Continuous Flow Glycolipid Synthesis Using a Packed Bed Reactor

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Abstract: Glycolipids are a class of biodegradable biosurfactants that are non-toxic and based on renewables, making them a sustainable alternative to petrochemical surfactants. Enzymatic synthesis allows a tailor-made production of these versatile compounds using sugar and fatty acid building blocks with rationalized structures for targeted applications. Therefore, glycolipids can be comprehensively designed to outcompete conventional surfactants regarding their physicochemical properties. However, enzymatic glycolipid processes are struggling with both sugars and fatty acid solubilities in reaction media. Thus, continuous flow processes represent a powerful tool in designing efficient syntheses of sugar esters. In this study, a continuous enzymatic glycolipid production catalyzed by Novozyme 435® is presented as an unprecedented concept. A biphasic aqueous–organic system was investigated, allowing for the simultaneous solubilization of sugars and fatty acids. Owing to phase separation, the remaining non-acylated glucose was easily separated from the product stream and was refed to the reactor forming a closed-loop system. Productivity in the continuous process was higher compared to a batch one, with space–time yields of up to 1228 ± 65 µmol/L/h. A temperature of 70 °C resulted in the highest glucose-6-O-decanoate concentration in the Packed Bed Reactor (PBR). Consequently, the design of a continuous biocatalytic production is a step towards a more competitive glycolipid synthesis in the aim for industrialization.

Keywords: continuous flow; heterogenous biocatalysis; packed bed reactor; glycolipid; biphasic system; Candida antarctica Lipase B

1. Introduction

Surfactants are amphiphilic molecules that are widely applied in the industry, as well as in our everyday life. In 2017, the global surfactant market had a revenue of USD 43.6 billion [1]. Surfactant sales are predicted to reach USD 66.4 billion in 2025 [1]. The majority of surfactants are still produced using fossil-based resources, albeit many precursors and petroleum-based surfactants show high ecotoxicity and low biodegradability [2].

On the other hand, biosurfactants can be produced from renewables and, in contrast to most of their petrochemical counterparts, they are biodegradable and non-toxic [3–8]. Therefore, they do not accumulate in the environment and pose a lower ecological burden. They present, in consequence, an ecofriendly alternative to petroleum-based surfactants. Environmental awareness and use restrictions of toxic detergents contributed to a growing demand in biosurfactants [1,2]. The Compound Annual Growth Rate for biosurfactants was predicted to be 5.6% from 2017 to 2022, resulting in a global market of USD 5.5 billion in 2022 [2].

The condensation reaction between a hydrophilic sugar moiety and a hydrophobic fatty acid or fatty alcohol results in highly surface-active molecules known as glycolipids, which are a class of biosurfactants [9–11]. Their production can be performed chemically, microbially and by enzymatic synthesis [12–14]. The performance of glycolipids is strongly
related to their structure [9,10]. Enzymatic synthesis offers a tailor-made production of a nearly unlimited number of glycolipids by choosing the appropriate head and tail groups depending on the applications, while microbial production is limited to the metabolism of the production host [14]. Enzymatically produced glycolipids already showed high surface-active potential and are able to outcompete conventional petroleum-based surfactants, such as SDS [10]. Moreover, biocatalysis is a powerful asset to a global bioeconomy and in relation to the fulfillment of the Sustainable-by-Design criteria released by the European Commission [15–18]. Lipases are applied as biocatalysts in glycolipid synthesis by reversing their hydrolytic activity when a reaction medium of low water is employed [19–21]. Hence, biocatalytic processes in non-common reaction media, organic solvents, ionic liquids and deep eutectic solvents, were developed [22–24]. However, those processes face some challenges, e.g., simultaneous solubilization of polar sugars and non-polar fatty acids [25], more specifically, biocatalysis in deep eutectic solvents and ionic liquids enable relatively low yields and reaction velocities as well as a difficult downstream processing, limiting their industrial application [26,27]. An optimal solvent system thus finds compromise between efficiently solubilizing the substrates and preserving the stability of the enzyme. Short-chain alcohols are described to cause conformational changes to Novozyme 435®; the biocatalyst used in this study [28–31]. These structural changes induce the partial or total loss of biocatalytic activity. Furthermore, ethanol is reported to dissolve the support material of the beads [31]. Nonetheless, Novozym 435® is one of the most used biocatalysts as it is characterized by high activity for various reactions and high stability [32].

