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Early-stage economic and environmental impact assessment for optimized bioprocess development: Monoterpenoid indole alkaloids

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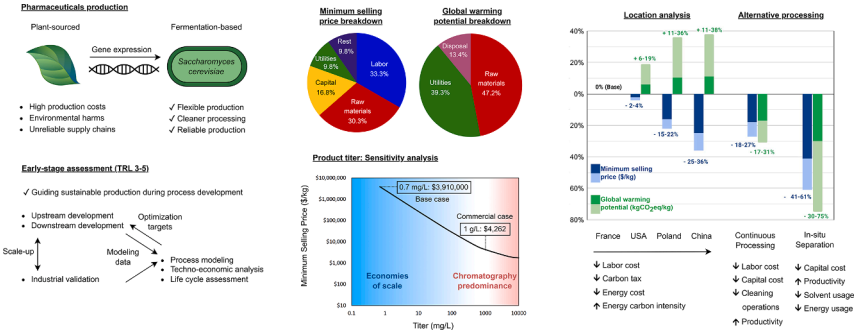
HIGHLIGHTS

- Microbial tabersonine production in *S. cerevisiae* is modeled in early-stage development.
- Base case 0.7 mg/L titer showed an MSP of 3,910,000 $/kg and GWP of 2,540 $kgCO_2eq/g$.
- At 1 g/L titer, MSP and GWP are respectively estimated as 4,262 $/kg and 6.36 $kgCO_2eq/g$.
- Chromatography costs are found predominant in higher titers (>100 mg/L).
- Plant location indicated a trade-off between economic and environmental performance.

GRAPHICAL ABSTRACT



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ABSTRACT

Microbial refactoring offers sustainable production of plant-sourced pharmaceuticals associated with high production costs, ecological harms, and supply chain dependencies. Here, microbial tabersonine production in *Saccharomyces cerevisiae* is modeled during early-stage development (TRL: 3–5), guiding decisions for process-scale economic and environmental optimization. The base-case 0.7 mg/L titer indicated a minimum selling price (MSP) of $3,910,000/kg and global warming potential (GWP) of 2,540 $kgCO_2eq/g$. The industrial process at 1 g/L resulted in an MSP of 4,262 $/kg and a GWP of 6.36 $kgCO_2eq/g$. Location analysis indicated a sustainability trade-off between France, USA, Poland, and China, with the written order of declining MSP and increasing GWP. Continuous processing promised reducing the MSP by 18–27 %, and the GWP by 17–31 %. In-situ product extraction during fermentation was estimated to lower the MSP by 41–61 %, and the GWP by 30–75 %. In addition to showcasing a combined TEA-LCA on biopharmaceuticals, the early-stage assessment approach guides bioprocess optimization.

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1. Introduction

Thanks to the great diversity of metabolic pathways plants host, they naturally synthesize a plethora of products currently commercialized in various industries such as pharmaceuticals, agrochemicals, food, and textile (Romero-Suarez et al., 2022). The pharmaceutical industry is particularly dependent on plant-based sources; for instance, 22.7 % of therapeutics approved by the US Food and Drug Administration (FDA) in 1981–2019 are comprised of plant-based natural products and their derivatives (Romero-Suarez et al., 2022).

However, these products are usually obtained via extraction processes utilizing plants directly as raw materials, which are associated with significant economic and environmental costs. Further, plants produce a mixture of diverse, yet structurally similar molecules, which necessitates a complex, thus expensive and laborious purification process. On top of that, they are usually found in minuscule amounts in their host plants, resulting in excessive consumption of plants as raw materials in addition to the other process resources. As the consumed plants are endemic that are raised in specific locations, this results in ecological risks in terms of land use and biodiversity, as well as market uncertainties associated with their supply chain (Romero-Suarez et al., 2022; J. Zhang et al., 2022). Accordingly, rampant exploitation of plant-based natural products has been identified as the most dominant cause for biodiversity loss in many regions of the world (Schulze et al., 2018). A particular case for this is monoterpenoid indole alkaloids (MIAs), that are traditionally obtained via extraction of endemic plants such as *Catharanthus roseus* and *Voacanga africana*, and used as therapeutics including anticancer and antipsychotic drugs (Romero-Suarez et al., 2022; J. Zhang et al., 2022). Consequently, current industrial production of MIAs is far from sustainable.

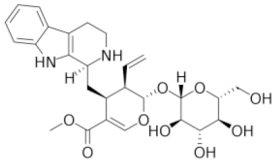
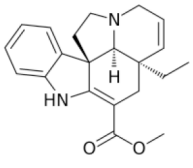
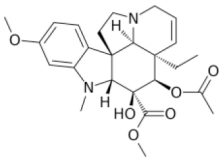
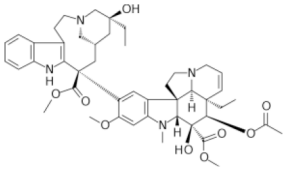
To address this challenge with an eco-design approach, the consortium for Refactoring Monoterpenoid Indole Alkaloid Biosynthesis in Microbial Cell Factories (MIAMi) [Grant agreement No: 814645] aims to develop novel MIA production routes using engineered yeast cell factories, coupled with a viable downstream processing to source MIA compounds via complete sustainable bioprocesses. Technologically, microbes, such as yeast, can grow faster compared to the plants (in a scale of hours for the former whereas months/years for the latter), can be metabolically engineered to finetune the product mixture to simplify the downstream processing, and can be cultivated via standardized and scalable fermentation-based manufacturing processes (Wang et al., 2019). By not exploiting endemic plants as raw materials, these process routes would mitigate the environmental setbacks, as well as reduce the supply chain costs and uncertainties (Kulagina et al., 2021; Romero-Suarez et al., 2022). Recent studies have focused to improve product titers for yeast-based MIA production by optimizing fermentation conditions, towards ultimately feasible bioproduction routes (Liu et al., 2021).

Albeit the remarkable benefits of the microbial production process, a sizable challenge in this quest is the human and financial resources to develop specific strains efficiently producing the relevant compounds in industrial standards, which takes an average of 6–8 years and $ 50 Million for a specific strain (Nielsen & Keasling, 2016). Furthermore, designing a corresponding downstream process and scaling-up the process from lab-scale to manufacturing scale tends to cost in the scale of $ 100 Millions (Crater & Lievense, 2018). In parallel, the global warming potential (GWP) metric is increasingly referred to measure the environmental performance of novel technologies (Galusnyak et al., 2022). Hence, modeling and analysis of the eventual microbial processes, during the development phase, would save considerable resources by determining the bottlenecks to guide the optimized technical aspects (Grasa et al., 2021). In that sense, process simulation software (e.g., SuperPro Designer®) can be used to model and, thus, evaluate the economic and environmental impacts of processes, respectively via techno-economic analysis (TEA) and life cycle assessment (LCA) (Ögmundarson et al., 2020). Combination of TEA and LCA for process optimization is being practiced increasingly commonly, and mostly for commodity chemicals (Meramo et al., 2022). Merging of these methods has shown to reduce risk of suboptimization of economic and environmental performance by providing respectively holistic sustainability assessments of chemicals (Meramo et al., 2022; Ögmundarson et al., 2020).

From the perspective of the field of sustainability assessment, there are very few examples of combined economic and environmental assessment for biopharmaceutical products, particularly via rigorous TEA and LCA. Amasawa *et al.* conducted a cost-benefit analysis on economic and environmental trade-offs for different monoclonal antibody (mAb) cultivation scenarios using Chinese hamster ovary cells. The economic aspects solely considered operational expenses (OPEX), while the environmental impacts are estimated via LCA (Amasawa et al., 2021). Bunnak *et al.* also compared upstream mAb production configurations by using cost of goods sold as an economic metric and applying LCA for the environmental assessment (Bunnak et al., 2016). Riazi *et al.* combined TEA and LCA to compare isostearic acid production routes based on traditional soybean oil extraction and tall oil upgrading processes (Riazi et al., 2019). Specifically on MIAs, the need for economic (Dusséaux et al., 2020) and environmental (Michailidou, 2023) assessments have also been highlighted in the literature. Hence, combining TEA and LCA for a microbial production process based on strain engineering to produce a pharmaceutical compound would also be a leading contribution to the field of impact assessment.

