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Research Paper

# Process modeling, environmental and economic sustainability of the valorization of whey and eucalyptus residues for resveratrol biosynthesis

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ABSTRACT

Biomass is one of the renewable resources with the greatest potential, not only because of the possibility of energy recovery but also because of its content in components of interest. In this context, the regions of Galicia and Portugal have large areas of land dedicated to forestry, agriculture and livestock, and the large amount of waste generated represents a cost for the producer. The importance of these facts has aroused great interest in society to focus its interest on improving the current situation while seeking a benefit, both environmental and economic, from existing resources. That is why the integration of biotechnological processes and biorefinery for their valorization are considered key aspects in the way of producing bioproducts and bioenergy. This research article proposes a process for producing resveratrol from whey from the dairy industry and eucalyptus residues from forestry exploitation. In order to evaluate its suitability, a techno-economic analysis and an environmental assessment have been carried out using the Life Cycle Assessment (LCA) methodology. The results obtained show the potential of these scenarios both from the economic point of view, by obtaining a minimum sale price of resveratrol to ensure the viability of the process below the market average, and from the environmental point of view, being eucalyptus residues those that result in a lower contribution to the environment per unit of resveratrol produced. Future research should focus on increasing the throughput of the production process to increase its profitability and on reducing energy requirements throughout the process, as these have been the main critical points identified. In addition, following the sensitivity assessment, it has been concluded that opting for renewable energy is the most sustainable option.

## 1. Introduction

The annual production of whey in the world amounts to 21,600 thousand tons, since 9 L of whey are obtained per kg of cheese produced. 66,990 tons of cheese were produced in Portugal on 2022, according to the European database (EUROSTAT), a value that has been increased since 2017 (61,950) and it is expected to be higher in the coming years. And this amount of production has ended up on 18.7 M kg of cheese whey produced in Portugal in 2020, according to TRIDGE database. This stream presents a characteristic high concentration of lactose (Arias et al., 2021; Carvalho et al., 2021; Lappa et al., 2019; Lopes et al., 2019). Its biological oxygen demand (BOD) content, around 1200–1800 mg/L, could result in significant impacts on aquatic ecosystems due to eutrophication caused by the direct discharge of the untreated stream (Cruz-Salomón et al., 2020; Zandona et al., 2021). In fact, it has been reported that the detrimental consequences of poorly treated cheese whey could imply a pollution capacity of more than 100 times greater than that caused by domestic wastewater (Rico et al., 2015). The first and most direct approach to the management of this stream is the recovery of its protein content (about 7 g / L), based on its fractionation through a series of ultrafiltration units (Buchanan et al., 2023; Yadav et al., 2014). However, as the lactose content is much higher (around 45 g/L), additional valorization alternatives to protein recovery can be proposed, such as the approach to their management as a source of fermentable sugars for the fermentation process. This fraction can be used to produce compounds with high added value, such as bioactive materials, bioplastics, biofuels and/or enzymes, among others (Buchanan et al., 2023; Guimarães et al., 2010; Nayak and Pal, 2013; Zhou et al., 2019).

Regarding forest residues, about 46 % of the total forest biomass is

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**Abbreviation**

| | |
|---|---|
| BC | Base case |
| CW | Cheese whey |
| ER | Eucalyptus residues |
| FE | Freshwater eutrophication |
| FET | Freshwater ecotoxicity |
| FRS | Fossil resource scarcity |
| GW | Global warming |
| HCT | Human carcinogenic toxicity |
| HNCT | Human non-carcinogenic toxicity |
| LU | Land use |
| ME | Marine eutrophication |
| MET | Marine ecotoxicity |
| MSP | Minimum selling price |
| RS | Renewable sources |
| SOD | Stratospheric ozone depletion |
| TA | Terrestrial acidification |
| TET | Terrestrial ecotoxicity |

managed as waste, representing 37.7 $m^3$ per hectare (Edzik et al., 2022; Tripathi et al., 2019). According to the *Instituto de Conservaçao da Natureza e das Florestas* the total amount of *Eucalyptus Globulus* harvested in the last campaigns amounts to 21,712,521, which corresponds, considering also the previous harvesting areas, 672,000 ha of land (Domingues et al., 2010) This represents about 47 % of the total *Eucalyptus Globulus* land harvested in the world (Teixeira et al., 2017). This huge land occupied by Eucalyptus, and its subsequent management to produce high added value products in the market, ends up in a high level of residues produced. The amount of residues coming from Eucalyptus harvesting and processing amounts to 0.7–4.3 Mg/ha/year (0.77–4,74 ton/ha/year), which appears as a significant management problem because, if left on the forest land (unmanaged), it could increase fire ignition and damage potential (Malico and Gonçalves, 2021). The most common practice for waste management is energy recovery, although its use has been considered for the production of particleboard, which would be produced from 50 % virgin material and 50 % residual material, complying with the requirements established in the EN 312 standard (Devasahayam et al., 2022; Iwakiri et al., 2017).

However, given the huge amount of wood products produced, new valorization strategies are required to valorize the volume of annual forest residues. Some studies have evaluated their valorization through biofuel production, although improvements are needed to reduce process costs, especially with regard to the pre-treatment of feedstocks (Goswami et al., 2023; Karthik et al., 2023; Romaní et al., 2019, 2016, 2014), others have relied on the fermentation process to produce bioactive compounds (Arias et al., 2023; Gaspar et al., 2019; Tavares et al., 2020) or even left directly on site in the forest area to favor the maintenance of soil fertility (Pergola et al., 2022).

Therefore, this research report proposes the use of these two residues, cheese whey and eucalyptus residues, for the development of a biotechnological process to produce resveratrol, an antioxidant with interesting biological properties for the food, pharmaceutical, and cosmetic sectors. It is a polyphenol with two phenolic rings linked together by an ethylene bridge (Gambini et al., 2015; Salehi et al., 2018). Briefly, microbial production of resveratrol is enabled by employing genetic engineering strategies to a given microorganism to express heterologous plant genes. Previously, a set of recombinant Saccharomyces cerevisiae strains was engineered to produce resveratrol from different carbon sources such as glucose (Costa et al., 2021), lactose (Costa et al., 2022) and xylose (Costa et al., 2022), which enabled the production of resveratrol using different agro-industrial wastes, such as eucalyptus wood and cheese whey (Fig. 1**A).** Resveratrol's market value is high, given its properties and multiple applications, so it is expected that the process of obtaining the valorization of these flows will be economically viable. To verify this, a large-scale techno-economic analysis of the process will be performed, through process modeling using the SuperPro Designer tool. In addition, to assess its environmental quality, a life cycle assessment (LCA) is also developed in this report, to obtain the environmental profile as well, as well as the contribution of each of the elements of the process to the environment.

