

PROPAGATION OF NON-ADHERENT HL-60 CELLS IN BATCH CULTURES MAINTAINED IN STATIC AND WAVE-TYPE AGITATED SYSTEMS

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Typically applied static (i.e. non-agitated) cultures do not provide sufficient conditions for efficient propagation of suspended non-adherent cells, in general. Feasibility of small-scale wave-type agitated single-use bioreactors for gentle agitation underlies applicability of such systems for scaling-up of fragile biomass of animal cells. The basic aim of the study was to compare the results of non-adherent HL-60 cell propagation performed referentially as the batch culture in typical static (i.e. non-agitated) disposable culture flasks (50 cm³ of culture medium) and in *ReadyToProcess* WAVE™25 bioreactor system (GE Healthcare) equipped with disposable culture bag (300 cm³ of culture medium) subjected to continuous wave-type agitation. The density and viability of HL-60 cells were significantly higher for the bioprocess subjected to wave-type agitation, than in the reference static culture. The values of the specific rate of glucose consumption *per* cell ($r_{\text{glc/cell}}$) exhibited by HL-60 cells maintained in the system with continuous wave-type agitation was significantly lower (i.e. up to more than 42%) than the values noted for the static culture, for exactly the same time-points of two compared cultures. The results of the studies undoubtedly and comprehensively confirmed the applicability of the studied disposable bioreactor with wave-induced agitation as the right platform for proceeding the propagation of non-adherent HL-60 cells and for providing the culture conditions required by HL-60 cells for sustainable metabolism.

Keywords: disposable (single-use) bioreactor, wave-type agitation, non-adherent cell culture, human HL-60 cells

1. INTRODUCTION

Nowadays, the biopharmaceutical industry is searching for technologies, which could reduce time and costs of *in vitro* bioprocesses with animal cells performed on a large scale. The single-use technology is commonly recognized as a solution which eliminates troublesome and time-consuming steps of bioprocessing, such as e.g. cleaning-in-process and cleaning validation as well. Another advantage of the application of a disposable culture bag for every new charge of cell culture is significant reduction of microbial or cross contamination of culture vessel. Such great merits are really beneficial for modern bioprocess industry, due to the possibility of scaling-up of cell cultures, as well as transforming a single-bioproduct profile

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of production into multi-bioproducts facilities. From the bioprocess scaling-up point of view it should also be noted that the single-use technology enables to reduce a capital cost of the plant constructions, contrary to typical bioreactor constructions made of glass or stainless steel (Gottschalk et al., 2012; Sette and Barbaroux, 2006; Shukla and Gottschalk, 2012). Currently offered commercial single-use culture bags are offered as ready-to-use culture vessels, which are flexible in their shapes, and they are usually made of multilayered films composed of: (i) polyethylene-based outer layer, which determines mechanical properties of the whole vessel; (ii) ethylene-vinyl-alcohol-based barrier layer, which prevents permeability of gases and liquids; (iii) ultra-low-density-polyethylene-based biocompatible inner layer for contact with biomass (Pollard and Kistler, 2016).

In wave-agitated bioreactors the oscillating movement of the apparatus's tray induces waves in the two-phase (i.e. gas phase and liquid broth) *in vitro* culture system closed inside the disposable polymer-based culture bag (Eibl et al., 2011). During the wave-induced agitation, the interfacial area formed between gas phase and culture medium is continuously renewed, and therefore the bubble-free surface aeration of the culture broth is successfully accomplished (Fig. 1) (Pilarek et al., 2018). Simultaneously, such a gentle wave-type agitation significantly limits the level of shear forces, as well as inhibits shear stress effects, which negatively influence fragile biomass in typical submerged *in vitro* culture systems supported with a mechanical stirrer or even air-lift aeration (Nienow et al., 2013).

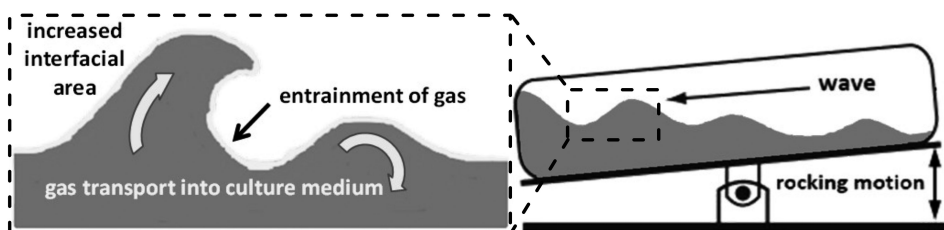


Fig. 1. Scheme of wave-type agitation by continuous rocking motion

The basic aim of the study was to compare the results of suspension cultures of non-adherent human HL-60 cells maintained independently as the batch culture in two systems: (i) typical static (i.e. non-agitated) disposable culture flasks (i.e. 50 cm³ of culture medium), and (ii) *ReadyToProcess* WAVETM25 bioreactor system (WAVE 25; GE Healthcare) equipped with disposable culture bag (i.e. 300 cm³ of culture medium) and subjected to continuous wave-type agitation. The effectiveness of two compared culture systems was analysed and discussed based on cell density and viability. The comparison of specific rate of glucose consumption calculated for the suspended cells maintained independently in both culture systems revealed which culture system provided the culture conditions preferred by HL-60 cells for sustainable metabolism. In our opinion, such investigations well-established by quantitative results are cognitively justified and welcomed by bioengineers due to the growing interest for performing bioprocesses in single-use bioreactors.

