Development of Environmentally Compatible Detergents Using Biosurfactants

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Abstract

Mannosylerythritol lipids (MELs) are biosurfactants with excellent characteristics, namely biodegradability, low toxicity, and biocompatibility. It can be used in several applications, such as in detergents.

During the development of the formulations, several tests (pH, viscosity, foam, and CMC determination) and several ingredients were evaluated to understand which formulations were the most suitable, thus we arrived at the final formulations of the manual dishwashing detergent (SLES+CB+MELs (5 g/L)) and glass cleaner (SDBS+MELs (2 g/L)).

The final dishwashing detergent formulation has a pH of 5.99, viscosity of 688.4 cP, and foam test of 5.25 cm which was held for 5 minutes. The final glass cleaner formulation has a pH of 8.11. For both detergents, the emulsification index at 24h was 47.69% and for the *Galleria mellonella* survival test, the results were not conclusive.

The formulations were subjected to accelerated stability tests for three months and both proved to be stable. About the performance tests performed, the hand dishwashing detergent needs improvement, as its cleaning ability is lower than the benchmark and an ecolabel certified formulation. The glass cleaner has a good cleaning ability similar to the market leader and also to the ecolabel certified formulation.

Finally, a questionnaire was conducted to obtain external opinions about the appearance and performance of the detergents.

Keywords: Biosurfactants; Mannosylerythritol Lipids; Detergents; Formulations; Performance

1. Introduction

The goods production industry has seen enormous growth in recent years, which has led to increased pollution and, consequently, environmental degradation. The household cleaning products industry is no exception.

Detergents Regulation (EC) No 648/2004 defines detergent as any substance or mixture designed for washing and cleaning operations that contain soaps and/or surfactants. They can be found in a variety of forms (liquid, powder, paste, bar, moulded piece) and are marketed for domestic, institutional, or industrial purposes [1]. It is expected that detergents can remove all dirt quickly, effectively, and safely.

The main constituent of detergents are surfactants (surface active agents), they are amphiphilic molecules with polar and non-polar domains that preferably break at the interface between liquid phases with different degrees of polarity [2].

Through particular and preferred interactions at surfaces and interfaces caused by hydrophilic and hydrophobic moieties in the same molecule, this property lowers the surface tension of liquids. While the polar component of a surfactant might be ionic (cationic or anionic), non-ionic, or amphoteric, the nonpolar component is frequently a chain of hydrocarbons [3].

A surfactant's efficiency is determined by reducing surface tension, which is the mechanical energy required to create one unit of new liquid surface area. The surface tension decreases with increasing surfactant concentration in the aqueous medium until the formation of micelles. The critical micellar concentration (CMC) is the concentration that corresponds to the point at which the surfactant reaches the lowest stable surface tension, this is the minimum concentration of surfactant necessary for the maximum reduction of surface tension. Micelles are usually formed when the critical micellar concentration is reached [4].

Other ingredients that usually are present are builders, the principal function is a sequestrating agent of Ca^{2+} and Mg^{2+} ions that could interfere with the surfactant, also enhance the surfactant performance, and provide an alkaline environment [5]. pH adjusters help to balance the pH of the formulation, making it either more basic or acidic considering the formulation type. Preservatives are needed to keep an adequate shelf life, otherwise, bacteria and fungi destroy the product [6].

To improve the appearance and the experience of the consumer, the fragrances give a nice smell to the product and could be used to mask odours caused by any of the other ingredients. Dyes are utilized to give a detergent an appealing colour. It also gives identity to a product, making it easier to distinguish it from other products.

1.1 Environmental impact of detergents

The environmental impact of detergents has focused on the discharge of industrial and domestic wastewater into receiving waters [7], which has various consequences on the fauna and flora of natural ecosystems. Detergents can alter water parameters such as pH, salinity, temperature, and turbidity, leading to a decrease in water quality. In general, one of the most common problems is caused by the formation of a foam layer on the water surface that decreases the oxygen rate in the water, so there will be poor oxygen adsorption by aquatic organisms. However, detergents are essential for human life and their manufacture and consumption cannot be eliminated, their impacts on human health, the environment, and biological treatment processes should be minimized. To control the adverse effects of detergents on the environment, there are several regulations at the European level that must be complied with. However, it is not enough, so bio-detergents should be used, they are more environmentally friendly and biodegradable, and have better cleaning characteristics than synthetic cleaning agents [8].