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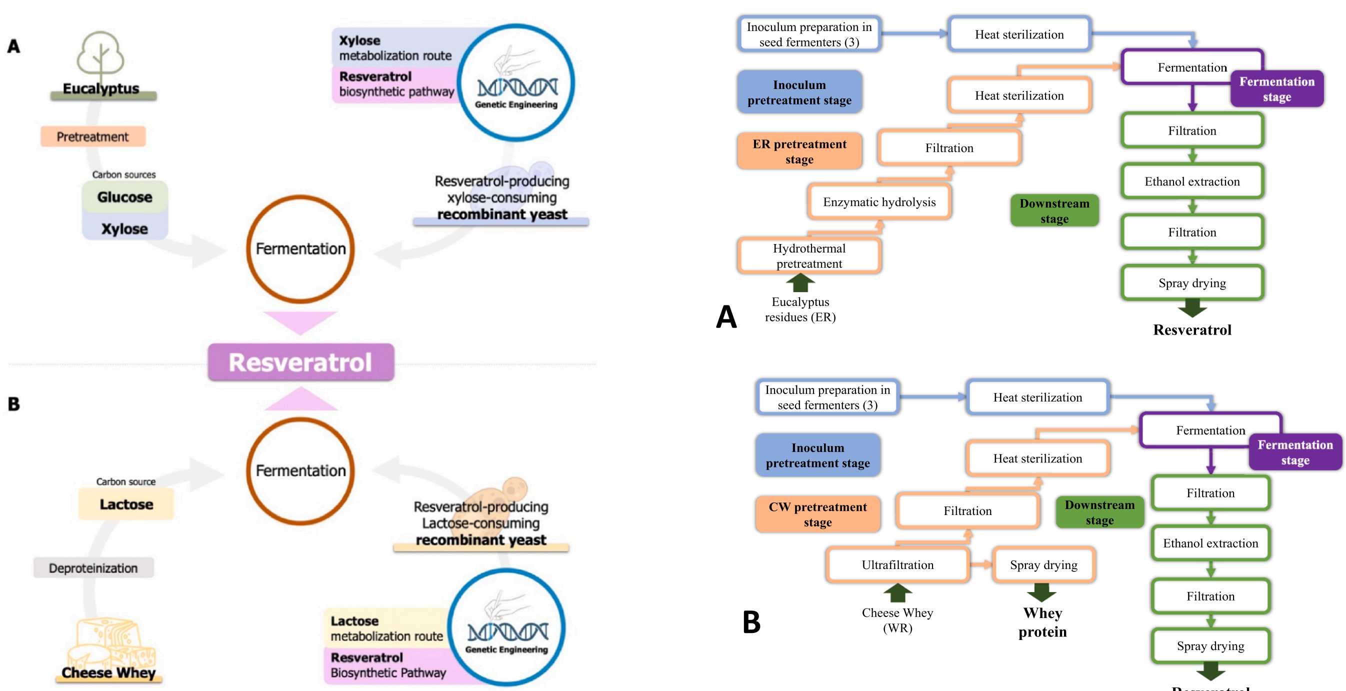


**Fig. 1.** Resveratrol production pathway (left figure) and process flow diagrams for eucalyptus residues (A) and cheese whey (B) valorization for resveratrol production (right figure).

applications, so it is expected that the process of obtaining the valorization of these flows will be economically viable. To verify this, a large-scale techno-economic analysis of the process will be performed, through process modeling (Costa et al., 2021, 2022) using the SuperPro Designer tool. In addition, to assess its environmental quality, a life cycle assessment (LCA) is also developed in this report, to obtain the environmental profile as well, as well as the contribution of each of the elements of the process to the environment.

## 2. Process description and modelling

To have a previous flow diagram about the process scheme, Fig. 1A and Figure 1**B** are included, in which the main stages are being defined for both scenarios. Process modeling has been based on considering a production capacity of 100 ton/batch of residual input material, given the values of the waste produced annually, its availability is guaranteed to consider this input production capacity. On the other hand, to consider the yields, operating conditions, chemicals needed and process efficiency in the production of resveratrol using these residues, the results of previous reports have been taken into account (Costa et al., 2022, 2021).

A summary of the process parameters and variables characterized by large-scale modeling within an input stream of 100 tons/batch from each of the side streams: ER (eucalyptus residues) and CW (cheese whey) is represented in Table 1. The characteristic details of each stage of the process are described below depending on the processed stream, considering that in the case of CW a first pretreatment is required for the recovery of the protein content, while for the ER, the first step is based on a release of fermentable sugars through a hydrothermal treatment followed by a simultaneous step of saccharification and fermentation (SSF). It is worth mentioning that both cellulose and hemicellulose fractions are considered sources of glucose and xylose, which will be used simultaneously in the fermentation process for the production of resveratrol.

The ER requires a pretreatment stage based on a hydrothermal pretreatment (considering a liquid–solid ratio of 8 kg water/kg ER) that takes place in a plug flow reactor (PFR-101, Fig. 1**SM**). Subsequently, a filtration stage is required for the separation of the solid and liquid fractions (BF-101**,** Fig. 1**SM**). This stage is followed by a simultaneous enzymatic and acidic saccharification (R-101, Fig. 1**SM**) (using Cellic CTec 2 as enzyme, 626 U/mL, 24 h, 45 °C and an acidic medium with sulfuric acid). The development of these processes allows a greater release of fermentable sugars, and therefore an improvement in the performance of the process. After the pretreatment stage, the process fermenter (FR-101, Fig. 1**SM**) operates in batch regime with 5 % solids, since this is the amount that gives the highest efficiency of the process, room temperature, constant agitation and 96 h. After the fermentation section, the fermentation broth is filtered to remove the biomass (RVF-102, Fig. 1**SM**). In addition, to purify resveratrol, ethanol is used as an extracting agent (V-103, Fig. 1**SM**). After separation of the remaining components present in the product (S-134, Fig. 1**SM**), the stream is treated in a spray drying stage (SDR-101, Fig. 1**SM**) to obtain resveratrol at 95 % purity.

For the case of CW (Fig. 2**SM**), an analogous process scheme is proposed, considering a fermentation time of 57 h and a lactose concentration in the fermentation medium of 100 g/L, with a difference in the pretreatment stage. In this case, the cheese whey is partially deproteinized in an incubation stage at 90 °C for 15 min (V-101, Fig. 2**SM**). This is an important pretreatment steam of CW valorization as a source of fermentable sugars since the protein content in the fermentation medium could imply a significant decrease in resveratrol production yield. In addition, the use of a centrifuge can accomplish protein separation (DC-101, Fig. 2**SM)** to perform the subsequent fermentation, for which a previous pasteurization stage (PZ-101, Fig. 2**SM**) at 60 °C for 1 h is required.

**Table 1**
Production capacity of the different scenarios assessed.

| Process | ER | CW |
|---|---|---|
| Batch time (h) | 99.25 | 60.25 |
| Number of batches per year | 79 | 131 |
| Batch size (kg resveratrol) | 183.19 | 16.87 |
| Resveratrol production (kg/year) | 14,163.85 | 2,210.50 |

## 3. Environmental assessment following the LCA methodology

The international standards ISO 14040 and ISO 14044 (European Commission, 2010; ISO, 2009) have been used as a guide for the development of the life cycle assessment, based on the definition and analysis of four methodological stages. For the first stage, the definition of the goal and scope of the system under study is required. In this case, the assessment of the environmental profile and potential impacts related to the valorization of ER and CW for the production of resveratrol. Regarding the scope, a "cradle-to-gate" approach has been chosen, covering all the stages from the extraction of raw materials to the production of the final product(s) (Arias et al., 2020). Besides, regarding the functional unit, as in the CW valorization scenario two products are obtained, protein concentrate and resveratrol, one production batch has been selected, in order to properly compare both scenarios.

