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RESEARCH

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Process scale-up simulation and techno-economic assessment of ethanol fermentation from cheese whey

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Abstract

Background Production of cheese whey in the EU exceeded 55 million tons in 2022, resulting in lactose-rich effluents that pose significant environmental challenges. To address this issue, the present study investigated cheese-whey treatment via membrane filtration and the utilization of its components as fermentation feedstock. A simulation model was developed for an industrial-scale facility located in Italy's Apulia region, designed to process 539 m^3/day of untreated cheese-whey. The model integrated experimental data from ethanolic fermentation using a selected strain of *Kluyveromyces marxianus* in lactose-supplemented media, along with relevant published data.

Results The simulation was divided into three different sections. The first section focused on cheese-whey pretreatment through membrane filtration, enabling the recovery of 56$\%_{w/w}$ whey protein concentrate, process water recirculation, and lactose concentration. In the second section, the recovered lactose was directed towards fermentation and downstream anhydrous ethanol production. The third section encompassed anaerobic digestion of organic residue, sludge handling, and combined heat and power production. Moreover, three different scenarios were produced based on ethanol yield on lactose ($Y_{E/L}$), biomass yield on lactose, and final lactose concentration in the medium. A techno-economic assessment based on the collected data was performed as well as a sensitivity analysis focused on economic parameters, encompassing considerations on cheese-whey by assessing its economical impact as a credit for the simulated facility, dictated by a gate fee, or as a cost by considering it a raw material. The techno-economic analysis revealed different minimum ethanol selling prices across the three scenarios. The best performance was obtained in the scenario presenting a $Y_{E/L} = 0.45$ g/g, with a minimum selling price of 1.43 €/kg. Finally, sensitivity analysis highlighted the model's dependence on the price or credit associated with cheese-whey handling.

Conclusions This work highlighted the importance of policy implementation in this kind of study, demonstrating how a gate fee approach applied to cheese-whey procurement positively impacted the final minimum selling price for ethanol across all scenarios. Additionally, considerations should be made about the implementation of the simulated process as a plug-in addition in to existing processes dealing with dairy products or handling multiple biomasses to produce ethanol.

Keywords Techno-economic analysis, Cheese whey, Ethanol, *Kluyveromyces marxianus*, SuperPro Designer, Fermentation, Bio-fuel, Waste

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Background

The growing climate crisis and globally unstable social conditions are commanding a shift from conventional production models towards more environmentally sound and robust approaches. Policies are being implemented to mitigate the anthropogenic causes of climate change, including measures to reduce waste and achieve carbon neutrality [1].

The European Union is a significant player in the global dairy industry, ranking among the world's leading milk producers. In 2022, Europe produced approximately 160 million tons of milk, of which 96% was cow milk. Italy accounted for 9% of the cow milk collected [2]. Notably, 82.5% of all milk harvested in Italy in 2021 was destined to the production of Protected Designation of Origin (PDO) cheeses and other derivatives [3]. Cheese whey (CW) is a by-product of cheese manufacturing. It is a yellow-greenish liquid rich in lactose, fats, and proteins formed after the curdling process [4]. In Italy, 10,432,000 tons of CW were produced in 2020, with most of it (61%) occurring in the northern regions of Lombardy, Emilia-Romagna, and Veneto. In contrast, the southern region of Apulia contributed with only 388,763 tons of CW during the same year. The majority of this amount (85%) derived from fresh cheese production, including mozzarella and burrata [5].Until a few decades ago CW disposal was not properly regulated. Indeed, CW was often discarded in waterbodies or used as fertilizer [6]. However, recognizing its significant environmental impact, primarily due to high biochemical and chemical oxygen demand levels [7], countries began regulating its disposal. Decision 97/80/EC from the European Union classified it as a by-product for disposal or reuse. CW contains roughly 55% milk nutrients, notably lactose (4–5%$_{w/v}$) and proteins (0.3–1%$_{w/v}$) [6, 8]. Several processing practices, including membrane filtration to obtain whey protein concentrate (WPC) [9–12], have been established since the late 70 s [13]. However, WPC production results in the generation of lactose-rich by-products that can be further valorized through concentration with fermentation being one of the possible cascade approaches [14, 15]. The deproteinized whey can be utilized as a feedstock, to produce biofuels, such as bioethanol [16, 17], 2,3-butanediol [18, 19], biogas [20, 21], and hydrogen [22, 23], along with other value-added products such as polyhydroxyalkanoates [24, 25]. The commercial production of bioethanol from CW-derived lactose via fermentation by *Kluyveromyces* spp. has already been applied, mostly by high-volume dairy manufacturers. These facilities include Carbery, which was founded in the late 1970s in Ireland, along with other examples in New Zealand and the United States [8, 26]. While bioethanol production has focused predominantly on plant-derived feedstocks [27–29], CW is attracting renewed academic interest [12] as a possible alternative.

To achieve the above goal, several strategies have been explored, including co-immobilizing β-galactosidases with conventional yeasts (e.g., *Saccharomyces* genus) [30, 31] or engineering yeast strains expressing the enzymes responsible for lactose hydrolysis and galactose metabolism [32, 33]. However, despite the potential benefits of these approaches, the resulting mutants tend to be unstable [34, 35]. Unconventional yeasts such as *Kluyveromyces* spp. can metabolize lactose, making them attractive candidates. Although typically showing a lower resistance to high concentrations of ethanol, ongoing research has made *Kluyveromyces* more suitable for industrial application [36, 37]. *Kluyveromyces marxianus* is a homothallic, respiro-fermentative, thermotolerant, and Crabtree-negative yeast [38]. Besides lactose, it grows well on a broad range of industrially relevant substrates, such as inulin and fructose [39]. Numerous industrial applications for this yeast have been attempted, including bioethanol generation from lactose, homologous enzyme production (e.g., β-D-galactosidase and pectinases), heterologous protein expression, as well as production of aromatic alcohols, single-cell proteins, bio-emulsifiers, and antioxidants [16, 17, 40–44]. Nevertheless, there are fewer economic feasibility studies on the industrial fermentation of CW to ethanol [45, 46] compared to research on lignocellulose or starch [47–49].

This study simulates a biorefinery capable of processing 539 m^3/day of CW to produce fuel-grade ethanol in the region of Apulia. The biorefinery model integrates three sections: (1) pretreatment via filtration to produce WPC; (2) fermentation and downstream processes; and (3) anaerobic wastewater treatment coupled to the combined generation of electricity, heat, and soil conditioner. Each section was simulated separately for three different scenarios (S1, S2, and S3) based on the experimental performance of *Kluyveromyces marxianus* DSM 7239 fermenting lactose to ethanol. Minimum Ethanol Selling Price (MESP) was calculated and sensitivity analysis was conducted to test the influence of external economic parameters on the final MESP and economic viability of the proposed process.

Methods

Strain maintenance and inoculum

Kluyveromyces marxianus DSM 7239 was purchased from Leibniz-Institut DSMZ—Deutsche Sammlung von Mikroorganismen und Zellkulturen. Cultures were maintained on either liquid YPD medium containing 10 g/l yeast extract, 20 g/l peptone, and 20 g/l D-glucose or YPDA (agar 15 g/l).

Pre-cultures were started from a single colony inoculated in sterile 10-ml tubes containing 2 ml YPD. Pre-cultures were incubated at 30 ℃ in a rotary shaker at 200 rpm for 16–18 h and inoculated in flasks at a final OD_{600} of 0.1.

Flask fermentation

Flask fermentation experiments were carried out in triplicates in 50-ml Erlenmeyer flasks, plugged with a metal cap and containing 20 ml Semi Synthetic Medium (SSM). The flasks were shaken at 200 rpm for 48 h at either 30 or 37 ℃. SSM was adapted from literature [50] and contained 5 g/l yeast extract, 0.7 g/l $MgSO_4$, 1 g/l KH_2PO_4, 0.1 g/l K_2HPO_4, and 5 g/l $(NH_4)_2SO_4$ (pH 5.5), it was supplemented with either 100 or 200 g/l lactose. At specific time points, samples were collected to measure OD_{600}, pH, and lactose content. Lactose and ethanol were quantified by high-performance liquid chromatography using a Waters Alliance 2695 separation module equipped with a Rezex ROA-Organic Acid H + (8%) 300 mm × 7.8 mm column (Phenomenex Inc., USA), coupled to a Waters 2410 refractive index detector and a Waters 2996 UV detector. Separation was carried out at 60 ℃ with 2.5 mM H_2SO_4 as mobile phase and a flow rate of 0.5 ml/min.

