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HAL Authorization

1 **Eco-design approach for an innovative production process of low molar mass**
2 **dextran**

3

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6

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15 **Highlights**

- 16 • Low molar mass dextrans are directly synthesized by DSR-M enzyme without acid hydrolysis and
17 fractionation
- 18 • A high-fidelity polymerization model PolyEnz was developed for reaction description
- 19 • Process modeling and Life Cycle Assessment are coupled for process eco-design
- 20 • Sucrose concentration is the most sensitive factor for global performances
- 21 • The methodology identifies and quantifies the most promising production routes for further
22 investigation

23

24

25 **Abstract**

26 An approach to early-stage eco-design of an enzyme-based process has been developed by coupling process
27 modeling, Life Cycle Assessment (LCA) and flowsheet design, in order to evaluate the real advantages of the
28 direct synthesis of low molar mass dextrans (5 - 25 kg.mol⁻¹) from sucrose substrate. This approach identifies
29 the most promising development pathways and crucial unit operations that require, as a matter of priority,
30 further investigation and experimentation. Process modeling is based on a comprehensive and multi-fidelity
31 building of Life Cycle Inventories (LCI) to establish all material and energy inputs and outputs of the processes
32 involved with a flexible and satisfactory level of accuracy. This essentially binds (i) a high-fidelity
33 polymerization model, namely PolyEnz, for the description of the synthesis of dextrans from sucrose using
34 the DSR-M enzyme following a non-processive mechanism, (ii) flexible-fidelity models for description of
35 subsequent purification, separation and drying steps, (iii) upstream processes in the value chain and life cycle
36 system using real-world data fromecoinvent data sets. Three process benchmarks were constructed and
37 compared to determine the most appropriate purification processes and operation conditions at a larger
38 scale. In addition, various process triggers, including the initial concentration and type of substrate, the type
39 of process water, the use of size exclusion chromatography for separation, and the amount of freeze drying
40 used for the last production stage were subject to a sensitivity analysis with the criteria being the overall
41 energy demand, the potential environmental damages evaluated by ReCiPe endpoint and the global warming
42 potential.

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45 **Key words**

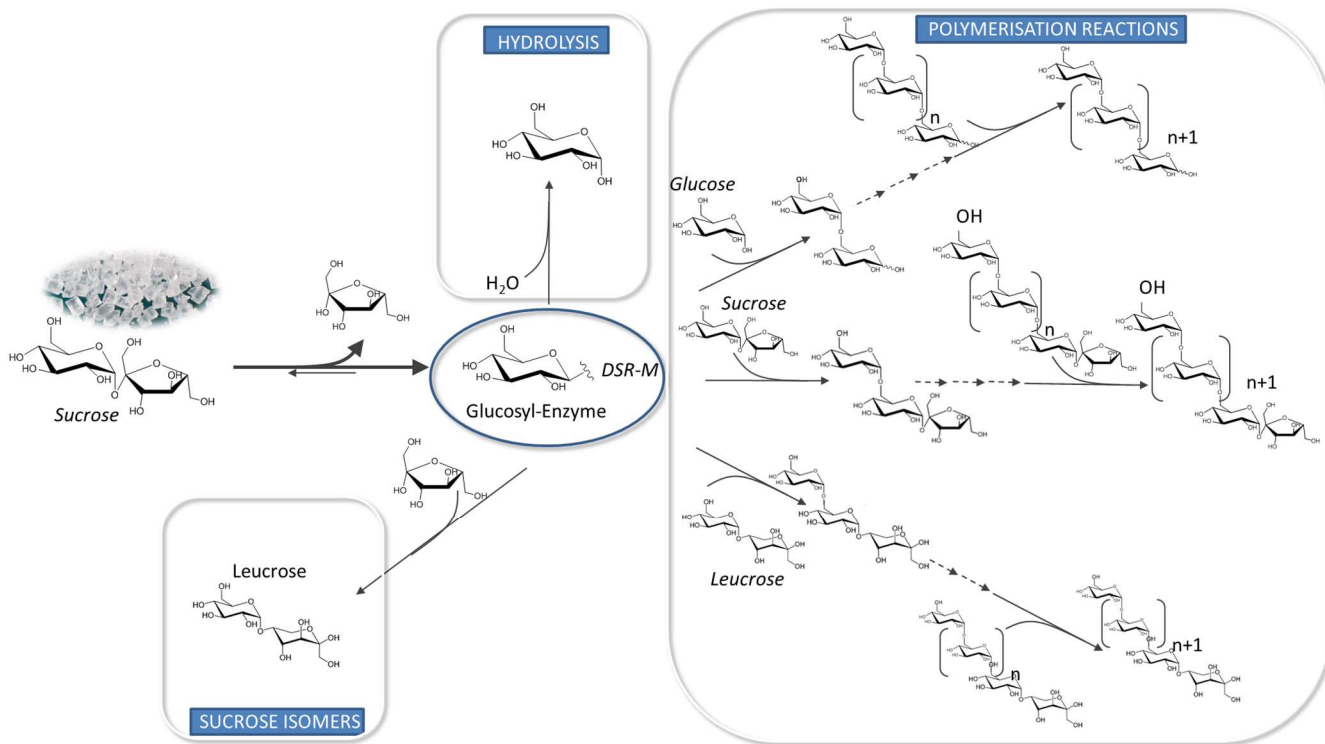
46 Dextranase, Life Cycle Assessment, process modeling, polymerization modeling

47 **1 Introduction**

48 Dextran is a homopolysaccharide of D-glucosyl units produced from sucrose, a cheap and abundant agro-
49 resource, by lactic acid bacteria, such as *Leuconostoc*, *Streptococcus*, *Lactobacillus*, or *Weissella* sp. They
50 generally have a very high molar mass (10^5 – 10^8 g.mol⁻¹) and contain a majority of α -1,6 osidic linkages. Those
51 containing more than 95% α -1,6 osidic bonds were the first microbial polysaccharides to find industrial
52 applications in the 1950's, essentially for pharmaceutical applications or in fine chemistry. Due to their low
53 viscosity in saline solutions and their limited antigenic characteristics, they are frequently used in the medical
54 field, for example, as blood plasma extenders, in the prevention of post-operative shocks, for healing burns,
55 or as anti-coagulant agents¹. In analytical chemistry, they factor in the composition of versatile
56 chromatographic supports (Sephadex® gels)². However, these applications concern dextran fractions with
57 controlled molar mass between 1,000 g.mol⁻¹ and 70,000 g.mol⁻¹ produced by the acid hydrolysis of native
58 dextran chains obtained via the fermentation of sucrose by lactic acid bacteria such as *L. mesenteroides* NRRL
59 B-512F, followed by successive alcohol precipitation steps. To the best of our knowledge, these processes
60 are still relatively costly today and of low or medium yield^{2,3}.

61 In lactic acid bacteria, the enzymes involved in dextran formation are called dextransucrases and are
62 classified in glycoside hydrolase family 70 (GH70; www.cazy.org)⁴. These enzymes work following a two-step
63 mechanism. First, a covalent glucosyl-enzyme intermediate is formed, with the concomitant release of the
64 fructosyl moiety issued from sucrose. Then, the glucosyl unit is transferred onto various molecules containing
65 hydroxyl functions (Figure 1): (i) water leading to the hydrolysis of sucrose, (ii) sucrose itself at the beginning
66 of the reaction, (iii) gluco-oligosaccharides, which grow as soon as they are available in the reaction medium,
67 and (iv) fructose released leading to the isomerization of sucrose into leucrose. Secondly, leucrose can itself
68 play the role of acceptor molecule (Figure 1). The growing gluco-oligosaccharide chains rapidly lead to high
69 molar mass polymers of thousands or millions of glucosyl residues primarily linked via α -1,6 osidic bonds. As
70 the dextran molecules contain either glucose, sucrose or leucrose at their reducing end, they will be named
71 G_i, G_jS and G_kL respectively, in this study.

72



73
74 **Figure 1. Reactions catalyzed from sucrose by dextransucrase DSR-M issued from *Leuconostoc citreum* NRRL B-1299.**

75

76 Recently, a new dextransucrase gene of interest was discovered in the genome of the strain *Leuconostoc*
 77 *citreum* NRRL B-1299. Indeed, after gene cloning and heterologous expression in *E. coli*, the resulting enzyme,
 78 called DSR-M, was shown to use sucrose to produce only low molar mass dextrans of approximately 10^3
 79 $\text{g}\cdot\text{mol}^{-1}$, exclusively composed of α -1,6 osidic linkages⁵. This enzyme is the sole dextransucrase known to date
 80 to produce only low molar mass dextrans without the concomitant synthesis of higher molar mass polymers
 81 and with a non-processive mechanism of polymerization⁶. Moreover, the dextran size can be finely
 82 modulated according to the initial sucrose concentration ranging from $50 \text{ g}\cdot\text{kg}^{-1}$ to $500 \text{ g}\cdot\text{kg}^{-1}$ and/or the
 83 temperature of the reaction ranging from 20°C up to 45°C . Therefore, size-controlled dextrans, i.e., with
 84 controlled molar mass ranging from $5 \text{ kg}\cdot\text{mol}^{-1}$ to $25 \text{ kg}\cdot\text{mol}^{-1}$, can be produced in a single step (without acidic
 85 hydrolysis and alcohol fractionation) with advantageous yields of approximately 80%⁷. As these dextran
 86 fractions are particularly well suited for industrial applications, it was tempting to develop an approach of
 87 process eco-design in order to identify the most promising development pathways and crucial unit operations
 88 that require, as a matter of priority, further investigation and experimentation.

89 It is now widely recognized that new processes or technologies need to be evaluated for their environmental
 90 performances via, for example, a life cycle assessment (LCA) in the early stages of design before they are
 91 placed on the market. Industry and stakeholders have also begun to incorporate the assessment of potential
 92 environmental impacts as a new criterion, in addition to the cost, to evaluate the coherence of any
 93 innovation. Indeed, the eco-design of a process or product implies the integration of the environmental

94 performances over the life cycle of the studied process, as well as other technical or economic performance
95 criteria. The purpose of such an approach is to explore the real benefit of a new process at a larger scale, to
96 identify from a theoretical or practical point of view the most promising development pathways, and to focus
97 funding on crucial process units or steps requiring, as a matter of priority, further investigation and
98 experimentation.

99 The LCA methodology is based on a comprehensive analysis that assesses the cumulative environmental
100 impacts of a process, product, or service, taking into account all the stages of its life cycle, namely, extraction
101 of natural resources, manufacturing, transportation, use and maintenance, end-of-life, and disposal. The LCA
102 methodology is guided by ISO 14040–14044^{8,9}, which is comprised of the following four steps: (1) goal and
103 scope definition, (2) life cycle inventory (LCI), (3) life cycle impact assessment (LCIA), and (4) result
104 interpretation; it can therefore be used as a tool for diagnosis and decision-making.

105 Building an LCI or quantification of all inputs and outputs of the processes involved in the life cycle of a system
106 is by far the most crucial step for determining the relevance of the end results of an LCA. The inventory is
107 divided into foreground and background processes. The foreground usually refers to a new system, e.g., an
108 innovative technology or process for which new experiments need to be conducted and/or high-fidelity
109 models need to be developed. Conversely, the background refers to support processes (energy,
110 transportation, and extraction of natural resources), which are considered to be upstream in the value chain
111 and life cycle system for which data actually exist as generic datasets, e.g. ecoinvent or ELCD^{10,11}. Several
112 studies have already pointed out the importance of an adaptive modeling approach for scaling and LCA in
113 the early stages of design in biotechnology. Examples include engineering tools for the development of some
114 biocatalytic processes¹², simulation-based design applied to bio-resins¹³, and tools for the LCA assessment of
115 succinic acid production from biomass¹⁴.

116 The application of LCA to biotechnology is relatively recent, even though the concept has been widely
117 adopted in various sectors of production and the economy¹⁵. For example, the environmental assessment of
118 biofuels with the development of decision-making supports for public institution funding grants, as well as
119 European Community regulations^{16,17,18}, and LCAs in the growing bioplastics sector can be mentioned^{19, 20}.
120 Despite the accelerated growth of the biotechnology industry, the situation is different for low tonnage
121 products, such as pharmaceuticals (drugs and additives), food components (proteins, dietary supplements,
122 and vitamins), oleochemicals (lubricants, surfactants, and coatings), molecular platforms (used to synthesize
123 various chemicals), and materials and molecules obtained from bio-resources rather than fossil resources. In
124 general, reported LCA studies in the above cited sectors are usually based on near full-scale/industrial
125 processes to compare their environmental performance to older technologies, for example, LCAs on wood
126 coatings from biological resources¹⁵, the industrial production of enzymes for pharmaceutical applications²¹,
127 and innovation in the production of active pharmaceutical ingredients and basic chemicals²². However, a

128 huge effort is needed for environmental performance assessments and improvements in the case of
129 innovative, low technology readiness level (TRL) processes in these fields.

130 In this context, the present study aims to perform comprehensive modeling for a detailed environmental
131 analysis and eco-design of the production of dextran with a controlled molar mass (10–20 kg.mol⁻¹) from
132 sucrose via the DSR-M enzyme. The originality of this study is twofold: (i) new synthesis and separation
133 pathways are proposed and studied from the viewpoint of design using a multi-fidelity modeling approach,
134 i.e., an adaptively rigorous and more flexible and practical method, therefore broadening the scope for
135 extension to other application areas in biotechnology, and (ii) the process of dextran synthesis and
136 purification is investigated from both energetic and environmental points of view with integrated LCA to
137 allow a thorough assessment of the overall performance and a coherent process design.