2. MATERIALS AND METHODS

2.1. HL-60 cells

HL-60 is a continuous line of non-adherent human promyelocytic leukemia white blood cells, which have been originally isolated from blood of a 36-year-old woman and established as a continuous line of non-adherent cells in USA (at the M. D. Anderson Hospital, Houston, US), in 1979. HL-60 cells are commonly used for laboratory research on *in vitro* blood cell formation and physiology. The cells applied in the present study were supplied by ATCC (US) as referentially certified HL-60 cell line.

In all performed experiments, HL-60 cells were maintained in the culture medium composed as a mixture of:

- 89% of Roswell Park Memorial Institute 1640 medium (RPMI) containing 2.0 g dm^{-3} of glucose,
- 10% of fetal bovine serum (FBS),
- 1% of antibiotic/antimikotic mixture (PenStrep).

All applied liquid media were supplied by Gibco (US) as certified media approved for animal cell cultures.

2.2. Single-use bioreactor system supported with wave-type agitation

The *ReadyToProcess* WAVETM25 system (WAVE 25, supplied by GE Healthcare Bio-Sciences AB, Sweden), was applied to *in vitro* culture of non-adherent HL-60 cells under conditions supported with continuously wave-type agitation. The schematic diagram of WAVE 25 setup is presented in Fig. 2. WAVE 25 was equipped with a disposable pre-sterilized polymer-based 2.0-litre CellbagTM (GE Healthcare, US), which is characterized by recommended working volume ranging from 0.1 dm^3 to 1.0 dm^3 of culture broth. The thermostatic (up to $40 \text{ }^\circ\text{C}$) rocking tray performed continuously oscillating movements of CellbagTM, with parameters ranging from 2° to 12° in case of oscillation angle (α) and from 2 min^{-1} to 40 min^{-1} in case of oscillation frequency (ω). The control unit (CBCU) allowed to control crucial culture conditions, such as: pH level and dissolved oxygen (DO) concentration (both *via* optical fibres connected independently with two miniaturized spectrophotometric sensors of pH and DO built-in inside the bottom of CellbagTM), concentration of O_2 (0–50%) and CO_2 (0–15%) in the inlet gas mixture (gas_{in}), as well as the total gas_{in} flow rate ($0.1\text{--}1.0 \text{ dm}^3 \text{ min}^{-1}$).

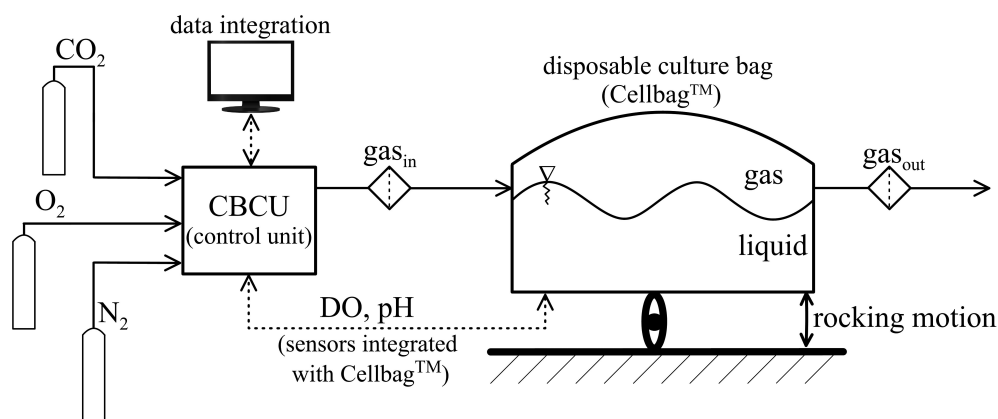


Fig. 2. Schematic diagram of *ReadyToProcess* WAVETM25

2.3. Culture of HL-60 cells in disposable systems

Typical static, i.e. non-agitated, batch cultures of HL-60 cells were referentially performed in classical disposable culture 75 cm^2 flasks (NEST Biotechnology, US) filled with 50 cm^3 of culture medium. All cultures maintained in non-agitated flasks were incubated at $37 \text{ }^\circ\text{C}$ in an incubator with CO_2 -enriched (5.0%) atmosphere, and the initial concentration of HL-60 cells was $10^5 \text{ cells cm}^{-3}$. The non-agitated cultures were maintained continuously for 7 days, and the samples of culture medium were harvested daily.

The batch cultures subjected to wave-type agitation were performed in 2.0-litre CellBagTM flexible bags filled with 300 cm^3 of culture medium, which were fixed in WAVE 25 bioreactor system. Every culture system was stabilized to obtain constant temperature of $37 \text{ }^\circ\text{C}$, as well as 100% saturation of the culture medium with O_2 from sterilely dosed ($0.5 \text{ dm}^3 \text{ min}^{-1}$) gas mixture (5% CO_2 + 21% O_2 + 74% N_2) prior to inoculation and starting point of culture. The initial concentration of HL-60 cells was $10^5 \text{ cells cm}^{-3}$.

