost. Product Purification also uses electricity significantly but less than Fermentation, contributing to 11.89% of the electricity costs. The total electricity cost amounts to €0.34 per kilogram of rhamnolipids produced.
- **Steam (High Pressure)**: High-pressure steam usage is highest in Product Purification (0.00797 MT/kg), costing €0.22 per kilogram of rhamnolipids, accounting for 59.46% of the total steam costs. Solvent Recovery and Reuse also utilize a notable amount, contributing 37.49% to the steam costs. The overall cost for steam is €0.36 per kilogram of rhamnolipids.

- **Cooling Water**: Cooling water is required across all sections, with the highest use in Product Purification, which costs €0.03 per kilogram of rhamnolipids. This section, along with Solvent Recovery and Reuse, dominates the cooling water costs, each accounting for over 30% of the total. The total cooling water cost is €0.08 per kilogram of rhamnolipids.
- **Chilled Water**: Only the Fermentation section uses chilled water (0.51378 MT/kg), with a total cost of €0.18 per kilogram of rhamnolipids, representing the entirety of the cost for this utility.
- **Steam LP (Low Pressure)**: Low-pressure steam is exclusively used in the Solvent Recovery and Reuse section, with a minimal cost impact of €0.01 per kilogram of rhamnolipids, constituting the total low-pressure steam costs.

The data indicates that high-pressure steam and electricity are the most significant contributors to utility costs in rhamnolipid production. Specifically, the Product Purification stage is the most energy-intensive in terms of high-pressure steam consumption, which aligns with the energy demands for processes like distillation. Conversely, chilled water and low-pressure steam have a lesser impact on the total utility costs.

4.4.Labor requirements and cost

Table 8 provides an overview of the labour costs associated with the production of rhamnolipids. It details the labour hours required per kilogram of rhamnolipids produced and the corresponding costs based on an hourly rate, which, for the purpose of this analysis, considers only the cost of operators. It's important to note that the hourly labour cost can vary significantly depending on the country and its respective labour rates.

Table 8: Labor Cost Analysis per Kilogram of Rhamnolipids in Production Process

Section Name	Unit Cost (€/h)	Amount (h/kg rhamnolipids produced)	Cost (€/kg rhamnolipids produced)	%
Media Preparation	23[26]	0.02595	0.59676	10.63
Fermentation	23	0.04408	1.0139	18.07
Product Purification	23	0.13857	3.1872	56.8
Solvent Recovery and Reuse	23	0.03538	0.81376	14.5
TOTAL		0.24398	5.61163	100

- **Media Preparation**: Requires 0.02595 hours of labour per kilogram of rhamnolipids, amounting to €0.59676 and constituting 10.63% of the total labour cost.
- **Fermentation**: Involves more labour at 0.04408 hours per kilogram, resulting in a cost of €1.0139, which is 18.07% of the total labour cost.

- **Product Purification**: This is the most labour-intensive step, requiring 0.13857 hours per kilogram and incurring a cost of €3.1872, thus accounting for 56.8% of the total labour cost.
- **Solvent Recovery and Reuse**: Demands 0.03538 hours of labour per kilogram with a cost of €0.81376, representing 14.5% of the overall labour cost.

The total labour hours required for the production of a kilogram of rhamnolipids are 0.24398, with an associated cost of €5.61163. This table underscores Product Purification as the most labour-costly part of the process, which could be a target for automation or process optimization.
The labour costs are highly dependent on the scale of production. In larger-scale operations, the cost per unit of product typically decreases due to the efficiencies gained from economies of scale. Moreover, the distribution of labour is determined by the complexity and requirements of each procedure. Optimizing labour allocation and improving process efficiencies can lead to significant cost savings, particularly in the Product Purification section, which is the most labour-intensive. This analysis is essential for strategic planning, budgeting, and identifying cost-saving opportunities in the production of rhamnolipids.

5. Hotspot Analysis

5.1.Environmental Hotspot Analysis

The Life Cycle Assessment (LCA) analysis presented herein was conducted using the SimaPro software version 9.5 (PRé Sustainability, The Netherlands), incorporating the Ecoinvent 3.8 database (Ecoinvent, Switzerland) to model the environmental impacts associated with the production process of rhamnolipids. The data input for this analysis was derived from the mass and energy balances outlined in the previous section. Furthermore, the assembly of the process was systematically divided according to the process sections previously mentioned, ensuring a detailed and structured assessment of each stage within the production lifecycle.

