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Enzymatic synthesis of *N*-alkanoyl-*N*-methylglucamide surfactants: solvent-free production and environmental assessment

Yasser Gaber,*[a,b] Ulrika Törnvall,[a] Cecilia Orellana-Coca,[a,d] Magdy Ali Amin[c] and Rajni Hatti-Kaul[a]

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A biocatalysis based method for the solvent-free production of *N*-alkanoyl-*N*-methylglucamide (MEGA) surfactants was developed and used as a case study for the evaluation of different environmental assessment tools, such as the freeware package EATOS (Environmental Assessment Tool for Organic Synthesis). In order to also consider energy usage and process facilities, *e.g.* heating, stirring and vacuum, a complementary tool was needed; hence the EcoScale method and the use of an energy monitoring socket were also exploited. The solvent-free method followed by a simple hydrolysis step gave a final amide yield of 99% and a product essentially free of remaining substrate, *N*-methylglucamine (MEG). The latter is important since MEG can potentially be converted to carcinogenic nitrosamines. The absence of solvent in the reaction medium was also found to result in a significantly reduced potential environmental impact. The environmental tools used in this study were further scrutinized, and even if they represent some of the best freely available tools for evaluation of early stage process development, some points for further improvements are suggested.

Introduction

Growing concerns about the environmental burden arising from the production of chemicals have led to calls for the development of less harmful and energy-efficient processes.[1] Surfactants comprise a large share of the total amount of chemicals produced today. The global surfactant market was 18 million metric tons in 2003 and is estimated to be about 22 million tons in 2012.[2] Non-ionic surfactants based on a carbohydrate as the head group, *e.g.* *N*-alkanoyl-*N*-methylglucamides (MEGAs), are of special interest since they are biodegradable, non-toxic, mild to the skin and stable under alkaline conditions. MEGA (known also as fatty acid glucamide) is produced on an industrial scale for detergent preparations especially for dishwashing purposes.[3,4] The electrical neutrality of MEGA gives it a significant role in pharmaceutical formulations containing proteins.[5]

The chemical synthesis of MEGA typically involves reductive amination of glucose with methylamine to yield the substrate *N*-methylglucamine (MEG), which is then linked *via* an amide bond to an alkyl chain, usually a fatty acid with chain length of more than eight carbons. The amidation reaction between MEG and a fatty acid ester can be catalyzed by a variety of metal salts. The commonly used catalyst is sodium methoxide,[6] which is hazardous and needs careful handling due to risk of explosion and, furthermore, has to be used under completely dry conditions to keep its activity.[6,7] The amidation may also be achieved using long-chain δ-lactones or ω-epoxy fatty acid esters without added catalyst, due to the enhanced reactivity of these substrates.[8,9] In addition, the enzymatic method with *t*-amyl alcohol used as solvent, as described by Maugard *et al.*,[10] provides increased yield as well as a safer alternative compared to the chemical synthesis. Selectivity of the reaction in both chemical and enzymatic routes is mainly a function of the molar ratio of amine to fatty acid methyl ester where higher amine ratio promotes amidation over esterification.[10]

Chemical reactions involving both polar and non-polar compounds generally require a suitable solvent. Ionic liquids can provide an appropriate reaction medium for this purpose especially after the recent achievements in this field. Use of ionic liquids containing dicyanamide anion has been described for esterification of sucrose with lauric acid using Novozym®435 (N435), *i.e.* immobilized *Pseudozyma* (formerly *Candida*) *antarctica* lipase B.[11,12] However, due to the relatively high cost of ionic liquids, and a wish to make the process even greener, an attempt has been made in our laboratory to develop a solvent-free method for enzymatic production of MEGA from renewable resources. Even if the yield is lower than for both the chemical method and the enzymatic method described above, we considered it relevant to perform an assessment of the environmental impact for these alternative methods, also addressing other parameters such as amount of waste generated.

Traditionally, evaluation of different chemical production processes has mainly been performed based on the chemical yield, *e.g.* the E-factor and atom efficiency methods. Such methods were in focus for R&D chemists until the last decade, when Sheldon[13] directed the attention towards the nature of the produced waste, by introducing the concept of the unfriendliness

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[a] *Department of Biotechnology, Center for Chemistry and Chemical Engineering, Lund University, P.O. Box 124, SE-221 00, Lund, Sweden. E-mail: yasser.gaber@biotek.lu.se; Fax: +46-46-222-4713; Tel: +46-46-222-7363*
[b] *Department of Microbiology, Faculty of Pharmacy, Beni-Suef University, Beni-Suef, 62111, Egypt*
[c] *Microbial Biotechnology Center, Faculty of Pharmacy, Cairo University, Kasr El-Aini, Cairo, Egypt*
[d] *Centro de Biotecnologia, Universidad Mayor de San Simon, Cochabamba, Bolivia*