Process design and model assumptions

The simulation model was created using SuperPro Designer (SPD) v.12.03 by Intelligen [51].Three different simulations were developed, each corresponding to a section in the overall process: pretreatment (F), fermentation and downstream processing (F+D), and wastewater treatment by anaerobic digestion (WWT). The simulations were run separately. Each section was connected to the other two by using the outputs of one as inputs in the next. The obtained results served for a Techno-Economic Analysis (TEA). The simulated plant was assumed to have the capability of handling 539 m^3/day of untreated whey serum based on the latest data on whey serum availability (1065 ton/day) [5] in Apulia, where the plant location was assumed.

The composition of the whey feedstock used for the simulation was assumed based on industry specifications and published data [52] (Fig. 1).

Sections F and F+D were modeled in batch mode with 24-h batches for the former and 173-h cycling every 28 h for the latter (scenario S1) or 174-h cycling every 29 h (scenarios S2 and S3). The WWT section, instead, was modeled in continuous operating mode. Each section was operated for 7920 h/year.

The pH during fermentation was assumed to be unmonitored, as in all conditions tested experimentally the pH remained stable around 4.5–5, and residual salts present in the final fermentation feed stream (0.25–0.50%$_{w/v}$) were assumed to have no effect on microbial performance [53, 54].

To model the reactions, *K. marxianus* composition was assumed based on published data [55]: $CH_{1.76}O_{0.66}N_{0.158}$ for fermentation and biomass growth, or $CH_{1.76}O_{0.66}N_{0.158}S_{0.0035}$ for residual biomass degradation in WWT.

Scenarios S1, S2, and S3 were built on a common design (Fig. 2). The common characteristics for each

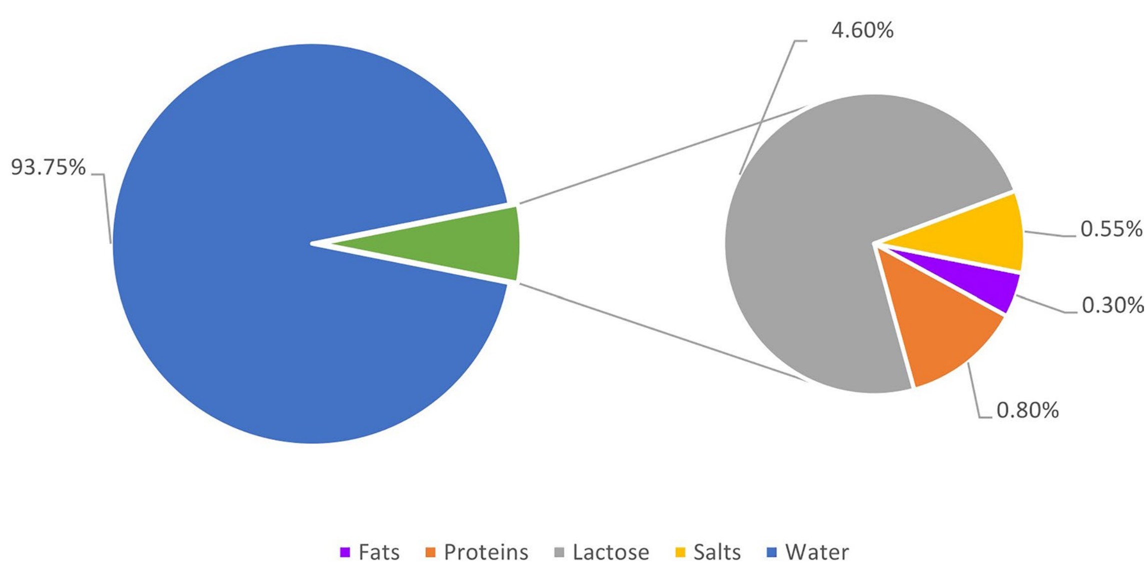

Fig. 1 Cheese whey composition used for the simulation

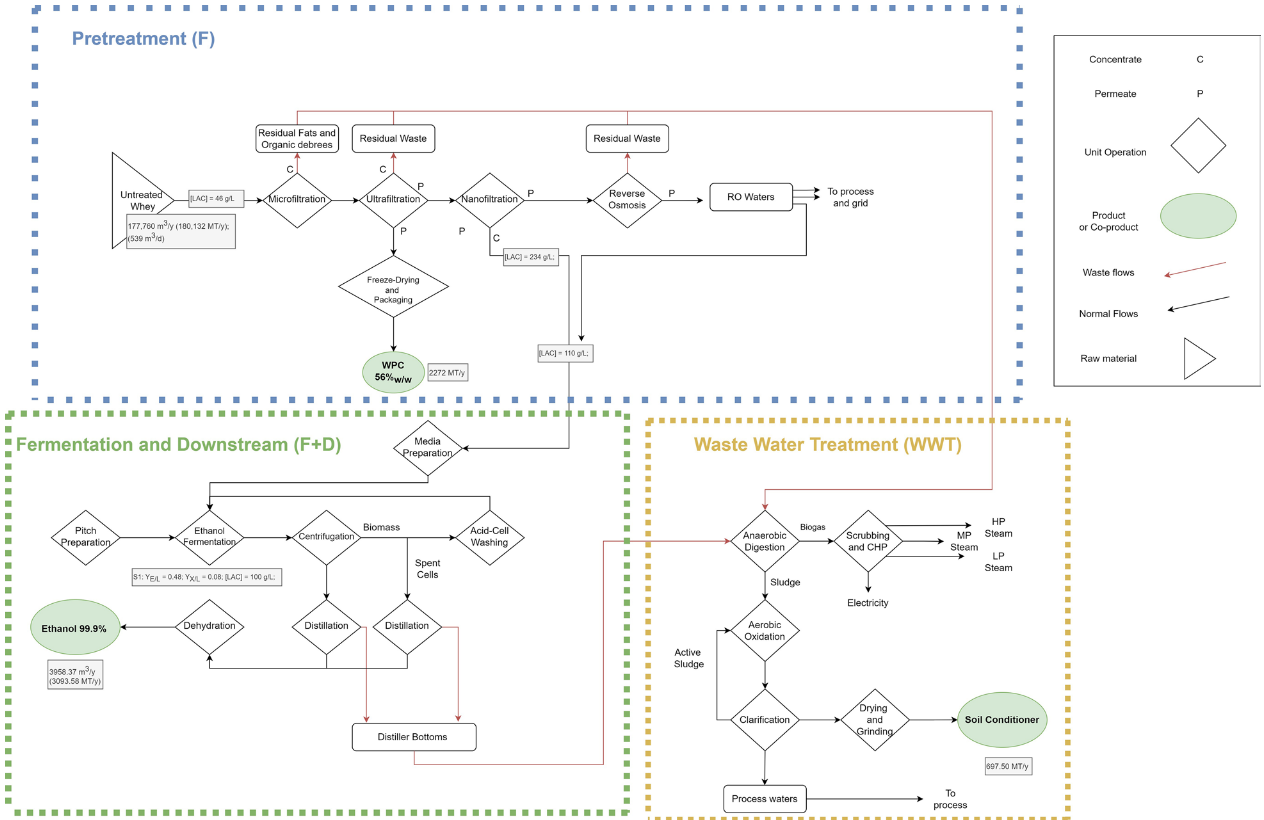

Fig. 2 Block flow diagram of the overall process for scenario S1

Table 1 Sizing and flows of membrane filtration procedures in the F section

Unit procedure	Flow (l/m^2 h)	Membrane area (m^2)
MF (microfiltration)	50	431.01
UF (ultrafiltration)	30	682.56
NF (nanofiltration)	30	546.27
RO (reverse osmosis)	20	633.95

section across scenarios, as well as the main differences between scenarios, are described hereafter.

Filtration section

Filtration was modeled to process a daily whey intake of 539 m^3 through microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO) based on published data [9, 10, 56, 57] and the SPD database of processes. The model was designed based on a previously developed patent [58] and data regarding each operation unit are reported in Table 1. In MF, fat and residual solids were removed from raw whey, and then concentrated (2.23 m^3/h, 3%$_{dw/v}$ fats) before being fed to the anaerobic digester (AD) in the WWT section. Afterwards, the filtrate was passed through the UF unit, where proteins were removed (1 m^3/h, 16%$_{dw/v}$ proteins), spry-dried, and packaged (20-kg plastic bags) to produce 56%$_{dw/v}$ WPC. The filtrate was led through a NF module, resulting in a lactose-rich concentrate (3.84 m^3/h, 21.7%$_{w/w}$ lactose). Depending on the scenario, the concentrate was either diluted with sterile water generated by RO to attain an appropriate concentration of lactose within the fermentor (100 g/l, S1) or used as such (200 g/l, S2 and S3). Instead, the permeate, was processed by RO to produce demineralized water, which was subsequently recirculated within the plant. The generated water was directed towards steam production, feed dilution of concentrated lactose, and preparation of growth media; whereas, organic waste streams were directed to WWT.

