Production of Candidate Natural Matrix Reference Materials for Micro-Analytical Techniques

R. Zeisler, Chemical Science and Technology Laboratory, National Institute of Standards and Technology, Gaithersburg, MD 20899, U.S.A., and
A. Fajgelj, E. Zeiller, Laboratories Seibersdorf, International Atomic Energy Agency, POB 100, A-1400 Vienna, Austria

Introduction

As analytical techniques have become more sensitive, they require ever smaller samples for the determination of natural and pollutant elements that are investigated in biological and environmental materials. Concurrent with this development, new procedures are employed using solid samples for the analysis rather than dissolutions of samples to either determine concentrations in the bulk sample or the distribution of elements in smaller components of the bulk sample. Many of the techniques with these capabilities are nuclear related, such as X-ray fluorescence, proton induced X-ray emission, including their application in microprobes, and also neutron activation analysis. In addition, many other techniques that were developed using sample dissolutions now have capabilities for solid sample introduction; these include atomic absorption and inductively-coupled plasma optical emission and mass spectrometry. Other probe techniques, such as spark source and laser ablation mass spectrometry, electron- and ion-microprobe X-ray emission spectrometry, etc., may also find applications in biological and environmental studies.

Unfortunately, this wide variety of effective techniques is confronted with two major problems: Many of these techniques are affected in their accuracy by the matrix composition, i.e., standards for calibration and quality assurance must match the analyzed samples as closely as possible, and essentially no natural matrix certified reference materials (CRMs) have been produced for small sample analysis. The latter is to some extent due to the inhomogeneity of the CRMs at the sample sizes commonly used; although CRMs made of artificial composites, such as alloys and glasses are homogeneous and are used to some degree by the techniques discussed.

Homogeneity is considered to be the most vital prerequisite for a CRM; more stringent requirements exist for the analysis of small subsamples. Environmental CRMs, but also plant and tissue materials typically consist of several different solid phases which have characteristic physical properties (e.g. grain size, particle geometry, density etc.) in addition to widely varying concentrations of trace elements in these different solid phases. Many of the natural matrix CRMs are prepared from bulk samples by grinding and milling them to a certain particle size, which is expected to provide a more homogeneous material; however, recommended sample sizes for biological and environmental reference materials are found to be more than 100 mg. Natural materials that are prepared to a small particle size or occur at a small particle size, best would be a narrow size distribution of particles not larger than 10μm, probably fulfill the requirements for homogeneity.

Recent CRMs were produced to near ideal particle size distributions by air-jet milling, among others are NIST SRM 1570a, Spinach and IAEA-375, Cabbage [1]. Figure 1 shows that the latter two materials have a peak particle size of about 10μm and form fine, free flowing powders. These should be very homogeneous, however, their homogeneity at small sample size has not been systematically evaluated. Other natural reference materials may not have been produced to similar small particle sizes and distributions when, for example, only conventional milling and sieving is employed to arrive at a more homogeneous material.

Since the milling of materials is costly and has some drawbacks, such as possible contamination and limited success in providing small and rather homogeneous size particles, as well as loss of significant amounts of material during such preparations, natural materials that already occur as small particles such as air particulate matter, certain sediments, and cellular biological materials may form the basis of the required reference materials. To explore this approach, the IAEA Analytical Quality Control Services (AQCS) has introduced a single cell algae and an air particulate matter candidate CRM for studies in this CRP on reference materials for microanalytical nuclear techniques. The nature of these materials, i.e., naturally occurring particles, may provide ideal model reference material for the techniques discussed. We describe here the production of the materials and preliminary tests, the evaluation for the micro-analytical techniques is part of the CRP's output.

Preparation of Microalgal Biomass

The IAEA AQCS has begun a project on candidate reference materials that would provide different concentration levels of measurands in the same matrix to allow analysts to check the overall concentration range encountered in their daily work. It was found that algal biomass would provide a good model for such reference materials and a cooperative project was initiated with the Trebon Institute of Microbiology. One of the samples appeared to be of particular use for the current CRP because of the elevated concentrations of toxic metals in the material.

