**Maximization of poly(3-hydroxybutyrate) production in fed-batch cultures of *A. vinelandii* based on the variation of the agitation rate**

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**Highlights**

* Specific growth rateis improved by high kLa
* PHB productivity was affected by the volumetric oxygen transfer coefficient
* Variation of kLa allowed to define maximal PHB productivity
1. **Introduction**

The poly-hydroxybutyrate (PHB) is a polyethylene-like bioplastic naturally synthetized by several classes of microorganisms, as a source of energy and carbon unit [1]. This material, which is a promising candidate for the replacement of fossil-based plastic, is already present at an industrial scale. However, the maximization of its production process is the goal to achieve in order to reduce production costs. *Azotobacter vinelandii* is strictly aerobic gram-negative soil bacteria, able to produce PHB in a carbon substrate-rich environment and under oxygen-limitation conditions [2]. The production of this polymer is particularly interesting in this species, because it can accumulate up to 85% of its dried biomass as PHB [3]. It is known that agitation rate affects the PHB production by *A*. *vinelandii* [2]. In this work, the PHB productivity under fed-batch cultivation was evaluated to different volumetric oxygen transfer coefficient (kLa). Thus, it was possible to determine an adequate kLa range for scale-up the production of PHB.

**2. Methods**

Batch cultures of *Azotobacter vinelandii* OP were performed in a 3 L bioreactor (30 g L-1 sucrose as carbon source) at 600 rpm during 30 h. After of this time, the cultures were fed by a single medium feeding pulse of the carbon source and the agitation speed was varied between 400 and 1000 rpm. The biomass evolution and the PHB production were evaluated. The kLa was estimated at different agitation rate using dynamic method.

1. **Results and discussion**

The maximal accumulation of biomass was 13.3 g L-1, obtained at an agitation speed of 800 rpm (Table 1). A highest agitation speed increased the specific growth rate, reaching a value of 0.069 h-1 to 1000 rpm. During fed-batch cultivation, a change in the agitation speed affected the PHB accumulation, obtaining the highest value (79.1 % w w-1) to 600 rpm.

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| --- | --- | --- | --- | --- |
| **Agitation speed (rpm)** | **kLa (h-1)** | **µ (h-1)** | **Xmax (g L-1)** | **PHB (% w w-1)** |
| 400 | 30.3 | 0.011 ± 0.001 | 9.45 ± 0.14 | 66.0 ± 2.6 |
| 600 | 65.6 | 0.021 ± 0.001 | 11.50 ± 0.24 | 79.1 ± 1.5 |
| 800 | 103.6 | 0.062 ± 0.002 | 13.25 ± 0.49 | 54.7 ± 3.5 |
| 1000 | 188.0 | 0.069 ± 0.001 | 11.80 ± 0.05 | 46.3 ± 3.0 |

Table 1: Parameters obtained in the fed-batch cultures of *A*. *vinelandii*

Figure 1 shows the influence of kLa on the PHB volumetric productivity. A maximal PHB productivity of 0.28 g L-1 h-1 was obtained at a kLa of 104 h-1. Overcoming the kLa of 104 h-1 the PHB productivity decreased until 0.18 g L-1 h-1. Under the conditions evaluated, the PHB productivity obtained to 100 h-1 was similar to previous studies [2,3].



**Figure 1.** Relationship between the PHB volumetric productivity and kLa values in *Azotobacter vinelandii* OP. The kLa values evaluated correspond to the agitation speed of 400, 600, 800 and 1000 rpm.

**4. Conclusions**

In fed-batch cultures of *Azotobacter vinelandii*, the PHB productivity was affected by the volumetric oxygen transfer coefficient. The highest PHB productivity was obtained in the cultures performed to 104 h-1 (800 rpm). In order to develop a bioprocess to produce PHB, it was possible to identify the optimal oxygen transfer conditions for further bioprocess scale-up.

**References**

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