Fermentation and downstream section

Section F+D included an initial seed train for microbial biomass production. The reactions used for biomass growth and ethanol fermentation were modeled stoichiometrically following published guidelines [27, 59–61] (Table 2). Biomass growth was modeled assuming a yield on glucose ($Y_{X/G}$) of 0.5 [62, 63]. The operation lasted

Table 2 Chemical equations used in section F+D

Reaction	Equation
F+D, *K. marxianus* growth on glucose	$C_6H_{12}O_6 + 0.27CO(NH_2)_2 + 2.63O_2 \rightarrow 3.38CH_{1.76}O_{0.66}N_{0.158} + 2.88CO_2 + 3.52H_2O$
F+D, lactose hydrolysis	
F+D, fermentation (S1)	$2.000C_6H_{12}O_6 + 0.081CO(NH_2)_2 + 0.275O_2 \rightarrow 1.029CH_{1.76}O_{0.66}N_{0.158} + 3.566C_2H_5OH + 3.919CO_2 + 0.547H_2O$
F+D, fermentation (S2)	$2.000C_6H_{12}O_6 + 0.041CO(NH_2)_2 + 3.686O_2 \rightarrow 0.515CH_{1.76}O_{0.66}N_{0.158} + 2.600C_2H_5OH + 6.325CO_2 + 3.822H_2O$
F+D, fermentation (S3)	$2.000C_6H_{12}O_6 + 0.041CO(NH_2)_2 + 1.457O_2 \rightarrow 0.515CH_{1.76}O_{0.66}N_{0.158} + 3.343C_2H_5OH + 4.839CO_2 + 1.593H_2O$

24 h at 32 ℃ and the number of seed reactors composing the seed train was set to achieve a biomass pitch concentration of 5 g/l [64] for the fermentation. The first seed fermentor was sized at either 44 l (S1) or 33 l (S2 and S3). Each step in the seed train increased in volume by a factor of 10, and every inoculum was assumed to make up $10\%_{v/v}$ of the next seed step. The seeds were supplied with 20 g/l glucose and 10 g/l urea in water. Ethanol fermentations for each scenario were modeled stoichiometrically as process equations (Table 2) using experimentally derived *K. marxianus* ethanol and biomass yields on lactose ($Y_{E/L}$ and $Y_{X/L}$, respectively) as constraints. Fermentation medium contained up to 200 g/l lactose (depending on the scenario), water, and urea (in stoichiometric concentrations as N-source). The fermentation was set to last for 24 h at 37 ℃ (S2 and S3) or 30 ℃ (S1), under microaerophilic conditions [17, 65]. The fermented broth was then sent to a disk stack centrifuge (95% solid removal). The obtained supernatant was distilled and the biomass flow was split to recycle part of the available biomass volume. Notably, the specific volumes varied across scenarios, as detailed in the following sections. Biomass was washed with H_3PO_4 ($0.60\%_{v/v}$) to remove potential microbial contaminants [66, 67] and inoculated with freshly produced biomass in the fermentor to reach the aforementioned pitch concentration. The remaining volume of available biomass after centrifugation was sent for a second distillation to recover residual ethanol contained within cells. Finally, the distiller bottoms and wastewaters from the different operations were directed towards WWT, while distilled ethanol underwent dehydration in a granular activated carbon (GAC) column to achieve fuel-grade $99.9\%_{v/v}$ concentration [68].

Wastewater treatment section

This section converted organic waste streams originating from the previous units into biomethane-enriched biogas and soil conditioner. The model was set to operate continuously, employing an anaerobic digestor with a hydraulic retention time of 30 days under mesophilic conditions (39 ℃). The obtained biogas underwent desulfurization via GAC filtration (98% efficiency) and combustion in a 100-bar steam generator. The latter was coupled to a steam expander modeled on an industrial SIEMENS SST-200 turbine to produce electricity and High pressure (HP = 35 bar), medium pressure (MP = 5 bar), and atmospheric pressure (LP) steam. Produced steam was consumed within the model as a heat vector. The sludge obtained from the AD was sent to an aerobic oxidation (AO) tank that degraded residual organic substances present in the AD sludge. The resulting waters were then processed through a decanter, which separated the AO sludge from reusable waters. The latter were recycled as a heat exchange vector and for non-sterile operations. The sludge was assumed to be split at a 1:1 ratio between re-seeding the AO tank to maintain a constant supply of activated sludge and being processed through an air-drying and grinding procedure to produce a soil conditioner (packaged in 20-kg paper bags). Specific functioning of single unit operations and chemical reactions were adapted from the literature [69] and SPD database of processes.

A block flow diagram of the process is outlined in Fig. 2, while the chemical equations for the simulation of the AD section are detailed in table S1 in the supplementary materials. Additionally, the SPD process flow diagrams for S2, which is used as a reference scenario, are provided in Figure S3 in the supplementary materials.

Scenario description and main differences

Three different scenarios (S1, S2, and S3) based on the $Y_{E/L}$, $Y_{X/L}$, and lactose concentration in fermentation medium ([LAC]) were considered. The scenarios were generated by stoichiometrically balancing the fermentation equations while keeping $Y_{E/L}$ and $Y_{X/L}$ as fixed constraints (Table 2). S1 and S2 employed experimentally derived yields, whereas S3 showcased a prospective and desirable scenario for a potentially improved strain. This strain, achievable through genetic engineering or adapted lab evolution, could ferment ethanol at high lactose concentrations and temperatures, chanelling most of the C-uptake into fermentation. This scenario assumes an achievable, but lower than theoretical maximum, $Y_{E/L}$ and $Y_{X/L}$. Key parameters related to each scenario are detailed in Table 3.

Table 3 Main parameters for each scenario

Scenario	$Y_{E/L}$ (g/g)	$Y_{X/L}$ (g/g)	[LAC] (g/l)	Fermentation temperature (°C)
S1	$Y_{E/L}=0.48$	$Y_{X/L}=0.08$	100	30
S2	$Y_{E/L}=0.35$	$Y_{X/L}=0.04$	200	37
S3	$Y_{E/L}=0.45$	$Y_{X/L}=0.04$	200	37

To reach the required [LAC], certain procedures were modified in each scenario. In S1, the fermentor was fed lactose-rich feed originating from NF ([LAC]: 234 g/l), diluted with 35% effluent from RO (total volumetric flow to the medium preparation vessel: 8.18 m^3/h, [LAC]: 110 g/l). These steps ensured a final [LAC] of 100 g/l after the addition of inoculum and urea. In S2 and S3, the NF concentrate remained undiluted prior to being sent to the medium preparation vessel (total volumetric flow: 3.84 m^3/h, [LAC]: 234 g/l) and eventually reached a [LAC] of 200 g/l. Moreover, in S2 and S3, the amount of cells headed for recycling was adjusted to 34% and 33% of total biomass volume, respectively. This value was slightly lower than the one (37%) in S1 used to obtain the appropriate pitching concentration for fermentation.

For each scenario, two filtering options were considered: polymeric membrane filters and ceramic filters. The membranes were modeled as consumables on SPD and, thus, no strict physical properties for the utilized materials were specified aside from lifetime and costs (Table 4).

Economic model assumptions and techno-economic analysis (TEA)

For TEA, construction of a new plant was assumed. Total Capital Investment (TCI) and OPerating EXpense (OPEX) were calculated using SPD unless stated otherwise. Selected input parameters and formulae relevant for the calculations are listed in Table 5. To determine the MESP, a discounted cash flow rate of return [70–72] analysis with a Discount Rate (DR) of 7% as a base case was performed.

