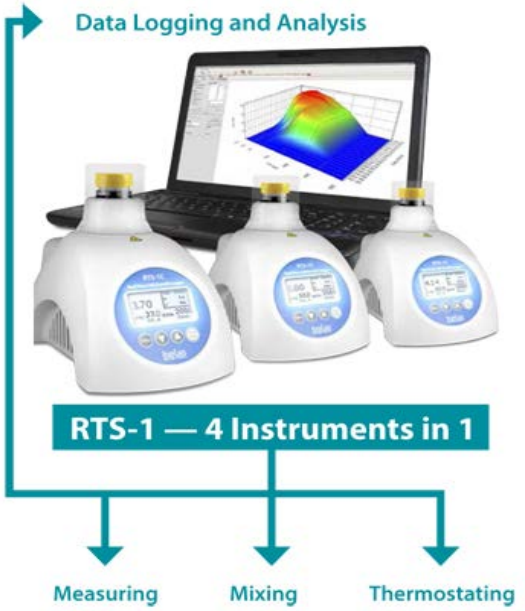
Reverse-Spin® Technologie

Description

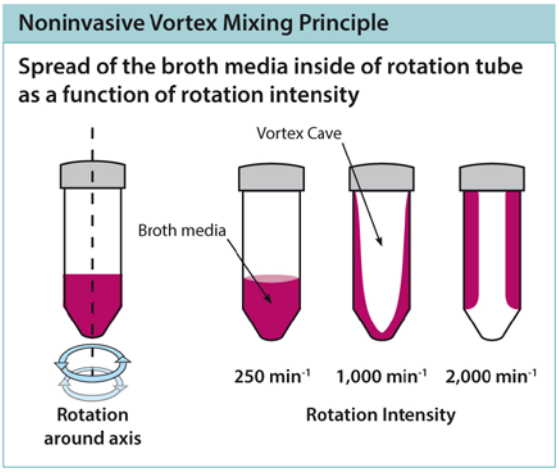
Reverse– Spinner — is a thermostating device with very low energy consumption that realizes innovative type of mixing, where liquid (cells in liquid medium) is mixed by the tube rotation around its axis, leading to highly efficient Vortex Type Mixing (VTM).

Absence of agitators inside the reactor gives opportunities to use Reverse Spinner as a rotating spectrophotometer (spectra–cells), which measures optical density in the reactor in Real–Time. Software makes it possible to set optimal parameters of fermentation, registers and logs all parameters (mixing intensity, temperature of the process, optical density and cell concentration, speed of the growth, etc.).



Noninvasive Vortex Mixing Principle

Initiation of the Vortex Type Mixing (VTM) and depth of the Vortex cave depend on 1) angular speed of RS–Reactor 2) time from initiating rotation of RS–Reactor 3) growth media viscosity 4) temperature. These parameters, also, determine the angular speed of rotating Vortex Layer (VL) and transition state from the Irrotational Vortex (IRV), when angular speed of the VL is proportional to the radius, to the Rotational Vortex, when the angular speed of the VL is the same and VL looks like a monolithic Vortex cavity. Common rules regulating Vortex type mixing processes may be stated as follows: the more time has passed since Vortex formation, the more obvious is a transition from IRV to the RV. The concept of the Reverse–Spin mixing is based on these assumptions.