1.2 Biosurfactants

The detergent industry has been adapting to technological advances and at the same time concerned with environmental problems. One option may be to replace surfactants with biosurfactants. Biosurfactants are compounds of microbial origin with diverse structures and surface properties [2] and are divided into 5 classes. The majority of the currently utilised are glycolipids, which have a low molecular mass [3].

They have aroused interest due to their unique properties such as high biodegradability, low toxicity, great structural diversity, and effectiveness at extreme temperatures [9]. Additionally, they can possess lower CMC values than synthetic surfactants, improving their efficiency in various applications [10]. Biosurfactants play important roles in industrial [11], pharmaceutical [12], and environmental applications [13], such as detergency, foaming, wetting [14], emulsification, bioremediation [15], stabilization [16], lubrication, dispersion, and solubilization of hydrophobic compounds, thanks to their structural diversity and functional properties.

The commercial availability of microbial biosurfactants is quite limited. Large-scale production of biosurfactants can be difficult to achieve at competitive costs [10]. There are markets interested in exploring the biosurfactant industry, as there is a growing receptivity among consumers for environmentally friendly compounds [17].

1.2.1 Mannosylerythritol lipids (MELs)

MELs are one of the most promising biosurfactants, glycolipids mainly produced by *Moesziomyces* spp., contain 4-O-β-D-mannopyranosyl-meso-erythritol or 1-O-β-D-mannopyranosylerythritol as the hydrophilic group and fatty acid and/or an acetyl group as the hydrophobic moiety [9]. MELs generally have one or two acetyl groups at C-4 and/or C-6 of the mannose moiety. Based on the degree of acetylation at the C-4 and C-6 positions, and their order of appearance on the thin layer chromatography, MELs are classified as MEL-A diacetylated, the most common, while MEL-B and MEL-C are monoacetylated at C-4 and C-6, respectively. MEL-D has a completely deacetylated structure, generally, it can only be derived by enzymatic synthesis of MEL-B [18]. The production of MELs isn't associated with growth, so MELs are only produced when the yeast is in a stationary phase [19]. MELs have been applied in many fields, due to their exceptional interfacial properties, biocompatibility, self-assembling properties, antimicrobial activities, and biochemical functions.

The main objective of this work was the development of new detergent formulations using MELs as an active ingredient and the characterization of these formulations.

2. Materials and methods

2.1 Development of formulations

The development of formulations was performed under classic methods [6], [20]. In order to obtain a good and environmentally friendly formulation several ingredients were tested, such as anionic, non-ionic and amphoteric surfactants and viscosity modifiers, using different mixing profiles and temperatures. The formulations were built using a base formulation, they were characterised through various tests, such as pH, viscosity, foam, and surface tension to define the critical micellar concentration. A reference formulation was also created, for both formulations, that uses decyl glucosidase instead of MELs. For final formulation was used a mixing procedure was started at 40ºC gradually increasing to 60°C and 500 rpm.

2.2 Characterization of developed formulation

2.2.1 pH

The pH was measured with help of a pH Meter (744, Metrohm), at room temperature.

2.2.2 Viscosity

The viscosity is measured with a viscometer (Brookfield DV-II+ Pro). The measurements were performed at room temperature, using the CPE-52 spindle and 20 rpm.

2.2.3 Foam test

The Ross-Miles method was adapted from the Kruss Benchmark test following the well-established ASTM D 1173-07 Ross-Miles standard for foam analysis [21]. In a measuring cylinder containing 25 mL of the solution in the test, 125 mL of the same solution was dropped from a height of 40 cm. The foam was measured at the instant when all the solution was added and after 5 minutes.

2.2.5 Emulsification potential

The emulsifying potential of the final detergent formulations was determined with the use of kerosene. The emulsification was measured by mixing equal volumes (4 mL) of kerosene and the final formulations. The mixture was vortexed for 2 minutes at 2400 rpm and left to stand for 24, 96 and 168 hours at room temperature (25°C).

2.2.6 *Galleria mellonella* survival assay

Galleria mellonella larvae were raised in insectaries at a temperature of 25°C in the darkness, along with a pollen grains diet. A solution with the dissolved hand dishwashing detergent was placed in the petri dishes at a concentration of 3 mL/L. This experiment was performed in triplicate and on each plate 10 larvae (90 ± 10 mg) were placed in the dark at 25°C. For one month the larval health score was evaluated. The health score consists of several parameters such as larval activity, cocoon formation, melanisation, and survival. The controls utilized were water and commercial detergents.

