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A bioassay of pollutants from animal house air by batch microcalorimetry

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Abstract

Batch microcalorimetry (BMC) was used to screen the effects of five compounds found in the air of animal houses on *Escherichia coli* broth cultures (ATCC 11229) in a short-term bioassay.

The microcalorimetric assay is described. A standard method of characterization of the power–time curve is proposed. Characteristics of the curve (e.g. peak time, peak height) are evaluated and related to the concentration of the test compounds used. The relationship is described by cubic polynomial regression. The procedure allows the establishment of minimum effective concentrations (D_{min}) which are (in mmol l^{-1}) for acetone 68, butyric acid 13.6, dimethylamine 19.5, formaldehyde 0.14 and phenol 2.27. Microcalorimetry offers the opportunity to determine the toxic effects of compounds on different metabolic processes of microorganisms.

Knowledge of the typical concentration of compounds in the air of animal houses allows assessment of their likely impact on animals by extrapolation from the results of BMC. Phenol provides a convenient yardstick against which the hazard of other compounds may be measured. The results indicate that butyric acid and dimethylamine present a greater hazard than phenol and acetone.

Bacterial short-term assays may not completely replace experiments involving animals but they allow an estimation of the potential hazard of compounds which have not yet been tested and may thus reduce the necessary amount of experiments with animals.

INTRODUCTION

In modern systems of animal husbandry, the importance of the composition of the house air for the health and welfare of animals is of increasing concern [1]. However, knowledge of the biological activity of the compounds in the air of livestock buildings is limited [2]. At the same time there is growing interest in methods by which the biological effect of such compounds can be assessed without resource to the use of animals in toxicity assays [3]. Microcalorimetry provides a general analytical tool for the characterization of metabolic processes in microorganisms [4–6]; the technique offers quick and reliable means for testing chemical toxicity using microorganisms, e.g. *Escherichia coli*.

To date, 136 different trace gases have been identified in the air of animal houses, but of these the concentration of only a few have been measured [7]. The following contribution describes the use of *Escherichia coli* broth cultures and batch microcalorimetry (BMC) in a short-term assay for screening the effects of five substances found in the air of animal houses. Quantitative data were available for four of the five compounds.

MICROCALORIMETRIC TEST MODEL

A batch microcalorimeter (model "Triflux", Thermanalyse Moirans France) of the heat-flux type, equipped with three pairs of reaction chambers, was used. The chambers contained close fitting vessels, made of stainless steel, which could be removed for preparation of the test solutions. The temperature of the microcalorimeter was kept at 37°C. The power–time curves (p–t curves) were recorded with a three channel recorder. *Escherichia coli* strain ATCC 11229 was used as the test organism. The inocula were prepared from *E. coli* broth cultures, which were incubated overnight and diluted to a final viable count of about 2×10^5 CFU ml^{-1} (CFU means colony forming unit) in the test medium. The growth and test media were prepared according to Eklund [8] and contained glucose as the energy source. The test compounds were of analytical grade, and sterilized water of a standardized hardness (WSH) of 17°dh (= German graded hardness) was used for all solutions [9].

Test procedure

The reaction vessels were loaded with 5 ml of double concentration broth medium and 5 ml of WSH containing the test compound. The vessels were prewarmed to 36°C for about 15 min prior to insertion into the chambers of the calorimeter. In the preheating zones of one of each pair of chambers, pipettes containing the inocula of 100 μl were placed just above the vessels. A schematic sectional diagram of one of the chambers of the BMC is given in Fig. 1. Thermal equilibrium and the establishment of stable baselines of the p–t curves were achieved after 4 to 6 h, and the inocula were then injected simultaneously. The fluctuation of the baselines caused by the inoculation process stabilized after ≈ 1 h.

Evaluation of the p–t curve

In addition to recording the p–t curve, oxygen content, pH value, optical density (by transmission) and glucose concentration in the broth were also measured. The number of viable bacteria was determined in the inoculum and in the broth at the end of each experiment. Figure 2 shows the measurements of heat flow rate (p–t curve), optical density (T), pH and dissolved oxygen content (O_2) for the control of the pure medium.