For the production of algal biomass, the Trebon Institute of Microbiology's technology of sloped open bioreactors was used [2, 3]. Briefly described, a six millimeter thick layer of a suspension of the algae in a water based medium runs down meandering lanes made of glass sheets in a steel frame. At an 1.7% inclination the flow rate is 0.7 $m \bullet s^{-1}$. At the lower end of the slope the suspension is collected and conveyed via a storage tank to a pump which transfers it back to the upper part of the cultivation surface. The system is operated during the day when the algae are exposed to sunlight, at night the suspension is kept in the aerated storage tank.

The building materials used for algal growth are sunlight, carbon dioxide, and biologically essential elements. The latter are supplied with mineral salts dissolved in the water based balanced nutrient medium in which the algae are suspended. During algal growth, gaseous carbon dioxide is supplied into the suction port of the circulation pump as well as into tubes lying in the troughs connecting individual lanes of the meandric culture system. The scheme of the open bioreactor is shown in Fig. 2.

Microalgal Biomass at Natural Levels of Toxic Elements: A one-batch culture regime was used for algal growth, starting at a density of about 1 g of algal dry weight per liter. Mineral nutrients are added daily (according to their consumption) in the proportion corresponding to the content of elements in the algal cells. Thus, optimum balanced composition of the nutrient medium in a growing culture is maintained. After the harvesting density of about 30 $g \bullet L^{-1}$ is attained, the suspension is thickened by a plate separator to a concentration of 80 - 100 $g \bullet L^{-1}$, and stored in a cooled tank from which it is then continuously fed to a spray-drier, the last step of the downstream processing line. A gently flowing dark green powder with about 5% of moisture content is the end product.

Microalgal biomass enriched by selected heavy metals: 5 mg of Pb, Cd, Ni, As, Cr, and 1 mg of Hg were added to 1 liter of the standard nutrient medium at the start of the outdoor growth cycle described above. Fourteen kg of spray-dried chlorella biomass were prepared with elevated levels of these elements.

Microalgal biomass with reduced levels of toxic metals: For controlled production of algal biomass with very low or defined content of heavy metals, a modular photoreactor without any metal parts and with artificial light sources was designed and constructed (Fig. 3).

To attain high microalgal productivity and highly efficient utilization of electrical energy entering the culture system, the following parameters had to be kept:

(i) optimal geometry of the cultivation space so that light energy losses are minimized,
(ii) high culture surface/volume ratio which allows to operate with high biomass concentration,
(iii) intensive mixing of algal suspension which limits wall adhesion and increases frequency of cell and dark periods,
(iv) light sources with high conversion efficiency of the electrical energy to the energy of photosynthetic active radiation (PhAR = 400-700 nm),
(v) homogeneous irradiance of the culture area, not surpassing the value of about 550 $\mu mol \bullet m^{-2} \bullet s^{-1}$

The modular photoreactor (culture volume 15 - 150 L) consists of planparallel organic glass sheets with meanderic channels irradiated homogeneously from both sides by fluorescent lamps with high energy conversion efficiency (η for PhAR = 0.28). Due to low thickness of the algal layer (12 mm) and pulsed algal flow (PTFE membrane pump), intensive turbulence and a high harvesting density of 30-40 $g \bullet L^{-1}$ as well as high electrical energy conversion efficiency was attained. To attain low levels of toxic elements, the culture medium was prepared from purified water and ultra pure chemicals. In addition, the whole apparatus was placed under a clean air supply (Class 10 HEPA filter) during the production of the algal biomass. Production rates diminished from the previous test conditions and only 3 kg was obtained for the candidate reference material.

Preparation of Air Particulate Matter

The IAEA AQCS is developing an air particulate matter reference material to be used for quality assurance of measurements in air pollution studies. Of special importance of such a reference material is its match in composition and physical parameters with the real world samples. In this case a particle size distribution is of special importance and it is expected that such material would have a particle size distribution peak at around 10 μm diameter and similar distribution on both sides of the distribution curve. It was also expected that a collection in the center of Vienna would yield a material contaminated with heavy metals because of industries distributed throughout the city and the heavy traffic.

