Comparison of motility of *N. gruberi* amoebae on different substrata (motility expressed as μm/sec ± SD)

Substrate used	Concentration of NaCl in medium (mM) 0	0.1	1.0	10
Glass	0.16±0.08	0.22±0.11	0.61±0.24	1.26±0.23
Plastic	0.21±0.09	0.32±0.20	0.84±0.28	1.25±0.29
0.5% Agar	0.22±0.07	0.28±0.16	0.50±0.35	1.25±0.55
Oil (FC 75)	0.21±0.12	0.14±0.07	0.79±0.27	1.11±0.22

attention has been focussed on the wetability or non-wetability of the substrate[4]. Few reports concern the use of liquid-liquid interfaces yet recently it was found that amoebocytes from the horseshoe crab, *Limulus*, could locomote equally well on an oil-water interface as on a solid substrate although information on relative speeds was lacking[5].

In the case of the soil protozoon *Naegleria gruberi* the speed of amoeboid movement on glass can be modulated experimentally by a factor of 4 with changes in ambient electrolyte concentration[6,7]. We report here experiments that sought for the first time to compare locomotory rates of a eukaryote cell, *Naegleria*, crawling across glass, agar, plastic and fluorocarbon oil surfaces under a range of defined salt conditions.

Amoebae were deposited onto the experimental substrate by gravity and their motility measured as described in the previous paper[7]. A glass substratum was provided by circular coverslips set up conventionally in a Prior chamber[8]. The plastic substrate was prepared from untreated polystyrene petri dishes cut to the same form as the circular glass coverslips and used similarly. The agar substrate was prepared by pouring 0.5% agar in deionised water onto the bottom glass coverslip of a topless Prior chamber 24 h before use. The Prior chamber was modified to allow a reasonable volume of fluorocarbon oil FC75 (3M Company, London, England) to be accommodated in the water-oil substrate experiments. The density of the fluorocarbon liquid was stated to be 1.75 g/cm^3 at 25 °C. During these experiments, which were conducted at room temperature, the composition of the aqueous phase within the tissue culture chamber was changed by perfusion with deionized water or electrolyte until the specific conductivity of the eluant matched that of the solution entering the chamber.

It can be seen from the table that no significant differences were recorded in the speed of locomotion of *Naegleria* amoebae at a given concentration of NaCl regardless of which of the 4 experimental substrates were employed. These results are in marked contrast to those obtained with vertebrate fibroblasts which are able neither to locomote across moist agar[9] nor to spread on unmodified polystyrene, a substrate of low wetability[4].

Given the chemical heterogeneity of soil and the wide geographical distribution of *Naegleria* it is unlikely that this amoeba should demonstrate (restricted) specificity towards locomotory substrates. Nonetheless the interstices of soil should present (unless acid conditions prevail) one common feature to an amoeba, namely a water covered particulate surface bearing negative charge[10]. It is likely therefore that for this cell moving through its natural environment interactions with the substrate will be dominated by those nonspecific forces occurring between closely apposed plates of like charge separated by weak electrolyte. However it should be stressed that artificial substrates in the form of thin films on glass of substances which have a strong and particular affinity for the *Naegleria* cell surface can be prepared. Such experimental substrates, for example polylysine[11] and concanavalin A[12], severely impede amoeboid locomotion.

The results obtained with fluorocarbon oil are at first sight surprising but studies on vertebrate cell spreading on protein oil/water interfaces have shown that leucocytes and macrophages will spread on fluid substrata having viscosities about 10,000 times lower than those required for fibroblast spreading[13]. Thus there is probably a great difference between various cell types regarding the type of interface required for spreading and/or locomotion.

Lack of substrate specificity may not be a ubiquitous character of movement of amoebae. It has been shown that some genera e.g. *Sappinia* do not extend well on glass but do so on agar surfaces[14].

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A bioreactor for continuous treatment of waste waters with immobilized cells of photosynthetic bacteria

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Summary. A bioreactor for assaying immobilized cells of photosynthetic bacteria for hydrogen production under optimal operating conditions was made. High and lasting H_2-photoevolutions were obtained using waste waters as substrates.

