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Volumetric Oxygen Mass Transfer Coefficient Determination and Hydrodynamic Optimization of Polyhydroxyalkanoate Production with Vegetal Oil as Carbon Source

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The effects of aeration rate and agitation speed on the production of Polyhydroxyalkanoates (PHA's) by *Burkholderia cepacia* B27 in a batch fermenter were investigated. Central composite design (CCD) technique was used to develop an empirical model to find the best hydrodynamic conditions to demonstrate the high influence of rheological culture over biomass production and PHA accumulation. The agitation speed and aeration rates were between 120 to 515 rpm and 4.3 to 22 $L\ min^{-1}$, respectively. This values range was selected due to the aeration and agitation restriction of bioreactor. A dynamic method was applied to measure the volumetric oxygen mass transfer, which has a critical influence on biomass development on the overall process. Results revealed the agitation was the most important factor to PHAs accumulation. Furthermore, other parameters including bacteria growth was affected by the mass transfer, dissolved oxygen and broth viscosity, therefore agitation speed and aeration rate had a significant effect over strain and its production efficiency. Maximum PHA's concentration reached was 8.72 $g\ L^{-1}$ at 24 h of fermentation time with aeration rate and agitation speed at 13.5 $L\ min^{-1}$ and 310 rpm, respectively.

1. Introduction

The problems associated with plastic accumulation in the environment and rapid-depletion of natural resources used in their production are motivating factors for research into sources and tools for alternatives to petroleum-based polymers (Amache et al. 2013, Mendez et al. 2014). There are different biopolymer alternatives to replace petroleum based polymers among them the PHA's, a family of biodegradable and biocompatible polyesters, synthesized and stored in intracellular granules by bacteria cells that use the biopolymer as a carbon and energy source under aerobically and usually under stress conditions. PHA's have been produced in different scales via fermentation processes as environmentally friendly and sustainable plastics with high potential application in packaging, biomedical and agricultural industry (Bugnicourt et al. 2014, Obruca et al. 2014).

However wider use of PHA's is limited mainly by their high production cost compared with petrochemical-derived plastics. With the aim of commercializing PHA's, a substantial effort has been devoted to reducing production costs through the development of improved bacterial strains and a more efficient fermentation process (Yang et al. 2006). Mathematical modelling is an useful tool for optimization process reported by numerous authors (Mendez et al. 2016, Novak 2015), likewise Response Surface Methodology (RSM) is a collection of mathematical and statistical techniques that is applied for the modeling and analysis of various processes in which the response of interest is influenced by several factors and the response is optimized (Dayana Priyadharshini et al.2016, Lakshmikandan et al. 2014, Jabeen et al. 2015).

Central Composite Design (CCD) used in RSM, is more advantageous over the classical approach in terms of the information gained and the accuracy of the experiment conducted (Ahmadi et al. 2006). Obtaining informative results depends on finding the effects between key parameters of the particular process and the combined effects of these controllable factors. The supply of oxygen as oxygen transfer rate (OTR) is chosen as the controlling footstep in many industrial bioprocess systems and also in its scale-up (Zafar et al. 2012a).

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It is an important parameter for the design and operation of aeration and agitation systems of bioreactors. The aeration supplies, the oxygen demand of microbial population, and its efficiency depends on oxygen solubilization and diffusion rate into the medium broth along with the bioreactor capacity. The study of agitation and aeration rates on growth and PHA's production is an important activity since oxygen is considered a limiting factor for PHA's accumulation inside many bacterial cells (Alejandra de Almeida et al. 2010).

Oxygen transfer as a function of agitation and aeration to bioreactor vessel plays an important role, in order to obtain the appropriate volumetric oxygen transfer coefficient ($K_L a$) that can be correlated with the PHA's productivity in defined culture medium. The rationale of $K_L a$ values indicate a certain mass-transfer capability that can cope with the oxygen demand of the culture. It often serves as the scale-up criteria to compare the efficiency of bioreactors and mixing devices (Yaoyu Feng et al. 2002, Bandaiphet et al. 2006, Prasertsan 2006).

