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Utilization of specific power input measurements for optimization of culture conditions in shaking flasks

S. Lotter, J. Büchs*

Biochemical Engineering, RWTH Aachen University, Worringerweg 1, Aachen 52056, Germany

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Abstract

Shaking flasks are often used in early stages of bioprocess development, particularly in screening purposes. Their advantages include ease of handling and low cost. The direction of bioprocess development is strongly influenced by the performance of microorganisms at these early stages. Therefore shaking conditions must be monitored carefully for limitations, e.g. oxygen limitations, or 'out of phase' conditions. Product formation of the xanthan producing bacterium *Xanthomonas campestris* is severely impaired by 'out of phase' shaking conditions. This could be shown for different shaking diameters and filling volumes. The dimensionless phase number could successfully be applied to prevent unsuitable 'out of phase' conditions. Cultures of *Pseudomonas putida CA3* grown at 'in phase' and 'out of phase' hydrodynamic states, but otherwise identical conditions, also shows a lower optical density for 'out of phase' conditions. With help of power consumption measurements these unsuitable shaking conditions can be detected and avoided.
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Keywords: Shaking flasks; Bioprocess design; Specific power consumption; Viscous fluids; Out-of-phase conditions

1. Introduction

For stirred bioreactors a variety of possibilities exist for measuring or calculating the volumetric power consumption P/V_L. Shaking bioreactors, however, have rarely been examined in terms of power input. Sumino et al. [1] carried out measurements in 200 ml shaking flasks whereas Kato et al. [2] investigated large cylindrical vessels with high filling volumes up to $V_L = 4000$ ml used in plant cell culture. Both found the power input to be of the same order of magnitude as in stirred tanks. Büchs et al. [3,4] examined a wide range of shaking conditions, leading to a correlation for the calculation of the power consumption for given shaking conditions in unbaffled shaking flasks. For viscous fluids a sudden decrease in power consumption was measured in combination with a change in flow regime. An increasing amount of fluid remained at the bottom of the flask, not contributing to power consumption and also reducing mixing and oxygen transfer capacities. These 'out of phase' conditions were described with help of the phase number Ph (Eq. (1)) which is derived from those power consumption measurements. The influence of gravity can be neglected if the ratio of radial and axial acceleration, the Froude number Fr_a (Eq. (3)), is high enough:

$$Ph = \frac{d_0}{d}(1 + 3\log Re_f) > 1.26 \tag{1}$$

$$Re_f = \frac{\rho n d^2}{\eta}\frac{\pi}{2}\left(1 - \sqrt{1 - \frac{4}{\pi}\left(\frac{V_L^{1/3}}{d}\right)^2}\right)^2 \tag{2}$$

$$Fr_a = \frac{(2\pi n)^2 d_0}{2g} > 0.4 \tag{3}$$

Similar 'out of phase' conditions exist for low viscosities in baffled flasks and in large shaking flasks [5]. Apart from the flask diameter d, one of the factors which strongly influences fluid movement in the shaking flask, and thus the phase number, is viscosity. Higher liquid viscosities increase the probability of the liquid rotating 'out of phase'. Thus shaking conditions for the cultivation of microorganisms, which increase viscosity, such as antibiotics fermentations, have to be set carefully.

The model organism, the bacterium *Xanthomonas campestris*, is used to show how growth and product formation are influenced by the 'out of phase' phenomenon in small shaking flasks and viscous liquids. This organism

* Corresponding author. Present address: Biochemical Engineering, RWTH Aachen University, Worringerweg 1, Aachen 52056, Germany. Tel.: +49-241-8025546; fax: +49-241-8022265.
E-mail address: buechs@biovt.rwth-aachen.de (J. Büchs).

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Nomenclature

d	flask diameter (m)
d_0	shaking diameter (m)
$Fr_a = (2\pi n)^2 d_0/2g$	axial Froude number
K	consistency index (N s^m/m^2)
m	flow behaviour index
n	shaking frequency (l/s)
$Ne = P/(\rho n^3 d^4 V_L^{1/3})$	power number
OTR	oxygen transfer rate (mol/(l h))
OTR_{max}	maximum oxygen transfer rate (mol/(l h))
P	power (W)
$Ph = (d_0/d)(1 + 3\log Re_f)$	phase number
P/V_L	power consumption (kW/m^3)
$Re = (\rho n d^2)/\eta$	flask Reynolds number
$Re_b = Re(\pi/2)[1 - \sqrt{(1 - (4/\pi)(V_L^{1/3}/d)^2)}]^2$	film Reynolds number
T	temperature (°C)
V_F	(nominal) flask volume (m^3)
V_L	filling volume (m^3)
Greek symbols	
$\dot{\gamma}$	shear rate (l/s)
η	dynamic viscosity (Pa s)
ρ	density (kg/m^3)
τ	shear stress (N/m^2)

produces xanthan, in aqueous solution a highly viscous biopolymer, which is used in food production and oil industry. The rheological behaviour of these fermentation broths is similar to antibiotic fermentations [8]. During fermentation of *X. campestris* both broth viscosity and non-newtonian behaviour of the broth increase [9] while shaking conditions have an effect on product formation in shaking flasks [10]. If oxygen is limited, product formation of *X. campestris* decreases in stirred bioreactors [11], leading to lower final viscosities. This effect can also be observed in shaking flasks [12] since xanthan concentration in baffled flasks is higher than in unbaffled flasks.