138 **2 Materials and methods**

139 **2.1 Early-stage integrated process modeling and LCA - a methodology for eco-design**

140 The concept of eco-design entails the notion of the life cycle of the studied process and implies consideration
141 in the early design stages of the environmental impacts generated directly by the process and indirectly by
142 the overall life cycle system (or background processes). In this sense, eco-design needs to consider (i) a
143 complete definition of the process with extended boundaries to its life cycle, (ii) an ecological choice of raw
144 materials and energy resources, (iii) a global spirit of waste reduction via revalorization and reuse, and (iv)
145 case-specific identification and adjustments of key operating parameters to maximize the overall efficiency
146 and to minimize the environmental footprint.

147 As illustrated in Figure 2, the methodology is first composed of a multi-level and multi-fidelity process
148 modeling structure comprised of unit operations and their layout with a judicious degree of complexity. At
149 this level, the process needs to be envisaged and placed at an industrial scale, so that the modeled system is
150 as close as possible to a real industrial process. In the case of ongoing development and a low TRL for an
151 innovative process, scale-up should be considered in direct feedback with the LCA guidelines, as illustrated
152 in Figure 2, by studying the environmental impacts generated by the material and energy flows at the desired
153 scale and by checking whether the overall process configuration remains the same at a larger scale of
154 production.

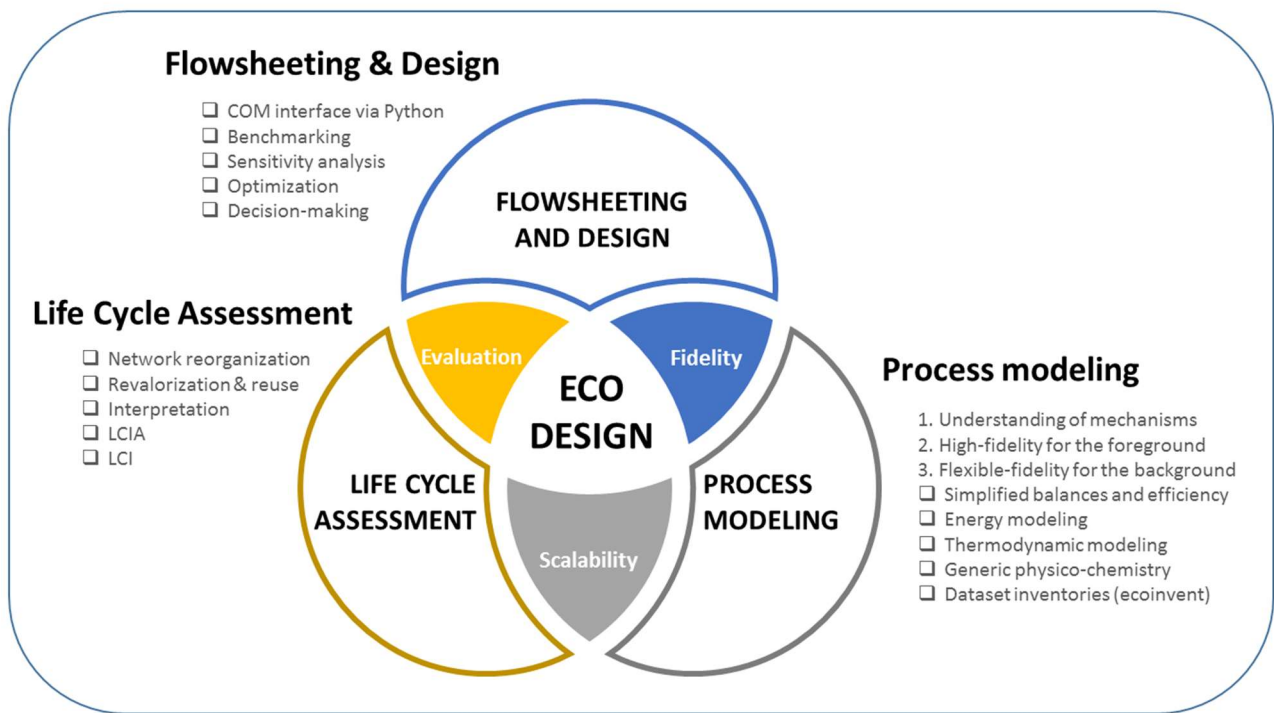


Figure 2. Venn diagram of integrated LCA-process modeling for eco-design.

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158 A process model provides material and energy inventories at the boundaries of the studied unit operation
 159 and can be associated with an LCA software package with general LCA databases for the background
 160 component of the life cycle system (e.g., raw material extraction, energy production, chemical production,
 161 transportation, and waste treatment). This type of simulation tool for the eco-design of biotechnologies does
 162 not currently exist on the market for the following reasons: (i) the complexity related to high fidelity modeling
 163 of certain unit operations, their parameterization, and their combination with other operations; (ii) the
 164 difficulty of fully quantifying elementary process flows, i.e., the release and emission of substances and
 165 residues to the environment; (iii) the diversity of unit operations and their modes of operation involved in a
 166 process, from the raw material to the final product, including core transformation processes (e.g., reactors),
 167 processes for utilities (e.g., steam, electricity, and water), and waste treatment and advanced separation
 168 units used in biotechnology that are not generally available in simulation software (e.g., chromatography,
 169 advanced driers, and filtration); and (iv) several technical difficulties associated with coupling or integrating
 170 process simulators into LCA software. However, note that examples of such integrated tools in the fields of
 171 biomass engineering and wastewater engineering have already been proposed and investigated in the
 172 literature^{14, 23, 24}.

173 When only laboratory-scale experimental data (TRL2-4) exists for an innovative biotechnology process, the
 174 core transformation process needs to be accurately modeled at high fidelity with parameterizations and the
 175 possibility of a scale-up analysis, while the background operations can be related more flexibly with lower

176 fidelity and higher genericity. Nevertheless, the technical compatibility between the foreground and
177 background processes needs to be verified. The core transformation model for the foreground production
178 unit is the most decisive step because it characterizes the productivity, and therefore this model needs to be
179 predictive to reflect its sensitivity to key influential operating conditions. For example, regarding dextran
180 synthesis, the polymerization reaction needs to be formulated and resolved with relevant thermodynamic
181 and kinetic parameters to clearly describe the performance according to the main driving conditions (e.g.,
182 the temperature, concentrations, and pH).

183 The coupled methodology for the process modeling–LCA framework in biotechnology includes the following
184 steps (Figure 3).

- 185 1. A description and understanding of the main transformation unit (the leading foreground process of
186 dextran synthesis in this work) based on representative laboratory experiments.
- 187 2. High-fidelity modeling, characterization, and parameterization of the synthesis model and
188 identification of the main process inducers.
- 189 3. Flexible-fidelity development or the use of background unit operations potentially involved in the
190 overall life cycle system and integration with an LCA calculator (in this work, the Umberto software
191 for flowsheeting and LCA). At this level, the models provide essentially accurate mass and energy
192 balances per operation, as well as efficiency calculations with the required degree of accuracy. They
193 may also involve advanced aspects such as in-depth thermodynamic modeling and the integration of
194 hydraulics, if necessary.
- 195 4. Benchmarking or comparative analyses of alternative process flowsheets and an assessment of the
196 LCA results building the global process flowsheet and linking with the background processes of the
197 ecoinvent database for a global calculation of the LCI following the LCA methodology. Different unit
198 operations are used and different configurations of recycling and waste treatment, as well as energy
199 integration, can be involved and evaluated. At this level, different process goals are defined and
200 evaluated together with the environmental performance via LCIA.
- 201 5. LCA results interpretation, sensitivity analysis, and decision-making. Predictive inventories are
202 generated and compared to provide design and performance insights. A predictive tool with
203 appropriate interconnections between the unit operations (feasibility and fidelity) and their
204 respective environmental footprints throughout the LCIA allows the performances to be studied, as
205 well as the influences of the key parameters of the process, the choice of unit processes involved,
206 and the choice of raw materials, energy sources, and transportation in the background system.

207 This methodology was specifically applied to dextran synthesis and conditioning. The key operation is the
208 enzymatic synthesis of size-controlled dextrans, for which experiments were conducted at the lab-scale.

209 Then, a global process, including product separation and conditioning, was built based on the lab-scale assays
210 and theoretical modeling.

211 Accordingly, the relevance of the unit operations and their interconnections in the overall flowsheet were
212 explored and evaluated by means of mass and energy balances, life cycle inventories (elementary flows such
213 as natural resource consumption and substance emissions), and associated environmental impacts. These
214 results were used in a comparative manner to identify the main impact contributions from the design, raw
215 materials, utilities, or operating conditions and to provide indications for a technically relevant and
216 environmentally friendly production path.

217 **2.2 LCA application**

218 In this study, the environmental impacts were calculated according to the LCA method on the basis of 1 kg
219 of produced dextran (one functional unit). The Python scripts for the unit processes were implemented in
220 the Umberto® 5.6 LCA software, linked with the ecoinvent 2.2 database, and laid out in process flowsheets
221 (representing the foreground system for the LCA). In addition, regarding the raw materials, utilities, and
222 waste treatment options, the corresponding standard lifecycle inventories of ecoinvent were selected and
223 linked to the foreground system to calculate the overall mass and energy balances and provide a global LCI
224 of the process. With regard to the foreground processes (the studied processes), only the operation and
225 functioning aspects were considered, i.e., the consumption of raw materials and utilities, while the
226 infrastructure (buildings and equipment) was not included due to the lack of relevant data. However, the
227 contribution of infrastructure and of the equipment maintenance was investigated through a sensitivity
228 analysis using generic datasets from ecoinvent database.

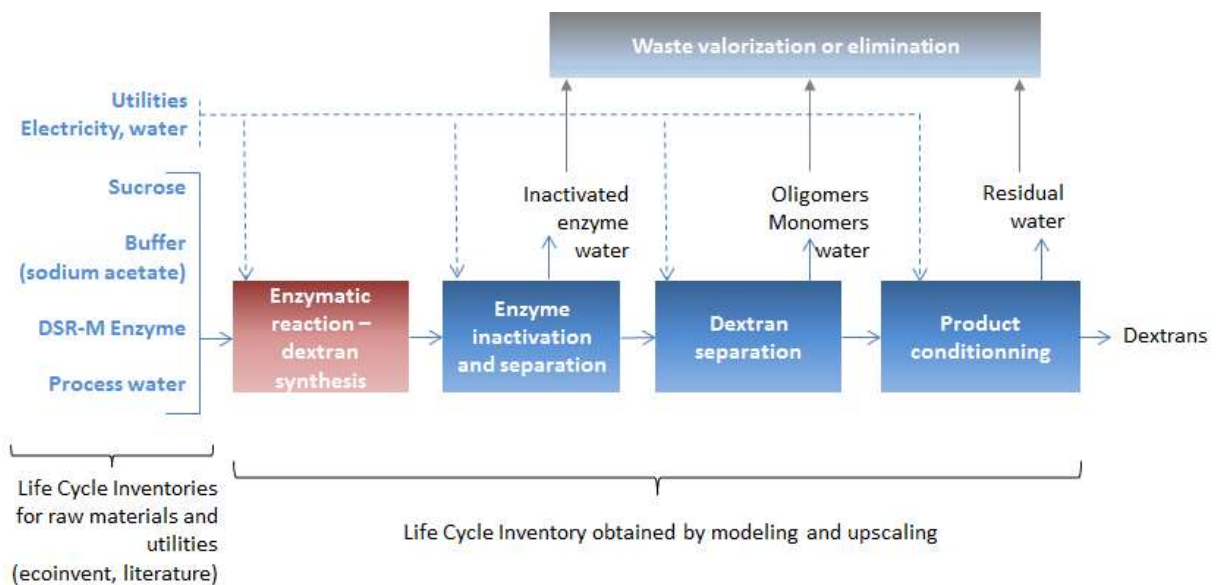
229 The environmental impacts were calculated using the ReCiPe method²⁵, which was used at the midpoint level
230 with an hierarchist perspective, as well as at the endpoint level (damage oriented) focusing on three
231 categories of damage: human health, ecosystem quality, and resources (fossil and metal depletion). With
232 regard to the endpoint impact, the average of the three perspectives (hierarchist, individualist, and
233 egalitarian) was used to weigh the impacts (40% for human health, 40% for ecosystem quality, and 20% for
234 resources). In addition, the impact of climate change was calculated using the IPCC 2013 method²⁶.

235

236 **2.3 Process description for the synthesis and conditioning of dextran polymers**

237 The production of high-quality size-controlled dextrans involves the following main steps (Figure 3),
238 applicable at the laboratory or industrial scale:

- 239 1. Synthesis of dextran, primarily based on a single in-vitro enzymatic polymerization using sucrose as
 240 a substrate (a cheap and abundant agro-resource) and dextransucrase expressed recombinantly by
 241 *E. coli* (details of the experimental synthesis protocol and its specificities are provided in Section 2.3);
 242 2. Inhibition of the enzyme and separation from the reaction medium;
 243 3. Separation of dextran polymers from oligomers and monosaccharides;
 244 4. Conditioning of the dextran mixture as a fine-powdered product; and
 245 5. Recovery or treatment of waste effluents.



