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Biological Computing Using Perfusion Anodophile Biofilm Electrodes (PABE)

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This paper presents a theoretical approach to biological computing, using biofilm electrodes by illustrating a simplified Pavlovian learning model. The theory behind this approach was based on empirical data produced from a prototype version of these units, which illustrated high stability. The implementation of this system into the Pavlovian learning model, is one example and possibly a first step in illustrating, and at the same time discovering its potential as a computing processor.

Keywords: artificial intelligence, biological computing, neurone-like, transistor-like, unit and connected assemblies, Pavlovian association learning.

1 INTRODUCTION

We have recently described what we now term as perfusion anodophile biofilm electrodes (PABES) and have demonstrated their potential for basic binary type computing [1]. In particular we have shown that the biofilm system can maintain a dynamic steady state under one (of many possible) particular set of physicochemical conditions and then switch to a new steady state in response to changes in one of the parameters of the physicochemical environment (set by the operator) leading to a new condition. We also proposed how interconnecting units might be configured into logic gates (AND, OR, XOR) in order to perform basic binary logic operations [1]. Connections could be made via electrical or fluidic links. A schematic representation of the PABE unit and the type of physicochemical changes it will respond to is shown in Greenman *et al*. [1], and the mathematical modeling for these units is described below.

1.1 Theoretical modeling of PABES

Some aspects of growth can be modeled using mechanistic equations of the type described by Monod [2]. Anodophilic biofilm cells exist in a non accumulative steady state. Even though the numbers retained in the biofilm remain constant, cells are nevertheless produced and shed into the perfusate. The growth rate (speed) and yield of new cells (biofilm + perfusate cells) can be modelled using Monods equations:

$$\mu = \frac{\mu_{max} \times S}{K_S + S} \tag{1}$$

where: $\mu =$ specific growth rate (h^{-1})

$\mu_{max} =$ maximum specific growth rate (limiting substrate supplied in excess)

$S =$ substrate concentration

K_s is Monod's constant = is the substrate utilisation constant, which is numerically equal to the substrate concentration when $\mu = \frac{1}{2}\mu_{max}$

For PABES in optimum substrate-limiting conditions the growth rate in the biofilm is given by:

$$\mu\left(h^{-1}\right) = \frac{\text{production rate of cells in perfusate (units} = \text{cfu/mL} \times \text{mlh}^{-1})}{\text{biofilm population (units} = \text{cfu/biofilm)}} \tag{2}$$

1.2 Substrate utilization rate

For PABEs in well-mixed continuous flow:
Substrate in (QSo)= substrate out (QS)+ substrate utilisation K_1ES/Ks + S

$$\Rightarrow \frac{K_1 E S}{Ks + S} \tag{3}$$

where : $Q =$ = volumetric flow rate of bulk fluid

$So =$ initial substrate concentration

$S =$ final substrate concentration

$K_1 =$ rate of reaction (where Vmax $= K_1 E$)

$Ks =$ Monod's (or Michaelis-Menten) constant

$E =$ cell (or enzyme) concentration

In terms of electrical output (Coulombs): Substrate breaks down and is transformed into carbon dioxide, protons and electrons. The theoretical maximum yield from 1 molecule of acetate is 8 electrons, according to the

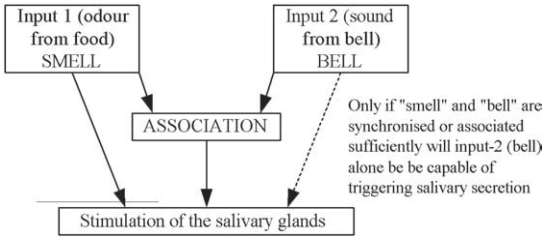

FIGURE 1
Simplified representation of Pavlovian feeding reflex in mammals (e.g. dog).

half-rate equation shown below:

$$CH_3COOH + 2H_2O \rightleftharpoons 2CO_2 + 8H^+ + 8e^-$$

In practice, electron abstraction efficiency can be as high as 96% [3, 4]. Using the aforementioned equations it would be possible to theoretically model some aspects of individual units.

2 A SIMPLE LEARNING PROBLEM TO SOLVE

We now propose to demonstrate (in a Gedanken model) how PABES may be configured to produce associated learning, such as that described by Pavlov (1927) and shown in simplified manner in Figure 1 [5]. Starting with the simplest case we can think of (smell of food, associated sound of bell, switch-on of salivation), we can reduce the original behavioural response to the action of single series of neurones. The challenge is to simulate the effects of smell (which can always switch on salivation), bell (which alone does not switch on salivation) and association of the two (learning cycle) such that the bell alone can now trigger the response. Features of this learning function include the number of associations, their duration, the cycle or frequency of repeats and the final loss of associated reflex following a long period of no association.

