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Scale-up of *Streptomyces* species cultivations based on the morphological response to the energy dissipation rate

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Abstract

Filamentous microorganisms exhibit complex morphologies that influence product formation and are affected by various bioprocess parameters. Consistent morphology is therefore essential for comparable results during scale-up. This study investigates the scale-up of *Streptomyces* species (*Streptomyces* spp.) cultivations from shake flasks to stirred-tank reactors while maintaining comparable morphology. Shake flask cultivations demonstrated that variations in hydromechanical stress, quantified by the energy dissipation rate, strongly affect the morphology of *Streptomyces* spp., confirming its relevance for scale-up. A scale-up based on the average energy dissipation rate led to altered morphology. Alternatively, the maximum energy dissipation rate was investigated, as it reflects hydromechanical stress more accurately. Since prior determination or measurement is unfeasible, computational fluid dynamics (CFD) simulations were applied for systematic quantification. Scale-up based on these simulations resulted in improved morphological consistency across scales, suggesting a new scale-up parameter. To overcome oxygen limitations, pure oxygen aeration was implemented, further enhancing morphological comparability.

KEYWORDS

computational fluid dynamics simulation, filamentous microorganisms, hydromechanical stress, maximum energy dissipation rate, morphology

Abbreviations: CFD, computational fluid dynamics; CTR, carbon dioxide transfer rate; HPLC, high-performance liquid chromatography; OTR, oxygen transfer rate; RAMOS, respiratory activity monitoring system; *S. albobacillus*, *Streptomyces albobacillus*; *S. bobilli*, *Streptomyces bobilli*; *S. coeruleorubidus*, *Streptomyces coeruleorubidus*; *Streptomyces* spp., *Streptomyces* species.

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1 | INTRODUCTION

Streptomyces spp. are the largest genus within the Actinomycetota, comprising over 700 described species.^{1,2} These gram-positive, aerobic bacteria are ubiquitous in soil environments.³ Due to their extensive genomic capacity and metabolic versatility, *Streptomyces* spp. exhibit a broad range of secondary metabolites^{2,3} including pigments,⁴ siderophores,⁵ and most notably, numerous pharmaceutically relevant products.^{3,6} This remarkable metabolic versatility makes *Streptomyces* spp. one of the most promising bacterial genera for application in biotechnology.

In contrast to most bacteria, *Streptomyces* spp. do not proliferate by binary cell division into coccoid or rod-shaped cells, but rather exhibit a complex, filamentous growth resembling that of fungi.^{7,8} Their life cycle begins with the germination of spores, from which germ tubes emerge and differentiate into a branching network of hyphae. Hyphal elongation occurs exclusively at the apical tips, leading to the formation of a vegetative mycelium. Under specific, often nutrient-limiting conditions, an aerial mycelium develops, which eventually differentiates into chains of spores.^{7,9} The macroscopic and microscopic morphology of filamentous microorganisms is highly variable and influenced by both genetic and environmental factors. Depending on the species and cultivation conditions, growth in submerged cultivations may occur as dispersed mycelia, as compact pellets, or as intermediate morphologies such as clumps.¹⁰ Several bioprocess parameters critically affect the morphology of filamentous microorganisms. These include the composition of the medium,¹¹ as well as osmolality,¹² pH value,¹³ temperature,¹⁴ the presence of additives such as talc,⁸ or the inoculum concentration.¹⁴ Furthermore, process conditions such as oxygen availability and hydromechanical stress have a significant impact on morphology.^{14,15} The two major types of morphologies, pelleted and dispersed, exhibit specific advantages and disadvantages in biotechnological processes but there is no universally optimal morphology since the preferred growth form is highly product- and process-specific. However, it is well established that morphology has a profound influence on microbial productivity,^{11,14,16} affecting not only the product formation and secretion but also the extent of posttranslational modifications.¹⁷ Therefore, consistent and precise control of morphology is essential to ensure reproducibility, scalability, and overall performance of microbial processes.

In bioprocess development, shake flasks are widely used due to their low operational effort and high throughput.¹⁸ At industrial scale, however, cultivations are conducted in bioreactors, with the stirred-tank reactor having become the predominant system, accounting for approximately 90% of all conducted bioprocesses.¹⁵ Scale-up from shake flasks to stirred-tank reactors is challenging due to the transition from a laminar flow regime in shaken systems¹⁹ to a turbulent flow regime in stirred systems,²⁰ resulting in different hydrodynamics. This difference affects critical process conditions, including oxygen availability, mixing time, and hydromechanical stress.^{15,21,22} These parameters are also frequently used as scale-up parameters, although the choice of an appropriate scale-up parameter varies depending on the specific process.

Hydromechanical stress represents a relevant scale-up parameter for bioprocesses involving shear-sensitive organisms, such as animal cell

cultures and filamentous microorganisms. The latter, including *Streptomyces* spp., are particularly sensitive due to their complex morphology.^{10,14} Hydromechanical stress refers to the mechanical forces acting on cells as a result of fluid movement, arising from flow-induced shear, turbulence, mixing, and aeration.^{20,23} A prevalent hypothesis to quantify hydromechanical stress attributes cell damage to eddies similar in size to the cells,²⁴ with the smallest eddies calculable via Kolmogorov's theory.²⁵ Given the larger cell size of filamentous microorganisms compared to unicellular organisms, this explains their sensitivity. For a more quantitative assessment, the volumetric power input or the energy dissipation rate is commonly used.^{19,26} In most studies, the average volumetric power input or average energy dissipation rate is used,^{27,28} representing an average value over the liquid phase. However, these average values do not account for spatial heterogeneities^{29,30} and are inadequate for systems with significant local fluctuations, such as stirred-tank reactors. This limitation is particularly critical in cultivations with shear-sensitive organisms,¹⁹ like filamentous microorganisms, where local peaks in hydromechanical stress can affect morphology. Thus, the maximum power input, the maximum energy dissipation rate, or the quotient of maximum and average values provide a more accurate measure of hydromechanical stress.²⁰

Although the maximum energy dissipation rate or its ratio to the average is useful for quantifying hydromechanical stress, reported values in literature for the ratio vary widely due to process-specific factors, but also due to the lack of determination.³¹ Aeration further complicates the issue by significantly affecting energy dissipation rates.³² While a few empirical correlations exist,²⁰ they are system-specific and not applicable to the reactor studied here. Consequently, estimating the maximum energy dissipation rate remains uncertain. Nowadays, computational fluid dynamics (CFD) simulations enable a detailed analysis of hydromechanical conditions and can be applied to quantify energy dissipation rates.^{29,33} Eslahpazir Esfandabadi et al. demonstrated this by using CFD-based modeling to more accurately describe hydromechanical stress in stirred-tank reactors during *Aspergillus niger* cultivations.³⁴ More recently, Zhou et al. developed a CFD model for *Streptomyces atratus* in a 5 L stirred-tank reactor, focusing primarily on the dynamic changes of the broth viscosity during cultivation due to biomass growth and its effects on the production of ilamycin E, while also incorporating aspects of energy input.³⁵ CFD simulations have also been conducted for shake flasks, including analyses of energy dissipation rates, by both Dinter et al.³⁶ and van Valderen et al.³⁷ In both cases, the results were consistent with the empirical data reported by Büchs et al. in 2000. Despite their potential, CFD simulations require considerable expertise, computational resources, and effort to be implemented effectively, which is why their application in industrial process development remains limited.

Although various *Streptomyces* spp. have been extensively studied, systematic and quantitative scale-up approaches remain limited. Many review and research articles highlight hydromechanical stress as a key parameter for scaling up filamentous microorganisms, including *Streptomyces* spp. However, this knowledge is rarely implemented in practice, likely due to the complexity in its determination.

Power input and energy dissipation rate are often referenced, but usually only as average values. For example, Zhou et al. reported the influence of stirrer speed on *Streptomyces kanasensis*, but based their scale-up primarily on oxygen availability.³⁸ Gamboa-Suasnavart et al. studied the scale-up of *Streptomyces lividans* using average volumetric power input and observed morphological differences between shake flasks and stirred-tank reactors, which were not further addressed.^{17,39}

This study aims to develop a scale-up strategy for *Streptomyces* spp. from shake flasks to stirred-tank reactor. The energy dissipation rate was selected as the scale-up parameter, as the literature shows that it quantifies hydromechanical stress imposed on filamentous microorganisms, which are particularly sensitive to it.^{23,40} Both average and maximum values are evaluated, and two novel stirrers are employed to modify their ratio. Additionally, simulations for the stirred-tank reactor are carried out to develop a deeper and more quantitative understanding of the process. The insights gained serve as a valuable basis for subsequent cultivations, with the goal of achieving comparable morphology of various *Streptomyces* spp. in both scales.

2 | MATERIALS AND METHODS

2.1 | Microorganisms

This study involved three different *Streptomyces* spp. obtained from the culture collection of the Leibniz Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institute (Jena, Germany), specifically *Streptomyces coeruleorubidus* DSMZ 40145 (*S. coeruleorubidus*), *Streptomyces albobacillus* DSMZ 20368 (*S. albobacillus*), and *Streptomyces bobili* DSMZ 40481 (*S. bobili*), which were previously identified as *Streptomyces galilaeus*.⁴¹

Spore solutions were prepared similarly to Hobbs et al.⁴² and Finger et al.⁴ Briefly, a spore solution was spread on agar plates, consisting of 20 g L⁻¹ soy flour (Reformhaus, Zarentin, Germany), 20 g L⁻¹ mannitol, and 20 g L⁻¹ agar (both from Carl Roth GmbH + Co. KG, Karlsruhe, Germany), and incubated at 30°C for 7 to 10 days, depending on the species. Afterward, the spores were harvested by scraping off and suspending them in deionized water. The mycelium was removed by filtration with a 40 µm cut-off cell strainer (pluriSelect Life Science, Leipzig, Germany). The spore concentration was measured using a particle sizer (Multisizer 4e, Beckmann Coulter, Brea, USA) and adjusted to 10⁸ mL⁻¹. The spores were stored at -80°C in 25% glycerol (Carl Roth GmbH + Co. KG, Karlsruhe, Germany).

2.2 | Medium composition

Medium composition was adapted from Pakula medium⁴³, as previously described.⁴⁴ The used chemicals were sourced from Carl Roth GmbH + Co. KG (Karlsruhe, Germany), Merck KGaA

(Darmstadt, Germany), or VWR International (Radnor, USA). For all cultivations, the medium consists of 15 g L⁻¹ glucose, 0.1 M 2 (-N-morpholino)-ethanesulfonic acid, 7.6 g L⁻¹ (NH₄)₂SO₄, 2.6 g L⁻¹ KH₂PO₄, 0.50 g L⁻¹ MgSO₄ · 7H₂O, 0.45 g L⁻¹ citric acid, 0.30 g L⁻¹ urea, 0.23 g L⁻¹ CaCl₂ · 2H₂O, 0.05 g L⁻¹ NaCl, 0.04 g L⁻¹ ZnSO₄ · 7H₂O, 8.0 mg L⁻¹ CuSO₄ · 5H₂O, 6.8 mg L⁻¹ CoCl₂ · 6H₂O, 6.0 mg L⁻¹ Fe₂(SO₄)₃ · H₂O, 4.0 mg L⁻¹ MnSO₄ · 7H₂O, 2.0 mg L⁻¹ H₃BO₃, and 0.01% v/v Tween 80. The pH value of the medium was set to 6.7 by adding 5 M NaOH, and Tween 80 was added after pH value adjustment. Subsequently, the medium was sterilized by filtration through a 0.22 µm cut-off filter (Millipore, Merck KGaA, Darmstadt, Germany). The medium was stored as two-fold concentrated stock at room temperature, with glucose added freshly just before the experiment.

