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Research Article

Jet aeration as alternative to overcome mass transfer limitation of stirred bioreactors

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In industrial biotechnology increasing reactor volumes have the potential to reduce production costs. Whenever the achievable space time yield is determined by the mass transfer performance of the reactor, energy efficiency plays an important role to meet the requirements regarding low investment and operating costs. Based on theoretical calculations, compared to bubble column, airlift reactor, and aerated stirred tank, the jet loop reactor shows the potential for an enhanced energetic efficiency at high mass transfer rates. Interestingly, its technical application in standard biotechnological production processes has not yet been realized. Compared to a stirred tank reactor powered by Rushton turbines, maximum oxygen transfer rates about 200% higher were achieved in a jet loop reactor at identical power input in a fed batch fermentation process. Moreover, a model-based analysis of yield coefficients and growth kinetics showed that *E. coli* can be cultivated in jet loop reactors without significant differences in biomass growth. Based on an aerobic fermentation process, the assessment of energetic oxygen transfer efficiency [$\text{kgO}_2 \text{ kW}^{-1} \text{ h}^{-1}$] for a jet loop reactor yielded an improvement of almost 100%. The jet loop reactor could be operated at mass transfer rates 67% higher compared to a stirred tank. Thus, an increase of 40% in maximum space time yield [$\text{kg m}^{-3} \text{ h}^{-1}$] could be observed.

Keywords: Aerobic bioprocess / Energetic efficiency / Jet loop reactor / Mass transfer performance / Productivity

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1 Introduction

To achieve techno-economic success, many sectors in industrial biotechnology, such as industrial enzyme production and production of commoditized intermediates demand both large production volumes and low production cost. Thus, both aspects, investment needs as well as operating costs play a major role when deciding on future production scenarios. Stirred tank reactors (STRs) are commonly used and highly valued for their benefits regarding easy operability. At cultivation volumes larger than 500 m³, the traditionally used aerated stirred tank reactors become excessively expensive, if applicable at all. Costs are increased due to mechanically limited volumetric power inputs (up to 5 kW m⁻³) and low energy efficiencies for the required air compression. Therefore, bubble columns and airlift loop re-

actors are often used instead of stirred tank reactors at volumes larger than 500 m³. However, volumetric power inputs and subsequently oxygen mass transfer rates (OTRs) of such apparatuses are limited [1]. If the gas liquid mass transfer is the limiting parameter for product formation, the generated OTR sets the boundary for the achievable space-time yield in the reaction system.

As known from chemical engineering the jet loop reactor (JLR) economically provides high power inputs ($>5 \text{ kW m}^{-3}$) in large-scale operations [1, 2]. JLRs are an efficient alternative to STRs and offer an easier scale up [3]. In coalescence inhibited systems a larger gas liquid interface can be generated with a JLR, compared to a STR. By overcoming mass transfer limitations, significantly higher space time yields can be achieved [4, 5]. Beside the application in the chemical industry, JLRs are frequently used for the biocatalytic treatment in wastewater applications [6, 7] and due to their high mass transfer performance, JLRs can be operated at high biomass concentrations [8].

Over the last decades several attempts have been made to apply JLR principles in fermentation processes [9–15]. Although the general applicability was shown, their commercial application in biotechnological production processes as mentioned above has not been realized yet or was not reported in literature.

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Abbreviations: DCW, dry cell weight; JLR, jet loop reactor; OTR, oxygen transfer rate; OUR, oxygen uptake rate; STR, stirred tank reactor; STY, space time yield

Bacterial growth systems that yield high biomass concentrations are of considerable interest for industrial biotechnology, e.g. in enzyme production. Most processes are operated in fed batch mode to control the specific growth rate (μ) (or specific substrate uptake rate q_s) and to avoid phenomena such as overflow metabolism, catabolite repression, and/or oxygen limitation [16]. In contrast to continuous (chemical) processes where the JLR with its fixed internals can be operated constantly at ideal process parameters, an operation in fed batch mode is more challenging due to altering filling levels, changing media properties and increasing power demands for mixing and mass transfer. As a consequence, occurring micro environmental heterogeneities arising from high local substrate and oxygen consumption might decrease the obtainable yields and productivity by the induction of anaerobic genes and the formation of anaerobic metabolites [17–19]. The paper aims to investigate the application and potential benefits of JLR usage compared to STR based on an industrial fed batch process. The presented study is motivated to compare the process performance of a STR to a JLR at the same specific power input for a chemical conversion system and a bacterial cultivation system. The study focusses on the oxygen mass transfer OTR [$\text{mmol L}^{-1} \text{h}^{-1}$], its energetic efficiency E [$\text{kgO}_2 \text{ kW}^{-1} \text{h}^{-1}$] and the achievable space time yield (STY) [$\text{kg m}^{-3} \text{h}^{-1}$] over process time. Potential negative effects for the microbial cultivation in a JLR compared to a STR are monitored.