In a biological system the neuron produces an output along its axon (firing pattern) if the collective effect of its inputs reaches a threshold. The axon from one neuron can influence the dendrites of another neuron via junctions (synapses). Some synapses may generate a positive (stimulatory) effect in the dendrite encouraging its neuron to fire, whilst others produce a negative (inhibitory) effect reducing the propensity to fire. A single neuron may receive inputs from 1000's of synapses and the total number of synapses in the human brain has been estimated to be of the order of 10^{15} [6]. Learning and memory reside within the circuits formed by the interconnections between the neurons (i.e. at the synapses) [6].

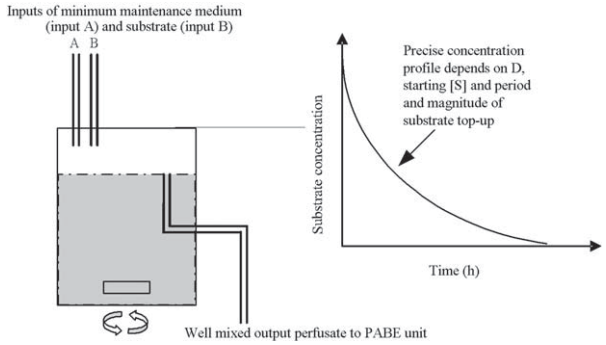

FIGURE 2
A chemical reservoir of substrate may be used to hold memory.

2.1 Memory based on substrate storage in a dynamic dilution reservoir

The fluid input into a PABE unit is fed from a reservoir of perfusate containing minimal maintenance medium with or without substrate, depending on the reservoir inputs (see Figure 2). The reservoir is a small continuous flow, stirred tank reactor to effect changes in the profile of substrate concentration with time. The dilution rate (D) is given by:

$$D = \frac{f}{V}\left[h^{-1}\right] \tag{4}$$

where: f = totality of liquid substrates flow rate (ml/h)
V = volume of the liquid in the vessel (typically measured in ml.).

The concentration profile measured at the output depends on the substrate concentration (e.g. acetate), which varies depending on the ratio of substrate stock or buffer diluent that are mixed together upon entering the reservoir. A simple case would be a quick pulse of strong substrate. The concentration profile thereafter can be both empirically measured and theoretically modelled as an exponential decay [7]. The rate or frequency of pulse additions into the memory vessel can now be calculated that would be needed to maintain the output concentration above or below threshold limits to maintain or activate PABES.

Increasing D of the maintenance substrate flow rate (without further addition of substrate) will decrease the residence time of the substrate molecules and 'memory' is more rapidly lost, requiring frequent top-up of substrate to keep the 'memory' in store. Decreasing D of the maintenance substrate flow rate will decrease the rate of substrate wash-out and lengthen the period of memory. Other dynamic mixing systems could also be applied for practical purposes to control the profile of substrate.

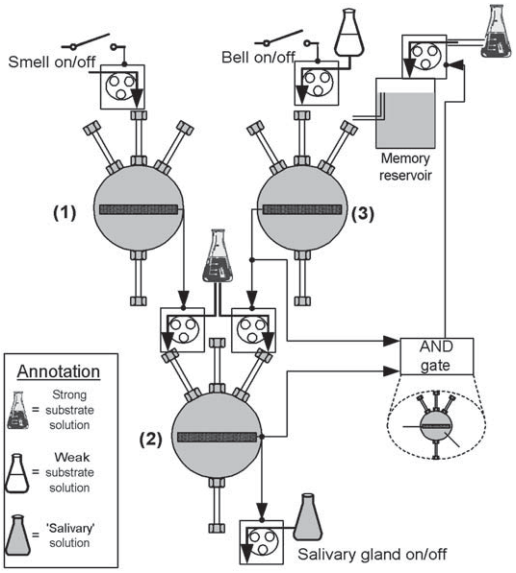

FIGURE 3
Implementation using memory, 3 PABE units, plus one unit for the AND gate.

3 POSSIBLE IMPLEMENTATION AS A SOLUTION TO THE PROBLEM

To implement the learning behaviour, the input we call 'smell' is represented by a switch. The switch may operate an actuator that brings substrate (akin to smell molecules) into a PABE unit (Unit-1), which senses the molecules and is switched on (see Figure 3).

When PABE Unit-1 is ON it then activates Unit-2. This link could be a direct electrical link to Unit-2, whereby the output from Unit-1 changes the electro-potential of Unit-2 from suboptimum to optimum. Alternatively, the output of Unit-1 switches a relay or actuator, which then switches ON a substrate stream into Unit-2. As a result, Unit-2 becomes active and switches ON the final pump labeled 'salivary gland'. Thus, whenever 'smell' is ON, it always switches ON the 'salivary gland'.