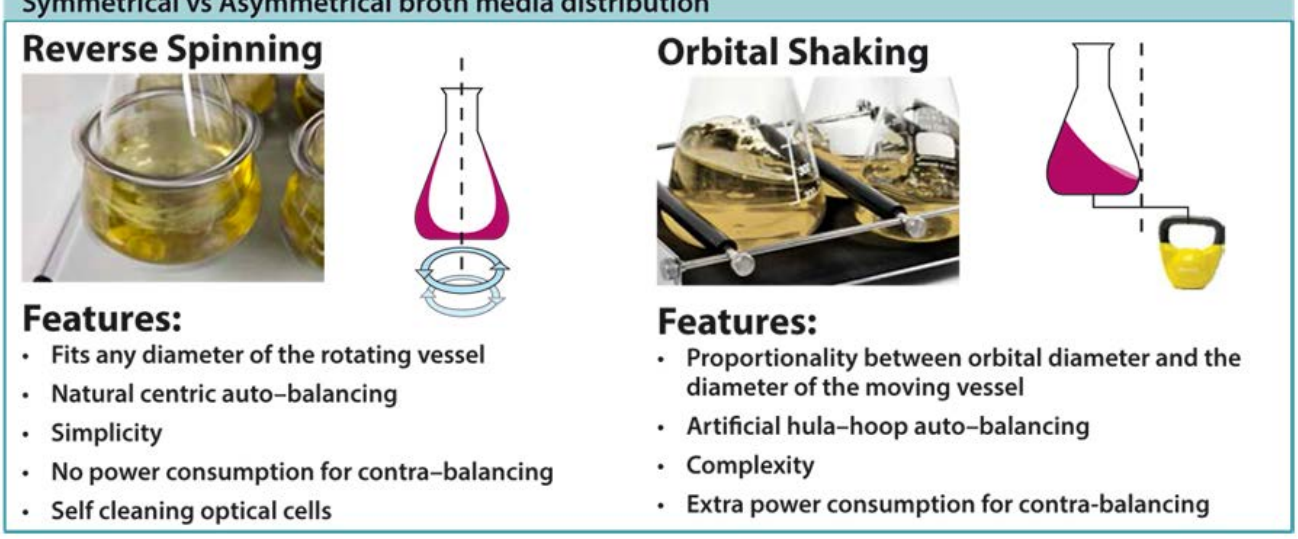


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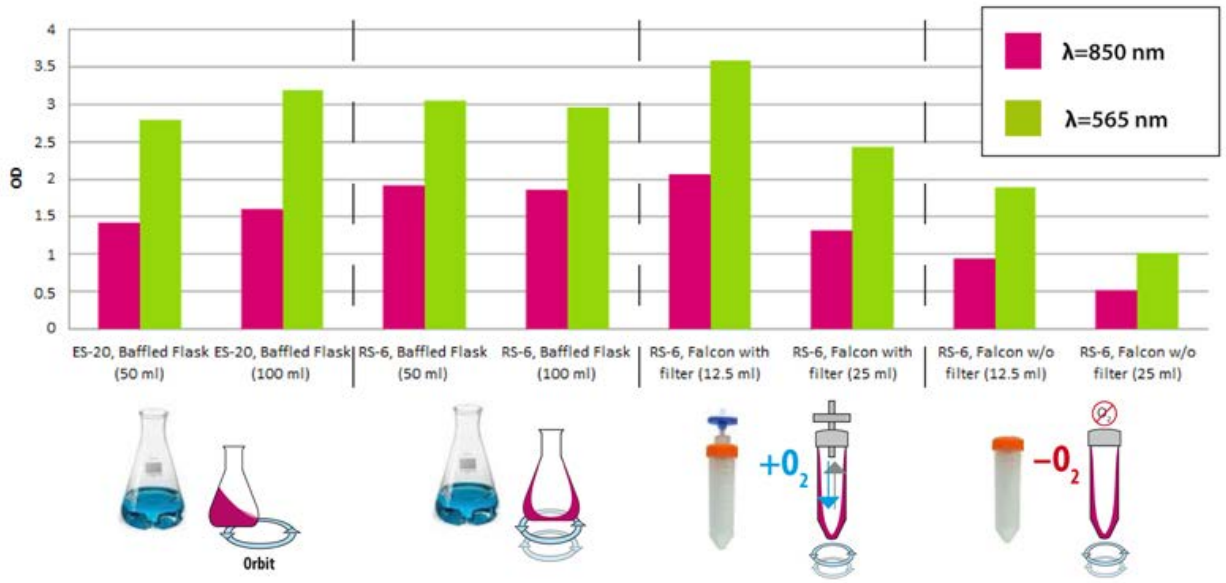
Fig 1. Aeration effect on E.coli BL21 growth using different growth techniques