2 Materials and methods

2.1 Description of JLR and STR

Both JLR and STR were based on an Infors Labfors 5 glass fermentation unit with a total volume of 7.5 L and a H:D ratio of 3 (Fig. 1). Controlled and monitored process variables were temperature, airflow, agitator- (STR), and pump speed (JLR) as well as dissolved oxygen levels and feed rates. The pH-value was measured via a pH sensors with a total length of 425 mm

(EasyFerm, Hamilton, Switzerland). The pH-value was maintained at a value above 7 by the addition of NH_4OH (10%) via a peristaltic pump (Infors HT, Switzerland) and was controlled by a one sided PI controller. The setpoint temperature of 37°C was measured by an PT100 sensor and maintained via the double jacket of the vessel. The integrated heating cartridge controlled the temperature of the thermostating liquid (1% w/w diethylene glycol). Cooling was provided by a thermostat (FL601, Julabo, Germany). A mass flow controller (red-y smart controller GSC-C, Vögtlin, Switzerland) controlled the inflow of air. The dissolved oxygen level was measured by an optical oxygen sensor with a total length of 425 mm (VisiFerm, Hamilton, Switzerland) calibrated on 100% air saturation in fresh medium at 37°C at 1 atm. Oxygen and carbon dioxide levels in the off gas were measured with gas analyzers (BlueInOne Ferm, Bluesens, Germany). For the determination of k_L values by the static method, oxygen levels were measured by an optical oxygen sensor with a total length of 120 and 425 mm (PST3, Presens, Germany). The sensor was calibrated to 100% air saturation (at 1 atm) in fresh air at offgas temperature. A peristaltic pump (Infors HT, Switzerland) added feed solution via the reactor head (STR) or via a feeding pipe into the nozzle outlet (JLR). The vessel itself and the containers for feed and pH-control agents were set up on balances (Sartorius, Germany) for the estimation of liquid levels and feed rates. The STR was agitated via a mechanically sealed agitator shaft, which was powered by an electric motor ($\text{BG56} \times 75\text{SI}$, Dunkermotoren, Germany). Mixing and dispersion was done by two six-bladed Rushton turbines with a diameter of 54 mm. The stirrers were installed at a distance of 135 and 55 mm from the reactor bottom. The reactor was equipped with three baffle plates with a width of 15 mm. The STR was supplied with pressurized air via a J-formed open pipe sparger at the reactor bottom. The stirrers were installed at a distance of 135 and 55 mm from the reactor bottom. The reactor was equipped with three baffle plates with a width of 15 mm. The STR was supplied with pressurized air via a J-formed open pipe sparger at the reactor bottom.

One of the Infors Labfors 5 fermentation units was retrofitted to a JLR. The JLR was equipped with an ejector nozzle without swirl device (H804, Schlick, Germany). The diameter of the liquid nozzle orifice was 1 mm. A gear pump (P72380, Gather, Germany) powered the liquid jet. The nozzle was mounted

Figure 1. Overview of reactor setup STR (A) and JLR (B). Increase of initial filling level (3.5 L) by feed and base addition (pH control) monitored by scales. Aeration rate set by mass flow controller. Dissolved oxygen level and pH value measured by internal sensors (quality indication, record, control (QIRC) $_{p\text{O}_2, \text{pH}}$). Temperature measured with PT100 and maintained via double jacket (temperature indication, record, control). Measurement of oxygen and carbon dioxide concentration in vent line by offgas sensor (QIRC $_{p\text{O}_2, p\text{CO}_2}$). Power input STR by stirrer and baffle plates. Power input JLR by pump and nozzle. Bubble deflection at baffle plate.

centrally in downflow configuration and plunged into the liquid phase at an immersion depth of 310 mm. The pressure between the discharge side of the gear pump and the injector had been measured via a manometer (Wika, Germany). The reactor was operated in injector mode (supply with pressurized gas). Pressure between the mass flow controller and the injector was measured via a pressure sensor (Wika, Germany). A baffle plate installed above the liquid outlet prevented gas entrainment.

2.2 Calculation of power input

For comparison of JLR and STR the power input to the liquid phase was calculated. The hydraulic power input via the nozzle was set by adjusting the pressure drop over the nozzle and the flowrate of the pumped liquid (Q_L). For the evaluated small-scale setup pressure losses in the pipeline and hydrostatic effects caused only a negligible pressure drop (≤ 100 mbar). Therefore, the pressure drop for calculation of power input was estimated by the difference between the liquid pressure in front of the liquid nozzle (p_{jet}) and the headspace pressure of the reactor (Eq. (1)). Nozzle pressures from 3.0 bar to 6.0 bar and liquid flow rates from 1.15 to 1.60 L min⁻¹ led to an absolute power input between 5.75 W and 16.00 W. The liquid flow Q_L was determined by volumetric analysis at each nozzle pressure beforehand.

$$P_{drop, jet} = (p_{jet} - p_{reactor}) Q_L \quad (1)$$

For the STRs it was aspired to refer the actual power consumption from torque measurements at the agitator shaft. The torque measurement for the STR in 3.5 L scale was not showing reliable and reproducible results. As an alternative, the hydraulic power input was calculated by Eq. (2) and the loss in power input by aeration was considered by a modified power number Po_b . The respective correlation published by Liepe et al. [17] (Eq. (3)) was explicitly developed for aerated reactors powered by two Rushton turbines with a stirrer to reactor diameter ratio (d_s/d_R) of 0.3 and installation distances of the stirrers ($\Delta h_s/d_s$) larger than 1.

