Calorimetric & Respirometric Monitoring of Metabolism: Some Examples

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INTRODUCTION

Simultaneous measurements of the rates of heat production and oxygen utilisation by living tissues provides a direct quantitative means of detecting subtle changes in metabolic state in the face of altered physiological and environmental conditions. The ratio of calorimetrically measured heat flux to the respirometrically measured oxygen flux is often called the "calorimetric-respirometric ratio" or CR ratio. This can be used to partition total metabolic energy flux into its aerobic and anaerobic components by comparison of the experimental CR ratio with the theoretical "oxycaloric equivalent" for fully aerobic respiration. Such information is most important when evaluating the physiological effect of environmental stress.

Industrialisation of many processes has lead to an increasing amount of synthetic and natural waste products expelled into the environment The consequences of this can often be fatal as seen by the many examples of polluted rivers, waste land and the atmosphere. This large amount of waste can be seen to be harmful to the ecosystem and has lead to an imbalance of the natural flora and fauna. There is a need, therefore to study the effects of pollutants and to evaluate quantitatively their toxicological effects. The combined use of microcalorimetry and respirometry provides a useful means of monitoring such effects.

EXAMPLES

Dormancy and hibernation are common among many organisms during their lifecycles. These metabolic and physiological changes are beginning to be better understood from a biochemical point of view. Using brine shrimp (Artemia) embryos, the role of intracellular pH in metabolic switching was investigated during anaerobic dormancy (Ref. 1). Artemia embryos placed in a ThermoMetric perfusion vessel coupled to the Cyclobios Twin-Flow respirometer

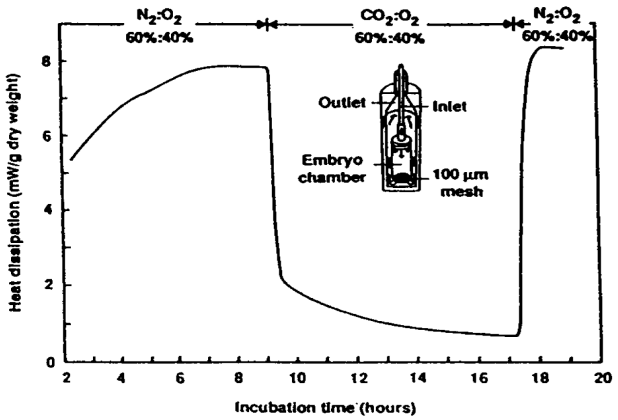

Fig L. Heat dissipation by Artemia embryos under varying aerobic/aerobic acidosis conditions (Ref 1).

exhibited an increase in heat flux as a function of embryonic development. They reached steady state after 7h under aerobic conditions *(Fig 1)*. At steady state the CR ratio (calorimetric heat change per respirometric oxygen uptake) was -495 kJ/mol O_2 for fully aerobic carbohydrate metabolism which is close to the oxycaloric equivalent of -478 kJ/mol for carbohydrate catabolism. Exposure of Artemia to CO_2 under aerobic conditions resulted in a cessation of development and a decrease in heat flux to 9% of control levels after 8h. Oxygen flux decreased simultaneously, that is the CR ratio remained approximately equal to the oxycaloric equivalent, indicating fully aerobic metabolism. Removal of CO_2 resulted in a rapid increase in heat flux to control levels.

