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Kinetic Study of Acetic Acid (Vinegar) Production from Start Fruit Juice in a Bioreactor

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A two stage fermentation process for production of acetic acid using star fruit juice with *Saccaromycescerevisiae* and *Acetomonasacetii* was conducted in a 2 L stirred tank bioreactor. The effect of inoculum size (5%,10%,15%) and agitation speed (250 rpm, 300 rpm, 350 rpm) on ethanol and acetic acid production of the juice were evaluated. Kinetic parameters on specific growth rate, yield, doubling time (i.e. ì,Yp/s. t_d,) of the fermentation process were also recorded. The optimum condition obtained from this batch fermentation was at 250 rpm of agitation speed and 5% inoculums sizes with the maximum product yield ($Y_{ethanol/glucose}$) of 0.859 g/g and ($Y_{Acetic\ acid/Ethanol}$) of 0.342 g/g. The maximum specific growth rate, ì was 0.097h^{-1} for *Saccharomyces cerevisiae* while 0.085h^{-1} for *Acetobacteraceti* using initial glucose concentration of 20%. Using Central Composite Design experiment, for optimization of acetic acid production, the R_2 value of 0.8372 was achieved and the highest acetic acid production obtained was 2.76 %TA while highest ethanol obtained was 17.8% or equivalent to 140.64 g/L. The above findings can be further utilized in fermentation process to produce vinegar in large amount from our tropical star fruit, *Averrhoacarambola* juice.

Key words: Star fruit vinegar, bioreactor, agitation speed, inoculums size.

Many natural vinegar such as apple cider vinegar, rice vinegar, malt vinegar, wine vinegar and many others are found in the market that are produced through fermentation process using specific substrates. In Malaysia there is abundance of tropical fruits found which can be used as substrate to produce fruit vinegar with good flavor. Many variety of vinegar are used widely as condiment and flavour enhances in cooking dishes and also used in salads and other vegetable dishes. The abundance of *Averrhoacarambola (*Carambola orstar fruit) found in Malaysia with their strong and good pleasant flavor offers a potential substrate that can be fermented with *Saccharomyces cerevisiae* and *Acetobacteracetii* to produce fruit vinegar. Vinegar production methods can vary with the use of wood cracks (Orleans Process) and surface culture (Generator Process) or trickling bioreactor system to submerged fermentation[6] are commonly used, however the use of stirred tank bioreactor may offer a better and effective system for fermentation of vinegar. Thus a kinetic study of acetic acid (vinegar) from star fruit juice in a 2 L stirred tank bioreactor was undertaken using different size inoculums of the microbial culture and different agitation speed of the reactor on the specific growth rate, doubling time of culture and yield of acetic acid (i.e. ì, t_d, $Y_{p/s}$) of the fermentation process.

Many fermentation parameters such as temperature, pH, agitation speed, dissolved oxygen level in bioreactor have a significant effect in the growth and metabolic production of acetic

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acid using microorganisms. Growth kinetic study of the microbial cultures can be used to estimate the cost effective production of acetic acid in large scale. Wood[13]has explained that vinegar can be made from any non-toxic raw material that furnishes a juice or solution containing fermentable sugars. Narain*et al*.[9] has studied the physical and chemical composition of ripe Carambola and has found out that the pulp contains 90% of moisture content, total sugars increased from 2.91% to 5.60% and reducing sugar from 2.80% to 5.04% for the green to ripe stage of the fruit. The high value of sugar indicates that Carambola can be used as suitable substrate for producing vinegar, however, glucose can also be added to further increase the yield of vinegar in the fermentation process. *Saccharomyces cerevisiae* yeast is most commonly used in ethanol industry[8]to produce ethanol whereby subsequently ethanol then can be further fermented or oxidized to acetic acid (vinegar) by *Acetobacteracetii*.

MATERIALS AND METHODS

A study was undertaken in a 2 L stirred tank bioreactor (B Braun) using different size inoculums of the microbial culture and different agitation speed of the reactor on the kinetics of specific growth rate, yield, doubling time (i.e. ì, $Y_{p/s.}$ $_{td}$) of the fermentation process in vinegar production.