During cultivation of *Pseudomonas putida CA3*, the second model organism, broth viscosity remains low. Therefore large shaking flasks are used to create 'out of phase' conditions.

2. Materials and methods

2.1. Cultivation of Xanthomonas campestris and Pseudomonas putida CA3

X. campestris NRRL B 1459 (DSM 1706, 'German Collection of Microorganisms and Cell Cultures') was maintained on agar plates at $T = 4$ °C, and transferred to a fresh plate every 3 weeks. For experiments the bacterium was transferred to a fresh agar plate and grew for 24 h at $T = 30$ °C before being transferred to the liquid preculture. Here a 250 ml shaking flask with a filling volume of $V_L = 25$ ml was used on an orbital shaker (Labshaker, Kühner AG, Switzerland) with a shaking diameter of $d_o = 5$ cm and a shaking frequency of $n = 200$ l/min at 30 °C. After 24 h the main cultures were inoculated 1% from the main preculture. For the main culture the following conditions were kept constant for each experiment: flask size ($V_F = 250$ ml), temperature ($T = 30$ °C), shaking frequency ($n = 200$ l/min), amount of inoculum (percentage of filling volume V_L) and medium composition, whereas shaking diameter d_o and filling volume V_L were varied.

The following medium for *X. campestris* is recommended by DSMZ and had the following components: glucose (10 g/l), yeast extract (20 g/l), $CaCO_3$ (10 g/l). Agar agar (2 g/l) is added for cultivation on plates. All substances were heated separately.

P. putida CA3 was grown on a synthetic medium containing glucose (15 g/l), a buffer solution, a trace element solution and a salt solution. The buffer solution consisted of K_2HPO_4 (22.9 g/l), KH_2PO_4 (4.8 g/l) and Na_2HPO_4 (1.7 g/l), whereas the trace element solution contained: $ZnSO_4{\cdot}7H_2O$ (0.0072 g/l), $Fe_2(SO_4)_3$ (0.04 g/l), $MnSO_4{\cdot}H_2O$ (0.0085 g/l), $CuSO_4{\cdot}5H_2O$ (0.0012 g/l), H_3BO_3 (0.0003 g/l), and $CoSO_4{\cdot}7H_2O$ (0.0028 g/l). The salt solution consisted of $Na_2MoO_4{\cdot}2H_2O$ (0.000024 g/l), $(NH_4)_2SO_4$ (10.57 g/l), Na_2SO_4 (0.142 g/l), NTA (0.764 g/l), $MgSO_4{\cdot}7H_2O$ (0.308 g/l), and $CaCl_2{\cdot}2H_2O$ (0.0029 g/l).

The trace element solution and the salt solution can be prepared together. Glucose and buffer solution have to be prepared and heated separately. These concentrations given above are final concentrations of the medium after mixing the different solutions. The preculture for *P. putida CA3* was cultivated for 24 h at 30 °C in a 500 ml shaking flask with a filling volume of $V_L = 50$ ml of the above medium using a shaking diameter of $d_o = 5$ cm and a shaking frequency of $n = 200$ l/min. The experiments were inoculated with 1% of the main culture volume.

2.2. Monitoring of growth and rheological measurements

Growth of *X. campestris* was monitored directly by measuring the oxygen transfer rate (OTR) [13] and indirectly by measuring viscosity. The oxygen transfer rate can be measured for six special 250 ml shaking flasks in parallel, allowing for duplicates. The head space of these flasks is modified to hold an oxygen sensor. However, both the bottom of the flask and mass transfer conditions are identical to normal shaking flasks. Some normal shaking flasks with the same filling volume are cultivated in parallel to take samples for viscosity measurements.

Broth rheology was studied in a cone and plate rheometer (Paar Physica Messtechnik, Germany, cone no. MK 91/6) at shear rates between $\dot{\gamma} = 10$ and 3000 l/s and a fixed temperature of $T = 22$ °C. Unless otherwise specified viscosities are always given at a shear rate of $\dot{\gamma} = 300$ l/s. The rheological data were fitted using the power law equation:

$$\tau = K\dot{\gamma}^m \quad (4)$$

Short videos of the rotating liquid were taken automatically in intervals of 5 min to observe, if and when an 'out of phase' state occurred. Photos of the fluid movement were extracted from these videos. A small ball which followed the acceleration of the shaking machine was placed in another flask to indicate the direction of the centrifugal acceleration for each photo.

Growth of *P. putida CA3* was monitored by optical density from a 1:60 dilution at 580 nm in a Kontron (Unikon 922) photometer. The dry biomass concentration was determined gravimetrically.