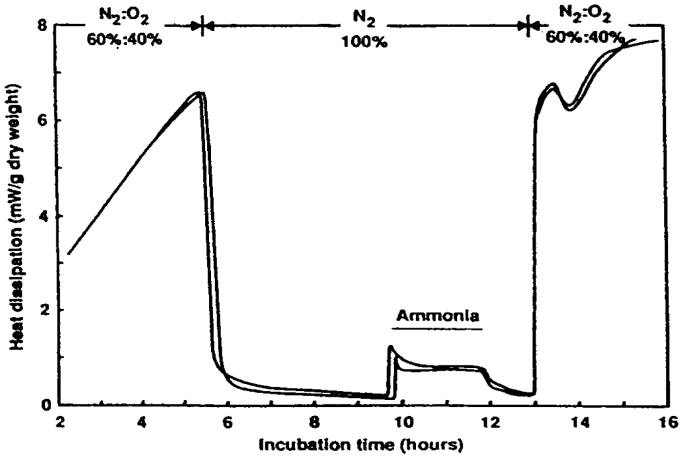

Fig 2. Heat dissipation by Artemia embryos under aerobic and anaerobic conditions. Dormancy was interrupted by addition of ammonia which was later removed (Ref 2).

In other experiments, oxygen was replaced by 100% nitrogen during the aerobic development of Artemia. An immediate drop in heat output was observed which declined to 2.4 +/- 0.4% of control values *(Fig 2)*, thus showing the degree to which oxygen is required for metabolic activity.

The internal pH is the primary regulatory factor which governs the metabolic pathway used by the Artemia. Addition of ammonia to Artemia under anaerobic dormancy resulted in an increased anoxic heat output as a consequence of the change in biochemical pathway utilised. However, since oxygen was not present for mitochondrial metabolism to function normally, the heat flux was not as high as control values. Nevertheless, a five fold increase was observed compared to the anaerobic dormant condition. Reinstatement of the oxygen presence caused a rapid bi-phasic increase in heat output to normal pre-an-

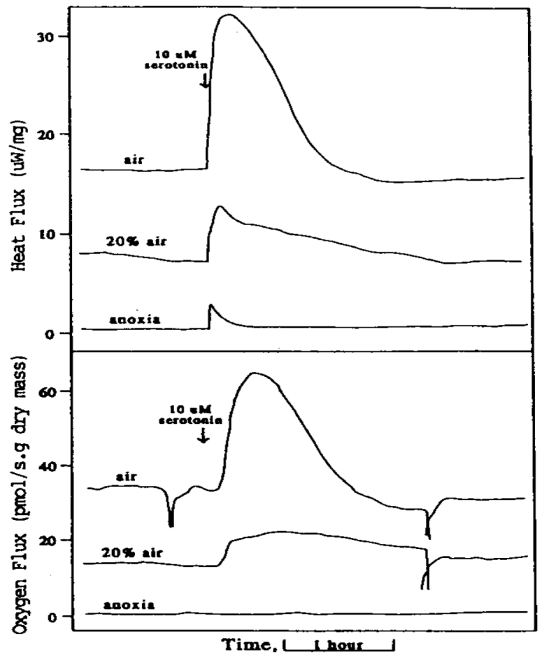

Fig 3. Heat and oxygen flux of Mytilus edulis gills under different conditions of air saturation.

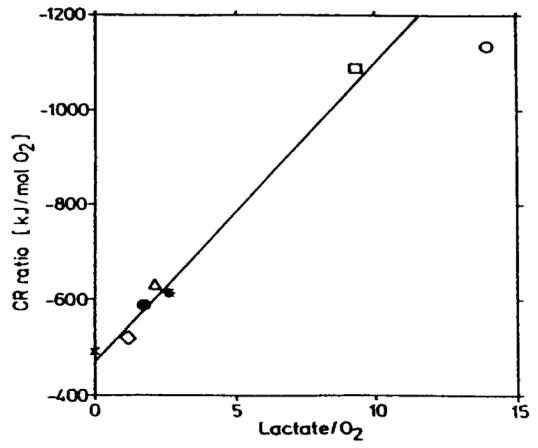

Fig 4. Calorimetric - respirometric (CR) ratio as a function of the molar lactate/O2 ratio in cultured or isolated mammalian cells (Ref 4). Hamster brown adipocytes (x); mouse macrophage hybridoma 2C11-12 (◊); human neutrophils activated for oxidative burst (•) and resting (o); human T-lymphoma cells CCRF-CEM during growth (✹) and under non-growing conditions (□); LS-L929 fibroblasts (△).

oxic levels.