249
 250 **Figure 3. General process diagram for high-quality dextran synthesis, purification, and drying.**

251
 252 Each step is based on one or more unit operations chosen for their proven technical relevance at the
 253 laboratory scale, as advised by experts and selected using a heuristic approach²⁷. The scale of production is
 254 defined in agreement with the current production capacities of dextran; a synthesis batch reactor with a
 255 nominal volume of 100 L (the reaction volume) was considered.

256 The sucrose initially enters the batch reactor with the enzymes where the polymerization reaction is
 257 conducted under the same conditions as at the laboratory scale, i.e., 30°C, atmospheric pressure, and pH
 258 5.75. At the end of the reaction, the enzymes are inactivated at 95°C over 5 min in the same container. The
 259 reaction medium is transferred for the enzyme separation operation, which may consist of membrane
 260 filtration.

261 The solution obtained after inactivation and the exclusion of the enzymes contains dextrans (polymers),
262 fructose, and other monosaccharides and oligosaccharides. At this level, the dextrans need to be separated
263 from the other small compounds to obtain a product containing polymers in the desired size range. The
264 separation can be carried out via size exclusion chromatography or via an appropriate membrane separation
265 process. The dextran aqueous fraction is then treated for the precipitation of dextran via a thermal/cryogenic
266 process, which may be lyophilization or spray drying. The effluents are obtained with a composition
267 depending on the efficiency of the unit operation used. The inactivated enzyme is separated into an aqueous
268 phase, which also contains all the other molecules in small amounts. The dextran separation generates an
269 aqueous solution containing monosaccharides and oligosaccharides, the concentrations of which depend on
270 the type of separation. From an LCA perspective, the design needs to take into account the associated
271 treatment processes, which may be elimination (e.g., waste water treatment plants and incineration), open
272 loop valorization (e.g., monosaccharides and oligosaccharides in another process), or closed loop recycling
273 (e.g., the reuse of residual water in the process).

274 Relevant modeling levels are necessary for the description of multiple-fidelity unit operations; these levels
275 include the following:

- 276 • A rigorous base model to simulate the enzymatic synthesis of size-controlled dextran polymers that
277 represents the critical stages of production, ensuring the required quality of the final product; and
- 278 • Flexible-fidelity modeling blocks for the other unit operations including mass and heat balances,
279 calculations of the utility requirements, hydraulic and efficiency models (for the chromatography,
280 filtration, and membrane processes), and a thermodynamic model (for drying).

281 Python scripts were therefore developed in the present study to generate predictive material and energy
282 flows for each unit operation. A full description of these modeling blocks is provided in Section 2.5 as well as
283 in the Supplementary Information.

284

285 **2.4 Laboratory-scale experiments with the DSR-M enzyme**

286 The DSR-M $\Delta 1$ enzyme is recombinantly produced in *E. coli* as previously described⁶. Briefly, the DSR-M $\Delta 1$
287 gene is cloned in a PET-55-DEST vector (Novagen) in fusion with a 6xHis tag at the C-terminal extremity and
288 a Strep-tag at the N-terminal extremity. The transformed *E. coli* BL21 star DE3 cells are grown in Erlenmeyer
289 flask at 21°C with agitation (150 rpm) in a modified ZYM5052 culture medium (with the following changes:
290 0.1% lactose, 0% glucose, and 1% glycerol)²⁸ supplemented with ampicillin (100 $\mu\text{g}\cdot\text{mL}^{-1}$). After 26 h of
291 incubation, the cells are harvested via centrifugation, newly suspended in a lysis buffer (50 mM sodium
292 acetate with pH 5.75) at a final $\text{OD}_{600\text{ nm}} = 80$, and disrupted via sonication. After centrifugation (30 min at
293 11,000 rpm), recombinant enzymes are recovered in the soluble fraction of the crude cell extract, ready for

294 dextran syntheses. The enzyme activity of these crude cell extracts is estimated using the 3,5-dinitrosalicylic
295 method²⁹, for which one unit of DSR-M activity is defined as the amount of enzyme that catalyzes the
296 production of 1 μmol of fructose per minute at 30°C from 100 $\text{g}\cdot\text{L}^{-1}$ sucrose in a 50 mM sodium acetate buffer
297 with pH 5.75. The level of enzyme production is about 20,000 units per liter of culture under these conditions.

298 Dextran syntheses were performed at 30°C in a volume of 250 mL with sucrose concentrations varying from
299 100 $\text{g}\cdot\text{L}^{-1}$ to 400 $\text{g}\cdot\text{L}^{-1}$ in a 50 mM sodium acetate buffer with pH 5.75 and using 1 $\text{U}\cdot\text{mL}^{-1}$ of enzyme
300 (corresponding to 0.014 $\text{mg}\cdot\text{mL}^{-1}$ of DSR-M proteins). For the kinetic analyses, 500- μL samples were
301 withdrawn at regular time intervals until total sucrose consumption. Reactions were stopped via a 5-min
302 incubation at 95°C.

303 To assay the sucrose depletion and determine the production of the glucose, fructose, and leucrose by-
304 products, the reaction media were analyzed via high performance liquid chromatography on a Dionex
305 UltiMate 3000 system and using an Aminex[®] HPX-87K carbohydrate analysis column (300 \times 7.8 mm; Bio-Rad).
306 The column oven temperature was set to 65°C. The compounds were separated isocratically using ultrapure
307 water as an eluent at a flow rate of 0.6 $\text{mL}\cdot\text{min}^{-1}$.

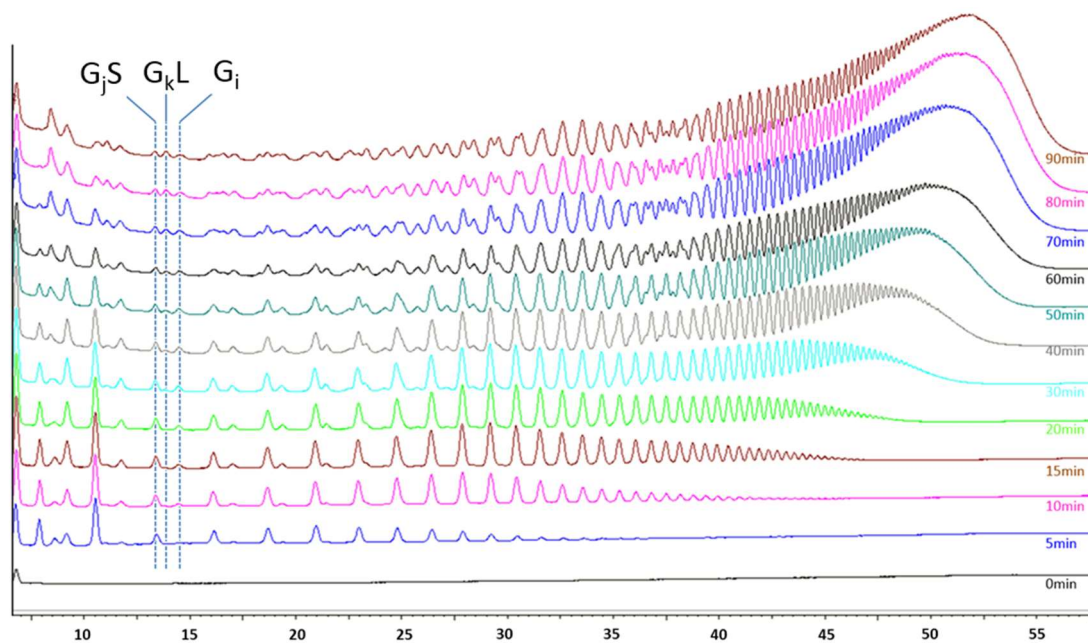
308 The size and concentration of the dextran were determined via high performance size exclusion
309 chromatography on a Fisher Ultimate 3000 system using two Shodex OH-Pak SB-802.5 and SB-805 columns
310 in series coupled with a Shodex OH-Pak SB-G guard column and placed in an oven at 70°C. Elution was
311 performed using 0.45 M NaNO_3 and 1% ethylene glycol (vol.) as eluent at a flow rate of 0.3 $\text{mL}\cdot\text{min}^{-1}$. The
312 samples were diluted to 20 $\text{g}\cdot\text{L}^{-1}$ of dry matter, also in 0.45 M NaNO_3 and 1% ethylene glycol. The weight-
313 average molar masses at peak apex of synthesized dextrans were determined using a calibration curve with
314 standards of fructose, sucrose, and dextrans of 1.5, 11.3, 39.1 and 68.4 $\text{kg}\cdot\text{mol}^{-1}$ (Sigma-Aldrich), at 10 $\text{g}\cdot\text{kg}^{-1}$
315 each.

316 Monitoring of dextran production versus time were performed thanks to HPAEC PAD (high performance
317 anion exchange chromatography with pulsed ampero-metric detection) analyses using a CarboPac TM PA100
318 analytical column (5 mm \times 250 mm) coupled with a CarboPacTM PA100 guard (5 mm \times 50 mm). A gradient
319 of Na acetate in 150 mM NaOH was applied at a flow rate of 1 $\text{mL}\cdot\text{min}^{-1}$ as follows: 0–2 min, 0 mM; 2–110
320 min, 0–225 mM; 110–130 min, 225–450 mM. Detection was performed using a Dionex ED40 module with a
321 gold working electrode and an Ag/AgCl pH reference. The samples were diluted in water to a final dry matter
322 of 1 $\text{g}\cdot\text{L}^{-1}$ before analysis.

323 These analyses revealed the simultaneous presence of three different populations of dextrans, namely G_i ,
324 G_jS and G_kL , evolving in parallel during the synthesis as illustrated in Figure 4. These three populations of
325 dextrans are involved in the three possible polymerization pathways described in section 1.2, where G_i is the
326 dextran chains formed from elongation of free glucose, G_jS correspond to dextran polymerized from sucrose
327 and G_kL form Leucrose. The three polymerization pathways will be discussed in Section 2.5.1 throughout the

328 introduction of the PolyEnz model. As shown in Figure 5 for a representative synthesis experiment, the
329 dextran solution after the inactivation and exclusion of enzymes contains dextran polymers, fructose and
330 traces of glucose and other oligosaccharides with low polymerization degree.

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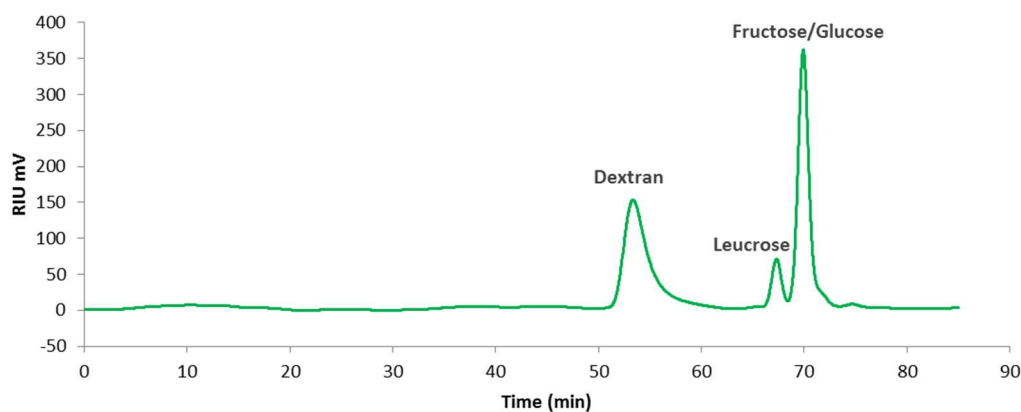


332

333 **Figure 4. Representation of the three different dextran populations, G_i , G_jS , and G_kL , using an HPAEC-PAD analysis on the**
334 **synthesis products at different time intervals obtained from an initial sucrose concentration of 200 g.L^{-1} .**

335

336



337

338 **Figure 5. SEC analysis of the reaction products after enzyme exclusion based on an initial sucrose concentration of 200 g.L^{-1} .**

339

340 The dextran product was purified from monosaccharides (glucose and fructose) and short oligosaccharides
341 (polymerization degree <3) using a 2-L DOWEX™ MONOSPHERE™ Ca^{2+} chromatography column with
342 deionized and degassed water as eluent. The flow rate was set to 15 mL.min^{-1} and the temperature to 50°C .