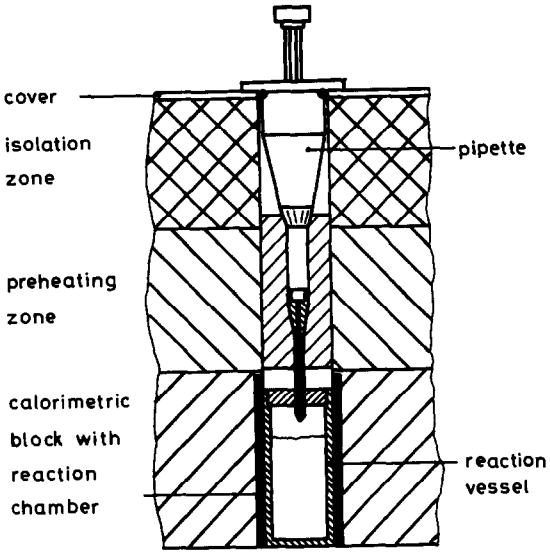

Fig. 1. Schematic sectional diagram of one chamber of the batch microcalorimeter with pipette.

The heat production showed two peaks. The first was the lower and occurred after a short lag phase. After a short decrease in heat flow rate, when the oxygen content was also low, a second distinctly higher peak was observed, and coincided with the utilization of $\approx 50\%$ of the available glucose. This second peak was followed by a steady decrease of heat flow rate, and the baseline was reached about 18 h after inoculation.

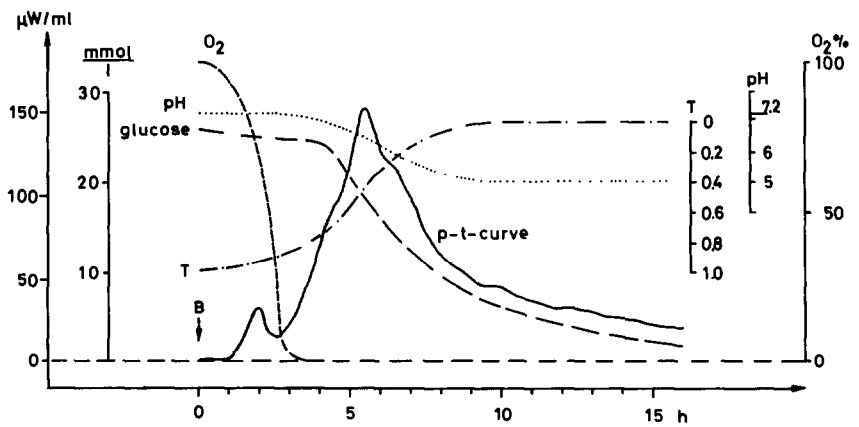

Fig. 2. Heat flow rate ($p-t$ curve, $\mu W\ ml^{-1}$), dissolved oxygen (O_2, %), glucose concentration (mmol l^{-1}), pH and optical density (T) in *E. coli* broth culture in the batch microcalorimeter (BMC). Mean of 5 to 7 runs, $p-t$ curve $n = 20$. B = injection. From [7].

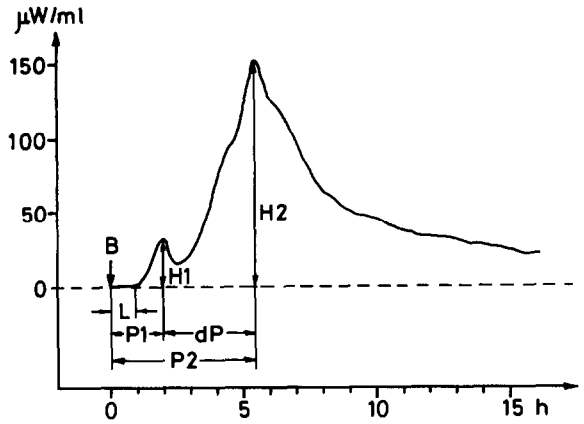

Fig. 3. Characteristics of the $p-t$ curve (for details see text).

The following characteristics of the $p-t$ curves were evaluated as descriptions of the effect of the test compounds on the heat flow rate (see also Fig. 3).

