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PSE PRESS

Process design for a novel fungal biomass valorisation approach

Theresa Rücker[a*], Matteo Gilardi[a*], Thomas Brück[b], and Bernd Wittgens[a]

[a] SINTEF Industry – P.O. Box 4760 Torgarden, NO-7465 Trondheim, Norway
[b] Technical University of Munich – Werner Siemens-Chair of Synthetic Biotechnology, Lichtenbergstr. 4, 85748 Garching, Germany
* Corresponding Author: theresa.rucker@sintef.no, matteo.gilardi@sintef.no - These authors contributed equally to this work.

ABSTRACT

The European Union is transitioning towards a circular and low-carbon economy, emphasizing renewable biological resources. This study explores the production of high-value compounds like chitosan from fungal biomass and presents a potential design for a sustainable biorefinery process, contributing to the diversification and optimisation of biomass feedstock utilisation. The process simulation includes dedicated sub-models for each unit operation, based on laboratory data and integrated into a comprehensive process flow sheet using COCO-COFE. The productivity of the simulated plant results in 2 500 tons of triglyceride oils and 1 800 tons of chitosan that can be produced from 15 000 tons of *Aspergillus niger*. On-site acetic acid production meets 45% of the total plant's demand, significantly reducing the amount of additional acetic acid to be purchased as raw material. Additionally, large-scale enzyme consumption and the substantial heat demand for biomass processing are key economic and environmental factors that need to be central in successful process design.

Keywords: waste valorisation, process design, sustainable product development, biomass conversion, data-driven modelling.

INTRODUCTION

The European Union is shifting towards a circular and low-carbon economy, which focuses on renewable biological resources [1]. A promising alternative to conventional plant biomass is the utilisation of non-plant biomass, such as microbial, fungal, and animal-derived materials. One example is the production of high-value compounds, such as chitosan, from fungal biomass [2]. A significant advantage of valorising non-plant biomass is the reduction in competition with food production. Unlike plant biomass, which often requires arable land and can lead to biodiversity loss and land use changes, non-plant biomass can be sourced from waste streams and by-products, minimising competition with food and environmental impact [3]. Innovative technologies are essential to diversify and optimise the use of available, underutilised, and more sustainable biomass feedstocks. An integrated strategy that can replace multiple conventional products derived from fossil feedstocks or unsustainable agricultural practices, such as palm oil production, is urgently needed.

A promising new conversion process focuses on the upcycling the residue of the fungus *A. niger*, generated in large quantities during citric acid production (over 2 million tonnes produced yearly, globally [4]).This biomass is expected to reach a scale of 3.3 million tons by 2028 [4] and holds potential for higher-value applications beyond its current use as low-value animal feed. This study investigates the design of a biorefinery process, which comprises parallel production of two value-added products, namely yeast oil as a greener alternative to palm oil and non-animal-derived chitosan.

Yeast oil can be obtained from a fermentation process of solubilised *A. niger* biomass (hydrolysate) followed by a hydrolysis process. The resulting oil is based on triglycerides showing relative palmitic, stearic, oleic, and linoleic acid contents similar to the ones observed in conventional plant-based oils, such as palm and coconut oil. The oil can be used directly or hydrolysed by chemicals (acid or base) or enzymes to yield free glycerol and a mix of free fatty acids resembling closely those of rapeseed and sunflower oil. Thus, yeast oil can be valorised as biobased chemical in a variety of applications such as

cosmetics formulations, biofuel production, the food industry, and the pharmaceutical sector. It is noteworthy, that downstream processing of the raw yeast oil obtained from fermentation and hydrolysis can lead to the production of specific additives for cosmetics (stearates), as well as coating applications (alkyd resins). The main expected benefits are the high oil purity (no need for solvent traces removal), reduced specific greenhouse gas emissions with respect to conventional plant-based oils, good recycling potential, biodegradability, and sustainability.

Chitosan, which can be derived from chitin fraction of the fungal biomass, and its higher-value derived products, such as chitosan oligosaccharides (CHOS), are non-toxic and biocompatible. Moreover, a part of these derivatives is characterised by a low molecular weight, which guarantees their water-solubility and enhances the spectrum of possible applications. Remarkably, CHOS have a wide variety of interesting properties for applications in food, agriculture, medicine, pharmaceuticals, and cosmetics [5].