The fig. 1 shows a comparative data of the biomass yield obtained for the E.coli night culture cultivated in LB medium in Erlenmeyer flasks (Shaker–Incubator ES-20, BioSan) and in test-tube reactors, RTS-1. Biomass yield is presented in the optical densities measured at 600 nm and 850 nm wavelengths. The results obtained for a tube rotating around its own axis at a speed up to 2,000 revolutions per minute.

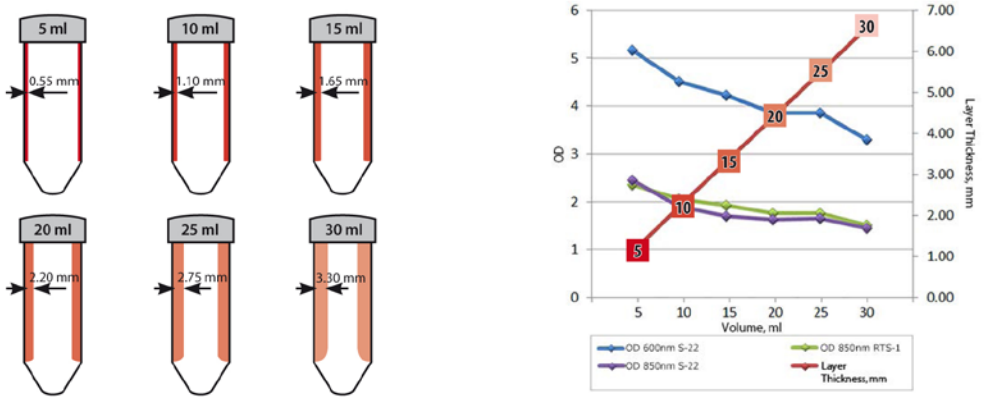
The experimental data suggests that biomass yield obtained using the Reverse Spin Technology is not lower than biomass amount obtained by traditional methods of cultivation and, as is the case of cultivation in Erlenmeyer flasks, depends on the volume of medium in the flask, all other parameters being equal.

Next, we concentrated our efforts on the development of correct cell concentration measurement technology in Real–Time. As you may know, the final concentrations of E. coli cells in LB medium significantly exceed OD=1.0 at λ=600 nm, which requires stopping the process of growing cells, sterile sampling and dilution. This makes the process of growing cells and controlling their concentration very difficult to reproduce.

The problem lies in the fact that the turbidimetric coefficients unlike molar extinction coefficients — are not linear. The behaviour of light in dense cell suspensions (see fig. 5 on page 4) is very interesting and at more than 2 OD at 600 nm it is almost impossible to measure the concentration of cells directly (unless you measure the Rayleigh scattering).

We approached this problem from a different side. It is known that the shorter the optical path is, the more

Fig 2. The effect of Media Volume on it's Layer Thickness (mm) during Reverse-Spin cycle



#	V, ml	RTS-1, ODonline 850nm	RTS-1, Layer Thickness, mm	RTS-1, Optical Path, mm	S-22, ODoff-line 850nm, 1mm cuvette	S-22, ODoff-line 600nm, 1mm cuvette	Cells Yield, 600 nm
1	5	2.45	0.55	1.10	2.35	5.17	25.8
2	10	1.92	1.10	2.20	2.05	4.51	45.1
3	15	1.71	1.65	3.30	1.92	4.22	63.3
4	20	1.62	2.20	4.40	1.75	3.85	77.0
5	25	1.65	2.25	5.50	1.75	3.85	96.2
6	30	1.45	3.30	6.60	1.50	3.30	99.0