2.3 | Precultures

Precultures were prepared by inoculating modified Pakula medium, see Section 2.2, with 10⁶ mL⁻¹ spores and cultivated in unbaffled 250 mL shake flasks containing 20 mL of medium. The further cultivation conditions were set to a temperature of 30°C, a humidity of 80%, a shaking frequency of 350 rpm, and a shaking diameter of 50 mm (Climo-Shaker ISF1-X, Adolf Kühner AG, Birsfelden, Switzerland). To prevent pellet formation and achieve a higher homogeneity, 600 mg of glass beads with a diameter of 2.7 ± 0.27 mm (BioSpec Products, Bartlesville, USA) were added to each flask⁴⁵. The precultures were incubated for 3 days, after which cells were harvested by centrifugation at 4000 rpm for 10 min (Rotina 35R, Andreas Hettich GmbH, Tuttlingen, Germany). The supernatant was discarded, and the cell pellet was resuspended in deionized water.

2.4 | Main cultures

Main cultures were conducted in modified Pakula medium, see Section 2.2, and inoculated with cells from the preculture. The optical density at the beginning of the cultivation (OD_{start}), measured at 600 nm using a photometer (Genesys 20, Thermo Fisher Scientific, Darmstadt, Germany), was set to 0.2.

Shake flask cultivations were conducted in unbaffled 250 mL shake flasks with a filling volume of 20 mL. The temperature was set to 30°C, the shaking frequency to 350 rpm, and the shaking diameter to 50 mm (Climo-Shaker ISF1-X, Adolf Kühner AG, Birsfelden, Switzerland). An in-house built respiratory activity monitoring system (RAMOS) was used to monitor the oxygen transfer rate (OTR) during the cultivations.⁴⁶ All shake flask cultivations were conducted in biological duplicates or triplicates.

Cultivations in bioreactors were performed in a 2 L stirred-tank reactor (Biostat, Sartorius, Göttingen, Germany), which was equipped with four baffles and one stirrer. Three different stirrers were used, one standard six-bladed Rushton turbine (diameter 52.6 mm, blade height 10.5 mm, blade width 14.3 mm) and two newly designed ones (small: diameter:

34 mm, blade height: 6.8 mm, blade width: 8.5 mm; large: diameter: 80 mm, blade height: 16 mm, blade width: 20 mm), see Supporting Information S1: Supplement 5. OTR and carbon dioxide transfer rate (CTR) were determined from the exhaust gas analysis (DASGIP GA4, Eppendorf SE, Hamburg, Germany). To keep the conditions as comparable as possible to those in the shake flask, the pH value was measured during cultivations (EasyFerm Plus K8 225, Hamilton, Hoechst, Germany) but not controlled. Similarly, the dissolved oxygen tension was measured (VisiFerm DO 225, Hamilton, Hoechst, Germany) but not controlled. The filling volume was 1 L, and the temperature was controlled at 30°C. Unless otherwise specified, aeration was performed with air. The aeration rate was kept constant at 60 L h⁻¹, corresponding to 1 vvm, and the stirrer speed was kept constant. The values used for the stirrer speed in the different cultivations are explained in more detail in Section 3.3. To prevent foam formation, 0.1 mL of antifoam (Plurafac LF 1300, BASF, Ludwigshafen, Germany) was added at the start and, if needed, during cultivations. Bioreactor cultivations were performed once due to the high workload.

2.5 | Offline measurements

During the cultivations, samples were taken at regular time intervals and analyzed using the following offline measurement methods.

For morphology documentation, microscopic images of the culture broth were taken with a microscope (Nikon Eclipse E600, Nikon Corporation, Tokyo, Japan), equipped with a camera (Sony α 6000, Nikon Corporation, Tokyo, Japan) with a 10× objective, providing a total magnification of 100×. Pellet diameter was measured with an objective micrometer (2 mm scale, 0.01 mm divisions; Ernst Leitz Wetzlar GmbH, Wetzlar, Germany).

The pH value was additionally measured offline with a pH meter (InLab Solids, Mettler Toledo, Columbus, USA; HI 221, Hanna Instruments, Thermo Fisher Scientific Inc., Schwerte, Germany).

To determine the cell dry weight gravimetrically, 1 mL of culture broth was added to a pre-weighed reaction vessel, centrifuged for 10 min at 14,000 rpm (Sigma 1-15PK, Sigma Laborzentrifugen GmbH, Osterode, Germany), and the supernatant was removed. The reaction vessel containing the cell pellet was dried at 80°C for 48 h and weighed again to determine the cell dry weight from the weight difference.

The glucose concentration was quantified using high-performance liquid chromatography (HPLC). The supernatant after centrifugation for the determination of the cell dry weight was sterile filtered using a 0.2 µm cut-off filter (VWR International, Radnor, USA) and stored at -20°C until the measurement. HPLC analysis was performed with a Thermo Fisher Ultimate 3000 (Thermo Fisher Scientific Inc., Waltham, USA), equipped with an ERC RefractoMax 520 detector (IDEX Health & Science LLC, Oak Harbor, USA) and a Rezex ROA-Organic Acid column (300 × 7.8 mm, Phenomenex, Torrance, USA). The flow rate of the mobile phase, 25 mM H₂SO₄ (Carl Roth GmbH + Co. KG, Karlsruhe, Germany), was set to 0.8 mL min⁻¹ with a column temperature of 85°C.

2.6 | Calculations

The volumetric power input (P/V) and the energy dissipation rate ε are mathematically related through the liquid density ρ , see Equation (1).

$$\varepsilon = \frac{1}{\rho} \times \left(\frac{P}{V} \right). \quad (1)$$

Assuming a constant Newton number Ne , defined in Equation (2),⁴⁷ in the turbulent flow regime, it can be used to obtain an expression for the average energy dissipation ε_θ , see Equation (3). It includes the power input P , the stirrer speed n , the stirrer diameter d , the liquid volume V , and the liquid density.

$$Ne = \frac{P}{\rho \times n^3 \times d^5}. \quad (2)$$

$$\varepsilon_\theta = Ne \times \frac{n^3 \times d^5}{V}. \quad (3)$$

The Newton number depends on the flow regime within the reactor. To assess the flow regime, the Reynolds number Re is used, see Equation (4), also including the dynamic viscosity η . In the turbulent regime, typically defined as $Re > 10^4$, a constant Newton number of 4.9 is usually observed for stirred-tank reactors equipped with a six-bladed Rushton turbine.^{47,48}

$$Re = \frac{n \times d^2 \times \rho}{\eta}. \quad (4)$$

In biological systems, stirred-tank reactors are typically operated with aeration, which affects the volumetric power input and the energy dissipation rate in the system. For processes with one stirrer, this influence is accounted for by using Equation (5), which includes the gravitational acceleration (g) and the superficial gas velocity v_G . This velocity is calculated from the ratio of the volumetric gas flow rate Q_G to the reactor cross-sectional area A_R , as shown in Equation (6).⁴⁹ This equation was selected because, in contrast to other literature correlations,^{50–52} it was derived from experiments conducted in both water and higher-viscosity media, thereby better reflecting the properties of actual fermentation broths more accurately.

$$\frac{(P/V)_{\theta, \text{aerated}}}{(P/V)_{\theta, \text{unaerated}}} = \frac{\varepsilon_{\theta, \text{aerated}}}{\varepsilon_{\theta, \text{unaerated}}} = \frac{1}{\sqrt{1 + 750 \times (v_G / \sqrt{g \times d})}}. \quad (5)$$

$$v_G = \frac{Q_G}{A_R}. \quad (6)$$

For shake flasks, based on the assumptions of Clark and Vermeulen,⁵³ a modified Newton number Ne' was introduced, see Equation (7).⁵⁴ Since it is a shaken system, the shaking frequency n_s and the inner flask diameter d_0 are included in the calculation.

$$Ne' = \frac{P}{\rho \times n_s^3 \times d_0^4 \times V^{1/3}}. \quad (7)$$

This leads to expressions for the average energy dissipation rate, as shown in Equation (8).

$$\varepsilon_\theta = Ne' \times \frac{n_s^3 \times d_0^4}{V^{2/3}}. \quad (8)$$

For estimating the modified Newton number, Equation (9), empirically derived by Büchs et al., is used.⁵⁵ The Reynolds number for shake flasks is calculated analogously to the stirred-tank reactor with adjusted input parameters, according to Equation (10).⁵⁴

$$Ne' = 70 \times Re^{-1} + 25 \times Re^{-0.6} + 1.5 \times Re^{-0.2}. \quad (9)$$

$$Re = \frac{n_s \times d_0^2 \times \rho}{\eta}. \quad (10)$$

The turbulent regime in shake flasks is reached at Reynolds numbers above 60,000.¹⁹ In contrast to stirred-tank reactors, shake flasks operate at substantially lower Reynolds numbers and predominantly in the laminar flow regime. Under these conditions, the energy dissipation rate is distributed uniformly across the liquid phase, such that the average energy dissipation rate can be considered approximately equivalent to the maximum energy dissipation rate $\varepsilon_{\max}$, as previously reported in literature.⁴⁰ Therefore, Equation (8) applies to calculate the maximum energy dissipation rate. In the turbulent regime, the maximum energy dissipation rate in shake flasks can be calculated using Equation (11) based on the liquid height h and the liquid velocity at the shake flask wall u . Peter et al. empirically derived an expression for the liquid height that depends on the Froude number Fr , given in Equations (12) or (13),¹⁹ also including the shaking diameter d_s .

$$\varepsilon_{\max} = 0.1 \times \frac{u^3}{h} = 0.1 \times \frac{(\pi \times n_s \times d_0)^3}{h}. \quad (11)$$

$$Fr > 0.4 : h = 1.11 \times d_s^{0.18} \times d_0^{-0.11} \times n_s^{0.44} \times V^{0.34}. \quad (12)$$

$$Fr < 0.4 : h = 1.31 \times d_s^{0.28} \times d_0^{0.02} \times n_s^{0.9} \times V^{0.35}. \quad (13)$$

The Froude number is calculated according to Equation (14).¹⁹

$$Fr = \frac{(2 \times \pi \times n_s) \times d_s^2}{2 \times g}. \quad (14)$$

2.7 | Simulations

Simulations were performed using the commercially available CFD software from SimVantage GmbH (<https://simvantage.com/>), which uses the lattice Boltzmann method to calculate the fluid flow field and

a Lagrangian Parcel Model to calculate the gas-phase dynamics. Turbulence is modeled via large eddy simulation, where the Smagorinsky-Lilly Model was used with a Smagorinsky constant of 0.16. Each simulation starts from resting fluid and simulates until a quasi-steady state is reached. More details on the implementation and additional validation of the solver used in this work can be found in previous publications.^{56–59} A spatial resolution of 1.182 mm per grid node was chosen, which was deemed sufficient to recover the flow field and power characteristics. The temporal resolution was defined via a Courant number of 0.05 at the stirrer blade tips. This simulation setup is compared to literature data in terms of power input for Rushton Disc Turbines across all simulated operating ranges. A detailed analysis of the results is found in Supporting Information S1: Supplements 11 and 12. The steady-state power characteristics show excellent agreement across the whole operating range including flooded conditions in low stirred operating conditions. The reactor dimensions, including its components, as well as the process parameters set during the cultivations, see Section 2.4, were entered into the software. Since the liquid density, dynamic viscosity, and surface tension of the medium were also needed as input parameters, they were measured using a density viscometer (DMA 4100M, Anton Paar GmbH, Graz, Austria) and a drop volume tensiometer (DVT50, Krüss GmbH, Hamburg, Germany), with the temperature set to 30°C during the measurements. The simulated time range was chosen to include at least 50 stirrer rotations for each simulation case. Complete three-dimensional output data was collected during the transient simulation every second. Stirrer speeds ranging from 100 to 1000 rpm were simulated for the three stirrers used. The post-processing analysis was performed using the software ParaView (Version 5.11.0., Sandia National Laboratories, Kitware, Los Alamos National Laboratory). The calculation of the different energy dissipation rates is carried out according to the following Equations (15–20):