The objective of this study is determinate the $K_L a$ for fermentation process through dynamic method, likewise, investigate the optimum combination of aeration and agitation through statistical optimization using central composite design and surface response that would yield the highest biomass and PHA's production by *Burkholderia cepacia* B27.

2. Materials and methods

2.1. Microorganism and Fermentation Process

Burkholderia cepacia B27 was obtained from genetically modified process of random mini-Tn5 transposon mutagenesis (Florez et al 2014), to increase the accumulation of intracellular PHA's, using vegetable oil as carbon source .The strain was maintained in cryovials with L.B., and 40% of glycerol; culture conditions and fermentation process, were developed according to previous research (Méndez et al. 2016) with a bioreactor BioFlo®/CelliGen® 115 with capacity of 7 L (5 L of work volume), and control system of aeration and agitation. The reactor dimensions has: 7.7 cm diameter Rushton impeller, 17.3 cm of internal diameter, 4 baffles with 1.6 cm width and operating conditions given by 22.6 cm of height of medium. The concentration of vegetal oil (20 $g\ L^{-1}$) and ammonium sulfate (2.8 $g\ L^{-1}$), was the same for each experiment.

2.2. Experimental design

Central composite design (CCD) and response surface methodology (RSM) were performed to study the significant effects and interactions between aeration (X_1) and agitation (X_2), selected as independent variables (Santos Danyelle et al. 2014) for oxygen transfer coefficient, ammonium consumption, PHA's and biomass production considered as responses (Y). The mathematical relationship of the responses (Y) to independent variables X_1 and X_2 were analyzed using the Eq(1) quadratic polynomial equation (Dayana Priyadharshini et al. 2016) :

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 \quad (1)$$

Where β_0 is a constant term; β_1 *and* β_2 are linear regression coefficients; β_{11} *and* β_{22} are quadratic regression coefficients and β_{12} is the interactive regression coefficient. The range of independent variables under study given in Table 1, were determined according to the stirring capacity of the engine (between 100 to 515 rpm) and the air flow depending on compression capacity (between 4 to 23 $L\ min^{-1}$). Experimental data were analyzed using statistical software STATGRAPHICS Centurion XVI.

2.3. Measurement of oxygen uptake rate (OUR) and volumetric mass transfer coefficient ($K_L a$)

The measurement of dissolved oxygen was made with a gas phase oxygen sensor InPro 6950i G (Mettler Toledo, Urdorf, Switzerland). The first step according to Doran, (2013) was the determination of critical concentration of oxygen in culture media which is related with strain respiration starts to be limited. Subsequently as suggested by Nadja Ranchenkova et. al, (2014) to estimate the volumetric oxygen transfer coefficient, the dynamic method was applied. It consists of aeration until the dissolved oxygen concentration is stabilized; then suspending the oxygen supply generating a decrease in concentration due to the respiration rate of the microorganisms present in the reactor.

Table 1: Central composite design to evaluate the oxygen consumption of the B. B27 strain to PHA's production

Experiment	Agitation (rpm)	Airflow $(L\ min^{-1})$
1	310	13.5
2	120	5.0
3	500	5.0
4	310	22.6
5	310	4.3
6	105.1	13.5
7	514.8	13.5
8	120	22.0
9	500	22.0
10	310	13.5

The next step, is evaluate the increase of dissolved oxygen concentration in the medium with respect to the time, represented by Eq(2).

$$C = -\frac{1}{K_L a} * \left(\frac{dC}{dt} + x * O_2\right) + C^* \tag{2}$$

where C is the dissolved oxygen concentration, $x * O_2$ is the respiration rate, $K_L a$ is the oxygen transfer coefficient, $\frac{dC}{dt}$ shows the concentrations changes versus time. Determine the constant $K_L a$ for the reactor and proposed conditions, is possible plotting C vs$\left(\frac{dC}{dt} + x * O_2\right)$, where the negative inverse of the slope is the volumetric oxygen transfer coefficient.