Regarding the second stage of the methodology, it focuses on the definition and development of life cycle inventories, which include all the data related to the process scheme under evaluation. SuperPro modeling, together with the bibliographic values used for the estimation of large-scale reaction yields, has been used as the basis for acquiring all the necessary data, both materials, energy, waste streams and emissions. It should be noted that the data included in the inventories are compiled on the basis of the selected functional unit.

The development of the environmental assessment is the third step of the methodology, based on the evaluation of the inventory data using characterization factors for each impact category. For this study, two methodologies have been considered: ReCiPe MidPoint (Huijbregts et al., 2017), based on 18 impact categories of which 12 have been selected, as they are considered as the most significant for the environmental assessment, and ReCiPe EndPoint, which provides 3 damage categories integrated in a single score (Table 1**SM**), which is useful for a global comparison between the two proposed scenarios. The fourth stage is the interpretation of the results obtained, as well as the identification of the main hotspots of the environmental profile, the main contributors. In addition, it is at this stage where sensitivity evaluations are also developed, with the objective of providing an improvement scenario with reduced environmental loads.

## 4. Techno-economic analysis

The economic feasibility of the valorization of ER and CW for resveratrol production has been evaluated through the development of economic analyses considering the associated costs of equipment purchase, materials, labor and utilities, in order to acquire the expected total direct and indirect costs of the plant, the variable costs associated with the annual production and the expected revenues obtained from the sale of resveratrol and/or other co-products obtained, such as protein concentrate in the case of CW. In addition, in order to compare the scenarios under evaluation, the minimum selling price of resveratrol has been estimated, according to the unit production costs obtained, which takes into account all the related monetary expenses of the whole process scheme.

To calculate equipment purchase costs, published equations based on actual volume and major equipment characteristics have been considered (Smith, 2005; Towler and Sinnott, 2021), In addition, as these tabulated values to be used in the equations are not updated to the year of evaluation, 2023, the CEPCI (Chemical Engineering Plant Cost Index) indexes have been taken into account to update the costs to the present date. In addition, some other assumptions and considerations have been evaluated for the development of the techno-economic evaluation: a period for construction of 30 months and 4 months for start-up, a useful life of the project of 30 years, an inflation of 4 % and an income tax of 25

%. With respect to the operating capacity of the facility, it operates 11 months per year, using 1 month for the maintenance of the facilities.

## 5. Results

### 5.1. Process modelling

SuperPro Designer modelling has been used for obtaining the equipment characteristics of the entire process scheme, considering a production capacity of 100 ton/batch of input residual material. To this end, the most important equipment characteristics are shown in Table 2SM, for the case of using ER as residual input and when using CW as feedstock. As previously mentioned, the ER require a more complex pretreatment than in the case of CW. In fact, the pretreatment for the cheese whey is developed with the aim of co-producing WPC that could be sold, thus increasing the functionality and potentiality of the proposed biotechnological procedure. Nevertheless, this required pretreatment for the ER implies a certain benefit when comparing the two production schemes proposed, as it is with this feedstock with which a higher amount of resveratrol is produced per batch process, as it has been shown in Table 2SM

Just to remark, the main differences between the two scenarios are that, firstly, the pretreatment stage, while in the case of the eucalyptus residues a more intensive stage is required for the release of the fermentable sugars, including hydrothermal and enzymatic hydrolysis, for the cheese whey, the process is simpler, only requiring the filtration steps for the separation of the protein and the concentration of the sugars for the subsequent fermentation step. Secondly, in the case of the downstream stage, while for the purification of the resveratrol the steps required are the same, in the case of the eucalypts residues an additional step is included, the anaerobic digestion, aiming to recover the energetic content from the lignin available on the eucalyptus residues. This anaerobic digestion has been modelled in SuperPro Designer tool, considering the equation provided by Achinas et al. (2016) and considering as conversion efficiency the one determined in the research done by Klassen et al. (2017), 88 %, as lignin is considered an appropriate substrate for energy valorization (Achinas et al., 2016; Klassen et al., 2017). Besides, in order to evaluate the input and output flows on the anaerobic digestion unit, mass and energy balance has been developed, estimating as theoretical biogas composition 25.7 % of methane, 63.9 % of carbon dioxide and 10.41 % of ammonia, as main compounds. Regarding the operational conditions, atmospheric pressure, and a temperature of 303.15 K have been considered, with a heat efficiency of 45 %, a value selected based on the research article developed by Mortier et al. (2016) (Mortier et al., 2016).

### 5.2. Environmental evaluation

#### 5.2.1. S01-Eucalyptus residues

The life cycle inventory of the scenario for the ER valorization, S01-ER, is included in Table 3**SM**. The large-scale modeling tool in SuperPro Designer has been used to estimate all data related to mass and energy balances, including emissions and waste streams.

Once the LCI has been developed, the ReCiPe Midpoint methodology has been used to obtain the environmental profile of S01-ER, depicted in Fig. 2**A**. As can be seen, two main contributors could be identified, with steam carrying the largest environmental load in the highest impact categories evaluated. The reason behind this huge impact is the result of the amount of steam required per procedure per batch (around $8.18\ 10^5$ MJ/batch), which is produced from fossil energy sources. To decrease the steam requirements, the lignin content of ER and the biomass produced within the process are valorized through anaerobic digestion, which allows to produce $3.25{\cdot}10^5$ MJ steam/batch. This recovery will imply about 40 % reduction in external steam. Electricity requirements also have some contribution to the toxicity-related impact categories, namely GW, TET and FRS, while the use of enzymes for the pretreatment section has certainly a significant burden on the ME, LU and SOD impact categories. With respect to electricity, its production from fossil resources is the main reason for its contribution, while the related background activities required for their production of enzymes (mainly energy requirements) are the reason behind their environmental burdens on the above-mentioned impact categories.

#### 5.2.2. S02 - Cheese whey

The life cycle inventory data used of S02- CW is included in Table 4SM, considering as a functional unit a complete batch process in which not only resveratrol is produced, but also protein concentrate in the form of 57 % w/w powder.

The environmental profile of the valorization of CW is depicted in Fig. 2**B.** The energy requirements of the process are the major contributors in most of the impact categories, with steam being the main hotspot, contributing more than 50 % of the profile for the categories related to GW, ecotoxicities, FRS and human health-related toxicities. On the other hand, as previously mentioned, the impact derived from whey is important given its organic matter content, promoting a significant contribution in the eutrophication category, EM. In addition, since whey is a co-product with commercial value, the associated production process presents an associated impact, which also implies having certain significant impacts in the SOD, LU and MRS categories. On the other hand, as far as electricity is concerned, its contribution is lower compared to the previous process. The reason for this fact has a double justification: (1) the pretreatment stage with a lower number of equipment and (2) the implementation of a pasteurization process using steam.

For this reason, the sensitivity analyses to be developed subsequently should focus on the reduction of steam consumption, or on replacing fossil resources to obtain it, as well as on improving the productive yield of the process, in order to obtain a greater volume of products per unit of raw material that enters the production system.

### 5.3. Sensitivity assessments for reducing the environmental loads

In order to improve the environmental impact associated with the scenarios evaluated, sensitivity analyses have been carried out in relation to the main critical points identified in the previous sections. Accordingly, the sensitivity analyses to be developed subsequently should focus on the reduction of steam consumption, or on the substitution of fossil resources to obtain steam, as well as on improving the productive yield of the process.

