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National Aerospace Laboratory NLR

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COMPACT OPTICAL SENSOR FOR REAL-TIME MONITORING OF BACTERIAL GROWTH FOR SPACE APPLICATIONS

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ABSTRACT

During long-duration manned space-missions complex chemical and biological processes need to be managed accurately for the recycling of human wastes and to produce human consumables. As a result, there is an increasing interest in how the characteristics of microbes are influenced by micro-gravity. Compact optical instrumentation allows for real-time and non-invasive measurement of bacterial growth parameters during flight experiments. In close collaboration, the National Aerospace Laboratory NLR of the Netherlands and Bioclear Environmental Biotechnology developed and tested an on-line optical biomass sensor successfully. The sensor concept is based on a turbidity measurement technique operating in the VIS-blue part of the light spectrum with use of blue LED sources. A diagnostic tool has been developed using compact spectrometers and optical fibres to characterise bacterial cultures. As a result a few sensor applications operating at different colours and sensor layouts are discussed in the paper.

INTRODUCTION

The recycling of human waste and production of human consumables are almost mandatory necessities for the success of long-term manned space missions. For that purpose complex biological and chemical processes are incorporated in the life support systems onboard the spacecraft. These processes need to be managed accurately. Applied biotechnological recycling technologies, e.g. biological air filters (BAF), make use of microbial activities (1). Consequently, there is an increasing interest in the micro-gravity influence on bacterial growth.

Recently several microbiological growth experiments have been performed in a micro-gravity environment onboard a space laboratory. Up to now the bacterial growth parameters obtained with these experiments were analyzed after flight in the usual laboratory environment on ground. This type of research will benefit considerably from in-flight real-time monitoring and online data recording.

The present paper covers a period of two years of research that was started with laboratory investigations on a compact optical turbidity sensor, because of its interesting features for micro-gravity experiments (2). The turbidity sensor concept has been developed into a sensor for real-time bacteria growth measurement. Recently the sensor has been used for the determination of growth characteristics of *Xanthobacter Autotrophicus GJ10* (3). In the sensor concept, two techniques are effectively combined, i.e. simultaneous measurement of transmission (Optical Density) and scattering measurement.

In the present paper a short discussion is given of the sensor concept. In order to optimize the operation of the sensor for new applications a diagnostic tool was developed. Finally, a few applications and sensor configurations are discussed such as a Wireless Turbidity Sensor (WTS) prototype, a Turbidity sensor on a Random Positioning Machine (RTS) and an in-line (ITS) version for continuous biomass production monitoring for an experimental bioreactor. Applications of the sensor for

milking machines and hydraulic oil systems are currently under investigation.

NOMENCLATURE

List of acronyms

3T	3T B.V. Measurement, Control & Data-registration, Enschede.
ATS	Autonomous Turbidity Sensor
BAF	Biological Air Filter
Bioclear	Bioclear Environmental Biotechnology, Groningen.
DESC	Dutch Experiment Support Centre
FOTON	Russian unmanned satellite
ITS	In-line Turbidity Sensor
LED	Light Emitting Diode
Melissa	Micro-Ecological Life Support Alternative
OD	Optical Density
RPM	Random Positioning Machine
RTS	Turbidity sensor on the RPM
WTS	Wireless Turbidity Sensor
WTS	Wireless Turbidity sensor
XA GJ10	bacterium *Xanthobacter Autotrophicus GJ10*

List of symbols

α	absorptivity function
β	scattering function
$\Delta\lambda$	part of wavelength spectrum
λ	wavelength of the transmitted light
[c]	concentration of scatters (e.g. cells)
d	transmission light path
D	detection function
I	intensity function
R	ratio of scattering- and transmission signal
R*	geometrical mean of ratios R
S	detected signal

List of subscripts

λ	referring to the wavelength
λ_1	lower wavelength value
λ_2	upper wavelength value
det	referring to the detector
medium	referring to the medium without cells
cells	referring to the cells in the sample

