facts

Safe and efficient bacterial disinfection with bio-based lactic acid

Jungbunzlauer

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Introduction

Towards green biocides

Hygiene and disinfection are an indispensable part of our daily life. We use anti-bacterial hand-soaps, surface cleaners and laundry detergents on a day-to-day basis. Biocides protect many products from germ formation and therefore contribute towards making our world safer, cleaner and more liveable. However, established biocides such as polychlorinated phenoxy phenols or isothiazolinones also pose risks such as harmful effects on human health or environmental toxicity. In addition, many conventional actives are synthetic chemicals, while there is also a growing demand for bio-based solutions. Green alternatives, e.g. lactic acid produced by a natural fermentation process, may overcome these drawbacks by combining natural origin, effectivity, safe handling and environmental friendliness.

The goal of this report is to explore the anti-microbial potential of lactic acid and to prove its suitability as a genuine green alternative in the aforementioned sense. Pure lactic acid and its mixtures with surfactant were therefore tested according to standard protocols. The biocidal performance of lactic acid was evaluated by screening its effect on four different germs. Jungbunzlauer's L(+)-lactic acid is obtained from fermentation based on natural and renewable resources. It is approved by ECOCERT as raw material from natural origin for the use in detergents and personal care. Before presenting and discussing the scientific results, a short overview of the relevant regulations will be outlined.

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Regulatory background

Regulation (EU) No 528/2012, the Biocidal Products Regulation (BPR), in force since September 2013, provides a detailed framework on the use of biocidal products and on putting them on the market.[1] It makes sure that only products approved by this regulation as biocides may enter the EU market. The regulation concerns 22 product types (PT) which can be classified in four main groups: disinfectants (PT 1-5), preservatives (PT 6-13), pest control (PT 14-20) and others (PT 21-22).

Furthermore, starting from September 1st 2015, only substances from listed suppliers are allowed to be used and claimed as a biocidal active substance in the final formulation (Article 95 list). This requirement will presumably create a protected market made up of only a few players because the hurdles for registration are quite high (time-consuming, financial and human resources).

At the same time, some conventional anti-microbial actives may disappear from the market because they do not fulfil the regulation's criteria. However, this circumstance may open the door for new substances to fill the gap and to satisfy the demand for safe and sustainable solutions. In this respect, organic acids such as L(+)-lactic acid represent a great potential.

Another relevant regulation is Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP). Since June 1st 2015, it also applies to mixtures (it had previously applied to pure substances only).[2] As a consequence, final formulations and consumer products have to be labelled according to the specified criteria (e.g. pH of the mixture and concentration of the active substance). This affects the use of biocides as well. Table 1 lists common anti-bacterial ingredients. As can be seen, the limits vary a lot: for the widely used Triclosan, concentrations up to 10% are label-free while lactic acid needs labelling starting from 1%.

Still, as we will show in the following chapter, low (label-free) use levels must not necessarily be a hurdle to the use of green biocides. Furthermore, common anti-microbial actives are often associated with detrimental effects to human health (e.g. sensitising the skin) or the environment (e.g. aqua toxicity, bio-accumulation), even at small concentrations.

Table 1: Use level depending labelling requirements for biocidal active substances according to Regulation (EC) No 1272/2008 (CLP), pH of formulation > 2

Substance	Categorisation	Labelling
Triclosan	c ≥ 10% Skin, eye irritant	
Chloromethylisothiazolinone/ Methylisothiazolinone (3/1)	c ≥ 0.6% Skin corrosive	
	0.06% ≤ c < 0.6% Skin, eye irritant	
	c ≥ 0.0015% Skin sensitising	
Lactic Acid	c ≥ 3% Eye corrosive	
	1% ≤ c < 3% Skin, eye irritant	

Anti-bacterial performance of lactic acid

The performance of common disinfectants such as triclosan has often been analysed and reported. Its performance even at low concentration is not in doubt. Yet there is a rising concern about its safety and impact on human health and the environment worldwide.[3–6]

The increasing demand for disinfectants and the concern about their origin is obvious in the personal and home care market. We therefore analysed the performance of lactic acid from natural origin, pure and combined with sodium laureth sulfate (SLES), based on the DIN EN 1040 tests. In the EN1040 test, a suspension of bacteria is added to the considered solution and the extent of bacteria reduction is assessed after a defined period of time. The biocidal performance was tested versus four (instead of two) common germs *Pseudomonas aeruginosa* (DSM 939), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 10536) and *Enterococcus hirae* (ATCC 10541) at 20°C.

After mixing bacteria and test solution, the composition was allowed to react for five minutes at 20°C. Subsequently, the bactericidal potential was immediately neutralised by the dilution-neutralisation method. In order to pass this basic disinfection test, at least a 99.999% or log 5 reduction of the gram positive and gram negative bacteria has to be achieved. In tests without further notice, the standard five minute contact or reaction time was applied.