2.3. Measurement of power consumption and oxygen transfer capacity

For determination of volumetric power input the torque sensor technique as described by Büchs et al. [5] was applied. The oxygen transfer capacity was determined as mentioned in Hermann et al. [6].

3. Results and discussion

3.1. 'Out of phase' conditions in small shaking flasks

During cultivation of *X. campestris* in 250 ml shaking flasks (shaking diameter $d_o = 5$ cm) broth viscosity increases strongly (Fig. 1), corresponding well to literature data for stirred tank fermentations [9,11,14] as well as cultivation in shaking flasks [12]. Higher viscosity also means higher concentration of xanthan (not measured) in the fermentation broth. Over time the non-newtonian behaviour of the fermentation broth also increases, shown by the steeper slope of the curves. After 24 h the final broth viscosity of $\eta \approx 120$ mPa s is reached.

Fig. 2A shows product generation of *X. campestris* at different shaking diameters with otherwise constant shaking conditions. Viscosity at a constant shear rate of $\dot{\gamma} = 300$ l/s is plotted versus culture time. High shaking diameters such as $d_o = 5$ and 7 cm lead to a final and constant viscosity of $\eta > 100$ mPa s after about 24 h of fermentation. This indicates the end of fermentation. Fermentation takes longer

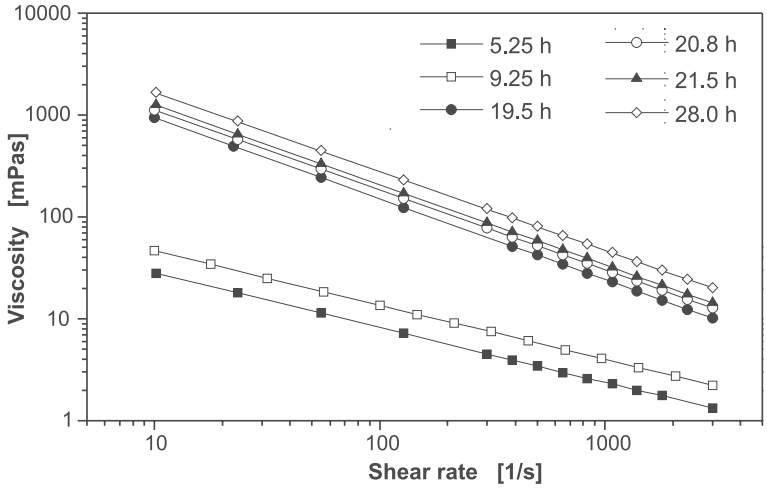

Fig. 1. Viscosity development of *Xanthomonas campestris* during fermentation in 250 ml shaking flasks (shaking diameter $d_o = 5$ cm, shaking frequency $n = 200$ l/min, filling volume $V_L = 25$ ml). Viscosity given at a shear rate of $\dot{\gamma} = 300$ l/s.