Figure 4 showcases a stacked column chart breaks down the relative environmental impacts of different steps in the rhamnolipid production process across six categories: global warming, terrestrial ecotoxicity, land use, mineral resource scarcity, fossil resource scarcity, and water consumption. Each column represents an impact category, and the coloured segments show the percentage contribution from each process step: Media Preparation, Fermentation, Product Purification processing, and Solvent Recovery.

In terms of global warming potential, Media Preparation is the primary contributor, indicating significant emissions are associated with the production and procurement of raw materials used in media, such as agricultural inputs and associated processing. This is followed by the Product Purification process, which suggests that the purification and extraction steps are also energy-intensive, likely due to the use of heat, electricity, and potentially environmentally impactful chemicals. Solvent Recovery and Fermentation have smaller but still notable impacts, with Fermentation contributing the least. This might be due to the efficiency of the bioreactors used or possibly the management of emissions from the fermentation process itself.

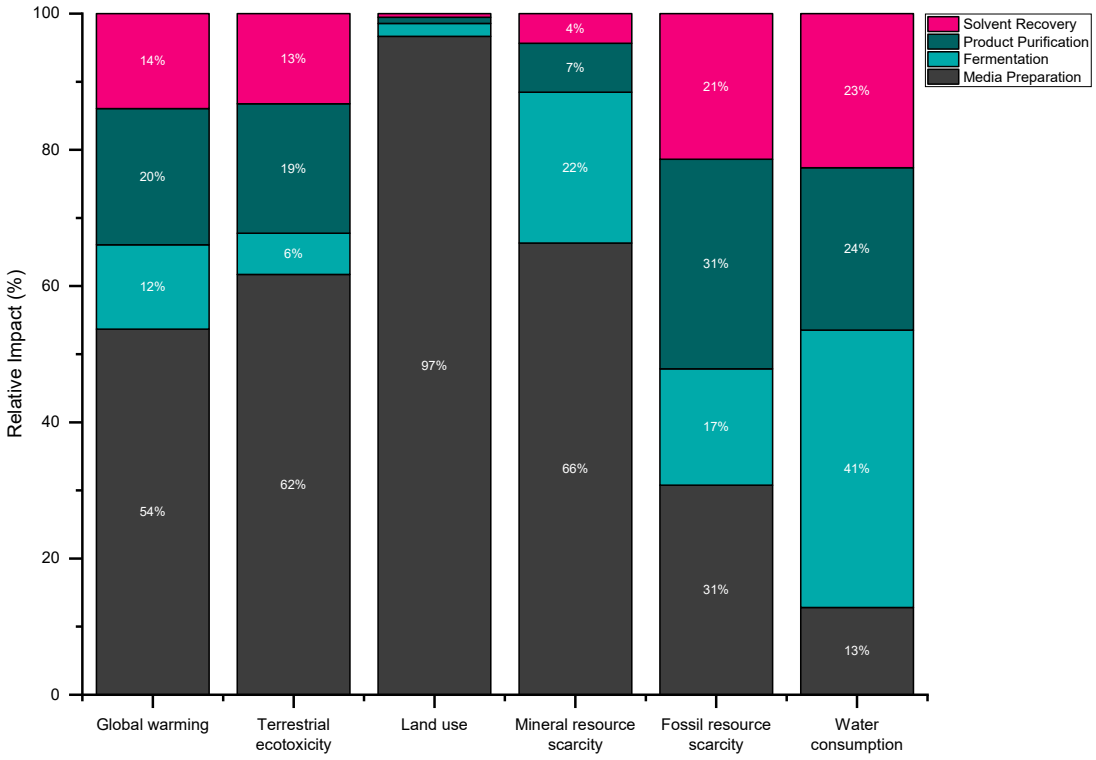

Figure 4: Relative Contribution of Process Steps to Environmental Impact Categories in Rhamnolipid Production

For terrestrial ecotoxicity, Media Preparation alone accounts for most of the impact, indicating the use of agricultural products or chemicals that may have downstream effects on terrestrial habitats. This underscores the potential benefits of exploring alternative, less ecotoxic media components or improving the agricultural practices associated with raw material production.