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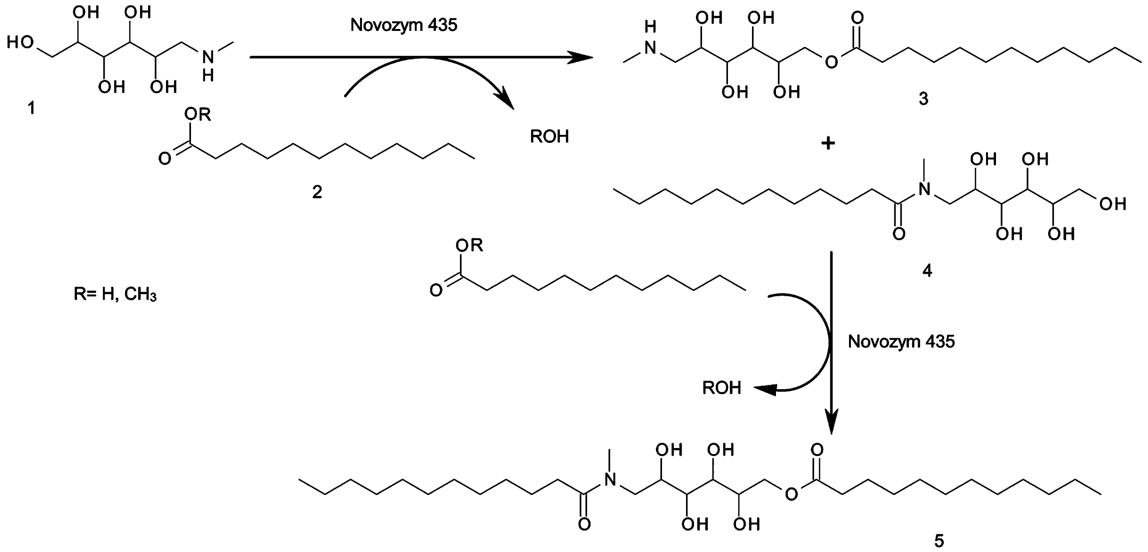

Scheme 1 Schematic representation of lipase catalysed reaction between MEG (1) and fatty acid or fatty acid methyl ester (2) to yield ester (3) and/or amide (product) (4), which are further acylated into amide-ester (by-product) (5) in the presence of excess fatty acid.

quotient; Q. The importance of studying processes from a life cycle perspective has since then gained increasing attention, and performing a life cycle assessment (LCA) is nowadays often the method of choice for environmental evaluations within the chemical industry. However, performing an entire LCA is unfortunately seldom possible for completely new synthesis routes due to lack of important information.

In order to facilitate environmental assessments in early phase process development, a number of computational tools have been developed, such as the GSK's green technology guidance,[14] Environmental, Health and Safety (EHS) assessment tool,[15] FLASC,[16] and GREENSCOPE.[17] More recently the strategic planning tool SWOT was also introduced for environmental assessment purposes.[18] However, one freely available alternative, in a user friendly java archive file, is the Environmental Assessment Tool for Organic Synthesis (EATOS), which was introduced in 2002 by Eissen and Metzger.[19] This method takes into account easily available information such as data from material safety data sheets (for substrates and by-products), prices as well as risk phrases of the substrates, for a rapid and reliable quantification of the E-factor and the potential environmental impact (PEI). The EATOS software has already been successfully applied for the evaluation of a number of different processes.[20–22] Since a few features are not included in the EATOS evaluation, such as process facilities (heating, stirring, and vacuum, *etc.*) and process time, performing a complementary assessment, by *e.g.* the EcoScale method[23] or measurements of energy requirements,[24] might in some cases be needed for comparison of different production processes.

In the present work, both the EATOS and the EcoScale methods were used, along with energy requirement calculations, to evaluate the environmental impact of the solvent free enzymatic production of MEGA compared to the reported solvent-based methods. Furthermore, simple means of conversion of the main by-product, the amide-ester, quantitatively to the amide product was also investigated in order to increase the product yield.

Results and discussion

In this study, Novozym®435 (N435) was used as the catalyst for solvent-free synthesis of *N*-lauroyl-*N*-methylglucamide (amide, 4, Scheme 1). The concentrations of the substrates, *i.e.* lauric acid, methyl laurate and *N*-methylglucamine (MEG), and solvents used in the reactions, together with the achieved yields, are given in Table 1. MEG is solubilized in melted lauric acid by ion pair formation.[25] The presence of excess MEG in the reaction mixture promotes the amidation over esterification;[25] however, since the lauric acid to MEG molar ratio determines the viscosity of the mixture, this ratio needs to be optimized for the enzymatic reaction. The optimal lauric acid to MEG molar ratio was determined for the amidation reaction using 4 wt% N435 under solvent-free conditions starting from 8 : 1 up to 2 : 1 at 90 °C (Fig. 1). The maximum lauric acid : MEG molar ratio that could be used under experimental conditions without

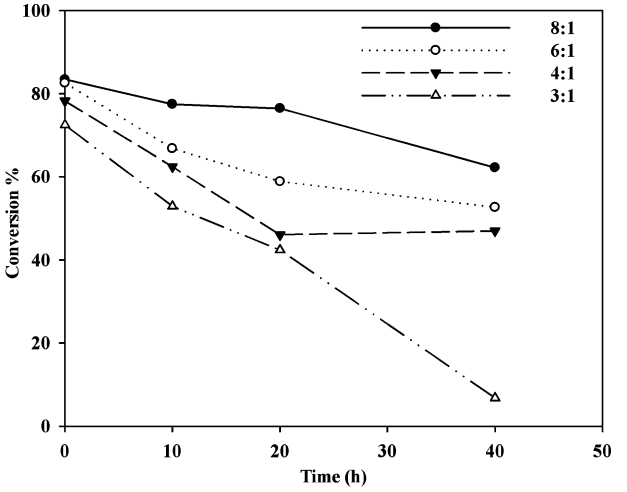

Fig. 1 Conversion of lauric acid in solvent free reactions using different molar ratios of lauric acid and MEG. Reactions were performed in a thermomixer at 90 °C, 900 rpm, using Novozym®435 (4 wt%).

