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Insight from Bioanalysis about the Behavior of Microbial Communities

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Abstract

In this article, a different method of investigating bacterial proliferation is suggested. It is based on the detection of enzyme activity as opposed to the traditional physical approaches. It is shown how to monitor microbial ureolytic activity in real time and online using flow analysis (applied to a model experimental biosystem). A solenoid micropumper and microvalve system controlled by an Arduino microcontroller make up the fully automated bioanalytical flow system that was designed. Dedicated flow-through optoelectronic detectors constructed of paired light emitting diodes are used to carry out photometric detection based on the Nessler reaction. With a detection limit below 0.44 U mL-1, a high sensitivity in the linear range of response (up to 200 mV U1 mL), and reasonably high throughput, the developed bioanalytical system enables discrete assaying of microbial urease in a wide range of activity up to 5.4 U mL-1 (9 detection per hour). The suggested differential measurement method allows for the removal of interfering effects from substrate and products of biocatalyzed reactions as well as other components of the medium used for bacterial expansion (i.e., a difference between peaks register for sample with and without external addition of urea is treated as an analytical signal). In order to control the growth of urease-positive bacteria strains (Proteus vulgaris, Klebsiella pneumoniae, and Paracoccus yeei), the developed bioanalytical system was successfully used. This included examining the effects of different microbial cultivation conditions, such as temperature, the makeup of the culture medium, and the quantity of substrate necessary to induce bacterial enzymatic activity. The created bioanalytical flow system can be used to detect the decline phase as well as estimate the parameters of the lag and log phases of microbial growth based on metabolic activity.

Keywords: Bacterial development; Urease production and monitoring flow analysis

Introduction

Investigations into microbial development are fundamentally significant in a variety of human endeavours, including clinical and medical diagnostics, healthcare, the food business and food safety, agriculture, and environmental management, among others. Research on the dynamics of bacterial proliferation, ideal culture conditions, stress, toxic and antibiotic effects, etc. can benefit from understanding the nature of bacterial growth through the assaying and monitoring of microbial biocatalytic activity. A recent assessment of real-time/online method advancements for tracking bacterial growth was published. Several electrochemical techniques based on impedance, capacitance, and conductivity measurements have been developed for such bioanalytical purposes. These methods provide monitoring of non-specific changes in the ionic [1-3] strength or pH of culture media brought on by a microbial population's metabolic products. Due to the high cost of bioreagents as well as the lengthy and arduous multistep bioanalytical procedures, methods based on gene analysis are rarely used for real-time monitoring development. In reality, it is much more common to employ traditional techniques based on optical density detection, such as turbidimetry, dynamic light scattering, or fluorescent probes. It should be noted that practically all of these methods are concentrated on determining the number, concentration, or biomass of bacterial populations rather than their viability as evidenced by metabolic (biocatalytic) activity. Since enzyme activity detection techniques are fundamentally kinetic in nature, several of them have been converted to flow analysis format. For kinetic-catalytic methods of analysis, where highly repeatable conditions of reagent transport (dosing, mixing/stirring) and exact control of reaction/incubation periods are important, flow approaches are particularly alluring. Numerous advantages of modern flow analysis techniques include: mechanisation, automation, and independent optimization of each step of multistep analytical procedures, high repeatability of microprocessor-controlled operations (sampling, mixing, incubation, etc.), ease of repetition of analytical procedures with changes in step parameters (like reagent concentration, time of reaction, etc.), increase in sample throughput, and elimination of an analyst's errors as we can perform the analysis ourselves. Particularly when flow analysis systems are downscaled to meso/microfluidic format, the consumption of reagents and samples is minimised. Pumps, valves, and detectors are examples of modular devices used in the design of flow systems. These devices are reasonably priced, especially when compared to the price of sophisticated analytical apparatus. Still unmatched in terms of simplicity and adaptability are flow approaches. Modern flow analysis principles, applications, and advancements have recently been evaluated. Furthermore, flow analysis systems can be created naturally as real-time/online process monitors that operate in both the discrete and continuous modes of measurements.

Materials and Method

Such flow monitors are helpful in a variety of industries, including the food sector, biomedical systems for hemodialysis control, pharmaceutical and environmental investigations, etc. It is suggested in this work that the two aforementioned skills (process monitoring and enzyme assaying) be combined for the sake of microbiological research. The ability to monitor variations in [4-7] biocatalytic activity online in real-time offers up new possibilities for using the flow analysis concept to regulate diverse biotechnological and biological processes.

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This paper presents the bioanalytical technique for tracking ureolytic bacteria activity. To the best of the authors' knowledge, this is the first instance of bacterial growth monitoring in a flow analysis format as well as study on microbial growth by monitoring its metabolic activities.

Parts testing

a. Materials and reagents

Sigma Aldrich provided urease (EC 3.5.1.5) from Canavalia ensiformis (Type IX, powder, 75, 265 units/G, Product No. U4002) (Germany). Avantor Performance provided urea, elements of media for bacteria cultivation, and other analytical-grade reagents (Poland). In the flow system, 0.10 M phosphate buffer with a pH of 7.0 was used as a carrier. In this investigation, the following bacterial strains were used: Proteus vulgaris ZGB1, Klebsiella pneumoniae 2102 and Paracoccus yeei CCUG 32054. The Institute of Microbiology at the University of Warsaw has a collection of all the strains of bacteria (Poland). Stock cultures that were raised in brain heart infusion (BHI) broth (Oxoid, UK) are kept in 15% glycerol at a temperature of 80 °C. On BHI agar (BHI enriched 1.5% agar), strains were cultivated. Each strain was chosen as a single colony, inoculated in a medium such as Christensen's or Luria-Bertani media supplemented with 0.5% urea, and then incubated at 30 °C for 18 hours while being shaken (120 rpm). Depending on the experiment, these overnight bacterial cultures were diluted 1:50 into fresh Christensen's medium or Luria-Bertani medium.

