Classification of the strain and growth phase of cyanobacteria in potable water using an electronic nose system

H.W.Shin, E.Llobet, J.W.Gardner, E.L.Hines and C.S.Dow

Abstract: An electronic nose comprising an array of six commercial odour sensors has been used to monitor not only different strains, but also the growth phase, of cyanobacteria which is normally called blue green algal. A series of experiments were carried out to analyse the nature of two closely related strains of cyanobacteria, *Microcystis aeruginosa* PCC 7806 that produces a toxin and PCC 7941 that does not. The authors have constructed a measurement system for the testing of the cyanobacteria in water over a period of up to 40 days. After some pre-processing to remove the variation associated with running the electronic nose in ambient air, the two different strains, and their growth phase, were classified with principal components analysis, multilayer perceptron (MLP), learning vector quantisation (LVQ), and fuzzy ARTMAP. The optimal MLP network was found to classify correctly 97.1% of unknown non-toxic and 100% of unknown toxic cyanobacteria. The optimal LVQ and fuzzy ARTMAP algorithms were able to classify 100% of both strains of cyanobacteria. The accuracy of MLP, LVQ and fuzzy ARTMAP algorithms with the four different growth phases of toxic cyanobacteria was 92.3%, 95.1% and 92.3%, respectively.

1 Introduction

The release of various chemical pollutants from industry, automobiles and homes, into the environment probably causes global environmental problems, such as acid rain, the greenhouse effect, ozone layer depletion, water enrichment [1]. The enrichment of water by inorganic plant nutrients is fast becoming another severe problem in the quality of water and a common source of odour pollution in the environment [2, 3]. In 1989, widespread toxic cyanobacterial (blue-green algae) blooms occurred in lakes within the United Kingdom, with associated animal poisonings and human health problems. When present in large concentrations in lakes and reservoirs, the cyanobacteria blooms cause serious nuisance. They clog up water treatment filters, impart unpleasant tastes to drinking water, and produce offensive smells resulting from bloom decay. One of the major problems associated with cyanobacteria is that they can produce toxins that are effective against cattle, wildfowl, fish, and human beings. A wide programme of research on cyanobacteria was commissioned in 1990 [4].

So far water analysis has been carried out mostly by the use of analytical instruments that are based on liquid chromatography or optical microscopy in the liquid phase. These instruments may give precise analytical data, but in most cases they need long times for sample analysis. Such a disadvantage makes clear the importance of the electronic nose approach, i.e. employing gas sensors as their counterpart and the need for new trials to see whether gas sensors can detect, and classify, odour components in water. The electronic nose technology has been applied within the food and drink industry [5] over the past 10 years. In recent years research has been directed towards health and safety. Previous attempts to identify micro-organisms with an electronic nose were made by Craven *et al.* [6] and Gibson *et al.* [7].

In this paper the use of an electronic nose based on a 6 MOS sensor array is described for the analysis of cyanobacteria cultures grown in water. The strain and growth phases of cyanobacteria were classified using principal component analysis (PCA), multilayer perceptron (MLP) neural network, learning vector quantisation (LVQ), and fuzzy ARTMAP paradigms.

PCA is a useful classical pattern recognition technique to show clearly the groupings of data-sets on a dimensionless PCA plot. In PCA, a set of linearly correlated variables is transformed into a set of uncorrelated variables (principal components) such that the first few components usually explain most of the variation in the data-set.

Artificial neural networks (ANNs) have been widely applied to data analysis in electronic nose systems since a back-propagation neural network was first applied to the output of an electronic nose in 1990 [8]. ANNs which mimic the architecture of the biological olfactory system do not require an explicit description of how the problem is to be solved. They learn from the data and are configured during a training period. They can cope with highly non-linear data and so unlike PCA can be made to cope with noisy or drifting sensor data. This is especially true of the MLP ANNs trained using back-propagation (BP). Multilayer networks are more powerful than single-layer

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networks although more computational intensive. The MLP network is one of the most popular neural network architectures and is suited to a wide range of applications. It consists of a set of sensory units that constitute the input layer, one or two hidden layers, and an output layer. NeuralWorks Professional II/Plus software provides a comprehensive framework for rapidly implementing neural networks, training methods, and flexibility to build new functions. It includes a comprehensive set of network architecture and training rules for the design of the network that best fits the application.