accurately it is possible to measure the concentration of cells, even at high densities (up to 10 OD). For this purpose, test tubes containing different volumes of medium are intensely rotated (2,000 min-1) and as a result, a monolayer of medium is generated, which thickness is directly proportional to the volume of culture medium in the tube (see fig. 2 below). Previously, we achieved linearity in the data, when measuring cell concentrations from 1–10 OD in the optical path of 1 mm, therefore correction coefficients were introduced in the program of RTS-1, which allows to measure concentrations of cells in a wide range. The algorithm for determining the concentration of bacterial cells in Real–Time includes the formation of the monolayer at given intervals and cell concentration measuring process. The process takes 5–10 seconds and then initially set parameters for the cell growth automatically restore. The graph shows that the cell concentration range optimal for measurement is 5–30 ml of culture medium in the reactor.

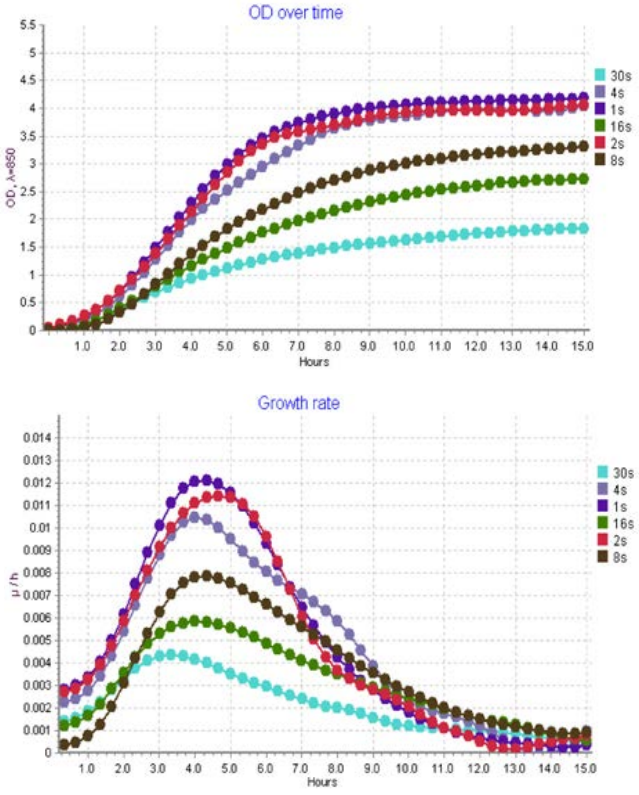


Fig 3. Influence of Frequency of Reverse Spinning on the Growth kinetics and Growth Rate (ΔOD(λ=850nm)/Δt) vs Time of fermentation (hrs). Legend of experiment: Real Time Cell Growth Logger was used — RTS-1 with 850 nm LED, Volume of LB media in 50 ml Falcon = 15 ml approx., Reverse Spin Frequency (RSF) 1, 2, 4, 8, 16, 30 sec -1 , Measurements frequency (MF) is 10 min-1 approx., Rotation speed of reactor = 2,000 rpm , temperature 37°C , Diameter of filters` pores (for aeration) = 0.25 µm.

