Optimization of Process Variables of Citric Acid Production Using *Aspergillus Niger* In A Batch Fermentor

K. Anand Kishore, M .Praveen Kumar, V. Ravi Krishna and G. Venkat Reddy

***Abstract*—Citric acid Production using *Aspergillus Niger*-NCIM 705 is influenced by the Process variables such as Initial sucrose concentration, initial p^H, nutrient concentration, additives, stirrer speed, incubation period, fermentation temperature, Air and O_2/N_2 supply. The Present work deals with, the study on the effect of Stirrer speed and Oxygen flow rate on the yield of Citric acid, Substrate consumption and biomass generation and the Optimization of the same variables using Genetic algorithm,. It is found that stirrer speed of 230 rpm was the most favorable speed producing highest amount of Citric acid (30.7 g/l) with initial sucrose concentration of 60 g/l. Lower amounts of citric acids were found to be produced with other speeds (250-310 rpm).The oxygen flow rate of 1.0 lpm was observed to he most favorable flow rate giving maximum amount of Citric acid (26.6 g/l) with initial sucrose concentration of (60 g/l).. Lower Citric acids were found to be produced with other flow rates (1.5 -2.5 lpm).**

***Index Terms*— Citric acid, optimization, genetic algorithm, *Asperigillus niger*.**

I. Introduction

One of the most important fungi used in the industrial microbiology *Aspergillus niger* has been employed for many years in the production of citric acid. Citric acid is produced from bulk hydrated materials and as a by-product of sugar production by Asergillus niger [2]. However the demand for citric acid production is increasing faster than its production and hence more economical processes are required [3]. The growth and production of citric acid will greatly be affected by medium composition, fermentation parameters and stimulators. Citric acid (2-hydroxy2, -propanetricarboxylic acid) productivity by *Aspergillus niger* can be improved by optimizing the fermentation parameters such as Initial sucrose concentration nitial p^H utrient concentration Additives Stirrer speed. Incubation period Fermentation temperatureAir and $O2/N_2$ supply[1]

A genetic algorithm (GA) is a search technique used in computer science to find approximate solutions to optimization and search problems. Genetic algorithms are a particular class of evolutionary algorithms that use techniques inspired by evolutionary biology such as inheritance, mutation, and recombination. Two elements are required for any problem before a genetic algorithm can be used to search for a solution: First, there must be a method of representing a solution in a manner that can be manipulated by the algorithm. Traditionally, a solution can be represented by a string of bits, numbers or characters. Second, there must be some method of measuring the quality of any proposed solution, using a fitness function. For instance, if the problem involves fitting as many different weights as possible into a knapsack without breaking it, a representation of a solution might be a string of bits, where each bit represents a different weight, and the value of the bit represents whether or not the weight is added to the knapsack. The fitness of the solution would be measured by determining the total weight of the proposed solution: The higher the weight, the greater the fitness, provided that the solution is possible.

II. Previous Work

M. Y. Lu," I. S. Maddox a & J. D. Brooks b [2] were investigated as kumara and taro were excellent substrates for citric acid production by solid-substrate fermentation using Aspergillus Niger- Conversely, potato was a poor substrate, although it supported profuse fungal growth. A kinetic analysis of citrate production from kumara showed an overall reactor productivity of 0.48 g citrate/kg wet weight kumara per hour, with a yield of 0.54 on a weight basis (g citrate produced/g starch used). Maximum citrate production rates were observed after 2 to 3 days of fermentation, while the fungal growth rate was still high. The optimum moisture content of the kumara for citrate production was 65% (w/w) or above, while metal ions were shown not to be inhibitory to the process.

Atsushi Suzuki, somsak sarangbin, kohtaro kirimura, and shoji usami [3] were reported as citric acid production from starch by Aspergillus Niger was studied by the shake and semi-solid culture methods. From a practical viewpoint, direct production of citric acid from corn- and potato starch was examined using the semi-solid culture method. When cultivated in a semi-solid culture using bagasse as a carrier, produced 107.4 and 92.9 g/l of citric acid, approximately 1.14 and 1.09 times as much as from 200 g/l of corn- and potato starch, respectively.