The lifetime of the project was assumed to be 20 years, the year of analysis was set to 2021, and all economic parameters and pricing were evaluated on the basis of a US Dollar to Euro conversion average for the year of analysis [73]. A 4% inflation rate was assumed. The year of the analysis was chosen to reflect a more standardized market condition, unaffected by external geopolitical disturbances (e.g., wars, pandemics) that could have introduced fluctuations and made the analysis less informative. For the construction of the plant, a period of 30 months from the year of analysis was assumed, along with a 4-month period for start-up. The plant was hypothesized to work at 100% capacity from the start-up. The latter assumption might be arguable as unrealistic, as it is typically challenging to reach full capacity immediately. Delays could arise due to standardization of procedures and adjustments in plant operation. Not anticipating a ramp-up phase could introduce risks to the facility's adaptability. It is, nonetheless, believed that the assumption aligns with the goal of the study of estimating possible MESPs for a fully operational facility. The co-products, namely 56%$_{dw/v}$ WPC and soil conditioner (35%$_{w/w}$ solids) were assumed to be sold at a constant price of 1.00 and 1.24 €/kg, respectively. The price chosen for the soil conditioner was decided assuming the absence of any inorganic contaminants, as well as compliance with EC regulations 2019/1009 (Ex. 2003/2003) and D.lgs. 75/2010, along with any subsequent modifications, thereby meeting quality certification standards [74]. CW was assumed to not generate a cost for the plant in the base case analysis (see Sensitivity Analysis section for details). Pricing for equipment was calculated based on standard quotations and cost models given by the software. Transport costs were not directly accounted for in the economic model, as they were assumed to be included in the purchasing prices or gate fees for CW under Delivered At Place (DAP) terms. All surpluses and deficits generated within the process for utilities (e.g., heat transfer agents such as cooling waters and steam) were accounted for by handling them as credits or operating costs, respectively. The generated credits, as well as costs, were calculated using annual production rates and multiplying them by the amount corresponding to the normal purchase price of the utility given by SPD. Surplus RO water was quoted by Italian market standards at 0.02 €/l (Table 5).

Project lifetime analysis

Due to current economic and social instability, as well as rapid technological advancements, shorter process obsolescence times could impact the facility, forcing it to readapt to market conditions sooner than expected. Therefore, the potential for a shorter prospected lifetime for the project due to market conditions was investigated. To this end, three different time periods for the project lifetime were chosen: 10, 15, and 20 years (reference), each with a corresponding depreciation timeframe of 7, 10, and 15 years, respectively.

Sensitivity analysis

The sensitivity analysis was set to reveal how specific economic variables impacted the final MESP in the model. The variables selected for the analysis were deemed to be the ones most likely to impact the economic feasibility of the process and included CW

Table 4 Raw materials, consumables, labor and utilities cost components used for the analysis

Cost item	Price (€)	Unit	Lifetime (y)	Source
Raw materials and consumables				
Paper bags	0.01	entity (100 g)		α
Glucose	0.37	kg		[92]
H3PO4(85% w/w)	0.68	kg		α
Urea	0.76	kg		[93]
Water	1.83	m^3 (STP)		[94]
Plastic bags	0.01	entity (200 g)		α
NaOH	0.600	kg		α
Consumables				
RO membrane	12.41	m^2	5	Lifetime[95]; Priceα
NF membrane (POL) (CER)	200[1] 590[1,2]	m^2	5 20	Lifetime[96]; Price[97, 98]
UF membrane (POL) (CER)	110[1] 520[1]	m^2	5 20	Lifetime[96]; Price[99, 100]
MF membrane (POL) (CER)	100[1] 210[1]	m^2	5 20	Lifetime[96]; Price[97, 100]
Dft GAC packing (G)	2.54	kg	18 cycles[3]	α
Dft GAC packing (L)	3.31	kg	5	α
2000-ml shake flasks	1.49	item		α
Membrane disposal	4.14	m^2		[99]
Utilities				
Std. power (Electricity)	0.09	kW/h		[77]
Steam (MP)	26.48	MT		α
Steam (LP)	24.82	MT		α
Steam (HP)	29.79	MT		α
Wastewater treatment	0.24	m^3		[94]
Glycol	0.66	MT		α
Labor				
Operator	20.91[4]	h		[101]
QC analyst	25.05[4]	h		[101]
Supervisor	25.47[4]	h		[101]

α: Internal price quotation from SPD; [1]:costs readapted to year of analysis with CEPCI indexes ($C_{2021} = C_{year} * \frac{I_{2021}}{I_{year}}$); [2]:price refers to generic flat sheet ceramic membrane for lack of better data; [3]single cycle lasting 3 h; [4]labor cost calculated as net labor cost for the year 2021 +benefits, benefits calculated with standard SPD multipliers for set type of laborer. Operator net labor cost = 9.09 €/h, CCNL level E1; QC analyst net labor cost = 10.89 €/h; CCNL level C1; supervisor net labor cost = 12.13 €/h, CCNL Level B2

price. Currently, Italy does not have a unified national strategy or policy for handling CW, which can lead to variability in CW pricing. CW is generally considered a by-product of the dairy industry, when treated and used for animal or human feeding in accordance with EC 853/2004 and 183/2005 regulations. When regarded as waste (D.lgs 152/06, EWC 020203), its regulation falls under regional and local legislation in observance of EC 1069/2009 on health rules as regards animal by-products and derived products not intended for human consumption. In Apulia, a gate fee approach is applied to promote urban waste recycling (D.C.R. 68 14/12/2021 and D.G.R. 2251 29/12/2021), classifying CW as part of the organic fraction and allowing it to be fed to anaerobic digestion plants for biomethane production. Other regions leave CW subject to the free market, enabling third parties to purchase it as raw material for processing into final products. Due to such a diverse legal and political framework, both options were accounted for in the model by including the highest price on the Italian market for industrial-use whey (0.025 €/kg) [75] as the most extreme range limit. These data served as a benchmark to assess either a gate fee paid to the company (handled as a credit in the economic evaluation) or a price paid by the company (raw material cost) for ethanol production.

Table 5 Main economic parameters used for TEA

DFC (direct fixed costs)	$\boldsymbol{DFC = DC + IC + OC}$
DC (direct costs)	$DC = \sum (x_i * PC)$ $PC = Listedeq.purchasecost + unlistedEq.purchasecost$ [a] $x_i = costfactor$
Piping	$0.07 * PC$
Instrumentation	$0.05 * PC$
Insulation	$0.02 * PC$
Electrical facilities	$0.07 * PC$
Buildings	$0.15 * PC$
Yard improvement	$0.05 * PC$
Auxiliary facilities	$0.07 * PC$
Installation[a]	$0.5 * UnlistedEqPC + 0.5 * listedEqPC$
IC (INDIRECT COSTS)	$IC = \sum (x_i * DC)$
Engineering construction	$0.07 * DC$ $0.15 * DC$
OC (OTHER COSTS)	$OC = \sum x_i * (IC + DC)$
Contractors contingency	$0.02 * (DC + IC)$ $0.05 * (DC + IC)$
WC (working capital)	Capital needed for 30 days of plant operation
SU (start-up cost and validation)	$0.05 * DFC$
TCI (total capital investment)	$\mathbf{TCI} = \boldsymbol{DFC} + \mathbf{WC} + \mathbf{SU}$
Operating costs	$OPEX = Rawmaterials + labordependent + facilitydependent + laboratory/QC/QA + consumables + wastetreatment/disposal + utilities - credits$ $OPEX = Rawmaterials + labordependent + facilitydependent + laboratory/QC/QA + consumables + wastetreatment/disposal + utilities - credits$
Facility dependent	$Maintenance + depreciation + Misc.$
Maintenance	Internal SPD model depending on single equipment PC
Depreciation	Internal SPD straight-line model depending on single equipment PC (15 year depreciation; salvage value = 0.05 * DFC)
Misc	$Misc. = Insurance + localtaxes + factoryexpenses$
Insurance	$0.01 * DFC$
Local taxes	$0.02 * DFC$
Factory expenses	$0.05 * DFC$
Labor dependent	Internal SPD model adjusted for Italian labor standards specific for set work position[95]
Laboratory/QC/QA	$15\% * labor$ dependent
Consumables[b]	$Consumables = \sum consumable_{i(q.ty)} * price_i$
Utilities[b]	$Utilities = \sum utility_{i(q.ty)} * price_i$
Waste treatment/disposal[b]	$Wastetreatment/disposal = \sum waste_{i(q.ty)} * price_i$
credits[b]	$Credit = \sum producedutility_{i(q.ty)} * sellingprice_i$

[a] Unlisted equipment purchase costs are calculated as 5% of the listed equipment purchase costs; [b]prices for a single element of the group are listed in Table 4

- Discount rate (DR): depending on the nature of the investment, different DRs can be used to calculate either the minimum selling price or the net present value of a set project [76]. In this study, a DR of 7% was applied for the MESP calculation. Additionally, DRs of 5% and 11% were considered as possible alternatives to explore scenarios of less and more profit-oriented investment planning or to reflect a higher perceived risk by investors, who may demand higher returns for their investments.
- Electricity price: the cost of electricity required to run the facility was based on records from EUROSTAT for the year 2020 [77]. The impact on the MESP was investigated within ± 100% of the quoted price. Said interval was supposed to reflect a scenario, whereby energy prices surged due to external factors and caused fluctuations in the market (+ 100%) or, conversely, whereby the facility met all the energy requirements internally, not needing electricity from the grid (− 100%).
- Co-product selling price: an interval of ± 10% on the cumulative revenues from co-products sales was investigated to account for potential fluctuations in market value and selling prices.
- Equipment purchase cost: to stay within a flexibility window on equipment quotes given by the software, a ± 30% interval was evaluated on listed equipment purchase costs.