#### 5.3.1. S01 - Eucalyptus residues

The main hotspots identified for S01-ER have been steam requirements in the first place, followed by cheese whey in some impact categories, such as ME, LU and SOD, and almost followed by electricity requirements. Since major contributors on the environmental profile are detected, a sensitivity assessment has been proposed with the objective of providing improvement scenarios with reduced environmental loads.

In the case of steam requirements, the first scenario has been based on the valorization of the lignin and the biomass from the fermentation stage. This valorization is developed through the inclusion of an anaerobic digestion unit, which allows to obtain $3.25{\cdot}10^5$ MJ steam/batch, which is a significant amount that allows to reduce by almost half the steam requirements based on fossil resources. As can be seen in Fig. 3**A**, this valorization implies a reduction of about 8 % for ecotoxicity categories, 13 % for GW and 26 % for FRS impact category, which is the highest reduction value obtained when compared to the base scenario. The other alternative to be evaluated is the use of steam from renewable resources, thus avoiding the depletion and environmental consequences of fossil fuel use. In this case, the reduction values increase, as expected, with a reduction of about 25 % for the TA, FE and HCT impact categories, 53 % for GW and 61 % for the FRS impact category. On the other hand, it is worth mentioning that the using steam coming from renewable resources could imply a greater impact on other impact categories, such as LU, SOD and HCNT, given the associated production process, which involves more occupied land than the use of fossil sources and certain emissions that could cause non-carcinogenic human health impacts and ozone depletion. However, given the significant benefits achieved by this modification in the production source, these certain increased impacts are certainly offset.

With respect to electricity, a similar approach has been considered, as the use of renewable resources for its production is analyzed. In this regard, significant reduction loads are obtained in all the impact categories when comparing to the base case. The range of improvement goes from an 8 % of reduction of the ME impact category to 60 % on the HCT category, with a 34 % of improvement over the GW, a mean reduction of 38 % over the ecotoxicity-related categories and a 31 % for the case of the fossil resources consumption (FRS). Given these values, it could be also concluded that the replacement of the use of fossil resources by the use of renewable resources implies a substantial improvement in the environmental profile of the process. For this reason, in order to observe the improvement from the point of view of both electricity and heat consumption, we have chosen to show the reduction profile when both utilities are obtained from renewable resources, thus observing the best environmental profile, i.e. the one with the lowest impact, among all those evaluated, with the only exception in the LU category, where the renewable base is expected to involve a greater land occupation. It is thus demonstrated that betting on renewable resources should be considered as a direct practice in the search for a more sustainable production profile.

#### 5.3.2. S02 - Cheese whey

The hotspots for the S02-CW scenario are the electricity requirements and to a lesser extent, the cheese whey. To this end the sensitivity analysis has been based on (1) the reduction on the electricity requirements by 20 %, (2) the use of renewable resources for electricity production, (3) the reduction in the amount of raw material per unit of product produced by 20 % (the fact that it is based on a laboratory scale process actually implies a significant improvement when scaling up to an industrial process capacity) and (4) the combined use of renewable resources for electricity production and the reduction of the cheese whey input flow.

As expected, the use of electricity from renewable resources implies the lowest environmental loads, in all impact categories, with the exception of the TET and LU impact categories, where the scores obtained are not as low compared to the others (Fig. 3**B**). The range of environmental impact reduction ranges from 6 % for the ME category to 84 % for HCT, with respect to the baseline scenario, when renewable resources are used. The percentage decrease is significantly reduced for the case of reducing the amount of electricity requirements by 20 %, going from 1 % to 18 % for the ME and HCT impact categories, respectively. A sensitivity assessment of cheese whey has also been considered, evaluating a reduction of its input by 20 %, based on the fact that a higher productivity and efficiency of the process could be obtained so that a lower amount of cheese whey input will be necessary to obtain the same amount of product as in the base scenario. In this sense, significantly reduced impacts are obtained for the SOD, ME and LU impact categories, for which a reduction of 11 %, 13 % and 17 %, respectively, is achieved.

When considering all the improved sensitivity scenarios, an optimized one has been evaluated, for which impact reductions are obtained in a range from 10 % for the FRS impact category to 30 % for the impact categories related to human toxicity, namely HCT and HNCT. The latter sensitivity scenario is the one with the best environmental profile.

### 5.4. Techno-economic analysis

The techno-economic scores obtained for the two proposed valorization scenarios are represented in Table 2, including equipment costs, fixed capital, variable operating costs, expected revenues and estimated minimum sales prices of the main product to guarantee the viability of the process. As can be seen, the total capital investment of the eucalyptus waste valorization scenario is almost double compared to that of cheese whey. The rationale behind this is mostly given the higher amount of equipment required for the pretreatment section, which increases the total equipment costs, having an important effect over the total capital costs. Besides, the need to include high temperature and pressure operating conditions, such as the case of hydrothermal pretreatment of eucalyptus residues prior to their enzymatic and acid hydrolysis, implies a higher need for labor costs, that is almost two times higher for the eucalyptus residues in comparison to the score obtained for the cheese whey valorization scheme. On the other hand, with respect to revenues, just as with eucalyptus residues a higher amount of resveratrol is obtained per process per batch, a higher profit is obtained when compared to the cheese whey scenario, whose revenues from the sale of both protein concentrate and resveratrol produced are almost ten times lower. These facts imply that the minimum selling price of resveratrol by using CW as raw material is more than twice that of the ER valorization scheme, so as to categorize the scenario as feasible in economic terms.

**Table 2**
Economic parameters obtained by performing the economic evaluation of the biotechnological production process of resveratrol using ER and CW as substrates.

| Economic Parameters | S01-ER | S02-CW |
|---|---|---|
| 1. Total Plant Direct Cost (TDC) [€] | 21,923,000 | 12,984,000 |
| 2. Total Plant Indirect Cost (TIC) [€] | 12,058,000 | 7,141,000 |
| *Fixed Capital (FC = TDC + TIC) [€]* | *33,981,000* | *20,135,000* |
| Equipment cost (EC) [€] | 8,780,000 | 5,220,000 |
| *Total Investment (EC + FC) [€]* | *42,761,000* | *25,355,000* |
| Labor Cost [€/year] | 1,154,533 | 787,229 |
| Material Cost [€/year] | 74,435 | 22,661 |
| Utilities Cost [€/year] | 1,967,994 | 275,094 |
| Annual Operation Cost [€/year] | 7,886,000 | 3,862,000 |
| Revenues [€/year] | 13,363,691 | 2,263,660 |
| MSP [€/kg resveratrol] | 531.12 | 1,747.22 |

On the other hand, when comparing the minimum selling prices obtained with the actual selling prices of resveratrol in the market according to values reported by BOCSCI Inc., BULK and Sigma-Aldrich, the selling price ranges from 750 €/kg to 4,677 €/kg. Taking into account the average value between those (2713 €/kg), the estimates for both valorization scenarios are clearly below, which allows forecasting their economic viability.