Land use is overwhelmingly dominated by the Media Preparation phase, reflecting the space required to grow the biological inputs, like crops for vegetable oil and glucose. This impact could be mitigated by sourcing materials from more sustainable agriculture with higher yield per area or by using waste streams as alternative feedstocks.
Interestingly, Mineral resource scarcity is relatively low across all process steps, indicating efficient use of these resources or low reliance on minerals in the production process.

For fossil resource scarcity, both Media Preparation and the Product Purification process are major contributors, which could be attributed to the energy sources used being derived from fossil fuels, as well as the possible use of petrochemical-derived solvents in the purification stage. These insights point to opportunities for using alternative energy sources and solvents that are less dependent on fossil resources.

When it comes to water consumption, Fermentation takes the lead, which might reflect the water required for the biological processes, as well as the cleaning and maintenance of bioreactors. This highlights an area where water use efficiency can be significantly improved, perhaps through the recycling of process water or the use of water-saving technologies.

By addressing the key areas identified—such as energy use in Fermentation and Media Preparation, reliance on agricultural products for Media Preparation, fossil resource use in Product Purification processing, and water use across the process—there is a notable opportunity to enhance the sustainability of rhamnolipid production.

Figure 5 show a Sankey diagram visualizing the flow of various resources used in the rhamnolipid production process and their respective contributions to the global warming potential category. The width of the streams is proportional to the amount of CO_2 equivalent emissions. Starting from the left, inputs such as Vegetable Oil, Steam, and Glucose, among others, lead into process stages (Media Preparation, Fermentation, Product Purification processing, Solvent Recovery) and culminate in the final impact on the production of rhamnolipids.

Table 9 enumerates the environmental impact of the rhamnolipid production process previously described.

Figure 5 show a Sankey diagram visualizing the flow of various resources used in the rhamnolipid production process and their respective contributions to the global warming potential category. The width of the streams is proportional to the amount of CO_2 equivalent emissions. Starting from the left, inputs such as Vegetable Oil, Steam, and Glucose, among others, lead into process stages (Media Preparation, Fermentation, Product Purification processing, Solvent Recovery) and culminate in the final impact on the production of rhamnolipids.

Table 9: Quantitative Environmental Impact of Rhamnolipid Production by Process Step per kg of rhamnolipids produced.

Category	Unit	Total	Media Preparation	Fermentation	Product purification	Solvent Recovery
Global warming	kg CO_2 eq	13.423	7.206	1.660	2.683	1.872
Terrestrial ecotoxicity	kg 1,4-DCB	3.586	2.212	0.217	0.682	0.475
Land use	m^2a crop eq	5.654	5.465	0.107	0.052	0.030
Mineral resource scarcity	kg Cu eq	0.040	0.026	0.009	0.003	0.002
Fossil resource scarcity	kg oil eq	2.954	0.909	0.505	0.909	0.632
Water consumption	m3	2.631	0.337	1.072	0.627	0.595

Starting with the inputs on the left, Vegetable Oil stands out as a significant contributor, its substantial flow signalling a high impact on global warming. This is likely due to the energy-intensive processes involved in its production, including cultivation, harvesting, and processing of the oil crops. Steam is the next prominent contributor, reflecting the energy consumption and the associated carbon emissions from possibly non-renewable energy sources used to generate steam.

As we follow the streams towards the right, Media Preparation shows a considerable share of the total emissions, suggesting that the manufacturing and preparation of media components are responsible for a large fraction of the overall global warming potential. It is evident that both the origin of materials and the energy used in this stage have substantial room for improvement from a sustainability perspective.