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Table 1 Substrate concentrations, solvents and catalysts used in reactions reported in literature; A[6] and B[10] and reactions presented in this work (C–I)

Entry	Lauric/ mmol	Methyl laurate/ mmol	MEG/ mmol	Molar ratio[a]	Catalyst (g)	Solvent (g)	Amide yield (%)	Time (h)
A[b]	0	500	500	1 : 1	Sod. Methoxide	Propane-1,2-diol (18.9)	95	3
B	0	3.50	3.50	1 : 1	N435 (0.1)	*t*-amyl alcohol (8)	80	20
C	3.50	3.50	3.50	2 : 1	N435 (0.08)	0	34	40
C$_h$[c]	3.50	3.50	3.50	2 : 1	N435 (0.08)	0	99	40
D	0.87	1.34	1.75	1.25 : 1	N435 (0.03)	0	58	40
E	0	1.40	1.40	1 : 1	N435 (0.02)	*t*-amyl alcohol (0.24)	56	40
F	0.70	0.70	1.40	1 : 1	N435 (0.02)	*t*-amyl alcohol (0.24)	20	40
G	4.80	0	0.60	8 : 1	N435 (0.04)	0	5	10
H	1.75	5.28	1.78	4 : 1	N435 (0.07)	0	6	10
I	1.80	0	0.60	3 : 1	N435 (0.02)	0	12	10

[a] Lauric acid + methyl laurate to MEG ratio. [b] 21 ml of 25% sodium methoxide was used as catalyst, the solvent used is not recycled. [c] C$_h$ is reaction C after hydrolysis in alkaline medium.

viscosity limitations was 3 : 1. Due to the high lauric acid ratio the amide yield was relatively low after 10 h (12%) and the rest was mainly the by-product amide-ester (5, Scheme 1).

In order to increase the amine molar ratio in the reaction mixture without addition of a solvent and without increasing the viscosity, methyl laurate, *i.e.* the ester of lauric acid with a more reactive carbonyl group and lower melting point than lauric acid (5 °C *versus* 44 °C), was introduced to the system. Methanol, instead of water, is formed when using methyl laurate; this will however quickly evaporate under the reaction conditions used. When using only MEG and methyl laurate as substrate, the MEG precipitated and only a minor formation of amide was observed. Hence, some lauric acid is needed in the system, most probably since it accounts for the ion pairing with MEG. A reaction mixture composed of lauric acid, methyl laurate and MEG in molar ratio 0.5 : 0.75 : 1, which corresponds to 1.25 acylating agents to one MEG (Reaction D, Table 1), apparently has some advantages; the molar ratio is close to the optimum 1 : 1 ratio which means higher atomic efficiency of the process, and half of the amine is available at the beginning of the reaction for nucleophilic attack of the acylated enzyme complex which enhances the formation of amide. Although the reaction mixture of reaction D had a high viscosity (see below), a final amide yield of up to 77% was achieved after 80 h, representing a concentration of 64% (w/w) in the final reaction mixture (Fig. 2), with the main by-product at the end of the reaction being the amide-ester.

The use of excess acylating agent (lauric and methyl laurate) to MEG in a molar ratio of 2 : 1 (Reaction C, Table 1), resulted in a lower yield (34% after 40 h) and a larger proportion of amide-ester in the final product (Fig. 3). But on the other hand for this reaction, 94% of the MEG substrate was converted into amide and amide-ester within 20 h and almost complete conversion was achieved in 40 h. A final product free of MEG is important since MEG is a source of nitrosoamines; a potential carcinogen.[3]

As the viscosities of the reaction mixtures were observed to increase with time, the rheological behaviour of the final reaction mixtures of entries C, D and I was investigated by determination of the apparent viscosities at different shear rates at 80 °C. The apparent viscosity of all reaction mixtures decreased with increasing shear rate (Fig. 4), indicating shear thinning (pseudoplastic) behaviour. As expected, entry D showed the highest apparent viscosity; 51.8 Pa s (at a shear rate of 77.5 1/s), while in case of entry C and I the measured apparent

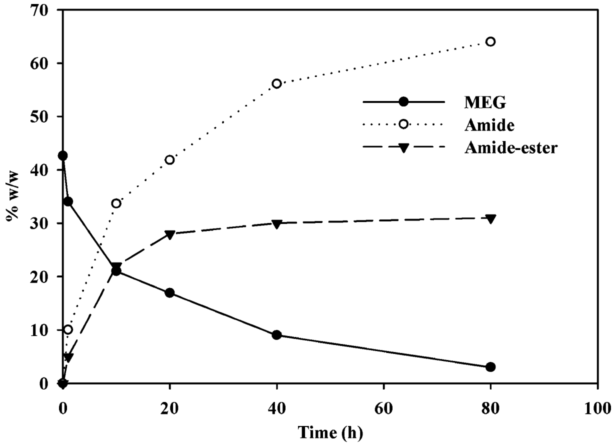

Fig. 2 Amidation reaction (entry D, Table 1). Reactions were performed in a thermomixer at 90 °C, 900 rpm, using Novozym®435 (4 wt%).

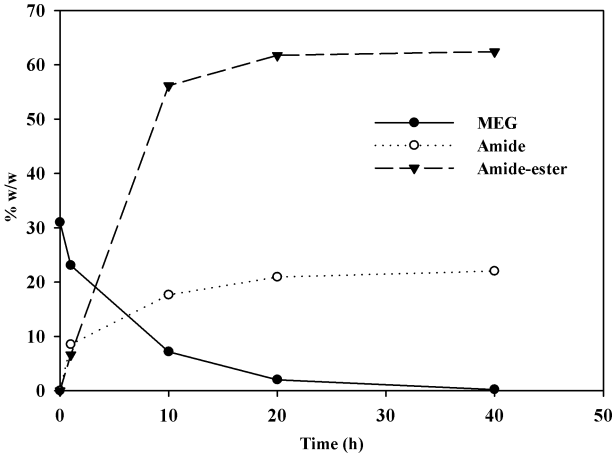

Fig. 3 Amidation using an excess of acylating material (methyl laurate and lauric acid) to MEG (entry C, Table 1) allows complete conversion of MEG after 40 h reaction. Reactions were performed in a thermomixer at 90 °C, 900 rpm, using Novozym®435 (4 wt%).