Considering some specific MIA compounds and their bioproduction in plants, a particular one, strictosidine, serves as the platform molecule that is essentially requiring glucose and tryptophan for its natural synthesis. Strictosidine is modified by the plant metabolism via a series of chemical reactions into more than 3,000 natural MIA molecules (Stander et al., 2020). A commercially significant MIA molecule is vinblastine, which is used to treat various cancers including Hodgkin's

Table 1
Chemical Formula and Structure of Mentioned MIA Compounds.

Molecule	Strictosidine	Tabersonine	Vindoline	Vinblastine
Formula	$C_{27}H_{34}N_2O_9$	$C_{21}H_{24}N_2O_2$	$C_{25}H_{32}N_2O_6$	$C_{46}H_{58}N_4O_9$
Structure				
Market Price	n/a (no established market)	2,500 $/kg (GlobalReach Business Solutions, 2019)	3,000 $/kg (ImportGenius.Com, 2022)	100,000 $/kg (ImportGenius.Com, 2022)

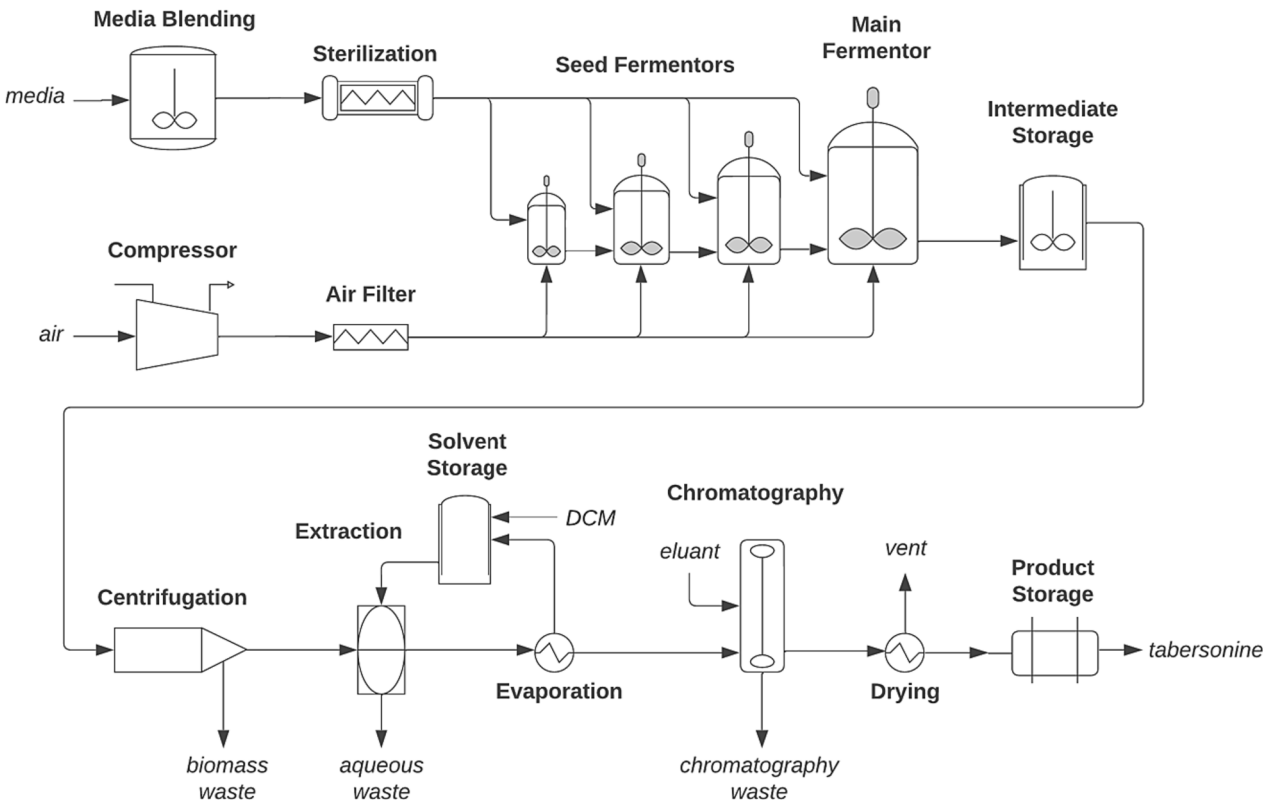


Fig. 1. A simple process flow diagram visualizing the microbial tabersonine production process.

lymphoma, lung cancer, bladder cancer, brain cancer, and testicular cancer (J. Zhang et al., 2022), and listed as an essential medicine by WHO (World Health Organisation, 2021). Regarding the metabolic pathway, vindoline is a precursor MIA molecule in vinblastine production (J. Zhang et al., 2022). Similarly, vindoline is produced via a series of reactions from its MIA precursor, tabersonine (J. Zhang et al., 2022). Recently, an optimized yeast strain efficiently producing vindoline from tabersonine with 88 % conversion was reported (Kulagina et al., 2021). Hence, an efficient process complimentarily producing tabersonine *de novo* from glucose is a fundamental step towards sustainably obtaining vinblastine. From an economic perspective, as being a precursor for anticancer therapeutics, tabersonine has also a market value of 2500 $/kg (GlobalReach Business Solutions, 2019). Chemical formula, structure, and market price of the MIA compounds mentioned in this paragraph are provided in Table 1, with the order of formation considering the metabolic pathway.

In the present work, the production process of tabersonine from glucose using a yeast strain developed by the MIAMi consortium is evaluated during the development stage (Technological Readiness Level (TRL): 3–5). During the conceptual process model construction, the input from upstream (fermentation) and downstream development research outcomes were implemented into the simulations. By conducting rigorous TEA and LCA, bottlenecks for the overall process route were determined, and optimization ideas were developed. The outcomes, optimization strategies, and relevant economic and environmental trade-offs are elaborated. As being the first combined TEA-LCA study considering a biopharmaceutical product based on strain engineering, this work is also a guiding contribution to the impact assessment field.

2. Materials and methods

2.1. Workflow and methodology

To estimate the economic and environmental impact of a full-scale microbial production process for tabersonine, simulations are performed based on the experimental data, documented by the upstream and downstream development teams, as well as the industrial partners of the MIAMi consortium (see supplementary information for the collaborative workflow). Based on the experimental outcomes, the upstream data mainly considers the fermentation stage including the operating conditions, media composition and preparation, and content of the product outlet stream. Technical data for the downstream processing include the selected equipment, operating conditions, technical requirements, and recovery and purification capacities. Based on the provided data, simulation models are designed in SuperPro Designer® to conduct the impact assessments. Economic impacts are estimated based on the simulation models via TEA. The modeling results including material and energy flows and required utilities are used as a basis for the LCA. The learnings, in terms of economic and environmental improvement points and optimization possibilities, are regularly conveyed to the relevant teams to improve the process development. Reciprocally, the models are regularly updated with new data by progressing experimental developments as well as outcomes from upscaling. Hence, the overall production process is coherently developed in an efficient and targeted direction, while the process simulation models are continuously validated.