2.3 Performance test

The hand dishwashing detergent performance test was based on SOFW-Journal, 128 Jahrgang, 5-2002 [22]. The soil composition was margarine (17%), olive oil (17%), flour (24%), and water (42%). In each dish was placed 5g of soil.

The 5L reservoir was placed 90 centimetres from the washbasin where the detergent is between the centre and the wall of the washbasin. In dishwashing, solution-soiled plates are washed by hand, with the help of a brush, until the foam layer collapses. When the foam layer permanently breaks down on the surface of the wash bath, the wash bath is considered exhausted, and the endpoint has been reached. The number of dishes washed was accounted for. The test was performed with hot water (37ºC) and cold water (21ºC).

2.4 Accelerated stability

The accelerated stability study ensures that the product maintains its physical appearance, chemical, aesthetic properties, functionality, and microbiological characteristics when stored under normal conditions. The detergent samples were stored in a refrigerator and climatic chambers with 24h control of temperature and humidity. The test conditions were 4ºC; 25ºC / 60% relative humidity (RH) and 40ºC / 75% RH, this study lasts for three months. At the beginning of the study, a measurement is carried out at the end of each month. For the manual dishwashing detergent, the parameters analysed were organoleptic (colour, aspect, odour), pH, viscosity and, lastly, microbiologic control for diverse bacteria, yeasts, and moulds, only in the beginning and in the third month. The acceptance limit was stipulated to be within ± 20% variation from the initial test value.

3. Results and Discussion

Several formulations have been tested to obtain good environmental and technical performance. Some surfactants were tested, anionic (SCS, SDBS, SLES) and non-ionic (DG and MELs). These surfactants were chosen due to their low environmental impact.

After some tests, it was possible to arrive at a formulation which is described in table 1.

Table 1. Composition of hand dishwashing detergent.

Composition (% of active matter)	Concentration (% w/w)
SLES (30%)	28
MELs (65%)	0.5
CB	1
Sodium Chloride	3
Water	to 100
Sodium Benzoate	0.01

This formulation has a pH of 5.99 and a viscosity of 688.4 cP. This pH is not irritating to the skin; however, it can slightly decrease the cleaning ability of the detergent. The viscosity of this detergent is higher than the viscosity of existing detergents on the market but still has an acceptable value.

Regarding the foam test, the developed formulation was compared with the benchmark and another detergent with ecolabel certification, the results were presented in Figure 1.

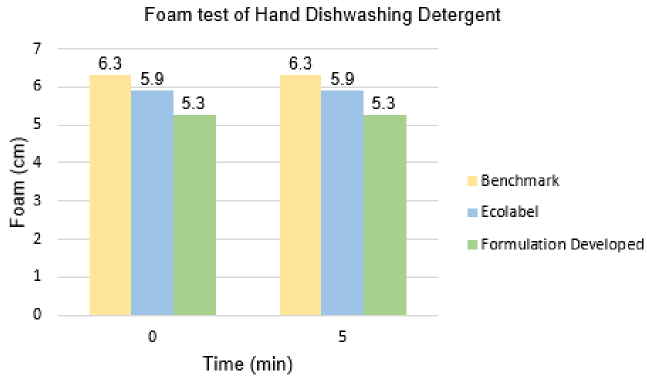

Figure 1. Foam test of hand dishwashing detergent final formulation comparing with for benchmark, ecolabel detergents.

The developed formulation had lower foam formation, having a difference of 1.05 cm to the benchmark, however, the foam is stable during the test time.

About the emulsification index, the most frequently analysed parameter is EI24, representing the emulsion in liquid that remains after 24h. Longer experimental times, 96 and 168 h were evaluated to understand the performance of the formulation over a longer time (Figure 2). Water was used as a negative control, and no emulsion formed. The benchmark had the highest emulsification at EI24 64.1% but decreased in the following days.

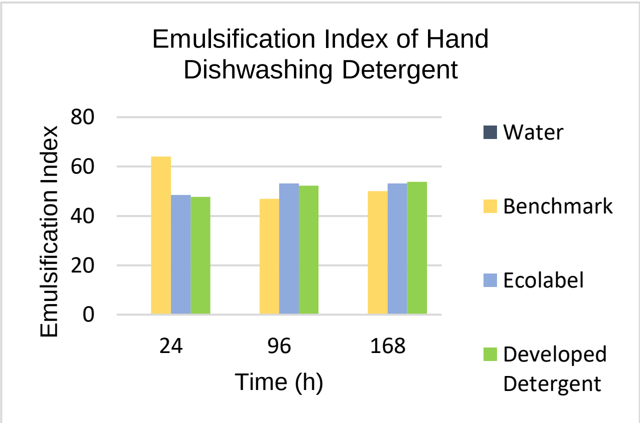

Figure 2. Emulsification index of hand dishwashing detergent: benchmark, ecolabel and developed formulation for 24h, 96h e 168h. Water as used was control.

