Manuel Ughetti^{1,2}

Daniel Jussen¹

Peter Riedlberger¹

¹Research Group Chemical Engineering, Institute of Chemistry and Biotechnology, Zurich University of Applied Sciences, Wädenswil, Switzerland

²Buss ChemTech AG, Pratteln, Switzerland

Technical Report

The ejector loop reactor: Application for microbial fermentation and comparison with a stirred-tank bioreactor

Ejector loop reactors (ELR) are successfully used in industrial chemical processes for gas/liquid reactions. They achieve higher mass transfer rates compared to the stirred-tank reactor (STR) at comparable specific power input. Insufficient oxygen transport and shear stress induced growth inhibition are limiting parameters during microbial fermentation. Due to its better mass transfer characteristics, the ELR was expected to have beneficial effects on biomass and recombinant protein production. One concern, however, was whether the ELR's shear stress characteristics would have a negative effect. This study evaluated the suitability of using the Buss-Loop[®] Reactor (BLR), one of the most advanced ELR technologies, as a bioreactor. The well-studied STR was used as a reference. A lab scale BLR was adapted for microbial fermentation. Mass transfer rates and specific power inputs were within the same order of magnitude in the ELR and the reference STR. Maximum $k_L a$ values of 207 and 205 h⁻¹ at power inputs of 6.9 and 9.7 W/L were measured in the ELR and STR, respectively. During batch fermentation of *Escherichia coli* K12 MG1655, maximum cell densities were higher in the ELR (OD600 of 22) than in the STR (OD600 of 18). Green fluorescence protein (GFP) production with pGS1 was comparable; however, more GFP was released into the media in the ELR. This indicates higher cell disruption compared to the STR. Despite this drawback of the first prototype, our work clearly demonstrates the potential of the ELR as a system for microbial fermentations.

Keywords: Ejector loop reactor / *Escherichia coli* / Microbial Fermentation / Oxygen transfer rate / Stirred-tank reactor

Received: August 14, 2017; *revised:* December 24, 2017; *accepted:* January 11, 2018

DOI: 10.1002/elsc.201700141

1 Introduction

In biotechnology, the stirred-tank reactor (STR) has been established as the standard for lab, pilot and industrial scale fermentations [1, 2]. One of the crucial parameters in most bioreactor processes is the oxygen supply during fermentation at every scale [3, 4] which can be limiting for the process performance [5]. In a STR, an increase in the oxygen transfer rate (OTR) is achieved, in addition to increasing the volumetric gas flow or oxygen concentration, by increasing the stirring rate, although this results in a corresponding increase in shear stress [6, 7].

Correspondence: Dr. Peter Riedlberger (peter.riedlberger@zhaw.ch), Research Group Chemical Engineering, Institute of Chemistry and Biotechnology, Zurich University of Applied Sciences, Einsiedlerstrasse 31, 8820 Wädenswil, Switzerland

Abbreviations: BLR, Buss loop reactor; ELR, ejector loop reactor; GFP, green fluorescence protein; STR, stirred-tank reactor

Furthermore, STRs suffer from large differences in energy dissipation between different mixing zones. This effect is exacerbated with increasing scale [8, 9]. Furthermore, scaling up on power per unit volume, necessary to achieve similar mass transfer rates, can become mechanically limiting at industrial scale [10].

Analogously, a high mass transfer rate and homogeneously mixed system are crucial in chemical processing of gas/liquid reactions [11]. For this type of reaction, an alternative to the STR concept, in which mixing and dispersing is achieved by a liquid jet generated by an ejector, is widely used and applied in industry. It has been shown that these so called Venturi or ejector loop reactors (ELR) favour higher mass transfer rates compared to the STR at comparable power inputs [12–14]. Loop reactors with liquid jets in downflow mode especially feature favourable mass transfer characteristics [15–18]. Additionally, the scale up of the ELR can be carried out on a constant power input basis. This leads to superior mass transfer characteristics in comparison to the STR at industrial scale [19]. The operating principle of an

Figure 1. Schematic drawings of the mixing and aeration principles of both reactors: (A) Aeration is achieved through the top mounted ejector, primary mixing inside the ejector and secondary mixing in the vessel by the deep jet of reaction mass in the case of the ejector loop reactor and (B) mixing by agitation and aeration via dip tube in the case of the stirred-tank reactor. Photos of the two lab scale bioreactors: (C) Custom made ejector loop reactor mounted onto a rack; (D) Stand-alone stirred-tank reactor KLF 2000 (Bioengineering AG, Wald, Switzerland).