The developed process simulation consists of dedicated sub-models for each unit operation, which are based on laboratory data and implemented as plug-ins and further integrated in a comprehensive process flow using COCO-COFE v3.6, a license-free CAPE-open simulation software developed by AmsterChem. The process is sized based on the fungal waste biomass generated in an industrial-size citric acid production plant located in Germany. The mass and energy balances from the simulation are exploited for a preliminary feasibility study of the new proposed process. In particular, this study shows the energy consumption associated with all the involved unit operations and the Key Performance Indicators (KPIs) of the novel fungal biomass valorisation process.

MODELLING

Block Flow Diagram

The process comprises six primary steps, as displayed in Figure 1. Initially, the fungal biomass undergoes enzymatic solubilisation, yielding a sugar-rich aqueous solution. This hydrolysate is first utilised for on-site acetic acid production. In a subsequent main fermentation step, a lipid-rich yeast biomass is cultivated, utilizing residual glucose and acetic acid as carbon sources. All nutrients, such as salts and inerts, are for simplicity not displayed in the model. The lipids produced are then released during the hydrolysis step, resulting in the separation of the product into three distinct phases: solid, aqueous, and oil. Concurrently, the non-solubilised, chitin-rich fraction from the enzymatic solubilisation of the fungal residues is subjected to a combination of enzymatic and chemical treatments to produce chitosan and CHOS.

Sub-models

Specific sub-models have been developed for the characterisation of each unit operation. A data-driven approach has been adopted where needed. According to this methodology, the conversion and yield of the reactions involved in the process are defined using correlations that contain adjustable parameters, which are fitted directly to the available experimental data. Therefore, the developed sub-models are only valid within the tested ranges of operating conditions and types of feedstocks.

We emphasise, that the process simulation approach and the criteria adopted to define representative molecular formulas for complex species are using well-established and thoroughly calibrated methods based on previous work [6].

Enzymatic solubilisation

The process can be characterised as a hydrolysis reaction, where glucan polymers are broken down to form simple glucose monomers with the addition of water

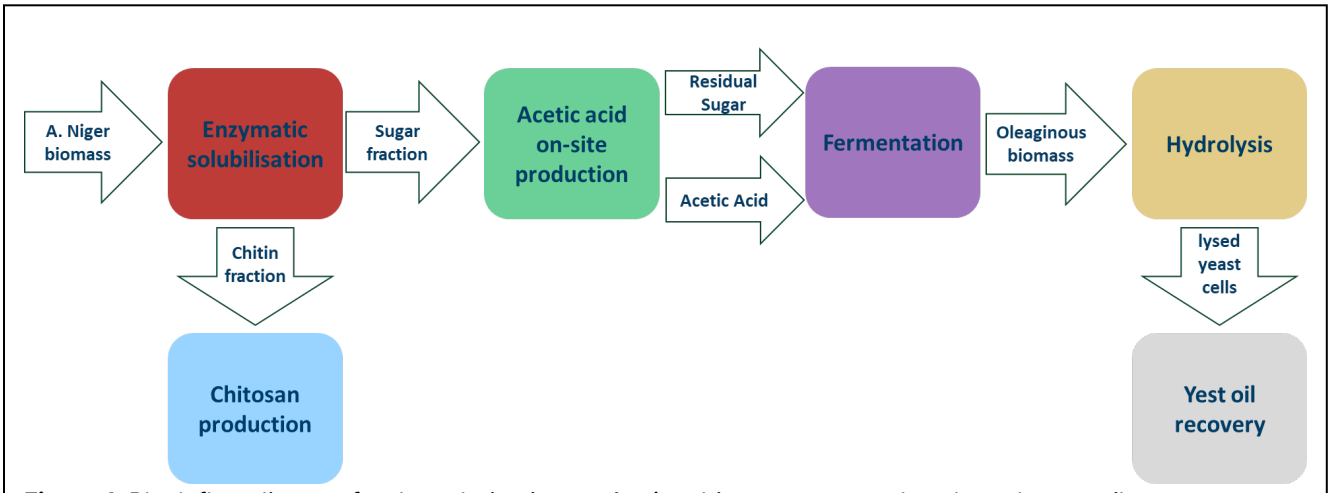

Figure 1: Block flow diagram for the valorisation og *A. niger* biomass to non-plant-based yeast oil.

molecules (glucan + water → glucose) and complex proteins into their shorter oligopeptides or their amino acid constituents, here simplified as protein + water → amino acids, as described by reactions (1) and (2), respectively.

