KINETICS OF *AZOSPIRILLUM BRASILENSE* UNDER DIFFERENT AIR FLOW RATES IN A BUBBLE COLUMN REACTOR AND ALTERNATIVE CARBON SOURCES.

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Introduction. *A. brasilense* is a rod shaped, free-living, nitrogen fixing and PHB accumulating rhizospheric microorganism, of the alfa-proteobacteria family; it shows a respiratory metabolism, although some strains possess the ability to grow on anaerobic conditions (1). Since 1973 *Azospirillum* is the most studied genus of the plant growth promoting rhizobacteria (2). Actually, its commercial use as biofertilizer began to spread in different countries including Mexico due to their ability to increase biomass yields of cereals such as maize, wheat, rice, sorghum, oats and forage grasses (3,4,5). There are few studies focused on optimizing growth and biomass production of *A. brasilense* as inoculant (6), some modifying the culture medium base (NFb) with malic acid as carbon source or nitrogen source. Until now the maximum concentration reached was at the order of 10^9 CFU/ml on agitated batch culture (7,8) and 10^{10} CFU/ml on fed-batch (6). The objective of the present work is to explore strategies that improve the biomass production (over 10^9 CFU/ml) of *A. brasilense*, modifying the carbon source and knowing the effect of different air flow rates.

Methods. Strains: Mexican industrial strains of *A. brasilense* named *start* and *calf* was used (Biofabrica Siglo XXI SA de CV).
Alternative carbon source in shaken flasks: Cultures were performed in 500ml Erlenmeyer shake flasks containing 100ml of NFb medium, (malic acid as control), sodium succinate, sodium gluconate and glycerol were tested at a concentration of 5 g/l, 30°C and 150rpm.
Air flow rates in column bubble reactor: Reactor cultures were performed in a 6 liter column bubble reactors, using a working volume of 5 liters. Culture medium was NFb. Cultures were inoculated at 10% of the volume reactor. Temperature was controlled at 30°C. Airflow were evaluated at 0.1, 0.5 and 1.0 volumes of air per volumes of culture medium (vvm).

Results. Growth of *A. brasilense* on alternative carbon sources. The maximum OD_{600nm} achieved for both strains of *A. brasilense* after 28h of culture was obtained with sodium gluconate (4.59 au for *start* and 4.92 au for *calf*). However, this optical density does not correspond to viability ($3.5x10^7$ CFU/ml for *start* and $4.3x10^{10}$ CFU/ml for *calf*). This is probably due to the intracellular polyhidroxybutiyrate (PHB) accumulated. It is noteworthy that the higher specific growth rates were obtained with malic and succinate acid (30% over sodium gluconate).
Growth of *A. brasilense* under different air flow rates. The air flow which supports the growth of *A. brasilense* was 0.1 vvm. In this flow was yielded the highest optical density and biomass measured as CFU/ml ($2.8X10^9$ *start* and $8.6x10^{10}$ *calf*) for both strains. These results are consistent if we considered the physiology and the environment of this microaerophilic organism.

Conclusions. In terms of biomass production it was found that changing the carbon source to sodium gluconate, results in an increase of biomass at the end of the culture (at least for *calf*). Respect to growth in the bubble column reactor *A. brasilense* is favored at low air flows, reaching the order of 10^{10} for *calf* (over an order on agitated batch cultures) which is a good indicator to try a fed-batch culture considering these parameters.

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