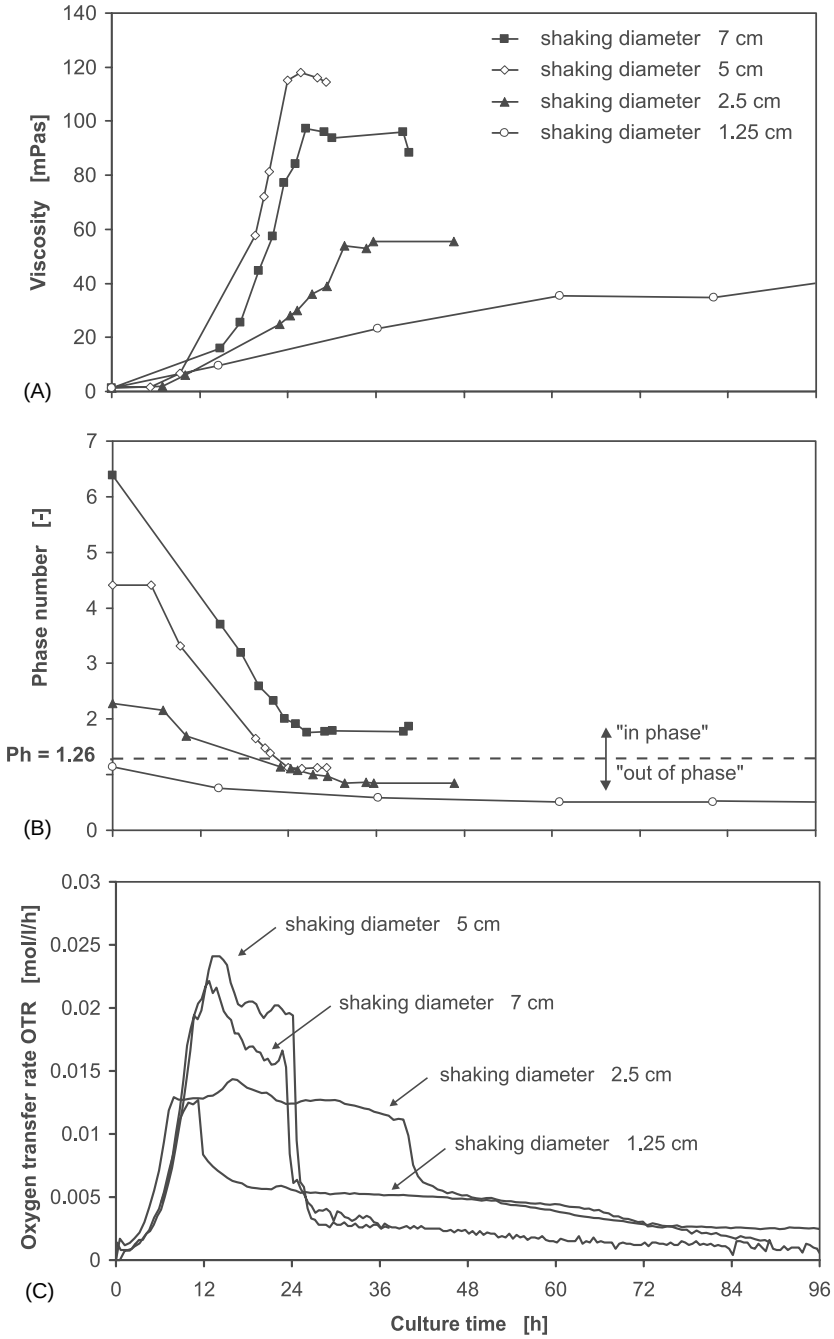

Fig. 2. Fermentation of *Xanthomonas campesteris* in 250 ml shaking flasks (shaking frequency $n = 200$ l/min, filling volume $V_L = 25$ ml, different shaking diameters d_o). Viscosity given at a shear rate of $\dot{\gamma} = 300$ l/s. (A) Viscosity development. (B) Phase number *Ph*. (C) Oxygen transfer rate (OTR).

(about 30 h) to reach constant broth viscosity for a smaller shaking diameter ($d_o = 2.5$ cm) and a lower final viscosity of $\eta = 60$ mPa s is reached. Growth at a shaking diameter of $d_o = 1.25$ cm takes even longer; after 96 h of cultivation a constant viscosity is not yet reached. Therefore, the shaking diameter strongly influences growth conditions for viscous fermentations. It should be noted that in a vast majority of publications found in all types of journals no information is given on the applied shaking machine and its orbital shaking diameter.

The phase number *Ph* (Eq. (1)) indicates the flow regime in shaking flasks. Fig. 2B shows an application of the phase number to the fermentations mentioned above. Values below $Ph = 1.26$ are considered 'out of phase', above $Ph = 1.26$ 'in phase'. The reduction of the phase number with increasing culture time is a result of the increase in broth viscosity. Whereas for the two larger shaking diameters ($d_o = 7$ and 5 cm) the phase number indicates an 'in phase' regime until the end of the fermentation, cultivation at smaller shaking diameters ($d_o = 2.5$ and 1.25 cm) partially takes place in the

'out of phase' region. Especially the fermentation with the smallest shaking diameter remains completely in the 'out of phase' region. These cultivations in the 'out of phase' region also show lower final viscosities.

The oxygen transfer rates develop similarly over time during the first phase of the fermentation (Fig. 2C). Almost without a lag time the oxygen transfer rates increase exponentially for all fermentations. This indicates that medium and inoculum are equivalent for all fermentations. Depending on the shaking diameter a different maximum oxygen transfer rate is OTR_{max} reached. For both large shaking diameters ($d_o = 7$ and 5 cm) the OTR increases during the first 12 h up to $OTR_{max} \approx 0.024$ mol/(l h), dropping slightly during the next 12 h (the fermentation for $d_o = 7$ cm always has a lower OTR than the fermentation for $d_o = 5$ cm, the reason for this is not clear) in order to drop sharply after 24–26 h. This sharp decrease is due to the depletion of the carbon source and indicates the end of the fermentation. At the same time the broth viscosity ceases to increase, as shown in Fig. 2A.

For the shaking diameter $d_o = 2.5$ cm the oxygen transfer rate also increases exponentially to a maximum of $OTR_{max} \approx 0.12$ mol/(l h) and remains at this level until the carbon source is depleted after about 40 h of fermentation (Fig. 2C). The constant OTR indicates an oxygen limitation between 12 and 40 h of cultivation. For the shaking diameter $d_o = 1.25$ cm there is a sharp drop in OTR after 12 h followed by a slowly decreasing OTR for the remaining fermentation time. The low OTR level after 12 h is again a indication of oxygen limitation, because this level remains above the value which indicates the depletion of the main carbon source. In addition to this, product is still generated (increasing viscosity in Fig. 2A). Reduced mass transfer in viscous liquids could be the reason for the slow decrease of OTR after 12 h. The sudden drop of the OTR after 12 h, however, must have a more severe cause, which will be discussed later.