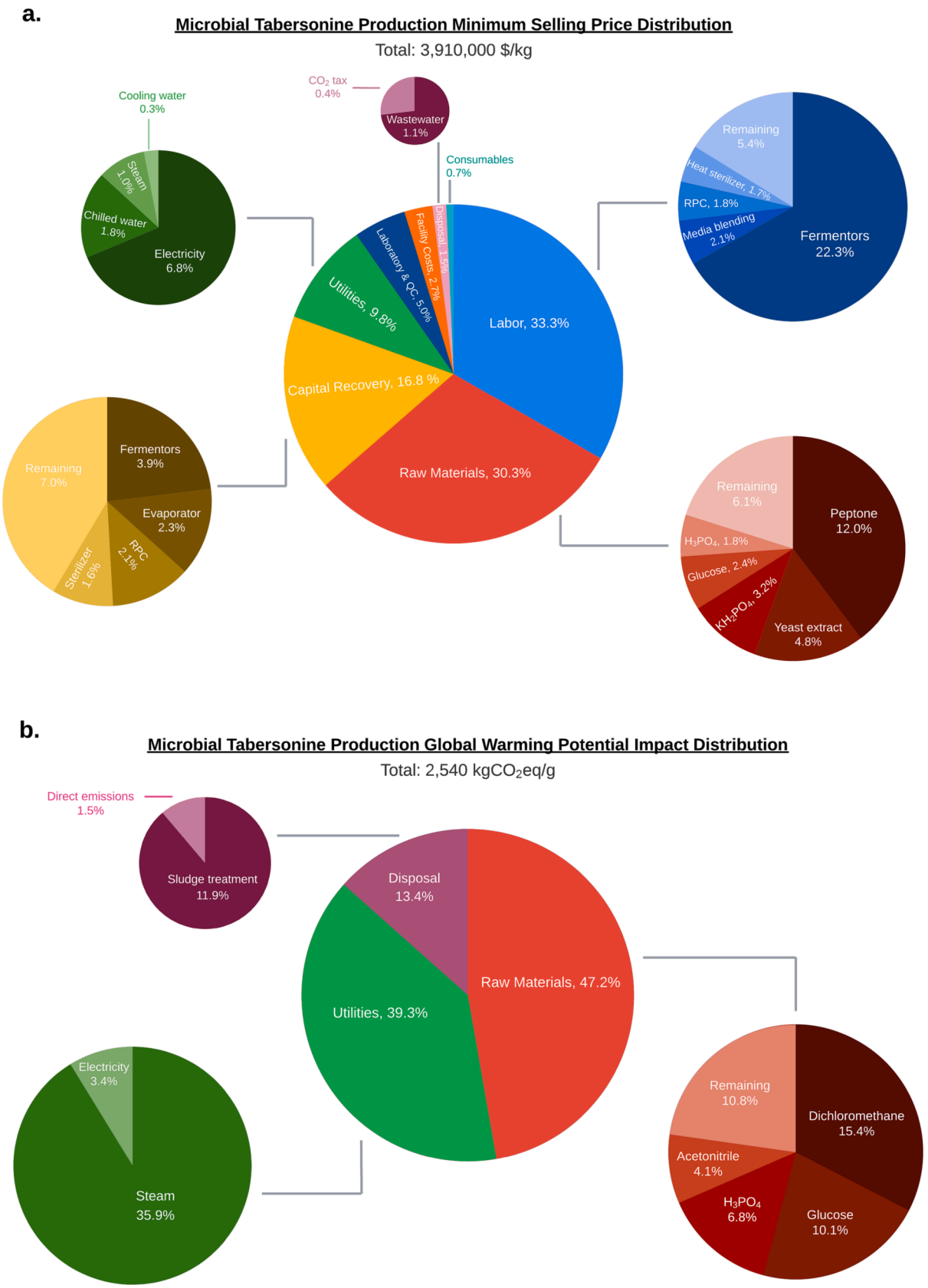


Fig. 2. A) minimum selling price ($/kg) breakdown for microbial tabersonine production process. rpc abbreviation stands for the reversed-phase chromatography unit. b) global warming potential ($kgco_2eq/g$) breakdown for the base case of the microbial tabersonine production process.

2.2. External data

As described, the process simulations are based on the provided technical data (see supplementary information for more details). As informed by the strain engineering teams, fermentation takes place at 30 °C at ambient pressure in 180 mL fermentors, with nutrient media containing 10 g/L yeast extract, 20 g/L BactoTM peptone, and 75 g/L glucose among other compounds. The product of fermentation stage contains 0.7 mg/L extracellular tabersonine, in addition to the metabolic side products and unused raw materials. Downstream processing data are provided by the project partner Axyntis Group. Separation of the biomass particles from the fermentation broth is handled by centrifugation. The target compounds from the aqueous phase are extracted to an organic phase by liquid–liquid extraction using dichloromethane (DCM) of 3 times as the feed volume. Tabersonine is recovered in the organic phase. The organic phase is dried by rotary evaporation at 20 mbar. Reversed-phase chromatography is performed to purify tabersonine from its similar compounds. Here, octadecylsilane is used as the resin and a mixture of 79.92 % water, 20 % acetonitrile, 0.08 % formic acid is used as the eluant. Tabersonine is obtained with 47.2 % recovery and 99 % purity. Eluant consumption is 13 times the chromatography bed volume, and the product is obtained in an eluant of 0.6 bed volume. The mixture is dried under 20 mbar to obtain the product.

2.3. Process modeling

The process flow diagram of the simulation configuration developed in SuperPro Designer® is illustrated in Fig. 1. Components of the nutrient media are introduced to the blending unit. The mixture is heat sterilized before feeding to fermentors. In parallel, air is filtrated prior to entering the fermentors. The fermentation stage consists of 4 fermentation units with respective sizes of 50 L, 500 L, 5 m^3 and 50 m^3. The scale of 50 m^3 is decided to benefit from the economies of scale while not risking mass dispersion problems (Meyer et al., 2017). The resulting broth is introduced to an intermediate storage unit, to ensure continuous downstream processing. The subsequent centrifuge unit removes the solid particles, mainly the biomass. Tabersonine and its similar side products are extracted from the aqueous phase to the organic phase (comprised of DCM) at the liquid–liquid extraction unit. This unit is coupled with an evaporation unit, enabling solvent recycling. The stream is introduced to the chromatography column purifying tabersonine from its similar side products. The resulting stream is dried under vacuum and stored at 4 °C as recommended. Cleaning procedures based on SuperPro Designer® involving a caustic wash (with 2 wt% NaOH), acidic wash (with 5 wt% H_3PO_4) and steam are implemented in the equipment operating in batch mode. Thermodynamic parameters of materials are used as in the databank of SuperPro Designer®. For molecules with no available data, thermodynamic properties are estimated via molecular modeling in ChemDraw® and ProPred®. Strictosidine aglycone is used as the model compound to simulate the metabolic side products of the tabersonine production in thermodynamic estimations, in coordination with the strain engineering team.

2.4. Techno-economic analysis

The TEA is initially conducted for the model described above (Fig. 1), which is considered as the base case. Modified versions of the base case are subsequently designed to analyze the impact of certain parameters and alternative processing strategies.

Regarding equipment costing, the purchased cost of fermentors is estimated by The National Renewable Energy Laboratory (NREL) data (Davis et al., 2016), based on vender prices. Cost of the heat sterilizer, air filters, product storage unit, and the auxiliary equipment are estimated by SuperPro Designer®. Prices for the rest of the equipment are estimated via guidelines described in Rules of Thumb in Chemical Engineering Practice (Woods, 2007) (see supplementary information for further details).

Pricing of raw materials are referred to The European Commission for glucose (The European Commission, 2022a), Zauba.com (Zauba.Com, 2021) for tryptophan, monopotassium phosphate, yeast extract, bacto peptone, leucine, formic acid, dichloromethane, acetonitrile and phosphoric acid, ECHEMI.com (ECHEMI.Com, 2022) for ammonium sulfate, dichloromethane, formic acid and sodium hydroxide, ChemAnalyst.com (ChemAnalyst.com, 2022) for ammonium sulfate and sodium hydroxide, and ImportGenius.com (ImportGenius.Com, 2022) for Kromasil C18 chromatography resin (see supplementary information for more details).

Considering different locations, currency exchange rates of 1.14 EUR/USD and 0.15 CNY/USD are applied when necessary (Financial Times, 2022). Regarding electricity prices, 0.22 $/kWh for France (European Commission - Eurostat, 2022), 0.17 $/kWh for Poland (European Commission - Eurostat, 2022), 0.084 $/kWh for China (Global Petrol Prices, 2022) and 0.075 $/kWh for USA (Energy Information Administration, 2022) are used. Carbon prices are applied as 87 $/MT-$CO_2$ for France and Poland based on the Emission Trading System (ETS) of the European Union, whereas no carbon pricing is applied for China and USA (The World Bank, 2022). Facility costs including the equipment maintenance and property insurance are estimated based on NREL guidelines (Davis et al., 2016). Labor rates for different locations are based on IHS data (Arne, 2016), assuming one supervising engineer per six plant operators (54.9 $/h for France, 25.6 $/h for Poland, 10.8 $/h for China, 56.0 $/h for USA). Laboratory and quality control (QC) costs are estimated as 15 % of labor expenses.

Plant lifetime is considered 30 years, including 2.5 years of construction. France is selected as the base plant location, being the location of the MIAMi project partner Axyntis Group. Economic analyses are based on 2021 prices. The interest rate for the internal rate of return is considered 5 % among selected locations. Minimum selling price (MSP) calculations are assessed by determining the product price over the plant lifetime to result in a zero net present value.

