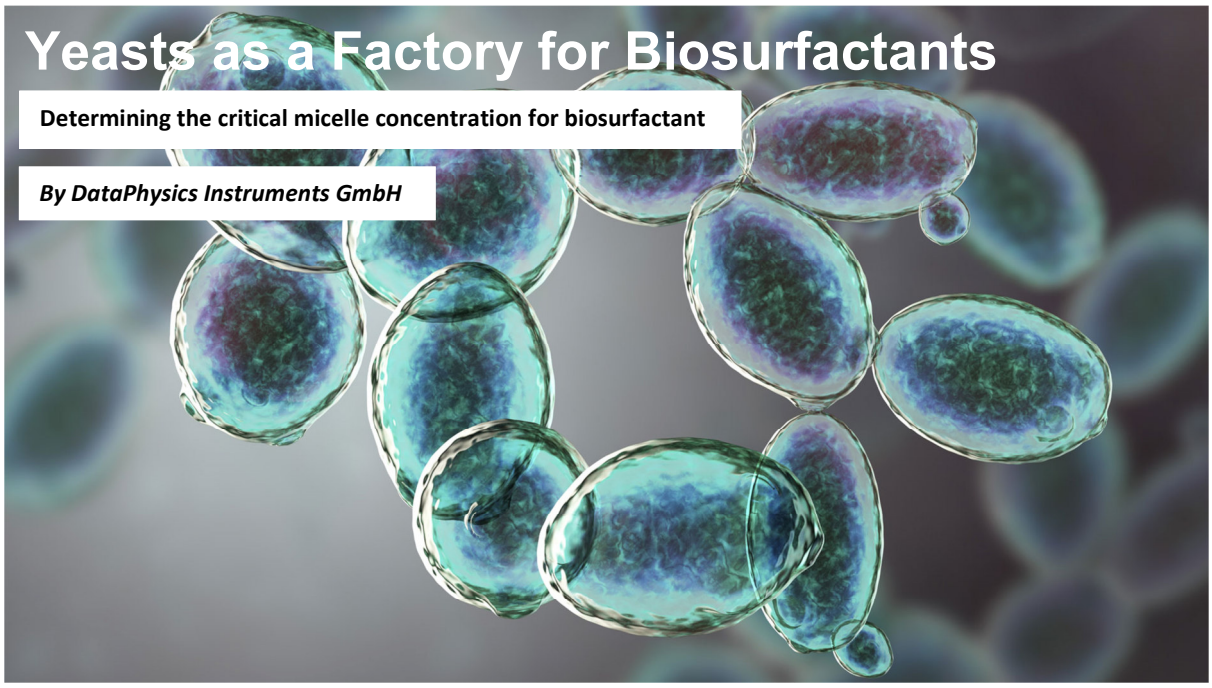
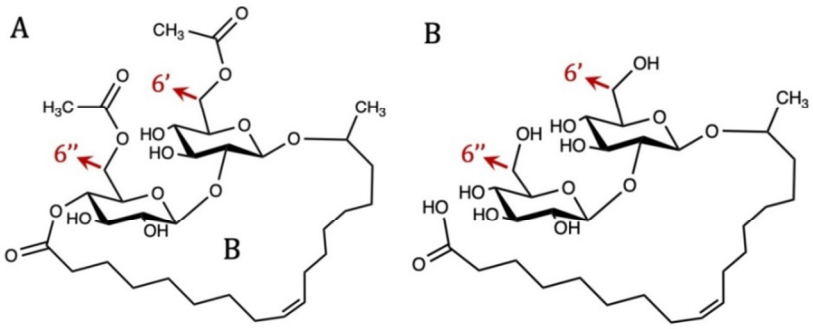
How CMC measurements can help characterize and evaluate new biosurfactants



Sophorolipids are glycolipids most commonly produced by a kind of yeast species *Starmerella bombicola*, including a hydrophobic fatty acid tail of 16 or 18 carbon atoms and a hydrophilic carbohydrate head comprising of sophorose, a glucose-derived di-saccharide. The sophorose motive can be acetylation at the 6'- and/or 6''- positions, resulting in a mixture of non-, mono-, and di-acetylated molecules (**Picture 1**).



Picture 1: *Sophorolipids produced by Starmerella bombicola; (A) diacetylated lactonic sophorolipid; (B) non-acetylated open-chain sophorolipid*

Employing sophorolipids leads to a strongly lowered surface-tension and antimicrobial activity making them attractive for various applications, for example as a renewable biosurfactant and in antimicrobial formulations. In 2015, scientists found a unique long-chain sophorolipid containing a 13-hydroxydocosanoic acid as the lipid tail (named 22:0-SL)