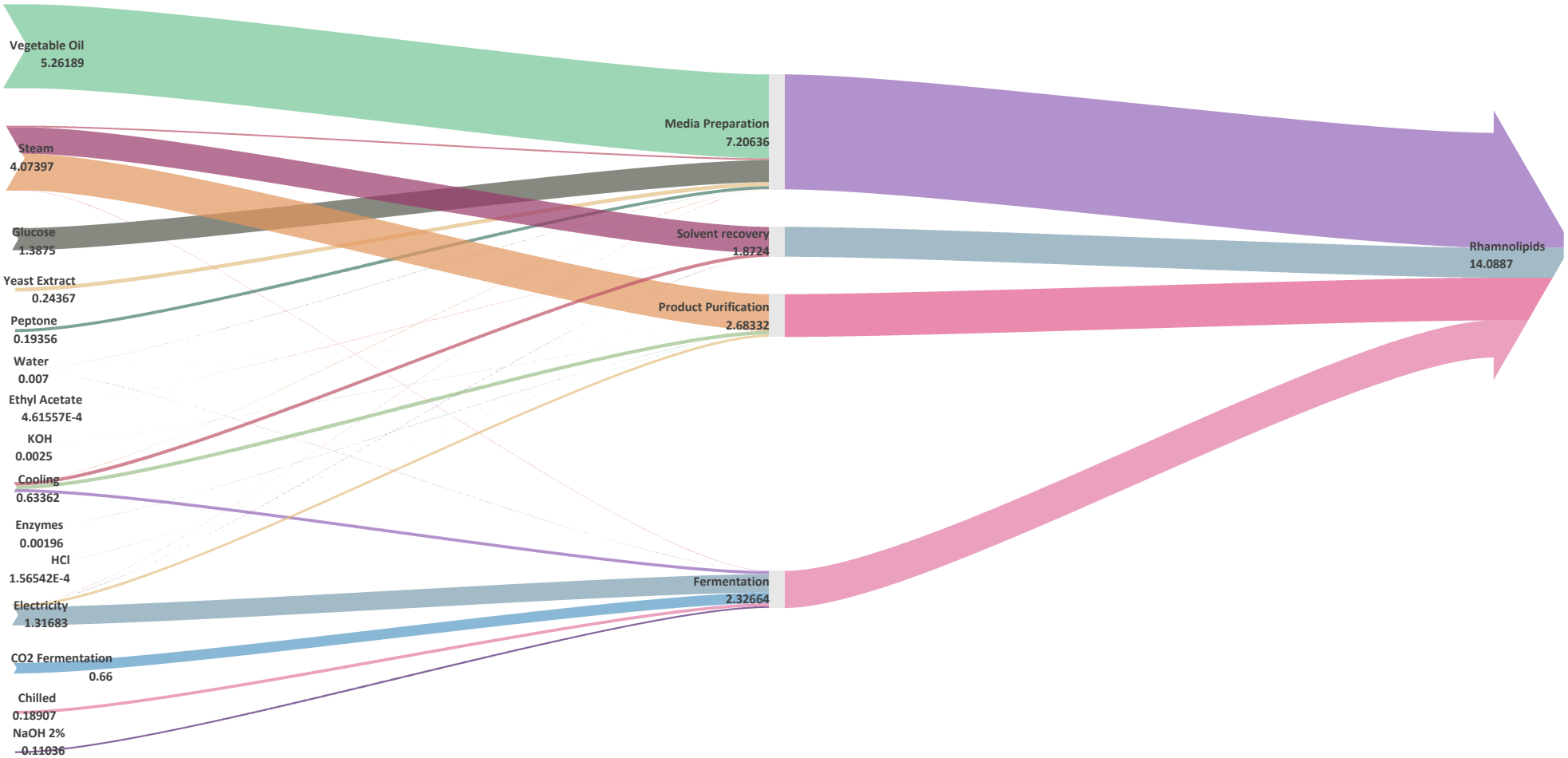

Figure 5: Flow of Resources Contributing to Global Warming Potential in the production of 1kg of rhamnolipids [kgCO_2 eq]

The Product Purification process is another major contributor, indicative of the energy required for product purification and extraction. Solvent use and waste management in this phase could be responsible for a significant amount of emissions. The representation of Solvent Recovery underscores its role in global warming potential, which could be due to the use of energy in the recovery and distillation processes.

Fermentation, while having a visible stream, is shown as less impactful than other stages. This might imply that the process is relatively efficient or that the emissions from fermentation are managed better compared to other stages. Nonetheless, it still represents a non-negligible impact and warrants consideration for further efficiency improvements.

The Sankey diagram ends with the final impact on rhamnolipids, summarizing the contributions from each process step to the total global warming potential. This visual representation emphasizes the need for targeted interventions in the Media Preparation and Product Purification processes to reduce the carbon footprint of rhamnolipid production. Transitioning to low-impact raw materials, renewable energy sources, and greener solvents could be potential strategies to achieve a more sustainable production process.