ELR is presented in Fig. 1A. Gas is induced into the ejector at the top of the reaction vessel and is mixed entirely within the motive fluid. The finely dispersed gas/liquid mixture is then injected into the reaction vessel. Secondary mixing is thus generated and a high interfacial area maintained. The multiphase reaction mixture is

recirculated by a specially suited centrifugal pump, optionally through a heat exchanger, back to the ejector [20]. In the 1980s and 90s, similar systems were tested for whey fermentation [21] and for production of xanthan [13] or cellulose [22]. Industrial scale yeast production [1] was also carried out. However, the STR has become the established predominant reactor system for microbial cultivation while the ELR has been further developed and enhanced for chemical processes. The most versatile design of a commercial ELR is claimed to be the Buss-Loop® Reactor (BLR) developed by Buss ChemTech AG, Pratteln, Switzerland (formerly Buss AG) [16, 23]. This reactor type, was designed for process intensification [24] and is now used for a range of reactions including hydrogenation, alkylation, phosgenation and amination [17]. While the BLR has never been formally evaluated for microbial cultivation, novel reactor concepts, such as the ELR, could provide new process opportunities for biotechnology, especially in the light of process intensification and green chemistry movements [25]. Due to its ease of scale up and superior mass transfer characteristics for chemical processes, this study investigated the BLR as a promising novel bioreactor for microbial cultivation. The OTR and performance of the BLR for microbial fermentation at laboratory scale were evaluated in reference to an STR.

2 Materials and methods

2.1 Bioreactors

A 1.5 L ecoclave (Type 2 glass pressure vessel, Büchi AG, Flawil, Switzerland) was modified to construct the prototype ELR used for the study (Fig. 1C). A custom made cover with integrated ejector and sterile process connection ports was constructed. An adapter with two Ingold sockets for optical oxygen (InPro6860i, Mettler Toledo, Greifensee, Switzerland) and pH electrodes was fabricated and mounted between the glass vessel wall and the bottom of the reactor. For circulation of the culture broth, an HMR060 multiphase centrifugal pump (Fink Chem+Tec GmbH, Leinfelden-Echterdingen, Germany) was used. Finally, a sampling and discharge line, with two diaphragm valves and two orthogonal valves for steam sterilization, was installed at the discharge port of the circulation pump.

A KLF 2000 (Bioengineering AG, Wald, Switzerland) with a double stage Rushton turbine stirrer was used as the reference STR (Fig. 1B & 1D).

2.2 Power input characterization

Determining power input for both systems was done on a theoretical basis and is given as specific power input (P/V). In the ELR, the energy required to mix the liquid and gas phases is introduced by the pump. Therefore, the power input was calculated for the desired impeller speed using the manufacturer's pump specification sheet with the aid of pump affinity laws as well as pump and system curves according to Sterling Fluid Systems [26]. In STRs, the power input for mixing and dispersion is generated by the agitator's impeller. The calculation was

performed according to Barradas et al. [27] in whose work the same type of STR was used.

2.3 Oxygen transfer characterization

For OTR characterisation, $k_L a$ values were determined at different volumetric air flow rates and power inputs according to the physical dynamic method [28]. Accordingly, the change in dissolved oxygen (DO) was monitored after switching the feed gas from nitrogen to air. Prior to aeration, the reactor's headspace was purged with air until the majority of nitrogen bubbles had disappeared. Due to this additional air purging, the disadvantage of back mixing nitrogen described by Baier [20] was reduced. The low liquid surface area of the unmixed reactor prevented a significant increase of DO prior to the measurement. In the ELR, the pump drive was varied between 15 and 30 Hz, corresponding to an impeller speed of 864–1728 rpm, an impeller tip speed of 2.8–5.6 m/s and a calculated P/V of 0.9 to 6.9 W/L. For evaluating the $k_L a$ in the STR, the impeller was operated between 400 and 1200 rpm, corresponding to an impeller tip speed between 1.0 to 3.0 m/s and 0.4 to 9.7 W/L P/V values. Aeration rates were set to 0.25, 0.5, 1.0 and 2.0 vvm in both reactors. $k_L a$ measurements were carried out twice for consistency.