The candidate reference material IAEA-396, Vienna Urban Dust, has been collected at the Vienna General Hospital (AKH) by the AQCS staff three times (Sept 1994 , May 1995 and Dec. 1995). Where the dust from electrostatic filters was collected with vacuum cleaners. Approximately 9 kg of raw dust was collected in three batches. The particle size distribution of the original sample is presented in Figure 4. Subsequently, the material was sieved through a set of sieves down to 70 μm and the joint sample was homogenized with a V-shaped homogenizer for 4 hours. Part of the sieved material was then air jet milled at the Agency's Laboratories Seibersdorf to obtain a more homogeneous distribution of smaller particles.

Particle size measurements to determine distributions in the original material as well as the progress in air jet milling were performed using a Mastersizer X (Malvern, Herrsching, Germany) laser light scattering instrument. As a feeding source for the material a MSX 14 sample suspension unit was used. Approximately 100 mg of the sample is needed for measurement. Samples were suspended in water with added surfactant (few drops of detergent) and preconditioned for 15 minutes. Ultrasound was applied to destroy eventual agglomerates and samples were measured using a 100 mm lens which covers a particle size range of 0.5 to 180 μm.

Results and Discussion

Microalgal biomass: In its cooperation with the Trebon Institute of Microbiology, AQCS has prepared a set of three intercomparison materials, IAEA-391 to 393, from the microalgal biomass samples produced by the above processes. The intended production of a set of materials with different elemental content but the same matrix has been accomplished. Relative concentration values are given in Table 1., taking the concentrations in the "Natural Level" material IAEA-392 as unity (because real concentrations should not be published before the completion of the current intercomparison). It should be noted that the administration of equal amounts of each heavy metal results in very different uptakes by the algae; the uptakes have been generally higher than expected. The algae probably show the highest uptake for mercury, which was administered at factor five lower concentration, and it was also accumulated above "natural level" in the laboratory environment in the "depleted" material IAEA-391. Essential trace elements show small variations among the materials, a factor ten enrichment of boron in IAEA-391 may be of some significance. In view of the diminished growth rate in the "ultra clean" preparation, the trace elements in this material must be studied in more detail to determine potential deficiencies for algal growth.

The "Elevated Level" material, IAEA-393, was viewed to be of interest to many of the microprobe techniques since some techniques would not be able to determine elements at the much lower levels of the two other materials. This material has been delivered to all CRP participants.

Figure 5 shows the single cell algae *scenedesmus obl.* as it exists in the IAEA-392 material. The uniformity of the particles as well as their size (approximately 8 μm) may provide the ideal physical properties for a homogeneous CRM suitable for the micro-analytical and solid sampling techniques. The participants of the CRP will use different sample sizes in their techniques and it may even be possible to calculate homogeneity at a given sample size from the analyses of individual cells.

Air particulate matter: The collected material and its products were initially characterized by XRF and INAA as well as particle size measurements. The bulk material and its fractions showed contemporary element concentrations as found in monitoring studies of Vienna urban particulate matter [4]. It should be noted that the lead level is still significant although more than an order of magnitude lower than in the NIST Standard Reference Material SRM 1648, Urban Particulate Matter, which was collected about two decades earlier in the US.

The particle size measurement (Fig. 4) show that after the third air jet milling a very well-defined material can be obtained. The material was regarded sufficiently homogeneous, further improvements may be limited with this technology since already the previous milling was close to the final product. The particle size mean diameter is 3.7 μm and particles are distributed between 0.2 and 15 μm. To assay also the potential progress achieved by the milling process for the homogeneity of trace elements, the sieved bulk material, designated IAEA-396A/S, and the three times jet milled fraction, designated IAEA-396A/M, has been incorporated in the CRP's investigations.

References

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Figure Captions

Fig. 1 Particle size distribution in IAEA 359, Cabbage, and NIST SRM 1570a, Spinach, after preparation by air-jet milling.