The transition from batch to continuous flow processes is thus a crucial technological pivot for the development of more efficient and cost-effective biocatalytic processes when aspiring to industrialization [33]. Continuous flow is considered beneficial to avoid enzyme inhibition and offers the possibility for in line downstream processing [34,35]. Moreover, flow reactors provide advantages for multiphase systems [34,36,37], such as considerably shorter reaction times and a substantial increase in the space–time yield [38]. Indeed, up to 650-fold higher space–time yields were reported for continuous production compared to batch processes [38,39]. In case of glycolipid production, continuous processing allowed for a biphasic system and thus dissolution of sugar in the aqueous phase and the vinylated fatty acid in organic solvents by avoiding short-chain alcohols as co-solvents for modulating solubilities.

The aim of this study is to evaluate the potential of continuous flow production for the enzymatic synthesis of glycolipids. Using a model reaction that converts glucose and vinyl decanoate to glucose monodecanoate catalyzed by Candida antarctica Lipase B (Novozym 435®), a biphasic system made of water and 2-methyl-2-butanol (2M2B) is investigated. The latter is chosen as an organic solvent because it proves, from previous research, to be relevant for glycolipid synthesis in batch process [40] and is compatible with applications in food and pharmaceuticals [41].

2. Results

A flow rate of 0.5 mL/min was chosen for all the following experiments after screening 2 mL/min (9 µmol/h), 1 mL/min (17 µmol/h) and 0.5 mL/min (29 µmol/min) due to the higher glucose monodecanoate production at the longer residence time (13.2 min). After flushing the column three times with the void volume, the glycolipid concentration in the solvent flow was analyzed at different time points. At an operation time of 1 h, quasi-steady-state conditions were attained (Figure 1). The process was stable for at least 72 h; no loss of activity of Novozym 435® was observed over this time period.
2.1. Influence of Temperature

Different temperatures were evaluated to find the optimal operating parameters. An increase in the temperature from 50 °C to 70 °C resulted in an increase in production from 29.50 ± 6.65 µmol/h to 36.84 ± 1.97 µmol/h (Figure 2), ending up in a biocatalyst productivity of 7.37 ± 0.39 µmol/h/g and a space–time yield of 1228 ± 65 µmol/L/h at 70 °C. A further increase in temperature caused a significant drop of productivity to 12.11 ± 1.33 µmol/h (80 °C) and 7.89 ± 2.51 µmol/h (90 °C).

Figure 1. Glucose monodecanoate concentration in relation to operation time of the packed bed reactor.

Figure 2. Influence of temperature on glucose monodecanoate production. a, b show statistically significant differences (one-way ANOVA, Tukey’s test, p < 0.05).
2.2. Ratio of the Aqueous Phase to Organic Phase

By varying the relative flow rates of the substrate streams, the best ratio of the aqueous phase to organic phase was obtained. Starting from the same flow rate for both feed solutions (0.25 mL/min each, total flow rate of 0.5 mL/min), it was evaluated whether the relative flow rates had an effect on glycolipid productivity. An increase in the relative flow rate of the organic phase to 75% (0.375 mL/min) resulted in higher glycolipid production, while higher flow rates for the aqueous phase led to a significant decrease in glycolipid production (Figure 3).

![Figure 3](image_url)

Figure 3. Influence of relative flow rates of glucose and fatty acid feed on glucose monodecanoate production. a, b show statistically significant differences (one-way ANOVA, Tukey’s test, p < 0.05).

2.3. Sugar Concentration

Different sugar concentrations were evaluated in order to increase process performance. At half of the initial concentration, glycolipid production remained stable (1120 ± 22 µmol/L/h), while a further decrease in the sugar concentration to 0.65 M resulted in a decrease in the space–time yield to 470 ± 165 µmol/L/h (Figure 4). A total of 2.6 M of the sugar solution had a water activity $a_w$ of 0.941. The water activity of the sugar solution increased with decreasing the sugar concentration to $a_w$ of 0.974 for 1.3 M and 0.990 for 0.65 M.