2.4. Measurement of biomass and polymer concentration

Biomass and polymer concentration was quantified by centrifugation according to the methodology proposed by Méndez Daniel et al., (2016). Sample was taken from culture media and centrifuged with successive washes, then dried and weighed, to know the biomass concentration. For polymer concentration, a digestion procedure was made with sodium dodecyl sulphate (SDS) as reagent with thermal process, allowing the separation of the polymer from biomass. The samples are immersed in a thermostated bath to 70°C for one hour, then is used conventional gravimetric method.

Table 2: The CCD matrix showing actual experimental values along with the predicted values of bioprocess under different condition of agitation and aeration.

Exps.	$K_L a$ Exp (s^{-1})	$K_L a$ rsm* (s^{-1})	Biomass Exp$(g\ L^{-1})$	Biomass rsm* $(g\ L^{-1})$	Polymer Exp $(g\ L^{-1})$	Polymer rsm* $(g\ L^{-1})$
1	0.012	0.011	12.00	11.89	8.72	8.20
2	0.030	0.030	6.94	7.42	1.99	3.97
3	0.014	0.022	7.33	5.83	3.59	1.60
4	0.022	0.023	9.24	9.47	2.00	2.11
5	0.021	0.019	4.83	5.88	2.05	1.69
6	0.034	0.032	11.38	10.61	9.59	8.42
7	0.007	0.008	11.43	12.77	6.11	7.58
8	0.040	0.039	5.30	6.21	2.40	4.07
9	0.013	0.013	13.00	12.37	4.00	2.16
10	0.012	0.011	12.34	11.89	7.60	8.22

*rsm= response surface methodology

3. Results and discussion

From previous research carried out by Méndez Daniel et al., (2016), it was considered that the strain *B.* B27 is in exponential phase at the 24th hour of fermentation, a factor of great importance because at this stage, *B.*

B27 has its higher oxygen consumption and therefore was the appropriate phase to estimate the oxygen transfer coefficient. Table 2, shows the response of the principal variables depending on experimental design proposed.
In terms of the experimental design used, a stepwise regression analysis to estimate the two factors evaluated (agitation and aeration rates) on K_La was made, resulting in a polynomial equation of second order. The main interaction and quadratic effects of variables for K_La determination is represented in Equation 3:

$$K_La = 5.31450e^{-2} - 1.34304e^{-4} * X_1 - 1.71794e^{-3} * X_2 + 1.58670e^{-7} * {X_1}^2 - 1.73684e^{-6} * X_1 * X_2 + 9.08905e^{-5} * {X_2}^2 \quad (3)$$

where K_La is Oxygen Transfer Coefficient (s^{-1}), X_1 and X_2 are the factors agitation speed (RPM) and aeration rate ($L\ min^{-1}$), respectively. The equation 3 had a correlation coefficient (R^2) value of 98 %. It is possible estimate the K_La within the experiments limits with a high reliability (Figure 1). Pareto analysis suggested that agitation was the most important effect over K_La values, likewise aeration had the least effects within the mass transfer compared the interactions and quadratic effects showed in Equation 1.

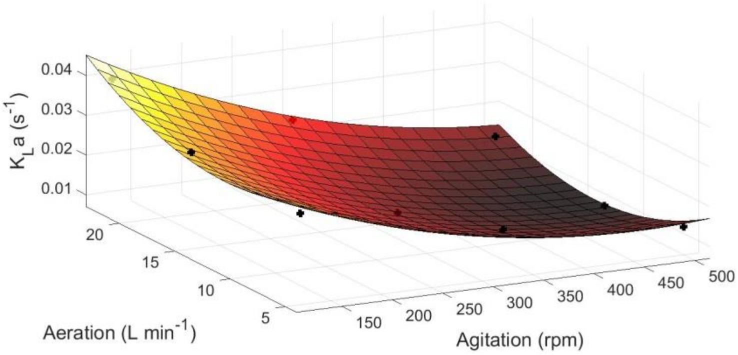

Figure 1: 3D response surface plot showing the effect of agitation and aeration factors in Oxygen Transfer Coefficient (K_La)