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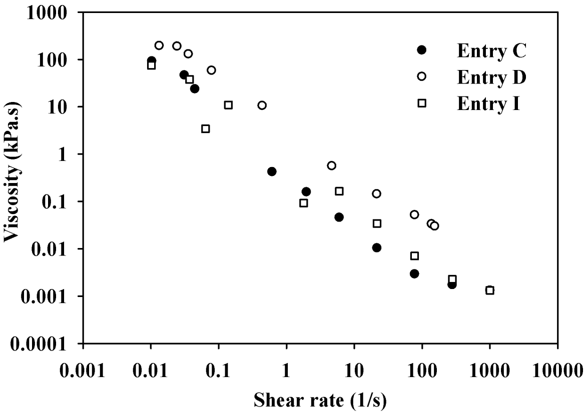

Fig. 4 Rheological profiles of final reaction mixtures of entries C, D and I (Table 1) at 80 °C. Log-log coordinate is used.

viscosities were 2.92 and 7.07 Pa s, respectively, at the same shear rate. Increasing the shear rate to 1000 1/s further improved the rheology of the mixtures (apparent viscosity was 1.33 Pa s for both entries C and I). This indicates the successful use of methyl laurate in entry C to increase the amine molar ratio while keeping the viscosity almost constant. A condensation process catalyzed by N435 for production of a polyester, with comparable viscosity at 80 °C to that reported here, has been described earlier, indicating the potential of scaling-up.[26]

Since the environmental benefits of the solvent-free amidation process would improve even further if the formed amide-ester (5) could be considered as a useful by-product, experiments were performed to verify if it could be hydrolysed to amide, based on the fact that the ester bond is much more susceptible to alkaline hydrolysis than the amide bond. By suspending the final product mixture in aqueous medium at pH 12.5 and stirring at room temperature for 30 min, the amide-ester was indeed found to be completely converted to the amide, *i.e.* compound 4 (Fig. 5), with an amide yield of 99% (Reaction C_h, Table 1). The kinetics of hydrolysis of the amide-ester was measured by monitoring its concentration at 5 min time intervals. A plot of the logarithmic concentrations of amide-ester *versus* time (in minutes) fits pseudo first order kinetics where the slope of the straight line equals the rate constant *k* (data not shown). Stability of amide-ester under alkaline condition expressed as $t_{\frac{1}{2}}$ (half life) was determined to be 4.1 min based on the equation $t_{\frac{1}{2}} = \ln 2/k$. This result is in line with the findings of Stjerndahl and Holmberg.[27] The lauric acid liberated during this hydrolysis step can be recovered and recycled back to the reactor.

Alternatively, the amide-ester could find use as a bleach activator in detergents, cleansers and disinfectants.[28] In this case, fractional crystallization using a suitable solvent can be employed to separate the amide-ester by making use of its higher hydrophobicity and molecular weight. Due to these possibilities, the environmental assessment using EATOS was performed considering the amide-ester as a useful by-product instead of treating it as waste.

Environmental assessment of MEGA production using EATOS

The traditional production process for *N*-lauroyl-*N*-methyl glucamide using sodium methoxide as catalyst,[6] enzymatic synthesis with *t*-amyl alcohol as the solvent described earlier by Maugard *et al.*,[10] and the solvent-free enzymatic methods described in the present work, were evaluated using the EATOS software. The reported yield for the chemical process is 95%, with residual 5% MEG in the final product (entry A, Table 1).[6] For the enzymatic synthesis, the yield was 80% when using 1 : 1 molar ratio of fatty acid methyl ester and MEG, and 10 ml *t*-amyl alcohol (entry B, Table 1).[10] It is important to note, that the method developed by Maugard *et al.*, was the first report on enzymatic synthesis of MEGA and hence not optimized in terms of solvent. The data from these experiments are used in this report to help investigate the EATOS and EcoScale assessment tools. One low-solvent reaction (entry E, Table 1), and two solvent-free reactions with different molar ratios of lauric acid, methyl laurate and MEG (entries C and D, Table 1) were used in the evaluation.

The mass index S^{-1} and potential environmental impact EI_{in} ($EI_{in} = S^{-1} \times Q_{in}$, where Q_{in} is the environmental quotient assigned by EATOS for the input chemicals), are shown in Fig. 6 for the chemical method and four different enzymatic reaction conditions. Performing the reaction with a relatively low amount of *t*-amyl alcohol as solvent and a substrate molar ratio of 1 : 1 (reaction E) apparently reduces the feedstock

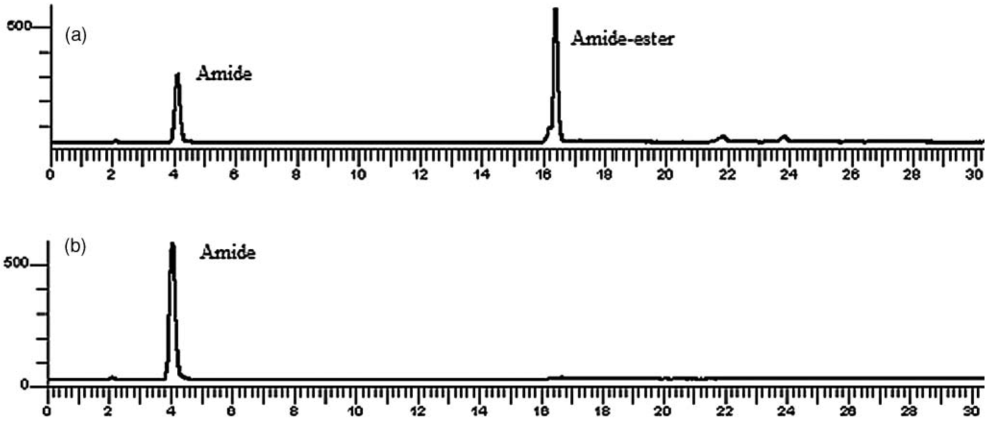

Fig. 5 Reaction mixture composition: (a) after completed amidation reaction using excess of acylating substrate (entry C), and (b) after hydrolysis of amide-ester to yield amide and lauric acid. Since the detection threshold of lauric acid in ELSD detectors is high, this peak is not seen in the HPLC chromatogram.