Superficial gas velocities between $0.94 \cdot 10^{-3}$ m s⁻¹ and $4.71 \cdot 10^{-3}$ m s⁻¹ led to a calculated reduction of the power number from 5.6 to values between 4.77 and 3.29. For minimal agitation (540 rpm) and aeration rates (1 L min⁻¹) the hydraulic power input was calculated to be 5.32 W. At maximum agitation (1050 rpm) and aeration rates (5 L min⁻¹) the hydraulic power input was increased up to 16.20 W.

$$P_s = m * P o_b * \rho_L * d_s^5 * n_s^3 \quad (2)$$

$$P o_b = \frac{P o_0}{\sqrt{1 + 490 * \frac{u_g}{\sqrt{g * D_R}}}} \quad (3)$$

In relation to the hydraulic power input the maximum pneumatic power was accounted for <2% of the total value. As air flow rates in JLR and STR were nearly the same for the compared cultivations, the pneumatic power input was neglected within this study.

2.3 OTR determination by sodium sulfite oxidation test

Reference values for the maximum achievable OTRs at $Q_{g,max}$ and P/V_{max} ($K_L a \rightarrow \max$) are generated by the static sulfite oxidation method. The maximum mass transfer performance ($OTR_{\Delta pO_{2max}}$) is determined at dissolved oxygen levels near zero ($\Delta pO_2 \rightarrow \max$).

$$OTR = k_L a (\Delta pO_2) \quad (4)$$

Details on the chemical reaction and the experimental procedure were published by Schulz and Gaden [20]. For evaluation of the obtained mass transfer rates attention must be paid on the reaction kinetics for oxidation of sodium sulfite. In dependence of the added catalyst the order of the reaction and the turnover rate is increased and the reaction can be dislocated from the bulk phase into the boundary layer of gas and liquid. The respective regime can be characterized by Ha numbers, whereas operation in the nonenhanced region ($Ha < 0.3$), should be applied for determinations of mass transfer capacity. Measurements at Ha numbers above the transition regime ($0.3 < Ha < 3.0$) should be applied if the specific gas liquid interface is of major interest [21].

2.4 Fermentation media

All substrates, buffer components, and macro elements for the preculture medium were pre dissolved in 0.4 L of demineralized water, trace elements, and vitamins were added from a respective stock solutions. After an adjustment to a pH-value of 7 with 50% w/w NaOH the concentrated medium was filled up with demineralized water to a final concentration of 5.5 g L⁻¹ Glucose H₂O, 0.72 g L⁻¹ Tricine, 8.25 g L⁻¹ MOPS, 13.3 g L⁻¹ KH₂PO₄, 4.0 g L⁻¹ (NH₄)₂HPO₄, 1.2 g L⁻¹ MgSO₄ 7H₂O, 1.7 g L⁻¹ C₆H₈O₇, 0.0084 g L⁻¹ EDTA, 0.0025 g L⁻¹ CoCl₂ 6H₂O, 0.015 g L⁻¹ MnCl₂ 4H₂O, 0.0015 g L⁻¹ CuCl₂ 4H₂O, 0.003 g L⁻¹ BH₂NaO₄, 0.0025 g L⁻¹ Na₂MoO₄ 2H₂O, 0.0013 g L⁻¹ Zn(CH₃COO)₂ 2H₂O, 0.1 g L⁻¹ Fe III-citrate, 0.0045 g L⁻¹ Thiamine HCl. The completed preculture medium was sterile filtered (pore size 0.2 μm) and stored at room temperature. All macro elements for the initial fermentation medium with a final volume of 3.5 L were prepared and pre dissolved in 2 L demineralized water to yield a final concentration of 13.3 g L⁻¹ KH₂PO₄, 4.0 g L⁻¹ (NH₄)₂HPO₄, 1.2 g L⁻¹ MgSO₄ 7H₂O. The concentrated solution was heat sterilized at 121 °C for 30 min. Trace elements and vitamins were added from a sterile filtered (pore size 0.2 μm) stock solution to yield a final concentration 1.7 g L⁻¹ C₆H₈O₇, 0.0084 g L⁻¹ EDTA, 0.0025 g L⁻¹ CoCl₂ · 6H₂O, 0.015 g L⁻¹ MnCl₂ 4H₂O, 0.0015 g L⁻¹ CuCl₂ 4H₂O, 0.003 g L⁻¹ BH₂NaO₄, 0.0025 g L⁻¹ Na₂MoO₄ 2H₂O, 0.0013 g L⁻¹ Zn(CH₃COO)₂ 2H₂O, 0.1 g L⁻¹ FeIII-citrate, 0.0045 g L⁻¹ Thiamine HCl. 0.3 g L⁻¹ Struktol® was added to prevent foam formation. The feed solution with a final concentration of 600 g L⁻¹ Glucose, 20 g L⁻¹ MgSO₄ 7H₂O was heat sterilized at 121 °C for 30 min. Trace elements were added from a sterile filtered (pore size 0.2 μm) stock solution to yield a final concentration of EDTA, 0.0025 g L⁻¹ CoCl₂ 6H₂O, 0.015 g L⁻¹ MnCl₂ 4H₂O, 0.0015 g L⁻¹ CuCl₂ 4H₂O, 0.005 g L⁻¹ BH₂NaO₄, 0.004 g L⁻¹ Na₂MoO₄ 2H₂O, 0.016 g L⁻¹ Zn(CH₃COO)₂ 2H₂O,

0.040 g L⁻¹ FeIII-citrate. NH₄OH (10% w/w) was used as pH control agent.

2.5 Description of the bioprocess

As model organism, a wild-type *Escherichia coli* K12 (ATCC 25404, NCIMB 11290) was chosen. The cultivation started at an initial volume of 3.5 L. No initial batch phase was conducted. The inoculation density (0.5 g L⁻¹) was chosen to fit within the minimal dosing rate of the peristaltic feed pump (>1 mL h⁻¹). The cell growth was adjusted to a specific growth rate μ of 0.5 h⁻¹ by an exponential feed profile. Substrate limitation led to a constant substrate concentration c_s in the broth (under detection limit of HPLC).