This is not the case for Unit-3, which is labeled as 'bell'. When the 'bell' switch is ON, it adds weak substrate into the perfusate to partially activate Unit-3. However, this weak activity gives an output that is insufficient to switch ON the substrate supply to Unit-2, and hence this unit is OFF (effectively the 'salivary gland' pump is OFF). Although the output from Unit-3 is insufficient to activate Unit-2, it is nevertheless sufficient to activate one of the two inputs into the AND gate. The implementation of an AND gate using a single PABE unit has already been described [1].

The other input to the AND gate is obtained from the electrical output of PABE Unit-2.

In associative learning, both ‘smell’ and ‘bell’ are switched ON at the same time. This means that Unit-2 (giving one input to the AND gate) and Unit-3 (giving the other input to the AND gate) are both ON simultaneously. The AND gate is now switched ON and its output is used to switch ON a strong substrate solution to dose the vessel called ‘memory reservoir’. Volume from this reservoir is displaced into Unit-3. If the period of association is sufficient, the concentration of ’memory’ substrate will build-up in the reservoir and the output concentration combined with the weak substrate entering Unit-3, are sufficient to produce a high output which will fully switch ON Unit-2.

On the next occasion, when the ‘bell’ alone is switched ON, the combination of output from the weak substrate pump and the ‘memory reservoir’ output are together sufficient to fully switch ON Unit-3. This is then capable of switching ON Unit-2 with a resultant effect of switching ON salivary secretion. The decay of ‘memory’ depends on (a) the dilution rate of the ‘memory reservoir’ vessel and (b) the frequency of addition of substrate to the ‘memory vessel’. The latter depends on co-activation of the AND gate by Units-2 and -3. If ‘bell’ is not used for a long period of time, the memory may decay to below threshold. This period may be ‘set’ by the operator by controlling ‘memory’ dilution rate (i.e. D). Another period of associative learning (smell and bell ON simultaneously) would then be required before ‘bell’ alone could switch ON the ‘salivary’ system. It should be noted that all PABE units, including the ‘memory reservoir’ throughout the experiment are constantly perfused with maintenance nutrient buffer and are all in continuous readiness (viable steady-state) to respond to changes in conditions.

4 DISCUSSION

The envisaged microbial processing units, are a symbiotic mix between the natural biological cells (the anodophiles) and artificial systems (electrodes, actuators, pumps and chemical solutions) in which the microbial cells individually mediate critical information-processing functions. In the simple example given, the processes are achieved by programming the units to have 2 different types of input (electrical and fluidic) and 3 electrical output states; OFF, ON and INT (intermediate-partially ON) e.g. Unit-3. In this way, whereas ON or OFF are hard-thresholds, which either activate or have no-effect on different parts of the system, the INT output can be used as either. In the Pavlovian learning example given above, the same INT output from Unit-3 denotes low/weak substrate conditions therefore having no effect on the next stage - Unit-2 - and at the same time activates a different

part of the system - the AND gate. The processing described is fairly trivial and possibly undervalues the number of states that could be realized and applied to achieve useful computing. Nevertheless this is perhaps a first step in the direction of developing such biological computing systems.

The described PABE model would still be considered to be operating in binary mode, even though the multitude of inputs and states of output may be suggesting otherwise. In other words, the operation is effectively 'learning' by association between a particular state and a conditional stimulus. The PABE units are on the other hand potentially capable of multiple inputs and, more importantly, multiple outputs of different forms. As described by Greenman *et al*. [1] and partially illustrated by Unit-3 in the Pavlovian learning example, the various (multiple) forms of input and output can also have multiple states, which increase the level of computational complexity that may be achieved. It may be set up to have only one state and associate with multiple stimuli or furthermore to have multiple states associating with multiple stimuli. The totality of input and output states in these units may be advantageously employed to possibly attempt to solve problems that cannot be solved by conventional computers.

A more useful expression of their processing power may lie in solving different types of problems particularly those involving multi-logic processing. Their information-processing virtuosity traces ultimately to the fact that they possess macromolecules, most notably proteins (e.g. substrate transport receptors or membrane transporters), that can recognize specific molecular objects in their environment (e.g. acetate, the main substrate) in a manner that uses shape and depends sensitively on physiochemical context [8]. The number of interacting physicochemical parameters that can be both controlled by the operator and would be expected to interact with the biofilms is huge. For the purposes of computing, the number of environmental (milieu) factors that could conceivably be used to encode the input signals is also virtually boundless. Thus, for each of the named parameters listed in Table 1, it is likely that 8 incremental states or separations of parameter magnitude can be recognized (resolved). For example, for a particular substrate (e.g. acetate), 8 different concentrations (all rate-limiting) would be expected to be resolvable into 8 different states, each being reproducible and repeatable over time. Since there are many such parameters (Table 1), the total number of discernable yet reproducible/repeatable states (i.e. specified states) could be as many as 8^{13}.