M. Papagianni1, M. Mattey, B. Kristiansen [4] were reported as the relationship between Aspergillus Niger

morphology and citric acid production was investigated in two reactor systems with different configurations, a tubular loop and a stirred tank bioreactor, with operating volumes of 6 and 8 dm3, respectively. Morphology was quantified by image analysis. In each system, morphology, characterized by the parameter P (mean convex perimeter of clumps), and citric acid production, were agitation-dependent and closely linked. Increased agitation caused a reduction of clump sizes and results when both reactors demonstrate that the parameter P should not exceed a threshold value in order to achieve increased productivities. The results obtained from the two reactors were in agreement, both qualitatively and quantitatively. Also, relationships valid for one system accurately described the results obtained from the other system, demonstrating the validity of the relationship between morphology and productivity for the particular fermentation, regardless of the reactor type. Previous attempts to evaluate the use of loop con®gurations as scale-up tools and their performance as bioreactors, neglected the morphology of the producer micro-organisms.

Mihir lal saha, yasuzo sakai, and fuji takahash.[5] were reported that the use of methanol or ethanol in the magneto-biotechnological technique was found to be enhance citric acid production in a magnetic drum contactor operation with Aspergillus Niger. Continuous and repeated-batch fermentations were conducted with the addition of 2% ethanol. Continuous fermentation for 50 d gave a better citric acid yield (85%) and average productivity (3.8 g/l) than repeated-batch fermentation over 60 d (65%, 2.3 g/l).

Luciana P.S. Vandenberghe Carlos R. Soccol a, Ashok Pandey, J.-M. Lebeault [6], were reported Solid-state fermentation was carried out to evaluate three deferent agro-industrial wastes, sugar cane bagasse, husk and cassava bagasse for their efficiency in production of citric acid by a culture of Aspergillus Niger. Cassava bagasse best supported the mould's growth, giving the highest yield of citric acid among the tested substrates. Results showed the fungal strain had good adaptation to the substrate (cassava bagasse) and increased the protein content (23 g/kg) in the fermented matter. Citric acid production reached a maximum (88-g/kg dry matter) when fermentation was carried out with cassava bagasse having initial moisture of 62% at 26°C for 120 h.

Sikander Ali, Ikram-ul-Haq, M.A. Qadeer and Javed Iqba [7] were investigated that sixteen different cultures of Aspergillus Niger were isolated from different soil samples. These isolates of Aspergillus Niger were evaluated for citric acid fermentation in shake flask. Sucrose salt media was used and the volume of fermentation medium was kept at 25 ml. The cultural conditions such as pH (3.5), temperature (30°C), incubation period (8 days) and sugar concentration (15%), were optimized.

III. Experimental

A 1.2 liter capacity fermentor made of glass [Scigenics (India) Pvt. Ltd.] equipped with standard control and instrumentation was used for the citric acid fermentation. The fermentor equipped with a flat blade impeller with three blades. Two 500 ml bottles were provided to the fermentor for the addition of acid and base, one silicon tube was provided for the addition of sterilized silicon oil to control foaming. The fermentor has arrangements for measuring pH and temperature by digital pH controller and digital temperature sensor. Cooling water supply was provided to maintain the temperature in the fermentor at the desired level. There are provisions for supplying Air, N_2 and O_2 at desired flow rates. The experimental set up is shown in Fig. 2.

Fermentor was thoroughly cleaned with water and sterilized in an autoclave for 20 minutes. The sterilized fermentor was placed in the main assembly and tube connections were given for water and air. Then the sterilized medium containing vegetative inoculum was transferred to the fermentor from the conical flask after 24 hours of incubation. Thus the system was ready for the process. The power was switched on. The experimental conditions maintained were; agitator speed 200 rpm, fermentation temperature 30^0C and air flow rate 1-lpm. Periodically samples were collected from the fermentor and analyzed for citric acid, sucrose and biomass concentrations.