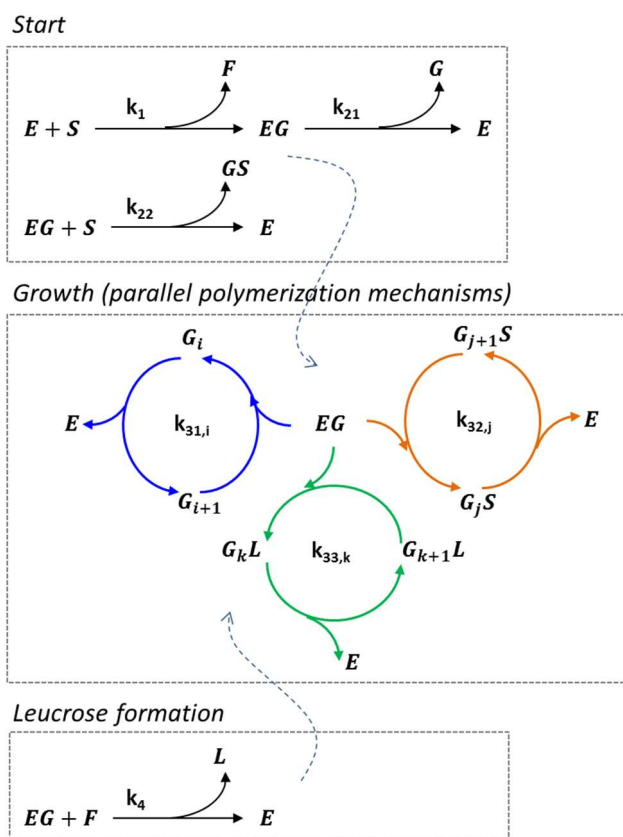
343 Aliquots of 15 mL were taken until total polysaccharide recovery. The dextran was then dried via
 344 lyophilization using a Telstar LyoBeta 2.0. The freezing temperature was set to -70°C . The primary drying was
 345 performed at -30°C under 0.2 mBar with the secondary drying at $+20^{\circ}\text{C}$ under 0.2 mBar. The total freeze-
 346 drying time was approximately 49 h.

347 2.5 Predictive modeling of dextran synthesis using the DSR-M enzyme in a batch reactor

348 2.5.1 PolyEnz model

349 The enzymatic polymerization of glucosyl units to form dextran chains is a key step in the process for which
 350 rigorous modeling is required. To this end, experiments were conducted at the laboratory scale, as described
 351 in Section 2.3, to generate the data necessary for the development of a kinetic-based predictive model for
 352 dextran polymerization using the DSR-M enzyme, namely, the PolyEnz model. This model provides a
 353 comprehensive description of the enzymatic polymerization of glucosyl units from a sucrose substrate via
 354 the non-processive mechanism illustrated in Figure 6. More specifically, the PolyEnz model describes and
 355 determines in a predictive manner (i) the pathway of synthesis for different initial concentrations of sucrose,
 356 (ii) the three simultaneous polymerization mechanisms during the growth phase, (iii) the size distribution and
 357 the degree of polymerization of the resulting polymers, and (iv) the accurate amount of side products.

358



359

360 **Figure 6. Mechanism for dextran synthesis from sucrose using the DSR-M enzyme: E = enzyme, S = sucrose, F =**
361 **fructose, EG = glucosyl-enzyme complex, G_i = growing dextran (glucosyl chains only), G_jS = growing dextran (elongated sucrose),**
362 **and G_kL = growing dextran (elongated leucrose).**

363

364 The previous analyzes in Figure 4 showed the simultaneous growth of the three populations of dextran
365 polymers using the DSR-M enzyme: the first represents glucosyl residues bonded together (G_i), the second
366 represents glucosyl residues bonded together with a sucrose molecule at the reducing end (G_jS)⁶, and the
367 third represents glucosyl residues bonded together with a leucrose molecule at the reducing end (G_kL). The
368 indices i, j, and k in Figure 6 indicate the number of glucosyl residues bonded to the corresponding acceptor.
369 Accordingly, the dextran product is composed of the three above-mentioned polymer formations, where i is
370 varied from 1 to n while j and k are varied from 1 to n-2 to obtain the same length of polymer whatever the
371 acceptor. Here, n represents the maximum polymerization degree. As illustrated in Figure 6, glucose released
372 in the starting phase reacts with the EG complex to form G_2 polymers, which are again polymerized later
373 during the growth phase to form G_i (the left polymerization mechanism in Figure 6). Conversely, the GS
374 molecules released during the starting phase are polymerized in parallel via consecutive reactions with the
375 EG complex to form G_jS polymers (the right polymerization mechanism in Figure 6). Leucrose, when
376 produced, also acts as an acceptor and induces the third polymerization chain (the bottom growth chain in
377 Figure 6) resulting in the formation of G_kL polymers. As a result, during dextran synthesis using DSR-M, three
378 polymerization paths occur simultaneously.

379 The start phase in Figure 6 is comprised of 3 reactions, while the growth phase results in the formation of G_i ,
380 G_jS , and G_kL and involves $3n - 6$ chain reactions. Only one reaction is needed to represent the formation of
381 leucrose. Therefore, the synthesis schema involves overall $3n-2$ reactions for which $3n-2$ kinetic constants
382 need to be defined and fitted. The overall system of reaction rate equations describing the dynamic mass
383 balances in the batch reactor and the corresponding kinetic data are summarized in Appendix A.

384 *2.5.2 Batch reactor utilities and mixing*

385 The reactions take place in a batch stirred tank reactor with free enzymes, in which the mixing is maintained
386 via mechanical agitation. A batch system (with temperature control via a heating/cooling jacket) is the most
387 commonly used reactor in biocatalysis¹². A reactor volume of 138 L (according to the standard DIN28136) can
388 be used for a solution of 100 L (the nominal volume). Details related to the calculation of the utilities (mixing
389 and heating) in a batch reactor are provided in the Supplementary Information (Table A.1).

390

391 **2.6 Flexible-fidelity modeling of unit operations for purification and separation**

392 *2.6.1 Inactivation of the DSR-M enzyme*

393 After synthesis, the enzymes need to be inactivated to prevent side reactions. This can be achieved by heating
394 the mixture to 95°C for 1 h in the reactor. Inactivation by heating the mixture at 95°C for 1 h in the reactor
395 was preferred to other methods such as medium acidification or basification, in order to prevent the risk of
396 osidic linkage cleavage, and thus of the deterioration of the product. Table A.2 in the Supplementary
397 Information offers an overall evaluation of the utility requirements for agitation and heating in a batch
398 reactor for enzyme inactivation.

399 *2.6.2 Ultrafiltration for enzyme separation*

400 Ultrafiltration (UF), a pressure-driven membrane process, is often used throughout downstream processing
401 for different applications in the production of bio-based chemicals. The process is smooth, fast, and
402 comparatively inexpensive. High concentration factors and high yields (85-99%) could be achieved²⁷ with the
403 condition to countering fouling issues. UF membranes are available with different cut-off limits for the
404 separation of molecules ranging from 1 kDa up to 300 kDa. The typical filtrate flux in UF ranges from 25
405 L.m⁻².h⁻¹ (LMH) to 150 LMH. Typical transmembrane pressures (TMP) in UF are within 0.2–4 10⁵ Pa. UF can
406 be carried out in biochemistry in varying modes of operation³⁰ such as direct flow filtration or tangential flow
407 filtration (TFF) in batch, feed batch, or diafiltration modes, which are chosen according to the context and
408 the separation objectives.

409 To separate out the enzymes, direct filtration (dead-end) in batch mode was chosen, the objective being to
410 recover all the solution containing the saccharide molecules and to eliminate the enzymes. Given the enzyme
411 properties (size 140 kDa), a polyethersulfone membrane with a molar mass cut-off of 50 kDa (not retaining
412 the produced dextrans) could be used due to its low-to-moderate binding properties to proteins and its
413 repellency with regard to saccharides³¹. The concentration of the enzyme in the reaction media was very low,
414 approximately 0.01 g.L⁻¹. A filtration flux of 30 L.m⁻³.h⁻¹ is expected, which represents a third of the maximal
415 flux for this membrane. A temperature of 40°C can improve the efficiency of the filtration by reducing the
416 viscosity of the solution. Heating of the solution may not be necessary because the previous operation
417 (enzyme inactivation) takes place at 95°C. Water can be used for the backwashing of the membranes with a
418 flux higher than the filtration flux, for 1 min, and with a relatively high frequency, e.g., every 15 min. In-place
419 cleaning and membrane sanitation occur at the end of the batch filtration with a hydrogen peroxide
420 solution³². The determination of the main operating conditions for the enzyme separation by UF, as well as
421 the corresponding utility requirements, is given in the Supplementary Information (Table A.3).

422 2.6.3 Dextran separation and concentration via UF

423 UF can be used successfully for the fractionation of polysaccharide mixtures, as is widely acknowledged in
424 the literature³³. The most commonly used filtration modes are diafiltration for fractionation and batch
425 filtration for concentration both with tangential flow. In addition to the molar mass cut-off, the separation is
426 conditioned by the chemical properties of the membranes, i.e., their hydrophobic characteristics. For
427 example, it is known that polyethersulfone membranes exhibit repellency with respect to proteins and
428 polysaccharides, therefore avoiding solute adsorption on the membrane surface and pores³³. Optimal
429 combinations of the hydrophobicity of a material and the molar mass cut-off can be selected (e.g., from
430 laboratory-scale experiments) to achieve efficient separation. A membrane with rather hydrophobic
431 properties and a cut-off size of 3 kDa can satisfy the current separation objective.

432 An optimal temperature for UF operation is observed near 40°C. Indeed, a high temperature decreases the
433 viscosity of the fluid by promoting the permeate flux while reducing the retention of molecules and therefore
434 the recovery of the product^{33,34,35}. With regard to the transmembrane pressure, a high pressure considerably
435 increases the permeate flux but, at the same time, decreases the efficiency of the separation. If fractionation
436 is the main objective, the membranes need to operate at low pressure. Fouling is prevented by backwashing.
437 For example, for hollow fiber UF in tangential mode, a frequency of a few minutes with a washing duration
438 of several seconds has been tested and has provided good results³³.

439 The goal of separation in this study is to eliminate monosaccharides and oligosaccharides with an overall size
440 of less than 3 kDa from the solution. All polymers of higher sizes constitute the so-called dextran product.
441 The purity of the obtained product depends on the type of membrane used and the filtration mode. The
442 usual filtration mode used for the size-dependent separation of molecules is tangential: diafiltration with
443 constant or variable volume (in a feed tank) and recirculation of the retentate. Assuming that the selectivity
444 of the chosen membrane is satisfactory (under operating conditions of a temperature of 40°C, a TMP of 1.10^5
445 Pa, and a permeate flux of $30 \text{ L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$) with a negligible permeability for medium-sized dextrans, the purity
446 (fraction of polymers) of the product depends on the dilution conditions³⁶ (the calculation is presented in the
447 Supplementary Information).

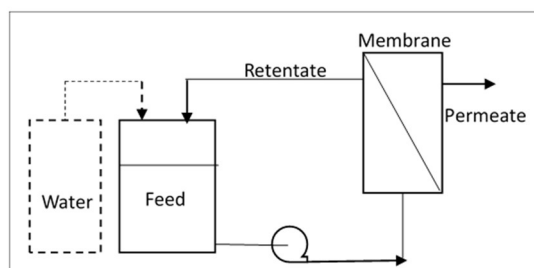
448 The washing of the membranes consists of a backflush with water at a reverse transmembrane pressure
449 higher than the filtration pressure. Membrane sanitation can be performed with a solution of hydrogen
450 peroxide (and optionally 0.1 M NaOH). All operations are considered at 40°C.

451 The characteristics of the product, the utilities necessary for filtration and cleaning (energy, water, and
452 reactant consumption), and the effluent produced (permeate composition) are calculated according to the
453 model presented in the Supplementary Information (Tables A.4 and A.5).

454 In the case of solutions that are too dilute, a concentration operation could be envisaged. Concentration
455 operations use the principle of UF or nanofiltration in tangential flow in batch mode, i.e., the retentate is

456 recirculated in the feed tank with no addition of water. The same operating parameters are used in terms of
457 the temperature, permeate flux, retentate flux, and TMP. However, the membrane molar mass cut-off needs
458 to be adapted to the dextran size, a smaller cut-off size is preferred, e.g., 1 kDa. The general scheme of UF in
459 separation mode or concentration mode is shown in Figure 7.

460



461

462 **Figure 7. Tangential flow filtration (TFF) set-up in concentration mode (solid lines) or separation mode (includes dotted area).**

463

464 2.6.4 Chromatography for dextran purification

465 Column chromatography has been shown to be an extremely efficient technique for separating saccharide
466 molecules in biological extracts. There are several weaknesses in industrial-scale chromatography, such as
467 designing and scaling difficulties, the dilution of the obtained product, and its high cost. Nevertheless, this
468 process is still applied in the case of high-value products^{27,37}.

469 Size exclusion chromatography can be used for the purification of dextrans. In a chromatography column
470 packed with porous particles of an ion-exchange resin, the dextran molecules are not retained but are rinsed
471 with the elution solvent (e.g., water). Conversely, the monomers and oligomers (small molecules) are
472 retained in the porous media and are eluted later. A maximum resolution can be achieved with sample
473 volumes ranging from 0.5% to 2% of the total volume of the column. However, volumes up to 5% can result
474 in acceptable separation and, based on preliminary laboratory experiments, this value was chosen in this
475 study. The scale-up follows the operating conditions of the laboratory; in addition, similar geometry and
476 hydrodynamics are adopted for the operation of the column. The proposed process is based on a single
477 column of 200 L, which is 10 times longer than its diameter (for hydrodynamic reasons); however, several
478 columns could also be implemented in parallel. The dextran polymers are eluted with two column void
479 volumes, while the monomers and small oligomers are recovered in the following four void volumes. The
480 temperature in the column is maintained at approximately 50°C to reduce the viscosity of the liquid. A
481 detailed determination of the main operating parameters, as well as the utility requirements, is given in the
482 Supplementary Information (Table A.6).