2.4 Batch fermentation

E. coli K12 MG1655, with the pGS1 plasmid for intracellular production of green fluorescence protein (GFP) under control of the araBP-promotor, was used for all cultivations. Cultures were grown in a HSG medium (14.9 g/L glycerol, 13.5 g/L soy peptone, 7.0 g/L yeast extract, 2.5 g/L NaCl, 2.3 g/L K_2HPO_4 , 1.5 g/L KH_2PO_4 , 0.249 g/L $MgSO_4 \cdot H_2O$) supplemented with 100 mg/L carbenicillin and 500 ppm polypropylene glycol 2000. Seed cultures were grown over-night in a HSG medium supplemented with 5.0 g/L glycerol in shake flasks at 37°C and 150 rpm. From the over-night cultures, a volume of 50 mL was centrifuged at $4000 \times g$ for 5 min, decanted, resuspended in 25 mL fresh media and used as inoculum in the ELR and STR with 1.2 and 2.0 L fermentation volumes, respectively. Process parameters were kept constant throughout an entire cultivation period to eliminate the influence of dynamic changes in aeration or agitation on the outcome of the experiments. Thereby, the fermentations were carried out at 37°C, 2.0 vvm aeration and mixing speeds at 6.9 W/L (30 Hz at pump's frequency converter) and 5.6 W/L (1000 agitator's rpm). The pH was controlled at 7.0 with 3 N NaOH and 85% H_3PO_4 . For induction, 0.2% L-arabinose was added in the early exponential phase once OD600 had reached 3.5 after approx. 4 h of cultivation. Experiments were carried out twice for consistency.

2.5 Analysis

Biomass formation was tracked by means of measuring optical density at 600 nm (OD600) [29].

For determining relative fluorescence intensity, each cultivation sample was centrifuged at $8000 \times g$ for 6 min. The clear

supernatant was used for fluorescence measurement of the culture supernatant (F_S). The cell pellet was resuspended in deionized water and the cells were disrupted by ultrasonic treatment (Bandelin Electronics, Sonoplus HD 3200) at 20% amplitude for 5 min. The cell debris solution was centrifuged ($8000 \times g$, 6 min) and the cell internal fluorescence intensity (F_C) was measured from the decanted supernatant. Fluorescence was analyzed with a Spark 10M (Tecan Group AG, Männedorf, Switzerland) multi-plate reader in black, flat-bottom, 96-well plates at 440 and 509 nm excitation and emission wavelengths, respectively. Samples were diluted 1:10 to a final volume of 200 μ L. The sum of the fluorescences F_S and F_C was defined as F_{tot} .

The glycerol content in the media was measured according to Kuhn et al. [30]. Accordingly, the glycerol was transformed in a two-step reaction to 3,5-diacetyl-1,4-dihydrolutidine which was quantified photometrically.

3 Results and discussion

3.1 Power input and oxygen transfer rate

Figure 2 depicts the comparison of the $k_L a$ values determined within the ELR (2A) and STR (2B). In the ELR, the highest $k_L a$ value of 207 h^{-1} was measured, as expected, at both maximum aeration and mixing intensity. A near linear increase in $k_L a$ was observed for both parameters, while the $k_L a$ reached a plateau at approx. 201 h^{-1} and 5.6 W/L in the STR. Further increase

Figure 2. Surface plots of measured mass transfer coefficients in the ejector loop reactor (A) and the stirred-tank reactor (B) at different aeration rates and mixing intensities/specific power inputs. Presented data are mean values of double determinations.

Figure 3. Comparison of the fermentation curves between the ejector loop reactor (A) and the stirred-tank reactor (B). Cultivations were run at almost equal levels with 207 h⁻¹ with the ejector loop reactor (corresponding to 6.9 W/L at 30 Hz circulation pump's frequency) and 201 h⁻¹ with the stirred-tank reactor (corresponding to 5.6 W/L at 1000 agitator's rpm). Also shown is cell density expressed in optical density (solid dots), glycerol concentration in the medium (solid triangles), sum of the culture supernatant and the cell internal fluorescence intensities (○), separate fluorescence intensity of the culture supernatant (triangles) and dissolved oxygen concentration (dotted line). Error bars represent standard deviations from double determinations.

of the power input or aeration rate in the STR did not yield a significant increase in the $k_L a$. The maximum $k_L a$ of 205 h⁻¹ was achieved at 9.7 W/L. These results are in line with literature on STR performance, as a plateau is observed for both parameters at the flooding point [31].