Lag phase (L): time from inoculation to the start of heat production (min);

Peak time (P): time from inoculation to primary (P1) or secondary (P2) peak of heat production (min);

Peak height (H): height of the primary (H1) or secondary (H2) peak above the baseline, heat flow rate ($\dot{Q}$) ($\mu W\ ml^{-1}$);

Peak time difference (dP): time difference (min) between primary and secondary peaks (P2 − P1).

The peak time difference (dP) gives consistent and reliable results with low variation coefficients of $\pm 3.7\%$ ($n = 20$) and satisfactory sensitivity towards the test compounds, as shown in previous investigations [7].

RESULTS

Figures 4–8 show examples of the influence of five different compounds on the $p-t$ curve of *E. coli* broth cultures. The control is the average of 20 runs, the others the average of six. Two distinct patterns of response were observed. Acetone, dimethylamine and formaldehyde seem to act predominantly by delaying the growth phase (longer peak times). Butyric acid, phenol and acetone acted rather on heat flow rate (decrease in peak heights).

Increasing concentrations of acetone (Fig. 4) reduced H2 significantly, leaving H1 almost unaltered. Similarly, P1, P2 and dP were also longer with increasing concentration. Both the peak heights (H1 and H2) were affected by butyric acid (Fig. 5), whereas both peak times were only longer at higher concentrations (> 13.6 mmol l^{-1}). Increasing amounts (> 19.5 mmol l^{-1}) of dimethylamine markedly altered the shape of the $p-t$ curve (Fig. 6). The

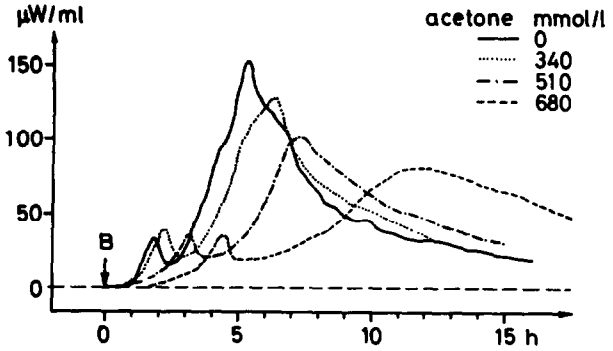

Fig. 4. The *p-t* curves of *E. coli* broth cultures in the batch microcalorimeter influenced by different concentrations of acetone. Control is 0 mmol l^{-1}; $n = 20$ runs. Others, mean of six runs.

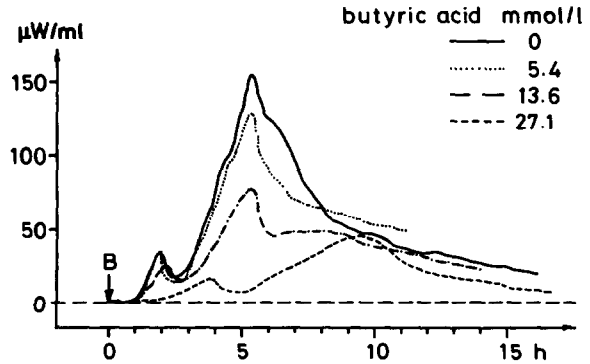

Fig. 5. The $p-t$ curves of *E. coli* broth cultures in the batch microcalorimeter influenced by different concentrations of butyric acid. Control is 0 mmol l^{-1}; $n = 20$ runs. Others, mean of six runs.

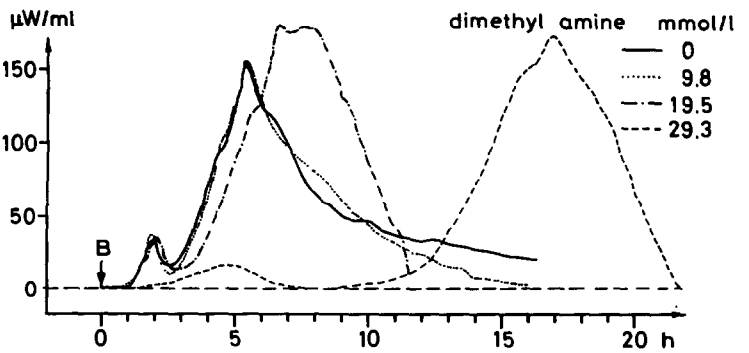

Fig. 6. The $p-t$ curves of *E. coli* broth cultures in the batch microcalorimeter influenced by different concentrations of dimethylamine. Control is 0 mmol l^{-1}; $n = 20$ runs. Others, mean of six runs.