$$C_6H_{10}O_5 + H_2O \rightarrow C_6H_{12}O_6 \quad (1)$$

$$C_{5.35}H_{9.8}N_{1.5}O_{2.45} + H_2O \rightarrow C_{5.35}H_{11.8}N_{1.5}O_{3.45} \quad (2)$$

The conversion rates of these reactions characterise the efficiency of the glucan/protein solubilisation. Lab-scale experiments reached an overall percentage of solubilised fungal biomass of 73%(w/w). Moreover, a comparable degree of solubilisation was observed for glucan and protein fractions. Therefore, assuming that no chitin is solubilised, the corresponding solubilisation factor for both glucan and protein is 85.3%(w/w).

Chitosan production

The chitosan synthesis involves both deacetylation and depolymerisation of chitin. During deacetylation, the chitin polymer reacts with NaOH (chitin polymer + sodium hydroxide → chitosan polymer + sodium acetate) according to expression (3). A chitosan polymer, with a degree of polymerisation equal to the one of the reacting chitin polymer is formed. The conversion (equal to the degree of deacetylation) is 84%.

$$C_{40040}H_{65067}N_{5005}O_{25026} + 5005\,NaOH \rightarrow C_{30030}H_{55057}N_{5005}O_{20021} + 5005\,NaC_2H_3O_2 \quad (3)$$

In the depolymerisation the complex chitosan polymer is broken down into shorter-chain oligomers (chitosan polymer + water → chitosan oligomer). Given that experimental observations only provide the average degree of polymerisation of the product (without details on the chain length distribution), a simplified model based on the literature has been developed (conversion is set to 100%), see equation (4) [7].

$$C_{30030}H_{55057}N_{5005}O_{25026} + 384\,H_2O \rightarrow 385\,C_{78}H_{145}N_{13}O_{53} \quad (4)$$

Acetic acid on-site production

The sugar-rich hydrolysate fraction is used for on-site production of acetic acid (AA) by fermentation. The AA will further be used as a co-feed carbon source in the main fermentation, where the AA and the residual sugar from the initial hydrolysate is exploited to grow oleaginous biomass. An inoculum flow equal to 4% of the mass of glucose in the hydrolysate is introduced in the medium to carry out the fermentation. Oxygen in the form of air is added as an input with 20% excess [8]. The conversion is defined considering the overall aerobic fermentation reaction of sugar to AA (glucose + oxygen → AA + carbon dioxide + water), described in equation (5). Based on the available literature, the average conversion is set to 85% [8,9]. The remaining 15% is the percentage of hydrolysate left after the acetic acid production step, to be exploited as the sugar source for the main fermenter. As a result, the AA to glucose molar ratio in the output stream is equal to 18:1.

$$C_6H_{12}O_6 + 2\,O_2 \rightarrow 2\,CH_3COOH + 2\,CO_2 + 2\,H_2 \quad (5)$$

Fermentation

The chemical formula for the main fermentation (initial cell mass + hydrolysate + AA + oxygen → oleaginous biomass + carbon dioxide + water) is implemented as described in reaction (6). All nutrients, such as salts and inert species in the medium, are for simplicity not displayed in the model. The initial cell mass chemical formula represents the overall chemical composition of the medium that takes part in the reaction. Nitrogen is added to close the mass balance.

$$C_{5.35}H_{9.8}O_{2.45}N_{1.5} + 1.5\,C_6H_{12}O_6 + 40.5\,C_2H_4O_2 + 4.85\,O_2 \rightarrow C_{67.95}H_{112.65}O_{8.81}N_{1.04} + 27.39\,CO_2 + 38.58\,H_2O + 0.23\,N_2 \quad (6)$$

An external AA input is added to the system since the AA to glucose ratio available from the on-site production is not sufficient to meet the fermentation reaction stoichiometry, which requires an input ratio of at least 27:1. Based on experimental data created in the project, the conversion rates were set to 98% for glucose and 70% for AA.