According to Eq. (5) the OTR in mmol L⁻¹ h⁻¹ is calculated by the airflow Q_g , the filling level (V_L) and the molar volume of the gas phase (V_M). The content of oxygen and carbon dioxide at the air in- and outlet was accounted by $Y_{O_2,in}$, $Y_{CO_2,in}$, $Y_{O_2,out}$ and $Y_{CO_2,out}$, respectively.

$$OTR = \frac{Q_g}{V_L \cdot V_M} \cdot \left(\frac{Y_{O_2,in}}{100} - \frac{Y_{O_2,out}}{100} \cdot \frac{100 - Y_{O_2,in} - Y_{CO_2,in}}{100 - Y_{O_2,out} - Y_{CO_2,out}} \right) \quad (5)$$

For estimation of the maximum achievable OTR at $k_{La,max}$ and $\Delta p_{O_2,max}$ (Eq. (4)) the feed rate and therefore oxygen consumption was increased until full oxygen depletion was reached (Section 3.1). To evaluate the reactor performance for strictly aerobic conditions, fedbatch fermentations with a minimal p_{O_2} level of 20% were performed (Section 3.2). The feed rate was increased with a rate of 0.5 h⁻¹. The p_{O_2} value declined with increasing feed rate until the predefined value of 20% was reached. The exponentially increasing oxygen demand was ensured by regulation of P/V and Q_g until a p_{O_2} value of 20% at an absolute power input of 16 W at Q_g 0.3 m³ h⁻¹ was reached. In the following the feed rate ($Q_{f,g}$) was hold constant to run the reactor on the maximum oxygen transfer rate OTR($Q_{f,g}$) achievable above p_{O_2} : 20% (Fig. 2).

2.6 Description of process model

With respect to the monitored parameters, occurring metabolic variability for the carbon limited fed batch fermentation can be implied by a different pattern for biomass growth, byproduct formation, and oxygen consumption. Without significant differences in the aforementioned aspects the process data should be describable with the same set of kinetic parameters. The following equations determine a simple unstructured model for a constantly fed batch culture, where the growth-limiting substrate is the energy source. Emerging differences in growth and substrate utilization would lead to deviations between the model output and process data [22] and therefore imply an imperative for a more detailed investigation.

Figure 2. Scheme of feed rate control over process time to maintain aerobic conditions, i.e. $p_{O_2} \geq 20\%$ at P/V_{max} ; $Q_{g,max}$ (Section 3.2).

The carbon containing compound is expressed in C-mol. Biomass and carbon dioxide were assumed to be the only products synthesized. In the following, the biomass and the substrate concentrations, X and S were modeled by dynamic equations.

$$\mu = \chi (\sigma - k_D X) \quad (7)$$

$$\frac{dX}{dt} = \mu X - \frac{F X}{W} \quad (8)$$

$$\frac{dS}{dt} = \sigma X + \frac{F (S_F - S)}{W} \quad (9)$$

With basic Monod kinetics, the solution of this model led to the oxygen uptake rate (OUR), which is given directly from the stoichiometry.

$$OUR = \beta \sigma X \quad (10)$$

The working volume W increased with the exponential substrate feed rate F and the base addition that resulted from the pH control.

2.7 Offline analytics

Microbial growth was monitored using the absorbance signal at 600 nm (OD_{600}) determined by spectral photometry (Bio-photometer D30, Eppendorf, Germany). One unit of OD_{600} was corresponded to 0.25 g L⁻¹ DCW (dry cell weight). To identify unwanted substrate or metabolite accumulation the medium composition was monitored via HPLC analysis for organic acids, carbohydrates, and ethanol (Aminex HPX-87 C, Biorad, USA, Gemini C18, Phenomenex, USA).

3 Results and discussion

In order to validate the mass transfer performance of a JLR compared to a STR obtainable for a chemical conversion system,

Table 1. OTR [$\text{mmol L}^{-1} \text{h}^{-1}$] and E [$\text{kgO}_2 \text{ kW}^{-1} \text{h}^{-1}$] for chemical pO_2 depletion (Na_2SO_3 Test), fermentation until $\text{pO}_2 \sim 0\%$ ($\text{Ferm}_{\Delta\text{pO}_2\text{max}}$), aerobic fermentation ($\text{Ferm}_{\text{aerob}}$). JLR and STR operated at $P/V: 4.6 \text{ kW m}^{-3}$, $Q_g: 0.3 \text{ m}^3 \text{h}^{-1}$

	OTR [$\text{mmol L}^{-1} \text{h}^{-1}$]	E [$\text{kgO}_2 \text{ kW}^{-1} \text{h}^{-1}$]
JLR		
Na_2SO_3 Test	230	1.63
$\text{Ferm}_{\Delta\text{pO}_2\text{max}}$	230	1.63
$\text{Ferm}_{\text{aerob}}$	120	1.10
STR		
Na_2SO_3 Test	105	0.74
$\text{Ferm}_{\Delta\text{pO}_2\text{max}}$	78	0.56
$\text{Ferm}_{\text{aerob}}$	72	0.50

fed batch fermentations with *E. coli* were performed. A JLR and a STR were compared on the basis of the maximum achievable oxygen transfer rate ($\text{OTR}_{\Delta\text{pO}_2\text{max,bio}}$) at the same power input $P/V: 4.6 \text{ kW m}^{-3}$ and aeration rate $Q_g: 0.3 \text{ m}^3 \text{h}^{-1}$ (Section 3.1). The achievable mass transfer performance for a strictly aerobic fermentation process was tested (Section 3.2) and yield coefficients (Y_{XS} , Y_{OS} , Y_{OX}) for an aerobic fermentation process were determined (Section 3.3). The improvement of the JLR compared to the STR was evaluated over process time by a process model and energy consumption and the achieved productivity were assessed by the energetic oxygen transfer efficiency (E_{O_2}) and the obtained STY (Section 3.4).