For otherwise constant shaking conditions the maximum OTR increases with increasing shaking diameter [7]. This effect is also visible for fermentations of *X. campestris*. Here the level of OTR during fermentation corresponds to the final broth viscosity (Fig. 2A and C). For cultivations with significant oxygen limitation ($d_o = 2.5$ and 1.25 cm) the final viscosity is much lower than for unlimited ($d_o = 7$ and 5 cm) fermentations. This is similar to results of Galindo et al. [12], where baffled flasks with high oxygen transfer rates show higher yields than unbaffled Fernbach flasks. Suh et al. [11] show that during oxygen limitation in stirred bioreactors lower mean molecular weights, and thus lower viscosities are reached.

To prevent oxygen limitation and thus lower productivity the filling volume V_L can be reduced [7,15]. However, for a shaking diameter of $d_o = 2.5$ cm the final viscosity does not increase (Fig. 3A) although the filling volume has been reduced from $V_L = 25$ to 10 ml. The time course of the increase in viscosity is different for both filling volumes. For the smaller filling volume viscosity increases strongly during the first 12 h and then increases slowly during the next 24 h.

The reason for the slow increase in viscosity and the unexpectedly low final viscosity for the filling volume $V_L = 10$ ml can be found if the oxygen transfer rate is considered. Fig. 3B shows both the OTR and the phase number for the two filling volumes. An oxygen limitation is observed for the larger filling volume ($V_L = 25$ ml) at a level of about 0.013 mol/(l h) and the corresponding phase number drops into the 'out of phase' region after 15 h. Both the oxygen transfer and the phase number curves are different for the smaller filling volume. The OTR increases exponentially during the first 12 h of cultivation. As intended by reducing the filling volume the OTR_{max} is much higher than for the larger filling volume and an oxygen limitation does not occur. Nevertheless, the oxygen transfer rate drops to a third of its original value from $OTR_{max} = 0.027$ mol/(l h) to $OTR \approx 0.09$ mol/(l h) within 2 h and decreases further during the remaining fermentation time. Since a low OTR level is reached, the resulting oxygen limitation causes the xanthan production to slow down, decreasing the slope of the viscosity curve (Fig. 3A) at about the same time as the OTR drops. The phase number depicted in Fig. 3B indicates that the cultivation with the smaller filling volume ($V_L = 10$ ml) takes place in the 'out of phase' region after 12 h of fermentation. The phase number for the larger filling volume ($V_L = 25$ ml) also decreases below the critical phase number of $Ph = 1.26$ after 16 h, although the oxygen transfer rate is not affected. However the phase number (Eq. (1)) depends on the broth viscosity, which is a function of the average shear rate $\dot{\gamma}$ for pseudoplastic fluids such as xanthan solutions. Most likely the true average shear rate is higher than the assumed value of $\dot{\gamma} = 300$ l/s for these shaking conditions.

Photos of the rotating liquid (now shown) taken during the fermentation show a complete change in fluid movement at 12 h of fermentation for the filling volume of $V_L = 10$ ml. Whereas at the beginning of the cultivation the liquid rotates along the flask wall following the centrifugal acceleration, with the bottom of the flask remaining dry, after 12 h of fermentation the flow regime changes. Now the liquid is located at the bottom of the flask while the flask wall is dry. This 'out of phase' state is preserved until the end of the fermentation. These observations are in full agreement with the results shown in Fig. 3. Because of a slight increase in viscosity to $\eta \approx 30$ mPa s (Fig. 3A, $V_L = 10$ ml) the flow regime in the shaking flask changes completely, leading to cultivation in the unsuitable 'out of phase' region with low oxygen transfer rates and little mixing.

The same kind of sudden drop in oxygen transfer has been shown for the shaking diameter $d_o = 1.25$ cm (Fig. 2C) and a filling volume of $V_L = 25$ ml. Here the reduced oxygen transfer rate and the low final broth viscosity are also caused by the transition from the 'in phase' to the 'out of phase' flow regime. In both cases, $V_L = 25$ ml, $d_o = 1.25$ cm (Fig. 2B)