Learning vector quantisation (LVQ) is an improved supervised learning technique with a self-organising feature map. The LVQ method has also been extended by Sakuraba *et al.* [9] to handle fuzzy data. The neural network architecture of LVQ is essentially the same as that of the Kohonen network (Fig. 1). The hidden layer in this network is a Kohonen layer, and it carries out the learning and classifying. The LVQ scheme consists of LVQ1 and LVQ2 algorithms. LVQ1, is the basic LVQ learning algorithm that helps all PEs (Processing elements) to take an active part in the learning. LVQ2 is a fine tuning mechanism, which refines class boundaries. Therefore the output from LVQ2 is ,the final encoded version of the original input signal applied to LVQ1.

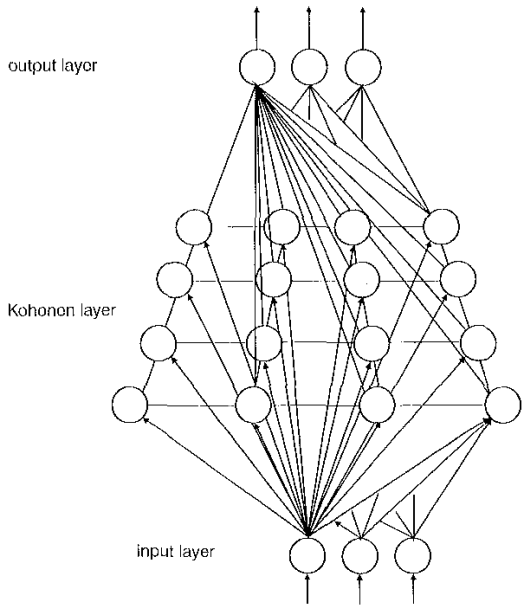

Fig. 1 *Schematic diagram of a LVQ with Kohonen layer*

Finally, fuzzy ARTMAP carries out supervised learning, like the back-propagation MLP does. But unlike back-propagation, it is self-organising, self-stabilising and suitable for incremental learning. Fuzzy ARTMAP is able to deal with uncertainty, which is a key element in any measurement system, and generally shows superior performance in learning compared with MLP. Fig. 2 shows the schematic architecture of a fuzzy ARTMAP neural network that consists of two ART modules interconnected by an associative memory and internal control structures. The orienting subsystem and the gain control are the two major subsystems. The orienting subsystem is responsible for generating a reset signal and the gain control sums the input signal. Inhibitoy paths are denoted by a minus sign and other paths are excitatory. One of the main advantages of fuzzy ARTMAP is that it is able to perform online learning without forgetting previously learnt patterns. This is very important from a practical viewpoint because the real dataset used to train the network may be increased during the development phase by adding new measurement when the system is applied in an industrial setting.

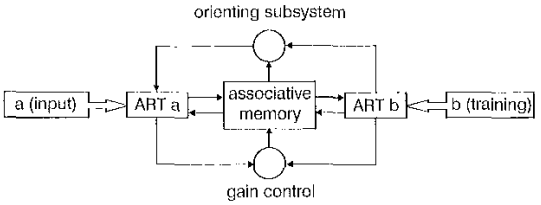

Fig. 2 *Architecture of a fuzzy ARTMAP neural network*

2 Experimental procedures

For the measurement of cyanobacteria samples, it was necessary to grow blue green algae. The growth medium used was BG-11, which allows us to grow the blue green algae found in rivers. The medium was made using analytical grade chemicals and double distilled water, and sterilised by autoclaving at 15 lb inch ^{2} for 30 minutes. Table 1 shows the composition of a BG-11 medium.

Table 1: Composition of the BG-11 medium

Chemicals	g l^{-1}
$NaNO_3$	1.5
$K_2HPO_4{\cdot}3H_2O$	0.04
$MgSO_4{\cdot}7H_2O$	0.075
$CaCl_2{\cdot}2H_2O$	0.036
Citric acid	0.006
$FeNH_4$ citrate	0.006
$Na_2Mg{\cdot}EDTA$	0.001
Na_2CO_3	0.02
Trace metal mix A_5+Co*	1 (ml l^{-1})
*Trace metal mix A_5+Co	
H_3BO_3	2.86
$MnCl_2{\cdot}4H_2O$	1.81
$ZnSO_4{\cdot}7H_2O$	0.222
$Na_2Mo{\cdot}O_4{\cdot}2H_2O$	0.390
$CuSO_4{\cdot}5H_2O$	0.079
$Co(NO_3)2{\cdot}6H_2O$	0.049