### 5.5. Comparison between scenarios

Besides the techno-economic analysis, also a comparison has been developed considering the environmental loads and scores obtained for each process alternative, considering both MidPoint and EndPoint calculation methodologies. It is worth mentioning that for this

**Table 3**
Environmental parameters obtained by performing the ReCiPe MidPoint methodology of the biotechnological production process of resveratrol using ER and CW as substrates, allocated to kg of resveratrol produced.

| Impact category | Unit | S01-ER | S02-CW |
|---|---|---|---|
| GW | kg $CO_2$ eq | $7.95{\cdot}10^{2}$ | **$1.89{\cdot}10^{3}$** |
| SOD | kg $CFC_{11}$ eq | $4.58{\cdot}10^{-4}$ | **$1.51{\cdot}10^{-3}$** |
| TA | kg $SO_2$ eq | 2.93 | **5.63** |
| FE | kg P eq | 0.19 | **0.31** |
| ME | kg N eq | $7.55{\cdot}10^{-2}$ | **0.22** |
| TET | kg 1.4-DCB | $9.74{\cdot}10^{2}$ | **$2.49{\cdot}10^{3}$** |
| FET | kg 1.4-DCB | 6.42 | **8.78** |
| MET | kg 1.4-DCB | 7.77 | **12.24** |
| HCT | kg 1.4-DCB | 10.54 | **16.67** |
| HNCT | kg 1.4-DCB | $3.09{\cdot}10^{2}$ | **$4.17{\cdot}10^{2}$** |
| LU | $m^2$a crop eq | 70.20 | **$7.58{\cdot}10^{2}$** |
| FRS | kg oil eq | $2.48{\cdot}10^{2}$ | **$5.94{\cdot}10^{2}$** |

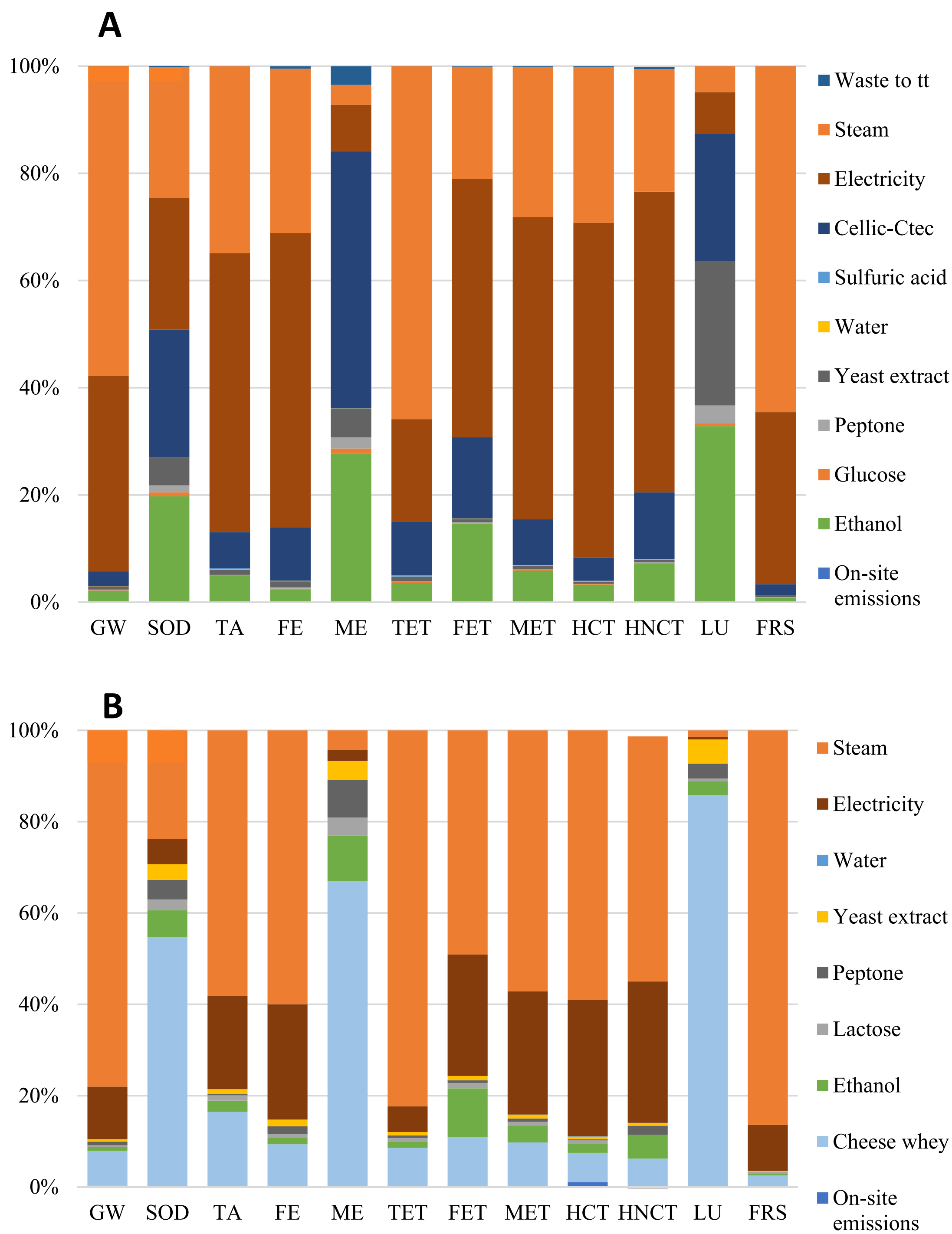


**Fig. 2.** Environmental profile of resveratrol production using ER as substrate (A) and cheese whey as substrate (B).

comparison several functional units have been considered, in order to be aware of the potential environmental impacts depending on the (1) complete batch process, (2) amount of products obtained per process and (3) total resveratrol obtained per batch process.

Table 3 show the environmental scores of the base case scenarios considering the entire batch process as a functional unit with a raw material input of 100 tons/batch. The alternative leading to the highest environmental load for each of the assessed impact categories is highlighted in bold. In this first approximation, for all categories, the cheese whey valorization scenario leads to the highest environmental loads, given the lower resveratrol production by batch process, compared to the eucalyptus waste valorization scheme. It is thus demonstrated that the pre-treatment stage of the eucalyptus waste does not have a major impact on the environmental loads obtained, thus making this valorization scenario the best alternative from an environmental point of view.

Given the results obtained, it has been considered to evaluate the environmental loads by modifying the functional unit, considering the comparison between both recovery scenarios considering the amount of resveratrol produced by batch process (Fig. 4**(A)**). As excepted, a similar trend is obtained, given the significant contribution of the resveratrol production capacity on the profile. For all impact categories, CW still has a higher impact, with the SOD, ME, TET, LU and FRS categories showing a large difference, with impacts increasing by more than 60 % compared to the ER scenario. The only categories where impact loads are closer are FET and HCNT, with a difference of -around 30 % between the scenarios, but still a significant variation.