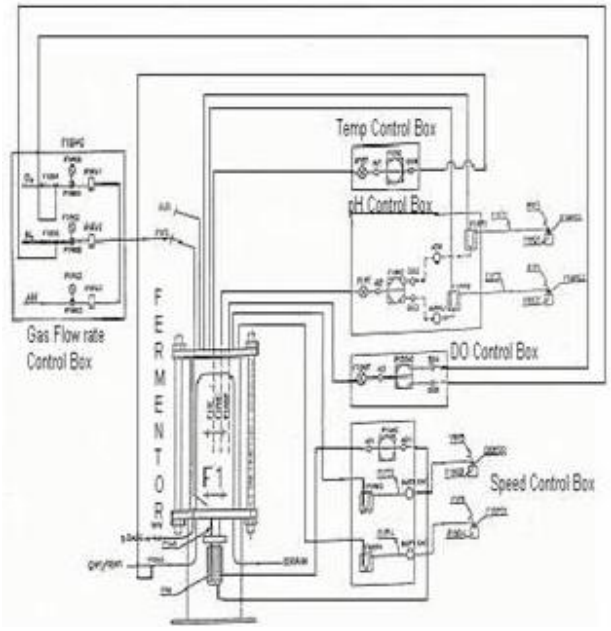

Fig. 1 Exponential set up

IV. Estimation Methods

A. *Estimation of Citric Acid*

Citric acid was estimated gravimetrically, using pyridine– acetic anhydride method as reported by Marrier and Boulet (1958). One ml of the diluted culture filtrate along with 1.30 ml of pyridine was added in the test tube and swirled briskly. Then 5.70 ml of acetic anhydride was added in the test tube. The test tube was placed in a water bath at 32C for 30 min. The absorbance was measured on a spectrophotometer (405 nm) and citric acid contents of the sample were estimated with

reference (run parallel, replacing 1.0 ml of the culture filtrate with distilled water) to the standard. The % of citric acid was determined.

B. Estimation of Sucrose

Concentration of sucrose was estimated by DNS method. A single beam UV scanning spectrophotometer (Systronic made and model-117) was used for measuring color intensity. One drop of concentrated HCl solution was added to 1 ml of the sucrose solution in a flask. The flask was heated to 90^0C for 5 minutes to allow hydrolysis. Three drops of 5 N KOH solution were added to neutralize the acid, because the DNS method must be applied in an alkaline condition to develop the red brown color which represents the presence of reducing sugars. Then the DNS reagent was added and the colour intensity was measured at 220nm. The color intensity is proportional to the concentration of sugar.

C. Estimation of Biomass

50 ml of sample was filtered through a free weighed filter paper. After filtration it was dried in an oven at 80 ^{0} C. cool it in desiccators then measure the final weight. The difference between initial and final weights was the biomass. .

V. Results and Discussion

In the present study, two parameters, namely Stirrer speed and O_2 flow rate are studied and the same are optimized using Genetic algorithm. Stirrer speed was varied from 170 to 310rpm and O_2 flow rate was varied between 0.5 to 2.5 lpm, keeping all other parameters such as Fermentation temperature=30^{o}C, Incubation period=24 hours, Initial sucrose concentration=60g/l, pH=6.0, Culture medium (Potato dextrose agar medium).