Average energy dissipation rate ε_θ :

$$\varepsilon_\theta = \int_0^\infty \varepsilon \times \frac{p(\varepsilon)}{100} d\varepsilon. \quad (15)$$

Median of energy dissipation rate $\varepsilon_{50\%}$:

$$\int_0^{\varepsilon_{50\%}} \frac{p(\varepsilon)}{100} d\varepsilon = 0.5. \quad (16)$$

Maximum energy dissipation rate $\varepsilon_{\max}$:

$$\varepsilon_{\max} = \max\{\varepsilon_1, \varepsilon_2, \dots, \varepsilon_n\}. \quad (17)$$

Ninetieth percentile value of energy dissipation rate $\varepsilon_{90\%}$:

$$\int_0^{\varepsilon_{90\%}} \frac{p(\varepsilon)}{100} d\varepsilon = 0.9. \quad (18)$$

Ninety-fifth percentile value of energy dissipation rate $\varepsilon_{95\%}$:

$$\int_0^{\varepsilon_{95\%}} \frac{p(\varepsilon)}{100} d\varepsilon = 0.95. \quad (19)$$

Ninety-ninth percentile value of energy dissipation rate $\varepsilon_{99\%}$:

$$\int_0^{\varepsilon_{99\%}} \frac{p(\varepsilon)}{100} d\varepsilon = 0.99. \quad (20)$$

3 | RESULTS AND DISCUSSION

3.1 | Variation of energy dissipation rate in shake flask

The effect of hydromechanical stress, quantified by the energy dissipation rate, on the morphology of *Streptomyces* spp. was investigated. Three representative *Streptomyces* spp., *S. coeruleorubidus*, *S. albobacilis*, and *S. bobili*, were selected for cultivation. According to Equation (8), the shaking frequency, the inner shake flask diameter, and the liquid volume

all influence the average energy dissipation rate. In this study, three different shaking frequencies, 250, 300, and 350 rpm, were investigated. For all conditions tested, the Reynolds number remained below 60,000, indicating a laminar flow regime. Under these conditions, average and maximum energy dissipation rates are considered equal. OTR, used to monitor microbial growth, along with microscopic images of the microorganisms at the end of the growth phase, is shown in Figure 1 for varying shaking frequencies.

For *S. coeruleorubidus*, an increase in the OTR was observed during the first 27 to 30 h of cultivation. After a short lag phase, an exponential growth phase was observed, followed by a linear increase in OTR, reaching a maximum of approximately 20 mmol L⁻¹ h⁻¹ for all three shaking frequencies. Subsequently, the OTR decreased sharply, reflecting glucose depletion. For the lowest shaking frequency of 250 rpm, a slight delay of 2 h was observed before the maximum OTR was reached. Microscopic images show that this species grew in pellets under the set cultivation conditions, with pellet diameter varying depending on the shaking frequency. At 250 rpm, pellets up to 500 μm in diameter were observed,

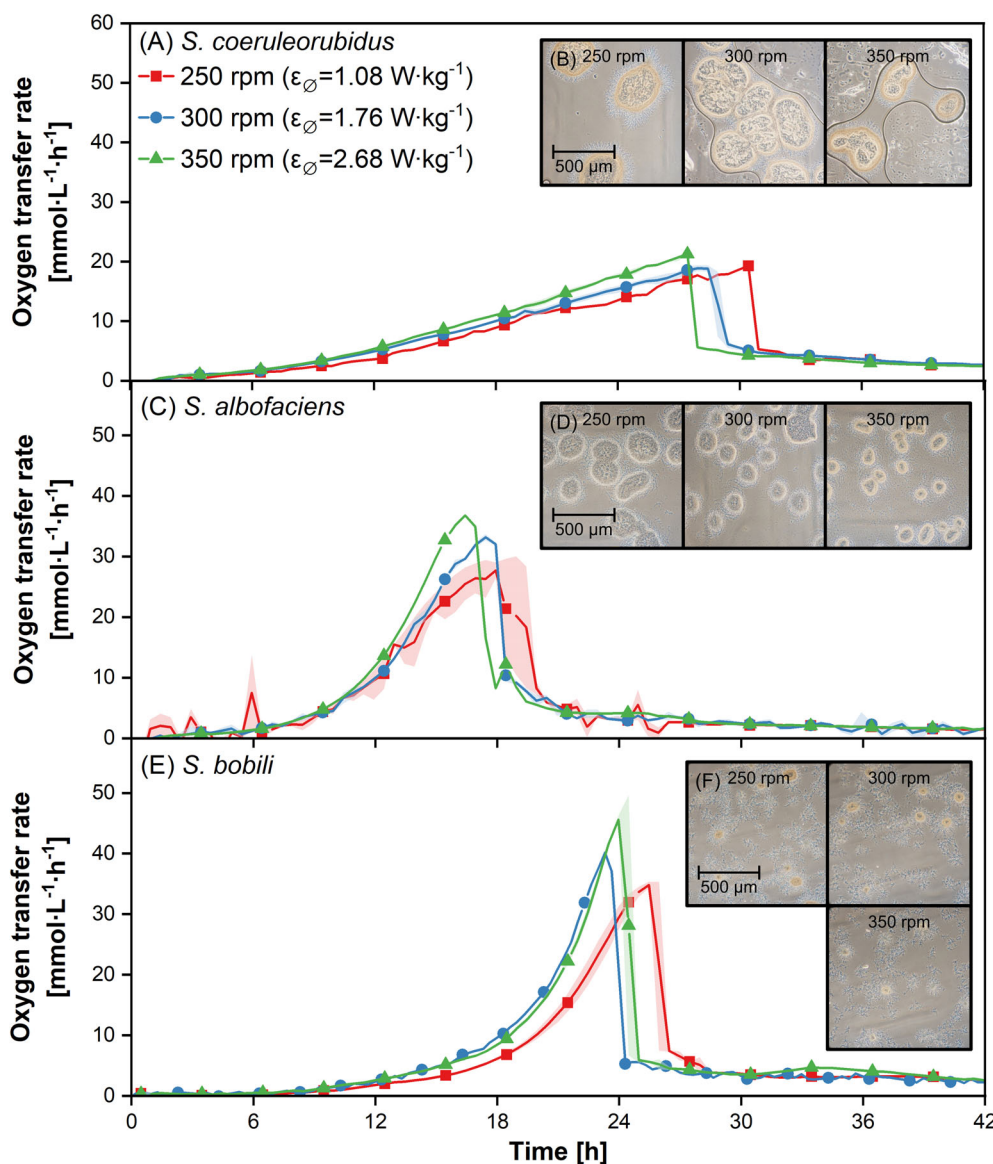


FIGURE 1 Effect of varied average energy dissipation rate on *Streptomyces* species by changing the shaking frequency. (A, B) *Streptomyces coeruleorubidus*. (C, D) *Streptomyces albobacilis*. (E, F) *Streptomyces bobili*. Cultivations were conducted in shake flasks and at the same time for comparison. Three different shaking frequencies were tested: 250, 300, and 350 rpm. The average energy dissipation rate is calculated according to Equation (8) and displayed in the figure. Under the set conditions, $\varepsilon_{\phi} = \varepsilon_{\max}$ is valid. (A, C, E) Oxygen transfer rate was monitored over time with an in-house-built respiratory activity monitoring system device. For clarity, only every sixth data point of OTR is plotted as a symbol. The data presented are mean values derived from biological duplicates (except for *S. coeruleorubidus* at 250 rpm, and *S. albobacilis* at 350 rpm, which are measured once). The shaded areas indicate minimum and maximum values for the duplicates. (B, D, F) Microscopic images were taken at the end of the growth phase at 100× magnification.

whereas at the highest shaking frequency of 350 rpm, pellet diameters reached only about 250 μm . A similar trend was observed for the other *Streptomyces* spp., both in terms of OTR and morphology. *S. albobacillus* exhibited an exponential growth phase lasting 16–18 h, followed by a decline in OTR. For the lower shaking frequencies, a delay of approximately 2 h was also noted, along with a slightly reduced maximum OTR of 28 $\text{mmol L}^{-1} \text{h}^{-1}$ compared to 37 $\text{mmol L}^{-1} \text{h}^{-1}$ at the highest shaking frequency. This species grew in pellets, with diameters decreasing from 300 μm at 250 rpm to about 100 μm at 350 rpm. For *S. bobilli*, following the lag phase, an exponential growth phase was observed from around 6 h up to 24 h. The lowest shaking frequency of 250 rpm caused a slight delay and a lower maximum OTR of 35 $\text{mmol L}^{-1} \text{h}^{-1}$ compared to 46 $\text{mmol L}^{-1} \text{h}^{-1}$ at higher shaking frequencies. Morphological comparison was more challenging for this species, as it showed less pronounced pellets and more dispersed growth. However, the results suggest that pellet diameter decreases with increasing shaking frequency.

Overall, the variation in maximum and average energy dissipation rates through changes in shaking frequency had only a minor effect on OTR, as growth curves were very similar, differing mainly in a time shift for the lowest shaking frequency. This time shift can be explained by the different morphologies observed. All three species showed a clear reduction in pellet diameter with increasing shaking frequency. Larger pellets likely experienced stronger nutrient limitations in their core regions, which may contribute to growth delays. In the center of the pellets, a highly dense and distinctly colored structure was observed, which has been described in the literature as a nutrient-limited region, often containing partially dead or lysed cells.⁶⁰ The effect of reduced pellet diameter with increasing shaking frequency has also been demonstrated for *Streptomyces pristinaespiralis* at shaking frequencies between 250 and 350 rpm⁶¹ and for other filamentous microorganisms.^{62,63} For a more systematic investigation of this effect, abiotic experiments demonstrated that the size distribution of oil droplets varied with shaking frequency.¹⁹ In summary, the results confirm that hydromechanical stress affects the morphology of filamentous microorganisms, as demonstrated here for the investigated *Streptomyces* spp. However, a complete transition from pellet growth to purely dispersed growth was not observed under the set cultivation conditions. Achieving this morphological change would likely require a higher energy dissipation rate, which is not achievable in shake flask cultivations.

3.2 | Scale-up based on average energy dissipation rate

After confirming that hydromechanical stress affects the morphology of *Streptomyces* spp., a scale-up from a shake flask to a stirred-tank reactor was performed. In a first step, the scale-up was carried out based on the average energy dissipation rate. For this, the average energy dissipation rate in the shake flask at 350 rpm was calculated according to Equation (8), and the corresponding stirrer speed in the stirred-tank reactor was adjusted using Equations (3) and (5). To ensure reliability and to rule out any influence of the inoculum during evaluation, a parallel cultivation was conducted in shake flasks.

Figure 2 shows the courses of the OTR as well as microscopic images at the end of the growth phase.

