developing biochemical processes is advantageous in relation to industrial and forest wastes disposal and for the process economy. The objective of this work was to study the influence of agitation, aeration and germination time of spores of *Aspergillus niger* on the production of citric acid in bioreactor, from hemicellulosic hydrolysate of eucalyptus waste. Tests for setting time of inoculum, agitation and aeration were performed on bench fermentor (MULTIGEN New Brunswink Sc. Co.), with throughput of 0.6 L, equipped with temperature control, pH, agitation and aeration, and dissolved oxygen meter. The study was done according to a 2^3 full factorial design and results were statistically analyzed using the software Design-Expert 6.0.6. With respect to the production of citric acid, it was observed that it was favoured by increasing agitation, aeration and time of cultivation of the spores. The highest values of ($Y_{P/S}$) were obtained with the agitation of 400 rpm and aeration of 1.5 vvm, as well as the ability of cells to produce citric acid ($Y_{P/X}$). It can be observed a better cell growth in the condition of greater agitation speed. When agitation speed of 200 rpm was used no clear variations in morphology or increasing in biomass was observed. The lowest conversion factors were obtained in the lower level of agitation speed (200 rpm). This fact could be due to the micelial morphology of the fungus developed under such condition of agitation (filamentous mycelium with long and poor branched hyphae) and not due to the oxygen transfer limitation. The statistical analysis revealed a significant model to describe this bioprocess. Also, the significance to the terms agitation and aeration confirmed the existence of curvature of the model. According to the graph of surface response it can be seen the existence of a region of higher concentration of citric acid (14.3 g/L) when the largest aeration and agitation are employed, within the range of values studied. Concerning the time of inoculum, the statistical analysis indicates that it has no significant effect on the product formation.

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Qualitative estimation of polyhdroxybutyrate in *Alcaligenes* spp. NCIM 5085 by transmission electron microscopy and FTIR (Fourier Transform Infrared Microscopy)

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Poly-β-hydroxybuyrate (PHB) is a carbon-energy storage material which is accumulated intracellularly in variety of microorganism under nutrient starved conditions. Solid PHB is a biodegradable thermoplastic polymer and is utilizable in various ways similar to many conventional plastics. *Ralstonia eutropha* (*Alcaligenes eutrophus*), a Gram-negative bacteria accumulates PHB as insoluble granules inside the cells when nutrients other than carbon are limited. In this report effort has been made to analyze PHB granule synthesis inside *Alcaligenes* spp. NCIM 5085 by Transmission electron microscopy (TEM) and qualitative estimation of PHB has been carried out by FTIR (Fourier Transform Infrared Microscopy) which provide better precision compared to other conventional techniques previously applied for PHB determination such as GC, Gravimetric analysis, HPLC and cell carbon analysis.

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Evaluation of sorghum straw hemicellulosic hydrolysate for xylitol production

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Forage sorghum (*Sorghum bicolor* L. Moench) is a member of the sorghum family and is closely related to grain sorghum. Forage sorghum, best adapted to warm regions and particularly noted for its drought tolerance compared to corn warm-season, is cultivated mainly in the South and Central-West Regions of Brazil. Research on the use of lignocellulosic materials has shown the feasibility of using hemicellulosic hydrolysates in biotechnological processes, such as the biotechnological production of xylitol, ethanol and enzymes. The objective of this work was to evaluate the use of forage sorghum straw hemicellulosic hydrolysate for xylitol production by the yeast *Candida guilliermondii*. Sorghum straw was collected at a local farm in Cascavel, Paraná. Hemicellulose acid hydrolysis was performed in 350 L AISI 316 stainless steel reactor at 121 °C, 10 min, employing 100 mg H_2SO_4/g raw material (solid—liquid rate of 1/10, w/w). Sugars, xylitol and acetic acid concentrations were determined by HPLC with IR detector and phenolic compounds were estimated by UV—vis spectrometry. Batch fermentation was carried out in triplicate in 125 mL-Erlermeyer flasks containing 50 mL of the three-fold concentrated hydrolysate, previously autoclavated at 115 °C for 15 min and supplemented with rice bran extract (20 g/L), $(NH_4)_2SO_4$ (2 g/L) and $CaCla_2{\cdot}2H_2O$ (0.1 g/L). Initial cells concentration was 1 g/L. The flasks were left under agitation (200 rpm) at 30 °C for 72 h. The initial pH was 5.5 previously adjusted by the addition of NAOH solution. Sugars analysis of hemicellulosic hydrolysate showed a high xylose content regarding others sugars (D-xylose 18.84 g/L; D-glucose 2.97 g/L; D-arabinose 2.09 g/L). Acetic acid (3.41 g/L) concentration did not exceed the commonly found values for sugar cane bagasse hydrolysate obtained at the same conditions, evidencing the possibility of utilizing sorghum straw hemicellulosic hydrolysate in biotechnological processes that requires pentoses, such as xylitol or ethanol by xylose fermenting yeasts. The high concentration of xylose together with the low concentrations of inhibitors suggest that sorghum straw hemicellulosic hydrolysate can be feasible for xylitol production. The highest xylitol yield (0.44 g/g), corresponding to 48% of the theoretical based on xylose, and the highest productivity (0.19 g/L/h) were obtained after 72 h of fermentation. However, the lower yields on xylitol attained with *C. guilliermondii* in comparison with those previously reported with this yeast grown on other hemicellulosic

hydrolysates make necessary further studies to establish an adequate acid hydrolysis as well as an adequate detoxification process.