Cyanobacteria produce toxic substances when the cyanobaterial population forms a bloom. There are many factors leading to blooms of cyanobacteria such as physical factors, temperature, light and the nutrient. Planktonic cyanobateria are very similar to most photosynthetic plankton in that they require a certain minimum average light intensity for growth. Light is usually provided in the laboratory by a bank of fluorescent lamps (cool white, daylight, or warm white). The intensity for stock culture maintenance can vary enormously, but a lower intensity than 500 lux is appropriate to cyanobacteria [10]. The optimum temperature for cyanobacteria is generally suggested to be between 15–30 C. Rates of nutrient supply may be of more importance than actual nutrient concentrations. Actually, the requirements for nutrient substances are variable depending on the cyanobacteria species. In this experiment, 3.5 l of medium (1.5 l of head-space) was placed in a standard 5 l glass jar. A small number of cells (contained in 100 ml of inoculum) from the reference store (master culture) were

inoculated into the medium. The maintenance of a particular cyanobacteria culture is very important because the bacteria culture will be useless if all the cells die, do not grow well, or if the culture is contaminated with different microorganisms.

The number of cells and cell size are sensitive indicators of the physiological status of cells. They change as the cells go through the different stages of growth. Thus cell size and number can be used to determine optimum culture conditions or growth phase. Small samples of liquid were extracted and analysed by the commercial CellFacts instrument (Microbial System Ltd.), and then it was possible to measure the size and distribution of bacteria. The CellFacts instrument uses electrical flow impedance determination both to count and size particles and cells in water quickly and easily by using real-time online sampling techniques. Cyanobacteria cells are passed through a 30mm orifice in an electrolytic fluid, the displacement creating voltage pulses that can be measured and counted. The range of cell volume is 0.1 to 450mm^3 and information about individual cells in a population can be gathered and automatically down-loaded to a personal computer.

Fig. 3 shows the layout of the measurement system for the testing of the cyanobacteria (blue-green algae) in water. The system consisted of three main stages: the odour sampling system, a customised FOX 2000 unit (Alpha MOS SA) and the CellFacts instrument. The sampling system and the modified FOX 2000 are controlled through custom designed software written in LabVIEW and run on an IBM PC compatible computer.

Liquid samples were extracted and analysed by the commercial CellFacts instrument and gas samples from each headspace were introduced to the electronic nose system. Gas flow was directed through one of three routes by the action of three solenoid valves. Each vessel had a solenoid valve (Lee Company, Manuf. No. LFAA1200118H) associated with it. At any one time, only one valve was powered up. Software run on the PC ultimately controlled the solenoid valves by an I/O card (National Instruments LPM-16) and therefore controlled gas flow. This subsystem therefore had three channels for gas to flow through from the input to the output with one vessel per channel. The gas pathways for all channels were designed to be of equal length and it was important to preserve identical gas flow characteristics across all channels in order to reduce any inter-channel variation. The sampling system was operated in a cyclic fashion, whereby a set sequence of timed valve actuation was repeated for a predetermined number of times. It consists of sampling the head space of three identical vessels which contain reference (medium) and odour samples. The reference vessel, when selected, allowed the sensor array to stabilise its response to a known odour. Next, one of the sample vessels was selected and the change in sensor response was observed and recorded. It was possible to set up the sampling system to activate any channel at a specific time using a LabVIEW program. The sequence of channel activation that was adopted in the LabVIEW program for each cycle was; channel 1 (reference), channel 2, channel 1, channel 3.

The electronic nose system also has a gas volumetric flow-rate sensor, a diaphragm vacuum pump (KNF Neuberger, Manuf. No. NMP30KNDC) and some analogue op-amp interfacing circuitry, that converts the resistance of the gas sensors into a DC voltage (0 to 10V) for input to the NI data acquisition card in a PC. The electrical signals from the six commercial gas sensors (Alpha MOS SA) were conditioned and then fed into a 12-bit ADC in the LPM-16 card and recorded by a LabVIEW program. The target gases for six gas sensors (model No.) were as follows: nonpolar compounds (FIS SP12), hydrocarbons (FIS SP11), polar compounds (FIS SP31, FIS SP AQ2), alcoholic compounds (FIS ST MW2) and heteroatom/chloride/aldehyde (FIS ST41).