On the other hand, it should be noted that the cheese whey valorization process also provides another product. Fig. 4**(B)** includes the comparison evaluating the process scenarios considering as functional unit the total products obtained, only resveratrol in the case of ER valorization and resveratrol and protein for the CW scenario. As the

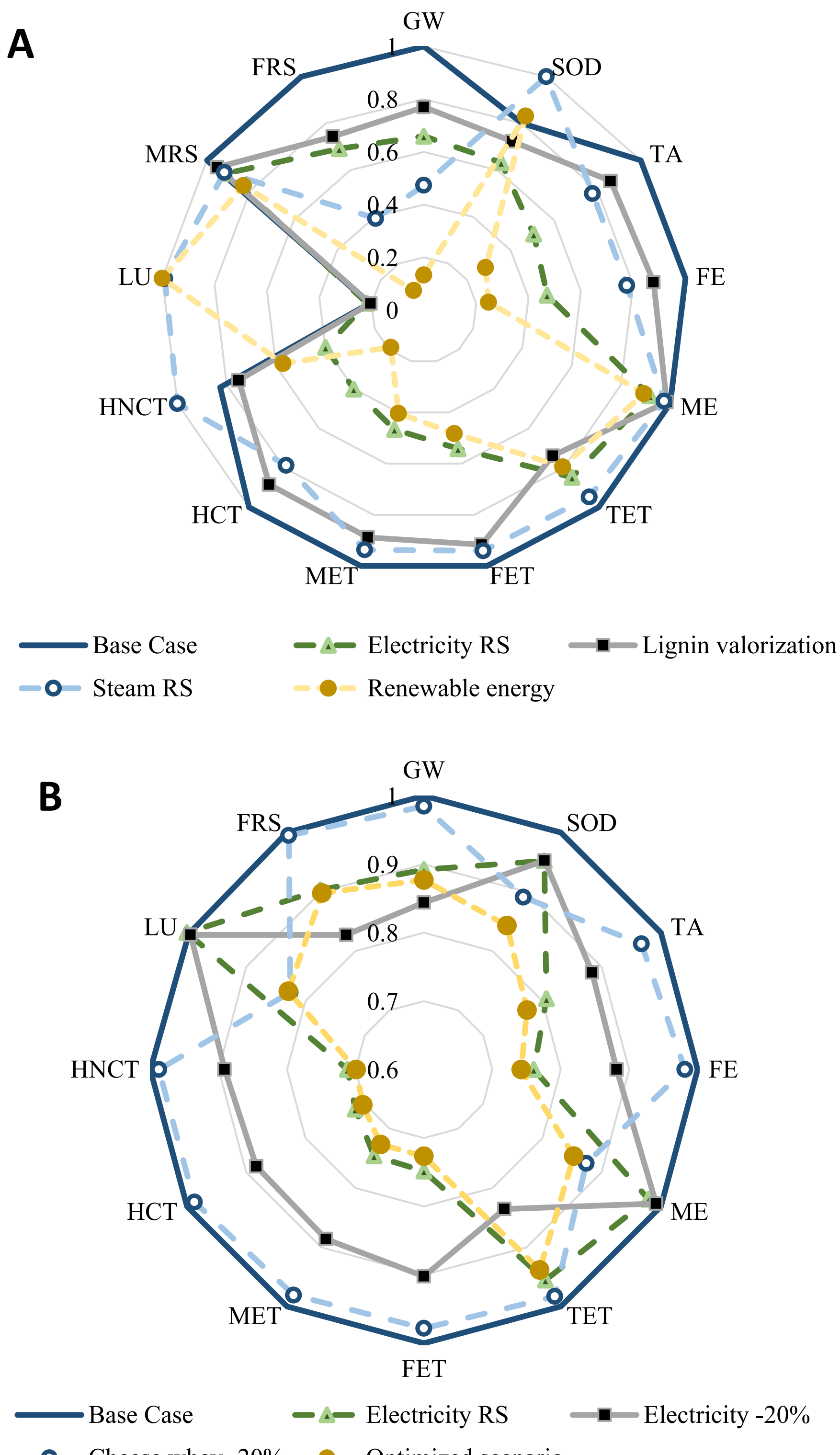


**Fig. 3.** Sensitivity assessment of the environmental profile of ER valorization for resveratrol production considering the use of renewable resources for the production of electricity and steam (A) and sensitivity assessment of the environmental profile of CW valorization for resveratrol production considering a reduction in the use of electricity by 20%, the use of renewable resources for electricity production, and on cheese whey input by 20% (B).

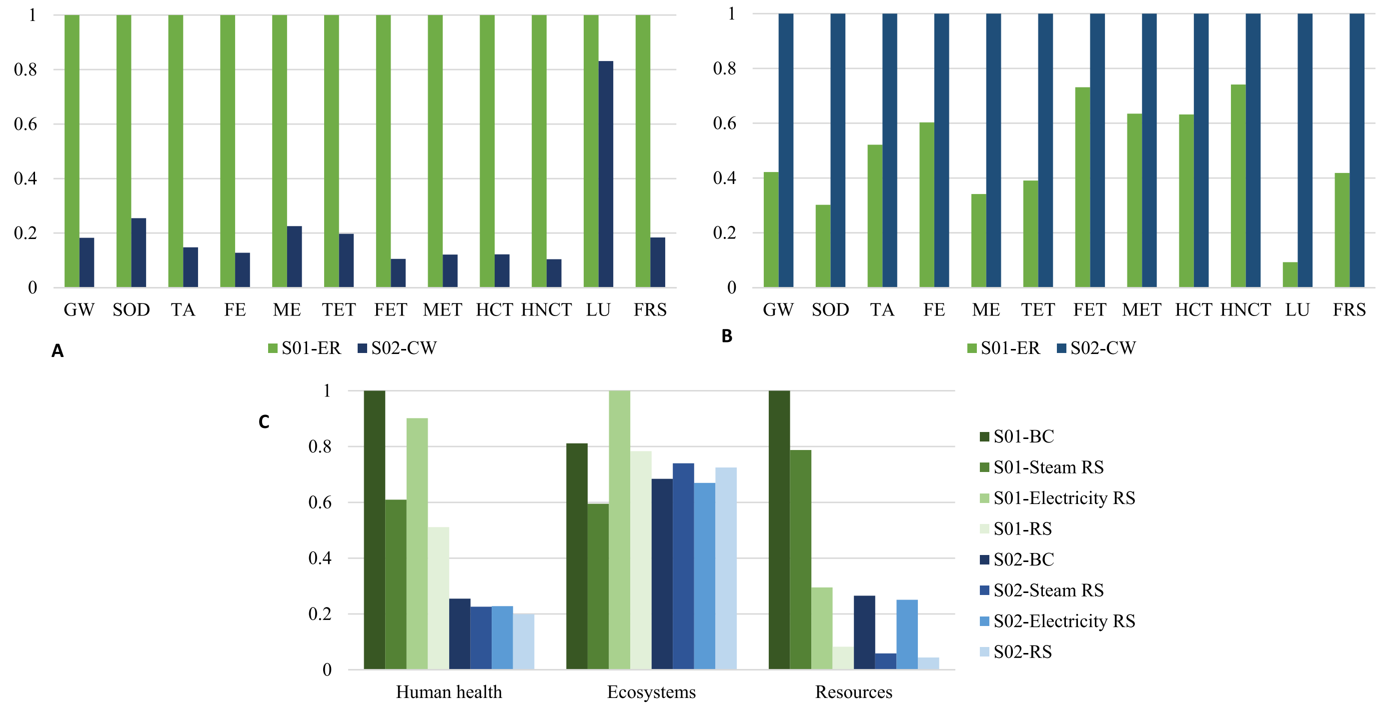


**Fig. 4.** Comparison of the environmental loads of the assessed scenarios using the ReCiPe MidPoint methodology (A) per batch (B) per kg of resveratrol and (C) comparing alternative scenarios.

amount of protein achieved within the pre-treatment stage of cheese whey valorization is very high, the environmental loads will be reduced per kg of production obtained. As it could be observed that this fact affects significantly on the comparison in the scenarios, as for this case, it is the eucalyptus waste that carries the highest environmental loads.