3.1 Determination of $\text{OTR}_{\Delta\text{pO}_2\text{max}}$

For a rough estimation of the expectable OTRs at P/V_{max} , $Q_{g,\text{max}}$ and dissolved oxygen levels near zero ($\Delta\text{pO}_2 \text{ max}$), OTR_{max} was determined by chemical oxygen depletion (Section 2.3). $\text{OTR}_{\Delta\text{pO}_2\text{max,SO}_4}$ values of $230 \text{ mmol L}^{-1} \text{h}^{-1}$ (JLR) and $105 \text{ mmol L}^{-1} \text{h}^{-1}$ (STR) were determined at a volumetric power input of 4.6 kW m^{-3} and an aeration rate of $0.3 \text{ m}^3 \text{h}^{-1}$ (Table 1). For estimation of the maximum OTR achievable in a biological system ($\text{OTR}_{\Delta\text{pO}_2\text{max,bio}}$) fed batch fermentations were conducted in JLR and STR (Section 2.5). Further on, the feed rate and therefore oxygen consumption was increased until complete oxygen limitation was reached at 4.3 h for STR and 6.6 h for JLR (Fig. 3A). At these time points $\text{OTR}_{\Delta\text{pO}_2\text{max,bio}}$ values of $78 \text{ mmol L}^{-1} \text{h}^{-1}$ and $230 \text{ mmol L}^{-1} \text{h}^{-1}$ were determined for STR and JLR respectively (Fig. 3B).

For the JLR comparable values for $\text{OTR}_{\Delta\text{pO}_2\text{max,SO}_4}$ and $\text{OTR}_{\Delta\text{pO}_2\text{max,bio}}$ were determined. For the STR a mass transfer rate increased by 30% was obtained by the chemical depletion method compared to the fermentation process. The obtained enhancement factor of 1.3 implies operation in the transition regime at $0.3 < \text{Ha} < 3.0$ [21, 23] (Section 2.3). Due to differences in the physicochemical properties (coalescence, O_2 solubility) of $0.5 \text{ M Na}_2\text{SO}_4$ solution and the fermentation broth a comparison of the absolute values would be insufficient. Therefore the ratio of the obtained $\text{OTR}_{\Delta\text{pO}_2\text{max,SO}_4}$ values for STR and JLR were compared with the ratio of the identified $\text{OTR}_{\Delta\text{pO}_2\text{max,bio}}$ values. Whereas an improvement of 120% for the JLR was determined

for the chemical depletion method an even higher improvement of 195% for the $\text{OTR}_{\Delta\text{pO}_2\text{max,bio}}$ values was determined for the JLR during fermentation.

It was shown that the unicellular *E. coli* can be cultivated in JLR and STR without significant differences in microbial growth. Although after the start of the exponential feed, small amounts of acetate were formed (0.2 g L^{-1} JLR, 0.28 g L^{-1} STR) and recycled within the first two hours of fermentation. With oxygen consumption approaching $140 \text{ mmol L}^{-1} \text{h}^{-1}$ and pO_2 levels below 5% (5.6–6.7 h, Fig. 3A and B), 1.5 g L^{-1} acetate were formed in the JLR. Therefore, for the evaluated JLR it can be stated that although maximal OTRs of $230 \text{ mmol L}^{-1} \text{h}^{-1}$ were achievable, it is implied that the threshold for a reliable operation with respect to the prevention of prevalent acetate formation and partial oxygen limitation is lower.

3.2 Determination of $\text{OTR}_{\text{aerob}}$ ($Q_{f,g}$, P/V , Q_g)

The fermentation experiments described above were set up to run in each of the reactor systems until their maximum mass transfer capacity were reached. This is indeed interesting for characterization, however it does not depict a realistic fermentation process. Thus, a fermentation process where oxygen limitation was prevented by regulation of the applied feed rate (Section 2.5) was set up. The feed rate was again increased exponentially with a rate of 0.5 h^{-1} until a pO_2 level of 20% was reached at P/V_{max} and $Q_{g,\text{max}}$. Subsequently, the feed rate was held constant to run the reactors on the maximum oxygen transfer rate above a pO_2 level of 20% ($\text{OTR}_{\text{aerob}}$ ($Q_{f,g}$, P/V , Q_g)). In Fig. 4 the achieved DCW, formed acetate and OTRs for JLR and STR are plotted. The experiments were performed in duplicates.

