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Relationship between shear energy input and sedimentation properties of exopolysaccharide-producing *Streptococcus thermophilus* strains

Florian Häffele[a,*], Susann Mende[b], Doris Jaros[b], Harald Rohm[b], Hermann Nirschl[a]

[a] Institute of Mechanical Process Engineering and Mechanics (MVM), Karlsruhe Institute of Technology (KIT), Straße am Forum 8, 76131 Karlsruhe, Germany
[b] Chair of Food Engineering, Institute of Natural Materials Technology, Technische Universität Dresden (TUD), Bergstraße 120, 01062 Dresden, Germany

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ABSTRACT

Besides fermentation, the production of bacterial starter cultures includes another crucial step, namely, the separation of the bacteria cells. This separation is most commonly carried out with disc stack separators and needs to be adjusted to the respective strain to obtain a high cell recovery rate. Exopolysaccharides (EPS) produced by several starter cultures, however, have a large negative impact on the separation properties of the cells. These EPS can be divided into cell-bound capsular EPS or free EPS that are released into the surrounding fermentation medium. To improve the separation step, shear forces were applied after fermentation with a gear ring disperser to simulate the impact of a homogenizer and the influence on the separation properties of six *Streptococcus thermophilus* strains was examined. In case of capsular EPS, the sedimentation velocity of the bacteria increased due to shearing off the capsular EPS layer. Shearing media with free EPS resulted in a viscosity decrease and, hence, in a higher sedimentation velocity, as was determined using a disc centrifuge and a LUMiSizer. Sediment compression as measured with the LUMiSizer was also affected by the shearing step. The results of this study suggest that a defined shear treatment of EPS producing bacterial starter cultures leads to improved separation properties and, hence, higher bacteria yields. We assume that both EPS types affect separation efficiency of the bacteria cells, free EPS because of increased media viscosity and capsular EPS because they act like a friction pad.

1. Introduction

The importance of starter cultures that are able to produce exopolysaccharides (EPS), for example several species of lactic acid bacteria (LAB), has significantly increased in recent years. In case of fermented dairy products, EPS influence product viscosity and mouthfeel. In case of yogurt and related products, LAB utilize lactose and produce lactic acid. A consequence of the decreasing pH is that the main milk protein fraction, the caseins, change their state and aggregate, such that the viscosity increases drastically [1,2]. This effect is enhanced when the respective bacteria are able to produce EPS during fermentation. EPS are long-chained polysaccharides that consist of either identical monosaccharides (homo-polysaccharide) or different monosaccharides (hetero-polysaccharide), such as glucose, galactose, or rhamnose [3,4]. Due to their high water-binding capacity, EPS have a large influence on the viscosity of the entire system. For instance some EPS are very ropy and able to interact with each other or the cells in the medium leading to an increase in viscosity. Examples of EPS that are, in isolated form, used in the food and pharmaceutical industries are dextran, a homopolysaccharide from *Leuconostoc mesenteroides*, or xanthan, a heteropolysaccharide from *Xanthomonas campestris* [5]. Furthermore, it is important to know that EPS produced by LAB are either attached to the cell wall (therefore denoted as capsular EPS) or released into the surrounding medium (free EPS). The capability of producing either EPS type is strain-dependent, a combination of both EPS types is also possible [6].

Industrial production of starter cultures consists of two main process steps, fermentation under specific conditions, such as pH, temperature, oxygen availability, and nutrient content, and subsequent separation of the bacteria from the fermentation broth, which is usually done with disc stack separators. Here, the influence of EPS on the viscosity of the fermentation broth makes the separation step difficult. Another complicating factor is that different starter cultures are usually produced in the same manufacturing line, which is why separation conditions should be adjusted to the respective strain. Both EPS characteristics and type (free, capsular or both) have a specific influence on the viscosity of the fermentation broth and, hence, on the sedimentation properties of the bacteria. Empirical observations indicate that the application of a

* Corresponding author.
E-mail address: florian.haeffele@kit.edu (F. Häffele).

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Table 1
Capsular EPS production and average cell chain length of unsheared Streptococcus thermophilus strains.

Strain	Presence of capsular EPS	Cocci per cell chain
ST-C	+	1–5
ST-D	−	2–19
ST-E	−	1–4
ST-G	+	1–8
ST-H	+	1–6
ST-I	+	1–5

homogenization step before cell separation results in improved sedimentation properties of the cultures, but also that this improvement depends on the EPS type produced. The aim of this study is to systematically investigate the effect of a homogenization step on the separation properties of EPS producing lactic acid bacteria.

2. Materials and methods

2.1. Materials

Six different *Streptococcus thermophilus* (ST) strains dispersed in fermentation broth were investigated. The samples were delivered as frozen pellets by Chr. Hansen A/S (Horsholm, Denmark), stored at −20 °C and, before use, defrosted overnight in a refrigerator. The strains differed in average cell chain length and in their ability to produce capsular EPS (Table 1).