The working parameters of the wave-type agitated cultures are shown in detail in Table 1. Every culture performed in WAVE 25 system was maintained continuously for 7 days, and the samples of culture medium were harvested daily.

Table 1. Operating parameters defining conditions of the wave-type agitated cultures of HL-60 cells performed in WAVE 25

Operating parameter	Value	Unit
angle of oscillations (α)	6	[°]
frequency of oscillations (ω)	20	[min ⁻¹]
gas flow rate (Q_G)	0.5	[dm ³ min ⁻¹]
volume of culture medium (V_L)	0.3	[dm ³]
concentration of O ₂ in inlet gas mixture (C_{O_2})	21	[%]
concentration of CO ₂ in inlet gas mixture (C_{CO_2})	5	[%]
temperature (T)	37	[°C]

2.4. Analytical methods

All samples harvested simultaneously from both culture systems were subjected to the following tests:

- (i) counting (manually) HL-60 cells pre-stained with 0.4% trypan blue solution in the Bürker-Türk hemocytometer for determination of cell density, as well as the viability of cells in culture medium;
- (ii) the PrestoBlue test for determination of the metabolic activity of the cells;
- (iii) the BioMaxima-glucose test for quantitatively determination of the glucose concentration in culture medium and further calculation of the specific glucose consumption rate;
- (iv) the BioMaxima-LDH test for determination of the activity of lactate dehydrogenase (LDH) in culture medium, as the quantitative parameter correlated with negative influence of hydrodynamic conditions of culture system forced on the cells suspended in culture medium. All spectrophotometric measurements were performed using GENESYS 20 UV-VIS spectrophotometer (ThermoFisherScientific, US), and Eclipse TS100 reverse microscope (Nikon, JP) was applied for all microscopic analyses.

To determine the viability of HL-60 cells, the sample of culture medium harvested from culture vessel was equimolarly mixed with 0.4% trypan blue aqueous solution (ThermoFischerScientific, US), and then incubated at room temperature for 3 minutes to distinguish the living (i.e. unstained) cells from the dead (i.e. stained) ones. Then the stained cells and the total number of cells were counted manually under microscope. The density (X) and the viability (Z) of HL-60 cells were finally calculated using the following equations:

$$X = \frac{x}{k} d \cdot 5 \cdot 10^5 \text{ [cell cm}^{-1}\text{]} \quad (1)$$

$$Z = \frac{z}{x} \cdot 100\% \quad (2)$$

$$x = z + m \quad (3)$$

where: x is the total number of cells counted in the measuring grid of the hemocytometer, k is the number of squares of measuring grid occupied by cells, d is the dilution of the sample, z is the number of alive (i.e. unstained) cells and m is the number of dead (i.e. stained) cells.

To monitor the metabolic activity of the HL-60 cells suspended in culture medium, a resazurin-based PrestoBlue assay (ThermoFischerScientific, USA) was used. 0.1 cm³ PrestoBlue reagent was added to 0.9 cm³ suspension of the cells. Simultaneously, 0.9 cm³ of pure culture medium (i.e. without the cells) and 0.1 cm³ PrestoBlue reagents were mixed, as the reference sample. All samples were incubated at room temperature for 10 minutes, and then measurements of specific absorbance were carried out at the analytical wavelength $\lambda = 570$ nm versus absorbance of samples measured at $\lambda = 600$ nm as reference. The metabolic activity of the cells (a_M) was finally calculated using the following equation:

$$a_M = 37.04 \cdot A_W \text{ [}\mu\text{kat dm}^{-3}\text{]} \quad (4)$$

where A_W is the specific absorbance of the sample.

To monitor the daily changes of glucose concentration in the culture medium, a BioMaxima-glucose test (BioMaxima, PL) was applied. 20 μ l of the filtered culture medium was mixed with 1.0 cm³ of the specific BioMaxima-glucose reagent. Referentially, 1.0 cm³ of BioMaxima-glucose reagent was mixed with 20 μ l of double-distilled water to be used as the blank sample. All samples were incubated at room temperature for 20 minutes. The measurements with spectrophotometer were carried out at the analytical wavelength $\lambda = 500$ nm. Values of the daily changes of glucose concentration in the culture medium (ΔC_{glc}), as well as X , were used to calculate values of the specific glucose consumption rate *per cell* ($r_{\text{glc/cell}}$) as follows:

$$r_{\text{glc/cell}} = \frac{\Delta C_{\text{glc}}}{\Delta t X} \text{ [g h}^{-1}\text{ cell}^{-1}\text{]} \quad (5)$$

where Δt is the time interval between two consecutive measurements.