3.2 Batch fermentations

With 6.9 W/L in the ELR and 5.6 W/L in the STR, the cultivations were carried out at comparable $k_L a$ values of 207 and 201 h⁻¹ (Fig. 2). Figure 3 depicts the fermentation results of the ELR (3A) and the STR (3B). Similar cultivation curves were obtained for each reactor type. In both reactors, the cells grew exponentially until induction after 4-h cultivation time. During this time, OD600 increased from 0.3 to 3.6 and from 0.2 to 4.1 in the ELR and the STR, respectively. After induction, the cell densities increased linearly until maximum OD600 values of 22.1 in the ELR and 18.2 in the STR were obtained after 10 h.

Glycerol concentration decreased inversely proportionate with OD600 and was depleted after 10 h. These results are consistent with literature, such as Moresi et al. [21], who outlined that

the use of an ejector and a circulatory pump did not affect the microbial growth of *Kluyveromyces fragilis*. The maximum growth rate in the ELR was 1.02 ± 0.14 h⁻¹ and was 14.6% higher than in the STR which achieved a maximum growth rate of 0.89 ± 0.04 h⁻¹. The minimum recorded DO value during fermentation of 40 ± 12 % was 1.6 times higher in the ELR compared to 25 ± 7 % in the STR. These findings were unexpected as the fermentations in both reactors were carried out at analogous $k_L a$ conditions. It has previously been reported that the physical dynamic method yields lower $k_L a$ values than methods that measure oxygen consumption [28] due to back mixing of bubbles with elevated nitrogen content [20]. This effect may be more prominent in an ELR as fine bubbles with elevated nitrogen content can remain in the reactor longer. Thus, actual mass transfer in the ELR could have been higher than measured. Similar higher $k_L a$ values were also reported by Meyer & Charles in a less advanced loop fermenter in comparison to an STR [22]. Regarding GFP production, comparable F_{tot} values of $3.5 \cdot 10^5 \pm 0.50 \cdot 10^5$ RFU and $3.6 \cdot 10^5 \pm 0.29 \cdot 10^5$ RFU were reached after 22 h in the ELR and the STR, respectively. However, F_s was 29% higher in the ELR than in the STR, with values of $1.8 \cdot 10^5 \pm 0.10 \cdot 10^5$ RFU and $1.4 \cdot 10^5 \pm 0.41 \cdot 10^5$ RFU, respectively. This higher portion of GFP released into the media in the ELR is a likely indicator of higher cell disruption, caused by local energy dissipation. Cell damage by shear stress is a well-known problem in microbial fermentation [32]. There are two possible shear hotspots in the ELR - the pump head (with a small housing and relatively large impeller) and the outlet of the ejector jet. The pump impeller tip speed was considerably higher in the ELR with 5.6 m/s than at the agitator tip with 2.5 m/s. This corresponds to a maximal shear rate of $60'500$ s⁻¹ in comparison to $33'600$ s⁻¹ in the ELR and STR, respectively. Shear rates were calculated according to Cristi [33]. This is partially caused by the higher pump impeller diameter (62 mm) at lab scale compared to the Rushton turbine (48 mm) in the STR. Thus, this local high shear stress may impair microbial growth, despite the fact that the overall mean power input is lower in the ELR. Nevertheless, oxygen dependent maturation of GFP is an additional explanation for the higher GFP signal in the ELR. However, this explanation does not cover the changes in the ratio of intra- and extracellular GFP. For further optimization, the pump impeller design and the ratio of reactor volume to circulation flow should be reconsidered. To provide sufficient mixing, the recirculation rate must be greater than 20 h⁻¹ [24]. A recirculation rate of 275 h⁻¹ was used in the prototype ELR during fermentation, however. Additionally, lower shear ejector jets, optimized for the cell diameter, could be considered. Further optimization of the shear stress applied to the cells is thus feasible.

4 Concluding remarks

This study compared the ejector loop reactor with the stirred-tank reactor for use as a bioreactor at laboratory scale. Oxygen transfer rates were determined in terms of $k_L a$ values for both systems. Within the experimental range for power input and aeration, the $k_L a$ value approached a plateau in the STR. In contrast, the $k_L a$ had a linear correlation to power input and aeration in the ELR, thus indicating the potential for a further increase in

oxygen mass transfer. This is consistent to the findings in the field of chemical process technology, as superior gas transfer by the ELR was reported which yielded increased performance for gas/liquid reactions [17, 18, 23, 24]. In this study, the ejector loop concept was successfully transferred to a microbial fermentation process. By the cultivation of *E. coli*, with intracellular recombinant GFP production, comparable fermentation results were obtained in the STR and ELR. Interestingly, higher OD600 values, growth rates, minimum DO saturations and equal GFP fluorescence intensities were obtained in the ELR in relation to the STR. This is in agreement with studies of the early loop reactors from the 1980's and 90's which reported that the circulation pump and the ejector in ejector-type loop reactors did not affect microbial growth [13, 21, 22]. However, the release of GFP into the growth media was higher in the ELR compared to STR, thus indicating higher cell perforation due to shear stress. Considering the results of this study, the scale up potential of the ELR and the potential for further optimization, it can be concluded that we have successfully demonstrated that the ELR is a promising system for microbial fermentations. The application is not limited to batch fermentations, as carried out in this study, as the ELR is also particularly suited to fed-batch and continuous processes.