The developed detergent and ecolabel detergent showed at EI24 a similar value of around 48%. The emulsification for the developed detergent increases slightly over the days.

To understand the impact of the developed detergent in ecotoxicological terms, a survival assay using *G. mellonella* was made, this preliminary study was important to comprehend their impact on the environment during the use and disposal phases. The results obtained in this assay were not as expected, because the larvae started cocoon formation early, on day 8 they already had their full cocoon, which was expected to happen only after day 20. This may have happened because they were exposed to some stress during the preparation of the study. As a consequence of the early cocooning, the butterflies that hatched were small, some did not hatch. In Figure 3 it is possible to observe the survival rate during the study, in none of the conditions there was a 100% survival rate as expected, at least in the case of water. The detergent developed was the one that showed the lowest survival rate (90%); however, it is not possible to understand the death of the larvae was caused by the detergent.

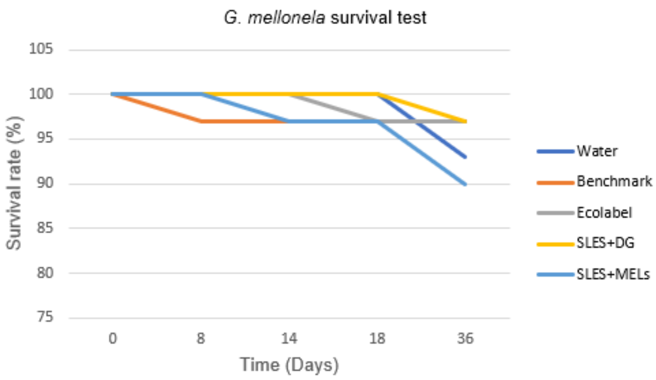

Figure 3. Assessment of hand dishwashing detergents on *G. mellonela* survival test.

3.1 Performance

The developed detergents efficiency was evaluated, by assessing their performance during the cleaning process, which is one of the most crucial qualities. The effectiveness of the product under test was evaluated based on its capacity to remove soil and keep a clean surface, compared to a reference product (in this case a benchmark product and a detergent with ecolabel certification).

The first step was determining the dosage of developed detergent that should be used, several quantities were used in 5 litres of hot water, first 5 mL of detergent, then 7 mL and finally 10 mL, the results obtained were presented in table 2. This dosage was used in the following experiments.

Considering these results, it is already possible to understand that the formulation needs some improvements because the established dosage is more than double the dosage established for the reference detergents. This problem did not affect the following tests as the defined dosage for each detergent was used.

Table 2. Developed detergent dosage determination, at 37°C.

Developed Detergent	
Water Temperature= 37°C	
Dosage in 5 L (mL)	**Clean plates**
5 ml	6
7ml	7
10 ml	10

After that, was proceeded to evaluate the performance of the developed detergent by comparing the performance with the benchmark and with an ecolabel certified detergent, at 37°C (Table 3). It was possible to notice that there is no variation in the number of dishes cleaned, which is a positive result, however as already mentioned the detergent developed requires a higher dosage and is therefore less effective.

The ability to clean in cold water, namely at 22°C, was also evaluated because the life cycle of hand dishwashing detergents has the biggest environmental impact in the use phase, which contributes 86-98% due to the energy required to heat the water [23]. The results were shown in table 3, the performance of the developed detergent and the ecolabel certified detergent decreases slightly, which is normal because grease removal is less effective at lower temperatures, however, the benchmark was able to maintain its performance.

Table 3. Performance tests of hand dishwashing detergent, at 37ºC and 22°C.

	Benchmark	Ecolabel Certification	Developed Detergent
Dosage in 5 L (mL)	4	4	10
Clean Plates (37°C)	10	10	10
Clean Plates (22°C)	11	8	9

Through this test, it was possible to realize that the detergent developed needs some improvements to be able to simultaneously increase performance and reduce the dosage used, in this case, the concentration of anionic surfactants should be higher, as this type of surfactant is the main responsible for foam formation. The two main options would be to increase the concentration of SLES, as this surfactant is somewhat irritating, it might not be the best solution, or use another anionic surfactant with a synergic effect.