Carbon limitation was achieved and glucose accumulation was successfully prevented. The acetate and glucose levels for STR1, STR2, and JLR1 were staying under the detection limit of the HPLC. For the process running in JLR2 about 0.8 g L^{-1} acetate was formed until a fermentation time of 3.6 h was reached. Beside the elevated acetate concentrations, no glucose was found for JLR2. Controlling pO_2 by P/V and Q_g in the highly dynamic phase of the fermentation process was more difficult with the JLR than for the STR. The result implies that the interaction of mixing and mass transfer is a critical aspect in case of the JLR. However, after entering the constant feed phase the amount of acetate formed in JLR2 was continuously reduced. The acetate level was falling under the detection limit of the HPLC after 4.6 h process time (Fig. 4A).

The STR reached its maximum capacity (at pO_2 20% and P/V_{max} and $Q_{g,\text{max}}$) at a feed rate $Q_{f,g}$ of 18 g h^{-1} and an OTR of $60 \text{ mmol L}^{-1} \text{h}^{-1}$ at a fermentation time of 4.1 h. The fermentation process conducted with the JLR reached a pO_2 level of 20% (P/V_{max} and $Q_{g,\text{max}}$) at a process time near 5 h, a feed rate of 28 g h^{-1} and an OTR of $120 \text{ mmol L}^{-1} \text{h}^{-1}$ (Fig. 4B). Further on, the feed rate for the STR and JLR was set to a constant value of 18 g h^{-1} (STR) and 28 g h^{-1} (JLR) to maintain strictly aerobic conditions above a pO_2 level of 20%. The measured values for OTR at pO_2 of 20% were consequently lower than the previously analyzed $\text{OTR}_{\Delta\text{pO}_2\text{max,bio}}$ as the driving force (ΔpO_2) was reduced (Eq. (4)).

Figure 3. Process data for fed-batch fermentation of *E. coli* for STR (process time 4.6 h) and JLR (process time 7.0 h). (A) Dissolved oxygen levels (pO₂) in [%] and feed rate Q_{f,g} in [g h⁻¹]. (B) Oxygen transfer rate (OTR) in [mmol L⁻¹ h⁻¹] and dry cell weight (DCW) in [g]. Simulated curves indicated as (SIM).

Figure 4. Process data for fed-batch fermentations of *E. coli* in JLR and STR controlled at pO₂ above 20%. (A) Obtained dry cell weight (DCW) in [g] compared to calculated model output DCW SIM. Abundant acetate over process time in [g L⁻¹]. (B) Oxygen transfer rate (OTR) in [mmol L⁻¹ h⁻¹] compared to calculated model output OTR SIM.

As it can be seen in Fig. 4B differences in the analyzed process parameters (see OTR for JLR1 and JLR2) have been found. The variations were originated by a high distortion of the monitored variable pO₂ by high gas holdups and therefore influenced the settings of P/V and Q_{f,g}. As a result, the shift from exponential to a constant feed at pO₂ = 20% at P/V_{max} and Q_{max} (Section 2.5) occurred at different process times (t).

3.3 Determination of Y_{OS}, Y_{XS}, Y_{OX}, and DCW_{SIM}, OTR_{SIM}

In order to validate whether the enhanced mass transfer performance and concomitantly the higher formation of DCW for JLR was specific for the applied reactor technology, yield parameters for oxygen Y_{OS} (g g⁻¹), Y_{OX} (g g⁻¹), and substrate Y_{XS} (g g⁻¹) consumption were calculated.

Over the entire fermentation process, almost constant values have been measured. Finally, for both reactors values of Y_{XS} = 0.3 g g⁻¹, Y_{OX} = 1.3 g g⁻¹, and Y_{OS} = 0.4 g g⁻¹ were determined (Fig. 5). Therefore, it is expected that no significant losses in yield

due to overflow metabolism and oxygen limitation occurred in either reactor. Formation and recirculation of the small amounts of acetate seen for JLR2 was not affecting the achieved overall Y_{XS}. Discrepancies for Y_{OS} (JLR) at q_{Feed} < 10 g h⁻¹ might be explained by an adaption phase to aerobic, substrate limited growth after inoculation. Some scattering of the biomass yield Y_{XS} and Y_{OX} values around the linear trend line can be attributed to errors in DCW determination. Standard deviations in OD₆₀₀ analysis up to 2% were observed for dilution factors of 100. For visualization, the DCWs plotted in Fig. 5B and C are indicated with a respective standard deviation of 2%. The variance in the obtained results is within the expectable uncertainties.

To further check the consistency of the obtained data (Y_{XS}:0.3, Y_{OX}:1.3, Y_{OS}:0.4) for STR and JLR, the unstructured model described in Section 2.6 was used to simulate the fermentation experiments conducted. The same set of model parameters for the Monod kinetics (μ_{max}:3.6 h⁻¹, K_s:0.024 g L⁻¹) and the assumption for the cell death rate (k_D:0.023 L g⁻¹ h⁻¹) yielded a satisfactory agreement of the model output to the process data. Considering the high uncertainties regarding monod derived parameters for *E. coli* described by other authors [24], the

Figure 5. (A) $r_O(Q_{f,g})$ for STR and JLR. Y_{OS} as slope of regression line. (B) $DCW(\Sigma Q_{f,g})$ for STR and JLR. Y_{XS} as slope of the regression line. (C) $\Sigma r_O(DCW)$ for STR and JLR. Y_{OX} as slope of the regression line. Error bars reflecting scattering of 2% by high dilution factors during OD₆₀₀ measurement.

optimized model parameters are in accordance with existing data found in the literature [25, 26]. All described experiments have been checked by simulation. An exemplary dataset for JLR and STR has been included within Fig. 3A and B and Fig. 4A and B. Based on the experimental results as well as on the model consistency check, no significant discrepancies for the cultivated *E. coli* in JLR and STR have been found. This statement is made under the restriction for operation under carbon limited and oxygen unlimited conditions. For the description of microbial growth under excess of glucose and/or oxygen limitation the implementation of a model considering overflow metabolism is needed [27].