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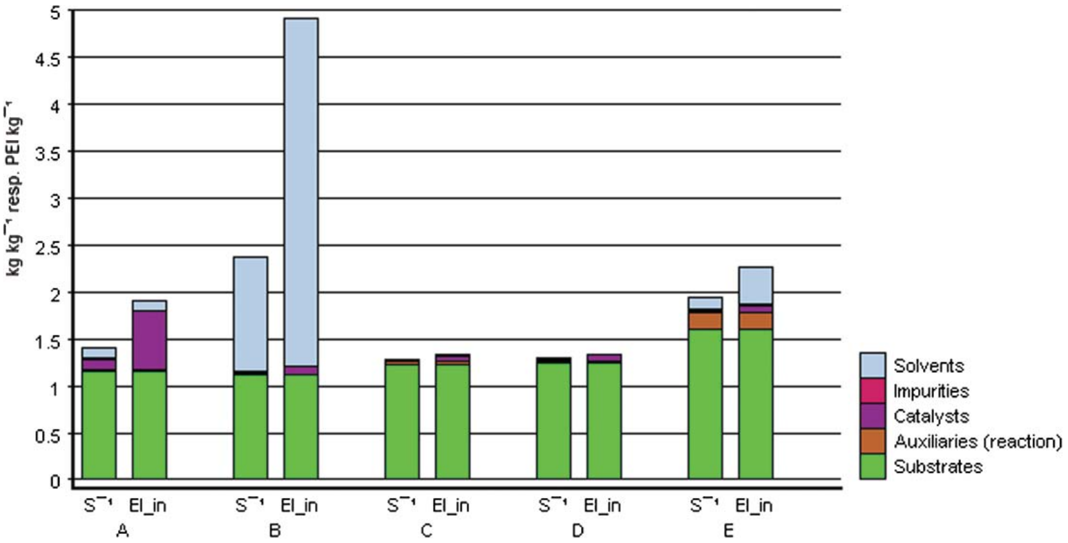

Fig. 6 Comparison of the mass index (S^{-1}) and the environmental impact of process feedstock (EI_{in}) for five different reactions from Table 1. Solvent recycling for entry B is assumed to be 80%. The amide-ester was considered a useful by-product in the calculations. Water used in the hydrolysis reaction was not included in the calculations.

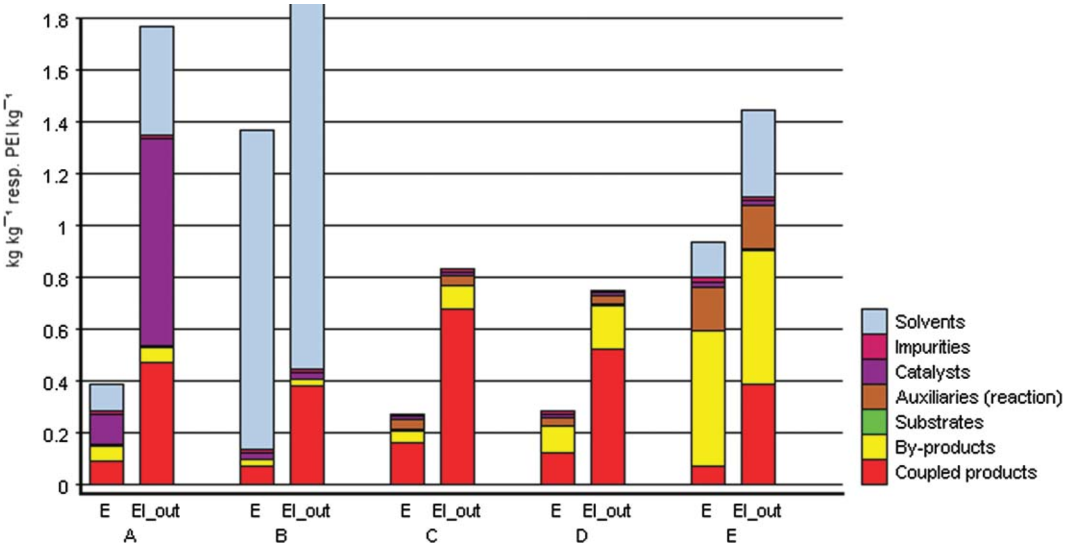

Fig. 7 Environmental assessment of the waste produced during five reactions (entries A–E in Table 1). Parameters considered in the assessment are human toxicity, ecotoxicity, accumulation, and biodegradability, whenever data is available. The EI_{out} bar of reaction B is cut in order to visualize the different contributions to the bars; its value is 3.5 PEI kg^{-1}. Water was not included in the calculations.

environmental impact by 54% compared to the solvent-based method (reaction B). The complete exclusion of the solvent from the process further improves the greenness of the method; the solvent-free enzymatic processes (reactions C and D) score considerably better than methods using a solvent (Fig. 6). The EI_{in} of reaction C is 1.3 EATOS units compared to 4.9 for reaction B, which corresponds to more than 73% reduction in the potential environmental impact. The difference is explained by the fact that *t*-amyl alcohol, contributing to 75% of the EI_{in} value for reaction B, is assigned a Q_{in} value of 3 by EATOS, based on its price and risk phrases, the latter indicates that it is flammable, harmful and irritating to respiratory system and skin. Under the premise of considering the amide-ester a useful by-product as discussed above, the solvent-free enzymatic processes (reactions C and D) are more environmentally friendly than the traditional process (reaction A), with almost 30% reduction in the environmental impact of the feed (Fig. 6).