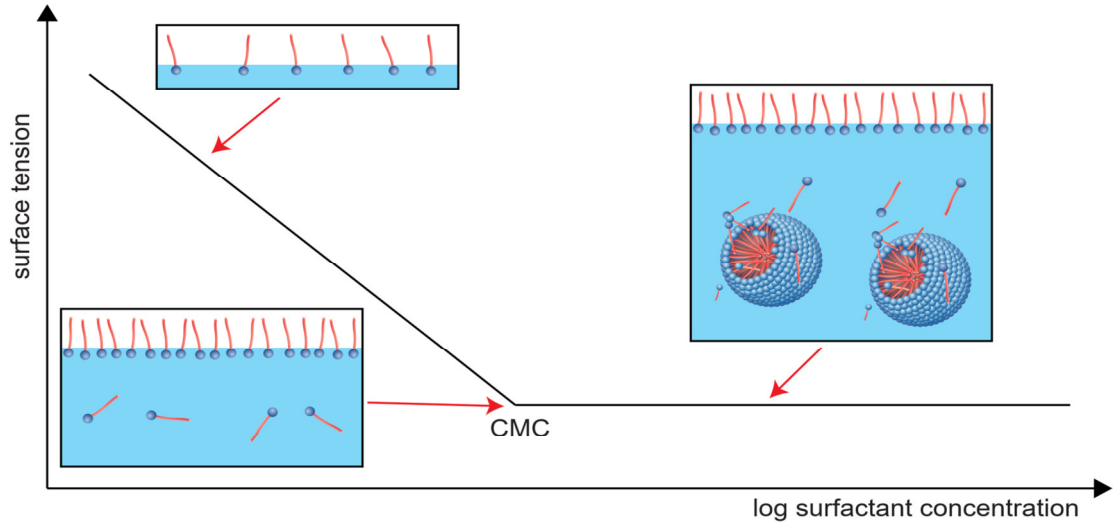
which is produced by the yeast *Pseudohyphozyma bogoriensis*. Due to a similar structure with two commercial oleochemicals (behenic acid and erucic acid), 22:0-SL has drawn extensive attention from scientists around the world. However, few studies focused on isolating and separating different 22:0-SL derivatives in order to study their interfacial chemistry until now. Recently, Solaiman et al. have proposed the first example of a convenient method (low-temperature crystallization) for isolating monoacetylated 22:0-SL from a raw mixture 22:0-SL and properly analyzing it regarding its interfacial chemical properties such as the critical micelle concentration (CMC).

The Critical Micelle Concentration (CMC)

The critical micelle concentration CMC is the **surfactant concentration above which micelles are formed**. It can be determined automatically for surfactant solutions by measuring the **surface tension at different concentrations**. Below the CMC the surface tension decreases with increasing surfactant concentration as the number of surfactant molecules at the interface increases. **Above the CMC**, in contrast, **the surface tension of the solution is constant** because the interfacial surfactant molecule concentration does not change any more.

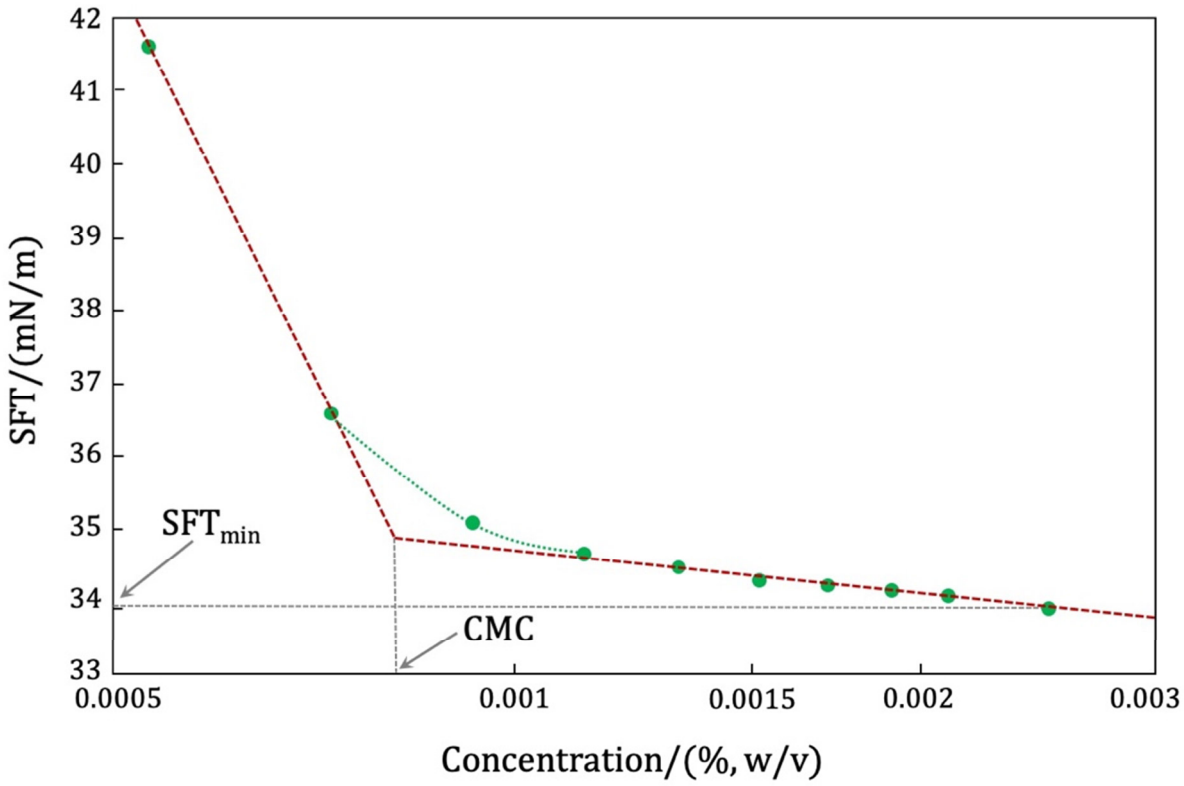
In a logarithmic representation of the surface tension versus the surfactant concentration there are two linear regimes below and above the CMC. An **extrapolation of respective regression** lines yields the CMC at the intersection. The **CMC can be determined automatically** with a Tensiometer of the DCAT series using a liquid dosing unit LDU 25.