2.5. Life Cycle assessment

Based on the mass and energy balances provided by the process models, a Life Cycle Assessment (LCA) was conducted to assess the environmental impacts of microbial tabersonine production, guiding on the eco-design objectives. SimaPro version 9.1.0.8 (SimaPro, n.d.) was used for the LCA modeling. The impact assessment method used is ReCiPe 2016 (Huijbregts et al., 2017). The results of the conducted LCA focus on global warming potential ($kgCO_2eq$) and water consumption (m^3) impact categories. The LCA is conducted following the guidelines of the International Reference Life Cycle Data System (ILCD) (European Commission, 2010), according to the ISO 14040 and 140,444 standards (International Organization for Standardization (ISO), 2006a, 2006b). For background processes in the LCA modeling, the Ecoinvent 3.6 was used (Wernet et al., 2016). The system boundary of the study was set as cradle-to-factory gate.

3. Results and discussion

3.1. Base case: Economic and environmental evaluation

The TEA for the base case process is based on the base process described above (Fig. 1). The MSP is estimated as 3,910,000 $/kg. With the current titer of 0.7 mg/L, the minimum selling price (MSP) is substantially higher than the market price of tabersonine (2,500 $/kg). The breakdown of MSP, with itemized contributions, is illustrated in Fig. 2a. As seen, the MSP is mainly contributed by the OPEX (83.2 %) whereas the fraction of capital recovery is relatively small (16.8 %). Since CAPEX estimates are based on the purchase cost of equipment, the distribution of equipment prices is also provided. As in the case of most bioprocesses, fermentors and the chromatography unit (note that the chromatography

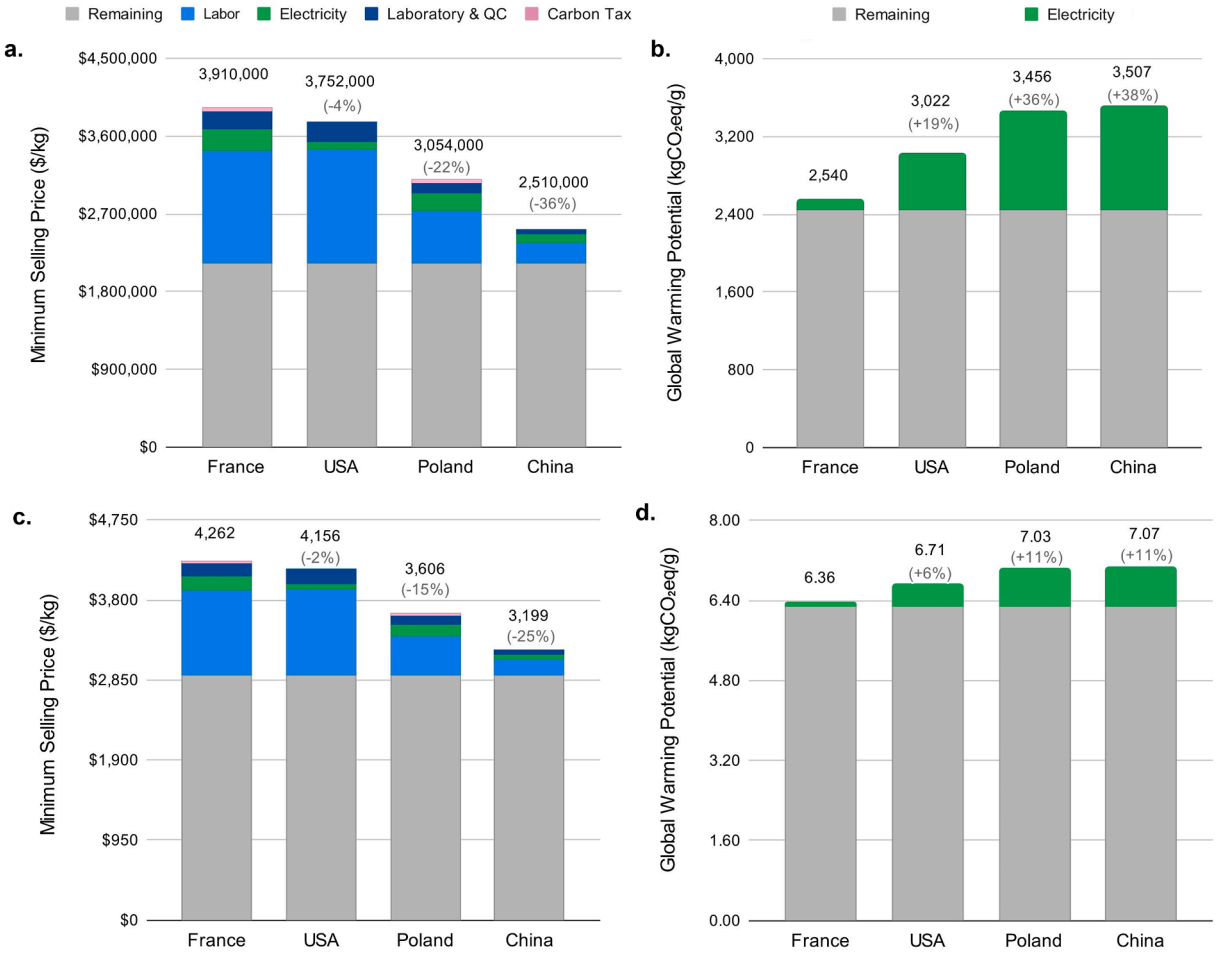


Fig. 3. Impact of plant location on a) Minimum selling price ($/kg) at 0.7 mg/L tier, b) Global warming potential ($kgCO_2eq/g$) at 0.7 mg/L titer, c) Minimum selling price ($/kg) at 1 g/L titer, d) Global warming potential ($kgCO_2eq/g$) at 1 g/L titer,

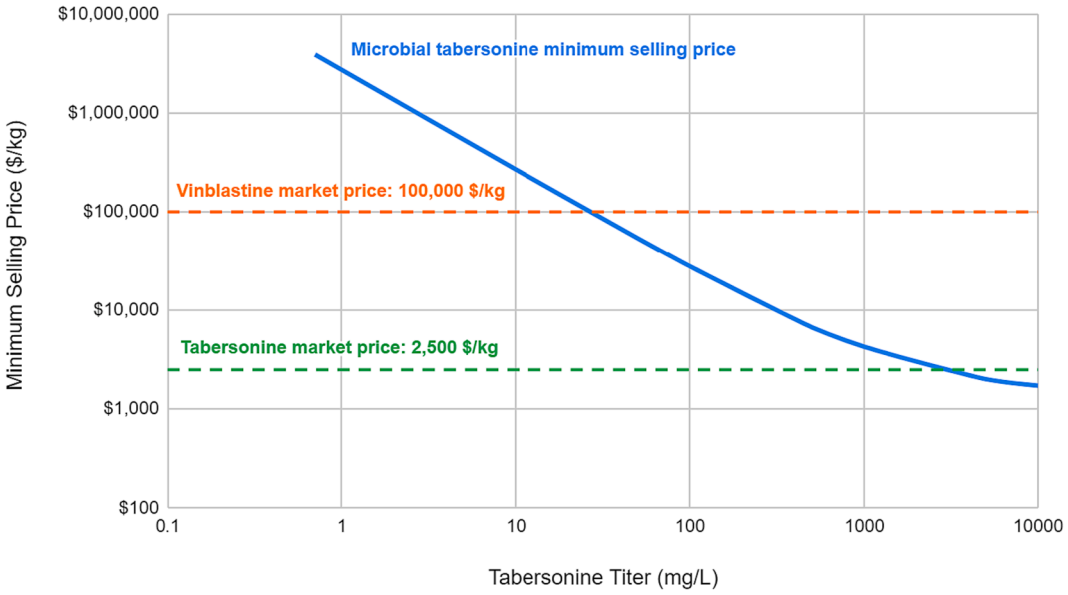


Fig. 4. Sensitivity analysis: Impact of tabersonine titer on minimum selling price ($/kg).