Comparison of the E-factors clearly shows that reaction C is less mass intensive than the other reactions (Fig. 7). For each kilogram of product and useful by-product (amide and amide-ester), only 0.26 kg waste is produced, which is 29% less than the amount of waste produced in the chemical method (0.38 kg) and one fifth of the waste for reaction B (1.36 kg). To take into account also the nature of the waste, EATOS can consider up to ten different environmental parameters like *e.g.* human toxicity, ecotoxicology, accumulation, biodegradation, air pollution and ozone creation for each unit mass of waste. These parameters are given a certain weight, normalized and combined in the environmental impact of the waste (EI_{out}), which is similar to Sheldon's environmental quotient EQ. To exemplify; in reaction

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B the solvent *t*-amyl alcohol (gray bar, Fig. 7) is assumed to be 80% recyclable and the waste generated is 1.23 kg per kg product. This amount represents 90% of the total waste produced. The MSDS for *t*-amyl alcohol reports that it is harmful (Xn sign), which is interpreted by EATOS to a human toxicity value of 4. Based on the median effective concentration for daphnia (EC_{50}), EATOS assigns its ecotoxicity to 4. Since the accumulation risk for *t*-amyl alcohol is low ($\log_{o/w} = 0.77$), $Q_{accumulation}$ is assigned only 1, and finally the degradability half life is 3.3 days, which is interpreted to a $Q_{degradability}$ of 1. Each of these four factors will contribute by 25% to the final Q_{out} ($0.25 \times 4 + 0.25 \times 4 + 0.25 \times 1 + 0.25 \times 1 = 2.5$), *i.e.* the environmental quotient assigned by EATOS for the waste produced. To calculate the contribution of the solvent to EI_{out}, the waste generated is multiplied by the Q_{out} to give 3.079 PEI kg^{-1} ($1.2318 \times 2.5 = 3.0794$, EATOS units), which represent 87% of the total EI_{out} value for reaction B.

In Fig. 7, it is seen that reaction C has an EI_{out} of 0.82, which corresponds to 53 and 76% reduction compared to reactions A and B, respectively. The catalyst used in reaction A, *i.e.* sodium methoxide, contributes with 45% to the total EI_{out} value (violet bar), due to its human toxicity. The high red bar in EI_{out} for reactions A and B is explained by the fact that the coupled by-product, methanol, is assigned with a Q_{out} value of 5.5. The EI_{out} value of reaction B is 3.5, but the graph was cut in order to visualize the columns in a better way.

Energy and process work-up assessment

Enzymatic reactions often require less energy than traditional organic synthesis[29,30] however this may not always be the case. A comparison between a new enzymatic method and the current process should therefore also address energy requirements during processing, including solvent condensation, stirring and vacuum. A method for considering the process auxiliaries is the ECO method (Ecological and Economic Optimisation), which along with the objectives included in EATOS also takes into account the energy demand for the supply of reactants, solvents and auxiliaries, the performance of the reaction, work-up, application of the products and disposal of the waste, as reported by Kreisel and co-workers.[24,31] The energy factor was determined by the authors for various ionic liquids using the life cycle assessment software Umberto, by which detailed material- and energy-flow systems can be constructed and analysed, with data derived from the data-base Ecoinvent. However, since this software and the database are not freely available, their evaluation was beyond the scope of this study. Instead, the EcoScale environmental assessment tool,[23] freely available as an online tool, was used. The online tool is linked to the ChemExper database, and only the CAS number is therefore required for automatic extraction of the reagents prices and safety values. However, since the online calculator does not support input of materials without a CAS number, *e.g.* like N435, the calculations were performed manually in the present study. The EcoScale penalties/points for the given reactions are shown in Table 2, where 100 would represent the ideal process free of environmental impact. The obtained result shows that reaction C after hydrolysis (C_h) has the best EcoScale points (95.5) followed by entry A (83.5), where the penalty given for usage of sodium methoxide (–10) greatly reduces its score. The tool does not consider the drawbacks of solvent condensation and vacuum energies, and it is easy to realize that the results are based mainly on the achieved yields. In addition, the method does not distinguish between smaller process optimizations like *e.g.* reduced reaction time.

Table 2 EcoScale comparison[a] of two reactions from literature and the developed methods in this study (see Table 1) for synthesis of MEGA surfactant. The ideal value for a process is 100

	A	B	C	C_h	D	E
Yield penalty	–2.5	–10	–33	–0.5	–21	–22
Safety						
t-amyl alcohol (F)	—	–5	—	—	—	–5
Sod. Methoxide (T, F)	–10	—	—	—	—	—
Technical setup						
(Inert) gas atmosphere	–1	—	—	—	—	—
Heating, > 1 h	–3	–3	–3	–3	–3	–3
Workup and purification						
Crystallization and filtration	—	–1	–1	–1	–1	–1
EcoScale	**83.5**	**81**	**63**	**95.5**	**75**	**69**

[a] Some parameters considered in EcoScale, which are not applicable in this comparison, *i.e.* price/availability and complicated addition and removal of solvent, are not shown for simplicity. F: Flammable (penalty, –5) T: Toxic (penalty, –5).

The energy demand during the process can be measured by an energy monitoring socket[24,31] and was performed for two different reactions on a 50 ml scale (Fig. 8). Reaction B was carried out according to the method described by Maugard *et al.*,[10] with good reproducibility, and reaction C with molar ratios as given in Table 1. As seen in Fig. 8, the energy demands per gram of product were 17% and 66% lower for reactions C and C_h, respectively, than for reaction B.