The biofilm cells are the primary processing component, acting on the chemical substrate to produce an electrical output that is directly proportional to their ultimate output, i.e. growth. Proteins (enzymes and receptors) may assume numerous molecular shapes. Within the cell (under physiological conditions) a subset of molecular shapes is favoured. Moreover, under different physiological states the whole cell expresses different genes and gives rise to a different proteomic landscape. This allows the whole cell

to fuse the information concerning the physical world into the single output (growth). It does this in a highly complex non-linear manner that would require a large number of conventional processors to simulate. Biological cells are replete with receptors that convert signals representing macrofeatures of the external environment into 'internal' signals that are susceptible to control by meso- and microlevel processing (by enzymes, pathways, protein expression etc.) [8]. Biomolecular architectures are sharply different to silicon designed systems since their complexity is inherent. For making PABES practical, the amount of computational work performed at the meso- or micro levels should be as much as possible since (a) this is thermodynamically favoured (in contrast to macroscopic signals from the electrode onwards, which are thermodynamically costly), and (b) the non-linear nature of complexity that is available at this level. The switch from one physiological state to another in response to even a single parameter change in the physiocochemical environment (real world) is highly complex and non-linear. It is here that powerful context-sensitive input-output computing transformations reside.

The output from a PABE is the totality of activity from a collection of about $10^7 - 10^8$ microbial cells. Although each cell is exposed to almost identical physicochemical conditions their growth cycle and division points are asynchronous. Although there is indeterminacy at the level of any one cell, this gives way to predictability at the level of the whole biofilm and a system with self-organising dynamics (the PABE has the potential to 'hold' thousands of different recognition-action, input-output transforms) may be advantageous to perform more complex operations than systems with programmable architectures. PABES can be networked by using wires or liquid flows and synapse-like inputs strengthened or inhibited by changing physicochemical environment by either electrical or fluid links. Changes can be induced in one part of the network by designing connections, and modulating these connections through a learning process.

TABLE 1
Different states of the PABE-physicochemical domain

Parameter	Possible parameter increments	Total states
C/E limiting substrate (e.g. acetate)	8	8^1
Types of C/E substrate	8	8^2
Other types of limiting nutrients (e.g. S, P, N, Mg^{2+}, K^+, Fe^{2+} etc.)	8	8^3
Binary/tertiary mixes of substrates	8	8^4
Osmolarity (NaCl)	8	8^5
Temperature	8	8^6
pH	8	8^7
Reversible competitive inhibitors	8 types, 8 concentrations	8^9
Flow rate of perfusate medium	8	8^{10}
Electrode overpotential	8	8^{11}
Number of PABE units	8×8	8^{13}

A systematic machine approach whereby PABES are integrated with conventional in silico devices could be explored and used to produce an interesting processing engine for solving certain types of computing tasks. Sculpting the desired functionality by coding of the input signals and gaining knowledge of the operational conditions that fuse the main inputs into the main output (growth) is the main challenge. Therefore, the ultimate goal of our work is to create a wide repertoire of high-complexity functions for implementing input-output transforms that cannot easily be done by current digital computers with programmable architectures. In our system a large portion of the complexity and initial processing is natural and endogenous to the living cell. There is less need for an outside programmer since programmable functionality can be molded through designed or natural adaptive procedures, maintainance functions, set parameters and feedback. Further desired functionality may also be achieved by using genetically engineered anodophiles. Engineered genetic regulatory functions of whole cells, for example, to emulate the functionality of semiconductor devices have already been described including the genetic toggle switch in *E. coli* [9] and the repressilator [10], a synthetic oscillatory network of transcriptional regulators. Over 140 'BioBricks' (DNA genetic sequences available from MIT registry of standard biological parts) [11] including sequences for AND, NOT, NAND and oscillators are now available to help genetic engineers programme microbes to better sense, interpret or report the state of the cell interacting with its environment. Thus, PABE units may represent an ideal computing substrate on which to base these types of genetic circuits.

Consider a change from null to any other condition and then a change back to the original null state. During this process, the cells perform 1000's of automatic back-calculations or adjustments (by default) in order to (fairly) rapidly return to the null- condition, so that the operator can set the next computational 'problem'. This is, at the moment, non-transparent to the human operator and accessing this micro/meso level of complexity, may succeed in achieving a high level of computing power.

In addition to the high level of computational capability, the transformation of substrate(s) into electrons allows the possibility for the electrical output of many units to be sufficiently strong to drive low-power actuators (or co-processors) giving the system energy autonomy, which may be ideally utilised for some applications, including small scale robots.

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