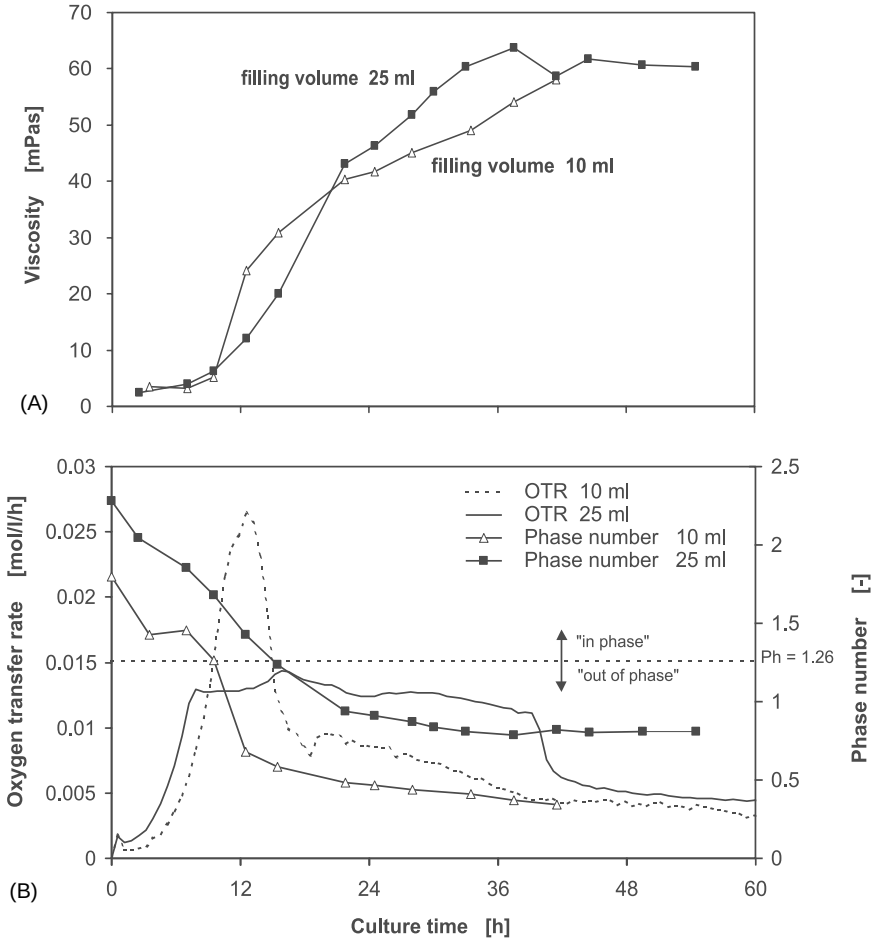

Fig. 3. Fermentation of *Xanthomonas campestris* in 250 ml shaking flasks (shaking frequency $n = 200$ l/min, shaking diameter $d_o = 2.5$ cm, different filling volumes V_L). Viscosity given at a shear rate of $\dot{\gamma} = 300$ l/s. (A) Viscosity development. (B) Oxygen transfer rate OTR and phase number *Ph*.

and $V_L = 10$ ml, $d_o = 2.5$ cm (Fig. 3B), the phase number was clearly below the critical value of $Ph = 1.26$. The calculation of the phase number (with estimated viscosities) before carrying out the experiments could, therefore, have pointed towards using different shaking conditions and thus preventing problems during cultivation.

For the filling volume $V_L = 25$ ml and shaking diameter $d_o = 2.5$ cm the phase number (Figs. 2B and 3B) also indicates cultivation in the 'out of phase' region although only growth or OTR (Figs. 2C and 3B) seem to be affected by oxygen limitation. The limitation, however, is at least partly caused by a change in liquid flow (Fig. 4). Here the centrifugal acceleration points to the left, which is also the direction of the maximum liquid height. A thin layer of lime ($CaCO_3$) sticks to the wall while the liquid rotates along the flask wall. After 18 h of fermentation the lime layer has increased, therefore the maximum liquid height must have decreased, leading to a smaller gas/liquid mass transfer area. During the next 27 h of fermentation the maximum liquid height drops further. At the same time the liquid is slightly shifted counterclockwise, liquid rotation and centrifugal acceleration are no longer 'in phase' (phase number $Ph \leq 1.26$). This fermentation is also affected by the change in flow regime.

3.2. 'Out of phase' conditions in large shaking flasks

For shaking flasks with a nominal volume of at least 2000 ml, 'out of phase' conditions can occur even for waterlike viscosities [5]. Measurements of power consumption (Fig. 5, full symbols) show that with slowly increasing shaking frequency the power consumption also increases. If, however, the shaking machine is accelerated quickly, a low level of power input is reached (Fig. 5, empty symbols), beyond a specific shaking frequency. Reducing the shaking frequency below a critical value ($n = 180$ l/min) leads to a sudden increase in power input reaching the standard level again.

It should be emphasized that this kind of 'out of phase' phenomenon is different from the phenomenon described above for small flasks at elevated viscosities. In the initially described case an increase of the shaking frequency will result in to 'in phase' operation. No hysteresis is observed for newtonian fluids. Whereas, in the latter case of large

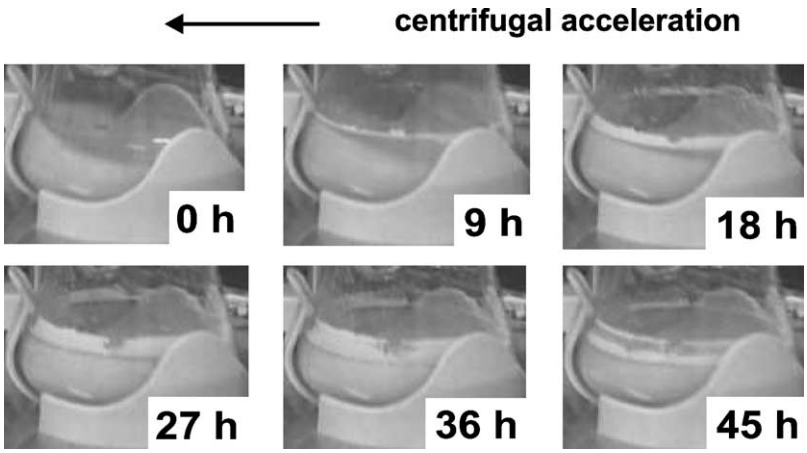

Fig. 4. Flow regime of *Xanthomonas campestris* during fermentation in 250 ml shaking flasks with small ($V_L = 25$ ml) filling volumes (shaking frequency $n = 200$ l/min, shaking diameter $d_o = 2.5$ cm).