TRANSMISSION MEASUREMENTS

Optical laboratory techniques for bacteria growth measurement are since a long time based on the observation that bacterial cultures become visibly turbid when they multiply. As such the bacteria density, or biomass, is related to a measure for the turbidity. Usually the biomass is obtained by measurement of the Optical Density (OD) at a predefined wavelength e.g. with use of a photo-spectrometer. The OD value is calibrated to the bacteria concentration by counting the bacteria number. For these measurements, as for instance for the growth of the bacteria *Xantobacter Autrophicus GJ10*, blue light is used at λ = 450nm. The OD is defined as the logarithm of the ratio of the undisturbed light intensity and the intensity of the light transmitted through the absorbing material. According to Beer's law, the detected signal obtained after transmission through the absorbing material can be expressed as an exponential function as follows:

$$S_{\det} = I_\lambda \cdot e^{-\alpha_\lambda \cdot d} \cdot D_\lambda \qquad [1]$$

with, at specified wavelength λ:

$S_{det,\lambda}$	detected signal
I_λ	input intensity
α_λ	absorptivity
d	transmission light path
λ	wavelength of the transmitted light
D_λ	detector function

For a culture medium with, and without growing bacteria, the detected signals, according to [1], are respectively:

$$S_{\det,sample} = I_\lambda \cdot e^{-(\alpha_{\lambda,cells}\cdot[c]+\alpha_{\lambda,medium})\cdot d} \cdot D_\lambda \qquad [2]$$

and

$$S_{\det,medium} = I_\lambda \cdot e^{-\alpha_{\lambda,medium}\cdot d} \cdot D_\lambda \qquad [3]$$

with, at the specified wavelength λ:

$S_{\lambda,det,\ sample}$	detected sample signal
$S_{\lambda,det,\ medium}$	detected medium signal
$\alpha_{\lambda,cells}$	absorptivity of the cells per unit concentration
$\alpha_{\lambda,medium}$	absorptivity of the medium
[c]	bacterial concentration

From its definition the Optical Density (OD) is calculated from the ratio of [2] and [3], in order to compensate for random errors and characteristic instrument component parameters, such as the output spectrum of the light source, the spectral sensitivity of the detector and absorptivity of the medium:

$$OD_\lambda = -^{10}\log(S_{\det,sample} / S_{\det,medium}) \approx \alpha_{\lambda,cells} \cdot [c] \cdot d \qquad [4]$$

Thus the measured OD_λ can be approximated by a linear function of the cell concentration for small values of $\alpha_{\lambda,cells}.[c].d$.

In a photometer (see figure 1) a light source, for instance a halogen lamp, emits light that is transmitted through a sample, of which the OD is measured. A detector collects the transmitted light. An optical wavelength bandfilter in front of the light source let pass a part of the wavelength spectrum. Within the transmitted spectral band the transmission is assumed to depend on the sample concentration only, provided that the other parameters have been kept constant.

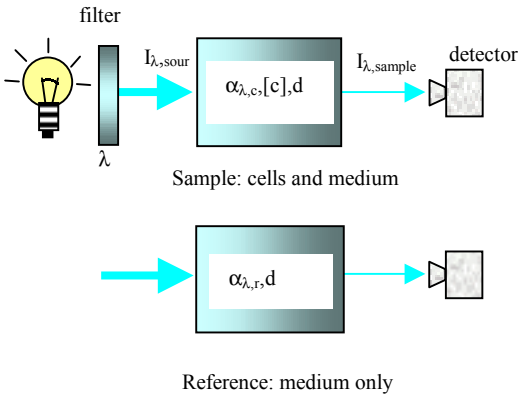

Figure 1. Optical Density (transmission) measurements with a photo-spectrometer using a selected spectrum from source and detector. The OD is calculated from a reference (medium only) and a sample measurement.

The linear measurement range is limited to densities: OD <1, due to increasing concentration and the influence of forward scattering. Since bacteria growth can extent to a period of a few days and may reach high optical densities, measuring bacteria growth using a photo-spectrometer has its limitations. It requires frequent manual extraction of measurement samples, dilution of samples, in case the OD<1 and the taking of reference samples. In addition it is sensitive to pollution of the optical transmitted surfaces.