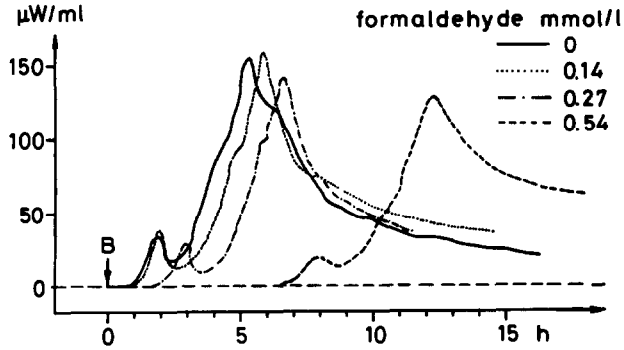

Fig. 7. The $p-t$ curves of *E. coli* broth cultures in the batch microcalorimeter influenced by different concentrations of formaldehyde. Control is 0 mmol l^{-1}; $n = 20$ runs. Others, mean of six runs.

secondary peak was characterized by a high, wide plateau which fell sharply to the baseline. Both P1 and P2 were longer at concentrations above 29.3 mmol l^{-1}, whereas concentrations in the medium of more than 35 mmol l^{-1} completely halted heat production.

The main distinguishing feature of exposure to formaldehyde (Fig. 7) was a remarkable increase in the lag phase and in P1 and P2 at higher concentrations (0.54 mmol l^{-1}). Under the influence of phenol (Fig. 8), P1 was longer and H2 was increased. Table 1 gives the complete set of data for phenol as an example of the method. Control values for 20 runs are also shown.

The relationship between the concentration of the test substance and the parameters of the $p-t$ curve was not linear. The different modes of actions were best described by a cubic polynomial regression. Minimum (D_{min}), median (D_{50}) and maximum effective doses (D_{max}) can be calculated from regression lines of best fit [7]. The parameter D_{min} seems to be a suitable one for evaluating the substances. Table 2 compares the D_{min} values for the

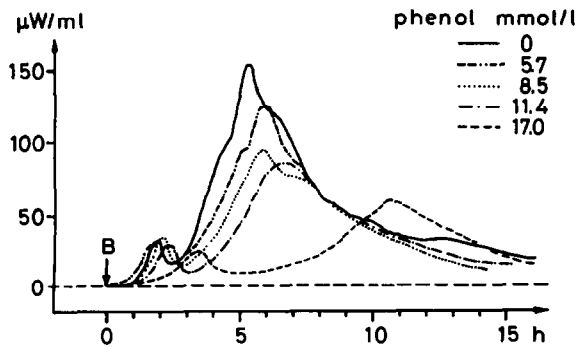

Fig. 8. The $p-t$ curves of *E. coli* broth cultures in the batch microcalorimeter influenced by different concentrations of phenol. Control is 0 mmol l^{-1}; $n = 20$ runs. Others, mean of six runs.

TABLE 1

Influence of phenol on $p-t$ curve parameters P1, P2 and dP

Phenol concentration (mmol l^{-1})	Parameters of the $p-t$ curve					
		P1 (min)		P2 (min)	dP (min)	
	n [a]	$\bar{x}$ [b]	s [c]	$\bar{x}$ [b] s [c]	$\bar{x}$ [b]	s [c]
0.0	20	113	7.7	326 ± 11.7	212	7.8
1.1	7	110	5.1	331 ± 3.4	222	5.6
2.3	4	108	4.9	334 ± 6.7	236	9.0
3.4	5	113	5.4	352 ± 11.5	239	8.0
5.7	6	114	7.6	362 ± 15.0	248	9.0
8.5	7	132	6.8	362 ± 6.4	230	2.9
11.4	8	150	11.5	407 ± 22.7	257	26.0
17.0	4	210	8.8	648 ± 31.5	438	23.0

[a] Number of runs. [b] Mean value. [c] Standard deviation.