For *S. coeruleorubidus*, a very similar course of OTR was observed in the shake flask and the stirred-tank reactor. After a lag phase of approximately 6 h, the OTR increased nearly exponentially, reaching a maximum of about 35 and 43 $\text{mmol L}^{-1} \text{h}^{-1}$, respectively, followed by a sharp decrease due to glucose depletion after 25 h in the shake flask and 2 h later in the stirred-tank reactor. However, a major difference was observed for the morphology: While large pellets with diameters of several hundred micrometers were present in the shake flask, showing diffusion-limited cores, only dispersed growth was observed in the stirred-tank reactor. This observation was made throughout the entire exponential growth phase, see Supporting Information S1: Supplement 2a. This trend was confirmed for the other two *Streptomyces* spp. *S. albobacillus* exhibited a very similar OTR course for both scales, increasing during the first 15 h to a maximum of 45 $\text{mmol L}^{-1} \text{h}^{-1}$, followed by a drop with a small secondary peak. Supporting Information S1: Supplements 1, 3, and 4 revealed a decrease in pH value during the exponential growth phase, with a slight increase following the glucose depletion. Especially for this species, a significant acidification of the medium was observed, suggesting the formation and subsequent metabolization of acidic by-products after glucose depletion. Morphologically, *S. albobacillus* showed pellet formation in shake flasks, while in the stirred-tank reactor, only dispersed growth was present. For *S. bobilli*, an exponential growth phase was observed following a lag phase of 12 h, with a maximum OTR of 40 and 50 $\text{mmol L}^{-1} \text{h}^{-1}$ in the shake flask and stirred-tank reactor, respectively, followed by a sharp decline. Similar to the observations for *S. coeruleorubidus*, the maximum OTR occurred with a delay of about 2 h between the two scales. This shift may be attributed either to differences between the two scales or to minor biological and experimental variability. While pellet formation mixed with dispersed cells was visible in shake flasks, only dispersed growth occurred in the stirred-tank reactor.

All in all, all three species exhibited a consistent trend: Although scale-up based on average energy dissipation rate resulted in highly similar OTR courses, the morphology differed between shake flask and stirred-tank reactor. These findings are consistent with previous reports in the literature. For example, in *Streptomyces lividans* cultivations, scale-up based on average volumetric power input, which is equivalent to the average energy dissipation rate, since they differ only by the factor liquid density, resulted in comparable online parameters but led to altered morphology.^{17,27} For instance, the pellet diameter decreased from 370 μm in the shake flask cultivation to 245 μm in the bioreactor cultivation, even though the average power input remained constant at 0.2 W m^{-2} , which corresponds to an average energy dissipation rate of 200 W kg^{-1} .¹⁷ In that case, the morphological shift negatively impacted the comparability of product formation. In another study, average volumetric power input was varied during *Aspergillus niger* cultivations in stirred-tank reactors, which led to a significant reduction in pellet diameter. However, pellets were still present, but stirrer speeds were only tested up to 500 rpm, corresponding to a volumetric power input of 443 W m^{-3} according to their calculations.³⁴ This equals an average energy dissipation rate of 0.442 W kg^{-1} and is therefore lower than the energy dissipation rate in our experiments, where

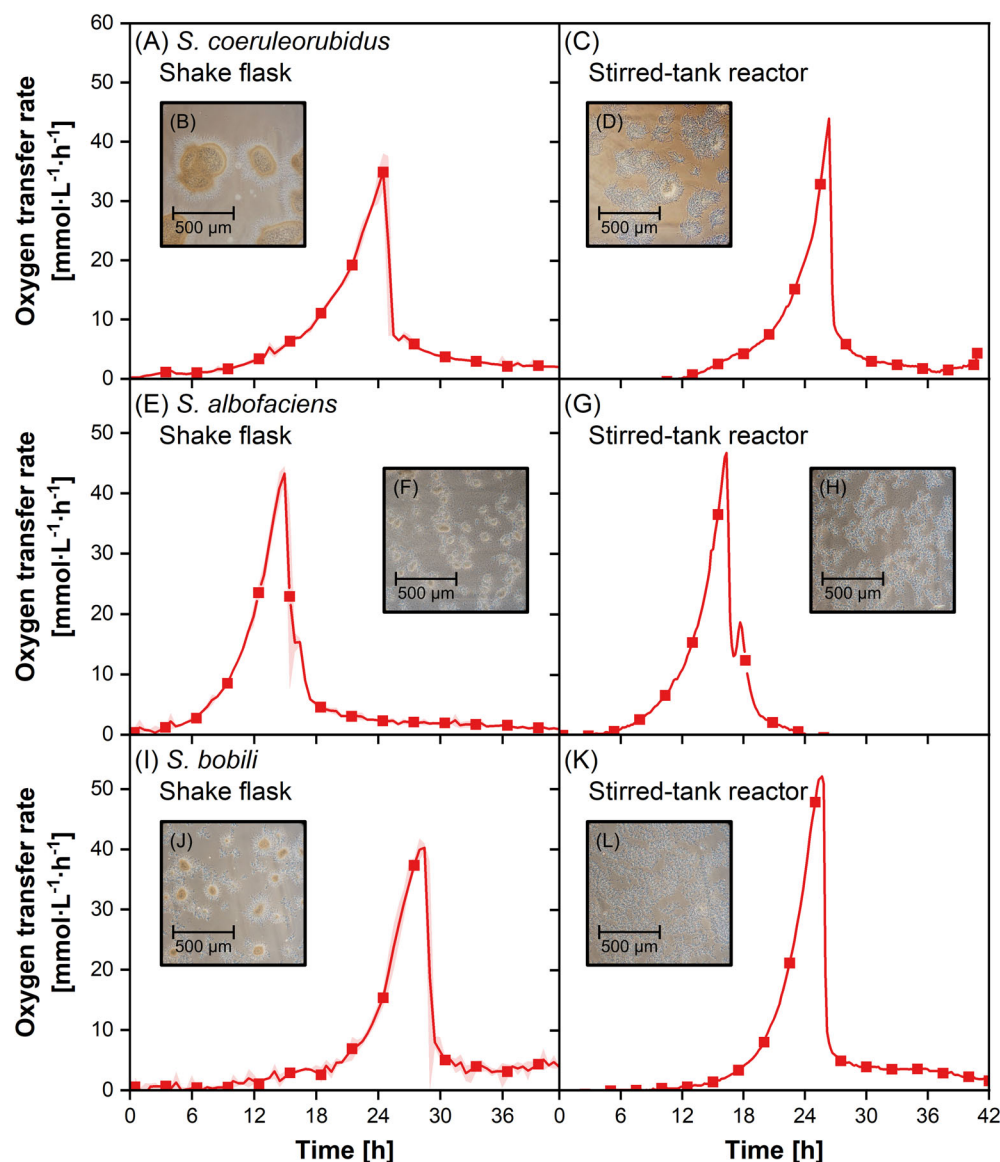


FIGURE 2 Scale-up of *Streptomyces* species, based on average energy dissipation rate. (A–D) *Streptomyces coeruleorubidus*. (E–H) *Streptomyces albobacillus*. (I–L) *Streptomyces bobili*. Cultivations were conducted in shake flasks and in a stirred-tank reactor at the same time for comparison. (A, E, I) Oxygen transfer rate in shake flasks was monitored over time with an in-house-built respiratory activity monitoring system device. For clarity, only every sixth data point of OTR is plotted as a symbol. The data presented are mean values derived from biological triplicates. The shaded areas indicate the standard deviations. (C, G, K) Energy dissipation rate was adjusted by setting the stirrer speed to 854 rpm and the aeration rate to 60 L h^{-1} to achieve the same average energy dissipation rate of 2.68 W kg^{-1} as in the shake flask, calculated according to Equations (3), (5), and (8). A standard six-bladed Rushton turbine (diameter: 52.6 mm, blade height: 10.5 mm, blade width: 14.3 mm) was used. The time-resolved course of OTR was measured. For clarity, only every fifteenth point of OTR is plotted as a symbol. (B, D, F, H, J, L) Microscopic images were taken at the end of the growth phase at $100\times$ magnification. Further data are provided in Supporting Information S1: Supplements 1, 3 and 4.

the stirrer speed was also considerably higher at a comparable reactor size. It can therefore be concluded that scale-up based on the average energy dissipation rate is not suitable for filamentous microorganisms to achieve a comparable morphology between shake flasks and stirred-tank reactors. High local peaks in hydromechanical stress in the stirred-tank reactor likely result in pellet destruction. As this maximum stress is not captured by the average energy dissipation rate, there is a need for an alternative scale-up parameter that accounts for this.

3.3 | CFD simulation of energy dissipation rate

Due to the lack of sufficient data in literature, the energy dissipation rate was examined systematically via CFD simulations. Simulations were performed across a stirrer range from 100 to 1000 rpm in 100 rpm increments.

Figure 3 shows the probability distribution and the cumulative distribution function of energy dissipation rate for an example case at 500 rpm using the standard Rushton turbine. The probability distribution is asymmetric and positively skewed, closely resembling a log-normal distribution. A high probability density was observed at low energy dissipation rates, particularly below 1 W kg^{-1} . The highest probability occurred at 0.1 W kg^{-1} . Following this peak, a long right-hand tail extended toward high values, reaching up to 75 W kg^{-1} , see Equation (17). The average energy dissipation rate in this case was 0.766 W kg^{-1} , see Equation (15). Both values are marked as vertical dashed lines in the diagram. Additionally, three percentile values, the 90th, the 95th, and the 99th, were selected to consider values with more statistical robustness than the maximum energy dissipation rate, see Equations (18–20). These three percentile values correspond to 1.684, 3.451, and 9.929 W kg^{-1} , respectively, and are also marked as vertical dashed lines in the diagram. In a stirred-tank reactor, energy is