Practical application

The ejector loop reactor is a promising system for microbial fermentations. This reactor type is suitable for batch, fed-batch as well as continuous processes. With this reactor system, higher gas-liquid mass transfer rates are obtainable at equal specific power input compared to the stirred-tank reactor. In addition, this reactor type features ease of scale up, allowing process performance optimized at lab scale to be maintained at industrial scale.

Nomenclature

$k_L a$	[h ⁻¹]	Volumetric mass transfer coefficient
F_C	[RFU]	Fluorescence intensity of cell disruption supernatant
F_S	[RFU]	Fluorescence intensity of culture supernatant
F_{tot}	[RFU]	Sum of F_C And F_S
P/V	[W/L]	Specific power input
DO	[%]	Dissolved oxygen
OD600	[-]	Optical density at 600 nm wavelength
OTR	[kg/(m ³ h)]	Oxygen transfer rate
RFU	[-]	Relative fluorescence units
vvm	[L/(L min)]	Volume, air per volume, medium per minute

We thank Walter Krebs, Rebecca Buller and Christin Peters for their scientific support. This study was carried out in cooperation between the ZHAW and Buss ChemTech AG, the commercial manufacturer of the ELR, who partially financed the work. We thank Norbert Drefs and Thomas Blocher for fruitful discussions.

The authors have declared no conflict of interest.

5 References

- [1] Chmiel, H., *Bioprozesstechnik*, 3rd ed., Spektrum Akademischer Verlag, Heidelberg 2011.
- [2] Hortsch, R., Stratmann, A., Weuster-Botz, D., New milliliter-scale stirred tank bioreactors for the cultivation of mycelium forming microorganisms. *Biotechnol. Bioeng.* 2010, 106, 443–451.
- [3] Ukkonen, K., Veijola, J., Vasala, A., Neubauer, P., Effect of culture medium, host strain and oxygen transfer on recombinant Fab antibody fragment yield and leakage to medium in shaken *E. coli* cultures. *Microb. Cell Fact.* 2013, 12, 73.
- [4] Junker, B. H., Scale-up methodologies for *Escherichia coli* and yeast fermentation processes. *J. Biosci. Bioeng.* 2004, 97, 347–364.
- [5] Mori, H., Yano, T., Kobayashi, T., Shimizu, S., High density cultivation of biomass in fed-batch system with do-stat. *J. Chem. Eng. Japan* 1979, 12, 313–319.
- [6] Pérez, S. J. A., Porcel, R. E. M., López, C. J. L., Sevilla, F. J. M. et al., Shear rate in stirred tank and bubble column bioreactors. *Chem. Eng. J.* 2006, 124, 1–5.
- [7] Müller, J. M., Risse, J. M., Jussen, D., Flaschel, E., Development of fed-batch strategies for the production of streptavidin by *Streptomyces avidinii* based on power input and oxygen supply studies. *J. Biotechnol.* 2013, 163, 325–332.
- [8] Paul, E. L., Atiemo-Obeng, V. A., Kresta, S. M., Fukushima, C. et al., *Handbook of Industrial Mixing*, John Wiley & Sons, Inc., Hoboken, NJ, USA 2003.
- [9] Riedlberger, P., Brüning, S., Weuster-Botz, D., Characterization of stirrers for screening studies of enzymatic biomass hydrolyses on a milliliter scale. *Bioprocess Biosyst. Eng.* 2013, 36, 927–935.
- [10] Botton, R., Cosserat, D., Charpentier, J. C., Operating zone and scale up of mechanically stirred gas-liquid reactors. *Chem. Eng. Sci.* 1980, 35, 82–89.
- [11] Lee, S.-Y., Pang Tsui, Y., Succeed at gas/liquid contacting. *Chem. Eng. Prog.* 1999, 95, 23–49.
- [12] Warnecke, H.-J., Hubmann, P., Volumetric mass transfer coefficients of gas liquid jet loop reactors by oxidation of hydrazine. *Chem. Eng. Commun.* 1989, 78, 131–138.
- [13] Zaidi, A., Ghosh, P., Schumpe, A., Deckwer, W.-D., Xanthan production in a plunging jet reactor. *Appl. Microbiol. Biotechnol.* 1991, 35, 330–333.
- [14] Evans, G. M., Biñ, A. K., Machniewski, P. M., Performance of confined plunging liquid jet bubble column as a gas-liquid reactor. *Chem. Eng. Sci.* 2001, 56, 1151–1157.
- [15] Duveen, R. F., Leuteritz, G., Der BUSS-Schleifenreaktor in der Öl- und Fetthärtungsindustrie. *Fette, Seifen, Anstrichm.* 1982, 84, 511–515.
- [16] Dutta, N. N., Raghavan, K. V., Mass transfer and hydrodynamic characteristics of loop reactors with downflow liquid jet ejector. *Chem. Eng. J.* 1987, 36, 111–121.
- [17] Dirix, C. A. M. C., van der Wiele, K., Mass transfer in jet loop reactors. *Chem. Eng. Sci.* 1990, 45, 2333–2340.
- [18] Cramers, P. H. M. R., Beenackers, A. A. C. M., van Dierendonck, L. L., Hydrodynamics and mass transfer characteristics of a loop-venturi reactor with a downflow liquid jet ejector. *Chem. Eng. Sci.* 1992, 47, 3557–3564.