This assessment has demonstrated the importance on the selection of the functional unit for evaluating the environmental profiles of the alternatives under evaluation. For these case scenarios, as various products are obtained, considering resveratrol production as functional unit, or the amount of products obtained per batch process as functional unit, as previously assessed, are the best options for assessing the environmental profiles, in order to be accurate on the comparison of the scenarios.

Finally, the ReCiPe Endpoint methodology has also been used to compare the two alternatives, providing 3 damage categories (human health, resources and ecosystems), which allows a global view of the impacts related to each scenario. To this end, Fig. 4**(C)** provides the endpoint results obtained for the eucalyptus (S0 1) and cheese whey (S02) waste valorization scenarios. As could be seen, the base scenario (BC) for both alternatives leads to the highest impact on the damage categories, with the variation being more significant with the optimized alternatives for S0 1-ER valorization than for S02-CW.

Furthermore, as identified for the mid-point characterization model, the fact that renewable resources are used to produce energy requirements, both steam and electricity, implies an improvement on the profile, with impact reductions in the range of 10–50 % for the human health damage category, 18 %-40 % for ecosystems one and 21 % to 85 % for the resource category in the case of S01 -ER valorization. In the case of cheese whey (S02), the variations between the base case and the optimized scenarios are not as significant in the case of the human health and ecosystem damage categories, while for resources the variations are significant, demonstrating once again that renewability in the use of energy resources is the most appropriate improvement.

## 6. Conclusions

This study has considered the biotechnological valorization of eucalyptus and cheese whey residues for the production of resveratrol, and, in the case of cheese whey, a protein concentrate is also obtained. Process modeling has been used to propose a large-scale production model of the two alternatives for techno-economic and environmental evaluation. In this regard, it has been shown that both scenarios are feasible in economic terms, since the minimum selling price obtained for the products is lower than the real average market price. However, energy requirements are the hot spots of the environmental profiles of both alternatives. Future research should encourage the use of renewable energy sources and also the improvement of process performance and yield. When comparing the two alternative scenarios, it could be concluded that the use of cheese whey as a raw material leads to reduced environmental burdens and increased profits. However, it should be noted that a lower amount of resveratrol is obtained compared to the valorization of eucalyptus residues. Therefore, both options have advantages and disadvantages that must be considered in the decision-making process.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.wasman.2023.10.030.

## References


Achinas, S., Euverink, W., Jan, G., 2016. Theoretical analysis of biogas potential prediction from agricultural waste. Resour.-Effic. Technol. 143–147.

Arias, A., González-García, S., González-Rodríguez, S., Feijoo, G., Moreira, M.T., 2020. Cradle-to-gate Life Cycle Assessment of bio-adhesives for the wood panel industry. A comparison with petrochemical alternatives. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2020.140357.

Arias, A., Feijoo, G., Moreira, M.T., 2021. Process and environmental simulation in the validation of the biotechnological production of nisin from waste. Biochem. Eng. J. 174, 108105 https://doi.org/10.1016/J.BEJ.2021.108105.

Arias, A., Feijoo, G., Moreira, M.T., 2023. Process modelling and environmental assessment on the valorization of lignocellulosic waste to antimicrobials. Food Bioprod. Process. 137, 113–123. https://doi.org/10.1016/J.FBP.2022.11.008.

Buchanan, D., Martindale, W., Romeih, E., Hebishy, E., 2023. Recent advances in whey processing and valorisation: Technological and environmental perspectives. Int. J. Dairy Technol. https://doi.org/10.1111/1471-0307.12935.

Carvalho, P., Costa, C.E., Baptista, S.L., Domingues, L., 2021. Yeast cell factories for sustainable whey-to-ethanol valorisation towards a circular economy. Biofuel Res. J. 8 https://doi.org/10.18331/BRJ2021.8.4.4.

European Commission, 2010. ILCD Handbook : Specific guide for Life Cycle Inventory data sets. EUR 24709 EN. Eur 24709 En.

Costa, C.E., Møller-Hansen, I., Romaní, A., Teixeira, J.A., Borodina, I., Domingues, L., 2021. Resveratrol Production from Hydrothermally Pretreated Eucalyptus Wood Using Recombinant Industrial Saccharomyces cerevisiae Strains. ACS Synth. Biol. 10, 1895–1903. https://doi.org/10.1021/ACSSYNBIO.1C00120.

Costa, C.E., Romaní, A., Teixeira, J.A., Domingues, L., 2022. Resveratrol production for the valorisation of lactose-rich wastes by engineered industrial Saccharomyces cerevisiae. Bioresour. Technol. 359, 127463 https://doi.org/10.1016/J.BIORTECH.2022.127463.

Cruz-Salomón, A., Ríos-Valdovinos, E., Pola-Albores, F., Lagunas-Rivera, S., Cruz-Rodríguez, R.I., Cruz-Salomón, K. del C., Hernández-Méndez, J.M.E., Domínguez-Espinosa, M.E., 2020. Treatment of cheese whey wastewater using an expanded granular sludge bed (EGSB) bioreactor with biomethane production. Processes 8. https://doi.org/10.3390/PR8080931.

Devasahayam, S., Dyer, L., Wronka, A., Kowaluk, G., 2022. Upcycling Different Particle Sizes and Contents of Pine Branches into Particleboard. Polym. 2022, Vol. 14, Page 4559 14, 4559. https://doi.org/10.3390/POLYM14214559.

Domingues, R.M.A., Sousa, G.D.A., Freire, C.S.R., Silvestre, A.J.D., Neto, C.P., 2010. Eucalyptus globulus biomass residues from pulping industry as a source of high value triterpenic compounds. Ind. Crop. Prod. 31 (1), 65–70.

Gambini, J., Inglés, M., Olaso, G., Lopez-Grueso, R., Bonet-Costa, V., Gimeno-Mallench, L., Mas-Bargues, C., Abdelaziz, K.M., Gomez-Cabrera, M.C., Vina, J., Borras, C., 2015. Properties of Resveratrol. In Vitro and In Vivo Studies about Metabolism, Bioavailability, and Biological Effects in Animal Models and Humans. Oxid. Med. Cell. Longev. https://doi.org/10.1155/2015/837042.

Gaspar, M.C., Mendes, C.V.T., Pinela, S.R., Moreira, R., Carvalho, M.G.V.S., Quina, M.J., Braga, M.E.M., Portugal, A.T., 2019. Assessment of Agroforestry Residues: Their Potential within the Biorefinery Context. ACS Sustain. Chem. Eng. 7 https://doi.org/10.1021/acssuschemeng.9b03532.

Goswami, G., Mukherjee, M., Katari, J.K., Datta, S., Das, D., 2023. Lignocellulosic bio-butanol production: challenges and solution. Adv. Dev. Biobutanol Prod. 261–277 https://doi.org/10.1016/B978-0-323-91178-8.00009-6.

Guimarães, P.M.R., Teixeira, J.A., Domingues, L., 2010. Fermentation of lactose to bio-ethanol by yeasts as part of integrated solutions for the valorisation of cheese whey. Biotechnol. Adv. https://doi.org/10.1016/j.biotechadv.2010.02.002.