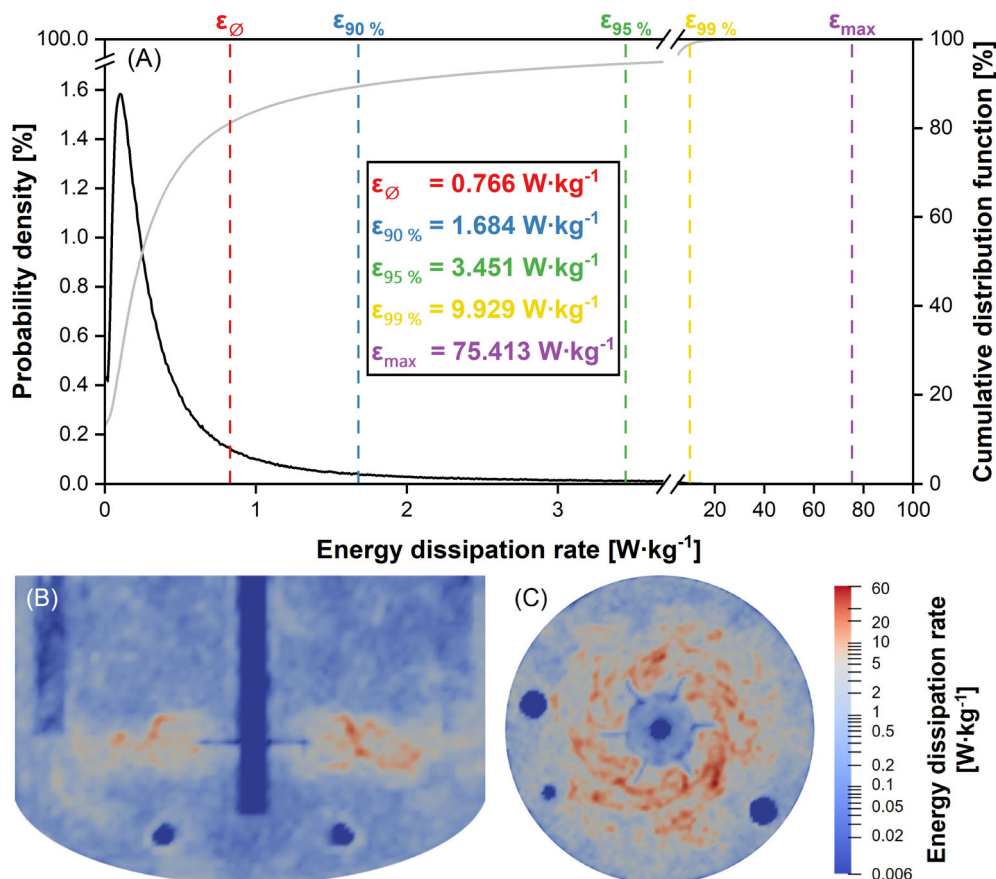


FIGURE 3 Computational fluid dynamics simulation of energy dissipation rate from simulations for the standard Rushton turbine operated at 500 rpm. (A) Probability distribution and cumulative distribution function of energy dissipation. Mean values were determined by averaging the last three recorded time points. Probability density (black) and cumulative distribution function (gray) are displayed. Average and maximum energy dissipation rates, along with the 90th, 95th, and 99th percentile values, are displayed by vertical dashed lines. (B, C) Logarithmic scale of the energy dissipation rate. (B) Cross-section in the XY plane. (C) Cross-section in the XZ plane. The simulations were performed using the CFD software from SimVantage GmbH (<https://simvantage.com>) for a 2 L stirred-tank reactor with a filling volume of 1 L. The stirrer speed was set to 500 rpm. All geometrical parameters of the reactor, internal components, and the stirrer were included as input. A standard six-bladed Rushton turbine (diameter: 52.6 mm, blade height: 10.5 mm, blade width: 14.3 mm) was used. The aeration at 60 L h⁻¹ and the properties of the medium were considered in the simulation. The simulation data were calculated every second, with a total simulation time of at least 5 s and at least 50 rotations.

introduced into the system via the stirrer, resulting in a highly localized input and consequently an inhomogeneous distribution of the energy dissipation rate. High dissipation values occur in the region surrounding the stirrer, while significantly lower values dominate the rest of the liquid volume. Since the regions of high energy dissipation rates are small relative to the total volume, this explains the high probability of low dissipation values observed in the distribution.

The choice of stirrer strongly influences process conditions such as hydromechanical stress, oxygen availability, and mixing time. To reduce hydromechanical stress while maintaining adequate oxygen supply and mixing efficiency, two new stirrers were designed. In preliminary experiments, the standard Rushton turbine caused pronounced changes in morphology, likely due to high maximum energy dissipation rates. Since most equations and correlations in the literature are derived for Rushton turbines, their application to new geometries is limited and complicates quantitative analysis. Therefore, the new stirrers were designed to retain the geometric proportions of

the standard Rushton turbine while varying in size, see Supporting Information S1: Supplement 5.

The quantitative analysis was repeated for all simulated stirrer speeds and for the two newly designed Rushton turbines. Results are presented in Figure 4. Due to the wide range of values, only the range relevant to scale-up is shown. The complete data set is provided in Supporting Information S1: Supplement 6. A horizontal dashed line indicates the average energy dissipation rate in a shake flask under standard cultivation conditions at 350 rpm, calculated according to Equation (8). In shake flasks, the maximum energy dissipation rate can be simplified as the average dissipation rate in the laminar regime.

For all turbines, the simulated energy dissipation rate increased with stirrer speed. This observation was also reported by Zhou et al., who performed CFD simulations in a 5 L stirred-tank reactor at stirring speeds between 250 and 650 rpm.³⁵ A comparison of turbines at identical stirrer speeds revealed that the simulated energy dissipation rates for the large Rushton turbine were higher, attributable to the

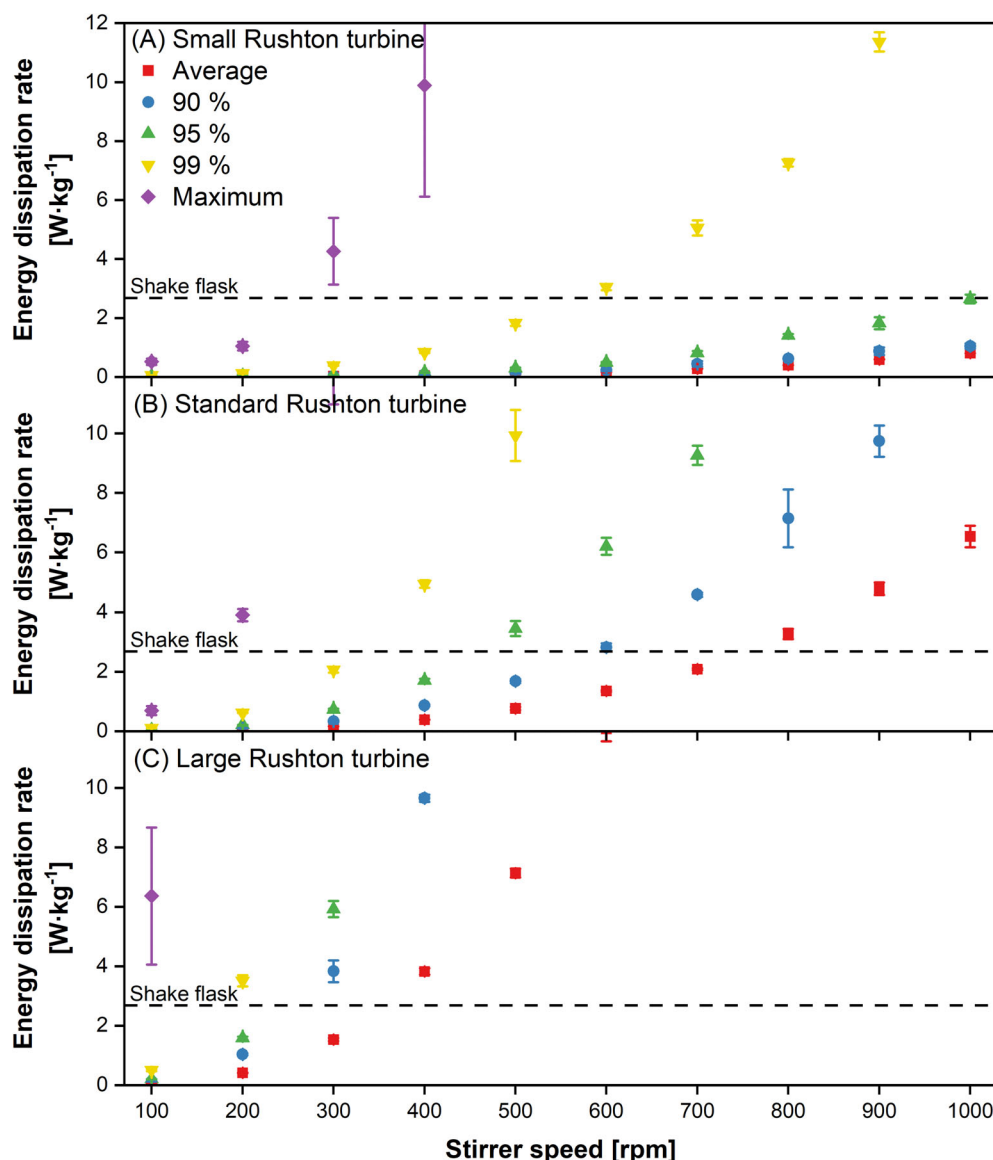


FIGURE 4 Simulation results of energy dissipation rates for the three different Rushton turbines across varying stirrer speeds. (A) Small Rushton turbine. (B) Standard Rushton turbine. (C) Large Rushton turbine. The simulations were performed using the computational fluid dynamics software from SimVantage GmbH (<https://simvantage.com>) for a 2 L stirred-tank reactor with a filling volume of 1 L. The stirrer speeds ranged from 100 to 1000 rpm in 100 rpm increments. All geometrical parameters of the reactor, internal components, and the stirrer were included as input. Three different six-bladed Rushton turbines with varying dimensions were used: Small Rushton turbine (diameter: 34 mm, blade height: 6.8 mm, blade width: 8.5 mm), standard Rushton turbine (diameter: 52.6 mm, blade height: 10.5 mm, blade width: 14.3 mm), and large Rushton turbine (diameter: 80 mm, blade height: 16 mm, blade width: 20 mm). The aeration at 60 L h^{-1} and the properties of the medium were considered in the simulation. The simulation data were calculated every second, with a total simulation time of at least 5 s and at least 50 rotations. Mean values were determined by averaging the last three recorded time points. Average and maximum energy dissipation rates, along with the 90th, 95th, and 99th percentile values, were calculated. The error bars indicate the standard deviations. The average energy dissipation rate in the shake flask is calculated according to Equation (8) and displayed in the figure as a horizontal dashed line. Under the set conditions, $\varepsilon_0 = \varepsilon_{\max} = 2.68 \text{ W kg}^{-1}$ is valid. This figure presents a process-relevant excerpt of the simulation data. A full representation of the energy dissipation rate profile across all regions is provided in Supporting Information S1: Supplement 6.

stronger impact of its larger blades on the flow regime. To determine the stirrer speed corresponding to the same hydromechanical stress as in the shake flask, the intersection point between the simulated energy dissipation rate curve and the horizontal line representing the shake flask value was used. Since it was not feasible to test all stirrers and scale-up parameters due to the extensive laboratory effort

required, a single stirrer and scale-up parameter based on the simulation results had to be selected. To identify the most suitable stirrer, the ratio of maximum to average simulated energy dissipation rate was calculated for each turbine and compared. The large and standard Rushton turbines showed similar values of 90 and 83, respectively, whereas the small Rushton turbine exhibited a markedly high ratio

of 211. Even at the same power input, lower maximum energy dissipation rates result when the energy is distributed over a larger swept volume. Therefore, small stirrers generate higher maximum energy dissipation rates at the same average energy dissipation rate, since the energy dissipation is more localized. Due to this high ratio, the small Rushton turbine was considered unsuitable and excluded from further investigations. Based on the simulation results, the large Rushton turbine was selected for subsequent experiments, based on the assumption that it ensures adequate oxygen supply while inducing comparatively low hydromechanical stress at low stirrer speeds.

In terms of choosing a scale-up parameter, the average energy dissipation rate is not suitable to characterize hydromechanical stress, particularly when working with filamentous organisms, since it fails to account for local maxima, as shown in Section 3.2. The simulation-derived values for the average energy dissipation rate were slightly higher, on average by about 19%, than those calculated using Equations (3) and (5), although the overall order of magnitude remained comparable. This discrepancy may be explained by inaccuracies in the consideration of aeration with the applied equation, with alternative formulations also reported in the literature. While the raw value of the maximum energy dissipation rate appears to be a promising candidate for comparison during scale-up, it is a highly localized value, only affecting a small region of the liquid volume. Therefore, even small turbulent fluctuations or the current angular position of the stirrer can influence the exact numerical value of the maximum energy dissipation rate. As it reflects a potentially singular, statistically non-reproducible event, it cannot be considered a robust scale-up parameter. Given these limitations, a more statistically robust value was sought, so the three percentile values were considered, which indicates that a specific percentage of all reached energy dissipation rates is below this threshold. At this point, it is not possible to definitively predict which percentile value will prove most effective. Nevertheless, the 95th percentile of the energy dissipation rate, $\varepsilon_{95\%}$, was chosen for further application. This percentile is commonly used in statistics and appears in other areas of bioprocess engineering, such as mixing time analysis.