TURBIDITY MEASUREMENTS

In industry and laboratories, particle concentrations of turbid suspensions are usually measurements with use of light scattering. A white light source is illuminating a suspension that contains the scattering particles (see figure 2). The scattered light is collected on a detector. The sensor signal is integrated function, proportional to light source intensity, detector response, scatter properties of the particles and the particle concentration:

$$S = \int_{\lambda_1}^{\lambda_2} I_\lambda \cdot \beta_\lambda \cdot [c] \cdot D_\lambda \, d\lambda \qquad [5]$$

With, at specified wavelength λ:

S	detected signal
I_λ	input intensity function
β_λ	scatter function
[c]	concentration of scatters
D_λ	detector function
λ_1	lower wavelength value
λ_2	upper wavelength value

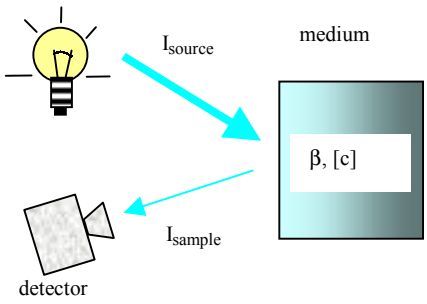

Figure 2. Scattering (turbidity) measurements using a (white) light source and detector. Instrumentation errors are not compensated for.

In order to be able to measure the concentration, the output of the light source, the detector sensitivity and particle scatter properties must be constant. Note that for long duration measurements this is usually not the case. Frequent maintenance is a disadvantage of online turbidity monitoring with use of this concept. In addition the signal response is poor for low concentration values, since fewer scatters are present in the medium. Source and/or detector degradation errors are not compensated for. It requires regular calibration using a turbidity standard solution, such as Formazine.

IMPROVED TURBIDITY SENSOR

The improved turbidity sensor concept is based on the dual ratio turbidity measurement. It applies simultaneous transmitted and scattered light measurement. Based on this concept the Dutch firm 3T BV (Enschede, The Netherlands) originally started with the development of a real-time turbidity sensor for a high turbid application. Accordingly, the sensor has been adapted for bacterial growth measurements under micro-gravity conditions onboard a space laboratory (2).

From an instrumental point of view the sensor has several advantages[1,2] with respect to single OD- or scattering measurements. It is well suited for real-time monitoring and-data analysis. It compensates for instabilities in the light source and detector circuitry and it is insensitive to pollution of optical surfaces. It can be build solidly with compact dimensions and inexpensive components (3).

The turbidity sensor (see figure 3) consists of two Light Emitting Diodes (LED) and two photodiodes that are located around a medium under investigation. The LEDs are operated one by one, producing a transmission and an under 90 degrees scattered signal collected by the photodiodes.

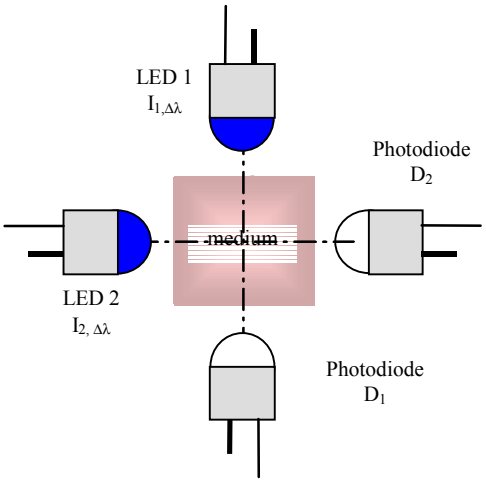

Figure 3. Turbidity sensor concept using two LED's and two photodiodes placed around a medium. The LED's are operated one after each other to measure both ratios of the transmitted and scattered light using the photodiodes. The dual ratio R is calculated from the ratio's thus compensating for instrumentation errors.*

The light intensity $I_{\Delta\lambda}$, the detector sensitivity $D_{\Delta\lambda}$, and specific transmission $\alpha_{\Delta\lambda}$ and scattering properties $\beta_{\Delta\lambda}$ are assumed constant over the LED's spectral bandwidth $\Delta\lambda$, which is normally in the order of 60 nm to 80 nm. The cell concentration is [c] and the dimensions of the sensor [d]. The amplification factors are neglected since they only add linear terms to the equations. Each measurement cycle produces four signals: two transmission and two scattering signals.

[1] Note that the scattered light intensity is usually a few orders of magnitude weaker than the transmitted light intensity. The dual ratio method is only valid when the scatter intensity is above detection limit.

[2] R* also compensates for (inhomogeneous) window fouling. Pollution adds additional linear terms in [5],[6],[7],[8],[9]. The ratios compensate therefor until the windows are completely blocked.