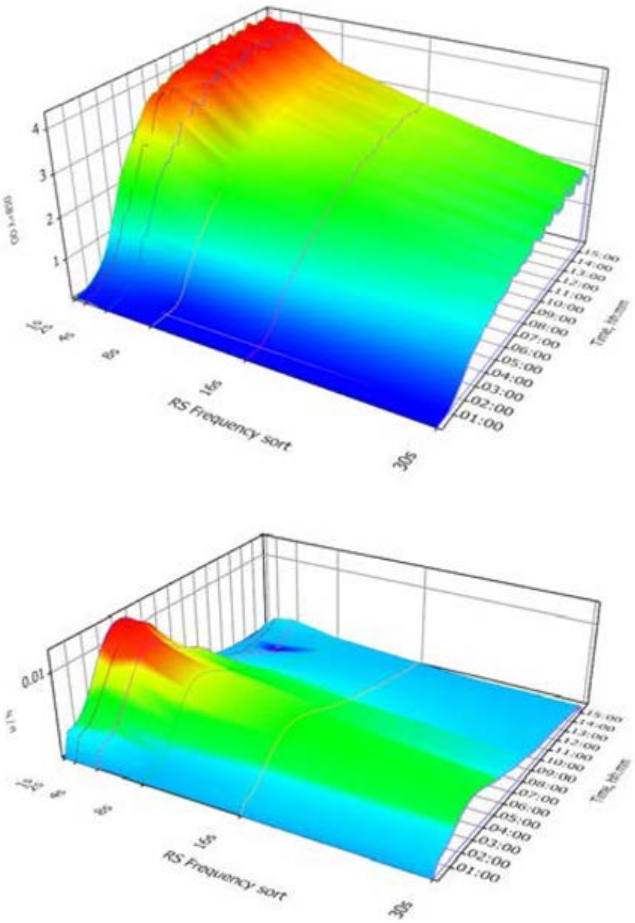


Fig 4. Influence of Frequency of Reverse Spinning on the Growth Kinetics vs Time of fermentation of E.coli BL21 in a 3D model.

Results obtained indicate that the maximum rate of cell division is detected at a frequency of 1 Reverse Spin per second (1 sec-1) at a speed of 2,000 rpm. The increase of pause between reverse spins reduces cell growth rate, reaching 50% of the maximum value, when RS freq. = 30 sec (see fig 3.).
For a better visual representation of the results, the data of three factor interdependence experiments are presented in the form of the 3D graph (see fig. 4). Display of the experimental results in the 3D format has one more advantage that the obtained data provides a clear visual tool for the analysis of the complex interrelated processes of cell growth and allows to find the optimal and reproducible parameters obtained for the output of the cell material.

Growth rate versus time data, presented in the graph, was obtained during 20 hours long fermentation process and optical density was measured in a 10 minutes interval. The optical density was determined in the monolayer of growing cells and growth media formed as a result of a Vortex (as described in the legend to fig. 3). Volume of the culture medium is taken into account when calculating the length of the optical path of the rotating tube that allows to calculate the optical density in standard values familiar for biotechnologists (λ=600 nm, optical path: 10 mm).
Classical 2D data view of cell growth versus time obtained at the endpoint or during cell growth when cell density was measured at intervals of 1–4 hours do not provide such opportunity.