The regression analysis between dependent (K_La value) and independent variables (Agitation and aeration rates) reveals that the mass transfer is strongly affected by agitation speed, aeration rate, (p value < 0.05) and different agitation rates also had a strong affect in K_La values. Furthermore, the relations between this factors showed an inverse behavior over K_La. When reactor works with high agitations the aeration should be low, in the same way, high aeration and low agitation improve the overall productivity. The mass transfer is reduced at measured time for high biomass concentration reached, might be due to initial conditions with high values of agitation and aeration. According to Zafar et al. (2012b) in addition to agitation and aeration rates, many other factors such mixing, broth viscosity, product formation, and biomass may also influence the mass transfer during the bioprocess; this behavior could be explained for the increase of biomass and PHA's contain (Figure 1A). The Oxygen Transfer Coefficient is affected for high cell concentration the mass transfer of the Oxygen.
In order to study the effect of the factors evaluated (aeration and agitation) on biomass and PHA's production, experiments were fitting using Response Surface Methodology approach with a quadratic polynomials, represented in Equations 4 and 5 respectively.

$$Biomass = 2.36563 - 7.01701e^{-3} * X_1 + 1.24992 * X_2 - 2.40938e^{-6} * {X_1}^2 + 1.13220e^{-3} * X_1 * X_2 - 5.32433e^{-2} * {X_2}^2 \quad (4)$$

$$Polymer = -4.68506 - 3.33066e^{-3} * X_1 + 1.96114 * X_2 + 4.63146e^{-6} * {X_1}^2 - 7.28275e^{-2} * {X_2}^2 \quad (5)$$

Where $Biomass$ is the concentration of *B*. B27 $(g\ L^{-1})$, $Polymer$ is the concentration of PHA's $(g\ L^{-1})$, X_1 and X_2 are the factors agitation speed (RPM) and aeration rate ($L\ min^{-1}$), respectively. The equations 4 and 5 had a correlation coefficients (R^2) values of 90 % and 87.8 %, respectively. Pareto analysis suggest that the most important effect for variation on value of Polymer accumulation was agitation as mains effects. However, a mayor significant negative quadratic effect of aeration rates on mass transfer is reported between all of those effects showed in Equation 5.

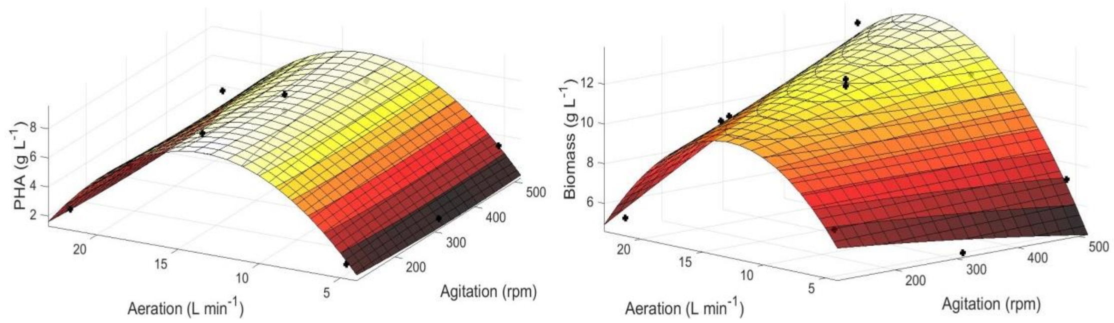

Figure 2: 3D response surface plots showing the effect of agitation and aeration factors in Biomass (A) and PHA's production (B)

According to the results, the experimental condition that maximizes PHA's production was 105.1 rpm and 13.5 $L\ min^{-1}$ (Experiment 6). This could be due the bacteria genetical modification to increase PHA's accumulation which could have a negative effect on cell wall strength, therefore a higher agitation could break it, and cause the microorganism decease. Additionally, *B.* B*27* was not limited or stressed by oxygen resulting in a higher productivity. Moreover, in the case of low aeration experiments: 2, 3 and 5 there was a low biopolymer accumulation, which is consistent with the research carried out by Chun Ping Xu et. al, (2005) due to a reasonable aeration rate ensures an increased dissolved oxygen level that is required for an optimal biopolymer production.

