**Development of an external pH monitoring system for a 10 L bioreactor**

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**Highlights**

* External pH monitoring system avoids electrode insertion in the bioreactor cover.
* A nylon cilyndrical device and a peristaltic pump are needed in addition to pHmeter.
* Broth pH can be continuously and accuratelly monitored in simple biorreactors.

**1. Introduction**

A new bioreactor was designed specifically for culturing shear stress sensitive cells and avoid problems caused by adherent filamentous fungi growth (Domingos et al., 2017). This bioreactor can also be used for other bioprocesses using yeasts and bacteria. It was named *Low Shear Aerated and Agitated Bioreactor* (LSAAB). Once the bioreactor was developed without internal prominent parts to avoid micelial adherence, no electrode or sensor holes were provided in its cover. However, in many cases pH must be monitored and controlled in order to ensure cell growth and product formation during a bioprocess. Domingos et al. (2017) verified that pH increases and it must be controlled during the basidiomycete *Ceriporiopsis subvermispora* culturing in LSAAB for optimizing biomass production. During 2,3‑butanediol production by *Klebsiella oxytoca* the pH decreases due to organic acids synthesis and excretion (Tsvetanova, Petrova, Petrov, 2014) and it must be controlled for better enzyme activity. Therefore, in the present work it was proposed a strategy of external broth circulation through a device containing the pH electrode inserted, providing broth pH monitoring in LSAAB. The system was tested with water and during the yeast *Saccharomyces cereviseae* growth.

**2. Methods**

*Electrode holder*: The device was first drawn, in order to enable its machining. Nylon was used, an appropriate material for both machining and further sterilizations. A cylindrical configuration was addopted, with a diameter 8 mm larger than that of the pH electrode, for a free broth flow. This device was attached to the bioreactor and the peristaltic pump using silicone hoses.

*pH mesuring and liquid circulation*: pH was mesured with a Sppencer Scientific SP3611 model pHmeter, with an armored electrode inserted in the holder. Liquids were pumped with a VELP Scientifica SP311 model peristaltic pump.

*Tests with water*: The system was first tested with water in order to verify pH reading stability as a function of pump flow rate (4.5, 19.0, 39.0, 58.0, 78.0, 92.0 e 112.0 mL/min). Samples were collected and had their pH measured separatelly with the same pHmeter. Following, it was investigated the time for pH change detection by the electrode after acid (5 mol/L HCl) addition, for each pump flow rate above and under agitation speeds of 140 and 200 rpm. For 200 rpm it was also performed tests with aeration (0.8 vvm). Acid was added at the liquid surface in the bioreactor.

*pH monitoring during fermentation*: *Saccharomyces cereviseae* was used to carry out the culturing in LSAAB for the external pH monitoring system avaliation. Culturing medium (10 L) consisted of (in g/L): glucose (20.0), (NH4)2SO4 (5.0), KH2PO4 (3.0), MgSO4 (1.5) and yeast extract (6.0). Initial pH was adjusted to 4.5. Temperature and aeration were 25 oC and 0.8 vvm, respectivelly. Substrate and cell concentrations were measured by DNS and optical density methods, respectivelly.

**3. Results and discussion**

The cylindrical device was attached to the bioreactor and showed to be proper for liquids (water and culturing medium) circulation using flow rates from 4.5 to 112.0 mL/min. Using water, on line pH readings remained constant after 1 minute from the adjusting for flow rates from 19.0 to 112.0 mL/min, indicating an adequate fluid dynamics accross the system. Collected samples and on line pH readings showed a difference from 0.01 to 0.04 pH units, considering all flow rates tested. Considering the simmulation of microrganism actuation, pH readings remained constant after 2.0 minutes from acid addition, for flow rates from 58.0 to 112 mL/min, at 140 rpm agitation. At 200 rpm the time was 1.0 min for flow rates 92.0 and 112.0 mL/min, showing the effect of agitation on the whole broth pH change. Aeration did not affect the time for obtaning on line constant pH readings. This facts indicate that a flow rate about 90 mL/min and an agitation of 200 rpm are sufficient for monitoring and controlling broth pH in LSAAB using the proposed system. It can be considered that a delay of 1.0 minute from acid or alkali addition to pH correction in the whole broth do not compromise the bioprocess.

However, regarding the yeast cultivation, the differences between samples pH and on line pH readings varied from 0.1 to 0.15 pH units. Although higher than those obtained for water, such values represent a maximum of 3.75% of the desired pH value. It should be mentioned that broth samples were cooled and had their pH mesured from 3 to 15 hours after collected. Due to the intense metabolism of the yeast some changes in the broth could have occurred, even during cooling. Therefore, it can be considered that the proposed external pH monitoring system does not interfere in the actual broth pH inside the LSAAB. Further investigations include carrying out fermentations requiring pH adjustment.

**4. Conclusions**

The external pH monitoring system proposed for LSAAB is adequate for obtaining the actual broth pH value during fermentation processes and its control in simple bench bioreactors, without insertion of an electrode in the bioreactor cover. Best results are obtained with an agitation of 200 rpm and a minimum of 90 mL/min of broth circulation flow rate, considering the geometric characteristics of LSAAB, like liquid height, vessel internal diameter and “L” shaped stirrer.

**References**

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2. F. Tsvetanova, P. Petrova, K. Petrov, Biotechological Prod. and Proc. Eng. 98 (2014) 2441-2451.