2.2. Shear treatment

A T25 Ultra-Turrax (UT) gear ring disperser (IKA-Werke GmbH & Co. KG, Staufen, Germany) was used for specific shearing of the ST-containing fermentation media. 30 ml of the sample were filled into a test tube (h = 200 mm, d_i = 28 mm) and sheared at either 5,000, 11,000, 19,000, or 24,000 rpm. At each stirring speed, samples were taken (about 0.3 ml with a syringe) after 10 s, 20 s, 30 s, 60 s, and 120 s of shearing.

2.3. Sedimentation velocity analysis (LUMiSizer)

The sedimentation velocity distribution of unsheared and sheared samples was measured with an optical analytical centrifuge (LUMiSizer, LUM GmbH, Berlin, Germany). This centrifuge allows the simultaneous measurement of twelve samples using a rectangular polycarbonate cuvette (2 × 8 mm base area) for each sample. The cuvettes are inserted into the LUMiSizer in horizontal position and irradiated lengthwise with parallel NIR light. The NIR light transmitted through the cuvette is detected with a CCD sensor. The resulting time and place resolved transmission profiles are then evaluated by using the STEP technology (Space and Time resolved Extinction Profiles) and provide information on the sedimentation properties of the samples. More information regarding the working principle of the LUMiSizer and the STEP technology can be found in [7–9].

The measurements were carried out at a rotational speed of 3600 rpm, which corresponds to a mean relative centrifugal force (rcf) of 1720*g* (mean distance from rotor was 120 mm). Approximately 0.3 ml diluted sample (ratio 1:2 with physiological NaCl solution) were filled into each cuvette. The sedimentation velocity distribution was calculated with the SEPVIEW program using the constant position method. For this, the mean transmission (averaged over ± 0.5 mm relating to the radial position) at three radial positions close to the bottom of the cells (123, 125, and 127 mm) was used. For each sample, two measurements were carried out.

2.4. Sedimentation velocity analysis (Disc Centrifuge)

The sedimentation velocity distribution of the unsheared and sheared samples was also measured with a DC24000 disc centrifuge (CPS Instruments Europe, Oosterhout, Netherlands). Here, the sample is injected centrally into a stirring vertical disc and deposited through a density-gradient with known viscosity and density. After a certain time, the particles pass a laser beam at the outer edge of the pellucid disc and the absorption-time spectrum of the sample is measured. By calibrating the centrifuge with a calibration standard with known particle size and sedimentation behavior, the absorption-time spectrum of the sample can be transferred to the sedimentation velocity distribution. More detailed information regarding the working principle of the disc centrifuge can be found in [10].

The stirring speed was set to 5850 rpm, which corresponds to rcf = 1720*g*. The measuring range was set from 400 nm to 5 µm and, because of the highly concentrated sample, a dilution with physiological NaCl solution (ratio 1:20) was necessary. As a density gradient, a combination of a 3 and a 7% (w/w) sucrose solution was used. Therefore 1.6 ml of the 7% (w/w) sucrose solution was injected into the disc followed by a combination of 1.4 ml of the 7% (w/w) and 0.2 ml of the 3% (w/w) sucrose solution. The next step included 1.2 ml (7%) and 0.4 ml (3%) and so forth. At the end solely 1.6 ml of the 3% (w/w) sucrose solution was injected. The density gradient was prevented from evaporating by adding 0.5 ml dodecane, followed by the recommended 15 min equilibration time. The PVC calibration standard had an average particle size of 0.483 µm (PVC Calibration Standard: Lot 149 - CPS Instruments Europe, Oosterhout, Netherlands) and was dispersed in deionized water. The injected sample (and calibration) volume was 0.1 ml. One calibration run was used as the basis for eight measurements.

2.5. Assessment of energy input

The energy input from the shear treatment with the Ultra-Turrax was approximated according to [11,12]. The Reynolds number Re (-) near the rotor was calculated by

$$Re = \frac{n \cdot d_R^2 \cdot \varphi_l}{\eta_l} \quad (1)$$

where n (1/s) is the rotational speed, d_R (m) stirrer diameter, ρ_l (kg/m^3) fluid density, and η_l (kg/m.s) apparent viscosity of the cell containing fermentation broth. As stated by [12,13], the flow pattern generated by the Ultra-Turrax is similar to that caused by a disc stirrer. Zlokarnik [11] characterized many different types of stirrers, including the disc stirrer, and plotted a typical dependency of Newton number on the Reynolds number for each stirrer. With this characteristic, it is possible to assign a Newton number Ne (-) to the calculated Reynolds number. Consequently, the input performance of the Ultra-Turrax is calculated by:

$$P = Ne \cdot n^3 \cdot d_R^5 \cdot \varphi_l \quad (2)$$

Finally, the volume-specific energy input E_V (J/m^3) due to shearing can be calculated:

$$E_V = \frac{P \cdot t}{V} \quad (3)$$

2.6. Sediment compression analysis

Sediment compression of the samples was measured with the LUMiSizer. The cuvettes were filled with approximately 2 ml undiluted sample. LUMiSizer speed was varied between 1500 rpm and 4000 rpm in steps of 500 rpm. As the maximum sedimentation velocity of the cultures is a few hundred µm/s only, the measurements were run at least overnight and at 1500 rpm for up to 20 h (+30 min for each