The activity of LDH in the culture medium was determined according to the manufacturer procedure of a BioMaxima-LDH test (BioMaxima, PL). 10 μ l of filtered ($\varphi = 0.2$ μ m) culture medium was added to 1.0 cm³ of Biomaxima-LDH reagents, and the absorbance of the reaction mixture was spectrophotometrically monitored in 1-minute intervals. Simultaneously, the mixture of 1.0 cm³ of LDH reagents and 10 μ l of double-distilled water was used as the reference blank sample. Measurements were carried out at the analytical wavelength of the method, i.e. $\lambda = 340$ nm. The LDH activity (a_{LDH}) was finally calculated from the following equations:

$$a_{\text{LDH}} = \frac{V_t}{\varepsilon l V_s} \Delta A \cdot 10^5 \text{ [U dm}^{-3}\text{]} \quad (6)$$

$$a_{\text{LDH}} = 267.2 \cdot \Delta A \text{ [}\mu\text{kat dm}^{-3}\text{]} \quad (7)$$

where ΔA is the absorbency change *per minute*, V_t is the total volume of reaction mixture (i.e. 1.01 cm³), ε is the molar coefficient of NADH absorbency at $\lambda = 340$ nm (i.e. $6.3 \cdot 10^2$ m² mol⁻¹), l is the optical path length (i.e. 1.0 cm³), and V_s is the volume of the sample of culture medium (i.e. 0.01 cm³).

3. RESULTS AND DISCUSSION

The idea of application of litre-scale disposable bioreactors for *in vitro* cultures of animal cells originated over twenty years ago. To date, few wave-type agitated disposable bioreactor systems, which utilize effectiveness of waves generated by continuous oscillating movement of culture vessel for continuous aeration and mixing of culture medium, have been readily and successfully applied in the wide range of bioprocesses focused mainly on propagation of biomass of isolated human, mammalian and insect cells (Clicke et al., 2011; Imseng et al., 2014; Marks, 2003; Sather et al., 2009; Wang et al., 2012), which is briefly shown in Table 2. All bioprocesses introduced in Table 2 were performed in culture bags with the total volume ranging from 1.0 to 2.0 litres because such culture bag volumes are the most frequently utilized for the first stage of robust scaling-up of submerged batch cultures of animal cells from millilitre-scale of

disposable vessels (e.g. culture flasks) into litres and beyond. There is no literature data on propagation of HL-60 cells in disposable bioreactor systems. Nevertheless, based on the data available in published literature (see Table 2), we decided to perform cultures of HL-60 cells in WAVE 25 bioreactor system supporting wave-type agitation under operating parameters (i.e. $\alpha = 6^\circ$ and $\omega = 20 \text{ min}^{-1}$) defined in Table 1 (see *Materials and Methods* section).

Table 2. Some applications of various wave-type agitated disposable bioreactors for *in vitro* propagation of human (h), mammalian (m) and insect (i) cells

Bioreactor (manufacturer)	Cell line	Operating parameters	Reference
WAVE (GE Healthcare; US)	HEK 293 EBNA (h)	$\alpha = 7 [^\circ]$ $\omega = 18 [\text{min}^{-1}]$	Sather et al., 2009
	S2 (i)	$\alpha = 8 [^\circ]$ $\omega = 22 [\text{min}^{-1}]$	Wang et al., 2012
	CHO/dhFr- (m)	$\alpha = 6 \div 8 [^\circ]$ $\omega = 20 \div 27 [\text{min}^{-1}]$	Clicke et al., 2011
Cultibag RM, (Sartorius; DE)	Sf-9 (i)	$\alpha = 6 [^\circ]$ $\omega = 18 \div 32 [\text{min}^{-1}]$	Imseng et al., 2014
Biowave (Sartorius; DE)	HEK 293 EBNA (h)	$\alpha = 6 [^\circ]$ $\omega = 30 [\text{min}^{-1}]$	Marks, 2003

In every bioprocess with isolated cells, the DO, as well as the pH of the culture medium are strictly critical process parameters. So, in the first step of the experiments the basic culture conditions, i.e. DO and pH levels were analyzed for the batch cultures of HL-60 in the static and wave-type conditions. What is also important to know, both measurements were performed using the same DO spectrophotometric mini-sensor, which was successfully transferred from the WAVE 25 system into the 75 cm² culture flask. For process development and scale-up, transfer of the processes between systems and sites is necessary. Therefore, it is essential to ensure comparability between DO and pH readings in different systems.

The level of DO concentration, as well as the pH level, both monitored during the whole time periods of independently maintained batch cultures of HL-60 cells: as the typical static culture and as the wave-type agitated bioprocess, performed in the two compared disposable culture systems, are presented in Fig. 3. In the case of DO characteristics, it can be clearly seen that the WAVE 25 system supported stable level of DO in culture medium in contrast to significant decrease of DO level observed from 2nd day of the batch culture of HL-60 cells maintained in the non-agitated disposable culture flask. Based on the observed effects in DO level it can be concluded that the WAVE 25 disposable system provided stable conditions for aerobic metabolism for all 7 days of HL-60 cell cultures without any noticeable decrease below the constant value of maximum DO concentration of the culture medium. In the case of the pH level, its value decreased monotonically from the 2nd day of the culture irrespective of culture conditions, but the level of pH was still in the range of values preferred by HL-60 cells, i.e. in the range of pH 7.0–7.4. It means that the buffer system applied in RPMI medium efficiently prevented any significant changes of pH level during 7-day cultures of HL-60 cells regardless of the studied type of culture system.