3 Results and discussion

3.1 Classification of cyanobacteria strain

Each measurement cycle is based on four elements (medium sample – cyanobacteria A medium – cyanobacteria B). The average values per cycle and per element were taken to reduce the large number of data points over several hundred cycles. Fig. 4 shows the typical short-term responses of the sensor array from the initial stage of cyanobacteria samples. Sensor responses showed some diurnal variation with the room temperature (but not sensor chamber temperature) and perhaps air humidity. The target temperature of the sensor chamber was set to 45 C and the temperature fluctuation of the main chamber was around –0.3 C during the test and it seemed satisfactory to perform the biological experiment. The temperature and the humidity of the sensor chamber were stable every experiment, thus these values can be excluded from the data classification process.

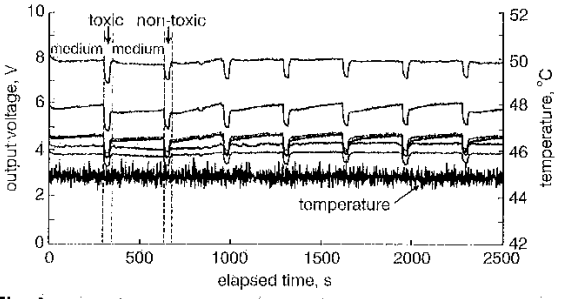

Fig. 4 *Typical sensor responses from medium, toxic cyanobacteria, medium and nontoxic cyanobacteria with cycles*

Numerous signal pre-processing algorithms have been employed in the application of electronic noses [11–15]. Several of the most promising pre-processing techniques

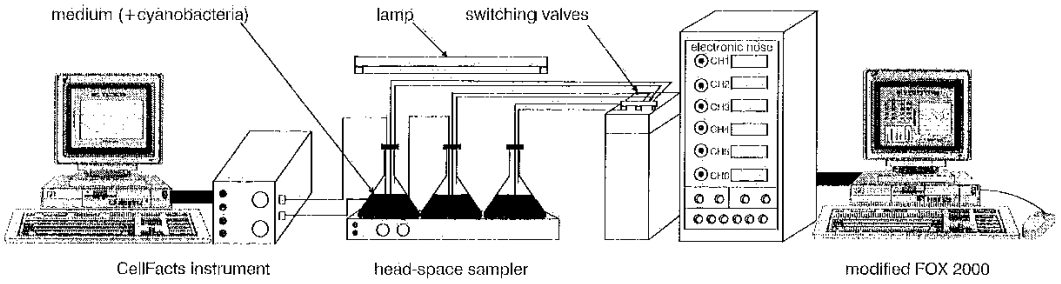

Fig. 3 *Schematic diagram of electronic nose measurement system to collect data from water samples*

were employed here in order to optimise the classification process in this experiment. Table 2 shows the sensor signal pre-processing algorithms, which have been applied. Each sensor, i, produces a time-dependent signal, $x_{ij}^{a}(t)$, in response to odours as shown in Fig. 4 and it is often convenient to remove the time dependence of the signal output. We settled upon the difference and fractional difference algorithms with autoscaling and normalisation techniques. Autoscaling and sensor normalisation give equal weighting to each sensor and thus compensate for differences in the magnitudes of the signals. The resulting pre-processed data were used for PCA, MLP, LVQ and fuzzy ARTMAP neural networks. All the neural network analyses were performed using the software package, NeuralWorks Professional II/Plus (NeuralWare Inc., USA) [16].

Table 2: Examples of signal processing algorithms used in experiment

Signal processing algorithms	Formula
Difference signal (signal from cyanobacteria a - signal from medium b)	$x_{ij} = x_{a,ij}^{a} - x_{b,ij}^{b}$
Relative signal	$x_{ij} = x_{a,ij}^{a}/x_{b,ij}^{b}$
Fractional difference	$x_{ij} = (x_{a,ij}^{a} - x_{b,ij}^{b})/x_{b,ij}^{b}$
Normalisation of sensor range	$k_{ij} = (x_{ij} - x_{ij,\min})/(x_{ij,\max} - x_{ij,\min})$
Autoscaling	$k_{ij} = (x_{ij} - \bar{x}_{ij})/s_j$

i = sensor, j = odour, s = population standard deviation, $\bar{x}$ = average value