3.4 | Scale-up based on maximum energy dissipation rate

Based on the scale-up conditions defined in Section 3.3, specifically using the 95th percentile value of the energy dissipation rate with the large Rushton turbine, cultivations were carried out in both shake flasks and the stirred-tank reactor. A stirrer speed of 212 rpm was selected to achieve the targeted energy dissipation rate. The OTR and the morphology at the end of the exponential growth phase in both scales were compared. The results are shown in Figure 5 and additional fermentation data can be found in Supporting Information S1: Supplements 7–9.

For all *Streptomyces* spp., the OTR in shake flasks showed a similar trend to earlier experiments, with an initial exponential increase to 35–45 mmol L⁻¹ h⁻¹, depending on the species, followed by a sharp

drop due to glucose depletion. The time point of glucose depletion also differed by a few hours between species. For the stirred-tank reactor, the OTR courses were nearly identical to the courses of the shake flask during the first 12–15 h, again showing an exponential increase. However, the OTR then entered a plateau at around 10 mmol L⁻¹ h⁻¹ and remained at this level until dropping due to glucose depletion after 35–45 h, depending on the species, much later than in the shake flask. These distinct OTR courses can be attributed to the low stirrer speed applied during these cultivations, which was applied to reach a comparably low maximum energy dissipation rate like in shake flask cultivations. However, this also resulted in reduced oxygen transfer to the liquid phase, leading to oxygen limitation already at an OTR of approximately 10 mmol L⁻¹ h⁻¹. This limitation is also reflected in the dissolved oxygen tension, which reached 0% at the onset of the plateau phase and only increased again after the end of growth due to glucose depletion.

More crucial, however, is the evaluation of microscopic images, as the primary goal was to achieve comparable morphology. For *S. coeruleorubidus*, pellet formation was observed in shake flasks, with maximum pellet diameters around 200 µm. Similar morphology and pellet diameter were observed in the stirred-tank reactor. This observation was consistently made throughout the exponential growth phase, while a shift toward a more dispersed morphology became apparent during the stationary phase, see Supporting Information S1: Supplement 2b. For *S. albobacis*, a mixture of dispersed growth and pellets around 100 µm in diameter was seen in the shake flask, and a comparable morphology with pellets and mycelia was observed in the reactor. *S. bobili* showed a mixed morphology of pellets and dispersed growth in shake flasks, which was likewise seen in the stirred-tank reactor.

Overall, scale-up based on the 95th percentile of the energy dissipation rate $\varepsilon_{95\%}$ resulted in significantly more comparable morphology between the shake flask and stirred-tank reactor. While scale-up based on average energy dissipation rate failed to produce pellet formation in the reactor, distinct pellet formation with a similar diameter was visible here. Due to the promising results, the 95th percentile of the energy dissipation rate $\varepsilon_{95\%}$ is proposed as a new parameter to describe maximum energy dissipation more statistically reliably and with greater clarity, especially in comparison to literature values where it often remains unclear whether a percentile value or a single peak value is meant. Furthermore, the 95th percentile of the energy dissipation rate $\varepsilon_{95\%}$ is suggested as a scale-up parameter for shear-sensitive organisms, such as filamentous microorganisms, to achieve a comparable morphology. To the best of our knowledge, this approach has not been applied in literature to date. However, the equivalent hydromechanical stress came at the cost of oxygen limitation, which cannot be avoided under the selected process conditions. It can also not be excluded that the low oxygen availability affects morphology.

3.5 | Effect of oxygen availability

In the previous scale-up experiment, a stirrer speed of 212 rpm was chosen to ensure a hydromechanical stress comparable to that of the

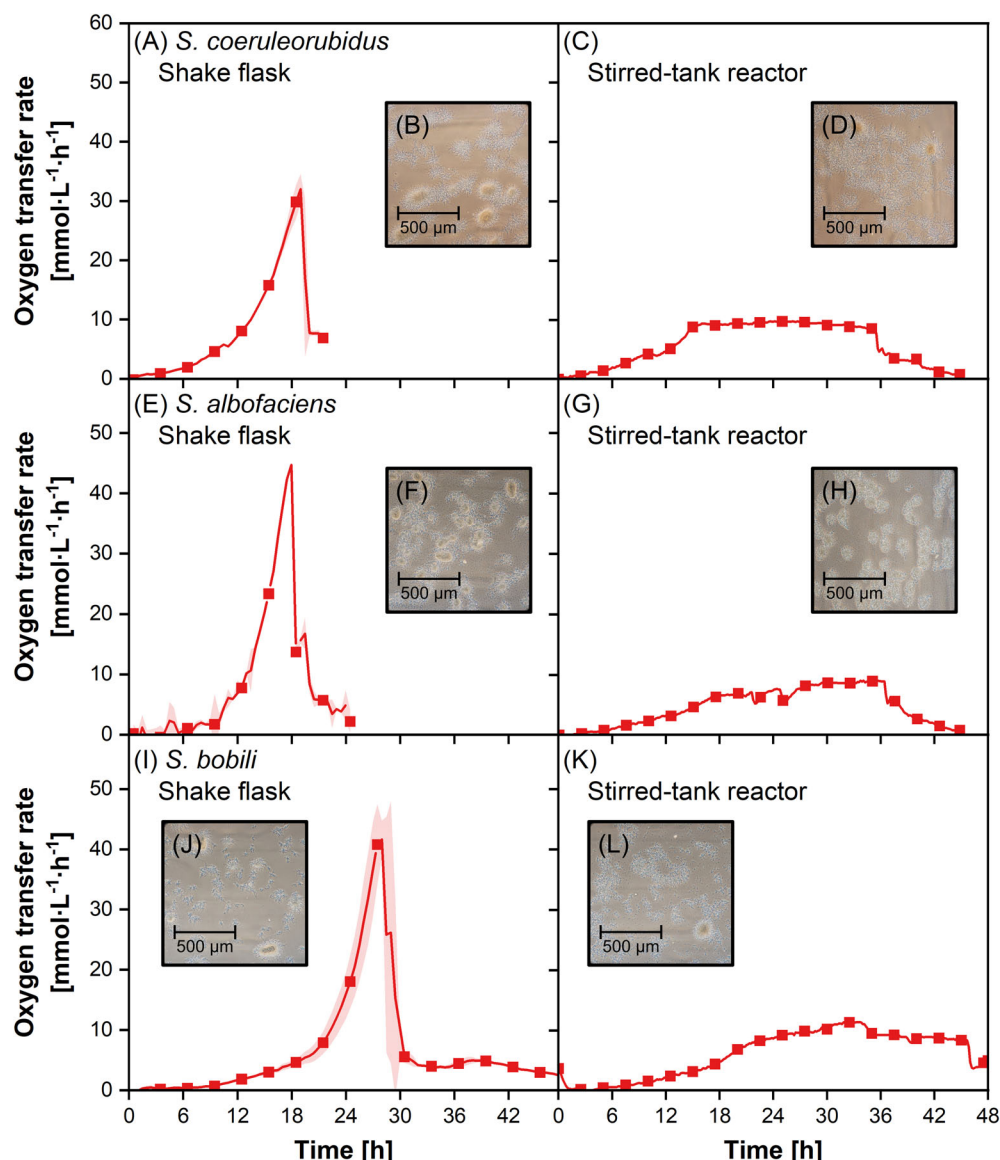


FIGURE 5 Scale-up of *Streptomyces* species, based on the maximum energy dissipation rate, represented by the 95th percentile of energy dissipation rate. (A–D) *Streptomyces coeruleorubidus*. (E–H) *Streptomyces albobacillus*. (I–L) *Streptomyces bobili*. Cultivations were conducted in shake flasks and in a stirred-tank reactor at the same time for comparison. (A, E, I) Oxygen transfer rate in shake flasks was monitored over time with an in-house-built respiratory activity monitoring system device. For clarity, only every sixth data point of OTR is plotted as a symbol. The data presented are mean values derived from biological triplicates. The shaded areas indicate the standard deviations. (C, G, K) Energy dissipation rate was adjusted by setting the stirrer speed to 212 rpm and the aeration rate to 60 L h^{-1} to achieve the same maximum energy dissipation rate as in the shake flask. For the shake flask, according to Equation (8), $\epsilon_{\theta} = \epsilon_{\max} = 2.68 \text{ W kg}^{-1}$ is valid under the set conditions. For the stirred-tank reactor, the maximum energy dissipation rate, represented by the 95th percentile of energy dissipation rate, was determined according to the simulations, displayed in Figure 4. A large six-bladed Rushton turbine (diameter: 80 mm, blade height: 16 mm, blade width: 20 mm) was used. The time-resolved course of OTR was measured. For clarity, only every fifteenth point of OTR is plotted as a symbol. (B, D, F, H, J, L) Microscopic images were taken at the end of the growth phase at 100 $\times$ magnification. Further data are provided in Supporting Information S1: Supplements 7–9.

shake flask. However, this low speed resulted in oxygen limitation. To investigate whether this oxygen limitation influences morphology, a cultivation was performed under the same hydromechanical stress but without inducing oxygen limitation. Since changes in stirrer speed or aeration rate would have affected the energy dissipation rate, pure oxygen was used as inlet gas. Data recorded during this cultivation, conducted using only one representative species, *S. coeruleorubidus*,

and microscopic images are provided in Figure 6 and Supporting Information S1: Supplement 10. Due to the high oxygen content in the inlet gas, the dissolved oxygen tension could no longer be measured, as it exceeded the sensor's measurement range. A dilution of the exhaust gas with nitrogen was required for off-gas analysis, since the sensor had a maximum detection limit of 50%. As a result, the system became sensitive to fluctuations, and the OTR appeared noisy.

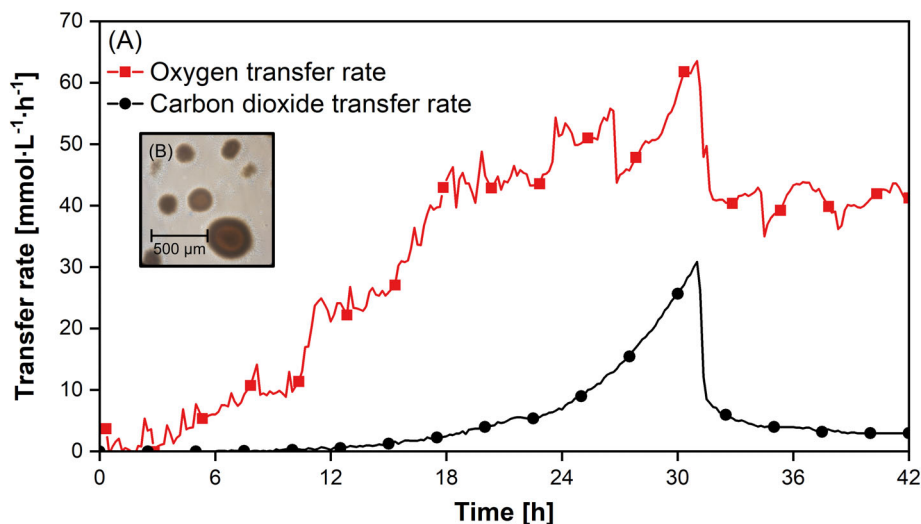


FIGURE 6 Scale-up of *Streptomyces coeruleorubidus* on pure oxygen, based on the maximum energy dissipation rate, represented by the 95th percentile of energy dissipation rate. Cultivations were conducted in shake flasks and in a stirred-tank reactor. Energy dissipation rate was adjusted by setting the stirrer speed to 212 rpm and the aeration rate to 60 L h^{-1} to achieve the same maximum energy dissipation rate as in the shake flask. For the shake flask, according to Equation (8), $\epsilon_0 = \epsilon_{\text{max}} = 2.68 \text{ W kg}^{-1}$ is valid under the set conditions. For the stirred-tank reactor, the maximum energy dissipation rate, represented by the 95th percentile of energy dissipation rate, was determined according to the simulations, displayed in Figure 4. Pure oxygen was used for aeration. A large six-bladed Rushton turbine (diameter: 80 mm, blade height: 16 mm, blade width: 20 mm) was used. (A) Display of gas transfer rates. For clarity, only every fifteenth point is plotted as a symbol. (B) Microscopic images were taken at the end of the growth phase at $100\times$ magnification. Further data are provided in Supporting Information S1: Supplement 10.