The final impact on the economic viability of the project for every variable was calculated as a variation percentage on base MESPs for each scenario.

Results and discussion

Experimental data on *K. marxianus* performance

Different flask experiments were set up and performed to better understand the ability of *K. marxianus* DSM 7239 to grow on the lactose present in CW and its fermentation to ethanol. Four conditions (A1, A2, B1, and B2) were tested with respect to temperature and [LAC] (Table 6).

Under conditions A1 and B1, cells were grown in SSM supplemented with 100 g/l (A1) or 200 g/l (B1) lactose, at 30 ℃ for 48 h. Under conditions A2 and B2, cells were grown in SSM supplemented with 100 g/l (A2) or 200 g/l (B2) lactose, at 37 ℃ for 48 h. The rationale behind raising substrate concentrations and temperatures was to identify the best condition for industrial application. The higher temperatures could reduce the need for thermal control utilities during the fermentation, while higher substrate concentrations could result in lower working volumes. A comparison of conditions A1 and A2, revealed that the substrate in A2 was depleted already after 24 h. The faster lactose utilization was probably caused by a higher temperature influencing the metabolic rate of the yeast; however, an elevated $Y_{E/L}$ and low $Y_{X/L}$ at 24 h were recorded for A1, suggesting a more efficient fermentation. A comparison of conditions B1 and

Table 6 Main conditions and results of flask fermentation trials

Condition	T	[LAC] g/l 0 h	[LAC] g/l 24 h	[LAC] g/l 30 h	[Ethanol] g/l 24 h	[X] g/l 24 h	$Y_{X/L}$ g/g 24 h	$Y_{E/L}$ g/g 24 h
A1	30	100	1.02	0	47.84	8.31	0.08	0.48
A2	37	100	0	0	6.89	8.94	0.09	0.06
B1	30	200	65.44	45.72	52.11	8.14	0.06	0.38
B2	37	200	6.52	0	68.78	8.11	0.04	0.35

Table 7 Primary outputs of the different sections in the process

Process	Batch (h)	Cycle (h)	Product	Unit	S1	S2	S3
Pretreatment	24	24	WPC (56%$_{W/W}$)	MT/y	2,272	2,272	2,272
Fermentation	173.52$_{(S1)}$ 173.96$_{(S2, S3)}$	28.4$_{(S1)}$ 28.8$_{(S2,S3)}$	Ethanol (99.9%)	m^3/y	3,958.37	2,889.26	3,707.40
Wastewater treatment	24	24	Soil conditioner	MT/y	697.50	544.57	581.69
			Biogas (of which % of biomethane)	kg/h	318.46 (36)	265.36 (37)	262.74 (38)
			Electricity	kw/h	350	297	302
			HP steam	m^3/h (MT/h)	13.80 (0.17)	11.59 (0.15)	11.78 (0.15)
			MP steam	m^3/h (MT/h)	163 (0.44)	139 (0.37)	141 (0.38)
			LP steam	m^3/h (MT/h)	1133 (1.72)	1462 (0.96)	1487 (0.97)

B2, confirmed the trend for faster substrate utilization at a higher temperature. Whereas all available substrate was consumed between 24 and 30 h in B2, it did so only after 30 h in B1. Even though the B2 condition led to a lower $Y_{E/L}$ at 24 h than B1, its lower $Y_{X/L}$ and faster lactose uptake suggested a more efficient fermentation. Therefore, A1 and B2 were identified as the two best conditions and the corresponding data were implemented in the model described in this study to construct scenarios S1 and S2.

The yields found in these trials, especially for conditions A1 and B2, were consistent with previous reports. For instance, Silveira et al. [65] reported ethanol yields close to the theoretical for *Kluyveromyces marxianus* UFV-3 grown in CW permeate at up to 240 g/l [LAC] and 30 ℃, achieving yields equal to 0.3–0.4 g/g [65]. For longer fermentation times (72 h), Das et al. (2016) reported $Y_{E/L}$ of 0.332 and $Y_{x/L}$ of 0.021 g/g at 200 g/l [LAC] and 35 ℃ with *Kluyveromyces marxianus* NCIM 3217 grown in CW powder-derived medium with limited oxygen [43].

Equipment sizing, energy integration, and outputs: highly concentrated feeds allow for similar productivities at smaller plant sizes

The three scenarios were compared in terms of equipment sizing, main outputs, and energy requirements for heat and electricity.

The primary outputs, such as yearly ethanol production, soil conditioner, WPC, electricity, biogas, and steam, obtained through the simulations are reported in Table 7 for every section and scenario. The final ethanol titer obtained during fermentation (S1 = 48 g/l; S2 = 71 g/l; S3 = 91 g/l) exceeded the threshold (40 g/$l_{w/v}$) for economic viability and energy efficiency in subsequent downstream applications [28]. In terms of yearly anhydrous ethanol production, S1 (3,958.37 m^3) and S3 (3,707.40 m^3) performed better than S2 (2,889.26 m^3). However, the higher volumes of process water required to dilute the lactose feed in S1 imposed the use of four fermentors; whereas, S2 and S3 necessitated only two. This made the S1 process less appealing due to higher capital costs. Although S1 appears to be more efficient in terms of ethanol production, it is important to note that its productivity is still close to that of S3. This highlights that an enhanced ability of the microorganism to handle higher substrate concentrations significantly benefits overall process efficiency.

Fats, lactose, and proteins in the anaerobic digestor feed derived from section F remained constant across scenarios: 68.24 kg/h, 107.08 kg/h, and 18.62 kg/h, respectively. Instead, those originating from section F+D, varied due to lower fermentation volumes in S2 and S3. The latter, in

Table 8 Electricity requirements and production for the different sections and scenarios

	S1	S2	S3
Produced (MW/y)	2,774.97	2,353.73	2,392.85
Required (MW/y)			
F	8,496.46	8,496.46	8,496.46
F + D	704.90	758.04	551.30
WWT	955.23	682.89	684.52
Tot	10,156.60	9,937.40	9,732.28

fact, required less biomass (22.97 and 22.36 kg/h, respectively) than S1 (44.86 kg/h) for the fermentation pitch, along with allocation of fewer cells to recycling. Consequently, the three scenarios generated different amounts of biomass for disposal by the anaerobic digestor: 79.56 kg/h (S1), 41.77 kg/h (S2), and 41.83 kg/h (S3). For the same reason, the amount of residual ethanol found in waste streams destined to the anaerobic digestor (distillation bottoms, acid wash, and rinsing) was slightly lower in S2 and S3 (28.86 and 37.85 kg/h, respectively) than in S1 (41.20 kg/h). The same trend was observed for water associated with the waste feed: 13,216.07 kg/h (S1), 8882.93 kg/h (S2), and 8777.32 kg/h (S3). This, in turn, resulted in slightly lower biogas productivities, as well as reduced electricity and steam generation for scenario S2 and S3 compared to S1 (Table 7).

In all scenarios, energy demand surpassed the amount generated by WWT (Table 8). Hence, part of the required electricity was obtained from the grid: 7381.63 MW/y (S1), 7583.66 MW/y (S2), and 7339.43 MW/y (S3).

As already mentioned, water and steam produced in the model were recycled as heat vectors and for media preparation, particularly in the case of RO processed water. In the model, reusable water partially satisfied internal water requirements and even generated a surplus in some instances. All scenarios produced a yearly surplus of RO water: 41,370.42 m^3 (S1), 79,554.39 m^3 (S2), and 79,314.00 m^3 (S3). Conversely, water derived from sludge treatment operations covered only part of the demand, thereby forcing uptake from the grid: 2,093,971.00 m^3/year (S1), 2,263,998.49 m^3/year (S2), and 2,137,009.67 m^3/year (S3). HP steam was utilized for drying operations; whereas, MP and LP steam were employed for distillation and thermal regulation of streams.