483

484 2.6.5 Drying pharmaceutical grade dextran polymers

485 Almost all biotechnology and pharmaceutical processes include a drying stage to improve the stability of the
486 final product. Lyophilization and spray drying are two widely used techniques for drying heat sensitive
487 materials. In fact, the elimination of water allows the degradation and the mobility of the product in the solid
488 state to be decreased³⁸.

489 Spray drying or atomization of liquid droplets is a continuous process consisting of a liquid feed spray in a hot
490 medium, where the liquid content (water) of the droplets is continuously evaporated to the desired moisture
491 level of the particles or until a crystalline state of the powder with the desired texture is obtained.
492 Lyophilization is often used at lab/pilot scales for a wide range of products. A freeze-dried product has a solid,
493 friable, and porous appearance and is characterized in particular by its great avidity for water. Lyophilization
494 (freeze drying), associated with the manipulations that lead to this state, is a process based on the use of
495 both cold (brutal freezing) and dehydration via sublimation of the ice formed. Indeed, lyophilization can be
496 decomposed into two main phases: the freezing phase (requiring cooling) and the dehydration phase
497 (requiring heating and vacuum pumping). The latter is subdivided into two stages, i.e., the sublimation and
498 desorption phases. Schematically, it can be said that the product is first frozen and then the remaining solvent
499 on the surface of the product is removed via sublimation. Details concerning the thermodynamic modeling
500 of the drying stage are given in the Supplementary Information (Table A.7).

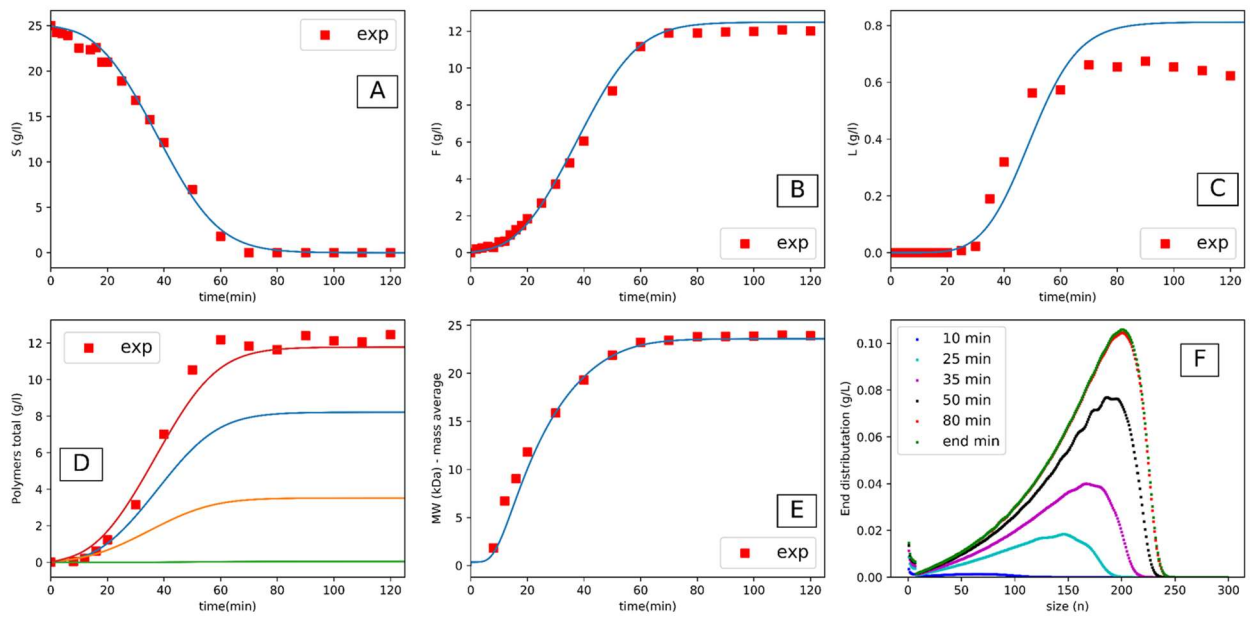
501 **3 Results and discussion**

502 **3.1 Predictive description of dextran synthesis using the DSR-M enzyme for different substrate** 503 **concentrations**

504 The experimental results for dextran synthesis at the laboratory scale and the simulations obtained using the
505 PolyEnz model (Section 2.5) are presented in Figures 8 and 9. Several series of curve-fittings and numerical
506 optimizations were carried out on the provided experimental data for initial sucrose concentrations between
507 10 g.L⁻¹ and 400 g.L⁻¹ to determine the kinetic constants and their correlations as a function of the initial
508 sucrose concentration. Table 1 summarizes the concentration of the obtained dextran, as well as the average
509 molar mass of the produced dextran polymers, as a function of the initial selected sucrose concentration.

510 To demonstrate the predictive behavior of the PolyEnz model, the results of two different initial
511 concentrations of sucrose, 25 g.L⁻¹ and 200 g.L⁻¹, were compared, as illustrated in Figures 8 and 9,
512 respectively. The concentration profiles of sucrose, fructose, and leucrose over time are shown in the top
513 rows of these figures. Meanwhile, the graphs on the bottom left show the formation of the dextran polymers
514 over time, namely, G_i, G_jS, and G_kL. The increase in the total dextran concentration (the red curve) results
515 from the addition of these three components.

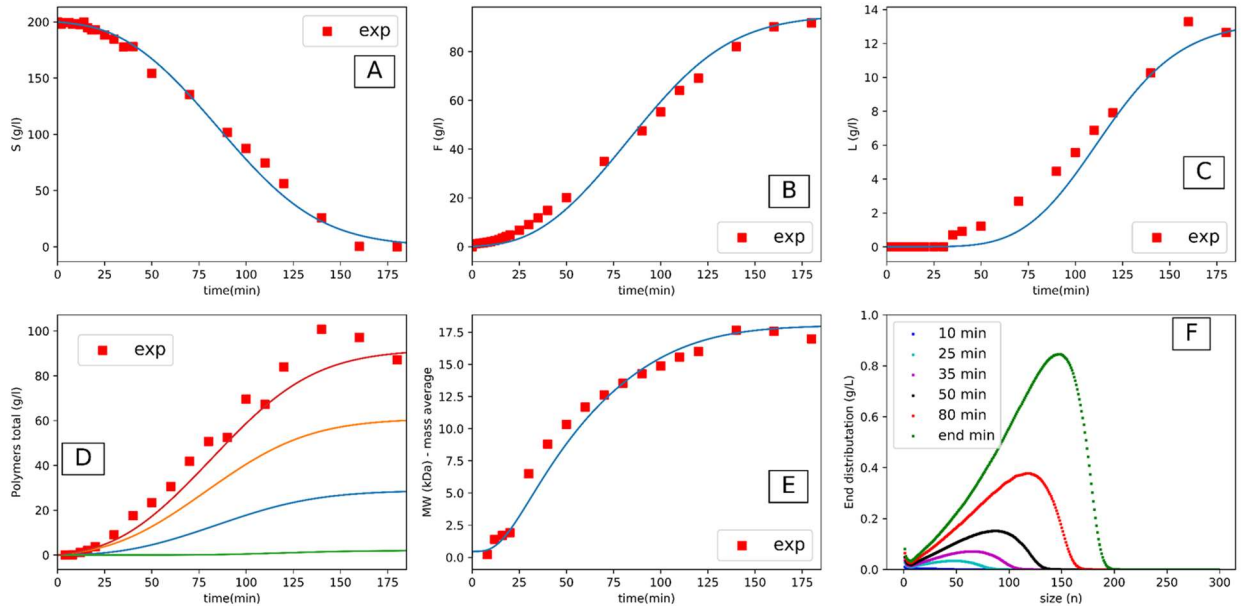
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517

518 **Figure 8. The PolyEnz model compared to the experimental data for dextran synthesis using the DSR-M enzyme with an initial**
 519 **sucrose concentration of 25 g.L⁻¹: (A) sucrose concentration; (B) fructose formation; (C) leucrose formation; (D) dextran**
 520 **polymerization; (E) evolution of the average dextran molar mass over time; and (F) dextran length distribution over time.**

521



522

523 **Figure 9. The PolyEnz model compared to the experimental data for dextran synthesis using the DSR-M enzyme with an initial**
 524 **sucrose concentration of 200 g.L⁻¹: (A) sucrose concentration; (B) fructose formation; (C) leucrose formation; (D) dextran**
 525 **polymerization; (E) evolution of the average dextran molar mass over time; and (F) dextran length distribution over time.**

526

527 The bottom middle graphs indicate the average molar mass of the polymers over time, and the graphs on
528 the bottom right indicate the length distribution of the polymers over time. A satisfactory agreement can be
529 observed when comparing the model results to the experimental data (the red squares).

530 For a given initial substrate concentration, the PolyEnz model allows a predictive description of the
531 polymerization quality, as shown in Table 1, in terms of mass average molar mass (M_w) and concentration of
532 dextrans in the batch synthesis reactor ($17.9 \text{ kg}\cdot\text{mol}^{-1}$ and $88 \text{ g}\cdot\text{L}^{-1}$, respectively, for an initial sucrose
533 concentration of $200 \text{ g}\cdot\text{L}^{-1}$, for instance). Additionally, in order to reflect the influence of the initial sucrose
534 concentration on the quality and dispersity of the final dextran products after oligomer separation and cut-
535 off at $3 \text{ kg}\cdot\text{mol}^{-1}$, Table 1 also provides data on the quality and dispersity of the end dextrans ($M_w = 19.9$
536 $\text{kg}\cdot\text{mol}^{-1}$, $M_n = 16.8 \text{ kg}\cdot\text{mol}^{-1}$, and $\text{PDI} = 1.18$, for an initial sucrose concentration of $200 \text{ g}\cdot\text{L}^{-1}$).

537 The sucrose concentration can be chosen simply depending on the desired length of dextran. However, from
538 a quantitative point of view, the production of dextran increases with increasing initial substrate
539 concentration while, in parallel, the length of the polymers decreases with increasing initial substrate
540 concentration. For example, to produce 1,000 kg of “20-kDa dextran” from a $200\text{-g}\cdot\text{L}^{-1}$ sucrose solution (Table
541 1), 11,364 L of the substrate solution needs to be synthesized and then treated given a final dextran
542 concentration of $88 \text{ g}\cdot\text{L}^{-1}$. Conversely, fixing the same amount of dextran, i.e., 1,000 kg, but aiming for a
543 slightly different dextran quality with an average molar mass of $25 \text{ kg}\cdot\text{mol}^{-1}$, which can be obtained from a
544 $100\text{-g}\cdot\text{L}^{-1}$ sucrose solution as shown in Table 1, 21,277 L of the substrate solution needs to be synthesized
545 and further separated throughout the process; the cost in this case will be nearly double that of the previous
546 case from both the treatment and energy perspectives. Therefore, the size of the future plant depends not
547 only on the desired product quantity but also on the type of dextran.

548 The product obtained from $200\text{-g}\cdot\text{L}^{-1}$ sucrose (highlighted in Table 1) has a mass average molar mass of
549 approximately $20 \text{ kg}\cdot\text{mol}^{-1}$, after separation and purification, when estimated at the peak apex of the
550 chromatogram from size exclusion chromatography coupled with refractive index detection. The production
551 of dextran using DSR-M directly from $200\text{-g}\cdot\text{L}^{-1}$ sucrose is further investigated via the eco-design approach.
552 This dextran size is of pharmaceutical interest (eye drops, blood volume expander, organ preservation,
553 vaccines, see www.dextran.com) as well as a carrier for biophysical probes after functionalization (FITC-dextran
554 20, Biotin-dextran 20, DEAE-dextran 20, see www.finabio.net).

555 **Table 1. Influence of initial substrate concentration on dextran quality in batch synthesis reactor and in end product after**
 556 **purification.**

Sucrose concentration (g.L ⁻¹)	Synthesis time (min)	Dextran concentration (g.L ⁻¹)	Synthesis product			
			in batch reactor	Final dextran product after cut-off at 3 kDa		
			M _w ⁽¹⁾ (kg.mol ⁻¹)	M _w ⁽¹⁾ (kg.mol ⁻¹)	M _n ⁽²⁾ (kg.mol ⁻¹)	PDI ⁽³⁾
10	80	4.8	20.7	24.7	21.1	1.17
25	90	12	24.0	27.1	23.5	1.15
50	100	23	24.6	27.3	23.8	1.15
100	120	47	22.5	24.9	21.4	1.16
200	200	88	17.9	19.9	16.8	1.18
300	300	130	14.5	16.2	13.6	1.19
400	400	175	11.5	12.9	10.9	1.18

557 ⁽¹⁾ Mass average molar mass

558 ⁽²⁾ Number average molar mass

559 ⁽³⁾ Polydispersity index

560

561 **3.2 Variants for product separation and purification at an industrial scale: Benchmarks 1–3**

562 Three flowsheets were compared, namely, Benchmarks 1, 2, and 3, to indicate the choice and combination
 563 of the most relevant operations for the optimal production of high-quality dextran (Figure 10). For the three
 564 benchmarks studied in this section, the comparative analysis is based on an initial substrate solution of 100
 565 L with an initial sucrose concentration of 200 g.L⁻¹ giving the same quality of dextran-20. As explained
 566 previously in Section 3.1, this choice was motivated by the mass average molar mass of the final product in
 567 this case (M_w = 19.9 kg.mol⁻¹ after purification, Table 1) and by the considerable savings made in terms of
 568 treatment and energy compared with the use of lower sucrose concentrations (< 200 g.L⁻¹). The separation
 569 steps, shown in Figure 10, were organized to converge to the same quality of products (See Section 2.6 and
 570 Supplementary Information A.). By ensuring the same quality of dextrans at the end of process after
 571 purification (M_w = 19.9 kg.mol⁻¹, M_n = 16.8 kg.mol⁻¹, PDI = 1.18, Table 1), the comparison becomes
 572 environmental and energy-wise, as presented later in this section.