Next, cell density and cell viability were analyzed in HL-60 cells cultured in both studied systems. The comparison of the density of HL-60 cells, as well as their viability, in samples of culture media simultaneously harvested from the reference (i.e. non-agitated) system and WAVE 25 bioreactor is presented in Fig. 4. In the case of the cell density characteristics (i.e. Fig. 4A), it turned out, that from the 3rd day of cultures the values of such parameter were considerably higher for the cultures subjected to wave-type

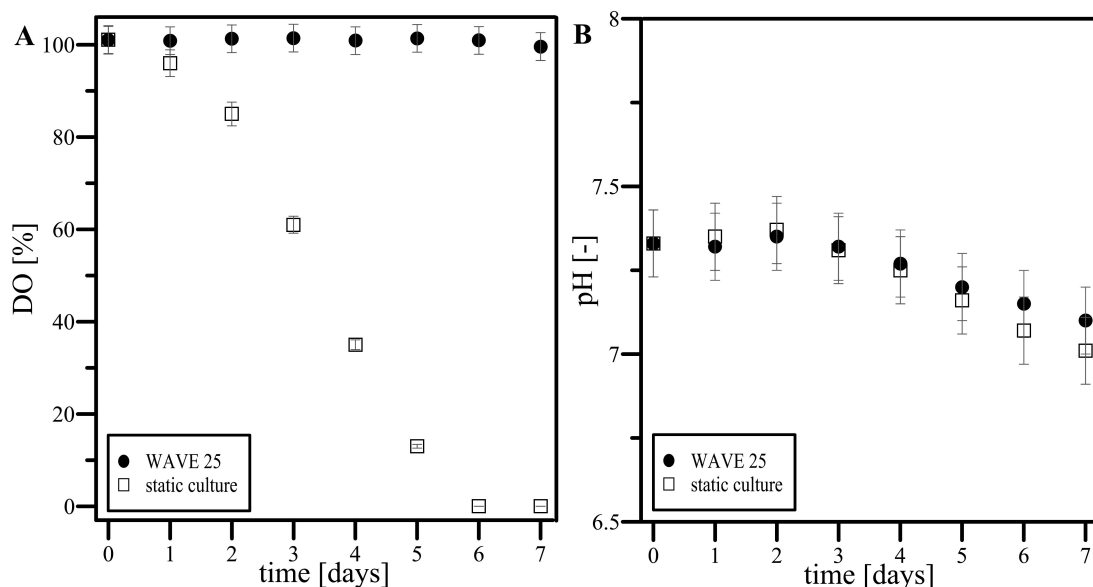


Fig. 3. The time-courses of DO (A) and pH levels (B) measured for cultures of HL-60 cells performed in WAVE 25 (•) and in non-agitated culture flasks (□)

agitation, than for the non-agitated static cultures. A similar tendency was also reported for the viability of HL-60 cells calculated for the static and WAVE 25 systems. Such results of cell density and cell viability of non-adherent HL-60 cells confirmed high potential of applicability of disposable bioreactor system supported with wave-type agitation, as a right platform to perform submerged cultures of suspended non-adherent cells.

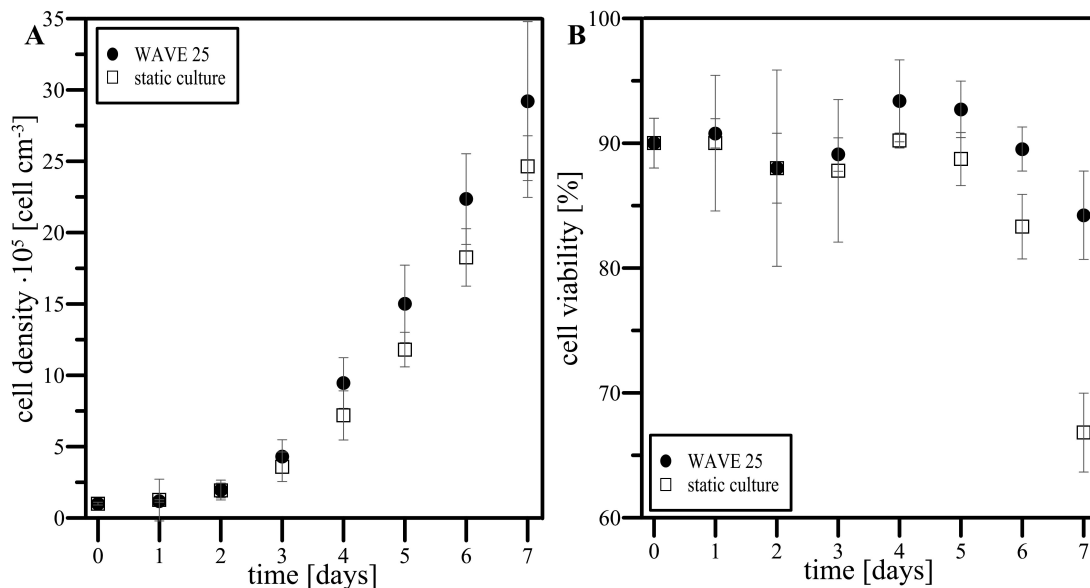


Fig. 4. The comparison of values of the cell density (A) and the viability (B) of HL-60 cells cultured in wave-type agitated WAVE 25 system (•) and in the reference system without any agitation (□)