When LED1 is on:

$$S_{1,\Delta\lambda} = I_{1,\Delta\lambda}.e^{-\alpha[c]d}.D_{1,\Delta\lambda} \qquad [5a]$$

$$S_{2,\Delta\lambda} = I_{1,\Delta\lambda}.\beta_{\Delta\lambda}[c].D_{2,\Delta\lambda} \qquad [5b]$$

When LED 2 is on:

$$S_{3,\Delta\lambda} = I_{2,\Delta\lambda}.e^{-\alpha[c]d}.D_{2,\Delta\lambda} \qquad [6a]$$

$$S_{4,\Delta\lambda} = I_{2,\Delta\lambda}.\beta_{\Delta\lambda}.[c].D_{1,\Delta\lambda} \qquad [6b]$$

The ratio's $R_1$and R_2 (obtained from [5a], [5b], [6a], and [6b]) of the scattered and transmitted light signals collected by the two photodiodes produce two signals that compensates for instabilities of the LED:

For LED 1:

$$R_{1,\Delta\lambda} = S_{2,\Delta\lambda}/S_{1,\Delta\lambda} = \beta_{\Delta\lambda}[c]/e^{-\alpha[c]d}.D_{2,\Delta\lambda}/D_{1.\Delta\lambda} \qquad [7]$$

For LED 2:

$$R_{2,\Delta\lambda} = S_{4,\Delta\lambda}/S_{3,\Delta\lambda} = \beta_{\Delta\lambda}[c]/e^{-\alpha[c]d}.D_{1,\Delta\lambda}/D_{2,\Delta\lambda} \qquad [8]$$

Calculation of the geometrical mean value of the two ratios compensates for both detector sensitivity variations, which yields:

$$R^*_{\Delta\lambda} = \sqrt{R_{1,\Delta\lambda}.R_{2,\Delta\lambda}} = \beta_{\Delta\lambda}[c]\, e^{\alpha[c]d}$$

$$\approx \beta_{\Delta\lambda}[c] + \alpha_{\Delta\lambda}\beta_{\Delta\lambda}[c]^2 d + \alpha^2{}_{\Delta\lambda}\beta_{\Delta\lambda}[c]^3 d^2 + \ldots \text{etc}$$

$$= a_1 + a_2[c] + a_3[c]^2 + \ldots \text{etc} \qquad [9]$$

The dual ratio, $R^*_{\Delta\lambda}$ is expressed here as an exponential expansion series, compensates for the instrumentation errors. $R^*_{\Delta\lambda}$ is a polynomial function of the cell concentration [c]. The coefficients a_i are sensitive for the specific transmission and scattering properties of the cells, that considered to be constant. Note that $R^*_{\Delta\lambda}$ depends on selected wavelength (=LED color) and cell geometry. It means that the sensor has to be calibrated for mutually different type of cells of which the growth must be measured. Therefor the sensor signal R^* must be measured for various known cell concentrations. Figure 4 presents an example.

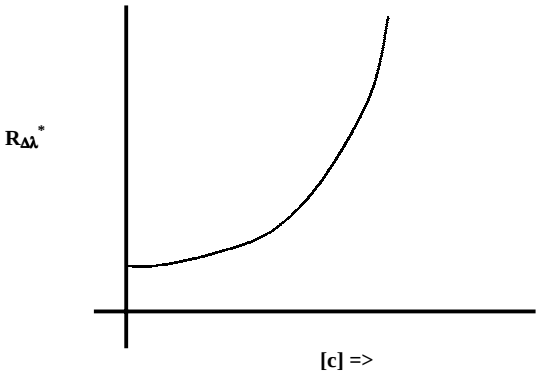

Figure 4. The turbidity sensor is calibrated using known cell concentrations [c] in the operational range, measuring the response $R_{\Delta\lambda}^*$ of the sensor.

General approach for LED selection

For each new application of the improved turbidity sensor, a spectral characterization test must be performed to select the LED color. For this reason a new diagnostic tool was developed, that is described in the next section. After selection of the LED sensor calibration is performed to relate the sensor signal to know sample concentrations (see figure 5).

The advantage of applying the improved turbidity sensor for real-time measurements with respect to the more traditional sample extraction methods is that it is insensitive for instrumentation errors by exploiting the dual ratio. It is compact and cost effective, has a large dynamic range and is spectrally selective, making multi-parameter analysis a possibility. The pro and cons of the discussed techniques are summarized in table 1 below.