The *Averrhoacarambola* or star fruit that was sterilized were used as a substrate in the experiment. The yeast, *S. cerevisiae*was used to ferment the sugar in the star fruit juice to ethanol and followed by *Acetobacteraceti* for conversion of the ethanol to acetic acid. Both the cultures were supplied by the Department of Biotechnology Engineering laboratory at IIUM. For the experiment a 2 L fermentorwith 1 L volume of fruit juice was used . The optimization design experiment were used using Design Expert software with Central Composite design having 9 experimental runs with two parameters , agitation speed (250 rpm, 300 rpm, and 350 rpm) and inoculums sizes (5%, 10%, and 15%) of each culture (S*Saccharomyces cerevisiae* and *Acetobacteracet*iseparately) containing $2.5x10^7$ cell/ml juice substrate were used. *Saccharomyces* yeasts were grown in Potato Dextrose broth for 48 hr and harvested by centrifugation at 5000rpm/15 min for their yeasts cells and similarly for *Acetotobacter* were grown in Glucose Yeast Calcium medium and the bacterial cells were harvested by centrifugation at 5000rpm/ 20min. The yeasts and bacterial cells were dispersed in star fruit juice containing the appropriate concentration of cells per ml. The parameters chosen were based on previous study from shaker flask optimization by AzyanHazwani[2] and Noorhidayah[10] which used (5%, 10%, 15% inoculums size and 150, 200, 250 rpm agitation speed). Design of experiment was modified using a stirred tank bioreactor with fermentation process temperature of 30^oC and supply of air at 0.5vvm provided. The yeast inoculum were first inoculated into the fruit juice and fermented for 48 hr and followed by *Acetobacteraceti* for another 48 hr.

During fermentation sample of the fermented juice were collected at intervals of 12 hr for analysis of reducing sugar, ethanol production, acetic acid production, total cell number, Optical Density and Cell Dry Weight. For reducing sugar the DNS method was used according to the method described by Mohd Nasir[5]. Ethanol analysis was conducted by using potassium dichromate and acetic acid using the titration method according to AOAC method[1]. Total cell number (TCN) were determined by using the counting chamber method[11]. Cell dry wt were determined by centrifuging 10 ml juice at 5000 rpm for 15-20 min and drying the pellets inan oven at 80^0C overnight until a constant wt was obtained after placing in a dessicator.

RESULTS AND DISCUSSION

Alcoholic Fermentation

For ethanol production it was found that run 9 with fermentation process condition of 10% inoculums size and 250 rpm agitation speed produced highest ethanol percentage of 17.8 % at 36h fermentation period (Table 1). Thus, the amount of culture inoculum used and aeration at 250 rpm agitation in a bioreactor play a great role in influencing greater production of ethanol by the yeast culture during fermentation of the star fruit juice.

Fermentation of Acetic acid from star fruit juice

Our results showed the highest % TA (titratable acidity) of acetic acid production

obtained during fermentation was 2.76% acid using 250 rpm aeration and 5% inoculums size culture. The result obtained here is much higher as compared to that obtained by Mohd Nasir[5] with 1.63%TA using a process conditions of (300 rpm, 0.5vvm, 20% glucose concentration and 10% of inoculums sizes.

Based on ANOVA analysis it was found that agitation speed (model A) and inoculum size (model B) did not have much effect independently on the yield of acetic acid, however, the interaction of agitation speed and inoculums size did have effect on acetic acid production (Table 2). It can be clearly seen that the model was significant with the value of Probability, P to be higher than the F value (Prob> F) which is 0.0205. For any model to be significant; the value of probability to be higher than F-value (Prob> F) has to be lower than 0.0500. This indicates that the model A (agitation) is not significant, B (inoculums sizes) is not significant

Table 1. Production of ethanol from fermentation of star fruit juice using different inoculums sizes and agitation (rpm) speed in 2L bioreactor

Run	Glucose (%)	Aeration (vvm)	Agitation (rpm)	Inoculum Sizes (%)	Ethanol (%)	Acetic Acid (%TA)
1	20	0.5	250	5.0	17.6	2.76
2	20	0.5	350	5.0	5.17	1.02
3	20	0.5	250	15.0	11.26	0.78
4	20	0.5	350	15.0	9.98	1.50
5	20	0.5	250	10.0	17.8	1.32
6	20	0.5	350	10.0	6.15	1.50
7	20	0.5	300	5.0	10.03	1.30
8	20	0.5	300	15.0	9.7	1.08
9	20	0.5	300	10.0	13.66	1.26

Table 2. ANOVA results for response surface factorial experiment of model A (agitation) and model B (inoculum size of cultures)

Source	Sum of Squares	DF	Mean Square	F Value	Prob> F	
Model	2.12	3	0.71	8.57	0.0205	*significant*
A	0.12	1	0.12	1.42	0.2867	*not significant*
B	0.49	1	0.49	5.97	0.0584	*not significant*
AB	1.51	1	1.51	18.32	0.0079	*significant*
Residual	0.41	5	0.083			
C. Total	2.53	8				

Table 3. Kinetics of Acetic Acid Fermentation Using Bioreactor

Run (std)	μ *S. Cerevisiae* (h^{-1})	t_d*S. cerevisiae* (h)	$Y_{P/S}$ (g/g) (Ethanol)	$Y_{P/S}$ (g/g) (Acetic acid)	μ *A. aceti* (h^{-1})	t_d*A. aceti* (h)	Productivity (Ethanol g/g.h)	Productivity (A. acid g/g.h)
1	0.085	8.15	0.859	0.342	0.097	7.16	2.318	0.287
2	0.069	10.05	0.141	0.277	0.048	14.44	0.419	0.121
3	0.075	9.24	0.534	0.045	0.072	9.63	1.85	0.130
4	0.065	10.66	0.305	0.132	0.078	8.89	1.635	0.225
5	0.072	9.63	0.550	0.021	0.071	9.76	3.91	0.183
6	0.086	8.06	0.240	0.310	0.064	10.83	1.335	0.156
7	0.088	7.88	0.222	0.520	0.08	8.66	1.31	0.135
8	0.072	9.63	0.246	0.760	0.088	7.88	2.19	0.150
9	0.07	9.9	0.488	0.131	0.075	9.24	2.24	0.270

but however, the interaction of AB value is found to be significant.