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Growth of *Bacillus thuringiensis* var. *israelensis* in glycerol derived from biodiesel industry aiming the production of bioinsecticide

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The search for renewable and ensuring sustainable development energy sources led to the implementation of biodiesel in the energy matrix of various countries as an alternative fuel. In general, for every 100 kg of biodiesel produced 10 kg of glycerol is obtained, which is impure and of low economic value. The microbial conversion of glycerol by biotechnological processes into higher added value products is a promising alternative to this waste disposal. The biological insecticide production from *Bacillus thuringiensis* var. *israelensis* in large scale is still an expensive process due to the high cost of the fermentation broth. The biological insecticides have several advantages over chemical insecticides, such as harmless to animals and humans and no environmental pollution. In this work, it was employed the glycerol from the biodiesel production as a fermentation medium component for *B. thuringiensis* var. *israelensis* growth. The work focused on an appropriate treatment for the glycerol, as well as the influence of some nutrients of the culture medium, in order to allow the characteristic development of the bacterium, as regards the production of toxins against larvae of *Aedes aegypti*. The assays were carried out in 1000 mL *Erlenmeyer* flasks containing 200 mL of medium and incubated (New Brunswick Sci. Co. Innova model 4000 shaker) at 30 °C under agitation speed of 180 rpm. The growth was monitored by microscopic observations. The larvicidal activity was determined by bioassays, using *Aedes aegypti* larvae of the 4th instar of development. It was found that the most appropriate procedure for treatment of the glycerol was: preliminary decanting, acidification with phosphoric acid to pH 7 followed by decanting and heating to 70 °C followed by a new settlement. This treatment allowed both the growth and production of toxins by the bacterium. With respect to nutrients, it was necessary to add yeast extract to the medium to allow growth of the bacterium. Moreover, ammonium sulfate and calcium chloride influenced the culture with regard to the occurrence of the different phases that characterize the growth of *B. thuringiensis* var. *israelensis*, in the endotoxins production process. From the tested media, only that one without yeast extract did not allow the growth and production of toxins by the bacterium. The best results of larvicidal activity was obtained when the medium contained no NH_3SO_4 and contained $CaCl_2$ at a concentration of 0.24 g/L, in addition of yeast extract (12 g/L).

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Influence of the oxygen transfer rate (OTR) on the alginate production and molecular mass of the polymer in cultures of *Azotobacter vinelandii* under oxygen-limited and non-oxygen-limited conditions

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Alginates form an important family of polysaccharides used for a wide variety of applications as thickener, stabilizer, gelling agent, and emulsifier in the food as well as textile and pharmaceutical industries. The alginate production by fermentation using bacteria as *Azotobacter vinelandii* could be a feasible strategy for the synthesis of this polymer. The previous studies have qualitatively revealed the importance of oxygen transfer rate (OTR) and dissolved oxygen tension (DOT) in the production of alginate; however, no systematic study has been reported regarding the independent influence of OTR and DOT on the molecular mass of the alginate produced by *A. vinelandii*. Therefore, we reported here the influence of OTR (manipulated through the agitation rate) and keeping the DOT constant by gas blending, on alginate production and its composition by a wild type strain (ATCC 9046). Cells were grown in a bioreactor with 2.0 L of Burk's media. The DOT, pH and agitation were kept constant throughout the cultivation. The conditions evaluated were 0.5 and 5% of DOT, 500 and 700 rpm. The results revealed that in the cultures conducted under non-limited oxygen conditions (5% of DOT) a change in the agitation rate (from 500 to 700 rpm) did not affect the profiles of the OTR and OTR_{max}, reaching in both cases a maximal value of OTR between 80 and 100 mmol/l/h. In contrast, in the cultures developed under oxygen limitation (0.5% of DOT) the profiles of OTR and OTR_{max} were clearly different when the agitation rate was changed. In the cultures carried out at 500 rpm the OTR_{max} was 20 mmol/l/h; whereas in the cultures conducted at 700 rpm the OTR_{max} was 80 mmol/l/h. Under oxygen limitation (0.5%) an increase in the OTR_{max}, improved alginate yield on biomass, achieving a maximal close to 0.8 g algin/g biomass when the OTR_{max} was 80 mmol/l/h. On the other hand, the molecular mass of the alginate decreased at higher OTR and the maximum (450 kDa) was obtained in the cultures in which the OTR_{max} was 20 mmol/l/h. Overall, our results show that under oxygen limitation, the alginate yield and the molecular mass of the polymer are determined by the OTR, independently of the DOT of the culture.

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