TABLE 2

Minimum effective doses (D_{min}) of five compounds deduced from the $p-t$ curve parameters P1, P2, H1, H2 and dP

Compound	D_{min} (mmol l^{-1}) for the $p-t$ curve parameters				
	P1	P2	H1	H2	dP
Acetone	102	170	272	272	68
Butyric acid	13.6	13.6	13.6	5.42	13.6
Dimethylamine	19.5	19.5	29.2	19.5	19.5
Formaldehyde	0.27	0.14	0.14	0.27	0.14
Phenol	8.51	2.27	17.0	2.27	2.27

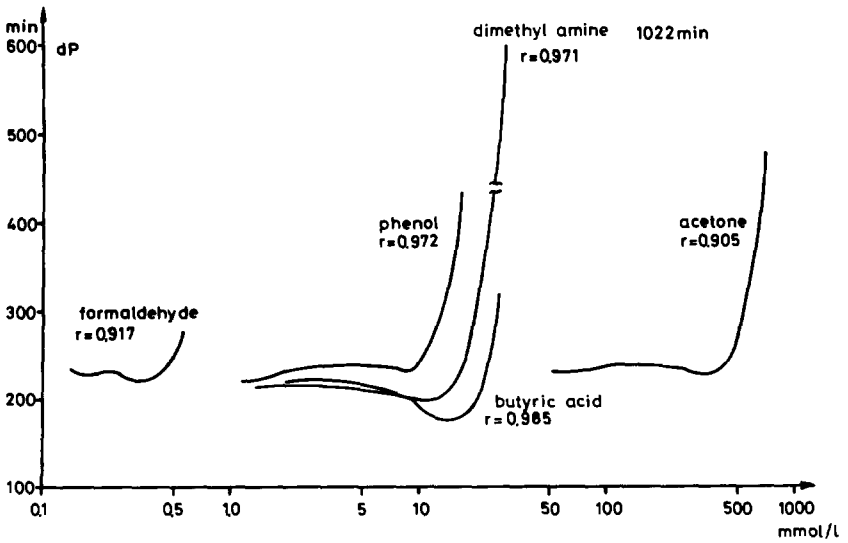

Fig. 9. Range of effective concentrations (mmol l^{-1}) of the five test compounds based on parameter dP (min). Cubic polynomial regression lines on a semi-logarithmic scale.

parameters P1, P2, H1, H2 and dP for each of the test compounds. Estimates of D_{min} for dP were usually the lowest except for H2 and butyric acid.

Figure 9 summarizes the range of effectiveness of the five test compounds on a semi-logarithmic scale based on the parameter dP and calculated by cubic polynomial regression. The length of the curves and the mode of action differ with the nature of the compounds. Acetone and phenol showed the widest spread of concentration range with more than one order of magnitude, followed by butyric acid and dimethylamine. Formaldehyde acts in a relatively small range of concentration.

COMPARISON WITH OTHER BIOASSAYS

There are very few microcalorimetric results available which can be compared with the results presented here. Values of D_{min} for acetone (BMC: 68 mmol l^{-1}) and phenol (BMC: 2.27 mmol l^{-1}) were in good agreement with the results reported by Schauerte [10], who found that 64.6 mmol l^{-1} of acetone was the highest concentration which did not produce an effect in his bio-calorimeter using *Serratia marcescens*, and by Weppen and Schuller [11], who reported a minimum effective concentration of 2.3 mmol l^{-1} for phenol analysed by using a twin calorimeter and *Acinebacter calcoaceticus*.

Comparisons of the microcalorimetric method with the Microtox test (MTT) are given in Fig. 10. The MTT [12] determines the effective concentration which diminishes the light emission of the test culture (*Photobacterium phosphoreum*) by 50% within 15 min (EC50). The MTT was more

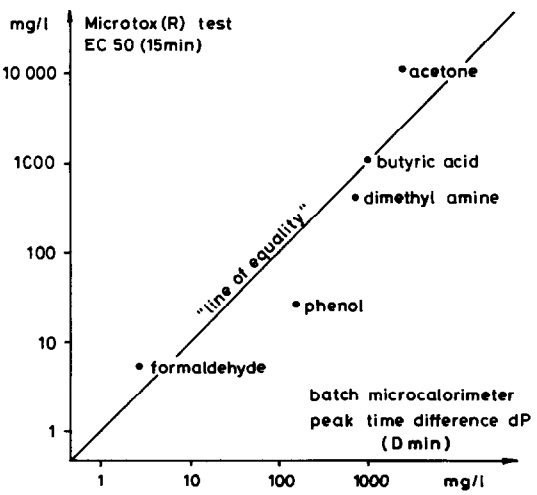

Fig. 10. Comparison of batch microcalorimetry (D_{min}) and Microtox test (EC50 15 min) results.