Then, metabolic activity of HL-60 cells, when they were cultured in the non-agitated system and in WAVE 25 bioreactor, was considered. We decided to simultaneously analyze two parameters, the metabolic activity of intracellular oxidoreductases (based of the PrestoBlue assay), as well as the specific glucose consumption rate, i.e. $r_{\text{glc}/\text{cell}}$ (based on results of the BioMaxima-glucose assay), which enabled us to obtain more complete information on metabolism of HL-60 cells maintained in two compared culture

systems (Sonnaert et al., 2015). The values of the quantitative parameters characterizing metabolic activity of cells in both compared culture systems are presented in Fig. 5.

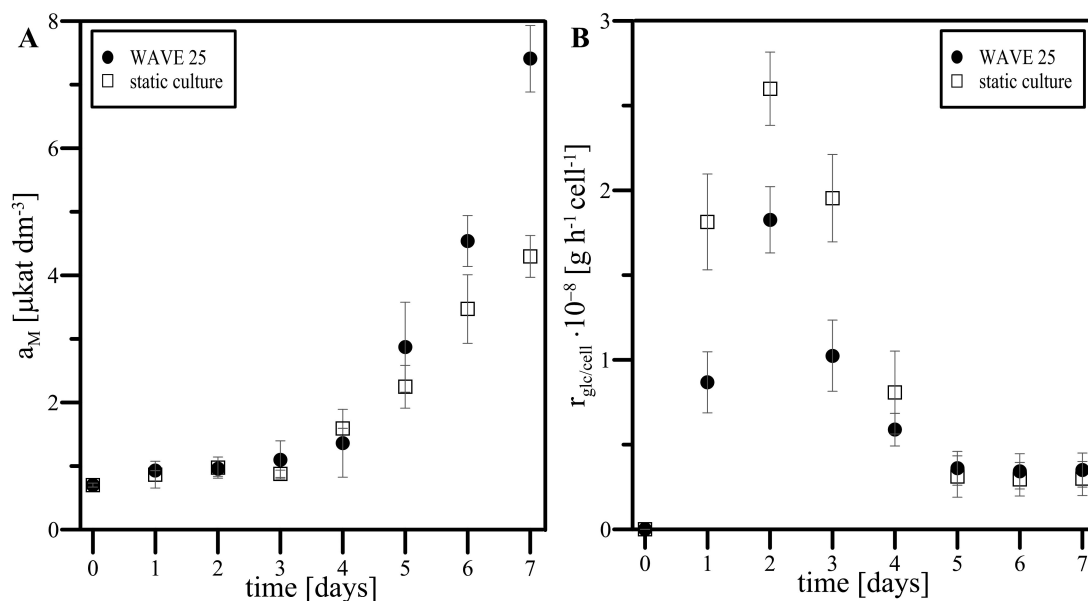


Fig. 5. Values of a_M (A) and $r_{\text{glc/cell}}$ (B) calculated individually for subsequent days of cultures of HL-60 cells performed in WAVE 25 (•) and in the reference non-agitated culture system (□)

The values of a_M of HL-60 cells determined according to PrestoBlue assay for the cells maintained in WAVE 25 system were higher than those of cells maintained referentially in the static system, starting from 3rd day of compared cultures (see Fig. 5A). The significant shift in a_M value determined for HL-60 cells cultured on 7th day in WAVE 25 may be interpreted as hypothetical spontaneous differentiation of the proliferating cells induced by low level of unconsumed monosaccharide, or other ingredients of the RPMI culture medium. Low values of $r_{\text{glc/cell}}$ reported for HL-60 cells maintained in WAVE 25, in contrast to significantly higher values of $r_{\text{glc/cell}}$ which characterized the cells in static cultures (see Fig. 5B), may be interpreted as the confirmation of the advantageous culture conditions supported by WAVE 25 in the case of submerged maintaining of suspended non-adherent cells. The values of $r_{\text{glc/cell}}$ calculated for the system with continuous wave-type agitation were more than 42% lower than the values noted for the static culture. Simultaneously observed higher values of cell density and lower values of the $r_{\text{glc/cell}}$ parameter, which characterized bioprocess in WAVE 25, indicated the more sustainable metabolism of HL-60 cells suspended and continuously wave-type agitated, which finally resulted in efficient propagation of the cells at lower level of metabolic assimilation of glucose exhibited by the cells.