Numerous procaryotes, belonging to physiologically and taxonomically different groups, are able to produce H_2 (Zajic et al.). Under suitable conditions, photosynthetic bacteria of the family *Rhodospirillaceae* can photo-metabolize organic substrates forming CO_2 and H_2 (Gest et al.[2]) according to the equation:

$$C_xH_yO_z + (2x-z)H_2O \rightarrow x\,CO_2 + (\tfrac{1}{2}y + 2x - z)H_2 \quad (1)$$

In view of the advantages offered by the use of immobilized cells in processes like H_2-production from organic substrates, which need the involvement of multienzymatic systems, we assayed the efficiency of agar-trapped *Rhodopseudomonas palustris* cells. For this purpose we set up a device which realizes optimal operating conditions for the immobilized cells and allows a continuous supply of substrate.

Materials and methods. Organism and culture conditions. *Rhodopseudomonas palustris*, strain 42 OL of the culture collection of the 'Centro di Studio dei Microrganismi Autotrofi, Firenze', was grown anaerobically in the medium described by Ormerod et al.[3] and modified by us by replacing ammonium sulphate and biotin with sodium-glutamate (1 g/l) and yeast extract (1 g/l) respectively. All the cultures were grown in completely filled glass bottles incubated at 30 °C and 4000 lux (supplied by incandescent lamps).

The cells were collected in the exponential phase of growth and resuspended in a solution of the same composition as the growth medium except that sodium-glutamate was omitted. Then the cells were N-starved by incubation in the light and 30 °C under an argon atmosphere for 36 h.

Cell immobilization. 50 mg dry weight of starved cells were centrifuged, resuspended in phosphate buffer (0.05 M; pH 7) and then added to 40 ml of a 4% agar solution kept at 48 °C. The suspension was allowed to solidify inside the loom (fig. 1c) forming the central mobile component of the reactor (fig. 1d). The loom (55 × 205 mm) has, along the inner edge, several little holes which allow the anchorage, after solidification, of the agarized panel. In this way the agar layer (4 mm thick) can sustain itself without a large support surface. The immobilization was performed inside an anaerobic box (Biolife Laboratory Equipment) under an atmosphere of argon.

Description of the reactor. The reactor (fig. 1d) is a rectangular plexiglass chamber (80 × 50 × 220 mm; vol. 680 cm^3) containing the agarized panel (fig. 1c). Anaerobic conditions are assured by a closing slab (fig. 1 a and b) which is tightened on the top of the chamber. The reactor is provided with an inlet and an outlet for the substrate solution to allow a continuous flow. A silicon septum for gas sampling is also present. The H_2 and CO_2 produced are collected into a variable volume container through a tube placed on the closing slab.

Table 1. H_2-photoproduction from 10 mM malate by 50 mg dry wt of *Rhodopseudomonas palustris* immobilized cells (temperature = 30 °C; incident light intensity = 69.5 cal/h/100 cm^2)

Average H_2-production rate (30 days)	40 µl/mg cells dry wt/h
Conversion of malate into H_2[a]	60%
Photosynthetic efficiency[b]	1.2%

[a] According to equation (1).
[b] Calculated by the relation $\frac{\Delta G^{\circ\prime}H_2 - \Delta G^{\circ\prime}S}{LE}$, in which $\Delta G^{\circ\prime}H_2$ and $\Delta G^{\circ\prime}S$ are the free energies of the hydrogen formed and of the substrate converted, respectively; LE is the light energy incident on the immobilized cells.