![Figure 4](image_url)

Figure 4. Influence of glucose concentration on glucose monodecanoate production. a, b show statistically significant differences (one-way ANOVA, Tukey’s test, p < 0.05).
2.4. Sugar Recycling

To reverse the hydrolytic activity of the lipase, high sugar concentrations are necessary to obtain high glycolipid titers. Therefore, we investigated if a closed-loop system for the sugar solution was possible, and thus refed it to the reactor for another cycle. In doing so, no loss in glycolipid production was observed over at least 3 cycles, as no statistically significant difference was found between the respective titers (Figure 5).

![Figure 5](image_url)

**Figure 5.** Recycling of the aqueous phase. Glucose containing aqueous phase was reusable as glucose feed solution over at least three cycles. a, b show statistically significant differences (one-way ANOVA, Tukey’s test, \( p < 0.05 \)).

3. Discussion

In this study, a continuous process for enzymatic glycolipid synthesis was investigated for the first time. We demonstrated that glucose monodecanoate can be synthetized in a biphasic system working in flow. The downstream processing of biphasic synthesis is rather advantageous as the remaining sugar can be easily separated from the product stream by phase separation.

The highest glycolipid production obtained in this study was achieved faster than in batch production. In parallel studies, Arcens et al. reached space–time yields of \( 525 \mu\text{mol/L/h} \) in a 10 mL scale-batch process in acetonitrile, while the present continuous process reached \( 1228 \pm 65 \mu\text{mol/L/h} \) for the enzymatic synthesis of glucose monodecanoate [42]. Furthermore, with \( 30 \text{mL/h} \), we processed a significantly larger reaction volume than a standard stirred tank reactor operating with \( 10 \text{mL} \) of reaction mixture over 3 days. This shows the superiority of continuous processing in terms of scale up, as even with comparably small reactors (our void volume was 6.6 mL), large volumes can be processed. Additionally, the batch production of glycolipids in hydrophilic Deep Eutectic Solvent (DES) had lower productivity with \( 25 \mu\text{mol/L/h} \) at 50 °C [25]. Moreover, for glycolipid synthesis in DES, an additional pretreatment step using sonication was necessary in order to improve fatty acid distribution [25]. Space–time yields of the continuous synthesis are, on average, 1.5 to 3 times higher than those achieved for ionic liquids for the production of sugar esters by Katsoura et al. (410 \( \mu\text{mol/L/h} \)) in 1-butyl-3-methylimidazolium tetrafluoroborate at 60 °C and Galonde et al. (760 \( \mu\text{mol/L/h} \)) in 1-butyl-1-methylpyrrolidinium tri-fluoromethanesulfonate at 80 °C [43,44]. Csajagi et al. already reported the beneficial effects of continuous flow for another lipase catalyzed reaction. As a matter of fact, the reaction rates of the acylation of racemic alcohols were up to 3 times higher [45]. Additionally, Novak et al. reported an increase in productivity by the application of a flow reactor in isoamyl acetate synthesis [46]. The superiority of continuous flow over batch processes is based on several aspects. On the one hand, the shorter residence times result in the reduced...
inhibition of the enzymes; on the other hand, continuous processes result in better mixing, higher mass transfer and improved temperature control [34–36,38,47].

Increasing the temperature from 50 °C to 70 °C resulted in higher glucose monodecanoate production. Similar results are reported from batch processes for which higher reaction rates were observed with increasing temperature [22,48–50]. At 90 °C, we observed significantly lower glycolipid production, which is in agreement with the literature. Gumel et al. reported a temperature optimum for glycolipid synthesis in organic solvents at 70 °C [50], and Šabeder et al. observed the thermal deactivation of Novozym 435® at temperatures of 90 °C and higher [22].

Lipase-catalyzed transesterifications are sensitive to water content in batch processes as water shifts the equilibrium towards the hydrolysis of the ester [21,22,49–53]. Water activities of $a_w < 0.2$ are reported to be most suitable for sugar ester production [21,54]. The water activity of the applied glucose solution in the continuous process was 0.94–0.99 with a significantly lower glycolipid production at the lowest glucose concentration ($a_w = 0.99$) tested. Water activity of the glucose solution is strongly related to the glucose concentration as glucose strongly interacts with water molecules and, hence, less water is freely available as solvent or reagent [55]. However, we observed higher glycolipid production rates than in batch processes with low water activity. This is most likely due to the much shorter residence time in the packed bed reactor (13 min) compared to the one in batch processes (~several hours to days). The reduced detrimental influence of water on the transesterification of glucose is in agreement with the study by Novak et al. who produced isoamyl acetate synthesis with *Candida antarctica* Lipase B in an aqueous–organic solvent system at higher reaction rates than in batch process [46].