The wave-type agitation of culture systems induced mixing, and thus homogeneity of cells suspension, in culture vessel subjected to oscillation movement. But simultaneously, agitation may cause negative effects of hydrodynamic shear stress, which affects destructively fragile cells suspended in culture broth. Such negative effects (resulting from e.g. collisions of cells, or collisions of cells with the inner walls of culture vessel) are intensified in more dense suspensions of cells, and if the agitation is too intense, progressive limitation of cell propagation (i.e. the cell growth rate) in culture systems might be observed (Odeleye et al., 2014). The activity of LDH, i.e. a_{LDH} , was monitored to check the potentially negative influence of culture conditions on physiology of HL-60 cells maintained under continuous wave-type agitation (Pilarek et al., 2017). LDH is an intracellular enzyme which is rapidly released into the media from even slightly damaged cells, and thus it is widely recognized as a specific biomarker for cellular cytotoxicity and cytolysis. The comparison of values of a_{LDH} detected in samples of culture media harvested daily from both studied culture systems is presented in Fig. 6. Much higher values of a_{LDH} were detected in samples from the static system, compared to rather low values of the parameter noted for the culture performed in WAVE 25,

what unequivocally revealed the negative influence of the static, non-agitated system, than in the WAVE 25 bioreactor under wave-type agitation defined by the applied operating parameters of the bioreactor. We hypothesize that this effect could have resulted from increased sedimentation of cells in non-agitated disposable culture flask which probably resulted in the mass transfer limitation (please see also Fig. 3A).

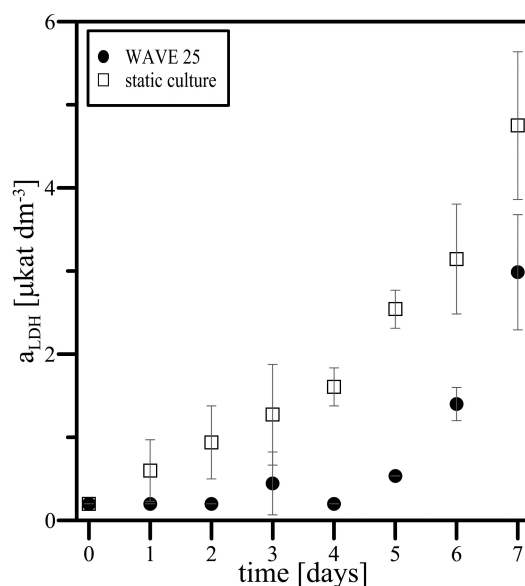


Fig. 6. Values of a_{LDH} determined for samples of RPMI medium harvested from cultures of HL-60 cells performed in WAVE 25 (•) and the reference system (□)

4. CONCLUSIONS AND OUTLOOK

Summarizing, the following conclusions can be drawn based on the results of the experiments presented and discussed above:

- the WAVE 25 disposable system provided stable conditions for aerobic metabolism without any noticeable decrease below the constant value of the maximum DO concentration noted for the culture medium for 7 days of HL-60 cell cultures;
- considerably higher values of cell density, as well as cell viability were observed for the HL-60 cell cultures performed in disposable culture bag subjected to wave-type agitation in the WAVE 25, than for the non-agitated static cultures in disposable culture flasks;
- more sustainable metabolism of suspended HL-60 cells, and more efficient propagation of the cells at lower level of metabolic assimilation of glucose exhibited by the cells, were revealed for wave-type agitated cultures in the WAVE 25 bioreactor, in comparison to non-agitated culture flasks;
- much lower values of a_{LDH} detected in the cultures performed in the WAVE 25 bioreactor, compared to high level LDH activity which have characterized non-agitated system, unequivocally revealed that lack of agitation in the static system had strong negative influence on the HL-60 cells, as opposed to the same cells in cultures performed in the WAVE 25 bioreactor under wave-type agitation.

Thus, the advantages of the wave-type agitated culture system applied as a platform for propagation of non-adherent cells were unequivocally indicated. Apart from the proved general applicability of the wave-type agitation performed in the WAVE 25 bioreactor for the efficient propagation of HL-60 cells under sustainable metabolism, further studies on optimization of the operating parameters defining wave-type agitation in the WAVE 25 must be performed, to fully characterize biomass yields of HL-60 cells possible to reach in the disposable bioreactor system supported with oscillating movements.

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SYMBOLS

A_W	specific absorbency of the sample
a_{LDH}	activity of lactate dehydrogenase, $\mu\text{kat dm}^{-3}$
a_M	metabolic activity of the cells, $\mu\text{kat dm}^{-3}$
C_{CO_2}	CO_2 concentration in the gas phase, %
C_{O_2}	O_2 concentration in the gas phase, %
d	dilution of the sample
k	number of square occupied by cells
l	optical path length, cm
m	number of dead cells, cells
$r_{\text{glc/cell}}$	specific glucose consumption rate, $\text{g h}^{-1} \text{cell}^{-1}$
T	temperature, °C
Q_G	gas flow rate, $\text{dm}^3 \text{min}^{-1}$
V_L	volume of the culture medium, dm^3
V_s	volume of the sample, cm^3
V_t	the volume of the reaction mixture, cm^3
X	density of HL-60 cells in the culture medium, cell cm^{-3}
x	number of the cells counted in hemocytometer, cells
Z	viability of the cells, %
z	number of alive cells, cells

Greek symbols

α	angle of oscillations, °
ΔA	absorbency change per minute
ΔC_{glc}	glucose concentration change, g dm^{-3}
Δt	time interval between two measurements, h
ω	frequency of oscillations, min^{-1}
ε	molar coefficient of NADH absorbency at $\lambda = 340 \text{ nm}$, $\text{m}^2 \text{mol}^{-1}$
φ	average filter pore diameter, μm
λ	wavelength, nm