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The effect of the pure substances in the modified DIN EN 1040 test is shown in Table 2. As expected there is no impact on the bacteria by the surfactant. Lactic acid at moderate concentrations results in a good effectiveness against gram positive bacteria. Yet the double membrane of gram negative bacteria lowers its efficacy. Finally, higher concentrations succeed in achieving a log 5 reduction of the cultures of *S. aureus*. However, such highly concentrated solutions might not be seen as favourable by the market as these will be accompanied by a corrosive labelling, posing potential risks to users and treated surfaces.

Table 2: Performance of substances in a modified DIN EN 1040 test (five minutes contact time); n. d. = not determined; colour represents pass (green; log >5 reduction) or fail (red) of the test

Log reduction (EN 1040)	P. aeruginosa	S. aureus	E. coli	E. hirae
10% Lactic Acid	> 5	> 5	> 5	< 5
5.0% Lactic Acid	> 5	< 5	> 5	< 5
2.5% Lactic Acid	> 5	< 5	> 5	< 5
1.0% Lactic Acid	> 5	< 5	n.d.	n.d.
3.0% SLES	< 5	< 5	n.d.	n.d.

In a next step we tested the biocide activity of lactic acid combined with a surfactant. Most anti-microbial products contain surfactants, of which the anionic SLES is one of the most widely used. The results of these experiments, again following the above presented modified DIN EN 1040 protocol, are presented in Table 3. Interestingly, we find a pronounced synergy between the two components: disinfectant efficacy is demonstrated for a broad range of concentrations. Even below 1% active matter, a log 5 reduction of the bacteria is achieved. Obviously, the surfactant helps the lactic acid to overcome and penetrate the bacterial cell walls. As a consequence, the lactic acid can fulfil its entire biocidal potential and the cell perishes subsequently.

Table 3: Performance of substances in a modified DIN EN 1040 test (five minutes contact time); colour represents pass (green; log >5 reduction) or fail (red) of the test

Log reduction (EN 1040)	P. aeruginosa	S. aureus	E. coli	E. hirae
5.0% Lactic Acid + 3.0% SLES	> 5	> 5	> 5	> 5
2.9% Lactic Acid + 2.9% SLES	> 5	> 5	> 5	> 5
1.8% Lactic Acid + 1.8% SLES	> 5	> 5	> 5	> 5
0.9% Lactic Acid + 0.9% SLES	> 5	> 5	> 5	> 5
0.6% Lactic Acid + 0.6% SLES	> 5	> 5	> 5	> 5

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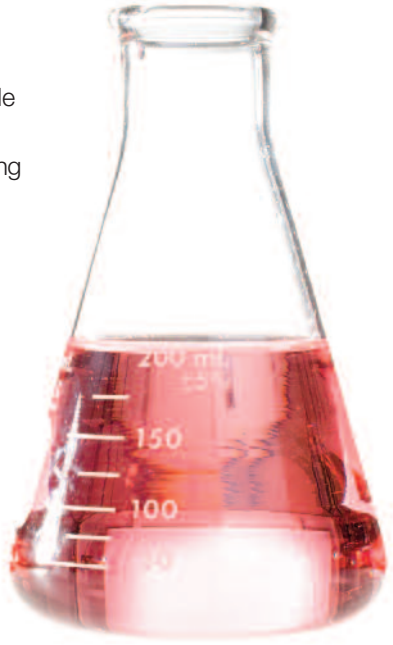
This is a remarkable result – and not only in terms of efficiency and the beneficial coaction of lactic acid and SLES, which certainly merits more profound scientific examination. This synergy is also highly noteworthy from a market point of view. As lactic acid/SLES solutions below 1% (pH above 2) are still effective, these simple mixtures represent label free disinfectant formulations in line with CLP Regulation. In practice it is, of course, more complex (shorter contact times, more challenging cleaning environment), but these first data already provide valuable information and may help to boost further investigations and business development towards green sanitising solutions.



In order to get closer to a realistic cleaning situation, we performed additional tests with a modified DIN EN 1040 protocol (shorter contact times) and a modified DIN EN 1276 approach.

The contact times of disinfection cleaners in classical household applications are typically shorter than five minutes. But even a reduction of the contact time down to 30 seconds still showed a stable biocidal performance for our test solutions. The modified DIN EN 1040 test was also passed by a low concentration combination of 0.9% of lactic acid and 0.9% of SLES yielding bacteria reduction of 99.999% within half a minute.

To study the anti-microbial efficacy upon clean, 0.3 g/L bovine albumin, or dirty, 3.0 g/L bovine albumin, conditions, a test upon a modified DIN EN 1276 protocol was performed. Products passing this test might be applied as a sanitiser for food applications. Apart from the presence of bovine albumin, this suspension test is carried out analogously to the above described DIN EN 1040. We tested the lactic acid/SLES solutions against four common germs: *Pseudomonas aeruginosa* (DSM 939), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 10536) and *Enterococcus hirae* (ATCC 10541) at 20°C. To pass this disinfection test, at least a 99.999% or log 5 reduction of the gram positive and gram negative bacteria has to again be achieved. The contact time is five minutes.