However, CTR showed a more reliable trend. Assuming a respiratory quotient of 1, consistent with all previous cultivations and known from literature for growth on glucose,⁶⁴ the CTR can be considered representative of the OTR.

The CTR course was similar to previous oxygen-unlimited OTR courses of *S. coeruleorubidus* cultivations, with an exponential growth phase reaching a maximum of $31 \text{ mmol L}^{-1} \text{ h}^{-1}$, followed by a decline due to glucose depletion. Notably, growth began only after a prolonged lag phase of 12–15 h, and the maximum CTR was reached after 31 h. This delay suggests that cultivation with pure oxygen imposed oxidative stress on the cells, negatively affecting growth. A similar effect has been reported for *Streptomyces clavuligerus*, where growth was also delayed under elevated oxygen conditions.⁶⁵ Priorly, it was unclear whether growth could occur under high dissolved oxygen tension, as the literature does not unequivocally confirm that all *Streptomyces* spp. can tolerate such conditions. Additionally, a lack of carbon dioxide may impair growth, as it is essential for carboxylase reactions and involved in both primary and secondary metabolic pathways of *Streptomyces* spp.⁶⁶

In terms of morphology, pellet formation was observed with almost no dispersed growth. These pellets were significantly larger than those observed in previous stirred-tank reactor and shake flask cultivations. They also exhibited a pronounced central region, likely nutrient-limited and containing mainly dead cells. This core appeared much darker than in previous experiments. Since the pellets in this experiment were significantly larger than in previous experiments, it can be concluded that oxygen availability, as already described in the literature,¹⁰ affects the morphology of filamentous microorganisms. This effect was confirmed here for the

investigation of *S. coeruleorubidus*. The larger pellet diameter suggests that oxygen availability was significantly higher than in all previous cultivations.

However, for future applications, it is questionable whether such a setup is feasible, as fermentation with pure oxygen poses significant safety risks. Furthermore, reports in the literature indicate that elevated oxygen concentrations can lead to oxidative stress, altering both the product spectrum and product titer in *Streptomyces* spp. cultivations,⁶⁷ which would negatively impact comparability during scale-up. A potential alternative for future experiments and industrial applications could be controlling the oxygen content in the inlet gas by maintaining a constant dissolved oxygen tension, rather than continuously supplying pure oxygen. This would reduce the cells' exposure to oxidative stress, which, as observed here, contributed to a prolonged lag phase. However, even this setup would face the problem that oxygen availability in shake flasks could not be directly reproduced. Unlike hydromechanical stress, which can be maintained at a constant level, oxygen availability follows a dynamic course throughout the cultivation due to oxygen depletion during growth. The results show that both process conditions, hydromechanical stress and oxygen availability, affect the morphology of filamentous microorganisms, making it difficult to completely separate their individual influences on morphology. Practical implementation of such a separation is challenging because changing one condition almost inevitably alters the other. Nevertheless, for hydromechanical stress, the scale-up parameter $\epsilon_{95\%}$, the 95th percentile of the energy dissipation rate, could be successfully established, leading to more comparable morphology in scale-up of filamentous microorganisms. Further investigation on oxygen availability in this context will be crucial but is beyond the scope of the presented work.

4 | CONCLUSION

This study aimed to establish a reliable scale-up strategy for *Streptomyces* spp. from shake flask to stirred-tank reactor. Given the decisive influence of filamentous microorganisms' morphology on overall process performance, comparable morphology across scales was the primary objective.

Initial experiments demonstrated that hydromechanical stress, quantified via the energy dissipation rate, strongly affects morphology. Shake flask cultivations at increasing shaking frequencies showed a clear trend toward smaller pellet diameter, confirming the energy dissipation rate as a suitable scale-up parameter. A first scale-up attempt based on average energy dissipation rate failed to achieve comparable morphology from shake flask cultivations, resulting in a shift from pelleted to dispersed growth. As this likely alters the product formation, an alternative scale-up parameter was pursued. The maximum energy dissipation rate appeared to be a more promising parameter. However, due to a lack of reliable literature data on maximum energy dissipation rates in stirred-tank reactors, CFD-based simulations were conducted to gain systematic and reliable quantification. These simulations revealed strong fluctuations in the maximum value, making it statistically unreliable. As a result, a more robust parameter, the 95th percentile of the energy dissipation rate $\varepsilon_{95\%}$, was introduced as a reliable quantification of maximum energy dissipation rate and as a novel scale-up parameter for filamentous cultivation processes. Using this scale-up parameter, subsequent cultivations in the stirred-tank reactor achieved significantly improved morphological comparability to the shake flask. A further experiment with pure oxygen in the inlet gas shows that oxygen availability also critically affects pellet diameter. Thus, oxygen content represents an additional important process condition influencing morphology.

This suggests that morphology can be further optimized by precisely controlling the oxygen content of the inlet gas. Moreover, microscopy has so far been applied only qualitatively, but quantitative image analysis with sufficient images would enable a more robust and objective characterization. Our findings highlight the importance of systematically analyzing process conditions that influence morphology, an approach likely to be valuable for other filamentous microorganism processes. It would be of great interest to investigate whether maintaining comparable morphology results in the same product titer in real production processes or in cultivations of filamentous microorganisms producing a quantifiable product during future process development.

AUTHOR CONTRIBUTIONS

Gesa Brauneck: conceptualization (lead); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); visualization (lead); writing—original draft preparation (lead). **Anton Brass:** investigation (supporting); methodology (supporting). **Artiom Boreiko:** investigation (supporting); methodology (supporting). **Marvin Ueßeler:** investigation (supporting); methodology (supporting). **Marcel Mann:** conceptualization (supporting); supervision (equal). **Johannes Pastoors:** supervision (supporting). **Valentin Steier:** supervision (supporting).

Philipp Eibl: formal analysis (supporting); resources (supporting). **Christian Witz:** formal analysis (supporting); resources (supporting). **Dorothea M. Schütterle:** conceptualization (supporting). **Ivan Schlembach:** conceptualization (supporting). **Miriam A. Rosenbaum:** project administration (equal); conceptualization (supporting); resources (supporting). **Jorgen Magnus:** conceptualization (supporting); project administration (equal); resources (lead); supervision (equal).

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CONFLICT OF INTEREST STATEMENT

Philipp Eibl and Christian Witz are employees of SimVantage GmbH, which granted free access to its simulation software used in this study. Gesa Brauneck used this software for CFD simulations. This affiliation did not influence the research process or the interpretation of the results. All other authors declare no competing interests.

DATA AVAILABILITY STATEMENT

Additional data from cultivation experiments supporting the findings of this study are available in Supplements 1–12 of Supporting Information S1. The numerical data from Figures 1–6, as well as from Supporting Information S1: Supplements 1, 3, 4, and 6–11, are provided in tabulated form in Supporting Information S2. Where duplicate or triplicate measurements were performed, mean values were calculated. Standard deviations are shown for triplicates, and deviations from the maximum and minimum values for duplicates. These are indicated by error bars or shaded areas, as stated in the respective figure captions. For the simulations, both input data and the resulting output files are provided as ZIP archives under https://git.rwth-aachen.de/dominik.engel1/data_brauneck_scale-up-of-streptomyces-species-cultivations. Data analysis was carried out as described in Section 2.