Subscripts

CO_2	carbon dioxide
O_2	oxygen

Abbreviations

CHO/dhFr	adherent hamster ovary cell line
DO	dissolved oxygen
FBS	fetal bovine serum
HEK 293 EBNA	adherent human embryonic kidney cell line
HL-60	non-adherent human promyelocytic leukemia white blood cell line
LDH	lactate dehydrogenase (EC 1.1.1.27)
PenStrep	mixture of antibiotic and antimycotic agents
RPMI	Roswell Park Memorial Institute 1640 medium
Sf-9	adherent/suspension <i>Spodoptera frugiperda</i> ovary cell line

S2 cell line derived from *Drosophila melanogaster* S2 embryos
WAVE 25 ReadyToProcess WAVE™ 25 bioreactor system

REFERENCES

- Clicke M.F., Mölleryd C., Zhang Y., Lindskog E., Walsh K., Chotteau V., 2011. Study of a recombinant CHO cell line producing a monoclonal antibody by ATF or TFF external filter perfusion in a WAVE Bioreactor™. *BMC Proc.*, 5, P105. DOI: 10.1186/1753-6561-5-S8-P105.
- Eibl R., Werner S., Eibl D., 2009. Disposable bioreactors for plant liquid cultures at litre-scale. *Eng. Life Sci.*, 9, 156–164. DOI: 10.1002/elsc.200800102.
- Gottschalk U., Brorson K., Shukla A.A., 2012. The need for innovation in biomanufacturing. *Nat. Biotechnol.*, 30, 489–492. DOI: 10.1038/nbt.2263.
- Im seng N., Steiger N., Frasson D., Sievers M., Tappe A., Grell er G., Eibl D., Eibl R., 2014. Single-use wave-mixed versus stirred bioreactors for insect-cell/BEVS-based protein expression at benchtop scale. *Eng. Life Sci.*, 14, 264–271. DOI: 10.1002/elsc.201300131.
- Marks D.M., 2003. Equipment design considerations for large scale cell culture. *Cytotechnology*, 42, 21–33. DOI: 10.1023/A:1026103405618.
- Nienow A.W., Scott W.H., Hewitt C.J., Thomas C.R., Lewis G., Amanullah A., Kiss R., Meier S.J., 2013. Scale-down studies for assessing the impact of different stress parameters on growth and product quality during animal cell culture. *Chem. Eng. Res. Des.*, 91, 2265–2274. DOI: 10.1016/j.cherd.2013.04.002.
- Odeleye A.O.O., Marsh D.T.J., Osborne M.D., Lye G.J., Micheletti M., 2014. On the fluid dynamics of a laboratory scale single-use stirred bioreactor. *Chem. Eng. Sci.* 111, 299–312. DOI: 10.1016/j.ces.2014.02.032.
- Pilarek M., Godlewska K., Kuźmińska A., Wojasiński M., Dąbkowska K., 2017. Enhanced chondrocyte proliferation in a prototyped culture system with wave-induced agitation. *Chem. Process Eng.*, 38, 321–330. DOI: 10.1515/cpe-2017-0025.
- Pilarek M., Sobieszuk P., Wierzchowski K., Dąbkowska K., 2018. Impact of operating parameters on values of a volumetric mass transfer coefficient in a single-use bioreactor with wave-induced agitation. *Chem. Eng. Res. Des.*, 136, 1–10. DOI: 10.1016/j.cherd.2018.04.012.
- Pollard D., Kistler C., 2016. Disposable bioreactors, In: Larroche C., Sanroman M.A., Du G., Pandey A. (Eds.), *Current Developments in Biotechnology and Bioengineering: Bioprocess, Bioreactors and Controls*. Elsevier, Amsterdam, 353–380.
- Sather D.N., Armann J., Ching L.K., Mavrantoni A., Selhorn G., Caldwell Z., Yu X., Wood B., Self S., Kalams S., Stamatatos L., 2009. Factors associated with the development of cross-reactive neutralizing antibodies during Human Immunodeficiency Virus type 1 infection. *J. Virol.*, 83, 757–769. DOI: 10.1128/JVI.00283-09.
- Sette A., Barbaroux M., 2006. Properties of materials used in single-use flexible containers: requirements and analysis. *BioPharm Int.*, 6, 1–8.
- Shukla A.A., Gottschalk U., 2012. Single-use disposable technologies for biopharmaceutical manufacturing. *Trends Biotechnol.*, 31, 147–154. DOI: 10.1016/j.tibtech.2012.10.004.
- Sonnaert M., Papantoniou I., Luyten F.P., Schrooten J.I., 2015. Quantitative validation of the PrestoBlue metabolic assay for online monitoring of cell proliferation in a 3D perfusion bioreactor system. *Tissue Eng. Part C*, 21, 519–529. DOI: 10.1089/ten.TEC.2014.0255.
- Wang L., Hu H., Yang J., Wang F., Kaisermayer C., Zhou P., 2012. High yield of human monoclonal antibody produced by stably transfected *Drosophila* Schneider 2 cells in perfusion culture using wave bioreactor. *Mol. Biotechnol.*, 52, 170–179. DOI: 10.1007/s12033-011-9484-5.

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