Table 2. H_2-photoproduction from industrial waste waters by 50 mg dry wt of *Rhodopseudomonas palustris* immobilized cells

	Sugar refinery wastes	Straw paper mill effluents
H_2 production rate averaged over all the experimental period (35 days) (µl H_2/mg dry wt/h)	30	35
H_2 obtained from 1 l of effluent (l)	0.78	2.2
Fraction of initial COD converted into H_2 (%)	43	28

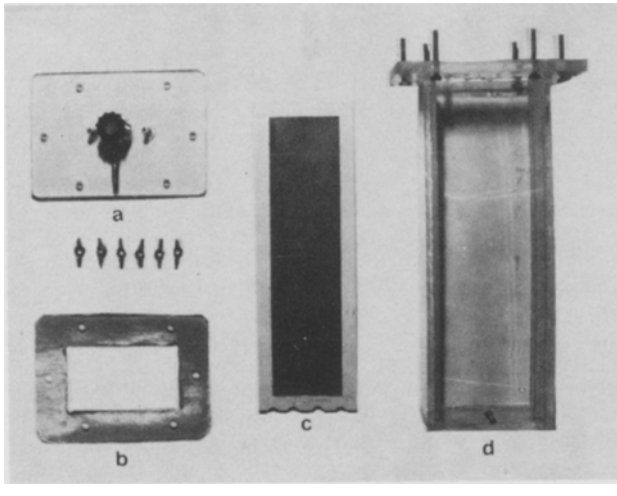

Fig. 1. Bioreactor components: *a* closing slab; *b* silicon packing; *c* loom with agar trapped cells; *d* bioreactor chamber.

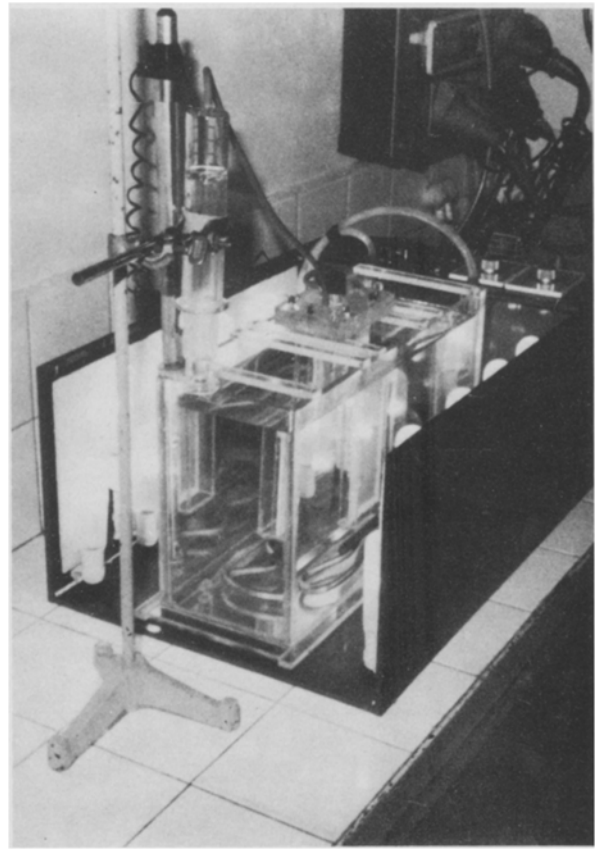

Fig. 2. View of the apparatus employed for the H_2-production assays under controlled conditions of temperature and light intensity.

Experimental conditions. For H_2-production assay, the reactor was kept in a thermostat-controlled (30 °C) water bath illuminated from 2 sides with tungsten lamps at 5500 lux (fig. 2).

The chamber can be operated as a continuous flow reactor by feeding the substrate solution with a peristaltic pump at a predetermined rate. Nevertheless in the experiments reported here the reactor was filled with 600 ml of substrate solution (10 mM malate or industrial waste waters). The solution was replaced when the H_2-production rate declined owing to exhaustion of the substrate.

H_2-determination. H_2 in the gas phase was monitored by gas-chromatography. Aliquots (1 ml) were withdrawn from the atmosphere of the reactor and injected into a Fractovap (Carlo Erba s.p.a.) gas-chromatograph having a thermal conductivity detector and provided with a silica-gel (510/1100 mesh cm^{-2}) column (2 m × 6 mm). Nitrogen was used as the carrier gas at a flow rate of 60 ml min^{-1}; the column oven was set at 125 °C and the bridge current was 120 mA. The results were quantitated by relating the peak areas to a calibration curve.