In order to maintain high space–time yields, glucose concentrations of 1.3 M were necessary in the feed solution. However, we showed that the aqueous phase was reusable after phase separation without the significant loss of productivity. Therefore, a glucose solution can be applied in a closed-loop system, which increases process efficiency and atom economy.

4. Materials and Methods

2-methyl-2-butanol (purity ≥ 99%) was purchased from SIGMA Aldrich Chemie GmbH (Taufkirchen, Germany). D-(+)-Glucose (purity ≥ 98%) was acquired from Carl Roth GmbH & Co. KG (Karlsruhe, Germany). Lipase formulation, Novozym 435®, was purchased from STREM Chemicals Europe (Bischheim, France). Vinyl decanoate (purity > 99%) was acquired from Tokyo Chemical Industry Co., Ltd. (TCI Europe, Zwijndrecht, Belgium). All chemicals were used without further purification.

4.1. Enzymatic Synthesis of Glucose Monodecanoate in Continuous Packed Bed Mode Operation

The transesterification of glucose and vinyl decanoate to glucose monodecanoate was conducted in an XK16 column from Cytiva (Fisher scientific GmbH, Schwerte, Germany). The column was loaded with 5 g of commercially immobilized *Candida antarctica* Lipase B Novozym 435®. The experiments were initiated by flushing the column 3 times with the void volume at a flow rate of 5 mL/min for 4 min. The flow rate was set to 0.5 mL/min (50% of glucose solution (2.6 M in ddH₂O) and 50% of vinyl decanoate solution (2.0 M in 2M2B)) using an Äkta™ start from GE healthcare (Uppsala, Sweden) as a pumping system (Figure 6). Feed solutions were maintained at 50 °C using a NeoMag magnetic stirrer from neoLab (Heidelberg, Germany). The column temperature was controlled at 50 °C using an E100 Thermostat water bath from Lauda (Lauda-Königshofen, Germany).
Glucose monodecanoate was quantified by reversed-phase chromatography using a Kinetex EVO C18 (2.6 µm, 250 mm × 4.6 mm) from Phenomenex (Aschaffenburg, Germany) for phase separation and subsequently analyzed by HPLC-ELSD.

The residence time was calculated as follows:

\[ \tau = \frac{\varepsilon}{q} \]

where \( \varepsilon \) is the void fraction and \( q \) is the flow rate. \( \varepsilon \) was estimated according to Arcos et al. (2000), resulting in \( \varepsilon \approx 6.6 \text{ mL} \) and a residence time of 13.2 min at a flow rate of 0.5 mL/min.

Biocatalyst productivity was calculated as follows:

\[ \text{Biocatalyst productivity} = \frac{n(\text{glucose monodecanoate})}{t \times m(\text{Novozym} 435)} \]

where \( t \) is the total reaction time and \( n \) is the produced moles of glucose monodecanoate.

4.2. Influence of Temperature

Different temperatures were accessed in order to optimize the productivity. Hereby, the temperature of the column was set to 50 °C, 70 °C and 90 °C.

4.3. Influence of Glucose Concentration

The glucose concentration was varied from 2.6 M to 0.65 M in order to evaluate the lowest glucose concentration possible without reducing the productivity. The ratio of glucose to vinyl decanoate was kept constant, so the vinyl decanoate concentration was varied accordingly from 2 M to 0.5 M.

4.4. Recycling of Glucose Solution

After the column, the glucose containing aqueous phase and the 2M2B phase containing glycolipid and fatty acid were separated by centrifugation and the aqueous phase was reused as glucose feed for another run.

4.5. Quantification

Glucose monodecanoate was quantified by reversed-phase chromatography using a Kinetex EVO C18 (2.6 µm, 250 mm × 4.6 mm) from Phenomenex (Aschaffenburg, Germany) with an accompanying guard column (4 mm × 3.0 mm ID) of the same phase and an Agilent
(Waldbronn, Germany) 1260 series liquid chromatograph equipped with a quaternary pump, an autosampler and a column oven. HPLC analysis was performed according to Hollenbach et al. [25]. Briefly, an evaporative light scattering detector from BÜCHI Labortechnik (Essen, Germany) was used for detection. Mobile phase was a gradient of acetonitrile (A) and water (B) with a total flow rate of 1 mL/min.