Hydrolysis of the yeast biomass

The hydrolysis is split into the release of triglyceride (oleaginous biomass → Tripalmitin + Tristearin + Triolein + Trilinolein + non-lipid biomass), which is presented in reaction (7), and the hydrolysis (non-lipid biomass + water → amino acid + sugar + lipid biomass), displayed in equation (8). Based on experimental data from project partners the overall conversion rate is set to 68%.

$$C_{67.95}H_{112.65}O_{8.81}N_{1.04} \rightarrow 0.403\,C_{57}H_{104}O_6 + 0.249\,C_{57}H_{110}O_6 + 0.045\,C_{57}H_{98}O_6 + 0.216\,C_{51}H_{98}O_6 + 1.855\,C_{9.3}H_{9.61}O_{1.8}N_{0.56} \quad (7)$$

$$C_{9.3}H_{9.61}O_{1.8}N_{0.56} + 2.5\,H_2O \rightarrow 0.2\,C_{13.42}H_{10.52}O_{0.014}N_{0.8} + 0.8\,C_{5.35}H_{9.8}O_{2.45}N_{0.5} + 0.39\,C_6H_{12}O_6 \quad (8)$$

The hydrolysis process yields a multiphase mixture with three fractions: (1) a solid fraction of dead cell mass and residual oleaginous biomass; (2) an organic liquid phase rich in lipids; (3) an aqueous phase, containing glucose, unreacted AA, and proteins.

Yeast oil separation

The modelled separation involves three compound splitters (Sep-1 to Sep-3): 83% of the aqueous phase is recycled to the main fermenter, 100% of the produced triglyceride oil is separated, and the residual mixture is split to remove solids, with the aqueous solution being recycled back to the main fermentation.

Flowsheet development

The sub-models described in section 2.2. have been integrated in the COCO-COFE process simulator. COCO (CAPE-OPEN to CAPE-OPEN) is a free-of-charge CAPE-OPEN compliant steady-state simulation environment. COFE (CAPE-OPEN Flowsheet Environment) is a user-friendly graphical user interface integrated in COCO for chemical flowsheeting provided with sequential solution algorithm using automatic tear streams. COFE displays properties of streams, deals with unit-conversion and provides plotting facilities. The system requirements to run COFE are Windows XP or higher.

A snapshot of the flowsheet is depicted in Figure 2. The Vapor-Liquid equilibrium in the process was simulated using the Raoult equation. Relevant physico-chemical properties, such as the chemical formula, ideal gas heat of formation, ideal gas entropy of formation, ideal gas heat capacity and vapor pressure, have been retrieved to characterise all species in the process simulator. In the absence of data or correlations from the literature, the vapor pressure of high molecular weight molecules such as enzymes, oleaginous yeast, and cell mass has been set to a fixed fictitious constant value of 0.000001. The reasonable implication of this simplifying assumption is that no vaporisation of the mentioned species occurs at the operating temperatures and pressures of interest for the investigated process.

The simulation was carried out assuming that the plant is treating 15 000 ton/year of dry *A. niger* biomass, which corresponds to the total annual amount of fungal waste generated by a citric acid industrial production site located in Germany. The fungal biomass feedstock contains 68%(w/w) glucan, 13%(w/w) chitin, 9%(w/w) amino acids and 10%(w/w) inert components. The latter one is a fraction of fatty acids and other components which do not react in the studied process and therefore are not displayed in the model. The *A. niger* biomass is diluted with water to a dry matter content of 15%(w/w) in the feed stream and preheated with steam to 121°C for 1 hour (autoclave sterilisation). After cooling, a dedicated enzyme cocktail is added to hydrolyse the glucan and protein present in the fungal biomass. Two dedicated fixed-conversion reactors are modelled to describe the protein (R100a deproteinisation) and glucan conversion (R100b glucan solubilisation), respectively. The residence time for the two enzymatic hydrolysis steps is set to 16 h and 40 h, respectively. Both reactors operate at a constant temperature of 50°C. The stream leaving R100b contains the sugar-rich hydrolysate and a residual solid fraction, containing chitin and unreacted glucan. These two fractions are separated in a filter (F-101). In this work, 100% of the filtrate is used for AA production.