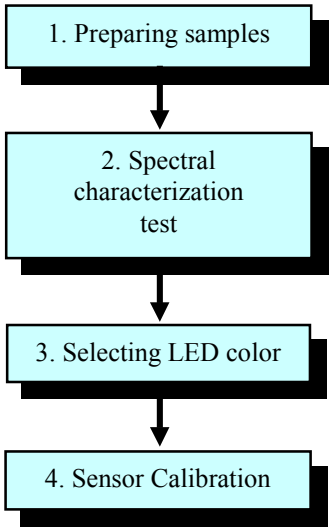

Figure 5. General approach for selecting the LED color for a new application of the improved turbidity sensor.

Table 1. Comparing optical techniques for real-time bacteria growth measurements.

Method	Advantages	Disadvantages
Transmission (Optical Density)	• Compensates for instrumentation errors • Linear with the cell concentration for OD<1 • Accurate and spectrally selective	• Requires reference measurements • Limited range: non- linear above OD >1 dilution of samples required • Off-line: manual extraction of samples required • Laboratory environment • Sensitive to pollution
Scattering (turbidity)	• On-line measurements possible • Coarse: less spectrally sensitive • Industrial environments: robust and cost effective • Large turbidity value applications	• Does not compensate for instrumentation errors • Needs regular calibration with standard solutions • Limited range: weak signal at low turbidities • Sensitive to pollution
Improved turbidity Sensor (Dual ratio of transmission and scattering)	• On-line measurement and control applications • Robust and in-sensitive to pollution and instrumentation errors • Small dimensions and cost effective • Large range: small and large turbididty applications • Spectrally selective, possibly allowing for multi-parameter analysis using more than one sensor • Spectrally applicable: new diagnostic tool	• Non-linear response: calibration required • Spectral characterization test needed

DIAGNOSTIC INSTRUMENTATION

From expression [9] it is clear that R^* is spectrally selective and sensitive for the scattering properties of the cells. Therefore, for a new application the medium needs to be spectrally characterized with diagnostic instrumentation for the selection of the LED spectrum to be used for the sensor (see figure 6).

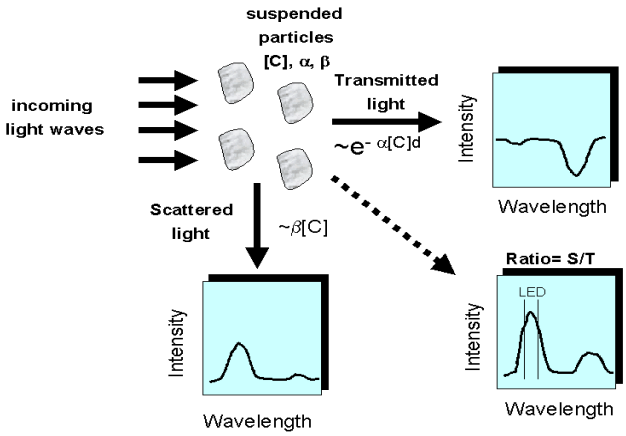

Figure 6. A light source illuminates a medium with suspended particles, it leads to spectrally sensitive transmission and scattering. The maximum ratio gives the LED color to be selected for the improved turbidity sensor.

For this reason a diagnostic instrument has been developed that explores the dual ratio technique by calculating R_λ^*, to compensate for instrumentation errors (see figures 7 and 8). It encompasses a halogen light source, an optical switch, a sample holder and two photo-spectrometers (λ = 530 nm to λ = 1180 nm) mutually connected via optical fibers. A cuvette containing the culture sample is placed in the sample holder. The optical switch adjusts the light the port 1 or 2. Port 3 is used for dark signal measurement. The transmitted and the 90 degrees scattered light intensities are transferred via fibers to the two photo-spectrometers. The integration time for the scattering and transmitted light is automatically adjusted in the software to obtain an optimum signal response. The dark current and integration time are compensated for. A high value of R_λ^* indicates high scattering and low transmission. Which is a suitable choice for the LED spectrum to be used for the turbidity sensor. With the diagnostic instrumentation a successful characterization test was carried out for the selection of the spectrum for the *Rhodospirillum Rubrum* measurement (see Application IV)