Fig. 2 Outdoors biomass cultivation plant

Fig. 3 Closed system cultivator

Fig. 4 Particle size distribution in bulk samples of air particulate matter as collected and after air-jet milling

Fig. 5 Single cell algae *Chlorella vulg. Böhm Borns*

Table 1. Depletion (IAEA-391) and enrichment (IAEA-393) factors for the microalgal biomass materials as compared to the "normal" level material IAEA-392.

Element	IAEA-391 "depleted"	IAEA-392 "natural level"	IAEA-393 "enriched"
Al	0.2	1	3
As	0.2	1	1000
B	10	1	1
Ca	0.1	1	1
Cl	0.3	1	0.8
Cd	<<1	1	20000
Co	2.7	1	1
Cr	0.2	1	100
Cu	2.3	1	0.5
Fe	0.6	1	3
Hg	10	1	10000
K	1	1	1
La	0.4	1	3
Mg	3.5	1	2
Mn	0.5	1	2
Mo	5	1	0.3
Na	1.4	1	0.5
Ni	<<1	1	300
Pb	<<1	1	400
S	1.0	1	2
Sc	0.3	1	3
Sb	0.3	1	3
Se	2	1	2
Zn	0.3	1	1

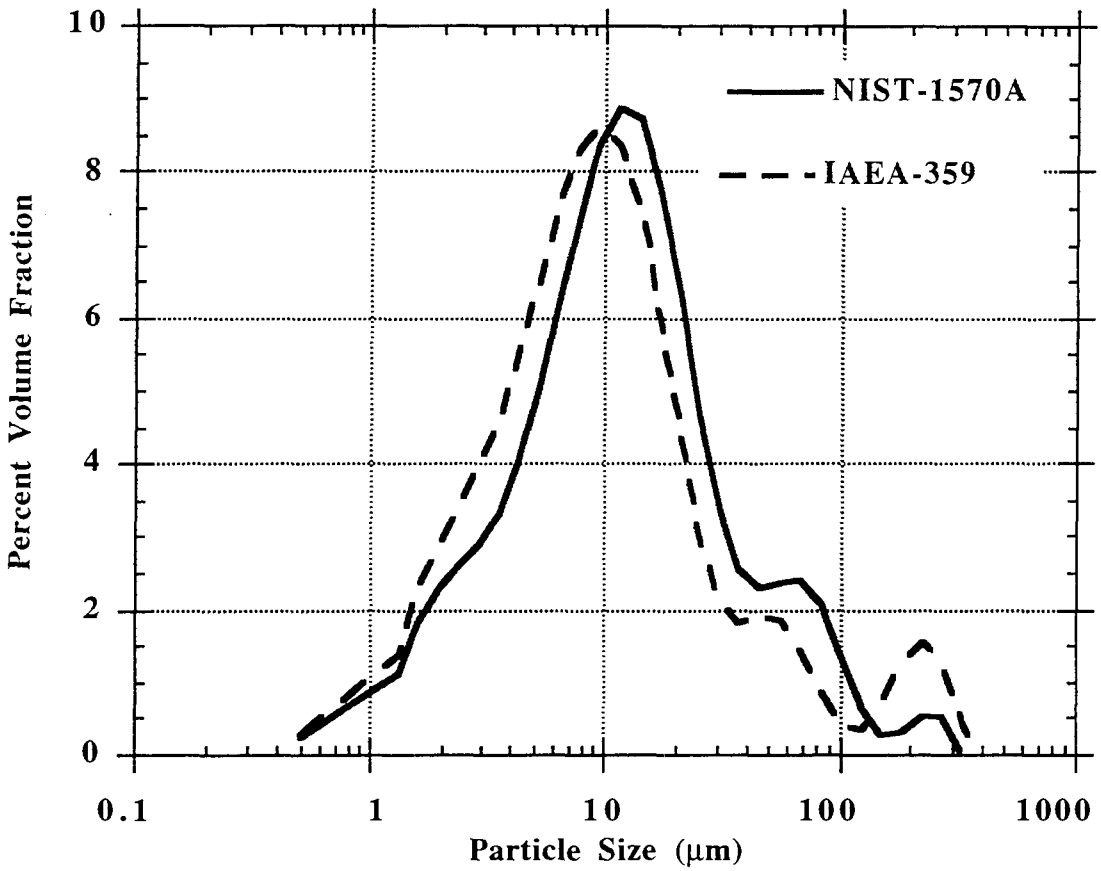

Figure 1. Particle size distribution in IAEA-359, Cabbage, and NIST SRM 1570a, Spinach, after preparation by air-jet milling.