The 3D response surface and contour plots response surface are used to further investigate the interactive effects of each independent variable. Results from Fig. 1 of contour plot showed agitation of 250 rpm and inoculums size of 5% has greater effect on the acetic acid production. It is possible 250 rpm agitation provide sufficient aeration for growth of the culture and the 5% inoculum has ample concentration of cells to produce acetic acid.

Kinetic Study of Acetic Acid Production

Results on growth kinetic (Table 3) showed the optimum condition obtained from this batch fermentation was at 250 rpm stirrer's agitation speed and 5% inoculums sizes with the maximum product yield ($Y_{ethanol/glucose}$) of 0.859 g/g and ($Y_{Acetic\ acid/Ethanol}$) of 0.342 g/g. The maximum specific growth rate, ì was 0.097h^{-1} for *Saccharomyces cerevisiae* while 0.085h^{-1} for *Acetobacteraceti* at an initial glucose concentration of 20%.

The range of the specific growth rate; µ for this study is consistent with findings of other previous study in bioreactor[3,7]. values of the µ or the specific growth rates were between in the range of 0.048 h^{-1} to 0.178 h^{-1} respectively. The value of yield coefficient for ethanol production; $Y_{P/S}$ (g/g) observed by Veny and Hasan[12] was in the range of 0.88 – 0.815 g/g glucose with the initial glucose concentration of 20 g/L being used. This is consistent with the value of $Y_{P/S}$ (g/g) obtained in this study which is in the range of 0.141 g/g – 0.859 g/g.

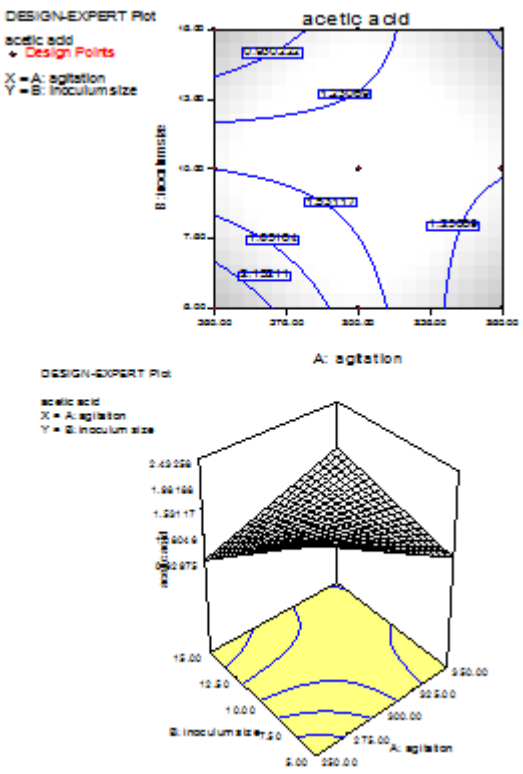

Fig. 1. Response surface described by the model equation to acetic acid yield value over independent variables agitation (rpm) and inoculums % (v/v). (i) Upper: Contour plot surface response (ii) Lower: 3D plot surface response

CONCLUSION

In conclusion, optimum bioreactor setting parameters are important to ensure the best culture condition prevail to optimize production of acetic acid in fruit juice.In stirred tank bioreactor, the best condition for the *Saccharomyces cerevisiae and Acetobacteraceti* to grow and produce optimum acetic acid in star fruit juice is at stirrer agitation speed of 250 rpm and inoculums size of 5%. The highest specific growth rate, $\mu$and yieldobtained were 0.859 and 0.342 g/g, respectively. The maximum concentration of acetic acid production was obtained in Run 1 with 2.76 %TA and highest ethanol production was showed by run 5 which gave 17.8% ethanol or yield of 150.34 g/L star fruit juice. at 36 hours fermentation. The TCNper mL of *S. cerevisiae is found* in run 5 at 36 hour fermentation having 2.1×10^8 cells concentration per mL juice substrate. The acetic acid value in run 5 is low due to the high ethanol concentration present which is eventually converted to acetic acid by the*Acetobacteraceti* which preceded for another 48 hr fermentation period. Previous studies[2,4,5]have shown that the production of vinegar from star fruit juice is achievable and is consistent to the findings of our study too.

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