Industrial Microbiology MBIO 4510
PROJECT ABSTRACTS

Dilution effect on high initial sucrose concentration for optimal production of citric acid by *Aspergillus niger*.

Group 1: Audrey Javellana, Ashley Peape, and Nicole Mwaura

The objective of this experiment was to optimize the citric acid production, of *Aspergillus niger,* by varying the dilution effect on a high initial sucrose concentration. This was done by replicating the directions in the MBIO 4510 Lab 2 manual for preparation of media, except that we used a initial sucrose concentration of 60% rather than 30% (this was because we wanted to maintain a high initial sucrose concentration after dilution). At approximately 32 hours, the original inoculum was diluted by a factor of 1 (undiluted), 3/4, 2/3, ½ (optimum dilution grade), 1/3, ¼ with deionized water adjusting to pH 2.0 with 6N HCl using a pH meter. All other procedures are as indicated in the MBIO 4510 lab manual.

The results indicated that the ½ dilution was the optimal dilution factor for citric acid production. It also showed that 1/3 and ¾ dilutions also resulted in high levels of citric acid. These diluted samples all produced more than twice as much product as the undiluted sample. We also observed a significant decrease in biomass in our diluted samples. This is as expected since citric acid is a primary metabolite and the factors needed for high citric acid excretion are below the optimal conditions for biomass growth.

Determination of % Sucrose Concentration w/v in media leading to Optimal Citric Acid production by *Aspergillus niger*

Group 2: Tran K., Vu J., Szulc A.
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Based on the present knowledge surrounding citric acid production by *Aspergillus niger*, varying the environmental parameter of sucrose concentration in the media will allow for the determination of optimal citric acid production by the process of a small-scale liquid fermentation. Media sucrose concentrations of 2.5% 10% 14% 22% and 30% w/v respectively were used. *Aspergillus niger* was incubated in the media, then later the samples were centrifuged and biomass mass was separated from the supernatant containing the citrate. A spectrophotometric analysis of the samples containing the citrate allowed for the quantification of citrate produced. Citric acid excretion by *Aspergillus niger* occurs under suboptimal conditions, therefore by measuring the citrate produced in regards to sucrose concentration in media, we are able to determine at which concentration we get maximum citrate production. An optimal sucrose concentration for citric acid production was at 10%, with a range with similar production between 10%-22% was observed. A low sucrose concentration of 2.5% showed only a fraction of the citrate produced, while a maximum sucrose concentration of 30% showed a slowed production of citric acid. Yet actual biomass of *Aspergillus niger* steadily increased with increasing media sucrose concentration, with almost a 75g/L difference between 2.5% and 30% w/v media sucrose. With the obtained data we assumed that the growth of the organism increased with an increase in carbon source, meanwhile the optimal condition for secretion was at 10% w/v media sucrose concentration.

Effect of Manganese on Citric Acid Production of *Aspergillus niger*

Group 4: Gillian Stangl and Carter Van Alystne

The citric acid production of *Aspergillus niger* is dependant upon many environmental factors, including the concentration of manganese (Mn^{2+}) in the medium. The objective was to examine the effect of manganese on the accumulation of citric acid produced by *A. niger*. By varying the concentration of Mn^{2+} in the media (1mg/ml-5mg/ml), the production of citric acid increased as the concentration increased. This was determined using a spectrometer to detect the presence of NADH, which is produced proportionally to citric acid. According to the results, in order to maximize citric acid production, a higher concentration of manganese should be added to the media.

The Positive Effect of Aeration on *Aspergillus niger* Citric Acid Production

Group #8 : Amy Nikkel, Arianne Blanco

This purpose of this experiment was to study the growth of *Aspergillus niger*, and to optimize its production of citric acid through the process of fermentation. This was done by varying environmental conditions, and the parameter in study was oxygen transfer rate, i.e. aeration. A series of identical tubes were setup with a basic *Aspergillus niger* medium, which were then inoculated with a spore suspension of *Aspergillus niger*. The tubes were then incubated at 28 degrees Celsius using a rotor for aeration. Duplicated tubes were then removed from on a daily basis and incubated at 28 degrees Celsius. Each tube was incubated for a total of 4 days, but aeration was varied by removing tubes at 0, 1, 2, 3, and 4 days of rotation. At the end of the sampling period, the biomass for each time point was taken. Based on the following equation, [citrate + NADH $\rightarrow$ malate + acetate + NAD^+], the presence of citrate was measured for, by measure absorbance of NADH at λ=350 nm. It was expected that increased aeration would result in increased production of citric acid. However, experiments and calculations appeared to show that this did not occur. As aeration increased, slight decreases in mM of citrate produced per grams per litre of biomass was observed. It was then concluded that aeration did not increase citrate production.