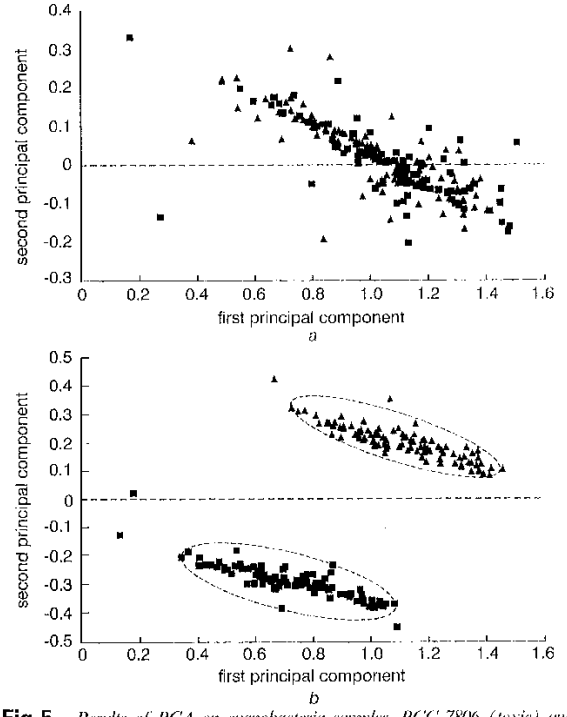

Fig. 5 *Results of PCA on cyanobacteria samples, PCC 7806 (toxic) and PCC 7941 (non-toxic)*
▲ PCC 7806
■ PCC 7941
a normalised difference model
b normalised fractional difference model, that separates the data into two distinct groups.

Fig. 5 shows two examples of PCA results for cyanobacteria samples. The feature-sets employed were the normalised difference model and the normalised fractional difference model, which showed good results for the classification of standard chemicals in previous experiments. Fig. 5*a* shows that PCA is unable to separate out the two cyanobacteria types. The target classes are indicated by different captions. But this is dramatically improved through the use of a normalised fractional difference algorithm, which is able to produce two distinct clusters of the toxic and non-toxic cyanobacteria (Fig. 5*b*). The stretched clusters indicate that sensor drift occurs over time and is mainly in the first component. Despite the sensor drift the PCA of the normalised fractional difference algorithm exhibited good classification performance without any overlap between the two clusters.

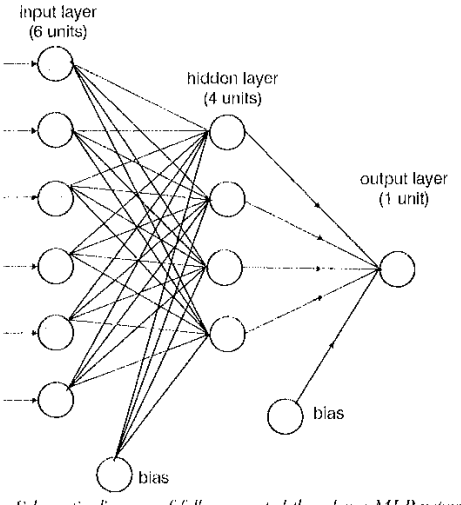

Fig. 6 *Schematic diagram of fully connected three-layer MLP network with 6 input, 4 hidden neurones and 1 output*

The MLP ANN was used to cope with the nonlinear and 'drifting' data. The training technique used is the BP delta learning rule. The BP is a commonly used supervised training algorithm and MLPs are usually trained using BP. The architecture of this neural network was a three-layer MLP network with six inputs, four units in the hidden layer and one output. The neural network was trained using the data set which had been pre-processed using the difference model and the fractional difference model. Fig. 6 shows schematically the fully connected three layer MLP network used. For brevity the network of Fig. 6 is referred to as a 6-4-1 network. Inputs and output were considered as a layer alternatively which constitute the network architecture. A tanh function was used for the transfer function in the network because some inputs and outputs have negative values. The target values were set to provide binary output, i.e. +1 for toxic bacteria and −1 for non-toxic bacteria. The network training parameters were set throughout to values of learning rate 0.3 and the number of epochs was 16. An epoch here is the number of sets of training data presented to the network (learning cycles) between weight updates. The best MLP set of parameters was found to classify correctly 97.1% of the unknown non-toxic bacteria samples and 100% of the unknown toxic bacteria samples on the basis of a set of 378 training vectors and 202 test vectors (35% of cross validation) as shown in Fig. 7.