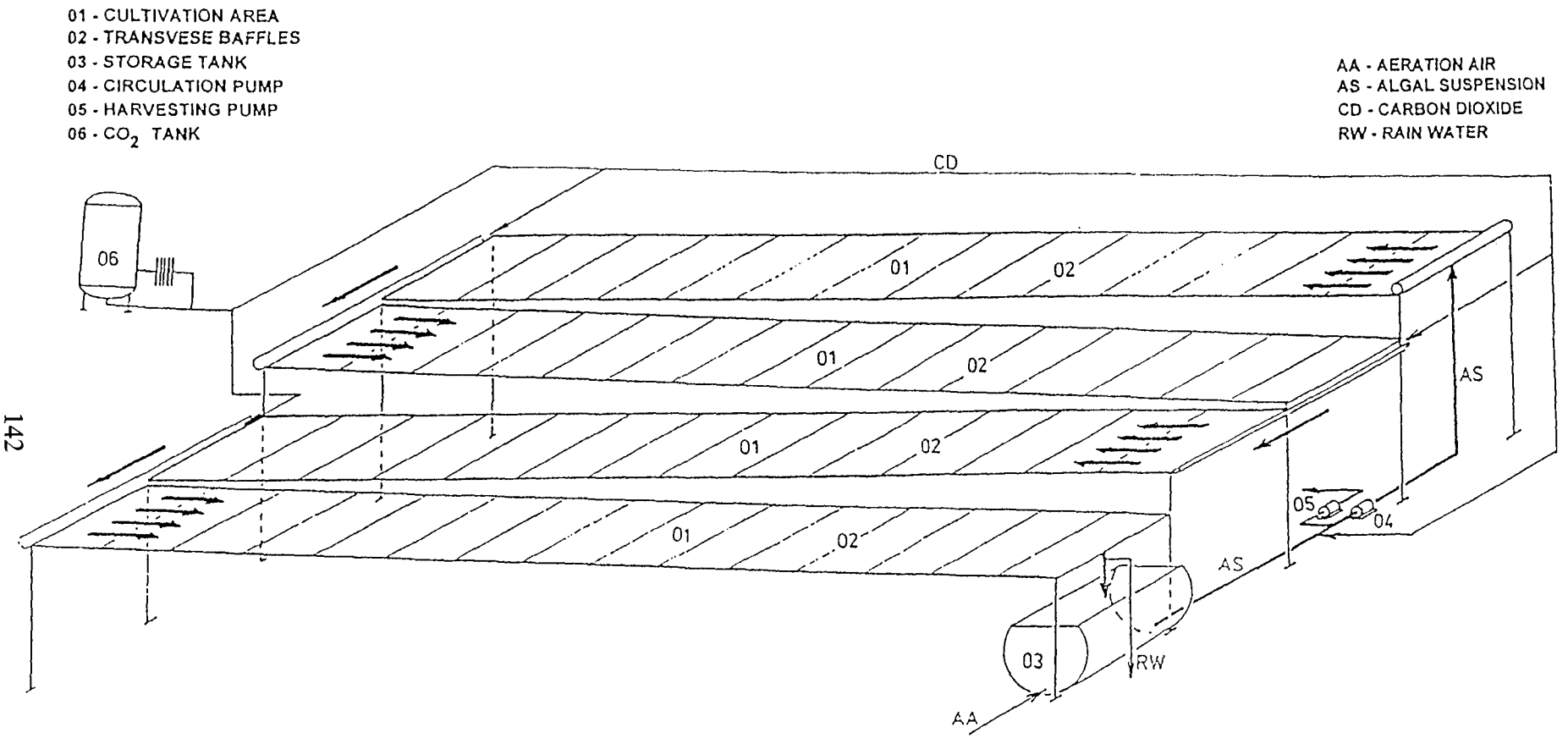

Fig. 2 : SCHEME OF AN OUTDOOR SLOPED BIOREACTOR

A. DIAGRAM OF CULTIVATION MODULE

VARIANT I.