DCAT 15 with LDU 25



Pure 6'-monoacetylated 22:0-SL was isolated from a mixture of non-, mono-, or/and di-acetylated 22:0-SL produced from *Pseudohyphozyma bogoriensis* by crystallization at low temperature (≤ −18 °C). High Pressure Liquid Chromatography (HPLC) and Liquid Chromatography/Quadrupole-Time of Flight Mass Spectroscopy (LC/Q-TOF-MS) data both indicate the successful separation, and specific component of two separated parts: "CRYSTAL" fraction composed of 6'-Ac_1-22:0-SL (100%) and "Hx-PRCP" fraction composed of 6', 6''-Ac_2-22:0-SL (89.2%); 6''-Ac_1-22:0-SL (6.6%); 6'-Ac_1-22:0-SL (4.2%).

The CRYSTAL and Hx-PRCP fractions were studied regarding their surface-active properties by using a DCAT 11 tensiometer from DataPhysics Instruments with automatic liquid-dosing unit LDU. They determined the minimum-surface-tension (SFT_{min}) and critical-micelle-concentration (CMC) values of different components by measuring the SFT values as a function of surfactant concentration (**Picture 2 and Table 1**).



Picture 2: *Determination of minimum-surface-tension (SFT_{min}) and critical-micelle-concentration (CMC)*

Table 1: *Minimum-surface-tension (SFT_{min}) and critical-micelle-concentration (CMC) of various Sophorolipids (SL)*

Chemical	MW (g/mol)	SFT_{min} (mN/m)	CMC[a]	
			mg/mL	mM
6'-Ac_1-22:0-SL[b]	723	34.6 ± 1.0	0.010	*0.014*
6', 6''-Ac_2-22:0-SL[c]	765	34.9 ± 1.0	0.014	*0.018*
6', 6''-Ac_2-16:0-SL[d]	662	35	> 0.2	*> 0.3*
6', 6''-Ac_2-18:0-SL[d]	690	35	0.035	*0.05*

[a] CMC values obtained by calculation using the indicated MW are shown in italics.
[b] "CRYSTAL" fraction composed of 6'-Ac_1-22:0-SL (100%)
[c] "Hx-PRCP" fraction composed of 6', 6''-Ac_2-22:0-SL (89.2%); 6''-Ac_1-22:0-SL (6.6%); 6'-Ac_1-22:0-SL (4.2%).
[d] Overwhelmingly lactonic (≥92%) form (Ashby et al., 2008)

The data show that the lengthening of the lipidic moiety increases the tail hydrophobicity (from 6', 6''-Ac_2-16:0-SL to 6', 6''-Ac_2-22:0-SL), thus decreasing the CMC values (from > 0.3 mM to 0.018 mM). However, sophorolipids with different chain-lengths and compositions all exhibited similar SFT_{min} values. Hence, compared to SFT_{min}, the CMC value is more responsive to the length of the hydrophobic tail. The antimicrobial activity of surfactants also depends on the CMC: below the CMC values, surfactants disperse in the solution and exert the antimicrobial function; above the CMC values, surfactants are engaged in the micelles and no longer available to participate in the microbial devastation.

Overall, the authors applied low-temperature crystallization method to preferentially separate 6'-Ac_1-22:0-SL from a mixture 22:0-SL for the first time. The experiments showed that the CMC values of sophorolipids were much affected by the length of the hydrophobic tail. Having goof antimicrobial activity and surface activity, 22:0-SL is a promising candidate to develop better bio-based antimicrobial surfactants for biomedical applications.

Dynamic Contact Angle measuring devices and Tensiometer (DCAT-11) equipped with a liquid-dosing unit (LDU) (both DataPhysics Instruments GmbH, Germany) were used in this research.

For more information, please refer to the following article:

Low-Temperature Crystallization for Separating Monoacetylated Long-Chain Sophorolipids: Characterization of Their Surface-Active and Antimicrobial Properties; Daniel K. Y. Solaiman, Richard D. Ashby, Alberto Nuñez, Nicole Crocker; *J. Surfact. Deterg.* **2020,** 23, 553-563; DOI: 10.1002/jsde.12396