Steam produced by combustion of internally generated biogas met only partially the demand in scenario S1, requiring 0.019 MT/h of HP steam and 7.65 MT/h of LP steam as additional inputs from the grid, while creating 0.2 MT/h of MP steam as surplus. In S2, a deficit

Table 9 TCI cost items for each scenario

TCI cost item (€)	S1	S2	S3
Equipment purchase cost	14,907,000.00	11,034,000.00	11,096,000.00
Installation	4,363,000.00	3,254,000.00	3,258,000.00
Process and piping	1,044,000.00	772,000.00	777,000.00
Instrumentation	746,000.00	552,000.00	555,000.00
Insulation	298,000.00	220,000.00	222,000.00
Electrical	1,044,000.00	772,000.00	777,000.00
Buildings	2,236,000.00	1,655,000.00	1,665,000.00
Yard improvement	746,000.00	552,000.00	555,000.00
Auxiliary facilities	1,044,000.00	772,000.00	777,000.00
Engineering	1,850,000.00	1,371,000.00	1,378,000.00
Construction	3,642,000.00	2,761,000.00	2,775,000.00
Contractor's fee	639,000.00	475,000.00	477,000.00
Contingency	1,595,000.00	1,186,000.00	1,192,000.00
Working capital	337,613.61	243,512.26	237,453.27
Start-up costs	1,707,000.00	1,272,000.00	1,271,000.00
TCI	36,198,613.61	26,891,512.26	27,012,453.27

Table 10 OPEX cost items for each scenario

OPEX cost item	S1	S2	S3
Raw materials	397,330.60	238,201.28	230,700.28
Labor-dependent	926,391.33	928,427.67	928,687.71
Depreciation	2,049,000.00	1,507,000.00	1,508,000.00
Other facility-dependent	4,074,000.00	3,018,000.00	3,031,000.00
Laboratory/QC/QA	118,000.00	118,000.00	118,000.00
Consumables	46,737.00	43,611.00	43,585.00
Waste treatment/disposal	10,830.00	7,898.00	7,573.00
Utilities	2,332,460.74	1,460,495.87	1,401,440.02
Credits	− 827,408.47	− 1,591,087.86	− 1,586,280.10
OPEX	9,127,341.20	5,730,545.96	5,682,705.91

of 3.27 MT/h for LP steam and a surplus of 0.031 and 0.34 MT/h for HP and MP steam, respectively, was reported. Finally, in S3, a deficit of 3.18 MT/h for LP steam and a surplus of 0.23 and 0.15 MT/h for MP and HP steam, respectively, were observed. This indicates that the increased fermentation temperatures and the reduced number of fermentors used in scenarios S2 and S3 led to a lower requirement for thermal regulation utilities, which was subsequently reflected in lower associated expenses in the OPEX for these scenarios.

Techno-economic analysis reveals S3 to be the best scenario

To assess the economic viability of the process and identify the best performing scenario, previously generated data on TCI (Table 9) and OPEX (Table 10) were analyzed and compared. The analysis aimed to identify, which cost item had the greatest impact on the process, highlighting areas for potential improvement or intervention. Finally, MESPs were calculated and compared for each scenario.

Scenarios S2 and S3 presented overall lower OPEX and TCI compared to S1. This disparity primarily driven by lower equipment purchase costs in S2 and S3 due to the reduced number of fermentors and secondarily by lower utility costs associated with these scenarios. Consequently, lower facility-dependent expenses (mainly maintenance and insurance) and depreciation costs were reflected in lower MESP in scenarios S2 (1.88 €/kg) and S3 (1.43 €/kg) compared to S1 (2.57 €/kg). It is clear that utilizing a microorganism capable of fermenting higher quantities of lactose can significantly improve the economic performance of the process. This improvement can be further amplified by achieving higher YE/L, lower YX/L, and increased fermentation temperatures. The use of different membrane materials as consumables for filtration in section F had no appreciable bearing on the calculated MESP. In fact, the higher nominal price for ceramic membranes was balanced by their longer life span over a projected 20-year base case. This result pointed also to a lower impact of section F on MESPs. Taking S2 as a reference, section F+D was responsible for the highest capital costs (Fig. 3), chiefly due to expenses related to the seed trains and fermentors equipment purchase costs.

Elevated equipment purchase costs influenced also the OPEX by raising depreciation costs and facility-dependent expenses, such as insurance and maintenance. These items were, in fact, the most significant components of OPEX for all scenarios (Fig. 4). Maintenance costs were calculated using the standard equipment purchase cost multiplier provided by SPD for each quoted piece of equipment due to the absence of more specific data. To mitigate the OPEX associated with the process, improved data on maintenance, such as direct quotes or stochastic prediction models [78] could be considered. Moreover, as shown in Fig. 4, the impact on OPEX resulting from the annual purchase of consumables could be considered negligible compared to other cost items. Another important consideration involves the process-associated credits. As shown in Table 10, the credits for scenarios S2 and S3 are significantly higher than those for S1, enhancing their performance in terms of OPEX and contributing to a lower MESP. This improvement is primarily due to the greater surpluses of RO water (resulting from a reduced need for water in NF lactose feed dilution) and steam (stemming from a lower demand for thermal regulation utilities due to higher fermentation temperatures) in scenarios S2 and S3.

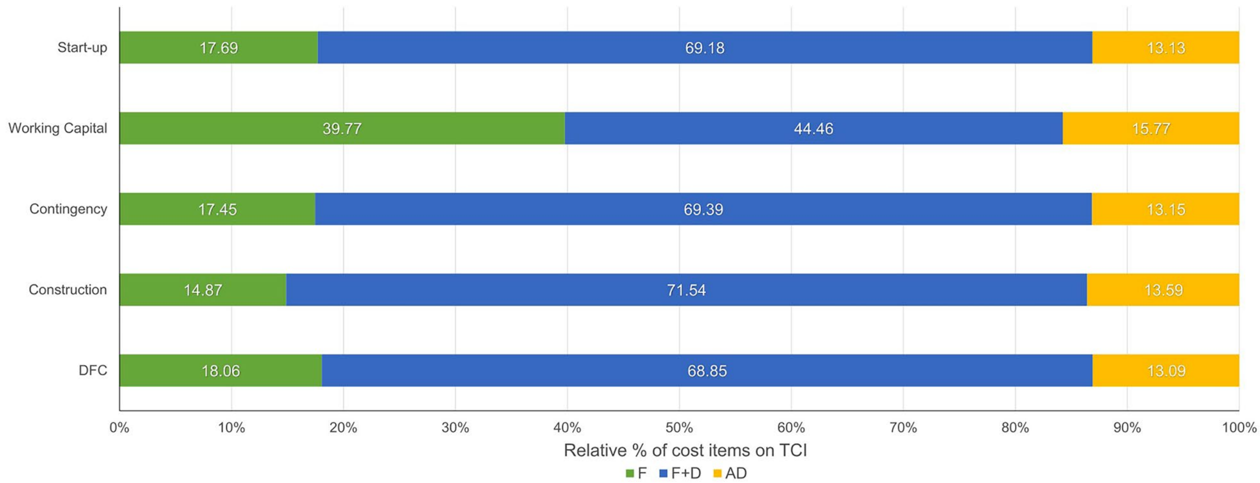

Fig. 3 Relative percentages of TCI items for S2 as a function of the different model sections