4. Conclusion

The aeration and agitation values that maximizes PHA's production by *Burholderia cepacia B27* was 105.1 rpm and 13.5 $L\ min^{-1}$ due to bacteria is not limited by oxygen and is not subjected to a strong mechanical stress.
The agitation was the most influential parameter, affecting negatively the oxygen mass transfer according to the results evaluated in the response surface method. On the other hand, aeration has a greater influence within the polymer production than agitation. Then, it is recommended when the agitation speed is high (around 500 rpm), the aeration rate should be in the range between 15 to 20 $L\ min^{-1}$. The results indicate that high values of oxygen mass transfer coefficient have an inverse behavior as regards to PHA's production and biomass growth. The results obtained in this work are an important tool to carry out the scale up of the bioprocess taking into account constant $K_L a$ criteria.

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References

Ahmadi, M., Vahabzadeh, F., Bonakdarpour, B. & Mehranian, M. 2006, "Empirical modeling of olive oil mill wastewater treatment using loofa-immobilized Phanerochaete chrysosporium", *Process Biochemistry,* vol. 41, no. 5, pp. 1148-1154.

Alejandra de Almeida, Andrea M. Giordano, Pablo I. Nikel & M. Julia Pettinari 2010, "Effects of Aeration on the Synthesis of Poly(3-Hydroxybutyrate) from Glycerol and Glucose in Recombinant Escherichia coli", *Applied and Environmental Microbiology,* vol. 76, no. 6, pp. 2036-2040.

Amache, R., Sukan, A., Safari, M., Roy, I. & Keshavarz, T. 2013, "Advances in PHAs production", *Chemical Engineering Transactions,* vol. 32, no. Francis 2011, pp. 931-936.

Bandaiphet, C. & Prasertsan, P. 2006, "Effect of aeration and agitation rates and scale-up on oxygen transfer coefficient, kLa in exopolysaccharide production from Enterobacter cloacae WD7", *Carbohydrate Polymers,* vol. 66, no. 2, pp. 216-228.

Bugnicourt, E., Cinelli, P., Lazzeri, A. & Alvarez, V. 2014, "Polyhydroxyalkanoate (PHA): Review of synthesis, characteristics, processing and potential applications in packaging", *Express Polymer Letters,* vol. 8, no. 11, pp. 791-808.

Chun Ping Xu, Jayanta Sinha, Jun Tae Bae, Sang Woo Kim & Jong Won Yun 2005, "Optimization of physical parameters for exo-biopolymer production in submerged mycelial cultures of two entomopathogenic fungi Paecilomyces japonica and Paecilomyces tenuipes", *Letters in Applied Microbiology,* vol. 42, pp. 501-506.

Danyelle K.F. Santos, Yana B. Brandao, Raquel D. Rufino, Juliana M. Luna, Alexandra A. Salgueiro, Valdemir A. Santos, Leonie A. Sarubbo, 2014, “Optimization of cultural conditions for biosurfactant production from Candida lipolytica”, *Biocatalysis and Agriculture Biotechnology*, vol. 3, pp. 48-57.

Dayana Priyadharshini, S. & Bakthavatsalam, A.K. 2016, "Optimization of phenol degradation by the microalga Chlorella pyrenoidosa using Plackett-Burman Design and Response Surface Methodology", *Bioresource technology,* vol. 207, pp. 150-156.

Doran, P.M. 2013, *Bioprocess engineering principles,* 2. ed. edn, Academic Press [u.a.], Amsterdam [u.a.].