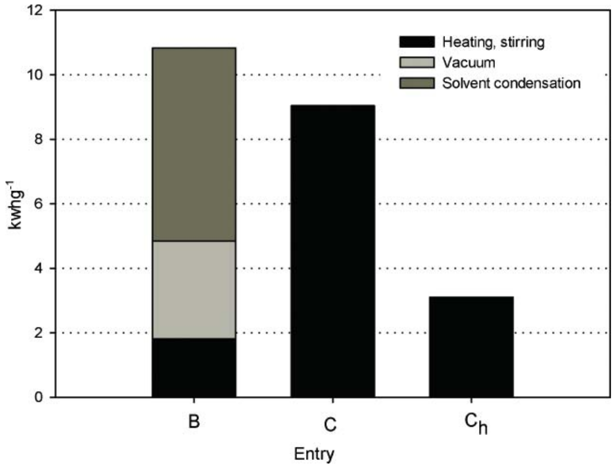

Fig. 8 Energy demand per gram of product for entries B (20 h reaction time), C (40 h reaction time) and C_h (after hydrolysis treatment of C). Experiments were performed at 90 °C in a 50 ml reactor, under reflux and 700 mbar vacuum for reaction B.

Evaluation of the performance of the environmental assessment tools

Lack of data, time and money are the main obstacles to performing a more thorough environmental investigation (such

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Table 3 Useful free computational tools for assessment of early stage R&D chemical synthesis[a]

	EATOS[19]	EHS[15]	EcoScale[23]
Availability	Downloadable Java-archive file	Downloadable Excel file	Web site
Purpose	Calculates the unfriendliness quotient Q and environmental quotient EQ values	Calculates what is equivalent to unfriendliness quotient Q values.	Calculates the process cost, safety, and work-up/purification
Advantages	Theoretically evaluates any process. Informative and versatile outputs (S^{-1}, E, EI_{in}, EI_{out}).	No manual data feeding. Discriminating.	Automatic data feeding is possible. Considers process work-up and energy.
Limitations	Manual data feeding. Non-discriminating in some aspects. A familiarity about the tool is a must before use.	Restricted to a small list of chemicals (mainly organic solvents).	Non-discriminating. Oversimplified.

[a] Refer to Results and discussion section and respective reference for more details.

as an LCA) for early stage process developments. EATOS was developed to address environmental improvements at early phase research and is an excellent tool to evaluate changes in the production method as well as to detect critical parts of the process where further efforts are needed in terms of improving the environmental parameters. However, for the thorough assessment of process developments, R&D chemists may need to also consider complementary tools. Especially in the light of the ongoing climate debate, the comparison between a traditional chemical method and a suggestion for an improved method using a catalyst, *e.g.* an enzyme, would lack relevance if energy demand and process conditions are not implemented in the assessment. We therefore used the EcoScale method, but found that it does not consider some factors, *e.g.* the length of heating since all processes involving heating longer than 1 h get three penalty points. Likewise, reactions utilizing *t*-amyl alcohols are all assigned five solvent-related penalty points, even if one reaction uses 30 times more *t*-amyl alcohol than the other (like *e.g.* entries B and E). Many methods are also considered more environmentally friendly based on reduced energy requirements during process, but this does not take into account the energy needed for production/regeneration of the catalyst. Similarly, processes using supercritical carbon dioxide can be expected to show a very good profile using EATOS, however the energy consumption and risks associated with the use of pressurized gas are concealed behind this good profile. A single environmental assessment tool incorporating all these issues would certainly be beneficial.

Prices are supposed to reflect environmental and social costs in a good way,[19] and EATOS uses the prices of the substrates to assign the environmental impact of their production, which can be expected to be justified *e.g.* for comparison between using a petroleum-based chemical and a chemical otherwise treated as waste, but it does not necessarily provide an overall fair comparison between, for example, non-renewable and renewable raw materials. The possibility to introduce a certain penalty depending on the origin of the substrate would be a good additional option for those cases where the prices are suspected not to reflect the true environmental impact. In addition, the price for a certain chemical might vary from time to time and between different suppliers and countries, *e.g.* the price of lauric acid is 80 €/kg from a laboratory chemicals supplier (VWR International, Stockholm, Sweden) and 1 €/kg from a large scale raw material supplier (AAK, Karlshamn, Sweden). This difference did not result in any variation in the potential environmental impact using EATOS, possibly reflecting a lack of sensitivity in the method.

Another perspective that is not included in the here presented environmental assessments is the space occupied by the materials, *i.e.* the volumetric productivity. In our case, reactions C and D can be run in 6 times less reactor volume than reaction B and this productivity influences the overall economics of the reaction. Furthermore, in the mass balance regions of EATOS, *i.e.* reaction, isolation, pre-waste treatment and waste treatment, we suggest the inclusion of substrate pretreatment, for consideration of items such as molecular sieves used to dry the starting substrates in our reaction. The automation of program feeding by linking each chemical to a database *via* its CAS number, as utilized in the EcoScale tool, further simplifies the use and should be possible also for other environmental tools.

The pros and cons of the EATOS and EcoScale used in this study, as well as the EHS tool, are summarized in Table 3.

Experimental

Materials

Novozym®435 (N435, *Candida antarctica* lipase B immobilized on an acrylic resin), was a gift from Novozymes A/S (Bagsvaerd, Denmark). Analytical grade methanol and lauric acid (>99% purity) were purchased from Merck (Darmstadt, Germany), while methyl laurate was from Fluka (Buchs, Switzerland). *N*-methyl-glucamine and *t*-amyl alcohol were purchased from Sigma-Aldrich (St Louis, USA). All other chemicals used were of analytical grade.