Huijbregts, M.A.J., Steinmann, Z.J.N., Elshout, P.M.F., Stam, G., Verones, F., Vieira, M., Zijp, M., Hollander, A., van Zelm, R., 2017. ReCiPe2016: a harmonised life cycle impact assessment method at midpoint and endpoint level. Int. J. Life Cycle Assess. 22, 138–147. https://doi.org/10.1007/S11367-016-1246-Y/TABLES/2.

Iso, 2009. ISO 14040:2006. Environmental management - Life Cycle Assessment -. Principles and Framework.

Iwakiri, S., Trianoski, R., Chies, D., Tavares, E.L., França, M.C., Lau, P.C., Iwakiri, V.T., 2017. USE OF RESIDUES OF FORESTRY EXPLORATION OF Pinus taeda FOR PARTICLEBOARD MANUFACTURE. Rev. Árvore 41. https://doi.org/10.1590/1806-90882017000300004.

Karthik, V., Periyasamy, S., Varalakshmi, V., Pauline, J.M.N., Suganya, R., 2023. Biofuel: A prime eco-innovation for sustainability. Environ. Sustain. Biofuels 267–284. https://doi.org/10.1016/B978-0-323-91159-7.00006-0.

Klassen, V., Blifernez-Klassen, O., Wibberg, D., Winkler, A., Kalinowski, J., Posten, C., Kruse, O., 2017. Highly efficient methane generation from untreated microalgae biomass. Biotechnol. Biofuels 10, 1. https://doi.org/10.1186/s13068-017-0871-4.

Lappa, I.K., Papadaki, A., Kachrimanidou, V., Terpou, A., Koulougliotis, D., Eriotou, E., Kopsahelis, N., 2019. Cheese whey processing: Integrated biorefinery concepts and emerging food applications. Foods. https://doi.org/10.3390/foods8080347.

Lopes, A.C.A., Eda, S.H., Andrade, R.P., Amorim, J.C., Duarte, W.F., 2019. New alcoholic fermented beverages-potentials and challenges, in: Fermented Beverages: Volume 5. The Science of Beverages. https://doi.org/10.1016/B978-0-12-815271-3.00014-2.

Malico, I., Gonçalves, A.C., 2021. Eucalyptus globulus coppices in portugal: Influence of site and percentage of residues collected for energy. Sustainability 13 (11), 5775.

Mortier, N., Velghe, F., Verstichel, S., 2016. Biotransformation of Agricultural waste and by-products: The food, feed, fibre, fuel, (4F) economy. Elsevier, pp. 69–124.

Nayak, J., Pal, P., 2013. Transforming waste cheese-whey into acetic acid through a continuous membrane-integrated hybrid process. Ind. Eng. Chem. Res. 52 https://doi.org/10.1021/ie3033729.

P̧ Edzik, M., Tomczak, K., Janiszewska-Latterini, D., Tomczak, A., Rogozí Nski, T., 2022. Management of Forest Residues as a Raw Material for the Production of Particleboards. For. 2022, Vol. 13, Page 1933 13, 1933. https://doi.org/10.3390/F13111933.

Pergola, M.T., Saulino, L., Castellaneta, M., Rita, A., Pecora, G., Cozzi, M., Moretti, N., Pericolo, O., Pierangeli, D., Romano, S., Viccaro, M., Ripullone, F., 2022. Towards sustainable management of forest residues in the southern Apennine Mediterranean mountain forests: a scenario-based approach. Ann. for. Sci. 79 https://doi.org/10.1186/s13595-022-01128-w.

Rico, C., Muñoz, N., Fernández, J., Rico, J.L., 2015. High-load anaerobic co-digestion of cheese whey and liquid fraction of dairy manure in a one-stage UASB process: Limits in co-substrates ratio and organic loading rate. Chem. Eng. J. 262, 794–802.

Romaní, A., Ruiz, H.A., Pereira, F.B., Teixeira, J.A., Domingues, L., 2014. Integrated approach for effective bioethanol production using whole slurry from autohydrolyzed Eucalyptus globulus wood at high-solid loadings. Fuel 135. https://doi.org/10.1016/j.fuel.2014.06.061.

Romaní, A., Ruiz, H.A., Teixeira, J.A., Domingues, L., 2016. Valorization of Eucalyptus wood by glycerol-organosolv pretreatment within the biorefinery concept: An integrated and intensified approach. Renew. Energy 95. https://doi.org/10.1016/j.renene.2016.03.106.

Romaní, A., Larramendi, A., Yáñez, R., Cancela, Á., Sánchez, Á., Teixeira, J.A., Domingues, L., 2019. Valorization of Eucalyptus nitens bark by organosolv pretreatment for the production of advanced biofuels. Ind. Crops Prod. 132 https://doi.org/10.1016/j.indcrop.2019.02.040.

Salehi, B., Mishra, A.P., Nigam, M., Sener, B., Kilic, M., Sharifi-Rad, M., Fokou, P.V.T., Martins, N., Sharifi-Rad, J., 2018. Resveratrol: A double-edged sword in health benefits. Biomedicines. https://doi.org/10.3390/biomedicines6030091.

Smith, R., 2005. Chemical process: design and integration.

Tavares, C.S., Martins, A., Miguel, M.G., Carvalheiro, F., Duarte, L.C., Gameiro, J.A., Figueiredo, A.C., Roseiro, L.B., 2020. Bioproducts from forest biomass II. Bioactive compounds from the steam-distillation by-products of Cupressus lusitanica Mill. and Cistus ladanifer L. wastes. Ind. Crops Prod. 158, 112991 https://doi.org/10.1016/J.INDCROP.2020.112991.

Teixeira, D., Carrilho, M., Mexia, T., Köbel, M., Santos, M.J., Santos-Reis, M., Rosalino, L. M., 2017. Management of Eucalyptus plantations influences small mammal density: Evidence from Southern Europe. For. Ecol. Manage. 385, 25–34.

Towler, G., Sinnott, R., 2021. Chemical engineering design: principles, practice and economics of plant and process design.

Tripathi, N., Hills, C.D., Singh, R.S., Atkinson, C.J., 2019. Biomass waste utilisation in low-carbon products: harnessing a major potential resource. Npj Clim. Atmos. Sci. 2 https://doi.org/10.1038/s41612-019-0093-5.

Yadav, J.S.S., Bezawada, J., Elharche, S., Yan, S., Tyagi, R.D., Surampalli, R.Y., 2014. Simultaneous single-cell protein production and COD removal with characterization of residual protein and intermediate metabolites during whey fermentation by K. marxianus. Bioprocess Biosyst. Eng. 37, 1017–1029. https://doi.org/10.1007/S00449-013-1072-6.

Zandona, E., Blažić, M., Režek Jambrak, A., 2021. Whey utilisation: Sustainable uses and environmental approach. Food Technol. Biotechnol. https://doi.org/10.17113/ftb.59.02.21.6968.

Zhou, X., Hua, X., Huang, L., Xu, Y., 2019. Bio-utilization of cheese manufacturing wastes (cheese whey powder) for bioethanol and specific product (galactonic acid) production via a two-step bioprocess. Bioresour. Technol. 272 https://doi.org/10.1016/j.biortech.2018.10.001.