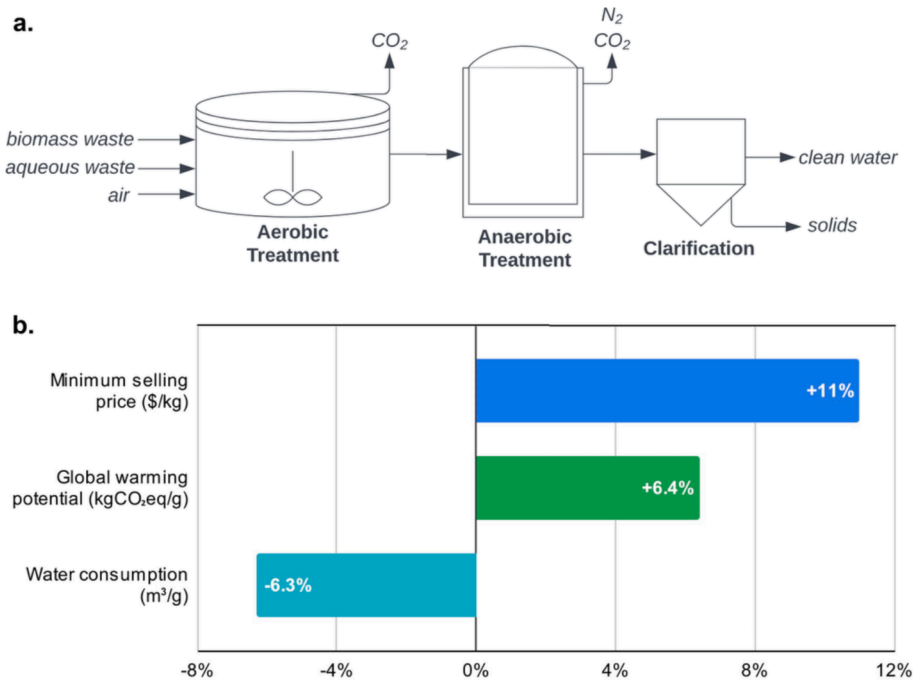


Fig. 5. A) a simple process flow diagram visualizing the water treatment system b) impact of the water treatment system to the economic and environmental performance for the base case.

column in the base case is 80 L due to the low titer, whose size and thus cost is expected to increase in parallel with the strain optimization) are substantial contributors. Labor and raw materials are the main fractions of the OPEX as well as the MSP. As expected, the units operating in batch mode, such as the fermentors, media blending unit, the reversed-phase chromatography (RPC), and heat sterilizer dominate labor expenses. Fermentors, particularly, comprise around two-thirds of labor expenses, and approximately a fifth of the whole MSP overall. Components of the yeast nutrient media (peptone, yeast extract, KH_2PO_4, glucose, leucine, tryptophan) make up 83.7 % of the raw material costs, corresponding to 25.4 % of the overall MSP. Utility costs are mainly contributed by the electricity, making up 6.8 % of the overall MSP.

Results of the Life Cycle Assessment (LCA) for the base case show an impact of 2,540 kg CO_2 eq per gram of tabersonine (kgCO_2eq/g). As detailed in Fig. 2b, drivers of the environmental impacts are the intensive use of chemicals (47.2 %), as well as high consumption of utilities in the production process (39.3 %), which altogether account for 86.5 % of the GWP. This is a common challenge seen in the few available LCAs for fine chemicals (Amasawa et al., 2021)(Kong et al., 2021). Regarding individual contributions, steam accounts for around two-fifths of the GWP overall. This outcome particularly exemplifies the contrast between economic and environmental process aspects, given that steam is estimated to contribute 1.0 % of the MSP (see Fig. 2a) whereas 35.9 % of the GWP. The steam required for solvent recycling during the extraction comprises 22.5 % of the overall GWP, indicating a major environmental hotspot. Moreover, the current extraction solvent DCM comprises 15.4 % of the overall GWP as a raw material. Therefore, investigating environmentally more benign solvents or optimizing solvent consumption for more effective yield could enhance the process performance. Finally, disposal is estimated to comprise 13.4 % of the overall GWP, that is chiefly driven by the sludge treatment whereas direct CO_2 emissions account for 1.5 % of the overall GWP.

3.2. Impact of different plant locations

Location dependent parameters, namely, labor, laboratory and quality control (which is estimated as 15 % of labor), electricity price, and carbon tax comprise a significant fraction of the MSP when combined, making around the half of it with 45.5 %. Simultaneously, the geographical location is a significant parameter for the environmental performance as well, due to the regionality of the energy mix. Therefore, the impact of different possible plant locations was investigated. The base case considered France as the plant location. Poland, China, and USA are selected as different plant locations to study the regional aspects.

Comparing France and Poland, though both being EU countries, labor rates and electricity prices are significantly lower in Poland compared to France. Selecting the location as China further reduces the labor rates and electricity prices as well as cuts the costs for carbon pricing. USA also does not apply carbon tax and electricity price is lower than China; however, the labor rate is slightly higher than France.

Economic performance in terms of MSP by different plant locations are provided in Fig. 3a. As illustrated, switching the location to Poland or China reduces the MSP respectively by 22 % and 36 %, with lower labor rates, electricity prices, and, for China, also lower carbon taxes. On the other hand, selecting USA as the plant location reduces the MSP by 4 % compared to France, with lower electricity prices and no carbon taxes, however with slightly higher labor costs.

Effect of regionality in terms of environmental performance is illustrated in Fig. 3b. Results show that locating the production in France or USA is beneficial to reduce the GWP impacts. Moving the location from France to China would increase the GWP by 38 % due to the substantial difference in electricity footprint. It is also noted that the environmental cost by countries in terms of GWP resulted in the opposite trend of MSP as visualized in Fig. 3a and Fig. 3b, showing the tradeoff between economic and environmental performance. This argument, though, needs to be explored further due to potential tradeoffs in environmental impacts considering the use of nuclear energy in France and the USA, potentially leading to higher ionizing radiation impacts compared to the other countries. Also, it needs to be stated that the average electricity mix for the USA varies between states, so a state-based analysis can guide towards more specific conclusions.