The solid fraction (chitin and glucan) is pre-cooled to 25 °C in Cooler_chitin and chilled to 4 °C in another heat exchanger, named Chiller_chitin. The solid mixture is mixed with NaOH, which is also cooled to the same temperature in Chiller_NaOH, and then fed to the deacetylation reactor (R200-Deacetylation) and, subsequently, to the depolymerisation vessel (R200-Depolymerisation). The overall residence time inside the two vessels is 48 h. The mixture leaving R200-Depolymerisation is centrifugated to separate the water-insoluble compounds, including chitin and remaining glucan. For the centrifuge, a separation efficiency of 90% is assumed. The chitin-rich solid fraction can be utilised as animal feed. The chitosan-rich water stream is dried at 130°C using steam as utility. The dryer is designed to achieve 98% moisture removal. The dried CHOS product

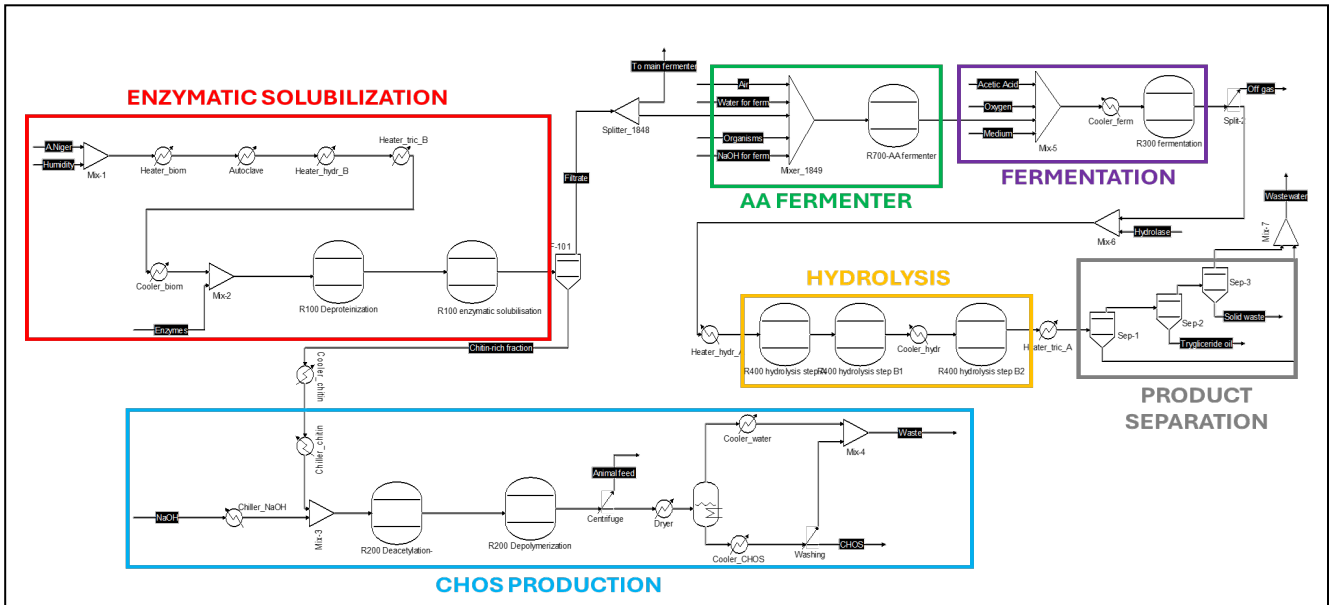

Figure 2: Process flowsheet implemented in COCO-COFE.

is cooled down to ambient temperature in Cooler_CHOS and sent to a washing step to remove residual impurities before storage.

The glucose-rich hydrolysate is mixed with all the other inputs required for fermentation in Mix-5: these streams are the fermentation organisms, additional water, NaOH for pH control, and air to provide oxygen. The mixed input enters a vessel where AA is produced (R500-AA fermenter). The residence time inside R500 is set to 50 hours. The output stream contains AA and glucose; thus, it represents the feed for the main fermenter. The additional feedstocks for the main fermentation medium, oxygen, and supplementary AA are mixed in Mix-6 and cooled down to 28 °C in Cooler_ferm. The residence time inside the fermenter is 72 h. Downstream the fermenter, a CO_2-rich off-gas is released, and the product is mixed with enzymes for the hydrolysis step. Finally, the mixture is heated up to 45 °C. The preheated stream is conveyed to hydrolysis.

In reactors (R400 hydrolysis step A and R400 hydrolysis step B), the non-lipid portion of the biomass is hydrolysed, producing glucose. Hydrolysis takes place in two vessels in series, operating at a temperature of 45 °C and 37 °C, with a residence time of 20 h and 6 h, respectively. Finally, the product is heated back to 45 °C in a process-process heat exchanger (Heater_tric) and sent to the product separation.

All residence time and operating conditions selected to model each process step represent the optimal conditions resulting from lab-scale tests carried out by our partners in VALUABLE.