So far, no negative influence on the unicellular *E. coli* for pumping the broth via the outer loop and the decompression at the nozzle tip has been identified. However, as it was reported by other authors, shear tolerance of the cultivated microorganism seems to be of major importance for comparable growth in JLR and STR [9, 12, 15].

3.4 Calculation of $E_{O_2}(P/V, Q_g, p_{O_2})$ and STY

To assess the energy consumption for mass transfer, JLR and STR were compared based on energetic oxygen transfer efficiencies E_{O_2} [$\text{kg kW}^{-1} \text{h}^{-1}$] and the results have been listed in Table 1. E_{O_2} is defined as the mass of oxygen transferred in relation to the energy supplied to the reactor (Eq. (11)).

$$E_{O_2} = \frac{OTR}{P/V} = \frac{k_L a \Delta p_{O_2}}{P/V} \quad (11)$$

To target the maximum mass transfer capacity achievable, the volumetric power input and the aeration rate were kept at constant values of 4.6 kW m^{-3} and $0.3 \text{ m}^3 \text{ h}^{-1}$ for the first set of experiments (Section 3.1). The chemical depletion method yielded efficiencies of $0.74 \text{ kg kW}^{-1} \text{h}^{-1}$ (STR) and $1.63 \text{ kg kW}^{-1} \text{h}^{-1}$ (JLR). For the fermentation process where the feed rate was increased until oxygen depletion was reached ($\Delta p_{O_2 \text{ max}}$), maximum efficiencies of $0.56 \text{ kg kW}^{-1} \text{h}^{-1}$ (STR) and $1.63 \text{ kg kW}^{-1} \text{h}^{-1}$ (JLR) and therefore an improvement of 190% in favor of the JLR was determined. Again, a direct comparison of both media formulations (chemical and fermentation) is questionable due to coalescence and solubility effects, but both reactors showed results in the same range for both methodologies.

For the aerobic fermentation, average values for $E_{O_2}(t)$ are depicted over process time (Fig. 6A). The E_{O_2} values were increasing with increasing process time until the maximum feed rate was reached. For a given feed rate the obtained efficiency $E = k_L a(\Delta p_{O_2})/(P/V)$ mainly depended on the setting of the power input (P/V , $k_L a$) and the actual oxygen driving force (Δp_{O_2}) within the reactor. Maximum values of $0.51 \text{ kg kW}^{-1} \text{h}^{-1}$ for STR and $1.0 \text{ kg kW}^{-1} \text{h}^{-1}$ for JLR (Fig. 6A) were achieved. As already stated before, the driving force for experiments presented in Section 3.2 was at least 20% lower than for the experiments performed in Section 3.1. The maximum improvement of the JLR compared to STR for the conducted aerobic fermentation with $p_{O_2} > 20\%$ was determined to be 96%. Therefore, it can be stated that the operation at elevated p_{O_2} levels (lower driving force) reduced the achieved oxygen transfer efficiency.

As mentioned before the energetic mass transfer efficiency sets the boundary for the achievable space time yield. The JLR offers higher mass transfer rates at the same power input at comparable yields for oxygen consumption (Y_{OX} , Y_{OS}) and substrate utilization (Y_{XS}). Consequently, also higher STYs were obtained (Fig. 6B) in JLR as higher feed rates could be applied until the maximum capacity of the reactor was reached. Maximum values about $2.5 \text{ kg m}^{-3} \text{h}^{-1}$ (JLR) and $1.75 \text{ kg m}^{-3} \text{h}^{-1}$ (STR) have been reached after the exponential feed phase respectively. The STY in JLR remained above the values achieved by the STR ($1.9 \text{ kg m}^{-3} \text{h}^{-1}$ JLR, $1.3 \text{ kg m}^{-3} \text{h}^{-1}$ STR), although the decline of STY over process time was more pronounced in JLR due to faster increasing liquid volumes.

However, for the evaluated fermentation process the JLR was achieving a higher oxygen mass transfer performance at identical power inputs and therefore it was capable to generate more biomass over process time compared to the STR.

Considering the improvements in efficiency for the evaluated JLR a significant reduction in operating costs would be achieved. For lab scale applications, mass transfer efficiencies between 1.0 and $1.8 \text{ kgO}_2 \text{ kW}^{-1} \text{h}^{-1}$ were also reported by Moresi et al. [9]. As according to Langhans [28] these values also can be generated in large scale, the JLR approach seems promising. However, with respect to savings in operating costs reliable estimations based on lab scale data are very difficult. Due to the importance of such data a parallel study regarding energy consumption for scalable pilot reactors and auxiliary aggregates is in preparation.