Citric acid production is increased at higher pH in *Aspergillus niger*

Group 9: Stephanie Armstrong and Lucy Yan

Aspergillus niger is a fungus often used in industrial fermentation processes to produce citric acid. A number of studies have been conducted using this organism to determine optimal conditions for citric acid accumulation. The effects of pH on citric acid production is recognized, but not well understood, so our objective was to optimize citric acid production by *Aspergillus niger* by varying environmental pH. After incubating the organism with four basic media of varying initial pH, citrate production was measured by coupling the reactions of citratase and malic dehydrogenase and observing with a spectrophotometer the corresponding decrease in absorbance as NADH was transformed into NAD+. It was found that increased pH corresponded to increased citric acid production, which would lead one to conclude that higher pH is optimal for citric acid production.

Methanol increases the production of citric acid by *Aspergillus niger*

Group 10: Yosha Jayasundra, Natalie Middlestead, Sara Frank

Citric acid is used in many medical products and also in food preservation. Citric acid is produced in a reaction involving citrate lyase and malate dehydrogenase. In this experiment, the amount of citric acid

produced when Aspergillus niger is grown with varying amounts of methanol is measured using absorbance. The change in absorbance is measured using a spectrophotometer at 340nm. Citrate combines with NADH producing malate, acetate and NAD+. NADH absorbs at 340nm while NAD+ does not. The amount of citric acid produced can be influenced by many factors such as methanol, aeration and pH. Methanol is added to media giving final concentrations of 0%, 1%, 2%, 3%, and 4%. When produced industrially, a greater yield of citric acid is desired. The results of the experiment show that at a 4% final concentration of methanol more citric acid is produced and released into the surrounding media by Aspergillus niger. This may be due to methanol increasing the membrane permeability which aids in excretion of citric acid into the medium. In general, as methanol concentration increased, so did the amount of citric acid produced.

The Negative Effect of Increasing Spore Inoculum Density on Citric Acid Production by *Aspergillus niger*

Group 11: By Ainsley Winter and Hsueh Yee Seow

Citric acid is used widely for a variety of applications. In the microbial industry, *Aspergillus niger* is a fungus commonly used to produce citric acid. However, not much research has been done on the effect of spore inoculum density on citric acid production by *Aspergillus niger*. We carried out an experiment to characterize the optimization of citric acid production by *Aspergillus niger* based on spore inoculum density. We varied the spore inoculum densities in small-scale liquid fermentation units by using spores within the range of $10^3 – 10^6$ spores/ml. After 5 days, citric acid production was measured by coupling reactions by citrate lyase and malic dehydrogenase and measuring the decrease in absorbance at 340nm. The results were correlated with the spore inoculum density. We also obtained the biomass of *Aspergillus niger* and correlated the effect of biomass to citric acid production. Our results show that as spore inoculum density increases, the fungal biomass of *Aspergillus niger* increases from $10^3 – 10^6$ spores/ml and citric acid production decreases. In conclusion, to optimize citric acid production, fewer spores should be inoculated into media.

Inhibitory Effects of Citric Acid Supplementation on *Aspergillus niger* Citric Acid Productivity and Correlative Enhancement on Cellular Biomass

Group 12: Tobin Verbeke, Daniel Papetti and Mila Sailer

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The production rate of citric acid by *Aspergillus niger* was measured. Addition of 250µl of a 1.5 molar citrate solution was added to a growing media of *Aspergillus niger* at times, 0, 24, 48, 72, and 96 hours. Final citrate concentrations were measured by spectrophotometry at 196 hours. The results show an increase in citrate production if citrate addition occurs at a later stage of growth and therefore suggests the inhibition of citric acid productivity by adding citric acid to the media. These results were correlated to the biomass in each media, measured by determination of culture mass after centrifugation and drying of all media at 196 hours. It was found that the average biomass of *Aspergillus niger* was lower if additional citrate occurred at a later time during growth. These findings suggest that the addition of citrate causes an increase in *Aspergillus niger* growth. It was hypothesized that citrate causes more rapid growth, which in turn causes a decrease in citrate production and metabolism. If citrate is added at

later stages, the fungus remains in stationary phase for longer periods of time, produces metabolic intermediates such as citrate and expels them extra-cellularly in preparation for growth.

The Effect on Citric acid Production of an *Aspergillus niger* Culture when Administering a Daily Ammonia Boost

Group 13: Ainsley Little, Kristen Bouchard

Citric acid production from *Aspergillus niger* is known to be most favorable at growing conditions that are sub-optimal. In this experiment, daily ammonium nitrate boosts were added to select *Aspergillus niger* spore cultures to determine the effect on citric acid production. Previous research indicates that increased nitrogen concentrations from sources such as ammonium nitrate improve the growth conditions for *Aspergillus niger*. Therefore, it was hypothesized that daily ammonium nitrate boosts would decrease the production of citric acid. The results obtained did in fact indicate that *Aspergillus niger* supplemented with daily ammonium boosts (containing 0.008 g of ammonium nitrate) lead to a continuous decrease in citrate production. Citric acid production from *Aspergillus niger* spore cultures that received no ammonium nitrate boosts produced 0.37 mM of citrate whereas spore cultures that received 4 days of consecutive boosts produced only 0.15 mM of citrate. In addition, no correlation between ammonium nitrate boosts and biomass were observed.