The LVQ method was applied to the same electronic nose data to classify type and growth phase of cyanobacteria (see next Section 3.2). The target values were set to provide 1 for toxic bacteria and 0 for non-toxic bacteria. The same training and test sets used for the MLP were applied to LVQ algorithms. Initially the network was trained with a learning rate of 0.06 and the conscience factor equal to 1.

The best LVQ set was found to classify correctly 100% of the unknown non-toxic bacteria samples and 100% of the unknown toxic bacteria samples on the basis of a set of 378 training vectors and 202 test vectors as shown in Fig. 8.

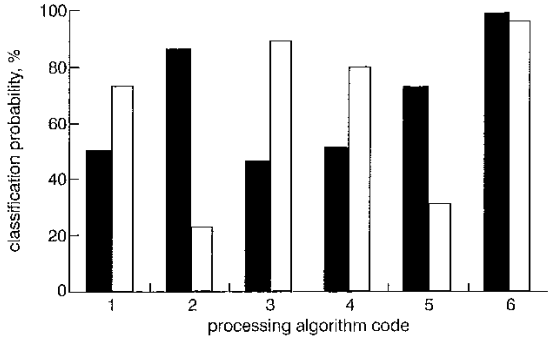

Fig. 7 *Bar chart showing the percentage of correctly classified toxic and non-toxic bacteria for 6 pre-processing algorithms*
1: difference; 2: difference autoscaling; 3: difference normalisation; 4: relative; 5: relative autoscaling; 6: relative normalisation
■ toxic
□ non-toxic

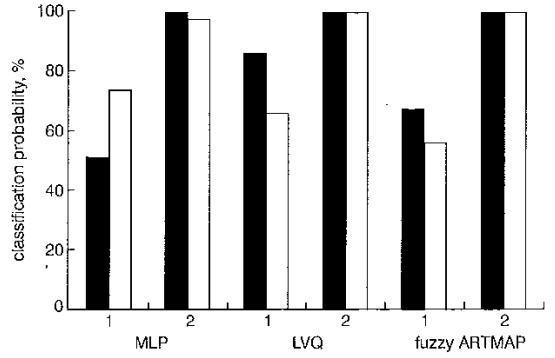

Fig. 8 *Bar chart showing the MLP, LVQ and fuzzy ARTMAP classification probability (%) of correctly classified toxic and non-toxic bacteria for 2 representative pre-processing algorithms*
1: difference normalisation; 2: fractional difference normalisation
■ toxic
□ non-toxic

The fuzzy ARTMAP network was trained with cyanobacteria dataset like the MLP analysis. The value of the recode parameter b was set to 0.5 and a previously learnt category has been slowly re-coded. This allows the established categories to be modified if there is a persistent attempt to do so. The baseline vigilance was set to 0, which allows for very coarse categories and the match-tracking system will refine these categories only if necessary. The fuzzy ARTMAP was able to classify 100% of the cyanobacteria samples. During the training process 25 'internal nodes' were committed.

3.2 Prediction of the cyanobacteria growth phases

Cellular culture growth phase influences the odours released from cyanobacteria cultures. The population was counted at intervals, a plot of the typical cyanobacterial growth curve that shows the growth of cells over a period of time can be drawn. The design, topology, training methods and testing methods of each neural network employed to predict the growth phase were identical to those used for the classification of the cyanobacteria strains. PCA and three supervised classifiers, MLP, LVQ and fuzzy ARTMAP were used to predict the cyanobacteria into the observed four growth phases. Once again the normalised fractional difference model, which showed good results for the classification of cyanobacteria strains, was used.

A series of measurements was performed on toxic cyanobacteria cultures. There were three separate experimental runs on each cyanobacteria culture. Figs. 9–11 show the growth curve from the CellFacts instrument and the corresponding PCA result from the output of the electronic nose system to four different growth phases of three cyanobacteria cultures. The growth curves show the cell counts and sizes against the elapsed time. The true phases, lag, growth, stationary and late stationary (labelled I to IV) were obtained by inspecting the growth curves and locating the changes in the slope against elapsed time. The growth curves and the PCA plots in Figs. 10 and 11 show very similar results compared to the result in Fig. 9. This can be explained by the size of the cells taken from the master culture because the initial cell size of cyanobacteria culture in Fig. 9 was 2.2mm whereas the others were 3mm. There is some overlap of the response vectors in each PCA plot, corresponding to the transition periods of cyanobacteria cultures.