573 Benchmark 1 combines the synthesis and purification/concentration processes currently used at the
 574 laboratory scale, which includes, as shown in Figure 10(a), polymerization (the enzymatic synthesis of
 575 dextran), enzyme inactivation via heating and separation (diafiltration–frontal ultrafiltration), ion-exchange
 576 chromatography to separate the small oligomers from the dextran polymers, and finally freeze drying to
 577 obtain excellent pharmaceutical quality dextran with relevant textures after drying. Benchmark 2 follows
 578 nearly the same production pattern except that an intermediate UF with TFF is introduced prior to the freeze
 579 dryer to concentrate the solution eluted from the chromatography column. Reducing the moisture content

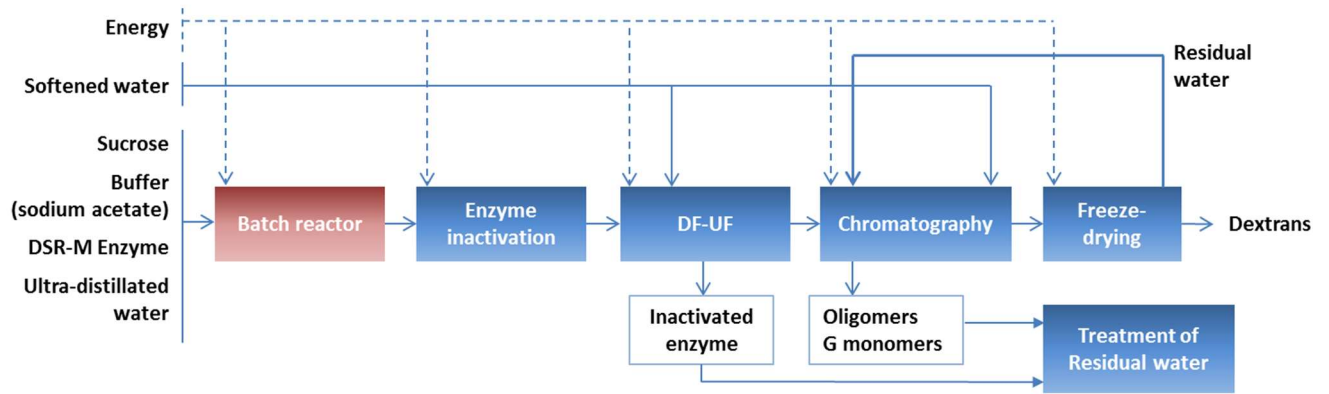
580 reduces the high energy requirements during freeze drying (Figure 10(b)). In both Benchmarks 1 and 2, the
581 use of chromatography for separation induces a marked dilution of the dextran solution due to the elution
582 step. This step directly impacts the energy requirements of the final drying phase. Therefore, an exclusive
583 multi-stage filtration strategy is proposed in Benchmark 3 (Figure 10(c)), instead of separation via ion-
584 exchange chromatography, for the removal of small oligosaccharides and monosaccharides, that is,
585 molecules with molar mass of less than $3 \text{ kg}\cdot\text{mol}^{-1}$.

586 In all scenarios, ultra-distilled water is used in the batch reactor during the enzymatic synthesis of the
587 dextrans and softened water is used as process water in the other operations (elution in chromatography,
588 cleaning of membranes, and membrane operation). A water reuse strategy is put in place for the entire
589 process. The condensed water in the freeze-drying operation is theoretically pure and can be used, e.g., as
590 an elution solvent in chromatography. The accumulated residual water is considered to have been treated
591 prior to release to the environment, as indicated in the process flowsheets (Figure 10).

592 The inventory data including the ecoinvent processes considered in LCA modelling are detailed in
593 Supplementary Information, Table C.1 and C.2.

594 Table 2 presents the overall performance results for the three benchmarks compared in terms of yield,
595 specific energy, and water consumption and the life cycle impacts per kg of dextran. The environmental
596 impacts are presented in Table 2 as the total endpoint and endpoints for human health, ecosystems, and
597 resources (the ReCiPe method) and as the global warming potential for their climate change impacts (kg eq-
598 CO_2). For a similar production yield (0.42 kg of dextran per kg of sucrose) in Table 2, significant discrepancies
599 are observed in terms of the energy and water consumption. Details concerning the distribution of the energy
600 and water consumption per unit process are shown in Figure 11. The main differences between the three
601 benchmarks occur at the separation and conditioning stage, owing to its high energy and water consumption
602 and its environmental impact.

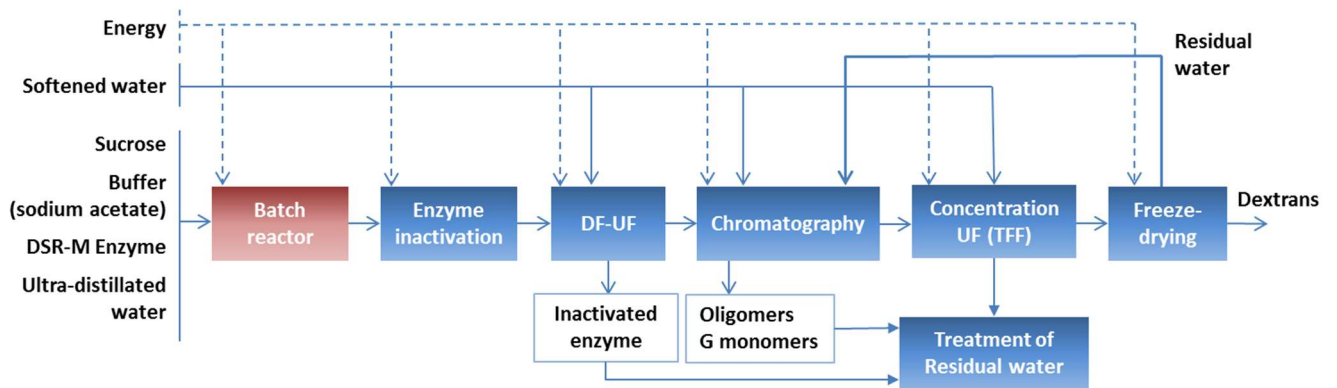
603 The freeze dryer (lyophilizer) represents the highest energy demand, followed by chromatography, while
604 chromatography is the primary consumer of water. As shown in Figure 11(c), the replacement of
605 chromatography by TFF in Benchmark 3 results in a radical reduction in the utility demands: 35 times less
606 energy and 20 times less water is needed in Benchmark 3 compared to Benchmark 1. The intermediate
607 Benchmark 2 involves a concentration step via TFF to reduce the water content of the chromatography
608 effluent; the chromatography elution step strongly dilutes the dextran solution. In this way, less water needs
609 to be removed in the last stage of freeze drying, followed by a marked reduction in energy consumption: 10
610 times less energy in Benchmark 2 compared to Benchmark 1. Conversely, more water is needed for the
611 chromatography due to the lower amount of recycling from the lyophilization to the chromatography; in this
612 case, a fraction of the water is rejected in the form of the TFF permeate to a wastewater treatment system.
613 As a result, the water consumption in Benchmark 2 is 1.5 times higher than in Benchmark 1.



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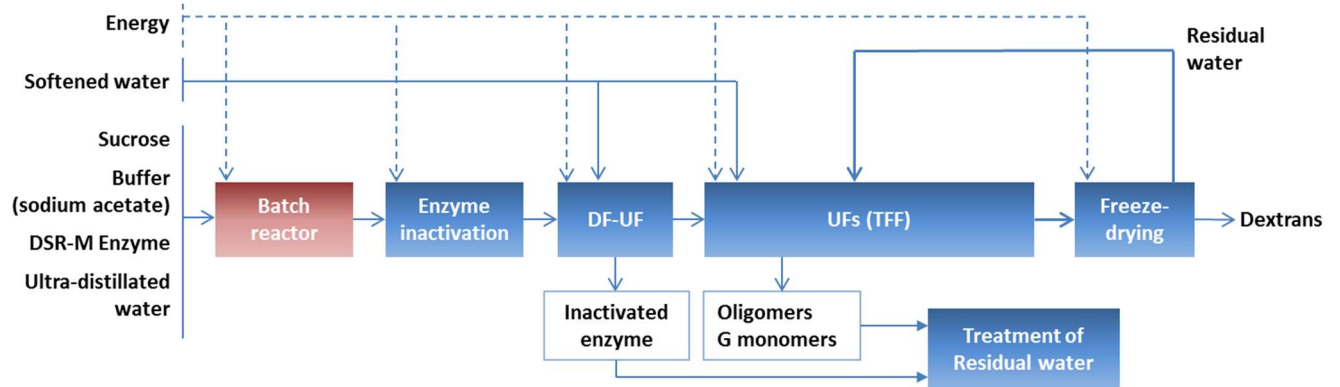
(a) Benchmark 1



616

617

(b) Benchmark 2



618

619

(c) Benchmark 3

Figure 10. Overall process diagram and its alternatives: (a) Benchmark 1: separation via chromatography/freeze drying; (b) Benchmark 2: separation via chromatography, TFF ultrafiltration, and freeze drying; and (c) Benchmark 3: separation via standalone TFF ultrafiltration and freeze drying.

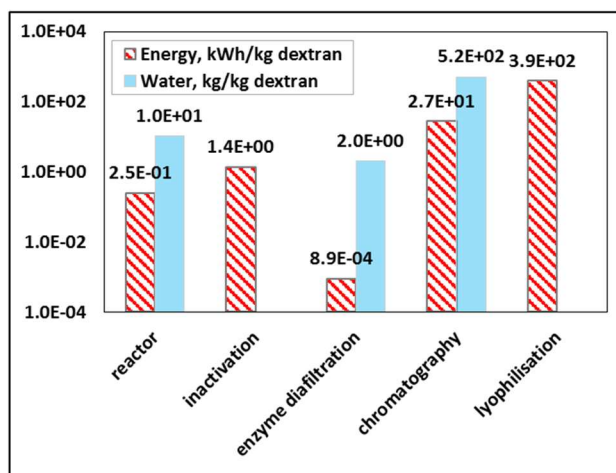
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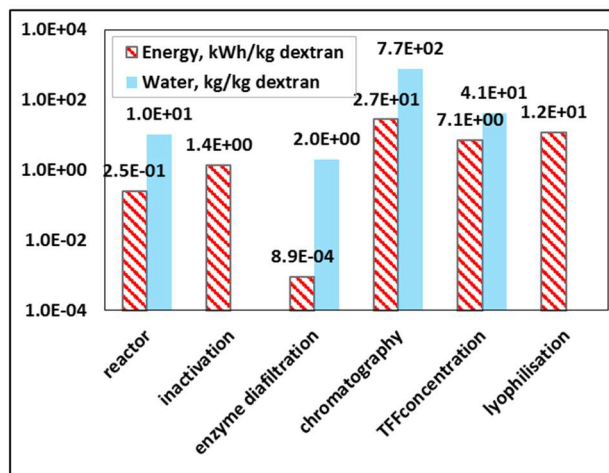
625 **Table 2: Overall utility demand, production yield, and environmental assessment for Benchmarks 1, 2, and 3 (results are given for**
 626 **a dextran production chain based on a 100-L initial solution with a sucrose concentration of 200 g.L⁻¹).**

	Benchmark 1	Benchmark 2	Benchmark 3
Specific energy required, kWh.kg ⁻¹ dextran product	419	48	12
Specific water consumption, kg.kg ⁻¹ dextran product	528	820	26
Product yield, kg dextran product.kg ⁻¹ sucrose	0.42	0.42	0.42
Total ReCiPe endpoint impact^(*) (per FU⁽¹⁾)	4.093	0.710	0.322
Ecosystem quality ⁽²⁾	0.853	0.214	0.142
Human health ⁽³⁾	1.970	0.298	0.108
Resources	1.270	0.199	0.007
Climate change - IPCC2013, kg eq-CO₂ (per FU)	38.94	6.20	2.40

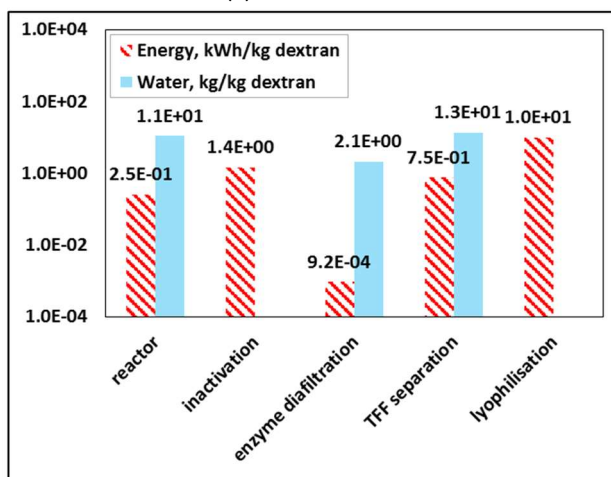
627 ^(*) Normalized impact in points, ⁽¹⁾ Functional unit (FU): 1 kg of dextran polymer obtained, ⁽²⁾ Main damage observed: climate
 628 change, ⁽³⁾ Main damages observed in decreasing order: climate change, particulate matter formation, human toxicity, and ionizing
 629 radiation