Behaviour of Light in the Environment of Different Densities

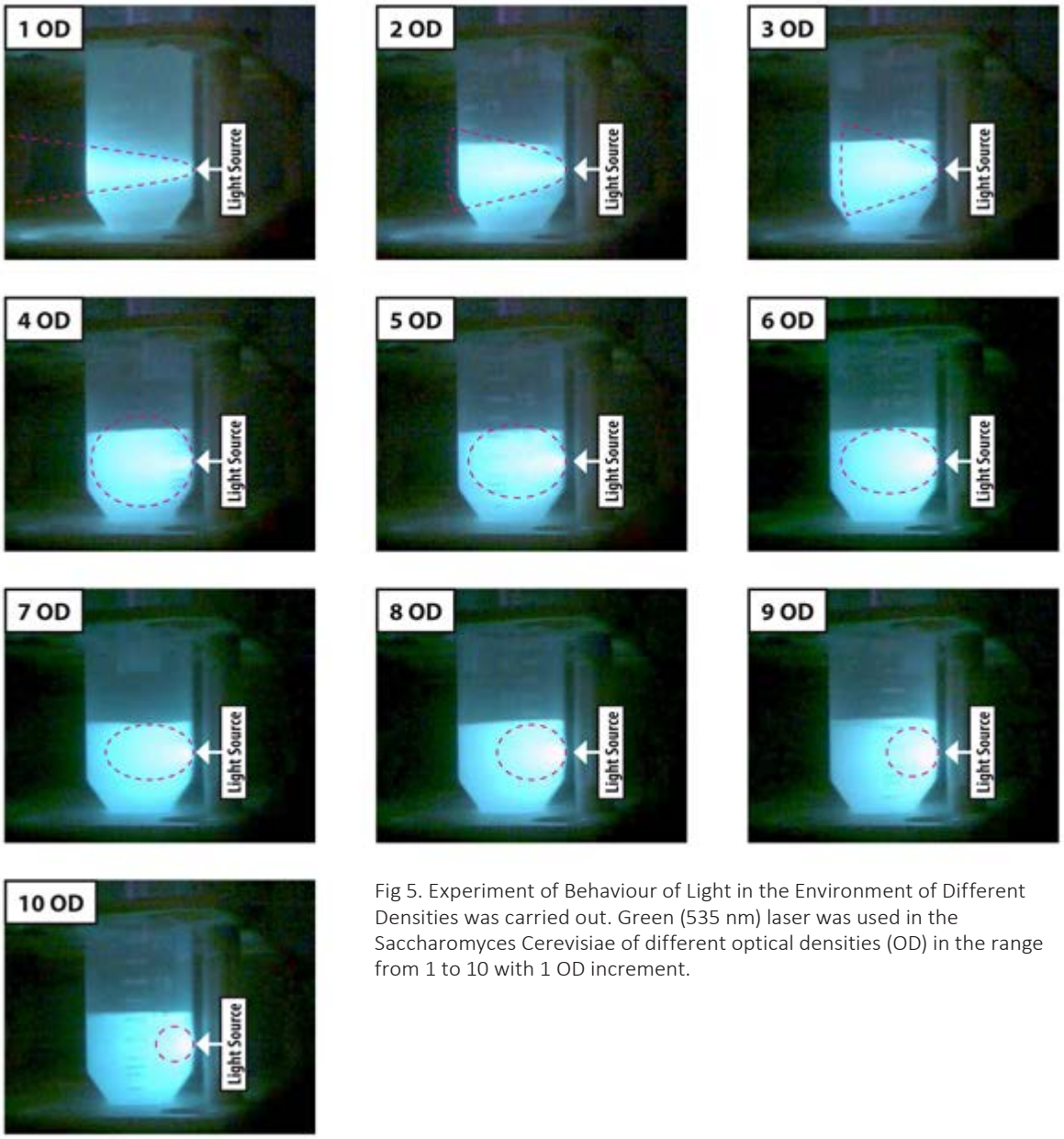


Fig 5. Experiment of Behaviour of Light in the Environment of Different Densities was carried out. Green (535 nm) laser was used in the Saccharomyces Cerevisiae of different optical densities (OD) in the range from 1 to 10 with 1 OD increment.

Conclusion

RTS-1 — Reverse Tube Spin cultivation based on the new method of external agitation of cultivation media, has been shown to be efficient for cultivation of aerobic microorganisms and cell growth logging.
Effective growth of E. coli on LB media has been demonstrated under extremely high speed rotation of the reactor (2,000 rpm).
To increase an OD measurement range we investigate near infrared (IR) spectra and showed that a 850 nm wavelength is sufficient to measure increased cell concentrations. Such wavelength shift (from traditional 600 nm to 850 nm) strongly expanded a range of correct OD measurements.
Moreover, we propose a new technology of a non-contact high biomass measurements during fermentation based on a formation of a thin layer of cultivation media, giving a correct data of bacteria concentration in a rotating reactor.
As a result, proposed RTS-1 technology excludes sampling and dilution procedure that is especially dangerous for harmful bacteria, pathogens or microorganisms living in extreme conditions, like Thermophilus. Results will be published in next issue of BioSan Analytica Journal.

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