5.2.Economic Hotspot Analysis

Figure 6 maps out the flow of operational expenses associated with the production of rhamnolipids. Labour costs immediately stand out as the dominant expense, suggesting that manual processes or high workforce requirements are significantly driving up costs. To improve efficiency, automating certain steps or optimizing labour scheduling could be effective strategies. Additionally, as production scales up, the labour cost per unit of product often decreases due to economies of scale, which suggests that increasing production volume could also help dilute these costs.
Downstream processing is another substantial cost driver, likely due to energy consumption, the use of solvents, and other materials required for purification.

Investigating more cost-effective purification methods, could potentially reduce these expenses. Energy efficiency measures in these stages or transitioning to less expensive or renewable energy sources could also be beneficial.

The contributions of Media Preparation and Fermentation to the overall OPEX are noteworthy as well. For Media Preparation, sourcing cheaper or more locally available raw materials without compromising quality could help reduce costs. Fermentation costs could be driven down by optimizing fermentation conditions to increase yield or by employing more efficient bioreactors that consume less energy and resources

Utility costs, including electricity, steam, chilled water, and cooling water, while less significant than labour and materials, still present opportunities for cost savings. For example, implementing energy recovery systems, such as heat exchangers, could utilize the excess heat generated by the process, thereby reducing the need for additional heating or cooling.

Given that scale-up typically leads to lower fixed costs per unit, expanding the production scale could address some of the economic hotspots identified in this analysis. However, it is crucial to ensure that scale-up efforts do not inadvertently