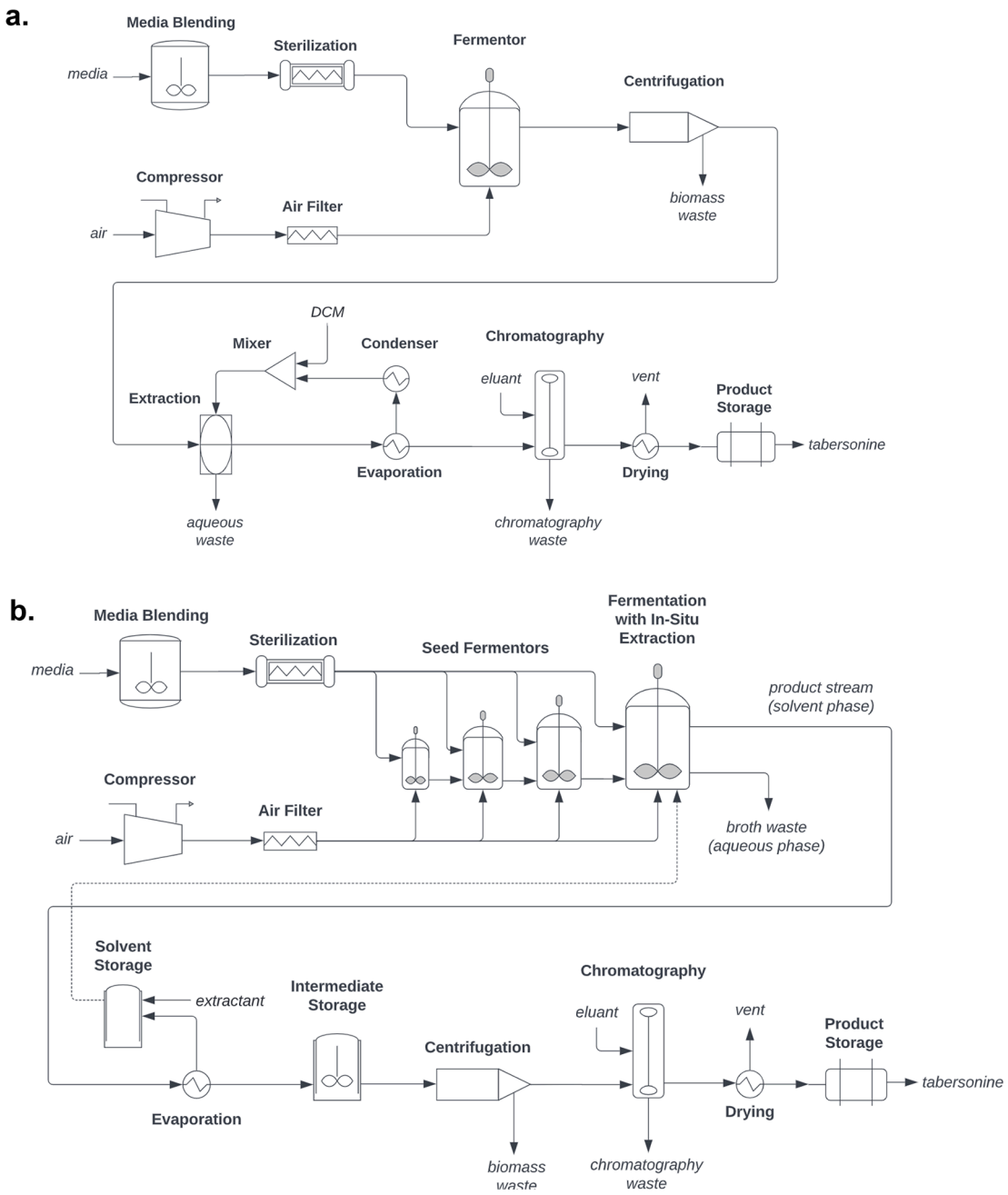


Fig. 6. A) process flow diagram for the continuous microbial tabersonine production process, b) process flow diagram for the microbial tabersonine production process featuring a fermentation unit with in-situ product removal, c) minimum selling price by process case at 0.7 mg/L, d) Minimum selling price by process case at 1 g/L, e) Global warming potential by process case at 0.7 mg/L, and f) Global warming potential by process case at 1 g/L.

3.3. Impact of product titer on process performance

As the market price for tabersonine is substantially lower than the MSP due to the currently small tabersonine concentration, the impact of tabersonine titer on the economic performance is investigated. The consolidated process models and simulations are used to foresee scenarios that would result in an economically viable and environmentally sustainable scenario, providing targets to the experimental workflow. The outcomes of this sensitivity analysis are visualized in Fig. 4. Due to the economies of scale (since more product is produced utilizing the same process), the MSP shows a linear decrease until a scale of 100 mg/L with the reducing labor, nutrient media, utility and capital recovery costs in addition to the other minor contributors. In contrast, with titers reaching to g/L scale, the advantages diminish due to the dominating chromatography costs that are spent to purify per quantity of tabersonine – namely, resin, acetonitrile and formic acid. Ultimately, the MSP reaches 1,700 $/kg (within the vicinity of product price) with a titer of 10 g/L. Higher titers were not investigated since the 10 g/L titer corresponds to c.a. 35 % of the global tabersonine market; hence, higher titers were not considered economically realistic utilizing the process in this scale. All in all, the analysis reveals that a titer in the scale of g/L should be achieved to promise an economically viable process for tabersonine.

To note, as a breakthrough, microbial vinblastine production using S. cerevisiae has been demonstrated recently, via complete refactoring of vindoline and catharanthine pathways followed by their semisynthesis

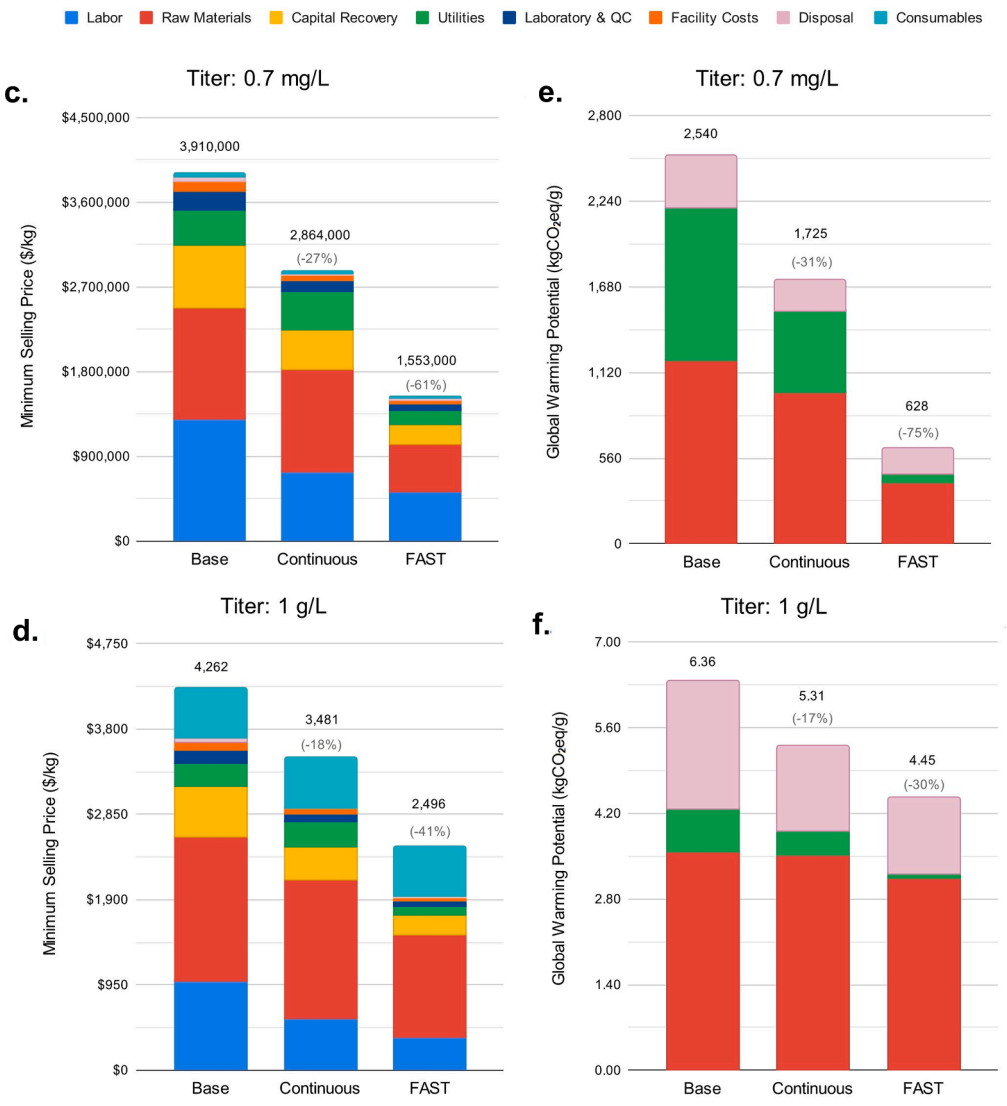


Fig. 6. (*continued*).

to vinblastine (J. Zhang et al., 2022). With a similar molecular structure to tabersonine (see Table 1), considering the same upstream and fermentation design combined with a similar subsequent downstream processing, the proposed process design can be also considered as a benchmark for microbial vinblastine production. Therefore, with a market price of 100,000 $/kg (ImportGenius.Com, 2022), titers in the scale of 10 – 50 mg/L (see Fig. 4) could achieve commercial viability in case of microbial vinblastine production according to the outcomes of this study.

As explored, titers in the scale of g/L are promising for economic viability for microbial tabersonine production. Hence, the case of 1 g/L is analyzed to describe the process when the commercially relevant titer is reached. Compared to Fig. 2a, though most cost contributors significantly diminish per produced tabersonine due to increased fermentation performance, the chromatography costs (equipment purchase, resin, eluant) proportionally raise with the increased tabersonine amount since that needs to be purified. In numbers, the chromatography column, chromatography resin, and the eluant including acetonitrile and formic acid respectively comprise 1.6 %, 13.1 % and 19.1 % of the overall cost cumulatively making 33.8 % of the MSP in the 1 g/L case - which were respectively 2.1 %, 0.7 % and 0.9 %, thus, cumulatively 3.7 % in the base case. Accordingly, chromatography is detected as a sizable contributor of the overall cost in such commercially viable titers, in line with many cases in the bioprocess industry (Roque et al., 2020). Dependence of MSP by different locations at a titer of 1 g/L is provided in Fig. 3c. As visualized, location-dependent costs constitute a relatively smaller fraction in this case. Moving the plant from France to USA, Poland, or China reduces the overall cost respectively by 2 %, 15 %, and 25 % - compared to the respective 4 %, 22 %, and 36 % of the base case.

Complementing the economic analysis, environmental performance of the process with 1 g/L titer is estimated via LCA. This scenario resulted in a GWP of 6.36 $kgCO_2eq/g$. Moreover, the environmental impact of this case is also investigated by different plant locations as provided in Fig. 3d. Since the relative importance of electricity is lower in this case, the difference in GWP by different plant locations is estimated to be slight, with all results being in the range of 6.36 – 7.07 $kgCO_2eq/g$.