Table 4: Performance of substances in a modified DIN EN 1276 test (five minutes contact time); colour represents pass (green; log >5 reduction) or fail (red) of the test

Log reduction (EN 1276)	P. aeruginosa	S. aureus	E. coli	E. hirae
Clean conditions (0.3 g/L BSA)				
2.9% Lactic Acid + 3.0% SLES	> 5	> 5	> 5	> 5
0.9% Lactic Acid + 0.9% SLES	> 5	> 5	> 5	> 5
Dirty conditions (3.0 g/L BSA)				
2.9% Lactic Acid + 3.0% SLES	> 5	> 5	> 5	> 5
0.9% Lactic Acid + 0.9% SLES	> 5	> 5	> 5	> 5

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This test protocol was successfully passed by the lactic acid and surfactant combinations (including one label free combination). Table 4 displays the two examined mixtures passing both the low and high bovine serum albumin contaminations of the suspension test.

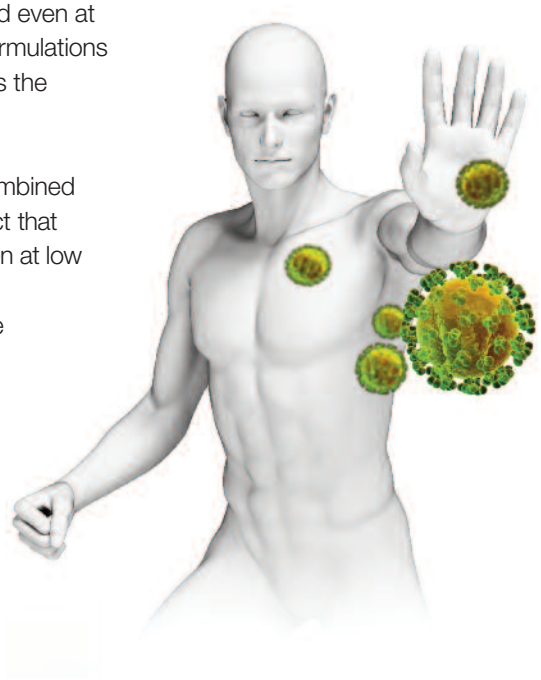
We can thus show that aqueous lactic acid/SLES solutions are highly effective bactericides – even at low concentrations and for demanding test conditions. Whether and how these findings may be transferred to complex formulations remains to be explored. However, first promising insights were gained and will eventually help to establish more natural, safe and sustainable biocidal products on the market.

Conclusion

We have investigated the biocidal performance of lactic acid and its mixtures with SLES according to established DIN EN standard protocols. Four common germs were selected to evaluate the anti-bacterial properties of the subset solutions. As a first result, we see that the sole use of lactic acid is only effective at higher concentrations beyond 5%. However, beside economic considerations regarding high amounts of active substance, such elevated levels of lactic acid entail the stricter corrosive labelling as required by CLP Regulation. Though this may not imply a hurdle to industrial or institutional applications, today's consumers are quite sensitive towards safety risks and hazardous labelling on end user products.

A potential solution to this issue is disclosed through the second major result following from our studies. While single substances (lactic acid, surfactant) only provide anti-bacterial action to a limited extent, the combination of both results in a drastic enhancement of the biocidal performance. Due to this favourable synergy, comprehensive disinfection claims (reducing 99.999% of all bacteria) are supported even at lactic acid concentrations down to 1% and below. This, in turn, means that end formulations may be developed bearing less strict abelling or even no labelling at all – as long as the effectivity of the lactic acid/surfactant mixture is still provided.

In summary, we have demonstrated that lactic acid is an efficient biocide when combined with the frequently used SLES. Fermentation derived lactic acid is a natural product that meets market and consumer demands for green, safe and sustainable solutions. Even at low concentrations, it is effective yet harmless and readily biodegradable. Furthermore, Jungbunzlauer's L(+)-lactic acid is an ECOCERT approved raw material for the use in detergents and personal care products. A claim of disinfection, safety and naturalness no longer has to be a contradiction.



Literature

[1] Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products
[2] Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures
[3] The Scientific Committee on Consumer Products, SCCP/1040/06 2006.
[4] Bundesinstitut für Risikobewertung, BfR, Stellungnahme 2006, Nr. 030/2006.
[5] K. L. Rule, V. R. Ebbett, P. J. Vikesland, Environ. Sci. Technol. 2005, 39, 3176–3185.
[6] R. U. Halden, Environ. Sci. Technol. 2014, 48, 3603–3611.

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About Jungbunzlauer

Jungbunzlauer is one of the world's leading producers of biodegradable ingredients of natural origin. We enable our customers to manufacture healthier, safer, tastier and more sustainable products. Due to continuous investments, state-of-the-art manufacturing processes and comprehensive quality management, we are able to assure outstanding product quality. Our mission "From nature to ingredients®" commits us to the protection of people and their environment.

Jungbunzlauer lactic acid for biocide applications is produced by fermentation of natural, renewable resources and is therefore a good alternative to synthetic chemicals used as antibacterial actives. It is efficient in removing bacteria when combined with surfactant and it is compatible with most other components in common formulations. Jungbunzlauer lactic acid is available as aqueous solution at different concentrations.

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