RESULTS

The Key Performance Indicators (KPIs) of the simulated plant include the productivity of triglyceride oil and chitosan (product targets), the on-site productivity of AA as an intermediate for the biomass conversion process, as well as the overall consumption of raw materials (the ones for which a major effect on the plant operating costs is expected), and the total steam demand (main utility for this plant). The KPIs are shown both on an absolute basis (see Table 1) and on a relative basis, assuming 1 kg of dry *A. niger* biomass as a reference (see Table 2).

Table 1: List of the Key Performance Indicators of the simulated plant on an absolute basis. AN – *Aspergillus niger*.

Indicator	Value	Unit
Triglyceride oil productivity	2446.5	ton/year
Chitosan productivity	1813.5	ton/year
On-site AA productivity	5221.9	ton/year
Extra demand for AA	6297.4	ton/year
Total heat duty (LP steam)	8.88	MW

The results show that about 2 450 tons of triglyceride oils and 1 800 tons of chitosan can be produced starting from 15 000 tons of *A. niger* biomass. The productivity of yeast oil is rather limited, considering that the glucan content in the biomass feedstock is 76%. However, this result can be justified considering that a significant portion of the glucan from the *A. niger* biomass is consumed to generate the AA needed as a co-carbon source for fermentation. On-site AA productivity turns out to cover 45% of the total AA demand of the plant: this is expected to have a positive effect on the operating costs, leading to a significant reduction of the amount of AA to be purchased.

Table 2: List of the Key Performance Indicators of the simulated plant (relative values per unit of feedstock). AN – *Aspergillus niger*

Indicator	Value	Unit
Triglyceride oil productivity	0.16	kg/kg AN
Chitosan productivity	0.12	kg/kg AN
On-site AA productivity	0.35	kg/kg AN
Extra demand for AA	0.42	kg/kg AN
Total heat duty (LP steam)	18.67	MJ/kg AN

The overall enzyme consumption represents a considerable challenge when considering a large-scale operation. Therefore, enzyme purchase cost is expected to be a key driver in the economic assessment of the plant.

Table 3 gathers a breakdown of the energy consumption associated with the single unit operations involved in the process.

Table 3: List of the energy requirements (and utility type) associated with each unit operation. C.W. - cooling water

Unit operation	Duty (kW)	Utility
Heater_biom	8337.95	steam
Cooler_biom	1730.39	C.W.
Cooler_ferm	4779.41	C.W.
Cooler_hydr	2421.24	C.W.
Cooler_chitin	88.58	C.W.
Chiller_chitin	72.36	Electricity*
Chiller_NaOH	0.76	Electricity*
Dryer	2891.88	steam
Cooler_water	3012.99	C.W.
Cooler_CHOS	137.55	C.W.

*Electricity consumption for the refrigeration cycle

The overall steam and cooling duty demands are 11.23 MW and 12.17 MW, respectively. Electricity consumption for the refrigeration cycle is a minor consumption. It is important to mention that the real electricity demand to run the plant will be higher since circulation pumps to overcome pressure drops must also be considered. However, based on the outcomes of the current

energy balance, the main conclusion is that the main operating cost associated with utilities is undoubtedly the one linked with the steam demand for the initial preheating of the fungal biomass to 121°C upstream of the autoclaving step.

CONCLUSIONS

In the present work, two key milestones of the preliminary feasibility study of the novel process developed in the VALUBALE project, namely process design and simulation, and estimation of KPIs, have been accomplished.

The proposed process offers a sustainable solution for transforming an industrial waste (i.e., *A. niger* biomass) into multiple high-value products. The main novelties are enhanced sustainability and the promotion of a circular economy by converting waste into valuable resources.

In this study, we established a novel integrated bioconversion of acetic acid and sugar to sustainable yeast oil production. Moreover, by integrating on-site acetic acid production through fermentation, the need for external acetic acid purchase is significantly reduced. As a result, the cost gap compared to plant-based lipids is expected to be considerably reduced.

The results achieved in this work will be exploited as a basic framework to carry out a detailed Techno-Economic Assessment (TEA), Life-Cycle Assessment (LCA) and social acceptance assessment of the novel VALUABLE process. The main target will be to compare its economic and environmental impact with benchmark processes for oil production based on plant biomass and chitosan production based on crustacean waste. More specifically, palm oil production and chitosan production from shrimp cells will be used as reference cases.

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