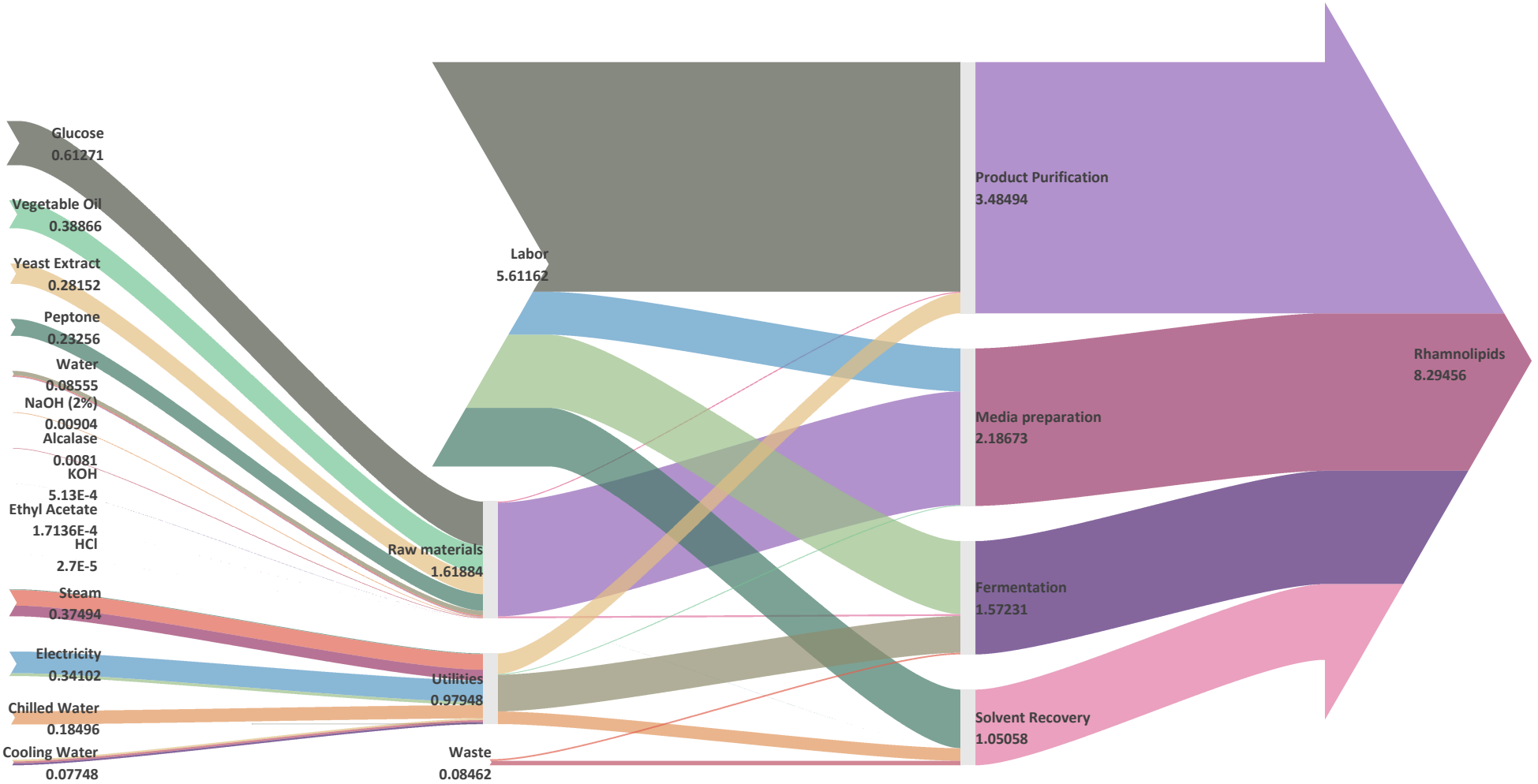

Figure 6: Operational Expenditure (OPEX) Distribution per Kilogram of Rhamnolipids Production [€/kg Rhamnolipids produced]

increase the environmental footprint or lead to diminishing returns in efficiency improvements.

5.3. Identified hotspots and possible strategies to approach each hotspot.

5.3.1. Environmental Hotspots:

1. **Global Warming Potential**

    1. **Hotspot**: Media Preparation is the leading cause of CO_2 equivalent emissions due to the energy and resources required to produce and process raw materials.

    Solutions: Switching to suppliers of raw materials who use sustainable practices can significantly lower emissions [10,13]. Implementing. renewable energy, like solar or wind power, specifically for these energy-intensive steps, and optimizing logistics and manufacturing to be more energy-efficient can further mitigate this impact.

2. **Terrestrial Ecotoxicity**

    1. **Hotspot**: The significant impact is driven by the use of chemicals and agricultural products in Media Preparation.

    2. **Solutions**: Employing alternative materials with a lower ecological footprint or ensuring that current materials are produced and disposed of in a manner that minimizes environmental harm [1,4,10]. Integrating closed-loop systems to recycle waste can also reduce this impact.

3. **Land Use**

    1. **Hotspot**: Dominated by Media Preparation due to the agricultural space required for growing feedstock.

    2. **Solutions**: Using waste materials or by-products as feedstock can reduce the need for dedicated crop land. Also, sourcing from vertical farms or hydroponic systems could diminish the land use per unit of raw material.

4. **Fossil Resource Scarcity**

    1. **Hotspot**: The extraction and processing activities in Media Preparation and Product Purification rely heavily on fossil resources.

    2. **Solutions**: Incorporating bio-based or recycled solvents and materials and considering the lifecycle approach in selecting materials can help conserve fossil resources. Process integration that allows for the reuse of solvents within the process can also reduce demand.

5. **Water Consumption**

    1. **Hotspot**: Fermentation requires significant amounts of water for biological processes and equipment cleaning.

    2. **Solutions**: Water management strategies such as condensate recovery from steam and reusing process water after treatment can drastically reduce the water footprint. Monitoring systems can also optimize water use, ensuring that only the necessary amounts are used.

5.3.2. Economic Hotspots:

1. **Labour Costs**

    1. **Hotspot**: This is the most substantial part of OPEX, due to manual processes or a large workforce.

    2. **Solutions**: Automation of routine tasks and optimization of staffing, such as shift rotations and task management, as well as scale-up of the facilities[27], can make labour use more efficient. Training workers for multiple roles may also improve flexibility and efficiency.

2. **Product purification Costs**

    1. **Hotspot**: Purification and extraction are resource-intensive, involving costly materials and energy.

    2. **Solutions**: Investing in new technologies for purification that are less reliant on expensive chemicals or energy can lower costs. Additionally, improving the yield and purity of the product can reduce the need for repeated processing.