3.4. Impact of water treatment plant addition

As described, a significant amount of water has been consumed throughout the fermentation process. In addition, sludge treatment is responsible for 11.9 % of the overall carbon footprint as shown in Fig. 2b, around half of which is resulted from the disposal streams from the centrifugation and extraction units combined. Hence, to improve the environmental performance of the process in terms of freshwater

consumption and with possible carbon footprint reductions, the addition of a water treatment plant is evaluated. Fig. 5a depicts the water treatment system modeled in SuperPro Designer®. As seen, the biomass waste and extraction waste streams, respectively released from the centrifuge and the liquid–liquid extraction units (see Fig. 1), are merged and supplied into an aerated tank for aerobic digestion and nitrification. Via aerobic digestion, organic material is converted into carbon dioxide, water, and ammonia (Misailidis & Petrides, 2021). Simultaneously, ammonia is converted into nitrite by nitrification bacteria (Intelligen Inc., 2021). At the unaerated tank, nitrite is converted to nitrogen by denitrification bacteria (Bernhard, 2010). Finally, biomass particles and ashes are separated at the clarification unit. Though a fraction of water is lost in the final unit, due to the water production by the microorganisms in the initial units, an overall 46.5 m^3 clean water is obtained. As 44.6 m^3 freshwater is used per batch of fermentation (which with the other ingredients adds up to 50 m^3 media volume), the water treatment plant completely recovers the required water for the fermentation. However, since 48.3 m^3 water is also consumed in the cleaning operations, the 46.5 m^3 clean water production corresponds to 50.1 % of the overall freshwater needs.

The impacts of this scenario on the overall process performance is illustrated in Fig. 5b. As seen, addition of a water treatment system would increase the MSP by 11 %. The water treatment units mainly add to the labor and carbon tax expenses while the impacts on capital expenses and utilities are relatively small. Accordingly, since these are usually location dependent parameters, this scenario would add to the MSP by 9 % in USA, 8 % in Poland, and 3 % in China for comparison (see supplementary information for more details).

Regarding the LCA results, as provided in Fig. 5b, adding the water treatment process decreases the overall water footprint by 6.3 % from 46.0 to 43.1 m^3/g but also increases the GWP by 6.4 %, from 2,540 to 2,704 $kgCO_2eq/g$. This shows a trade-off between the two impact categories. To note, though the GWP by the sludge treatment decreased in this case, the overall GWP increased mainly due to the increase in direct CO_2 emissions released by the microbial digestion as indicated in Fig. 5a, in addition to the utilities required for the treatment units. Furthermore, this case study demonstrates that, though fermentation processes are notorious for direct freshwater consumption (Bunnak et al., 2016; Yuan et al., 2021), the overall water footprint may lie in other parts of the production process, which can be determined LCA. In this case, the water footprint is mainly contributed by two raw materials, namely phosphoric acid by 48.3 % and glucose by 14.6 %, whereas the fraction of direct water consumption is estimated as 12.7 %. As a result, adding a water treatment system reduced the direct water consumption by 50.1 %, which corresponded to a 6.3 % reduction in the overall water footprint. To target more significant reductions in the water footprint, different feedstocks for the nutrient media or different agents for process cleaning can be investigated.

3.5. Alternative processing: The fully continuous process

According to the results of the base case, as visualized in Fig. 2a, labor is a sizable fraction of the MSP. Hence, as continuous processes tend to require approximately half the labor cost of the corresponding batch mode (Peters et al., 2004), the case of a hypothetical fully continuous operation is evaluated. Continuous fermentation operation with *S. cerevisiae* have been reported in previous studies (Su et al., 2020; X. Zhang et al., 2021). Nevertheless, the feasibility of fully continuous operation requires to be verified for the specific production and scale. Accordingly, the purpose of this case study is to assess the potential impacts of such continuous operation.

The process flow diagram of the continuous process is provided in Fig. 6a. Different from the batch case (see Fig. 1), seed fermentors are not used in the process, considering that solely the nutrients will be continuously supplied to the fermentor. Also, the intermediate storage unit between the fermentation and centrifugation units, whose purpose in the base case was to enable continuous operation for the subsequent downstream units, is not used in this case. Regarding the recycling of the fermentation solvent, considering fully continuous operation, the storage unit is replaced with a condenser and mixer for the make-up. The process is based on the equivalent fermentation capacity of the base case. As two main fermentors of 50 m^3 volume with 96 h residence time operated in staggered mode in the batch case, the continuous process likewise involves two fermentors of 50 m^3 volume with 96 h residence time, operating in parallel. As transferring times and cleaning procedures are not included in the continuous case, the overall production also increased 10 % compared to the batch case.

Economic impact of the continuous process in terms of MSP is comparatively provided in Fig. 6c for 0.7 mg/L titer and Fig. 6d for 1 g/L titer. For the former, the continuous process reduced the overall production costs by 27 % compared to the batch case, mainly via reduced labor -also noting that laboratory & qc are estimated as 15 % of labor cost- and capital expenses. The outcomes are in line with previous findings, as such economic advantages of continuous bioprocessing have been previously reported (Mahal et al., 2021; Yang et al., 2019). For the commercially estimated case of 1 g/L titer, as visualized in Fig. 6d, the difference in MSP is 18 % between the batch and continuous cases, since both cases consume similar amounts of raw materials (the little advantage of continuous case is mainly due to reduced consumption of cleaning agents) and chromatography resin as consumable. Accordingly, dominant chromatography costs in higher titers that make the overall MSP difference relatively smaller.

The impact of continuous processing on the environmental performance is comparatively displayed for 0.7 mg/L titer in Fig. 6e and for 1 g/L titer in Fig. 6f. Accordingly, continuous processing promises to reduce the GWP by 31 % (from 2,540 $kgCO_2eq/g$ to 1,725 $kgCO_2eq/g$) for the current titer and 17 % (from 6.36 $kgCO_2eq/g$ to 5.31 $kgCO_2eq/g$) for the commercial titer, demonstrating its improvement on the ecodesign objectives. The advantages are mainly driven by the absence of cleaning procedures, particularly steam-in-place (SIP) and clean-in-place (CIP). The absence of SIP reduces steam consumption, thus, improving the performance by less utilities consumption. Regarding CIP, the impact of raw materials decreases by the reduced H_3PO_4 and NaOH cleaning solutions consumption and, correspondingly, the cut of their addition to the sludge treatment impacts after the procedure. The outcomes are in line with the literature, as the significance of CIP/SIP procedures on the environmental impacts of fermentation processes have been previously reported via LCA (Pietrzykowski et al., 2013).

3.6. Alternative processing: Fermentation with in-situ product extraction

As a relatively novel concept, fermentation units with in-situ product separation by extraction promise increased productivity and process intensification. The process equipment with various unit designs have been successfully verified at a laboratory scale (Teke & Pott, 2021). Upscaling the concept towards the commercial scale, the biotechnology company DAB.bio in The Netherlands reported productivity increased to 2.4 times and solvent consumption reduced by 93 % with their novel design of Fermentation Accelerated by Separation Technology (FAST) fermentation system (Pappas & Oudshoorn, 2021) (see supplementary information for the schematic design). In the FAST process, the fermentation operation proceeds in batch mode. The in-situ extraction operation is handled by continuously supplying the extractant from the bottom of the reactor, continuously binding with the product. The extractant containing the product is continuously removed from the top of the reactor. The extractant volume inside the reactor was reported to be 7 % of the conventional mixer-settler case (*vide supra*). Thus, with the significantly reduced solvent flow rate, the subsequent downstream equipment is downsized.

Moreover, the centrifugation unit is downscaled, since minimal biomass exists in the product outlet stream – as the product is not in the aqueous phase, in this case. Based on this information, the fermentation

Table 2
Parameters of Selected Solvent Alternatives.