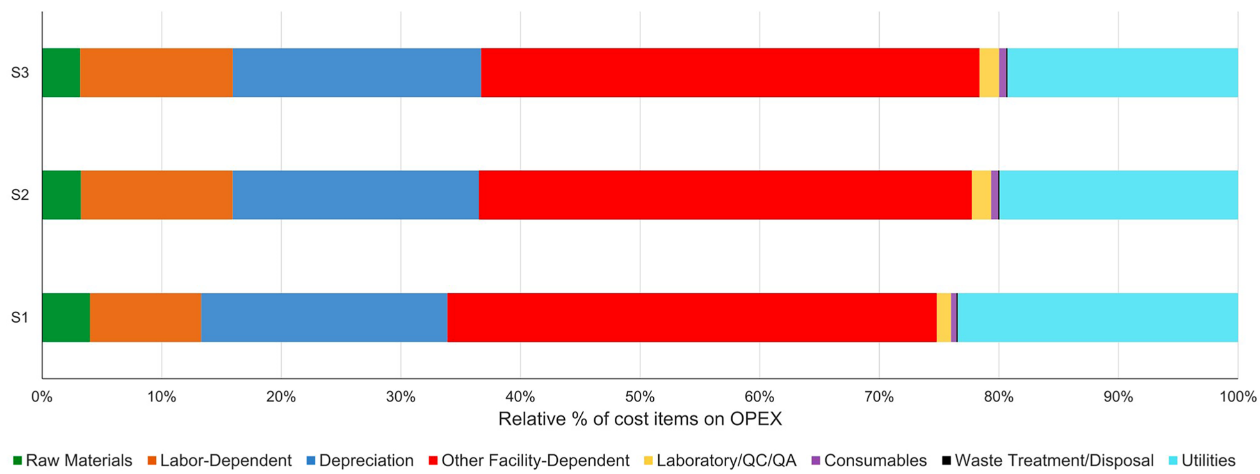

Fig. 4 OPEX composition and weight of cost items for each scenario

All scenarios returned higher MESPs than current ethanol market quotations in Italy, in line with other reports. In a study using a similar approach [46], which did not involve a pretreatment step and sought to sell biomethane to the grid coupled with the fermentation of a mixture of rye and CW to ethanol, a MESP higher than existing market quotations was also found (1.89 vs 0.49 $/l). Another study [79] integrating napa cabbage residues with CW to produce 32 m^3/year of ethanol within a small cheese manufacturing plant, reported a higher MESP than the market price (3.02 vs 0.26 $/l) prior to split-off cost allocation. This study demonstrated how, when integrated, the process lowered waste handling expenses and achieved a break-even point in only a few months. To the best of our knowledge, there are no studies evaluating the economic feasibility of industrial ethanol production from CW, especially as a stand-alone process. A comparison of the simulated MESPs with those for other feedstocks, such as 1G- or 2G-derived ethanol, could be made. It is important to state that the processes discussed here differ fundamentally in pretreatment and fermentation strategies due to the varying composition of the feedstock used. Generally, current ethanol market prices are linked to 1G-derived ethanol, commonly obtained from easily fermentable substrates such as corn and sugar-beet which require minimal pretreatment compared to both the process at hand and 2G-derived ethanol. Reported MESPs for 1G-derived ethanol have ranged from 0.32 €/kg [80] in 2014 to 0.63 €/kg [81] in 2016, depending on process configuration and substrate

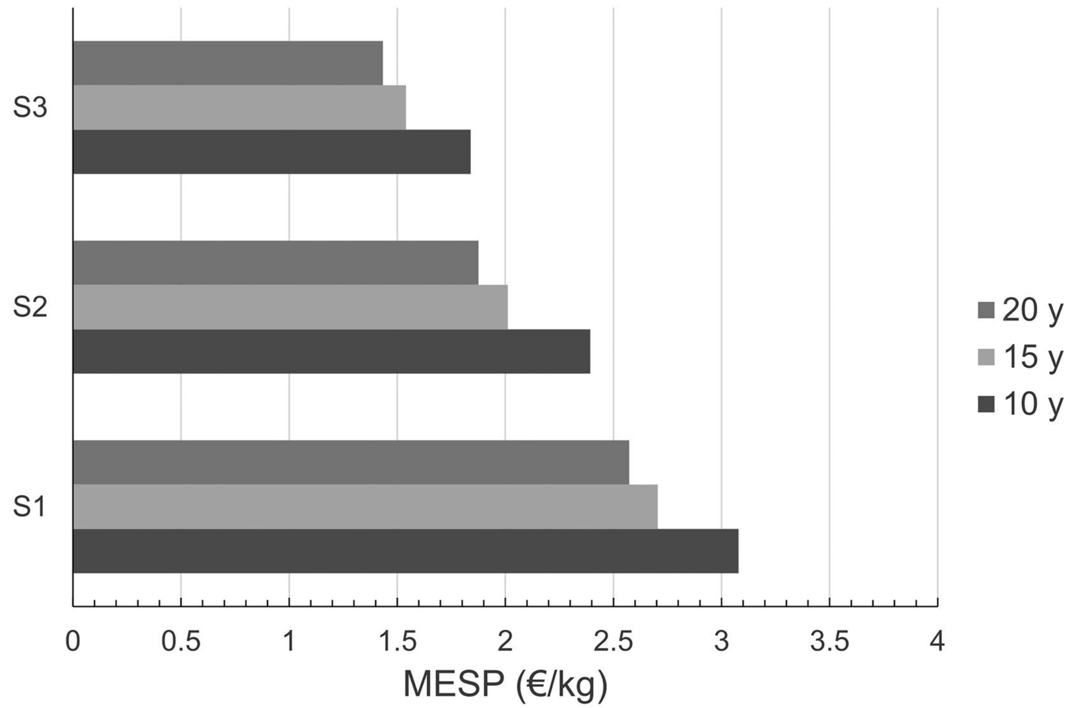

Fig. 5 Impact of a different process lifetime on final calculated MESPs

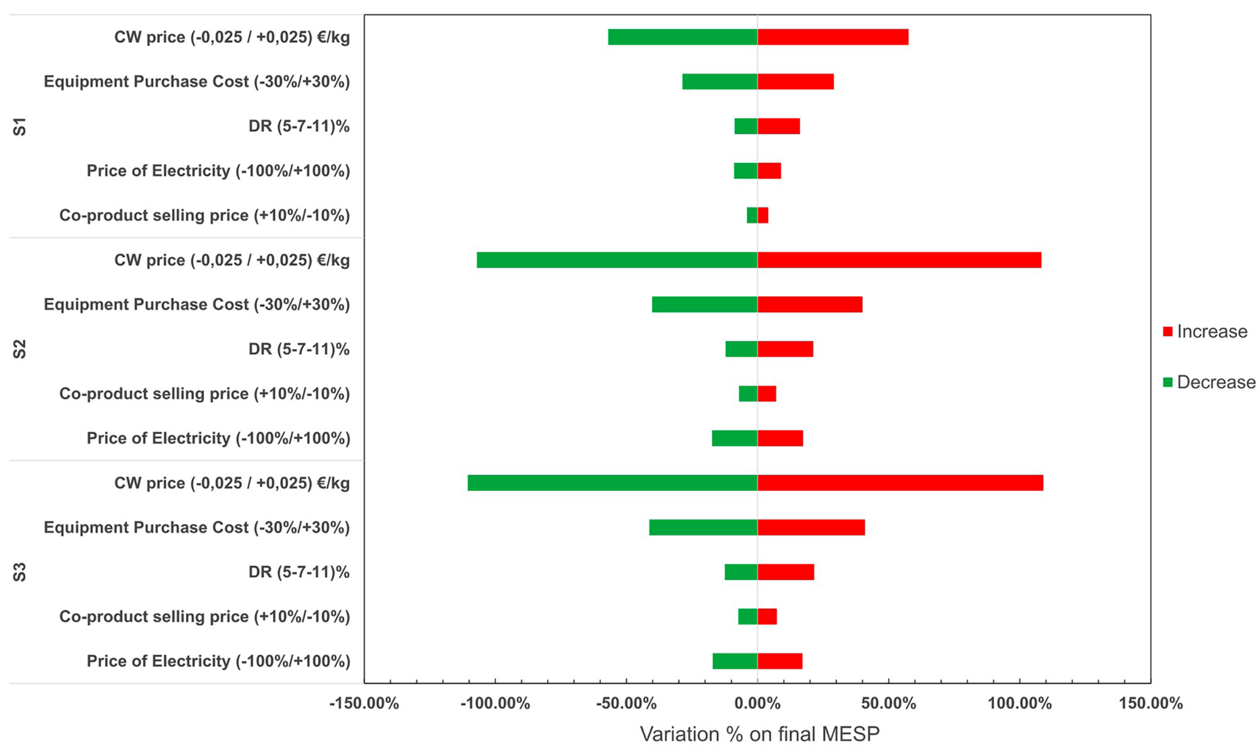

Fig. 6 Sensitivity analysis on a selected number of economic parameters and their impact on the calculated MESPs

used. For 2G-derived ethanol, ethanol selling prices vary significantly based on the producing system, with recent reports ranging around 0.15–0.93 €/kg, which highlights the dependence of MESP on the final fuel output volume [48].

Project lifetime and depreciation: shorter lifetimes allow for more flexibility at the expense of slightly higher MESPs

As expected, shorter project lifetimes led to higher MESPs (Fig. 5). This was caused by elevated depreciation costs over shorter periods, such as 10 years depreciation for 15 years lifetime and 7 years depreciation for 10 years lifetime compared to the 20 year lifetime option. Transitioning from a projected 20-year operational lifespan to 15 years had a lower impact on MESPs than switching from 15 to 10 years. Adopting a shorter lifespan may better reflect the accelerated pace of environmental policies, technology, and market conditions, potentially rendering some projects more appealing to short-term risk investors.

Sensitivity analysis: a gate fee approach for CW makes the process competitive

CW price and gate fees were shown to have the most significant impact on the variability of calculated MESP for each scenario (Fig. 6).

Scenario S1 was the least sensitive to CW price. The final calculated MESP for this scenario varied from + 58% to − 57%, resulting in a MESP ranging from 1.11 to 4.06 €/kg. Conversely, scenarios S2 and S3 were more sensitive to CW price given its strong bearing on the OPEX. Scenario S2 varied between + 108% and − 107%, resulting in a MESP of − 0.13 €/kg. The latter reflected a process that was always profitable, as it achieved lower than market prices for ethanol when the credit option for CW was evaluated. Moreover, a MESP of 3.91 €/kg was attained when CW was treated as a process cost. The same trend was observed for S3, which varied between + 109% and − 110%, and whose MESP ranged from − 0.15 to 2.99 €/kg.