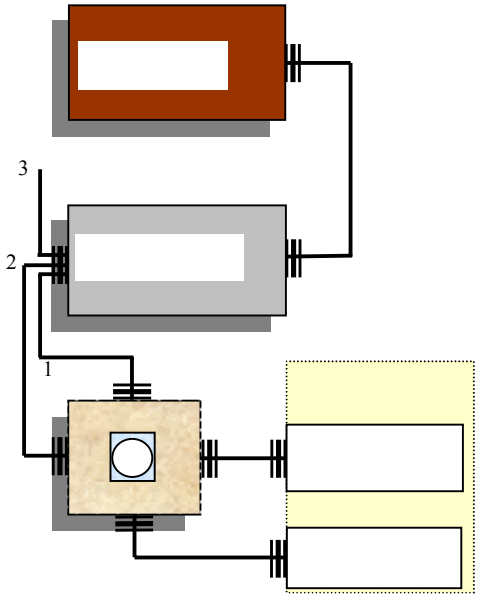

Figure 7. Diagnostic Instrument for new applications of the turbidity sensor using a light source, an optical switch and two photo-spectrometers (λ = 530 nm to λ = 1180 nm) exploring the dual ratio technique to compensate for instrumentation errors.

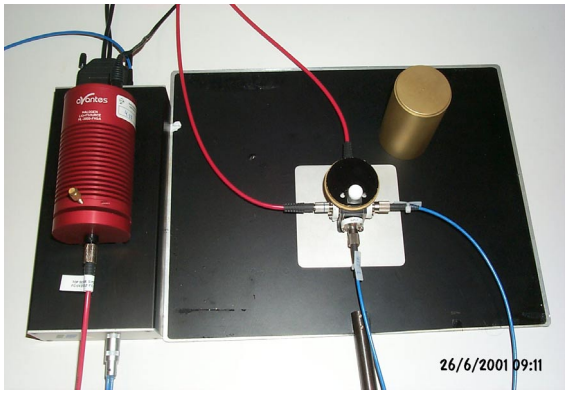

Figure 8. Diagnostic instrument with from left to right: a light source, an optical switch, a sample holder with cuvette and protection cover. Mutually connected via optical fibers.

APPLICATIONS

Application I: The on-line Biomass Turbidity Sensor.

A prototype dual ratio turbidity sensor was used successfully for measurement the growth curve of the bacterium *Xanthobacter autotrophicus GJ10.* The experiment was carried out at Bioclear premises (Bioclear B.V, Environmental Technology) and has been reported previously (3). See figure 9 for a photograph of the set-up and figure 10 for the test results. A square cuvette (with air chamber) is placed in a sample holder. The sensor uses blue LED's which emit at λ = 450 nm. The culture is kept homogeneous with use of a magnetic stirrer. A dark cover prevents external light disturbing the measurements.

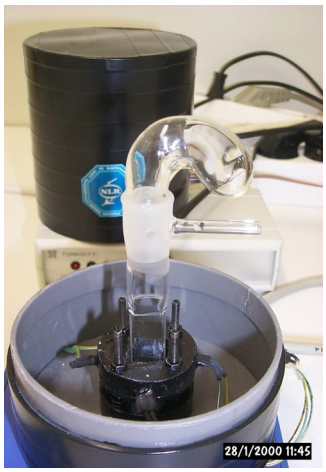

Figure 9. Set-up used for the on-line test of the XA GJ10 Bacterium at Bioclear premises.

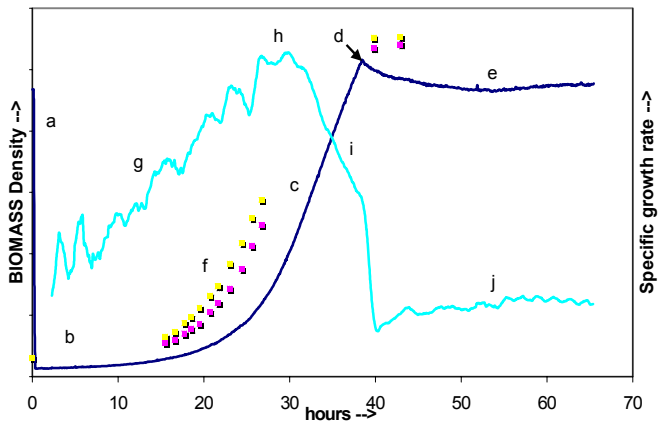

Figure 10. Test results with the improved turbidity sensor showing exponential growth of the XA GJ10 bacterium (c) and specific growth rate (g). Off-line reference measurements (f) show a comparable biomass density and specific growth rate.

The set-up is placed in a temperature-controlled room. The sensitivity appeared adaquate and the derived specific growth rate corresponds to those derived from the control experiments. Interesting feature, yet unexplained, is a sudden signal drop at the end of the exponential growth ((d) in figure 10), when the bacteria culture is running out of nutrient. Apparently it's optical properties changes. By repetition the experiment has been shown to be reproducible.