3.2 Accelerated stability study

The stability study was used to observe and predict the changes that may occur in products over time and predict the shelf life. This study increases the rate of chemical degradation and physical change of the detergents by using exaggerated storage conditions. The conditions are 4ºC, 25ºC/60%RH and 40ºC/75%RH.

All organoleptic parameters (aspect, colour, odour) remained constant over the three months of the study, in all conditions. Concerning the pH, the variations were minimal in all conditions throughout the study, however, the sample with the highest variation was 40ºC/ 75% RH, which was expected as it is the sample that is under the most stress (Figure 4).

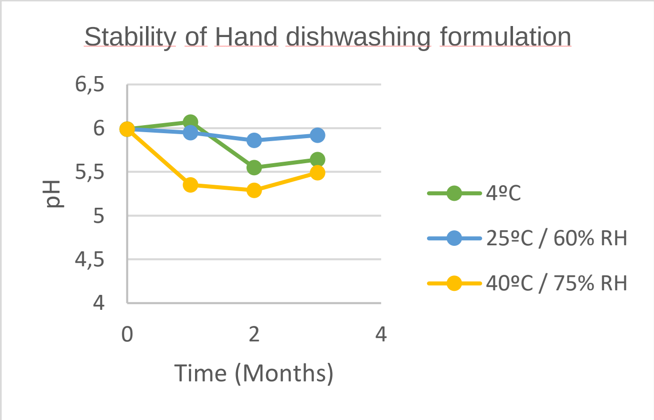

Figure 4. Stability of hand dishwashing detergent over 3 months for pH.

Regarding viscosity, there was a high variation in the first month, this variation happened due to the short waiting time at room temperature before the measurements (Figure 5). In the following months, this time increased, so in the following measurements, the variations were not significant. Regarding the microbiological analysis, there was no growth of any microorganism both at the beginning and at the end of the study, namely various bacteria, moulds and yeasts, which revealed the effectiveness of the preservative, sodium benzoate, and the application of correct dosage.

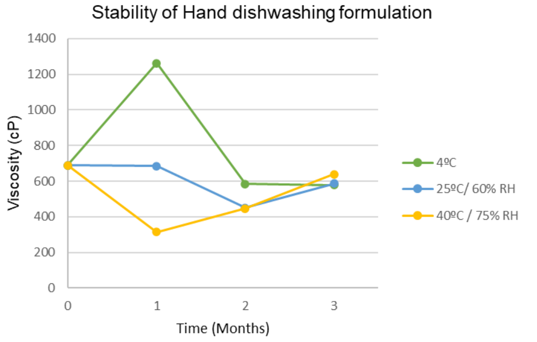

Figure 5. Stability of hand dishwashing detergent over 3 months for viscosity.

At the end of the 3 months, all organoleptic and physic-chemical parameters were in accordance with the specifications and accordance with the acceptance criteria, for the samples tested under the three different conditions. There was some variation in the pH and viscosity results at the various checkpoints, however, the results are within the acceptance criteria.

3.3 Questionnaire

It was relevant to have an external opinion about the products, in both terms of appearance and performance, so a questionnaire was prepared to compare the formulated detergent with the benchmark and other with the ecolabel certification. The questionnaire was carried out by five people, which independently used the three detergents twice. The opinions obtained were in line with the results obtained in the performance tests carried out.

The respondents prefer the traditional aspect of detergent. The evaluation of the detergent during the washing shows the benchmark was the favourite, followed by the performance of the ecolabel certified detergent and lastly, the formulated detergent, the respondents said this detergent foamed less. However, the appearance of the crockery at the end of the wash was equal for all detergents. The preferred detergent, for all the participants, was the benchmark.

4. Conclusions and Future perspectives

Most of the detergents contain surfactants made of petroleum and using them is not the most environmentally friendly practice. Alternatives to the traditional surfactants are beginning to appear such as MELs which is a biosurfactant. One of the objectives was the development of new detergent formulations using MELs as an active ingredient.

After some tests it was possible to arrive at a formulation, however, this needs further improvement. The dishwashing detergent formulation could have a slightly higher pH, around 7-8 and a higher foaming power. The performance can also be considerably improved, the SLES concentration should be higher or SLES should have been used in synergy with another anionic surfactant.

Regarding future perspectives, the formulations developed can be improved and subsequent characterisation and performance. Or it would be interesting to use the MELs in other formulations, for example, laundry detergents or multipurpose detergents. Another option could be in the developed formulations to use other biosurfactants, like sophorolipids or rhamnolipids and compare them, to understand which would be the most viable.

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