- [19] van Dierendonck, L. L., Zahradník, J., Linek, V., Loop Venturi reactora feasible alternative to stirred tank reactors? *Ind. Eng. Chem. Res.* 1998, 37, 734–738.
- [20] Baier, F. O., *Mass Transfer Characteristics of a Novel Gas-Liquid Contactor, the Advanced Buss Loop Reactor*, ETH, Zürich, 2001.
- [21] Moresi, M., Bartolo Gianturco, G., Sebastiani, E., The ejector-loop fermenter: description and performance of the apparatus. *Biotechnol. Bioeng.* 1983, 25, 2889–2904.
- [22] Meyer, H., Charles, M., Cultivation of a filamentous bacterium in a deep jet fermentor. *Biotechnol. Bioeng.* 1982, 24, 1905–1909.
- [23] Cramers, P. H. M. R., *Hydrodynamics and Mass Transfer Characteristics of Liquid Driven Jet Reactors*, ETH, Zürich, 1997.
- [24] Laneaster, M., Hoyle, B., *Clean Technology for the Manufacture of Speciality Chemicals*, 1st ed., The Royal Society of Chemistry, Cambridge 2001.
- [25] Storhas, W., *Bioverfahrensentwicklung*, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany 2013.
- [26] *Sterling Fluid Systems, Basic Principles for the Design of Centrifugal Pump Installations*, 7th ed., Sterling SIHI, 2003.
- [27] Platas Barradas, O., Jandt, U., Minh Phan, L. Da, Villanueva, M. E. et al., Evaluation of criteria for bioreactor comparison and operation standardization for mammalian cell culture. *Eng. Life Sci.* 2012, 12, 518–528.
- [28] Garcia-Ochoa, F., Gomez, E., Oxygen transfer rate determination: chemical, physical and biological methods. *Encycl. Ind. Biotechnol. Bioprocess, Biosep. Cell Technol.* 2010, 1–21.
- [29] Wang, J., Zhang, Z., Liu, H., Sun, F. F. et al., Construction and optimization of trans-4-hydroxy-L-proline production recombinant E. coli strain taking the glycerol as carbon source. *J. Chem. Technol. Biotechnol.* 2016, 91, 2389–2398.
- [30] Kuhn, J., Müller, H., Salzig, D., Czermak, P., A rapid method for an offline glycerol determination during microbial fermentation. *Electron. J. Biotechnol.* 2015, 18, 252–255.
- [31] Stanbury, P. F., Whitaker, A., Hall, S. J., *Principles of Fermentation Technology*, 2nd ed., Butterworth-Heinemann, Burlington 1995.
- [32] Lange, H., Taillandier, P., Riba, J.-P., Effect of high shear stress on microbial viability. *J. Chem. Technol. Biotechnol.* 2001, 76, 501–505.
- [33] Chisti, Y., Shear sensitivity, in: *Encyclopedia of Industrial Biotechnology*, John Wiley & Sons, Inc., Hoboken, NJ, USA 2009.