When whey was assessed as a gate fee, MESPs for S2 and S3 fell below the time of study market price of 0.70 €/kg [82]. The required gate fee to reach a MESP equal to 0.00 €/kg for the different scenarios was calculated. In S1, a gate fee of 0.0438 €/kg of whey was needed, which was beyond the limit set for sensitivity analysis. In contrast, for S2 and S3, the required gate fee was 0.0233 and 0.0225 €/kg, respectively, which was within the limit. To reach a MESP of 0.70 €/kg of ethanol, S1 required a gate fee of 0.032 €/kg; whereas, S2 and S3 necessitated gate fees of 0.0147 and 0.0114 €/kg, respectively. These findings further confirmed S2 and S3 as the best performing scenarios within the current policy context of the study.

In line with what has been already mentioned for TCI and OPEX, most of the expenses were related to the purchased equipment, either directly as base purchase costs or indirectly in the form of depreciation and maintenance. This dependency also emerged also from sensitivity analysis, where even a variation in equipment purchase costs led to a significant change in MESP for all scenarios. Scenario S3 proved to be the most sensitive, with the base MESP decreasing by 13.88% when equipment purchase costs were reduced by 30%, reaching a MESP of 0.84 €/kg. Conversely, Scenario S1 was the least sensitive, showing only a ± 2.9% change in final MESP with a ± 30% variation in equipment purchase costs. Changes in the selling price of co-products did not result in substantial variations (± 4%) in MESPs for S1, although they changed by ± 7.09% and ± 7.34% in S2 and S3, respectively. The price of outsourced electricity impacted scenarios S2 and S3 more than S1. Even if the process met internally all its needs for electricity, or if the electricity price surged due to external factors (± 100%), the final MESP changed by ± 9% for S1 and ± 17% for S2 and S3. This highlights how reducing dependence on the grid could improve the economic viability of the process. Potential strategies to achieve this include optimizing AD parameters or co-digesting with other compatible waste sources in order to increase the biogas production and, consequently, electricity generation.

The use of different DRs emerged as another important factor in calculating MESPs. The different DRs analyzed in S1 and S2 yielded a variation of − 9% to + 17% and of − 12% to 20% in S3. Many studies focusing on biofuels or bioproducts utilize DRs between 7 and 11% [83–85], although typical DR's ranges from 5 to 20% are used for private and corporate investments, DRs of only 2% have also been applied when considering government-mandated investments [86]. In the present study, adopting a relatively high DR (11%) that could reflect in a higher risk protection for the investors or higher demanded returns made the operations economically challenging for all scenarios in terms of MESPs: 3.06 €/kg for S1, 2.38 €/kg for S2, and 1.83 €/kg for S3.

Conclusions and future perspectives

In this study, the potential for valorizing CW through ethanol fermentation using a simulated biorefinery integrated model based on *K. marxia*nus was assessed. The facility was assumed to be located in the Apulian region of Italy and designed to handle 539 m^3/day of CW. Different scenarios based on $Y_{E/L}$, $Y_{X/L}$ and initial [LAC] during fermentation were investigated. MESPs resulting from TEA indicated that the best performing scenarios were those capable of handling higher concentrations of substrate in the fermentation feed. This led to lower costs associated with equipment such as fermentors. The higher fermentation temperatures in S2 and S3 (37 ℃), combined with a reduced number of fermentors, led to a decreased need for thermal regulation utilities compared to S1 (30 ℃). This resulted in lower associated expenses for S2 and S3. Further improvement was observed when higher substrate concentrations were combined with a higher $Y_{E/L}$ and lower $Y_{X/L}$, resulting in the initial MESP dropping from 2.57 €/kg (S1) to 1.88 €/kg (S2) and 1.43 €/kg (S3). Sensitivity analysis conducted on all scenarios confirmed the economic sustainability of the model, which strongly depended on the strategy employed during raw material acquisition and management. Market-advantageous outcomes were observed only with a gate fee approach. If such an approach was no longer supported by current policies, process improvement would be necessary. Section F+D was the most expensive in terms of both OPEX and TCI for all scenarios. As shown by sensitivity analysis, a reduction in equipment purchase costs by 30% resulted in a noticeable drop in the final calculated MESP for all scenarios. Therefore, further optimization of the process should involve exploring the potential advantages of fed-batch or continuous fermentation setups. These approaches could enhance productivity and reduce the scale of required equipment. Achieving results similar to those in scenario S3, while ideally reducing fermentation time, would enable faster batch processing and increase overall process efficiency. However, achieving this goal, requires better data describing the kinetics [87] of fermentation and biomass growth of the selected microorganism. Moreover, a plant that already owns part of the required equipment (e.g., ethanol fermentation plants, distilleries, waste treatment plants) could mitigate the costs associated with TCI and OPEX by repurposing expensive items such as fermentors and distillation towers, thereby implementing the current process as a plug-in addition. Nonetheless, commercializing ethanol in a different market could provide more economic leeway for a company trying to profit from the hypothesized process alone. According to internal intelligence from Italbiotec srl (personal communication), the market price of solvent-grade ethanol or ethanol biofuel for household heating could be as much as 1.20 €/kg. However, due to current model limitations, this potential cannot be readily assessed. A higher level of detail in the simulation is needed to evaluate the impurities generated during the process and their concentration in the final product. This would help determine the feasibility of classifying the ethanol as food-grade (EU 2019/787) or pharmaceutical-grade (as per European Pharmacopoeia), thereby granting access to new market segments. Considering the scale of the plant, larger sizing would help lower MESPs by taking advantage of economies of scale, as demonstrated for 1G- and 2G-derived ethanol [29, 48, 49, 88]. Another option would involve the use of alternative waste biomass in combination with CW to produce ethanol, maximizing production and diversifying the obtained co-products. Similar approaches are reported in the studies by Utama et al. [79] and Cunha et al. [89], where co-fermentation of napa cabbage residuals and corn cobs, respectively, achieved promising ethanol yields [79, 89]. Additionally, Cunha et al. [90] and Gibbons and Westby [91] demonstrated the use of eucalyptus wood and stillages to enhance ethanol production when combined with CW [90, 91]. However, scaling up the process presents several challenges. While the unit operations used in the simulations are based on established technology that integrates well, scaling up involves logistical challenges related to handling and transporting large volumes of CW, which may be susceptible to spoilage and contamination. To address these issues, a well-organized supply chain is essential for managing the scale of operations effectively. Moreover, implementing the proposed model could address the problem of CW disposal in Italy, particularly in the Apulia region, while also contributing to sustainable biofuel production. This would help valorize a typically underutilized waste material and support circular economy principles and environmental policies.

Abbreviations

CW	Cheese whey
WPC	Whey protein concentrate
MESP	Minimum ethanol selling price
YPD	Yeast peptone dextrose
YPDA	Yeast peptone dextrose agar
SSM	Semi-synthetic medium
SPD	SuperPro Designer
TEA	Techno-economic analysis
UF	Ultrafiltration
NF	Nanofiltration
MF	Microfiltration
RO	Reverse osmosis
$Y_{E/L}$	Ethanol yield on lactose
$Y_{X/L}$	Biomass yield on lactose
GAC	Granular activated carbon
AD	Anaerobic digestor
AO	Aerobic oxidation
HP	High pressure steam
MP	Medium pressure steam
LP	Atmospheric pressure steam
[LAC]	Lactose concentration
OPEX	Operating expense
TCI	Total capital investment
DR	Discount rate
CCNL	Contratto Collettivo Nazionale di Lavoro
QC	Quality control
DFC	Direct fixed costs
DC	Direct costs
IC	Indirect costs
OC	Other costs
WC	Working capital
SU	Start-up costs and validation
EWC	European Waste Code
1G	First generation
2G	Second generation
SPD	SuperPro Designer

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13068-024-02567-5.

Additional file 1.

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Author contributions

M.C., I.P., M.B. and G.A. designed the study. C.D. performed wet-lab experiments. M.C. performed in silico simulation, data analysis, techno-economical analysis and sensitivity analysis. I.P., G.A., M.B., M.J. and S.M. supervised the study. I.P. and G.A. provided funding. M.C. drafted the manuscript. G.A., I.P., and M.B. revised the manuscript. All authors have read and approved the final manuscript.

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Availability of data and materials

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information Files. Should any raw data files be needed in another format, they are available from the corresponding author upon reasonable request. No datasets were generated or analyzed during the current study.

Declarations

Competing interests

The authors declare that they have no competing interests

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