Application II: Turbidity sensor (RTS) for the Random Positioning Machine (RPM).

The experiment has been developed in close co-operation with the Dutch Experiment Support Centre (DESC, at the Free University of Amsterdam, The Netherlands) and Fokker Space (Leiden, The Netherlands). A sample holder is mounted on the platform of the Desktop RPM of Fokker Space. (Figure 11).

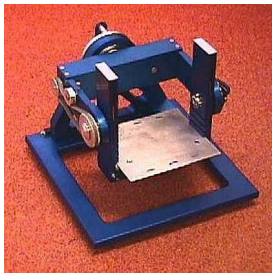

Figure 11. The Desk-Top Random Positioning Machine (RMP) of Fokker Space

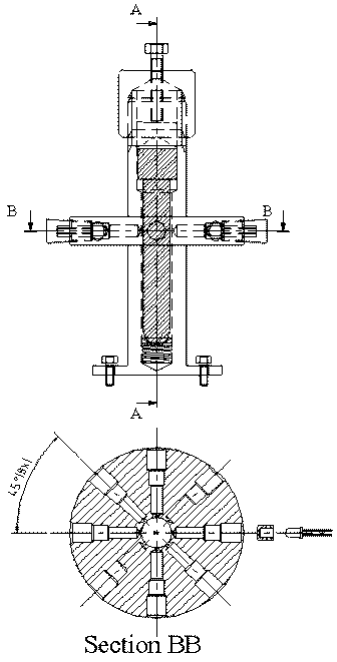

Figure 12. RTS sample holder with double sensor design

The sample holder (see figure 12) is equipped with a double turbidity sensor, to avoid changing LEDs. The three dimensional random rotations of the RPM around the Earth's gravity vector "simulate" a micro-gravity environment in the centre of the machine. Disposable laboratory tubes can be placed in the sample holder. A spring and cap keeps the tube in position (see figure 9b). The sensor is optimised for two specimen (e.g. E-coli and yeast cells). The interesting application of this layout is that two-parameter analysis might be possible or that scattering can be measured simultaneously at different angular orientations with respect to the incident light beam. A reference experiment is carried out placing a sample holder on a shaker table. It will be operational in September 2001.

Application III: Wireless/autonomous turbidity sensor (WTS/ATS) for a FOTON flight

One of the problems, when placing a disinfected sensor probe in an experiment (bio)container, is that it requires a sterile, leak tight, feed through for wires or optical fibres to operate the sensor. A solution to this problem is the design of a Wireless Turbidity Sensor (WTS) inside the experiment bag (figure 13). In co-operation with IDENTO Electronics (Ens, The Netherlands) a prototype of the WTS (figure 14) has been build using their RF techniques, developed for agricultural applications. The sensor is placed in a small glass box and an external RF field supplies the required energy. The measurement data are digitally transmitted to the reader. The distance between the RF field supply- and data transfer antenna, and the sensor is currently limited to a few centimetres.

At present the development of an autonomous version of the Turbidity Sensor (ATS) is going on. It leaves out the RF antenna for energy supply and data transfer. Instead it will use a battery for energy, a memory for data storage and a temperature sensor to start the sampling. The aim for the near future is application of the ATS in a bacterial growth experiment accommodated in an autonomous experiment box on a FOTON flight in 2002/2003. The experiment is prepared and set up in co-operation with Bioclear (Bioclear B.V, Environmental Technology) and the European Space Agency (ESA).

Application IV: An in-line turbidity sensor (ITS) for a Melissa bioreactor

A latest development, in co-operation with ESA, is the application of the turbidity sensor to control the biomass production of the bacterium *Rhodospirilum rubrum* in an experimental bioreactor of Melissa (4) (see figure 15).