Effect of Stirrer speed

The variations of citric acid produced, sucrose consumed, and biomass generated plotted against time at each stirrer speed were shown in Figures 2-7 and this indicate that the citric acid production, sucrose consumption, and biomass generation were gradually increased with fermentation time and simultaneously sucrose concentration was decreased. It was observed in above figures that during initial stages, substrate consumption was low but after 96 hours of fermentation, its consumption increased and citric acid production gradually increased

For the comparison purpose, variations in citric acid produced, sucrose consumed and biomass concentration were plotted against Stirrer speed at 168 hour and were shown in Figure-8. The figure shows the highest production of citric acid (30.71 g/l) is obtained at 230rpm stirrer speed. The citric acid concentration varied from 17.2 to 30.7 g/l as stirrer speed varied from 170 to 230 rpm. As citric acid production increased, fast increment in sucrose consumption, due to acid formation and gradual increase in the concentration of biomass was observed because the microorganism was in exponential growth phase.

Effect of Oxygen flow rate:

The variation of citric acid produced, sucrose consumed and biomass concentration was plotted against Oxygen flow rate which was shown in **Figure 14**. From this figure, it was observed that with increase in concentration of citric acid, substrate consumption increased significantly up to Oxygen flow rate of 1.0 lpm. Further increase in flow rate caused decrease in citric acid production and substrate consumption. At Oxygen flow rate 1.0lpm, maximum citric acid (26.6 g/l) was obtained.

Optimization was done using **genetic algorithm** (one of the optimization techniques.). Algorithm is given below

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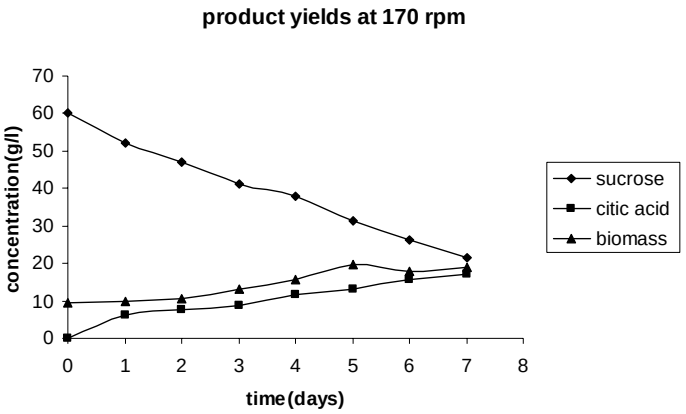