flasks at low viscosity an increasing shaking frequency will cause the 'out of phase' flow regime. Depending on the operating conditions a significant hysteresis is observed [5]. Similar transitions of the flow regime have been described for rotating partially filled horizontal vessels [16,17].

Fig. 5 shows that at the same shaking frequency two completely different levels of power consumption are possible. This occurs because of different hydrodynamic regimes: 'in phase' and 'out of phase' movement of the liquid. 'Out of phase' conditions in large flasks at waterlike viscosities are possible if the flask is quickly accelerated. This may occur, if:

- the shaking machine has a strong engine;
- the load of the machine is low (small number of flasks placed on the shaker table);
- or shaking flasks which were removed from the shaking table (e.g. for sampling) are placed back on the running machine.

In the last case flasks with the same filling volume can be observed at 'in phase' and 'out of phase' states on a single shaking machine at the same time. Fig. 6 shows an example during the cultivation of *P. putida CA3*. Here the flask on the right has been removed from the tray and placed back onto the running tray leading to 'out of phase' conditions. Instead of rotating along the flask wall as in the 'in phase' case on the left, most of the liquid remains in the middle, while a small wave is rolling through the flask at a positions opposite to the direction of the centrifugal acceleration. An analogy to this behaviour can be found for rotating partially filled horizontal vessels [16,17]. Removing the flask on the right of Fig. 6 once at the beginning of the fermentation and replacing it on the running shaker is the only difference during cultivation. Nevertheless, the courses of the growth curves for 'in phase' and 'out of phase' conditions in Fig. 7 are different. In both cases at the beginning the optical density increases exponentially. After 12 h of cultivation, however, the slopes become linear with a steeper slope for the 'in phase' case.

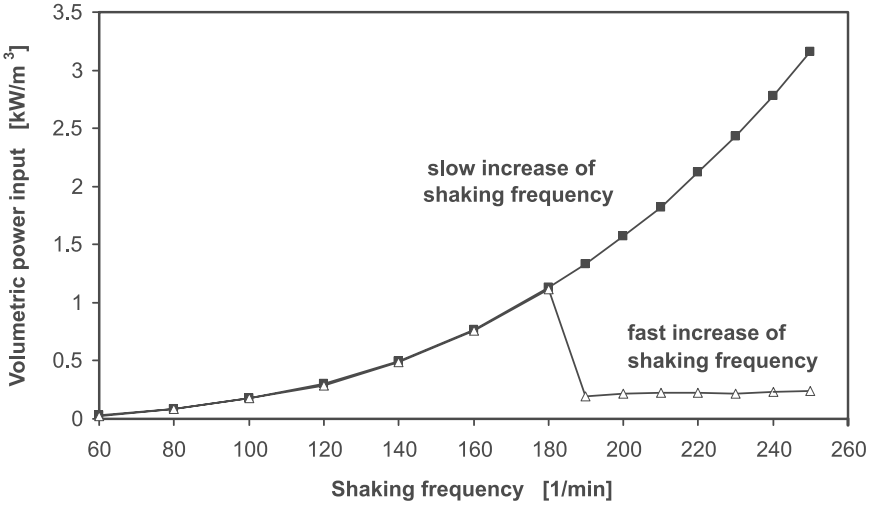

Fig. 5. Power consumption in a 2000 ml shaking flask filled with $V_L = 200$ ml of water at a shaking diameter of $d_o = 2.5$ cm and different shaking frequencies n (full symbols, slowly increasing shaking frequency; empty symbols, fast acceleration to high shaking frequency ($n \geq 200$ l/min)).