Results and discussion. With the device described a good and lasting H_2-production both from malate and waste waters was obtained (tables 1 and 2). Indeed, the rate of H_2-evolution was remarkably stable over a 30-day-period (30–40 µl H_2 per mg of cells dry wt per h). These values are much higher than those reported by Bennett and Weetal[4] for agar trapped *Rhodospirillum rubrum* on malate (0.7 µl H_2 per mg of cells wet weight per h). Apart from the difference in the bacterial species used, the geometry of our agarized panel maximizes the contact between immobilized cells and substrate solutions so that the limitations due to diffusion of substrates and products are significantly reduced.

Under the experimental conditions employed, the final conversion of substrate into H_2 by *Rhodopseudomonas palustris* immobilized cells was 60%. High H_2-production and high COD (Chemical Oxygen Demand) removal were also obtained utilizing waste waters as substrates for the photo-dissimilative activity of the immobilized cells (table 2). A yield of 0.78 l H_2 from 1 l of sugar refinery wastes (initial COD = 1200 ppm) and 2.2 l H_2 from 1 l of straw paper mill effluent (initial COD = 5600 ppm) were achieved. The H_2-photoproductions thus obtained would allow an appreciable energy recovery; in fact the hydrogen produced from 1 l of straw paper mill effluent allows the recovery of 6.2 Kcal.

In summary, the reactor described in this paper is easy to build and to operate. Moreover, it attains high rates of H_2-production from various organic substrates. Hence it can be conveniently utilized for investigating the factors determining the efficiency of photosynthetic reactors employing immobilized cells in the recovery of hydrogen from waste waters.

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Immunohistochemical localization of galactocerebroside in kidney, liver, and lung of golden hamster[1]

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Summary. Localization of galactocerebroside in kidney, liver, and lung of hamster was studied by the immunoperoxidase method using an affinity-purified specific antibody. Epithelial cells of the following anatomical sites were labelled with the antibody: distal tubuli, ascending limbs of Henle's loops, and collecting tubuli in kidney; periportal bile ducts and hepatic parenchyma in liver; bronchioli and alveoli in lung. The existence of galactocerebroside in these 3 organs was also confirmed by chemical analysis.

Galactocerebroside (GC), a biosynthetic precursor of sulfatide, is one of the major glycosphingolipids of the white matter of brain and known to be a useful cell surface marker for myelin sheath and oligodendroglial cells[2]. Chemical analyses have shown the presence of GC in kidney[3], intestine[3], spleen[4], and lung[5], but the precise histological location of this glycolipid in organs other than brain remains obscure. We previously reported an affinity chromatographic technique for preparing highly purified specific antibody against glycosphingolipids[6]. It is imperative to use such a purified antibody for unequivocal immunohistochemical identification of glycolipid antigens in cells and subcellular structures. The present paper describes the specific cellular distribution of GC in kidney, liver, and lung of hamster, determined by the immunoperoxidase method using this purified anti-GC antibody.

Materials and methods. Anti-GC antibody was raised in rabbits and purified by affinity chromatography as described before[6]. This anti-GC antibody was previously shown by the immunoperoxidase method to react selectively with the myelin sheath, oligodendroglial cells, and epithelial cells of choroid plexus in brain[7]. The antibody preparation used had a complement fixation titer of 1:200. Kidney, liver, and lung of 8-week-old male golden hamsters, which had been sacrificed under anesthesia with Nembutal (5 mg/animal), were immediately frozen at −70 °C and cut with a cryostat into 4-µm-thick sections. They were fixed in 100% acetone for 10 min at room temperature, dried in air, and then exposed to anti-GC rabbit antibody without further dilution for 30 min at 37 °C. After washing, the tissue sections were incubated for 30 min with goat antirabbit IgG labelled with horse raddish peroxidase (E-Y Laboratory, lot No. 1101B) diluted 1:30, followed by washing with PBS. They were immersed in a freshly prepared solution of 0.05% diaminobenzidine hydrochloride and 0.01% hydrogen peroxide in 0.05 M Tris buffer, pH 7.6 (Graham and Karnovsky's reagent) for 3–8 min to reveal peroxidase activity. After washing without