Fig 2: product yields at 170 rpm

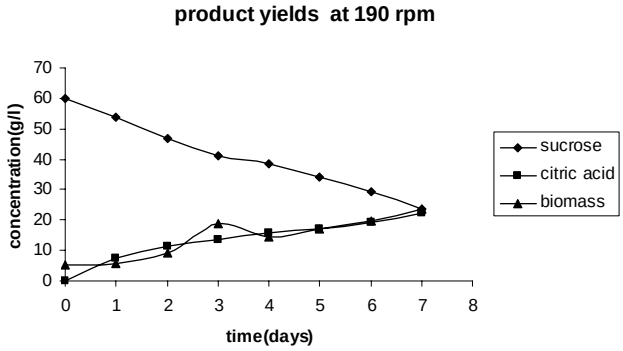

Fig. 3 Product yields at 190 rpm

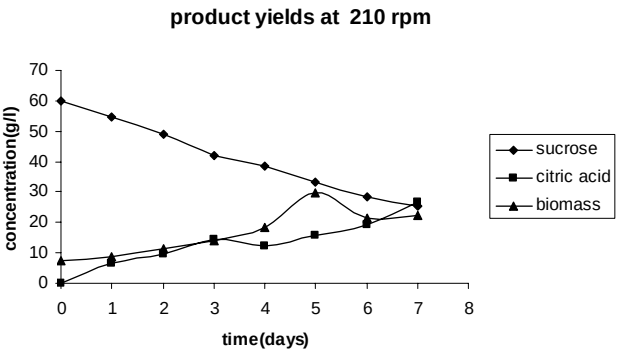

Fig.4: product yields at 210 rpm

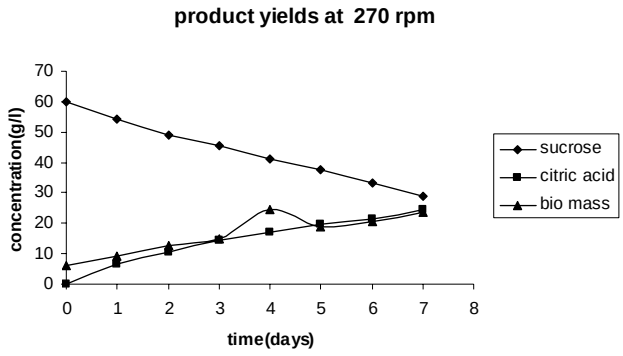

Fig. 5: product yields at 270 rpm

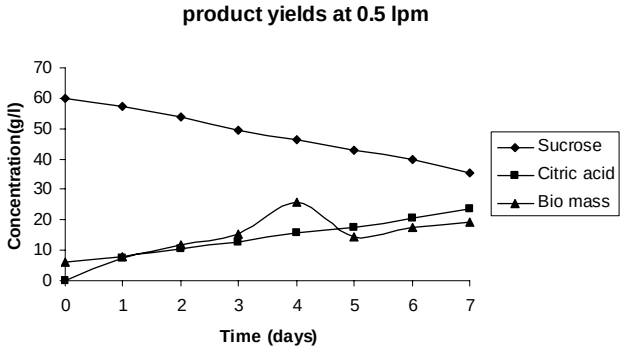

Fig 9: product yields at 0.5 lpm

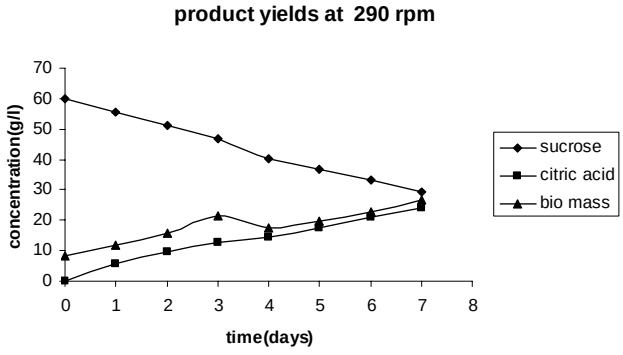

Fig 6: product yields at 250 rpm

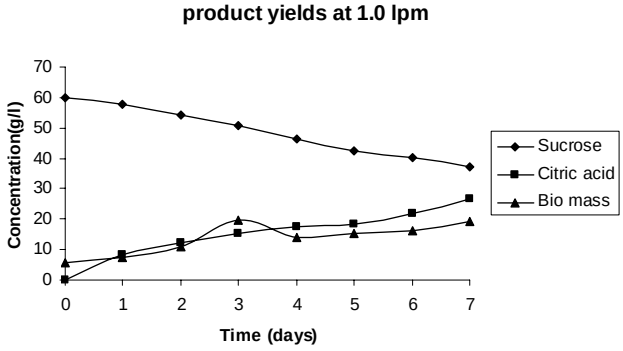

Fig 10: product yields at 1.0 lpm

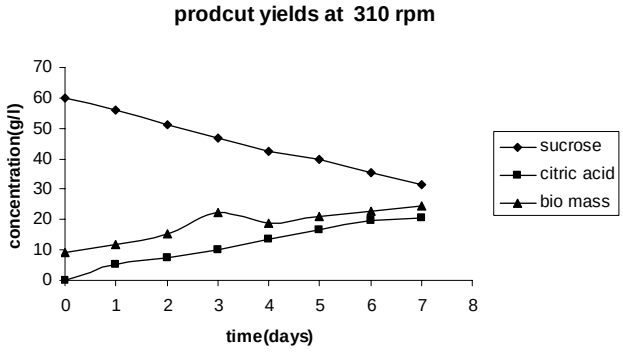

Fig 7: product yields at 310 rpm

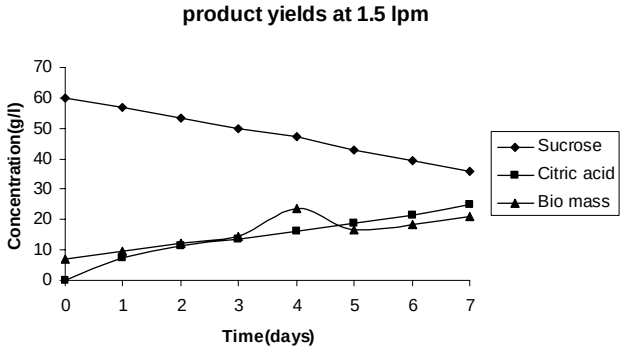

Fig 11: product yields at 1.5 lpm

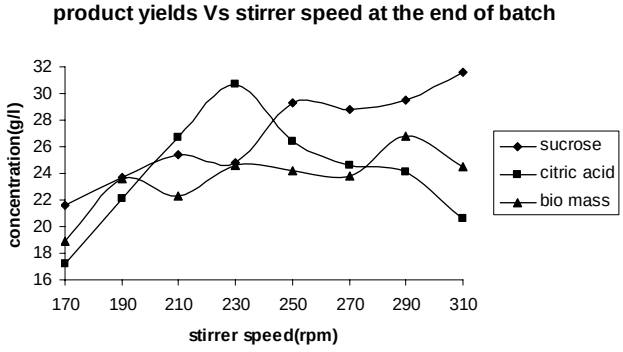

Fig 8: product yields Vs Stirrer speed at the end of the batch

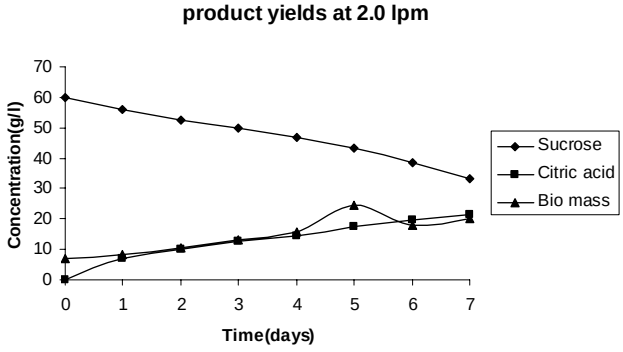

Fig 12: product yields at 2.0 lpm

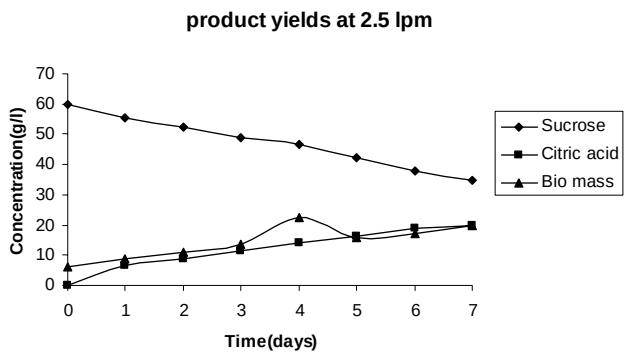

Fig 13: product yields at 2.5 lpm

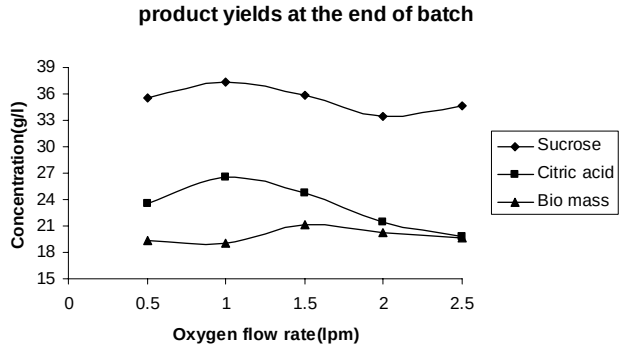

Fig 14: product yields at the end of batch for O_2 flow rate

Optimization by Genetic Algorithm

1. Take the experimental data between the input (Stirrer speed and Oxygen flow rate and output (citric acid yield).
2. Using curve fit technique; find the mathematical equation (relation) between input and output.
3. Optimize the output by controlling the input using the genetic algorithm.