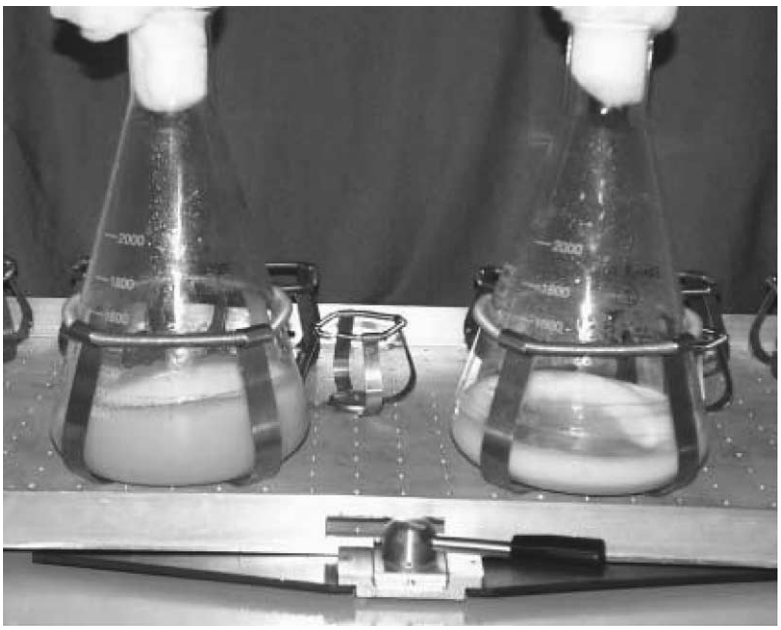

Fig. 6. Fermentation of *Pseudomonas putida CA3* in 2000 ml shaking flasks filled with $V_L = 200$ ml at a shaking diameter of $d_o = 2.5$ cm and a shaking frequency of $n = 230$ l/min at 'in phase' (left) and 'out of phase' (right) conditions. After inoculation the right flask war removed from the shaker table and immediately afterwards replaced on the running machine.

The reason for the linear increase of the optical density from 12 h is an oxygen limitation. With the method used by Duetz et al. [18] for the same microorganism oxygen transfer rates of roughly OTR = 0.017 mol/(l h) ('in phase') and OTR = 0.0108 mol/(l h) ('out of phase') can be derived from the two slopes. Those values correspond nicely with the maximum oxygen transfer capacity measured by the sulfite method [6]. At 'in phase' conditions a maximum oxygen transfer capacity of $OTR_{max} = 0.0135$ mol/(l h) is reached, for 'out of phase' conditions only $OTR_{max} = 0.0119$ mol/(l h) are possible. Therefore growth at 'out of phase' conditions is slower and results in a lower final dry biomass concentration of 5.9 g/l compared to 7.5 g/l for 'in phase' conditions. The two different types of fluid movement, therefore, cause different levels of oxygen transfer capacity which lead to slower growth for 'out of phase' conditions.

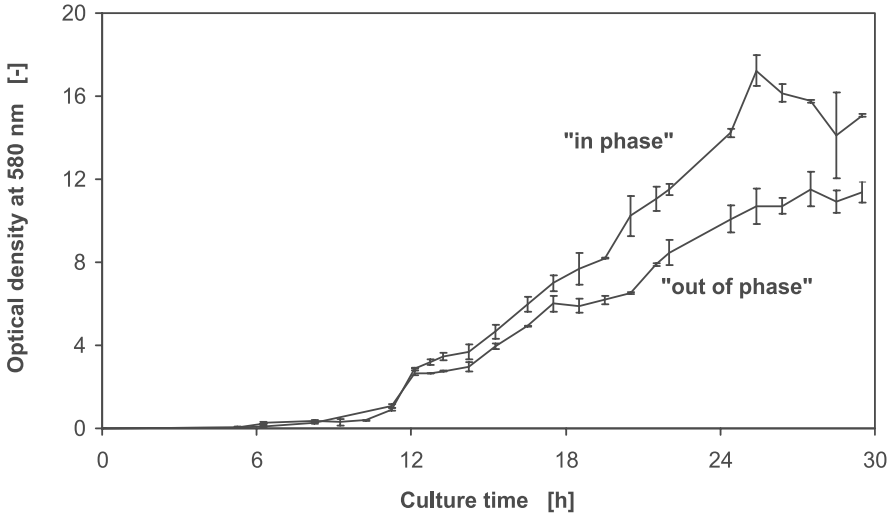

Fig. 7. Optical density of *Pseudomonas putida CA3* in 2000 ml shaking flasks filled with $V_L = 200$ ml at 'in phase' and 'out of phase' conditions (shaking diameter of $d_o = 2.5$ cm and a shaking frequency of $n = 230$ l/min). For each measurement two samples were taken from different flasks. Final dry biomass concentration: 7.5 g/l for 'in phase' conditions 5.9 g/l for 'out of phase' conditions.

4. Conclusion

'Out of phase' shaking conditions have been shown for both, viscous fermentations in 250 ml shaking flasks and low-viscosity fermentations in 2000 ml shaking flasks to strongly reduce growth or product formation. For *P. putida CA3* this is probably due to lower oxygen transfer capacity at those unsuitable shaking conditions. *X. campestris* reaches lower maximum viscosities, therefore producing less xanthan if cultivated at oxygen limitation. Both shaking diameter d_o and filling volume V_L have a direct influence on the occurrence of an oxygen limitation during cultivation, caused by a change in flow regime. These parameters, shaking diameter d_o and filling volume V_L, strongly influence the flow regime in the shaking flask, leading to unsuitable 'out of phase' conditions if not selected with care. For viscous fermentations the flow regime can be predicted with help of the phase number *Ph* (Eq. (1)) which has been derived from power consumption measurements.

Acknowledgements

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