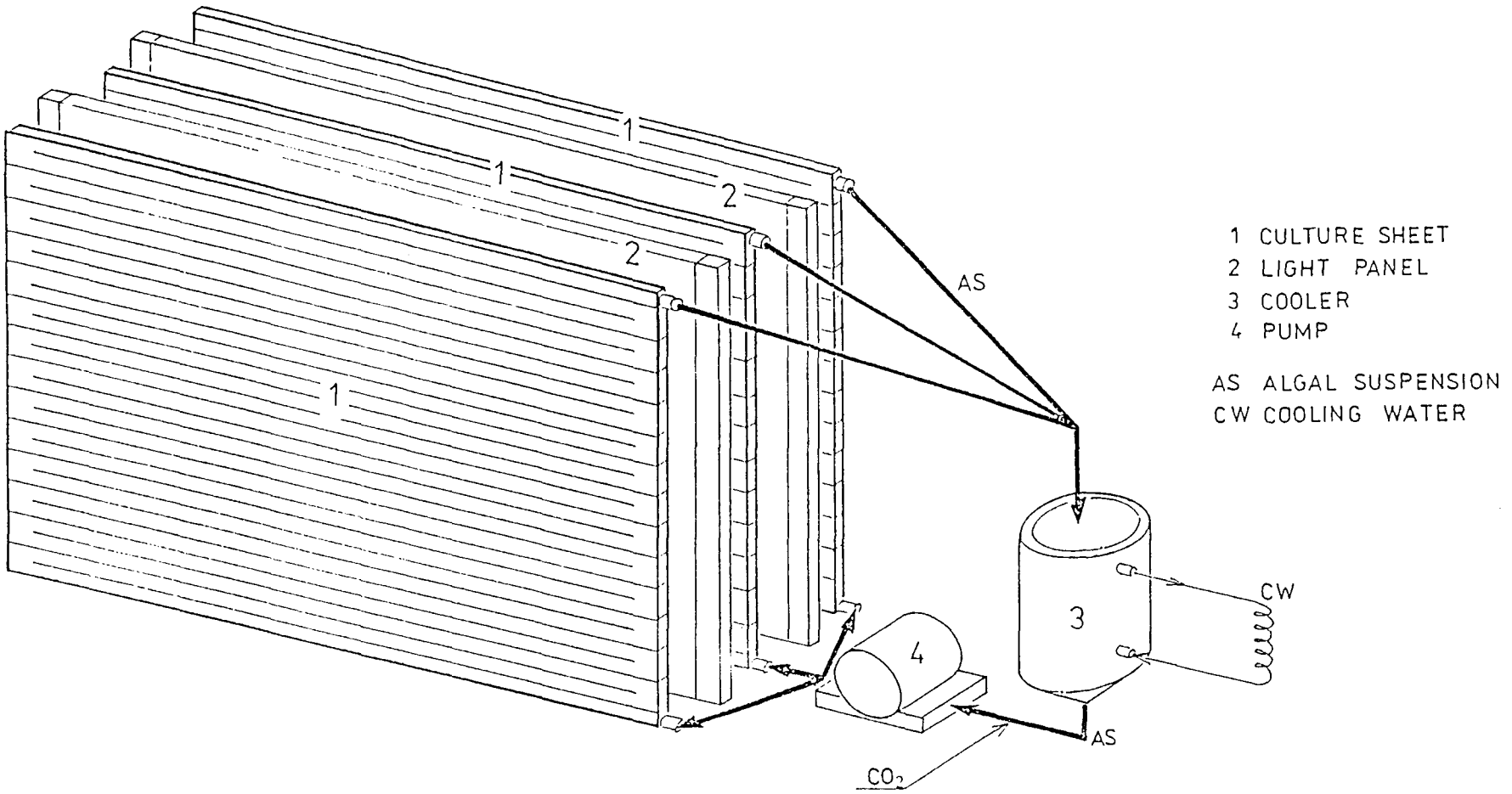

Fig.3 : SCHEME OF A MODULAR PHOTOBIOREACTOR WITH ARTIFICIAL IRRADIATION

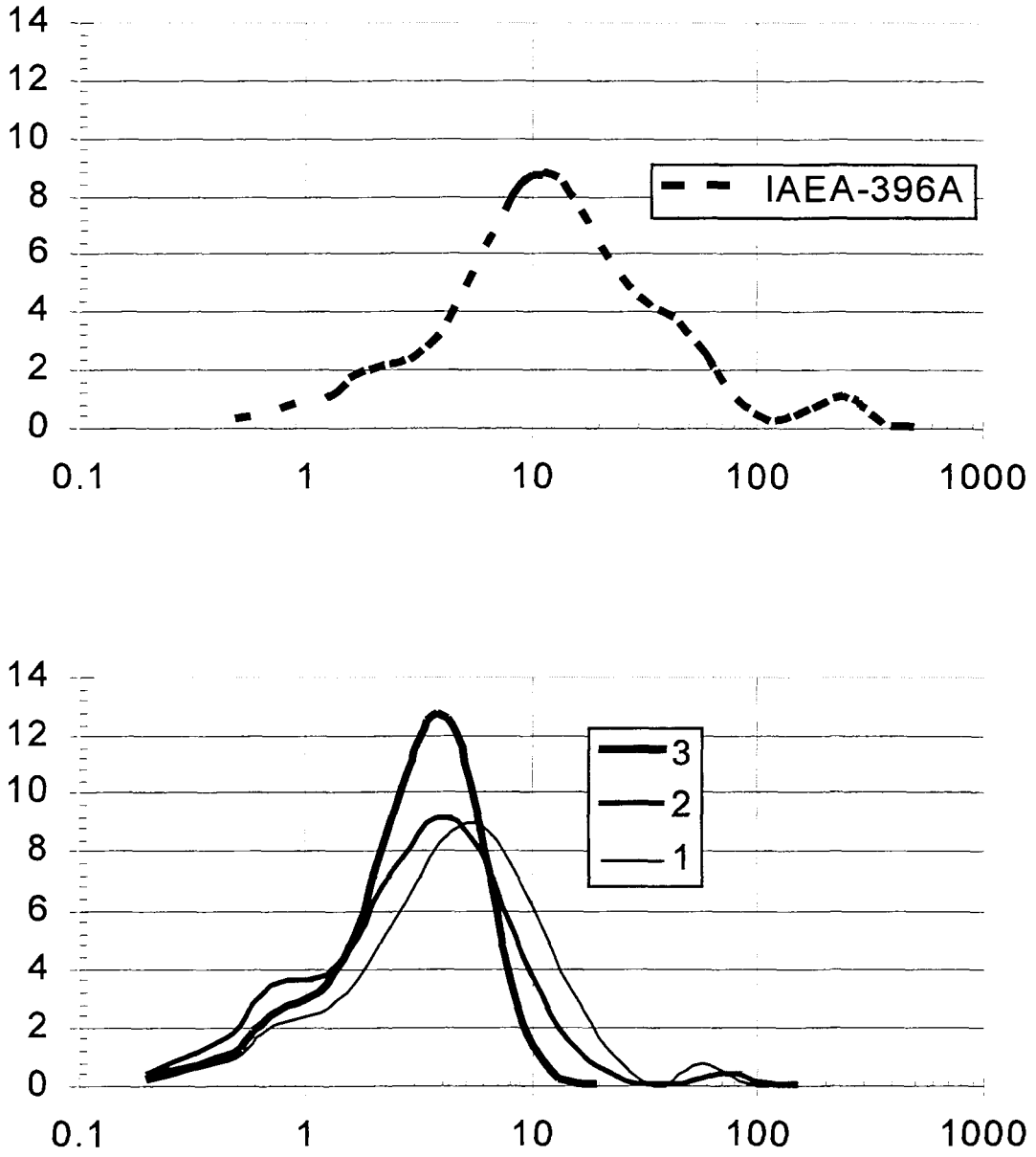

Figure 4. Particle size distribution in bulk samples of air particulate matter, as collected at the Vienna General Hospital (AKH), and after one to three passes through the air-jet mill.