4.6. Water-Activity Measurement

Water activity was measured using a LabMaster-aw neo from Novasina (Lachen, Switzerland). The temperature was set to 50 °C.

4.7. Statistical Analysis

OriginPro software 9.7 (version 2020) (OriginLab Corporation, Northampton, MA, USA) was used for raw data treatment and statistical analysis. The Results are presented as mean ± standard deviation ($n = 3$). Statistical analysis was performed by one-way ANOVA and Tukey’s test, and the results were considered significant if the $p$-value was <0.05, if not stated otherwise.

5. Conclusions

In this study, the continuous enzymatic production of glycolipids was investigated for the first time. It was demonstrated that an aqueous–organic two-phase system is suitable for continuous glycolipid production by Candida antarctica Lipase B. This system relies on a closed loop of sugar solution that drastically enhances the performances of our process and is seemingly facile to scale up. Space–time yields of the presented continuous process were comparatively higher than in the batch process, e.g., 50 times higher than in hydrophilic DES in batch and twice as high as in acetonitrile in batch. Consequently, the design of a continuous biocatalytic production is a step towards a more competitive glycolipid synthesis and a relevant approach for potential industrialization. For the further optimization of the continuous process, different reactor set-ups should be compared to the presented packed bed reactor, e.g., a rotating bed reactor or a coil reactor, to investigate how reactor design can influence performance through potentially higher mass and heat transfer.

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References

1. Banat, I.M.; Carboué, Q.; Saucedo-Castañeda, G.; de Jesús Cázares-Marinero, J. Biosurfactants: The green generation of speciality chemicals and potential production using Solid-State fermentation (SSF) technology. Bioresour. Technol. 2021, 320, 124222. [CrossRef] [PubMed]
2. Farias, C.B.B.; Almeida, F.C.G.; Silva, L.A.; Souza, T.C.; Meira, H.M.; da Silva R de, C.F.S.; Luna, J.M.; Santos, V.A.; Converti, A.; Banat, I.M.; et al. Production of green surfactants: Market prospects. Electron. J. Biotechnol. 2021, 51, 28–39. [CrossRef]
3. Hirata, Y.; Ryu, M.; Oda, Y.; Igarashi, K.; Nagatsu, A.; Furuta, T.; Sugiura, M. Novel characteristics of sophorolipids, yeast glycolipid biosurfactants, as biodegradable low-foaming surfactants. J. Biosci. Bioeng. 2009, 108, 142–146. [CrossRef] [PubMed]
4. Baker, I.J.A.; Matthews, B.; Suárez, H.; Krodkiewska, I.; Furlong, D.N.; Grieser, F.; Drummond, C.I. Sugar fatty acid ester surfactants: Structure and ultimate aerobic biodegradability. J. Surfactants Deterg. 2000, 3, 1–11. [CrossRef]

5. Lima, T.M.S.; Procópio, L.C.; Brandão, F.D.; Carvalho, A.M.X.; Tótola, M.R.; Borges, A.C. Biodegradability of bacterial surfactants. Biodegradation 2011, 22, 585–592. [CrossRef]

6. Johann, S.; Seiler, T.B.; Tiso, T.; Bluhm, K.; Blank, L.M.; Hollert, H. Mechanism-specific and whole-organism ecotoxicity of mono-rrhamnolipids. Sci. Total Environ. 2016, 549–549, 155–163. [CrossRef]

7. Dörjes, J. Experimentelle Untersuchungen zur Wirkung von Rohöl und Rohöl/Tensid-Gemischen im Ökosystem Wattenmeer. XVI. Zusammenfassung und Schlussfolgerungen. Senckenberg. Marit. J. Mar. Sci. 1984, 16, 267–271.