Many other aquatic organisms such as the common mussel (Mytilus edulis) have also been examined for simultaneous oxygen and heat flux measurements (Ref 2). The gills of Mytilus were carefully excised and placed in the TAM perfusion vessel under aerobic, hypoxic and anoxic conditions (Ref 3). Cilia on the gills propel water across the gills to provide oxygen and nutrition. The rate of ciliary activity is principally governed by the balance of the rate of formation and utilisation of ATP. Hence, factors that influence the rate of formation of ATP will invariably affect the rate of ciliary activity.

Results from stimulated ciliary activity simultaneously monitored in the TAM connected to the Cyclobios Twin-Flow respirometer *(Fig 3)* suggest that the rate of utilisation of oxygen and heat output are directly related to the rate of ciliary activity and that the energy metabolism of intact gills is mainly aerobic. Therefore, the presence of pollutants and other such chemicals could be added in order to observe the toxicological effects of these agents.

A number of mammalian cells have also been examined for information on the CR ratio (Ref 4). It has been suggested that a number of cell types always retain a degree of anaerobic metabolism in addition to the normal aerobic contribution. Simultaneous measurement of the oxygen uptake and heat flux has revealed the extent to which this occurs in living cells.

Results from a number of different mammalian cell types *(Fig 4)* have shown quantitative differences in anaerobic metabolism. This suggests that different metabolic pathways are utilised in the generation of ATP. Hence, it can be further suggested that the CR ratio can be used to differentiate between different cell types under the same physiological conditions.

CONCLUSION

The above examples have demonstrated the usefulness of both microcalorimetric and oxygen measurements in establishing the effects of different environmental factors upon metabolism. Indeed, the results have shown quantitative differences between different cell types as well as the degree to which the anaerobic contribution exists.

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NOTE

Thermometric AB collaborates with Cyclobios (Austria) in the improvement of Calor-respirometric systems.

EXPERIMENTAL

A Thermal Activity Monitor (TAM 2277) equipped with a flow-through measuring cylinder (2277-202) was used (Fig. 1). The tubings of the cylinder are made of 24 carat gold with an internal diameter of 1 mm. An external solution, with or without bacteria, was pumped via a peristaltic pump (flow-rate: 20 mL/h) through the tube. In the measuring cylinder the solution passed firstly through a heat-exchange coil for temperature equilibration before reaching the measuring position. Here the heat evolution in 0.6 mL of the tubing system was registered by Pellier elements. Calibration range for these experiments was 0 - 300 µW. The limit of detectability was 0.5 µW and the base line stability was ± 0.3 µW. The thermostat was set to 30°C as operating temperature.

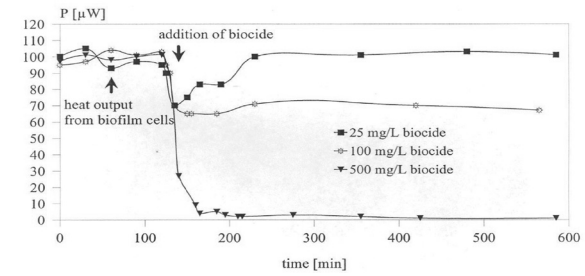

Figure 2. Influence of biocide concentration (GLU) on an aerobic culture biofilm with Vibrio natriegens (P = thermal power) [5].

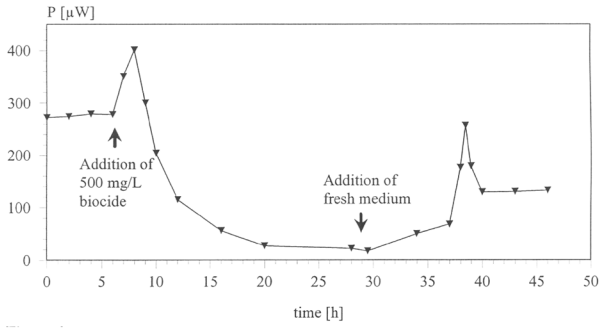

Figure 3. Influence of biocide concentration (GLU) on an anaerobic mixed culture biofilm with SRB and COT (P = thermal power) [5].