Enzymatic synthesis of MEGA in 4 ml scale

N-Methylglucamine (MEG), lauric acid and/or methyl laurate were mixed in 4 ml open vials (except for reaction mixtures containing *t*-amyl alcohol where the vials were closed with lids) and heated to 90 °C in a KTMR-133 thermomixer (HLC, Bovenden, Germany). N435 (4 wt%), previously dried over silica gel for 48 h, was added and the reactions were carried out in the thermomixer at 90 °C and 900 rpm, under normal atmospheric pressure. Water was removed from methyl laurate and solvents prior to running the reaction by using molecular sieves (3 Å,

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10% w/w), shaking the mixture overnight at room temperature, and finally storing the chemicals in the presence of silica gel.

Amidation in 50 ml scale

The substrates methyl laurate and MEG were mixed in a 50 ml reactor in 1 : 1 ratio for reaction B, and methyl laurate, lauric acid and MEG were used at 1 : 1 : 1 ratio for reaction C. The temperature was set to 90 °C using an oil bath, and stirring was achieved using a magnetic bar. Reaction B was performed under reflux and vacuum of 700 mbar. The energy consumption was estimated by an energy monitoring socket (Energy Check 3000, Voltcraft, Germany) positioned in series between the power supply and the reactor, cryostat and vacuum pump.

Rheology measurement

Rheology measurements were performed at 80 °C for final reaction mixtures of entries C, D and I under controlled stress at different shear rates using a Physica UDS 200 rheometer (Paar Physica, Stuttgart, Germany) equipped with a cone (50 mm and 1° angle) and plate geometry.

Hydrolysis of the amide-ester by-product

The mixture of amide and amide-ester (100 mg) after the completed reaction (reaction C) was subjected to alkaline hydrolysis at pH 12.5 in aqueous medium (2 ml) by addition of 0.05 M NaOH and stirring for 30 min at room temperature °C to convert the amide-ester into amide.

HPLC analysis

Samples were withdrawn from the reaction mixtures, diluted and analyzed using a PerkinElmer HPLC system (Boston, USA) equipped with an Alltech 3300 evaporative light scattering detector ELSD (Grace, Deerfield, USA). The ELSD was set to 38 °C and filtered air was used as nebulizing gas at a flow rate of 1.3 l min^{-1}. A LiChroCART® 125–4 mm analytical column packed with a LiChrospher® 100 RP-C18, 5 µm (Merck, Darmstadt, Germany) was used for the separation with the temperature maintained at 40 °C. Elution (1 ml min^{-1}) involved an isocratic flow of 25 : 75 water–methanol during 5 min, linear gradient from 25 : 75 to 5 : 95 for 10 min, holding for 10 min, linear gradient back to 25 : 75 within 5 min and a final equilibration time of 10 min. The water phase contained 0.05% TFA (v/v). Retention times for MEG, amide, lauric acid and amide-ester were 2.1, 4.1, 8.4 and 16.4 min, respectively. Quantification of the compounds was performed using standards produced in-house, purified by flash chromatography, and confirmed by HPLC, infrared spectroscopy and mass spectroscopy. Conversion of lauric acid was analyzed by acid titration using 0.05 M KOH as titrant, and phenolphthalein as indicator.

Mass spectrometry

Identification of the amide product and amide-ester by-product was performed using the same HPLC system and gradient as described above, but connected to a hybrid QSTAR Pulsar quadrupole TOF mass spectrometer (PE Sciex Instruments, Toronto, Canada), equipped with an electrospray ionization source. MS and MS/MS were recorded in the mass range of m/z 100–2000 and 50–2000, respectively, with the turbospray operating at 350 °C in both negative and positive mode. Nitrogen was used as the collision gas with the collision energy set at 30 eV. Data were assessed using the Analyst® QS software (PE Sciex Instruments, Toronto, Canada).

Environmental assessment

Data required to feed the EATOS software depended mainly on the material safety data sheets available from *e.g.* Sigma Aldrich. Prices were taken from VWR International (Stockholm, Sweden) except for N435 which has a current selling price of 900 €/kg. Water used in the hydrolysis experiment was not included in the calculations, as discussed by Sheldon.[13] The accumulation part of the Q_{out} values for amide-ester was based on the $\log_{o/w}$ value, which was calculated using the OSIRIS tool.[32]

Conclusions

The final product obtained from the enzymatic solvent-free process developed in this study is clean in terms of solvent and catalyst residuals in contrast to the product of the chemical reaction, which contains the solvent used and the remains of the catalyst. In addition, the process can be performed with no residual MEG in the final product without the risk of using hazardous sodium alkoxides. The preliminary environmental assessment shows that even if the developed method (Entry C) gives a lower yield than the traditional methods, it provides benefits in terms of lower environmental impact. With a lower yield, consideration of the nature of the by-product becomes essential. In our case it was found that the by-product could be quantitatively converted into product by a simple one-step reaction, and it was hence included in the calculations as a useful by-product instead of being treated as waste.

The environmental assessment tool EATOS was found to be an excellent software for a fast and easy evaluation of different process options during the early stage development of more environmentally benign methods for chemical production. The main strength with EATOS is in establishing if a certain alternative process will significantly reduce the amount of waste produced and decrease the environmental impact of the waste and the materials used in the process, as well as to pinpoint which parts of a process that contribute the most to the total environmental impact. There is a potential to further improve the software by integration of process conditions like work-up and special setting requirements, as well as energy requirements, similar to the EcoScale system, however with the suggested improvements mentioned in the discussion section. In addition, the evaluated tools do not include the perspective of different substrate sources, *e.g.* if it is derived from renewable (lauric acid or methyl laurate) or non-renewable (*t*-amyl alcohol) raw material. The development of more environmentally friendly processes for production of chemicals is a highly important topic, and the discussed tools are certainly helpful for this purpose. However, before performing the environmental assessment, different available assessment tools need to be carefully scrutinized in order to ensure that all important aspects are incorporated.

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