(a) Benchmark 1



(b) Benchmark 2



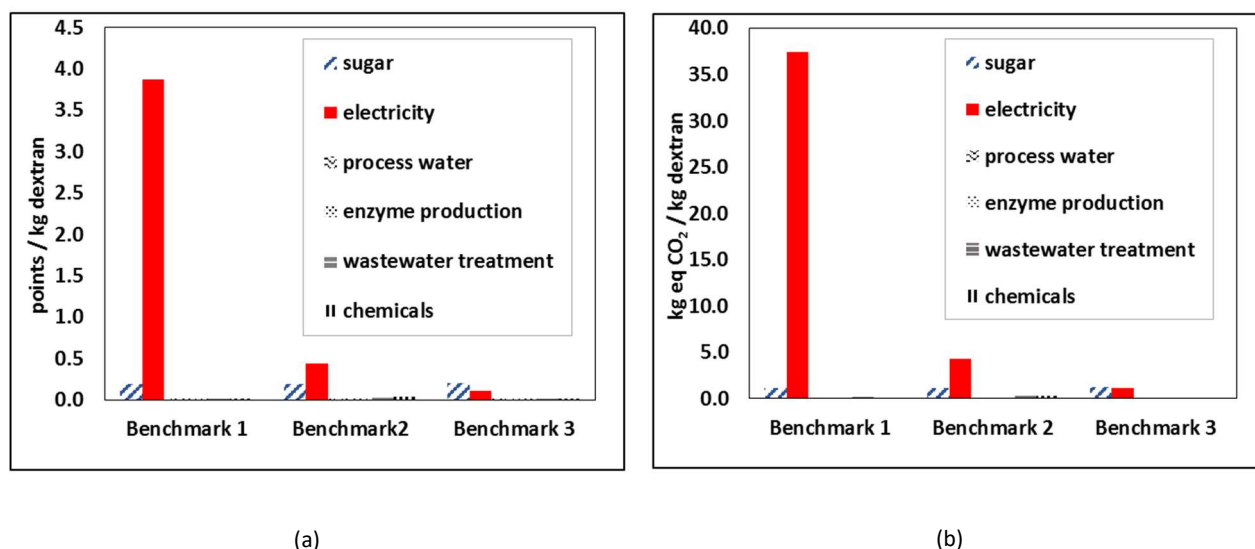
(c) Benchmark 3

630 **Figure 11. Contribution of specific energy and water requirements per kg dextran product for (a) Benchmark 1, (b) Benchmark 2,**
 631 **and (c) Benchmark 3 (results are given on a logarithmic scale, and for a dextran production chain based on a 100-L initial solution**
 632 **with a sucrose concentration of 200 g.L⁻¹).**
 633

634

635 The LCA results are summarized in Table 2 and Figure 12. The total ReCiPe endpoint score is shared between
 636 the three areas of protection, namely, human health, ecosystem quality, and resources. Unambiguously,
 637 Benchmark 3 presents the best environmental performance, followed by Benchmark 2 and, with a much
 638 lower performance, Benchmark 1. The same ranking is obtained for the climate change impact category.
 639 Therefore, the detailed results of the ReCiPe method (presented in the Supplementary Information B.)
 640 demonstrate that climate change is the main contributor to the potential damages to human health and
 641 ecosystem quality. The contribution analysis of the LCA results shown in Figure 12 highlights again the net
 642 impact of energy consumption for all the benchmarks, corroborating the results previously presented in
 643 Figure 11. These results were obtained in a French economic context considering the French electricity mix
 644 as an electricity type close to reality. Note that, due to the different energy configurations in Europe, the
 645 choice of electricity is country dependent and therefore the origin of the electricity significantly influences
 646 the type and amount of the resulting impacts. The second largest contributor to the environmental impacts
 647 is the production of sugar, which is used as a raw material in the synthesis of dextran. Because the production
 648 yields of the three benchmarks are similar, sugar production has identical impacts in each benchmark.
 649 Negligible impacts are generated by the water treatment (process water and wastewater), enzyme
 650 production, and the production of chemicals consumed in the process.

651



652 **Figure 12. Life cycle impact assessment (LCIA) for different utilities and services: (a) the total normalized ReCiPe endpoint**
 653 **and aggregated impacts and (b) the IPCC2007 climate change impact (results are given for a dextran production chain based on**
 654 **an initial substrate solution of 100 L with an initial sucrose concentration of 200 g.L⁻¹).**

655

656 The low contribution of enzyme production is explained by the small quantity necessary for the synthesis of
 657 dextran, i.e. $1.4 \cdot 10^{-5}$ kg of enzyme for 1 kg of dextran, and by the small corresponding culture medium volume
 658 (maximum 500 L for 1 t of dextran produced with non-optimized culture in Erlenmeyer flask, a value that will
 659 be reduced with large-scale productions in fermenter).

660 3.3 Sensitivity analysis and identification of the main production factors

661 As indicated in Table 3, various factors related to production, in particular the initial concentration of the
662 substrate, the type of substrate, the type of water, the use of chromatography, and the amount of freeze
663 drying expressed by the ratio of the freeze-dried moisture content to the total water content of the solution
664 at the dextran separation stage were subjected to an additional sensitivity analysis to indicate the main
665 factors favoring energy-efficient production with a reduced impact on the environment. The relative
666 sensitivities in Table 3 are reported in terms of the global energy demand and the ReCiPe total endpoint to
667 cover all categories of potential environmental damage.

668 The choice of the sucrose concentration as a substrate is undoubtedly the most significant determining factor
669 in the entire production chain because it directly determines the quantity and length of the dextran polymers
670 and, consequently, the scale of the process and the number of separation and drying steps involved. Earlier
671 in Section 3.1, the study of the influence of the initial substrate concentration on the quality of the dextran
672 in the batch polymerization reactor, as shown in Table 1, was intended to emphasize the importance of
673 avoiding low concentrations of sucrose as a substrate due to the resulting large energy demands for
674 separation and drying. The sensitivity of the results to the initial concentration of sucrose provided in Table
675 3 is further evidence of the importance of this choice. In fact, given the size of the targeted dextran polymers
676 and based on the same production yield, the optimal substrate concentration to be synthesized should be
677 selected first, as explained in Section 3.1. Figure 13 also offers benchmark simulations for sucrose
678 concentrations between 25 g.L⁻¹ and 400 g.L⁻¹ to better illustrate the direct effect of the substrate
679 concentration on the different performance indicators in terms of utilities and environmental impacts for 1
680 kg of dextran produced. Figure 13 includes the results of Benchmarks 1 and 3; however, those of Benchmark
681 2 are not indicated because the performance of Benchmark 2 was delimited by those of the other two
682 benchmarks. It can easily be seen that the utility requirements and the environmental footprints are very
683 sensitive to the choice of the initial substrate solution due to the different solution volumes to be processed
684 in the different unit operations on the basis of the same dextran yield.

685 As shown in Table 4, to achieve a targeted production yield, for example, 1 t of dextran per year, depending
686 on the initial substrate concentration, a different number of batches of equal size (e.g., reactors for 100 L of
687 solution) need to be involved. Assuming that the synthesis and inactivation operations involve a one-day
688 batch reactor operation (the separation/conditioning operations last for one day) and that 200 days per year
689 are actually productive, the production of 1 t of dextran will naturally require several lines of production in
690 parallel, as suggested in Table 4, independent of the benchmark. Note that, for syntheses using different
691 sucrose concentrations, the sizes of the obtained dextrans are different (Table 1) and, therefore, the present
692 comparison needs to be regarded from the perspective of the specific use of the targeted dextrans and their
693 market.

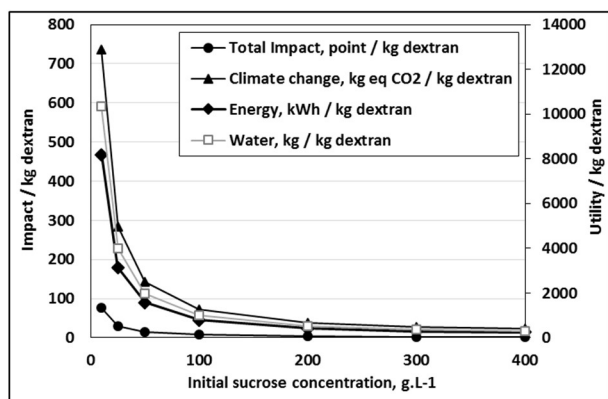
694

695 **Table 3. Relative sensitivity of the energy costs and environmental impacts to certain production factors in dextran synthesis and**
 696 **separation.**

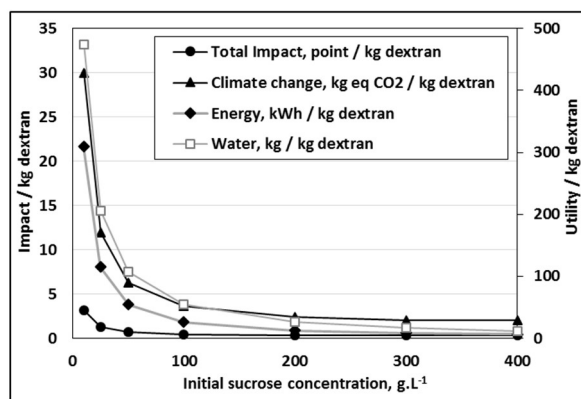
Factor	Range	Relative sensitivity coefficients	
		Energy demand	Total ReCiPe Endpoint
Sucrose concentration	25–400 g.L ⁻¹	3.02	2.95
Freeze-drying use	5–95%	0.93	0.87
Chromatography use	(1) Yes, (2) No	0.56	0.39
Substrate type	(1) Commercial, (2) Residual	0.39	0.12
Water type	(1) Ultra-distilled, (2) Softened, (3) Tap water	<0.01	0.04

697

698



(a) Benchmark 1



(a) Benchmark 3

699 **Figure 13. Sensitivity of the overall performances to the initial sucrose concentration: (a) Benchmark 1 and (b) Benchmark 3**
 700 **(the performance indicators involved are the total ReCiPe-endpoint impact and the climate change impact on the left axis and**
 701 **the energy demand and the total water consumption on the right axis).**

702

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708 **Table 4. Influence of the substrate solution on the process scale to produce 1 t of dextran polymer (for a batch reactor with 100 L**
 709 **of solution).**

S_0 (g.L ⁻¹)	Batches to be processed	Number of production lines	
10	2381	12	710
25	952	5	712
50	476	3	713
100	238	2	714
200	119	1	714
300	79	1	715
400	60	1	716

717

718 In the second row of Table 3, the amount of moisture removal achieved via lyophilization, namely, via freeze
 719 drying, becomes crucial. Note that at least a small amount of freeze drying needs to be maintained at the
 720 end of the chain to achieve the high texture quality of the obtained dextran powder. Nevertheless, the
 721 majority of the water content can be removed prior to the freeze drying via filtration because freeze drying
 722 is expensive at an industrial scale.

723 Of the existing drying technologies, freeze drying (lyophilization) is one of the most energy-intensive, as
 724 acknowledged in the literature, with an average specific energy demand (E_s) of 2 kWh per kg of water
 725 removed; other widely used technologies have lower specific energy demands, e.g., 1.3 kWh.kg⁻¹ for vacuum
 726 drying, 1.6 kWh.kg⁻¹ for spray drying, and 1.9 kWh.kg⁻¹ for air drying^{38,39}. Using a more energy-efficient drying
 727 technology can significantly reduce the amount of energy consumed in all the studied benchmarks. In
 728 addition, a lateral sensitivity analysis was performed for a specific energy demand of drying ranging from 1.3
 729 kWh to 2 kWh per kg of water removed. In summary, for all of the benchmarks, the total energy consumption
 730 could be improved by 1.3-fold for $E_s = 2$ and by 0.9-fold for $E_s = 1.3$ with respect to the theoretical value used
 731 in the modeling. However, note that the improvement yielded from the selection of a more energy-efficient
 732 drying technology remains relatively minor compared to the energy demand associated with the dextran
 733 separation strategy implemented in the three benchmarks, i.e., the replacement of intensive water-
 734 consuming chromatography by membrane separation (Benchmark 3) or, to a lesser extent, by combining
 735 chromatography with a concentration step (Benchmark 2). The purpose of the latter was to minimize the
 736 water content prior to the final drying step.