Although the JLR has shown a superior mass transfer efficiency and increased STY in the evaluated lab setup the time

Figure 6. (A) Average energetic oxygen transfer efficiency (E_{O_2}) in $[\text{kgO}_2 \text{ kW}^{-1} \text{ h}^{-1}]$ of JLR and STR over process time. (B) Average space time yield (STY) in $[\text{kg m}^{-3} \text{ h}^{-1}]$ of JLR and STR over process time. Variation in duplicates indicated by error bars.

constants for mass transfer and mixing remain a critical aspect. For efficient operation of the process pump and liquid circulation in the external loop of an JLR, separation of the major fraction of gas from the fermentation broth is required. Without further modifications and an assumed total gas separation, the remaining oxygen at a dissolved oxygen level of 20% and an oxygen demand of $120 \text{ mmol L}^{-1} \text{ h}^{-1}$ will be consumed in a few seconds. Time constants that can be easily met in lab scale can become critical in large scale, as for a wild-type *E. coli* K12 already 15 seconds under anaerobic conditions can lead to the induction of anaerobic pathways [19].

In the evaluated scale, the wild-type *E. coli* was not sensitive to variations between JLR and STR with respect to the obtainable yield and biomass growth. However occurring bioreactor inhomogeneities as they can arise in large scale are known to decrease substrate yield and productivity [17]. Especially, if recombinant protein production is considered yields might be significantly affected by arising pO_2 gradients in the respective apparatus [18, 29]. Due to the absence of nonaerated regions and the lower OTR at the aspired pO_2 of 20% the situation is assumed to be less critical for the STR.

4 Concluding remarks

Based on the findings described above it can be stated that the evaluated JLR outperforms a standard stirred tank bioreactor. For the applied fermentation of *E. coli*, no adverse effects of the external loop and decompression at the nozzle tip were seen. The improvement in maximum energetic oxygen transfer efficiency for JLR compared to STR (>100%) obtainable for a chemical conversion system was confirmed. It can be summarized that the $OTR_{\max}$ values in a JLR are significantly improved compared to a STR operated at identical volumetric power input and aeration rates. For a strictly aerobic bioprocess (realistic case with $pO_{2,\min}$ above 20%) the improvement of the energetic oxygen transfer efficiency for JLR compared to STR was 96%. Due to its favorable mass transfer efficiency, significantly higher space time yields were obtained by the JLR. Conclusively it can be stated that the obtained values for space time yields and energy consumption, imply potential for a reduction of operating- and investment costs by JLRs compared to STRs. Although it is noted that the performance of the JLR should be reassessed if an application for an alternative bioprocess is considered.

Practical application

To achieve techno-economic success, many sectors in industrial biotechnology, such as industrial enzyme production and production of commoditized intermediates demand both large production volumes and low production cost. Thus, investment needs as well as operating costs play a major role when deciding on future production scenarios. Whenever the achievable space time yield is determined by the mass transfer performance of the reactor, energy efficiency plays an important role to meet these requirements. JLRs are an efficient alternative to the mostly applied STRs. By overcoming mass transfer limitations, significantly higher space time yields can be achieved. Due to their high mass transfer performance JLRs are frequently used in the chemical industry and for the biocatalytic treatment in wastewater applications. The paper aims to investigate the application and potential benefits of JLR usage in industrial biotechnology.

The authors have declared no conflict of interest.

Nomenclature

D	[m]	reactor diameter
d	[m]	diameter
DCW	[g]	dry cell weight
E_{O_2}	$[\text{kgO}_2 \text{ kW}^{-1} \text{ h}^{-1}]$	energetic oxygen transfer efficiency
F_b	$[\text{L h}^{-1}]$	flow rate base
g	$[\text{m s}^{-2}]$	gravitational acceleration
h	[m]	installation height
H	[m]	reactor height
Ha	[-]	Hatta number
k_D	$[\text{L g}^{-1} \text{ h}^{-1}]$	death rate
k_{La}	$[\text{h}^{-1}]$	mass transfer coefficient O_2
K_s	$[\text{g L}^{-1}]$	monod constant
m	[pc]	number of stirrers
m	$[\text{g g}^{-1} \text{ h}^{-1}]$	maintenance rate
n_s	$[\text{s}^{-1}]$	stirrer speed
OTR	$[\text{mmol L}^{-1} \text{ h}^{-1}]$	oxygen transfer rate
OUR	$[\text{mmol L}^{-1} \text{ h}^{-1}]$	oxygen uptake rate
p	[Pa]	pressure
P	[W]	power input

Po	[-]	power number
pO ₂	[%]	dissolved oxygen O ₂
Q _{f,g}	[g h ⁻¹]	feed rate glucose
Q _g	[L min ⁻¹]	aeration rate
Q _L	[L min ⁻¹]	liquid flow rate
u _g	[m s ⁻¹]	superficial gas velocity
V	[m ³]	volume
W	[L]	working volume
X	[g L ⁻¹]	concentration biomass
Y	[-]	molar fraction
Y _{OS}	[-]	yield oxygen, substrate
Y _{XS}	[-]	yield biomass, substrate
Y _{OX}	[-]	yield oxygen biomass

Greek symbols

α	[-]	stoichiometric coefficient ammonia
β	[-]	stoichiometric coefficient oxygen
δ	[-]	stoichiometric coefficient water
Δ	[-]	delta, difference
ε	[-]	stoichiometric coefficient carbon dioxide
μ	[h ⁻¹]	growth rate
ρ	[kg m ⁻³]	density
σ	[h ⁻¹]	spec. substrate consumption rate
χ	[-]	stoichiometric coefficient biomass

Indices

abs	absolute
0	unaerated
b	aerated / modified
bio	determined by fermentation
drop	pressure drop
F	feed
in	input
jet	liquid jet
M	molar
m	maximal
min	minimal
out	output
R	reactor
s	stirrer
SO ₄	determined in sulfite system

5 References

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