8. Poremba, K.; Gunel, W.; Lang, S.; Wagner, F. Toxicity testing of synthetic and biogenic surfactants on marine microorganisms. Environ. Toxicol. Water Qual. 1991, 6, 157–163. [CrossRef]

9. Hollenbach, R.; Völz, A.R.; Höfert, L.; Rudat, J.; Ochsenreither, K.; Willenbacher, N.; Syldatk, C. Interfacial and Foaming Properties of Tailor-Made Glycolipids—Influence of the Hydrophilic Head Group and Functional Groups in the Hydrophobic Tail. Molecules 2020, 25, 3797. [CrossRef]

10. Hollenbach, R.; Oeppling, S.; Delavault, A.; Völz, A.R.; Willenbacher, N.; Rudat, J.; Ochsenreither, K.; Syldatk, C. Comparative study on interfacial and foaming properties of glycolipids in relation to the gas applied for foam generation. RSC Adv. 2021, 11, 34235–34244. [CrossRef]

11. Garofalakis, G.; Murray, B.S.; Sarney, D.B. Surface activity and critical aggregation concentration of surfactants with different sugar headgroups. J. Colloid Interface Sci. 2000, 228, 391–398. [CrossRef] [PubMed]

12. Babu, P.S.; Vaidya, A.N.; Bal, A.S.; Kapur, R.; Juwarkar, A.; Khanna, P. Kinetics of biosurfactant production by Pseudomonas aeruginosa strain BS2 from industrial wastes. Biotechnol. Lett. 1996, 18, 263–268.

13. Siebenhaller, S.; Grüninger, J.; Syldatk, C. Enzymatic Synthesis of Glycolipid Surfactants. In Lipid Modification by Enzymes and Engineered Microbes; Elsevier: Amsterdam, The Netherlands, 2018; pp. 293–313.

14. Grüninger, J.; Delavault, A.; Ochsenreither, K. Enzymatic glycolipid surfactant synthesis from renewables. Process Biochem. 2019, 87, 45–54. [CrossRef]

15. Amadio, A.; Malyska, A.; Markouli, C.; Salinas, S.; Sanfelix, J.; VanHumbeeck, T. Mapping Study for the Development of Sustainable-by-Design Criteria. Eur. Comm. Dir. Res. Innov. 2021, 1, 19–35. Available online: https://op.europa.eu/en/publication-detail/-/publication/1679c200-a314-11eb-9585-01aa7ed71a1/languageen (accessed on 17 May 2022).

16. Rodríguez-Padrón, D.; Puente-Santiago, A.R.; Balu, A.M.; Muñoz-Batista, M.J.; Luque, R. Environmental Catalysis: Present and Future. ChemCatChem 2019, 11, 18–38. [CrossRef]

17. Wohlgemuth, R. Bio-based resources, bioprocesses and bioproducts in value creation architectures for bioeconomy markets and beyond—What really matters. EFB Biocon. J. 2021, 1, 100009. [CrossRef]

18. Wohlgemuth, R.; Twardowski, T.; Aguilar, A. Bioeconomy moving forward step by step—A global journey. N. Biotechnol. 2021, 61, 22–28. [CrossRef]

19. Zaks, A.; Klibanov, A.M. The effect of water on enzyme action in organic media. J. Biol. Chem. 1988, 263, 8017–8021. [CrossRef]

20. Arroyo, M.; Sánchez-Montero, J.M.; Sinisterra, J.V. Thermal stabilization of immobilized lipase B from Candida antarctica on different supports: Effect of water activity on enzymatic activity in organic media. Enzym. Microb. Technol. 1999, 24, 3–12. [CrossRef]

21. Chamoufau, F.; Coulon, D.; Girardin, M.; Ghoul, M. Influence of water activity and water content on sugar esters lipase-catalyzed synthesis in organic media. J. Mol. Catal. A Chem. 2001, 11, 949–954. [CrossRef]

22. Šabeder, S.; Habulin, M.; Knez, Z. Lipase-catalyzed synthesis of fatty acid fructose esters. J. Food Eng. 2006, 77, 880–886. [CrossRef]

23. Ganske, F.; Bornscheuer, U.T. Optimization of lipase-catalyzed glucose fatty acid ester synthesis in a two-phase system containing ionic liquids and t-BuOH. J. Mol. Catal. B Enzym. 2005, 36, 40–42. [CrossRef]

24. Pöhlein, M.; Ulrich, J.; Kirschhöfer, F.; Nusser, M.; Muñoz-Goll, C.; Kannengiesser, B.; Brenner-Weiß, G.; Luy, B.; Liese, A.; Syldatk, C.; et al. Lipase-catalyzed synthesis of glucose-6-O-hexanoate in deep eutectic solvents. Eur. J. Lipid. Sci. Technol. 2015, 117, 161–166. [CrossRef]