Solvent	Boiling Point (°C)	Density (kg/m^3)	Hansen Parameters Dispersion Bonds	Polar Bonds	Hydrogen Bonds	Price ($/kg)	GWP ($kgCO_2eq/kg$)	Bacterial Toxicity*	Yeast Toxicity*
Dichloromethane	40	1323	17	7.3	7.1	0.6 (ECHEMI.Com, 2022; Zauba.Com, 2021)	3.6 (Wernet et al., 2016)	0.65–6.5 g/L (Byers & Sly, 1993)	2.5–5 g/L (Ramel et al., 1996)
Methyl *tert*-butyl ether	55	886	Thermo Fisher Scientific, MIT (Fisher Science Education, n.d.)			1.0 (Zauba.Com, 2021)	Not found	10–15 g/L (Roslev et al., 2015)	740 mg/L (Roslev et al., 2015)
Ethyl acetate	77	750	15.8	5.3	7.2	1.3 (Indiamart, 2022)	2.8 (Wernet et al., 2016)	10 g/L (Wilbanks & Trinh, 2017)	> 6.41 g/L (Fan et al., 2019)
2-methyl tetrahydrofuran	80	902	16.8	4.8	4.6	1.7 (Bangalore Ashok et al., 2022)	0.2 (Clarke et al., 2018)	1 g/L (Sigma-Aldrich, 2022)	Not found

*In toxicity columns, ">" is to indicate that the relevant study reports undisrupted microbial growth at the corresponding concentration. The values given in a range is to indicate that growth inhibition is observed to start in the corresponding range.

with in-situ product extraction, referred to as the "FAST" case, is hypothetically modeled with a productivity of 2.4 times and a solvent consumption of 7 % of the base case. To note, the FAST process requires a solvent that is not toxic to the organism -in this case, yeast- and has a density lower than water so that it can travel to the top of the unit when sparged from the bottom. Since dichloromethane satisfies neither criteria, a different solvent would be required to actualize this process. Accordingly, this case is studied to assess the FAST process's possible impact on the overall performance. Alternative solvents, that can be utilized in this production scheme, are investigated as an outlook of this project and detailed in section 3.7.

The process flow diagram of the modeled process is provided in Fig. 6b. Different from the base case, the product leaves the fermentation unit in the solvent phase, while the aqueous phase containing the broth is removed after the operation. The solvent is sparged from the bottom of the FAST reactor during the operation.

The economic performance of the FAST process is comparatively provided in Fig. 6c for the 0.7 mg/L titer and Fig. 6d for the 1 g/L titer. As visualized in Fig. 6c, the FAST process resulted in a dramatic 61 % decrease in the MSP for the current titer. The remarkable difference is mainly due to the increased productivity, resulting in a relative decrease in all costs per product as more tabersonine is produced using a system of similar scale. The reduction in MSP is highest in capital recovery and facility cost subcategories, thanks to the downsized equipment and the lack of extraction unit. For the commercial case of 1 g/L titer, as illustrated in Fig. 6d, the FAST process still remarkably reduces the MSP by 41 % compared to the base case. The smaller reduction in MSP compared to the lower titer case is due to the chromatography costs at higher titers that is required per amount of product, reducing the advantage of increased productivity. Accordingly, as visualized in Fig. 6d, raw material and consumable are the closest among the cost subcategories comparing the base and the FAST process cases. The closer raw material costs are due to the chromatography eluant components, including acetonitrile and formic acid, while the consumable costs are solely contributed by the chromatography resin. To note, licensing terms that can be applied to the fermentation unit are not covered in this assessment.

Regarding the environmental performance, the FAST process is estimated to diminish the GWP by 75 % (from 2,540 $kgCO_2eq/g$ to 628 $kgCO_2eq/g$) for 0.7 mg/L titer and 30 % (from 6.36 $kgCO_2eq/g$ to 4.45 $kgCO_2eq/g$) for 1 g/L titer, as respectively visualized in Fig. 6e and Fig. 6f, indicating it as a very promising option considering the ecodesign objectives. Like the MSP, the reduced environmental costs are mainly driven by the increased productivity, resulting in reduced impacts among different categories. The environmental benefits of the FAST process outweigh its economic advantages in lower titers, thanks to the diminished extraction solvent consumption. Accordingly, DCM consumption as raw material combined with the steam usage for its recycling comprise a sizable fraction of the overall GWP for the base case. In contrast, these contributions are relatively minor for the MSP (*vide supra*). Also, similar to the MSP, the closer result in higher titers is due to the increasing relative importance of the chromatography chemicals, such as acetonitrile and formic acid in this case.

3.7. Solvent alternatives to replace dichloromethane

Several aspects of the extraction solvent choice have been encountered and discussed throughout the assessment. As described in section 3.1, a third of the product's entire GWP is related to the solvent, including the required recycle energy (22.5 %) and the addition of solvent make-up (15.4 %) for the base case. Regarding the addition of a water treatment system, as explained in section 3.4, DCM cannot be used due to its bacterial toxicity. Moreover, as explained in section 3.6, DCM is not workable with the FAST process due to its toxicity to yeast and also having a higher density than water - as it cannot travel to the top of the reactor when sparged from the bottom. Hence, solvent alternatives with less environmental impact, consumption, and toxicity would potentially optimize the environmental performance while considering the compatibility with recommended guidelines for safe and sustainable by design chemicals (The European Commission, 2022b).

A field study on different solvent alternatives was conducted as an outlook. To begin with, Hansen solubility parameters are standard to predict solvent properties and interpret their compatibility in specific applications. Accordingly, solvents with close Hansen solubility parameters, namely, dispersion bonds (δD), polar bonds (δP), and hydrogen bonds (δH), tend to work in closely related applications (Novo & Curvelo, 2019).

Thus, Table 2 is prepared with the possible solvent alternatives to replace DCM. To note, since evaporation is used to recycle the most of the solvent in the recycling system, solvents with boiling points higher than water are not considered. One of the potential alternatives is methyl *tert*-butyl ether (MTBE). Though the specific Hansen parameters for MTBE were not found in the literature, a study by Thermo Fisher Scientific cross-referring to the Massachusetts Institute of Technology (MIT) suggested MTBE as a possible less toxic substitute to DCM (Fisher Science Education. n.d). The price of MTBE is in a similar range to DCM, and it is promising if the water treatment system is considered concerning its higher tolerance of bacterial toxicity. More studies (e.g., LCA) are required for MTBE to estimate its possible impact on environmental performance.

Ethyl acetate (EtOAc) appears to be a promising alternative with less microbial toxicity. Its compatibility can be studied for the water

treatment plant and FAST process cases, for instance, via growth inhibition tests. With a density lower than water, EtOAc can also be sparged from the bottom to the top of the FAST reactor. EtOAc also promises to reduce the GWP if used in a similar amount with the DCM. To estimate such environmental advantages accurately, the required extractant volume per broth for EtoAc should be determined to make a fair comparison. To note, though the price of EtOAc is more than twice of DCM per weight, in terms of volume, that is equivalent to 1.3 times with its lower density. Still, it might have a sizable impact on economic performance, and thus, the corresponding trade-offs should be analyzed if considered in future phases of this project.

As sourced from lignocellulosic biomass, 2-methyl tetrahydrofuran (2-MeTHF) appears to be very promising to reduce the GWP of the process (Clarke et al., 2018), with its 94 % less GWP than DCM in terms of weight. Similar to the EtOAc case, as it is a relatively costly option, the overall trade-offs should be analyzed prior to switching to 2-MeTHF. This option might not be compatible with the water treatment case due to its bacterial toxicity. To be considered for the FAST case, its toxicity to yeast should be investigated.

Hence, MTBE is suggested to be a promising and relatively economical option if the water treatment plant is considered. With its less toxicity for yeast and bacteria, EtOAc can be compatible with water treatment and FAST process cases. EtOAc can also improve the environmental performance, but the economic trade-offs should be assessed since it is a costly solvent. If improving the environmental performance is prioritized, 2-MeTHF can be very promising with its relatively smaller carbon footprint; however, its higher price should also be considered. To note, the technical performance of all the alternatives should be analyzed (e.g., determining the exact required amount for desired product recovery) to draw more concrete conclusions for further assessments.

4. Conclusions

Offering a sustainable alternative to plant-sourced routes, microbial tabersonine production at 0.7 mg/L titer indicated an MSP of $3,910,000/kg and GWP of 2,540 $kgCO_2eq/g$. The commercially relevant 1 g/L titer suggested the respective values as 4,262 $/kg and 6.36 $kgCO_2eq/g$. Different plant locations (France, USA, Poland, China) resulted in a trade-off between economic and environmental performance. Continuous processing is estimated to lower the MSP by 18–27 %, and the GWP by 17–31 %. In-situ product extraction offered reducing the MSP by 41–61 %, and the GWP by 30–75 %. Combined economic-environmental assessment is showcased to optimize bioprocesses from early-stage development.

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

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2023.130005.

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