4. Consider population size=20; chromosome length=8, Input minimum= 170 rpm and 0.5 lpm l respectively; input maximum = 310 rpm and 2.5 lpm respectively
5. Generate the initial population (20 chromosomes) by random generation of 0's, 1's.
6. Decode the generations into actual values of input.
7. Find the fitness values (citric acid production) for the total population using equation from the curve fitting.
8. Arrange fitness values in the ascending order and arrange chromosomes in same order.
9. Find the difference error= abs (fit (1)-fit (population size)).
10. If error <=eps, stop the process, go to 20.
11. If error >= eps, go for next generation, step 5.
12. **Elitism**: copy the 10% of top previous population(2 chromosomes) to the next population
13. **Cross over**: select the two parents from the previous population using roulette wheel technique.
14. Apply cross over between these two parents using different marketing techniques. We will get two children for next population.
15. Generate total population using cross over technique.
16. **Mutation**: generate a random value. if the random value<2% of population size
17. Then select a chromosome and a bit from that chromosome randomly from the none population, and made it inverse (i. e if it is '0', made it '1' vice-versa).
18. Then we have new population of '20'.
19. Then go to step 6.
20. 1st chromosome of that population is the optimal input for the maximum production of citric acid.

A polynomial is developed using input (Stirrer speed, rpm) and output (Citric acid Yield gm/lit), relating input to the output. For stirrer speed versus citric acid production the following equation has been established. The polynomial has been used to get the optimum value of speed, using the genetic algorithm. The experimental results and the values generated by genetic algorithm have been plotted in figure15

$$Y = 0.292796 - 1.43324\,X + 0.018859\,X^2 - 7.34\,E^{-05}\,X^3 + 9.15\,E^{-08}\,X^4$$

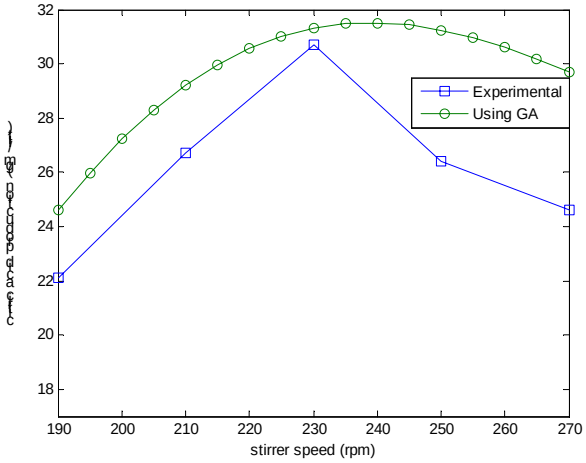

Figure 15: showing closeness of Equation approximated for GA, to optimize stirrer speed

Using Genetic algorithm, the optimum value of stirrer speed is 237.81 rpm

A similar polynomial is developed using input (Oxygen flow rate, lpm) and output (Citric acid Yield g/l), relating input to the output. For stirrer speed versus citric acid production the following equation has been established. The polynomial has been used to get the optimum value of oxygen flow rate, using the genetic algorithm. The experimental results and the values generated by genetic algorithm have been plotted in figure 16.