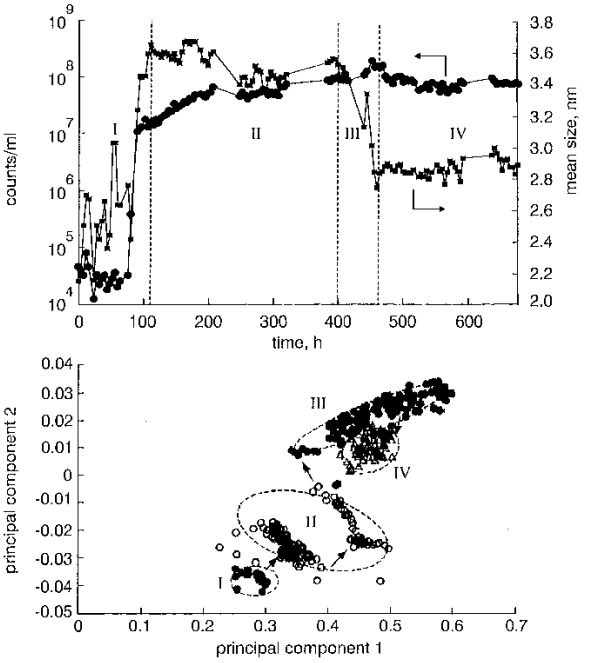

Fig. 9 *Growth phase plot and PCA results (culture 1)*
The four growth phases are lag, growth, stationary and late stationary (labelled I to IV)
a Growth phase plot of Cellfacts instrument showing the number of cell and cell size for cyanobacteria over a 700h period
—●— counts/ml
– ■ – mean size (mm)
b PCA results of the response of a six-element gas sensor based electronic nose to the headspace of cyanobacteria

The cyanobacteria data-set was divided into three test folds containing 48 measurements each (12 measurements per phase category) and the neural networks were trained using 144 vectors for each fold. Test vectors were selected at random from each phase without replacement. The networks had six inputs and four outputs since a 1-of-4 code was used to define the four different phases. The confusion matrix for the classification of the growth phases is shown in Table 3. The classification rates of MLP, LVQ and fuzzy ARTMAP were similar (92.3%, 95.1% and 92.4%, respectively) but fuzzy ARTMAP was the fastest to learn and judged to perform best because it self-organises and selects its own 'hidden neurons'.

To evaluate generalisation, MLP, LVQ and fuzzy ARTMAP were used for the prediction of unknown growth

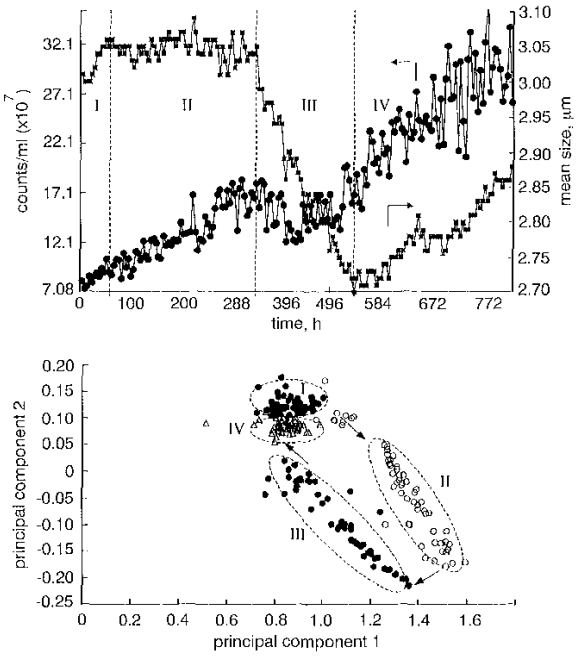

Fig. 10 *Growth phase plot and PCA results (culture 2)*
The four growth phases are lag, growth, stationary and late stationary (labelled I to IV)
a Growth phase plot of Cellfacts instrument showing the number of cell and cell size for cyanobacteria over a 800h period
● counts/ml
■ mean size (mm)
b PCA results of the response of a six-element gas sensor based electronic nose to the headspace of cyanobacteria

phases. The patterns in sets 1 (Fig. 9) and 3 (Fig. 11) were used for training and the patterns in set 2 (Fig. 10) were used to test the network. This led to a performance of 70% in the classification of the test patterns for LVQ and fuzzy ARTMAP and 48% for MLP. The MLP and LVQ network required typically 25,000 and 2,000 training cycles, respectively but fuzzy ARTMAP required only 150 training iterations, thus it was faster than the others.