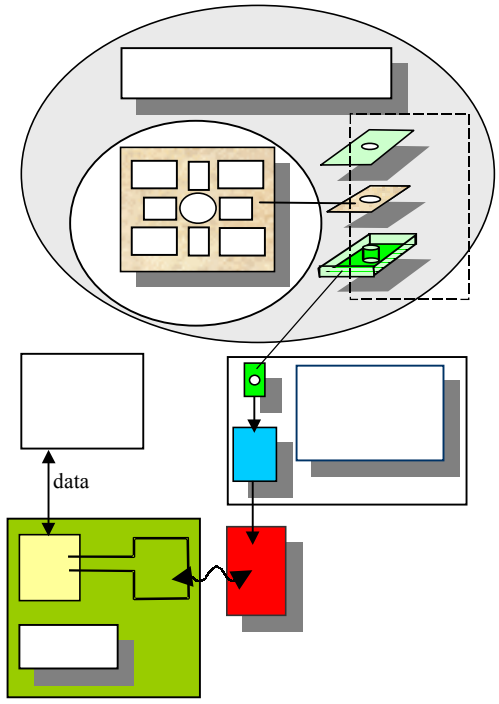

Figure 13. Concept of a miniature Wireless Turbidity Sensor (WTS) to be placed in a bioreactor (experiment bag). A RF field is used for power supply and data exchange.

Figure 14. Prototype wireless turbidity sensor using RF field for power and data transfer and remote operation electronics as developed in co-operation with IDENTO Electronics.

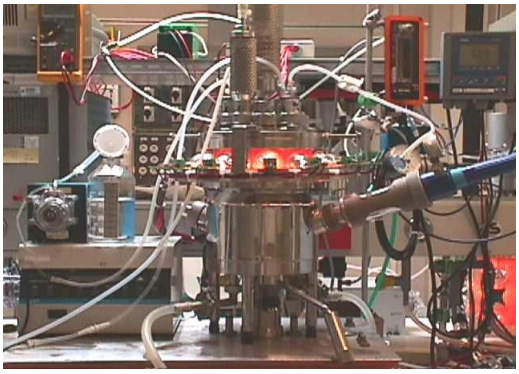

Figure 15. Experimental bioreactor set-up of Melissa

Melissa is a project for the development a biological life support system for long-duration manned space missions. In this set-up a real-time sensor with reservoir (to collect liquid) is placed in the overflow appendage of the bioreactor. Preliminary in-house laboratory experiments have verified that red LEDs are in this case well suited for the *Rhodospirillum Rubrum* population measurements (see figure 16). The sensor signal R^* will be correlated to the biomass dry-weight.

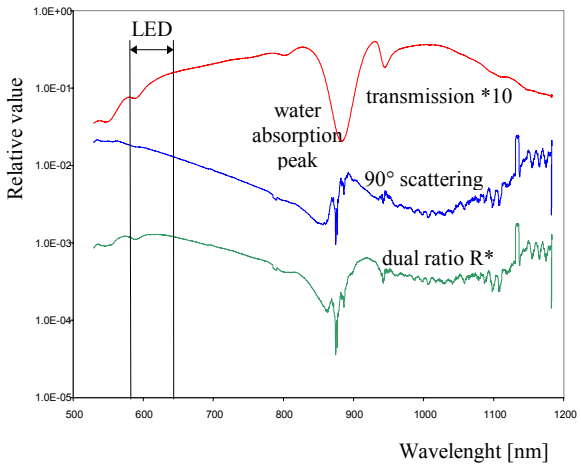

Figure 16. Spectral characterization test on the Rhodospirillum rubrum *using the diagnostic instrumentation. The transmission value is multiplied by a factor 10 to shift it upward in the figure. Note that the y-scale is logarithmic. The maximum dual ratio R_λ^* value is found around λ = 620 nm. In this case red LED's must be used for the improved turbidity sensor.*

SUMMARIZING CONCLUSIONS

For managing biological processes there is a demand for on-line and compact optical diagnostic sensors for earth- and space applications. Traditional techniques showed less suited for continuous, long-term monitoring (e.g. for control purposes) of bacteria growth. The dual ratio turbidity sensor, discussed in the present paper, copes with these problems. A determination of the growth rate on the *Xanthobacter Autotrophicus GJ10* showed its performance, although interpretation of the some results, obtained with the new technique is still open. The need to perform spectral analysis for selection of the LED color induced the development of a new diagnostic instrument. A first successful diagnostic test, using the instrument has been carried out on the *Rhodospirillum rubrum*. New applications of the sensor such as for the RPM and the Melissa experimental bioreactor are in progress. The sensor is cost effective, robust, accurate and reliable, and capable to perform real-time measurements. New applications of the sensor concept are foreseen in the future based on more in-depth interpretation of the results. Therefor more theoretical and practical research with respect to optical (scattering) properties of cells and particles will be required.

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