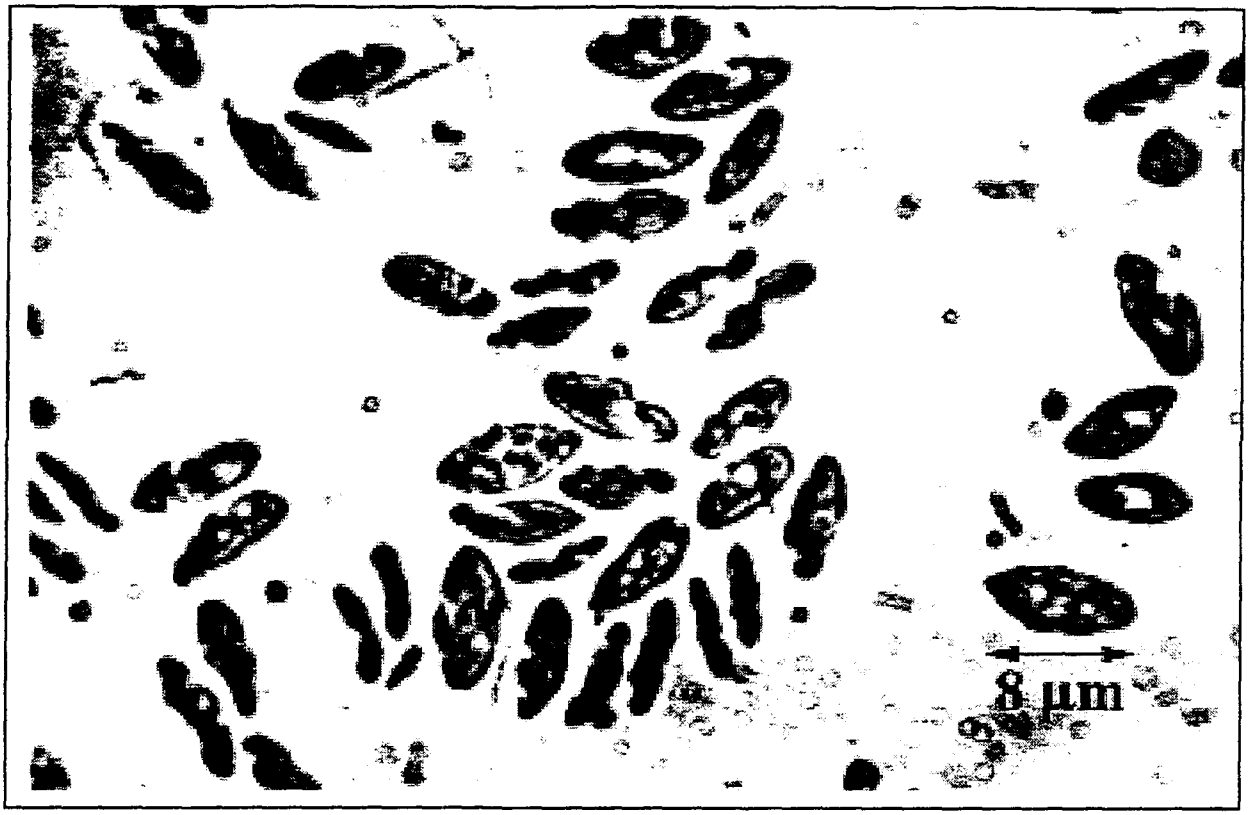

Figure 5. Single cell algae (*scenedesmus obl.*) in IAEA-392