Florez D. V., Suarez Z. R., Merchan N. A., Gutierrez L. C., Olmos L., Moreno N. C., 2014, “Mutagenesis studies for enhancement of PHA production by Burkholderia cepacia 2G57, isolated from colombian soils”, *International Symposium on Biopolymers Santos-Brazil*, 92.

Gumel A.M., Annuar M.S.M., Heidelberg T., 2012, “Biosynthesis and Characterization of Polyhydroxyalkanoates Copolymers Produced by P. putida Bet001 Isolated from Palm Oil Mill Effluent”, PLoS ONE, 7

Jabeen, H., Iqbal, S., Anwar, S. & Parales, R.E. 2015, "Optimization of profenofos degradation by a novel bacterial consortium PBAC using response surface methodology", *International Biodeterioration & Biodegradation,* vol. 100, pp. 89-97.

Lakshmikandan, M., Sivaraman, K., Elaiya Raja, S., Vasanthakumar, P., Rajesh, R.P., Sowparthani, K. & Jebasingh, S.E.J. 2014, "Biodegradation of acrylamide by acrylamidase from Stenotrophomonas acidaminiphila MSU12 and analysis of degradation products by MALDI-TOF and HPLC", *International Biodeterioration & Biodegradation,* vol. 94, pp. 214-221.

Mendez, D.A., Cabeza, I.O., Moreno, N.C. & Riascos, C.A.M. 2016, "Mathematical Modelling and Scale-up of Batch Fermentation with Burkholderia cepacia B27 Using Vegetal Oil as Carbon Source to Produce Polyhydroxyalkanoates", vol. 49, pp. 277-282.

Mendez, D.A., Quintero-Cerón, J.P., Váquiro, H.A., Solanilla, J. F., 2014, “Alginato de sodio en el desarrollo de películas comestibles”, Revista Venezolana de Ciencia y Tecnología de Alimentos, vol 5 no. 2, pp. 089-113

Nadja Radchenkova, Spasen Vassilev, Martin Martinov, Margarita Kuncheva, Ivan Panchev, Serafim Vlaev, Margarita Kambourova. 2014, “Optimization of the aeration and agitation speed of Aeribacillus palidus 418 exopolysaccharide production and the emulsifying properties of the product”, Process Biochemistry, vol. 49, pp. 576-582.

Novak, M. 2015, "Mathematical Modelling as a Tool for Optimized PHA Production", *Chemical and Biochemical Engineering Quarterly,* vol. 29, no. 2, pp. 183-220.

Obruca, S., Benesova, P., Petrik, S., Oborna, J., Prikryl, R. & Marova, I. 2014, "Production of polyhydroxyalkanoates using hydrolysate of spent coffee grounds", *Process Biochemistry,* vol. 49, no. 9, pp. 1409-1414.

Wang, Y., Yin, J. & Chen, G.Q. 2014, "Polyhydroxyalkanoates, challenges and opportunities", *Current Opinion in Biotechnology,* vol. 30, pp. 59-65.

Yang, J., Huang, J. & Ni, J. 2006, "Mathematical modeling of the batch fermentation of Zoogloea sp. GY3 used for synthesizing polyhydroxyalkanoates", *Journal of Chemical Technology & Biotechnology,* vol. 81, no. 5, pp. 789-793.

Yaoyu Feng, Zhimin He, Say Leong Ong, Jiangyong Hu, Zhigang Zhang & Wun Jern Ng 2002, *Optimization of agitation, aeration, and temperature conditions for maximum -mannanase production*.

Zafar, M., Kumar, S.S., Kumar, S.S., Dhiman, A.K., Kumar, S.S. & Dhiman, A.K. 2012, "Optimization of polyhydroxybutyrate (PHB) production by Azohydromonas lata MTCC 2311 by using genetic algorithm based on artificial neural network and response surface methodology", *Biocatalysis and Agricultural Biotechnology,* vol. 1, no. 1, pp. 70-79.