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REFERENCES

- Komaki H. Recent Progress of reclassification of the genus *Streptomyces*. *Microorganisms*. 2023;11:831.
- Nikolaidis M, Hesketh A, Frangou N, et al. A panoramic view of the genomic landscape of the genus *Streptomyces*. *Microb Genom*. 2023;9:mgen001028.
- Procópio REL, Da Silva IR, Martins MK, de Azevedo JL, de Araújo JM. Antibiotics produced by *Streptomyces*. *Braz J Infect Dis*. 2012;16(5):466-471.
- Finger M, Sentek F, Hartmann L, et al. Insights into *Streptomyces coelicolor* A3(2) growth and pigment formation with high-throughput online monitoring. *Eng Life Sci*. 2022;23(1):e2100151.
- Terra L, Dyson P, Ratcliffe N, Castro HC, Vicente ACP. Biotechnological potential of *Streptomyces* siderophores as new antibiotics. *Curr Med Chem*. 2021;7(28):1407-1421.
- Schatz A, Bugle E, Waksman SA. Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative bacteria. *Exp Biol Med*. 1944;55:66-69.
- Nielsen J. Modelling the morphology of filamentous microorganisms. *Trends Biotechnol*. 1996;14:438-443.
- Driouch H, Sommer B, Wittmann C. Morphology engineering of *Aspergillus niger* for improved enzyme production. *Biotechnol Bioeng*. 2010;105(6):1058-1068.
- Grimm LH, Kelly S, Krull R, Hempel DC. Morphology and productivity of filamentous fungi. *Appl Microbiol Biotechnol*. 2005;69(4):375-384.
- Böl M, Schrinner K, Tesche S, Krull R. Challenges of influencing cellular morphology by morphology engineering techniques and mechanical induced stress on filamentous pellet systems – a critical review. *Eng Life Sci*. 2021;21(3-4):51-67.
- Zhou Y, Du J, Tsao GT. Mycelial pellet formation by *Rhizopus oryzae* ATCC 20344. *Appl Biochem Biotechnol*. 2000;84-86:779-789.
- Wucherpfennig T, Hestler T, Krull R. Morphology engineering - osmolality and its effect on *Aspergillus niger* morphology and productivity. *Microb Cell Fact*. 2011;10:58.
- Carlsen M, Spohr AB, Nielsen J, Villadsen J. Morphology and physiology of an α -amylase producing strain of *Aspergillus oryzae* during batch cultivations. *Biotechnol Bioeng*. 1996;49(3):266-276.
- Vecht-Lifshitz SE, Magdassi S, Braun S. Pellet formation and cellular aggregation in *Streptomyces tendae*. *Biotechnol Bioeng*. 1990;35:890-896.
- Garcia-Ochoa F, Gomez E, Santos VE. Fluid dynamic conditions and oxygen availability effects on microbial cultures in STBR: an overview. *Biochem Eng J*. 2020;164:107803.
- Dittmann J, Tesche S, Krull R, Böl M. The influence of salt-enhanced cultivation on the micromechanical behaviour of filamentous pellets. *Biochem Eng J*. 2019;148:65-76.
- Gamboa-Suasnavart RA, Marín-Palacio LD, López-Griego L, Córdova-Aguilar MS, Valdez-Cruz NA, Trujillo-Roldán MA. Volumetric power input as a reliable parameter for scale-up from shake flask to stirred-tank bioreactor: production of a recombinant glycoprotein by *Streptomyces lividans*. *Rev Mex Ing Quím*. 2019;18(3):1085-1099.
- Brauneck G, Engel D, Grebe LA, et al. Pitfalls in early bioprocess development using shake flask cultivations. *Eng Life Sci*. 2025;25(1):e70001.
- Peter CP, Suzuki Y, Büchs J. Hydromechanical stress in shake flasks: correlation for the maximum local energy dissipation rate. *Biotechnol Bioeng*. 2006;93(6):1164-1176.
- Daub A, Böhm M, Delueg S, Mühlmann M, Schneider G, Büchs J. Characterization of hydromechanical stress in aerated stirred tanks up to 40 m³ scale by measurement of maximum stable drop size. *J Biol Eng*. 2014;8:17.
- Tan R-K, Eberhard W, Büchs J. Measurement and characterization of mixing time in shake flasks. *Chem Eng Sci*. 2011;66(3):440-447.
- Büchs J, Zoels B. Evaluation of maximum to specific power consumption ratio in shaking bioreactors. *J Chem Eng Jpn*. 2001;34(5):647-653.
- Neuß A, Borges JST, von Vegesack N, Büchs J, Magnus JB. Impact of hydromechanical stress on CHO cells' metabolism and productivity: insights from shake flask cultivations with online monitoring of the respiration activity. *N Biotechnol*. 2024;84:96-104.
- Rocha-Valadez JA, Galindo E, Serrano-Carreón L. The influence of circulation frequency on fungal morphology: a case study considering Kolmogorov microscale in constant specific energy dissipation rate cultures of *Trichoderma harzianum*. *J Biotechnol*. 2007;130(4):394-401.
- Kolmogorov AN. Dissipation of energy in the locally isotropic turbulence. *Proc R Soc Lond A Math Phys Sci*. 1991;434(1890):15-17.
- Sieck JB, Cordes T, Budach WE, et al. Development of a scale-down model of hydrodynamic stress to study the performance of an industrial CHO cell line under simulated production scale bioreactor conditions. *J Biotechnol*. 2013;164:41-49.
- Gamboa-Suasnavart RA, Marín-Palacio LD, Martínez-Sotelo JA, et al. Scale-up from shake flasks to bioreactor, based on power input and *Streptomyces lividans* morphology, for the production of recombinant APA (45/47 kDa protein) from *Mycobacterium tuberculosis*. *World J Microbiol Biotechnol*. 2013;29(8):1421-1429.
- Antonio Rocha-Valadez J, Estrada M, Galindo E, Serrano-Carreón L. From shake flasks to stirred fermentors: scale-up of an extractive fermentation process for 6-pentyl- α -pyrone production by *Trichoderma harzianum* using volumetric power input. *Process Biochem*. 2006;41(6):1347-1352.
- Morchain J, Gabelle J-C, Cockx A. A coupled population balance model and CFD approach for the simulation of mixing issues in lab-scale and industrial bioreactors. *Am Inst Chem Eng*. 2014;60(1):27-40.
- Villiger TK, Morbidelli M, Soos M. Experimental determination of maximum effective hydrodynamic stress in multiphase flow using shear sensitive aggregates. *AIChE J*. 2015;61(5):1735-1744.
- Bliatsiou C, Panckow RP, Nolte L, Böhm L, Maaß S, Kraume M. Characterisation of particle stress in turbulent impeller flows utilising photo-optical measurements of a flocculation system – PART 2. *Chem Eng Sci*. 2024;291:119853.
- Forl I, Machón V, Kadlec P. Distribution of energy dissipation rate in an agitated gas-liquid system. *Chem Eng Technol*. 1993;16(6):389-394.
- Lane G. Predicting the energy dissipation rate in mechanically stirred tank. In: Eleventh International Conference on CFD in the Minerals and Process Industries. 2015.
- Eslahpazir Esfandabadi M, Wucherpfennig T, Krull R. Agitation induced mechanical stress in stirred tank bioreactors – linking CFD simulations to fungal morphology. *J Chem Eng Jpn*. 2012;45(9):742-748.
- Zhou W, Zheng G, Wang J, Xin X, Zhuang L, An F. Computational fluid dynamics (CFD) modeling of Ilamycin E production in *Streptomyces atratus* SCSIO ZH16 submerged fermentation. Preprint. 2025. <https://doi.org/10.1101/2025.01.02.630827>.
- Dinter C, Gumprecht A, Menze MA, et al. Validation of computational fluid dynamics of shake flask experiments at moderate viscosity by liquid distributions and volumetric power inputs. *Sci Rep*. 2024;14:3658.
- van Valderen RD, Juarez-Garza BE, Klijn ME, Ottens M, Haringa C. Implicit large eddy simulations for hydrodynamic stress characterization in 125 mL shake flasks for stem cell cultures. *Biochem Eng J*. 2025;219:109734.
- Zhou Y, Han L-R, He H-W, et al. Effects of agitation, aeration and temperature on production of a novel glycoprotein GP-1 by *Streptomyces kanasensis* ZX01 and scale-up based on volumetric oxygen transfer coefficient. *Molecules*. 2018;23:125.
- Marín-Palacio LD, Gamboa-Suasnavart RA, Valdez-Cruz NA, et al. The role of volumetric power input in the growth, morphology, and production of a recombinant glycoprotein by *Streptomyces lividans* in shake flasks. *Biochem Eng J*. 2014;90:224-233.
- Klöckner W, Büchs J. Advances in shaking technologies. *Trends Biotechnol*. 2012;30(6):307-314.

41. Saygin H, Ay H, Guven K, Cetin D, Sahin N. *Streptomyces Cahuitamycinicus* sp. nov., isolated from desert soil and reclassification of *Streptomyces galliaeus* as a later heterotypic synonym of *Streptomyces bobili*. *Int J Syst Evol Microbiol*. 2020;70(4):2750-2759.
42. Hobbs G, Frazer CM, Gardner DCJ, Cullum JA, Oliver SG. Dispersed growth of *Streptomyces* in liquid culture. *Appl Microbiol Biotechnol*. 1989;31:272-277.
43. Pakula TM, Salonen K, Uusitalo J, Penttilä M. The effect of specific growth rate on protein synthesis and secretion in the filamentous fungus *Trichoderma reesei*. *Microbiology*. 2005;151:135-143.
44. Brauneck G, Heykena MS, Schütterle DM, et al. Fluorescence-based online monitoring enables differentiation in co-cultures of untagged *Streptomyces* species and *Trichoderma reesei*. *Appl Biochem Biotechnol*. 2025;197:7276-7295.
45. Kieser T, Bibb MJ, Buttner MJ, Chater KF, Hopwood DA. *Practical Streptomyces Genetics*. The John Innes Foundation; 2000.
46. Anderlei T, Büchs J. Device for sterile online measurement of the oxygen transfer rate in shaking flasks. *Biochem Eng J*. 2001;7:157-162.
47. Nienow AW. Stirring and stirred-tank reactors. *Chem Ing Tech*. 2014;86(12):2063-2074.
48. Rushton JH, Costich EW, Everett HJ. Power characteristics of mixing impellers. *Chem Eng Prog*. 1950;46:467-476.
49. Möckel H-O, Wolleschensky E, Drews S, Rahner H-J. Modelling of the calculation of the power input for aerated single- and multistage impellers with special respect to scale-up. *Acta Biotechnol*. 1990;10(3):215-224.
50. Henzler H-J. Verfahrenstechnische Auslegungssunterlagen für Rührbehälter als Fermenter. *Chem Ing Tech*. 1982;54(5):461-476.
51. Michel BJ, Miller SA. Power requirements of gas-liquid agitated systems. *AIChE J*. 1962;8(2):262-266.
52. Nagata S. *Mixing: Principles and Applications*. John Wiley & Sons, Ltd.; 1975.
53. Clark MW, Vermeulen T. Incipient vortex formation in baffled agitated vessels. *AIChE J*. 1964;10(3):420-422.
54. Büchs J, Maier U, Milbradt C, Zoels B. Power consumption in shaking flasks on rotary shaking machines: I. Power consumption measurement in unbaffled flasks at low liquid viscosity. *Biotechnol Bioeng*. 2000;68(6):589-593.
55. Büchs J, Maier U, Milbradt C, Zoels B. Power consumption in shaking flasks on rotary shaking machines: II. Nondimensional description of specific power consumption and flow regimes in unbaffled flasks at elevated liquid viscosity. *Biotechnol Bioeng*. 2000;68(6):594-601.
56. Bernauer S, Schöpf M, Eibl P, Witz C, Khinast J, Hardiman T. Characterization of the gas dispersion behavior of multiple impeller stages by flow regime analysis and CFD simulations. *Biotechnol Bioeng*. 2021;118(8):3058-3068.
57. Bernauer S, Eibl P, Witz C, Khinast J, Hardiman T. Analyzing the effect of using axial impellers in large-scale bioreactors. *Biotechnol Bioeng*. 2022;119(9):2494-2504.
58. Eibl P, Rustige S, Witz C, Khinast J. LBM for two-phase (bio-)reactors. *Advances in Chemical Engineering*. Vol 55. Elsevier; 2020:219-285.
59. Witz C, Treffer D, Hardiman T, Khinast J. Local gas holdup simulation and validation of industrial-scale aerated bioreactors. *Chem Eng Sci*. 2016;152:636-648.
60. Wittler R, Baumgartl H, Lübbers DW, Schügerl K. Investigations of oxygen transfer into *Penicillium chrysogenum* pellets by microprobe measurements. *Biotechnol Bioeng*. 1986;28(7):1024-1036.
61. Mehmood N, Olmos E, Goergen J-L, et al. Oxygen supply controls the onset of pristnamycins production by *Streptomyces pristinaespiralis* in shaking flasks. *Biotechnol Bioeng*. 2011;108(9):2151-2161.
62. Roa Engel CA, van Gulik WM, Marang L, van der Wielen LAM, Straathof AJJ. Development of a low pH fermentation strategy for fumaric acid production by *Rhizopus oryzae*. *Enzyme Microb Technol*. 2011;48(1):39-47.
63. Engelbert K, Deffur C, Cairns TC, et al. Adjusting *Aspergillus niger* pellet diameter, population heterogeneity, and core architecture during shake flask cultivation. *Biotechnol Biofuels Bioprod*. 2025;18(1):62.
64. Heyman B, Lamm R, Tulke H, Regestein L, Büchs J. Shake flask methodology for assessing the influence of the maximum oxygen transfer capacity on 2,3-butanediol production. *Microb Cell Fact*. 2019;18(1):78.
65. Ribeiro RMMGP, Esperança MN, Sousa APA, Neto ÁB, Cerri MO. Individual effect of shear rate and oxygen transfer on clavulanic acid production by *Streptomyces clavuligerus*. *Bioprocess Biosyst Eng*. 2021;44(8):1721-1732.
66. Zhu T, Wang L, Wang W, et al. Enhanced production of lipstatin from *Streptomyces toxytricini* by optimizing fermentation conditions and medium. *J Gen Appl Microbiol*. 2014;60(3):106-111.
67. Dmitrieva ME, Malygina EV, Belyshenko AY, et al. The effects of a high concentration of dissolved oxygen on actinobacteria from Lake Baikal. *Metabolites*. 2023;13(7):830.

SUPPORTING INFORMATION

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