3. **Media Preparation Costs**

    1. **Hotspot**: Raw materials for media preparation are a substantial expense.

    2. **Solutions**: Long-term contracts with suppliers for bulk purchasing, local sourcing, and researching alternative, less expensive inputs can all contribute to reduced costs.

4. **Fermentation Costs**

    1. **Hotspot**: Significant expenses are associated with the energy for maintaining bioreactor conditions and resources.

    2. **Solutions**: Advancing process controls to optimize fermentation conditions, improving yield, and reducing waste. Using adaptive

bioreactor systems that can adjust to varying feedstock qualities can increase efficiency[11,28,29].

5. **Utility Costs**

    1. **Hotspot**: The need for electricity, heating, and cooling is a notable operational cost.

    2. **Solutions**: On-site generation of renewable energy, such as solar panels for electricity or biogas for heating, can reduce utility costs. Implementing energy recovery systems to capture and reuse energy from process streams can also provide significant savings.

6. **Solvent Recovery Costs**

    1. **Hotspot**: Although only a moderate impact on OPEX, improvements in solvent recovery can contribute to both economic and environmental benefits.

    2. **Solutions**: Enhancing recovery rates through better process design and implementing closed-loop systems can minimize solvent losses. Additionally, using solvents that are easier and less costly to recover or recycle can reduce both operational costs and environmental impact [13].

These detailed hotspots and solutions show the interconnectivity between environmental sustainability and economic efficiency. Addressing these areas can help make the production of rhamnolipids more sustainable and cost-effective, especially when combined with a strategy to scale-up operations judiciously. The scale-up must be managed to maintain control over the process and avoid unforeseen increases in costs or environmental burdens. By incrementally increasing production volume, a company can carefully monitor the effects of scale on both OPEX and environmental impact, making adjustments as needed to optimize the balance between the two.

6. Conclusions

The analysis of both environmental and economic aspects of the rhamnolipid production process has revealed a multifaceted view of the operation's sustainability. It's clear that while the production of rhamnolipids holds promise as a bio-based product, there are several hotspots that, if not addressed, could compromise both its ecological benefits and economic viability.

In the environmental dimension, Media Preparation is identified as a principal contributor to the process's global warming potential and terrestrial ecotoxicity. The dependency on energy-intensive operations and substantial agricultural inputs, which often involve the use of fertilizers and pesticides, emphasizes the need for interventions. Adopting renewable energy in this phase and selecting raw materials from less intensive agricultural systems or even from waste streams can significantly mitigate these impacts.

The Downstream processing phase is also pinpointed as a significant environmental pressure point, mainly contributing to the global warming potential through its energy demands and to fossil resource scarcity, likely through the utilization of fossil-fuel-derived solvents. This phase holds potential for improvement, such as optimizing purification techniques to decrease energy consumption or innovating towards bio-based or recycled solvents to alleviate the dependence on fossil resources.

Economically, the analysis sheds light on labour as the predominant operating cost, representing around 67% of the overall operational cost. This underscores the potential for substantial gains scale-up and through the automation of certain production steps, thus reducing reliance on manual labour. Enhanced training for workforce optimization and the introduction of more efficient workflow management systems could also optimize labour costs.

Furthermore, energy and material costs in the Downstream processing emerge as a significant economic burden. Here, the focus on process improvements such as the employment of energy-efficient machinery, the introduction of solvent recovery systems, and the enhancement of the overall yield and purity of rhamnolipids can drive considerable financial benefits. In the realm of utility costs, though less significant, the implementation of measures such as energy recovery systems, particularly for heat, and the adoption of renewable energy sources for steam generation could offer notable savings.

The concept of scaling up production is recurrently identified as a solution for both the environmental and economic hotspots. An increase in production volume could reduce the environmental impact per unit by spreading the fixed costs over a larger output and achieving more efficient resource utilization, provided that the scalability is carefully managed to avoid increased waste or inefficiency.

In conclusion, this analysis highlights the intricacy of balancing environmental sustainability with economic efficiency. For the rhamnolipid production to be sustainable in the long term, it's crucial to embrace innovation and efficiency at all stages of production. By adopting a holistic approach that considers both the environmental and economic impacts of each step of the production process, SECRETed production processes could move towards a more sustainable and profitable future.

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