TABLE 3

Comparison of the BMC results with occupational health standards at the workplace (MAK) [a]

Test substance	BMC in water		MAK [a] in air	
	D_{min} (mmol l^{-1})	Rank	μmol l^{-1}	Rank
Acetone	68	1	41.3	1
Dimethylamine	19.5	2	0.4	2
Formaldehyde	0.14	4	0.04	4
Phenol	2.27	3	0.2	3

[a] MAK = German occupational health standards at the workplace [16].

sensitive than BMC for dimethylamine and phenol in particular, while the converse was true for formaldehyde and acetone; butyric acid gave similar results by either method. The consistency of the techniques is remarkable, because *E. coli* is assumed to be relatively resistant against environmental influences [13]. It seems, therefore, that the described microcalorimetric technique might be even more sensitive if other test organisms were used. The MTT might also show increased sensitivity if light emission were to be evaluated at 30 min instead of 15 min.

Our own MTT results also show good agreement with other bioassays, which are commonly used for water quality testing and employ *Pseudomonas putida, Daphnia magna* and the fish *Leuciscus idus melanotus* [14]; similarly good correspondence with the rat test (*Rattus rattus*, oral LD50) was found [15].

Table 3 compares the German occupational health standards for an eight hour working day (MAK) [16] with the BMC results for four compounds. The ranking of compounds for toxicity is identical.

SIGNIFICANCE OF BMC FOR ASSESSING AIR QUALITY IN ANIMAL HOUSES

Knowledge of the typical concentration of compounds in the air of animal houses allows assessment of their likely impact on animals by

TABLE 4

Hazard ratio of BMC results and animal house air concentrations of the test compounds

Test substance	BMC in water D_{min} (mmol l^{-1})	Conc. in air (μmol m^{-3})	Hazard ratio BMC/conc.
Acetone	68	5.68 [a]	1:12000
Butyric acid	19.5	6.80 [b]	1:2000
Dimethylamine	0.14	440.00 [c]	44
Phenol	2.27	0.46 [b]	1:5000

[a] From ref. 19. [b] From ref. 20. [c] From ref. 21.

extrapolation from the results of BMC (Table 4). The ratio of D_{min} to concentrations in the air provides an estimate of the potential hazard of each compound. These ratios indicate that butyric acid and dimethylamine are more hazardous than phenol and acetone.

Phenol provides a convenient yardstick against which the hazard of other compounds may be measured. Dimethylamine is about 300, 115, and 40 times more hazardous than acetone, phenol and butyric acid respectively. This method of evaluation requires further work to prove its general applicability.

CONCLUSION

These investigations show that it is possible to characterize the antimicrobial effect of chemical compounds by the aid of batch microcalorimetry. The method seems to be particularly useful at low concentrations. Microcalorimetry offers the opportunity to determine the toxic effect of compounds on different metabolic processes of microorganisms. A proposal is made for a standard method of characterization of the p–t curve.

A disadvantage of the batch microcalorimetric technique is the equilibration time of up to 6 h. During a normal working day no more than three runs can usually be made. On the other hand, the technique is simple to handle. A great advantage of the method in comparison with, for example, the Microtox test is the ability to use different test microorganisms, not only bacteria.

The results also indicate the potential utility of batch microcalorimetry as a microbial short-term assay for evaluating air compounds in animal houses and their likely hazard to farm animals. The results are comparable with those of other biological short-term assays [7,14]. The method seems worthy of further investigation. However, the range of the results may support the suggestion of applying two or more short-term tests involving different organisms in a "test battery" [14,17,18].

Bacterial short-term assays of substances which are relevant in the air of animal houses may not replace experiments involving animals, but they allow an estimation of the potential hazard of substances which have not yet been tested, and may thus reduce the necessary amount of experimentation involving animals.

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