737 For the experiments, refined commercial grade sucrose was used as a raw material for the dextran synthesis.
 738 However, other sucrose-rich raw materials could be envisaged, for example, molasses (the residual substrate
 739 in Table 3). Estimating the process performance using molasses instead of sugar could help identify the
 740 potential value of this resource and guide future laboratory-scale experimental designs for process

741 adaptation. Sugar beet molasses contains up to 50% sugar and other compounds (e.g., monosaccharides and
742 salts). A replacement was therefore calculated on the basis of 3 kg of molasses per kg of sugar. The impact
743 contribution of the raw material is reduced by a factor of 1.56 (for all impact categories), resulting in a total
744 impact reduction of up to 36% for Benchmark 3, where the raw material contribution was predominant. In
745 this scenario, the performance of Benchmark 2 could be improved by up to 10%, while Benchmark 1 should
746 be discarded due to its huge energy consumption via freeze drying (calculation details in Supplementary
747 Information D.). Similar assessments were also conducted with different sucrose sources, e.g., from
748 sugarcane, without significant changes in the initial performances (e.g., the transport from remote sugar
749 factories offsets the environmental benefit of sugar production on other continents).

750 Process water consumption is very important, particularly in Benchmarks 1 and 2 (Table 2); however, its
751 contribution to the total impact remains very low, at less than 1% (Figure 12). The type of water treatment
752 can affect the environmental performances of consumer processes. Simulations were performed with tap
753 water in a batch reactor (replacing ultrapure water) for synthesis and softened water for other operations.
754 As expected, the results were not significantly different considering the relatively low contribution of the
755 water treatment to the overall impact. Water as a natural renewable resource is not considered in current
756 LCIA methods even though its consumption represents a real environmental and societal issue. This is why
757 water consumption was calculated as an independent indicator in the present study.

758 As specified in Section 2.4, the infrastructure was not included in the LCA. However, an estimate of the
759 infrastructure contribution (buildings and equipment) is possible using the generic data available in the
760 ecoinvent database. The construction of a chemical plant for the production of organic chemicals (ecoinvent
761 2.2 database) generally contributes little to the life cycle impact of the produced substances; the operation
762 stage represents approximately 95% of the life cycle impacts. According to the generic data, the
763 infrastructure would likely contribute less than 3% to the impacts calculated per functional unit (1 kg of
764 dextran produced) for the three benchmarks and for any initial sucrose solution. According to the results in
765 Table 4, the infrastructure impact is much larger when 1 t of dextran is produced from 25-g.L⁻¹ sucrose
766 solutions than when 1 t of dextran is produced from 200-g.L⁻¹ solutions. However, the relative contribution
767 to the environmental impact remains at the same level due to the higher total impact of diluted solutions
768 (Figure 13) (more details are presented in Supplementary Information D.).

769 The cleaning and backwashing operations for the membranes and the chromatography resin were included
770 in the process operations, and thus in the system inventory (Supplementary Information A.). The influence
771 of equipment maintenance over the lifetime of the infrastructure was assessed by sensitivity analysis using
772 generic data from ecoinvent (details of the inventory are presented in Supplementary Information C.).
773 Replacement of used membranes and of used chromatography resin was considered with a maximum
774 frequency of 1 year to evaluate the upper limit of their contribution. The impact contribution for the three

775 benchmarks was less than 2% for membrane and resin replacements taken altogether (details are presented
776 in Supplementary Information D.).

777 Finally, the LCA results were analyzed relative to common organic chemicals provided by the chemical
778 industry to validate the order of magnitude of the results. In the LCA database (ecoinvent), an average score
779 is calculated for organic chemicals derived from abiotic resources as a ReCiPe endpoint of 0.27 points and
780 climate change for 2 kg eq-CO₂ per 1 kg of substance produced. The impact results for Benchmark 3 were of
781 the same order of magnitude, which constitutes a validation support for the approach proposed in this study.
782 In addition, the unusually high impacts assessed for Benchmark 1 prove that this option cannot be adapted
783 in its basic form at an industrial scale.

784 **4 Conclusions**

785 A new process for the production of size-controlled dextran polymers was proposed in this study.
786 Experimental studies at the laboratory scale have demonstrated the feasibility of enzymatic synthesis from
787 an aqueous sucrose solution while using the patented DSR-M enzyme. Experiments provided synthesis
788 kinetics and demonstrated that the dextran characteristics (the size distribution of the polymers) strongly
789 depend on the initial sucrose concentration and the reaction time. In the laboratory, the separation and
790 purification of dextran polymers requires a few operations, in particular chromatography, prior to the final
791 step of drying the dextran polymers to fine regularly structured solid crystals. This promising production path
792 needs to be evaluated from the viewpoint of the overall technical and energetic performance and
793 environmental perspectives before being implemented at an industrial scale. In this study, an eco-design
794 approach was applied to the synthesis and purification of dextran polymers based on multi-fidelity process
795 modeling, scale-up principles, and LCA.

796 The synthesis of dextrans via the DSR-M enzyme was represented by a multilayer polymerization model,
797 namely, PolyEnz, via a non-processive mechanism. The PolyEnz model predicted (i) the pathway of synthesis
798 for different initial concentrations of sucrose, (ii) three simultaneous polymerization mechanisms during the
799 growth phase, (iii) the size distribution and the degree of polymerization of the resulting polymers over time,
800 and (iv) the exact amount of byproducts requiring additional purification steps. The model satisfactorily fitted
801 experimental data obtained for various sucrose concentrations in the range of 10–400 g.L⁻¹.

802 Three process benchmarks were proposed and compared to determine the appropriate and reasonable
803 purification and scale-up processes. Various production factors, including the initial concentration and type
804 of substrate, the type of process water, the use of ion-exchange chromatography for size exclusion
805 separation, and the amount of freeze drying used for the final stage of production were subject to a sensitivity

806 analysis with the criteria being the overall energy demand, the total aggregated potential environmental
807 damages expressed in terms of the ReCiPe total endpoint, and the global warming potential.

808 The substrate concentration appears to be a determining factor because it directly shapes the production
809 scale (and the related investment) for the same targeted production yield. It was clearly observed that the
810 synthesis needs to be orientated according to the choice of the targeted dextran length and that, overall, the
811 use of low concentrations of sucrose of less than $100 \text{ g}\cdot\text{L}^{-1}$ is not favored because it requires an additional
812 number of batch operations and purification steps with their associated extra energy requirements. The
813 latter again showed the importance of the rigorous modeling of dextran synthesis as a function of the initial
814 substrate concentration to substantially guide all the subsequent separation and purification steps. Via a
815 comparative analysis (Benchmarks 1–3), ion-exchange chromatography and membrane filtration were
816 compared, both standalone and combined, for the separation of dextran polymers from smaller saccharides
817 with molar mass less than 3 kDa.

818 Even though its effectiveness is widely acknowledged, ion-exchange chromatography naturally causes a
819 significant dilution of the product and the water content must then be removed via intensive drying facilities.
820 The use of ion-exchange chromatography at an industrial scale would therefore induce a marked increase in
821 the energy consumption for the final drying stage. The replacement of chromatography by membrane
822 filtration can overcome this bottleneck. A new process layout, as suggested in Benchmark 3, used TFF instead
823 of chromatography and resulted in a radical reduction in the utility requirements, a nearly 35-fold smaller
824 energy demand and 20-fold less elution by water. Optimal environmental performances and the lowest utility
825 demands were reported for Benchmark 3. A further reduction in the energy consumption is possible via the
826 optimization of the operating conditions or via the replacement of freeze drying with more efficient drying
827 approaches. Replacing commercial sugar with a sucrose-rich residue, such as molasses, could also improve
828 the environmental footprint; nevertheless, the feasibility of such a replacement needs to be studied
829 experimentally.

830 The benefits of an LCA-based performance study on the synthesis and purification of dextrans in the early
831 stages of process design was demonstrated in this study with the aim of identifying and quantifying the most
832 promising production routes and research and development requirements. With regards to biotechnology,
833 it is very much a priority today to show, first and foremost, the potential and behavior of an innovative
834 process at a larger scale and then to highlight the main challenges at that larger scale, which are usually met
835 at the separation and purification stage.

836

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841 Houmani are also acknowledged for their helpful participation to the project.

842 **Conflicts of interest**

843 There are no conflicts to declare.

844

845 **Appendix A. Rate equations for the synthesis of dextran polymers using the DSR-M**
 846 **enzyme**

847 Notations

848 E: Enzyme, EG: Glucosyl-enzyme complex, S: Sucrose, G: Glucose, F: Fructose, L: Leucrose, G_i: Glucosyl chains
 849 only, G_jS= Elongated sucrose with glucosyl chains, G_kL: Elongated leucrose with glucosyl chains.

850

851
$$\frac{d[S]}{dt} = -k_1[E][S] - k_{22}[EG][S]$$

852
$$\frac{d[F]}{dt} = k_1[E][S] - k_4[EG][F]^2$$

853
$$\frac{d[EG]}{dt} = k_1[E][S] - k_{21}[EG] - k_{22}[EG][S] - k_4[E][EG] - \sum_{i=1}^{n-1} k_{31,i}[G_i][EG] - \sum_{j=1}^{n-3} k_{32,j}[EG][G_jS]$$

 854
$$- \sum_{k=0}^{n-3} k_{33,k+1}[EG][G_kL]$$

855
$$\frac{d[G]}{dt} = k_{21}[EG] - k_{31,1}[EG][G] \quad (i = 1)$$

856
$$\frac{d[G_i]}{dt} = k_{31,i-1}[EG][G_{i-1}] - k_{31,i}[EG][G_i] \quad (i = 2, \dots, n-1)$$

857
$$\frac{d[G_n]}{dt} = k_{31,n-1}[EG][G_{n-1}] \quad (i = n)$$

858
$$\frac{d[G_1S]}{dt} = k_{22}[EG][S] - k_{32,1}[EG][G_1S] \quad (j = 1)$$

859
$$\frac{d[G_jS]}{dt} = k_{32,j-1}[EG][G_{j-1}S] - k_{32,j}[EG][G_jS] \quad (j = 2, \dots, n-3)$$

860
$$\frac{d[G_{n-2}S]}{dt} = k_{32,n-3}[EG][G_{n-3}S] \quad (j = n-2)$$

861
$$\frac{d[L]}{dt} = k_4[EG][F]^2 - k_{33,1}[EG][L]$$

862
$$\frac{d[G_1L]}{dt} = k_{33,1}[EG][L] - k_{33,2}[EG][G_1L] \quad (k = 1)$$

863
$$\frac{d[G_kL]}{dt} = k_{33,k}[EG][G_{k-1}L] - k_{33,k+1}[EG][G_kL] \quad (k = 2, \dots, n-3)$$

864
$$\frac{d[G_{n-2}L]}{dt} = k_{33,n-2}[EG][G_{n-3}L] \quad (k = n-2)$$

865 According to the reaction rates when using the DSR-M enzyme and in accordance with the notations and rate
 866 equations mentioned above, the following mathematical correlations for kinetic constants have been
 867 identified to fit the experimental kinetic data at varying initial concentration of substrate $[S_0]$. Indeed,
 868 decreasing trends with the initial sucrose concentration were taken into account and curve-fitted to reflect
 869 the inhibition of the substrate at higher concentrations of initial sucrose solution due to the influence of
 870 water content on the structure of sucrose in solutions under these conditions.

871 **Table A1. Kinetic correlations for synthesis of dextrans using DSR-M enzyme**

Nature of reaction	Kinetic correlation	Descriptions and units
Formation of Glucosyl-enzyme complex (EG)	$k_1 = \left(\frac{[S_0]+d}{a+b.[S_0]+c[S_0]^2} \right)$	k_1 in $L.mol^{-1}.min^{-1}$ and $[S_0]$ in $mol.L^{-1}$ $a = 3.543 \times 10^{-5}$, $b = 2.066 \times 10^{-4}$, $c = 3.780 \times 10^{-3}$, $d = 1.1090 \times 10^{-2}$
Hydrolysis of Glucosyl-enzyme complex (EG)	$k_{21} = 1.001$	k_{21} in min^{-1}
Formation of elongated sucrose (GS)	$k_{22} = 3.277 [S_0]^{-0.2110}$	k_{22} in $L.mol^{-1}.min^{-1}$ and $[S_0]$ in $mol.L^{-1}$
Size-dependent dextran polymerization	Slow polymerization ⁽¹⁾ ($\ell < 8$): $k_{3,\ell} = 6\,565 (\ell) + 46$ Fast polymerization ⁽²⁾ ($\ell \geq 8$): $k_{3,\ell} = k_{hp} \cdot \left(\frac{n-(\ell-8)}{n} \right)^2$ with $k_{hp} = a' \cdot \exp(b' \cdot [S_0]) + c'$	$k_{31,i} = k_{32,j} = k_{33,k} = k_{3,\ell}$ in $L.mol^{-1}.min^{-1}$ and $[S_0]$ in $mol.L^{-1}$ n : maximum polymerization degree k_{hp} : maximum polymerization kinetics $a' = 2.782 \times 10^5$, $b' = -3.749$, $c' = 1.293 \times 10^4$
Formation of Leucrose (L)	$k_4 = 49.90 [S_0]^{-0.9964}$	k_4 in $L^2.mol^{-2}.min^{-1}$ and $[S_0]$ in $mol.L^{-1}$

872 ⁽¹⁾ The degree of polymerization $\ell = 8$ represents the length (ℓ) of the polymer below which the kinetics increases linearly
 873 but at relatively lower values.

874 ⁽²⁾ Above the $\ell = 8$ polymer length, the DSR-M enzyme suddenly accelerates elongation, as longer polymers are easier
 875 to capture and to synthesize. However, at this point the value of $k_{3,\ell}$ should decrease for an increase of ℓ because the
 876 molar number of dextran polymers decreases considerably with rapid growth of the length of polymers.

877

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