Either an anaerobic enrichment culture (containing SRB and COT) or an aerobic culture of Vibrio natriegens (DSM 759) were externally incubated at 30 °C as described elsewhere [5]. The respective culture solutions were continuously pumped (20 mL/h) through the flow-through calorimeter. Biofilm formation was traced by replacing the bacterial culture solution by a sterile one. This occurred at the end of the logarithmic growthphase

of the various test cultures (5 h for Vibrio natriegens, 48 h for the enrichment). The remaining heat output could be exclusively attributed to attached cells on the surface of the gold tubing in the measuring cylinder. Biocide was added to the continuous culture assays after the start of the experiment at about 46 h in case of Vibrio natriegens and at 120 h in case of the enrichment. At these times a constant heat output had indicated that a mature biofilm had developed. Control experiments aiming at the detection of surviving cells in the biofilm were run by replacing the biocide-containing by biocide-free nutrient solution followed by continuous heat-output recording.

RESULTS

A stable biofilm was obtained on the inner surface of the gold tubing of the flow-through cylinder with Vibrio natriegens after 46 h and for the enrichment culture after 120 h. The heat output amounted with Vibrio to 100 µW (Fig. 2), in case of the enrichment to 270 µW (Fig. 3).

With the Vibrio-biofilm and GLU the lowest biocide concentration resulted only in a slight and transient decrease of microbial activity (Fig. 2). With 100 mg/L GLU microbial activity decreased somewhat but remained during the whole experiment at around 70 µW. At the highest concentration microbial activity rapidly decreased to 3 µW and remained at

that level. If in the latter case the biocide-containing medium was replaced by a biocide-free one, metabolic activity became restored to 85 μW after 10 h (not shown). Obviously, several bacteria survive the biocidal action (probably those which are deeply embedded in the biofilm and thus are protected) and start to regrow.

The biofilm of the enrichment culture was tested in the same way. In these experiments the

biocide concentrations 25 and 100 mg/L remained without any influence on microbial activity (not shown). Only at a concentration of 500 mg/L GLU microbial activity (after a short transient increase) was strongly reduced to 3 μW after 23 h (Fig. 3). After nutrient solution exchange, biofilm regrowth also occurred. A heat output of 110 μW was noted after 18 h of consecutively culturing.

DISCUSSION

For testing biocide efficacy many techniques are in use. However, plate counts and MPN-techniques remain to be the most widely used ones because of their low cost and the unequivocal results concerning the killing effect of the biocidal agents. The results presented here indicate that microcalorimetry has considerable advantages over those techniques. Although the latter is primarily more expensive than the classical methods, it may replace the former, because it allows to measure rapidly, even online, an inhibition of microbial activity by biocidal action. Furthermore, in combination with the classical techniques it becomes possible to differentiate between killing or inhibition as a result of a biocidal action. Even a remaining effect, due to adsorbed biocide to the biofilm matrix, becomes detectable. Thus, the time span until a further biocide application becomes necessary, can be determined. This may be of considerable importance for industries such as paper industry, where biocide actions can only reduce but not totally remove microbial biofilm-bound activity.

CONCLUSION

Microcalorimetry allows by rapid and online tests to screen biocides for their efficacy under a manifold of conditions. However, until now, it is not possible to test biofilms on other surfaces than gold with the flow-through system. Because of the direct response of the instrument to any heat evolution, the experimental time becomes considerably reduced. In times, where economical considerations together with environmental responsibility are of utmost importance, the novel technique will surely f nd its place in the biocide business.

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