25. Hollenbach, R.; Bindereif, B.; van der Schaar, U.S.; Ochsenreither, K.; Syldatk, C. Optimization of Glycolipid Synthesis in Hydrophilic Deep Eutectic Solvents. Front. Chem. 2020, 8, 382. [CrossRef] [PubMed]

26. Komba Ibenga, S.K.; Fabre, J.F.; Bikanga, R.; Moulongui, Z. Atypical Reaction Media and Organized Systems for the Synthesis of Low-Substitution Sugar Esters. Front. Chem. 2019, 7, 587. [CrossRef]

27. Truppo, M.D. Biocatalysis in the Pharmaceutical Industry: The Need for Speed. ACS Med. Chem. Lett. 2017, 8, 476–480. [CrossRef]

28. Mangiagalli, M.; Ami, D.; de Divitiis, M.; Brocca, S.; Catelani, T.; Natalello, A.; Lotti, M. Short-chain alcohols inactivate an immobilized industrial lipase through two different mechanisms. Biotechnol. J. 2022, 2100712. [CrossRef]

29. Mangiagalli, M.; Carvalho, H.; Natalello, A.; Ferrario, V.; Pennati, M.L.; Barbiroli, A.; Lotti, M.; Pleiss, J.; Brocca, S. Diverse effects of aqueous polar co-solvents on Candida antarctica lipase B. Int. J. Biol. Macromol. 2020, 150, 930–940. [CrossRef]

30. José, C.; Briand, L.E. Deactivation of Novozym®435 during the esterification of ibuprofen with ethanol: Evidences of the detrimental effect of the alcohol. React. Kinet. Mech. Catal. 2010, 99, 17–22. [CrossRef]

31. José, C.; Bonetto, R.D.; Gambaro, L.A.; Guauque Torres, M.D.P.; Foresti, M.L.; Ferreira, M.L.; Briand, L.E. Investigation of the causes of deactivation-degradation of the commercial biocatalyst Novozym®435 in ethanol and ethanol-aqueous media. J. Mol. Catal. B Enzym. 2017, 71, 95–107. [CrossRef]
32. Ortiz, C.; Ferreira, M.L.; Barbosa, O.; Dos Santos, J.C.S.; Rodrigues, R.C.; Berenguer-Murcia, Á.; Briand, L.E.; Fernandez-Lafuente, R. Novozym 435: The “perfect” lipase immobilized biocatalyst? Catal. Sci. Technol. 2019, 9, 2380–2420. [CrossRef]

33. Alcántara, A.R.; Dominguez de María, P.; Littlechild, J.A.; Schürmann, M.; Sheldon, R.A.; Wohlgemuth, R. Biocatalysis as Key to Sustainable Industrial Chemistry. ChemSusChem 2022, 15, 202102709.

34. Burek, B.O.; Dawood, A.W.H.; Hollmann, F.; Liese, A.; Holtmann, D. Process Intensification as Game Changer in Enzyme Catalysis. Front. Catal. 2022, 2, 1–18. [CrossRef]

35. Lindeque, R.M.; Woodley, J.M. Reactor selection for effective continuous biocatalytic production of pharmaceuticals. Catalysts 2019, 9, 262. [CrossRef]

36. Strniša, F.; Urbiš, T.; Znidaršič-Plazl, P.; Plazl, I. Process Intensification and Miniaturization of Chemical and Biochemical Processes. Comput. Aided Chem. Eng. 2019, 46, 1801–1806.

37. Znidaršič-Plazl, P. The Promises and the Challenges of Biotransformations in Microflow. Biotechnol. J. 2019, 14, 1800580. [CrossRef] [PubMed]

38. Tamborini, L.; Fernandes, P.; Paradisi, F.; Molinari, F. Flow Bioreactors as Complementary Tools for Biocatalytic Process Intensification. Trends Biotechnol. 2018, 36, 73–88. [CrossRef]

39. Jones, E.; McClean, K.; Housden, S.; Gasparini, G.; Archer, I. Biocatalytic oxidase: Batch to continuous. Chem. Eng. Res. Des. 2012, 90, 726–731. [CrossRef]

40. Delavault, A. Lipase-Catalysed Synthesis of Glycolipids-Utilization of Innovative Media, Reactors and Substrates. Repository KITOpen. 2021. Available online: https://publikationen.bibliothek.kit.edu/1000136597 (accessed on 17 May 2022).