$$Y = 0.04881 + 78.0754X - 76.925X^2 + 30.12222X^3 - 4.23333X^4$$

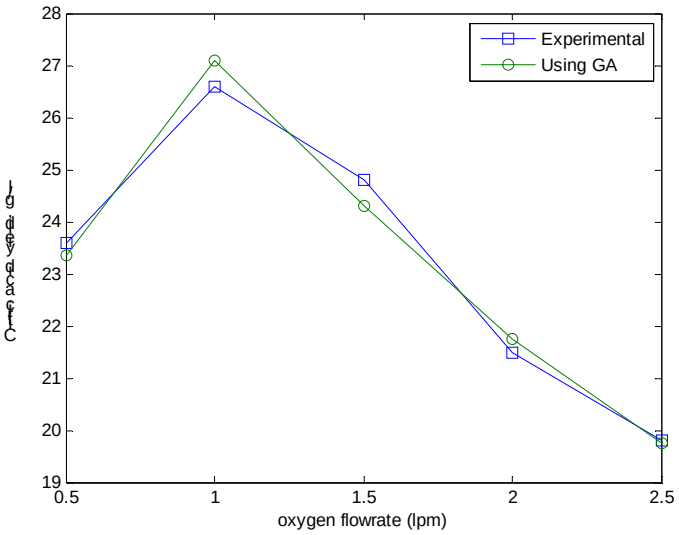

Fig.16 showing closeness of Equation approximated for GA, to optimize Oxygen flow rate.

Using Genetic algorithm the optimum value of oxygen flow rate is 0.9219 lpm

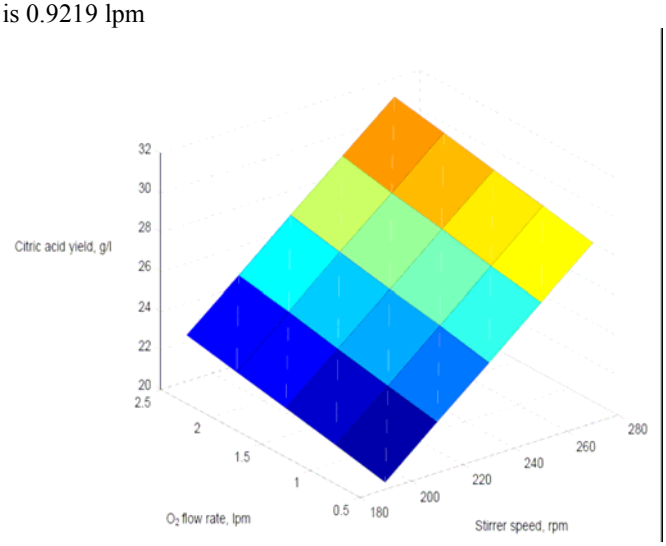

Fig 17: Linear model of Citric acid yield in terms of stirrer speed and O_2 flow rate

VI. Conclusion

Based on the results of this study, the following conclusions can be made

Increasing stirrer speed enhanced the mechanical forces on the fungal cells. Agitation also determines the mixing conditions in the reactor which greatly influence the sucrose mass transfer. It indicates that stirrer speed was found to be the most important factor affecting citric acid yield in our experiment. Higher stirrer speeds resulted in higher citric acid yield. However, it was observed that with the stirrer speed higher than 230 rpm, micro-organism *(Aspergillus Niger)* may be severely damaged by shear stress which resulted in poor biomass development (Dawson et al. 1986; Sanjay and Sharma, 1994). Thus, a stirrer speed of 230 rpm is considered to be the optimal rate for maximum citric acid yield.

Proper agitation intensity is important for the maintance of a suitable oxygen supply to the mould growth.If the aeration rate is too high, the partial pressure of dissolved CO_2 in the medium can become too low. Carbon dioxide is important as a substrate for pyruvate carboxylase which replenishes the supply of oxaloacetate for citrate synthesis. Citric acid production was significantly decreased when the aeration rate was increased beyond 1.0 lpm. Therefore 1.0 lpm oxygen flow rate was optimized for citric acid production in stirred fermentor.

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