Table 4 shows the results of the generalisation tests of MLP, LVQ and fuzzy ARTMAP network in growth phase classification, in terms of patterns correctly classified/numbers of patterns. It was difficult to recognise the growth phase of an unknown culture that had a different trend from other cultures used for training. It was found that the majority of errors in the generalisation test occurred in lag phase and the boundaries between the different growth phases, this was due to the implementation of a hard boundary. This is not the case with fuzzy ARTMAP that sets a non-crisp boundary but has similar error in the phase boundaries.

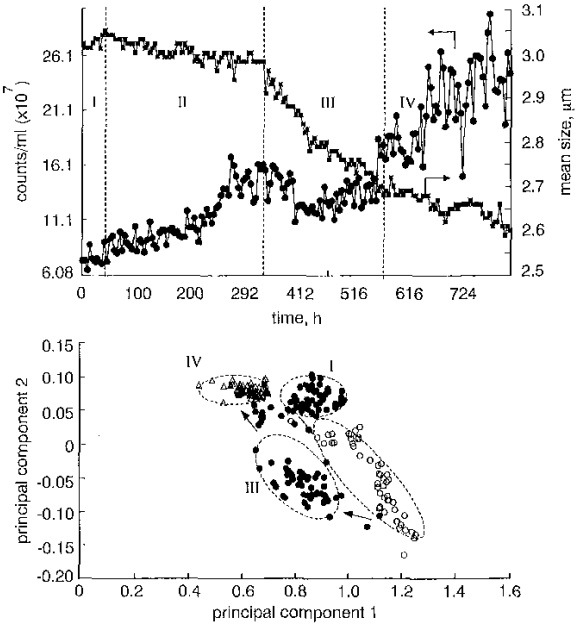

Fig. 11 *Growth phase plot and PCA results (culture 3)*
The four growth phases are lag, growth, stationary and late stationary (labelled I to IV)
a Growth phase plot of Cellfacts instrument showing the number of cell and cell size for cyanobacteria over a 800h period
● counts/ml
– ■ – mean size (mm)
b PCA results of the response of a six-element gas sensor based electronic nose to the headspace of cyanobacteria

4 Conclusions

This study shows the potential application of an electronic nose to the analysis of the quality of potable water.

Table 3: Confusion matrix showing the best performance of growth phase classification of cyanobacteria using a normalised fractional difference model with MLP, [LVQ] and (fuzzy ARTMAP)

	Actual phase			
Predicted	Lag	Growth	Stationary	Late stationary
Lag	140 [136] (140)	8 [16] (20)	0	4 [4] (16)
Growth	[4]	136 [128] (120)	0	0
Stationary	4 [4] (4)	0	144 [144] (144)	0
Late stationary	0	(4)	0	140 [140] (128)

Table 4: Results of the generalisation test of MLP, [LVQ] and (fuzzy ARTMAP) network in growth phase classification, in terms of patterns correctly classified/numbers of patterns

	Growth phase				Classification rate (%)
Training/tested sets	Lag	Growth	Stationary	Late stationary	
	0/50	50/50	2/50	44/50	48
1, 3/2	[3/50]	[45/50]	[45/50]	[45/50]	70
	(20/50)	(48/50)	(47/50)	(44/50)	70

An electronic nose system has been used for the continuous monitoring of the growth of cyanobacteria over a period of 40 days. Several pre-processing techniques were explored to remove the noise factor associated with running the electronic nose in ambient air, and the normalised fractional difference method gave the best PCA plot. Three supervised neural networks, MLP, LVQ and fuzzy ARTMAP, were used and compared for the classification of both two strains and four different growth phases of cyanobacteria (lag, growth, stationary and late stationary). Our best results show that the toxic strain of cyanobacteria was correctly predicted with an accuracy of 100% and that the growth phase of the toxic cyanobacteria was correctly predicted for 70% of all unknown samples using LVQ and fuzzy ARTMAP. The training iterations of fuzzy ARTMAP was found to be typically more than an order of magnitude less than those for the MLP and the LVQ network. Therefore fuzzy ARTMAP was chosen to be the best algorithm overall.

The majority of the classification error is associated with the different growth trend of the cyanobacteria culture and so it could be reduced significantly if the neural network was trained online. Even so the errors occurring in the boundaries between the different growth phases is difficult to reduce, even in fuzzy ARTMAP, so further work is needed on boundary identification.

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