41. Khaled, N.; Montet, D.; Pina, M.; Griaille, J. Fructose oleate synthesis in a fixed catalyst bed reactor. Biotechnol. Lett. 1991, 13, 167–172. [CrossRef]

42. Arcens, D.; Grau, E.; Grelier, S.; Cramail, H.; Peruch, F. Impact of Fatty Acid Structure on CALB-Catalyzed Esterification of Glucose. Eur. J. Lipid. Sci. Technol. 2020, 122, 2–7. [CrossRef]

43. Galonde, N.; Brostaux, Y.; Richard, G.; Nott, K.; Jerôme, C.; Fauconnier, M.L. Use of response surface methodology for the optimization of the lipase-catalyzed synthesis of mannosyl myristate in pure ionic liquid. Process Biochem. 2013, 48, 1914–1920. [CrossRef]

44. Katsoura, M.H.; Polydera, A.C.; Katapodis, P.; Kolisis, F.N.; Stamatis, H. Effect of different reaction parameters on the lipase-catalyzed selective acylation of polyhydroxylated natural compounds in ionic liquids. Process Biochem. 2007, 42, 1326–1334. [CrossRef]

45. Csajági, C.; Szatzker, G.; Rita Toke, E.; Úrge, L.; Darvas, F.; Poppe, L. Enantiomer selective acylation of racemic alcohols by lipases in continuous-flow bioreactors. Tetrahedron Asymmetry 2008, 19, 237–246. [CrossRef]

46. Novak, U.; Lavric, D.; Znidaršič-Plazl, P. Continuous lipase B-catalyzed isoamyl acetate synthesis in a two-liquid phase system using Corning® AFR™ module coupled with a membrane separator enabling biocatalyst recycle. J. Flow. Chem. 2016, 6, 33–38. [CrossRef]

47. Britton, J.; Majumdar, S.; Weiss, G.A. Continuous flow biocatalysis. Chem. Soc. Rev. 2018, 47, 5891–5918. [CrossRef]

48. Arcos, J.A.; Hill, C.G.; Otero, C. Kinetics of the lipase-catalyzed synthesis of glucose esters in acetone. Biotechnol. Bioeng. 2003, 42, 104–110. [CrossRef]

49. Cauglia, F.; Canepa, P. The enzymatic synthesis of glucosylmyristate as a reaction model for general considerations on “sugar esters” production. Bioresour. Technol. 2008, 99, 4065–4072. [CrossRef]

50. Gumel, A.M.; Annuar, M.S.M.; Heidelberg, T.; Chisti, Y. Lipase mediated synthesis of sugar fatty acid esters. Process Biochem. 2011, 46, 2079–2090. [CrossRef]

51. Burek, B.O.; Dawood, A.W.H.; Hollmann, F.; Liese, A.; Holtmann, D. Process Intensification as Game Changer in Enzyme Catalysis. Front. Catal. 2022, 2, 1–18. [CrossRef]

52. Cheng, Y.; Tsi, S. Effects of water activity and alcohol concentration on the kinetic resolution of lipase-catalyzed acyl transfer in organic solvents. Enzym. Microb. Technol. 2003, 32, 362–368. [CrossRef]

53. Delavault, A.; Opochenska, O.; Lanieque, L.; Soergel, H.; Muhle-Goll, C.; Ochsenreither, K.; Syladt, C. Lipase-catalyzed production of sorbitol laurate in a “2-in-1” deep eutectic system: Factors affecting the synthesis and scalability. Molecules 2021, 26, 2759. [CrossRef] [PubMed]

54. Lee, S.H.; Koo, Y.M.; Ha, S.H. Influence of ionic liquids under controlled water activity and low halide content on lipase activity. Korean J. Chem. Eng. 2008, 25, 1456–1462. [CrossRef]

55. Zuorro, A. Water activity prediction in sugar and polyol systems using theoretical molecular descriptors. Int. J. Mol. Sci. 2021, 22, 11044. [CrossRef] [PubMed]