Instrumentation

The paired LEDs (5 mm diameter, transparent lens, flat format) that were purchased from Optosupply were used to build the photometric ammonia detector (Hong Kong). Both a light emitting 395 nm LED and a light-detecting 405 nm LED have been employed. These LEDs were attached to a mechanically drilled PEEK block to create a flow-cell with an internal volume of 70 L and an optical path [6-10] length of 1.0 cm. Similar optoelectronic detectors for flow analysis systems may now be produced using rapid prototyping technology, it was recently demonstrated. The Arduino platform was used to control the 4.0 mA current that powered the detector. Using a laboratory multimeter (Keysight, model U1231A, USA) working in voltmeter mode and connected with data storage software (supplied by the manufacturer) on a PC through IR-Bluetooth®RS232 interface, the analytical signal (electromotive force) produced by the detector was measured and recorded. Such an ammonia optoelectronic detector's analytical performance and operating theory under flow analysis settings have both been extensively discussed elsewhere.

Results and Discussion

The monitoring of activity in many biological species and systems could be done using a variety of enzyme activity assays. Enzyme assays are typically based on the measurement of the amount of product produced during a biocatalyzed reaction after a predetermined reaction period. Typically, synthetic nitrophenyl or fluorescein derivatives of natural substrates are created as artificial chromogenic or fluorogenic enzyme substrates for such experiments. Due to the absence of synthetic enzyme substrates and the byproducts of their biocatalytic degradation in natural samples, this approach is rather straightforward from an analytical standpoint. However, for at least two reasons, it is much more difficult to detect ureolytic activity, which was used in this study as a model for microbial metabolic activity. First off, there are no synthetic chromogenic or fluorogenic substrates for urease, an almost exclusively natural enzyme. Because of this, urease activity tests must be based on the detection of urea hydrolysis byproducts. The presence of urea and the byproducts of its biocatalyzed hydrolysis in the culture media is the second issue. Additionally, due to the ureolytic activity of developing bacteria, their levels in the monitored medium alter during cultivation. As a result, it is required to use a different measurement methodology when observing the growth of urease-positive bacteria. Such a method is also helpful for the elimination of effects resulting from the inherent colour of the culture medium during spectrophotometric detection. Fortunately, repeating measurements under various conditions is not a problem for tests carried out in the flow analysis format. The created monitor is based on a modified multicommutation flow analysis (MCFA) system produced specifically for the purpose of detecting urease activity , which consists of micropumps and microvalves connected to an optoelectronic detector made of paired LEDs. The article includes a diagram of the monitor's layout. The shown MCFA system enables the incubation of sampled microbial culture segments with or without the inclusion of an external enzyme substrate (urea). Two analytical signals that are required for measurements in the differential mode are acquired in this manner. P2 micropump delivers segments of 0.020 M urea or carrier buffer without urea to the enzyme reaction coil (ERC), depending on the position of the V1 microvalve. P1 micropump delivers segments of microbial culture medium or carrier buffer (0.10 M phosphate buffer pH = 7.0) to the ERC (depending on the position of V2 microvalve). After stopping in the ERC for a 5-minute incubation period (with or without exogenous urea), the produced bacteria segment is pumped to the flow-through detector cell along with Nessler reagent using pump P1. The detector is then rinsed with a section of 0.1 M hydrochloric acid after the position of the V3 microvalve is modified. Technically speaking, buffer used for ERC washing is removed using a V4 microvalve. The supplementary material accompanying this study displays a complete graphical portrayal of the programme managing the functioning of the MCFA system. Examples of solenoid-based flow analysis system operation-controlling programmes, including genuine executive programmes developed in Arduino C++, are provided elsewhere. Proteus vulgaris in its stationary phase of growth was employed as the culture for initial investigations using actual microbiological samples. It was discovered that the upper determination limit of the flow analysis equipment had been surpassed because of the high enzyme activity and significant ammonia signals present in the investigated culture medium. As a result, portions of the culture media from the sampled cultures were used in all subsequent studies at a fivefold dilution.

The monitor's use in microbiological research

The investigation of the impact of nutrient presence on the microbial growth could serve as an example of the working theory and practical application of the produced monitor. In contrast to the 0.5% beginning concentration typically used for the development of urease positive bacteria, urea at a 2.0% initial concentration was used to cultivate the Proteus vulgaris strain in Christensen's medium.

Conclusions

This work describes a completely automated flow analysis method for tracking the development of urease-positive microbial species. This cutting-edge, extremely cost-effective bioanalytical system is built with low-cost solenoid micropumps and microvalves that are controlled by an Arduino microcontroller. It is also equipped with a remarkably affordable optoelectronic flow-through detector made of paired light emitting diodes.

Conflict of Interest Statement

The authors reaffirm that they are not aware of any personal or financial conflicts that might have appeared to affect the research described in this paper.

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